Light or Dark Preference of *Peromyscus leucopus* in a Y-maze Choice Experiment

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Abstract: *Peromyscus leucopus*, or white-footed mouse, is a small mammal that has a large range in North America. This mouse is also usually the most common mammal in any given area. *P. leucopus* are nocturnal mammals. They have many predators, and we looked at certain aspects of how *P. leucopus* try to escape these predators. We looked at whether *P. leucopus* would rather escape into a tunnel that is lighted or a dark tunnel. We tested this by capturing wild *P. leucopus*, running them through a Y-maze, and recording whether they went down the dark or light tunnel of the maze. We hypothesized that mice will go down the dark tunnel significantly more often than the light tunnel. Our results suggest that a Y-maze can be used for choice experiments concerning small mammals.

Keywords: *Peromyscus leucopus*, Y-maze, Visual Discrimination

Introduction

The system we used for the purposes of this study are wild members of the genus *Peromyscus.* Members of this genus are mice that are generally nocturnal, widely distributed across North America, and are often the most common animal in the area (Baker, 1968). We selected *Peromyscus* in part due to this wide range and abundance that mean these mice should not be as challenging to capture when compared to other, less common mammals. These mice are also abundant enough to obtain adequate samples for statistical analyses without impacting wild populations (Kirkland and Layne, 1989). Another reason *Peromyscus* was chosen as the system is because the mice of this genus average below 225 mm in total length (Baker, 1968), making them relatively small and thus more easily handled than larger mammals (Kirkland and Layne, 1989). *Peromyscus* are also easily trapped in live traps and several species are able to adapt to life in a laboratory (Blair, 1968). There are many species in the genus *Peromyscus* (Baker, 1968), but for the purposes of this research, we focused on *P. leucopus.* These species in particular is found in a large number of different biomes, which is why they are the primary focus of this study (Blair, 1968). Knowing how abundant this species is, we reasoned that it should not be difficult to find this species within the area we conducted our research in, Lake Carl Blackwell, Stillwater, OK.

This study made use of a maze shaped like a Y, also known as a Y-maze. The maze used in this study was made of PVC pipe and had the additions of LED lights in either arm of the Y-maze, switches to turn these lights on and off, a dimmer to control the intensity of the lights, and a camera set up at the fork of the tunnels (Figure 1). The camera was linked wirelessly to a smart phone so that we could record which tunnel of the maze the mouse went down. The maze has an infrared light at the fork of the tunnels, and the camera is

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able to pick up on this light to allow us to see everything. Infrared light is used because it does not interfere with the visual discrimination of the mice but still allows us to see what is going on so that we may record data.

The primary question that we addressed is how useful is a Y-maze in ascertaining the ability of *P. leucopus* to distinguish between a light and a dark tunnel? In other words, how useful is a Y-maze in finding out how *Peromyscus* uses visual discrimination to choose between a light or dark tunnel? This question in turn leads to our second question: what is the preference of mice when faced with a light tunnel and a dark tunnel? Other studies have already made use of Y-mazes, wild-caught animals, and lights. Marcos Gorresen et al. (2015) used a Y-maze to test whether wild-caught bats were able to perceive reflected UV light and if the bats were able to perceive UV light under dim lighting conditions. Another study used a Y-maze to test the premise that infra-red light didn't affect behavioral response in Mexican free-tailed bats when compared with visible lights such as white, red, and far-red lights, (Mistry and McCracken 1990). Other studies even made use of the Y-maze in ways that went beyond testing only visual perception. A study by Chase (1983) tested if it was visual or acoustic cues that guided bats' escape from a Y-maze. Most of these studies incorporated a question regarding the ability of bats to orient towards the light, similar to our own question of the ability of *Peromyscus* to orient towards light. Although these studies had bats as their system, not mice, there are also studies done on *Peromyscus* and light perception. Two studies by Serge Daan and Colin Pittendrigh, for example, focused on the circadian cycles of nocturnal rodents, including *Peromyscus*, when kept in constant darkness (Daan and Pittendrigh, 1975A) and when they were kept in continuous light under varying intensities, also adding in the variable of giving the rodents either heavy water or tap water (Daan and Pittendrigh, 1975B). Barry and Francq (1982) studied which intensity of light was preferred by *P. leucopus*; however, this study did not utilize a Y-maze. There are studies using light and a Y-maze, and there are studies using light and *Peromyscus*, but there are not many studies that utilize both *Peromyscus* and a Y-maze and the studies that do so may not be testing visual discrimination. For instance, one study used both a Y-maze and *P. leucopus;* however, they were placing ticks in the maze and seeing which mouse they would go to and what that had to do with the mouse's sex and body mass (Dallas and Fore, 2013). This study did not address response to light.

We set out to investigate the usefulness of a Y-maze in testing the visual discrimination of *Peromyscus* when given a choice between a light tunnel and a dark tunnel. In addition, we also originally set out to find at what level of light intensity do mice stop orienting towards the light, and what is the consistency of the response to the light and the dark tunnels? We also originally wanted to find out at what intensity of light the mouse started making random choices, which would indicate that the mice did not perceive the light well enough anymore for the light to impact their choice. However, this question had to be dropped due to the mice not wanting to run enough trials to gather a full set of data at all the different intensities of light.

Methods

We collected *P. leucopus* at an area assigned to the Integrative Biology Department at Lake Carl Blackwell, Stillwater, OK. We used Sherman® Live traps baited with rolled oats and peanut butter. The traps are mechanical, measure 3 inches by 3 inches by 9 inches, and are made of aluminum, but insulated cotton nesting material was also added to each trap. On a trapping session, a maximum of 150 traps were set around wood piles. The traps were set at around 3 P.M., then left out overnight. The traps were all picked up the next morning while it was still dark out. All traps were brought back to the field building. Traps were also soaked in a 5% bleach solution after each use to disinfect them.

We did capture members of species that were not the target of this study. These species include *Neotoma floridana, Sigmodon hispidus,* and *Reithrodontomys fulvescens.* If trapped animals were identified as members of these species, they were taken back to the field house, weighted and their sex determined, and then released without running the maze.

The same evening the animals are trapped, the species, sex, and mass of mice was recorded. An individual was placed in a bag and, using the bag plus animal method, a Pesola® scale was used to determine and record the weight of an individual in grams. To answer our questions, we ran the mice through a Y-maze with one arm with the light off and one arm with the light on. The arm of the maze that was lighted was chosen randomly with a dice. A mouse was considered to have chosen a particular arm of the maze when most of its body had entered a certain tunnel. Each time a mouse ran through the maze, we recorded what tunnel of the Y-maze the mouse went down and whether or not that tunnel was the lighted tunnel or the dark tunnel. For the trials that we started off doing with the different light intensities, the tunnel with the light on was chosen at random by rolling a dice and the different numbers were assigned to each of the 5 possible light intensities. The number 6 on a dice was not attributed to a light intensity.

Figure 1 - Y-maze with camera and LED lights. Picture taken by Jimmy Lovett.

The Y-maze was made of a 26.5 cm entry tunnel that separates into three tunnels (Figure 1). The outer two tunnels are each equipped with an LED light at the top of the tunnel, which was powered by a 9-volt battery. Each LED's light intensity was adjusted using the potentiometer attached to

each light. The ends of these two tunnels are capped so that animals cannot escape the Y-maze. The middle tunnel is comparatively short and has a Geekpro camera mounted onto the tunnel within a hollow Styrofoam ball. All tunnels are made up of PVC that is 2 inches in diameter.

Results

Originally, each mouse was to be individually run through the Y-maze 5 times, with 5 minute breaks in between each trial. However, it was discovered that mice will start to exhibit habituation and will not run the full set of trials. Our methods were then changed so that each mouse was only run once through the maze with the light being set at 40 lux.

For the twenty mice that we ran through the maze only once, we used the open-source software package "R" to analyze our data with binomial statistical tests. On average, around 80% of mice picked the dark tunnel over the light tunnel (Figure 2). Our data resulted in the p value 0.01182, showing us that a statistically significant number of mice chose the dark tunnel. A test was also run to make sure that the mice running the maze were not making their choice based on following the scent of the mouse that had ran before it. There was not a statistical significant relationship indicating that mice were following the scent of the mouse that ran the maze just prior to them.

Discussion

We had to change methods due to animals not behaving how we wanted them to. However, knowing that wild caught *P. leucopus* will not run five nearly consecutive trials is still valuable information. We can

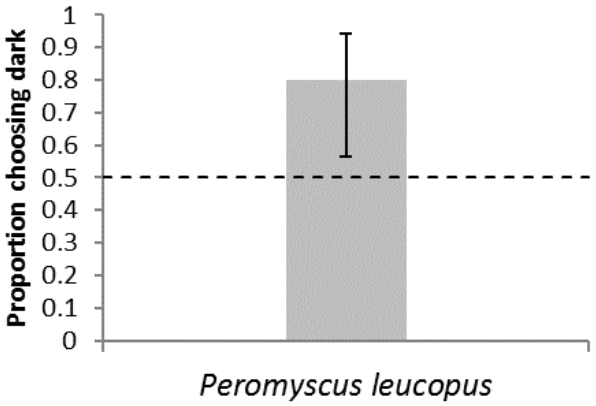

Figure 2 - Proportion of mice choosing dark arm of Y-maze with 95% confidence intervals.

use that knowledge and apply it to the study following this one that will take place in Tar Creek, OK. We now know that we have to run mice through just once, but we can change the light intensities so that after five mice have run through the maze at five different light intensities on multiple mice.

Our data supports the hypothesis that wild-caught *P. leucopus* tend to choose the dark tunnel of a maze rather than the light tunnel of a maze. Knowing this, other studies can build off of our study and find out why members of this genus do not orient towards light. This study may contribute both to studies trying to figure out what actions *Peromyscus* may take when escaping predation in the wild, and other studies concerning the escape response of rodents and other mammals. Our findings also show that a Y-maze is a good apparatus to be used in choice experiments concerning small mammals. Additionally, other studies may learn from our study by looking at how our mice did not want to complete five trials. This will be helpful to other studies when they are creating their methods.

This study is foundational for a study to be conducted by graduate student Jimmy

Lovett. In Lovett's study, the same Y-maze used in this study will be used with the same target species, *P. leucopus*. Some of these mice will be from a superfund site, and some will be from a reference site. Much like with this study, Lovett's study will be used to test the response of these mice when placed in front of a dark and a light tunnel. The light will also be of varying intensities in different trials with different mice. The mice will be run through once, but the intensity of light will be different for different mice. These data will result in finding at what point of light intensity to mice not perceive the light well enough for the light to impact their choice of either a light or dark maze. This will help to show to what extent contaminates from the superfund site have affected response to light in mice.

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