Available online at www.sciencedirect.com
SCIENCE DIRECT
Pharmacology, Biochemistry and Behavior 82 (2005) 522 – 530
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

"Coffee, Tea *and* Me": Moderate doses of caffeine affect sexual behavior in female rats

Fay A. Guarraci *, Anastasia Benson

Department of Psychology, Southwestern University, Georgetown, TX 78626, USA

Received 8 August 2005; received in revised form 10 October 2005; accepted 18 October 2005
Available online 22 November 2005

Abstract

The present study evaluated the effects of acute caffeine administration on paced mating behavior and partner preference in ovariectomized rats primed with estrogen and progesterone. In Experiment 1, female rats were tested for paced mating behavior following acute administration of caffeine (15 mg/kg). Caffeine shortened the latency to return to a male following an ejaculation. Although this dose of caffeine did not alter the likelihood of leaving a male after receiving sexual stimulation, locomotor activity did increase significantly. Experiment 2 evaluated the dose response characteristics of caffeine (7.5, 15, 30 mg/kg) administration on paced mating behavior. Replicating Experiment 1, caffeine at the lower doses shortened the latency to return to a male following an ejaculation. Finally, to determine whether the effects of caffeine (15 mg/kg) on contact-return latency reflect a change in sexual motivation or merely an inability to inhibit locomotion, rats were tested for partner preference (intact male vs. estrous female) following caffeine administration (Experiment 3). Although caffeine did not disrupt preference for a sexual partner, caffeine selectively increased visits to the male when physical contact was possible. Collectively, these results suggest that the effects of caffeine on female mating behavior may reflect an increase in both sexual motivation and locomotor activity.

Keywords: Paced mating behavior; Partner preference; Locomotion; Psychomotor stimulants

1. Introduction

Caffeine is the most widely used psychoactive substance in the world (Fredholm et al., 1999). The main pharmacological action of caffeine is the blockade of adenosine receptors (Cauli and Morelli, 2005; Fredholm et al., 1999). Although there are 4 types of adenosine receptors (A_1, A_{2A}, A_{2B} and A_3), the stimulant properties of caffeine are mediated through blockade of A_1 and A_{2A} receptors (Cauli and Morelli, 2005; Fredholm et al., 1999; Halldner et al., 2004).

Similar to traditional drugs of abuse, the reinforcing properties of caffeine have been demonstrated in laboratory settings. For example, caffeine is self-administered by animals under certain circumstances (Griffiths and Woodson, 1988a,b), albeit less reliably than cocaine or amphetamine. Furthermore, animals will readily prefer places associated with caffeine administration (Bedingfield et al., 1998; Tuazon et al., 1992). Many studies have investigated the ability of psychomotor stimulants (e.g., amphetamine, cocaine, and caffeine) to enhance the reinforcing properties of drugs of abuse and natural rewards (Fiorino and Phillips, 1999a,b; Mendrek et al., 1998; Piazza et al., 1990). Similar to the effects of amphetamine (Mendrek et al., 1998; Piazza et al., 1990), acute pretreatment with caffeine enhances the self-administration of other drugs of abuse such as cocaine (Comer and Carroll, 1996; Schenk et al., 1994). Pretreatment with a psychomotor stimulant, such as amphetamine (Lett, 1989) or caffeine (Bedingfield et al., 1998; Tuazon et al., 1992) also facilitates the development of a conditioned place preference associated with the administration of other drugs of abuse. In terms of the effects of psychomotor stimulants on natural rewards, previous experience with amphetamine (Fiorino and Phillips, 1999a,b) or caffeine (Soulairac and Soulairac, 1978; Zimbardo and Barry, 1958) has been shown to facilitate sexual behavior in male rats as measured by shorter latencies to engage in sexual behavior. Although acute administration of amphetamine (Agmo and Picker, 1990) or caffeine (Zimbardo and Barry, 1958) shortens mount and intromission latencies in males, psychomotor stimulants have been shown to disrupt female

* Corresponding author. Tel.: +1 512 863 1747; fax: +1 512 863 1846.
E-mail address: guarracf@southwestern.edu (F.A. Guarraci).

0091-3057/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2005.10.007

sexual behavior. Specifically, moderate to high doses of amphetamine (ED_{50} ~2.6 mg/kg) interfere with the display of sexual receptivity in ovariectomized, hormone-primed rats (Michanek and Meyerson, 1977). Furthermore, a low dose of amphetamine (1 mg/kg) disrupts the appetitive aspects of female sexual behavior (Guarraci and Clark, 2003a). Surprisingly, there are no studies, to date, investigating the effects of caffeine on female sexual behavior.

Sexual behavior in the female rat is characterized by both receptive and proceptive behaviors. Receptive behavior is defined by the lordosis posture, a dorsoflexion of the female rat's back in response to a mount by a male rat (Beach, 1976). Female rats also engage in proceptive or soliciting behaviors including hopping, darting, ear wiggling, and pacing of sexual stimulation (Erskine, 1989). If a sexually receptive female is given the opportunity, she will approach and withdraw from a sexually vigorous male, thereby controlling the timing of the receipt of sexual stimulation (i.e., mounts, intromissions, and ejaculations). This pattern is known as paced mating behavior. The pacing of sexual stimulation by the female can be observed under naturalistic conditions and has also been studied in laboratory settings [for review see, (Blaustein and Erskine, 2002; Erskine, 1989)].

The partner preference paradigm is used commonly to evaluate the appetitive aspects of sexual behavior (Avitsur and Yirmia, 1999; Bakker, 2003; Paredes and Alonso, 1997; Paredes and Vazquez, 1999). Partner preference tests typically allow an experimental animal to make a choice between two stimulus animals; one that is a sexual partner and one that is not. In female rats, preference for a male is most robust when sexual interactions are limited, suggesting that the distal cues of the partner (auditory, visual and olfactory) are sufficient for the display of partner preference (Clark et al., 2004).

The present study tested the effects of acute caffeine administration on the display of paced mating behavior and partner preference in sexually receptive female rats. We hypothesized that caffeine would increase a female rat's motivation to approach a male rat during mating. Operationally, this would be expressed as a decrease in the latency to return to the male following sexual stimulation (Erskine, 1992). Because caffeine is less aversive and produces fewer anxiogenic effects than amphetamine (Dringenberg et al., 2000; Goudie, 1979; Guarraci and Clark, 2003a; Kunin et al., 2001), we also hypothesized that caffeine would not increase the female rat's likelihood of retreating from the male following sexual stimulation as was observed following amphetamine administration (Guarraci and Clark, 2003a). Finally, we hypothesized that caffeine would increase preference for a male stimulus rat during a test of partner preference.

2. Methods

2.1. Animals

One hundred eight (Experiment 1: 15; Experiment 2: 40; Experiment 3: 53) female Long–Evans rats (*Rattus norvegicus*), weighing 250–300 g were obtained from Harlan Sprague–Dawley (Indianapolis, IN) and used as experimental rats. Sexually experienced male (350–500 g) and female (250–350 g) Long–Evans rats were used as stimulus rats during behavioral testing. Rats were housed in hanging plastic cages within a light- and temperature-controlled vivarium and maintained on a reversed 12:12-h light–dark cycle (lights off at 1000 h). All experimental procedures occurred during the dark portion of the cycle under dim red light. Food and water were available ad libitum. Experimental rats were weighed once per week. Experimental and stimulus female rats were ovariectomized (OVX) under Nembutal (sodium pentobarbital, 50.0 mg/kg, i.p.) anesthesia one week prior to behavioral testing. All guidelines for the care and use of animals set by the United States Public Health Service (*Guide for the Care and Use of Laboratory Animals*, (Public Health Services, 1996)) were followed. In addition, all procedures using animals described in this manuscript were approved by the Southwestern University Institutional Animal Care and Use Committee.

2.2. Drugs and vehicle

All hormone injections were administered subcutaneously in the flank. Experimental and stimulus female rats received 10.0 μg of estradiol benzoate (EB) 48 h and 1.0 mg of progesterone (P) 4 h prior to each mating test. These doses of EB and P have been shown to produce high levels of receptivity and paced mating behavior in OVX rats (Zipse et al., 2000). Both hormones were delivered in a sesame seed oil vehicle. Caffeine free base (7.5, 15, and 30 mg/kg) was administered i.p. in a physiological saline vehicle. All hormones and drugs were purchased from Sigma Chemical Company (St. Louis, MO).

2.3. Behavioral procedure

2.3.1. Sexual receptivity

Approximately 1 week after ovariectomy, the sexually naïve experimental rats were tested for sexual receptivity. This test was conducted in a clear Plexiglas arena (30.5 cm long × 30.5 cm wide × 35.6 cm high) with wood shavings covering the floor. A single male rat was placed in the arena for a 5-min period and was permitted two intromissions with an OVX stimulus female rat (primed with EB and P) to ensure sexual vigor. Following the 5-min period, an experimental rat was placed into the arena with the male rat. The test was complete when the experimental rat received 10 mounts with or without intromissions. Lordosis responses (LR) were scored on a 4-point scale [0–3; (Hardy and DeBold, 1971, 1972)]. The percentage of times the experimental rat exhibited lordosis in response to a sexual stimulation (lordosis quotient [LQ] = number of LR scores of 2 or 3/number of mounts × 100) was calculated. Because we were interested in paced mating behaviors in sexually receptive rats, only the experimental rats that were sexually receptive (operationally defined as an LQ ≥ 60) were included in the study and further tested for paced mating behavior. Of the 108 rats, 4 did not exhibit sexual

receptivity on one of the three mating tests (receptivity test, baseline test of paced mating behavior or post-drug test of paced mating behavior).

2.3.2. Paced mating behavior

Approximately 1 week after sexual receptivity testing, the experimental rats were tested for baseline paced mating behavior. In studies of paced mating behavior it is necessary to conduct a baseline test to ensure that the display of paced mating behaviors are similar between groups of rats before introducing a manipulation because of the variation in the levels of baseline behavior observed in different cohorts of rats. Paced mating behavior was observed in a clear Plexiglas arena (91.4 cm long × 30.5 cm wide × 35.6 cm high) with wood shavings covering the floor. The arena was divided into three equally sized separate compartments using two clear partitions (30.5 cm wide × 35.6 cm high), which had a 5.0 cm hole in each bottom corner.

The experimental and male rats were acclimated to the paced mating arena on two separate occasions (15 min each) prior to any behavioral testing. The experimental rats were allowed to explore the entire extent of the arena during acclimation sessions. During the acclimation sessions for the males, a single male rat was placed in an outer compartment of the arena and tapped gently on the nose if he attempted to exit through the small holes in the Plexiglas divider (Emery, 1986; Erskine, 1985). Because the holes were too small for most males to fit through, only two sessions were needed to train the males.

Five minutes before the start of the mating test, an experimental rat was confined to the center compartment, with opaque Plexiglas partitions (30.5 cm wide × 35.6 cm high) in place on either side, blocking the clear Plexiglas partitions. A stimulus male was confined to one of the outer compartments, the location of which was determined randomly. The stimulus male was allowed one intromission with a stimulus female to ensure his sexual vigor. The mating test was started when one opaque partition was removed, allowing the experimental rat access to this stimulus male rat through the clear Plexiglas partition (Brandling-Bennett et al., 1999). The mating test was complete when the experimental rat received an ejaculation, left the male rat's compartment, and then retuned to him. At this point the test timer was stopped, the opaque partition was replaced and the experimental rat was once again confined to the center compartment.

LQs and LRs were recorded during the paced mating test. The contact-return latency and percentage of exits in response to each type of sexual stimulation were also calculated. *Contact-return latency* refers to the time elapsed between receiving sexual stimulation (i.e., mount, intromission, ejaculation), leaving the male rat's compartment and re-entering the male rat's compartment. More specifically, if multiple sexual stimulations are received during a visit to the male stimulus rat, contact-return latency can only be calculated on the last stimulation received before the experimental rat left the male rat's compartment. *Percentage of exits* refers to likelihood that the experimental rat left the male rat's compartment following the receipt of mating stimulation (i.e., mount, intromission or ejaculation). More specifically if an experimental rat received 2 mounts while she was in the male rat's compartment and then left, the likelihood of leaving the male after mounts is 50%. In addition, the percentage of time the experimental rat spent with the male, the number and rate of other proceptive behaviors (hops, darts and ear wiggling), rejection behaviors (kicks and defensive postures), and arena crossings (sum of entries and exits from the male compartment) were quantified. All mating tests were recorded with digital video cameras (Sony DCR-HC65) for off-line analysis of behaviors.

2.3.3. Partner preference contact condition

Approximately 1 week after sexual receptivity testing, the experimental rats were tested for baseline partner preference. Preference tests were conducted in tri-compartment arenas, which were identical to the paced mating arenas. Immediately prior to each partner preference test, the arena was cleaned with ethanol (70%) and fresh bedding was added. An experimental rat was then placed in the center compartment and two stimulus rats (a sexually vigorous male and an OVX hormone-primed female) were confined individually to each of the outer compartments on either side of the experimental female. The position (left or right) of the male and female stimulus rats was altered randomly between tests. Each male was allowed one intromission with a stimulus female to ensure his sexual vigor. All rats were allowed a 5-min period with the opaque Plexiglas partitions in place before the start of the test.

The test began when both opaque partitions were removed, allowing the experimental rat access to both stimulus rats through the clear Plexiglas partitions. After 10 min, both opaque partitions were replaced. Compartment entries were scored when all four paws of the experimental rat passed through the holes in the clear Plexiglas partition into a compartment. When ejaculations were received, we waited for the experimental rat to leave the male rat's compartment and return to him, at which point the test timer was stopped, the opaque partitions were replaced, and the experimental rat was once again confined to the center compartment. The stimulus rats were replaced, and after 5 min the test was resumed.

The number and timing of entries/exits and the time spent in each compartment (i.e., male, female, neutral) were measured. In addition, the number and rate of proceptive behaviors, rejection behaviors, and arena crossings were quantified. Acclimation sessions were identical to those described previously for paced mating behavior (see 2.3.2.).

2.3.4. Partner preference no-contact condition

Preference tests in the No-Contact Condition were conducted following identical procedures and in identical arenas to those described previously for the Contact Condition (see 2.3.3.), except that the stimulus rats were housed behind hardware cloth partitions (30.5 cm wide × 35.6 cm high) inserted in the middle of each side compartment. The hardware

cloth partitions allowed the transmission of visual, auditory and olfactory cues but prohibited mating.

2.3.5. Experiment 1: acute caffeine administration and paced mating behavior

Following a baseline paced mating behavior test, experimental rats were randomly assigned to one of two drug treatment groups. All behavioral measures (e.g., contact-return latency, percentage of exits, LQ, arena crossings, proceptive behaviors) were comparable between randomly assigned groups, as determined by statistical evaluation of baseline measures (all t's<1.0; p's>0.1). One week after the baseline test, the rats were administered EB+P and received either physiological saline ($n=7$) or 15 mg/kg caffeine ($n=7$) 30 min prior to a second paced mating test. The 15 mg/kg dose of caffeine was chosen because it produces significant stimulant properties (Nehlig et al., 1992; Waldeck, 1975). Experimenters were unaware of group assignment during behavioral testing.

2.3.6. Experiment 2: dose response of acute caffeine administration and paced mating behavior

Experiment 2 evaluated the dose response characteristics of acute administration of caffeine on paced mating behavior. Experimental rats were randomly assigned to one of four drug treatment groups. All behavioral measures (e.g., contact-return latency, percentage of exits, LQ, arena crossings, proceptive behaviors) were comparable between randomly assigned groups, as determined by statistical evaluation of baseline measures (all F's<1.0; p's>0.1). One week after the baseline test, the groups received saline ($n=10$), 7.5 mg/kg ($n=10$), 15 mg/kg ($n=10$), or 30 mg/kg ($n=10$) caffeine 30 min prior to a second paced mating test.

2.3.7. Experiment 3: acute caffeine administration and partner preference

A partner preference test was conducted to determine whether the shorter contact-return latencies following ejaculations observed in rats receiving caffeine in Experiments 1 and 2 reflect an alteration in sexual motivation or a general increase in locomotor behavior. We hypothesized that general increases in locomotor behavior would disrupt a female's ability to prefer the male stimulus to the female stimulus (Guarraci and Clark, 2003a). If no such disruption is observed or if female rats administered caffeine display an enhanced preference for the male stimulus, we would have additional support for caffeine affecting sexual motivation. Experimental female rats were randomly assigned to either the No-Contact condition ($n=26$) or the Contact condition ($n=27$). Following a baseline partner preference test, rats in each condition (No-Contact and Contact) were randomly assigned to drug treatment groups. All behavioral measures (e.g., male/social preference, LQ, arena crossings, proceptive behaviors) were comparable between randomly assigned groups, as determined by statistical evaluation of baseline measures (all F's<1.3; p's>0.1). One week after the baseline test, rats received either saline ($n=13$; $n=12$) or 15 mg/kg caffeine ($n=14$; $n=14$) 30 min prior to a second partner preference test.

2.4. Data analysis

For Experiment 1, all behavioral measures collected during tests of paced mating (baseline and drug test, separately) were analyzed with independent t-tests comparing randomly assigned groups (saline and caffeine). Specifically, independent t-tests were calculated on contact-return latencies and percentage of exits (mounts, intromissions and ejaculations separately), as well as LQ, LR, percentage of test time spent with the male, the rate of proceptive behaviors, the rate of rejection behaviors, test duration, the rate of arena crossings, and body weight. For Experiment 2, all behavioral measures collected during tests of paced mating (baseline and drug test, separately) were analyzed with one-way analysis of variance (ANOVA) comparing groups (saline, 3 doses of caffeine). LSD post hoc analyses were used to evaluate any statistically significant ANOVAs.

In Experiment 3, male partner preference was evaluated by calculating a preference score for time spent in the vicinity of the male [time with male/(time with male + time with female)]. A social preference score was also calculated to evaluate preference to spend time in the vicinity of the stimulus rats vs. alone [time with male+time with female/total test time]. A multivariate analysis of variance (MANOVA) was used to evaluate the effects of drug treatment (saline or caffeine) and test condition (No-Contact vs. Contact) on the male preference score, social preference score, and arena crossings. A MANOVA is useful for determining the effects of multiple independent variables (lesion and test condition) on multiple dependent measures (male preference, social preference, and arena crossings; (Clark et al., 2004)). One-way ANOVAs were also calculated on LQ, LR, the rate of proceptive behaviors, the rate of rejection behaviors, and body weight to evaluate drug treatment effects.

Because the data from the baseline tests were used to match statistically the drug treatment groups on all behavioral measures, the statistical analyses for the baseline tests are not reported.

3. Results

3.1. Experiment 1: acute caffeine administration and paced mating behavior

One experimental rat failed to display sexual receptivity during baseline paced mating and was therefore excluded from statistical analysis. There was a statistically significant difference between the drug treatment groups on contact-return latencies following ejaculations [$t(10)=4.35$, $P<0.01$] (Fig. 1 Top). That is, rats receiving caffeine returned to the male rat following ejaculations faster than did rats receiving saline. However, no statistically significant differences were observed between the drug treatment groups on percentage of exits [t's<1] (Fig. 1 Bottom). Although no differences were observed in the frequency of exiting the male rat's compartment after sexual stimulation, rats receiving caffeine made significantly more arena crossings per minute than did the rats

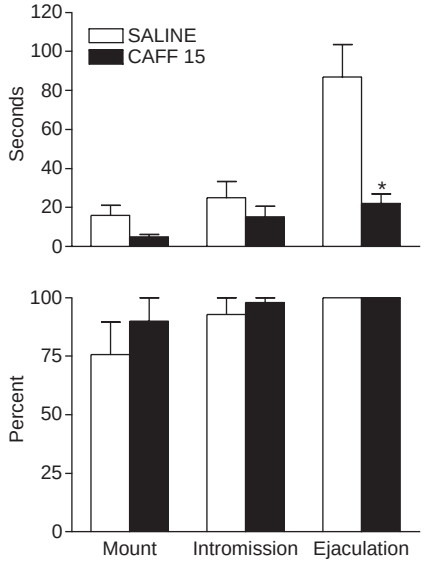

Fig. 1. Caffeine decreased the latency to return to the male rat following an ejaculation (TOP: SALINE $n=7$; CAFF 15 mg/kg $n=7$), but did not affect the likelihood of withdrawing from the male following sexual stimulation during a test of paced mating behavior (BOTTOM: SALINE, $n=7$; CAFF 15 mg/kg, $n=7$). Data are expressed as means ± SEM. NOTE: n's=number of rats in each group. * significantly different from saline ($p<.05$).

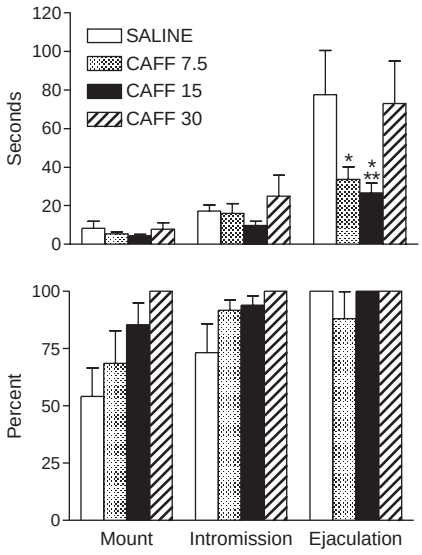

Fig. 2. Rats receiving the two lower doses of caffeine (7.5 and 15 mg/kg) exhibited decreased latency to return to the male rat following an ejaculation when compared to rats receiving saline or 30 mg/kg caffeine during a test of paced mating behavior (TOP: SALINE $n=9$; CAFF 7.5 mg/kg $n=10$; CAFF 15 mg/kg $n=9$; CAFF 30 mg/kg $n=6$). However caffeine did not affect the likelihood of withdrawing from the male following sexual stimulation (BOTTOM) during the paced mating test. ** significantly different from 30 mg/kg caffeine ($p<.05$).

receiving saline [$t(12)=3.15$, $P<0.008$] (Table 1). Few, if any rejection behaviors were observed in either group (data not shown). No other statistically significant differences were observed between the drug treatment groups [t's<1.9] (Fig. 1; Table 1).

3.2. Experiment 2: dose response of acute caffeine administration and paced mating behavior

Three experimental rats failed to display sexual receptivity during sexual receptivity testing and were therefore not tested for paced mating behavior. During paced mating testing, the video recordings of three animals were lost due to equipment malfunction. Therefore, the data from these animals were excluded from statistical analysis. A statistically significant difference between drug treatment groups was observed on contact-return latency following an ejaculation [$F(3, 29)=3.23$, $P<0.03$]. Post-hoc comparisons indicated that contact-return latencies following an ejaculation were significantly different between the rats receiving either 7.5 mg/kg or 15 mg/kg caffeine and the rats receiving saline [$P<0.05$]. Consistent with the results from Experiment 1, rats receiving caffeine (7.5 or 15 mg/kg) returned to the male following ejaculations faster than did the rats receiving saline (Fig. 2 Top). In addition, rats receiving 15 mg/kg caffeine returned to the male following ejaculations faster than did the rats receiving 30 mg/kg caffeine [$P<0.05$]. However, no statistically significant differences were observed between the drug treatment groups on percentage of exits [F's<2.2] (Fig. 2 Bottom). A statistically significant difference between drug treatment groups was observed on arena crossings [$F(3,$

Table 1
Behaviors observed during mating tests

	LQ	Proceptive behaviors/minute	Arena crossings/Minute	Test duration (seconds)	% of time with male
Experiment 1					
SALINE ($n=7$)	100±0.0	2.8±0.5	5.5±1.0	459.3±153.7	24.4±10.1
15 mg/kg CAFFEINE ($n=7$)	100±0.0	2.5±0.8	12.0±1.8*	294.1±80.8	31.0±6.5
Experiment 2					
SALINE ($n=9$)	100±0.0	2.7±0.8	6.4±1.4	398.4±122.0	43.3±5.7
7.5 mg/kg CAFFEINE ($n=10$)	98.5±1.4	2.6±0.7	11.2 ± 1.2*	244.7±67.4	38.7±6.9
15mg/kg CAFFEINE ($n=9$)	100±0.0	0.7±0.3	14.1±1.8*	256.0±57.2	44.8±6.2
30 mg/kg CAFFEINE ($n=6$)	100±0.0	2.1±0.7	9.7±1.3	687.8±304.8	26.9±5.9
Experiment 3					
SALINE NO-CONTACT ($n=12$)	N/A	1.8±0.5	6.5±0.6	600	N/A
CAFFEINE NO-CONTACT ($n=14$)	N/A	1.2±0.4	7.0±0.6	600	N/A
SALINE CONTACT ($n=13$)	94.0±4.0	2.0±0.5	7.9±0.6	600	N/A
CAFFEINE CONTACT ($n=14$)	99.5±3.7	2.0±0.4	10.8±0.6*	600	N/A

Means are reported ± standard error of the mean. An asterisk indicates a significant difference compared to the SALINE group ($p<.05$).

30)=4.93, $P<0.02$]. Post-hoc comparisons indicated that arena crossing per minute were significantly different between the rats receiving either 7.5 or 15 mg/kg caffeine and the rats receiving saline [$P<0.05$]. Rats receiving either 7.5 or 15 mg/kg caffeine made significantly more arena crossings per minute than the rats receiving saline (Table 1). Few if any rejections behaviors were observed in any group (data not shown). No other statistically significant differences were observed between the drug treatment groups [F's<2.4] (Fig. 2; Table 1).

3.3. Experiment 3: acute caffeine administration and partner preference

The MANOVA revealed a significant effect of test condition [$F(3, 47)=41.66$, $P<0.0001$]. However only trends were observed for the effect of drug treatment group [$F(3, 47)=2.29$, $P=0.09$] and for the drug treatment by test condition interaction [$F(3, 47)=2.31$, $P=0.08$] with male preference, social preference, and arena crossings as dependent measures (Fig. 3). To follow up the significant effect of test condition, univariate ANOVAs were calculated to reveal a significant effect of test condition on male preference [$F(1, 49)=20.92$, $P<0.0001$], social preference [$F(1, 49)=98.36$, $P<0.0001$] and arena crossings [$F(1, 49)=22.43$, $P<0.0001$]. These results indicate that experimental rats tested in the No-Contact condition displayed greater preference for the male than experimental rats tested in the Contact condition (Fig. 3 Top). Similarly, experimental rats tested in the No-Contact condition also displayed a greater preference to be social (spending more time with either male or female stimulus rats than alone) compared to experimental rats tested in the Contact condition (Fig. 3 Middle). However, experimental rats made more arena crossings if they were tested in the Contact condition compared to the rats tested in the No-Contact condition (Table 1).

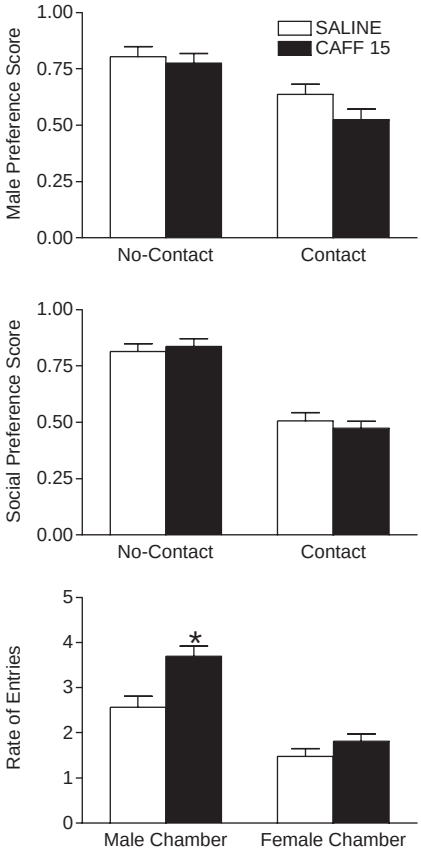

Fig. 3. Caffeine administration had no affect on either the male preference score [Stud Male Time/Stud Male Time + Estrous Female Time] (TOP) or the social preference score [Stud Male + Estrous Female/Total Test Time] (MIDDLE) when tested in either the No-Contact (SALINE, $n=13$; CAFF15 mg/kg, $n=14$) or the Contact Condition (SALINE, $n=12$; CAFF 15 mg/kg, $n=14$). However, caffeine selectively increased entries into the male rat's compartment when experimental rats were tested in the Contact Condition (BOTTOM).

Univariate ANOVAs also revealed significant effects of drug treatment group on arena crossings [$F(1, 49)=6.94$, $P<0.01$] as well as a significant interaction between drug treatment group and test condition on arena crossings [$F(1, 49)=5.56$, $P<0.02$]. These results indicate that although experimental rats administered caffeine displayed more arena crossings than experimental rats administered saline, the effect of caffeine was most pronounced in the Contact condition [$P<0.01$] (Table 1). Few if any rejections behaviors were observed in any group (data not shown). No other significant differences were observed [F's<2.3] (Table 1).

Although the nature of the preference for the male using the male preference score (time with male/time with male+time with female) was unaffected by caffeine, it is possible that caffeine has more subtle effects on sexual motivation in the partner preference paradigm. In addition to time spent with the male, the rate of visits to one stimulus animal versus the other may also be an indication of motivation. To determine if caffeine affected this measure of motivation, we analyzed the effect of drug treatment on the rate of visits to the male stimulus vs. the female stimulus. Because the increase in arena crossings following caffeine administration was most pronounced in the Contact condition, only this condition was analyzed. A significant effect of drug treatment was observed only for the rate of visits to the male stimulus rat [$F(1, 24)=6.52$, $P<0.02$]. Experimental rats administered caffeine visited the male stimulus more than did rats administered saline (Fig. 3 Bottom). However, no significant differences between drug treatment groups were observed for the rate of visits to the female stimulus rat [$F<1.1$]. Experimental rats administered caffeine visited the female stimulus at a similar rate as rats administered saline (Fig. 3 Bottom).

4. Discussion

The results of the present study demonstrate that caffeine altered paced mating behavior in OVX rats primed with estrogen and progesterone. Specifically, moderate doses of caffeine (7.5 and 15 mg/kg) shortened the latency to return to the male following an ejaculation during paced mating behavior (Experiments 1 and 2). However, a higher dose of caffeine (30 mg/kg) failed to increase arena crossings and failed to affect sexual behavior during tests of paced mating. Increases in arena crossings following caffeine administration were found to be more robust during tests of partner preference

when physical contact was unrestricted (Contact condition) than when physical contact was restricted (No-Contact condition). The preference for the male (i.e., a sexual partner) or the preference to be social was unaffected by caffeine administration (15 mg/kg) in either condition. However, the rate of entries specifically into the male stimulus rat's chamber, was greater following caffeine administration than saline in the Contact condition. Because caffeine administration did not increase the rate of entries into the female stimulus rat's chamber, when compared to saline administration, one effect of caffeine appears to be directed specifically towards a sexual stimulus.

The acute effects of caffeine observed on paced mating behavior in Experiments 1 and 2 (i.e., shorter return latency following ejaculations) may reflect the locomotor stimulating effects of caffeine. That is, increases in locomotion could have produced faster contact-return latencies. However, a number of observations suggest that not all of the effects of acute caffeine on mating behavior can be attributed to an increase in locomotor activity. First, the contact-return latencies following mounts and intromissions were unaffected by any of the doses of caffeine tested in the present experiments (7.5, 15, or 30 mg/kg). If the increase in arena crossings following caffeine shortened contact-return latencies directly, then all of the contact-return latencies would have been equally affected, not just those following ejaculations. In contrast, latencies to return to the male following mounts or intromissions were not shorter in the caffeine treated rats when compared to the saline treated rats. Second, although rats that received caffeine made significantly more arena crossings than rats that received saline during the partner preference test (Contact condition), only entries into the male stimulus rat's chamber were increased. This result suggests that locomotor activity was not increased in general, but that the increase in locomotion was directed toward a particular stimulus (i.e., the male). Third, if the stimulant properties of caffeine produced non-specific increases in activity that interfered with a female rat's ability to prefer one stimulus to another stimulus, partner preference would have been disrupted (Guarraci and Clark, 2003a). That is, rats receiving caffeine would have visited the male and female stimulus rats at an equal rate and would have spent an equal amount of time with the stimulus rats. No such disruption was observed. Instead, rats receiving caffeine demonstrated a robust preference for the male during partner preference sessions in the No-Contact condition, similar to the preference observed in rats receiving saline.

Consistent with our hypothesis, caffeine increased a female rat's motivation to approach a male rat during mating, operationally defined as a decrease in the latency to return to the male following sexual stimulation (Erskine, 1992). However, only return latencies following ejaculations were affected by the lower doses of caffeine (7.5 and 15 mg/kg). Mounts and intromissions may not have been affected by caffeine because an ejaculation is the most rewarding stimulations received by the female during mating (Dominguez-Salazar et al., 2005; Gonzalez-Flores et al., 2004; Paredes and Vazquez, 1999) and therefore more sensitive to the reward-enhancing effects of caffeine. Additional research is necessary to investigate this hypothesis.

We also hypothesized that caffeine would not increase percentage of exits the way amphetamine administration had (Guarraci and Clark, 2003a) because caffeine is not anxiogenic (Bhattacharya et al., 1997) or aversive (Tuazon et al., 1992) at low doses. Only the highest dose of caffeine (30 mg/kg) tended to increase percentage of exits following sexual stimulation during paced mating behavior, although not significantly. It is possible that this higher dose of caffeine, which has been shown to be aversive (Tuazon et al., 1992) and moderately anxiogenic (Bhattacharya et al., 1997; Kurt et al., 2003), produced some mild avoidance of the male, similar to what is observed following amphetamine administration (Guarraci and Clark, 2003a). This mild avoidance of the male may have also contributed to the failure of caffeine at 30 mg/kg to increase arena crossings. Furthermore, the stimulation of locomotor activity has been shown to be less consistent following the administration of more than 25 mg/kg of caffeine (Anden and Jackson, 1975).

The present results from the partner preference tests are consistent with previous findings (Clark et al., 2004). Female rats preferred a sexual partner (i.e., intact male) to a non-sexual partner (i.e., estrous female) when physical contact was restricted (No-Contact). However, female rats tended to spend an equal amount of time with both stimulus partners (male and female) when physical contact was unrestricted (Contact). Inconsistent with our hypothesis, rats administered caffeine did not show an enhanced preference for the male when tested in the No-Contact condition. It is possible that we did not see enhanced preference for the male in the caffeine treated rats during the No-Contact test because male preferences scores were close to a ceiling in the present study (SALINE: 0.80; CAFFEINE: 0.78).

Previously, we have reported that psychomotor stimulant administration (i.e., amphetamine) can facilitate female mating behavior (Guarraci and Clark, 2003a). However, this effect was only observed in rats treated chronically with amphetamine and tested for paced mating behavior following a three-week withdrawal period. This chronic drug regimen produced shorter contact-return latencies following mounts, in rats sensitized to amphetamine, when compared to rats that received chronic saline. In the current study, caffeine facilitated female mating behavior after acute administration but only following an ejaculation. Because chronic exposure to caffeine has typically produced effects that are opposite to the effects of acute caffeine administration (Jacobson et al., 1996; Kuzmin et al., 2000), it is unlikely that chronic administration of caffeine will affect paced mating behavior in the same way as acute administration. Future studies are necessary to confirm this hypothesis and to investigate why responses to mounts and ejaculations are differentially affected by caffeine and amphetamine.

It is possible that caffeine may be altering neurotransmission in the hypothalamus to affect female sexual behavior (i.e., paced mating behavior and partner preference). Immunohisto-

chemical techniques have identified A_{2A} receptors in the hypothalamus, although they are not as densely localized as in the striatum (dorsal or ventral) (El Yacoubi et al., 2001; Parkinson and Fredholm, 1990). Because lesions of the medial preoptic area (mPOA) of the hypothalamus *increase* the latency of a female rat to return to a male rat following an ejaculation (Guarraci et al., 2004; Yang and Clemens, 2000) and *disrupt* partner preference in the Contact condition (Guarraci and Clark, 2003b), it is possible that the blockade of A_{2A} receptors in the mPOA plays a role in regulating female approach to a male during mating behavior. Our lab is currently testing this hypothesis.

Understanding how psychomotor stimulants affect intrinsically motivated behaviors, such as sexual behavior, is important for our understanding of the neurobiological mechanisms of drug addiction. The current study is the first to demonstrate that the commonly used stimulant, caffeine, can affect female mating behavior. The present study provides additional evidence that psychomotor stimulants enhance natural rewards. Although changes in locomotor activity cannot be easily separated from changes in approach behaviors, more information about how locomotor activity alters the measures of female sexual behavior allows us to better understand the multiple components of paced mating behavior.

In conclusion, the acute effects of caffeine on paced mating behavior and partner preference may reflect both locomotor stimulating effects and increased approach behavior towards a sexual stimulus (i.e., an indication of sexual motivation). The effects of caffeine on female sexual behavior may be mediated through the blockade of adenosine receptors in the mPOA. Although it is difficult to distinguish the locomotor stimulating properties from the reinforcing properties of stimulant drugs, the present study utilizes two paradigms that better isolate the specific effects of caffeine on intrinsic motivation. Defining the contributions of locomotion and motivation to the display of paced mating behavior will advance our understanding of the neurobiology of female sexual behavior.

Acknowledgements

We thank Brittany Mason and Matthew Gilbert for technical assistance and Drs. Russell Frohardt, Jesse Purdy, and Traci Giuliano for comments on the manuscript.

References

Agmo A, Picker Z. Catecholamines and the initiation of sexual behavior in male rats without sexual experience. Pharmacol Biochem Behav 1990; 35:327–34.

Anden NE, Jackson DM. Locomotor activity stimulation in rats produced by dopamine in the nucleus accumbens: potentiation by caffeine. J Pharm Pharmacol 1975;27:666–70.

Avitsur R, Yirmiya R. The partner preference paradigm: a method to study sexual motivation and performance of female rats. Brain Res Protoc 1999;3:320–5.

Bakker J. Sexual differentiation of the neuroendocrine mechanisms regulating mate recognition in mammals. J Neuroendocrinol 2003;15:615–21.

Beach FA. Sexual attractivity, proceptivity, and receptivity in female mammals. Horm Behav 1976;7:105–38.

Bedingfield JB, King DA, Holloway FA. Cocaine and caffeine: conditioned place preference, locomotor activity, and additivity. Pharmacol Biochem Behav 1998;61:291–6.

Bhattacharya SK, Satyan KS, Chakrabarti A. Anxiogenic action of caffeine: an experimental study in rats. J Psychopharmacol 1997;11:219–24.

Blaustein JD, Erskine MS. Feminine sexual behavior: cellular integration of hormonal and afferent information in the rodent forebrain. San Diego, CA: Elsevier Science; 2002.

Brandling-Bennett EM, Blasberg ME, Clark AS. Paced mating behavior in female rats in response to different hormone priming regimens. Horm Behav 1999;35:144–54.

Cauli O, Morelli M. Caffeine and the dopaminergic system. Behav Pharmacol 2005;16:63–77.

Clark AS, Kelton MC, Guarraci FA, Clyons EQ. Hormonal status and test condition, but not sexual experience, modulate partner preference in female rats. Horm Behav 2004;45:314–23.

Comer SD, Carroll ME. Oral caffeine pretreatment produced modest increases in smoked cocaine self-administration in rhesus monkeys. Psychopharmacology (Berl) 1996;126:281–5.

Dominguez-Salazar E, Camacho FJ, Paredes RG. Prenatal blockade of androgen receptors reduces the number of intromissions needed to induce conditioned place preference after paced mating in female rats. Pharmacol Biochem Behav 2005;81:871–8.

Dringenberg HC, Wightman M, Beninger RJ. The effects of amphetamine and raclopride on food transport: possible relation to defensive behavior in rats. Behav Pharmacol 2000;11:447–54.

El Yacoubi M, Ledent C, Parmentier M, Ongini E, Costentin J, Vaugeois JM. In vivo labelling of the adenosine A2A receptor in mouse brain using the selective antagonist [3H]SCH 58261. Eur J Neurosci 2001;14: 1567–70.

Emery DE. Effects of endocrine state on sociosexual behavior of female rats tested in a complex environment. Behav Neurosci 1986;100:71–8.

Erskine MS. Effects of paced coital stimulation on estrus duration in intact cycling rats and ovariectomized and ovariectomized-adrenalectomized hormone-primed rats. Behav Neurosci 1985;99:151–61.

Erskine MS. Solicitation behavior in the estrous female rat: a review. Horm Behav 1989;23:473–502.

Erskine MS. Pelvic and pudendal nerves influence the display of paced mating behavior in response to estrogen and progesterone in the female rat. Behav Neurosci 1992;106:690–7.

Fiorino DF, Phillips AG. Facilitation of sexual behavior and enhanced dopamine efflux in the nucleus accumbens of male rats after *d*-amphetamine-induced behavioral sensitization. J Neurosci 1999a;19: 456–63.

Fiorino DF, Phillips AG. Facilitation of sexual behavior in male rats following *d*-amphetamine-induced behavioral sensitization. Psychopharmacology 1999b;142:200–8.

Fredholm BB, Battig K, Holmen J, Nehlig A, Zvartau EE. Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. Pharmacol Rev 1999;51:83–133.

Gonzalez-Flores O, Camacho FJ, Dominguez-Salazar E, Ramirez-Orduna JM, Beyer C, Paredes RG. Progestins and place preference conditioning after paced mating. Horm Behav 2004;46:151–7.

Goudie AJ. Aversive stimulus properties of drugs. Neuropharmacology 1979;18:971–9.

Griffiths RR, Woodson PP. Caffeine physical dependence: a review of human and laboratory animal studies. Psychopharmacology (Berl) 1988; 94:437–51.

Griffiths RR, Woodson PP. Reinforcing properties of caffeine: studies in humans and laboratory animals. Pharmacol Biochem Behav 1988;29: 419–27.

Guarraci FA, Clark AS. Amphetamine modulation of paced mating behavior. Pharmacol Biochem Behav 2003a;76:505–15.

Guarraci FA, Clark AS. Partner preference is altered in female rats with lesions of the medial preoptic area. Horm Behav 2003b;44.

Guarraci FA, Megroz AB, Clark AS. Paced mating behavior in the female rat following lesions of three regions responsive to vaginocervical stimulation. Brain Res 2004;999:40–52.

Halldner L, Aden U, Dahlberg V, Johansson B, Ledent C, Fredholm BB. The adenosine A1 receptor contributes to the stimulatory, but not the inhibitory effect of caffeine on locomotion: a study in mice lacking adenosine A1 and/or A2A receptors. Neuropharmacology 2004;46:1008–17.

Hardy DF, DeBold JF. Effects of mounts without intromission upon the behavior of female rats during the onset of estrogen-induced heat. Physiol Behav 1971;7:643–5.

Hardy DF, DeBold JF. Effects of coital stimulation upon behavior of the female rat. J Comp Physiol Psychol 1972;78:400–8.

Jacobson KA, von Lubitz DK, Daly JW, Fredholm BB. Adenosine receptor ligands: differences with acute versus chronic treatment. Trends Pharmacol Sci 1996;17:108–13.

Kunin D, Gaskin S, Borjas MB, Smith BR, Amit Z. Differences in locomotor response to an inescapable novel environment predict sensitivity to aversive effects of amphetamine. Behav Pharmacol 2001;12:61–7.

Kurt M, Bilge SS, Kukula O, Celik S, Kesim Y. Anxiolytic-like profile of propofol, a general anesthetic, in the plus-maze test in mice. Pol J Pharmacol 2003;55:973–7.

Kuzmin A, Johansson B, Semenova S, Fredholm BB. Differences in the effect of chronic and acute caffeine on self-administration of cocaine in mice. Eur J Neurosci 2000;12:3026–32.

Lett BT. Repeated exposures intensify rather than diminish the rewarding effects of amphetamine, morphine, and cocaine. Psychopharmacology 1989;98:357–62.

Mendrek A, Blaha CD, Phillips AG. Pre-exposure of rats to amphetamine sensitizes self-administration of this drug under a progressive ratio schedule. Psychopharmacology 1998;135:416–22.

Michanek A, Meyerson BJ. A comparative study of different amphetamines on copulatory behavior and stereotype activity in the female rat. Psychopharmacology 1977;53:175–83.

Nehlig A, Daval JL, Debry G. Caffeine and the central nervous system: mechanisms of action, biochemical, metabolic and psychostimulant effects. Brain Res Brain Res Rev 1992;17:139–70.

Paredes RG, Alonso A. Sexual behavior regulated (paced) by the female induces conditioned place preference. Behav Neurosci 1997;111:123–8.

Paredes RG, Vazquez B. What do female rats like about sex? Paced mating. Behav Brain Res 1999;105:117–27.

Parkinson FE, Fredholm BB. Autoradiographic evidence for G-protein coupled A2-receptors in rat neostriatum using [3H]-CGS 21680 as a ligand. Naunyn-Schmiedebergs Arch Pharmacol 1990;342:85–9.

Piazza PV, Deminiere JM, le Moal M, Simon H. Stress- and pharmacologically-induced behavioral sensitization increases vulnerability to acquisition of amphetamine self-administration. Brain Res 1990;514:22–6.

Public Health Services, US. Guide for the care and use of laboratory animals institute of laboratory animal resources commission on life sciences. Washington, D.C.: National Academy Press; 1996.

Schenk S, Valadez A, Horger BA, Snow S, Wellman PJ. Interactions between caffeine and cocaine in tests of self-administration. Behav Pharmacol 1994;5:153–8.

Soulairac A, Soulairac ML. Relationships between the nervous and endocrine regulation of sexual behavior in male rats. Psychoneuroendocrinology 1978;3:17–29.

Tuazon DB, Suzuki T, Misawa M, Watanabe S. Methylxanthines (caffeine and theophylline) blocked methamphetamine-induced conditioned place preference in mice but enhanced that induced by cocaine. Ann N Y Acad Sci 1992;654:531–3.

Waldeck B. Effect of caffeine on locomotor activity and central catecholamine mechanisms: a study with special reference to drug interaction. Acta Pharmacol Toxicol 1975;36:1–23.

Yang LY, Clemens LG. MPOA lesions affect female pacing of copulation in rats. Behav Neurosci 2000;114:1191–202.

Zimbardo PG, Barry III H. Effects of caffeine and chlorpromazine on the sexual behavior of male rats. Science 1958;127:84–5.

Zipse LR, Brandling-Bennett EM, Clark AS. Paced mating behavior in the naturally cycling and the hormone-treated female rat. Physiol Behav 2000;70:205–9.