Differential Effects of Chronic vs. Acute Ethanol Exposure on Memory in C57BL/6J Mice

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Abstract

The field of psychology often studies several different aspects of behavior. Imbedded within the field of psychology we find the field of psychopharmacology which typically studies the effects of various drugs. Ethanol, or alcohol, is a psychoactive drug that is widely used, and which consumption is more frequent during adolescents. Adolescents is one of the most crucial time periods in brain development and the use of psychoactive drugs, like ethanol, can disrupt the process. This study was aimed to evaluate the effects of ethanol on memory in adolescent mice. Specially, to examine the differential effects of acute vs. chronic ethanol exposure. The methods used included the exposure of C57BL6 mice to chronic, acute, or no alcohol, and behavioral testing included the open field test, novel object recognition, and the Morris water maze. The results showed that there was no significant different in activity or anxiety as well as in recognition memory among the three conditions. However, there was a significant difference in spatial recognition memory in mice exposed to chronic, vs. acute or no alcohol. These findings, suggesting that chronic alcohol exposure has detrimental effect on spatial learning and memory in animals, should be further explored at the biochemical and molecular levels.

Keywords: mice, novel object recognition, ethanol, memory, Morris water maze

Introduction:

The field of psychology often studies several different aspects of behavior. Imbedded within the field of psychology we find the field of psychopharmacology, which typically studies the effects various drugs. Ethanol, or alcohol, is a psychoactive drug that is widely used, and which consumption usually increases in adolescents, one of the most crucial periods in a person's life. During this time the brain is still developing, and neurons are still being generated to allow the brain to improve its plasticity and cognitive ability (Oliveira et al, 2015). At moderate to high doses, ethanol exposure can have severe effects, including a decrease in a person's speed of processing, impaired attention, and disruption of memory recall (Sircar et al., 2006).

One area of the brain areas that plays a key role in memory function and anxiety, as well as other important functions such as emotional regulation, is the hippocampus. The amnesic effects that people often experience due to ethanol exposure closely resemble the amnesia seen after hippocampal damage. This suggests that the hippocampus also plays a role in alcohol-induced amnesia (Garcia-Moreno et al., 2012). During adolescents, this area of the brain has been reported to be especially vulnerable to the effects of alcohol. In some studies, that have been conducted in rats, it has been reported that there is a loss in the number of neurons in this brain region and an overall loss in the volume of the hippocampus after alcohol exposure (Oliveira et al., 2015). The overall reduction of neurons in the area of the hippocampus due to ethanol exposure is believed to be the cause of the memory impairment in some research (Garcia-Moreno et al., 2012).

The purpose of this study was to examine the differential effects of chronic vs. acute ethanol exposure on C57BL/6J mice using behavioral measures. Our intent was to explore the

differences that exist between mice that were chronically or acutely exposed to ethanol. We expected to find that mice who were chronically exposed to ethanol would show more severe memory deficits when subjected to behavior testing than mice in the acute exposure or control groups.

Methods

Subjects

Eighteen C57BL/6J male mice, 6 weeks old, were housed individually (1/cage), under 12/12 light/dark cycle (lights on at 7am, off at 7pm), with ad lib food, and modified water schedules (explained later). All experiment procedures followed NIH guidelines and were approved by the Saint Francis University Institutional Animal Care and Use Committee (Protocol No. 00036).

Apparatus/ Equipment

Behavioral equipment: To measure learning and memory the Open Field Test (Seibenhener and Wooten 2015), 3 Chamber Novel Object Recognition Test (Huang and Hsueh 2014), and Morris Water Maze (Voorhees and Williams 2006) were implemented.

Open Field Test:

This test provided information about the activity levels of each mouse, as well as some indication about the mouse's anxiety levels. On day 1, all mice were observed in a series of trials, beginning with a non-automated activity box. In this test, mice were allowed to wander freely for 5 minutes inside a box marked with four rows of four squares (box dimension being 24.5in x 24.5in x 17.5 in), and the number of squares crossed was recorded (Seibenhener and

Wooten 2015). Mice typically possess an instinctive tendency to remain close to the walls of the box, where they feel more secure and are less vulnerable to prey. However, when mice spend more time in the center of the box, they are believed to exhibit less anxious behaviors, because they are not frightened by a condition that placed them out in the open (in the eye of potential danger).

Novel Object Recognition:

On day 2 and 3 of testing, the 3 chambered novel object recognition test was implemented. The 3 chambered novel object recognition test is commonly used to assess recognition memory. The purpose of this task in the current project was to see if exposure to ethanol would have an effect on ability to recognize novel or new objects. This test consisted of two steps. In the first part on day one, a mouse was placed in a non-automated box of three interconnected empty chambers with dimensions of 24.75in x 9in x 9in, and allowed to explore it freely for one minute. After one minute, two identical Legos or conical tubes were placed into the two outermost chambers (Legos, dimensions of 2.5in x 2.5in x 1.5in, or conical tubes, length of 4.5in and diameter of 1 in, were randomly chosen for each mouse). Each mouse was allowed to explore the chambers (and objects) again for 4 minutes. On day 2 of testing, the mice were again allowed time to habituate to the 3 chambers for one minute. Then, a Lego and Tube were placed into the two outermost chambers, creating a condition in which one object in familiar and one is novel. The time spent in the chamber with each object was recorded. This test is based on the natural tendency of mice to prefer to explore the novel object over the one they previously had contact with. A mouse displaying “normal” behaviors would spend more time with the novel object. A mouse that spends equal time with both objects (or less time with the novel object) shows a decrease in memory function (Huang and Hsueh 2014).

Morris Water Maze:

On days 4-10 of testing the Morris Water Maze was used. The dimensions of the pool apparatus were 43 inches in diameter by 6 inches in height. The water was approximately 4 inches deep and the platform was 3.75 inches in height allowing the platform to rest just under the level of the water. The platform was placed in one of the quadrants and kept in the same quadrant over the 7 days of the experiment. Each mouse was placed into a randomly selected quadrant each day for a 4-minute trial period and the time it took to reach the platform was measured. If the mouse did not reach the platform within the 4-minute period, they were placed on the platform by the researcher for approximately 15 seconds. The first 7 days of the test were used to measure learning. Mice that demonstrated a shorter distance/less time to reach the platform, learned where the platform was located. On day 11 of testing, the probe test was conducted. This stage includes the removal of the platform from the water and the time each mouse spends in the quadrant the platform was previously in is assessed. This test measures memory, meaning that if the mice spend an increased amount of time in the quadrant where the platform had been, it shows memory of its location (Voorhees and Williams 2006).

Procedure:

Eighteen male mice were chosen for this study. These mice were randomly allocated into three groups with 6 mice in each condition; chronic ethanol exposure, acute ethanol exposure or the control group. Mice that received chronic or acute ethanol exposure were on a 10-hour water/14-hour ethanol schedule, meaning that at 7am each morning they were given a bottle that contained only water, at 5pm the bottle was switched to one that contained ethanol. Chronic ethanol exposure consisted of mice being exposed daily to ethanol for 14 hours during what mostly consisted of their dark cycle (7pm-7am dark/7am-7pm light). Acute ethanol exposure

consisted of mice being exposed to ethanol once a week for 14 hours (these hours were primarily during their dark cycle). The control group received no ethanol throughout the experiment and only consumed water. The length of ethanol exposure lasted three weeks. During the first week, mice were exposed to 1% ethanol solution, the second week consisted of exposure to a 5% ethanol solution, and in the third week mice were exposed to 10% ethanol solution.

After the three-week alcohol consumption period all 18 mice underwent a series of behavioral testing. On day one of testing mice experienced the Open field test. In this test all 18 mice were placed one at a time into a box with a grid on the bottom. Mice were monitored for 5 minutes. During these five minutes, mice were allowed to explore the box freely and behavior was recorded. After each mouse completed their trial the box was cleaned with 10% ethanol, mice were returned to their home cage and the next mouse was placed in the box.

On day two of testing all mice were exposed to the Novel Object Recognition chamber. The chamber is broken up into 3 boxes, where the mice has a "door" that grants them access to roam freely between the three boxes. During this procedure 2 objects were randomly selected for the mice to be exposed to. They were either exposed to two identical Lego blocks or two identical tubes. Mice were given one minute to habituation to the 3 chambered box, then a four-minute test period began. On day 3 of testing the second part of the Novel Object Recognition test was conducted. In this phase all mice were exposed to one tube and one Lego block which were placed in the outermost chambers of the box. Again, mice were given a one-minute habituation time and four-minute test period. In this phase the time that the mice spent with each object was recorded.

On days 4-10 of the procedure mice were tested using the Morris Water Maze. During this procedure a small pool was filled with water and mice were placed into one of the four

quadrants and the object is for the mice to swim and find the platform that is hidden just below the surface of the water. Each mouse was exposed to one 4-minute trial. If a mouse did not find the platform before the trial ended the mouse was placed on the platform and stayed there for 15 seconds. During this test the time it took each mouse to reach the platform was recorded. On day 11 of testing each mouse underwent the Morris Water Maze probe test. On this day the platform is removed from the water and the amount of time that each mouse spent in the quadrant that the platform was previously in was measured.

Results

Results suggest that there are some differences in the effect of chronic or acute ethanol exposure on learning and memory.

Specifically, the novel object recognition test shows that when mice were chronically exposed to ethanol, the majority spent about equal time with each object or spent more time with the familiar object. Only one mouse within the chronic exposure group spent a significantly larger amount of time with the novel object (Figure 1).

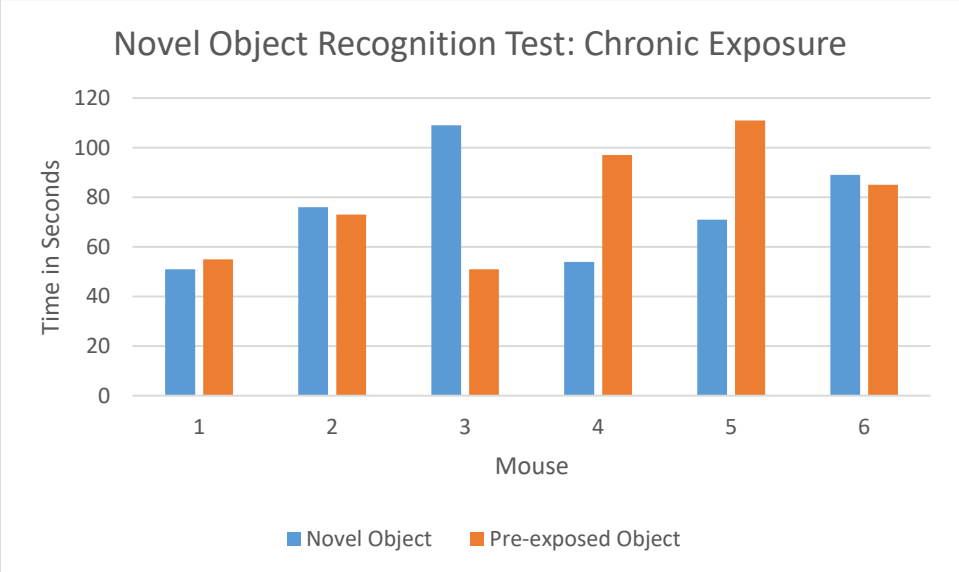

Figure 1: The majority of mice who experienced chronic exposure spent more time or equal time with the pre-exposed object.

When looking at this same test we see that mice who were acutely exposed to ethanol show slightly different behavioral patterns. Specifically, we see that 50% of the mice spent more time with the novel object, and the other half spent more time with the familiar object, as shown in figure 2.

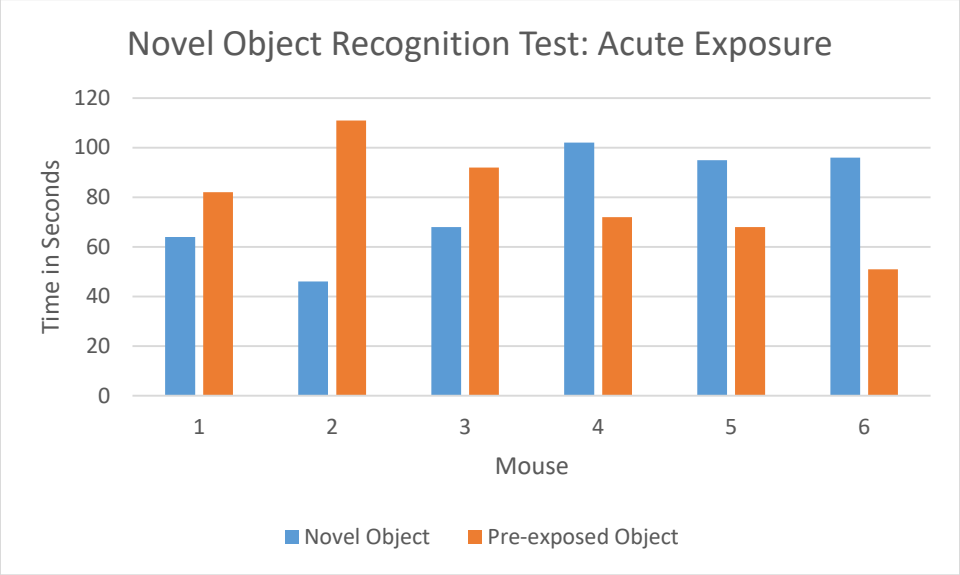

Figure 2: 50% of the mice spent more time with the novel object. The other 50% spent more time with the pre-exposed object.

When looking at the control group, we see similar results as we seen with the acute exposure mice. Half of the mice spent a significantly higher or equal amount of time with the novel object, and the other half spent more time with the familiar object (see Figure 3).

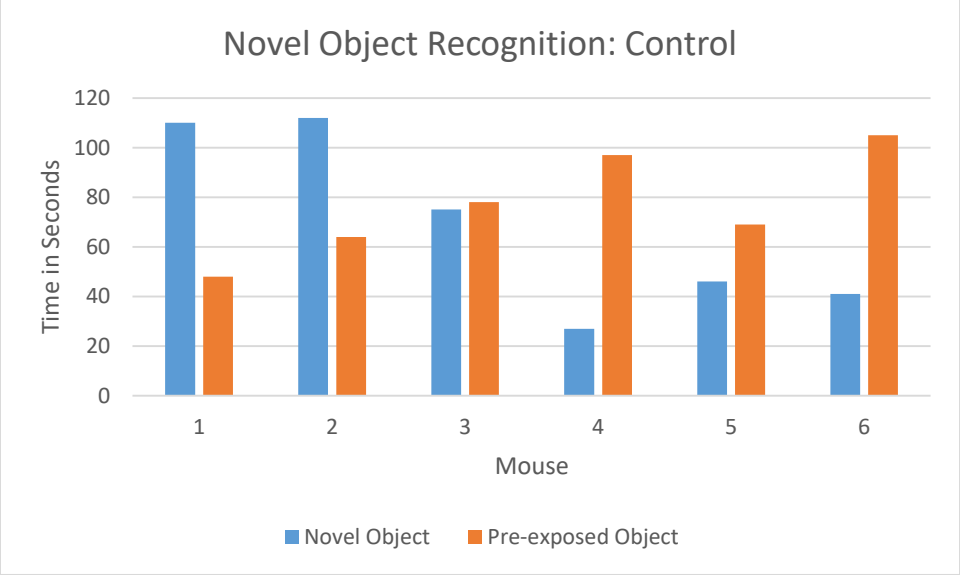

Figure 3: Results from Novel Object Recognition test control group.

The Morris Water Maze test results is used to demonstrate learning and memory. When examining the control groups data in this study, it was found that over the course of the 7 days the time it took the mice to find the platform decreased continually over the given period of time. The trend shows a decrease in time that it took each mouse to find the platform as shown in figure 4.

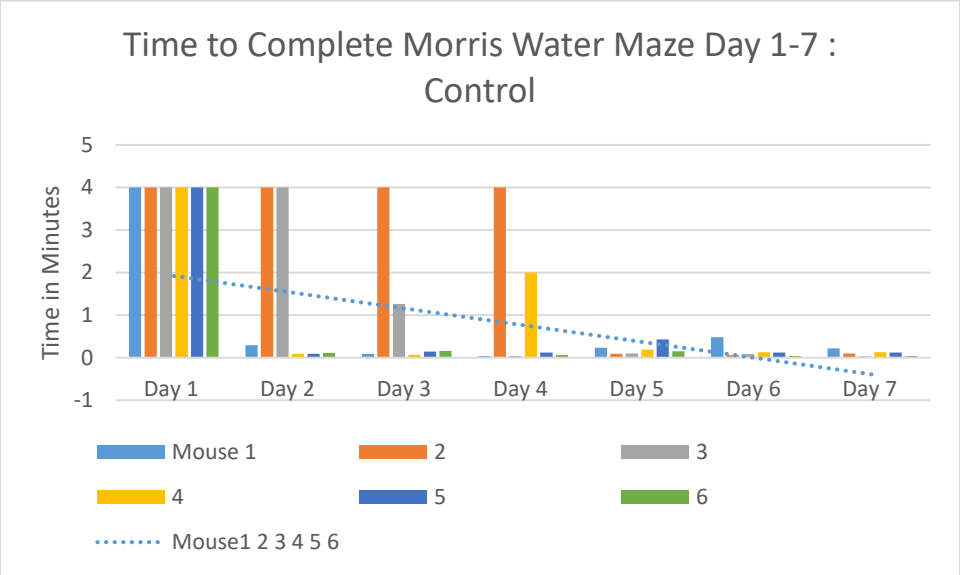

Figure 4: Continual decline in the time it took each mouse to locate the platform during the Morris Water Maze Learning Test.

When examining the data from the acute ethanol exposure group, we see similar results. We can still see a continual decline in the amount of time it takes for the mouse to reach the platform. It is worth noting that once the mice in the acute group located the platform their time to reach the platform was about half of the control times (Figure 5).

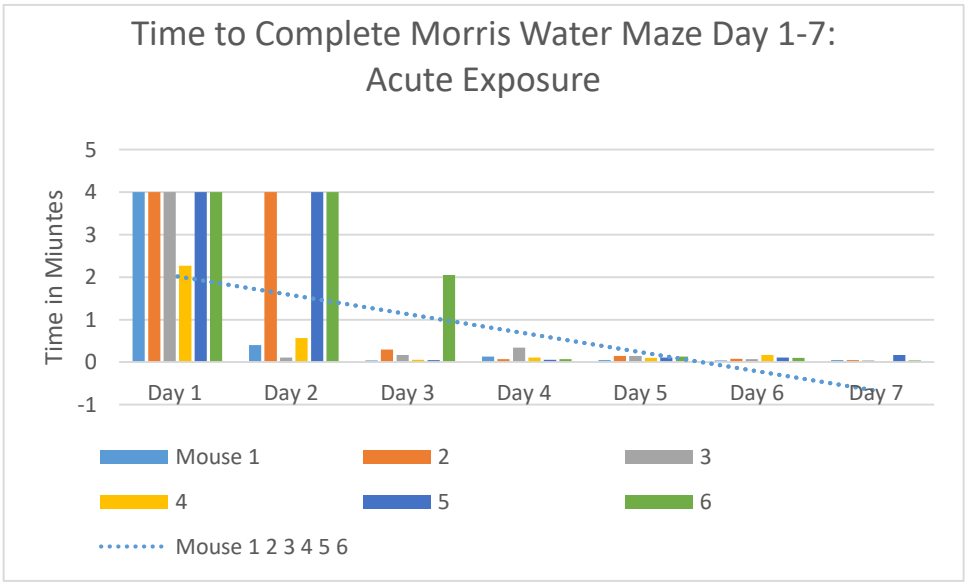

Figure 5: Results from acute ethanol exposure mice during the Morris Water Maze testing. Shows a downward trend in time which indicated learning.

When examining the chronic ethanol exposure group data, we see slightly different results when compared to both the control and acute ethanol exposure groups. While still continuing to see a downward trend in the data, the chronic ethanol exposure group overall took longer to learn where the platform was located. Over the first few days of Morris Water Maze testing, the times it took some mice to reach the platform varied greatly among each mouse. As seen in figure 6, this difference mostly between days 1 and 2 of testing.

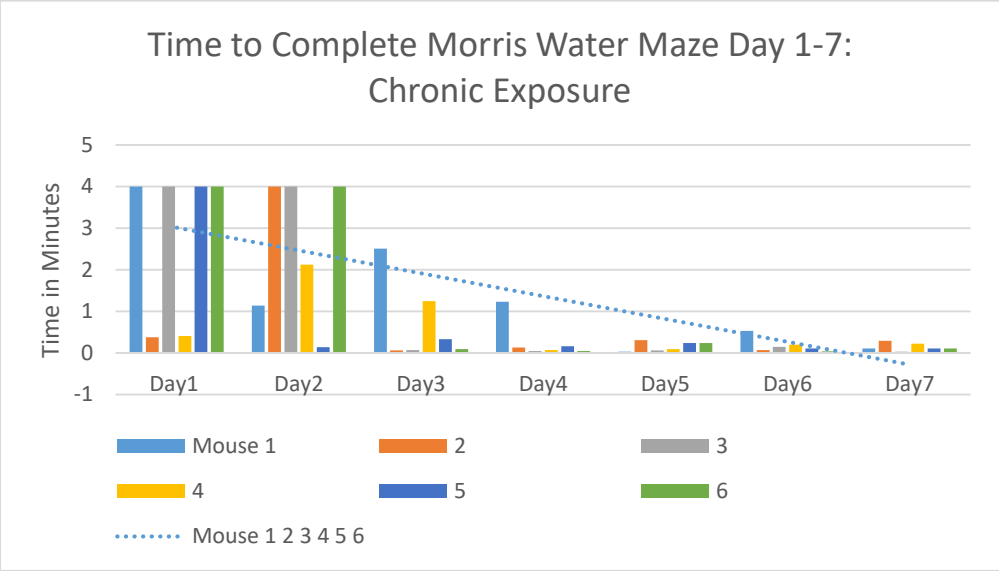

Figure 6: Chronic ethanol exposure mouse data, shows a downward trend, with more sporadic time differences between days.

On day 8 of Morris Water Maze testing the probe test was completed as seen in figure 7. On this day the platform was removed from the water and spatial memory was tested. Memory is tested by measuring the amount of time that each mouse spends in the quadrant that the platform was previously in. The data from the probe test do no indicate any significant differences between any condition. Mice in all conditions spent a significantly higher amount of time in the quadrant that the platform was in compared to any another quadrant. It is worth mentioning that on average, the chronic condition spent less time in the quadrant compared to both the acute ethanol exposure and control condition (Chronic: 1.05, Acute: 1.40, Control: 1.08).

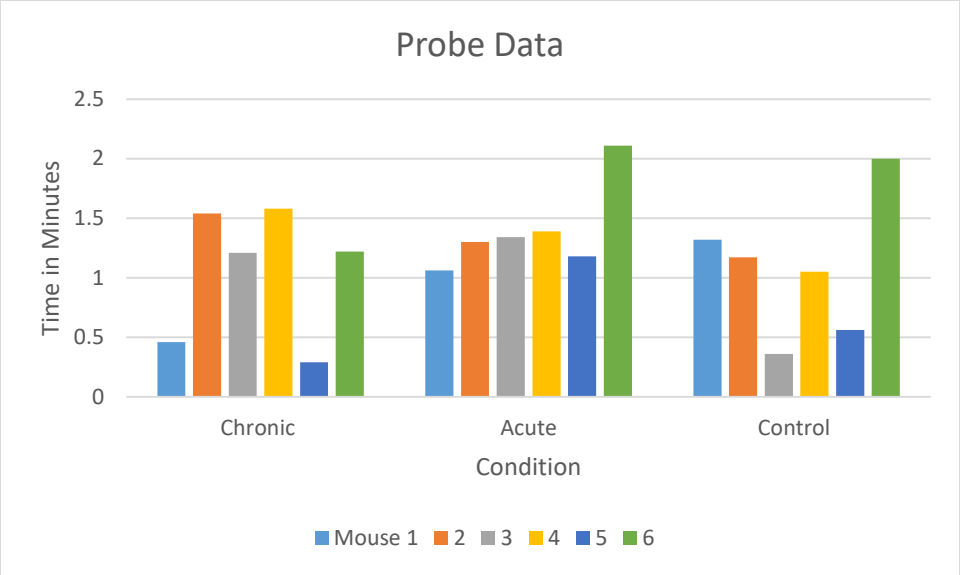

Figure 7: No significant difference between the amount of time that each condition spent in quadrant that the platform was previously in.

Chronic or acute ethanol exposure did not seem to have an effect on the activity of each mouse when they were subjected to the open field test. When examining the number of grid crosses shown in figure 8, there was no significant difference in the number of crosses across conditions. These results show that ethanol did not decrease the activity of the chronic exposure group which could have been expected based on the known effects of ethanol.

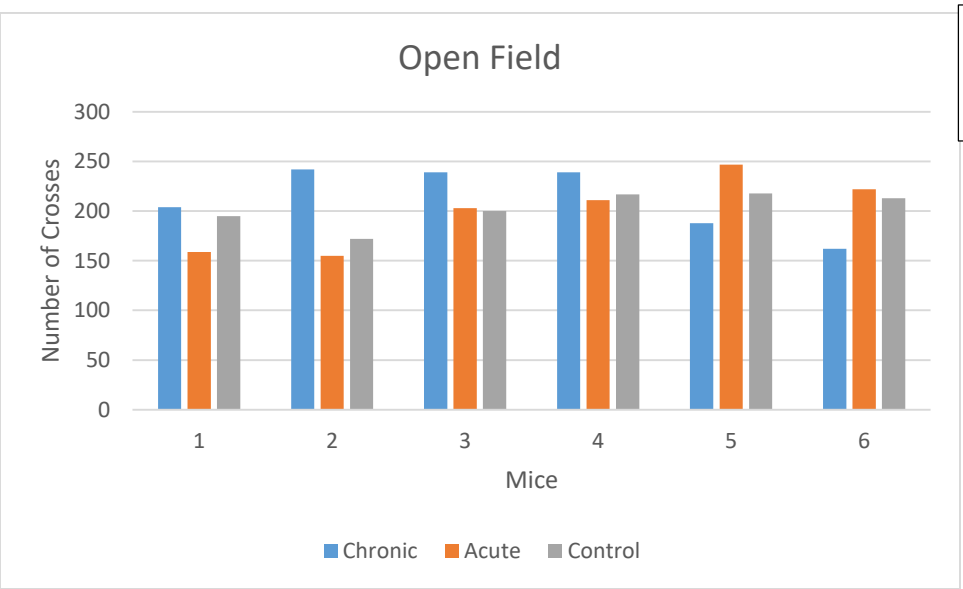

Figure 8: No significant difference in number of grid crosses in open field test

Additionally, there was no significant difference in the amount of time that mice spent in the middle section of the open field test. Figure 9 shows there was no significant difference across conditions, but it is worth mentioning that the mice in the chronic exposure group spent a slightly higher amount of time in the middle section of the box, (yet, not enough to reach significance). This effect could mean that the ethanol had a slight, but not significant, ability to decrease anxiety.

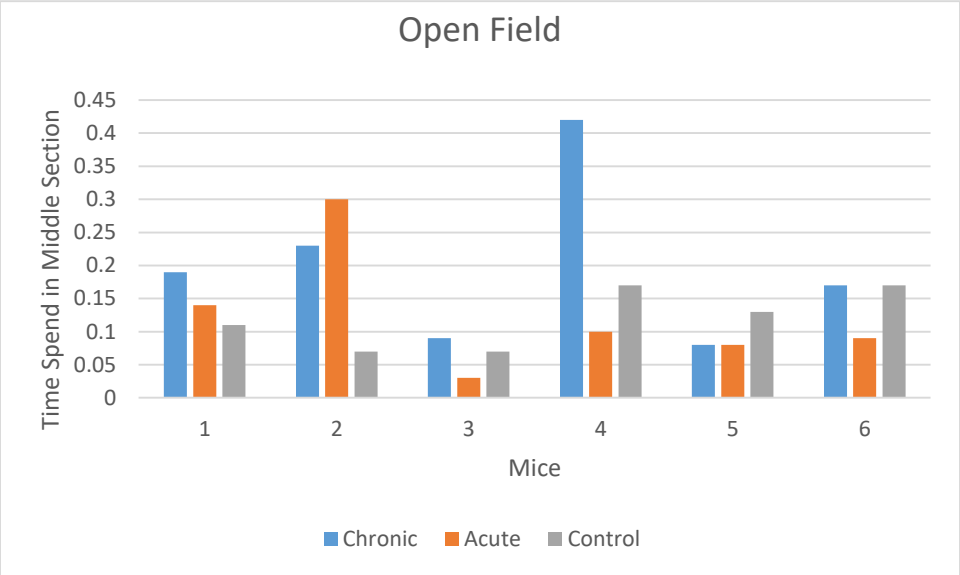

Figure 9: No significant difference in amount of time spent in the middle section of open field test.

Discussion

Based on the results demonstrated above, it can be concluded that there are some differences between chronic ethanol exposure and acute ethanol exposure on learning and memory in mice. In relation to the Novel Object Recognition test, the results indicated that there is no significant difference between each condition and the time spent with the novel object or the familiar object. The findings in the current study relating to the novel object recognition test contradict research conducted by Strgier. His research reported that when the Novel Object Recognition test was performed, the chronic ethanol treated mice preformed worse than controls, meaning that they spent more time with the familiar object that the novel object (Strgier et al

2015). In the current study there is a possible explanation for the fact that there was no significant difference between any of the conditions. When examining data from day 1 and day 2 of testing we determined that many of the mice in the study showed a chamber or side preference. Specifically, on day one of testing it was expected that mice would spend around 50% of the time with each object. However, for most mice that was not that case, and they spent more time in one side of the chamber or the other, and that same preference was demonstrated on day 2 of testing.

When examining the results of the Morris Water Maze, which measures spatial memory, current findings are supported by that of other studies. Acheson et al (2013) found that animals who were treated with ethanol took an increased amount of time to reach the platform than control animals did. This evidence supports the findings in the current study when comparing the chronic ethanol exposure mice to the control mice. The chronic ethanol exposure group took more time to reach the platform than the control mice did on just about any day of the study. When comparing the control with the acute ethanol exposure group in the current study the data show some slight differences, though. At the begging of testing it took the acute ethanol exposure group longer to locate the platform than it did the controls, however, by day 7 of the test, the acute ethanol exposure group was locating the platform faster than both the chronic ethanol exposure group and the control group. Therefore, we see that there is some learning deficit shown within the conditions, especially when looking at those mice who were chronically exposed to ethanol.

While the results show some significance in determining the effects of chronic versus acute ethanol exposure, there are some limitation that should be considered. First, the computer software that is necessary to track the movement of the mice during the Morris Water Maze was

not available to us. Therefore, we were unable to tell the distance that the mice traveled in order to reach the platform, meaning we were unable to show any repetitive behavior in swimming patters and if there was a difference between the groups. Additionally, this study is open to human error since all data was recorded by the human eye and could thus have small errors in data analysis. Also, it should be noted that the Morris Water Maze is sometimes a stressful environment for mice and could have played a role in the results of the data. It is thus recommended that future research extends the findings of the current study, by investing in the software to examine the distance swam by mice. To summarize, the current study points to important behavioral trends among mice chronically and acutely exposed to ethanol, which indicates some learning and memory impairment. The findings indicate that humans who are chronic alcohol users have a higher chance of being affected by learning and memory impairment than people who consume alcohol on occasion or not all.

References

Acheson, S., Bearison, C., Risher, M., Abdel wahab, S., Wilson, W., Swartzwelder, H. (2013). Effects of Acute or Chronic Ethanol Exposure During Adolescence on Behavioral Inhibition and Efficiency in a Modified Morris Water Maze Task. *PLoS ONE,* 8(10). Retrieved from ProQuest.

Garcia-Moreno, L.M., Cimadevilla, J.M. (2012). Acute vs Chronic Ethanol Intake: Effects on Spatial and Non-Spatial Memory in Rats. *Alcohol*. 46:757-762. Retrieved from ProQuest.

Huang, T. and Hsueh, Y. (2014). Novel Object Recognition for Studying Memory in Mice. *Bio-protocol* 4(19): e1249.

Olivera, A., Pereira, M., da Silva Santana, L., Fernandes, R., Teixeria, F., … do Socorro Ferraz Maia, F. (2015). Chronic Ethanol Exposure During Adolescence Through Early Adulthood in Female Rats Induces Emotional and Memory Deficits Associated with Morphological and Molecular Alterations in the Hippocampus. *Journal or Psychopharmacology,* 29(6), 712-724. Retrieved from SAGE.

Seibenhener, M. L., & Wooten, M. C. (2015). Use of the Open Field Maze to Measure Locomotor and Anxiety-like Behavior in Mice. *Journal of Visualized Experiments*: JoVE, (96), 52434. Retrieved from NCBI.

Siggins, G.R., Martin, G., Roberto, M., Nie, Z., Madamba, S., De Lecea, L. (2003). Glutamatergic Transmission in Opiate and Alcohol Dependence. *Annals of the New York Academy of Sciences*. Retrieved from Google Scholar.

Sircar, R., Sircar, D. (2006) Repeated Alcohol Treatment in Adolescent Rats Alters Cortical NMDA Receptors. *Alcohol*: 39. 51-58. Retrieved from ProQuest.

Stragier, E., Martin, V., Davena, E., Poilbout, C., Mongeau, R., Corradetti, R., Landumey, L. (2015). Brain Plasticity and Cognitive Functions After Ethanol Consumption in C57BL/6J Mice. *Translational Psychiatry*. 5. Retrieved from ProQuest.

Voorhees, C. V., & Williams, M. T. (2006). Morris water maze: procedures for assessing spatial and related forms of learning and memory. *Nature Protocols*, 1(2), 848–858. Retrieved from NCIB.