Published in final edited form as:

Neurotoxicology. 2012 October ; 33(5): 985–995. doi:10.1016/j.neuro.2012.04.016.

Sex and Rearing Condition Modify the Effects of Perinatal Lead Exposure on Learning and Memory

D. W. Anderson, K. Pothakos, and J.S. Schneider

Department of Pathology, Anatomy and Cell Biology, Thomas Jefferson University, Philadelphia, PA 19107.

Abstract

Developmental lead (Pb) exposure is associated with cognitive impairments in humans and rodents alike. In particular, impaired spatial learning and memory, as assessed using the Morris water maze (MWM), has been noted in developmentally Pb-exposed rats. Although sex and rearing environment can influence MWM performance in normal animals, the interactions of sex and rearing environment on the impact of developmental Pb exposure on hippocampal-dependent processes has not been well characterized. The present study examined the effects of perinatal exposure (i.e., gestation through weaning) to different levels of Pb (250, 750 and 1,500 ppm Pb acetate in food) in males and females raised in a non-enriched environment (standard cage with 3 animals and no toys) or an enriched environment (large cage containing a variety of toys that were changed twice weekly). Testing in the MWM began at postnatal day 55. Behavioral outcomes were influenced by sex and rearing environment, with complex interactions with Pb exposure. In non-Pb exposed control animals, beneficial effects of environmental enrichment on spatial learning and memory were observed in males and females, with greater effects in females. Pb exposure in females mitigated at least some of the benefits of enrichment on learning, particularly at the lowest and highest exposure levels. In males, enrichment conferred a modest learning advantage and for the most part, Pb exposure did not affect this. However, in males with the highest Pb exposure, enrichment did help to overcome detrimental effects of Pb on learning. In females, any potential benefit to reference memory contributed by enrichment was muted by exposure to Pb and for the most part, this was not reproduced in males. Thus, there are complex interactions between sex, environment, and Pb exposure on spatial learning and memory. Environmental manipulation is a potential risk modifier of developmental Pb exposure and interacts with other factors including sex and amount of Pb exposure to affect the functional influences of Pb on the brain.

Keywords

Lead; Sex; Environment; Learning; Memory

© 2012 Elsevier B.V. All rights reserved.

Please address correspondence to: Dr. J.S. Schneider, Dept. of Pathology, Anatomy and Cell Biology, Thomas Jefferson University, 1020 Locust Street, 521 JAH, Philadelphia, PA 19107. Phone: 215-503-0370; Fax: 215-923-3808; jay.schneider@jefferson.edu.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Conflict of Interest Statement: The authors declare that there are no conflicts of interest.

1. Introduction

Childhood lead (Pb) poisoning impairs a variety of cognitive processes including various aspects of learning and memory (Bellinger et al., 1991, Lanphear et al., 2000, Surkan et al., 2007). Numerous studies have attempted to model this effect of Pb in rodents, with the majority of studies focusing on hippocampal-associated spatial learning and memory processes. While most studies examined only males (Cao et al., 2008, Gilbert et al., 2005) a few studies have examined both sexes simultaneously (Jett et al., 1997, Xu et al., 2009). Since sex-related differences in the performance of spatial learning and memory tasks have been found in humans (Astur et al., 1998, Duff and Hampson, 2001, Newhouse et al., 2007, Rizk-Jackson et al., 2006) and rodents (Frye, 1995, Frye and Sturgis, 1995, Galea et al., 1996), utilizing both males and females in animal studies would seem to be important. Sex-dependent differences in cognitive performance have been described and may be related to different learning strategies used by males and females during performance of spatial tasks. For example, males tend to guide navigation using geometric configuration of the environment and have a preference for a place-learning strategy during navigation, whereas females seem to attend more to landmark cues (Blokland et al., 2006, Dalla and Shors, 2009, Mendez-Lupez et al., 2009, Sandstrom et al., 1998, Saucier et al., 2002, Saucier et al., 2008, Williams and Meck, 1991). These differences in strategy may be particularly relevant in regard to performance of the Morris water maze, a task in which spatial learning is guided by distal cues (Morris, 1984, Vorhees and Williams, 2006). For example, impaired navigation in the Morris water maze following unilateral entorhinal cortex lesions was found to be sex dependent; males showed a persistent water maze deficit whereas similarly lesioned females were only slightly impaired relative to sham controls, and performed significantly better than lesioned males (Roof et al., 1993). A recent meta-analysis of water maze studies reported male advantages in spatial working and reference memory for rats across different strains, protocols, ages and rearing environments (Jonasson, 2005). Developmental Pb exposure has been shown at least in some studies to have detrimental effects on Morris water maze performance, but this effect may depend on several variables including age, sex, level of Pb exposure and developmental period during which Pb was present. Nonetheless, the interactive effects of sex and Pb exposure on behavioral outcome have been minimally explored and the extent to which developmental Pb exposure results in sex-dependent effects on water maze performance are not completely clear.

Rearing environment also plays an important role in influencing behavioral outcomes. For example, children growing up in low socioeconomic (SES) environments appear to perform less well on a variety of cognitive measures than children from higher socioeconomic backgrounds (Bradley and Corwyn, 1999, Bradley et al., 2001, Brooks-Gunn and Duncan, 1997, Farah et al., 2006, McCloyd, 1988). This is not particularly surprising since low SES children may be exposed to less environmental complexity, have fewer toys and books and less exposure to social and cultural activities than higher SES children. One method for modeling the effects of differential rearing in animals has been to manipulate the environmental complexity of the housing environment. In the extreme situation, environmental complexity is manipulated by housing animals individually in a single small cage, often referred to as “isolation” rearing (Farah et al., 2006). Housing in an “enriched” environment typically involves housing several animals together in a large cage and exposing them to various and novel objects and toys that they can explore as they interact with their environment and with each other (van Praag et al., 2000). This provides for enhanced social interaction as well as complex inanimate stimulation (Rosenzweig et al., 1978). Intermediate between these two situations is a “non-enriched” or “social enrichment” environment in which animals are housed in small groups in cages containing standard bedding and no environmental enhancements, having only the opportunity for social interaction. Animals reared in non-enriched environments have less well-developed brains

(according to a variety of anatomical and neurochemical measures) than animals reared in more complex environments (van Praaget al., 2000) and exposure to enriched environments improves learning and memory performance in normal animals (Will et al., 2004) and also increases functional recovery from different types of brain damage including that from traumatic (Kolb and Gibb, 1991, Rose et al., 1993a, Rose et al., 1993b, Will et al., 1977) or ischemic (Dahlqvist et al., 2004, Farrell et al., 2001, Gobbo and O'Mara, 2004) injury. Environmental enrichment may also ameliorate some of the negative effects of Pb exposure on cognition and neurochemistry in rats, although the limited studies performed to date on this subject have only examined male animals, used relatively high Pb exposures, and compared only the extremes of “enriched” or “isolation” reared animals (Caoet al., 2008, Guilarte et al., 2003, Schneider et al., 2001).

In view of the limitations of previous work, the present study was performed to examine the potential interactions between sex, rearing environment and the potential neurotoxic effects of different levels of Pb exposure on spatial learning and memory utilizing the Morris water maze task.

2. Methods

2.1. Animals

The use of animals was in compliance with NIH Guidelines for the Care and Use of Laboratory Animals and the study was approved by the institutional animal care and use committee at Thomas Jefferson University. Long Evans dams (Harlan Laboratories) were fed Pb-containing food (RMH 1000 chow with or without added Pb acetate: 0 ppm, 250 ppm, 750ppm or 1500 ppm) for ten days prior to breeding and remained on the same diet through weaning. Litters were culled to equal numbers of pups to standardize litter size, with an aim of having eight pups per litter. Equal numbers of males and females were maintained wherever possible and were exposed to Pb from gestation through lactation (i.e., to postnatal day 21). At weaning 1 male and 1 female was taken from each litter and combined with animals from other litters to form behavioral cohorts. Rats were either housed 3 to a cage (47.6 × 25.9 cm) with no added stimuli (non-enriched) or 6 to a cage (61 × 43.5 cm) containing a variety of toys, climbing and nesting materials, and tunnels that were changed twice per week (enriched group). All cages contained a 50:50 mix of Alpha-Dri:Beta-Cob bedding material (Shepherd Specialty Papers), to a standard depth of 1cm. Other than the differences already described, animals were handled in exactly the same manner and were exposed to a 12h:12h light:dark cycle for the duration of the study. At day 55 and following three days of handling, the Morris water maze protocol was initiated.

2.2. Water Maze Apparatus

The water maze apparatus was located in a dimly lit (35 lux) room and consisted of a circular white pool (diameter 171 cm; height 60 cm) that was filled 40 cm deep with water maintained at a temperature of 20 ± 1 °C. A square transparent escape platform (12 × 12 cm), submerged 2 cm below the water surface, was located in the same position of a specific quadrant of the maze throughout training and testing. The pool was enclosed in an area defined by white walls on two sides and a white opaque curtain on the two other sides. Prominent geometric figures (dark in color) to be used as spatial cues were permanently affixed on all four surfaces surrounding the pool. The experimenter was positioned behind the curtain during testing and could observe the animal on a laptop computer connected to a camera located above the pool. Data were collected and analyzed using AnyMaze software (Stoelting, Inc.).

2.3. Behavioral Procedure

The rats were moved from their housing room to a room adjacent to that containing the water maze 30 min before the start of testing. The experimenter lowered each rat to the starting position with the animal facing the pool wall. Four starting positions (approximately equidistant from the platform) were used in a pseudorandom order during the acquisition period. Each rat was given 4 trials per day, and each trial lasted 90 sec or until the rat found the escape platform, whichever came first. If the rat was unable to find the platform, the experimenter guided it to the platform. Once the rat was on the platform it was left there for 30 sec. The rat was then removed from the platform and placed at a new starting position for the next trial. There were 4 or 7 days of testing during the acquisition period (for males and females respectively).

2.4. Spatial Learning and Memory Probe Measures

During the acquisition period, latency to the escape platform, the path length and path efficiency (the ratio of the shortest possible path length to actual path length) were measured (Lucas et al., 2011, Savic et al., 2009, Vorhees and Williams, 2006). The criterion used for determination of learning during the acquisition phase was the ability to reach the platform in 20 seconds or less. The rats were tested for reference memory 24 hours after the last acquisition trial. During that memory probe trial, the rats were left to swim in the maze for 90 sec from a novel starting position without the presence of the escape platform. During the memory probe, the percent time spent in quadrant of the pool that previously contained the platform, the percent of time spent in the platform quadrant, the percent of time spent in the platform position annulus, the path efficiency to the center of the annulus and the swim speed were recorded. Values were calculated both for the first 30 sec and the whole 90 sec period probe period.

2.5. Blood and Brain Lead Measurements

Blood and brain tissue were collected at the time of euthanasia and analyzed for Pb levels by either graphite furnace atomic absorption with Zeeman background correction (ESA Labs, MA) for blood Pb level, or inductively coupled plasma mass spectrometry (ICP-MS) for brain Pb levels, at the University of Pennsylvania, School of Veterinary Medicine Toxicology Laboratory. Additionally, blood samples were obtained from dams prior to Pb exposure and at parturition and weaning. Blood Pb levels were also obtained from a sample of pups at birth, day 7, 14 and 21 (weaning).

2.6. Statistical analyses

The acquisition measures from the Morris water maze were analyzed using a linear mixed model analysis with full factorial fixed effects and a 1st order autoregressive correlation structure for covariance and Bonferroni post hoc analyses where appropriate, with the average values of the days of training being the within subjects factor and the different Pb exposure levels and living conditions being the between subjects factors. The memory probe measures were analyzed with a Generalized Linear Model followed by ANOVA and Bonferroni post hoc corrected multiple comparisons. SPSS 17 software was used for the analyses.

3. Results

3.1 Animal Characteristics

There were no significant differences in the body weights of Pb exposed animals and control animals at the end of the study (age 60 and 63 days old for males and females respectively) (Table 1). Mean blood Pb levels for Pb-exposed animals, measured at the end of the study,

were not significantly different from controls (i.e., all animals had blood Pb levels at or below detectable limits, i.e., $<1 \mu\text{g/dl}$). Maternal blood Pb levels showed concentration and time-dependent changes as did blood Pb levels of offspring (Figure 1). Brain Pb levels for control groups were not significantly different regardless of rearing environment or sex. Brain Pb levels, measured at the conclusion of the study (age 60 and 63 days old for males and females respectively), in Pb-exposed groups increased significantly with increasing levels of Pb exposure (female: ($F_{(6,33)} = 48.36, p < 0.0001$); male: ($F_{(6,33)} = 34.09, p < 0.0001$) (Table 2). There was no significant effect of environment (enriched vs. non-enriched) on brain Pb levels at any given Pb exposure level.

3.2. Behavioral Analysis

3.2.1. Acquisition Phase—In general, all animals improved their performance over the 4 day (male) or 7 day (female) training period, as measured by a reduction in the mean time required to locate the hidden platform on each day (Figures 2 and 3, respectively). Similarly, path efficiency improved over the training period, with all animals increasing their path efficiency over the acquisition period (Figures 2 and 3).

In male rats, there was an overall significant main effect of environment, but not Pb exposure, on both latency to the target ($F_{(1,122)} = 45.79, p = 0.0001$) and path efficiency ($F_{(1,122)} = 19.04, p = 0.0001$). Enriched male rats located the platform significantly faster than non-enriched animals regardless of level of Pb exposure, and this effect was primarily observed during the first 2 days of learning (Figure 2). Using escape latency as a measure of learning, the learning profiles of enriched animals exposed to Pb were not significantly different from the learning profiles of enriched control animals (Figure 2). Similarly, the learning profiles of the non-enriched animals exposed to Pb were not significantly different from the learning profiles of the non-enriched control animals. Overall analysis of path efficiency across the 4 acquisition days found that only at the 750ppm and 1500ppm Pb exposure levels did animals reared in the enriched environment have a significant advantage over animals reared in the non-enriched environment ($t = 0.73, p = 0.049$ and $t = 0.15, p = 0.0001$ respectively).

Female rats also had an overall significant effect of environment on both latency to the target ($F_{(1,160)} = 47.01, p = 0.0001$) and path efficiency ($F_{(1,188)} = 37.41, p = 0.0001$). An overall main effect of Pb exposure on path efficiency was also observed ($F_{(1,189)} = 3.32, p = 0.021$). Control animals reared in the enriched environment showed reduced latencies to reach the platform on all days, except the last training day, compared to animals reared in the non-enriched environment ($t = -11.62, p = 0.0001$) (Figure 3A). Enriched Pb-exposed female rats also often took significantly less time to locate the platform, compared to non-enriched animals (250 ppm Pb ($t = 120.7, p = 0.001$) and 750 ppm Pb ($t = -7.47, p = 0.005$)), except for animals exposed to 1,500 ppm Pb (Figure 3A). Path efficiency was greater in control enriched animals compared to non-enriched animals over the first 6 acquisition days but the groups did not differ on this measure on the last acquisition day (Figure 3B). The advantage provided by enrichment was significantly reduced in animals exposed to 250 ppm Pb ($t = 0.98, p = 0.011$), and 750 ppm Pb ($t = 0.14, p = 0.0001$), and completely absent in animals exposed to 1,500 ppm Pb (Figure 3B).

Path efficiency for reaching the platform was also analyzed using a two-slope linear regression model, taking into account the biphasic nature of the curves. This analysis also showed significant differences between control and Pb-exposed animals. Enriched male rats exposed to 1,500 ppm Pb had a significantly different slope during the first 3 days of acquisition compared to enriched control males ($q = 3.896, p < 0.001$), but the groups did not differ during the last day of acquisition. All other enriched male groups performed similarly to controls. Non-enriched males, regardless of level of Pb exposure, were no different from

non-enriched control males, during any part of the acquisition phase. Enriched female rats exposed to 1,500 ppm Pb had a significantly different learning slope during the first 3 days of acquisition compared to enriched control females ($q=3.786$, $p<0.001$), but as with the males, were no different from controls during the later phase of acquisition (days 4–7). Non-enriched female rats exposed to Pb were no different from controls during the first phase of acquisition, however, non-enriched females exposed to 750 ppm or 1,500 ppm Pb had a learning curve that was significantly different from controls during the later phase of acquisition ($q=2.448$, $p<0.005$ and $q=2.521$, $p<0.05$ respectively).

3.2.2. Probe Trial—Performance on probe trials was analyzed for the following measures: (i) percent time in the northeast (NE) quadrant (i.e., the quadrant in which the platform was originally located during acquisition); (ii) percent time in the platform annulus; (iii) path efficiency to first entry into the platform annulus; and (iv) swim speed. All analyses were performed using the data from the first 30 seconds of the probe trial.

In male rats there was a significant main effect of environment on the percent of time spent in the NE quadrant during the probe test ($F_{(1,87)} = 4.88$, $p=0.03$), however the difference in performance between enriched and non-enriched animals was significant only for the 750 ppm Pb exposure group ($t=-12.98$, $p=0.003$; Figure 4A). There was no main effect of either environment or Pb exposure level on the percent time male rats spent in the platform annulus (Figure 4B). While there were no statistically significant differences between enriched and non-enriched groups at any given Pb exposure, males exposed to 250ppm and 1,500ppm Pb showed a trend toward a greater percent of time spent in the platform annulus in enriched compared to non-enriched animals ($t=2.64$, $p=0.077$ and $t=2.72$, $p=0.051$ respectively). There was a significant main effect of environment on path efficiency to the first entry into the platform annulus during the first 30s of the probe trial ($F_{(1,84)} = 3.95$, $p=0.05$). The main effect of Pb exposure on this measure was not statistically significant ($F_{(3,84)} = 0.737$, $p=0.533$) (Figure 4C). There was however a significant interaction between Pb exposure and environment ($F_{(3,84)} = 3.67$, $p=0.015$). Non-enriched males with 250 ppm Pb exposure were the only Pb exposure groups that had significantly lower path efficiency compared to non-enriched controls. Within a Pb exposure group, non-enriched animals in the 250 ppm and 1500 ppm groups (but not the 750 ppm group) had significantly lower path efficiencies compared to enriched animals in the same exposure groups ($t=0.30$, $p=0.018$ and $t=0.25$, $p=0.03$ respectively; Figure 4C). Analysis of swim speed showed that there was a significant main effect of Pb exposure ($F_{(3,87)} = 3.54$, $p=0.018$), but not of environment on swim speed. In enriched male rats, only 1,500 ppm Pb-exposure resulted in a statistically significant reduction in swim speed when compared to control ($t=-0.46$, $p=0.01$; Figure 4D). There were no differences in swim speed in the non-enriched group regardless of Pb exposure.

In female rats there was a significant main effect of both Pb exposure ($F_{(1,103)} = 3.92$, $p=0.011$) and environment ($F_{(1,103)} = 4.62$, $p=0.034$) on the percent of time spent in the NE quadrant during the first 30s seconds of the probe test. There was also a significant main effect of environment ($F_{(1,103)} = 14.36$, $p=0.0001$) and Pb exposure level ($F_{(3,103)} = 2.9$, $p=0.039$) on the percent time female rats spent in the platform annulus during the first 30 seconds of the probe trial. Non-enriched control and 750 ppm Pb-exposed rats spent significantly less time in the platform annulus than their enriched counterparts ($t=-2.25$, $p=0.02$ and $t=-3.09$, $p=0.015$ respectively; Figure 5B). There were main effects of environment ($F_{(1,99)} = 13.05$, $p=0.0001$) and Pb exposure ($F_{(1,99)} = 2.69$, $p=0.05$) on path efficiency to the first entry into the platform annulus during the first 30s of the probe trial in female rats. Significant differences in path efficiency between enriched and non-enriched rats were observed at the 250 ppm and 750 ppm exposure levels ($t=0.268$, $p=0.046$ and $t=0.316$, $p=0.006$ respectively) with 1,500 ppm exposure level trending towards significance

($t=0.194$, $p=0.059$; Figure 5C). There was no overall main effect of either environment or Pb exposure on swim speed in female rats, but there was a significant interaction of Pb-exposure on environment ($F_{(3, 103)} = 3.46$, $p=0.019$) on swim speed in female rats. Non-enriched female rats exposed to 250 ppm Pb swam slightly but statistically significantly slower than enriched female rats exposed to 250 ppm Pb ($t=-0.051$, $p=0.011$; Figure 5D).

4. Discussion

The present results suggest that there are complex interactive effects between sex, rearing environment and developmental Pb exposure on spatial learning and memory, as assessed by performance in the Morris water maze. In control animals, beneficial effects of environmental enrichment on spatial learning were observed in both males and females, with effects from enrichment greater in females. The data also suggest that in females, the combination of being raised in a non-enriched environment and being exposed to Pb during development can result in slower and less efficient spatial learning compared to female animals raised in a non-enriched environment without Pb exposure. In comparison, there was an advantage to enrichment early during task acquisition in males but thereafter, there were few differences between enriched and non-enriched males whether exposed or not exposed to Pb during development. In females, Pb exposure also appeared to mitigate at least some of the benefits of environmental enrichment on spatial learning, particularly at the lowest and highest exposure levels. In males, being raised in an enriched environment conferred some spatial learning advantage (although not as much as in females) and for the most part, Pb exposure did not seem to blunt this modest advantage. However, in males with the highest Pb exposure, environmental enrichment did help to overcome detrimental effects of Pb on spatial learning.

In control (non-Pb exposed) animals, both non-enriched and enriched males reached the learning criterion (escape latency ≤ 20 sec) by the third day of training. Enriched females also reached this criterion by the third day of training but non-enriched females did not reach this criterion until the 6th day of training. Thus, there were no differences in spatial learning ability for males or females raised in the enriched environment but females reared in the non-enriched condition learned the task more slowly and adopted a less efficient escape strategy than males. These data are consistent with previous reports that male rodents acquire spatial navigation tasks more rapidly than females, but in the current study, this was only true for animals raised in the non-enriched environment. However, our “non-enriched” housing condition is similar to the “standard” housing condition used in other studies. Sex-related differences in spatial learning have been reported before and generally have tended to be small and have most typically been seen early during training (Beiko et al., 2004). However, relatively few studies simultaneously assessed the performance of males and females. A recent meta-analysis of sex differences in rodent models of learning and memory (Jonasson, 2005) reported significant male advantages in spatial learning and reference memory with lesser effects of rearing environment and pre-training on spatial learning. Our data suggest that rearing environment has an important and sex-dependent contribution to spatial learning ability that can be modified by developmental Pb exposure. Others have suggested that the male advantage in spatial learning ability can be affected by rearing conditions and manipulation of specific task parameters and that males and females may differ on Morris water maze performance because they make use of different types of cues when forming spatial memories (Roof and Stein, 1999). The issue of sex difference in spatial learning ability is complex and not easily attributable to specific neural mechanisms.

Beiko et al. (2004) have suggested that sex differences in the acute stress response may underlie sex differences in Morris water maze performance. In particular, female rats may have a greater acute stress response than males early in water maze training when sex

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

differences in performance have been most frequently reported (Frye, 1995, Warren and Juraska, 1997) and this increased stress response may impair water maze performance in females during early training (Beikoet al., 2004). We did not measure stress responsivity of the animals in the current study but our data suggest that being raised in the enriched environment modulated the acute stress response in females, essentially eliminating a sex difference in spatial learning ability. Non-enriched females exposed to 250 ppm or 1,500 ppm Pb behaved somewhat differently from control animals: non-enriched animals in the 250 ppm group never reached learning criterion and non-enriched animals in the 1,500 ppm group were delayed in reaching criterion. These results could be related to effects of Pb on acute stress responses in female rats. The effects of maternal Pb on offspring corticosterone levels (i.e., increased corticosterone levels measured at postnatal day 30) have been described in both male and female rats (Yu et al., 1996), as have exacerbated effects of a mild stressor in Pb-exposed animals (Virgolini et al., 1999, Virgolini et al., 2004), although the latter effect was not examined in males and females in the same study. It is possible though that a Pb-induced alteration of stress responsivity in non-enriched females was greater than in males and contributed to the apparent spatial learning deficit in these animals. Male rats raised in an enriched environment, have faster adaptation/habituation of pituitary-adrenocortical and sympathetic-adrenomedullary responses and reduced ACTH, corticosterone and adrenaline responses to acute stress (Moncek et al., 2004). Although the extent to which this may also occur in females is not entirely clear, nor are the ways in which developmental Pb exposure might interact with such phenomena, the combined effects of Pb and stress have been well-documented and are potentiated in female rats (Cory-Slechta et al., 2004).

On at least some measures of reference memory, potential benefits contributed by rearing in the enriched environment were attenuated by exposure to Pb and this effect differed somewhat for males and females. Few animal studies have systematically assessed the influence of sex on outcome from Pb exposure but in those studies that have, sex-related differences have been described. Female rats exposed to Pb through gestation and lactation were more impaired in reference memory than male rats with similar Pb exposures (Jett et al., 1997). De Souza Lisboa et al. (2005) reported that exposure to Pb during both pregnancy and lactation led to depressive-like behavior (detected in the forced swimming test) in female but not male rats. The influence of sex as an effect modifier of childhood Pb poisoning has also received little systematic attention. Few clinical studies have directly examined the influence of sex on outcome following childhood Pb exposure and in the ones that have, the findings have been mixed (Bellinger et al., 1990, Dietrich et al., 1987, McMichael et al., 1992, Rabinowitz et al., 1991). However, accumulating evidence suggest that childhood Pb exposure may have a different impact on the male brain compared to the female brain. We have recently described interactive effects of Pb and sex on gene expression patterns in the rat hippocampus, a structure involved in learning and memory (Schneider et al., 2011). Although the functional significance of these changes in the hippocampal transcriptome remains to be determined, the data suggest that the response of the brain to a given Pb exposure may vary depending on sex (Schneider et al., 2011).

Non-linear dose-response effects are a common observation in toxicology and pharmacology and particularly in the area of Pb neurotoxicity. For example, the functional impact of low levels of Pb on IQ scores in Pb-exposed children is greater than the proportional impact at higher levels of exposure (Lanphear et al., 2005; Rothenberg and Rothenberg, 2005). In rats, hippocampal long-term potentiation, synaptic release of glutamate, and calcium-dependent release of GABA follow a “U-shaped” dose-response curve (Lasley and Gilbert, 2002, White et al., 2007). In particular, “U-shaped” curves reflect an apparent reversal or inversion in an effect of an otherwise toxic agent at a low or intermediate region of the dose continuum (Davis and Svendsgaard, 1990). In the current

study, effects on a number of behavioral measures were observed at the lowest and highest levels of Pb exposure whereas similar effects were not observed at the intermediate level of exposure. Such results suggest that different mechanisms may be affected by low and high levels of Pb exposure than are affected at intermediate levels of exposure. At low levels of exposures, Pb may effectively interfere with a variety of physiological process that could result in functional changes at the behavioral level, due to well-known effects of Pb in the nanomolar and picomolar ranges on a variety of physiological mechanisms relevant to neurotransmission (ex., Braga et al., 1999, Markovac and Goldstein, 1988). At higher (intermediate) levels of exposure that are toxic but do not overwhelm the system, homeostatic adjustments may occur in response to the exposure leading to an apparent loss of toxic effect which may more likely reflect effective compensatory responses becoming active. At still higher exposure levels, compensatory responses may become overwhelmed and toxic responses prevail over potential protective responses (Davis and Svendsgaard, 1990).

Few studies have examined the interaction of sex and environmental manipulation on cognitive performance and this is the first study, to the best of our knowledge, that has examined the interactions of sex, environment, and Pb exposure on aspects of cognition. Additionally, most studies employing environmental manipulation, including a previous one from this laboratory (Schneider et al., 2001) have examined differences between environmental extremes (i.e., isolation rearing vs. enrichment). While such studies may provide important information, their translational relevance may be limited since in nature, postnatal development does not occur in isolation, although it may occur under non-enriched circumstances. In rodent studies, environmental enrichment typically encompasses a combination of social enrichment (group housing) and addition of stimuli (ex., toys, climbing and nesting material that are changed on a regular schedule) to a large enclosure and animals housed in this manner are compared with animals housed singly in a small cage with no added stimuli. Some studies however, have compared outcomes from rats raised under enriched conditions, as described above, with rats raised under social enrichment only (i.e., animals raised in small groups with no added stimuli to the environment: the “non-enriched” group of the current study). In one such study, differences in rearing conditions affected outcomes on standard behavioral tests and in almost all instances, “low enriched” rats (housed in groups of 6 in a standard cage with one shelter, nest material and 1 or 2 toys) had measures intermediate between “non-enriched” (housed in a groups of 5–6 in a standard cage without any enrichment objects) and “high enriched” (housed in a large cage in groups of 11 with climbing materials, toys and nesting materials rearranged and changed regularly) rats (Hoffmann et al., 2009). In another study in which rats were housed 3 to a cage with no toys and compared with rats housed similarly but with added toys, social and environmental factors were found to independently alter behavioral responses as well as neurochemistry and both conditions were found to be beneficial compared to rats raised in isolation (Zakharova et al., 2009). In the current study, we found that housing environment was an effect modifier for behavioral outcome and interacted with sex to modify the influence of Pb exposure. If sex interacts with the environment to influence cognitive outcomes and also modulates the impact of Pb on the brain, then a more complete understanding of sex-environment-Pb interactions is needed and may have important clinical and educational implications for Pb poisoned individuals.

Socioeconomic status (SES) is modeled in animals by raising animals in housing environments of different complexity. A child’s SES clearly affects the likelihood of exposure to Pb but it may also influence the outcome from Pb exposure. There are several concomitants of poverty that not only increase the likelihood of a child being exposed to Pb, but also of absorbing more Pb. For example, dietary deficiencies that increase the absorption of Pb, such as a diet deficient in calcium, iron, protein, and/or zinc, may increase

vulnerability to the adverse effects of Pb, and this is expected to be more frequently present in economically disadvantaged children. However, apart from enhancing the ease with which Pb may gain access to the brain, there is evidence that SES or its analog in animals, environmental enrichment or impoverishment, may modify the neurotoxic effects of a given level of Pb once it is in the brain. In an experimentally enriched environment, animals are exposed to more sensory and social stimuli than are present in a non-enriched environment represented by standard rodent housing conditions. Animals reared in non-enriched environments have less well-developed brains (according to a variety of anatomical and neurochemical measures) than animals reared in more complex environments (van Praaget al., 2000) and exposure to enriched environments not only improves learning and memory performance in normal animals but also increases functional recovery from different types of brain damage (Willet al., 2004) as well as ameliorates some of the negative effects of Pb exposure on cognition and neurochemistry in rats (Guilarte and McGlothlan, 2003, Schneider et al., 2001). Thus, as suggested by the current study, environmental manipulation is a potential risk modifier that may modulate the impact of developmental Pb exposure and potentially interacts with other factors such as sex and individual genetic makeup to affect the molecular, neurochemical and functional influences of Pb on the brain.

Acknowledgments

This work was supported by NIH grant ES015295. The funding source had no role in the design, conduct or interpretation of the study. The authors thank Rebecca Schray, William Mettil, Emmanuel Decamp and Leslie Walinchus for providing technical support throughout the study.

REFERENCES

- Astur RS, Ortiz ML, Sutherland RJ. A characterization of performance by men and women in a virtual Morris water task: a large and reliable sex difference. *Behav Brain Res.* 1998; 93:185–190. [PubMed: 9659999]
- Beiko J, Lander R, Hampson E, Boon F, Cain DP. Contribution of sex differences in the acute stress response to sex differences in water maze performance in the rat. *Behav Brain Res.* 2004; 151:239–253. [PubMed: 15084440]
- Bellinger D, Leviton A, Sloman J. Antecedents and correlates of improved cognitive performance in children exposed in utero to low levels of lead. *Environ Health Perspect.* 1990; 89:5–11. [PubMed: 2088755]
- Bellinger D, Sloman J, Leviton A, Rabinowitz M, Needleman HL, Waternaux C. Low-level lead exposure and children's cognitive function in the preschool years. *Pediatrics.* 1991; 87:219–227. [PubMed: 1987535]
- Blokland A, Rutten K, Prickaerts J. Analysis of spatial orientation strategies of male and female Wistar rats in a Morris water escape task. *Behav Brain Res.* 2006; 171:216–224. [PubMed: 16647766]
- Bradley, RH.; Corwyn, RF. Parenting. In: Tamis-Lemonda, CLB., editor. *Child Psychology: A Handbook of Contemporary Issues.* New York: Psychology Press; 1999. p. 339-362.
- Bradley RH, Corwyn RF, McAdoo HP, Coll CG. The home environments of children in the United States part I: variations by age, ethnicity, and poverty status. *Child Dev.* 2001; 72:1844–1867. [PubMed: 11768149]
- Braga MF, Pereira EF, Marchioro M, Albuquerque EX. Lead increases tetrodotoxin-insensitive spontaneous release of glutamate and GABA from hippocampal neurons. *Brain Res.* 1999; 826:10–21. [PubMed: 10216192]
- Brooks-Gunn J, Duncan GJ. The effects of poverty on children. *The Future of Children.* 1997; 7:55–71. [PubMed: 9299837]
- Cao X, Huang S, Ruan D. Enriched environment restores impaired hippocampal long-term potentiation and water maze performance induced by developmental lead exposure in rats. *Dev Psychobiol.* 2008; 50:307–313. [PubMed: 18335502]

- Cory-Slechta DA, Virgolini MB, Thiruchelvam M, Weston DD, Bauter MR. Maternal stress modulates the effects of developmental lead exposure. *Environ Health Perspect.* 2004; 112:717–730. [PubMed: 15121516]
- Dahlqvist P, Ronnback A, Bergstrom SA, Soderstrom I, Olsson T. Environmental enrichment reverses learning impairment in the Morris water maze after focal cerebral ischemia in rats. *Eur J Neurosci.* 2004; 19:2288–2298. [PubMed: 15090055]
- Dalla C, Shors TJ. Sex differences in learning processes of classical and operant conditioning. *Physiology & behavior.* 2009; 97:229–238. [PubMed: 19272397]
- Davis JM, Svendsgaard DJ. U-Shaped dose response curves: Their occurrence and implications for risk assessment. *J Toxicol Environ Health.* 1990; 30:71–83. [PubMed: 2192070]
- de Souza, Lisboa S F.; Gonzalves, G.; Komatsu, F.; Salci Queiroz, CA.; Aparecido Almeida, A.; Gastaldello, Moreira EN. Developmental lead exposure induces depressive-like behavior in female rats. *Drug Chem Toxicol.* 2005; 28:67–77. [PubMed: 15720036]
- Dietrich KN, Krafft KM, Bornschein RL, Hammond PB, Berger O, Succop PA, et al. Low-level fetal lead exposure effect on neurobehavioral development in early infancy. *Pediatrics.* 1987; 80:721–730. [PubMed: 2444921]
- Duff SJ, Hampson E. A sex difference on a novel spatial working memory task in humans. *Brain Cogn.* 2001; 47:470–493. [PubMed: 11748902]
- Farah MJ, Shera DM, Savage JH, Betancourt L, Giannetta JM, Brodsky NL, et al. Childhood poverty: Specific associations with neurocognitive development. *Brain Res.* 2006; 1110:166–174. [PubMed: 16879809]
- Farrell R, Evans S, Corbett D. Environmental enrichment enhances recovery of function but exacerbates ischemic cell death. *Neuroscience.* 2001; 107:585–592. [PubMed: 11720782]
- Frye CA. Estrus-associated decrements in a water maze task are limited to acquisition. *Physiology & behavior.* 1995; 57:5–14. [PubMed: 7878125]
- Frye CA, Sturgis JD. Neurosteroids affect spatial/reference, working, and long-term memory of female rats. *Neurobiol Learn Mem.* 1995; 64:83–96. [PubMed: 7582815]
- Galea LA, Kavaliers M, Ossenkopp KP. Sexually dimorphic spatial learning in meadow voles *Microtus pennsylvanicus* and deer mice *Peromyscus maniculatus*. *The Journal of experimental biology.* 1996; 199:195–200. [PubMed: 8576690]
- Gilbert ME, Kelly ME, Samsam TE, Goodman JH. Chronic developmental lead exposure reduces neurogenesis in adult rat hippocampus but does not impair spatial learning. *Toxicol Sci.* 2005; 86:365–374. [PubMed: 15788721]
- Gobbo OL, O'Mara SM. Impact of enriched-environment housing on brain-derived neurotrophic factor and on cognitive performance after a transient global ischemia. *Behav Brain Res.* 2004; 152:231–241. [PubMed: 15196790]
- Guilarte TR, McGlothan JL. Selective decrease in NR1 subunit splice variant mRNA in the hippocampus of Pb2+exposed rats: implications for synaptic targeting and cell surface expression of NMDAR complexes. *Brain Res Mol Brain Res.* 2003; 113:37–43. [PubMed: 12750004]
- Guilarte TR, Toscano CD, McGlothan JL, Weaver SA. Environmental enrichment reverses cognitive and molecular deficits induced by developmental lead exposure. *Ann Neurol.* 2003; 53:50–56. [PubMed: 12509847]
- Hoffmann LC, Schutte SR, Koch M, Schwabe K. Effect of "enriched environment" during development on adult rat behavior and response to the dopamine receptor agonist apomorphine. *Neuroscience.* 2009; 158:1589–1598. [PubMed: 19110038]
- Jett DA, Kuhlmann AC, Farmer SJ, Guilarte TR. Age-dependent effects of developmental lead exposure on performance in the Morris water maze. *Pharmacol Biochem Behav.* 1997; 57:271–279. [PubMed: 9164582]
- Jonasson Z. Meta-analysis of sex differences in rodent models of learning and memory: a review of behavioral and biological data. *Neurosci Biobehav Rev.* 2005; 28:811–825. [PubMed: 15642623]
- Kolb B, Gibb R. Environmental enrichment and cortical injury: behavioral and anatomical consequences of frontal cortex lesions. *Cereb Cortex.* 1991; 1:189–198. [PubMed: 1822732]

- Lanphear BP, Dietrich K, Auinger P, Cox C. Cognitive deficits associated with blood lead concentrations <10 microg/dL in US children and adolescents. *Public Health Rep.* 2000; 115:521–529. [PubMed: 11354334]
- Lanphear BP, Hornung R, Khoury J, Yolton K, Baghurst P, Bellinger DC, et al. Low-level environmental lead exposure and children's intellectual function: an international pooled analysis. *Environ Health Perspect.* 2005; 113:894–899. [PubMed: 16002379]
- Lasley SM, Gilbert ME. Rat hippocampal glutamate and GABA release exhibit biphasic effects as a function of chronic lead exposure level. *Tox Sci.* 2002; 66:139–147.
- Lucas MM, Lenck-Santini P-P, Holmes GL, Scott RC. Impaired cognition in rats with cortical dysplasia: additional impact of early-life seizures. *Brain.* 2011; 134:1684–1693. [PubMed: 21602270]
- Markovac J, Goldstein GW. Picomolar concentrations of lead stimulate brain protein kinase C. *Nature.* 1988; 334:71–73. [PubMed: 3386747]
- McCloyd VC. Socioeconomic disadvantage and child development. *Am Psychol.* 1988; 53:185–204.
- McMichael AJ, Baghurst PA, Vimpani GV, Robertson EF, Wigg NR, Tong SL. Sociodemographic factors modifying the effect of environmental lead on neuropsychological development in early childhood. *Neurotoxicol Teratol.* 1992; 14:321–327. [PubMed: 1454040]
- Mendez-Lupez M, Mendez M, Lupez L, Arias JL. Spatial working memory learning in young male and female rats: Involvement of different limbic system regions revealed by cytochrome oxidase activity. *Neurosci Res.* 2009; 65:28–34. [PubMed: 19463868]
- Moncek F, Duncko R, Johansson BB, Jezova D. Effect of environmental enrichment on stress related systems in rats. *J Neuroendocrinol.* 2004; 16:423–431. [PubMed: 15117335]
- Morris R. Developments of a water-maze procedure for studying spatial learning in the rat. *J Neurosci Methods.* 1984; 11:47–60. [PubMed: 6471907]
- Newhouse P, Newhouse C, Astur RS. Sex differences in visual-spatial learning using a virtual water maze in pre-pubertal children. *Behav Brain Res.* 2007; 183:1–7. [PubMed: 17629971]
- Rabinowitz MB, Wang JD, Soong WT. Dentine lead and child intelligence in Taiwan. *Arch Environ Health.* 1991; 46:351–360. [PubMed: 1772260]
- Rizk-Jackson AM, Acevedo SF, Inman D, Howieson D, Benice TS, Raber J. Effects of sex on object recognition and spatial navigation in humans. *Behav Brain Res.* 2006; 173:181–190. [PubMed: 16887201]
- Roof RL, Stein DG. Gender differences in Morris water maze performance depend on task parameters. *Physiol Behav.* 1999; 68:81–86. [PubMed: 10627065]
- Roof RL, Zhang Q, Glasier MM, Stein DG. Gender-specific impairment on Morris water maze task after entorhinal cortex lesion. *Behav Brain Res.* 1993; 57:47–51. [PubMed: 8292254]
- Rose FD, al-Khamees K, Davey MJ, Attree EA. Environmental enrichment following brain damage: an aid to recovery or compensation? *Behav Brain Res.* 1993a; 56:93–100. [PubMed: 8397858]
- Rose FD, Davey MJ, Attree EA. How does environmental enrichment aid performance following cortical injury in the rat? *Neuroreport.* 1993b; 4:163–166. [PubMed: 8453055]
- Rosenzweig MR, Bennett EL, Hebert M, Morimoto H. Social grouping cannot account for cerebral effects of enriched environments. *Brain Res.* 1978; 153:563–576. [PubMed: 698794]
- Rothenberg SJ, Rothenberg JC. Testing the dose-response specification in epidemiology: Public health and policy consequences for lead. *Environmental Health Perspect.* 2005; 113:1190–1195.
- Sandstrom NJ, Kaufman J, Huettel SA. Males and females use different distal cues in a virtual environment navigation task. *Brain Res Cogn Brain Res.* 1998; 6:351–360. [PubMed: 9593991]
- Saucier DM, Green SM, Leason J, MacFadden A, Bell S, Elias LJ. Are sex differences in navigation caused by sexually dimorphic strategies or by differences in the ability to use the strategies? *Behav Neurosci.* 2002; 116:403–410. [PubMed: 12049321]
- Saucier DM, Shultz SR, Keller AJ, Cook CM, Binsted G. Sex differences in object location memory and spatial navigation in Long-Evans rats. *Animal Cognition.* 2008; 11:129–137. [PubMed: 17562087]
- Savic MM, Milinkovic MM, Rallapalli S, Clayton T Sr, Joksimovic S, Van Linn M, et al. The differential role of alpha1- and alpha5-containing GABA(A) receptors in mediating diazepam

- effects on spontaneous locomotor activity and water-maze learning and memory in rats. *Int J Neuropsychopharmacol.* 2009; 12:1179–1193. [PubMed: 19265570]
- Schneider JS, Anderson DW, Sonnenahalli H, Vadigepalli R. Sex-based differences in gene expression in hippocampus following postnatal lead exposure. *Toxicol Appl Pharmacol.* 2011; 256:179–190. [PubMed: 21864555]
- Schneider JS, Lee MH, Anderson DW, Zuck L, Lidsky TI. Enriched environment during development is protective against lead-induced neurotoxicity. *Brain Res.* 2001; 896:48–55. [PubMed: 11277972]
- Surkan PJ, Zhang A, Trachtenberg F, Daniel DB, McKinlay S, Bellinger DC. Neuropsychological function in children with blood lead levels <10 microg/dL. *Neurotoxicology.* 2007; 28:1170–1177. [PubMed: 17868887]
- van Praag H, Kempermann G, Gage FH. Neural consequences of environmental enrichment. *Nat Rev Neurosci.* 2000; 1:191–198. [PubMed: 11257907]
- Virgolini MB, Cancela LM, Fulginiti S. Behavioral responses to ethanol in rats perinatally exposed to low lead levels. *Neurotoxicol Teratol.* 1999; 21:551–557. [PubMed: 10492389]
- Virgolini MB, Volosin M, Fulginiti AS, Cancela LM. Amphetamine and stress responses in developmentally lead-exposed rats. *Neurotoxicol Teratol.* 2004; 26:291–303. [PubMed: 15019963]
- Vorhees CV, Williams MT. Morris water maze: procedures for assessing spatial and related forms of learning and memory. *Nat Protoc.* 2006; 1:848–858. [PubMed: 17406317]
- Warren SG, Juraska JM. Spatial and nonspatial learning across the rat estrous cycle. *Behav Neurosci.* 1997; 111:259–266. [PubMed: 9106666]
- White LD, Cory-Slechta DA, Gilbert ME, Tiffany-Castiglioni E, Zawia NH, Virgolini M, et al. New and evolving concepts in the neurotoxicology of lead. *Toxicol Appl Pharmacol.* 2007; 225:1–27. [PubMed: 17904601]
- Will B, Galani R, Kelche C, Rosenzweig MR. Recovery from brain injury in animals: relative efficacy of environmental enrichment, physical exercise or formal training (1990–2002). *Prog Neurobiol.* 2004; 72:167–182. [PubMed: 15130708]
- Will BE, Sutter AR, Offerlin MR. Effects of methamphetamine and enriched experience on behavioral recovery after brain damage (author's transl). *Psychopharmacology (Berl).* 1977; 51:273–277. [PubMed: 403543]
- Williams CL, Meck WH. The organizational effects of gonadal steroids on sexually dimorphic spatial ability. *Psychoneuroendocrinology.* 1991; 16:155–176. [PubMed: 1961837]
- Xu J, Yan HC, Yang B, Tong LS, Zou YX, Tian Y. Effects of lead exposure on hippocampal metabotropic glutamate receptor subtype 3 and 7 in developmental rats. *J Negat Results Biomed.* 2009; 8:5. [PubMed: 19374778]
- Yu SY, Mizinga KM, Nonavinakere VK, Soliman KF. Decreased endurance to cold water swimming and delayed sexual maturity in the rat following neonatal lead exposure. *Toxicol Lett.* 1996; 85:135–141. [PubMed: 8644125]
- Zakharova E, Miller J, Unterwald E, Wade D, Izenwasser S. Social and physical environment alter cocaine conditioned place preference and dopaminergic markers in adolescent male rats. *Neuroscience.* 2009; 163:890–897. [PubMed: 19580849]

Highlights

- Sex and rearing environment modified effects of Pb on learning and memory.
- Pb counteracted some effects of enrichment primarily in females
- Effects of Pb on learning/memory were often seen at lowest and highest exposures

Fig. 1. Blood lead levels during the perinatal exposure period. (A). Maternal blood lead levels. Prior to lead exposure all animals had equivalent lead levels. After 10 days of lead exposure and prior to being paired with a male for breeding, there was a dose-dependent increase in blood lead levels. Blood lead levels still showed a dose-dependent increase immediately post-partum and immediately post-weaning. (B). Male blood lead levels from birth to weaning. (C) Female blood lead levels from birth to weaning. Statistical comparisons are indicated on the graphs, with significance levels as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$.

A. Escape Latency

B. Path Efficiency

Fig. 2.

Water maze task acquisition data for male rats. *Escape Latency*: All groups of animals acquired a spatial map by the fourth day of training as defined by an escape latency of less than 20 seconds (learning criterion is shown by the dotted line in each graph). Enriched animals acquired a spatial map faster than non-enriched animals and virtually all enriched groups reached criterion by the completion of the second day of training. Non-enriched groups required 3 days of training to reach criterion. By day 3, all lead-exposed non-enriched animals had escape latencies similar to matched enriched animals, with the exception of 1,500 ppm lead-exposed non-enriched animals. *Path Efficiency*: All groups of animals regardless of treatment showed greater path efficiency (a measure of the efficiency

of the path taken by the animal from the release point to the location of the hidden platform) over time. All lead-exposed and non-enriched animals performed worse than matched enriched animals at all time points. Group Sizes: Enriched Animals - Control (n=18), 250ppm Pb-exposure (n=10), 750ppm Pb-exposure (n=10), and 1500ppm Pb-exposure (n=11); Non-Enriched Animals: Control (n=11), 250ppm Pb-exposure (n=10), 750ppm Pb-exposure (n=13), and 1500ppm Pb-exposure (n=12). All data are shown as group mean $\pm$ S.E.M. Statistical differences are between enriched and non-enriched groups within a given level of lead exposure. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, and **** $p<0.0001$.

A. Escape Latency

B. Path Efficiency

Fig. 3.

Water maze task acquisition data for female rats. *Escape Latency*: All females acquired a spatial map by the 7th day of training as defined by an escape latency of less than 20 seconds, with most groups of animals at or below criterion by the 5th day of training (learning criterion is shown by the dotted line in each graph). In all groups, except those exposed to 1,500 ppm Pb, enriched animals acquired the task faster than non-enriched animals. The non-enriched 250 ppm lead-exposed group was the only group that did not reach learning criterion by day 7. *Path Efficiency*: All groups of animals showed improved path efficiency (a measure of the efficiency of the path taken by the animal from the release point to the location of the hidden platform) over time. There was little difference in the

performance of non-enriched and enriched animals in the 250 ppm and 1,500 ppm exposure groups with some residual benefits from enrichment in the 750 ppm exposure group. All data are shown as group mean $\pm$ S.E.M. Statistical differences are between enriched and non-enriched groups within a given exposure level. Group Sizes: Enriched Animals - Control (n=20), 250ppm Pb-exposure (n=16), 750ppm Pb-exposure (n=10), and 1500ppm Pb-exposure (n=20); Non-Enriched Animals: Control (n=17), 250ppm Pb-exposure (n=5), 750ppm Pb-exposure (n=12), and 1500ppm Pb-exposure (n=11). * p<0.05, ** p<0.01, *** p<0.001 and **** p<0.0001.

Fig. 4.

Water maze probe trial performance of male rats. **(A)** Percent of time spent in the Northeast (NE) quadrant during the first 30 seconds of the probe test. Only the non-enriched animals exposed to 750 ppm lead performed significantly differently than matched enriched animals. **(B)** Percent of time spent in the NE (platform) annulus during the first 30 seconds of the probe test. There was no significant effect of lead exposure or rearing environment on this measure. **(C)** Path efficiency of first entry into the NE (platform) annulus during the first 30s of the probe test. Non-enriched animals exposed to either 250 ppm or 1,500 ppm lead (but not 750 ppm lead) were impaired on this measure compared to matched enriched animals. **(D)** Swim speed within the first 30s of the probe test. Statistically significant but not functionally meaningful differences were observed within enriched environment at 1500ppm Pb exposure. Group Sizes: Enriched Animals - Control (n=18), 250ppm Pb-exposure (n=10), 750ppm Pb-exposure (n=10), and 1500ppm Pb-exposure (n=11); Non-Enriched Animals: Control (n=11), 250ppm Pb-exposure (n=10), 750ppm Pb-exposure (n=13), and 1500ppm Pb-exposure (n=12). All data are shown as group mean $\pm$ S.E.M. Statistical

differences between enriched and non-enriched groups within a given exposure level are defined as follows: ♦ $p < 0.05$, ♦♦ $p < 0.01$. Statistical differences within environment are defined using linking bars: ** $p < 0.01$.

Fig. 5. Water maze probe trial performance of female rats. **(A)** Percent of time spent in the Northeast (NE) quadrant during the first 30 seconds of the probe test. There was no significant effects within lead exposure or rearing environment on this measure. **(B)** Percent of time spent in the NE annulus during the first 30 seconds of the probe test. Non-enriched 750 ppm-exposed and control animals were significantly impaired on this measure compared to matched enriched animals. **(C)** Path efficiency of first entry into the NE annulus during the first 30s of the probe test. Non-enriched animals exposed to either 250 ppm or 750 ppm lead (but not 1,500 ppm lead) were impaired on this measure compared to matched enriched animals. **(D)** Swim speed within the first 30s of the probe test was statistically slower in the 250 ppm, non-enriched group compared to matched enriched animals. Group Sizes: Enriched Animals - Control (n=20), 250ppm Pb-exposure (n=16), 750ppm Pb-exposure (n=10), and 1500ppm Pb-exposure (n=20); Non-Enriched Animals: Control (n=17), 250ppm Pb-exposure (n=5), 750ppm Pb-exposure (n=12), and 1500ppm Pb-exposure (n=11). All data are shown as group mean $\pm$ S.E.M. Statistical differences between

enriched and non-enriched groups within a given exposure level are defined as follows: ♦
p<0.05, ♦♦ p<0.01.

Table 1

Body weights at study conclusion.

	Body Weight (g)			
	0 ppm	250 ppm	750 ppm	1500 ppm
Male Impoverished	352.2 ± 8.3	340.8 ± 12.9	308.5 ± 22.9	328.8 ± 5.2
Male Enriched	365.5 ± 12.2	355.7 ± 4.8	355.8 ± 10.5	328.0 ± 6.5
Female Impoverished	255.2 ± 4.1	227.5 ± 11.0	221.0 ± 5.1	224.2 ± 8.7
Female Enriched	215.8 ± 9.2	219.2 ± 4.3	225.7 ± 8.9	210.0 ± 4.5

There were no significant differences in body weights within gender regardless of lead exposure levels or rearing environmental.

Table 2

Brain lead levels at study conclusion.

	Brain Lead Levels (ppb)			
	0 ppm	250 ppm	750 ppm	1500 ppm
Male Impoverished	4.5 ± 0.4	15.6 ± 3.5	36.2 ± 6.8 **	71.0 ± 6.3 ***
Male Enriched	3.6 ± 1.2	9.0 ± 0.9	20.7 ± 2.8 **	60.5 ± 5.1 ***
Female Impoverished	6.4 ± 3.6	12.6 ± 2.2	18.5 ± 1.9	48.7 ± 6.1 ***
Female Enriched	6.2 ± 1.3	14.4 ± 1.3 *	16.4 ± 1.3 *	58.3 ± 2.6 ***

Significant differences within a gender and rearing environment group between lead-exposed and control animals are indicated (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).