Spring 2009

The Effects of Housing Environment On Drug Addiction in Mice

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THE EFFECTS OF HOUSING ENVIRONMENT ON DRUG ADDICTION IN MICE

A thesis submitted to

Regis College
The Honors Program
in partial fulfillment of the requirements
for Graduation with Honors

by

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May 2009

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Acknowledgements

I would like to thank my thesis advisor, Dr. Mark Basham, my thesis reader, Dr. Jose Lafosse, and my honors program advisor, Dr. Thomas Bowie for their constant guidance and generous willingness to help and encourage me throughout this process. I would also like to thank my family for their moral support and for their assistance during the research process. Finally, I would like to thank SPARC for the grant that they provided; without it, this project would not have been possible.

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Abstract

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The Effects of Housing Environment on Drug Addiction in Mice

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In 2006, 9.6% of Americans ages 12 and over were either dependent on or were abusing alcohol or illicit drugs (National Institute on Drug Abuse, 2008). Furthermore, the cost of alcohol, tobacco, and illicit drug abuse to companies and hospitals is over $500 billion per year (National Institute on Drug Abuse, 2008). A cost effective treatment that decreases drug reward would benefit medical patients taking potentially addictive drugs, recovering drug addicts, and companies and hospitals that expend money as a result of drug abuse. This study examined the effects of housing environment (both social grouping and cage enrichment) on ethanol and morphine addiction in mice. The results did not show that mice in a social and enriched environment would show the least preference for both morphine and ethanol. However, the extremely large effect sizes and past finding suggest that a larger subject population and the addition of a tactile stimulus to the experiment may show that housing environment does affect drug addiction; this could be a monumental contribution to the medical and corporate fields.

The Effects of Housing Environment on Drug Addiction in Mice

According to the National Institute on Drug Abuse, approximately 23,600,000 Americans (ages 12 or older) needed treatment for an alcohol or illicit drug problem in 2006. The susceptibility and resistance to drug addiction in humans and animals has been a great source of interest in the scientific community (Xu, Hou, Gao, He, & Zhang, 2007; Cabib et al., 1996). Reducing or even eliminating drug addiction as a result of environmental changes would be extremely helpful to individuals fighting drug addiction or taking potentially addictive medications. This study aims to investigate the effect that housing and social environments have on addiction to drugs, specifically, morphine and alcohol.

Opium, an extract of the poppy plant, has been a popular drug since 1500 B.C. when the Egyptians used it for its analgesic properties (Meyer & Quenzer, 2005). Morphine is the principle active ingredient in opium and is a natural analgesic. Heroin is a semisynthetic narcotic which is converted into morphine once past the blood and brain barrier. However, heroin is more potent than direct injections of morphine because it is lipid soluble and therefore passes into the brain and onto opioid receptors faster (Meyer & Quenzer, 2005). The relaxation and occasional euphoria experienced from narcotics and the reward circuit that they work on makes them extremely addictive; this is why morphine is no longer extensively used in medical settings and why heroin is illegal (Meyer & Quenzer, 2005).

Morphine has such a strong effect on anxiety that stress levels increase as a result of morphine withdrawal. One study concerning withdrawal and anxiety found that rats

that experienced morphine withdrawal showed a significant increase in acoustic startle response (Harris & Gewirtz, 2004). Because of its analgesic properties, though, more morphine can relieve the stress induced by withdrawal. The increased anxiety from morphine withdrawal as well as the drug's analgesic properties reportedly occur in humans as well (Harris & Gewirtz, 2004).

Other researchers suggest that humans have a lifetime of stress experiences whereas laboratory rats do not; therefore stress experienced by rats is quantitatively different from the stress felt by humans (Haller, 2001). The anxiety reducing effects of anxiolytics observed in rats may therefore not be observed in humans because of the additional stress they may have due to a lifetime of experiences; the additional stress in humans may override some anxiety reducing effects of anxiolytics. Further research into the relationship between stress and efficacy of anxiolytics is therefore necessary in order for it to be applicable to humans.

Many studies suggest that socially isolated rats will seek more morphine than rats that are housed in groups (Panskepp, 1980; Schenk, Hunt, Klukowski, and Amit, 1987). Rats that are socially isolated experience a decreased analgesic effect of morphine as compared to those that are housed in groups, possibly because social isolation desensitizes opiate receptors (Panskepp, 1980; Schenk, Hunt, Klukowski, and Amit, 1987). Therefore, as a result of the stress caused by social isolation the rat will seek more morphine to experience euphoria and eliminate the stress. Similar results have been found in mice; isolated conditions can make mice less sensitive to morphine and therefore mice seek more of the drug (Broseta, Rodriguez-Arias, & Aguilar, 2005).

In contrast to social isolation studies, few experiments have been conducted concerning the effect of enriched environments on morphine addiction. Of these few studies, some have shown no effect of enriched cage environment on morphine preference in rats (Paolone et al., 2003; Hill & Powell, 1976). However, a more recent study reported that an enriched environment does reduce morphine induced conditioned place preference in C57BL/6 mice (Xu, Hou, Gao, He, & Zhang, 2007). Interestingly, another recent study found that prenatal stress enhances morphine addictive behavior in adult mice offspring but that enriched environment counteracts it (Yang et al. 2006). Therefore, an enriched environment could be a condition important in the prevention of drug addictive behavior.

Due to its sedative properties, alcohol is also an addictive substance. As a central nervous system (CNS) depressant, when alcohol diffuses across the blood-brain barrier, the effects experienced include an increase in relaxation, decrease in anxiety, and impairment of self perception which could result in more outgoing behavior (Meyer & Quenzer, 2005). Among other factors, alcohol's addictive properties make it the second most used psychoactive drug in the United States following caffeine (Meyer & Quenzer, 2005).

Although there has been a lot of research conducted concerning morphine induced conditioned place preference in C57BL/6 mice, there has been very little research reported concerning C57BL/6 mice and alcohol induced conditioned place preference. The majority of the studies that have been reported, however, support the claim that C57BL/6 mice become addicted to ethanol. In comparing the intake of alcohol between

C57BL/6 and DBA/2J mice, ethanol intake is significantly higher in the C57BL/6 strain than in the DBA/2J strain (Mittleman, Van Brunt, & Matthews, 2003; Meliska, Bartke, McGlacken, & Jensen, 1994). Additionally, wild type C57BL/6 mice exhibit greater ethanol induced conditioned place preference, as compared to those injected with an opioid-modulator (Marchand et al., 2005).

Similar to humans, in mice, stress can lead to an increase in alcohol consumption. Stress caused by social defeat leads to an increase in the amount of alcohol intake by C57BL/6 mice (Croft, Brooks, Cole, & Little, 2004). Similarly, severe social and physical stress in mice results in a significant increase in alcohol consumption (Hilakin-Clarke & Lister, 1992). Additionally, stress caused by post-weaning social isolation resulted in a significant increase in alcohol consumption in male C57BL/6 mice (Advani, Hensler & Koek, 2007). Although there are some studies examining social stress, there appears to be few, if any, studies addressing the effect of the stress induced by cage environments and alcohol preference in mice.

The conflicting results that studies report concerning the effect that enriched environment has on morphine preference is perplexing. C57BL/6 mice are naturally social animals (Lindzey, Winston, & Roberts, 1965; Sankoorikal, Kaercher, Boon, Lee, & Brodkin, 2006); therefore, they should stress less in a group housed environment. In the same way, a naturalistic environment full of obstacles and objects to maneuver would seem to be the preferred and therefore less stressful environment for mice; in fact, one study found that mice placed in enriched environments were more tranquil and less jumpy than those put in standard environments (Van de Weerd et al., 2002). Given the

fact that that findings suggest that mice are social and are more tranquil in enriched environments, this study will investigate whether mice that are born and raised in social and enriched environments as opposed to standard cages will become more stressed when put into isolated and/or standard cages post-weaning and therefore develop a greater preference for sedative drugs like morphine and alcohol. Although there are many studies that have examined morphine preference and isolation (Panskepp, 1980; Schenk, Hunt, Klukowski, and Amit, 1987; Broseta, Rodriguez-Arias, & Aguilar, 2005) or morphine preference and enriched environments (Paolone et al., 2003; Hill & Powell, 1976; Xu, Hou, Gao, He, & Zhang, 2007), none tested both simultaneously. Because of this, the current study also aims to manipulate both physical environment and social conditions at the same time to see if there is an additive effect of these conditions. It is possible that the interaction between both variables (social grouping and environment) will result in preference for an environment that is exceedingly natural (social AND enriched) or may result in more stress from an extremely unnatural setting. Given that previous studies have found that mice are naturally social animals and are more tranquil in enriched environments, the first hypothesis is that the enriched and social group will exhibit the least preference for morphine as compared to the enriched and isolated group, the standard and social group, and the standard and isolated group (as exhibited by conditioned place preference).

There are few studies that have examined the effect of environment (grouping or enrichment) on alcohol preference (Croft, Brooks, Cole, & Little, 2004; Hilakin-Clarke &

Lister, 1992; Advani, Hensler, & Koek, 2007). Therefore, this study will also examine the effect of post-weaning cage and social environment on alcohol addiction. Again, because mice are instinctually social animals that seem less stressed in enriched environments, the second hypothesis is that mice in the enriched and social environment will exhibit the least preference for alcohol as compared to the enriched and isolated group, standard and social group, and the standard and isolated group (as exhibited by conditioned place preference).

Method

Subjects

Eleven (7 male, 4 female) adult (at least 6 weeks of age) C57BL/6J mice from Jackson Laboratory weighing 16-31 g at the time of testing were used in the experiments. Five other mice died before or after the preconditioning session therefore their data was not included in the results. The mice had free access to food and water except during preconditioning, conditioning, and postconditioning sessions. The mice experienced 12-hour light/dark schedules (6:00AM on, 6:00PM off) in their housing at the Regis University Animal Care Facility. The Regis University Institutional Animal Care and Use Committee approved the experimental protocol of this study.

Drugs

Morphine HCl (Sigma) and ethanol were dissolved in saline (0.9%). The ethanol was diluted to a 20% v/v solution and injected intraperitoneally in a volume of 12.5 ml/kg

(dose = 2mg/kg). The morphine dose was 5 mg/kg mouse and was injected intraperitoneally in a volume of 10 ml/kg.

Conditioned Place Preference Apparatus

The conditioned place preference apparatus consisted of two boxes, one with white walls and the other with black walls, both of equal dimensions (53 X 53 X 30 cm) connected by a hollow tube 8.5 cm in length and 5 cm in diameter. The flooring was the same in both boxes and neither box had a ceiling. For the conditioning sessions, the mouse was placed in either black or white compartment and the entry way into the rest of the apparatus was blocked.

Housing

All of the mice used for this experiment were born and raised in enriched plastic cages (36 X 31 X 18 cm) until weaning. These cages contained one large PVC tube, two balls, one hanging object, and one smaller PVC tube. When the pups were weaned from their mother's milk (21 days of age) the researcher moved them into one of four different housing conditions. The housing conditions were as follows: enriched and social (enriched cage with the same maneuverable objects as in the birth cage, 4 mice per cage), enriched and isolated (enriched cage with the same maneuverable objects as in the birth cage, 1 mouse per cage, 4 cages), standard and social (standard plastic cage, 4 mice per cage), and standard and isolated (standard plastic cage, 1 mouse per cage, 4 cages). The mice lived in these housing conditions for the duration of the experiment.

Procedure

After three weeks in these housing conditions, a pre-conditioning test was used to determine that the conditioned place preference apparatus was unbiased. During this test, the researcher placed each mouse into the connecting tube of the conditioned place preference apparatus and measured the time spent in each box for 10 minutes. The placement into the connecting tube was counterbalanced so that there would be no bias towards the box the mouse was facing.

One day after the pre-conditioning test, conditioning sessions were performed. The ethanol-induced conditioned place preference conditioning sessions had 2 phases. The first phase consisted of 8 conditioning sessions (2 session per day) over a span of 4 days in which each mouse was injected with either saline solution or ethanol solution in the morning and either ethanol or saline solution in the evening. Every mouse received counterbalanced saline and ethanol injections each conditioning day; if a mouse received a saline injection in the morning, it received an ethanol injection in the evening and vice versa. Two minutes after the injection, the researcher confined the mouse to either the white box (saline paired) or the black box (ethanol paired) for 15 min (the interconnecting tube was blocked). After the four days of conditioning, a post conditioning test was performed on the fifth day of testing in which the researcher placed each mouse into the apparatus for 10 min and measured the time spent in each box.

During the second phase of ethanol-induced conditioned place preference which was approximately 2 wks after the post-conditioning test of the first phase, the researcher injected each mouse in the morning with ethanol, waited approximately 2 min and confined the mouse to the black box for 15 min (the interconnecting tube was blocked).

There were 3 conditioning sessions (1 session per day) in this phase of the experiment. Again, the researcher performed a post-conditioning test on the fourth day of testing in which she placed each mouse into the connecting tube of the conditioned place preference apparatus for 10 min and measured the amount of time each mouse spent in each box.

The morphine-induced conditioned place preference portion of the experiment took place approximately 2 months after the post-conditioning test of the last session of the second phase of the alcohol-induced conditioned place preference portion of the experiment. During this portion, the researcher injected each mouse with morphine and immediately confined the mouse to the black box for 15 min. This portion of the experiment consisted of 3 conditioning sessions (1 session per day). The researcher performed a post-conditioning test in which the researcher recorded the amount of time each mouse spent in each box for a total of 10 min.

Results

Across all conditioning sessions, the mice in the enriched conditions spent significantly more time in the black box than the mice in the standard conditions ($F(1)= 6.82$, p-value= 0.035). Additionally, the mice in the social conditions spent significantly more time in the black box than the mice in the isolated conditions ($F(1)=6.97$, p-value=0.033). However, there was not a significant difference in time spent in the black box between different conditioning sessions, $F(1) = 0.232$, $p = 0.645$. Similarly, there was no interaction between housing condition and conditioning session on time spent in the black box, $F(1) = 0.05$, $p = 0.829$, nor was there an interaction between social

condition and conditioning session, F(1) = 0.172, p = 0.691. Finally, there was not a significant three way interaction between all three variables, F(1) = 0.074, p = 0.794.

However, following the first conditioning session, the size of the effect of the enriched and social environment is very large (d= 1.78). Similarly, the effect size of the enriched and social environment for the second ethanol post conditioning session is also large (d= 0.77). Finally, the effect size of the enriched and grouped environment for the morphine post conditioning session is also very large (d= 1.06).

Figure 1. Percentage of time spent in black box by the enriched groups across the four conditioning sessions.

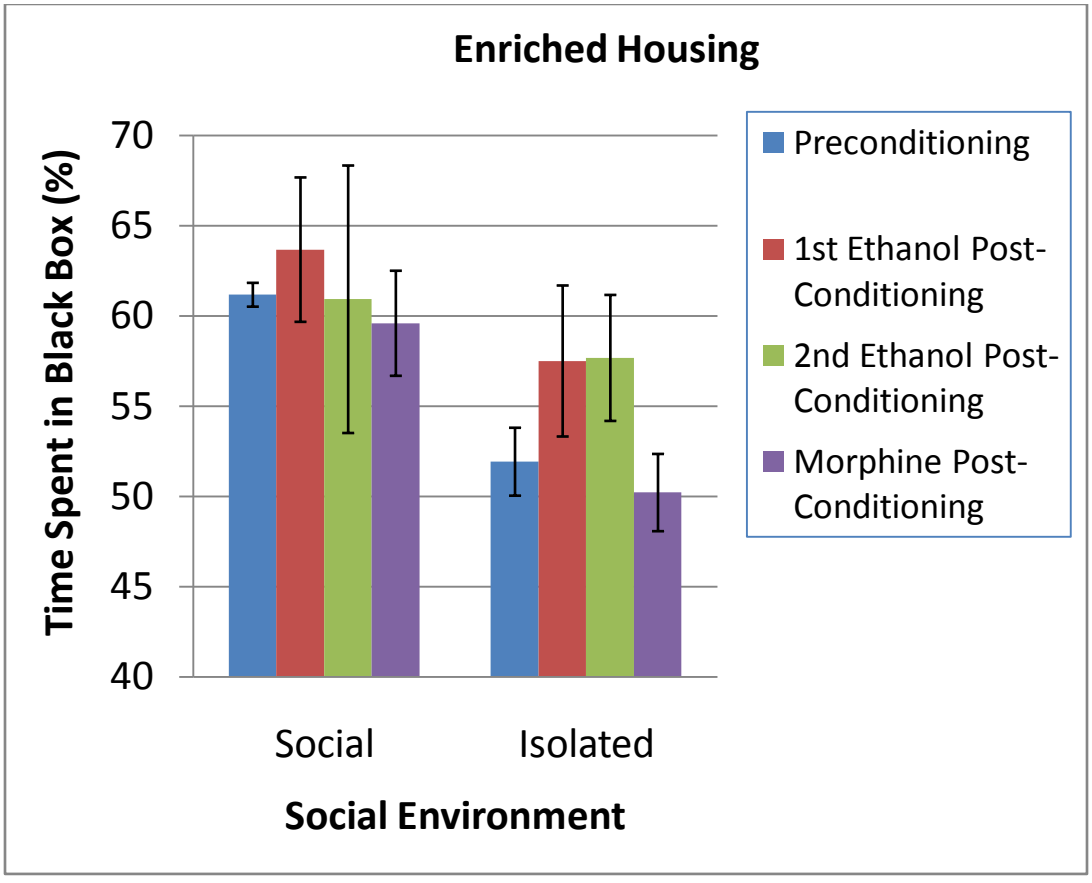
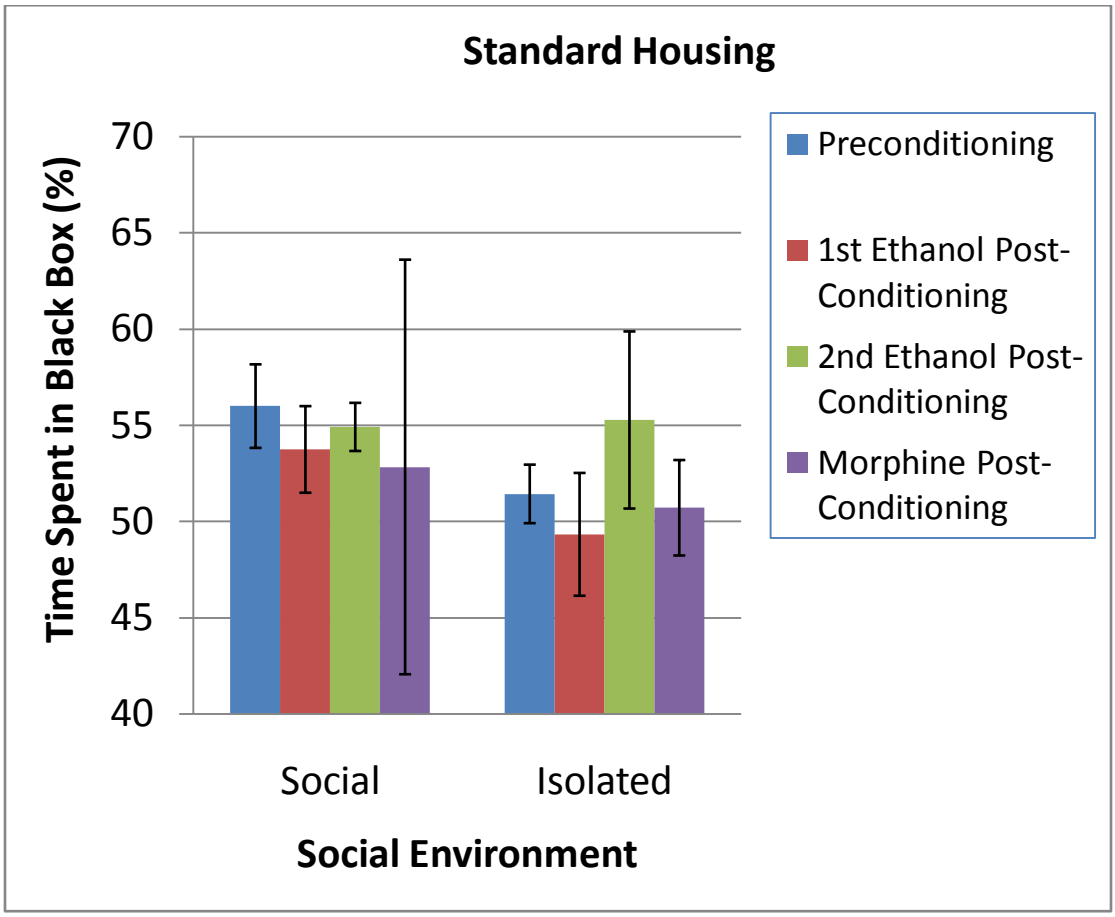


Figure 2. Percentage of time spent in black box by the standard groups across the four conditioning sessions.



Discussion

The finding did not support the hypothesis that the mice in the enriched and social conditions would exhibit the least amount of morphine and ethanol addiction. Although the mice in the enriched groups as compared to the standard groups and the social groups as compared to the isolated groups spent significantly more time in the drug-paired box across all pre- and post-conditioning sessions, the reason for these findings is unclear and therefore should be addressed in future studies. However, the huge effect sizes

comparing the enriched and social group to the other three housing groups from both ethanol and morphine conditioning sessions suggest that with a larger test subject population, a significant difference would have been observed.

The conditioned place preference apparatus used in the current study had uniform flooring throughout to prevent bias towards particular flooring. Previous research suggests, however, that flooring in one compartment must be different from another in order for conditioned place preference to occur. For example, in one study, the researchers used a mixture of textured floor in the ethanol and morphine paired compartments and a lack thereof in the saline paired compartments and they observed ethanol and morphine induced conditioned place preference (Font, Aragon, and Miquel, 2006). Therefore, one follow-up question from this study is, would the researchers observe drug induced place preference if the drug paired compartment would have the smooth flooring as compared to grid or perforated flooring? Additionally, why is the tactile sense so important in conditioning? Since mice can tell the difference between light and dark, why are visual cues not enough to result in significant drug induced conditioned place preference? Finally, for human addicts that overdose in novel areas, is tactile sensation a factor?

Although the results show no significant differences among groups within conditioning sessions, past studies (Yang et al. 2006; Panskepp, 1980; Schenk, Hunt, Klukowski, and Amit, 1987; Hensler & Koek, 2007) do show that preference for morphine and alcohol is affected as a result of environmental changes. A future study examining the additive effect of environmental housing on mice using tactile and visual

stimuli in the conditioning process has great potential in reducing the magnitude of drug addiction.

Why is this important? A discovery in the prevention of drug addiction could revolutionize the medical field; medications that have been prohibited or put out of use because of their highly addictive characteristics can be reinstated. The money that hospitals and companies can save on expensive treatment plans or lost productivity could be used for other needs. Because heroin and morphine are so similar in structure and narcotic effect, the results of future experiments concerning enriched environment and social grouping in minimizing drug induced conditioned place preference are valuable in that they could provide a method of treatment for recovering drug addicts. Incorporation of stimulating environments would therefore help those recovering from narcotic addiction and would also act as a preventative measure for those that have not yet been exposed to narcotics. If an enriched environment and social housing decreases morphine/heroin preference, the possibility for relapse is significantly reduced, and the recovery rate of a drug addict can increase greatly. More importantly, if the results in future experiments support that stimulating post-natal environments reduce preference for a drug, the incidence of narcotic addiction could be reduced if not eliminated. This is especially important today's society in which drug addiction in poorer cities is an ever-present concern. Potential results pointing to the importance of the enriched and social environment could eliminate the fall of many living in impoverished situations to drug addiction. The absence of drug addiction in our society as a result of small environmental housing changes can lead to immense positive social reform.

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