Environmental Enrichment and Reinstatement of Alcohol Addiction in Mice

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The purpose of the current study was to investigate the effects of environmental enrichment (EE) on the blocking of reinstatement of conditioned place preference (CPP) for ethanol. Mice acquired CPP for ethanol in four trials. After a test for CPP, mice were conditioned to extinguish the CPP. During this period, half were placed into EE and half remained in a standard environment (SE). The results indicate that mice developed CPP for ethanol.
Environmental Enrichment and Reinstatement of Alcohol Addiction in Mice

Alcohol addiction is a widespread affliction in today’s society. The process of alcohol addiction includes: sensitization, withdrawal, and relapse. During sensitization, the alcohol user experiences mild euphoria, in which alcohol becomes a positive reinforcer. This positive reinforcement often leads to frequent drug seeking. After prolonged exposure, if the user decides to stop drinking alcohol, the user experiences withdrawal, which can include dysphoria, anxiety, and irritability. Because of these negative states, the user is motivated to reduce the discomfort by drinking alcohol, which becomes a negative reinforcer. Also, if the user revisits or continues to live in the environment in which the alcohol was consumed, relapse may occur due to conditioned learning.

Specific cues of the environment can become associated with alcohol, so contact with these cues can trigger drug expectancy, resulting in drug seeking and relapse (Jupp & Lawrence, 2010). For example, mice chronically exposed to ethanol in the elevated plus maze (EPM) display elevated anxiety when placed into the EPM during ethanol withdrawal compared to mice not exposed to ethanol in the EPM (Cole, Littleton, & Little, 1999). Thus, environmental cues that elicit drug-expectancy without presentation of the drug can produce physiological changes in mice. In the case of alcoholism, the negative experience of anxiety associated with environmental cues may trigger relapse.

Although several treatment options exist for alcohol addiction, only a few recovering alcoholics who complete treatment successfully maintain abstinence (McKay, 2007). Some of the treatment options that are available include: social support, prescription drugs, and various forms of psychotherapy, including group therapy.
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Social support and guidance programs such as Alcoholics Anonymous (AA) can be effective at first, but then patients run the risk of relapse and drop out. On average, nearly 33% of the participants drop out within the first month and a small minority (25%) of the initial participants continue to attend the meetings after one year (Westley, 2002). Additionally, research of the efficacy of AA’s methods has shown that positive outcomes are unrelated to AA’s specific practices or spiritual mechanisms. Rather, recovery is attributed to the enhancing effects of self-efficacy, coping skills, and motivation that the program attempts to instill in participants (Kelly, Magill, & Stout, 2009). Thus, AA’s success may lie in its general feature of encouragement of a positive life experience rather than specific processes.

Currently, a few pharmacological treatments exist for alcoholism, but most have been ineffective in sustaining abstinence from alcohol (McKay, 2007). Likewise, the treatments can produce counterproductive effects such as tolerance and dependence (McKay, 2007). Also, each individual responds differently to the available treatment options. For example, antidepressants are only effective in treating alcohol addiction if the patient has a comorbid diagnosis with depression (McKay, 2007).

Additionally, some researchers assert that alcohol addiction is a behavioral problem and therefore can only be solved through behavioral treatment. An example in which addiction has been framed as more of a behavioral problem than a drug problem has been demonstrated in a study of Vietnam War veterans. In this study, it was found that veterans who became addicted to heroin overseas were not addicted when they came back home to the United States (Robins, Helzer, & Davis, 1975). Further, Kalant (2009) states that neurobiological mechanisms in addiction are useful in understanding the brain and identifying possible pharmacological interventions for those who may have a genetic predisposition for alcoholism, but that the
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treatment of alcoholism must involve understanding the behavioral causes of addictive behavior rather than mechanisms by which it is expressed.

Since human models for drug addiction pose ethical problems for experimental research, animal models are often used to study possible treatment options. One such treatment that has been studied in animals is called environmental enrichment. In rodents, environmental enrichment typically involves grouping several animals (8-12) in a large cage with several novel objects. In these environments, the animal is granted the opportunity to explore and socially interact (Chapillon, Patin, Roy, Vincent, & Caston, 2002).

Numerous studies have concluded that environmental enrichment is a preventive and restorative measure for several abnormalities, such as genetic deficiencies, brain trauma, and prenatal stress (Chapillon et al., 2002). Manipulation of the environment in which one develops and inhabits has been shown to modify behavioral, physical, and neuronal processes. For example, several clinical studies have documented that offspring of mothers who experience stress during pregnancy oftentimes display low birth weight and long-term behavioral abnormalities (Chapillon et al., 2002). Behaviorally, prenatal stress rat models demonstrate decreased ambulation in an open field arena and increased anxiety in the EPM (Chapillion, et al., 2002). When these animals are subsequently placed in an enriched environment, however, they demonstrate decreased anxiety and increased ambulation (Chapillon, et al., 2002). These effects are supported by neuronal research, which demonstrates that enriched environments can promote an increase in total brain weight, an increase in the thickness of the cerebral cortex, greater neuron density in the hippocampus, more synaptic connections, and an increase in the total number of dendritic branches (Chapillion et al., 2002).
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Further, living in EE has been shown to ameliorate the effects of daily stress. For example, when mice experienced chronic restraint stress (6 hours/day) and lived in EE, they demonstrated less impairment in spatial recognition memory, as indicated by the results in the Y-maze, compared to stressed mice living in standard environmental conditions (Chen, Mao, Zhou, Hu, Wang, & Ma, 2010).

Although numerous studies have investigated the effects of environmental enrichment for a range of abnormalities, a limited number have studied its treatment possibilities for drug addiction. Further, of these studies, only a few have studied the effects of environmental enrichment after contact with the drug; instead, most have focused on environmental enrichment as a preventive measure for drug addiction by implementing the procedure before subsequent contact with the drug. The few research studies that have covered this area have produced favorable results. One such study by Solinas et al. (2008) examined the effects of environmental enrichment on recovery from cocaine addiction and found that cocaine-addicted rats living in an enriched environment during abstinence from the drug demonstrated less affinity for cocaine in a conditioned place preference test than the rats living in standard environmental conditions (Solinas, Chauvet, Thiriet, Rawas, & Jaber, 2008). Further, environmental enrichment prevented relapse, as evidenced by reduced drug-seeking behavior when cocaine was reinstated (Solinas, et al., 2008).

Because research has demonstrated environmental enrichment as an effective treatment for attenuating cocaine addiction, it is both relevant and important to study whether environmental enrichment would attenuate alcohol addiction. In the current study, it was expected that alcohol exposure would induce conditioned place preference and would decrease holeboard task performance. It was also expected that EE would induce greater holeboard
exploration and that reinstatement would be less likely for mice that experienced EE compared to mice that experienced SE.

**Method**

**Subjects**

Subjects were 25 male mice, which were obtained from the local We Luv Pets store in Marietta, OH.

**Materials**

**Housing**

Animals were grouped by threes in standard home cages. Initially, all cages had standard environmental (SE) conditions, which consisted of bedding and free access to standard food and water. During the extinction phase of the study, half of the cages were switched to an enriched environmental (EE) condition, which consisted of a much larger home cage, with free access to standard food and water, and the addition of toys (i.e. colorful balls, ladders, tubes, etc) which were changed every three days. Figure 1 demonstrates the placement of the animals.

**Testing apparatuses**

Conditioned place preference (CPP) chambers were constructed from standard fish tanks. Each tank was sectioned into two areas. One area had white walls and bedding covering the floor. The other area had black and white striped walls and gravel paper covering the floor.

The holeboard apparatus was a wooden square divided into 16 equal squares, containing four holes in the floor, and propped up by wooden legs.

On test days, a video camera was placed over each CPP chamber and holeboard to record the activity of the animals for subsequent data analysis.
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Ethanol

Absolute ethanol was dissolved in a saline vehicle (0.9% NaCl) to make a 20% v/v solution and was administered (12.5 mL/kg, IP) 10 minutes prior to placing the mice in the CPP chamber.

Procedure

Conditioned Place Preference and Holeboard Tests

The CPP test was performed to detect preference for ethanol. The CPP test consisted of placing each animal into the CPP chamber for 5 minutes and recording how many seconds it spent in each area. If a mouse had one or more of its paws on the separation line, then the area that had the greater number of paws was counted as the area the mouse was in. All CPP tests were video recorded.

The holeboard task was performed to measure exploratory behavior. The holeboard task consisted of placing an animal in the center of the holeboard and recording the number of head dips for 5 minutes. All holeboard tests were video recorded.

Acclimation and Baseline Test

Mice were placed into SE home boxes for seven days for purposes of acclimation and weight gain. Animals were handled and weighed during this phase. On the following day, mice performed the CPP test (Day 0; baseline test; see Figure 2) to detect any inherent differences in area preference.

Acquisition of CPP

For the next eight days, mice underwent one CPP session per day. The ethanol group received an injection of ethanol, and on alternate days, received an injection of saline. The control group received an injection of saline at every session. After the injection, the animal was
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placed into its SE home cage for 10 minutes. The animal was then placed into one of the areas of the CPP chamber for 5 minutes. During this time, there was a partition in the chamber to prevent the animal from moving to the other side. The same animals were always placed into the same side of the chamber. The placement was counterbalanced within the alcohol and saline groups.

Conditioned Place Preference and Holeboard Tests

On the day following the last session, the CPP test was performed to detect preference for alcohol and the holeboard task was performed to detect the effect of alcohol on exploratory behavior.

Environmental Enrichment and Extinction of CPP

After the tests, half of the mice remained in SE and half of the mice switched to EE, in which novel toys were introduced into the cages. Toys were replaced every three days to maintain the novelty aspect.

For the next eight days, mice underwent extinction of CPP. During extinction, an ethanol-exposed animal was given an injection of saline, returned to its SE home box for 10 minutes, and placed into its drug-paired chamber for 5 minutes. The partition was in place during this time. On alternating days, an ethanol-exposed animal was given an injection of saline, returned to its SE home box for 10 minutes, and placed into its saline-paired chamber for 5 minutes. Saline-exposed animals underwent the same procedure, alternating areas each day, just like in the acquisition phase.

Holeboard Test

The holeboard test was conducted to detect the effect of EE on exploratory behavior.
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Reinstatement of CPP Test

The day after the holeboard test, reinstatement was measured. Each ethanol-exposed animal was given an injection of ethanol, returned to its home box for 10 minutes, and, with the partition removed, was placed into the CPP chamber for 5 minutes to measure activity (time spent in each area). Each saline-exposed animal was given an injection of saline, returned to its home box for 10 minutes, and was placed into the CPP chamber for 5 minutes to measure activity (time spent in each area).

Results

A t-test revealed that there was no inherent significant difference between the two chamber areas, \( t(24) = 0.92, p = 0.95 \). Results for the CPP tests were analyzed using a multivariate ANOVA. As shown in Figure 3, the baseline CPP test revealed that there was no inherent significant difference in area preference between the alcohol group and saline group, \( F(1,24) = .005, p = .944 \). As shown in Figure 4, after the exposure phase, alcohol-exposed mice demonstrated significantly greater CPP compared to control mice, \( F(1,24) = 11.01, p = .003 \).

Given that CPP was established, it was expected that CPP would persist in the CPP retest for alcohol-exposed mice housed in SE, but would diminish for alcohol-exposed mice housed in EE. However, this hypothesis was not confirmed; as shown in Figure 6, alcohol-exposed mice housed in EE were not significantly different from alcohol-exposed mice housed in SE, \( F(1,24) = 1.42, p = .268 \).

Results for the holeboard tests were analyzed using a multivariate ANOVA. As shown in Figure 5, after alcohol exposure, alcohol-exposed mice elicited significantly fewer head dips than control mice, \( F(1,24) = 4.63, p = .042 \). Following abstinence from alcohol, it was expected that mice housed in EE would elicit a significantly greater number of head dips than mice housed in
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SE, but this hypothesis was not confirmed. As shown in Figure 7, control mice housed in EE did not show any significant difference in head dipping behavior compared to control mice housed in SE, $F(1,24) = 2.58, p = .054$, although there was a trend for head dipping differences.

Discussion

As indicated by acquisition of conditioned place preference (CPP), alcohol-exposed mice developed a preference for alcohol. Also, alcohol exposure led to poorer performance in the holeboard test, as previous research would predict. These results indicate that the amount of alcohol and duration of exposure were sufficient to induce CPP to alcohol.

The hypothesis that alcohol-exposed mice housed in environmental enrichment (EE) would have less affinity for alcohol than alcohol-exposed mice housed in a standard environment (SE) was not confirmed. However, this finding may be due to the short duration of EE, which may not have allowed enough time for EE to have an effect on the observed behaviors (i.e., head dipping and CPP) animals. Effective duration has been found to be 1 to 4 weeks for most strains (Amaral, Vargas, Hansel, Izquierdo, & Souza, 2008) and the animals in this study were in EE for only 8 days. This explanation would also account for the finding that EE mice were not significantly different from SE mice in exploratory behavior in the holeboard test. This finding was not expected because it contradicts previous research demonstrating that EE induces greater exploratory behavior.

There are several instances of human error that need to be noted. The data was collected by video recording and then coded by the author, which could have introduced an implicit bias. An improvement to the study would be to include a blind procedure, in which assistants who are naïve to the experimental question could perform the coding. Further, if multiple assistants coded
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the same data, this method would also improve inter-rater reliability. Better yet, an automated system would collect the most accurate data.

There were also a number of issues with the subjects. Because the strain was unknown, it is difficult to conclude that the results obtained are representative of other laboratory mice of known strain. Also, the subjects were all male and juvenile, so the results cannot be generalized to adult animals and females. It would be interesting to see if different results could be obtained with adult mice and females.

Future studies could model the current study, but lengthen the duration of EE. Also, the number of animals housed together in the SE/EE environments may play a role in the enrichment experience; the number of animals housed together did not change in the current study, but could be manipulated in future work. An increased number of animals housed together would enhance an enriched environment.

Additionally, it would be important for future studies to add a CPP test for extinction. This would allow the efficacy of the extinction procedure to be accessed. In the current study, there was no test for extinction, so it was unclear whether the CPP response was extinguished.

Researching possible avenues for treatment for alcohol addiction is an important and worthwhile endeavor because alcohol addiction is fairly ubiquitous. Currently, the popular treatment option of AA is less than ideal and any positive outcomes may be due to a general positive experience (Westley, 2002). If it can be shown that the experience of novel environments can attenuate alcohol addiction, self-empowering treatment options can be developed for humans that focus on having the client explore novel activities. Doing these activities in lieu of or in combination with prescription drugs may prove to be an effective approach to combating the chronic relapsing nature of alcohol addiction.
Figure 1. Number of animals in each group.
Figure 2. Procedure for the current study.
Figure 3. Mean time spent in CPP chamber at baseline. Differences between the two groups (alcohol: n=12; saline: n=13) were not significant. The dashed line indicates the mark where time spent in each area would be equal.
Figure 4. Mean time spent in CPP chamber following alcohol exposure. Alcohol-exposed mice (n=12) demonstrated significantly greater CPP compared to control mice (n=13). * Indicates significant difference. The dashed line indicates the mark where time spent in each area would be equal.
Figure 5. Mean number of head dips after exposure phase. Alcohol-exposed mice (n=12) exhibited significantly less exploration than control mice (n=13). * Indicates significant difference.
Figure 6. Average time spent in CPP chamber following EE (Reinstatement). Alcohol-exposed mice housed in EE (n=6) were not significantly different from alcohol-exposed mice housed in SE (n=6). The dashed line indicates the mark where time spent in each area would be equal.
Figure 7. Mean number of head dips for each condition (following EE). Control mice housed in SE (n=6) were not significantly different from control mice housed in EE (n=7) in either the alcohol or saline condition.
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References


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