2013-01-01

Behavioral Markers of Chronic Low-Level Lead Exposure in Young Mice

Mayra Gisel Flores Montoya
University of Texas at El Paso, mgflores3@miners.utep.edu

BEHAVIORAL MARKERS OF CHRONIC LOW-LEVEL LEAD EXPOSURE IN YOUNG MICE

MAYRA GISEL FLORES MONTOYA

DEPARTMENT OF PSYCHOLOGY

APPROVED:

_____
Edward Castaneda Ph.D., Chair

_____
Rosa Maldonado Ph.D

_____
Laura O'Dell Ph.D.

_____
Christina Sobin Ph.D.

_____
Benjamin C. Flores Ph.D.
Dean of the Graduate School

BEHAVIORAL MARKERS OF CHRONIC LOW-LEVEL LEAD EXPOSURE IN YOUNG MICE

By

MAYRA GISEL FLORES MONTOYA, B.A.

THESIS

Presented to the Faculty of the Graduate School of

The University of Texas at El Paso

in Partial Fulfillment

of the Requirements

for the Degree of

MASTER OF ARTS

Department of Psychology

THE UNIVERSITY OF TEXAS AT EL PASO

May 2013

ACKNOWLEDGMENTS

I would like to acknowledge my deepest appreciation for my mentor and Committee Chair Dr. Christina Sobin who has been extremely supportive during my undergraduate and graduate career. Also, I would like to acknowledge the assistance of Benjamin Valencia in the completion of the behavioral testing of animals. This research was made possible by grants from the National Institute of Child Health and Human Development (NICHD), National Institutes of Health, (R21HD060120, CS, PI); the National Center for Research Resources, a component of the National Institutes of Health (5G12RR008124); the Center for Clinical and Translational Science, The Rockefeller University, New York, New York; the Paso del Norte Health Foundation, El Paso, Texas; the University Research Institute, University of Texas, El Paso; and from the J. Edward and Helen M. C. Stern Professorship in Neuroscience, University of Texas, El Paso (CS). The funders had no role in the design, implementation, data analysis, or manuscript preparation for this thesis.

DEDICATION

I dedicate this thesis to my mother Rosa Maria Montoya Chavez, to my father Victor Manuel Flores Hernandez and to my sister Diana Margot Flores Montoya. They are the people that taught me strong values such as responsibility, commitment, hard work, and caring about my community. All of their support, love, and the values that they taught me made possible the completion of this thesis.

ABSTRACT

Behavioral markers of chronic low-level lead exposure in young mice were examined in the present study. Three groups of C57BL/6J mice (N = 35) were exposed from birth to PND 28 to one of three lead acetate levels: 0 ppm (control), 30 ppm (low-dose), or 330 ppm (high-dose). On PND 28, and immediately prior to sacrifice, mice were tested on the novel odor recognition task [(NODR) (memory task)], on the open field task [(OF) (exploratory behavior, habituation, and anxiety behavior task)], and on the nose poke task [(NP) (exploratory behavior task)]. Blood lead levels were analyzed with inductively coupled plasma mass spectrometry (ICP-MS). ANOVA and ANCOVA models were used to examine possible behavioral differences between groups. When significant differences between groups were found, general linear models were run to test for linear or quadratic associations between blood lead level and behavior. For the NODR task, the lead-exposed groups had diminished short-term olfactory recognition memory as compared to the control group. A simple regression analysis revealed a significant small negative association between blood lead level and memory. No significant quadratic association was found. For the OF task, the low-dose group had increased exploratory activity as compared to the other groups. No significant linear or quadratic associations between blood lead level and exploratory activity were found. No significant differences between groups were found for the NP task. Olfactory memory and exploratory activity are behavioral markers for the effects on brain of chronic exposure to low-level lead.

TABLE OF CONTENTS

LIST OF TALBES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

Lead is a heavy metal that is particularly toxic for children. Lead is prevalent in urban areas and underserved children are at greater risk of exposure (Bernard & McGeehin, 2003). More than 9,600 industries in United States release from 10 to 10,000 pounds of lead into the environment every year (EPA, 2006). For children living in close proximity to these facilities, air-borne lead might present the greatest danger. For other children additional sources of exposure include lead-based paint in old housing, imported toys, drinking water contaminated by lead pipes, pottery, inexpensive cookware, cosmetics, and children's jewelry (CDC, 1991).

Clinical studies have provided convincing evidence that children with blood lead levels below 10 µg/dL have diminished neurocognitive function and motor dexterity. More specifically, the deficits associated with chronic low-level lead exposure include but are not limited to working memory, attention, problem solving, and motor dexterity (Bellinger & Needleman, 2003; Canfield et al., 2003; Franko, Palome, Brown, Kennedy & Moore, 2000; Gilbert & Weiss, 2006; Jusko et al., 2007; Landrigan et al., 2006; Lanphear, Burgoon, Rust, Eberly & Galke, 1998; Lanphear et al., 2005; Needleman & Gatsonis, 1990; Needleman, Schell, Bellinger, Leviton & Allred, 1990; Needleman, Riess, Tobin, Biesecker & Greenhouse, 1996; Schnaas et al., 2000; Wasserman et al., 2000). The brain mechanisms underlying the described neurocognitive deficits are not understood. Currently the only approach to intervention is source removal, and since lead is ubiquitous in the environment, this intervention is not a practical solution.

Animal models are needed to understand the brain changes that result from chronic low-level lead exposure. In order to develop an animal model of chronic low-level lead exposure behavioral tests that are sensitive to the brain changes that occur following chronic exposure to low-level lead must be identified. Very few studies thus far have examined the effects on behavior of early chronic low-level lead exposure. Below is a summary of these studies presented in chronological order.

Studies Examining Behavioral Markers of Chronic Low-Level Lead Exposure in Animals

Kasten-Jolly, Pabello, Bolivar, and Lawrence study (2012). The behavioral markers of chronic low-level lead exposure suggested in this study were memory impairments and decreased exploratory activity in mice. BALB/c female and male adult mice were mated from post-natal days (PNDs) 60-90. From gestational day eight until PND 21 of the pups half of the dams drank distilled water (control group) and half of the dams drank water contaminated with 20 parts per million (ppm) of lead (experimental group). Dams gave birth to 66 mice, that were exposed or not to lead via drinking dams' milk, lead exposed (n = 36); controls (n = 30). Mice were tested for long-term visuo-spatial memory, exploratory activity, locomotor activity (age of testing was not reported), and for aggressive behavior (tested at 10 months old).

Mice were tested for long-term visuo-spatial memory in the Morris Water Maze. This maze consisted of a pool that was 1.5 m in diameter. The water of the pool was made opaque with white paint. The opaque water hid a platform that was located 1.5 cm below the surface of the water. For this test, mice were trained to locate the hidden platform and stay on it for a period of time. After mice were successfully trained they were removed from the pool, and the platform was removed as well. After a delay, mice were placed back in the pool. If mice had an unaffected

memory, they were expected to spend more time swimming around the area in which the platform was previously located.

Mice were trained for eight trials per day (for four consecutive days) to locate the platform (which had distal visuo-spatial cues). Mice were allowed to locate the platform in a period of 60 sec. Once they identified it, they were left to stay on it for 30 sec. If mice did not reach the platform within the allotted 60 sec the experimenters placed them manually on the platform and allowed them to stay on it for 30 sec. After each trial, mice had a 45 min rest period. In the interval of trial four and five mice were allowed to rest for 60 min. On testing day four, one hour after the last trial was conducted, the platform was removed and mice were tested for 60 sec. An Accutrak (automated tracking system) was used to record behavior.

Mice were tested for exploratory behavior in a dim light chamber that had multiple compartments. These compartments (65 cm x 55 cm x 55 cm) were equipped with 16-beam automated activity monitors. Beam breaks were recorded by a PC computer and Digiscan software. First, mice were acclimated to the testing room for 1 hr. After acclimation, they were placed in a mouse cage for 5 min and then placed back in the arena. Then they were left to explore the arena freely for 15 min. The Digiscan software recorded total distance traveled, resting time, rotations, habituation, speed, and percentage of time spent in the center of the apparatus.

For examining locomotor activity mice were tested on the rotarod task (RR). The RR consisted of a machine that had at its center a rubber barrel that rotated automatically at the speed set by the experimenter. This machine recorded the time (in minutes and seconds) that the mice spent on it. Mice were tested for three consecutive days on sessions that lasted 20 min.

First, they were acclimated to the rubber barrel for 1 hr and then they were tested. During the first 60 sec the speed of the rubber barrel increased from 0 rotations per minute (rpm) to 15 rpm. The speed was maintained to 15 rpm for the following 110 sec. On the last 10 sec, the speed of the rubber barrel was decreased from 15 rpm to 0 rpm. A software (Smartrod windows version 1.7) recorded the time that passed since the mouse was placed on the rubber barrel until it fell from it.

Aggressive behavior was tested using the resident-intruder test. Individually housed 10 month old mice were exposed to an intruder (a mouse that was not their litter-mate) in their home cages. Behavior was video-taped for 10 min. The behaviors examined were biting, chasing or wrestling. The number of aggressive behaviors and the time that passed before the first aggressive encounter occurred were also recorded. Behavior within the cage when the mice were interacting with their litter-mates was also examined.

Behavioral tests were analyzed using two-way ANOVAS, a student t-test and a mixed model ANOVA. For the Morris Water Maze no significant differences between the groups were found. However, it was found that the majority of mice (regardless of their exposure group) froze at the beginning of the task. For this reason, post hoc analyses were conducted and mice that swam less than 10 sec on the first probe trial were excluded from the study. These analyses revealed that lead-exposed mice spent less time than control mice swimming around the area where the platform was previously located. It was also found that the lead-exposed mice moved more between zones than controls. This may mean that the lead-exposed mice had diminished visuo-spatial learning memory as compared to control mice.

For the OF task, it was found that the lead-exposed groups had reduced travel distances, reduced time spent moving and reduced average speed as compared to the control group. It was also found that males had increased activity as compared to females regardless of their exposure group.

With regard to anxiety, it was found that female lead-exposed mice travelled less distance and spent less time in the center of the apparatus as compared to controls. This may suggest that female lead-exposed mice were more anxious than control mice.

For the RR task, no significant differences between groups were found. For the resident-intruder test, it was found that lead-exposed mice were less aggressive towards the intruder, but were more aggressive towards their litter-mates as compared to control mice. To conclude, BALB/c mice chronically exposed to low-levels of lead had memory impairments, decreased exploratory behavior, increased aggression towards their litter-mates, increased anxiety (only females), and no locomotor deficits.

Azzaoui, Ahami, and Khadmaoui study (2009). The behavioral markers of chronic low-level lead exposure suggested in this study were long-term object recognition memory impairments and increased exploratory activity in adult rats. Fourteen Wistar rats (3 months old) were tested. The experimental group (n = 7) drank distilled water contaminated with 20 ppm of lead nitrate, whereas the control group (n = 7) drank distilled water for 90 days. After exposure, rats were tested on the OF task (exploratory activity task) and on the novel object recognition task (NOR task) (see description of the task on page 6 below). Rats were then sacrificed and their brains were extracted for lead examination.

The OF task was used to examine exploratory behavior. For this task, rats were tested in an open top wooden square arena (100 cm x 100 cm x 40 cm) covered with white plastic. The floor of the arena was marked creating 25 squares. Every 14 days from the beginning to the end of the intoxication period, rats were placed on the center of the arena and behavior was videotaped for 7 min. Ratings were conducted by a rater blind to experimental condition who counted the number of times that the rats crossed the squares.

The NOR task was used to examine rodent's long-term object recognition memory. This task consisted of a training phase and a testing phase. For the training phase, the rodent was placed in a square arena and was exposed to two identical objects that were placed in the two upper corners of the arena. Then the rodent was allowed to explore the two objects for a period of time. After exploration, the rodent was removed from the arena, and was placed in a mouse cage. After a delay, the mouse was tested. For the testing phase, the mouse was placed back in the square arena but this time the square arena had on one of its upper corners one of the objects presented on the training phase of the task (a familiar object), and on its other upper corner a novel object. If object recognition memory was unaffected, the rodent was expected to spend more time exploring the novel object than the familiar object.

Every 14 days alternatively with the OF task, rats were tested on the NOR task. They were tested in a square arena (40 cm x 50 cm x 50 cm) that had walls covered with a black plastic layer. Each rat was first habituated to the arena for 5 min, and then it was removed from it. After a 24 hr delay, two identical objects were placed on the two upper corners of the arena. Each rat was allowed to freely explore the objects for 5 min. After this time passed, rats were removed from the arena. Twenty four hours later, one of the objects was replaced by a novel

object and each rat was placed back in the testing arena for 5 min. The time that the rats spent exploring each object was recorded.

The statistical analyses used for the OF task were one-way ANOVA and repeated measures one-way ANOVA. For the statistical analyses of the NOR task a discrimination ratio was calculated by dividing the time that each rat spent exploring the novel object by the total time spent exploring both objects [Tnovel/(Tnovel+Tfamiliar)]. One-way ANOVA and repeated measures one-way ANOVA were used to test for possible differences between treatment groups with regard to the discrimination ratio.

Significant differences between groups were found. The lead-exposed group had significantly greater amounts of exploration and had diminished long-term object recognition memory as compared to the control group. Specifically, for the OF task, from week 1-10 of exposure the lead-exposed group and the control group did not display significant differences with regard to exploratory activity nor with regard to locomotor activity. However, on week 11 (last week of testing) the lead-exposed group displayed significantly higher exploratory activity as compared to the control group.

For the NOR task, from week 2-6 no significant differences between groups were found with regard to the discrimination ratio. However, it was found that from week 8-10 of exposure, the lead-exposed groups had a significant diminished discrimination ratio as compared to the control group, that is, they spent exploring the novel object for less time as compared to the control group. To conclude, the results suggested that behavioral markers of chronic low-level lead exposure included increased exploration of an environment, and long-term object recognition memory impairments during late adulthood.

Leasure et al. study (2008). The behavioral markers of chronic low-level lead exposure suggested in this study were decreased exploratory activity, increased exploration after receiving an injection of amphetamine, and locomotor deficits (only in male mice). Five-week old female C57BL/6 mice were individually housed in a cage with male mice. Dams gave birth to six to nine mice per sex per group (exact sample size was not reported). Two models of lead exposure were tested, including a gestational lead exposure model (GLE model) and a postnatal lead exposure model (PLE model). For the GLE model, dams were exposed to one of four lead acetate levels: 0 ppm (control), 27 ppm (low-dose), 55 ppm (moderate-dose), or 109 ppm (high-dose). They were exposed to lead two weeks before they were mated, and until PND 10 of the pups. For the PLE model, the experimental dams received 27 ppm (low-dose) or 55 ppm (moderate-dose) of lead acetate from birth of the pups until PND 21. Control dams of the PLE model received tap water.

After exposure, and when the pups matured to one year, mice from the GLE model but not the PLE model were tested behaviorally. Mice from the GLE model were tested on the OF task, on the RR task, and on running wheel activity. The OF task consisted of an enclosed Optovarimax behavioral monitor (40 cm x 40 cm x 40 cm) equipped with 16 infrared photo receptor beams that were used to record locomotor activity. Activity was quantified by measuring the number of times that mice crossed the laser beams each 5 min. Mice were acclimated to the OF arena for 15 min and then 30 min of exploratory activity were recorded. After two weeks, mice were acclimated again to the OF for 15 min, then they were removed from it and received a 3 mg/kg d-amphetamine sulfate injection. After this, they were immediately placed back in the OF, and their behavior was recorded for three hours.

Injections that range from 1 mg/kg to 5.0 mg/kg of d-amphetamine are typically given to animals to test their motor responsiveness to this drug challenge, for example with regard to locomotor activity, rearing and sniffing (Fray et al., 1980). Thus, in this study amphetamine was administered to lead-exposed and unexposed animals in order to examine whether groups differed in their responses to the amphetamine challenge. Differences between groups would indicate that the sensitivity to d-amphetamine was altered as a result of chronic exposure to lead, perhaps suggesting that one or various d-amphetamine pathways were altered as well.

Mice were also tested on the RR task (see pages 3 and 4). They were placed on top of a stationary rubber barrel and were trained to stay on it. Once they learned how to stay on it, the rubber barrel was activated at 5 rpm for 90 sec. After this, mice were tested for three trials with a one-hour interval between trials. On each trial the speed of the rubber barrel was set at 5 rpm and each 30 sec the speed was incremented by 0.1 rpm. The latency to fall from the rubber barrel was recorded and averaged for the three trials to obtain a total score for each mouse.

Mice were also tested on running wheel activity. For this test, mice were individually placed in a running wheel (Whatman-type) that rotated as fast as the mouse moved. A computer program recorded the number of meters travelled. Mice were placed for five consecutive days in individual cages that contained water, food and a running wheel. Four weeks after they were exposed to the running wheels, mice from the GLE model were sacrificed and blood was extracted for lead analysis. Mice from the PLE model were also sacrificed and blood was also extracted for lead analysis.

Possible differences between groups with regard to blood lead levels were examined by two-way ANOVA (Lead Exposure x Sex). Behavioral data were analyzed by one-way ANOVA

and by one-way repeated measures ANOVA. For mice from the GLE model, it was found that blood lead levels were significantly higher for the three experimental groups as compared to the control group on PNDs 0, 10, and 21. However, these differences disappeared on PND 30. It was also found that one year old lead-exposed male mice, but not female mice, weighed significantly more than controls. For mice from the PLE model, it was found that blood lead levels for the two experimental groups were significantly higher as compared to the control group on PNDs 7, 14, 21, and 45. However, on PND 60 these differences disappeared. No significant differences in weight were found among mice from the PLE model.

For the OF task, significant differences between groups in the first 30 min of novel exploration were found. Specifically, the low-dose and the high-dose lead-exposed male mice, but not female mice, explored significantly less the OF than control mice. Interestingly, the low-dose lead-exposed male mice explored significantly less the OF than the high-dose lead-exposed male mice. After amphetamine was administered, it was found that for the first 30 min of exploration lead-exposed male mice displayed significantly increased exploratory activity as compared to control mice. With regard to running wheel activity no significant differences between groups were found. For the RR task it was found that the lead-exposed male mice had significantly shorter latencies to fall from the rubber barrel as compared to control mice. Remarkably, the GLE male mice in the low-dose group had significantly poorer performance than the GLE male mice in the high-dose group. To conclude, it was suggested that chronic low-level lead exposure caused decreased exploration in a novel environment, increased exploration after receiving an injection of amphetamine, and locomotor impairments in male mice.

Ferguson and Bowman study (1990). The behavioral markers of chronic low-level lead exposure suggested in this study were a delay to habituate to a novel environment, and increased

exploratory activity after familiarization with the environment. Eight rhesus monkeys were exposed either to lead (experimental group) or sodium (control group). The experimental group (n = 4) and the control group (n = 4) were exposed to lead or sodium via nasogastric intubation. Specifically, from PND 9 to PND 29, the experimental group was given 0.7-mg of lead/kg of body weight dissolved in a milk formula. From PND 42 to PND 365 the experimental group was given 3.0-mg of lead/kg of body weight as described previously. For the control group the quantity and time of exposure to sodium was equivalent to that of the lead-exposed groups.

The testing apparatus consisted of an OF that was equipped to be a playroom (2.4 m x 2.0 m x 2.2 m). It had on its inside wire mesh ledges, ramps, and a bar that traversed the room. Chalk lines were marked on the floor of the OF creating 27 sectors of equal size. From 3-12 months of age, monkeys were habituated to the testing arena. After this, they were allowed to mature to four years old and then they were tested.

Monkeys had six test sessions once a week. On each test session they were transported in their home cage to the entrance of the OF. A stopwatch was used to record the time that took for the monkeys to get into the OF. Monkeys were given a maximum of 600 sec to enter the OF, if they exceeded this time they were pushed into it. Once they entered the OF they were left in it for 15 min and behavior was recorded. The behaviors analyzed were latency to enter the OF, number of urinations and defecations (measures of anxiety), number of sectors entered, and frequency and duration of 17 behaviors per 5 min (described on page 12 below). After 15 min of exploration, the monkeys were placed back in their home cage. An Apple IIE microcomputer was used to record their behavior. After testing, blood was collected via femoral venipuncture and atomic absorption spectrometry was used to determine blood lead levels.

Data were analyzed using repeated measures ANOVA. At age four (age of testing) the blood lead level of all monkeys was < 5 µg/dl. Only five out of the 17 behaviors recorded happened frequently enough to be analyzed by repeated measures ANOVA. These behaviors were time of inactivity, environmental exploration (manipulation of the environment with their hands, mouth and/or feet), self-directed behavior (play activity), locomotion, and number of vocalizations.

Significant differences between the experimental group and the control group were found with regard to latency to enter the OF, time of inactivity, and environmental exploration. Specifically, it took significantly more time for the experimental group than for the control group to enter the OF. It was also found that the experimental group had significantly shorter periods of inactivity and displayed more manipulation of the environment with their hands and feet as compared to the control group. These results suggested that exposure to chronic low-level lead can result in delayed adaptation to a novel environment and increased exploratory activity.

Reiter, Anderson, Laskey, and Cahill study (1975). The behavioral markers of chronic low-level lead exposure suggested in this study were decreased exploratory activity before and after receiving an injection of amphetamine, a significant delay in the development of the righting reflex (explanation below), and a significant delay on the time at which rats opened their eyes.

Two generations of Sprague-Dawley rats were pre-treated for 40 days with 0 ppm (control), 5 ppm (low-dose), or 50 ppm (high-dose) of lead acetate dissolved in drinking water. After pre-treatment, rats were mated, and the first generation of pups (F1) was exposed to lead via drinking dams' milk. When the pups were weaned they continued the same lead treatment for

180 days. The F1 generation of rats was mated on PND 90 and their offspring (F2 generation) received the same exposure to lead. One hundred and fifty pups of both the F1 and F2 generations were tested for the startle response, the righting reflex, and the PND at which their eyes were completely opened was recorded. Additionally, five pups from 9-11 litters of the F1 and F2 generations (exact sample size was not reported) were tested for exploratory activity.

For testing the startle response, a clicking sound was made (using a toy clicker) behind the head of the rats. If the rat was healthy, it was expected to produce a muscle jerk in one of its limbs (this reflex is present usually at PND 12). For testing the righting reflex, the rats were held from the back of the neck and tail and were turn upside down, and dropped on a table. If the rat was healthy it was expected to turn right in mid-air and land with all four paws on the table.

For testing locomotor activity, from PND 120 to PND 125 rats were placed in a residential maze with access to food and water *ad libitum*. Locomotor activity was detected by eight phototransistor infrared light emitting diode pairs. Activity counts were recorded every hour on a Xerox minicomputer. After five days of testing, rats were removed from the maze and they received a 4 mg/kg d-amphetamine injection, 20 min after this, they were placed back in the maze.

T-tests were used to examine possible differences between groups. No differences between the F1 and F2 generations were found. For this reason, the data collected from both generations of rats were analyzed together. It was found that the lead-exposed animals had a significant delay in the time at which they opened their eyes, and a significant delay in the development of the righting reflex as compared to control animals. It was also found that during the first four days of testing, the low-dose and the high-dose lead-exposed groups had decreased

exploratory activity as compared to the control group. No differences between the experimental groups and the control group with regard to the development of the startle response were found. On day five, and after the amphetamine injection, both lead-exposed groups had decreased exploratory activity as compared to the control group. These results suggested that rats exposed to chronic low-level lead can have delays in development, decreased exploratory activity, and decreased sensitivity to amphetamine.

Suggested Behavioral Markers of Chronic Low-Level Lead Exposure

The studies described suggested several behavioral markers of chronic low-level lead exposure. Some of the findings were consistent between studies, and some were not. The consistencies identified were in memory. Two of the studies identified that chronic low-level lead exposure affected memory (Azzaoui et al., 2009; Kasten-Jolly et al., 2012).

Findings were inconsistent with regard to exploratory activity and locomotor activity. In two of the studies increased exploratory activity was suggested as a behavioral marker of chronic low-level lead exposure (Azzaoui et al., 2009; Ferguson & Bowman, 1990) whereas two additional studies suggested decreased exploratory activity (Kasten-Jolly et al., 2012; Leasure et al., 2008). Additionally, delayed adaptation to a novel arena was suggested as a behavioral marker of chronic low-level lead exposure (Ferguson & Bowman, 1990).

Mixed results were also found with regard to exploratory activity after receiving an injection of amphetamine. One study identified increased exploratory activity in lead-exposed animals (Leasure et al., 2008), whereas another study identified decreased exploratory activity (Reiter et al., 1975). With regard to locomotor activity one study suggested locomotor

impairments in lead-exposed animals (Leasure et al., 2008) however another study did not suggest locomotor impairments (Kasten-Jolly et al., 2012).

It may be that some of the behavioral findings were inconsistent because some moderator variables were influencing behavior. These moderator variables may be the species tested (mice, rats, and monkeys), duration of exposure to lead, and the PND at which animals were tested. Despite inconsistent findings these studies provided a useful starting point because they suggested that chronic low-level lead exposure produced changes in specific behaviors that have been associated with discrete brain regions and pathways. It is important to note, however, that none of these studies examined behavioral markers of chronic low-level lead exposure in young animals.

To consider the age at which animals are tested is very important in order to develop an animal model of chronic low-level lead exposure that is relevant to human child lead exposure. Equally important is to consider the choice of the animals to be tested. For our studies, we use C57BL/6J mice for two reasons. First, the C57BL/6J mouse genome has been completely mapped. In previous child studies, our laboratory has shown that the PEPT2*2 haplotype is associated with increased blood lead burden in young children (Sobin, Gutierrez, & Alterio, 2009; Sobin, Parisi, Schaub, Gutierrez, & Ortega, 2011). A mouse model will be needed to explore this important genetic vulnerability factor by, for example, creating a peptide transporter 2 transgenic mouse. The second reason is that, very similar to humans, the levels in blood of a key lead-binding protein, delta-aminolevulinic acid dehydratase, are stable during development in mice, but not in rats (Davis & Avram, 1978; Onalaja & Claudio, 2000). For this reason, rat models of early lead exposure are of questionable relevance to the problem of lead exposure in children.

The purpose of the present study was to examine in a mouse model the sensitivity of three mouse behavioral tests to chronic low-level lead exposure. The tests were selected because each requires no training period which is necessary in order to test young mice; furthermore, each behavioral test challenges brain pathways that are implicated in child studies of chronic low-level lead exposure.

It was hypothesized that there would be significant differences between exposure groups with regard to memory and exploratory activity. That is, for the NODR task, it was predicted that the experimental groups would have diminished memory. Specifically, as the level of lead in the blood increased, memory would decrease as quantified by the discrimination ratio of the NODR task. For the OF task and the NP task, differences between groups with regard to exploratory activity were predicted. Because findings reported in the literature with regard to exploratory activity were inconsistent, predictions of the directions of the effects of lead exposure in exploratory activity were not made. Thigmotaxis (the amount of time that mice spent walking along the walls of the arena) was also measured to examine whether anxiety might have confounded the results.

CHAPTER 2

METHODS

Pilot Studies

Animals. The pilot studies were conducted in accordance with the US Public Health Service Policy on Humane Care and Use of Laboratory Animals and had the approval of the Institutional Animal Care and Use Committee (IACUC) at the University of Texas at El Paso (UTEP). For pilot testing, mice (N = 19) of the strain C57BL/6J were used from our established colony housed at the Bioscience research Building at UTEP. Animals had *ad libitum* access to food and water. Mice were handled approximately twice per week prior to testing during cage cleaning and food replenishment.

Behavioral tasks.

Novel object recognition task (NOR task). (See page 6).

Object selection. The objects selected for pilot testing of the NOR task were guided by personal communications with researchers experienced with the NOR task including Cheryl A. Frye, at The University at Albany, and Bruce McEwen, at The Rockefeller University. Objects were selected for their size, shape, color and textural interest. Mice were exposed to seven objects of different colors, sizes, and shapes. These objects were food toys (a grape and an onion), an animal toy (a rhino), toy cars (a van and a truck), a small cup and a black ball.

Six female and male mice (3 months old) were used for pilot testing. Mice were removed from their original cages and were housed individually for a period of approximately one week prior to testing. At the start of pilot testing, each mouse was habituated for 5 min to a square

arena surrounded by opaque Plexiglas walls (8 in x 8 in x 8 in). After habituation, mice were returned to their individual cages for 3 min, and then they were placed back in the square arena. This time the square arena had on its upper center a single object (one of the objects that were described previously). Two experimenters used a timer to record the time (in seconds) that each mouse spent exploring each object during a period of 3 min. Exploration was quantified when the mouse stepped on the object, or when it had its nose and eyes directed to the object with a proximity of at least 2 cm.

It was found that on average the objects explored for the greatest amount of time were the rhino toy, and the truck toy (see Table 1). It was noticed that mice were exploring the truck toy for a longer period of time as compared to the other objects because they were able to move it. Since movement could be a confounding variable, it was decided to select instead the rhino toy (the first most-explored object) and the grape cluster toy (the third most-explored object) for pilot testing of the NOR task.

NOR task pilot testing in arena with bedding. Five male mice (3 months old) were tested on the NOR task. They were placed in the same square arena that was described previously but this time the square arena contained floor bedding from the mice's home cages. This was done in order to reduce the stress that a novel environment may produce in mice. First, mice were habituated to the arena for 5 min. Then they were removed from it and placed back in their mouse cage. After 3 min they were trained. For the training phase, mice were placed back in the square arena and exposed to two identical objects (two grape toys or two rhino toys). These objects were placed on the upper left and upper right corners of the arena. Mice were allowed to explore them for 10 min. After this time elapsed, mice were removed from the arena and placed back into their individual cages. Five minutes later, they were tested.

For the testing phase, mice were exposed to a familiar object (one of the two identical objects that were presented on the training phase of the task) placed on the upper left corner of the arena, and to a novel object (either the grape toy or the rhino toy) placed on the upper right corner of the arena. Mice were allowed to explore the objects freely for 3 min. Two experimenters used a timer to record the time that mice spent exploring each object. One experimenter focused only on recording the time exploring the familiar object, and the other experimenter focused only on recording the time exploring the novel object. A discrimination ratio score was calculated to examine if mice spent more time exploring the novel object as compared to the familiar object. This ratio was calculated by dividing the time exploring the novel object by the sum of the time exploring the novel object and the familiar object [Tnovel/(Tnovel+Tfamiliar)] (Bevins & Besheer, 2006).

A discrimination ratio higher that 0.50 would mean that the mice spent exploring more time the novel object as compared to the familiar object, and a discrimination ratio lower than 0.50 would mean that the mice spent more time exploring the familiar object as compared to the novel object. A one sample t-test was used to examine if the discrimination ratio differed significantly from the hypothetical 0.50. It was found that the discrimination ratio was 0.478. This meant that mice explored the novel object 47.8% of the time, and the familiar object 52.2% of the time (see Table 2). The discrimination ratio did not differ significantly from the hypothetical 0.50 (M = 0.478), t = -2.35, p = 0.83.

For the purpose of examining recognition memory we expected to obtain a discrimination ratio that was significantly higher than 0.50. However this goal was not accomplished. It was noticed that during testing the mice frequently dug into the home bedding and it was suspected

that this distracted the mice from exploring the target objects. For this reason, we decided then to conduct an additional set of pilot tests without bedding in the square arena.

NOR task pilot testing in arena without bedding. Six female and male mice (3 months old) were tested on this task. The testing and rating procedures were identical to that of the NOR task that were described previously (see pages 18 and 19) the only difference being that the square arena did not have bedding on its floor. The discrimination ratio of the novel object was calculated and a one sample t-test was run to examine if the discrimination ratio differed significantly from the hypothetical 0.50. In this case, it was found that the discrimination ratio was 0.617. This meant that mice explored the novel object 61.7 % of the time, and the familiar object 38.3% of the time (see Table 3). This discrimination ratio did not differ significantly from the hypothetical 0.50 (M = 0.617), t = 2.45, p = 0.06.

Since the discrimination ratio did not differ significantly from the hypothetical 0.50, and in order to improve the effect size for this test, we attempted a modification. This modification was to transform the task from an object recognition memory task to an olfactory recognition memory task.

Novel odor recognition task (NODR task). Eight female and male mice (3 months old) were tested on this task. This task follows a similar protocol to that of the NOR task (Bevins & Besheer, 2006) and to that of the simple odor recognition task (Johns Hopkins University, 2011).

Odor selection. The odors selected were liquid pure orange extract and liquid pure almond extract of the brand McCormik (edible food-quality products). These odors were selected because orange and almond are known to be attractive odors for mice and because the former is citrus and the latter is sweet they may be easily differentiated from one another (Johns Hopkins

University, 2011; Mandairon, Poncelet, Bensafi, & Didier, 2009). The extracts were poured into 2 oz. spray bottles.

NODR task pilot testing. Mice were first habituated to the square arena (see pages 17 and 18) for 5 min, and after a 3 min delay they were exposed to odors. For the training phase, mice were placed in the arena and were exposed to two identical odors (either orange or almond) placed on the upper left and upper right corners of the arena. Two furry toy mice of the brand Zanies were sprayed with either orange (sprayed eight times) or with almond (sprayed five times). The amount of times that the furry toy mice were sprayed differed because the intensity of the odors differed. It was determined that these numbers of spray pumps per odor yielded equivalent odor intensity.

The scented furry toy mice were attached to the arena with Velcro. Mice were allowed to freely explore both furry toy mice for 10 min. For the training phase, four mice were exposed only to orange, and four mice were exposed only to almond. After exploration, mice were removed from the arena, placed in an individual cage for 3 min, and then they were tested.

For the testing phase, mice were placed back in the square arena. This time the arena contained a familiar odor (the same odor that was presented on the training phase of the task) and a novel odor (either orange or almond). Two experimenters used a timer to record the time that mice spent exploring each odor. One experimenter focused only on recording the time that mice spent exploring the familiar odor, and the other experimenter focused only on recording the time that mice spent exploring the novel odor. A discrimination ratio was calculated as described for the NOR task (see page 19).

A one sample t-test was used to examine if the discrimination ratio differed significantly from the hypothetical 0.50. It was found that the discrimination ratio was 0.682. This meant that mice explored the novel odor 68.2% of the time, and the familiar odor 31.8% of the time (see Table 4). The discrimination ratio differed significantly from the hypothetical 0.50, (M = 0.682), t = 2.45, p = 0.04. Because the NODR task showed a significant effect on recognition memory and produced a slightly larger effect size than the NOR task, it was decided to use the NODR task as a test for examining memory as a behavioral marker of chronic low-level lead exposure in mice.

In addition to the NODR task, two behavioral tasks were used. These were the open field task (OF) and the nose poke task (NP). Both of them are relatively simple tasks, both had been tested previously in our laboratory in studies of chronic low-level lead exposure, and had been used by other researchers to assess behavioral changes following lead exposure.

Main Study

Animals. Breeders of the strain C57BL/6J were purchased from Jackson Laboratories and housed at the Bioscience research building at UTEP under standard laboratory conditions with access to food and water *ad libitum*. Adult mice were mated on PND 30, and 79 mice were obtained. Thirty-five mice were randomly selected for behavioral testing. Immediately after testing, mice were anesthetized and sacrificed. Heart blood was extracted for determination of quantity of lead in blood using ICP-MS. (The remaining mice were anesthetized and sacrificed, and their brains were extracted for immunohistochemical studies).

Lead exposure. At birth, dams and litters were randomly assigned to one of three lead acetate exposure levels. The control group (n = 11) was exposed to 0 ppm of lead acetate, the

low-dose group (n = 11) was exposed to 30 ppm of lead acetate, and the high-dose group (n = 13) was exposed to 330 ppm of lead acetate given in dams' drinking water. Natural unculled litters were used. Each exposure group included 2-3 litters. Exposure continued until PND 28, time at which mice were tested and sacrificed.

Behavioral tasks.

Novel odor recognition task. This task was conducted following the same protocol as that described for the pilot test of the NODR task (see pages 20-22). Minor changes were made to ensure timely completion of the experiment.

Experimental setting. Three square arenas surrounded with opaque walls (8 in x 8 in x 24 in) were used to test the mice in three sequential phases. A camera was placed on top of the arenas and behavior was recorded. Each square arena had on its floor a beige mat made of odorless synthetic material that provided a slip-proof walking surface for the mice.

The three square arenas and three mouse cages were placed on a table. The first square arena was used for habituation. A mouse cage was placed next to the square arena and served as a waiting station between the habituation phase and the training phase of the task. The second square arena was used for the training phase of the task. A mouse cage was placed next to the square arena and served as a waiting station between the training phase and the testing phase of the task. The third square arena was used for the testing phase of the task. A mouse cage was placed next to the square arena, and mice were released in this cage after completion of the experiment.

The three square arenas and two of the mouse cages (waiting station cages) had a timer placed in front of them. This was done in order to monitor the time that mice spent in each of the

square arenas and in each of the mouse cages. The timer for the first square arena was set at 5 min, the timer for the second square arena was set at 10 min, and the timer for the third square arena was set at 5 min. The two timers that were placed in front of the two mouse cages were set at 5 min.

Experimental procedure. A mouse was introduced into the first square arena for habituation, and immediately the timer of this arena was set at 5 min. When the alarm turned on, the mouse was removed from the arena and placed in the mouse cage adjacent to the arena. The timer of this arena was set at 5 min. At minute 4:30 the experimenters prepared the square arena so that the training phase of the task was initiated. Two furry toy mice of the brand Zanies were sprayed with either orange (sprayed eight times) or almond (sprayed five times) and were attached to the upper left and upper right corners of the arena using Velcro. In order to counterbalance the odors, for the training phase of the task half of the mice in each treatment group were only exposed to orange, and half of the mice in each treatment group were only exposed to almond.

By the time the timer of the mouse cage (waiting cage) was turned on, the training arena was ready. The mouse was picked up from the mouse cage and placed immediately in the training arena. The timer of this arena was set at 10 min. When the timer of the training arena turned on, the mouse was picked up and placed in the adjacent mouse cage (waiting cage). The timer of this mouse cage was set at 5 min. At minute 4:30 the experimenters prepared the testing arena so that the testing phase of the task was initiated.

For the testing phase new furry toy mice were sprayed with two different odors. One furry toy mouse was sprayed only with the familiar odor, that is, with the odor that the mouse

was exposed to on the training phase of the task (either orange or almond) and was placed on the upper left corner of the arena. Another furry toy mouse was sprayed with a novel odor, that is, with an odor that the mouse was not exposed to on the training phase of the task (either orange or almond) and was placed on the upper right corner of the arena. When the timer of the waiting cage turned on, the mouse was immediately removed from the waiting cage and placed in the testing arena. The timer of this arena was then set at 5 min. Once the timer of the testing arena turned on, the mouse was picked up and placed on the third mouse cage where it was individually contained and removed from the experimental area. Video cameras were placed above the training and testing arenas and all behaviors were recorded. Arenas were cleaned with 10% isopropyl alcohol immediately after removing the mice and all timers were turned off and reset to avoid disturbing the mice.

Open field task. This task was used to examine rodent's exploratory activity. The apparatus consisted of a square arena (16 in x 16 in) surrounded by clear Plexiglas walls. Four quadrants were marked on the floor of the arena with white masking tape. Mice were placed on the lower right corner of the arena and were allowed to explore the area freely for 5 min. After this time elapsed, mice were removed from the arena and the floor of the arena was cleaned with 10% isopropyl alcohol. Behavior was recorded using a video camera that was mounted at the level of the arena. At a later time, raters trained to reliability and raters blind to experimental condition watched the video recordings. All procedures for raters training and rating of behavioral videos are described on pages 26 and 27 below.

After training was complete, raters counted three behaviors during three different rating sessions. In the first rating session raters measured exploratory activity by counting the number of times that the mouse crossed into a different quadrant with all four paws. In the second rating

session, raters measured exploratory activity by counting the number of leg stands (when the mouse stood on its hind legs). In the third rating session raters measured anxiety by quantifying thigmotaxis (see page 16).

Nose poke task. Exploratory activity was also examined with this task. The apparatus consisted of a square arena (16 in x 16 in) surrounded by clear Plexiglas walls. This arena had an elevated platform with 16 equally spaced holes. Each mouse was placed on the bottom right corner of the platform and was allowed to freely explore it for 3 min. After this time elapsed, each mouse was removed from the arena and the floor of the arena was cleaned with 10% isopropyl alcohol.

A video camera was mounted at the level of the arena and exploratory behavior was recorded. Raters blind to experimental condition watched the video recordings and examined two behaviors during two different rating sessions. In the first rating session raters counted the number of times that the mouse poked the nose into the holes deeply enough so that the level of the eyes reached the level of the platform (deep dips). In the second rating session raters counted the number of times that the mouse poked the nose into the holes to any depth (any type of dips).

Raters' training for behavior ratings of video recordings. For the NODR task four raters were trained to reliability. Two of the raters were blind to experimental condition, and two of the raters were the experimenters. The four raters were trained by watching five video recordings of the NODR task on a (60 in x 45 in) projection screen. A line demarcating 2 cm from the objects was super-imposed on the screen. Using a timer, two raters (a rater blind to experimental condition and one experimenter) recorded only the time that the mouse spent exploring the upper left furry toy mouse. The other two raters (a rater blind to experimental

condition and one experimenter) recorded only the time that the mouse spent exploring the upper right furry toy mouse. The same approach was used for both phases of the task. “Exploration” was defined as the mouse coming to within 2 cm of the furry toy mouse; if the mouse stepped on the furry toy mouse this also counted as exploration. After training, raters conducted the behavioral ratings of the NODR task.

For the OF task and the NP task two raters blind to experimental condition were trained to reliability. The raters watched five video recordings of each task on the (60 in x 45 in) projection screen and counted the behaviors that were described previously (see pages 25 and 26). After training, raters conducted the behavioral ratings of the OF and NP tasks.

Reliability of ratings.

Novel odor recognition task. A simple regression analysis revealed that the reliability of the ratings for the time that mice spent exploring the novel odor was strong and significant (r = .98), $p < .001$. The same was true for the reliability of the ratings for the time that mice spent exploring the familiar odor (r = .94), $p < .001$.

Open field task. A simple regression analysis revealed that the reliability of the ratings for the total number of quadrants crossed was strong and significant (r = .99), $p < .001$. The same was true for the reliability of the ratings for the total number of leg stands (r = .99), $p <.001$, and for thigmotaxis (r = .94), $p < .001$.

Nose poke task. A simple regression analysis revealed that the reliability of the ratings for the number of deep dips was strong and significant (r = .92), $p < .001$. The same was true for the reliability of the ratings for the number of any type of dips (r = .96), $p < .001$.

Blood extraction. Immediately after the behavioral testing was completed, mice were anesthetized with Avertin (from 5 ml to 10 ml depending on body weight). To examine if mice were completely anesthetized, corneal reflex and paw pinch were used. Once mice were completely unresponsive heart blood was extracted and full-body transcardial perfusion was performed.

Analyses of blood lead levels. The quantity of lead in blood was determined in micrograms per deciliter (μg/dL) using ICP-MS.

Statistical analyses. Data was analyzed using SAS Version 9.3 Statistical Software. One-way ANOVA was run to examine differences between groups with regard to blood lead levels. To determine whether weight and sex should be included as control factors in the statistical analyses, one-way ANOVA was run to test for differences between groups with regard to weight, and t-tests were run to test differences between males and females with regard to the behavioral measures of each task. Sex was included in the statistical models only for behavioral tasks in which significant differences between males and females were found. Body weight was co-varied only for those tests in which it could influence behavioral performance (e.g. movement).

ANOVA and ANCOVA models were used to test for significant differences between treatment groups with regard to each of the behaviors measured on the three behavioral tasks (NODR task, OF task, and NP task). When significant differences between groups were found, secondary analyses using ANOVA or ANCOVA models were run to examine possible differences between groups by each minute of the test. When significant differences between groups were found, general linear regression analyses were used to test for a linear trend, and if

the results suggested any curvature a quadratic trend was run to test for it. The significance of the effects was determined by Type III sum of squares, and was assumed at $p < .05$.

Novel odor recognition task. A discrimination ratio was calculated to examine the time that mice spent exploring the novel odor as compared to the familiar odor (see page 19). One sample t-tests were used to test if the discrimination ratio of each group differed significantly from a hypothetical 0.50 (Bevins & Besheer, 2006). One-way ANOVA was used to examine possible differences between groups with regard to the discrimination ratio.

Open field task.

Quadrants crossed. Two-way ANCOVA (Lead Exposure x Sex) controlling for weight was used to examine possible differences between groups with regard to the total number of quadrants crossed.

Leg stands. One-way ANCOVA controlling for weight was used to examine possible differences between groups with regard to the total number of leg stands.

Thigmotaxis. One-way ANCOVA controlling for weight was used to test for possible differences between groups with regard to total thigmotaxis.

Nose poke task.

Deep Dips. One-way ANCOVA controlling for weight was used to test for differences between groups with regard to the total number of deep dips.

Any Dips. One-way ANCOVA controlling for weight was used to test for differences between groups with regard to the total number of any dips.

CHAPTER 3

RESULTS

Sample Characteristics

Thirty-five C57BL/6J mice were included in this study. The control group included 11 mice (3 females; 8 males). The low-dose group included 11 mice (5 females; 6 males). The high-dose group included 13 mice (6 females; 7 males).

Blood Lead Levels

One-way ANOVA revealed significant differences between groups with regard to blood lead levels, $F = 187.21$, $p < .001$. Post hoc analyses with Tukey's pairwise comparison tests revealed that the mean blood lead level of the low-dose group ($M = 3.00$, $SEM = 0.57$) was significantly higher than that of the control group ($M = 0.03$, $SEM = 0.57$), $p < .001$, and significantly lower than that of the high-dose group ($M = 14.15$, $SEM = 0.53$), $p < .001$. Differences in blood lead levels between the control group and the high-dose group were statistically significant, $p < .001$ (see figure 1).

Body Weight

One-way ANOVA revealed significant differences between groups with regard to body weight, $F = 6.35$, $p < .01$. Post hoc analyses with Tukey's pairwise comparison tests revealed that mice in the low-dose group weighted significantly less ($M = 12.51$, $SEM = 0.44$) than mice in the control group ($M = 14.74$, $SEM = 0.44$), $p < .01$. Also, mice in the high-dose group weighted significantly less ($M = 13.44$, $SEM = 0.41$) than mice in the control group, $p < .05$. No

differences in weight between the low-dose group and the high-dose group were found (see figure 2).

Testing for Differences between Males and Females in Behavioral Performance

Novel odor recognition task.

Time exploring both odors (novel plus familiar) during the testing phase of the task. T-test did not suggest significant differences between males and females on the total time that they spent exploring both the novel odor and the familiar odor during the testing phase of the task (see table 5).

Discrimination ratio. T-test did not suggest significant differences between males and females on the discrimination ratio (see table 5).

Open field task.

Quadrants crossed. T-test revealed significant differences between males and females on the total number of quadrants crossed in the open field task, $t(33) = -2.19$, $p < .05$. Specifically, females ($M = 55.93$, $SEM = 5.94$) crossed significantly more quadrants than males ($M = 43.24$, $SEM = 2.59$), $p < .05$ (see table 5).

Leg stands. T-test did not suggest significant differences between males and females on the total number of leg stands (see table 5).

Thigmotaxis. T-test did not suggest significant differences between males and females on total thigmotaxis (see table 5).

Nose poke task.

Deep dips. T-test did not suggest significant differences between males and females on the total number of deep dips (see table 5).

Any dips. T-test did not suggest significant differences between males and females on the total number of any dips (see table 5).

Testing for Lead Exposure Group Differences in Behavioral Performance

Novel odor recognition task.

Time exploring both odors (novel plus familiar) during the testing phase of the task. One-way ANOVA did not suggest significant differences between groups with regard to the total amount of time that mice spent exploring both the familiar odor and the novel odor during the testing phase of the task (see tables 6 and 7).

Discrimination Ratio. T-tests did not suggest that the discrimination ratio of the low-dose group differed significantly from the hypothetical 0.50 (M = 0.46, SEM = 0.08), t= -0.55, p = 0.59. Also, the discrimination ratio of the high-dose group did not differ significantly from the hypothetical 0.50 (M = 0.48, SEM = 0.07), t = -0.26, p = 0.80. On the other hand, the discrimination ratio of the control group differed significantly from the hypothetical 0.50 (M = 0.72, SEM = 0.08), t = 3.18, p < .01.

One-way ANOVA revealed significant differences between groups with regard to the discrimination ratio, $F(2, 32) = 3.46$, p <.05. Post hoc analyses with Tukey's pairwise comparison tests revealed that the low-dose group and the high-dose group had significantly smaller discrimination ratios than the control group (see ratios above), p < .05. No significant

differences between the low-dose group and the high-dose group were found (see tables 6 and 7; see figure 3).

Because significant differences between the lead-exposed groups and the control group with regard to the discrimination ratio were found, simple regression analyses were run to test for a linear trend and for a quadratic trend. For the linear trend a very small significant negative association was found, as blood lead level increased, the discrimination ratio decreased, β= -0.014, r^2 = 0.12, $F(1, 33) = 4.32$, $p < .05$ (see table 16; see figure 4). For the quadratic trend, no significant association between blood lead level and the discrimination ratio was found (see table 17).

Open field task.

Quadrants crossed. T-tests revealed that females crossed significantly more quadrants than males. Additionally, one-way ANOVA revealed significant differences in weight between exposure groups. Since the variable measured in this task was total amount of area traversed by the animal ("distance"), and because significantly lower weight could cause animals to have less motoric stamina, a two-way ANCOVA was run to test for main effects of lead exposure group and of sex on the total number of quadrants crossed while controlling for weight. This analysis revealed a significant main effect of lead exposure on the total number of quadrants crossed, $F(2, 28) = 4.02$, $p < .05$, and a significant main effect of sex on the total number of quadrants crossed, $F(1, 28) = 4.10$, $p = 0.05$. However, no significant interaction between lead exposure and sex on the total number of quadrants crossed was found, and weight did not influence significantly the number of quadrants crossed.

Post hoc analyses with Tukey's pairwise comparison tests revealed that the low-dose group crossed significantly more quadrants (M = 62.29, SEM = 5.32) than the control group (M = 42.06, SEM = 5.65), $p < .05$, and that the low-dose group crossed significantly more quadrants than the high-dose group (M = 44.93, SEM = 4.38), $p < .05$. No significant differences between the control group and the high-dose group were found (see tables 8 and 9; see figure 5). Thus, the effect of increased activity was only found among the low-dose mice.

To further test these results, and examine when differences might have occurred during this behavioral test, secondary analyses with two-way ANCOVA were run to test for differences on the number of quadrants crossed during each minute of exploration.

Significant group differences were identified for min 2 and 3 of exploration. Specifically, for min 2, a significant main effect of lead exposure on the total numbers of quadrants crossed was found, $F(2, 28) = 5.20$, $p < .05$. However, no significant main effect of sex on the number of quadrants crossed was found, nor was a significant interaction found, and weight did not influence significantly the number of quadrants crossed. For min 3, a significant main effect of lead exposure on the number of quadrants crossed was found, $F(2, 28) = 4.31$, $p <. 05$. Also, a significant main effect of sex on the number of quadrants crossed was found, $F(1, 28) = 5.41$, $p < .05$. However, no significant interaction between lead exposure and sex was found, and weight did not influence significantly the number of quadrants crossed. No significant main effects of lead exposure and sex, or interactions were found for min 1, 4 and 5.

For min 2, post hoc analyses with Tukey's pairwise comparison tests revealed that the low-dose group (M = 16.04, SEM = 1.81) crossed significantly more quadrants than the control group (M = 8.76, SEM = 1.92), $p < .05$, and crossed significantly more quadrants than the high-

dose group (M = 9.05, SEM = 1.49), $p < .01$. No significant difference between the control group and the high-dose group was found. For minute 3 post hoc analyses with Tukey's pairwise comparison tests revealed that the low-dose group (M = 12.74, SEM = 1.44) crossed significantly more quadrants than the control group (M = 6.89, SEM = 1.52), $p < .05$, and that the low-dose group crossed significantly more quadrants than the high-dose group (M = 8.05, SEM = 1.18), $p < .05$. No significant difference between the control group and the high-dose group was found (see tables 8 and 9; see figures 6 and 7).

Because significant differences between groups with no significant interactions were found for the total number of quadrants crossed, and for min 1, 2, and 3, simple regression analyses were run to test for a linear trend and for a quadratic trend for these outcome variables. No significant linear or quadratic associations between blood lead level and the number of quadrants crossed were found (see tables 16 and 17).

Leg stands. One-way ANCOVA revealed a significant main effect of lead exposure on the total number of leg stands, $F(2, 31) = 7.41$, $p < .01$, and revealed that weight did not influence significantly the total number of leg stands. Post hoc analyses with Tukey's pairwise comparison tests revealed that the low-dose group (M = 44.74, SEM = 2.83) displayed a significantly higher number of leg stands than the control group (M = 30.10, SEM = 2.89), $p < .01$, and displayed a significantly higher number of leg stands than the high-dose group (M = 32.03, SEM = 2.41), $p < .01$. No significant difference between the control group and the high-dose group was found (see tables 10 and 11; see figure 8).

Secondary one-way ANCOVA analyses were run to test for group differences by minute of exploration. Significant differences between groups were found for min 1, 2 and 3, $F(2, 31) =$

4.29, $p < .05$, $F(2, 32) = 7.56$, $p < .01$, $F(2, 31) = 8.32$, $p < .001$, respectively. Weight did not influence significantly the number of leg stands. No significant differences between groups for min 4 and 5 were found.

For min 1, post hoc analyses with Tukey's pairwise comparison tests revealed that the low-dose group ($M = 10.73$, $SEM = 0.79$) displayed a significantly higher number of leg stands than the control group ($M = 7.36$, $SEM = 0.79$), $p < .01$, and displayed a significantly higher number of leg stands than the high-dose group ($M = 8.15$, $SEM = 0.73$), $p < .05$. No significant difference between the control group and the high-dose group was found. For min 2, the low-dose group displayed a significantly higher number of leg stands ($M = 9.68$, $SEM = 0.77$) than the control group ($M = 5.84$, $SEM = 0.79$), $p < .01$, and displayed a significantly higher number of leg stands than the high-dose group ($M = 6.06$, $SEM = 0.65$), $p < .01$. No significant difference between the control group and the high-dose group was found.

For min 3, the low-dose group displayed a significantly higher number of leg stands ($M = 10.05$, $SEM = 0.83$) than the control group ($M = 6.49$, $SEM = 0.85$), $p < .001$, and displayed a significantly higher number of leg stands than the high-dose group ($M = 5.69$, $SEM = 0.71$), $p < .001$. No significant difference between the control group and the high-dose group was found. No significant differences between groups for min 4 and 5 were found (see tables 10 and 11; see figures 9–11).

Because significant differences between groups with no significant interactions were found for the total number of leg stands, and for min 1, 2, and 3, simple regression analyses were run to test for a linear trend and for a quadratic trend for these outcome variables. No significant

linear or quadratic associations between blood lead level and the number of leg stands were found (see tables 16 and 17).

Thigmotaxis. One-way ANCOVA did not suggest significant differences between groups with regard to the total time spent close to the walls (thigmotaxis), (see tables 12 and 13).

Nose poke task.

Deep dips. One-way ANCOVA did not suggest significant differences between groups with regard to the total number of deep dips (see tables 14 and 15).

Any dips. One-way ANCOVA did not suggest significant differences between groups with regard to the total number of any dips (see tables 14 and 15).

CHAPTER 4

DISCUSSION

The present study was designed to examine behavioral markers of chronic low-level lead exposure in young C57BL/6J mice. Mice were tested on a memory task (NODR task), and on two exploratory activity tasks (OF and NP tasks). It was hypothesized that the lead exposed groups would have diminished short-term olfactory recognition memory as compared to the control group in the NODR task, and that the lead exposed groups would display differences in exploratory activity in the OF and NP tasks.

For the NODR task, as hypothesized, the lead exposed groups had significantly diminished short-term olfactory recognition memory as compared to the control group. For the OF task, also as hypothesized, there were significant differences between the groups. Specifically, the low-dose group had increased levels of horizontal activity (quadrants crossed) and increased levels of vertical activity (leg stands), as compared to the control group, and as compared to the high-dose group. For the NP task significant differences between groups were not observed with regard to exploratory activity.

Behavioral Markers of Chronic Low-Level Lead Exposure

Novel odor recognition task. It was found that the lead-exposed groups had a significantly lower discrimination ratio as compared to the control group. Specifically, during the testing phase of the task, the low-dose group and the high-dose group spent significantly less time than the control group exploring the novel odor as compared to the familiar odor. Importantly, mice of the three treatment groups did not differ in the time that they spent exploring both objects (novel plus familiar) during the testing phase of the task, suggesting that

there were no significant differences between groups with regard to overall exploratory activity displayed during this task. In other words, lead-exposed mice were not simply moving less overall than control mice.

The discrimination ratios observed for the three groups were 0.46 (low-dose), 0.48 (high-dose), and 0.72 (controls). Thus, the low- and high-dose groups explored the novel odor and the familiar odor for approximately equal amounts of time whereas the control group spent significantly more time exploring the novel odor as compared to the familiar odor. The discrimination ratios of the low- and high-dose groups did not differ (results from post hoc tests were not statistically significant).

The linear negative association between blood lead levels and the discrimination ratio was significant but very small, $\beta = -0.014$, $r^2 = 0.12$, $p < .05$, bringing into question whether there was a dose-response relationship between lead exposure as measured by blood lead level, and the discrimination ratio. Because the variance explained by the model was very small it can be concluded that lead exposure may diminish the discrimination ratio regardless of blood lead level (ranging from 2.15 µg/dL to 20.31µg/dL). A model of short-term olfactory memory will be described next in order to contextualize the present findings.

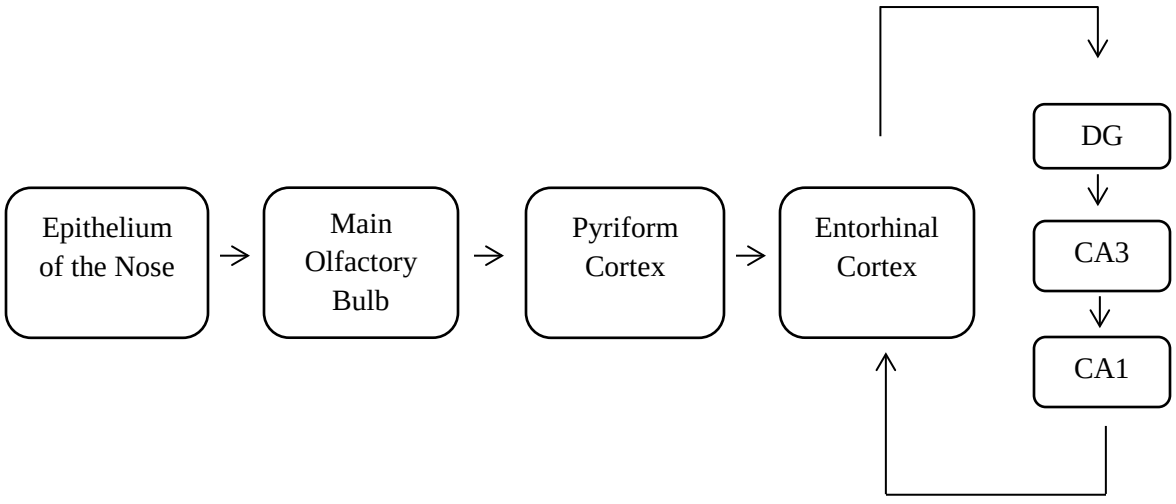
Short-term olfactory recognition model. Memory has been described as a process that is composed of three stages: acquisition, consolidation and retrieval. Acquisition refers to the stage at which the animal first encounters the stimuli, consolidation refers the stage at which the information is saved, and retrieval refers to the stage at which the information can be accessed in memory (Abel & Lattal, 2001). Thus, in the context of the NODR task, when mice were in the training phase (exposed to two identical odors) they were undergoing the acquisition stage. Then

when they passed to the waiting phase (in the waiting cages) they were undergoing the consolidation stage. Finally, when they started the testing phase (exposed to the novel and familiar odors) they were undergoing the retrieval stage. These phases will be described based on the neural substrates that may be controlling them and the potential effects that chronic low-level lead exposure may have on them.

In a review paper written by Brennan and Keverne (1997) a pathway of how olfactory information reaches the hippocampus was described. Olfactory stimuli first reaches the epithelium of the nose of the animal where multiple odor receptors receive the stimuli; once they receive it, the odor receptors send the information via the main olfactory bulb to the pyriform cortex. After this, the information travels to the entorhinal cortex, and then to the hippocampus.

It has been suggested that once the information reaches the hippocampus, it travels first to the dentate gyrus (DG), then to area CA3, and then to area CA1 of Ammon's horn. After this, the information travels back to the enthorinal cortex forming a loop. It has been proposed that this loop, known as the "reverberating circuit," mediates short-term memory (Hebb, 1949) (see model below).

Short-term Olfactory Recognition Memory Model



Possible mechanisms mediating discrimination ratio group differences in lead-exposed and control mice. Based on this model of short-term olfactory recognition memory, it may be that chronic low-level lead exposure affected the transmission of olfactory information somewhere along the pathway from the epithelium of the nose to the hippocampus.

It has been found that G-coupled olfactory receptors that are located in the olfactory sensory neurons of the epithelium of the nose of animals are responsible for odor detection. In fact, mammals have more than 1,000 odor receptors (Alberts, Johnson, & Lewis, 2002). One possibility is that chronic low-level lead exposure disrupted the reception of the information received by one or various olfactory receptors that are located within the epithelium of the nose of the mice. This in turn may have impaired the transmission of odor information from the epithelium to the hippocampus.

Recently, it has been suggested that the expression of the vomeronasal receptors that send information to the accessory olfactory bulb (that receives information from animal pheromones) were impaired as a result of chronic low-level lead exposure (Kasten-Jolly et al., 2012). However, whether chronic low-level lead exposure also alters the expression of odor receptors that send information to the main olfactory bulb is a question that remains to be investigated.

Another possibility is that lead exposure (regardless of quantity) affected the reverberating circuit that controls short-term memory within the hippocampus, and this may explain why lead-exposed groups had diminished short-term olfactory recognition memory. It may be that the transmission of information within the reverberating circuit was affected by an alteration of the neuroimmune system.

Recently, studies from our laboratory have shown that low-level lead exposure resulted in loss of microglial cells and disruption of the neuroimmune system in DG (Sobin et al., in press).

In these same studies, DG volume was shown to be significantly reduced in low- and high-dose animals. Thus, the same processes that resulted in loss of microglia and diminished DG volume may have also disrupted normal transmission of information within the reverberating circuit of the hippocampus, thus, affecting the consolidation and/or retrieval of olfactory information.

Findings in past studies vs. present study. The behavioral results that are reported in the literature are consistent with the hypothesis that chronic low-level lead exposure may diminish memory. Specifically, a recent study reported that chronic low-level lead-exposed BABL/c mice had diminished long-term visuo-spatial memory (Kasten-Jolly et al., 2012). Another study reported that chronic low-level lead-exposed Wistar rats had diminished long-term object recognition memory (Azzaoui et al., 2009).

Open field task. The meaning of mouse behavior in the OF task has been interpreted in many ways. For example, researchers have used it as a measure of exploratory activity, as a measure of memory related to habituation, as a measure of motor activity, and also as a measure of "emotionality," and specifically, anxiety (Gould, Dao, & Kovacsics, 2009; Walsh & Cummis, 1976). For the purposes of this discussion, the results guided how differences on this task were interpreted, as described below.

Open field exploration may involve memory. It is known that animals naturally move more in a novel environment than in a familiar environment. It has been hypothesized that animals display this increased activity in a novel environment because they need to gain knowledge about it (Light et al., 2011). As the amount of time that animals spend in the novel environment increases, animals gain knowledge about it, and gradually decrease their exploration because they start to habituate to it (Elliot & Grunberg, 2005). Simply stated, the

novel environment becomes more familiar through time and familiarity is seen through reduced exploration (movement in the novel space).

It has been suggested that when animals are exposed to a novel arena for less than 10 min, the test should be used to examine exploratory activity in reaction to novelty rather than "baseline" motor activity (Blizard et al., 2007; Gould et al., 2009). Also, it has been suggested that animals gain knowledge about a novel environment by forming associations between relevant environmental cues (Eichenbaum et al., 1999). Thus, learning and memory may be important cognitive processes involved in the exploration of a novel environment and habituation to it in paradigms less than 10 min.

In our data, significant differences between groups were not found at the beginning of the task (during min 1) perhaps because the strength of the natural instinct to explore a novel environment was equally strong among animals regardless of lead exposure. Differences however were observed during min 2 and 3. Specifically, the low-level lead-exposed group displayed a larger amount of activity (exploration) as compared to the other groups. It may be that the habituation to the novel environment was less efficient among the low-level lead-exposed animals and thus these animals required more time to gain knowledge about the arena. Significant differences between groups were not found during min 4 and 5 of exploration. This may mean that by the last two minutes of the task, the lead-exposed group had become equally habituated to the arena as the other groups.

Linear or quadratic associations between blood lead level and exploration (number of quadrants crossed) were not found, suggesting that there was not a dose-response association between the variables; rather the low-level lead-exposed animals were the only ones to show an

increase in activity during min 2 and 3 of the OF task. It is important to note that the lack of differences on this task in the high-dose group may not necessarily mean that higher levels of lead exposure do not cause damage for the developing brain with regard to exploratory activity. It may mean instead, that the effects on exploration of a novel environment in higher-dose lead-exposed animals might be better captured with a different behavioral task.

Also in the OF task, the low-dose group displayed more exploratory activity as measured by the number of leg stands. Specifically, during min 1, 2, and 3 of exploration the number of legs stands displayed by the low-dose group were higher than those displayed by the control group and higher than those displayed by the high-dose group. No significant difference between the control group and the high-dose group was found. This provides additional evidence that it took longer for the low-dose group to gain knowledge about the novel environment as compared to the control group and as compared to the high-dose group.

Linear or quadratic associations were not found between blood lead level and the number of leg stands. These results again suggested that the OF task was more sensitive to the effects of lower levels of exposure than higher levels of exposure in these animals.

Together the results of the OF task suggested that the low-dose group had increased exploratory activity as compared to the control group and as compared to the high-dose group. It may be important to note that, only for min 1 of exploration significant differences between groups were found with regard to the number of leg stands but not with regard to the number of quadrants crossed. This may mean that during the first minute of exploration, the low-dose group needed to supplement their horizontal activity (quadrants crossed) with vertical activity (leg stands) in order to gain knowledge about the novel environment.

The low-dose group needed to maintain a higher level of exploration of the novel environment through min 2 and 3 of the task and this was manifested in both crossing a higher number of quadrants and displaying a higher number of leg stands than the other groups.

Importantly, significant differences between groups were not found with regard to thigmotaxis, suggesting that the low-dose group was not more active simply as a result of increased anxiety.

Possible brain mechanisms mediating increased exploratory activity in low-dose exposed mice. Given the results of the NODR task, it may be that deficits in memory mediated the increase in exploratory activity in the low-level lead-exposed mice. Memory has been suggested to be reliant on long-term potentiation (LTP) within the hippocampus. LTP is a signal that influences synaptic plasticity, that is, when an animal is repeatedly exposed to a stimulus the transmission of the signal between two stimulated neurons is strengthened (Lynch, 2004). It has been found that when LTP is induced in the DG of animals, those animals that explored a novel environment had enhanced LTP as compared to those that did not (Davis, Floretta, & Derrick, 2004). Importantly, LTP in hippocampus has been found to be impaired as a result of chronic-low-level lead exposure (Liu et al., 2012). This suggests that impairments in LTP in hippocampus may have accounted for increased exploratory activity of the low-level lead-exposed group in the OF task.

It has also been suggested that dopaminergic neurons that project to the hypothalamus, the limbic system and the striatum are involved in exploratory behavior (Kelly, Cador, & Stinus, 1990). Evidence that the dopaminergic system may be disrupted as a result of chronic low-level lead exposure is that animals exposed to chronic low-levels of lead had decreased dopamine binding in the nucleus accumbens (Pokora, Riechfield, & Cory-Slechta, 1996) and had a

decreased number of active dopamine neurons in the substantia nigra and in the ventral tegmental area (Tavakoli-Nezhad & Pitts, 2004). This may mean that disruption of dopaminergic systems in several brain areas that are related to exploratory behavior may have produced increased exploratory activity in the low-dose lead-exposed mice tested in the present study.

Findings in past studies vs. present study. The present study suggested that chronic low-level lead produced increased exploratory activity in the OF task. Consistent with the findings in the present study, two studies that tested rats and monkeys identified increased exploratory activity in lead-exposed animals (Azzaoui et al., 2009; Ferguson & Bowman, 1990), and identified a delayed adaptation to a novel environment (Ferguson & Bowman, 1990). On the other hand, two mouse studies reported decreased exploratory activity in the OF task displayed by the lead-exposed groups (Kasten-Jolly et al., 2012; Leasure et al., 2008).

Inconsistencies in the results between past studies and the present study with regard to exploratory activity may be due to differences in methodology, that is, the species tested and the post-natal days at which animals were tested differed. It may be that for the OF task differences in methodology may influence more strongly inconsistencies between studies in behavioral outcomes as compared to other behavioral tasks. In fact, it has been reported that exploratory behavior in the OF task is particularly influenced by species tested, age at which animals are tested, previous behavioral experience, shape and color of the arena, and light among others (Gould et al., 2009).

Additionally, in two of the studies animals received an amphetamine injection before being tested for exploratory activity in the OF task. In one of the described studies, it was found that mice had increased exploratory activity after the amphetamine injection (Leasure et al., 2008), and in another study decreased exploratory activity was found (Reiter et al., 1975). Also,

differences in these results may due to the fact that the species tested were different. Regardless of inconsistencies in these findings, both studies suggested that chronic low-level lead exposure may have altered one or several dopamine brain pathways that are followed by d-amphetamine, for example, the dopamine mesolimbic pathway (Yehuda & Wurtman, 1975). This provides additional evidence that the dopaminergic system may be altered as a result of chronic low-level lead exposure.

Nose poke task. Significant differences between groups with regard to the number of poked holes were not found in the NP task. It may be that by the time that the mice reached the NP arena they were fatigued, and this may have obscured the effects of chronic low-level lead in exploratory activity. Specifically, the first task to be administered was the NODR task. This task required 5 min of habituation, 10 min of exploration, and 10 min of waiting time. The second task to be administered was the OF task, this task required 5 min of exploration. In total, the mice were tested for 30 min prior to the NP task perhaps fatiguing the mice and limiting the extent to which exposure effects could be observed.

Strengths and Limitations

The present study is unique because behavioral markers of chronic low-level lead exposure in young mice have rarely been examined. Importantly, we administered a short-term memory task. In past studies, short-term memory tests such as the Morris Water Maze and foot-shock tests were used for testing lead-exposed rodents' memory. Our short-term memory task was suggested to be non-invasive because it induced a minimum of stress in the experimental animals. This was suggested by the results in the OF task that did not yield significant differences between groups with regard to thigmotaxis.

A weakness of the present study is that for the NODR task, the odor molecules of the familiar odor and the novel odor may have quickly dispersed in the air, causing mixing of odor molecules and thus yielding a small effect size with regard to the association between blood lead level and the discrimination ratio. Another weakness (as mentioned earlier) is that the three behavioral tasks were administered to mice consecutively in one day. It may be that this fatigued the mice, and thus obscured possible behavioral differences that might have been otherwise captured with the NP task.

Conclusions and Future Directions

As suggested in the present study, chronic low-level lead exposure may diminish short-term olfactory recognition memory and may produce increased exploratory activity in C57BL/6J mice. Consistent with this findings child studies have suggested working memory and short-term memory alterations as a result of chronic low-level lead exposure (Lanphear, Dietrich, Auinger, & Cox, 2000; Min et al., 2007; Surkan et al., 2007). Also perhaps consistent with these findings it has been suggested that children that are exposed to chronic low-levels of lead have diminished attention (Chiodo, Jacobson, & Jacobson, 2004; Chiodo et al., 2007; Min et al., 2007; Surkan et al., 2007).

For future behavioral studies, improvements could be made with regard to the methodology used for behavioral testing. For example, for the NODR task, mice could be tested in a bigger arena so that the scented furry toy mice are separated enough so that the odor molecules do not get combined with each other. Also, for the NODR task shorter periods of testing might better capture the effects (e.g. exposing mice to odors for 3 min during the testing phase of the task).

To further explore the possible detrimental effects of chronic low-level lead exposure in consolidating environmental cues in memory, mice could be tested in an apparatus that has a novel box and a familiar box. This apparatus would consist of two connected exploratory boxes divided by a slide door. Each box could have different environmental cues that could allow differentiation of the boxes. Animals could first be habituated to one of the boxes for 5 min (with the slide door closed). Then they could be removed from the familiar box and after a delay they would be placed back in it. The slide door would be immediately opened and the animals would be allowed to freely explore the novel and familiar boxes for 10 min. If mice have intact memory they would be expected to spend more time exploring the novel box as compared to the familiar box.

REFERENCES

Abel, T., & Lattal, K. M. (2001). Molecular mechanisms of memory acquisition, consolidation and retrieval. *Current Opinion in Neurobiology, 11,* 180-187. Doi:10.1016/S0959 4388(00)00194-X.

Alberts, B., Johnson A., & Lewis J. (2002). Signaling through G-protein-linked cell-surface receptors. *Molecular Biology of the Cell. 4th edition*. New York: Garland Science.

Azzaoui, F. Z., Ahami, A. O. T., & Khadmaoui, A. (2009). Impact of lead sub-chronic toxicity on recognition memory and motor activity of wistar rat. *Pakistan Journal of Biological Sciences, 12*, 173-177. Doi: 10.3923/pjbs.2009.173.177.

Bellinger, D. C., & Needleman, H. L. (2003). Intellectual impairment and blood lead levels. *The New England Journal of Medicine, 349,* 500-502. Doi: 10.1056/NEJM200307313490515.

Bernard, S. M., & McGeehin, M. A. (2003). Prevalence of blood lead levels >or= 5 micro g/Dl among US children 1 to 5 years of age and socioeconomic and demographic factors associated with blood of lead levels 5 to 10 micro g/Dl, Third National Health and Nutrition Examination Survey, 1988-1994. *Pediatrics, 112,* 1308-1313. Doi: 10.1289/ehp.10424.

Bevins, R. A., & Besheer, J. (2006). Object recognition in rats and mice: a one-trial nonmatching-to-sample learning task to study 'recognition memory.' *Nature Protocols, 1*, 1306–1311. Doi:10.1038/nprot.2006.205.

Blizard, D. A., Takahashi, A., Galsworthy, M. J., Martin, B., & Koide, T. (2007). Test standardization in behavioural neuroscience: A response to stanford. *Journal of Psychopharmacology, 21,* 136-139. Retrieved from http://0-search.ebscohost.com.lib.utep.edu/login.aspx?direct=true&db=a9h&AN=24682967&site=ehost-live&scope=site.

Brennan, P. A., & Keverne, E. B. (1997). Neural mechanisms of mammalian olfactory learning. *Progress in Neurobiology, 51,* 457-481. Doi:10.1016/S0301-0082(96)00069-X.

Canfield, R. L., Henderson, C. R., Jr., Cory-Slechta, D. A., Cox, C., Jusko, T. A., & Lanphear, B. P. (2003). Intellectual impairment in children with blood lead concentrations below 10 micrograms per deciliter. *The New England Journal of Medicine, 348,* 1517-1526. Doi: 10.1056/NEJMoa022848.

Centers for Disease Control and Prevention, Preventing lead poisoning in young children: A statement by the centers for disease control. (1991). *Report of the CDC Preventing lead poisoning in young children*. Retrieved from http://wonder.cdc.gov/wonder/prevguid/p0000029/p0000029.asp.

Chiodo, L. M., Jacobson, S. W., & Jacobson, J. L. (2004). Neurodevelopmental effects of postnatal lead exposure at very low levels. *Neurotoxicology and Teratology, 26,* 359-371. doi: 10.1016/j.ntt.2004.01.010.

Chiodo, L. M., Covington, C., Sokol, R. J., Hannigan, J. H., Jannise, J., Ager, J., . . . Delaney Black, V. (2007). Blood lead levels and specific attention effects in young children. *Neurotoxicology and Teratology, 29,* 538-546. doi: 10.1016/j.ntt.2007.04.001.

Davis, J. R., & Avram, M. J. (1978). Developmental changes in delta-aminolevulinic acid dehydratase (ALAD) activity and blood reticulocyte percent in the developing rat. *Mechanisms of Ageing and Development, 7,* 123-129. doi: 10.1016/0047-6374(78)90058.

Davis, C.D., Floretta, L.J., & Derrick B.E. (2004). Novel environments enhance the induction and maintenance of long-term potentiation in the dentate gyrus. *The Journal of Neuroscience, 24,* 6497-6506. Doi: 10.1523/1JNEUROSCI.497003.2004.

Eichenbaum, H., Dudchenko P., Wood E., Shapiro M., & Tanila H. (1999). The hippocampus, memory and place cells: is it spatial memory or memory space? *Neuron, 23,* 209-226. Doi: 10.1.1.214.6953.

Elliott, B. M., & Grunberg, N. E. (2005). Effects of social and physical enrichment on open field activity differ in male and female Sprague–Dawley rats. *Behavioural Brain Research, 165,* 187-196. Doi:10.1016/j.bbr.2005.06.025.

Environmental Protection Agency (2006). Documentation for the final 2002 point source national emissions inventory. *Report of the EPA Documentation for the Final 2002 Point Source National Emissions Inventory.* Retrieved from ftp://ftp.epa.gov/EmisInventory/2002finalnei/documentation/point/2002nei_final_point source_documentation0206.pdf.

Ferguson, S. A., & Bowman, R. E. (1990). Effects of postnatal lead exposure on open field behavior in monkeys. *Neurotoxicology and Teratology, 12,* 91-97. Doi: 10.1016/0892 0362(90)90118-V.

Fjerdingstad, E.J., Danscher G., & Fjerdingstad E., (1974). Hippocampus: selective concentration of lead in the normal rat brain. *Brain Research, 80,* 350-354.

Franko, E., Palome, J., Brown, M., Kennedy, C., & Moore, L. (2000). Blood lead levels in young children-United States and selected states, 1996-1999. *Morbidity and Mortality Weekly Report, 49,* 1133-1137. Retrieved from http://www.cdc.gov/mmwr/preview/mmwrhtml/mm4950a3.htm.

Fray, P.J., Sahakian, B.J., Robbins, T.W., Koob, G.F., & Iversen, S.D. (1980). An observational method for quantifying the behavioral effects of dopamine agonists: Contrasting effects of d-amphetamine and apomorphine. *Psychopharmacology, 69,* 253-259. Retrieved from http://link.springer.com/article/10.1007%2FBF00433091#.

Gilbert, S. G., & Weiss, B. (2006). A rationale for lowering the blood lead action level from 10 to 2 [mu]g/Dl. *Neurotoxicology, 27,* 693-701. Doi: 10.1016/j.neuro.2006.06.008.

Gould, D.T., Dao D.T., & Kovacsics C.E. (2009). The open field test. *Mood and Anxiety Related Phenotypes in Mice. Neuromethods, 42,* 1-20. Doi: 10.1007/978-1-60761-303-9_1.

Hebb, D. O. (1949). *The organization of behavior; a neuropsychological theory*. New York: Wiley.

Johnes Hopkins University (2011). Simple odor recognition protocol. *Neurogenetics & Behavior Center*. Retrieved from http://nbc.jhu.edu/behavioral_tasks/tasks/simple_odor_recognition_protocol.html

Jusko, T. A., Henderson, C. R., Jr., Lanphear, B. P., Cory-Slechta, D. A., Parsons, P. J., & Canfield, R. L. (2007). Blood lead concentrations < 10 mg/Dl and child intelligence at 6 years of age. *Environmental Health Perspectives, 116*, 243-248. Doi: 10.1289/ehp.10424.

Kasten-Jolly, J., Pabello, N., Bolivar, V. J., & Lawrence, D. A. (2012). Developmental lead effects on behavior and brain gene expression in male and female BALB/cAnNTac mice. *Neurotoxicology, 33,* 1005-1020. Doi: 10.1016/j.neuro.2012.04.017.

Kelley, A.E, Cador M., & Stinus L. (1990). Exploration and its measurement. *Psychopharmacology Neuromethods, 13*, 95-144. Doi: 10.1385/0-89603-129-2:95.

Landrigan, P. J., Trasande, L., Thorpe, L. E., Gwynn, C., Lioy, P. J., D'Alton, M. E., . . . Susser, E. (2006). The national children's study: A 21-year prospective study of 100, 000 American children. *Pediatrics, 118*, 2173-2186. Doi: 10.1542/peds.2006-0360.

Lanphear, B. P., Burgoon, D. A., Rust, S. W., Eberly, S., & Galke, W. (1998). Environmental exposures to lead and urban children's blood lead levels. *Environmental Research, 76,* 120-130. Doi: 10.1006/enrs.1997.3801.

Lanphear, B. P., Dietrich, K., Auinger, P., & Cox, C. (2000). Cognitive deficits associated with blood lead concentrations <10 microg/Dl in US children and adolescents. *Public Health Reports, 115*, 521-529. Retrieved from http://0-search.ebscohost.com.lib.utep.edu/login.aspx?direct=true&db=a9h&AN=5027664&site=ehost-live&scope=site.

Lanphear, B. P., Hornung, R., Khoury, J., Yolton, K., Baghurst, P., Bellinger, D. C., . . . Roberts, R. (2005). Low-level environmental lead exposure and children's intellectual function: an

international pooled analysis. *Environmental Health Perspectives, 113*, 894-899. Doi: 10.1289/ehp.7688.

Leasure, J. L., Giddabasappa, A., Chaney, S., Johnson, J. J. E., Pothakos, K., Yuen Sum, L., & Fox, D. A. (2008). Low-level human equivalent gestational lead exposure produces sex-specific motor and coordination abnormalities and late-onset obesity in year-old mice. *Environmental Health Perspectives, 116*, 355-361. Doi: 10.1289/ehp.10862.

Light, K. R., Grossman, H., Kolata, S., Wass, C., & Matzel, L. D. (2011). General learning ability regulates exploration through its influence on rate of habituation. *Behavioural Brain Research, 223*, 297-309. Doi:10.1016/j.bbr.2011.04.050.

Liu, M.C., Liu, X.Q., Wang, W., Shen, X.F., Che, H.L., Guo, Y.Y. . . Luo, W.J. (2012). Involvement of microglia activation in the lead induced long-term potentiation impairment. *Public Library of Science*, 7, e43924. Doi:10.1371/journal.pone.0043924.

Lynch, M.A. (2004). Long-term potentiation and memory. *Physiological Reviews, 84*, 87-136. Doi: 10.1152/physrev.00014.2003.

Mandairon, N., Poncelet J., Bensafi., & Didier A. (2009). Humans and mice express similar olfactory preferences. *Plos One, 4*, 1-5. Doi:10.1371/journal.pone.0004209.

Min, J.-Y., Min, K.-B., Cho, S.-I., Kim, R., Sakong, J., & Paek, D. (2007). Neurobehavioral function in children with low blood lead concentrations. *Neurotoxicology, 28*, 421-425. Doi:10.1016/j.neuro.2006.03.007.

Needleman, H. L., & Gatsonis, C. A. (1990). Low-level lead exposure and the IQ of children. A meta-analysis of modern studies. *Jama, 263,* 673-678. Doi: 10.1001/jama.1990.03440050067035.

Needleman, H. L., Schell, A., Bellinger, D., Leviton, A., & Allred, E. N. (1990). The long-term effects of exposure to low doses of lead in childhood. An 11-year follow-up report. *The New England Journal of Medicine, 322,* 83-88. Doi: 10.1056/NEJM199001113220203.

Needleman, H. L., Riess, J. A., Tobin, M. J., Biesecker, G. E., & Greenhouse, J. B. (1996). Bone lead levels and delinquent behavior. *Jama, 275,* 363-369. Doi: 10.1001/jama.1996.03530460029021.

Onajala, A. O., & Claudio L. (2000). Genetic susceptibility to lead poisoning. *Environmental Health Perspectives, 108,* 23-28. Retrieved from http://www.jstor.org/stable/3454630.

Pokora, M.J., Riechfield, E.K., & Cory-Slechta, D.A. (1996). Preferential vulnerability of nucleus accumbens dopamine binding sites to low-level lead exposure: Time course of effects and interactions with chronic dopamine agonist treatments. *Journal of Neurochemistry, 67,* 1540-1550. Doi: 10.1046/j.1471-4159.1996.67041540.

Reiter, L. W., Anderson, G. E., Laskey, J. W., & Cahill, D. F. (1975). Developmental and behavioral changes in the rat during chronic exposure to lead. *Environmental Health Perspectives, 12,* 119-123. Retrieved from http://www.jstor.org/stable/3428220.

Schnaas, L., Rothenberg, S. J., Perroni, E., Martinez, S., Hernandez, C., & Hernandez, R. M. (2000). Temporal pattern in the effect of postnatal blood lead level on intellectual

development of young children. *Neurotoxicology and Teratology, 22,* 805-810. doi: 10.1016/S0892-0362(00)00101-X.

Sobin, C., Gutierrez, M., & Alterio, H. (2009). Polymorphisms of delta-aminolevulinic acid dehydratase (ALAD) and peptide transporter 2 (PEPT2) genes in children with low-level lead exposure. *Neurotoxicology, 30,* 881–887. Doi: 10.1016/j.neuro.2009.08.006.

Sobin, C., Parisi, N., Schaub, T., Gutierrez, M., & Ortega, A. (2011). Δ-Aminolevulinic acid dehydratase single nucleotide polymorphism 2 and peptide transporter 2*2 haplotype may differentially mediate lead exposure in male children. *Archives of Environmental Contamination and Toxicology, 61,* 521-529. Doi: 10.1007/s00244-011-9645-3.

Sobin, C., Montoya, M. G. F., Parisi, N., Schaub, T., Cervantes, M., & Armijos, R. X. (in press). Microglial disruption in young mice with early chronic lead exposure. *Toxicology Letters,* (0). Doi:10.1016/j.toxlet.2013.04.003.

Surkan, P. J., Zhang, A., Trachtenberg, F., Daniel, D. B., McKinlay, S., & Bellinger, D. C. (2007). Neuropsychological function in children with blood lead levels <10 [mu]g/Dl. *Neurotoxicology, 28,* 1170-1177. doi: 10.1016/j.neuro.2007.07.007.

Tavakoli-Nezhad, M., & Pitts D.K., (2004). Postnatal inorganic lead exposure reduces midbrain dopaminergic impulse flow and decreases dopamine D1 receptor sensitivity in nucleus accumbens neurons. *Journal of Pharmacology and Experimental Therapeutics, 312,* 1280-1288. doi: 10.1124/jpet.104.076166.

Walsh, R. N., & Cummins, R. A. (1976). The open-field test: A critical review. *Psychological Bulletin, 83,* 482-504. doi:10.1037/0033-2909.83.3.482.

Wasserman, G., Liu, X., Popovac, D., Factor-Litvak, P., Kline, J. K., Waternaux, C.,. . . Graziano, J. H. (2000). The Yugoslavia prospective lead study: contributions of prenatal and postnatal lead exposure to early intelligence. *Neurotoxicology and Teratology*, *22*, 811-818. doi: 10.1016/S0892-0362(00)00106-9.

Yehuda, S., & Wurtman, R. J. (1975). Dopaminergic neurons in the nitro-striatal and mesolimbic pathways: Mediation of specific effects of d-amphetamine. *European Journal of Pharmacology*, *30*, 154-158. doi: 10.1016/0014-2999(75)90094-1.

TABLES

Table 1

Mean Time Exploring the Objects (During 3 Min)

Object	*Exploration Mean (sec)*
Grape	**16.64**
Onion	**16.13**
Cup	**7.39**
Black ball	**9.14**
Truck	**18.86**
Rhino	**19.35**
Orange Van	**13.36**

Note. The three objects that were explored the most are in boldface.

Table 2

Novel Object Recognition Task in Arena with Bedding

Mouse	***Sex***	***Familiar Object (sec)***	***Novel Object (sec)***	***Discrimination Ratio***
1	*Male*	9.97	11.33	0.532
2	*Male*	7.65	31.47	0.804
3	*Male*	14.56	9.39	0.392
4	*Male*	7.40	5.39	0.421
5	*Male*	11.94	3.76	0.239
Mean		**10.30**	**12.26**	**0.478**

* The discrimination ratio differed significantly from the hypothetical 0.50 ($p < .05$).

Table 3

Novel Object Recognition Task in Arena without Bedding

Mouse	***Sex***	***Familiar Object (sec)***	***Novel Object (sec)***	***Discrimination Ratio***
1	*Female*	4.82	8.45	0.637
2	*Female*	6.77	24.82	0.786
3	*Female*	9.36	7.40	0.442
4	*Male*	2.53	5.47	0.684
5	*Male*	3.49	4.50	0.563
6	*Male*	6.88	9.83	0.588
Mean		**5.64**	**10.07**	**0.617**

* The discrimination ratio differed significantly from the hypothetical 0.50 ($p < .05$).

Table 4

Novel Odor Recognition Task

Mouse	***Sex***	***Familiar Object (sec)***	***Novel Object (sec)***	***Discrimination Ratio***
1	*Female*	1.34	2.86	0.681
2	*Female*	0.00	2.03	1.000
3	*Female*	19.24	18.40	0.489
4	*Female*	0.75	1.06	0.586
5	*Male*	0.34	2.22	0.867
6	*Male*	1.22	5.37	0.815
7	*Male*	4.51	2.41	0.348
8	*Male*	2.68	5.54	0.674
Mean		**3.76**	**4.98**	**0.682***

* The discrimination ratio differed significantly from the hypothetical 0.50 ($p < .05$).

Table 5

T-tests Results of Males and Females Tested on Three Behavioral Tasks

Task	***Sex***	***n***	***df***	***Mean***	***SEM***	***T***	***P***
Novel Odor Recognition							
Exploration Novel Odor and Familiar Odor							
	Females	*14*	*33*	7.50	1.37	0.18	0.86
	Males	*21*		7.81	1.12		
Discrimination Ratio							
	Females	*14*	*33*	0.51	0.07	0.59	0.56
	Males	*21*		0.57	0.06		
Open Field							
Quadrants Crossed							
	Females	*14*	*33*	55.93	5.94	-2.19	**<.05**
	Males	*21*		43.24	2.59		
Leg Stands							
	Females	*14*	*33*	36.00	2.17	-0.26	0.79
	Males	*21*		35.02	2.63		
Thigmotaxis							
	Females	*14*	*33*	102.60	5.26	-0.79	0.43
	Males	*21*		97.16	4.31		
Nose Poke							
Deep Dips							
	Females	*14*	*33*	22.50	1.34	0.79	0.44
	Males	*21*		24.20	1.51		
Any Dips							
	Females	*14*	*33*	34.50	1.79	0.56	0.58
	Males	*21*		35.97	1.78		

Table 6

Means and Standard Error of the Means of Lead Exposed Groups Tested on the Novel Odor Recognition Task

Variable	*Lead Exposure*	*n*	*Mean*	*SEM*	*P*
Exploration Novel Odor and Familiar Odor					
	0 ppm	*11*	7.88	1.57	0.99
	30 ppm	*11*	7.51	1.57	
	330 ppm	*13*	7.68	1.44	
Discrimination Ratio					
	0 ppm	*11*	0.72	0.08	**<.05**
	30 ppm	*11*	0.46	0.08	
	330 ppm	*13*	0.48	0.07	

Table 7

One-way ANOVA Testing for Lead Exposure Group Differences on the Novel Odor Recognition Task

Variable	***Source of Variation***	***df***	***Sum of Squares***	***Mean Squares***	***F***	***P***
Exploration Novel Odor and Familiar Odor						
	Model	*2*	0.73	0.36	0.01	0.99
	Error	*32*	866.06	27.06		
Discrimination Ratio						
	Model	*2*	0.47	0.23	3.46	**<.05**
	Error	*32*	2.18	0.07		

Table 8

Least Square Means and Standard Error of the Means of Lead Exposed Groups Tested on the Open Field Task (Number of Quadrants Crossed)*

Quadrants Crossed	*Lead Exposure*	*n*	*Mean*	*SEM*	*P*
Total					
	0 ppm	*11*	42.06	5.65	**<.05**
	30 ppm	*11*	62.29	5.32	
	330 ppm	*13*	44.93	4.38	
Min 1					
	0 ppm	*11*	13.23	0.94	0.14
	30 ppm	*11*	15.80	0.88	
	330 ppm	*13*	13.83	0.73	
Min 2					
	0 ppm	*11*	8.76	1.92	**<.05**
	30 ppm	*11*	16.04	1.81	
	330 ppm	*13*	9.05	1.49	
Min 3					
	0 ppm	*11*	6.89	1.52	**<.05**
	30 ppm	*11*	12.74	1.44	
	330 ppm	*13*	8.05	1.18	
Min 4					
	0 ppm	*11*	7.25	1.48	0.59
	30 ppm	*11*	9.35	1.40	
	330 ppm	*13*	7.88	1.15	
Min 5					
	0 ppm	*11*	5.93	1.25	0.29
	30 ppm	*11*	8.35	1.18	
	330 ppm	*13*	6.12	0.97	

* Least square means are means adjusted for sex and weight.

Table 9

Two-way ANCOVA Testing for Lead Exposure Group Differences Controlling for Sex and for Weight on the Open Field Task (Number of Quadrants Crossed)

Quadrants Crossed	***df***	***Model F***	***Effects***	***Effects F***	***P***
Total	(2, 28)	2.52*****	lead	4.02	**<.05**
			sex	4.10	**0.05**
			lead*sex	0.47	0.63
			weight	0.23	0.64
Min 1	(2, 28)	0.89	lead	2.09	0.14
			sex	0.98	0.33
			lead*sex	0.12	0.89
			weight	0.48	0.49
Min 2	(2, 28)	2.64*	lead	5.20	**<.05**
			sex	3.75	0.06
			lead*sex	0.33	0.72
			weight	0.47	0.50
Min 3	(2, 28)	2.94*	lead	4.31	**<.05**
			sex	5.41	**<.05**
			lead*sex	1.21	0.31
			weight	0.45	0.51
Min 4	(2, 28)	1.11	lead	0.52	0.59
			sex	1.91	0.18
			lead*sex	0.60	0.55
			weight	0.04	0.84
Min 5	(2, 28)	0.82	lead	1.28	0.29
			sex	0.92	0.35
			lead*sex	0.02	0.98
			weight	0.00	0.99

*****$p < .05$

Table 10

Least Square Means and Standard Error of the Means of Lead Exposed Groups Tested on the Open Field Task (Number of Leg Stands)*

Leg Stands	*Lead Exposure*	*n*	*Mean*	*SEM*	*P*
Total					
	0 ppm	*11*	30.10	2.89	**<.01**
	30 ppm	*11*	44.74	2.83	
	330 ppm	*13*	32.03	2.41	
Min 1					
	0 ppm	*11*	7.36	0.79	**<.05**
	30 ppm	*11*	10.73	0.79	
	330 ppm	*13*	8.15	0.73	
Min 2					
	0 ppm	*11*	5.84	0.79	**<.01**
	30 ppm	*11*	9.68	0.77	
	330 ppm	*13*	6.06	0.65	
Min 3					
	0 ppm	*11*	6.49	0.85	**<.001**
	30 ppm	*11*	10.05	0.83	
	330 ppm	*13*	5.69	0.71	
Min 4					
	0 ppm	*11*	5.79	1.00	0.44
	30 ppm	*11*	7.67	0.98	
	330 ppm	*13*	7.15	0.83	
Min 5					
	0 ppm	*11*	4.73	1.02	0.51
	30 ppm	*11*	6.50	1.02	
	330 ppm	*13*	4.85	0.94	

* Least square means are means adjusted for weight.

Table 11

One-way ANCOVA Testing for Lead Exposure Group Differences Controlling for Weight on the Open Field Task (Number of Leg Stands)

Leg Stands	***df***	***Model F***	***Effects***	***Effects F***	***P***
Total	(2, 31)	6.39****	lead	7.41	**<.01**
			weight	0.01	0.94
Min 1	(2, 31)	3.30*	lead	4.29	**<.05**
			weight	0.19	0.66
Min 2	(2, 31)	5.51**	lead	7.56	**<.01**
			weight	0.36	0.55
Min 3	(2, 31)	8.02***	lead	8.32	**<.001**
			weight	0.93	0.34
Min 4	(2, 31)	0.87	lead	0.83	0.44
			weight	0.02	0.88
Min 5	(2, 31)	0.63	lead	0.68	0.51
			weight	0.02	0.89

*****$p < .05$; ** $p < .01$;*** $p < .001$

Table 12

Least Square Means and Standard Error of the Means of Lead Exposed Groups Tested on the Open Field Task (Thigmotaxis)*

Thigmotaxis	***Lead Exposure***	***n***	***Mean***	***SEM***	***P***
Total					
	0 ppm	*11*	112.12	6.26	0.06
	30 ppm	*11*	94.66	6.13	
	330 ppm	*13*	92.47	5.21	

* Least square means are means adjusted for weight.

Table 13

One-way ANCOVA Testing for Lead Exposure Group Differences Controlling for Weight on the Open Field Task (Thigmotaxis)

Thigmotaxis	***df***	***Model F***	***Effects***	***Effects F***	***P***
Total	(2, 31)	2.02	lead	2.98	0.06
			weight	0.60	0.45

Table 14

Least Square Means and Standard Error of the Means of Lead Exposed Groups Tested on the Nose Poke Task (Number of Dips)*

Type of Dips	***Lead Exposure***	***n***	***Mean***	***SEM***	***P***
Deep Dips					
Total					
	0 ppm	*11*	24.31	1.97	0.57
	30 ppm	*10*	23.05	2.03	
	330 ppm	*12*	23.21	1.72	
Any Dips					
Total					
	0 ppm	*11*	34.40	2.39	0.70
	30 ppm	*10*	37.89	2.46	
	330 ppm	*12*	34.22	2.08	

* Least square means are means adjusted for weight.

Table 15

One-way ANCOVA Testing for Lead Exposure Group Differences Controlling for Weight on the Nose Poke Task (Number of Dips)

Type of Dips	***Source of Variation***	***df***	***Sum of Squares***	***Mean Squares***	***F***	***P***
Deep Dips						
Total						
	Model	*3*	183.72	30.62	0.81	0.57
	Error	*29*	980.75	37.72		
Any Dips						
Total						
	Model	*3*	38.89	19.44	0.35	0.70
	Error	*29*	1678.99	55.97		

Table 16

Simple Regression Analyses Testing for Linear Associations between Blood Lead Level and Behavior

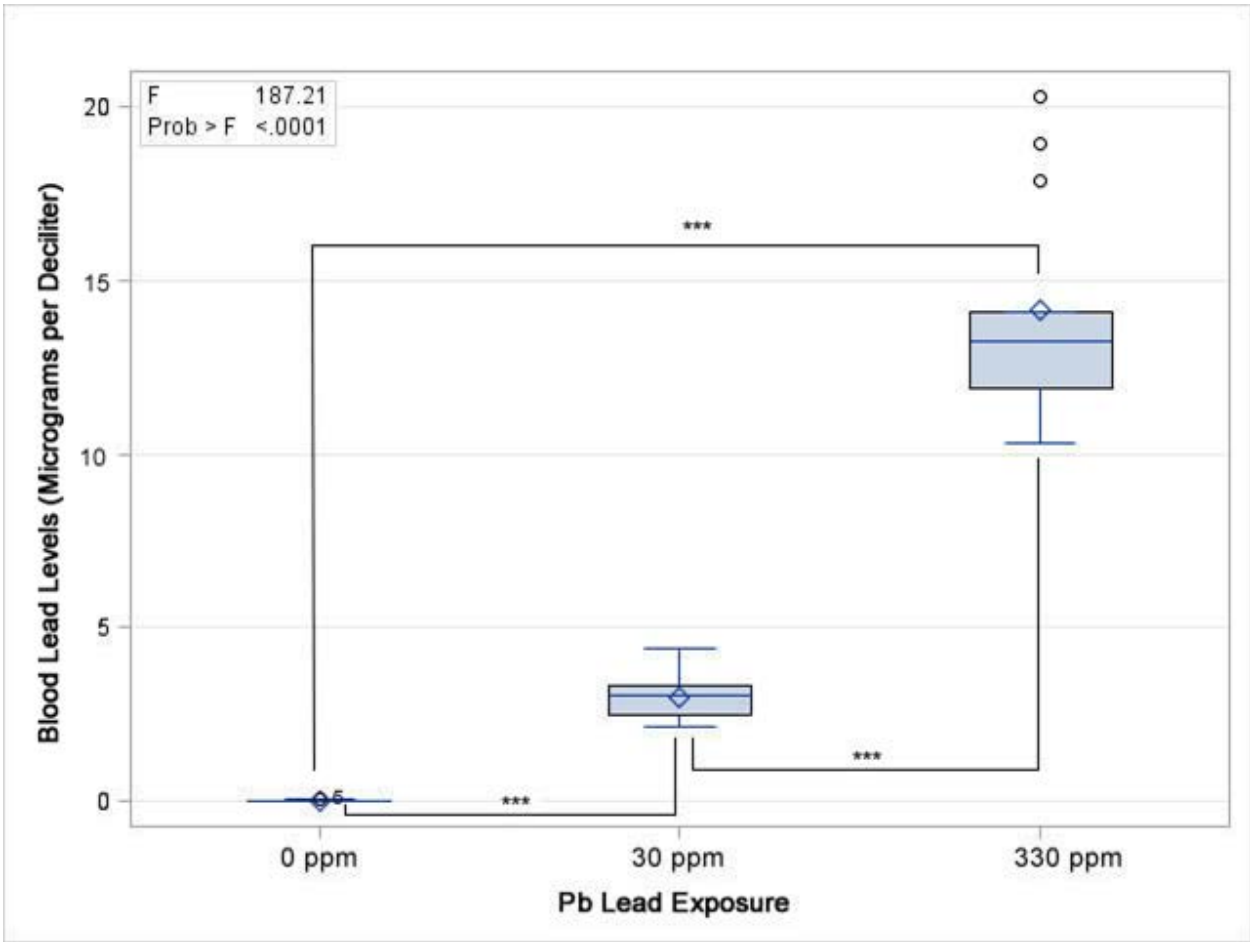
Task	***df***	r^2	***F***	***β***	***P***
Novel Odor Recognition					
Discrimination Ratio	(1, 33)	0.116	4.32	-0.014	**<.05**
Open Field					
Quadrants Crossed					
Total	(1, 33)	0.004	0.12	-0.161	0.73
Min 2	(1, 33)	0.018	0.59	-0.122	0.45
Min 3	(1, 33)	0.006	0.20	-0.058	0.66
Leg Stands					
Total	(1, 33)	0.013	0.42	-0.181	0.52
Min 1	(1, 33)	0.004	0.13	-0.027	0.72
Min 2	(1, 33)	0.014	0.47	-0.050	0.49
Min 3	(1, 33)	0.084	0.091	-0.143	0.09

Table 17

Simple Regression Analyses Testing for Quadratic Associations between Blood Lead Level and Behavior

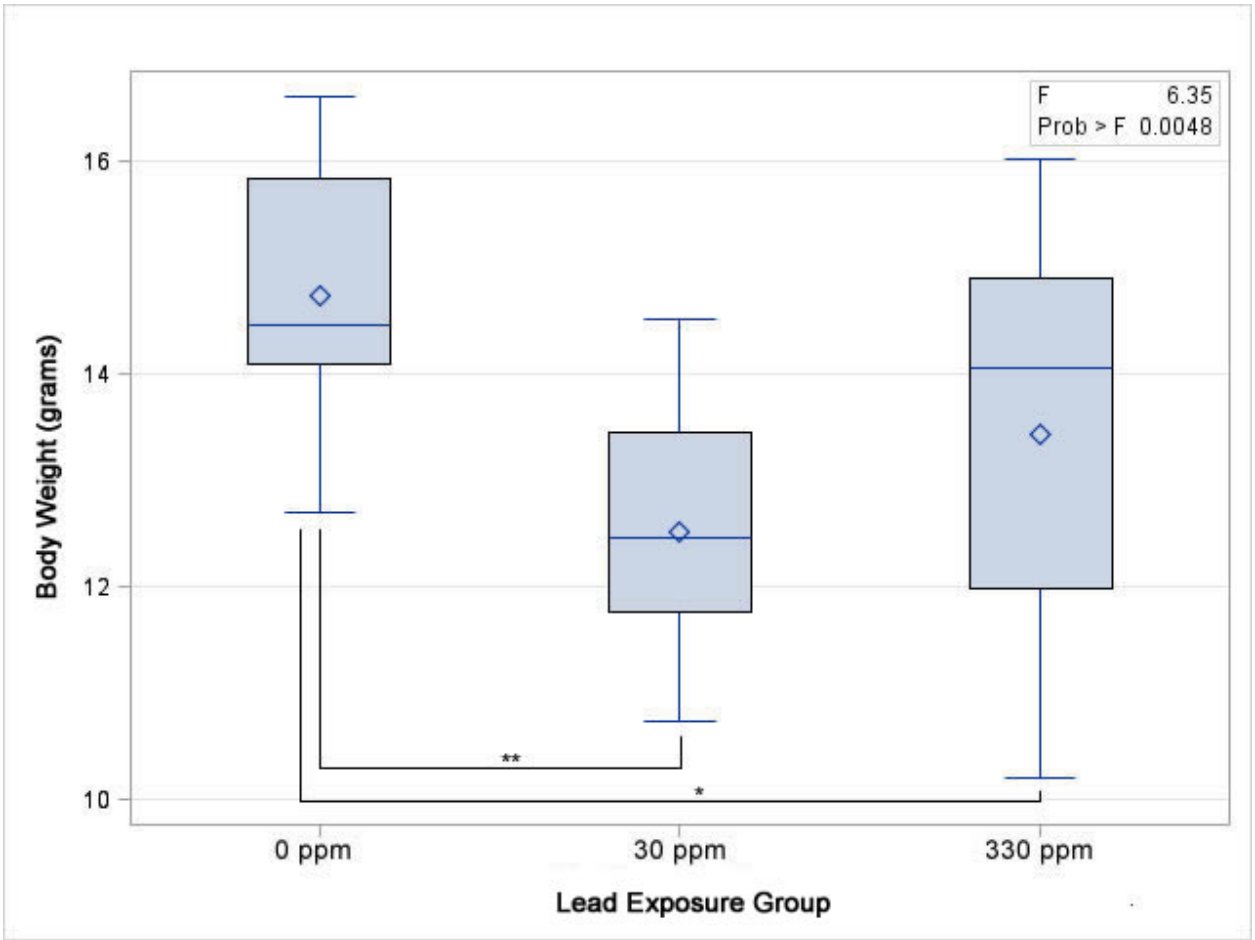
Task	***df***	r^2	***F***	***β***	***P***
Novel Odor Recognition					
Discrimination Ratio	(2, 32)	0.12	2.28	-.001	0.12
Open Field					
Quadrants Crossed					
Total	(2, 32)	0.035	0.59	-0.10	0.31
Min 2	(2, 32)	0.062	1.05	-0.04	0.36
Min 3	(2, 32)	0.047	0.80	-0.03	0.46
Leg Stands					
Total	(2, 32)	0.042	0.71	-0.06	0.49
Min 1	(2, 32)	0.066	1.13	-0.02	0.34
Min 2	(2, 32)	0.015	0.24	-0.002	0.79
Min 3	(2, 32)	0.136	2.30	-0.02	0.12

FIGURES



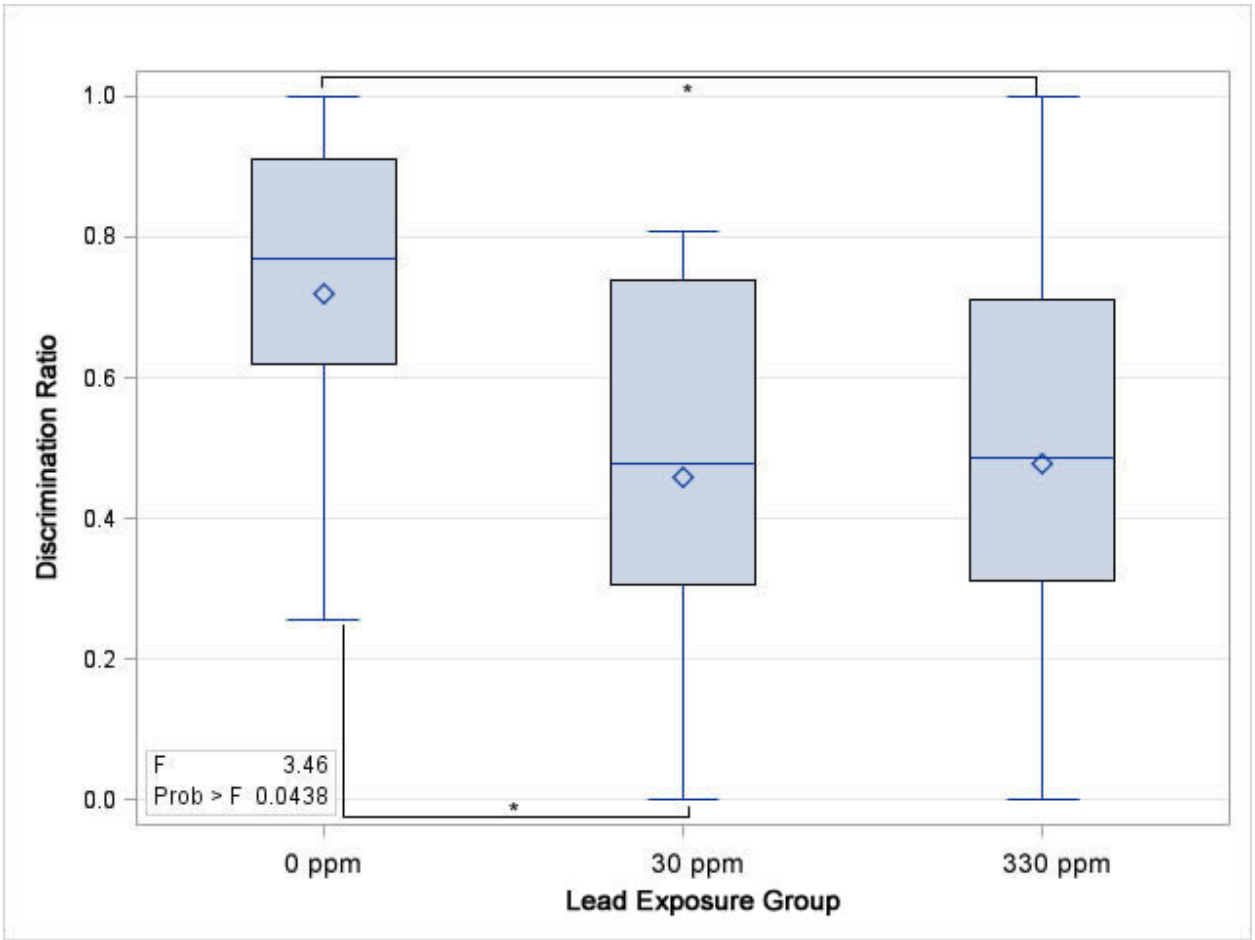
**p* < .05; ***p* < .01; ****p* < .001

Figure 1. Group Differences in Blood Lead Levels.



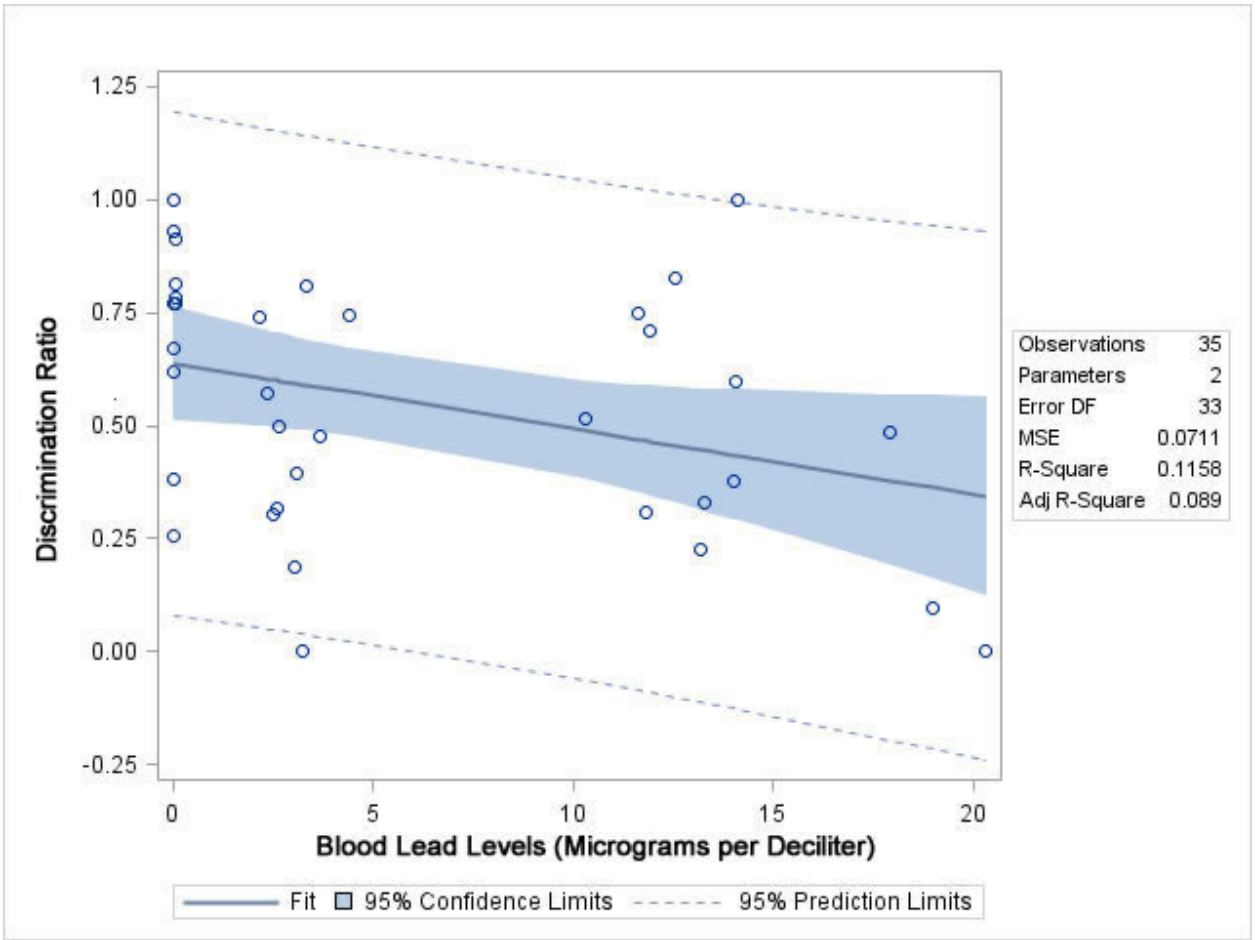
***p* < .05; **** p* < .01; ***** p* < .001

Figure 2. Group Differences in Body Weight



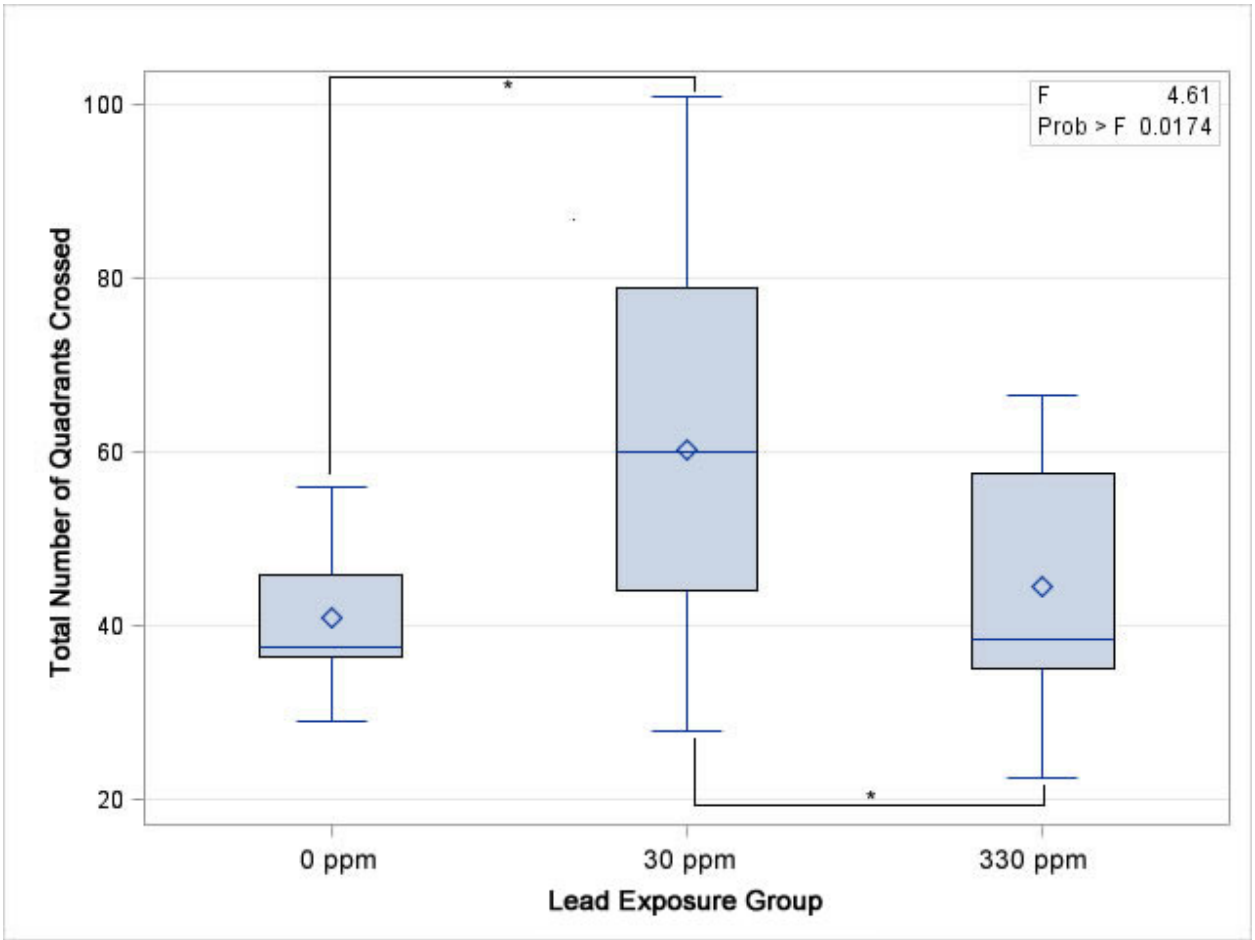
***p* < .05; ****p* < .01; *****p* < .001

Figure 3. Novel Odor Recognition Task Lead Exposure Group Differences on the Discrimination Ratio



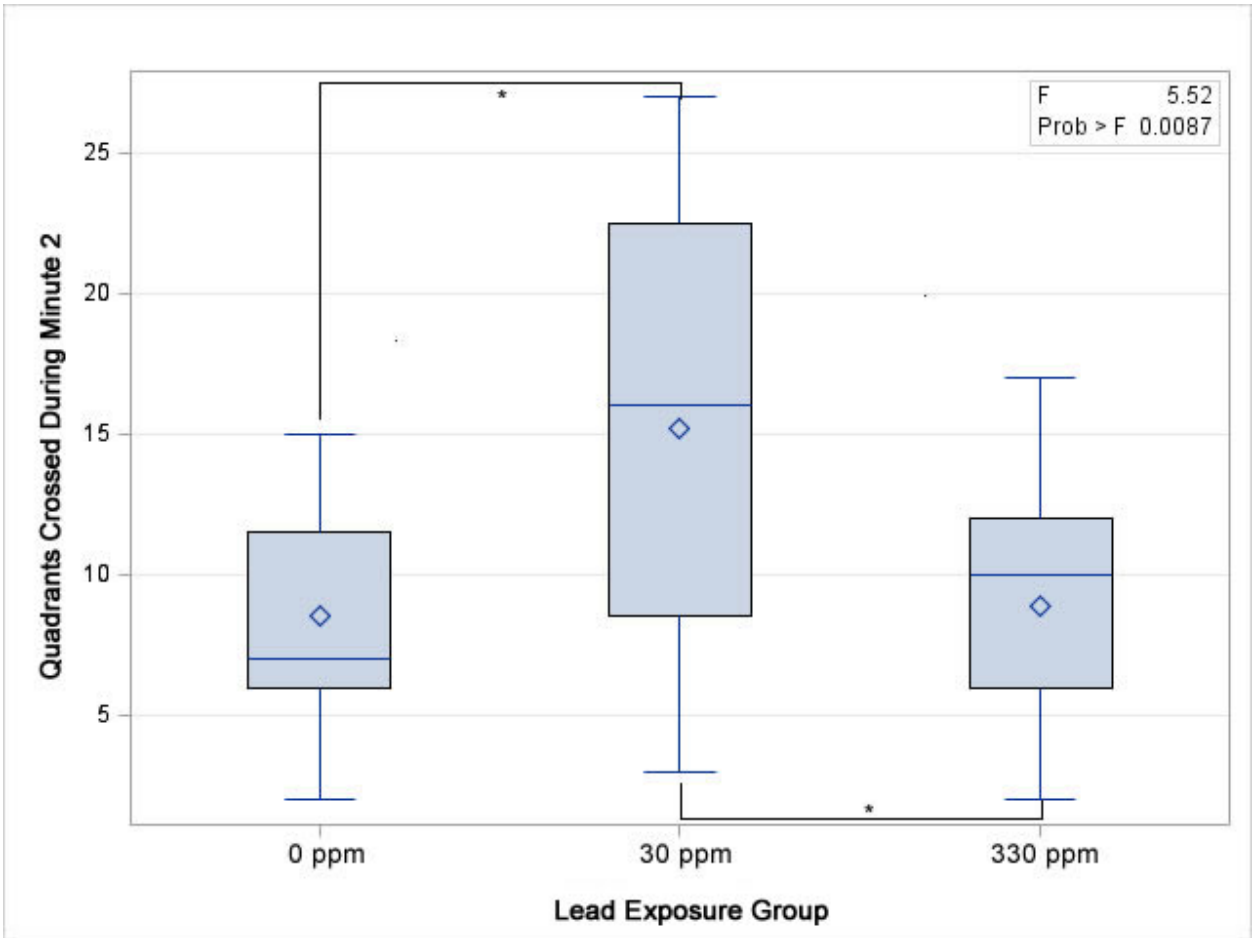
*****$p < .05$

Figure 4. Novel Odor Recognition Task Linear Association between Blood Lead Level and the Discrimination Ratio



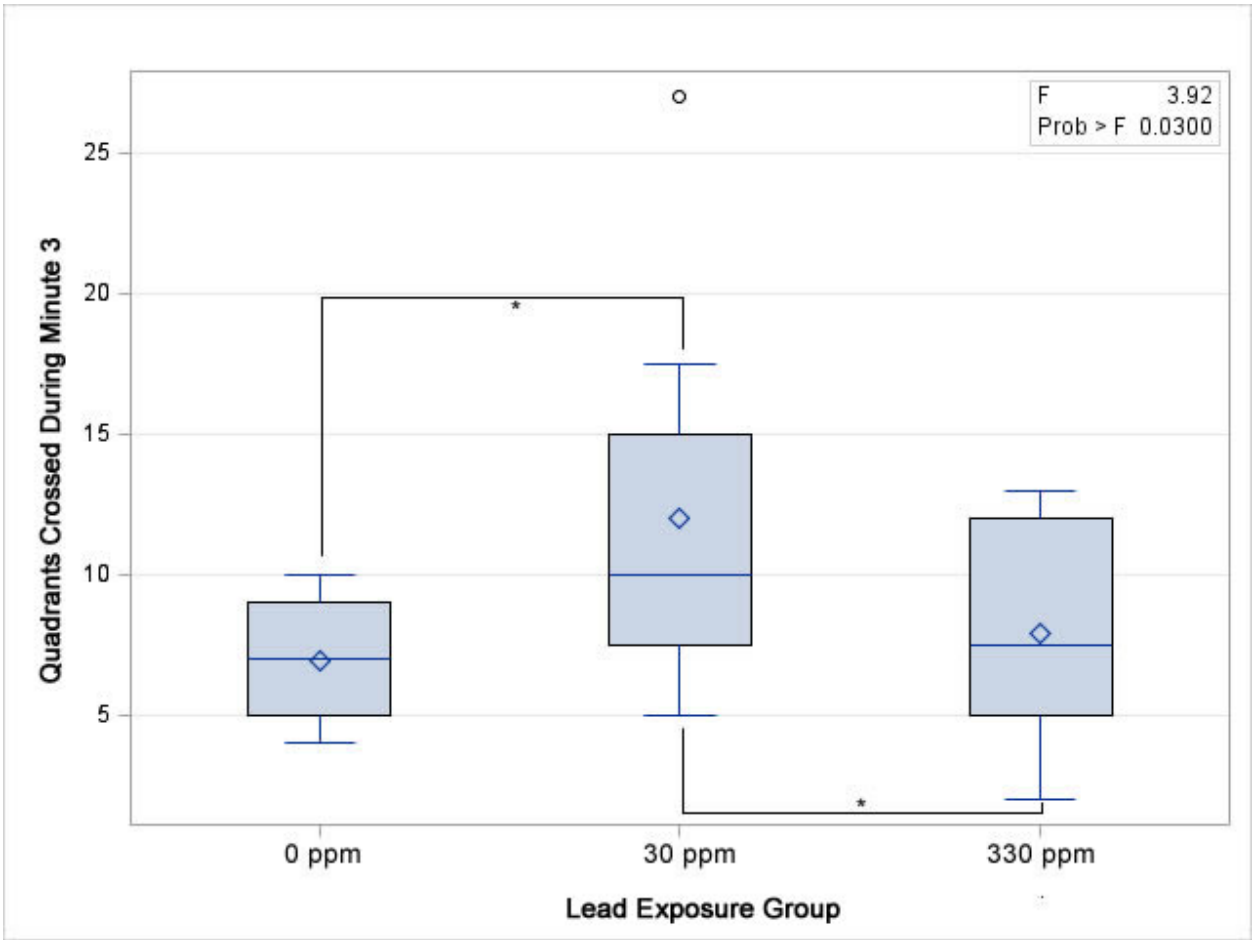
p* < .05; * *p* <.01; ***** *p* < .001

Figure 5. Open Field Task Main Effect of Lead Exposure on the Total Number of Quadrants Crossed



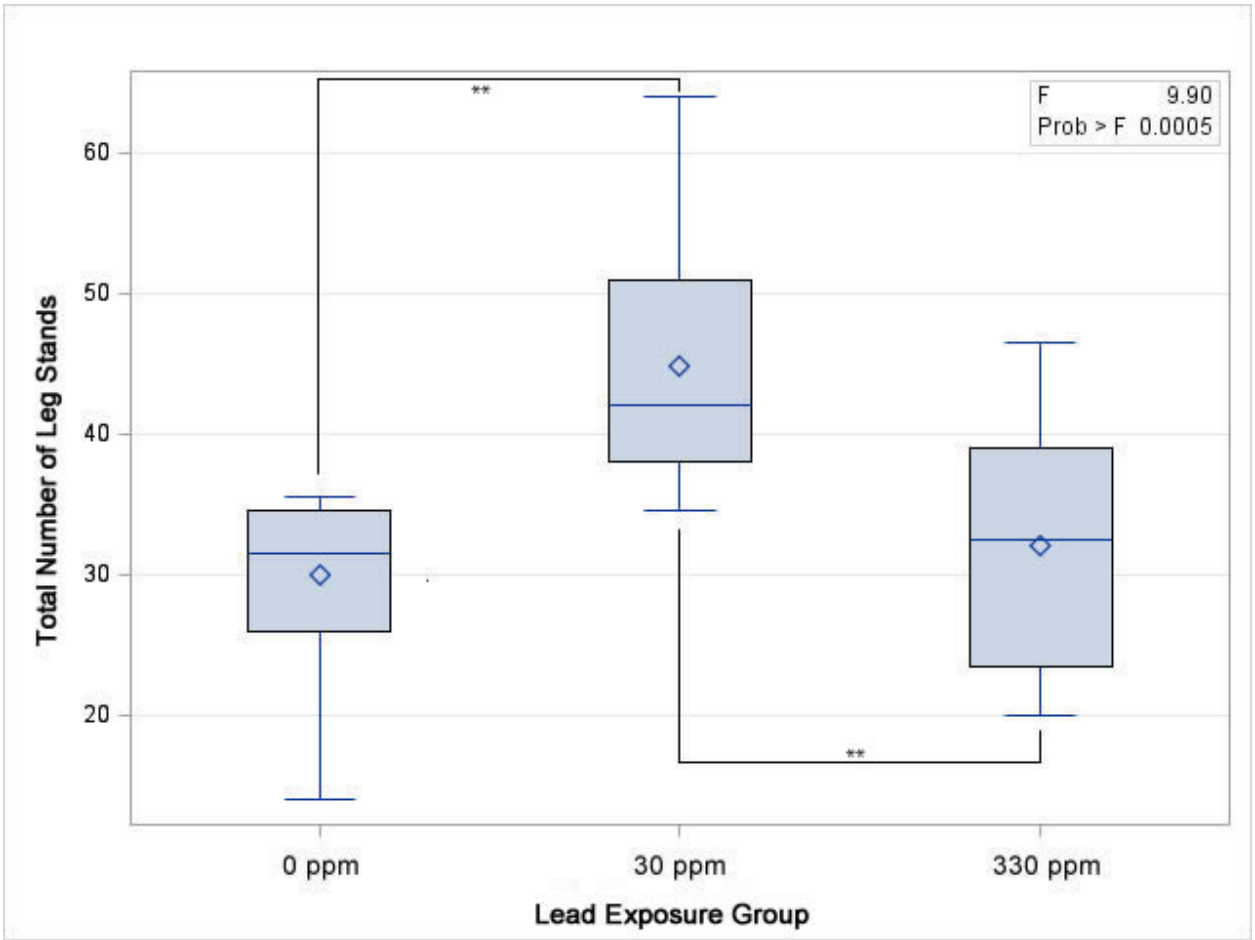
***p* < .05; ****p* < .01; *****p* < .001

Figure 6. Open Field Task Main Effect of Lead Exposure on the Number of Quadrants Crossed During Min 2



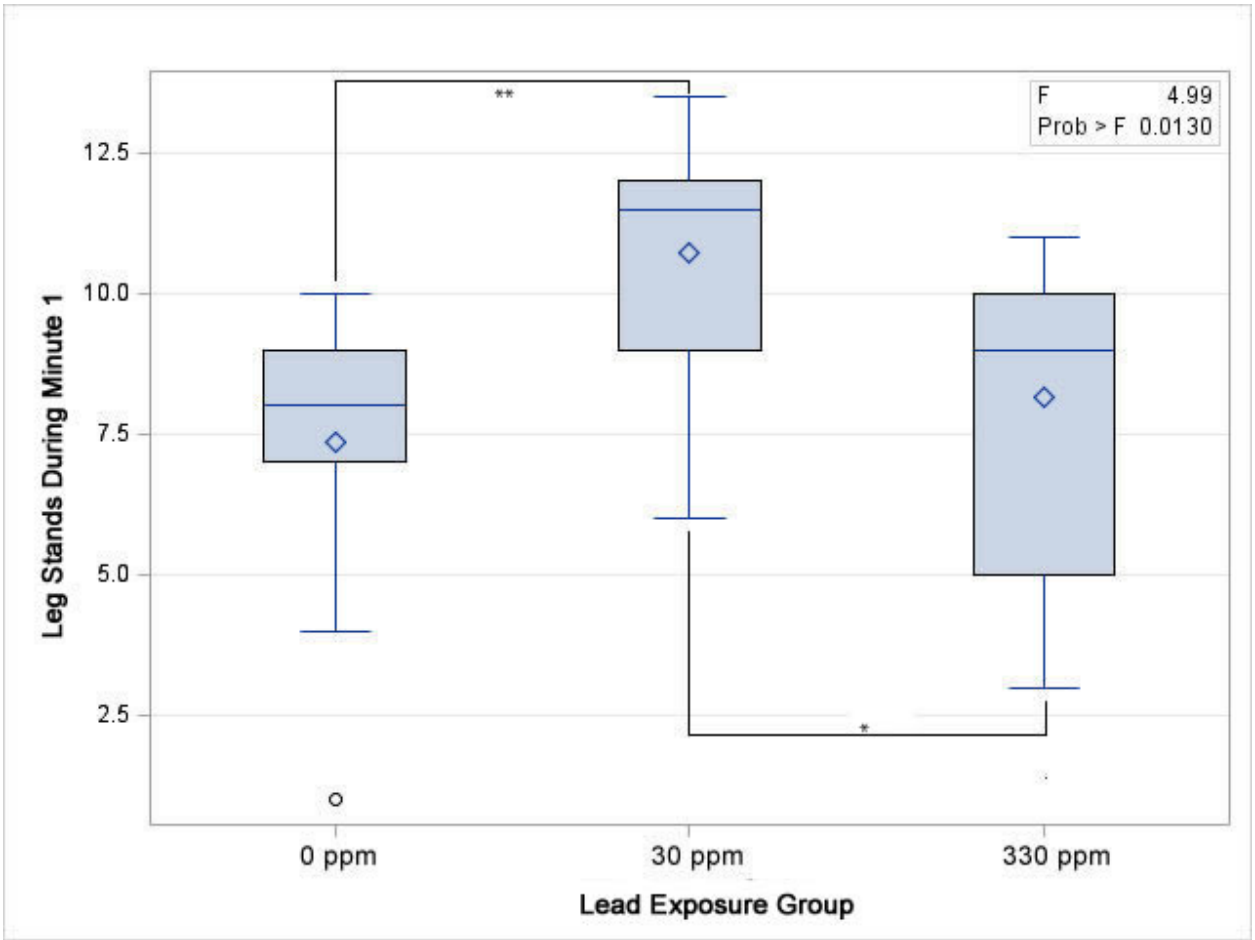
***p* < .05; ****p* < .01; *****p* < .001

Figure 7. Open Field Task Main Effect of Lead Exposure on the Number of Quadrants Crossed During Min 3



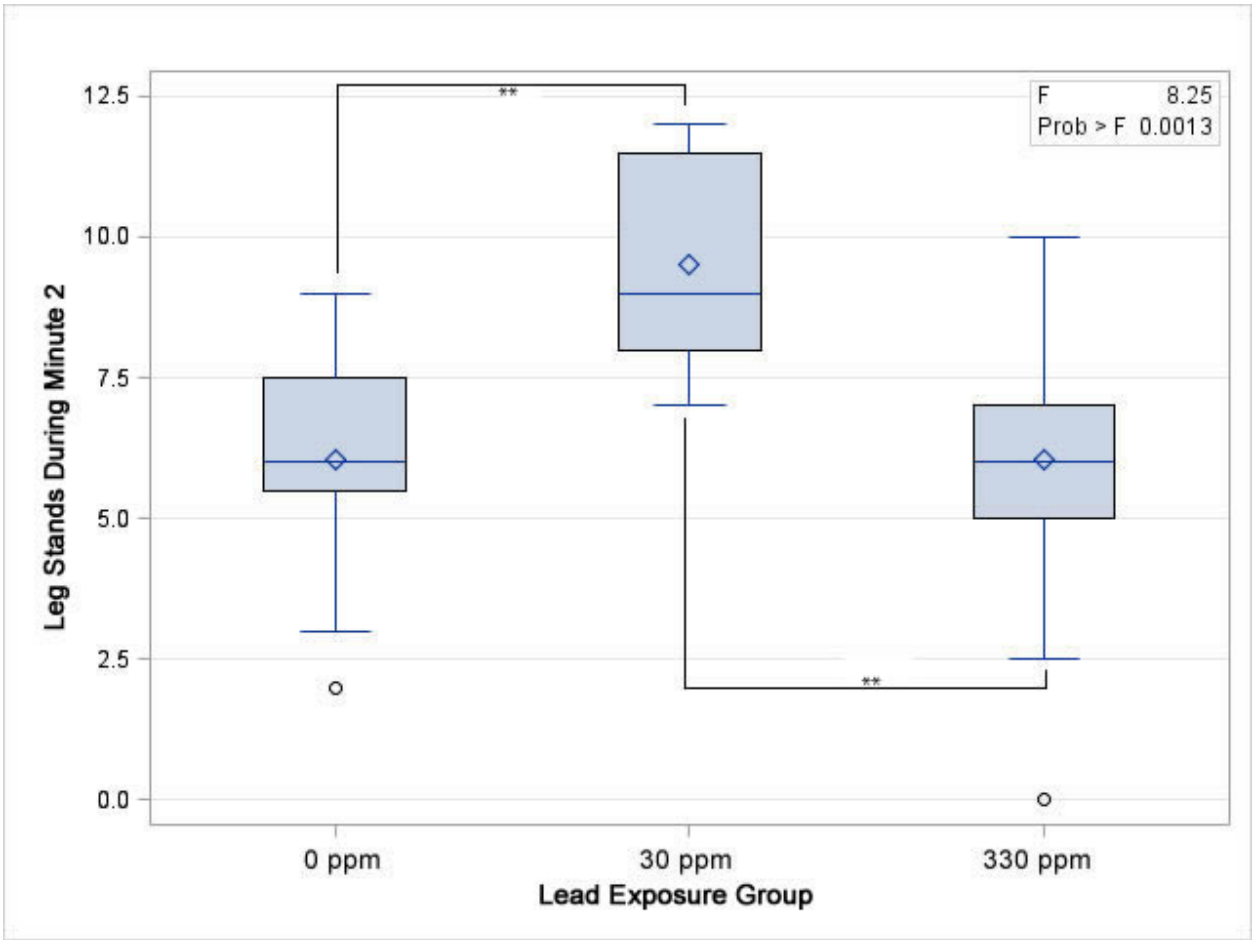
**p* < .05; ***p* < .01; ****p* < .001

Figure 8. Open Field Task Main Effect of Lead Exposure on the Total Number of Leg Stands



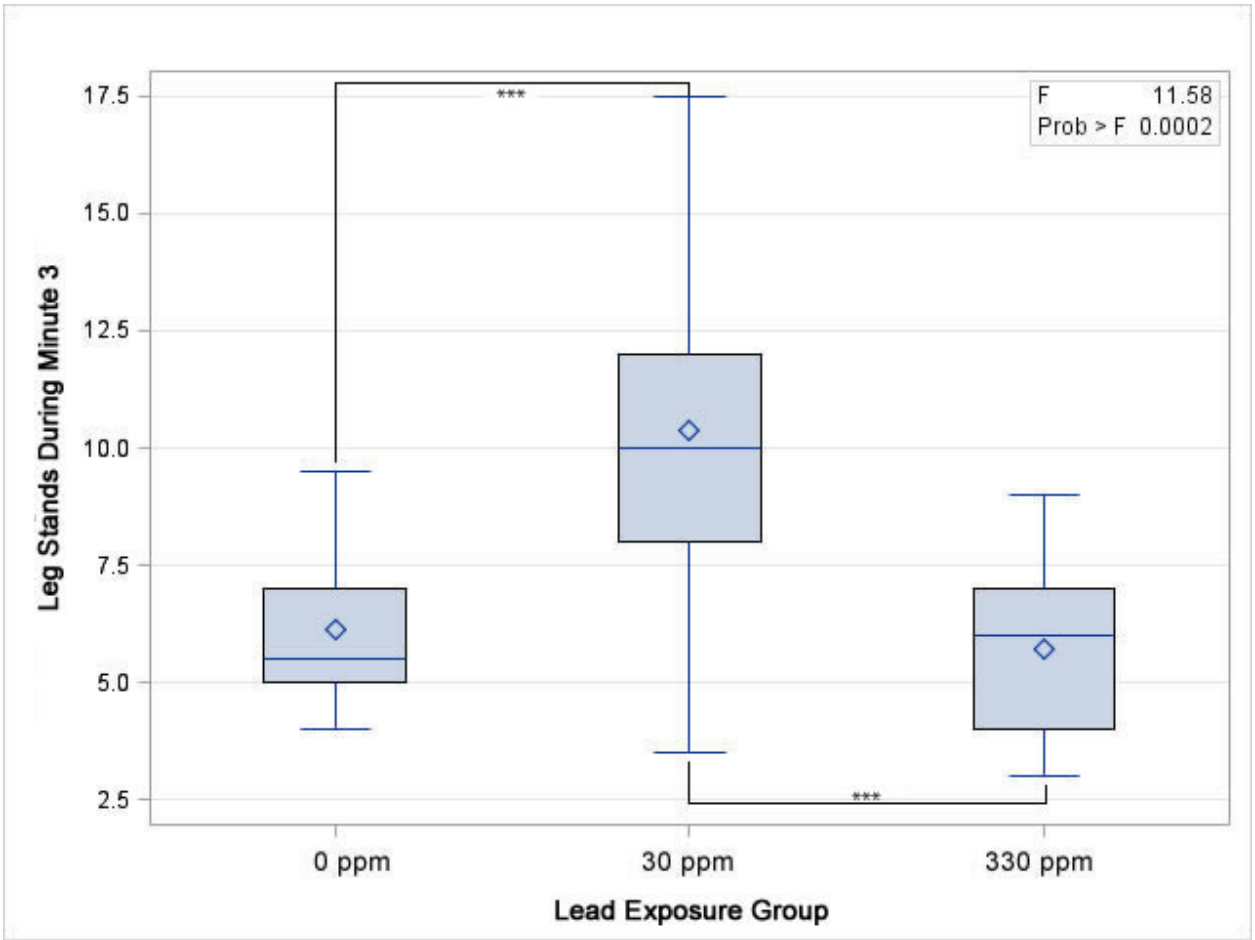
***p* < .05; ****p* < .01; *****p* < .001

Figure 9. Open Field Task Main Effect of Lead Exposure on the Number of Leg Stands During Min 1



**p* < .05; ***p* < .01; ****p* < .001

Figure 10. Open Field Task Main Effect of Lead Exposure on the Number of Leg Stands During Min 2



***p* < .05; ****p* < .01; *****p* < .001

Figure 11. Open Field Task Main Effect of Lead Exposure on the Number of Leg Stands During Min 3

CURRICULUM VITA

Mayra Gisel Flores Montoya was born in Chihuahua, Mexico. The second daughter of Rosa Maria Montoya Chavez and Victor Manuel Flores Hernandez, she graduated from the High School COBACH 3, Chihuahua, Mexico, in the fall of 2005 and entered The University of Texas at El Paso in the fall 2006, with the Chihuahua-UTEP Scholarship. While pursuing a Bachelor's Degree in Psychology at the University of Texas at El Paso she worked in the Laboratory of Neurocognitive Genetics and Developmental Neurocognition, under the mentorship of Dr. Christina Sobin, laboratory head. In the fall of 2011, she was accepted into the Experimental Psychology M.A. Program at the University of Texas at El Paso and continued working in the Laboratory of Neurocognitive Genetics and Developmental Neurocognition. She completed her Master's degree in May 2013 and is second author on the manuscript entitled "*Microglial Disruption in Young Mice with Early Chronic Exposure to Lead"* published in 2013 in the *Toxicology Letters*. In spring 2013, she was accepted into the doctoral program in Psychology, with sub-specialization in Social/Cognitive/Neuroscience at the University of Texas at El Paso. She will continue her investigation of memory impairment in mouse models of early chronic low-level lead exposure.

Permanent Address: 3500 Sun Bowl, apt 62

El Paso, Texas 79902

Or

mgflores3@miners.utep.edu