The Influence of Environmental Conditions on Ethanol Consumption in Rats

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Abstract

The following study examined the effect that environmental living conditions have on alcohol consumption. 25 male Sprague-Dawley rats were placed into either an isolated environmental condition or an enriched environmental condition in order to assess the amount of alcohol consumed in each group. For those in the experimental group, toys were switched periodically throughout the experiment and interaction with other rats provided socialization. In the control group, rats were kept alone in hanging wire cages throughout the course of the experiment. As expected, rats living in the isolated environment consumed significantly more alcohol than rats living in the enriched environment. Furthermore, rats living in the enriched environment showed significantly fewer signs of relapse than rats living in the isolated environment. The current study concludes that future researchers should conduct similar studies, with an emphasis placed on both male and female rats, as well as increase awareness in human populations with regards to the potentially devastating effects of alcohol consumption, especially in impoverished environments where increased alcohol consumption is more likely.

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Learning experiences provided by social interactions impact nearly all aspects of a person's development. Some studies suggest that evolution seems to have equipped infants with a capacity for entering social relationships with peers (Nash & Hay, 2003). As children grow into adolescence, an increased availability of extracurricular activities increases opportunities for social development (Schaefer et. al, 2011). Increased socialization through these activities may certainly be a positive experience on an individual, but there are also times when peers influence each other negatively. Furman and Buhrmester found that during adolescence, peers begin to surpass parents as sources of intimacy and support (1992) and some of these peers may partake in breaking the law (Berndt & Murphy, 2002). One increasingly common violation of the law among teens is the consumption of alcohol. Recent findings suggest a heightened prevalence among adolescents to become intoxicated via the consumption of alcohol, despite the enforcement of alcohol control policies (Paschall, Grube, & Kypri, 2009). This indicates a tendency among adolescents to consume alcohol at early ages.

Previous research has emphasized the importance of environmental and genetic factors with regards to the consumption of alcohol among adolescents. Dawkins, for example, found that an increased inclination to drink might be the result of the location in which a person lives. Adolescents from rural settings were significantly more likely to consume alcohol than those from urban or suburban neighborhoods (Dawkins, 1996). Further research suggests that living in a disadvantaged community significantly increases the likelihood of an adolescent's consumption of alcohol (De Hann, Boljevac, & Schaefer, 2010). One explanation for this finding may be due to risk factors, genetic variables associated with an increased risk of disease, which are activated in indigent environments (Kendler, Gardner, & Dick, 2011). A longitudinal study conducted on over

1,700 identical twins confirmed the role of genetics on premature alcohol consumption. After running statistical analyses, it was found that genetic factors influencing alcohol consumption significantly increased between the ages of sixteen and nineteen years (Rose, Dick, Viken, & Kaprio, 2001). Regardless of the cause, an alarming finding states that alcohol consumption during adolescence may lead to an increased likelihood of developing an alcohol use disorder (Winters & Lee, 2008) and other studies have shown that consuming alcohol may lead to a future criminal record (Stenbacka & Stattin, 2007). These studies demonstrate the critical importance of understanding the use and misuse of alcohol in young people.

Since studying the patterns of alcohol intake throughout the lifespan of humans is both impractical and unethical, it is necessary to observe environmental influences on alcohol consumption in animal models. Researcher Mark Rosenzweig discovered that rats placed in an enriched environment developed thicker cortexes than rats placed in an impoverished environment (1966). Researchers interested in the consumption of alcohol among adolescents have used this seminal study to determine if rats that live in an enriched environment will be less inclined to consume alcohol than those in an impoverished environment. This is known as the EC/IC paradigm. Studies with Wistar rats suggest that both motherhood and living condition may impact alcohol consumption. In one particular experiment, a sample of male rats was separated from their mothers either zero, fifteen, or three hundred and sixty minutes during the postnatal period (PN 1 to 20). The rats were then randomly selected to live in an enriched or an impoverished environment and ethanol consumption was measured over a five-week period. Results indicated that there is a negative correlation between maternal separation and ethanol consumption. Thus, the amount of ethanol that rats consume during adolescence and adulthood significantly increases as the number of days that rats spend with their mother decreases (Daoura, Haaker, & Nylander, 2010). However,

aside from the research conducted by Daoura and Haaker, experiments observing alcohol consumption with regard to the EC/IC paradigm simultaneously among rat samples are few and far between.

The current study seeks to measure adolescent drinking behavior and relapse in male rats that were raised in either enriched or impoverished environments. Research has demonstrated that rats can become addicted to alcohol in the same way that humans can. After voluntarily consuming alcohol for a few months, rats were significantly more likely to show signs of addiction to alcohol (i.e. drinking during the inactive light phase when drinking is minimal) than prior to the experiment (Spanagel & Holter, 1999). Furthermore, in the same way that studies have shown that adolescents that consume alcohol are more likely to develop an alcohol use disorder (Winters & Lee, 2008), studies conducted on rat models suggest that adolescent consumption of alcohol leads to a greater likelihood of relapse (Schramm-Sapyra et. al, 2008). It would thus be interesting to see if this data is consistent when rats are placed in either enriched or isolated environmental conditions. Deehan and his research team found that rats living in an isolated environmental condition craved ethanol significantly more than rats living in enriched conditions after ethanol fading was conducted (2007). Thus, the current study ultimately hypothesizes that rats living in an enriched environment will drink significantly less ethanol than rats living in impoverished environmental conditions. Furthermore, rats living in impoverished environments will show greater signs of relapse than rats living in enriched environmental conditions.

Method

Subjects

A total of 25 male Sprague-Dawley rats were used in this experiment, with 10 rats being placed in the isolated environmental condition and 15 being placed in the enriched environmental condition. The rats were obtained from Harlan Laboratories, Inc. and were housed on PN21. All of the animals were kept on a 14:10 light/dark cycle (lights on at 7:30 AM), and were given ad libitum access to food (Global Diet 2018 Chow; Harlan) and tap water throughout the experiment. The Institutional Animal Care and Use Committee at Lycoming College approved all procedures.

Materials

Rats living in the enriched environmental condition were placed in a cage purchased from PETCO Animal Supplies, Inc., where they were provided non-chewable toys that were switched every three days. The rats living in the isolated environmental condition were housed in hanging wire cages. For liquid consumption, standard plastic bottles were used with a capacity for 237 ml of fluids. Water and ethanol were filled daily to 100 ml, and fluid consumption was measured using graduated cylinders after fluid presentation. Ethanol solutions were prepared with tap water and 95% medical grade ethanol and arranged according to the two-bottle paradigm, in which each bottle was composed of either 3, 6, 9, or 12% (v/v) ethanol solution. For the first three days, 10% sucrose (w/v) was added to each ethanol solution.

Procedure

The rats began the experiment as adolescents on PN28. For rats living in the enriched environmental condition, fluid intake was measured twice a day (at 9:00AM and 9:00PM) and body weight and food intake were measured once a day. Rats living in the isolated condition were weighed once a week. Food consumption was monitored by administering a premeasured amount of food (approximately 25 grams) and weighing the remaining food the following morning. Animals were allowed access to the ETOH solution, water, and food at ad libitum at night,

whereas water was administered ad libitum during the day. Animals living in the enriched condition were housed as a group during the day and then moved to individual plastic cages at night. Fluids contained 10% sucrose for three days, and phase changes occurred every 3 days (3,6,9,12% v/v). The position of the 2 bottles was counterbalanced throughout the course of the experiment to avoid potential preferences due to specific bottle location (1/2 received ETOH on the left and 1/2 received ETOH on the right). During the deprivation phase, the ETOH/sucrose bottle was removed for three nights. The dependent variables of this experiment were ethanol consumption (mL), water consumption (mL), and food consumption (g).

Results

First, a paired samples t-test was performed to test for the differences between two related dependent variables, sucrose mixed with tap water and tap water alone, $t(17) = 5.477$, $p < .0001$. It then became important to make sure that there were no differences in sucrose preference between animals living in either the enriched environment or isolated environment, with sucrose preference being the amount of water that the animal drank over the total fluid. As expected, an independent samples t-test demonstrated that there was no significant difference in sucrose preference between rats in either living condition ($p > .05$). A repeated measures Analysis of Variance (ANOVA) was calculated in order to examine acquisition data in the experiment, specifically allowing for data to be collected on the same animal each time the amount of ethanol increased in the study (i.e. 3% to 6% to 9% to 12%). The repeated measures ANOVA also analyzed differences in consumption among animals living in either the enriched or isolated environment at each of the four time points. Overall, the pattern of alcohol consumption during acquisition changed over time, $F(3) = 13.113$, $p < .0001$. Specifically, as the percent ethanol increased throughout the experiment, rats continued to consume less ethanol than they had at the previous percentage (See Fig. 1). A paired samples t-test

was calculated and it was found that, overall, rats consume significantly more ethanol after a deprivation period when compared to a baseline $p > .05$ (See Fig. 2). It then became of interest to see if rats living in either the enriched or isolated environment consume more alcohol after deprivation. An independent samples t-test was calculated and there was no significant difference between ADE1 and ADE2 between groups, $p > .05$.

Discussion

The results of the current study reject the hypothesis that rats living in an enriched environment will drink significantly less ethanol than rats living in an isolated environment. There were no significant group differences among rats living in either milieu. These findings make sense, as recent research has suggested that stressful situations significantly increases the likelihood that a rat will consume alcohol as opposed to rats living in an enriched environment (Daoura, Haaker, & Nylander, 2010). A rat living in an enriched environment may not be under any more stress than a rat living in an isolated environment. The second hypothesis – rats living in impoverished environments showed greater signs of relapse after a deprivation period than rats living in an enriched environmental condition - was also rejected in this experiment. This was also expected, as previous researchers have discovered that, overall, rats are significantly more likely to consume alcohol after a deprivation period (Spanagel & Holter, 1999). Furthermore, rats that consume ethanol during the adolescent period are significantly more likely to consume more alcohol after deprivation than rats that do not consume ethanol during this time period (Schramm-Sapyra et. al, 2008).

The current study, like any other, was not without its limitations. The primary issue with this experiment was the number of researchers involved in the study. Twenty-six researchers all trained in different ways with regards to fundamental scientific skills may lead to a slight

inaccuracy in the data produced. A second problem that logically follows with having so many researchers is that data may not have been collected exactly at the twelve-hour time interval. It is possible that some researchers forgot about the experiment, or simply chose not to collect data on any given day.

Despite the fact that there were limitations to this experiment, these are minor points that do not discount the importance of the results. The current study found similar results compared to other studies presently found in the literature by suggesting that living in an isolated environment has no impact on alcohol consumption when compared to living in an enriched environment. These findings may have a positive impact on humans, as they suggest that, regardless of the region, high levels of alcohol consumption can be an issue. Perhaps this awareness could start in the high school setting, where courses or presentations could lead to a decreased likelihood to consume alcohol in large quantities. Future research should continue with similar methodologies to see if this finding continually produces similar results. Specifically, researchers should study the effect that environmental living conditions plays on females, as the sample in this study was composed of males alone. It may be of further interest to see if other species of rat aside from Sprague-Dawley produce similar results as those found in the current study.

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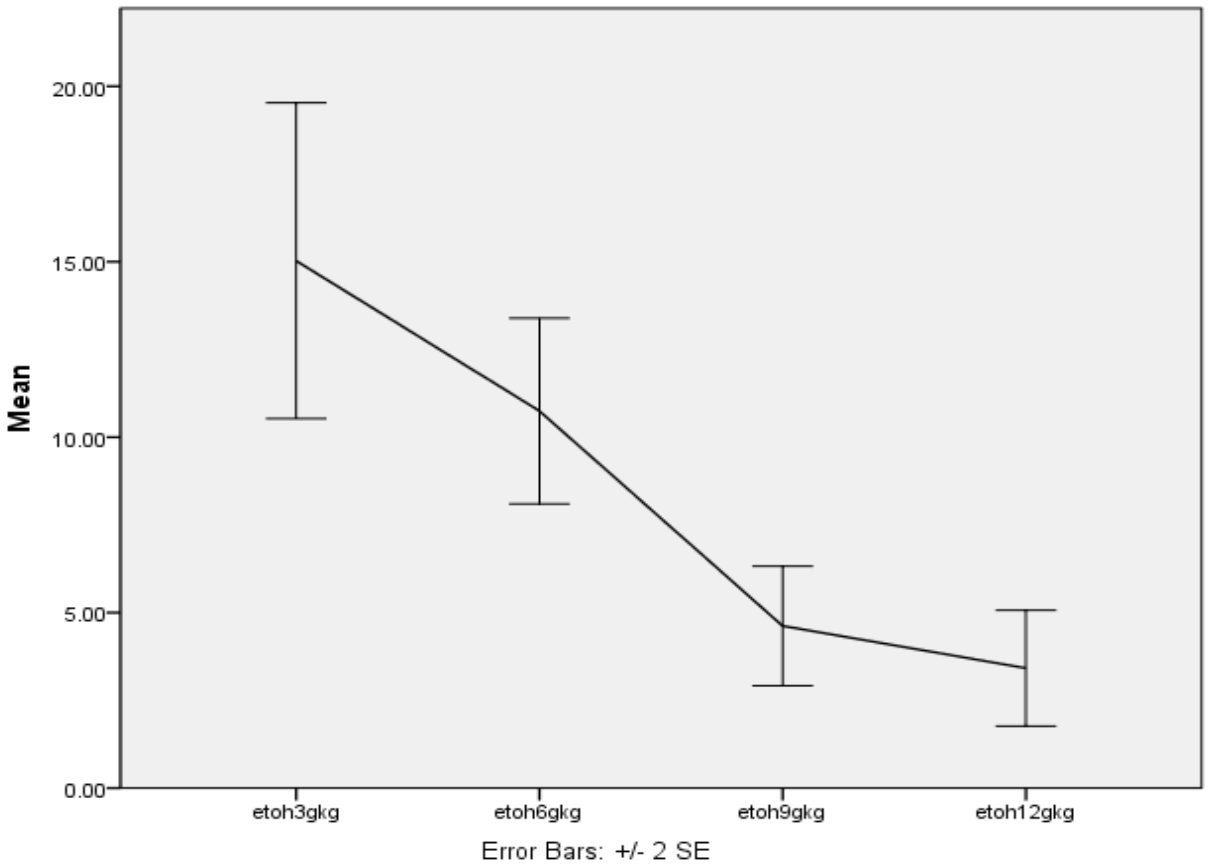
Figure Captions

Figure 1. Alcohol Consumption Across Each Time Interval. This figure represents the amount of alcohol consumed with regards to each time the amount of ethanol increased in the study (i.e. 3% to 6% to 9% to 12%). If independent samples t tests were ran, it is likely that there would be a significant difference between the 3% and the 12% time interval, though this cannot be stated until

these tests are run. The error bars attached to each column represent standard errors.

Figure 2. Deprivation Period Data. Mean values representing alcohol consumption at baseline, deprivation period number one, and deprivation period number two. Overall, rats consume significantly more ethanol after a deprivation period when compared to a baseline. Standard errors are represented in the figure by the error bars attached to each column.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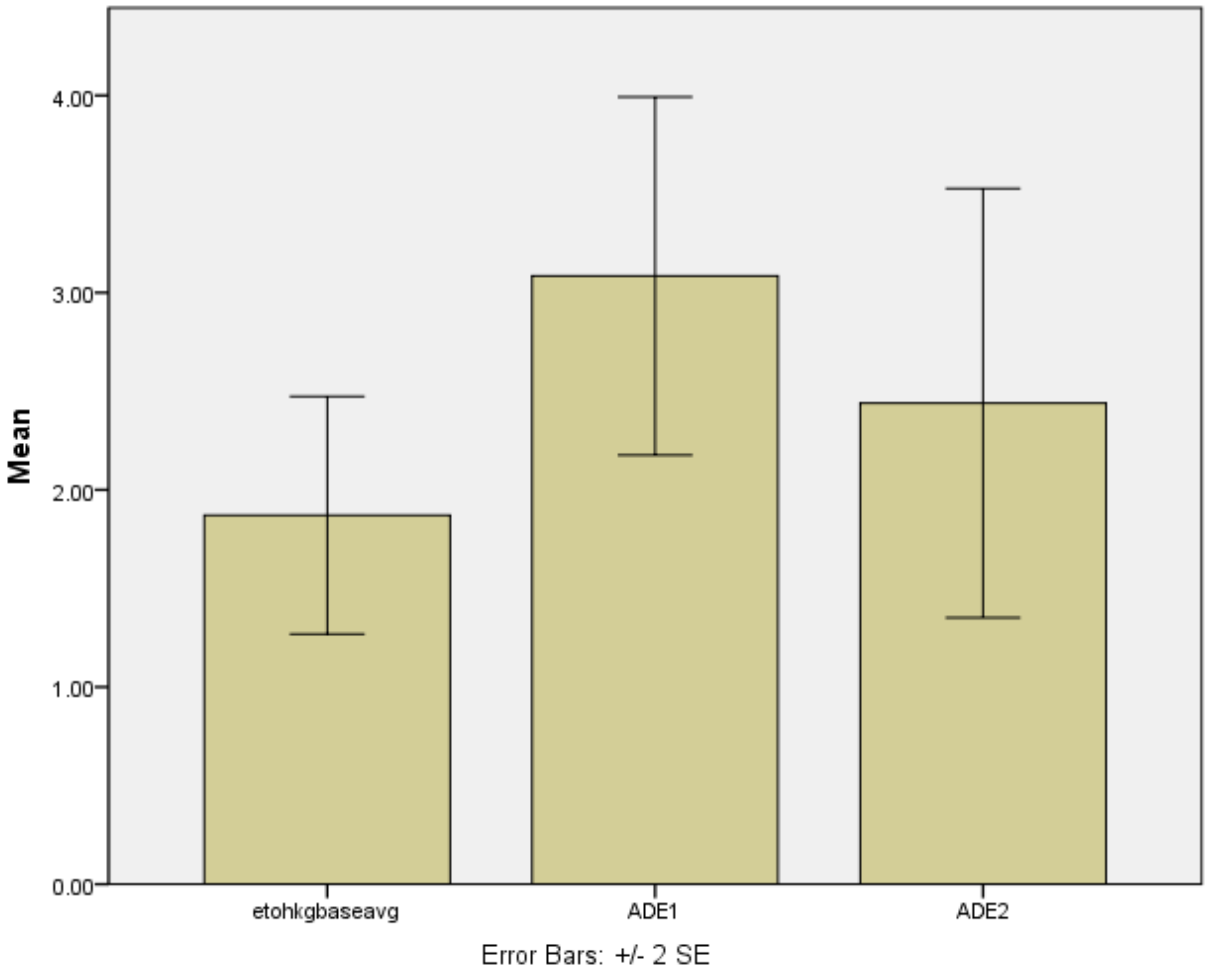
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