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Remember that party last night? Examining the effects of binge-like alcohol on memory

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Abstract

Alcohol is a widely available drug that is often abused. Studies have shown alcohol-induced memory loss in humans and animals; however, the large amount of alcohol often necessary to observe such loss and use of involuntary drinking paradigms in animals makes translation difficult. The current study was designed to look at voluntary binge-like drinking behavior and memory in rodents. We anticipated decreases in working memory function following consumption of binge-like alcohol in mice and expected a greater deficit in mice experiencing acute withdrawal during the memory task. The present study explored drinking behaviors in adult C57BL/6J mice (18 male, 18 female) using an intermittent access 2-bottle choice paradigm to alcohol (3-20% v/v). Mice were separated into three conditions: water, alcohol, and alcohol withdrawal. Each group had access to a secondary (drug) bottle every 24 hours. The amount of liquid consumed from each bottle was recorded daily. Approximately 24 hours after the last drinking day, working memory was assessed using the novel object recognition (NOR) test. Results showed a significant main effect of concentration, where all mice consumed significantly more alcohol when exposed to higher concentrations. Results also showed a significant main effect of gender, where females drank significantly more alcohol than males. There was also a significant interaction of concentration and sex, where females drank more alcohol than males at higher concentrations. There were no significant findings for the NOR. These results highlight the need for more research into the memory-impairing effects of alcohol consumption and withdrawal.

Alcohol is one of the most commonly abused drugs in the United States. It is easy to get while underage and there are minimal laws regulating its consumption. A survey conducted in 2015 reported that 86.4% of people 18 years old and above had drank alcohol in their lifetime, and 26.9% reported that they had binge drank in the past month (Alcohol Facts and Statistics, 2015). In a survey conducted on college students, it was reported that of those who reported drinking alcohol, 51% of them experienced blacking out from drinking (Alcohol Alert, 2004). It is not uncommon for adults to consume alcohol, including drinking to the point of it becoming unhealthy. The danger of alcohol is seen in its aftermath, as an estimated 88,000 people die in alcohol-related deaths annually (Alcohol Facts and Statistics, 2015). Binge drinking is the consumption of large amounts of alcohol in short periods of time and is often defined by the criteria of 5 or more drinks for a male and 4 or more drinks for a female (Alcohol Facts and Statistics, 2015). In humans, immediate visual memory and working memory are often impaired through binge drinking (Vinader-Caerols, Montañés, & Monleón, 2017). Excessive alcohol intake, such as that seen with binge drinking, results in neurodegeneration - or damage to neurons or nerve cells in the brain (Morris, Smith, & Nixon, 2010). Excessive alcohol consumption is a growing problem and the effects of such drinking habits require more intense research.

Due to alcohol consumption being commonplace, it is important to look at how it affects the brain directly. Multiple areas of the brain are changed as a result of alcohol consumption. Alcohol primarily acts on ion channels and the receptors of neurons in order to alter brain function. A neuron is a nerve cell, and these nerve cells contain receptors for specific chemicals, neurotransmitters, in the brain that allow these neurons to communicate with each other (Owens & Kriegstein, 2002). These changes, caused by alcohol acting on

ion channels and neuron receptors, are associated with the inhibition of glutamatergic NMDA receptors and calcium channels, excitation of GABA receptors, glycine receptors, and increasing potential of serotonergic receptors (Van Skike *et al.,* 2016; Vengeliene *et al.,* 2008). As alcohol interacts with these various sites, there is a release of neurotransmitters such as dopamine, glutamate, and noradrenaline (Vengeliene *et al.,* 2008). These neurotransmitters are important factors in human behavior, including emotional responses and actions. Alcohol consumption clearly changes the chemical composition of the brain and can lead to changes in behavior - effects that are important to study because of the negative impacts they can have on an individual.

One of the negative effects often associated with heavy alcohol consumption is the decline in memory. The main areas of the brain involved in memory include the hippocampus, the medial prefrontal cortex, and the amygdala. The hippocampus retrieves detailed memories through the perirhinal–lateral entorhinal cortex and parahippocampal–medial entorhinal cortex feedback pathways (Preston & Eichenbaum, 2013). The medial prefrontal cortex receives information from the hippocampus and collects information about interrelated memories (Preston & Eichenbaum, 2013). For recognition-specific memory, the hippocampus works with the perirhinal and medial frontal cortices for object-in-place memory as well as recency recognition memory (Barker & Warburton, 2011). The amygdala is involved in emotional memory (Preston & Eichenbaum, 2013). Specifically, blockade or inactivation of the hippocampus is thought to underlie memory disruption.

Importantly, alcohol is known to alter areas of the brain such as the hippocampus, and alcohol-induced memory deficits have been increasingly studied in humans. Specifically, there is a correlation between increased drinking behavior and a decline in

working memory function (Lechner, Day, Metrik, Leventhal, & Kahler, 2016). There is a decrease in working memory function in conjunction with increased alcohol consumption as a result of reduction in posterior alpha power function (Boissoneault, Frazier, Lewis, & Nixon, 2016). Posterior alpha power function is a component of an EEG, specifically the frequencies of 8 to 12 Hz, which has been found to be sensitive to different task demands (Boissoneault, Frazier, Lewis, & Nixon, 2016), therefore as the posterior alpha power decreases, the performance of the specified task also decreases. Another example of alcohol affecting memory is Korsakoff's syndrome. Severe and chronic alcohol consumption to the point of alcoholism and malnutrition can lead to Korsakoff's syndrome, which is characterized by memory impairment similar to that seen with Alzheimer's disease. In Korsakoff's patients, working memory deficits were found to be related to brain damage in the hippocampus (Pitel *et al.*, 2008). Hippocampal brain damage appears to be one of the mediating factors between alcohol consumption and memory deficits.

Due to ethical considerations with conducting alcohol-related human studies, memory deficits arising as a result of heavy alcohol consumption have often been researched further in non-human models. One such non-human model used in alcohol research is the mouse model. Similar to what has been shown with human studies, mice that have consumed sufficiently high quantities of alcohol suffer from brain neurodegeneration in areas such as the hippocampus, which leads to memory deficits, and more specifically leads to the inability to identify a novel object in the Novel Object Recognition test (Golub *et al.,* 2015). In such animal binge-drinking models, neurotoxicity occurs in the hippocampus and the surrounding cortex (Leasure & Nixon, 2010). When binge alcohol exposure occurs at a young age, spatial deficits occur, producing deficits in

a Morris water maze (Wagner, Zhou, & Goodlett, 2014). Alcohol-induced memory impairment is more likely to occur in an individual if they have experienced this impairment before (Miller, Merrill, DiBello, & Carey, 2018). Mice that have consumed alcohol use a different portion of their hippocampus to perform spatial memory tasks as compared to mice that have not consumed alcohol, and this effect is thought to be due to alcohol's suppressive effects on the neurons that are involved in place-learning strategy (Sun & Tang, 2018). In general, animals that experienced chronic alcohol consumption had different reactions to object novelty and were unable to effectively perform memory tasks (Meunier, Demeillier, Célérier, & Maurice, 2006). Similar to human memory deficits, mice that consume alcohol often exhibit spatial and working memory deficits that stem from hippocampal damage.

Mice are frequently used in alcohol use research. Mice can be bred to consume larger amounts of alcohol, and they are easily housed and handled (Holleran & Winder, 2017). Oftentimes humans are not viable options for substance abuse research due to the ethical guidelines that would be broken in order to perform experimental research. For voluntary consumption of alcohol, which relates most accurately to drinking habits found in humans, a two-bottle choice paradigm is often used. As illustrated by Meunier, Demeillier, Célérier, & Maurice (2006), the mouse has a water bottle and an alcohol bottle, and the mouse can drink out of either bottle for a set amount of time. While there are studies that utilize non-voluntary alcohol consumption, voluntary consumption is best for comparison to alcohol use in humans. This is due to human alcohol consumption itself being voluntary and the effects of voluntary (vs. involuntary) consumption on the brain. In voluntary drinking, there are more natural relationships between the amount of alcohol

consumption and withdrawal effects (Holleran & Winder, 2017). In sum, mice that are voluntarily consuming alcohol are more likely to have behavior and brain changes more similar to those that occur in humans voluntarily consuming alcohol.

In typical memory research with animals, the most commonly utilized tasks are the Morris water maze and the novel object preference test. The Morris water maze includes placing the mice on a platform floating in water and training them to determine an escape route; the time it takes them to get to the escape location is measured (Wagner, Zhou, & Goodlett, 2014). It is believed that the greater the animal's capacity for memory, the shorter amount of time to escape. Following binge-like alcohol consumption, while still exposed to alcohol, the mice took longer to escape the maze (Wagner *et al.,* 2014). The novel object preference test utilizes three phases in which the animal is habituated to the testing arena, then is introduced to two identical objects, then is introduced to a novel object along with a familiar object (Golub *et al.*, 2015). The animal is expected to spend more time with the novel object than with the familiar object. Decreased time spent with the novel object is often seen in animals that have previously experienced binge-like alcohol consumption (Golub *et al.,* 2015). This task represents an accurate test of hippocampal function (Stackman *et al.*, 2016). It is important to study the impact of alcohol on these memory tasks in non-human animals because it relates to working and spatial memory function due to hippocampal changes from alcohol seen in humans.

Due to the high prevalence rate of binge-like alcohol use in humans, a critical step in treating individuals dependent on this substance is understanding the impact alcohol has on memory. The use of an animal model allows researchers to determine the direct effects of alcohol on memory in a controlled manner. This research is designed to build upon the

knowledge already known about the interactions between alcohol and memory. Many previous animal studies have utilized involuntary high-dose alcohol paradigms to investigate alcohol-induced memory impairment (Golub *et al.,* 2015); however, as detailed in the paragraphs above, passive administration of drugs often results in differing changes in the neurochemistry compared to voluntary (active) administration of the same drugs. Thus, it is not known exactly how voluntary binge-like alcohol consumption and withdrawal impacts memory in tasks such as the novel object recognition, and this research would help to add more evidence in that field. Utilizing this memory task in a two-bottle choice intermittent access paradigm would add to the preclinical literature because there is a lack of information on the specific effects of voluntary binge-like alcohol consumption on memory. Specifically, this research investigated the novel object recognition test for memory function following binge-like alcohol consumption or withdrawal from chronic binge-like alcohol consumption. It was hypothesized that memory function will be significantly worse for mice undergoing acute alcohol withdrawal during the novel object recognition task.

Method

Subjects

Jackson Laboratory (Bar Harbor, ME) provided both male and female mice of the C57BL/6J genetic strain (n=18 males, 18 females). The mice were all at least 8 weeks of age at the outset of the experiment. All mice were housed individually, and food and water were freely available to all mice for the duration of the experiment. On a weekly basis, each mouse's weight was recorded. All animal care was performed in accordance with the

National Institutes of Health Office of Laboratory Animal Welfare and the Institutional Animal Care and Use Committee at Butler University protocol.

Drugs and Chemicals

Sigma (St. Louis, MO) provided 190 proof alcohol for the experiment. Tap water was used to mix concentrations of alcohol solutions.

Drinking Model

For the two-bottle choice intermittent access paradigm, mice had unlimited access to food and a bottle of water at all times. Mice were presented with a second bottle containing increasing concentrations of alcohol (3-20% v/v) for exactly 24 hours on Monday, Wednesday, and Friday during the week. On Tuesday, Thursday, Saturday, and Sunday during the week, the mice only had access to a bottle of tap water. The bottles were weighed at the same time each day to measure consumption. The positions of the two bottles were switched every day that the mice had access to the alcohol bottle and the fluids were refreshed every 4-5 days. This paradigm was repeated throughout each week for 4 weeks total, increasing the concentration of alcohol every week (on Monday).

Novel Object Recognition Task

At the completion of the experiment, the mice were assessed based on the novel object recognition task. The mice in all groups were assessed the same day, but the mice in the alcohol withdrawal group had the bottle containing alcohol removed 24 hours prior to the test, whereas the mice in the alcohol group still had access to alcohol on test day. The amount of time spent exploring the novel object for each mouse was compared across groups.

Statistical Analysis

To account for natural evaporation and leaking, two control bottles were placed in a cage without any mice. The average daily weight difference of those bottles was subtracted from the weight differences of the experimental bottles. The adjusted raw consumption values were transformed in terms of percent alcohol composition, density of alcohol, and each individual mouse's weight. This transformed data was converted to g/kg/day over each concentration's time period. A 2 (male and female; between subjects) x 3 (group: alcohol, alcohol withdrawal, and water; between subjects) x 4 (concentration; within subjects) mixed factor ANOVA was performed to evaluate consumption and preference data. Novel object recognition test data were evaluated through a 3 (alcohol, alcohol withdrawal, and water) x 2 (male and female) between-subjects ANOVA.

Results

Alcohol Consumption

All alcohol bottles were weighed after each consumption day to collect consumption levels for each mouse at each concentration examined. A three-way ANOVA revealed no interaction of concentration and group, sex and group, nor concentration and sex and group. The three-way ANOVA also revealed no main effect of group. There was a significant main effect of concentration, $F(5, 117) = 181.5$, $p<0.0001$, where all mice consumed significantly more alcohol when exposed to higher concentrations (6%, 10%, 20%) compared to the lower concentration (3%). There was also a significant main effect of sex, $F(1, 117) = 204.7$, $p<0.0001$, where the female mice consumed significantly more alcohol than the male mice. There was a significant interaction of concentration and sex, $F(5, 117) = 21.67$, $p<0.0001$, where females drank more alcohol than males at higher concentrations of alcohol. Refer to Table 1 for these results.

Novel Object Recognition Task

The novel object recognition task results were examined using a 3 x 2 between subjects ANOVA. There was no significant main effect of group for the quadrant containing the novel object, nor the quadrant with the familiar object. See Table 2 and Table 3 for these results. The mice did not spend a significantly different amount of time with the novel object, regardless of whether they were control, going through withdrawal, or still consuming alcohol at the time of the task. There was also no significant interaction of sex and group for the quadrant containing the novel object, nor the quadrant with the familiar object. The sex of the mice did not change the proportion of time spent in each object's quadrant.

Discussion

The current study aimed to determine the effects of binge-like alcohol consumption on working memory in C57BL/6J mice in order to further understand the relationship in human models. C57BL/6J mice were used because of their genetic predisposition for higher alcohol consumption than other strains of mice (Rhodes *et al.*, 2005). A two-bottle choice intermittent access paradigm was utilized because it has been shown to best resemble human binge drinking behavior (Meunier, Demeillier, Célérier, & Maurice, 2006). The memory task utilized in this study was the novel object recognition task, which has been used in previous studies to test the working memory of mice (Stackman *et al.*, 2016). Results from the novel object recognition task showed no significant group differences, regardless of whether the mice were undergoing alcohol withdrawal or still had access to alcohol at the time of the test.

Alcohol consumption levels were examined to determine if there were concentration, sex, or group differences in preference for alcohol. There was a significant main effect of sex on alcohol consumption, where female mice drank more than male mice (Figure 1). This is consistent with the literature, where female mice consume more alcohol per body weight compared to male mice (Yoneyama *et. al*, 2008). This finding suggests that consumption data varies depending on gender and is not generalizable across genders. There was also a significant main effect of concentration on alcohol consumption, where more alcohol was consumed at higher concentrations (6%, 10%, 20%) compared to lower concentrations (3%), which is again consistent with findings from previous studies (Klein *et al.*, 2004). This finding demonstrates that mice are more likely to consume higher amounts of alcohol if there is a higher concentration of alcohol in each drink. Along with the two significant main effects, there was also a significant interaction of concentration and sex in which the female mice consumed more alcohol than the male mice at higher concentrations of alcohol. At lower concentrations, the male mice consumed more alcohol per body weight, but that trend flipped when the alcohol was presented at higher concentrations. In the literature, this trend is consistently reiterated (Klein*et al.*, 2004). There was no significant interaction of concentration and group, which is consistent with expectations, as the two alcohol consuming groups had no differences in the consumption phase of the experiment. There was no significant interaction of sex and group, which fits expectations because each group had the same number of male and female mice. There was also no significant interaction of concentration and sex and group, which was expected because the groups were consistent in sex and concentration.

The data for the novel object recognition task were examined to determine differences across groups for the familiar object and the novel object. There was no significant main effect of group; the mice spent the same amount of time in the quadrant with the familiar object as they did in the quadrant with the novel object, regardless of the condition they were in. Refer to Figure 2 and Figure 3 for these results. Whether the mice never had access to alcohol (control group), were still consuming alcohol, or were going through alcohol withdrawal did not have an impact on the proportion of time spent in each object's quadrant. This does not mean that the working memory of the mice was not impaired, as even the mice in the control group did not differentiate their time based on whether the object was familiar or novel. This is not consistent with findings in the literature; other studies have found that mice who do not have a memory impairment will spend more time with the novel object than the familiar object (Golub *et al.,*2015). These results show that the novel object recognition task did not accurately test the mice's working memories, despite previous studies showing that it does. This suggests that further research needs to be performed in order to determine the memory-impairing effects of alcohol and alcohol withdrawal. Along with this data, there was also no significant interaction of sex and group, which displays that the sex of the mice did not impact the amount of time spent in each object's quadrant when group is also taken into consideration. This result shows that there were no further factors involved in the amount of time spent in the different quadrants. These additional results reiterate the importance of further research in order to fully understand the memory-impairing effects of binge-like alcohol consumption and alcohol withdrawal. This information needs to be extrapolated from animal models first in order to

effectively assume the memory impairments that would occur in human models. This would allow for the best design of similar human studies.

The limitations of this study could give explanations as to why there were not significant data found for the novel object recognition task. First, it should be noted that this was the first run using the novel object recognition task in this laboratory. Although we based our methods off of previous literature, we cannot rule out the possibility that this new task resulted in inconsistencies. Second, this study utilized a voluntary drinking paradigm, which does not allow for data collection of when each mouse last consumed alcohol. It is not known when the mice who were in the alcohol withdrawal group consumed alcohol last, and thus it is impossible to determine if they were going through withdrawal at the exact time of the task. Along with that, it is not known if the mice in the alcohol group had drunk alcohol close to the time of the task or if they had abstained prior to testing. Knowing the specific blood alcohol levels of each mouse during the time of the novel object recognition task would be beneficial in determining the exact implications of the results. Another limitation in this study is the amount of time that the mice were tested during the novel object recognition task. The mice were tested for five minutes, which may not have been enough time for them to fully become familiar with the objects for the habituation phase, nor long enough for the time spent in each quadrant during the test phase to be characteristic of their memory.

In order to combat these limitations, further research needs to be performed, in which physiological withdrawal is tested before the novel object recognition task is executed. This would ensure that the mice in the alcohol withdrawal group were going

through significant withdrawal, making them substantially different from the alcohol group. Further research could also involve other memory tasks besides the novel object recognition task. Due to nonsignificant data for the novel object recognition task, it could be beneficial for a different working memory test, such as a Morris water maze, to be used in its place. This would allow for a different evaluation of working memory, which could lead to different results due to the variation in the operational definitions of working memory function.

In conclusion, although the current study did not obtain the statistically significant differences expected, several limitations including the voluntary alcohol consumption paradigm used likely contributed to this. Future studies should examine to what extent physical and psychological dependence occurs following voluntary binge-like alcohol consumption in mice. Results from rodent models such as the one utilized in the current study can provide valuable information about human memory following alcohol consumption due to the similarities in the drinking paradigm and the neuroanatomical structures that are similar in both mice and humans. This research is important because of the prevalence of binge-drinking in the population (Alcohol Facts and Statistics, 2015). Obtaining a better understanding of the mechanisms underlying alcohol-induced memory deficits using preclinical models is also a necessary step toward later development of novel pharmacological treatments to aid humans suffering from memory loss due to chronic, high-dose alcohol consumption.

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Table 1

ANOVA for alcohol consumption of C5BL/6J mice. Significant results of P<0.0001 found for main effects of concentration and sex. Significant results of P<0.0001 found for interactions of concentration and sex.

	SS	**DF**	**MS**	**F (DFn, DFd)**	**P value**
Concentration	18597	5	3719	F (5, 117) = 181.5	P<0.0001
Sex	4195	1	4195	F (1, 117) = 204.7	P<0.0001
Group	16.70	1	16.70	F (1, 117) = 0.8150	P=0.3685
Concentration x Sex	2220	5	444.1	F (5, 117) = 21.67	P<0.0001
Concentration x Group	23.99	5	4.798	F (5, 117) = 0.2341	P=0.9468
Sex x Group	66.21	1	66.21	F (1, 117) = 3.231	P=0.0748
Concentration x Sex x Group	124.9	5	24.97	F (5. 117) = 1.219	P=0.3046
Residual	2397	117	20.49		

Table 2

ANOVA for amount of time spent in zone 1, the zone with the novel object. No significant results found.

Treatment	$F(2,30) = 0.4186$	$p = 0.662$
Sex	$F(1,30) = 1.9960$	$p = 0.171$
Interaction	$F(2,30) = 2.7912$	$p = 0.077$

Table 3

ANOVA for amount of time spent in zone 3, the zone with the familiar object. No significant results found.

Treatment	F(2,30) = 0.4186	p = 0.662
Sex	F(1.30) = 1.9660	p = 0.171
Interaction	F(2,30) = 2.7912	p = 0.077

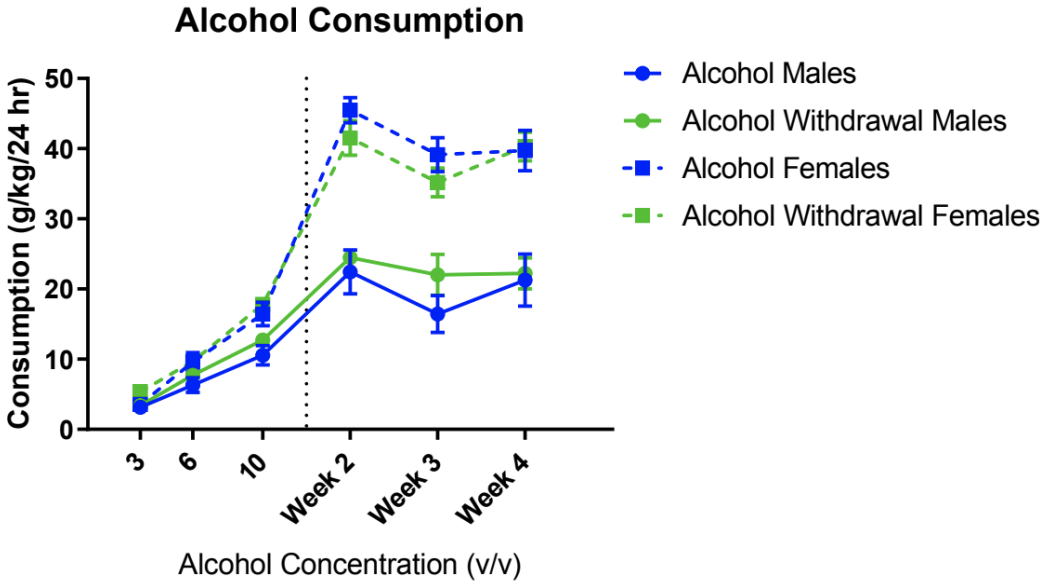

Figure 1: Average alcohol consumption for male and female C5BL/6J mice. There was a significant increase in alcohol consumption at higher levels of alcohol, and in female mice.

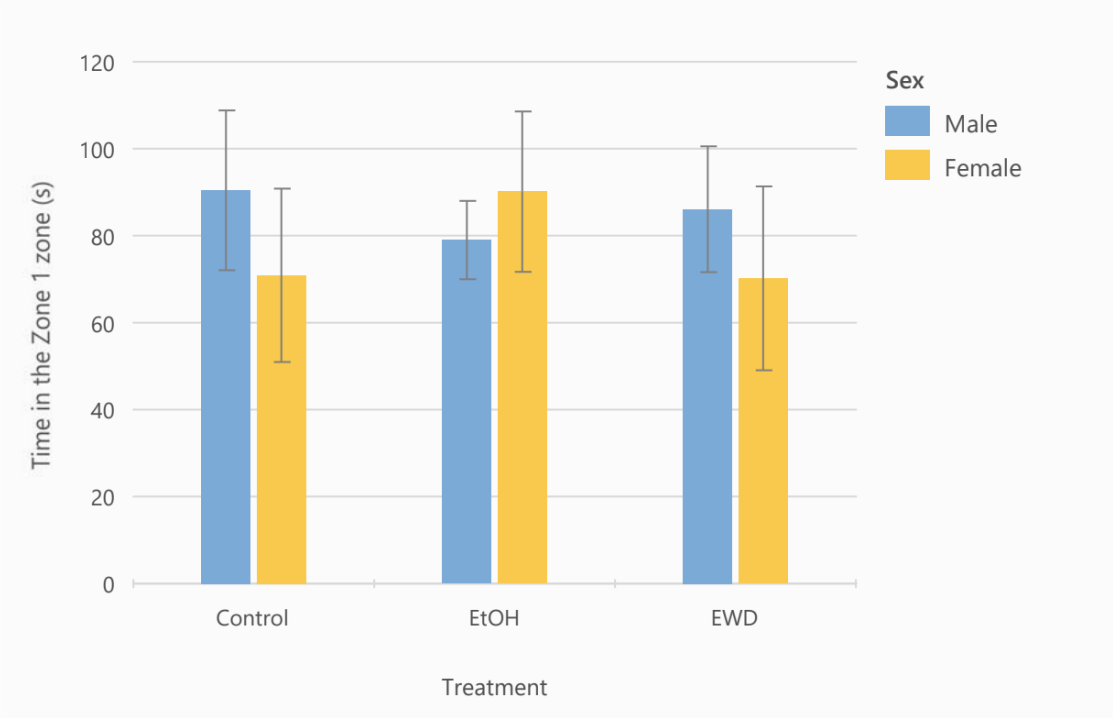

Figure 2: Amount of time spent in zone 1, the zone with the novel object, for male and female C5BL/6J mice. There were no significant differences between sex nor group.

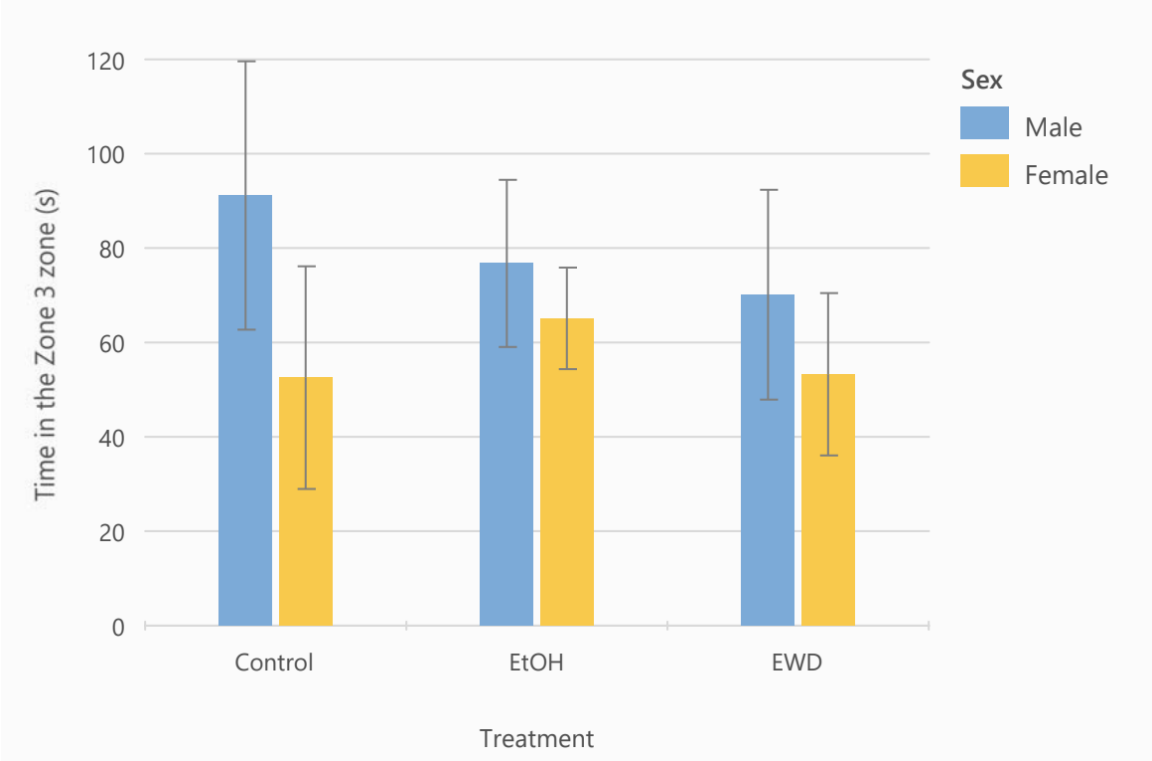

Figure 3: Amount of time spent in zone 3, the zone with the familiar object, for male and female C5BL/6J mice. There were no significant differences between sex nor group.