INFLUENCE OF ENRICHMENT ON THE BEHAVIOR OF NEW ZEALAND WHITE RABBITS AND CYNOMOLGUS MACAQUES IN A RESEARCH SETTING DURING THEIR QUARANTINE PERIOD

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Dedication:

This thesis project is dedicated to my family and friends. John and Suzanne Sheehy, my parents, who helped create an environment where I was able to pursue my passion of working with animals, their support and words of encouragement has had a tremendous impact on my professional and academic success. My close family members and friends have provided lots of support and encouragement to follow my dreams. This is also dedicated to my pets and all of the different animals I have worked with, as they inspire me to always do better to have a positive impact on their welfare. My dogs, Jackson, Raven, and Rufus, and my cats, Simba, Timon, and Pumbaa have been the most loyal companions and have spent many nights by my side throughout this academic experience.

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Abstract:

Enrichment is an excellent tool used to refine animal husbandry practices in laboratory animal medicine. When animals first arrive at a facility, the animals are given a quarantine period to ensure they are healthy, and to allow the animals to acclimate to the facility. By offering environmental enrichment and food enrichment at different frequencies, we can observe how different levels of enrichment influences the behavior of the animals. In this experiment, New Zealand white rabbits (*Oryctolagus cuniculus*) and cynomolgus macaques (*Macaca fascicularis*) were observed to see how various types of enrichment promote animal welfare and behavior and this is illustrated by the presence of positive behaviors being observed and the absence of negative behaviors. For each species, an ethogram was created to measure the behaviors of the animals, and noninvasive observations were taken daily while the animals were undergoing their quarantine period. We also monitored fecal output to observe the animals' physiological health. Enrichment was given to the animals in three different treatment groups. Group 1 received the base enrichment, one form of environmental enrichment, changed weekly, and received food enrichment once weekly. Group 2 received two forms of environmental enrichment, the same device as Group 1, in addition to another device. Group 3 received both types as enrichment as Group 2, as well as a daily food enrichment. For each species, we predicted that Group 3 animals, will show more positive, friendly behaviors and less negative, stereotypic behaviors. The data suggests that there was statistical significance between the different variables for the rabbits. The data suggests that there was statistical significance between most of the variables for the macaques, with the exception of fecal output and displaying stereotypic behaviors, which

were not significant. We concluded that both environmental and food enrichment had a positive influence on the behavior of the animals during their quarantine period, as seen by the observations of their behavior.

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Introduction:

Different types of animal models are used in research settings for a variety of purposes. Two animal models that will be observed for this research are New Zealand white rabbits (*Oryctolagus cuniculus*) and cynomolgus macaques (*Macaca fascicularis*). Many studies have looked to determine the effects of enrichment on laboratory dogs, nonhuman primates, and even rabbits, but these studies have not focused on how the enrichment can affect the acclimation period prior to use in a laboratory setting (Meidam et al., 2021). When the animals arrive at the laboratory facility, they are given several days to adjust to their new living arrangements prior to being used on a study. This period is the quarantine and acclimation period. The facility uses the quarantine period to ensure that the animals are healthy when they arrive, but it doubles as an acclimation period as the animals are able to adjust to their new surroundings.

Acclimating to a new environment can be stressful, this stress can be observed through the animal's health and behavior. When animals arrive at a laboratory facility, they are given some time to acclimate to their new surroundings for a period of time prior to being used in research activities. The transportation process to the laboratory environment can be extremely stressful for animals, the stress can lead to changes in the cardiovascular, endocrine, immune, central nervous, and reproductive systems, allowing for some period of acclimatization following transportation is suggested to restore homeostasis in these animals (Obernier and Baldwin, 2006). In a laboratory setting, animals may experience pain based on the type of the experiment, as long as it is scientifically justifiable. While the animals are in quarantine and acclimating to their

environment, they are not exposed to any invasive procedures and should not experience any pain or distress. The prior experiences of the animal may influence their behavior as well but can be difficult to determine what the animal may have experienced.

Environmental enrichment and food enrichment have both been used to promote species-specific behaviors and allow for animals to display natural behaviors. After Congress amended the Animal Welfare Act in 1985 to promote the psychological well-being of primates, the scientific community began using environmental enrichment to improve the well-being of captive animals (Beaver, 1989). Enrichment is beneficial to the welfare of the animals as it is a tool that the animals can control; this helps prevent the animals from experiencing boredom in their environment and also decreases the likelihood of the animals displaying stereotypic behaviors (Poggiagliolmi et al., 2011). The frequency of the enrichment provided can influence the behavior of the animals and may potentially limit stress experienced by the animal during stressful experiences, such as transferring into a research laboratory and acclimating to their new environment.

Rabbits and cynomolgus macaques display behaviors very differently from one another, but the behaviors they display could potentially give insight to animal's current welfare state. As animals benefit from receiving environmental and food enrichment, it is predicted that when enrichment is offered at a higher frequency, it will have a positive influence on the behavior of the animals during their acclimation period to a laboratory environment. This is expected be seen by the presence of positive behaviors and the absence of negative behaviors.

The goal of this experiment is to identify if environmental enrichment and food enrichment can positively influence the welfare of the animals, specifically New Zealand White rabbits and cynomolgus macaques, during their acclimation process to the laboratory environment. This will be tested by offering different types of enrichment at different frequencies to the animals. I hypothesize that as the animals receive enrichment they are exposed to positive human interaction, which will not only enhance the acclimation of the animals to the laboratory environment, but also to humans in the research setting, potentially making the animals less fearful and less stressed while used for various study purposes.

New Zealand White Rabbit:

As animal behavior will be observed for this experiment, it is important to understand certain behaviors that rabbits display. Rabbits are lagomorphs, and there are lot of behaviors that are specific to rabbits. Rabbits are a prey species so even humans can cause the animals to respond negatively. The body language of rabbits can be useful in determining the animal's welfare state. Enrichment has been found to improve the function and structure of whole brain networks in rabbits (Illa et al., 2018). While rabbits are not as charismatic as other research models such as dogs, their faces, ears, and body posture can be used to indicate how the animal is feeling and how it reacts to different stimuli. When frightened, a rabbit may run away, freeze, or even attack with its teeth and claws, a rabbit can learn to use these behaviors if humans respond to them, teaching the rabbit to use aggression to defend itself (Crowell-Davis, 2007). Rabbits will display certain behaviors such as thumping and freezing in response to stressors in the

environment. Thumping is typically seen as an aggressive behavior in rabbits. Freezing is response that the rabbit does that is associated with fear. Rabbits can be seen twitching their noses, or sniffing, to display curious behaviors.

Rabbits interact with enrichment in different ways, they can observe it and smell it, they can chew on enrichment, toss it around, nudge it, and potentially avoid or ignore it. There are several dependent variables that will be used to measure the rabbits' health and behavior. In a study comparing food and environmental enrichment, food was a stronger enrichment device than non-food objects (Harris et al., 2001).

In order to evaluate the effect of enrichment on rabbit acclimation, I will make several behavioral and physiological assessments. Fecal output is one of the variables that will be measured to see if the rabbits are physiologically healthy. Rabbits that are eating and drinking water at a healthy amount will produce healthy amounts of stool. Typically, in laboratory rabbits, rabbits that do not have feces in their litter pans have an underlying health issue that needs to be addressed, it could be not eating or drinking, or it can be due to another cause. In another study, the concentration of glucocorticoid metabolites (GCM) in rabbit feces has been suggested as a non-invasive indicator of stress, found that environmental enrichment reduced GCM concentrations before and after the animals were exposed to different stressors (Buijs et al., 2011). For this study, the amount of feces will be used to identify that the animal is healthy, and it assumes the animal is eating and drinking normally.

The rabbit's position in the cage while the observer is away from the cage is important to note and measure as the animal's position might change when the observer

approaches the cage. The position in the cage will be measured by identifying if the animal is in the front, middle, or back of the cage. Animals that are at the front of the cage seem to be comfortable with their surroundings and are interacting with their environment. Animals in the back of the cage are often seen in states of relaxation or fear, some animals are relaxed and laying down, while others are trying to hide. When enrichment is provided to the animals, it is placed in the front of the cage, to encourage the animals engage in species-specific behaviors.

Thumping is a unique behavior that rabbits display, this behavior was described by Charles Darwin as a loud stamp on the ground to signal to their comrades; but rabbits and other animals may thump when angry (Black and Vanderwolf, 1969). Not only is this behavior seen as an aggressive way to communicate with others, but it is also seen as a part of the fear or pain response (Black and Vanderwolf, 1969). By observing the animals for thumping, it can provide insight to the animal's welfare state as the animal may be in a state of fear or experiencing pain. Thumping is a stereotypic behavior that the rabbits display in response to stress in the environment.

Freezing is another behavior that will be measured in this experiment. Rabbits are prey animals, and the freezing response is essentially a coping mechanism that they use in stressful situations. Low frequency and duration of freezing indicates low fear response in rabbits (Verga et al., 2007). This behavior can be a stereotypic behavior in rabbits, if the animal is constantly freezing for extended periods of time (Poggiagliolmi et al., 2011).

Movement is important to observe in rabbits because the type of movement can indicate what the animal is experiencing. If the animal is relaxed and laying down, it may not move at all. If an animal is in the front of the cage, and then moves to the back of the cage when approaches, this movement can be a fear response. Or the animal may be moving in the cage by interacting with its environment.

Interacting with enrichment is another way to tell if an animal is comfortable in its environment. In order for the rabbits to interact with the enrichment, they have to be offered it first. By providing environmental enrichment specific to rabbits is one way to improve the living conditions of these laboratory animals and ultimately the welfare of them (Baumans, 2005). This can be observed by the animal nudging the enrichment, sniffing it, tossing it, and chewing it. Enrichment allows for the animals the opportunity to interact with its surroundings and have some sort of control while in the cage, this gives the animal better welfare (Hansen and Berthelsen, 2000). Chewing is a behavior that can be observed when a rabbit is interacting with its enrichment, this behavior is seen in high frequencies, as rabbits need to chew in order to maintain good dental health (Huls et al., 1991).

Sniffing or nose twitching is a sign that the animal is interacting with its environment in a curious way, usually detecting scents (Jenkins, 2001). This behavior can be done with other behaviors, but it has also been observed while the animals are relaxed and laying down.

Rabbits can be quite curious, and this can be seen by their exploratory behavior. Rabbits will investigate their enrichment prior to physically engage in it, but rabbits also

investigate people in the room. I predict that animals that come to the front of the cage to investigate the observer are displaying exploratory behaviors.

The cage size can influence the rabbit's behavior, but during these studies, the rabbits were housed in the same sized cage for the observations. Enrichment can help prevent stereotypic behaviors in rabbits, such as cage manipulations, tearing the liner, loosening the water valve, destroying parts of the cage if they are able to do so (Buijs et al., 2011). Rabbits that have environmental enrichment devices to interact with have positive welfare, as the enrichment allows for the animal to display species-specific behaviors (Coda et al., 2020).

Cynomolgus Macaque:

Cynomolgus macaques are interesting research models and have such individualized personalities. Their intelligence and individualization can create challenges when assessing their behavior, especially during quarantine, as some individuals acclimate rather quickly to the daily routines within the laboratory environment. There are several variables that will be observed to analyze the behavior of the cynomolgus macaques. Cynomolgus macaques share many behavioral similarities with rhesus macaques, and it is not uncommon to reference literature based on these types of monkeys (Teng et al., 2021). The National Centre for the Replacement Refinement & Reduction of Animals in Research (NC3Rs) sponsors The Macaque Website. The Macaque Website is a great resource to learn about the behavior of macaques, this site has a database on the common behaviors, postures, vocalizations, social behaviors, and the potential reason for the monkey to display them.

To understand how the cynomolgus macaques respond to their enrichment during the acclimation period, I will observe several behavioral and physiological measurements. There are no peripheral biomarkers associated with environmental enrichment, so it is important to observe behavior (Nader et al., 2012). Fecal output is important to make sure that the animals are physiologically healthy, and it is not uncommon for stressed monkeys to have loose stool. In a study with rhesus macaques, after being in new groups, animals showed signs of loose stool as a response to the stressful transition (Goo and Sassenrath, 1980). Some monkeys even smear their stool on the walls of the cage, this is a stereotypic behavior.

The position of the monkey in the cage is important as a behavioral measure. These animals are vigilant, they tend to always be awake when humans are around and they can be in the front, middle or back of the cage (NC3Rs). Monkeys utilize the space of the cage very differently than other animals, but typically use the top corners of the back of the cage to retreat and feel safe.

Cynomolgus macaques are very social animals, and can communicate with their facial expressions, body language, and vocalizations. There are several facial expressions that macaques use. These are seen in affiliative or interactive expressions, neutral or withdrawn expressions, and even aggressive expressions. These can be done together with other behaviors than what they are typically intended for depending on the context. Lip smacking represents peaceful intentions and is the facial expression that we anticipate seeing the most of. Displaying bared teeth is an example of an aggressive behavior. (NC3Rs).

Body posture or body language of the macaques can be used to identify if the animal is alert or relaxed. This behavior can be interpreted by the observer to see if the animal is alert when the observer interacts with the animal. Relaxed animals can still interact with the observer, but typically they can do so without appearing startled or moving quickly.

Interacting with enrichment is important for the monkeys as we want to limit stereotypic behaviors. Usually, these animals are seen playing with their toys, actively watching their movie, and they quickly eat their food. Different textures of the food enrichment are good ways to maintain novelty. Some of the monkeys interact with their food prior to eating it, they pick it up, smell it and look at it prior to tasting it, while others will quickly shove the food in their cheek pouch and then investigate it prior to swallowing it.

Displaying stereotypic behavior is undesired, but it does happen. The goal with this is to identify any individuals displaying stereotypical behavior and then trying to identify the cause. Some types of stereotypic behaviors that are common in macaques include smearing stool on the wall, repetitive motion and flips, self-mutilation, overgrooming, cage shaking, bar biting, etc. If these behaviors are seen, it will be noted, and the specific behavior will be identified.

Displaying affiliative behavior is desired. This can be through facial expressions, body language, vocalizations, and even just the animal being comfortable enough to eat in the presence of humans. Some animals will even come to the front of the cage and wait for their food to be given to them.

Materials and Methods:

All behavioral research on rabbits and cynomolgus macaques described in this study was conducted at IIT Research Institute (IITRI), a fully AALAC-accredited institution, and was reviewed and approved by IITRI's IACUC.

The goal of this research is to see how offering novel environmental and food enrichment can help animals acclimate to the laboratory environment. This idea is based on the human-animal bond: by offering enrichment to the animals, the animals are exposed to positive human interaction, which can prevent fearful responses that could adversely influence future unavoidable interactions with research technicians (Poggiagliolmi, et al., 2011).

The animals were acquired under alternate IACUC-approved protocols and used for the present noninvasive study during their acclimation period to the facility. Three different rabbit rooms and two different cynomolgus macaque rooms were observed for this experiment. A scoresheet was created as a paper-based ethogram for each species observed for this study.

The animals were given one full day to acclimate to their environment prior to being observed and offered enrichment at different frequencies. At IITRI, all animals are provided with at least one form of environmental enrichment. All of the rabbits are offered food enrichment at least once a week. The nonhuman primates are offered fresh produce with their laboratory grade nonhuman primate biscuits twice a day and are offered a novel food enrichment daily.

For this experiment, different groups of animals will receive more enrichment during their acclimation period to see if the additional enrichment helps the animals acclimate to the facility more efficiently. After the data is collected, it will be analyzed, and the findings will be discussed.

New Zealand White Rabbits:

The rabbits are housed individually in standard laboratory racks that contain cages specifically designed for rabbits. The cages are suspended over litter pans that are lined with absorbent liners. These liners are changed by animal care personnel every Monday, Wednesday, and Friday. The animals were scored prior to the liners being changed, as the rabbits' fecal output was measured. The racks are changed every fourteen days. The toy is changed with the rack rotations, this is to maintain novelty, as studies have shown that time spent interacting with the toy will decrease over time (Johnson et al., 2003). The rabbits have ad libitum access to a laboratory grade rabbit diet and an automatic watering system that provides a constant supply of fresh water during this acclimation period. After the animals arrive, each individual is given a number to identify them. Each animal will be assessed using a paper-based ethogram.

The first rabbit room was treated as a baseline. This room had a total of twenty-seven female rabbits. All the animals received one form of environmental enrichment, which was a standard enrichment device, a plastic dumbbell, after two weeks, this toy was rotated with another device. These animals received food enrichment on Wednesdays. The ethogram designed for this room was used as a trial to practice scoring the animals. This ethogram included fecal output, position in cage when person

approaches the cage, thumping, freezing, and activity- exploring the cage. The animals were given one day to acclimate to the facility and then they were scored by one observer, once a day, for six days.

After observing these animals, the ethogram was modified to include other behaviors that were seen (Appendix A). A legend was created and used for scoring the behaviors (Appendix B). The addition of the rabbit's position in the cage when the person is away from the cage, interacting with enrichment, and sniffing or nose twitching. Activity- exploring cage was modified to separate movement, and curiosity or exploratory behaviors, as some animals would move, but not because they were curious.

The second rabbit room was treated as Rabbit Experiment 1. This room included twenty-two males and twenty-two females, forty-four animals in total. These animals were separated into groups. Groups 1 and 2 each had seven males and seven females, Group 3 had eight males and eight females. Group 1 was the control, where the animals received one toy, dumbbell, and weekly food enrichment. Group 2 received the same toy as Group 1, but also a cardboard tube filled with hay. Group 3 received the toy, tube stuffed with hay, and a daily food enrichment. The daily food enrichment was often a vegetable, either a piece of kale or a slice of zucchini, on days when all animals received food enrichment, Group 3 received an apple slice in addition to what the other animals received. The hay for the cardboard tubes was refilled on the days that the liners were changed. The daily food enrichment and the hay refill occurred before scoring the animals. These animals were given one day to acclimate to the facility and then were

scored by one observer, once a day, for eleven days. The time the room was observed was recorded, along with any notes.

The third rabbit room was treated as Rabbit Experiment 2. This room included twenty-eight females and they were divided into three groups. Group 1 had ten females. Groups 2 and 3 each had nine females. The groups were treated the same way as Rabbit Experiment 1. These animals were time-mated females that were given one day to acclimate to the facility, and then were scored by one observer, once a day, for seven days. The time the room was observed was recorded, along with any notes.

The data from these experiments was then translated from paper into a Microsoft Excel spreadsheet for the data to be analyzed. The mean for each group number was calculated per day for each variable. These averages were plotted on scatterplots to visually compare the group averages over time. The data was also analyzed in the statistical program Stata for mixed effects ordered logistic regression. For each variable, if the Omnibus (Prob > Chi^2) was less than 0.05 the data is considered statistically significant. The P values for each group were also calculated, and if less than 0.05, suggest that the data for those groups are statistically significant for each variable. Sex and trial day are two fixed variables that were also analyzed during the statistical analysis.

Cynomolgus macaques:

Cynomolgus macaques at IITRI are housed in rack systems specifically designed for nonhuman primates. The racks are changed every fourteen days. The racks are

hooked up to an automatic watering system. Each rack has four cages, two on the top of the rack, and two on the bottom, and each animal gets their own home cage. The racks are designed with dividers, so that the animals can see the individuals in the cage next to them. When pair housed, the dividers are opened so that the two animals have complete access to both cages. Another unique feature about nonhuman primate cages is the squeeze bar that allows for the back of the cage to brought up forward, reducing the space of the cage.

At IITRI, the animal care personnel wash the room down daily, check the water valves of the watering system, feed the monkeys the morning produce with biscuits, offer a novel food enrichment, provide a movie or music, and then offer the evening produce with biscuits. The novel food enrichment offered can be any treat, it can be a frozen treat, peanut butter, jelly, yogurt, fruit snacks, freeze-dried mealworms, freeze-dried banana chips, apple sauce, raw or cooked pasta, popcorn, rice, or even the rinds and peels of the fruit. Each animal is given one toy, but the room will have two different toys, that alternate, so when the animals are pair housed, they can each play with a toy, and the toys are different to maintain novelty and prevent boredom. The main environmental enrichment devices include a variety of toys, such as several types of approved dog toys, mirror balls filled with sand, the dumbbells similar to the rabbits, and other small toys that the monkeys can hold and manipulate. The movie provided is changed every three days, to maintain novelty but also to allow the animals to enjoy their cartoons. This creates background noise which can help dilute the excess noise from other parts of the

facility, such as the cage wash equipment, construction noise, and the sounds from other animals in the facility.

The ethogram developed for the cynomolgus macaques includes fecal output, position in cage, facial expression, body posture, interact with enrichment, display stereotypic behavior, and display affiliative behavior (Appendix C). A legend was created and used for scoring the behaviors of the animals (Appendix D).

The first room of cynomolgus macaques was treated as Monkey Experiment 1. This room included forty young macaques, twenty males and twenty females. These animals scored on a paper-based ethogram, after one day of being at the facility, by one observer, once a day, for twenty days. The observer noted the date and time, as well as any notes or additional information about the experiment. After scoring the animals for the first time, they were pair housed and monitored closely for any signs of aggression. The animals arrived with tattoos which are used to identify the individuals while in pair housing. There were five racks of males and five racks of females, the first three racks for each were in Group 1. Then the fourth rack was Group 2, and the fifth rack was Group 3. For this room, each cage is given a toy, and the toys alternate so that the two monkeys can play with two different toys. All the animals received the food enrichment with breakfast, and then Group 3 would receive an additional food enrichment prior to being observed. Group 1 received the environmental enrichment that would be offered, just the two toys that were changed weekly, and the daily movie or music. Group 2 received everything that Group 1 received, but also an additional form of environmental enrichment, such as a PVC pipe or wooden branch, also changed weekly. Group 3

received everything Group 2 received, but also an additional daily novel food item, sometimes it was the same item, to prevent the other monkeys from becoming jealous.

The second room of cynomolgus macaques was treated as Monkey Experiment 2. This room had eight male monkeys. These were older individuals and because of their size and temperament, it was decided that these animals would remain single housed to prevent any fights, as they would be single housed anyways when on study. These monkeys were divided into three groups, the four monkeys in the top cages were Group 1. Group 2 had two monkeys on the bottom of one rack, and Group 3 had two monkeys on the bottom of the other rack. These groups were treated the same way as Monkey Experiment 1. After these animals had one day to acclimate, they were scored by one individual, once a day, for fifteen days. The observer noted the date and time and any additional information, such as the specific enrichments Groups 2 and 3 received.

This data was then translated from paper into Microsoft Excel spreadsheet to be analyzed. The mean for each group number was calculated per day for each variable. These averages were plotted on scatterplots to visually compare the group averages over time. The raw data was also analyzed in the statistical program Stata. The data was analyzed using a mixed effects order logistical regression. For each variable, if the Omnibus (Prob > Chi^2) was less than 0.05 are considered statistically significant. The P values for each treatment group were also calculated, and if less than 0.05, suggest that the data for those groups are statistically significant for each variable. Sex and trial day are fixed effects, their P values were also calculated, and if less than 0.05, suggests that the data is statistically significant for those variables.

Results:

New Zealand White Rabbits:

Table 1. Rabbit Results

		P Values				
Variable	Prob > Chi^2	Group 1	Group 2	Group 3	Sex	Trial Day
Fecal Output	0.0025	0.262	0.065	0.113	0.406	0
Position Away	0		0.008	0.269	0.014	0.012
Position Approached	0.0004	0.29	0.795	0.001	0.96	0.026
Thumping	0.0039	0.04	0.038	0.037	0.757	
Freezing	0	0.015	0.482	0.019	0.667	0
Movement	0	0.137	0	0	0.176	0
Interact w Enrichment	0		0.01	0		0
Sniffing	0		0	0	0.731	0
Curiosity	0		0.015	0	0.3	0

Fecal output Omnibus value was 0.0025.

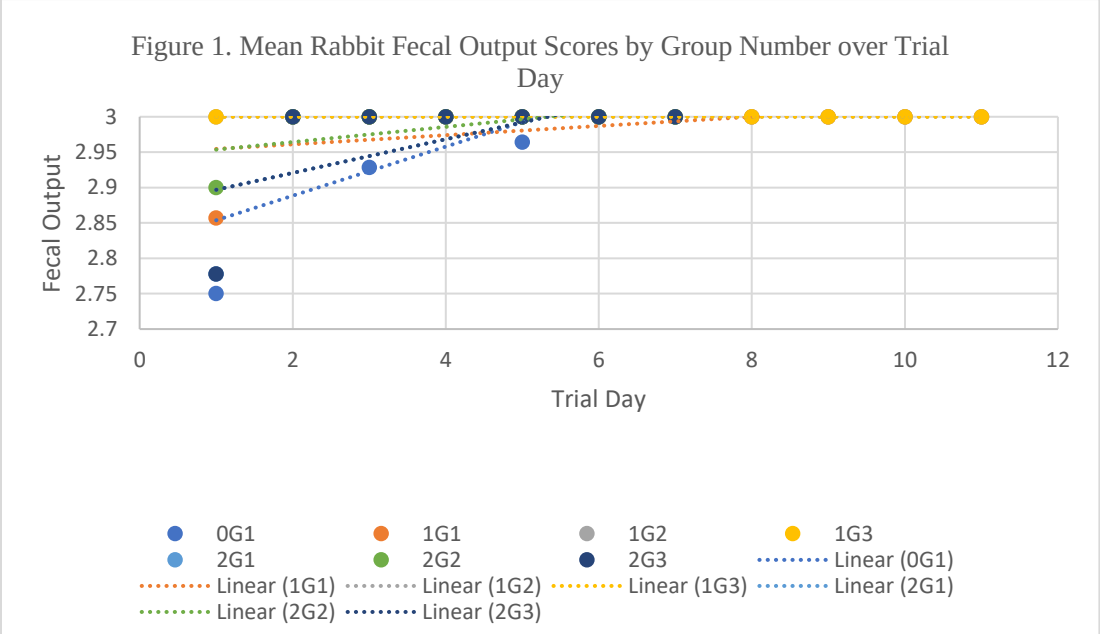

Figure 1. Mean Rabbit Fecal Output Scores by Group Number over Trial Day.

Position away Omnibus value was 0.0000.

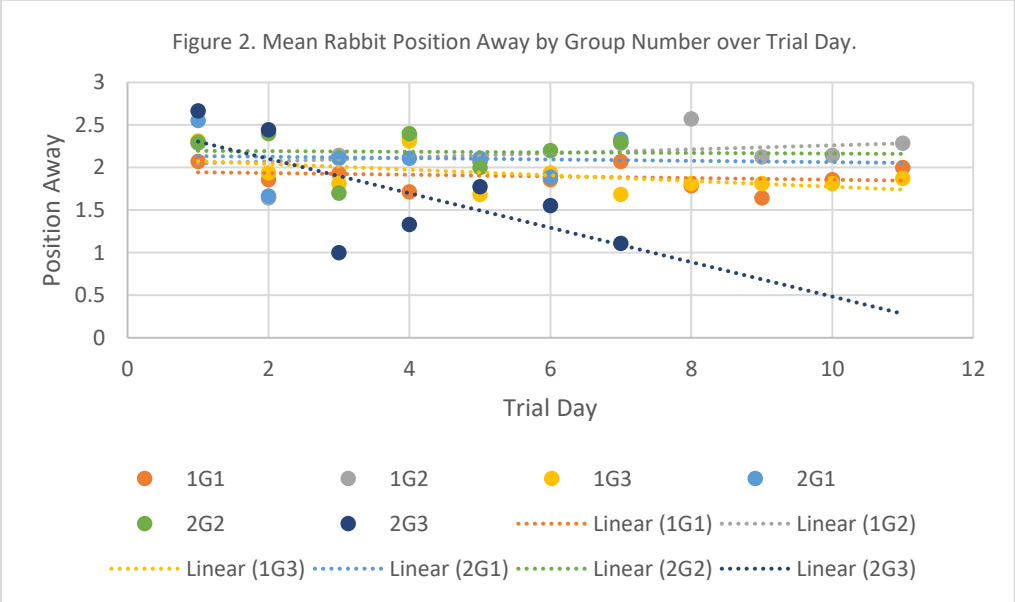

Figure 2. Mean Rabbit Position Away by Group Number over Trial Day.

Position approached Omnibus value was 0.0004.

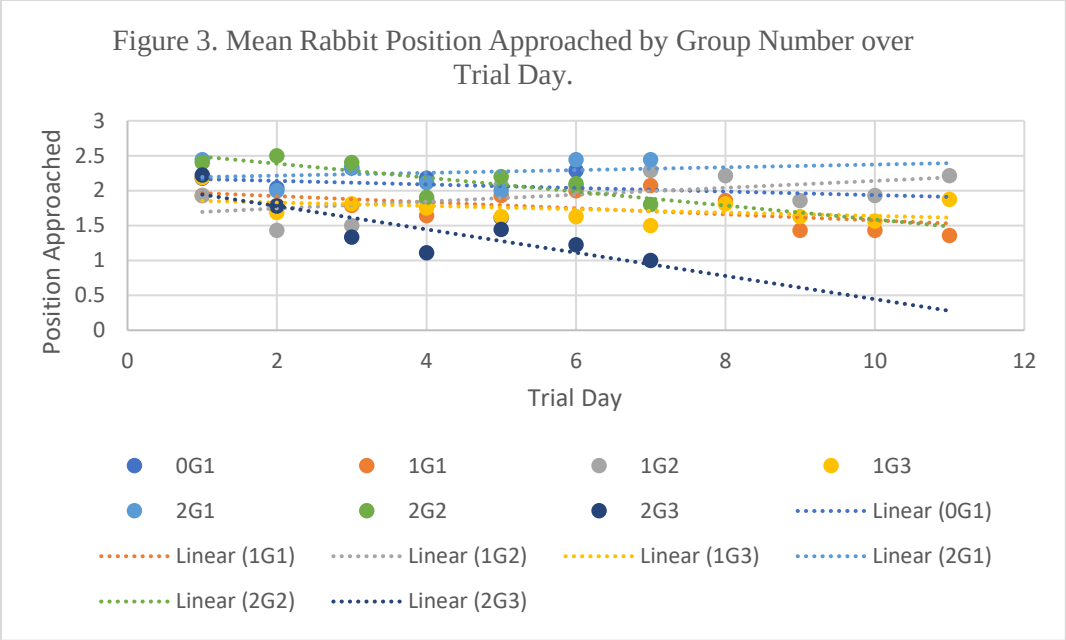

Figure 3. Mean Rabbit Position Approached by Group Number over Trial Day.

Thumping Omnibus value was 0.0039.

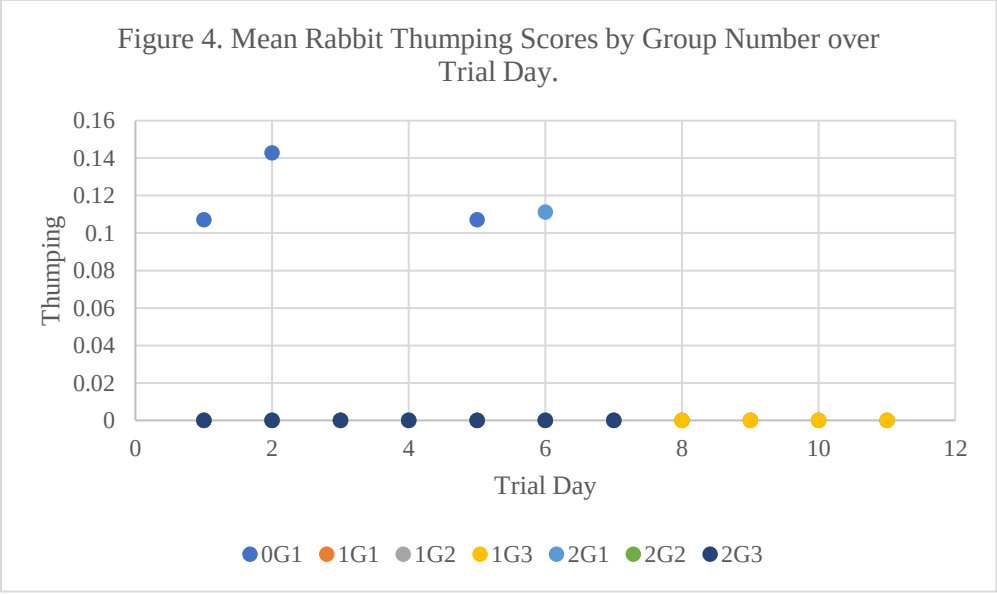

Figure 4. Mean Rabbit Thumping Scores by Group Number over Trial Day.

Freezing Omnibus value was 0.0000.

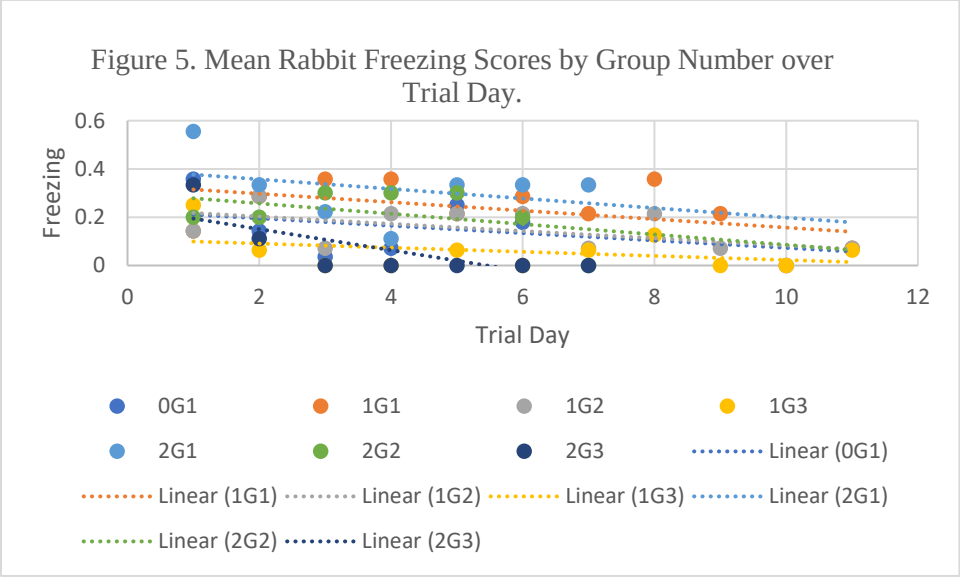

Figure 5. Mean Rabbit Freezing Scores by Group Number over Trial Day.

Movement Omnibus value was 0.000.

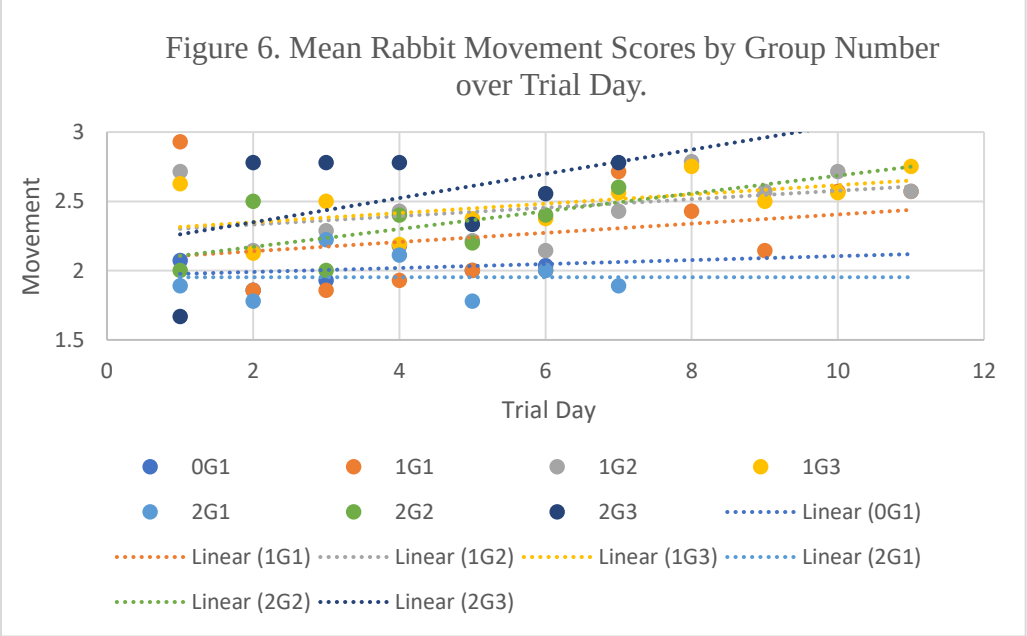

Figure 6. Mean Rabbit Movement Scores by Group Number over Trial Day.

Interact with enrichment Omnibus value was 0.0000.

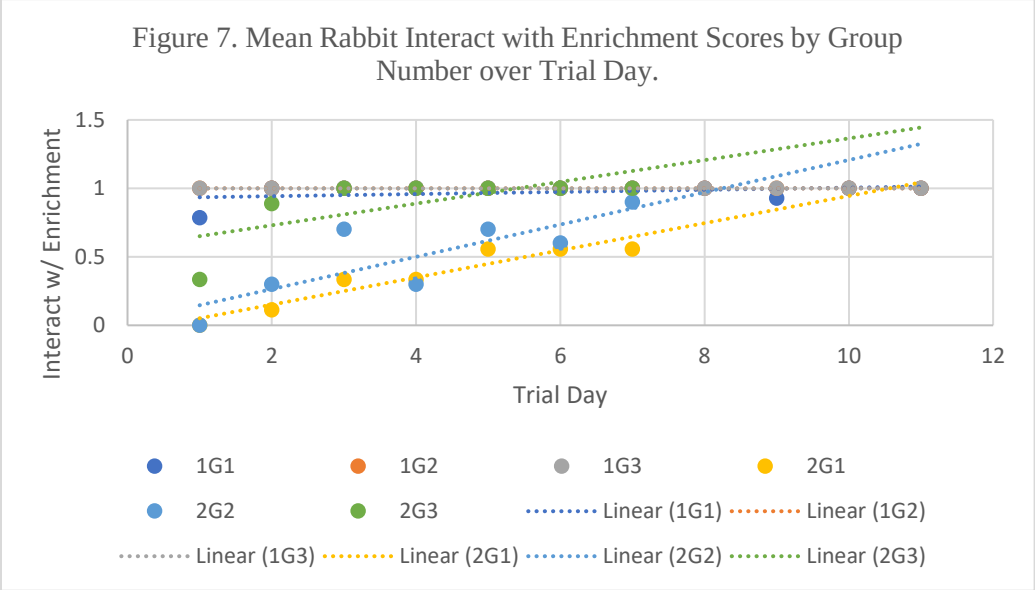

Figure 7. Mean Rabbit Interact with Enrichment Scores by Group Number over Trial Day.

Sniffing Omnibus value was 0.0000.

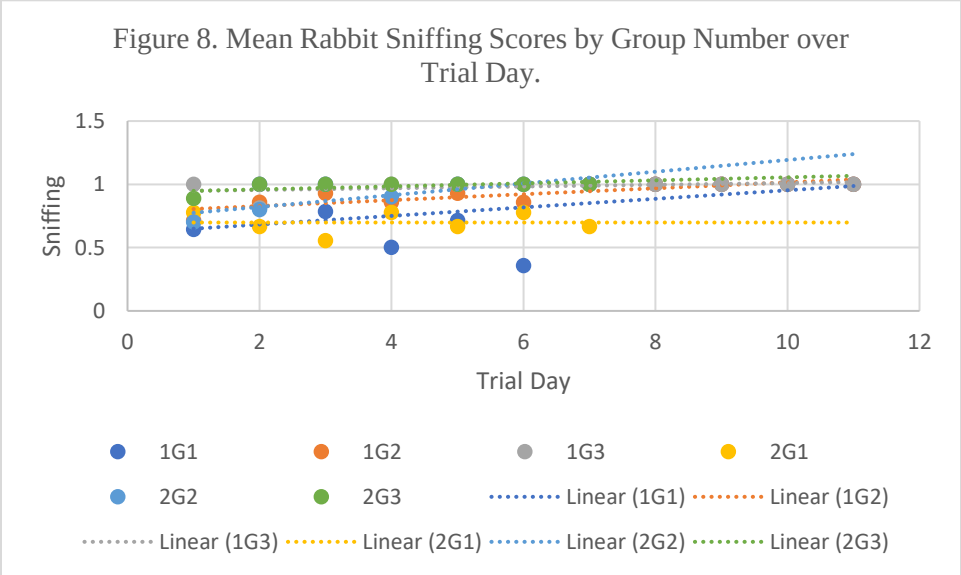

Figure 8. Mean Rabbit Sniffing Scores by Group Number over Trial Day.

Curiosity Omnibus value was 0.0000.

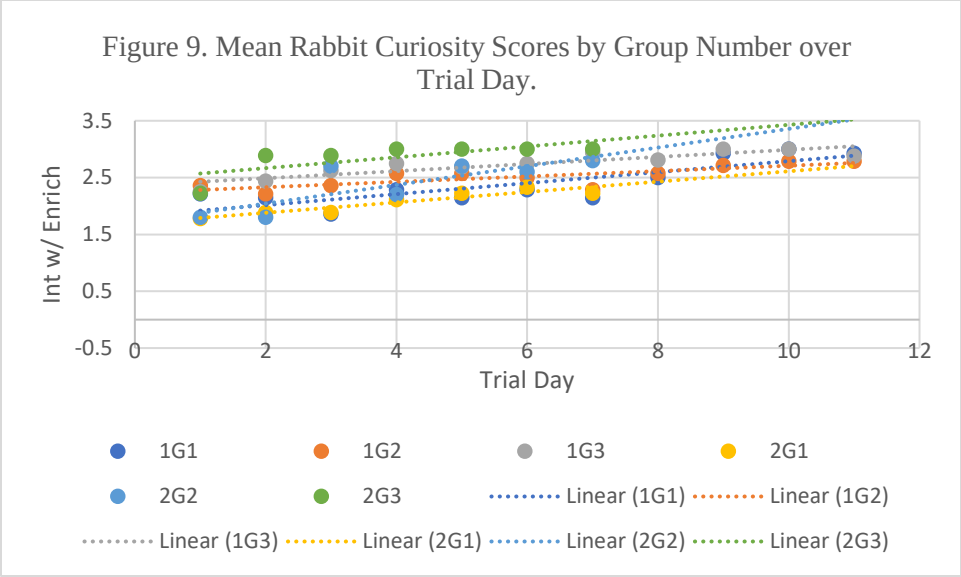

Figure 9. Mean Rabbit Curiosity Scores by Group Number over Trial Day.

Cynomolgus Macaques:

Table 2. Macaque Results

		P Values				
Variable	Prob > Chi^2	Group 1	Group 2	Group 3	Sex	Trial Day
Fecal Output	0.9842		0.603	0.603	0.876	0.974
Position	0		0.222	0	0.042	0
Facial Expression	0		0.045	0	0.031	0
Body Posture	0.0006		0.561	0	0.035	0.561
Interact w Enrichment	0		0.003		0.001	0
Display Stereotypic	0.7638		0.744		0.304	0.959
Display Affiliative	0		0.07	0.005	0.055	0

Fecal output Omnibus value was 0.9842.

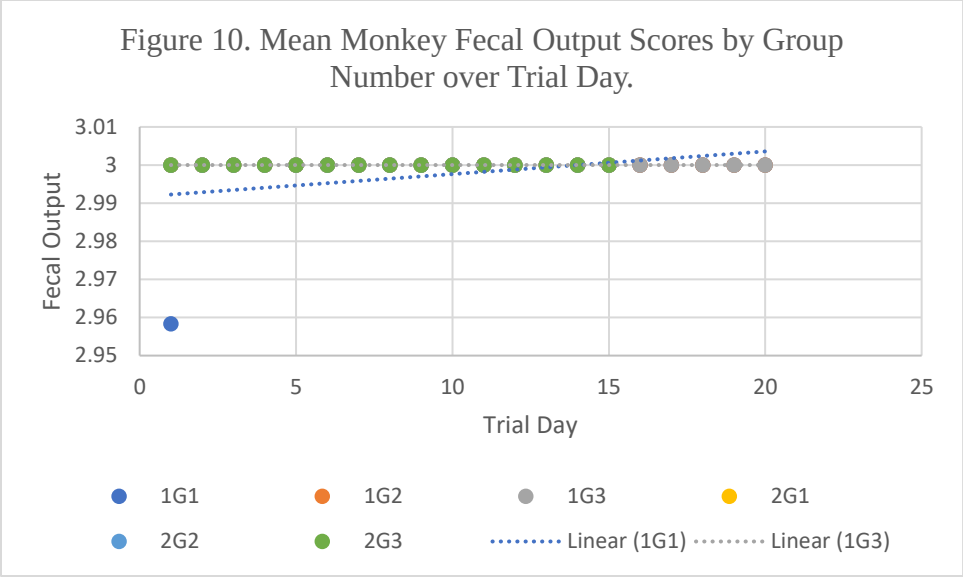

Figure 10. Mean Monkey Fecal Output Scores by Group Number over Trial Day.

Position in cage Omnibus value was 0.0000.

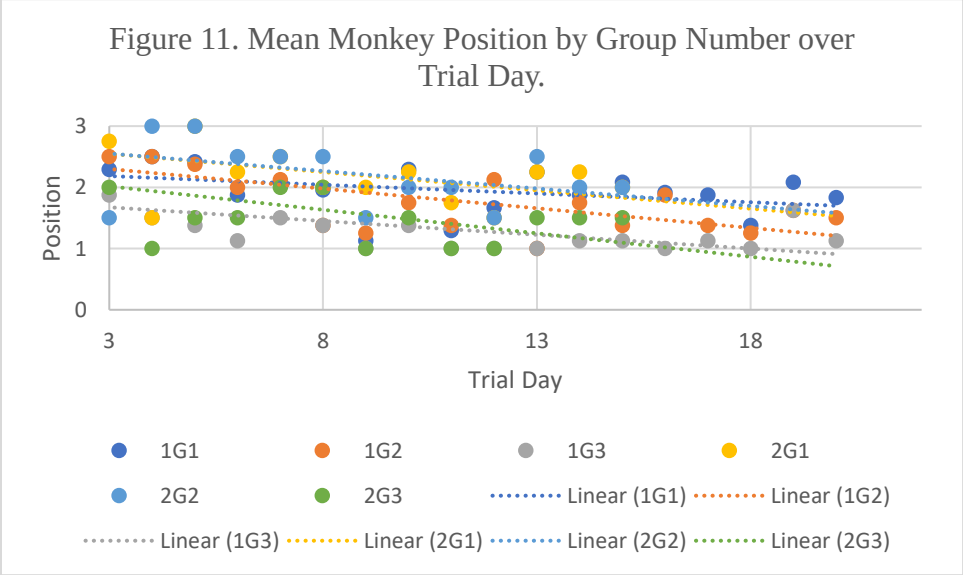

Figure 11. Mean Monkey Position by Group Number over Trial Day.

Facial expression Omnibus value was 0.0000.

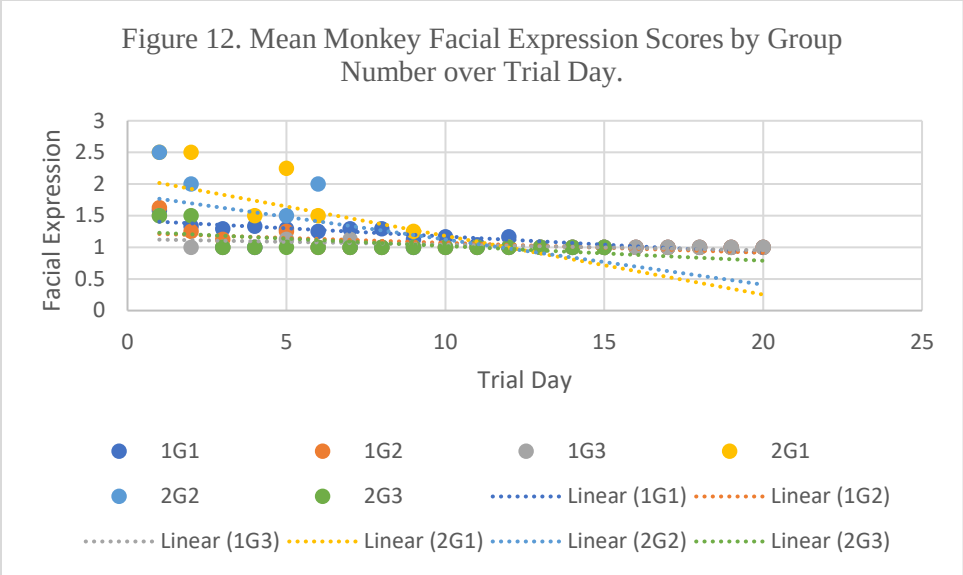

Figure 12. Mean Monkey Facial Expression Scores by Group Number over Trial Day. Body posture Omnibus value was 0.0006.

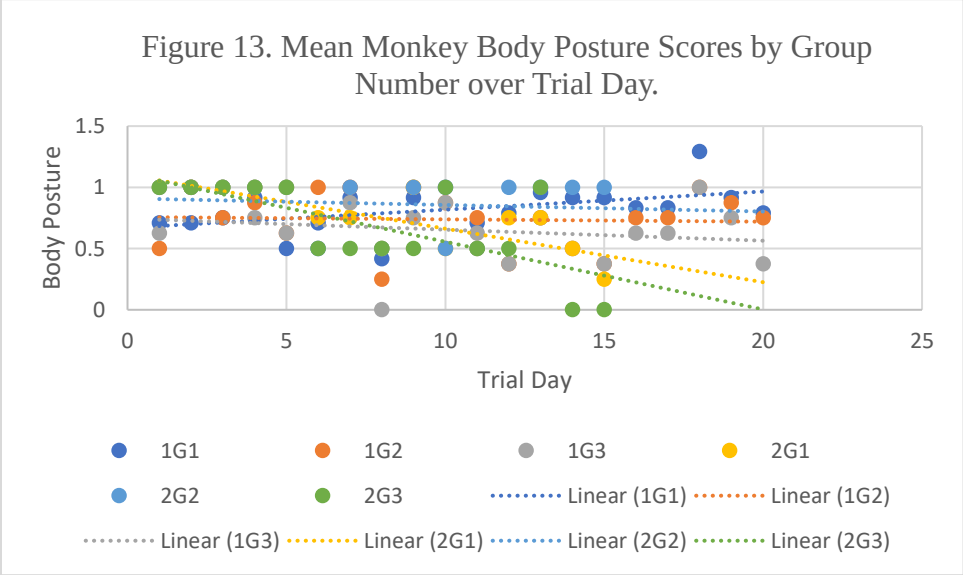

Figure 13. Mean Monkey Body Posture Scores by Group Number over Trial Day. Interact with enrichment Omnibus value was 0.0000.

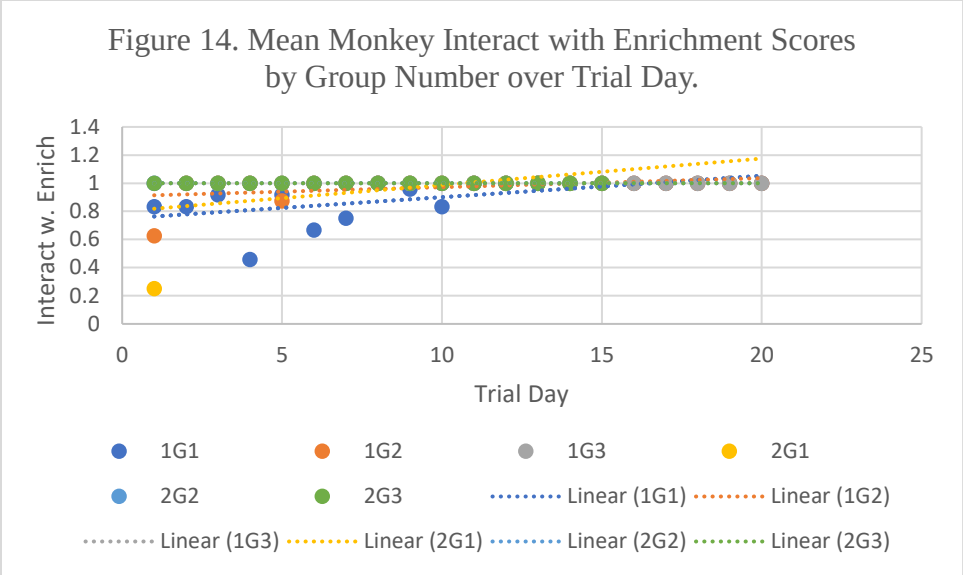

Figure 14. Mean Monkey Interact with Enrichment Scores by Group Number over Trial Day.

Display stereotypic behavior Omnibus value was 0.7638.

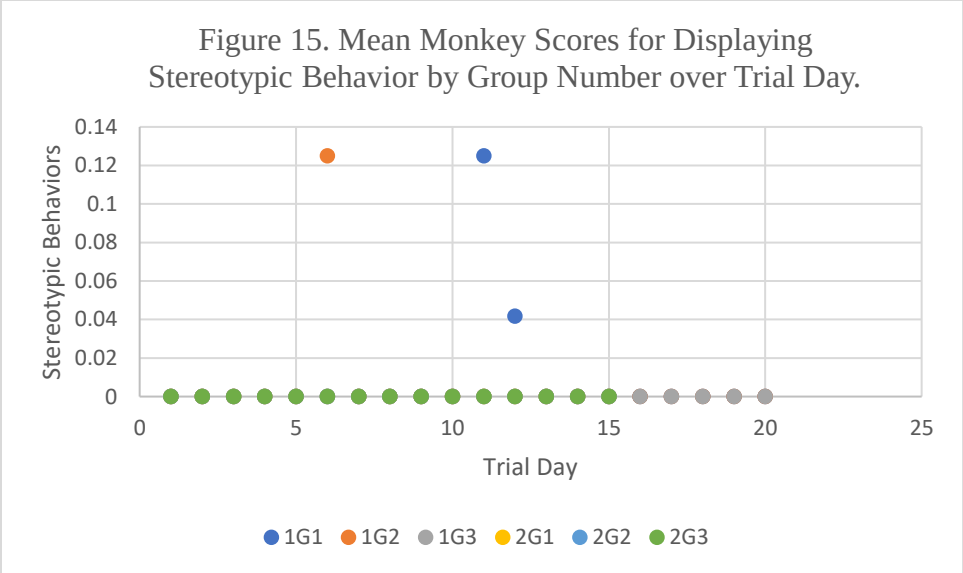

Figure 15. Mean Monkey Scores for Displaying Stereotypic Behavior by Group Number over Trial Day.

Display affiliative behavior Omnibus value was 0.0000.

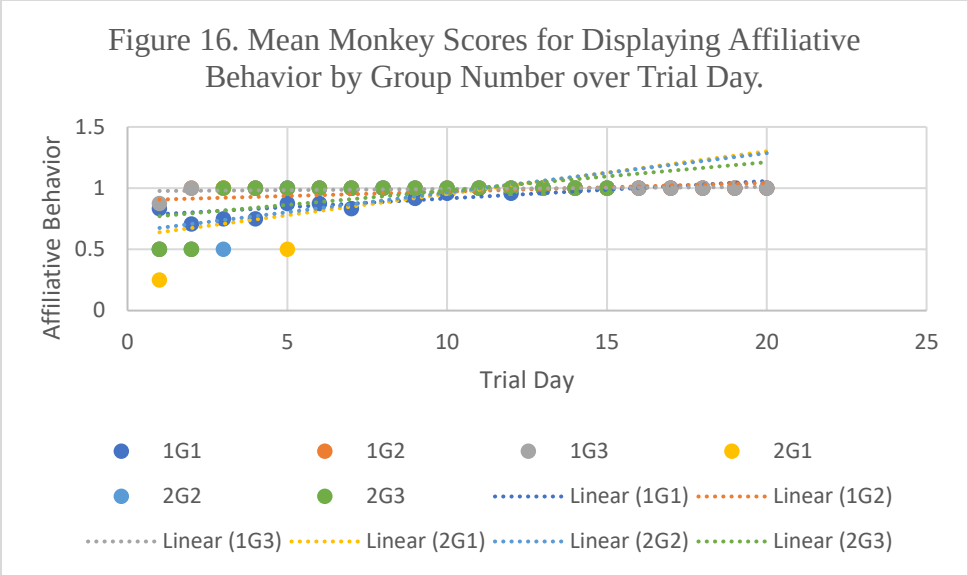

Figure 16. Mean Monkey Scores for Displaying Affiliative Behavior by Group Number over Trial Day.

Discussion:

The objection of this experiment was to see if there is an influence on the behaviors of animals during their quarantine and acclimation period after providing enrichment at different frequencies. To test this experiment, we have to provide enrichment to the animals. *The Guide for the Care and Use of Laboratory Animals*, often referred to as, *The Guide*, is an excellent resource for the industry standards to provide excellent care for animals in a laboratory setting. *The Guide* provides different types of enrichment that are appropriate for each species covered in it, and the enrichment provided is intended to promote species-specific behaviors (2010). Enrichment can increase desired behaviors such as playing, affiliation, foraging, exploration, and reduce undesired behaviors such as self-injury, over grooming, and stereotyped repetitive movements (Chamove, 1989). For developing an enrichment program for laboratory

animals, it has been noted to be familiar with species-typical behaviors, as well as age, activity patterns, and sex of the subjects to help decide which enrichment techniques are most appropriate for the animals (Bloomsmith et al., 1991). When it comes to providing enrichment to the animals, the costs of the enrichment, the time it takes to provide the enrichment, how the animals interact with the enrichment, and what the enrichment does for the animal are important to consider (Bloomsmith et al., 1991). For this experiment, we are investigating how offering various types of environmental and food enrichment at different frequencies can influence behavior of the animals. The amount of time it took to distribute enrichment for this study was not measured, but when it was not a considerable amount of time to provide animals with additional enrichment. One benefit that was observed when providing additional animals with enrichment was the reduction of food waste, as less produce was spoiled prior to use.

New Zealand White rabbits and cynomolgus macaques behave very differently from one another, but they can benefit from the same types of both environmental enrichment devices and food enrichment. Some examples of these enrichment devices that are safe for both species include, plastic dumbbell toys, toys that rattle and make noise, mirror toys, loose timothy hay, and a variety of fruits and vegetables. These enrichment devices are commonly used at IITRI for both the rabbits and cynomolgus macaques. There are some types of enrichment that are safe for one species, but not the other, for example, one of the rabbit toys, Nylabone keys are good for the rabbits, but not durable enough to give to the cynomolgus macaques unsupervised, as they easily destroy the toy, and it can be a hazard. The macaques benefit from having a movie playing in the

animal housing room, this provides them with something that stimulates them mentally as they can focus on the screen and also hear the movie, I have observed some individuals even use their body language and facial expressions towards the DVD player to interact with the movies, the noise from the movies may startle the rabbits and cause stress to them.

In this experiment, environmental enrichment and food enrichment were offered to see how the animals respond and the potential impacts on animal welfare. The rabbits and the monkeys in this behavioral experiment were observed during their quarantine and acclimation period prior to being animal models for toxicology studies. By offering enrichment at a higher frequency, this helps the animals associate humans with positive experiences. By associating humans with positive experiences, the animals might be easier to handle and work with for the research for which they are used. This helps both the researchers and the animal models, as both parties would be less stressed throughout the experiment.

New Zealand White Rabbits:

The rabbits used in this experiment were ordered for different toxicology studies, so the sex and the ages of the animals were specific to the research being done for those studies. The first room of rabbits contained twenty-seven females that were young juveniles. The second room contained twenty-two males and twenty-two females, and these rabbits were also young juveniles. The third room contained twenty-eight females which were time mated and were in various stages of gestation when they arrived.

After observing and collecting data from the baseline group (Group 0) of rabbits, the ethogram was modified to include several other behaviors, as the first ethogram did not capture different types of behaviors that represent positive welfare, such as sniffing and curiosity. The position of the rabbits before and after being approached was also collected to see if the animals' position would change when approached. Not only were more variables added to the ethogram, but three different treatment groups were also created for each experiment. Group 1 received one toy, that was changed with the rack rotation. Group 2 received the same toy as group one, as well as a cardboard tube, stuffed with hay. Group 3 received both environmental enrichment devices, as well as a daily food enrichment in the form of a vegetable. The hay for Groups 2 and 3 was replenished on the days that the cage liners were changed, Mondays, Wednesdays, and Fridays. The Group 0 rabbit data was used as a reference in Stata when comparing the other groups.

Fecal output was measured to ensure the animals were physiologically healthy. It is not uncommon for the animals to be stressed in the first couple of days after arriving to the facility. During the acclimation period, using the animal's fecal output is a good way to measure that the animal is eating and drinking normally. If the fecal output score is low, then that could indicate that there is something wrong with the animal or maybe something in the environment is stressing the animal out. For this variable, the desired score would be a 3, this indicates the animal had healthy stool. Some individuals did have lower scores, but the lowest score, 1, was only observed on the first day of data collection, which was the animal's second day at the facility (Figure 1.).

The results from the data analysis indicated that fecal output was statistically significant from the Omnibus value. Groups 2 and 3 were also statistically significant. Sex had no significant significance on this data set. The trial day was statistically significant. This is meaningful information as it confirms the rabbits were healthy when they arrived at the laboratory and also that the food enrichment had no negative effect on the rabbits' stool. The rabbits having healthy stool supports our hypothesis because the healthy stool is a positive indicator of the animal's physiological health (Table 1.).

The position of the rabbits in the cage when the observer is away from the cage was measured. This was measured to see if there would be a change in position when the rabbits are approached. Interestingly enough, the data confirms that there was statistical significance for the position of the rabbits when the observer is away from them. This difference can be because the rabbits that receive daily food enrichment (Group 3) spent more time towards the front of the cage, in anticipation of receiving the enrichment. The animal's position in the cage when the observer is away from the animal does not indicate positive or negative welfare, as the animal is living in that space, and the animals has the choice of deciding where to be in the cage. The rack style of caging used to house the rabbits had the water valve located in the back of the cage, and the feed hopper is located on the front of the cage. This cage design may be used to identify if an animal's position changed because they wanted to get to their food or water.

The data suggests that sex of the animals had a statistically significant influence on the position of the rabbits when the observer is away from the cage. The trial day also

had a statistically significant influence on the rabbits, as each day, the rabbits become more familiar with the observer (Table 1.).

Figure 2. Shows the relationship between each treatment group's average score for position away over time. The trend lines show that the groups did have some slight change in position over time, coming towards the front of the cage.

The position of the rabbits in the cage when the observer approaches the cage was measured. The data suggests that there is statistical significance between the three different groups. This statistical significance is meaningful as the variation suggests that the Group 3 rabbits were at the front of the cage when approached. This is meaningful as the rabbits were not retreating to the back of the cage, which supports our hypothesis that we would see an increase in positive behaviors and a decrease in negative behaviors. If a rabbit was retreating to the back of the cage when approached, that might indicate that the animal is in a state of distress.

The data suggests that the Group 3 rabbits, which received daily enrichment, displayed positive behaviors, by being in the front of the cage, and not hiding in the back of the cage when approached. When the cages were approached, some of the rabbits would react by coming forward or moving towards the back of the cage, some rabbits would not react by continuing with what they were doing. Some rabbits would be playing with a toy or relaxing in the back of the cage, prior to being approached and they would not react to being approached. This supports our hypothesis since the animals continued to engage in behaviors when approached. It is an indicator that the animals were in a positive welfare state, as they did not experience fear or distress when approached. The

rabbits that came forward when approached were indicators of positive welfare because the rabbits were displaying curious behaviors, which is desired. Sex was not a statistically significant fixed effect for this data set. The trail day was statistically significant (Table 1.).

Figure 3. illustrates the relationship between the average group position when approached by the observer over the trial days. The negative slopes indicate that the rabbits come closer to the front of the cage as time goes on. This supports our hypothesis as the average group scores would move towards the front of the cage, representing that the rabbits were not fearful of the observer, and possibly suggests the rabbit was interacting with the observer when they moved closer to the front of the cage.

Thumping was one of the negative behaviors that was on the ethogram. We did not want to see any thumping behavior in the rabbits, and if we did, we hoped to see a decrease in this behavior over time. The Omnibus value suggests that thumping was statistically significant. We observed low frequencies of this behavior, which supports our hypothesis. Thumping behavior that was observed happened at very low frequencies, and there were several external factors that could have had an influence on that behavior in the select few rabbits. Building maintenance and construction was occurring during the time of this study, and the noise could have been the cause of the thumping behavior. This may explain why the P value for thumping was unable to be calculated from this data set. During the observations, thumping was observed the most in the baseline group of rabbits, which received just one enrichment device with weekly food enrichment, interestingly enough, the other individual rabbit that was observed thumping was in

Group 1 of experiment 2. The other groups of rabbits did not display thumping behavior in the presence of the observer (Table 1.).

Figure 4. illustrates the average thumping score for each group over time. Thumping was a behavior that did not happen frequently, but the absence of thumping is a sign of positive welfare. This supports our hypothesis, as the frequency of thumping did not increase, representing the absence of negative behaviors.

Freezing was another negative behavior that was on the ethogram. Freezing is a natural response in rabbits, as they are a prey animals. The Omnibus value suggests that freezing was statistically significant. As the frequency of freezing decreases, it is an indicator of positive welfare (Verga et al., 2007). Throughout the experiment, the amount of times freezing was observed decreased. Freezing may have also been influenced by external factors such as noise from construction. Over time, the frequency of freezing decreased, which supports our hypothesis that we would see a decrease of negative behaviors. The data suggests that sex was not likely to influence freezing behavior, as the P value was not statistically significant. The trial day was significant on the influence of this data set (Table 1.).

Figure 5. Supports our hypothesis because the negative trendlines illustrate how freezing behavior happened less frequently over time. Group 3 trendlines show animals with more enrichment opportunities displayed fewer freezing behaviors.

Movement of the rabbits in their cage was observed. In this experiment, movement represents the activity level of the rabbits. Some of the rabbits were relaxed

and laying down, these rabbits were given a 1, rabbits that were moving around were given a 2, and rabbits that were very active in their cage and interacting with their environment were given a 3. The Omnibus value suggests that movement was statistically significant. The Groups 2 and 3 rabbits had the highest movement levels, which we predicted as they had more enrichment devices, so they were seen playing and moving more often than the rabbits with only one enrichment device. Group 3 had the most statistical significance. A higher activity level is an indicator of positive welfare, as it represents that the animal is not freezing, and that the animal is engaging in exploratory behavior within their cage. During these observations, there were no stereotypic repetitive movements, such as constantly digging in the corner of the cage or moving repeatedly in a circle over and over again. Sex was not statistically significant on the impact of movement for this data. The trial day was statistically significant (Table 1.).

Figure 6. visually represents the average group score for the rabbits' movement by trial day. This supports our hypothesis as movement increased overtime, and that the animals that received more enrichment had higher scores for this variable.

Another positive behavior that we observed in the rabbits was interacting with enrichment. We wanted to make sure that the rabbits used the enrichment provided to them. It is important to offer enrichment to the animals' cages, so the animals do not get bored and engage in stereotypical behaviors. The cardboard tubes stuffed with hay were a favorite device among the rabbits, Groups 2 and 3 for each experiment had more instances of interacting with enrichment. This could be because rabbits can interact with that device in so many ways. The rabbits enjoyed getting the hay out of the tubes as well

as destroying the cardboard tubes. The Omnibus value suggests that interacting with enrichment was statistically significant. A lot of the rabbits interacted with enrichment right away, and many of them still interacted with enrichment when being observed. The rabbits that interact with enrichment when being observed indicates that the animals were not afraid of humans enough to stop playing with the enrichment while being observed. Interacting with enrichment is a positive behavior, and by providing more opportunities for enrichment, then the rabbits have more chances to interact with enrichment. Offering enrichment at a higher frequency encourages the rabbits to display more play and exploratory behaviors. The rabbits interacting with their enrichment is a positive behavior that we expected to see when offering enrichment at higher frequencies. It is interesting to see that the time mated rabbits did not interact with enrichment as much as the younger rabbits did, this could be due to the cage size relative to the pregnant rabbit's size. The pregnant rabbits did however interact with enrichment more and more each day. The trial day was significant for this data set (Table 1.).

Figure 7. Represents the mean for the rabbits interacting with enrichment scores. Groups 2 and 3 from experiment 1 interacted with enrichment each day, this supports our hypothesis because offering enrichment during quarantine period can benefit the welfare of the animals by having positive impacts on their behavior.

Sniffing is a natural, positive behavior for rabbits. The data suggests that sniffing was statistically significant. This represents that the rabbits tended interact with their environment. Rabbits that were relaxed and laying down could still engage in sniffing while not needing to move around, some were observed sniffing their environmental

enrichment and food enrichment. We wanted to see the rabbits sniffing, as sniffing is a positive behavior, and we did see an increase in sniffing when food enrichment was provided. The rabbits that received daily food enrichment had the highest scores for sniffing, as they would investigate their food enrichment. Offering food enrichment at higher frequencies is an excellent way to encourage sniffing behavior in rabbits. This supports our hypothesis as sniffing is an exploratory behavior which has a positive influence on the rabbit's welfare. Groups 2 and 3 were statistically significant. The trail day was significant for this data set (Table 1.).

Figure 8. Illustrates the relationship between the groups average sniffing score over time. This supports our hypothesis as it shows a positive trend over time, and the animals with more enrichment had higher scores of the positive sniffing behavior.

Curiosity is another natural behavior for rabbits. The data suggests that curiosity was statistically significant. Group 3 had a higher level of statistical significance than Group 2. This suggests that rabbits that got enrichment at a higher frequency tended to be more curious than rabbits that did not. Rabbits display curiosity as an exploratory behavior, and by actively exploring their environment, it is a desired behavior. Curiosity is a positive indicator for welfare because active exploring is associated with greater activity, which decreases the risks of osteoporosis and gastrointestinal stasis (Coda et al.,2020). This supports our hypothesis because interacting with enrichment is displaying positive behaviors (Table 1.).

Figure 9. Illustrates the average group curiosity scores over time. This supports our hypothesis because the positive trendlines show that curiosity increased overtime, but the animals with the most enrichment in Group 3 had the highest level of curiosity.

Cynomolgus Macaques:

The purpose of using cynomolgus macaques in this research was to determine how enrichment helps the animals' welfare while they are acclimating to their new surroundings. By offering various types of enrichment at various frequencies, we can identify if enrichment influences the behavior of the animals and promotes positive welfare. During this experiment, environmental enrichment devices within the cage were changed weekly, and for Group 3, food enrichment was given daily. For each of these experiments, the animals were also given a DVD to watch during the day after the cages were cleaned.

The cynomolgus macaques used in experiment 1 included forty monkeys, twenty males and twenty females that were between two to three years old and were not previously used for biomedical research. These younger animals appeared to be docile and were displaying more signs of affiliative behavior to one another through their dividers. These animals were pair housed on trial day 1, but after they were scored. Every individual responded well to being housed in pairs. The animals have their identification numbers tattooed on the front of their bodies, so the individuals can be easily identified for the remainder of the observations. The benefit to having the animals pair housed is that they get the additional enrichment from being with a conspecific. Another benefit to

being pair housed is the animals get an additional environmental enrichment device, as they get to play with each other's toy.

The eight males in experiment 2 were much older adults that have been used previously at other laboratory animal facilities prior to arriving at IITRI. These animals were not displaying affiliative behaviors with one another through the dividers, instead they were displaying aggressive and dominant behaviors in attempt to establish a hierarchy amongst themselves. In wild cynomolgus macaques, the males have a higher social rank than the females, and the highest-ranking males get priority of breeding opportunities and resources (Bayne, 2005). Even though these animals were single housed, they were still able to interact with conspecifics within the same animal housing room, which is why they have to establish a hierarchy. It was clear that some of the individual monkeys were previously in stressful situations, as seen by signs of overgrooming, scar tissue from previous altercations or procedures. The social hierarchy of individual monkeys can influence physiological health (Sapolsky, 2005). To avoid any fights, these individuals were to remain single housed, as they would be single housed when their study began.

The monkeys were housed in stainless steel racks, that could accommodate four single housed monkeys. Two monkeys would be on the top and two monkeys would be on the bottom, and if allowed, then they could interact with one another, and the divider can be opened so they can use both cages on top or the bottom of the rack, these racks do not allow for the animals to go to the cage above or below them. In my experience, it is not uncommon for the monkeys to unlock their squeeze bar and get themselves trapped in

a tight space at the front of their cage. This usually happens if the animals are bored and trying to entertain themselves. They find the locks for the squeeze bar and unlock them and then accidently get themselves locked in a smaller portion of the cage. When they do this, they put themselves in a position where they have less space and may feel more vulnerable. Macaques also display a variety of stereotypic behaviors, such as bar chewing, cage shaking, overgrooming, self-injury, and constantly back flipping in circles, that are undesired in a laboratory setting, but the animals display the behavior in an attempt to cope with their environment. Different types of environmental enrichment and food enrichment help prevent these stereotypic behaviors.

One external factor that could have had an influence on the animals during their quarantine period would be the days that they were tested for tuberculosis (TB test). The tuberculin indicator is injected intradermally in the eyelid to see if the animals have tuberculosis. For this to occur, the animals are anesthetized, and the procedure was completed. On these days, the data was collected later in the day, so that the animals had time to wake up from the anesthesia, which was ketamine. As the primary care provider for these animals, I was the individual who was anesthetizing them, and the animals may have associated me with a negative experience.

The fecal output of the monkeys was observed to make sure the animals were physiologically healthy. The desired score for this variable was a three. The data, Omnibus and P values, suggests that the fecal output was not significant between the different treatment groups (Table 2.). Figure 10. Illustrates how after trial day 1, the monkeys had the best score for fecal output. This is useful to know because it represents

that the animals were healthy when they arrived, and that food enrichment did not cause any loose stool or negative effect on the fecal output. In another study, the behavioral and physiological responses to environmental disturbances, live and recorded explosions (to mimic construction noise), in cynomolgus macaques found that there was an increase in fecal cortisol and immunoreactive cortisol metabolites, and the animals reacted with vertical flight and vocalizations (Westlund et al., 2012).

The position of the cynomolgus macaques in their cages was observed to see how the animals would react and where they would go in their cage when being observed. Three was the back of the cage, two was the middle of the cage, and one was the front of the cage. For the monkeys, it seemed that the animals' position in cage had a positive impact on their welfare. This can be seen by the group averages over time trending towards the front of the cage (Figure 11.). The data suggests that there was statistical significance on the position of the animals (Table 2.). One thing that was interesting to see was that the monkeys would use the vertical space and position themselves towards the top of the cage, especially the animals that were housed in the bottom cages of the racks. It is also important to note that the racks have the feed ports on the front of the rack, and the monkey's produce and biscuits were served through those ports. Depending on the environmental enrichment device, the monkeys would find ways to push them through the feed port, if they could fit. This added an additional layer of fun to the monkeys, as it was a mental exercise that includes trying to get the device out of their cage. The water valves were located on the back sides of the cages. The monkeys had the best access of viewing their DVD when closer to the front of the cage.

Facial expressions were scored from 1 to 3. The desired score, 1, represents affiliative facial expressions, such as lip-smacking behaviors, these interactive behaviors are positive. 2 represents facial expressions that are associated with shy and withdrawn behaviors. The undesired score, 3, represents aggressive facial expressions and warning behaviors, such as a barred tooth open mouth display. The data suggests that there is statistical significance between the facial expressions of the monkeys. The data suggests that Group 3 had the most statistical significance from Group 1. Sex and trial day were statistically significant (Table 2.). This could be because the animals get familiar with the observer, especially because the observer was the source of the enrichment. This supports our hypothesis because affiliative facial expressions are a positive behavior. Figure 12. Represents the group averages for facial expression scores of the cynomolgus macaques over time. It shows how on average Group 3 had the most affiliative facial expressions. It is interesting to see by day 13, every group had scored a 1 for affiliative facial expressions.

Body posture was measured to be either alert or relaxed. Alert body posture represents the animal is ready at all times, vigilant, and constantly aware of its surroundings. Relaxed body posture represents that the animal is comfortable but not necessarily concerned with being observed. The Omnibus value suggests the body posture for Group 3 was statistically significant (Table 2.). Figure 13. Represents the mean body posture for each group of monkeys over time. The trendlines show most of the groups get more relaxed over time, except for Group 1, from Experiment 1. This is also seen by the P value for the trial day was not statistically significant (Table 2.).

In a study that looked at the social status of monkeys, it was found that dominant and subordinate monkeys displayed different patterns of brain glucose metabolism in their home cage, including areas associated with vigilance, stress, and anxiety (Gould et al., 2017). This can help to explain why the Group 1 monkeys displayed more alert behavior over time, and it can be because those individuals may be more subordinate than the other monkeys in Groups 2 and 3. This could be because they do not have the additional enrichment devices that Groups 2 and 3 had. Perhaps, Group 3 animals felt the most dominate due to receiving the daily food enrichment. This supports our hypothesis that the animal's with the daily enrichment will show more positive behaviors and less negative behaviors than the animals that receive enrichment less frequently.

Interacting with enrichment is a positive behavior we want to see the animals performing. Food enrichment can be combined with environmental enrichment. Coating tubes and toys in yogurt or apple sauce and sprinkling dry treats onto that and freezing it is one way to incorporate environmental and food enrichment into one device. In another experiment, sprinkling food on fleece provided an excellent foraging opportunity for the cynomolgus macaques (Lam et al., 1991). This experiment found that the monkeys spent up to twenty-seven minutes per hour foraging in the fleece, foraging is one activity that wild macaques do to spend their time (Lam et al., 1991). The data from our experiment suggests that interacting with enrichment is statistically significant, as the animals with more enrichment devices have more opportunities to interact with them. The Omnibus value is statistically significant. The data suggests that sex and trial day are also statistically significant (Table 2.). This supports our hypothesis as interacting with

enrichment is a way for animals to express normal behaviors, which is a positive sign for animal welfare.

Figure 14. Illustrates the mean group scores for interacting with enrichment, and it is interesting to see that the trendlines were positive, which indicates that the animals did not get bored with the enrichment. The environmental enrichment was rotated out weekly, and the animals were pair housed with a conspecific, and had the daily DVD, so there were a lot of opportunities for the animals to engage in positive behaviors and interacting with enrichment.

A negative behavior that was observed was if the animals displayed stereotypic behaviors. Stereotypic behaviors for macaques can be displayed in a few ways, a repetitive motor behavior that occurs at least three times in quick succession, self-inflicted hair plucking, repetitive perch patting, pacing or stereotypic locomotion (Shively et al. 2005). We did not see many instances of stereotypic behavior. Figure 15. Illustrates how stereotypic behavior was only observed on a few occasions, with animals from Experiment 1, Groups 1 and 2. The stereotypic behaviors observed during this experiment were repetitive behaviors which included bar shaking, bar biting, and cage shaking. On these days, it was noted that there was facility maintenance with loud noise, this might be the reason why the animals were displaying stereotypic behaviors. This type of stress is acute stress, as it did not go on for extended periods of time. The data suggests that displaying stereotypic behavior is not statistically significant (Table 2.). This could be because we did not observe enough stereotypic behaviors, but in our hypothesis, we established we wanted to see a decrease in negative behaviors.

Display affiliative behaviors. Shively et al., found that subordinated females hypersecreted cortisol, were insensitive to negative feedback, had suppressed reproductive function, received more aggression, engaged in less affiliation, and spent more time alone than dominants (1995). In this study, there was one female in the first monkey room that took quite a few days longer than the rest of the animals to show affiliative behaviors. This was interesting as the females are typically smaller, and this individual would grab her food right away, I thought of her as a troublemaker and a female that was trying to establish a hierarchy with me. Figure 16. Illustrates the relationship between the mean group scores for displaying affiliative behavior over time. It is interesting to see how Group 3 animals displayed affiliative behaviors earlier than Groups 1 and 2.

In another study, researchers were trying to see if sex and age of cynomolgus macaques increases the animal's affinity for animal care personnel, they found that the effects vary by sex but not age (Nishimoto et al., 2015). This is interesting as the second group of monkeys was only males, but the males were ordered based on their study protocol, not for behavioral traits. The data from this study suggests that sex is not statistically significant on the effect of displaying affiliative behaviors as the P value was 0.055 (Table 2.). This P value is almost statistically significant. The significance of Group 3 was higher than that of Group 2, supporting our hypothesis that animals receiving enrichment more frequently will display more positive behaviors. This can be because the daily food enrichment is a reward for the monkeys.

Conclusion:

The goal of this research was to see the influence of enrichment has on animal welfare and behavior for laboratory animals during their quarantine and acclimation period. The animal models used for this experiment were New Zealand White rabbits and cynomolgus macaques, as both are common research models for toxicology research. The use of animals in biomedical research is only possible with consent from the general public, even though it is scientifically justified, there must be an ethical argument that justifies the use of animals in biomedical research (Ringach, 2011). The Three R's of Research include, reduction, replacement, and refinement (Russell and Burch, 1959). The definition of refinement has changed over the years, but it now includes the promotion of wellbeing to minimize distress (Tannenbaum and Bennet, 2015). Enrichment is a type of refinement as it gives the animals the opportunity to enjoy the freedom to express normal behaviors. By offering enrichment that is safe for the animals, The Five Freedoms of Animal Welfare are respected, as enrichment helps the animals to also maintain their freedom from discomfort, freedom from fear and distress, freedom from hunger, thirst, and malnutrition, and freedom from pain, injury, and disease.

The animals were placed into different treatment groups and were observed for several days until they were randomized for their toxicology studies. The Group 3 animals were the animals that received the highest frequency of enrichment. These animals were also the animals that displayed the most amount positive behaviors and displayed the least number of negative behaviors. Offering enrichment at a higher frequency increases the amount of positive human interaction and strengthens the human-animal bond. The enrichment is positive for the animals as they get to engage in species-

specific behaviors and behave in a natural way. This improves animal welfare as the animals are not engaging in stereotypical behaviors which can be indicators of poor welfare. We can conclude that both environmental and food enrichment have had a positive influence on the welfare of the animals during their quarantine period, and by offering enrichment at higher frequencies, we can refine husbandry practices and improve the welfare of the animals in our care.

As this was a pilot study, there are several directions for which this research can develop. Future works for this type of behavioral research include modifying the ethogram and applying to other animal models in research to see how other animals are influenced by enrichment. In a future experiment, changing the types of enrichment devices offered could lead to measuring which enrichment items are preferred by the animals. Another way to expand this research further would be to develop a scoresheet for the animals to see how the animals respond to handling during different procedures that might be necessary for the experiments that they participate in. Another way to use this research would be to incorporate the ethograms into standard operating procedures for training, as this can give new staff a unique way to learn more about the animals they are caring for and to help identify if there have been any changes in the animal's behavior.

Appendices:

Appendix A. Rabbit Ethogram

Rabbit Scoresheet – Well-being Room Number _____ Date _____

Rabbit number	Fecal output – 1-3 (1 low 3 high)	Position in cage when person is away from the cage (door, back, middle)	Position in cage when person approaches the cage (door, back, middle)	Thumping (+ or -)	Freezes, ears erect (+ or -)	Movement – 1 = none 2= some 3 = relaxed	Interact w/ enrichment (+ or -)	Sniffing / Nose twitching (+ or -)	Curiosity / exploratory behavior – 1 = none 2 = some 3 = displays behavior

Appendix B. Rabbit Legend

Legend 1. Rabbit Ethogram

Fecal Output; 1 = none, 2 = low, 3 = good

Position; 1 = front of cage, 2 = middle of cage, 3 = back of cage

Thumping; + = thumps while being monitored, - = does not thump while being monitored

Freezing; + = freezes while being monitored, - = freeze while being monitored

Activity; 1 = no movement, 2 = some movement, 3 = relaxed / laying down

Interact with enrichment; + = interacts with enrichment, - = does not interact with enrichment

Sniffing / Nose twitching; + = nose twitching when looking, - = nose not twitching

Curious behavior; 1 = none, 2 = some, 3 = displays behavior

Rabbit Figure Legend

0G1 = Experiment 0 Group 1 (Baseline)

1G1 = Experiment 1 Group 1

2G1 = Experiment 2 Group 1

Appendix C. Cynomolgus Macaque Ethogram

Cynomolgus Macaque Scoresheet Date______ Room Number______ Notes:

Animal ID	Fecal Output	Position in Cage	Facial Expression	Body Posture	Interact with enrichment	Display stereotypic behavior	Display affiliative behavior

Appendix D. Cynomolgus Macaque Legend

Legend 2. Cynomolgus Macaque Ethogram

Observation, potential scores that are recorded and what the scores represent.

Fecal Output; 1 = none, 2 = low, 3 = good

Position; 1 = front of cage, 2 = middle of cage, 3 = back of cage

Facial expression; 1 = affiliative/ interactive, 2 = shy / withdrawn, 3 = aggressive / warning

Body posture; A = 1 = alert while being monitored, R = 0 =Relaxed while being monitored

Interact with enrichment; + = 1 = interacts with enrichment, - = 0 = does not interact with enrichment

Display stereotypic behavior; + = 1 = does display behavior, - = 0 = does not display behavior

Display affiliative behavior; + = 1 = does display behavior, - = 0 = does not display behavior

Macaque Figure Legend

1G1 = Experiment 1 Group 1

2G1 = Experiment 2 Group 1

Appendix E. Stata Data

Stats_4_28_2022.pdf

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