Lead Exposure and (n-3) Fatty Acid Deficiency during Rat Neonatal Development Affect Subsequent Spatial Task Performance and Olfactory Discrimination

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ABSTRACT Docosahexaenoic acid [22:6(n-3), DHA] is important for optimal infant central nervous system development, and lead (Pb) exposure during development can produce neurological deficits. Long-Evans strain rats were fed either an (n-3) deficient [(n-3) Def] diet to produce brain DHA deficiency, or an adequate [(n-3) Adq] diet through 2 generations. At the birth of the 2nd generation, the dams were subdivided into 4 groups and supplied drinking water containing either 5.27 mmol/L (Pb) or sodium (Na) acetate until weaning. Rats were killed at 3 wk (weaning) and 11 wk (maturity) for brain Pb and fatty acid analysis. Spatial task and olfactory-cued behavioral assessments were initiated at 9 wk. Rats in the (n-3) Def group had a 79% lower concentration of brain DHA compared with the (n-3) Adq group with no effect of Pb exposure. At weaning, Pb concentrations were 7.17 ± 0.47 nmol Pb/g of brain (wet weight) in the (n-3) Adq-Pb group and 6.49 ± 0.63 nmol Pb/g of brain (wet weight) in the (n-3) Def-Pb group. At maturity, the brains contained 1.30 ± 0.22 and 1.07 ± 0.12 nmol Pb/g (wet weight), respectively. In behavioral testing, significant effects of both Pb and DHA deficiency were observed in the Morris water maze probe trial and in 2-odor olfactory discrimination acquisition and olfactory-based reversal learning tasks. Both lactational Pb exposure and (n-3) fatty acid deficiency led to behavioral deficits with additive effects observed only in the acquisition of 2-odor discriminations. J. Nutr. 135: 1019–1026, 2005.

KEY WORDS: • (n-3) fatty acid deficiency • neonatal development • spatial learning • olfactory discriminations • lead toxicity • Pb

Many infants receive low levels of (n-3) fatty acids via unsupplemented formulas and may also have exposure to environmental lead (Pb). Dietary intakes of (n-3) fatty acids and particularly docosahexaenoic acid (DHA) were demonstrated to be low for inner-city pregnant women (1). In addition, the rates of breast-feeding are lower in ethnic minorities and in women of lower socioeconomic status (2). Children living in these circumstances, particularly those living in older, urban housing are also at a much higher risk for Pb poisoning (3).

Maintenance of tissue DHA, the most abundant PUFA in the nervous system, is important for optimal infant develop-
1–30) were shown to have impairments in long-term memory storage (13). Additionally, it was shown that maternal Pb exposure or exposure during lactation can produce deficits in associative learning by inducing damage to the amygdala and nucleus accumbens (14). Collectively, these studies suggest a sensitive period for Pb toxicity during the lactational period. At birth, rats are at an earlier stage of neurodevelopment relative to primates; however, the period up until weaning at 3 wk of age encompasses much of the period of early brain maturation observed in human infants that is relevant for Pb exposure.

In this study, the effects of (n-3) fatty acid deficiency and Pb exposure as well as their interaction were examined on behavioral measures of brain function. An interaction may be expected to occur because DHA exerts effects on mechanisms such as ion channels (15), neurotransmitter systems (16), and signal transduction systems (17), mechanisms that are proposed as principal mediators of the effects of Pb (18). Another connection between DHA deficiency and Pb effects is that in both cases, the hippocampus has been implicated as one important site of action (6,19,20). The coexistence of these 2 risk factors for nervous system damage/dysfunction may then have a worse outcome than either one alone. Conversely, the addition of DHA to the diet may afford some protective effect to those exposed to Pb.

To test these hypotheses, a 2 x 2 factorial design was employed with (n-3) fatty acids and Pb as the variables. Figure 1 presents a schematic representation of the study design. Male Long-Evans rats were made deficient in brain DHA by deprivation of all sources of dietary (n-3) fatty acids throughout gestation, lactation, and postnatal life. This approach is based on models developed using oils rich in (n-6) fatty acids (6,8,9,21,22), and behavioral assessments aimed at determining the effects of fatty acid manipulation on spatial task (6,7) and olfactory-cued reversal learning (8,9). These end-points were selected because they have proven to be sensitive to manipulations of both brain DHA (6–9) and Pb (14,23–26). Motor activity, the elevated plus maze, and the visible trials in the Morris water maze served as controls. Rat pups from each dietary group were exposed to Pb during lactation by giving their dams free access to drinking water containing 0.2% lead acetate or sodium acetate as a control. The dose level of 0.2% Pb acetate was selected on the basis of several studies in which comparable or higher doses were used to expose fetuses and/or neonatal rats for the study of behavioral or morphological changes at the adult stage (25,27–31). The effect of an (n-3)–deficient diet and Pb exposure on the fatty acid compositions of several tissues is presented in an accompanying paper (32).

**MATERIALS AND METHODS**

**Animals and study design.** This experimental protocol was approved by the Animal Care and Use Committee of the NIAAA, NIH. Female Long-Evans rats (3 wk old) were obtained from Charles-River; they were divided into 2 groups of 60 each, and fed a diet either deficient in (n-3) fatty acids [(n-3) Def] or one in which α-linolenic acid and DHA [(n-3) Adq] were added (see next section). The rats were maintained at our animal facility under conventional conditions of controlled temperature (23 ± 1°C) and illumination (12-h light:dark cycle). Rats were allowed free access to food and water.

At 11 wk of age, the dams were paired with 12-wk-old, Long-Evans proven male breeders for 1 wk. After the mating period, the females continued to consume (n-3) Adq or (n-3) Def diets and were housed 3/cage until a few days before the estimated time of delivery when they were housed individually. As soon as the dams delivered their pups, the (n-3) Adq and (n-3) Def groups were each subdivided into 2 groups to receive drinking water containing either 0.2% lead acetate or a solution of sodium acetate (Aldrich Chemical). The 0.2% lead acetate trihydrate solution was made by diluting a stock solution of 40 g in 100 mL water by 200-fold, which provided 5.27 mmol/L of Pb as verified by atomic absorption spectrophotometry. The 4 groups were designated as (n-3) Adq-Na, (n-3) Adq-Pb, (n-3) Def-Na and (n-3) Def-Pb to identify their diet and treatment with either Pb or Na. The rat pups were nursed by dams fed either the Pb or control drinking water until weaning at 21 d of age. At weaning, 10 litters from each of the 4 groups were selected on the basis of time of birth (within a 48-h period) and male pups were selected within each litter to control for the mean body weight of the group. A sample of pups was killed at this time for analysis of Pb and fatty acids in brain tissue. Blood samples were also collected from pups at 3 and 6 wk of age for commercial whole-blood Pb analyses by atomic absorption spectrophotometry (Antech Diagnostics). After weaning, the pups consumed the same diets as their dams but were switched to tap water. One male pup was selected from each dam for a sequence of tests as follows: motor activity, elevated plus maze, Morris water maze. This sequence began at 9 wk of age. This group was used for both brain fatty acid composition and Pb analysis. A separate group of male pups was used for the olfactory-based tasks with water limitation beginning at 7 wk of age and behavioral assessment at 9 wk of age.

**Experimental diets.** The custom-pelleted experimental diets were obtained commercially (Dyets) and were based on the AIN-93 formulation (33) with several modifications as previously described (6,9) and presented in detail in a supplementary table.4 Both the (n-3) Adq and (n-3) Def diets contained 10% fat but the (n-3) Adq diet contained (g/100 g diet) coconut oil, 7.45; safflower oil, 1.77; flaxseed oil, 0.48; and DHASCO (Martek Bioscience), 0.3 g/100 g diet. The (n-3) Def diet contained only coconut oil at 8.1 and safflower oil at 1.9 g/100 g diet.

**Brain tissue analyses.** After termination of the behavioral experiments, the rats were killed by decapitation, weighed, and the

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4 A supplemental table is available with the online posting of this paper at www.nutrition.org.
brains were divided in half along the midline and quickly frozen (−80°C) for subsequent analyses for lipid composition and Pb concentration. For Pb analysis, frozen brain samples were transferred to quartz tubes, weighed, treated with 100 μL of 0.6% Mg(NO₃)₂ as an ashing aid, and dried on a heating block before being placed in a muffle furnace at 400°C overnight. The next day, they were treated twice with 1 mL of 5% nitric acid (Optima brand, Fisher #A467–2) and diluted in 2 mL of 5% nitric acid. All Pb determinations were accomplished using graphite furnace atomic absorption spectrometry. Measurements were made at the 283.3 nm line on a Perkin-Elmer Model 5100Z system, and Zeeman background correction was used. NIST SRM 157b bovine liver was analyzed as a control material; excellent accuracy was obtained: a mean value of 0.63 ± 0.03 nmol Pb/g (dry weight) was obtained compared with the certified value of the material of 0.62 ± 0.02 nmol Pb/g.

For lipid composition, brain samples were extracted with chloroform-methanol containing BHT (50 μg/sample) by a modification of the method of Folch et al. (34) to obtain the total lipid extract, and transmethylated by BF₃-methanol according to the method of Morrison and Smith (35) as modified by Salem et al. (36). Capillary GC analysis of FAME was as described by Salem et al. (36).

Spatial task assessments. Behavioral assessments, including the elevated plus maze (37), motor activity, and the Morris water maze, were performed as previously described (6,7). The spatial task was chosen for this experiment because it was shown to be sensitive to manipulations of both (n-3) fatty acids (6–8,38,39) and Pb exposure (12,40).

Olfactory discriminations and reversal learning. The go, no-go procedure as used by Slotnick et al. (41) clearly showed that when rats were trained for multiple 2-odor discrimination tasks, they showed positive transfer across problems and were using a type of strategy to allow for the nearly errorless learning shown in later trials. The go, no-go procedure also allows the examination of active avoidance, i.e., the ability of the animal to refrain from responding. Studies showed that lead toxicity can lead to hyperactivity (42,43). Because increased hyperactivity in humans may contribute to learning difficulties (44), the go, no-go procedure required active avoidance as a part of the learning strategy. The go, no-go paradigm was used successfully in various response inhibition tasks (45,46) and tests of higher learning in rodents (4,6,9,47).

Several olfactory-based behavioral tasks were given to all 4 groups of rats including olfactory discrimination problems using 4 pairs of odors, a reversal learning task, and a memory task for the set of odors given previously. Beginning at 7 wk of age, rats were subjected to water limitation (48). Water limitation was used because access to water for an animal that is highly motivated to receive it is a reliable and noninvasive positive reinforcement. The nature of this test allowed for an increase in the connection between the odorant stimulus and the reinforcement by placing the water tube inside the odor sampling port. After 10 d of water limitation, rats were trained in an olfactometer using standard operant procedures as described previously (9,48). Olfactometer design and hardware function were as previously described (47). The Eight Channel Liquid Dilution Olfactometers with digital interfaces were purchased from Knosys. Rats were trained using a go, no-go discrimination procedure described previously in detail (49).

Olfactory tasks. The rats were first given 4 sets of 2-odor discriminations (termed “pre” indicating prereversal). Odors were counterbalanced and the number of errors incurred to reach a criterion of 85% correct responding was measured. The 4 pairs of odors used were as follows: Problem 1, garlic and vanilla; Problem 2, root beer and pineapple; Problem 3, lemon and anise; Problem 4, onion and pepper.

For the reversal problems, 2 novel odors (cherry and banana) were counterbalanced and presented to the rats for an initial discrimination referred to as original learning. The number of errors incurred in reaching a criterion of 85% correct responding was recorded. After initial exposure, the significance of the S+ and S− odors was reversed each time that the rat achieved an 85% correct response. The reversals were continued until the rats reached asymptotic performance (8–11 reversals).

The memory task was given after the completion of the reversals (termed “post” indicating postreversal) and consisted of repeating the original four 2-odor discrimination problems that the rats had learned before the reversal tasks. The number of errors to criterion pre- and postreversal tasks was compared. The time elapsed between the memory task and the first presentation of these problems was 5 wk.

Statistical analysis. All data were expressed as means ± SEM and significance was determined by 2-way ANOVA with interactions for diet and Pb exposure effects using SPSS for Windows statistical software (release 11.5.1; SPSS) except as noted in the text. For the Morris water maze probe trial, the number of crossings into the 4 regions was also examined by 1-way ANOVA within each treatment group. Repeated-measures analyses were completed when appropriate. The Linear Mixed Models procedure in SPSS was used for statistical analyses of olfactory reversals to allow for incomplete repeated-measures analysis. When the F-test was significant, multiple comparisons among groups were done using Tukey's HSD test. Homoscedasticity of variances were examined by the Levene's test of equality of error variances function in SPSS. For variables with heteroscedastic variances, the individual data points were rank transformed and the analyses were repeated. The statistical findings after rank transformation analyses did not alter the conclusions of the primary analysis. Differences were considered significant at P < 0.05.

RESULTS

Growth. The dams fed the (n-3) Adq and (n-3) Def diets did not differ in weight gain during the premating period; however, the (n-3) Adq dams were significantly heavier in the later stage of gestation; this may have been a result of increased (P < 0.05) litter size (Table 1). Body weight at the time of pup weaning did not differ between the (n-3) Adq and (n-3) Def groups, but was lower in the rats exposed to Pb (P < 0.001). This difference in body weight at weaning was probably a reflection of the observation (although not quantified) that the dams exposed to Pb ate and drank less. The difference in consumption by the dams was reflected in their pups, which were visibly smaller and seemed to develop more slowly. At weaning, the pups from the dams administered Pb had an ~50% reduction in body weight compared with control pups for both the (n-3) Adq-Pb and (n-3) Def-Pb groups (Table 1). Pb administration was discontinued at weaning (21 ± 1 d), but the Pb-exposed rat pups remained lower in weight such that at 11 wk of age (at the time when the spatial task studies were completed), the (n-3) Adq-Pb and (n-3) Def-Pb rats had 22–24% lower body weights than rats in the (n-3) Adq-Na and (n-3) Def-Na groups (P < 0.001 for Pb exposure). Other than the obvious difference in size and weight during their early growth period, the Pb-exposed pups did not exhibit other obvious differences from the Na controls.

There were no differences in brain weight at 3 or 11 wk of age, although there was a tendency for a diet × Pb exposure interaction at 3 wk of age (P = 0.053) and a tendency for an effect of Pb exposure at 11 wk of age (P = 0.073) (Table 1).

Tissue analyses. Pb exposure through lactation significantly increased brain and blood Pb concentrations in the rats. The control rats had very low background concentrations of Pb for both weaning and adult groups. At weaning, brain concentrations of Pb for the (n-3) Adq-Pb and (n-3) Def-Pb groups were 7.17 ± 0.47 and 6.49 ± 0.63 nmol Pb/g, respectively. After weaning and 8 wk without further exposure to Pb, the brain Pb concentrations of Pb for the (n-3) Adq-Pb and (n-3) Def-Pb groups were 1.30 ± 0.22 and 1.07 ± 0.12 nmol Pb/g, respectively. Brain Pb of mature pups had fallen to 16.5–18% of that of the 3-wk-old pups. There was no effect of diet on brain Pb concentrations. In the blood of Pb-exposed pups, levels of Pb were 18.7 ± 4.05 μmol/L at the end of the
exposure period (3 wk) and by 6 wk of age, Pb blood levels were decreased to 0.74 ± 0.04 μmol/L with behavioral testing occurring at 9 wk.

The brain concentration of DHA was significantly higher for the (n-3) Adq groups in the weanling and 11-wk-old pups (32). For weanlings, although the mean DHA concentration was 30% greater in the (n-3) Def-Pb–treated group compared with the (n-3) Def-Na–treated group, this effect was not significant (P = 0.086). The deficit in DHA concentration was compensated by large increases in the (n-6) fatty acid docosapentaenoic acid [DPA (n-6), 22:5(n-6)]. At 3 wk of age, a diet × Pb exposure interaction was detected for the concentration of brain DPA (n-6); this fatty acid was the highest in the (n-3) Def-Na pups followed by the (n-3) Def-Pb pups. Brain DPA (n-6) concentrations in the (n-3) Adq-Na and (n-3) Adq-Pb pups were much lower than in the (n-3) Def pups, but not different from each other. At 11 wk of age, only the main effect of diet remained significant for DPA (n-6) concentrations in brain. The ratios of DPA (n-6) to DHA were not significant. There were 20% fewer crossings in the other regions than in the A region for the (n-3) Def-Na and (n-3) Def-Pb, these differences from the other regions were not significant. There were ~20% fewer crossings in the other regions than in the A region for the (n-3) Def-Na and (n-3) Adq-Pb groups and 25% fewer for the (n-3) Def-Pb group. Analysis by 2-way ANOVA for diet and Pb exposure effects with interaction indicated a significant main effect of Pb for an increased number of crossings into areas B, C, and D.

Spatial task assessments. In the motor activity testing, the 4 groups did not differ in either the moving distance (P = 0.81) or moving time (P = 0.42) (data not shown). Diet and Pb exposure did not affect the elevated plus maze in either the number of visits or the time spent in the open arm due to variation within groups (data not shown).

In the Morris water maze, the groups did not differ in swimming speed or the escape latency in the visible trial, and the number of rats that reached the platform in the visible trial did not differ, indicating no difficulties with sensory and motor components of the spatial task.

With the hidden platform, the escape latency or swimming time did not differ between diets or Pb exposure or diet (Fig. 2). A main effect of time was detected because the escape latency (P < 0.001) and swimming time (P < 0.001) decreased with increasing sessions. By d 4, the escape latency and swimming time reached a minimal plateau for all rats. Therefore, the results of the previous sessions (d 3) were analyzed separately and a diet × Pb exposure interaction was detected for both escape latency (P = 0.013) and swimming time (P = 0.023). However, in ad hoc tests, only the (n-3) Adq-Na and (n-3) Def-Na were significantly different from each other; the intermediate points were not different from either of the other 2 groups. The (n-3) Adq-Na rats were faster than the (n-3) Def-Na rats in both assessments by Tukey’s HSD test (P < 0.05).

In the probe trial (Fig. 3) assessed on d 5, for the (n-3) Adq-Na group, the number of crossings of the former platform position (region A) was greater (by 70%) than that in region D (P < 0.005). The number of crossings in the regions B and C for group (n-3) Adq-Na was ~45% lower than in Region A. In contrast, although the crossings in Region A were always higher for the treatment groups (n-3) Def-Na, (n-3) Adq-Pb and (n-3) Def-Pb, these differences from the other regions were not significant. There were ~20% fewer crossings in the other regions than in the A region for the (n-3) Def-Na and (n-3) Adq-Pb groups and 25% fewer for the (n-3) Def-Pb group. Analysis by 2-way ANOVA for diet and Pb exposure effects with interaction indicated a significant main effect of Pb for an increased number of crossings into areas B, C, and D.

### TABLE 1

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<th>(n-3) Adequate</th>
<th>(n-3) Deficient</th>
<th>P-Value</th>
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<tr>
<td></td>
<td>Na control</td>
<td>Pb exposed</td>
<td>Na control</td>
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<tr>
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<td>26.7 ± 2.3</td>
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<td>1.30 ± 0.04</td>
<td>1.26 ± 0.02</td>
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<tr>
<td>11 wk</td>
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<td>409 ± 17</td>
<td>491 ± 13</td>
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<tr>
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<td><strong>Dams</strong></td>
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<td>Weaning weight</td>
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<td>276 ± 15</td>
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1 Values are means ± SEM, n = 5 for 3 wk, 10 for 11 wk.
2 Significant main effect of diet.
3 Significant main effect of Pb exposure.
4 NS, not significant, P ≥ 0.05.

**FIGURE 2** Effect of (n-3) Adq and (n-3) Def diets and Pb exposure on (A) escape latency and (B) swimming time in the Morris water maze. Values are means ± SEM, n = 8–10. Means without a common letter differ, P < 0.05.
(P ≤ 0.03 for all 3 areas) but not for A (P = 0.07). Thus, introduction of Pb exposure led to poorer performance in the probe trial.

Olfactory discrimination. For the original learning and retention tests, the total number of errors for each 2-odor problem according to time, diet, and Pb exposure is presented in Figure 4. A 2-way, repeated-measures ANOVA analysis was performed to evaluate dietary (n-3) fatty acids and Pb exposure at the 2 time points (“pre” and “post” reversal task, as defined earlier). There were no interaction effects and no effect of time. In all 4 problems, there were significant main effects of (n-3) fatty acids (P < 0.001 for all problems) and Pb exposure (P < 0.001 for all problems) with more errors made in groups with (n-3) Def diets and those exposed to Pb. This indicates an additive effect of (n-3) fatty acid deficiency and Pb exposure on errors made in acquiring 2-odor discriminations. In Pb-exposed rats only, t tests indicated that the (n-3) Adq group made significantly fewer errors than all other groups and the (n-3) Def-Na group made fewer errors than the Pb-exposed groups across time (Fig. 5). The (n-3) Adq-Pb and (n-3) Def-Pb groups did not differ from each other. All groups made fewer errors with continued testing.

DISCUSSION
The purpose of this study was to investigate (n-3) fatty acid deficiency, Pb exposure during lactation, and the interaction of (n-3) deficiency and Pb with respect to their effects on

In the olfactory-cued reversal tasks, a diet × Pb exposure interaction (P < 0.001) and a main effect of time (P < 0.001) were evident after Linear Mixed Models analysis. Multiple comparisons testing by Tukey’s HSD indicated that the (n-3) Adq-Na group made significantly fewer errors than all other groups and the (n-3) Def-Na group made fewer errors than the Pb-exposed groups across time (Fig. 5). The (n-3) Adq-Pb and (n-3) Def-Pb groups did not differ from each other. All groups made fewer errors with continued testing.
spatial task performance and olfactory discriminations. Each of these topics will thus be considered in sequence.

**Spatial task performance.** The escape latency in the Morris water maze reflects an aspect of cognition and spatial learning (50,51). There was no evidence for an additional effect of Pb beyond that related to the diet on escape latency in the maze. Escape latency was demonstrated previously to be increased with Pb exposure (23,25); however, the Pb effect was shown to be age dependent with increased escape latencies during testing at a young age (postnatal d 21) but not at older ages (26). Additionally, the lack of a Pb exposure effect in the present study may have been more difficult to observe because performance was already adversely affected by (n-3) fatty acid deficiency and the resulting low concentration of brain DHA. The probe trial results suggest that both an (n-3) Adq diet and a lack of exposure to Pb were necessary for the optimal spatial retention performance and that an (n-3) def diet and Pb exposure in particular were associated with a decline in spatial retention.

The lack of significant differences in the motor activity testing, the elevated plus maze, and the visible trial portion of the Morris water maze indicate that the (n-3) deficiency and Pb exposure were not associated with any gross deficits in arousal, anxiety level, sensory function, and motor ability. This was demonstrated previously in (n-3) deficiency studies (6,7,38,52), but it was suggested (42) that gestational and lactational exposure to Pb results may increase anxiety level as elicited by the elevated maze test.

**Olfactory discrimination.** The olfactory discrimination results clearly indicated negative effects of Pb exposure and an (n-3) Def diet. The reversal learning results indicate that the (n-3) Def diet exacerbated the adverse effect of Pb; however, the presence of the (n-3) fatty acids in the (n-3) Adq-Pb group could not prevent the strong adverse effect of Pb exposure. From inspection of all of the olfactory discrimination data, it is apparent that the magnitude of the effect of (n-3) fatty acid deficiency was similar to that of early Pb exposure on olfactory task performance.

The effects of (n-3) Adq diets compared with (n-3) Def diets in the olfactory results of the present study successfully replicate findings from this laboratory that were presented and discussed previously (8,9,52). Briefly, a loss of DHA in neuronal aminophospholipids with (n-3) deficiency may result in a deficit in learning of a discrimination problem that can be reduced by additional training. In contrast, the effect of Pb exposure on olfactory discriminations has not been investigated thoroughly. To date, only Hilson and Strupp (24) and Garavan et al. (14) have demonstrated significant impairment with Pb exposure on olfactory reversal tasks. The latter study determined that the impairment was associated with a bias in response and an inability to associate cues and/or actions with consequences. In the olfactory discriminations of the present study, Pb exposure elicited an effect of a magnitude similar to that of the (n-3) fatty acid deficiency. Pb neurotoxicity likely involves several possible mechanisms that, as suggested previously, may result in a common functional neurobehavioral-cognitive impairment (53).

**Interaction between Pb and DHA deficiency.** One goal of this study was to evaluate a possible interaction between brain DHA status and the neurotoxicity of Pb as measured by behavioral means. For escape latency, both of the Pb-exposed groups performed like the control group, the (n-3) Adq-Na; thus, possible interactive effects could not be evaluated (Fig. 2). In the probe trial (Fig. 3) of the Morris water maze and in the olfactory reversal task (Fig. 5) in which the Pb-exposed groups performed worse than the Na control groups, there was also no evidence of higher brain DHA concentrations sparing the Pb exposed rats from impairment in the acquisition of these tasks. The original learning of four 2-odor discriminations did provide evidence that performance in (n-3)-deficient rats was worse with exposure to Pb compared with DHA-adequate rats (Fig. 5). The “memory retention” trial of the four 2-odor discriminations produced exactly the same results as the original learning trial, confirming this finding. It was remarkable that essentially identical results were obtained for this set of problems when given again after 5 wk because Bodyak and Slotnick demonstrated that rodents can retain essentially perfect memory for a set of 8 odors for up to 30 d (47). It therefore appeared that none of the rats retained information from the first presentation of these olfactory discrimination problems. Differences obtained among groups in the performance of this task cannot then be ascribed to effects on recall but rather appear to reflect effects on associative learning or impaired inhibitory control.

**Achievement of desired experimental conditions.** The brain concentrations of DHA in the weanling and adult rats in the (n-3) Def-Na or (n-3) Def-Pb groups were ~80% lower than those of their (n-3) Adq-Na or (n-3) Adq-Pb counterparts. This compares favorably with other attempts to produce rats with brain DHA deficiency from this laboratory in which spatial tasks and olfactory discriminations were performed (6,7,9,39).

The brain concentrations of Pb at weaning were between the concentrations reported by other investigators using a similar dose of Pb. For instance, one study showed a brain Pb concentration of 1.93 ± 0.10 nmol/g after lactational and postweaning exposure to 0.2% lead acetate in the drinking water when examined at the adult stage at 14 mo of age (54). Another study showed concentrations as high as 17.6 ± 0.14 nmol/g in the hippocampus in 17- to 23-d-old animals (28). This difference in brain Pb content may be explained in part by the observation that Pb distribution across various brain areas can vary considerably (55). In this study, a portion of the Pb was retained in the brain at the time of killing at 11 wk of age, which is in contrast to previous reports of no residual Pb remaining in the brain in adults after neonatal and/or lactational exposure (12,28,56). Blood levels of ~0.97 µmol/L can lead to functional impairment of the brain in rodents (28).

In humans, the current blood Pb level for treatment intervention is ≥0.48 µmol/L (3); however, acute or chronic Pb ingestion can result in blood concentrations >14.5 µmol/L. Before regulations restricting environmental Pb contamination, blood Pb levels between 4.3 and 39.8 µmol/L in humans occurred more frequently (57), but similarly high levels continue to occur as indicated by a case report of a child presenting with blood levels of 26.5 µmol/L (58). In the Pb-exposed rats in the present study, blood Pb levels at 6 wk of age had fallen markedly to ~4% (0.74 ± 0.04 µmol/L) of their concentration at the termination of Pb intake at weaning.

In the present study, the body weight of the Pb-exposed rats at 3 wk of age was 56% less than the Na-control rats. This body weight difference was much reduced after discontinuation of Pb exposure but persisted to 11 wk of age (22% less than Na-controls). The initial loss in body weight is attributed to a decrease in food intake either due to limited milk production, the inability of the Pb-exposed pups to feed normally, or an alteration in maternal interactions with the pups. Limited milk production by the dams subsequent to Pb exposure has not been reported to our knowledge, but remains one possibility. Subsequent to weaning at 3 wk of age, the catch-up in body weight in the Pb-exposed rats entails a more rapid rate
of weight gain after weaning and thus a likely greater food intake. Previous studies using similar dose levels of Pb in rats either did not report body weight (28,29,31,54) or reported no differences between body weights of Pb-exposed animals and controls (25,30). However, lower body weights in rats exposed to high Pb levels were reported previously (59,60) as well as in those chronically exposed to lower Pb levels (24). Hilson and Strupp suggested previously that Pb at up to 1.45 mmol/L did not lead to undernutrition but rather stunting, perhaps due to an effect on growth hormone (24).

One important issue then is the possibility that the effects of Pb on behavior were due to early malnutrition. Our data cannot rule out the possibility that the compromised nutrition during the lactational period had an effect on the behavioral outcomes measured herein. However, Huang et al. (61) found that early malnutrition induced by large litter sizes resulted in no differences in spatial navigation tasks even though the malnourished group had a 37% decline in body weight. Similarly, Hall (62) found that early protein malnutrition did not lead to changes in hippocampal-related behaviors such as radial arm maze and spontaneous alternation. Analyses of the mechanisms underlying Pb effects on behavior in studies that produced only a small and transitory change in body weight indicated a specific deficit in associative ability (14) and no difference in inhibitory control (24,63). Moreover, protein malnourishment during prenatal development produced no difference in a go, no-go task in the rat offspring even though both the dams and their offspring had lower body weight than controls (64). Therefore, it is likely that the early decrease in body weight in Pb-exposed rats did not by itself cause the adverse effects on behavioral performance. In any case, increased Pb levels were demonstrated to be associated with lower birth weight in infants (65), indicating that growth impairment is a relevant effect of Pb toxicity.

In summary, these findings may indeed have relevance not only in developing countries in which infant nutrition may be compromised and restrictions on Pb use and environmental contamination lacking, but also in present day Western societies because dietary intake of (n-3) fatty acids is extremely low (1,66) as reflected in Western breast milk (67–69), and many older, urban buildings still contain Pb-based paints (3,58).

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LITERATURE CITED


