The Effects of Lead Exposure During Early Development on Fentanyl Addictive Behavior in Mice

(2022-2023 URA Application Materials)

Introduction

Lead exposure is a critical concern in public health because lead (Pb) is a neurodegenerative chemical that affects healthy neuron firing, resulting in many physical and mental disabilities including ADHD, Alzheimer's, and Parkinson's disease (Bressler, 2002; Hong, 2015; Liu, 2013). People most at risk for Pb exposure typically have disadvantaged Social Determinants of Health (SDOH), which are the societal conditions in one's environment that affect health (Office of Disease Prevention and Health Promotion, n.d.; Bellinger, 2008). Examples of SDOH that may affect Pb exposure are income, race, and location. These three SDOH are a matrix and can be traced back to the history of redlining, in which POC applying for home loans were racially discriminated against by the Federal Housing Administration. From redlining, the racial wealth gap and segregation of POC into older, inner cities occurred. In these cities, Pb pipelines deliver drinking water and Pb paint covers walls, as Pb was a popular building material in the early to mid-1900s. This matrix can be seen in the physical overlapping maps of redlining in Cleveland, Ohio (*Image 1* in appendix) and elevated blood lead levels (BLL) of children in Cleveland, Ohio (*Image 2* in appendix) (Kirwan Institute, 2017; Cuyahoga County Board of Health, 2022). These patterns are not unique to Cleveland but are reflected throughout the country.

Substance use disorders (SUDs), which are cognitive, behavioral, and physiological symptoms where one continues to use a substance despite significant substance-related consequences, are also critical concerns in public health with over 932,000 deaths from a drug overdose since 1999 (Center for Disease Control and Prevention, 2022). As 75% of drug overdose deaths involve opioids, the opioid epidemic is a current issue that public health departments are addressing (Center for Disease Control and Prevention, 2022). Fentanyl, a synthetic opioid, is 50 times stronger than heroin, 100 times stronger than morphine, and is one of the leading causes of overdose with over 150 deaths occurring every day (Center for Disease Control and Prevention, 2022). Similar to Pb exposure, people most at risk for SUDs have disadvantaged SDOH (Hansen, 2022). Evidence shows that SUDs and learning disabilities like ADHD are comorbid (Wilens, 2007). Because of underlying neurological effects of Pb on learning and neuron firing, which is seen in consequential developments of learning disabilities, could this comorbidity of ADHD and SUDs be determined, in part, by Pb exposure?

Current research suggests that there are effects of Pb on SUDs. Female injection heroin users were shown to have 1.8 times higher tibial Pb concentrations, which is the concentration of Pb in bone where Pb accumulates over time, than age-adjusted community-dwelling females (Fishbein, 2008). While this data shows some relationship between Pb and SUDs, the investigators did not administer drugs or Pb and the findings thus cannot be used to determine a causal relationship. In a study with male and female rats, chronic developmental Pb exposure was shown to increase μ-opioid receptor (MOR) levels in brain regions that are associated with addiction (Albores-Garcia 2021). This data supports the notion that Pb alters the same circuits and neurochemicals that are targeted by drugs of abuse. In a study with male and female mice, developmental Pb exposure in amounts similar to midwestern inner cities (BLL < 10 μg/dL) was shown to have no effect on initial alcohol self-administration but it did increase the amount of alcohol consumed during relapse (Rangel-Barajas, 2020). The results of this study show that Pb may impact some but not all phases of the cycle of addiction.

In the proposed study, I will investigate the causal effects of Pb on addictive behaviors, specifically fentanyl consumption in mice. With human models of study, there are many confounding factors, such as SDOH other than Pb exposure and Adverse Childhood Experiences (ACEs). ACEs are events in one's childhood that perpetuate violence, abuse, neglect, instability, and more (Centers for Disease Control and Prevention, 2022). To evaluate the relationship of Pb and FUD with minimal variables, I will be using a mouse model. There are not many studies currently investigating this

relationship with animal models, and much less with fentanyl as the drug of choice. With this study, I aim to continue the initiative of investigating environmental public health concerns with behavioral neuroscientific animal models.

Statement of Goals for the Project (Hypothesis)

The goal for this project is to address the question, "Does lead exposure during early development increase risk for fentanyl addictive behavior in mice?" My hypothesis is that if mice are exposed to Pb during critical early postnatal development periods, then they are at a higher risk of fentanyl addictive behavior. This addictive behavior will be measured by aversion resistance, which would be the continuous consumption of solution with fentanyl with the addition of quinine, which is extremely bitter tasting. Results would support this hypothesis if Pb-exposed mice show a significantly higher preference for fentanyl solution instead of water compared to control mice over the course of the experiment, even with the quinine taste aversion addition to the fentanyl mixture.

My long-term goal is to utilize my interests in behavioral neuroscience and public health to conduct research using animal models that provides insight into the role of adverse environmental conditions. This type of work allows us to manipulate conditions in ways that are not possible in human subjects research. I think this is a unique perspective, as most public health research is based in human models. Furthermore, I hope to lead my research and address major public health issues with DEI initiatives to support underserved populations in our society.

Methodology

Subjects

Male and female wildtype C57BL/6J mice will be used in this study (16 control and 16 experimental).

Mice are widely used to model addictive behavior in animals and the Radke lab has established a mouse model of oral fentanyl consumption (Monroe et al., 2021). Sex will be included as a variable in this study as previous studies have shown that there are sex differences in the development of SUDs and sex differences in the expression of learning disabilities caused by Pb exposure in humans. Conceptions of female hormone cycles imposing confounding factors on behavioral neuroscientific studies have been debunked within the past decade, with new studies encouraging the use of female mice to have well-rounded and generalizable results (Shansky, 2019).

Developmental Lead Exposure

The following procedure for Pb exposure in mice has been previously reported (Rangel-Barajas, et al., 2020). See *Diagram* 1 in the appendix for an illustrated timeline. Mice are weaned from their mothers on postnatal day (PND) 21 and randomly assigned to the experimental Pb treatment group or control no-Pb treatment group. The experimental group water supply is Pb dissolved in glacial acetic acid (0.5%) and distilled water. The control group water supply is glacial acetic acid (0.5%) and distilled water. Mice are given these water supplies for 3 weeks (PND21-PND42). Then, mice are individually housed and given normal reverse osmosis drinking water in order to not confound taste of water solution in the paradigm until they reach adulthood and begin the fentanyl drinking task on PND60.

Blood samples are collected during the third week of Pb exposure to confirm blood lead levels (BLL). The optimal BLL of the experimental group is 2.5-10 µg/dL, based on studies that aimed to model BLL experienced by children of inner cities of midwestern United States, such as Cleveland, Ohio (Rangel-Barajas, et al., 2020; Cuyahoga County Board of Health, 2022). These measurements are to ensure the groups received effective treatments for the experiment.

There will be 8 control male and 8 control female mice. Control mice will be raised under normal housing conditions, without Pb exposure. These mice will follow the same DID drinking procedure timeline as the experimental mice.

Drinking in the Dark Paradigm

The drinking in the dark (DID) paradigm uses two fluid bottles placed on an animal's home cage to measure preference for a substance. Because mice are nocturnal and are most active in the dark, this task encourages binge-like consumption of alcohol and other drugs.

Mice are housed individually and two solutions are offered in the home cage: reverse osmosis filtered drinking water and fentanyl citrate (10 μg/mL). See *Image 3* in the appendix for an illustration of the paradigm. Bottle weights are measured before they are put in cages (initial) and after they are taken off (final) the cage.

Mice will consume fentanyl vs. water 5 days/week for two weeks (10 sessions). See *Diagram 2* for an illustration of the experimental timeline in the appendix.

To control for any cage side preference , which is different than solution preference, the bottles will switch sides before each session. To control for spillage due to the handling of bottles and for any involuntary leakage from the bottles, there will be two home cages without mice. These cages will be referred to as "dummy cages."

Aversion Resistance

One way to model the symptoms of SUDs in mice is to test aversion resistance. Aversion resistance is characteristic of SUDs and is defined by the continuous consumption of a substance, despite the presence of an aversive or negative consequence.

Aversion resistance will be assessed in this study by adding quinine, a very bitter tasting solute, to the fentanyl solution in escalating concentrations (0, 100, 250, 500 μM) starting on session 11. If the mice continue to show preference to fentanyl solution over water, even with the addition of quinine to the fentanyl solution, then they are displaying aversion resistance. Quinine was chosen as the additive that measures aversion resistance because it has been a successful aversive substance without confounding taste with fentanyl in previous studies with mice (Monroe, 2020).

Analysis

The amount of solution consumed by each subject will be determined by the difference between the initial weight and the final weight of the solution bottle and corrected for the animals body weight. Variation in solution consumption due to bottle handling will be accounted for by subtracting the average solution difference from dummy cages. Consumption is calculated as ug of fentanyl consumed per kg of body weight. Preference is calculated as (fentanyl consumption – water consumption)/(total consumption). Consumption and preference will be averaged across groups.

A two-way ANOVA test will be conducted to determine the effects of sex and Pb exposure.

Expected Results and Interpretation

I expect the results to be different between sexes, with females showing higher preference for fentanyl over water compared to males. I expect this because females typically show higher aversion resistance than males. I also expect the Pb-treated group to show higher preference for fentanyl over water compared to no-Pb treatment. I expect this because I am hypothesizing that Pb exposure increase addictive behavior.

I would interpret these results as females exposed to Pb during development to be at higher risk of FUD compared to males with Pb environmental conditions or females without Pb environmental conditions.

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Appendix

Image 1: Redlining in Cleveland, OH (Kirwan Institute, 2017)

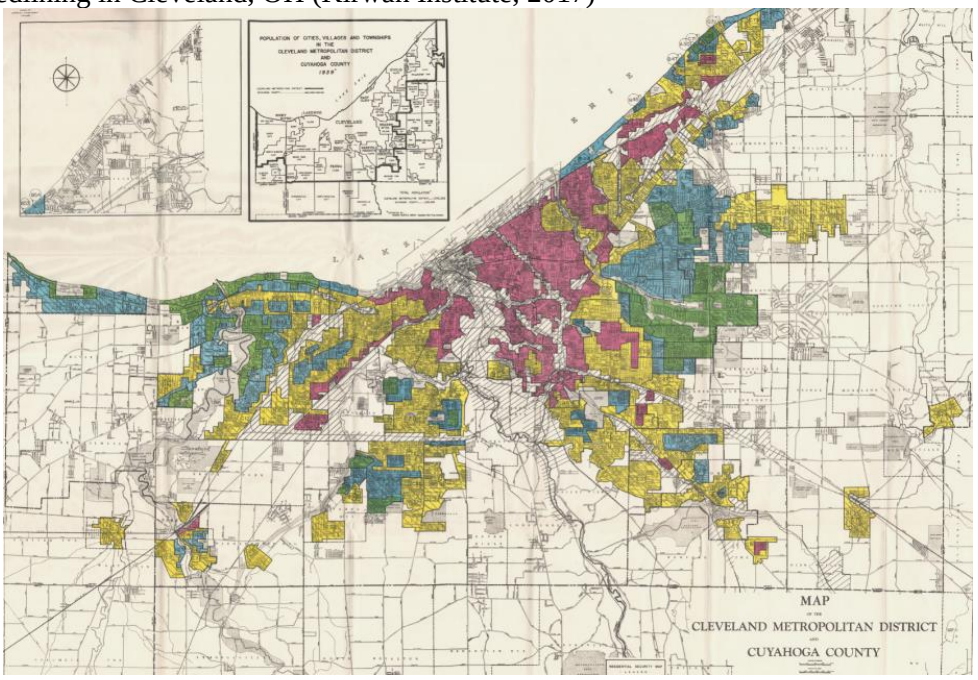


Image 2: Elevated Blood Lead Levels (BLL) in children less than 6 years old (Cuyahoga County Board of Health, 2022)

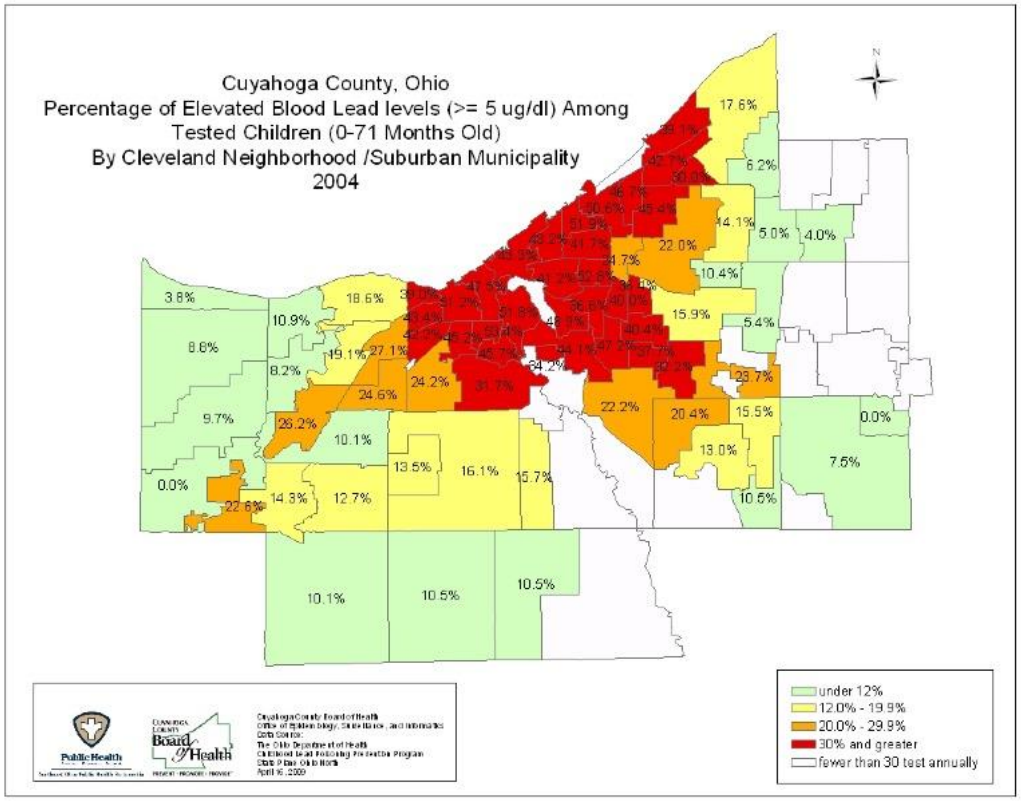
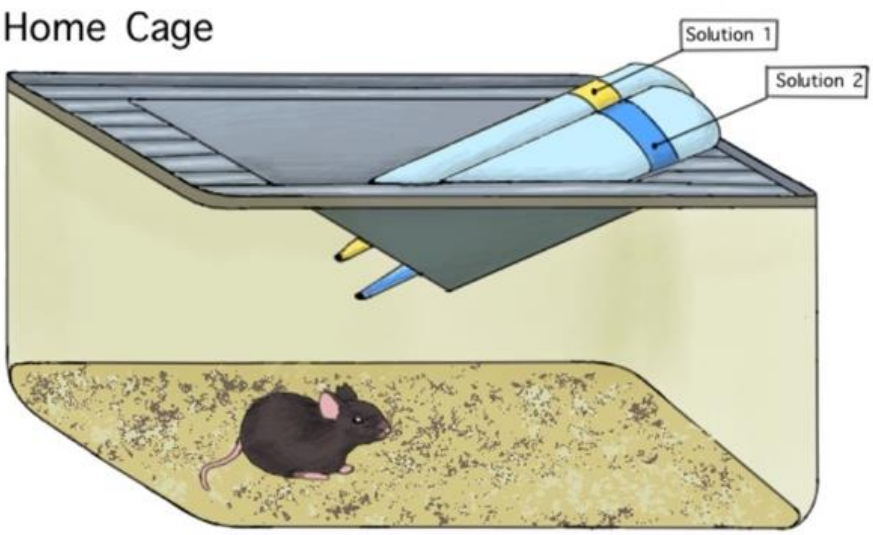


Image 3: Illustration of the Drinking in the Dark Paradigm



Chloe Thach, 2022

Diagram 1: Lead (Pb) Administration Timeline, based on a study by Rangel-Barajas, et al., 2020

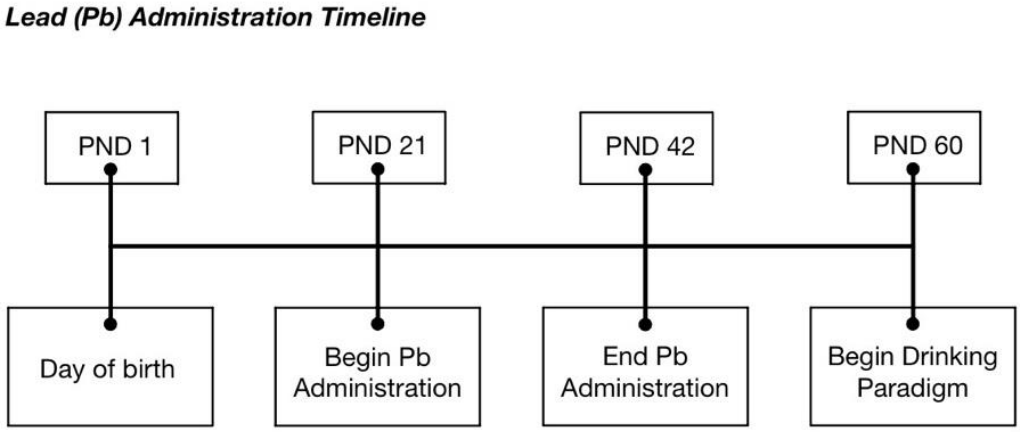


Diagram 2: Experimental Timeline