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Olfactory recognition memory is disrupted in young mice with chronic low-level lead exposure

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HIGHLIGHTS

- Olfactory memory was examined in mice with and without early chronic lead exposure.
- Blood lead levels in exposed mice were 2.02–20.3 micrograms per deciliter.
- In males, as blood lead level increased olfactory memory decreased.
- In females, a non-linear effect was observed at lowest levels of exposure.

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ABSTRACT

Chronic developmental lead exposure yielding very low blood lead burden is an unresolved child public health problem. Few studies have attempted to model neurobehavioral changes in young animals following very low level exposure, and studies are needed to identify tests that are sensitive to the neurobehavioral changes that may occur. Mechanisms of action are not yet known however results have suggested that hippocampus/dentate gyrus may be uniquely vulnerable to early chronic low-level lead exposure. This study examined the sensitivity of a novel odor recognition task to differences in pre-adolescent C57BL/6J mice chronically exposed from birth to PND 28, to 0 ppm (control), 30 ppm (low-dose), or 330 ppm (higher-dose) lead acetate (N = 33). Blood lead levels (BLLs) determined by ICP-MS ranged from 0.02 to 20.31 μg/dL. Generalized linear mixed model analyses with litter as a random effect showed a significant interaction of BLL × sex. As BLLs increased olfactory recognition memory decreased in males. Among females, non-linear effects were observed at lower but not higher levels of lead exposure. The novel odor detection task is sensitive to effects associated with early chronic low-level lead exposure in young C57BL/6J mice.

1. Introduction

The dangers of developmental lead exposure are well-documented and there is widespread recognition that even low-level exposure alters neurobehavior in young children. Child studies have suggested many neurobehavioral functions altered by early chronic low-level lead exposure. These include but are not limited to memory and learning, visual attention, abstract problem-solving, cognitive set-shifting, and motor dexterity (Bellinger and Needleman, 2003; Canfield et al., 2003; Franko et al., 2000; Gilbert and Weiss, 2006; Jusko et al., 2008; Landrigan et al., 2006; Lanphear et al., 1998, 2005; Needleman et al., 1990, 1996; Schnaas et al., 2000; Sobin et al., 2015; Wasserman et al., 2000). The mechanisms by which low-level lead disrupts neuro-development however are not yet known and few animal models of early chronic low-level lead exposure have been proposed. In order to advance knowledge in this area, neurobehavioral tests that are sensitive to the effects of early chronic low-level lead exposure in animals are needed.

Of the neurocognitive disruptions identified in low-level lead-exposed children, changes in memory may have the most profound implications for life-long brain health. The brain regions critical for memory and learning, in particular the hippocampus/dentate

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gyrus regions, overlap neurogenesis pathways. Early disruption of these regions and pathways has the potential to alter neural pathway formation (Schafer et al., 2012), memory function, learning during development (Schinder and Gage, 2004) and neurogenesis during adulthood and aging (Jessberger et al., 2009), perhaps increasing vulnerability to cognitive decline and dementia.

Only a few past studies have examined memory in rodents with early chronic low-level lead exposure. For example, in adulthood, BALB/c mice chronically exposed to 20 ppm lead acetate delivered in dam's drinking water had diminished memory (object recognition memory task) as compared to controls (Azzaoui et al., 2009). In a similar study, as compared to controls, adult Wistar rats chronically exposed to 20 ppm of lead in dam's drinking water had diminished spatial memory (water maze) (Kasten-Jolly et al., 2012) (blood lead levels were not reported). A recent study in our laboratory of pre-adolescent C57BL/6J mice showed that as blood lead level (BLL) increased, exploration of a novel environment decreased (Flores-Montoya and Sobin, 2014).

In the current study, the sensitivity of a novel odor detection paradigm to the effects of early chronic low-level lead exposure was examined in pre-adolescent C57BL/6J mice. It was hypothesized that from lowest to highest levels of lead exposure, as BLL increases, olfactory recognition memory decreases.

2. Method

2.1. Animals

All animal procedures had prior approval of the Institutional Animal Care and Use Committee (IACUC) and were carried out in accordance with the US Public Health Service Policy on Humane Care and Use of Laboratory Animals (National Research Council, 2011). C57BL/6J mice were purchased from Jacksons Laboratories and housed in the Bioscience Research Facility at the University of Texas at El Paso (UTEP). Mice were group housed by sex in ventilated cages (22.22 cm × 36.83 cm × 13.97 cm) with *ad libitum* access to food and water. The animal holding room had a temperature of 20°–26° C, relative humidity of 30–70 percent, and a 12 h light-dark cycle.

Dams were mated beginning at post-natal day (PND) 40 using harem breeding. Two females were placed with one male, checked daily, and housed separately after vaginal plug was identified. Ten dams were mated with five sires. Nine of ten dams were successfully impregnated. Gestation durations were between 19 and 21 days. Prior studies suggested that early chronic low-level lead exposure may alter stress-responsive neuroimmune processes (Sobin et al., 2013) thus, to avoid stressing dams and pups, unculled litters were planned with sex and litter (as a random effect) controlled in all analyses. Seven dams produced litters ranging in size from 3 to 6 pups, N = 33, including 13 females and 20 males. Two remaining litters of one pup each were not included. Each litter was assigned to one of three lead treatments, either 0 ppm, control (n = 10, 2 females; 8 males), 30 ppm, low-dose (n = 10, 5 females and 5 males), and 330 ppm, higher-dose (n = 13, 6 females and 7 males). No animals died during the course of the study.

2.2. Lead exposure

Pups were exposed to lead via dams' milk. From PND 0 to PND 28 dams were given either lead-treated water (30 ppm or 330 ppm 99.4% lead acetate crystals, Sigma–Aldrich, St. Louis, MO) or sodium-treated water (30 ppm).

2.3. Behavioral testing

Recognition memory was tested at PND 28 with a novel odor recognition (NODR) task. The protocol was based on those used in previously published protocols (Bevins and Besheer, 2006; Simple Odor Recognition Protocol, 2011). This task was adapted from a novel object recognition memory task (NOR task) (Bevins and Besheer, 2006). The original task included a training phase and a testing phase. During the training phase, mice were placed in a square arena and allowed to explore two identical objects located in the upper corners of the arena. The testing phase then follows an inter-trial interval (ITI). A familiar object was replaced with a novel object. Mice were returned to the arena and allowed to freely explore the familiar and novel objects. Mice with intact memory spend more time exploring the novel as compared to the familiar object. For the current study, odors rather than objects were used to maximize possible group differences. The odors selected were those published in previous mouse behavioral protocols (Simple Odor Recognition Protocol, 2011).

All testing occurred between 10:00 a.m. and 1:00 p.m. Three identical square Plexiglas arenas (8 in × 8 in × 24 in) equipped with a timer were used for habituation (10 min), training (10 min), and testing (5 min) phases, with 5 min inter-trial intervals (ITI) between each phase. During the ITI, mice were returned to a holding cage with home bedding.

For the habituation phase, animals were placed in the empty arena and allowed to freely explore. For the training phase, animals were placed in the second arena with two identically scented vehicles. Orange or almond food-grade edible natural liquid flavors (McCormic®) were sprayed on 1" mouse-shaped felt objects positioned in the upper left and right arena corners approximately 4 cm from each wall. For the testing phase, the familiar scented object was replaced with a novel (orange or almond) scented object. Fixed visual cues in the testing room external to the testing arena were asymmetrical and to accommodate this, the location of the novel odor was fixed to the upper right corner; "familiar" and "novel" orange or almond odors were counterbalanced. All arenas were cleaned with 10% isopropyl alcohol after each trial. Each mouse was returned to the home cage when testing was completed.

Video cameras placed over the top of the arenas recorded all mouse activity during testing. Video recordings were later scored by four raters trained to reliability and blind to experimental condition. Exploration was recorded when the mouse nose was oriented towards and within a 2 cm proximity to the odor vehicle. Inter-rater reliability was determined after rater training and during and after test scoring. All post-training and scoring reliabilities exceeded 0.90.

2.4. Blood collection

Immediately after behavioral testing, mice were anesthetized with Avertin (5–10 mL). Animals were sexed and weighed after they were unresponsive to corneal touch and paw pinch tests. Heart blood was extracted (50 μL of blood per animal) via syringe puncture at the heart apex. Blood samples were refrigerated and processed for inductively coupled plasma mass spectrometry (ICP-MS) analysis within 72 h of sample collection.

2.5. Inductively coupled plasma mass spectrometry (ICP-MS) analysis of blood lead

The detailed method for the measurement of BLL was previously described (Sobin et al., 2011). Briefly, an Agilent 7500ce ICP-MS with an octopole reaction system and a CETAC ASX520 autosampler was used. A Micro Mist U-series nebulizer