Lead and its effects on Zebrafish

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Abstract

When ingested or inhaled lead can cause serious damage to a person's health, primarily to their brain. This experiment shows the effects that lead can have on our brain specifically our learning and memory. Zebrafish were used in this experiment because they have similar functions to humans. Eight test were done, four control and four experimental. The control group was raised in normal 7ph water conditions whereas the experimental were exposed as embryos in 10uM Pb concentrated water.We used a t-maze to determine the zebrafish cognitive flexibility. Collecting data and observing each group to see if they chose the “correct” way. The zebrafish exposed to lead showed signs of hesitation and were indecisive of when choosing a direction, many showed signs of perseveration. Results like these are able to show use how lead can affect all.

Introduction

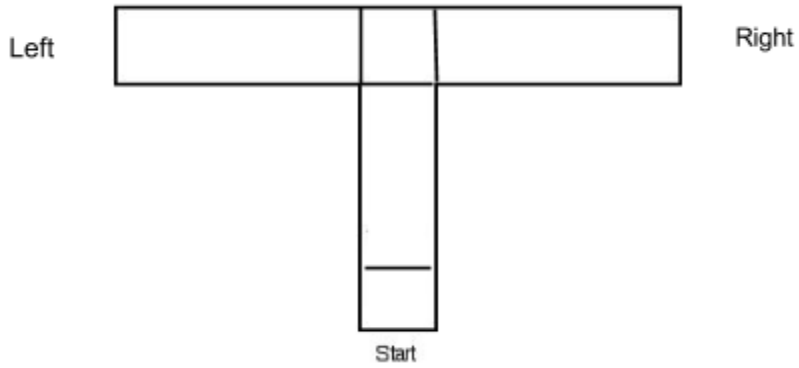
Lead is a useful and a common metal that has been used by humans for thousands of years. It is also a very dangerous poison particularly for children, when accidentally inhaled or ingested(Environmental Health Sciences Core Center). Low levels of lead in children can cause problems, such as: Learning disabilities, Attention Deficit disorder, behavior issues, nervous system damage, speech and language impairment, decreased muscle growth, decreased bone growth, and kidney damage. High levels can be life threatening, causing seizures, unconsciousness, and death.Lead can even cause serious health issues for adults. In adults low levels of lead can cause high blood pressure, digestive issues, nerve disorder, memory and concentration problems, muscle and joint pain, and can increased chance of illness during pregnancy. This can cause the fetus to have brain damage or even lead to death.("Public Health”) Lead is known to affect your brain, specifically the hippocampus, which is the part of the brain that stores your memories.It has been known for some time that lead is potent inhibitor of the NMDA receptor, a protein known to play an important role in brain development and cognition. In study we demonstrate that lead exposure decreased the amount of NMDA receptor gene and protein in a part of the brain called the hippocampus("Why Use the Zebrafish in Research?"). This change is associated with impairments of nerve communication in the brain and of learning. This study is testing the effects it has on the zebrafish cognitive flexibility and how it affects the way the fish can learn and make decisions("Why Use the Zebrafish in Research?"). So how will the exposure to lead at an early age affect the cognitive flexibility of a zebrafish? If zebrafish are exposed to lead at an early age their cognitive flexibility will be negatively affected compared to zebrafish not exposed to lead.

Materials and Methods

- 1 T- maze unit/group (3 students)
- 4 L Dechlorinated water
- 1 L holding tank (1 L beaker) for each fish being tested (renew water once each day)
- 1 plastic pipet
- marking tape/pens to label tanks
- 1 Fish Net
- 4- L graduated beaker or flask
- 1-2 floor mats
- 1 plastic table covering

- 1 Mini Aquarium Heater/ fish tank or beaker

Setup:



Methods:

Student Roles:

We had three students with each student have specific role. The three roles are:

1) Start Box: students will open and close the gate to release the fish into the T-Maze raceway.
2) Netting: student will be in-charge of netting the fish out of the maze and returning it to the start box.
3) Arm Gate, Data, and Time: student will lower the gate of the incorrect area once the fish has entered the correct side, then the student will record the data on the data sheet as well as keep track of time the fish is in the start box and correct arm of the T-maze.

Procedure:

Each day is labeled to the process of learning being tested. Day 1: Training (shaping a skill set), Day 2: Testing (developing improvement in function), and Day 3: Learning test

Fish Acclimation

Place individual fish into the T-Maze for 5-minute acclimation period. During this time there are no gates (except for the starting block) inserted into the T-maze.

Left-Right Discrimination

1. Place individual fish into starting block.
2. After 15 seconds, open starting block to allow fish to enter raceway. Close gate.
3. Allow fish to swim down raceway. If it enters **Right** arm lower the gate on the **Left** arm and record arm choice of your data sheet. Allow the fish to remain undisturbed for 45 seconds representing positive reinforcement. Then **GENTLY** net it and place it back into the starting block for 15 seconds.
4. If fish chooses **Left** side, net **IMMEDIATELY** and place back into starting block, stir up the water in starting block with a stirring rod LIGHTLY. This will show the fish positive punishment. Record choice on data sheet.
5. Continue conducting trials until maximum of 20 trials or a successful string of 5 correct choices in a string of 6 trials occurs. This is Reaching Criteria.
6. Upon Reaching Criterion, place the fish back into the starting block and switch the correct gate from the **LEFT** to the **RIGHT**. Repeat above procedure until fish reaches criterion. Now that is has successfully achieved what is termed a reversal task and is a measure of cognitive flexibility.

7. When all the trials are completed, drain water and rinse the t-maze and all gates with distilled water and allow to dry overnight.

Results

When analyzing the results there were some zebrafish had more cognitive skills than others. The independent variable in this experiment was the lead that was given to the zebrafish in the experimental group.The dependent variable was the learning process the fish went through.

To help show that our data was statistically significant we used a two-sample t-test. The analytical data taken during this experiment showed the longest string of correct trials (figure 3), perseveration(Figure 6), number of trials needed to successfully reverse the task (figure 13), the first time the correct way (figure 9), and number of trials between 1 and 2 (figure 12). When all this data was put into the t-test it came out that the majority were not statistically significant. The only one that came out significant was the number of trials needed to successfully reverse the task (figure 13) on day three. Though this number was ruled out due to there not being enough data collected. Looking at it all we realized that the qualitative data was a lot more valuable in showing how lead exposed zebrafish behave differently than zebrafish not exposed to lead this can be shown in tables 1 and 2.

Discussion

This experiment was successful in seeing whether lead affects how a zebrafish reacts. Though in order to be conclusive on the cognitive flexibility of a zebrafish that has been exposed to lead, this experiment needs more zebrafish and more time for the experiment. With such a short time there was a restriction of eight subjects, four control, and four lead exposed. The hypothesis stated that if a zebrafish was exposed to lead then the cognitive flexibility of the zebrafish would be negatively affected compared to zebrafish not exposed to lead. It could not be determined whether this hypothesis is true or not because there was not enough evidence to conclude that lead affects the cognitive flexibility of a zebrafish.The experiment's final data failed to support this hypothesis. Some limitations may have been if the Zebrafish were bias toward either the RIGHT or LEFT. It also occurred to us that we were restricted to the amounts of trials we were able to use. 20 trials were not enough for most of the zebrafish to both learn and relearn. This made the quantitative data collected not significant when collected in the t-test.With how the data was collected we noticed that the zebrafish exposed to lead did just as well as the ones not exposed. Nevertheless, looking at the qualitative data, there were observations of some of the symptoms that lead can cause. When the lead exposed Zebrafish would finally enter the t-maze they would immediately show hesitation, and painstakingly chose right/left and continually chose this way even with punishment (observed in fish 2 and 3 (Pb)). This is called perseveration which is the repetition of a particular response, such as a, word, phrase, or gesture, despite the absence or cessation of a stimulus, usually cause by a brain injury or other organic disorder.Hesitation was also observed during this experiment, the lead exposed zebrafish would take a long time to leave the starting gate, we would eventually have to gently force them out with a stirring rod, once the gate was closed behind them they would attempt to stay in the middle raceway as long as possible. They would then be pushed all they way to the end of the raceway with gate where they would then just sit in the middle of the RIGHT/LEFT arms. They were so hesitant to make any choice that would end with them getting a punishment.The last action we observed in the zebrafish exposed to lead was indecisiveness to which way they wanted to choose, often leading us to have to force them to make a choice

because they wouldn't leave the middle raceway (observed in fish 1 (Pb)).Overall, we could determine that there were some differences in how exposed zebrafish compared to zebrafish not exposed to lead reacted to the t-maze. Based on that we are able to see the possible effects of lead exposure on humans.

Websites

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