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A Behavioral and Voltammetric Study of the Effects of Pb Exposure in Mice as a Model of Autism Spectrum Disorder

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A Behavioral and Voltammetric Study of the Effects of Pb Exposure in Mice as a Model of Autism Spectrum Disorder

by

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DEDICATION

I dedicate this thesis to my late grandfathers, great men that taught me the value of hard work, I miss you both every day.

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To Dr. Parastoo Hashemi, for being the best mentor and providing me with amazing opportunities to grow as a person and young scientist.

To the Hashemi Lab, for your unwavering friendship and guidance, I could not have asked for better people to spend this time with.

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To my parents, for your unwavering love and support of my ambitions as well as being the voices of reason on good and bad days. I could not have done any of this without you.

ABSTRACT

Despite being a known toxin for several centuries, lead (Pb^{2+}) is still utilized in commercial products including leaded gasoline, paint, and modern cosmetics. This presence has caused Pb^{2+} to pollute the environment, including public drinking water sources. The recent outbreaks of elevated levels of Pb^{2+} in the drinking water in Flint, Michigan and Washington D.C. have caused large populations to be exposed to the heavy metal for months at a time, raising a public health concern over the implications of widespread, long-term Pb^{2+} exposure. Young children are most susceptible to the effects of Pb^{2+} toxicity, exhibiting symptoms of cognitive and behavioral impairments. These symptoms are closely related to those exhibited by autism spectrum disorder (ASD) patients. ASD is of unknown etiology but is thought to be caused by a mixture of genetic factors and environmental toxins. ASD is also linked to abnormal serotonin functionality due to a large population being treated with selective serotonin reuptake inhibitors (SSRIs) to decrease anxiolytic behaviors. Serotonin (5-HT) is a neurotransmitter heavily involved in development, mood, and sleep. Despite the connection between ASD-like symptoms and Pb^{2+} exposure, there have been no studies chronicling the effects of Pb^{2+} exposure on the serotonergic system *in vivo.* We hypothesize that serotonergic neurotransmission will be altered as a direct result of Pb exposure. *In vivo* measurements require high temporal resolution, selectivity, and sensitivity; all achieved with fast-scan

cyclic voltammetry (FSCV) and microelectrodes. Due to Pb^{2+} exposure greatly affecting and serotonin's critical role in proper development, the medial prefrontal cortex (mPFC) was chosen due to its dense population of serotonin neurons as well as being the last region to fully develop. In this study, mature mice were exposed to Pb^{2+} in three experimental paradigms: acute, chronic, and perinatal studies. Behavioral tests were performed on the perinatal cohort prior to voltammetric measurements to observe any behavioral alterations as a result of Pb consumption. It was found that Pb^{2+} affected the amplitude, but not the reuptake of evoked 5-HT in the acute cohort. There were no significant changes in voltammetric measurements nor behaviors after chronic and perinatal exposures. This work has shown that acute Pb^{2+} exposure does alter serotonergic neurotransmission in the mPFC, but symptoms were not observed after long-term exposure to 15 ppb Pb^{2+}.

PREFACE

This Thesis is based on the following refereed publication:

Parke, B., West, A., Tavakoli, N., Hashemi, P. “A Behavioral and Voltammetric Study of the Effects of Pb Exposure in Mice as a Model of Autism Spectrum Disorder” *In Preparation*

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LIST OF ABBREVIATIONS

ASD ..... Autism Spectrum Disorder

BBB..... Blood-brain Barrier

Ca^{2+} ..... Calcium

CFM ..... Carbon Fiber Microelectrodes

DATs..... Dopamine Transporters

EPA..... Environmental Protection Agency

FSCV ..... Fast Scan Cyclic Voltammetry

mPFC..... medial Prefrontal Cortex

NETs..... Norepinephrine Transporters

OCTs ..... Organic Cation Transporters

Pb^{2+}..... Lead

SERTs ..... Serotonin Transporters

SSRIs..... Serotonin Selective Reuptake Inhibitors

5-HT..... Serotonin

CHAPTER 1

A BEHAVIORAL AND VOLTAMMETRIC STUDY OF THE EFFECTS OF PB EXPOSURE IN MICE AS A MODEL OF AUTISM SPECTRUM DISORDER

1.1 Introduction

Lead (Pb^{2+}) has been a well-documented toxin since the 2nd century B.C. but has been heavily present in commercial products such as leaded gasoline and paint up until the mid-20th century and is currently present in modern cosmetics[1, 2]. Its commercial use has polluted topsoil and drinking water sources with Pb^{2+}[3]. Recently, several public water systems have struggled to keep Pb^{2+} levels in drinking water at the 15-ppb limit set forth by the Environmental Protection Agency (EPA), most notably: Flint, Michigan and Washington D.C[4, 5]. These large populations have been chronically exposed to Pb^{2+} at elevated levels for months at a time. As a result, these outbreaks have raised public health concern for the short and long-term health effects of Pb^{2+} exposure.

The effects of Pb^{2+} on the body are well-known[2]. Pb^{2+} can mimic Fe^{2+} in red blood cells, causing anemia[6]. This can subside with the administration of a chelating agent, but not completely. When chronically consumed, Pb^{2+} gets stored in bones and teeth, which can leech into the bloodstream over time, prolonging the effects of Pb^{2+} exposure after treatment. Additionally, Pb^{2+} readily crosses the blood-brain barrier (BBB)[7]. In the brain, Pb^{2+} has been shown to

mimic and compete with Ca^{2+} during action potentials, disrupting neurotransmission[8]. It has been shown that this phenomenon can increase normal firing of neurotransmitters and decrease evoked signals[9]. Altering homeostatic neurotransmission can lead to unknown, downstream effects that could manifest as observed behavioral changes.

There is ample evidence that developing brains are more susceptible to the effects of Pb^{2+} exposure than adults[10-12]. Before the age of seven, synapses and learning pathways are forged in the brain and disrupting this process causes prolonged long-term effects[13]. Children exposed to Pb^{2+} suffer from cognitive and behavioral deficits including altered social skills, aggressive behaviors, hyperactivity, and low IQ's. These behaviors are like those exhibited by patients with autism spectrum disorder (ASD), forging a possible connection. ASD is of unknown etiology but is thought to be caused by a combination of genetic factors and environmental toxicity[14]. ASD is characterized by social and learning impairments and developmental delays. A concrete connection was made between the presentation of ASD behaviors and Pb^{2+} exposure by Yassa et al., where children diagnosed with ASD and elevated blood Pb^{2+} levels were administered a chelating agent[15]. This treatment resulted in significant decreases in blood Pb^{2+} levels as well as ASD behaviors. A large population of ASD patients exhibit anxiolytic behaviors, which are efficaciously treated by selective serotonin reuptake inhibitors (SSRIs)[16]. Serotonin (5-HT) is a neuromodulator responsible for regulating sleep and mood, amongst others, but is mostly known for its implication in neuropsychiatric diseases: depression and anxiety[17].

Additionally, approximately 30% of ASD patients exhibit elevated blood serotonin levels, further implicating the neurotransmitter in ASD[18]. The connection between ASD and Pb^{2+} exposure is clear, however there have been no studies regarding serotonergic disruptions. Therefore, we hypothesize that serotonergic neurotransmission is disrupted as a result of Pb^{2+} exposure.

The common cognitive and behavioral symptoms of Pb^{2+} exposure and ASD indicates the possibility of serotonergic neurotransmission is altered as a result of Pb^{2+} exposure. Pb^{2+} causes an inflammatory response, resulting in neuroinflammation. Samaranayake et al. has shown that neuroinflammation increases histamine levels in the brain while concurrently suppressing serotonin levels[19, 20]. There is currently no literature describing alterations to serotonin neurotransmission *in vivo*, but an *in vitro* study has shown that Pb^{2+} has a high affinity to bind to the 5-HT_{2A} autoreceptor[21]. 5-HT_{2A} controls the overall level of serotonin in the synaptic space and is associated with learning and memory functions in the mPFC[22]. Additionally, the mPFC is one of the last brain regions to fully develop and is densely populated with serotonin neurons, making it the ideal region to observe the effects of Pb^{2+} exposure in terms of developmental disruptions[13]. Since neurotransmission occurs on a sub-second time scale, a method to observe discrete changes as a result of Pb^{2+} exposure must have high temporal resolution. Additionally, *in vivo* methods require selectivity and sensitivity in a complex matrix. One such method, fast scan cyclic voltammetry (FSCV), embodies the desired attributes for *in vivo* observation utilizing

microelectrodes. In this study, FSCV will be used to observe changes in serotonin neurochemistry in the mPFC as a result of Pb^{2+} exposure.

It is important to note that West et al. has previously determined that there are two types of serotonin neurons that project to the mPFC[23]. The two types of serotonin neurons are distinguished by the types of transporters expressed to take up 5-HT from the synapse. The first type expresses dopamine, norepinephrine, and organic cation transporters (DATs, NETs, and OCTs, respectively), henceforth referred to as “non-SERTs”. These transporters take up 5-HT non-specifically, therefore quickly, resulting in a single reuptake event, shown in figure 1A. The second population of serotonin neurons express serotonin transporters (SERTs). SERTs have a high affinity for serotonin, therefore they reuptake 5-HT slower than the non-SERTs. The first population of serotonin neurons are heavily present throughout all layers of the mPFC, but the second population are more densely populated in layers 5-6, shown in figure 1C. Both populations of serotonin neurons being present in layers 5-6 and taking up serotonin at two different rates results in a double peak, shown in figure 1B, where the first reuptake is due to the non-SERTs and the second reuptake by SERTs. This information is important in studies, like this, that report FSCV measurements taken in the mPFC.

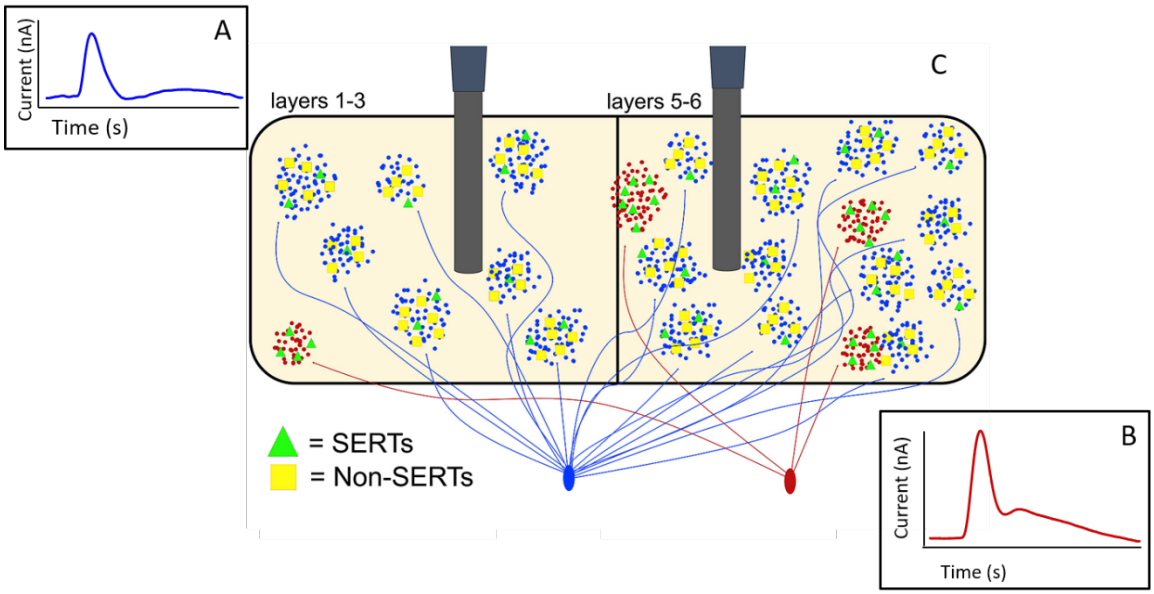

Figure 1.1 Serotonergic Projections to the mPFC: this figure is a representation of the two types of serotonergic neurons projecting into the different layers of the mPFC, reported by West et al. [23].

Overall, this study aims to determine the effects of Pb^{2+} exposure on the serotonergic system in the mPFC *in vivo*. An acute exposure paradigm was employed in mature mice to determine short-term effects on serotonin neurotransmission in real-time. Long-term Pb^{2+} exposures were mimicked with one-week chronic and perinatal exposures to determine the effects on both behavior and serotonin neurochemistry.

1.2 Methodology

1.2.1 Study Design

The Guide for the Care and Use of Laboratory Animals, as accepted by the Institutional Animal Care and Use Committees of the University of South Carolina (Institution Approval # A3049-01), was followed in all animal care and procedures. C57BL/6 male and female mice were purchased from Jackson

Laboratories (Bar Harbor, ME) at 6 weeks old. Mice were group housed on a 12-hour light/dark cycle at the University of South Carolina and had access to food and water *ad libitum*. Three separate exposure paradigms: acute, chronic, and perinatal to Pb^{2+} were followed. For the acute exposure, mice were chosen at random between 7-10 weeks old, with no regard for the estrous cycle of female mice. These mice underwent stereotaxic surgery and FSCV was utilized to examine serotonin neurochemistry. After control files of evoked 5-HT were taken, animals were given an injection of 1.5, 15, or 150 ppb lead acetate in saline (5 mL/kg) (Sigma Aldrich) intraperitoneally. Serotonin neurochemistry was observed for 60 minutes after the acute injection. The chronic cohort of mice were exposed to either normal water from the animal care center, filtered with a reverse osmosis filter, or water from the animal care center containing 15 ppb lead acetate (Sigma Aldrich). These mice were exposed to Pb^{2+} for 7 days total, then were given Pb^{2+}-free water to drink and stereotaxic surgeries were performed within a week. As for the perinatal cohort of mice, two adult breeders were housed together with drinking water containing 15 ppb Pb^{2+} acetate, as previously described. They were exposed to this drinking water until their pups were weaned into groups at 21 days old. Both the breeders and pups were then given normal drinking water from the animal care center. Behavioral tests were administered to the pups at 8 weeks old and stereotaxic surgeries followed within 1-2 weeks.

1.2.1.1 Behavioral Tests

The three-chamber sociability, marble burying, and olfactory habituation/dishabituation tests were chosen to examine whether the mice perinatally exposed to Pb^{2+} exhibited ASD-like behaviors. The elevated zero maze test was performed to determine additional anxiety caused by the other tests.

1.2.1.2 Three-Chamber Sociability Test

A custom built three chamber apparatus was utilized with dimensions according to commercially available equivalents from Ugo Basile. The cage cups used were purchased from Ugo Basile. The perinatal and chronic cohorts were given this test. Each mouse was chosen at random to be the test subject for each trial. In a three-chamber enclosure, the mouse was restricted to the center chamber to habituate for 5 minutes, then allowed to roam in any chamber for 10 minutes. After which, a familiar or novel mouse was put into a cage cup in either the left or the right chamber, which alternated with each test subject. The test subject could roam with the doors open for 10 minutes with a familiar mouse and 10 minutes with a novel mouse.

1.2.1.3 Olfactory Habituation/Dishabituation

For the olfactory habituation/dishabituation test, the mice were singly housed without food or water for the 45-minute habituation period and the test itself. The mice were exposed to 5 scents: 18.2 MΩ Millipore water, two non-social odors, almond and orange extract, and two social odors. The non-social odor solutions were prepared by adding 100 µL of almond extract to 10 mL of Millipore water,

and was repeated for the orange extract solution. When preparing the cotton swabs for testing, three swabs were prepared for each odor per mouse. Each swab received 50 µL of odorant and was stored in an Erlenmeyer flask and covered with parafilm. The social odors were prepared by swabbing the bottom of two cages of mice of the same sex, which hadn't been cleaned for a minimum of two days. The mice were exposed to each odor for two minutes and dishabituated for one minute. This was repeated three times per odor beginning with water, then the non-social odors, finishing with the social odors. Noldus Ethovision was used to record and score the time spent sniffing the novel and familiar mice. A t-test was performed to determine the statistical significance of time spent with the novel vs. familiar mouse as well as time sniffing the mouse in the chamber cage vs. being away from it.

1.2.1.4 Marble Burying Test

For the marble burying test, biofresh comfort bedding was used because the cellulose fibers promote burying. Each mouse was singly housed without water or food access for the duration of the habitation and test. A ten-minute habituation period was allowed prior to the introduction of 12 marbles. The marbles were placed in a grid pattern while the mouse was temporarily removed from the cage. The mice were returned to the cage with marbles in it for a ten-minute test. After which, the marbles were scored as buried, partially buried, or not buried. Partially buried marbles were determined as visible but had bedding on top of it. This test was hand-scored by two researchers, blind to the identity of

each mouse. The two cohorts were averaged, and the groups were compared with a t-test.

1.2.1.5 Observation of Repetitive Behaviors

Mice were singly housed during this test without access to food or water. They were given a fifteen-minute habituation period in a clean cage and the repetitive behaviors of grooming, rearing, climbing, and digging were observed and scored by hand for fifteen minutes. The researchers were blind to the identity of each mouse prior to data analysis. The two groups, exposed and control mice, were averaged and the groups compared with a t-test.

1.2.1.6 Elevated Zero Maze Test

The elevated zero maze behavioral test was performed to test for anxiolytic behaviors that could have affected the outcome of other behavioral tests. An elevated zero apparatus was used from Maze Engineers (Boston, MA). The mice were placed at one of the four points where the raised edges meet the open maze, chosen at random. Their behavior was observed for 5 minutes after being placed onto the maze. Noldus ethovision was used to record and score the test. The time spent in open vs closed arms was scored and compared using a t-test.

1.2.2 Microelectrode Fabrication

Carbon fiber microelectrodes (CFMs) were made in house by aspirating carbon fibers into glass capillaries and pulled to a tight seal with a glass pipette puller. Exposed carbon fibers were cut to 150 micrometers for serotonin measurements. An electrical connection was forged by inserting a wire coated

with silver colloidal paint into the glass capillary. Finally, a thin layer of Nafion was electropolymerized onto the carbon surface and cured in a 70 ºC oven for 10 minutes prior to use *in vivo*. This process has been extensively described by Hashemi et al. [24].

1.2.3 Animal Surgeries

All animal procedures were performed in agreement with IACUC guidelines at the University of South Carolina, accredited by AAALAC. Male and female C57BL/6 mice (Jackson Laboratories, Bar Harbor, ME, USA) aged 7-12 weeks and weighing between 17.0 – 30.0 grams were used. Mice were chosen at random, with no regard for the female estrous cycle, and injected with a 25% w/v solution of urethane intraperitoneally to induce and maintain anesthesia. Body temperature was maintained at 37 ºC with a heating pad (BrainTree Scientific). Stereotaxic coordinates for the mPFC were taken in reference to bregma. A Nafion-coated CFM was lowered into the mPFC (+1.70 AP, -0.20 ML, -2.00 DV) for serotonin measurements. A stainless-steel stimulation electrode (SE) was lowered into the medial forebrain bundle (MFB) (-1.58 AP, -1.00 ML, -4.80 DV). A pseudo Ag/AgCl reference electrode, made by electroplating AgCl onto a Ag wire, was placed in a contralateral hemisphere.

1.2.4 Fast-scan Cyclic Voltammetry (FSCV)

The serotonin waveform is generated by a PCIe-6341 DAC/ACD card (National Instruments) and scanned at 1000 Vs^{-1} from 0.2 V to 1.0 V to -0.1 V and back to the resting potential of 0.2 V at a frequency of 10 Hz using a potentiostat (Dagan Corporation). In order to find a stable serotonin signal [in vivo]

the working electrode will be moved in the dorsal-ventral plane until a classic serotonin signal is found in the mPFC. A minimum of four control files are taken prior to any pharmaceutical or toxin exposure to observe the baseline of evoked serotonin for each animal. An acute dose of lead acetate (Sigma Aldrich) was given to a cohort of healthy, unexposed mice at a dose of 1.5, 15, or 150 ppb Pb^{2+}. Acute effects of Pb^{2+} on the serotonin system were observed at 10-minute increments for 60 minutes. A cohort of mice chronically exposed to 15 ppb of Pb^{2+} acetate in their drinking water was given 10 mg/kg of escitalopram (ESCIT) after control files were taken and the effect of the SSRI was observed for 60 minutes at 10-minute increments.

1.2.5 Histology

After the surgery is complete, a large voltage of approximately 13 V is applied to the working electrode *in vivo* causing it to break and form a lesion where serotonin measurements were taken. The lesion allows for visualization of the electrode location during histology. The mouse was euthanized by cervical dislocation followed by decapitation, in accordance with IACUC, and the brain was harvested. The harvested brain was stored in paraformaldehyde (PFA) at 0 ºC for a minimum of three days. At least 24 hours prior to slicing the brain, it was transferred to a 30% w/w sucrose solution (Sigma Aldrich) to fill in the capillaries. A cryotome was used to maintain the tissue between -20 ºC and -25 ºC while slicing 30 micrometer thick slices. The slices with the brain regions of interest were transferred to a glass microscope slide and kept for staining and

examination under microscopes to determine the exact placement of the working and stimulation electrodes.

1.3 Results

1.3.1 Acute Exposure to Lead

The baseline responses of evoked serotonin in the mPFC of healthy, mature mice were recorded and then given bolus injections of Pb^{2+} at 1.5, 15, and 150 ppb. The effects of these acute exposures on serotonin neurochemistry were observed for 60 minutes post-injection. Since FSCV signals in the mPFC vary with electrode placement, results were grouped as single and double peak responses to each dose of Pb^{2+}, shown in figures 2 and 3.

1.3.2 Evoked Serotonin in the mPFC layers 1-3

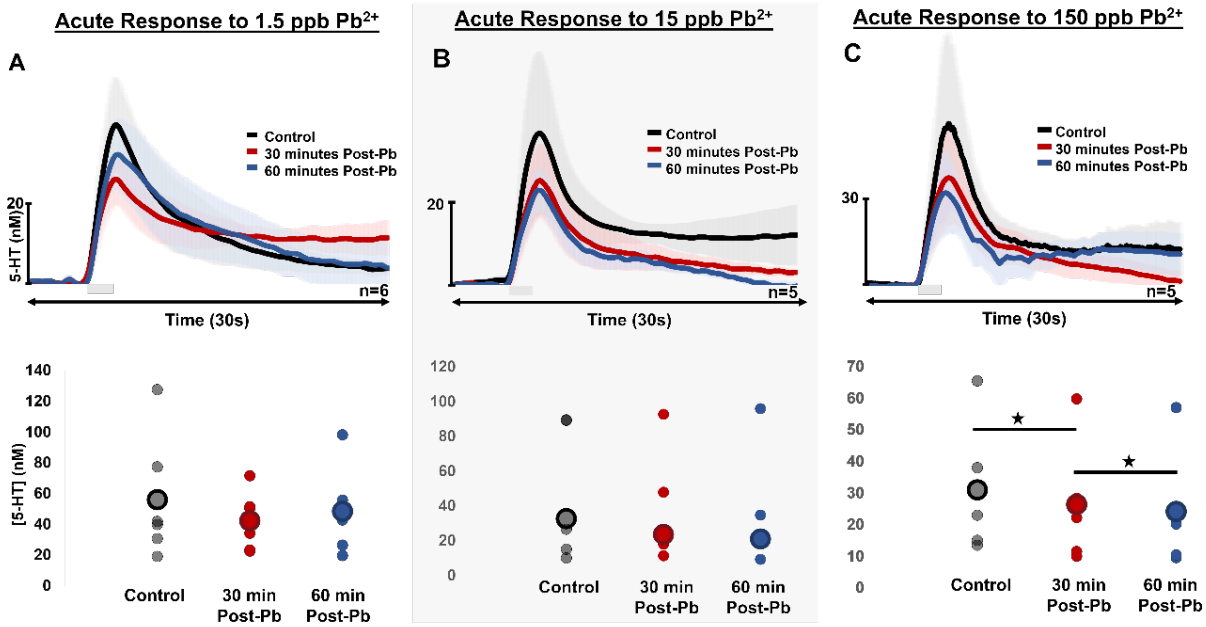

Figure 1.2 Response to an Acute Dose of Pb^{2+} in Layers 1-3 of the mPFC: Evoked serotonin (stimulation represented by the gray bars) release prior to and after acute doses of Pb^{2+} at 1.5, 15, and 150 ppb in layers 1-3 of the mPFC. Each animal was given a bolus of Pb^{2+} after four control files were collected. Row A shows the evoked 5-HT concentration (nM) vs. time plots of each dose. The faded colors of each trace represent the standard error of the mean (SEM), and the sample sizes are below each curve. Row D shows the maximum amplitude reached by the traces in Row A. Here, the largest points are the averages of the animals and the smaller points the individual animals themselves. The largest points are the averages of each animal, represented by the smaller points. Columns A, B, and C represent the responses to 1.5, 15, and 150 ppb Pb^{2+}, respectively.

In layers 1-3 of the mPFC, the acute exposure of 1.5 ppb Pb^{2+} caused evoked 5-HT to slightly decrease after 30 minutes, but almost recover to the control level, figure 2 column A. A decrease in amplitude was also observed after exposure to 15 ppb Pb^{2+} without recovering to control levels, figure 2 column B. These changes in amplitude are not significantly different ($p=0.24$ and 0.35, respectively). Significant decreases in amplitude were observed after a dose of

150 ppb Pb^{2+} and persisted after 60 minutes (p=0.03), shown in figure 2 column C.

Changes in reuptake are quantitatively represented by the time (s) it took each curve to decay by one-half ($t_{1/2}$). There were no significant changes in reuptake mechanisms for any of the dose responses, outlined in table 1, although slightly slower reuptakes were consistent across all doses after 30 and 60 minutes.

Table 1.1 shows the time (s) it took each reuptake curve in figure 2, row A to decay by one-half ($t_{1/2}$) with their respective SEM, with significance conducted via a t-test.

$t_{1/2}$ (s)						
	Control Avg ± SEM (nM)	30 min Post-Pb Avg ± SEM (nM)	60 min Post-Pb Avg ± SEM (nM)	P-value (0,30)	P-value (0,60)	P-value (30,60)
1.5 ppb Pb	11.8 ± 1.3	16.2 ± 3.2	14.6 ± 3.3	0.23	0.24	0.72
15 ppb Pb	11 ± 1.5	12.3 ± 1.9	12.3 ± 1.7	0.17	0.088	0.95
150 ppb Pb	12.9 ± 1.5	13.5 ± 2.1	14.3 ± 2.3	0.48	0.24	0.57

1.3.3 Evoked Serotonin in the mPFC layers 5-6

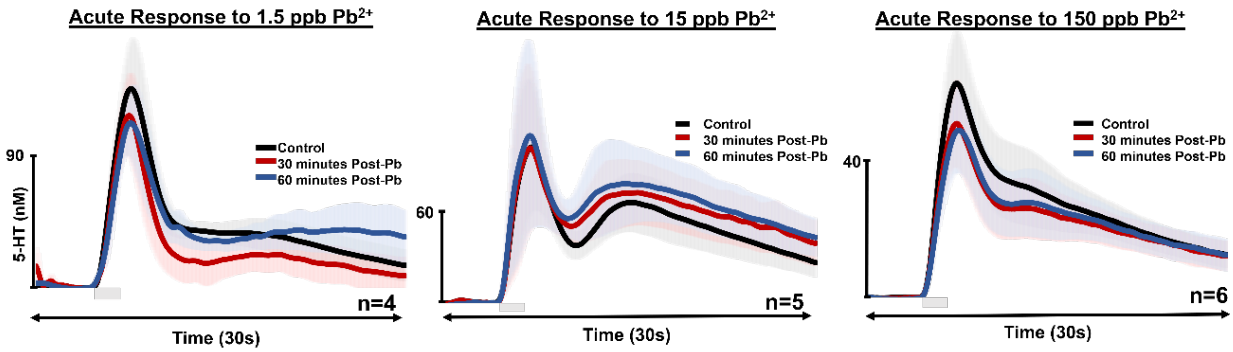

Figure 1.3 Response to an Acute Dose of Pb^{2+} in Layers 5-6 of the mPFC: Evoked 5-HT signals (stimulation represented by the gray bar) in response to an acute dose of either 1.5, 15, or 150 ppb Pb^{2+} in the mPFC layers 5-6. Each animal was given a bolus of Pb^{2+} after four control signals were collected. The concentration (nM) vs. time (s) curves are the average of several animals, denoted at the bottom of each curve, with the faded colors representing the SEM. Significance testing was performed on the amplitude differences via t-tests.

When the working electrode was placed in layers 5-6 of the mPFC, the response to an acute dose of Pb^{2+} was slightly different than when placed in layers 1-3. As previously mentioned, there are two different projections of serotonin neurons in the mPFC that are layer-specific, resulting in two distinct peaks. Shown in figure 3, the amplitude of evoked serotonin for both peaks slightly decreased after acute doses of 1.5 and 150 ppb Pb^{2+}, but the decreases were not statistically significant (p= 0.29 and 0.09 ,respectively). There was no change in the amplitude of the first peak, but a slight increase over 60 minutes was observed in the second peak of the 15 ppb Pb^{2+} dose response, but this was also not statistically significant (p= 0.81).

Changes in reuptake mechanisms for each peak were calculated via a t1/2 value. For each of the first peaks in figure 3, no changes in reuptake were observed. After doses of 1.5 and 15 ppb Pb^{2+}, there were slight delays in reuptake via the SERTs, but not statistically significant (p= and , respectively). No significant change in the second reuptake for the 150 ppb Pb^{2+} dose was observed.

1.3.4 Chronic Exposure to Lead

Mice were exposed to 15 ppb Pb^{2+} in their drinking water for one week while control mice received lead-free drinking water from the Animal Care Center. The evoked serotonin signals are shown in figure 3. The chronic mice had a slightly lower maximum amplitude than the control mice, but this was not significantly different with p=0.5, determined by a t-test. The reuptake of 5-HT by the transporters were compared by calculating the time it took to decay by one-half

($t_{1/2}$). The $t_{1/2}$ values for the control and exposed mice were 9.6 and 9.8 s, respectively. These values were determined to not be significantly different (p=0.7) via a t-test. It is important to note that the evoked serotonin signals for the exposed mice were acquired after exposure, and the control signals for each animal prior to exposure could not be acquired.

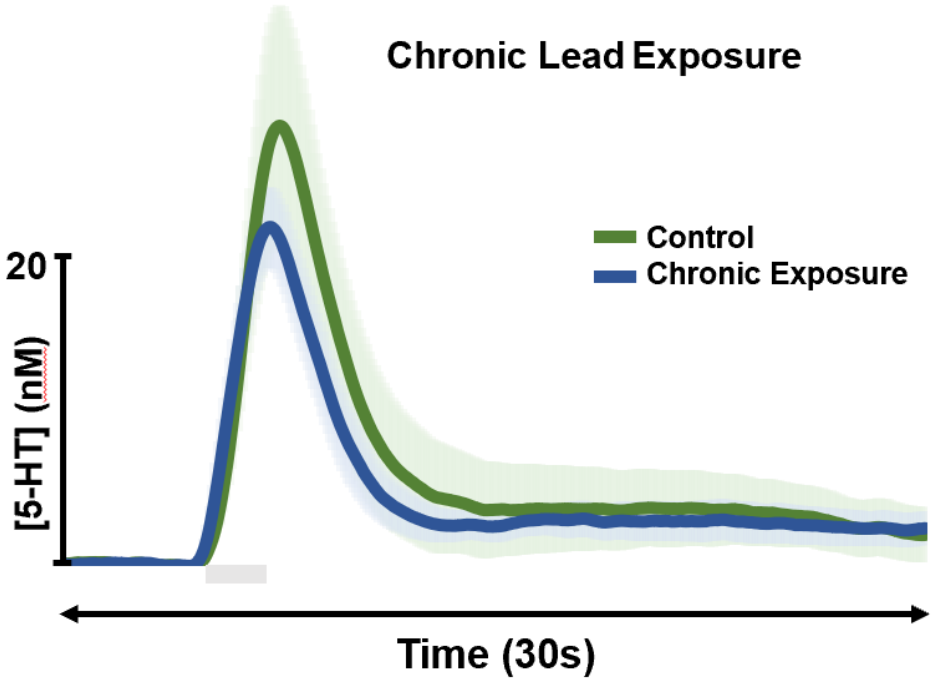

Figure 1.4 Response to a Chronic Exposure to Pb^{2+}: Chronic 1-week exposure to 15 ppb Pb^{2+} in drinking water. The stimulated release of serotonin (stimulation denoted by the gray bar) in control and chronically exposed animals. Control animals are shown in green (n=6), and chronic exposure animals are shown in blue (n=6). There was no significant difference between the maximum amplitudes (p=0.5) nor the reuptake (p=0.7) of 5-HT.

1.3.5 Perinatal Exposure to Lead

At 8 weeks of age, mice that were peritoneally exposed to 15 ppb Pb^{2+} were administered behavioral tests to determine whether the exposure caused observable cognitive and behavioral deficits, like those observed in young

children, shown in figure 5. No significant behavioral changes were observed between the control and exposed mice. Although, the exposed mice climbed significantly less than the controls, determined by a t-test (p=0.002).

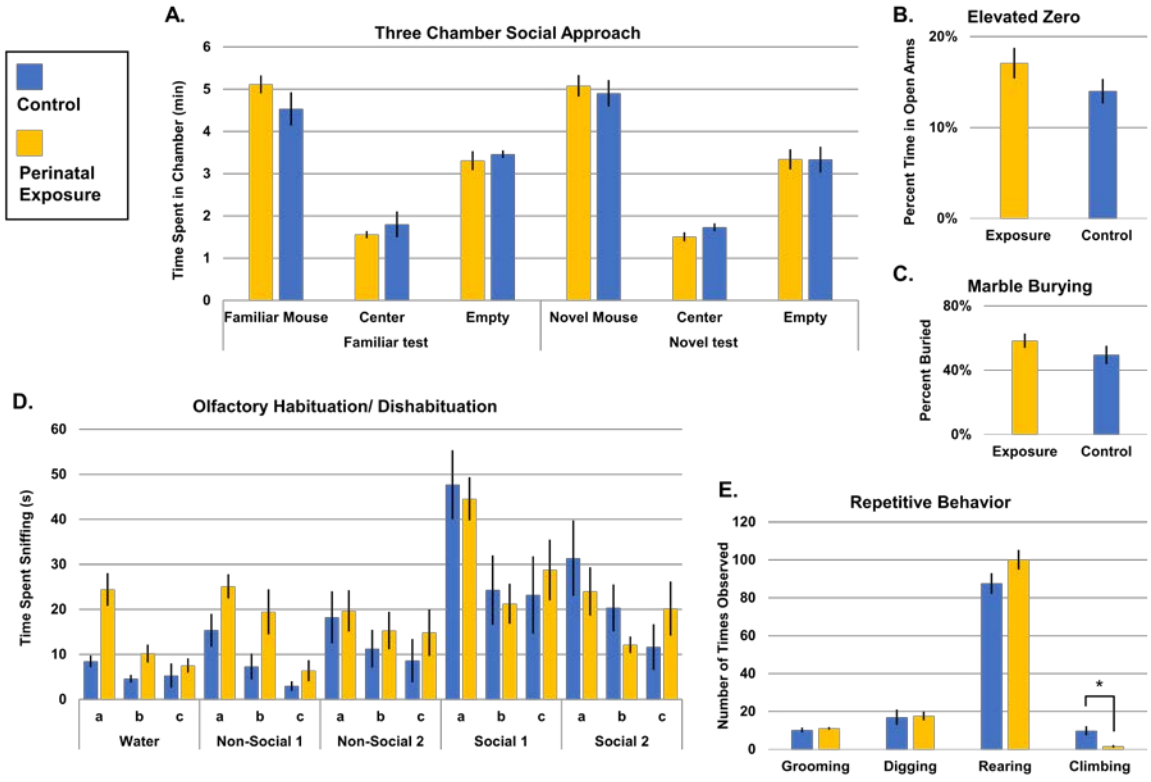

Figure 1.5 Behavioral Tests After a Perinatal Exposure to Pb^{2+}: Behavioral test results between control mice (blue, n=18) and those perinatally exposed to 15 ppb Pb^{2+} (yellow, n=22). A depicts the Crawley Three-Chamber Social test for social deficits. B shows the elevated zero maze test for anxiolytic behaviors. C shows the results of the marble burying test, for repetitive, compulsive behaviors. D depicts the results of the Olfactory Habituation/Dishabituation test with five scents to determine social deficits. E shows the repetitive behaviors observed in the open field test. A t-test determined that the only statistically significant change in behavior was repetitive climbing (p=0.002), denoted by a black star.

After behavioral testing was done, FSCV was performed to observe any changes in serotonin neurochemistry, shown in figure 6. Slightly higher amplitudes of evoked 5-HT were observed in the exposed mice, although this was determined to be statistically insignificant (p=0.3). No statistical change in

reuptake mechanisms were observed between the two groups, quantified by the $t_{1/2}$ values for each curve's decay (p=0.1).

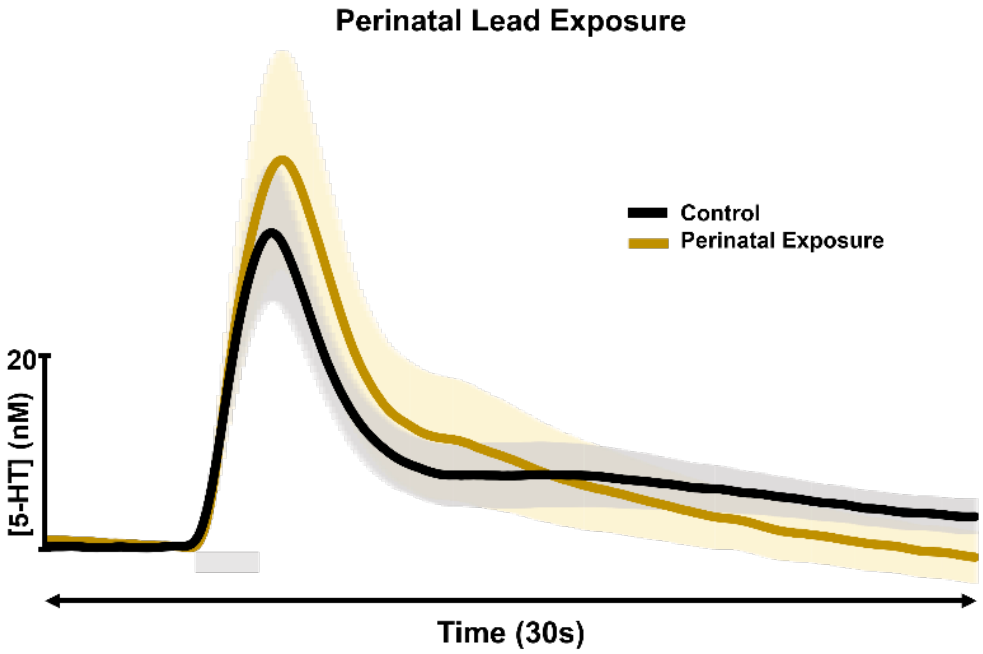

Figure 1.6 Response to a Perinatal Exposure to Pb^{2+}: Evoked serotonin (stimulation denoted by the gray bar) of both control mice (black, n=7) and those perinatally exposed to 15 ppb Pb^{2+} (gold, n=7).

1.4 Discussion

1.4.1 Functional Disruptions of Autoreceptors and Transporters by Pb^{2+}

The acute dose of 150 ppb Pb^{2+} caused significant decreases in the amplitude of evoked 5-HT, without altering reuptake mechanisms of the transporters. All other doses caused decreases in amplitude, albeit insignificant, also without altering reuptake. Disruptions in reuptake are caused by malfunctioning transporters, but since reuptake was not altered, the transporters are not being significantly disturbed by the presence of Pb^{2+}.

Alternatively, the consistent decrease in evoked serotonin after acute Pb^{2+} exposure implicates the autoreceptors and Ca^{2+} competition as the cause of dampening serotonin levels. Pb^{2+} has an affinity to bind to G-coupled protein receptors (GPCRs), which are the type of receptor the 5-HT_{2A} autoreceptors are in the mPFC[21]. 5-HT_{2A} autoreceptors are abundantly expressed in the mPFC, therefore Pb^{2+} could be binding and falsely signaling excessive 5-HT in the synapse, causing the neurons to cease firing. Pb^{2+} also readily competes with and mimics Ca^{2+} function during action potentials, causing an increased or decreased release of 5-HT into the synapse, depending upon the mechanism of disruption by Pb^{2+}[9]. This could add to the fact that serotonin isn't dampened significantly, because firing is varied, causing some animals to have slight increases or significant increases in evoked serotonin levels.

1.4.2 Inflammation caused by Pb^{2+} exposure

Pb^{2+} has been extensively shown to induce neuroinflammation, therefore, indirectly altering serotonin neurochemistry[20]. The administration of Pb^{2+} in brain slices causes glial activation, a hallmark of a neuroinflammatory response. Each animal has their own inflammatory response to Pb^{2+} exposure, which could explain a lack of statistical significance in the evoked serotonin decreases after acute Pb^{2+} exposure.

Our lab has previously shown that evoked serotonin proportionally decreases with increased levels of histamine after pharmacological induction of neuroinflammation, implicating the H3 receptor in the tight regulation of serotonin[19]. Since Pb^{2+} easily crosses the blood brain barrier, inducing

neuroinflammation, this could explain the dampening of evoked serotonin after an acute dose of Pb^{2+}. It seems that the brain was able to return to serotonergic homeostasis at the lowest doses, but not at the highest, alluding to an inflammatory response that is both animal and dose dependent.

1.4.3 Chronic and Perinatal Exposure of Pb^{2+}

No statistically significant alterations in the evoked release nor reuptake of serotonin were found in either the chronic and perinatal cohorts. With this method of exposure, a limitation becomes apparent in the voltammetry. Each animal has their own baseline of evoked serotonin, but this cannot be determined prior to Pb^{2+} exposure. Additionally, the location of the electrode cannot be precisely replicated in each animal, therefore a control level could not be determined. No drastic difference was observed between the exposed and control animals, but the levels of evoked serotonin were comparable, so the conclusion can be drawn that there were no alterations to serotonin neurotransmission.

Based on the results of the acute exposure paradigm, we expected the long-term exposure to cause more dramatic alterations in serotonin neurochemistry. However, this is logically explained by the body's tight regulation of serotonin neurochemistry, resulting in a compensation to maintain homeostasis after being exposed to a toxin over a long period of time. Additionally, the dose of 15 ppb Pb^{2+} was much lower than previously reported studies that caused behavioral changes, therefore the body's compensation mechanisms were able to overcome the chronic exposure and retain normal behaviors. As a major conclusion, a long-

term exposure of 15 ppb Pb^{2+} is not enough to cause significant behavioral and neurochemical changes in mature mice.

1.5 Conclusion

The administration of an acute dose of Pb^{2+} caused evoked serotonin signals to decrease in the mPFC of mature mice, but not all doses caused significant decreases. This could be due to two known mechanisms of Pb^{2+} in the brain: binding to serotonin autoreceptors and competing or mimicking Ca^{2+}. The lack of statistical significance could also be attributed to Pb^{2+} being able to increase or decrease serotonin signaling, resulting in variable responses by each animal. Overall, low doses of Pb^{2+} can be quickly recovered from by the body as shown by the recovery to control levels in the 1.5 ppb dose.

Although no statistical significance was observed between control animals and those exposed to Pb^{2+} chronically and perinatally, this highlights a limitation with FSCV. A baseline of evoked serotonin for each animal is imperative to determine the effects of exposure to a toxin. As the animals in this study were exposed to Pb^{2+} prior to voltammetric measurements, the baseline was not able to be determined. These results could also indicate that chronic exposure low enough doses of Pb^{2+} does not significantly impact serotonin neurochemistry. Chronic exposures to higher doses of Pb^{2+} for longer periods of time could be useful to determine a minimum time frame and dose that will definitively cause long-term damage.

The serotonergic system seems to be well-defended against the effects of Pb^{2+} exposure. In the cases of children exposed to high levels of Pb^{2+} in drinking

water for several months, like those in Washington D.C. and Flint, Michigan, the long-term effects of exposure could be significantly disrupting other signaling pathways in the brain to cause behavioral changes.

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