5-11-2006

# Female Hormonal Influences on Stress- and Drug-induced Reinstatement of Extinguished Amphetamine-induced Conditioned Place Preference

Melissa Elaine Bleile
*University of Missouri-St. Louis*, mbleile01@charter.net

# FEMALE HORMONAL INFLUENCES ON STRESS- AND DRUG-INDUCED REINSTATEMENT OF EXTINGUISHED AMPHETAMINE-INDUCED CONDITIONED PLACE PREFERENCE

**Melissa E. Bleile, M.A.**

**May 2006**

**A Dissertation Submitted to the Graduate School**

**of the University of Missouri-St. Louis**

**in Partial Satisfaction of Requirements for the**

**Doctor of Philosophy Degree in Psychology**

**Advisory Committee:**

**George T. Taylor, Ph.D.**

**Chairperson**

**Carl Bassi, Ph.D.**

**Michael G. Griffin, Ph.D.**

**Jennifer Siciliani, Ph.D.**

## ACKNOWLEDGEMENTS

I thank God for giving me the strength, knowledge, and courage needed to complete this dissertation and Ph.D. degree.

I thank my children, Jessica and Keith, my reasons for living, for giving me their love and support, and enduring my pursuit of the Ph.D., even at their own great sacrifice.

I am grateful to my mentor and Chairperson, Dr. George T. Taylor, for his encouragement and guidance, and for prodding me when I needed it.

I was fortunate to have Dr. Michael G. Griffin as a boss and a friend throughout this process. I thank him for always being concerned about me through my battle with back pain and everything else.

Thank you to my committee members, Dr. Jennifer Siciliani and Dr. Carl Bassi, for their time and advice.

Thank you to Michael Howe, School of Optometry, for taking time to build the CPP apparatus, and special thanks to John Hancock, III, manager, and Larry Hinkle, Animal Welfare Unit for their help and patience.

Thank you to Dr. Mark Tubbs of the University of Missouri-St. Louis, and Dr. Judy McGee and Dr. Daniel Sparling of Maryville University for the opportunities to teach during graduate school, their support and encouragement, and for being great role models.

Many thanks to my parents, other family members, friends, and fellow graduate students for their encouragement, prayers, and belief in me even when I did not believe in myself.

May God bless you all.

This research was supported in part by the Psychology Department of the University of Missouri-St. Louis.

# TABLE OF CONTENTS

# LIST OF TABLES

## LIST OF FIGURES

## ABSTRACT

Women may experience greater drug sensitivity than men, and estrogen appears to increase drug sensitivity in women and experimental animals. The literature is contradictory and unclear on gender differences in response to stress and the interaction of stress and estrogen. The purpose of this experiment was to assess the effects of female sex hormones on drug sensitivity. Of particular interest was the interaction of these hormones with the primary factors causing relapse in human drug addicts, stress or drug re-exposure, on drug-seeking behavior.

Female rats were ovariectomized and received replacement hormones to control circulating hormone levels. Animals received estradiol benzoate (EB-only), progesterone (PROG-only), both hormones (EB + PROG), or vehicle only (VEH). These four groups of animals were tested for amphetamine (AMPH)-induced conditioned place preference (CPP) and the rate of CPP extinction before and after a challenge. The challenge came in the form either of AMPH re-exposure or restraint stress.

Conditioned place preference (CPP) is an animal paradigm commonly used as a measure of drug seeking in animals. The results of this experiment indicated that 1 mg/kg body weight AMPH produced a clear CPP in all groups, based on increased times spent in the drug-paired compartment. However the different hormone treatments did not differentially influence the acquisition or magnitude of CPP. Hormone treatments, however, influenced extinction of CPP. Using overall times, animals treated with EB-only spent the most time in the drug-paired compartment during extinction, while the PROG-only group spent the least. The EB-only and the EB + PROG groups did not achieve the criterion for

extinction. The PROG-only group met this criterion on day 4 and VEH group on day 6 of the extinction phase. These findings suggest that EB treatment with or without PROG results in greater resistance to extinction as compared to treatments with PROG-only or VEH.

Using a challenge following extinction indicated that both stress and drug challenges induced reinstatement of CPP. However, there were no statistically significant differences between the two challenge conditions in the magnitude of CPP reinstatement. The different hormone regimens did not significantly impact reinstatement in the challenge phase, nor was there a statistically significant interaction between hormone regimen and challenge condition.

During the second extinction phase, the two challenge conditions produced different rates of extinction. Collapsing across hormone treatment, the animals receiving the drug challenge met the criteria for extinction, whereas those administered the stress challenge did not. This indicates that CPP for the animals receiving the stress challenge did not completely re-extinguish during the five days of the second extinction phase. The suggestion is that the effect of a single stress exposure on drug-seeking behaviors may be longer lasting than a single drug challenge. The effects of stress during extinction 2 were the greatest in the EB + PROG group. The effects of drug during extinction 2 were least in the VEH group. Neither the EB-only or PROG-only groups differed from each other.

These findings provide evidence for the hypothesis that estrogen enhances and progesterone suppresses resistance to extinction of a drug preference response. Also, both stress and drug exposure are significant triggers for the reinstatement of extinguished drug-seeking behavior, and the effects of stress may be more prolonged than drug exposure. It may also suggest that drug treatment of women should be tailored to hormone status.

## INTRODUCTION

Drug addiction is a major public health concern, affecting millions of Americans. It is a chronic and relapsing disorder characterized by a compulsion to take the drug and a loss of control in limiting intake (American Psychiatric Association, 1994). As of 2000, an estimated 14 million Americans were currently using illicit drugs, and of these, 3.5 million were dependent on them. In addition, 12.6 million people were heavy users of alcohol, and 8.2 million were dependent on alcohol (SAMHSA, 2000).

One aspect of drug addiction is prolonged craving that lasts for years, even after long periods of abstinence. The recidivism rate for problem drug users is very high, and relapse is a major problem in the treatment of drug abuse. Between 60% and 80% of cocaine users resume cocaine use within 3 weeks following outpatient treatment (Kosten, Gawin, Kosten, & Rounsaville, 1993; Weiss, Martinez-Raga, Griffin, Greenfield, & Hufford, 1997). Determining the factors influencing recidivism could improve the treatment of addicted individuals and decrease relapse rates. The two most effective events for reinstating drug-seeking behaviors after their extinction in animals, and triggering drug craving or relapse in humans, are re-exposure to the drug and exposure to acute stress.

There is evidence that women are more sensitive to the effects of drugs and stress, and that these differences are hormone driven (Justice & DeWit, 1999; Lukas, Sholar, Lundahl, Lamas, Kouri, Wines, Kragie, & Mendelson, 1996; Robbins, Ehrman, Childress, & O'Brien, 1999). Therefore, if changes in hormone levels alter sensitivity to stress and drugs, then women may need more intensive treatment and prevention programs teaching them how to cope with stressful situations in a manner and setting sensitive to women.

Historically, several models have evolved to explain drug addiction and to suggest methods to treat the addicted individual, including the learning and conditioning model. Animal paradigms have been critical for understanding mental illnesses, including addiction. These paradigms have been particularly valuable in evaluating the rewarding qualities of addictive drugs. One animal paradigm, the conditioned place preference (CPP), is based on the learning and conditioning model of addiction.

## DRUG ADDICTION

The World Health Organization (WHO) once defined drug addiction as "a state of periodic or chronic intoxication detrimental to the individual or society, produced by the repeated consumption of a drug. Its characteristics were described as an overpowering desire or need (compulsion) to continue taking the drug, because of either psychological or physical dependence on the effects of the drug, and a tendency to increase the dose or frequency of use" (Grilly, 2002, p. 119). More recently, the addiction concept has been applied to other behaviors including problem gambling, eating disorders, excessive spending, exercise, and sexual preoccupation (Walters, 1999). Because the term "addiction" is used so widely, it has fallen out of favor with drug researchers and the medical community, and it has been replaced with diagnoses of drug dependence or substance abuse.

Some researchers refer to drug addiction as a behavior, not a condition (McKim, 2003; Peele, 1985). Peele (1985) defines addiction as a compulsion to act that is beyond the user's self-control. The notion is that people use and abuse drugs because of their rewarding properties. In other words, most abused drugs induce euphoria in the user, and the user continues using drugs to achieve euphoria. McKim (2003) describes addiction as abnormal behavior. But unlike Peele (1985), McKim (2003) also recognizes that something abnormal,

such as a disease state, may be influencing addictive behaviors. People are expected to seek experiences that cause pleasure and avoid pain but addicted individuals seem to do so compulsively, even at their own destruction.

Doweiko (2002) summarizes a continuum of drug use, ranging from total abstinence from drug use to clear addiction to drugs, with criteria for four levels of drug use including rare or social use, heavy social use or early problem use, heavy problem use, and addiction. Doweiko's criteria for addiction resemble those of the Diagnostic and Statistical Manual of Mental Disorders (DSM) category of drug dependence.

The term "addiction" is still used by many readers because it is ingrained in our language and widely understood. The terms "addiction" and "dependence" are therefore used interchangeably in this thesis.

## Models of Addiction

Several models have evolved to explain addiction. Some models emphasize physiological mechanisms and include the medical model and the neurobiological model. Others are from a more behavioral perspective, including the sociocultural model, the cognitive model, and the conditioning model. The animal paradigm used in the current experiment is based on the conditioning model of addiction and measures drug-seeking behavior as an important aspect of addiction.

### Medical Model of Addiction

The medical model of drug addiction emphasizes the physiological symptoms of addiction. Addiction is regarded as a disease. As with other disease states, the treatment often includes hospitalization and drug therapy. The diagnosis is based on the presence of tolerance, when more of the drug is required to produce the same effects as with initial drug

use. The other hallmark symptom is the presence of physiological or psychological withdrawal symptoms.

The origin of the medical or disease model of addiction is credited to Benjamin Rush in the United States and to Thomas Trotter in Great Britain (Meyer, 1996). They believed that since alcohol had such a profound effect on the nervous system, and excess drinking caused a nervous system imbalance, defining alcoholism as a disease was a logical conclusion.

In the middle of the 20th century, the medical model gained prominence with the Alcoholics Anonymous movement. E.M. Jellinek, one of A.A.'s most influential theorists, further refined the medical model of alcoholism, reserving the disease category for users who displayed tolerance, withdrawal symptoms, loss of control over drinking, and inability to abstain from drinking (Meyer, 1996). The disease model was subsequently applied to other drugs of abuse, including opioids and cocaine.

During the latter part of the 20th century, medical research has supported the obvious influence of many risk factors such as family history, life style, environment, and others on many diseases, including alcoholism and drug addiction. Alcoholism was formally declared a disease by the World Health Organization in 1951, and by the American Medical Association in 1953. Later, addictions to all drugs were identified as diseases.

Addiction also is formally identified in the DSM. The DSM-IV (1994) defines and differentiates between substance dependence and substance abuse. Substance abuse is defined as a maladaptive pattern of recurrent drug use that leads to failure to fulfill responsibilities, drug-related legal problems, and continued drug use despite recurrent interpersonal and other problems caused by drug use (DSM-IV, p. 183). Dependence is

considered to be a more serious diagnosis. In addition to the criteria for abuse, dependence includes additional criteria of tolerance, withdrawal, and failed attempts to decrease or control drug use (DSM-IV, p. 181. Criteria for the diagnosis of substance dependence or substance abuse are refined in each edition of the DSM. The International Statistical Classification of Diseases and Related Health Problems (ICD) is a catalog of diseases issued by the World Health Organization. The ICD-10 (1993) distinguishes between harmful use and dependence, with criteria for each similar to criteria in the DSM-IV.

**Neurobiological Model of Addiction**

Another physiological model of addiction, the neurobiological model, is based on evidence that many drugs of abuse directly or indirectly activate a system in the brain that is known to be associated with natural experiences of pleasure. Many classes of drugs that acutely affect different brain neurotransmitter systems are known to be addictive. However, most abused drugs directly or indirectly increase dopamine activity, suggesting a common action among these drugs. The dopamine brain reward system hypothesis is the primary focus of this model.

Olds and Milner (1954) were the first to identify a brain reward system. They were searching for the area of the brain that is the stimulus for hunger by placing electrodes in the brains of rats. Serendipitously, they identified sites in the brain where electrical stimulation appeared to be reinforcing.

Initially, a number of brain regions were found to produce rewarding effects, but most of these sites were discovered to be linked through a common neural pathway, the medial forebrain bundle (MFB); (Koob & Nestler, 1997). Although other brain systems may produce rewarding effects as well, the MFB produces the most robust rewarding effects. Several

neurotransmitters may be involved in the rewarding effects from brain stimulation, but dopamine appears to be the neurotransmitter essential for reward. Stimulation activates a descending component of the MFB that synapses at the ventral tegmental area (VTA) to the ascending mesolimbic dopamine system. The mesolimbic dopamine pathway synapses with the nucleus accumbens and the prefrontal cortex. This pathway is activated naturally by many rewarding stimuli, such as satiety from hunger and sexual contact. All drugs that have high abuse potential increase the release of dopamine in the nucleus accumbens either directly such as cocaine, or indirectly such as nicotine (Picciotto, 1998). The mesolimbic dopaminergic system is the critical system for reinforcement and this body of evidence has evolved into the dopamine brain reward system hypothesis (Koob & Nestler, 1997).

Psychostimulant drugs such as cocaine and amphetamine are powerful monoamine reuptake inhibitors. The monoamines include the neurotransmitters dopamine, norepinephrine, epinephrine, and serotonin. The mechanism of action for the psychostimulants is interaction with monoamine transporter proteins located on nerve terminals, which normally terminate a monoamine signal by transporting the monoamine back into the presynaptic terminals. They thus increase the synaptic concentrations of monoamines and increase their stimulation of postsynaptic neurons. Their rewarding properties are attributed primarily to the increased dopamine activity in the mesolimbic pathway (Koob & Nestler, 1997).

**Sociocultural Model of Addiction**

Traditionally a discussion of models of addiction is divided into physiological and psychological theories. The latter are those that emphasize environmental influences, including social interactions or conditioning.

The sociocultural model emphasizes that social and cultural factors, including beliefs and attitudes, influence drug-taking behavior. Beliefs and attitudes about drugs and their usage are certainly related to the problems associated with them. Bales (1946) was one of the first to study the influence of sociocultural factors on alcohol use. He compared drinking patterns of Jewish-Americans and Irish-Americans and the incidence of problem alcohol use among them. Jewish children were introduced to wine as an important sacramental part of religious ritual, but showed low rates of alcoholism. Irish Americans, on the other hand, were forbidden to drink in their youth and were exposed to men who considered drinking to excess with friends an important part of comradery. Irish-American men were much more likely to progress to a level of drinking that produced problems in their physical and mental health. Bales also described how the amount of conflict between groups, such as socioeconomic classes, races, and religious groups influenced the risk of problem drug use.

Other researchers have examined the influence of sociocultural factors on the tendency to develop problem drug use. For example, Oetting and Donnermeyer (1998) developed a model of drug addiction from a perspective of primary socialization. The primary socialization model states that normal and deviant behaviors are learned social behaviors. The norms for social behavior, including drug use, are learned through interaction with primary socialization sources. This model focuses on adolescence when deviant behavior, including drug use, is typically the most problematic.

**Cognitive Models of Addiction**

The cognitive models of addiction are based on evidence that cognitive biases are associated with emotional disorders such as depression and anxiety, and are likely associated with drug addiction as well. Cognitive biases are erroneous or variant beliefs or expectations

(McCusker, 2001). The Cognitive Social Learning Theory (Marlatt & Gordon, 1985) proposes that a drug user may have cognitive biases related to two factors, his confidence in his ability to abstain, and expectations of the effects of the drug. Craving is considered to be reciprocally related to the individual's confidence in his ability to abstain, in that high craving would undermine this confidence and challenge his coping skills.

Another example of a cognitive approach to explaining addiction is the Cognitive Labeling Model (Drummond, 2001). The cognitive labeling model is based on Schacter and Singer's (1962) cognition-arousal theory of emotion. According to Schacter, the feeling of an emotion depends on sensory feedback related to the body's response to an event, and the person's perceptions and thoughts concerning the environmental event that evoked the body's response. Applied to drug craving, an addict may cognitively interpret the physiological effects of drug cues as drug craving. For example, a smoker may interpret the physiological changes caused by nicotine withdrawal as a need for a cigarette.

The sociocultural and cognitive models of addiction have been examined in many experiments. They are best adapted to research in humans, and it is difficult to create an animal paradigm to test them. The other psychological model of addiction, the conditioning model, has been widely examined and supported in both human and animal research. This model is the focus of the research summarized here and the current dissertation experiment.

**Conditioning Model of Addiction**

Conditioning models can be used to explain the perpetuation of substance abuse, the high rate of relapse after substance withdrawal, and drug craving. They are built on Pavolvian conditioning, in which environmental stimuli are paired with subjective states believed to trigger craving. For example, the drinker who often drinks in a certain bar over

time associates the sights, smells, and sounds in the bar atmosphere with the consumption of alcohol. These visual, olfactory, and auditory cues then elicit the response of drinking or craving for a drink. Drug use is often associated with rituals that become conditioned drug cues.

Some conditioning models explain drug craving and relapse in the sense that conditioning results in the unconscious adaptations of the body that counteract drug effects. Two theorists who have promoted this type of model are Abraham Wikler (1948) and Shepard Siegel (1998).

Wikler (1948) introduced the conditioned withdrawal model that proposes that drug-related cues, such as the sight of drug-taking paraphernalia or places in which drugs had previously been taken, would result in drug withdrawal. As with classical types of conditioning, it is not the direct effects of the drugs that are conditioned, but the adaptive responses to them. For example, an animal can be conditioned to withdraw an extremity following a noise stimulus when that noise stimulus has been previously paired with a foot shock. It is not the direct effect of the shock, the sensation of pain, that is conditioned, but the adaptive responses to it, withdrawal of the extremity. Thus, drug-related cues that have been conditioned trigger the adaptive responses of the body to the presence of drug, even before the drug is administered. These adaptive responses can cause negative emotions in the user. For example, depression is a reported feeling in the withdrawal of stimulants that produce euphoria. Relapse thus occurs due to the need of the addict to relieve the negative state resulting from drug withdrawal.

Similarly, Siegel's (1998) model of conditioned opponent processes supports a theory of homeostasis to explain drug craving and tolerance. Drugs alter homeostasis, and the body

compensates to diminish these alterations with opponent processes. Opponent processes may include increased drug metabolism or up- or down-regulation of receptors. Over time, these opponent processes gain strength and lead to tolerance. Thus, Siegel's model predicts that the conditioned stimulus or drug cue results in a conditioned response that is the opposite of the drug's effect, and the addict takes more drug to obtain relief from this negative state.

Another conditioning model promotes the notion of drug craving being a pleasurable experience rather than being a negative withdrawal experience, particularly with stimulant drugs such as cocaine or AMPH (Stewart, de Wit, & Eikelboom, 1984). The model proposes that repeated pairings of drug-related environmental cues with positive unconditioned drug effects result in positive conditioned responses to the drug-related cues themselves. A process of positive reinforcement rather than negative reinforcement thus drives drug seeking. Evidence for this is seen in animal research. Animals seek places previously associated with drugs that cause euphoria. The model posits that the conditioned incentive effects of drugs activate neural mechanisms that mimic the activity of the drugs themselves. The activation of positive affective states by the presence of the drug cues is involved in the continuation of drug taking behavior (Stewart, de Wit, & Eikelboom, 1984).

Near-death overdoses support the conditioning models (Gutierrez-Cebollada, de la Torre, Ortuno, Garces, & Cami, 1994). Often what these victims report is that the drug dosage that currently resulted in toxicity had been well tolerated in the past. The usual difference is that the overdose occurred in a novel situation, such as in a different place or with different people. In a sample of heroin addicts admitted to the hospital for overdose, 52 percent of these subjects indicated that the last injection of heroin took place in an unusual

setting. This evidence supports the notion that the body had fewer cues to unconsciously trigger its opponent processes, and thus overdose resulted.

Similarly, veterans who had ready access to heroin in Vietnam often became addicted in Vietnam and showed withdrawal symptoms. About 50 percent of the soldiers in Vietnam used narcotics, and about 20 percent of those men became addicted there (Robins, Helzer, & Davis, 1975). However, upon returning to the United States, most of those addicted soldiers did not show craving for the drug or withdrawal symptoms.

More evidence for the conditioning models is observed in drug users who compulsively self-inject (Levine, 1974.) These individuals appear to find positive reinforcement simply by injecting themselves, independent of their addiction to the drugs themselves. The needles become positive reinforcers, likely because of their conditioned association with the positive effects of the drugs. These individuals will self-inject saline when given the opportunity and experience pleasure from doing so.

The high recidivism rate observed in individuals who have undergone in-patient drug treatment may provide evidence for the conditioning models as well. Once the person returns to their original environment and social groups, they often start using drugs again. The key to continued abstinence might be for them to avoid the drug and the environment associated with it, or to retrain the habit of drug use through behavior modification. Alternatively, repeated exposure to environmental cues previously associated with drug use without the reinforcement of drug administration is another mode of addiction treatment based on the conditioning model. Whether the addicted individual relapses to experience pleasure or to avoid the negative withdrawal state, evidence supports the importance of conditioned cues in triggering relapse.

## Amphetamine and Other Psychoactive Drugs

Psychoactive drugs can be classified by their influence on the central nervous system. The primary categories are stimulants, depressants, and hallucinogens and marijuana, and the drugs within these categories vary in their abuse potential (US Code Title 21). The Controlled Substances Act of 1970 distinguished five levels of abuse potential for controlled drugs, Schedule I through Schedule V. Schedule I drugs have the highest abuse potential and no currently accepted medical use in treatment in the U.S. Legal use of these drugs is primarily in research. Drugs categorized as Schedule II, III, IV, and V have current accepted medical uses, but Schedule II drugs have high abuse potential, and Schedule III and IV have low to moderate potential for abuse. Schedule V drugs have the lowest potential for abuse, and in some states, these drugs may be dispensed without a prescription. These drug schedules describe controlled drugs only. Other drugs and substances, including alcohol, have the potential to be addictive, especially when used for purposes not intended by their manufacturers. For example, volatile inhalants, such as gasoline and glues have legitimate uses, and preventing their misuse is difficult.

Explanations for reasons that some drugs are more likely to be abused than others coincide with the models of addiction. For example, according to the medical model drugs that have higher abuse potential would be those that rapidly cause tolerance and induce the user to take higher doses of the drug for the desired effects. In addition, the highly-abused drugs should cause physical withdrawal symptoms during abstinence. This concept is not entirely consistent with the data because alcohol, for example, has potential for being addictive, but tolerance develops rather slowly. Physical dependence on alcohol generally requires months to years to develop (Grillly, 2002). Similarly, the psychostimulants cocaine

and methamphetamine have high abuse potential but may cause few or no physical withdrawal symptoms (Satel. S.L., Price, L.H., Palumbo, J.M., et al., 1991a; Weddington, W.W., Brown, B.S., Haertzen, C.A., 1990).

Some drugs vary also in their abuse potential depending on how they are administered and the dosage used. Administration influences the peak plasma levels and the amount of drug that reaches the brain. Absorption by most drugs is immediate for intravenous or inhalation routes, more slowly for nasal route, and still slower for oral or transcutaneous routes. As an example, natives living near the Andes Mountains have chewed the leaves of the coca plant for thousands of years without developing dependence, and snorted cocaine may not be highly addictive when used in small doses. However, when smoked in its freebase form, cocaine yields a much more intense effect and is markedly more addictive (Hatsukami, 1996).

Amphetamine (AMPH) is categorized as a psychomotor stimulant and is a Schedule II drug that is abused by some people. Chemically, several isomers of the molecule exist, including dextroamphetamine, or *d*-amphetamine (d-AMPH), the *d*-isomer. Generally d-AMPH has more potent CNS stimulant effects than the other isomers, e.g, *l*-amphetamine (Fawcett & Busch, 1998). Even more potent, methamphetamine (METH) is preferred by illicit AMPH abusers because of its longer half-life and greater capacity to cross the blood-brain barrier (Albertson, Derlet, & van Hoozen, 1999). The chemical structure of AMPH is similar to that of the neurotransmitters norepinephrine and dopamine, and as a result, it is classified as a catecholamine agonist (King & Ellinwood, 1997). AMPH stimulates neurons to release dopamine stores and inhibits the reuptake of dopamine (Hyman & Nestler, 1996). AMPH is readily self-injected by laboratory animals, even at doses as low as 0.01 mg/kg

(Carroll & Lac, 1997; Pierre & Vezina, 1997). Various dosages of intraperitoneal (I.P.) AMPH, as low as 0.3 mg/kg, have also resulted in significant CPP (Gilbert & Cooper, 1983).

The research cited and the current dissertation experiment herein focuses on psychostimulants, particularly AMPH. Also, unless otherwise specified, d-AMPH is the form of AMPH discussed in the literature review and the current dissertation experiment.

## ANIMAL PARADIGMS USED TO STUDY ADDICTION

Animal paradigms have been critical in clarifying basic principles of disease. Results from animal studies have proven valuable in explaining physiological mechanisms underlying disorders, as well as developing treatments for the disorders. The human condition of drug addiction is not easily modeled in an animal paradigm because addiction is a complex set of behaviors. However, some animal paradigms for addiction have been developed that have good face validity. These paradigms test animal models of various aspects of addiction including drug reward value.

Criteria have been established to evaluate animal models used to study addiction and other psychological and behavioral disorders. Prominent among these are those proposed by McKinney and Bunney (1969). They suggested that the validity of the animal model should be evaluated on its similarity to the human disorder with respect to four criteria. These are etiology, symptomatology, biochemistry, and response to treatment. Common animal paradigms used to model various aspects of addiction include self-administration, electrical brain stimulation, choice paradigms, conditioned reinforcement, and conditioned place preference (CPP).

## Self-Administration

The primary route used in drug self-administration studies is the intravenous route in which an intravenous catheter is inserted into a large vein of an animal. The animal learns that a behavioral task such as a lever press results in administration of an intravenous dose of drug (Bozarth, 1987). Variables traditionally measured are frequency of bar pressing for the drug (presses per min), duration of the presses, and latency to bar press (the amount of time between individual bar presses.

This method of studying drug reinforcement is based on the principles of operant conditioning. Intravenous drug self-administration can be shown to control behavior just as traditional reinforcers such as food (Spealman & Goldberg, 1978). Intravenous self-administration is often used to measure drug reinforcement. It has good face validity. Also, the correlation between intravenous self-administration by animals and addiction liability in humans is strong (Griffiths & Balster, 1979). In other words, drugs that tend to be more addictive in humans also are more reliably self-administered by laboratory animals.

## Electrical Brain Stimulation

As Olds & Milner (1954) discovered, electrical stimulation of certain areas of the brain is rewarding. Animals develop a strong conditioned place preference for an environment where electrical brain stimulation has been administered (Duvauchelle & Ettenberg, 1991; Ettenberg & Duvauchelle, 1988). Lab animals are implanted with brain electrodes most frequently in the medial forebrain bundle (MFB), an axonal projection from the hindbrain to the forebrain and cortex. The cell bodies for some of the neurons in the MFB are found in the substantia nigra and VTA, two regions of dopamine-containing cell

bodies in the brainstem. The projections from the VTA are known as the mesocortical and mesolimbic projections, since they project to the neocortex and limbic system, respectively.

One animal paradigm using this technique is intracranial self-stimulation (ICSS), in which an animal learns to perform some task such as lever pressing to receive electrical stimulation (Wise, 1996). Intensity, pulse width, frequency, and train duration of the electrical signal can be manipulated. When each of these is held constant except frequency measured in Hz, rate of responding measured in bar presses per min produces a dose-response curve. Low doses of ICSS fail to establish lever pressing above chance levels. As doses increase, so does lever pressing, until finally a point is reached where further stimulation increases do not cause further increases in lever pressing.

Shifts in this dose-response curve up or down reflect changes in the effectiveness of ICSS, or synergism or antagonism of the rewarding impact of the stimulation (Wise, 1996). For example, administration of dopamine antagonists diminishes ICSS responding (Hunt, Altrens, & Jackson, 1994). Both dopamine and opiate antagonists cause right shifts in the ICSS rate-frequency curve (Wise, 1996). In other words, to keep the animal bar-pressing at the same rate over time, a greater intensity of stimulation is required. This is evidence of diminishing brain stimulation reward.

Alternatively, injections of dopamine agonists have been found to increase electrical stimulation (Redgrave, 1978). Experimental work also has shown that injections of drugs such as cocaine increase extracellular dopamine concentrations in the nucleus accumbens while increasing the rewarding quality of intracranial stimulation. In other words, dopamine agonists lower the voltage threshold at which animals will work for electrical stimulation of the brain (Wise, 1996). Most drugs of abuse cause a left shift in the ICSS rate-frequency

curve. The animal will bar-press at a consistent rate for a lower frequency of stimulation. Brain stimulation thresholds are lowered with amphetamines, cocaine, heroine, morphine, nicotine, phencyclidine, cannabis, and, possibly, alcohol (Wise, 1996).

Despite the effects seen in studies with drugs of abuse and ICSS, there is controversy regarding its use for measuring drug abuse liability (Bozarth, 1987). It is unclear what is being measured. The lowering of voltage thresholds makes intuitive sense, since the drug activates the reward pathway, lower stimulation is required to receive a rewarding amount of stimulation. However, using rate measurements as the dependent variable may not be reliable because rates vary considerably within an animal and over the course of a testing session, i.e., with fatigue (Bozarth, 1987).

## Choice Paradigms

In choice paradigms, an animals is presented with a choice between two stimuli, such as a drug versus other rewarding stimuli, e.g. food (Woolverton & Balster, 1981) or other drugs (Johanson & Schuster, 1975). Choice procedures allow the comparison of the incentive value of drugs. The percentage of trials in which the drug is chosen out of the total number of trials is measured and can be used as a measure of the relative incentive value of the drug (Markou, Weiss, Gold, Caine, Schulteis, & Koob, 1993). Drugs abused by humans, such as cocaine and heroin, have been shown to be reliably preferred over food by lab animals in this paradigm (Johanson, 1976).

## Conditioned Reinforcement Paradigm

The conditioned reinforcement paradigm is based on classical conditioning and the conditioning/learning model of addiction. It is used to assess the incentive value of normally neutral stimuli through association with a drug. In this paradigm associations can be made

between a rewarding drug and any type of stimulus, for example a visual or auditory stimulus (Bozarth, 1987).

In the typical procedure, an animal is trained in an operant box with two levers. One lever is active, and pressing of this lever results in the presentation of a formerly neutral stimulus followed by administration of a drug. The other lever is inactive, producing neither the neutral stimulus nor the drug. The animal learns to choose the active lever. During the testing phase, the active lever again results in the presentation of the drug-paired stimulus, but it is followed by a saline injection. The percentage of responses on the active lever provides a measure of the reinforcement value of the drug.

**Conditioned Place Preference Paradigm**

Conditioned place preference (CPP) is a specific type of conditioned reinforcement paradigm. In the CPP paradigm, the animal's location within an apparatus and the environmental cues associated with that location are reinforced by the association with a rewarding drug. Numerous treatments, such as food and sucrose, and drugs such as stimulants, opiates, hypnotics, produce CPP (Swerdlow, Gilbert, & Koob, 1989). These results have been consistent with results of other useful measures of reinforcement. There is good concordance between drugs that produce CPP and drugs that are self-administered by animals (Bardo & Bevins, 2000). CPP is a valid measure of the seeking of rewards, and perhaps the positive reinforcement value of drugs of abuse. CPP is a less invasive procedure than intravenous self-administration paradigms. Intravenous self-administration carries risks such as infection, the intravenous catheter becoming dislodged or nonpatent, and death due to the necessity of anesthesia to insert the catheter. Studies using CPP examine acquisition and

extinction of the response, as well as the reinstatement of extinguished CPP resulting from stress (Erb, Shaham, & Stewart, 1996) or drug exposure (Wang, Luo, Zhang, & Han, 2000).

**Conditioning of CPP**

The apparatus used in CPP is a box with at least two compartments with distinct environmental cues. Several conditioning trials are performed in which the animal is injected with a drug and confined to one specific compartment of the box. These trials are alternated with trials in which the animal receives no drug and is confined to another compartment. In the testing phase when drug is no longer administered, the animal is allowed to freely roam the apparatus. The amount of time that the animal spends in the compartment associated with the drug injection, versus the other compartment(s), is measured. If more time is spent in the compartment associated with the drug, CPP is said to have been acquired, and the drug is presumed to have rewarding properties. CPPs in animals have been demonstrated for most drugs that are addictive in humans (Bozarth, 1987). The paradigm assesses the incentive-motivational value of a drug infusion in the absence of acute effects of the drug that could influence performance, such as lever pressing.

Numerous studies have revealed that animals show CPP for environments associated with AMPH injections. The CPP effects of d-AMPH and l-AMPH are dose dependent (Gilbert & Cooper, 1983). Various dosages of intraperitoneal d-AMPH, including 0.3 mg/kg, 1 mg/kg, and 3 mg/kg, all resulted in significant CPP, with the higher dosages having greater CPP effects. In the same study (Gilbert & Cooper, 1983), l-AMPH also exhibited dose-dependent CPP effects. Dosages of 1 mg/kg, 3 mg/kg, and 10 mg/kg intraperitoneal l-AMPH each resulted in significant CPP compared to saline, with the higher dosages having greater CPP effects. Experiments such as these confirm findings from other measures of the

rewarding properties of AMPH, such as the self-injection paradigm, and confirm the validity of CPP as a measure of the rewarding properties of AMPH.

**Extinction of CPP**

The CPP paradigm is also used in studies of extinction and reinstatement following extinction, both of which reflect the incentive value of a drug (Davis and Smith, 1976). In the extinction paradigm, animals that show preference for a drug via CPP are tested under similar conditions without drug administration. The degree to which a drug is resistant to extinction is determined by several measurements. These measures include the duration of extinction responding, for example, the numbers of trials in which CPP is still observed, the percentage of time spent in the drug-paired compartment during the extinction trials, and the probability of reinstatement (Markou, 1993). Reinstatement refers to a response that reoccurs after it has previously been extinguished. For example, when a food reward is paired with an auditory tone, an animal may salivate to the tone even when food is no longer paired with it. After repeated presentations of the tone without the food reward, the conditioned response of salivation extinguishes. Reinstatement of salivation can occur with a subsequent presentation of food along with the auditory tone.

It is important to emphasize that extinction of a conditioned response is a measure of performance rather than learning or memory. When responding stops, it does not suggest that the connection between the stimulus, response and reinforcement has been forgotten. It merely indicates responding no longer occurs (Restle, 1975). In the case of self-injection or CPP, an animal stops working to obtain drug reinforcement. If the CPP to a drug requires more time to extinguish than another drug, it may or may not indicate that the animal has learned the CPP task better. Such a finding would suggest that the animal is willing to work

harder for one drug as compared to another. Similarly, for an animal to learn to work for reinforcement, the reinforcer must be sufficient for reliable learning. However, the rate of learning does not depend upon how rewarding is the reinforcer (Restle, 1975).

In studies using the CPP paradigm, group data are most commonly used (Mueller & Stewart, 2000). The criterion generally used to determine extinction is when the difference between the time spent in the drug-paired compartment and the saline-paired compartment is no longer statistically significant (Mueller & Stewart, 2000). In other studies extinction is defined as when the difference in the time spent in the drug-paired compartment post drug exposure and pre drug exposure is no longer statistically significant (Itzhak & Martin, 2001).

**Reinstatement of CPP**

Extinction and its reinstatement have good face validity in modeling abstinence and relapse in humans. People who have abused a drug often experience relapse, thus presenting cycles of drug use and abstinence (Wikler, 1973). Also, evidence shows that addicts whose treatments have included extinction therapy, or exposure to drug cues without drug administration, experience less craving and fewer withdrawal signs (Childress, McLellan, & O'Brien, 1986). In addition to exposure to drug related cues, the most important environmental events that may lead to relapse in humans and reinstatement of drug-seeking in animals are re-exposure to drug and exposure to a stressful situation.

**Drug priming and reinstatement.** Previous studies have examined the effects of priming doses of drugs in reinitiating drug self-administration (Ranaldi, Pocock, Zereik, & Wise, 1999; Schenk & Partridge, 1999). Often a single dose of a drug such as cocaine is enough to re-establish intravenous self-administration. The effect of drug primes on reinstatement is dose-dependent, in that higher priming doses result in more prolonged

attempts by the animal to obtain the drug, compared to lower priming doses (deWit & Stewart, 1981).

More recently, drug priming has been examined for its capacity to reinstate CPP. Following the extinction of morphine-induced CPP, rats exposed to morphine or AMPH showed preference for the former drug-paired compartment (Wang et al., 2000). CPP was established with 10 training doses of 4 mg/kg morphine. Extinction of CPP was observed after seven trails with no drug injection. Priming injections of either 0.25 mg/kg of morphine or 0.25 mg/kg of AMPH re-initiated CPP in which the animals showed a significant preference for the former drug-paired compartment.

Other studies have revealed that single priming doses of rewarding drugs other than the drug initially used to establish CPP can reinstate CPP. Cocaine-induced CPP was reinstated after extinction by single injections of cocaine, methamphetamine, and methylphenidate (Itzhak, & Martin, 2001). In this study, CPP was established with 20 mg/kg of cocaine. Extinction of CPP was observed after eight days of only saline injections. Single priming doses of 15-mg/kg cocaine, 0.5 mg/kg of methamphetamine, and 20-mg/kg methylphenidate each resulted in reinstatement of CPP at or near the pre-extinction level.

Priming injections of morphine, heroin, and cocaine re-established extinguished morphine-induced CPP (Lu, Xu, Ge, Yue, Su, Pei, & Ma, 2002). Morphine-induced CPP was established following 6 doses of morphine (10 mg/kg). Extinction of CPP was determined after 21 days of saline injections. Single priming doses of 1 mg/kg morphine, 0.1 mg/kg heroin, and 3-mg/kg cocaine all re-established CPP for the morphine-paired compartment.

The results of these experiments support the conclusion that a subsequent drug exposure is a significant factor in the reinstatement of extinguished drug-seeking behavior. The extinction and reinstatement of drug-seeking behavior is a useful animal paradigm that models the human condition of relapse following abstinence. The suggestion is that even a single drug experience following abstinence can restore drug use to its former level.

**Stress and reinstatement**. Along with drug priming, acute exposure to stress may also be effective in reinstating drug-seeking behaviors in animals showing extinction (Erb et al., 1996). Following the extinction of lever pressing for intravenous self-administration of cocaine, animals received a priming dose of cocaine or exposure to intermittent foot shock used as an acute stressor, alternating on a daily basis for four days. The lever pressing during reinstatement testing resulted in saline infusions only, and the lever pressing behavior was used as a measure of drug seeking behavior. A third group received neither foot shock nor cocaine, and was used as a control for measuring lever-pressing reinstatement. Both cocaine and foot shock significantly increased lever pressing compared to controls, and foot shock produced significantly more lever pressing than cocaine priming.

Stress also results in reinstatement of extinguished CPP. For example, animals exhibited CPP after 10 days of training with 4 mg/kg morphine injections, and extinction of CPP was observed after 9 days of no injections (Wang et al., 2000). On the following day, rats were given intermittent foot shock for 15 min. Recurrence of CPP was observed with time spent in the previous morphine-paired compartment similar to the original CPP.

Even a conditioned stimulus previously paired with foot shock can reinstate extinguished cocaine-induced CPP (Sanchez & Sorg, 2001). Groups of rats were trained on a conditioned fear task by presenting an odor either paired with foot shock or unpaired. Other

groups of rats were trained with an auditory tone, either paired with foot shock or unpaired. The animals receiving paired foot shock were given trials where odor or tone presentation was followed by foot shock. The unpaired group was given similar presentation of odor or tone with foot shock but in random order. The following day, animals began CPP training using cocaine 12 mg/kg. CPP was established, then allowed to extinguish. The next day animals were once again exposed to the odor or auditory stimulus. Both the odor and the auditory stimulus resulted in significant reinstatement of CPP in the paired group as compared to the unpaired group. Thus, conditioned and unconditioned stressors are capable of reinstating extinguished CPP.

Stress that occurs before initial drug exposure may increase vulnerability to drug-seeking as well. Two groups of animals, one of which received tail pinches for 1 min 4 times per day for 15 days, were implanted with self-injection catheters and allowed to nose-poke for an AMPH injection of 1.75 mg/kg. The animals that had received tail pinches had a significantly greater intake of AMPH than the control group over a five-day period (Piazza, Deminiere, le Moal, & Simon, 1990).

These studies indicate that stress, whether conditioned or unconditioned, is a significant factor in re-initiating extinguished drug-seeking behavior, and may increase vulnerability for drug use. One question raised by such studies is that animals who have experience with drug taking may be more sensitive to stress-induced reinstatement of drug seeking than other previously learned responses (Ahmed & Koob, 1997). Animals trained to work to earn food rewards show little evidence of stress-induced reinstatement following extinction of these behaviors. The suggestion is that drug exposure and reinstatement of drug-seeking behaviors may uniquely vulnerable to stressful events (Sinha, 2001).

# GENDER, OVARIAN HORMONES, AND ADDICTION

## Gender Differences in Drug Sensitivity

Gender and the influence of gonadal hormones are important considerations in the study of addiction. In general, men have a higher prevalence of drug dependence than women (Anthony, Warner, & Kessler, 1994). However, these gender differences in drug involvement may be due to greater exposure to drugs and opportunities to use them as opposed to differences in the tendency to become dependent on drugs (Van Etten, Neumark, & Anthony, 1999). Most research on stimulant addiction has used cocaine. Women begin using cocaine at an earlier age, report higher rates of cocaine use, and also report shorter periods of abstinence than men (Lynch & Carroll, 1999). Women are also more likely to relapse after cessation of cigarette smoking than men (Ward, Klesges, Zbikowski, Bliss, & Garvey, 1997). Similar findings could be expected with amphetamine addiction.

### Animal Studies

Results from animal studies support the hypothesis that there are gender differences in drug responses and that gonadal hormones are involved in these differences. Female rats acquire cocaine self-administration at a faster rate than males (Lynch & Carroll, 1999). Male and female rats were allowed to lever-press for an infusion of intravenous cocaine (0.2 mg/kg), and a criterion was established to determine self-administration acquisition. This acquisition criterion was met within a 30-day maximum by 70% of the female rats, compared to only 30% of the males. Of the rats that met this criterion, female rats required a mean of 7.57 days, and males required 16.67 days.

Tolerance develops rapidly to the euphoria induced by AMPH (Julien, 2001). However, some effects of drugs are not decreased, but are increased by repeated drug

administration (Robinson & Berridge, 1993). This is known as sensitization, and it is found with some of the effects of psychostimulants, including AMPH. Behaviors showing sensitization include locomotor hyperactivity, stereotyped behavior, and rotational behavior, and these behaviors often reveal gender differences (Becker, Robinson, & Lorenz, 1982). Male and female rats were injected with 1.56 mg/kg and 1.25 mg/kg AMPH, respectively. The doses differed to compensate for sex differences in AMPH metabolism in the liver and brain, and the levels of AMPH in the brain were measured. Brain AMPH levels were similar in males and females, but females exhibited significantly more rotations. Other animal studies have reported that females display greater increases in rotational behavior following AMPH administration than males (Hyde & Jerussi, 1983; Robinson, Becker, & Presty, 1982; Robinson, Becker, & Ramirez, 1980).

Sex differences in AMPH-induced locomotor activity are also reported. For example, Schneider & Norton, (1979) used three different doses of AMPH, 0.25, 0.5, and 1.0 mg/kg. Each AMPH dose increased activity in rats as measured in a residential maze compared to saline controls. The increased activity was greater in females than males at all three AMPH doses.

In another experiment, female rats showed greater locomotion and stereotypy than did males following repeated administrations of AMPH (Camp & Robinson, 1988). Females received lower doses of AMPH than males to ensure similar levels of AMPH in the brain. Injections of AMPH were given daily for ten days. Stereotypy increased in both males and females over the course of the ten days, but females showed greater enhancements with successive injections than did males. Locomotion increased over the ten days as well, and again females showed increased locomotion compared to males. Similarly, female rats

showed greater stereotypy responses to 1.5 mg/kg and 5 mg/kg doses of AMPH and were slower to recover to normal activity levels than males (Beatty & Holzer, 1978).

**Human Studies**

Women have a more rapid progression to cocaine addiction than men (Sinha & Rounsaville, 2002) and higher frequency of AMPH use (Holdcraft & Iacono, 2004). Also, greater physiological responses to drug administration are often reported by female drug abusers compared to male drug abusers. In laboratory experiments, these have included prolonged cardiovascular effects after consuming similar dosages relative to body weight (Lukas et al., 1996).

In a study of cocaine-addicted outpatients, subjects were exposed to cocaine cues including a video of cocaine use and handling of cocaine paraphernalia (Robbins et al., 1999). They were asked to rate their feelings of being "high," withdrawal responses, and cravings before and after exposure to these cues. Women reported significantly higher ratings of craving than men. Women also showed a trend toward higher ratings of feeling high and symptoms of withdrawal, although these were not statistically significant.

Other studies have suggested that among non-treatment seeking cocaine-addicted individuals, women reported higher ratings of craving than men (Elman, Karlsgodt, & Gastriend, 2001). Researchers administered questionnaires to cocaine-addicted males and females who had not used cocaine in at least 12 hours. In addition to showing more present craving for cocaine, the women had lower scores on the desire to stop using cocaine. This experiment also measured responsivity to drug-related cues such as viewing a video of cocaine being used or handling drug paraphernalia, and women had greater responses than men.

## Estrogen-Stimulant Interactions

Several factors have been suggested to explain the gender differences observed in responses to drugs, primarily among those who are abusing the drugs (Sinha & Rounsaville, 2002). These include differences in personality, coping responses to stress, social burdens and support, and the physiological effects of abused drugs. However, the primary explanation is that variances in cycling hormone levels, including estrogen and progesterone, influence drug effects in females. The focus, however, has been on estrogen. This is based in part on variation in the effects of drugs including AMPH throughout the menstrual cycle in women and the estrous cycle of rats. The conclusions commonly point to estrogen facilitating the actions of drugs based on the findings of animal and human studies.

### Animal Studies

Drug responses vary throughout the estrous cycles of female animals, and estrogen is likely a key factor in these varied responses, including drug-seeking behavior (Lynch, Roth, Mickelberg, & Carroll, 2001). One paradigm used to reveal hormone effects is to measure drug-related behavior over the estrous cycle of gonadally intact females. More commonly, females are ovariectomized (OVX) and tested with or without replacement doses of hormones. The reason is to know fairly precisely which hormones and the amount of the hormones that is in the current circulation of the animal.

In one such study, female rats were either OVX or gonadally intact (Lynch et al., 2001). They were then implanted with intravenous catheters and trained to self-inject cocaine. Groups were OVX with or without estrogen benzoate (EB), and intact with or without tamoxifen, an estrogen antagonist. Using a criterion measure of self-administration, 70% of the OVX + EB animals and 80% of the intact females receiving no tamoxifen

acquired cocaine self-administration. Only 30% of the OVX animals without EB restoration and 50% of the intact animals with tamoxifen reached criterion. These data suggest that estrogen may underlie sex differences in drug self-administration. The mechanism through which estrogen enhances the reactivity to stimulant drugs may be increasing dopamine (DA) activity in the brain. Estrogen treatments in OVX rats increased DA turnover in the striatum (Di Paolo, Rouillard, & Bedard, 1985), and enhanced AMPH-stimulated striatal DA release (Xiao & Becker, 1998).

Numerous studies have also found that female gonadal hormones differentially influence other stimulant-induced behaviors such as locomotion and stereotyped behavior (Becker et al., 1982; Chiodo, Caggiula, & Saller, 1981; D?az-Véliz, Baeza, Benavente, Dussaubat, & Mora, 1994; Joyce , Montero, & Van Hartesveldt, 1984). Females in estrus make significantly more rotations than females in diestrus, and OVX rats given EB replacement reliably display increased AMPH-induced behaviors such as locomotion, rotation, postural deviation, and stereotypy compared to OVX females without EB restoration. These behaviors are commonly observed following AMPH administration, especially in high doses, and are measured as an indication of AMPH sensitivity.

Amphetamine increased locomotor activity, and this stimulating effect was greater in females than in males (Savageau & Beatty, 1981). Castration of males had no effect on AMPH-induced locomotor behavior, however OVX blocked the stimulating effect of AMPH on activity in females. Similarly, gonadectomy decreased AMPH-induced stereotypy in females, but not in males (Camp, Becker, & Robinson, 1986). Males also displayed greater locomotor activity induced by AMPH (0.25 mg/kg) when treated with EB prior to AMPH administration than males not treated with EB (West & Michael, 1986).

Female rats, for instance, were ovariectomized either prior to or well after puberty to investigate whether lifelong exposure to EB contributes to enhanced responsive to AMPH as adults (Forgie & Stewart, 1994a). Subgroups of these animals received EB or VEH throughout the experiment. Locomotor activity was measured following six doses of AMPH, each administered every third day. The animals ovariectomized early in life had lower AMPH-induced activity levels than those ovariectomized later in life, in both the EB and the control conditions. Also, EB-treated rats had higher levels of AMPH-induced activity and showed greater sensitization to repeated AMPH doses than did the oil-treated rats, regardless of age at ovariectomy. The results of these studies provide further evidence that it is the current estrogen levels rather than the historical levels that increase responsiveness to cocaine and AMPH.

In addition to influencing behavioral responses to stimulants, ovarian hormones also influence striatal dopamine release following exposure to stimulants (Becker & Cha, 1989; Becker & Rudick, 1999; Peris, Decambre, Coleman-Hardee, & Simpkins, 1991; Xiao & Becker, 1998). Repeated administrations of cocaine (10 mg/kg) resulted in sensitization of stereotypic and locomotor responses. This sensitization and dopamine release were enhanced in OVX females administered EB-only, but not those administered EB + PROG or PROG-only (Peris et al., 1991). Similarly, pretreatment with EB-only, but not PROG-only enhanced striatal dopamine release following AMPH compared to controls (Becker & Rudick, 1999). A study of intact female rats revealed greater AMPH-stimulated striatal dopamine release and stereotyped behaviors of rats in estrus compared to those in diestrus (Becker & Cha, 1989). These studies suggest that estrogen enhances behavioral sensitization to stimulant drugs due to increased dopamine release following exposure to these drugs.

**Human Studies**

The subjective and behavioral effects of AMPH were examined during the follicular and luteal phases of two menstrual cycles in women (Justice & DeWit, 1999, Justice & DeWit, 2000b). Recall that estrogen levels are highest in women during the follicular phase, and progesterone levels are highest during the luteal phase of the menstrual cycle. The menstrual phases of the women were confirmed by assays of plasma estrogen and progesterone levels. The effects of AMPH were greater during the follicular phase than the luteal phase. During the follicular phase, subjects were more likely to report feeling energetic, euphoric, and craving more drug after oral doses of AMPH than during the luteal phase. Also, during the follicular phase, plasma levels of estrogen were positively correlated with subjective ratings of euphoria and energy levels. Estrogen titers were not related to the responses to AMPH during the luteal phase.

Pretreatment with estradiol benzoate (EB) increases the effects of AMPH (Justice & deWit, 2000a). Transdermal EB patches were applied to women during the early follicular phase of the menstrual cycle, with the result of increased magnitude of the subjective effects of AMPH. The ratings of "pleasant stimulation" were elevated, and "wanting more" were decreased. The dose of EB used in this study raised plasma levels to supraphysiological levels, nearly ten times those measured in normal control subjects. Although these findings are important in demonstrating the influence of estrogen, they leave open the question of the influence of physiological dosages of EB.

In another study, oral administration of AMPH to healthy women volunteers produced differential effects by menstrual cycle phase (White, Justice, & de Wit, 2002). Women participated in four experimental trials, two during the follicular phase, and two

during the luteal phase. Women in the follicular phase had increased reports of heightened mood responses to AMPH compared to females in the luteal phase. Estrogen levels were positively correlated with stimulant effects. Progesterone levels were negatively correlated with stimulant effects of AMPH during the follicular phase, although progesterone levels were very low during this phase.

In contrast, progesterone may attenuate the effects of stimulants such as cocaine in both males and females (Sofuoglu, Babb, & Hatsukami, 2002; Sofuoglu, Mitchell, & Kosten, 2004). Female cocaine users received a single 200 mg oral dose of PROG or placebo within three to nine days after the beginning of their menses, and two hours later were given three deliveries of 0.4-mg/kg smoked cocaine (Sofuoglu et al., 2002). Heart rate changes and the subjective responses to cocaine were attenuated by PROG as compared to placebo. Similar conclusions were found in a study of male and female cocaine users (Sofuoglu et al., 2004). The same 200 mg dose of PROG attenuated the subjective responses to intravenous cocaine in both males and females.

These studies suggest a role for estrogen and progesterone in the physiological effects and the subjective euphoric feelings induced by stimulant drugs in women. However, there are several possible criticisms of these studies. As mentioned previously, ethical considerations prevent using drug naïve humans. Participants are volunteers who admit to being drug users. The amount of experience with and tolerance to such drugs cannot be controlled, and undoubtedly plays a role in these studies. In addition, evaluation of hormone levels can be uneven because often subjects must agree to blood draws or saliva collections and not be currently using hormone replacement therapy or oral contraceptives. Also, it may be difficult to obtain female participants in both the luteal and follicular phases.

**Organizational-Activational Model of Hormones**

Current hormone levels, i.e., hormone amounts circulating in the bloodstream at the moment, play an influential role in the sex differences observed in drug sensitivity and other behaviors. Most studies in this literature examined the influence of current hormone levels. However, current hormone levels are not the only determinant of sex differences in drug responses. Fetal brains are organized to be sexually dimorphic in drug responses, as they are in other behaviors. It is suggested that gonadal hormones influence behavior at two different time periods during the animal's development, fetal and post-pubertal periods. These have been designated as organizational and activational (Arnold & Breedlove, 1985).

Organization effects are those in which hormones act to organize neural systems. Organization occurs during prenatal development, or very early in life, whereas activational effects occur later in life. Organizational effects are permanent, whereas activational effects are generally temporary and short term. Therefore, gender differences in the effects of gonadal hormones can be related to nervous system organization early in life, or to activational effects throughout adulthood.

Activational effects of hormones are those that presumably activate neural pathways that are already formed. The methodology used by behavioral endocrinologist to distinguish activational from organizational effects on a sexually dimorphic behavior is to compare gonadectomized animal with or without hormone replacement. If the behavior does not differ between the groups, one can conclude that the sex differences are not due to current levels of hormone. If, however, the behaviors differ, one can conclude that both current hormones and their activational effects play a role. The current dissertation experiment examines only the influence of current hormone levels, thus emphasizing the activational

influence of hormones. Still it is possible that prenatal organization also influences the behavior studied here. Only another experiment that manipulates perinatal hormones and pubertal hormones would clearly distinguish only activational effects.

## STRESS AND ADDICTION

Stress exposure is a powerful influence on the risk of relapse in humans and reinstatement of drug seeking in animals (Erb et al., 1996; Sinha, 2001; Sinha, Fuse, Aubin, & O'Malley, 2000; Want et al., 2000). Both chronic and acute stress increase the risk of relapse. Findings with humans also point to stress increasing drug craving. Drug abusers often report stress and negative mood changes as reasons for relapse to drug use.

### Animal Paradigms Used to Model Stress

Researchers have used various methods to create stress in an animal. Physiological stressors have included electric shock to the tail or foot (Wang et al., 2000), cold (Bhanagar, Mitchell, Betito, Boksa, & Meany, 1995; Klenerová, Jurcovicová, Kaminský, Šída, Krejcí, Hlinák, & Hynie, 2003), tail pinch (Piazza, Deminiere, le Moal, & Simon, 1990), ether (Tinnikov, 1999), forced exercise (Tinnikov, 1999), and food restriction (Takahashi, Singer, & Oei, 1978). Psychological stressors have included social stress (Taylor, Bardgett, Farr, Womack, Komitowski, & Weiss, 1993; Taylor, Weiss, & Rupich, 1987), social defeat (de Jong et al., 2005), crowded housing (Laviola, Adriani, Morley-Fletcher, & Terranova, 2002), noise (Katz, Roth, Schmaltz, & Sible, 1980), and restraint (Hidalgo, Armario, Flos, Dingman, & Garvey, 1986). Some studies have also examined the effects of conditioned stress, where a formerly neutral stimulus such as a visual, auditory or olfactory stimulus is paired with a known stressor such as foot shock (Sanchez & Sorg, 2001).

**Restraint Stress and Corticosterone**

A common method to induce stress in animals in the literature is restriction of movement, described as either restraint or immobilization. Although some researchers use the terms interchangeably (Murison, 1983), immobilization and restraint are quite different with different intensities of stress induced. Immobilization is the situation where an animal cannot move at all, accomplished, for example, by taping the limbs and head to a board. Restraint limits movement, and the most common method of restraint is placing the animal in a small apparatus (Campmany, Pol, & Armario, 1995). Duration of time the animal is in one of these settings ranges typically from 0.5 to 2 hours (Flores, Hernandez, Hargreaves, & Bayer, 1990; Pitman, Ottenweller, & Natelson, 1988).

Measures of corticosterone (CORT) titers are generally accepted as the marker of stress. Restraint reliably increases CORT levels. For example, adult male rats were restrained for varying lengths of time in small plastic tubes (Hidalgo et al., 1986). Rats assigned to stress were restrained for 0, 1, 12, 24 or 48 hours. The animals were decapitated immediately following restraint, and trunk blood was analyzed for serum CORT levels. All stressed groups had significantly higher CORT levels than the control group, and each stressed group differed from each other. The group restrained for 1 hour had the highest CORT levels, followed by those restrained for 12, then 48 then 24 hours. The explanation for the latter findings was adaptation to the stressor in the 48-hour group.

Another experiment reported elevated CORT levels after rats were restrained in a plastic tube for two hours (Pitman et al., 1988). A control group was kept in their home cages. The restraint procedures were repeated for 21 days. Blood samples were collected on all animals on days 1, 3, 5, 7, 10, 14, 17, and 21. These samples were collected both before

the stress administration (basal levels) and one hour after the stress procedure (post-stress levels). Post-stress CORT levels were significantly elevated in the experimental animals on day one compared to the control group and remained elevated over the remaining days of the experiment. Moreover, the CORT response of the experimental animals showed no habituation over the 21 days of the experiment.

Many other studies have reported CORT elevations following restraint (Bauer, Perks, Lightman & Shanks, 2001; Crine, Louis, Sulon, & Legros, 1983; Keim & Sigg, 1976). Serial CORT levels obtained from animals restrained in small tubes for 240 min indicated that CORT levels were highest after 30 min of restraint, then gradually decreased over another 90 min, and plateaued for the remaining 120 min. The latter values remained significantly elevated from baseline (Flores et al., 1990).

The results of these studies support the conclusion that restraint is a significant stressor for animals. Restraint reliably increases CORT levels and CORT response typically habituates over the time restraint is applied (Flores et al., 1990).

**Animal Studies of Stress-Stimulant Interactions**

Stress may influence drug sensitivity in terms of relapse in humans or reinstatement of drug seeking in animals, as well as other drug behaviors. Sensitization is observed with repeated doses of stimulants (Robinson & Berridge, 1993), and also occurs following exposure to stressors, and this phenomenon was described and termed “stress-induced cross-sensitization” (Antelman, Eichler, Black & Kocan, 1980).

Since that time, numerous studies have examined the interaction of stress and stimulants on behavior (de Jong, Wasilewski, van der Vegt, Buwalda, & Koolhaas, 2005; Herman, Stinus, & le Moal, 1984), and occasionally studies have reported conflicting

findings. Some studies suggest that stress suppresses general activity and locomotion (Papp, Muscat, & Wilner, 1993). However, most studies suggest that stress increases stimulant behavioral effects, including locomotion (Herman et al., 1984).

Animals receiving repeated foot shock displayed an increased locomotor response to 0.75 mg/kg AMPH, compared to non-shocked controls (Herman et al., 1984). Stressed animals received foot shocks every min for 20 min daily over 10 days. Locomotor activity was the behavioral measure. After the tenth shock trial, the animals receiving AMPH had significantly greater locomotor activity than controls. Thus, stress appears capable of enhancing the behavioral effects of AMPH.

Findings also suggest stress-induced cross sensitization to AMPH can occur with a single exposure to a stressor (de Jong et al., 2005). Social defeat was used as an acute stressor by placing rats in the cage of an aggressive male rat. Subjects were then placed in a protective cage but remained in close proximity to the aggressive rat. During this time, they could maintain auditory, olfactory and visual contact with the aggressive rat. Groups of experimental animals were exposed to AMPH challenges at three different time intervals following the social defeat, and using three dosages of AMPH, 0, 0.25, or 1 mg/kg. After the AMPH administration, locomotor activity in the form of distance traveled in an open field was measured. For stressed animals both doses of AMPH significantly increased the distance traveled as compared to saline controls three days following social defeat, and the 1 mg/kg dose produced greater locomotor activity. AMPH did not show these effects two or three weeks following the single exposure to social defeat. Thus, acute stress increased behavioral sensitivity to AMPH, but the effect was short lasting (de Jong et al., 2005).

Similar findings were reported following a single exposure of restraint stress for 30 min (Pacchioni, Gionino, Assis, & Cancela, 2002). Rats restrained in a Plexiglas restraining cylinder were compared to control animals left in their home cages. Restraint was administered 30 min after 0.5 mg/kg IP AMPH. The locomotor behavior of the animals was measured 24 hr later. The animals subjected to restraint had significantly greater locomotion measurements than control animals.

Chronic stress can also induce behavioral sensitization to AMPH in drug-naïve rats (Piazza et al., 1990). Animals subjected to daily tail pinches for 15 days showed a significantly greater locomotor response to a single dose of AMPH (1 mg/kg) on the day following the last stress day compared to non-stressed controls. Similarly, chronic stress in the form of restraint potentiated AMPH-induced locomotor effects (Bisagno, Grillo, Piroli, Giraldo, McEwen, & Luine, 2004). Intact female rats restrained in Plexiglas restraint for 6 hr for 21 consecutive days displayed greater locomotor effects of chronic 2.6 mg/kg IP AMPH than nonstressed controls.

Mixed findings were reported in a study comparing unpredictable to predictable stress (Haile, GrandPre, & Kosten, 2001). Chronic unpredictable stress was created by presenting different types of stressors randomly twice each day for a period of 10 days. Predictable stress was created by 60 min of restraint every day for 10 days. Control animals were left in home cages and not stressed. Following 7.5 mg/kg doses of IP cocaine, locomotor activity was recorded. The unpredictable stress enhanced locomotor activity following cocaine compared to nonstressed controls, however predictable stress did not result in significant enhancement of locomtion.

Most studies report that stress exposure results in increased behavioral responses to AMPH (Deroche, Piazza, Casolini, Maccari, le Moal, & Simon, 1992). Conflicting results are occasionally observed, and several factors have been suggested to explain these findings, including the severity of the stressor, the number of exposures to the stressor, or the duration of the stress exposure (de Jong et al., 2005; Haile et al., 2001; Will, Der-Avakina, Pepin, Durkan, Watkins, & Maier, 2002).

**Stress and Drug Craving in Humans**

In a study of individuals addicted to cocaine, participants reported increased drug craving following each of two commonly used stress-inducing tasks (Sinha, Catapano, & O'Malley, 1999). After each of these tasks, they rated their cocaine craving and emotional state on a standardized questionnaire. Cocaine craving significantly increased after each of the stress inducing tasks. Also, positive emotional ratings decreased and negative emotional ratings increased.

Similarly, cocaine addicted individuals participated in a laboratory trial in which they were exposed to stress, drug cues, and neutral relaxing imagery conditions (Sinha et al., 2000). Significant increase in subjective cocaine craving occurred in the stress and drug imagery, but not with the neutral-relaxing imagery. Similar increases in physiological measures including heart rate and salivary CORT levels also were observed with the drug cues and stress imagery scripts as compared to the neutral-relaxing script.

An acute social stressor altered the effects of a low oral dose of methamphetamine (METH) in healthy male volunteers (Söderpalm, Nikolayev, & de Wit, 2003). Subjects were assessed in two separate trials, one in a stress condition, and one in a relaxed situation. Following the acute stressor or relaxation trial, subjects were administered 10 mg METH or

placebo solubilized in a beverage. Early in the trials, stress appeared to dampen or have no effect on the stimulant effects of METH based on the subjective ratings of the participants. However, stress significantly increased the rating of "want more drug." At later time points, METH produced its typical increases in stimulant and euphoric effects and decreases in sedative effects, regardless of whether subjects were stressed or not.

Following commonly used stress-inducing tasks, ratings of negative emotions and the desire to use METH or cocaine increased in METH and cocaine-addicted individuals (Harris, Reus, Wolkowitz, Mendelson, & Jones, 2005). The measures were repeated in subjects who returned for further evaluations, for a maximum of four stress sessions. Repetition decreased the ratings of negative emotions, but the desire for drug did not change over these sessions.

The collective evidence from these studies suggests that stress increases drug craving and may be a significant risk factor for relapse in drug-addicted humans. The exact physiological mechanism of the influence of stress is unclear, but a suggestion is that individual differences in hypothalamic-pituitary-adrenal (HPA) axis function may influence vulnerability to drug addiction (Oswald, Wong, McCul, Zhou, Kuwabara, Choi, Brasic, & Wand, 2005). A criticism of human studies is that these subjects have varied experience with drug use and different degrees of addiction. In addition, the motivation of human study participants may be unknown. These factors can be controlled in animal studies.

## OVARIAN HORMONES AND STRESS

Studies of gender differences in response to stress, as well as the interaction of estrogen and stress, have produced inconsistent and sometimes contradictory findings, especially in studies of humans. The findings in animal research are relatively consistent. Female rats have higher baseline levels of CORT and greater CORT responses to stress than

do males (Aloisi, Ceccarelli, & Lupo, 1998; Laviola et al., 2002). However, the conclusions are less consistent in human studies. Some findings suggest similar stress responses in men and women (Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999), while others suggest greater responses in one gender or the other (Kirschbaum, Wust, & Hellhammer, 1992).

## Estrogen-Stress Interactions

### Animal Studies

Rodents show sexual dimorphism in their physiological responses to stress, as measured by plasma CORT levels. For example, male and female rats were either subjected to 30 min of restraint stress or left in their home cage as a control group (Aloisi et al., 1998). They were then decapitated and trunk blood was used for hormonal assays. Corticosterone levels were higher in females than in males in both the control and the restraint groups.

Basal CORT levels were higher in female mice than in males as found in plasma assays (Laviola et al., 2002). These female and male mice also were exposed to two different housing conditions. One condition was the normal noncrowded housing and the other condition was nine days of crowded housing. After a 24-hour period of individual housing female mice showed much higher CORT levels than males. These findings support the conclusion that female rats have higher baseline levels of CORT and greater CORT responses to stress than do males.

In another study, male and female rats were exposed to 30 min of restraint and serum hormone assays including adrenocorticotropic hormone (ACTH) and CORT levels (Farabollini, Albonetti, Aloisi, Facchinetti, Grasso, Lodi, Lupo & Muscettola, 1993). These hormones are measured to determine the level of functioning throughout the HPA system.

The pituitary releases corticotropic hormone which stimulates the pituitary to release ACTH. ACTH acts primarily on the adrenals to stimulate the release of corticosteroids. Compared to nonstressed controls, restrained animals had higher levels of ACTH, but no gender differences in these levels. Gender differences were found in CORT levels in both restrained and control groups with females having higher levels of CORT. Similar results were reported later by Aloisi et al. (1998).

There are conflicting findings suggesting that estrogen may inhibit the hypothalamic-pituitary-adrenal (HPA) stress response in animal models. For example, Young, Altemus, Parkinson, and Shastry (2001) implanted female rats with pellets containing CORT and either injected them with 100 ?g/kg tamoxifen, an estrogen antagonist, or vehicle only. The following day, all animals were exposed to a 30-min restraint stress, immediately followed by blood collection. ACTH increased in response to restraint stress in controls. The CORT pellets blunted the ACTH response to stress, presumably as a result of negative feedback in the HPA axis. Tamoxifen produced a significant increase in ACTH secretion. There was no interaction between CORT and tamoxifen treatments on the ACTH response to stress. The CORT response to stress was blunted in the rats implanted with CORT pellets as expected, and tamoxifen attenuated the CORT response to stress in CORT treated rats. These findings suggest that estrogen may inhibit responsiveness of the HPA axis to stress.

In summary, female experimental rodents typically have higher baseline levels of CORT and greater CORT responses to stress (Aloisi et al., 1998; Farabollini et al., 1993). The females also have increased ACTH levels following stress (Young et al., 2001). In addition to the endocrine responses, females typically have greater behavioral responses to stress, for example (Herman et al., 1984). However, the interaction of ovarian hormones and

stress responses is equivocal. Some findings suggest that endocrine and behavioral responses to stress may be diminished in the presence of estrogen (Young et al., 2001).

**Human Studies**

Some studies suggest greater stress responses in women, or mixed results. In one such study, stress reactions among four groups of participants were compared. Men, women in the follicular phase of the menstrual cycle, women in the luteal phase of the menstrual cycle, and women using oral contraceptives were subjected to a standard social stress test (Kirschbaum et al., 1999). Heart rate, ACT titers, salivary and serum cortisol (CORT) titers, and subjective stress ratings were collected at baseline and post-stress test. All measures increased significantly following the stress test. Men displayed higher ACTH responses than all three groups of women, supporting the idea that men have a greater hypothalamic drive. The salivary CORT results revealed the following pattern: luteal > men > follicular > contraceptive. The interpretation was that women in the luteal phase when estrogen levels are characteristically lower had CORT responses similar to men under stressful conditions.

Estrogen influences cardiovascular responses to stress differently in healthy postmenopausal women with or without hormone replacement therapy (HRT) (Matthews, Flory, Owens, Harris, & Berga, 2001). Participants performed experimental tasks such as preparing a speech to recite to the experimenter. Several cardiac function parameters were measured including systolic blood pressure, pulse pressure, EKG, and heart rate. The women taking HRT had significantly lower systolic blood pressure and pulse pressure during the stressful tasks than women not taking HRT. These findings and others (Lindheim, Legro, Bernstein, Stanczyk, Vijod, Presser, & Lobo, 1992) suggest that estrogen can have a protective role against stress-induced cardiovascular reactions in women.

Men may display a greater response to stress than women, as measured by changes in serum CORT levels. For example, the CORT responses of healthy men and women to two psychological stressors were examined (Kirschbaum et al., 1992). The first stressor was public speaking, and the second was a serial subtraction task. CORT levels in both men and women increased significantly after exposure to both stressors from baseline measurements. Men, however, experienced larger increases than women.

Stress reactivity was compared among three groups of postmenopausal women: those receiving HRT treatment with EB-only, EB + PROG, or no HRT (Burleson, Malarkey, Cacioppo, Poehlmann, Kiecolt-Glaser, Berntson, & Glaser, 1998). CORT titers were measured at baseline and following a brief social stress test. Women on either type of HRT had higher CORT levels than the control group.

Women who were taking oral contraceptives and those who did not were exposed to a stress-inducing task either during the premenstrual phase or the midcycle phase of their menstrual cycle (Marinari, Leshner, & Doyle, 1976). Following the task, participants rated their affective state, and blood samples were collected for plasma CORT assays. The women who were both taking contraceptives and were in the premenstrual phase had significantly greater CORT responses to the task than the other three groups of women, which did not differ from each other. No group differences in subjective affective ratings were obtained.

One explanation suggested for these conflicting results is that estrogen may influence corticosterone-binding protein levels, and some researchers suggest that in studies of stress reactivity, simultaneous measurement of free and total CORT levels should be collected (Kirschbaum et al., 1999).

**Estrogen-Stress-Stimulant Interaction**

The interaction of the three factors, estrogen, stress, and stimulants on drug-seeking behavior has not been examined systematically, although there are several relevant reports in the literature. The interaction among gender, mild stress, and AMPH, was examined with AMPH-induced locomotion as the behavioral measure (West & Michael, 1988). Exploratory behavior and AMPH-enhanced locomotor activity of rats was influenced by mild stress such as handling. Groups of female and male rats were handled daily for five days. Animals in each group received 0.25 mg/kg AMPH or saline. The animals that were handled displayed greater activity following AMPH than those that were not handled, but this cross-sensitization did not show sex differences. In other words, the three-way interaction of handling, drug, and sex was not statistically significant.

Groups of adult rats were subjected to a social stress as adolescents (McCormick, Robarts, Gleason, & Kelsey, 2004). Several weeks later, a subset of these animals was tested for locomotor activity and response to nicotine. The animals were injected with saline or nicotine daily for five days, and activity was measured. Adolescent social stressors increased locomotor response to nicotine in adulthood compared to controls. However, this stress x nicotine interaction was significant only for females, and not for males.

The conclusions from these studies suggest that there may be complex interactions among gender or ovarian hormones, stress, and drug exposure. One implication is that stress has a sexually dimorphic influence on drug-seeking and the perpetuation of drug addiction.

## THE CURRENT EXPERIMENT

### Overview

The present dissertation experiment examined the interaction of ovarian hormones and stress exposure, as well as repeated drug exposure, on reinstatement of drug-seeking behaviors in female rats. One purpose was to assess the interaction among female sex hormones, stress and repeated AMPH exposure, with special emphasis on conditioned place preference (CPP), an animal model of relapse. Groups of ovariectomized (OVX) females were used to examine the CPP response to AMPH of animals receiving different hormone replacements. The animals received estrogen benzoate (EB-only), progesterone (PROG-only), both hormones (EB + PROG), or vehicle only (VEH). The predicted outcome was that OVX females receiving EB-only would show greater CPP responses to AMPH, be more resistant to CPP extinction, and would have different CPP reinstatement responses when exposed to stress or drug than ovariectomized females receiving EB + PROG, VEH, PROG-only.

There were six phases to the experiment. The **baseline** phase was used to adapt the animals individually to the apparatus before drug treatments began. During the **conditioning** phase, AMPH was used to establish CPP. This was followed by a single **test for CPP acquisition**. **Extinction** began the next day and was used to return the animal to baseline preferences, that is the same time spent in the drug-paired compartment as during baseline. No drug was administered during extinction. After extinction, the animals were given a **challenge**, and the animals were tested for reinstatement of CPP. Last, a **second extinction** phase began the day following the challenge administration. The primary data collected were

times spent in the drug-paired compartment during baseline, CPP acquisition test, extinction, challenge, and second extinction phases.

## Materials and Methods

### Subjects

Subjects for the experiment were 64 ovariectomized (OVX) female Long-Evans rats, between the ages of 60 and 96 days of age (M = 66.20 ? 8.76), and weighing between 164 and 244g (M = 201.03 ? 17.26) on the first day of the experiment. Animals were bred and maintained by the University of Missouri-St. Louis animal unit, and were housed individually in large, flat-bottom polycarbonate cages, measuring 50 x 40 x 20 cm. Water and LabDiet 5001 commercial rodent chow were available ad libitum during the entire study. Animals were maintained on a 12-h light/dark cycle (lights on at 2300h and off at 1100h) under controlled temperature (20? C to 22? C) and humidity (50 ? 5 %). Body weights were recorded weekly throughout the study for overall health monitoring purposes and to ensure proper drug dosages.

### Surgery

All animals were OVX one week prior to the start of AMPH conditioning trials. Rats were anesthetized with Halothane, purchased from Sigma Chemical Company (St. Louis, MO). An adequate plane of anesthesia was assured by verifying that a toe pinch failed to elicit a reflex reaction. Ampicillin 100 mg/kg, purchased from Henry Schein Company (Port Washington, NY) and prepared for injection in a solution of isotonic saline, was administered SC prophylactically.

The hair on the abdomen was shaved, and the skin was cleansed with Betadine scrub and water, followed by Betadine solution. The cleansed surgical area was dried with sterile

gauze and covered with a 10 x 14 cm Op-Site transparent, self-adhesive drape. A 1-cm incision was made in the lower abdomen. The skin and muscle were incised to expose the abdominal cavity. The ovaries were ligated and removed. The muscle and deep tissues were apposed using plain gut simple continuous sutures, Ethicon 4-0, and the skin was apposed with 4.0 nylon sutures in a simple interrupted suture pattern. Autoclaved instruments were used for the first procedure of each day. Prior to each subsequent procedure instruments were wiped clean of blood and tissues and soaked in glutaraldehyde, followed by a saline rinse.

Analgesia was provided in a two-fold manner. For local analgesia, 1% Bupivicaine was infiltrated at the incision site, using 0.5 – 1.0 cc. For systemic analgesia, 0.025 mg/kg Buprenorphine was injected SC after surgery, and again every 12 hours for 24 hours as needed. Buprenorphine and Bupivicaine were also purchased from Henry Schein Co. (Port Washington, NY). A heating pad was used beneath clean drapes to prevent hypothermia during the ovariectomies. Additional analgesia was provided by having 200-mg/ml acetaminophen in the drinking water for a total of 3 days. Rats were closely monitored for 72 hours after surgery for potential complications. The rats recovered in clean cages containing autoclaved bedding for seven days, after which time they were housed in their routine bedding and behavioral testing began. Skin sutures were removed 7 to 10 days post-op. Daily observations were recorded on a post-op form until the sutures were removed.

**Drugs**

D-amphetamine (AMPH) was purchased from Sigma Chemical Company (St. Louis, MO) and prepared for injection in a solution of isotonic saline. A dose of 1 mg/kg bwt was

injected SC in a volume of 1 ml/kg bwt. In previous experiments, doses of 1-mg/kg AMPH and lower have successfully induced CPP (Gilbert & Cooper, 1983).

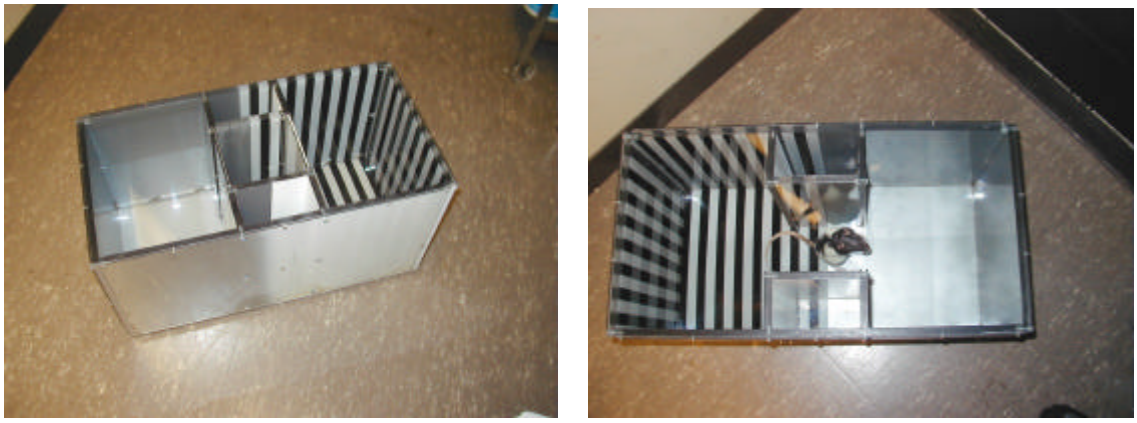
Estrogen benzoate (EB) and progesterone (PROG), purchased from Sigma Chemical Company (St. Louis, MO), were suspended in olive oil and injected SC. The dose of EB administered was 20 ?g/kg bwt suspended in 1 ml/kg of olive oil. The dose of PROG administered was 100 ?g /kg bwt suspended in 1 ml/kg of olive oil. The logic of the 20-?g/kg dose of EB and the 100-?g /kg dose of PROG also used in other studies was to restore circulating levels of the hormones to those found in young adult female rats (Antus, Hamar, Kokeny, Szollosi, Mucsi, Nemes, & Rosivall, 2003; Butcher, Colins, & Fugo, 1974; Niyomchai, Russo, Festa, Akhavan, Jenab, & Quiñones-Jenab, 2005). Hormone injections were administered daily throughout the study, starting on the third day following ovariectomies. The VEH group received daily injections of 1 ml/kg olive oil only.

**Apparatus**

The animals were trained and tested for CPP in a rectangular Plexiglas box, constructed from descriptions in the literature (e.g., Lu et al., 2002). The box was 60 cm x 30 cm x 30 cm, with 2 equal size side compartments (22 cm x 30 cm), and a small central start box (13 cm x 13 cm). The floors and walls of the compartments were visually different. One of the large compartments had black and white stripes 2 cm wide on all 4 walls and the floor, and the other compartment was solid gray. The start box, located in the center of the apparatus, had opaque guillotine doors on the two sides leading into the main compartments. The color of the two guillotine doors matched those two compartments of the apparatus. The start box was divided in half, such that the walls and floor had the same color pattern as the corresponding larger compartments. Therefore, the entire apparatus was essentially divided

into two halves, with half being striped and the other half solid. The apparatus was positioned in the rear of the dimly lit 2.67 m x 5.39 m experimental room. Stopwatches were used to measure time spent in each compartment of the apparatus. Hand-held counters were also used for counting transitions from one compartment to another in the CPP apparatus.

A commercially available flat-bottom restrainer (Braintree Scientific, Inc, Braintree MA) was used to deliver restraint stress to the animals. The tubular plastic cylinder, 6.35cm in height x 15.24cm in length had slits in the top that allowed adjustment of the restraint space to the size of each individual animal.



**CPP Apparatus: Guillotine doors closed and doors open with rat inside**

**Flat-bottom restrainer: Empty and with restrained rat**

## Procedures

### Baseline Phase

This phase served three purposes. The first was for the animals to become accustomed to daily handling, transporting, and being in the experimental room. The second purpose was for the animals to investigate the CPP apparatus, and to become accustomed to being placed into it. The third purpose was to establish baseline performance to determine if the animal had an unconditioned preference for one compartment over another before drug exposure began.

For a baseline trial, an animal was placed in the start box with the guillotine doors closed. After 10 sec both guillotine doors were lifted, and the rat was allowed to investigate the CPP apparatus for 20 min, after which the animal was returned to the home cage. This procedure was repeated on subsequent days for a total of three baseline days for each animal. On each of these days, the time spent by the animal in each of the two compartments of the apparatus was recorded to assess for unconditioned place preference. Recall that since the start box was divided in half with the halves matching the color pattern of the larger compartments, an animal was always determined to be in one compartment or the other. The start box was not a neutral area. An animal was considered to be in a compartment by the position of its nose, whether it was beyond the striped half of the start box or the solid half. In addition, the numbers of transitions made between the compartments by each animal were recorded as a measure of general activity level, and these were compared among the four hormone groups. The apparatus was cleaned with a mild soap and water solution and dried thoroughly between trials.

**Conditioning Phase**

During the eight days of the conditioning phase, each rat received four administrations of AMPH once per day every other day. Immediately after drug administration, the animal was confined for 20 min to one compartment of the CPP. On the other four days spaced between the drug exposure days, the animal was administered isotonic saline vehicle and confined to the opposite compartment. The compartment that was paired with AMPH was counterbalanced within and between groups. All animals began day one on the striped side of the apparatus, thus the order of injections was counterbalanced also, such that half the animals in each group received AMPH on day one and the other half received saline on day one.

One purpose of the baseline phase was to determine if an animal had an unconditioned preference for one side of the apparatus. An unconditioned preference was defined as spending more than 60% of the total time over the 3 days of the baseline phase in one compartment of the apparatus. The numbers of animals meeting this criterion were 22 of the 64, and the preferred compartment was used for saline administration. That is, the less preferred compartment of the apparatus was used for AMPH exposure.

All other animals were randomly assigned to receive AMPH in the striped vs. the solid compartment. Assigning drug to the less preferred compartment, referred to as the biased method (Bardo & Bevins, 2000), is a typical procedure used in studies measuring CPP (Lynch, 1991). Conversely, some studies have used an unbiased method, in which half of the animals in a study receive drug in the preferred compartment, and the other half in the less preferred compartment (Pelloux, Costentin, & Dueterte-Boucher, 2004). Valid arguments have been made for each procedure, such as the risk of a ceiling effect in the case of the

unbiased method. In the biased method, a valid question is whether an animal has acquired CPP or simply reduced its aversion to the non-preferred compartment (Bardo & Bevins, 2000).

Although CPP has been induced after only one or two AMPH exposures (Bardo, Valone, & Bevins, 1999; Pelloux et al., 2004), previous experiments typically expose animals to the experimental drug for four administrations over days (Gilbert & Cooper, 1983). That precedent was followed in this experiment. Placing the animal in the apparatus immediately following the administration of AMPH is also the typical procedure used in experiments measuring CPP (Gilbert & Cooper, 1983; Pelloux et al., 2004). AMPH peaks rapidly, and although no stereotypical behaviors were noted in the animals in this experiment, informal observation suggested the animals urinated and defecated more often in the CPP apparatus to AMPH than to saline. Presumably, the animals were experiencing drug effects while in the apparatus.

**CPP Acquisition Testing**

The day following the last day of the drug exposure phase, the animals were tested for CPP acquisition. No AMPH or saline was injected. Each rat was placed in the start box of the apparatus with the guillotine doors closed. After 10 sec, both guillotine doors were lifted, and the animal was allowed to freely move among the compartments of the apparatus for 20 min. The amount of time spent in each compartment was measured during the trial, determined by the position of the nose. Again, recall that the apparatus essentially had two halves, striped or solid. Therefore an animal was determined to be in the striped or solid half at all times.

Times in the drug-paired compartment, as well as difference scores were also obtained for each animal. The latter scores were determined by the time spent in the AMPH-associated compartment at CPP acquisition testing relative to the average time spent in the same compartment during baseline. These measures were used in the statistical analyses to compare CPP acquisition among the four hormonal groups.

In addition, the numbers of transitions made between the compartments by each animal were recorded as a measure of general locomotor activity, and these were compared among the four hormone groups. A transition was counted each time the animal's nose crossed the central line dividing the striped half of the apparatus from the solid half.

**Extinction Phase 1**

During the first extinction phase, animals were given daily 20-min trials in the CPP apparatus, using the same procedures used for CPP acquisition testing. That is, no AMPH or saline was injected on any of the ten days of extinction. Each rat was placed in the start box of the apparatus with the guillotine doors closed. After 10 sec, both guillotine doors were lifted, and the animal was allowed to move about the apparatus for 20 min. These procedures were repeated daily for ten days. Previous reports indicate that eight consecutive days of no drug exposure has resulted in extinguished cocaine-induced CPP (Itzhak & Martin, 2001), therefore, ten days was presumed to be adequate time for extinction to occur. The criterion used to determine extinction was when the difference in the times in the drug-paired compartment post drug exposure and pre drug exposure were no longer statistically significant (Itzhak & Martin, 2001). The amount of time in each compartment was measured during each daily trial. Difference scores were also obtained for each animal on each day, that is, time in the AMPH-associated compartment on each day of extinction, relative to the

time in the same compartment during baseline. These measures were obtained on each of the ten days for each of the four hormone groups and were used in the statistical analyses to compare CPP extinction among the four hormonal groups.

In addition, the numbers of transitions made between the compartments by each animal were recorded as a measure of general locomotor activity. Mean numbers of transitions during the ten days of extinction were calculated for each animal as a single value during extinction, and these values were compared among the four hormone groups.

**Challenge Phase**

The day following the final day of the extinction phase the animals received either a stress or a drug challenge. For animals in the drug challenge groups, a single dose of AMPH (1 mg/kg) was administered, and the animal was immediately placed in the CPP apparatus for 20 min. The animals in the stress groups were confined for 90 min in the restrainer, during which time each animal was given a sham injection of saline 1 ml/kg. Immediately upon removal from the restrainer, the animal was placed in the CPP apparatus for 20 min. The procedures for this challenge test were the same as used during CPP acquisition testing and extinction. That is, after 10 sec, the guillotine doors were lifted and the animal was allowed to move freely between the two compartments. Time spent in the drug-paired compartment was recorded and difference scores were calculated for each animal. The latter was the time spent in the AMPH-paired compartment relative to time spent in the same compartment on the tenth day of the first extinction phase. These measures were used in the statistical analyses to compare CPP reinstatement among the four hormonal groups, between the two challenge condition groups, and for the interaction of the two variables. Once again, the numbers of transitions made between the compartments by each animal were recorded as

a measure of general locomotor activity. These were compared among the four hormone groups, the two challenge groups, and the hormone by challenge interaction.

**Extinction Phase 2**

Beginning the day following the challenge administration, animals were tested for a second extinction of the presumably reinstated CPP. The procedures for this extinction phase were identical to the first extinction phase, except that this phase was only five days. Once again, the numbers of transitions made between the compartments by each animal were recorded as a measure of general locomotor activity. Mean numbers of transitions during the five days were calculated for each animal as a single measure during the second extinction, and these were compared among the four hormone groups, the two challenge groups, and the hormone by challenge interaction.

The Institutional Animal Care and Use Committee (IACUC) of the University of Missouri-St. Louis approved all procedures prior to the start of the experiment. Table 1 on p. 59 contains an overview of the experimental phases.

## Hypotheses

**CPP Acquisition Testing**

**Hypothesis 1.1.** Based on findings that AMPH induces CPP at dosages lower than the 1 mg/kg dose used in this research project (Gilbert & Cooper, 1983), all groups of animals were expected to meet the criterion of CPP during the CPP acquisition tests. That is, animals in each hormone treatment group were predicted to spend more time in the drug paired compartment at acquisition testing relative to baseline. Data used to test this hypothesis were the times spent in the drug-paired compartment at CPP acquisition testing and the times spent in the same compartment during baseline. A mean was calculated over

**Table 1**

Overview of Experiment Phases

| Phase | Days of Experiment | Procedures |
|---|---|---|
| Baseline | Days 1 - 3 | Daily hormone injections began prior to beginning baseline, 3 days post-ovariectomy; free exploration of CPP apparatus |
| Conditioning | Days 4 – 11 | Daily hormone injections continued; alternating daily administrations of AMPH 4x and saline 4x with confinement in alternating sides of CPP apparatus |
| CPP Acquisition Testing | Day 12 | Daily hormone injections continued; free exploration of CPP apparatus |
| Extinction 1 | Days 13 – 22 | Daily hormone injections continued; free exploration Of CPP apparatus |
| Challenge | Day 23 | Daily hormone injections continued; single dose of AMPH (1 mg/kg) or restraint stress (90min) followed by free exploration of CPP apparatus |
| Extinction 2 | Days 24 – 28 | Daily hormone injections continued; free exploration of CPP apparatus |

the three days of the baseline phase to obtain a single time score for each animal during baseline. Therefore two time measurements were used, the mean times spent in the drug-paired compartment over the three days of baseline and the times spent in the same compartment at CPP acquisition testing. These times were used to test Hypotheses 1.1 and 1.2.

**Hypothesis 1.2.** Another prediction for CPP acquisition testing was that there would be differences in the strength of CPP among the hormone groups. Based on evidence that estrogen enhances and progesterone depresses the rewarding properties of AMPH (Justice & de Wit, 1999), animals receiving EB-only were expected to have the greatest increase in time spent in the AMPH-associated compartment compared to baseline. This was expected to be followed by the group receiving EB + PROG and the VEH group. These two groups were expected to show similar results due to EB and PROG having opposing effects. Animals receiving PROG-only were expected to spend the least amount of time in the drug-paired compartment during acquisition testing, relative to the time at baseline.

**Extinction 1**

**Hypothesis 2.1.** A prediction for the first extinction phase was that there would be a statistically significant main effect of day. Specifically, the prediction was for a decrease by all groups over the ten days of this phase in the time spent in the previously AMPH-paired compartment. However, a statistically significant hormone group x day interaction was also expected. That is, the hormone groups would differ in rates of extinction.

The criterion used to determine extinction was the absence of statistical significance between the time spent in the drug-paired compartment during the extinction trials and the time spent in the same compartment at baseline. Animals receiving EB-only were expected to

experience the most protracted extinction. The day that this occurred was expected to be later for the animals receiving EB-only and earlier for the other groups, with the animals receiving PROG-only being the earliest.

These results provide assessment of hormonal group differences in rates of extinction, and rate of extinction of CPP provides an additional measure of drug sensitivity. The assumption is that the more days that are required for CPP to extinguish, the more sensitive the animal is to the drug effects. This hypothesis was once again based on evidence that estrogen enhances and progesterone depresses the rewarding properties of AMPH (Justice & de Wit, 1999). The times spent in the drug-paired compartment on each of the ten days of extinction were compared to baseline, therefore these times were the data used to test Hypotheses 2.1 and 2.2.

**Hypothesis 2.2.** A further prediction for the first extinction phase was that hormone treatments would have a statistically significant influence on time spent in the AMPH-paired compartment on the final day of extinction. Again reflecting the working concept that estrogen enhances and progesterone dampens drug sensitivity, it was predicted thatanimals receiving EB-only would spend more time in the AMPH-paired compartment on the last day of extinction than the other hormone groups as a result of CPP being more firmly established. The animals receiving PROG-only would spend the least time in the AMPH-paired compartment on this final day of extinction phase 1.

**Challenge**

**Hypothesis 3.1.** For the challenge phase, both stress and drug re-exposure challenges were expected to result in statistically significant increases in time spent in the AMPH-paired compartment for all hormone treatment groups (Itzhak & Martin, 2001; Wang et al., 2000).

For each animal, the time spent in the drug-paired compartment at challenge and the time spent in the same compartment on the last day of extinction were used to calculate a difference score. These difference scores were the data used to test Hypotheses 3.1, 3.2, 3.3, and 3.4.

**Hypothesis 3.2.** An additional expectation for the challenge phase was that the time in the previously AMPH-paired compartment would differ based on hormone treatments. In other words, there would be a significant main effect of hormone treatment. Among the hormone groups, CPP reinstatement was expected to be greatest in the animals receiving EB-only, as further evidence of estrogen enhancing the rewarding properties of AMPH (Justice & de Wit, 1999). Also, animals receiving EB +PROG or VEH would show greater reinstatement than those receiving PROG-only, regardless of challenge condition.

**Hypothesis 3.3.** Drug challenge was expected to result in greater reinstatement of CPP than the stress challenge, regardless of hormonal treatment. That is, the differences in the amount of time spent in the drug-paired compartment following the challenge and the time spent in the same compartment on the last day of extinction were expected to be significantly greater in the four drug groups than the four stress groups.

**Hypothesis 3.4.** The hormone group x challenge condition interaction was also expected to be statistically significant. That is, there would be different hormonal effects observed in the drug challenge group than in the stress challenge group. For example, animals receiving EB-only were expected to have less effect of the stress because estrogen has been found to attenuate stress responses (Young et al., 2001). However the EB was expected to potentiate the effects of the drug exposure (Sell, Scalzitte, Thomas & Cunningham, 2000), and the animals receiving the drug challenge would have the greatest

effects if they were also administered EB-only compared to the animals administered the other hormone treatments.

**Extinction 2**

**Hypothesis 4.1.** Over the five days of the second extinction phase, the hypothesis was for a statistically significant decrease for all groups in the time spent in the AMPH-paired compartment. Therefore, a statistically significant main effect for day was expected. Difference scores, or the difference between the time spent in the drug-paired compartment on challenge day and each day of extinction 2 and the time spent in the same compartment during baseline were the data used to test Hypotheses 4.1, as well as 4.2, 4.3, 4.4.

**Hypothesis 4.2.** The hormone groups were expected to differ in the rate of extinction during the second extinction phase. Animals receiving EB-only were expected to experience the most extended extinction. The measurement used to compare the hormone groups was the day in which the times spent in the drug-paired compartment were no longer statistically different from the times at baseline. The day that this occurred was expected to be later for the animals receiving EB-only than for the other groups. Among these latter groups, it was anticipated that the animals receiving PROG-only would reach extinction the earliest. Therefore, a main effect of hormone group was predicted to be significant, as well as the hormone group x day interaction. This prediction was based, once again, on evidence that estrogen can attenuate stress responses (Young et al., 2001), while potentiating the effects of drug exposure (Sell et al., 2000).

**Hypothesis 4.3.** Drug challenge was anticipated to produce more lengthy extinction than the stress challenge as well. Thus, the hypothesis was that there would be a main effect

for challenge condition, and that the challenge condition x day interaction would be statistically significant for the extinction 2 data.

**Hypothesis 4.4.** The two-way interaction of hormone group and challenge condition was predicted to be statistically significant. Animals receiving EB-only were expected to have less effect to the stress, followed by EB + PROG, VEH, and finally PROG-only (Young et al., 2001). However, the EB was expected to magnify the effects of the drug exposure. The animals receiving the drug challenge would have the greatest effects if they were administered EB-only than any of the other hormone regimens (Sell et al., 2000). It was also anticipated that the three-way interaction of these factors (day, hormone group, and challenge condition) would be statistically significant.

**Other Measures.**

**Transitions.**

**Hypothesis 5.1.** Note again that a transition refers to each time the animal's nose crossed the central line dividing the striped half of the apparatus from the solid half. Mean numbers of transitions made by each animal were calculated for the three days of baseline, the ten days of the first extinction, and the five days of the second extinction, resulting in one transition score for each of the experimental phases: baseline, CPP acquisition testing, extinction 1, challenge, and extinction 2. The challenge phase was the only phase expected to reveal group differences in the number of transitions made between compartments due to the stimulating effects of AMPH on locomotion (Schneider & Norton, 1979). Thus, the prediction was for a statistically significant main effect of experimental phase in the transition data.

**Hypothesis 5.2.** The numbers of transitions for the animals receiving the drug challenge were expected to be the greatest due to the stimulant effects of AMPH, whereas the animals receiving the stress challenge were expected to have attenuated locomotion (Papp et al., 1993). Therefore, a main effect of challenge condition was anticipated.

**Hypothesis 5.3.** The EB-only animals were expected to have the greatest sensitivity to the stimulating effects of AMPH, followed by EB + PROG, VEH, and finally PROG-only (Sell et al., 2000). Animals receiving the stress challenge were expected to have attenuated locomotor activity, and the EB-only group of animals would show less attenuation, thus have a greater number of transitions than the other hormone groups (Papp et al., 1993; Young et al., 2001). Thus, in the transition data, the two-way interactions, phase x hormone group and challenge condition x hormone group, were predicted to be statistically significant.

**Body Weights.**

**Hypothesis 6.** Weekly body weights were obtained on all animals during the experiment, including day 1 of baseline, day 5 of conditioning, day 5 of extinction, and the day of the challenge presentation. Body weights were expected to increase over the four weeks of the experiment for a statistically significant main effect of experiment week. A statistically significant main effect of hormone group and a statistically significant hormone group x week interaction were anticipated. The animals receiving VEH were expected to gain the most weight and the fastest, followed by the PROG-only group and the EB + PROG group. The EB-only group was expected to gain the least weight. These predictions were based on the known effects of hormones on weight (Jensen, Vestergard, Hermann, Gram, Eiken, Abrahamsen, Brot, Kolthoff, Sørensen, Beck-Nielsen, Nielsen, Charles, & Mosekilde, 2003; Writing group for the PEPI trial, 1995).

## Statistical Analyses

### CPP Acquisition Testing

A 2 x 4 factorial ANOVA was used to analyze the acquisition test data with main factors of phase (baseline and acquisition testing) and of hormone group (EB-only, EB + PROG, PROG-only, and VEH). Recall that the animals were not further divided into groups for stress or drug re-exposure until the challenge phase. All time data is expressed in min and hundredths of min.

### Extinction 1

The important data during Extinction 1 were the days on which the times in the drug-paired compartment were no longer significantly different from the times during baseline. The data used were the raw times in the drug-paired compartment measured in min and hundredths of min. The data were analyzed in a 4 x 12 repeated measures factorial ANOVA, with main factors for hormonal group (EB-only, EB + PROG, VEH, or PROG-only) and day as the repeated factor. The days factor included a single baseline value, the single acquisition testing data, and the 10 days of extinction for a total of the 12 days used in the analysis.

### Challenge

The challenge phase data were absolute difference scores in min and hundredths of min, or the differences in the amount of time spent in the drug-paired compartment following the challenge and the time spent in the same compartment on the last day of extinction. These were analyzed with a 2 x 4 factorial ANOVA, with main factors of challenge condition (drug or stress) and hormone group (EB-only, EB + PROG, VEH, or PROG-only).

**Extinction 2**

Data from extinction 2 were absolute difference scores in min and hundredths of min relative to baseline. These data were analyzed with a 4 x 2 x 6 repeated measures factorial ANOVA, with main factors of hormonal group (EB-only, EB + PROG, VEH, or PROG-only), challenge condition (stress vs. drug), and day (challenge day and days 1 through 5 of the extinction 2 phase) as the repeated measure.

Also for the extinction 2 analyses, a grouping variable was created to include 8 groups: EB-only with drug challenge, EB + PROG with drug challenge, PROG-only with drug challenge, VEH with drug challenge, EB-only with stress challenge, EB + PROG with stress challenge, PROG-only with stress challenge, and VEH with stress challenge. This grouping variable was used to compare the rate of extinction among the eight groups in a 8 x 6 repeated measures factorial ANOVA, with main factors of group and day.

**Transitions**

The numbers of transitions, or mean number of transitions, made by the animals during each of the experimental phases were analyzed with a 4 x 5 repeated measures factorial ANOVA. The main factors were hormone group (EB-only, EB + PROG, PROG-only, or VEH) and experimental phase as the repeated measure (baseline, CPP acquisition testing, extinction 1, challenge, and extinction 2.)

To evaluate group differences after being divided into eight groups with the challene, the transition data during the challenge and extinction 2 phases were analyzed with a 4 x 2 x 2 factorial ANOVA. The main factors were hormone group (EB-only, PROG-only, EB + PROG, or VEH), challenge condition (stress or drug), and experimental phase as the repeated measure (challenge and extinction 2).

**Body Weights**

Body weight data were analyzed with a 4 x 4 factorial ANOVA with main factors of hormone regimen (EB-only, EB + PROG, PROG-only, or VEH) and experimental week as the repeated measure. Weights were collected weekly during the experiment for a total of four values. Challenge condition was not a factor in the analysis of the body weight data because the last weight measurements were collected on the day of the challenge, prior to the administration of the challenge.

**Simple Main Effects and Post-hoc Tests**

In the event of statistically significant F values on interactions between main factors, further analyses were used to examine the simple main effects (Kirk, 1982). Follow-up analyses were also conducted with nonsignificant interactions if required to address *a priori* hypotheses. Tukey's Honestly Significant Difference (HSD) post-hoc tests (Gravetter & Wallnau, 2004) were performed when appropriate for a statistically significant F value. The confidence interval for all statistical tests was $p \leq 0.05$. Analyses were conducted with statistical software, SPSS 13.0 (SPSS, Inc.)

## Results

**CPP Acquisition Test**

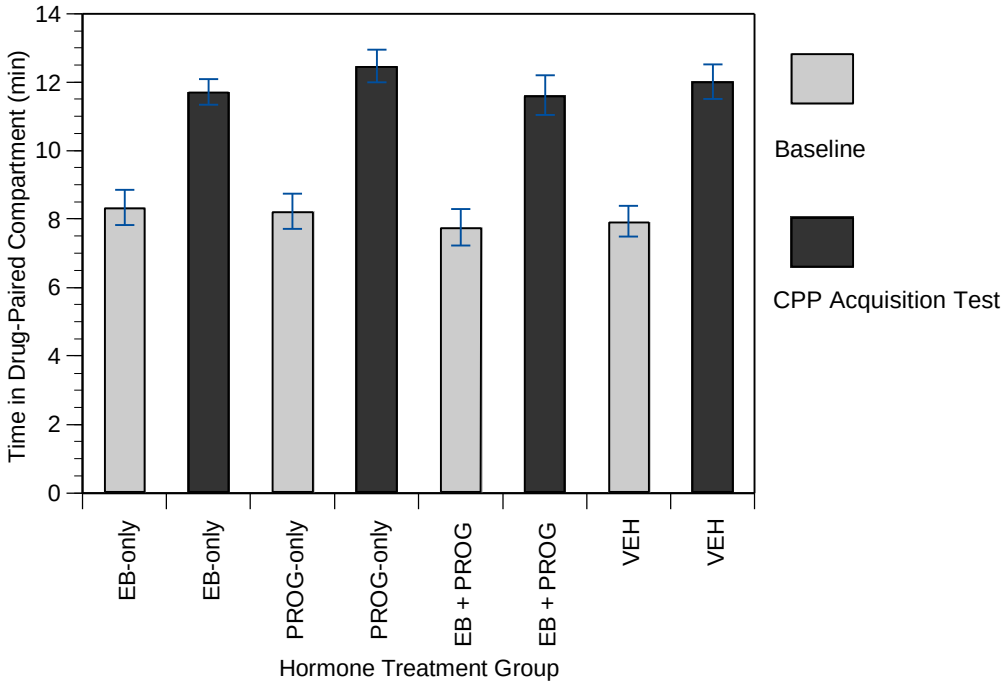
The results for the baseline vs. CPP test data are depicted in Table 2 on p. 69 and Figure 1 on p. 70. A 4 x 2 factorial ANOVA with main effects of hormone group (EB-only, EB + PROG, VEH, or PROG-only) and phase (baseline and CPP acquisition testing) revealed a statistically significant main effect of phase for the time spent in the drug-paired compartment, [$F(1, 120) = 124.63$, $p < 0.001$]. With all groups combined, more time was spent in the drug-paired compartment during CPP acquisition testing than during baseline.

**Table 2.** Mean times in drug-paired compartment during baseline and at CPP acquisition testing for each of the four hormone groups

| Hormone Group | Baseline | CPP Acquisition Testing | Difference Scores |
|---|---|---|---|
| EB-only | 8.23 ? 0.51 | 11.72 ? 0.38 | 3.55 ? 0.48 |
| PROG-only | 8.22 ? 0.51 | 12.47 ? 0.48 | 4.24 ? 0.51 |
| EB + PROG | 7.76 ? 0.54 | 11.62 ? 0.58 | 3.86 ? 0.57 |
| VEH | 7.93 ? 0.44 | 12.01 ? 0.50 | 4.08 ? 0.48 |
| Total | 8.03 ? 0.25 | 11.95 ? 0.24 | 3.93 ? 0.25 |

Data are means of times in min and hundredths of min in the drug-paired compartment ? SEM. The hormone group x phase interaction was not statistically significant, nor was the main effect of hormone group. Therefore no post-hoc comparisons were performed. The phase main effect was statistically significant, $p < 0.001$, with more time spent in the drug-paired compartment during CPP testing as compared to baseline. Tukey's HSD tests revealed that the differences between the baseline and CPP acquisition test data for each of the four hormone groups were also statistically significant, $p < 0.01$.

**Figure 1.** Mean times in drug-paired compartment during baseline and at CPP acquisition testing for each of the four hormone groups




Data are means of times in min in the drug-paired compartment ? SEM. The hormone group x phase interaction was not statistically significant. There were also no statistically significant hormone group differences. The phase main effect was statistically significant, $p < 0.001$, as were the differences between baseline and CPP acquisition test data for each of the four hormone groups, $p < 0.01$.

The results revealed no statistically significant main effect of hormone group, [F (3, 120) = 0.59, n.s.], and the hormone group x phase interaction was also not statistically significant, [F (3, 120) = 0.22, n.s.]

Tukey's HSD tests were used to compare baseline and CPP acquisition test data for each hormone group to test the *a priori* hypothesis that all four hormone groups of animals would display CPP. The differences between the baseline and CPP acquisition test data for each of the four groups were statistically reliable, each $p < 0.01$.

**Extinction**

The mean times in the drug-paired compartment for each of the four hormone groups on each of the days of extinction 1 are displayed in Table 3 on the following page. The data from the first extinction phase are also depicted in Figures 2 – 4 on subsequent pages.

The times in the drug-paired compartment for the extinction phase were analyzed with a 4 x 12 repeated measures factorial ANOVA with main effects of hormone group (EB-only, EB + PROG, VEH, and PROG-only) and 12 phase days including baseline, CPP acquisition testing, and the 10 days of extinction 1. The results revealed a statistically significant main effect of phase day, [$F (11, 720) = 8.34$, $p < 0.001$]. A statistically significant main effect of hormone group was also detected, [$F (3, 720) = 4.25$, $p < 0.01$]. The hormone treatments were significantly different when the extinction 1 data were collapsed over the twelve days. Tukey's post hoc revealed that times in the drug-paired compartment for EB-only = VEH > PROG-only, and the EB + PROG group did not significantly differ from any other group.

The hormone x phase day interaction was not statistically significant, [F (33, 720) = 0.64, n.s.] One-way ANOVAs were computed for the effect of phase day within each

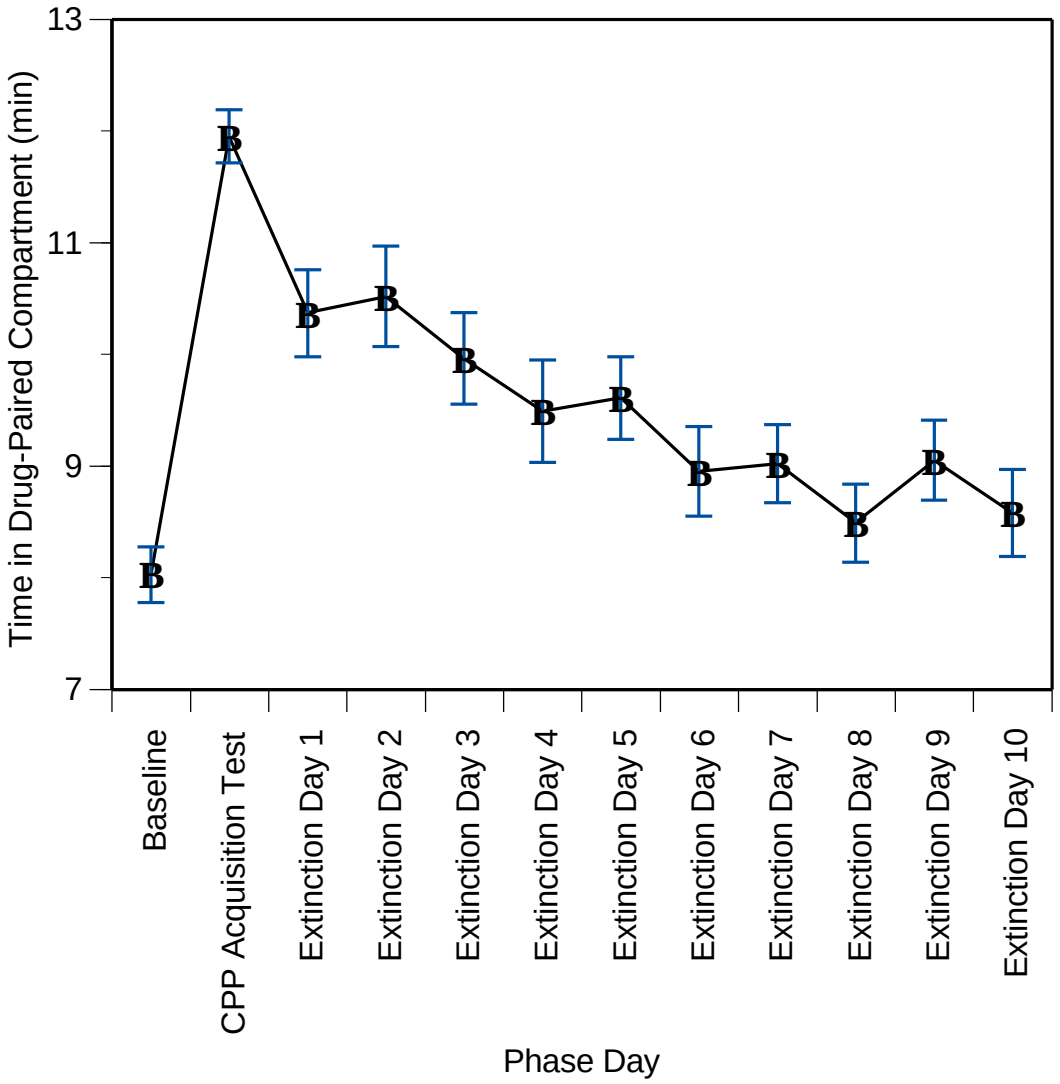
**Table 3.** Mean times in the drug-paired compartment during baseline, CPP acquisition testing, and the ten d

each of the four hormone groups

| Hormone Group | Baseline | CPP Test | Extinction Day 1 | Extinction Day 2 | Extinction Day 3 | Extinction Day 4 | Extinction Day 5 | Extinction Day 6 | Extinction Day 7 | Extinction Day 8 | Extir D |
|---|---|---|---|---|---|---|---|---|---|---|---|
| EB-only | 8.23 ? 0.51 | 11.72 ? 0.38 | 10.41 ? 0.78 | 10.47 ? 0.86 | 10.18 ? 0.65 | 10.18 ? 0.47 | 9.58 ? 0.63 | 9.23 ? 0.60 | 9.70 ? 0.56 | 9.29 ? 0.72 | 9.( ? 0.( |
| PROG-only | $8.22^{A}$ ? 0.51 | $12.47^{B}$ ? 0.48 | $10.79^{AB}$ ? 0.87 | $9.62^{AB}$ ? 0.88 | $9.49^{AB}$ ? 0.85 | $7.68^{A}$ ? 1.00 | $9.02^{AB}$ ? 0.77 | $8.30^{A}$ ? 0.92 | $8.11^{A}$ ? 0.84 | $7.73^{A}$ ? 0.75 | 7. ? 0 |
| EB + PROG | 7.76 ? 0.54 | 11.62 ? 0.58 | 9.82 ? 0.77 | 10.48 ? 1.08 | 10.12 ? 1.12 | 10.52 ? 1.01 | 10.25 ? 0.96 | 9.39 ? 0.75 | 8.46 ? 0.67 | 8.43 ? 0.70 | 9 ? 0 |
| VEH | $7.93^{A}$ ? 0.44 | $12.01^{C}$ ? 0.50 | $10.47^{ABC}$ ? 0.76 | $11.49^{BC}$ ? 0.76 | $10.04^{ABC}$ ? 0.67 | $9.59^{ABC}$ ? 0.82 | $9.58^{ABC}$ ? 0.62 | $8.90^{AB}$ ? 0.62 | $9.79^{ABC}$ ? 0.65 | $8.54^{AB}$ ? 0.66 | 9. ? 0. |
| Total | $8.03^{A}$ ? 0.25 | $11.95^{B}$ ? 0.24 | $10.37^{AB}$ ? 0.39 | $10.52^{AB}$ ? 0.45 | $9.96^{AB}$ ? 0.41 | $9.49^{AB}$ ? 0.46 | $9.61^{AB}$ ? 0.37 | $8.95^{AB}$ ? 0.40 | $9.02^{AB}$ ? 0.35 | $8.49^{A}$ ? 0.35 | 9.05 ? 0.3( |

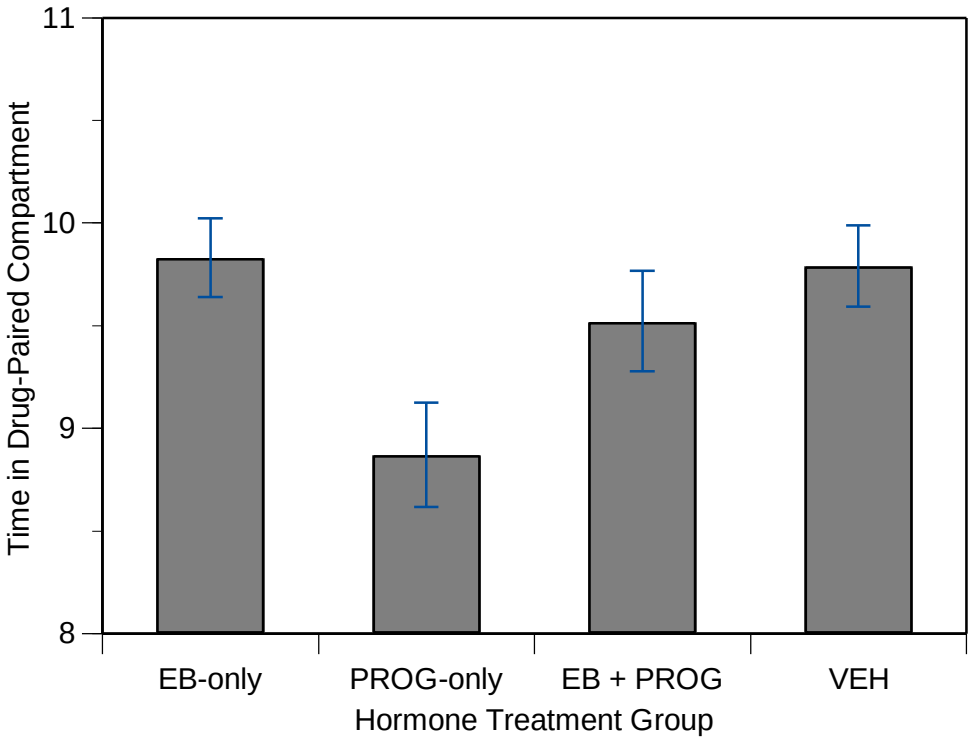
Data are means of times in min and hundredths of min in the drug-paired compartment ? SEM. Data in each column (phase day) t
alphabetical superscripts differ at $p = 0.05$. A main effect was found for hormone group, but the hormone group x phase day intera
significant. Data in each row (hormone group) that do not share capitalized alphabetical superscripts differ at $p = 0.05$. A statistic
phase day was revealed, and individual one-way ANOVAs revealed statistically significant phase day effects in the hormone group

**Figure 2.** Mean times in the drug-paired compartment on each of the twelve phase days: baseline, CPP acquisition testing, and extinction 1.



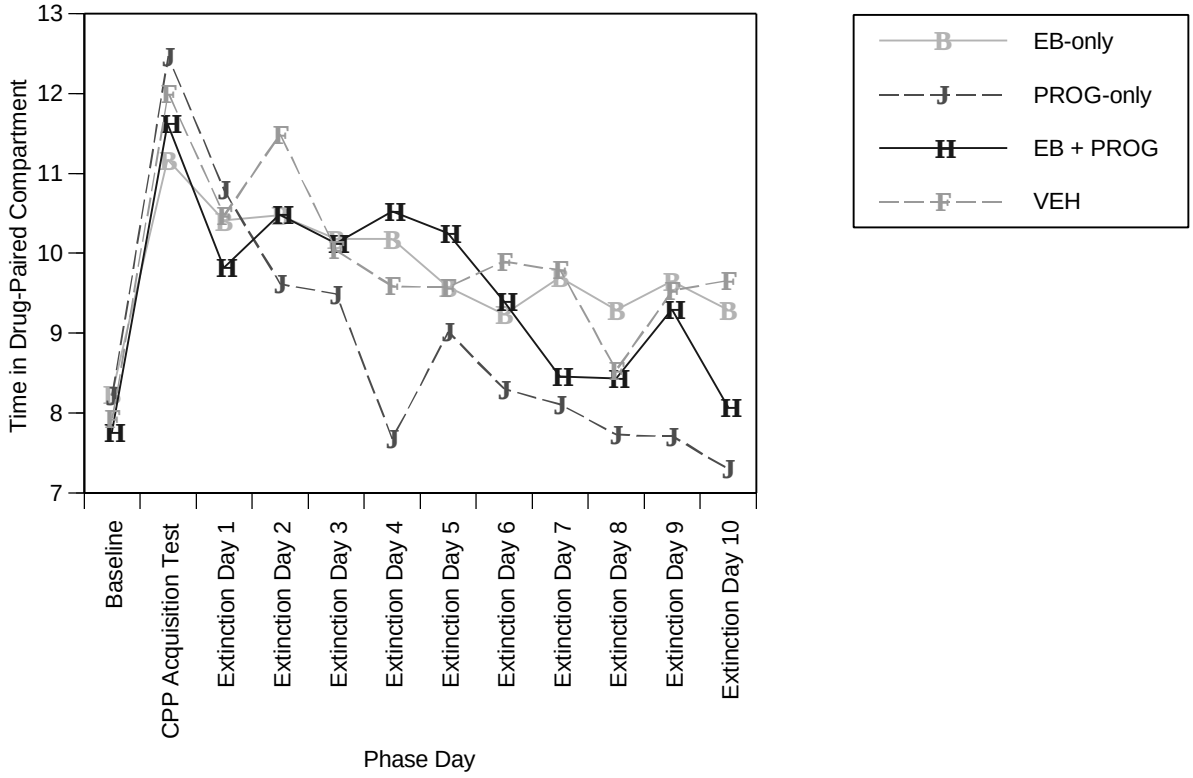

Data are means of times in min in the drug-paired compartment for each of the phase days: baseline, CPP acquisition testing, and the ten days of extinction 1, collapsed to include all four hormone groups. A statistically significant main effect of phase day was revealed, $p < .01$. The results of Tukey's HSD post-hoc comparisons are displayed in Table 3 on p. 82.

**Figure 3.** Absolute mean times in the drug-paired compartment for each of the four hormone groups including baseline, CPP acquisition testing, and extinction 1.




Data are means of times in min in the drug-paired compartment ? SEM for each of the four hormone groups, collapsed across phase days: baseline, CPP acquisition testing, and the ten days of extinction 1. A statistically significant main effect of hormone group was detected, $p < .01$, such that EB-only = VEH > PROG-only. EB + PROG did not significantly differ from any other hormone group.

**Figure 4.** Mean times in the drug-paired compartment at baseline, CPP acquisition testing and during each of the ten days of extinction for each of the four hormone groups.




Data are means of times in min ± SEM spent in the drug-paired compartment for each of the four hormone groups, at each of the twelve phase days: baseline, CPP acquisition testing, and the ten days of extinction 1. The hormone group x phase day interaction was not statistically significant.

hormone group to test the *a priori* hypothesis that the hormone groups would display different rates of extinction. The results of these analyses for the EB + PROG, PROG-only, and VEH groups were statistically reliable, [F (11, 180) = 1.96, p < 0.05, 3.33, p < 0.001, and 3.12, p < 0.01 respectively]. The EB-only group did not achieve statistical significance, [F (11, 180) = 1.75, n.s.]

Subsequent analyses of each group were conducted to determine on which days the times differed from each other within each hormone group. The Tukey's post hoc-test of the EB + PROG group revealed that no days differed at the .05 level. The difference between baseline and CPP test only approached statistically significance, p < 0.10. No other comparisons between days during extinction of the EB + PROG hormone group approached statistical significance. The results of the Tukey's post-hoc comparisons for the PROG-only and the VEH groups are displayed in Table 3 on p. 82.

The hormone groups were hypothesized to differ in the amount of time in the drug-paired compartment on the last day of extinction. A one-way ANOVA tested the effect of hormone group on this day only, and the results were not statistically significant, [F (3, 60) = 2.02, n.s.]

**Challenge**

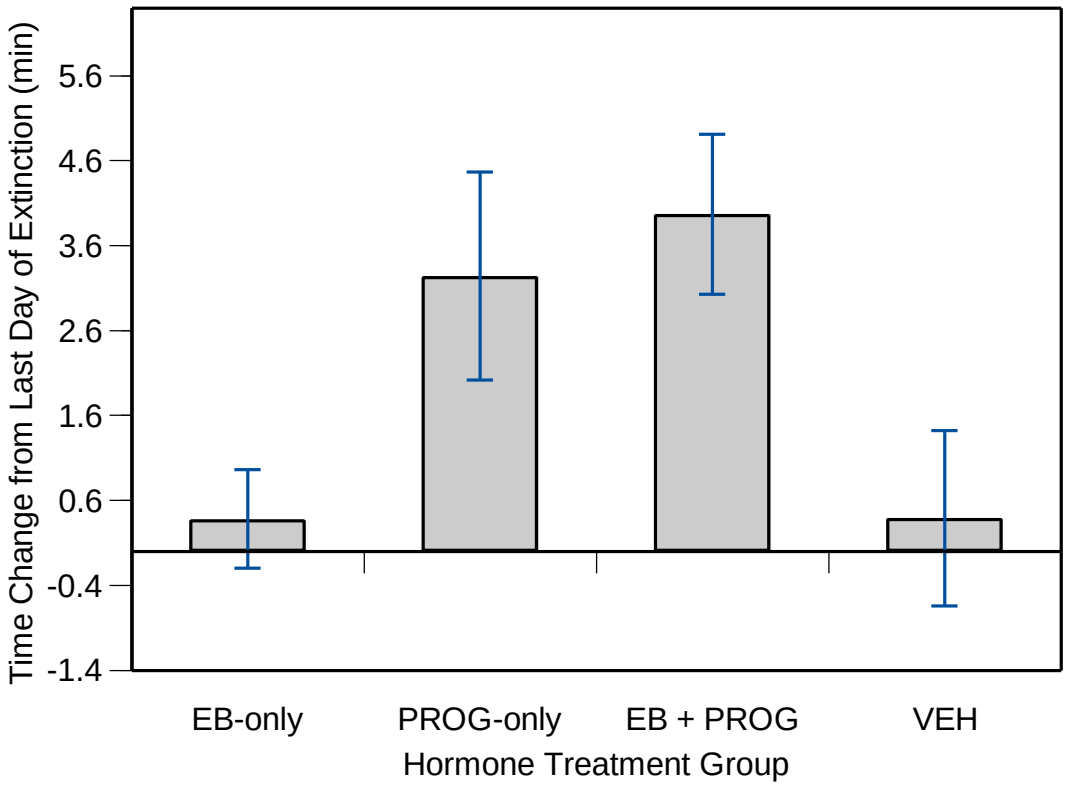
The time in the drug-paired compartment during the challenge phase relative to the time in the same compartment on the last day of extinction appear in Table 4 on the following page and in Figures 5 – 7 on subsequent pages. These time change scores were analyzed with a 2 x 4 factorial ANOVA. The main factors were hormone regimen (EB-only, EB + PROG, VEH, or PROG-only) and challenge condition (drug vs. stress).

**Table 4.** Challenge phase time change scores: times in drug-paired compartment during challenge phase minus times at last day of extinction.

| Hormone | Drug Challenge | Stress Challenge | Total (Mean) |
|---|---|---|---|
| EB | 0.80 [a] ± 0.87 | -0.34 [a] ± 0.81 | 0.38 [a] ± 0.58 |
| PROG | 3.88 [b] ± 1.75 | 2.61 [ab] ± 1.80 | 3.24 [ab] ± 1.22 |
| EB + PROG | 4.88 [b] ± 1.57 | 3.06 [b] ± 1.08 | 3.97 [b] ± 0.95 |
| VEH | -0.21 [a] ± 0.91 | 1.00 [a] ± 1.91 | 0.39 [a] ± 1.04 |
| Total | 2.34 ± 0.74 | 1.66 ± 0.74 | 2.00 ± 0.52 |

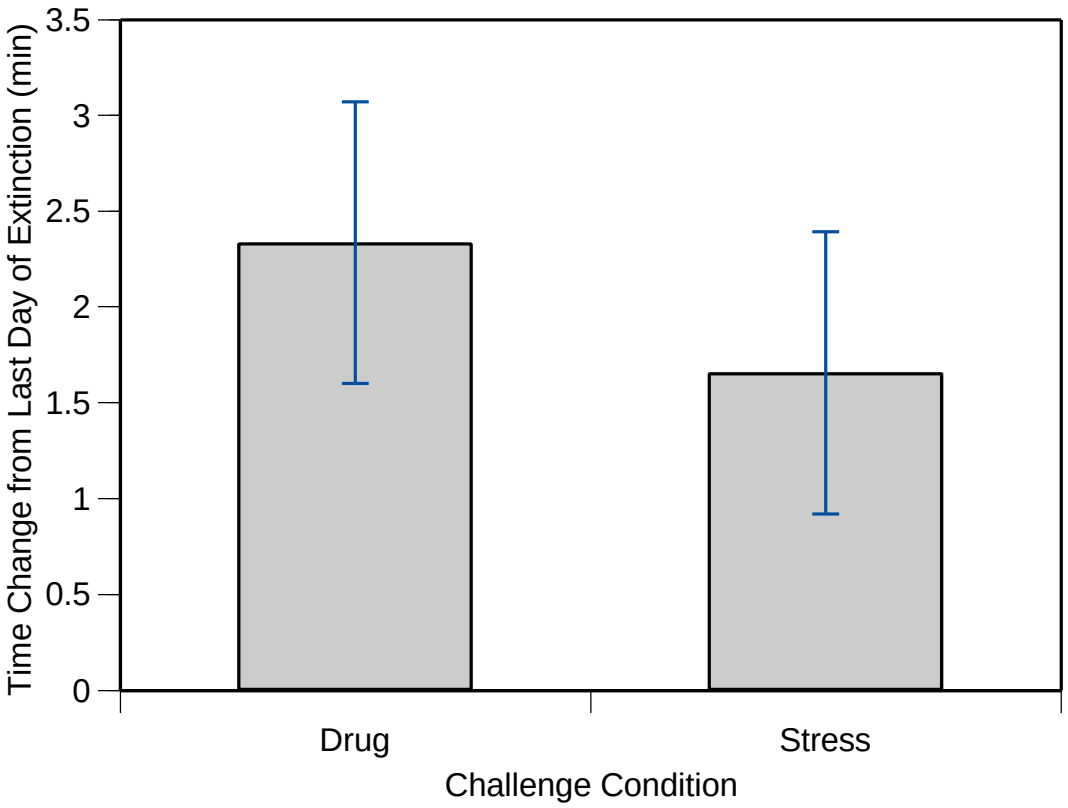
Data are mean difference scores, i.e. time in the drug-paired compartment during the challenge phase, minus the time in the same compartment in min and hundredths of min on the final day of extinction 1, ± SEM. Data in each column (phase) that do not share lowercase alphabetical superscripts differ at $p \leq 0.05$, except in the total column, in which the groups differ at $p < 0.10$. The challenge condition effect was not statistically significant overall, nor within each individual hormone group.

**Figure 5.** Time change scores at challenge phase for each hormone group.



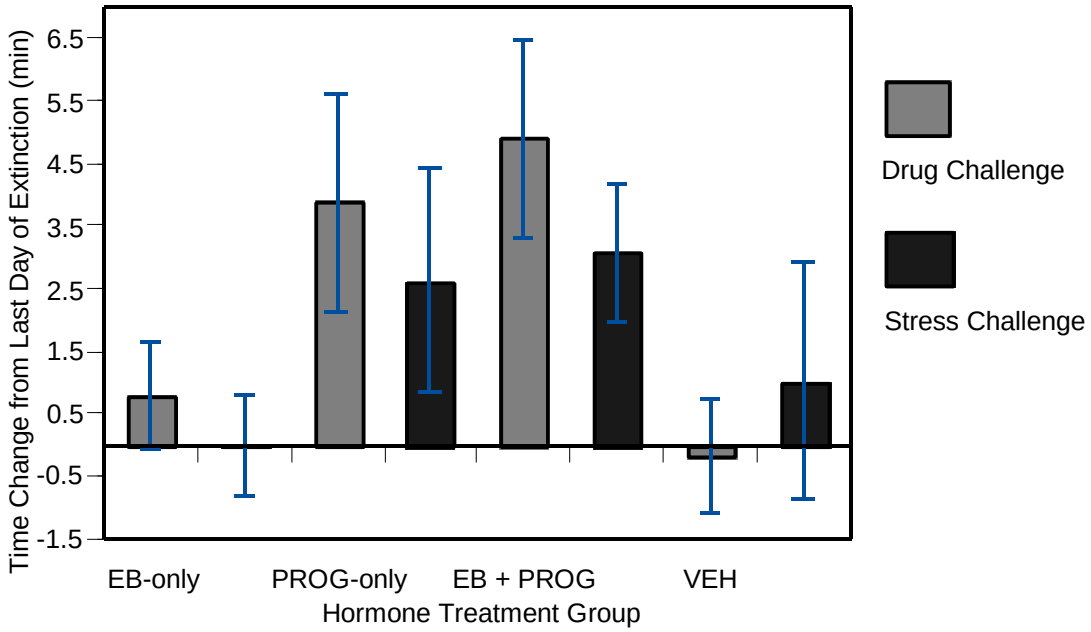

These data are mean difference scores, i.e., the times in min in drug-paired compartment on challenge day minus the times on the last day of extinction 1 for each hormone group ± SEM. These data are collapsed to include both challenge conditions. The main effect of hormone group was statistically significant, $p < 0.05$. However, Tukey's HSD post-hoc test revealed differences that only approached statistical significance, $p < 0.10$, such that EB + PROG > VEH = EB-only. The PROG-only group did not significantly differ from any other hormone group.

**Figure 6.** Time change scores at challenge phase for each challenge condition.




These data are mean time change scores, i.e., times in min and hundredths of min in drug-paired compartment on challenge day minus the time on the last day of extinction 1 for each challenge condition ± SEM. These data are collapsed to include all four hormone groups. The challenge condition main effect was not statistically significant.

**Figure 7.** Time change scores at challenge phase for the challenge condition x hormone group interaction.




These data are mean time change scores, i.e., times in min in drug-paired compartment on challenge day minus times on the last day of extinction 1 for each challenge condition ± SEM. These data display the hormone group x challenge condition interaction, which was not statistically significant. Tukey's HSD post-hoc tests revealed no challenge condition differences among any of the four hormone groups, however hormone group differences were found in each of the two challenge conditions. The results of these Tukey's HSD analyses are displayed previously in Table 4.

The analysis revealed a significant main effect for hormone group, [F (3, 56) = 3.59, p < 0.05]. Tukey's post-hoc tests of the four hormone groups only, i.e., with the two challenge conditions combined, revealed that none of the hormone groups differed at p < 0.05. However, several group comparisons approached statistical significance, p < 0.10, such that EB + PROG > VEH = EB-only. The PROG-only group did not significantly differ from any of the other hormone groups.

There was no statistically significant main effect for the challenge condition, [F (1, 56) = 0.47, n.s.] The hormone x challenge condition interaction was also not statistically significant, [F (3, 56) = 0.44, n.s.]

Tukey's HSD tests were used to address *a priori* hypotheses about group differences. The effects of the two challenge conditions were compared for each of the four hormone groups individually, and none were found to be statistically significant.

Tukey's HSD tests were also used to compare the effects of the four hormones for each of the two challenge conditions. These revealed several statistically significant differences within the drug challenge group, p < .05, such that EB + PROG = PROG-only > EB-only = VEH. Statistically significant hormone group differences were also uncovered within the stress challenge group, p < .05, such that EB + PROG > EB = VEH, but no other hormone groups differed. Therefore, the effect of drug challenge was greater in the EB + PROG and the PROG-only hormone groups. The effect of the stress challenge was greatest in the EB + PROG group.

**Extinction 2**

Data analyzed for the second extinction phase were difference scores, i.e., the times in the drug-paired compartment on the single day of the challenge phase and the five days of

the second extinction phase following the challenge phase minus times at baseline. The data from extinction 2 are illustrated in Tables 5 and 6 on pp. 93 and 94 respectively, and in Figures 8 – 14, on pp. 95 – 101 respectively. A 2 x 4 x 6 factorial ANOVA was used with main factors of challenge condition (stress vs. drug), hormone treatment (EB-only, EB + PROG, VEH, or PROG-only) and phase day as the repeated measure (challenge day and days 1 through 5 of the second extinction phase).

There was no statistically significant main effect for hormone for these data, i.e. collapsed to include both challenge conditions and all six days, [$\underline{F}$ (3, 336) = 0.47, n.s.] The challenge condition main effect, i.e., collapsed to include all four hormone groups and the six days, however, was statistically significant, [$\underline{F}$ (1, 336) = 18.36, $\underline{p} < 0.001$]. This result indicates the stress challenge condition resulted in more time in the drug-paired compartment over the extinction 2 phase relative to baseline than the animals exposed to the drug challenge. There was a statistically significant main effect for phase day as well, [$\underline{F}$ (5, 336) = 5.34, $\underline{p} < 0.001$]. Tukey's HSD revealed decreased difference scores over the five days of the second extinction phase.

The three-way interaction was not statistically significant, [$\underline{F}$ (15, 336) = 0.39, n.s.] Neither were any of the two-way interactions statistically significant, i.e., the hormone x challenge interaction, the hormone x day interaction, or the challenge x day interaction, [$\underline{F}$ (3, 336) = 0.46, $\underline{F}$ (15, 336) = 0.65, $\underline{F}$ (5, 336) = 0.62, respectively, each n.s.]

To test the *a priori* hypothesis that the challenge conditions would display different rates of extinction, a one-way ANOVA was conducted to test the effect of phase day for each of the two challenge conditions, collapsed across hormone groups. The results for the drug challenge groups were statistically significant, [ $\underline{F}$ (5, 186) = 5.04, $\underline{p} < 0.001$], but not for the

**Table 5.** Time change scores: times in drug-paired compartment during challenge and extinction 2 phase minus times at baseline for each challenge conditions, collapsed across the hormone groups.

| | Phase Day | | | | | | |
|---|---|---|---|---|---|---|---|
| Challenge Condition | Challenge | Extinction 2 Day 1 | Extinction 2 Day 2 | Extinction 2 Day 3 | Extinction 2 Day 4 | Extinction 2 Day 5 | Total (Mean) |
| Drug | $2.33^{A}$ ± 0.61 | $1.22^{AB}$ ± 0.68 | $-0.03^{B}$ ± 0.62 | $0.28^{AB}$ ± 0.53 | $-0.74^{B}$ ± 0.46 | $-1.00^{B}$ ± 0.39 | $^{a}0.34$ ± 0.24 |
| Stress | 2.79 ± 0.84 | 2.99 ± 0.69 | 2.56 ± 0.59 | 1.53 ± 0.80 | 1.29 ± 0.66 | 0.55 ± 0.69 | $^{b}1.95$ ± 0.29 |
| Total | $2.56^{A}$ ± 0.52 | $2.10^{AB}$ ± 0.99 | $1.26^{ABC}$ ± 0.45 | $0.90^{ABC}$ ± 0.48 | $0.27^{BC}$ ± 0.42 | $-0.22^{C}$ ± 0.37 | 1.15 ± 0.19 |

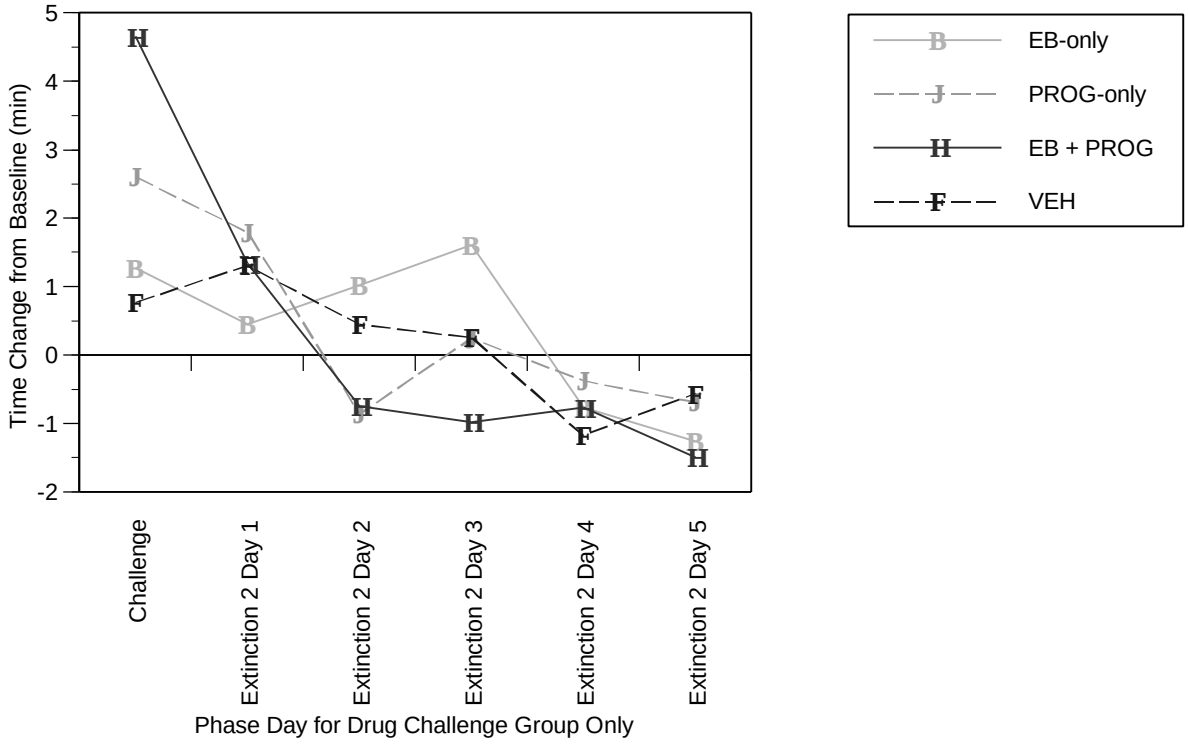
Data are means of time change scores in min and hundredths of min ? SEM. Data in each row that do not share capitalized alphabetical superscripts differ at $p = 0.05$. Data in the totals column that do not share lowercase alphabetical superscripts differ at $p = 0.05$. Statistically significant main effects were found for phase day and for challenge condition. ANOVA revealed a statistically significant effect of phase day within the drug challenge group, but not the stress challenge group.

**Table 6.** Time change scores: times in drug-paired compartment by each of the 8 groups during challenge and extinction 2 phase minus times at baseline.

| | | Phase Day | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Hormone Group | Challenge Condition | Challenge | Extinction 2 Day 1 | Extinction 2 Day 2 | Extinction 2 Day 3 | Extinction 2 Day 4 | Extinction 2 Day 5 | Total (Mean) |
| EB | Drug | 1.27 ± 1.33 | 0.45 ± 1.25 | 1.02 ± 1.56 | 1.61 ± 1.08 | -0.77 ± 0.96 | -1.26 ± 0.49 | 0.39$^{AB}$ ± 0.47 |
| EB | Stress | 1.62 ± 1.18 | 2.91 ± 1.12 | 4.04 ± 0.77 | 1.58 ± 1.40 | 2.12 ± 1.31 | 0.86 ± 0.94 | 2.19$^{AB}$ ± 0.46 |
| EB | Total | 1.45 ± 0.86 | 1.68 ± 0.87 | 2.53 ± 0.93 | 1.60 ± 0.86 | 0.67 ± 0.87 | -0.20 ± 0.58 | 1.29 ± 0.34 |
| PROG | Drug | 2.61 ± 1.29 | 1.80 ± 1.06 | -0.86 ± 0.90 | 0.25 ± 1.19 | -0.37 ± 0.86 | -0.68 ± 0.78 | 0.46$^{AB}$ ± 0.44 |
| PROG | Stress | 2.14 ± 1.48 | 1.85 ± 1.85 | 1.30 ± 1.37 | 1.06 ± 2.07 | 0.99 ± 1.50 | 1.17 ± 1.45 | 1.42$^{AB}$ ± 0.64 |
| PROG | Total | 2.38 ± 0.95 | 1.82 ± 1.03 | 0.22 ± 0.84 | 0.65 ± 1.16 | 0.31 ± 0.86 | 0.24 ± 0.83 | 0.94 ± 0.39 |
| EB + PROG | Drug | 4.66 ± 1.12 | 1.34 ± 2.03 | -0.74 ± 1.52 | -0.98 ± 1.21 | -0.66 ± 1.23 | -1.49 ± 1.18 | 0.35$^{AB}$ ± 0.63 |
| EB + PROG | Stress | 3.91 ± 1.97 | 4.21 ± 1.66 | 2.62 ± 1.47 | 2.61 ± 1.74 | 1.39 ± 1.62 | 0.38 ± 1.65 | 2.52$^{B}$ ± 0.68 |
| EB + PROG | Total | 4.28 ± 1.10 | 2.77 ± 1.32 | 0.94 ± 1.11 | 0.81 ± 1.12 | 0.37 ± 1.02 | -0.55 ± 1.01 | 1.44 ± 0.47 |
| VEH | Drug | 0.77 ± 0.83 | 1.31 ± 1.13 | 0.45 ± 0.91 | 0.26 ± 0.70 | -1.17 ± 0.74 | -0.56 ± 0.57 | 0.18$^{A}$ ± 0.34 |
| VEH | Stress | 3.49 ± 2.11 | 2.98 ± 0.72 | 2.28 ± 1.02 | 0.86 ± 1.32 | 0.64 ± 0.98 | -0.21 ± 0.69 | 1.67$^{AB}$ ± 0.52 |
| VEH | Total | 2.13 ± 1.15 | 2.15 ± 0.68 | 1.37 ± 0.70 | 0.56 ± 0.73 | -0.27 ± 0.64 | -0.39 ± 0.43 | 0.92 ± 0.32 |

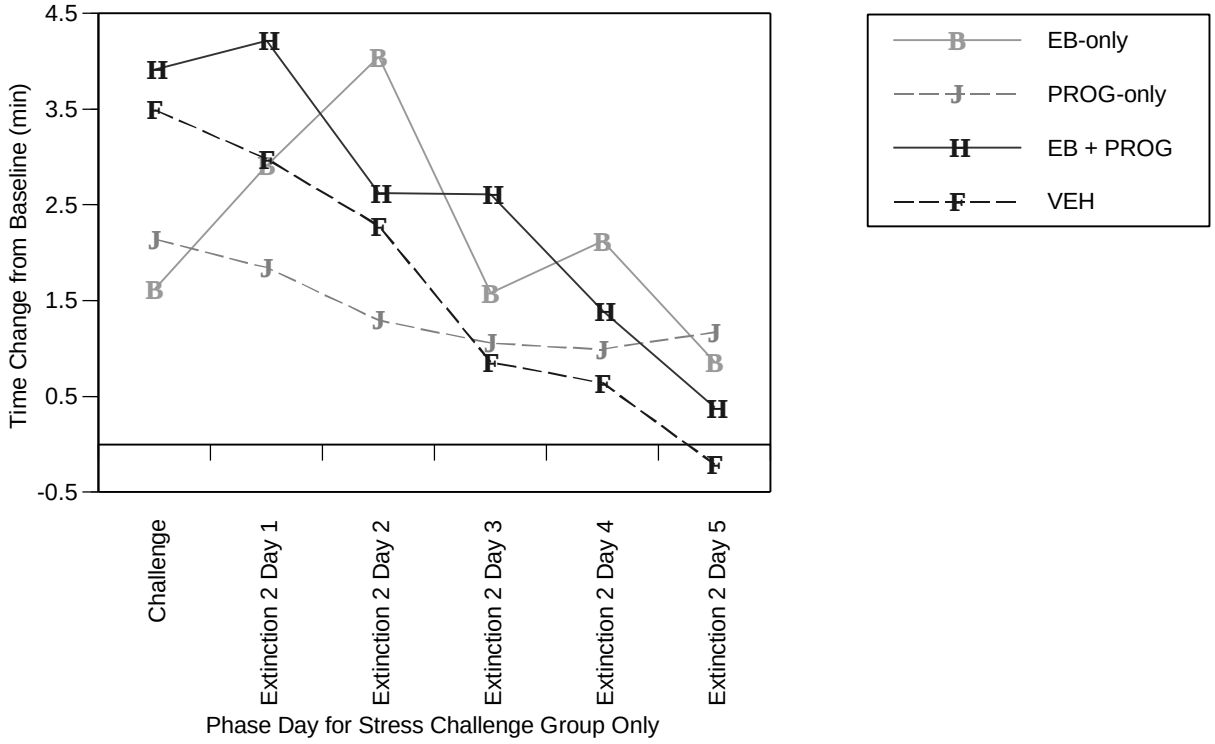
Data are means of time change scores in min and hundredths of min ? SEM. Statistically significant main effects were found for phase day and for challenge condition, but not for hormone group, as indicated in Table 5 on p. 93. Statistically significant main effects were also found for the hormone/challenge group. Data in the total column that do not share alphabetical superscripts differ at $p = 0.05$ in Tukey's post-hoc tests.

**Figure 8.** Mean time change scores (drug challenge groups only): times in drug-paired compartment during challenge day and extinction 2 days 1 through 5 minus times at baseline for each of the 4 hormone treatment groups that were exposed to the drug challenge.



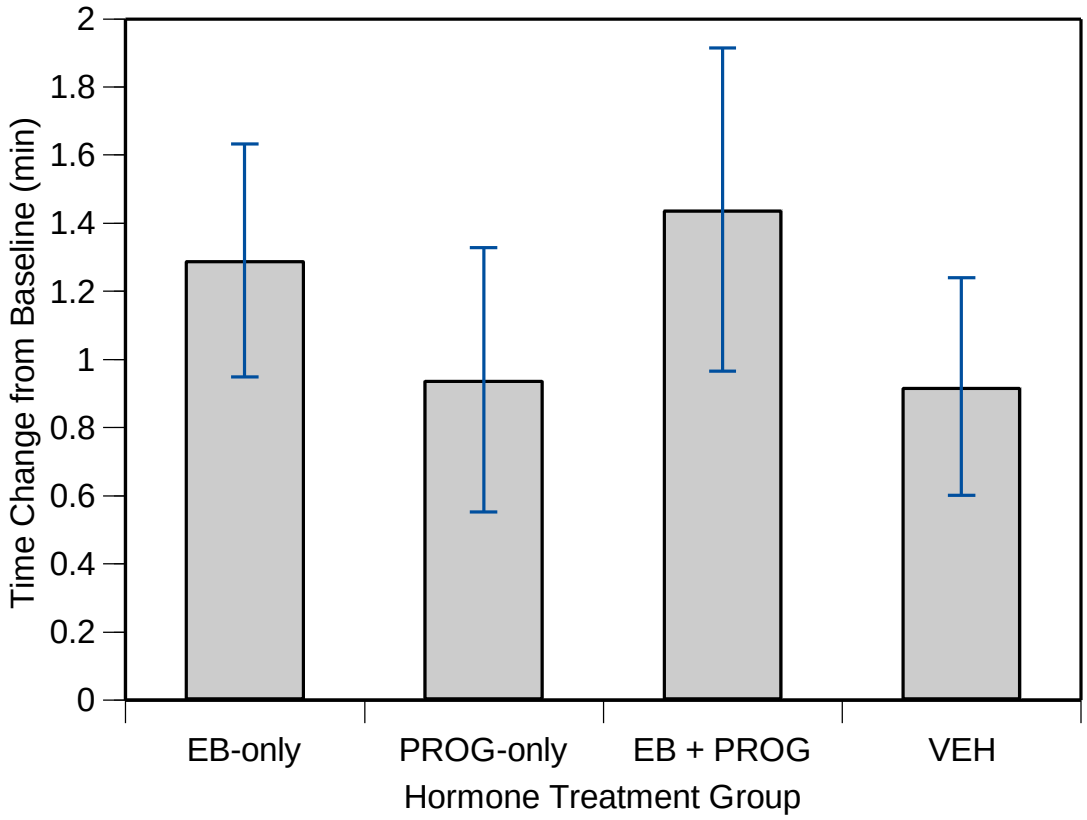

These data are means of times in drug-paired compartment minus times at baseline in min for each of the four hormone groups that were exposed to the drug challenge only. The data include the challenge phase and the five days of extinction 2. Statistically significant main effects were found for hormone-challenge group and for phase day, but the group x phase day interaction was not statistically significant. The results of Tukey's HSD tests are displayed in Table 6 on p. 94.

**Figure 9.** Mean time change scores (stress challenge groups only): times in drug-paired compartment during challenge day and extinction 2 days 1 through 5 minus times at baseline for each of the 4 hormone treatment groups that were exposed to the stress challenge.



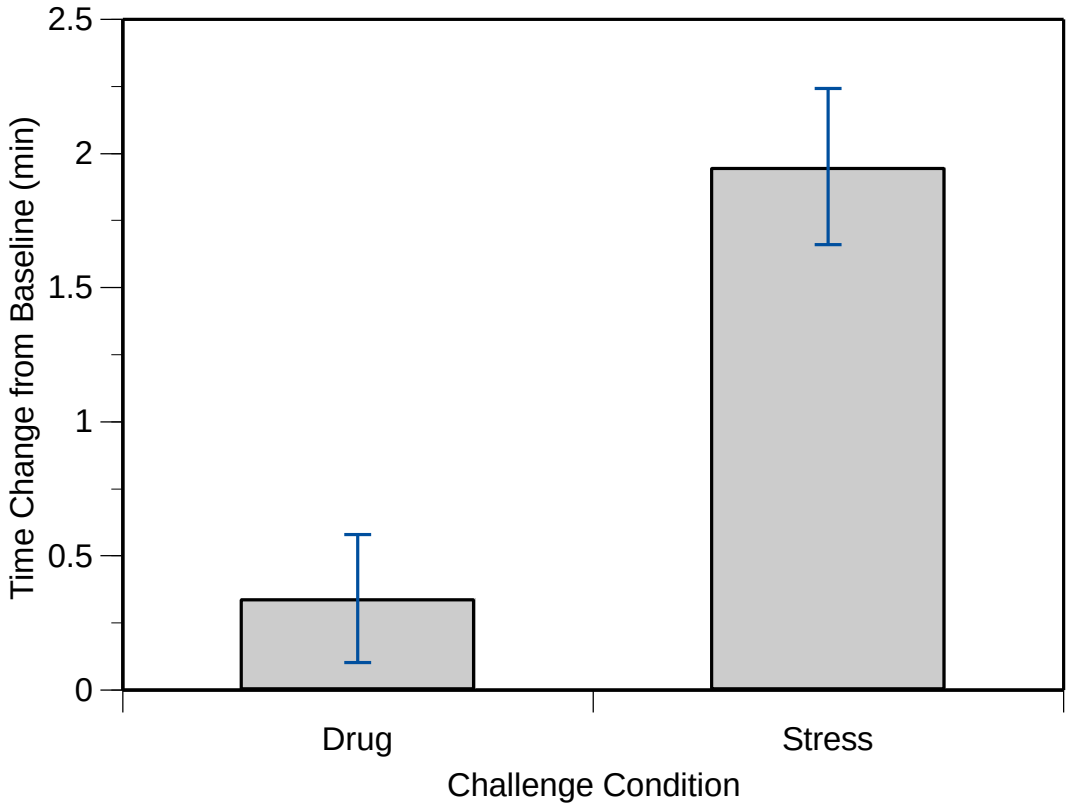

These data are means of times in drug-paired compartment minus times at baseline in min for each of the four hormone groups that were exposed to the stress challenge only. The data include the challenge phase and the five days of extinction 2. Statistically significant main effects were found for hormone-challenge group and for phase day, but the group x phase day interaction was not statistically significant. The results of Tukey's HSD tests are displayed in Table 6 on p. 94.

**Figure 10.** Mean time change scores : times in drug-paired compartment during challenge day and extinction 2 days 1 through 5 minus times at baseline for each hormone group.



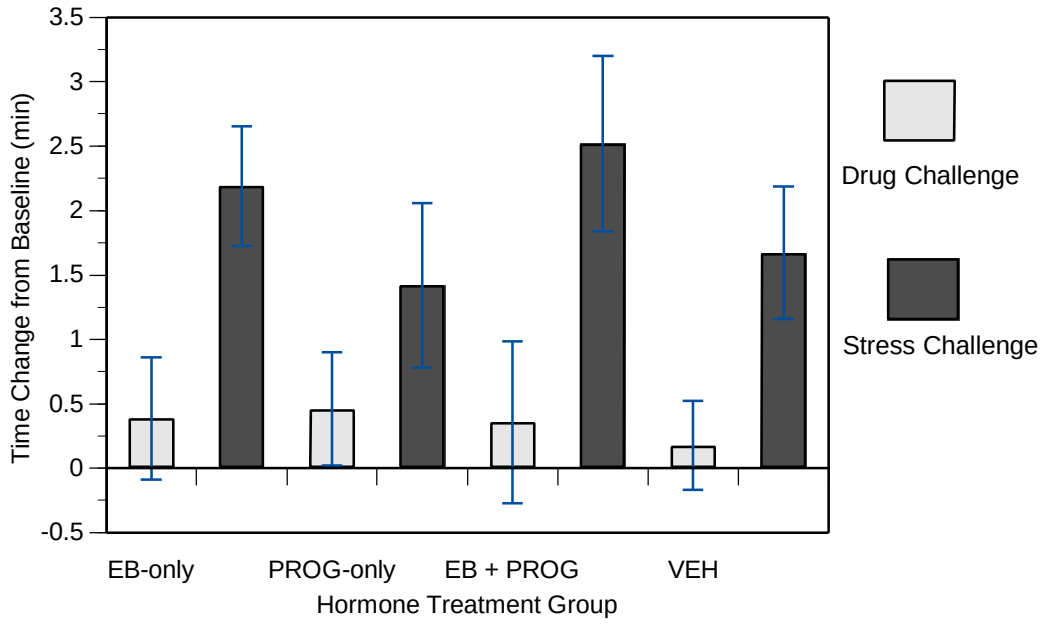

These data are the mean time change scores: times in min in the drug-paired compartment during challenge day and extinction 2 days 1 through 5 minus times at baseline ± SEM. This graph displays the main effect of hormone, collapsed across both challenge conditions and all phase days. The hormone regimen effect was not statistically significant.

**Figure 11.** Mean time change scores : times in drug-paired compartment during challenge day and extinction 2 days 1 through 5 minus times at baseline for each challenge condition.



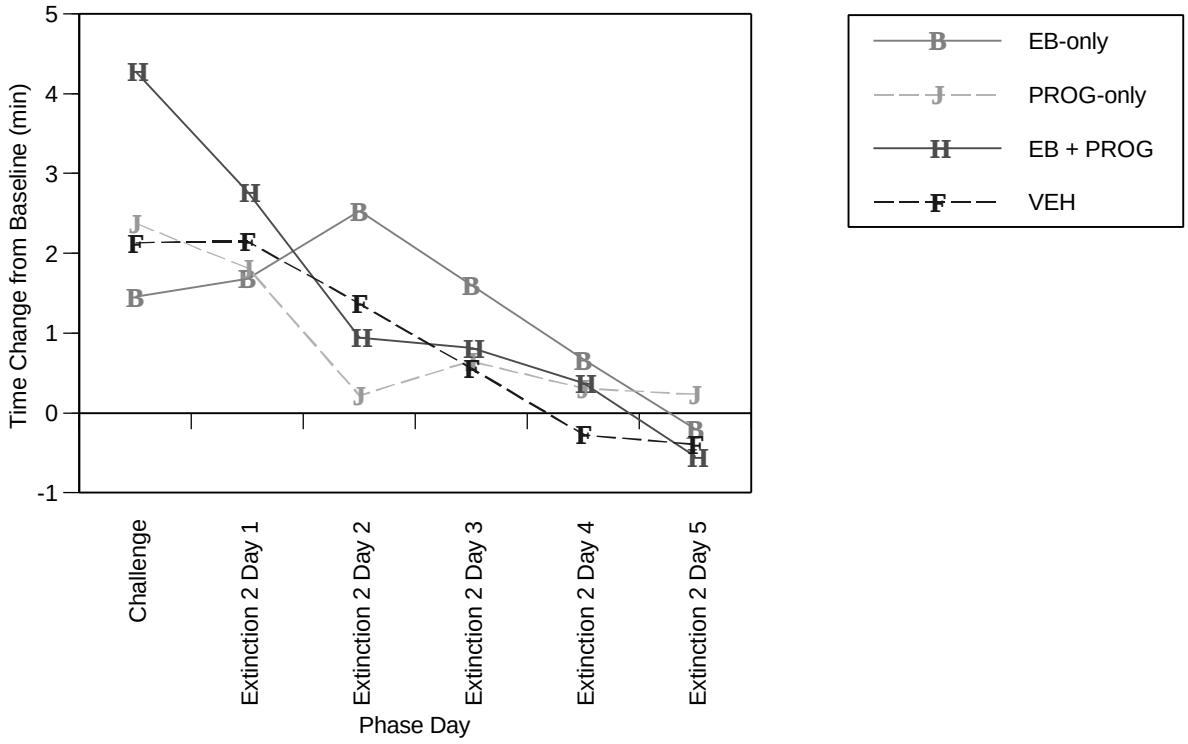

These data are the mean times in min ± SEM during challenge day and extinction 2 days 1 through 5 minus times at baseline. This graph displays the main effect of challenge condition, collapsed to include all four hormone treatments and all phase days. The challenge condition main effect was statistically significant, $p < 0.05$.

**Figure 12.** Mean time change scores: times in drug-paired compartment during challenge day and extinction 2 days 1 through 5 minus times at baseline for each challenge condition for each of the 8 groups.



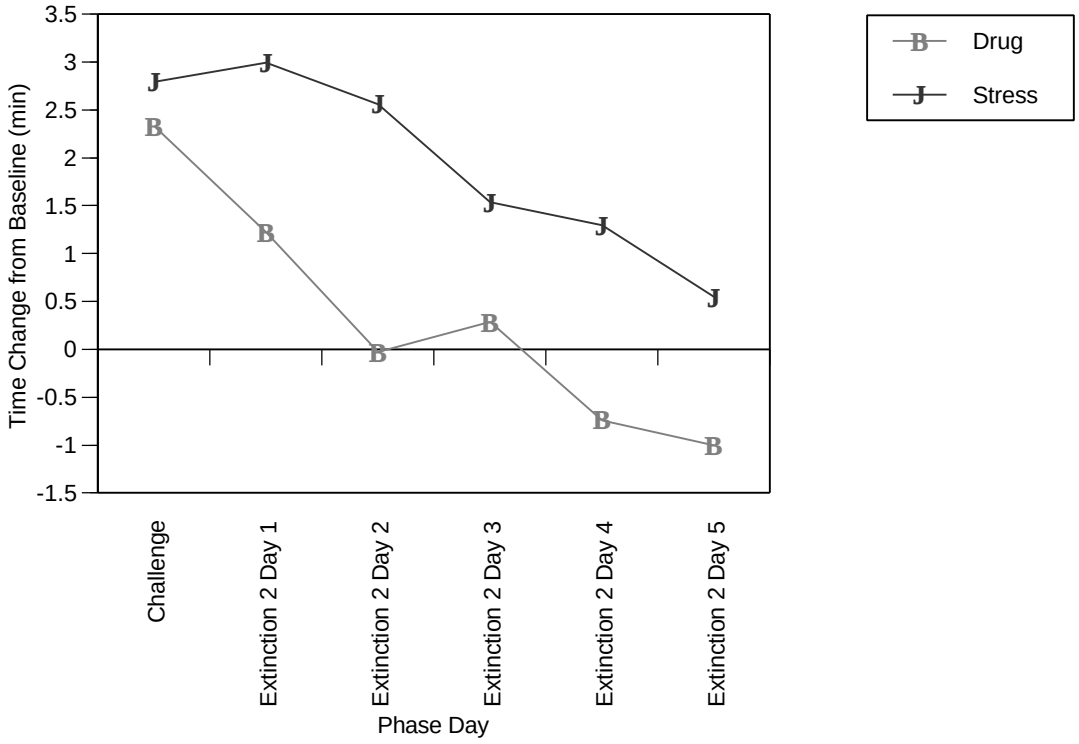

These data are the mean times in min ± SEM during challenge day and extinction 2 days 1 through 5 minus times at baseline. This graph displays the interaction between hormone treatment and challenge condition, collapsed to include all phase days. The hormone x challenge interaction was not statistically significant.

**Figure 13.** Mean time change scores: times in drug-paired compartment during challenge day and extinction 2 days 1 through 5 minus times at baseline for each of the four hormone groups.




These data are the mean times in min during challenge day and extinction 2 days 1 through 5 minus times at baseline for each of the four hormone groups. This graph displays the interaction between hormone treatment and phase day, collapsed to include both challenge conditions. The hormone x phase day interaction was not statistically significant.

**Figure 14.** Mean time change scores: times in drug-paired compartment during challenge day and extinction 2 days 1 through 5 minus times at baseline for each of the two challenge conditions.




These data are the mean times in min during challenge day and extinction 2 days 1 through 5 minus times at baseline for each of the two challenge groups. This graph displays the interaction between challenge condition and phase day, collapsed across all four hormone treatments. The challenge x phase day interaction was not statistically significant. However, one-way ANOVAs revealed that the effect of phase day was statistically significant for the drug challenge group, but not for the stress challenge group. The results of the Tukey's post-hoc tests for the drug challenge group are illustrated in Table 6 on p. 94.

stress challenge groups, [F (5, 186) = 1.92, n.s.] Tukey's post-hoc test for the drug challenge group revealed that times in the drug-paired compartment on the day of the challenge were significantly greater than on days 2, 4 and 5 of the second extinction phase. No other days differed at the $p < 0.05$ level.

An additional 8 x 6 factorial ANOVA was performed with main effects of group (EB drug, EB stress, EB + PROG drug, EB + PROG stress, VEH drug, VEH stress, PROG drug, and PROG stress) and phase day. This analysis revealed statistically significant main effects of group and phase day, [F (7, 336) = 3.02, p < .01, 5.34, p < 0.001, respectively], but the group x phase day interaction was not statistically significant, [F (35, 336) = 0.54, p = n.s.] Tukey's post-hoc test revealed EB + PROG + stress > VEH + drug, but no other group differences were statistically significant.

**Transitions**

The transition data and the results of the Tukey's post-hoc tests are displayed in Tables 7 and 8 on pp. 103 and 104. The number of transitions made by the animals were analyzed with a 4 x 5 factorial ANOVA to test for the main effect of hormone treatment (EB-only, EB + PROG, VEH, or PROG-only), the main effect of phase (baseline, acquisition testing, extinction 1, challenge, and extinction 2), and the hormone x phase interaction.

This first analysis collapsed the data across the challenge conditions. During baseline, extinction, and extinction 2, a mean was calculated to give a single transition score for each of the 5 phases. The hormone group x experimental phase interaction was not statistically significant, [F (12, 300) = 0.76, n.s.] A significant main effect for hormone group was revealed, [F (3, 300) = 3.97, p < 0.01.], such that VEH = PROG > EB + PROG. The EB-only group did not significantly differ from any other group. The data also revealed a

**Table 7.** Transitions made during each of the experimental phases for each of the four hormone groups. The the challenge and extinction 2 phases are collapsed across both challenge conditions.

| | Experimental Phase | | | | | |
|---|---|---|---|---|---|---|
| Hormone Group | Baseline | CPP Acquisition Test | Extinction 1 | Challenge | Extinction 2 | Total (Mean) |
| EB-only | 32.10 ± 3.69 | 52.13 ± 3.56 | 6.91 ± 2.31 | 52.31 ± 8.37 | 50.28 ± 4.00 | $46.75^{AB}$ ± 2.28 |
| PROG-only | 27.54 ± 2.86 | 49.06 ± 4.53 | 46.65 ± 4.31 | 66.13 ± 12.91 | 49.44 ± 4.18 | $47.76^{B}$ ± 3.27 |
| EB + PROG | 28.63 ± 3.72 | 45.25 ± 5.86 | 40.42 ± 4.76 | 42.31 ± 7.97 | 33.33 ± 2.94 | $37.99^{A}$ ± 2.43 |
| VEH | 31.73 ± 2.89 | 57.13 ± 6.00 | 54.34 ± 5.47 | 52.44 ± 10.03 | 53.59 ± 4.52 | $49.84^{B}$ ± 2.91 |
| Total | $30.00^{a}$ ± 1.64 | $50.89^{b}$ ± 2.54 | $47.08^{b}$ ± 2.22 | $53.30^{b}$ ± 5.00 | $46.66^{b}$ ± 2.17 | |

Data are means of numbers of transitions made between apparatus compartments ? SEM. Data that do not share alphabetical super Tukey's HSD test. Uppercase letters indicate hormone group differences within the total column. Lowercase letters indicate exper within the total row.

**Table 8.** Transitions made during the challenge and extinction 2 phases for each of the four hormone groups and both challenge conditions.

| Hormone Group | Challenge Condition | Experimental Phase: Challenge | Experimental Phase: Extinction 2 | Total (Mean) |
|---|---|---|---|---|
| EB-only | Drug | 79.00[B] ± 9.27 | 52.68 ± 6.43 | 65.84 ± 6.42 |
| EB-only | Stress | 25.63 ± 3.32 | 47.88 ± 5.07 | 36.75 ± 4.10 |
| PROG-only | Drug | 108.38[C] ± 14.18 | 51.18 ± 5.23 | 79.78 ± 10.38 |
| PROG-only | Stress | 23.88 ± 1.88 | 47.70 ± 6.83 | 35.79 ± 4.60 |
| EB + PROG | Drug | 62.13[A] ± 11.77 | 31.38 ± 5.01 | 46.75 ± 7.34 |
| EB + PROG | Stress | 22.50 ± 4.63 | 35.28 ± 3.31 | 28.89 ± 3.21 |
| VEH | Drug | 87.50[B] ± 8.30 | 50.35 ± 5.35 | 68.93 ± 6.76 |
| VEH | Stress | 17.38 ± 3.31 | 56.83 ± 7.54 | 37.10 ± 6.46 |
| Total | Drug | 84.25 ± 6.07 | 46.39 ± 3.06 | 65.32 ± 4.13 |
| Total | Stress | 22.34 ± 1.72 | 46.92 ± 3.13 | 34.63 ± 2.35 |

Data are means of numbers of transitions made between apparatus compartments ? SEM. The challenge condition was statistically significant during the challenge phase only, within each hormone group and in the total challenge phase data, $p < 0.05$. Hormone group simple effects were only significant for the drug challenge condition during the challenge phase, and those data that do not share alphabetical superscripts differ at $p = 0.05$ in the Tukey's HSD test.

significant main effect for experimental phase, [F (4, 300) = 9.71, p < 0.001.] Tukey's post-hoc test revealed that the mean number of transitions during baseline was significantly reduced relative to the other four phases. None of these latter four phases were significantly different from each other.

The transition data during the challenge and second extinction phase were also analyzed to examine the effect of the challenge condition, in addition to the two-way and three-way interactions among challenge condition, hormone, and experimental phase. The three-way interaction was not statistically significant, [F (3, 112) = 1.82, p = n.s.] The effect of challenge condition was statistically significant, [F (1, 112) = 75.00, p < 0.001], with drug challenge inducing more transitions than stress.

The challenge condition x experimental phase interaction was also statistically significant, [F (1, 112) = 77.58, p < 0.001.] Simple main effects revealed that within the challenge phase, the challenge condition effect was statistically significant, [F (1, 112) = 152.57, p < 0.001], and the drug challenge group had significantly more transitions than the stress challenge group. Within the extinction 2 phase, the challenge condition effect was not significant, [F (1, 112) = 0.01, n.s.]

The hormone x challenge condition interaction was not statistically significant when collapsed to include both the challenge and extinction 2 phases, [F (3, 112) = 2.29, n.s.] However, during the challenge phase only, the hormone x challenge condition interaction was statistically significant, [F (3, 112) = 3.81, p = 0.05.] Within the animals that received the drug challenge, the hormone effect during the challenge phase was statistically significant, [F (3, 112) = 7.36, p < 0.01], such that the PROG-only > VEH = EB-only > EB + PROG. Within the animals that received the stress challenge, the hormone effect during the

challenge phase was not statistically significant, [F (3, 112) = 0.25, n.s.] The challenge condition was statistically significant for each hormone group during the challenge phase, and drug challenge produced significantly more transitions than the stress challenge. The results for the EB-only, EB + PROG, VEH, and PROG-only groups were [F (1, 112) = 28.35, 15.63, 48.94, 71.05, respectively, each $p < 0.01$]. Within the second extinction phase, the hormone x challenge condition interaction was not statistically significant, [F (3, 112) = 0.30, n.s.]

In summary of the challenge and extinction 2 transition data, the drug challenge induced more transitions than stress as expected. But this challenge condition difference was only significant during the challenge phase and not during the extinction 2 phase. Thus, the effect of the challenge on transitions was short-term. The drug exposure had different effects among the four hormone treatments during the challenge phase only.

**Body Weights**

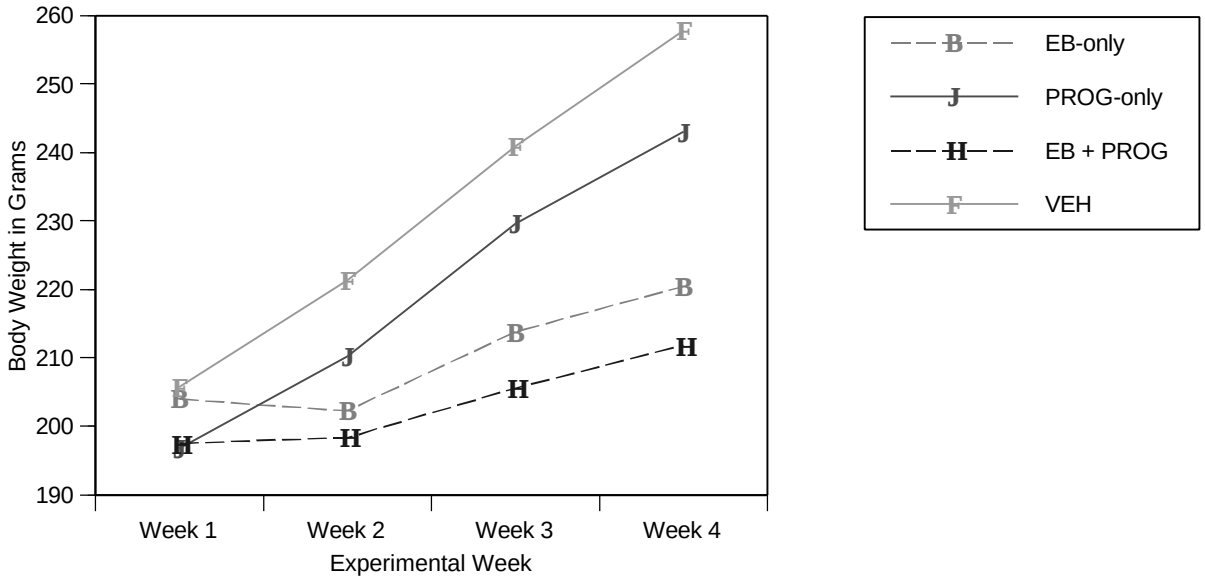
The body weight data are presented in Table 9 on p. 107 and Figure 15 on p. 108. Body weights were recorded on each animal weekly throughout the experiment. Data analyzed include values obtained weekly during the experiment for each animal. A 4 x 4 repeated-measures factorial ANOVA was used with main factors of hormone group (EB-only, EB + PROG, VEH, and PROG-only) and experimental week. The results for the week main effect achieved statistical significance, [F (3, 240) = 40.06, $p < 0.001$]. Based on the results of Tukey's HSD, body weights increased with each week of the experiment, regardless of hormone treatment. The hormone group main effect was also statistically significant, [F (3, 240) = 28.950, $p < 0.001$], and Tukey's HSD revealed that VEH > PROG-only > EB-only = EB + PROG.

**Table 9.** Mean body weights for each hormone group during each of the four weeks of the experiment.

| Hormone Group | Experiment Week | | | | |
|---|---|---|---|---|---|
| | Week 1 | Week 2 | Week 3 | Week 4 | Total (Mean) |
| EB-only | $204.06^{a}$ ± 4.57 | $^{A}202.31^{a}$ ± 4.53 | $^{A}213.19^{b}$ ± 3.77 | $^{B}220.44^{b}$ ± 4.49 | $^{A}210.00$ ± 2.31 |
| PROG-only | $196.81^{a}$ ± 4.24 | $^{A}210.25^{b}$ ± 4.46 | $^{B}229.56^{c}$ ± 4.70 | $^{C}243.00^{d}$ ± 4.46 | $^{B}219.91$ ± 2.13 |
| EB + PROG | 197.50 ± 3.98 | $^{A}198.38$ ± 4.12 | $^{A}205.56$ ± 5.11 | $^{A}211.81$ ± 4.51 | $^{A}203.31$ ± 2.29 |
| VEH | $205.75^{a}$ ± 4.40 | $^{B}221.38^{b}$ ± 4.63 | $^{C}240.94^{c}$ ± 5.49 | $^{D}257.81^{d}$ ± 5.17 | $^{C}231.47$ ± 3.46 |
| Total | $201.03^{a}$ ± 2.16 | $208.08^{a}$ ± 2.43 | $222.31^{b}$ ± 2.92 | $233.27^{c}$ ± 3.23 | 216.17 ± 1.56 |

Data are means of body weights ? SEM. Data that do not share alphabetical superscripts differ at $p = 0.05$ in the Tukey's HSD test. Uppercase letters in the columns indicate hormone group differences within each week. Lowercase letters in the rows indicate differences in weeks within each hormone group.

**Figure 15.** Mean body weights for each hormone group during each of the four weeks of the experiment.




These data are mean body weights for each hormone treatment group during each the four phases in which weights were measured. The hormone group x week interaction was statistically significant. There were no differences in body weights among the four hormone groups during week 1. However, there were statistically significant differences among the four hormone treatment groups during each of the other weeks. The results of Tukey's HSD post-hoc tests are displayed in Table 9 on p. 107.

The hormone group x week interaction was also statistically significant, [$F$ (9, 240) = 3.48, $p < 0.001$.] Simple main effects were examined. The effect of week within the EB-only, EB + PROG, VEH, and PROG-only hormone groups were [$F$ (3, 240) = 3.42, $p < 0.05$, 2.17, n.s., 24.80, $p < .01$, and 19.96, $p < 0.01$, respectively.] The results of Tukey's post-hoc tests indicating the differences in weeks for each hormone group are displayed in Table 9 on p. 107.

Within the baseline, conditioning, extinction, and challenge weeks of the experiment, the results of the effect of hormone on body weight were [$F$ (3, 240) = 0.99, n.s., 4.95, $p < 0.01$, 12.24, $p < 0.01$, and 21.20, $p < 0.01$, respectively.] Thus, there were no hormone group differences in body weights during the baseline week. However, during the remaining three weeks, differences in body weights among the four hormone groups were statistically significant. The consistent finding during each of these weeks was that body weights were the highest for the VEH group and lowest for the EB + PROG group. Specifically, during the conditioning phase, the weights for VEH > EB-only = PROG-only = EB + PROG. During extinction, the weights for VEH > PROG-only >EB-only = EB + PROG, and during challenge, the weights for VEH > PROG-only >EB-only > EB + PROG.

## Discussion

### Overview

This study was designed to examine the interaction of female sex hormones and stress and attraction to an environment formerly associated with amphetamine (AMPH) injections. The primary measure was conditioned place preference (CPP). The methodology included two stages of CPP extinction, which are intended as an animal model of abstinence and relapse in humans. The animals were ovariectomized (OVX) rats receiving replacement

doses of EB-only, PROG-only, EB + PROG, or VEH. It was predicted that OVX females receiving EB-only replacement would show greater CPP responses to AMPH and be more resistant to the initial CPP extinction than OVX animals in the other groups. The animals administered EB were expected to have greater responses to a drug challenge and to display more resistance to a second extinction than animals in the other hormone treatment groups. Other secondary hypotheses were proposed prior to the experiment.

Results supported some hypotheses and failed to support others. For example, extinction of the initial CPP was significantly influenced by hormone treatment, particularly estrogen. The criterion used to define extinction was that the time in the drug-paired compartment was to be similar to the time during baseline, i.e., the day at which there were no statistically significant differences between these times. Based on this criterion, the EB-only and the EB + PROG groups never achieved extinction over the ten days of the first extinction test. The PROG-only and VEH groups met the criterion on day 4 and day 6, respectively, of the extinction phase.

The overall time in the drug-paired compartment during the ten days of the extinction 1 phase revealed that animals administered EB-only spent the most time in the drug-associated compartment, while the PROG-only group spent the least time. These findings suggest that EB treatment with or without PROG resulted in greater resistance to extinction as compared to the other hormone treatments. Resistance to extinction can be used as a measure of drug reward, and the suggestion is that EB enhances and PROG-only attenuates drug reward (Justice & de Wit, 1999; Markou et al., 1993; Sofuoglu et al., 2004). Results from some studies suggest that PROG counters the enhancing effects of EB (e.g., Jackson,

Robinson, & Becker, 2006), and others suggest that PROG may have little or no influence on the effects of EB (Russo et al., 2003; Sofuoglu et al., 2004).

Following the extinction phase, animals were assigned to one of two challenge conditions**.** Groups received either a single exposure with restraint stress or a single AMPH injection, followed immediately by a test for CPP reinstatement. Both the stress and drug challenges resulted in reinstatement of CPP as predicted (Itzhak & Martin, 2001; Wang et al., 2000). However there were no statistically reliable differences between the two challenge conditions in the magnitude of CPP reinstatement. The different hormone regimens also did not significantly impact reinstatement in the challenge phase, nor was there a statistically significant interaction between hormone regimen and challenge condition.

Following the test of drug- or stress-induced reinstatement of CPP, re-extinction of CPP was measured for another five-day period. The two challenge conditions resulted in different rates of extinction during this second extinction phase. Recall that the criterion used to measure extinction was that the time in the drug-paired compartment was to be similar to the time during baseline, i.e., the day at which there were no statistically significant differences between the two times. Collapsing across hormone treatment, the animals receiving the drug challenge met the criterion for extinction on day2, whereas the groups receiving the stress challenge never met the criterion in the 5 days of the second extinction phase. Results indicated that CPP for the animals receiving the stress challenge did not completely re-extinguish the response in the five days of extinction 2. The effects of stress during extinction 2 were the greatest in the EB + PROG group. The effects of the drug challenge were least among the VEH group. The EB-only and the PROG-only groups did not differ. These findings suggest that females with ovarian hormone levels similar to

estrous levels experience the most significant impact of stress on drug-seeking behaviors. Conversely, females with metestrous levels of hormones are least affected by a drug challenge.

The following is a summary and discussion of the hypotheses proposed and tested in each phase of the experiment.

**CPP Acquisition Test**

As predicted, all four hormone treatment groups achieved statistically significant CPP. That is to say, times in the drug-paired compartment were reliably higher at acquisition testing than times during baseline. No previous study has specifically compared AMPH-induced CPP among different hormone treatments of females. However it was anticipated that the hormone groups would differ because current sex hormone levels influenced responses to stimulants in other studies (Becker et al., 1982; Díaz-Véliz et al., 1984; Forgie & Stewart, 1994a; Lynch et al., 2001). Estrogen increases behavioral responses to AMPH (Becker et al., 1982; Díaz-Véliz et al., 1984; Forgie & Stewart, 1984a), and it also increases self-injection of stimulants, including cocaine (Lynch et al., 2001). The magnitude of cocaine-induced CPP in OVX females also was potentiated by EB + PROG treatment and diminished by PROG-only treatment in a previous study (Russo, Festa, Fabian, Gazi, Kraish, Jenab & Quiñones-Jenab, 2003). However, in the present study there were no differences among the different hormone treatments in the amount of time in the drug-paired compartment.

The 1-mg/kg bwt dose of AMPH was chosen because it resulted in significant CPP in other studies (e.g., Gilbert & Cooper, 1983). This dose of AMPH resulted in a clear CPP, but the different hormone treatments did not differentially influence the acquisition of CPP.

However, the effects of this dosage of AMPH may have obscured subtle hormonal influences, or there may have been a ceiling effect related to the amount of time spent in one compartment or the other. Because the CPP effects of AMPH are dose dependent, the hormone groups may have differed from each other if a lower AMPH dosage was used (Gilbert & Cooper, 1983).

In addition, hormone dosages used could have been partly responsible. Some animal and human studies reporting significant hormonal influences used pharmacologic dosages of hormones (e.g., Justice & deWit, 2000a), that is estrogen and progesterone dosages were not the physiological levels induced by the dosage in the current study

**Extinction 1**

The expectation was that the effect of time, or phase day, would differ among the hormone groups. That was the result obtained. Resistance to extinction of drug-seeking behavior has been used as a measure of the rewarding properties of a drug in that an animal may be willing to work for a longer period of time to obtain a more rewarding drug versus a less rewarding one (Markou et al., 1993).

Data from all groups combined indicated that animals spent less time in the drug-paired compartment on the final days of extinction 1 compared to CPP acquisition testing or to the initial days of extinction.

Researchers have used different terminology to describe the four stages of the estrous cycle of female rats (e.g., Loscher, Wahnschaffe, Rundfeldt, Honack, & Hoppen, 1992). I have adopted the terminology described by Carter (1992), i.e., metestrus, diestrus, proestrus and estrus phases. Metestrus is a phase of reduced hormonal and behavioral activity following ovulation when both estrogen and progesterone levels are low. Diestrus is the pre-

ovulatory phase associated with the beginning of the rise in estrogen secretion. Proestrus is the stage leading up to ovulation when both estrogen and progesterone levels are elevated, producing behavioral estrus and preparing for ovulation. Estrus is when ovulation occurs and both estrogen and progesterone levels are decreasing, although progesterone is elevated relative to estrogen.

There were also significant differences among the four hormone regimens in the time in the drug-paired compartment over the entire extinction phase. When times were averaged over all days of extinction, the groups administered EB-only and VEH similarly spent the most time in the drug-paired compartment, and the PROG-only group spent the least. The combined EB + PROG group did not differ from any other groups. The most direct implication is that progesterone administered alone enhanced the rate of CPP extinction. These extinction data support findings in the literature that progesterone diminishes the response to stimulant drugs (Justice & de Wit, 1999; Russo et al., 2003). These data are also similar to findings suggesting that PROG may have little or no effect on the facilitating effects of EB (Russo et al., 2003; Sofuoglu et al., 2004) because the EB + PROG group did not differ from the EB-only group.

The animals administered VEH are more similar to males than to intact females, at least in terms of circulating ovarian hormone levels. Because this control group of OVX animals did not differ from those administered EB-only or EB + PROG, this suggests males would have similar extinction responses to AMPH as intact females in late diestrus and proestrus phases. The literature is rife with reports of gender differences in responses to AMPH in intact animals, with females typically found to display greater responses than males (Becker et al., 1982; Camp & Robinson, 1988; Lynch & Carroll, 1999). The

implication of my data is that these gender differences would be moderated when females are in diestrus**;** this is supported by the findings of several previous studies (e.g., Becker et al., 1982; Díaz-Véliz et. al, 1994).

Within-group changes were used to evaluate the prediction that the four hormone groups would display different rates of extinction. The VEH and the PROG-only groups of animals showed an extinction effect, i.e., both groups spent less time in the drug-paired compartment over days. The EB-only and EB + PROG groups failed to exhibit clear CPP extinction according to the criterion, i.e., the decrease in time in the drug-paired compartment was not statistically reliable. The data clearly suggest a decline over days, however the decline was not statistically significant. The VEH group met the extinction criteria on day 6, and the PROG-only group met these criteria on day 4. These data suggest that EB maintained CPP with or without PROG (Russo et al., 2003; Sofuoglu et al., 2004).

**Challenge Phase**

Following extinction, the four hormone treatment groups were further divided to be administered a challenge. Indeed, one unique contribution of this experiment was the direct comparison of the two types of challenge. Previous studies have examined the effects of either drug exposure (Ranaldi et al., 1999; Schenk & Partridge, 1999; Wang et al., 2000) or stress (Erb et al., 1996; Sanchez & Sort, 2001; Wang et al., 2000) on drug-seeking behaviors. No known study has directly compared the capacity of the two challenge conditions to reinstate CPP. One study did test the ability of both stress and drug exposure to reinstate CPP (Wang et al., 2000). These results indicated that both drug exposure and stress reliably reinstated drug-seeking behaviors, but the magnitude of the reinstated CPP was not compared between the two challenge groups. In a more direct comparison between stress

and drug, Erb et al. (1996) compared the capacity of foot shock and drug challenge to reinstate an extinguished bar press response established previously with cocaine self-administration. Foot shock induced significantly more lever presses than cocaine. The reinstated lever pressing produced an injection of saline only.

For the challenge, half of the animals in each hormone treatment group were exposed to AMPH, and the other animals were exposed to restraint stress. Time-change scores were calculated based on time in the drug-paired compartment on the final day of extinction 1. EB was predicted to enhance the reinstatement of CPP following drug re-exposure and to inhibit stress-induced CPP reinstatement. However, the main effect of hormone treatment was not statistically reliable in these challenge phase data, nor was the hormone x challenge condition interaction.

Both drug and stress challenge conditions resulted in statistically significant reinstatement of CPP. On the day that the challenge was presented, the times in the drug-paired compartment were significantly greater compared to the last day of extinction 1. These findings confirm that drug and stress challenges produced CPP reinstatement, as in other studies (Itzhak & Martin, 2001; Sanchez & Sorg, 2001; Wang et al., 2000).

It was anticipated that drug challenge would result in greater time changes than stress challenge. This was based on a) the assumption that drug exposure would induce more drug seeking than would stress and b) because the drug exposure was the same stimulus originally paired with the compartment during the conditioning phase. Although stress is known to reinstate extinguished drug-seeking behaviors (Itzhak & Martin, 2001; Sanchez & Sorg, 2001; Wang et al., 2000), it was predicted that its effects would be less than that of the drug challenge. There were, however, no statistically significant differences between stress and

drug challenge in the time-change data in the present experiment. The implication is that a single stressful experience or a single drug exposure has equal potential to trigger a relapse after a period of abstinence in human drug users.

The hormone main effect was statistically significant during the challenge test, but not as predicted. The measure was the change of times in the formerly drug-associated box from that on the last day of the first extinction phase. The logic of this measure is that it reflects sensitivity to challenge, i.e., the greater the time change, the greater the sensitivity. The EB + PROG group had the greatest time change scores overall, and the VEH and EB-only groups had the smallest time change scores. Recall that the hormone main effect combines the two challenge conditions. Still, these results suggest that, contrary to expectation, estrogen magnified the reinstatement to former drug-associated cues only when combined with progesterone.

There is considerable evidence in experimental animals that EB magnifies the effects of drugs (Becker et al., 1982; Forgie & Stewart, 1994a; Lynch et al., 2001). Estrogen may also magnify the effects of stress (Shansky, Glavis-Bloom, Lerman, McRae, Benson, Miller, Cosand, Horvath, & Arnsten, 2004). Evidence exists as well that progesterone may have anxiolytic effects (Bitran, Klibansky, & Martin, 2000; Drury & Gold, 1978; Rupprecht, Koch, Montkowski, Lancel, Faulhaber, Harting, & Spanagel, 1999; and White & Uphouse, 2004), suggesting PROG would diminish stress-induced reinstatement of drug seeking. Therefore, the statistically significant hormone main effect among these challenge phase data is contrary to the findings of those studies. However, some studies have found that the combined EB + PROG treatment to not differ from EB-only (e.g., Sell et al., 2000).

One possible explanation for these contradictory data and lack of statistical significance is that a ceiling effect may have occurred in the amount of time in the drug-paired compartment, particularly in the case of animals administered EB-only. Another possibility is that the effects of the drug or stress exposure overwhelmed the influence of hormone treatment. However, based on the literature, EB with or without PROG would be expected to induce the greatest reinstatement of CPP following the challenge exposures, and VEH and PROG would be expected to induce the least reinstatement.

Another consideration is that because EB-only magnified the original CPP, one suggestion is that women with high estrogen levels may be more susceptible to addiction. While this may be the case, these data suggest that progesterone slows the rate of extinction of a formerly established drug response. Extinction in this experiment models human withdrawal and abstinence, and the groups administered progesterone were slower to extinguish. The implication is that progesterone may extend the time period to extinguish a drug response in women. No known reports exist in the literature comparing the effects of hormone treatment on drug- and/or stress-induced reinstatement. Although the effects of estrogen on gender differences in drug sensitivity have been studied extensively, the effects of progesterone have not.

Simple main effects also revealed that the EB + PROG group had the greatest time-change scores among the groups receiving the drug challenge and among the groups receiving the stress challenge. Further analysis of simple main effects revealed that the VEH and EB-only groups had the lowest time change scores among both the drug and stress challenge groups. The consistency of these findings across challenge modalities is notable. Rather than pointing to estrogen, however, the findings point to the role of progesterone.

PROG increased the effects of both the stress and drug challenges, with or without EB. These findings suggest the need for more studies examining the influence of progesterone on reinstatement of drug-seeking behaviors and relapse in humans.

A fundamental question is whether the influences on drug taking and reinstatement in females are a result of current levels of ovarian sex steroids or from early, fetal effects. A partial answer can be offered based on the findings of the VEH groups. The findings of the VEH groups that revealed low time-change scores also suggest that it is current hormone levels and not fetal organizational effects that influence these reinstatement behaviors.

**Extinction 2**

An effect of the challenge condition was uncovered during the second extinction phase (extinction 2). This is another unique aspect of this experiment in that no other study has examined a second extinction of drug seeking following reinstatement induced by a challenge. Some studies have presented a challenge at various lengths of time following extinction. Wang et al. (2000) reported that a stressor could reinstate drug-seeking following two weeks of extinction, and Shaham, Adamson, Grocki, and Corrigall (1997) reported that drug-exposure can reinstate drug seeking following three weeks of extinction.

The stress challenge groups over all days of extinction 2 experienced greater time change scores relative to baseline than the drug challenge groups. These results suggest that although the initial impact of stress on drug-seeking behaviors is no different than drug exposure, the effect of stress is longer lasting than drug exposure. The implication is that following a period of abstinence, a human drug user exposed to stress may be more vulnerable to relapse than one exposed to a single drug administration. The results of laboratory studies of human drug users indicate that stress and exposure to drug or drug-

related cues are similar in their ability to induce drug craving (Sinha et al., 2000**).** No known reports in the literature compared the capacity of the two to induce drug-seeking with the exception of Erb et al (1996). In this experiment, animals were trained to lever press for a self-injection of cocaine, and that behavior was allowed to extinguish. Animals were then challenged with either a dose of drug administered by the experimenter or exposure to foot shock. The results revealed that foot shock increased lever pressing compared to a drug challenge (Erb et al., 1996). These results support the finding that stress may have a greater impact on drug-seeking behavior than drug exposure, as was revealed in the extinction 2 data.

The rates of extinction during extinction 2 for the two challenge conditions appear to be different. Within group comparisons of all drug groups indicated the drug challenge promoted reduced time change scores relative to times at baseline beginning on day 2 of extinction 2. Lower scores are an indication of diminishing CPP. No similar pattern was observed in the stress group. The suggestion is that the CPP response in stress groups did not extinguish within the five days of this phase. This is an interesting finding, and counter to the hypothesis proposed *a priori* that drug re-exposure would have a greater impact on reinstatement and subsequent extinction than would stress exposure. The implication is that following a period of abstinence, a human drug user would be more vulnerable to continued drug use over a greater period of time than an abstinent drug user exposed to a single drug administration. In other words, stress may be more likely to prolong a relapse than a single drug exposure would.

In another analysis of the extinction 2 data, comparison of the eight groups (EB-only + drug, EB-only + stress, EB + PROG + drug, EB + PROG + stress, VEH + drug, VEH +

stress, PROG-only + drug, and PROG-only + stress) revealed statistically significant differences among these groups when data were combined for each group across all days of the extinction 2 phase. The greatest time change scores were in the EB + PROG + stress group and least for the VEH + drug group. The EB + PROG + stress group spent more time in the drug-paired compartment relative to times at baseline compared to the VEH + drug group. No other groups differed significantly from the others. Applied to intact females, proestrus would be the phase when the animal is most vulnerable to the effects of stress, and metestrus would be the phase when the animal is less sensitive to drug effects. Applied to humans, women would be most vulnerable to the effects of stress during the luteal phase, and less sensitive to drug effects during the follicular phase.

These results are interesting but different than what was hypothesized. Stress resulted in prolonged extinction compared to drug, however the prediction was that the impact of stress would be most influenced by EB-only rather than the combination of EB +PROG. The combined EB + PROG facilitated drug seeking in animals exposed to stress. Most reports in the literature for both human and non-human animals suggest that estrogen has the greatest influence on stress reactions (Herman et al., 1984; Matthews et al., 2001; Young et al., 2001). There is also considerable evidence that progesterone may have anxiolytic properties and diminish many behavioral stress responses (Bitran et al., 2000; Drury & Gold, 1978; Rupprecht et al., 1999; White & Uphouse, 2004).

Also, the effects of drug were expected to be magnified by EB-only and reduced by PROG-only (Forgie & Stewart, 1994a; Justice & de Wit, 1999; Lynch et al., 2001). However the current data revealed VEH + drug displayed the lowest time-change scores with none of the other hormone and drug groups differing from each other.

It is puzzling that EB-only, PROG-only, or EB + PROG treatments did not reliably differ in the drug challenge groups. A possible explanation is that the data revealed substantial within-group variability in some measures, and this variability surely had an impact on the absence of statistical significance. Several analyses were repeated eliminating outliers, however, the conclusions were the same, with no significant differences among groups. This variability may have occurred due to environmental effects that proved difficult to control, including noises outside of the experiment room. It is also possible that individual differences in factors such as fearfulness may have had considerable influence on these measures. Ceiling effects may have continued to have an impact in the second extinction data as well.

Although the 3-way interaction of challenge condition, hormone treatment, and phase day was not statistically significant, some interesting trends are noted in the data. Among the drug challenged groups, EB + PROG showed the most rapid and dramatic drop in time-change scores. This group had the highest time change scores on the day of challenge, but appear to have extinguished rapidly. Although having had lower time change scores on the day of the challenge, the PROG-only group also dropped rapidly on the first day of extinction 2. The VEH and EB-only groups had a much more gradual decline in time-change scores. These findings again point to the important role of progesterone.

Among the stress challenged groups, the EB + PROG group had greater time change scores throughout this phase, but showed a similar rate of extinction as the VEH group. The EB-only group and the PROG-only groups were affected the least by the stress challenge on the challenge day, but the EB-only group spent more time in the drug-paired compartment on the first day of the second extinction phase than on the day of the challenge, and was slow to

decrease in time after that. The PROG-only group demonstrated a slow, gradual decrease in time-change scores over the extinction 2 phase.

These data, although not statistically significant using conservative tests, suggest that PROG with or without EB hastens the rate of extinction following reinstatement, and EB-only retards the rate. These data support findings of other studies that EB facilitates drug responses and PROG diminishes them (Justice & deWit, 1999). Examination of the literature on general extinction from food and other non-drug reinforcers reveal no studies comparing the influences of hormones including progesterone.

The suggestion is that intact rats in proestrus and women in the early luteal phase with high progesterone and estrogen levels may have the greatest response to drug or stress challenge but that they would extinguish faster or achieve abstinence quickly. Also, intact rats in estrus and women in the late luteal phase with relatively high progesterone and low estrogen would have a greater sensitivity to remission to a drug re-exposure but be relatively immune to stress. Again, progesterone would increase the rate of extinction and a return to abstinence. Intact rats in proestrus and women in the follicular phase are likely to require longer to develop complete abstinence to the drug after undergoing a drug re-experience or a stress exposure, but the effects are less dramatic among those undergoing a drug re-exposure.

**Transitions**

The transition data were used as a measure of locomotion and as another measure of drug sensitivity because AMPH increases locomotor behavior (Becker et al., 1982; Chiodo et al., 1981). The results of the hormone group effect were not as predicted, with the VEH and PROG-only groups completing the most transitions and EB + PROG completed the fewest. The greater number of transitions suggests greater sensitivity to the stimulating effects of

AMPH. This stimulating effect was expected to be magnified by EB-only and diminished by PROG-only (Becker et al., 1982; Chiodo et al., 1981; Montero & Van Hartesveldt, 1984). The acute effects of AMPH would have been most apparent during the challenge phase.

One possible explanation for these data is to consider transitions as a measure of novelty seeking as opposed to locomotion. Novelty seeking may possibly induce more transitions. Novelty seeking has been used as a model of sensation seeking in humans (Piazza, Deminiere, leMoal, &Simon, 1989), a characteristic found in many drug addicts (Zuckerman, 1979). Novelty seeking would likely be diminished in stressed animals. Therefore, if EB magnified the effects of stress, then these transition data would be predicted. Previous research has indicated that novelty-seeking may predict AMPH CPP (e.g., Klebaur & Bardo, 1999). This particular study categorized animals as high or low novelty seekers based on the amount of time spent exploring novel objects. High novelty seekers displayed greater AMPH CPP. Therefore, if transitions reflect novelty-seeking, then these data reflected expected hormone effects. AMPH may decrease novelty-seeking, and PROG would diminish the effects of AMPH as these data indicate.

The phase main effect was also statistically significant. The fewest transitions were completed during baseline, and the other phases, including CPP acquisition testing, extinction, and challenge, did not significantly differ. The baseline measurements used were an average of three separate days. It is likely that the animals were frightened in a novel environment during baseline, despite collecting these data over three days, and therefore were less active during baseline than during the latter phases. It was anticipated that the numbers of transitions would be significantly different during the challenge phase because of

the stimulating effects of AMPH, however the challenge phase was not reliably different from any phase other than baseline.

However, during the challenge phase the hypothesis that drug exposure would result in greater numbers of transitions than the stress exposure was supported. This effect was significant during the challenge phase, but not during extinction 2. This suggests that the effects of stress may be prolonged in terms of drug-seeking behaviors (Wang et al., 2000), but not in terms of the stimulating effects of AMPH, including locomotion. Stress exposure increased locomotion in previous studies (Hermal et al., 1984), but decreased locomotion in others (Papp et al., 1993). In studies that revealed stress-induced cross-sensitization to AMPH, the findings generally suggest that this effect is short-term as was revealed in this experiment (de Jong et al., 2005).

Also, during the challenge phase only, the hormone x challenge condition interaction was statistically significant, suggesting an influence of hormone on the stimulating effects of AMPH. Interestingly, among the drug-challenge group, the most transitions were in the PROG-only group and the least were in the EB + PROG group. It was anticipated that EB-only would have magnified the stimulating effects of drug, and PROG-only would have diminished them (Justice & de Wit, 1999; Sell et al., 2000).

The hormone effect was not significant for the stress-challenge group, although it was anticipated that this interaction would have been significant. EB was predicted to diminish the effects of the stress challenge (Young et al., 2001). The effect of challenge condition was statistically significant among all four hormone groups, with AMPH inducing more transitions than the stress challenge. Conflicting results have been reported on the interaction of gender, stress, and stimulants. One study reported that behavioral sensitization of stress

and AMPH-induced locomotion revealed no sex differences (West & Michael, 1988), whereas another experiment reported sex differences (McCormick et al., 2004).

Based on the findings of the current experiment, it appears that the drug-induced increases in transitions were opposite to the predicted outcome, in that PROG-only enhanced locomotor sensitization induced by AMPH re-exposure and EB + PROG hampered this sensitization (Justice & de Wit, 1999; Sell et al., 2000). The VEH and EB-only treatments produced similar effects that were different than PROG-only as well as EB + PROG. The findings that AMPH induced fewer transitions in the EB-only group as compared to the VEH group suggest that males may be more likely than females to display behavioral sensitization from repeated AMPH doses. This finding is counter to other studies that reported greater behavioral sensitization in females (Becker et al., 1982). Spontaneous locomotor activity in the absence of psychostimulants was also reported to be greater in OVX animals treated with EB as opposed to vehicle only (Ohtani, Nomoto, & Douchi, 2001). Females showed higher levels of locomotor activity than males as well (Forgie & Stewart, 1994b).

Some studies have reported no gender differences in locomotor activity, in the absence of amphetamine (Renner, Bennett, & White, 1992). Animals allowed to freely explore an open field with small stimulus objects. There were no gender differences in activity level or investigation of the stimulus objects. Baseline locomotor activity was not increased by estrogen in OVX animals implanted with either EB-filled silastic implants or empty implants in another study (Febo, Jiménez-Rivera, & Segarra, 2002). The majority of studies report that females have greater locomotor activity than males, and that this increased activity may be related to estrogen, but some conflicting findings have been reported, as were revealed in the current experiment.

**Body Weights**

The body weight data supported the hypotheses initially proposed. Body weights significantly increased during the four weeks of the experiment as expected, since these animals had not reached expected adult weights. The hormone effect was statistically significant as well when comparing weights during all four weeks combined. The VEH animals were the heaviest, followed by PROG-only, then EB-only and EB + PROG. The EB-treated females gained the least weight with or without PROG.

The hormone x week interaction was also statistically significant. That is, the hormone groups of animals gained weight at different rates. There were no hormone group differences in body weight at the start of the experiment, i.e., at baseline. The effect of week on weight was not significant for the EB + PROG group. In other words, this group did not have a statistically significant change in weight. The other hormone groups did display significant weight gain. The VEH group gained the most weight, followed by the PROG-only, EB-only, and EB + PROG. Estrogen replacement decreases food intake and weight gain in OVX animals (Albert, Jonik, Gorzalka, Newlove, Webb, & Walsh, 1991; Asarian, & Geary, 2002; Geary & Asarian, 1999; Wade & Zucker, 1970).

Similar findings that EB decreases weight gain are reported in studies of post-menopausal women (Jensen et al., 2003; Writing group of the PEPI trial, 1995). The findings of the current experiment support those of these previous experiments, in that oophorectomized women may gain more weight if they are not administered hormone replacements that includes EB.

**Conclusions**

There is evidence in the data supporting the hypothesis that estrogen enhances and progesterone suppresses drug responses. This is especially apparent in the first extinction phase data. EB-only slowed CPP extinction, whereas PROG-only hastened it. Extinction is a model for abstinence in the drug-addicted human. All individuals receiving drug treatment should receive education about the influence of drug cues and subsequent drug exposure on the potential for relapse. But women, because of their higher levels of estrogen throughout the menstrual cycle, may have prolonged drug craving during abstinence compared to men, thus making them more vulnerable to relapse.

Also, as reported in other studies, both stress and drug exposure are significant triggers for the reinstatement of extinguished drug-seeking behavior (Itzhak & Martin, 2001; Wang et al., 2000). An experimental challenge with drug or stress did not differ in the magnitude of reinstated CPP. The effects of stress-induced reinstatement have appeared to be more prolonged than drug exposure. Interestingly, the effect of drug exposure and stress challenge on reinstatement and subsequent extinction was influenced by hormone regimen, but not as was predicted. Based on the findings reported in the literature, EB-only should have enhanced the effects of AMPH and PROG-only should have diminished them. It is unclear why the results of the current study did not produce such findings. Possible explanations for the lack of statistical significance in some of the challenge phase data include a ceiling effect or that the effects of the drug or stress exposure overwhelmed the influence of hormone treatment.

These findings suggest that women may require special care during drug addiction treatment. If estrogen enhances drug sensitivity, especially in terms of protracted extinction, women may need more intense therapy and longer in-patient treatment than men to increase

the likelihood of sustained abstinence. Women should also be informed that during the follicular phase of their menstrual cycle when estrogen titers are the highest, it is possible that they will experience greater craving and be more vulnerable to relapse if exposed to drug.

Stress management techniques should also be taught as part of drug treatment because drug exposure and stress are key factors in triggering relapse, and stress may have prolonged influence on the potential for relapse. Special care should also be taken in hormone replacement treatment for post-menopausal women or those who have had hysterectomies and a history of drug addiction. Careful dosing of hormones and possibly administering the lowest possible dosages to these women may be important to decrease the potential for relapse.

**Future Research Considerations**

Future experiments using this design could be done using various dosages of AMPH to possibly uncover dose-response measures in CPP acquisition and extinction. Lower AMPH dosages may reveal significant differences among the four hormone treatments in the test of CPP acquisition. Also, a longer extinction 1 phase should be done. If data had been collected for more days during this phase, the EB-only and EB + PROG groups may have met the defined criteria for extinction, and this extinction data may reveal dose-response effects if several different dosages of AMPH were used as well.

Other possibilities would be to employ models for acute and chronic stress by exposing animals to the stress paradigm prior to AMPH exposure, and varying the length of time or the number of days that the animals were restrained. Varying the length of time would provide dose-response measures for stress on reinstatement. Dose-response measures

could also be obtained by administering varying dosages of AMPH during the drug challenge as well.

To distinguish whether transitions are truly a measure of locomotion or novelty seeking, time spent in each compartment between transitions would have been informative. Increased novelty seeking would be expected to display greater times in each compartment between transitions as opposed to rapidly moving between the compartments.

Also, there was considerable variability in some of the obtained measures. Care was taken to control lighting levels, temperature, and noise in the experimental room. However, outside noises were occasionally noted, and could have influenced some of the behaviors of some of the experimental animals. Sound-proofing of rooms could be beneficial in any subsequent experiments in this field.

Also, to evaluate the influence of menstrual cycle on relapse in humans, data could be collected at drug treatment facilities. Women who have re-entered treatment due to a recent relapse could provide information on phase of menstrual cycle at the time of the relapse as well as the trigger for the relapse, e.g., if stress or drug cue related.

Much more research is needed to examine the influence of progesterone on AMPH-related behaviors, especially reinstatement induced by stress or drug re-exposure based on the findings of this study. Studies should also be conducted to examine the influence of hormones, particularly progesterone, on other general extinction behaviors, e.g. food rewards.

## REFERENCES

Ahmed, S.H., & Koob, G.F. (1997). Cocaine- but not food-seeking behavior is reinstated by stress after extinction. Psychopharmacology, 132, 289-295.

Albert, D.J., Jonik, R.H., Gorzalka, B.B., Newlove, T., Webb, B., & Walsh, M.L. (1991). Serum estradiol concentration required to maintain body weight, attractivity, proceptivity, and receptivity in the ovariectomized female rat. Physiology & Behavior, 49, 225-231.

Albertson, T.E., Derlet, R.W., & van Hoozen, B.E. (1999). Methamphetamine and the expanding complications of amphetamines. Western Journal of Medicine, 170, 214-219.

Aloisi, A.M., Ceccarelli, I., & Lupo, C. (1998). Behavioural and hormonal effects of restraint stress and formalin test in male and female rats. Brain Research Bulletin, 47 (1), 57-62.

American Psychiatric Association. (1994). Diagnostic and Statistical Manual of Mental Disorders (4th ed., pp. 181-183). Washington, DC: American Psychiatric Association.

Anthony, J.C., Warner, L.A., & Kessler, R.C. (1994). Comparative epidemiology of dependence on tobacco, alcohol, controlled substances, and inhalants: basic findings from the National Comorbidity Survey. Experimental and Clinical Psychopharmacology, 2, 244-268.

Antelman, S.M., Eichler, A.J., Black, C.A., & Kocan, D. (1980). Interchangeability of stress and amphetamine in sensitization. Science, 207, 329-331.

Antus, B., Hamar, P., Kokeny, G., Szollosi, Z., Mucsi, I., Nemes, Z., & Rosivall, L. (2003). Estradiol is nephroprotective in the rat remnant kidney. Nephrology Dialysis Transplantation, 18, 54-61.

Arnold, A.P., & Breedlove, S.M. (1985). Organizational and activational effects of sex steroids on brain and behavior: a reanalysis. Hormones and Behavior, 19, 469-498.

Asarian, L. & Geary, N. (2002). Cyclic estradiol treatment normalizes body weight and Restores physiological patterns of spontaneous feeding and sexual receptivity in Ovariectomized rats. Hormones and Behavior, 42, 461-471.

Bales, R.F. (1946). Cultural differences in rates of alcoholism. Quarterly Journal of Studies on Alcohol, 6, 480-499.

Bardo, M.T. & Bevins, R.A. (2000). Conditioned place preference: what does it add to our preclinical understanding of drug reward? Psychopharmacology, 153, 31-43.

Bardo, M.T., Valone, J.M., & Bevins, R.A. (1999). Locomotion and conditioned place preference produced by acute intravenous amphetamine: role of dopamine receptors and individual differences in amphetamine self-administration. Psychopharmacology, 143, 39-46.

Bauer, M.E., Perks, P., Lightman, S.L., & Shanks, N. (2001). Restraint stress is associated with changes in glucocorticoids immunoregulation. Physiology & Behavior, 73, 525-532.

Beatty, W.W. & Holzer, G.A. (1978). Sex differences in stereotyped behavior in the rat. Pharmacology, Biochemistry, & Behavior, 9, 777-783.

Becker, J.B. & Cha, J.H. (1989). Estrous cycle-dependent variation in amphetamine-induced behaviors and striatal dopamine release assessed with microdialysis. Behavioural Brain Research, 35, 117-125.

Becker, J.B., Robinson, T.E., & Lorenz, K.A. (1982). Sex differences and estrous cycle variations in amphetamine-elicited rotational behavior. European Journal of Pharmacology, 80, 65-72.

Becker, J.B. & Rudick, C.N. (1999). Rapid effects of estrogen or progesterone on the amphetamine-induced increase in striatal dopamine are enhanced by estrogen priming: a microdialysis study. Pharmacology, Biochemistry & Behavior, 64, 53-57.

Bhatnagar, S., Mitchell, J.B., Betito, K., Boksa, P., & Meaney, M.J. (1995). Effects of chronic intermittent cold stress on pituitary adrenocortical and sympathetic adrenomedullary functioning. Physiology & Behavior, 57 (4), 633-639.

Bisagno, V., Grillo, C.A., Piroli, G.G., Giraldo, P., McEwen, B., & Luine, V.N. (2004). Chronic stress alters amphetamine effects on behavior and synaptophysin levels in female rats. Pharmacology, Biochemistry, & Behavior, 78, 541-550.

Bitran, D., Klibansky, D.A., & Martin, G.A. (2000). The neurosteroid pregnanolone prevents the anxiogenic-like effect of inescapable shock in the rat. Psychopharmacology, 151, 31-37.

Bozarth, M.A. (1987). An overview of assessing drug reinforcement. In M.A. Bozarth (Ed.), Methods of assessing the reinforcing properties of abused drugs (pp. 635-658). New York: Springer-Verlag.

Burleson, M.H., Malarkey, W.B., Cacioppo, J.T., Poehlmann, K.M., Kiecolt-Glaser, J.K, Berntson, G.G., & Glaser, R. (1998). Postmenopausal hormone replacement: effects on autonomic, neuroendocrine, and immune reactivity to brief psychological stressors. Psychosomatic Medicine, 60, 17-25.

Butcher, R.L., Colins, W.E., & Fugo, N.W. (1974). Plasma concentrations of LH, FSH, prolactin, progesterone, and estradiol-17? throughout the 4-day estrous cycle of the rat. Endocrinology, 91, 1704-1708.

Camp, D.M., Becker, J.B., & Robinson, T.E. (1986). Sex differences in the effects of Gonadectomy on amphetamine-induced rotational behavior in rats. Behavioral and Neural Biology, 46, 491-495.

Camp, D.M. & Robinson, T.E. (1988). Susceptibility to sensitization. I. Sex differences in the enduring effects of chronic d-amphetamine treatment on locomotion, stereotyped behavior and brain monoamines. Behavioural Brain Research, 30, 55-68.

Campmany, L., Pol, O., & Armario, A. (1995). The effects of two chronic intermittent stressors on brain monoamines. Pharmacology, Biochemistry, & Behavior, 53 (3), 517-523.

Carroll, M.E., & Lac, S.T. (1997). Acquisition of IV amphetamine and cocaine self-administration in rats as a function of dose. Psychopharmacology, 129, 206-214.

Carter, C.S. (1992). Neuroendocrinology of sexual behavior in the female. In J.B. Becker, S.M. Breedlove, & D.Crews (Eds.), Behavioral endocrinology (pp. 71 – 96). Boston: MIT Press.

Childress, A.R., McLellan, A.T., & O'Brien, C.P. (1986). Abstinent opiate abusers exhibit conditioned craving, conditioned withdrawal, and reductions in both through extinction. British Journal of Addiction, 81, 655-660.

Chiodo, L.A., Caggiula, A.R., & Saller, C.F. (1981). Estrogen potentiates the stereotypy induced by dopamine agonists in the rat. Life Sciences, 28, 827-835.

Crine, A.F., Louis, F., Sulon, J., & Legros, J.J. (1983). Changes in total serum immunoreactive neurophysins and corticosterone levels after restraint stress in rats. Psychoneuroendocrinology, 8 (4), 447-450.

Davis, W.M., & Smith, S.G. (1976). Role of conditioned reinforcers in the initiation, maintenance and extinction of drug–seeking behavior. Pavlov Journal of Biological Science, 11 (4), 222-236.

de Jong, J.G., Wasilewski, M., van der Vegt, B.J., Buwalda, B., & Koolhaas, J.M. (2005). A single social defeat induces short-lasting behavioral sensitization to amphetamine. Physiology & Behavior, 83, 805-811.

Deroche, V., Piazza, P.V., Casolin, P., Maccari, S., le Moal, M., & Simon, H. (1992). Stress-induced sensitization to amphetamine and morphine psychomotor effects depend on stress-induced corticosterone secretion. Brain Research, 598, 343-348.

deWit, H. & Stewart, J. (1981). Reinstatement of cocaine-reinforced responding in the rat. Psychopharmacology, 75, 134-143.

D?az-Véliz, G., Baeza, R., Benavente, F., Dussaubat, N., & Mora, S. (1994). Influence of the estrous cycle and estradiol on the behavioral effects of amphetamine and apomorphine in rats. Pharmacology, Biochemistry, & Behavior, 49(4), 819-825.

Di Paolo, T., Rouillard, C., & Bedard, P. (1985). 17 beta-Estradiol at a physiological dose acutely increases dopamine turnover in rat brain. European Journal of Pharmacology, 117, 197-203.

Doweiko, H.E. (2002). Concepts of chemical dependency (5th ed). Pacific Grove, CA: Brooks/Cole.

Drummond, D. C. (2001). Theories of drug craving, ancient and modern. Addiction, 96, 33-46.

Drury, R. & Gold, R.M. (1987). Differential effects of ovarian hormones on reactivity to electric footshock in the rat. Physiology & Behavior, 20, 187-191.

Duvauchelle, C.L. & Ettenbert, A. (1991). Haloperidol attenuates conditioned place preferences produced by electrical stimulation of the medial prefrontal cortex. Pharmacology, Biochemistry & Behavior, 38 (3), 645-650.

Elman, I., Karlsgodt, K.H., & Gastriend, D.R. (2001). Gender differences in cocaine craving among non-treatment-seeking individuals with cocaine dependence. American Journal of Drug and Alcohol Abuse, 27 (2), 193-202.

Erb, S, Shaham, Y, & Stewart, J. (1996). Stress reinstates cocaine-seeking behavior after prolonged extinction and a drug-free period. Psychopharmacology, 128, 408-412.

Ettenberg, A. & Duvauchelle, C.L. (1988). Haloperidol blocks the conditioned place preferences induced by rewarding brain stimulation. Behavioral Neuroscience, 102 (5), 687-691.

Farabollini, F., Albonetti, M.E., Alosi, A.M., Facchinetti, F., Grasso, G., Lodi, L, Lupo, C. & Muscettola, M. (1993). Immune and neuroendocrine response to restraint in male and female rats. Psychoneuroendocrinology, 18 (3), 175-182.

Fawcett, J. & Busch, K.A. (1998). Stimulants in psychiatry. In: Schatzberg, A.F. & Nemeroff, C.B. (eds.). Textbook of Psychopharmacology (2nd ed., p. 506). Washington, D.C.: American Psychiatric Press, Inc.

Febo, M., Jiménez-Rivera, C.A., & Segarra, A.C. (2002). Estrogen and opioids interact to Modulate the locomotor response to cocaine in the female rat. Brain Research, 943, 151-161.

Flores, C.M., Hernandez, M.C., Hargreaves, K.M., & Bayer, B.M. (1990). Restraint stress-induced elevations in plasma corticosterone and ß-endorphin are not accompanied by alterations in immune function. Journal of Neuroimmunology, 28, 219-225.

Forgie, M.L. & Stewart, J. (1994a). Effect of prepubertal ovariectomy on amphetamine-induced locomotor activity in adult female rats. Hormones and Behavior, 28, 241-260.

Forgie, M.L., & Stewart, J. (1994b). Sex differences in the locomotor-activating effects of amphetamine: role of circulating testosterone in adulthood. Physiology & Behavior, 55 (4), 639-644.

Geary, N. & Asarian, L. (1999). Cyclic estradiol treatment normalizes body weight and test meal size in ovariectomized rats. Physiology & Behavior, 67 (1), 141-147.

Gilbert, D. & Cooper, S.J. (1983). ?-phenylethylamine, d-amphetamine, and l-amphetamine-induced place preference conditioning in rats. European Journal of Pharmacology, 95, 311-314.

Gravetter, F.J. & Wallnau, L.B. (2004). Statistics for the behavioral sciences (6th ed). Belmont, CA: Wadsworth/Thomson Learning.

Griffiths, R.R., & Balster, R.L. (1979). Opioids: Similarity between evaluations of subjective effects and animal self-administration results. Clinical Pharmacology and Therapeutics (25), 611-617.

Grilly, D.M. (2002). Drugs and human behavior (4th ed.). Boston, MA: Allyn & Bacon.

Gutierrez-Cebollada, J., de la Torre, R., Ortuno, J., Garces, J.M., & Cami, J. (1994). Psychotropic drug consumption and other factors associated with heroin overdose. Drug and Alcohol Dependence (35), 169-174.

Harris, D.S., Reus, V.I., Wolkowitz, O.M., Mendelson, J.E., & Jones, R.T. (2005). Repeated psychological stress testing in stimulant-dependent patients. Progress in Neuro-Psychopharmacology & Biological Psychiatry, 29, 669-677.

Hatsukami, D.K., & Fischman, M.W. (1996). Crack cocaine and cocaine hydrochloride: are the differences myth or reality? The Journal of American Medical Association, 276 (19), 1580-1588.

Herman, J.P., Stinus, L., & le Moal, M. (1984). Repeated stress increases locomotor response to amphetamine. Psychopharmacology, 84, 431-435.

Hidalgo, J. Armario, A., Flos, R., Dingman, A., & Garvey, J.S. (1986). The influence of restraint stress in rats on metallothionein production and corticosterone and glucagons secretion. Life Sciences, 39, 611-616.

Holdcraft, L.C. & Iacono, W.G. (2004). Cross-generational effects on gender differences in psychoactive drug abuse and dependence. Drug and Alcohol Dependence, 74, 147-158.

Hunt, G.E., Atrens, D.M., & Jackson, D.M. (1994). Reward summation and the effects of dopamine D1 and D2 agonists and antagonists on fixed-interval responding for brain stimulation. Pharmacology Biochemistry and Behavior, 48 (4), 853-862.

Hyde, J.F. & Jerussi, T.P. (1983). Sexual dimorphism in rats with respect to locomotor activity and circling behavior. Pharmacology, Biochemistry, & Behavior, 18, 725-729.

Hyman, S.E., & Nestler, E.J. (1996). Initiation and adaptation: A paradigm for understanding psychotropic drug action. American Journal of Psychiatry, 153, 151-162.

Itzhak, Y. & Martin, J.L. (2001). Cocaine-induced conditioned place preference in mice: induction, extinction, and reinstatement by related psychostimulants. Neuropsychopharmacology, 26 (1), 131-134.

Jackson, L.R., Robinson, T.E., & Becker, J.B. (2006). Sex differences and hormonal influences on acquistion of cocaine self-administration in rats. Neuropsychopharmacology, 31, 129-138.

Jensen, L.V., Vestergard, P., Hermann, A.P., Gram, J., Eiken, P., Abrahamsen, B., Brot, C., Kolthoff, N., Sørensen, O.H., Beck-Nielsen, H., Nielsen, S.P., Charles, P., & Mosekilde, L. (2003). Hormone replacement therapy dissociates fat mass and bone mass, and tends to reduce weight gain in early postmenopausal women: a randomized controlled 5-year clinical trial of the Danish osteoporosis prevention study. Journal of Bone and Mineral Research, 18 (2), 333-342.

Johanson, C.E. (1976). Pharmacological and environmental variables affecting drug preference in Rhesus monkeys. Pharmacological Reviews, 27 (3), 343-355.

Johanson, C.E. & Schuster, C.R. (1975). A choice procedure for drug reinforcers: cocaine and methylphenidate in the rhesus monkey. Journal of Pharmacology and Experimental Therapeutics, 193, 676-688.

Joyce, J.N., Montero, E., & Van Hartesveldt, C. (1984). Dopamine-mediated behaviors: characteristics of modulation by estrogen. Pharmacology, Biochemistry, & Behavior, 21, 791-800.

Julien, R.M. (2001. A primer of drug action. New York: Worth Publishers.

Justice, A.J.H. & deWit, H. (2000a). Acute effects of estradiol pretreatment on the response to d-amphetamine in women. Neuroendocrinology, 71, 51-59.

Justice, A.J.H. & deWit, H. (2000b). Acute effects of d-amphetamine during the early and late follicular phases of the menstrual cycle in women. Pharmacology, Biochemistry, and Behavior, 66, 509-515.

Justice, A.J.H. & deWit, H. (1999). Acute effects of d-amphetamine during the follicular and luteal phases of the menstrual cycle in women. Psychopharmacology, 145, 67-75.

Katz, R.J., Roth, K.A., Schmaltz, K., & Sible, M. (1980). Interaction of stress and morphine in the rat using a classical conditioning design. Behavioral and Neural Biology, 28, 366-371.

Keim, K.L. & Sigg, E.B. (1976). Physiological and biochemical concomitants of restraint stress in rats. Pharmacology, Biochemistry & Behavior, 4, 289-297.

King, G.R., & Ellinwood, E.H. (1997). Amphetamines and other stimulants. In: Substance abuse: A comprehensive textbook (3rd ed., Lowinson, J.H., Ruiz, P., Milman, R.B., & Langrod, J.G., eds.). New York: Williams & Wilkins.

Kirk, R.E. (1982). Experimental design: procedures for the behavioral sciences (2nd ed.). Pacific Grove, CA: Brooks/Cole Publishing Co.

Kirschbaum, C., Kudielka, B.M., Gabb, J., Schommer, N.C., & Hellhammer, D.H. (1999). Impact of gender, menstrual cycle phase, and oral contraceptives on the activity of the hypothalamus-pituitary-adrenal axis. Psychosomatic Medicine, 61, 154-162.

Kirschbaum, C., Wust, S., & Hellhammer, D. (1992). Consistent sex differences in cortisol responses to psychological stress. Psychosomal Medicine, 54, 648-657.

Klebaur, J.E. & Bardo, M.T. (1999). Individual differences in novelty seeking on the playground maze predict amphetamine conditioned place preference. Pharmacology, Biochemistry & Behavior, 63, 131-136.

Klenerová, V., Jurcovicová, J., Kaminský, O., Šída, P., Krejcí, I., Hlinák, Z. & Hynie, S. (2003). Combined restraint and cold stress in rats: effects on memory processing in passive avoidance task and on plasma levels of ACTH and corticosterone. Behavioural Brain Research, 142, 143-149.

Koob, G.F., & Nestler, E.J. (1997). The neurobiology of drug addiction. The Journal of Neuropsychiatry and Clinical Neuroscience, 9, 482-497.

Kosten, T.A., Gawin, F.H., Kosten, T.R., & Rounsaville, B.J. (1993). Gender differences in cocaine use and treatment response. Journal of Substance Abuse Treatment, 10, 63-66.

Laviola, G., Adriani, W., Morley-Fletcher, S., & Terranova, M.L. (2002). Peculiar response of adolescent mice to acute and chronic stress and to amphetamine: evidence of sex differences. Behavioural Brain Research, 130, 117-125.

Levine, D.G. (1974). "Needle freaks": Compulsive self-injection by drug users. American Journal of Psychiatry, 131 (4), 297-300.

Lindheim, S.R., Legro, R.S., Bernstein, L, Stanczyk, F.Z., Vijod, M.A., Presser, S.C., & Lobo, R.A. (1992). Behavioral stress responses in premenopausal and postmenopausal women and the effects of estrogen. American Journal of Obstetrics & Gynecology, 167 (6), 1831-1836.

Loscher, W., Wahnschaffe, U., Rundfeldt, C., Honack, D., & Hoppen, H. (1992). Regional Alterations in brain amino acids during the estrous cycle of the rat. Neurochemistry Research, 17, 973-977.

Lu, L., Xu, N.J., Ge, X., Yue, W., Su, W.J., Pei, G., & Ma, L. (2002). Reactivation of morphine conditioned place preference by drug priming: role of environmental cues and sensitization. Psychopharmacology, 159, 125-132.

Lukas, S.E., Sholar, M., Lundahl, L.H., Lamas, X., Kouri, E., Wines, J.D., Kragie, L., & Mendelson, J.H. (1996). Sex differences in plasma cocaine levels and subjective effects after acute cocaine administration in human volunteers. Psychopharmacology, 125, 346-354.

Lynch, M.R. (1991). Scopolamine enhances expression of an amphetamine-conditioned place preference. NeuroReport, 2, 715-718.

Lynch, W.J., & Carroll, M.E. (1999). Sex differences in the acquisition of intravenously self-administered cocaine and heroin in rats. Psychopharmacology, 144, 77-82.

Lynch, W.J., Roth, M.E., Mickleberg, J.L., & Carroll, M.E. (2001). Role of estrogen in the acquisition of intravenously self-administered cocaine in female rats. Pharmacology, Biochemistry and Behavior, 68, 641-646.

Lynch, W.J. & Carroll, M.E. (2000). Reinstatement of cocaine self-administration in rats: sex differences. Psychopharmacology, 148, 196-200.

Marinari, K.T., Leshner, A.I., & Doyle, M.P. (1976). Menstrual cycle status and adrenocortical reactivity to psychological stress. Psychoneuroendocrinology, 1, 213-218.

Markou, A., Weiss, F., Gold, L., Caine, S., Schulteis, G., & Koob, G. (1993). Animal models of drug craving. Psychopharmacology, 112, 163-182.

Marlatt, G.A., & Gordon, J.R. (1985). Relapse prevention: theoretical rationale and overview of the model In: Marlatt, G.A. & Gordon, J.R. (Eds). Relapse Prevention: Maintenance Strategies for Addictive Behaviors (pp. 1-70). New York: Guilford Press.

Matthews, K.A., Flory, J.D., Owens, J.F., Harris, K.F., & Berga, S.L. (2001). Influence of estrogen replacement therapy on cardiovascular responses to stress of healthy postmenopausal women. Psychophysiology, 38, 391-398.

McCormick, C.M., Robarts, D., Gleason, E., & Kelsey, J.E. (2004). Stress during adolescence enhances locomotor sensitization to nicotine in adulthood in female, but not male, rats. Hormones & Behavior, 46, 458-466.

McCusker, C.G. (2001). Cognitive biases and addiction: an evolution in theory and method. Addiction, 96, 47-56.

McKim, W.A. (2003). Drugs and behavior: an introduction to behavioral pharmacology, (5th ed.). Upper Saddle River, NJ: Prentice Hall.

McKinney, W.T., & Bunney, W.E. (1969). Animal models of depression. Archives of General Psychiatry, 21, 240-248.

Meyer, R. E. (1996). The disease called addiction: emerging evidence in a 200-year debate. The Lancet, 347, 162-166.

Mueller, D., & Stewart, J. (2000). Cocaine-induced conditioned place preference: reinstatement by priming injections of cocaine after extinction. Behavioural Brain Research, 115, 39-47.

Murison, R.C.C. (1983). Time course of plasma corticosterone under immoblisation stress in rats. IRCS Medical Science: Psychology and Psychiatry, 11, 20-21.

Oetting, E.G., & Donnermeyer, J.F. (1998). Primary socialization theory: the etiology of drug use and deviance. Substance Use & Misuse, 33 (4), 995-1026.

Ohtani, H., Nomoto, M., & Douchi, T. (2001). Chronic estrogen treatment replaces striatal Dopminergic function in ovariectomized rats. Brain Research, 900, 163-168.

Olds, J., & Milner, P. (1954). Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain. Journal of Comparative and Physiological Psychology 47, 419-427.

Oswald, L.M., Wong, D.F., McCaul, M., Zhou, Y., Kuwabara, H., Choi, L., Brasic, J., & Wand, G.S. (2005). Relationships among ventral striatal dopamine release, cortisol secretion, and subjective responses to amphetamine. Neuropsychopharmacology, 30, 821-832.

Pacchioni, A.M., Gioino, G. Assis, A., & Cancela, L.M. (2002). A single exposure to restraint stress induces behavioral and neurochemical sensitization to stimulating e ffects of amphetamine. Annals of the New York Academy of Sciences, 965, 233-246.

Papp, M., Muscat, R, & Wilner, P. (1993). Subsensitivity to rewarding and locomotor stimulant effects of a dopamine agonist following chronic mild stress. Psychopharmacology, 110, 152-158.

Peele, S. (1985). The Meaning of Addiction. Lexington, KY: D.C. Heath and Company.

Pelloux, Y., Costentin, J., & Duterte-Boucher, D. (2004). Differential effects of novelty exposure on place preference conditioning to amphetamine and its oral consumption. Psychopharmacology, 171, 277-285.

Peris, J., Decambre, N., Coleman-Hardee, M.L., & Simpkins, J.W. (1991). Estradiol enhances behavioral sensitization to cocaine and amphetamine-stimulated striatal [$^{3}$H]dopamine release. Brain Research, 566, 255-264.

Piazza, P.V., Deminiere, J.M., le Moal, M., & Simon, H. (1990). Stress- and pharmacologically-induced behavioral sensitization increases vulnerability to acquisition of amphetamine self-administration. Brain Research, 514, 22-26.

Piazza, P.V., Deminiere, J.M., le Moal, M., & Simon, H. (1989). Factors that predict Individual vulnerability to amphetamine self-administration. Science, 245, 1511-1513.

Picciotto, M.R. (1998). Common aspects of the action of nicotine and other drugs of abuse. Drug and Alcohol Dependence, 51, 165-172.

Pierre, P.J., & Vezina, P. (1997). Predisposition to self-administer amphetamine: the contribution of response to novelty and prior exposure to the drug. Psychopharmacology, 129, 277-284.

Pitman, D.L., Ottenweller, J.E., & Natelson, B.J. (1988). Plasma corticosterone levels during repeated presentation of two intensities of restraint stress: chronic stress and habituation. Physiology & Behavior, 43, 47-55.

Ranaldi, R., Pocock, D., Zereik, R., & Wise, R. (1999). Dopamine fluctuations in the nucleus accumbens druing maintenance, extinction, and reinstatement of intravenous d-amphetamine self-administration. The Journal of Neuroscience, 19 (10), 4102-4109.

Redgrave, P. (1978). Modulation of intracranial self-stimulation behaviour by local perfusions of dopamine, noradrenaline and serotonin within the caudate nucleus and nucleus accumbens. Brain Research, 155, 277-295.

Renner, M.J., Bennett, A.J., & White, J.C. (1992). Age and sex as factors influencing spontaneous exploration and object investigation by preadult rats (*Rattus norvegicus*). Journal of Comparative Psychology, 106 (3), 217-227.

Restle, F. (1975). Learning: animal behavior and human cognition. New York: McGraw-Hill.

Robbins, S.J., Ehrman, R.N., Childress, A.R., & O'Brien, C.P. (1999). Comparing levels of cocaine cue reactivity in male and female outpatients. Drug and Alcohol Dependence, 53, 223-230.

Robins, L.N., Helzer, J.E., & Davis, D.H. (1975). Narcotic use in Southeast Asia and afterward. Archives of General Psychiatry, 32, 955-961.

Robinson, T.E. & Berridge, K.C. (1993). The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Research Review, 18, 247-291.

Robinson, T.E., Becker, J.B., & Presty, S.K. (1982). Long-term facilitation of amphetamine-induced rotational behavior and striatal dopamine release produced by single exposure to amphetamine: sex differences. Brain Research, 253, 231-241.

Robinson, T.E., Becker, J.B., & Ramirez, V.D. (1980). Sex differences in amphetamine-elicited rotational behavior and the lateralization of striatal dopamine in rats. Brain Research Bulletin, 5, 539-545.

Rupprecht, R., Koch, M., Montkowski, A., Lancel, M., Faulhaber, J., Harting, J., & Spanagel, R. (1999). Assessment of neuroleptic-like properties of progesterone. Psychopharmacology, 143, 29-38.

Russo, S.J., Festa, G.D., Fabian, S.J., Gazi, F.M., Kraish, M., Jenab, S., & Quiñones-Jenab, V. (2003). Gonadal hormones differentially modulate cocaine-induced conditioned place preference in male and female rats. Neuroscience, 120, 523-533.

SAMHSA (Substance Abuse and Mental Health Services Administration), 2000. The National Household Survey on Drug Abuse (NHSDA).

Sanchez, C.J., & Sorg, B.A. (2001). Conditioned fear stimuli reinstate cocaine-induced conditioned place preference. Brain Research, 908, 86-92.

Satel, S.L., Price, L.H., & Palumbo, J.M. (1991a). Clinical phenomenonology and neurobiology of cocaine abstinence: A prospective inpatient study. American Journal of Psychiatry, 148, 1712-1716.

Savageau, M.M. & Beatty, W.M. (1981). Gonadectomy and sex differences in the behavioral responses to amphetamine and apomorphine of rats. Pharmacology, Biochemistry, & Behavior, 14, 17-21.

Schacter, S., & Singer, J.E. (1962). Cognitive, social and physiological determinants of emotional state. Psychological Review, 69, 379-399.

Schenk, S. & Partridge. (1999). Cocaine-seeking produced by experimenter-administered drug injections: dose-effect relationships in rats. Psychopharmacology, 147, 285-290.

Schneider, B.F., & Norton, S. (1979). Circadian and sex differences in hyperactivity produced by amphetamine in rats. Physiology & Behavior,22, 47-51.

Sell, S.L., Scalzitti, J.M., Thomas, M.L., & Cunningham, K.A. (2000). Influence of ovarian hormones and estrous cycle on behavioral response to cocaine in female rats. The Journal of Pharmacology and Experimental Therapeutics, 293 (3), 879-886.

Shaham, Y., Adamson, L.K., Grocki, S., & Corrigall, W.A. (1997). Reinstatement and spontaneous recovery of nicotine seeking in rats. Psychopharmacology, 130, 396-403.

Shansky, R.M., Glavis-Bloom, C., Lerman, D., McRae, P., Benson, C., Miller, K., Cosand, L., Horvath, T.L., & Arnsten, A.F.T. (2004). Estrogen mediates sex differences in stress-induced prefrontal cortex dysfunction. Molecular Psychiatry, 9, 531-538.

Siegel, S., & Allan, L.G. (1998). Learning and homeostasis: Drug addiction and the McCollough Effect. Psychological Bulletin, 124 (2), 230-239.

Sinha, R., & Rounsaville, B.J. (2002). Sex differences in depressed substance abusers. Journal of Clinical Psychiatry, 63 (7), 616-627.

Sinha, R. (2001). How does stress increase risk of drug abuse and relapse? Psychopharmacology, 158, 343-359.

Sinha, R., Catapano, D., & O'Malley, S. (1999). Stress-induced craving and stress response in cocaine dependent individuals. Psychopharmacology, 142, 343-351.

Sinha, R., Fuse, T., Aubin, L.-R., & O'Malley, S.S. (2000). Psychological stress, drug-related cues and cocaine craving. Psychopharmacology, 152, 140-148.

Söderpalm, A., Nikolayev, L., & de Wit, H. (2003). Effects of stress on responses to methamphetamine in humans. Psychopharmacology, 170, 188-199.

Sofuoglu, M., Babb, D.A., & Hatsukami, D.K. (2002). Effects of progesterone treatment on smoked cocaine response in women. Pharmacology, Biochemistry, & Behavior, 72, 431-435.

Sofuogul, M, Mitchell, E., & Koste, T.R. (2004). Effects of progesterone treatment on cocaine responses in male and female cocaine users. Pharmacology, Biochemistry, & Behavior, 78, 699-705.

Spealman, R.D., & Goldberg, S.R. (1978). Drug self-administration by laboratory animals: control by schedules of reinforcement. Annual Review of Pharmacology and Toxicology (18), 313-339.

Stewart, J., de Wit, H., & Eikelboom, R. (1984). Role of unconditioned and conditioned drug effects in the self-administration of opiates and stimulants. Psychological Review, 91, 251-268.

Swerdlow, N.R., Gilbert, D., & Koob, G.F. (1989). Conditioned drug effects on spatial preference: critical evaluation. In: Boulton, A.A., Baker, G.B., & Greenshaw, A.J. (eds.) Neuromethods: Psychopharmacology. Clifton, New Jersey: Humana Press.

Takahashi, R.N., Singer, G., & Oei, T.P.S. (1978). Schedule induced self-injection of d-amphetmaine by naïve animals. Pharmacology, Biochemistry, & Behavior, 9, 857-861.

Taylor, G.T., Mardgett, M., Farr, S., Womack, S., Komitowski, D., & Weiss, J. (1993). Steroidal interactions in the ageing endocrine system: absence of suppression and pathology in reproductive systems of old males from a mixed-sex socially stressful rat colony. Journal of Endocrinology, 137, 115-122.

Taylor, G.T., Weiss, J., & Rupich, R. (1987). Male rat behavior, endocrinology, and reproductive physiology in a mixed-sex, socially stressful colony. Physiology & Behavior, 39, 429-433.

Tinnikov, A.A. (1999). Responses of serum corticosterone and corticosteroid-binding globulin to acute and prolonged stress in the rat. Endocrine, 11 (2), 145-150.

U.S. Code: Title 21, Chapter 13, Subchapter I, Part B. Schedules of controlled substances.

Van Etten, M.L., Neumark, Y.D., & Anthony, J.C. (1999). Male-female differences in the earliest stages of drug involvement. Addiction, 94 (9), 1413-1419.

Wade, G.N., & Zucker, I. (1970). Development of hormonal control over food intake and Body weight in female rats. Journal of Comparative and Physiological Psychology, 70 (2), 213-220.

Walters, G.D. (1999). The addiction concept. Boston, MA: Allyn & Bacon.

Wang, B., Luo, F., Zhang, W.T., & Han, J.S. (2000). Stress or drug priming induces reinstatement of extinguished conditioned place preference. NeuroReport, 11 (12), 2781-2784.

Ward, K.D., Klesges, R.C., Zbikowski, S.M., Bliss, R.E., & Garvey, A.J. (1997). Gender differences in the outcome of an unaided smoking cessation attempt. Addictive Behaviors, 22 (4), 521-533.

Weddington, W.W., Brown, B.S., & Haertzen, C.A. (1990). Changes in mood, craving, and sleep during short-term abstinence reported by male cocaine addicts. A controlled, residential study. Archives of General Psychiatry, 47, 861-868.

Weiss, R.D., Martinez-Raga, J., Griffin, M.L., Greenfield, S.F., Hufford, C. (1997). Gender differences in cocaine dependent patients: a 6 month follow-up study. Drug and Alcohol Dependence, 44, 35-40.

West, C.H.K. & Michael, R.P. (1988). Mild stress influences sex differences in exploratory and amphetamine-enhanced activity in rats. Behavioural Brain Research, 30, 95-97.

West, C.H.K. & Michael, R.P. (1986). Time-dependent modulation by estrogen of amphetamine-induced hyperactivity in male rats. Pharmacology, Biochemistry, & Behavior, 25, 919-923.

White, T.L., Justice, A.J.H., & de Wit, H. (2002). Differential subjective effects of d-amphetamine by gender, hormone levels and menstrual cycle phase. Pharmacology, Biochemistry, and Behavior, 73, 729-741.

White, S. & Uphouse, L. (2004). Estrogen and progesterone dose-dependently reduce disruptive effects of restraint stress on lordosis behavior. Hormones & Behavior, 45, 201-208.

Wikler, A. (1948). Recent progress in research on the neurophysiologic basis of morphine addiction, American Journal of Psychiatry, 105, 329-338.

Wikler, A. (1973). Dynamics of drug dependence: implications of a conditioning theory for research and treatment. Archives of General Psychiatry, 28, 611-616.

Will, M.J., Der-Avakian, A., Pepin, J.L., Durkan, B.T., Watkins, L.R., & Maier, S.F. (2002). Modulation of the locomotor properties of morphine and amphetamine by uncontrollable stress. Pharmacology, Biochemistry, & Behavior, 71, 345-351.

Wise, R.A. (1996). Addictive drugs and brain stimulation reward. Annual Review of Neuroscience, 19, 319-340.

Woolverton, W.L. & Balster, R.L. (1981). Effects of antipsychotic compounds in rhesus monkeys given a choice between cocaine and food. Drug and Alcohol Dependence, 8, 69-78.

World Health Organization (1992): International Statistical Classification of Diseases and Related Health Problems ($10^{th}$ ed.). Geneva, Switzerland, World Health Organization.

The Writing Group for the PEPI Trial. (1995). Effects of estrogen or estrogen/progestin regimens on heart disease risk factors in postmenopausal women: The postmenopausal estrogen/progestin interventions (PEPI) Trial. JAMA, 273, 199-208.

Xio, L. & Becker, J.B. (1998). Effects of estrogen agonists on amphetamine-stimulated striatal dopamine release. Synapse, 29, 379-391.

Young, E.A., Altemus, M., Parkison, V., & Shastry, S. (2001). Effects of estrogen antagonists and agonists on the ACTH response to restraint stress in female rats. Neuropsychopharmacology, 25 (6), 881-891.

Zuckerman, M. (1979). Sensation seeking: Beyond the optimal level of arousal. Clifton, NJ: Lawrence Erlbaum Associates.