The Effects of Environmental Enrichment on Cognitive Functions in Fancy Mice, *Mus musculus*

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Category: Animal Systems (AS)

Division II

Abstract

Cognitive function is the ability to process thought. Cognition primarily refers to memory, the ability to learn new information, speech, and reading comprehension. Previous research has shown that mice varying in ages were able to improve their cognition because of the environmental enrichment placed in their cages. In this experiment the ages of the mice were unknown but it was inferred that the ages ranged from 1 to 2 years old based on their appearance and vigor. Eight mice were separated into two groups: female and male. The mice were again divided; two females and two males were housed in an enriched environment which exposed them to various stimuli in their environments while the other two females and males were not exposed to enriched conditions and instead placed in a standard laboratory environment. For eight weeks, the mice were assessed in two hand-built mazes for 16 trials, 8 tests per maze. After the eighth week, the results showed that there was improvement in neither the enriched females nor the enriched males. Though the hypothesis was proven wrong the results were compromised due to all the health issues and deaths that occurred in the female group (both the enriched and controlled).

Introduction

Mice are the most commonly used animal in a laboratory setting. In many research facilities mice and other rodents do not have the opportunity to engage in enrichment activities. Because mice play an essential role in biomedical research related to the human system, understanding the similarities between humans and mice is pertinent. Since the genetic makeup between a human and mouse brain is 90 percent identical (Serendip 1), testing the intelligence in assessment trials can help to see if environmental enrichment will increase the intelligence of

mice. Since the foundation of the idea that environmental enrichment may create improvement in the cognition of an animal, researchers since then have always wanted to prove or disprove this theory through conducting their own experiment. This ideology has a great impact on humans because if it is clear that environmental enrichment has an effect on animals this theory could possibly be proven with humans. Understanding how enrichment affects the intelligence of mice may help contribute to comprehending the possible effects of enrichment on humans.

Literature Review

Previous Research

For decades research has been trying to conclude that environmental enrichment has a direct impact on a rodent's intelligence and their ability to learn tasks. The level of intelligence is not only ascertained from a mouse's genetics but also the quality of their living conditions. Donald Hebb was a psychologist; he sought to understand how the function of neurons influenced psychological processes such as learning. He was the first to develop the concept that an enriched environment has an effect on the brain. "Hebb first described how increasing the complexity of a laboratory setting improved its subsequent behavior in learning tasks" (Benefiel, Dong, & Greenough, 2005, p. 95). In Hebb's early work, he brought laboratory rats to his home where they were treated as companion animals, the rats were frequently out of their cage and had free run of his home. At maturity these home-raised rats scored better than laboratory-reared rats on the Hebb-Williams maze (Hebb & Williams, 1946). Hebb concluded that "the richer experience of the pet group during development made them better able to profit by new experiences at maturity" (Hebb, 1949) which he explains is the one of the characteristics of human intelligence. Hebb's students repeated the same research in a laboratory setting and they

came to a similar conclusion, "that a more stimulating rearing environment enhanced performance on complex learning tasks" (Benefiel et al., 2005, p. 95). It was also found that mice being raised in enriched conditions have increase functioning within four general areas (a) neurochemical, often in terms of alterations in neurotransmitters, (b) physiological, generally related to improved adaptive functioning, (c) neuroanatomical, including changes in the mass of the brain and the neural connections within it, and (d) behavioral systems (Curtis & Nelson, 2003).

Senescence is the condition or process of deterioration with age. This condition is found in humans and other mammals and is commonly accompanied by weakening in the functions of learning and memory (Frick et al., 2003). The decline in brain functioning may result from depreciation of brain regions such as the hippocampus and neocortex. Although there have been numerous drug treatments developed, there have also been a handful of studies that suggest that simple adding enrichment into the animal's environment could improve the cognition and relieve aged-related memory dysfunction(Frick et al., 2003). "More recent work in adult rodents indicates that enrichment initiated at nearly any point in the lifespan can improve spatial and nonspatial memory" (Frick et al., 2003, p. 187). Spatial memory is part of memory responsible for recording information about one's environment. There have been many studies of observing rats that are reared in differential conditions which has involved spatial problem-solving tasks with various types of mazes and most studies have found improved learning in environmental enriched mice (Curtis & Nelson, 2003).

Environmental Enrichment

"Environmental enrichment is the intentional manipulation of a captive animal's surroundings to affect its physical and mental well-being in a positive way" (Royer, 1995, p. 1). There are many forms of environmental enrichment from sensory manipulation, such as adding new smells, to cognitive enrichment, such as adding new objects into the cage. "Environmental enrichment typically involves housing rats or mice in groups and allowing them to play with novel toys, traverse obstacles, and/or exercise in running wheels" (Frick et al., 2003, p. 187). There are two types of enrichment: intervention and supplementation. "Enrichment as supplementation assumes that if the organism is left to natural course of events adequate development will occur, while enrichment as intervention implies that non-enriched environments is inadequate for insuring normal development" (Curtis & Nelson, 2003, p. 4).

The Human Brain

The brain is one of the most important organs in the human body. The brain is composed of billions of neurons, each working in unison to provide humans the capacity to reason, to express emotions, and to understand the world around us. "The brain is made up of three main parts: the forebrain, the midbrain, and the hindbrain. The forebrain consists of the cerebellum, thalamus, and hypothalamus. The midbrain consists of the tectum and tegmentum. Lastly the hindbrain is made of the cerebellum, pons, and medulla. The cerebrum or cortex is the largest part of the human brain; the cerebrum is associated with higher brain function such as thought and action" (Kinser, Grobstein, & Mawr, 2000, p. 1). The cerebral cortex is divided into four parts: frontal, occipital, parietal, and temporal lobe. These four components of the cerebral cortex associate with many features that process and develop the brain's ability to learn and its intelligence (Kinser et al., 2000, p. 2). The frontal lobe associates with reasoning, planning, parts of speech, movement, emotions, and problem solving; the parietal lobe associates with

movement, orientation, recognition, and perception of stimuli; the occipital lobe associates with visual processing, and the temporal lobe associates with perception and recognition of auditory stimuli, memory, and speech (Kinser et al., 2000, p. 2).

Comparisons of a Human and Rodent Brain

Researchers are not allowed to physically study human patients so using an alternate that is closely related to the human system internally is fundamentally important. There are minor differences between humans and mice but the similarities far outweigh the differences. Although a mouse brain is not as complex as a human brain is, it functions the same. "The relevance of knowledge gained from other species to human processes is fairly well established, and many nonhuman animals share cognitive processes that have characteristics in common with many conceptions of human intelligence" (Curtis & Nelson, 2003, p. 17). In addition, mechanisms of memory formation at the molecular level are quite similar in human and nonhuman animals (Curtis & Nelson, 2003, p. 17). It was found that global network properties of the brain transcriptome are highly preserved between species (Miller, Horvath, & Geschwind, 2010, p. 1). Furthermore, the genes that are responsible for building and operating the brain are 90 percent identical between the human and mouse genome (Serendip 1).

Because mice and humans have similar characteristics of the brain, understanding how environmental enrichment affects cognitive functions of mice can open the window to comprehending how enrichment can affect human cognitive functions. Many researchers had the same conclusion, that environmental enrichment has a direct impact on a rodent's intelligence and their ability to learn. This conclusion can lead to further research and understanding of the relationship between enrichment and intelligence.

Materials and Methods

Eight black fancy mice were collected from the Small Animal Care room at John Bowne High School. Although the ages of the mice were unknown they all seem to be around the ages, it is believed that they are all around a year to about 1 and half years old, this was inferred because of appearance and their activeness. The eight mice were divided into 2 groups; one group held four females and the second group held four males. The mice were then categorized into four sub-groups; they were dividedly evenly among 4 cages so there was one pair (of the same sex) in each cage. Cage One and Two held the males and Cage Three and Four held the females. The mice held in Cage One (Male 1 and Male 2) and Cage Three (Female 1 and Female 2) were housed in an 18 inches by 12 inches cage with environmentally enriched conditions. To make the Cage One and Three environmentally enriched I added one exercise wheel (per cage), CritterTrail tubes, and chew blocks. The mice held in Cage Two and Four were also housed in a 18 inches by 12 inches cage and were known as the controlled group because their cages did not hold any stimulating objects, such as the exercise wheel and the tubes, the cages only contained wood shavings and food pellets.

Two hand built mazes were used to assess the run times for each individual mouse. Both Maze One and Maze Two were built using a plastic container for the outer exterior (although Maze 2 was built using a longer plastic container), cardboard for the interior walls of the mazes, and wood shavings, provided by John Bowne High School, to cover the base of the maze. To assess the mice, each mouse was placed individually at the starting position of each maze and timed from the point of position until they completed the mazes by reaching the desired treat (located at the end of the maze). The treat used in this study was an apple flavored fritter

covered with less than a teaspoon of Skippy All Natural Peanut Butter. To ensure that each mouse liked the treat being used and would recognize the scent of the peanut butter; the treat was placed in each cage immediately before each testing. It is unknown that placing the treat immediately before testing would make the mice want the treat, but it would allow them to become accustomed to the smell and therefore not be frightened to go near the treat.

Test 4: Maze 2			
Cage 1	Enriched	Male 1	33.5 seconds
Cage1	Enriched	Male 2	1 minute & 1.2 seconds
Cage 2	Controlled	Male 3	37.9 seconds
Cage 2	Controlled	Male 4	3 minutes & 42.5 seconds
Cage 3	Enriched	Female 1	10 minutes
Cage 3	Enriched	Female 2	4 minutes & 9.0 seconds
Cage 4	Controlled	Female 3	31.0 seconds
Cage 4	Controlled	Female 4	20.3 seconds

Table 1.The data table used to record the run times of each individual mouse in Test 4 of Maze 2.

There were a total of sixteen trials, eight trials per maze. On Fridays' each mouse ran through Maze 1 and on Monday's each mouse ran through Maze 2. After every mouse had finished the maze, their times were recorded in a data table as shown in the Table 1. Sometimes the mice were not able to be assessed on the proper days, but this was not a factor for the experiment. Before testing officially had begun; one of the females from Cage 4 died and therefore was immediately replaced. As the experiment progressed there were more complications with the female group. Female 1 from Cage 3 died before Test 6 had begun; it is believed she died from complications due to her old age. Because the experiment was almost completed, Female 1 was not replaced with a new female. Then two weeks later Female 3 from Cage 4 died before Test 7: Maze 2 had begun; she was also not replaced.

During the experiment the mice were also observed every Wednesday. The mice were observed to see if the mice in Cage One and Three were interacting with the objects that were placed inside their cages. I wanted to see them interact with the exercise wheel, the tubes, and the wood blocks because if they were interacting with the objects then it could be predicted that they would have a shorter time in the maze tests. To examine the effects of the stimulating objects in the enriched cages versus the controlled group, a line graph was drawn and analyzed after every three trials for both Maze 1 and Maze 2. Every three test results for both the females and the males were compared to each other to see which sex, if any, is performing better. The results would also show if the males and females housed in the enriched conditions were performing better versus the males and females housed in the standard laboratory cages.

I hypothesized that the mice housed in the enriched environment would have a significant decrease in the running times while the controlled group would stay consistent. It was believed the stimulating objects placed in the enriched cage would have an effect on the intelligence of the mice and by placing the mice individually in the maze it would have been shown if their memory and their sensory abilities were improving or diminishing.

Results

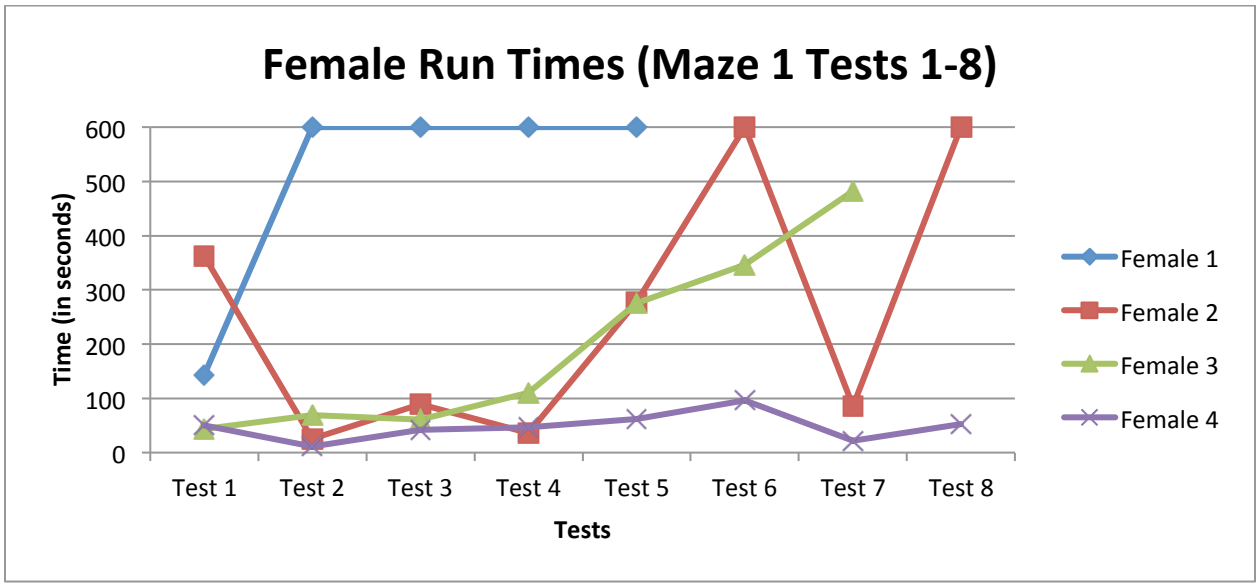

Figure 2. Displays the decreases and increases of the run times for both the enriched and controlled group of females from Test 1 to Test 8 of Maze 2.

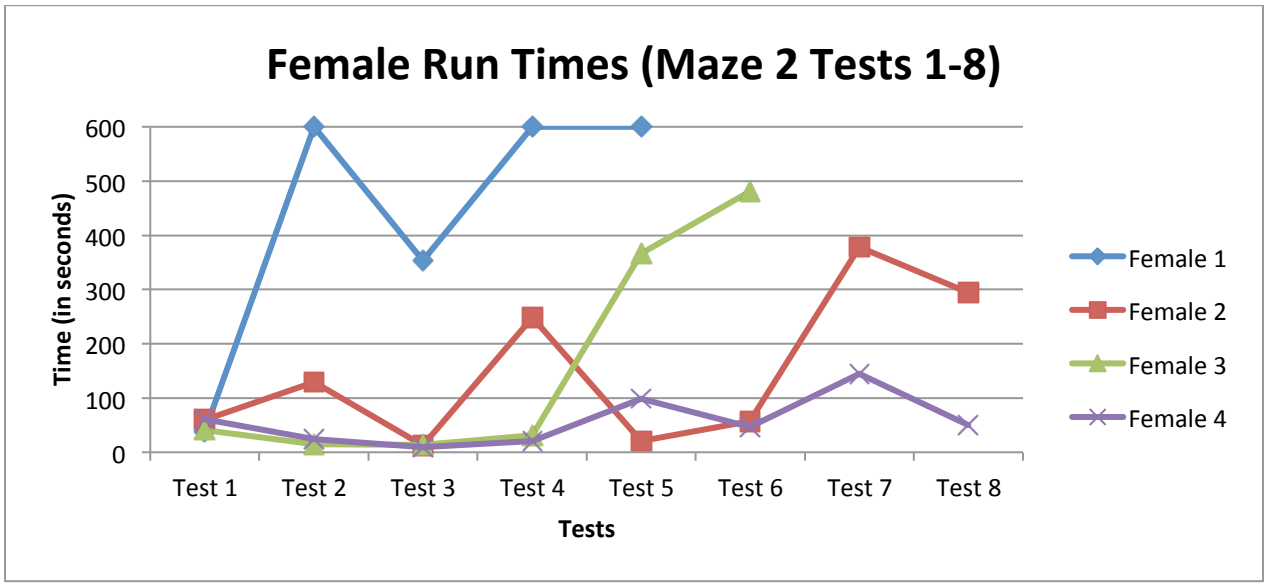

Figure 4. Displays the differences of the run times for the enriched and controlled females from Test 1 to Test 8 of Maze 1.

The two hand-built mazes have shown that the environmental enrichment did not affect the mice the way that it was hypothesized. There was no consistent drop in time for neither the

males nor the females. In Test 1 Maze 1 all the mice had a run time less than 200 seconds, except for Female 2 which took 6 minutes and 2.9 seconds. The next test for Maze 1 Female 1's time had drastically increased from under 200 seconds to the maximum time limit of 600 seconds or 10 minutes. While Female 1's time had increased over 458.3 seconds, the other females had decreased in time; Figure 2 shows that after Test 2 the majority of the Female group's time had declined to less than 100 seconds. As the experiment progressed the Female group's run times had been increasing. Female 1 had been consistent with the maximum time limit of 600 seconds in Maze 1 until she died (after Test 5 was completed). Although at first Female 2, Female 3, and Female 4's time had decreased, Figure 2 shows a slight increase in time for Test 3. After Test 3, Female 3's time had continued to increase until she had died after Test 7: Maze1. Female 4, who was also housed in the controlled cage had been rather consistent, her run times have never passed 100 seconds. Female 2, who was housed in the enriched conditions, began to rise in her run times and then fluctuated from Test 6 to Test 8.

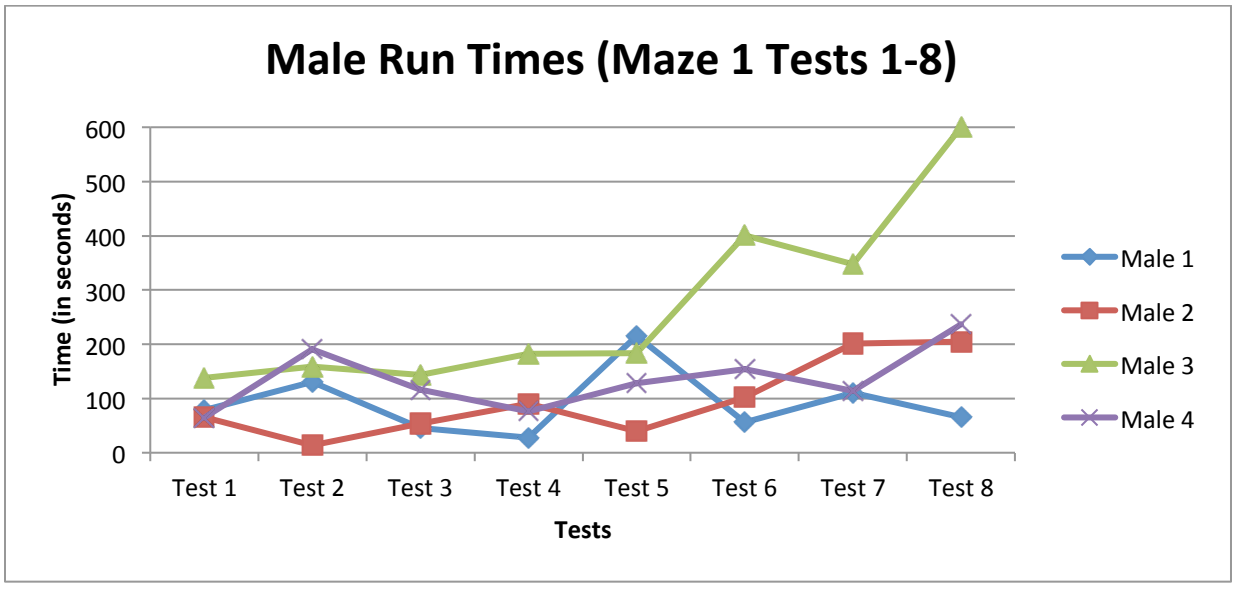

Figure 1. Illustrates the differences of the run times for the enriched and controlled males from Test 1 to Test 8 of Maze 1.

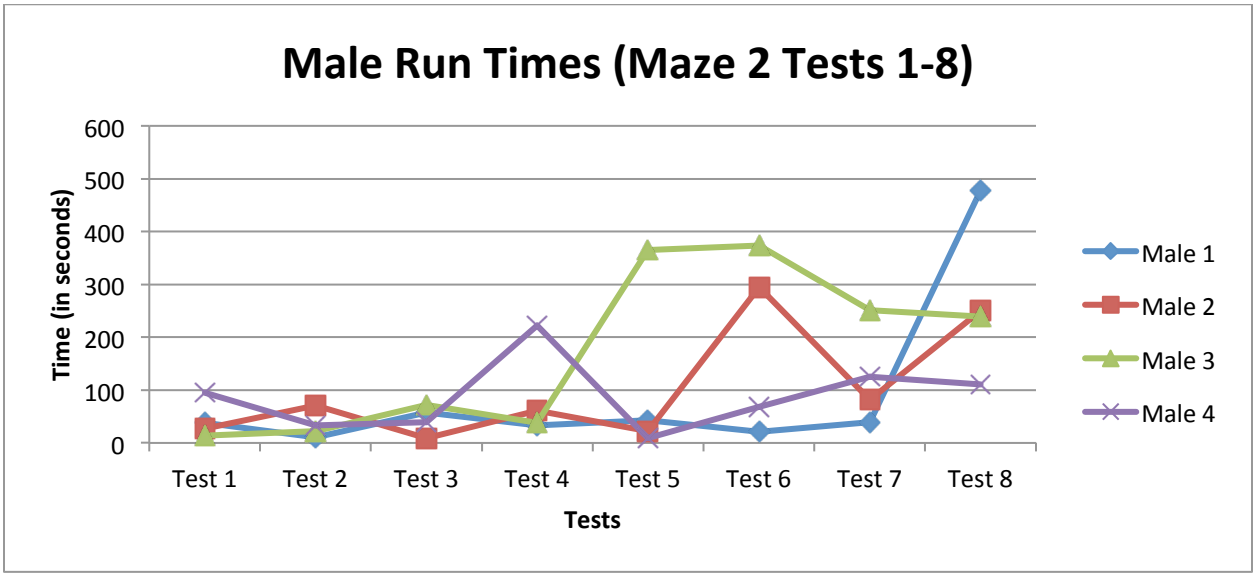

Figure 3. Illustrates the decreases and increases of the run times for both the enriched and controlled group of males from Test 1 to Test 8 of Maze 2.

In Maze 2, Female 1 again was rather constant with her time of 600 seconds except for the drop in Test 3. After Test 1 there was a drop in time for the controlled group but an increase in the enriched group. Throughout the experiment Figure 4 displays that there is a great deal of variation for Female 2, unlike in Maze 1 Female 2 was less stable for Maze 2. Unlike Female 2, Female 3's time was decreasing from 41.0 seconds to 20.3 seconds, but then her time suddenly started to increase from 20.3 seconds to 8 minutes and 0.4 seconds. Again, Female 4 had the best performance in the mazes, Figure 4 illustrates that her run times had never passed 100 seconds except for the slight spike in time in Test 7.

The male group in Maze 1 was more stable with their times than the female group. At the beginning of testing all the males stayed under 200 seconds until Test 5 when Male 1 had a spike in his time. From Test 1 to Test 2, Male 1's time had increased and then after Test 2, there was a decreasing trend until the spike he had in Test 5. Male 2, who was housed in the enriched environment, he was having more an increasing trend than decreasing. From Test 1 to Test 2 there was drop in Male 2's time from 65.1 seconds to 13.4 seconds, that is a 51.7 seconds

subtraction; after Test 2, Male 2's time was increasing until the drop in Test 5, but then his time began to increase again. Male 3 was the most consistent for his time because his time had continuously increased throughout the whole experiment. Lastly, Male 4 was also rather stable with his time; he did not have drastic variations in his time. Figure 1 shows that after Test 2, Male 4's time is decreasing until Test 5 when his time starts to increase.

In Maze 2, again, all the males started with a low time, they all started under 100 seconds and all of the males continued to have a run time less than 100 seconds until Male 4 had a drastic rise in time in Test 4. After the rise in Male 4's time in Test 4, he had a decrease in Test 5 and then again his time began to increase from Test 5 to Test 7. Male 3 started with the lowest run time of all the males, but then his time started to increase until Test 3. After, his time did decline slightly in Test 4 but then radically elevated in Test 5. Finally after Test 6 his time was starting to diminish. Male 2's time had fluctuated throughout the whole experiment in Maze 2. Unlike Male 2, Male 1's time had been the most consistent until Test 8 when there was a drastic increase in his time from 38.9 seconds to almost 8 minutes.

Discussion and Conclusion

The two hand-built mazes were employed to detect any significant changes in the intelligence of the eight mice. Both mazes were built with the same materials; however the first maze was built with a more complicated path. The mazes were used to assess if the enriched mice can use their memory and sensory abilities to travel through the maze at a quicker pace versus the controlled mice each time they ran through the maze because of the environmental enrichment placed in their cages. This study shows that both mazes detected no environmental effect for cognitive function in the male group. Both Male 1 and Male 2 were housed in the

enriched cage but as shown in Figures 1 and 2 there is no significant decline in time. Although the figures show that there has been a decrease between tests, the decline is not consistent; there are always spikes in the run times. These findings suggest that the environmental enrichment toys placed in the cage had no stimulating effect on the males or Male 1 and Male 2 were not interacting with the objects and therefore would have no decrease in their run times. As time progressed the mice did start to interact with the stimuli in their cages but there still was no improvement with their times. Male 3 and Male 4 were housed in the standard laboratory conditions and their time was inconsistent, but since they were the controlled group it did not matter that their time was not consistent.

The Female group was very inconsistent due to all the deaths and health issues concerned with that group. The Female group could only be observed until Test 5 because before Test 6 began Female 1 died due to her age. After Female 1 had passed, two weeks later Female 3 had also died due to unidentified issues. When the times of the certain females were increasing dramatically, such as Female 1 and Female 3, it was inferred that were becoming ill and may possibly die. Also halfway through testing, Female 2 had developed a skin irritation, so her trend for both mazes was increasing. The experiment was almost finished when it was discovered that there may be a possible infestation of mites and lice and all the rodents in the Small Animal Care room may be infected. The news of all the mice being infected with mites and lice can be the reason why Female 2 developed a rash and why Female 3 died. After all the deaths and health concerns the testing was finished on March 20th, 2013. There were many factors that had affected the experiment. The hypothesis may have been proven if additional enrichment was placed in the cages. Also the experiment could have further been improved if the position of enrichment placed in the cages was switched every day and the positions were

repeated every week; switching the position of the enrichment could have increased the cognitions of the mice at a more rapid rate because it would require their memory and make them process new information. For future research, experimenters should not only record the run times of each mouse but also calculate the mistakes each mouse takes before they finish the maze. Calculating the mistakes could show that there is actual improvement even though the time the mice take does not decline. In this experiment the mice were of varying ages, so it was unknown if the run times that were recorded were not improving because of the enrichment or the ages, so for further research each group of mice should be of equivalent ages so age range will not be a factor. When testing was completed, it could not be determined which gender group was performing better in the mazes due to all of the inconsistencies and fluctuations in both groups. It was hypothesized that both the female and male enriched group would perform better in the mazes. It was believed that as the experiment continued the enriched mice would have a gradual decrease in their time because the enriched environment would enhance their cognition. The enriched males and females both did not have any stable decline in their run times as the experiment progressed, therefore the hypothesis was proven wrong.

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Acknowledgements

This work was supported by the Agriculture Department of John Bowne High School. I would like to thank the Assistant Principal of the Agriculture Department, Mr. Perry, for funding and his encouragement of this experiment. Finally I would like to recognize my Agriculture teacher, Ms. Gottfried for allowing me to conduct this experiment and for instilling the steps to correctly completing the study.