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Sexual-incentive motivation and paced sexual behavior in female rats after treatment with drugs modifying dopaminergic neurotransmission

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Abstract

The effects of the dopamine receptor agonist apomorphine, the dopamine releaser amphetamine, and the dopamine receptor antagonist cis(*Z*)-flupenthixol on sexual-incentive motivation and on paced-mating behavior were studied in female rats. Apomorphine, in the doses of 0.125 and 0.5 mg/kg, showed a tendency to reduce incentive motivation. Ambulatory activity was inhibited, evidenced both by diminished distance moved and reduced velocity of movement. Amphetamine (0.25 and 1 mg/kg) and flupenthixol (0.25 and 0.5 mg/kg) failed to modify incentive motivation while stimulating and reducing ambulatory activity, respectively. In the mating test, apomorphine enhanced the latency to enter the male's half and reduced the number of proceptive behaviors. However, these effects were associated with the appearance of stereotyped sniffing. Amphetamine increased the propensity to escape from the male after a mount without having other effects. Flupenthixol augmented the duration of the lordosis posture. Neither amphetamine nor flupenthixol affected sniffing. These data show that facilitated dopaminergic neurotransmission stimulates neither paced female sexual behavior nor sexual-incentive motivation. Dopamine receptor blockade has slight consequences. It is concluded that dopamine is not a transmitter of major importance for unconditioned female sexual motivation and behavior.

Keywords: Female sexual behavior; Lordosis; Pacing; Sexual-incentive motivation; Dopamine

1. Introduction

During the last several years, some indirect data have suggested that dopamine may be of importance for the regulation of female sexual behavior. Dopamine concentration in the nucleus accumbens of rats (Mermelstein and Becker, 1995; Pfaus et al., 1995) and hamsters (Kohlert et al., 1997), as well as in the female rat preoptic area (Matuszewich et al., 2000), increases during sexual activity. It appears that at least the accumbens release occurs immediately before the female receives an intromission (Jenkins and Becker, 2003). The increase in the nucleus accumbens, but not in the preoptic area, is particularly evident in paced-mating tests. Based on these observations, Becker et al. (2001) suggested that accumbens dopamine release is important for sexual motivation and reward. Although attractive, this proposal runs into considerable problems if a substantial amount of earlier pharmacological studies is taken into account. In fact, several kinds of pharmacological manipulations leading to reduced dopaminergic activity stimulate lordosis behavior in estrogen-primed rats (reviewed in Ahlenius, 1993; see also Ahlenius et al., 1972; Caggiula et al., 1979; Everitt et al., 1974, 1975; Everitt and Fuxe, 1977; Herndon et al., 1978). To the contrary, facilitation of dopaminergic neurotransmission by a dopamine releaser or dopamine receptor agonists inhibit lordosis (Eliasson and Meyerson, 1976; Everitt et al., 1974; Michanek and Meyerson, 1977a, 1982). It appears that the dopamine D_2 receptor is mediating these effects because D_1 agonists and antagonists do not modify receptivity (Grierson et al., 1988). There is, then, substantial pharmacological evidence showing that enhanced dopaminergic activity has an inhibitory effect on lordosis behavior, whereas reduced activity in dopaminergic systems has a facilitatory effect. The processes of determining the intensity or ease of activation of a response are frequently conceptualized as motivation. Since lordosis is a sexual response, its ease of activation must be considered an indicator of sexual

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motivation (Pfaff and Ågmo, 2002). Therefore, the reports summarized above suggest that, at least, some aspect of sexual motivation is inhibited by dopamine.

Concerning dopaminergic effects on other putative indices of sexual motivation, for example, proceptive behaviors, data are scarce, and results are mixed. One study reported that intraventricular 6-OH-dopamine reduced proceptivity (Caggiula et al., 1979), while another study from the same group failed to detect any effect (Herndon et al., 1978). Infusion of 6-OH-dopamine into the ventral tegmental area left both lordosis and proceptivity unaffected (Hansen et al., 1991). There was no locomotor response to amphetamine in the lesioned animals, showing that the mesolimbic dopamine system was severely affected by the lesion. Thus, available data suggest that dopamine is of slight importance for proceptive behaviors. In agreement with this proposal is a report showing that electrical stimulation of the ventral tegmental area fails to modify proceptivity (Hasegawa et al., 1991).

Although a majority of data suggest an inhibitory role of dopamine in female sexual behavior, there are a few reports showing facilitation. Apomorphine and dopamine itself stimulated lordosis when infused into the preoptic area or ventromedial hypothalamus in animals primed with estrone, a rather unusual way to induce receptivity. Dopamine antagonists had the opposite effect (Foreman and Moss, 1979). In another study, dopamine agonists were ineffective in the preoptic area while facilitating lordosis after infusion into the ventromedial nucleus (Apostolakis et al., 1996). A D_1 receptor agonist was recently reported to facilitate lordosis after infusion into the ventral tegmental area, whereas an antagonist had the opposite effect (Petralia and Frye, 2002). Finally, apomorphine, as well as specific D_1, but not D_2 agonists, enhanced lordosis after infusion into the 3rd ventricle (Mani et al., 1994). In view of the contradictory observations, it is impossible to arrive to a final conclusion as to the importance of dopamine in the control of female sexual behavior. Moreover, only parts of the sequence of events constituting this behavior were included in the earlier studies.

Lordosis is the final element of female rat sexual behavior. Before the female can receive the tactile stimulation provided by the mounting male, which is necessary for activating the lordosis circuit (Pfaff et al., 1973), she needs to have detected a male, approached him, and eventually activated his mounting behavior. About 90% of sexual interactions are initiated by the female while the male initiates only about 3% of such interactions (McClintock and Adler, 1978). This shows that female precopulatory behaviors are key determinants of sexual encounters. The mechanisms controlling the occurrence and intensity of approach behaviors are normally called motivation. In addition, any stimulus able to activate approach behaviors is defined as a positive incentive (Bindra, 1969, 1974, 1978). Motivation activated by the presence of an incentive is considered as incentive motivation. In the case of the female rat, it has repeatedly been shown that the male indeed functions as a positive incentive (reviewed in Ågmo, 1999; Pfaff and Ågmo, 2002). Neither the standard mating test (male and female put together in a small enclosure) nor paced mating (where the female moves freely while the male is confined to one part of the testing environment) is ideally suited for evaluating the intensity of approach behaviors, that is, sexual-incentive motivation (discussed in Pfaff and Ågmo, 2002). There are, however, alternative procedures that reliably evaluate this aspect of sexual behavior (Ågmo, 2003a; Meyerson and Lindström, 1973; Vega-Matuszczyk and Larsson, 1991).

In the present series of experiments, we analyzed the role of dopamine in the control of sexual-incentive motivation, paced-mating behavior, and receptivity and proceptivity. We employed a procedure designed for the evaluation of sexual-incentive motivation, independently of copulatory behavior (Ågmo, 2003a), in addition to tests of paced sexual behavior. Two doses each of the dopamine receptor agonist apomorphine, the dopamine releaser amphetamine, and the receptor antagonist cis(*Z*)-flupenthixol were employed. During the test for sexual-incentive motivation, several parameters of spontaneous activity were registered besides variables related to sexual motivation. Because dopamine agonists may induce stereotyped behaviors, particularly sniffing (e.g., Havemann et al., 1986), even when administered in low doses, we also quantified sniffing behavior during part of the paced-mating test.

2. Methods

2.1. Animals

Male (about 300 g upon arrival) and female (250 g) Wistar rats were bought from B&K, Sollentuna, Sweden. They were housed in pairs in large Macrolon cages and were given free access to food (B&K low protein rodent pellets) and water. The animal room was maintained at 21 ± 1 °C, with a relative humidity of $55 \pm 10\%$. Lights were off between 1100 and 2300 h.

Females were ovariectomized under anesthesia with Hypnorm/Dormicum (Janssen-Cilag, Oslo, Norway, and Roche, Basel, Switzerland, respectively; doses were 0.2 mg/rat of fentanyl, 3 mg/rat of fluanisone, and 3 mg/rat of midazolam). Two males, to be used as incentive animals, were castrated under the same anesthesia.

All experimental procedures were approved by the local ethics committee and the use and care of animals were in agreement with the European Community directive 86/609/EEC and applicable local laws.

2.2. Apparatus

The general setup of the sexual-incentive motivation test has been described in considerable detail earlier (Ågmo,

2003a). Basically, it consisted of an oval arena, (100 × 50 × 45 cm high) with two openings (25 × 25 cm) diagonally opposed on the long walls. Cages containing the incentive animals could be fitted to these openings. The front of the incentive animal cages was made of wire mesh (1-mm wire, mesh size 12 × 12 mm), allowing the experimental and incentive animals to see, smell, and hear each other. Although limited physical contact was possible through the meshes, no copulatory interaction could occur. A virtual area (30 × 20 cm) adjacent to each incentive animal cage was defined as the incentive zone. A video track system (Ethovision Pro, Noldus, Wageningen, The Netherlands) determined the position of the experimental subject's point of gravity, with a frequency of 5 Hz. In addition, it calculated the distance moved during the test, the mean velocity of movement, and the time moving. The system was set in such a way that nonambulatory movements, for example, grooming, were not registered.

Paced sexual behavior was observed in rectangular arenas (40 × 60 × 40 cm high), divided in halves by a transparent Plexiglas screen. Three regularly spaced openings of adjustable size in the screen allowed the female free passage between the halves. Because of its larger size, the male could not pass through the openings. A video camera was placed exactly in front of the observation cage, and all tests were recorded on tape.

2.3. Design and procedure

Before beginning experiments, the females were familiarized to the incentive motivation test arena during three sessions of 10 min each, separated by 24 h. During these sessions, the incentive animal cages were empty. Then, the females, as well as the males, were accustomed to the mating tests. The females were given an injection of estradiol benzoate (25 μg/rat) and, 48 h later, of progesterone (1 mg/rat). Both steroids were purchased from Sigma (St. Louis, MO, USA), dissolved in peanut oil and injected subcutaneously in a volume of 0.2 ml/rat. These doses of the steroids induce paced sexual behavior of an intensity similar to that seen in intact, cycling females (Brandling-Bennett et al., 1999; Zipse et al., 2000). Some researchers have employed low doses of estrogen when stimulation of sexual behavior was expected or wanted, and high doses when inhibition was predicted or desired. In addition to begging the question, this means testing the animals in an unnatural state. Although such a procedure could facilitate the detection of drug effects, we preferred to employ females in a state similar to physiological estrus. This would presumably increase the physiological significance of any drug effect.

Training was performed 4–6 h after the progesterone injection. Although the females rapidly learn how to enter into and escape from the male's side, this learning should preferably precede drug treatment. Most studies of paced mating include some form or another of training to attain a stable level of sexual performance. The female was put on one side of the mating test arena, and about 5 min later, a sexually inexperienced male was introduced on the other side. Sexually inexperienced males were used for three reasons. First, they are easily trained to remain on their side of the test cage rather than trying to pass through and get stuck in the openings. Second, it was considered desirable to use males and females of about the same age. Finally, sexual behavior is only marginally modified by experience, and after two tests with two ejaculations at each, the behavior has already reached a stable level (Cruz-Casallas et al., 2000). The training test lasted until the male had achieved two ejaculations. In case a male did not intromit within 15 min, or did not ejaculate within 30 min of the first intromission, it was replaced with another, and the test continued until the female had received two ejaculations. An identical test was performed 2 weeks later. Only males that achieved two ejaculations on at least one of these tests were used as studs in the experiment. Data from the training tests were not used because they are unrelated to the purpose of this study.

After training, each female was assigned a particular male and, on future tests, this and only this male was the stud. This means that the same partner was used after treatment with the different doses of each drug. The rationale for this was that any drug-induced change in female behavior should be more easily detected with a constant than with different males. Whether repeated copulation with the same male modifies the female's behavior is unknown, but any possible effect of this should have been balanced out between drug doses.

After the completion of pacing training and familiarization to the incentive motivation test arena, the experiment was started. Before each experimental session, the females received the hormonal treatment described above. After the appropriate drug treatment, a 10-min sexual-incentive motivation test with the incentives castrated male and intact male was performed. Immediately thereafter, the experimental subject was transferred to another room where the paced-mating test was performed. This means that the preinjection time for the mating test was about 13 min (range 11–15 min) longer than for the sexual-incentive motivation test.

During tests, behavioral items were recorded on line using the EthoLog software (Ottoni, 2000, downloaded from http://www.geocities.com/CapeCanaveral/Lab/2727/ethohome.html). The following parameters of sexual behavior were registered or calculated for the female: latency to enter the male's half (time from introduction of the male until the female had four paws on the male side); proportion of exits after mount (number of times the female left, that is, four paws inside her own half, the male's half within 10 s of a mount or without having showed any behavior other than locomotion if exit occurred after this time/total number of mounts received); proportion of exits after intromissions and ejaculation (calculated as indicated for the mount); return latency after mount (this is the time between the female's exit from the male's compartment following a mount and

her return); return latency after intromission and ejaculation (calculated as for mounts); and total number of crossings between compartments. We also recorded the number of female rejections. In agreement with other studies of paced mating (Coopersmith et al., 1996), the incidence of such behaviors was low. Too low for statistical analysis, in fact. No instance of aggressive behavior was recorded in the present series of experiments. In addition, the presence or absence of lordosis when mounted was recorded, and the lordosis quotient was calculated (total number of lordosis displayed/mounts received; Hardy and DeBold, 1971; Kuehn and Beach, 1963). Finally, the number of proceptive behaviors (ear wiggling and hop darting) and the frequency and duration of sniffing were recorded during the first 5 min of the test. This was done from the videotapes by an observer blind to the animals' treatment. The reasons for limiting the observation of these behaviors to the initial part of the tests were (a) the proceptive behaviors have been shown to be important for the activation of male copulatory behavior (Hlinak and Madlafousek, 1977; Madlafousek and Hlinak, 1983; Madlafousek et al., 1976) and are consequently most frequent during the early stages of sexual interaction. Thus, we observed these behaviors during the period they have an established biological significance. (b) The purpose of quantifying sniffing was to evaluate whether drug-induced behaviors incompatible with sex affected the females' propensity to enter the male's half. A 5-min observation period was arbitrarily considered sufficient for attaining the purpose. Lordosis duration was also determined from the videotapes. The frame where dorsiflexion began was taken as the starting point. The tape was then advanced, frame by frame, until the dorsiflexion had disappeared completely. Knowing that the camera recorded 25 frames per second, the lordosis duration (number of frames between the beginning and end of lordosis divided by 25) was determined with a precision of 1/25th of a second.

Items of male sexual behavior were also recorded: mount and intromission latency (the time from the female's entry into the male's half until the first mount and intromission, respectively); ejaculation latency (time from the first intromission until ejaculation); postejaculatory interval (time from ejaculation until the following mount); number of mounts (only mounts with pelvic thrusting were recorded); and number of intromissions. The mating test was ended at the end of the first postejaculatory interval; if the female did not enter the male's half within 15 min; if no intromission occurred within 15 min of the female's entry; or if the ejaculation latency was >30 min.

We have previously reported that performance in the sexual-incentive motivation test, as well as in sex behavior tests, is stable over a large number of repeated tests (Ågmo, 2002, 2003a). Thus, repeated testing should not affect present results. It is not known if the motivation test affects the paced-mating test that immediately followed. Despite extensive use of this procedure, we have never observed such an effect, though.

2.4. Drugs

Apomorphine HCl (Sigma) was dissolved in physiological saline containing 0.1% (v/w) ascorbic acid. The solution was kept on ice until injection and always used within 90 min of preparation. Two doses of apomorphine were included, 0.125 and 0.5 mg/kg. This drug was administered subcutaneously 10 min before the sexual-incentive motivation test. D-Amphetamine sulfate (Sigma) and cis(*Z*)-flupenthixol × 2 HCl (Lundbeck, Copenhagen, Denmark) were dissolved in physiological saline and injected intraperitoneally. Preinjection times were 40 and 30 min, respectively. Amphetamine was given in the doses of 0.25 and 1 mg/kg, and flupenthixol in the doses of 0.25 and 0.5 mg/kg. The doses of all drugs we employ here have previously been used in similar studies in the male rat. A low dose of each drug was found to have modest or no motor effects, whereas the high dose normally had clear effects on motor functions (Ågmo, 2003b). The injection volume was always 1 ml/kg body weight, and the doses indicated were based on the salt. Drug administration was counterbalanced so that all subjects received both doses of each drug plus saline. Each drug was administered to the 12 females.

Doses were similar with those used in several earlier studies in the male in this laboratory (Ågmo and Fernández, 1989; Ågmo and Picker, 1990; Ågmo and Villalpando, 1995) and in the lower range of doses employed in earlier studies of females (Eliasson and Meyerson, 1976; Everitt and Fuxe, 1977; Everitt et al., 1975; Michanek and Meyerson, 1977a; Sietnieks and Meyerson, 1985). Preinjection times were similar with those used in the studies just mentioned. The interval between each drug treatment was sufficiently long for avoiding any effect of a previous dose being carried over to a following treatment (Ågmo and Soria, 1999).

2.5. Statistics

The preference score [time spent in the intact male's incentive zone/(time in this zone + time in the castrated male's incentive zone)], distance moved, mean velocity of movement, and time moving were analyzed with one-factor ANOVA for repeated measures. The factor was dose. Frequency and total duration of sniffing, as well as the mean duration of each sniffing episode, were analyzed in the same way. The time spent in the incentive zones and the number of visits to them were evaluated with two-factor ANOVA for repeated measures on both factors. The factors were dose and incentive (intact vs. castrated male). Since there was a large neutral zone available for the experimental subjects, neither the times spent in the incentive zones nor the number of visits to them were mathematically dependent on each other, making these analyses possible.

A preference for a particular incentive should manifest itself both as a preference score significantly larger than 0.5 (absence of preference) and a significantly longer time spent in that incentive area than in the other. This double criterion

becomes especially important when drug-induced change in preference is the object of study. A larger preference score following one or more doses of a drug can be a result of either increased time spent in the active male's incentive zone, reduced time spent in the castrated male's incentive zone, or a combination of both. Conversely, a reduced preference score may be a result of either reduced time in the active male's incentive zone, increased time in the castrated male's incentive zone, or a combination of both. It is not evident that the motivational significance of the different possibilities is the same. Thus, although the preference score probably is the best indicator of the intensity of sexual-incentive motivation, *changes* in preference score between one dose and another become meaningful only when combined with data on changes in time spent in the incentive zones.

The proportion of females displaying lordosis, as well as the proportion of males displaying mount, intromission, and ejaculation, were analyzed with Cochran's Q test. This was the only choice for the nominal variable absence–presence of the respective behavior. In case of significance, the McNemar test for the significance of changes was used for comparing each dose with saline. The lordosis quotient and the proportion of mounts, intromissions, and ejaculations, followed by escape, were analyzed by Friedman's ANOVA, followed by the Wilcoxon test in case of significance. Here, the distribution of data deviated substantially from normality, excluding the employment of parametric tests. In the case of the lordosis quotient and the proportion of exits after ejaculation, most females had a value of 1, whereas several females had a value of 0 in the case of proportion escapes after mount. Return latencies, the lordosis duration, the number of proceptive behaviors, and the number of crossings between halves, as well as the male latencies and number of mounts and intromissions, were analyzed with one-factor ANOVA for repeated measures. A posteriori comparisons were performed with the Tukey HSD test.

In the case of apomorphine, where only a few animals displayed sexual behavior after the 0.5 mg/kg dose, repeated-measures analyses produced small *n*s making the test's power rather small. Therefore, one-factor ANOVAs for independent measures were employed in addition to the repeated-measures analyses. This is indicated where appropriate. The latency to enter the male's compartment and the number of proceptive behaviors had nonhomogenous error variances, as shown by Hartley's F_{max} test. Therefore, the nonparametric Friedman ANOVA and the Kruskall-Wallis one-way ANOVA were employed instead of the parametric tests. These tests were also used for the number of mounts and intromissions displayed by the males copulating with apomorphine-treated females. About half the animals had a value of 0 after the 0.5 mg/kg dose, making the distribution highly skewed.

Before performing the data analysis described above, we evaluated any possible effect of treatment order. This was done by using order of treatment as an additional factor in the ANOVAs, as recommended by Winer et al. (1991). No significant effect of treatment order was found (all $P>.2$), and this issue is not further mentioned.

All tests were two-tailed, and an alpha >.05 was considered nonsignificant.

3. Results

3.1. Apomorphine

3.1.1. Sexual incentive motivation

Due to a computer failure, data from one animal were lost after treatment with apomorphine, 0.5 mg/kg. Because of the repeated-measures design, data from this animal could not be included in the analyses. The following account is therefore based on data from 11 subjects.

The preference score (Fig. 1A) was unaffected by treatment with apomorphine according to ANOVA, $F(2,20)=1.36$, NS. Although the score did not differ significantly between treatments, apomorphine nevertheless seemed to have some effect. While the preference score was above chance level (0.5) after saline, it was not so when the animals were treated with either 0.125 or 0.5 mg/kg apomorphine. The apparent reduction of sexual-incentive motivation after apomorphine becomes somewhat more evident when the time spent in the incentive zones is analyzed. There was an effect of incentive, $F(1,10)=14.09$, $P<.01$, while that of apomorphine dose was of borderline significance, $F(2,20)=3.42$, $P=.053$. There was no interaction Dose × Incentive, $F(2,20)=1.63$, NS. However, as can be seen in Fig. 1B, the time spent in the intact male's incentive zone was larger than that spent in the castrated male's incentive zone only after saline when the respective means were compared with the Tukey HSD test.

ANOVA of the number of visits revealed a difference between incentives, $F(1,10)=13.75$, $P<.01$, as well as a dose effect, $F(2,20)=7.70$, $P<.01$. The interaction incentive-dose was nonsignificant, $F(2,20)=1.96$, NS. When the doses were compared with the Tukey test, it became apparent that the total number of visits to the incentives (i.e., active + castrated male) was reduced after both doses of apomorphine compared with saline. However, comparisons of the interaction means with the Tukey test revealed that only the number of visits to the intact male was reduced, only after the 0.5 mg/kg dose. It is noteworthy, though, that the subjects made more visits to the intact male than to the castrated only after saline. There was no dose effect on the number of visits to the castrated male. Data are summarized in Fig. 1C.

The reduced number of visits suggested a drug-induced inhibition of ambulatory activity. Analysis of the distance moved confirmed this. There was a significant effect, $F(2,20)=8.76$, $P<.01$, and the Tukey test determined that both doses diminished ambulatory activity (Fig. 1D). The reduction of the distance moved was a consequence of a

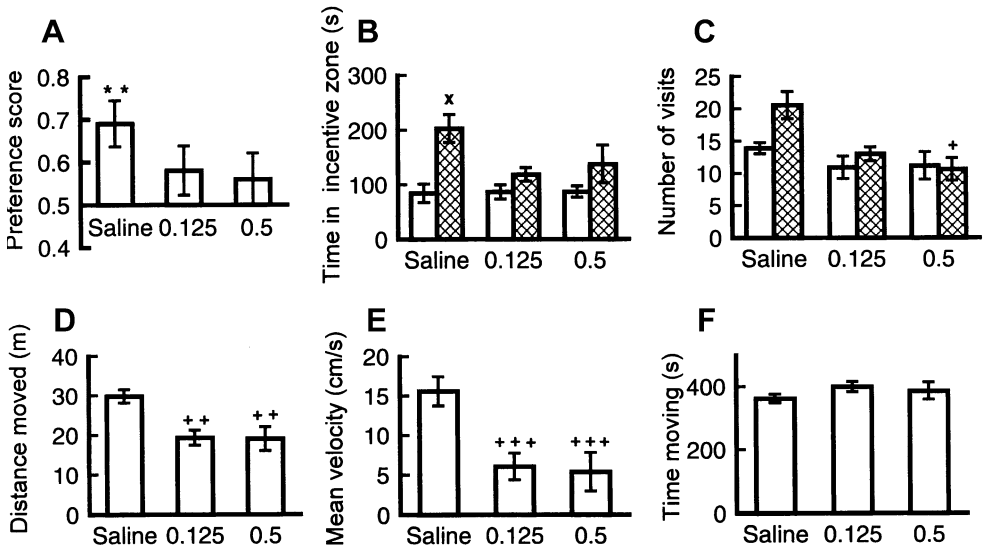

Fig. 1. Mean (± S.E.M.) of preference score (A), time spent in the incentive zones (B), number of visits to the incentive zones (C), distance moved (D), velocity of movement (E), and time moving (F) in female rats ($n = 11$) treated with varying doses of apomorphine. The dose (under the abscissa) is given in milligrams per kilogram body weight. White bars: castrated male incentive; hatched bars: intact male incentive. Asterisks indicate a significant difference from no preference, that is, a preference score of 0.5, $^{**}P < .01$. The multiplication sign indicates a significant difference from the castrated male, $^{x}P < .05$. Plus signs indicate a significant difference from saline, $^{+}P < .05$, $^{++}P < .01$, $^{+++}P < .001$.

drastic reduction in velocity of movement, $F(2,20) = 79.76$, $P < .001$ (both doses reduced it; Fig. 1E). There was no effect on time moving, $F(2,20) = 1.11$, NS (Fig. 1F). Thus, whereas apomorphine had ambiguous effects on parameters of sexual-incentive motivation, the drug had a strong inhibitory effect on ambulatory activity.

3.1.2. Female sexual behavior

The test was ended before the first ejaculation in five of the saline-treated animals, in six animals after treatment with apomorphine, 0.125 mg/kg, and in 10 animals after apomorphine, 0.5 mg/kg. The reason was that the latency to enter the male's half exceeded the cutoff limit of 15 min (three animals treated with 0.5 mg/kg), or the intromission latency exceeded 15 min (two animals after saline, five animals after 0.125 mg/kg, and six after 0.5 mg/kg), or the ejaculation latency exceeded 30 min (three animals after saline, one after 0.125 mg/kg, and one after 0.5 mg/kg). In no case the test was ended because of a return latency exceeding the established criteria.

Analysis of the number of proceptive behaviors revealed a significant effect of apomorphine dose, $\chi^2(2) = 21.04$, $P < .001$. A posteriori comparisons established that both doses reduced proceptivity. In fact, proceptive behavior was almost absent after the 0.5 mg/kg dose (see Table 1).

Of the females entering the male's half and being mounted by the male all displayed lordosis with a lordosis quotient close to 1. Lordosis duration was not modified by apomorphine, $F(2,24) = 0.69$, NS, when analyzed with ANOVA for independent observations. The employment of this analysis permitted the inclusion of data from all animals displaying lordosis. The lack of drug effect on lordosis duration was confirmed when data from the four females showing lordosis after all treatments were evaluated with ANOVA for repeated measures, $F(2,6) = 1.11$, NS. The latency to enter the male's half was modified by apomorphine, both when analysis was made for independent observations, $\chi^2(2) = 10.14$, $P < .01$, and when only animals entering after all treatments were included in a repeated-measures analysis, $\chi^2(2) = 6.89$, $P < .05$. In both cases, the 0.5 mg/kg, as well as the 0.125 mg/kg, dose prolonged the latency to enter. There was no drug effect on any other aspect of pacing when all data were included in analyses for independent measures (all $P > .12$). Dependent-measure analyses, where only animals displaying a particular behavior were included, gave similar results ($P > .20$). However, the number of crossings through the division was much reduced by apomorphine, $F(2,22) = 11.12$, $P < .001$. The Tukey test established that the dose of 0.5 mg/kg reduced this number, whereas the lower dose was ineffective. Data concerning female sexual behavior are illustrated in Table 1.

In summary, apomorphine seemed to reduce the females' willingness to engage in sexual activity and/or affected the male's interest to mount. After sexual interaction was initiated, though, the drug did not modify any parameter of female sexual behavior. The reduced ambulatory activity observed in the sexual-incentive motivation test was evident also in the mating test.

3.1.3. Relationship between ambulatory activity and the propensity to escape from the male

Due to the low number of females displaying sexual behavior after apomorphine, 0.5 mg/kg, no meaningful analysis could be performed.

Table 1
Paced sexual behavior in female rats treated with saline or two doses of apomorphine

Behavior	Saline	Apomorphine 0.125	Apomorphine 0.5
Proceptive behaviors	16.7 ± 2.45 (12)	4.2 ± 1.32 (12)*	0.3 ± 0.2 (12)**
Latency to enter	17 ± 3 (12)	119 ± 34 (12)*	234 ± 72 (9)**
Lordosis quotient	1.00 ± 0.00 (12)	0.95 ± 0.05 (10)	0.97 ± 0.08 (5)
% Displaying lordosis	100 (12)	100 (10)	100 (5)
Lordosis duration	1.79 ± 0.36 (12)	1.96 ± 0.15 (10)	1.38 ± 0.14 (5)
% Escape after mount	28 ± 8 (12)	24 ± 7 (10)	12 ± 5 (5)
% Escape after intromission	65 ± 10 (10)	37 ± 13 (7)	27 ± 18 (3)
% Escape after ejaculation	100 ± 0 (7)	67 ± 21 (6)	50 ± 50 (2)
Return latency mount	66 ± 20 (12)	98 ± 52 (9)	101 ± 55 (3)
Return latency intromission	57 ± 13 (10)	95 ± 35 (7)	117 ± 58 (3)
Return latency ejaculation	99 ± 21 (7)	255 ± 102 (6)	74 ± 74 (2)
Crossings through division	25.2 ± 4.25 (12)	15.4 ± 3.2 (12)	5.2 ± 1.8** (12)

Data are mean ± S.E.M. Lordosis quotient is based on data from females having received at least one mount, and lordosis duration is based on females having displayed at least one lordosis. In case multiple lordoses were shown by the same female, the mean duration was calculated, and the data above is, therefore, the mean of means. The percent escape, as well as return latencies are likewise based on data from females having received at least one mount, intromission, etc. The *n* for each parameter at each dose is shown in parenthesis after the standard error. Latencies are in s. Asterisks indicate significant difference from saline according to Tukey's HSD or the Wilcoxon *T* test.

* $P < .05$.

** $P < .01$. *

3.1.4. Drug effects on behavior incompatible with mating

Sniffing was affected by apomorphine. There was an effect on sniffing frequency, $F(2,22) = 19.09$, $P < .001$, on total duration of sniffing, $F(2,22) = 55.32$, $P < .001$, as well as on the mean duration of each episode of sniffing, $F(2,22) = 6.99$, $P < .01$. In fact, animals given the 0.5 mg/kg dose spent most of the observation period sniffing, and this behavior was rarely interrupted by other activities. Data are shown in Fig. 2A–C.

3.1.5. Male sexual behavior

Despite the fact that the male partner was untreated, there were several changes in male sexual behavior as a consequence of treating the female with apomorphine. The reduced proportion of males displaying mounts (Cochran's $Q(2) = 9.75$, $P < .01$) and intromissions, $Q(2) = 6.73$, $P < .05$, is partly, but not entirely explained, by the fact that three females never entered the male's half. The number of mounts, $\chi^2(2) = 7.45$, $P < .05$, and intromissions, $\chi^2(2) = 7.66$, $P < .05$, was also reduced. Mount [ANOVA for independent measures, $F(2,24) = 2.01$, NS; dependent measures, $F(2,6) = 4.04$, NS] and intromission latencies [independent measures, $F(2,17) = 2.43$, NS; no animal intromitted under all three treatments] were unaffected by apomorphine. Data are shown in Table 2.

3.2. Amphetamine

3.2.1. Sexual incentive motivation

There was no drug effect on the preference score, $F(2,22) = 1.56$, NS, and the score remained significantly above chance after both doses (Fig. 3A). Similarly, the time spent in the incentive zones was not modified, $F(2,22) = 1.26$, NS, whereas the incentives differed, $F(1,11) = 140.51$, $P < .001$. There was no interaction between dose and incentive, $F(2,22) = 1.98$, NS. Data are shown in Fig. 3B. The number of visits to the incentives was modified by amphetamine, $F(2,22) = 75.15$, $P < .001$. There was also a difference between incentives, $F(1,11) = 55.07$, $P < .001$, but no interaction Dose × Incentive, $F(2,22) = 1.02$, NS (see Fig. 3C). The Tukey test revealed that the number of visits to the intact male was larger than the number of visits to the castrated male after all treatments. Furthermore, the animals made more visits to both incentives after amphetamine, 1 mg/kg, than after saline. The drug also increased the distance moved, $F(2,22) = 102.44$, $P < .001$, enhanced the velocity of movement, $F(2,22) = 5.13$, $P < .05$, and increased the time moving, $F(2,22) = 33.34$, $P < .001$. The distance moved was augmented by both doses, whereas only the 1 mg/kg dose affected the velocity of movement and the time moving. Data are shown in Fig. 3D–F. In summary, amphetamine was unable to modify sexual-incentive motivation, even at doses having multiple effects on motor functions.

3.2.2. Female sexual behavior

The test was ended before the first ejaculation in four cases after all treatments. This was due to excessive intromission latency in two animals after all treatments, and to excessive ejaculation latency in two others, again after all doses.

All females displayed lordosis after all treatments, with a lordosis quotient of 1.0. There was no significant drug effect on lordosis duration, $F(2,22) = 2.86$, NS. The proportion of escapes following mount was increased, $\chi^2(2) = 10.95$, $P < .01$. A posteriori comparisons showed that only the 1 mg/kg dose was effective. There was no statistically significant effect on any other item of pacing or on proceptivity (all $P > .20$). However, the number of crossings between halves was modified, $F(2,22) = 3.73$,

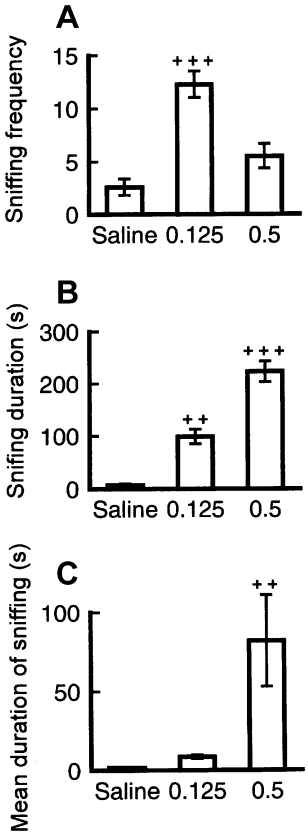

Fig. 2. Sniffing behavior in female rats treated with varying doses of apomorphine. (A) The mean ± S.E.M. number of sniffing episodes during the first 5 min of a paced mating test. (B) Total duration (mean ± S.E.M.) of sniffing during the first 5 min of the test. (C) Mean duration of each sniffing episode. The graph shows the mean ± S.E.M. of the individual means. The apomorphine dose is given in milligrams per kilogram body weight under the abscissa. Plus signs indicate a significant difference from saline, $^{++}P<.01$, $^{+++}P<.001$.

$P<.05$. Only the 1 mg/kg dose was effective. Data are shown in Table 3.

The main effect of amphetamine was to increase motor activity, as shown by the enhanced number of crossings between halves. The drug failed to affect lordosis behavior, and the effect on paced female sexual behavior was limited to a modest increase in the tendency to escape from the male after a mount.

3.2.3. Relationship between ambulatory activity and the propensity to escape from the male

The increase in locomotor activity could be the underlying cause for increased escape after mount. The number of crossings between halves was 28.5 ± 6.9 (mean ± S.E.M.) after saline versus 51.2 ± 10.9 after amphetamine, 1 mg/kg, that is, an increase of 79.6%. If the crossings were randomly distributed over the test session, the probability of crossing during any fixed interval should also increase by about 80%, provided that the length of the observation period was constant. The criterion for regarding an escape as mount-induced is one such fixed interval. In fact, considering a random distribution of exits and taking into account the time spent in the male's half and the total number of exits, a female would exit about every 31.6 s. To consider an exit as mount-induced, we determined that it should occur within 10 s of a mount. The random probability to exit after a mount would then be 10/31.6, that is, 31%. The proportion of escapes after mount was 32%, quite close to random escape, in the saline-treated subjects. There was no significant difference in total test duration between animals treated with saline, amphetamine, 0.25 mg/kg, or amphetamine, 1 mg/kg. Thus, the probability to escape after a mount should increase with about 80% after treatment with amphetamine, 1 mg/kg, since the number of exits increased that much. The observed increase was 75%, from 32% to 56% escapes. Taking into account the higher number of crossings after amphetamine, 0.5 mg/kg, random distribution of exits would be one every 17.6 s. Within 10 s following a mount, the random probability of escape would be 57%, not much different from the observed value. Incidentally, the Pearson correlation between the number of crossings between halves and the proportion of escape after mount in saline-treated animals was 0.74, $P<.01$, showing that activity level is strongly associated with the mount-induced propensity to escape. Due to the higher baseline after intromission, the importance of increased ambulatory activity for intromission-induced escape should be less. Furthermore, the fact that the intromission-induced escape is above random suggests that the intromission itself is a contributing factor, perhaps even a determining factor. Indeed, it appears that sensory feedback from the vaginocervical area is important because denervation or anesthesia of this area reduced escapes to the level seen after mounting (Bermant and Westbrook, 1966; Erskine, 1992). Thus, contrary to the mount, escape after intromission is not a random event but a direct consequence of vaginocervical stimulation. Ambulatory activity should therefore have a much smaller effect on escape after intromission than after the mount. In fact, there is no correlation between activity

Table 2
Sexual behavior in male rats copulating with females treated either with saline or apomorphine in two doses

Behavior	Saline	Apomorphine 0.125	Apomorphine 0.5
% mounting	100	83	41 *
N of mounts	20.0 ± 6.8	13.1 ± 4.4	8.4 ± 4.3
% Intromitting	83	58	25 *
N of intromissions	6.5 ± 1.1	5.5 ± 1.5	1.2 ± 0.7 *
% ejaculating	58	50	16
Mount latency[a]	11 ± 4	47 ± 31	92 ± 39
Intromission latency[a]	67 ± 44	80 ± 58	291 ± 136
Ejaculation latency[a]	653 ± 200	880 ± 206	1049 ± 632
PEI[a]	473 ± 159	379 ± 68	517 ± 252

Data are mean ± S.E.M., analyzed with ANOVA for independent measures. Asterisks indicate significant difference from saline. For further details, see note to Table 1.

[a] Based on data from animals displaying the behavior.

* $P<.05$.

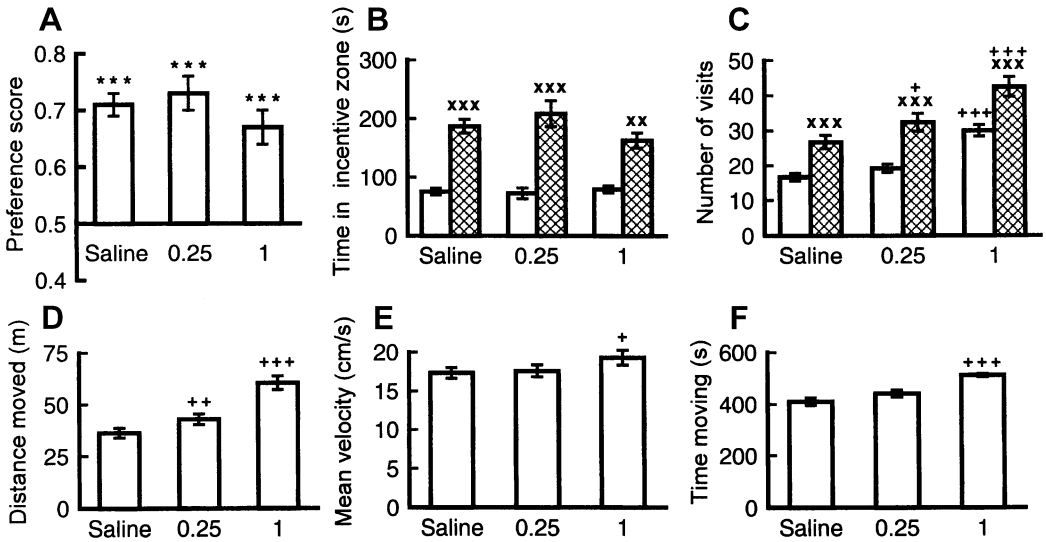

Fig. 3. Mean (± S.E.M.) of preference score (A), time spent in the incentive zones (B), number of visits to the incentive zones (C), distance moved (D), velocity of movement (E), and time moving (F) in female rats ($n = 12$) treated with two doses of amphetamine. The doses (under the abscissa) are given in milligrams per kilogram body weight. White bars: castrated male incentive; hatched bars: intact male incentive. Asterisks indicate a significant difference from no preference, that is a, preference score of 0.5, $^{***}P < .001$. The multiplication signs indicate a significant difference from the castrated male, $^{xx}P < .01$, $^{xxx}P < .001$. Plus signs indicate a significant difference from saline, $^{+}P < .05$, $^{++}P < .01$, $^{+++}P < .001$.

and escape after intromission in saline-treated animals (Pearson's $r = -.26$, NS). Amphetamine failed to significantly affect escapes after intromission.

3.2.4. Drug effects on behavior incompatible with mating

Amphetamine failed to significantly modify sniffing in the doses employed here [frequency, $F(2,22) = 0.87$, NS; total duration, $F(2,22) = 2.97$, NS; mean duration, $F(2,22) = 3.30$, NS]. However, there was a nonsignificant tendency ($P = .056$) for each sniffing episode to be shorter after the largest dose.

3.2.5. Male sexual behavior

The lack of effect of amphetamine on female sexual behavior would suggest that the untreated males' behavior also should remain unaffected. This was indeed the case. There was no significant drug affect on any item of male sexual behavior (all $P > .10$, data not shown).

3.3. Flupenthixol

3.3.1. Sexual incentive motivation

There was no effect on the preference score, $F(2,22) = 0.24$, NS. It remained well above chance level after both doses of flupenthixol. The time spent in the incentive zones was equally unaffected by the drug, $F(2,22) = 0.15$, NS. There was a substantial difference between the incentives, $F(1,11) = 105.55$, $P < .001$. On all occasions, the animals spent more time with the intact male than with the castrated male. The interaction Dose × Incentive was nonsignificant, $F(2,22) = 0.05$, NS. Concerning the number of visits to the incentives, the drug effect was significant, $F(2,22) = 14.77$,

Table 3
Paced sexual behavior in female rats treated with saline or two doses of amphetamine

Behavior	Saline	Amphetamine 0.25	Amphetamine 1
Proceptive behaviors	9.7 ± 1.55 (12)	9.7 ± 2.6 (12)	6.3 ± 1.88 (12)
Latency to enter	18 ± 8 (12)	7 ± 2 (12)	7 ± 1 (12)
Lordosis quotient	1.0 ± 0 (12)	1.0 ± 0 (12)	1.0 ± 0 (12)
% Displaying lordosis	100 (12)	100 (12)	100 (12)
Lordosis duration	1.55 ± 0.12 (12)	1.20 ± 0.13 (12)	1.27 ± 0.11 (12)
% Escape after mount	32 ± 10 (12)	30 ± 8 (12)	56 ± 11 * (12)
% Escape after intromission[a]	52 ± 10 (10)	70 ± 13 (10)	68 ± 10 (10)
% Escape after ejaculation[a]	100 ± 0 (8)	100 ± 0 (8)	100 ± 0 (8)
Return latency mount	17 ± 5 (12)	22 ± 10 (12)	17 ± 5 (12)
Return latency intromission[a]	49 ± 15 (10)	45 ± 25 (10)	19 ± 3 (10)
Return latency ejaculation[a]	159 ± 73 (8)	62 ± 12 (8)	67 ± 12 (8)
Crossings through division	28.5 ± 6.93 (12)	32.7 ± 10.17 (12)	51.2 ± 10.95 * (12)

Data are mean ± S.E.M. Asterisks indicate significant difference from saline. For further details, see Table 1.

[a] Based on data from animals displaying the behavior.

* $P < .05$.

$P<.001$, and there was also a difference between incentives, $F(1,11)=21.31$, $P<.01$, but no interaction Dose-×Incentive, $F(2,22)=1.87$, NS. A posteriori comparisons of the means with the Tukey test showed that the number of visits was larger to the intact male than to the castrated male after saline but not after flupenthixol, independently of dose. Furthermore, the number of visits to the intact but not to the castrated male was reduced after flupenthixol, 0.5 mg/kg.

The distance moved was reduced, $F(2,22)=18.30$, $P<.001$, after both doses. An identical effect was found on the time moving, $F(2,22)=18.12$, $P<.001$. Again, both doses were effective. The mean velocity of movement was not modified, $F(2,22)=0.61$, NS. Data from the sexual-incentive motivation test are displayed in Fig. 4.

3.3.2. Female sexual behavior

The observation of three animals was ended before the first ejaculation after saline, of two animals after flupenthixol, 0.25 mg/kg, and of three animals after 0.5 mg/kg. Too long intromission latency was the cause in one animal each after saline and the 0.5 mg/kg dose, while two animals failed to intromit after 0.25 mg/kg. Another two animals failed to ejaculate within 30 min of the first intromission after saline as well as after flupenthixol, 0.5 mg/kg.

All females displayed lordosis, with a lordosis quotient of 1 after all treatments. Lordosis duration was increased by flupenthixol, $F(2,22)=3.60$, $P<.05$. Only the 0.5 mg/kg dose was effective according to the Tukey test. Neither pacing behavior nor proceptivity was affected by the drug (all $P>.13$). To the contrary, the number of crossings between halves was modified, $F(2,22)=4.95$, $P<.05$. Both doses reduced the number of crossings. Data are shown in Table 4.

3.3.3. Relationship between ambulatory activity and the propensity to escape from the male

Flupenthixol reduced the number of crossings from 20.2 ± 5.1 after saline to 10.5 ± 1.6 after the 0.5 mg/kg dose, that is, a reduction of 48%. The length of the test was not affected by the drug. Thus, if we consider escape after mount as a random event, the proportion of escapes after flupenthixol, 0.5 mg/kg, should be reduced by a similar amount. This was almost the case because flupenthixol reduced the proportion of escapes after mount by 42.8%. Again, if we use total time on the male's side and the number of exits to calculate the probability of a random escape during the 10 s following a mount, it was 22% after saline versus 12% after flupenthixol, 0.5 mg/kg. Observed values were 21% and 12%, respectively. However, the reduction in escape after mount was not significant. This may be due to the low proportion of mounts that were followed by escape after treatment with saline. A low baseline makes it difficult to obtain a significant reduction. In fact, only six animals escaped at all after mount when treated with saline. This means that a reduction of escapes was mathematically impossible in half of the sample. Therefore, we selected the five animals with the proportion of escape after mount above the median after saline treatment. A t test revealed that these animals indeed escaped less after flupenthixol, 0.5 mg/kg, than after saline $t(4)=4.03$, $P<.05$ (mean ± S.E.M. after saline was 0.41 ± 0.11 vs. 0.21 ± 0.09 after flupenthixol). Escape after intromission was not modified in these same animals,

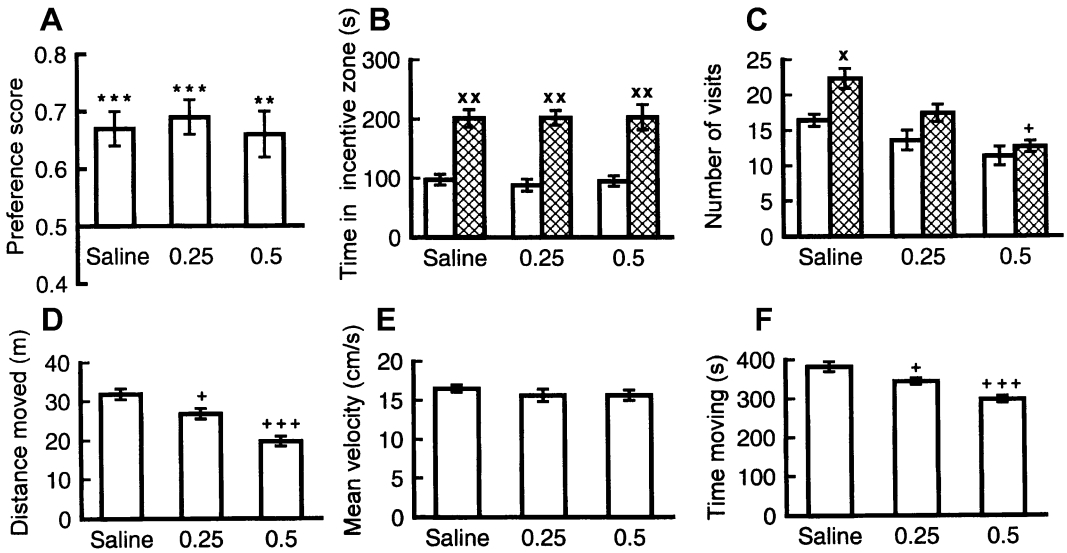

Fig. 4. Mean (± S.E.M.) of preference score (A), time spent in the incentive zones (B), number of visits to the incentive zones (C), distance moved (D), velocity of movement (E), and time moving (F) in female rats ($n=12$) treated with two doses of cis(Z)-flupenthixol. The doses (under the abscissa) are given in milligrams per kilogram body weight. White bars: castrated male incentive; hatched bars: intact male incentive. Asterisks indicate a significant difference from no preference, that is, a preference score of 0.5, $^{**}P<.01$, $^{***}P<.001$. The multiplication signs indicate a significant difference from the castrated male, $^{x}P<.05$, $^{xx}P<.01$. Plus signs indicate a significant difference from saline, $^{+}P<.05$, $^{+++}P<.001$.

Table 4
Paced sexual behavior in female rats treated with saline or two doses of cis(*Z*)-flupenthixol

Behavior	Saline	Flupenthixol 0.25	Flupenthixol 0.5
Proceptive behaviors	9.5 ± 1.75 (12)	6.3 ± 1.31 (12)	6.2 ± 1.66 (12)
Latency to enter	6 ± 2 (12)	9 ± 2 (12)	7 ± 2 (12)
Lordosis quotient	1 ± 0 (12)	1 ± 0 (12)	1 ± 0 (12)
% Displaying lordosis	100 (12)	100 (12)	100 (12)
Lordosis duration	1.22 ± 0.06 (12)	1.71 ± 0.23 (12)	2.16 ± 0.40 *
% Escape after mount[a]	21 ± 8 (10)[b]	9 ± 4 (10)[b]	12 ± 5 (11)[b]
% Escape after intromission[a]	44 ± 12 (11)	30 ± 8 (10)	42 ± 12 (11)
% Escape after ejaculation[a]	100 ± 0 (9)	100 ± 21 (10)	100 ± 50 (9)
Return latency mount[a]	14 ± 6 (10)	24 ± 11 (10)	44 ± 21 (11)
Return latency intromission[a]	59 ± 38 (11)	17 ± 5 (10)	32 ± 10 (11)
Return latency ejaculation[a]	123 ± 53 (9)	134 ± 25 (10)	157 ± 52 (8)[c]
Crossings through division	20.2 ± 5.11 (12)	11.2 ± 1.6 * (12)	10.5 ± 1.6 * (12)

Data are mean ± S.E.M. Asterisks indicate significant difference from saline. For further details, see Table 1.
[a] Based on data from animals displaying the behavior.
[b] One or two females did not receive any mount without intromission.
[c] Of the nine females receiving an ejaculation, one did not return to the male before cut-off time. No return latency could therefore be obtained.
* $P < .05$.

$t(4) = 0.72$, NS. Another illustration of the association between activity and escape is given by a Pearson correlation of .94, $P < .001$, between the number of crossings and proportion of escape after mount in the saline condition (all animals included).

3.3.4. Drug effects on behavior incompatible with mating

Flupenthixol did not modify sniffing [frequency, $F(2,22) = 1.00$, NS; total duration, $F(2,22) = 1.30$, NS; mean duration, $F(2,22) = 0.78$, NS].

3.3.5. Male sexual behavior

There was no effect of flupenthixol on any behavioral item (all $P > .25$, data not shown).

4. Discussion

Although apomorphine showed a tendency to reduce preference for the active male, there was no significant reduction of the preference score. However, the females did not spend more time in the vicinity of the active male than they did in the vicinity of the castrated male after both doses of apomorphine. This observation is indicative of reduced sexual-incentive motivation. Moreover, data from the paced-mating test showed that the latency to enter the male's half was increased by the drug, another observation suggesting reduced sexual motivation. Proceptive behaviors were also reduced and, insofar as these reflect sexual motivation in one way or another, this would reinforce the idea of an inhibitory effect. Nevertheless, the large drug effect on general activity observed both in the test for sexual-incentive motivation and in the paced-mating test obscures any interpretation in terms of sexual motivation. Furthermore, it is possible that the rather intense stereotyped sniffing observed after the 0.5 mg/kg dose was incompatible with the manifestation of behaviors related to the male. It may be noted that enhanced sniffing after treatment with apomorphine has been reported many times (e.g., Bianchi et al., 1986; Ljungberg and Ungerstedt, 1977). In sum, although apomorphine had clear behavioral effects, it failed to stimulate any aspect of female sexual behavior. To the contrary, many were inhibited. Present data do not, however, allow for a definitive conclusion as to the cause of this inhibition.

Lordosis was unaffected by apomorphine. All animals that were mounted displayed lordosis with a lordosis quotient close to 1 and with a lordosis duration equal to that obtained after treatment with saline. This contradicts earlier studies where apomorphine, in doses similar with those used here, reduced lordosis (Eliasson and Meyerson, 1976; Michanek and Meyerson, 1982). A possible explanation is that our data are based on females voluntarily seeking sexual contact with a male, whereas the females in the earlier reports were forcibly exposed to mounting males. It has already been reported that experimental manipulations may have completely different effects in a paced-mating test and in a standard mating test (Whitney, 1986).

Males showed different sexual behavior when copulating with females treated with apomorphine, 0.5 mg/kg, than when copulating with saline-treated females. The main effect was a reduced tendency to mount and intromit. Thus, the females were less efficient in activating sexual behavior in the males. This is probably a consequence of the almost total absence of proceptive behaviors combined with almost constant sniffing. A drug-induced reduction of attractiveness as a contributing factor cannot be excluded. Nevertheless, this observation illustrates the important fact that drug treatment of one member of a copulating pair can affect the behavior of the other member.

Amphetamine failed to modify sexual-incentive motivation but had a small effect on paced-mating behavior. The proportion of mounts followed by escape from the male was increased by the drug. Interestingly, an almost identical effect was reported after ibotenic acid lesion of the nucleus

accumbens core (Guarraci et al., 2002) and after electrolytic lesions destroying most of the accumbens (Rivas and Mir, 1990). These lesions also increased the number of crossings between the male's and the female's halves. A similar effect was observed after amphetamine treatment in the present experiment. Receptivity and sexual motivation were unaffected by accumbens lesion (Rivas and Mir, 1990) just as they were by amphetamine. It seems, then, that the lesions of the nucleus accumbens and systemic amphetamine have quite similar effects on female sexual behavior. It may be worth noting that lesions of the accumbens enhance locomotor activity in the same way as systemic or intra-accumbens amphetamine does (Kelly and Roberts, 1983; Kubos et al., 1987; Maldonado-Irizarry and Kelley, 1995; Sharp et al., 1987). Moreover, the motor stimulating effect of systemic amphetamine injection is abolished after the selective destruction of dopaminergic neurons in the accumbens, suggesting that at least some behavioral actions of amphetamine are mediated by this nucleus (Carey, 1983). The similarities of the effects observed after accumbens lesion and amphetamine treatment can easily be understood, taking into account that dopamine has been suggested to inhibit intra-accumbens circuitry (Akaike et al., 1983; Manns et al., 2003; White and Wang, 1986).

Concerning the stimulatory effects of accumbens lesion and amphetamine injection on the tendency to escape after a mount, one explanation is that both treatments enhance the aversive effects of cutaneous stimulation. There is, indeed, independent evidence showing that accumbens lesion enhances reactivity to environmental stimuli, among these, tactile (Albert and Brayley, 1979; Fernández-Espejo and Mir, 1990; Lee et al., 1983). Amphetamine has also been shown to enhance reactivity to tactile stimuli (Davis, 1980; Handley and Thomas, 1979). The effect of accumbens lesion, as well as of amphetamine, on escape after mounts may be a direct result of this increased reactivity to tactile stimuli. The fact that the effect is specific to mounts is easily explained when taking into account that intromission and ejaculation produce intense vaginocervical stimulation in addition to cutaneous, rendering the latter of marginal importance. It is interesting to note that apomorphine does not affect reactivity to tactile stimuli (Szechtman, 1988) and does not modify the tendency to escape after a mount.

A more parsimonious explanation for the increased escapes after mount is the increased ambulatory activity. Such an explanation is substantiated by the calculations showing that randomly distributed escapes from the male would show an increase similar with that found after amphetamine.

Amphetamine was found to reduce receptivity in some studies (Michanek and Meyerson, 1977a,b, 1982). No such affect was observed in the present experiments, where the lordosis quotient, as well as lordosis duration, was unaffected. This discrepancy is most likely due to the differences in amphetamine dose. Indeed, in a dose-effect study, it was found that the ED_{50} for lordosis inhibition by amphetamine was 2.6 mg/kg (Michanek and Meyerson, 1977a), far above the largest dose employed here. We did not consider it appropriate to use doses beyond 1 mg/kg because already this dose has strong stimulatory effects on ambulatory activity. Potential effects of larger doses of amphetamine on pacing or receptivity would, therefore, inevitably be confounded with nonspecific motor effects, making interpretation of the results ambiguous. The conclusion that amphetamine, in a dose having significant facilitatory effects on ambulatory activity, failed to modify incentive motivation, pacing, and receptivity is firmly supported by our data, and it could probably not be reinforced by the employment of larger amphetamine doses.

Apomorphine and amphetamine were found to have different, sometimes even opposite, effects on some behavioral items. For example, amphetamine stimulated ambulatory activity while apomorphine reduced it. Contrasting results with these drugs have been obtained in many other studies. It has been proposed that the different effects are a result of actions at different brain sites. Indeed, as mentioned above, there is compelling evidence for a primary role of the mesoaccumbens system in the behavioral effects of amphetamine, whereas many of the effects of apomorphine, particularly the induction of stereotyped behaviors, can be attributed to actions within the striatum (Costall et al., 1977a,b; Kelly et al., 1975). Regarding female sexual behavior, the striatum seems to be of modest importance. In fact, striatal lesion has, as its only effect, a modest decrease in the proportion of females escaping from the male after ejaculation (Jenkins and Becker, 2001). This observation suggests that activity in striatal neurons should be involved only in this aspect of paced-mating behavior. Since apomorphine failed to have any effect on escapes after ejaculation, we suggest either that such effects were masked by the stereotyped behavior activated by the drug or that nondopaminergic intrastriatal neurons control postejaculatory escape.

Flupenthixol failed to affect sexual-incentive motivation or any parameter of paced mating, except the number of crossings and the associated reduction of escape after mounts in some animals. However, the duration of lordosis was increased. This coincides with earlier reports where depletion of dopamine with 6-OH-dopamine or treatment with dopamine antagonists produced exactly the same effect (Everitt et al., 1974; Herndon et al., 1978). Since lordosis was displayed after every mount in the present experiments, no facilitatory effect of flupenthixol on the lordosis quotient was possible. Nevertheless, the increased lordosis duration is an indicator of enhanced lordosis behavior. Thus, the data obtained with flupenthixol show that reduced dopaminergic activity inhibits neither sexual-incentive motivation nor the intensity of female sexual behavior. If anything, the latter is facilitated.

It could be argued that the effects on ambulatory activity observed after the largest dose of all drugs may obscure effects on incentive motivation and/or copulatory behavior.

This is certainly possible. However, the lower dose of the drugs failed to modify indices of motricity or had marginal, albeit statistically significant, effects on it. It is unlikely that this could have obscured any effect on incentive motivation or copulatory behavior. In the case of the larger dose, it appears that stereotyped sniffing after apomorphine was more detrimental than effects on ambulatory activity per se. Concerning amphetamine and flupenthixol, they had opposite effects on ambulatory activity while leaving sexual behavior, as well as sexual-incentive motivation, essentially unaffected. This argues against any overshadowing of effects on these behaviors. If changes in ambulatory activity overshadowed some drug effect on motivation or on paced mating, then changes in activity in one direction should reasonably have reinforced this while changes in the other direction should have reduced it. This did not occur.

Present results do not support a stimulatory role for dopamine neither in the control of sexual-incentive motivation nor in the paced-mating behavior or receptivity. Furthermore, there are data showing that dopamine does not contribute to the positive effect (reward) produced by sexual activity in the female rat (Garcia-Horsman and Paredes, in press). Thus, available experimental data suggest that dopamine is involved neither in unconditioned sexual-incentive motivation and paced mating nor in the affective consequences of sexual behavior. The hypotheses concerning a role for dopamine in these processes are, as mentioned in the Introduction, based on inferences from studies of dopamine release in the nucleus accumbens in association with sexual activity. However, all kinds of behavioral events, in addition to sex, release dopamine in this nucleus, for example, tail pinch or restraint stress (Doherty and Grattton, 1992), foot-shock (Takahashi et al., 1998), social defeat (Tidey and Miczek, 1996), eating and drinking (Yoshida et al., 1992), etc. Therefore, it is likely that accumbens dopamine release has some general function. It cannot be associated with reward or approach behaviors, as once believed, because aversive events producing withdrawal rather than approach are just as efficient as rewarding ones for stimulating accumbens dopamine release. We suggest that sex-associated release may be important for the impact of conditioned sexual incentives, perhaps through dopamine-enhanced general arousal. Such a proposal coincides nicely with current views on the function of accumbens dopamine (Berridge and Robinson, 1998; Hall et al., 2001; Ikemoto and Panksepp, 1999).

Dopamine is also released in the medial preoptic area during copulation. Interestingly, this release is only evident in a non-paced-mating situation (Matuszewich et al., 2000), that is, a situation where the female receives intense sensory stimulation from the male's massed mounts and intromissions. Interestingly, intense vaginocervical stimulation activates the fos protein in the preoptic area and other brain sites, and this activation is blocked by a dopamine antagonist (Quysner and Blaustein, 2001). Taken together, these observations suggest that preoptic dopamine release mediates at least some of the consequences of the massive sensory stimulation received during non-paced-mating. There is no indication, though, that preoptic dopamine controls any aspect of paced mating, simply because no dopamine release is observed in this situation.

It appears that dopamine is not a transmitter of primary importance for any aspect of unconditioned female sexual behavior. Its role in the control of sexual motivation and behavior by conditioned sexual incentives remains to be established. Of course, it could be argued that some dopaminergic drug could be effective if infused in a sufficiently large dose at some presently unknown brain site. The force of that argument is somewhat reduced when considering that it can be applied to any negative finding independently of whether the drug was administered systemically or intracerebrally.

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