THE EFFECTS OF DEVELOPMENTAL LEAD POISONING ON THE ADULT RAT'S FREEZING AND EXPLORATION BEHAVIORS IN A HOLE BOARD TEST

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Human lead (Pb) exposure has been a well-established environmental contaminant since antiquity. However, within the last 70 years, developmental Pb-exposure has been confirmed as a neurotoxicant (See for Review Lidsky & Schneider, 2003).

The CDC currently recommends that children should not have a blood lead level (BLL) that exceeds 5 μg/dL. Notably, for every 10 μg/dL a child has, there experience a 5-7 point reduction in their Intelligence Quotient (IQ) (See for Review Neuwirth, 2018a).

Research on the long-term economic costs and social-developmental effects of Pb-exposed children that a loss of a single IQ point has been estimated to translate into an individual lifetime loss of income/earnings ranging between $43,044-$124,382, and for the economy, a loss ranging between $110-$318 billon dollars within the U.S. (Grosse et al., 2002).
Developmental Pb-exposure has been reported to decrease GABAergic signaling (Neuwirth, 2014; 2017; 2018b; 2019).

A reduction in proper central nervous system inhibition has been associated with increased brain excitation (Neuwirth, 2014; 2017; 2018b; 2019).

The results of such aberrant neurodevelopmental problems have been associated with: autism, ADHD, behavioral impulsivity, emotional dysregulation and irritability, poor decision making, and intellectual disability (Neuwirth, 2014; 2017; 2018b; 2019).
HOLE BOARD TEST:

- The hole board test has the following problems:

  - It is debated to be either a test for fear or exploration.

  - When the walls are clear, the rat under testing can be easily visually distracted artificially inflating fear.

  - It is difficult to explain what is meant by head pokes as being exploratory, when typical aversive bright light conditions induce fear and when combining too many behaviors to study at once (Labots et. al., 2015).

  - The hole board test can be a good assessment of inhibitory control or lack thereof.

  - Thus, research investigating fear vs. exploration within the same hole board test require further study and clarification.
In the field of behavioral neuroscience, often times more than one behavioral test is required to assess complex behaviors (i.e., using an open field for locomotor activity, prior to using an elevated plus maze to test for anxiety).

This can inadvertently result in increased time to gather data, as well as, creating the potential for carry-over effects from one test to another; thereby, reducing the translation and generalizability of the data.

The present study tries to use a slight modification of a hole board test for fear to then reduce, as best as possible, the need to change the behavioral apparatus to test exploratory behaviors.
HYPOTHESES:

- The hole board test will be able to show a clear difference in fear vs. exploration head poke behaviors if an olfactory cue is used in the exploration condition.

- Developmentally Pb-exposed rats will show reduced fear behavior when compared to Control rats.

- Developmentally Pb-exposed rats will show a reduced exploration behavior when compared to Control rats.
METHODS:
DEVELOPMENTAL Pb-EXPOSURE MODEL

Treatment Groups
Control Rats

Perinatal Pb-Exposed 1,000 ppm Rats

1 Month Prior to Breeding
3 Weeks Breeding
Pups Born PND 0
3 weeks Until Weaning PND 22
PND 22-55 2-Day Hole Board Testing

Control Water

Duration of Pb-exposure in Drinking Water

Control Water

Analyze Data
SUBJECTS & SAMPLES SIZE

- $N = 15$ Long Evans Hooded Rats were tested between postnatal days 30-55.

- Control rats ($n = 7$)

- Perinatal Pb-exposed 1,000 ppm rats ($n = 8$)

- Only male rats were used since the litters did not have many females to set up age-matched comparisons.
Day 1
Head Pokes
Test for Escape Behaviors
Assess Fear Behavior

Day 2
Head Pokes
Test for Exploration Behaviors
Assess Cognitive Behavior

No Olfactory Cues

4 Different Olfactory Cues

Vanilla
Orange
Lemon
Almond

Tested under bright white light 300 Lux as an Anxiogenic stimulus

24 hrs later Rats should habituate

Re-tested under bright white light 300 Lux as an Anxiogenic stimulus
HOLE BOARD TEST PROCEDURES & PARAMETERS:

- Each test was 10 minutes in duration.

- A behavioral video tracking software (Anymaze, Stoelting, Inc.) was used to capture and analyze all rat behaviors.

- Head pokes into any of the 16-holes were manually scored by pressing a key for each observed behavior resulting in a head poke frequency.

- Head poke duration into any of the 16-holes were scored by the duration of time that the key was pressed.

- These procedures were identical for Day 1 (Fear) and Day 2 (Exploration).
Repeated Measures ANOVA revealed a significant Within-Subjects Effect of Minutes $F_{(9)} = 1.948, p < 0.05^#, \eta_p^2 = 0.13$

Effect of Day $F_{(1)} = 8.124, p < 0.01^{**}, \eta_p^2 = 0.385$

And a significant Between-Subjects Effect of Treatment $F_{(1)} = 12.675, p < 0.01^{‡‡}, \eta_p^2 = 0.494$
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ANOVA revealed a significant effect of Treatment $F_{(1)} = 11.09$, $p < 0.001##$, $\eta^2_p = 0.299$

A significant effect of Condition $F_{(1)} = 8.81$, $p < 0.01**$, $\eta^2_p = 0.253$

However, there was no significant Treatment X Condition interaction $F_{(1,1)} = 0.802$, $p = 0.379$ n/s
Developmentally Pb-exposed rats showed an initial difference in the first 2-minutes of Day 1 with more hyper-activity and reduced freezing response to a novel test environment when compared to Control rats.

During Day 2, across the entire 10-minutes of the test, Pb-exposed rats showed a consistent suppression of exploratory behavior when compared to Control rats.

The overall effects when comparing Day 1 vs. Day 2 revealed a significant effect of Test condition, which indicated that the Hole Board manipulation was strong enough to alter the behavior under study and that these manipulations were sensitive enough to assess and test the differences in both fear and exploratory behaviors within the same testing apparatus.

Thus, this testing paradigm may be useful as it decreases the need to over test, reduces testing conditions, and prevents the potential carry-over effects inherent to most behavioral neuroscience testing approaches.
CONCLUSION:

- This study shows that by using the same test apparatus and modifying the stimuli, another dimension of behavior can be assessed within the same apparatus reducing carry-over effects, and maximizing sensitivity of testing across different behavioral dimensions.

- Developmental Pb-exposure causes persistent GABAergic problems that manifest as hyper-excitability with reduced freezing on Day 1 and cognitive deficits with decreased exploration on Day 2.

- The current modified version of the hole board test was shown to be sensitive enough to parse out the effects of developmental Pb-exposure within- and between- Day 1 for fear and Day 2 for exploration behaviors.
However, the Perinatal lead-exposed rats exhibited difficulty in shifting from their anxiogenic responses, showed little habituation, and a delayed onset to sensorimotor dependent exploration of the novel odors.

The data suggest that perinatal lead poisoning impairs sensory processes required for contextual adaptations, efficiency, and ongoing environmental changes directed by the prefrontal cortical through goal-directed behaviors.

This study shows that rather than using the hole board independently to test either fear or exploration, that using this 2-Day paradigm, it can be used for both dimensions of behavior.
REFERENCES:


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