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Amphetamine modulation of paced mating behavior

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Abstract

The present study evaluated the effects of acute and repeated, intermittent amphetamine administration on paced mating behavior in ovariectomized (OVX) rats primed with estrogen and progesterone. In Experiment 1, female rats were tested for paced mating behavior following acute administration of amphetamine (1.0 mg/kg). Amphetamine increased the likelihood that a female would withdraw from a male following a mount or an intromission. Although this dose of amphetamine did not alter sexual receptivity or the latency to return to a male after sexual stimulation, locomotor activity was increased significantly. Experiment 2 evaluated the dose response characteristics of acute amphetamine (0.5, 1.0 and 2.0 mg/kg) administration on paced mating behavior. In agreement with Experiment 1, amphetamine at all doses increased the likelihood that a female would withdraw from a male following sexual stimulation. In Experiment 3, female rats were tested for partner preference (sexually active male vs. estrous female) following acute amphetamine administration. Amphetamine treatment augmented both social and sexual preferences. In Experiment 4, female rats were administered estrogen (20 μg/kg) and amphetamine (1.0 mg/kg) for 3 weeks and tested for paced mating behavior 1 and 4 weeks later, amphetamine free. Repeated intermittent exposure to amphetamine shortened the latency to return to a male after receiving a mount on the test conducted 1 week after the final drug injections. Collectively, these results suggest that the acute effects of amphetamine on paced mating behavior may reflect a reduction in social and sexual behaviors and an increase in locomotor activity, whereas the effects of repeated exposure may reflect a change in incentive motivation.

Keywords: Motivation; Individual differences; Novelty; Locomotion; Dopamine; Partner preference

1. Introduction

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 been studied in laboratory settings (for reviews, see Blaustein and Erskine, 2002; Erskine, 1989).

Recent findings suggest that dopamine, in particular dopamine in the nucleus accumbens (NAc), may be involved in paced mating behavior. Mermelstein and Becker (1995) reported that paced mating behavior is accompanied by increases in extracellular dopamine levels in the NAc and adjacent caudate putamen. Furthermore, the dopamine release in the NAc is sensitive to the individual female rat's preferred pacing interval (Becker et al., 2001b) and lesions of the NAc alter the pattern of mating behavior (Guarraci et al., 2002; Jenkins and Becker, 2001; Rivas and Mir, 1990).

Amphetamine is a psychomotor stimulant that is used recreationally by humans. Research has shown that the reinforcing properties of amphetamine are related to increases in extracellular dopamine levels in the forebrain (Jones et al., 1995; Schmitz et al., 2001). Amphetamine not only acts as a positive reinforcer (Bevins et al., 1997; Piazza et al., 1990; Pierre and Vezina, 1997), but also has been shown to enhance the reinforcing properties of drug (Horger et al., 1992; Piazza et al., 1990; Pierre and Vezina, 1997; Valadez and Schenk, 1994) and natural rewards (e.g., food, sex; Fiorino and Phillips, 1999b; Nocjar and Panksepp, 2002). Previous experience with amphetamine has been

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shown to facilitate (1) the acquisition and rate of sexual behavior in sexually naïve male rats (Fiorino and Phillips, 1999a,b), (2) the acquisition of drug self-administration (Mendrek et al., 1998; Piazza et al., 1990), and (3) the development of a conditioned place preference associated with drug administration (Lett, 1989).

In addition to being a positive reinforcer and psychomotor stimulant, amphetamine is known to have aversive properties (Goudie, 1979; Kunin et al., 2001) and is a potent anxiogenic (Dringenberg et al., 2000). Doses of amphetamine that support self-administration readily induce a conditioned taste aversion (Goudie, 1979; Kunin et al., 2001) and decrease open-arm exploration on an elevated plus-maze (Dringenberg et al., 2000). The effects of amphetamine on social behavior are not well understood. In male rats, File and Hyde (1979) reported that 2.0 mg/kg amphetamine *reduced* social interaction in both a low- and high-anxiety condition, whereas Guy and Gardner (1985) reported that 1.5 mg/kg amphetamine *increased* social interaction in male rats in both conditions. Differences in locomotor activity could not account for these discrepancies because in both reports, amphetamine produced a significant increase in locomotor behavior. To date, few studies have examined the effects of amphetamine on social behavior in female animals (cf., Beatty et al., 1982).

The effects of amphetamine on sexual behavior have also been examined primarily in male rats (Agmo and Picker, 1990; Everitt and Stacey, 1987; Fiorino and Phillips, 1999b). For example, acute amphetamine administration shortens mount and intromission latencies in sexually naïve males (Agmo and Picker, 1990). It is known that in ovariectomized (OVX), hormone-primed rats, moderate to high doses of amphetamine (ED_{50} ~ 2.6 mg/kg) interfere with the display of sexual receptivity (Michanek and Meyerson, 1977). Furthermore, sexual experience increases sensitivity to the stimulant properties of amphetamine in OVX, hormone-primed hamsters (Bradley and Meisel, 2001). Specifically, six weekly mating episodes can cross-sensitize with amphetamine (Bradley and Meisel, 2001). Given the effects of amphetamine on male sexual and social behavior and the role of dopamine in paced mating behavior, the present study tested the effects of acute and repeated, intermittent amphetamine administration on the display of paced mating behavior and other appetitive aspects of mating in sexually receptive female rats.

2. Methods

2.1. Animals

One hundred thirty-two (Experiment 1: 47; Experiment 2: 40; Experiment 3: 18; Experiment 4: 27) 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. Each rat was individually housed in a hanging wire cage 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 female rats were weighed once per week. Experimental and stimulus female rats were ovariectomized under Brevital (sodium methohexital, 50.0 mg/kg ip) anesthesia 1 week before 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 Dartmouth College 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 before each mating test. These doses of EB and P 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. *d*-Amphetamine sulfate (0.5, 1.0, 2.0 mg/kg) was administered intraperitoneally in a physiological saline vehicle. All hormones and drugs were purchased from Sigma, 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 (39.4 cm long × 22.9 cm wide × 31.1 cm high) with wood shavings covering the floor. A single male rat was placed in the arena for 5 min 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. Only two rats did not exhibit sexual receptivity on this test.

2.3.2. Paced mating behavior

Approximately 1 week after sexual receptivity testing, the experimental rats were tested for baseline paced mating behavior. Paced mating behavior was observed in a clear Plexiglas arena (112.4 cm long × 37.5 cm wide × 31.7 cm high) with wood shavings covering the floor. The arena was divided into three equally sized separate compartments using two clear partitions (36.5 × 31.7 cm) that 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) before any behavioral testing. During the 15-min 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 it 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. The experimental rats were allowed to explore the entire extent of the arena during acclimation sessions.

Five minutes before the start of the mating test, an experimental rat was confined to the center compartment, with opaque Plexiglas partitions (each 36.5 × 31.5 cm) in place on either side, blocking the clear Plexiglas partitions. The opaque partitions prevented access to the two male rats, each of which was confined individually to one of the outer compartments on either side of the experimental female (e.g., Brandling-Bennett et al., 1999). Each male was allowed one intromission with a stimulus female to ensure his sexual vigor.

At the start of the mating test, one opaque partition was removed, allowing the experimental rat access to a single male rat through the clear Plexiglas partition. During the mating test, the experimental rat had access to only one male at a time.

The mating test was complete when the experimental rat received 10 mounts with intromissions, including ejaculations when they occurred. 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 partition was replaced and the experimental rat was once again confined to the center compartment. The test was resumed immediately (< 5 s) on removal of the other opaque partition, allowing the experimental rat access to the second male rat. Paced mating tests were terminated when the experimental rat returned to the male rat after receiving a 10th intromission. Although a female that receives five intromissions during a paced mating test may exhibit the physiological changes requisite for pseudopregnancy (for a review, see Erskine, 1995), we used a criterion of 10 intromissions to allow most rats to receive an ejaculation during the paced mating test (Brandling-Bennett et al., 1999; Guarraci et al., 2002; Zipse et al., 2000).

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 before the experimental rat re-enters the male rat's compartment following mating stimulation (i.e., mount, intromission, ejaculation). *Percentage of exits* refers to the rate of withdrawals by the experimental rat from the male rat's compartment following mating stimulation. 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.

2.3.3. Experiment 1: Acute amphetamine and paced mating behavior

Following a baseline paced mating behavior test, experimental rats were assigned to one of two groups matched for comparable levels of sexual receptivity and paced mating behaviors. Although sexual experience (six 10-min sessions) has been shown to enhance the sensitivity to amphetamine in female hamsters (Bradley and Meisel, 2001), in studies of paced mating behavior it is necessary to conduct a baseline test to "match" groups on measures of paced mating behavior before introducing a manipulation because of the variation in the levels of baseline behavior observed in different cohorts of rats. Therefore, amphetamine effects on paced mating behavior were not tested in sexually naïve rats. One week after the baseline test, the rats were administered EB + P and received either saline ($n = 24$) or 1.0 mg/kg amphetamine ($n = 23$) 15–20 min before a second paced mating test. The 1.0 mg/kg dose of amphetamine was chosen because it produces neither stereotyped motor patterns nor decrements in sexual receptivity (for a review, see Grilly and Loveland, 2001; Michanek and Meyerson, 1977). Experimenters were unaware of group assignment. The procedures of this experiment were replicated in a second study and the data were pooled.

2.3.4. Experiment 2: Dose response of acute amphetamine and paced mating behavior

Experiment 2 evaluated the dose response characteristics of acute administration of amphetamine on paced mating behavior. Experimental female rats were assigned to one of four groups matched for comparable levels of sexual receptivity and paced mating behaviors at baseline. One week later, the groups received either saline ($n = 10$) or 0.5 mg/kg ($n = 10$), 1.0 mg/kg ($n = 10$), or 2.0 mg/kg ($n = 10$) amphetamine 15–20 min before a second paced mating test.

2.3.5. Experiment 3: Acute amphetamine and partner preference

A partner preference test was conducted to determine whether the reduction in time spent with the male following amphetamine administration observed in Experiments 1 and 2 reflects an alteration in sexual motivation or a general decrease in social behavior. Because a sexually receptive female rat demonstrates a preference to be near a sexually active male vs. an estrous female when physical contact between the rats is limited, a no-contact partner preference procedure was used (Clark et al., 2003). Following a baseline partner preference test, groups of rats were matched for comparable levels of sexual receptivity and partner preference and received either saline ($n=10$) or 1.0 mg/kg amphetamine ($n=8$) 15–20 min before a second partner preference test.

2.3.6. Partner preference

Preference tests were conducted in tricompartment arenas identical to the paced mating arenas except that the stimulus rats were housed behind hardware cloth partitions (36.5 × 31.5 cm high) inserted in the middle of each side compartment. The hardware cloth partitions allowed limited contact and the transmission of visual, auditory, and olfactory cues but prohibited sexual contact. Acclimation sessions for the experimental and stimulus rats were identical to those described previously for paced mating behavior (see Section 2.3.2). Immediately before each test, the testing arena was cleaned with ethanol (70%) and fresh bedding was added. An experimental female rat was then placed in the center compartment with two opaque partitions in place. The stimulus rats (a sexually vigorous male and an OVX hormone-primed female) were placed individually into the side compartments. The position of the stimulus rats was varied between experimental rats. All rats were allowed a 5-min period with the opaque Plexiglas partitions in place before the start of each test.

The test began when both opaque partitions were removed. The experimental rats were able to move freely throughout the testing arena for the 10-min test. Compartment entries were scored when all four paws of the experimental rat passed through the holes in the clear Plexiglas partition into a compartment. The number and timing of entries/exits (sum of entries and exits from each compartment), and the time spent in each compartment (male, female, neutral) was measured. In addition, proceptive behaviors (hop, darts, and ear wiggles) were quantified.

2.3.7. Experiment 4: Repeated, intermittent amphetamine and paced mating behavior

2.3.7.1. Sensitization. Because the interpretation of the results of Experiments 1–3 may be confounded by locomotor stimulating effects of amphetamine, we used a procedure that has been used previously to reduce the contribution of locomotor stimulating effects of amphetamine to the display of other behaviors (Fiorino and Phillips, 1999a,b). Specifically, we modified a protocol used to evaluate the effects of repeated cocaine on turning behavior in OVX rats (Becker et al., 2001a; Hu and Becker, 2003). Rats were assigned to one of two drug treatment groups (saline-repeated: $n=13$ or amphetamine-repeated: $n=14$) matched for comparable levels of sexual receptivity and paced mating behaviors. For 3 weeks, all experimental rats received daily injections of 20 μg/kg EB followed 30 min later by injections of 1.0 mg/kg amphetamine or saline for 4 consecutive days followed by 3 days off each week (for timing and pattern of drug/hormone administration protocol, see Becker et al., 2001a; Hu and Becker, 2003). Estrogen or amphetamine/saline was not administered during drug holidays. In total, all of the experimental rats received 12 injections of EB paired with either amphetamine or saline during the 3-week period. Immediately after receiving each injection of EB, the rats were placed into the paced mating arena with access to all three compartments. To evaluate locomotor sensitization, arena crossings were recorded on the first and final day of injections (Days 1 and 12). On Days 1 and 12, locomotor activity was recorded for 5 min immediately *before* the administration of amphetamine/saline (baseline activity test). Locomotor activity was also measured for the 30 min immediately *following* amphetamine/saline injections (drug activity test). This measure of sensitization was used instead of an amphetamine challenge because it has been shown that even a single injection of amphetamine can produce sensitization of the dopamine system (Robinson, 1984). Therefore, to ensure that the experimental rats receiving saline were not sensitized for the paced mating test, no challenge dose of amphetamine was administered. The experimental rats were primed with EB + P and tested for paced mating behavior 6 and 28 days after the final drug injections. These time points were chosen to evaluate both the early and late phases of sensitization development. Based on the findings in male rats (Fiorino and Phillips, 1999a,b), repeated exposure to amphetamine was predicted to increase the incentive value of sexual stimulation, operationally defined as a reduction in contact–return latencies following sexual stimulation (Erskine, 1992).

2.3.7.2. High vs. low responders to novelty. A growing body of literature suggests that individual differences in sensitivity to novelty predict differences in sensitivity to psychomotor stimulants in terms of self-administration and locomotor stimulating effects (Bardo et al., 1999; Piazza et al., 1990). In addition, response to novelty has also been shown to predict reactivity to stressors (Bardo et al., 1999; Bevins et al., 1997; Piazza et al., 1990) and acquisition of schedule-induced polydipsia and conditioned feeding-induced activity (Hooks et al., 1994). It is not known whether these differences in novelty responses predict differences in responses to other natural rewards, such as sex. Therefore, before any mating tests in Experiment 4, response to novelty

was evaluated to determine whether individual differences in reactivity to novelty predict individual differences in paced mating behavior. Response to novelty was defined as the number of arena crossings made during the first 15-min exposure to the paced mating arena (i.e., the first acclimation session). The experimental rats were classified as high and low responders to novelty based on a median split. Behaviors displayed on the baseline paced mating test and baseline locomotor activity on Day 1 of drug injections were analyzed using this grouping factor to determine whether the reaction to novelty predicted individual differences in paced mating behavior or locomotor activity.

2.4. Data analysis

Paced mating behavioral data (Experiments 1, 2, 4) were analyzed separately within the baseline and drug tests for a main effect of drug treatment group. A full factorial ANOVA comparing the behavioral data between drug treatment groups and across stimuli could not be conducted because an insufficient number of rats in both treatment groups received all three types of stimulation. Therefore, one-way ANOVAs on contact–return latencies and percentage of exits (mounts, intromissions, and ejaculations separately), LQ, LR, percentage of test time spent with the male, the rate of proceptive behaviors (hops, darts, and ear wiggling), the rate of rejection behaviors (kicks and defensive postures), test duration, the rate of arena crossings, and body weight were also calculated. 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. Student–Newman–Keuls tests were used for post hoc analyses.

Partner preference (Experiment 3) was evaluated by calculating a preference score for time spent near the sexually active male [time with male/(time with male + time with female)]. A social preference score was also calculated to evaluate preference to spend time near 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 on the male preference score, social preference score and locomotor activity. Because the baseline partner preference test was conducted to match groups for preferences, these data are not reported.

Sensitization (Experiment 4) was evaluated using a repeated measures ANOVA (Drug Group × Day) to compare arena crossings made during the first and the final day of drug injections. Baseline and drug locomotor activity data were analyzed separately. Simple main effects were used for post hoc analysis of significant interactions between drug group and the repeated measure factor day. Response to novelty was determined by calculating the median split in rate of arena crossings during the initial 15-min acclimation exposure to the paced mating arenas. The rats were classified as high responders if they fell above the median split and low responders if they fell below (Exner and Clark, 1993; Hooks et al., 1991; Piazza et al., 1990; Pierre and Vezina, 1997). Baseline paced mating behaviors and arena crossings during the baseline activity test were analyzed using one-way ANOVAs with the groups from the median split as a factor. The alpha level was set at $P < .05$.

3. Results

3.1. Experiment 1: Acute amphetamine and paced mating behavior

There were no significant differences between the drug treatment groups on contact–return latencies [F's < 2.1] (Fig. 1A). There was a significant main effect of drug group on percentage of exits following mounts [$F(1,39) = 10.68$, $P < .002$] and following intromissions [$F(1,45) = 4.63$, $P < .04$] (Fig. 1B). Rats receiving amphetamine were more likely than rats receiving saline to exit the male rat's compartment after mounts and after intromissions. There

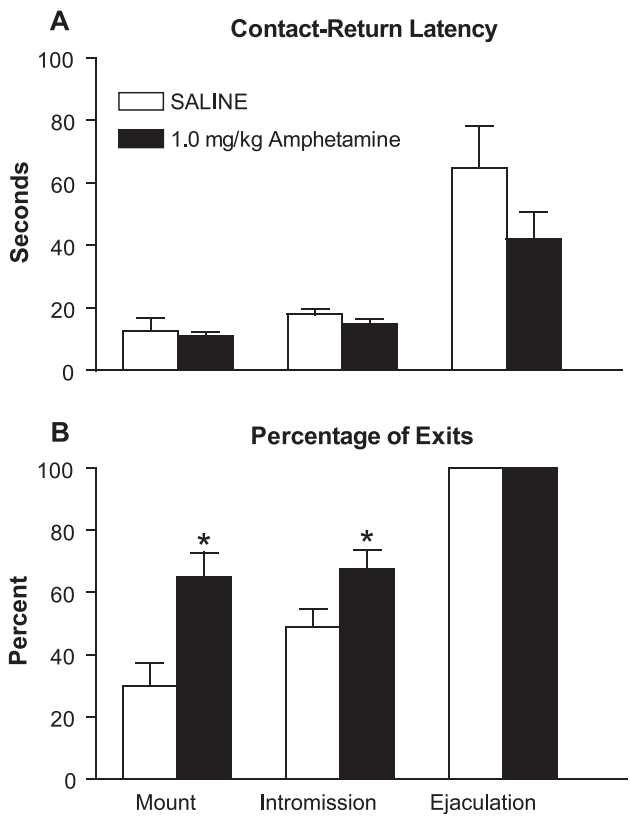

Fig. 1. Amphetamine did not affect the latency to return to the male rat following sexual stimulation (A: saline, $n = 13–24$; Amphetamine, $n = 18–23$), but did increase the likelihood of withdrawing from the male following mounts and intromissions (B: saline, $n = 19–24$; Amphetamine, $n = 20–23$) during the paced mating test. Data are expressed as means ± S.E.M. n = range of the number of rats in each group that received each type of stimulation. * Significantly different from saline ($P < .05$).

Table 1
Behaviors observed during paced mating tests

	LQ	Rejection behaviors/min	Proceptive behaviors/min	Arena crossings/min	Test duration (s)	Percent of time with male
Experiment 1						
Saline ($n=23$)	99.5 ± 0.5	0.05 ± 0.03	2.0 ± 0.4	4.13 ± 0.4	362.6 ± 56.4	64.4 ± 4.2
1.0 mg/kg amphetamine ($n=24$)	97.2 ± 1.6	0.09 ± 0.05	1.6 ± 0.5	6.63 ± 0.8*	454.7 ± 4 9.0	51.4 ± 4.3*
Experiment 2						
Saline ($n=10$)	98.3 ± 1.7	0.03 ± 0.02	2.5 ± 0.7	4.0 ± 0.7	389.2 ± 132.4	68.7 ± 4.7
0.5 mg/kg amphetamine ($n=10$)	96.2 ± 2.7	0.03 ± 0.02	2.4 ± 0.5	4.6 ± 0.8	407.6 ± 55.3	58.7 ± 5.1
1.0 mg/kg amphetamine ($n=10$)	99.3 ± 0.7	0.01 ± 0.01	1.2 ± 0.3	5.4 ± 0.8	514.9 ± 70.0	41.9 ± 4.7*
2.0 mg/kg amphetamine ($n=10$)	84.1 ± 4.2*	0.1 ± 0.01	0.3 ± 0.1*	5.2 ± 0.6	630.2 ± 90.3	53.7 ± 4.1*
Experiment 4						
Saline-repeated ($n=14$)	99.4 ± 0.6	0.03 ± 0.01	4.9 ± 1.0	5.0 ± 0.5	660.8 ± 84.0	27.7 ± 5.5
Amphetamine-repeated ($n=13$)	100 ± 0.0	0.02 ± 0.01	3.8 ± 0.6	4.8 ± 0.8	903.8 ± 128.0	26.5 ± 4.9

Data are expressed as means ± S.E.M.
* Significant difference compared to the saline group ($P<.05$).

was also a significant main effect of drug group on percentage of test time spent with the male [$F(1,45)=4.66$, $P<.04$] (Table 1). Rats receiving amphetamine spent significantly less of the test time with the male compared to the rats receiving saline. Finally, there was a significant main effect of drug group on the rate of arena crossings [$F(1,46)=8.24$, $P<.006$], indicating that rats receiving amphetamine made significantly more arena crossings per minute than the rats receiving saline. No other statistically significant differences were observed between the drug treatment groups.

3.2. Experiment 2: Dose response of acute amphetamine and paced mating behavior

There were no significant differences between the drug treatment groups on contact–return latencies [F's <1.8] (Fig. 2A). There was a significant main effect of drug

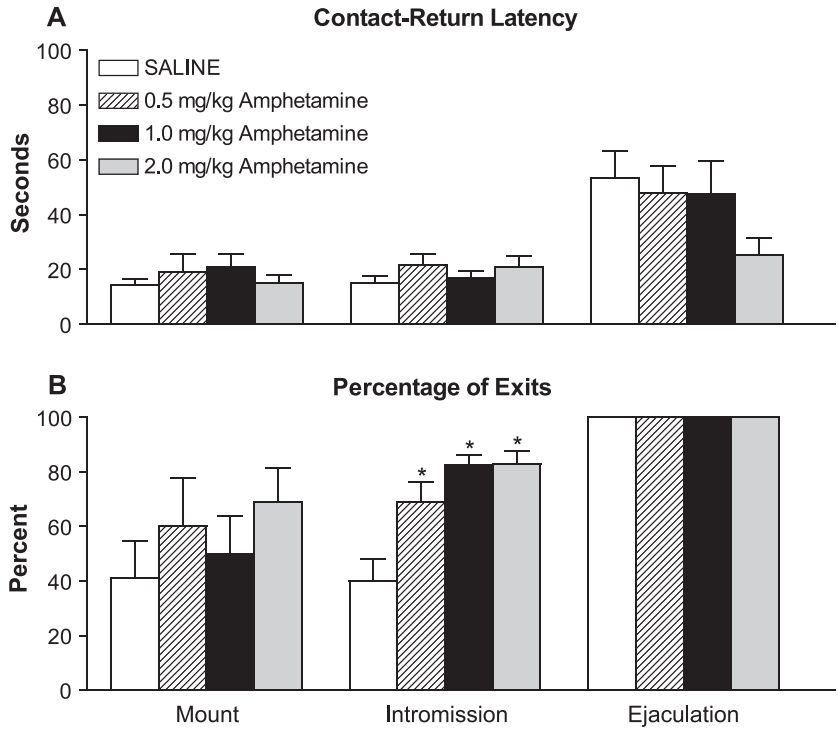

Fig. 2. Contact–return latencies were unaffected by amphetamine (A: saline, $n=6$–10; 0.5 mg/kg, $n=6$–10; 1.0 mg/kg, $n=8$–10; 2.0 mg/kg, $n=6$–10), whereas all doses of amphetamine tested increased the likelihood of withdrawing from the male following intromissions (B: saline, $n=9$–10; 0.5 mg/kg, $n=6$–10; 1.0 mg/kg, $n=9$–10; 2.0 mg/kg, $n=8$–10). * Significantly different from saline ($P<.05$).

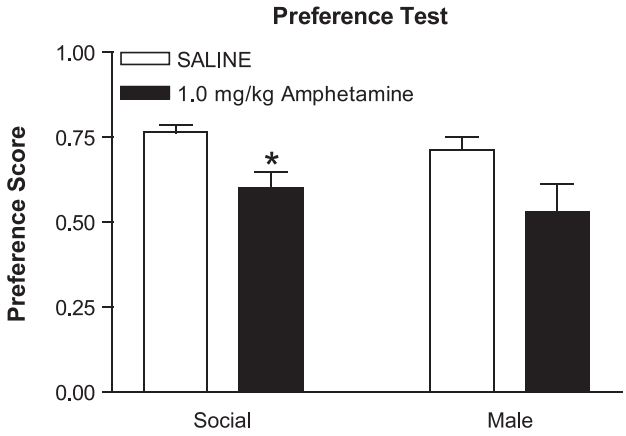

Fig. 3. Acute exposure to amphetamine reduced the social preference score (sexually active male + estrous female/total test time) and tended to reduce the male preference score (sexually active male time/sexually active male time + estrous female time) (saline, $n=10$; 1.0 mg/kg, $n=8$). * Significantly different from saline ($P<.05$).

group on percentage of exits following intromissions [$F(3,36)=10.23$, $P<.0001$] (Fig. 2B). Rats receiving any dose of amphetamine were more likely than rats receiving saline to exit the male rat's compartment after intromissions. There was also a significant main effect of drug group on LQ [$F(3,36)=7.00$, $P<.001$] (Table 1). Consistent with previous findings (Michanek and Meyerson, 1977), rats receiving 2.0 mg/kg of amphetamine displayed significantly reduced levels of sexual receptivity compared to all other groups. There was a significant main effect of drug group on proceptive behaviors [$F(3,35)=5.00$, $P<.005$]. Post hoc analyses indicated that rats receiving 2.0 mg/kg of amphetamine displayed significantly less proceptive behaviors per minute compared to the rats receiving saline or 0.5 mg/kg of amphetamine. Finally, there was a significant main effect of drug group on percentage of test time spent with the male [$F(3,36)=5.67$, $P<.004$]. The rats receiving 1.0 mg/kg amphetamine spent less of the test time with the male compared to the rats receiving saline. No other group differences were observed.

3.3. Experiment 3: Acute amphetamine and partner preference

The MANOVA revealed a significant effect of drug group on social preference, male preference and arena crossings [$F(3,14)=6.36$, $P<.006$]. The rats receiving amphetamine spent significantly less time near the stimulus rats than the saline-treated rats [$F(1,16)=10.95$, $P<.004$] (Fig. 3). The reduction in preference for the male in the amphetamine-treated rats only approached statistical significance [$F(1,16)=4.15$, $P=.05$]. Amphetamine-treated rats made significantly more arena crossings (5.6 ± 0.5 vs. 3.9 ± 0.5 crossings/min) compared to the saline-treated rats [$F(1,16)=5.86$, $P<.03$]. Few proceptive behaviors were observed (data not shown).

3.4. Experiment 4: Repeated amphetamine and paced mating behavior

3.4.1. Sensitization

There was a significant main effect of day on arena crossings made during the 5-min baseline activity test [$F(1,25)=7.80$, $P<.01$] (Fig. 4A). Both groups of rats made significantly more arena crossings on the final day of injections when compared to the first day.

A significant main effect of day [$F(1,25)=39.11$, $P<.0001$], drug group [$F(1,25)=15.41$, $P<.05$] and interaction of day by drug group [$F(1,25)=21.06$, $P<.0001$] were observed on arena crossings made during the 30-min drug activity test (Fig. 4B). On the final day of injections, the rats receiving repeated amphetamine made significantly

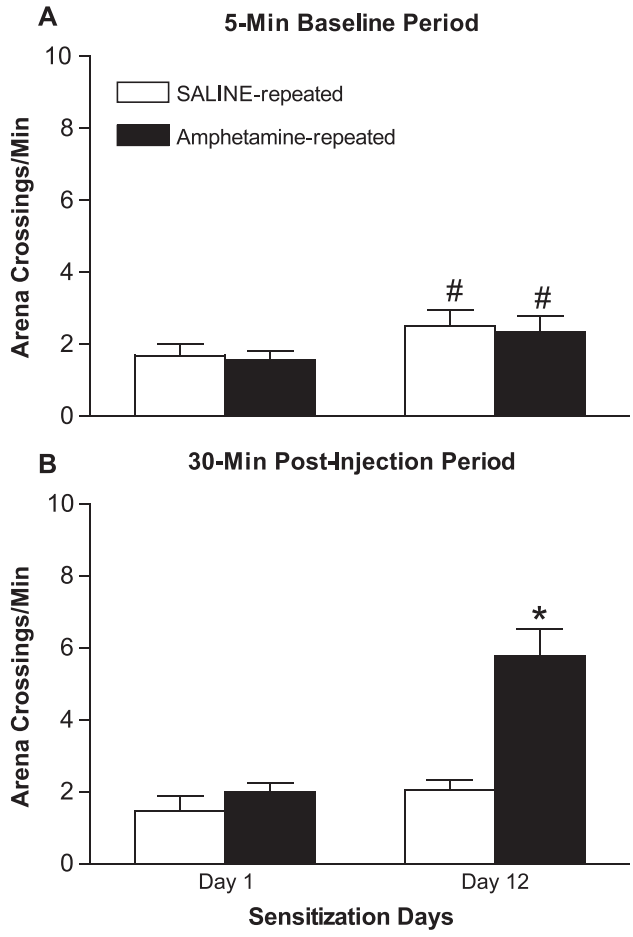

Fig. 4. Repeated, intermittent exposure to amphetamine or saline increased arena crossings during the baseline period 5 min before drug injections on Day 12 (A: saline-repeated, $n=13$; Amphetamine-repeated, $n=13$). On the test conducted 30 min following drug injections, only repeated exposure to amphetamine increased arena crossings on Day 12. * Significantly different from saline ($P<.05$). #Significantly different from Day 1 ($P<.05$).

more arena crossings than the rats receiving repeated saline injections [$F(1,25) = 56.53$, $P < .0001$].

3.4.2. Paced mating behavior

There was a significant main effect of drug treatment group on contact–return latencies following mounts [$F(1,21) = 7.05$, $P < .02$] (Fig. 5A) on the paced mating test conducted 1 week after the final drug injections. The rats receiving repeated amphetamine returned to the male rat's compartment more quickly after mounts than the rats receiving saline. No other statistically significant differences were observed between the groups (Table 1). No drug treatment group effects were observed 4 weeks after the final drug injections (data not shown).

3.4.3. High vs. low responders to novelty

There was a significant main effect of group on arena crossings made during the 5-min baseline activity test on Day 1 [$F(1,25) = 8.95$, $P < .006$] of the drug injections. The high responders made almost twice as many arena crossings than the low responders on Day 1 (10.8 ± 1.4 vs. 5.2 ± 1.2). No other significant differences were observed between the high and low responders (data not shown).

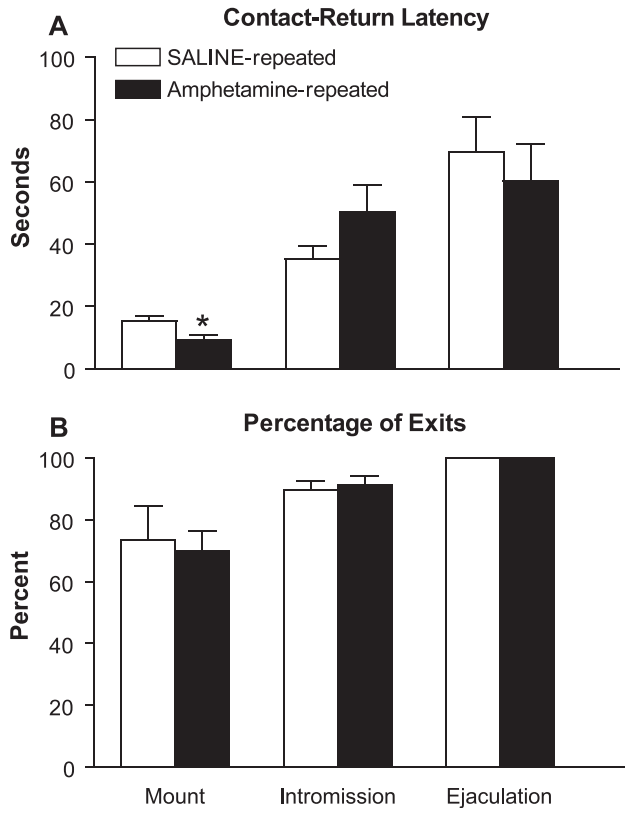

Fig. 5. Repeated exposure to amphetamine reduced contact–return latencies following mounts (A: saline-repeated, $n = 10–13$; Amphetamine-repeated, $n = 12–13$), whereas percentage of exits (B: saline-repeated, $n = 12–13$; Amphetamine-repeated, $n = 12–13$) were unaffected during the paced mating test conducted 1 week following the final drug injections. * Significantly different from saline ($P < .05$).

4. Discussion

The results of the present study demonstrate that in OVX hormone-primed rats, amphetamine alters the pattern of paced mating behavior. Female rats tested under the influence of 1.0 mg/kg amphetamine are more likely than rats receiving saline to exit a male rat's compartment after receiving a mount or an intromission (Experiment 1). In Experiment 2, the increase in percentage of exits is accompanied by a reduced level of sexual receptivity and lower rate of proceptive behaviors in rats receiving a higher dose of amphetamine (2.0 mg/kg). In Experiment 3, acute amphetamine reduced both the male and social preference scores in a partner preference test. To minimize the confounding effects of the locomotor stimulating effects of amphetamine on paced mating behavior, a group of female rats was treated repeatedly with amphetamine and then tested for paced mating behavior, drug free after a 1- and 4-week-long abstinence period (Experiment 4). Although repeated treatment with amphetamine did not affect the likelihood of exiting the male's compartment following sexual stimulation, the latency to return to the male following mounts was shorter in rats receiving repeated amphetamine than in the rats receiving saline when tested a week after the final drug injections. Finally, response to novelty did not predict differences in paced mating behavior.

4.1. Acute effects of amphetamine

The acute effects of amphetamine observed on paced mating behavior in Experiments 1 and 2 (i.e., an increase in percentage of exits) may reflect the locomotor-stimulating effects of amphetamine. That is, increases in locomotion produced increases in withdrawal responses. However, several observations suggest that not all of the effects of acute amphetamine on paced mating behavior can be attributed to locomotor activity alone. First, the lowest dose of amphetamine tested (0.5 mg/kg) increased withdrawal responses during paced mating behavior despite the lack of effect observed on locomotor activity. Second, the rats receiving 2.0 mg/kg also displayed less proceptive behaviors and reduced sexual receptivity, behavioral changes that are independent of locomotor activity. Third, rats receiving 1.0 mg/kg amphetamine also spent less time with the male rat (Experiments 1 and 2), consistent with a reduction in sexual motivation. These results taken together suggest that acute amphetamine administration may have reduced a sexually receptive female's motivation to interact with a male (increased likelihood of withdrawal responses, spent less time with the male, displayed less proceptive behaviors, and reduced sexual receptivity). However, the effects of amphetamine that may be independent of locomotor activity effects may not be entirely sexual in nature. Data from pilot studies indicate that when given the opportunity to interact with one other rat, rats receiving

amphetamine spend significantly more time alone regardless of the stimulus rat's sex (unpublished results). In Experiment 3, rats receiving amphetamine spent more time alone than their saline-treated counterparts. The decrease in social preference observed in Experiment 3 and the reduction in time spent interacting with a male or female rat in pilot studies are consistent with the observation that amphetamine decreases social behaviors (File and Hyde, 1979).

Alterations in locomotor activity as measured by arena crossings in paced mating arenas were not always observed following amphetamine administration in the present study. For example, the rats receiving any dose of amphetamine in Experiment 2 did not demonstrate a significant increase in arena crossings when compared to rats receiving saline. One possible explanation for this finding is that EB + P-primed rats exhibit an elevated level of locomotor activity during paced mating behavior. Results from a pilot study indicate that sexually receptive rats permitted to interact with a male in a paced mating arena make more arena crossings (3.0 crossings/min) than sexually receptive female rats permitted to interact with a stimulus female in a paced mating arena (1.5 crossings/min; unpublished findings). Although administration of 1.0 mg/kg amphetamine in this pilot study increased arena crossings in both groups (5.3 and 3.6 crossings/min, respectively), the magnitude was greater when the experimental rat was tested with a stimulus female. Therefore, locomotor activity levels, tested under conditions that allow for paced mating approach a ceiling. The results of Experiment 4 are consistent with this interpretation. After 12 injections of amphetamine, the sensitized locomotor response (when mating is *not* possible) produced a level of locomotor activity (5.7 crosses/min) comparable to that observed during paced mating behavior (Experiments 1, 2, and 4: 4.4–6.6 crosses/min with and without amphetamine).

Understanding how alterations in locomotor behavior are reflected as changes in specific measures of mating behavior is necessary to interpret the results from experiments that include manipulations that alter general levels of locomotor activity. The current study is the first to demonstrate that the sensitized locomotor response to amphetamine and the locomotor response observed during paced mating behavior are similar, at, or near a ceiling of locomotor activity when measured in paced mating arenas. Recently, it has been shown that unconditioned sexual incentive motivation in the male rat is *not affected* by amphetamine (Agmo, 2003), although previous studies found that amphetamine shortened mount and intromission latencies in sexually naïve males (Agmo and Picker, 1990). Although changes in locomotor activity cannot easily be separated from changes in approach behaviors, the more information we have about how locomotor activity alters or confounds the measures of female sexual behavior, the better we will understand the components of paced mating behavior.

4.2. Amphetamine sensitization

A paradigm that has been useful in dissociating the locomotor and motivational effects of amphetamine involves repeated intermittent exposure to amphetamine followed by a period of abstinence, and then behavioral tests in a drug-free state (i.e., the sensitization paradigm). Sensitization of the dopaminergic system has been shown to augment the incentive value of other reinforcers without the confounding influence of locomotor effects (Fiorino and Phillips, 1999a,b). Specifically, repeated intermittent exposure to amphetamine facilitates sexual behavior in males, such that the latency to mount is shorter and number of ejaculations is greater in sexually naïve male rats exposed to 4 weeks of amphetamine and tested for mating behavior drug free, than in nonsensitized rats (Fiorino and Phillips, 1999a,b). We hypothesized that repeated amphetamine 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). In Experiment 4, we found that rats treated repeatedly with amphetamine displayed shorter contact–return latencies after mounts than saline-treated rats, when tested drug free, 1 week after the final drug injections. The results of Experiment 4 are consistent with the results from studies of male sexual behavior illustrating that amphetamine can facilitate the incentive value of sexual stimulation (Fiorino and Phillips, 1999a,b). It is possible that contact–return latencies following intromissions and ejaculations were not affected in Experiment 4 because vaginocervical stimulation renders these more intense sexual stimuli more aversive. This is indicated by the longer contact–return latencies typically observed following ejaculations as well as the further lengthening of contact–return latencies following intromissions and ejaculations throughout the course of a mating episode (Coopersmith et al., 1996; Peirce and Nuttal, 1961). Unlike repeated amphetamine effects on male sexual behavior (Fiorino and Phillips, 1999a,b), when dopamine sensitization in the NAc is greatest (at least 14 days after final injection; Kalivas et al., 1993; Robinson and Becker, 1986), the effects of repeated amphetamine treatment on paced mating behavior are no longer evident (Experiment 4, 28-day test). Because percentage of exits were close to maximum in both the saline- and amphetamine-treated groups following repeated injections, we were unable to evaluate any effects of repeated amphetamine administration on percentage of exits.

4.3. Individual differences

Individual differences in response to novelty are related to differences in goal-directed behaviors (e.g., drug self-administration; Bardo et al., 1999), therefore in Experiment 4, we tested whether individual differences in response to novelty predicted differences in paced mating behavior.

Although differences in locomotor activity between the high and low responders continued to be observed after two exposures to the paced mating arenas on Day 1 of injections, no group differences in paced mating behavior were observed. Not all goal-directed behaviors have been shown to be sensitive to this distinction in response to novelty. For example, response to novelty does not predict differences in conditioned place preference associated with amphetamine administration (Erb and Parker, 1994). One possibility that Erb and Parker (1994) discuss is that high baseline levels of locomotor activity in the high responders may be particularly advantageous for acquiring active behaviors (i.e., drug self-administration), whereas high baseline levels of locomotor activity may be detrimental for acquiring passive behaviors. Because paced mating behavior requires both active (exits and approaches) and passive behaviors (time away), the effect of a high baseline level of locomotor activity on paced mating behavior may have been masked, thus adding support to the possibility that individual differences in response to novelty do not reflect differential sensitivity to reward.

5. Mechanisms

The effects of acute and repeated intermittent amphetamine on paced mating behavior may reflect changes in dopamine neurotransmission in specific forebrain regions. As mentioned previously, extracellular levels of dopamine increase in the NAc during paced mating behavior in the female rat (Becker et al., 2001b; Mermelstein and Becker, 1995). The increase in the percentage of exits in rats receiving amphetamine observed in the present study is similar to the effects of the NAc core lesions on paced mating behavior, that is, an increase in withdrawal from the male following less intense sexual stimulation (Guarraci et al., 2002). Collectively, these results suggest that augmented dopamine transmission in regions of NAc (e.g., shell) that were spared in the previous experiment (Guarraci et al., 2002) are involved in producing withdrawal from the male following sexual stimulation. In contrast, the time course of repeated amphetamine effects on paced mating behavior does not appear to coincide with the time course of dopamine sensitization in the NAc. Contact–return latencies were affected by repeated amphetamine exposure after a week of abstinence but not after 4 weeks, a time when dopamine sensitization in the NAc occurs (Kalivas et al., 1993; Robinson and Becker, 1986). More research will be necessary to understand the mechanisms involved in producing the short-lived effects of repeated amphetamine on paced mating behavior.

In conclusion, the acute effects of amphetamine on paced mating behavior and partner preference may reflect both the locomotor-stimulating effects of amphetamine as well as a reduction in sexual and social behaviors. In female rats treated repeatedly with amphetamine and tested drug free, there is a suggestion that approach behavior is altered. 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.

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References

Agmo A. Lack of opioid or dopaminergic effects on unconditioned sexual incentive motivation in male rats. Behav Neurosci 2003;117:55–68.

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

Bardo MT, Valone JM, Bevins RA. Locomotion and conditioned place preference produced by acute intravenous amphetamine: role of dopamine receptors and individual differences in amphetamine self-administration. Psychopharmacology (Berl) 1999;143:39–46.

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

Beatty WW, Dodge AM, Dodge LJ, White K, Panksepp J. Psychomotor stimulants, social deprivation and play in juvenile rats. Pharmacol Biochem Behav 1982;16:417–22.

Becker JB, Molenda H, Hummer DL. Gender differences in the behavioral responses to cocaine and amphetamine. Implications for mechanisms mediating gender differences in drug abuse. Ann N Y Acad Sci 2001a; 937:172–87.

Becker JB, Rudick CN, Jenkins WJ. The role of dopamine in the nucleus accumbens and striatum during sexual behavior in the female rat. J Neurosci 2001b;21:3236–41.

Bevins RA, Klebaur JE, Bardo MT. Individual differences in response to novelty, amphetamine-induced activity and drug discrimination in rats. Behav Pharmacol 1997;8:113–23.

Blaustein JD, Erskine MS. Feminine sexual behavior: cellular integration of hormonal and afferent information in the rodent forebrain. Hormones, Brain and Behavior, vol. 1. San Diego, CA: Elsevier Science; 2002. p. 139–213.

Bradley KC, Meisel RL. Sexual behavior induction of c-Fos in the nucleus accumbens and amphetamine-stimulated locomotor activity are sensitized by previous sexual experience in female Syrian hamsters. J Neurosci 2001;21:2123–30.

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.

Clark AS, Kelton MC, Guarraci FA, Clyons EQ. Hormone and test condition, but not sexual experience, modulate partner preference in female rats. Society for Neuroscience 2003;33:402.12.

Coopersmith C, Candurra C, Erskine MS. Effects of paced mating and intromissive stimulation on feminine sexual behavior and estrus termination in the cycling rat. J Comp Psychol 1996;110:176–86.

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.

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

Erb SM, Parker LA. Individual differences in novelty-induced activity do

not predict strength of amphetamine-induced place conditioning. Pharmacol Biochem Behav 1994;48:581–6.
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.
Erskine MS. Prolactin release after mating and genitosensory stimulation in females. Endocr Rev 1995;16:508–28.
Everitt BJ, Stacey P. Studies of instrumental behavior with sexual reinforcement in male rats (*Rattus norvegicus*): II. Effects of preoptic area lesions, castration, and testosterone. J Comp Psychol 1987;101:407–19.
Exner M, Clark D. Behaviour in the novel environment predicts responsiveness to *d*-amphetamine in the rat: a multivariate approach. Behav Pharmacol 1993;4:47–56.
File SE, Hyde JR. A test of anxiety that distinguishes between the actions of benzodiazepines and those of other minor tranquilisers and of stimulants. Pharmacol Biochem Behav 1979;11:65–9.
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.
Goudie AJ. Aversive stimulus properties of drugs. Neuropharmacology 1979;18:971–9.
Grilly DM, Loveland A. What is a ''low dose'' of *d*-amphetamine for inducing behavioral effects in laboratory rats? Psychopharmacology 2001;153:155–69.
Guarraci FA, Megroz AB, Clark AS. Effects of ibotenic acid lesions of the nucleus accumbens on paced mating behavior in the female rat. Behav Neurosci 2002;116:568–76.
Guy AP, Gardner CR. Pharmacological characterisation of a modified social interaction model of anxiety in the rat. Neuropsychobiology 1985;13:194–200.
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;78:400–88.
Hooks MS, Jones GH, Smith AD, Neill DB, Justice JB. Individual differences in locomotor activity and sensitization. Pharmacol Biochem Behav 1991;38:467–70.
Hooks MS, Jones GH, Juncos JL, Neill DB, Justice JB. Individual differences in schedule-induced and conditioned behaviors. Behav Brain Res 1994;60:199–209.
Horger BA, Giles MK, Schenk S. Preexposure to amphetamine and nicotine predisposes rats to self-administer a low dose of cocaine. Psychopharmacology 1992;107:271–6.
Hu M, Becker JB. Effects of sex and estrogen on behavioral sensitization to cocaine in rats. J Neurosci 2003;23:693–9.
Jenkins WJ, Becker JB. Role of the striatum and nucleus accumbens in paced copulatory behavior in the female rat. Behav Brain Res 2001;121:119–28.
Jones SR, Garris PA, Kilts CD, Wightman RM. Comparison of dopamine uptake in the basolateral amygdaloid nucleus, caudate-putamen, and nucleus accumbens of the rat. J Neurochem 1995;64:2581–9.
Kalivas PW, Sorg BA, Hooks MS. The pharmacology and neural circuitry of sensitization to psychostimulants. Behav Pharmacol 1993;4:315–34.
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.
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.
Mermelstein PG, Becker JB. Increased extracellular dopamine in the nucleus accumbens and striatum of the female rat during paced copulatory behavior. Behav Neurosci 1995;109:354–65.
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.
Nocjar C, Panksepp J. Chronic intermittent amphetamine pretreatment enhances future appetitive behavior for drug- and natural-reward: interaction with environmental variables. Behav Brain Res 2002;128:189–203.
Peirce JT, Nuttal RL. Self-paced sexual behavior in the female rat. J Comp Physiol Psychol 1961;54:310–3.
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.
Pierre PJ, Vezina P. Predisposition to self-administer amphetamine: the contribution of response to novelty and prior exposure to the drug. Psychopharmacology 1997;129:277–84.
Public Health Services. U.S. guide for the care and use of laboratory animals. Institute of Laboratory Animal Resources Commission on Life Sciences. Washington, DC: National Academy Press; 1996.
Rivas FJ, Mir D. Effects of nucleus accumbens lesion on female rat sexual receptivity and proceptivity in a partner preference paradigm. Behav Brain Res 1990;41:239–49.
Robinson TE. Behavioral sensitization: characterization of enduring changes in rotational behavior produced by intermittent injections of amphetamine in male and female rats. Psychopharmacology 1984;84:466–75.
Robinson TE, Becker JB. Enduring changes in brain and behavior produced by chronic amphetamine administration: a review and evaluation of animal models of amphetamine psychosis. Brain Res 1986;396:157–98.
Schmitz Y, Lee CJ, Schmauss C, Gonon F, Sulzer D. Amphetamine distorts stimulation-dependent dopamine overflow: effects on D2 autoreceptors, transporters, and synaptic vesicle stores. J Neurosci 2001;21:5916–24.
Valadez A, Schenk S. Persistence of the ability of amphetamine preexposure to facilitate acquisition of cocaine self-administration. Pharmacol Biochem Behav 1994;47:203–5.
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.