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Methamphetamine enhances sexual behavior in female rats

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ABSTRACT

The present study evaluated the effects of methamphetamine (MA) on sexual behavior in female rats. In Experiment 1, ovariectomized, hormone-primed rats were injected with MA (1.0 mg/kg, i.p.) or saline prior to a test for mate choice wherein females could mate with two males simultaneously. Female rats treated with saline returned to their preferred mate faster after receiving intromissions and visited their preferred mate at a higher rate than their non-preferred mate. In contrast, MA-treated female rats spent a similar amount of time with their preferred and non-preferred mate and failed to return to their preferred mate faster than to their non-preferred mate following intromissions. Two weeks later, the females received the same drug treatment but were tested for partner preference wherein females could spend time near a male or female stimulus rat. All subjects spent more time near the male stimulus than the female stimulus. However, the MA-treated rats visited the male stimulus more frequently and spent less time near the female stimulus than the saline-treated rats. Similar to Experiment 1, female rats in Experiment 2 were tested for mate choice and then two weeks later tested for partner preference; however, females received three daily injections of MA (1.0 mg/kg, i.p.) or saline. Females treated chronically with MA returned to both males faster following intromissions than females treated with saline, independent of preference (i.e., preferred mate and non-preferred mate). Furthermore, MA-treated rats were more likely to leave either male (i.e., preferred or non-preferred mate) than saline-treated rats after receiving sexual stimulation. Although MA-treated subjects spent more time near the male stimulus than the female stimulus, they spent less time near either when compared to saline-treated subjects. The present results demonstrate that MA affects sexual behavior in female rats partly by increasing locomotion and partly by directly affecting sexual behavior.

1. Introduction

Methamphetamine (MA) is a commonly abused psychomotor stimulant (Darke et al., 2008). Use of MA has increased over the last decade—in both the number of users (Lorvick et al., 2006; Semple et al., 2004a) and the diversity of user-demographics (Brecht et al., 2005). For example, MA is now one of the most common drugs used by high school students and is widely available in urban, suburban, and rural communities across the United States (Maxwell, 2005; Springer et al., 2007). Unlike other psychomotor stimulants, MA is purported to have robust and distinct effects on sexual behavior (Leavitt, 1969; Rawson et al., 2002). Some MA users take MA to enhance sexual pleasure (Semple et al., 2004a). Specifically, while under the influence of MA, users report experiencing enhanced sexual pleasure, enhanced sexual confidence, and enhanced sexual performance compared to when they are not using MA (Semple et al., 2004a, b). In addition, MA users are also more likely to engage in sexual risk taking than non-drug users (Semple et al., 2004a,b). For example, individuals who use MA (including male heterosexual, male homosexual, and female heterosexual users) are more likely to report having participated in anal intercourse and having more sexual partners within the previous 12-month period than non-drug users (Molitor et al., 1998, 1999). The use of MA is also associated with low rates of condom usage (Semple et al., 2004a,b) and high rates of prostitution (Molitor et al., 1998, 1999). As a potential consequence of engaging in high-risk sexual behavior, MA users are more likely to have contracted a sexually transmitted infection in their lifetime than non-drug users (Lorvick et al., 2006; Molitor et al., 1998, 1999; Semple et al., 2004a,b). Finally, one study (Lorvick et al., 2006) compared women who inject MA regularly to women who inject other drugs of abuse, such as heroin. Lorvick et al. (2006) found that women who inject MA were more likely to report having unprotected anal intercourse, having sex for money or drugs, and having more than five sexual partners within the previous six month period than women who inject other drugs.

Although the findings from surveys of drug users suggest a relationship between sexual behavior and MA use, few empirical

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studies have investigated the effects of MA (in animals or humans) on female sexual behavior. Sexual motivation can be studied using animal models, such as rats in laboratory settings (Guarraci, 2010). During mating, female rats display a number of complex behavioral patterns that allow them to control their sexual interactions with male rats [reviewed in (Erskine, 1989)]. For example, a female rat will mate with multiple males simultaneously and pace the receipt of sexual stimulation by retreating from the males between sexual contacts (Calhoun, 1948, 1962; McClintock and Adler, 1977; McClintock et al., 1982; Robitaille and Bovet, 1976). The female rat's ability to control the number and timing of her sexual contacts by approaching and withdrawing from a male (known as paced-mating behavior) (McClintock and Adler, 1977) ensures optimal fertility for the female (Coopersmith and Erskine, 1994; Erskine et al., 1989). When female rats are given the opportunity to pace the receipt of sexual stimulation from two or more males simultaneously, they will display a preference for one male over another by spending more time with him and returning to him more quickly after receiving sexual stimulation (Lovell et al., 2007). A female rat's preference for a particular male is consistent across repeated encounters with the same pair of males (i.e., preferring the same male 70% of the time). Female rats also display solicitation behaviors (i.e., hopping, darting, presenting and ear wiggling), which "solicit" the attention of male partners, and indicate a female rat's motivation to mate (Beach, 1976; Erskine, 1989). Together, the ability to control the temporal pattern of mating with multiple males, the display of solicitation behaviors and the expression of the lordosis reflex represent the full repertoire of mating behavior in the female rat (Beach, 1976; Erskine, 1989).

One empirical study, which investigated the effects of MA administration on sexual behavior in female rats, found that repeated MA administration increased receptive (i.e., the lordosis reflex) and solicitation behaviors (Holder et al., 2009). However, this study did not measure female sexual motivation in a paradigm that allows a female rat to control her interaction with multiple potential sexual partners simultaneously. The present study was designed to investigate the effects of acute and chronic MA administration on several different aspects of female sexual behavior by using both a mate choice test as well as a partner preference paradigm in order to gain insight into the potential for MA to enhance sexual motivation. The mate choice test, wherein a female mates with two males simultaneously, allows for the expression of a preference for one male over another. The partner preference paradigm allows for the expression of a sexual preference over a social preference.

2. Method

2.1. Animals

A total of 53 (Experiment 1: 20; Experiment 2: 33) sexually naïve female Long–Evans rats (200–300 g) were used as experimental subjects. Sexually experienced male (400–600 g) and female (200–400 g) Long–Evans rats were used as stimulus animals. All rats were purchased from Harlan Sprague–Dawley (Indianapolis, IN) and were housed in hanging plastic cages with aspen wood shavings for bedding. Food and water were available ad libitum. Female rats were housed three to a cage. Male rats were housed two to a cage. All rats were weighed weekly. Temperature and humidity in the animal colony were monitored daily. The lights in the colony were maintained on a reversed 12:12 h light–dark cycle (lights off at 10:00 a.m.). All of the behavioral procedures took place during the dark cycle under dim red light.

At least one week before any mating tests took place, all female rats were bilaterally ovariectomized (OVX) under Nembutal (sodium pentobarbital; 50.0 mg/kg i.p.) anesthesia after pretreatment with atropine sulfate (2.5 mg), which reduces respiratory distress.

2.2. Drugs and hormones

Experimental female subjects and female stimulus rats received 10.0 μg of estradiol benzoate (EB) 48 h and 1.0 mg of progesterone (P) 4 h prior to each mating test. All hormone injections were administered subcutaneously in the flank. Both hormones were delivered in a sesame seed oil vehicle. The doses of EB and P used in the present study produce high levels of sexual receptivity in OVX rats (Zipse et al., 2000).

Experimental female subjects received either saline (.9%) or MA (1.0 mg/kg i.p.), which was dissolved in .9% physiological saline. All hormones and drugs were purchased from Sigma Chemical Company, St. Louis, MO.

2.3. Behavioral test procedure

2.3.1. Experiment 1: acute methamphetamine and female sexual behavior

2.3.1.1. Acclimation. All rats were acclimated to the mating chambers on two separate occasions for 15 min each prior to any mating tests. Each mating chamber consisted of a Plexiglas arena (101.0 cm long × 32.0 cm high × 37.0 cm wide) divided into three equal compartments using clear Plexiglas dividers. Each of the dividers had a 5.0 cm hole in each of the two bottom corners. Wood shavings covered the floor of each compartment. During acclimation sessions for male rats, a single male rat was placed in each of the outer compartments and was tapped lightly on the nose if he attempted to exit through the holes in the partition. During acclimation sessions for the female subjects, a single female rat was placed alone in the arena and allowed to move freely between the three compartments.

2.3.1.2. Baseline mate choice test. Approximately one week after acclimation, female subjects were given the opportunity to mate with two male rats simultaneously. Prior to the start of each mating test, a female subject was placed into the center compartment of the mating chamber, confined with solid opaque dividers, and allowed to acclimate for 5 min. The dividers prevented the female subject from entering either of the two side compartments, each of which held one male rat. The location of each male rat (on the left or right) was randomly assigned.

The mating test began when the opaque dividers were removed, thereby providing access to both male rats simultaneously. The mating test ended when the female subject received an ejaculation from and returned to each of the two male rats. At this point, the dividers were replaced and all of the rats were returned to their home cages.

During each mate choice test, the type and timing of sexual stimulations (i.e., mounts, intromissions, and ejaculations), solicitation behaviors (i.e., hops and ear wiggles), rejection behaviors (i.e., kicks, squeaks, and defensive postures), and the number and timing of entries and exits into each compartment were recorded. Compartment entries were scored when all four paws of the female subject passed through the holes in the partition. Time spent in each compartment and percentages of time spent with each of the male rats were calculated. The male rat that the female subject spent the greatest amount of time with was classified as the preferred mate. The lordosis response (LR) of the female subjects to each sexual stimulation was scored on a 4-point scale (0–3) and the lordosis quotient (LQ) was calculated as the percentage of lordosis responses of 2 or 3 (Hardy and DeBold, 1972). In addition, contact-return latency and percentage of exits in response to each type of sexual stimulation received from each male rat were calculated. Contact-return latency is the time elapsed between receiving sexual stimulation, leaving the male's compartment, and re-entering the male's compartment. If multiple sexual stimulations are received during a visit to a male rat, contact-return latency can only be calculated for the last stimulation received before the female subject left the male's

compartment. Percentage of exits is the likelihood that the female subject left the male's compartment following the receipt of sexual stimulation.

2.3.1.3. Post-drug mate choice test. One week after the Baseline Mate Choice Test, female subjects were given another opportunity to mate with two male rats (each of which they had never mated with before) simultaneously following a procedure identical to that described for the Baseline Mate Choice Test. However, 20 min prior to this mating test, each female subject received an i.p. injection of either saline or MA (1.0 mg/kg). This dose and pretreatment schedule were used because it has been shown to produce no stereotypy (Milesi-Hallé et al., 2005, 2007). Assignment to either the saline-treatment group or the MA-treatment group was based on matching groups of female subjects for comparable levels of mating behavior observed during the Baseline Mate Choice Test. There were no statistical differences between the groups (data not shown) prior to drug treatment.

2.3.1.4. Post-drug partner preference test. Two weeks after the Post-Drug Mate Choice Test, all female subjects were tested for partner preference. Partner preference tests were conducted in areas identical to those described previously for the mate choice tests, following a similar protocol except that the stimulus rats used in this test included a sexually vigorous male rat on one side and an OVX hormone-primed female rat on the other. In addition, the stimulus rats were housed behind wire mesh partitions (37 cm wide × 32 cm high) inserted in the middle of each side compartment. The wire mesh partitions allowed the transmission of visual, auditory and olfactory cues but prohibited mating. Either saline or MA (1.0 mg/kg) was administered 20 min prior to the Partner Preference Test. Drug treatment was identical to the treatment administered during the Post-Drug Mate Choice Test. Immediately prior to each partner preference test, the arena was cleaned with ethanol (70%) and fresh bedding was added. Opaque Plexiglas partitions (each 37 cm wide × 32 cm high) were inserted to block the clear Plexiglas partitions. A female subject was then placed in the center compartment and the two stimulus rats (a sexually vigorous male and an OVX hormone-primed female) were confined individually to each of the outer compartments of the arena on either side of the female subject. The position (left or right) of the male and female stimulus rats varied randomly between tests. All rats were allowed a 5-min period to acclimate with the opaque Plexiglas partitions in place before the start of the test.

The test began when both opaque partitions were removed, allowing the female subject access to both stimulus rats through the clear Plexiglas partitions. After 10 min, both opaque partitions were replaced. Compartment entries were scored when all four paws of the female subject passed through the holes in the clear Plexiglas partitions.

Immediately after each test for partner preference, each female subject was tested for locomotor behavior in an open field. Line crossings were recorded during a 10-min period in a clear Plexiglas arena (101.0 cm long × 37.0 cm wide × 32 cm high) with lines marking the floor of the arena every 5.0 cm. Line crossings were counted when all four legs of the female subject crossed any line.

Immediately after each test for locomotor behavior, each female subject was tested to confirm expression of high levels of sexual receptivity. Tests took place in a clear Plexiglas arena (37 cm long × 37 cm wide × 32 cm high) with wood shavings covering the floor. Each female subject was placed into the arena with a sexually vigorous male. The test was complete when the female subject received 10 mounts with or without intromissions. The LR to each stimulation was scored, and LQ was calculated as described previously.

2.3.2. Experiment 2: chronic methamphetamine and female sexual behavior

All protocols and procedures were identical to those described for Experiment 1, except that the 33 experimental female subjects in Experiment 2 were given saline or MA chronically. Saline or MA was injected once per day at approximately 1:00 p.m, for three consecutive days. Daily injections started two days prior to the Post-Drug Mate Choice Test, with the last dose occurring 20 min before the mating test. Daily doses of saline or MA took place again two weeks later starting two days before the Post-Drug Partner Preference Test, with the last dose occurring 20 min before the test. This chronic regimen is similar, but not identical, to the protocol used by Holder et al. (2009).

2.4. Data analysis

2.4.1. Mate choice test

The total time spent by each female subject in each of the outer compartments was calculated to determine the percentage of time spent with each male stimulus rat. The preferred mate was defined as the male with whom the female spent more time with during the mating test. A repeated measures analysis of variance (ANOVA) was used to determine the effect of drug treatment (i.e., saline or MA) as it interacts with preference (i.e., preferred or non-preferred mate) on all mating behaviors (e.g., percentage of test time spent with each male stimulus rat, contact-return latency, percentage of exits, solicitation behaviors). All significant interactions between drug treatment and preference were followed up with Tukey's post-hoc tests. The alpha level was set at $p<.05$.

2.4.2. Partner preference test

The total time spent in each of the outer compartments was calculated for each female subject to determine the percentage of time spent with each stimulus rat. Entries into each outer compartment were also summed. Repeated measures ANOVAs were calculated on both of these behaviors using drug treatment group (i.e., saline or MA) as the between-subjects factor and preference (i.e., male or female partner) as the repeated measures factor.

3. Results

3.1. Experiment 1: acute methamphetamine and female sexual behavior

3.1.1. Mate choice test

3.1.1.1. Visits and time. All mating behaviors (e.g., contact-return latencies, percentage of exits, and time spent with mates) observed during the Baseline Mate Choice Test were used to assign female subjects to two matched groups (i.e., saline or MA). Because both groups were matched and no significant differences were found between subjects assigned to the saline-treatment group and subjects assigned to the MA-treatment group, the baseline data are not shown.

Repeated measures ANOVAs were calculated on all mating behaviors observed during the Post-Drug Mate Choice Test, using drug treatment (saline or MA) as the between-subjects factor and preference (preferred vs. non-preferred mate) as the repeated measures factor. One female subject from the saline-treatment group was not receptive (LQ = 0) on the Post-Drug Mate Choice Test and, therefore, her data were not included in any statistical analyses (saline group: n = 7; MA group: n = 12). A significant main effect of preference [$F(1,17) = 5.19$, $p = .04$] was observed on the number of visits to the male stimulus rats. Post-hoc comparisons indicated that saline-treated subjects made significantly more visits to their preferred mate than to their non-preferred mate ($p<.05$); however, MA-treated subjects visited their preferred and their non-preferred mate at the same rate (Fig. 1 TOP). A significant main effect of preference [$F(1,17) = 18.71$, $p<.0001$], as well as a significant interaction between drug treatment and preference [$F(1,17) = 4.89$, $p = .04$] were observed on time spent with the male stimulus rats. Post-hoc comparisons indicated that saline-treated subjects spent

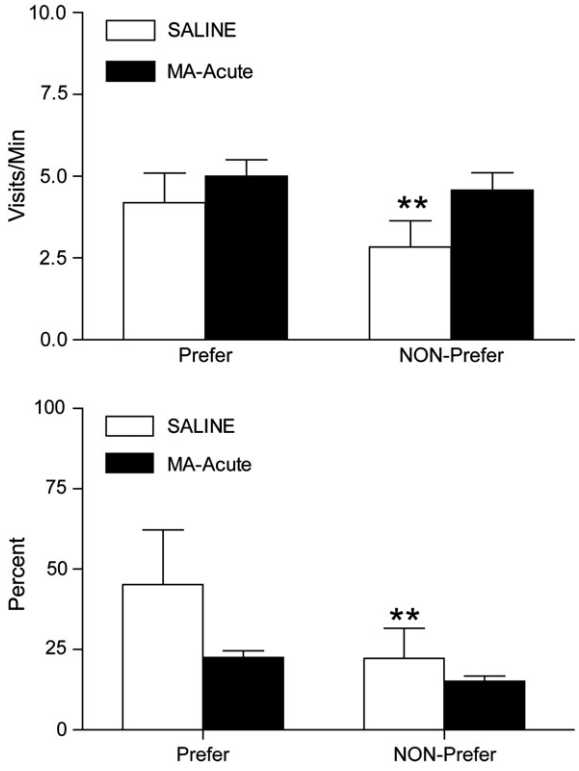

Fig. 1. TOP Subjects in the saline-treatment group made more visits to their preferred mate than to their non-preferred mate. However, subjects treated with MA visited both their preferred mate and their non-preferred mate at the same rate (MEANS ± SEM; SALINE: n = 7; METHAMPHETAMINE 1 mg/kg: n = 12). BOTTOM Subjects in the saline-treatment group spent more time with their preferred mate than their non-preferred mate. However, subjects treated with MA spent the same amount of time with their preferred mate and their non-preferred mate. A significant difference within treatment group between preference (Preferred vs. NON-Preferred) is denoted by a double asterisk ** ($p<.05$).

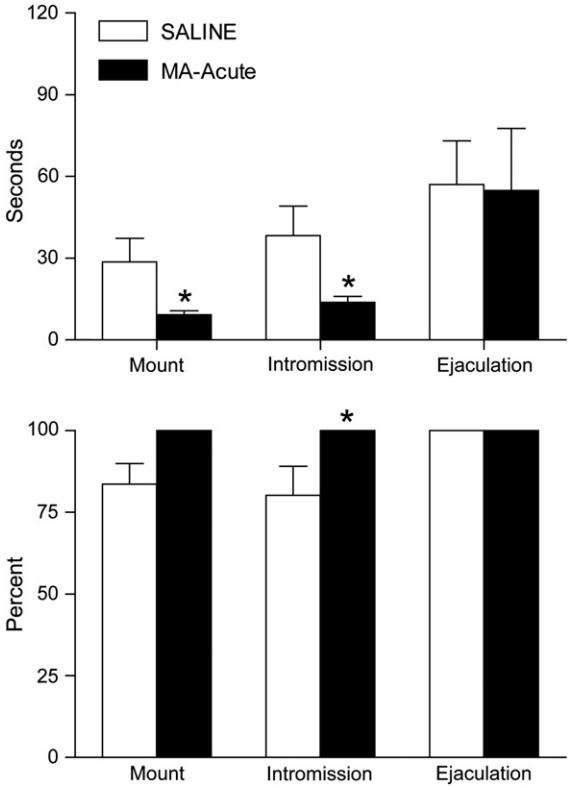

Fig. 2. TOP Subjects treated with MA returned to their non-preferred mate faster following intromissions than saline-treated subjects (MEANS ± SEM; SALINE: n = 7; METHAMPHETAMINE 1 mg/kg: n = 12). BOTTOM Subjects treated with MA were more likely to leave their non-preferred mate following an intromission than saline-treated subjects. A significant difference from saline-treatment group is denoted by an asterisk * ($p<.05$).

significantly more time with their preferred mate than their non-preferred mate ($p<.05$); however, MA-treated subjects spent the same amount of time with their preferred mate and their non-preferred mate (Fig. 1 BOTTOM).

3.1.1.2. Contact-return latencies. A significant main effect of drug treatment [$F(1,10) = 11.63$, $p = .007$] was observed on contact-return latency following mounts. Female subjects treated with MA returned to either their preferred mate or to their non-preferred mate faster than saline-treated subjects following mounts (Fig. 2 TOP; only NON-Preferred mate shown).

A significant main effect of drug treatment [$F(1,17) = 11.21$, $p = .005$], a significant main effect of preference [$F(1,17) = 8.18$, $p<.02$], as well as a significant interaction between drug treatment and preference [$F(1,17) = 4.67$, $p = .04$] were observed on contact-return latency following intromissions. Post-hoc comparisons indicated the female subjects treated with MA returned to their non-preferred mate significantly faster following intromissions than the saline-treated females ($p<.05$). However, MA-treated subjects did not return faster to their preferred mate following intromissions than saline-treated subjects ($p>.05$) (Fig. 2 TOP; only NON-Preferred mate shown). No other significant effects were observed (i.e., contact-return latency following ejaculations; all Fs < 3.5).

3.1.1.3. Percentage of exits. A significant main effect of drug treatment was observed on percentage of exits following mounts [$F(1,10) = 7.61$, $p = .02$]. Female subjects treated with MA were more likely to leave either their preferred mate or their non-preferred mate following mounts when compared to saline-treated subjects (Fig. 2 BOTTOM; only NON-Preferred mate shown). A significant main effect of drug treatment [$F(1,17) = 16.56$, $p = .005$] was observed on percentage of exits following intromissions. Female subjects treated with MA were more likely to leave either their preferred mate or their non-preferred mate following intromissions when compared to saline-treated subjects (Fig. 2 BOTTOM; only NON-Preferred mate shown). Nevertheless, the saline-treated subjects were more likely to leave their non-preferred mate than their preferred mate following intromissions. No other significant effects were observed (i.e., percentage of exits following ejaculations, solicitation behaviors; all Fs < 3.5).

3.1.2. Partner preference test

All female subjects included in the analysis of the Post-Drug Mate Choice Test were receptive after the test for partner preference. Therefore, all female subjects were included in the statistical analysis of the behaviors observed during the Post-Drug Partner Preference Test. Repeated measures ANOVAs were calculated on the number of visits to each of the stimulus rats and time spent with each of the stimulus rats and during the Post-Drug Partner Preference test. A significant main effect of drug treatment [$F(1,17) = 10.55$, $p = .005$], a significant main effect of preference [$F(1,17) = 58.97$, $p<.0001$] and a significant interaction between drug treatment and preference [$F(1,17) = 4.40$, $p = .05$] were observed on the number of visits to the stimulus rats. Post-hoc comparisons indicated that the subjects treated with MA made significantly more visits to the male stimulus rat than saline-treated subjects ($p<.05$), but visited the female stimulus rat at the same rate as the saline-treated subjects ($p>.05$) (Fig. 3 TOP). A significant main effect of drug treatment [$F(1,17) = 12.03$, $p = .005$] and a significant main effect of preference [$F(1,17) = 66.79$, $p<.0001$] were observed on time spent near the stimulus rats. Both

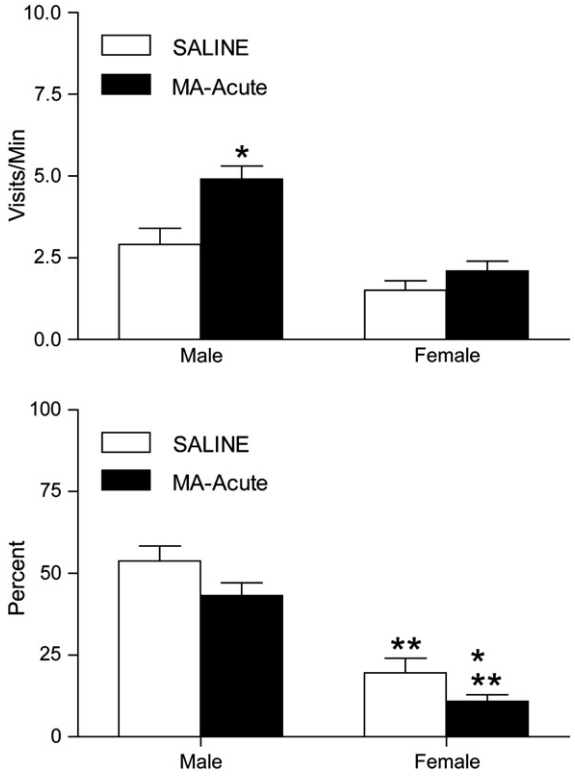

Fig. 3. TOP Subjects in both groups made more visits to the male stimulus rat than to the female. However, subjects treated with MA made more visits to the male stimulus rat than saline-treated subjects (MEANS ± SEM; SALINE: n = 7; METHAMPHETAMINE 1 mg/kg: n = 12). BOTTOM Subjects in both groups spent more time near the male stimulus rat than near the female. However, subjects treated with MA spent less time near the female stimulus rat than the saline-treated subjects. A significant difference from saline group is denoted by an asterisk * ($p<.05$). A significant difference within treatment group between preference (male vs. female) is denoted by a double asterisk ** ($p<.05$).

groups of subjects spent more time near the male stimulus than the female stimulus; however, subjects treated with MA spent significantly less time in the vicinity of either stimulus rat than saline-treated rats (Fig. 3 BOTTOM). Furthermore, MA-treated subjects spent significantly less time near the female stimulus rat than the saline-treated subjects ($p<.05$).

3.1.3. Open field test

During the open field test, there was a significant main effect of drug treatment on total line crossings [$t(17) = 5.38$ $p = .03$]. (MEANS ± SEM of the number of line crossings in 10 min; saline: 199.33 ± 16.20; MA: 401.2 ± 36.82).

3.2. Experiment 2: chronic methamphetamine and female sexual behavior

3.2.1. Mate choice test

3.2.1.1. Visits and time. All mating behaviors (e.g., contact-return latencies, percentage of exits, and time spent with mates) observed during the Baseline Mate Choice Test were used to assign female subjects to two matched groups (saline and MA). Because both groups were matched and no significant differences were found between subjects assigned to the saline-treatment group and subjects assigned to the MA-treatment group, the baseline data are not shown.

Repeated measures ANOVAs were calculated on all mating behaviors observed during the Post-Drug Mate Choice Test, using drug treatment (saline or MA) as the between-subjects factor and preference (preferred vs. non-preferred mate) as the repeated measures factor. Two female subjects (one from each treatment group) were not receptive (LQ = 0) on the Post-Drug Mate Choice Test and therefore their data were not included in any statistical analyses, leaving 31 subjects for data analysis (saline group: n = 15; MA group: n = 16). A significant main effect of drug treatment [$F(1,29) = 8.27$, $p = .007$] and a significant main effect of preference [$F(1,29) = 16.31$, $p<.0001$] were observed on the number of visits to the stimulus rats. Both groups of subjects made more visits to their preferred mate than to their non-preferred mate (Fig. 4 TOP). However, MA-treated subjects made more visits to both their preferred mate and their non-preferred mate when compared to the saline-treated subjects. A significant main effect of preference [$F(1,17) = 18.71$, $p<.0001$] was observed on time spent with the stimulus rats, indicating that both groups of subjects spent more time with their preferred mate than their non-preferred mate (Fig. 4 BOTTOM). A significant main effect of drug treatment [$F(1,29) = 11.49$, $p = .005$] and a significant main effect of preference [$F(1,29) = 13.62$, $p = .005$] were observed on solicitation behaviors displayed near the stimulus rats. Both groups of subjects displayed more solicitation behaviors near their preferred mate. However, when compared to saline-treated subjects, MA-treated subjects displayed significantly less solicitation behaviors toward either their preferred or non-preferred mate (MEAN ± SEM number of solicitation behaviors per min; Saline: Preferred .83 ± .14 and NON-Preferred .33 ± .09; MA: Preferred .33 ± .11 and NON-Preferred .09 ± .04).

3.2.1.2. Contact-return latencies. A significant main effect of drug treatment [$F(1,25) = 7.15$, $p = .03$] and a significant main effect of preference [$F(1,25) = 4.33$, $p = .04$] were observed on contact-return latency following intromissions. Both groups of subjects returned to their preferred mate faster than their non-preferred mate. However, when compared to saline-treated subjects, MA-treated subjects

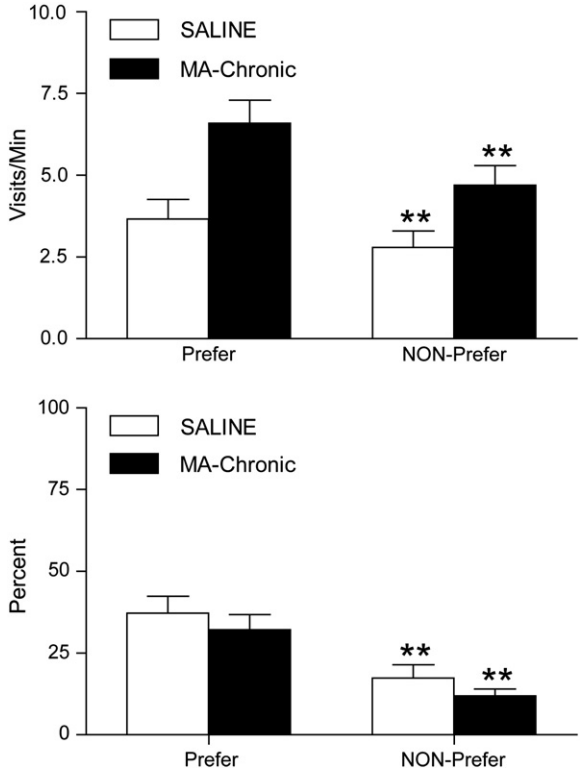

Fig. 4. TOP Subjects in both groups made more visits to their preferred mate than to their non-preferred mate. However, subjects treated with MA made more visits to both stimulus rats (MEANS ± SEM; SALINE: n = 15; METHAMPHETAMINE 1 mg/kg × 3 doses: n = 16). BOTTOM Subjects in both groups spent more time with their preferred mate than their non-preferred mate. However, subjects treated with MA spent less time with either stimulus rat. A significant difference within treatment group between preference is denoted by a double asterisk ** ($p<.05$).

returned to either their preferred or non-preferred mate faster following intromissions (Fig. 5 TOP; only NON-Preferred mate shown; $p>.05$). A significant main effect of drug treatment [$F(1,19)=7.08$, $p=.03$] was also observed on contact-return latency following ejaculations, indicating that MA-treated subjects returned to either their preferred or non-preferred mate following ejaculations faster than saline-treated subjects (Fig. 5 TOP; only NON-Preferred mate shown).

3.2.1.3. Percentage of exits. A significant main effect of drug treatment [$F(1,28)=9.15$, $p=.005$] was observed on percentage of exits following intromissions. Female subjects treated with MA were more likely to leave either their preferred mate or their non-preferred mate than saline-treated subjects following intromissions (Fig. 5 BOTTOM). No other significant effects were observed (e.g., contact-return latency following mounts, percentage of exits following ejaculations; all Fs<4.3).

3.2.2. Partner preference test

Four female subjects (saline group: n=2; MA group: n=2) were not sexually receptive after the Post-Drug Partner Preference Test (LQ=0) and, therefore, were excluded from all statistical analyses. The two rats that were not receptive during the Post-Drug Mate Choice Test were not tested. Seven female subjects (saline group: n=2; MA group: n=5) died after the Post-Drug Mate Choice Test and therefore were not tested in the partner preference test. This leaves the data from 20 female subjects (saline group: n=9; MA group: n=11) available for analysis from the Post-Drug Partner Preference Test. Repeated measures ANOVAs were calculated on the number of visits to each of the stimulus rats and time spent with each of the stimulus rats during the Post-Drug Partner Preference Test. A significant main effect of preference [$F(1,18)=4.80$, $p=.04$] was observed on the number of visits to the stimulus rats. Both groups of subjects made more visits to the male stimulus than to the female stimulus. However, MA-treated subjects made slightly more visits to both stimulus rats, when compared to saline-treated subjects (Fig. 6 TOP). A significant main effect of drug treatment [$F(1,18)=5.85$, $p=.04$] and a significant main effect of preference [$F(1,18)=4.90$, $p=.04$] were observed on time spent near the stimulus rats. Post-hoc comparisons indicated that saline-treated subjects spent significantly more time with the male stimulus than the female stimulus ($p<.05$); however, subjects treated with MA spent a similar amount of time with both stimulus rats (Fig. 6 BOTTOM).

3.2.3. Open field test

During the open field test, there was a significant main effect of drug treatment on total line crossings [$F(1, 18)=3.03$ $p=.04$] (MEANS ± SEM of the number of line crossings in 10 min; saline: 255.13 ± 16.92; MA: 432.5 ± 46.1).

4. Discussion

The results of the present study indicate that during a mate choice test, female rats treated acutely with a low dose of MA returned to their *non-preferred* mate faster following mounts and intromissions than female rats treated with saline. In contrast to saline-treated subjects, who made more visits to their preferred mate, acute MA-treated subjects visited their preferred mate and their non-preferred mate at the same rate. Finally, subjects in the acute MA-treatment

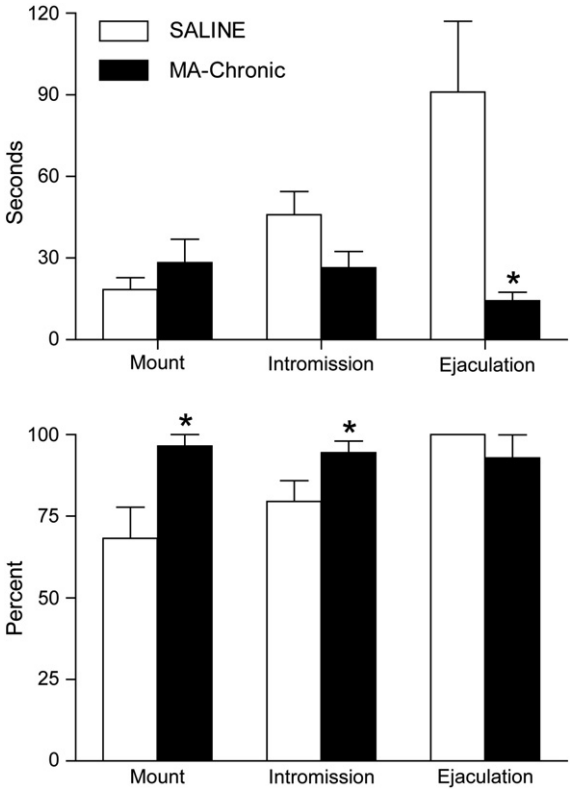

Fig. 5. TOP Subjects treated with MA returned to their non-preferred mate faster following an ejaculation than saline-treated subjects (MEANS ± SEM; SALINE: n=15; METHAMPHETAMINE 1 mg/kg×3 doses: n=16). BOTTOM Subjects treated with MA were more likely to leave their non-preferred mate following a mount or an intromission than saline-treated subjects. A significant difference from saline-treated group is denoted by an asterisk * ($p<.05$).

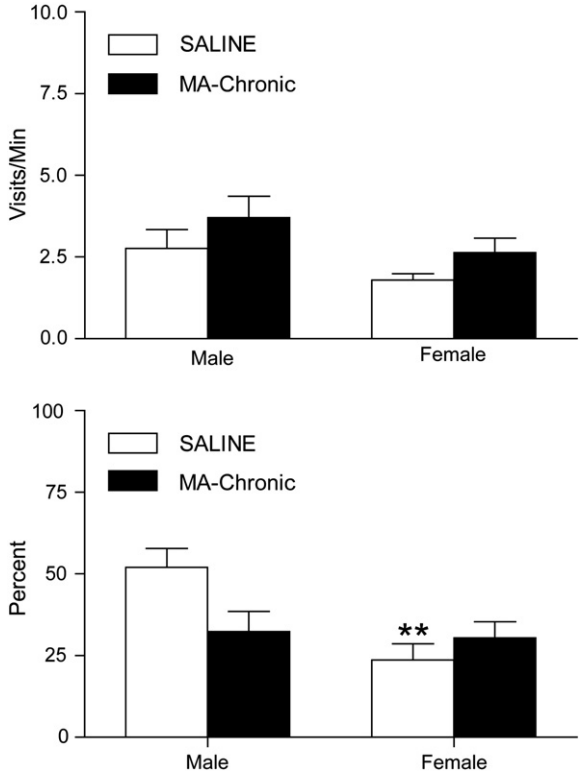

Fig. 6. TOP Subjects in both groups made more visits to the male stimulus rat. However, subjects treated with MA made more visits to both stimulus rats than saline-treated subjects (MEANS ± SEM; SALINE: n=9; METHAMPHETAMINE 1 mg/kg×3 doses: n=11). BOTTOM Subjects treated with saline spent more time near the male stimulus rat than near the female. However, subjects treated with MA spent the same amount of time near both stimulus rats. A significant difference from saline group is denoted by an asterisk * ($p<.05$). A significant difference within treatment group between preference (male vs. female) is denoted by a double asterisk ** ($p<.05$).

group were more likely to leave either their preferred mate or their non-preferred mate following mounts and intromissions than subjects in the saline-treatment group. These results indicate that female rats given MA acutely do not discriminate between their preferred mate and their non-preferred mate with the same sensitivity as female rats given saline.

Previous studies of sexual behavior in female rats led Erskine to hypothesize that contact-return latency reflects female sexual motivation and that shorter latencies indicate an enhanced drive to engage in sexual contact (Erskine, 1989). Recent research provides support for Erskine's idea. When female rats are given the opportunity to control a mating encounter with multiple male rats simultaneously, they display a distinct preference for one male over others, spending more time with one of the male rats (Ferreira-Nuño et al., 2005, 2010; Lovell et al., 2007; Zewail-Foote et al., 2009). Furthermore, female rats return to their preferred mate (i.e., the male with whom they spend more time) faster than they return to their non-preferred mate after receiving sexual stimulation (Lovell et al., 2007; Zewail-Foote et al., 2009). Given these observations, it is likely that the shorter contact-return latency after sexual stimulations in the acute MA-treated subjects reflects enhanced sexual drive to engage in sexual contact. Nevertheless, acute MA treatment may be capable of differentially enhancing the rewarding properties of some sexual stimulation (e.g., intromissions) and not others (e.g., ejaculations).

To confirm that the failure to discriminate between the preferred mate and the non-preferred mate is not a disruption of female sexual motivation caused by acute MA administration, female subjects were tested for partner preference when one stimulus rat was a sexual partner (e.g., a sexually vigorous male) and one was not (e.g., a female). Acute MA-treated subjects spent about the same time near the male stimulus as saline-treated subjects. However acute MA-treated subjects spent significantly less time near the female stimulus when compared to the saline-treated subjects. Furthermore, acute MA-treated subjects visited the male stimulus rat more frequently than saline-treated subjects, but visited the female stimulus rat at the same rate as saline-treated subjects. Therefore, the acute MA-treated subjects displayed enhanced discrimination between a sexual partner and a non-sexual partner during the partner preference test.

The psychomotor stimulant properties of MA make it difficult to tease apart the effects of acute MA administration on sexual behavior from the effects on locomotor behavior. Because acute MA treatment increased open-field activity, it is clear that MA has stimulant properties at the dose regimen used in the present study. However, if acute MA administration had *any* specific effects on sexual behavior (above what is an artifact of enhanced locomotion) only a subset of sexual behaviors would be affected. In contrast, if the locomotor stimulating effects of acute MA prevented the animals from inhibiting behavior, than all sexual behaviors would be affected equally by MA. For example, if all of the effects of acute MA administration were due to the stimulant properties of MA, then the latency to return to the *preferred* mate after all sexual stimulations (i.e., mounts, intromissions and ejaculations) would be shorter in the MA-treatment group than in the saline-treatment group. In addition, the latency to return to the *non-preferred* mate following ejaculations would also be shorter in the MA-treatment group than in the saline-treatment group.

However, the results of the present study demonstrated that acute MA treatment only affected some measures of sexual behavior. For example, acute MA administration only shortened latency to return to the *non-preferred* mate following mounts and intromissions (not following ejaculations), suggesting that the effects of MA are specific. Females treated with acute MA still displayed the characteristic "stair-step" response to increasing levels of sexual stimulation (mounts< intromissions<ejaculations) for contact-return latencies with the *preferred mate*. Because females treated with acute MA spent more time with a male stimulus than a female stimulus suggests they are capable of controlling and directing their behavior despite enhanced locomotion. Finally, females treated acutely with MA made more visits to the *male* stimulus than females treated with saline. However, females treated acutely with MA did NOT make more visits to the *female* stimulus than females treated with saline during the partner preference test. Therefore, the effects of acute MA on sexual behavior may be due to enhanced sexual motivation, and not entirely due to increases in uncontrolled locomotion.

Although an increased likelihood of leaving a male after sexual stimulation could indicate enhanced aversive motivation (Guarraci and Clark, 2006), it could also indicate enhanced locomotion. Because all stimulations delivered by preferred and non-preferred mates were affected, we cannot conclude that the effect of acute MA on percentage of exits reflects alterations in sexual motivation. The effects of acute MA on percentage of exits were not specific to a particular response (i.e., to a type of mate or to a type of sexual stimulation); therefore, these effects could be artifacts of increases in locomotor behavior or alterations in sensitivity to sexual stimulation in general.

The effects of chronic MA treatment were less specific than the effects of acute MA treatment. Unlike females treated acutely with MA, females treated chronically with MA visited both their preferred mate *and* their non-preferred mate more than females treated with saline. Female rats in the chronic MA-treatment group returned faster following intromissions than females in the saline-treatment group independent of preference (returning faster to both their preferred mate and their non-preferred mate than the saline-treatment group). Finally, female rats treated chronically with MA were also more likely to leave following intromission independent of preference. Therefore, the effects of chronic MA are more general and less specific than the effects of acute MA.

Chronic treatment with MA also had more robust and less specific effects on partner preference than acute treatment with MA. Females treated chronically with MA did not display a preference for the male stimulus rat over the female stimulus rat. Specifically, females receiving chronic MA treatment spent a similar amount of time with both stimulus rats and visited both of the stimulus rats at the same rate. These results indicate that the effects of chronic exposure to MA on sexual behavior may be more sensitive to enhanced locomotor behavior than the effects of acute exposure.

Although the most recent work by Holder and Mong (2010) tested the effects of MA in a paradigm that is very similar to the mate choice test used in the current study, there are a number of differences between the two studies in terms of methodologies. Nevertheless, the results are consistent and complementary. Holder and Mong (2010) tested chronic exposure of MA on paced-mating behavior with one male rat, whereas the current study investigated acute and chronic MA treatment when female rats had the opportunity to control sexual contact with two male rats simultaneously. In addition to a different strain of rats (Sprague–Dawley), Holder and Mong (2010) used a higher dose of MA (5.0 mg/kg). Furthermore, we tested the effects of MA in the partner preference test. Finally, they observed mating behavior 4 h after the final MA injection, whereas we observed mating behavior 20 min after the final MA injection. As a consequence of this difference in pre-injection time, MA-treated subjects displayed less locomotor behavior in the study by Holder and Mong (2010). Despite these methodological differences, they reported similar results. Specifically, they also found that MA-treated subjects took less time to return to a male rat following intromissions. In contrast to the present study, they found that MA-treated subjects were less likely to leave a male rat following intromissions and displayed more solicitation behaviors. Although more solicitation behaviors were observed when saline-treated subjects were interacting with their preferred mate than when they were interacting with the non-preferred mate, MA treatment actually decreased solicitation behavior in the current study. One possible explanation for these inconsistencies between studies is that solicitation behaviors, in particular hops and ear wiggling, do not typically occur while the female is

moving between compartments of the arena; therefore, the increase in locomotor behavior may have prevented or superseded the display of solicitation behaviors. Furthermore, percentage of exits may be more sensitive to alterations in locomotor behavior than contact-return latency. Because contact-return latency was affected in both studies, but locomotor behavior was only affected in the present study, it is likely that the effects on contact-return latency observed in the present study are not merely the consequence of enhanced locomotion.

Recently, Holder and Mong (2010) also reported that expression of the cytoskeleton-associated protein, spinophilin, which is associated with structural plasticity of dendritic spines, increased in the medial amygdala in female rats treated with MA and tested for paced-mating behavior. This potential localization of the effects of MA to the medial amygdala is consistent with our recent findings that lesions of the posterodorsal region of the medial amygdala (MePD) alter the sensitivity of female rats to sexual stimulation received during a mate choice test (Guarraci, 2010). Specifically, female rats with MePD lesions preferred one mate to another, but they failed to return to their preferred mate faster than their non-preferred mate. The females with MePD lesions also visited both potential mates at a similar rate and received a similar number of stimulations from both mates. These results indicate that the potential usefulness of a female rat's preference for one male over another depends on the MePD. Without a functioning MePD, mate choice has reduced behavioral consequences (e.g., no increase in the receipt of sexual stimulation from the preferred mate, no shorter contact-return latencies after receiving sexual stimulation from the preferred mate). Together, these results support a critical role for the MePD mediating the effects of MA on sexual motivation. Research in our lab is currently underway to determine if the effects of MA on sexual motivation can be directly localized to increases in dopamine neurotransmission in the MePD using intracranial infusions of MA immediately prior to a test of mate choice and partner preference.

In conclusion, the present study demonstrates that although acute administration of MA increases locomotion, MA-treated female rats *are capable of controlling* locomotion and consequently displaying enhanced sexual motivation. Specifically, acute MA administration produces directed hyper-locomotion, not just a general increase in locomotion during mating tests. Because the effects of chronic MA on female sexual behavior are less specific, it is likely that chronic exposure to MA produces more robust locomotor effects that may be incompatible with the expression of enhanced sexual motivation. Similar to the results of surveys of drug users that indicate that MA enhances sexual motivation as well as increases sexual risk taking (Lorvick et al., 2006; Molitor et al., 1998, 1999), female rats treated with MA may be less discriminating about how and with whom they mate but are more interested in sex than saline-treated rats. By using both the mate choice and the partner preference paradigms, we have been able to demonstrate enhanced (by visiting the male more than the female stimulus), but less discriminating interest in sex (by spending the same amount of time with both mates/failing to prefer one male over another) in MA-treated female rats.

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