Epigenetically Heritable Effects of Maternal Behavior in Rats

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Introduction

Mental health is now becoming a greater issue today. Depression affects over 300 million individuals, or approximately 4 percent of the world's population (Health Poverty Action, 2018). Researchers have discovered that mental health difficulties can lead to a wide range of physical health difficulties, which is one of the concerns associated with mental health problems. People with mental illnesses, for example, are more susceptible to infections and non-communicable diseases. Anxiety and depression are two major neuropsychiatric illnesses that affect people all over the world (Merikangas et al., 2010).

It is no wonder that specialists are working hard to figure out what is producing such a large population effect. Research is being done on parenting practices and hormone expression in the brain during development. Human studies on the association between mother and daughter interactions and the daughter's anxiety and depression, as well as the association between estradiol and progesterone levels and stress and anxiety, have been reported (Crowell et al., 2017).

Human research, though useful, have several drawbacks, including a lack of controlled settings and invasive brain investigations. Because of these limitations, model species such as rats are crucial in learning more about the mental health difficulties that individuals face. Evidence shows that alterations are handed down epigenetically from generation to generation (Champagne et al., 2008 and Curley et al, 2003). It is encouraging to know that cognitive enrichment may happen over the course of a person's life.

Pup retrieval has been utilized to measure mother behavior as well as the degree of maternal care provided to offspring (Berretta et al., 2019). To assess rats within a cohort on their

level of maternal care, researchers employed latency to collect pups from their own litter, pups from another mother, and a mixed ratio of pups. The more quickly a mother recovers her pups, the more likely she is to participate in other parental activities like grooming or nursing (Dias et al., 2015). Slower mothers are more prone to engage in inattentive behaviors such as self-grooming and cage exploration rather than interact with their pups (Dias et al., 2015).

Pups nurtured by moms who are fast to retrieve them are more likely to retrieve their own pups later in life (Weaver et al., 2004). Pup born to a mother who is slow to collect her pups but raised by a mother who is quick to retrieve her pups will inherit the maternal traits of the mother who reared them (Champagne, 2008). Cross-fostering shows that mother behavior is not entirely driven by genetics, and that environmental factors may have a significant impact. Evidence has been discovered that early maternal care variables can cause epigenetic alterations that can last a lifetime (Weaver et al., 2004).

Rats' physical and behavioral changes have been demonstrated to be influenced by environmental enrichment. Inanimate object enrichment and social enrichment are two types of environmental enrichment (Rosenzweig and Bennett, 1996). Keeping enrichment, such as huts and tunnels, play enrichment, such as chew blocks, scavenging enrichment, such as concealing food and allowing the rats to escape their cages, and social enrichment, such as housing many rats together, are all examples of these variables. Environmental enrichments, when combined, can amplify the effects of maternal behavior and environmental enrichment. Reduced anxiety, tension, and anhedonia, as well as increased resilience and motivation, boost welfare (Sparling et al., 2018).

This paper will look at how Good and Bad mothers, as well as their pups, perform on four distinct behavioral tests, some of which are exposed to environmental enrichment. These

assessments were chosen because they assess behavioral characteristics connected to maternal behavior and can be altered by environmental enrichment (Zuene et al., 2016 and Tarantino et al., 2011). The elevated plus maze (EPM) is a tool for determining anxiety levels (Ravenelle et al., 2014, Pritchard et al., 2013). The novel object preference (NOP) test assesses non-spatial memory, whereas the object location memory (OLM) test assesses spatial memory (Birch et al., 2013). Finally, the force swim test (FST) assesses adaptation and resilience (West, 1990).

Pups with moms that have quicker retrieval rates and live in environmentally enriched housing are expected to be less nervous, resilient, and have superior spatial and non-spatial memory, according to prior study. Pups whose moms either did not recover their pups or did so slowly and are kept in control cages with no additional environmental enrichment would perform the worst. In control cages, pups from lousy moms are expected to be more nervous, less resilient, and have poor spatial and non-spatial memory.

Methods and Materials

All procedures were approved by the Longwood Institutional Animal Care and Use Committee (IACUC).

Housing

Animals were pair-housed using the Innorack IVC Rat Rack and IVC Caging System (Innovive, San Diego, CA). Housing and behavior rooms were maintained at ~30-50% humidity, temperature of 21℃ and on a 12/12 (7AM- 7PM) light cycle. Standard Diet (Vendor, Glen Forrest, WA) and tap water were provided *ad libitum*.

In addition to the above conditions, animals housed in the “Enriched Environment” condition were given a nestlet, a chew toy, and a place to hide (Figure 1). Enrichment objects were rotated every four days.

Animal Subjects

Thirty-two rats were raised from birth in the lab and sorted equally into four conditions at weaning: Good Mothers + Enriched Environment; Good Mothers + Control Environment; Bad Mothers + Enriched Environment; Bad Mothers + Control Environment. One rat did not survive to adolescence, leaving thirty-one rats to be tested. All thirty-one rats participated in behavioral testing (below) as adolescents at approximately 35 days old. At 120 days old, sixteen rats were mated and did not participate in behavioral testing until after they had weaned their pups. The remaining 15 rats participated in a second round of behavioral testing as Nulliparous females between the ages of 138-142 days old.

Behavioral Tests

Five behavioral tests were used in this study. A Pup Recognition Test was used to categorize if a mother rat was “good”, “average”, or “bad” at maternal behavior. The remaining four tests – Elevated Plus Maze, Object Location Maze, Novel Object Preference Test, and Forced Swim Test – assessed anxiety/boldness, spatial memory, non-spatial memory, and resilience in rats.

Mating

A total of six male laboratory rats were bought from (Taconic Biosciences, Germantown, NY). Six F1 generation females were chosen for the initial round of mating; owing to mating, these rats did not undergo any nulliparous testing. During the female rat's estrous cycle, a male and female rat were housed in a cage that was nearly identical to the control groups in the F1 generation. For a total of five days, these animals were kept together. Following the completion of the mating procedure for group one, additional six female rats were picked based on when

they were born and which category they fell into (Good Enriched, Good Control, Bad Enriched, or Bad Control). Male rats were chosen depending on which females they had previously married with, with males who had previously mated with a GC marrying with one of the other three groups, and so forth. All mother rats were given permission to give birth and care for their offspring.

Pup Recognition Test

To assess whether a mother rat was a "good" or "bad mother, we used a pup recognition test when the mother rat's pups were between 6-9 days old. For this test, there were three conditions: a mother rat would be presented with either 8 of her own pups (OWN), 4 OWN and 4 from another mother (ALIEN), or 8 ALIEN pups for 15 minutes. For each trial, mothers were assessed for the latency to retrieve the first, fourth, and eighth pups, if she nursed the pups, groomed, the pups, or self-groomed during the trial (Figure 2).

To initiate the test, mothers were taken from their home cage and placed in a test cage in the behavioral testing room to acclimate for 5 minutes. During this time, her pups remained in the home cage and were placed under a under a heat lamp. Also, during the acclimation period, researchers placed the appropriate number of pups into a Pyrex bowl (measurements; FIG). To identify them, OWN pups were marked parallel lines and ALIEN pups were marked with an X using an "odor-free" marker (Brand, City, State). Following acclimation, the bowl of pups was placed in the test cage and the researcher left the room. The mother rat then had 15 minutes to interact with the pups.

Elevated Plus Maze

Anxiety was assessed using the Elevated Plus Maze (Noldus, Leesburg, VA). Rats were removed from their homes and placed in a new cage with access to water before being

transported to the testing room. The rats were placed in a 12in section of the Elevated Plus Maze after a 20-minute acclimation period in the testing room. The experiment started right away and finished within five minutes of exploration (Figure 3). After the 5-minute trial, the rats were returned to their home cages. The duration spent by rats in each zone of the apparatus was measured using video recordings of the trial. When the rat moved from one zone to another, the next zone's time began when the rat's feet entered the arm.

Novel Object Recognition Test

The rat's non spatial memory was tested using the Novel Object Recognition Test. Rats were taken out of their home cages with access to water before being transported to the testing area. The rats were then placed in an open field maze with two identical items positioned on the opposite side of the open field for the first part of the test (Figure 5). Each rat had five minutes to explore the objects. The rats were withdrawn and returned to their home cage after the first stage, and the testing arena which was known as the open field maze and the objects were cleaned with one of the items being replaced with a new object. The rat was brought back into the maze for the second time and given five minutes to explore both known and new objects. Then the rats were returned to their home cage at the end of the test and the maze was cleaned. Students used video recording to track time spent examining familiar and unfamiliar objects. Exploring was defined as smelling and engaging with the object or the surroundings around it.

Object Location Memory Task

The rats' spatial memory was tested using the Object Location Memory Task. The rats were removed from their home cages, placed in new cages with access to water, and transported

to the testing room. After that, rats were placed in an open field maze with two identical items on the opposite side of the open field (Figure 4). The rat had 5 minutes to investigate the objects . The rats were then removed and returned to their home cage after the first stage, and the testing arena, which was an open field maze, was cleaned. One object was left in its original location in the testing stage, while the other was put in a new side of the open field maze. The rat was placed in the field and was given 5 minutes to investigate both the familiar and unfamiliar object locations. The students used video recordings to track time spent studying known and moved things as a discriminating measure. Exploring was characterized as smelling and interacting with an object or its environment.

Forced Swim Test

Rat were placed in a 29 in X 12in X 16in fish tank containing 12 inches of water for 5 minutes. Latency to dive, number of dives, time spent swimming, and time spent floating were assessed as measures of coping strategies.

Video Data Analysis

Following the completion of each test, the films of each trial were examined to determine each rat's behavior. Following the inter-rater liability requirement, at least two individuals were timed each specific item of data that required to be gathered. Time was gathered for OLM depending on the amount of time the rat spent with each object in each location. Every time the rat sniffed, touched, or sniffed the wall near the item, it was tallied. NOP was timed in a same way, with the amount of time spent on each object around all the data. The quantity of time spent on the closed and open arms was measured using EPM at three intervals: 60s, 120s, and 300s. When she was facing the open arm and at least one paw was on it, the rat was deemed “on” it. The number of droppings, time spent floating, swimming, diving, latency to float, and latency to

dive were all recorded for FST. After one second of not attempting to swim, a rat was regarded to be “floating.”

Results

Except for the Force Swim Test, which employed percentage Z tests, the data from each behavioral test was examined using an independent samples t-test.

Pup Recognition Test

The average time spent retrieving the first pup in each condition (eight of their own pups, four of their own, four from another mother, and eight from another mother) was calculated. These averages were used to categorize the moms into groups based on prior Dr. Franssen Lab research. Each maternal rat was classified as a “Good Mom”, or “Bad Mom” based on tendencies discovered in prior studies.

Elevated Plus Maze

The rats in the F1 generation spent substantially more time in the closed arm than in the open arm. For the excellent moms, however, there were no differences in the amount of time spent in the closed arm vs the open arm (M = 97, SD = 51, SEM =27) and the bad mothers (M = 67, SD = 45, SEM= 23) (Figure 6).

Novel Object Preference

The length of time spent with the new and novel object was assessed in seconds and compared between groups for the Novel Object Preference test. For the F1 Generation both good and Bad mother did not demonstrate a significant different in time spend with novel object. The F1 from good mother demonstrated (M= 29, SD= 7). As for the bad mother (M=27, SD=6) (Figure 8).

Object Location Memory Test

When comparing the groups, it was found that good mothers vs bad mothers did not have any significant different in the amount of time spend with stationary object. For the stationary object goof mom spend (M= 26, SD= 8, and SEM= 4) and for bad mom they spend a average of (M= 44, SD= 9 and SEM=5) (Figure 10, 9).

Force Swim Test

In the F1 generation, there were no notable variations in the amount of time spent floating or swimming. Although there was a significate different in the time it took for the F1 Nulliparous Female Rats to dive, which was good mother demonstrated an average (M = 259, SD = 71) and bad mothers demonstrated an average of (M= 274, SD= 27) (Figure 11). Although there was not a significate different in the amount of time it took for both bad and good mother rats to float. The rats from good mom had an average data of (M = 69, SD = 44) and rats from bad mom had an average of (M= 79, SD=50) (Figure 12).

Discussion

These findings are the first from this intergenerational, longitudinal research. Even though the study is still in its early stages, several substantial behavioral variations across groups are already apparent. More definitive findings within each age group can be predicted when values are increased with subsequent trials and generations. However, substantial disparities across groups are crucial to highlight at this early stage of study. Disparities in memory tasks (Novel Object Preference and Object Location Memory) within the F1generation, for example, indicate differences in memory capacity between Good and Bad mothers.

According to the findings of the current study, which are discussed further below, animals can be cognitively enriched at three points during their lives: maternal care as pups, environmental enrichment during the developmental maturation period, and later in life because

of pregnancy, parturition, and parenthood. The current findings show that developmental stage and motherhood have an influence on spatial and non-spatial memory in the rat model. Previous study has revealed that moms had enhanced spatial and non-spatial memory when compared to non-mothers (Kinsley and Lambert, 2008).

Pup Recognition Test

Latency to retrieve the first pup in a particular trial was revealed to be a critical predictor of Good or Bad mothers among the behaviors studied. In the 8:0, 4:4, and 0:8 circumstances, the time it took to recover the first pup was averaged. To see if the rats in this study fast or slow retrievers were, researchers compared their timings to those of rats in prior experiments.

Elevated Plus Maze

The F1 generation of Nulliparous female rats spent substantially less time in open arms than in closed arms, as predicted. This is typical of rats, as prior study has shown that rodents are more cautious in open areas (Lezak et al., 2017). However, early evidence shows that the kind of mother has no effect on anxiety and boldness since there were no significant differences between the Good and Bad moms. This is an unexpected conclusion because it was expected that Good moms would be more courageous than Bad mothers. Our prediction, on the other hand, is since moms spend more time on open arms than non-mothers, indicating boldness and low nervousness (Kinsley and Lambert, 2008; Love et al., 2005). That research compared mothers to non-mothers and did not differentiate between different types of mothers, such as good vs. bad mothers. Our data suggest that while parenthood may have an influence on boldness and anxiety, anxiety is not distinguished at Good/Bad levels once a mother.

Novel Object Preference Test

The F1 generation spend more time with the novel object thank the familiar object. This result suggests that Good moms have better non-spatial memory than Bad mothers. This is in line with earlier research that found moms had stronger non-spatial memory than non-mothers (Kinsley and Lambert, 2008;love et al., 2005).

Object Location Memory Task

Similar to the new item choice test, good moms spent more time exploring the relocated object, albeit there was no statistically significant difference between the two groups, which is consistent with research that show rats utilize spatial memory to detect and recall object locations (Vogel-Cierna and Wood, 2014). The time spent with each object by the Bad moms, on the other hand, did not change significantly, showing that Good mothers are better at detecting the relocated object and had superior non-spatial memory than the Bad mothers. This lack of significance in the Bad mom's group might possibly be owing to the small sample size of only four people in the Bad mothers group. As the number of values increases, the data may maintain the present trend or demonstrate that there is no significant difference between Good and Bad moms in terms of spatial memory.

Force Swim Test

When looking at time spend swimming versus time send floating, the F1 generation females shown no significate difference between Good mothers and Bad mothers. When it came to the rats' diving attempts, however, Bad moms made more attempts than Good mothers.

Figures

A. **B.**

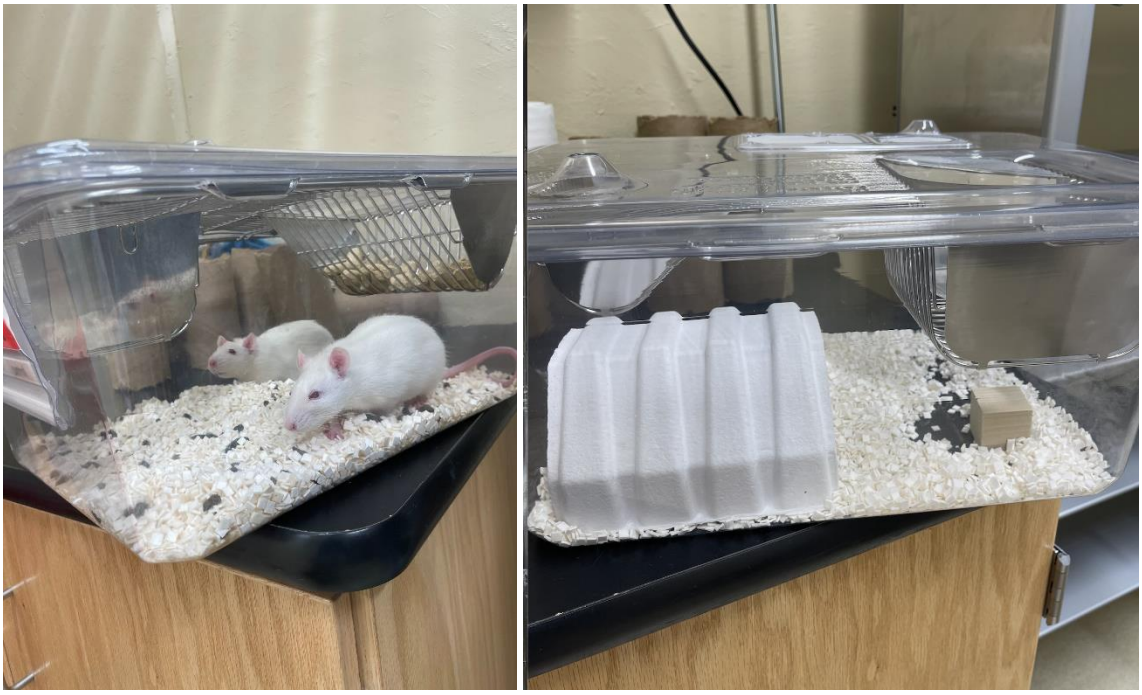


Figure 1. Demostrates Standard vs. Enriched Conditions. In the control cage, rats are kept in pairs and have unrestricted access to food and water. In the enriched condition the rats are kept in pairs in an enriched cage with unlimited food and water, as well as three pieces of enrichment: a cardboard tunnel, chewing log, and square of nestlet.

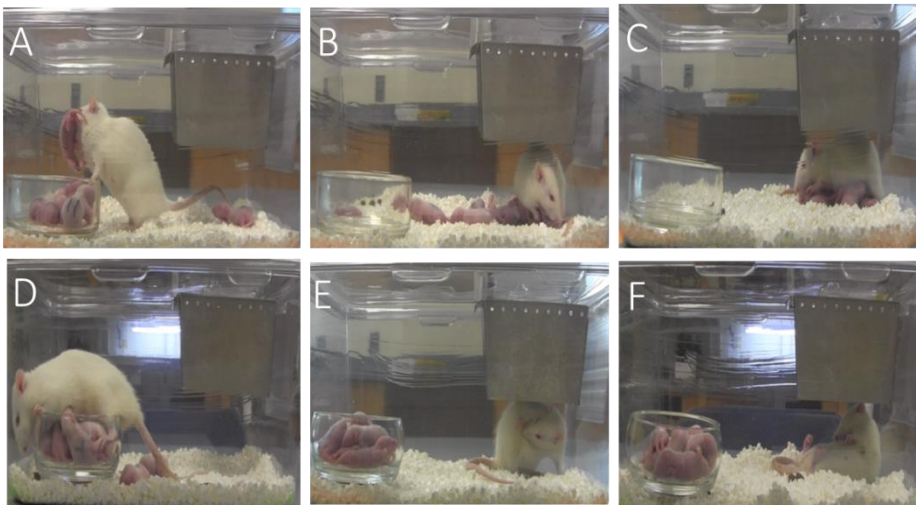
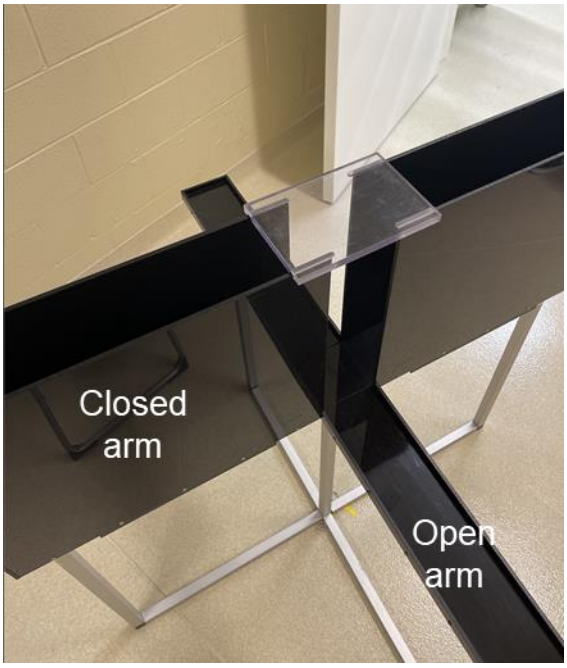


Figure 2. Maternal Responses to the Maternal Pup Recognition and Retrieval Test. A demonstrated maternal rat retrieving pup. B, rat grooming the pups. C and D shows maternal rat nursing pups. E, rat is self-grooming in which we can infer that's a anxiety behavior. F, maternal rat is sleeping.

Figure 3.

Elevated plus Maze.



A. **B.**

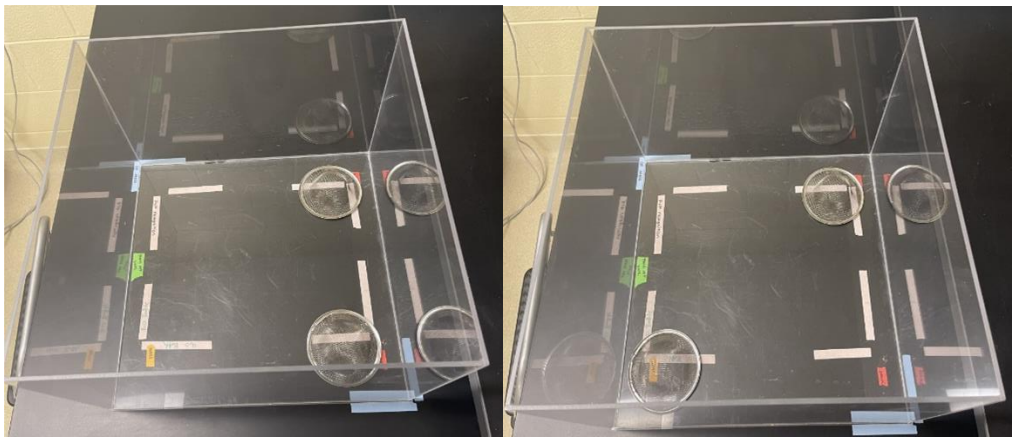


Figure 4. Open field maze set up for the Object Location Memory Test. (A) demonstrated both items are identical and are in the container's opposite corners. (B) Both items remain the same, but one has been relocated to the cage's diagonal corner.

A. **B.**

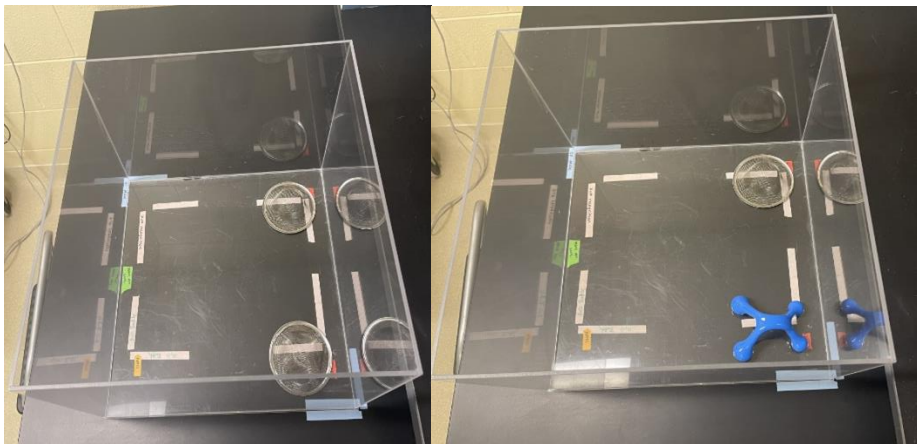


Figure 5. Open field maze set up Novel Object Preference Test. (A) demonstrated both items are identical and are in the container's opposite corners. (B) One of the objects has been replaced with a new, clean object that the rat has never seen before.

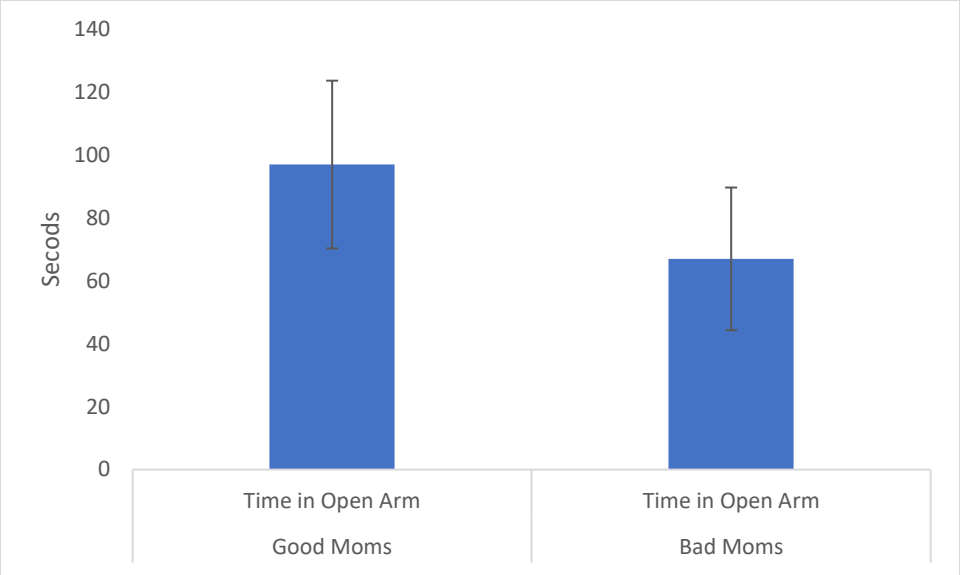


Figure 6. Time Spend in Open and Closed Arm on Elevated Plus Maze for F1 Nulliparous Female Rats. In the closed arm, each group spent considerably more time. The error bars are standard errors of the mean.

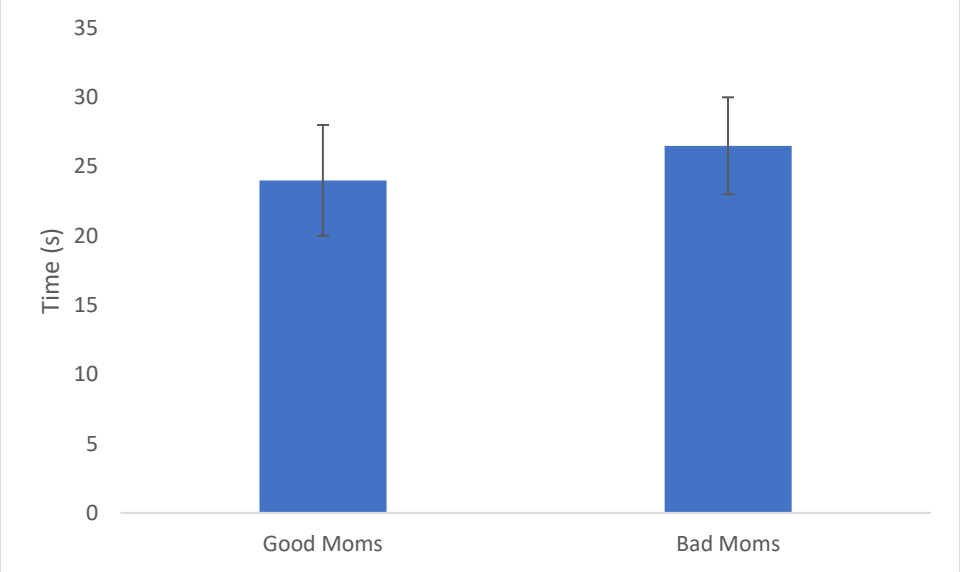


Figure 7. Novel Object Preference Test: Good vs Bad Moms Time Spend with Novel Object F1 Nulliparous Female Rats.

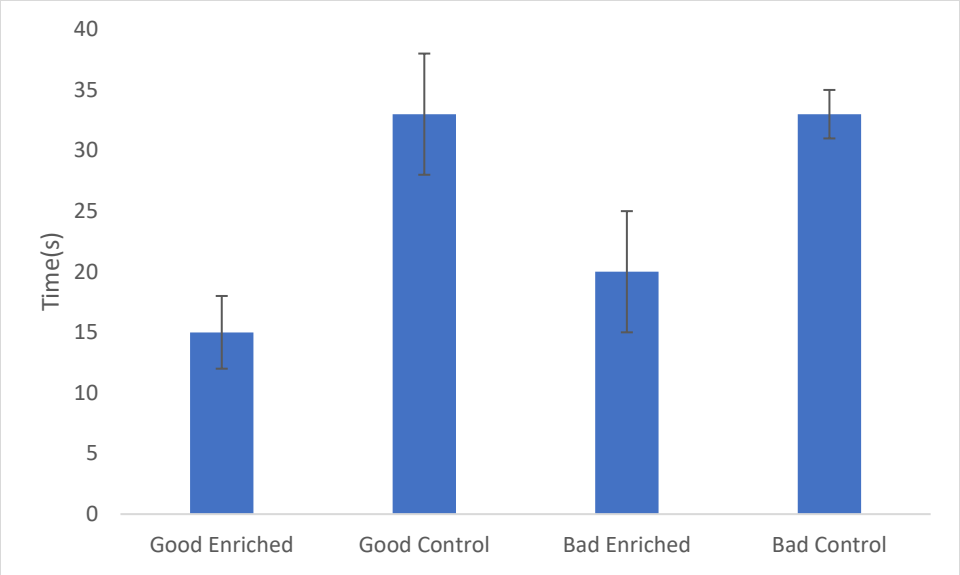


Figure 8. Novel Object Preference: Time Spend with Novel Object F1 Nulliparous Female Rats

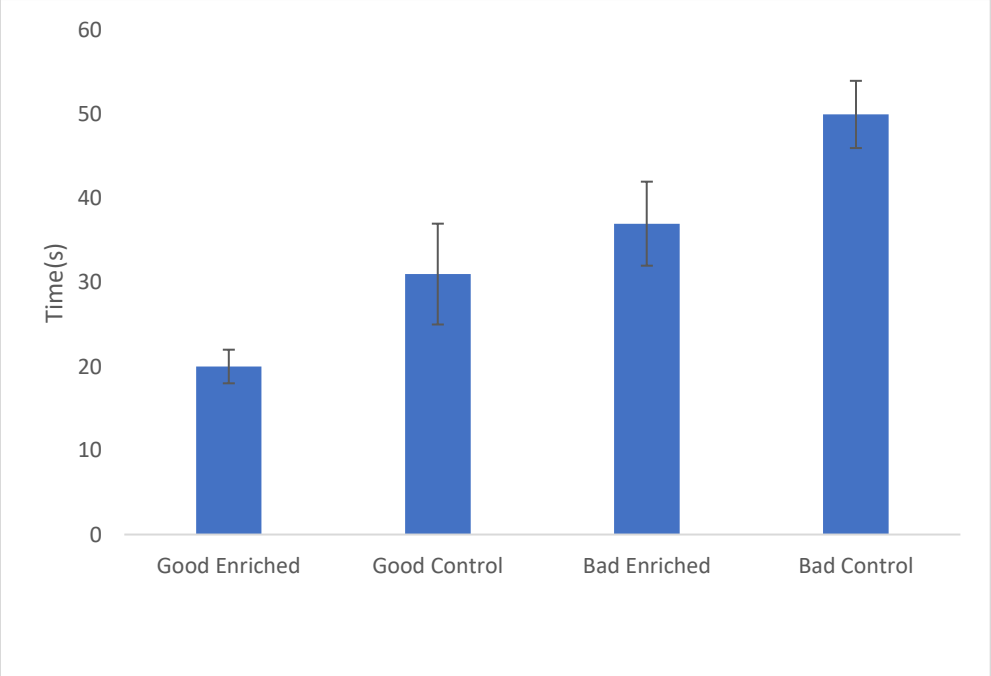


Figure 9. Overall Time Spend with Stationary Object for Object Location Memory Test for F1 Nulliparous Female Rats.

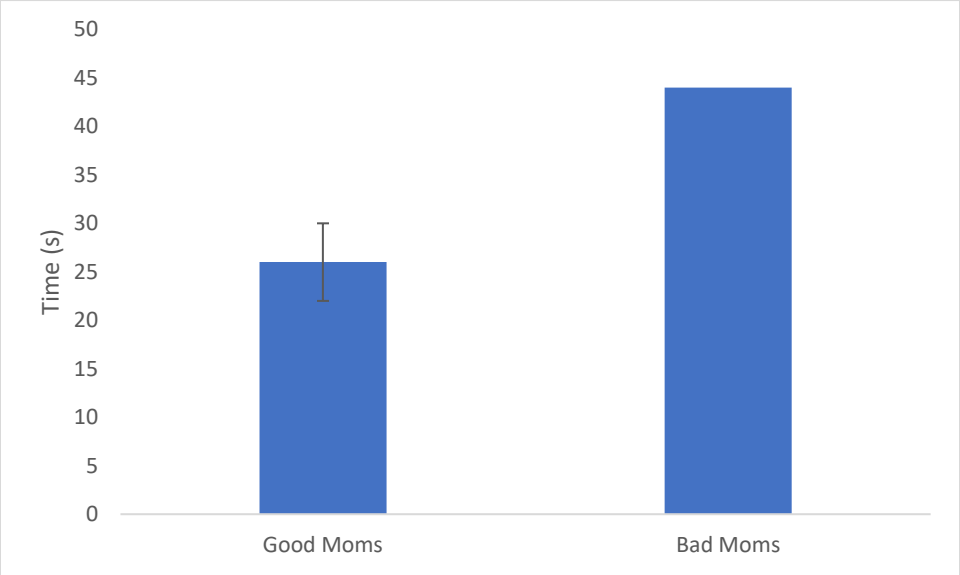


Figure 10. Good vs Bad Moms Overall Time Spend with Stationary Object for Object Location Memory Test F1 Nulliparous Female Rats.

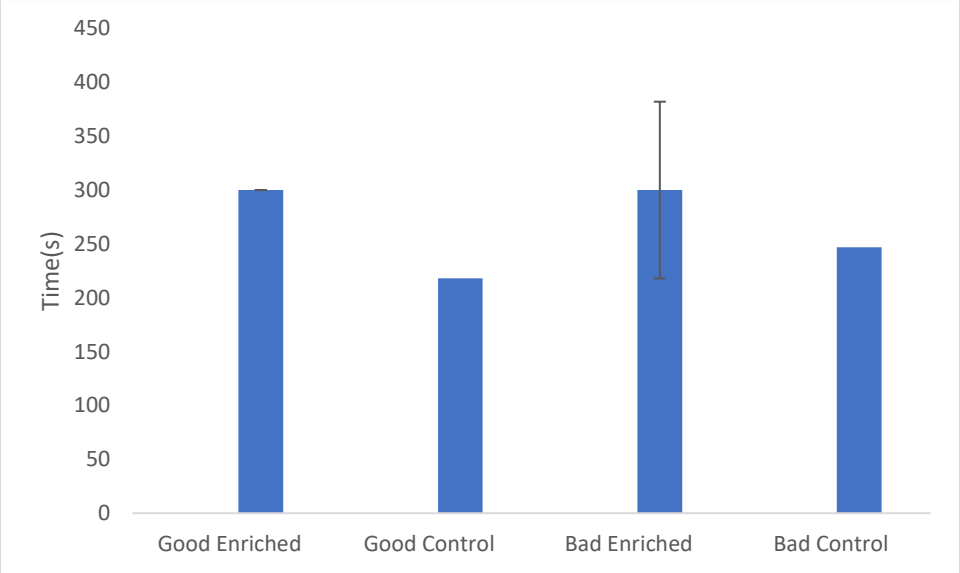


Figure 11. Force Swim Test: Latency to Dive F1 Nulliparous Female Rats.

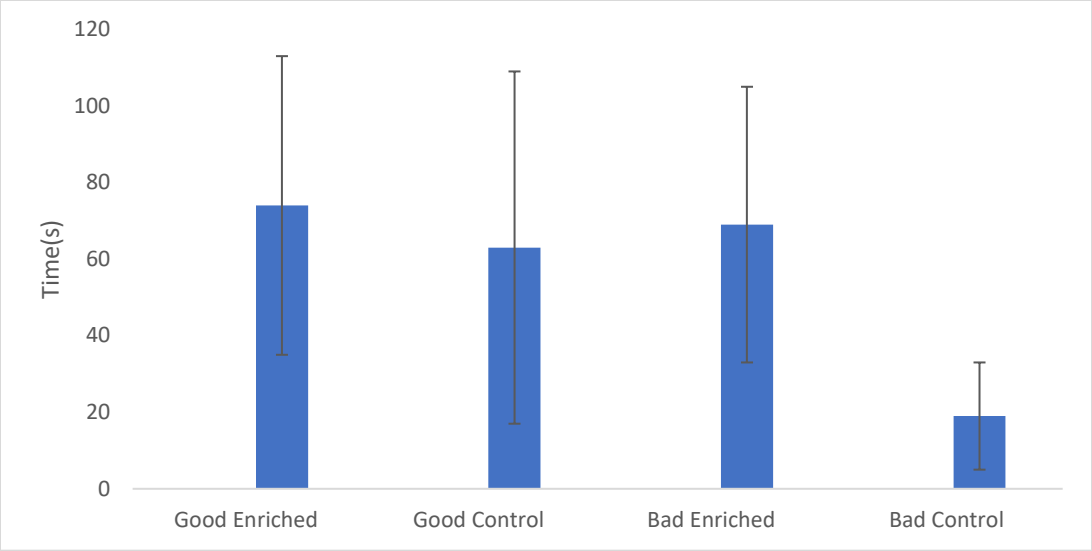


Figure 12. Force Swim Test: Time Spend Floating F1 Nulliparous Female Rats.

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