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Greater Sensitivity to the Stimulating and Anxiety-Like Effects of Methamphetamine in the HIV-1 Transgenic Rat

Marley D. Kass
Seton Hall University

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**GREATER SENSITIVITY TO THE STIMULATING AND ANXIETY-LIKE EFFECTS
OF METHAMPHETAMINE IN THE HIV-1 TRANSGENIC RAT**

by
Marley D. Kass

**Department of Psychology
Seton Hall University**

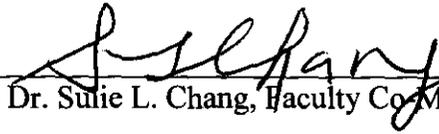
**A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master
of Science in Experimental Psychology with a Concentration in Behavioral Neuroscience**

April, 2009

Approved By:



Dr. Michael Vigorito, Faculty Co-Mentor



Dr. Julie L. Chang, Faculty Co-Mentor



Dr. Amy Hunter, Committee Member



Dr. Kelly Goedert, Committee Member



Dr. Janine Buckner, Director of Graduate Studies

*This thesis is dedicated to my grandparents, Grandma Pearlle and Papa Maxie and
Grandma Rainy and Papa Milton, for their endless love and support. Thank you for all
of the encouragement that you have given to me.*

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Abstract

Methamphetamine (METH) abuse and the Human Immunodeficiency Virus (HIV) are highly comorbid illnesses, and over the past decade this comorbidity has come to be known as a double epidemic (Chang, Ernst, Speck, & Grob, 2005). METH can aggravate and promote the neuropathological deformations caused by HIV, resulting in severe cognitive and motor deficits. Among the HIV population, those who use METH have poorer prognosis and develop HIV-related pathologies sooner than nonusers (Cloak, Chang, Ernst, Barr, Huitron-Resendiz, Sanchez-Alavez, et al., 2004).

Highly active antiretroviral therapies (HAART) can slow the onset of profound neurological impairments such as HIV-associated dementia (HAD) and minor cognitive/motor disorders (MCMD). However, METH users will succumb to more severe cognitive and motor impairments, and at a faster rate, than nonusers despite the use of HAART. Because of this, new treatments will need to be developed to adequately treat the clinical profiles exhibited by METH users who are infected with the HIV virus. The study of METH use and HIV in an animal model may be of use to better develop treatment. Recently, a noninfectious HIV type 1 (HIV-1) transgenic (Tg) rat that displays many of the immune irregularities and clinical abnormalities seen in HIV patients was created (Reid, Sadowska, Denaro, Rao, Foulke, Hayes, et al., 2001). The HIV-1 Tg rat may be a useful animal model for evaluating the effects of METH in the presence of continuous HIV infection on brain function and behavior.

The present set of experiments set out to establish an animal model of HIV-1 and METH use, and extrapolate findings to HIV-infected METH users. In Experiment One, Sprague-Dawley (SD) rats were used to develop a behavioral sensitization (BS) paradigm

and examine drug context effects associated with BS of METH-induced behavior.

Additionally, a modified context pre-exposure facilitation effect (CPFE) paradigm was implemented to assess the effects of METH on one-trial fear conditioning. Experiment Two utilized the HIV-1 Tg rat to evaluate the interactions between METH and the HIV-1 virus on BS, drug context effects, and the CPFE.

The HIV-1 Tg rat exhibited an augmented behavioral response to the psychoactivating properties of METH and increased sensitivity to a stressful event (i.e. a footshock). The maladaptive responses seen in the HIV-1 Tg rat are likely mediated by neuroalterations associated with the virus. These studies indicate that the HIV-1 Tg rat may have a greater sensitivity to the stimulating and anxiety-like effects of METH. The differential effects of METH in HIV-1 Tg rats and normal controls have serious implications for the HIV-infected METH using population.

Introduction

With the advent of highly active antiretroviral therapies (HAART) in the late 1990s, the human immunodeficiency virus (HIV) has become a manageable and chronic illness. HIV-infected individuals with access to HAART, and that are compliant with treatments, now have better prognosis and long life expectancies (Anthony, Ramage, Carnie, Simmonds, & Bell, 2005; McArthur, 2004; Whelan, 2000). HAART can reduce peripheral viral load and retard the progression of HIV. However, HAART is unable to permeate the blood-brain barrier (BBB), leaving HIV untreated in the central nervous system (CNS). Thus, there are still high rates of neurological disorders, such as HIV-associated dementia (HAD) and minor cognitive/motor disorders (MCMD), among the HIV-infected population (Arendt, Hefter, & Jablonowski, 1993; Goodkin, Wilkie, Concha, Hinkin, Symes, Baldewicz, et al., 2001; McArthur, 2004).

There is a substantial comorbidity between substance addiction and HIV. In fact, substance use is a major cause for propagation of the virus (Cass, Harned, Peters, Nath, & Maragos, 2003; Cloak, Chang, Ernst, Barr, Huitron-Resendiz, et al., 2004). The abuse of psychostimulants, and methamphetamine (METH) in particular, is notably high in the HIV population (Chang, et al., 2005; Cloak, et al., 2004; Flora, Lee, Nath, Hennig, Maragos, & Toberek, 2003). HIV and METH have some shared mechanisms of neural degeneration, and thus lead to greater brain damage if both toxins are present in the CNS (Cass, et al., 2003; Chang, et al., 2005; Cloak, et al., 2004; Flora, et al., 2003).

Accordingly, HIV-infected METH users exhibit accelerated disease progression and more profound impairments. Because the drugs used in HAART are unable to cross the BBB, HAART is ill equipped for treating brain dysfunction in this population. Thus, in

addition to treating the immune dysfunction, attention has been directed towards developing adequate treatments for the range of neurological deficits emerging in METH-using HIV patients.

Currently, very little is known about the interactions between HIV and METH. To develop efficacious treatments, the effects of METH need to be studied in the presence of continuous HIV infection, and this task requires the use of animal models. To accurately reflect how HIV and METH affect brain function and behavior in humans, a model needs to integrate an addiction paradigm with an animal model of HIV that parallels the progression of HIV in the HAART era. Experiment One was concerned with developing a behavioral sensitization (BS) paradigm of drug addiction, and identifying how METH affects a simple form of learning known as the context pre-exposure facilitation effect (CPFE) in normal Sprague-Dawley (SD) rats. In Experiment Two, the paradigms used in the first study were adapted and integrated with an animal model of HIV-1, the HIV-1 transgenic (HIV-1 Tg) rat.

Comorbidity of METH and HIV

METH is a highly addictive stimulant that is related to amphetamine (AMPH), but is more potent, and has longer lasting effects on the CNS (The National Institute on Drug Abuse [NIDA], 2008). Although NIDA's (2008) *Monitoring the Future Survey* indicated that METH use among 8th, 10th, and 12th grade students is declining, it was still estimated that about 1.9 million Americans, age 12 and older, had abused METH at least once in 2006. Short and long term effects of METH use can vary from increased wakefulness and activity, decreased appetite, hyperthermia, or irregular, rapid heartbeat to extreme weight loss, dental problems, anxiety, insomnia, or psychosis (NIDA, 2008).

METH can be taken intra-orally, intra-nasally, intrapulmonary, or intravenously (i.v.). Since METH use can undoubtedly lead people to engage in dangerous behaviors, all users, despite route of administration, are at risk for becoming infected with and transmitting HIV. Specifically, altered inhibitions may draw users towards precarious sexual behavior which can spread the virus (Chang, et al., 2005; Ferris, Mactutus, & Booze, 2007; NIDA, 2008). Despite the chancy behavior all types of users engage in, i.v. users are most vulnerable to contracting the virus from injecting with contaminated needles. As such, in the United States i.v drug use is the second most risky behavior directly associated with the transmission of HIV (Ferris, et al., 2007).

The Joint United Nations Programme on HIV/AIDS ([UNAIDS], 2006) has estimated that between 33 and 46 million people are living with HIV globally. In recent years, the lifespan of those infected has been extended due to treatments using HAART, and the incidence rate has plateaued. Thus, the worldwide population of people living with HIV continues to grow, along with the various neurological disorders and illnesses that are emerging in these populations (Ferris, et al., 2007; Goodkin, et al., 2001; McArthur, 2004; UNAIDS, 2006).

The Effects of HIV and METH on the CNS

The effects of METH and HIV on brain function and behavior will vary with the level and duration of CNS exposure. Both neurotoxins exert their effects on similar neurotransmitter systems, particularly the monoamines. Minimal changes will occur in the CNS when exposure is low. However, with higher levels and longer durations of exposure, METH and HIV can produce analogous neurodegeneration. Additionally, each toxin is believed to aggravate CNS alterations associated with the other. For instance,

METH compromises the BBB, leaving the CNS vulnerable to foreign toxic agents. Consequently, METH use contributes to the virus's entry into the CNS, leading to an accelerated pathogenesis of clinical syndromes (King, Eugenin, Buckner, & Berman, 2006). Additionally, HIV causes changes in dopamine (DA) systems, possibly leading to greater sensitivity to METH in the HIV-infected brain.

CNS changes associated with METH. METH is a potent, indirectly acting sympathomimetic amine (i.e., it stimulates the sympathetic nervous system) that, with extreme exposure, can be toxic to monoaminergic systems (Seidon & Sabol, 1995). Research has shown that METH can be extremely toxic to DAergic and serotonergic (5-HTergic) systems (Bowyer & Holson, 1995; Davidson, Gow, Lee, & Ellinwood, 2001; Frost & Cadet, 2000). However, not all METH exposure results in neurotoxic events, which are characterized by a reduction in brain aromatic monamines, and last longer than several hours (Bowyer & Holson, 1995). Furthermore, cognitive deficits can be exhibited in the absence of neurotoxic events. In rodent research, for instance, it has demonstrated that METH can impair performance in an object recognition (OR) task without reducing DA or 5-HT transporter (DAT; SERT) binding (Belcher, O'Dell, & Marshall, 2006).

The reinforcing and behavioral effects of METH are highly attributed to an excess release of DA which occurs because of the drug's ability to block reuptake and cause DATs to run in reverse (Fleckenstein, Gibb, & Hanson, 2000; Kuczenski, Everall, Crews, Adame, Grant, et al., 2007; Suzuki, Mizuo, Nakazawa, Funae, Fushiki, Fukushima, et al., 2003). Thus, the addictive properties of METH are assumed to emanate from DAergic neuronal activity. Specifically, METH causes changes in the factors that regulate

DAergic activity, such as DATs and the DA-synthesizing enzyme tyrosine hydroxylase (TH) (Baucum, Rau, Riddle, Hanson, & Fleckenstein, 2004; Tsuchida, Akiyama, Sakai, Ujike, Li, & Kuroda, 1996).

CNS changes associated with HIV. People living with HIV-1 can be afflicted with various, and sometimes extreme, neurological disorders as the infection progresses (Starling, Wright, Arbuthnott, & Harkiss, 1999). After crossing the BBB, the virus can directly and indirectly lead to CNS abnormalities manifested as MCMD, HAD, or other disturbances in cognition and affect (Barak, Weidenfeld, Goshen, Ben-Hur, Taylor, & Yirmiya, 2002; King, et al., 2006; Lawrence & Major, 2002). Despite its ability to cause apoptosis, HIV-1 does not appear to infect neurons. Instead, the virus infects microglia and other non-neuronal mediums which spread the infection. Thus, the virus initiates a cascading chain of events which eventually lead to neuronal dysfunction (Gurwell, Nath, Sun, Zhang, Martin, Chen, et al., 2001; Lawrence & Major, 2002; Pocernich, Sultana, Mohammad-Abdul, Nath, & Butterfield, 2005).

Viral proteins, such as the envelope glycoprotein 120 (gp120) or trans-activator (tat) protein, are shed into the extracellular space, cerebrospinal fluid (CSF), and sera (Gurwell, et al., 2001; King, et al., 2006). Respectively, tat and gp120 can be taken up by non-infected cells and interfere with normal monoamine transmission and stimulate the release of toxic substances from immune cells (Aksenova, Silvers, Aksenov, Nath, Ray, Mactutus, et al., 2006; Bansal, Mactutus, Nath, Maragos, Hauser, & Booze, 2000; Barak, et al. 2002; Pocernich, et al., 2005). HIV-1 proteins tat and gp120 activate maladaptive immune responses. This leads to neuroinflammation, and eventually HIV-associated encephalitis (HIVE), which produces dramatic changes in cognition.

Volumetric basal ganglia and hippocampal reductions, accompanied by enlarged ventricles, are often identified in HIV patients (Barak, et al., 2002; Ferris, et al., 2008; Iyer, Brooke, & Sapolsky, 1998). Such neuropathological deformations are associated with deficits in certain motor, spatial discrimination, and memory tasks, and, when presented, are reminiscent of subcortical dementias, such as Parkinson's disease (PD). Similar to PD patients, HIV-1 patients exhibit behavioral inflexibility (i.e., failure to change set), among other deficits, which indicates dysfunction in the DA systems.

METH Addiction

It is well documented that those who use METH may experience any combination of the following: bursts of energy, loss of appetite, a sense of well being, confidence, elevated awareness, increased sexual performance, and euphoria (NIDA, 2008). Despite the pleasurable effects of METH, and great potential for abuse, only a relatively small percentage of the population uses METH. Furthermore, not everyone that uses METH continues usage and develops full blown addiction. This is because the stimulating and rewarding properties of METH can be regulated by numerous genetic and environmental factors. Thus, the potential for METH use, and further abuse, is contingent upon such interactions (Caprioli, Celentano, Paolone, & Badiani, 2007; Crombag, Badiani, Chan, Dell'Orco, Dineen, & Robinson, 2001; Crombag, Badiani, Maren, & Robinson, 2000). METH addiction can be understood from an incentive sensitization perspective, because genetic and environmental components of drug taking behavior are integrated in the incentive sensitization theory of addiction.

According to the incentive sensitization theory of addiction, repeated exposure to drugs of abuse can cause changes in neural pathways that normally regulate the

attribution of incentive salience to stimuli, and this process is initially mediated by individual differences and environmental factors (Robinson & Berridge, 2008). In animals, neural sensitization occurs with repeated stimulant administration and the environment surrounding drug administration modulates the expression of neural sensitization as BS (Robinson, Browman, Crombag, & Badiani, 1998). There is a distinction between drug-liking and drug-wanting (i.e., incentive salience). Liking is described as the pleasurable effects of stimuli- stimuli that are sought after because they have subjective pleasurable effects. Wanting, on the other hand, is behavior that seeks out stimuli whether or not they are subjectively pleasurable. Typically, liking and wanting occur together, but they can become dissociated when drugs are the stimuli being sought out. For example, a drug may be consumed initially because of the pleasurable effects (liking), but with repeated exposure drug-seeking behavior may increase (i.e., enhanced incentive salience) even though the hedonic value has diminished or even been eliminated. This increased drug-seeking behavior is “wanting” in the absence of “liking”. Wanting a stimulus without liking it, is viewed as excessive and compulsive addictive behavior. Evidence suggests that there are separate liking and wanting neural pathways in the brain (see Berridge & Kringlebach, 2008 for review). The neural pathways that are involved in drug-wanting are DAergic and are prone to sensitization effects, and through associative mechanisms, the sensitized drug-wanting gets associated to surrounding cues. The neural pathways involved in drug-liking, however, develop tolerance. Incentive sensitization is not easily reversible and hypothesized to persist for extended periods of time, maintaining a compulsive drug-wanting, even after use has stopped.

Associative stimulus-response (S-R) learning is believed to regulate the expression of neural sensitization. However, the flexibility of drug-seeking and –taking behaviors indicates that additional motivational processes are involved. S-R habits promote drug consumption, but the core problem lies within sensitization of neural networks that support drug-wanting. Environmental stimuli can acquire incentive properties through Pavlovian conditioning. Thus, animal studies reveal incentive sensitization if BS is produced, and is also able to facilitate approach behavior, instrumental transfer, and conditioned reinforcement (Robinson & Berridge, 2008). Respectively, these behaviors demonstrate the drug-seeking behavior, drug-taking behavior, and cravings that are seen in humans (Crombag, Badiani, Maren, & Robinson; 2000; Robinson, et al., 1998).

Conditioning of environmental stimuli to the incentive salience attributes of a drug elucidates why users engage in numerous activities to obtain and self-administer drugs. More importantly, it explains why METH users seek and take METH despite the negative outcomes, i.e., contracting HIV, that are associated with it. Conditioned contextual cues can elicit drug-wanting and perpetuate compulsive drug-taking behaviors (Crombag, Badiani, Chan, Dell’Orco, Dineen, & Robinson, 2000). Drug-induced dysfunction in frontal brain structures contributes to pathological behavior in addicts. With compromised executive function, decision making will be impaired and a user will be more likely to succumb to incentive salience that has been attributed to contextual cues. The high concordance of METH addiction and HIV may, in part, be mediated by neuroalterations associated with both toxins that, when together, produce greater incentive sensitization.

Dependent Measures in Animal Models of Drug Use and Addiction

The stimulating effects of METH in animals are thought to be reflected by a range of behaviors. Therefore, in animal models METH exposure is measured via physiological parameters such as feeding, drinking, diuresis, and motorical behaviors (Badiani, Mundl, & Cabilio, 1993). Commonly used dependent measures distinguish between hyperlocomotion and stereotypies. Locomotor activity (i.e., rearing and traveling) is among the many physiological and behavioral processes that DAergic systems regulate (Brown, Bay, Kiyatkin, 2007), and METH can induce psychomotor activation through increasing DA neural transmission (Brennan, Johnstone, Fitzmaurice, Lea, & Schenk, 2007; Brown, et al., 2007; Nakayama, Kitaichi, Ito, Hashimoto, Takagi, Yokoi, et al., 2007). Stereotypic responses are elicited by stimulants, such as AMPH, METH, and cocaine (COC), and are characteristic of moderate and high doses (Gentry, Ghafoor, Wessinger, Laurenzana, Hendrickson, & Owens, 2004; Kuczenski & Segal, 1999). METH-induced stereotypies consist of focal movements such as biting/chewing/gnawing, licking, sniffing, and head weaving (Davidson, et al., 2007; Gentry, et al., 2004; Milesi-Halle, McMillan, Laurenzana, Byrnes-Blake, & Owens, 2007; Segal & Kuczenski, 1987). Stereotypies are often intense in that they appear extremely rapid and repetitive (Abekawa, Ohmori, & Koyama, 1997; Kucenski & Segal, 1999). Further, an absence of locomotion often accompanies extremely intense stereotypy, and repetitive head movements may seem to be fixated or confined to a small area.

After stimulant administration, rodents tend to exhibit certain combinations of these behaviors, which can often be categorized as one of two distinct behavioral profiles

(Segal & Kuczenski, 1987). One pattern is characterized by high levels of locomotion (Subgroup 1 [S₁]), while the other consists of a multiphasic pattern with intense and continuous stereotypy (Subgroup 2 [S₂]) (Kuczenski & Segal, 1999; Kuczenski, et al., 1995; Milesi-Halle, et al., 2007; Segal & Kuczenski, 1999). While both genres of behavior (i.e., locomotion and stereotypies) are caused by changes in DA function, they may be attributable different neural mechanisms (e.g., Abekawa, et al., 1997; Brennan, et al., 2007; Brown, et al., 2007; Davidson, et al., 2002; Gentry, et al., 2004; Kuczenski & Segal, 1999; Segal & Kuczenski, 1987). Hyperlocomotion may be elicited via alterations in the mesolimbic DAergic system and stereotypies are attributed to changes in the nigrostriatal DAergic system (Abekawa, et al., 1997; Bartlett, Hallin, Chapman, & Angrist, 1997; Brennan, et al., 2007; Kuczenski, et al., 1995; Segal & Kuczenski, 1987).

METH-induced BS: An Animal Model for Addiction

In animal models of drug use and addiction, an augmentation of the psychomotor activating effects elicited by AMPH, METH and COC is thought to parallel the progressive, stimulant-induced addiction seen in humans (Abekawa, et al., 1997; Davidson, Lazarus, Xiong, Lee, & Ellinwood 2002; Zhang, Kitaichi, Fujimoto, Nakayama, Shimizu, Iyo, & Hashimoto, 2006). The phenomenon of an escalating and long lasting hyperactive response that can be produced by repeated, intermittent drug exposure is known as BS (Badiani, Browman, & Robinson, 1995; Browman, Badiani, & Robinson, 1998; Crombag, et al., 2001; Ostrander, Hatman, Badiani, Robinson, & Gnegy, 1998). The effects of stimulants that can cause BS include their psychomotor activating effects as well as their rewarding effects (Robinson, et al., 1998). The behavior sensitizing effects of these drugs are thought to be mediated through the

mesolimbic DAergic system, which stimulates brain regions responsible for the rewarding properties of psychostimulants. In animals, BS is demonstrated by an increase in hyperlocomotion or stereotypic behavior that emerges with repeated drug administrations.

The development of BS, and its corresponding neurochemical profiles, can be modulated by many things. The environment surrounding drug administration (e.g., Badiani, Oates, Day, Watson, & Robinson, 1999; Ostrander, Badiani, Day, Norton, Watson, Akil, et al., 2003), dose being used (e.g., Browman, et al., 1998) and individual differences such as sex (e.g., Milesi-Halle, et al., 2007) or stress (e.g., Caprioli, et al., 2007) are among these mediating factors. Thus, BS paradigms are consistent with the incentive sensitization approach to drug use and addiction in that the expression of BS is modulated by drug-context, and reflects neural sensitization of incentive salience.

Environmental modulation of BS. The environment in which psychostimulant drugs are administered can be largely involved in the psychomotor activating effects of those substances. Specifically, the context surrounding drug administration has the potential to enhance or eliminate the development of BS (Crombag, et al., 2000; Crombag, et al., 2001; Fraioli, Crombag, Badiani, & Robinson, 1999; Paolone, Palpoli, Marrone, Nencini, & Badiani, 2003). Additionally, some studies have implicated that environmental modulation of stimulant-induced neural alterations also occurs (Ostrander, et al., 1998; Ostrander, et al., 2003; Uslaner, Badiani, Day, Watson, Akil, & Robinson, 2001).

Environmental novelty mediates stimulant induced psychomotor responses and BS (Fraoli, et al., 1999; Ostrander, et al., 2003). When rodents receive unsignalled i.v.

infusions of AMPH or COC in their home cages, BS does not develop. However, when unsignalled i.v. infusions of AMPH or COC are immediately preceded by placement into a novel context BS will occur (Browman, et al., 1998; Crombag, et al., 1996). When rats are habituated to a novel context for several hours prior to drug administration BS is abolished (Crombag, et al., 2001). These findings suggest that incentive sensitization is manifested behaviorally only when the drug context contains novel cues that drug-wanting can be attributed to. In addition to novelty, environmental distinctness is crucial towards modulating psychostimulant effects. A contextually distinct environment can promote the induction of BS and at a greater rate than a drug context that is discretely different from the home cage (Crombag, et al., 2000). To clarify, a contextually distinct test environment shares no characteristics with the home environment, and a discretely distinct test environment only differs from the home environment with respect to discrete cues such as odor, texture, scent, and light. The more distinct contextual cues there are in a drug environment, the more the environment has the ability to enhance BS (Crombag, et al., 1996; Crombag, et al., 2000; Ostrander, et al., 1998; Robinson, et al., 1998). Thus, the environment surrounding drug administration is thought to produce robust sensitization through facilitating associative learning. This is because the environment in which a stimulant is administered becomes a conditioned stimulus (CS) and can elicit a conditioned response (CR) in the absence of the drug. For example, when rodents are administered a sensitizing regimen of AMPH in a novel context and then later given saline in that context, a conditioned drug response will emerge (Crombag, et al., 2001). The CRs seen in animals reflect human drug cravings that are elicited by cues which have been associated with previous drug-taking. Thus, demonstrating a conditioned drug

response in BS animal paradigms is necessary because it confirms what the incentive sensitization theory predicts: neural sensitization of drug-wanting can be attributed to contextual cues, which in turn will come to elicit drug-wanting.

Contextual Fear Conditioning

CPFE Paradigm

Contextual fear conditioning paradigms are commonly used to study the neurobiology of associative learning and emotional behavior. After an animal is placed in an operant chamber, an aversive unconditional stimulus (US) (e.g., a footshock) is given. Later, when the animal is brought back to the operant chamber, it exhibits freezing behavior which demonstrates a memory for the shock-context. However, if the US given at the same time that the animal is placed in operant chamber, and then the animal is immediately removed, it will not freeze to the shock-context when it is brought back at a later time. This phenomenon has been termed the immediate shock deficit (ISD), and has been found even with multiple immediate shock exposures and when the US intensity is increased (Landeira-Fernandez, DeCola, Kim, & Fanselow, 2006).

The ISD occurs because the animal is not able to form a context-shock association. If the animal is context pre-exposed to the shock-context prior to receiving the shock, then the ISD will be abolished, and a context-shock association will be evident by freezing behavior (Landeira-Fernandez, et al., 2006). Immediate versus delayed startle also produces similar effects on contextual fear in rodents (Kiernan & Cranney, 1992). These studies have shown that context pre-exposure facilitates contextual fear conditioning to an immediate aversive US, and in this fashion the phenomenon has been termed the CPFE. The CPFE is believed to rely upon the same mechanisms, i.e., the

hippocampus, which support declarative memory in humans (Rudy, Huff, & Matus-Amat, 2004). A traditional CPFE procedure consists of three phases: context pre-exposure, immediate shock, and a test for memory of the shock-context (Kenney & Gould, 2008; Matus-Amat, Higgins, Barrientos, & Rudy, 2004).

Context pre-exposure. Rats are taken from their home cage and transferred to the conditioning context in an enclosed transport apparatus, which prevents the rats from identifying any external visual cues (Matus-Amat, et al., 2004). Animals are left in the context for a short period of time (e.g., two, five, or ten minutes) to explore, and after this they are returned to their home cages. When multiple exposures are given after the initial context pre-exposure, animals are transferred back and forth between the context and the home cage and left in each for extremely brief durations (e.g., 40 seconds). Multiple exposures are given so that the transport apparatus and process serve as retrieval cues for placement in the context (Matus-Amat, 2004).

Context learning occurs during this phase, and this is where the animal integrates all the features of the context into a unitary, conjunctive representation. The elaboration of features into a conjunctive representation is believed to be dependent on communication between the cortex and the hippocampus (Kenney & Gould, 2008; Rudy, et al, 2004).

Immediate shock exposure. Immediate shock exposure occurs some time after context pre-exposure. In some paradigms, immediate shock exposure is delayed for varying periods of time before delivering a footshock, such that context pre-exposure and shock exposure occur within the same day (Barrientos, O'Reilly, & Rudy, 2002; Landeira-Fernandez, et al., 2006). Other procedures vary the delay between context pre-

exposure and immediate shock by one or more days (Kenney & Gould, 2008). During this phase, rodents receive an immediate footshock and are then returned to their home cages.

Lesioning and pharmacological manipulations have indicated that *N*-methyl-D-aspartate (NMDA) receptors in the hippocampus and basolateral amygdala (BLA) are both essential for memory of the shock-context. However, the hippocampus and BLA are involved with distinct processes needed to form the context-shock association. The hippocampus interacts with the cortex and retrieves the context memory that was formed during context pre-exposure. The BLA is involved with the fear response produced by the shock. Through connections with the BLA, the hippocampus then forms a conjunctive representation whereby it attaches the memory of the shock to the memory of the context (Kenny & Gould, 2008; Matus-Amat, 2007). Thus, the ISD occurs when animals have not been given context pre-exposure because they never formed a memory of the context to which the shock was associated.

Test for memory of the shock-context. Contextual fear is assessed 24 hours (or more) after immediate shock exposure. Animals are returned to the shock-context and if they have associated the footshock with the context they exhibit fear. Freezing, which is easily identifiable because the rodent will be immobile, is a natural defensive response that rodents engage in when they are in a threatening environment (Rudy, et al., 2004). Therefore, freezing behavior is the most commonly used dependent variable to assess memory during this phase. Animals that remember the shock-context will exhibit increased freezing behavior compared to animals with no memory of the shock-context (i.e., controls given no context pre-exposure or no shock).

When intrahippocampal injections of nicotine are administered prior to this phase, animals exhibit stronger memory for the shock-context, as indicated by extended freezing durations (Kenney & Gould, 2008). Conversely, temporarily inactivating the hippocampus with muscimol prior to testing for memory reduces the CPFE (Rudy, et al., 2004). Thus, retrieval of the shock-context association is a hippocampal dependent task.

The Effects of METH on the CPFE

METH exposure in humans can lead to a variety of cognitive impairments, most of which include deficits in executive function. Learning and memory tasks that are supported by hippocampal function can also be affected by METH, but such impairments are not as consistently identified in METH users, and may be mediated by changes in affect. Animal studies have shown that stimulant-induced learning impairments may be related to dose and length of exposure, which is another reason why learning and memory impairments in humans vary considerably. For instance, performance in an OR task is impaired by two weeks of COC self-administration, and this impairment is more profound when animals are allowed extended access during two weeks of self-administration (Briand, Gross, & Robinson, 2008). This suggests that learning and memory deficits in humans may be mediated by repeated drug use. OR in animals is also impaired by sensitizing doses of METH, and this is independent of monoaminergic toxicity (Belcher, et al., 2006).

Like OR tasks, the CPFE can be used in animal studies to assess the effects of METH on hippocampal dependent tasks. Currently, the effects of METH on the CPFE have not been studied, and may not be as straightforward as the effects of METH on OR tasks because other limbic structures (i.e., the amygdala) are recruited during the CPFE.

METH has facilitative effects on a variety of other fear conditioning paradigms (e.g., Suzuki, Ishigooka, Watanabe, & Miyoka, 2002; Tsuchiya, Inoue, Izumi, Hashimoto, & Koyama, 1996; Tsuchiya, Inoue, & Koyama, 1996). Thus, it may be possible for METH to enhance fear responses in CPFE paradigm.

The effects of AMPH on one-trial fear learning indicate that the CPFE may be differentially affected by varying doses and the time of administration relative to fear conditioning. For instance, sensitizing doses of AMPH given prior to fear conditioning can lead to an increased fear response (e.g., Robinson, Becker, Young, Akil, & Castaneda, 1987), whereas slightly lower doses of AMPH (i.e., 1.0 – 2.0 mg/kg) given after training do not modulate fear conditioning (e.g., Lee, Berger, Stiedl, Spiess, & Kim, 2001). However, when sensitizing doses of AMPH (i.e., 4.0 mg/kg) are administered post-training animals will exhibit an enhanced fear response (e.g., Hamamura, Ichimaru, & Fibiger, 1997). Fear conditioning paradigms are similarly modulated by the time of COC administration, and a dose-effect analysis determined that lower doses produce enhanced fear, while moderate and higher doses lead to memory impairments (Wood, Fay, Sage, & Anagnostaras, 2007). In sum, low to moderate doses of METH, AMPH, and COC potentiate the fear response in classical fear conditioning paradigms. Thus, the CPFE may similarly be affected by METH.

The HIV-1 Tg Rat

The HIV-1 Tg rat was created from Fischer 344 (F344) and SD strain backgrounds, and contains a provirus with deleted *gag-pol* genes that is regulated by HIV-1 long terminal repeat (LTR). Viral proteins, such as *tat* and *gp-120*, have been

identified in tissue samples collected from HIV-1 Tg rats (Reid, et al., 2001). Thus, the HIV-1 Tg rat (Fig. 1) is the first noninfectious rat model of HIV-1 (Reid, et al., 2001).



Figure 1. Photograph of an HIV-1 Tg rat. *Note.* Opaque cataracts are manifested by rats with the severe phenotype.

Reid et al reported that the pathology of HIV-1 Tg rats mimics many of the immune irregularities and clinical abnormalities that were seen in HIV patients before the HAART era, such as extreme weight loss, skin lesions, and renal disease. Recently, in a colony of HIV-1 Tg rats, it was shown that about one quarter of the colony developed skin lesions. Further, severity of the lesions corresponded directly with cutaneous expression of functional HIV-1 transgenes (Cedeno-Laurent, Bryant, Fischelevich, Jones, Deng, Eng, et al., 2009). It was proposed that the HIV-1 Tg rat could be used as a model for immune-mediated skin diseases that are still seen in acquired immune deficiency syndrome (AIDS) patients, such as pruritus, xerosis, atopic-like dermatitis, psoriasis, and

eosinophilic folliculitis. In our laboratory, however, AIDS-related pathology has not been observed, and the HIV-1 Tg rat may serve as a better model of HIV patients undergoing HAART treatment.

At younger ages, HIV-1 Tg rats show some immunologic dysfunction and are lighter in body weight than transgenic (Tg) littermates and F344 controls, but do not show signs of anhedonia or wasting, and are essentially healthy. HIV-1 Tg rats were maintained until older ages, and when they were tested in an open field at 18 months of age they showed no signs of motor impairment compared to Tg and F344 controls (Kass, Callahan, O'Donnell, Ruggeri, Vigorito, & Chang, 2008). At 20 to 24 months of age, HIV-1 Tg rats begin to show signs of wasting and die sooner than Tg and F344 controls. The progression of the disease in the HIV-1 Tg rat, as observed in our laboratory, appears to parallel that seen in HIV-infected patients being treated with HAARTs.

When tested in a modified Morris water maze (MWM) at five months of age, HIV-1 Tg rats show poorer acquisition and reversal learning than Tg and F344 controls (LaShomb, Vigorito, & Chang, 2008). Additionally, the HIV-1 Tg rat exhibits extreme impairments in the MWM when the task requires new strategy learning (Vigorito, LaShomb, & Chang, 2007). The deficits exhibited by the HIV-1 Tg rat in the MWM are comparable to HAD-related deficits seen in HIV patients, such as spatial impairments and behavioral inflexibility. This suggests that some of the cognitive and motor impairments that emerge in humans can be seen in the HIV-1 Tg rat. The pathogenesis of HIV CNS disorders can be studied at level of behavior, rather than just neuronal dysfunction, in HIV-1 Tg rats.

Compared to F344 controls, HIV-1 Tg rats respond differently to the analgesic effects of morphine. Specifically, HIV-1 Tg rats demonstrate longer tail flick latencies than F344 controls after morphine treatment (Chang & Vigorito, 2006). This suggests there is a difference in the sensitivity to abusive drugs. Thus, the HIV-1 Tg rat may be a useful model in studying the concerted effects of METH and HIV-1 on brain function and behavior.

EXPERIMENT ONE

Experiment One consisted of two procedures that were executed simultaneously to evaluate 1) sensitization and drug context effects associated with METH treatment, and 2) if the CPFE is affected by METH in normal SD rats. In the sensitization and drug context effects procedures, METH-induced, stereotypic head movement was of extreme interest, and a quantitative scoring method was used to assess varying aspects of this characteristic behavior. Thus, experiment one set out, in part, to determine an optimal dose for eliciting the behavior in question, and the accuracy of the scoring method used to quantify it.

Stereotypies are more consistently elicited with higher doses of METH, and become more intense as dose increases. A time sampling procedure was used to score head movements in normal SD rats that were administered either a moderate or moderate-to-high dose of METH on five consecutive days. If the quantitative scoring method is accurate, then it should be sensitive to differences in dose-dependent, METH-induced responses. Further, behavioral effects post-injection vary with the course of drug action, and if counting head movements is a discriminative scoring method, then head movements recorded during a post-injection time sampling procedure should reflect the course of drug action. It would be expected to observe variations in the number of head movements counted at different time points of a drug session. Specifically, if the scoring method is accurate, and the chosen doses are able to elicit stronger stereotypic responses at the expense of hyperlocomotor responses, then the course of drug action should be illustrated graphically as an inverse U.

The sensitization and drug context effects procedures were also set up to evaluate if BS of METH-induced head movement will develop after five days of drug treatment, and to determine which of the two doses produces more pronounced BS. If BS occurs, then METH-induced, stereotypic head movement should increase over the five consecutive days of drug treatment. Moreover, if BS develops across five days of drug treatment, then this should later be confirmed during a challenge test. If SD rats that were exposed to METH during the five days of drug treatment developed BS, then they should exhibit a greater number of head movements in response to a low dose of METH than SD rats that received saline during the five days of drug treatment.

The neuroalterations associated with BS are believed to be relatively long lasting, and reflect the extent of drug exposure. If this is true, and BS is identified during the challenge test, then head movement scores should reflect drug history. Explicitly, SD rats given the moderate-to-high dose of METH during five days of drug treatment should display the largest number of head movements in response to a low challenge dose, followed, in order, by rats that were given the moderate dose of METH and rats that were given saline during five days of drug treatment. Additionally, if BS is associated with persisting changes, then drug history should be evident even with varying periods of withdrawal.

It is largely accepted that the acute and sensitizing properties of psychostimulants can be modulated by the environment surrounding drug administration. To study environmental modulation of the acute and sensitizing effects of METH, drug administration was consistently paired with one of two discretely distinct modified housing cages that were kept in a room outside of the vivarium. If the discrete contextual

manipulations used to create the modified housing cages are sufficient in demonstrating environmental modulation, then there should be a difference in the number of head movements exhibited in each context. The contexts were created to be discretely distinct from the home cage, as well as discretely distinct from each other, and the discrete cues that were manipulated are brightness, texture, and scent. There are no a priori hypotheses regarding which set of cues will augment, or lessen, the acute and sensitizing effects of METH. It is however hypothesized that the discretely distinct contexts should affect head movement in a noticeable fashion.

When drug administration is repeatedly paired with a specific context, the effects of the drug can become associated with the context surrounding administration. With enough pairings, the context surrounding drug administration can elicit a drug response in the absence of the drug. The circumstances necessary to produce conditioned responding to the drug-paired context were examined in experiment one. After five days of METH treatment SD rats were given an injection of saline in either the drug-paired context or a novel context. If conditioned responding to the drug-paired context occurs, then rats given saline in the context that they previously received METH in will exhibit a greater number of head movements than rats given saline in novel context.

Another goal of experiment one was to determine how, and if, a sensitizing regimen of METH treatment affects the CPFE. During the CPFE procedure, SD rats were context pre-exposed to an operant chamber in which they would later receive a shock. METH or saline was administered on the five consecutive days before or after subjects were context pre-exposed. Then all rats received immediate shock exposure, and were returned 24 hours later to assess memory of the shock-context. It was believed that

the CPFE would be dose-dependently lessened by METH treatment. However, it was unclear how time of drug treatment relative to context pre-exposure would affect the CPFE. Thus, it is expected that placement in the shock-context will elicit the most freezing behavior in saline-pretreated rats, followed, in dose order, by METH-pretreated rats. There are no expectations of how the time of drug treatment will affect memory for the shock-context.

Method

Animals

Twenty-four experimentally naïve, male SD rats obtained from Harlan Co. (Indianapolis, IN) were used as subjects. Animals ranged between seven and twelve weeks of age throughout testing. All animals were triple housed in clear, plastic rat cages (45.7 cm × 22.9 cm × 20.3 cm) with Harlan Teklad™ 1.8", corn-cob bedding. Food (Harlan Teklad™ Mouse/Rat Laboratory Diet 7102) and water were provided *ad libitum* through the duration of the study. The vivarium was maintained on a 12:12 hour light-dark cycle (8:00am – 8:00pm), and within recommended temperature ($22^{\circ} \pm 5^{\circ}$ C) and humidity ($50\% \pm 20\%$) conditions.

Throughout the study rat body weights were monitored. Body weights were measured daily at 10:00am \pm 1 hour, and prior to any experimentation. 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.

METH Conditioning Apparatus

On all drug treatment days, METH administration was paired with one of two different contexts. In each context there were three identical, home made chambers

which provided a total of six conditioning units. Distinct contexts were created with the application of three discrete contextual manipulations (i.e., brightness, texture, and scent) to rectangular housing cages (45.7 cm × 22.9 cm × 20.3 cm). Clear, 0.5 cm Plexiglas sheets (52.1 cm × 28 cm) were used as lids. Twenty-three breathing holes were drilled around the edges of the plexiglass sheets to maintain an oxygen flow in the cages. Clusters of 10 holes were drilled in diagonally opposite ends of the Plexiglas lids. Seventy percent ethanol alcohol was used to sanitize the conditioning units and the countertop they were mounted on before and after each use.

(A)

(B)

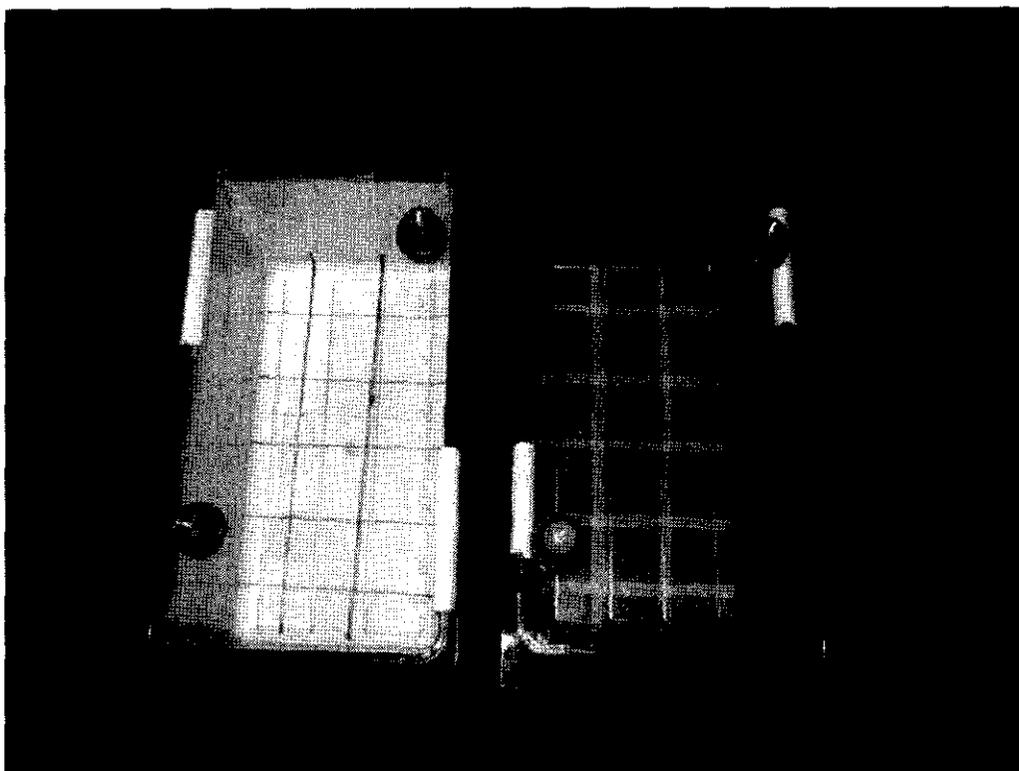


Figure 2. Drug-paired contexts. (A) Context B; white walls for high brightness, smooth surface, & mint scent; or (B) Context D; dark walls for low brightness, rough surface, & vanilla scent.

The two contexts will be distinguished by the most visually salient cue, i.e., brightness. In the bright context (Fig. 2A), which will be referred to as Context B from here on out, the surface of the plastic cage floor was left untouched and exposed providing a smooth texture on the inside. The outside of four walls (i.e., floor, left side, back side, and right side) was covered with white paper to create a bright environment. The outside of one wall (i.e., front side) was left uncovered so the experimenter could observe the subjects' behaviors. To maintain a mint scent within the cage, two miniature clay pots were placed on top of that unit's lid (i.e., one above each cluster of holes). Inside each clay pot were two cotton balls that had been dabbed with wintergreen flavored mouthwash (Pathmark™ Spring Mist) to create the mint scent.

In the dark context (Fig. 2B), which will be referenced as Context D, the surface of the plastic cage floor was scratched lengthwise (40.6 cm) five times using a blade cutter to provide a rough texture on the inside. To create a dark context, the floor of the cage was covered with navy paper on the outside, and the outside of three walls (i.e., left side, back side, and right side) was covered with a black garbage bag with the remaining front wall left uncovered to allow for observation. To maintain a vanilla scent within a chamber, the cotton balls in the clay pots were dabbed with vanilla extract (McCormick™ Pure Vanilla Extract).

On all drug treatment days, saline administration was paired with a context similar to the home environment. Three clear, plastic rat cages (45.7 cm × 22.9 cm × 20.3 cm), with Harlan Teklad™ 1.8", corn-cob bedding, were set up in the experimenting room beneath the observation table. After all saline treatment days, seventy percent ethanol alcohol was used to sanitize the cages and fresh bedding was added.

Shock Chamber

All CPFE procedures took place in two identical operant conditioning chambers (26.7 cm × 23.9 cm × 26.7 cm; Ralph Gebrands Instruments, Arlington, MA). The sides and hinged top of each chamber were made of 1.3 cm clear Plexiglas. The floor grid in each chamber contained 17 stainless steel rods (0.23 cm diameter), spaced 1.3 cm apart. The rods were wired to a generator and scrambler (ENV-416S Standalone Grid Shocker/Scrambler; Med Associates Inc., Albans, VT) that were controlled by MED PC computer software. Output signals sent by a metal lever on the outside of each chamber allowed the experimenter to present a two second, 1.2-mA electric shock. Seventy percent ethanol alcohol was used to sanitize the operant chambers before and after each subject.

White light bulbs (6 watt, 120 volt) that illuminated the chambers allowed the experimenter to observe and videotape the rats' behaviors. A video camera was mounted on a tripod with both of the operant chambers in view, and the experimenter observed the rats' behaviors on a monitor outside of the experimental room.

Drugs and Solutions

All animals used in this study received intraperitoneal (i.p.) injections of METH [(+)-methamphetamine hydrochloride, Sigma-Aldrich Co., St. Louis, MO] or saline, via 27½ gauge/1cc/syringes. METH was dissolved in sterile 0.9% saline immediately prior to injections. Throughout the drug treatment phase, METH was administered i.p. at a dose of 0.0mg/kg (saline), 2.0mg/kg, or 2.5mg/kg at a volume of 1 ml/kg. It was determined that intermediate doses of METH would be optimal for eliciting head movement stereotypy, the main dependent variable, and for inducing BS. These doses

were chosen based on previous research (e.g., Gentry, et al., 2004; Kuczenski & Segal, 1999; Segal & Kuczenski, 1987) which demonstrates that stereotypy is not consistently evoked below 2.0 mg/kg METH, and research that has used lower doses to attenuate the possibility of sensitization (e.g., Milesi-Halle, et al., 2007). In addition, the dosing regimen did not exceed 2.5 mg/kg because self injurious behaviors, convulsions, and mortality can be elicited from doses higher than 3.0 mg/kg METH.

On challenge test days and conditioned responding test days METH was administered at a dose of 0.5mg/kg and 0.0mg/kg at a volume of 1 ml/kg, respectively. This dose was chosen based on previous research (e.g., Brennan, et al., 2007; Itzhak, et al., 2002) that shows 0.5 mg/kg METH is an appropriate challenge amount, and can demonstrate the occurrence of BS. Intraperitoneal injections were administered at three-minute intervals, and immediately prior to placement in a METH conditioning unit.

Procedures

Due to the large number of animals required, this experiment was conducted over a 39 day time period (Table 1), and consisted of four, six-phase replications. The six phases: Drug treatment (five days), BS challenge test, test for conditioned responding to the drug-paired context, shock-context pre-exposure, immediate shock exposure, and the test for freezing to the shock-context were not completed in the same order for each replication. Phase order was varied based on the goals of each procedure, i.e., the sensitization and drug context effect procedure, and the CPFE procedure.

Sensitization and Drug Context Effects

Drug treatment (5 days). Throughout the drug treatment phase rats received injections of METH or saline. In an attempt to establish BS, behaviors from METH-treated rats were thoroughly observed.

Table 1.

Time Course for all Experimental Procedures and Testing Phases

	<i>Phase 1</i>	<i>Phase 2</i>	<i>Phase 3</i>	<i>Phase 4</i>	<i>Phase 5</i>	<i>Phase 6</i>
Replication 1	Drug treatment	Context pre-exposure	Immediate shock exposure	Test for freezing	BS challenge test	Test for conditioned responding
Days	1-5	8	15	16	38	39
Replication 2	Context pre-exposure	Drug treatment	Immediate shock exposure	Test for freezing	Test for conditioned responding	BS challenge test
Days	1	1-5	8	9	31	32
Replication 3	Drug treatment	Context pre-exposure	Immediate shock exposure	Test for freezing	Test for conditioned responding	BS challenge test
Days	1-5	8	15	16	17	18
Replication 4	Context pre-exposure	Drug treatment	Immediate shock exposure	Test for freezing	BS challenge test	Test for conditioned responding
Days	1	1-5	8	9	10	11

Note. The variability in time delay between phases 1 through 4 and phases 5 through 6 among replications is because all 24 rodents were tested in phase 5 and phase 6 on the same two days.

Each replication included six rats. Home cages were removed from the vivarium to transfer rats into the experimenting room. All lights, except one desk lamp (50 watt, 130 volt), remained off while the subjects were in the experimenting room. Rats were individually removed from their home cages and injected at three-minute intervals. The

saline-treated rats were always injected first, followed by the METH-treated rats. The same injection procedure occurred over the course of five consecutive days of drug treatment (Table 2). Immediately after receiving its injection, each rat was placed in its

Table 2.

Injection Time Course and Order

		Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Rat 6
Replication 1 & Replication 4	Injection Type	SAL	SAL	SAL	METH	METH	METH
	Context	<i>home</i>	<i>home</i>	<i>home</i>	<i>1</i>	<i>1</i>	<i>1</i>
	Injection Time	0 min	3 min	6 min	9 min	12 min	15 min
Replication 2 & Replication 3	Injection Type	SAL	SAL	SAL	METH	METH	METH
	Context	<i>home</i>	<i>home</i>	<i>home</i>	<i>2</i>	<i>2</i>	<i>2</i>
	Injection Type	0 min	3 min	6 min	9 min	12 min	15 min

Note. The drug treatment phase was five days long, and this table only demonstrates one day of drug treatment for each cycle.

designated training context. Thus, rats were removed from their home cages and immediately placed in a novel environment post-injection. The METH-treated rats were placed in one of two discretely distinct contexts (see Fig. 2) for observation while under the influence of the drug. The saline-treated rats were placed in standard rat cages and not observed.

Context B was paired with METH administration in replications one and four, and Context D was paired with METH administration in replications two and three. In other words, all METH-treated rats in replications one and four were immediately placed into a Context B conditioning unit after receiving injections, and all METH-treated rats in

replications two and three were immediately placed into a Context D conditioning unit after receiving injections. All saline-treated rats were immediately placed in a cage with dimensions and bedding identical to that of their home cages after receiving injections.

Once the rats were placed into their modified cages they were left undisturbed for two hours. A camera mounted in the ceiling provided an aerial view of the three METH conditioning cages. All drug treatment days were recorded on a commercial DVR that was connected to the ceiling camera. In addition to this, the experimenter remained in the room the entire time, and sat positioned in front of all three conditioning units. The experimenter then observed and recorded behaviors from the METH-treated rats. Behavior was scored for two-minute intervals, every 10 minutes, for up to 120 minutes post-injection.

The main dependent variable during the drug treatment phase was rodent head movements (Fig. 3). Through live scoring all lateral, circular, and diagonal head movements were tallied during each two-minute observation interval. The method of scoring did not differentiate between lateral, circular and diagonal movements. Rather, all movement was scored together providing a quantitative description of the total amount of head movements observed. To tally head movements, “tick marks” were entered in an open Microsoft Word document. When a two-hour drug session was completed, the experimenter revisited the Word document and used the word count tool to count the observed number of head movements during each observation interval.

To fully evaluate METH-induced stereotypic head movement, two doses were administered on treatment days. Replications one and two received a moderate dose (2.0

mg/kg) of METH, while replications three and four received a moderate-to-high (2.5 mg/kg) dose of METH.

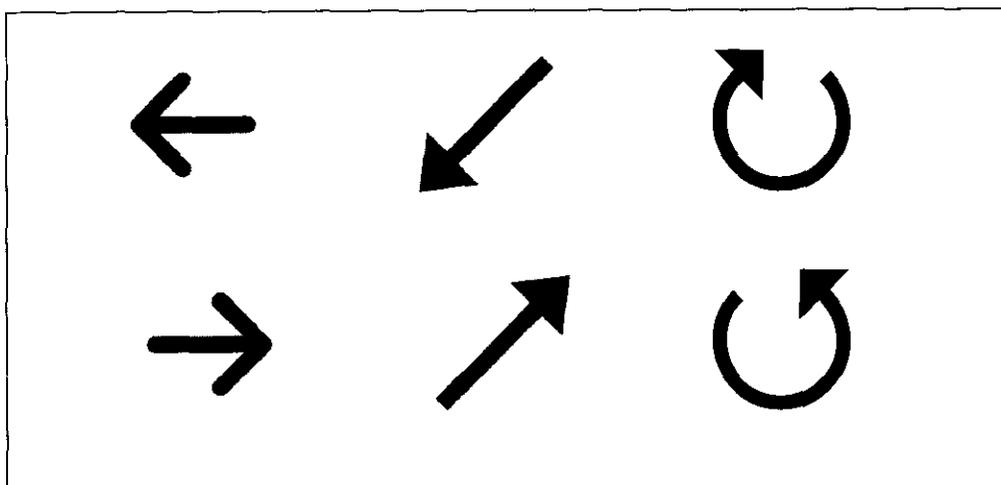


Figure 3. Examples of scored motion. Head movements were scored if they appeared to be moving to left/right, diagonally, circular, or any combination of these directions. According to this operational definition, head movements that appeared to be strictly vertical were not counted.

BS challenge test. This phase was used to examine any enduring effects the rats may have incurred from five days of drug treatment. The BS challenge test was conducted with saline- and METH-pretreated rats from all four replications over two consecutive days. The delays between the last day of drug treatment and the BS challenge test for replications one through four were five weeks, four weeks, two weeks, and one week, respectively (see Table 1).

During the drug treatment phase, in each replication six rats were administered injections daily, but only three rats were placed in modified cages and observed post-injection (i.e., only METH-treated rats were observed). To accommodate this procedure,

each replication was divided into two squads, consisting of three rats. Squads were separated according to drug history and running order. As such, saline-pretreated rats were always in squad 1, and METH-pretreated rats were always in squad 2.

The squads were transferred into the experimenting room in their home cages. Rats were individually removed from the home cage and administered 0.5 mg/kg METH via i.p. injections at three-minute intervals. To be consistent with the initial drug treatment schedule, the saline-pretreated rats (squad 1) were always injected and tested first, followed by the METH-pretreated rats (squad 2). Immediately after receiving an injection each rat was placed in a modified cage and left undisturbed for 30 minutes. Because saline-pretreated rats were not exposed to Context B or Context D during the drug treatment phase, they were placed in the context that their METH-treated counterparts had been trained in.

All BS challenge test sessions were recorded to a commercial DVR via a ceiling camera with an aerial view of the three chambers. The experimenter sat positioned in front of the test chambers and remained in the room for the duration of the session. During two-minute observation intervals at 10, 20, and 30 minutes post-injection the experimenter scored head movements. When the session was complete, the rats were placed in their home cage and transferred back to the vivarium. All modified cages and countertops were sanitized with 70% ethanol alcohol between squads.

This procedure was repeated four times during the two BS challenge days (i.e., four sessions per day for a total of eight thirty-minute test sessions). All METH pretreatment doses (i.e., 0.0 mg/kg, 2.0 mg/kg and 2.5 mg/kg) had to be tested together, thus replications one and four were tested on the first day, while replications two and

three were tested on the second day. To counterbalance for order, the BS challenge test and the test for conditioned responding were carried out on the same two days. Thus, on the first day replications two and three were tested in the conditioned responding phase, and on day two replications one and four were tested in the conditioned responding phase. That is, half the rats were given a low challenge dose first, followed 24 hours later by the test for conditioned responding, and the reverse order was true for the other half of the rats.

Test for conditioned responding to the drug-paired context. This phase was completed by all replications at the same time, and testing took place on two, consecutive days. Rats that received saline during drug treatment were not subject to testing since they had not been trained in either context. Two replications were tested for conditioned responding in the drug-paired context, while the other two were tested in a novel context. Replication order was varied based on testing context and drug history (Table 3).

Table 3.

Test for Conditioned Responding Phase: Test Order, Test Context, and Drug History

		Test Order	Test Context	Drug History
Test Day 1	Replication 2	1	Same	2.0 mg/kg METH
	Replication 3	2	Different	2.5 mg/kg METH
Test Day 2	Replication 1	1	Different	2.5 mg/kg METH
	Replication 4	2	Same	2.0 mg/kg METH

The home cage was removed from the vivarium and used to transfer the rats to the experimenting room. In addition to maintaining contextual congruency in the

experimenting room, testing was initiated during the same time of day (± 45 minutes) as drug treatment. Saline injections were administered at three-minute intervals. Immediately post-injection, each rat was placed into the drug-paired context or a novel modified cage. The experimenter remained in the room while each rat was left undisturbed in the drug-paired or novel context for 30 minutes. Head movement was scored during two-minute observation intervals at 10, 20, and 30 minutes post-injection. Once the session was completed, rats were removed from the modified cages and brought back to the vivarium in their home cage. All counters and test contexts were then sanitized with 70% ethanol alcohol. This procedure was repeated four times (i.e., two thirty-minute test sessions on two consecutive days).

CPFE

Context pre-exposure. During the context pre-exposure phase of testing (see Fig. 4 for CPFE summary), all rats were removed from their home cages and immediately

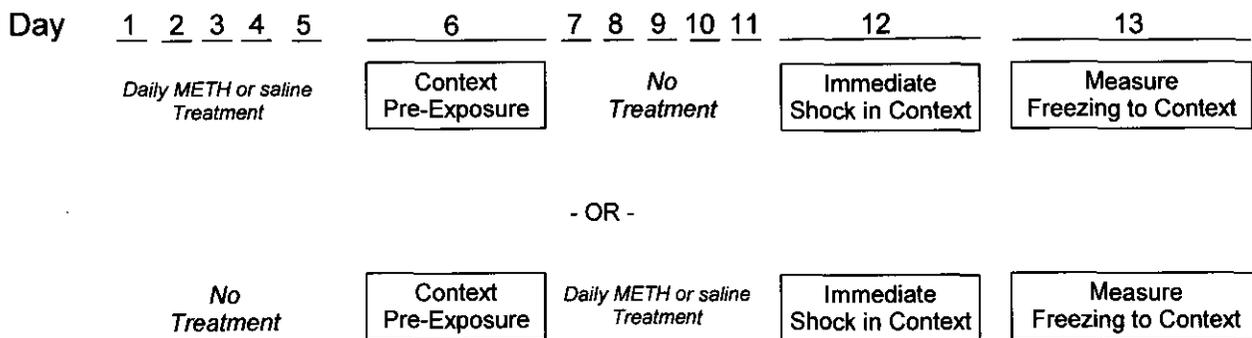


Figure 4. CPFE procedure summary. Rats were treated with saline or METH for five consecutive days either before or after context pre-exposure. Immediate shock exposure took place on day 12 for all rats, and was followed 24 hours later by the test for freezing to the shock-context.

placed in a transport carrier. For all CPFE procedures two identical, plastic fish tanks (25.4 cm × 16.5 cm × 14 cm) were used to transfer subjects from their home cages to the operant conditioning units. The base of each tank was lined with pine bedding (Northeastern Products Corp.; Warrensburg, NY). Each tank was wrapped from the base up with a black, plastic garbage bag to block any visual input outside of the transport carrier. It was thought that blocking external cues would strengthen the association of the carrier itself being a predictor of further placement in the operant chamber.

Upon entrance into the experimenting room, the carrier was set down, and left untouched for 120 seconds. After 120 seconds of being in the covered fish tank, the subject was quickly removed and placed into the operant chamber for 120 seconds. After 120 seconds the subject was removed and transferred back to the fish tank as quickly as possible. This process was repeated five consecutive times for each rat. After the fifth exposure, the rat would be transferred back to its home cage.

Drug treatment (5 days). During this phase, rats received i.p. injections of METH or saline once a day, on five consecutive days. Time of drug treatment was varied such that half the subjects were treated prior to being context pre-exposed, and the other half were treated after. That is, the context pre-exposure and drug treatment phases were varied between the four replications. The environment surrounding drug administration was completely separate from that of all other CPFE procedures (see sensitization and drug context effects procedures). Animals were always drug-free during the context pre-exposure, immediate shock exposure and the test for freezing phases.

Immediate shock exposure. In this procedure, the transport from the home cage to the operant chamber was identical to that of the transport during context pre-exposure.

Upon entry in the experimenting room, the transport carrier was set down in the same place it was set down during context pre-exposure. Immediately after setting the fish tank down, the animal was removed and placed into the operant chamber as quickly as possible. Upon closing the chamber's door, the experimenter pushed a lever which elicited a two-second, 1.2 mA footshock. When the shock terminated the animal was removed from the chamber, placed back into the transport apparatus, and returned to its home cage.

Test for freezing. Twenty-four hours after immediate shock exposure all subjects were tested for freezing to the shock-context. The transport procedures from the home cage to operant chamber and vice versa were identical to the procedure used during context pre-exposure and immediate shock exposure. After entering the experimenting room, the subject was removed from the transport apparatus and immediately placed in the operant chamber, where it was left undisturbed for six minutes. The experimenter then exited the room and closed the door.

A video camera projected images onto a screen outside of the experimenting room. All freezing test days were recorded on this monitor. In addition to recording these sessions, the experimenter scored each six-minute session live on the screen with the Etholog 2.2 behavioral observation transcription tool (Ottoni, 2000). The primary dependent variable was time spent freezing. Thus, sessions were scored in a binary fashion where the subject was observed as freezing or not freezing. Freezing behavior was defined as the absence of any and all movement except breathing. Therefore, any observed behavior that could not be categorized according to this operational definition was scored as not freezing.

Results

Sensitization and Drug Context Effects

Drug Treatment (5 days)

Although all METH-treated rats received five days of treatment in the drug-paired context, we were unable to observe behavior from the 2.0 mg/kg METH-treated groups on the fifth day. Thus, all subsequent statistical analyses only include the first four days of treatment. In addition, all drug treatment analyses are only applicable to METH-treated rats, because saline-treated rats were not observed and scored during this experimental phase.

To evaluate METH-induced stereotypic head movement, data from the drug treatment phase were analyzed with a dose (2) × context (2) × day (4) × observation interval (12) mixed ANOVA, with dose and context as between-groups factors, and day and observation interval as within-groups factors. Figure 5 shows the mean (\pm SE) number of head movements for all groups and all conditions. The top graph illustrates head movement scores that were recorded across 12 observation intervals on four consecutive treatment days from rats that were administered 2.0 mg/kg METH in Context B and Context D. The bottom graph represents 12 observation intervals post-injection on four consecutive days of 2.5 mg/kg METH administration in Context B and Context D. To better evaluate the results, however, subsequent graphs re-plot the data to show main effects and simple interactions.

BS. The 4-way ANOVA yielded a significant main effect of day, $F(3, 24) = 8.59$, $p < .001$, $\eta^2 = .52$, such that head movement scores from day 1 [$M = 48.23$, $SD = 2.56$] were significantly less, on average, than head movement scores from day 4 [$M = 64.88$,

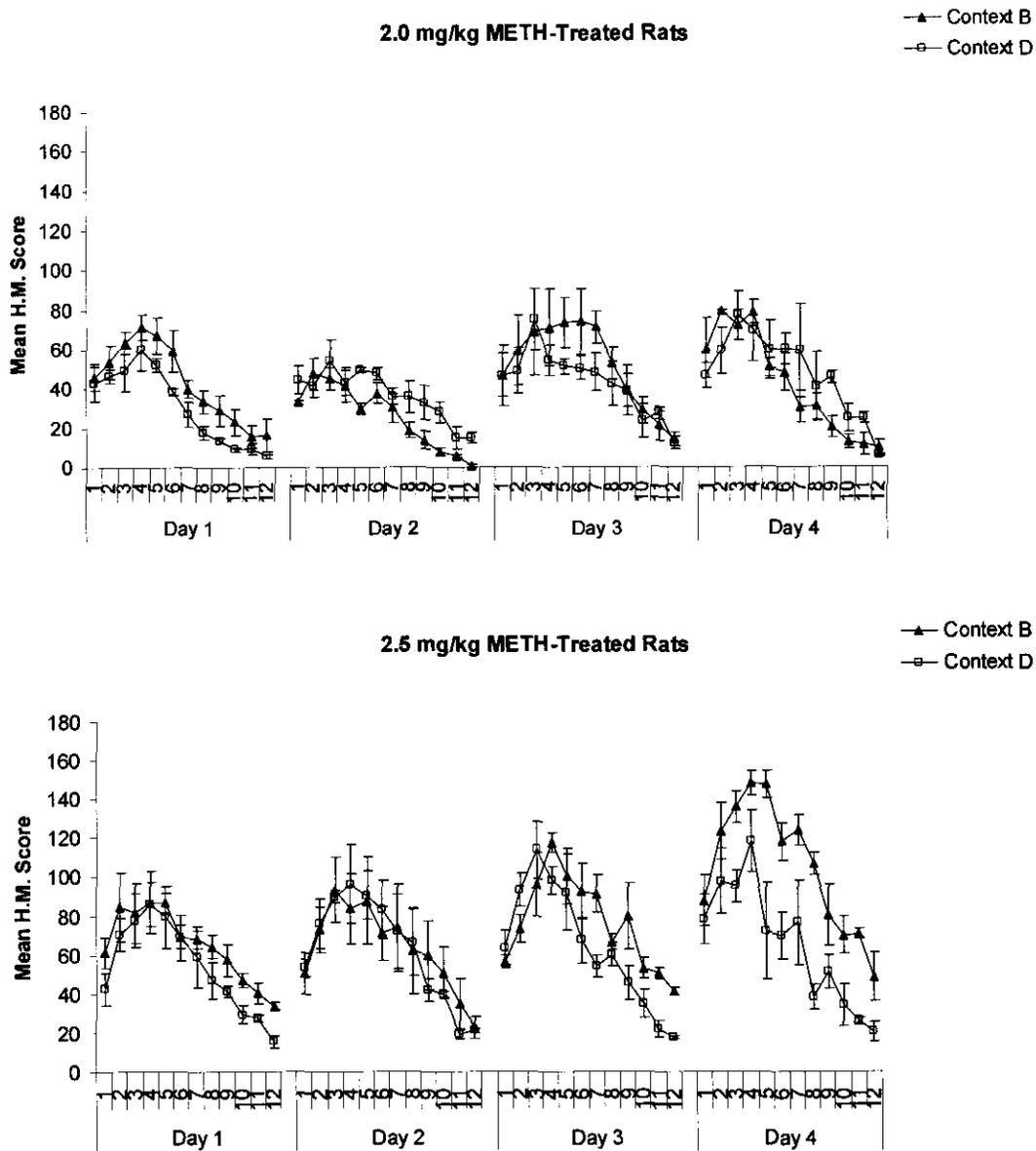


Figure 5. Mean (\pm SE) head movement scores recorded from dose \times context groups during two-minute observation intervals that took place every 10 minutes, up to 120 minutes post-injection across four treatment days.

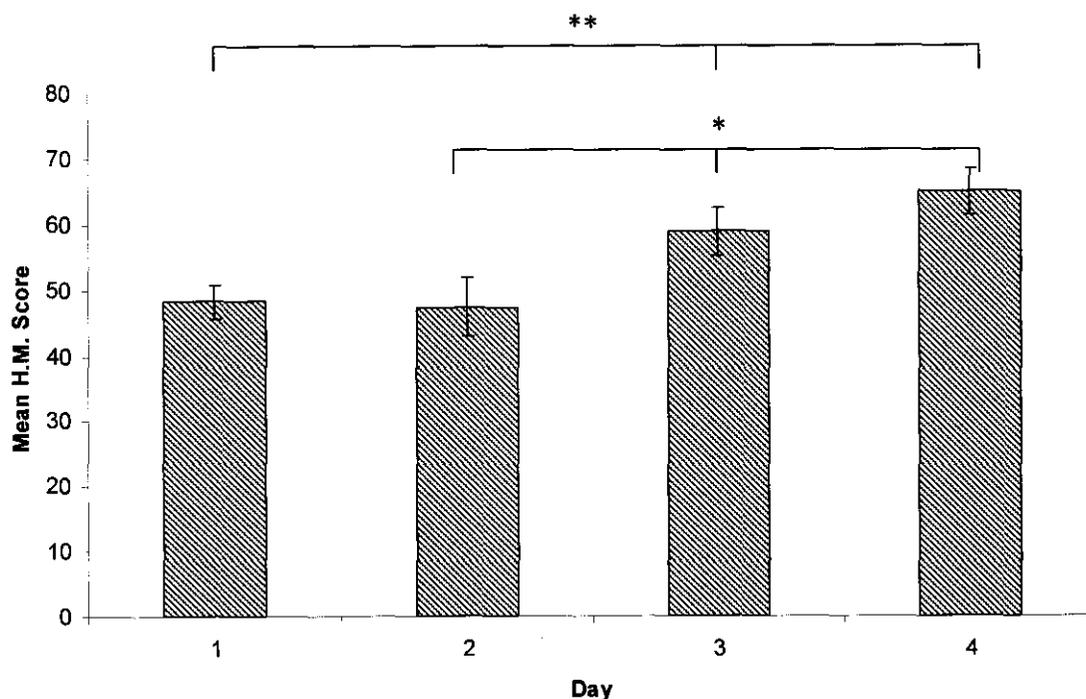


Figure 6. Mean (\pm SE) head movement score per day. Head movement scores from days 3 and 4 are significantly greater than head movement scores from day 1. This demonstrates that BS of METH-induced head movements has occurred by day 3 and continues to increase on day 4. Note. * indicates $p < .05$; ** indicates $p < .01$

$SD = 3.5$]. The main effect of treatment day is plotted in Figure 6. Planned comparisons were used to determine the number of treatment days it takes for the increase of METH-induced head movement (i.e., BS) to occur. These comparisons indicate that sensitization begins to develop on day 3 of treatment, such that the mean head movement score [$M = 58.72$, $SD = 3.71$], on average, was significantly ($p < .05$) greater than that of day 1 [$M = 48.23$, $SD = 2.56$].

Dose response curve. The overall ANOVA yielded a significant main effect of dose, $F(1, 8) = 2.18$, $p < .01$, $\eta^2 = .80$, such that head movement scores from 2.0 mg/kg METH-treated rats [$M = 40.2$, $SD = 3.68$], on average, were significantly less than head

movement scores from 2.5 mg/kg METH-treated rats [$M = 69.34$, $SD = 3.68$] (Fig. 7A). Moreover, the dose \times day interaction did not reach significance [$F(3, 264) = 2.12$, $p > .05$], indicating the different dose responses remained constant over days. Specifically, sensitization rates from 2.0 mg/kg and 2.5 mg/kg METH-treated rats were not affected by dose (Fig. 7B).

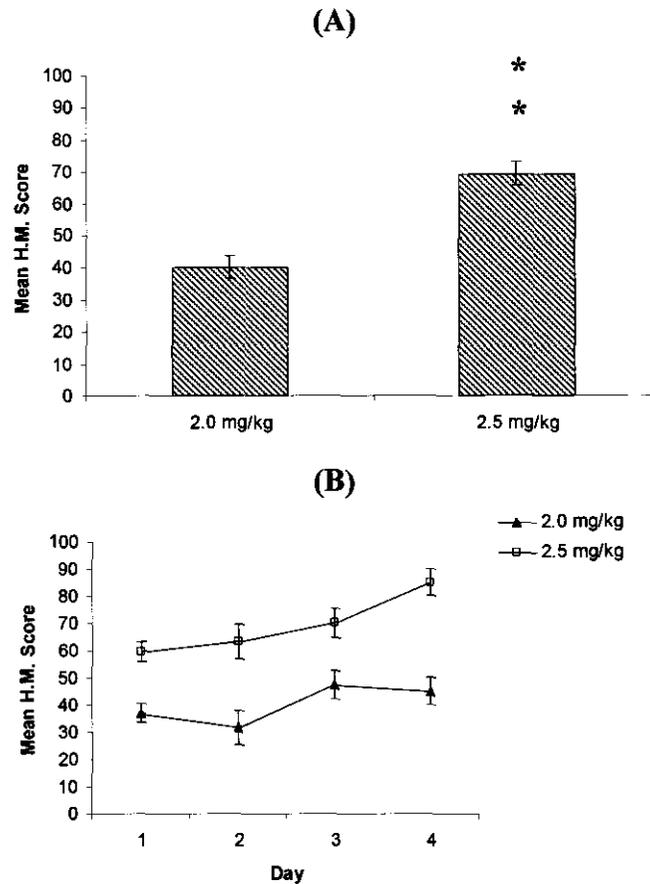


Figure 7. Mean (\pm SE) head movement scores from 2.0 and 2.5 mg/kg METH-treated rats. This figure illustrates that (A) the method used to measure stereotypic head movement was sensitive to a dose response and (B) that dose did not modulate sensitization rates. Note. ** indicates $p < .01$

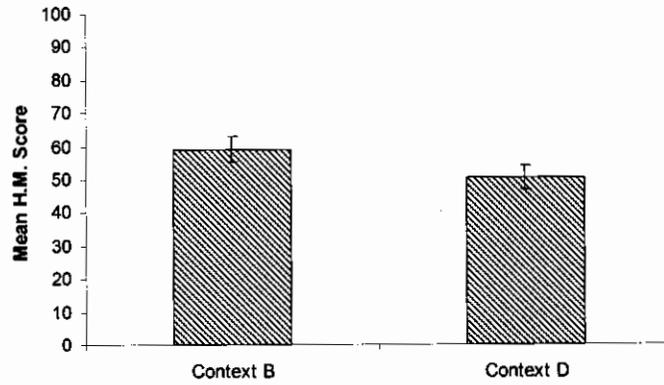
Context. Although a main effect of context did not reach significance [$F(1, 8) = 2.8, p > .05$] (Fig. 8A), there was a trend towards a day \times context interaction, $F(3, 24) = 2.71, p < .1, \eta^2 = .25$ (Fig. 8B), and a significant day \times dose \times context interaction, $F(3, 24) = 3.23, p < .05, \eta^2 = .29$ (Fig. 8C). Separate ANOVAs were calculated for each treatment day to assess the reason for the 3-way interaction.

A dose (2) \times context (2) \times observation interval (12) mixed ANOVA was calculated for day one, and yielded a significant main effect of dose, $F(1, 8) = 19.06, p < .01, \eta^2 = .70$, such that the average head movement score from 2.5 mg/kg METH-treated rats [$M = 59.4, SD = 3.6$] was significantly greater than that of 2.0 mg/kg METH-treated rats [$M = 37.06, SD = 3.6$]. A significant main effect of context, $F(1, 8) = 5.31, p < .05, \eta^2 = .40$, was also revealed, such that the average head movement score from subjects which received METH in Context B [$M = 54.13, SD = 3.62$] was significantly higher than the average head movement score from subjects which received METH in Context D [$M = 42.33, SD = 3.62$].

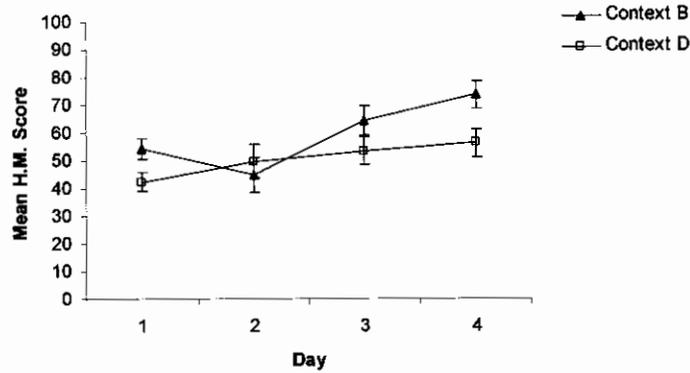
The ANOVA for day two also yielded a significant main effect of dose, $F(1, 8) = 12.57, p < .01, \eta^2 = .61$, [2.5 mg/kg dose: $M = 63.06, SD = 6.3$; 2.0 mg/kg dose: $M = 31.47, SD = 6.3$], but the effect of context was not significant, [$F(1, 8) = .31, p > .05$]. The results on day three were the same as day two, with a significant main effect of dose, $F(1, 8) = 9.3, p < .05, \eta^2 = .54$, [2.5 mg/kg dose: $M = 70.01, SD = 5.24$; 2.0 mg/kg dose: $M = 47.42, SD = 5.24$], but a non-significant effect of context, [$F(1, 8) = 2.04, p > .05$].

On day four, however, there was a significant main effect of dose, $F(1, 8) = 32.92, p < .01, \eta^2 = .81$, [2.5 mg/kg dose: $M = 84.9, SD = 4.94$; 2.0 mg/kg dose: $M = 44.85, SD = 4.94$], and a significant main effect of context, $F(1, 8) = 6.19, p > .05, \eta^2 = .43$.

(A)



(B)



(C)

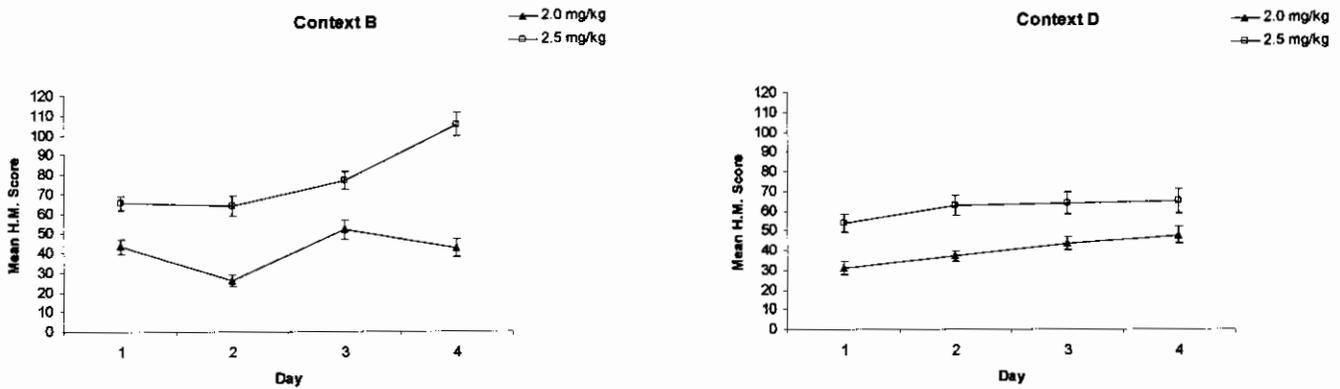


Figure 8. Mean (\pm SE) head movement scores from (A) Context B and Context D, (B) Context B and Context D over four days of drug treatment, and (C) context \times dose groups over four days of drug treatment.

.45, such that the average head movement score from subjects which received METH in Context B [$M = 73.56$, $SD = 4.94$] was significantly higher than the average head movement score from subjects which received METH in Context D [$M = 56.19$, $SD = 4.94$]. A significant dose \times context interaction was also revealed, $F(1, 8) = 10.7$, $p < .05$, $\eta^2 = .57$, such that context had no effect between the 2.0 mg/kg METH-treated rats [Context B, $M = 42.11$, $SD = 6.98$; Context D, $M = 47.58$, $SD = 6.98$]. However, context did have an effect between the 2.5 mg/kg METH-treated rats, such that the average head movement score from rats trained in Context B [$M = 105$, $SD = 6.98$] was significantly higher than the average head movement score from rats trained in Context D [$M = 64.81$, $SD = 6.98$].

BS Challenge Test

The head movement data from the BS challenge test were analyzed with a training dose (3) \times context (2) \times observation interval (3) mixed ANOVA, with training dose and context as between-subjects factors, and observation interval as a within-subjects factor. Figure 9A depicts the mean (\pm SE) number of head movements exhibited during three post-injection observation intervals from saline-pretreated, 2.0 mg/kg and 2.5 mg/kg METH-pretreated rats that received a low challenge dose of METH. Effects of interest are isolated and re-plotted in subsequent graphs.

BS. The 3-way ANOVA yielded a significant main effect of training dose, $F(2, 18) = 29.07$, $p < .01$, $\eta^2 = .76$, such that rats that were pretreated with METH during five days of drug treatment exhibited, on average, a significantly greater number of head movements than rats that received saline during five treatment days (see Fig. 9B).

Drug history. Planned comparisons revealed that the average head movement

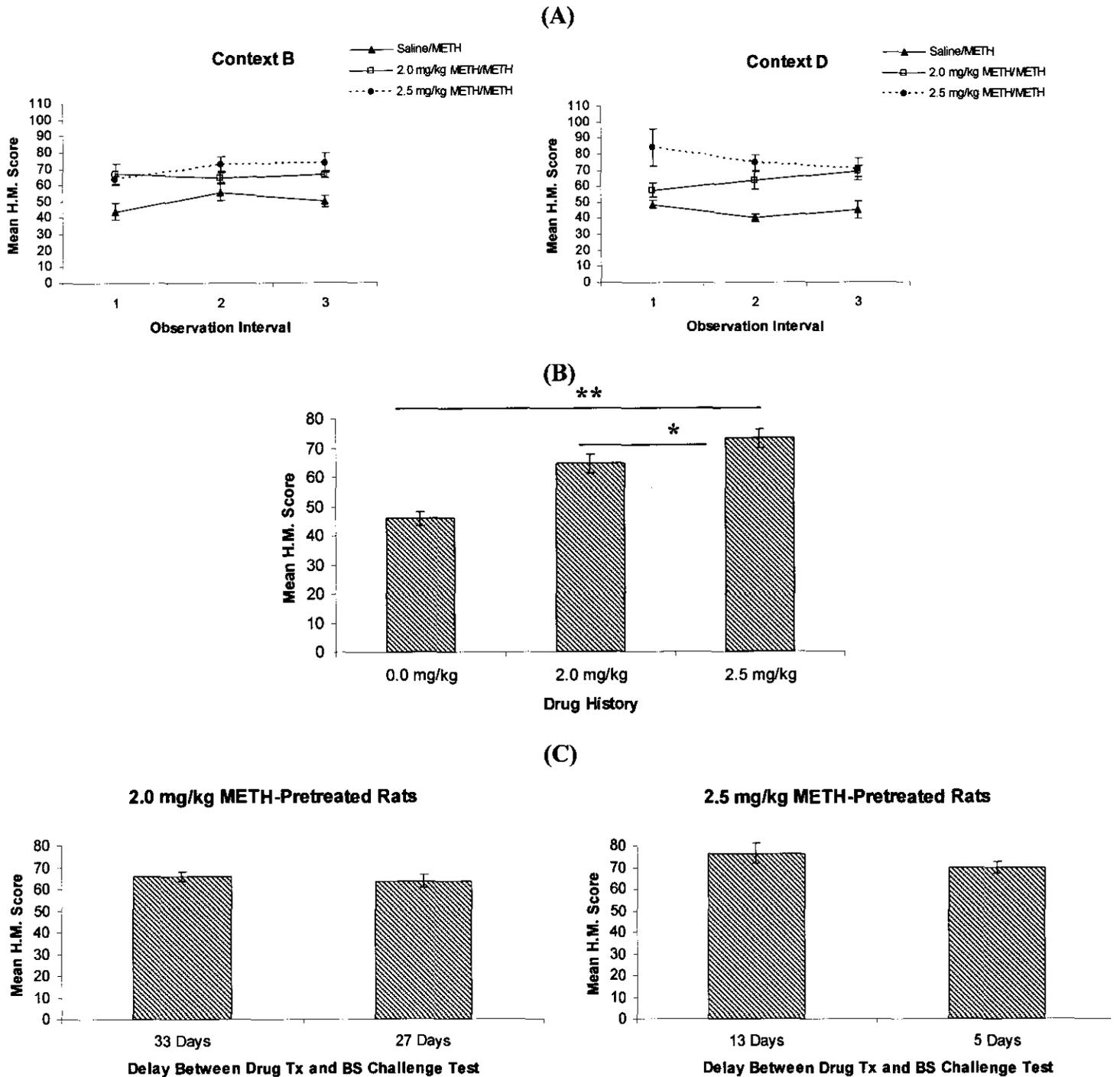


Figure 9. Mean (\pm SE) number of head movements exhibited in response to a low challenge dose from (A) drug pretreatment \times context groups during two-minute observation intervals every 10 minutes, up to 30 minutes post-injection, (B) drug pretreatment groups, and (C) METH-pretreatment groups with different delays between the last day of drug treatment and the BS challenge test. Note. * indicates $p < .08$; ** indicates $p < .01$

score for rats that were not pre-exposed to METH [$M = 45.78, SD = 2.21$] was significantly ($p < .01$) less than the average head movement score from rats pre-exposed to 2.0 mg/kg METH [$M = 64.61, SD = 3.12$], and significantly ($p < .01$) less than the average head movement score from rats pre-exposed to 2.5 mg/kg METH [$M = 73.06, SD = 3.12$]. Rats pre-exposed to 2.5 mg/kg and 2.0 mg/kg demonstrated a trend ($p < .08$) in head movements based on drug history (see Fig. 9B).

Context. The 3-way ANOVA did not yield a main effect of context [$F(1, 18) = .03, p > .05$]. However, a trend towards a dose \times context \times observation interval interaction was detected, $F(4, 36) = 2.52, p < .06, \eta^2 = .22$. To determine how the independent levels were being mediated, separate ANOVAs were calculated for each observation interval.

A training dose (3) \times context (2) between subjects ANOVA was calculated for observation interval one and demonstrated no context effect [$F(1, 23) = 1.39, p > .05$], but did reveal a trend towards a training dose \times context interaction, $F(2, 23) = 2.81, p < .09, \eta^2 = .24$. This trend suggests that training dose and context were modulating METH-induced responding for rats pre-treated with 2.5 mg/kg, since the average head movement score for rats trained in Context B [$M = 63.33, SD = 6.27$] was less than that of rats trained in Context D [$M = 84, SD = 6.27$]. Stereotypic head movement from saline-pretreated rats, and 2.0 mg/kg METH-pretreated rats did not appear to be modulated by context.

The ANOVAs calculated for observation intervals two and three revealed no context effect [observation interval one; $F(1, 23) = .72, p > .05$; observation interval two;

$F(1, 23) = .01, p > .05$], or training dose \times context interaction, [observation interval one; $F(2, 23) = 1.33, p > .05$: observation interval two; $F(2, 23) = 1.12, p > .05$].

Test for Conditioned Responding to the Drug-Paired Context

To test for conditioned responding to the drug-paired context, groups were separated by training dose and testing context (i.e., same or different) and means were calculated (Fig. 10). A training dose (2) \times context (2) \times observation interval (3) mixed

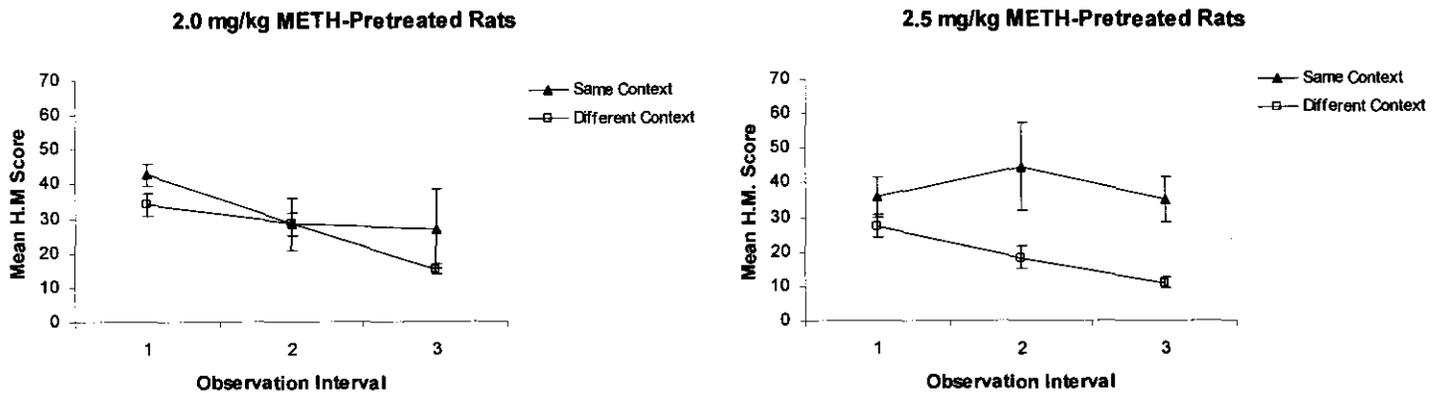


Figure 10. Mean (\pm SE) head movement scores recorded from 2.0 and 2.5 mg/kg METH-pretreated rats after receiving an injection saline in the drug-paired context during two-minute observation intervals that took place every 10 minutes, up to 30 minutes post-injection.

ANOVA was calculated, with training dose and context as between-subjects factors, and observation interval as a within-subjects factor.

The 3-way ANOVA yielded a significant main effect of testing context, $F(1, 8) = 21.01, p < .01, \eta^2 = .72$, such that the mean head movement score for rats that were administered saline in the drug-paired context [$M = 35.56, SD = 2.03$] was significantly

higher than that of rats that were administered saline in a novel context [$M = 22.39$, $SD = 2.03$] (Fig. 11A). An effect of training dose did not reach significance [$F(1, 8) = .05$, $p >$

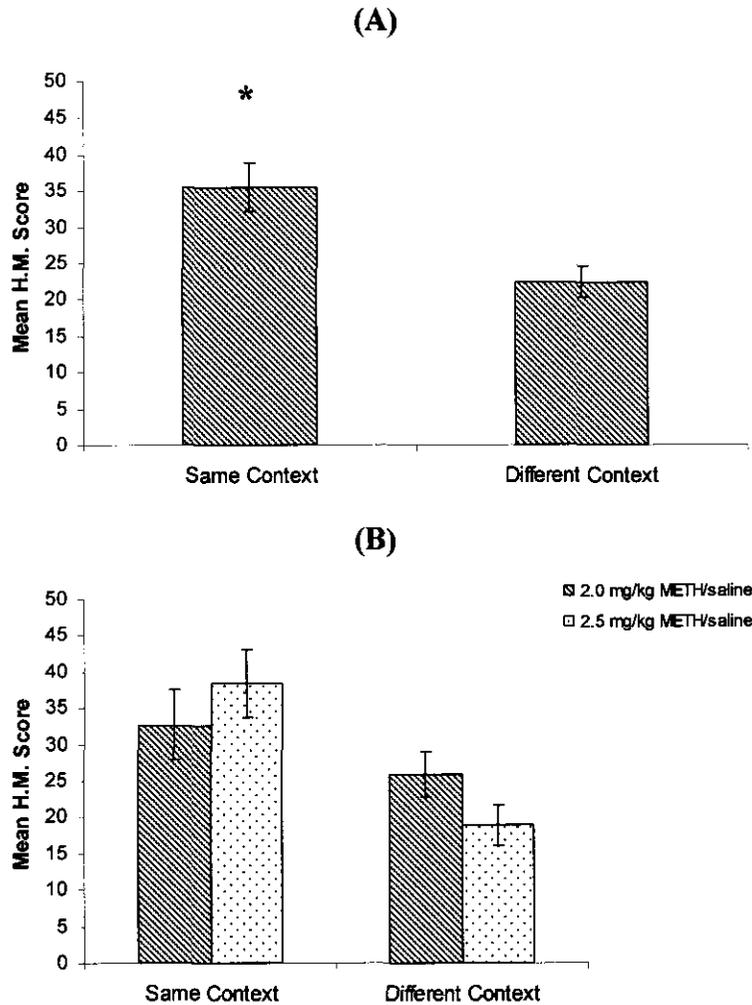


Figure 11. Test for conditioned responding to the drug context. Mean (\pm SE) head movement scores from (A) testing context groups after saline administration, and (B) pre-treatment dose \times testing context groups. Note. * indicates $p < .01$

.05]. However, a trend towards a training dose \times context interaction was revealed, $F(1, 8) = 4.95$, $p < .06$, $\eta^2 = .38$ (Fig. 11B). This trend demonstrates that both doses tested in

the same context responded higher than both doses tested in the different context. However, of the rats tested in the same context, on average the 2.0 mg/kg METH pre-treated rats [$M = 32.67$, $SD = 2.87$] responded less than the 2.5 mg/kg METH pre-treated rats [$M = 38.44$, $SD = 2.87$].

CPFE

Freezing behavior was analyzed with a drug treatment (2) dose (2) \times time of

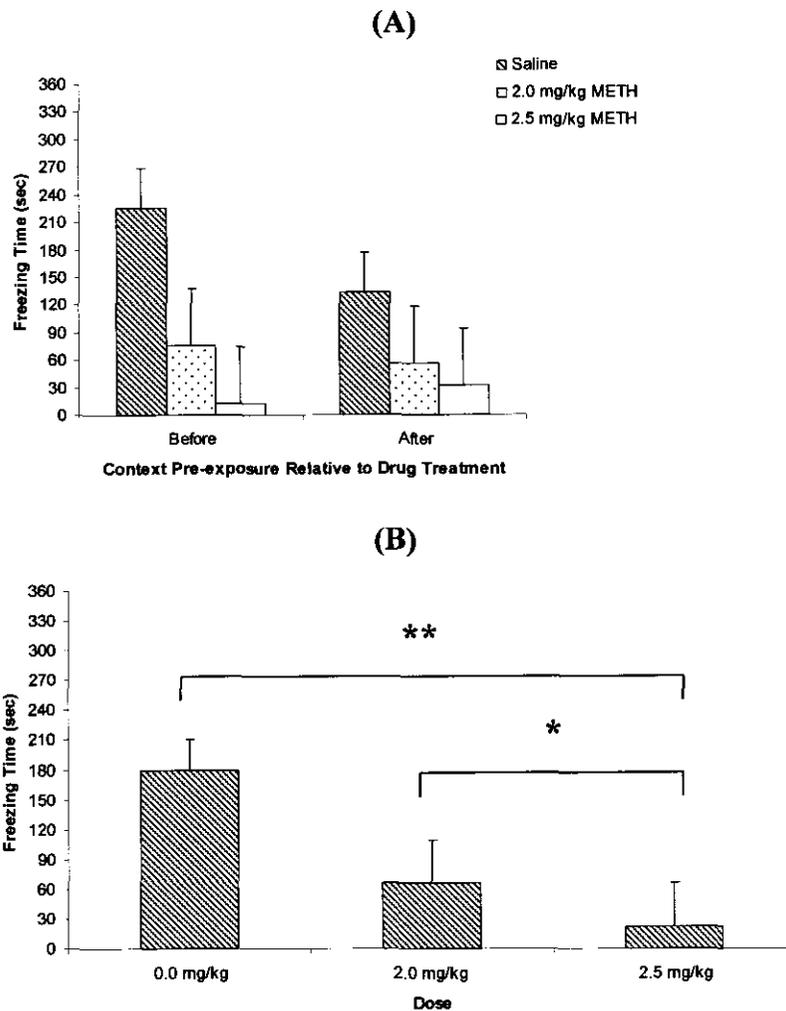


Figure 12. Mean (\pm SE) time (seconds) spent freezing to the shock-context from (A) dose \times time of context pre-exposure groups, and (B) dose alone groups. Note. * indicates $p < .1$; ** indicate $p < .01$

treatment (2) between subjects ANOVA. Figure 12A displays the mean (\pm SE) time (seconds) spent freezing to the shock-context from 2.0 mg/kg and 2.5 mg/kg METH-treated and saline-treated rats that were context pre-exposed either before or after drug treatment.

The ANOVA yielded a significant main effect of drug treatment, $F(1, 16) = 10.498, p < .01, \eta^2 = .396$, such that METH-treated rats, on average, froze significantly less time [$M = 44.25, SE = 29.524$] than the saline-treated rats [$M = 179.53, SE = 29.524$]. There was also a marginally significant effect of dose, $F(1, 16) = 3.047, p = .1, \eta^2 = .16$, such that rats pretreated with 2.0 mg/kg METH froze more to the shock context, on average, than rats pretreated with 2.5 mg/kg METH (Fig. 12B). No other effects or interactions reached significance ($ps > .05$).

Conclusions

Drug Treatment (5 days)

If head movement scores reflect the occurrence of BS, then they will exhibit an increase over treatment days. The main effect of day (see Fig. 6) demonstrates that BS of stereotyped head movement was produced in METH-treated rats. BS emerged on day three of drug treatment, and was further enhanced on day four. These results suggest that BS begins to occur after very few METH exposures, and, once it has developed, may be heightened with each successive METH treatment.

Stereotypic head movement emerges, and becomes more intense, with increasing doses of METH. Therefore, if the method used to score stereotypic head movement in this experiment is sensitive to dose response effects, then an effect of dose should be observed. An effect of dose was in fact identified, demonstrating that these data are

consistent with research (e.g., Kuczenski & Segal, 1999; Segal & Kuczenski, 1987; Takahashi, et al., 2000) that has shown stereotypies are characteristic of moderate and high METH doses, and become more pronounced as the METH dose increases. In addition, these data indicate that the novel method used to score stereotypic head movement is sensitive to dose, thus may be an accurate measure of the stimulating effects of METH.

On day one of drug treatment, the context surrounding drug administration appears to have modulated the stimulating properties of METH (see Fig. 8). Specifically, on day one when drug treatment was paired with Context B, METH-induced stereotypic head movements were more intense than when METH administration was paired with Context D, suggesting that Context B enhanced the acute effects of METH. Moreover, the context also modulated the sensitizing effects of METH, and possibly enhanced BS in rats trained in Context B, although the difference in head movements between Context B and Context D was not consistent across all four treatment days. These results are consistent with previous demonstrations that the acute and sensitizing effects of METH can be affected by the context surrounding drug administration (e.g., Crombag, et al., 2000; Crombag, et al., 2001; Fraioli, et al., 1999; Paolone, et al., 2003).

BS Challenge Test

The number of head movements exhibited by METH-pretreated rats in response to a low challenge dose was significantly greater than the response seen in saline-pretreated rats. This indicates that BS of METH-induced head movement developed after five days of METH treatment and can persist after a period of abstinence. Moreover, BS was consistent with drug history, such that 0.5 mg/kg METH evoked the largest

stereotypic response in 2.5 mg/kg METH-pretreated rats, followed in order by 2.0 mg/kg METH-pretreated and saline-pretreated rats (see Fig. 9).

These data are consistent with current views that BS may reflect METH-induced CNS changes which can be relatively long lasting. The delay between the last day of drug treatment and the BS challenge test was varied between one and five weeks, and even after a five week withdrawal period, METH-pretreated rats exhibited significantly more stereotypic head movement than saline-pretreated rats. In addition, when METH-pretreated rats were separated according to training dose, the stereotypic head movements from rats given a low challenge dose after the shortest delay were comparable to that of rats with the longest delay (see Fig. 9C). Thus, BS was not dependent on length of withdrawal. Taken together, five days of METH treatment may have led to neuroalterations that reflect the initial level of drug exposure, and be relatively long lasting.

Test for Conditioned Responding to the Drug-Paired Context

When rats were administered saline in a context that had previously been paired with five days of METH administration, they exhibited a conditioned drug response. This was apparent when the number of head movements from rats given saline in the drug-paired context was compared to that of rats given saline in a novel context (see Fig. 11). Specifically, saline administration in the drug-paired context led to a greater number of head movements than when saline was given to METH-pretreated rats in a novel context. Further, drug-free behavior was consistent with drug history; of the rats that were injected with saline in the drug-paired context, 2.0 mg/kg METH-pretreated rats displayed less head movements than 2.5 mg/kg METH-pretreated rats. This finding

suggests that conditioned responding to the drug-paired context reflects, not only the response that was once elicited in that context, but also the magnitude of that response. These results illustrate that through associative conditioning, a once neutral context (i.e., the modified cages) can elicit behavior that is reminiscent of a drug-induced response in the absence of the drug.

It is noteworthy that the head movements observed during the test for conditioned responding to the drug-paired context were qualitatively different from the head movements that were observed during five days of METH treatment. Head movement scores that were recorded during this phase of testing do not represent the stereotypic head movement which is elicited by METH. Instead, they likely reflect increased locomotion. Thus, there was a conditioned effect of METH, but the conditioned responding was not stereotypic.

CPFE

Results from this study demonstrate that five days of treatment with moderate and moderate-to-high doses of METH can dose-dependently attenuate the CPFE. Rats that were treated with saline froze significantly more to the shock-context than rats that were treated with METH, suggesting that METH impaired memory of the shock context. Moreover, 2.0 mg/kg METH-treated rats exhibited longer freezing times than that of 2.5 mg/kg METH-treated rats, indicating that memory deficits associated with higher doses of METH may be more profound (see Fig 12B). The impairment seen in this study was not dependent on the time of drug treatment relative to context pre-exposure. In other words, the CPFE was equally attenuated when five days of drug treatment preceded or

followed context pre-exposure. These findings indicate that five days of METH treatment can produce neuroalterations which lead to memory deficits.

The nature of the memory impairment identified in this study remains unknown. Memory impairment was established, but it is unclear if the impairment resulted from acquisition or retrieval deficits. To elaborate, it is possible that the context memory, shock memory, or conjunctive representation, where memory of the shock is attached to memory of the context, were impaired. It will be extremely difficult to parse out which phase of the CPFE has been compromised, because to do this, METH will need to be administered before and after all three phases of the CPFE paradigm, i.e., context pre-exposure, immediate shock exposure, test for freezing to the shock-context. This is problematic, as METH-induced hyperactivity will clearly interfere with freezing behavior, the main dependent variable during the test for freezing to the shock-context.

Discussion

The quantitative scoring method used in the present study appears to accurately reflect the stimulating and sensitizing effects of METH. It is well established that METH-induced, stereotypic head movement becomes more intense with escalating doses, and the results of this study demonstrate that exact dose response. Specifically, the number of head movements observed during a post-injection time sampling procedure was dose-dependently affected. In addition, the scoring method was sensitive to the course of drug action. That the measure was successful in demonstrating a dose response effect and the course of drug action implies it may be a useful tool in future studies concerned with METH-induced responding.

This study was able to establish an effective sensitization paradigm, as well as conditioned responding to the drug-paired context. However, there are some limitations of the procedures that were used which need to be addressed. To begin with, saline controls were not observed in, or paired with, Context B or Context D during the five days of drug treatment. Without comparing head movement data from METH-treated rats to that of saline-treated rats during the drug treatment phase, it is difficult to evaluate the robustness of the observed METH-induced responses.

That the saline-treated rats were never exposed to Context B or Context D confounds the results from the BS challenge test. While saline-pretreated rats exhibited significantly less head movement in response to a low challenge dose than METH-pretreated rats, it is unclear if this response was augmented by environmental novelty. BS was evident from a low dose of METH, and so the BS challenge test was successful in achieving the aforementioned goal. However, there may have actually been a larger effect of drug pretreatment than what was observed. Specifically, the novelty of the modified cages may have elicited exploratory behavior in the saline-pretreated rats, which was summed with the METH-induced hyperactivity.

With respect to the assessment of conditioned responding to the context, it is problematic that responding in the presence of a drug-paired context was not compared with responding in the presence of a saline-paired context, because, for practical reasons, saline-treated rats were not exposed to a distinct drug context during the drug treatment phase. For a more accurate assessment of conditioned responding, future studies would need to adapt the sensitization and drug context effects procedures such that METH- and saline-treated rats are given identical treatment during all phases.

The present study replicated the CPFE and demonstrated that METH attenuates the CPFE in normal SD rats. This result suggests that, in normal rats, a sensitizing regimen of METH is associated with neuroalterations that can be manifested as impaired context memory. Further, this impairment is independent of the time of drug administration. A limitation of the CPFE method used in the current study is its inability to elucidate what was actually impaired. METH may have attenuated the CPFE by disrupting storage or retrieval processes, or possibly both.

The current study was adapted in Experiment Two to address the effects of METH in the HIV-1 Tg rat. An animal model of HIV and METH use will be extremely valuable given the prevalence of METH use that exists in the HIV population, and the poor prognosis that is associated with it. Studying the effects of METH on unconditioned and conditioned behaviors in the HIV-1 Tg rat is a necessary step towards developing treatments for HIV-infected METH users.

EXPERIMENT TWO

The effects of METH in the presence of continuous HIV-1 infection were addressed in Experiment Two. The two procedures used in this study were adapted from Experiment One, and were executed simultaneously (Table 4) to evaluate the following in HIV-1 Tg rats and F344 controls; 1) sensitization and drug context effects associated with METH treatment, and 2) how the CPFE is affected by METH.

Table 4.

Time course for all Experimental Procedures and Testing Phases

	<i>Phase 1</i>	<i>Phase 2</i>	<i>Phase 3</i>	<i>Phase 4</i>	<i>Phase 5</i>	<i>Phase 6</i>	<i>Phase 7</i>	<i>Phase 8</i>
	Context pre-exposure	Habituation	Drug treatment	Immediate shock exposure	Test for freezing	BS challenge test	Test for conditioned responding	Tissue Collection
Days	1	2	3-7	8	9	10-11	10-11	12

Note. Twelve day procedure was replicated six times. Phases 6 and 7 of testing, i.e., the BS challenge test and test for conditioned responding, were alternated between days 10 and 11 of experimentation. These phases were systematically varied both between and within replications to ensure that all rats had the same extent of METH exposure prior to the BS challenge test and the test for conditioned responding.

Phase two, i.e., habituation, was added to the present study for several reasons. First, BS of METH-induced, stereotypic head movement did not emerge until day three of drug treatment in Experiment One. It is possible that head movements did not increase from day one to day two because the head movements that were scored on day one represented the sum of the effects of METH plus environmental novelty. Thus, on day

two, METH-induced head movements may have increased from day one, but this was not observed because the effects of environmental novelty were no longer present. If this were true, then METH-induced, stereotypic head movement would appear stable between days one and two, even if sensitization had occurred. A habituation phase was therefore added to eliminate any effects of environmental novelty, and ensure that only the stimulating properties of METH were measured on day one of drug treatment. If the animals habituate to the modified cages, then there will be an observed decrease in behavior on the habituation day. Moreover, if habituation is evident during phase two, then observed behavior on the first day of drug treatment can be attributed to the stimulating properties of METH alone.

Another purpose for adding the habituation phase was to assess baseline activity when placed in the modified cages. Any differences in drug-free activity elicited in Context B and Context D would be of relevance because it is hypothesized that the discretely distinct contexts will modulate the effects of METH. If differences in behavior exist prior to the onset of drug treatment this would need to be considered during later analyses of drug-induced behavior. For instance, if one context elicits greater exploratory behavior in a drug-free state, then it would be expected to see similar differences in behavior after METH-administration. In this case, however, the environment is not modulating the effects of METH. Instead, exploratory behavior elicited by the environment is being summed with METH-induced behavior. Thus, this would provide a similar problem to that of lack of habituation.

It was determined that baseline activity was also needed for comparison to the test for conditioned responding to the drug-context phase. In Experiment One, saline-treated

rats were never exposed to Context B or Context D during five days of drug treatment, and therefore they were not used during the test for conditioned responding. Instead of comparison to saline-controls, head movements from METH-treated rats that were given saline in the drug-paired context were compared to that of METH-treated counterparts that were given saline in a novel context. In the present study METH- and saline-treated rats were exposed to Context B and Context D during all five days of drug treatment. In this fashion, all METH-treated were administered saline in the drug-paired context because it was possible to compare their responses to saline controls. If all METH-treated rats are tested for a conditioned drug response in the drug-paired context, then this can be compared to behavior that was observed during habituation. Thus, the habituation phase was added, in part, to compare behavior that was observed before five days of drug treatment, to behavior that was observed after five days of drug treatment. This manipulation provides the opportunity to more definitively determine if METH-treated rats exhibit a conditioned drug response. Specifically, if METH-treated rats exhibit greater behavior during the test for conditioned responding than saline-treated rats *and* their own behavior prior to drug treatment, then they will have demonstrated conditioned responding to the drug-paired context.

Lastly, habituation was added to identify any strain differences in baseline activity. It is hypothesized that there will be no differences in behavior between HIV-1 Tg and F344 rats. If there are no strain differences in drug-free exploratory behavior, then any strain differences identified during the drug treatment, BS challenge test, and test for conditioned responding phases can be attributed to METH's effects while in the presence of the HIV-1 virus. In other words, differential drug responses between the

HIV-1 Tg and F344 rats could be attributed to interactions between the virus and METH, not motor effects of the virus alone.

In the present study, each replication consisted of two, four-rat squads that were run consecutively on all 12 days. For practical reasons, the post-injection time sampling procedure used during the drug treatment phase was cut down from 120 minutes to 90 minutes. Results from Experiment One indicate that a 90 minute observation period after METH administration is sufficient for demonstrating the time course of drug action, as well as long-term drug effects across days.

It is believed that HIV-1 Tg rats will be more sensitive to the stimulating effects of METH. If this is true, then HIV-1 Tg rats should exhibit a more robust acute response to METH administration, and possibly augmented sensitization. It is hypothesized that this will be independent of context effects. Re-stated, if HIV-1 Tg rats have a greater sensitivity to METH that is only associated with neuroalterations caused by the virus, then they will display a larger METH-induced behavioral response in Context B and Context D, compared to F344 rats. Further, if any context effects are identified, they will be the same for METH-treated HIV-1 Tg and F344 rats.

In line with the previous hypothesis, it is expected that conditioned responding to the drug-paired context will not differ between HIV-1 Tg rats and F344 controls. If METH-treated HIV-1 Tg rats exhibit a more robust acute response or augmented sensitization compared to METH-treated F344 rats, this difference can be attributed to interactions between the virus and METH if no differences are observed during the test for conditioned responding to the drug-paired context. If the conditioned drug responses of HIV-1 Tg and F344 rats can be dissociated, then this would suggest differential

context × drug interactions. It is believed that that the environment surrounding drug administration will have similar modulatory effects between strains. Similarly, associations between the METH-induced responses and the context that those responses are elicited in should be comparable, such that no strain differences in conditioned drug responses are identified.

In Experiment One, the home cage with all three rats was transferred from the vivarium to the experimenting room. In the present study, animals were individually transferred to the experimenting room in separate carriers, and the home cage was left in the vivarium. This was considered necessary to eliminate any associations between the home environment and the drug context. It is likely that an enhanced METH-induced response will be observed from abolishing home cage-drug context associations, such that stereotypic head movement scores from Experiment Two will demonstrate an increase compared to head movement scores from Experiment One.

Importantly, since it is expected that there will be no strain differences related to drug context effects and conditioned responding to the drug-paired context, it was essential to ensure that all animals had equal exposure to the experimenting room (located outside of the vivarium) and the modified cages. To do this, everything was executed at 2.5-minute intervals; animals were individually brought into the experimenting room at 2.5-minute intervals, given injections and placed in the modified cages at 2.5-minute intervals, and removed from the modified cages and returned to their home cages at 2.5-minute intervals. It was determined that this was optimal for maintaining an equal level of exposure to the drug-paired context.

Rodent head movements were the only dependent variable in Experiment One. However, in the present study both head movements and rearing events were scored during all sensitization and drug context effects procedures. The purpose of this addition was twofold. First, METH-induced stereotypy can increase at the expense of locomotor behavior (i.e., rearing) and vice versa. Therefore, if only the behavior that decreased happened to be chosen as the dependent variable, the drug effect may have been mistakenly interpreted as a tolerance effect rather than a sensitization effect. Because stereotypic and locomotor behaviors compete they need to be studied in unison to accurately demonstrate the behavioral effects of METH.

Second, the effects of METH in the HIV-1 Tg rat have not been characterized yet. The additional variable will better illustrate the HIV-1 Tg rat behavioral response profile, and identify any differences between HIV-1 Tg and F344 rats. In normal, rats, it has been well established that there is a wide range of individual difference in responsiveness to psychostimulant drugs (Kuczenski & Segal, 1999; Kuczenski, et al., 1995; Milesi-Halle, et al., 2007; Segal & Kuczenski, 1999). The present study is thus equipped to identify if METH-induced behavioral responses from HIV-1 Tg rats are similar to that of normal F344 rats, or if HIV-1 Tg rats exhibit a differential characteristic response profile.

In Experiment One, the CPFE was attenuated by five days of METH treatment, but time of drug treatment relative to context pre-exposure did not mediate this impairment. Because time of drug treatment had no effect on the CPFE, during phase one of the present study, all rats were context pre-exposed prior to drug treatment.

It is expected to replicate the findings of Experiment One, such that METH-treated F344 rats will show impairments similar to those seen in METH-treated SD rats.

Additionally, it is believed that there will be a difference between F344 and HIV-1 Tg rats. It is unclear however, what difference should be expected. It has been shown that HIV-1 Tg rats exhibit cognitive impairments in a modified MWM (LaShomb, et al., 2008; Vigorito, et al., 2007), so it may be possible for HIV-1 Tg rats to show global cognitive impairments. In this scenario, it would be expected that saline-treated HIV-1 Tg rats display memory impairments compared to saline-treated F344 rats. Further, METH would attenuate the CPFE even more so in HIV-1 Tg rats than F344 rats.

Conversely, in a fear conditioning paradigm it has been demonstrated that HIV-1 Tg rats exhibit greater fear conditioning as measured by freezing (LaShomb, et al., 2007). If this translates to the CPFE, then it would be expected that saline-treated HIV-1 Tg rats will exhibit greater memory of the shock-context than saline-treated F344 rats. METH *should* impair memory of the shock-context, as was demonstrated with SD rats in Experiment One. However, if HIV-1 Tg rats exhibit greater fear conditioning, then it is unclear how METH will affect their performance in the CPFE. Because the CPFE paradigm is more closely related to the fear conditioning paradigm than it is to the MWM, it is probable that strain differences will parallel those found in the fear conditioning paradigm, and not the MWM.

A tissue collection was added to the present study. This phase was added to measure the expression of HIV-1 viral proteins in various brain structures and to elucidate if METH-induced neuroalterations differ between HIV-1 Tg rats and F344 controls. Additionally, this will allow for direct correlations to be made between brain function and behavior. However, the results from this experimental phase will not be reported here. Therefore, the present study provides an analysis of the effects of METH

in the HIV-1 Tg rat at the behavioral level, and only inferences will be made regarding brain function.

Method

Animals

Twenty-four experimentally naïve, male HIV-1 Tg rats and 24 experimentally naïve, male F344 strain background controls were obtained from Harlan Co. (Indianapolis, IN) and used as subjects. It was determined that Tg littermate controls were not needed, and that F344 rats were a sufficient control group. Earlier studies that have implemented the HIV-1 Tg rat as an animal model of HIV have found differences between the HIV-1 Tg rat and Tg controls, but no differences between Tg controls and F344 controls. This suggests that any differences found in the HIV-1 Tg rat can be associated with the virus and not the transgenic process.

Animals ranged between eight and twelve weeks of age throughout testing. All animals were double housed in clear, plastic rat cages (45.7 cm × 22.9 cm × 20.3 cm) with Harlan Teklad™ 1.8", corn-cob bedding. Food (Harlan Teklad™ Mouse/Rat Laboratory Diet 7102) and water were provided *ad libitum* through the duration of the study. The vivarium was maintained on a 12:12 hour light-dark cycle (8:00am – 8:00pm), and within recommended temperature ($22^{\circ} \pm 5^{\circ}$ C) and humidity ($50\% \pm 20\%$) conditions. Throughout the study rat body weights were monitored. Body weights were measured daily at 10:00am ● 1 hour, and prior to any experimentation. 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.

Apparatuses

METH conditioning apparatus. On all drug treatment days, METH and saline administration were paired with one of two discretely distinct contexts (see Fig. 2). All contextual manipulations described in experiment one remained the same, except that squad one was always paired with Context B during early experimentation (i.e., 10:00 am – 12:00 pm, \pm 1 hour), and squad two was always paired with Context D during late experimentation (i.e., 12:30 pm – 2:30 pm, \pm 1 hour). With this minor adaptation the modified cages had four discretely distinct cues: Context B consisted of a bright surrounding, smooth surface, mint scent, and morning; and Context D consisted of a dark surrounding, rough surface, vanilla scent, and afternoon. A fourth cage was added to each context, thus there were a total of eight modified cages (i.e., four Context B cages and four Context D cages).

Shock chamber. All CPFE procedures took place in one operant conditioning chamber. The dimensions of the operant chamber and the computer software that was used to run the immediate shock program is described in experiment one.

Drugs and Solutions

All animals used in this study received i.p. injections of METH or saline, via 27½ gauge/1cc/syringes. METH was obtained from Sigma-Aldrich Co. (St. Louis, MO.) and dissolved in sterile 0.9% saline immediately prior to injections. Results from experiment one indicated that 2.5 mg/kg METH was an optimal dose for eliciting stereotypic head movement and studying sensitization and context effects associated with METH administration. Throughout the drug treatment phase, METH was administered at a dose of 0.0mg/kg (saline) or 2.5mg/kg at a volume of 1 ml/kg.

On challenge test days and conditioned responding test days METH was administered at a dose of 0.5mg/kg and 0.0mg/kg at a volume of 1 ml/kg, respectively. This dose was chosen based on previous research (e.g., Brennan, et al., 2007; Itzhak, et al., 2002), and the results from experiment one which demonstrate that 0.5 mg/kg METH is an appropriate challenge amount.

Procedures

The time course for all experimental procedures and testing phases consisted of six 12-day replications (see Table 4). A 12-day replication was further divided into eight

Table 5.

A. Sensitization and Drug Context Effects Procedures

	<i>Phase 1</i>	<i>Phase 2</i>	<i>Phase 3</i>	<i>Phase 4</i>	<i>Phase 5</i>	<i>Phase 6</i>	<i>Phase 7</i>	<i>Phase 8</i>
		Habituation	Drug treatment			BS challenge test	Test for conditioned responding	Tissue Collection
Days		2	3-7			10-11	10-11	12

B. CPFE Procedure

	<i>Phase 1</i>	<i>Phase 2</i>	<i>Phase 3</i>	<i>Phase 4</i>	<i>Phase 5</i>	<i>Phase 6</i>	<i>Phase 7</i>	<i>Phase 8</i>
	Context pre-exposure		Drug treatment	Immediate shock exposure	Test for freezing			Tissue Collection
Days	1		3-7	8	9			12

Note. A twelve-day replication consisted of two different experiments, (A) the sensitization and drug context effect procedures and (B) the CPFE paradigm, which were conducted simultaneously.

testing phases; 1) habituation, 2) context pre-exposure, 3) drug treatment (5 days), 4) immediate shock exposure, 5) test for freezing, 6) BS challenge test, 7) test for conditioned responding to the drug-paired context, and 8) tissue collection. Phases one, three, six, seven and eight comprised the sensitization and drug context effects procedures, (Table 5A), while phases two, three, four, five, and eight comprised the CPFE procedures (Table 5B).

With the exception of phases six and seven, phase order remained constant for all six replications. Phases six and seven of testing, i.e., the BS challenge test and test for conditioned responding, were alternated between days 10 and 11 of experimentation. These phases were systematically varied both between and within replications to ensure that all rats had the same extent of METH exposure prior to the BS challenge test and the test for conditioned responding.

Sensitization and Drug Context Effects

With the exception of replication three, which only had six rats (HIV-1 Tg, n = 3; F344, n = 3), all replications consisted of eight rats (HIV-1 Tg, n = 4; F344, n = 4), that were divided into two squads of four (squad one – METH-treated HIV-1 Tg, n = 1; METH-treated F344, n = 1; saline-treated HIV-1 Tg, n = 1; saline-treated F344, n = 1; squad two – METH-treated HIV-1 Tg, n = 1; METH-treated F344, n = 1; saline-treated HIV-1 Tg, n = 1; saline-treated F344, n = 1). In all sensitization and drug context effects procedures squad one was always run first (in the morning) and paired with Context B, while squad two was always run second (in the afternoon) and paired with Context D.

Room entry, injection administration, post-injection observations, and room exit followed the same time line during all sensitization and drug context effects procedures. Animals were individually removed from their home cages and transferred to the experimenting room at 2.5-minute intervals. After the fourth animal entered the experimenting room, injections began, and were administered at 2.5-minute intervals. Each animal was placed in its designated context immediately post-injection and left undisturbed for observation. The only difference between phases was that the post-injection observation period was varied depending on the specific procedure.

Animals were always run in the same order and paired with the same context. Order was counterbalanced within and between replications, such that the alternation of HIV-1 Tg rats and F344 controls was even (e.g., HIV-1 Tg rat → F344 rat → HIV-1 Tg rat → F344 rat; and F344 rat → HIV-1 Tg rat → F344 rat → HIV-1 Tg rat were counterbalanced). The experimenter remained in the room for the duration of each drug session and scored the number of head movements (see Fig. 3) and rearing events that were observed live during each observation interval. In addition, all sessions were recorded to a commercial DVR that was connected to a ceiling camera which provided an aerial view of the four units. All modified cages and countertops were sanitized with 70% ethanol alcohol before and after each use.

Habituation. Habituation always took place the day before the drug treatment phase. After receiving an i.p. injection of saline, each rat was immediately placed in Context B (squad one) or Context D and left undisturbed for 60 minutes. Behavior was scored during two-minute intervals, every 10 minutes, for up to 60 minutes post-injection. The session was over after the sixth observation interval for the fourth rat, at

which point animals were individually returned to their home cages at 2.5-minute intervals.

Drug treatment (5 days). During drug treatment, rats were administered saline or 2.5 mg/kg METH on five consecutive days. On each day, rats were left undisturbed for 90 minutes after receiving an injections and being placed in their designated training contexts. A session was terminated after the ninth observation interval for the fourth rat in a squad, at which point rats were individually returned to their home cages.

BS challenge test. All rats received a low challenge dose of METH during the BS challenge test. Saline-pretreated and METH-pretreated rats were administered 0.5 mg/kg METH in the context that was previously paired with five days of drug treatment. Behavior was scored for up to 60 minutes post-injection, and then rats were individually returned to their home cages. The duration of this session was extended such that it was twice the length of the BS challenge test that was used in Experiment One. This test was extended so that the course of drug action after a low challenge dose could be evaluated thoroughly in HIV-1 Tg rats and F344 controls.

Test for conditioned responding to the drug-paired context. All rats received a saline injection and were immediately placed in their designated training context; testing context always matched training context. Behavior was scored for up to 30 minutes post-injection. The length of the test for conditioned responding to the drug-paired context observation period was kept the same as that used in Experiment One. It was determined that 1) 30 minutes was a sufficient length of time to elicit a conditioned drug response, and 2) extending the session would result in decrease in behavior reflecting habitual processes, which were not of interest during this particular phase.

Tissue collection. On the twelfth, and last day of each replication animals were sacrificed in their running order, beginning at 9:30 am (\pm 1 hour). Rats were sacrificed by decapitation and the trunk blood, spleen, thymus, liver, spinal cord, and brain were collected. Cytokine levels in the serum were measured, and the spleen, thymus, and brain were weighed and body weight ratios were calculated. Over wet ice, the brain was dissected into the cerebellum, pituitary gland, hypothalamus, PFC, cortex, striatum, and hippocampus for RNA extraction.

CPFE

The experimenting room was identical to that described in Experiment One. The transfer to the experimenting room and then back to home cage were also identical, as was the carrier that was used to transfer each rat. The operant chamber was sanitized with 70% ethanol alcohol before and after each rat. Figure 13 depicts the CPFE procedure summary.

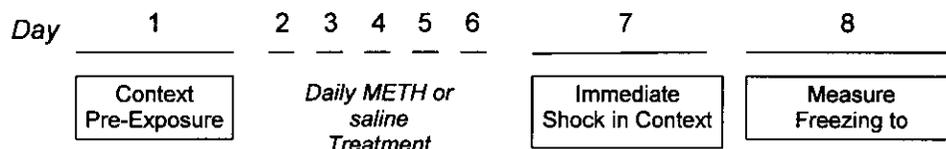


Figure 13. CPFE procedure summary. All rats were context pre-exposed on day one. During days two through six rats were treated with saline or METH. Immediate shock exposure occurred on day seven, and was followed by the test for freezing to the shock-context 24 hours later.

Context pre-exposure. Rats were individually transferred from their home cages to the experimenting room. After entering the room, the experimenter closed the door

behind her, and set the carrier down for 60 seconds. After 60 seconds, the experimenter removed the rat from the carrier and placed it in the operant chamber as quickly as possible. After 45 seconds in the operant chamber, the experimenter removed the rat and placed it back in the carrier as quickly as possible. This process was repeated eight consecutive times before the subject was transferred back to its home cage.

Drug treatment (5 days). Rats were administered saline or 2.5 mg/kg METH on five consecutive days. The context of drug administration was completely separate from that of all other CPFE procedures (see procedures for sensitization and drug context effects), and all rats were drug-free during context pre-exposure, immediate shock exposure, and the test for freezing.

Immediate shock exposure. Rats were individually transferred into the experimenting room, and placed in the operant chamber immediately after the carrier was set down. After the chamber door was closed, the experimenter immediately pressed a lever on the outside of the chamber to deliver a two-second, 1.0 mA footshock. Immediately after the shock terminated, the rat was removed from the operant chamber and returned to its home cage.

Test for freezing. Twenty-four hours after immediate shock exposure rats were individually transferred to the experimenting room, and left in the operant chamber for a six-minute observation period before being returned to their home cages. The test for freezing was described in detail in Experiment One. An additional manipulation, however, was that behavior was not scored in a binary fashion. Instead, freezing behavior, rearing, grooming and other exploratory movements that could not be categorized as rearing or grooming were scored via EthoLog 2.2.

Results

Due to the length of each replication, the animals tested in this experiment came from two different cohorts to maintain the same age during experimentation. To determine if there were any effects of cohort, preliminary analyses were conducted on data that was collected during each testing phase. These analyses revealed no cohort effects or interactions, indicating that any variance in behavior could not be attributed to differences between the litters. Because there were no a priori hypotheses regarding cohortal effects, this variable was removed from all subsequent analyses.

Sensitization and Drug Context Effects

Habituation

To assess baseline activity prior to drug treatment, as well as habituation to the context, means (\pm SE) were calculated for the number of rodent head movements (Fig. 14A) and rearing events (Fig. 14B). To identify any effects of context or strain on baseline activity each behavior was analyzed with a context (2) \times strain (2) \times observation interval (6) mixed ANOVA with context and strain as between-subjects factors, and observation interval as a within-subject factor.

There was a significant main effect of observation interval for number of head movements, $F(5, 210) = 3.753, p < .01, \eta^2 = .082$ (Fig. 15A), and rearing events, $F(5, 210) = 2.423, p < .05, \eta^2 = .055$ (Fig. 15B). Pairwise comparisons demonstrated that head movement scores and rearing events that were recorded during observation interval six were significantly ($p < .05$) less than that of observation intervals one, two, three, and five; and observation interval four were significantly ($p < .01$) less than that of observation interval one, indicating that all rats habituated to the drug-context. No other

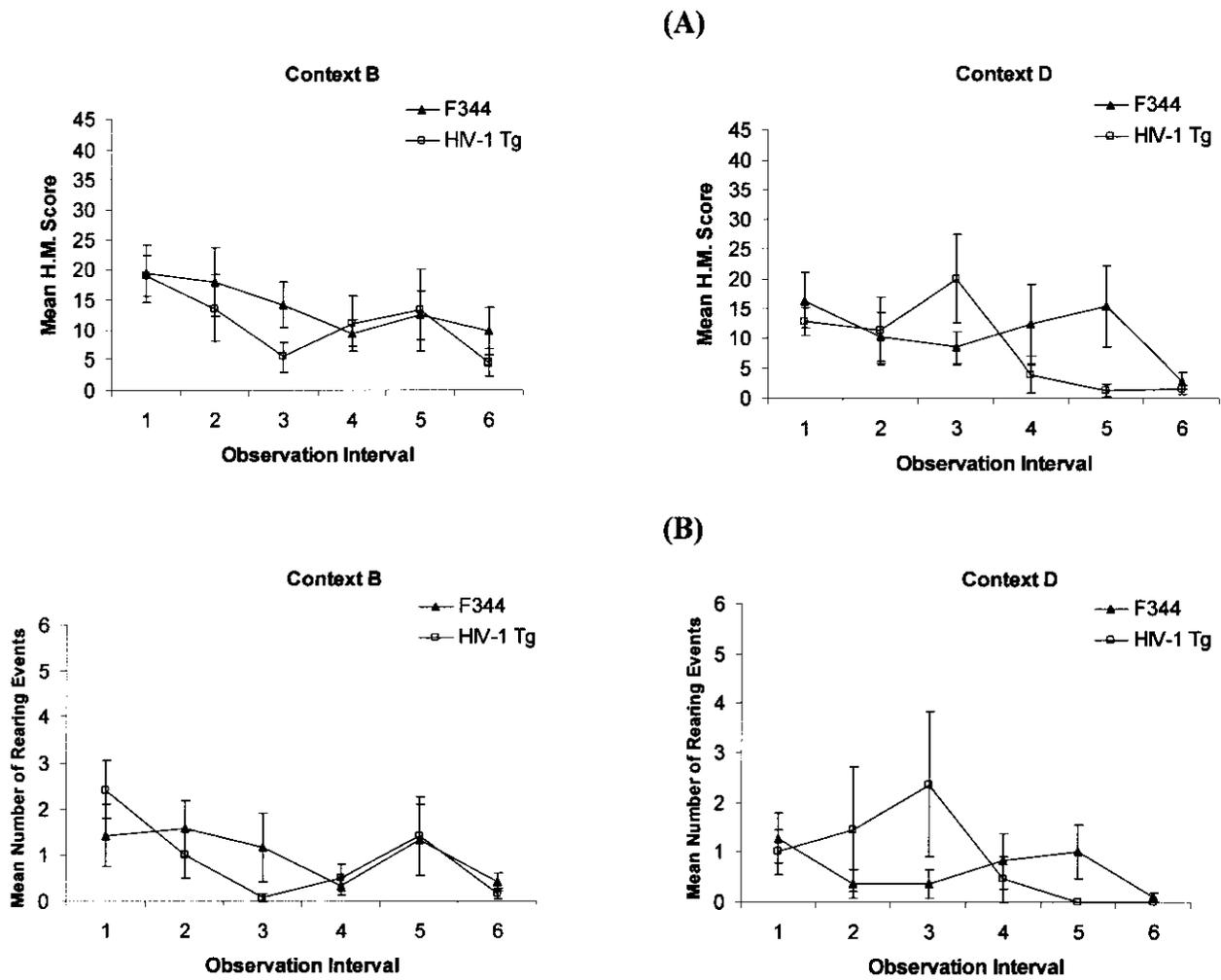


Figure 14. Mean (\pm SE) number of (A) head movements and (B) rearing events recorded from context \times strain groups during two-minute observation intervals that took place every 10 minutes, for up to 60 minutes after receiving an injection of saline.

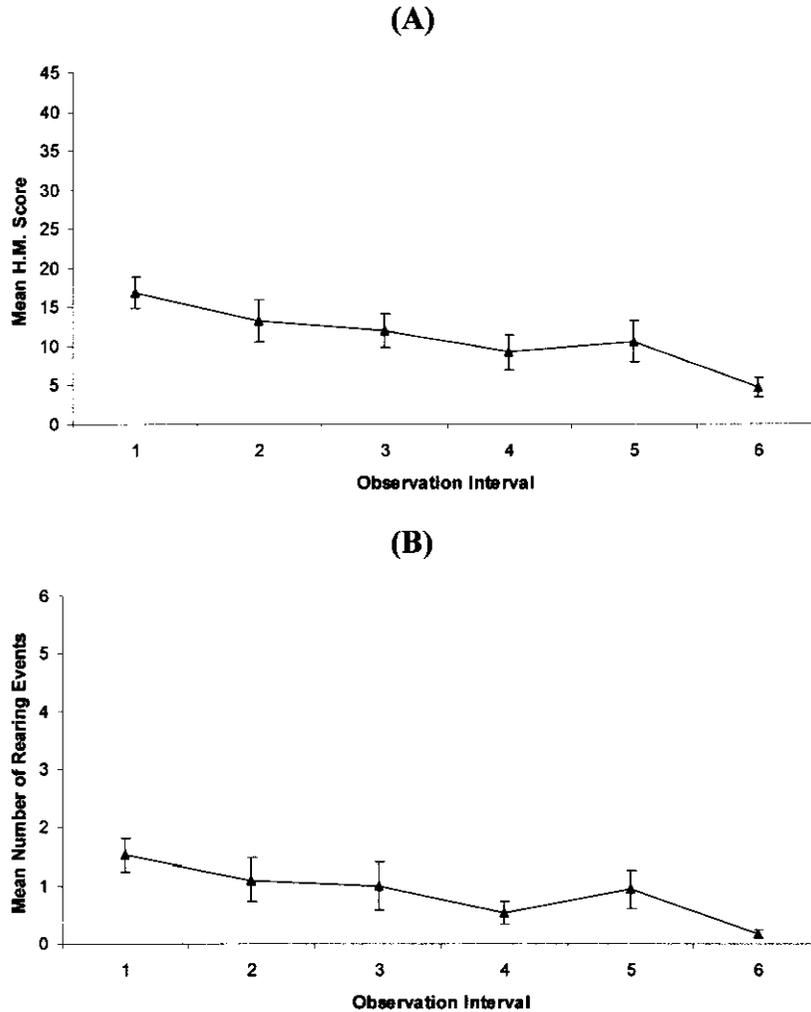


Figure 15. Mean (\pm SE) number of (A) head movements and (B) rearing events recorded during two-minute observation intervals that took place every 10 minutes, for up to 60 minutes after receiving an injection of saline.

effects or interactions reached significance ($ps > .05$), suggesting that basal levels of activity were not affected by strain or context.

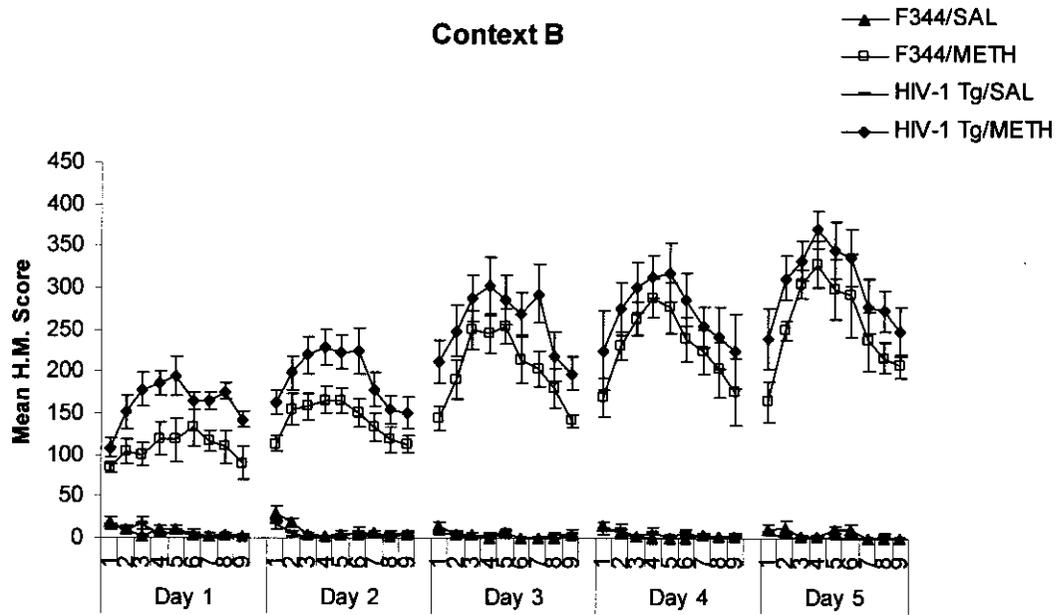
Drug Treatment (5 Days)

To identify any modulatory effects of strain or context on the psychoactivating and behavior sensitizing properties of METH administration, means (\pm SE) were

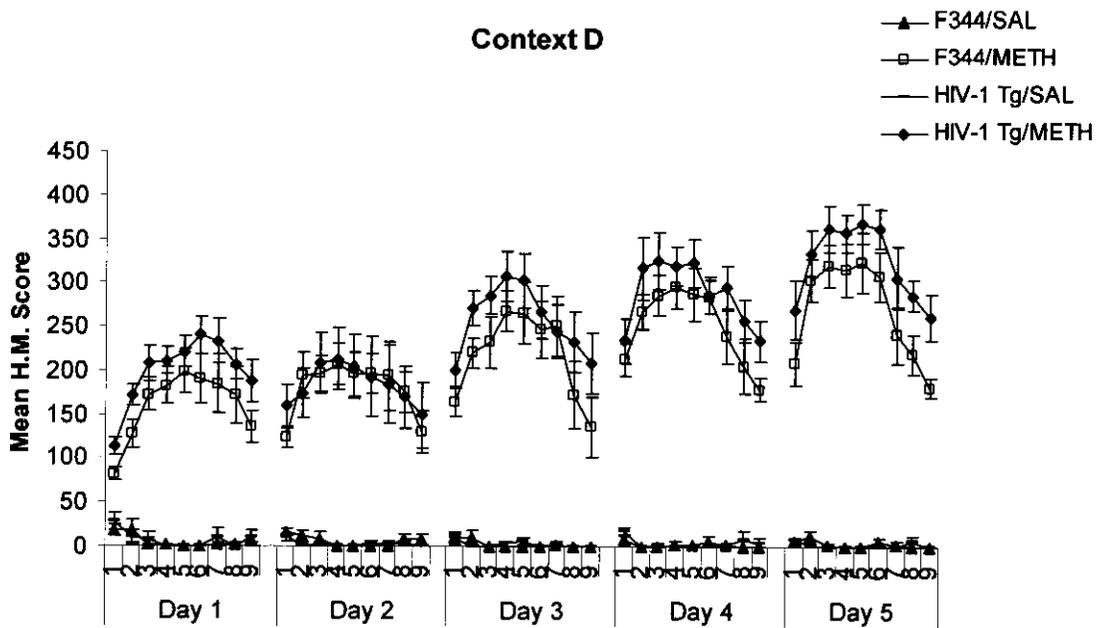
Figure 16. Mean (\pm SE) number of (A) head movements and (B) rearing events recorded from context \times strain \times drug groups during two-minute observation intervals that took place every 10 minutes, for up to 90 minutes post-injection.

(A)

Context B

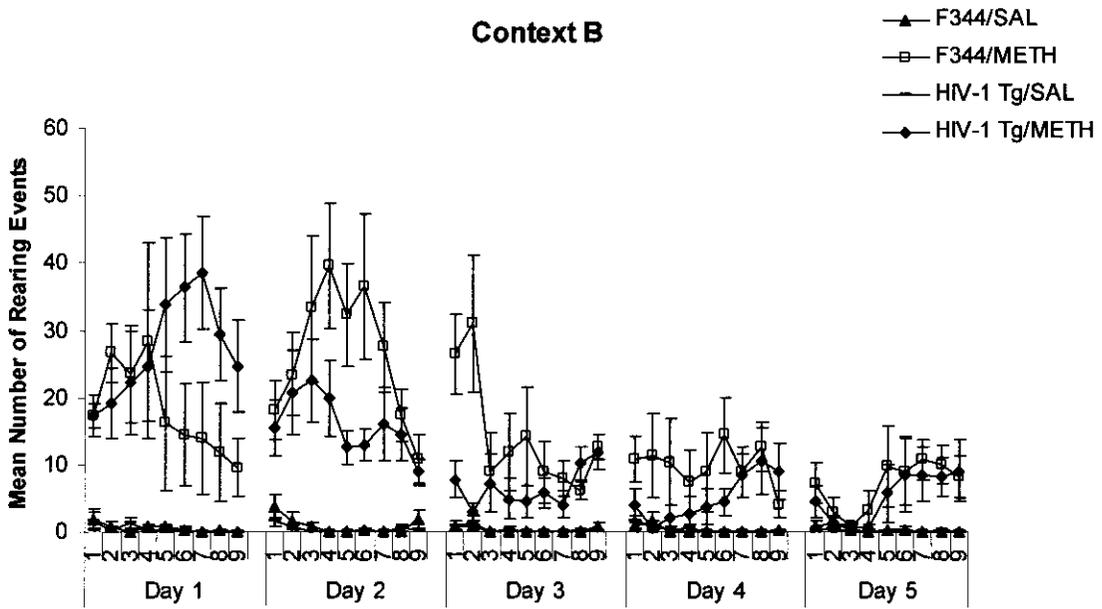


Context D

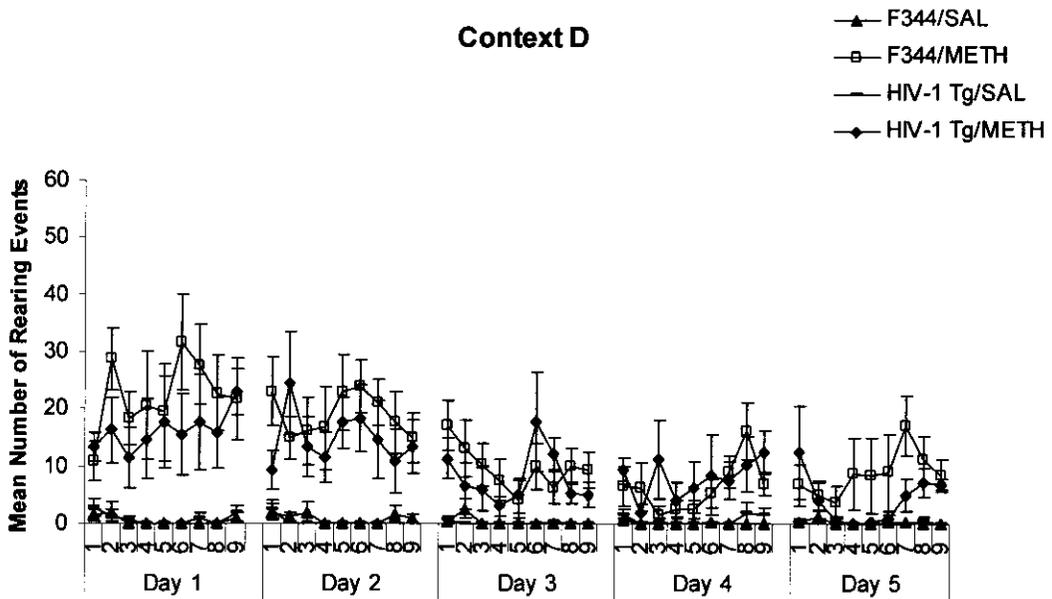


(B)

Context B



Context D



calculated for the number of rodent head movements (Fig. 16A) and rearing events (Fig. 16B). Each dependent variable was analyzed with a strain (2) × context (2) × drug (2) × day (5) × observation interval (9) mixed ANOVA, with context, strain and drug as between-subjects factors, and day and observation interval as within-subjects factors. All data were first analyzed with the 5-way ANOVAs followed by additional ANOVAs to evaluate predicted interactions and any other observed interactions of interest.

Psychoactivating and behavior sensitizing properties of METH administration.

The 5-way ANOVAs that were calculated for rodent head movements and number of rearing events revealed a significant main effect of drug [head movements; $F(1, 38) = 556.932, p < .001, \eta^2 = .936$; rearing events; $F(1, 38) = 121.332, p < .001, \eta^2 = .762$], such that animals that were treated with METH exhibited, on average, a significantly greater number of head movements (Fig. 17A) and rearing events (Fig. 17B) [head movements; $M = 222.14, SE = 6.367$; rearing events; $M = 12.591, SE = .765$] than that of animals treated with saline [head movements; $M = 4.388, SE = 6.678$; rearing events; $M = .38, SE = .802$]. Both 5-way ANOVAs also revealed a significant day × drug interaction [head movements; $F(4, 152) = 58.274, p < .001, \eta^2 = .605$; rearing events; $F(4, 152) = 15.293, p < .001, \eta^2 = .287$] (Fig. 18). To assess the day × drug interactions, the number of rodent head movements and rearing events were further analyzed with additional strain (2) × context (2) × day (5) × observation interval (9) mixed ANOVAs, for the METH- and saline-treated rats separately.

The separate ANOVAs revealed a significant main effect of day [METH-treated rats; $F(4, 160) = 61.395, p < .001, \eta^2 = .754$; saline-treated rats; $F(4, 144) = 7.073, p < .001, \eta^2 = .282$], such that the number of rodent head movements from METH-treated

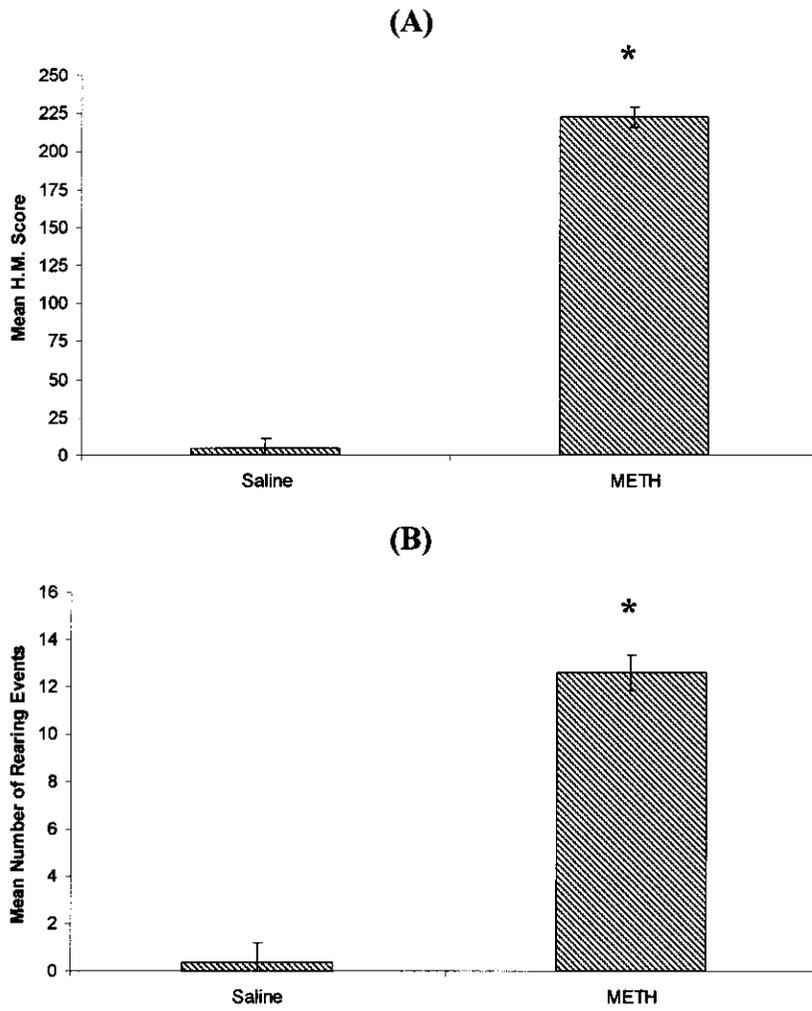
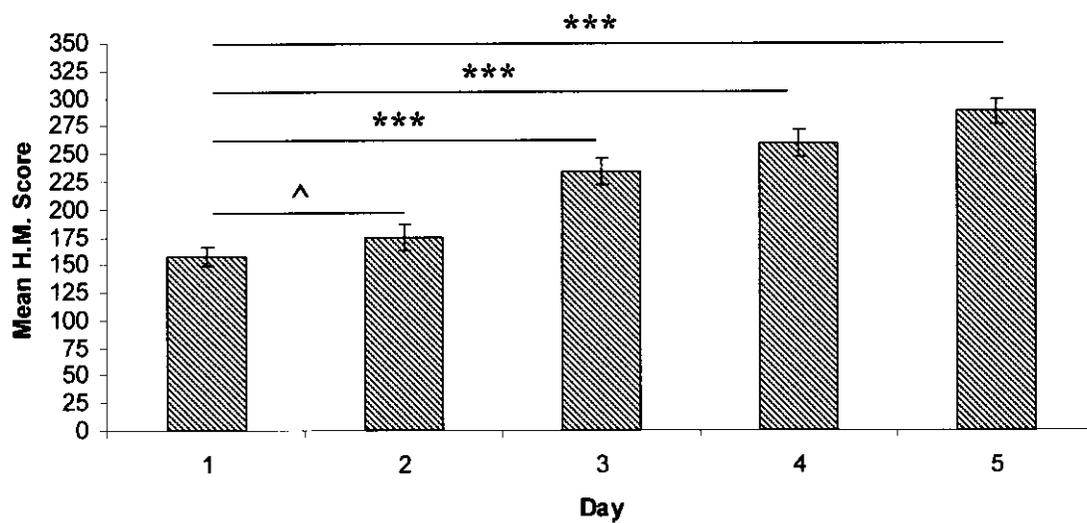


Figure 17. Mean (\pm SE) number of (A) head movements and (B) rearing events from saline- and METH-treated rats. Note. * indicates $p < .001$

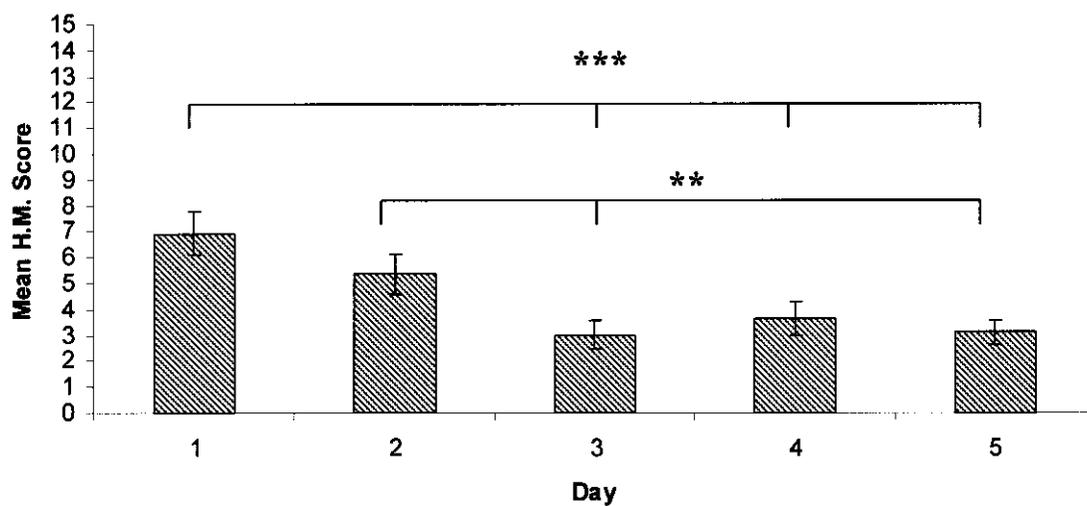
Figure 18. Mean (\pm SE) number of (A) head movements and (B) rearing events from METH- and saline-treated rats across five days of drug treatment. *Note.* ^ indicates $p < .06$; * indicates $p < .05$; ** indicates $p < .01$; *** indicates $p < .001$

(A)

METH-Treated Rats

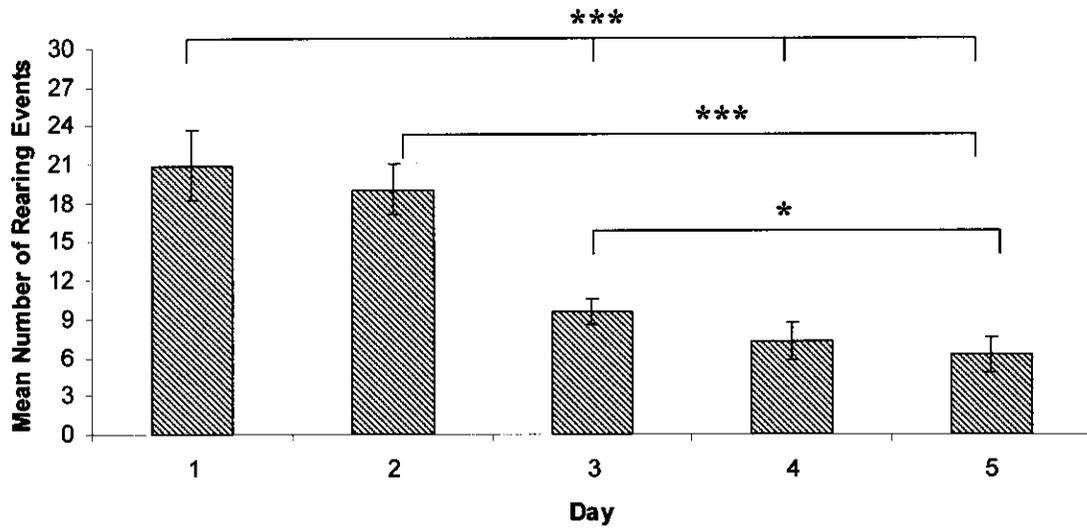


Saline-Treated Rats

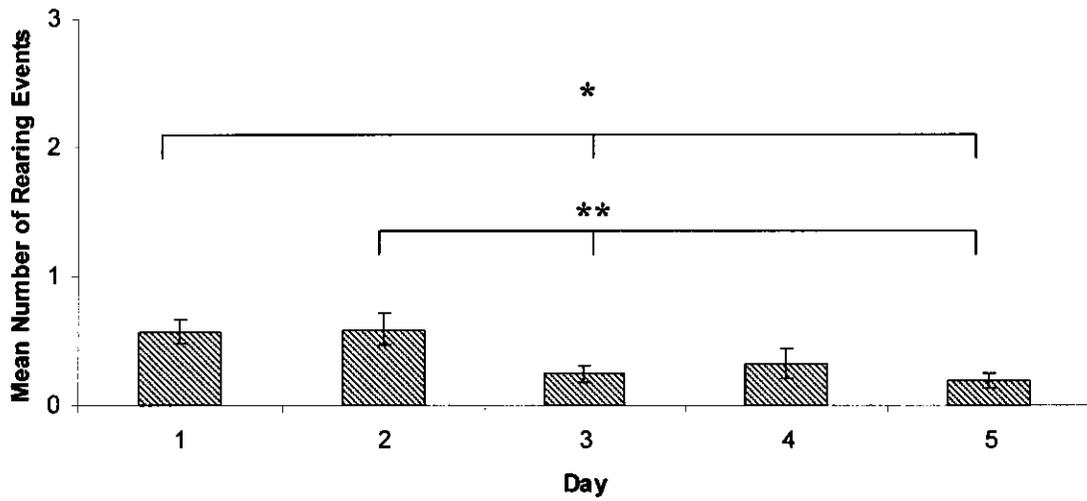


(B)

METH-Treated Rats



Saline-Treated Rats



rats significantly increased during five days of drug treatment, whereas that of saline-treated rats significantly decreased (see Fig. 18A). The separate ANOVAs that were calculated for the number of rearing events from METH- and saline-treated rats revealed a significant main effect of day [METH-treated rats; $F(4, 160) = 17.85, p < .001, \eta^2 = .472$; saline-treated rats; $F(4, 144) = 3.61, p < .05, \eta^2 = .167$], such that rearing significantly ($p < .05$) decreased between all days, except days one and two and four and five for METH-treated rats, whereas rearing on days three and five showed a significant ($p < .05$) decline from days one and two for saline-treated rats (see Fig. 18B).

The 5-way ANOVA for rodent head movements revealed a significant observation interval \times drug interaction, $F(8, 304) = 83.863, p < .001, \eta^2 = .688$. Figure 19A shows the pattern of head movements across observation intervals. Follow up ANOVAs illustrated that saline-treated rats exhibit a pattern of head movements that declines across observation intervals. Specifically, their overall pattern of head movements indicates that they are habituating to the context. This is evident because head movement scores recorded during observation interval one are significantly ($p < .01$) greater than that of all subsequent observation intervals, and head movement scores recorded during observation interval two are also significantly ($p < .01$) greater than all subsequent intervals. METH-treated rats exhibit a pattern of head movements which parallels the expected course of drug action, i.e., head movement scores significantly increase ($p < .001$) during observation intervals one through four, peak between observation intervals four and five ($p < .05$), and then significantly ($p < .001$) decline between intervals six through nine.

A significant day \times observation interval \times drug interaction, $F(32, 1216) = 3.969$, $p < .001$, $\eta^2 = .095$, indicated that these patterns of behavior changed over days (see Fig. 19B). The saline-treated rats appear to habituate both within and between treatment days because the mean head movement score recorded 10 minutes post-injection (i.e., interval one) declines each treatment day. Thus, the behavioral pattern discussed in reference to Figure 19A is consistent throughout five days of treatment, but has a lower starting point each day. In contrast, METH-induced head movements become sensitized on each consecutive treatment day, and METH-treated rats exhibit a head movement pattern that shifts upward each day. Head movement scores from METH-treated rats visually illustrate a curve that maintains the same width (i.e., the same course of drug action), but increases each day.

The 5-way ANOVA that was calculated for number of rearing events did not yield a significant observation interval \times drug interaction ($p > .05$), but did identify a significant day \times observation interval \times drug interaction, $F(32, 1216) = 2.618$, $p < .001$, $\eta^2 = .064$ (Fig. 20). Follow up ANOVAs found a significant main effect of observation interval, $F(8, 32) = 13.746$, $p < .001$, $\eta^2 = .433$, for saline-treated rats. Planned comparisons demonstrated that rearing exhibited by saline-treated rats during; observation interval one was significantly ($p < .001$) greater than all other observation intervals, observation interval two was significantly ($p < .01$) greater than that of observation intervals three through eight; and observation intervals four and five were significantly ($p < .05$) greater than that of observation interval nine. These data demonstrate that saline-treated rats habituated both within and between drug treatment

days. However, the lack of a day \times observation interval interaction ($p > .05$) indicates that the overall pattern of rearing was not consistent over days.

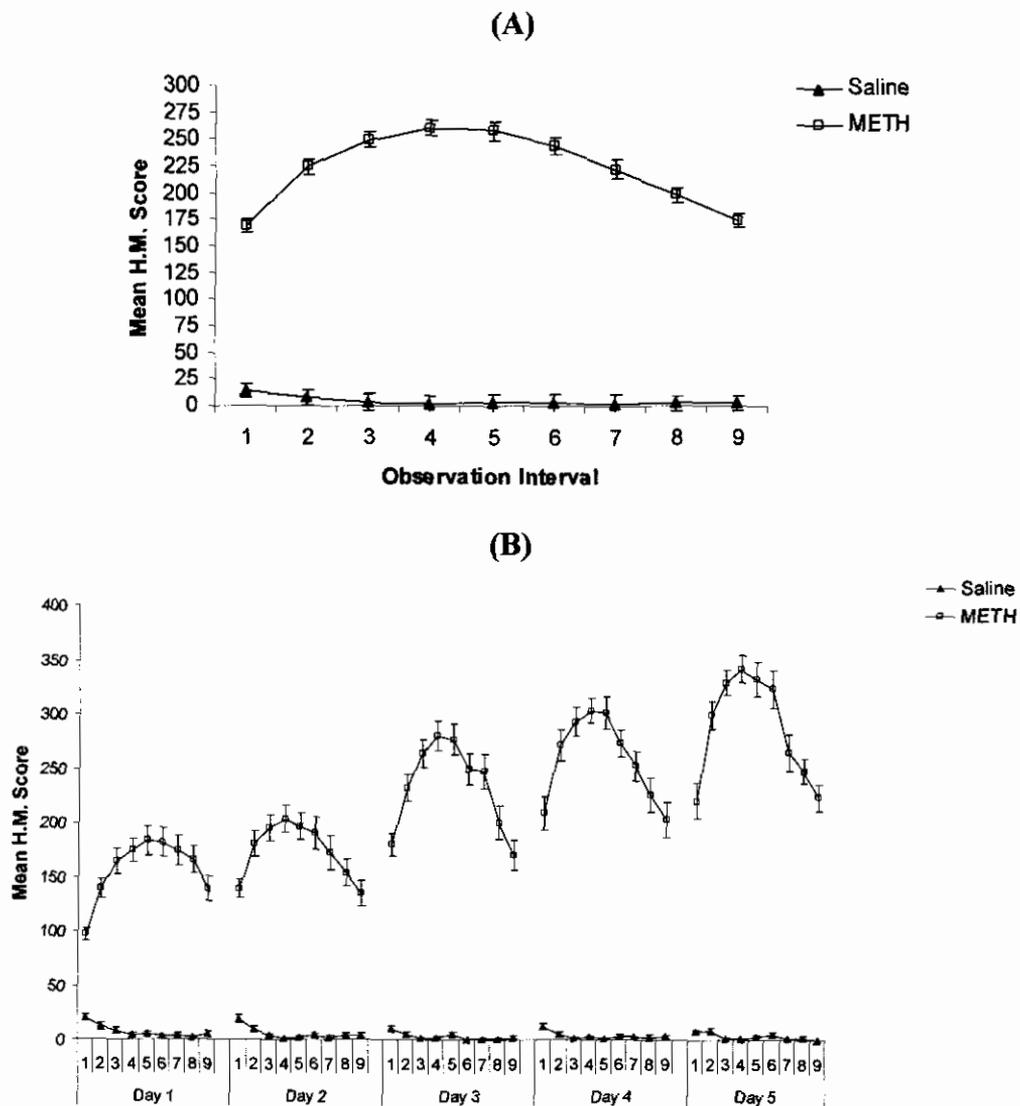


Figure 19. Mean (\pm SE) head movement scores from saline- and METH-treated rats demonstrating (A) an observation interval \times drug interaction, and (B) a day \times observation interval \times drug interaction.

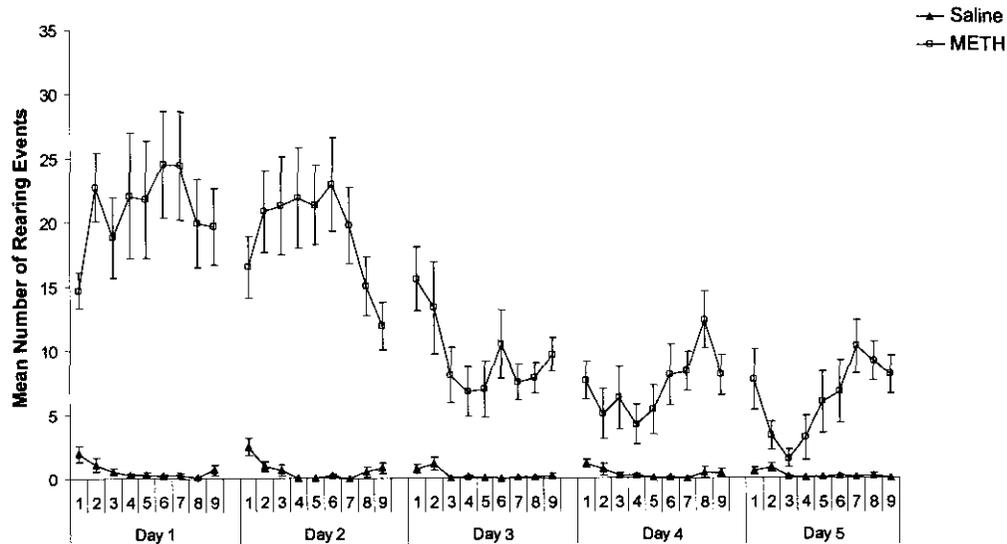


Figure 20. Mean (\pm SE) number of rearing events from saline- and METH-treated rats demonstrating a day \times observation interval \times drug interaction.

There was a significant day \times observation interval interaction, $F(4, 80) = 2.761, p < .001, \eta^2 = .121$, for the number of rearing events exhibited by METH-treated rats.

Pairwise comparisons demonstrated that rearing displayed during observation interval six was significantly ($p < .05$) greater than that of observation intervals three, four, and five, and rearing exhibited during observation interval seven was significantly ($p < .05$) greater than that of observation intervals three, four, and nine. The pattern of rearing exhibited by METH-treated rats illustrates the inverse relationship that exists between stereotypic head movements and locomotor behavior (compare with Fig. 19B). Rearing is increased by METH administration during earlier observation intervals, begins to decrease around the middle of the session, and finally increases again towards the end. Further, this pattern was consistent over drug treatment days, such that the number of rearing events exhibited on day one was significantly ($p < .001$) greater than days two through five, rearing on day two was significantly ($p < .001$) greater than days three through five, and

rearing seen on day three was significantly ($p < .05$) greater than day five. The decrease in rearing that was seen in METH-treated rats over days highlights that stereotypic head movements became sensitized at the expense of rearing.

Strain. The 5-way ANOVA that was used to analyze rodent head movements revealed a significant main effect of strain (Fig. 21A), $F(1, 38) = 5.126, p < .05, \eta^2 = .119$, such that head movement scores from F344 rats were, on average, significantly less than that of HIV-1 Tg rats [F344; $M = 102.818, SE = 6.524$: HIV-1 Tg; $M = 123.71, SE = 6.524$]. However, the 5-way ANOVA that was calculated for number of rearing events revealed no such effect (Fig. 21B), $F(1, 38) = 1.962, p > .05, \eta^2 = .049$.

The 5-way ANOVA also revealed a significant strain \times drug interaction (Fig. 22A), $F(1, 38) = 5.542, p < .05, \eta^2 = 1.27$, for number of head movements, but not rearing events, $F(1, 38) = 1.775, p > .05, \eta^2 = .045$ (Fig. 22B). Separate strain (2) \times context (2) \times season (2) \times day (5) \times observation interval (9) mixed ANOVAs were calculated for saline- and METH-treated rats, and demonstrated that on average, METH-treated F344 rats displayed significantly ($p < .05$) less METH-induced head movement than METH-treated HIV-1 Tg rats, and head movement scores from saline-treated F344 and HIV-1 Tg rats did not differ [METH-treated F344 rats; $M = 200.833, SE = 13.499$: METH-treated HIV-1 Tg rats; $M = 243.446, SE = 13.499$: saline-treated F344 rats; $M = 4.695, SE = .485$: saline-treated HIV-1 Tg rats; $M = 3.977, SE = .485$].

Interestingly, additional ANOVAs conducted on the saline-treated rats yielded significant day \times strain interactions [head movements; $F(4, 144) = 2.684, p < .05, \eta^2 = .13$ (Fig. 23A): rearing events; $F(4, 144) = 2.641, p < .05, \eta^2 = .128$ (Fig. 23B)]. To assess these interactions further, head movement scores and rearing events were analyzed

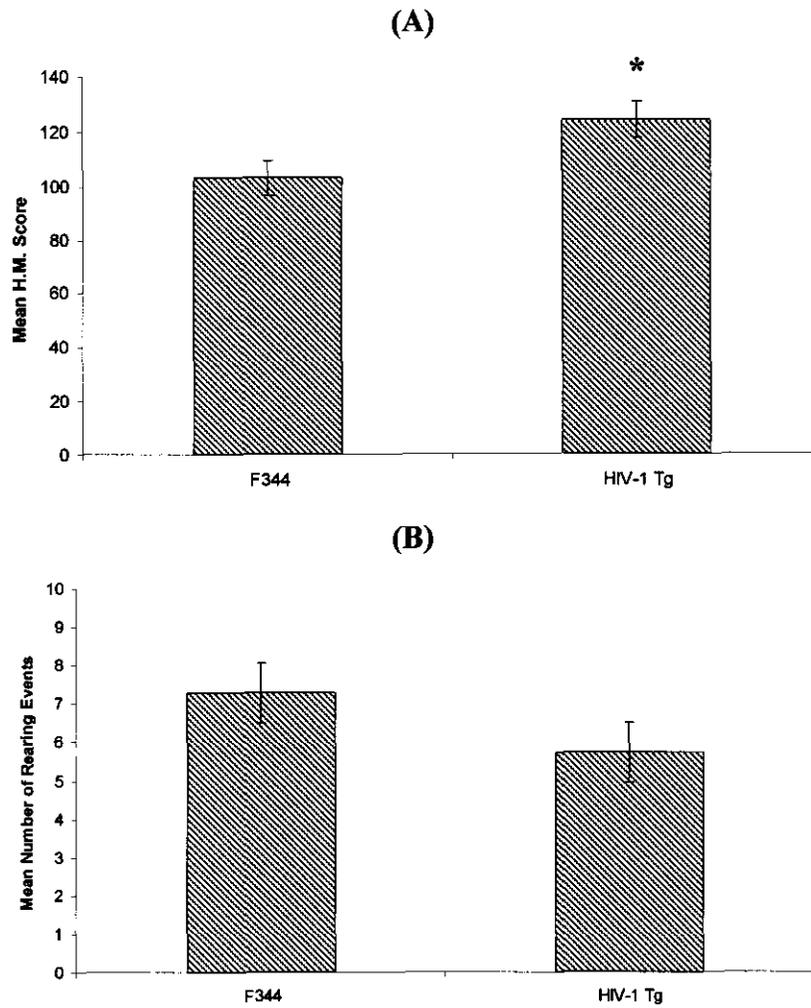


Figure 21. Mean (\pm SE) number of (A) head movements and (B) rearing events from F344 and HIV-1 Tg rats. Note. * indicates $p < .05$

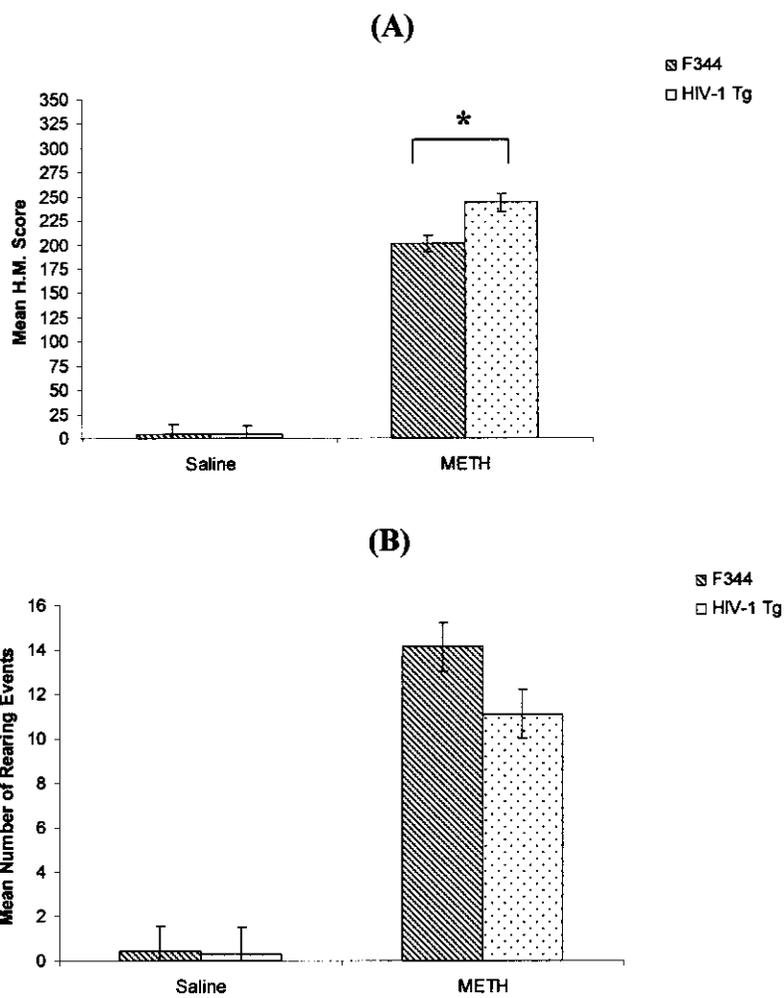


Figure 22. Mean (\pm SE) number of (A) head movements and (B) rearing events from strain \times drug groups. Note. * indicates $p < .05$

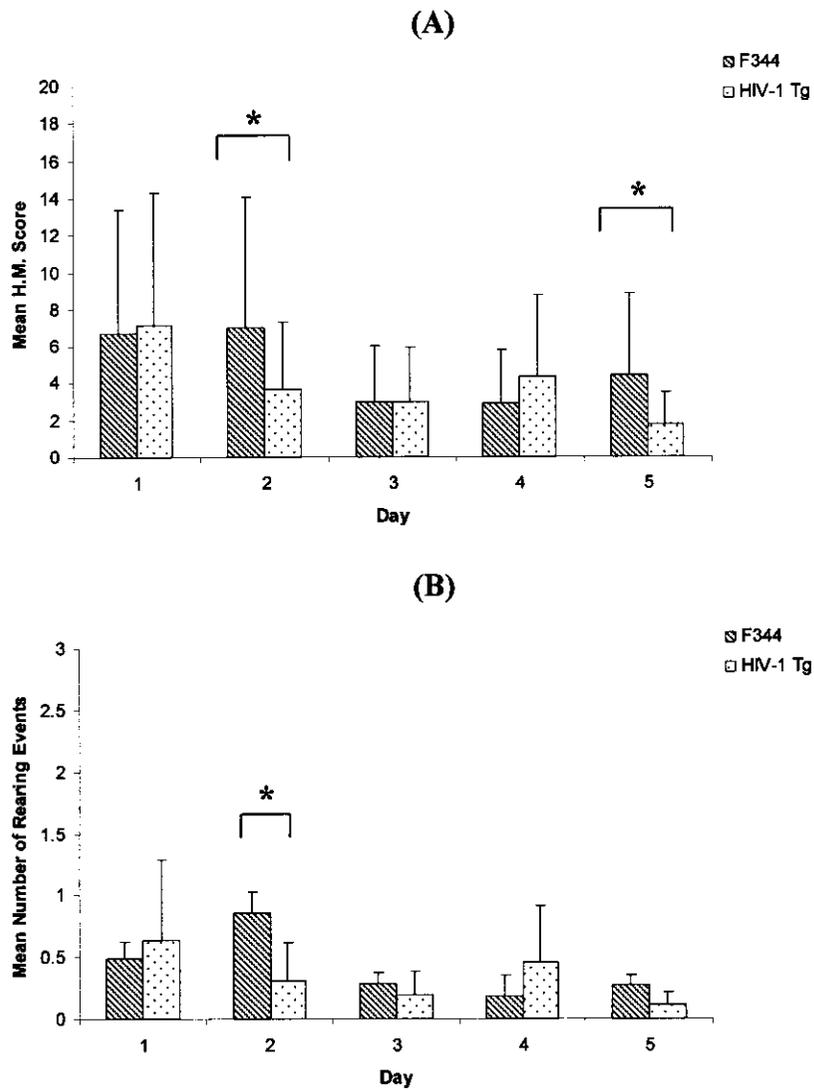


Figure 23. Mean (\pm SE) number of (A) head movements and (B) rearing events from saline-treated F344 and HIV-1 Tg rats across five days of treatment. Note. * indicates $p < .05$

with ANOVAs for each day separately, that is, a strain (2) × context (2) × observation interval (9) mixed ANOVA was calculated for each day of the drug treatment phase. A significant main effect of strain was revealed on day two [head movements; $F(1, 18) = 4.925, p < .05, \eta^2 = .215$: rearing; $F(1, 18) = 4.793, p < .05, \eta^2 = .21$], and day five [head movements; $F(1, 18) = 8.469, p < .01, \eta^2 = .32$], such that head movement scores and rearing events from F344 rats were greater than that of HIV-1 Tg rats.

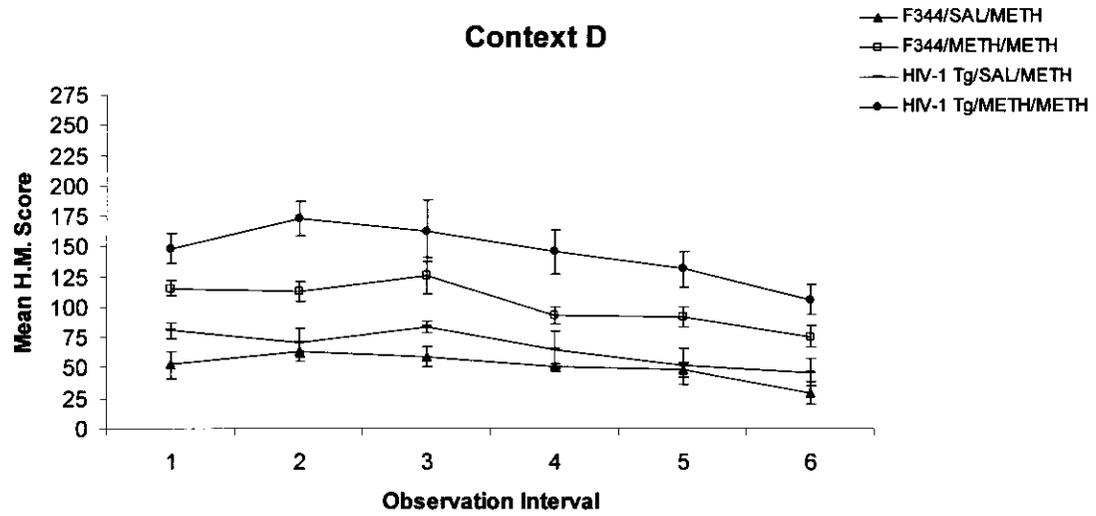
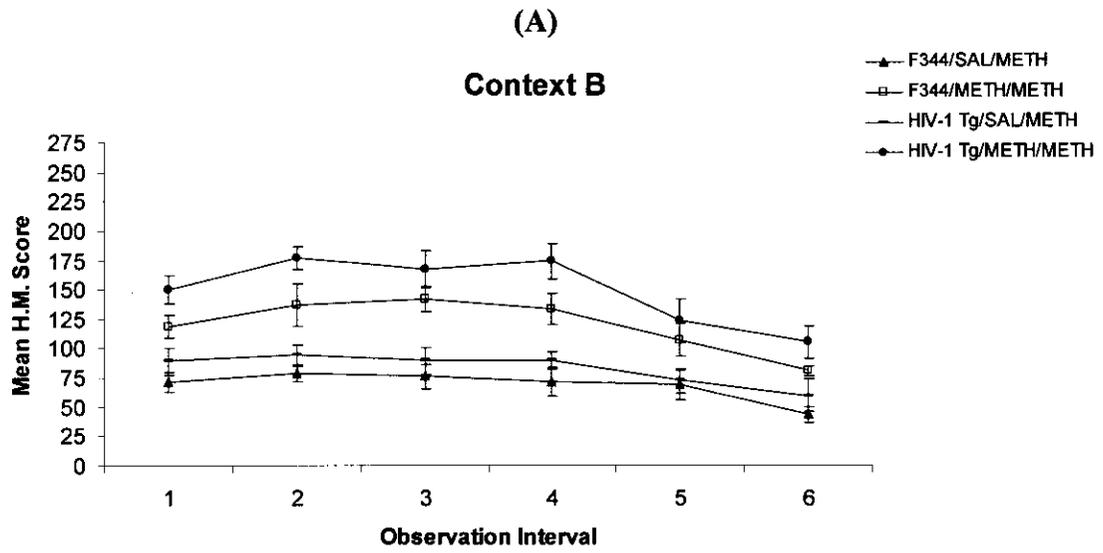
Context. The 5-way ANOVAs that were calculated for number of head movements and rearing events did not yield a main effect of context [head movements; $F(1, 30) = .87, p > .05, \eta^2 = .028$: rearing events; $F(1, 30) = .638, p > .05, \eta^2 = .021$].

BS Challenge Test

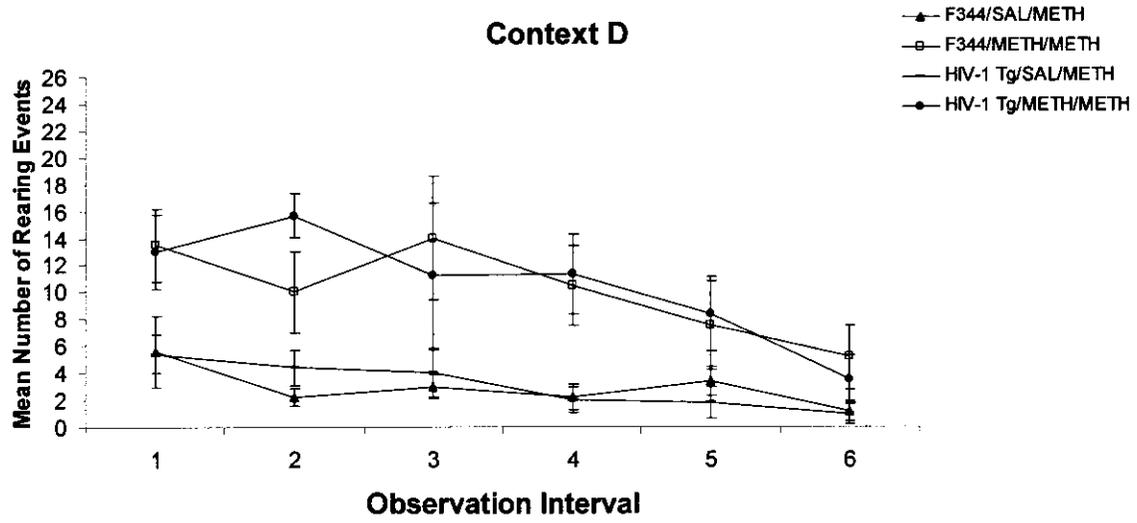
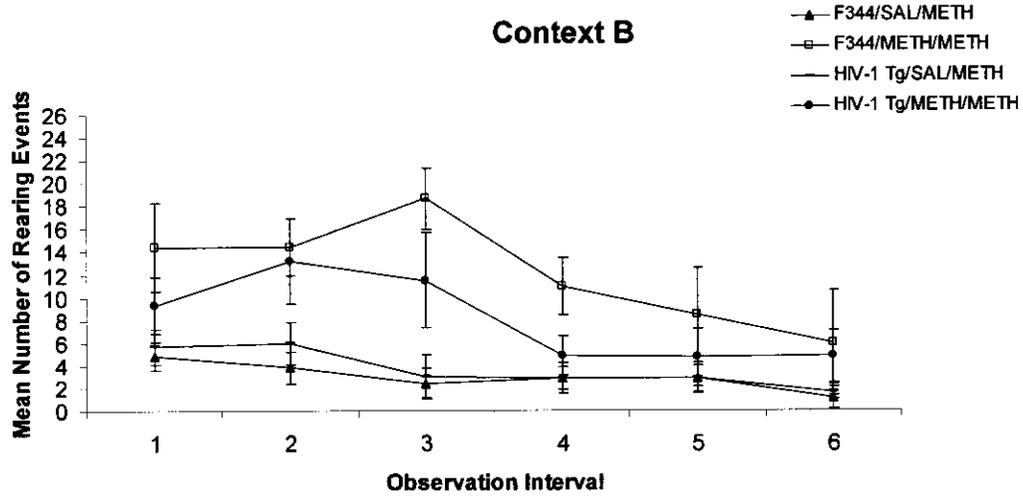
To determine if BS had occurred after five days of METH treatment, and whether or not strain and/or drug context had any modulating effects on the development of BS, means (\pm SE) were calculated for the number of rodent head movements (Fig. 24A) and rearing events (Fig. 24B). Each dependent variable was analyzed with a context (2) × strain (2) × drug pretreatment (2) × observation interval (6) mixed ANOVA, with context, strain, and drug pretreatment as between-subjects factors, and observation interval as a within-subjects factor. Due to experimental error, all data from one METH-pretreated F344 rat that was trained in Context B was discarded from the BS challenge test.

BS. The 4-way ANOVAs that were calculated for METH-induced head movements and number of rearing events revealed a significant main effect of drug pretreatment [head movements; $F(1, 37) = 117.015, p < 001, \eta^2 = .76$: rearing events; $F(1, 37) = 30.191, p < .001, \eta^2 = .449$], such that METH-pretreated rats exhibited, on

Figure 24. Mean (\pm SE) number of (A) METH-induced head movements and (B) rearing events that were recorded from context \times strain \times drug pretreatment groups during two-minute observation intervals, which took place every 10 minutes, for up to 60 minutes after administration of a low challenge dose of METH.



(B)



average, a greater number of METH-induced head movements (Fig. 25A) and rearing events (Fig. 25B) in response to a low challenge dose than that of saline-pretreated rats [head movements, METH-pretreated rats; $M = 129.003$, $SE = 4.020$: head movements, saline-pretreated rats; $M = 66.783$, $SE = 4.114$: rearing, METH-pretreated rats; $M = 10.200$, $SE = .894$: rearing, saline-pretreated rats; $M = 3.168$, $SE = .915$].

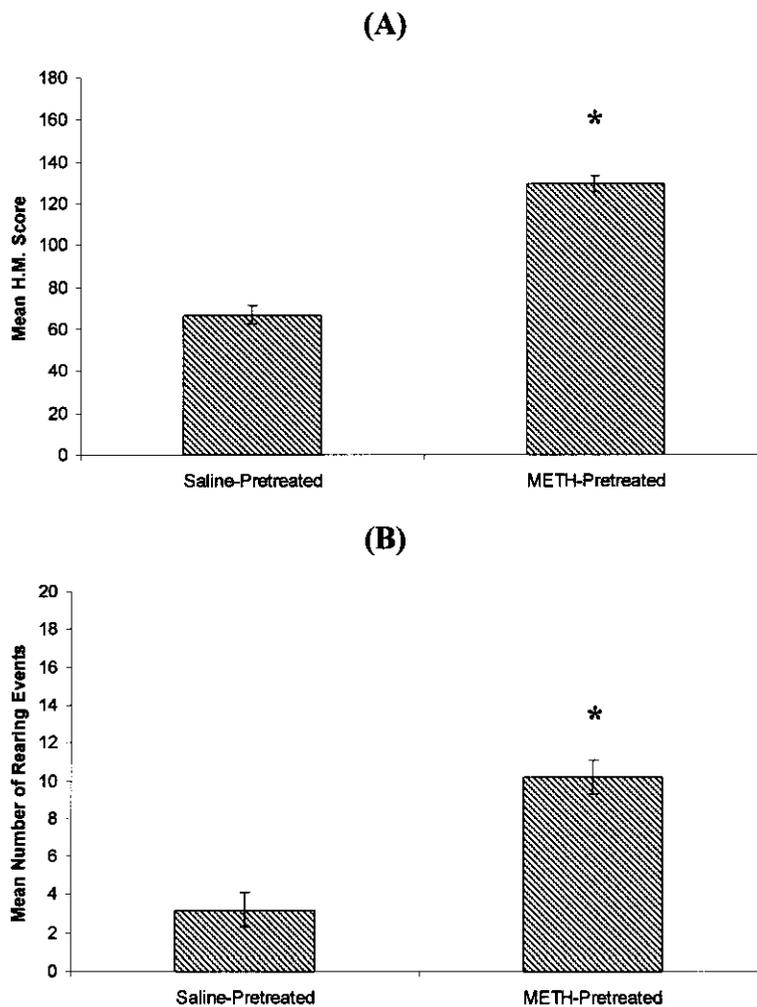


Figure 25. Mean (\pm SE) number of (A) METH-induced head movements and (B) rearing events exhibited in response to a low challenge dose from METH-pretreated and saline-pretreated rats. Note. * indicates $p < .001$

There was also a significant observation interval \times drug pretreatment interaction for METH-induced head movement (Fig. 26A), $F(5, 185) = 2.277, p < .05, \eta^2 = .058$, and number of rearing events (Fig. 26B), $F(5, 145) = 10.961, p < .01, \eta^2 = .229$. Separate

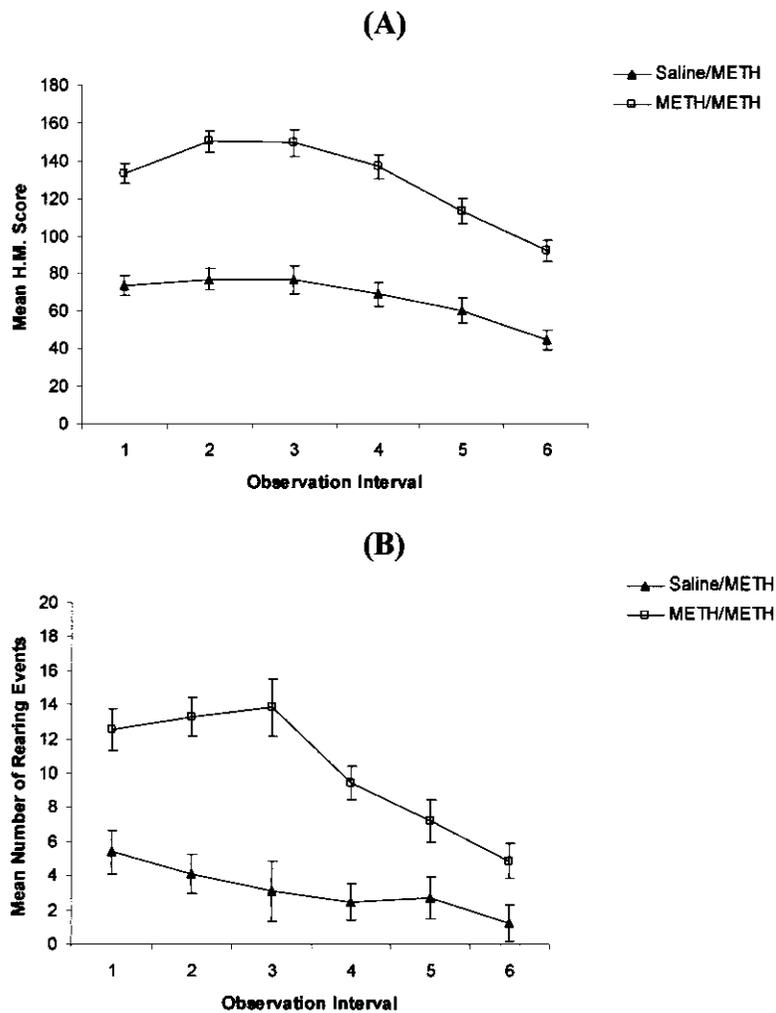


Figure 26. Mean (\pm SE) number of (A) head movements and (B) rearing events illustrating drug pretreatment \times observation interval interactions.

ANOVAs and follow-up paired comparisons confirmed that both behaviors decreased more across observation intervals in the METH-pretreated rats than in saline-pretreated

rats. This effect is largely due to the fact that the METH-pretreated rats showed greater increases in head movement and rearing and therefore had more to decrease. Moreover, the lower METH dose in this challenge test resulted in a more substantial reduction in the drug effect by the end of the observation period than seen with the original higher METH dose (compare with Fig. 19 and Fig. 20).

Strain. Analysis of METH-induced head movement also revealed a significant main effect of strain, $F(1, 37) = 19.586, p < .001, \eta^2 = .346$, such that, on average, METH-induced head movement exhibited by F344 rats in response to a low challenge dose of METH was significantly less than that of HIV-1 Tg rats [F344; $M = 85.165, SE = 4.114$; HIV-1 Tg; $M = 110.621, SE = 4.020$] (Fig. 27A). However, the ANOVA for rearing events yielded no effect of strain, $F(1, 37) = .305, p > .05, \eta^2 = .008$ (Fig. 27B).

A marginally significant strain \times drug pretreatment interaction was found for METH-induced head movement, $F(1, 37) = 3.322, p < .08, \eta^2 = .082$ (Fig. 28A), but not for rearing events, $F(1, 37) = .790, p > .05, \eta^2 = .021$ (Fig. 28B). Follow up context \times strain \times observation interval mixed ANOVAs were calculated for saline-pretreated and METH-pretreated rats to assess the interaction. Although HIV-1 Tg saline-pretreated rats exhibited, on average, greater METH-induced head movement than F344 saline-pretreated rats, an effect of strain did not reach significance, $F(1, 18) = 4.165, p = .056, \eta^2 = .188$. However, a significant main effect strain, $F(1, 19) = 16.735, p < .001, \eta^2 = .468$, was observed in METH-pretreated rats, such that HIV-1 Tg METH-pretreated rats, on average, exhibited a significantly greater number of head movements in response to a low challenge dose than that of F344 METH-pretreated rats.

Context. The 4-way ANOVA that was calculated for METH-induced head

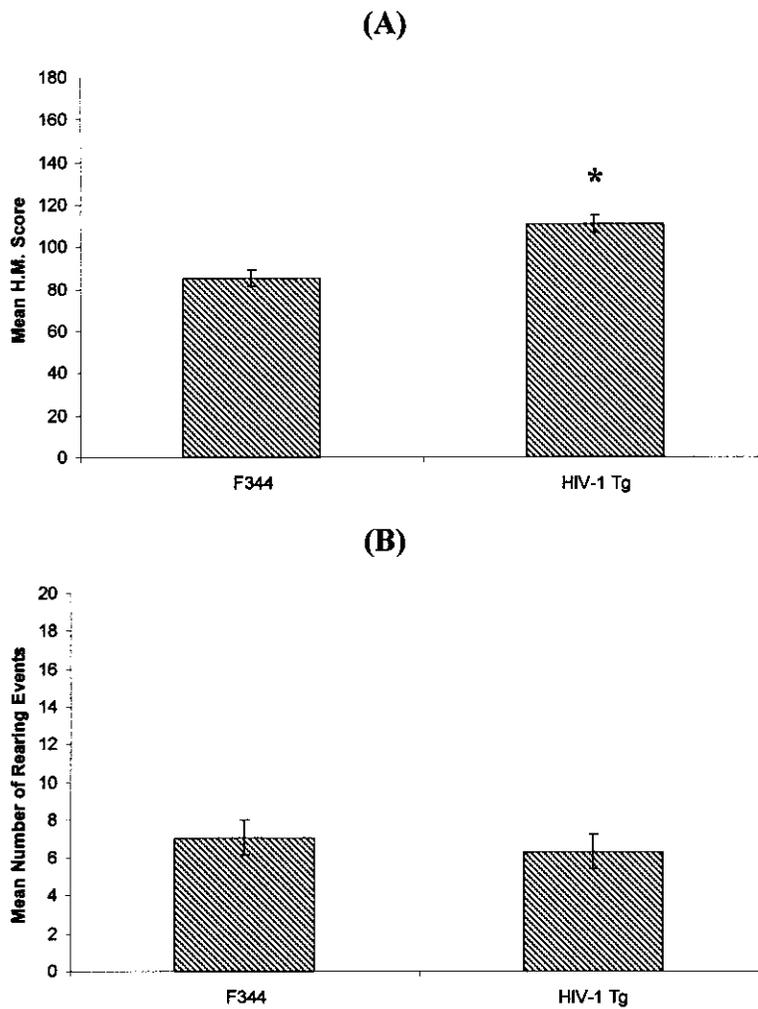


Figure 27. Mean (\pm SE) number of (A) METH-induced head movements and (B) rearing events exhibited in response to a low challenge dose from F344 and HIV-1 Tg rats. *Note.* * indicates $p < .001$

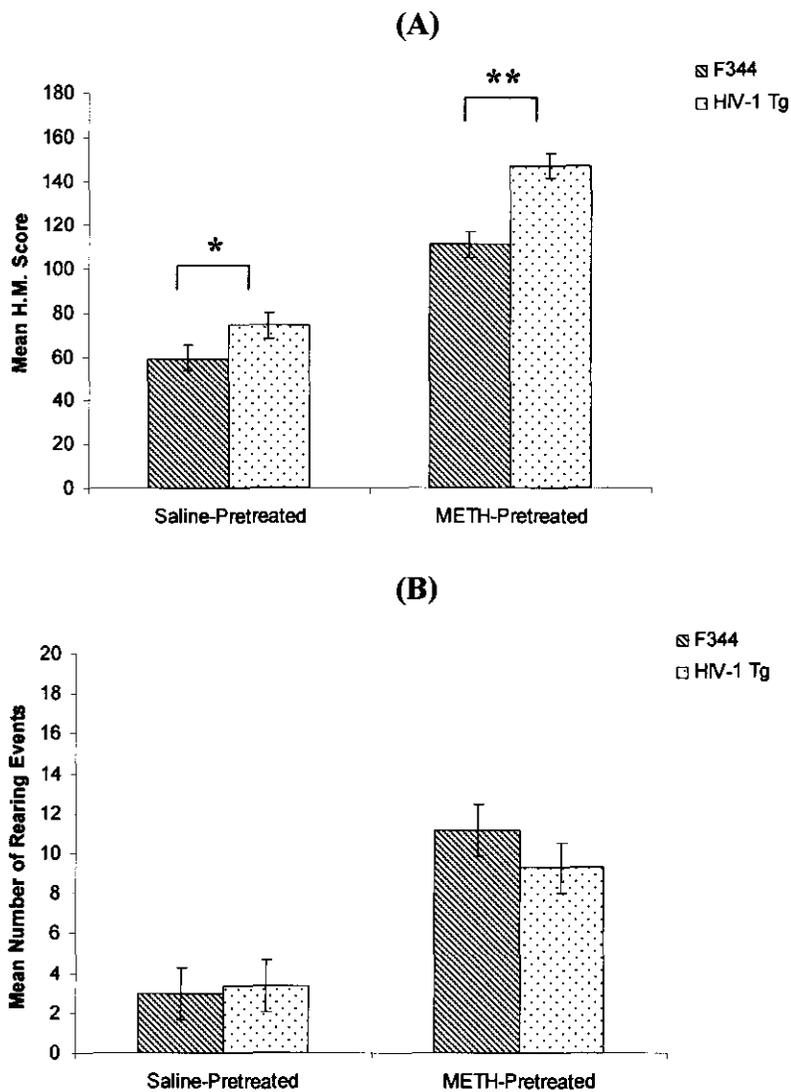


Figure 28. Mean (\pm SE) number of (A) METH-induced head movements and (B) rearing events exhibited by strain \times drug pretreatment groups after receiving a low challenge dose of METH. Note. * indicates $p < .06$; ** indicates $p < .001$

movement revealed a significant main effect of context, $F(1, 37) = 6.088, p < .05, \eta^2 = 1.41$, such that rats that received a low challenge dose in Context B exhibited, on average, significantly greater METH-induced head movement than that of rats that received a low challenge dose in Context D [Context B; $M = 104.989, SE = 4.020$: Context D; $M = 90.797, SE = 4.114$] (Fig. 29A). However, no effect of context was found from METH-

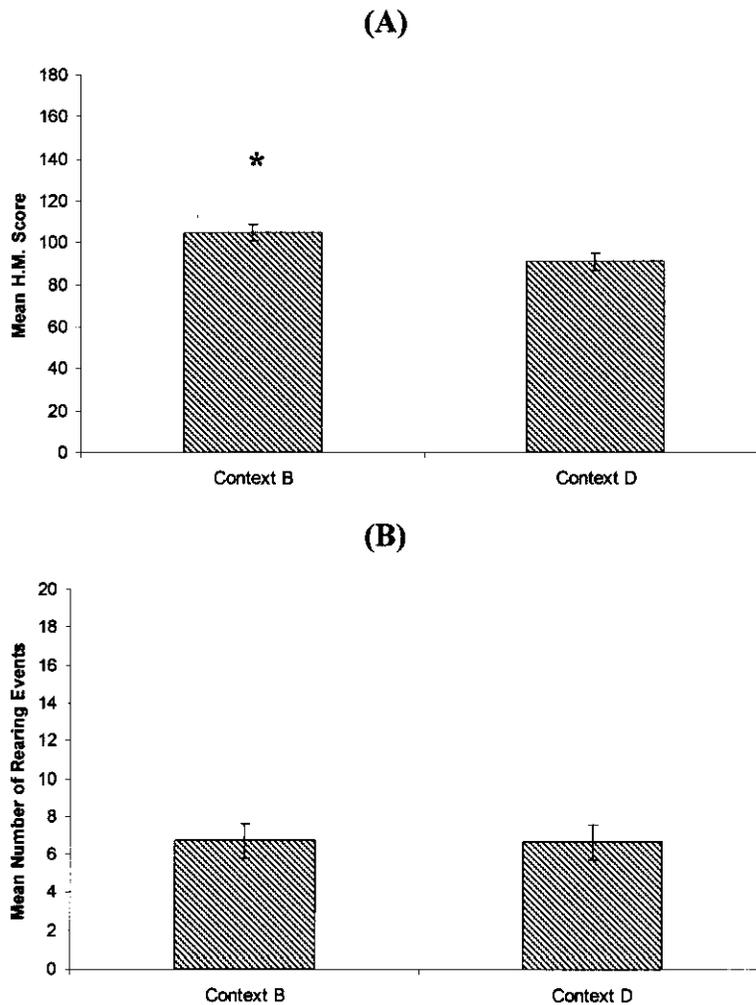


Figure 29. Mean (\pm SE) number of (A) METH-induced head movements and (B) rearing events exhibited in response to a low challenge dose from rats treated in Context B and Context D. Note. * indicates $p < .05$

induced rearing, $F(1, 37) = .001, p > .05, \eta^2 = .000$ (Fig. 29B).

Test for Conditioned Responding to the Drug-Paired Context

To determine if a conditioned drug response developed in METH-treated rats after five days of treatment, behavior was measured following saline injections in the training context. The mean (\pm SE) number of head movements (Fig. 30A) and rearing events (Fig. 30B) was analyzed with a context (2) \times strain (2) \times drug pretreatment (2) \times observation interval (3) mixed ANOVA, with context, strain, and drug pretreatment as between-subjects factors, and observation interval was a within-subjects factor.

Conditioned drug response. The overall ANOVA calculated for head movements revealed a significant main effect of drug pretreatment, $F(1, 38) = 56.253, p < .001, \eta^2 = .597.$, such that after receiving an injection of saline in the context which had previously been paired with METH or saline administration, METH-pretreated rats exhibited a greater number of head movements, on average, than that of saline-pretreated rats [METH-pretreated rats; $M = 38.319, SE = 2.518$: saline-pretreated rats; $M = 10.950, SE = 2.641$] (Fig. 31A). A significant main effect of drug pretreatment was also revealed in the ANOVA calculated for number of rearing events, $F(1, 38) = 13.073, p < .01, \eta^2 = .256$, such that after receiving an injection of saline in the context which had previously been paired with METH or saline administration, METH-pretreated rats exhibited a greater number of rearing events, on average, than that of saline-pretreated rats [METH-pretreated rats; $M = 2.833, SE = .372$: saline-pretreated rats; $M = .883, SE = .390$] (Fig. 31B). No other effects or interactions reached significance.

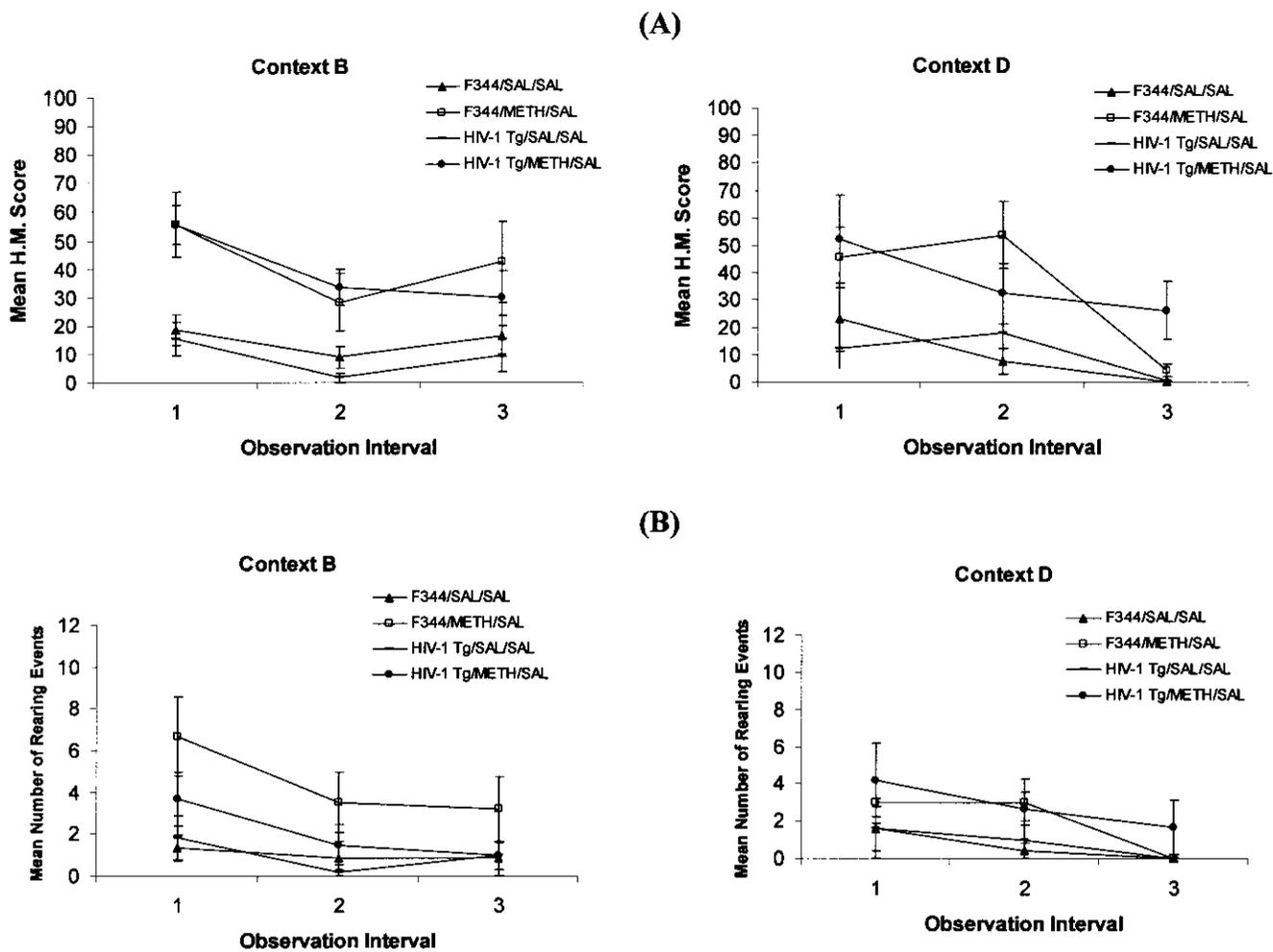


Figure 30. Mean (\pm SE) number of (A) head movements and (B) rearing events that were recorded during two-minute observation intervals every 10 minutes, for up to 30 minutes after saline administration from context \times strain \times drug pretreatment groups.

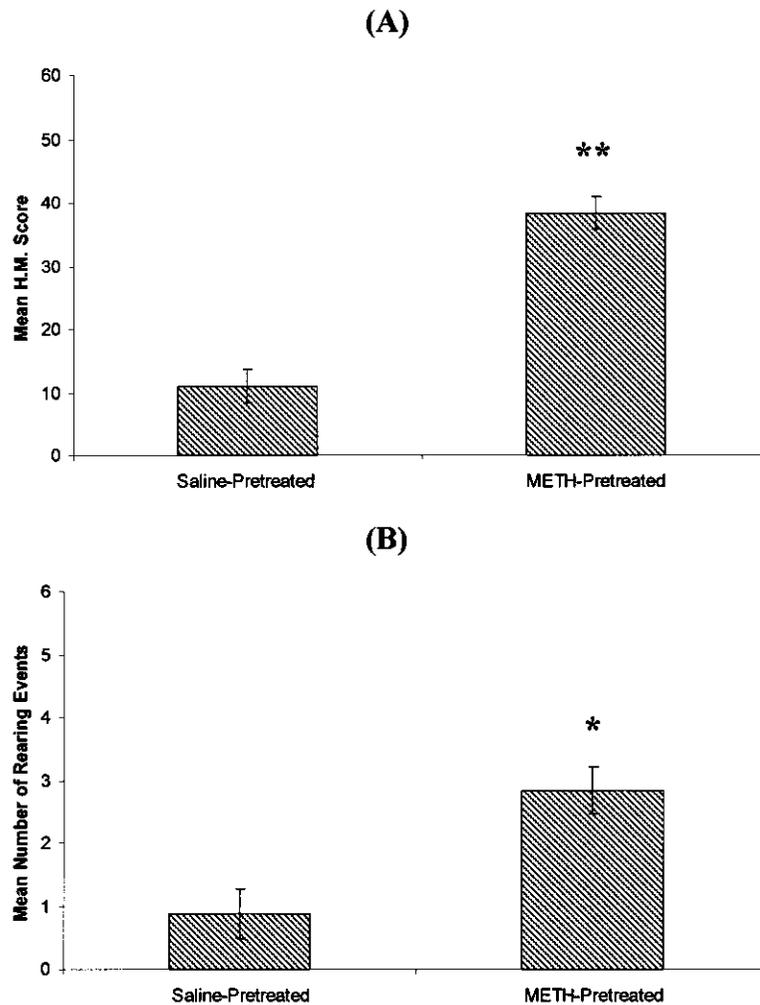


Figure 31. Mean (\pm SE) number of (A) head movements and (B) rearing events exhibited after receiving administration of saline in the drug-paired context from METH-pretreated and saline-pretreated rats. *Note.* * indicates $p < .01$; ** indicates $p < .001$

Taken together, these data suggest that a conditioned drug response may have occurred in METH-pretreated rats. However, it is possible that the saline-pretreated rats exhibited less behavior after receiving an i.p. injection of saline because they had habituated to that same procedure during the five days of drug treatment, whereas the METH-pretreated rats just never habituated. To further evaluate whether or not METH-pretreated rats displayed a conditioned drug response, or just never habituated to the drug-paired context, data that was recorded from the METH-pretreated rats during the habituation phase (i.e. prior to five days of METH treatment) was compared to data that was recorded from the METH-pretreated rats during the test for conditioned responding (i.e., after five days of METH treatment). The same analysis was also conducted for the saline-pretreated rats. Both dependent variables were then analyzed with context (2) × strain (2) × day (2) × observation interval (3) mixed ANOVAs that were calculated for METH-pretreated and saline-pretreated rats.

Both ANOVAs that were calculated for METH-pretreated rats revealed a significant main effect of day [head movement; $F(1, 20) = 51.030, p < .001, \eta^2 = .718$; rearing; $F(1, 40) = 10.821, p < .01, \eta^2 = .351$], such that the number of head movements (Fig. 32A) and rearing events (Fig. 33A) exhibited during the habituation phase were significantly less, on average, than that of the test for conditioned responding phase. Thus, responding in the METH-paired context increased from the first habituation exposure day to the last test after saline injection, supporting the hypothesis that there was a conditioned increase in motor behavior and not simply failure to habituate. Although an effect of day did not reach significance for the saline-pretreated rats, there was an observed decrease between the days before and after drug treatment in the number

of head movements (Fig. 32B) [habituation; $M = 13.994$, $SE = 1.987$: test for conditioned responding; $M = 10.95$, $SE = 1.789$] and rearing events (Fig. 33B) [habituation; $M = 1.183$, $SE = .291$: test for conditioned responding; $M = .883$, $SE = .243$] that were displayed.

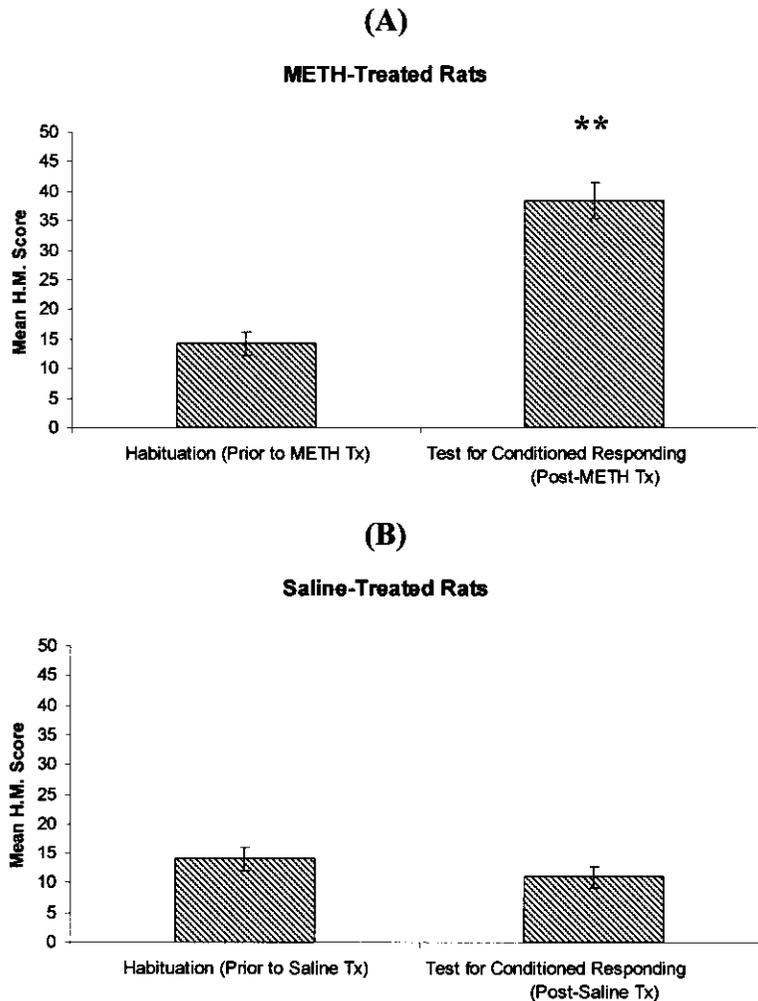


Figure 32. Mean (\pm SE) number of head movements from (A) METH-treated rats and (B) saline-treated rats after receiving administration of saline in the drug-paired context before and after the drug treatment phase. Note. ** indicates $p < .001$

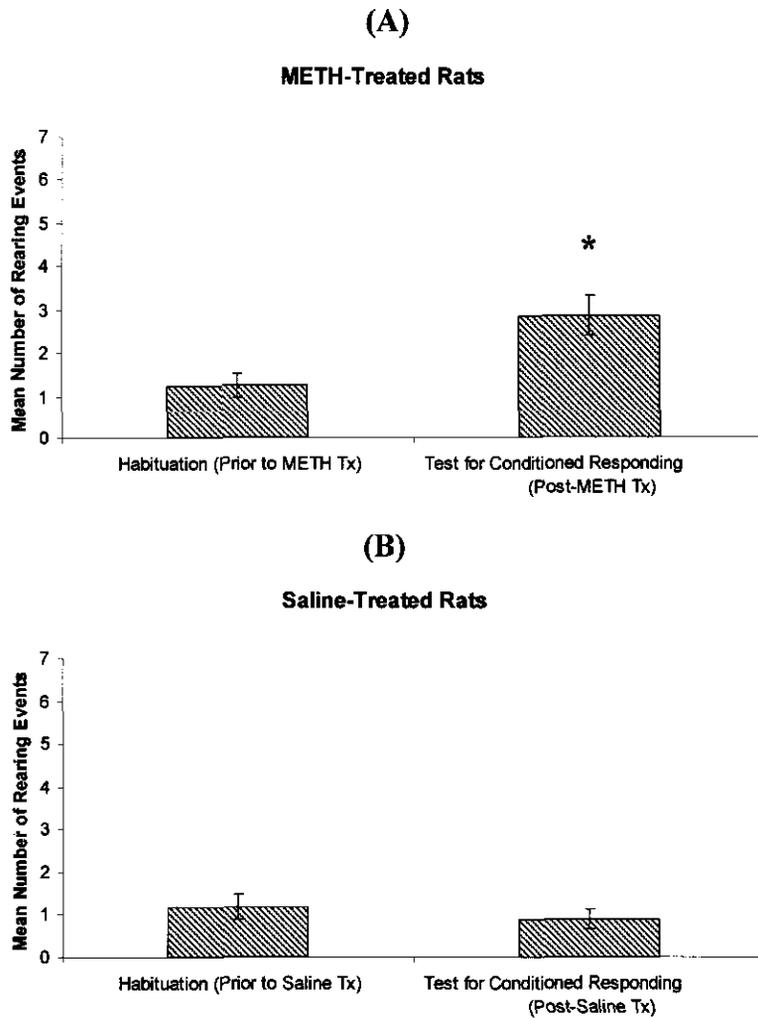


Figure 33. Mean (\pm SE) number of rearing events from (A) METH-treated rats and (B) saline-treated rats after receiving administration of saline in the drug-paired context before and after the drug treatment phase. *Note.* * indicates $p < .01$

CPFE

Test for Freezing

To assess the effects of METH in the presence of the HIV-1 virus on the CPFE, means (\pm SE) were calculated. Freezing time (seconds) was analyzed with a strain (2) \times

drug (2) ANOVA (see Fig. 34), and all other time (seconds) spent in movement (i.e., rearing, grooming, and other) was analyzed with a strain (2) \times drug (2) MANOVA (see Fig. 35).

Freezing. The ANOVA revealed a significant strain \times drug interaction, $F(1, 42) = 8.295, p < .01, \eta^2 = .165$, and no other effects or interactions reached significance. Additional ANOVAs were calculated for F344 and HIV-1 Tg rats to assess the interaction. A significant main effect of drug was found between saline- and METH-treated F344 rats (Fig. 36A), $F(1, 21) = 6.6, p < .05, \eta^2 = .239$, such that saline-treated rats froze significantly longer to the shock-context than METH-treated rats [saline-treated F344 rats; $M = 106.906, SE = 17.303$; METH-treated F344 rats; $M = 45.365, SE = 16.566$]. No effect of drug was found between HIV-1 Tg rats ($p > .05$) (Fig. 36B), although there was a trend such that freezing times from METH-treated HIV-1 Tg rats [$M = 108.605, SE = 15.84$] were longer than that of saline-treated HIV-1 Tg rats [$M = 74.69, SE = 16.54$].

When individual freezing times were plotted (Fig. 37) there did not appear to be a normal distribution. Thus, Mann-Whitney U s were calculated for F344 and HIV-1 Tg rats to follow up on the strain \times drug interaction that was revealed in the 2-way ANOVA. The mean rank for saline-treated F344 rats [$M = 15$] was significantly greater than the mean rank for METH-treated F344 rats [$M = 9.25$], $U = 33, p < .05$, and there was a trend such that the mean rank for METH-treated HIV-1 Tg rats [$M = 14.33$] was greater than the mean rank for saline-treated HIV-1 Tg rats [$M = 9.45$], $U = 38, p = .085$.

Movement in the shock-context. The MANOVA that was calculated for time spent in movement revealed a strain \times drug interaction for time spent rearing, $F(1, 42) =$

4.174, $p < .05$, $\eta^2 = .09$, and time spent engaging in other exploratory movement, $F(1, 42) = 6.26$, $p < .05$, $\eta^2 = .13$. Follow up analyses demonstrated that when placed in the shock-context METH-treated F344 rats spent significantly more time engaging in exploratory activity than saline-treated F344 rats, $F(1, 23) = 5.593$, $p < .05$, $\eta^2 = .21$, and METH-treated HIV-1 Tg rats, $F(1, 22) = 8.499$, $p < .01$, $\eta^2 = .279$. Similar trends were identified for rearing events.

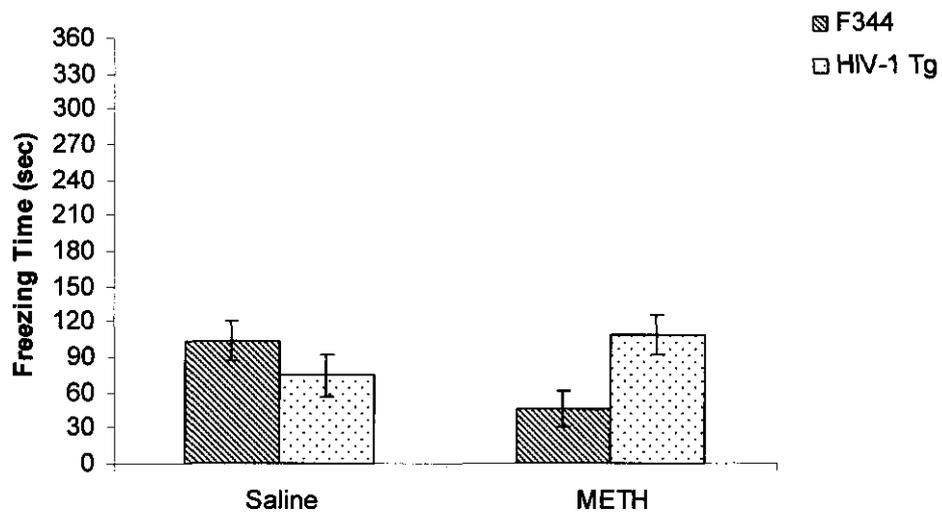


Figure 34. Mean (\pm SE) time (seconds) spent freezing to the shock-context from strain \times drug groups.

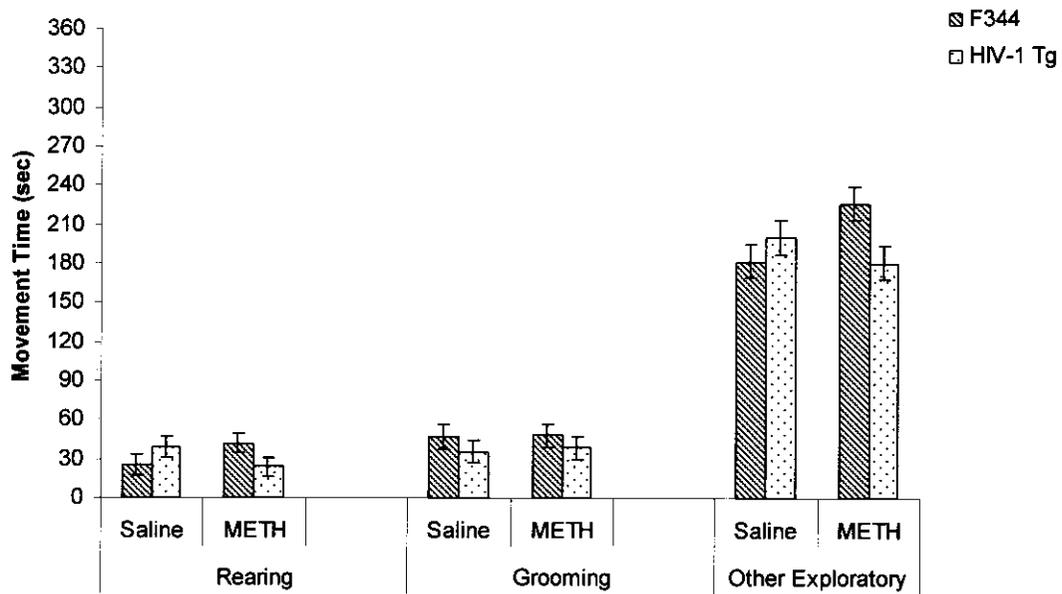


Figure 35. Mean (\pm SE) time spent rearing, grooming, and engaging in other exploratory behavior when placed in the shock-context 24 hours after receiving a shock from strain \times drug groups.

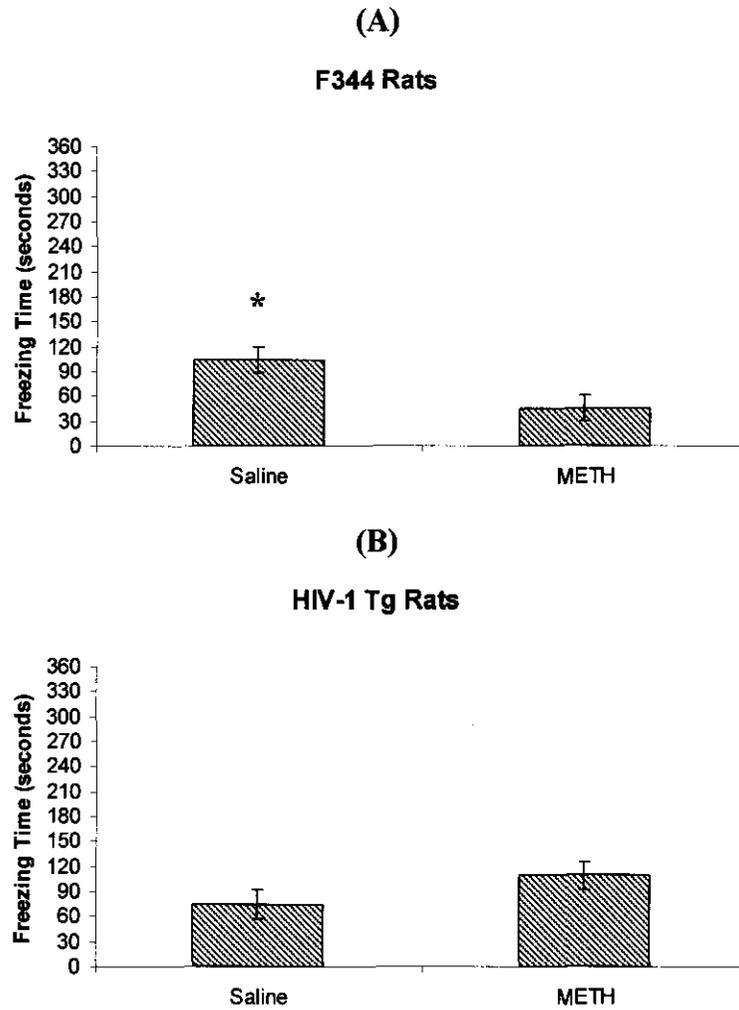


Figure 36. Mean (\pm SE) time (seconds) spent freezing to the shock-context from saline- and METH-treated (A) F344 and (B) HIV-1 Tg rats. Note. * indicates $p < .05$

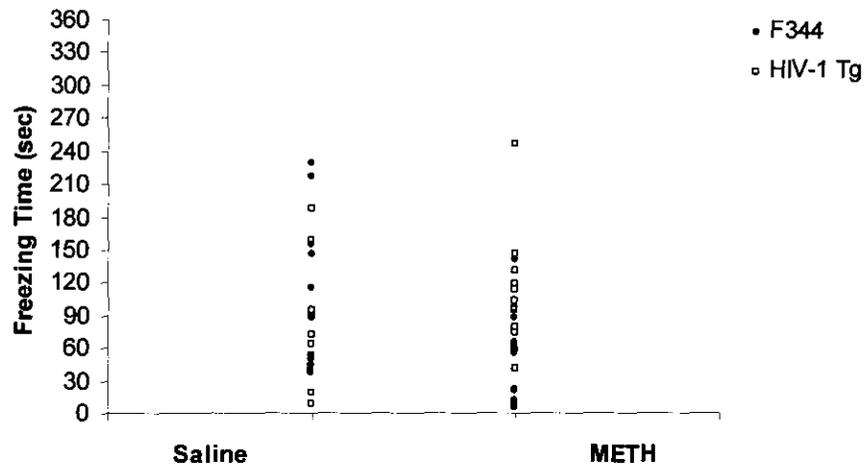


Figure 37. Distribution of freezing times from saline- and METH-treated F344 and HIV-1 Tg rats.

Conclusions

Sensitization and Drug Context Effects

Habituation

After receiving an injection of saline, HIV-1 Tg and F344 rats both habituated to the modified cages during a 60-minute time period. This was evident by the decline in the number of head movements and rearing events that were observed across six observation intervals. Importantly, there were no context effects during habituation indicating that Context B and Context D did not modulate drug-free exploratory behavior, and that habitual processes were also unaffected.

Of extreme interest, there were no differences in baseline behavior displayed by HIV-1 Tg rats and F344 controls. These findings demonstrate that at two to three months of age HIV-1 Tg rats do not have motor impairments compared to F344 rats. This coincides with the progression of motor disorders seen in HIV patients undergoing

HAART treatments; motor deficits do not emerge until much later after the virus has been contracted if HAART treatments are available. At younger ages (i.e., two to three months of age) HIV-1 Tg rats do not show signs of motor impairment, indicating a similar disease progression.

It is important that habituation was established prior to drug treatment, and it is also crucial that there were no modulatory effects of context or strain on drug-free behavior. It is assumed that habituating the animals to the modified cages prior to drug treatment eliminated the possibility of confounding results on day one of drug treatment. The effects of environmental novelty on exploratory behavior and the stimulating effects of METH were separated by acclimating animals to the procedure (i.e., transfer to the experimenting room and receiving an injection) and the modified cages. Likewise, it is meaningful that there were no strain or context effects because any differences observed during drug treatment can now be confidently attributed to interactions between METH and the environment and/or METH and the virus.

Drug Treatment (5 Days)

The effect of drug established the powerful behavioral action of METH, such that METH-treated rats exhibited significantly more head movements and rearing events than saline-treated rats. The powerful psychoactivating properties of METH were clearly evident in this study (Fig. 17). Of the METH-treated groups, HIV-1 Tg rats exhibited a more robust stereotypic response than F344 controls (see Fig. 22A), suggesting that HIV-1 Tg rats may be more sensitive to the acute effects of a moderate dose of METH. Interestingly, there was no effect of strain on METH-induced rearing behavior. However, it appears that F344 rats tended to engage in hyperactive rearing behavior more

often than HIV-1 Tg rats (see Fig. 22B). Given the distinct behavioral responses exhibited by HIV-1 Tg and F344 METH-treated rats, it is possible that METH differentially affects the HIV-1 Tg rat and normal F344 controls.

METH-treated HIV-1 Tg and F344 rats both developed BS over the five treatment days, such that stereotypic head movements increased each day. In fact, there was even a marginal effect between treatment days one and two, suggesting that sensitization may begin to occur after just one METH administration. Recall back to Experiment One, BS did not emerge in METH-treated SDs until day three of drug treatment. The results from Experiment Two indicate that one, 60-minute habituation session was sufficient in eliminating any confounding effects of environmental novelty, and that only METH-induced psychoactivation was measured on day one of drug treatment. BS of METH-induced, stereotypic head movement was accompanied by a dramatic depression in rearing, demonstrating competition between the two behaviors. This finding is consistent with research that demonstrates stimulant-induced behavior can occur at the expense of another behavior. Compared to F344 rats, stereotypic head movement was more robust in HIV-1 Tg rats on all drug treatment days, but the increased behavioral response was not accelerated between days. Thus, the sensitization rates did not differ between HIV-1 Tg and F344 rats. Additionally, the depression of rearing activity that was coincident with BS of METH-induced head movement was comparable between strains.

Behavior seen in saline-treated rats declined over five days of drug treatment, representing the continuation of habitual processes. Saline-treated rats appeared to habituate both between treatment days and within. In other words, not only did head movements and rearing events decrease between drug treatment days, but both behaviors

also declined over nine observation intervals on each day. The decrease in head movements and rearing was attributed to continued habituation because the behavioral patterns of saline-treated rats overtly displayed five of the 10 common characteristics of habituation (Rankin, Abrams, Barry, Bhatnagar, Clayton, Colombo, et al., 2008). Specifically, placement in the modified cages resulted in a progressive decrease in activity. Spontaneous recovery was evident because head movements and rearing events during the first few observation intervals were increased from the last few observation intervals of the previous day. Potentiation of habituation was demonstrated in that repeated habituation and spontaneous recovery training (i.e., each successive treatment day) resulted in accelerated behavior decrements. In other words, saline-treated rats habituated during a 90-minute session on day one, showed spontaneous recovery on day two and then habituated again. On day three, spontaneous recovery is evident again, but head movements and rearing did not recover completely, and this decremental pattern continues on days four and five (see Fig. 19 and Fig. 20).

Interestingly, there was an unexpected strain \times day interaction, indicating that HIV-1 Tg rats habituated at a faster rate during five days of saline treatment. Head movements and rearing events seen in saline-treated HIV-1 Tg rats on days two and five were less than that of saline-treated F344 rats. Thus, it appears that potentiation of habituation was larger in HIV-1 Tg rats than F344 rats.

BS Challenge Test

In response to a low challenge dose, METH-pretreated rats exhibited more head movements and rearing events than saline-pretreated rats confirming that BS had occurred after five days of drug treatment (see Fig. 25). It is noteworthy that BS was

evident by the larger number of rearing events exhibited by METH-pretreated rats because stereotypic head movements increased at the expense of this behavior during the drug treatment phase. Although only BS of stereotypic head movement emerged during five days of drug treatment, these results demonstrate that BS of both stereotypic head movement and rearing had actually occurred.

A main effect of strain illustrated that, in comparison to F344 rats, HIV-1 Tg rats exhibit a greater number of stereotypic head movements, but not rearing events, in response to a low challenge dose (see Fig. 27). Follow up analyses on a strain \times drug pretreatment interaction demonstrated that there was a small effect of strain between the saline-pretreated HIV-1 Tg and F344 rats, suggesting that drug-naïve HIV-1 Tg rats are more sensitive to the acute effects of a low dose of METH. Of extreme interest, it appears that BS of stereotypic head movement was augmented in METH-pretreated HIV-1 Tg rats. There was a moderate to large effect of strain found between the HIV-1 Tg and F344 METH-pretreated rats after receiving a low challenge dose. It is obvious that this effect is enhanced when comparison is made to the strain effect that was identified in saline-pretreated rats. Additionally, the effect of strain found between METH-pretreated HIV-1 Tg and F344 rats during the BS challenge test is amplified from the effect that was found in those same rats during five days of drug treatment. Taken together, the BS challenge test indicates that neuroalterations associated with BS may be exacerbated in the HIV-1 Tg rat.

These data demonstrate that the acute and long-term effects of METH may be intensified in HIV-1 Tg rats, and, importantly, this is independent of context effects. Rats given the challenge dose in Context B exhibited a greater number of stereotypic head

movements than rats that were administered the low dose in Context D, and there was no interaction with strain. This finding shows that the environment surrounding drug administration can modulate the stimulating properties of a low dose of METH.

Environmental modulation of stereotypic head movements was the same in HIV-1 Tg rats and F344 controls. This suggests that the differences between strains are a result of interactions between METH and the virus in the HIV-1 Tg rat.

Test for Conditioned Responding to the Drug-Paired Context

Head movements and rearing events that were observed in METH-pretreated rats after receiving an injection of saline in the drug-paired context were greater than that of saline-pretreated rats. Moreover, both dependent variables showed an increase from what was observed the day before METH treatment began. These results imply that conditioned responding to the drug-paired context was observed in METH-treated rats.

Because saline-treated rats habituated during drug treatment, it was expected to see a decrease in head movements and rearing events when this phase was compared to the day before five days of saline treatment. Both dependent variables did in fact show a decrease, but neither reduction was significant. The test for conditioned responding to the drug-paired context and the BS challenge test were alternated, such that half the rats were given saline in the drug-paired context the day before the BS challenge test and vice versa. It is possible that a significant reduction in activity was not found in saline-pretreated rats because half of them received the low challenge dose first. In fact, dishabituation, whereby a habituated response can be recovered when a novel stimulus is introduced, is a common characteristic of habituation (Rankin, et al., 2008), and may

have occurred in the rats that were given the low dose of METH on the day prior to the test for conditioned responding to the drug-paired context.

The conditioned drug responses found in METH-pretreated rats were not affected by strain. This indicates that the process whereby environmental stimuli can become associated with the effects of METH does not differ between HIV-1 Tg rats and F344 controls.

CPFE

Test for Freezing

Experiment Two replicated the findings of Experiment One in F344 rats, such that five days of METH treatment attenuated the CPFE in F344 rats. The freezing behavior seen in HIV-1 Tg rats was not as straightforward. Saline-treated HIV-1 Tg rats had freezing times that were comparable to saline-treated F344 rats, indicating that drug-naïve HIV-1 Tg rats do not exhibit memory deficits in the CPFE. However, freezing times from METH-treated F344 and HIV-1 Tg rats were not comparable. In fact, METH-treated HIV-1 Tg rats spent significantly longer time freezing to the shock-context than F344 rats. Moreover, freezing times from METH-treated HIV-1 Tg rats did not statistically significantly differ from either saline group. This suggests METH has differential effects on the CPFE in HIV-1 Tg rats and F344 controls. It is noteworthy that there was large variability in the observed freezing times. When nonparametric methods were used to analyze freezing behavior a marginal effect of drug was detected in the HIV-1 Tg rats. This effect was in the opposite direction seen in F344 rats. Specifically, it seems to be the case that the CPFE is attenuated by METH in F344 rats, but enhanced by METH in HIV-1 Tg rats.

The patterns of rearing and other exploratory movement in the shock-context provide further evidence supporting the notion that METH enhances the CPFE in HIV-1 Tg rats. The time that METH-treated F344 rats spent engaging in exploratory movement in the shock-context was greater than that of saline-treated F344 rats. This, in part, would be expected because the saline treated rats freeze longer and could therefore spend less time in exploratory activity. Similar results were not found in METH-and saline-treated HIV-1 Tg rats. Moreover, METH-treated F344 rats engaged in more exploratory behavior than METH-treated HIV-1 Tg rats, but not saline-treated HIV-1 Tg rats. In sum, the effects of METH on the CPFE in HIV-1 Tg and F344 rats are in opposition.

General Discussion

The quantitative scoring method that was used accurately reflected the time course of drug action, the stimulating and sensitizing effects of METH, and differential effects of METH in HIV-1 Tg rats and F344 controls. However, these results should not be taken at face value, and alternative scoring methods should be considered and implemented in future studies. For instance, accelerometers and motion detectors may be more sensitive to stereotypic head motions and provide an even more precise quantitative description of METH-induced stereotypy. If the findings of this study are replicated through the use of accelerometers or motion detectors, then not only would the present findings be supported further, but the scoring method would be validated.

The quantitative scoring method was successful in demonstrating the magnitude of METH-induced stereotypic head movements, and furthermore, identifying a greater stereotypic response in HIV-1 Tg rats. These results are no doubt useful for understanding the effects of METH in the presence of HIV-1. However, counting the number of stereotypic head movements does not adequately address the possibility of qualitative differences that exist between HIV-1 Tg rats and controls. For instance, differences in response characteristics can be inferred from the numeric representations of METH-induced stereotypy. In this fashion, F344 controls appear to exhibit a behavioral response profile that is more consistent with S_1 stimulant responses, while HIV-1 Tg rats display S_2 response profiles. This inference cannot be confirmed without qualitative scoring methods that reflect differences in METH-induced responding. Therefore, future studies will need to incorporate qualitative rating scales that are more suitable for describing behavioral differences and illustrating response profiles.

Respectively, S_1 and S_2 response profiles may initially be mediated by both mesolimbic and nigrostriatal DAergic systems (Kuczenski, et al., 1995; Segal & Kuczenski, 1987). However, through incentive sensitization S_1 and S_2 responses may become sensitized at the neuronal and behavioral levels, and attributed to the context surrounding drug administration. Thus, the results from the drug treatment phase may reflect activation of both the mesolimbic and nigrostriatal DAergic pathways, whereas results from the BS challenge test and test for conditioned responding to the drug-paired context may reflect activation of the mesolimbic DAergic system. Therefore, the greater number of stereotypic head movements seen in HIV-1 Tg rats during the drug treatment phase may be attributable to activational effects of METH in the nigrostriatal DAergic pathway that differ between HIV-1 Tg rats and F344 controls. According to incentive sensitization theory, the greater number of stereotypic head movements observed in the HIV-1 Tg rats during the BS challenge test may be associated with neuroalterations in the mesolimbic DAergic pathway that lead to a greater sensitivity to the incentive salience attributes of METH. In short, both DAergic pathways may be activated by METH, but only the mesolimbic DAergic pathway may be responsible for BS and drug context effects.

The differential effects of METH pretreatment on the CPFE suggest that the virus rendered HIV-1 Tg rats more sensitive to the anxiety-like effects of a moderate dose of METH that causes impairments in normal rats. This is particularly evident by the fact that moderate doses of METH attenuated the CPFE in two strains of normal rats, SDs and F344s. That memory of the shock-context was impaired in two strains of normal rats indicates that memory processes are affected differently by METH in HIV-1 Tg rats.

Taken together, the differential effects of METH on BS and the CPFE in the HIV-1 Tg rat suggest greater neural sensitization occurs in an HIV-infected brain.

Sensitization to AMPH, METH, and COC, which is usually marked by increasing psychosis, produces cross sensitization to stressful events in humans (Hamamura, et al., 1997; Peleg-Raibstein & Feldon, 2008; Robinson, et al., 1987; Suzuki, et al., 2002). It appears that the HIV-1 Tg rat may be hypersensitive to METH and psychological stress (i.e., a footshock). HIV-1 Tg rats exhibited greater stereotypic head movements in response to a low challenge dose indicating greater neural sensitization, and an augmented fear response in the CPFE suggesting that cross sensitization to stressful events was augmented. In normal rats, it has been identified that DAergic and 5-HTergic systems play a role in METH-induced emotional sensitivity to stress. Given that HIV-1 is toxic to monoaminergic systems, it is possible that neuroalterations associated with the virus may have made the HIV-1 Tg rat hypersensitive to METH-induced emotional states. Thus, augmented BS and enhanced fear responses in the HIV-1 Tg rat may be mediated by the same neural structures. Specifically, the hypofrontality and DAergic dysfunction that is associated with HIV-induced neuroalterations may lead to enhanced sensitivity to the stimulating, behavior sensitizing, and anxiety-like effects caused by METH. Taken together, these findings are consistent with the idea that HIV-1 and METH act additively on the same neural pathways.

It is possible that the prevalence of METH use and addiction in the HIV-1 community is partly mediated by a greater sensitivity to the stimulating and anxiety-like effects of the drug. Because HIV-1 Tg rats exhibited a more robust acute response and greater BS than F344 controls, it may be the case that HIV-1 potentiates incentive

sensitization. Thus, drug-wanting in HIV-infected individuals may increase at a faster pace than in non-infected users. In this event, the attribution of potentiated incentive sensitization to contextual stimuli would perpetuate drug-taking behavior faster in HIV-infected individuals. Chronic use of stimulants can produce long-lasting changes in brain function that result in enhanced vulnerability to psychological stressors (Hamamura, et al., 1997). During a period of withdrawal the increased sensitivity to anxiety events is believed to elicit relapse in humans and re-instatement of self-administration in rodent models. Thus, HIV-infected METH users may be more prone to relapse after a period of withdrawal because the over-reactivity to stressful events is heightened.

Cognitive processes associated with benign declarative memories and emotionally charged events may be differentially affected by METH in the HIV-1 Tg rat. To that end, a battery of anxiety-related and memory-related tasks will need to be utilized. If the anxiety-like effects of METH are enhanced in the HIV-1 Tg rat, then performance on other anxiety-related tasks, such as the elevated plus maze, should be similarly affected by METH. Additionally, there may be a dissociation between METH-induced stress and the effects of METH on attentional set-shifting or OR tasks in the HIV-1 Tg rat, and so these cognitive processes need to be evaluated in concert. Further studies implementing the HIV-1 Tg rat in animal models of addiction will be necessary to evaluate the complex drug-environment-stress interactions that were identified in the present study.

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Appendix

All of the abbreviations used throughout the text are listed below. Abbreviations are organized in alphabetical order and correspond with the page number where they first appeared in the text.

Acquired Immune Deficiency Syndrome (AIDS).....	18
Amphetamine (AMPH).....	2
Basolateral amygdala (BLA).....	15
Behavioral sensitization (BS).....	2
Blood-brain barrier (BBB).....	1
Central nervous system (CNS).....	1
Cerebrospinal fluid (CSF).....	5
Cocaine (COC).....	9
Conditioned response (CR).....	12
Conditioned stimulus (CS).....	12
Context pre-exposure facilitation effect (CPFE).....	2
Dopamine (DA).....	4
DA transporter (DAT).....	4
Fischer 344 (F344).....	17
5-HT transporter (SERT).....	4
Glycoprotein 120 (gp120).....	5
Highly active antiretroviral therapies (HAART).....	1
Human Immunodeficiency Virus (HIV).....	1
HIV-associated dementia (HAD).....	1

HIV-associated encephalitis (HIVE).....	5
HIV-1 transgenic rat (HIV-1 Tg rat).....	2
Immediate shock deficit (ISD).....	13
Intraperitoneal (i.p.).....	27
Intravenous (i.v.).....	3
Joint United Nations Programme on HIV/AIDS (UNAIDS).....	3
Long terminal repeat (LTR).....	17
Methamphetamine (METH).....	1
Minor cognitive/motor disorders (MCMD).....	1
Morris water maze (MWM).....	19
National Institute on Drug Abuse (NIDA).....	2
<i>N</i> -methyl-D-aspartate (NMDA).....	15
Object recognition (OR).....	4
Parkinson's disease (PD).....	6
Serotonin (5-HT).....	4
Sprague-Dawley (SD).....	2
Stimulus-response (S-R).....	8
Subgroup 1 (S ₁).....	10
Subgroup 2 (S ₂).....	10
Trans-activator protein (tat).....	5
Transgenic (Tg).....	19
Tyrosine hydroxylase (TH).....	5
Unconditional stimulus (US).....	13