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Effects of chronic caffeine pre-exposure on conditioned and unconditioned psychomotor activity induced by nicotine and amphetamine in rats

M.I. Palmatier^a, E.Y.K. Fung^b and R.A. Bevins^a

Three experiments examined the effects of chronic pre-exposure to caffeine on the subsequent conditioned and unconditioned locomotor activating effects of nicotine or amphetamine in rats. Rats were given daily intraperitoneal injections of caffeine anhydrous (0, 10 or 30 mg/kg base) for 30 days. Conditioning (environment–drug pairings) began after the last day of caffeine pre-exposure. Pre-exposure to 30 mg/kg of caffeine enhanced the acute and chronic locomotor effects of amphetamine (0.5 mg/kg). A similar enhancement of activity was not seen with the high (0.421 mg/kg base) or low dose (0.175 mg/kg) of nicotine. In a drug-free test, the distinct environment paired with amphetamine and the high dose of nicotine evoked increases in activity relative to controls. Caffeine pre-exposure did not affect expression of this conditioned hyperactivity. These effects of caffeine pre-exposure on amphetamine-induced activity could not be attributed to

non-specific effects of caffeine. *Behavioural Pharmacology* 14:191–198 © 2003 Lippincott Williams & Wilkins.

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Introduction

Acute nicotine challenge is characterized by depressed motor activity relative to saline-injected controls (0.210–0.421 mg/kg, Stolerman *et al.*, 1973, 1995; Bevins *et al.*, 2001). Tolerance to these locomotor depressant effects is observed by the second injection (Stolerman *et al.*, 1973; Clark and Kumar, 1983). This locomotor suppression tends to be replaced with hyperactivity after repeated daily exposure to nicotine (0.210–0.421 mg/kg; Clark and Kumar, 1983; Kita *et al.*, 1992; Ksir, 1994; Bevins *et al.*, 2001). Rats also show heightened activity relative to controls with repeated administration of amphetamine (e.g. Schoffelmeier *et al.*, 2002). In contrast to nicotine, amphetamine doses typically used to study behavioral activation do not induce an initial suppression of locomotor activity (Browman *et al.*, 1998; Fraioli *et al.*, 1999; 0.125–1.0 mg/kg in our laboratory, see later). In fact, the stimulant effects of amphetamine can be seen on the first administration (e.g. Browman *et al.*, 1998).

Conditioning to environmental cues reliably associated with the drug can contribute to the locomotor activating effects of nicotine and amphetamine (Vezina *et al.*, 1989; Reid *et al.*, 1996, 1998; Fraioli *et al.*, 1999; Bevins *et al.*, 2001). From this perspective, the effects of the drug on the nervous system are the unconditioned stimuli (USs), while the resulting behavioral changes (e.g. locomotor

hyperactivity) are the unconditioned responses (URs). When these unconditioned drug effects reliably occur in the presence of environmental cues (i.e. conditioned stimuli or CSs), the drug effects and cues can become associated via Pavlovian conditioning processes. For example, in our laboratory, nicotine-conditioned hyperactivity is evidenced as more activity relative to controls in the absence of nicotine but in the presence of environmental cues (context CS) that have been repeatedly paired with nicotine (Bevins *et al.*, 2001; Palmatier and Bevins, 2002; see Reid *et al.*, 1998 for an alternative testing protocol).

Chronic exposure to one drug might promote increased behavioral sensitivity to another drug (e.g. Vezina *et al.*, 1989; Liguori *et al.*, 1997; Fenu *et al.*, 2000; Beyer *et al.*, 2001; Lamarque *et al.*, 2001; Pontieri *et al.*, 2001; Cauli and Morelli, 2002). In one such study, repeated daily exposure to the dopamine (DA) D₂ receptor subtype agonist bromocriptine induced hyperactivity when rats were later challenged with the non-specific adenosine antagonist caffeine or the adenosine A_{2A} receptor subtype antagonist SCH 58261, regardless of test environment (Fenu *et al.*, 2000). Of particular interest in the present report is the potential for ‘cross-sensitization’ between caffeine and other drugs of abuse. Caffeine is prevalent in a wide variety of foods, beverages, and over-the-counter

medications. Fredholm *et al.* (1999) estimate that children aged 7–10 years ingest approximately 0.5–1.8 mg/kg caffeine/day, primarily from soft drinks and chocolate products. To our knowledge, longitudinal assessment of caffeine as a risk factor for susceptibility to later drug use has not been investigated in humans. However, some studies have identified a relationship between caffeine consumption and use of alcohol or other abused drugs (e.g. Istvan and Matarazzo, 1984; Brown and Benowitz, 1989; Kozłowski *et al.*, 1993). Further, caffeine intake is a potential risk factor for relapse to tobacco use (e.g. Cummings *et al.*, 1985; Krall *et al.*, 2002).

Recent studies using animal models have confirmed these purported observations of cross-sensitization. Caffeine pre-exposure can enhance the reinforcing (Shoab *et al.*, 1999) and discriminative (Gasior *et al.*, 2000, 2002) effects of nicotine, as well as the acute psychomotor effects of nicotine, cocaine and amphetamine (Gasior *et al.*, 2000). The purpose of the present research was to test the effects of chronic caffeine pre-exposure on the subsequent acute and chronic unconditioned locomotor activity induced by nicotine and amphetamine in rats. Although caffeine pre-exposure might enhance the acute ambulatory effects of amphetamine and nicotine (e.g. Gasior *et al.*, 2000), its chronic effects are unclear. Also, chronic drug exposure might be deleterious to associations formed between drugs and other cues (e.g. Cappel and Poulos, 1979; Iwamoto and Williamson, 1984; Kunin *et al.*, 1999, 2000; Palmatier and Bevins, 2001; but see Shoab *et al.*, 1994; Bevins and Palmatier, 2003). Thus, drug (US) pre-exposure in a Pavlovian drug-conditioning situation might facilitate or retard acquisition of conditioned associations. Accordingly, we also assessed how chronic pre-exposure to caffeine affects the acquired conditioned association between the context CS and the psychomotor effects of nicotine or amphetamine (i.e. context-dependent hyperactivity).

Method

Subjects

One hundred and fifteen naive male Sprague–Dawley rats from Harlan Industries (Indianapolis, Indiana, USA) were housed individually in hanging wire-mesh cages. The rats weighed 150–224 g on arrival. Food and water were continuously available in the home cage. The colony was maintained on a 12:12 light/dark cycle; all procedures were conducted in the light portion of the cycle. Each rat was handled for approximately 2 min daily for 3 days prior to the start of the experiment.

Apparatus

All pre-exposure injections took place in the home cage. Conditioning was conducted in eight cylindrical PVC activity chambers measuring 30.5 cm in diameter. Each chamber was fitted with two infrared emitter/detector

units located 4 cm above the wire-mesh floor that divided the chamber into four equal sections. Infrared beam-breaks were recorded automatically by a personal computer and served as the measure of activity.

Experiment 1A

This experiment sought to examine the effects of chronic caffeine pre-exposure on the unconditioned and conditioned psychomotor effects of 0.421 mg/kg nicotine. In our laboratory, this dose of nicotine produces robust conditioned hyperactivity on a drug-free test for conditioning (Bevins *et al.*, 2001; Palmatier and Bevins, 2002).

Procedure

Caffeine pre-exposure. At the start of each experiment, rats were randomly assigned ($n = 20$ per dose) to one of three doses of caffeine (0, 10 or 30 mg/kg). Each rat was injected i.p. with its assigned dose of caffeine once daily for 30 days. Injections occurred in the afternoon (16.30 ± 1 h).

Context-nicotine exposure (conditioning). Rats from each caffeine dose were randomly assigned to one of two conditions (Paired or Unpaired; $n = 10$ per condition). Nicotine conditioning began approximately 17 h after the last caffeine pre-exposure injection (day 31). Paired rats were injected with 0.421 mg/kg nicotine s.c. immediately before placement in the activity chambers. Unpaired rats were injected s.c. with saline immediately before placement. Conditioning continued once daily for 10 days. Locomotor activity (infrared beam-breaks) was recorded for the entire 30-min conditioning trial. To equate drug exposure, approximately 6 h after each conditioning trial rats assigned to the Unpaired conditions were given s.c. injections of nicotine in the home cage; Paired rats were injected with saline. This temporal separation produces no evidence of conditioned excitation or inhibition in the Unpaired condition. In fact, this protocol results in an activity profile in the Unpaired condition that is similar to that of rats never exposed to nicotine (see Experiment 2 of Bevins *et al.*, 2001).

Drug-free test for conditioning. Twenty-four hours after the last conditioning trial, rats were given a drug-free test. Prior to placement in the activity chambers for the 30-min test, all rats were injected s.c. with saline.

Experiment 1B

Experiment 1B assessed the effects of caffeine pre-exposure on the subsequent unconditioned and conditioned effects of a lower dose of nicotine (0.175 mg/kg) that has weak locomotor suppressing and activating effects and does not support conditioning (Bevins *et al.*, 2001). Using a lower nicotine dose might reveal unconditioned and conditioned effects of caffeine

pre-exposure potentially masked by a higher nicotine dose (i.e. ceiling effect).

Procedure

All experimental procedures were identical to Experiment 1A, except as follows. Based on findings from Experiment 1A (see later), only the 0 and 30 mg/kg caffeine pre-exposure doses were included in Experiment 1B. Also, the nicotine dose was reduced to 0.175 mg/kg. For each caffeine pre-exposure dose (0 or 30 mg/kg), eight rats received nicotine immediately before placement in the activity chambers (Paired) and seven rats received nicotine in the home cage (Unpaired).

Experiment 2

Experiment 2 assessed the generality of the results of Experiment 1A by examining the effects of chronic caffeine exposure on the later psychomotor effects of another stimulant (i.e. amphetamine). Given the diversity of locomotor conditioning protocols with amphetamine (e.g. Stewart et al., 1994; Ahmed et al., 1996; Bespelov and Zvartau, 1996; Arvanitogiannis et al., 2000; Sutton et al., 2000), we conducted a pilot study to examine amphetamine (0.125, 0.25, 0.5 and 1.0 mg/kg), using the conditioning protocol described for nicotine. Mean activity counts for the drug-free test for conditioning (11th session) are presented in Table 1. Based on these results, we selected the 0.5 mg/kg amphetamine dose for Experiment 2 because it supported robust conditioned hyperactivity after 10 trials (cf. the 0.421 mg/kg nicotine dose, Experiment 1A).

Procedure

Caffeine pre-exposure. Pre-exposure was similar to that used in Experiment 1B. During this phase, one animal was removed due to experimenter error. Thus, analyses included 20 rats in the 0 mg/kg condition and 19 rats in the 30 mg/kg condition.

Table 1 Experimental conditions and mean (± 1 SEM) activity counts on the drug-free test for activity (11th session) of the pilot experiment

Amphetamine dose	Group	Mean (± 1 SEM) activity counts
0.125 mg/kg	Paired ($n=8$)	447.60 (43.42)
	Unpaired ($n=8$)	328.90 (21.72)
0.25 mg/kg	Paired ($n=8$)	527.40 (63.36)
	Unpaired ($n=8$)	275.90 (27.20)
0.5 mg/kg	Paired ($n=8$)	591.40 (44.33)
	Unpaired ($n=8$)	313.30 (23.62)
1.0 mg/kg	Paired ($n=8$)	498.80 (31.40)
	Unpaired ($n=7$)	318.90 (39.91)

Using a two-way ANOVA, the main effect of amphetamine dose [$F(3,55)=1.13$, $P=0.34$] and the Group \times Dose interaction [$F(3,55)=1.91$, $P=0.14$] were not significant. However, there was a significant main effect of Group [$F(1,55)=62.17$, $P<0.001$], indicating that Paired rats displayed conditioned hyperactivity relative to Unpaired controls regardless of conditioning dose.

Context-amphetamine exposure (conditioning). Unless mentioned, the procedural details for context-amphetamine exposure were identical to those in Experiments 1A and 1B. Paired groups ($n=10$ per pre-exposure condition) received 0.5 mg/kg amphetamine i.p. immediately before 30-min exposure to the activity chambers; i.p. saline was administered in the home cage approximately 6 h later. Unpaired (0 mg/kg caffeine, $n=10$; 30 mg/kg caffeine, $n=9$) groups received similar treatment, except that saline was administered before placement in the activity chambers and amphetamine was given in the home cage.

Drug-free test for conditioning. The 30-min drug-free test was similar to that of Experiments 1A and 1B, except that saline was injected i.p.

Drugs

Amphetamine hydrochloride and caffeine anhydrous (Sigma, St. Louis, Missouri, USA) were dissolved in saline (0.9% NaCl) and injected i.p. at a volume of 2 ml/kg for caffeine and 1 ml/kg for amphetamine. (-)-Nicotine hydrogen tartrate salt (Sigma) was dissolved in saline and brought to a pH of 7.0 ± 0.2 with a dilute NaOH solution. Nicotine was injected s.c. at a volume of 1 ml/kg. Doses of caffeine and amphetamine are expressed as the salt form; nicotine doses are expressed as the base form.

Data analyses

Analyses of variance (ANOVAs) were used for overall comparisons. For example, omnibus ANOVAs including one within-subjects factor, Trial, and two between-subjects factors, Group (Paired or Unpaired) and Dose (e.g. 0, 10 or 30 mg/kg caffeine), were conducted as tests for caffeine pre-exposure effects on nicotine- or amphetamine-induced activity. Further, to assess the non-specific effects of caffeine exposure on activity, data from Unpaired groups were analyzed separately using Caffeine Dose as the between-subjects factor and Trial as the repeated measure. This maneuver provided a more sensitive test for possible non-specific effects of caffeine exposure on activity. Drug-free tests were analyzed with two-way ANOVAs in which Group and Dose were the between-subject factors. A two-tailed rejection criterion ($P \leq 0.05$) was used for all other analyses.

Results

Experiment 1A

Non-specific effects of caffeine pre-exposure

Caffeine can have anorectic effects. For example, Gans (1984) found that, relative to saline controls, body weight and food consumption were attenuated for rats chronically exposed to caffeine (see also Palmatier and Bevins, 2001). To test this possibility, body weight on the last day of caffeine pre-exposure (day 30) was compared for rats pre-exposed to each dose of caffeine (0, 10 or 30 mg/kg).

There were no significant differences in body weight between caffeine doses [$F(2,57) = 1.75$, NS].

Figure 1A shows locomotor activity across conditioning trials for Unpaired groups pre-exposed to caffeine or saline. There was a significant main effect of Trial [$F(9,243) = 53.59$, $P < 0.001$], denoting that activity decreased over trials. The main effects of Dose and the Dose \times Trial interaction were not significant [$F_s < 1$], indicating that, in our situation, caffeine pre-exposure did not affect later motor activity in a drug-free state.

Nicotine-induced activity (0.421 mg/kg)

Omnibus ANOVA examined nicotine-induced activity for the Unpaired and Paired groups across conditioning trials (Figs 1A, B). There were significant main effects of Trial [$F(9,486) = 10.08$, $P < 0.001$], and of Group [$F(1,54) = 25.48$, $P < 0.001$]. The Group \times Trial interaction was also significant [$F(9,486) = 143.48$, $P < 0.01$], indicating that, as conditioning progressed, activity for Paired rats increased relative to that for Unpaired controls. The main effect of Dose [$F(2,54) = 2.20$, NS] and the Group \times Dose interaction [$F(2,54) = 1.91$, NS], the Dose \times Trial and the Group \times Dose \times Trial interactions [$F_s < 1$] were not significant,

Drug-free test

Data from the drug-free test for conditioning are presented in Fig. 1C. A two-way ANOVA revealed a significant effect of Group [$F(5,54) = 65.79$, $P < 0.0001$], indicating nicotine-conditioned hyperactivity in the Paired rats. The main effect of Dose and the Group \times Dose interaction were not significant [$F_s(2,54) \leq 2.08$, NS].

Experiment 1B

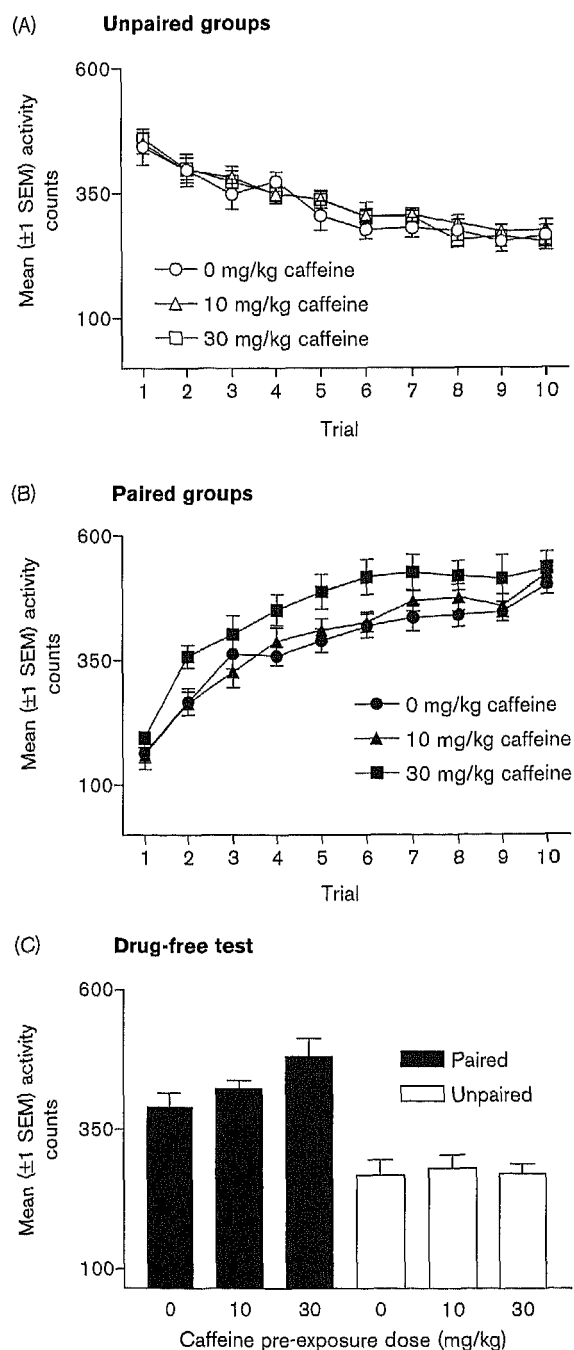
Non-specific effects of caffeine exposure

The body weight of rats on the last day of pre-exposure did not differ significantly across caffeine doses (0 versus 30 mg/kg) [$F(1,28) = 1.57$, NS]. Figure 2A shows mean activity counts for the two Unpaired groups. Analyses revealed a significant main effect of Trial [$F(9,117) = 7.78$, $P < 0.01$], indicating that activity in the Unpaired groups decreased over the 10 conditioning trials. The main effect of Caffeine Dose and the Dose \times Trial interaction were not significant [$F_s < 1$], confirming that chronic pre-exposure to caffeine does not affect motor activity in a non-specific manner.

Nicotine-induced activity (0.175 mg/kg)

Omnibus ANOVA examined activity for the Unpaired and Paired groups across conditioning trials (Figs 2A and 2B, respectively). There was a significant Group \times Trial interaction [$F(9,234) = 19.84$, $P < 0.001$], indicating that over the 10 conditioning trials, activity in Paired groups

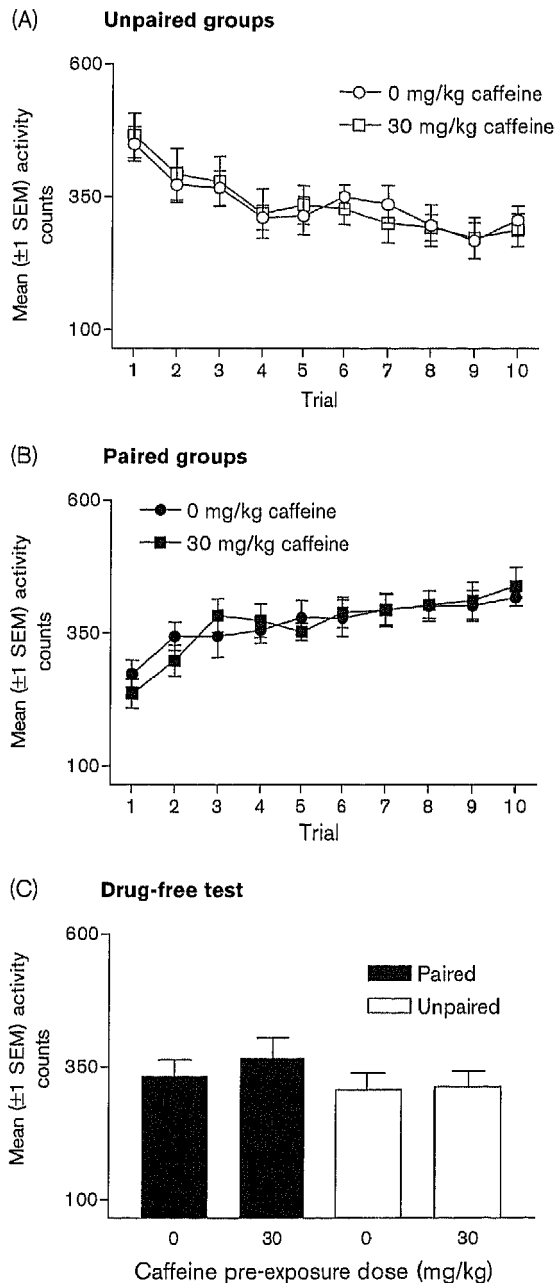
Fig. 1



Locomotor activity for rats treated with 0.421 mg/kg nicotine (Experiment 1A). (A) and (B) contrast caffeine pre-exposure conditions for Unpaired and Paired groups across conditioning trials, respectively. (C) Activity on the drug-free test for conditioning.

increased and activity in Unpaired groups decreased. The main effects of Group, Trial, and Dose were not significant [$F_s \leq 2.84$, NS]. The interactions in which Dose (0 or 30 mg/kg) was a factor were not significant either [$F_s \leq 1.26$, NS].

Fig. 2



Locomotor activity for rats treated with 0.175 mg/kg nicotine (Experiment 1B). (A) and (B) contrast 0 versus 30 mg/kg caffeine pre-exposure for Unpaired and Paired groups, respectively. (C) Activity data from the drug-free test for conditioning.

Drug-free test

Figure 2C shows activity from the drug-free test for conditioning. The two-way ANOVA revealed that the main effect of Group, Dose, and the Dose \times Group interaction were not significant [F 's ≤ 2.15 , NS]. No main effect of Group or Dose \times Group interaction indicates that 0.175 mg/kg nicotine did not support conditioned

hyperactivity in our situation and that caffeine pre-exposure did not alter this outcome.

Experiment 2

Non-specific effects of caffeine exposure

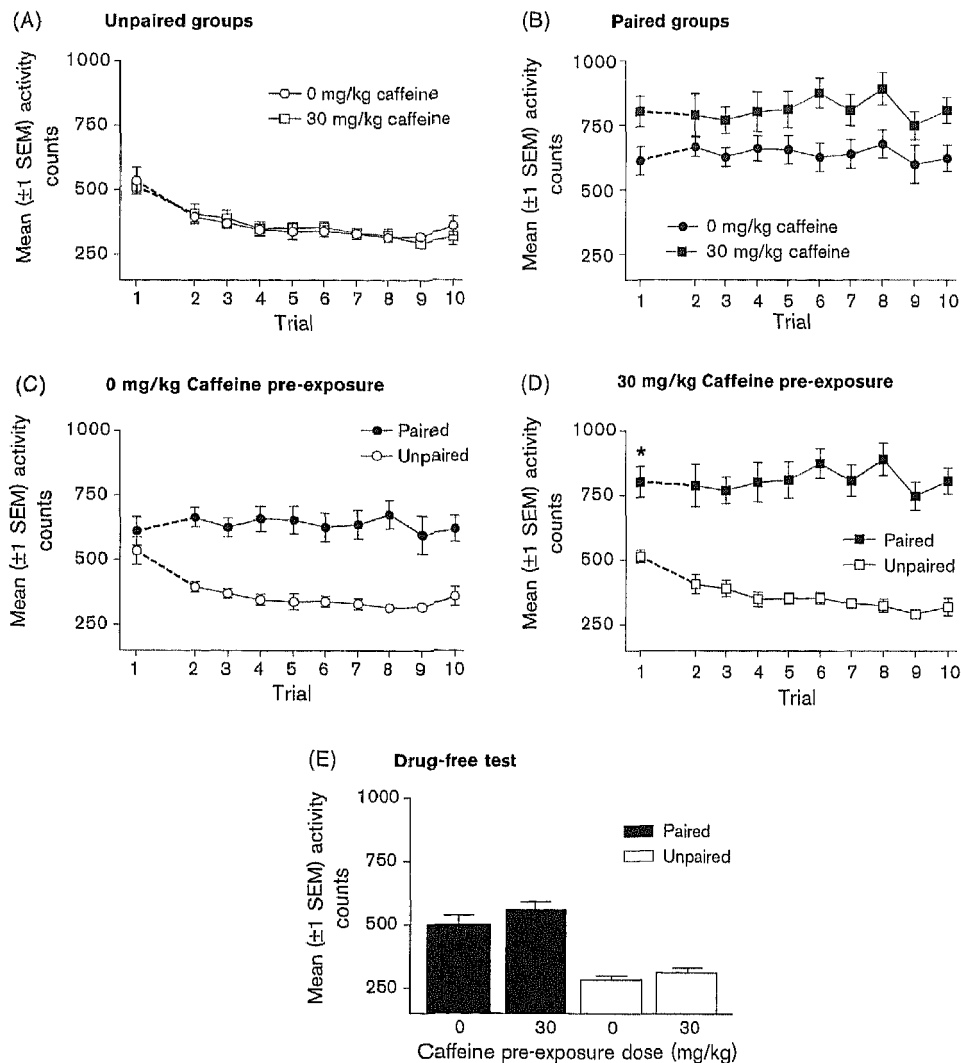
As in the previous experiments, caffeine pre-exposure did not alter body weight [$F(1,37) = 2.06$, NS]. On the first conditioning trial (day 31) an equipment failure resulted in the loss of data for 13 rats. Therefore, these data were analyzed separately from the remaining trials using a Student's t -test (Fig. 3A). For Unpaired rats, there was no difference in activity between caffeine pre-exposure doses on the first conditioning trial [$t < 1$]. Repeated-measures ANOVA examined possible non-specific effects of caffeine in Unpaired rats on subsequent trials (i.e. 2–10). For the Unpaired groups, there was a significant main effect of Trial [$F(8,136) = 3.92$, $P < 0.05$]. The main effect of Dose and the Dose \times Trial interaction were not significant [F 's < 1], indicating that caffeine pre-exposure did not have a non-specific effect on activity.

Amphetamine-induced activity (0.5 mg/kg)

Trial 1 data for caffeine pre-exposed (Paired $n = 7$, Unpaired $n = 6$) and non-pre-exposed (Paired $n = 5$, Unpaired $n = 8$) rats were analyzed separately with a two-way ANOVA. Given the decrease in statistical power due to loss of acute amphetamine data, combined with the previous findings that caffeine potentiates activity induced by amphetamine in an acute challenge (Gasior et al., 2000), statistical significance for this analysis was set at $P \leq 0.10$ (one-tailed). There was a significant main effect of Group [$F(1,22) = 12.06$, $P < 0.01$], and a significant Dose \times Group interaction [$F(1,22) = 4.07$, $P = 0.056$]. The main effect of Dose was not significant [$F(1,22) = 2.62$, NS]. Subsequent comparisons prompted by the Dose \times Group interaction examined amphetamine-treated rats (i.e. Paired groups) relative to comparable saline-treated (Unpaired) controls. The comparisons revealed that for non-pre-exposed rats, Paired and Unpaired groups were statistically similar [$t < 1$] (Fig. 3C, Trial 1). However, chronic caffeine pre-exposure potentiated acute amphetamine-induced activity [$t(11) = 4.21$, $P < 0.01$] (Fig. 3D, Trial 1).

Activity data illustrated in Figs 3C and D contrast Paired and Unpaired groups for non-pre-exposed and caffeine pre-exposed rats, respectively. Omnibus ANOVA for Trials 2–10 revealed a significant main effect of Trial [$F(8,280) = 2.75$, $P < 0.01$], of Group [$F(1,35) = 94.01$, $P < 0.001$], and of Dose [$F(1,35) = 4.78$, $P < 0.05$]. The Group \times Trial interaction [$F(8,280) = 2.90$, $P < 0.01$] was also significant, indicating that activity in Paired and Unpaired groups diverged across trials. The Dose \times Group interaction [$F(1,35) = 4.69$, $P < 0.05$] was also significant; however, the Dose \times Trial and Group \times Dose \times Trials interactions were not significant [F 's ≤ 1.22]. The main

Fig. 3



Locomotor activity for rats treated with 0.5 mg/kg amphetamine (Experiment 2). (A) and (B) contrast 0 versus 30 mg/kg caffeine pre-exposure for Unpaired and Paired groups, respectively. (C) and (D) contrast Paired versus Unpaired groups for rats pre-exposed to 0 and 30 mg/kg caffeine. (E) Activity data from the drug-free test for conditioning. * 30 mg/kg pre-exposed Paired rats were more active than comparable Unpaired controls on Trial 1.

effect of Dose and the Group \times Dose interaction indicates that pre-exposure to caffeine potentiated amphetamine-induced activity (i.e. only the pre-exposed Paired group). This enhancement in activity, relative to the comparable non-preexposed group, was consistent across trials.

Drug-free test

Figure 3E illustrates data from the drug-free test for conditioning. Analyses of these data revealed a main effect of Group [$F(1,35) = 77.97$, $P < 0.001$], indicating conditioned hyperactivity in Paired rats. The main effect of Dose [$F(1,35) = 2.62$] and the Group \times Dose interaction [$F < 1$] were not significant.

Discussion

Although caffeine can be a potent anorexic (see Gans, 1984), there were no significant differences in weight between rats pre-exposed to caffeine and those pre-exposed to saline in the present work. Caffeine pre-exposure did not have any non-specific effects on initial activity in a novel environment or habituation (decrease in activity) over trials, in Unpaired rats. Thus, differences in activity after chronic caffeine exposure cannot be attributed to non-specific effects on weight, environmental familiarization, or general motor activity.

Depending on the situation, nicotine produces locomotor suppression in rats; this suppression is replaced with

enhanced activity after repeated nicotine treatment (e.g. Clarke and Kumar, 1983; Kita *et al.*, 1992; Ksir, 1994; Bevins *et al.*, 2001). We replicated this pattern of activity in controls, and found that the general biphasic locomotor effects of nicotine were still present after pre-exposure to caffeine. Caffeine pre-exposure did not affect acute activity induced by 0.421 or 0.175 mg/kg nicotine (i.e. first conditioning trial for Paired rats). This outcome appears to contrast with previous caffeine pre-exposure research demonstrating enhanced hyperactivity after an acute nicotine challenge (Gasior *et al.*, 2000). In that study, rats were injected s.c. with nicotine (0.3 or 0.56 mg/kg salt; 0.105 or 0.196 mg/kg expressed as base, respectively), after which activity was monitored for 60 min. However, due to the procedural restrictions of equating activity tests with nicotine discrimination tests, activity for the first 15 min was not included in the analyses of that study. In contrast, we examined the effects of nicotine immediately after administration and for only 30 min. More importantly, Gasior *et al.* (2000) did not suspend caffeine exposure before the nicotine challenge test; rats had free access to caffeine in the home cage until just before the nicotine challenge. This manipulation opens the possibility that the acute psychomotor effect was due to the presence of both drugs at the time of testing. Further evidence for this possibility has recently been published. In a similar nicotine-discrimination study, Gasior *et al.* (2002) demonstrated that, regardless of the chronic exposure condition (i.e. caffeine-water or tap-water), pretreatment with an i.p. injection of caffeine (10, 17 or 30 mg/kg) enhanced generalization to a 'threshold' dose of nicotine (i.e. 0.05 mg/kg base). This finding clearly indicates that the co-presence of nicotine and caffeine can have important behavioral effects. Whether differences in testing protocol, or other procedural variations, such as nicotine dose, route of caffeine administration, or the co-presence of caffeine and nicotine in the rat at the time of testing, account for between-study differences will require further experimentation.

Caffeine enhances the psychomotor stimulant effects of amphetamine in an acute challenge (Gasior *et al.*, 2000). We confirmed this finding and extended it to include a chronic situation (i.e. repeated daily administration for 10 days). This outcome extends the literature demonstrating that chronic exposure to one drug of abuse can enhance the effects of another drug (e.g. Vezina *et al.*, 1989; Vezina and Stewart, 1990; Kuribara, 1999; Xu and Domino, 1999).

Potentiation of amphetamine-induced locomotor activity by caffeine might reflect changes in the underlying neural processes common to both drugs. There is converging evidence that the psychomotor effects of caffeine are mediated by the same mesolimbic dopamine structures

implicated in the psychomotor effects of amphetamine (e.g. Okada *et al.*, 1996, 1997; Afanas'ev *et al.*, 2000; Zahniser *et al.*, 2000). One possibility is that pre-exposure to caffeine alters the mesolimbic dopamine (DA) system, such that it is more sensitive to amphetamine. For example, contralateral rotational behavior elicited by caffeine in rats with unilateral 6-hydroxydopamine lesions of the nigro-striatal pathway is attenuated by the DA D₂ receptor antagonist, eticlopride (Garrett and Holtzman, 1995). Also, the locomotor hyperactivity produced by caffeine in rats is attenuated by systemic administration of the DA D₁ receptor subtype antagonist SCH 23390 and the DA D₂ receptor subtype antagonists eticlopride and sulpiride (Garrett and Holtzman, 1994). These alterations might depend on the antagonistic interaction between adenosine and dopamine receptors (e.g. Ferré *et al.*, 1992, see Fredholm *et al.*, 1999 for a review). That is, adenosinergic antagonism by caffeine might disrupt inhibitory effects of endogenous adenosine on DA D₁ and D₂ receptor affinity. However, the potential role for other neurotransmitter systems altered by chronic pre-exposure to caffeine should not be overlooked (e.g. Shi *et al.*, 1994; Jacobson *et al.*, 1996; Fredholm *et al.*, 1999; see Daly, 1993 for a review).

Amphetamine-conditioned hyperactivity in a drug-free test was unaffected by caffeine pre-exposure, despite the enhancement of amphetamine-induced activity. Similarly, nicotine-conditioned hyperactivity was unaffected by caffeine pre-exposure. Clearly, much more parametric work is required before concluding that caffeine pre-exposure cannot retard or enhance an association between environmental cues and the psychomotor effects of amphetamine or nicotine. Such variables include amphetamine and nicotine doses, conditioning trial parameters (e.g. number of trials, trial duration, injection to placement interval), and pre-exposure parameters (administration route, inter-dose interval, dose, duration, etc.).

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