Low Level Lead Exposure Impairs Attentional Set Shifting Task Performance Depending upon Sex and Developmental Periods of Exposure

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Introduction

Exposure to low levels of lead (Pb) impairs a variety of cognitive processes. Although children exposed to Pb developmentally present with a variety of cognitive impairments that include deficits in learning, memory, language, and executive functioning, experimental work on Pb toxicity in rats has focused mostly on learning and memory deficits and less on executive functions. However, detrimental effects on executive functioning could lead to or even underlie a variety of other cognitive problems attributed to Pb exposure. In this study, we examined the ability of Long Evans rats (control and Pb-exposed: 150ppm Pb-acetate in food given perinatally (gestation through weaning) or early postnatally (EPN, birth through weaning)) to acquire and perform an attention set shifting test (ASST) that requires animals to locate a food reward based on discriminating between digging materials and odors. The task consisted of simple (SD) and compound (CD) discriminations and reversals and intra-dimensional (ID) and extra-dimensional (ED) shifts followed by reversals.

Methods

Subjects: The use of animals complied 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 chow (RMH 1000) with or without added Pb acetate (0ppm or 150 ppm) for 10 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. One male and female were taken from each litter and combined with animals from other litters to form experimental cohorts. Rats were all housed 4 to a standard cage (47.6 x 25.9 cm) and were exposed to a 12h:12h light:dark cycle for the duration of the study. Animals exposed to Pb from gestation through lactation (i.e., to postnatal day (PND) 22) comprised the perinatal (Peri) exposure group. Other animals were exposed to the same levels of Pb but exposure started on PND 0 and continued to PND 22 (early postnatal exposure group (EPN)). At PND 22 all rats were placed on regular rat chow (RMH 1000) until completion of the study. Male rats (Cont N=6, EPN N=8, & Peri N=8) and female rats were tested (Cont N=4, EPN N=4, and Peri N=4).

***Food Restriction Protocol*:** At PND 60, rats were housed individually, baseline weights were taken and they were placed on a food restriction protocol of 12g (i.e. 4 food pellets) per day to maintain 80% body weight. Food restriction was implemented for 2 weeks to ensure stable motivation to search for and eat food rewards that would be used during testing. Pb-exposed rats showed no differences in weight vs. Control rats.

***Blood and Brain Lead Level Analyses*:** Blood lead levels (BLL) were determined by ESA Lead Care II analyzer and blood lead test kit (Magellan Diagnostics). At the time of behavioral testing, BLLs were below detection threshold given the time difference between lead exposure termination (PND 22) and euthanasia (PND 70-90). Brain lead levels are being analyzed.

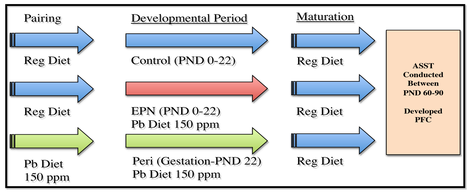
Digging Training (Day 1): Rats remained in their individual home cage and acclimated to the testing room for 1 hour prior to training (i.e. this step was completed for each of the test days that follow). Acrylic bowls containing a dusting of crushed FrootLoops (Kellogs) were placed into the home cage. Training began with half a FrooLoop place in the middle of the bowl and slightly covered with shredded paper. Rats dug to retrieve the FrootLoop reward. This process was repeated with increasing amounts of shredded paper to shape adequate digging behaviors. Training was completed when 6 consecutive trials of reliable digging were observed. FrootLoops were varied in each trial during training and testing to prevent satiation.

***Habituation (Day 1)*:** Rats were allowed to habituate to the testing environment for 60 minutes, given a 60 minute break and re-habituated again for an additional 60 minutes.

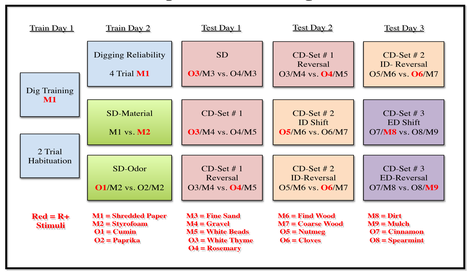
***Simple Discrimination (Day 2)*:** Rats explored both bowls with Fruit Loops covered in shredded paper to ensure reliable digging for 4 consecutive trials. Afterwards, rats were presented with a simple discrimination task (SD) with 6 consecutive trials (i.e. medium discrimination task) in which one bowl contained shredded paper only and the other contained styrofoam with a FrootLoop reward. Next the rats were presented with two bowls containing styrofoam with either cumin or paprika scents (i.e. odor discrimination) for 6 consecutive trials.

Complex Discrimination Day 3-5: Rats were trained to respond to either a specific odor or medium for 6 consecutive trials, then a reversal of either the odor or medium stimuli in a set. A new set of stimuli were presented and rats were trained to respond to a new odor or medium (i.e. intra-dimensional shift) for 6 consecutive trials, followed by another reversal. Lastly, a third set of stimuli were presented to the rats and they had to shift (e.g. odor-medium stimuli to medium-odor stimuli (i.e. extra-dimensional shift)).

Flowchart of Developmental Pb Exposure Paradigm

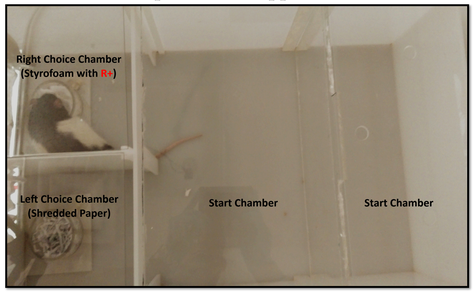


Experimental Design



Experimental Design: Rats were trained to dig and habituated to the test chamber on day 1. On day 2, rats were trained again for digging and once this was done reliably, they were taught to make a simple discrimination (i.e. SD) between digging materials and odors. Tests were split across 3 days due to EPN and Peri rats failing to maintain focus and inability to complete the task in a 1 day test session. Across the 3 test days rats learned complex discriminations (i.e. CD) and reversals for a given stimulus dimension (i.e. material or odor), followed by another complex discrimination and reversal of a new stimulus from the same dimension (i.e. intra-dimensional shift) and ten a final complex discrimination and reversal of a new stimulus from another dimension (i.e. extra-dimensional shift). At the start of test day 2 and 3 memory retention of the prior days complex discrimination reversal was assessed to ensure stable learning performance before proceeding to the next test condition. O# = odor stimulus; M# = material stimulus.

Experimental Apparatus



Data Analysis: Data were recorded using Anymaze video capture software (Stoelting, Co.) Latency to obtain reward, number of trials to reach criterion and number of errors made in reaching criterion were recorded.

Digging Shaping Procedure

Reward Presentation Order

Dig Training: Rats were trained to dig in bowls that incrementally increased with digging material to find varied FrootLoops as a reward.

Attention Set Shifting Task (ASST) Results

Developmental Effects of Pb Exposure on ASST Trials to Criterion in Males

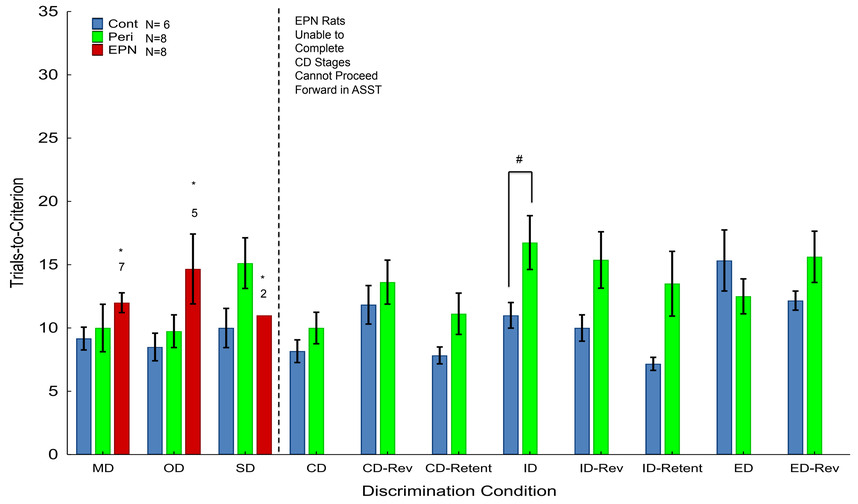


Figure 1. Time of Pb exposure during development produces different deficits in the ability for males to learn the ASST. Bar graph showing the number of trials to reach learning criteria (6 consecutive correct trials). Control male rats learned the task well and had the most difficulty at the ED shift stage. EPN rats had difficulty with learning SD with odor more than material and were unable to learn CD **(Note: * # indicates the number of EPN rats that completed the ASST stage)**. EPN male rats that could not learn CD could not proceed through ASST. Peri male rats were able to complete the ASST, required more trials to reach criterion at almost every stage, compared to Control male rats, and had the most difficulty performing the ID shift (ANOVA post hoc T-Test = -2.08. df (12), p<0.05#). **MD** = Material Discrimination ; **OD** = Odor Discrimination; **SD** = Simple Discrimination; **CD** = Complex Discrimination ; **CD-Rev** = Complex Discrimination Reversal; **CD-Retent** = Complex Discrimination Retention; **ID** = Intra-Dimensional Shift; **ID-Rev** = Intra-Dimensional Reversal; **ID-Retent** = Intra-Dimensional Retention; **ED** = Extra-Dimensional Shift; **ED-Rev** = Extra-Dimensional Reversal. Data are shown as ± SEM.

Developmental Effects of Pb Exposure on ASST Trials to Criterion in Females

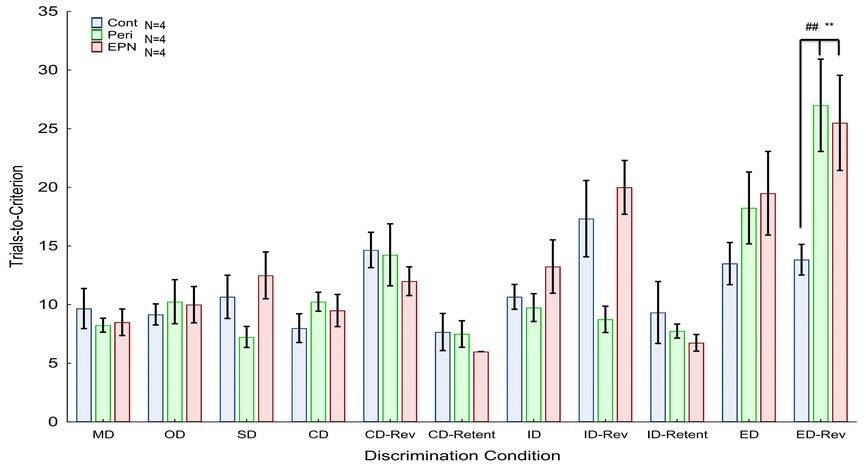


Figure 2: Time of Pb exposure during development results in different deficits in the ability for females to learn the ASST. Bar graph showing the number of trials to reach learning criteria (6 consecutive correct trials). Unlike EPN males, EPN females were able to complete the ASST test. All female rats irrespective of treatment were able to perform SD, CD, CD-Rev, and ID shifts without issue. Control and EPN female rats showed trends requiring more trials than Peri female rats to learn the ID-Rev. Control female rats had the least number of trials needed to learn at the ED and ED-Rev stages (ANOVA post hoc T-Test = -3.56, df (8), p<0.01##/**) (***ANOVA Discrimination Condition X Treatment Interaction Effect***, df (20), F (2.42), p<0.001***). **MD** = Material Discrimination ; **OD** = Odor Discrimination; **SD** = Simple Discrimination; **CD** = Complex Discrimination ; **CD-Rev** = Complex Discrimination Reversal; **CD-Retent** = Complex Discrimination Retention; **ID** = Intra-Dimensional Shift; **ID-Rev** = Intra-Dimensional Reversal; **ID-Retent** = Intra-Dimensional Retention; **ED** = Extra-Dimensional Shift; **ED-Rev** = Extra-Dimensional Reversal. Data are shown as ± SEM.

Summary

- Preliminary data show that timing of developmental Pb exposure produces different types of deficits in performance of an attention set shifting task. These observed cognitive differences are also dependent on the sex of the rat.
- Gender differences were noted at the ID stages between control males and females only.
- Notably, EPN males could not learn the ASST test based on their inability to learn SD and CD shifts which is a prerequisite skill for learning a CD. Moreover, they exhibit specific deficits in processing odor stimuli which may relate to the time of Pb exposure and postnatal development/maturation of olfactory pathways.
- In contrast, EPN females were able to learn the test and should increased difficulty in learning ED and ED-Rev shifts compared to controls.
- Peri male rats are able to learn SD and CD tasks and can perform ID and ED shifts. However, they had increased trials to learn at the CD-Retent, ID, ID-Rev, ID-Retent, and ED-Rev stages. In contrast, Peri female rats had less difficulty to learn ID tasks and more difficulty to learn ED and ED-Rev tasks.
- Pb exposure may result in different attention/executive-based cognitive deficits depending on the developmental window of exposure and sex whereby these effects may be evaluated through the ASST.

Conclusions

- Pb-exposed animals performed the task differently than controls. The exposure type (i.e. EPN vs. Peri) produced distinctive cognitive learning deficits.
- These cognitive deficits were further differentiated based upon the rat sex.
- Male rats with EPN Pb exposure were unable to learn an odor-based SD, whereas female EPN Pb exposed rats were able to complete all ASST tasks. EPN female rats had the most difficulty at the ED and ED-Rev stages compared to controls.
- Perinatally Pb exposed male rats learned the odor SD but had significant numbers of errors at the CD-Retent, ID, ID-Rev, and ID-Retent and ED Rev stages. Perinatally Pb exposed female rats were better at learning the ID-Rev shift than controls and EPN female rats and had the most difficulty in the ED and ED-Rev tasks.
- EPN males exhibit inabilities to learn and perform simple discriminations and thus cannot be tested fully to assess the formation of an attention set. EPN females can for attention sets and have deficits in extra-dimensional set shifting.
- Peri males exhibit deficits in intra-dimensional set shifting, whereas peri females exhibit deficits in extra-dimensional set shifting.
- These data suggest that low level Pb exposure may result in attention/executive dysfunction and in particular, may impair the ability to form, maintain, and shift response sets resulting in potential problems with cognitive flexibility.
- Lastly, different outcomes based on developmental period of Pb exposure and sex suggests the potential for different critical periods during which brain systems involved in different aspects of executive functioning may be affected.

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