

Seton Hall University
eRepository @ Seton Hall

Theses

Spring 5-2011

Effect of an Enriched Environment on Morphine Conditioned Place Preference in Rats

Gina E. DiFeo
Seton Hall University

Follow this and additional works at: <https://scholarship.shu.edu/theses>

 Part of the [Psychology Commons](#)

Recommended Citation

DiFeo, Gina E., "Effect of an Enriched Environment on Morphine Conditioned Place Preference in Rats" (2011). *Theses*. 20.
<https://scholarship.shu.edu/theses/20>

EFFECT OF AN ENRICHED ENVIRONMENT ON MORPHINE CONDITIONED PLACE
PREFERENCE IN RATS

by

Gina E. DiFeo

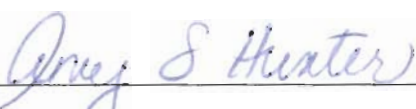
Department of Psychology

Seton Hall University

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science
in Experimental Psychology with a concentration in Behavioral Neuroscience

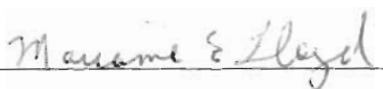
May, 2011

Approved By:




A handwritten signature in blue ink that reads "Amy S. Hunter". The signature is written in a cursive style and is positioned above a horizontal line.

Dr. Amy Hunter, Faculty Mentor



A handwritten signature in black ink that reads "Marianne E. Lloyd". The signature is written in a cursive style and is positioned above a horizontal line.

Dr. Marianne Lloyd, Committee Member



A handwritten signature in black ink that reads "Michael Vigorito". The signature is written in a cursive style and is positioned above a horizontal line.

Dr. Michael Vigorito, Committee Member



A handwritten signature in black ink that reads "Janine P. Buckner". The signature is written in a cursive style and is positioned above a horizontal line.

Dr. Janine Buckner, Director of Graduate Studies

Dedication

This thesis is dedicated to my parents, Denis and Cynthia DiFeo, for their unconditional love and support. Thank you for never giving up on me and for giving me the opportunity and encouragement to pursue my dreams. I love you and would not be where I am today without you both.

Acknowledgements

I would like to express my sincerest gratitude to my advisor and mentor Dr. Amy Hunter for all of her support and guidance given to me throughout my experience here at Seton Hall University. I'd also like to thank my thesis committee members, Dr. Marianne Lloyd and Dr. Michael Vigorito, for their guidance and suggestions in the completion of my thesis. Additionally, I would like to give a special thanks to Dr. Sulie Chang, whose generosity and support have made my thesis possible.

I would also like to thank all of my professors, as well as the entire faculty of the Psychology Department, for their wisdom and support during my time here in the graduate program. My work here would not be possible without the help given to me by the other members of Dr. Hunter's lab; thank you all for your help and assistance with my experiment. A special "thank you" to my fellow graduate students for their support and friendship, you have made my experience here at Seton Hall an enjoyable one.

Thank you also to all of my friends and family, who have supported and stood by me always while I have been pursuing my Master's degree. You have all helped me balance my personal and academic life, and I am eternally grateful to you all. I would especially like to thank my siblings, Jenifer, Elisa, Julie, and Anthony; you guys are the best and I want to thank you for always being there for me.

Table of Contents

APPROVED BY.....	i
DEDICATION.....	ii
ACKNOWLEDGEMENTS.....	iii
TABLE OF CONTENTS.....	iv
LIST OF TABLES.....	v
LIST OF FIGURES.....	vi
ABSTRACT.....	vii
INTRODUCTION.....	1
PILOT STUDY.....	19
METHOD.....	32
RESULTS.....	36
DISCUSSION.....	40
REFERENCES.....	47

List of Tables

Table 1.....	17
--------------	----

List of Figures

Figure 1.....	20
Figure 2.....	22
Figure 3.....	25
Figure 4.....	26
Figure 5.....	27
Figure 6.....	29
Figure 7.....	34
Figure 8.....	37
Figure 9.....	38
Figure 10.....	39

Abstract

One of the greatest problems plaguing our society today is the use and abuse of illegal substances. The use of opiates, such as morphine, is of particular concern due to the high rates of relapse in those addicted. The conditioned place preference (CPP) paradigm is a technique used to study the rewarding properties of drugs of abuse in rats, and is reliant on classical conditioning. One way to model environmental influences on drug abuse and addiction vulnerability is through an enriched environment (EE) paradigm, in which rats are housed in large cages with increased opportunity for exploratory behavior and social interaction. EE studies have been shown to have beneficial effects in the brain and on behavior in animal studies. Thus far, studies on drug addiction using both the EE and CPP paradigms have shown conflicting results. The goal of the present study was to investigate the effects of EE in rats on the acquisition, extinction, and stress-induced reinstatement of a morphine CPP. Male Sprague-Dawley rats were raised in either an enriched or standard environment for 6 weeks. Animals were given a 3 mg/kg dose of morphine during the conditioning phase of the experiment. Following a preference test to determine CPP acquisition, the preference for the drug-paired chamber was extinguished. One week after extinction, half of the animals were exposed to an unsignaled footshock stressor to produce stress-induced reinstatement of the drug-paired chamber. While both groups developed a morphine CPP, the magnitude of this preference was significantly greater in EE rats compared to rats reared in standard conditions (SE). The rates of extinction did not differ between the two groups of rats. Exposure to stress produced a trend towards reinstatement in SE rats. However, EE rats did not show this stress-induced reinstatement. These results suggest that raising rats in EE may have a protective effect against the reinstatement of the preference for the drug-paired chamber that occurred as a result of the

stress-inducing footshock. This is consistent with previous research indicating a beneficial effect of EE in the CPP model of drug abuse.

Effect of an Enriched Environment on Morphine Conditioned Place Preference in Rats

One of the greatest problems plaguing our society today is the use and abuse of illegal substances. Drug addiction has been classified as a chronic relapsing disorder characterized by compulsive drug-seeking and drug-taking behaviors, without regard for the negative consequences often associated with drug use (Jaffe, 1990). In the recent National Survey on Drug Use and Health done by the Substance Abuse and Mental Health Services Administration (SAMSHA, 2009), it is estimated that 21.8 million Americans 12 years of age or older, or approximately 8.7% of the population, are considered to have a substance abuse or dependence disorder. Drug addiction is associated with severely detrimental social and economical effects, which in turn have led to a number of research studies in which the sole purpose has been to seek out and directly examine the underlying behaviors associated with substance abuse disorders. As we move toward a better understanding of the neurobiological processes and brain alterations involved in drug addiction, we will hopefully be capable of developing more effective treatment options and better preventative measures in order to attenuate this growing epidemic in our society.

Neurobiology of Addiction

To better understand the procedures and results of behavioral studies on addiction, it is important to have knowledge of the biological mechanisms underlying these behaviors. We know that there is a genetic component involved in addiction (for reviews, see Compton, Thomas, Conway, & Colliver, 2005; Crabbe, 2002; Zhou, Proudnikov, Yuferov, & Kreek, 2010), and many studies are focused on the neurobiological aspects of drug addiction through the use of animal models. Research on animals commonly involves the investigation of the reinforcing effects of abused substances. Although there are several neural mechanisms involved in

reinforcement, the activities of dopaminergic neurons play an especially important role. The most important dopamine (DA) circuit in drug reinforcement is the mesolimbic dopaminergic system originating in the ventral tegmental area and sending axonal projections to the nucleus accumbens. The nucleus accumbens is located in the ventral striatum of the basal forebrain and its neurons project to the more ventral areas of the basal ganglia involved in learning. The release of dopamine in the nucleus accumbens is the primary effect of most drugs of abuse, as well as in the activity of most types of natural reinforcers. According to Kauer & Malenka (2007), it appears that the process of addiction begins in the mesolimbic dopaminergic system and then produces long-term changes in other brain regions that receive input from these neurons. The process begins with the strengthening of the synaptic connections of the inputs to the neurons of the ventral tegmental area which then project their excitatory dopamine outputs to their respective brain areas, most notably the nucleus accumbens. While the early reinforcing effects that encourage drug taking behavior takes place in the nucleus accumbens, the ventral region of the striatum, it is the changes that take place in the dorsal areas of the striatum that lead to the behaviors becoming habitual. In their review on the neural systems of reinforcement in drug addiction, Everitt & Robbins (2005) hypothesize that changing from voluntary drug use to habitual and compulsive drug use is represented by a transition at the neuronal level from precortical to striatal control over behavior as well as a progression from ventral to more dorsal domains of the striatum, which involves the innervations of the DA system. These transitions depend on the neuroplasticity in the cortical and striatal structures induced by chronic drug use. Two of the general types of structural plasticity have been observed in addiction studies: the first being changes in the size of cell bodies and the second is changes in dendritic spine arborizations or spine morphology (Russo et al., 2010). These morphological changes are the mediators of

addictive behavior. It has been theorized that continued exposure to drugs of abuse results in a pathological shift in the drug user's hedonic set point and an overall state of dysregulation of brain reward systems, resulting in the loss of control over drug intake and compulsive use (Koob & LeMoal, 2008). Through the use of animal models of addiction we are able to investigate the neurophysiological basis of behavior, as well as the perceptual and motivational aspects of addiction.

Animal Models of Addiction

Studying the development of drug abuse is difficult to do in human subjects; therefore nonhuman animal models are preferred by addiction researchers interested in the neurobiology behind the behaviors of addiction, with rodents being the most commonly studied. One of the reasons that animal models are preferred is that it is more difficult to study the neurobiology behind the acquisition of an addiction in humans. Additionally, animal models allow us to do neurobiological manipulations that can't be done in humans, and animal models also allow for greater experimental control. Studies on mice and rats allow us to probe the physiological aspects of addiction, from the stages of acquisition of drug addiction, escalation of drug use, extinction of the drug preference, and relapse, or reinstatement of drug use after a period of abstinence. One of the most widely used models in addiction research on rodents is the conditioned place preference paradigm.

Conditioned Place Preference

What is conditioned place preference?

The conditioned place preference (CPP) paradigm is a technique used to study the rewarding properties of drugs. It is important to note that there is a distinction between the reinforcing and the rewarding effects of drugs of abuse. According to Bardo and Bevins (2000),

the reinforcing properties of a drug are better measured through the operant drug self-administration paradigm, since it is clear to see what behaviors are more likely to occur in the presence of a drug using this model. In the CPP paradigm it is not clear as to what behaviors are reinforced since the drug is given passively to the animal, so it is thought to be more of a measure of the rewarding, or appetitive value of the drug. The methodology of CPP experiments can vary but the basic premise remains constant and is reliant on the theory of Pavlovian conditioning. In this type of conditioning, there is an unconditioned stimulus (US), which is capable of eliciting a particular response, being paired with a neutral stimulus. Through a series of pairings with the US, the initially neutral stimulus then becomes what is known as the conditioned stimulus (CS), and the two stimuli become associated with one another, allowing the previously neutral CS to now elicit the response of the US, with or without the presence of the US. The CS that gets paired with the US could be something discrete, such as a tone, or something less specific, such as a context.

When using the CPP paradigm to measure the rewarding properties of drugs it is important that the apparatus is devised in such a way that the two chambers are contextually distinct from one another. This can be accomplished through the use of various visual, olfactory, and/or tactile cues. One of the chambers becomes the CS as it becomes associated with the rewarding US of drug administration through the conditioning process. The CPP procedure can be considered a discrimination task since it involves both a CS+ and CS-. The conditioning stage of the CPP paradigm, known as acquisition, involves repeated pairings of the drug US to one chamber (CS+), alternated with exposure to other non-drug-paired chamber (CS-). Following the conditioning pairings, a CPP test is given in order to test for chamber preference. The animal is considered to have developed a preference, or CPP, for the drug if it spends an increased amount

of time in the drug-paired chamber relative to the time spent in the other chamber. If there is a preference for the drug-paired chamber it is assumed that the animal has learned the association between the environmental context of the chamber and the drug, and that the drug has rewarding properties. On the other hand, if there is a greater preference for the non-drug paired chamber, the drug could be acting as an aversive stimulus. Therefore, we can consider the CPP paradigm as also being capable of measuring the aversive properties of a given substance. The CPP test is performed in the absence of any drug injections, so as to eliminate any confounds involved with the rewarding properties of the chosen drug. (Aguilar, Rodriguez-Arias, & Minarro, 2009). The CPP paradigm allows us to study the various stages of drug addiction and can involve the acquisition, extinction, and reinstatement of a drug-induced preference. The acquisition phase can be viewed as the development of a preference for the drug and its rewarding values. Extinction is a way to model the loss of this preference for the drug and may help us study drug cravings and drug withdrawals. The reinstatement phase of the CPP paradigm is considered to be a model of drug relapse, which is often seen in addiction.

In addition to being used as a screening tool for the abuse potential of drugs, the CPP paradigm is also an excellent model to study neurotransmitters, brain areas, signaling pathways, and other mechanisms that are involved in mediating the rewarding, or aversive effects of drugs. The neurobiological mechanisms underlying the expression of a CPP have been the focus of a number of addiction research studies. The most basic mechanism appears to be the mesolimbic DA system, which originates in the ventral tegmental area (VTA) and terminates in different limbic structures such as the nucleus accumbens and hippocampus. Other brain regions and pathways thought to be involved in the behaviors seen in CPP for various drugs of abuse are the pre-frontal cortex (PFC) and amygdala (for review, see Hoffman, 1989; and Tzschentke, 2007).

Habituation and CPP

The first phase of the CPP is when the animals undergo a pre-conditioning, or habituation, phase which usually lasts one to three days, and is usually dependent upon the distinguishing characteristics of each chamber of the CPP apparatus; the more distinct they are from one another, the less number of days are needed for the habituation period. This habituation to the CPP apparatus allows free access to all chambers and is typically used as a means of eliminating novelty as a confounding variable in the study. Following the habituation days, a preference test is given to determine a baseline score. A baseline score is determined by measuring the amount of time spent in each of the side chambers of the CPP apparatus in order to see if one of the chambers is initially preferred over the other chamber. This baseline data can then be used to determine the drug-pairing chamber assignments and is also used for comparison with the post-conditioning CPP test probe in determining whether a CPP was developed.

Acquisition of CPP

After habituation and determining the chamber assignment protocol to be used, the conditioning phase begins. Conditioning typically involves alternating days of drug-pairing and vehicle-pairing confinement in the CPP apparatus, which is counterbalanced according to the design of the experiment. On days for drug-pairing the animal is injected with the drug and then immediately confined to the assigned drug-paired chamber of the CPP apparatus. The number of sessions of alternating drug- and saline-paired conditioning trials in order to produce a CPP is dependent upon the reward strength of the drug being used, but is usually anywhere between one and six sessions of drug-pairings. The day after the last day of conditioning, the post-conditioning CPP test is given, which entails placing the animal in the apparatus with free access to both chambers for the same amount of time used to obtain the baseline score. The amount of

time spent in each of the chambers is measured and if the animal spends more time in the drug-paired chamber it can be inferred that the animal has developed a CPP due to the rewarding effects of the drug.

Extinction of a CPP

Another aspect of drug addiction that can be modeled in the CPP paradigm is the process of extinction. Through the process of extinction we are able to directly observe the incentive motivational properties of the drug-paired context, or chamber. Extinction typically involves repeated exposure to the previously paired drug chamber in the absence of the drug followed by a probe test. The probe test consists of allowing the animal free access to both sides of the apparatus and measuring the time spent in each chamber. Extinction is considered to be a decrease in a learned response's intensity or frequency once the US has been removed (Pavlov, 1927), which is the drug used in the CPP procedure. This confinement and probe test sequence can be repeated until there is no significant difference between the times spent in each chamber, which is indicative of the CPP being extinguished.

Reinstatement of a CPP

After the extinction of the initial preference established by the drug, the CPP paradigm is also capable of providing an animal model of drug relapse, called the reinstatement model. This model was originally proposed by Stewart & de Wit (1987), where reinstatement of drug seeking is presumed to occur when a previously drug-reinforced behavior is resumed through exposure to drug or non-drug stimuli after extinction. Their original model was developed using the self-administration paradigm and more recently a reinstatement procedure based on the CPP paradigm has been developed. As described in a review article on the reinstatement of drug relapse, after extinction of the CPP has been established, tests for reinstatement of the CPP are

given after drug injection or exposure to a non-drug stimuli, usually a stressor (Shaham et al., 2003). The two most widely used models are the drug priming- and stress-induced reinstatement procedures in CPP models of relapse. These two methods of reinstatement appear to be mediated by different neural pathways, with stress-induced reinstatement involving the circuit from the medial PFC (mPFC) through the VTA to the shell of the nucleus accumbens; while drug-induced reinstatement involves a more direct pathway from the mPFC to the nucleus accumbens shell. Though mediated by different pathways, both stress- and drug-induced reinstatement involve similar neurotransmitter systems, such as DA, glutamate, opioid, corticotrophin-releasing factor (CRF), and noradrenaline (Aguilar et al, 2009).

Exposure to stress is known to be involved in drug abuse vulnerability in humans and has been utilized after extinction to induce CPP in rats. One of the most common forms of stressors used in the experiments is intermittent footshock exposure. Studies using footshock as a stressor to induce reinstatement of a CPP have been successful for cocaine (Lu et al., 2002), and for morphine (Wang, Fang, Liu, & Lu, 2006).

Through the use of the CPP paradigm in animals such as rats we are able to model various aspects of the addiction process, including the initial rewarding effects of drugs, abstaining from drug use, drug cravings and withdrawal, and relapse. A variety of experimental manipulations may be incorporated into the standard CPP paradigm which is ultimately a measurement of the context-drug associative learning that takes place in the procedure. The susceptibility and vulnerability of acquiring an addiction to drugs is thought to be influenced by a number of factors which can be modeled in the laboratory. An important method used to study addiction with the CPP paradigm in rats is modeling the environmental influences on drug abuse vulnerability. One way of modeling this in the laboratory is using different housing variations for

the rats included in the study, in order to assess the effects of environmental conditions on behavior and learning, both important aspects of addiction.

Environmental Enrichment

What is Environmental Enrichment?

Most behavioral studies using rats as subjects employ a social environmental (SE) housing condition, which is typically 2 rats in a standard sized cage, with no novel or inanimate stimuli. A modification on the SE housing conditions of rats being used in behavioral neuroscience studies is what has been termed environmental enrichment (EE). The typical environment of the enriched housing conditions is that of larger cages with a greater number of rats per cage in order to allow for more complex social interaction. The actual housing structure is complex as well and often consists of tunnels, toys, nesting materials, and running wheels, all of which are varied over the time span of the experiment (van Praag, Kempermann, & Gage, 2000). Though EE paradigms differ significantly among laboratories, EE rats are usually grouped together at approximately two months of age with anywhere between 4 and 12 rats per large cage, which usually consists of multiple levels to encourage optimal locomotion and exploratory activity. The optimal time at which to utilize the enriched environment in rodents is during adolescence when the brain is thought to be most plastic, but exposure of aged mice to an enriched environment can also produce neurological benefits (Brown et al., 2003; Kempermann, Kuhn, & Gage, 1998).

Effects of EE on the Brain

Early studies on the effects of EE on the brains of rats showed an overall increase in weight and thickness in the cerebral cortex, an increase in the size of the hippocampus, an increase in the diameter of the cortical capillaries, and an increase in the number of glial cells

and dendritic branching (Rosenzweig, 1966). Another early study revealed that there is a correlation between complexity of the EE and the increases in cerebral measures, and that it is possible to reverse the effects of an impoverished environment on cerebral effects by later exposing these rats to an EE (Rosenzweig, Krech, Bennett, & Zolman, 1962). According to van Praag et al. (2000), EE in rats has been shown to significantly promote neurogenesis in the dentate gyrus of the hippocampus as well as the pyramidal cells in areas CA1 and CA3 of the hippocampus.

Research has demonstrated that EE-reared rats show superior performance on a variety of learning and memory tasks. A study by Bruel-Jungerman, Laroche, & Rampon (2005) investigated whether the new neurons in the hippocampus resulting from an EE were actually involved in the improved memory performance of these rats. In order to test this they injected rats with 5-bromo-2'-deoxyuridine (BrdU), an immunostain marker that labels newborn dividing cells, in the dentate gyrus of the hippocampus in EE rats and assessed memory performance on an object recognition task. In order to determine that the BrdU+ cells were responsible for enhanced memory performance in EE rats, half of the EE rats were treated with an agent known to reduce neurogenesis. The EE rats not treated with this agent performed significantly better on the memory task than those who were treated with the agent. Their results confirmed that these new dentate granule cells produced during enrichment were indeed critically involved in the enhanced memory performance of the rats.

Another study set out to investigate the underlying physiological changes in the EE rat brain that cause increased neurogenesis in the hippocampus (Segovia, Yague, Garcia-Verdugo, & Mora, 2006). This study used microdialysis in the CA3 area of the hippocampus in EE rats compared with a control group of rats raised in an isolated condition, along with an assessment

of cognitive status using the Morris water maze test. Their results showed that the increase in neurogenesis parallels the improvements in the performance on the Morris water maze test. In addition, EE increased the levels of glutamate and GABA, implicating these neurotransmitter systems in the neurogenesis of hippocampal neurons in the EE rats.

Another important brain region that has been studied through the EE paradigm in rats is the PFC, a region involved in the physiological response to stress and an important component of the mesocortical DA system (Deutch, Clark, & Roth, 1990; Grobin & Detch, 1998). One study was concerned with dopamine transporter (DAT) function in the medial PFC (mPFC) in rats and how this activity is modified by EE (Neuegebauer et al., 2004). This study looked at DAT function in rats that were prenatally exposed to cocaine, which is known to cause alterations in mesocorticolimbic and nigrostriatal DA function, specifically decreased DAT density and function, which in turn increases levels of DA in the mPFC. What they found was that there was decreased DAT density in the mPFC of EE rats compared to isolated condition (IC) rats, but that mPFC DAT function was more efficient in the EE group. These results suggest that the typical effect of prenatal cocaine exposure on mPFC DAT function is attenuated by EE. From a behavioral standpoint, these results would suggest that the typical negative social impact that prenatal exposure to cocaine usually causes in rats could possibly be reversed or attenuated by placing them in an EE. Prenatal cocaine exposure may lead to aggressive, anxious, and anti-social behaviors, often associated with greater drug abuse vulnerability, and could possibly be prevented by later placing the rats in an EE during rearing or adolescence.

Although most research using the EE paradigm has focused on the hippocampus, spatial memory, and learning, there are other brain areas and behavioral models known to be altered by an enriched environment. Studies show that EE rats are more efficient at assimilating stimuli

from their environment than IC rats (Varty, Paulus, Braff, & Geyer, 2000), less sensitive to reward as measured by anticipatory behavior (van der Harst, Baars, & Spruijt, 2003), and less impulsive than IC rats (Wood, Siegel, & Rebec, 2006). It has also been seen that EE enhances learning about contextual cues and reduces the overall fear that is often associated with aversive events (Barbelivien et al., 2006). A study done by Leggio et al. (2005) assessed how EE rats processed spatial information as compared to standard rats and found that EE rats are more efficient at accelerated acquisition, as well as rapid transition from acquisition to consolidation of this spatial information. In the amygdala, the brain region responsible for emotion, EE has been shown to increase the proliferation of progenitor cells in addition to suppressing cell death in this structure in mice (Okuda et al., 2009).

Enriched Environment and Drug Addiction

One way to model the environmental factors of drug abuse vulnerability and the subsequent phases of addiction is through the EE paradigm. According to Caprioli, Celentano, Paolone, & Badiani (2007), there are three major ways in which the environment impacts drug use and addiction. The first is that some life experiences make one more likely to first develop drug addiction and increase the likelihood of relapse. The next is that there can be neutral cues in the environment which are capable of becoming associated with drugs, and therefore may later trigger one to seek drugs. The third way is when the environment in which one takes a drug alters the subjective, behavioral, and rewarding effects of that drug, causing it to later influence the individual to take the drug again (Caprioli et al., 2007). Since the environment is a major factor contributing to the chances of developing an addiction in humans, manipulation of an animal's environment provides researchers with an opportunity to study this aspect of addiction in the laboratory. In the preclinical research laboratory rodents are commonly used subjects and

the two primary addiction models employed are the self-administration and CPP paradigms.

Three different types of environmental manipulations are typically used: isolated environment (IE), SE, and EE. In an IE, animals are raised in an isolated setting or cage with no social interaction or enriching stimuli. In an SE, animals are housed 2-4 per cage with no enriching stimuli. In an EE, animals are exposed to enriching stimuli, as well as having social interaction. Although various types of commonly abused drugs and other addictive substances have been studied, the most extensive research has been on psychostimulants and opiates.

Environmental Manipulation and the Effects of Psychostimulants

Several studies have compared the effects of IE, SE, and EE on psychostimulant drugs and researchers have focused most of their behavioral studies on cocaine and amphetamine. In self-administration studies it has been shown that IE rats have increased self-administration compared to SE rats for cocaine (Schenk, Lacelle, & Amit, 1987). The CPP studies have shown that IE rats are less sensitive, or less likely to develop a CPP, than SE rats to the rewarding properties of cocaine (Berry & Marsden, 1994), amphetamine (Wongwitdecha & Marsden, 1995), and methamphetamine (Gehrke, Cass, & Bardo, 2006).

The effect of EE on self-administration of drugs has been investigated and consistently shows that EE rats self-administer less than IE rats for amphetamine (Bardo, Klebauer, Valone, & Deaton, 2001; Green, Gehrke, & Bardo, 2002) and for cocaine (Howes, Dalley, Morrison, Robbins, & Everitt, 2000). These results suggest that EE may have reduced the reinforcing effect of these drugs.

The CPP experiments have shown that EE rats have an increased sensitivity to the rewarding effects of amphetamine (Bardo, Bowling, Rowlett, & Manderscheid, 1995; Bowling & Bardo, 1994) and cocaine (Green et al., 2009). The study by Bardo et al. (1995) tested three

different doses of amphetamine (.1, .3, and 1.0 mg/kg) in EE and IE rats and found increased preference ratios at all doses in the EE rats. This suggests that at these low dosages of amphetamine, EE rats are more sensitive to the drug's rewarding effects.

In a study using cocaine, Green et al. (2009) tested for both self-administration and CPP in EE and IE rats. They found that the EE rats self-administered less cocaine than the IE rats, but the EE rats demonstrated a stronger CPP. One implication of this study is that EE may decrease addiction liability, as assessed by the reduction in self-administration, without decreasing the drug sensitivity, as measured by the increased CPP.

It is important to note that not all the findings of studies using cocaine are consistent. Zakharova, Miller, Unterwald, & Izenwasser (2009) studied the effects of cocaine on rats using six different environmental conditions, differing in levels of social and environmental enrichment. Their analyses showed that additional rats and/or increased environmental enrichment of the home cage decreased the cocaine CPP. These results suggest that the conditioned rewarding effects of cocaine are inversely related to the degree of enrichment. These conflicting results between Green et al. (2009) and Zakharova et al. (2009) could be due to the age of the animals or differences in experimental design and procedures. A summary of the results of these studies investigating the effects of EE exposure on CPP and self-administration for psychostimulants and opiates is provided in Table 1.

Environmental Manipulation and the Effects of Opiates

Studies of self-administration and opiates have shown that IE rats have increased self-administration compared to SE for both heroin (Bozarth, Murray, & Wise, 1989) and morphine (Alexander, Coombs, & Hadaway, 1978; Kostowski, Czlonkowski, Rewerski, & Piechoki, 1977). CPP studies have shown IE rats to be less sensitive or less likely to develop a CPP than

SE rats to the rewarding properties of morphine (Wongwitdecha & Mardsen, 1996).

After searching the literature, no studies were found applying the EE paradigm to opiate self-administration. However, studies have looked at EE with regards to opiate CPP. A study by Bardo, Robinet, & Hammer (1997) compared the effects of various doses of morphine (0, .1, 1, and 10 mg/kg) on EE and IE rats. They used a wide range of morphine doses since it was not clear what the optimal dose was yielding differences between EE and IE rats. As determined by the results of the CPP test, the morphine produced a dose-dependent preference for the drug-paired compartment in both the EE and IE rats; with the magnitude of the CPP being significantly greater in EE rats relative to IE rats. This suggests that the primary rewarding effects for all doses of morphine used was greater in the EE rats. These findings complement those using psychostimulants (Bowling & Bardo, 1994; Green et al., 2009) making it a possibility that EE may alter some neural mechanism activated similarly by repeated stimulation of psychostimulants and opiates.

Contradicting these findings in rats (Bardo et al., 1997), a study done by Xu, Hou, Gao, He, & Zhang (2007) found that mice reared in EE were less sensitive to the rewarding effects of morphine. The study compared EE and SE mice at a 5 mg/kg dose of morphine and found that the CPP was blocked by environmental enrichment, as the EE mice showed no CPP for the morphine paired compartment, while SE mice did. These contradictory results for mice and rats may have been due to differences in experimental design, but most likely is the result of species differences.

One study in rats examined the effects of EE on the sensitivity to various mu-opioids with different efficacies at the mu-opioid receptor (Smith et al., 2005). The substances used were morphine and levaorphanol (higher-efficacy drugs) and buprenorphine, butorphanol, and

nalbuphine (lower-efficacy drugs). They found that the higher-efficacy drugs produced a CPP in both EE and IE rats, but that the lower-efficacy drugs only produced a CPP in the EE rats. These results indicate that EE rats are more sensitive to the rewarding effects of low-efficacy mu-opioids and that the differences between the EE and IE rats may be mediated by the functional alterations in opioid receptor population cause by the environmental manipulation.

Another study examining the effects of EE in rats and the effects on opioid receptor systems was performed by Smith, Bryant, & Mc Clean (2003). They used spiradoline, a kappa-opioid receptor agonist in their CPP experiment on EE and IE rats. Kappa-opioid receptor agonists are known to have aversive stimulus effects and their results found that EE rats were more sensitive than IE rats to spiradoline, indicated by a higher aversion to the drug-paired compartment. These results demonstrate that in addition to mu-opioid receptors, the kappa-opioid receptor system is also sensitive to environmental manipulation. Through the use of the CPP paradigm, animals exposed to EE also show differences in the aversive effects of opiates, not only the appetitive ones.

Table 1.*Summary of Self-administration (SA) and Conditioned Place Preference (CPP) Findings in Rats.*

Authors	Paradigm	Drug	Effect
Howes, Dalley, Morrison, Roberts, and Everitt, 2000	SA	Cocaine	EE < IE
Bardo, Klebauer, Valone, & Deaton, 2001	SA	Amphetamine	EE < IE
Green, Gehrke, & Bardo, 2002	SA	Amphetamine	EE < IE
Kostowski, Czlonkowski, Rewerski, & Piechoki, 1977	SA	Morphine	IE > SE
Green et al., 2009	CPP	Cocaine	EE > IE
Zakharova, Miller, Unterwald, & Izenwasser, 2009	CPP	Cocaine	EE < IE and SE
Bardo, Bowling, Rowlett, & Manderscheid, 1995	CPP	Amphetamine	EE > IE
Bowling & Bardo, 1994	CPP	Amphetamine	EE > IE
Bardo, Robinet, & Hammer, 1997	CPP	Morphine	EE > SE
Smith et al., 2005	CPP	Morphine	EE > SE
Xu, Hou, Gao, He, & Zhang, 2007	CPP	Morphine	EE < SE *

Note. IE= Isolated Environment. SE= Social Environment. EE = Enriched Environment. *This study was performed on mice.

A review of the literature turned up no studies implementing both the EE and CPP paradigms with studies incorporating the three phases of morphine addiction discussed above (acquisition, extinction, and reinstatement). Given the variety of results seen in previous studies a pilot study was conducted using the conditioned place preference paradigm in order to determine

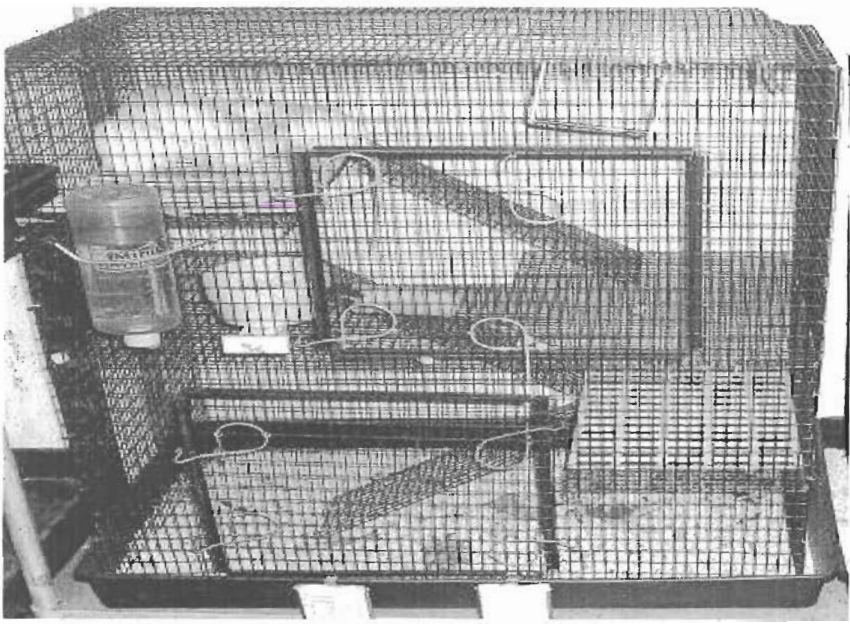
the effects of an enriched environment on morphine acquisition, extinction, and stress-induced reinstatement in rats.

Pilot Study

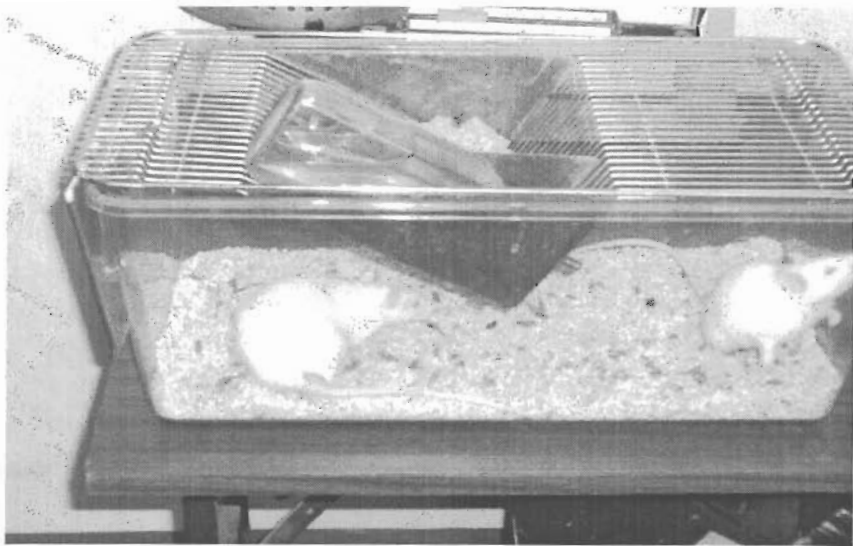
Methods

Subjects

Seventeen male Sprague-Dawley albino rats were used as the subjects. The rats were acquired at six weeks of age and one week after arrival were randomly assigned to either the SE (n=9) or EE (n=8) housing condition. Photographs of both EE and SE housing are shown in Figure 1. The rats were raised in their assigned environments for six weeks before being used as subjects in a fear conditioning and REM deprivation study (Nicaretta & Hunter, 2010). In that study, all rats were exposed to ten CS-US fear conditioning trials, after which half of the rats were exposed to a single six hour session of REM sleep deprivation and half were exposed to a control condition. Following conditioning, all rats were exposed to three separate days of extinction training, which consisted of ten trials of the CS being presented alone. Following this study, all of the rats were run in a visual version of the Morris water maze, consisting of four trials a day for four days. Data collection for the CPP pilot study began 19 weeks after the initial placement of the rats in their assigned housing conditions. Since arrival, the room housing the rats was kept on a 12:12 hour light-dark cycle, with lights on at 8am, and all rats received food and water *ad libitum*.



A.



B.

Figure 1. Photographs of EE (A) and SE (B) housing conditions.

In the present study the acquisition and extinction phases of the experiment consisted of four groups: EE + low dose of morphine, n=4, EE + high dose of morphine, n=4, SE + low dose of morphine, n=5, and SE + high dose of morphine, n=4. The reinstatement phase of the experiment consisted of 8 groups, with each of the four groups being divided into half receiving a footshock and the other half not receiving a footshock. All groups had two rats, except the SE + low dose of morphine + footshock group, which had three. All procedures were approved by the Seton Hall University Institutional Animal Care and Use Committee.

Drugs

Morphine sulfate salt pentahydrate was a generous gift from Dr. Sulie Chang, and was obtained from Sigma-Aldrich Chemical Corporation (St. Louis, MO, US). The morphine sulfate was dissolved in saline and administered subcutaneously in a volume of 1.0 ml/kg of body weight. The two dosages used in the present study were 3 mg/kg morphine for the low dose and 7 mg/kg morphine for the high dose.

Apparatus

The conditioned place preference (CPP) apparatus used, as shown in Figure 2, was a 30'' L x 12'' W x 12'' H Plexiglas box. This box was divided into three separate chambers. The two end conditioning chambers of the apparatus were 12'' L x 12'' W x 12'' H. The middle chamber of the apparatus was 6'' L x 12'' W x 12'' H. There were two guillotine doors dividing the middle chamber from each of the two end chambers that were used during the CPP testing, allowing the animals access to the entire chamber. The guillotine doors each had a 4'' H x 4 ¾'' W opening. In order to distinguish them from each other, one of the end chambers had a black double striped design against a white background and the other end chamber had a black circular bull's eye design against a white background. For the CPP experiments, two of these CPP boxes

were placed on a table in a dimly lit room, with a video camera mounted above them recording the animals' behavior.

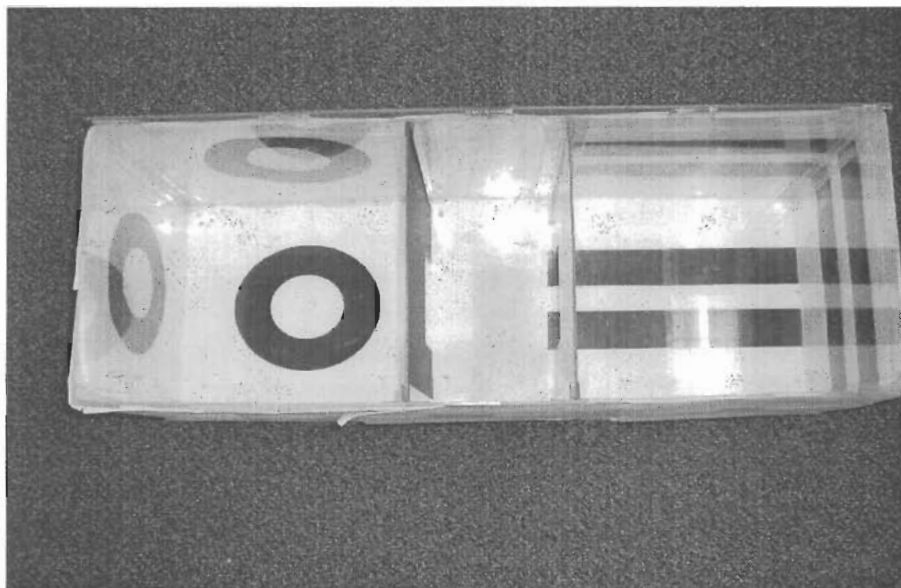


Figure 2. Photograph of the CPP apparatus.

The apparatus used for the stress-induced reinstatement phase of the study was the same as described by Silvestri (2005). Two conditioning chambers made up of Plexiglas sides and a metal grid floor were located in a dimly lit room with activity monitored by a video camera. The dimensions of each conditioning chamber were 9.1'' x 7.1'' x 9.3''. The stress-inducing footshocks were presented via the metal grid floors of the apparatus. Footshocks were produced via an ENV-414 shocker/distributor (MED Associates Inc., Georgia, VT). A computer program using MED-PC (MED Associates Inc., Georgia, VT) controlled the footshock presentations.

Procedure

A diagram of the procedure is shown in Figure 3. During the pre-conditioning phase, rats were given one 15-minute habituation session on the first day, then a 15-minute baseline testing

session on the second day. During each of these two sessions they were allowed free access to the entire chamber. In these sessions, rats were placed in the center compartment and the guillotine doors were placed in the opened position allowing access to both side chambers. Time spent in each side chamber and was measured using a stopwatch and sessions were recorded via the overhead video camera. Entrance into a chamber was recorded when both front and hind legs passed completely through a doorway. The time spent in each side chamber during the baseline testing session was used to determine a baseline score and those rats showing a preference or bias for one chamber were drug-paired to the non-preferred chamber for the conditioning phase. Those rats showing no preference for either chamber as indicated by their baseline score, were randomly assigned as to which chamber (circle or stripe) would be drug-paired.

Ten conditioning trials took place. During these trials, rats were weighed each morning and injected with either their assigned dose of morphine or saline and confined to the assigned chamber of the apparatus for 30 minutes. Morphine and saline administration alternated daily so that each rat received five conditioning trials with their assigned morphine dose and five conditioning trials with saline.

On the day following the last conditioning trial, the existence of a conditioned place preference for each rat was assessed. For this CPP test, rats were placed in the center compartment with both guillotine doors in the opened position. The rats were given free access to the entire chamber for 15 minutes and the time spent in each chamber was measured and summed over the 15 minute test period. A preference score was computed by taking the time spent in the morphine-paired chamber minus the time spent in the saline-paired chamber, as measured in seconds. A positive score indicated a preference for the morphine-paired chamber, while a negative score indicated a preference for the saline-paired chamber.

The extinction phase of the experiment began the day following the CPP test. The purpose of the extinction phase was to rid the rats of their preference for the drug-paired chamber. Extinction involved confinement to only the drug-paired chamber, in the absence of the drug for 30 minutes for two consecutive days. Every third day a probe test was given for each rat, which allowed them free access to the entire chamber for 15 minutes, as in the CPP test. The time spent in each side chamber was measured and recorded over this 15 minute period to see if the preference they had developed for the drug was extinguished. Overall, there were ten extinction sessions and five probe tests conducted in order to determine extinction of the preference.

The reinstatement phase of the experiment occurred exactly one week after the last day of the extinction phase. This phase used a form of stress-induced reinstatement, in which half of the rats were exposed to footshock stressor and the other half a control condition, where they were placed in the footshock chamber without being administered the footshock. During this part of the reinstatement phase, rats were placed in the footshock chamber, as described above, for 45 minutes and they either received or did not receive 10 unsignaled shocks of 0.8 mA of 0.5 second duration. Immediately following this 45 minute exposure to the chamber, rats were again placed in the CPP apparatus for 15 minutes of free access to the entire chamber. The time spent in each of the side chambers was measured and recorded to assess for preference.

DAYS	1 and 2	3-12	13	14-28	1 WEEK DELAY	35
PHASE	PRE- CONDITIONING Habituation (15 min) and Baseline Testing (15 min)	CONDITIONING 5 morphine, 5 saline pairings (30 min each)	CPP TEST (15 min)	EXTINCTION 10 training sessions (30 min each) and 5 probe tests (15min each)		REINSTATEMENT 35 min in footshock chamber followed by 15 min CPP test

Figure 3. Procedure of pilot study.

Results

No inferential statistical tests were calculated due to the small number of rats in each group.

Acquisition

The results from the acquisition phase of the experiment were measured by the CPP test and are shown in Figure 4. As shown, both the EE and SE rats administered the 3 mg/kg dose of morphine appear to have developed a CPP for the morphine, with the EE rats showing a stronger preference than the SE rats. For rats given the 7 mg/kg dose of morphine, a preference did not develop in either the EE or SE condition. The EE rats given the 7 mg/kg dose of morphine appear to show a slight aversion to the morphine-paired chamber.

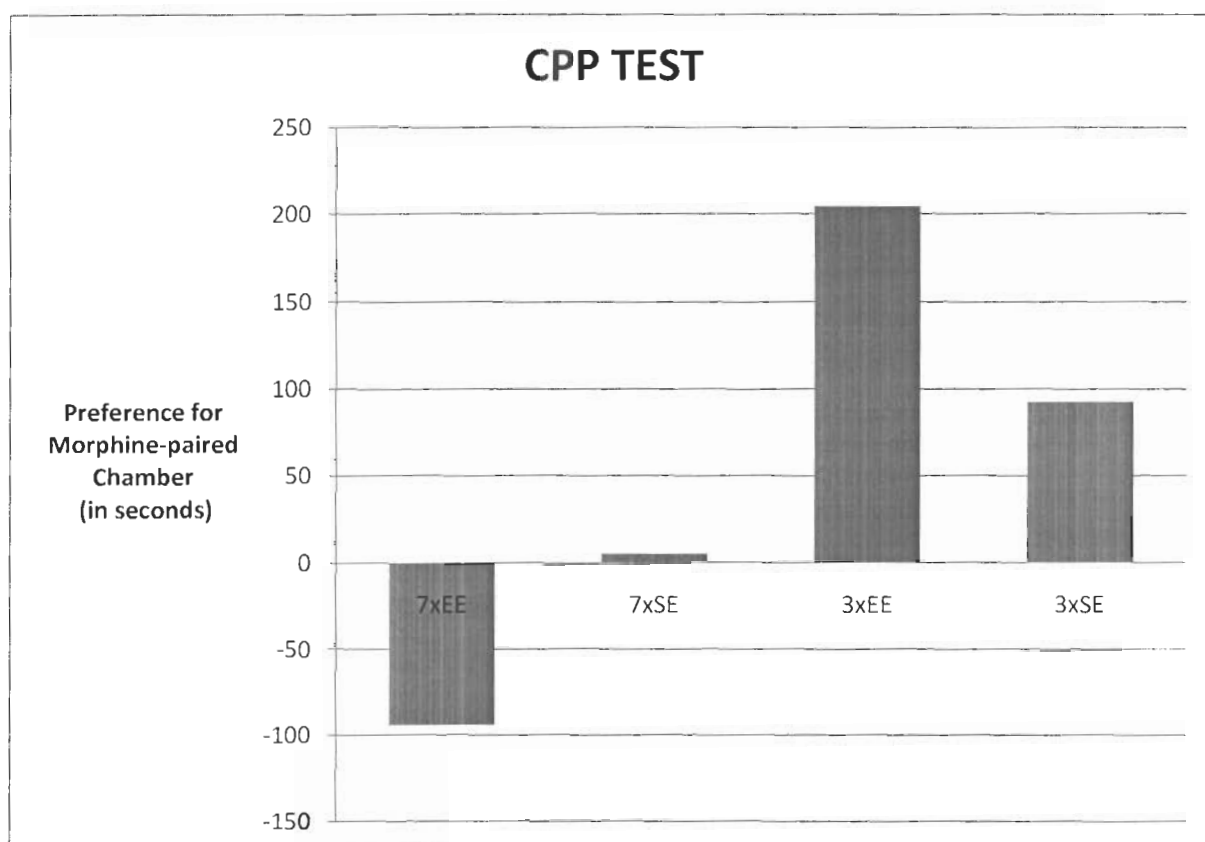


Figure 4. Results of pilot study CPP test.

Extinction

The results from the extinction phase of the experiment are shown in Figure 5. The results from the first probe indicate all four groups as showing a preference at this time for the morphine-paired chamber, with EE rats showing a stronger preference than the SE rats. It is interesting to note that the EE and SE rats given the 7 mg/kg dose of morphine, which did not show a preference during the CPP test, now appear to show a CPP to the morphine-paired

chamber. As can be seen in the graph, the CPP for morphine appears to be extinguished in all four groups by the day of the third probe. The results of the fourth probe test reveal an increase in the preference for the morphine-paired chamber in all four groups while this preference decreased by the fifth probe test. The reasons for the results of the fourth and fifth probes are unclear but could be due to confounding factors such as an increase in noise outside the testing room the day these probes took place.

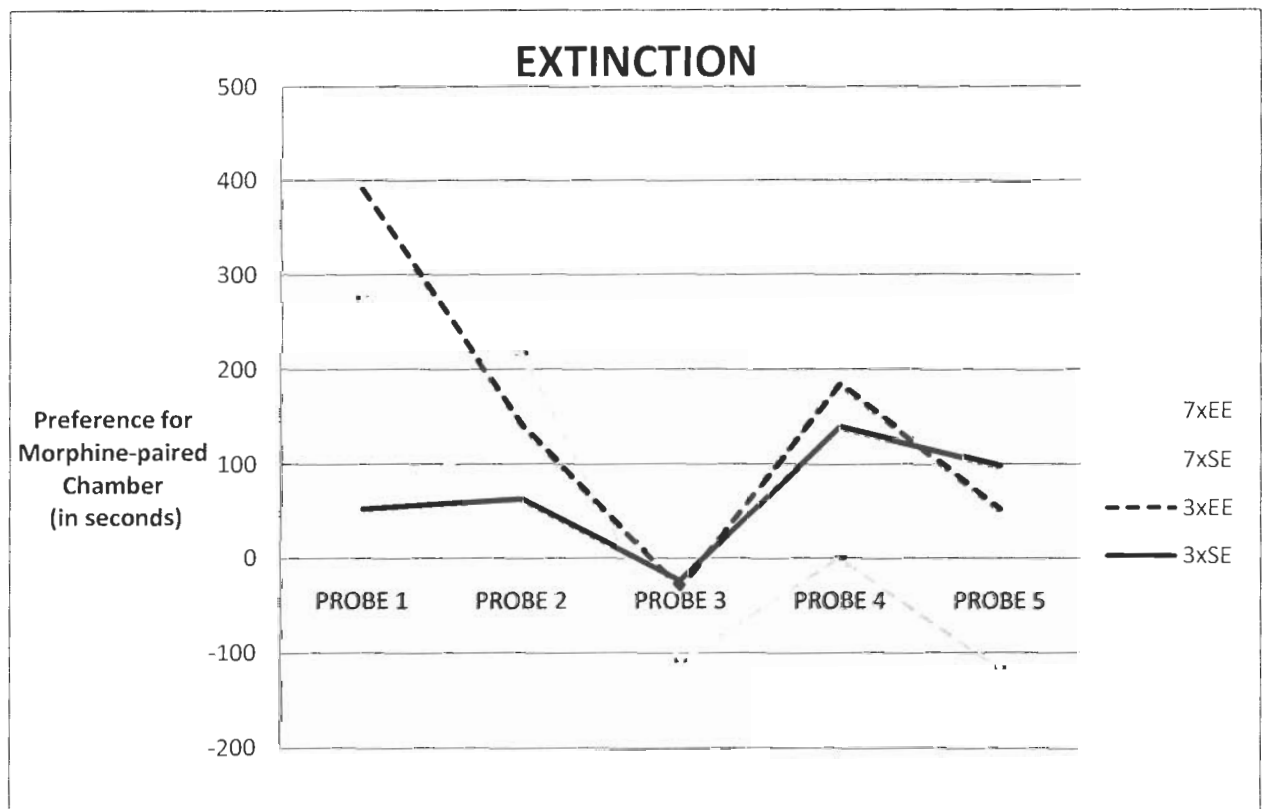


Figure 5. Results of pilot study extinction.

Reinstatement

The results from the reinstatement phase of the experiment are shown in Figure 6. All eight groups of rats seem to have reinstated their CPP for the morphine-paired chamber. The two groups showing the strongest preference are the SE rats at both doses of morphine that received the footshock as a stressor (7xSE_{shock} and 3xSE_{shock}). The SE rats given the 3 mg/kg dose of morphine plus the footshock showed a stronger preference than the SE rats given the 3 mg/kg dose of morphine without the footshock, indicating that the stress-inducing footshock was capable of reinstating the preference. This difference between the shock and no-shock condition can also be seen in the SE rats given the 7 mg/kg dose of morphine. For EE rats receiving the 3 mg/kg of morphine, the preference was reinstated both with and without the footshock, but there was a stronger preference for those in the no shock condition. In the EE rats at the 7 mg/kg dose of morphine, the preference was reinstated, though the strength of this preference did not seem to be dependent on the stress-inducing footshock, as it did at both doses in the SE condition.

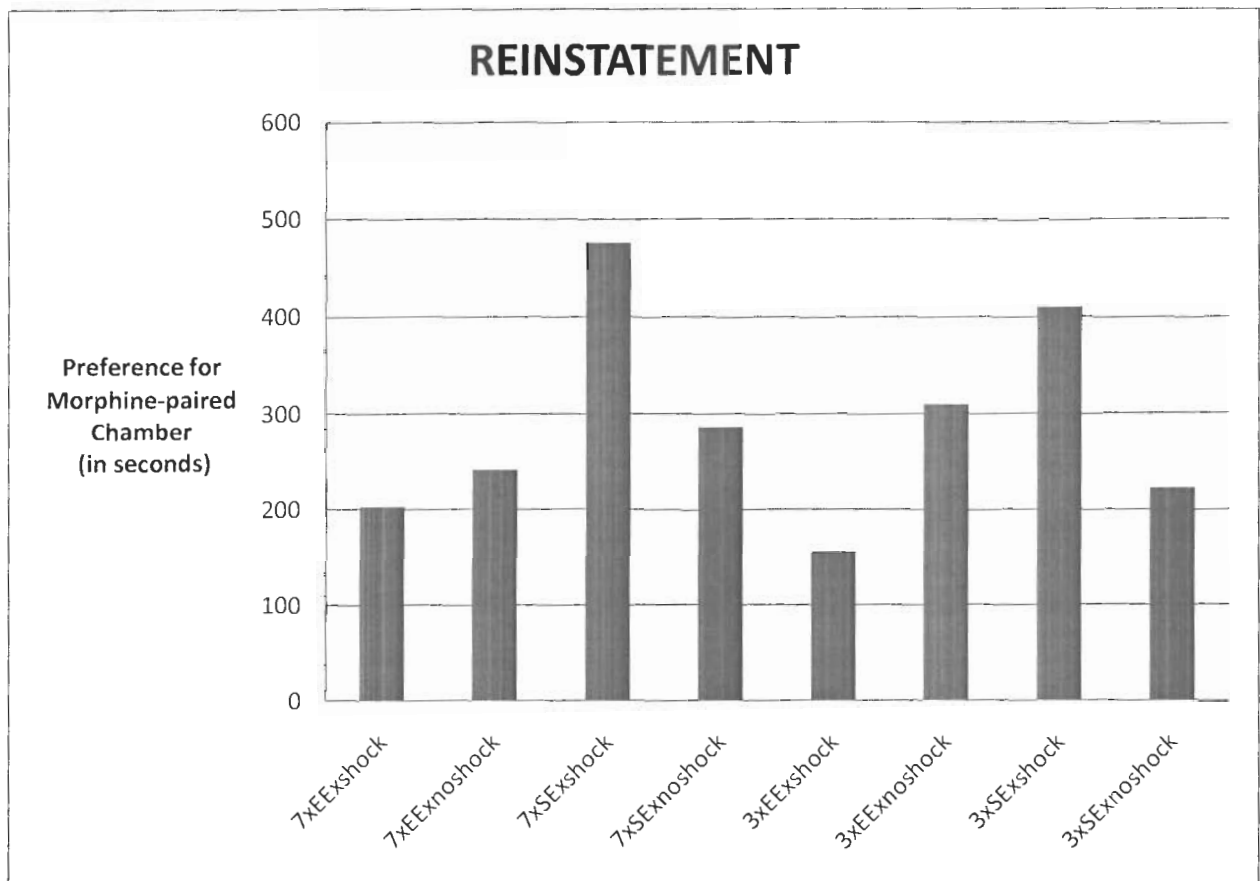


Figure 6. Results of pilot study reinstatement.

Discussion

With regards to the acquisition of a CPP for morphine, it appears that at the 3 mg/kg dose, the EE rats demonstrated a stronger preference than the SE rats did for the morphine paired context, which suggests that EE rats may be more sensitive to the rewarding effects of morphine at this dose. The fact that the 7 mg/kg dose of morphine did not induce a CPP in either the EE or SE rats may suggest that this dose may be too high when looking at the rewarding properties of the drug when associated with a particular context.

Since all four groups showed a preference for the morphine-paired chamber in the first

extinction probe, another possible explanation for this could be that the 7 mg/kg dose of morphine caused negative withdrawal symptoms, which were not induced by the 3 mg/kg dose, making the morphine-paired chamber slightly aversive at first to the rats given the 7 mg/kg dose during the CPP testing.

The results from the reinstatement phase allow us to assess how a stress-inducing event impacts the re-establishment of a preference, and allows us to compare this impact between the low and high doses of morphine, and, more importantly, comparing this impact on rats raised in an EE to rats raised in a SE. We have shown that the footshock does indeed reinstate the preference for the morphine-paired chamber, as evidenced by the stronger preference of the SE rats given the 3 mg/kg morphine dose with a footshock than those that did not receive the footshock. The same was also true for SE rats at the 7 mg/kg dose, further strengthening this argument. In order to examine the effects of an enriched environment, we can compare both doses of morphine in the SE condition receiving no shock to the EE rats receiving no shock. In comparing the no shock condition, there does not appear to be much difference between EE and SE rats at either of the doses. The effect of environmental influences on stress-induced reinstatement is shown in the results of those rats that received a footshock. The footshocked SE rats show a much stronger preference than the footshocked EE rats. This difference between the footshocked SE and EE rats was observed at both doses of morphine. These results suggest that at both the high and low morphine doses, raising rats in an enriched environment may have a protective effect against stress-induced reinstatement of the CPP. This is suggestive of environmental enrichment having an impact on structures involved in the stress systems of the brain, especially those involved in relapse to drugs and their rewarding properties.

Based on these results, the following changes to the study methods were made. First, two

extinction probe, another possible explanation for this could be that the 7 mg/kg dose of morphine caused negative withdrawal symptoms, which were not induced by the 3 mg/kg dose, making the morphine-paired chamber slightly aversive at first to the rats given the 7 mg/kg dose during the CPP testing.

The results from the reinstatement phase allow us to assess how a stress-inducing event impacts the re-establishment of a preference, and allows us to compare this impact between the low and high doses of morphine, and, more importantly, comparing this impact on rats raised in an EE to rats raised in a SE. We have shown that the footshock does indeed reinstate the preference for the morphine-paired chamber, as evidenced by the stronger preference of the SE rats given the 3 mg/kg morphine dose with a footshock than those that did not receive the footshock. The same was also true for SE rats at the 7 mg/kg dose, further strengthening this argument. In order to examine the effects of an enriched environment, we can compare both doses of morphine in the SE condition receiving no shock to the EE rats receiving no shock. In comparing the no shock condition, there does not appear to be much difference between EE and SE rats at either of the doses. The effect of environmental influences on stress-induced reinstatement is shown in the results of those rats that received a footshock. The footshocked SE rats show a much stronger preference than the footshocked EE rats. This difference between the footshocked SE and EE rats was observed at both doses of morphine. These results suggest that at both the high and low morphine doses, raising rats in an enriched environment may have a protective effect against stress-induced reinstatement of the CPP. This is suggestive of environmental enrichment having an impact on structures involved in the stress systems of the brain, especially those involved in relapse to drugs and their rewarding properties.

Based on these results, the following changes to the study methods were made. First, two

squads of 17 rats were run at separate times to increase the number of rats to eight or nine per group. Since the most important variable being investigated is the housing condition of the rats and how an EE affects the various stages of the CPP paradigm for morphine, only the 3 mg/kg dose of morphine was used. One reason for using the 3 mg/kg dose is that it was sufficient in inducing a CPP in both the EE and SE rats. Another reason for using the 3 mg/kg dose is that it was a high enough dose to observe the differences found between EE and SE rats' response to the stress-inducing footshock during the reinstatement phase. Finally, the 7 mg/kg morphine dose appeared to be somewhat aversive as indicated in the CPP test. The last change in the study is to the extinction phase of the experiment, in which six extinction sessions and three probe tests were administered. The reasons for this change was that the pilot study had shown this number of sessions and probe tests to be sufficient in extinguishing the initial preference and because of the inconsistent and unexplainable results observed in the fourth and fifth probe tests.

Method

Subjects

A total of 34 naïve male Sprague-Dawley rats were used as the subjects, and were run in two separate squads of 17 rats. The rats were acquired at six weeks of age and randomly assigned to either the SE (n=17) or EE (n=17) housing condition. Rats were raised in their assigned environments for at least six weeks prior to commencement of the study. The room housing the rats was kept on a 12:12 hour light-dark cycle, with lights on at 8am, and all rats received food and water *ad libitum*. All procedures were approved by the Seton Hall University Institutional Animal Care and Use Committee.

The acquisition and extinction phases of the experiment consisted of two groups: EE, n=17, and SE, n=17. The reinstatement phase of the experiment consisted of four groups: EE + no footshock, n=8, EE + footshock, n=9, SE + no footshock, n=8, and SE + footshock, n=9.

Drugs

Morphine sulfate salt pentahydrate was a generous gift from Dr. Sulie Chang, and was obtained from Sigma-Aldrich Chemical Corporation (St. Louis, MO, US). The morphine sulfate was dissolved in saline and a dose of 3 mg/kg was administered subcutaneously in a volume of 1.0 ml/kg of body weight.

Apparatus

The conditioned place preference (CPP) apparatus used, as shown in Figure 2, was the same as described in the pilot study. Two of these CPP boxes were placed on a table in a dimly lit room, with a video camera mounted above them recording the animals' behavior. A television in the room generated a background white noise.

The apparatus used for the stress-induced reinstatement phase of the study was the same

as described in the pilot study.

Procedure

A diagram of the procedure can be seen in Figure 7. During the pre-conditioning phase, rats were given one 15-minute habituation session on the first day, then a 15-minute baseline testing session on the second day. During each of these two sessions they were allowed free access to the entire chamber. During each, rats were placed in the center compartment and the guillotine doors were placed in the opened position allowing access to both side chambers. Time spent in each side chamber and was measured using a stopwatch and sessions were recorded via the overhead video camera. Entrance into a chamber was recorded when both front and hind legs passed completely through a doorway. The purpose of the habituation session was to acclimate rats to the apparatus and to remove novelty as a confounding factor. The time spent in each side chamber during the baseline testing session was used to determine a baseline score and those rats showing a preference for one chamber were drug-paired to the non-preferred chamber for the conditioning phase. Approximately seven of the EE rats showed an initial preference for one chamber, and only 2 of the SE rats showed an initial preference for one chamber. Those rats showing no preference for either chamber as indicated by their baseline score were randomly assigned as to which chamber (circle or stripe) would be drug-paired.

DAYS	1 and 2	3-12	13	14-22		29
PHASE	PRE- CONDITIONING Habituation (15 min) and Baseline Testing (15 min)	CONDITIONING 5 morphine, 5 saline pairings (30 min each)	CPP TEST (15 min)	EXTINCTION 6 training sessions (30 min each) and 3 probe tests (15min each)	1 WEEK DELAY	REINSTATEMENT 35 min in footshock chamber followed by 15 min CPP test

Figure 7. Timeline and procedure of experiment.

Ten conditioning trials took place. During these trials, rats were weighed each morning and injected with either morphine or saline and confined to the predetermined chamber of the apparatus for 30 minutes. Morphine and saline administration alternated daily so that each rat received five conditioning trials each of morphine and saline.

On the day following the last conditioning trial, the existence of a conditioned place preference was assessed. For this CPP test, rats were placed in the center compartment with both guillotine doors in the opened position. The rats were given free access to the entire chamber for 15 minutes and the time spent in each chamber was determined.

The extinction phase of the experiment began the day following the CPP test. The purpose of the extinction phase was to rid the rats of their preference for the drug-paired chamber. Extinction training sessions involved confinement to only the drug-paired chamber, in the absence of the drug for 30 minutes for two consecutive days. Every third day a probe test was given. During the probe test, each rat was allowed free access to the entire chamber for 15 minutes, as in the CPP test. The time spent in each side chamber was measured and recorded

over this 15 minute period. This sequence was repeated three times for a total of six extinction sessions and three probe tests.

The reinstatement phase of the experiment occurred exactly one week after the last day of the extinction phase. This phase used a form of stress-induced reinstatement, in which half of the rats were exposed to a footshock stressor. During this part of the reinstatement phase, rats were placed in the footshock chamber for approximately 35 minutes and received 10 unsignaled shocks (0.8 mA, 0.5 sec). Control rats were placed in the same chamber for 35 minutes but no footshocks were presented. Immediately following footshock exposure or the control condition, rats were again placed in the CPP apparatus for 15 minutes of free access to the entire chamber. The time spent in each of the side chambers was determined to assess for preference.

Results

CPP Test

Due to the amount of time rats spent in the center chamber of the CPP apparatus, the previously used dependent variable of preference scores showed a great degree of variability. Therefore, in order to reduce this variability, preference ratio scores were computed and used for statistical analyses. The preference ratio score was computed by dividing the time spent in the morphine-paired chamber by the time spent in the saline-paired chamber. A score of one on this measure indicates no preference for either chamber. A score greater than one on this measure indicates a preference for the morphine-paired chamber. Rats with preference ratio scores below one were dropped from all statistical analyses, as this indicated they did not acquire a preference for the morphine-paired chamber at the time of the initial CPP test following conditioning. A score below one could also indicate that those rats may have developed an aversion to the morphine-paired chamber. Using this as the criteria, a total of 12 of the initial 34 rats were dropped, leaving 11 EE rats and the 11 SE rats. The data from baseline testing revealed that there were no differences between the dropped rats and the non-dropped rats with respect to a pre-existing preference for one chamber. There were also no differences found during baseline testing between the EE and SE rats.

In order to assess the strength of the preference for the morphine-paired chamber, within subjects t-tests comparing the observed preference ratio score to 1 were calculated. Results indicated that there was a significant preference for the morphine-paired chamber in both the EE [$t(10) = 6.316, p < .05$] and SE [$t(10) = 4.853, p < .05$] groups (Figure 8). Calculation of Cohen's d revealed large effect sizes for EE ($d = 1.90$) and SE ($d = 1.46$) rats. A between-subjects t-test using the preference ratio scores indicated that EE rats had a significantly greater

preference than the SE rats for the morphine-paired chamber [$t(20) = 2.267, p < .05$]. This also had a large effect size ($d = .98$).

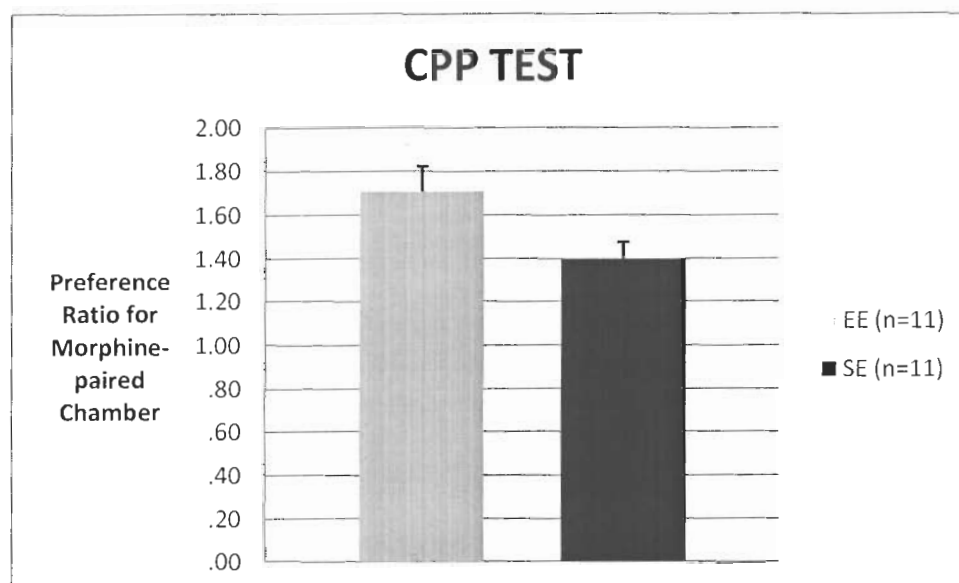


Figure 8. Results of CPP test.

Extinction

Calculation of a paired samples t-test revealed that the SE rats extinguished their preference from the CPP test by probe one [$t(10) = 4.018, p < .01$]; while the EE rats extinguished their preference from the CPP test by probe two [$t(10) = 2.271, p < .05$; Figure 9). Calculation of a repeated measures 2 x 3 (housing x probe trials) ANOVA revealed a trend toward a significant main effect of housing [$F(20) = 2.417, p = .051, \eta_p^2 = .177$], with EE rats showing a stronger preference for the morphine paired chamber. This ANOVA revealed no significant main effect of trials and no significant interaction (all p 's $> .551$).

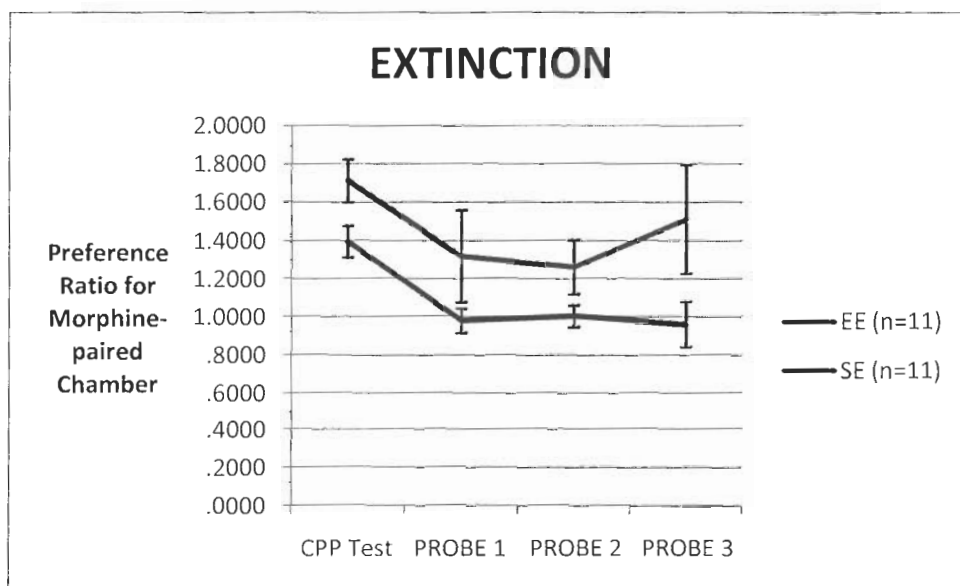


Figure 9. Results of extinction probe test.

Reinstatement

Calculation of a 2 X 2 (housing x shock) ANOVA on reinstatement data revealed no significant main effect of housing, no significant main effect of shock condition, and no significant interaction (all p 's > .120; Figure 10). Calculation of an independent samples t-test indicated a trend for the SE shock rats to reinstate their preference for the morphine-paired chamber [$t(9) = 2.074, p = .068$], indicating marginal effectiveness of the shock in reinstating the CPP. However, an independent samples t-test indicated that the shock had no significant effect on reinstatement of preference in the EE rats ($p > .1$).

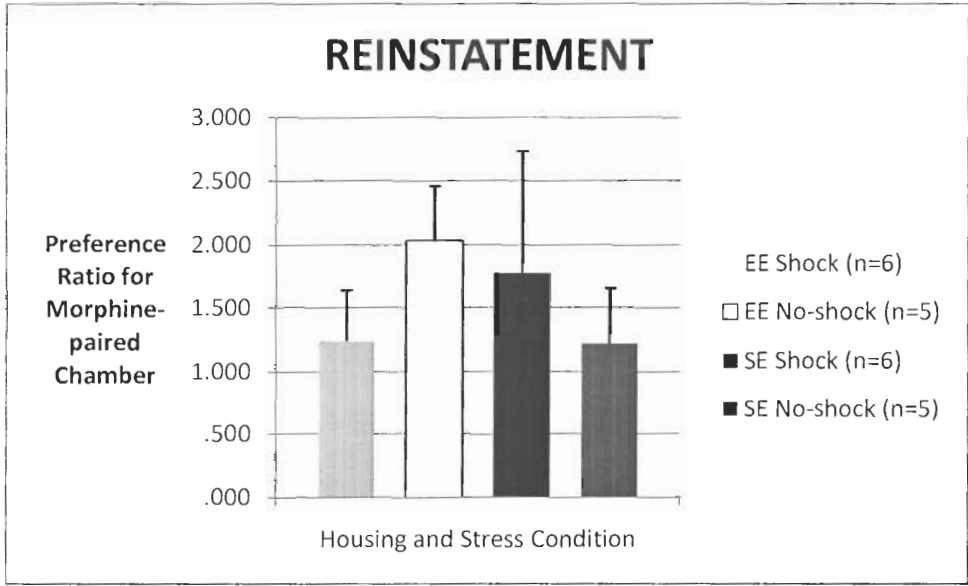


Figure 10. Results of reinstatement.

Discussion

The results of the current study indicated that a 3 mg/kg dose of morphine and a 10 day conditioning procedure was capable of inducing a CPP in most EE and SE rats. When comparing the EE and SE rats that demonstrated the morphine CPP, the magnitude of this preference was significantly greater in EE rats compared to SE rats. The SE rats extinguished their preference from the CPP test by probe one, while the EE extinguished their preference by probe two. This suggests similar extinction rates since the EE rats showed a stronger preference at the CPP test than the SE rats, which would necessitate additional trials to reach a similar level of extinction. A stress-inducing footshock produced a trend towards reinstatement of the preference for the morphine-paired chamber in SE rats. EE rats, on the other hand, did not show this preference induced by the footshock; neither the EE shocked nor EE non-shocked rats showed a significant reinstated preference for the morphine paired chamber.

As seen in both the pilot and present study, at a dose of 3 mg/kg of morphine, most of the EE and SE rats acquired a preference for the morphine-paired chamber following conditioning. Although, unlike the pilot study, not all of the rats in the present study developed this preference for the morphine-paired chamber, which could have been due to a number of factors including individual differences among the rats, an aversion to the morphine, or possibly that the dose of morphine was not high enough in order for them to develop a preference. Both studies also demonstrate that EE rats show stronger acquisition of this initial preference than SE rats. In the pilot study, both EE and SE rats required a longer amount of time to extinguish this initial preference than in the present study. Although in both studies EE and SE rats extinguished at similar rates, rats in the pilot study did not extinguish until the third probe test, while the SE rats were extinguished by the time of the first probe test and the EE rats extinguished by the second

probe in the present study. While the pilot study used older rats that had been subjected to numerous experimental procedures, the present study used naïve, younger rats. Therefore it is possible that the older rats used in the pilot study showed a learning deficit compared to the younger rats, as they took longer to extinguish the preference for the morphine-paired chamber. During reinstatement in the pilot study, it appeared that all groups reinstated their preference, and that SE rats were more susceptible to the footshock, as they showed stronger preference than the non-shocked SE rats. This effect of footshock was not seen in pilot study EE rats. In the present study, none of the groups showed a significant preference during reinstatement, although there was a trend seen in shocked SE rats to reinstate. The differences seen between the pilot and present study may be due to the differences in ages and exposure to other experimental procedures, as well as the variability inherent in the CPP. Additionally, it is difficult to compare the two studies since the low number of rats in the pilot study did not allow for inferential statistics to be performed.

Since the CPP paradigm is considered a technique to measure the rewarding properties of drugs, the results of the present study suggests that raising rats in a more socially stimulating and enriched environment may make them more sensitive to the rewarding properties of morphine than rats raised in a standard, or social, environment. These results are consistent with prior literature. For example, Bardo, Robinet, & Hammer (1997) used various doses of morphine and found that CPP magnitude was stronger at all doses in the EE rats compared to IE rats. It is important to note that this study differed from the present study in that they compared EE to IE, not SE, rats. While this may seem to be problematic to use as a comparison, it has been shown that SE rats are more sensitive and more likely to develop a morphine CPP than IE rats in numerous studies (Schenk et al., 1983; Wongwitdecha & Madsen, 1996; Coudereau et al. 1997).

This finding, together with the present results, indicates that as the level of enrichment in housing conditions is increased, the magnitude of morphine CPP also increases. This housing effect is also seen in CPP studies with psychostimulants. Studies on cocaine CPP have shown that EE rats show stronger CPP than IE rats (Green et al., 2009) and that SE rats show stronger CPP than IE rats (Berry & Mardsen, 1994). It would be useful for future studies to determine the specific component of the EE paradigm that contributes to the differences in sensitivity to drugs of abuse.

Besides EE rats showing an increased sensitivity to morphine, it is also possible that EE rats learned to associate the drug-paired chamber with the morphine better than the SE rats; in other words, that the EE rats learned this context (chamber)/US (morphine) association better than did the SE rats. It has been shown in numerous studies that raising rats in EE increases their performance on a variety of learning and memory tasks, especially spatial and contextual learning tasks, such as in a drug CPP paradigm. Rats that are raised in EE show a significant increase in neurogenesis in the hippocampus, especially in regions such as the dentate gyrus and the pyramidal cells in areas CA1 and CA3 (van Praag et al., 2000). It is these neuronal changes in the hippocampus that may be responsible for the fact that EE rats showed stronger acquisition of the morphine CPP than the SE rats, and this difference is possibly due to greater learning abilities of the EE rats.

Although the present study found differences in acquisition of morphine CPP in EE and SE rats, both groups appeared to show similar rates in extinguishing this preference for the morphine-paired chamber. Since extinction is considered to be a procedure involving new learning rather than unlearning of the original preference (Rescorla, 2001), the results suggest that EE and SE rats may differ in their learning abilities of specific tasks. The EE rats appeared

to show better learning in the acquisition phase than the SE rats, while both the EE and SE rats appeared to learn at similar rates in the extinction phase. Another possibility is that during conditioning and acquisition of the morphine CPP, the EE and SE rats differ in their sensitivity to the rewarding properties of morphine, rather than differing in their ability to learn the context-US association.

Reinstatement using the CPP paradigm is considered to be an animal model of relapse that is often seen in drug addiction. The present study used a stress-inducing footshock in an attempt to reinstate the preference for the morphine-paired chamber. Using footshock as a stressor to induce reinstatement in the CPP paradigm has been successful in previous studies (for example, Lu et al., 2002; Wang et al., 2006). There was a trend for the shocked SE rats to reinstate their preference for the morphine-paired chamber, while no such effect was seen in the non-shocked SE rats. One possible explanation for why rats did not reinstate their preference could be that the footshock was not stressful enough to them. This explanation could be ruled out by administering a stronger shock in future studies to determine if morphine preference would be reinstated. It is also possible that extinction was learned stronger than necessary for this type of reinstatement procedure to be successful. Since the SE rats were already extinguished by the first probe test and the EE rats were extinguished by the second probe in the present study, the additional extinction training sessions may have strengthened this learning to a point at which it rendered the stress-induced reinstatement procedure unsuccessful. To determine whether overlearning of extinction was a factor in the failure of reinstatement in the present study, a future study could use fewer extinction training sessions. Neither the shocked nor non-shocked EE rats showed a reinstated preference. These results may have important implications because it appears that raising rats in an EE may have a protective effect against these stress-inducing

footshocks. However, the possibility that EE rats did not reinstate because of these reasons mentioned above for the SE rats cannot be ruled out.

In a review of the reinstatement model of drug relapse, Shaham et al. (2003) state that there are two important brain systems involved in footshock-induced reinstatement: the noradrenaline system and, more importantly for the present study, the brain stress hormone corticotrophin-releasing factor (CRF) system. Unsignaled footshock has been shown to increase plasma corticosterone levels, which is a stress hormone controlled by the brain's hypothalamo-pituitary-adrenocortical (HPA) axis. It is possible that raising rats in an EE may have an impact on this HPA stress axis, which may be the brain system upon which EE exerts its protective effects. EE rats also appeared to be more resistant to the stress-inducing footshocks than the SE rats. Thus, raising rats in an EE may increase their ability to cope with stress, possibly due to a blunting of the HPA stress axis in the brain. Consistent with this explanation, Belz et al. (2003) have also demonstrated that EE is associated with lower levels of stress-response hormones in rats.

The main limitation to the present study is the low number of rats in each group, especially since approximately one-third of the rats used did not develop a morphine CPP and therefore were dropped from the statistical analyses. Another possible limitation of the study was the use of SE rats instead of IE rats for comparison. Some studies have shown that comparing EE and IE rats is a better option since these two populations exhibit the greatest between-group differences in sensitivity to psychotropic drugs (Bowling & Bardo, 1994). The reason for using SE, as opposed to IE, rats in the present study is primarily due to the default housing conditions in our laboratory. Although space limitations are also a factor, our laboratory uses SE housing as the standard for our rats since the guidelines of the National Research Council (2011) suggests

that the appropriate living environment for social animals, such as rats, is housing them in stable pairs or groups in order to account for the animals' social needs. Another reason for using SE as opposed to IE rats is that isolation housing of rats has proven to produce stress in the animals (Barnard & Hou, 1988), therefore SE rats would serve as a better control condition for the present study.

In a review of CPP, Bardo & Bevins (2000) give a thorough discussion of the major advantages and disadvantages in using this paradigm in preclinical studies of drug reward. The disadvantages mentioned include the fact that CPP is subject to interpretation due to the issue of novelty seeking, that it does not provide substantial graded dose-effect curves which can be applied pharmacologically, and that it lacks face validity as a protocol for human studies of addiction. Although CPP is a good model for contextual conditioning of drug effects that are important to the relapse and craving seen in addiction, it lacks a true discrete operant response. Even considering these disadvantages, the CPP paradigm is both an important and useful tool in providing information about the rewarding effects of contextual cues that are associated with a drug stimulus.

Future research that would expand on this study's results could be the addition of an IE group of rats. In doing so, this would allow for the investigation of whether it is the social or the environmental factors that contribute to the differences seen in housing conditions in drug sensitivity. Studies like these could also include another spatial learning task, such as the Morris water maze, in order to determine what role learning plays in morphine CPP in the different housing conditions. Similar studies to the present one could also increase the dose of morphine or increase the number of conditioning sessions in order to produce a stronger CPP. A stronger footshock could also be implemented in order to increase the probability of stress reinstating the

initial preference. Studies that look at EE and morphine CPP could also compare a stress-induced and drug-primed model of the reinstatement phase in order to determine if the effects seen for the stress-inducing footshock in the present study are also found in a drug-primed reinstatement. Results of studies such as these could determine if EE does in fact have a protective effect from the footshock stressor, as drug-primed reinstatement relies on different brain regions and systems. With the knowledge of an enriched environment's impact on important brain structures involved in the morphine CPP, specifically the acquisition, extinction, and reinstatement processes, future research could utilize the EE paradigm to investigate the behavioral effects of morphine addiction. Particular brain structures of interest would be the amygdala, hippocampus, nucleus accumbens, and the prefrontal cortex, as they are all affected by an enriched environment manipulation.

In summary, the present study demonstrated that a 3 mg/kg dose of morphine produced a CPP in both EE and SE rats, and that the strength of this preference was greater in the EE rats. The housing condition of the rats did not affect the rates at which this preference was extinguished. Using a stress-inducing footshock in an attempt to reinstate the morphine CPP, the study demonstrated that housing rats in an EE may have protective effects against this stressor. Further research is necessary to investigate how EE may influence the HPA stress axis in the brain, and the role that EE plays in blocking the footshock-induced reinstatement of morphine CPP. If we are able to locate the precise neural mechanisms underlying EE-induced changes in behavior, these findings could lead to the development of better pharmacological and behavioral therapies to treat opiate addiction and prevent relapse.

References

- Aguilar, M.A., Rodriguez-Arias, M., & Minarro, J. (2009). Neurobiological mechanisms of the reinstatement of drug-conditioned place preference. *Brain Research Reviews*, *59*, 253-277.
- Alexander, B., Coombs, R., & Hadaway, P. (1978). The effect of housing and gender on morphine self-administration in rats. *Psychopharmacology*, *58*(2), 175-179. doi: 10.1007/BF00426903.
- Barbelivien, A., Herbeaux, K., Oberling, P., Kelche, C., Galani, R., & Majchrzak, M. (2006). Environmental enrichment increases responding to contextual cues but decreases overall conditioned fear in the rat. *Behavioural Brain Research*, *169*(2), 231-238. doi:10.1016/j.bbr.2006.01.012.
- Bardo, M.T., & Bevins, R.A. (2000). Conditioned place preference: what does it add to our preclinical understanding of drug reward? *Psychopharmacology*, *153*:1, pp. 31-43.
- Bardo, M., Bowling, S., Rowlett, J., & Manderscheid, P. (1995). Environmental enrichment attenuates locomotor sensitization, but not in vitro dopamine release, induced by amphetamine. *Pharmacology, Biochemistry and Behavior*, *51*(2-3), 397-405. doi: 10.1016/0091-3057(94)00413-D.
- Bardo, M.T., Klebaur, J.E, Valone, J.M., & Deaton, C. (2001). Environmental enrichment decreases intravenous self-administration of amphetamine in female and male rats. *Psychopharmacology*, *155*, 278-284.

- Bardo, M.T., Robinet, P.M., & Hammer Jr., R.F. (1997). Effect of differential rearing environments on morphine-induced behaviors, opioid receptors and dopamine synthesis. *Neuropharmacology*, *36*(2), 251-259.
- Barnard, N., & Hou, S. (1988). Inherent stress: the tough life in lab routine. *Laboratory Animals*, *17*: 21-7.
- Belz, E. E., Kennell, J. S., Czambel, R., Rubin, R. T., & Rhodes, M. E. (2003). Environmental enrichment lowers stress-responsive hormones in singly housed male and female rats. *Pharmacology, Biochemistry and Behavior*, *76*(3-4), 481-486.
doi:10.1016/j.pbb.2003.09.005
- Berry, M.I., & Marsden, C.A. (1994). Rats reared in social isolation fail to show place preference to cocaine. *J. Psychopharmacology BAP Meeting Suppl.* A36
- Bowling, S., & Bardo, M. (1994). Locomotor and rewarding effects of amphetamine in enriched, social, and isolate reared rats. *Pharmacology, Biochemistry and Behavior*, *48*(2), 459-464. doi: 10.1016/0091-3057(94)90553-3.
- Bozarth, M., Murray, A., & Wise, R. (1989). Influence of housing conditions on the acquisition of intravenous heroin and cocaine self-administration in rats. *Pharmacology, Biochemistry and Behavior*, *33*(4), 903-907. doi: 10.1016/0091-3057(89)90490-5.
- Brown, J., Cooper-Kuhn, C., Kempermann, G., van Praag, H., Winkler, J., Gage, F., et al. (2003). Enriched environment and physical activity stimulate hippocampal but not olfactory bulb neurogenesis. *European Journal of Neuroscience*, *17*(10), 2042-2046.
doi:10.1046/j.1460-9568.2003.02647.x.

- Bruel-Jungerman, E., Laroche, S., & Rampon, C. (2005). New neurons in the dentate gyrus are involved in the expression of enhanced long-term memory following environmental enrichment. *European Journal of Neuroscience*, *21*(2), 513-521. doi:10.1111/j.1460-9568.2005.03875.x.
- Caprioli, D., Celentano, M., Paolone, G., & Badiani, A. (2007). Modeling the role of environment in addiction. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *31*, 1639-1653.
- Compton, W., Thomas, Y., Conway, K., & Colliver, J. (2005). Developments in the epidemiology of drug use and drug use disorders. *The American Journal of Psychiatry*, *162*(8), 1494-1502. doi:10.1176/appi.ajp.162.8.1494.
- Coudereau, J., Monier, C., Bourre, J., & Frances, H. (1997). Effect of isolation on pain threshold and on different effects of morphine. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *21*(6), 997-1018. doi:10.1016/S0278-5846(97)00094-8
- Crabbe, J. (2002). Genetic contributions to addiction. *Annual Review of Psychology*, *53*(1), 435-462. doi:10.1146/annurev.psych.53.100901.135142.
- Deutch, A., Clark, W., & Roth, R. (1990). Prefrontal cortical dopamine depletion enhances the responsiveness of mesolimbic dopamine neurons to stress. *Brain Research*, *521*(1-2), 311-315. doi: 10.1016/0006-8993(90)91557-W.
- Everitt, B. J. & Robbins, T.W. (2005). Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. *Nature Neuroscience*. Vol.1, no.11, 1481-1489.

- Gehrke, B., Cass, W., & Bardo, M. (2006). Monoamine-depleting doses of methamphetamine in enriched and isolated rats: Consequences for subsequent methamphetamine-induced hyperactivity and reward. *Behavioural Pharmacology*, *17*(5-6), 499-508. doi: 10.1097/00008877-200609000-00016.
- Green, T.A., Alibhai, I.N., Roybal, C.N., Winstanley, C.A., Theobald, D.E.H., Birnbaum, S.G., . . . Nestler, E.J. (2009). Environmental enrichment produces a behavioral phenotype mediated by low cyclic adenosine monophosphate response element binding (CREB) activity in the nucleus accumbens. *Biological Psychiatry*, *67*, 28-35.
- Green, T.A., Gehrke, B.J., & Bardo, M.T. (2002). Environmental enrichment decreases intravenous amphetamine self-administration in rats: dose-response functions for fixed- and progressive-ratio schedules. *Psychopharmacology*, *162*, 373-378.
- Grobin, A. & Deutch, A. (1998). Dopaminergic regulation of extracellular γ -aminobutyric acid levels in the prefrontal cortex of the rat. *The Journal of Pharmacology and Experimental Therapeutics*, *285*(1), 350-357. Retrieved from PsycINFO database.
- Hoffman, D. (1989). The use of place conditioning in studying the neuropharmacology of drug reinforcement. *Brain Research Bulletin*, *23*(4-5), 373-387. doi:10.1016/0361-9230(89)90224-4.
- Howes, S., Dalley, J., Morrison, C., Robbins, T., & Everitt, B. (2000). Leftward shift in the acquisition of cocaine self-administration in isolation-reared rats: Relationship to extracellular levels of dopamine, serotonin and glutamate in the nucleus accumbens and amygdala-striatal FOS expression. *Psychopharmacology*, *151*(1), 55-63. doi:10.1007/s002130000451.

- Gehrke, B., Cass, W., & Bardo, M. (2006). Monoamine-depleting doses of methamphetamine in enriched and isolated rats: Consequences for subsequent methamphetamine-induced hyperactivity and reward. *Behavioural Pharmacology*, *17*(5-6), 499-508. doi: 10.1097/00008877-200609000-00016.
- Green, T.A., Alibhai, I.N., Roybal, C.N., Winstanley, C.A., Theobald, D.E.H., Birnbaum, S.G., . . . Nestler, E.J. (2009). Environmental enrichment produces a behavioral phenotype mediated by low cyclic adenosine monophosphate response element binding (CREB) activity in the nucleus accumbens. *Biological Psychiatry*, *67*, 28-35.
- Green, T.A., Gehrke, B.J., & Bardo, M.T. (2002). Environmental enrichment decreases intravenous amphetamine self-administration in rats: dose-response functions for fixed- and progressive-ratio schedules. *Psychopharmacology*, *162*, 373-378.
- Grobin, A. & Deutch, A. (1998). Dopaminergic regulation of extracellular γ -aminobutyric acid levels in the prefrontal cortex of the rat. *The Journal of Pharmacology and Experimental Therapeutics*, *285*(1), 350-357. Retrieved from PsycINFO database.
- Hoffman, D. (1989). The use of place conditioning in studying the neuropharmacology of drug reinforcement. *Brain Research Bulletin*, *23*(4-5), 373-387. doi:10.1016/0361-9230(89)90224-4.
- Howes, S., Dalley, J., Morrison, C., Robbins, T., & Everitt, B. (2000). Leftward shift in the acquisition of cocaine self-administration in isolation-reared rats: Relationship to extracellular levels of dopamine, serotonin and glutamate in the nucleus accumbens and amygdala-striatal FOS expression. *Psychopharmacology*, *151*(1), 55-63. doi:10.1007/s002130000451.

- Hunter, A.S. & Nicaretta, J. (2010, April). *REM sleep deprivation and fear conditioning extinction: exposure to an enriched environment*. Poster presented at the annual meeting of the Eastern Psychological Association, Brooklyn, NY.
- Jaffe, J. (1990). Trivializing dependence. *British Journal of Addiction*, 85(11), 1425-1427.
doi:10.1111/j.1360-0443.1990.tb01624.x.
- Kauer, J.A. & Malenka, R.C. (2007). Synaptic plasticity and addiction. *Nature Reviews: Neuroscience*. 8, 844-858
- Kempermann, G., Kuhn, H., & Gage, F. (1998). Experience-induced neurogenesis in the senescent dentate gyrus. *The Journal of Neuroscience*, 18(9), 3206-3212.
- Koob, G.F. & Le Moal, M. (2008). Addiction and the brain anti-reward system. *Annual Review of Psychology*, 59, 29-53.
- Kostowski, W., Członkowski, A., Rewerski, W., & Piechocki, T. (1977). Morphine action in grouped and isolated rats and mice. *Psychopharmacology*, 53(2), 191-193.
doi:10.1007/BF00426491.
- Leggio, M., Mandolesi, L., Federico, F., Spirito, F., Ricci, B., Gelfo, F., & Petrosini, L. (2005). Environmental enrichment promotes improved spatial abilities and enhanced dendritic growth in the rat. *Behavioural Brain Research*, 163(1), 78-90.
doi:10.1016/j.bbr.2005.04.009.
- Lu, L., Xu, N., Ge, X., Yue, W., Su, W., Pei, G., & Ma, L.(2002). Reactivation of morphine conditioned place preference by drug priming: role of environmental cues and sensitization. *Psychopharmacology*, 159, 125-132.

- National Research Council (U.S.), Institute for Laboratory Animal Research (U.S.), & National Academies Press (U.S.). (2011). *Guide for the care and use of laboratory animals*. Washington, D.C.: National Academies Press.
- Neugebauer, N., Cunningham, S., Zhu, J., Bryant, R., Middleton, L., & Dwoskin, L. (2004). Effects of environmental enrichment on behavior and dopamine transporter function in medial prefrontal cortex in adult rats prenatally treated with cocaine. *Developmental Brain Research*, 153(2), 213-223. doi:10.1016/j.devbrainres.2004.09.001.
- Okuda, H., Tatsumi, K., Makinodan, M., Yamauchi, T., Kishimoto, T., & Wanaka, A. (2009). Environmental enrichment stimulates progenitor cell proliferation in the amygdala. *Journal of Neuroscience Research*, 87(16), 3546-3553. doi:10.1002/jnr.22160.
- Pavlov, I.P. (1927). *Conditioned Reflexes*. Oxford University Press, London.
- Rescorla, R.A. (2001). Experimental Extinction. In: Mowrer, R.R., Klein, S.S., eds. *Handbook of Contemporary Learning Theories*. Mahwah, N.J.: Erlbaum. P. 119-54.
- Rosenzweig, M. (1966). Environmental complexity, cerebral change, and behavior. *American Psychologist*, 21(4), 321-332. doi:10.1037/h0023555.
- Rosenzweig, M., Krech, D., Bennett, E., & Zolman, J. (1962). Variation in environmental complexity and brain measures. *Journal of Comparative and Physiological Psychology*, 55(6), 1092-1095. doi:10.1037/h0042758.
- Russo, S. J., Dietz, D.M., Dumitriu, D., Morrison, J.H., Malenka, R.C., & Nestler, E.J. (2010). The addicted synapse: mechanisms of synaptic and structural plasticity in nucleus accumbens. *Trends in Neuroscience*, 33(6), 1-10. doi: 10.1016/j.tins.2010.02.002

- Schenk, S. S., Hunt, T. T., Klukowski, G. G., & Amit, Z. Z. (1987). Isolation housing decreases the effectiveness of morphine in the conditioned taste aversion paradigm. *Psychopharmacology*, 92(1), 48-51. doi:10.1007/BF00215478
- Schenk S., Lacelle G., Gorman K., Amit Z. (1987). Cocaine self-administration in rats influenced by environmental conditions: Implications for the etiology of drug abuse. *Neuroscience Letters*, 81 (1-2), pp. 227-231.
- Segovia, G., Yagüe, A., García-Verdugo, J., & Mora, F. (2006). Environmental enrichment promotes neurogenesis and changes the extracellular concentrations of glutamate and GABA in the hippocampus of aged rats. *Brain Research Bulletin*, 70(1), 8-14. doi:10.1016/j.brainresbull.2005.11.005.
- Shaham, Y., Shalev, U., Lu, L., de Wit, H., & Stewart, J. (2003). The reinstatement model of drug relapse: History, methodology and major findings. *Psychopharmacology*, 168(1-2), 3-20. doi:10.1007/s00213-002-1224-x.
- Silvestri, A.J. (2005). REM sleep deprivation affects extinction of cued but not contextual fear conditioning. *Physiology & Behavior*, 84, 343-349.
- Smith, M. A., Bryant, P. A., & McClean, J. M. (2003). Social and environmental enrichment enhances sensitivity to the effects of kappa opioids: studies on antinociception, diuresis, and conditioned place preference. *Pharmacology, Biochemistry, and Behavior*, 76, 93-101.
- Smith, M.A., Chisholm, K.A., Bryant, P.A., Greene, J.L., Mc Clean, J.M., Stoops, W.W., & Yancey, D.L. (2005). Social and environmental influences on opioid sensitivity in rats:

- importance of an opioid's relative efficacy at the mu-receptor. *Psychopharmacology*, *181*, 27-37.
- Stewart, J. & de Wit, H. (1987). Reinstatement of drug-taking behaviors a method of assessing incentive motivational properties of drugs. In: Bozarth MA (ed) *Methods of assessing the reinforcing properties of abused drugs*. Springer-Verlag, New York, pp 211–227.
- Substance Abuse and Mental Health Service Administration. *Results from the 2009 National Survey on Drug Use and Health: Volume I- Summary of national Findings*. Rockville, MD: Office of Applied Studies; 2010.
- Tzschentke, T. (2007). Measuring reward with the conditioned place preference (CPP) paradigm: Update of the last decade. *Addiction Biology*, *12*(3-4), 227-462. doi:10.1111/j.1369-1600.2007.00070.x.
- van der Harst, J., Baars, A., & Spruijt, B. (2003). Standard housed rats are more sensitive to rewards than enriched housed rats as reflected by their anticipatory behaviour. *Behavioural Brain Research*, *142*(1-2), 151-156. doi:10.1016/S0166-4328(02)00403-5.
- van Praag, H., Kempermann, G., & Gage, F.H. (2000). Neural consequences of environmental enrichment. *Nature Reviews*, *1*, 191-198.
- Varty, G., Paulus, M., Braff, D., & Geyer, M. (2000). Environmental enrichment and isolation rearing in the rat: Effects on locomotor behavior and startle response plasticity. *Biological Psychiatry*, *47*(10), 864-873. doi:10.1016/S0006-3223(99)00269-3.
- Wang, J., Fang, Q., Liu, Z., & Lu, L. (2006). Region-specific effects of brain corticotropin-releasing factor receptor type 1 blockade on footshock-stress- or drug-priming-induced

- reinstatement of morphine conditioned place preference in rats. *Psychopharmacology*, 185, 10-28.
- Wood, D., Siegel, A., & Rebec, G. (2006). Environmental enrichment reduces impulsivity during appetitive conditioning. *Physiology & Behavior*, 88(1-2), 132-137.
doi:10.1016/j.physbeh.2006.03.024.
- Wongwitdecha, N. & Marsden, C. A. (1995). Isolation rearing prevents the reinforcing properties of amphetamine in a conditioned place preference paradigm. *European Journal of Pharmacology*, 279, 99-103.
- Wongwitdecha, N., & Marsden, C. (1996). Effect of social isolation on the reinforcing properties of morphine in the conditioned place preference test. *Pharmacology, Biochemistry and Behavior*, 53(3), 531-534. doi:10.1016/0091-3057(95)02046-2.
- Xu, Z., Hou, B., Gao, Y., He, F., & Zhang, C. (2007). Effects of environmental enrichment on morphine-induced reward in mice. *Experimental Neurology*, 204, 714-719.
- Zakharova, E., Miller, J., Unterwald, E., Wade, D., & Izenwasser, S. (2009). Social and physical environment alter cocaine conditioned place preference and dopaminergic markers in adolescent male rats. *Neuroscience*, 163, 890-897.
- Zhou, Y., Proudnikov, D., Yuferov, V., & Kreek, M. (2010). Drug-induced and genetic alterations in stress-responsive systems: Implications for specific addictive diseases. *Brain Research*, 1314, 235-252. doi:10.1016/j.brainres.2009.11.015.