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The Effects of Maternal Behavior and Environmental Enrichment on Behavior and Cognition in Sprague-Dawley Rats

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by

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This thesis has been read and approved by the following supervisory committee and submitted in its final draft to the Longwood Senior Thesis Committee in the conferment of Honors towards the degree of Bachelor in Biology.

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Table of Contents

List of Figures:

Abstract

Early environments critically impact the development of many organisms. Exposure to varying maternal care behaviors and environmental enrichments can cause persisting changes throughout a rat's life. The current study examines the effect of high and low levels of maternal care and environmental enrichment on anxiety, resilience, spatial memory, and non-spatial memory. Maternal rats were identified as Good or Bad mothers based on their pup retrieval speed. Their pups were then sorted by maternal care they experienced and placed into two housing conditions: enriched, and control (resulting in four conditions total). Each group was assessed on four behavioral tests including the elevated plus maze to measure anxiety, the novel object preference test and object location memory task to measure non-spatial and spatial memory respectively, and the forced swim test to measure resiliency based on level of escape behavior. Preliminary results found that Good mothers had better spatial and non-spatial memory than Bad mothers. Behavioral results from the adolescent pups showed pups raised by Good mothers who were in enriched housing had better non-spatial memory. Pups that had a Bad mother and lived in control housing exhibited deficits in spatial memory. In addition, the level of maternal care determined the level of resiliency in the adolescent pups. Overall, environmental enrichment and early maternal behavior had an influence on the memory and resiliency in rats.

Dedication

This thesis is dedicated to my parents, Hudson Bates and Helen Cunny, for always supporting me in all my endeavors and making me the researcher I am today.

Introduction

The early developmental environment is a critical period for cognitive development in nearly all mammalian species, including humans and rats (Elmadih & Abumadini, 2019, Szyf & Bick, 2013). Early life experiences can cause long lasting changes that can affect neurological, physical, and behavioral development of an individual. In particular, the relationship between a mother and her young is very impactful. The early-life interactions that human babies and rat pups have with their mothers has been shown to shape the offspring's neurodevelopment, which then translates into adult behaviors (Champagne & Curley, 2009, Curley et al., 2011). In particular, research done on early neurodevelopment in rats has shown that early life experiences can influence anxiety, resiliency, response to stress, learning, and memory (Sparling et al., 2018, Stairs et al., 2020, Segovia et al., 2008, Mileva & Bielajew, 2015, de Carvalho et al., 2015, West, 1990), all of which can affect the future maternal care behavior of the offspring (Zuena et al., 2016). What's more, a growing body of evidence suggests that the early-life interactions between a mother and offspring can have generational effects. In rats, it has been demonstrated that a rat's level of maternal care for their offspring is passed to their offspring. It has been reported that pups grow up and mother their pups in the same way that they were mothered (Francis et al. 1999, Champagne, 2008), a remarkable example of epigenetic inheritance (van Otterdijk et al., 2016, Francis et al., 2002). Findings such as these have dramatically altered the understanding of how traits are passed across generations. Rather than focusing exclusively on genetic traits, we now know that changes in gene expression and methylation can be caused by the environment. These methylation changes can then be passed from one generation to the next.

Maternal Behavior

Early maternal care behavior is one of the first early life experiences any animal receives. Rat mothers care for their pups by nursing them, keeping them warm, grooming and licking them, and retrieving them when they leave the nest. Pup retrieval has been used as a tool to help assess maternal behavior and the level of maternal care given to offspring (Berretta et al., 2019). Latency to retrieve pups of their own litter, pups of another mother, and a mixed ratio of pups has been used to compare rats within a cohort on their level of maternal care. The faster a mother retrieves the pups the more likely she is to engage in other maternal behaviors, such as grooming or nursing (Dias et al., 2015, Weaver et al., 2004). Mothers that are slower to retrieve their pups are more likely to engage in inattentive behaviors, like self-grooming and cage exploration, than to engage with the pups (Dias, et al. 2015, Weaver et al. 2004). Prior work in the Franssen Lab has used pup retrieval tests to allow researchers to sort mothers into two groups: Good moms, who retrieve the pups quickly and care for them and Bad moms, who retrieve their pups slowly or not at all (Franssen et al., in prep). The impact of having a Good or Bad mother can be seen across generations (Dias et al., 2015).

Female rat pups raised by Good mothers that are quick to retrieve them are more likely to be quick to retrieve their own pups later on (Dias et al., 2015). This includes cross fostering. If a pup is born to a mother that is slower to retrieve her pups but is raised by a faster retrieving mother, she will take on the maternal characteristics of the mother who raised her (Dias, et al. 2015, Francis et al. 1999, Champagne, 2008). Cross fostering demonstrates that maternal behaviors are not solely determined by genetics, and environmental influences can play a large role. Evidence has been found to suggest that factors of early maternal care can result in epigenetic changes, such as with the glucocorticoid receptor gene in the hippocampus, that can persist throughout life, and in some cases, across generations (Eşel, 2010, Weaver et al. 2004).

Housing Conditions

While maternal care behavior has been shown to cause both behavioral and neurological changes it is not the only way these changes can happen. Environmental enrichment has also been shown to influence neurological and behavioral changes. Environmental enrichment for rats typically consists of inanimate object enrichment and social enrichment (Rosenzweig & Bennett, 1996). These factors include housing enrichment such as huts and tunnels, play enrichment such as balls and chew blocks, scavenging enrichment such as hiding food and letting the rats out of their cages, and social enrichment such as housing multiple rats together. Environmental enrichment can reduce anxiety, stress, and anhedonia, while increasing resiliency and motivation (Sparling et al., 2018, Stairs et al., 2020, Segovia et al., 2008, Mileva & Bielajew, 2015, de Carvalho et al., 2015, West, 1990). In addition, environmental enrichment can help rats improve in various cognitive tasks, such as both spatial and nonspatial memory (Harris et al., 2009).

Good or Bad maternal behavior has been linked to specific behavioral traits that are similar to the traits that can be affected by environmental enrichment. Good moms are typically less anxious than Bad moms. The Meaney Lab has shown they can change Good mothers to Bad mothers by injecting a drug that increases the level of methylation on specific genes (Meaney, 2010, Champagne & Curley, 2009). Environmental enrichment has been shown to reduce the level of anxiety in rats (Zuena, 2016, Berretta et al., 2019). It is possible that by enriching a rat's environment, we could change the behaviors of the rat, which could change their maternal care behavior in the future.

Unsolved Questions

Despite the increasing amount of research that has been conducted on the individual effects of environmental enrichment and Good or Bad maternal care, there is still the question of to what extent these environmental influences can change behavior, especially when they are both acting together on the rat. Would it be possible for environmental enrichment to overcome negative effects of Bad maternal care on tests of anxiety, memory, and resiliency, and could the differences in behavior result in future maternal care differences? Environmental enrichment can affect many behaviors such as anxiety, stress responses, and memory. All of these behaviors have been linked to the maternal care behavior. Good mothers are typically less anxious and more relaxed while the opposite is true for Bad mothers (Zuena, 2016, Berretta et al., 2019). This suggests that if there are changes in these behaviors, then this may predict that their maternal care behaviors could have been altered. For example, if the offspring of a Bad mom has low anxiety and is relaxed then this could indicate that they will show Good maternal care behaviors in the future.

In order to assess behaviors the present study will examine how pups exposed to differing maternal care and environmental enrichment scored on four different behavioral tests. The following tests were selected because they measure behavioral traits that have been linked to maternal behavior and can be influenced by environmental enrichment (Meaney, 2010, Tarantino et al., 2011, Zuena et al., 2016, de Oliveira Soares et al., 2014, Sampedro-Piquero et al., 2013). The elevated plus maze (EPM) measures anxiety levels (Ravenelle et al., 2014, Pritchard et al., 2013). Good mothers are typically less anxious than bad mothers (Zuena, 2016, Berretta et al., 2019). Novel object preference test (NOP) measures non-spatial memory, while object location memory test (OLM) measures spatial memory (Birch et al., 2013, Stanford Medicine, 2021).

Good mothers typically have better spatial and non-spatial memory then Bad mothers (Liu et al., 2000). Lastly, the forced swim test (FST) measures resilience and adaptability (Stairs et al., 2020, West, 1990). Uchida et al. suggested that rats raised by Good mothers would have higher resiliency and adaptability then those raised by Bad mothers (2010). The more resilient rats would explore the tank more looking for an escape. This includes diving more and swimming more before deciding to float.

I predicted that pups with Good mothers who lived in an enriched housing condition will be less anxious, have higher resilience, and a superior spatial and non-spatial memory than the rats with Bad mothers who lived in control housing. On the other end of the spectrum I predict pups with Bad mothers and lived in control cages, with no added environmental enrichment, will be more anxious, less resilient, and have less advanced spatial and non-spatial memory when compared to the rats born to Good mothers and/or any of the rats living in enriched housing conditions.

Methods

Animals

Fifteen pregnant female Sprague-Dawley rats were ordered from Taconic Biosciences Inc. to serve as the parental generation (P) and stock for the new animal housing facility in Allen Hall at Longwood University. Of these, fourteen females delivered litters, and raised pups (F1 generation) through the weaning period. After weaning, thirty-two F1 generation females were pseudo-randomly selected (an even number from each litter) and sorted into four groups as described below.

Housing Conditions

Upon arrival, each pregnant female was housed individually in an Innovive IVC Rat Rack, using an IVC Rat Caging System (Innovive, US, Figure 1). After delivery she was housed with her pups. For the duration of the study, rats were provided food and water *ad libitum*, and animal room conditions provided constant temperature (70°F), humidity (35%), and light cycle (12/12). Cages were cleaned weekly.

All protocols were approved by the Longwood University IACUC and were in adherence with the Animal Welfare Act and the U.S. Department of Health and Human Services "Guide for the Care and Use of Laboratory Animals".

F1 Groups and Housing

On postnatal day 35, pups were weaned from their mothers, males culled, and thirty-two F1 females were sorted into four groups: 1) pups from Good mothers (those whose mothers who exhibited the highest levels of maternal retrieval) in enriched housing, 2) pups from Good mothers in control housing, 3) pups from Bad mothers (those whose mothers exhibited lowest levels of maternal retrieval) in enriched housing, 4) pups from Bad mothers in control housing (Figure 1). In all conditions, female siblings experienced a baseline level of enrichment where they were pair housed. Seven days after weaning, one female pup who had a Good mother, and was in a control cage died of unknown causes.

Enrichment for F1 Animals

For this experiment, the F1 enriched condition consisted of two additional forms of enrichment (in addition to pair housing). The first was enriched housing. Enriched cages had three types of enrichment: housing (a cardboard hut or cardboard tunnel), chew toy (wooden log or wooden block), and a nestlet (Figure 2). The enrichment schedule was adapted from Mann & Gervais study (2011). Like Mann & Gervais the enrichment was rotated once a week and moved around in the cages halfway through the week. At all times enriched cages contained the same three types of housing enrichment.

The second form of enrichment was exploration time outside of the home cage. For three weeks, beginning at age 35 days, F1 rats were put into exercise balls twice a week and allowed to explore one of the laboratory rooms for 20 minutes (Figure 3). Eight rats from the same condition (i.e., Good Enriched or Bad Enriched) rolled around at the same time, and were able to see, smell, and bump into one another's exercise ball.

Procedure for Testing for Behavioral Tasks

The P generation was presented with three different ratios of pups during a Maternal Behavioral Retrieval exercise to determine if they were Good or Bad mothers. This test was performed when the pups were between the ages of 5-7 days old.

All P generation and F1 generation rats were tested on four behavioral tasks: Elevated Plus Maze, Novel Object Preference Test, Object Location Memory Test, and Forced Swim Test. Each test occurred on different days, and no rat experienced two different tests on the same day. The P generation was tested as post lactational, between ten- and sixteen-days post weaning. F1 generation rats were tested as adolescents, between eight and ten weeks old.

Maternal Behavioral Retrieval

To determine if a P generation rat was a Good or Bad mother, each maternal rat participated in a retrieval task (Figure 4). The pup retrieval task was conducted in a similar manor of Franssen et al.'s previous research (in prep). Mother rats were moved from the Innovive rat rack to a Test Cage in a separate behavioral testing room. The Test Cage was identical to home cages except that food was removed. Mothers were placed alone in the test cage in the behavior testing room for a five-minute acclimation. During this time, her pups remained in the main housing room, kept under a heat lamp.

Following the five-minute acclimation period was the testing period. During the testing period, mothers were presented with pups in a Pyrex glass bowl. The pups presented were in one of three ratios: 8 of her own pups:0 pups from another mother (alien), 4 own:4 alien, and 0 own:8 alien. Each mother participated in three trials, presented with a different ratio each time. Mother rats were given five minutes to retrieve and care for the pups, at which time the trial ended, and mothers and pups were returned to their home cages. These ratios of pups were determined to be necessary to identify the maternal care behavior of each rat (Franssen et al., in prep).

Pup ratios were pseudo-randomly varied among all mothers tested. To identify which pups were own and alien, the researchers marked each pup with a scentless non-toxic marker. To protect pups, no mother or pups were tested within an hour and a half of their last trial. Between trials, the Pyrex bowls were cleaned with Alconox and water to remove the scents of the rats before them.

All trials were filmed from the side and later assessed for the mother's latency to retrieve one, four, and eight pups. The maternal behaviors nesting, nursing, and grooming, along with the non-maternal behaviors of exploring, self-grooming, and sleeping were also assessed. The average number of seconds to retrieve the first pup in each condition was used to categorize Good and Bad mothers.

Elevated Plus Maze

The elevated plus maze was set up and run in a similar fashion to Lezak et al. (2017). The elevated plus maze was 40 cm off the ground and consists of four plastic arms – two with high, protective walls, and two arms that are open to the room, set in the shape of a plus sign. The center space was open (Figure 5). To prevent the rat from using identifying wall makers, a curtain was hung from the ceiling around the maze, limiting the rat's ability to see the rest of the room.

Rats were given two minutes to acclimate to the room in a transfer cage with standard bedding and water before being placed into the maze. Each rat was placed halfway down one of the open arms facing the center and allowed to explore the maze for five minutes. Following trials, we analyzed video and recorded time spent in open arms and time spent in closed arms. After each trail, the maze was cleaned with Alconox and water to remove possible odor residues.

Novel Object Preference Test

The Novel Object Preference test was adapted from Lueptow and took place in an open field maze: a clear 26 cm by 26 cm by 25 cm plastic box (2017). In setting up the test, two identical objects were placed in corners that were adjacent to one another (Figure 6a). The rat being tested was gently placed equidistant from each object and released to begin the two-minute test period. During the test, rats were allowed to freely explore for two minutes. Next, the rat was removed from the maze, returned to a transfer cage, and moved out of the behavioral testing room for five minutes. During those five minutes, the open field maze and objects were cleaned with Alconox and water. For the second part of the test, a novel object was selected and replaced one of the familiar objects in the open field (Figure 6b). The novel object was pseudo-randomly placed on the right or left side of the maze for each animal's test. Once the maze was reset, the rat was placed back into the open field maze and allowed to explore the two objects for two minutes. Following all animal trials, video recordings of the trials were analyzed for time spent with the novel object and time spent with the familiar object. After each trial, the NOP task box was cleaned with Alconox and water and thoroughly dried.

Objects consisted of various small items including metal combination locks, plastic back massagers, glass containers, metal drain catchers, and metal containers. All items were used approximately the same number of times.

Object Location Memory Task

The Object Location Memory Task took place in the same open field maze as described in the Novel Object Preference Test. The object location memory task was conducted in the same manner as described in Vogel-Cierna & Wood (2014). Additionally, the task begins in the same way as the NOP task, with two identical objects placed in the corners of the maze (Figure 7a). The tested animal was first allowed five minutes to explore the maze and the identical objects. After five minutes, the rat was removed from the open field maze and returned to their home cage. One hour later, the rat was put back in the open field maze. Conditions were identical, except that one of the objects had been moved such that the two objects were now diagonal from one another (Figure 7b). The rat was allowed to explore the cage and objects for a total of five minutes. The amount of time rats spent with the familiar and moved objects was recorded.

Objects consisted of various small items including metal combination locks, plastic back massagers, glass containers, metal drain catchers, and metal containers. All items were used approximately the same number of times.

Forced Swim Test

The methodology of the forced swim test was adapted from Commons et al. (2017). Each rat was gently placed in a large fish tank filled with room temperature water (23ºC) to a height of approximately 40 cm. There was enough water that the rats were unable to stand and keep their heads out of the water and were also unable to touch the top of the tank to pull themselves out of the water. Each rat was monitored and allowed to swim for up to five minutes before being removed from the take and placed under a heat lamp to dry off. The following behaviors were measure: number of dives, latency for first dive, time spent swimming, and time spent floating. After each trial, all feces were removed from the tank to clean it for the next rat.

Data Analysis

At the conclusion of each behavioral task, a team of researchers analyzed data and scored behavior appropriate for the task. Teams of two or more first worked to achieve inter-rater reliability on a sample video before proceeding to score remaining videos. Raters were kept blind to whether the P generation rat was a Good or Bad mother until after data was collected and blind to the test condition of the F1 generation rats (i.e., Good enriched, Good control, Bad enriched, Bad control). The data for each behavioral test was analyzed using an independent samples t test, except for the Forced Swim Test which used a proportion Z test.

Results

Maternal behavior Pup Retrieval

The time spent to retrieve the first pup in each condition (eight of their own pups, four of their own, four of another mothers, and eight from another mother) was averaged. These averages were used to classify the mothers into Good or Bad groups based on previous research in the Franssen Lab (Franssen et al., in prep). The average retrieval speed of the first pup across the three testing conditions was used for the classifications of mothers because it was found that categorical behaviors, such as if the mother groomed the pups or not, often aligned with the retrieval speed. Figure 8 shows the data from the past three years of the Franssen's lab maternal rats. The green bars and red bars are mothers from the current study. In the current study, all rats that retrieved pups retrieved them fast enough to be considered Good mothers. The Bad mothers retrieved no pups in any of the conditions.

Elevated Plus Maze

In the P generation, rats spent significantly more time in the closed arm than the open arm ($p < .01$). However, there were no differences between the number of seconds spent in the closed arm verses the open arm for the Good mothers (M = 235, SEM = 15.4, M = 65.1, SEM = 15.4) and the Bad mothers ($p > .05$, M = 209, SEM = 26.6, M = 90.8, SEM = 26.6) (Figure 9).

The results were similar for the F1 generation. In every condition the rats spent significantly more time (s) in the closed arm then the open arm ($p < .01$), but there were no differences between how long the pups of Good mothers in enriched cages (M = 250, SEM = 12.0, M = 49.8, SEM = 12.0), pups of Good mothers in control cages (M = 251, SEM = 8.83, M = 49.3, SEM = 8.83), pups of Bad mothers in enriched cages (M = 250, SEM = 7.94, M = 51.4, SEM = 7.94), and pups of Bad mothers in control cages (M = 264.5, SEM = 8.60, M = 36.6, SEM = 8.60) spent in the closed arm verses open arms (Figure 10).

Novel Object Preference

For the novel object preference test, the amount of time spent with the new and novel object was measured in seconds and compared across groups. For the P generation, the Good mothers spent significantly more time with the novel object ($p < .05$, M = 25.9, SEM = 4.31) than the familiar object (M = 14.0, SEM = 3.82). The Bad mothers spent approximately the same amount of time exploring each object (Figure 11).

For the F1 generation, the pups raised by Good moms who were in the enriched housing spent significantly more time in seconds with the novel object ($p < .05$, M = 26.8, SEM = 4.99) than the familiar object (M = 13.6, SEM = 1.65). None of the other three conditions had significant differences (Figure 12).

Object Location Memory Test

In the P generation, the Good moms spent significantly fewer seconds with the familiar location object ($p < .05$, M = 32.1, SEM = 6.46) than the novel location object (M = 52.0, SEM = 8.87). There was no significant difference between the amount of time the Bad mothers spent with the familiar location object and the novel location object (Figure 13).

For the F1 generation, the pups raised by Bad mothers in control cages ($p < .05$, M = 54.9, SEM = 9.53) spent significantly more time with the familiar location object than the pups raised by Bad mothers in enriched cages (M = 34.8, SEM = 3.60), pups raised by Good mothers in control cages (M = 40.3, SEM = 8.29), and the pups raised by Good mothers in enriched cages (M = 37.8, SEM = 4.84) (Figure 14).

Forced Swim Test

There were no significant differences in the time spent floating or the time spent swimming in the P generation. The only notable difference was that the Bad mothers (75%) dove more than the Good mothers (30%) ($p = .06$) (Figure 15).

There were significant differences in the F1 generation when they were compared as pups of Good mothers versus pups of Bad mothers. The pups of Good mothers (93%) dove significantly more ($p < .05$) than the pups of Bad mothers (75%) (Figure 16). The pups of Good mothers also spent significantly more seconds swimming ($p < .05$, M = 232) than the amount of time the pups of Bad mothers spent swimming (M = 195) (Figure 17).

Discussion

These results represent the first findings of this on-going, longitudinal, and multigenerational study. Despite the early phase of research, it is striking that some significant behavioral differences among groups are already evident. Significant differences were noted in F1 animals related to enrichment vs non-enrichment for both the novel object preference task and the object location memory task. Differences in the forced swim test were related to maternal care behavior for F1 animals raised by Good versus Bad mothers.

The preliminary results of this study confirm that, in rats, enrichment and stimulating early environment, such as Good maternal care as pups, environmental enrichment as teens, and pregnancy and parenthood as adults, can contribute to enhanced cognition. In this study, the P generation is the only generation to experience the pregnancy and parenthood, but there is little information known about their life histories prior to arriving in the Franssen lab. All comparisons between the P generation and the F1 generation will have to take into consideration that their parentage and early environments are unknown. For the F1 and subsequent generations, the cognitive enrichment at all three stages will be carefully controlled and tracked. At the time of this writing, the F1 rats have received different levels of maternal behavior interaction and environmental enrichment.

Maternal Behavioral Retrieval

The time spent to retrieve the first pup in each of the conditions (eight own, four own and four alien, eight alien) was averaged together. These averages were compared to one another and previous data to decide which maternal rats would be considered a Good mother and a Bad mother (Figure 8). Ten rats were identified as Good mothers and four Bad mothers. We had no control over what type of mothers were ordered, but this did cause the number of rats in the Bad mom group to be lower than what was desired. The low number of Bad mothers could explain why there were some unexpected results for both the Elevated Plus Maze and Forced Swim Test in the P generation.

Elevated Plus Maze

For both the P and F1 generations, rats responded appropriately to the Elevated Plus Maze (EPM); rats spent significantly more time in the closed arms than in the open arms. This indicated that the test worked. In the EPM all rats should spend more time in the closed arms because rats naturally do not like to be in brightly lit, open spaces (Rao & Sadananda, 2016). The closed arms provide security and are considered to be the safer arms to the rats. It would have been very unusual if the rats spent significantly more time on the open arms.

All conditions spent significantly more time in the closed arms, but there were no significant differences in the P generation between the Good and Bad mothers (Figure 9). Similarly, there were no significant differences in the F1 generation related to either maternal behavior or environmental enrichment (Figure 10). This means that the rats in every condition seemed to have similar levels anxiety and boldness.

These results do not align with what some previous researchers have found (Pritchard et al., 2013, Sparling et al., 2018). However, not all of our findings are different from previous research. Research done by Kinsley & Lambert showed that mother rats were bolder and would spend more time on open arms of the EPM than non-mothers (2008). The findings in their study indicated that once a mother, there is less of a difference in state anxiety when categorized by their mothering skills (e.g. Good or Bad mothers). For the F1 adolescents, it will be interesting to determine if there are differences in anxiety/boldness when they become non-mother adults and/or lactating females as they will be closer in developmental stages and ages to when the P generation was tested.

An additional option would be to include another anxiety measure for trait anxiety. Trait anxiety is how anxious a rat or human is typically in their everyday life, while state anxiety is how anxious an individual is in that particular moment (Rao & Sadananda, 2016). It has been argued in some papers that the elevated plus maze measures a rat's state level of anxiety in that current moment (Goes et al., 2015). As more research is published examining both state and trait anxiety in rodents, there has been a movement to start measuring both state and trait to allow for a more complete understanding of the effects of environmental enrichment and early life experiences (Goes et al., 2015). These findings might be overlooked if just examining state level anxiety (Goes et al., 2015). In the future generations of this study, the addition of the Free Exploratory Paradigm test could be considered to provide a measurement for trait level anxiety. By adding in this test, we can make sure that we are fully understanding how environmental enrichment and early mothering behavior will affect anxiety.

Novel Object Preference Test

The Novel Object Preference task showed that Good mothers (P) and environmentally enriched adolescents with Good mothers (F1) spent significantly more time with the novel object (Figure 11 & 12). These rats demonstrated higher levels of non-spatial memory then any of the other groups. Previous research found that Good mothers had better non-spatial memory than Bad mothers, which supports the findings of the current study (Kinsley & Lambert, 2008; Love et al., 2005

Our results suggest that non-spatial memory is not genetically linked, but possibly epigenetically linked because the improved non-spatial memory was not present in the rats with Good mothers in control housing. Having multiple phases of a stimulating, enriched, environment appears to be necessary to improve non-spatial memory. In the F1 generation it was not sufficient to have just a Good mother (and have control housing) or enriched housing (and have a Bad mother) to see effects. Both a Good mother, and enriched housing were necessary to for improvements in non-spatial memory in the F1 generation to be present. However, the P generation of Good mothers (who were likely raised by Good mothers) had improved non-spatial memory after the stimulating, enriching, experience of raising pups. If this hypothesis of needing multiple stages of enriched environment is correct, then in the future, the F1 groups with a Good mother and control housing, and Bad mothers and enriched housing, should show improved non-spatial memory following parenthood, as this is considered to be an enriching experience.

Object Location Memory Task

The results of the object location memory task also showed that Good mothers had better spatial memory than Bad mothers. The Good mothers spent significantly more time with the novel location object than the stationary one (Figure 13). These are similar to the findings in previous research (Liu et al., 2000).

In the F1 generation, the adolescents in the control cages with Bad mothers spent significantly longer with the stationary location object than any of the other conditions (Figure 14). This shows that the rats who had Bad mothers and did not get to experience an enriched environment did not remember the locations of the objects after one hour. This could indicate that having a Good mother or having an enriched environment is enough enriching stimulation to allow for there not to be deficiencies in spatial memory because the rats with Bad mothers and enriched cages performed similarly to the rats of Good mothers.

Forced Swim Test

There was no difference in the amount of time spent swimming or floating in the P generation rats. There was a higher percentage of Bad mothers that dove (75%) than the Good mothers (30%), but that is likely linked to the fact that there were only four Bad mothers in the P generation compared to the ten Good mothers tested (Figure 15). This difference was not statistically significant. The unequal distribution of Good and Bad mothers caused the P generation to be slightly under powered, and in the case of the forced swim test we suspect that could explain why it appeared that a higher percentage of Bad mothers dove.

In the F1 generation the differences in swim behavior were linked to the maternal care behavior the rat experienced, not the type of housing the rat was exposed to. The pups of the Good mothers swam significantly more and floated significantly less than the pups of Bad mothers (Figure 16). In addition, a higher percentage of the adolescent pups of Good mothers dove (93%) than the pups of Bad mothers (75%) (Figure 17). These results can indicate that the adolescent pups of Good moms spend more time exploring and trying to escape from their environments before starting to float, potentially showing more resiliency in their behavior. This supports the findings in the Uchida et al. paper where they found resiliency and escape behavior to be linked to the type of early maternal care the rats experienced (2010).

Translational Human Applications

Both spatial and non-spatial memory seem to be affected by maternal behavior and environmental enrichment. Having a Good mother and enriched environment lead to rats that had the highest non-spatial memory. Interestingly, for spatial memory it showed that having either a Good mother or an enriched environment could cause increases in spatial memory. If this trend continues to persist in the next generations then the hypothesis could be made that environmental enrichment could be used to help improve spatial memory in offspring (rat or human) of parents who exhibit lower parental care.

This could be valuable when considering the argument for free or reduced cost for quality daycare and pre-K for all. Often parents with low socioeconomic status are the ones to struggle to be able to spend extended amounts of time with their children due to the long hours that they have to work (Mendelsohn et al., 2008). In addition, they struggle to afford quality childcare that exposes young children to attentive adults and stimulating environments (Laurin et al., 2015). A study done by Aronen et al. found that high spatial memory was one of the best indicators for academic success (2005). If environmental enrichment and stimulation can help counter the effects of having a parent with low parental care and raise skills like spatial memory, then instating some form of free or reduced cost childcare can help ensure all children are going into Kindergarten with the highest chance for succeeding.

Final Thoughts and Future Directions

These are the preliminary results of a longitudinal, multigenerational study. Even though it is too early to draw definitive conclusions about the effect of early maternal care behavior and environmental enrichment on anxiety, resiliency, and memory, there are some interesting trends and discrepancies with the previous research that should be noted. Out of the four behaviors that were measured it appeared the non-spatial and spatial memory seemed to be most affected by maternal behavior and environmental enrichment. While improved spatial memory was only present in the F1 generation of rats that had Good mothers and environmental enrichment, rats with a Good mother or environmental enrichment seemed to avoid having a deficit in non-spatial memory. This trend will be monitored to see if it persists in the next generations.

In addition there were results that did not match the primary literature that we will closely observe in the next generation of rats to see what happens. All rats had the same level of anxiety and boldness within their generation. Environmental enrichment typically decreases anxiety and increases boldness (Sparling et al., 2018), however in the current study we did not observe this trend. With the potential addition of the free exploratory paradigm and more generations, we hope to be able to better understand the current results.

In addition to the current findings, this lab aims to find the extent to which environmental enrichment can improve maternal care behavior in rats. Other studies have shown that maternal behavior, such as nursing, grooming, and retrieving, is inheritable. Pups with mothers that express high maternal behaviors are more likely to express high maternal behavior with their own pups (Cutuli et al., 2019, Weaver et al., 2004, Dias et al., 2015). Pups who had mothers that expressed low levels of maternal behavior are more likely to express low levels of maternal behavior for their own pups (Cutuli et al., 2019, Weaver et al., 2004, Dias et al., 2015). While the maternal care behavior that is expressed is inheritable, it is also highly dependent on the environment. The impact of the environment can cause epigenetic changes that are transgenerational (Meaney, 2010).

The ultimate goal of this multigenerational, longitudinal study is to see if environmental enrichment can be used to compensate for early life exposure to poor maternal behaviors. Environmental enrichment has been shown to be associated with behavioral traits of anxiety, fear, memory, and stress (Barbelivien et al., 2006, Sparling et al., 2018, Harris et al., 2009). The preliminary results of the current study show that there are behavioral changes between the groups of mothers and the offspring in enriched versus non-enriched environments. In the object location memory test, adolescent rats raised by Bad mothers, but who had environmental enrichment, preformed similarly to rats raised by Good mothers, and out preformed their control counterparts that had no enrichment. The rats raised by Good mothers in an environmentally enriched cage demonstrated better non-spatial memory in the novel object preference test, while the rats also raised by Good moms but were in control cages did not. These are changes that reflect the effect of environmental enrichment on long term behavior.

Early environmental enrichment has also been shown to change brain structures of rats, which can result in the positive impacts on anxiety and memory (Weaver et al. 2004, Francis et al. 2002, Sampedro-Piquero et al., 2013). If environmental enrichment can be used to rescue rats exposed to poor maternal behavior then this indicates that epigenetic change can happen. This allows environmental enrichment to cause persistent changes that can affect maternal behavior. The pups that had Bad mothers should, in turn, be Bad mothers and raise more Bad mothers, but if environmental enrichment can epigenetically change that, then pups of Bad mothers can become Good mothers, who will in turn raise Good mothers, breaking the previously believed genetic cycle. Once the F1 generation in this study has their own offspring then the full extent of the effect of environmental enrichment on maternal behavior can be analyzed.

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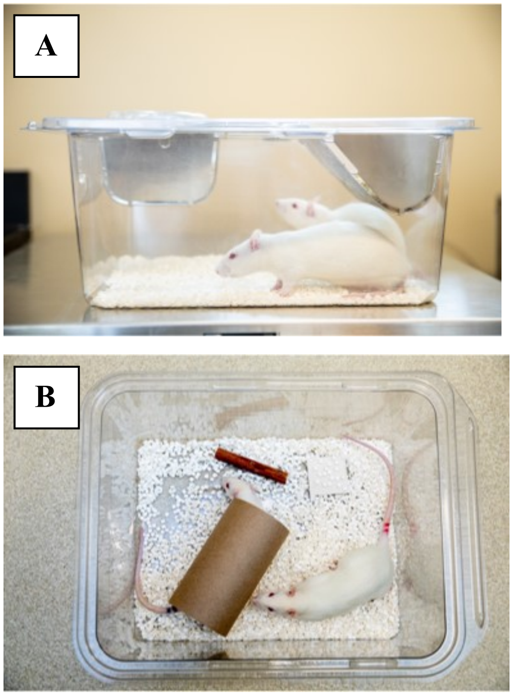
Figure 1

Housing Rack for All Cages

Note. P generation occupies the top three rows. Then going vertically down from left to right are columns containing pups with Bad moms and enriched cages, pups with Bad moms and control cages, pups with Good mothers and enriched cages, and pups with Good mothers and control cages.

Figure 2

Example of the Two Types of Enriched Cages



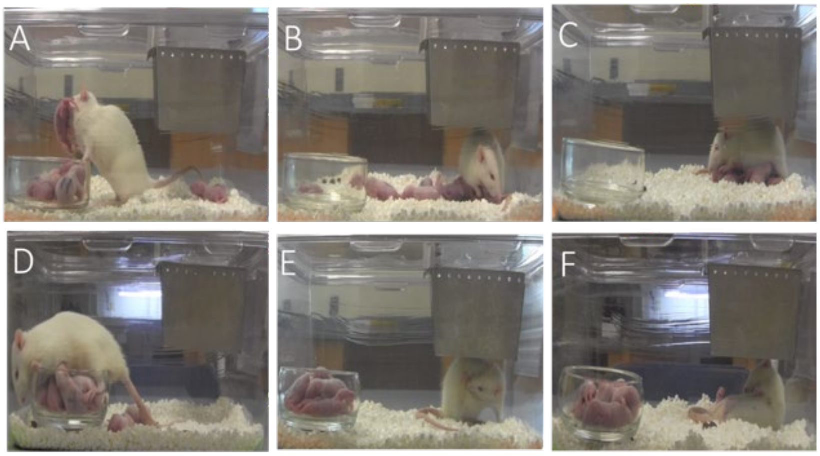
Note. Top picture (A): Control cage, rats are pair housed and given unlimited access to food and water. Bottom picture (B): Enriched cage, rats are pair housed, with unlimited access to food and water, and three pieces of enrichment: a cardboard tunnel, chewing log, and square of nestlet.

Figure 3

Enriched 40-day old Rat Rolling in Clear Plastic Balls

Figure 4

Maternal Responses to the Maternal Pup Recognition and Retrieval Test



Note. Panel A: Maternal rat retrieving pup. Panel B: Maternal rat grooming the pups. Panel C & D: Maternal rats nursing pups. Panel E: Maternal rat is self-grooming (anxiety behavior). Panel F: Maternal rat is sleeping.

Figure 5

Rat is in the Open Arm of the Elevated Plus Maze

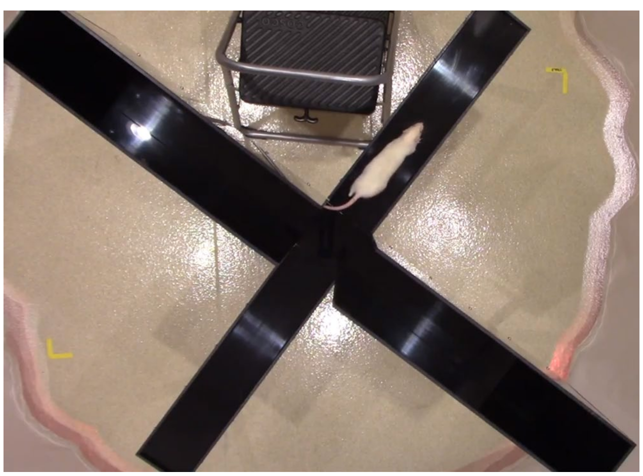
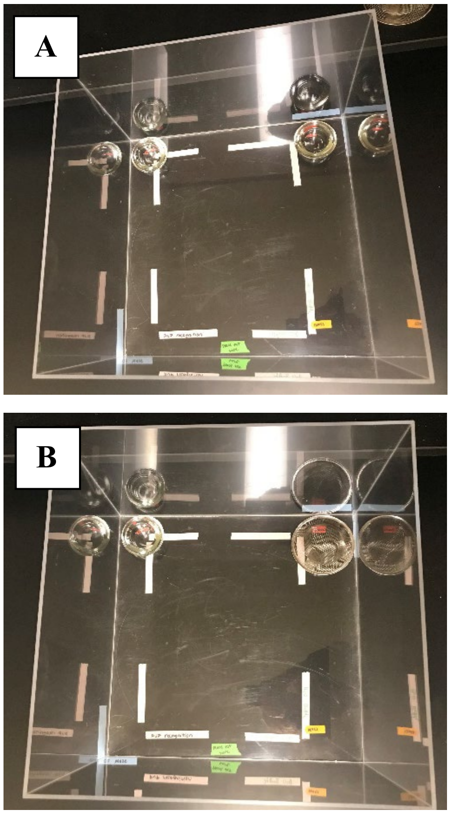


Figure 6

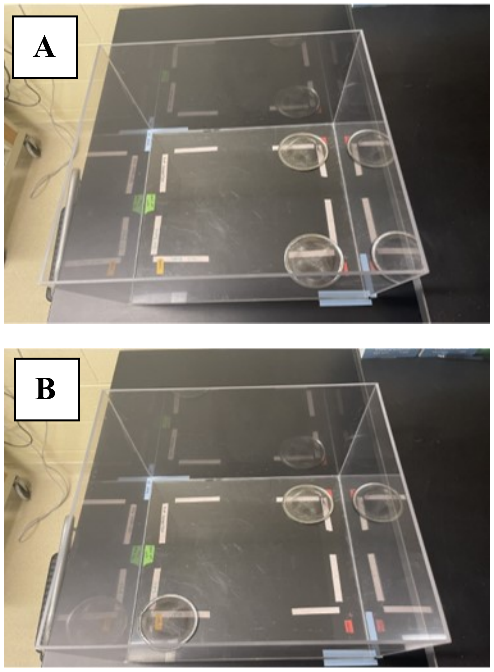
Open Field Maze Set Up for the Novel Object Preference Test



Note. Top panel (A): Both objects are the same and in adjacent corners of the container. Bottom panel (B): One object has been replaced with a clean novel object the rat has not seen before.

Figure 7

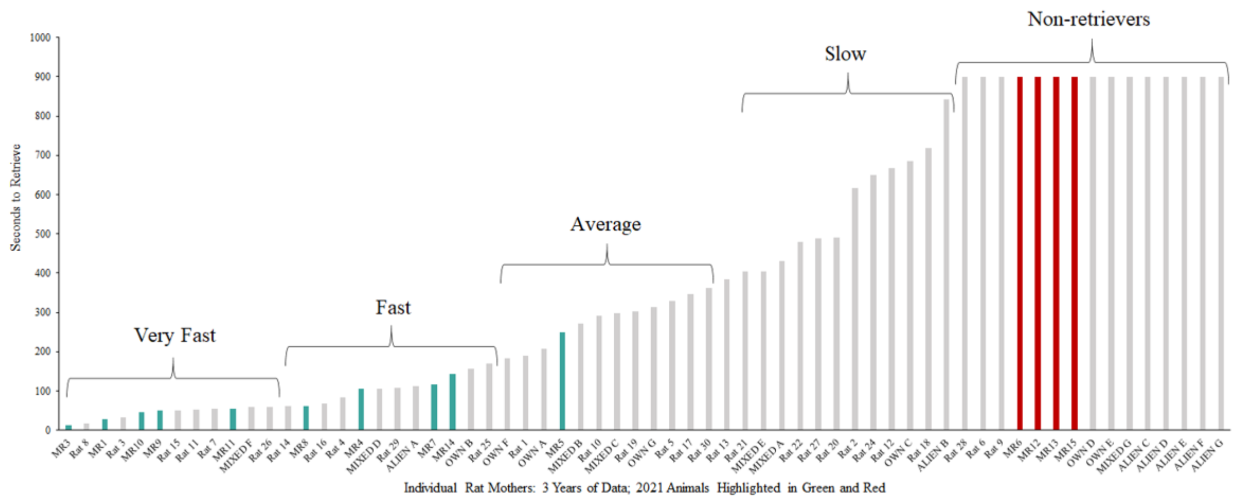
Open Field Maze Set Up for the Object Location Memory Test



Note. Top panel (A): Both objects are the same and in adjacent corners of the container. Bottom panel (B): Both objects are still the same, however one object has been moved to the diagonal corner of the cage.

Figure 8

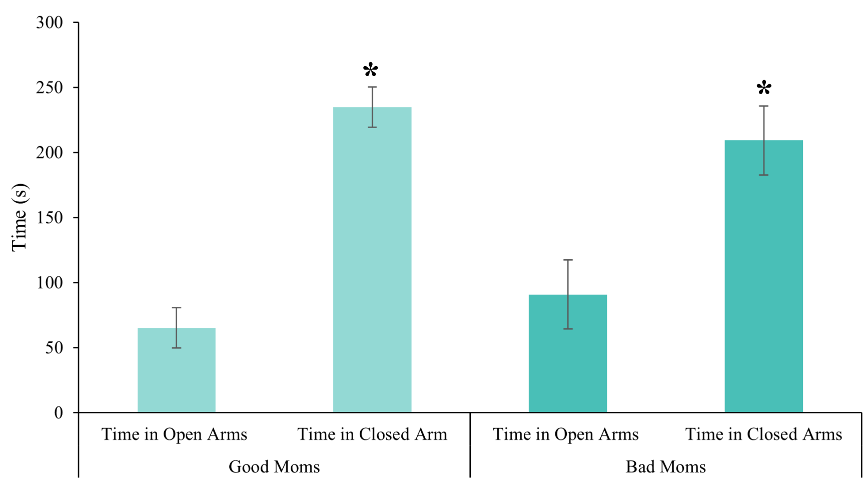
Latency to Retrieve 1st Pup Across All Trials



Note. This figure includes data from the past three years in the Franssen Lab. The rats in the current study are represented by the green and red lines. To be considered a Good mother, the maternal rat had to rank with the very fast, fast, or average retrievers. In the current study, all non-retrievers were classified as Bad.

Figure 9

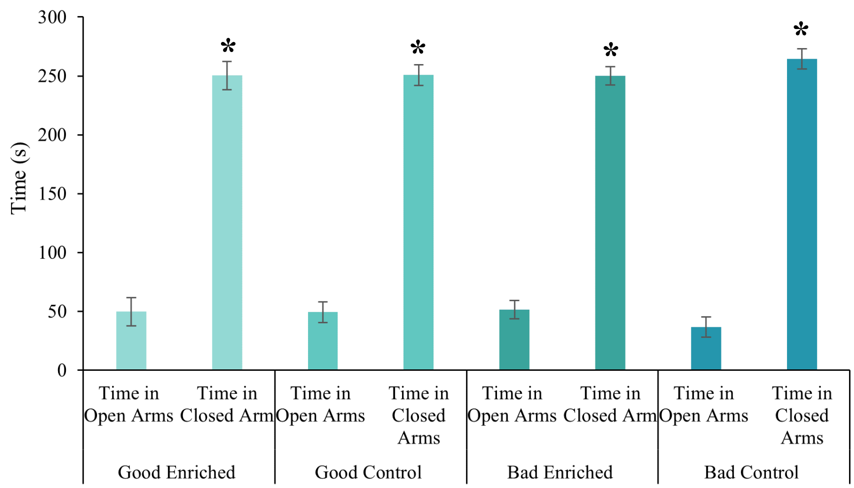
Time Spent in Open and Closed Arm on Elevated Plus Maze for P Generation



Note. Each group spent significantly more time in the closed arms ($p < .05$), but there was no significant difference between groups ($p > .05$). Error bars are ± standard error of the mean (SEM). Total N=14, Good Moms N=10, Bad Moms N=4.

Figure 10

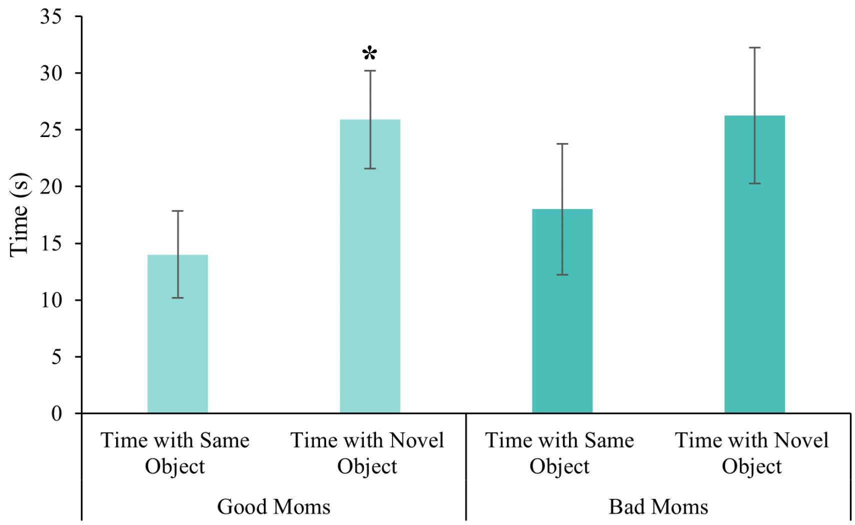
Time Spent in Open and Closed Arm on Elevated Plus Maze for F1 Generation



Note. Each group spent significantly more time in the closed arms ($p < .05$), but there were no significant differences between the four groups ($p > .05$). Error bars are ± standard error of the mean. Total N=31, Pups with Good mothers who are enriched N=8, Pups with Good mothers in standard housing N=7, Pups with Bad mothers who are enriched N=8, Pups with Bad mothers who are in standard housing N=8.

Figure 11

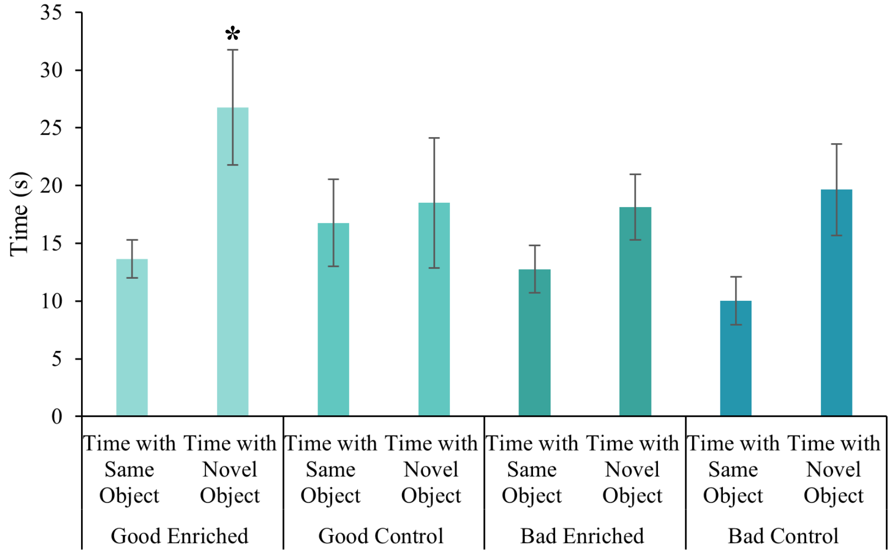
Time Spent Investigating Novel and Same Object on the Novel Object Preference Test for P Generation



Note. Good mothers spent significantly more time with the novel object than the same object (p < .05). Error bars are ± standard error of the mean. Total N=14, Good Moms N=10, Bad Moms N=4.

Figure 12

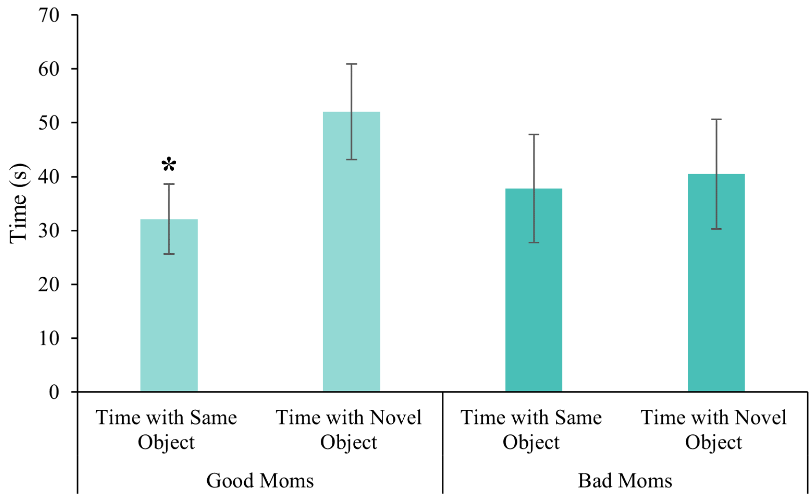
Time Spent Investigating Novel and Same Object on the Novel Object Preference Test for F1 Generation



Note. Pups raised by Good mothers in an enriched cage spent significantly more time with the novel object than the same object ($p < .05$). Error bars are ± standard error of the mean. Total N=31, Pups with Good mothers who are enriched N=8, Pups with Good mothers in standard housing N=7, Pups with Bad mothers who are enriched N=8, Pups with Bad mothers who are standard housing N=8.

Figure 13

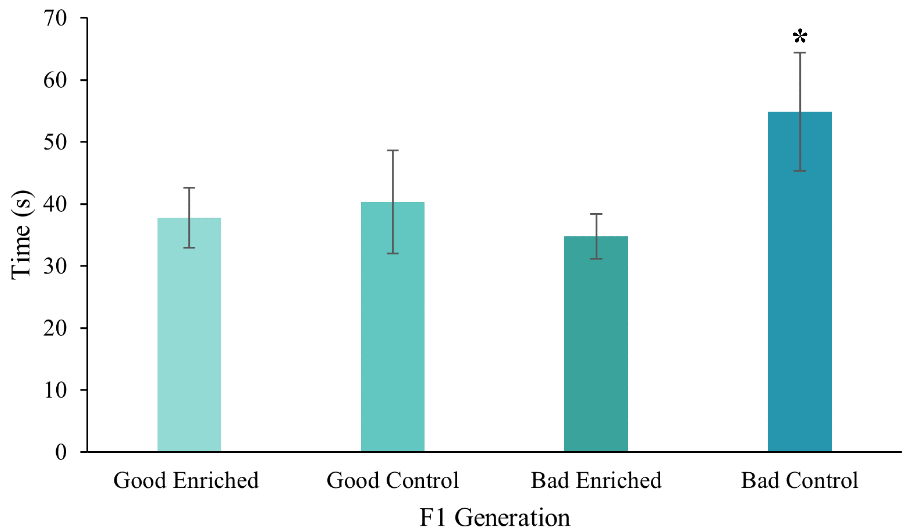
Time Spent Investigating Novel and Same Object Locations on the Object Location Memory Test for P Generation



Note. Good mothers spent significantly less time with the same object than the novel object ($p < .05$). Error bars are ± standard error of the mean. Total N=14, Good Moms N=10, Bad Moms N=4.

Figure 14

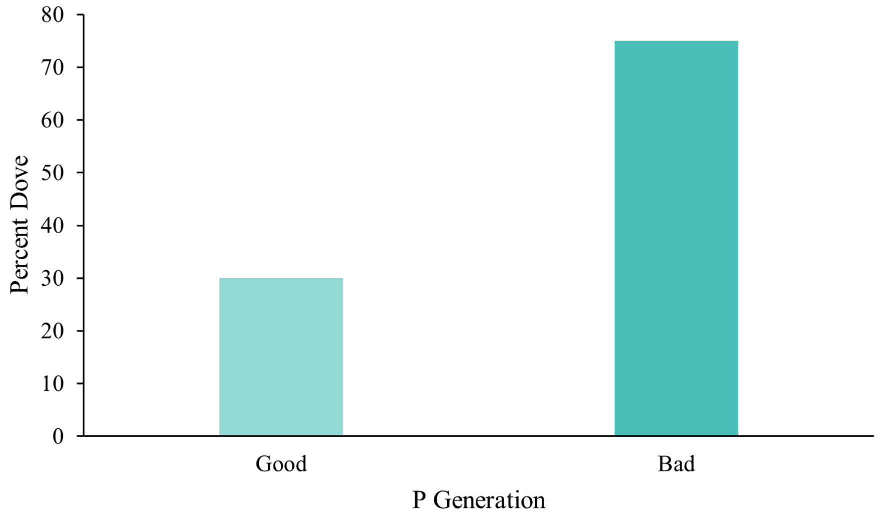
Time Spent Investigating the Same Object Locations on the Object Location Memory Test for F1 Generation



Note. Rats raised by Bad mothers and in control cages spent significantly more time with the same object than any other condition ($p < .05$). Error bars are ± standard error of the mean. Total N=31, Pups with Good mothers who are enriched N=8, Pups with Good mothers in standard housing N=7, Pups with Bad mothers who are enriched N=8, Pups with Bad mothers who are standard housing N=8.

Figure 15

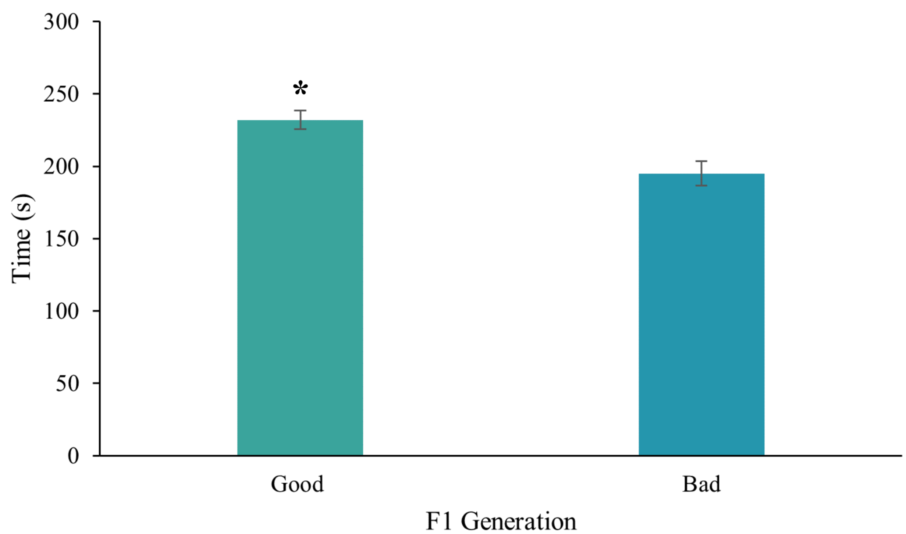
Percentage of Rats in Each Condition that Dove in the Forced Swim Test in the P Generation



Note. There was no significant difference between the percentage of Good and Bad moms that dove (p = .06). Total N=14, Good Moms N=10, Bad Moms N=4.

Figure 16

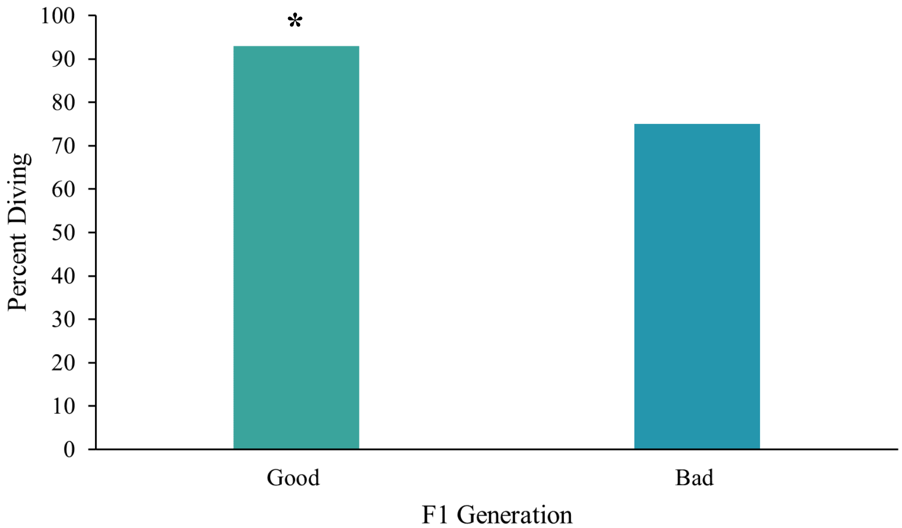
Time Spent Swimming in the Forced Swim Test in the F1 Generation



Note. Rats raised by Good mothers swam for a significantly longer amount of time than the rats raised by Bad mothers ($p < .05$). Error bars are ± standard error of the mean. Total N=31, Good Pups N=16, Bad Pups N=15.

Figure 17

Percentage of Rats in Each Condition that Dove in the Forced Swim Test in the F1 Generation



Note. Adolescent rats raised by Good mothers dove significantly more than adolescent rats raised by Bad mothers ($p < .01$). Total N=31, Good Pups N=16, Bad Pups N=15.