Epigenetically Heritable Effects of Maternal Behavior in Rats

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PRISM 2021

Abstract

Introduction

Nature vs. Nurture

The human brain is able to adapt to and learn from many differences and challenges within the environment, however these environmental challenges can cause psychiatric disorders in individuals who are more susceptible. The big question of nature vs. nurture tries to understand how the brain's responses to stress can be sculpted and manipulated (Zannas, Anthony S. and Anne E. West, 2013). Research has shown that prenatal stress can be passed onto future generations, leading to the idea that individuals who are bad moms raise bad moms and so forth (Roseboom et al. 2011). Children's psychopathology risk and behavior can be influenced by parental characteristics that include mental health, migrant status, and socioeconomic position (Melchior, Maria and Judith van der Waerden, 2016). It is essential for future progressions in neurological and psychological research to understand whether or not the environment impacts the brain's ability to cope with stress.

Epigenetics

Epigenetics focuses on how the environment and other outward stressors, not relating to genes, can manipulate the genes that are expressed or not expressed. Research today has brought to light the idea of epigenetics, and how the environment can play a role in various mechanisms of the brain, including stress responses, memory, and neuroplasticity (Stankiewicz et al., 2013; Wildemichael, 2014). Three mechanisms of epigenetics have been frequently studied: DNA methylation, histone modification, and microRNA activity (Stankiewicz et al., 2013).

Maternal Care

The development of cognitive, endocrine, and behavioral stress responses in the rat can be influenced by maternal care (Champagne). It has been observed that high grooming mothers raise pups that grow up to become high grooming mothers and vice versa, but the question remains: whether this behavior is genetically or epigenetically inherited. Two approaches can be used to determine the inheritance: swapping pups from one litter to another (Francis et al, 1999) and focusing on hormone expression and DNA Methylation (Champagne et al, 2001).

When comparing non-mothers to mother rats, the mother rats have improved spatial memory and non-spatial memory (Kinsley et al., 2008), less anxiety (Massimo et al., 2011; Pawulski et al., 2016), and improved resiliency (Franssen et al., 2012). In a previous study, mother rats were able to overcome various stressors including induced seizures more quickly and efficiently than rats who were never mothers (Franssen et al., 2012).

Enrichment

Previous research has shown that enriched environments can improve learning and memory, decrease anxiety (Livingston-Thomas et al., 2016), and stimulate neurogenesis (Kempermann, 2019). Many labs have used pair-housing, nestlets, bigger cages, and chew toys as ways to stimulate the rat's sensorimotor systems. These enrichment environments (EE) are known to modulate hippocampal neurogenesis and behavior, which can increase newborn neurons and enhance hippocampus-dependent cognition (Clemenson et al., 2015).

Approach

We proposed that the epigenetic effects of maternal behavior are more far-reaching than what was previously reported. Like parental care, we proposed that maternal behavior will have transgenerational epigenetic consequences for neuro-flexibility, memory, and anxiety. This research focused on identifying cognitive differences in rats with "Good" and "Bad" mothers using five behavioral tasks across generations and developmental stages. We hypothesized that rats who had "Good" mothers would have lower levels of anxiety, have better memory and neuro-flexibility, and be better mothers to their offspring.

Materials and Methods

Animal Subjects

Thirty-two rats (*Rattus norvegicus*) 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.

Rats were divided into four categories based on housing and their mother. The four categories were as follows: good enriched (GE), good control (GC), bad enriched (BE), and bad control (BC). A rat who was labeled as good had a good mother and vice versa. Housing varied between the good and bad rats through the use of enrichment. An enriched rat had a chew toy, tunnel, and nestlet while the control group rats did not have any neurostimulating items within their cages.

Behavioral Tests

The rats were tested based off of a generational time scale as well as a developmental one. Testing first began when these rats were nulliparous females. The five tests used during these trials were Pup Recognition (identification of good or bad mom), Object Location Maze (spatial memory), Novel Object Preference (non-spatial memory), Elevated Plus Maze (boldness/anxiety), and Forced Swim Test (neuroplasticity).

For the object location maze (OLM), a female rat was placed into the observation room for five minutes the day before her trial for a habituation period. This period was too ---. The maze consisted of a clear box that was 18in x 18in x 18in (Noldus, Leesburg, VA). The next day, the female rat was placed into the maze that contained two identical objects in the top two corners of the maze. She "trained" for ten minutes. After waiting at least an hour, she was placed back into the maze with the two identical objects only this time one was moved to a different corner. Data was collected on the time spent with the stationary versus the moved object in order to determine whether or not she exhibited good spatial memory.

Novel object preference (NOP) was used to test non-spatial memory. For this test, a female rat was placed into the observation room before her test began for five minutes for an acclimation period. After those five minutes, the rat was placed into the same maze that was used for OLM with two identical objects. She trained for ten minutes. After waiting at least an hour, she was placed back into the observation room where she was allowed to acclimate for another five minutes. Then she was placed into the same maze but this time with two different objects located in the same place. Data was scored based on the time they spent with each object.

Elevated plus maze (EPM) was used to test for anxiety and boldness. The maze consists of four arms, two open arms and two closed arms, that are both 12 inches long. The rats were allowed to acclimate in the room for 20 minutes in their transfer cage before the testing began. After the acclimation period, they were placed onto one of the open arms facing another open arm. They were kept in this maze for five minutes. Data was scored based on the time spent in each arm: more time spent in the closed arm was a sign of anxiety while more time spent in the open arm was a sign of boldness.

Forced swim test (FST) tests for neuroplasticity. The FST tank is a glass 29in x 12in x 16in fish tank. Water was filled 12in deep to ensure the rats were unable to escape from the tank. The rats did not have to undergo an acclimation period. They were placed into the middle of the FST tank belly down. They were tested for a total of five minutes. The number of droppings, dives, latency to dive, latency to float, time spent floating, and time spent swimming were all counted.

The last behavioral test ran was pup recognition. Using the F2 generation pups, we tested the F1 generation mothers on their latency to retrieve, grooming, self-grooming, and nursing tasks using three separate trials: 8 of their own pups (8:0), 4 of their own pups and 4 alien pups from another mother (4:4), and 8 alien pups (0:8). Mother rats in the F1 generation were allowed to acclimate in the testing cage for 5 minutes before testing. After five minutes, a cup containing 8 total pups (either 8:0, 4:4, or 0:8) was placed into the testing cage. Testing ran for 15 minutes. Data was collected on the latency to retrieve the first, fourth, and eighth pup as well as if they groomed, self-groomed, retrieved, and nursed.

Mating

Six male lab rats were ordered (Taconic Biosciences, Germantown, NY). Six F1 generation females were chosen to undergo the first round of mating; these rats did not undergo any nulliparous testing due to mating. A male and female rat were placed into a cage almost identical to the control groups in the F1 gen during the female rat’s estrous cycle. These animals were housed together for a total of five days. After the mating process was finished for group one, another six female rats were chosen according to when they were born and what category they fell into (GE, GC, BE, or BC). The males were selected based on which females they mated with prior, with a male who mated with a GC mating with one of the other three groups and so forth. All mother rats were allowed to give birth and care for her pups.

Video Data Analysis

Once each test was concluded, the videos of each trial were analyzed to see the behavior of each rat. At least two people were timing each specific piece of data that needed to be collected following the inter-rater liability standard. For OLM, time was collected based on the amount of time the rat would spend with each object in the different locations. Any time the rat was sniffing or touching the object or sniffing the wall near the object was counted. NOP was timed in a similar fashion with all the data being surrounded by the amount of time spent with each object. EPM data was collected on the amount of time spent on the closed and open arms in three intervals: 60s, 120s, and 300s. A rat was considered “on” the open arm when she was facing it and at least one paw was on that arm. For FST, the number of droppings, time spent floating, time spent swimming, number of dives, latency to float, and latency to dive were all counted. A rat was considered “floating” after one continuous second of not attempting to swim.

Results

Behavioral Tests

The good enriched had significantly longer latency to dive states when comparing them as teens to nulls(p=0.004), and there was a strong trend for bad enriched (p=0.058) (Figure 1).The good enriched showed a trend for having a shorter latency to float value and had a high value for the time spent floating (Figure 2). There was no significant difference in the amount of time spent on the closed arm between each of the four groups. There was a downward trend for the amount of time spent on the open arms when going from the Good Enriched to the Bad Control (Figure 3). For NOP, the total time spent with the objects decreased only for the good enriched. There was a significant increase in the time spent with both objects only for the bad control (p=0.05) (Figure 4).

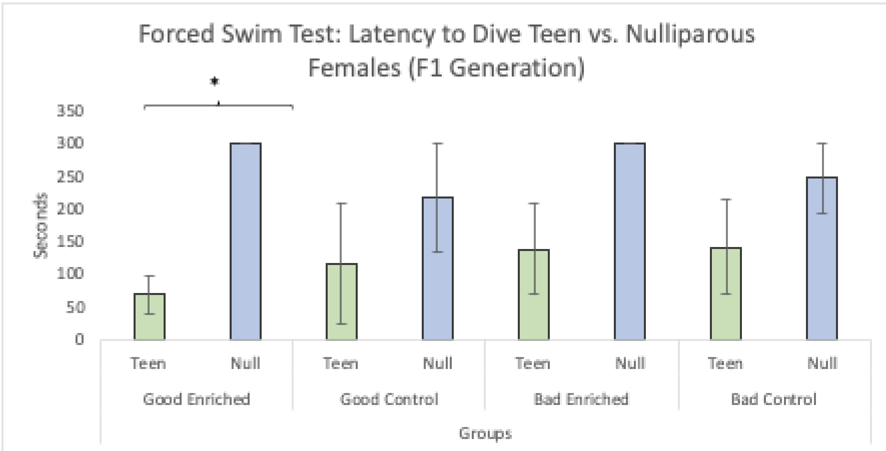

Figure 1. FST: Latency to Dive Teen vs. Nulliparous Females (F1 Generation). This figure shows the average latency to dive for each of the four categories of rats as teens and as nulls.

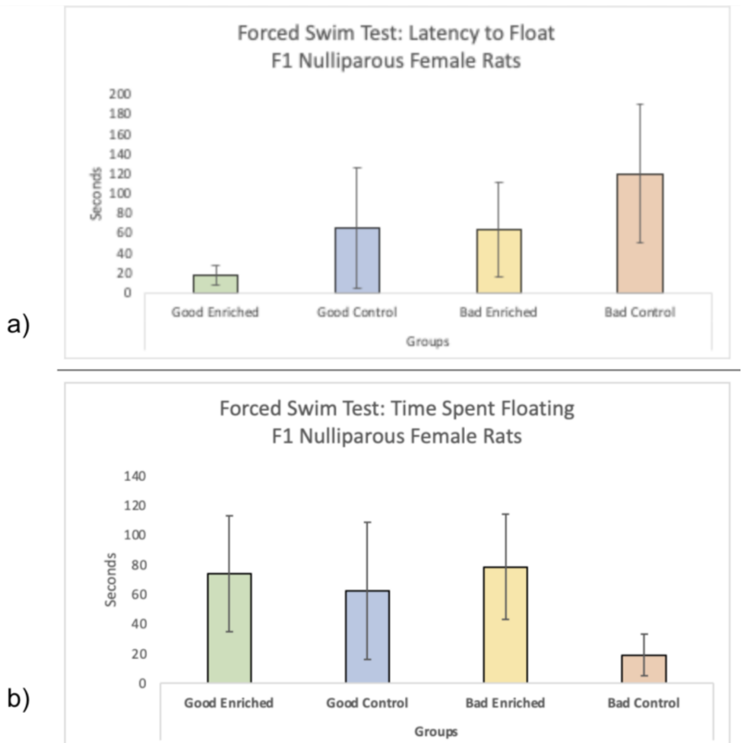

Figure 2. FST: Latency to Float and Time Spent Floating (F1 Generation). This figure shows the average latency to float (a) and time spent floating (b) for nulliparous females in all four categories.

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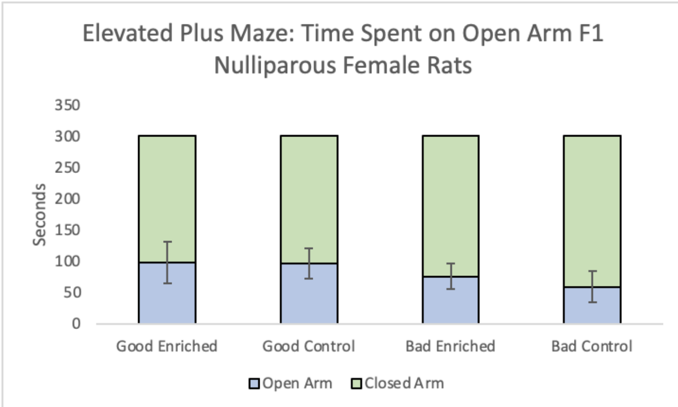

Figure 3. EPM: Time Spent on Open Arm. This figure shows the total time spent on the elevated plus maze with an emphasis on the time spent on the open arm for each of the four categories.

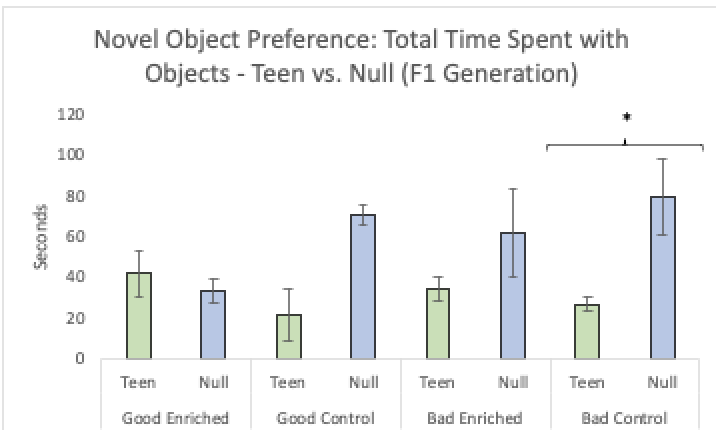

Figure 4. NOP: Time Spent with Objects Across Developmental Stages. This figure shows the total time spent with the objects for NOP for each of the four categories as teens and nulls.

Statistical Analyses Across Developmental Stages

When comparing the latency to dive during the FST for the Teen and Nulliparous developmental stages, the GE rats were the only ones to show a significant difference in the latency to dive when compared to the other three categories (Figure 5). The bad enriched showed a strong trend similar to the GE. The time spent with the stationary object for OLM differed across the developmental stages between each of the four categories of rats. The good enriched category was the only one to show a strong trend for decreasing the time they spent with the stationary object (p=0.086).

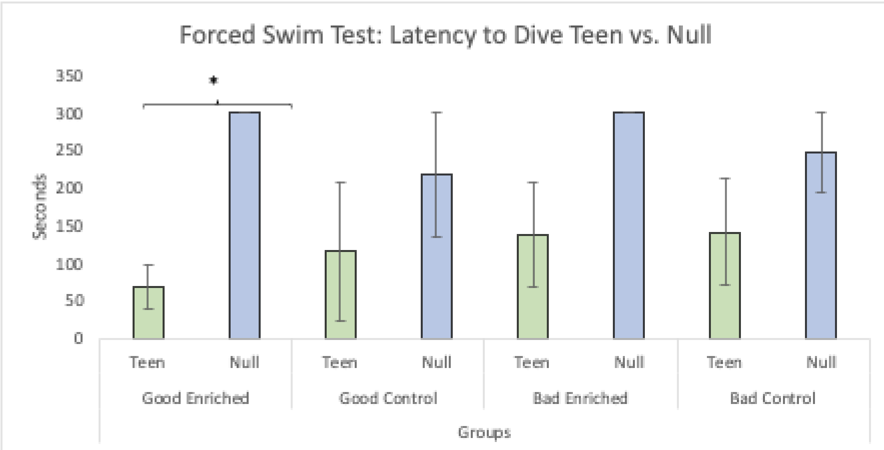

Figure 5. FST: Latency to Dive Across Developmental Stages. This figure shows the average latency to dive for each group during the teen and nulliparous developmental stages.

Discussion

Rats in the bad control category did not use effective coping strategies (i.e. floating) and are slow adopters, meaning they are not quick to identify their situation. Statistically, there is no difference between each of the four groups. The good enriched are better adopters, however they do not spend more time floating than the bad enriched category (p=0.94) which raises the question whether or not there is a constraint on how long these rats can float. Having two forms of enrichment benefits how quickly the rats are able to identify their situation and begin floating. Having a good mother is sufficient to improve coping strategies; however, having two forms of enrichment does not improve coping strategies more (Figure 2).

Rats in the good enriched category spent significantly less time with the novel object when compared to the other three groups (GEvsGC - p=0.02; GEvsBE - p=0.001; GEvsBC - p=0.04). It was then shown that the good enriched spent significantly less time with both objects as nulliparous females than the good control and bad control categories. There was a trend for them to spend significantly less time with both objects when compared to the bad enriched category. This could answer why they spent significantly less time with novel object; there was not a problem with the test, they just learned not to explore the maze as much (Figure 6).

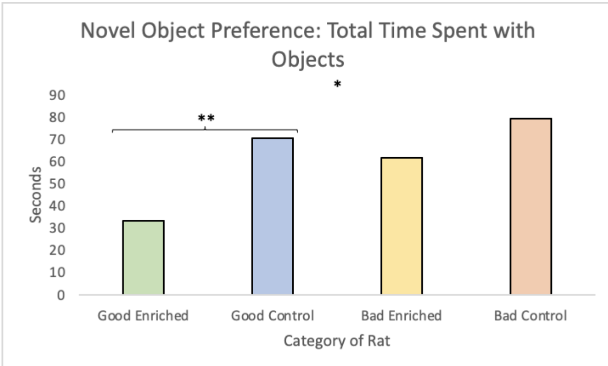

Figure 6. NOP: Total Time Spent with Objects. This figure shows the total time spent with both the same and novel object for all four categories of rats.

For OLM, there were differences in the amount of time spent with the stationary and moved objects across developmental stages. This could be due to the fact that they had already been in the maze once as teens, so they did not feel like they had to explore the cage as much or interact with the objects as much.

It was interesting to see that no category spent significantly more or less time on the open arms in comparison to another group for EPM. There was only a slight trend which showed that rats from good mothers, no matter the enrichment, spent more time on the open arms in comparison to the rats from bad mothers, with rats from the bad control group showing the least amount of time (Figure 3). This shows that having one form of enrichment, whether it be an enriched environment or having a good mother, can potentially make the rat more bold in comparison to no enrichment. Having a good mother in this instance is better than enrichment.

When collectively looking at the data, having a good mother only helps improve spatial memory ($p=0.0058$), while enrichment helps improve non-spatial memory and shows a trend for helping spatial memory ($p=0.0007$ & $p=0.0765$).

Potential limitations include having the one rat die before testing could be conducted which narrowed the data for the GC group. Also, many rats demonstrated behaviors that appeared to resemble learning the tests which could have skewed the data. More research can be done to determine whether or not this is genetically or epigenetically inherited. Future studies should control for the learned behaviors throughout each of the developmental stages.

Although there was not a lot of statistical significance found in the tests, a lot of trends pointed towards enrichment helping rats from bad mothers overcome their challenges. This can be applied to humans to show that enriching an environment may be able to improve a child's resilience and allow them to function as they would if they had a good mother.

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