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Age-Dependent Effects of Developmental Lead Exposure on Performance in the Morris Water Maze

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JETT, D. A., A. C. KUHLMANN, S. J. FARMER AND T. R. GUILARTE. *Age-dependent effects of developmental lead exposure on performance in the Morris water maze.* PHARMACOL BIOCHEM BEHAV **57**(1/2) 271–279, 1997.—The neurobehavioral toxicity of developmental exposure to lead (Pb) was investigated by conducting tests of spatial learning in the Morris water maze. Female Long–Evans rats were exposed to 0 or 250 ppm Pb acetate in the diet beginning 10 days prior to breeding and continued throughout gestation and lactation. Pups were weaned onto the same diets as the dams at postnatal day 20 (PN20). Increased levels of Pb were detected in the hippocampus of the 250 ppm Pb acetate group relative to controls. The highest concentration of Pb measured in the hippocampus was at PN21 with decreasing levels at older ages. In the Morris Water Maze, a statistically significant ($p < 0.03$; female rats) or near significant ($p < 0.07$; male rats) 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. This effect was only observed when rats were tested at PN21 and not at older ages. No significant effects of developmental Pb exposure were measured when rats were tested in a working memory paradigm of the Morris water maze at any age. These initial studies indicate an impairment of performance in the swim task in PN21 rats exposed to Pb during development. The age-dependent effect of Pb in this learning paradigm is consistant with previous studies in experimental animals and with the observation that children are more susceptible to Pb-induced cognitive deficits than adults. The Morris water maze may be useful for studying the effects of Pb on learning and memory, and their neurochemical basis. © 1997 Elsevier Science Inc.

Lead Development Rats Learning Morris water maze Age-dependence

EXPOSURE to environmental lead (Pb) is a major public health concern because of the global pervasiveness of this metal and its documented health effects. There is scientific evidence that chronic low-level exposure to Pb affects cognition in children (4,5,20), and there has been substantial progress made in identifying potential underlying neuronal mechanisms of Pb neurotoxicity (6,29). In our laboratory, we have recently demonstrated that *N*-Methyl-d-Aspartate (NMDA) and muscarinic cholinergic receptors are altered in the hippocampus of rats at 14 and 28 days of age, but not in older rats, that were exposed to Pb continuously during development (16). We have also found that protein kinase C (PKC) levels and activity were significantly altered in the hippocampus of Pb-exposed developing rats (8). In order to study further the mechanisms of Pb neurotoxicity and to correlate the neurochemical changes we observed with tests of learning and memory in rats, we initiated behavioral studies using the Morris water maze spatial learning task.

The Morris water maze was originally designed to test the ability of rodents to learn and memorize the location of a hidden platform in a pool of opaque water by its position relative to distal extramaze cues (22). This learning task was selected because a great deal of knowledge has been obtained on the neurochemical, neuroanatomical, and neurophysiological basis for the behaviors associated with this paradigm (21). For example, several neurotransmitter receptor antagonists have been shown to cause deficits in swim task performance when administered to rats (21). This was an important consideration in the selection of this behavioral test because the two most potent antagonists that impair spatial learning act at

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NMDA and muscarinic cholinergic receptors (21,24), the same receptor systems which we have shown to be altered in Pb-exposed rats (16). Besides the neurochemical effects of developmental Pb exposure, Pb also interferes with the induction of long-term potentiation (LTP) in the hippocampus (2,19), a cellular model of learning and memory (14,15). Impairment of hippocampal LTP pharmacologically (21), or by other means (28), has also been associated with impaired performance in the Morris water maze. Finally, performance in this swim task was originally shown to be highly sensitive to hippocampal lesions (22), an area known undergo morphological changes in rats exposed to Pb during development (1,17), and where we have measured Pb-induced neurochemical changes.

In the present study, we utilized the Morris swim task to assess the effects of Pb exposure on learning and memory in Long–Evans rats. An experimental protocol was used to provide continuous exposure of the pups to Pb in utero, during lactation, and from the diet after weaning. Performance in the water maze was assessed in control and Pb-treated rats at early and late stages of development. Two types of memory have been identified with this and other behavioral paradigms based on the interval of time required to learn a particular task. These are short-term or working memory, and long-term or reference memory. Working and reference memory in the swim task were assessed in different groups of rats. Different groups of rats were also used at different developmental stages. The goal was to determine if developmental exposure to Pb impairs learning of the Morris swim task at developmental time points when neurochemical changes in brain chemistry occurred in animals exposed to Pb in a similar fashion (16). We found that the ability of the Pb-exposed rats to learn the swim task was impaired at a time when neurochemical changes are present in the brain.

METHOD

Subjects

Adult female Long–Evans rats were housed individually and maintained at 23°C on a 12/12 h light/dark cycle. Female rats were randomly assigned to diets containing either 0 or 250 ppm Pb acetate. Food and water were allowed ad lib. The semi-purified diets (AIN-76) consisted of sucrose (50%), vitamin-free casein (20%), corn starch (15%), fiber (5%), AIN mineral mix (3.5%), AIN vitamin mix (1%), D,L-methionine (0.3%), and 250 ppm Pb acetate incorporated by the manufacturer (ICN Nutritional Biochemicals, Cleveland, OH). Diets were analyzed independently for verification of Pb content, and the levels measured did not differ more than 10% from the stated amount.

Dams were fed the diets beginning 10 days prior to breeding and continued throughout gestation and lactation. Litters were culled to 10 pups on postnatal day (PN) 1 and were weaned onto the same diets as the dams at PN20. Randomly chosen male and female rat pups from each litter were used for behavioral testing. A total of six different groups of rats (121 rats total) were tested in the swim tasks (see Table 1 for specific sample sizes within each group). Two groups were tested beginning at PN21, one group in the reference memory task, and one in the working memory task. Likewise two groups were tested beginning at PN56 and two groups at PN91. Thus, different rats were used in all behavioral tests. Upon completion of behavioral testing at each age, the rats were sacrificed by decapitation, and their brains were rapidly excised, weighed, dissected and frozen at −70°C. One hippocampus was used for Pb determination by a modified graphite furnace atomic absorption spectroscopy procedure (3).

Morris Water Maze Test

Behavioral testing of PN56 and PN91 rats was conducted in a round white pool 1.8 m in diameter and 0.7 m deep. A similar but smaller white pool (0.9 m in diameter and 0.5 m deep) was used for the PN21 age group because of the considerable difference in body size compared to the older age groups. This was done to circumvent the potential problem of exhaustion and greater task difficulty for the PN21 rats if they were tested in the larger pool. The dimensions of the pool used for the PN21 rats is very similar to that used in Morris swim task studies of pre- and post-weanling rats (18,27). The pools were filled to a depth of 30 cm with water made opaque with white, non-toxic water-based paint (Van Aken Int., Cucamonga, CA). Water temperature was maintained at 20 ± 2°C with aquarium heaters. The escape platform was a 25 cm^2 Plexiglas square for the small pool and a 80 cm^2 Plexiglas disc for the large pool. Both platforms were supported by adjustable Plexiglas stands that enabled them to be hidden 2–3 cm below the surface of the water. Testing was conducted in a room containing several extramaze visual cues. Results for each rat for each testing session were recorded on video tape and analyzed simultaneously using a digital tracking system that quantifies swimming time and distance (Videomex-V Image Analyzer, Columbus Instruments, Columbus, OH).

The general procedure for testing both reference and working spatial memory began by placing the rat on the hidden platform for 20 s. A trial was started by placing the rat in the pool facing the wall in one of four quadrants delineated by marks at the four cardinal directions. Rats were allowed to swim to the hidden platform and the escape latency (time to find the hidden platform) and pathlength (distance traveled to the hidden platform) were recorded. Trial lengths of 30 s and 60 s were used for the PN21 and PN56-PN91 groups, respectively. If the platform was not found within the allotted time, the rat was manually placed onto the platform. This procedure was repeated with each rat from starting positions in all four quadrants. Rats were allowed to rest on the platform for 20 s between each trial (inter-trial interval). The 20 s inter-trial interval was used for the PN21 group despite the shorter trial length compared to older rats. This was done to allow for a greater relative period of rest for the PN21 rats and to minimize the potential for an age-related problem with swimming endurance. After the fourth trial, the rat was allowed to remain on the platform for 20 s before removal to a drying cage. A session of four trials was conducted each day from 9:00–11:00 A.M. for each rat, and rats were tested on consecutive days until the study was completed. A probe test was conducted to further characterize swim task performance after the initial training sessions. In this test, the platform was removed and the rat was allowed to swim freely for the original training session length of time (either 30 or 60 s based on the age of the rat). The rat always began this test in a quadrant adjacent to the one in which it was trained. Visual cue tests were performed by extending a large black flag above the water level from the submerged platform. This test was repeated in all four quadrants of the pool, and each trial began in the quadrant opposite to the one containing the platform. The maximum time allowed was the same as the original training sessions.

TABLE 1

AVERAGE BODY WEIGHT IN GRAMS AND PERCENT BRAIN TO BODY WEIGHT RATIO FOR RATS USED IN REFERENCE AND WORKING MEMORY COMPONENTS OF THE MORRIS WATER MAZE SWIM TASK

			Reference Memory			Working Memory		
Group	Sex		Body Weight (gm) †	Brain/Body Weight (%) ‡	*N*	Body Weight (gm) §	Brain/Body Weight (%) ‡	*N*
PN21	M	Control	44.6 ± 2.2	2.1 ± 0.10	3	91.6 ± 5.7	1.7 ± 0.10	4
		Pb	33.1 ± 1.5*	2.7 ± 0.20	6	66.7 ± 2.8*	2.1 ± 0.10*	6
	F	Control	38.5 ± 2.7	2.2 ± 0.05	4	80.3 ± 5.4	1.9 ± 0.10	5
		Pb	32.2 ± 1.3*	2.7 ± 0.20	6	62.2 ± 2.4*	2.1 ± 0.10	6
PN56	M	Control	285.9 ± 7.4	0.6 ± 0.02	5	319.5 ± 7.8	0.6 ± 0.03	5
		Pb	239.2 ± 6.5*	0.7 ± 0.05	6	273.3 ± 11.2*	0.6 ± 0.03	5
	F	Control	200.9 ± 4.6	0.9 ± 0.04	5	224.7 ± 10.5	0.8 ± 0.10	5
		Pb	180.3 ± 7.0*	0.9 ± 0.04	6	203.9 ± 11.9	0.8 ± 0.04	5
PN91	M	Control	399.1 ± 14.1	0.5 ± 0.07	4	432.5 ± 14.6	0.5 ± 0.02	5
		Pb	326.9 ± 12.4*	0.5 ± 0.03	5	329.7 ± 22.4*	0.6 ± 0.03	5
	F	Control	247.3 ± 8.2	0.7 ± 0.03	5	233.3 ± 12.6	0.8 ± 0.02	5
		Pb	234.8 ± 6.5	0.8 ± 0.01	5	238.3 ± 11.4	0.7 ± 0.02	5

The mean ± SEM are presented for each treatment group.

†Body weight of rats at the end of the acquisition phase of the reference memory swim task. Actual ages of the rats were PN25, PN63, and PN98 for the PN21, PN56 and PN91 treatment groups, respectively.

‡Body weight/Brain weight × 100. Body weights used in this ratio were taken when brains were collected (ages of rats were PN30, PN68, and PN103 for the PN21, PN56 and PN91 treatment groups, respectively).

§Body weight of rats at the end of the working memory swim task. Actual ages of the rats were PN30, PN63 and PN97 for the PN21, PN56 and PN91 treatment groups, respectively.

*Significantly different from control rats in same age and sex group, one-way ANOVA, $P < 0.05$.

Reference Memory

In tests of reference memory, the hidden platform remained in the same location throughout each phase of the experiment. During the acquisition phase, the platform was placed in the center of the quadrant, 15 cm from the edge of the pool, and it was moved to the opposite quadrant for the reversal phase. Testing was conducted in daily sessions of four trials each. The rats were tested in a pseudorandomized order that ensured a different rat was first to swim on a given day, and control and Pb-exposed rats were alternated. The starting positions for each rat were also chosen randomly for each trial such that the rat started from all four quadrants during a session. Each phase of the experiment was terminated when control rats reached a latency criteria of ≤10 s. This criteria was experimentally determined in preliminary studies and is similar to that used in other swim task studies. A probe test and a visual cue test were performed on the last day of both the acquisition and reversal phases.

Working Memory

In tests of working memory, each rat was required to find the hidden platform located in a new position each day. The location of the hidden platform was changed daily in a pseudorandomized fashion such that different rats were tested in all quadrants on a given day, and all rats were trained in each quadrant at least twice during the experiment. The distance of the platform from the edge of the pool was also varied in an attempt to negate any search strategies employed by the rats. As in reference memory tests, the order of the rats tested, and the starting positions were randomized to ensure that these factors were evenly distributed within the experimental design. Working memory experiments were terminated when the rats reached the ≤10 s criteria in the last of four daily trials. A probe test was conducted daily after the trials, with post-training intervals ranging from 10 to 30 min. These post-training intervals were randomized according to the existing study design. A visual cue test was performed on the day following termination of the testing.

Statistical Analysis

Escape latency data from male and female rats in both reference and working memory paradigms were analyzed using one-way repeated measures analysis of variance (RANOVA), with Treatment as the main effect, and Day as the repeated measure. A one-way RANOVA was also used for working memory probe data since these tests were conducted daily. Probe data from reference memory and all visual cue and swim speed data were analyzed by a one-way ANOVA, as were body weight and brain Pb values. Post-hoc Student's t-tests were used for comparison of individual means.

RESULTS

Pb Exposure

Exposure to 250 ppm Pb acetate in the diet resulted in significant increases in Pb levels in the hippocampus of rats at all ages. Pb levels in the hippocampus of PN21, PN56 and PN91 Pb-treated rats were 1.73 ± 0.19, 1.02 ± 0.04, and 0.91 ± 0.05 g/g wet weight (mean ± SEM; $n = 4$). The average Pb level in the hippocampus of control rats was 0.12 ± 0.04 μg/g. This suggests that hippocampal Pb levels depend on the age of the animal in this model of Pb exposure. Rats that were exposed to Pb appeared to be in good general health relative to controls as judged by apparent behavioral signs of intoxication throughout the exposure periods (e.g. ataxia, lethargy, morbidity). A small to moderate (10–27%) reduction in body weight was observed in all Pb-treated rats except for PN56 females at the end of working memory tests, and PN91 females

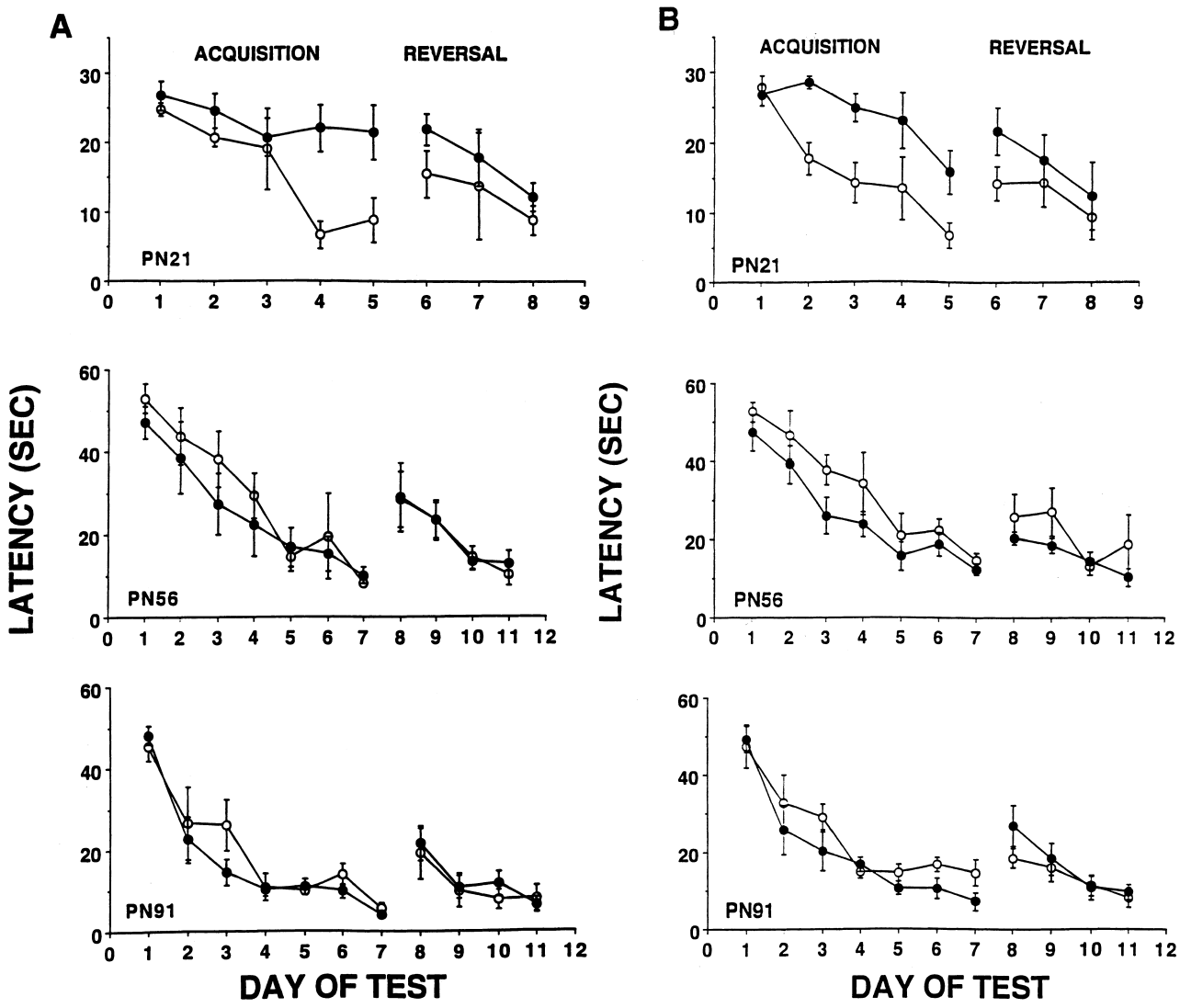

FIG. 1. Average escape latency (time in sec) to find the hidden platform during reference memory tests in male (A) and female (B) rats exposed to 0 (open circles) or 250 (filled circles) ppm Pb acetate in the diet and tested at PN21, PN56, and PN91. The platform remained the same position during the acquisition phase and was changed to the opposite quadrant during the reversal phase. Each point represents the mean ± SEM (see Table 1 for sample sizes of each age-treatment group).

at the end of both reference and working memory tests (Table 1). Generally, female body weight was least affected by the Pb exposure. Exposure to Pb had no significant effect on the whole brain wet weight to body weight ratio, except for a 24% increase in PN21 males used in the test of working memory (Table 1).

Reference Memory

Control male and female PN21 rats used in the reference memory test reached a latency criteria of ≤10 s in five days. Pb-exposed male rats that received testing at PN21 never found the platform in under 20 s during the acquisition phase (Fig. 1A) and statistical analysis indicated a near significant overall treatment effect [$F(1, 7) = 4.2, p < 0.08$], and a significant Day-Treatment interaction [$F(4, 28) = 2.8, p < 0.05$] due primarily to the 12.3-15-5 s longer escape latency on days 4–5 (Fig. 1A). The data analysis for female rats at PN21 indicate a more pronounced effect on swim task performance. A 10 s increase in escape latency was observed in Pb-exposed female rats after the first day of testing in the acquisition phase, and this was consistant until day 5 (Fig. 1B) and supported by an overall significant treatment effect [$F(1, 8) = 10.1, p < 0.02$]. The male and female rats tested at PN56 and PN91 reached the ≤10 s criteria in 7 days, and the control and Pb-exposed groups did not differ significantly in escape latency (Fig. 1A and B; also see Table 1 for sample sizes in each group).

The deficits in the reference memory component of the swim task observed in PN21 rats were supported by probe tests conducted at the end of the acquisition phase. Control and Pb-exposed males spent 12.7 ± 2.7 s and 8.2 ± 2.0 s, respectively, in the quadrant in which training occurred, however this 4.5 s difference was not statistically significant. The effects of Pb exposure on PN21 female performance in the

probe test were more dramatic. Control rats spent nearly twice the amount of time in the training quadrant: control = 16.0 ± 2.5 s, Pb = 8.6 ± 1.4 s (mean ± SEM), and these effects were significant [$F(1, 8) = 7.7$, $p < 0.03$]. The number of platform annulus crossings during the probe tests was also significantly less in PN21 females exposed to Pb [$F(1, 8) = 13.0$, $p < 0.01$]. The training quadrant time and annulus crossings were not significantly different between control and Pb-treated groups for males or females that received testing at PN56 and PN91, a result similar to the escape latency observed during the acquisition phase of the reference memory task. When the platform was placed in the opposite quadrant (reversal phase), control PN21 rats appeared to performed slightly better than Pb-treated rats on day 6 (Figs. 1A and B), but there were no overall significant differences treatment effects between control and Pb-exposed rats at this age or those tested at PN56 and PN91. All male and female rats were able to reach criteria at the new platform position in 3–4 days (Figs. 1A and B).

Working Memory

In the test of working memory, performance of PN21 rats during daily sessions was more variable than in reference memory, and there were no statistical differences overall in escape latency (average of four daily trials) during the nine day testing period (Figs. 2A and B). Male rats, however, had an overall escape latency of 17.0 ± 1.4 s for controls, and 22.4 ± 1.0 s for Pb-exposed rats [$F(1, 8) = 4.1$, $p < 0.08$], and Pb-exposed males had slower escape latencies on days 4 and 8 when analyzed alone (day 4: $F(1, 8) = 10.7$, $p = 0.01$, day 8: $F(1, 8) = 4.9$, $p < 0.06$) (Fig. 2B). Similar to the results obtained with tests of reference memory, there were no significant treatment effects in rats receiving the test at PN56 and PN91 (Fig. 2A and B). In all age and sex groups, the Pb-exposed animals were not impaired in probe tests conducted at the end of each daily working memory session. This is not surprising for the PN21 rats because when the escape latency data (during training) were analyzed using the fourth daily trial only, instead of the average of four trials, no significant effects of the Pb exposure were detected (data not shown).

Visual Cue Tests

All rats used in reference and working memory experiments were also administered a visual cue test. This procedure is believed to provide information on the possible non-specific effects involving motor, visual, or motivational abilities unrelated to learning and memory (21). The results of these tests are presented in Table 2. Generally, all rats were observed to swim directly to the platform as soon as the visual target (black flag) was detected. Rats used in reference memory tests were able to escape to the visible platform in 4.8–10.3 s at the end of acquisition, and control and Pb-exposed rats were not significantly different at any age (Table 2). Similarly, a cue test was conducted after the end of the working memory test and the escape latency averaged 6.2–19.7 s. There were also no significant differences observed between treatment groups during these tests.

Swim Speed

In an effort to further characterize the potential effects of Pb on other factors which may influence the ability of the rat to learn the spatial task, swim speed for each rat was measured during the probe tests (Table 3). Swim speed determined in PN21 male rats exposed to Pb was on the average 6.6 cm/s slower than control rats [$F(1, 7) = 9.9$, $p < 0.02$], but this may have been influenced by a relatively small sample size of control males at this age. On the other hand, female rats at this age had nearly identical swim speeds (Table 3). Swim speeds of rats tested at older ages were not significantly different between treatment groups except for Pb-exposed males at PN91 which surprisingly swam faster than controls [$F(1, 7) = 9.4$, $p < 0.02$]. We also measured swim speed on the first day of reference memory testing. Swim speed did not differ between Pb-exposed and control rats for any age-sex group (Table 3). In the daily probe tests during the working memory task, Pb exposure had no significant effect on swimming speed in any age-sex group except for a 3.4 cm/s decrease in PN91 females [$F(1, 8) = 12.3$, $p < 0.01$] (Table 3).

DISCUSSION

The findings from the present study suggest that exposure to Pb during development resulted in an age-dependent impairment in performance in the Morris water maze. Overall, the time required to find the hidden platform by control male and female PN21 rats during the acquisition phase of the reference memory component, decreased to under 10 s after five daily session of four trials each, whereas Pb-treated rats were considerably slower (Figs. 1A and B). These results were supported by the finding that Pb-exposed weanling rats spent less time than controls in the training quadrant during probe tests after the acquisition phase. The effect of developmental Pb exposure on the impaired learning in the reference memory test appeared to be more pronounced in female than in male rats at PN21. However, we are unable to conclude that female rats are more sensitive to the effects of Pb on swim task performance without conducting more tests and increasing sample size. Also, from the present study, we cannot determine if Pb-treated rats would have reached criteria, that is finding the hidden platform in $\leq$10 s, if testing was continued beyond the 5 days of the acquisition phase. The fact that PN21 rats in control and Pb-treated groups performed equally well after three days during the reversal phase of the reference memory task (Fig. 1a and b), suggests that PN21 Pb-treated rats may have eventually learned the task if additional sessions were performed during the acquisition phase. Therefore, it is possible that the effect of Pb exposure during development may be to delay the time it takes for the rat to learn the task.

Our data also indicates no significant differences in overall performance of Pb-treated PN21 rats during the nine days of the working memory testing, however, there was some indication that males might have been adversely affected by the Pb exposure. The overall treatment effects were near significance ($p < 0.08$), and males had noticeably longer escape latencies on days 4 and 8 (Fig. 2A). The lack of any indication of adverse effects from the Pb exposure in probe tests, and escape latency during the last of four daily trials suggests that the Pb-treated rats were able to locate the hidden platform as well as control rats at the end of the four trials, but the rate of learning during a daily session may have been impaired. It is generally thought that task difficulty in a variety of behavioral tests correlates positively with the sensitivity of the test for detecting small differences between control and manipulated treatment groups. It is therefore a reasonable assumption that the Pb effects should have been greater in the working versus reference memory component of the swim task, since the platform location is changed daily. A potential explanation for the lack of a difference in the working memory component between Pb and control PN21 rats is that at this age, the task may have been very

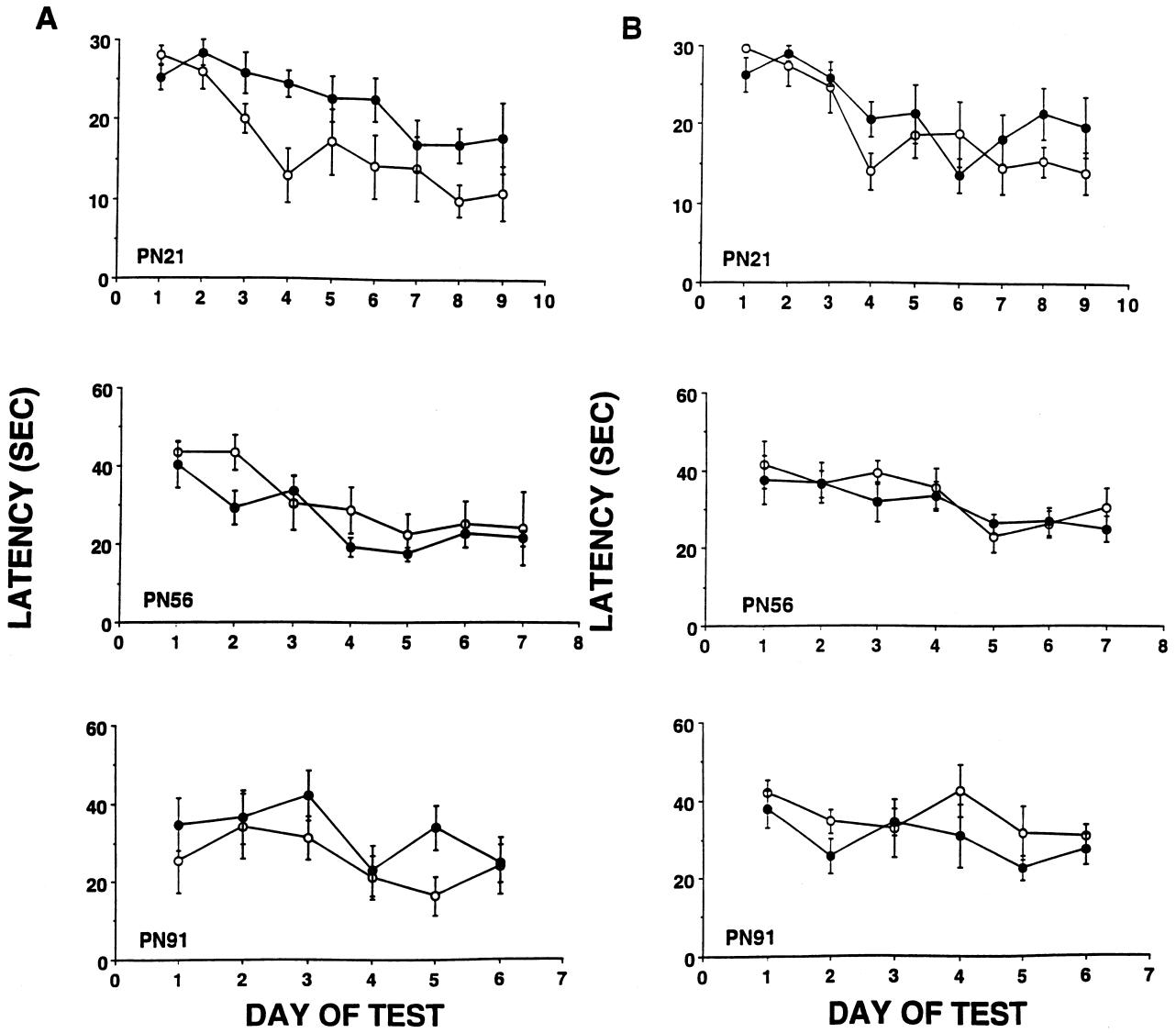

FIG. 2. Average escape latency (time in sec) to find the hidden platform during working memory tests in male (A) and female (B) rats exposed to 0 (open circles) or 250 (filled circles) ppm Pb acetate in the diet. The platform position was changed daily. Each point represents the mean ± SEM (see Table 1 for sample sizes of each age-treatment group).

difficult for the control rats. The evidence for this is that during the working memory, control rats were not able to find the platform in four daily trials in under 12 s, even after nine days of testing. Whereas, the control rats tested in the reference memory were well under the 10 s criteria in only 5 days. We do not have a clear explanation at this time but suggest that the sensitivity of a particular behavioral test to detect a difference may depend on several factors including age and sex of the subject, and the kind of behaviors involved in the task. Future studies in our laboratory will address these issues.

Chronic developmental exposure to Pb resulted in moderate reduction in body weight in nearly all groups of rats used in the Morris swim task (Table 1). One may argue that the effects of Pb observed in water maze performance may have been related to effects on body weight rather than specific effects on learning and memory. This possibility was addressed experimentally in this study, and there are several reasons why we believe the effects of Pb are primarily due to its effect on cognitive processes. First, the visual cue test results indicate that motor, motivational, and visual abilities did not differ significantly between the treatment groups (Table 2). If functional disabilities involving these parameters were present in Pb-exposed rats, they should have taken significantly longer to locate the visual platform. Second, we believe that swim speed is a good measure of motor and coordination abilities in the context of the swim task, and the general lack of any differences in swim speed in the reference and working memory tests (Table 3) indicates that differences in motor function could not have accounted for the differences in performance. We do recognize that some of the PN21 male rats exhibited lower swim speeds than controls (Table 3), however, female rats at this age showed impaired performance in the acquisition phase of the reference memory component and expressed no significant differences in swimming speed (see Table 3 and Fig. 2). Lastly, there is

TABLE 2

AVERAGE ESCAPE LATENCY FOR RATS IN THE VISUAL CUE TESTS AFTER REFERENCE AND WORKING MEMORY MORRIS SWIM TASKS

			Average Escape Latency (sec)			
Group	Sex		Reference Memory†	*N*	Working Memory‡	*N*
PN21	M	Control	5.0 ± 2.3	3	7.6 ± 1.9	4
		Pb	5.7 ± 0.7	6	6.2 ± 0.5	6
	F	Control	4.8 ± 0.7	4	7.9 ± 1.7	5
		Pb	6.4 ± 1.0	6	8.7 ± 1.0	6
PN56	M	Control	6.7 ± 1.2	5	11.1 ± 2.2	5
		Pb	9.0 ± 0.8	6	18.8 ± 3.5	5
	F	Control	7.8 ± 1.9	5	14.5 ± 2.8	5
		Pb	10.3 ± 4.7	6	11.6 ± 2.0	5
PN91	M	Control	6.3 ± 2.2	4	14.5 ± 2.3	5
		Pb	6.9 ± 1.6	5	14.8 ± 3.5	5
	F	Control	9.1 ± 2.0	5	13.6 ± 4.1	5
		Pb	7.6 ± 0.6	5	19.7 ± 5.1	5

The mean ± SEM are presented for each treatment group.
†Visual cue test was conducted after the acquisition phase of the reference memory test.
‡Visual cue test was conducted after the last day of the working memory test.

experimental evidence that loss in body weight is not correlated with poor performance in the Morris water maze. It has been reported that chronically malnourished male and female rats with 30–37% reductions in body weight performed similarly to age-matched controls in the Morris swim task (10).

The ontogeny of performance in the Morris water maze has been shown to occur early in development; the ages at which rats were able to locate the visual and hidden platforms were PN17 and PN20, respectively (27). Our results from both reference and working memory tests indicate that swim task performance may have been delayed in Pb-treated rats. Our finding that deficits in water maze performance were limited to PN21 rats is consistent with previous reports that children are more sensitive than adults to chronic low-level Pb exposure (4,23). The finding that Pb-induced deficits in swim task performance were not present in PN56 or PN91 rats suggests that older rats may be less susceptible, possibly due to compensatory mechanisms that occur with age. The present findings are also consistant with our previous neurochemical studies in which marked changes in NMDA and muscarinic cholinergic receptors were measured in the hippocampus of young but not adult rats chronically exposed to Pb during development (16). Also, in vitro

TABLE 3

AVERAGE SWIM SPEEDS OF RATS USED IN REFERENCE AND WORKING MEMORY MORRIS SWIM TASKS

			Reference Memory			Working Memory	
Group	Sex		Swim Speed on Day 1 of Test (cm/s)†	Swim Speed during Probe Test (cm/s)‡	*N*	Swim Speed during Daily Probe Tests (cm/s)	*N*
PN21	M	Control	21.5 ± 0.35	22.2 ± 1.01	3	17.2 ± 0.61	4
		Pb	18.7 ± 0.44	15.6 ± 1.38*	6	18.3 ± 0.51	6
	F	Control	21.1 ± 1.35	19.9 ± 1.69	4	19.0 ± 0.62	5
		Pb	19.5 ± 0.83	18.3 ± 0.80	6	18.2 ± 0.51	6
PN56	M	Control	21.1 ± 0.96	18.2 ± 1.87	5	20.1 ± 0.33	5
		Pb	20.8 ± 0.85	16.0 ± 1.72	6	18.7 ± 0.54	5
	F	Control	23.4 ± 0.44	21.7 ± 1.65	5	22.4 ± 0.48	5
		Pb	20.8 ± 0.97	17.7 ± 1.12	6	20.8 ± 0.48	5
PN91	M	Control	22.7 ± 0.20	17.5 ± 1.06	4	23.2 ± 0.73	5
		Pb	21.5 ± 0.61	21.5 ± 0.80*	5	22.2 ± 0.47	5
	F	Control	20.9 ± 1.24	18.8 ± 0.65	5	23.9 ± 0.59	5
		Pb	19.6 ± 0.84	19.6 ± 2.10	5	20.5 ± 0.49*	5

The mean ± SEM are presented for each treatment group. These values were calculated by dividing the distance traveled by latency (cm/s) during the specified test.
†First day of the acquisition phase of the reference memory test.
‡Probe test conducted after completion of the acquisition phase of the reference memory test.
*Significantly different from control rats in same age and sex group, $P < 0.05$. A one-way ANOVA was used to analyze data from reference memory tests; a one-way repeated measures ANOVA was used for daily probe tests of working memory.

studies from different laboratories have shown that inhibition of NMDA receptor function by Pb is greater in neuronal membrane preparations from young rat brain (11) or in young hippocampal neurons in culture (13) than from adult brain membranes or older hippocampal neurons in culture. Thus, there is supporting evidence that the effects of Pb on brain neurochemistry are more pronounced in younger rats (2,11–13). Therefore, children and experimental animals during development may, in part, be more sensitive to the effects of Pb on learning behavior because of intrinsic age-related differences in the molecular sites at which Pb interacts.

Another possible explanation for the age-dependence of Pb-induced deficits in swim task performance observed in the present study is the differential accumulation of Pb in hippocampal tissue. We observed that the level of Pb in the hippocampus of Pb-treated rats was 41–47% lower in PN56 and PN91 rats than in PN21 rats. The relative increase in Pb levels in the hippocampus of PN21 rats may be due to an immature blood-brain barrier and greater absorption of Pb in developing rats (9). It is plausible that the deficits observed in PN21 rats resulted from Pb levels above a threshold that was not reached in older rats. The age-dependence of Pb-induced deficits in swim task performance observed in the present study may be due to both developmental differences of Pb effects on neuronal targets, and the concentration of Pb achieved at the site of action. Future studies will address this important issue in Pb neurotoxicology.

Previous studies have shown that in vivo Pb exposure results in deficits in other measures of learning (26). Pb impairs spatial discrimination in monkeys (25), and deficits in active avoidance learning by rats were associated with dietary Pb exposure during development (2). Recently, it has been shown that post-weaning exposure of rats to Pb caused subsensitivity to the NMDA receptor non-competitive antagonist MK-801 in a standard operant food-reinforced drug discrimination paradigm (7). These studies suggest that Pb may interfere with the acquisition and processing of several different types of sensory information. In the present study, we have shown that developmental exposure to Pb caused impairment in performance in the reference but not the working memory component of the Morris water maze, and these deficits were restricted to young (PN21) rats. To our knowledge, this is the first evidence of Pb-induced changes in swim task performance in the Morris water maze, and provides a foundation for further in-depth research on the neurochemical basis of Pb effects on learning behavior.

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