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Intracranial infusions of amphetamine into the medial preoptic area but not the nucleus accumbens affect paced mating behavior in female rats

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Abstract

The present study evaluated the effects of intracranial administration of amphetamine (AMPH) on paced mating behavior and open field activity in sexually receptive female rats. In Experiment 1, AMPH (0.5 μl of 10 μg/μl) or vehicle was infused bilaterally into the medial preoptic area (mPOA). In Experiments 2 and 3, AMPH (0.5 μl of 40 μg/μl) or vehicle was infused bilaterally into the shell region of the nucleus accumbens (NAc) or core region of the NAc, respectively. In Experiment 1, infusions of AMPH into the mPOA increased the latency to return to the male following sexual stimulation without affecting locomotor activity in the open field test. However, when AMPH was infused 3.0 mm dorsal to the mPOA, no effects were observed. In Experiments 2 and 3, infusions of AMPH into the NAc shell or core significantly increased locomotor activity during the open field test but failed to affect most measures of paced mating behavior. Together these results suggest that amphetamine-stimulate dopamine release in the mPOA but not in the NAc alters paced mating behavior, confirming previous conclusions that the mPOA plays a critical role in female sexual behavior, whereas the NAc plays a relatively limited role.

Keywords: Sexual behavior; Dopamine; Sexual arousal; Motivation; Core; Shell; Locomotor behavior; Open field activity

1. Introduction

In female rats, sexual behavior can be 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 behaviors including hopping, darting, ear wiggling, and pacing of sexual stimulation. When given the opportunity, a sexually receptive female 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 of behavior is known as paced mating behavior (Blaustein and Erskine, 2002; Erskine, 1989).

The medial preoptic area of the hypothalamus (mPOA) and the nucleus accumbens (NAc) have been implicated in the control of female mating behavior. For example, lesions of the mPOA profoundly affect paced mating behavior. Electrolytic and neurotoxic lesions of the mPOA increase the latency for a sexually receptive female rat to return to a sexually vigorous male following sexual stimulation (Guarraci et al., 2004; Yang and Clemens, 2000). Furthermore, lesions of the mPOA also decrease the display of solicitation behaviors (e.g., hops and ear wiggles) but increase the display of rejection behaviors (e.g., kicking, rolling, boxing) (Guarraci et al., 2004; Hoshina et al., 1994). Finally, female rats with mPOA lesions fail to prefer a sexually vigorous male rat over a female rat during partner preference tests that allow mating or not (Guarraci and Clark, 2006).

Although not as effective as mPOA lesions, lesions of the NAc also alter the pattern of female mating behavior. Female rats with electrolytic lesions of the NAc fail to prefer a sexually

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vigorous male rat when given the opportunity to spend time with either a female rat or a male rat only when mating is possible, as well as display more rejection behaviors in response to male mount attempts (Rivas and Mir, 1991, 1990). In addition, lesions of the NAc alter paced mating behavior. Specifically, lesions of the NAc core, one of three anatomically distinct regions of the NAc (for review, (Heimer et al., 1995), increase the likelihood of withdrawing from the male rat after receiving mounts during paced mating behavior (Guarraci et al., 2002). Together, these results suggest that the mPOA and the NAc may be important for a female rat's tolerance of male sexual stimulation.

Additional findings suggest that dopamine, in particular dopamine in the NAc, may be involved in female mating behavior. Mermelstein and Becker (1995) reported that paced mating behavior is accompanied by increases in extracellular dopamine in the NAc and caudate putamen. Pfaus et al. (1995) found that presentation of a sexual partner, as well as copulation increase extracellular dopamine in the NAc. Furthermore, dopamine release in the NAc is sensitive to the individual female rat's preferred pacing interval (Jenkins and Becker, 2003).

Less is known about the role of incertohypothalimic dopamine than mesolimbic dopamine in female mating behavior. In female rats, extracellular dopamine in the mPOA increases significantly during non-paced copulatory behavior when compared to concentrations of dopamine measured during baseline or during paced copulatory behavior (Matuszewich et al., 2000). Unlike dopamine in the NAc (Pfaus et al., 1995), dopamine in the mPOA does not increase in response to the presentation of a sexual partner (Matuszewich et al., 2000). Furthermore, intra-mPOA infusions of the dopamine agonist apomorphine enhance sexual receptivity, whereas infusions of dopamine antagonists haloperidol or flupenthixol disrupt lordosis (Foreman and Moss, 1979).

Effects of systemic administration of the dopamine agonist amphetamine on female mating behavior are mixed. Moderate acute doses of amphetamine increase the likelihood of withdrawing from the male rat after receiving mounts and intromissions and decrease solicitation behaviors during paced mating behavior (Guarraci and Clark, 2003). Moderate to high acute doses of amphetamine disrupt the display of the lordosis posture (Michanek and Meyerson, 1977). Chronic exposure to amphetamine also alters paced mating behavior. Specifically, female rats that are sensitized to amphetamine return to the male rat faster after receiving mounts than rats that are not sensitized (Guarraci and Clark, 2003).

The present study was designed to localize the brain regions where systemic amphetamine may be acting and to evaluate the functional significance of dopamine release during paced mating behavior. Female rats received acute bilateral intracranial infusions of amphetamine into the mPOA or the NAc prior to a test of paced mating behavior. Because the NAc is comprised of anatomically distinct regions (Heimer et al., 1995) and lesions of the NAc core not the NAc shell affected paced mating behavior, different groups of rats received amphetamine infusions into the shell and core. As a behavioral control, open field activity was observed immediately following the mating test.

2. Methods

2.1. Animals

Eighty-seven (Experiment 1A mPOA $n=37$; 1B $n=10$; Experiment 2 NAc shell $n=18$; Experiment 3 NAc core $n=22$) naïve female Long–Evans rats (*Rattus norvegicus*) weighing between 200 and 250 g were used as experimental rats (Harlan Sprague Dawley, Indianapolis, IN). During mating tests, sexually experienced male (350–500 g) and female (250–350 g) Long–Evans rats were used as stimulus rats. All rats were housed in hanging plastic cages, within a light- and temperature-controlled vivarium. The male rats were pair-housed upon arrival and remained in these pairings for the duration of the experiments. The experimental and female stimulus rats were housed three per cage. The vivarium was maintained on a 12:12-h light–dark cycle (lights off at 1000 h). All experimental procedures were conducted during the dark portion of the cycle under dim red light. Food and water were available ad libitum. All rats were weighed once a week. Prior to any mating tests, the experimental and female stimulus rats were ovariectomized (OVX) under sodium pentobarbital (50.0 mg/kg ip) anesthesia following pretreatment with atropine sulfate (2.5 mg), which reduces respiratory distress. All animal care guidelines were followed from the United States Public Health Service *Guide for the Care and Use of Laboratory Animals*, Public Health Services (Public Health Services, 1996), and monitored by the Southwestern University Institutional Animal Care and Use Committee.

2.2. Hormone priming

All 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. Hormones were dissolved in a sesame seed oil vehicle, and injections were administered subcutaneously into the flank. All hormones were purchased from the Sigma Chemical Company (St. Louis, MO). These doses of EB and P were chosen because they have been shown to stimulate high levels of sexual receptivity and paced mating behavior in OVX rats (Zipse et al., 2000).

2.3. Surgery

Bilateral cannulae were implanted under sodium pentobarbital (50.0 mg/kg ip) anesthesia following pretreatment with atropine sulfate (2.5 mg). In Experiment 1, double-barreled 22-gauge stainless steel guide cannulae (Plastics One, Roanoke, VA) were implanted 2.0 mm dorsal to the mPOA (Experiment 1A; coordinates: 0.6 mm posterior to bregma, ±0.5 mm lateral to midline, and 6.1 mm ventral to the surface of the skull). For Experiment 1B, dorsal controls were included to ensure that effects observed following infusions of amphetamine into the mPOA were specific and not due to diffusion of the

amphetamine into regions just dorsal to the mPOA. Therefore cannulae were implanted 4.0 mm dorsal to the mPOA in Experiment 1B (coordinates: 0.6 mm posterior to bregma, ±0.5 mm lateral to midline, and 4.1 mm ventral to the surface of the skull). In Experiment 2, double-barreled 22-gauge stainless steel guide cannulae (Plastics One, Roanoke, VA) were implanted 2.0 mm dorsal to the NAc shell (coordinates: 1.7 mm anterior to bregma, ±0.5 mm lateral to midline, and 5.6 mm ventral to the surface of the skull). In Experiment 3, double-barreled 22-gauge stainless steel guide cannulae (Plastics One, Roanoke, VA) were implanted 2.0 mm dorsal to the NAc core, (coordinates: 1.7 mm anterior to bregma, ±1.5 mm lateral to midline, and 4.5 mm ventral to the surface of the skull). Implanting the guide cannulae at least 2.0 mm dorsal to our target brain areas reduced the likelihood of tissue damage in the mPOA and NAc caused by the barrels of the cannulae. All coordinates were based on the rat brain atlas of Paxinos and Watson (1998). Cannulae were secured to the skull with 3 jewelers' screws (Plastics One, Roanoke, VA) and dental cement (Ortho-Jet, Lang Dental Mfg. Co., Inc, Wheeling, IL). After surgery, stylets cut flush with the guide cannulae and dust caps sealed the openings of the guide cannulae.

2.4. Behavioral procedure

2.4.1. Sexual receptivity

Approximately one week after ovariectomy, experimental rats were tested for sexual receptivity in a clear Plexiglas arena (30.5 cm long × 30.5 cm wide × 35.6 cm high) with wood shavings on the floor. A stimulus male was placed in the arena for 5 min with a hormone-primed OVX stimulus female rat to ensure his sexual vigor. Once the male rat's sexual vigor was ensured, a hormone-primed experimental rat was placed into the arena with the male rat. Once the experimental rat received 10 mounts with or without intromissions, the test was complete. The lordosis response (LR) of the experimental rats to each mount was scored on a 4-point scale (0–3) (Hardy and DeBold, 1972, 1971), and a lordosis quotient (LQ) was calculated by dividing the number of LRs greater than 2 (2 and 3 considered lordosis postures, 0–1 not considered lordosis postures) by the total number of sexual stimulations (e.g.,10 mounts with or without intromissions) and multiplied by 100. Any LR that was 0 or 1 diminished the magnitude of the LQ. For example, if a female rat displayed 9 LRs > 2 and 1 LR < 1, she received an LQ of 90%. All of the experimental rats were sexually receptive (LQ > 90) and therefore proceeded to paced mating behavioral testing.

2.4.2. Acclimation

All rats were acclimated to the mating arenas on two separate sessions, each lasting 15 min. The mating arena consisted of a Plexiglas arena (101.0 cm long × 37.0 cm wide × 32.0 cm high) sectioned into three equal compartments using clear Plexiglas dividers. Each divider had a rectangular opening (5.0 cm wide × 8.0 cm high) in each of the two bottom corners. Wood shavings covered the floor. During acclimation sessions for the stimulus male rats, a single male was placed into each of the side compartments of the mating arena and the males were tapped lightly on the nose if they attempted to venture through one of the openings in the divider. In contrast, the experimental and stimulus female rats were placed alone in the mating arena and allowed to roam freely between the three compartments during their acclimation sessions.

2.4.3. Baseline paced mating behavior

Approximately one week after sexual receptivity testing and acclimation, the experimental rats were tested for baseline paced mating behavior. Five minutes before the start of each mating test, an experimental rat was placed into the central compartment of the mating arena and confined with solid opaque dividers that covered the sidewalls (36.2 cm wide × 31.1 cm high). The opaque dividers prohibited the experimental rat from entering either of the adjacent compartments, one of which contained a stimulus male rat. The location of the stimulus male rat was randomly determined. The mating test began when the opaque divider separating the male and female rats was removed. Upon removal of the opaque partition, the experimental female rat was able to move freely between the center compartment and the outer compartment, whereas the male rat was confined to his outer compartment. The mating test was complete when the experimental rat received an ejaculation from the male rat, left his compartment, and then returned to him.

2.4.4. Baseline open field activity

Because amphetamine has been shown to affect locomotor activity for up to 60 min following infusions into the NAc and hypothalamus (Brudzynski and Mogenson, 1986; Sharp et al., 1987; Stewart and Vezina, 1988), a test of open field activity was conducted immediately following each mating test. Line crossings were recorded during a 10 min-period in a clear Plexiglas arena (101.0 cm long × 37.0 cm wide × 32.0 cm high) with lines marking the floor of the arena every 5.0 cm. Line crossings were counted when all four legs of the rat crossed any line.

2.4.5. Intracranial infusions

Cannulae were implanted one week after the baseline paced mating test. The experimental rats were assigned to one of two groups (saline or amphetamine) matched for comparable levels of baseline paced mating behavior and open field activity. Following a 7–10 day post-operative recovery period, the experimental rats were tested for paced mating behavior and open field activity again. Prior to the start of this mating test, the experimental rats were transported to a room adjacent to the testing room and wrapped in a towel. In Experiment 1A, sterile double-barrel 22 gauge injectors (Plastics One, Roanoke, VA) that extended 2.0 mm beyond the tip of the guide were inserted for infusions into the mPOA. In Experiment 1B, injectors extended 1.0 mm beyond the tip of the guide cannulae. The injectors were attached to two 10.0 μl Hamilton gastight syringes with polyethylene tubing (PE 50) and mounted on a microinfusion pump (kd Scientific, Model 200, Stoelting, Wood Dale, IL). In Experiments 1A and B, 0.5 μl amphetamine

(10 μg/μl; Sigma Chemical Company, St. Louis, MO) or sterile saline vehicle were infused into the mPOA over 5 min (0.1 μl/min). The injectors remained in place for 5 min after infusions to ensure diffusion away from the injector tips. Pilot research determined this concentration to effect mating behavior without producing motor disruptions in open field test (unpublished observations). The experimental rats were placed into the mating arena immediately after the 5-min diffusion period.

In Experiment 2, sterile double-barrel 22 gauge injectors (Plastics One, Roanoke, VA) that extended 2.0 mm beyond the

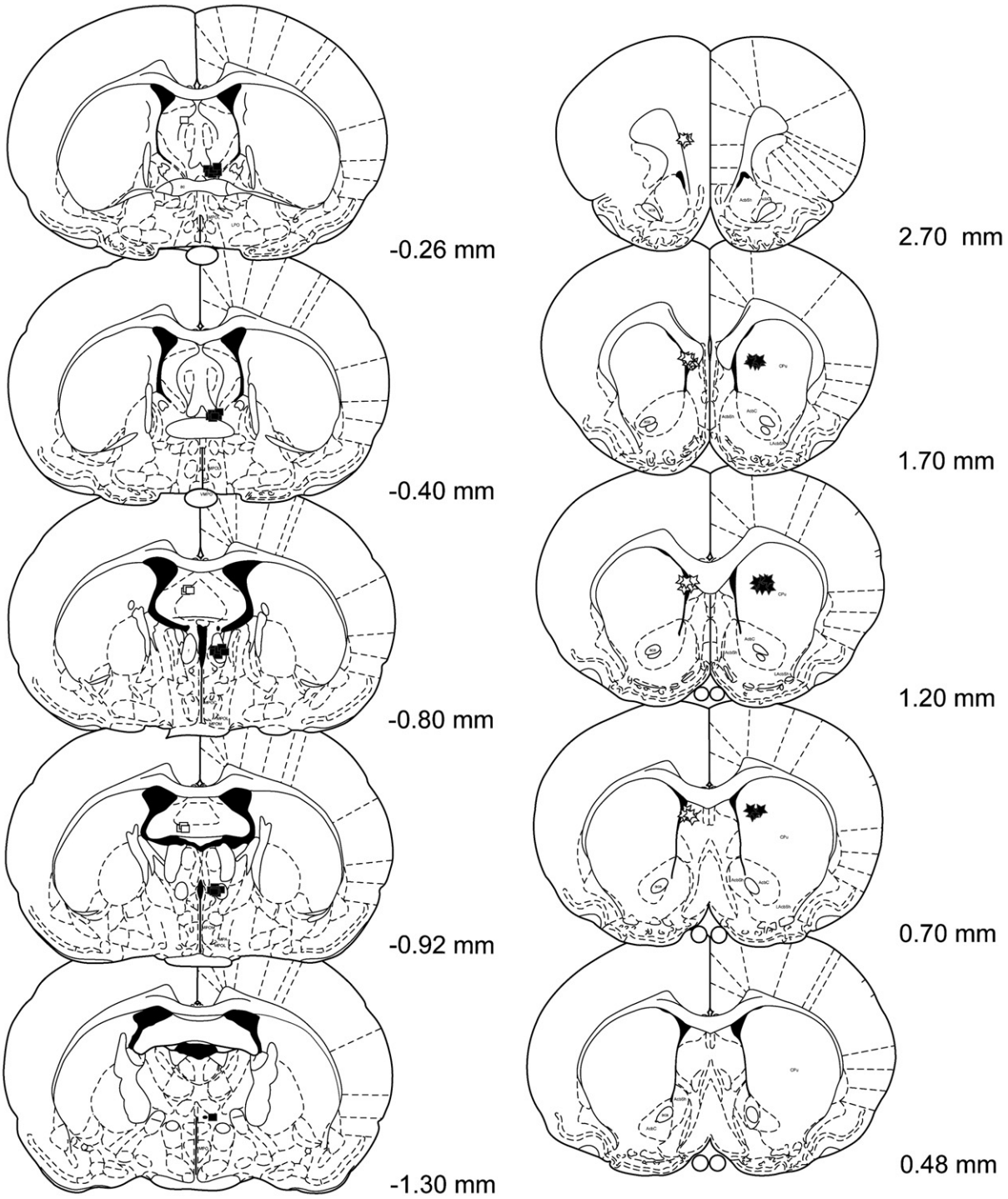

Fig. 1. Coronal sections through (left) the medial preoptic area (mPOA; 0.26–1.30 mm posterior to bregma) and (right) the nucleus accumbens (NAc; 2.7–0.48 mm anterior to bregma). All placements are bilateral but only one side is depicted. Solid squares represent accurate placements of cannulae tips 2.0 mm dorsal to the mPOA. Open squares represent accurate placements of the dorsal controls, which are 4.0 mm dorsal to the mPOA. Open stars represent accurate placements of cannulae tips 2.0 mm dorsal to the NAc shell. Solid stars represent accurate placements of cannulae tips 2.0 mm dorsal to the NAc core. From *The Rat Brain in Stereotaxic Coordinates* (4th ed.), by G. Paxinos and C. Watson, 1998, San Diego, CA: Academic Press. Copyright 1998 by Academic Press.

tip of the guide were inserted for infusions into the NAc shell. However, in Experiment 3, thinner 25 gauge sterile double-barrel injectors (Plastics One, Roanoke, VA) that extended 2.0 mm beyond the tip of the guide cannulae were inserted for infusions into the NAc core. Thinner injectors were used in Experiment 3 because the design of the double-barrel cannulae set 1.5 mm apart require more flexibility in the injectors (Plastics One, Roanoke, VA). In Experiments 2 and 3, 0.5 µl amphetamine (40 µg/µl; Sigma Chemical Company, St. Louis, MO) or sterile saline vehicle were infused into the NAc shell or NAc core over 5 min (0.1 µl/min). This concentration was used because previous experiments have shown it to affect behavior (operant responding) when infused into the NAc (Wyvell and Berridge, 2000).

2.5. Data analysis

During each mating test, LR, LQ, the type and timing of sexual stimulation (i.e. mount, intromission, ejaculation), number and rate of solicitation behaviors (i.e., hops/darts, ear wiggles), number and rate of rejection behaviors (i.e., kicks, defensive postures), the total mating test duration, and the percentage of time spent with the male stimulus were recorded. The number of entries and exits into the stimulus male rat's compartment were also recorded; compartment entries were scored when all four paws of the experimental rat passed through the openings in the clear Plexiglas dividers. Finally, contact–return latency and percentage of exits were calculated. *Contact–return latency* refers to the time elapsed between receiving sexual stimulation, leaving the male rat's compartment and re-entering the male rat's compartment. More specifically, if multiple sexual stimulations were received during a visit to the male, 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 the likelihood that the experimental rat left the male rat's compartment following the receipt of sexual stimulation. More specifically, if an experimental rat received 2 mounts while she was in a male rat's compartment and then left, the likelihood of leaving that male after mounts is 50%. All mating tests were recorded with digital video cameras (Sony DCR-HC65) for off-line analysis of behaviors.

Baseline measures of paced mating behavior and open field activity were used to determine if random assignment to drug treatment (saline or amphetamine) created groups that were matched equally for mating behavior and open field activity prior to amphetamine infusions using independent *t*-tests. Because there were no significant differences between treatment groups for baseline measures (mating and open field), these data are not shown [all ts<1.5]. Independent *t*-tests were also calculated on each behavioral measure observed during the post-infusion mating test. Line crossings made during five 2-min bins of the post-infusion open field test were analyzed with a repeated measures analysis of variance across time and between drug treatment groups (saline vs. amphetamine). Post-hoc simple effects tests were used to analyze differences between drug treatment groups during each individual 2-min bin.

2.6. Histology

Immediately following behavioral testing, experimental rats were deeply anesthetized with sodium pentobarbital (150.0 mg/

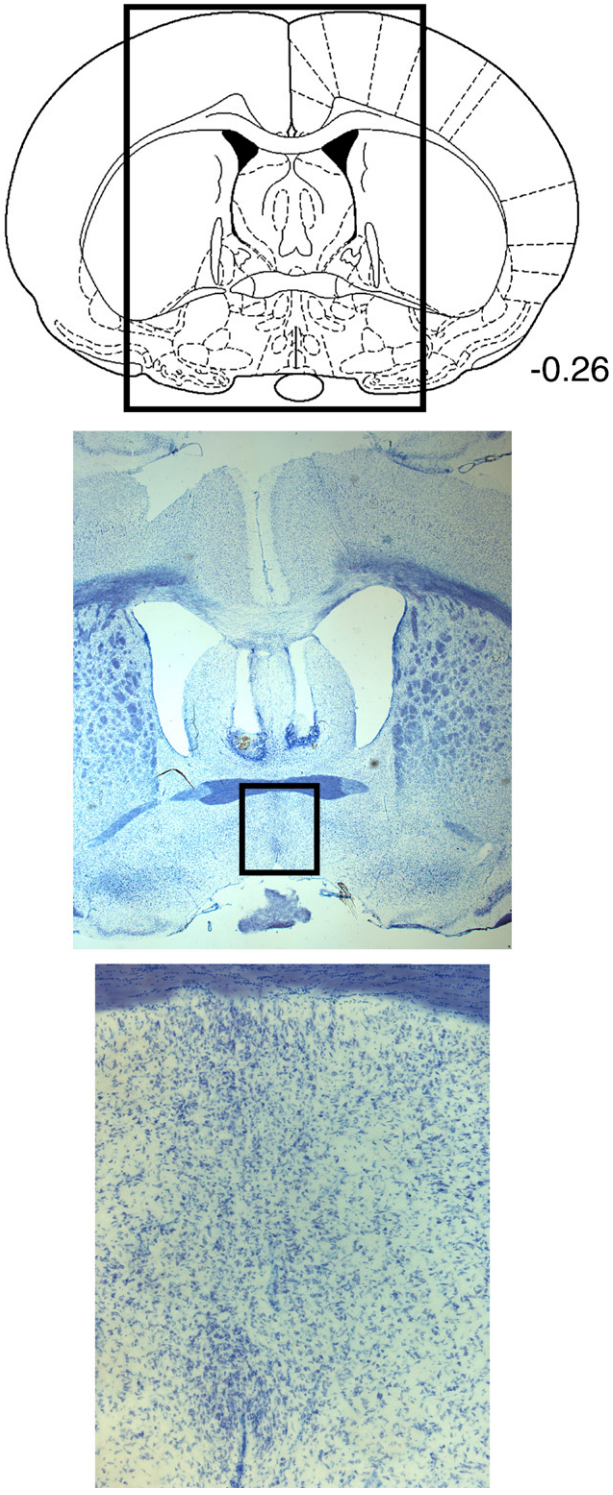

Fig. 2. A photomicrograph of a representative brain section 0.26 mm posterior to bregma of an experimental rat with cannulae placements accurately located ~2.0 mm dorsal to the mPOA (middle; magnification 12.5×). To ensure no gliosis occurred following AMPH infusions, the mPOA was examined at 100× magnification. As can be seen in the bottom photomicrograph, healthy cells were present in the mPOA with no gliosis.

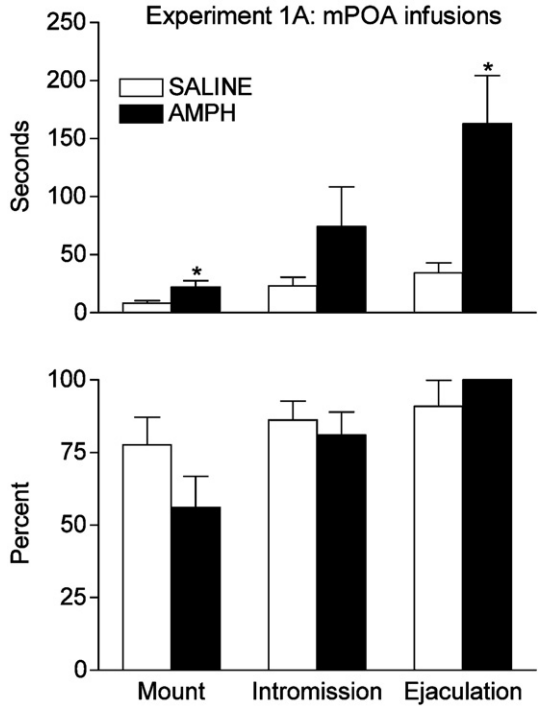

Fig. 3. Amphetamine infusions into the mPOA increased the latency to return to the male rat following sexual stimulation (top: saline $n=15$; AMPH 10 μg/0.5 μl $n=11$), but did not affect the likelihood of withdrawing from the male following sexual stimulation during the test of paced mating behavior (bottom). Data are expressed as means±SEM. NOTE: *n*s = number of rats in each group. *significantly different from saline ($P<.05$).

kg ip) and perfused with 0.9% (wt/vol) saline, followed by 4.0% (wt/vol) formalin–saline solution. Their brains were removed and fixed in a 20.0% (wt/vol) sucrose-formalin-saline solution for at least 24 h. Brains were then frozen and sectioned at approximately 30 μm and stained with thionin for microscopic examination of cannulae placements and to confirm that amphetamine was not exerting a cytotoxic effect on the brain areas of interest. Using ImagePro-Plus, the number of glial cells present in a 1.0 mm extent at the center of the areas of interest were counted.

3. Results

3.1. Experiment 1A

3.1.1. Histology

Cannulae placements were considered accurately located if both guide cannulae tips were microscopically verified to be no more than 2.5 mm dorsal to the surface of the mPOA (Fig. 1). Because cannulae were aimed 2.0 mm dorsal to the mPOA, no damage was produced by the guide cannulae. Furthermore, injectors were so thin and left in for such a short time (10 min) that no gliosis was produced by the injectors. Cells in the mPOA were examined under high power magnification for any signs of gliosis. No experimental rats sustained any damage in the mPOA. A photomicrograph of cannulae placements from a representative experimental rat with high power magnification of the mPOA can be seen in Fig. 2. All accurate cannulae placements were located between 0.26 and 1.3 mm posterior to bregma. Fig. 1 (LEFT) depicts the accurate placements from Experiment 1A. A total of 11 out of 37 experimental rats were eliminated from all statistical analyses. Four rats had placements that were too anterior, too posterior or unilateral to the mPOA. Five rats died during post-operative recover. Finally, the cannulae of two rats were clogged and injectors could not be inserted on test day.

3.1.2. Paced mating behavior

There were significant differences between amphetamine-treated rats and saline-treated rats on contact–return latencies following mounts [$t(17)=2.36$, $P<.04$] and ejaculations [$t(23)=$

Table 1
Behaviors observed during mating tests

	LQ	Solicitation behaviors/min	Rejection behaviors/min	Arena crossings/min	Percentage of time spent with male	Test duration (s)	Number of mounts	Number of intromissions
Experiment 1A: mPOA								
Saline $n=15$	100.0±0.0	6.2±1.8	0.03±0.02	7.1±0.6	35.4±8.2	292.3±55.8	4.7±1.5	4.3±0.7
Amphetamine $n=11$	97.3±2.6	3.3±0.8	0.25±0.11	2.9±0.6*	24.6±4.1	638.5±84.6*	3.1±1.0	4.4±0.6
Experiment 1B: dorsal mPOA								
Saline $n=3$	98.9±1.1	2.1±0.8	0.06±0.06	6.2±3.0	61.6±20.1	442.7±242.8	4.7±2.7	6.5±2.2
Amphetamine $n=4$	100.0±0.0	4.2±2.1	0.37±0.37	5.9±1.0	58.1±19.1	310.0±87.3	5.3±1.4	10.3±3.8
Experiment 2: shell								
Saline $n=10$	98.5±1.2	6.7±1.1	0.44±0.25	3.7±0.6	37.4±6.9	386.9±52.5	3.7±0.9	6.2±0.7
Amphetamine $n=8$	99.0±1.0	7.2±2.0	0.34±0.12	5.0±1.0	51.4±9.7	440.9±78.9	3.3±1.3	7.2±0.8
Experiment 3: core								
Saline $n=7$	98.8±1.2	2.2±0.5	0.16±0.1	5.6±0.1	53.0±11.1	241.4±40.3	4.9±1.2	6.0±0.6
Amphetamine $n=9$	98.1±1.3	1.0±0.3*	0.83±0.4	12.7±2.6*	48.5±8.3	553.7±260.0	8.2±4.8	6.3±1.2

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

3.03, $P<.03$] (Fig. 3 TOP). Experimental rats receiving amphetamine took longer to return the male rat's compartment after mounts and ejaculations than rats receiving saline. However, there were no significant differences between experimental rats treated with amphetamine and rats treated with saline on percentage of exits after sexual stimulation [ts<1.6] (Fig. 3 BOTTOM). There were significant effects of drug treatment on test duration [$t(24)=3.34$, $P<.005$] and arena crossings per minute [$t(24)=3.26$, $P<.005$] (Table 1). The amphetamine-treated rats took longer to complete the mating test (i.e., receive an ejaculation and return to the male) and they visited the male rat less frequently than the saline-treated rats. No other significant differences were observed during the mating test [ts<1.5] (Table 1). During the open field test, there was a significant main effect of time for line crossings [$F(4,96)=23.25$, $P<.0001$]. Both groups made less line crossings over time (Fig. 4 TOP). No other significant differences were observed during the open field test [Fs<1.0].

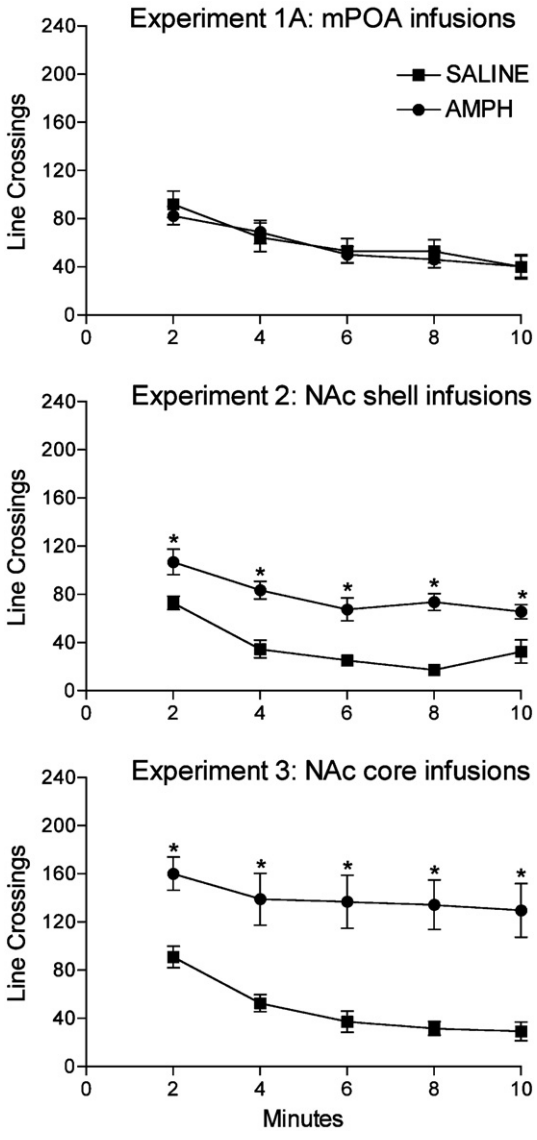

Fig. 4. Amphetamine infusions into the mPOA had no effect on line crossings in the open field test (top: saline $n=15$; AMPH 10 μg/0.5 μl $n=11$). Both groups made less line crossings over time. When infusions were made into the NAc shell (middle: saline $n=10$; AMPH 40 μg/0.5 μl $n=8$) or NAc core (bottom: saline $n=7$; AMPH 40 μg/0.5 μl $n=9$), amphetamine increased line crossings in all five 2-min bins during the open field test. However, fewer line crossings were made over time, independent of drug treatment.

3.2. Experiment 1B

3.2.1. Histology

Cannulae placements were considered accurately located if both guide cannulae tips were microscopically verified to be no more than 4.5 mm dorsal to the surface of the mPOA. Because cannulae were aimed 4.0 mm dorsal to the mPOA, no damage was produced by the guide cannulae. Furthermore, injectors were so thin and left in for such a short time (10 min) that no gliosis was produced by the injectors. All placements meeting these criteria were located between 0.26–1.3 mm posterior to bregma (Fig. 1 left). A total of 3 out of 10 experimental rats were eliminated from all statistical analyses because they died during post-operative recovery.

3.2.2. Paced mating behavior

There were no significant differences between experimental rats treated with amphetamine and rats treated with saline on any behaviors during the mating test (Table 1). During the open field test, there was a significant main effect of time for line crossings [$F(4,20)=10.89$, $P<.0001$]. Both groups made less line crossings over time (data not shown). No other significant differences were observed during the open field test [Fs<1.0].

3.3. Experiment 2

3.3.1. Histology

Cannulae placements were considered accurately located if both guide cannulae tips were microscopically verified to be no more than 2.5 mm dorsal to the surface of the NAc shell. Because cannulae were aimed 2.0 mm dorsal to the NAc shell, no damage was produced by the guide cannulae. Furthermore, injectors were so thin and left in for such a short time (10 min) that no gliosis was produced by the injectors. All placements meeting these criteria were located between 2.7 and 0.5 mm anterior to bregma. Fig. 1 (right) depicts the accurate placements from Experiment 2. All 18 experimental rats had cannulae accurately placed dorsal to the NAc shell.

3.3.2. Paced mating behavior

There were no significant differences between experimental rats treated with amphetamine and rats treated with saline on contact–return latencies or percentage of exits after sexual stimulation [ts<1.5] (data not shown). There were no significant differences between experimental rats treated with amphetamine and rats treated with saline on any other behaviors during the mating test (Table 1). During the open field test, there was a significant main effect of time for line crossings [$F(4,64)=24.64$, $P<.0001$] as well as a significant main effect of treatment [$F(1,16)=24.06$, $P<.0001$]. Although both groups made less line crossings over time, the amphetamine-treated rats made significantly more line

crossings during each 2-min bin of the open field test than saline-treated rats [$ts > 2.6$] (Fig. 4 middle).

3.4. Experiment 3

3.4.1. Histology

Cannulae placements were considered accurately located if both guide cannulae tips were microscopically verified to be no more than 2.5 mm dorsal to the surface of the NAc core. Because cannulae were aimed 2.0 mm dorsal to the NAc core, no damage was produced by the guide cannulae. Furthermore, injectors were so thin and left in for such a short time (10 min) that no gliosis was produced by the injectors. All placements meeting these criteria were located between 2.7–0.5 mm anterior to bregma. Fig. 1 (right) depicts the accurate placements from Experiment 3. A total of 6 out of 22 experimental rats were eliminated from all statistical analyses. Five rats had placements that were too anterior, too dorsal or unilateral to the NAc core. One rat died during post-operative recover.

3.4.2. Paced mating behavior

There were no significant differences between experimental rats treated with amphetamine and rats treated with saline on contact–return latencies or percentage of exits after sexual stimulation [$ts < 1.5$] (data not shown). However, there was a significant difference between amphetamine-treated rats and saline-treated rats on the rate of solicitation behaviors [$t(14) = 2.43$, $P < .03$] (Table 1). Rats treated with amphetamine displayed a lower rate of solicitation behaviors than rats treated with saline. In addition, there was a significant difference between amphetamine-treated rats and saline-treated rats on the rate of arena crossings [$t(14) = 2.55$, $P < .04$] (Table 1). Rats receiving amphetamine visited the male rat more frequently than the saline-treated rats during the mating test. No other significant differences between drug treatment groups were observed during the mating test [$ts < 1.4$] (Table 1). During the open field test, there was a significant main effect of time for line crossings [$F(4,56) = 9.07$, $P < .0001$] as well as a significant main effect of treatment [$F(1, 14) = 18.36$, $P < .001$]. Although both groups made less line crossings over time, the amphetamine-treated rats made significantly more line crossings during each of the 2-min bin of the open field test than saline-treated rats [$ts > 3.4$] (Fig. 4 bottom).

4. Discussion

The present study found that infusions of amphetamine into the mPOA increased the latency to return to the male rat after receiving sexual stimulation. Although female rats receiving intra-mPOA amphetamine infusions made significantly fewer arena crossings during the paced mating test than controls, the lack of an effect of amphetamine on line crossings during the open field test suggests that the decrease in arena crossings during the paced mating test was the result of altered mating behavior (i.e., avoidance of the male), not a general decrease in locomotor behavior. Another side-effect of avoiding the male following intra-mPOA amphetamine infusions was longer test durations. When infusions of amphetamine were made 3.0 mm dorsal to the mPOA, no effects were observed.

The effects of amphetamine infusions into the mPOA are similar to the effects of ibotenic acid lesions of the mPOA on paced mating behavior. Specifically, lesions of the mPOA increase the latency to return to the male after intromissions and ejaculations, increase the likelihood of leaving the male after intromissions, decrease percentage of time spent with the male, decrease solicitation behaviors and increase test duration (Guarraci et al., 2004; Yang and Clemens, 2000). Yang and Clemens (2000) interpreted these results to indicate that mPOA lesions may increase the female rat's sensitivity to the sensory input provided by sexual stimulation. In addition, we have found that female rats with mPOA lesions avoid a male rat during partner preference tests (when sexual contact is possible as well as when it is not) (Guarraci and Clark, 2006).

Disruption of the vasoregulatory output from the mPOA to the genital tissue may represent one mechanism underlying the avoidance of the male following of mPOA lesions. Giuliano and colleagues (Giuliano et al., 2001, 2002) found that electrical stimulation of the mPOA increases vaginal blood flow and vaginal wall tension. Thus, the mPOA may contribute to the genital blood flow and vaginal pressure necessary for the female to engage in the timely progression of mating. Hypersensitivity to pain may also contribute to the avoidance of the male rat following mPOA lesions. For example, Seta et al. (2001) found that naloxone-independent analgesia produced by cold-water stress activates the neurotensin system in the mPOA. In addition, Silva et al. (2004) reported that amino acid neurotransmission increases in the mPOA immediately following an sc injection of formalin into the paw. Because intra-mPOA amphetamine infusions produce results that are similar to neurotoxic lesions, amphetamine-stimulated dopamine release may be inhibitory in the mPOA. Based on anatomical and microdialysis studies, dopaminergic terminals arising from the rostral zona incerta (A13) and the rostral periventricular cell group (A14) (Bjorklund et al., 1975; Dahstrom and Fuxe, 1964) synapse near glutamatergic neurons in the mPOA (Hull and Dominguez, 2007). Limited information from electrophysiological studies in Japanese quail suggests that dopamine application in the mPOA hyperpolarizes a majority (80%) of intrinsic mPOA neurons but depolarizes others (10%) (Cornil et al., 2002). Furthermore, dopamine decreases the firing frequency of a majority of mPOA neurons (52%) but increases the firing frequency of others (24%). Future studies are necessary to determine if the mPOA neurons that control paced mating behavior are inhibited or excited by amphetamine-stimulated dopamine release.

Because dopamine in the mPOA facilitates male sexual behavior and genital reflexes (for review see, Hull and Dominguez, 2007), amphetamine-stimulated dopamine release in the mPOA may enhance female sexual behavior by increasing the female rat's sensitivity to physical contact. Consistent with this conclusion, intra-mPOA infusions of apomorphine increase the sensitivity to sexual stimulation when female rats are pretreated with low levels of estrone, which results in enhanced sexual receptivity (Foreman and Moss, 1979). Although

enhanced paced mating behavior could be operationally defined as shorter approach latencies, this may be an incorrect assumption. The interval between the receipt of sexual stimulation by the female is longer during paced mating behavior than during non-paced mating behavior, but it is paced mating behavior that results in optimal fertility for the female (Coopersmith and Erskine, 1994; Erskine et al., 1989). Therefore it is possible that sexual behavior in the female is enhanced, not disrupted, following intra-mPOA amphetamine infusions. More research is necessary to determine if longer than normal contact–return latencies are beneficial to reproductive success. Studies are currently underway to test this hypothesis.

Unlike the effects of intra-mPOA amphetamine infusions, infusions of amphetamine into the NAc (shell or core), failed to affect paced mating behavior. However, locomotor activity during the open field test was significantly increased following infusions of amphetamine into either the NAc shell or core. Although NAc core infusions had no effect on paced mating behavior, arena crossings were increased during the paced mating test, which most likely contributed to the decrease in solicitation behaviors. Given that the concentrations used in the present study were high enough to increase locomotor behavior in the open field test and in the mating test, it is unlikely that higher concentrations of amphetamine into the NAc shell or core would have been more effective. The results from NAc shell infusions are consistent with previous findings, which failed to observe an effect of NAc shell lesions on paced mating behavior (Guarraci et al., 2002). Although these null results indicate that amphetamine-stimulated dopamine in the NAc neither disrupted or facilitated female sexual behavior, the present results provide important information for our understanding of female mating behavior. First, the present data add to a growing body of evidence suggesting that the mesolimbic dopaminergic system is not critical for sexual motivation, especially in female rats (For review, Paredes and Agmo, 2004). For example, NAc core lesions produce only a modest effect on paced mating behavior, increasing the likelihood of leaving the male after mounts (Guarraci et al., 2002). In addition, Jenkins and Becker (2001) found that only half of their subjects with NAc lesions delayed approaching the male during paced mating behavior, whereas the other half of the experimental subjects were not different from the controls. Furthermore, systemic administration of drugs that alter dopaminergic neurotransmission failed to affect paced mating behavior or sexual incentive motivation in female rats (Ellingsen and Agmo, 2004). Second, despite a 2-fold increase in arena crossings per minute following amphetamine infusions (12.70 ± 2.60 vs. 5.64 ± 0.06), no effects were observed on paced mating behavior. These results suggest that it is possible to dissociate locomotor effects from effects on sexual behavior in tests of paced mating behavior. This dissociation is important for interpreting future studies of paced mating behavior that may be confounded by changes in locomotor behavior.

In conclusion, the present study found that amphetamine-stimulated dopamine release in the mPOA increases the latency to return to the male rat following sexual stimulation during paced mating behavior. This avoidance of the male rat could be a function of increased sensitivity to physical contact experienced during a sexual encounter. Although the effects of amphetamine-stimulated dopamine release on paced mating behavior are similar to the effects of mPOA lesions, future research is necessary to confirm that dopamine inhibits mPOA neurons. Amphetamine-stimulated dopamine release in the NAc (shell or core) had no effect on paced mating behavior. These null results are important because they support a growing body of research suggesting that the mesolimbic dopaminergic system is not critical to sexual motivation, despite the long standing importance of mesolimbic dopamine in other motivated behaviors (Paredes and Agmo, 2004).

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