2007

Does HIV-1 Affect Sensitivity to Addictive drugs? Methamphetamine-Induced Conditioned Place Preference in HIV-1 Transgenic Rats

Brent Foster Costleigh
Seton Hall University

Does HIV-1 affect sensitivity to addictive drugs? Methamphetamine-Induced Conditioned Place Preference in HIV-1 Transgenic Rats

By

Brent Foster Costleigh

A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science in Experimental Psychology with a concentration in Behavioral Neuroscience

Department of Psychology

Seton Hall University

August, 2007

Approved By:

Dr. Michael Vigorito, Ph.D. (Mentor, Committee Member & Graduate Director)

Dr. Kelly M. Goedert, Ph.D. (Committee Member)

Dr. Amy J. Silvestri, Ph.D. (Committee Member)

Dedication

This research is dedicated to the loving memory of Dale William Costleigh and Kevin William Costleigh.

Acknowledgements

I would like to express my sincerest gratitude and appreciation to the entire faculty of the Department of Psychology at Seton Hall University. I am grateful for all the knowledge you have imparted upon me throughout my academic career.

In particular, I would like to acknowledge the following people:

Dr. Michael Vigorito, my mentor and advisor during both my undergraduate and graduate studies, for he has been the most influential individual throughout all my academic and educational endeavors. I would like to thank him for all the wisdom, encouragement, and patience he has shown during the conduction and completion of this thesis; in additional to all of the numerous contributions (writing and data analysis) to this research, which have aided immeasurably to the culmination of this thesis.

Dr Amy Silvestri and Dr. Kelly Goedert, for their willingness to serve on my thesis committee and for all of the helpful comments and suggestions made in review of this thesis.

Dr. Sulie Chang, for her financial support, via the use of animals and drug compounds, without which, this research would not have been possible.

David Root for his experimental training and assistance in running pilot research in preparation for the research conducted for this thesis.

Abigail LaShomb and Shilpa Swarup for their assistance in the conduction of experimental procedures related to this research.

Abigail LaShomb, for her assistance in the scoring of raw data for this research.

Saroj Ailawadi, at Brookdale Community College's Bankier Library, for her continued assistance in locating and obtaining numerous articles, which aided immeasurably towards the completion of this thesis.

My fellow graduate students, David Root, Abilgail LaShomb, Paul Ladny, and Gregory Cvasa, for their mutual support and guidance throughout the completion of this graduate program. You have all greatly added to my knowledge and to my educational experience at Seton Hall; I can only hope that I have done the same for you. My best to all of you in your future endeavors.

My mother, Doris, brothers, Dale Jr., Jason, and Christopher, and the Farber's, Susan and Samuel for all their love, encouragement, and advice throughout the years.

Lastly, I am grateful for all the love and continued support of my fiancée Lindsay J. Farber. You have been a force of stability and guidance throughout my graduate studies and life. I love you, always!

Table of Contents

List of Figures

List of Tables

Abstract

The illicit use of methamphetamine (METH) among the gay and bisexual population has been correlated with the spread of the human immunodeficiency virus. The prevalence of METH use within this community has lead Halakitis and colleagues (2001) to characterize this phenomenon as a "double epidemic". Chang and Vigorito (2006) have recently posited that the HIV-1 infection may leave infected individuals more susceptible to the addictive properties of drugs of abuse, such as methamphetamine. The current study sought to test this hypothesis, using a recently created non-infectious animal model of HIV-1. An observation of greater sensitivity to METH-induced conditioned place preference (CPP) and behavioral sensitization (BS) in HIV-1 transgenic rats (HIV-1 Tg), relative to transgenic (FTg) and normal controls (F) would support the hypothesis. METH (3.25 mg/kg)-induced CPP failed to develop in all groups. Interestingly, a priming injection of METH (1.0 mg/kg) given just prior to a test for CPP induced the expression of drug-seeking behavior within the drug-paired compartment. Locomotor behavior increased in a dose dependant manner in response to two challenge doses of METH (0.5 and 1.0 mg/kg). However, no between group differences were observed in either behavioral measure suggesting no between group differences in BS. Consistent with previous reports, the HIV infection may have increased the animals' susceptibility to the toxic effects of the drug as evidenced by the HIV-1 Tg group experiencing a significantly greater anorexic effect compared to normal control. It is concluded that although the hypothesis on increased susceptibility to the addictive properties of drugs in HIV-1 cannot be completed rejected the results are not promising. Future research may be more productive examining how drugs of abuse interact with the HIV-1 infection to place the patient at greater risk for neurocognitive deficits.

Does HIV-1 affect sensitivity to addictive drugs? Methamphetamine-Induced Conditioned Place Preference in HIV-1 Transgenic Rats

The prevalence of methamphetamine (METH) use among the gay and bisexual population has risen dramatically throughout the last decade (Reback & Ditman, 1997). Its use has been correlated with the spread of the human immunodeficiency virus (HIV) (Reback & Ditman, 1997). The exact nature and characterization of this "double epidemic" requires further investigation (Halakitis, Parsons, & Stirrat, 2001).

Between 1981 and 2004, the Center for Disease Control, (CDC) reported 522,723 deaths that were directly attributed to acquired immunodeficiency syndrome (AIDS; Glynn, M.K., & Rhodes, P., 2005). At present, it is estimated that over 1,000,000 individuals are living with HIV in the United States (CDC, 2005). Although the advent of antiretroviral therapies (ARV), such as Highly Active Antiretroviral Therapy (HAART) have been very successful in slowing the progression of the HIV infection to AIDS and AIDS to death (CDC, 2005), the rate of new diagnoses remains alarmingly high. Based on thirty-five areas of reporting, the CDC (2005) estimates that 157,468 persons were diagnosed with HIV/AIDS between 2001 and 2004. The most frequently identified route to HIV infection was male-to-male sexual contact (men who have sex with men [MSM]), which represented 44% of all cases reported during that time. In contrast, heterosexual contact attributed for 34%, injection drug use (IDU) 17%, MSM/IDU 4%, and 0.6% being attributed to perinatal causes (CDC, 2005). All routes of infection have steadily declined between the years of reporting, save the MSM subgroup (CDC, 2005); MSM as a route of infection has remained stable during that time period (Morbidity and Mortality Weekly Report, 2006, June 2).

Research on the epidemiology of HIV has routinely indicated a strong relationship between HIV infection and the use of abused drugs (CDC, 1999; 2005). For instance, commonly abused psychostimulant drugs, such as the amphetamines (including methamphetamine) and cocaine have been associated with non-adherence to HAART therapy (Peretti-Wattel, Spire, Lert, Obadia, & VESPA Group, 2006) and an increase in the frequency of sexual risk taking behaviors (Hayaki, Anderson, & Stein, 2006; Semple, Patterson, & Grant, 2004a) and impulsivity (Semple, Patterson, & Grant, 2004b). This increase in risk taking behavior and impulsivity meets with increased gravity when the drug user carries an infectious disease (i.e. seropositive for HIV, among other sexual transmitted diseases – STD's) and engages in unsafe (high risk) sexual behaviors such as unprotected sex (Robinson & Rempel, 2006; Semple, et al., 2004b). High risk behaviors are especially common among MSM who engage in illicit methamphetamine use (Frosch, Shoptaw, Huber, Rawson, & Ling, 1996; Reback, Kamien, & Amass, 2007; Robinson & Rempel, 2006; Shoptaw, Reback, Pack, Yang, Rotherman-Fuller, Larkins, et al., 2005; Winters, Remafedi, & Chan, 1996).

Comorbidity of Methamphetamine Abuse and HIV Seroposivity

Methamphetamine is a highly addictive, potent central nervous system psychostimulant (Carlson, 2007; Meyer & Quenzer, 2005) and has frequently been characterized as a 'club drug'. Common routes of METH administration in human populations include intravenous injection, inhalation, and oral consumption. Psychostimulant drugs, such as METH have been shown to produce an increase sexual desire (libido), reduce anxiety, enhance physical sensations and perceptions, and induce behavioral disinhibition, which can result in an increase in the duration of sexual contact (Carlson, 2007; Frosch, et al., 1996; Halkitis, et al., 2001; Meyer & Quenzer,

2005; Kolb & Whishaw, 2003; Robinson & Becker, 1987). As reported by Frosch and colleagues (1996), "the physiological effects of amphetamines result in delayed ejaculation resulting in prolonged intercourse as well as decreased bodily secretions that facilitate sexual acts, resulting in 'raw genitalia'"(pg. 484); these outcomes increase the probability of HIV transmission. Furthermore, it has been reported that intravenous METH users are more impulsive than their non-injecting (e.g. smoking or snorting) counterparts leading to increased HIV transmission risk behaviors associated with the drug (i.e. needle sharing; Semple, et al., 2004b); however, non-injecting METH users may be more prone to transmitting the HIV infection as a result of unprotected sexual contact due to a perceived lack of vulnerability to HIV infection, because they do not administer METH intravenously (Semple, et al., 2004a).

As such, METH use has been strongly correlated with the commission of high risk sexual behaviors (Hayaki, et al., 2006; Semple, et al., 2004), this relationship has been shown to be especially common among the gay and bisexual (i.e. MSM) HIV seropositive (HIV+) community (Frosch, et al., 1996; Semple, et al., 2002a; 2004). For instance, Semple and colleagues (2002b) recently assessed sexual behaviors and found that HIV+ MSM's were more likely to have sex without a condom, seek out risky sexual partners, and more likely to be the insertive partner if they abused METH. Moreover, within the MSM community the prevalence of METH use has been directly attributable to its psychostimulant effects, that is, as an enhancer of sexual experiences as a strategy for the self-management of HIV-related symptoms (Robinson & Rempel, 2006; Semple, et al., 2002b).

Sexual Enhancement

Use of METH for the purposes of sexual enhancement is extremely common among the MSM population, especially those that are HIV+. In one study on the motivational factors related to METH use in HIV+ MSM, all subjects identified increased sexual pleasure as primary reason for their METH use (Semple et al., 2002b). In the same study the authors explicitly identified factors relating to METH's use in sexual enhancement. These factors included: making sex more pleasurable, encouraging sexual experimentation, and reducing anxiety concerning engaging in casual sex. Features of sexual enhancement were characterized by increased sensation, removal of pain related to anal sex, and enhancing sexual performance. Features associated with the encouragement of sexual experimentation included the quality of sexual experience, which was characterized as being more "aggressive, hard, rough, animalistic, wild, and manly" (pg. 152). This feature related to individuals also reporting feeling bolder and more adventurous during their sexual encounters, and willingness to engage in casual sex - a factor that may be attributed to METH's anxiolytic effects.

HIV-Related Symptom Self-Management

In the same study, Semple, et al. (2002b) also identified motivating factors of METH use associated with HIV-related symptom self-management such as coping with an HIV+ diagnosis, providing a temporary escape from the HIV+ diagnosis, subduing the physical symptoms related to HIV, coping with the "specter of death", as well as to aide in the management of negative self perceptions associated with being HIV+. In this study one individual characterized his use of METH as an escape tool: "Everywhere you go, you're reminded of HIV. Can I have one day when I'm not reminded that I'm HIV+? Meth gives me that" (p. 153). Moreover, Semple and

colleagues (2002b) found that thoughts of helplessness prevail in the HIV+ MSM population. As one subject reported, these depressive thoughts directly relate to continued and habitual drug use.

> When I found out that I was HIV+, I doubled my meth use. You go off and party blindly. Every day could be your last. Although it affects your health, you don't care because you're on your way out anyway (p. 153).

Overall the majority of subjects within this study reported using METH to alleviate HIV-associated symptoms, especially those relating to protracted fatigue. METH was reported to compensate for this fatigue by providing energy that allowed them to function better in their day-to-day lives.

Subjective self-reports are emblematic of HIV-associated symptom self-management techniques within the MSM population and are paralleled by those documented in a more recent study conducted by Robinson and Rempel (2006). For instance one subject reported using METH to relieve HIV-associated pain in his fingers: "I play the piano, and the pain in my fingers is so severe sometimes I can't stand it. The meth takes the pain away (p. 11)." Furthermore, several participants in this study additionally reported that an advantageous by-product of the drugs use was METH's property as an anti-(HIV-associated)-diarrheic.

As these studies clearly show, the subjective (self-reported) motivations for the use of METH within this population directly relates to their habitual drug use. Recent estimates posit that between 50 and 65% of individuals within the HIV+ MSM population habitually use METH (Shoptaw, Peck, Reback, & Rotheram-Fuller, 2003; Robinson & Rempel, 2006). Additionally, METH use has been directly linked with a decrease in the effectiveness of ARV's, which

consequentially increases viral load (Ellis, Childers, Lazzaretto, Latendre, Grant, & HIV Neurobehavioral Research Center Group, 2003), Thus, it is clear that this "double epidemic" needs to be further characterized.

Research has consistently shown there are marked discrepancies between the self-reported uses of METH within this population and the actual reasons for their use (Reinhard, Hinkin, Barclay, Levine, Marion, Castellon, et al., 2007); such inconsistencies are further correlated to poor adherence of ARV therapy (Levine, Hinkin, Marion, Keuning, Castellon, Lam, et al., 2006). As such, such self reports may not be relied upon and the actual reasons for their drug use must be tested, experimentally if possible. The use of animal in the study of addiction has permitted an analysis of such addictive behaviors devoid of such discrepancies. There are several neurobehavioral models of addiction and addictive behavior that have been studied in animals, which may help to explicate further, the relationship between the habitual use of METH and the HIV virus.

Neurobehavioral Models of Addiction

Addiction is characterized as a complex behavioral pattern of compulsive drug craving, seeking and use (Meyer & Quenzer, 2005; National Institute of Drug Abuse, 2006). Addictive behaviors persist regardless of severity of consequences that follow from these behaviors (NIDA, 2006). As stated by Robinson and Berridge (1993), there are three key features of addictive behavior that any model of addiction must address and be capable of explaining: 1. drug craving (intense 'wanting' of drugs), 2. persistence of drug craving or reinstatement after a period of abstinence, and 3. continued craving despite a reduction in the pleasurable effects of the drug (tolerance). Several neurobehavioral models of addiction have been postulated in an attempt to

explain the development of drug use and the maintenance of drug abuse in addiction. Most of these models have met with varied success when addressing the criterion put forth by Robinson and Berridge. The physical dependence, positive reinforcement, and incentive-sensitization models of addiction attempt to explain addictive behavior and to define neural substrates that subserve these behaviors. In this section, these models and their respective neural underpinnings will be articulated.

The Physical Dependence Model

According to the physical dependence model, individuals become physically dependant to the pharmacological effect of the drug after repeated or habitual use. Typically this dependence occurs via the development of drug tolerance. Tolerance is a weakened response to a particular drug after being repeatedly exposed to that drug. Meaning that once the drug has been repeatedly administered (or in some cases after a single administration – as in acute tolerance), it will require ever-increasing amounts of that same drug (or in some instances drugs of the same classification) to produce equal biological effects as compared to the amount that initially caused the desired effect. Tolerance can develop via biological routes such as changes in metabolic and/or pharmacodynamic factors (Robinson & Becker, 1986; Meyer & Quenzer, 2005). Metabolic tolerance results from an increased rate of metabolism of the drug. This increased rate of metabolism leads to a reduction in the amount of drugs available to target tissues. Pharmacodynamic tolerance results from receptor down-regulation; this is when the overall number of receptors for a specific drug agent decreases due to repeated activation. Thus, with fewer receptors available for the drug to bind, less of an effect is produced with the same amount of the drug (Carlson, 2007; Meyer & Quenzer, 2005; Kolb & Whishaw, 2003; Robinson &

Becker, 1986). Another form of tolerance is behavioral in nature. Behavioral tolerance develops via the acquisition of Pavlovian associations. After repeated administration of a drug in a specific environment or context, tolerance to the drug in that context develops, though outside this context the drug maintains its normal effectiveness (Carlson, 2007; Meyer & Quenzer, 2005; Kolb & Whishaw, 2003). In this form of tolerance, the physiological effect of the drug becomes associated with the context via Pavlovian associations (Seigel, 1985). Since the drug is predicted within this context, the body counteracts the effects of the drug resulting in a reduced effectiveness (tolerance); outside of this context the drug is not anticipated and hence its effects are not counteracted – no tolerance has been formed. Thus the physical dependence model is characterized by both physiological and behavioral correlates.

If the drug abuser attempts to refrain from using the drug she/he will experience highly aversive withdrawal symptoms. These withdrawal symptoms may also be referred to as abstinence syndrome. The experience of withdrawal symptoms is so unpleasant that the drug abuser breaks the period of abstinence to alleviate them. Thus, the drug abuser relapses – again compulsively taking the drug. Physical dependence creates a "cycle" in which the drug abuser may repeatedly attempt to abstain only to again start using to remove the withdrawal symptoms numerous times. Thus from a behavioral learning stand point, the removal of withdrawal symptoms (via the resumption of drug use) serves as a powerful negative reinforcer for the continued use of the drug. Furthermore, even if the drug abuser remains abstinent for a considerable period of time, simple contact with stimuli that are associated with the drug taking behavior (conditioned stimuli; e.g. needles) may elicit the withdrawal symptoms leading to resumption of the drugs usage (Carlson, 2007; Meyer & Quenzer, 2005; Kolb & Whishaw, 2003; Robinson & Berridge, 1993; Wise & Bozarth, 1987).

There are a number of problems or limitations to the account of drug abuse and addiction that the physical dependence model affords. Firstly some drugs of abuse such as METH fail to induce strong physical dependence; therefore, it fails to generalize to other typical drugs of abuse (Wise & Bozarth, 1987). Secondly, this model offers no conceptualization to account for initial drug use. Thirdly, this model cannot explain why when drug addicts have been completely detoxified and all withdrawal symptoms subside, there may still be drug relapse. Hence, it does not identify any motivational factors that may lead to a reinstatement of drug use (Carlson, 2007; Meyer & Quenzer, 2005; Robinson & Berridge, 1993; Wise & Bozarth, 1987). Lastly, it fails to address the fact that psychostimulants such as methamphetamine induces sensitization rather than tolerance. Sensitization is the reverse of tolerance in that with repeated use of the drug less and less amounts of the drug are required to produce the same effects (Meyer & Quenzer, 2005). Stated in another way, sensitization results in an increase in the drugs' effect with repeated administration (Robinson & Berridge, 2003). For instance, studies have consistently shown that animals treated with psychostimulants such as METH display behavioral sensitization, which is defined as increased arousal, attention, and motor behavior such as locomotion, exploration and approach in response to a lesser dose/concentration of the dug (Antoniou & Kafetzopoulos, 1991; Robinson & Becker, 1986; Robinson & Berride, 2003). Furthermore at higher dosages these effects may lead to intensely repetitive stereotyped behaviors such as intense grooming (Robinson & Becker, 1986; Wise & Bozarth, 1987). As was with tolerance, sensitization may also be learned via non-associative and associative means (Kolb & Whishaw, 2003; Robinson & Becker, 1986; Robinson & Berridge, 1993; 2000, 2001, 2003). For example, Anagnostarus and Robinson, (1996) have shown that psychostimulants such as amphetamine produce robust expression of behavioral sensitization effects in a context specific manner.

In response to the first two issues (i.e. failure to characterize the initial development of the drug dependency and failure of drugs such as METH to induce a strong physical dependence), it has been suggested that drugs such as METH may act as negative reinforcers by alleviating psychological distress rather than the physical distress that usually characterizes physical dependence (Wise & Bozarth, 1987) and may have been used initially to self-medicate preexisting medical conditions (e.g. pain, anxiety, depression) that existed independent of the symptoms that emerged after prolonged drug use (Robinson & Berridge, 1993; 2000). The self-reported uses of METH within the HIV+ MSM population would seem to support this view; however, these additions to the model still fail to address the reinstatement of drug use after long periods of abstinence. Additionally, it fails to completely characterize the initial development of drug use and dependency and the induction of psychostimulants' sensitizing effects (Meyer & Quenzer, 2005; Robinson & Berridge, 1993; 2000; Wise & Bozarth, 1987). Thus, as stated by Wise and Bozarth (1987), these "facts are inconsistent with the view that physical dependence is either a necessary or a sufficient condition for addiction (p. 470)."

The Positive Reinforcement Model

In contrast to the physical dependence model, the positive reinforcement model focuses on the rewarding or positively reinforcing properties of drugs of abuse. Positive reinforcers increase drug taking behavior because of the physiological states they induce rather than those they alleviate (Wise and Bozarth, 1987). Typically, a drug of abuse is taken to produce some hedonic effect, such as feelings of well-being or elevated mood. These euphoric feelings serve to positively reinforce the behavior which immediately preceded them – drug self-administration. Thus, the subjective sense of euphoria serves to reinforce additional drug usage. When the drug

user abstains from the drug she/he may experience an overwhelming urge (craving) to re-experience the euphoric effects induced by the drug. As such, these cravings ultimately lead to continued drug use and, after a period of abstinence, drug relapse (Meyer & Quenzer, 2005; Robinson & Berridge, 1993; 2000). The reinforcing euphoric effects produced by a drug of abuse stems from its complex pattern of pharmacokinetic interactions within the brain.

It has been clearly shown that the reinforcing properties of nearly all addictive drugs, including the psychostimulants, have the mesolimbic dopamine system as a common neural pathway (Pierce & Kumarensan, 2006). Dopaminergic neurons within this pathway originate from the ventral tegmental area (VTA) and innervate the nucleus accumbens (NAc), amygdala, hippocampus, medial prefrontal cortex (mPFC) and ventral pallidum. Changes within dopaminergic neurons modulate the stream of information throughout these interconnected brain areas, which compromise the mesolimbic pathway (Berridge & Robinson, 1998; Carlson, 2007; Kauer, 2004; Kelley & Berridge, 2002; Kolb & Whishaw, 2003; Meyer & Quenzer, 2005; Pierce & Kumarensan, 2006). The NAc in particular seems to be a significant dopaminergic hub within this pathway (Pierce & Kumarensan, 2006). Practically all addictive drugs initiate dopamine (DA) release within the NAc (Berridge & Robinson, 1998; Carlson, 2007; Kauer, 2004; Kelley & Berridge, 2002; Kolb & Whishaw, 2003). DA appears to be released within the NAc in response to all primary appetitive reinforcers (e.g. food & sex). As such, this fact has historically led to an improper characterization of DA as a "pleasure" neurotransmitter and the NAc as a pleasure center. DA release in the NAc, however, is not limited to pleasurable appetitive stimuli (Berridge, 2003; Berridge & Robinson, 1998; 2003; Kelley & Berridge, 2002). For example, Giorgi and colleagues (2003), showed increased DA release within the shell compartment of the NAc in response to a mild aversive stimulus (tail pinch), which correlated with an increased

expression of fear-related behaviors (freezing and self-grooming). Thus, it appears that DA release is associated with *emotionally salient stimuli*, whether it be appetitive or aversive.

As with the physical dependence model, the reinforcement model too has some problems and limitations that bar it from effectively explaining drug use and addiction. For instance, it fails to account for effects such as drug-induced tolerance and sensitization. The failure to account for these effects is exemplified in the observation that with continuous drug use, the abusers' cravings increase as the drug effect is reduced. Thus, the magnitude of the drug high does not correlate with the magnitude of drug craving. It also fails to explain why the strong negative consequences of drug use and addiction (e.g. loss of job, burden on limited financial resources) do not counteract the positive reinforcing effects of the hedonic valence (Meyer & Quenzer, 2005). Though, it is hypothesized that this failure is due to the significantly delayed temporal contingency between taking the drug and the negative consequences, as opposed to the immediate temporal contingency associated with the positive effects of the drug (Dennett, 2003). Additionally, this model cannot directly account for why some drug users are more sensitive to the reinforcing effects of the drug and become addicted, while others are not (Carlson, 2007; Meyer & Quenzer, 2005; Robinson & Berridge, 1993; 2000). Although, recently researchers have theorized that genetic differences may place an individual at greater risk for addition (Kalivas, 2003); such differences may result in alterations in dopaminergic functioning (Hanania, Gulley, Salaz, Larson, & Zahniser, 2004).

As the self-reports of the HIV+ MSM's clearly indicate, a major (at least initial) impetus for METH usage is for its hedonic effects. As one HIV+ MSM stated, "Meth makes you feel like puberty. Every touch is enhanced. On meth, orgasms are over the top. It's increased sensation on meth. Even a kiss is amazing. Everything tingles" (Semple, et al 2002b, p. 152). Thus according

to the positive reinforcement model the pleasurable effects produced by METH serve to reinforce continued usage within this population. However, from a learning theory point of view, positive reinforcement is simply a description of the behavioral effects and not an explanation of those effects. That is simply stating that addictive drugs serve as positive reinforcers fails to explain the phenomenon of addiction, it simply redefines it (Wise & Bozarth, 1987). As stated by Wise and Bozarth (1987), "A theory of addiction based on the concept of reinforcement would have to identify actions of drugs that are *operationally independent* of self-administration habits in order to offer insight as to *why* drugs are addictive" (p. 473). Thus reinforcement based models of addiction have proved woefully inadequate in their attempts to explain the entire gamut of addictive behaviors and addiction. Any effective model of addiction should be able to explain the entire spectrum of behavioral effects that have been identified in addiction (Wise & Bozarth, 1987; Robinson & Berridge, 1993; 2003).

The Incentive-Sensitization Model

Addressing the limitations of the physical dependency and positive reinforcement models, Robinson and Berridge (1993; 2000; 2001; 2003) put forth a neurobehavioral model of addiction that directly posits an explanation as to '*why* drugs are addictive'. As such, their incentive-sensitization model addresses the three aforementioned criteria that an effective model of addictive behavior must address: craving, reinstatement, and the disparity between a drugs' hedonic value and its craving. This model posits that addiction develops in three stages. The first stage is similar to the positive reinforcement theory, in that pleasure is activated as a consequence of taking the drug – a hedonic outcome (i.e. they like the drug). In the second stage, pleasure becomes associated with drug related stimuli as a result of Pavlovian conditioning,

which may include objects, places, acts, and events. In the third stage incentive salience is attributed to those cues that have been associated with drug use. This attribution of incentive salience results in increased craving (i.e. a want) for the drug (Berridge, 2004; Robinson & Berridge, 1993, 2000, 2001, 2003). Thus, the mere exposure to drug related cues increases the drug users craving for the drug; though the reason for the drug craving is oftentimes not consciously accessible to the addict as the operation of the mental representations are conceived to be under implicit behavioral control (Robinson & Berridge, 2003).

A key feature of the incentive-sensitization model that distinguishes it from all other models of addiction is the distinction it posits between drug 'liking' (hedonia) and drug 'wanting' (craving). Drug 'liking' is the product of positive affective reactions (i.e. some sensory pleasurable, euphoric or hedonic effect that a drug produces) and is the purpose for which the drug is initially taken (Berridge, 2003; Robinson & Berridge, 2000; 2001). As Berridge (2003) shows, 'liking' and positive affective reactions involve a complex subcortical network, which includes the nucleus accumbens shell, ventral pallidum, and brainstem, and is largely due to the activity of opioid neurons. Furthermore, this 'liking' network may induce the conscious experience of sensory pleasure via connections with other brain systems involved in explicit cognitive representations. However, the production of positive affective reactions does not explain why drug taking persists (i.e. drug craving), especially in instances when its hedonic impact is reduced. As such, it follows that 'liking', the positive affective reaction produced by a drug, is not equal to 'wanting' or craving a drug. 'Wanting' by itself is *not* a sensory pleasurable event and does *not* produce a hedonic state within an organism, nor does it increase the probability of positive affective reactions to the hedonic effect produced by the drug (Berridge & Robinson, 1998; 2003; Berridge, 2003; 2004). 'Wanting' or incentive salience is the

motivational incentive *valence* for the drugs' hedonic effect and it is by itself "essentially nonhedonic in nature" (Berridge, 2003, p. 115). As Robinson and Berridge (1993; 2000; 2003) assert, incentive salience is a process *of* reward, which serves to make the representations associated with the drug more attractive. As such, incentive salience is principally attributed to conditioned stimuli associated with the drug and reward cues. Reward cues and/or conditioned stimuli initiate 'cue triggered' 'wanting' for the associated reward. "Wanting' is further dissociable from 'liking' in the cortical pathway that subserves its mechanism of action. The 'wanting' or incentive salience system, exclusively activates the mesolimbic dopaminergic pathway, which as mentioned previously includes the dopamine neurons in the VTA that project to the NAc of the basal ganglia to the limbic system (expressly the hippocampus and frontal cortex; Berridge, 2003; Kolb & Whishaw, 2003; Robinson & Berridge, 1993; 2000; 2001; 2003). In summary, 'liking' and 'wanting' are dissociable processes which are governed by two different neural pathways and neurotransmitter systems. As a process 'liking' is purely hedonic while 'wanting' concerns the incentive salience of stimuli associated with the drug, which ultimately increases the motivational quality of the drug.

According to the incentive-sensitization model, what makes drugs *addictive* is their ability to permanently change or alter the attribution of incentive salience - the drugs incentive motivational quality (i.e. how much the drug is 'wanted'; Robinson & Berridge, 1993). This aspect of the model is the fundamental difference that differentiates it from both the positive reinforcement and the physical dependence models and is conceived to be the reason why drug craving persists even after long periods of abstinence. The permanent change in incentive salience is a direct response to the continued and repeated administration of a drug, which results in the *sensitization* of the NAc-related neural pathway; which, is specifically associated with

drug *'wanting'* not 'liking' (Robinson & Berridge, 1993; 2003). The process of sensitization is produced by means of dopaminergic neural adaptation, whereby drugs such as METH increase DA release in the NAc (Robinson & Berridge, 2000), and D1 receptors of neurons within the NAc become hypersensitive, which is believed to further potentiate the DA signal within the mesolimbic circuit (White & Kalivas, 1998). However, neural-sensitization is not solely associated with DA. Serotonin, acetylcholine, norepinephrine, GABA, glutamate and opioid systems have also been linked with the process of sensitization, which may further interact with brain regions associated with the mental representations of drug cues further enabling the change in incentive salience (Robinson & Berridge 2003). For example Tien and colleagues (2007) have recently demonstrated opioid receptors mediate the sensitization effects to METH, specifically finding that the mu-opioid receptor is critically involved in the regulation of METH induced increases in D2 receptor binding and gene expression of opioid peptides. Additionally, glutamate transmission within the NAc appears to be crucial for the development of psychostimulant drug-seeking behavior (Gergjikov & Beninger, 2006). Ultimately, these neural changes increase the amount of unconscious 'wanting' for the drug, not the amount of pleasure derived from the drug – 'liking' (Berridge, 2004). Thus, the drug user craves the drug more and more without deriving anymore (or even less!) pleasure from the drug (Robinson & Berridge, 1993). Moreover, sensitization is considered to be primarily non-associative in nature (Anagnostaras & Robinson, 1996) as compared to the learning processes involved in *incentive* sensitization, which (as mentioned previously) are associative (Robinson & Berridge, 2000; 2001; 2003). In summary, according to the incentive–sensitization model, with continued and repeated use of a drug the physiological effects and the associated mental representations of the drug are more readily

activated, which leads to an alteration in the incentive salience of the drug and consequentially the drug is craved (wanted) more and more.

The three stages of the incentive sensitization model fulfills all three criteria (development, maintenance, and reinstatement) which Robinson and Berridge (1993) assert any sufficient model of drug addiction must address in order to adequately explain addiction. Empirical support for this model stems from experimental findings of behavioral sensitization to the psychomotor effects of drugs utilizing animal models (Anagnostaras & Robinson, 1996; Robinson & Becker, 1986; Robinson & Berridge, 1993; 2003). Herein lays a common criticism of the incentive sensitization model, which is that it may not be directly applicable to addiction in humans. This criticism is especially concerned with the applicability of sensitization in humans. As Koob and Le Moal (1997), assert "there is little evidence of sensitization in drug-dependant people, and most clinical evidence points to tolerance, not sensitization" (p. 55-56). However, this criticism has been directly challenged by two studies demonstrating sensitization to the psychomotor effects of d-amphetamine in humans (Strakowski, Sax, Setters, & Keck, 1996; Strakowski & Sax, 1998). Strakowski and colleagues (1996) administered two doses of 0.25mg/kg d-amphetamine to drug-naïve subjects 48 hours apart, finding that second administration produced significantly greater responses in four behavioral indices (eye-blink rate, mood, activity/energy, amount of speech) as compared to the first administration. Likewise, Strakowski and Sax (1998) demonstrated that eye-blink rate and activity/energy progressively increased following three successive (48 hours apart) administrations of 0.25 mg/kg d-amphetamine, which replicates the basic findings of behavioral sensitization in animal models (Robinson & Becker, 1986). Thus, contrary to the criticism of the incentive sensitization model, behavioral sensitization to the psychomotor effects of drugs has been shown to be directly

applicable to a human population. Therefore, the processes outlined in the incentive sensitization model appears to be the best current model of addiction that explains the entire gamut of neural, behavioral and phenomenological aspects of addiction.

The incentive sensitization model may also be applied to the characteristic patterns of METH use within the HIV+ MSM population. Within this population, METH is initially used for the euphoric and pleasurable effects (Stage 1. activation of a hedonic state). As the self-reports suggest, a major impetus for METH use is for the drugs ability to enhance ones sexual experience as one subject stated: "Every touch is enhanced. On meth, orgasms are over the top. It's increased sensation on meth. Even a kiss is amazing. Everything tingles" (Semple et al., 2002, pg. 152). The euphoric effects of METH are also the property of the drug that relieves the negative affective and physical symptoms associated with HIV positivity, as illustrated by two HIV+ MSM subjects in a recent study: "I definitely used meth for managing my depression" and "Using helps avoid the emotional pain of HIV" (Robinson & Rempel, 2006, p. 11). The use of METH in the management of HIV-related symptom self-management also serves as a powerful drug cue that becomes associated with hedonia (Step 2: Pavlovian conditioning). Furthermore, as reported by Frosch and colleagues (1996) the use of METH as a "sex-drug" (i.e. sexual enhancement) within the MSM population causes the drug to become associated with ones sexual identity, serving as a powerful discriminative stimulus for the expression of their sexual behaviors. According to the viewpoint of the incentive-sensitization model, both of these factors leads to increased craving ('wanting') for METH (Step 3: attribution of incentive salience), which is often unconscious to the user. Additionally, when the HIV+ MSM METH user abstains from drug use for any period of time they report their HIV-associated symptoms worsen: "your

fatigue, nausea, body aches, and depression are 10 times worse" (Robinson & Rempel, 2006, p. 11), which further suggests that some neural-adaptation has occurred.

As the reported evidence and self-reports indicate, the incentive-sensitization model serves to more effectively and robustly explain the development and maintenance of drug abuse (METH addiction/dependency) in the HIV+ MSM population than either the negative reinforcement or positive reinforcement models. However, explaining the development and maintenance of drug addiction within this population fails to address the significantly disproportionate number of individuals who are MSM and HIV+ that are METH dependent as compared to MSM's who are HIV seronegative (HIV-). Reports suggest that MSM's are 5 to 6 times more likely to be METH dependent if they are HIV+ than if they are HIV- (Robinson & Rempel, 2006; Shoptaw, et al., 2003; Stall, Paul, Greenwood, Pollack, Bein, Crosby, et al., 2001). Such a significantly disproportionately higher number of dependencies to METH observed in individuals who are HIV+ is suggestive of an interaction between the HIV infection and addiction to METH (Chang & Vigorito, 2006; Robinson & Rempel, 2006).

Interactions between Methamphetamine and the HIV Infection

Chang and Vigorito (2006) have recently posited that such interactions could lead to an increased vulnerability to addiction in those infected with HIV. Synergetic interactions between the HIV infection and drugs of abuse could take place on a neurochemical level within the mesocorticolimbic dopaminergic pathway, because similarly to METH, the HIV infection yields pathological alterations upon neuronal functioning within these structures. However, the HIV infection does not target neurons directly, but indirectly. The HIV- type 1 infection appears to cause neurotoxic affects within the CNS via the shedding of neurotoxins and soluble viral

proteins, such as gp120 and Tat (Bansal, Mactttus, Nath, Maragos, Hauser, & Booze, 2000; King, Eugenin, Buckner, & Berman, 2006) and an elevation of excitatory amino acids (e.g. glutamate), proinflammatory cytokines and chemokines (Kopinsky, Bao, Lin, 2007).

Evidence suggests that HIV-associated proteins, such Tat (Trans-Activator of Transcription) and gp120 (glycoprotein 120) are neurotoxic within the same pathways as METH (Achat-Mendes, Ali, & Itzhak, 2005; Nath, Anderson, Jones, Maragos, Booze, Mactutus, Bell, Hauser, & Mattson, 2000). For instance, gp120 has been found to regulate the expression of the mu opioid receptor at the mRNA and protein levels of immune cells. This is significant as the mu opioid receptor has been found to be involved in the regulation of the aforementioned METH induced changes on D2 receptors and gene expression of endogenous opioid peptides (Tien, et al., 2007), which aides the neuronal changes resulting in sensitization. However, the effects of amphetamines on the immune system are not well understood. Given that glutamate transmission is integral in the development of drug seeking behavior (Gergjikov & Beninger, 2006) it is reasonable to assume that any increase in glutamatergic transmission, as is associated with gp120 and Tat neurotoxicity may serve to potentiate the mechanisms of incentive-sensitization within structures such as the NAc and its connections to cortical regions. Amphetamines (such as METH) potentiate the release of dopamine into the synaptic cleft (McCann, Wong, Yokoi, Villemagne, Dannals, Ricaurte, 1998) and Gp120 blocks DA uptake in mesocorticolimbic (Bennett, Rusyniak, Hollingsworth, 1995); thus, METH and gp120 may serve to produce a synergistic interaction on DA functioning in these neurons (Nath, Booze, Hauser, Mactutus, Bell, Cass, Maragos, & Berger, 1999). In addition, Maragos and colleagues (2002) found that the administration of METH and Tat results in a synergistic decrease in dopamine and its

metabolites and enhance the degradation of dopamine transporter ligand binding sites within the striatum, which suggests that METH and Tat interact to destroy dopaminergic terminals.

Synergistic effects between HIV and METH may be further increased during the later stages of the infection, when vascular changes enhance the ability of the HIV infection to migrate into the CNS and initiate excitation of excitotoxic pathways (Grant, Heaton, Dawson, Marcotte, 1999; Goodkin, Wilkie, Concha, Hinkin, Symer, Baldewicz, et al., 2001). For instance, HIV+ individuals who abuse METH seem to be more susceptible to immunosuppression leading to increased viral replication (Ahmed, 2002; Carey, Woods, Rippeth, Gonzolez, Heaton, & Grant, 2006) and the neurologically damaging effects of the virus (Nath, Anderson, Jones, Maragos, Booze, Mactutus, Bell, Hauserm, & Mattson, 2000; Rippeth, Heaton, Carey, Marcotte, Moore, Gonzalez, Wolfson, Grant, & HNRC Group, 2004), such as AIDS dementia complex (ADC). In summary, synergistic interactions between METH and the HIV infection have been reported; however, the exact mechanisms that permit such interactions are not fully understood. The HIV-associated proteins, gp120 and Tat are two possible routes to such interactions as their mechanisms of action have been shown to affect similar processes associated with METH addiction. To reiterate, Chang and Vigorito (2006) have suggested that these interactions may lead sufferers of the HIV infection to become more susceptible to drug addictions, such as the grossly disproportionate levels of METH addiction observed within the HIV+ MSM population as compared to HIV- MSM population.

Does HIV Infection Affect sensitivity to the addictive properties of Methamphetamine?

The current experiment looks to test the hypothesis that the HIV infection increases the vulnerability to METH addiction. However, as stated by Chang and Vigorito (2006) studying

"the impact of the HIV infection on the vulnerability to drug addiction is very difficult to determine from epidemiological and clinical studies because of the numerous confounding variables" (p. 100). Thus, to test this hypothesis a recently created non-infectious animal model of HIV-1 was used, as subjects.

Non-Infectious HIV-1 Transgenic Rat Model

Reid and colleagues (2001) created a non-infectious HIV-1 transgenic rat (HIV-1 Tg), which expresses all of the HIV-1 related genes, except those that make the virus infectious (gag and pol). Significantly, the genes that are expressed are sufficient to produce functional glycoprotein 120 (gp120) and transactivator of transcription (Tat) viral proteins. The viral encoded HIV envelope protein gp120 acts to alter glutamate transmission and induce cytokine production, which can injure neurons and alters the activation state of astrocytes and microglia (Ellis, Langford & Masliah, 2007). Tat causes glia cell activation and neurotoxicity within the CA3 region of the hippocampus (Chauhan, Turchan, Pocernich, Bruce-Keller, Roth, Butterfields, et al., 2003); in addition to dendritic loss and cell death (Bruce-Keller, Chauhan, Dimayuga, Gee, Keller, & Nath, 2003). The HIV-1 Tg rats express the neuropathology's and immune system irregularities that are characteristic of the human HIV-1 infection. On a clinical level these pathologies include: cataracts, respiratory difficulty, and neurological changes. The neuropathological changes include: reactive gliosis, lymphocyte infiltration, neuronal cell loss, and alterations in endothelial cells, which is associated with degradation of the blood-brain barrier. Similarly to HIV infected humans they also show a degeneration of peripheral nerves and skeletal atrophy (Reid et al., 2001).

Although, Reid et al (2001) have reported deficits in motor functioning (e.g. hind limb paralysis) within the HIV transgenic rats, no such deficits have been observed by this laboratory. The formation of cataracts in these animals ranges from mild to highly opaque; those born with high opaque cataracts are designated as HIV-1 Tg rats and their litter mates born without pronounced cataracts are designated littermates controls (FTg). This animal model of HIV-1 has been used extensively in a number of studies conducted within this laboratory and is the continuing subject of a number of other experiments currently underway. Briefly, these studies have found that the HIV transgenic rat model serves as a suitable model of HIV infection and differ from controls in that they display greater freezing in a conditioned fear paradigm (LaShomb, Vigorito, Root, & Chang, 2006), show learning impairments in the Morris water maze (Vigorito, LaShomb, & Chang, 2007), and express greater motivation to run in a wheel in short-term tests (Chang & Vigorito, 2006). HIV-1 Tg rats also show up-regulation of the mu opioid receptor (MOR) and a functional super sensitivity to morphine, a MOR receptor agonist (Chang & Vigorito, 2006, Chang, Beltran, & Swarup, 2007). At this time, no studies other than the original Reid et al (2001) article exist, which have investigated the effects of the HIV-1 infection on the brains of these animals. However, the behavioral research conducted within this laboratory suggests the brain of these transgenic animals is affected; indeed, the learning deficits observed in these animals are consistent with those associated with ADC in human patients.

In the present study the HIV-1 Tg rats were tested in two behavioral measures that are associated with drug use and vulnerability to drug addiction in laboratory animals, conditioned place preference (CPP) and behavioral sensitization (BS). The finding that HIV-1 Tg rats show greater sensitivity to CPP and BS would support the hypothesis that the HIV infection increases vulnerability to drug addiction.

Conditioned Place Preference (CPP)

In the CPP paradigm, a distinctive environment is repeatedly paired with the administration of a drug and a different environment is paired with a vehicle (e.g. saline). Typically, the two environments differ from one another on the basis of several sensory modalities (e.g. visual, tactile and olfactory); the distinctions between these two environments serve as contextual cues. During these repeated pairings a classically conditioned (Pavlovian) association is formed between the drug state (US) and the drug-paired contextual cues (CS). Animals are later tested by being presented with the opportunity to freely spend time in the drug-paired context or the vehicle-paired context and the time spent in each context is monitored. This test reflects the animals' motivation for the particular state associated with each context. During this test session the rewarding or aversive effect of the drug is determined by the amount of time spent in each context. If the animal spends significantly more time in the drug-paired context (CS+) the drug is said to be rewarding; conversely, if the animal spends significantly more time in the saline-paired compartment (CS-) the drug is said to be aversive (Meyer & Quenzer, 2005; Stolerman, 1992; van der Kooy, 1987). Although place preference has been observed with as few as one conditioning cycle (one drug-context pairing and one vehicle-context pairing), preference for the drug-paired context has been shown to be greater with increasing conditioning cycles (Bozarth, 1987). Thus, the acquisition of place preference is sensitive to the amount of drug pairings.

The CPP paradigm may be characterized as assessing drug seeking behavior (Bozarth, 1987). Drug seeking behavior is reflective of drug craving ('wanting') as it expresses the animals' desire to re-experience the previously experienced drug state, which produced a positive affective state. When drug-seeking behavior as is observed in the CPP paradigm is

analyzed via the incentive sensitization model, the repeated pairings of the drug within the distinct environment, serves to attribute the drug state to the contextual cues of that environment. Incentive salience is then attributed to those contextual cues, which serves to increase drug craving in the presence of the cues. In this way, when the animal is provided with the opportunity to freely choose the environment in which the drug was paired, as in a CPP test session, the animal will be motivated to seek out the context in which the drug was paired.

The mesolimbic dopaminergic pathway has been shown to be the neural substrate that underlies the induction and expression of CPP (Phillips & Fibiger, 1987). As can be expected, the basolateral amygdala and NAc play a significant role in the increase in incentive value of the context where the drug (e.g. psychostimulants, such as the amphetamines) is administered (Berridge & Robinson, 2003; Carr & White, 1983), thereby increasing the animals' motivation for seeking out the drug-paired context. A myriad of compounds have shown to produce place preference, such as morphine, heroin, ethanol, diazepam, nicotine, and the enkephalins, in addition to the psychostimulants, such as cocaine and the amphetamines (Bozarth, 1987). These drugs have been shown to produce place preference along a varied dose range. Amphetamines, such as METH in particular have been found to produce significant place preferences in dose regimens ranging from 0.5mg/kg (Goeders & Goeders, 2004) to as high as 4.0 mg/kg (Tokuyama, Takahashi, & Kaneto, 1996). However, at even higher doses, METH has been shown to produce neurotoxic effects to DA nerve terminals within the mesolimbic pathway (Achat-Mendes, Anderson & Itzhak, 2007). Such neurotoxic effects may effect the development of CPP. For instance, Achat-Mendes and colleagues (2005) found that a neurotoxic dose of METH (5.0mg/kg) significantly impaired CPP. In addition, it is of note that some drugs such as

the amphetamines have also been found to produce place aversions (van der Kooy, 1987).

Behavioral Sensitization (BS)

Behavioral sensitization is another typical measure used to study addiction in animal models. BS refers to the ability of certain drugs to produce increases in stereotypical hyper-active behavior (e.g. locomotor activity and repetitive grooming) after successive drug administrations (Wise & Bozarth, 1987). That is, behavioral responding increases over time (it sensitizes) while the drug dose remains the same or has been lowered (Robinson & Becker, 1986). This sensitization of the psychomotor effects of the drug has been shown to persist for months and even years (Robinson & Becker, 1986; Paulson, Camp, & Robinson, 1991).

Many drugs have shown to produce such increases in behavioral responding, but the psychostimulants, cocaine and METH, in particular have been shown to produce the most significant increases in locomotor activity (Wise & Bozarth, 1987). Increases in locomotor behavior are usually associated with the initial 'pre-phase' of hyper-activity, before behavioral stereotypy (e.g. repetitive grooming) occurs (Robinson & Becker, 1986). A typical behavioral sensitization procedure may be conducted in two ways: continuous or repeated intermittent. In the continuous administration paradigm, multiple relatively large doses of the drug (e.g. METH 10mg/kg; Ohmori, Abekawa, & Koyama, 1996) is repeatedly injected over the course of several days or is continuously administered via osmotic pump (e.g. METH 25mg/kg/day; Zhang, Lee, Xiong, Chen, Davidson, Wetsel, & Ellinwood, 2006). This paradigm serves to maintain elevated concentrations of the drug in the brain. In the repeated intermittent administration paradigm, relatively lows doses of the drug (e.g. METH 1.0-4.0 mg/kg; Iwasa, Kikucki, Hasegawa, Suzuki,

& Sato, 1996) are injected in discrete sessions, which may be daily or on some other variable schedule (Robinson & Becker, 1986).

The sensitization of behavioral responding reflects changes which occur on a neural level, the aforementioned processes of neural adaptation (Robinson & Becker, 1987). As the incentive-sensitization model explicates, these neuronal changes take place within the dopaminergic mesolimbic reward pathway and include an increase of DA release in the NAc (Robinson & Berridge, 2000), a hyper-sensitization of D1 receptors within the NAc further potentiating the DA signal within the mesolimbic circuit (White & Kalivas, 1998), and changes in the length of dendrites and number of dendritic spines from the NAc to the prefrontal cortex (Robinson & Kolb, 1997; 1999). All of these factors combine to affect information processing with the mesolimbic pathway (Robinson & Berridge, 2003). This sensitization process is primarily a non-associative one where the drug action on neural pathways serves to produce a direct behavioral response. However, excitatory Pavlovian associations as described in the CPP paradigm have been shown to increase a drug-induced psychomotor response when animals are tested in the environments (contexts) paired with the drug; in the same way an inhibitory associative process prevents the expression of the same psychomotor response within the environment that drug is not expected (Robinson & Berridge, 1993; 2003; Wang & Hsiao, 2003). Thus, increases in locomotor behavior are typically the greatest in the context which the drug was paired. For example, Wang and Hsiao (2003) showed that rats develop robust sensitization in a number of contexts (e.g. home cage or novel box) in response to 1.0mg/kg amphetamine (AMPH). Sensitization persisted when the same rats were subsequently challenged with 0.5mg/kg AMPH within the same context which they received the pretreatment; however,

sensitization was abolished when animals were challenged within a novel context (i.e. when the context was switched).

The Performed Experiment

The general aim of the current study is to evaluate if the HIV-1 virus affects the vulnerability to the development of addictive behaviors. The sensitivity to addictive drugs will be assessed in response to repeated-intermittent exposure to METH using the CPP and BS paradigms in the HIV-1 Tg animals and compared to the responding on the same measures in two control groups, a standard F344/Nhsd (F) group, and a group of F344/Nhsd animals which underwent the same transgenic process as the HIV-1 Tg group, but did not express the HIV-1 genome and associated proteins – FTg group. The latter group of animals is included in order to control for artifacts of the transgenic process. In the current experiment, all animal groups were initially exposed to six conditioning cycles, six exposures to METH paired with a distinctive environment and six exposures to saline paired with an alternative environment. Tactile contextual cues (i.e., smooth floor vs. textured floor) were used exclusively during the course of this experiment in order to control for differences in visual acuity as a result of very pronounced cataracts in the HIV-1 TG animals. Tests for place preference were conducted following the first three conditioning sessions (cycle 1) and again after the conclusion of the final three conditioning sessions (cycle 2) in order to map the development of place preference.

The drug administration schedule utilized for the acquisition of place conditioning phase is essentially a repeated-intermittent drug administration schedule that is used in studies of behavioral sensitization. Therefore, by observing the locomotor behavior of the rats during each drug-administration day it is possible to determine if locomotor behavior increases with repeated

drug administration, that is if there is behavioral sensitization (Robinson & Becker, 1987). If a sensitization effect is not observed during the repeated administration of the drug, a dose lower than the original training dose can be administered as a challenge test dose. Thus, following the conclusion of place preference testing, all animals will be administered two different challenge doses of METH to test for the expression of behavioral sensitization. If HIV-1 Tg rats experienced a greater sensitization process than control rats, then they should show greater locomotor behavior to the lower dose. During the challenge test dose phase, animals were given the opportunity to freely explore the entire apparatus (rather than be confined to the drug-paired compartment). This permitted further analysis of place preference under the influence of the drug – a pilot study within this laboratory has suggested that drug-seeking behavior (place preference) will be increased under the influence of the drug. Furthermore, this procedure permitted for an additional analysis of differences in locomotor responding within the drug-paired context and saline-paired context.

The hypothesis that HIV-1 increases the vulnerability to drug abuse anticipates that the HIV group will display greater drug-seeking behavior ('wanting') in tests of place preference and greater behavioral sensitization during METH challenge tests than both control groups. Consistent with the incentive sensitization model of addiction, it is anticipated that drug seeking behavior as measured by the CPP paradigm will increase for all groups and this will be expressed as an increase in place preference for the drug-paired context from the first CPP test to the second. It is anticipated that the HIV group will express significantly greater place preference for the METH-paired context. Additionally, the interaction between METH and the HIV-associated proteins Tat and gp120 may serve to increase the rate of place preference acquisition. Based on the aforementioned unpublished data, it is anticipated that place preference will be increased for

all groups during the METH challenge phase of this experiment. The differences anticipated between groups during the first two place preference tests should be further exacerbated during the two METH challenge tests, with the HIV group expressing greater place preference for the METH-paired context.

Increases in locomotor behavior are typically associated with the initial 'pre-phase' of hyper-activity, before behavioral stereotypy (e.g. repetitive grooming) occurs (Bozarth, 1987; Robinson & Becker, 1987). Thus for this experiment the analysis of locomotor behavior will be consigned to the first 15 minutes of each METH-challenge test session. During these test sessions it is anticipated that all animal groups will express increased locomotor activity, confirming the induction of behavioral sensitization. If the HIV-1 Tg group is more sensitive to the sensitizing effects of METH they will express significantly greater locomotor activity during the challenge tests on all indices of behavioral sensitization. However, the procedure employed in this study is not ideally set up to assess behavioral sensitization. Normally, in challenge tests of behavioral sensitization a control group that received only saline during the training phase would be included. This control group is necessary to provide evidence that the drug-treated animals respond more to the same challenge dose than the drug-naïve (saline) control group. This additional control group was not possible to include due to the scarcity of and financial expense associated with these animals. Nevertheless, we reasoned that if the HIV-1 rats are more vulnerable to the sensitizing effects of METH as compared to controls, then they should show a greater response to the challenge dose than the controls. If no between groups differences are observed, the requisite controls are not available to determine if behavioral sensitization has in fact occurred.

The main goal of the study was to investigate between group differences in METH-induced CPP. A secondary goal of the study was to look for evidence of between group differences in a measure of BS. To reiterate, the experimental procedure employed in this study allowed for this possibility, however, it is not ideal for detecting BS.

It is proposed that the incorporation of the HIV-1 genome in transgenic rats leads to an increased sensitivity to the addictive properties of METH and this effect will be reflected as increased motivation ('wanting') for METH as measured by CPP and increased psychomotor sensitization as measured by BS. This finding would support the hypothesis as originally proposed by Chang and Vigorito (2006) that drugs of abuse, such as METH interact with HIV-associated proteins (Gp120 and Tat) to produce a synergistic effect within the mesolimbic pathway leading to increased vulnerability to drugs of abuse in those with the HIV infection. Such an effect may ultimately lead human HIV+ sufferers to be more susceptible to viral replication (Ahmed, 2002; Carey, et al., 2006) and ADC (Nath, et al., 2000; Rippeth, et al., 2004) in addition to increasing the likelihood of engaging in risk taking behaviors (e.g. unsafe, unprotected sex). The increased vulnerability to drugs of abuse may partially explain why a decline in the number of new HIV infections is seen in all clinical subpopulations except in the MSM subgroup (CDC, 2005; MMWR, 2006, June 2). Such an effect would also help to further explicate the prevalence of METH use within the HIV+ MSM community, which has been reported to be around 50%, with some estimates nearing 60-65% (Shoptaw, et al., 2003; Robinson & Rempel, 2006) as compared to the approximately 10% in non-infected MSM population (Stall, et al., 2001).

Method

Animals

The animals used in this study were eleven male HIV-1 transgenic rats (HIV-1 Tg), nine male F344/Nhsd non-transgenic rats (F Tg), and ten male F344/Nhsd rats (F) obtained from Harlan, Co. (Indianapolis, IN). All animals were approximately thirty-three weeks of age at the start of the experiment. Prior to use in this study, these animals were tested in a study where the rats were housed with a running wheel and running behavior was monitored 24 hours per day. All animals were housed in pairs in clear plastic rat cages with Harlan Teklad 1.8" corn-cob bedding. Food (Purina™ Rat & Mice Chow 7001) and water were provided *ad libitum* through the duration of the study. The vivarium will be maintained on a constant 12:12 hour light-dark cycle (lights on 8:00am – 8:00pm) and at a constant temperature (24° ± 20° C) and humidity conditions. All experimental procedures were conducted during the light cycle, between 10:00am and 4:00pm and in accordance with the Seton Hall University Institutional Animal Care and Use Committee.

Conditioning Apparatus

Two identical, home-made place conditioning apparatus was used for training and testing throughout the duration of the experiment (Figure 1). The apparatuses were constructed to be similar in overall dimensions and design to commercially available chambers. The chamber consists of two outer compartments separated by one smaller inner compartment. The two inner walls separating the outer compartments from the inner compartment can be removed and replaced with walls containing access doors (16 x 16 x 16 cm) permitting free access to the entire apparatus during pre-conditioning and testing days. The dimensions of the two outer chambers

Conditioned Place Preference Apparatus

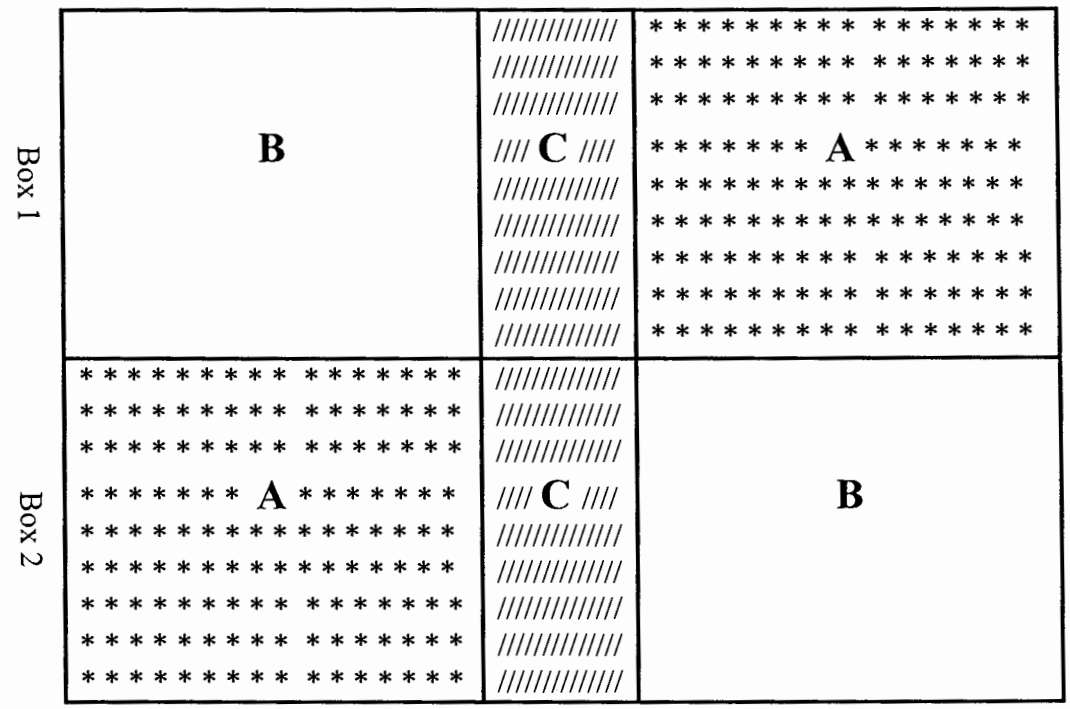


Figure 1. Two-dimensional depiction of the general layout of the two-box design for the conditioned place preference apparatus used throughout this study. Tactile cues within compartment A (perforations) and in compartment C (mesh grid) are indicated, but are not to scale.

are 31 x 31 x 31 cm; the dimension of the inner chamber is 31 x 16 x 31 cm. All walls were made of clear Plexiglas™ except for the inner walls separating the outer compartments from the inner compartment which were opaque. The outer compartment floors were made of sheet metal. To provide salient tactile discriminative cues, the floor was textured in one compartment and left smooth in the other compartment. The textured outer compartment (A-perforated) was created by punching holes (6-7mm) with a nail 7 times per 7.75 cm^2 and the other outer compartment (B-

smooth) floor remained smooth. The floor of the inner compartment (C-rough) was made of a rough plastic grating, purchased commercially at a local hardware store. Preliminary studies conducted in this laboratory have shown these cues to be sufficient to establish a conditioned place preference in HIV-1 Tg and control rats. The floors of compartments A and B were marked with sixteen, 7.75 cm^2 red gridlines which will be used to aide in scoring of locomotor activity. Each animal experienced all phases of the experiment within the same box.

Body Weight

Animals' body weights were taken daily prior to any experimental manipulations at approximately 10:00 am and were recorded throughout the course of experimentation. Evidence suggests that METH produces anorexic effects and thus body weight loss can be seen as a supplementary measure of reactivity to METH (Caul, Jones, & Barrett, 1988; Ginawi, Al-Majed, & Al-Suwailem, 2005a; 2005b; Robinson & Becker, 1986).

Drugs and Solutions

All animals used in this study received intraperitoneal (IP) injections of methamphetamine (METH) or saline, via 27½ gauge/1cc syringes. IP injections were used in this study even though they are not typically used by human drug abusers, because they are more convenient and accurate when administering drugs into small sized animals such as rats (Chiu, Ma, & Ho, 2005). METH was obtained from Sigma-Aldrich Co. (St. Louis, MO.) and dissolved in sterile 0.9% saline immediately prior to injections. METH and saline were administered IP at a dose of 3.25mg/kg, a dose that falls within the accepted range administered to induce behavioral sensitization (Iwasa, et al., 1996) and conditioned place preference (Tokuyama, et al.,

1996). During the drug treatment phase injections were made 20 minutes prior to all experimental sessions in order to permit the commencement of drug actions; during this time the animals waited in their home cages. Injections during the behavioral sensitization challenge test phase were made immediately preceding placement in the place conditioning apparatus ($t \leq 1$ min.).

Procedures

The time course for this experiment was conducted over a 21-day period (Table 1) and consisted of six phases: preconditioning (2-days), Phase 1 drug treatment (6-days), conditioned place preference test 1 (1-day), Phase 2 drug treatment (6-days), conditioned place preference test 2 (1-day), behavioral sensitization challenge (4-days). The design of this study was constructed similarly to that employed by Shimosato & Ohkuma (1999), so that drug treatment associated with the conditioning for place preference and for the development of behavioral sensitization will be conducted concurrently during two drug treatment phases of the overall procedure. Furthermore, the employment of two drug treatment phases and place preference tests permitted for a characterization of the acquisition for place preference in all animal groups, further characterizing any differences in the development of place preference between groups. Following the first conditioned place preference test, one day was devoted solely to scoring and interpretation of the data; on this day no experimental procedures were conducted and animals remained in their home cages.

After testing each animal, all compartments were washed thoroughly with 70% alcohol to avoid odor cues. All sessions were video recorded by a commercially available DVD recorder

Table 1.

Time Course for all Experimental Procedures and Testing Phases

Days	1-2	3-8	9	11-16	17	18-21
Phase	Preconditioning	Cycle 1 (3-drug & 3 saline pairings)	CPP Test 1	Cycle 2 (3-drug & 3 saline pairings)	CPP Test 2	Behavioral Sensitization Challenge (0.5 & 1.0 mg/kg tests)

Note. CPP test 2 also served as 0.0mg/kg drug dose for purposes statistical comparisons.

for later scoring by trained laboratory personnel. All scoring was conducted and transcribed utilizing the EthoLog 2.2 behavioral and timing transcription program (Ottoni, 2000).

The dependant variable for the purposes of the methamphetamine-induced place preference procedure was time spent in a compartment as measured by the presence of both forepaws entering that compartment - permitting the opportunity for the tactile feedback and discrimination of each compartment, based solely upon floor texture (i.e. perforated, smooth, or rough). Locomotor activity within compartments A and B served as the dependant variable of motor behavior for the measurement of methamphetamine-induced behavioral sensitization. Locomotor activity was measured by the number of times both of the animals ears crossed a grid mark (due to the design of the apparatus this measure will only be possible in compartments A and B). Locomotor behavior was assessed during the first 15 minutes (900 sec.) of the 40 minute challenge session to permit examination of the development of the drug effect and stereotypy. During this time, place preference was also assessed providing a measure of conditioned place preference under the influence of the methamphetamine. Pilot studies conducted within this

laboratory suggest the expression of CPP may be greater after receiving a priming injection of the drug.

Preconditioning Procedure. On days 1 and 2, all animals were acclimated to the conditioning apparatus and the existence of any initial unconditioned place preference was determined. A natural place preference was operationally defined as greater than 60% of the total session time spent in either compartment A or B (De Fonseca, et al., 1995). For this procedure the two center walls of the apparatus separating compartments A, B and C were removed and the walls containing the access doors were inserted. The animals were placed in the center compartment (C), and were permitted to freely explore the three compartments for 15 min. The amount of time spent in each compartment was measured. All animals were assigned to receive drug pairing with compartment A or B.

Drug Treatment (Place Conditioning and Induction of Behavioral Sensitization). The drug treatment phase began on day 3. This phase consisted of two, six-day conditioning cycles separated by two days, which were allotted for CPP testing. Thus, cycle 1 was conducted on days 3 through 8 and cycle 2 was conducted on days 11 through 16. During each cycle, rats received three drug context pairings and three saline context pairings - on alternating days. For example, if an animal received METH on days 1, 3, and 5 they received saline on days 2, 4, and 6. Therefore, across both cycles each animal experienced six context pairings (compartment A or B) with METH injections. Drug treatments always occurred under the same context for each animal. All animal groups were counter balanced for compartment and days. Hence, roughly an equal number of animals from each group received the drug context pairing in compartment A as

received the drug pairing in compartment B. Furthermore, half of the animals received their first METH injection on the first day and the remaining half will received their first METH injection on the second day. Thus beyond place conditioning, the overall design of the drug treatment schedule represents a context-specific, repeated intermittent drug administration schedule; such a treatment paradigm has been shown to induce robust behavioral sensitization in rats (Anagnostaras & Robinson, 1996; Robinson & Becker, 1986; Robinson & Berridge, 1993; 2000; 2001; 2003).

Place Preference Testing. The testing phase for place preference was carried out following the completion of each drug treatment cycle in a methamphetamine-free state. Each animal was tested once during each test. For testing, the removable walls separating the three compartments were replaced with the access doors. The animals were placed in the center compartment (C) and permitted to freely explore the entire apparatus for 15 min. The time spent in each compartment was recorded for each animal and the change in preference was calculated as the difference (in seconds) between the time spent in the drug paired compartment on the testing day and the time spent in the same compartment on the preconditioning day.

Behavioral Sensitization Challenge Testing. The behavioral sensitization challenge phase was conducted for four days immediately following the second conditioned place preference test (days 18 - 21). All animals were placed in the center compartment with the access doors inserted permitting access to all compartments for 40 minutes. Because it was not be possible to test all of the animals in a single day, the animals were divided into two groups and tested on alternate days. All animals received injections of both 1.0 and the 0.5 mg/kg METH solutions. The order

of the dosage was counterbalanced so that half the animals received 1.0 mg/kg on their first test day followed by 0.5 mg/kg on their second test day. The other half of the animals experienced the drug dosing sequence in the opposite order. One subgroup began challenge testing on day-18 and the other on day-19. Thus, one day of no testing occurred between the two methamphetamine challenge tests for all rats.

Statistical Analyses

Body weight reactivity to METH was measured during the drug treatment phase as the difference between pre-and post-drug exposure and proportion of total body weight loss following the completion of the METH treatments during this phase. Body weight data as analyzed using one-way ANOVA's to determine body weight variation between groups. Drug seeking behavior data (CPP) was measured by the change in the amount of time (sec.) spent in the drug-paired and saline-paired compartments during the CPP tests as compared to the time spent in the same compartments during the pre-conditioning phase (Bozarth, 1987; Stolerman, 1992). Additionally, a compartment preference score was generated by subtracting the difference between compartment A and B. Preference scores taken from all CPP tests were compared to the preference score obtained from the pre-conditioning phase. Measures of drug seeking behavior and locomotor behavior were analyzed using mixed analyses of variance (ANOVA). Following the determination of a significant F value, for all measures, post-hoc Bonferroni analyses were performed to assess specific group by group comparisons. Statistical significance was accepted at $p<0.05$. All calculations were performed using the SPSS statistical package.

Results

Baseline: Preconditioning Phase

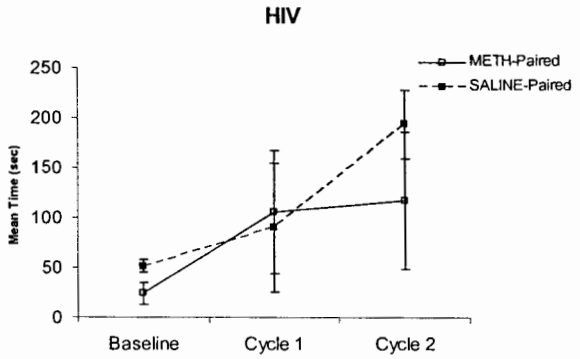
During the conditioning phase of the experiment, compartments A and B were used as the drug-and saline-paired compartments; therefore, it was necessary to determine if there were any natural preferences or aversions for any of these floor surfaces prior to conditioning sessions. The middle compartment (C) was not paired with any solution (drug or saline) and therefore the preference score only compared compartments A and B. A natural preference was defined as 60% or more time spent in either of the two compartments.

During the pre-conditioning phase no animal expressed a natural preference for either compartment A or B. However, several animals were reassessed on day two because they failed to move from the center compartment on day one. All animals spent a greater proportion of the total session time during the preconditioning phase in the center (C) compartment (AVG=89.05% ±1.45%), than in either A or B, the eventual METH-paired (AVG=6.45% ±1.10%) and saline-paired (AVG=4.52%±.60%) compartments. On the basis of these results, animals were randomly assigned to one of the two compartments (A or B) as the METH-paired compartment and the other compartment as the Saline-paired compartment.

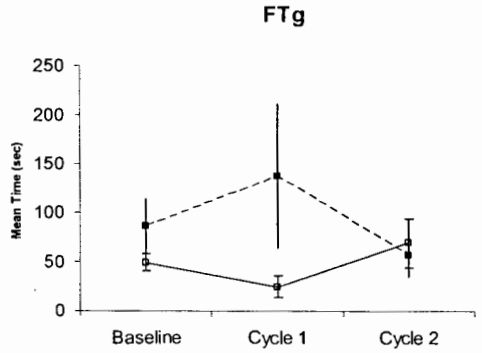
Methamphetamine-Induced Conditioned Place Preference

To test for the development of CPP, mean time spent in drug-and saline-paired compartments were calculated during tests for place preference following the first and second conditioning cycles and compared to the time spent in the same compartments during the pre-conditioning phase (baseline). Figure 2 displays the mean (± SE) time (sec.) spent in METH-and Saline-paired compartments during baseline and after the first and second conditioning cycles for

A



B



C

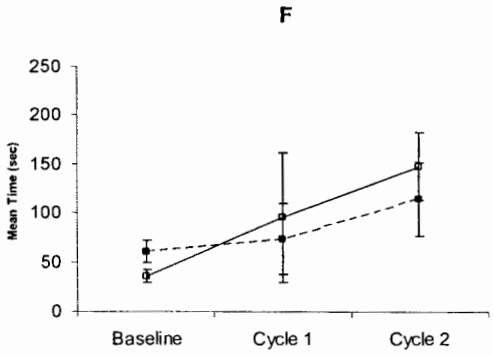
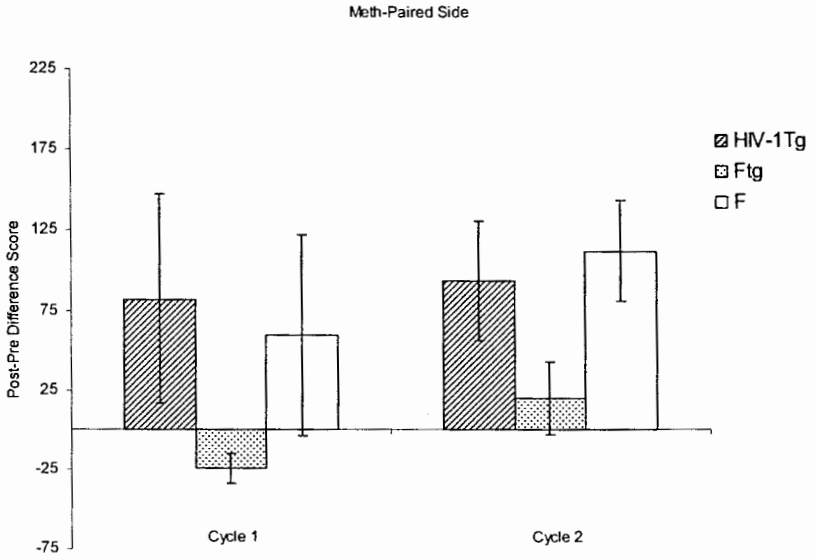


Figure 2. Mean (± SE) time (sec.) spent in METH-and Saline-paired compartments during baseline and after the first and second conditioning cycles for: HIV-1 Tg, FTg, & F groups.

the HIV-Tg, FTg, and F groups respectively. A group (3) x cycle (3) x side (2) mixed factors ANOVA showed no effect of groups, $F(2,27)=.751$, $p > .05$, or side, $F(1,27)=1.216$, $p>.05$, but significant main effect of cycle, $F(1,27)=16.335$, $p < .001$, with the time spent in each compartment increasing over cycle (cycle 2 was significantly greater than baseline, $p<.05$). A cycle x side interaction would suggest that this effect was due to the development of CPP; as time spent within the METH-paired compartment would be significantly greater compared to the time spent within the Saline-paired compartment. However, this interaction was not significant, $F(2,54)=.107$, $p > .05$. No other interactions were significant. The increase in time spent within the METH-and Saline-paired compartments was most likely due to a non-specific effect of repeated exposures to the testing apparatus. To confirm the failure in the development of CPP, the data was reanalyzed utilizing an alternative method, the calculation of a CPP preference score.

Preference scores for the drug-and saline-paired compartments during the first and second CPP tests were generated by subtracting the difference (in seconds) between the amount of time spent in those compartments during each test from the amount of time spent in the same compartment during the pre-conditioning phase (baseline) for each animal. Figure 3 displays the mean (±SE) preference scores for all groups following three (cycle 1) and six (cycle 2) conditioning cycles for both the METH-and Saline paired compartments. A group (3) x cycle (2) x side (2) mixed factors ANOVA yielded no significant main effects of cycle, $F(1,27)=.947$, $p=.231$, side, $F(1,27)=.995$, $p=.704$, or group, $F(2,27)=2.043$, $p>.05$; no significant interactions between cycle and group, $F(2,27)=.932$, $p>.05$, side and group, $F(2,27)=.971$, $p>.05$, or cycle and side, $F(1,27)=.997$, $p>.05$. Additionally, there was no significant group x cycle x side interaction, $F(2,27)=.901$, $p>.05$. The fact that there were no significant increases observed in preferences

A.



B.

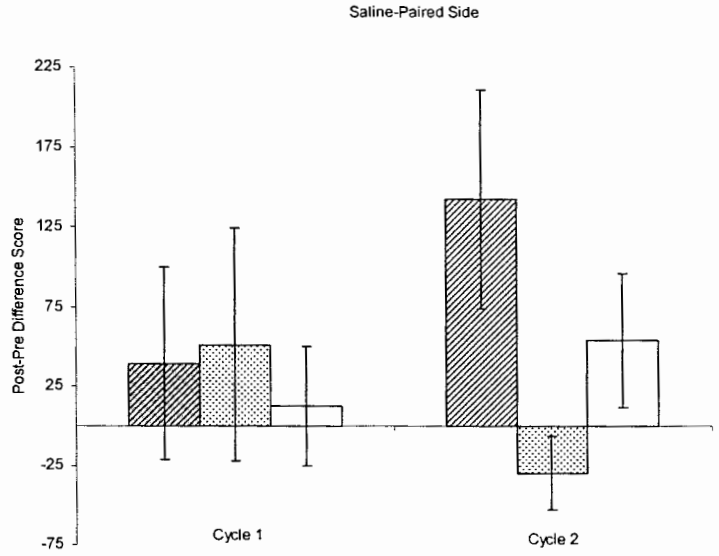


Figure 3. Mean (±SE) preference scores for all groups following three (cycle 1) and six (cycle 2) conditioning cycles. A. Preference for the METH-paired side (place preference) and B. Preference for the Saline-paired side (place aversion).

Table 2.

Time Spent in METH-and Saline-Paired Compartments during CPP Tests for Animals that met the 30% Criterion during the Cycle 2 CPP Test

		Cycle 1		Cycle 2	
Group	Animal	METH	Saline	METH	Saline
HIV-Tg	H22	118.89	690.40	74.24	334.95
HIV-Tg	H24	738.00	48.49	247.86	422.73
HIV-Tg	H29	16.07	5.94	79.89	685.89
HIV-Tg	H31	15.65	56.65	378.83	380.91
FTg	F29	94.54	175.09	208.28	118.51
FTg	F31	46.81	274.44	178.00	124.83
F	F41	2.43	0.00	171.47	149.19
F	F42	684.11	2.93	353.55	228.63
F	F43	25.65	313.20	29.11	389.07
F	F44	35.51	81.73	272.53	110.22

Note. All data reported in seconds (sec). Maximum possible time spent in either compartment during CPP tests = 900.00 sec.

scores for the METH-paired compartment from the first to the second CPP test is consistent with the previous analysis that METH-induced place preference failed to develop for all groups. The failure of METH-induced conditioned place preference to be observed can not be explained by the deployment of conditioned place aversion, because conditioned place aversion was also not observed. This is evidenced by no significant increase observed in preference scores for the saline-paired compartment. Furthermore, preference scores did not significantly differ from cycle to cycle for each side, which indicates the development of METH-induced place preference or aversion did not benefit from the additional conditioning cycles. Groups did not radically differ from one another throughout the place preference testing, as indicted by the observation that there were no significant differences between groups, $F(2,27)=2.043$, $p>.05$.

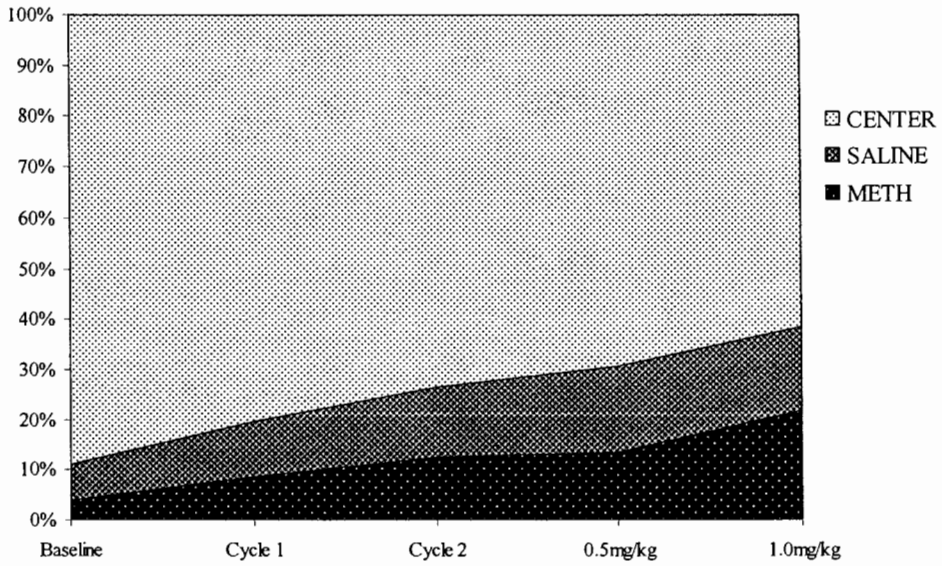


Figure 4. Mean proportion of total session time (sec).spent within each compartment (Center, Saline-paired & METH-paired) of the CPP apparatus for all animals throughout the course of the experiment.

As mentioned previously all animals spent a considerably greater amount of the test session time within the center compartment during the pre-conditioning phase; as shown in figure 4, this observation continued throughout subsequent tests for CPP: Cycle 1 (AVG=80.13%); Cycle 2 (AVG=73.67%). Such a strong preference for the center compartment may have had an effect upon the development of CPP. In order to evaluate this possibility, animals that spent less than 30% of the total test session time in the center compartment during the second cycle CPP test were eliminated. A total of ten animals met this criterion (Table 2): four HIV-Tg, two FTg, and four F animals. Statistical analyses of CPP test session data were repeated on the data obtain from these animals, but because only ten animals met this criterion between group comparisons

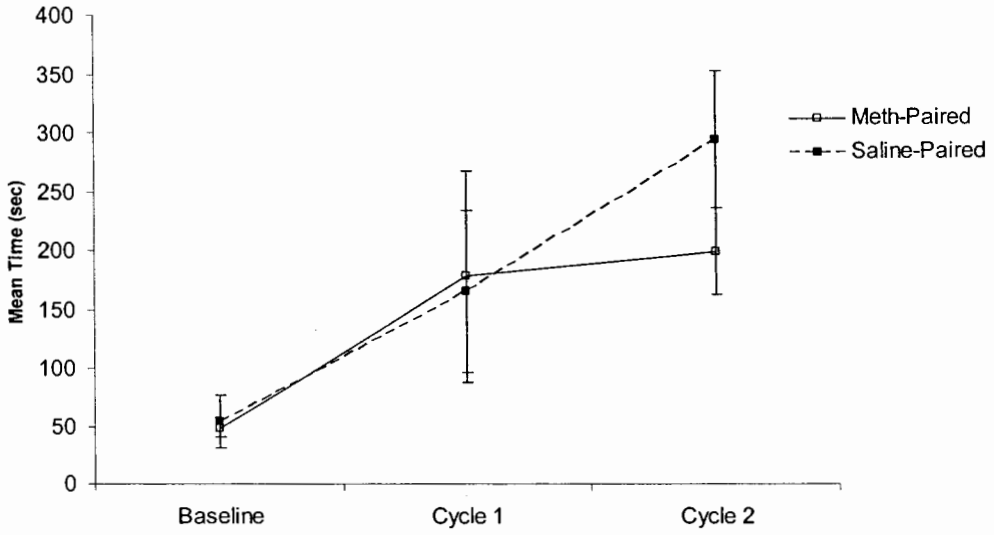


Figure 5. Mean (± SE) time (sec.) spent in METH-and Saline-paired compartments during baseline and after the first and second conditioning cycles for animals that met the 30% criterion of time spent outside the center compartment during the second cycle CPP test.

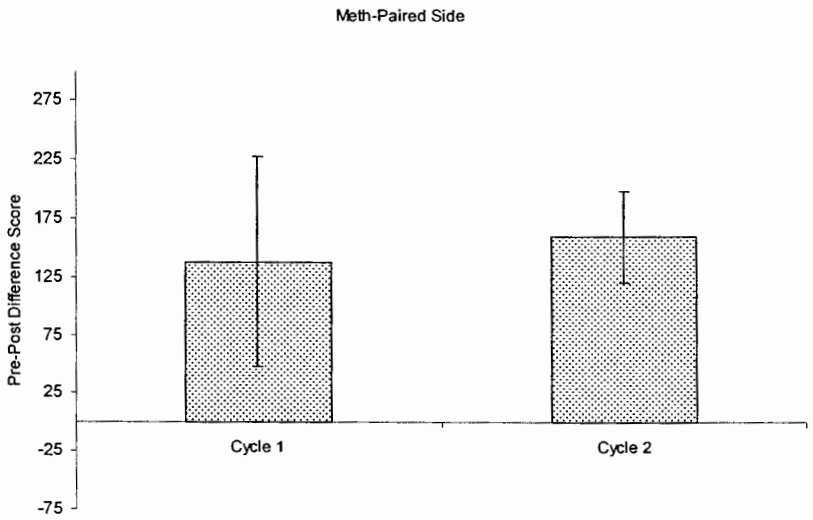
were not conducted. Figure 5 shows the mean (± SE) time (sec.) spent in METH-and Saline-paired compartments during baseline and after the first and second conditioning cycles for the animals that met this criterion. A cycle (3) x side (2) ANOVA was conducted to determine presence of a preference for the METH-paired compartment and if so, did that preference increased over cycles. This additional analysis revealed no significant differences from the previous analysis conducted on all animals. Specifically, there was a main effect of cycle, $F(2,18)=7.498$, $p=.004$, but not side, $F(1,9)=.612$, $p>.05$, nor was there a cycle x side interaction, $F(2,18)=.423$, $p>.05$. Likewise, there were no significant differences seen in the analysis of preference scores conducted with these ten animals in relation to the previous analysis conducted on all animals. Figure 6 shows the mean (±SE) preference scores for the animals that met this

criterion following three (cycle 1) and six (cycle 2) conditioning cycles for both the METH-and Saline-paired compartments. A cycle (2) x side (2) ANOVA revealed non-significant effects of cycle, $F(1,9)=1.769$, $p>.05$ and side, $F(1,9)=.000$, $p>.05$ with a non-significant cycle x side interaction, $F(1,9)=.667$, $p>.05$.

The second part of the study included treatment with two challenge doses of Methamphetamine to evaluate group differences in behavioral sensitization (see below for results of this part of the study). These challenge tests, however, provided an additional opportunity to evaluate evidence of CPP. During the challenge tests the rats were given free access to all three compartments as in the CPP test days. As a result, it is possible to compare place preference at the end of conditioning trials when testing was in the absence of a drug state (i.e., cycle 2 data reviewed above) with place preferences during the challenge tests when the rats were under the influence of methamphetamine. Because two challenge doses were tested (0.5 and 1.0 mg/kg), time spent in the METH-paired and saline-paired compartments under the influence of 0 mg/kg (end of cycle 2), 0.5 mg/kg (first challenge dose) and 1.0 mg/kg (second challenge dose) METH were compared.

A group(3) x dose(3) x side(2) mixed factors ANOVA indicated a main effect of dose, $F(2,26)=5.143$, $p=.013$ and marginal dose x side interaction, $F(2,26)=3.601$, $p=.062$, but no group effects (group, $F(2,27)=1.183$, $p > .05$; group x dose, $F(4,54)=.719$, $p > .05$; dose x side x group, $F(4,52)=2.066$, $p=.099$). Additionally, there was no main effect of side, $F(1,27)=.000$, $p>.05$. A significant main effect of dose indicated that the time spent in the METH- and Saline-paired compartments increased as a factor of drug dose. This dose effect may reflect the locomotor enhancing effect of the 1.0 mg/kg dose of METH. If the rats are more active as a result of the influence of the drug, then it is not surprising that they are spending more time in the

A.



B.

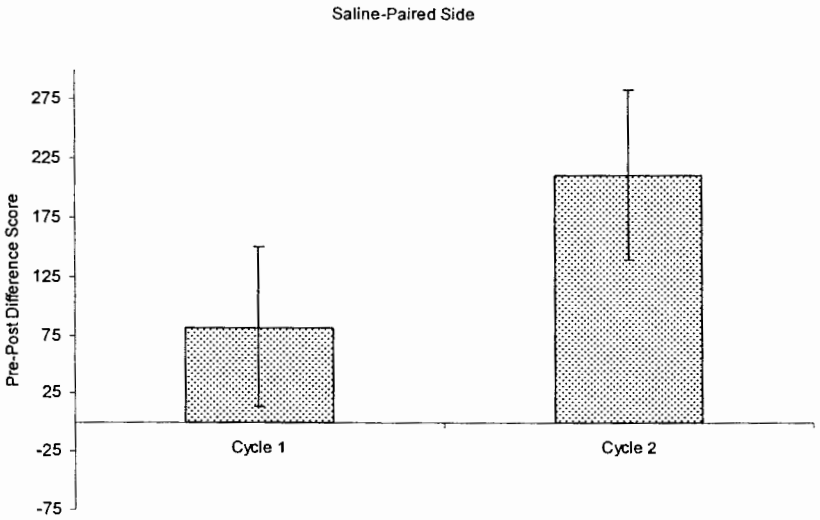


Figure 6. Mean (±SE) preference scores for all groups following three (cycle 1) and six (cycle 2) conditioning cycles. A. Preference for the METH-paired side (place preference) and B. Preference for the Saline-paired side (place aversion) for animals that met the 30% criterion of time spent outside the center compartment during the second cycle CPP test.

outer compartments. However, a significant dose x side interaction (Figure 7) indicated that the increase was greater in the METH-paired compartment than in the saline-paired compartment. Separate group (3) x dose (3) ANOVA's were subsequently conducted on the METH-and Saline-paired sides, to confirm and explicate this interaction. These analyses revealed that animals spent significantly greater time within the METH-paired side during the 1.0mg/kg challenge dose compared to the 0.5mg/kg dose, $p=.004$, an effect which was not observed between the same doses within the Saline-paired side, $p>.05$. Thus, the data confirmed the presence of the interaction, signifying that animals did prefer the METH-paired compartment to the Saline-paired compartment when challenged with a 1.0mg/kg dose of METH, which could not solely be explained by the locomotor enhancing effect of the drug. Thus, these results suggest that priming injections of METH (at least at certain dosages) does induce a weak expression of conditioned place preference for the drug-paired compartment. However, there were no significant differences observed between groups.

Behavioral Sensitization Challenge

METH-induced locomotor activity was determined by calculating the mean number of grid-crosses (crosses) within the METH-and Saline-paired compartments during baseline (0.0mg/kg), 0.5mg/kg and 1.0mg/kg challenge drug administrations. As mentioned previously, research suggests that locomotor activity should be increased in a compartment specific manner, where psychomotor sensitization in response to a challenge dose is observed only within the same context in which the initial training dose was administered and not in a novel context (i.e. context where the drug has not been administered previously – the saline-paired context). Thus,

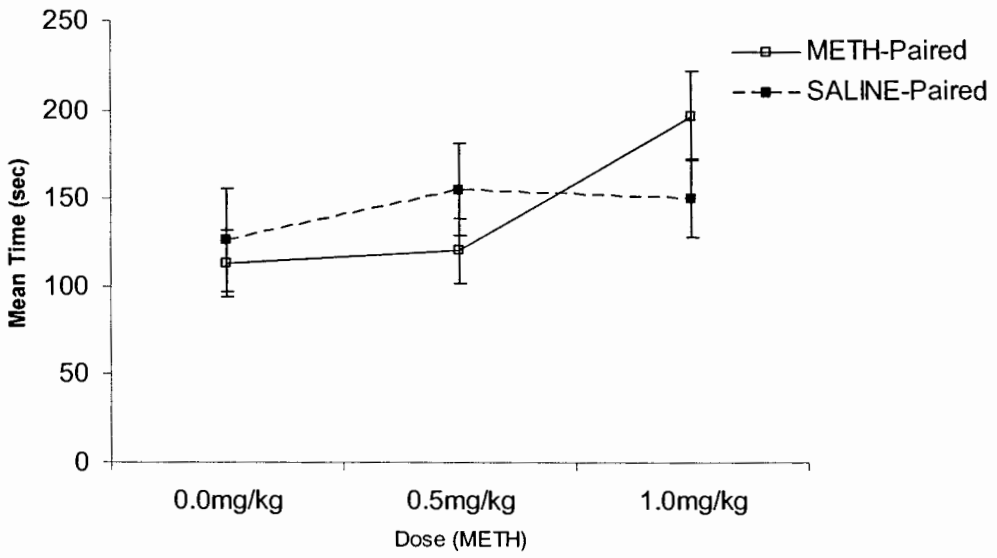


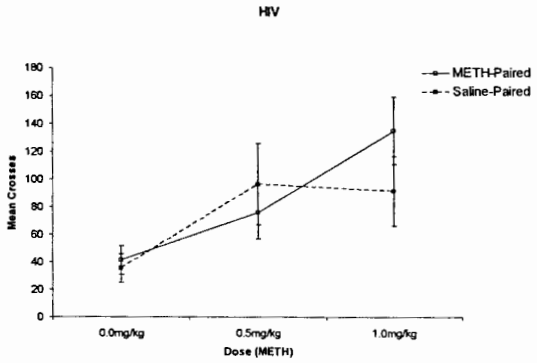
Figure 7. Mean (± SE) time (sec.) spent in METH-and Saline-paired compartments, showing dose x side interaction.

if this effect is present, locomotor activity should be significantly greater within the drug-paired context as opposed to the vehicle-paired context. This possibility was analyzed via a group(3) x dose(3) x side(2) mixed factors ANOVA. This analysis yielded a non-significant main effects of side, $F(1,27)=.013$, $p>.05$ and group, $F(2,27)=1.183$, $p>.05$, a non-significant side x group interaction, $F(2,27)=1.049$, $p>.05$, but a significant main effect of dose, $F(2,54)=40.913$, $p=.000$, a significant dose x side interaction, $F(2,54)=5.819$, $p=.008$ and marginally significant group x dose x side interaction, $F(4,54)=2.388$, $p=.063$. Figure 8 shows the mean (± SE) crosses within the METH-and Saline-paired compartments during 0.0, 0.5, & 1.0mg/kg challenges doses of METH for: A. HIV-1 Tg, B. FTg, & C. F groups. As this figure clearly shows, mean number of crosses increased for each group within the METH-and Saline-paired compartments as the drug

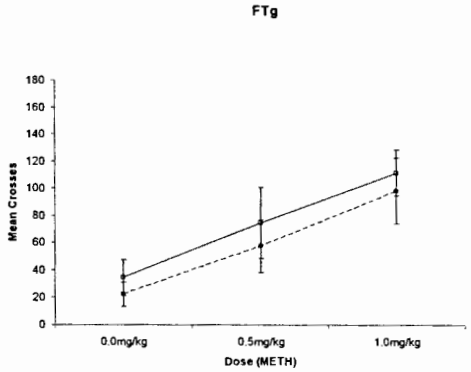
dose was increased, as indicated by the significant main effect of dose. However, the fact that there was a marginally significant group x dose x side interaction as well as a significant dose x side interaction suggests that group(s) may have increased locomotor activity differentially within the compartments. In order to determine if there were any group differences between the rates of METH-induced locomotor activity between the METH-and Saline-paired compartments, a separate group(3) x side(2) ANOVA was conducted for each challenge dose (i.e. 0.0, 0.5, & 1.0mg/kg). Analyses revealed no between group effects at any of the doses (0.0mg/kg: $F(2,27)=.688$, $p>.05$; 0.5mg/kg: $F(2,27)=1.665$, $p>.05$; 1.0mg/kg: $F(2,27)=.550$, $p>.05$). Group x side interactions were non-significant at the 0.0mg/kg dose, $F(2,27)=.101$, $p>.05$ and the 1.0mg/kg dose, $F(2,27)=.643$, $p>.05$; however, a marginally significant group x side interaction was revealed at the 0.5mg/kg dose, $F(2,27)=2.693$, $p=.086$.

In order to further explicate this interaction individual t-tests were conducted for each group comparing number of crosses within the METH-and Saline-paired compartments during the 0.5mg/kg challenge dose. As Figure 9 clearly shows, the F group animals made a greater number of crosses within the saline-paired compartment as compared to the METH-paired compartment, which approached significance $t(10)=-2.194$, $p=.056$. T-tests revealed no significant differences between the number of crosses made within the METH-and Saline-paired compartment with the HIV, $t(10)=-.798$, $p>.05$ and FTg, $t(10)=.878$, $p>.05$ groups. Thus, overall, the suggestion that locomotor responding would be greatest within the METH-paired context was not confirmed as responding was not found to differ markedly between the two contexts; save for the F group during the 0.5mg/kg challenge dose, where locomotor responding was greater in the Saline-paired context.

A.



B.



C.

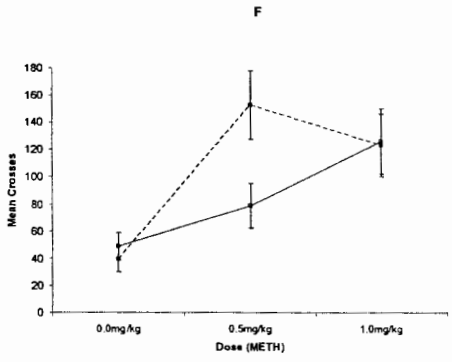


Figure 8. Mean (± SE) crosses within the METH-and Saline-paired compartments during 0.0, 0.5, & 1.0mg/kg challenges doses of METH for: HIV-1Tg, FTg, & F groups.

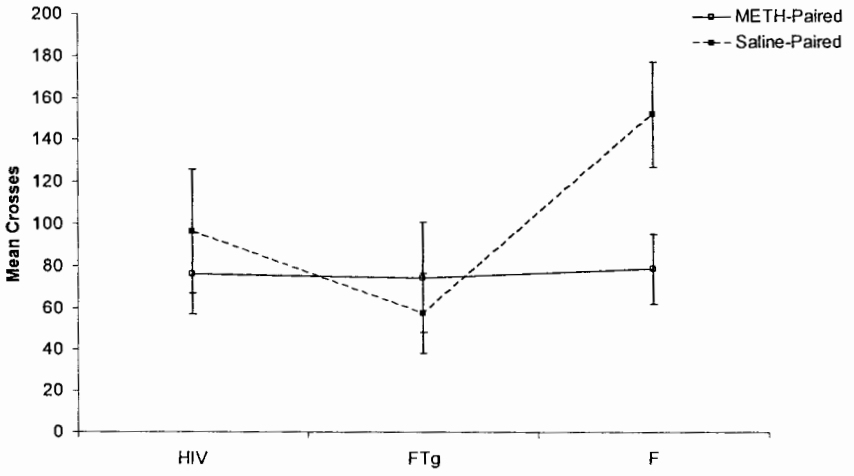


Figure 9. Mean (± SE) crosses within the METH-and Saline-paired compartments for each group during the 0.5mg/kg challenge dose test.

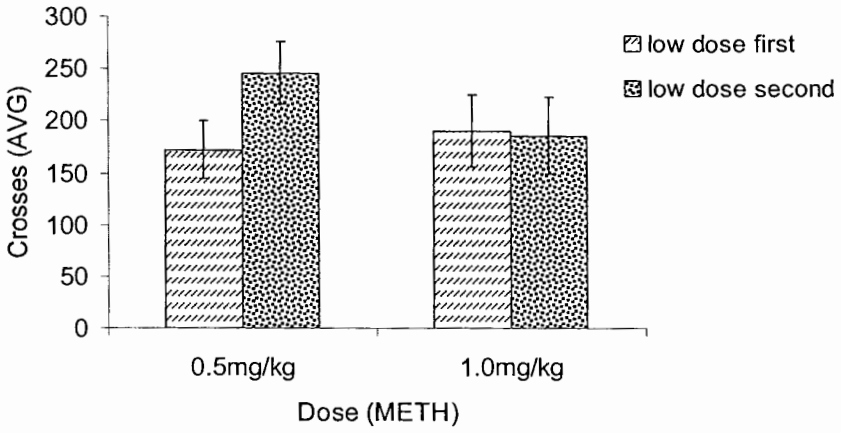


Figure 10. Mean (±SE) METH-induced locomotor behavior (crosses) as a factor of drug administration order; comparing the animals that received the low dose first to the animals that received the low dose second.

FTg=409 ± 4.11 and F=387 ± 4grams. A one-way ANOVA indicated a significant main effect of days $F(2,27)=41.272$, $p<.000$. Thus, all groups showed a significant decrease in total body weight following the six intermittent-repeated doses of 3.25mg/kg METH during the conditioning/drug-treatment phase of the experiment.

In order to determine between group differences, the proportion of total body weight loss was calculated for all animals in response to the six repeated-intermittent doses of 3.25mg/kg METH. As shown in figure 11, the proportion of total body weight loss averaged 8.9% (±.29) for the HIV-1 Tg animals, 7.9% (±.52) for FTg and 6.7% (±.62) for F animals. A one-way ANOVA indicated a significant between groups effect, $F(2,27)=5.686$, $p=.009$. Post Hoc Bonferroni t-tests revealed the total proportion of body weight loss was significantly greater in the HIV group compared to the F controls, $p=.007$. Differences in body weight loss were not significant between the HIV and FTG ($p>.05$) groups, nor the FTG and F ($p>.05$) groups. Thus, the results suggest that the HIV group was more reactive to the anorexic effects of METH.

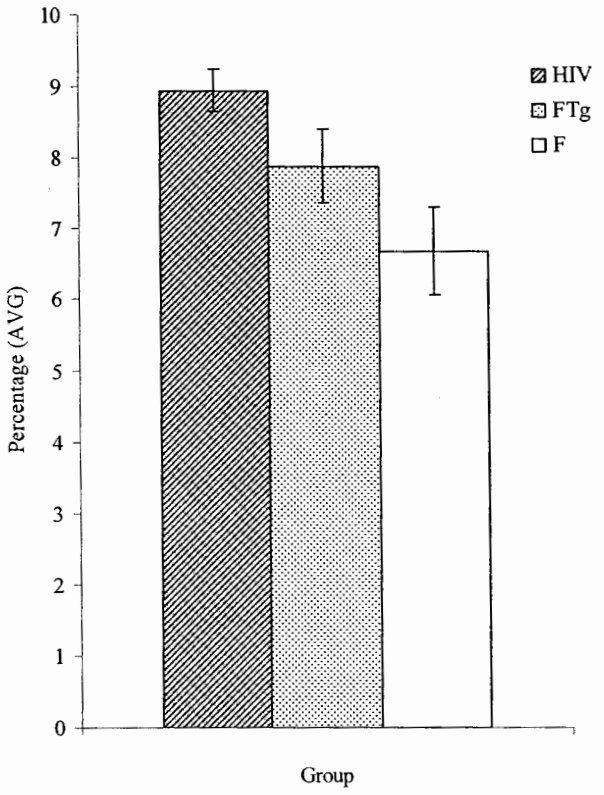


Figure 11. METH-induced anorexia represented as the mean (± SE) proportion of total body weight reduction for all groups follo175wing six repeated-intermittent drug exposures (3.25mg/kg each).

Discussion

Methamphetamine-induced conditioned place preference failed to develop following the two standard conditioning cycles. Furthermore, the HIV-1 Tg group did not differ from either control group during these standard tests for CPP using time or preference scores as the dependant variable. However, a weak CPP effect was observed following a 1.0mg/kg challenge dose of METH; as evidenced by animals spending significantly greater time within the METH-paired compartment during this challenge dose compared to the Saline-paired compartment. There were no significant differences in drug-seeking behavior observed between the HIV group and controls during this challenge dose. The administration of two challenge doses of METH produced increasing locomotor responding, which increased as a factor of drug dose. Again, there were no between groups differences seen in locomotor responding following these two challenge doses of the drug. Lastly, all groups experienced a significant anorexic effect in response to the repeated administration of METH; with the HIV-1 Tg group experiencing a significantly greater reduction in body weight compared to the F control animals.

Studies of CPP are not as straight forward as they seem and often require many pilot studies in order to establish the correct parameters (Bozarth, 1987; van der Kooy, 1987). Several parameters that may affect the development of conditioned place preference include: 1. establishing the optimal dose/drug concentration, 2. a natural preference for the center compartment, 3. the type of discriminative stimuli employed to differentiate compartments, and 4. the type of drug compound being assessed. Each of these parameters will be considered as they relate to the current study.

1. In typical studies of CPP, researchers develop a dose-response curve for the particular drug and animals they are assessing, which describes the relative biological and/or behavioral

effectiveness for given dose/drug concentration (Meyer & Quenzer, 2005). For instance, Suzuki and Misawa (1995) administered 0.25, 0.5, 2.0, 4.0, and 8.0 mg/kg I.P. of METH, with place conditioning resulting at the 2.0, 4.0, and 8.0 mg/kg dosages; however, it was found that only at the 2.0mg/kg dose was conditioning statistically significant. Developing such a dose-response curve would hence require many groups of animals, which was a luxury we did not have, due to the high cost of the HIV-1 transgenic animals. Had we had the requisite number of animals in order to plot a dose-response curve for METH as Suzuki and Misawa plotted, it would have permitted us the ability to identify the optimal dose required to effectively induce drug-seeking behavior within these animals. However, the dose used in this study has been shown in previous research (e.g. Tokuyama, et al., 1996) to produce robust drug-seeking behavior in tests of METH-induced place preference. When working with these animals in the future it would be prudent to develop a dose-response curve using the standard Fischer (F) control animals; doing so would permit an initial evaluation of dose effectiveness and demonstrate the development of place preference prior to any evaluation of between group differences in the transgenic animals.

2. In the current study, the strong preference observed for the center compartment may have been a contributing factor of the failure of place preference to develop. A preference for the center compartment is not unusual and most published papers do not report this preference for the middle compartment, since it is assumed that a reinforcing drug will produce a preference for a drug-paired compartment regardless of the initial low preference for that compartment (M. Vigorito, personal communication, June 7, 2007). However, we attempted to evaluate the possibility that the preference for the center compartment did contribute to the failure of CPP, by analyzing the data obtained from animals that entered the A and/or B compartment for at least 30% of the total test session time during the cycle 2 CPP test; place preference was still not

observed in the animals that met this criterion either. Thus, the failure to observe a conditioning place preference could not solely be attributed to a natural preference for the center compartment. Yet another possible explanation for the failure of place preference to be observed is the contextual cues used as discriminative stimuli to differentiate compartments in this study.

3. Studies of place preference generally utilize a combination of discriminative cues from different sensory modalities, such as visual (e.g. black/white boxes; black/white vertical or horizontal stripes), tactile (e.g. steel bar/wire mesh flooring), olfactory (almond/orange scents), and thermal variations (Bozarth, 1987). Although, it is not uncommon for CPP to be observed using discriminative cues from only one sensory modality; when this is done, the cues are usually visual in nature, but it is not unusual for the use of only tactile cues in establishing CPP. For instance, Davis and colleagues (2007) recently established morphine-induced CPP using only tactile cues (i.e. smooth Plexiglas and textured plastic) to differentiate the drug-and saline-paired compartments. It may be that the floor textures used in this study, which were employed in order to control for group differences in visual acuity were not sufficiently salient to establish CPP. However, unpublished research conducted within this laboratory using the same apparatus and discriminative cues showed significant and robust CPP using morphine; thus, it may be that METH is not as reinforcing as morphine and therefore, requires more salient discriminative stimuli. In deed, a brief review of the literature indicates that multiple discriminative cues (typically, visual and tactile) are employed when assessing METH-induced CPP; this may speak to the psychopharmacodynamic nature of methamphetamine.

4. All compounds that have rewarding properties produce CPP (Bozarth, 1987); however, some drugs of abuse may more readily produce a CPP than others. As shown in figure 13, morphine-and cocaine-induced CPP yielded dramatically more "hits" for published articles when

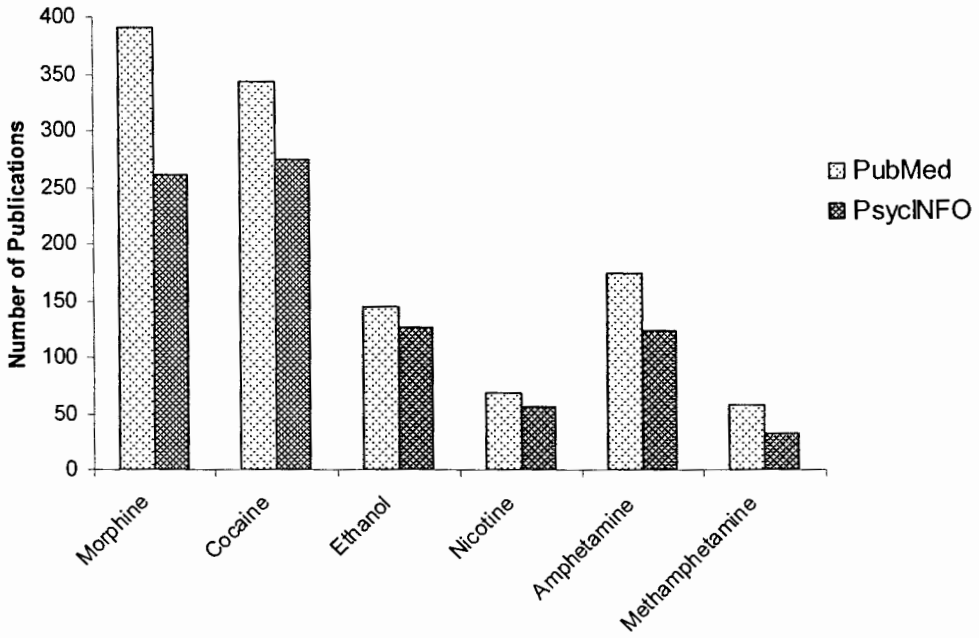


Figure 12. Research (i.e. number of publications) crossing a drug of abuse (e.g. "ethanol") and "conditioned place preference" as keywords using two popular search engines: PubMed and PsychINFO.

compared to other commonly studied drugs of abuse, such as ethanol, nicotine and amphetamine using two popular search engines (PubMed and PsychINFO). It is interesting to note that methamphetamine-induced CPP yielded the fewest number of "hits" for published articles in both search engines when compared to all other drugs of abuse searched. Taken by itself this provides no direct evidence for the ineffectiveness of METH to induce CPP, as it may only suggest that there is more interest (and funding) in assessing the addictive properties of morphine and/or cocaine. However, it may reflect an inherent difficulty in assessing the additive properties of METH using the CPP paradigm. As such, it is more likely that successfully conducted research (leading to published articles), which utilize CPP as a method of assessing the addictive

properties of a drug, if the drug of abuse in question readily induces a CPP. Thus, it may be the unique combination of psychopharmacodynamic properties associated with methamphetamine increase the complexity and difficulty of establishing the appropriate parameters required to use the CPP paradigm to assess the addictive properties of the drug.

It is interesting to note that a weak CPP was observed when animals were tested for place preference after being injected with 1.0mg/kg METH. This observation suggests that a significantly physiologically salient (0.5mg/kg did not induce such an effect) challenge injection of the drug induces stronger drug-seeking behavior within the drug-paired location. This context specific drug-seeking effect could not be explained by simple increases in locomotor responding, as the results showed no significant difference between the rate of locomotor behaviors in the drug-and saline-paired contexts. Thus, METH-induced increases in locomotor behavior were context unspecific. This finding confirms the observations made during the initial pilot investigations, which suggested that the expression of CPP was increased under the influence of the drug. However, this evidence stands in contrast to a number of reports where injections of the drug, made following the successful induction of CPP, diminish or abolish the expression of place preference. For instance, a recent study conducted by Cherng and colleagues (2007), which demonstrated that a single challenge dose of 1.0mg/kg METH abolished the expression of place preference in animals that had previously been conditioned using the same dose. It is generally accepted that a priming injection of a drug (or drugs within its pharmacological class) will reinstate drug-seeking behavior in animals that have undergone extinction sessions. However, this may be the first reported evidence demonstrating that an injection of a lower dose of the drug than was initially used to condition place preference actually *increases* the expression of drug-seeking behavior.

The main purpose of this study was to ascertain any group differences in CPP, but no such differences were found. With no (or very weak) evidence of a CPP, the conclusion that the HIV-1 animals are not more sensitive to the METH-induced CPP is difficult to evaluate; though the results of the study do not seem to suggest any differences even if a METH-induced CPP was observed. Moreover, it was found that METH increased locomotor activity in a dose dependant manner, but likewise there was no evidence to suggest a greater behavioral sensitivity in the HIV-1 Tg animals. As mentioned previously, the procedure employed in this study was not ideally set up to assess behavioral sensitization. The requisite drug-naïve control group that should only receive a vehicle was not available in this study. However, it was reasoned that a group that is more sensitive to the sensitizing effects of a drug would be more expected to increase locomotor behavior in response to a lower dose compared to a group that is less sensitive. These group differences were also not found.

The results of this study provide no evidence in support the hypothesis that the HIV-1 Tg rats are more sensitive the addictive properties of methamphetamine. However, the fact that the HIV-1 Tg rats displayed greater anorexic effects in response to the repeated administration of METH, suggests that these animals may be more sensitive to the toxic effects of the drug; a result that is consistent with other animal and human studies (Chang, Ernt, Speck, & Grob, 2005; Langford, Grigorian, Hurford, Adame, Crew, & Masliah, 2004; Maragos, et al., 2002; Turchan, Anderson, Hauser, Sun, Zhang, Liu, et al., 2001; Wang, Chang, Volkow, Telang, Logan, Ernst & Fowler, 2004). Further research conducted on this phenomenon may serve to more fully characterize the physiological effects associated with this "double epidemic" – the prevalence of illicit methamphetamine abuse within the HIV+, MSM community. In conclusion, the null results of this study do not permit us to completely reject the Chang and Vigorito's (2006)

hypothesis that the HIV-1 infection increases ones susceptibility to the addictive properties of drugs of abuse, but the results are not promising. Instead it may be more productive to examine how drugs of abuse interact with HIV-1 to put the patient at greater risk for neurobiological deficits. It has been reported that 50% or more of HIV-1 infected individuals experience some form of neurocognitive impairment (Ellis, Langford, & Masliah, 2007; Hinkin, Castellon, Atkinson, & Goodkin, 2001). Numerous reports suggest METH and HIV-associated proteins interact within the CNS to damage various sites associated with cognitive functioning (Chang, et al., 2005; Maragos, 2002; Langford, et al., 2004; Turchan, et al., 2001). Indeed, as reported by Rippeth and colleagues (2004), HIV+ individuals with a history of METH use were more likely to suffer from various neurocognitive impairments than non-users. Moreover, recent research conducted within this laboratory has suggested that the transgenic HIV-1 Tg rat, will serve as a suitable animal model of HIV-1 in which to study the neurocognitive deficits associated with the infection (Vigorito, LaShomb, & Chang, 2007).

References

Achat-Mendes, C., Ali, S.F., & Itzhak, Y. (2005). Differential effects of amphetamine-induced neurotoxicity on appetitive and aversive Pavlovian conditioning in mice. *Neuropsychopharmacology, 30, 6,* 1128-1137.

Achat-Mendes, C., Anderson, K.L., & Itzhak, Y. (2007). Impairment in consolidation of learned place preference following dopaminergic neurotoxicity in mice is ameliorated by N-acetylcysteine but not D1 and D2 dopamine receptor agonists. *Neuropsychopharmacology, 32, 3,* 531-541.

Ahmed, K. (2002). Additive drug increases HIV replication and mutation. *The Lancet: Infectious Diseases, 2,* 456.

Anagnostaras, S.G. & Robinson, T.E. (1996). Sensitization to the psychomotor stimulant effects of amphetamine: Modulation by associative learning. *Behavioral Neuroscience, 110,* 1397-1414.

Antoniou, K. & Kafetzopoulos, E. (1991). A comparative study of the behavioral effects of d-amphetamine and apomorphine in the rat. *Pharmacology, Biochemistry & Behavior, 39,* 61-70.

Bansal, A.K., Mactutus, C.F., Nath, A., Maragos, W., Hauser, K.F., & Booze, R.M. (2000). Neurotoxicity of HIV-1 proteins gp120 and Tat in the rat striatum. *Brain Research, 879,* 42-49.

Barr, M.C., Huitron-Resendiz, S., Sanchez-Alavez, M., Henricksen, S.J., & Phillips, T.R. (2003). Escalating morphine exposures followed by withdrawal in feline immunodeficiency virus-infected cats: A model for HIV infection in chronic opiate abusers. *Drug and Alcohol Dependence, 72,* 141-149.

Barroso, J., Burrage, J., Carlson, J., & Carlson Waag, B. (2006). Salivary Cortisol values in HIV-positive people. *Journal of the Association of Nurses in AIDS Care, 17, 3,* 29-36.

Bennett, B.A., Rusyniak, D.E., & Hollingsworth, C.K. (1995). HIV-1 gp120-induced neurotoxicity to midbrain dopamine cultures. *Brain Research, 705,* 168-76.

Berridge, K.C. (2003). Pleasures of the brain. *Brain and Cognition 52,* 106-128.

Berridge, K.C. (2004). Motivation concepts in behavioral neuroscience. *Physiology & Behavior, 81,* 179-209.

Berridge, K.C. & Robinson, T.E. (1998). What is the role of dopamine in reward: Hedonic impact, reward, learning, or incentive salience? *Brain Research Reviews, 28,* 309-369.

Berridge, K.C. & Robinson, T.E. (2003). Parsing reward. *Trends in Neuroscience, 26, 9,* 507-513.

Bozarth, M.A. (1987). Conditioned place preference: A parametric analysis using systemic heroin injections. In M.A. Bozarth (Ed.), *Methods of assessing the reinforcing properties*

Bruce-Keller, A.J., Chauhan, A., Dimayuga, F.O., Gee, J., Keller, J.N., & Nath, A. (2003). Synaptic transport of human immunodeficiency virus-Tat protein causes neurotoxicity and gliosis in rat brain. *Journal of Neuroscience, 23, 23,* 8417-8422.

Carey, C.L., Woods, S.P., Rippeth, J.D., Gonzalez, R., Heaton, R.K., & Grant, I. (2006). Additive deleterious effects of methamphetamine dependence and immunosuppression on neuropsychological functioning in HIV infection. *AIDS and Behavior, 10, 2,* 185-190.

Carlson, N.R. (2007). *Physiology of behavior, 9th ed.* Needham Heights, MA: Allyn and Bacon.

Carr, G.D. & White, N.M. (1983). Conditioned place preference from intra-accumbens but not intra-caudate amphetamine injections. *Life Sciences, 33,* 2551-2557.

Cass, W.A., Harned, M. E., Peters, L.E., Math, A., Maragos, W.F. (2003). HIV-1 protein Tat of methamphetamine-induced decreases in evoked overflow of dopamine in the striatum of the rat. *Brain Research, 984,* 133-142.

Caul, W.F., Jones, J.R., & Barrett, R.J. (1988). Amphetamine's effects on food consumption and body weight: The role of adaptive processes. *Behavioral Neuroscience, 102, 3,* 441-450.

Center for Disease Control CDC. (1999). *HIV/AIDS surveillance report 1999, 11, 2.* Retrieved January 31, 2007, from http://www.cdc.gov/hiv/stats/hasr1102.pdf

Center for Disease Control CDC. (2005). *HIV/AIDS surveillance report 2005, 16.* Retrieved January 31, 2007, from http://www.cdc.gov/hiv/topics/surveillance/resources/reports/2004report/pdf/2004SurveillanceReport.pdf

Center for Disease Control (CDC), Morbidity and Mortality Weekly Report (MMWR). (2006, June 2). *Epidemiology of HIV/AIDS --- United States, 1981-2005.* Retrieved January 31, 2007, from http://www.cdc.gov/mmwr/PDF/wk/mm5521.pdf

Chang, L., Ernst, T., Speck, O., & Grob, C.S. (2005). Additive effects of HIV and chronic methamphetamine use on brain metabolite abnormalities. *American Journal of Psychiatry, 162, 2,* 361-369.

Chang, S.L. & Vigorito, M. (2006). Role of HIV-1 infection in addictive behavior: A study of a HIV-1 transgenic rat model. *American Journal of Infectious Disease, 2, 2,* 98-106.

Chang, S.L., Beltran, J.A., & Swarup, S. (2007). Expression of the mu opioid receptor in the human immunodeficiency virus type 1 transgenic rat model. *Journal of Virology, 81, 16,* 8406-8411.

Cherng, C.G., Tsai, C.-W., Tsai, Y.-P., Ho, M.-C., Kao, S.-F., & Yu, L. (2007-In Press). Methamphetamine-disrupted sensory processing mediates conditioned place preference performance. *Behavioural Brain Research.*

Chuahan, A., Turchan, J., Pocernich, C., Bruce-Keller, A., Roth, S., Butterfields, D.A., Major, E.O., & Nath, A. (2003). Intracellular immunodeficiency virus Tat expression in astrocytes promotes astrocytes survival but induces potent neurotoxicity at distant sites via axonal transport. *Journal of Biological Chemistry, 278, 15,* 13512-12519.

Chiu, C.-T., Ma, T, & Ho, I.K. (2005). Attenuation of methamphetamine-induced behavioral sensitization in mice by systemic administration of naltrexone. *Brain Research Bulletin, 67,* 100-109.

Cloak, C.C. Chang, L., Ernst, T., Barr, M.C., Huitron-Resendiz, S., Sanchez-Alavez, M., & Henriksen, S. (2004). Methamphetamine and IAD: HMRS studies in a feline model of human disease. *Journal of Neuroimmunology, 147,* 16-20.

Davis, C.M., Roma, P.G., Dominguez, J.M., & Riley, A.L. (2007). Morphine-induced place preference in Fischer and Lewis rats: Acquisition and dose-response in a fully biased procedure. *Pharmacology, Biochemistry, and Behavior, 86,* 516-523.

De Fonseca, F.R., Rubio, P., Martín-Calderón, J.L., Caine, S.B., Koob, G.F., & Navarro, M. (1995). The dopamine receptor agonist 7-OH-DPAT modulates the acquisition and expression of morphine-induced place preference. *European Journal of Pharmacology, 274,* 47-55.

Dennett, D. C. (2003). *Freedom evolves.* New York, NY: Viking Press.

Domjan, M. (2006). *The principles of learning and behavior,* 5th ed. Belmont, CA: Wadsworth/Thompson Learning.

Ellis, R.J., Childers, M.E., Lazzaretto, D., Latendre, S., Grant, I., & HIV Neurobehavioral Research Center Group. (2003). Increased human immunodeficiency virus loads in active methamphetamine users are explained by reduced effectiveness of antiretroviral therapy [Abstract]. *Journal of Infectious Disease, 188, 12,* 1820-1826.

Ellis, R., Langford, D. & Masliah, E. (2007). HIV and antiretroviral therapy in the brain: Neuronal injury and repair. *Nature Reviews: Neuroscience, 8,* 33-44.

Everall, I., Salaria, S., Roberts, E., Corbeil, J., Sasik, R., Fox, H., Grant, I., Masliah, E., & HNRC Group. (2005). Methamphetamine stimulates interferon inducible genes in HIV infected brain, *Journal of Neuroimmunology, 170,* 158-171.

Flora, G., Lee, Y.W., Nath, A., Hennig, B., Maragos, W., & Toborek, M. (2003). Methamphetamine potentiates HIV-1 Tat protein-mediated activation of redox-sensitive pathways in discrete regions of the brain. *Experimental Neurology, 179,* 60-70.

Frosch, D., Shoptaw, S., Huber, A., Rawson, R.A., & Ling, W. (1996). Sexual HIV risk among gay and bisexual male methamphetamine abusers. *Journal of Substance Abuse Treatment, 13, 6,* 483-486.

Gavrilin, M.A., Mathes, L.E., & Podell, M. (2002). Methamphetamine enhances cell-associated feline immunodeficiency virus replication in astrocytes [Abstract]. *Journal of Neurovirology, 8,* 240-249.

Ginawi, O.T., Al-Majed, A.A., & Al-Suwailem, A.K. (2005[A]). NAN-190, a possible specific antagonist for methamphetamine. *Regulatory Toxicology and Pharmacology, 41, 2,* 122-127.

Ginawi, O.T., Al-Majed, A.A., & Al-Suwailem, A.K. (2005[B]). Ondansetron, a selective 5-HT3 antagonist, antagonizes methamphetamine-induced anorexia in mice. *Pharmacological Research, 51, 3,* 255-259.

Giorgi, O., Lecca, D., Piras, D., Driscoll, P, & Corda, M.G. (2003). Dissociation between mesocortical dopamine release and fear-related behaviours in two psychogenetically selected lines of rats that differ in coping strategies to aversive conditions. *European Journal of Neuroscience, 17, 12,* 2716-2726.

Glynn, M.K., & Rhodes, P. (2005, June, 14). *Estimated HIV prevalence in the United States at the end of 2003* [Abstract]. Paper presented at the 2005 National HIV Prevention Conference. Retrieved January 31, 2007, from http://www.aegis.com/conferences/NHIVPC/2005/T1-B1101.html

Goeders, J.E. & Goeders, N.E. (2004). Effects of oxazepam on methamphetamine-induced conditioned place preference. *Pharmacology, Biochemistry, and Behavior, 78, 1,* 185-188.

Goodkin, K., Wilkie, F.L., Concha, M., Hinkin, C.H., Symes, S., Baldewicz, T.T., Asthana, D., Fujimura, R.K., Lee, D., van Zuilen, M.H., Khamis, I., Shapshal, P., & Eisdorfer, C. (2001). Aging and neuro-AIDS conditions and the changing spectrum of HIV-1-associated morbidity and mortality. *Journal of Clinical Epidemiology, 54,* S35-S43.

Grant, I., Heaton, R.K., Dawson, L.K., & Marcotte, T.D. (1999). Abuse of methamphetamine and cocaine may enhance HIV associated neurotoxicity [Abstract]. *Archives of Clinical Neuropsychology, 14, 1,* 130.

Gravetter, F.J. & Wallnau, L.B. (2007). *Statistics for the behavioral sciences* (7th ed.). New York: Thompson Higher Education.

Hanania, T., Gulley, J.M., Salaz, D.O., Larson, G.A., & Zahniser, N.R. (2004). Role of the dopamine transporter in the differential cocaine-induced locomotor activation of inbred long-sleep and short-sleep mice. *Neuropsychopharmacology, 29,* 1814-1822.

Halkitis, P.N., Parsons, J.T., & Stirrat, M.J. (2001). A double epidemic: Crystal methamphetamine drug use in relation to HIV transmission among gay men [Abstract]. *Journal of Homosexuality, 41, 2,* 17-36.

Hayaki, J., Anderson, B., & Stein, M. (2006). Sexual risk behaviors among substance users: relationship to impulsivity. *Psychology of Addictive Behaviors, 20, 3,* 328-332.

Heaton, G.I., Dawson, R.K., & Marcotte, T.D. (1999, January). Abuse of methamphetamine and cocaine may enhance HIV associated neurotoxicity [Abstract]. *Archives of Clinical Neuropsychology, 14, 1,* 130.

Hinkin, C.H., Castellon, S.A., Atkinson, J.H., & Goodkin, K. (2001). Neuropsychiatric aspects of HIV infection among older adults. *Journal of Clinical Epidemiology, 54,* S44-S52.

Itzhak, Y., Martin, J.L., & Ali, S.F. (2002). Methamphetamine-induced dopaminergic neurotoxicity in mice: Long-lasting sensitization to the locomotor stimulation and desensitization to the rewarding effects of methamphetamine. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 26,* 1177-1183.

Iwasa, H., Kikuchi, S., Hasagawa, S., Suzuki, K., & Sato, T. (1996). Alteration of G protein subclass mRNAs in methamphetamine-induced behavioral sensitization [Abstract]. *Annals of the New York Academy of Sciences, 801,* 110-115.

Kalivas, P.W. (2003). Predisposition to addiction: Pharmacokinetics, pharmacodynamics, and brain circuitry. *American Journal of Psychiatry, 160,* 1-2.

Kapinsky, K.L., Bao, J., & Lin, Y.W. (2007). Neurobiology of HIV, psychiatric and substance abuse comorbidity research: Workshop report. *Brain, Behavior, and Immunity, 21,* 428-441.

Kauer, J.A. (2004). Learning mechanisms in addiction: Synaptic plasticity in the ventral tegmental area as a result of exposure to drugs of abuse. *Annual Reviews Physiology, 66,* 447-475.

Kelley, A.E. & Berridge, K.C. (2002). The neuroscience of natural rewards: Relevance to addictive drugs. *The Journal of Neuroscience, 22, 9,* 3306-3311.

King, J.E., Eugenin, E.A., Buckner, C.M., & Berman, J.W. (2006). HIV tat and neurotoxicity. *Microbes and Infection, 8,* 1347-1357.

Kolb, B. & Whishaw, I.Q. (2003). *Fundamentals of human neuropsychological, 5th ed.* New York, NY: Worth Publishers.

Koob, G.F. & Le Moal, M. (1997). Drug abuse: Hedonic homeostatic dysregulation. *Science, 278,* 52-58

Ottoni, E.B. (2000). EthoLog 2.2: a tool for the transcription and timing of behavioral observation sessions. *Behavioral Research Methods, Instrumentation & Computation, 32, 3,* 446-449.

Langford, D., Grigorian, A., Hurford, R., Adame, A., Crews, L., & Masliah, E. (2004). The role of mitochondrial alterations in the combined toxic effects of human immunodeficiency virus Tat protein and methamphetamine on calbindin positive-neurons. *Journal of Neurovirology, 10, 6,* 327-37.

LaShomb, A. L., Vigorito, M., Root, D.H., & Chang, S.L. (2006). *HIV-1 Transgenic Rats Display Greater Freezing in a Fear Conditioning Paradigm.* Poster presented at the Association for Psychological Science, 18th Annual Convention, New York, NY.

Levine, A.J., Hinkin, C.H., Marion, S., Keuning, A., Castellon, S.A., Lam, M.M., Robinet, M., Longshore, D., Newton, T., Myers, H., & Durvasula, R.S. (2006). Adherence to antiretroviral medications in HIV: Differences in data collected via self-report and electronic monitoring. *Health Psychology, 25, 3,* 329-335.

Magendzo, K. & Bustos, G. (2003). Expression of amphetamine-induced behavioral sensitization after short- and long-term withdrawal periods: Participation of μ- and δ-opioid receptors. *Neuropsychopharmacology, 28,* 468-477.

Maragos, W.F., Young, K.L., Turchan, J.T., Guseva, M., Pauly, J.R., Math, A., Cass, W.A. (2002). Human immunodeficiency virus-1 Tat protein and methamphetamine interact synergistically to impair striatal dopaminergic functioning. *Journal of Neurochemistry, 83, 4,* 955-963.

McCann, U.D., Wong, D.F., Yokoi, F., Villemagne, V., Dannals, R.F., & Ricaurte, G.A. (1998). Reduced striatal dopamine transporter density in abstinent methamphetamine and methcathinone users: evidence from positron emission tomography studies with [^{11}C]WIN-35,428. *The Journal of Neuroscience, 18, 20,* 8417-842.

Meyer, J.S. & Quenzer, L.F. (2005). *Psychopharmacology: drugs, the brain, and behavior.* Sunderland, MA: Sinauer Associates, Inc.

Nath, A., Anderson, C., Jones, M., Maragos, W., Booze, R., Mactutus, C., Bell, J., Hauser, K.F., & Mattson, M. (2000). Neurotoxicity and dysfunction of dopaminergic systems associated with AIDS dementia. *Journal of Psychopharmacology, 14, 3,* 222-227.

National Institute on Drug Abuse (NIDA) InfoFacts: Treatment approaches for drug addiction (August, 2006). Retrieved January 31, 2007, from http://www.drugabuse.gov/infofacts/treatmeth.html

Ohmori, T., Abekawa, T., & Koyama, T. (1996). The role of glutamate in behavioral and neurotoxic effects of methamphetamine. *Neurochemistry International, 29, 3,* 301-307.

Paulson, P.E., Camp, D.M., & Robinson, T.E. (1991). The time course of transient behavioral depression and persistent behavioral sensitization in relation to regional brain monoamine concentrations during amphetamine withdrawal in rats. *Psychopharmacology, 103,* 480-492.

Peretti-Watel, P., Spire, B., Lert, F., Obadia, Y., & the VESPA Group. (2006). Drug use patterns and adherence to treatment among HIV-positive patients: Evidence from a large sample of French outpatients (ANRS-EN12-VESPA 2003). *Drug and Alcohol Dependence, 82, 1,* S71-S79.

Pierce, R.C. & Kumarensan, V. (2006). The mesolimbic dopamine system: The final common pathway for the reinforcing effects of drugs of abuse? *Neuroscience and Biobehavioral Reviews, 30,* 215-238.

Phillips, A.G. & Fibiger, H.C. (1987). Anatomical and neurochemical substrates of drug reward determined by the conditioned place preference technique. In M.A. Bozarth (Ed.), *Methods of assessing the reinforcing properties of abused drugs* (pp. 275-290). New York, NY: Springer-Verlag.

Reback, C.J. & Ditman, D. (1997). *The social construction of a gay drug: Methamphetamine use among gay and bisexual males in Los Angeles.* City of Los Angeles, AIDS Coordinator.

Reback, C.J., Kamien, J.B., & Amass, L. (2007). Characteristics an HIV risk behaviors among homeless, substance-using men who have sex with men. *Addictive Behaviors, 32,* 647-654.

Reid, W., Sadowska, M., Denaro, F., Rao, S., Foulke, J., Hayes, N., et al. (2001). An HIV-1 transgenic rat that develops immunologic dysfunction. *Proc Natl Acad Sci, 98, 16,* 9271-9276.

Reid, W., Abdelwahab, S., Sadowska, M., Huso, D., Neal, A., Ahearn, A., et al. (2004). HIV-1 transgenic rats develop T cell abnormalities. *Virology, 321,* 111-119.

Reinhard, M.J., Hinkin, C.H., Barclay, T.R., Levine, A.J., Marion, S., Castellon, S.A., Longshore, D., Newton, T., Durvasula, R.S., Lam, M.N., & Myers, H. (2007). Discrepancies between self-report and objective measures for stimulant drug use in HIV: Cognitive, medication adherence and psychological correlates. *Addictive Behaviors,* in press.

Rippeth, J.D., Heaton, R.K., Carey, C.L., Marcotte, T.D., Moore, D.J., Gonzolez, R., Wolfson, T., Grant, I., & HNRC Group. (2004). Methamphetamine dependence increases risk of neurological impairment in HIV infected persons. *Journal of International Neuropsychological Society, 10,1,* 1-14.

Robinson, L. & Rempel, H. (2006). Methamphetamine use and HIV symptom self-management. *Journal of the Association of Nurses in AIDS Care, 17, 5,* 7-14.

Robinson, T.E. & Becker, J.B. (1986). Enduring changes in brain and behavior produced by chronic amphetamine administration: A review and evaluation of animal models of amphetamine psychosis. *Brain Research Reviews, 11,* 157-198.

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

Robinson, T.E. & Berridge, K.C. (2000). The psychology and neurobiology of addiction: An incentive-sensitization view. *Addiction, 95, 2,* S91-S117.

Robinson, T.E. & Berridge, K.C. (2001). Incentive-sensitization and addiction. *Addiction, 96,* 103-114.

Robinson, T.E. & Berridge, K.C. (2003). Addiction. *Annual Reviews Psychology, 54,* 25-53.

Robinson, T.E. & Kolb, B. (1997). Persistent structural modifications in nucleus accumbens and prefrontal cortex neurons produced by previous experience with amphetamine. *Journal of Neuroscience, 17, 21,* 8491-8497.

Robinson, T.E. & Kolb, B. (1999). Alterations in the morphology of dendrites and dendritic spines in the nucleus accumbens and prefrontal cortex following repeated treatment with amphetamine or cocaine [Abstract]. *European Journal of Neuroscience, 11, 5,* 1598-1604.

Roitman , M.F., Wheeler, R.A., & Carelli, R.M. (2005). Nucleus accumbens neurons are innately tuned for rewarding and aversive taste stimuli, encode their predictors, and are linked to motor output. *Neuron, 17, 45,* 587-597.

Seigel, S. (1985). Drug-anticipatory responses in animals. In White, L., Tursky, B., & Scwartz, B. (Eds.), *Placebo: Theory, Research and Mechanisms* (pp. 288-305). New York, NY: Guilford Press.

Semple, S.J., Patterson, T.L., & Grant, I. (2002). Motivations associated with methamphetamine use among HIV+ men who have sex with men. *Journal of Substance Abuse Treatment, 22,* 149-156.

Semple, S.J., Patterson, T.L., & Grant, I. (2004[A]). The context of sexual risk behavior among heterosexual methamphetamine users. *Addictive Behaviors, 29,* 807-810.

Semple, S.J., Patterson, T.L., & Grant, I. (2004[B]). A comparison of injection and non-injection methamphetamine-using HIV positive men who have sex with men. *Drug and Alcohol Dependence, 76,* 203-212.

Shimasota, K. & Ohkuma, S. (1999). Simultaneous monitoring of conditioned place preference and locomotor sensitization following repeated administration of cocaine and methamphetamine. *Pharmacology Biochemistry and Behavior, 66, 2,* 2850292.

Shoptaw, S., Peck, J., Reback, C.J., & Rotheram-Fuller, E. (2003). Psychiatric and substance dependence comorbidities, sexually transmitted diseases, and risk behaviors among methamphetamine-dependant gay and bisexual men seeking outpatient drug abuse treatment. *Journal of Psychoactive Drugs, 35, 1,* 161-168.

Shoptaw, S., Reback, C.J., Peck, J.A., Yang, X., Rotheram-Fuller, E., Larkins, S., Veniegas, R.C., Freese, T.E., & Hucks-Ortiz, C. (2005). Behavioral treatment approaches for methamphetamine dependence and HIV-related sexual risk behaviors among urban gay and bisexual men. *Drug and Alcohol Dependence, 78,* 125-134.

Stall, R., Paul, J.P., Greenwood, G., Pollack, L.M., Bein, E., Crosby, G.M., Mills, T.C., Binson, D., Coates, T.J., Catania, J.A. (2001). Alcohol use, drug, use, and alcohol-related problems among men who have sex with men: The Urban Men's Health Study. *Addiction, 96,* 1589-1601.

Strakowski, S.M., & Sax, K.W. (1998). Progressive behavioral response to repeated d-amphetamine challenge: Further evidence for sensitization in humans. *Biological Psychiatry, 44,* 1171-1177.

Strakowski, S.M., Sax, K.W., Setters, M.J., & Keck, P.E. Jr. (1996). Enhanced response to repeated d-amphetamine challenge: Evidence for behavioral sensitization in humans. *Biological Psychiatry, 40,* 872-880.

Stolerman, I. (1992). Drugs of abuse: Behavioural principles, methods and terms. *Trends in Pharmacological Sciences, 13, 5,* 170-176.

Suzuki, T. & Misawa, M. (1995). Sertindole antagonizes morphine-, cocaine-, and methamphetamine-induced place preference in the rat. *Life Sciences, 57, 13,* 1277-1284.

Theodore, S., Cass, W.A., & Maragos, W.F. (2006). Methamphetamine and human immunodeficiency virus protein Tat synergize to destroy dopaminergic terminals in the rat striatum. *Neuroscience, 137,* 925-935.

Tien, L.-T., Ho, I.-K., Loh, H.H., & Ma, T. (2007). Role of μ-opioid receptor in modulation pf preproenkephalin mRHA expression and opioid and dopamine receptor binging in methamphetamine-sensitized mice. *Journal of Neuroscience Research, 85,* 673-680.

Tokuyama, S. Takahashi, M. & Kaneto, H. (1996). The effect of ginseng extract on locomotor sensitization and conditioned place preference induced by methamphetamine and cocaine in mice. *Pharmacology Biochemistry and Behavior, 54, 4,* 671-676.

Turchan, J., Anderson, C., Hauser, F.F., Sun, Q., Zhang, J., Liu, Y., Wise, P.M., Kruman, I., Maragos, W., Mattson, M.P., Booze, R., & Nath, A. (2001). Estrogen protects against the synergistic toxicity by HIV proteins, methamphetamine and cocaine. *BMC Neuroscience, 2, 3.*

Van der Kooy, D. (1987). Place conditioning: A simple and effective method for assessing the motivational properties of drugs. In M.A. Bozarth (Ed.), *Methods of assessing the reinforcing properties of abused drugs* (pp. 229-240). New York, NY: Springer-Verlag.

Vanderschuren, L.J.M.J. & Kalivas, P.W. (2000). Alterations in dopaminergic and glutamatergic transmission in the induction and expression of behavioral sensitization. *Psychopharmacology, 151, 99-120.*

Vigorito, M., Lashomb, AL, & Chang SL (2007). Learning and memory in the HIV-1 transgenic rat, *Journal of Neuroimmune Pharmacology*, in press.

Wang, G.-J., Chang, L., Volkow, N.D., Telang, F., Logan, J., Ernst, T., & Fowler, J.S. (2004). Decreased brain dopaminergic transporters in HIV-associated dementia patients. *Brain, 127,* 2452-2458.

Wang, Y.C. & Hsiao, S. (2003). Amphetamine sensitization: Nonassociative and associative components. *Behavioral Neuroscience, 117, 5,* 961-969.

White, F.J. & Kalivas, P.W. (1998). Neuroadaptations involved in amphetamine and cocaine addiction. *Drug and Alcohol Dependence, 51,* 141-153.

Winters, K.C., Remafedi, G., & Chan, B.Y. (1996). Assessing drug abuse among gay-bisexual young men. *Psychology of Addictive Behaviors, 10, 4,* 228-236.

Wise, R.A. & Bozarth, M.A. (1987). A psychomotor stimulant theory of addiction. *Psychological Review, 94, 4,* 469-492.

Zhang, X., Lee, T.H., Xiong, X., Chen, Q., Davidson, C., Wetsel, W.C., & Ellinwood, E.H. (2006). Methamphetamine induced long term changes in $GABA_A$ receptor $\alpha 2$ subunit and GAD_{67} expression. *Biochemical and Biophysical Research Communications, 351, 1,* 300-305.