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Nicotine Elicits Methamphetamine-Seeking in Rats Previously Administered Nicotine

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Abstract

Research has indicated a high correlation between psychostimulant use and tobacco cigarette smoking in human substance abusers. The objective of the current study was to examine the effects of acute and repeated nicotine administration on responding for intravenous methamphetamine (0.03 mg/kg/infusion) in a rodent model of self-administration, as well as the potential of nicotine to induce reinstatement of previously extinguished drug-taking behavior in male Sprague-Dawley rats. In addition, it was assessed whether nicotine-induced reinstatement of methamphetamine-seeking behavior and nicotine-induced locomotor sensitization require that nicotine be temporally paired with the methamphetamine self-administration session or the locomotor activity chamber. Nicotine acutely decreased methamphetamine self-administration, but did not persistently alter responding during the maintenance of methamphetamine self-administration. However, following extinction of methamphetamine self-administration, nicotine administration reinstated methamphetamine-seeking behavior only in rats that had previously been administered nicotine. Nicotine-induced reinstatement and expression of locomotor sensitization were not dependent on a temporal pairing of nicotine with either the methamphetamine self-administration session or the locomotor activity chamber, respectively. These results indicate that nicotine may be acting, at least in part, through a non-associative mechanism to reinstate methamphetamine-seeking behavior.

Keywords

Methamphetamine; Nicotine; Self-Administration; Reinstatement

1. Introduction

Many individuals using illicit drugs also smoke tobacco cigarettes. Results derived from the National Household Survey on Drug Abuse (NHSDA) indicate that individuals who report

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Contributors

All authors were involved with the design of the experiments. Author NN conducted the behavioral experiments, statistical analysis and wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

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Conflict of interest

None

previous participation in a drug treatment program have a three-fold greater chance of being cigarette smokers compared to the general population (Richter et al. 2002). Moreover, a high percentage of individuals seeking treatment for methamphetamine abuse in Los Angeles County report previous tobacco use (Brecht et al. 2004). It is likely that both the behavioral and pharmacological interactions of methamphetamine and nicotine, derived from smoking tobacco cigarettes, contribute to the concomitant use of these substances. Further understanding of these interactions would aid in the development of more effective treatment strategies for methamphetamine dependence.

Several animal models have indicated a pharmacological interaction exists between amphetamine-like drugs and nicotine. In rats trained to discriminate amphetamine (1 mg/kg) from saline, nicotine administration results in partial substitution (Bardo et al. 1997). This suggests that amphetamine and nicotine share some common discriminative stimulus properties. In addition, studies have shown that the locomotor stimulant effect of nicotine cross-sensitizes with that of amphetamine. Animals pretreated with nicotine for 5 days display a sensitized locomotor response to a subsequent amphetamine challenge 3 weeks later (Schoffeleer et al. 2002). Furthermore, when mecamylamine, a non-specific nicotinic antagonist, is co-administered with amphetamine across a 5-day pretreatment regimen, the sensitized response to amphetamine administration 3 weeks later is attenuated (Schoffeleer et al. 2002). In addition, mecamylamine also blocks amphetamine-induced neurochemical sensitization as assessed by electrically evoked ^3H DA release in superfused nucleus accumbens slices. These studies suggest pharmacological manipulation of the nicotinic acetylcholinergic system alters amphetamine-induced locomotor sensitization, possibly through dopaminergic mechanisms.

While the effect of nicotine on the ongoing self-administration of amphetamine-like drugs has yet to be determined, the effect of nicotine on cocaine self-administration has been examined. Repeated nicotine pretreatment prior to cocaine self-administration sessions in rats results in a significant increase in progressive ratio break point compared to saline pretreatment controls (Bechtolt and Mark 2002). Following extinction, nicotine reinstates cocaine-seeking only in nicotine pretreated rats, but not in saline pretreated rats. However, acute nicotine has been shown to reinstate methamphetamine-seeking behavior, albeit to a lesser degree than methamphetamine itself (Hiranita et al. 2006). There is also evidence that mecamylamine decreases cocaine self-administration on a fixed ratio 1 schedule of reinforcement in rats (Levin et al. 2000). Further, administration of mecamylamine attenuates the escalation of cocaine self-administration observed in animals on an extended access self-administration schedule (Hansen and Mark 2007). These studies provide evidence that pharmacological manipulation of the nicotinic acetylcholinergic system can alter the reinforcing effects of cocaine and may play a role in psychostimulant-induced drug-seeking behaviors.

Given the high comorbidity of nicotine and methamphetamine dependence, it is of clinical value to determine the behavioral effects of concurrent nicotine administration and methamphetamine self-administration. An initial experiment was conducted to establish the dose-dependent effect of nicotine administration on methamphetamine self-administration following acquisition of methamphetamine self-administration using a within-subjects design. A second experiment was conducted to assess the effects of repeated nicotine (0.2 mg/kg) administration on the acquisition of methamphetamine self-administration and drug-induced reinstatement. This lower dose of nicotine was used in order to assess the effects of nicotine-induced reinstatement of extinguished methamphetamine-seeking at a dose that has been shown to affect locomotor activity in our laboratory, as well as others, but did not acutely alter methamphetamine self-administration (Ksir et al. 1987; Wooters et al. 2008). Lastly, the dose of nicotine (0.04 mg/kg) that resulted in decreased methamphetamine intake was subsequently used to assess the effect of repeated nicotine on acquisition of methamphetamine self-

administration and methamphetamine-induced reinstatement in rats. Given that nicotine can act as a conditional stimulus (CS) to signal the availability of response-contingent reward, (Bevins and Palmatier 2004; Chaudhri et al. 2006), this experiment also determined if nicotine-induced reinstatement of methamphetamine-seeking behavior and nicotine-induced locomotor sensitization require that nicotine be temporally paired with the methamphetamine self-administration session or the locomotor activity chamber, respectively.

2. Methods

2.1 Subjects

Male Sprague-Dawley rats (250-300g) obtained from Harlan Industries (Indianapolis, IN) were housed individually and allowed to acclimate to the colony for 7 days with *ad libitum* access to food (Purina Rat Chow) and water. Animals were handled for 3 days prior to the commencement of each experiment. The animal vivarium was maintained on a 12-hr/12-hr light/dark cycle at 24°C and 45% relative humidity. All experiments were conducted during the light phase. All procedures were approved by the University of Kentucky Institutional Animal Care and Use Committee and conformed to the 1996 edition of the *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health).

2.2 Drugs

d-methamphetamine (Sigma; St. Louis, MO) and S(–)-nicotine hydrogen ditartrate (Sigma; St. Louis, MO) were prepared in distilled saline (0.9% NaCl). The dose of methamphetamine was calculated using the salt weight and the doses of nicotine were calculated using the free base weight with the pH adjusted to 7.4.

2.3 Apparatus

Operant chambers (ENV-001, Med Associates St. Albans, VT) were enclosed in sound attenuating compartments (ENV-018, Med Associates St. Albans, VT) and controlled by MED-PC IV software (SG-502, Med Associates St. Albans, VT). A 5 × 4.2 cm opening that allowed access to a recessed food tray was located on the front panel of each chamber. Two metal response levers on either side of the food tray were located 7.3 cm above a metal-grid floor and a white cue light was centered above each response lever. Drug infusions (0.1 ml over 5.9 sec) were delivered via a syringe pump (Med Associates, PHM-100). A water-tight swivel allowed attachment of the catheter tubing from a 10-ml syringe to an acrylic head mount of the rat within the operant chamber.

Locomotor activity was recorded using an automated system (AccuScan Instruments Inc., Columbus, OH) comprised of clear acrylic chambers (42 × 42-cm square and 30 cm high) set inside metal frames containing a horizontal 16 × 16 grid of photo beam sensors which were located 2.5 cm apart and 7.0 cm above the chamber floor. Through a computer interface, Versamax System software recorded photobeam interruptions and calculated total distance traveled (cm) for the 60-min session.

2.4 Methamphetamine Self-Administration

Rats were first trained briefly to respond for sucrose reinforcement (45 mg sucrose pellets, NOYES Co., Inc., Lancaster, NH) as described previously (Harrod et al., 2001). After training, rats were allowed free access to food for the remainder of the experiment. One week after food training, rats were surgically implanted with a chronic indwelling jugular catheter. Rats were anesthetized (100 mg/kg ketamine, 5 mg/kg diazepam, i.p.) and implanted with a catheter into the right jugular vein that exited through a dental acrylic head mount. The head mount was affixed to the skull with metal jeweler screws. Daily infusions of heparinized saline (0.2 mg/

0.1 ml/rat/day) were given to maintain catheter patency. Rats were allowed to recover for at least 5 days before beginning methamphetamine self-administration.

Rats were allowed to self-administer methamphetamine (0.05 mg/kg/infusion) on a fixed ratio 1 (FR 1), 20-s signaled time out schedule of reinforcement during daily, 60-min sessions. Drug was infused following depression of one lever (active lever); responding on the second lever (inactive lever) was recorded, but had no programmed consequence. Each drug infusion was followed by a 20-s time out interval that began immediately following completion of the ratio requirement and was signaled by illumination of the lights above the response levers. During this interval, responding on either lever was without consequence and was not recorded.

2.5 Experiment 1. Dose Effect of Nicotine on Methamphetamine Self-Administration

Rats (n=12) were trained initially to self-administer methamphetamine (0.05 mg/kg/infusion) on a FR1 schedule of reinforcement as described above. In this experiment, the schedule of reinforcement was then incremented across sessions from a FR 1 to a FR 3, and then to a terminal FR 5 schedule until responding stabilized. Stable responding was operationally defined as less than 15% variability in the number of infusions earned across three consecutive sessions, a greater than 2:1 ratio of active to inactive responses and at least 10 infusions earned per session. Following acquisition of stable methamphetamine self-administration, nicotine (0, 0.1, 0.2 or 0.4 mg/kg, S.C.) was administered 15 min prior to the 60-min operant session. Each rat received each dose in a randomized order with 2 maintenance days (no pretreatments) between doses. Rats were transported daily in their home cage to the experimental room. Pretreatments were administered in the experimental room and the animals were returned to the home cage in the experimental room for the 15 min preceding the operant session. Immediately following the operant session, rats were returned to the colony room.

2.6 Experiment 2. Repeated Nicotine (0.2 mg/kg) Pretreatment on Methamphetamine Self-Administration and Reinstatement of Methamphetamine-Seeking Behavior

In order to assess the effect of nicotine on the acquisition of methamphetamine self-administration, rats were administered saline (n=5) or nicotine (0.2 mg/kg; n=7) 15 min prior to 14 consecutive methamphetamine self-administration sessions (60 min), beginning with the first session. This lower dose of nicotine was used in order to assess the effects of nicotine-induced reinstatement of extinguished methamphetamine-seeking at a dose that has been shown to affect locomotor activity in our laboratory, as well as others, but did not acutely alter methamphetamine self-administration (Ksir et al. 1987; Wooters et al. 2008). Self-administration sessions were conducted using a terminal FR1 schedule of reinforcement in order to minimize differences in responding that may result from increasing the ratio requirement. No maintenance sessions occurred between nicotine pretreatment sessions. Both groups of rats subsequently underwent extinction training for 14 sessions. Extinction sessions were identical to the self-administration sessions except saline was substituted for methamphetamine and no pretreatment was administered. Following extinction, each rat underwent 3 sessions during which reinstatement of methamphetamine-seeking behavior was assessed. Rats were pretreated with saline (S.C), nicotine (0.2 mg/kg, S.C.) or methamphetamine (0.5 mg/kg, S.C) 15 min prior to a non-reinforced operant session (60 min). Rats received each dose in a counterbalanced order with 2 extinction days (no pretreatments) between doses.

2.7 Experiment 3. Repeated Paired and Unpaired Nicotine (0.4 mg/kg) Pretreatment on Methamphetamine Self-Administration, Nicotine-Induced Reinstatement of Methamphetamine-Seeking Behavior, and Locomotor Activity

Starting on the first session, rats self-administered methamphetamine (0.05 mg/kg/infusion; FR1 schedule of reinforcement) during a 60-min operant session and 4 hr later underwent a

30-min locomotor session in a different experimental room. Both behavioral paradigms were conducted daily from for the entire duration of the experiment. In order to assess the conditioned stimulus properties of nicotine, one group was administered saline 15 min prior to both the operant and locomotor sessions (Group SAL-SAL, $n=9$); a second group was administered nicotine (0.4 mg/kg) 15 min prior to the operant session and saline 15 min prior to the locomotor session (Group NIC-SAL, $n=11$); and a third group was administered saline 15 min prior to the operant session and nicotine (0.4 mg/kg) 15 min prior to the locomotor session (Group SAL-NIC, $n=11$). Pretreatments were administered for 14 consecutive days (sessions 1-14). A higher dose of nicotine (0.04 mg/kg) was used in this study compared to the dose used in experiment 2 (0.02 mg/kg) so that robust locomotor sensitization to repeated nicotine administration could be observed. All groups of rats then underwent extinction training for 14 consecutive days (sessions 15-28). Extinction sessions were identical to the pretreatment sessions, except saline was substituted for methamphetamine in the self-administration paradigm and all pretreatment injections were saline in both paradigms. Following extinction, each rat was tested for reinstatement of methamphetamine-seeking behavior on 2 consecutive days (sessions 29-30). During these sessions, rats were pretreated with either saline or nicotine (0.4 mg/kg, S.C.) 15 min prior to the non-reinforced operant session (60 min) in a counterbalanced manner; the locomotor sessions during these days were identical to the extinction sessions. Following operant reinstatement sessions, animals underwent two additional days of extinction (sessions 31-32). Subsequently, animals were challenged with nicotine (0.4 mg/kg, sc) or saline 15 min prior to a locomotor session on 2 consecutive test days (sessions 33-34) with operant sessions identical to the extinction sessions.

2.8 Statistics

Data were analyzed with SPSS (Chicago, IL version 15) software. The dose and time course effects of nicotine (0, 0.1, 0.2 or 0.4 mg/kg) on methamphetamine self-administration were analyzed using 2-way ANOVAs with repeated measures. The effects of repeated nicotine (0.2 mg/kg) on methamphetamine self-administration and subsequent extinction were analyzed using separate 2-way ANOVAs with repeated measures, with pretreatment dose as a between-subject variable and session as a within-subject factor. Reinstatement was also analyzed using a 2-way ANOVA with repeated measures, with pretreatment dose as a between-subject variable and reinstatement dose as a within-subject factor. Separate 2-way ANOVAs were used to analyze the effects of paired vs. unpaired nicotine administration on methamphetamine self-administration, extinction, reinstatement, and locomotor activity, with pretreatment group as a between-subjects factor and session/challenge as a within-subject factor. All posthoc comparisons were made using paired or unpaired t-tests with correction for familywise error.

3. Results

3.1 Experiment 1: Dose Effect of Nicotine on Methamphetamine Self-Administration

Figure 1A shows that acute nicotine pretreatment significantly attenuated responding for methamphetamine as indicated by a significant main effect of dose upon analysis of active lever responses ($F(3,33)=4.449$, $p<0.01$) and infusions earned ($F(3,33)=4.442$, $p<0.01$). Pretreatment with lower doses of nicotine (0.1 or 0.2 mg/kg) had no effect on responding, while pretreatment with a higher dose of nicotine (0.4 mg/kg) significantly attenuated responding on the active lever and the number of methamphetamine infusions earned compared to saline pretreatment (both $p<0.05$). No significant change in inactive lever responding occurred at any dose.

To further examine the significant attenuation in responding following high doses of nicotine, a time course assessment of the operant session was analyzed in 5-min bins. A two-way ANOVA of active lever responses across time following nicotine (0.4 mg/kg) or saline

pretreatment revealed a significant drug $\times$ time interaction ($F(11,121)=2.88, p<0.01$). Post hoc t-tests indicated that nicotine (0.4 mg/kg) significantly decreased responding on the active lever only during the first 5 min compared to saline (Figure 1B).

3.2 Experiment 2. Repeated Nicotine (0.2 mg/kg) Pretreatment on Methamphetamine Self-Administration and Reinstatement of Methamphetamine-Seeking Behavior

Repeated pretreatment with nicotine (0.2 mg/kg) did not alter methamphetamine self-administration or responding during extinction sessions compared to saline controls (Figure 2A). A main effect of session was observed upon analysis of extinction ($F(13,130)=4.74, p<0.01$), with animals showing a significant decrease in responding on the previously active lever between the first and last day of extinction ($p<0.001$), regardless of pretreatment condition. Following extinction, all rats underwent 3 reinstatement sessions (Figure 2B). ANOVA revealed a significant interaction of pretreatment condition $\times$ reinstatement drug ($F(2,20)=12.20, p<0.001$). Subsequent analyses indicated that the nicotine challenge reinstated responding only in the nicotine pretreatment group ($p<0.05$), whereas the methamphetamine challenge resulted in reinstatement in both groups ($p<0.05$) compared to saline challenge.

3.3 Experiment 3. Repeated Paired and Unpaired Nicotine (0.4 mg/kg) Pretreatment on Methamphetamine Self-Administration, Nicotine-Induced Reinstatement of Methamphetamine-Seeking Behavior, and Locomotor Activity

There were no differences in methamphetamine self-administration or extinction of responding between the pretreatment groups (Figure 3A). Although the analysis indicated a significant main effect of day across the pretreatment sessions ($F(13,364)=2.59, p<0.005$), no significant difference was observed between the first and last pretreatment sessions. In addition, all groups showed a significant reduction in active lever responding across the extinction sessions ($F(13,364), p<0.001$); posthoc analysis indicated a significant difference between the first and last extinction sessions ($p<0.001$).

Following the extinction phase, each animal was tested for nicotine-induced reinstatement of methamphetamine-seeking behavior (Figure 3B). ANOVA revealed a significant treatment (SAL-SAL, NIC-SAL or SAL-NIC) $\times$ reinstatement challenge (nicotine or saline) interaction ($F(2,28)=9.77, p<0.001$). There were no significant differences in responding among pretreatment groups following the saline challenge. However, the SAL-SAL group exhibited a significant decrease in responding on the previously active lever following nicotine challenge compared to saline challenge ($p<0.05$). In contrast, active lever responses were significantly increased by the nicotine challenge in both the NIC-SAL and SAL-NIC groups ($p<0.05$ in each case). Thus, regardless of whether or not nicotine pretreatment was temporally paired with the operant session or given prior to the locomotor activity session, subsequent reinstatement of methamphetamine-seeking behavior by a nicotine challenge was observed.

Locomotor activity was analyzed as total distance traveled. In contrast to methamphetamine self-administration, the pretreatment regimen significantly altered locomotor activity across pretreatment sessions, as indicated by a significant group $\times$ session interaction (Figure 4A, left panel; $F(26,364)=7.99, p<0.001$). T-tests revealed that SAL-NIC rats showed less activity than SAL-SAL animals during sessions 1-3 ($p<0.05$) and more activity during sessions 13-14 ($p<0.05$). Across the extinction phase (Figure 4A; right panel), all animals exhibited decreased locomotor activity, regardless of pretreatment ($F(17,476)=11.81, p<0.001$); posthoc analysis confirmed a significant decrease in locomotor activity between the first and last extinction session ($p<0.01$).

Following the extinction phase, all rats were assessed for nicotine-induced locomotor sensitization (Figure 4B). An ANOVA revealed a significant pretreatment group (SAL-SAL,

NIC-SAL or SAL-NIC) $\times$ locomotor challenge (nicotine or saline) interaction ($F(2,28)=11.77$, $p<0.001$). There were no significant differences in locomotor activity among pretreatment groups following a saline challenge. The SAL-SAL group exhibited a significant decrease in locomotor activity following nicotine challenge compared to saline challenge ($p<0.05$). In contrast, activity was significantly increased by the nicotine challenge in both NIC-SAL and SAL-NIC groups ($p<0.05$ in each case). Thus, regardless of whether or not nicotine pretreatment was temporally paired with the locomotor activity sessions or the operant sessions, a nicotine challenge subsequently resulted in locomotor hyperactivity, a response indicative of sensitization.

4. Discussion

The aim of the current experiments was to characterize the effects of acute and repeated nicotine on methamphetamine self-administration and reinstatement of methamphetamine-seeking behavior. In Experiment 1, low doses of nicotine (0.1 and 0.2 mg/kg) had no effect on methamphetamine self-administration, while a higher dose of nicotine (0.4 mg/kg) decreased responding. Analysis of the time course indicated that the decrease was significant only during the beginning of the session. Although previous reports have shown that nicotine pretreatment (0.15 – 0.6 mg/kg) produces an inverted U-shape response curve on the number of cocaine infusions earned, these studies were conducted using a progressive ratio schedule of reinforcement (Bechtholt and Mark 2002). The progressive ratio breakpoint is likely a more sensitive measure for the detection of changes in the motivational component of drug taking behavior. Further experiments are needed to determine whether the use of an alternate schedule of reinforcement, such as progressive ratio, would uncover nicotine-induced alterations in the reinforcing effects of methamphetamine.

In Experiment 2, no differences in responding for methamphetamine were observed across 14 repeated administrations of nicotine. Furthermore, nicotine pretreatment during maintenance of methamphetamine self-administration did not significantly alter responding during the subsequent extinction phase. Following extinction, a challenge with systemic methamphetamine (0.5 mg/kg) significantly reinstated responding in both saline and nicotine pretreatment groups. Interestingly, a challenge with systemic nicotine (0.2 mg/kg) reinstated responding in rats that had previously received nicotine pretreatments. This study indicates nicotine administration reinstates lever responding for methamphetamine-seeking specifically in rats previously administered nicotine. These results are in contrast to previous reports that acute nicotine, at similar doses, reinstates extinguished methamphetamine-seeking behavior in rats with no prior nicotine exposure (Hiranita et al. 2006). This discrepancy could be attributed to methodological difference between these studies. In the study by Hiranita et al. five extinction sessions were conducted in the absence of cues and nicotine was administered 30 min prior to the reinstatement session. In the current experiments, the cues were presented during the fourteen extinction sessions and nicotine was administered 15 min prior to the reinstatement session. Due to the high comorbidity of tobacco smoking and methamphetamine use, it is likely that an individual seeking treatment for methamphetamine addiction has had previous exposure to nicotine. In light of the current results, nicotine-induced relapse to methamphetamine-seeking behaviors in individuals that quit using both substances at the same time warrants further examination.

Nicotine can also modulate motivated behavior through conditioning processes. Although nicotine has primarily been discussed as producing unconditioned stimulus effects, it is well documented that nicotine also produces interoceptive cue effects, which allow it to serve as a discriminative stimulus in operant drug discrimination assays and as a CS in Pavlovian conditioning procedures (Bardo et al. 1997; Besheer et al. 2004; Bevins and Palmatier 2004; Stolerman 1991). For example, the drug discrimination procedure shows that rats learn to

discriminate a nicotine-induced interoceptive cue state from a non-cue state following saline. Following nicotine, rats respond on one particular lever to obtain a non-drug reinforcer, whereas after saline, animals respond on the second response lever for the same reinforcer. Thus, rats learn that a nicotine interoceptive cue, or the absence of the cue, provides specific information about reinforced responding (Bardo et al. 1997; Stolerman 1991). Bevins and colleagues have extended these findings by demonstrating that nicotine-induced interoceptive stimuli can serve as a CS in an appetitive discrimination procedure (Besheer et al. 2004; Bevins 2009 for review). In those experiments, rats were administered nicotine or saline. On nicotine trials, the dipper contained sucrose solution, whereas on saline trials no solution was present. During testing, rats exhibited approach behavior (i.e., goal tracking) toward the dipper after injections of nicotine, but not saline, thus demonstrating that rats learn that the nicotine interoceptive cue signals sucrose availability. Taken together, these findings indicate that nicotine can alter goal-directed behavior and Pavlovian elicited behaviors by functioning as a discriminative cue or CS, respectively.

Given that nicotine can function as a CS, one possible explanation for the nicotine-induced reinstatement of methamphetamine-seeking is that nicotine (0.2 mg/kg) served as a cue for the availability of methamphetamine in the operant chamber. In order to address this question, nicotine administration was temporally separated from the methamphetamine self-administration session in Experiment 3. Compared to Experiment 2, a higher dose of nicotine (0.4 mg/kg) was used in order to enhance locomotor sensitization. While this difference in doses across experiments is a limitation of the current set of experiments, no significant difference between groups was observed on methamphetamine self-administration or extinction using the higher nicotine dose. This contrasts with the results of Experiment 1, where a decrease in responding was observed following the higher dose of nicotine (0.4 mg/kg). The most likely explanation for this difference is that rats were trained on an FR5 in Experiment 1 and an FR1 in Experiment 3. Based on the rate dependency hypothesis, the higher rate of responding in Experiment 1 may have been more susceptible to disruption by nicotine (Dews 1977).

Interestingly, Pavlovian conditioning can also be supported under conditions when a drug is used as the CS and a second drug is the US. Revusky and colleagues demonstrated that pentobarbital served as a conditioned stimulus (CS) following repeated pairings with amphetamine as the unconditioned stimulus (US). Following repeated CS (pentobarbital)-US (amphetamine) pairings, presentation of the CS elicited a robust and enduring conditioned response (CR) of increased heart rate (Reilly and Revusky 1992; Revusky et al. 1989), which was similar to the US effects of amphetamine in that procedure. Furthermore, drug onset cues induced by protracted, intravenous morphine injection can elicit a conditioned response related to the analgesic properties of the morphine US (i.e., compensatory CR; Kim 1999; Sokolowska et al. 2002). If the mechanism by which nicotine functions to reinstate methamphetamine-seeking behavior in rats previously administered nicotine is by serving as a cue for the availability of methamphetamine in the operant chamber, one would predict that nicotine-induced reinstatement would have occurred only in the NIC-SAL group. However, significant nicotine-induced reinstatement was observed in both the NIC-SAL and SAL-NIC groups. This finding suggests that the underlying mechanism of nicotine-induced reinstatement of methamphetamine-seeking in rats previously administered nicotine does not depend on nicotine acting as a cue signaling the availability of methamphetamine. The present results are in accord with previous research showing that nicotine and amphetamine can cross-potentiate the behavioral and neurochemical consequences of each other in a non-associative manner (Jutkiewicz et al. 2008; Kuribara 1999).

As expected, when nicotine was administered 15 min prior to the locomotor session across 14 consecutive sessions, an initial decrease in activity was observed, followed by an increase in

locomotor activity during the later sessions. This effect has been reported previously from our laboratory and others (Hentall and Gollapudi 1995; Ksir 1994; Wooters et al. 2008). Interestingly, following the extinction phase, when rats were administered saline prior to the locomotor sessions for 14 days, a nicotine (0.4 mg/kg) challenge produced hyperactivity in both nicotine pretreatment groups, regardless of whether they had previously received nicotine prior to the operant or locomotor session. Since no difference in locomotor activity was observed following the saline challenge, it is likely that the nicotine-induced hyperactivity reflects neurochemical sensitization independent of conditioned hyperactivity. Future work may reveal the neural mechanisms involved in the ability of repeated nicotine treatment to non-associatively enhance methamphetamine-induced hyperactivity and reinstatement, thus providing clues about potential new medications for the treatment of methamphetamine abuse.

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Figure 1.

(A) Dose Effect of Nicotine on Methamphetamine Self-Administration Low doses of nicotine pretreatment had no effect on active lever responses for methamphetamine, while the highest dose of nicotine tested (0.4 mg/kg) induced a significant attenuation in active lever responses and number of methamphetamine infusions earned (both $*p < 0.05$). (B) Nicotine Pretreatment on Methamphetamine Self-Administration: Timecourse. Nicotine pretreatment (0.4 mg/kg) decreased active lever responding for methamphetamine compared to saline pretreatment during the first 5 min of the operant session ($*p < 0.05$).

Figure 2.

(A) Repeated Nicotine (0.2 mg/kg) Pretreatment on Methamphetamine Self-Administration and Reinstatement of Methamphetamine-Seeking Behavior. Left panel: NIC pretreatment did not alter operant responding for methamphetamine compared to SAL pretreatment. Right panel: Extinction of operant responding was evident across sessions with no significant difference between groups. (# $p < 0.05$ compared to first extinction session). (B) Effect of Nicotine on Reinstatement of Methamphetamine-Seeking Behavior. Responses on the previously active lever were increased by NIC challenge in rats pretreated previously with NIC and were increased by METH challenge in rats pretreated previously with either SAL or NIC (* $p < 0.05$ compared to corresponding SAL challenge group).

Figure 3.

(A) Effect of Repeated Nicotine (0.4 mg/kg) Pretreatment Administered in a Paired or Unpaired Manner on Methamphetamine Self-Administration (FR1). Left panel: Treatment during acquisition had no effect on lever responding. Right panel: Extinction of operant responding was evident in all groups and no group differences were observed during the extinction phase ($\#p < 0.05$ compared to first extinction session). (B) Effect of Nicotine (0.4 mg/kg) on Reinstatement of Methamphetamine-Seeking Behavior. Responses on the previously active lever were decreased by nicotine challenge in SAL-SAL group ($\#p < 0.05$ compared to corresponding saline challenge group) and were increased by nicotine challenge in NIC-SAL and SAL-SAL groups ($*p < 0.05$ compared to corresponding saline challenge groups).

Figure 4.

Effect of Repeated Nicotine (0.4 mg/kg) Pretreatment on Locomotor Activity. Left panel: NIC pretreatment prior to the locomotor session (SAL-NIC group) initially decreased activity and subsequently increased activity compared to the SAL-SAL group (* $p < 0.05$). Right panel: During the extinction phase of the experiment, no group differences in locomotor activity were evident on any session; however, a significant decrease in activity between the first and last extinction session was observed (#, $p < 0.01$ compared to the first extinction session). (B) Effect of Nicotine-Sensitization Following the Extinction Phase. Activity was decreased by nicotine challenge in SAL-SAL group (# $p < 0.05$ compared to corresponding saline challenge group) and was increased by nicotine challenge in NIC-SAL and SAL-NIC groups (* $p < 0.05$ compared to corresponding saline challenge groups).