The Effects of Unstable Social Environment and Trait Anxiety on Extinction and Reinstatement Behaviors of Rats Addicted to Cocaine

Ryan Barnes

Spring 2004

Mentor: Dr. Barbara Sorg

Department of Veterinary Comparative Anatomy Pharmacology and Physiology

Honors Thesis

PASS WITH DISTINCTION
TO THE UNIVERSITY HONORS COLLEGE:

As thesis advisor for Ryan D. Barnes

I have read this paper and find it satisfactory.

Thesis Advisor

12 Feb '04
Table of Contents

I. Introduction
   a. Individual and societal effects of drug abuse (pg 1)
   b. Distribution tendencies of drug/cocaine users (pg 2)
   c. Extinction/Withdrawal and Reinstatement/Relapse (pg 3)
   d. The fight against drug use (pg 4)
   e. Stress and cocaine abuse (pg 5)
   f. Anxiety and drug use (pg 5)
   g. Proposal (pg 6)

II. Methodology
   a. Elevated plus maze (pg 8)
   b. Conditioned Place preference (pg 8)

III. Results (pg 9)

IV. Discussion (pg 10)
List of Figures

I. Figure 1: Means of Plus Maze Score

II. Figure 2: Anxiety Distributions

III. Figure 3: CPP Behavior: First 9 Days

IV. Figure 4: CPP Behavior: Last 4 Days
Précis

The topic of stress and drug addiction is widely studied because it is known that stress greatly increases chances of cocaine relapse in cocaine addicts. Using rat models, scientists have shown that stress reinstates cocaine-seeking behavior in rats. This reinstatement to cocaine-seeking behavior is dependant on the stress hormone corticosterone and brain stress hormone receptors. Repeated stressors alter the regulation of the stress system components but, to our knowledge, no one has studied the effects of repeated stressors on cocaine addiction.

Anxiety symptoms often accompany cocaine use and withdrawal. In rats, anti-anxiety drugs, such as benzodiazepines, decrease stress-induced reinstatement to cocaine, thus suggesting a role of anxiety in drug addiction.

A better understanding of the role of stress and trait anxiety in drug addiction may lead to effective behavioral and therapeutic treatments that can help drug addicts abstain from drug use. To better understand the role of stress and trait anxiety in cocaine addiction, we investigated the effects of an unstable social environment, meant to act as a repeated stressor, and trait anxiety on cocaine extinction rates and reinstatement behavior of rats. We used the elevated plus maze experiment to determine trait anxiety and then we performed conditioned place preference to determine the effects of unstable social environment and trait anxiety on cocaine addiction in rats.

We saw a significant effect of anxiety on cocaine-induced reinstatement as high anxiety rats reinstated less than low anxiety rats. Additionally, high anxiety rats in an unstable social environment failed to reinstate to an odor stressor while the low anxiety rats in an unstable social environment and control rats both reinstated to the odor stressor.
The effect was not strong enough for a significant difference between the groups, however.

Our results suggest that anxiety plays a role in cocaine-induced reinstatement of cocaine-seeking behavior. Anxiety seems to affect stress-induced reinstatement only when rats are in an unstable social environment. This may reflect different coping strategies of high and low anxiety rats in response to an unstable social environment. It is possible that high anxiety rats had a greater stress response to unstable social environment than low anxiety rats and this could affect stress-induced reinstatement.
Introduction

**Individual and Societal Effects of Drug Abuse**

Drug abuse manifests throughout the world and results in serious consequences for not only individuals but also, society as a whole. Society is affected with economic burdens due to loss of work productivity, drug related crime, health care costs, and social welfare costs. In 1998 alone, drug abuse exhausted a substantial $150 billion and experts predict $160 billion for 2000. These costs affect both taxpayers and the families of addicts, as they are responsible for the costs.

In addition to its detrimental effects on the economy and society, drug abuse harms the individual. Individuals and their families’ lives have been financially, socially, physically, and mentally ruined by drug addiction. Financially, drug addicts struggle to find jobs and are financially bankrupted through drug costs. Socially, they lose friends and family thus deteriorating their social network. Physically, they suffer from numerous physical and mental health problems. Cocaine abuse, in particular, leads to serious health side effects. The most prominent short-term side effects of cocaine abuse involve the cardiovascular system. Changes in the cardiovascular system that accompany cocaine use include hypertension, hyperkinesis, and rapid heartbeat. Other short-term side effects include tremor, anxiety, sweating, hyperthermia, loss of appetite, loss of sleep, mental confusion and loss of coordination. Consequences of chronic use of cocaine can be fatal, as it damages the cardiovascular system and may lead to arrhythmia, myocardial infarction, or congestive heart failure. In addition, long-term cocaine use disturbs the dopaminergic reward pathway, thus resulting in depression and lack of motivation. Other
undesirable consequences of long-term cocaine use include sleeplessness, paranoia, irritability, acute toxic psychosis, lung damage, and decreased sexual function including impotency, gynecomastia, galactorrhea, infertility, and amenorrhea. The harmful effects of cocaine use is evident from the number of hospital visits and car accidents due to cocaine use. By 1989, cocaine was the number one cause of emergency room visits in Washington DC, New York, Atlanta, Baltimore, Chicago, Indianapolis, Detroit, Los Angeles, New Orleans, and many other cities. Moreover, cocaine was present in more than 18% of major vehicle fatalities in New York between 1984 and 1987.

Distribution Tendencies of Drug/Cocaine Users

Considering its destructive effects, one would think that everyone would avoid illicit drug use. This is not the case, as a large number of people continue to abuse cocaine even with knowledge of the damage it can inflict. Since animals and human beings are pro-survival by nature, other factors must account for the suicidal use of drugs. Indeed, the distribution of cocaine abusers among the population is not random, but instead, certain groups or types of people have greater tendencies to abuse cocaine than other groups. This suggests that certain factors render people more susceptible to substance abuse. Transients and people with mental illness are more likely to abuse cocaine, as 30-40% of homeless and 29% of mentally ill have a substance abuse disorder. Additionally, 47% of schizophrenics and 24% of people with anxiety disorders abuse drugs. Unemployment, early use of marijuana, and involvement in crime increase probability of drug use. Race influenced probability of cocaine use in 1994 with a higher percentage of blacks and Hispanics using cocaine than whites (1.3%, 1.1%, .5%). People who dropped
out of high school, cohabited, collected unemployment or welfare, have been fired from a job, divorced, those who have not desired a college degree, and not had a full-time job are also more likely to abuse cocaine throughout their lifetime\(^2\).

Another factor that affects a person’s susceptibility to abuse cocaine is the user’s normal level of excitation\(^2\). The basal level of arousal of the user, controlled by the noradrenergic system, thyroxin, and other regulators, influences the behavioral response to cocaine. People already hyperexcited are less likely to continue abusing cocaine as they do not enjoy the high of cocaine as much as users who have low basal level of arousal.

**Reinstatement/Relapse**

Due to the damaging effects of drug use, many drug users seek treatment to quit cocaine use. Abstinence proves to be difficult, as physiological and psychological symptoms of withdrawal compel humans to relapse and animals to reinstate cocaine-seeking behavior. Such symptoms of cocaine withdrawal include drug cravings, agitation, anxiety, irritability, and fatigue. Factors that especially exacerbate the symptoms include stimuli that the user associates with drug use, such as a familiar location where drug use took place, needles, etc. In addition to these factors, stressful situations, such as relationship conflicts, greatly increase cocaine cravings\(^3\). Although many aversive symptoms persist during withdrawal, cocaine-seeking behavior in rats and humans decreases during extinction/withdrawal until a factor leads to reinstatement/relapse.

Many drug rehabilitation centers exist to help drug addicts quit drug use, but the patients’ right to leave limits these centers’ ability to adequately prevent relapse in all
patients. Moreover, rehabilitation centers support patients for a limited amount of time and the patients may not be ready to live outside of the rehab center after release.

The Fight Against Drug Use

An estimated 8.3 percent of the national population twelve and older were current illicit drug users in 2002, almost half of the 16.3% estimated in 1985\(^4\). Efforts to combat drug addiction are partly responsible for the decrease, but the prevalence of drug use is still high. Cocaine use, in particular, has decreased since 1985, but its devastating effects are still prominent in the 3.6 million chronic cocaine users\(^4\). Because of cocaine's powerful, destructive effects, it is an important topic in the field of drug abuse and has been studied extensively.

In efforts to diminish drug abuse, the United States government spends $15.1 billion each year. This money is used for prison, law enforcement, and efforts to prevent illegal drugs from entering the United States\(^6\). This effort has been only moderately successful, as illegal drugs continue to be smuggled into the United States. Moreover, the efforts to prevent importation of illegal drugs have elevated illegal drug costs, adding more costs for the economy. In response to this failure to eliminate entry of drugs, the government provides approximately one billion dollars each year, $968 million in 2003\(^{13}\), for drug abuse research in hopes that treatments to rid addicts of cravings will decrease drug use.
Stress and Cocaine Abuse

Research findings during the last 4 decades have revealed many important insights on brain mechanisms of addiction and factors that influence addiction. One such finding is the role stress plays in inducing drug cravings and its ability to influence reinstatement in animals and relapse in humans (the term relapse is used for human behavior, while reinstatement is an equivalent term used to describe animal behavior). This is most evident in cocaine-addicted rat models as they exhibit reinstatement behavior to a greater degree when presented with stressors such as foot-shock and restraint. Administration of glucocorticoid receptor antagonists eliminates this stress-induced increase in reinstatement behavior in rats, demonstrating that the mechanism occurs via stress hormones. Stress not only affects reinstatement in rats but also acquisition of cocaine self-administration. Unpredictable footshock increases acquisition of cocaine self-administration at low doses and glucocorticoids seem to be necessary for this acquisition of cocaine self-administration.

The effects of stress on reinstatement pertain to humans as well. There is evidence that humans increase drug use after stress as compared to non-stress situations. Indeed, relapse is usually predictive of cocaine-addicted humans with poor stress coping strategies. However, glucocorticoids do not appear to have an essential role in the reinforcing properties of cocaine in humans as it does in rats.

Anxiety and Drug Abuse

In addition to stress, anxiety has associations with drug abuse. Symptoms of anxiety develop during cocaine withdrawal in rats and humans. These anxiogenic symptoms can
be alleviated by benzodiazepines, such as valium, suggesting that the anxiogenic behavior is regulated by GABAergic receptors in the brain. Administration of benzodiazepines not only relieves anxiogenic systems but also attenuates cocaine self-administration in rats, thus demonstrating anxiety's involvement in cocaine abuse. This attenuation is thought to occur by regulation of stress hormones\textsuperscript{12}, which suggests an interactive role of anxiety and stress. These research findings strongly support the role of anxiety in drug reinstatement and relapse.

Despite the studies done with benzodiazepines and stress hormones, questions still remain about the role of anxiety in drug abuse. Trait anxiety is the basal level of anxiety of an animal or human, that is stable over time, and can be measured in rats using various methods. Since both humans and rats exhibit individual differences in trait anxiety, these methods may help us better understand anxiety's influence on drug abuse in different individuals.

Proposal

It is clear that acute stress and anxiety are involved in drug abuse relapse. However, to our knowledge, no one has examined the effects of repeated chronic stress on drug reinstatement of cocaine-seeking behavior in rats. Additionally, to our knowledge, no one has examined how trait anxiety influences reinstatement.

With evidence that stress induces cocaine reinstatement and that chronic stress leads to changes in components of the stress response we propose that chronic stress during extinction will affect extinction rates and/or reinstatement of conditioned place preference behavior. Additionally, with preliminary evidence from Dr. Sorg's lab that
increased basal anxiety levels cause an increase of reinstatement in response to stress, we propose that basal anxiety levels will affect extinction rates and/or reinstatement of conditioned place preference behavior. We will examine the effects of chronic stress and anxiety on drug addiction by using the conditioned place preference apparatus. Two tests of reinstatement will be conducted. A stressful stimulus and cocaine are both known to produce cocaine-seeking behavior in rats. Therefore, both of these types of stimuli will be tested in the present study.
Methodology

Forty-two rats were obtained from Harlan laboratories and were allowed free water and food access for 3 weeks before beginning the experiments.

We performed the elevated plus maze experiment to test basal anxiety levels of the rats. The elevated plus maze was made from black Plexiglas and was 10 cm in width and 50 cm in length, creating a central 10x10 cm neutral zone. The plus-maze was elevated 50 cm from the floor. Two of the arms were enclosed with black Plexiglas walls 40 cm high, with no ceiling. To begin the experiment, the rats were placed in a dark room for 30 minutes to adapt them to the lighting used for the plus maze test. Next they were placed on the plus maze and allowed to freely roam the platform for five minutes. The time spent in open and closed arms, and the numbers of entries into the open and closed arms were measured. Additionally, we considered the rat to be exploring the open arm when two feet were placed into the open arm while the hind paws remained in the neutral zone. To determine classification of rats as either high or low basal anxiety, we divided rats into high and low anxiety based on their distribution of scores.

The conditioned place preference (CPP) apparatus was made of Plexiglas with two main compartments measuring 35x25x36 cm high and one central (neutral) compartment measuring 12x16x36 cm high. One of the main compartments was made with black Plexiglas walls and a floor of wire mesh. The other main compartment had white Plexiglas walls with a floor of stainless steel rods spaced 25mm apart. The central compartment was made of gray opaque Plexiglas walls and floor. A computer recorded the time spent in each compartment. For the CPP experiment, rats were allowed to explore the three-compartment preference apparatus for 15 minutes. This procedure was
repeated one more day to determine the compartment that the rat preferred. The following
day, rats were conditioned to the non-preferred compartment by injecting them with
cocaine (12mg/kg) just prior to placing them in the compartment for 30 minutes. The
next day, rats were given saline (1mL/kg, ip) and confined to the preferred compartment
for 30 minutes. Alternating days of cocaine and saline were given for a total of 8 days.

After eight days of cocaine and saline injections, rats were tested to ensure that
they were conditioned to the cocaine-paired compartment (testing day). The rats were
placed in the neutral compartment and were allowed to choose between the 3
compartments for 15 minutes. Based on previous work in Dr. Sorg’s lab, a 20% increase
in time spent in the cocaine-paired compartment as compared to the initial preference day
was expected. If the rat failed to condition to the cocaine-paired compartment, their
results were discarded.

Before beginning the extinction phase, we separated one-half of the rats from
their cage partner (referred to shifter rats) and placed them in a cage previously inhabited
with a single rat. The singly caged rats adapted to their housing condition for 3 weeks
before the introduction of other rats into their cage. The remainder of the rats remained
with their previous partner and did not change cage partners. The rats placed with the
initially isolated rats were switched to a different cage containing a previously singly
housed rat each day during the extinction phase.

To allow for extinction of CPP, the rats were allowed to explore all 3
compartments for 15 minutes each day, without cocaine injection, until there was no
longer a significant difference in time spent in the cocaine-paired compartment as
compared with original preference day.
After the conditioned place preference behavior extinguished, stress induced reinstatement was done by placing a 37% solution of formaldehyde on two cotton swabs taped to the lid of the central compartment. This was considered an aversive stimulus to the rat. The rats were allowed to explore all three compartments for 15 minutes. They were then allowed one more extinction day to explore the compartment without any stressor. On the last day of testing they were examined for reinstatement by giving an injection of cocaine (10 mg/kg, ip) and allowing them to explore freely all 3 compartments.
Results

The time spent in the open arms plus the number of open explorations was used to measure basal anxiety levels of the rats. Figure 1 shows the mean values of high and low anxiety rats. The distribution of anxiety scores is shown in Figure 2.

Our requirements for CPP behavior consist of a 20% increase in time spent in the non-preferred compartment after conditioning. Six rats failed to exhibit CPP behavior and their results were eliminated as our experimental depends on rats learning CPP behavior.

As shown in figure 3, time spent in the cocaine-paired compartment significantly increased from the initial preference day to testing day (tested one day after training) in all four groups. The increased time spent in the cocaine-paired compartment persisted during extinction day 1 in all groups except the high anxiety shifter rats. The low anxiety control rats spent significantly more time in the cocaine-paired compartment during the second extinction day, as compared to the initial preference day, while the rest did not. There was no significant difference between initial preference day and the remaining extinction days (figure 3).

Following extinction, the presence of formaldehyde significantly reinstated CPP behavior in all groups except the high anxiety shifter rats. All groups extinguished cocaine-seeking behavior during the extinction day given between the two test days for reinstatement. In response to cocaine priming, low anxiety shifter and control rats significantly reinstated. High anxiety rats failed to significantly restate to cocaine-priming (figure 4).
Discussion

Thirty-six of 42 rats conditioned to the cocaine-paired compartment. Six rats, however, failed to condition to this compartment. We eliminated their data and their behavior was not considered in our results.

Unstable social environment failed to significantly alter extinction of CPP behavior. Trait anxiety, however, had a significant effect on reinstatement. High anxiety rats failed to reinstate to cocaine-priming injection. Additionally, there were differences when comparing the results within each group. High anxiety shifter rats failed to reinstate when presented with the stimulus formaldehyde while the other three groups significantly reinstated. This suggests that unstable social environment affects CPP behavior only in rats with high trait anxiety. Anxiety may play a role in this phenomenon by affecting the reaction of the rat to the changing environments. Unstable social environment may lead to a stress response in high anxiety rats while having no significant stress effect on low anxiety rats as they may better adapt to the situation.

Examining the influence of stress on behavior is especially challenging as various components comprise the stress system and many different factors can differentially regulate each component. This creates limitations for experiments studying stress, as it is impossible to control for each factor that can potentially affect the stress system. We chose the unstable social environment as a method to induce stress during extinction because this method has been shown to impact CRH, ACTH, and cortisol levels. However, we did not measure these parameters in this study and so cannot be sure that the unstable social situation acted as a stressor. Other types of stressors, such as footshock, restraint stress, and cold restraint, may modify the stress axis but in different
ways and could be examined to further understand the role of chronic stress in cocaine addiction.

Repeating our experiment with a difference stressor, such as footshock, would be especially interesting, as it would provide additional information on the role of trait anxiety on stress-induced reinstatement. A limitation of using unstable social environment as a stressor is that rats may react differently in terms of their stress response. Some rats might even find the unstable social environment enjoyable. Chronic unpredictable footshock, however, would most likely result in an aversive emotional response in all rats.

The significant effect of anxiety on cocaine-induced reinstatement is interesting as it suggests that rats respond to cocaine cues to different degrees depending on the anxiety levels of the rat. It would be interesting to see if this result is also relevant in humans drug addicts. We can test this by determining trait anxiety of human cocaine addicts and exposing them to cocaine cues while measuring their emotional response. If our results do relate to humans, we could expect that human drug addicts with high anxiety levels have a greater emotional response to cocaine cues than those with low anxiety levels and this could lead to greater likelihood of relapse.
References

Means of Plus Maze Score

Figure 1
Anxiety Distributions

Distribution of open time + open exploration scores. Rats that scored above 19 were labeled as low anxiety. Rats below 19 were labeled as high anxiety.

Figure 2
Conditioned place preference during preference day, initial testing day, and extinction days.

* Denotes significant difference compared to initial preference day

** Figure 3 **
CPP behavior - last 4 days

* Denotes a significant difference between final extinction day and stress reinstatement
+ Denotes a significant difference between final extinction day and cocaine reinstatement

Figure 4
REQUEST FOR PERMISSION TO INCLUDE YOUR HONORS STUDENT'S THESIS IN THE WSU RESEARCH EXCHANGE

Washington State University Libraries

Permission is requested for a non-exclusive license to post the Honors thesis described below in digital form in the Honors College community within the WSU Research Exchange <https://research.wsulibs.wsu.edu:8443/dspace/community>. Posting in the Research Exchange will make the material publicly available as part of the Washington State University Research Exchange digital repository of research-related documents. Additional information about Research Exchange can be viewed at <http://research.wsulibs.wsu.edu>.

Thesis Author Barnes, Ryan

Thesis Title The Effects of Unstable Social Environment & Trait Anxiety on Extinction & Reinstatement Behaviors of Rats Addicted to Cocaine

Date of Thesis Spring 2004

I grant a non-exclusive right to include this item in the Research Exchange. All other rights under copyright law are retained.

Permission granted by:

Barbara Sorg

Name (please print)

Signature (not computer generated)

Date of Signature

Please return the form with your written signature via fax, email, or postal service to:

Kay Vyhnanek, Scholarly Communication Librarian
120F Terrell Library / PO Box 645610
Washington State University
Pullman, WA 99164-5610 USA
Fax: +1-509-335-672