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Effects of Differential Housing Conditions on Ethanol Intake in Female Rats

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The purpose of the current study was to investigate if differential rearing conditions of rats in enriched conditions (EC) and isolated conditions (IC) would affect the consumption of ethanol. To facilitate ethanol consumption, sucrose fading and an ethanol deprivation paradigm were employed. Results showed that overall IC rats did not consume significantly more ethanol when compared to the EC rats. However, both groups consumed more ethanol following a period of deprivation. These findings have both basic science and clinical implications.

Keywords: *Alcohol deprivation effect, sucrose fading, development, ethanol*

Adolescent consumption of alcohol has become an important area of research due to the alarming increase of alcohol intake in this population over the years (Nixon & McClain, 2010). By twelfth grade, nearly 73% of adolescents have tried alcohol, and about half of these individuals between the ages 12 and 17 binge drink. Early exposure to alcohol has been directly related to developing an alcohol use disorder later in life (Clark, 2004). Alcohol also affects the developing adolescent brain in several ways. It causes neurodegenerative impairments which work to disrupt behavioral control systems, and therefore, potentially lead to addiction (Matthews, 2010). Adolescents may abuse alcohol because they are less sensitive to the negative effects from alcohol like lack of coordination or drowsiness, while being more sensitive to alcohol's positive effects like feeling more at ease in social situations (Masten, Faden, Zucker, & Spear, 2008). Other problems that early alcohol consumption can lead to are damage to the lungs and liver, impaired learning and memory, brain damage and cognitive deficits (Matthews, 2010; Nixon et al., 2010; Masten, 2008). Research is being conducted on what predisposes adolescents to drink. Researchers believe it is attributed to both genetic and environmental influences working together.

An individual's genetic makeup and environmental influences affect early alcohol intake. Genetics play a major role in patterns of alcohol intake seen in young individuals (Harden, Hill, Turkheimer, & Emery, 2007). Children of alcoholics are four to ten times more likely to become alcoholics themselves (Harden et al., 2007). Individuals who inherit certain genes may be more likely to start drinking at younger ages and develop alcohol problems. Pinpointing a genetic cause is not sufficient, however. Drinking behaviors are a complex interplay between the genes and environment around the individual. The gene-environment interaction is referred to as the amount one's genes interact with his/her environment, thus increasing the likelihood of developing a disorder (Dick et al., 2009). The environment can trigger or compensate for various genetic predispositions an individual has, control the expression of the genetic variables, or enhance the specific genetic predisposition (Harden et al., 2007). One example of an environmental influence is social interactions with peers which can influence and pressure individuals to drink. Some adolescents may be less resilient to peer pressure than others due to high self-esteem and/or secure attachment style (Harden et al., 2007).

However, environmental factors that potentially influence ethanol consumption are difficult to control in the human population. In contrast, animal models can control for environmental factors which complicate human studies. To date, several research lines have been developed to explore the contribution of environmental factors or external influences on developing alcohol problems. Scientists have accomplished this through manipulating environmental factors and rearing paradigms in rodent models. Bennette, Diamond, Krech, and Rosenizweig (1964) first demonstrated environmental effects on rodent brain development. The two test groups in their study were a social

condition group (SC), housed in groups of three and given no special treatment, and an environmental complexity and training group, which was a total of 10 rats housed in a big cage with toys to play with. The rats' brains were then studied at the end of the behavioral experiment. Results showed that the rats given the enriched experience had greater weight and thickness of the cortical tissue compared to the restricted littermates.

The general paradigm of enriched (EC) verses isolated (IC) rearing conditions can only be applied to rats but it can be used to "mimic" environmental factors that play a role in alcohol consumption in humans. The EC involves the handling of the rats, giving them toys/objects to interact with, and allowing them time in a social environment. The IC rats are given standard living conditions and no special treatment.

Many studies have been shown that EC/IC rearing conditions affect the response to drugs of abuse. In one study conducted by Green, Cain, Thompson, and Bardo (2003), environmental housing conditions were shown to decrease nicotine-induced hyperactivity in rats. In normal conditions, nicotine produced a period of hypoactivity. They used male Sprague-Dawley rats which were randomly assigned to an EC, SC, and IC groups. All the rats were injected with saline or nicotine before being placed in a locomotor apparatus. Locomotor activity was measured for a total of eight sessions. Results showed that the IC rats produced a nicotine dependent decrease in locomotor activity which was then followed by a period of hyperactivity. EC rats were found to be less sensitive to the effects of the locomotor stimulant that was produced by low doses of nicotine (0.8 mg/kg) than the IC rats. This shows that the environmental enrichment decreased sensitization to nicotine.

In another study, ethanol intake was assessed using the EC/IC/SC paradigm. Twenty-eight male Long-Evans were reared in EC, IC, or SC conditions while undergoing a sucrose/ethanol fading technique designed to facilitate ethanol consumption using operant chambers (Deehan, Cain, Kiefer, 2007). All the rats were then tested in operant chambers to determine ethanol preference and responses to 10% ethanol. Results demonstrated that EC and SC rats responded for 10% ethanol at a lower rate than the IC rats. Also, IC rats displayed a clear preference for the 10% ethanol when water was also available (Deehan et al., 2007). Similarly, other studies have also found that IC rats consumed more ethanol than EC/SC rats (Deatherage, 1972; Parker & Radow, 1974).

Another experiment looked at the effects of enriched housing conditions during neuro-development on the impact of the ethanol on Spontaneously Hypertensive Rats (SHR). One hundred and four female SHR were divided into a standard environment (SE) or enriched environment (EE). The rats went through four phases which included habituation, free choice between saccharin and water, force ethanol intake, and free choice between ethanol and water. Results showed that EE rats did not prefer to drink ethanol nor did they consume as much as the SE rats (Carvalho, Pandolfo, Pamplona, & Takahashi; 2008). In summary, differential rearing conditions including isolated and enriched groups can dramatically affect responses to drugs and ethanol in rats.

The purpose of the current study is to assess adolescent drinking during concurrent exposure to either enriched or isolated environmental conditions. Concurrent exposure to either EC/IC conditions with ethanol access differs from the majority of literature (e.g. Deehan et al., 2007), in which animals are raised in EC/IC environments as adolescents and given ethanol or drug access as adults. The current methodology of simultaneous EC/IC and ethanol exposure was employed to examine environmental effects on adolescent drinking behavior. Based on previous research, it was hypothesized that female Sprague-Dawley rats raised in the IC condition would consume more ethanol following a period of ethanol deprivation than rats raised in the EC condition.

Methods

Subjects

Twenty female Spargue-Dawley rats were used in this study. Subjects arrived to the laboratory on postnatal day (PND) 21 from Harlan Laboratories, Inc. Eleven rats were randomly assigned to an enriched condition (EC) and nine to the isolated conditioned (IC) upon arrival in the laboratory. Rats remained in their condition throughout the duration of the experiment (from 8:00 AM - 8:00 PM, see Methods for detail). The rats were allowed a standard laboratory diet consisting of Teklad Global 2018 Chow (Harlan Laboratories, Inc.) Rats were given ad libitum access to water. Food was restricted to the evening (from 8:00 PM – 8:00 AM) to allow for measurement of individual consumption. Laboratory conditions consisted of a 14:10 light/dark cycle and a constant temperature of 22.22° Celsius. All procedures were approved by the Institutional Animal Care and Use Committee at Lycoming College.

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Materials

Animals were presented with fluids using a two bottle choice procedure. Fluids consisted of either water, 10% sucrose (w/v) solution, or 95% medical grade ethanol. Fluids were presented in standard plastic bottles and measured with graduated cylinders. Animals were presented with water in both bottles (3 days) beginning PND 28 to facilitate habituation to the two bottle choice procedure.

Procedure

Housing conditions for the rats was as follows. EC animals were placed together in a cage with non-chewable toys (from 8:00 AM - 8:00 PM). At night (from 8:00 PM - 8:00 AM), EC rats were placed into separate plastic cages. The IC rats were placed into separate wire cages without toys for the entirety of the experiment. Body weight, food and fluid consumption were measured once per day (at 8:00 AM). EC rats were handled for approximately five minutes in the evening (at 8:00 PM).

Sucrose Fading Procedure. A sucrose fading procedure was used to facilitate ethanol consumption in adolescent rats using a two bottle choice procedure (beginning PND 31). Bottle placement was counterbalanced, with placement of bottles remaining consistent throughout the experiment. During the first phase of the procedure, bottles contained either water or 10% sucrose (3 days). Following, bottles contained water, or 3, 6, 9, 12% ethanol (v/v) (3 days each), respectively. Ethanol solutions were mixed in a 10% sucrose (w/v) solution. (Thus, a 10% sucrose (w/v) solution was mixed by measuring 10 grams of sucrose and adding water to equal 100 mL. A 3% ethanol solution was mixed by adding 3 mL of ethanol to 97 mL of the 10% sucrose solution to make 100mL). As animal consumption of ethanol dropped dramatically during the sucrose fading (5% sucrose (w/v) and 12 % ethanol v/v), the 10% sucrose (w/v) and 12% ethanol (v/v) solution was used for testing.

Ethanol Deprivation Procedure. An ethanol deprivation technique was used to assess the alcohol deprivation effect (ADE) for each rat (PND 58-PND 60). In our laboratory, the ethanol deprivation period occurred for three days during which water was presented to the animal in both bottles. Following, the animal was presented with a choice between the 10% sucrose (w/v) and 12% ethanol (v/v) solution, or water. The alcohol deprivation effect occurs when ethanol consumption increases from baseline (the day prior to ethanol deprivation) following the ethanol deprivation period.

Data Analysis

Statistical analyses were completed using SPSS Version 19 (IBM, 2010). Analyses were designed to specifically address the hypothesis of whether EC and IC animals would differ in the ethanol consumption following ethanol deprivation. Thus, baseline data from the two bottle choice of either water or 10 % sucrose (w/v) were analyzed to demonstrate that 1) fluid consumption levels in EC and IC animals were similar and 2) EC and IC animals did not differ in sucrose preference. Results from a 2(group) x 3(day) repeated measures ANOVA showed that group differences in baseline fluid consumption and sucrose preference were non-significant (p's > .05). Following, data were collapsed across group and a paired samples t-test was conducted to compare the differences between water and sucrose fluid consumption for days 1-3 of baseline fluid consumption for all animals. Given the repeated nature of these analyses, a Bonferonni correction was applied to determine significance. Similarly, alcohol deprivation effect data were subjected to a 2(group) x 3(day) repeated measures ANOVA to compare EC/IC differences in ethanol consumption. In these analyses, the last day of drinking was used as the baseline for comparisons. These analyses demonstrated that there were no EC/IC differences in ethanol consumption between groups (p's > .05). Following, data were collapsed across group and a paired samples t-test was conducted to compare differences in ethanol consumption between the day prior and the day following the deprivation period. Food consumption data for the ethanol deprivation effect period were analyzed to demonstrate that the increase in ethanol consumption following the deprivation period was not due to an overall increase in consummatory behaviors.

Results

Baseline Data

Baseline data was assessed using a paired samples t-test. Overall, rats consumed more of the 10% sucrose (w/v) solution on the three baseline drinking days compared to water [$t(13)=6.72$, $p<.0001$; $t(14)=6.989$, $p<.0001$; $t(19)=18.197$, $p<.0001$] (see Figure 1). Importantly, baseline differences in sucrose preference were non-

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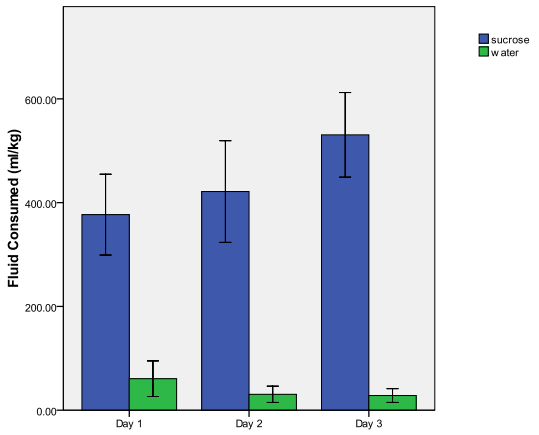

Figure 1. Overall fluid consumption in rats. Rats preferred sucrose over water during three baseline drinking days[$t(13)=6.72$, $p<.0001$; $t(14)=6.989$, $p<.000$; $t(19)=18.197$, $p<.0001$]. There were no differences in baseline sucrose or water consumed (mL/kg) for days 1, 2, and 3 between EC and IC animals (p's > .05).

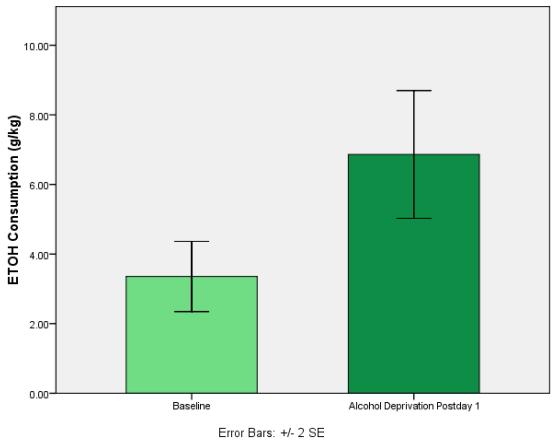

Figure 2. Both EC and IC rats consumed more ethanol (g/kg) following a period of ethanol deprivation [t(19)=-3.572, p=.002]. Group differences between EC and IC rats were non-significant (p's > .05).

significant between EC and IC groups (p's >.05, see Data Analysis section for description of statistical analysis).

Ethanol Deprivation Effect Data

A paired samples t-test was used to assess overall differences in ethanol consumption collapsed across EC/IC condition. These findings showed that rats consumed more ethanol following a period of deprivation [t(19)=-3.572, p=.002] (see Figure 2). Differences between EC and IC rats following ethanol deprivation were non-significant (p's > .05, see Data Analysis section for description of statistical analysis).

Food Consumption

Food consumption was assessed using a paired sample t-test. Following the ADE, differences produced in ethanol consumption were not due to increased consummatory behaviors, (p's > .05).

Discussion

The current study investigated how differential housing conditions affect the amount of ethanol consumption in female Sprague-Dawley rats. According to the results, the hypothesis that IC rats would consume more ethanol was not supported. There was no significant difference in ethanol consumption between the EC and IC groups.
However, the rats consumed more ethanol following a period of ethanol deprivation (also known as the alcohol deprivation effect (ADE)). Previous studies have found that ADE is not produced without multiple deprivation exposures (Rodd et al., 2008). In contrast, rats in the current experiment demonstrated an ADE following the first ethanol deprivation period, possibly due to the age differences (adolescent versus adult) in the animals (Anderson, Varlinskaya, & Spear, 2010). Interestingly, in a conditioned taste aversion paradigm, Spear and colleagues demonstrated that adolescent rats continue to consume ethanol in spite of the dysphoric effects of the drug, requiring a greater dose of ethanol to display CTA effects (Anderson, Varlinskaya, & Spear, 2010). The findings of Spear and colleagues support those in the current study in showing that adolescent animals are particularly susceptible to the reinforcing properties of ethanol, such that following a period of ethanol deprivation, they may consume more ethanol that adults. Future studies should address this issue in rodents reared in either an EC or IC environment.

Between the EC and IC rats, there was no significant difference in ethanol consumption following the period of ethanol deprivation. In contrast, multiple other studies have found that IC rats do consume more ethanol compared to EC/SC rats. For example, IC rats in similar studies preferred ethanol and responded for ethanol at higher rates than the EC and/or SC groups (Deehan et al., 2007; Deatherage, 1972; Parker & Radow, 1974). One factor that could contribute to the disparity in the

findings is the age of rats. In the current study, adolescent rats were studied, while other studies reporting differences in drinking behaviors between EC and IC rats did so in adult rats. There could be a difference between adolescent and adult ethanol consumption that leads to the EC/IC differences of ethanol intake. A follow up study should be completed with adult rats to see if similar results would be produced.

At baseline, all rats consumed more of the 10% sucrose (w/v) solution over water, showing that they prefer the sweeter solution over tap water. Similar findings were also found from a study showing that rats in general prefer sucrose mixtures over plain water when given the choice of both (Beck, Nash, Viernstein & Gordon, 1972). Importantly, between the EC and IC groups there were no significant differences produced in baseline sucrose preferences. While similar appetitive reward properties are desirable for the sucrose fading procedure and soundness of the experimental design, further research should replicate these findings in EC and IC groups differentially housed during adolescence.

The differences produced in ethanol intake following ethanol deprivation were not due to differences in consummatory behaviors. This is similar to results found by Garcia-Burgos, Gonzalez, Manrique and Gallo (2009) which showed that patterns of ethanol intake were not explained by hyperphagia. In their study, they found that the rats did not drink more because of an abnormally increased appetite. These results support those in the current study suggesting that rats drink more following a period of deprivation, possibly due to the appetitive nature of the drug.

Some limitations of the current study were the sample size and gender of the rats. However, assuming a medium effect size, the study was adequately powered to find differences between groups if they existed. Female rats were studied based on prior data showing a higher consumption of ethanol in females adolescents rats (Gilbertson et al., 2004). However, this focus precluded analysis of sex differences in drinking behavior following EC/IC exposure. Future studies should address sex differences in ethanol consumption following a period of deprivation in EC/IC exposure rats.

In conclusion, the results of the current study suggest that adolescent rats will consume more ethanol following a period of ethanol deprivation, independent of environmental rearing conditions.

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