

Effects of High-Fat Diet and Ethanol on Cognition and Behavior in Mice

Christopher Daniell¹, Shelby Bartlett², & Jessica A. Siegel³

The college lifestyle is associated with both a high-fat diet and alcohol consumption. While the isolated effects of diet and alcohol consumption on cognition and behavior are well studied, there is little known about the effect of both together. In this study, we created a mouse model of the college lifestyle in order to better understand the potential combined effects of a high-fat diet and alcohol consumption on cognition and behavior. Adult C57BL/6J female mice were exposed to either regular food or high-fat food and daily injections of either saline or ethanol. The Porsolt forced swim test (FST), open field, and novel object recognition test measured depression-like behavior, anxiety-like behavior and locomotion, and memory, respectively. In the Porsolt forced swim test, ethanol-exposed mice trended towards spending more time immobile indicating increased depressive-like behavior. Ethanol exposure also decreased distance moved by the mice in the center of the open field arena, suggesting increased anxiety-like behavior. The high-fat diet had no effect in the FST, but, it did increase distance moved in the open field. There were no effects of diet or ethanol exposure on memory in the novel object recognition test. In conclusion, it appears that ethanol can increase the risk for depression-like behavior and anxiety-like behavior in female mice, but contrary to our original hypothesis, there were no effects or interactions of high-fat diet on depressive behavior or cognition in the current study. The findings regarding ethanol's effects correspond to the current high rates of student depression and anxiety seen on college campuses.

INTRODUCTION

Modern day society has appropriately emphasized the importance of college student's health as it sets the groundwork for a future independent adult lifestyle. High-fat diets and alcohol, commonly available to college students, are known to have negative consequences on student health. Moreover, previous studies have linked Major Depressive Disorder and alcohol abuse in college students, suggesting students tend to drink to cope with preexisting depressive symptoms (Deykin et al. 1987). College students frequently experience episodes of depression and anxiety. A nation-wide survey of college students found that 30% of participants reported feeling "so depressed that it was difficult to function" at some point in the last year (NIMH 2009). Another study found that almost a third of college students report that stress and anxiety have negatively affected their academics (AMCHA 2007). A high-fat diet is also quite common among college students; around 25% of students gain at least five pounds during their first year at college (Anderson et al. 2003). While prior studies reveal the isolated effects of high-fat diet and alcohol consumption in college-age students, few studies have examined the combined effects of these lifestyle choices on behavior and cognition.

Previous research has shown that chronic ethanol exposure can increase depressive-like behavior (Ribeiro-

Carvalho et al. 2011) and impair cognition in mice (Beracochea and Jaffard 1985), an effect that seems to be age dependent. Long-term exposure to ethanol can decrease gamma-Aminobutyric acid receptor A (GABA_A) activity and this potentially contributes to increased depressive-like and anxiety-like behavior (Devaud and Alele 2004; Hirani et al. 2002; Olsen and Spigelman 2012; Rasmussen et al. 2001). The cognitive effects of ethanol can be partially explained by its antagonistic effect on the N-methyl-D-aspartate (NMDA) glutamate receptors (Moykkynen and Korpi 2012). Ethanol exposure impairs spatial memory in the Morris water maze in adolescent rats (Markwiese et al. 1998) and hippocampal long-term potentiation to a greater degree in adolescent rodents compared to adults rodents (Pyapali et al. 1999; Swartzwelder et al. 1995). These findings suggest that adolescents are more sensitive to the cognitive effects of ethanol than adults, and thus, early college-aged students may be at a high risk for ethanol-induced cognitive impairments (Moykkynen and Korpi 2012).

Previous research has demonstrated that a high-fat diet can increase depression-like behaviors and impair memory in rodents. For example, a high-fat diet increases depressive-like behavior of rats in the Porsolt forced swim test (FST) (Abildgaard et al. 2011). A high fat diet has also been shown to impair memory by altering hippocampal function (Francis and Stevenson 2011; Valladolid-Acebes et al. 2011).

Extensive literature has shown that psychologists are appropriately emphasizing the effects of poor diet and alcohol abuse on mental health. Research on rodents demonstrates that a high-fat diet and ethanol exposure can impair cognition and increase depression-like and anxiety-like behaviors. There are currently few data, however, on the potential combined effects of a high-fat diet and ethanol exposure on cognition and behavior. Therefore, the aim of this study was to explore both the isolated and combined effects of a high-fat diet and ethanol exposure on

¹ Department of Psychology, Sewanee: The University of the South; danielcm0@sewanee.edu; 735 University Ave, Sewanee, TN 37383

² Department of Psychology, Sewanee: The University of the South, now at The Pingry School, Martinsville, New Jersey. sbartlet@pingry.org; 131 Martinsville Road. Basking Ridge, NJ. 07920

³ Department of Psychology, Sewanee: The University of the South; jasiegel@sewanee.edu; 735 University Ave, Sewanee, TN 37383;

*To whom correspondence should be addressed: danielcm0@sewanee.edu

anxiety-like behavior, depression-like behavior, and cognition using a mouse model. Based on previous literature, we hypothesized that ethanol exposure would increase anxiety-like behavior in the open field test and that both high-fat diet and ethanol would increase depressive-like behavior in the FST and impair memory in the novel object recognition test. Moreover, we hypothesized that the effects would be most severe in the mice receiving a combination of both a high-fat diet and ethanol exposure.

METHODS

Subjects & Treatment

This study used twenty singly housed C57BL/6J adult female mice. Mice were ordered from The Jackson Laboratory (Bar Harbor, ME) at 8 weeks of age and handled 5 days a week for 4 weeks. The mice were housed in a ventilated room with a 12 hour light/dark cycle (lights on at 06:00). The mice were randomly assigned to four groups (5 mice per group): the control group received regular food and saline injections (Reg/Sal), the ethanol-only group received regular food and ethanol injections (Reg/Eth), the high-fat-only group received high-fat food and saline injections (HF/Sal), and the experimental group received high-fat food and ethanol injections (HF/Eth). The mice underwent treatment conditions for four consecutive weeks and were weighed daily. The mice were administered inter-peritoneal injections of ethanol or saline once a day for five days a week, for a total of four weeks. For the first two weeks of treatment, mice receiving ethanol were administered injections of 1.0 g/kg ethanol (20% ethanol solution). The dose of ethanol was increased to 1.5 g/kg (20% ethanol solution) for the final two weeks of treatment to model increased ethanol consumption, which is often observed in the college population. An increase in the dose also prevents adaptations due to tolerance to the lower dose of ethanol. The regular food was PicoLab Rodent Diet 20, #5053 (PMI Nutrition International, St. Louis MO, USA), and the high-fat food was PicoLab Mouse Diet 20, #5062 with 9.1% fat. Food and water were provided *ad libitum*. All manipulations and procedures were approved by Sewanee: The University of the South's Institutional Animal Care and Use Committee.

Open field test

The open field test occurred during week two of treatment to measure anxiety-like behavior and locomotion. Mice were placed in an isolated, clear Plexiglas arena (40 x 40 cm) for one 10-minute trial. Locomotor activity (total distance moved) and the distance moved and percent time spent in the center (defined as the inner 20 x 20 cm area of the arena) versus the periphery of the arena were measured using Anymaze video tracking software (Stoelting; Wood Dale, IL). The distance moved and percent time in center versus the periphery of the arena are standard measures of anxiety-like behavior in mice; the greater distance moved and percent time the animal spends in the center, the less anxious the animal (Clement et al. 2002).

Forced swim test (FST)

The FST occurred during week three of treatment (ethanol injections increased to 1.5g/kg) to measure depressive-like behaviors (Matsuo et al. 2010). Mice were placed in 1000 mL beaker filled with 700 mL of room temperature water for a 5-minute trial. Researchers recorded the time spent floating during the trial, with floating defined as all limbs and tails motionless. Percent time spent floating versus actively swimming was calculated. Increased time floating during the FST is interpreted as increased depression-like behavior or behavioral despair (Matsuo et al. 2010).

Novel object recognition test

The novel object recognition test occurred during week three of treatment. Prior to testing, the mice underwent two consecutive days of habituation, during which they were placed in the open field Plexiglas arenas for 5 minutes and allowed to explore. On test day, mice were placed in the habituated arenas with two objects placed in different corners of the arena. Mice were then allowed to explore the objects in two 10-minute trials. For the first trial, the objects were a toy ball and toy fish. During the second trial, the toy fish was replaced with the novel object, a toy car, and the familiar object (toy ball) remained in the arena. Between trials, there was a 5-minute inter-trial interval during which toys and testing arenas were cleaned with 70% isopropyl alcohol. Anymaze video tracking software (Stoelting; Wood Dale, IL) was used to track the time each mouse spent with each object. The difference between the percent time spent with the novel object and the percent time with the old object was calculated as a measure of object recognition memory (Antunes and Biala 2012).

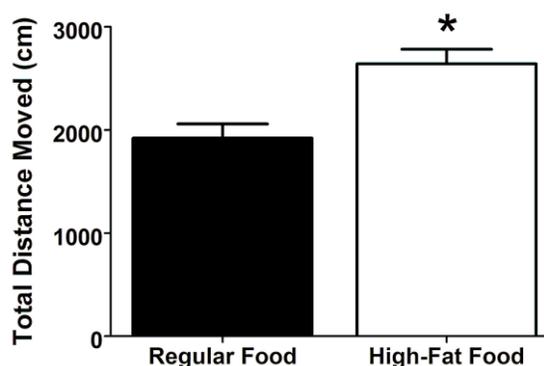


Figure 1. Total distance moved in the open field. Mice receiving a high-fat diet moved a greater distance in the open field arena compared to mice receiving a regular diet. * $p = 0.001$, high-fat > regular food.

Data collection & analysis

Analysis of Variance (ANOVA) was used to assess the effects of food and ethanol treatment on performance in the behavioral tests. Data was analyzed using SPSS analysis software. All statistical tests were conducted with a two-tailed significance alpha level of 0.05.

RESULTS

Weight gain

There were no differences between the groups in baseline weight prior to the start of the injections or food manipulation (Reg/Sal = 18.26 g ± 0.61; HF/Sal = 18.68 g ± 0.25; Reg/Eth = 19.68 g ± 0.42; HF/Eth = 19.18 ± 0.73). The ANOVA revealed the HF/Eth group gained the most weight throughout the duration of the study, however, this finding was not statistically significant (Reg/Sal = 1.74 g ± 0.34; HF/Sal = 2.52 g ± 0.52; Reg/Eth = 2.32 g ± 0.53; HF/Eth = 3.02 ± 0.80). There was no significant main effect of food treatment or ethanol treatment on weight gain, and there was no interaction between food treatment and ethanol treatment on weight gain throughout the duration of the study.

Open field test

The ANOVA revealed a main effect of food treatment on total distance moved in the arena ($F(1, 16) = 14.14, p = 0.001$). Mice fed the high-fat diet moved a greater distance in the open field compared to the mice fed with the regular diet (Figure 1). There was no main effect of ethanol treatment ($F(1, 16) = 1.96, p = 0.18$) or a significant ethanol treatment by food treatment interaction ($F(1, 16) = 1.94, p = 0.18$) on total distance moved in the open field test. However, there was a significant main effect of ethanol treatment on distance moved in the center of the open field arena ($F(1, 16) = 4.94, p = 0.041$). Mice receiving saline injections moved a greater distance in the center of the arena compared to mice receiving ethanol injections (Figure 2). There was also a significant main effect of food treatment on distance moved in the center of the arena ($F(1, 16) = 16.28, p < 0.01$), with the high-fat food mice moving a greater distance in the center of the open field compared to mice fed regular food. There was no interaction between ethanol treatment and food treatment on distance moved in the center of the arena ($F(1, 16) = 0.4, p = 0.54$). There was no significant effect of either ethanol treatment ($F(1, 16) = 0.05, p = 0.83$) or food treatment ($F(1, 19) = 0.66, p = 0.43$), or an interaction between the two ($F(1, 16) = 0.19, p = 0.67$), on percent time spent in center of the arena.

Forced swim test

The ANOVA demonstrated a trend toward a main effect of ethanol treatment on the percent time floating in the FST ($F(1, 16) = 3.78, p = 0.07$). Mice exposed to ethanol trended towards spending more time floating in the FST compared to mice exposed to saline (Figure 3). Food treatment, however, did not have a significant effect on percent time floating ($F(1, 16) = 1.48, p = 0.24$), and there was no significant food treatment by ethanol treatment interaction ($F = 0.1, p = 0.75$) in the FST.

Novel object test

The novel object recognition test did not reveal a statistically significant effect of food treatment ($F(1, 16) = 0.005, p = 0.95$) or ethanol treatment ($F(1, 16) = 0.04, p = 0.84$) on object memory, as measured by the percent time with the new object minus the old object (Reg/Sal = 2.78 ± 3.51; HF/Sal = 1.08 ± 2.02; Reg/Eth = 0.44 ± 1.15; HF/Eth = 2.46 ± 1.87). We also did not find a significant food treatment by ethanol treatment interaction ($F(1, 16) = 0.65, p = 0.43$).

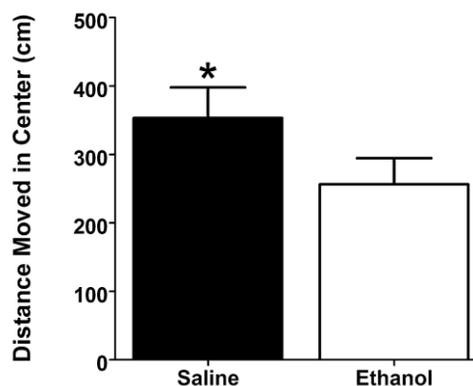


Figure 2. Distance moved in the center of the open field. Mice receiving ethanol injections moved less distance in the center of the open field arena compared to mice receiving saline injections, indicating increased anxiety-like behavior in ethanol-exposed mice. * $p = 0.041$, saline > ethanol.

DISCUSSION

The aim of this study was to investigate the effects of a high fat diet and ethanol exposure on cognition and behavior in mice. Results from the open field test indicated that ethanol exposure increases anxiety-like behavior in mice. Although results from the FST did not reach statistical significance, our study suggests a trend that ethanol exposure also increases depression-like behavior in mice. Both of these results confirm our hypotheses; we expected to find increased anxiety-like and depression-like behaviors in ethanol-exposed mice. A high fat diet significantly increased locomotor activity in the open field, although contrary to our original hypotheses, it did not affect anxiety, depression, or memory. Thus, our hypothesis that the combined effect of ethanol and high-fat diet would have the most severe effects on cognition and behavior was not supported by our study with mice.

The open field data show saline-exposed mice moved a greater distance in the center of the arena compared to the ethanol-exposed mice. This effect indicates that ethanol increased anxiety-like behavior in the open field test. Previous studies have shown that acute ethanol exposure initially

decreases anxiety-like behavior in the elevated plus maze test, which was likely due to the GABA_A receptor activating effects of ethanol (Morales-Mulia et al. 2012). In contrast, long-term ethanol exposure and withdrawal increases anxiety-like behavior by inducing changes in GABA_A receptor system (Olsen and Spigelman 2012). As the mice were exposed to ethanol 5 days a week for 4 weeks, it is likely that our study modeled chronic ethanol exposure and ethanol withdrawal during the time of testing.

Though our FST results did not reach statistical significance, our results support the literature suggesting ethanol increases risk for depression, a disorder estimated to affect 30% of the college student population (NIMH 2009). Moreover, our results help to understand the complex directionality of alcohol abuse and depression. While some studies suggest that students use alcohol to cope with preexisting depression (Deykin et al. 1987), our mouse model suggests that alcohol can also cause depressive-like symptoms. It is important to note that results from the FST were shy of statistical significance, but we believe this may in part be due to our small sample size.

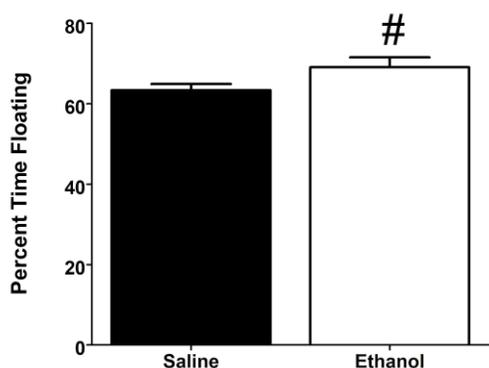


Figure 3. Percent time immobile in the Porsolt forced swim test. Mice receiving ethanol injections spent a greater percent time immobile in the forced swim test compared to mice receiving saline injections, indicating increased depression-like behavior in ethanol-exposed mice. #*p* = 0.07, ethanol > saline.

All groups performed similarly in the novel object recognition test. We do not, however, believe our non-significant results challenge current literature on the cognitive and behavioral effects of a high-fat diet and ethanol consumption (Abildgaard et al. 2011; Francis and Stevenson 2011; Markwiese et al. 1998; Valladolid-Acebes et al. 2011). The lack of an effect of high-fat diet on anxiety-like behavior, depression-like behavior, and object memory is likely explained by the fact that mice given a high fat diet did not gain a significant amount of weight over the duration of the study. As there was no significant weight gain, it is possible that the mice did not experience the cognitive consequences that were expected to follow. This explanation is further supported by the significant effects of a

high fat diet on total distance moved in the open field test. These findings suggest that the groups of mice given the high fat diet simply had more energy than the regular diet groups due to the increased caloric intake. This increase in caloric intake and energy also likely contributed to the increase in distance moved in the center of the arena among the high-fat diet mice, as they moved more overall compared to the regular food diet mice. Due to resource limitations, the mice were exposed to a high-fat diet for only four weeks and the amount of food consumed each day was not assessed. The limited duration of high-fat food exposure could explain the lack of an effect of food on weight, cognition, and behavior. Future studies should increase the duration of high-fat diet exposure on the mice and measure the quantity of food eaten each day by each mouse to ensure that the mice are indeed consuming more fat and calories each day in comparison to the mice fed a regular diet.

Ethanol’s lack of effect on memory in the novel object test may also be due in part to the small sample size of the study. It is possible that the doses of ethanol were too low to cause impairment to brain structures important for spatial memory such as the dentate gyrus of the hippocampus and entorhinal cortex (Albasser et al. 2010). Studies have shown that these regions are impaired by doses of ethanol representative of binge drinking, which equals over six times the dose we administered (Cippitelli et al. 2010). In this way, the amount of ethanol administered to the mice may have been sufficient to affect anxiety-like behavior in the open field test and depression-like behavior in the FST, yet, the dose may have been insufficient to cause impairments in object recognition memory.

There were several limitations of our study, most notably our small sample size. Each treatment group had five mice; it would be interesting to replicate our study with more mice in each treatment group to produce more robust behavioral outcomes. Also, our high-fat feed did not induce significant weight gain; future studies should increase the fat-content of the food or extend the treatment period. Finally, our model cannot account for the dynamic influences of peers, academics, and extracurricular pressures on college student behavior. Despite these limitations, we believe our study provides an adequate model to examine the interaction between high-fat diet and ethanol exposure (the “college lifestyle”) on behavior and cognition in mice. This information is very important for university health officials dealing with unhealthy diets and binge drinking patterns and the subsequent associated emotional and behavioral impairments.

In conclusion, this study utilized a mouse model to examine the effects of a high fat diet and ethanol exposure on measures of anxiety, locomotion, depression, and memory. Ethanol exposure increased anxiety-like behavior and depressive behaviors while the high fat diet increased locomotor activity in our study. Our findings suggest that college health care providers should consider ethanol intake as a potential contributor to students’ mental health issues and future research should continue to examine the combined effects of ethanol and high fat diet on behavior and cognition.

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