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Hanna J. Peterson
University of Dayton

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Abstract

The purpose of this study was to understand relapse behavior through a pre-clinical rodent model of relapse which models the important aspects of the human addiction and relapse condition, called the alcohol deprivation effect (ADE) model. It has been found to model alcohol addiction and relapse in rats and can therefore allow for further understanding of relapse behavior as well as allow for testing of the effects of various variables like stress or therapeutic drugs on relapse behavior. The model gives rats baseline access to ethanol and then allows them access to only water, called deprivation periods, and then gives them re-access to the ethanol, called re-access periods or ADE periods. There are repeated cycles of deprivation and re-access periods which has been shown to lead to relapse-like drinking behaviors upon re-access to the ethanol characterized by drinking significantly more ethanol (g/kg) in the re-access periods when compared to the rats' baseline ethanol intake. This investigation looked at the effectiveness of the ADE model in adolescent male, adult male, and adult female Long Evans rats by allowing the rats access to ethanol for a baseline period and then having three repeated cycles of deprivation and re-access periods. It was found that only the adult female Long Evans rats exhibited a significant ADE. These results differ from previously found patterns in adult male Long Evans rats which have been shown in other studies to display a robust ADE.

Dedication or Acknowledgements

I would like to thank my advisor, Dr. Tracy Butler, for her support and assistance throughout this project. I would also like to thank the University of Dayton Honors Program for funding this project.

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Introduction

In the United States, about 14% of the adult population has developed an alcohol dependence at some point in their life (Kessler et al 1994). Alcoholism is one of the most common forms of addiction but there are still not many effective treatments for people who suffer from this disease (Rasmussen et al 2014). If alcoholism goes untreated, the addict can be more likely to develop various long-term problems in the brain and body including, but not limited to, brain disease, liver disease, and nutritional deficits (Sutherland et al 2014). Alcoholism affects both males and females but male and female behavior and drinking patterns can differ. Males have been found to have a more continuous pattern of drinking and have less periods of abstinence from alcohol than females (Olenick and Chalmers 1991).

People may relapse for many reasons. Many people who use alcohol feel good when they are drinking because of the changes alcohol causes in the brain. People also drink alcohol under similar environmental conditions each time which creates a learned association between their environment and their craving for alcohol. Therefore, when people encounter these learned conditions even after they have stopped drinking, they may crave alcohol again and relapse (O'Brien et al 1998). Another reason for relapse can be stress. Stress can produce a negative affect which then leads the person to remember the good feeling they get from alcohol. This can lead to relapse to cope with the stress by blocking the stressful feeling with the good feeling the drug produces (Baker et al 2004). Current treatments for alcohol dependence include cognitive behavioral therapy, psychosocial therapy, and some FDA approved drugs including disulfiram, acamprosate, and naltrexone. Drugs (acamprosate or naltrexone) and therapy (cognitive behavioral) are often used together because the combination of the two often leads to better treatment results than if each was used alone (Feeney et al 2006). However, psychosocial therapy is associated with relapse rates of 40-70% and different drugs have varying effectiveness (Finney et al 1996). There is clearly still a need for more treatments that are not

associated with high relapse rates.

There are many differences between the male and female brain. Sex differences may be due to many biological factors, including levels of sex hormones estrogen, testosterone, and progesterone, the size of different brain structures, and differences at the level of synaptic patterns (Ciofi et al 2006). Studies have also noted behavioral differences in males and females relating to alcohol dependence and withdrawal, specifically. One study discovered that women had a lower incidence of withdrawal symptoms than men (Deshmukh et al 2003). Another study found that women sought treatment for their alcohol dependence earlier than men did; women sought treatment at 3.8 years after the onset of alcohol dependence but it took men 6.4 years after the onset of alcohol dependence to seek treatment (Schuckit et al 1998). These sex differences in alcohol dependence-related behaviors can have implications because it can lead to a better understanding of the neurobiology of alcohol dependence and addiction in general. This can then lead to better treatments for these conditions.

The National Longitudinal Alcohol Epidemiological Survey states that people who start to use alcohol before they are 14 years old have a rate of lifetime alcohol dependence that is four times higher than people who don't start to use alcohol until they are 20 years of age or older (Grant and Dawson 1997). There is evidence indicating that the earlier a person begins to drink alcohol, the higher their risk is of developing more alcohol and drug problems later in life (Grant et al 2006). However, not many adolescents become addicted to drugs or alcohol although many experiment with them. More research is needed into what causes and associations are present in adolescents who become addicted and adolescents who do not (Gladwin et al 2011).

Animal models for human conditions can be very useful when the model replicates an important part of the human condition that needs to be further studied. The animal must only exhibit the important characteristics of the many aspects of the human disease of alcoholism (McBride et al 2014). However, no animal model completely replicates or reproduces the human condition but studying animals still allows for an in depth study of the condition of interest. The animal brain, and rat brain more specifically, is a good

model for the human brain. Therefore, rats can be used to help us understand how brain chemistry leads to addiction or relapse and understand different causes and effects of addiction. Rodent models can also help develop new treatments for certain conditions and test those treatments to make sure they are safe before using them on humans.

The alcohol deprivation effect model (ADE) is a model used in laboratory animals that models relapse-like behavior. In the model, rats undergo long-term alcohol (ethanol) drinking with repeated cycles of ethanol deprivation. Rats then develop compulsive drinking behavior called the alcohol deprivation effect. The compulsive behavior and the ADE can be seen when, allowed re-access to ethanol after deprivation, rats drink high levels of ethanol despite aversive taste (a substance rats do not find palatable is added to the ethanol and they still drink it), they drink higher concentrations of alcohol compared to baseline, and they drink significantly more ethanol compared to baseline (Pildain et al 2013). Current research studies have varying lengths of time for the alcohol drinking phases and deprivation phases as well as varying lengths for the full study. Most studies are many months long, with some even being a year or longer, to ensure that a robust ADE is observed. However, in one study by Sinclair and Tiihonen (1988), a significant ADE in Long Evans rats, characterized by significantly more drinking over baseline during the ADE period, was demonstrated in only 16 days of baseline drinking followed by seven days of deprivation. This study was modeled after Sinclair and Tiihonen's study because their study used male Long Evans rats and used the same schedule of alcohol access and deprivation.

Sex differences have been found in the ADE model. In one study of the ADE model using alcohol-preferring (P) and high alcohol drinking (HAD) rats, the female rats drank the same amount, or more, at baseline than the male rats but as the rats were put through more cycles of ethanol deprivation and re-access to ethanol, the males showed a more robust ADE. This progressive increase in the magnitude of the ADE was not seen in females. This sex difference in ethanol consumption in rats may partially model the higher alcohol abuse and dependence in human males when compared to females (Rosenwasser et al 2014). In another study using Long Evans rats in an intermittent ethanol access model, similar differences in ethanol consumption patterns between males

and females were found. The male rats drank more from week 1 to week 5 but the females did not have this same pattern. The females reduced their drinking from week one to week five, although it was not a significant decrease. This increase in drinking in male rats and not female rats may be because the female rats already drink a lot at baseline so they cannot increase their ethanol drinking much more. However, the males drink less at baseline so they have room to increase their drinking compared to baseline (Morales et al 2015). This may indicate that the ADE model is better at modeling the drinking behavior in male rats rather than female rats but further investigation is still needed.

In one study that used the ADE model in adolescent Wistar rats, it was demonstrated that adolescent rats did not show an ADE with respect to ethanol consumption meaning that they did not significantly increase their ethanol intake after the deprivation period. However, they did show an increase in preference for ethanol over water after the deprivation period. In contrast to the adolescent rats in this study, the adult rats showed an ADE characterized by significantly increasing their ethanol intake following deprivation and significantly increasing their preference for ethanol following deprivation (Garcia-Burgos et al 2009).

This project investigated the ADE model to see if a robust ADE could be seen in the same amount of time as it was seen in the study done by Sinclair & Tiihonen (1988). It also investigated the ADE model to see if it works in adult female and adolescent male Long Evans rats as well as it does in adult male Long Evans rats.

Methods

Eight adult male and eight adult female Long Evans rats were received in the lab at post-natal day 56 and eight adolescent male Long Evans rats were received in the lab at post-natal day 21. All rats had an acclimation period of three full days after they were received and during this period, they were pair housed. The rats were kept on a 7am to 7pm light-dark schedule and received *ad libitum* access to food and water throughout the

acclimation and experiment. The cages were changed once per week throughout the duration of the experiment.

The adult male and adult female rats began the baseline period of ethanol drinking at post-natal day 60 and were separated to be single housed at this point. The adolescent rats began the baseline period of ethanol drinking at post-natal day 25 and were also separated to be single housed before the start of baseline drinking. The baseline drinking period was a total of 16 days and during this period, the rats received constant access to 10% ethanol solution. The position of the ethanol solution and water was switched every day and the rats were weighed every day. The amount of ethanol and the amount of water consumed by the rats was measured in grams each day 30 minutes after the ethanol was put on the cage and 24 hours after it was put on the cage. After 16 days, the ethanol solution was taken off and the first deprivation period began in which the ethanol was taken away from the rats for seven days. After the first deprivation period, the first re-access period began. During the re-access periods, the rats were given the 10% ethanol solution again for another 7 days in which the position of the ethanol and water bottles were switched every day and the rats were weighed every day. The ethanol and water consumption was measured in grams at 30 minutes after the bottles were put on the cages each day and again at 24 hours after the bottles were put on the cages. This cycle of seven days of deprivation and seven days of re-access was done three times total. At the end of the last re-access period, the rats were sacrificed. The grams of ethanol consumed per kilogram of body weight was calculated for each rat on each day, at both the 30 minute and 24-hour time points. A paired, one-tailed t-test was used to compare each rat's ethanol intake during the first day after deprivation to its intake during the last seven days of baseline drinking to determine if an ADE was present for each of the groups of rats.

Results

The data was assessed to determine whether an ADE was observed in any of the groups of rats. An ADE is said to have occurred if the rats drank statistically significantly more ethanol (g/kg) at the 24-hour time point during the first day of the re-access period when

compared to the average of the last seven days of ethanol consumption (g/kg) of the baseline period. A paired, one-tailed t-test was used to determine statistical significance within groups.

The female Long Evans rats were the only group to show a significant ADE at the 24-hour time point. The female ethanol intake (g/kg) during the first day of the first re-access period was significantly more when compared to baseline ethanol intake, $t(6) = 4.397$, $p= 0.0023$. The ethanol intake (g/kg) during the first day of the second re-access period was also significantly more when compared to baseline ethanol intake, $t(6) = 2.582$, $p=0.0208$. The ethanol intake was 3.051 g/kg (+/- 0.871 g/kg) during the first day of the third re-access period and was not significantly more when compared to baseline ethanol intake (2.539 g/kg +/- 0.513 g/kg), $t(6)= 0.5086$, $p= 0.3146$. These results can be seen in Figure 1. The adult male Long Evans rats had a relatively constant ethanol intake (g/kg) throughout the study. There was no statistically significant difference between the baseline ethanol intake and any of the re-access periods. The adult male rats slightly decreased their ethanol intake (g/kg) on the first day of the first re-access period when compared to baseline. At baseline, the average ethanol intake was 2.555 g/kg (+/- 0.460 g/kg). On the first day of the first re-access period, the adult male rats decreased their ethanol intake to 2.253 g/kg (+/- 0.591 g/kg). On the first day of the second re-access period, the adult male rats still had ethanol intake lower than baseline at 2.530 g/kg (+/- 0.838 g/kg). On the first day of the third re-access period, the adult male rats slightly increased their ethanol intake compared to baseline and it was 2.947 g/kg (+/- 0.659 g/kg). However, this increase was not enough to be significant. These results can be seen in Figure 2. The adolescent male rats had a lower ethanol intake at every re-access period when compared to baseline but this decrease from baseline ethanol intake was not statistically significant at any of the re-access periods. Their average baseline ethanol intake was 3.877 g/kg (+/- 1.047 g/kg). On the first day of the first re-access period, the adolescent male rats drank 2.944 g/kg (+/- 0.683 g/kg). On the first day of the second re-access period, the adolescent male rats drank 2.069 g/kg (+/- 0.688 g/kg). On the first day of the third re-access period, the adolescent male rats drank 2.240 g/kg (+/- 0.683 g/kg). These results can be seen in Figure 3. The total results can be seen in Figure 4.

Figure 1.

*p<0.05 vs. baseline

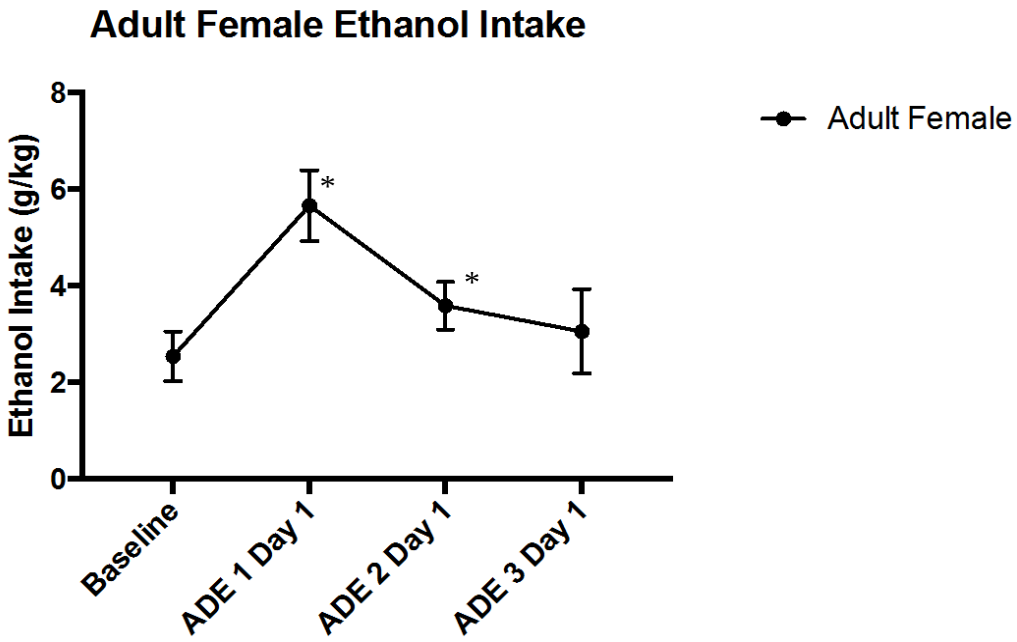


Figure 2.

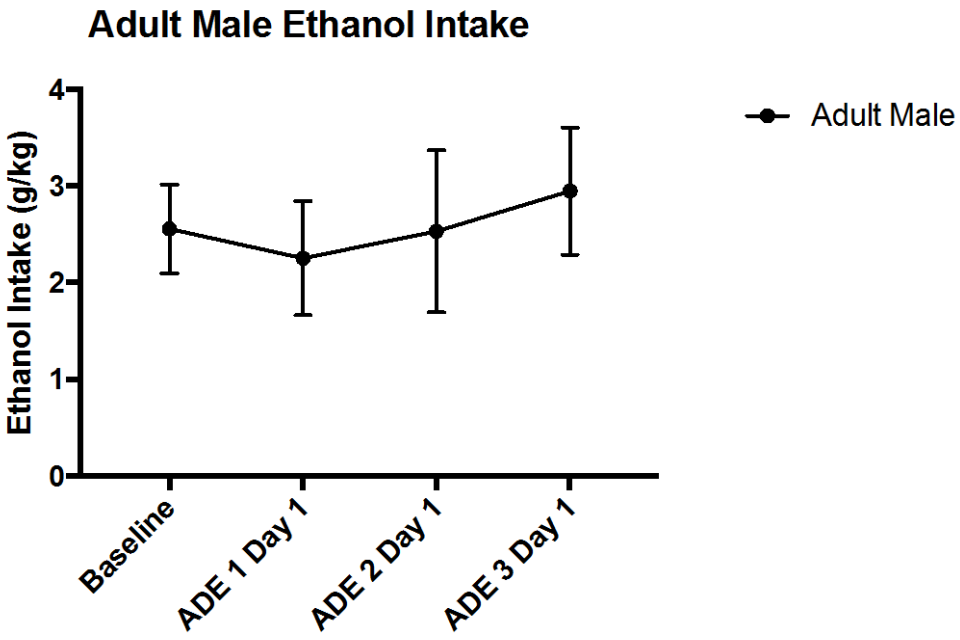


Figure 3.

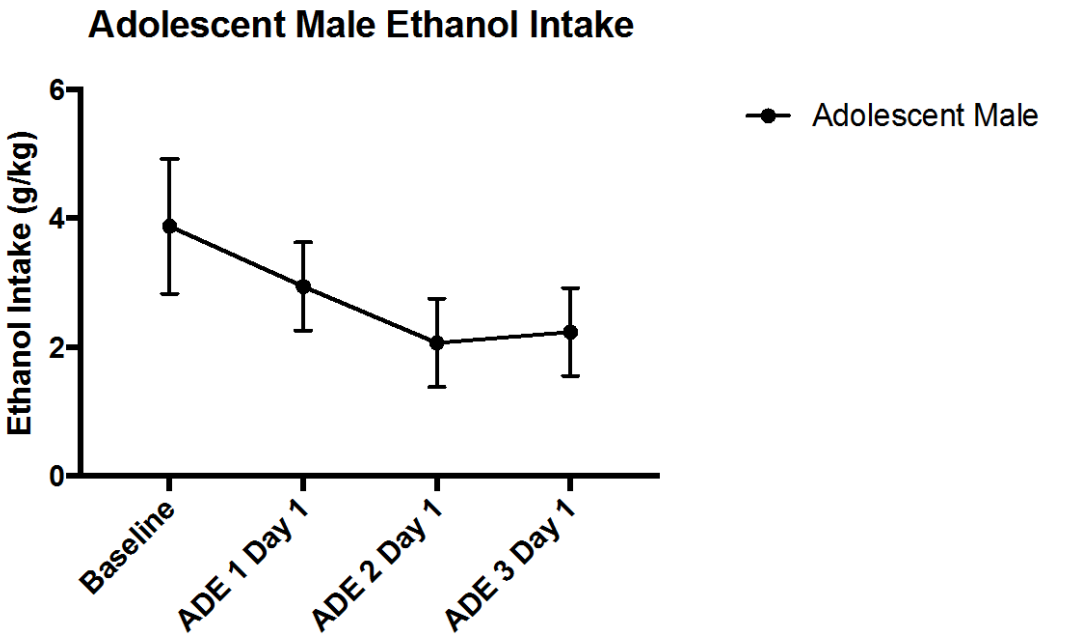
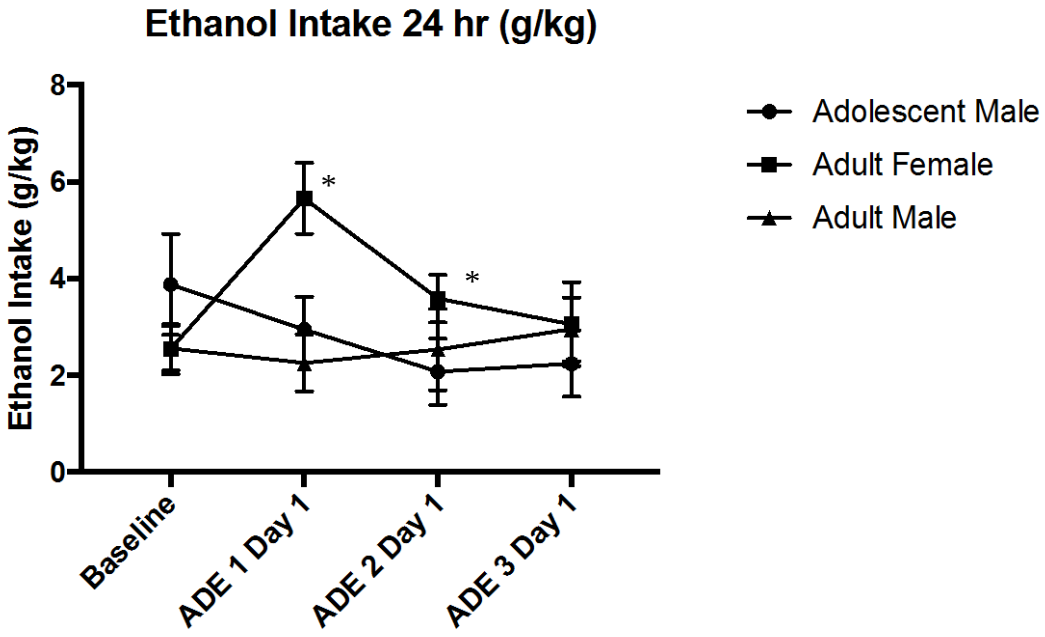


Figure 4.

*p<0.05 vs. baseline



Discussion

The results indicate that the only group to show an alcohol deprivation effect (ADE) was the adult females because they had a statistically significant increase in ethanol intake during the first and second re-access periods but not the third. Therefore, the adult females showed an ADE in the first and second re-access periods but not the third. The adolescent males decreased their ethanol intake and the adult males also decreased their ethanol intake except for a slight increase in the third re-access period that was not statistically significant. Therefore, neither the adolescent males nor adult males showed an ADE since an ADE requires a statistically significant increase in the ethanol intake during the first day of each re-access period when compared to the last week of baseline ethanol consumption.

In one study that used the ADE model in adult female alcohol-preferring (P) rats showed a robust ADE in the rats after six weeks of baseline access to ethanol and then two weeks of ethanol deprivation, two weeks of ethanol re-access, and two weeks of ethanol deprivation again (Engleman et al 2011). The Engleman study is different from this study because the rats had a longer baseline access to ethanol and double the time (two weeks) of deprivation and re-access periods. However, the Engleman study also used less cycles of deprivation and re-access which shows that female P rats display an ADE after just one cycle of baseline ethanol access, ethanol deprivation, and ethanol re-access. Similar results were found in this study. The female Long Evans rats displayed a robust ADE after just one cycle of baseline ethanol access, ethanol deprivation, and ethanol re-access. However, this study had a shorter baseline period and shorter deprivation and re-access periods, making this version of the ADE model more efficient if used in female Long Evans rats.

In the study done by Sinclair and Tiihonen (1988), adult male Long Evans rats exhibited a robust ADE in the same schedule of ethanol access as this study. One possible reason that the males did not show an ADE here is that the short schedule of baseline (16 days), deprivation (7 days each cycle), and re-access (7 days each cycle) was not long enough

for this specific adult male rat cohort to establish an ethanol dependence and therefore the male rats did not show relapse behaviors. Furthermore, this does not explain why the female rats did exhibit relapse behaviors since they were tested using the same schedule of ethanol access. Therefore, it is questionable whether the ethanol access schedule is the reason that the females showed an ADE while the males did not. These results may not be representative of typical relapse behaviors in rats due to a small sample size of only eight rats in each group. Also, differences in the male and female brain may account for some of the variation between their addictive and relapse behaviors that were demonstrated in this study.

In one study using the ADE model in adolescent male Wistar rats, it was found that the adolescent rats did not show an ADE and they progressively decreased their ethanol intake throughout the study (Garcia-Burgos et al 2009). Those results were mirrored in this study since the adolescent male Long Evans rats also progressively decreased their ethanol intake throughout the study and did not display an ADE.

In one study of the ADE model using both male and female alcohol-preferring (P) and high alcohol drinking (HAD) rats, the female rats drank the same amount, or more, at baseline than the male rats but as the rats were put through more cycles of ethanol deprivation and re-access to ethanol, the males showed a more robust ADE. This progressive increase in the magnitude of the ADE was not seen in females (Rosenwasser et al 2014). In another study using Long Evans rats in an intermittent ethanol access model, similar differences in ethanol consumption patterns between males and females were found. The male rats increased their ethanol intake from week one to week five but the females did not have this same pattern. The females reduced their drinking from week one to week five, although it was not a significant decrease. This increase in drinking in male rats and not female rats may be because the female rats already drink a lot at baseline so they cannot increase their ethanol drinking much more. However, the males drink less at baseline so they have room to increase their drinking compared to baseline (Morales et al 2015). The results of these two studies are different from the results of this study. In this study, females showed a robust ADE whereas males did not. However, after

the first re-access period, the females' ethanol intake did decrease. During the second re-access period the female ADE was of a lesser magnitude than the ADE shown during the first re-access period and then the females decreased their ethanol intake even further during the third re-access period when they did not show a significant ADE. In this study, the males did not increase their drinking and never showed a significant ADE. Their ethanol consumption was relatively constant throughout the study.

There are some limitations of this study. One is that the amount of ethanol consumed in grams was measured by using a scale. This required the bottles to be taken on and off the cages to weigh them and in the process, ethanol could sometimes leak out of the sipper and therefore falsely inflate the amount of ethanol the rats were consuming. Also, one of the female rats chewed through either her ethanol or water bottle every day and her data therefore had to be thrown out because there was no way to know how much ethanol or water she consumed and how much just leaked from her bottle. In contrast to this method of measuring the amount of ethanol consumption, one study used high-precision sensors that were attached to the bottles (Pildain et al 2013). This seems to obtain a more accurate measurement for how much ethanol the rats are consuming. That study also used irregular drinking and deprivation phases in which these phases were different lengths of time so no behavioral patterns were developed in the rats (Pildain et al 2013). This study did not use that mechanism and may have allowed the rats to develop behavioral patterns since the deprivation and access phases were the same length each time. However, these re-access and deprivation periods were only one week long and there were only three cycles so this may not have been a long enough time period for the rats to learn the schedule and adapt to it.

Conclusion

This study investigated the alcohol deprivation effect model using 16 days of baseline drinking and three cycles of seven days of ethanol deprivation and seven days of ethanol

re-access. It was found that the female Long Evans rats displayed a statistically significant ADE at the 24-hour time point for the first and second re-access periods but not the third. Both the adult and adolescent male Long Evans rats did not show a significant ADE. The conflicting results between this study and the study done by Sinclair and Tiihonen in 1988 which used the same ethanol access schedule, shows that the ADE model using this schedule of ethanol access requires more investigation before it can be said that it effectively and accurately models ethanol dependence and relapse. More investigation should also be done on the difference between the male and female brain and behavior because males and females consistently show differing ethanol dependence and relapse behaviors.

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