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Effects of caffeine on persistence and reinstatement of nicotine-seeking behavior in rats: interaction with nicotine-associated cues

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Abstract

Rationale—Caffeine and nicotine are the most commonly co-used psychostimulants. However, it is still unclear whether caffeine exposure enhances nicotine-seeking behavior.

Objective—The present study examined the effects of caffeine on nicotine-seeking in rats trained to self-administer nicotine with and without pre-session administration of caffeine.

Methods—Male Sprague–Dawley rats were trained to intravenously self-administer nicotine (0.03 mg/kg/infusion, freebase) on a fixed ratio 5 schedule of reinforcement and associate a stimulus cue with each nicotine administration. Five minutes before the sessions, the rats received an intraperitoneal administration of caffeine (5 mg/kg). Extinction tests were conducted under four conditions: pre-session caffeine administration, response-contingent presentation of nicotine cues, neither condition, or both conditions. Reinstatement tests were conducted after responding was extinguished by withholding pre-session caffeine, nicotine, and its cues. A separate group of rats trained without pre-session caffeine exposure was also subjected to the reinstatement tests.

Results—In the rats trained with pre-session caffeine exposure, continued caffeine administration sustained nicotine-seeking responses and interacted with nicotine cues to significantly delay the extinction of nicotine-seeking behavior. Readministration of caffeine after extinction effectively reinstated nicotine-seeking behavior. In caffeine-naïve rats, caffeine administration did not reinstate extinguished nicotine-seeking behavior but significantly potentiated the cue-induced reinstatement of nicotine-seeking.

Conclusion—These data demonstrate that caffeine administration sustained and reinstated nicotine-seeking behavior, possibly via its acquired discriminative-stimulus properties predictive of nicotine availability. These findings suggest that smokers who attempt to quit may benefit from stopping caffeine consumption.

Keywords

Caffeine; Conditioned stimuli; Extinction; Nicotine; Nicotine-seeking; Reinstatement; Self-administration

Introduction

Caffeine and nicotine are among the most widely used psychoactive substances. In the USA, most adults (approximate 90%) report regular caffeine use, mostly from coffee (70%), soda (16%), and tea (12%) (Frary et al. 2005). A recently emerging source of caffeine is the energy drink, which contains 50–505 mg caffeine per can or bottle (Aranda and Morlock 2006). The energy drink market has grown exponentially, with approximately 200 new brands launched in the USA during the 1-year period ending July 2007 (Packaged-Facts 2007). This fast-growing energy drink market will expose even more people to caffeine. Moreover, there is still a high prevalence of smoking in the USA, with approximately 22% of adults and 24% of youth current smokers (Centers for Disease Control and Prevention 2008). Importantly, a link has been established between caffeine use and tobacco smoking (Budney et al. 1993; Hettema et al. 1999; Istvan and Matarazzo 1984; Kendler et al. 2007; Kozlowski et al. 1993; Martin et al. 2008; Swan et al. 1996, 1997; Swanson et al. 1994). For example, coffee drinking and tobacco smoking temporally co-vary within individuals (Emurian et al. 1982; Istvan and Matarazzo 1984; Marshall et al. 1980; Nellis et al. 1982), and smoking episodes usually occur more often during the 20 min after drinking a cup of coffee than in the 20 min before (Emurian et al. 1982).

In laboratory research, however, both human and animal studies reported complex interactions between caffeine and nicotine. Concluding that caffeine exposure directly changes nicotine actions or tobacco smoking behavior is difficult. For example, some studies found an enhancing effect of caffeine on nicotine actions (Jessen et al. 2005; Jones and Griffiths 2003; Perkins et al. 1994; Ray et al. 1986; Rose and Behm 1991; Tanda and Goldberg 2000), whereas others reported that caffeine suppresses the effects of nicotine and tobacco smoking (Johnson et al. 2010; Kozlowski 1976; Rose and Behm 1991). Many studies have failed to find a significant effect of caffeine on the reinforcing, subjective, and discriminative effects of nicotine and smoking behavior (Bickel et al. 1992; Blank et al. 2007; Chait and Griffiths 1983; Duka et al. 1998; Marshall et al. 1980; Ossip and Epstein 1981; Perkins et al. 2005; Pritchard et al. 1995). Similarly, animal research has also produced equivocal results. Caffeine has been reported to enhance (Celik et al. 2006; Gasior et al. 2000, 2002; Shoaib et al. 1999; Sudakov et al. 2003), reverse, oppose (Bespalov et al. 1999; Cohen et al. 1991; Mumford et al. 1988; Palmatier and Bevins 2001), or have no effect on the responses to nicotine (Jaszyna et al. 1998; Justinova et al. 2009; Palmatier et al. 2003).

One important aspect of the interactions between caffeine and nicotine has received little experimental attention. Because of the close temporal relationship between smoking and caffeine consumption (i.e., smokers smoke while they consume caffeine or immediately following caffeine intake), an associative learning process could occur. As such, the pharmacological effects of caffeine may acquire the properties of an interoceptive cue (or an occasion setting) for smoking behavior and thereby become predictive of nicotine reinforcement. Caffeine exposure may thus serve as a reminder for nicotine consumption and thereby facilitate nicotine-seeking and trigger relapse to smoking behavior. To test this hypothesis, the present study used a rat model of nicotine self-administration and relapse to nicotine-seeking to examine whether pre-session caffeine administration during nicotine self-administration can affect the extinction and reinstatement of nicotine-seeking behavior. Specifically, during the nicotine self-administration phase, an intraperitoneal administration of caffeine at a dose (5 mg/kg) that does not change nicotine intake was administered 5 min before each session. The effects of pre-session caffeine administration on the extinction of nicotine-seeking behavior were examined by varying the experimental conditions (i.e., all combinations of pre-session caffeine or saline with the presence or absence of nicotine-associated cues) under which the animals underwent extinction. The effects of caffeine vs.

saline exposure during self-administration on the reinstatement of extinguished nicotine-seeking were examined by exposing animals to caffeine alone, nicotine-associated cues alone, and a combination of caffeine and cues during reinstatement testing. The caffeine dose (5 mg/kg) used in this study was selected based on the following facts. (1) Moderate per capita daily intake of caffeine in humans is approximately 280 mg, which is equivalent to 4–5 mg/kg, whereas intake of over 700 mg/day or 10 mg/kg is considered heavy caffeine consumption or sometimes referred to “caffeinism.” (2) Pretreatment with caffeine up to 5 mg/kg produced no change in nicotine self-administration or the discriminative-stimulus effects of nicotine (Perkins et al. 2005). (3) Caffeine at 5 mg/kg in rats did not alter locomotor activity, and the minimum dose that enhanced locomotor activity was 10 mg/kg (Antoniou et al. 1998). (4) Caffeine at 5 mg/kg did not interact with nicotine to alter locomotor activity when the nicotine dose was <1 mg/kg (Celik et al. 2006). In this study, the rats self-administered an average of 0.3–0.6 mg/kg nicotine during the 1-h sessions.

Materials and methods

Subjects

Male Sprague–Dawley rats (Charles River, Portage, MI), weighing 200–225 g upon arrival, were used. The animals were individually housed in a humidity- and temperature-controlled (21–22°C) colony room on a reversed light/dark cycle (lights on 20:00; lights off 8:00) with unlimited access to water. After 1 week of acclimation, the rats were placed on a food restriction regimen (20 g chow/day) throughout the experiments. Training and experimental sessions were conducted during the dark phase at the same time each day (9:00–14:00). All experimental procedures were performed in accordance with the *National Institutes of Health Guide for the Care and Use of Laboratory Animals* and approved by the University of Mississippi Medical Center Institutional Animal Care and Use Committee.

Self-administration apparatus

Sixteen standard operant conditioning chambers (Med Associates, St. Albans, VT) were used for the self-administration, extinction, and reinstatement tests. These chambers were located inside sound-attenuating cubicles. Each chamber was equipped with two retractable response levers on one side panel, with a 28 V (100 mA) white light above each lever. Between the two levers was a food pellet trough. A red house light and sonalert were located on the top of the same panel. Intravenous nicotine injections were delivered by a drug delivery system with a syringe pump (Med Associates, model PHM100—10 rpm). Experimental events and data collection were automatically controlled by an interfaced computer and software (Med Associates, Med-PC IV).

Food training

The rats were trained to press a lever for food reinforcement to facilitate the learning of operant responding for nicotine self-administration (see below). The food-training sessions began with the introduction of the two levers. Responses on the right lever were rewarded with the delivery of a food pellet (45 mg). Sessions lasted 1 h with a maximum of 45 food pellets available. Once the rats earned all of the 45 food pellets on a fixed ratio (FR) 1 schedule in a single session, the reinforcement schedule was increased to FR5. Successful food training, defined as 45 pellets earned on the FR5 schedule in a single session, was achieved within two to five daily sessions.

Surgery

After food training, the rats underwent intravenous catheter implantation surgery under isoflurane anesthesia (1–3% isoflurane in 95% O₂ and 5% CO₂). The catheters were

constructed of a 15-cm piece of Silastic tubing (0.3 mm inner diameter, 0.64 mm outer diameter; Dow Corning Corporation, Midland, MI) attached to a 22-gauge bent cannula. The latter was molded onto a durable polyester mesh with dental cement and became the catheter base. Through an incision on the rat's back, the base was anchored underneath the skin at the level of the scapulae, and the catheter passed subcutaneously to the ventral lower neck region and was inserted into the right jugular vein (3.5 cm). The animals were allowed at least 7 days to recover from surgery. During the recovery period, the catheters were flushed daily with 0.1 ml of sterile saline that contained heparin (30 U/ml) and Timentin (66.7 mg/ml, a combination of ticarcillin disodium, and clavulanate potassium) to maintain catheter patency and prevent infection. Thereafter, the catheters were flushed with heparinized saline before and after the experimental sessions throughout the experiments. On weekends when no experimental session was conducted, the catheters were flushed once daily.

Nicotine self-administration

After recovery from surgery, the rats were trained to intravenously self-administer nicotine (0.03 mg/kg/infusion, freebase). (–)-Nicotine hydrogen tartrate (Sigma-Aldrich, St. Louis, MO) was dissolved in physiological saline, the pH was adjusted to 7.0 ± 0.4 with 1 N sodium hydroxide solution (Sigma-Aldrich, St. Louis, MO), and the solution was sterilized by filtration through a 0.22- μ m syringe filter (Fisher Scientific, Pittsburgh, PA). During the training sessions, the animals were placed in the operant conditioning chambers and connected to the drug delivery system. The daily 1-h sessions were initiated by introducing the two levers and illuminating the red house light. Once the FR requirement on the active (right) lever was met, an infusion of nicotine was dispensed by the drug delivery system in a volume of 0.1 ml in approximately 1 s depending on the body weight of the rats. Each nicotine infusion was paired with the presentation of an auditory/visual stimulus that consisted of a 5-s tone and 20-s illumination of the lever light. The latter signaled a 20-s timeout period, during which time responses were recorded but not reinforced. Responses on the inactive lever had no consequence. An FR1 schedule was used for the first 5 days, an FR2 was used for days 6–8, and an FR5 was used for the remainder of the experiments. The rats received a total of 20 daily training sessions.

One group of rats ($n=28$) received an intraperitoneal administration of caffeine 5 min before each self-administration session, and another group of rats ($n=8$) received saline. Anhydrous caffeine (Sigma-Aldrich, St. Louis, MO) was dissolved in physiological saline and administered at a dose of 5 mg/kg in a volume of 1 ml/kg.

Extinction

The extinction tests were performed after the completion of the self-administration/conditioning phase. The rats were subjected to daily extinction sessions, during which saline was substituted for nicotine. The FR5 schedule and 20-s timeout period were still in effect for the saline infusions. All of the rats received ten extinction test sessions because our previous studies showed that nicotine-maintained responding was typically extinguished within seven to ten sessions (Liu 2010; Liu et al. 2008). Pre-session caffeine administration or response-contingent presentations of the nicotine-associated cues were scheduled depending on the different experimental conditions described in detail in experiment 1 below.

For the reinstatement tests described below, the extinction sessions were conducted without pre-session caffeine administration, nicotine delivery, or nicotine cue presentation. The criterion for extinction was three consecutive sessions in which the number of active lever responses per session was $\leq 20\%$ of the average across the last three self-administration sessions, which could be achieved within seven to ten sessions (Liu 2010; Liu et al. 2008).

Reinstatement tests

The reinstatement tests began 1 day after the final (tenth) extinction session. In these test sessions, responses on the active lever resulted in a saline infusion on an FR5 schedule. Responses on the inactive lever had no consequence. Pre-session caffeine or saline administration or presentation of the nicotine-associated cues was scheduled depending on the different experimental conditions described in details in experiments 2 and 3 below. The test sessions lasted 1 h.

Experiment 1 Effects of caffeine, nicotine cues, and their combination on the extinction of nicotine-seeking behavior in rats that received pre-session caffeine during nicotine self-administration (caffeine-experienced rats)

Rats used in this experiment were subjected to pre-session caffeine administration throughout the nicotine self-administration phase. The rats were then divided into four groups ($n=6-8$) for the extinction tests. In the extinction test sessions, responses on the active lever on the FR5 schedule resulted in the delivery of saline instead of nicotine infusions. The extinction sessions were conducted under four conditions: (1) pre-session saline + no cues, (2) pre-session caffeine + no cues, (3) pre-session saline + cues, and (4) pre-session caffeine + cues.

Experiment 2a Effects of caffeine, nicotine cues, and their combination on the reinstatement of extinguished nicotine-seeking in rats that received pre-session caffeine during nicotine self-administration (caffeine-experienced rats)

Rats ($n=7$) from group 1 of experiment 1 were used for these reinstatement tests. After the completion of extinction (under pre-session saline + no cues), three reinstatement test sessions were conducted in the following order: (1) with the pre-session caffeine but not nicotine cues, (2) without pre-session caffeine but with response-contingent nicotine cues, and (3) with both pre-session caffeine and nicotine cues. Two extinction sessions were inserted between the reinstatement tests to maintain the extinction baseline before each test session.

Experiment 2b Effects of caffeine, nicotine cues, and their combination on the reinstatement of extinguished nicotine-seeking in rats that received pre-session saline during nicotine self-administration (caffeine-naïve rats)

The rats ($n=8$) used for this experiment received pre-session intraperitoneal administration of saline instead of caffeine throughout the nicotine self-administration phase. After the completion of the ten daily extinction sessions conducted under the pre-session saline + no cues condition, the reinstatement test sessions began exactly as described for experiment 2a. There were three reinstatement sessions: (1) pre-session caffeine + no cues, (2) pre-session saline + cues, and (3) pre-session caffeine + cues. Two extinction sessions were performed before each reinstatement test to maintain the extinction baseline.

Statistical analyses

The data are expressed as the mean $\pm$ SEM number of lever responses and nicotine infusions earned. The active lever response data collected from the self-administration phase and extinction tests were separately analyzed using repeated-measures analysis of variance (ANOVA), with group (caffeine vs. saline during self-administration and test conditions in extinction) as the between-subjects factor and sessions as the within-subject factor. Similar repeated-measures ANOVAs were applied to the inactive lever response data. In the reinstatement tests, extinction baseline data were averaged across the two extinction sessions before each reinstatement test session. The reinstatement test data were analyzed using one-way ANOVA, followed by Fisher's protected least significant difference (PLSD) post hoc test to verify differences among individual means.

Results

Nicotine self-administration

In 20 daily 1-h self-administration training sessions, stable nicotine self-administration was established regardless of pre-session administration. Pre-session administration of caffeine at 5 mg/kg did not alter nicotine self-administration behavior (Fig. 1). Averaged across the final three sessions (sessions 18, 19, and 20), the rats trained with pre-session caffeine administration emitted a mean $\pm$ SEM number of responses of 77.6 $\pm$ 4.9 on the active lever and 17.3 $\pm$ 2.6 on the inactive lever. These rats earned 13.2 $\pm$ 0.9 infusions of nicotine. Similarly, the rats trained with pre-session saline administration made a mean $\pm$ SEM number of responses of 78.1 $\pm$ 5.2 on the active lever and 20.5 $\pm$ 4.8 on the inactive lever. These caffeine-naïve rats earned 13.0 $\pm$ 0.9 infusions of nicotine. A repeated-measures ANOVA of the number of active lever responses in the last three sessions revealed no significant effect of group [caffeine vs. saline, $F(1,34)=0.58$, $p=0.45$] and sessions [$F(2,68)=0.03$, $p=0.97$] and no significant group $\times$ session interaction [$F(2,68)=0.19$, $p=0.31$]. A similar analysis of the nicotine infusions and inactive lever responses in the last three sessions produced similar negative results (data not shown).

For the extinction tests, rats with pre-session caffeine treatment were divided into four groups ($n=6-8$ per group) in a pseudorandom manner. No difference was found among groups in the number of responses on the active lever [$F(3,24)=0.08$, $p=0.97$] and inactive lever [$F(3,24)=0.35$, $p=0.78$] and nicotine infusions [$F(3,24)=0.02$, $p=0.997$]. Detailed data are presented in Table 1.

Effects of caffeine, nicotine cues, and their combination on the extinction of nicotine-seeking behavior in rats that received pre-session caffeine during nicotine self-administration (caffeine-experienced rats)—Figure 2 shows the profiles of responses on the active lever during the ten daily extinction sessions. Although all animals showed a gradual decrease in responses on the active lever across sessions, significantly distinct profiles of lever-press responding were found between the different groups. A two-way repeated-measures ANOVA revealed a significant effect of group [$F(3,24)=3.38$, $p<0.05$] and session [$F(9,216)=32.52$, $p=0.0001$] but no significant group $\times$ session interaction [$F(27,216)=0.95$, $p=0.54$]. Further one-way ANOVA of the active lever responses averaged across the final three sessions showed a significant effect of group [$F(3,24)=3.46$, $p<0.05$], and subsequent Fisher's PLSD post hoc tests confirmed that the number of responses in the pre-session caffeine + no cues condition ($p<0.05$), pre-session saline + cues condition ($p<0.05$), and caffeine + cues condition ($p<0.001$) was significantly higher than in the pre-session saline + no cues condition. Although rats in the caffeine + cues group showed a higher level of nicotine-seeking compared with either the pre-session caffeine + no cues and pre-session saline + cues groups, the difference in the number of active lever responses failed to reach statistical significance ($p>0.05$). Throughout the extinction sessions, the inactive lever responses remained low, with no significant differences among groups [$F(3,24)=0.94$, $p=0.45$] or changes across sessions [$F(9,216)=1.15$, $p=0.13$]. These data demonstrate that continued pre-session caffeine exposure, response-contingent presentation of nicotine cues, and their combination significantly sustained responding on the active, previously nicotine-reinforced lever, delaying the extinction of nicotine-seeking behavior.

Effects of caffeine, nicotine cues, and their combination on the reinstatement of extinguished nicotine-seeking in rats that received pre-session caffeine during nicotine self-administration (caffeine-experienced rats)—As shown in Fig. 3, in the reinstatement test sessions, pre-session caffeine, response-contingent cue

presentation, and the combination of caffeine and cues effectively reinstated the extinguished responding on the active, previously nicotine-reinforced lever in rats ($n=7$) that received pre-session caffeine administration during nicotine self-administration. One-way ANOVA of the active lever responses revealed a significant effect of test condition [$F(3,24)=3.33, p<0.05$]. Subsequent Fisher's PLSD post hoc tests confirmed significant higher responses under the pre-session caffeine + no cues condition ($p<0.05$), pre-session saline + cues condition ($p<0.05$), and caffeine + cues condition ($p<0.01$) compared with extinction. No difference ($p>0.05$) was found in the magnitudes of response reinstatement among the three test conditions. Responses on the inactive lever remained low and indistinguishable from extinction baseline levels.

Effects of caffeine, nicotine cues, and their combination on the reinstatement of extinguished nicotine-seeking in rats that received pre-session saline during nicotine self-administration (caffeine-naïve rats)—Figure 4 shows the lever responses during the reinstatement tests conducted in rats ($n=8$) that received pre-session saline rather than caffeine in the nicotine self-administration phase. As opposed to the response-reinstating effect of caffeine observed in caffeine-experienced rats, pre-session caffeine administration (pre-session caffeine + no cues) in these caffeine-naïve rats did not reinstate extinguished nicotine-seeking behavior. However, as expected, representation of the nicotine cues (pre-session saline + cues) effectively reinstated active lever responses. Interestingly, pre-session caffeine significantly enhanced the effect of nicotine cues in the pre-session caffeine + cues condition, leading to a higher level of nicotine-seeking. One-way ANOVA of the active lever responses revealed a significant effect of test condition [$F(3,28)=11.09, p=0.001$]. Subsequent Fisher's PLSD post hoc tests confirmed a significant difference between the pre-session saline + cues condition ($p<0.05$) and pre-session caffeine + cues condition ($p<0.0001$) and the extinction condition and a significant difference between the caffeine + cues condition compared with both the pre-session caffeine + no cues condition ($p<0.0001$) and pre-session saline + cues condition ($p<0.05$). However, responses on the inactive lever remained at low levels that were indistinguishable among conditions.

Discussion

To our knowledge, this is the first report that investigated the effect of caffeine administration on nicotine-seeking behavior in extinction and reinstatement procedures. Several main findings were obtained. In the rats that had been trained to self-administer nicotine with pre-session caffeine administration, continued pre-session caffeine administration, especially together with nicotine-associated cues, sustained nicotine-seeking responding in the extinction sessions. After lever responding was extinguished by withholding pre-session caffeine administration, nicotine infusions (saline substitution), and its associated cue presentation, readministration of pre-session caffeine effectively reinstated nicotine-seeking behavior. In the rats that had been trained to self-administer nicotine without pre-session caffeine administration (saline control or caffeine-naïve group), caffeine administration did not reinstate nicotine-seeking responses but significantly enhanced the response-reinstating effect of nicotine cues.

To properly interpret the results, the issue of whether the effects of caffeine observed in this study resulted from general arousal or locomotor activation by caffeine needs to be addressed. Caffeine is a psychomotor stimulant that produces locomotor activation (Bedingfield et al. 1998; Garrett and Holtzman 1994). As such, a convenient explanation for the present data is that the ability of caffeine to increase nicotine-seeking responding and enhance the response-reinstating effect of nicotine cues may be attributable to general arousal/motor activation by caffeine. However, such an explanation is unlikely for the following reasons. First, pre-session caffeine administration did not alter nicotine self-

administering behavior. The lack of effect of caffeine on nicotine self-administration is consistent with human studies that showed that pretreatment with caffeine up to 5 mg/kg did not change the subjective effects and self-administration of nicotine (Perkins et al. 2005). Second, caffeine in rats trained without pre-session caffeine administration failed to reinstate nicotine-seeking behavior. Third, in all tests, caffeine did not change responding on the inactive lever. Fourth, caffeine at the dose (5 mg/kg) used in this study did not stimulate locomotor activity in a variety of behavior test paradigms (Antoniou et al. 1998; Cohen et al. 1991; Garrett and Holtzman 1994; Jaszyna et al. 1998). Therefore, the effects of caffeine observed in this study were not attributable to nonspecific psychomotor stimulation but rather specific for goal-directed lever-pressing behavior.

In the extinction tests conducted in the rats that had been trained to self-administer nicotine with pre-session administration of caffeine, continued pre-session caffeine administration sustained responses on the active, previously nicotine-reinforced lever, delaying the extinction of nicotine-seeking behavior. The ability of caffeine to support nicotine-seeking responding might be attributable to the discriminative-stimulus effect of caffeine. Arguably, pre-session caffeine administration in the nicotine self-administration training phase produced a specific interoceptive state that reliably predicted the availability of nicotine. Caffeine might have served to set an "occasion" under which the operant conditioning chamber context signaled nicotine availability. This argument is consistent with previous observations that showed that the discriminative-stimulus effect of a drug could be obtained without an explicit stimulus indicative of non-reinforcement in pigeons (Schaal et al. 1996) and that amphetamine could acquire the discriminative-stimulus effect in a food self-administration procedure in rats (Odum and Shahan 2004). Therefore, continued pre-session caffeine administration maintained a higher level of active lever responses compared with the extinction group without pre-session caffeine, delaying the extinction of nicotine-seeking behavior. These findings suggest that in caffeine-consuming smokers who try to quit smoking, the continued consumption of caffeine may be a risk factor for undiminished craving for tobacco. Therefore, caffeine-consuming smokers may benefit from simultaneous abstinence from caffeinated drinks during their attempt to quit smoking.

In the reinstatement tests conducted in the rats trained to self-administer nicotine with pre-session caffeine administration and after lever responding was extinguished by withholding pre-session caffeine, nicotine delivery, and its associated cues, readministration of pre-session caffeine effectively reinstated extinguished nicotine-seeking behavior. Pre-session caffeine is postulated to reinstate nicotine-seeking responses via its discriminative-stimulus properties. Such an occasion-setting effect of caffeine would have been more consolidated after extinction. During the extinction (i.e., saline substitution) sessions in which nicotine delivery and its associated cues were withheld, the chamber context in the absence of caffeine set an occasion for the lack of nicotine availability. As such, the self-administration and extinction sequence, albeit unlike standard discrimination training procedures, established pre-session caffeine as a discriminative stimulus. This is consistent with previous studies in which discriminative stimuli effectively reinstated extinguished drug-seeking behavior in animals trained to self-administer cocaine, heroin, ethanol, nicotine, and sucrose (Alvarez-Jaimes et al. 2008; Burbassi and Cervo 2008; Ciccocioppo et al. 2001; Gracy et al. 2000; Katner et al. 1999; Wing and Shoaib 2008). Notably, the response-reinstating effect of caffeine on nicotine-seeking is consistent with a human prospective (for up to 35 years) study (Krall et al. 2002) in which caffeine consumption significantly increased the risk of smoking relapse. Altogether, these results suggest that in clinical smoking cue extinction therapy for caffeine-consuming smokers, caffeine exposure should be included in the cue constellation that needs to be extinguished.

The finding that acute caffeine challenge did not reinstate extinguished nicotine-seeking responses in caffeine-naïve rats is in contrast with the results obtained from cocaine-trained rats in which a caffeine priming injection reinstated cocaine-seeking behavior in rodents (Green and Schenk 2002; Kuzmin et al. 1999; Schenk and Partridge 1999; Schenk et al. 1996; Worley et al. 1994). Two factors may underlie this discrepancy. The first is the different classes of drugs used (i.e., nicotine and cocaine). The second is the substantial difference in the test procedures because the previous studies tested the effect of caffeine using a so-called within-session paradigm in which the extinction and reinstatement tests were conducted in a single session. However, the issue of whether acute caffeine exposure produces differential effects in the abstinent subjects that used to take cocaine or nicotine deserves future investigation.

Interestingly, pre-session caffeine did not enhance the response-reinstating effect of nicotine cues in the caffeine-experienced vs. caffeine-naïve rats. The lack of interactive effects of pre-session caffeine as a discriminative stimulus and the discrete nicotine-conditioned cues is in contrast with the results obtained in rats trained to self-administer other drugs of abuse (e.g., cocaine and ethanol) or a natural reward (e.g., sucrose) (Bossert et al. 2006; Katner et al. 1999; Tsiang and Janak 2006). The discrepancy may be attributable to two possibilities. First, the nature of the employed discriminative stimuli was different, in which the discriminative stimuli used in the previous reports were contextual or olfactory stimuli, whereas the present study used a caffeine-induced interoceptive state to create a discriminative stimulus. Although not the focus of the present study, the issue of whether a pharmacological state can serve as a discriminative stimulus in exactly the same manner as non-pharmacological, sensory stimuli needs to be addressed in future research. Second, tolerance to the conditioned reinforcement (cue)-enhancing effect of caffeine may have developed after chronic administration during the self-administration training phase so that caffeine failed to facilitate the response-reinstating effect of nicotine cues. Such an interpretation is supported by both human and animal studies in which daily consumption of caffeine resulted in the development of tolerance to many of caffeine's effects (Holtzman and Finn 1988; Jaszyna et al. 1998; Robertson et al. 1981). After chronic exposure, caffeine failed to generalize to the discriminative-stimulus effects of nicotine (Justinova et al. 2009). In caffeine-naïve rats in the present study, pre-session caffeine administration significantly enhanced the response-reinstating effect of nicotine cues. Nonetheless, the lack of additive effect of caffeine and cues in the reinstatement tests in the rats trained with pre-session caffeine is similar to our recent data in which combined exposure to pre-session nicotine administration and nicotine cues reinstated nicotine-seeking responses to a level similar to that elicited by nicotine cues alone (Liu 2010). In the latter case, the self-administered nicotine acquired discriminative-stimulus properties that predicted the availability of further nicotine deliveries. Similar to caffeine, the conditioned reinforcement (cue)-enhancing effect of nicotine diminished after chronic exposure because of the development of tolerance. Moreover, such an account is supported by a previous report in which pre-exposure to caffeine for 30 days did not change the nicotine-conditioned hyperactivity in rats (Palmatier et al. 2003).

In summary, the present study demonstrated that continued caffeine exposure sustained and reinstated nicotine-seeking behavior in rats that had been trained to self-administer nicotine with pre-session caffeine administration. The motivational effect of pre-session caffeine may be attributable to the discriminative-stimulus properties of caffeine that were predictive of nicotine availability. Moreover, caffeine significantly potentiated the response-reinstating effect of nicotine-associated cues in rats without prior caffeine exposure. These results suggest that smokers may benefit from stopping caffeine consumption in their attempts to quit smoking.

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Fig. 1.

Lever responses in the final three sessions of the nicotine self-administration phase in rats that received an intraperitoneal administration of 5 mg/kg caffeine ($n=28$) or saline ($n=8$) 5 min before the sessions. The data are expressed as mean $\pm$ SEM responses

Fig. 2.

Extinction of active lever responding (nicotine-seeking behavior) under different test conditions in rats ($n=6-8$) that received pre-session caffeine (5 mg/kg, i.p.) in the nicotine self-administration phase. *SA* indicates lever responses averaged across the final three self-administration sessions. *Saline/-* represents the pre-session saline + no cues condition. *Caffeine/-* represents the pre-session caffeine + no cues condition. *Saline/cue* represents the pre-session saline + cues condition. *Caffeine/cue* represents the pre-session caffeine + cues condition. The data are expressed as mean $\pm$ SEM responses. * $p < 0.05$, significant main effect of group

Fig. 3.

Lever responses in the reinstatement tests in rats ($n=7$) that received pre-session caffeine (5 mg/kg) in the nicotine self-administration phase. *Caffeine* represents the pre-session caffeine + no cues condition. *Cue* represents the pre-session saline + cues condition. *Caffeine/cue* represents the pre-session caffeine + cues condition. The data are expressed as mean $\pm$ SEM lever responses. * $p < 0.05$, ** $p < 0.01$, significant difference from extinction baseline

Fig. 4.

Lever responses in the reinstatement tests in rats ($n=8$) that received pre-session saline in the nicotine self-administration phase. *Caffeine* represents the pre-session caffeine + no cues condition. *Cue* represents the pre-session saline + cues condition. *Caffeine/cue* represents the pre-session caffeine + cues condition. The data are expressed as mean $\pm$ SEM lever responses. * $p < 0.05$, *** $p < 0.0001$, significant difference from extinction and caffeine conditions; + $p < 0.05$, significant difference from cue condition

Table 1

Similar profiles of caffeine-experienced rats before extinction test

Extinction groups	Number of responses		Nicotine infusions	Body weight (g)
	Active lever	Inactive lever		
Saline/–	80.8±10.6	16.0±5.9	13.7±1.8	365±25
Caffeine/–	77.7±15.3	15.5±5.5	13.4±2.8	348±30
Saline/cue	73.3±8.8	14.9±5.8	13.1±1.7	359±21
Caffeine/cue	78.3±6.7	19.3±3.0	13.2±1.0	369±35

These rats received pre-session administration of caffeine (5 mg/kg, i.p.) in the nicotine self-administration training phase. The data were averaged across the final three self-administration sessions (days 18, 19, and 20) and expressed as mean±SEM