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# Biological Sex Differences in the Monodelphis Domestica: Investigating Behavioral Responses in Environmental Enrichment and Conditioned Place Preference Paradigms

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# BIOLOGICAL SEX DIFFERENCES IN THE MONODELPHIS DOMESTICA: INVESTIGATING BEHAVIORAL RESPONSES IN ENVIRONMENTAL ENRICHMENT AND CONDITIONED PLACE PREFERENCE PARADIGMS

A Thesis

by

KATELYNN M. RENTERÍA

Submitted in Partial Fulfillment of the

Requirements for the Degree of

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May 2022

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# ABSTRACT

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The *Monodelphis domestica*, commonly known as the grey short-tailed opossum, has become an increasingly frequented animal model for areas of psychological study, including comparative psychology. Despite the growing interest in utilizing the *Monodelphis* as an animal model, several areas of research remain limited in what has been studied. This includes associative learning and the effects of environmental enrichment on the *Monodelphis*. This study novelly incorporates the two using environmental enrichment with the conditioned place preference (CPP) paradigm to both diversify associative learning literature and expand existing knowledge on the *Monodelphis domestica*. The CPP paradigm included interactive objects as environmental enrichment to investigate biological sex differences in *Monodelphis* behavioral responses.

# DEDICATION

To my family, for their unwavering and never-ending support of my endeavors. My father, Reynaldo G. Rentería, my mother, Patricia L. Rentería, and my younger brother, Reynaldo G. Rentería II, have watched this journey with love and support in their hearts. I am undoubtedly where I am today because you have all been right by my side every step of the way. Thank you for believing in me.

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# CHAPTER I

# INTRODUCTION

The field of psychology continues to grow as topics are diversified using non-traditional research models, evolving paradigms, and investigating increasingly varied variables. Using animal models for comparative and translational studies is frequently occurring, with one such animal model being the *Monodelphis domestica*. The *Monodelphis domestica*, commonly known as the grey short-tailed opossum, has been utilized in several areas of psychological research, such as social behavior, motor behavior, and physiological development, among others. The grey-short tailed opossum has become frequently used in marsupial biology studies, so much so that it is now referred to as the laboratory opossum (VendeBerg, 1997). Despite its growing popularity for research use, information is still limited in several areas, including environmental enrichment and associative learning. In particular, the effect of environmental enrichment on *Monodelphis* behavior and the use of the *Monodelphis* in the associative learning conditioned place preference paradigm. Very little literature on the effect of enrichment on the behavior of the *Monodelphis* exists, and the use of the *Monodelphis* in conditioned place preference has been investigated once before. Thus, a preliminary study to investigate whether enrichment impacts *Monodelphis* behavior was conducted for expansion of previous studies, and following subsequent findings, environmental enrichment was novelly implemented in the conditioned place preference paradigm to investigate enrichment's effectiveness in this associative learning paradigm, and expand *Monodelphis* associative learning research.

## ***Monodelphis domestica*** **as a Research Animal Model**

Originating in South America, the grey short-tailed opossum is one of the smallest marsupials, with adult body length ranging from 10 to 15 cm., adult males weighing between 90 and 155 grams, and adult females weighing between 80 and 100 grams (Moore, n.d.). The *Monodelphis* breeds well in captivity, with entire colonies produced for research purposes over a period of several years (VandeBerg, 1997). The gestation period ranges from 14 to 15 days, and litters can have between 1 and 15 offspring, with an average of 9 per litter (Moore, n.d.). Both adult male and female *Monodelphis* reach sexual maturity at 5 to 7 months of age (Moore, n.d.).

*Figure 1: Monodelphis domestica (gray short-tailed opossum).* Photo taken by Phil Myers, Museum of Zoology, University of Michigan-Ann Arbor.

The *Monodelphis* has been used in both observational studies gathering quantitative data, as well as for biomedical research investigating their brain chemistry and physiological makeup. Because of its marsupial characteristics, the *Monodelphis* is often investigated for gestation studies, gene mapping, or specific research involving X-linked gene expression between sexes

(VandeBerg, 1997). Despite its growing use in marsupial-related studies, the *Monodelphis* has been investigated for topics comparative to human research, such as ultraviolet radiation induced skin and eye cancers and dietary induced hypercholesterolemia. The *Monodelphis* even has the distinction of being the only naturally existing mammal besides humans to be susceptible to melanoma in response to solitary ultraviolet radiation (VandeBerg, 1997).

There is a large range of studies targeting the *Monodelphis*, from postnatal lung development to motor behavior in response to temperature changes (Modepalli et al., 2018; Corriveau-Parenteau et al., 2019). Studies such as these seek both to expand *Monodelphis* literature and diversify existing psychological research by using a non-traditional animal model. There are examples of observational research generating quantifiable data from the *Monodelphis*, including the investigation of photic preference conducted by Seelke et al. (2014). Researchers placed opossums into a circular testing arena where one side was “dark” and void of light (0 lux) and other sides had light (~1.4, 40, or 400 lux). By placing the animal into the apparatus and recording the duration of time spent in each side, significant differences were found in whether or not the opossums preferred the dark or the light (Seelke et al., 2014). Mean duration of time spent in each side was used in determining photic preference, and it was found that the opossums spent significantly longer in the dark sides rather than the light, reflective of the opossums’ nocturnal disposition (Seelke et al., 2014). This study noted that its lack of experimental manipulation opens doors for further research to build upon their findings. Existing experimental studies utilizing the *Monodelphis* range from behavioral studies to physiological investigation. Topics such as *Monodelphis* food preference have been investigated using surgical procedures such as occluding access to the vomeronasal organ, demonstrating the versatility of the laboratory opossum in observable, behavioral, and manipulated studies (Halpern et al., 2005).

Sex differences in the *Monodelphis* have been investigated previously, with the context varying from X-linked gene expression to innate behaviors that are distinguishable between biological sex (VandeBerg, 1997). Biological sex differences in particular have been investigated alongside an array of topics, including social behavior and non-associative learning (Gil et al., 2019). By socially isolating opossums and then exposing them to a non-familiar, same-sex partner once every 24 hours for 3 days, Gil et al. (2019) was the first research group to find statistically significant sex differences in nonsocial behavior duration. Studies like Gil et al. (2019) demonstrate the variation in behavior that exists between sexes in the *Monodelphis*, and as the laboratory opossum becomes more frequently used for both broad and niche research topics, there is importance in continuing to diversify existing literature with a non-traditional model as well as adding to existing *Monodelphis* research.

## Environmental Enrichment in the *Monodelphis*

Environmental enrichment refers to physical objects or additions to an environment that can be interacted with to increase behavior or intellectual activity frequencies (American Psychological Association, n.d.). Environmental enrichment has been implemented in countless animal model research, yet in the *Monodelphis,* close to no literature exists on the effect of environmental enrichment on laboratory opossum behavior. Research has established that environmental enrichment not only promotes welfare and natural behavior in animal models, but that when enrichment is novel and adds an interactive, sensory experience, it has the ability to affect the animal at the neurochemical level by enhancing neural plasticity (Reynolds et al., 2010). A beneficial addition to comparative studies, animal models that are receptive to enhanced neural plasticity and who are affected by their environment in that way are used in studies such as the rehabilitation of neurodevelopment disorders (Reynolds et al., 2010).

Literature is plentiful when enrichment is implemented in traditional animal models, namely in rats and mice. One article was able to be found involving *Monodelphis* behavior in response to enriched environments, by implementing an enriched floor pen and observing if the *Monodelphis* exhibited stereotypic behaviors following enrichment exposure. Wilkinson et al. (2010) used a within-subjects sample of 5 male opossums aged 10 – 12 weeks. All opossums were housed in a standard rat cage for a time, with behaviors being recorded during their nocturnal active hours, then the process was repeated using an enriched floor pen complete with tubes, climbing wires, and other interactive facets of the environment. When quantified, behaviors such as sniffing the air, manipulating their nests, and interacting with the enrichment apparatus compromised a significant amount of time during the data collection period (Wilkinson et al., 2010). The only manipulation of this study was the implementation of enrichment, and subsequent findings reflect that enrichment does affect laboratory opossum behavior. Being that the sample size was small and comprised of all males, further studies to remedy those limitations may yield replicative results.

## Conditioned Place Preference in the *Monodelphis*

Conditioned place preference (CPP) is a subtopic of associative learning, with associative learning being defined as the process of acquiring new, lasting information due to the connections between elements in our surroundings (APA Dictionary of Psychology, n.d.). In other words, elements like stimuli in our environment prompt the mind to make associations (acquiring new information) and remember the connection (lasting information); a topic discussed largely in psychology from the work of Pavlov to modern applications of classical conditioning. A form of classical conditioning, CPP has been utilized with several traditional animal models to gauge to ability for the mind to be conditioned using varying reinforcers. CPP uses its own specialized apparatus comprised on a three-compartment chamber that can be closed to create two chambers, so the apparatus changes from free range to intentional conditioning with the reinforcer in one chamber and non-associative exploratory behavior in the opposite chamber (Prus et al., 2009). CPP is commonly associated with drug conditioning, often used in animal models to comparatively draw similarities between animals and humans for drug and/or alcohol use (Raw et al., 2018). However, CPP is noted to be compatible with food or other rewarding stimuli. The goal of utilizing conditioned place preference is to condition an animal to associate and "remember" the conditioning to a certain stimulus they experienced by traveling back to the chamber where their conditioning took place during your pre-test period (Prus et al., 2009).

Only one piece of literature investigates CPP being used with the *Monodelphis* as an animal model, using Froot Loops cereal as the reinforcer for classical conditioning. Ramirez (2018) conducted a 10-day protocol pertaining to two days of pre-tests, one on each day, no testing the third, conditioning using Froot Loops in the CPP apparatus for 5 days, and subsequent post-testing on the ninth and tenth days. Each pre- and posttest was conducted for 15 minutes, with conditioning trials in the conditioning chamber for 30 minutes followed by 10 minutes in the non-conditioning side of the apparatus (Ramirez, 2018). Following data collection and analyses, Ramirez found that the Froot Loops had the opposite effect of conditioning than originally intended. Preference scores for which chamber the animal visited during pre- and posttests were evaluated, and trends were found where the animals actually spent less time in their conditioning chamber during posttests after enrichment exposure, raising questions as to the effectiveness of conditioned place preference on *Monodelphis* associative learning, or the effectiveness of that particular food reinforcer on laboratory opossum conditioning.

Conditioned place preference has been utilized across other traditional animal models, following a pattern of roughly week-long conditioning paradigms. Kang et al. (2017) utilized conditioned place preference in a mouse model to investigate chronic inflammatory pain when the anterior cingulate cortex is inhibited and does not elicit excitatory neuronal activity that would worsen chronic pain symptoms. Using an eight-day paradigm of pre-testing on Day 1, followed by 6 days of conditioning, then a final day of posttests, researchers were able to see significant differences between pre- and post-conditioning in immunohistochemistry expressions.

## Bridging Environmental Enrichment and Conditioned Place Preference

Environmental enrichment has shown to be effective in impacting the neural plasticity of animal models, and neural plasticity plays a key role in the retention of learning and memory (Reynolds et al., 2010; American Psychological Association, n.d.). Wilkinson et al. (2010) has demonstrated that environmental enrichment significantly impacts gray-short tailed opossum behavior, though the sample was comprised of five all-male subjects. This study aims to investigate the impact of environmental enrichment on gray-short tailed opossum behavior in both sexes, targeting biological sex differences between the two. With environmental enrichment having shown to significantly impact gray short-tailed opossum behavior in previous literature and based on this study's findings, environmental enrichment was novelly implemented into the associative learning conditioned place preference paradigm to investigate the effectiveness of enrichment on associative learning in the *Monodelphis domestica*.

# CHAPTER II

## ENVIRONMENTAL ENRICHMENT IN THE *MONODELPHIS*

In order to investigate the effect of environmental enrichment on gray short-tailed opossum behavior, duration of mobility was utilized as the dependent variable, meaning the target behavior investigated was the amount of time the opossum spent moving around the apparatus. A total of 6 enrichment objects were introduced to 5 opossums each, with a pre-test baseline and post-test period used to compare mobility before and after enrichment exposure. Manual key scoring was conducted after testing to analyze potential object preference among the opossums tested, and mobility duration and other related variables were recorded in real-time using ANYmaze video capturing software. Independent t-tests were conducted to compare pre- and posttest scores, as well as investigate potential object preference in the *Monodelphis*.

### Methods

#### Subjects

Five gray short-tailed opossums were used to investigate the effect of enrichment on mobility, targeting biological sex differences. The five consisted of three females and two males, and all were siblings from the same litter, having the same biological mother and father. All subjects were roughly 2.5 months of age, making them younger than adults since the

*Monodelphis* reaches sexual maturity between 5 to 7 months of age (Moore, n.d.). The three females were housed together in the same cage, and the two males shared a cage separate from the females.

## Materials & Apparatus

The animals were placed into a clean cage with bedding identical to the cage they were housed in, with the clean cage serving as the apparatus where the experiment would take place. ANYmaze video tracking software was used to record the apparatus and all trials, and within ANYmaze the apparatus was split into two zones; the right side of the cage served as the "right stimulus zone," and the left as the "left non-stimulus zone."

*Figure 2: Empty Cage.* Apparatus for Enrichment.

*Figure 3: Enrichment in Right Stimulus Zone.* Apparatus for Enrichment.

Enrichment objects were placed into the right side of the cage for every enrichment trial, and if the animal moved the object to the non-stimulus zone during testing, the object was promptly moved back to the stimulus zone. Six total enrichment objects were used, with one object per trial. Enrichment objects included a metal feeder that hung on the inside of the cage, a red, transparent, plastic cylinder, a red, transparent, plastic tunnel, a red, transparent, plastic bottle with openings to crawl into, a small plastic bone, and an olfactory stimulus made with lime-scented extract diluted into 100 mL of water, with a few drops pipetted onto filter paper taped to a microscope slide (not pictured).

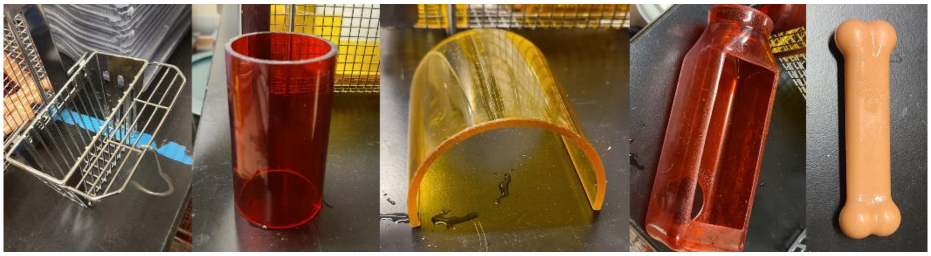

*Figure 4: Enrichment Objects*. Metal Feeder, Red Cylinder, Yellow Tunnel, Red Bottle, Bone

Videos, key scoring, and data from the experiment were recorded and exported using ANYmaze video capture software, and statistical analyses were conducted using IBM SPSS Statistics 26.

## Procedure

The order of animals tested was randomized, the only caveat being that males were tested prior to females, since the possible odor of the females in the apparatus may have affected male behavior as a confounding variable. The order in which enrichment objects were presented to each animal was randomized to prevent a possible ordering effect. Randomization was conducted using GraphPad, an online randomization tool.

All animals were introduced to the clean cage apparatus for 10 minutes as an acclimation period, followed immediately by a 10-minute baseline period to record mobility duration prior to any enrichment exposure. Following baseline, the first enrichment object was introduced, and the animal was able to interact with the object for a total of 10 minutes per enrichment object trial. After each enrichment trial, a 5-minute inter-trial interval was implemented as a cool-down period for the animals to spend in an empty cage before introducing the next enrichment object, in order to remedy a possible carryover effect. Following all 6 enrichment trials, a 10-minute post-trial was conducted, where no enrichment was present, reflecting the baseline period absent of any objects.

During testing, ANYmaze tracking software recorded each animal's duration of mobility, time spent in the stimulus and non-stimulus zones, and other variables relating to the animal's movement and zone occupancy. After testing had concluded, ANYmaze was used to manually score each animal's interaction frequencies with the enrichment items. When re-scoring videos, when the animal interacted with the object (i.e. touched the object with its nose, climbed the objected, bit the object, made physical contact with the object, etc.), a key (i.e. the letter K on the keyboard) was pressed for the duration of the time the animal interacted with the object.

## Methods of Analysis

The dependent variable analyzed in relation to enrichment object exposure was the duration of mobility for each trial, including acclimation, baseline, six enrichment exposure trials, and posttest periods. Mobility duration refers to the amount of time the animal was mobile during the trial period throughout the entire apparatus. Independent t-tests were conducted for all trials with biological sex as the independent variable and mobility duration per trial as the dependent variable, with statistical significance defined as $p \leq 0.05$.

In order to determine if a significant object preference existed among subjects, ANYmaze video tracking software was used for the researcher to manually press a key on the computer's keyboard when an animal interacted with each enrichment object, creating object interaction frequency data. An independent t-test was conducted to determine if any significant differences in means for object interaction frequency occurred between males and females, and a table recounting which object had the highest values for several independent variables related to object interaction was manually calculated. All statistical analyses were conducted using IBM SPSS Statistics 27.

## Results

### Biological Sex Differences in Mobility

An independent samples t-test ($\alpha = 0.05$, two-tailed) was conducted to test for biological sex differences in mobility duration in response to environmental enrichment exposure. Significant differences were found in two out of nine trials, with an approach to significance in one and biological sex trends in all three. In the third trial, the trial in which the first enrichment object is introduced, an approach to significance is present. Females ($M = 374.20$, $SD = 49.63$) had an approach to significantly higher duration of mobility than males ($M = 340.80$, $SD = 4.10$), $t(3) = 0.90$, p = 0.08, Cohen's d = -0.823. In the fifth trial, during the third enrichment object exposure, females ($M = 416.13$, $SD = 61.76$) had significantly higher mobility durations than males ($M = 207.0$, $SD = 287.6$), $t(3) = -1.32$, $p < 0.05$, Cohen's $d = -1.205$. Lastly, in the sixth enrichment trial, females (*M* = 414.20, *SD* = 35.35) had significantly higher levels of mobility than males (*M* = 281.75. *SD* = 261.42), *t*(3) = -0.944, $p < 0.05$, Cohen's *d* = -0.862. Results of the independent t-test are shown in Table 1. All three trials exhibited a trend in higher female mobility duration in response to enrichment object exposure over males, with Figures 5-7 illustrating sex differences in mobility in Trials 3, 5, and 6, respectively.

Table 1

*Independent Samples t-test Comparing Biological Sex Differences in Mobile Duration*

*Independent Samples Test*

| | | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | 95% Confidence Interval of the Difference | |
| | | F | Sig. | t | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | Lower | Upper |
| Mobile Duration Trial 1 | Equal variances assumed | 2.536 | .209 | -1.059 | 3 | .367 | -58.8167 | 55.5169 | -235.4964 | 117.8630 |
| | Equal variances not assumed | | | -1.256 | 2.873 | .301 | -58.8167 | 46.8113 | -211.5946 | 93.9613 |
| Mobile Duration Trial 2 | Equal variances assumed | .007 | .937 | -.017 | 3 | .987 | -1.0167 | 58.9799 | -188.7169 | 186.6836 |
| | Equal variances not assumed | | | -.017 | 2.197 | .988 | -1.0167 | 59.5857 | -236.5311 | 234.4978 |
| Mobile Duration Trial 3 | Equal variances assumed | 6.663 | .082 | -.901 | 3 | .434 | -33.4000 | 37.0579 | -151.3348 | 84.5348 |
| | Equal variances not assumed | | | -1.160 | 2.041 | .364 | -33.4000 | 28.8024 | -154.9856 | 88.1856 |
| Mobile Duration Trial 4 | Equal variances assumed | 2.669 | .201 | -.449 | 3 | .684 | -24.4333 | 54.3936 | -197.5382 | 148.6715 |
| | Equal variances not assumed | | | -.394 | 1.496 | .742 | -24.4333 | 61.9363 | -398.3280 | 349.4613 |
| Mobile Duration Trial 5 | Equal variances assumed | 56.683 | .005 | -1.321 | 3 | .278 | -209.1333 | 158.3680 | -713.1309 | 294.8642 |
| | Equal variances not assumed | | | -1.013 | 1.062 | .488 | -209.1333 | 206.4029 | -2496.3865 | 2078.1199 |
| Mobile Duration Trial 6 | Equal variances assumed | 152.021 | .001 | -.944 | 3 | .415 | -132.4500 | 140.2757 | -578.8699 | 313.9699 |
| | Equal variances not assumed | | | -.712 | 1.024 | .604 | -132.4500 | 185.9732 | -2366.2602 | 2101.3602 |

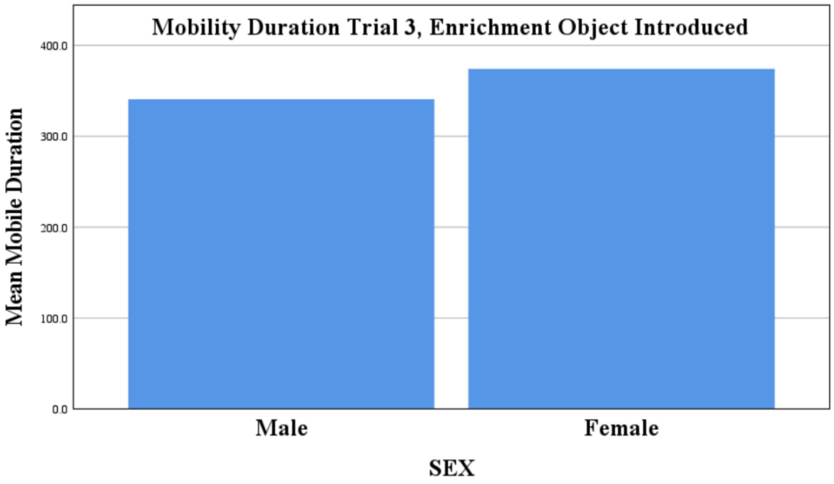

*Figure 5: Mobility Duration Trial 3.* Female Mobility > Male Mobility

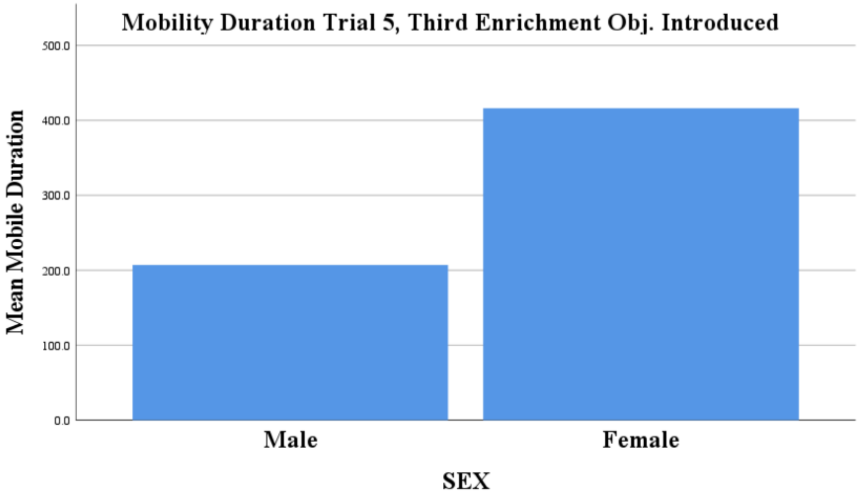

*Figure 6: Mobility Duration Trial 5.* Female Mobility > Male Mobility

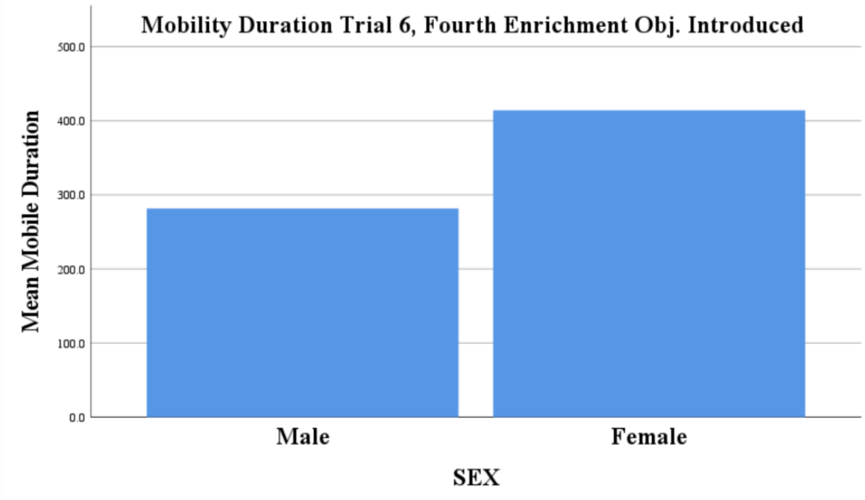

*Figure 7: Mobility Duration Trial 6.* Female Mobility > Male Mobility

## Object Preference

A repeated measures ANOVA was conducted to detect significant differences in enrichment object preference by comparing object interaction frequency means of all 6 objects with one another. No significance differences were found in comparing enrichment object interaction frequencies ($F(5) = 1.933$, $p = 0.148$) as shown in Table 3. Findings also reflect no significant differences found in the interaction of object interaction frequency and biological sex ($F(5) = 0.920$, $p = 0.495$), also shown in Table 3. Table 4 reflects pairwise comparisons from the repeated measures ANOVA conducted, and a significant value of the yellow tunnel showing higher interaction frequencies compared to the red bottle were found ($p < 0.05$). It is important to note that the F-statistic tied to ANOVA did not reflect significance in object preference, so this value is not significant in that aspect. Following these results, the plastic, transparent red bottle and the plastic bone were selected for the subsequent conditioned place preference experiment due to their interactive capabilities; the bottle provided a space for the animals to crawl in and out, and the bone could be moved due to its size of less than 6 inches.

Table 2

*Means of Object Interaction Frequencies*

*Group Statistics*

| | SEX | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| Red Bottle Interaction Frequency | 1 | 2 | 60.50 | 26.163 | 18.500 |
| | 2 | 3 | 23.67 | 19.502 | 11.260 |
| Feeder Interaction Frequency | 1 | 2 | 39.00 | 15.556 | 11.000 |
| | 2 | 3 | 28.00 | 10.440 | 6.028 |
| Yellow Tunnel Interaction Frequency | 1 | 2 | 69.00 | 19.799 | 14.000 |
| | 2 | 3 | 40.00 | 20.664 | 11.930 |
| Red Cylinder Interaction Frequency | 1 | 2 | 71.50 | 40.305 | 28.500 |
| | 2 | 3 | 36.67 | 21.127 | 12.197 |
| Olfactory Stimulus Interaction Frequency | 1 | 2 | 33.50 | 6.364 | 4.500 |
| | 2 | 3 | 29.00 | 3.606 | 2.082 |
| Bone Interaction Frequency | 1 | 2 | 53.50 | 6.364 | 4.500 |
| | 2 | 3 | 41.00 | 10.817 | 6.245 |

Table 3

*Within-Subjects Repeated Measures for Enrichment Frequencies & Biological Sex Interaction*

*Tests of Within-Subjects Effects*

Measure: Enrichment Object Interaction Frequencies

| Source | | Type III Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Noncent. Parameter | Observed Power[a] |
|---|---|---|---|---|---|---|---|---|---|
| Enrichment Items | Sphericity Assumed | 2393.644 | 5 | 478.729 | 1.933 | .148 | .392 | 9.666 | .490 |
| | Greenhouse-Geisser | 2393.644 | 1.976 | 1211.623 | 1.933 | .226 | .392 | 3.819 | .258 |
| | Huynh-Feldt | 2393.644 | 5.000 | 478.729 | 1.933 | .148 | .392 | 9.666 | .490 |
| | Lower-bound | 2393.644 | 1.000 | 2393.644 | 1.933 | .259 | .392 | 1.933 | .168 |
| Enrichment Items * Biological Sex | Sphericity Assumed | 1139.244 | 5 | 227.849 | .920 | .495 | .235 | 4.600 | .242 |
| | Greenhouse-Geisser | 1139.244 | 1.976 | 576.667 | .920 | .448 | .235 | 1.818 | .145 |
| | Huynh-Feldt | 1139.244 | 5.000 | 227.849 | .920 | .495 | .235 | 4.600 | .242 |
| | Lower-bound | 1139.244 | 1.000 | 1139.244 | .920 | .408 | .235 | .920 | .106 |
| Error(Enrichment Items) | Sphericity Assumed | 3714.556 | 15 | 247.637 | | | | | |
| | Greenhouse-Geisser | 3714.556 | 5.927 | 626.749 | | | | | |
| | Huynh-Feldt | 3714.556 | 15.000 | 247.637 | | | | | |
| | Lower-bound | 3714.556 | 3.000 | 1238.185 | | | | | |

a. Computed using alpha = .05

Table 4

*Repeated Measures Pairwise Comparisons of Object Interaction Frequencies*

*Pairwise Comparisons*

Measure: Object Interaction Frequencies

| (I) Enrichment Items | (J) Enrichment Items | Mean Difference (I-J) | Std. Error | Sig.[b] | 95% Confidence Interval for Difference[b] Lower Bound | 95% Confidence Interval for Difference[b] Upper Bound |
|---|---|---|---|---|---|---|
| Red Bottle | Feeder | 8.583 | 10.121 | .459 | -23.627 | 40.794 |
| | Yellow Tunnel | -12.417* | 2.651 | .018 | -20.852 | -3.981 |
| | Red Cylinder | -12.000 | 12.851 | .419 | -52.896 | 28.896 |
| | Olfactory Stimulus | 10.833 | 8.134 | .275 | -15.052 | 36.718 |
| | Bone | -5.167 | 11.657 | .688 | -42.264 | 31.930 |
| Feeder | Red Bottle | -8.583 | 10.121 | .459 | -40.794 | 23.627 |
| | Yellow Tunnel | -21.000 | 9.428 | .112 | -51.004 | 9.004 |
| | Red Cylinder | -20.583 | 7.697 | .075 | -45.079 | 3.913 |
| | Olfactory Stimulus | 2.250 | 4.614 | .659 | -12.432 | 16.932 |
| | Bone | -13.750 | 9.691 | .251 | -44.592 | 17.092 |
| Yellow Tunnel | Red Bottle | 12.417* | 2.651 | .018 | 3.981 | 20.852 |
| | Feeder | 21.000 | 9.428 | .112 | -9.004 | 51.004 |
| | Red Cylinder | .417 | 12.537 | .976 | -39.480 | 40.313 |
| | Olfactory Stimulus | 23.250 | 7.372 | .051 | -.210 | 46.710 |
| | Bone | 7.250 | 11.518 | .574 | -29.407 | 43.907 |
| Red Cylinder | Red Bottle | 12.000 | 12.851 | .419 | -28.896 | 52.896 |
| | Feeder | 20.583 | 7.697 | .075 | -3.913 | 45.079 |
| | Yellow Tunnel | -.417 | 12.537 | .976 | -40.313 | 39.480 |
| | Olfactory Stimulus | 22.833 | 11.716 | .146 | -14.453 | 60.119 |
| | Bone | 6.833 | 17.076 | .716 | -47.509 | 61.175 |
| Olfactory Stimulus | Red Bottle | -10.833 | 8.134 | .275 | -36.718 | 15.052 |
| | Feeder | -2.250 | 4.614 | .659 | -16.932 | 12.432 |
| | Yellow Tunnel | -23.250 | 7.372 | .051 | -46.710 | .210 |
| | Red Cylinder | -22.833 | 11.716 | .146 | -60.119 | 14.453 |
| | Bone | -16.000 | 5.893 | .073 | -34.753 | 2.753 |
| Bone | Red Bottle | 5.167 | 11.657 | .688 | -31.930 | 42.264 |
| | Feeder | 13.750 | 9.691 | .251 | -17.092 | 44.592 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Yellow Tunnel | -7.250 | 11.518 | .574 | -43.907 | 29.407 |
| | Red Cylinder | -6.833 | 17.076 | .716 | -61.175 | 47.509 |
| | Bone | 16.000 | 5.893 | .073 | -2.753 | 34.753 |

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

# CHAPTER III

# ENVIRONMENTAL ENRICHMENT IN THE CONDITIONED PLACE PREFERENCE PARADIGM

To investigate the effectiveness of environmental enrichment in the conditioned place preference paradigm using the *Monodelphis* as an animal model, 12 opossums randomized into an experimental and control group underwent a 7-day protocol, with one day dedicated to pre-testing, 5 days of conditioning, and one day of post-testing respectively. The experimental group was exposed to the same randomized enrichment object for every consecutive day of conditioning, while the control group did not receive any enrichment exposure. Animals were randomized into which chamber of the CPP apparatus would be their conditioning chamber, and preference scores based on that randomization were created using the time spent in the conditioning chamber and total time spent in the entire apparatus. Pre-test and post-test preference scores were compared to investigate potential biological sex differences in the conditioned place preference paradigm with environmental enrichment.

## Methods

### Subjects

Twelve gray short-tailed opossums were used to investigate the effectiveness of enrichment in the conditioned place preference paradigm, targeting biological sex differences.

The 12 opossums were randomized into an experimental and control group, with three males and three females per group, each group consisting of 6 animals each. Some animals were siblings from the same litter, though not all were related, and all subjects were roughly 9 months of age, making them sexually mature adults, since the *Monodelphis* reaches sexual maturity between 5 to 7 months of age (Moore, n.d.). All animals were housed separately, with two having their own individual cages and the other housed with another same-sex opossum, though a plastic divider kept the two apart and no interaction between the two ever took place.

### Materials & Apparatus

The conditioned place preference apparatus consisted of three chambers, two identical in size and shape (40 cm x 40 cm x 35 cm), and a third connecting chamber that could be closed off for conditioning purposes, made of black plexiglass with a solid metal base. Environmental enrichment was provided in the form of a red, transparent plastic bottle, and a small plastic bone. As in Ramirez (2018), each animal had its own set of mats that would cover the floor of each chamber recurringly throughout the 7-day protocol, with each animal having its own set of mats for 12 mats total. One mat was made of a black mesh, dubbed the “black chamber,” and the white mat was softer, dubbed the “white chamber.” The mats were both different colors and different textures to serve as cues for the *Monodelphis* during and after conditioning, as reminiscent of the CPP paradigm. A small, plastic, see-through cage was used as the holding apparatus before the animal was released into the apparatus for pre- and posttests, as per Ramirez (2018). All trials were recorded using ANYmaze video capturing software, and all analyses were run using IBM Statistics SPSS 27. All randomizations were conducted with GraphPad, an online randomization tool.

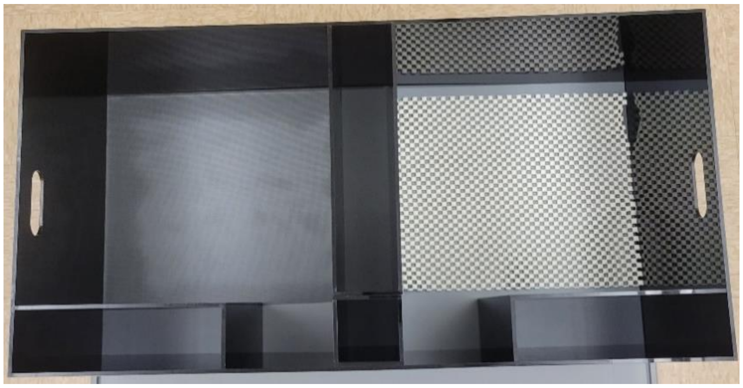

*Figure 8: Conditioned Place Preference Apparatus.* Empty, with “black chamber” on left, “white chamber” on right.

### Procedure

Both the control and experimental groups underwent the exact same protocols, the only difference being the experimental group received enrichment during the conditioning trials and the control group did not. On the first day, two sets of pre-tests occurred, one serving as a 15-minute acclimation period for the animal to get accustomed to the new apparatus, and another after a 2-hour inter-trial interval to serve as a 15-minute habituation to allow the animal to explore the apparatus thoroughly. Prior to being placed into the apparatus, the animal was placed into a small holding box for 30 seconds, as per Ramirez (2018). During the next 5 days, conditioning trials were conducted. A randomization tool was used to determine which chamber the animal’s conditioning would take place, in order to eliminate the color or texture of the mat as a confounding variable. During conditioning trials, the animal was placed into their assigned conditioning chamber for 30 minutes, immediately followed by a 10-minute trial in their nonassigned conditioning chamber. Experimental group subjects only received enrichment during the 30-minute assigned conditioning trial in their assigned chamber. Following five days of conditioning, posttests were conducted in the same manner as the pre-tests, with a 15-minute post-test, 2-hour inter-trial interval, and a final 15-minute posttest. The 30-minute, 10-minute, and 15-minute durations for testing were conducted as per Gil et al. (2013), and replicated from Ramirez (2018). The apparatus was cleaned with ethanol in between each trial and animal, and all trials were recorded and quantified using ANYmaze video capture software.

*Figure 9: Monodelphis domestica in holding box.*

*Figure 10: Monodelphis domestica in open three-chamber CPP.* Pre-test, Acclimation Trial.

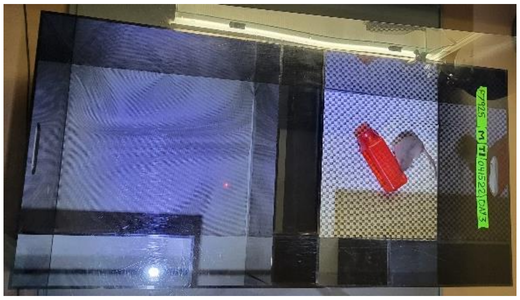

*Figure 11: Monodelphis domestica in closed two-chamber CPP.* Conditioning Trial, Day 3 of 5.

## Methods of Analysis

To determine preference of chamber in relation to the conditioned place preference paradigm, a preference score was calculated by taking the amount of time spent in the conditioning chamber (C) and dividing that by the total amount of time spent in the apparatus, or in both the non-conditioning and conditioning chambers (C / N + C). This method of preference score calculation was used in the conditioned place preference paradigm of both Ramirez (2018) and Gil et al. (2013). Preference scores were calculated for both pre-tests and posttest for both the experimental and control groups, and the scores were input into IBM SPSS Statistics for analyzation, with statistical significance defined as $p \leq 0.05$.

A one-way between subjects Analysis of Variance (ANOVA) and Tukey's HSD test were conducted to analyze the dependent variable of preference scores in relation to biological sex, experimental condition group, and pre- and posttest trials.

# Results

## Preference Scores

A one-way between subjects ANOVA was conducted with biological sex, experimental condition, and pre- and post-test trials as the independent variables, and preference scores as the dependent variable. Tukey's HSD test revealed no significant differences between pre-test 1 and pre-test 2, nor post-test 1 or post-test 2, nor the pre-tests compared to post-tests. When looking at all pre-tests and posttests, a significant interaction was found between biological sex and experimental condition $F(1, 48) = 7.29$, $p < 0.05$, $\eta^2 = .186$, shown in Table 5. Trends reflecting that potential biological sex difference were seen across pre- and posttests, with females exhibiting higher preference scores than males, illustrated in Figures 12-15. A repeated measures ANOVA was conducted to compare control and experimental preference scores in pre- and posttests.

Table 5

*One-way ANOVA Comparing Interactions of Biological Sex, Experimental Conditions, and Pre/Posttests Preference Scores*

*Tests of Between-Subjects Effects*

Dependent Variable: Preference Score

| Source | Type III Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared | Noncent. Parameter | Observed Power[b] |
|---|---|---|---|---|---|---|---|---|
| Corrected Model | .268[a] | 15 | .018 | 1.342 | .235 | .386 | 20.127 | .651 |
| Intercept | 11.543 | 1 | 11.543 | 866.568 | .000 | .964 | 866.568 | 1.000 |
| Sex | .013 | 1 | .013 | 1.003 | .324 | .030 | 1.003 | .163 |
| Experimental Condition | .001 | 1 | .001 | .076 | .784 | .002 | .076 | .058 |
| Pre/Post Trial | .024 | 3 | .008 | .604 | .617 | .054 | 1.813 | .161 |
| **Sex * Condition** | .097 | 1 | .097 | **7.290** | **.011** | .186 | 7.290 | .745 |
| Sex * Pre/Post Trial | .038 | 3 | .013 | .959 | .424 | .083 | 2.878 | .237 |
| Condition * Trial | .083 | 3 | .028 | 2.089 | .121 | .164 | 6.266 | .484 |
| Sex * Condition *Trial | .011 | 3 | .004 | .267 | .849 | .024 | .800 | .095 |
| Error | .426 | 32 | .013 | | | | | |
| Total | 12.238 | 48 | | | | | | |
| Corrected Total | .694 | 47 | | | | | | |

a. R Squared = .386 (Adjusted R Squared = .098)

b. Computed using alpha = .05

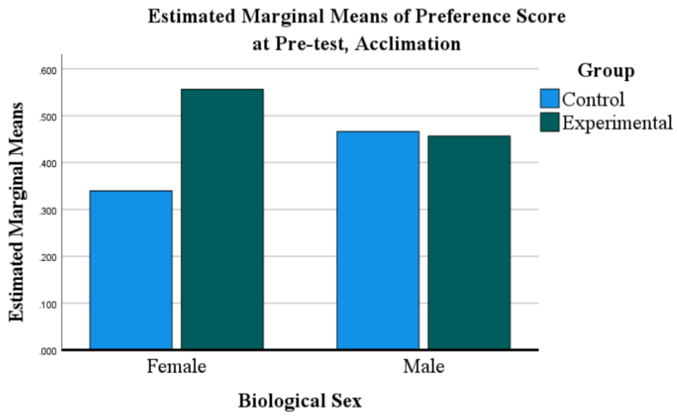

*Figure 12: Pre-test, Acclimation*. Experimental Female Preference Score > Male Preference Score

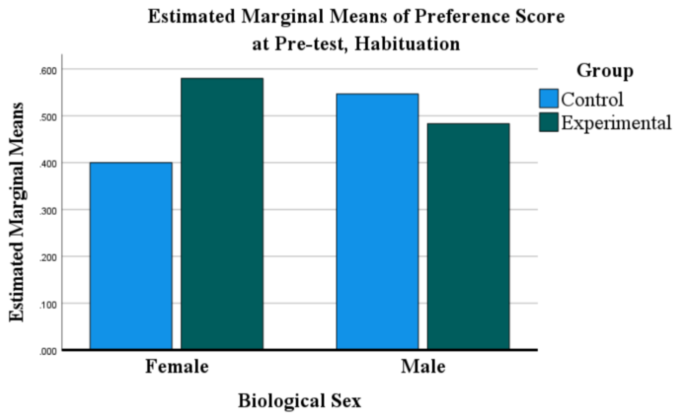

*Figure 13: Pre-test, Habituation*. Experimental Female Preference Score > Male Preference Score

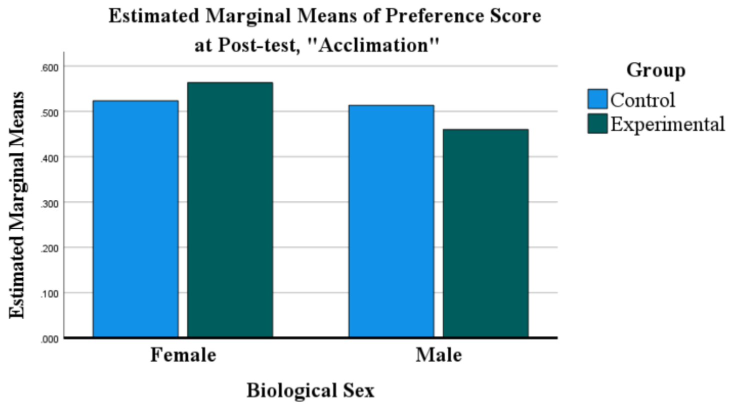

*Figure 14: Posttest, Acclimation*. Female Preference Score > Male Preference Score

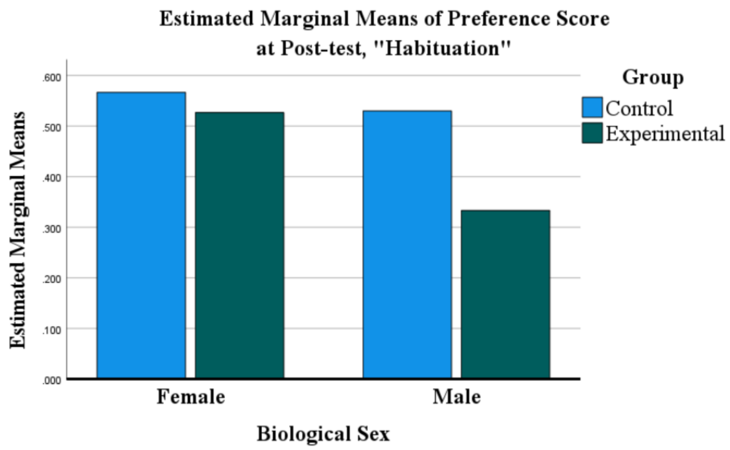

*Figure 15: Posttest, Habituation*. Female Preference Score > Male Preference Score

A repeated measures ANOVA was conducted to compare biological sex and experimental condition in relation to preference scores. No significant differences were found in relation to a biological sex, experimental condition, and pre/posttest preference score interaction, and Table 6 reflects means from each experimental group in relation to biological sex and pre-test 1, pre-test 2, posttest 1, and posttest 2. Figures 16-17 graphically illustrate female and male preference score means in each pre- and posttest trial, respectively. Control females exhibited a non-statistically significant increase in preference scores between pre- and posttests in the control group, which was not exposed to enrichment during conditioning. Control male preference scores did not significantly change from pre-tests to posttests. Experimental female exhibited higher pre- and posttest preference scores than experimental males, though their preference scores slightly decreased after enrichment conditioning, which was reflected in experimental male pre- and posttest preference scores as well.

Table 6

*Repeated Measures Test Means for Biological Sex, Experimental Condition, and Pre/Post Trial Preference Score Interaction*

*8. SEX * GROUP * Pre_Post_PrefScore*

Measure: Preference Scores

| | | | | | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| SEX | GROUP | Pre/Post Pref. Score | Mean | Std. Error | Lower Bound | Upper Bound |
| Female | Control | Pre-test, Acclimation | .340 | .096 | .120 | .560 |
| | | Pre-test, Habituation | .400 | .031 | .329 | .471 |
| | | Posttest, Acclimation | .523 | .060 | .385 | .662 |
| | | Posttest, Habituation | .567 | .064 | .420 | .714 |
| | Experimental | Pre-test, Acclimation | .557 | .096 | .336 | .777 |
| | | Pre-test, Habituation | .580 | .031 | .509 | .651 |
| | | Posttest, Acclimation | .563 | .060 | .425 | .702 |
| | | Posstest, Habituation | .527 | .064 | .380 | .674 |
| Male | Control | Pre-test, Acclimation | .466 | .096 | .246 | .687 |
| | | Pre-test, Habituation | .547 | .031 | .475 | .618 |
| | | Posttest, Acclimation | .513 | .060 | .375 | .652 |
| | | Posttest, Habituation | .530 | .064 | .383 | .677 |
| | Experimental | Pre-test, Acclimation | .457 | .096 | .236 | .677 |
| | | Pre-test, Habituation | .483 | .031 | .412 | .555 |
| | | Posttest, Acclimation | .460 | .060 | .322 | .598 |
| | | Posttest, Habituation | .333 | .064 | .186 | .480 |

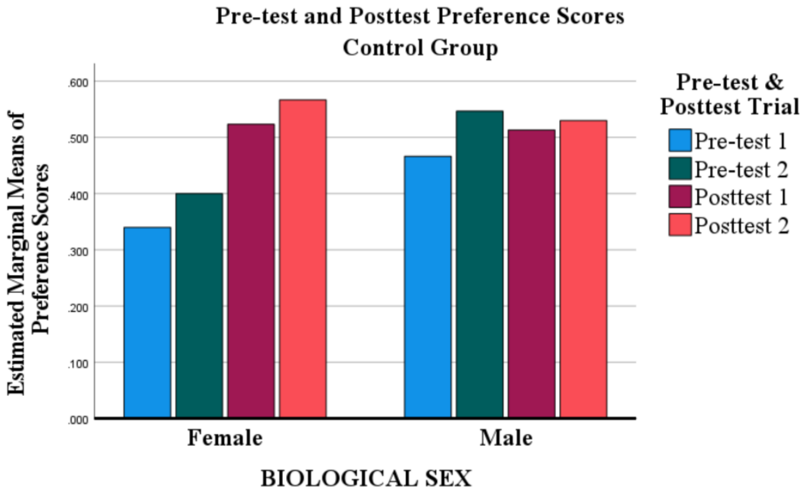

*Figure 16: Control Group Pre-test and Posttest Preference Scores.* Female Posttest Preference Scores > Female Pre-test Preference Scores.

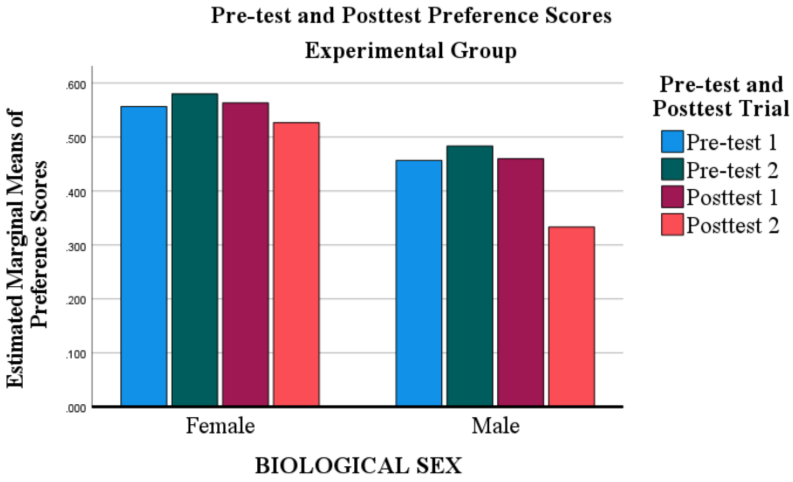

*Figure 17: Experimental Group Pre-test and Posttest Preference Scores.* Female Pre-test and Posttest Preference Scores > Male Pre-test and Posttest Preference Scores

# CHAPTER IV

# CONCLUSIONS AND DISCUSSION

## Conclusions

### The Effect of Enrichment on *Monodelphis* Behavior & Biological Sex

When examining biological sex differences, it was found that females exhibited higher duration of mobility than their male counterparts. This helps to answer the question that environmental enrichment does have an effect on gray short-tailed opossum behavior. Because females exhibited statistically higher levels of mobility than males on more than one occasion, the question of enrichment's effectiveness on male opossums specifically emerges, or if this effectiveness could be due to their young age or siblinghood. Further research is needed to replicate and verify higher levels of female *Monodelphis* mobility in response to enrichment, but a larger sample size may be able to determine if the effect can be seen in male opossums, opossums of varying age, or opossums of different litters.

### The Effectiveness of Enrichment in the Conditioned Place Preference Paradigm

With no significant differences within or between pre-tests and posttests, findings indicate that environmental enrichment is not significantly effective for conditioning using the conditioned place preference paradigm. When analyzing pre-test 1, there should have been little to no difference in preference scores across all pre-test acclimation periods, since conditioning had not yet been implemented and the apparatus was novel to the subjects. The same is true for the pre-test habituation period. Had environmental enrichment been effective in acting as a reinforcer for conditioned place preference conditioning, experimental group posttest preference scores would have indicated significant differences both between the control and within the experimental group compared to pre-test preference scores.

## Biological Sex Differences in Conditioned Place Preference

Findings indicated a significant interaction between biological sex and the experimental condition subjects were assigned to, in relation to preference scores. This refers to females in the experimental group having significantly higher preference scores than males in both the experimental and control groups. Since it was established that enrichment was not an effective reinforcer for associative learning, the significant interaction across all trials may indicate biological sex differences on another variable, i.e. exploratory behavior, among countless others.

There was an apparent trend favoring higher female preference scores across most pre- and posttests, and a decreasing trend in male preference scores through pre- and posttest trials, the opposite of what would be expected from a reinforcer conditioning paradigm. These trends of female preference even before independent variable exposure may have ties to females exhibiting higher levels of mobility than males when exposed to enrichment; the hypothesis that a novel item or environment may prompt behavior differences arises. The decreasing trends in male preference is similar to the findings of Ramirez (2018), which may prompt that habituation rather than conditioning is occurring with repeated reinforcer exposure. Further studies to verify the possibilities are needed, with a potentially larger sample size and characteristically diverse sample of laboratory opossums.

## Discussion

When identifying the significance of the studies conducted, it is important to refer back to the versatility of the *Monodelphis* as an animal model and what these findings could mean when applied comparatively to humans, or other animal models alike. The *Monodelphis* has already distinguished its unique physiological relation to humans in cases like melanoma susceptibility (VandeBerg, 1997). Despite findings in such niche areas of study, literature investigating other aspects of the *Monodelphis* remains limited, so there could be even more similarities that lead to *Monodelphis* studies being translated to human needs and research that have yet to be investigated and discovered. As a marsupial model, the *Monodelphis* is often investigated for X-linked gene research, and with possible biological sex differences being investigated and concluded, this study and others like it that aim to investigate biological sex differences may yield translational findings for other marsupial models, or even be used comparatively to human research (VandeBerg, 1997).

Despite the *Monodelphis* emerging in popularity as a non-traditional animal model, several areas of research utilizing it for study remain limited to non-existent. Findings from studies like this one that seek to answer a question, then takes a possible answer to that question and applies it to another realm of study and paradigms serves to diversify literature from several angles. With the *Monodelphis* being a translational model not just for other marsupials, but for humans as well, it is imperative to keep the influx of information and discoveries into the field of psychology constant by diversifying literature with this non-traditional animal model, and continuing to add to existing *Monodelphis* knowledge so that we may understand it better from an array of perspectives. This study's limitations serve as a possible starting point for future replicability attempts, and with hypotheses forming based on findings to similar research, there is still much to answer and discover about the emerging laboratory opossum.

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# BIOGRAPHICAL SKETCH

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