THE EFFECTS OF ISOLATION REARING IN ADOLESCENCE ON BEHAVIORAL SENSITISATION TO METHAMPHETAMINE IN ADULTHOOD

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(Psychology)

Submitted as a St. Mary's Project

in Partial Fulfillment of the Graduation Requirements

for the Degree of Bachelor of Arts in Psychology

April, 2006

St. Mary's College of Maryland

St. Mary's City

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Abstract

Environmental stress has been shown to play a significant role in addictive behavior in both animal models and in humans. The present study investigates whether the stress of social isolation in adolescence increases measures of behavioral sensitization to methamphetamine in adult rats. Male Sprague-Dawley rats were reared either in isolation (n=11) or housed two to a cage (n=12) beginning in adolescence. At the onset of adulthood, rats were randomly assigned to receive either methamphetamine (n=15) or saline (n=8), and locomotor activity was measured in activity chambers at baseline, over five chronic days, and at a later test day. Results reveal that rats reared in isolation in adolescence do not exhibit higher levels of sensitization to methamphetamine than non-isolated rats. In conclusion, the current study does not show that isolation rearing increases sensitization to methamphetamine.

The Effects of Isolation Rearing in Adolescence on Behavioral Sensitization to Methamphetamine in Adulthood

One measure of addictive behavior commonly used in animal models is behavioral sensitization. Behavioral sensitization to a psychostimulant drug such as methamphetamine is characterized by increased locomotor activity after repeated injections of the drug (Higashi et al., 1989; Paulson & Robinson, 1995; Szumlinski et al., 2000). Environmental stress has been shown to play a significant role in initial drug addiction and relapse in both animal models and in humans (Antelman et al., 1980; Cabib & Puglisi-Allegra, 1996; de Jong, 2004; Shalev et al., 2000; Sinha, 2001). Therefore, it is no surprise that stressors of several kinds – from defeat/threat to food deprivation to intermittent shock – have been shown to enhance sensitization to psychostimulant drugs in rats (Covington & Miczek, 2001; de Jong et al., 2005; Lu et al., 2003; Miczek et al., 1999; Shalev et al., 2000; Stewart, 2000).

Neurological modifications are believed to accompany the locomotor changes, although whether these behavioral changes are an observable reflection of the underlying neurological changes is still under debate. The areas of the brain involved in stress are the nucleus accumbens (NA) and the prefrontal cortex (PFC), areas of the brain also associated with the mesolimbic-dopamine reward pathways involved in drug addiction (Fibiger, 1996; Marinelli & Piazza, 2002; Robinsion & Berridge, 2001; Sinha, 2001; Stewart, 2000). Stressful stimuli are known to increase dopamine (DA) transmission in the mesolimbic area (Cabib & Puglisi-Allegra, 1996). Stress can also act as a co-activator of the dopamine stress pathway and of brain reward pathways, enhancing the positive effects of drugs and leading to increased drug administration (Marinelli and Piazza, 2002; Sinha, 2001). Chronic activation of the DA pathway has been shown to lead to "state-related" changes in the brain reward pathways (Sinha, 2001). Stress acts as a primer that preps the reward system, facilitating later drug addiction through increased sensitivity to the reinforcing properties of drugs (Fibiger, 1996; Marinelli and Piazza, 2002; Sinha, 2001).

The effects of stress on addictive behavior to a psychostimulant drug have been demonstrated behaviorally in rats. For example, the presentation of the mild stress stimulus of tail pressure and the administration of amphetamine are interchangeable in their ability to induce sensitization in male Sprague-Dawley rats (Antelman et al., 1980). Animals briefly exposed to an aggressive opponent in a social defeat stress task exhibited significantly increased locomotion, meaning greater sensitization to cocaine, compared to non-stressed rats treated with cocaine (Miczek, 1999). While these studies involved repeated exposures to stressful stimuli, de Jong et al. (2005) demonstrated that a single encounter with an aggressive male was sufficient enough to induce behavioral sensitization to amphetamine for a short period of time. In addition, studies investigating the effects of environmental stressors on the self-administration of psychostimulants have found many links between both chronic and acute stressors and increased levels of drug usage and relapse (Lu et al., 2003). Although there is a mixture of findings, higher intake of both high and low psychostimulant doses has been shown in studies using social isolation for four to six weeks after PD 21 as a chronic stressor (Lu et al., 2003). Therefore, stressful stimuli, whether experienced chronically or in a single, major event, can enhance behavioral sensitization to a psychostimulant.

While most research investigates effects of stress on addictive behaviors in adulthood, there is much less literature investigating the effects of stress factors in postnatal and adolescent rats on addictive behaviors in adulthood. Such research is potentially important because events early in human adolescence, such as measures of parental bonding and caring, have been shown to affect later health and illness in midlife, including depression and drug abuse (Canetti et al., 1997; Russek and Schwarts, 1997). One particular study, however, did examine the effects of early postnatal rearing conditions on behavioral sensitization to cocaine and amphetamine in adulthood in rats. Daily maternal separation during PNDs 1-14 was shown to enhance sensitization to amphetamine and NAcc DA and behavioral sensitization to cocaine (Brake, 2004). Although most research focuses on the effects of stress on behavioral sensitization, very little of this literature investigates the effects of stress during adolescence on behavioral sensitization in rats.

Such experiments investigating the effects of stress on drug addiction in adolescent animals can be used as models of later adult addiction in humans. The current study therefore aimed to investigate behavioral sensitization to methamphetamine in adulthood in rats reared in isolated or social conditions. It was hypothesized that isolated rats would show higher levels of sensitization to methamphetamine than non-isolated rats. Such findings would demonstrate the significant effects of adolescent stress – notably stress due to social isolation – on drug addiction in adult rats. Such results would also encourage further investigation into the significance of social isolation during the human adolescent period on later drug addiction in adulthood.

Methods

Procedure

Isolation Phase

Pregnant adult female Sprague-Dawley rats were obtained at ED17 (n=3). Animals were singly housed in a colony room with a 12 h light/dark cycle (lights on at 7:00 am). After birth, male neonatal Sprague-Dawley rats were reared with the mother until weaning at 21 days of age, while female neonatal rats were sacrificed by hypothermia on PD7. At day 21, the approximate beginning of rat adolescence, isolation began. The socially housed group (n = 12) were singly housed and kept from any social contact, while the non-socially housed group (n=12) were housed two or three to a cage. All rats received free access to both food and water throughout all experimentation.

Behavioral protocol

Upon reaching adulthood (approximately 56 days of age), prior to testing, all animals were housed individually. Rats were handled at least two days in advance prior to testing. A subset of rats from each condition (isolated and non-isolated) was randomly assigned to receive either methamphetamine (n=15 per groups) or saline (n=8 per group), and was administered the assigned treatment throughout the entire sensitization procedure. On the first day of testing (baseline phase), animals were allowed to acclimate to the testing chamber (43.2 x 43.2x 30.5 cm, Med Associates, St. Albans, VT) for 30 minutes, during which locomotor activity was measured. After this time, baseline measurements of locomotor activity to injections of methamphetamine (0.5 mg/kg, i.p.) or saline (1 ml/kg, i.p.) were measured in the activity chambers. Activity counts were recorded whenever the rat interrupted one of the 16 evenly spaced IR transmitters and receivers positioned around the periphery of the chamber. Locomotor activity was measured in blocks of 15 minute intervals for a total of 2 hours following injections for all rats. Three days after baseline measurements were recorded, chronic administration (chronic phase) began and continued for 5 consecutive days. During this time, after a 30 minute habituation period to the chamber, activity counts were measured for 2 hours after each drug administration of methamphetamine (0.5 mg/kg, i.p.) or saline (1 ml/kg, i.p.) during the 5 day period for all rats in all four conditions. On the fourteenth day after the last chronic injection was administered, the test phase took place, during which rats were given a single challenge dose of either methamphetamine (0.5 mg/kg, i.p.) or saline (1 ml/kg, i.p.), and locomotor activity was again measured for 2 hours after the 30 minutes habituation period.

Results

Results for all statistical measurements involving a repeated-measures Analysis of Variance (ANOVA) are reported using the Huynh-Feldt corrected F and p values, and uncorrected degrees of freedom. A 3-way ANOVA performed on the total activity counts from baseline to test day for both housing and treatment revealed that total activity counts did significantly increase from baseline to test day, $F(1, 19) = 7.57$, $p = 0.013$. Also, the total activity counts for the methamphetamine-treated group was significantly higher than the counts for the saline group, $F(1, 19) = 23.73$, $p = .001$ (see Figure 1). However, no other significant main effects or interactions were found for housing under either treatment group, all p values > 0.064.

A 3-way mixed ANOVA was calculated separately for the saline and drug groups on the 8 blocks of 15 minute activity count increments measured during the baseline and test days to provide a closer analysis of possible differences between locomotor activity in the social and non-social groups. As seen in Figure 2, the ANOVA showed that for the methamphetamine-treated group, there was a significant increase in block activity counts from baseline to test day, $F(1, 13) = 6.02$, $p = 0.029$, showing that the animals were behaviorally sensitized to methamphetamine. The ANOVA also revealed a significant main effect of block on activity count, meaning that there was a significant decrease in activity counts between the 8 15-minute blocks recorded for the methamphetamine group, $F(7, 13) = 46.39$, $p = .001$. There was also a significant interaction between the day of testing and block counts for the drug group, $F(7, 13) = 5.58$, $p = 0.006$. As can be seen in Figure 2, the peak of drug effect appears to occur earlier on the test day than the baseline day. No other significant differences or interactions for the methamphetamine group were found in the 3-way ANOVA, including no differences or interactions involving housing, all p values > 0.72. The ANOVA calculated for the saline group on the 8 blocks of 15 minute activity count increments during the baseline and test days revealed a significant main effect of block on activity counts for the control group, $F(7, 13) = 7.03$, $p = 0.001$. This can be seen in Figure 3 by the decrease in activity counts across time. No other significant main effects or interactions were found, all p values > 0.085.

A 3-way mixed ANOVA was also performed on activity counts during the five days of chronic injections for the methamphetamine group. Again, activity counts were measured in 8 15-minute blocks. The ANOVA revealed a significant main effect of block on activity counts, meaning that collapsed across the five chronic injection days, the activity levels for the methamphetamine-treated animals significantly decreased over the 2-hour recording time, $F(7, 13) = 55.82$, $p = .001$ (refer to Figures 4-9). The ANOVA also revealed a significant interaction between the chronic test day 1 through 5 and the eight 15-minute blocks of activity counts, $F(28, 13) = 2.48$, $p = 0.008$. However, since this effect did not interact with housing, $F(4,13) = 0.790$, $p = 0.344$, it was not explored further. No other significant main effects or interactions were found between variables, all p values > 0.27.

The percent change in locomotor activity from baseline measurements to test phase measurements was calculated as a measure of percent sensitization. As show in Figure 10, an independent-samples t-test revealed that there was not a significant difference in percent sensitization from baseline to test day between social and non-social animals administered methamphetamine, $t(13) = -.85$, $p = 0.414$. Likewise, there was not a significant difference in percent sensitization from baseline to test day between saline animals in the social and non-social groups, $t(6) = -.90$, $p = 0.405$ (refer to Figure 10).

Animals were categorized as either sensitized if they had percent sensitizations above 50%, or as non-sensitized (below 50%). Fisher's exact test was performed separately for both drug and saline groups to determine if there was a difference in the proportion of sensitized animals in the social and non-social groups. For the methamphetamine group, the difference in the proportion of sensitized animals in the social (37.5 %) and non-social (57.1 %) groups was not significant, $p = 0.619$. For the saline group, the difference in sensitized animals for social (50.0 %) and non-social (75%) was also non-significant, $p = 1.00$.

Discussion

Contrary to the hypothesis, housing did not have a significant effect on activity level in methamphetamine-treated animals. Therefore, the present study demonstrates that rats reared in isolated conditions in adolescence do not show higher levels of sensitization to methamphetamine than non-isolated rats. Although results do confirm previous findings that animals are behaviorally sensitized, i.e., they exhibit significantly greater locomotor activity after repeated injections of the drug compared to their own baseline, few other significant findings of interest are revealed (Higashi et al., 1989; Paulson & Robinson, 1995; Szumlinski et al., 2000). Measures of percent sensitization in the different housing groups indicate that social isolation in adolescence does not alter behavioral sensitization to methamphetamine in adulthood.

There are several possible explanations for the failure of social isolation in adolescence to induce increased sensitization to methamphetamine compared to non-isolated animals. Although social isolation is known to be a mild stressor to rats, perhaps in adolescence is not severe enough to produce measurably different levels of sensitization in rats (Brake et al., 1997; Howes et al., 2000). Most important, however, is the small sample size that is used in this experiment, a factor that is due primarily to limited resources. The total sample size of only 23 rats may not be large enough to produce significant findings. Increasing the sample size would also likely increase the power of the experiment and provide a more accurate representation of the total population. The results indicate a large standard deviation around the mean, which demonstrates that there is a wide range of variability in the activity levels of the animals throughout the testing days. High variability in the data can reduce the likelihood of finding a consistent pattern in the results, and thus reduces the chances of finding significant results. Another factor that may contribute to the lack of significant findings is error on the part of the researcher. While the methods are standardized as much as possible, there may be differences in the handling practices between the different cohorts, likely due to the cohorts being tested at different times.

Despite the lack of any effect of housing on activity counts, there was a significant 2-way interaction between the testing day – at baseline and at the final test day – and block counts for the methamphetamine group. As seen in Figure 2, this can likely be explained by differences in the peak activity counts at baseline and test day, showing that for both housing groups the peak effects of the drug occurred earlier on the final test day then on the baseline day. This earlier peak may be the result of the emergence of behavioral sensitization in the methamphetamine-treated animals on the test day. Alternatively, perhaps the 15-minute block was too long of a time increment to record the beginning effects of the drug on the animals on the final test day, an explanation as to why the initial shape of the test day plots differ from the baseline plots in Figure 2. As for the significant main effect of block on activity counts for both the saline and methamphetamine groups, this is an expected result, given the normal activity patterns of rats in novel environments. Upon first entering the chamber, the rat may slowly begin to explore before settling down, and the effects of the drug wear off and is cleared from the body over the two-hour testing period.

Although the test day did have a main effect on activity level in the drug group during the baseline and the final test phases, this was not the case during the chronic injection days. The chronic phase began three days after the baseline measurements were recorded, whereas the test phase occurred 14 days after the final chronic injection day. Therefore, there was a sufficient amount of time in between baseline and test days for the emergence of behavioral sensitization. However, behavioral sensitization is not likely to be apparent after only a week's time. For example, animals tested after only 3 or 7 days after pretreatment injections of amphetamine failed to exhibit behavioral sensitization or sensitization-related changes in DA release. However, animals tested after 14 and 28 days did show enhanced motor activity and enhanced amphetamine-stimulated DA-release (Paulson & Robinson, 1995). Behavioral sensitization was shown to be time-dependent, with its emergence appearing only after withdrawal-related symptoms had disappeared. Therefore, the lack of the test day to have a main effect of activity levels throughout the chronic phase is validated by the available literature.

Although the present study was unable to support the hypothesis that socially isolated rats would show higher levels of sensitization to methamphetamine than non-isolated rats, there is a large body of literature demonstrating that stress does play a role in addictive behavior. Environmental stressors of several kinds, including defeat/threat exposure, food restriction, and even daily maternal separation, have been shown to enhance behavioral sensitization in rats. Therefore, further investigation into how adolescent stressors affect addictive behavior in adult rats is encouraged. In future research, larger sample sizes are also encouraged to increase the power of the study and the possibility of revealing significant findings. Animal models of addictive behavior are useful tools for investigating drug addiction in humans, and continuing investigations into how social stressors in adolescence affect addictive behavior are important to understanding drug addiction as a whole.

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Appendix

Research on human addictive behavior has shown a positive correlation between exposure to environmental stressors and increased vulnerability to drug addiction and relapse (Lu et al., 2003). Animal models of drug addiction have supported this speculation that stress plays a significant role in addictive behavior (Antelman et al., 1980; Cabib & Puglisi-Allegra, 1996; de Jong, 2004; Shalev et al., 2000; Sinha, 2001). One measure of addictive behavior commonly used in animal models is behavioral sensitization. Behavioral sensitization to a psychostimulant drug such as methamphetamine is characterized by a progressive increase in locomotor activity after repeated injections of the drug (Brady et al., 2005; Higashi et al., 1989; Paulson & Robinson, 1995; Szumlinski et al., 2000). Studies investigating the phenomenon of behavioral sensitization have suggested that the emergence of sensitization is time-dependent. In one study, animals administered a challenge dose of amphetamine 7 days after their last pretreatment dose did not exhibit sensitization, whereas animals tested 14 and 28 days after their last pretreatment dose did show sensitization (Paulson & Robinson, 1995). Sensitization was also shown to be persistent in some cases, with the animals remaining sensitized to the psychomotor activating effects of the drug for up to a year (Paulson & Robinson, 1995).

The assumption of increased motor activity levels as a model of addictive behavior is that the neural changes that occur with enhanced locomotor activity are, in part, the same changes responsible for the positive effects of drugs, such as euphoria and mood enhancement (Robinson & Berridge, 2001). This assumption stems from evidence showing that, in addition to hypersensitivity to the challenge dose, sensitized animals exhibit significantly enhanced dopamine (DA) release from the mesolimbic dopaminergic neurons that innervate the nucleus accumbens (NAcc) (Higashi et al., 1988; Paulson & Robinson, 1995; Robinson & Berridge, 2001; Thomas et al., 2001).

Although addictive behavior is highly complex and believed to alter a variety of neurological circuits, a majority of research focuses on the role of the mesolimbic DA system and its structures, notably the NAcc, the ventral tegmental area (VTA), and the prefrontal cortex (PFC) (Fibiger, 1996). The mesolimbic DA system is termed the reward pathway, as most naturally rewarding behaviors, such as eating, drinking, and copulation, have been shown to stimulate DA release from the system into its target structures (Fibiger, 1996; Paulson & Robinson; 1995; Sinha, 2001). It is also this system, however, that is affected during drug administration, as an increase in DA release is observed during the administration of psychostimulants and other drugs (Fibiger, 1996; Paulson & Robinson; 1995; Sinha, 2001). More specifically, the development of behavioral sensitization is mediated in the VTA. Evidence for the VTA's role in sensitization to a psychostimulant in rats has been demonstrated when injections of glutamate receptor antagonists into the VTA blocks the expression of sensitization (Thomas et al., 2001). The actual expression of behavioral sensitization is mediated in the NAcc, as can be measured during the challenge dose of the drug both behaviorally, through increased locomotor activity, and neurologically, as an increase in the amount of DA released into the NAcc compared to baseline measurements (Higashi et al., 1989; Paulson & Robinson; 1995; Sinha, 2001; Szumlinski et al., 2000). Both DA and glutamate antagonists have been shown to block the expression of sensitization (Robinson & Berridge, 1993).

Behavioral Sensitization: A Model of Craving

Research suggests that most of the neurological changes that occur during behavioral sensitization take place in the mesolimbic DA system and its component structures. Contrary to tolerance, in which the drug taker becomes desensitized to the positive effects of the drug, in behavioral sensitization there is an enhancement in the concentration of released DA (Higashi et al., 1989; Paulson & Robinson; 1995; Sinha, 2001; Szumlinski et al., 200). Therefore, behavioral sensitization is a reversal of tolerance. This reversal of tolerance in rats is believed to model many aspects of addictive behavior in humans, including the development of craving for the drug (Brady et al., 2005; Thomas et al., 2001). Drug craving in humans is the persistent wanting, or craving, for the drug long after drug administration has ceased, and is believed to be a major contributing factor to the relapse of drug addicts (Robinson & Berridge, 1993; Sinha, 2001).

Similar to the phenomenon of behavioral sensitization in rats, humans are believed to become neurologically sensitized to the craving/wanting of a drug, a speculation contrary to the popular layman's belief that addicts seek out drugs because they are sensitized to the positive mood enhancing effects of the drug. Therefore, craving for a drug does not denote liking for a drug, and re-exposure to drugs and drug cues can therefore elicit the sensitized wanting for the drug, despite the negative effects drug addiction has on most other components of the addict's life. Craving usually precedes relapse and the resulting drug-seeking and drug-taking behavior (Robinson & Berridge, 1993). However, re-exposure to drugs and drug-related cues are not the only risk factors that increase the likelihood of relapse. Research has shown that exposure to environmental stressors can also play a significant role in susceptibility to drug abuse and relapse (Robinson & Berridge, 1993; Sinha, 2001). In fact, stressors of several kinds – from defeat/threat to food deprivation to intermittent shock – have been shown to enhance sensitization to psychostimulant drugs in rats (Covington & Miczek, 2001; Jong et al., 2005; Lu et al., 2003; Miczek et al., 1999; Shalev et al., 2000; Stewart, 2000).

Stress and Increased Vulnerability to Drug Addiction: Theoretical Models

Theoretical models have attempted to explain why stress tends to increase human vulnerability to drug abuse. The stress coping model of addiction, for instance, proposes that drug abuse results from a combination of the positively reinforcing properties of drugs, namely mood elevation, and a reduction of the negative reinforcement of stress (Sinah, 2001). For most addicts, however, the latter effect predominates as addicts tend to build tolerance to the positively reinforcing properties of drugs. A similar theory referred to as the tension reduction and self-medication hypothesis states that individuals seek drugs as a means to reduce stress brought on by emotionally difficult experiences. Such theories propose that those with poor coping mechanisms to stressful environments, such as parental substance abuse and peer pressure or isolation, have a higher risk of seeking drugs to alleviate acute and chronic stress (Sinha, 2001).

Human studies provide evidence that supports the connection between stress and increased addictive behaviors in certain individuals. For instance, those with a history of sexual abuse are at higher risks for drug abuse that begins at a younger age than those without such a history (Sinha, 2001). Along a similar line, there is a high correlation between those with substance abuse problems and those suffering from psychiatric disorders involving chronic stress experiences, such as anxiety and affective disorders. A study investigating the effects of parental bonding on mental health in adolescence found that those participants with optimal parental bonding reported less emotional distress and an overall better sense of well-being than those who reported less than optimal parental bonding (Canetti et al. 1997). The level of reported parental care in adolescence has also been shown to affect mental and physical health in adulthood. Russek & Schwatz (1997) investigated the effects of parental caring in adolescence on mental and physical health in adulthood. Results showed that 91% of the participants that rated their parental relationships as non-caring had some kind of diagnosed disease, including alcoholism. This is compared to 45% of those participants who rated their parental relationship positively (Russek & Schwatz, 1997). Investigations into the effects of stress on human vulnerability to drug addiction are relevant to the understanding of addictive behavior as a whole.

Neurological Mechanisms of Stress

Studies on the neurological mechanisms underlying stress models of drug addiction reveal possible explanations as to why individuals exposed to acute or chronic stressors exhibit increased vulnerability to addiction. The areas of the brain involved in stress are the nucleus accumbens (NA) and the prefrontal cortex (PFC), areas of the brain also associated with the mesolimbic-dopamine reward pathways involved in drug addiction (Fibiger, 1996; Marinelli & Piazza, 2002; Robinson & Berridge, 1993; Robinsion & Berridge, 2001; Sinha, 2001; Stewart, 2000). Chronic activation of the DA pathway has been shown to lead to "state-related" changes in the brain reward pathways (Sinha, 2001). Stressful stimuli are known to increase dopamine (DA) transmission in the mesolimbic area, acting as co-activators of the dopamine stress pathway and of brain reward pathways (Cabib & Puglisi-Allegra, 1996; Marinelli and Piazza, 2002; Sinha, 2001). Much as addicts experience tolerance to the positive properties of drugs, stressful stimuli alone can decrease the rewarding effects of DA activation. Therefore, stress can act as a primer that preps the reward system, facilitating later drug addiction through increased sensitivity to and desire for the reinforcing properties of drugs (Fibiger, 1996; Marinelli and Piazza, 2002; Sinha, 2001).

Controllable vs. Uncontrollable Environmental Stressors

An interesting finding of stress research is that this increased vulnerability to drug addiction is partly dependent on the level of control the organism has over its environment. In shock-yoked experiments, two mice subjects are shocked, but only one mouse has control over the shock duration (Cabib & Puglisi-Allegra, 1996). This gives one mouse control over both subjects' environments, while leaving the yoked animal helpless. As would be expected in stressful conditions, both subjects experience an increase in DA released in the mesolimbic area upon initial exposure to the shocks. Results reveal that a neurochemical difference begins to develop between those subjects given some measure of control over the environmental stressors compared to those animals exposed to stressors that are uncontrollable. More specifically, subjects given control over shock duration continue to have increased DA released in the mesolimbic area, while yoked subjects exhibit a decrease in DA release over time. This highlights the importance of the environment on the mesolimbic response in stressful situations (Cabib & Puglisi-Allegra, 1996).

When we are first exposed to environmental stress, we experience an increase in mesolimbic DA release that constitutes the normal coping mechanism. Should this coping mechanism prove successful against the stressor, increased DA release continues for as long as we experience that stressful stimulus, promoting the continuation of the appropriate coping behavior. If the coping behavior against stressful stimuli repeatedly fails, mesolimbic DA release diminishes, and helplessness or the initiation of detrimental coping mechanisms may ensue. Such a behavioral and neurochemical response models states of chronic depression in humans (Cabib & Puglisi-Allegra, 1996). Research suggests that inappropriate coping mechanisms, such as drinking and drug use, are common means of dealing with chronic environmental stressors (Sinha, 2001). Those who use drugs and alcohol as coping mechanisms for stress are at a higher risk for dependence than those who use drugs and alcohol in social situations alone (Sinha, 2001). Therefore, a blunted mesolimbic DA response occurs only for those chronically stressed individuals, or those exposed to a single stressful experience, who are unable to appropriately cope with and control environmental stressors (Cabib & Puglisi-Allegra, 1996).

Environmental Importance on Addictive Behavior

Although many individuals have experience with drugs, most do not become addicted. Therefore, it is important to investigate the abundance of factors that may influence why one individual becomes an addict and another with a similar initial drug history does not. Research has shown that environmental enrichment in adolescent rats affects both their addictive behavior later in adulthood and the mesolimbic DA system (Bardo et al., 2001). Self-administration, in which the animal is given some measure of control over the number of drug dosages it receives over time, is an additional method used as a measure of addictive behavior in animals. Self-administration more closely resembles human drug-taking behavior because the animal has the ability to administer its own drug doses, as opposed to an outside researcher administering the dose.

In one experiment, adolescent rats were raised in three different environmental conditions: an enriched condition (EC), in which animals were given both social contact and a range of novel objects, a social condition (SC), in which the animals had social contact alone, and an isolated condition (IC), in which the animals were raised alone without social contact or objects. After reaching adulthood, all subjects were allowed to self-administer amphetamine on a fixed-ratio (FR) schedule 3hours a day for five consecutive days, followed by one day of self-administration on a progressive-ratio (PR) schedule for five hours. Results indicated that environmental enrichment during adolescent development affected amphetamine self-administration in adulthood. More specifically, both EC and SC rats earned fewer amphetamine self-infusions (0.03 mg/kg per infusions) than IC rats on the FR schedule. However, only EC rats exhibited a significant decrease in amphetamine administration rate compared to IC rats. In addition to highlighting the importance of both social contact and, most importantly environmental enrichment, in adolescence, such results suggest that environmental enrichment may decrease the rewarding effects of the drug. Such enrichment in adolescence may, therefore, serve as a protective mechanism in amphetamine self-administration (Bardo et al., 2001).

Stress and Addiction: Self-Administration and Conditioned Place Preference

There is an abundance of research on the effects of various environmental stressors on addictive behavior as measured by means other than behavioral sensitization. In animal laboratory experiments, stress is usually defined as forced exposure to some adverse condition that the animal would normally avoid. Based on this definition, environmental stressors can be separated into two groups: those that involve the addition of some unpleasant event or stimuli, such as restraint, tail pinch, foot shock, or social defeat, and those that involve the removal of an environmental condition that is required for the animal's normal psychological and physiological functioning. Such stressors in this category include social isolation, maternal deprivation, and food deprivation. Stressors may also be experienced chronically or in a single acute episode (Lu et al., 2003).

The basic scheme used in such studies investigating the role of various environmental stressors on addictive behavior is whether a particular stressor increases drug self-administration. While behavioral sensitization is believed to model aspects of human craving and self-administration is believed to more closely model the human drug-seeking experience, the reinstatement procedure is argued to model drug relapse in humans (Lu et al., 2003). During the reinstatement procedure, animals are first trained to self-administer the drug by lever-pressing. This behavior is subsequently extinguished, as the drug is replaced by saline or completely removed, and the animal's lever-pressing ceases. The final step involves measuring the ability of the drug or other non-drug stimuli to reinstate the drug-seeking behavior previously observed during the drug self-administration phase (Lu et al., 2003). Therefore, the reinstatement procedure models the tendency of human relapse in that, after the initial drug-taking experience has ceased, the animal is re-exposed to the drug, to certain drug-related cues, or to environmental stressors to determine if such cues can cause the reinstatement of drug-seeking behavior.

Drug self-administration studies have revealed mixed results. Rats exposed to intermittent shock as a stressor revealed that shock can increase cocaine self-administration when the shock is given both with and independent of lever pressing for food (Goeders & Guerin, 1996). Whether this is a result of chronic shock altering the reinforcing properties of the drug is not known, however (Lu et al., 2003). Similar results have been obtained in experiments in which the subject is exposed to a social defeat/threat stressor. This stressor involves introducing an intruder rat into the cage of another rat, a procedure that has been shown to induce physiological and behavioral stress responses (Kabbaj et al., 2001). Results show that prior exposure to such defeat/threat conditions can decrease the initiation time for self-administration at low doses of cocaine, but not for higher doses (Kabbaj et al., 2001). This defeat/threat stressor increases cocaine self-administration resembling binges when the animal is given free access to low doses of the drug (Kabbaj et al., 2001; Lu et al., 2003). More research is necessary to solidify the effects of such additive stressors on self-administration in rats.

Other forms of environmental stressors include the removal of conditions that would occupy the normal healthy environment of the animals. Maternal separation is a form of stress believed to affect addictive behavior in adult rats. Although contradictory results have been obtained, such varied outcomes are likely a factor of difference in the length of separation time, and the age at which maternal separation begins (Lu et al., 2003). While Matthews et al. (1999) suggested that maternal separation did not affect the dose-response curve for cocaine self-administration, Kosten et al. (2000) claimed that maternal separation does in fact increase cocaine self-administration acquisition (Lu et al., 2003). Similar discrepancies can be found in studies using social isolation as an environmental stressor, with results depending on the age at which the subjects are isolated. In isolation rearing, the rats are housed separately and devoid of social contact immediately after weaning, while in isolation housing, subjects are houses alone in adulthood. Results indicate that isolation rearing, but not isolation housing, can moderately enhance self-administration initiation at low cocaine doses only (Lu et al., 2003). As with shock and defeat/threat stressors, results suggest that these environmental stressors do affect drug self-administration, but additional research is necessary to determine the underlying mechanisms for such changes.

Unlike the previously presented stressors, food deprivation has consistently been shown to affect psychostimulant self-administration. Both acute food deprivation for a 24 hour period and chronic food restriction over the course of several days or weeks have been shown to consistently and significantly increase the initiation and maintenance of cocaine self-administration (Cabeza de Vaca & Carr, 1998; Carroll, 1985). Researchers suggest that the stress of chronic or acute food deprivation induces such an increase because this particular stressor enhances the rewarding effects of the drugs. This means that such increases in self-administration are only possible with drugs that act as positive reinforcers, such as psychostimulants and opiates, but not oral cocaine or injected THC (Cabeza de Vaca & Carr, 1998). Although there is consistent evidence that acute and chronic food deprivation significantly increases psychostimulant administration during the initiation and maintenance phases, the neuronal mechanisms for such an effect are not fully understood and require further investigation.

Research suggests that certain environmental stressors do in fact cause reinstatement of the drug-seeking behavior. Intermittent footshock over wide ranges of shock durations and doses was shown to be as, if not more, effective as priming injections of cocaine in reinstating drug-seeking behavior in rats (Erb et al., 1996; Lu et al., 2003). Although results are not as consistent as footshock, rats that were food deprived during training and again during the extinction phase exhibited a reinstatement of cocaine-seeking behavior in some conditions. In one study, acute food restriction reinstated cocaine-seeking only in those previously exposed to the food restraint condition during self-administration training (Carroll, 1985). On the other hand, a single period of 24 hours of food deprivation was shown to be sufficient to reinstate cocaine-seeking in rats not previously exposed to food restraint conditions (Shalev et al., 2000; Lu et al., 2003).

In addition to using self-administration as a measure of addictive behavior in animals, conditioned place preference (CPP) is a procedure used to measure the reinforcing properties of unconditioned stimuli. In CPP, animal subjects are given drug injections in one environment and a vehicle injection in another environment. The rats are trained to associate the different injections with the appropriate environment, and when administered a drug-free test, prefer the drug-paired environment over the non-drug-paired environment. Results show that acute and chronic food deprivation and acute restraint enhances psychostimulant CPP, but additional research is required for such confirmation (Bell et al., 1997; Lu et al., 2003). The downfall to using CCP as a measure of addictive behavior is that the animals are administered the drug and do not have direct control over the drug administration, as they do in self-administration studies.

Recently, reinstatement was also demonstrated in CCP, where the animal’s conditioned behavior of entering the environment initially paired with cocaine administration is extinguished. Footshock was shown to be as sufficient as priming injections of cocaine in reinstating drug-seeking behavior in rats (Lu et al., 2001). Conditioned fear, in which an unconditioned fear-inducing stimulus, such as footshock, is paired with a neutral conditioned stimulus, can also serve as an environmental stressor that reinstates cocaine CPP after extinction (Lu et al., 2003; Sanchez & Borg, 2001). Thus far, few other forms of environmental stressors have consistently shown the ability to reinstate drug-seeking behavior in rats in either self-administration conditions or CPP.

Stress and Behavioral Sensitization

The effects of stress on addictive behavior in rats have been demonstrated using behavioral sensitization to a psychostimulant drug. As previously mentioned, sensitized animals exhibit a progressive increase in locomotor activity after repeated injections of a psychostimulant, such as cocaine or methamphetamine (Antelman et al., 1980; Brake et al., 2004; de Jong et al., 2005; Miczek et al., 1999). Antelman et al. (1980) took a slightly different approach when they investigated whether the presentation of tail pressure as a mild stress stimulus was interchangeable with the administration of amphetamine in its ability to induce sensitization in male Sprague-Dawley rats. Stereotyped behavior measurements were compared between one group that received repeated amphetamine injections, and another that was subjected to repeated stress by tail pinching followed by a single dose of amphetamine. Results indicated that there were no significant differences in stereotyped behavior measurements between the two groups, indicating that repeated stress by tail pressure was able to induce behavioral sensitization to a later amphetamine injection (Antelman et al., 1980). In turn, Antelman et al. (1980) also showed that a single injection of amphetamine could induce sensitization of tail pressure behavior, showing that stress by tail pressure and amphetamine were interchangeable in their ability to induce sensitization. Such results suggest the power that stress has on behavior, in general. Not only may a single stressor sensitize individuals to later stressors, but repeated exposure to stress may lead to increased vulnerability and sensitization to the effects of the drug (Antelman et al., 1980).

Other forms of stress have been shown to induce increased sensitization to a psychostimulant drug, including social stressors. Miczek et al. (1999) briefly exposed rats to an aggressive opponent in a social defeat stress task. The social defeat task was both unpredictable and uncontrollable, and shown to be stressful by the increase in characteristic stressful behavior, such as certain grooming behaviors, and increased measurements in stress-related hormones. Results indicated that those animals briefly exposed to an aggressive opponent exhibited significantly increased locomotion, meaning greater sensitization to cocaine, compared to non-stressed rats treated with cocaine (Miczeket al., 1999). While this study involved repeated exposure to a stressful stimulus, de Jong et al. (2005) demonstrated that a single encounter with an aggressive male was sufficient to induce behavioral sensitization to amphetamine for a short period of time. In addition, studies investigating the effects of environmental stressors on the self-administration of psychostimulants have found many links between both chronic and acute stressors and increased levels of drug usage and relapse (Lu et al., 2003). Although there is a mixture of findings, higher intake of both high and low psychostimulant doses has been shown in studies using social isolation for four to six weeks after PD 21 as a chronic stressor (Lu et al., 2003). Therefore, stressful stimuli, whether experienced chronically or in a single, major event, can enhance behavioral sensitization to a psychostimulant.

While most research investigates the effects of stress on addiction in adulthood, there is much less literature investigating the effects of stress factors in postnatal and adolescent rats on drug addiction in adulthood. Events early in human adolescence, such as measures of parental bonding and caring, have been shown to affect later health and illness in midlife, including depression and drug abuse (Canetti et al., 1997; Russek and Schwarts, 1997). One particular study, however, did examine the effects of early postnatal rearing conditions on behavioral sensitization to cocaine and amphetamine in later adulthood in rats. They observed that rearing conditions in the postnatal period were related to differences in behavioral responses to stress and psychostimulants (Brake et al., 2004). More specifically, daily maternal separation during PNDs 1-14 was shown to enhance sensitization to amphetamine and NAcc DA and behavioral sensitization to cocaine. However, postnatal rats that were handled daily during PNDs 1-14 did not show this increased sensitization. Possible explanations for such observed differences are related to differences in the maternal treatment of pups: handled pups were observed to receive more attention (increased licking, grooming, arched-back nursing) than maternally separated pups, suggesting that maternal care is an important factor in later behavioral sensitization to cocaine and amphetamine (Brake, 2004).

Experiments investigating the effects of adolescent stress on addictive behavior in adult animals could be used as models of later adult addiction in humans who have suffered adolescent stress. The current study therefore aims to investigate behavioral sensitization to methamphetamine in adulthood in rats reared in isolated or social conditions. It is hypothesized that isolated rats will show higher levels of sensitization to methamphetamine than non-isolated rats. Such findings would demonstrate the significant effects of adolescent stress – notably stress due to social isolation – on drug addiction in adult rats. Such results would also encourage further investigation into the significance of social isolation during the human adolescent period on later drug addiction in adulthood.

Figures and figure captions

Figure 1. Total activity counts as a function of test day, housing, and treatment group.

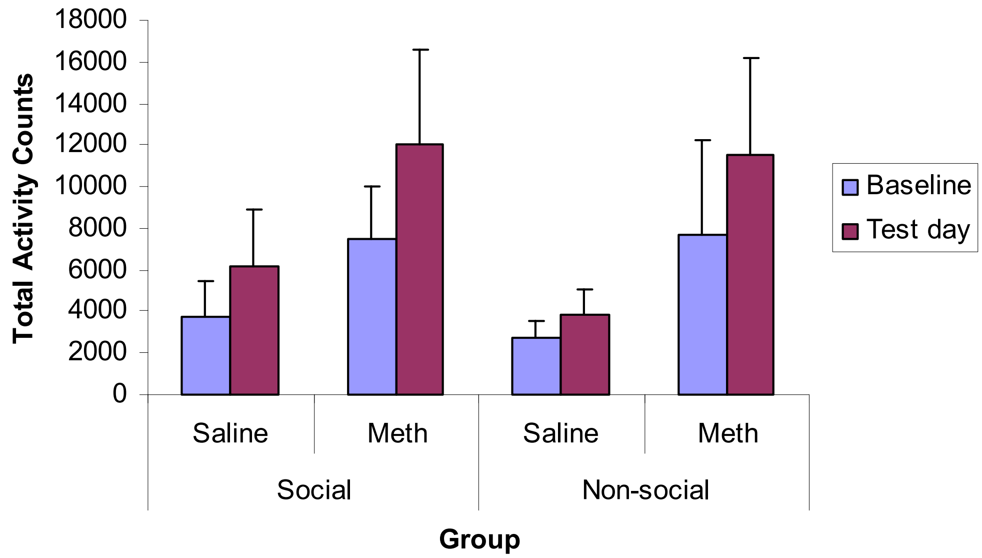

Figure 2. Mean total activity counts as a function of test day, housing, and time for the methamphetamine drug group. Activity counts were measured in 8 blocks of 15 minute increments.

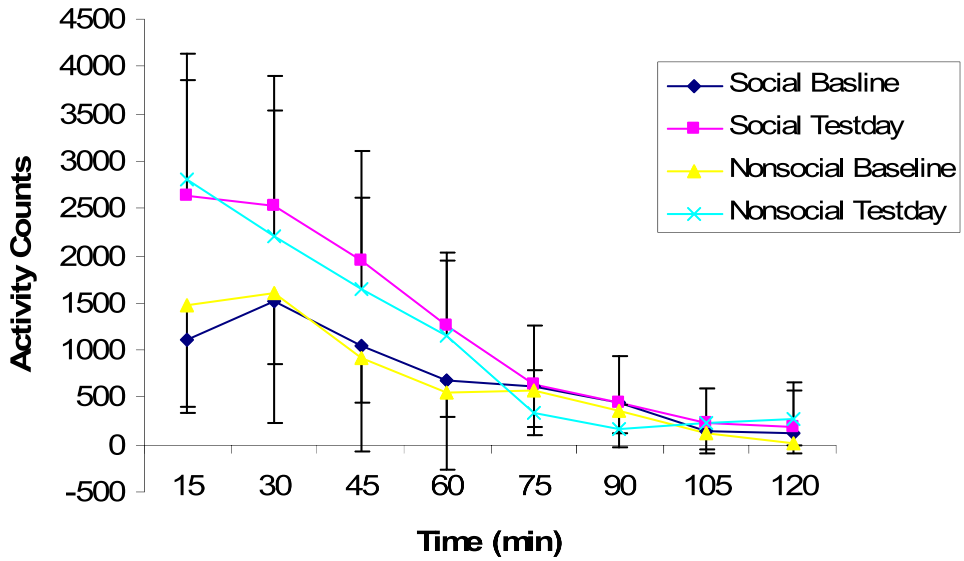

Figure 3. Mean total activity counts as a function of test day, housing, and time for the saline control group.

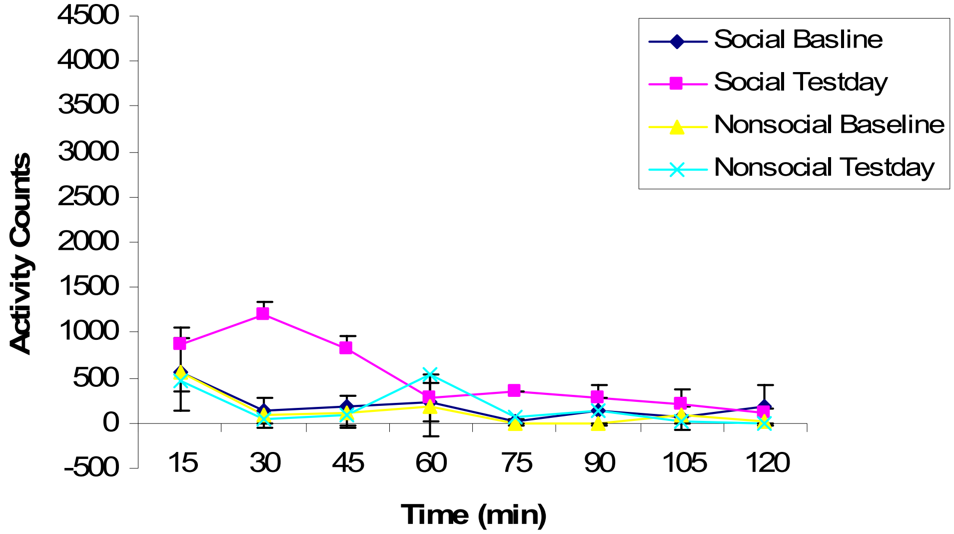

Figure 4. Collapsed total mean activity counts for both social and non-social housing groups as a function of time for chronic injection days 1 through 5 for the methamphetamine group only.

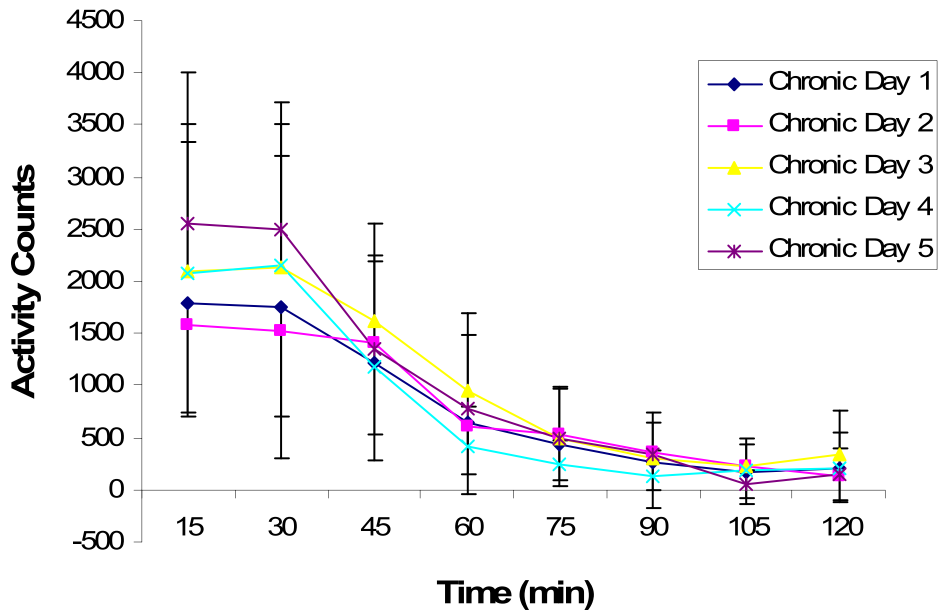

Figure 5. Mean total activity counts as a function of housing and time on chronic injection day 1 for the methamphetamine group only.

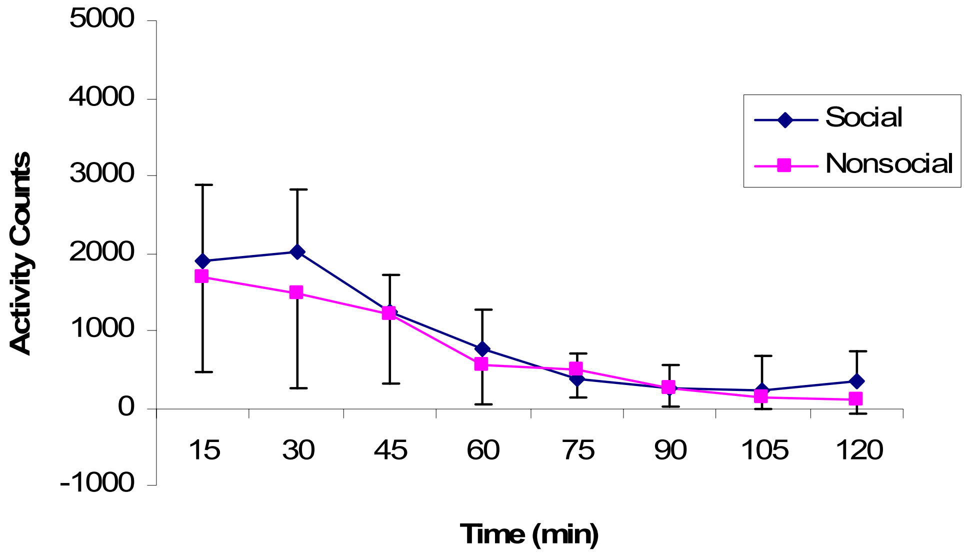

Figure 6. Mean total activity counts as a function of housing and time on chronic injection day 2 for the methamphetamine group only.

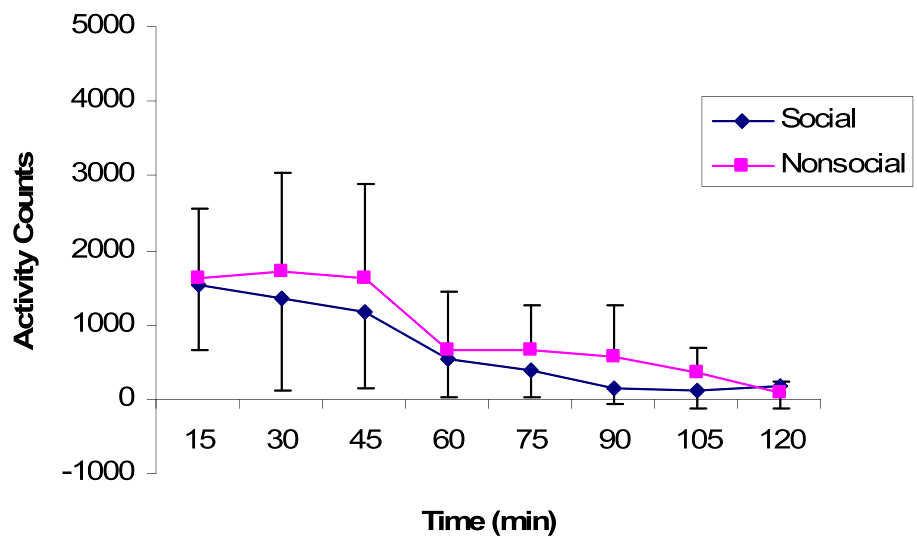

Figure 7. Mean total activity counts as a function of housing and time on chronic injection day 3 for the methamphetamine group only.

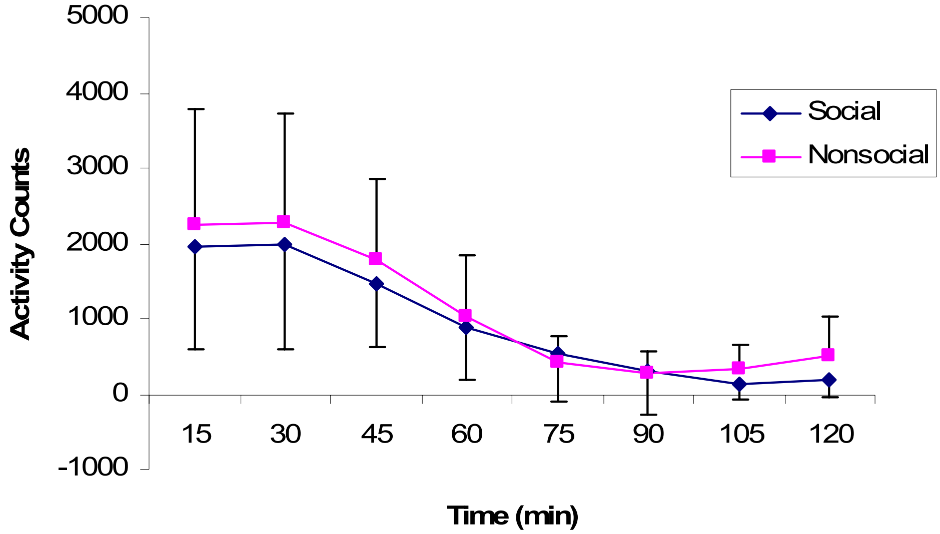

Figure 8. Mean total activity counts as a function of housing and time on chronic injection day 4 for the methamphetamine group only.

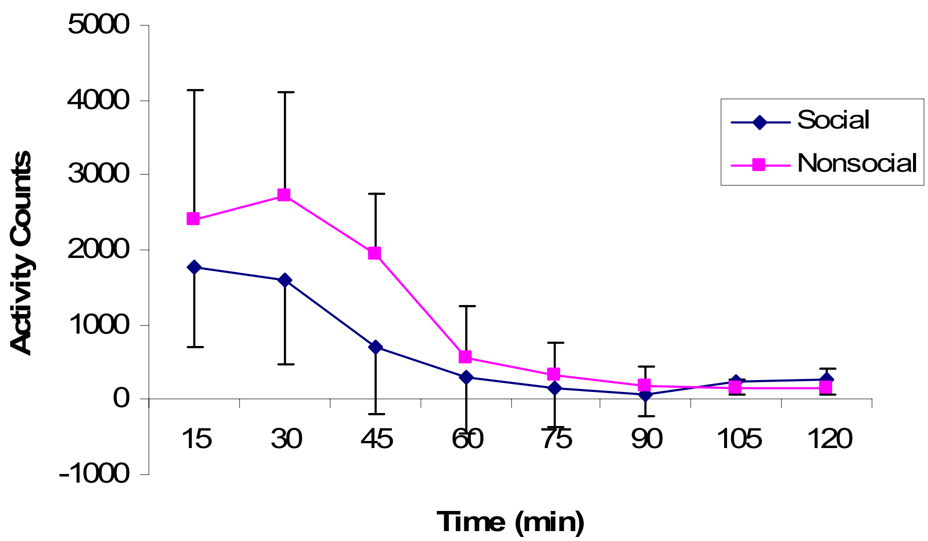

Figure 9. Mean total activity counts as a function of housing and time on chronic injection day 5 for the methamphetamine group only.

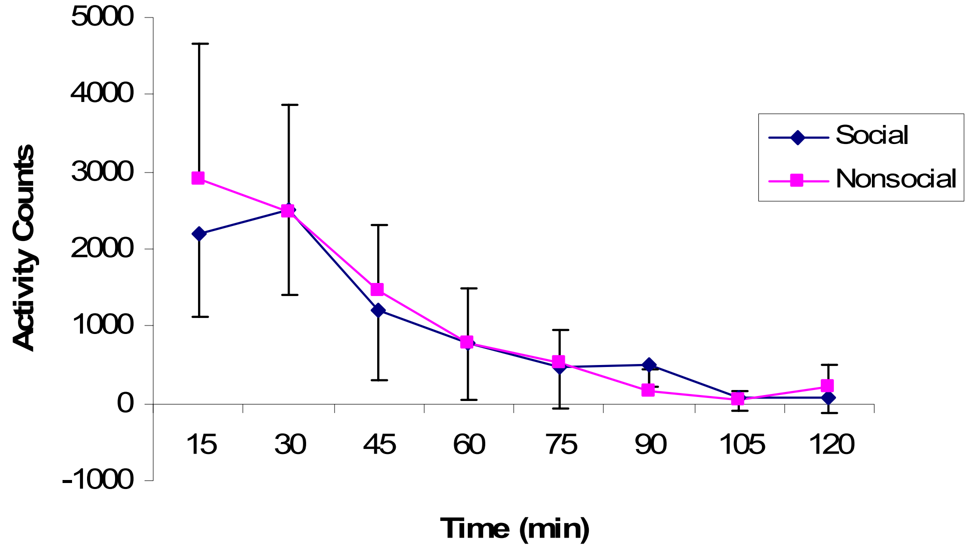

Figure 10. Percent sensitization (%) as a function of housing and treatment group. Percent sensitization is the percent change in locomotor activity from baseline measurements to test phase measurements.