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Factors influencing fluoxetine-induced sexual dysfunction in female rats

Sarah Adams, Danyeal Heckard, James Hassell, and Lynda Uphouse

Department of Biology, Texas Woman's University, Denton, Texas 76204

Danyeal Heckard: danyealheckard@yahoo.com; James Hassell: hassell_jamese@yahoo.com

Abstract

Treatment with selective serotonin reuptake inhibitors, such as fluoxetine, produces sexual side effects with low sexual desire being the most prevalent effect in females. In few studies have preclinical models for such antidepressant-induced sexual dysfunction been fruitful. In the current manuscript, the effects of fluoxetine on multiple measures of female sexual motivation and sexual receptivity were examined. Ovariectomized, Fischer rats were primed with 10 μ g estradiol benzoate and 500 μ g progesterone. Partner preference, active investigation of the male, and measures of sexual behavior were examined after injection with 15 mg/kg fluoxetine. Factors (pretesting for sexual behavior, size of the test arena, non-contact time with a male) that differ among experiments designed to study antidepressant-induced female rat sexual dysfunction were studied.

The male preference ratio was not affected by fluoxetine treatment but active investigation of the male was reduced; lordosis behavior was inhibited and pretesting for sexual receptivity amplified fluoxetine's inhibition; size of the testing arena or non-contact experience with the male had no effect. Regardless of test condition, when given the opportunity to escape from the male, fluoxetine-treated females displayed escape behavior. Measures of male preference and active investigation, but not lordosis behavior, appeared to be affected by fluoxetine's impact on activity. The collective data provided a behavioral profile of fluoxetine-induced sexual dysfunction. These findings reinforce the value of multiple measures when attempting to model antidepressant-induced female sexual dysfunction.

Keywords

sexual receptivity; sexual motivation; partner preference; active investigation; lordosis; ovariectomized; proceptivity; escape behavior

1.0 Introduction

Selective serotonin reuptake inhibitors (SSRIs) are among the most prescribed classes of antidepressants and are also associated with a high incidence of sexual side effects [1-3]. In

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Department of Biology, Texas Woman's University, Denton, Texas 76204. Tel.: +940-898-2356; fax: 940-898-2382, LUphouse@mail.twu.edu.

Department of Biomedical Science, Meharry Medical College

Department of Biology, University of South Dakota, Vermillion, SD

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many cases, the development of these sexual side effects contributes to patients stopping their medication prior to relief from symptoms of depression [2-5]. Although antidepressant-induced sexual side effects occur in males and females, strategies to reduce the sexual side effects have been less successful in females than in males [6-8]. In part, this reflects the difficulty in precisely identifying the nature of the sexual dysfunction in females. Symptoms of antidepressant-induced sexual dysfunction in females often fall within the category of low sexual motivation [e.g. low desire, low arousal, lack of satisfaction [2, 9, 10]] that has been difficult to assess in animal models.

Although multiple models of female sexual motivation have been used in rodents to differentiate sexually receptive from non-sexually receptive females [11-14], their utility in modeling antidepressant-induced female sexual dysfunction has been limited. Female rodent sexual behavior includes appetitive, precopulatory and consummatory behaviors [14, 15]. Consummatory behavior, which is commonly measured as the lordosis quotient or lordosis to mount ratio, has been the most frequently assessed behavior following treatment with antidepressants and is reported to decline after acute or repeated treatment with the antidepressant, fluoxetine [16-18]. However, in models of female rodent sexual motivation, such as the partner preference paradigm, antidepressant-induced effects have seldom been reported [17-19]. In this paradigm, the female's preference for spending time near a sexually active male, relative to a social incentive, is considered to reflect the female's sexual motivation [13]. When the effect of the SSRI, fluoxetine, was examined, fluoxetine did not reduce the female's preference for spending time near the male even though sexual receptivity (lordosis to mount ratio) was reduced [18]. However, in the experiment by Sarkar et al. [18], the female was tested for sexual receptivity immediately before the measurement of partner preference so it is possible that this pretesting influenced the female's behavior in the partner preference paradigm. In addition, Sarkar et al. studied two doses of fluoxetine: 10 mg/kg which may have been too low for detection of deficits in sexual motivation and 20 mg/kg which may have produced locomotor side effects that influenced the measure of sexual motivation. Therefore, the following experiment was designed to examine the female's behavior in the partner preference paradigm at an intermediate dose of fluoxetine and in the absence of a pretest for sexual receptivity. In addition to the assessment of the male preference ratio, the female's active investigation while near the male was examined as has been previously recommended [20]. Sexual receptivity was measured after completion of the partner preference testing.

Portions of these data were submitted at the 2011, Society for Neuroscience Annual Meeting [21].

2.0 Materials and General Methods

2.1 Subjects

Adult female Fischer rats were purchased from Charles River Laboratories (Wilmington, MA) and housed 2-3 per cage in polycarbonate cages (45.72 × 24.13 × 21.59 cm) with food and water available ad lib. Rats were housed in rooms maintained at 25°C and 55% humidity and with a 12 h-12 h light/dark cycle with lights off at noon.

2.2 Materials

Estradiol benzoate, progesterone, fluoxetine (methyl[3-phenyl-3-[4-(trifluoromethyl)phenoxy]propyl]ammonium chloride), and sesame seed oil were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). Isoflurane (AErrane®) was purchased from Butler Schein Animal Health (Dublin, OH). All other supplies came from Fisher Scientific (Houston, TX).

2.3 General Methods

All procedures were conducted according to PHS policy and were approved by the IACUC at Texas Woman's University.

2.3.1 Surgical procedures and treatment of animals—Two weeks after arrival at TWU, females (150-200 g) were anaesthetized with AErrane® and ovariectomized as previously described [22]. Two weeks after ovariectomy, rats were injected subcutaneously (sc) with 10 µg estradiol benzoate. Two days later, rats were injected sc with 500 µg progesterone. The hormonal priming regimens were based on prior studies showing acute and subchronic effects of fluoxetine under these conditions [18, 23]. Testing ensued approximately 4-6 hr after the progesterone injection. Females were sexually naïve on the day of testing. Fluoxetine was prepared in distilled water at a concentration of 10 mg/ml. Females were injected intraperitoneally (ip) with 15 mg/kg fluoxetine or the water vehicle in a volume of 1.5 ml/kg. A dose of 15 mg/kg was chosen because, in a previous study [18], when the effects of 10 and 20 mg/kg fluoxetine on the male preference ratio were examined, we suspected that the 20 mg/kg dose may have produced interfering behavior while the 10 mg/kg may have been too low for detection of effects of fluoxetine.

2.3.1. Sexual receptivity testing—In some experiments, females were pretested for sexual behavior before injection with fluoxetine. Pretesting occurred in the home cage of a sexually-active male. For sexual behavior testing after fluoxetine, independent of any intervening treatment, receptivity testing always occurred 40 min after the injection with fluoxetine (See Table 1). Sexual receptivity was measured as the lordosis/mount (L/M) ratio (number of lordosis responses divided by number of male mounts). Lordosis quality (relative degree of arching of the back) was scored as previously described [22]. A minimal arching of the back was scored as 1.0 and a normal reflex arch was scored as 3.0; an intermediate arch was scored as 2.0; and an exaggerated reflex was scored as 4.0. Proceptivity [presence of hopping and darting [14]], a measure of sexual motivation, was scored as present or absent. If the female showed a single hop/dart sequence during the respective scoring interval (e.g. before or after fluoxetine), the female was scored as proceptive for that interval.

2.3.2. Statistical analyses—Parametric data were compared by Student's t-test or ANOVA. Other data were evaluated by Fisher's Exact Test or correlations were examined. Post hoc comparisons after ANOVA were performed with Tukey's test. Data were analyzed with SPSS v.15 or v.17 and the statistical reference was Zar [24].

2.4 Specific Procedures

2.4.1 Experiment 1: Partner preference testing and examination of active investigation

2.4.1.1 Adaptation to the testing apparatus: The testing apparatus was a 3-compartment, clear Plexiglas chamber (91.44 × 31.75 × 31.11 cm). On each end of the chamber, a wire mesh cloth separated the main area from an area measuring 16.51 cm long where the incentive male or incentive female could be placed. On the day of the estradiol benzoate injection and the following day, females were adapted to the testing apparatus for 30 min/day. The wire mesh cloth was in place but no incentive animals were present. On the day of testing, test females received an additional 5 min adaptation just before initiation of the experimental procedures.

2.4.1.2 Partner preference testing and active investigation: After the test female's 5 min adaptation period, the incentive male and incentive female (ovariectomized female without

hormonal priming) were placed behind one of the wire mesh barriers. The positions of the incentive male or female were varied and were counterbalanced between experimental groups. The test female was placed into the center of the apparatus and allowed to move freely and spend time near either the male or the female. Behavior was recorded on videotape for 10 min for later analysis. The test female was then removed, injected ip with either distilled water or 15 mg/kg fluoxetine, and returned to the home cage. Thirty min later, rats were again placed into the center of the apparatus for another 10 min period.

In the partner preference paradigm, females can choose between spending time in the vicinity of a sexual (intact male) or a social (castrate male or ovariectomized female) stimulus. An increase in time spent near the sexual incentive relative to time spent near the social plus sexual incentive is thought to reflect the female's sexual motivation/arousal [13]. Seconds spent near the incentive male (TWM) or incentive female (TWF) were determined for the 10 min period before and after injection. The test female was counted as being near the incentive male or female when all four of her paws were inside the 12.7 cm area adjacent to the wire mesh barrier separating the incentive animal from the main apparatus. TWM plus TWF were computed as the test female's total social time (TST). TWM divided by TST was used as the male preference ratio.

Active investigation, as described by Hawcock et al. [20] was scored when the test female engaged in sniffing, licking, chewing, or climbing of the wire mesh divider separating the test female from the incentive animal. Time spent investigating the male (TIM) and incentive female (TIF) were measured and summed for total investigation time (TTI). TIM/TTI was used as the male investigation preference ratio.

The number of times the test female crossed the center of the apparatus was recorded as an index of activity.

2.4.1.3 Sexual behavior testing after partner preference: At the completion of partner preference testing (40 min after injection with fluoxetine), the incentive female was removed from the chamber; the wire mesh barrier between the incentive male and test female was removed; and the incentive male was allowed access to the female in the arena for 10 min of nonpaced mating (e.g. the female could not leave the chamber). Sexual receptivity, lordosis quality, and proceptivity were measured as described in the General Methods (2.3.1).

2.4.1.4 Statistical analysis: Two different individuals scored each videotape. Time with either the male or the female was analyzed by repeated measures ANOVA with before or after fluoxetine as the repeated factor and treatment as the independent factor. For the male preference ratio, the ratio of TWM/TWF was computed within each animal and then analyzed by repeated measures ANOVA. The data for each of these variables by the two scorers were compared by Pearson's rho ($r = 0.82$ for all measures) and the data were then averaged for the statistical analysis. Post hoc comparisons were made with Tukey's test. L/M ratios and lordosis quality data were compared with Student's t-test. Proceptivity data were examined with Fisher's Exact Test procedures.

2.4.2 Experiment 2—Effect of testing environment (test area) on sexual behavior after fluoxetine

2.4.2.1 Adaptation to the testing apparatus: All females were hormonally primed with 10 μ g estradiol benzoate and 500 μ g progesterone and pre-adapted to the partner preference apparatus as described in Experiment 1. On the day of testing, females were assigned to be tested in either the partner preference chamber (large area) or in the home cage of a sexually

active male. Females that were to be tested in the large arena received an additional 5 min adaptation immediately before behavioral testing occurred.

2.4.2.2 Testing procedures: On the day of the experiment, every female was pretested in the home cage of a sexually active male until the male had accomplished 10 mounts. L/M ratios and lordosis quality were monitored as described above. Only females with a pretest L/M ratio ≥ 0.70 were included in the remaining procedures. Immediately after the pretest, rats were injected with either ultra-pure water or 15 mg/kg fluoxetine and returned to their home cage. Forty min after injection [for consistency with receptivity testing after the partner preference testing (2.4.1.3)], sexual behavior testing occurred either in the single central portion of the partner preference arena ($91.44 \times 31.75 \times 31.11$ cm) (no incentive animals present but wire mesh barriers were in place) or in the home cage of a sexually active male cage ($45.72 \times 24.13 \times 21.59$ cm) for 10 min. L/M ratios and lordosis quality were monitored as described in the General Methods (2.3.1).

After this 10 min test for sexual behavior, rats that had been tested in the arena were then given an additional 10 min of testing where the female was able to escape from the male. In this test, one of the wire mesh barriers was completely removed and the other was raised high enough for the test female (but not the male) to exit the central area into a 16.51 cm long space (burrow) behind the wire mesh barrier within the 2-compartment chamber. The female could then choose to remain with the male or escape to the burrow. Time the female spent in the burrow away from the male was monitored.

2.4.2.3 Statistical analysis: Data were compared by two-way, repeated measures ANOVA with time before and after injection as the repeated factors. Type of injection and testing area were the independent factors. L/M ratios, lordosis quality scores, and time spent away from the male were compared by two-factor ANOVA. Post hoc comparisons were made with Tukey's test.

2.4.3 Experiment 3—Effects of pretest and arena experience on sexual behavior

2.4.3.1 Testing procedures: All females were hormonally primed and pre-adapted as described for Experiment 1 (2.4.1). Prior to injection, females were either pretested for sexual behavior in the home cage of a sexually active male or were not pretested but remained in their home cage for a comparable time period. Pretest procedures were as described in Experiment 2 (2.4.2). After this pretest or home cage experience, rats were injected with 15 mg/kg fluoxetine and returned to their home cage. Females were assigned to one of the following conditions: (1) Pretest-preference arena exposure with male incentive behind wire mesh barrier; (2) Pretest-no arena exposure; (3) No pretest-preference arena exposure with male incentive behind wire mesh barrier; and (4) No pretest-no arena exposure. For all conditions, sexual behavior testing occurred in the central portion of the preference testing arena 40 min after injection with fluoxetine.

For rats in the no arena conditions, females remained in their home cages for 40 min after injection with fluoxetine. Sexual behavior testing occurred for 10 min in the central compartment of the partner preference arena. L/M ratio and lordosis quality were recorded as described above (2.3.1). For rats in the arena condition, 25 min after injection with fluoxetine, females were adapted for 5 min in the partner preference apparatus (without a male incentive present). A male incentive was then placed behind the wire mesh barrier and the female remained in the central compartment for 10 min. Although the male was present behind the barrier during this 10 min arena time, partner preference testing was not performed (e.g. no incentive female was present). At the end of this 10 min arena experience, the wire mesh barrier was removed and the male and female were allowed to

interact (nonpaced mating) for 10 min. L/M ratios and lordosis quality were recorded for 10 min. After this 10 min sexual behavior test, the remaining wire mesh barrier was elevated to produce a 2-compartment chamber so the female could escape the compartment with the male (e.g. enter the burrow) and females were tested for 10 min of paced mating. Time spent in the burrow was recorded.

2.4.3.2 Statistical analysis: Data were compared by two-factor ANOVA with presence or absence of a pretest and plus or minus the 10 min period in the test arena as independent factors. Post hoc comparisons were made with Tukey's test. For time in the burrow, data were compared by one-way ANOVA.

2.4.5 Experiment 4—Effects of 20 mg/kg fluoxetine with and without a pretest

2.4.5.1 Treatment of animals: In a final experiment, ovariectomized rats were hormonally primed as in the preceding experiments. Females either received a 10 mount pretest or remained in their home cage for the same period time. Females were then injected with 20 mg/kg fluoxetine and returned to their home cage. Forty min later, females were tested for sexual behavior in the home cage of a sexually active male.

2.4.5.2 Statistical analysis: L/M ratios and lordosis quality were compared by Student's t-test.

3.0 Results

3.1 Partner preference testing and examination of active investigation

3.1.1 Partner preference—Time near the male (TWM), time near the female (TWF), and the total time spent near either incentive animal (TST) are shown in Table 2. Overall, the major change after fluoxetine was an increase in time near the female and a decrease in time near the male incentive while, for water-treated rats, time with either the male or the female incentive declined after the injection. Since TWM declined for both groups, there was a significant effect of the test interval (ANOVA for time, $F_{1,14} = 16.24$, $p = 0.001$) but no other effects were significant. However, for TWF, where water-treated rats declined and fluoxetine-treated rats increased, there was a significant test interval by treatment interaction ($F_{1,14} = 7.92$, $p = 0.01$) and a significant difference between water-treated and fluoxetine-treated rats during the 10 min interval following injection (Tukey's $q_{14,1} = 4.72$, $p = 0.05$). After injection, there was an increase in total social time in fluoxetine-treated rats and a decrease in water-treated rats leading to a significant interaction between test interval and treatment (ANOVA for interaction between time and treatment, $F_{1,14} = 4.92$, $p = 0.04$). However, none of the post hoc comparisons were significant.

In agreement with a prior study [18], fluoxetine failed to significantly reduce the male preference ratio ($p > 0.05$; see Figure 1). Given the respective changes in TWM and TWF, an effect on the male preference ratio might have been expected. However, behavior of fluoxetine-treated rats varied with some of the females spending most of their time near the female incentive and some spending a majority of their time near the male incentive. Five of the 8 fluoxetine-treated females spent more time with the female than the male. However, 4 of the 8 rats injected with fluoxetine never crossed the center portion of the apparatus. Instead, these rats settled near either the male or the female incentive and remained there for the entire test interval. Two of these settled near the female and two settled near the male. As a consequence, the male preference ratio (computed within individual subjects) ranged from 0 to 1.0.

Activity (as indicated by the number of times females crossed the center of the apparatus) was significantly reduced by fluoxetine [(See Table 3) ($F_{1,14} = 8.48$, $p = 0.01$ for treatment)]. The number of crossings by fluoxetine and water-treated females did not differ during the first 10 min in the apparatus while treatment effects emerged following injection. There were significant effects of the test interval ($F_{1,14} = 52.79$, $p = 0.001$) as well as the interaction between test interval and treatment ($F_{1,14} = 20.54$, $p = 0.001$). The interaction resulted exclusively from the decline in fluoxetine-treated rats (Tukey's $q_{14,4} = 11.8$, $p = 0.05$).

3.1.2 Active Investigation—Fluoxetine-treated rats showed significantly less active investigation of the male than did the water-treated rats (See Figure 2). As expected from the hormonal priming with estradiol benzoate and progesterone, prior to injection, females spent more time in active investigation of the male than of the female incentive. After injection, active investigation of both male and female incentives declined for both treatments but this was considerably more robust for fluoxetine-treated than for water-treated rats.

For time investigating the male, there were significant effects of treatment ($F_{1,14} = 4.83$, $p = 0.04$), time of testing ($F_{1,14} = 62.81$, $p = 0.001$) and their interaction ($F_{1,14} = 4.70$, $p = 0.04$) with post injection declines evident for both water and fluoxetine-treated rats (respectively, $q_{14,4} = 5.75$ and 10.09 , $p = 0.05$). After injection, there was a significant difference between water and fluoxetine-treated animals ($q_{14,4} = 5.53$, $p = 0.05$). Although investigation of the female incentive also declined after injection ($F_{1,14} = 8.24$, $p = 0.01$), neither treatment nor the time by treatment interaction were significant ($p > 0.05$). This reflected the relatively low amount of female investigation time that was present before injection.

Therefore, the post-injection decline in total investigation time resulted primarily from a reduction in time spent investigating the male (Table 4). For total investigation time, there were significant effects of the test interval ($F_{1,14} = 65.57$, $p = 0.001$) and treatment ($F_{1,14} = 9.13$, $p = 0.009$) but not their interaction ($p > 0.05$). After injection, total investigation time declined in both water and fluoxetine-treated rats (Tukey's $q_{14,4} = 6.47$ and 9.72 , respectively, $p = 0.05$) but was more substantial in fluoxetine-treated rats. Consequently, water and fluoxetine-treated rats differed significantly after injection ($q_{14,4} = 5.06$, $p = 0.05$).

Because fluoxetine-treated rats spent less time in total active investigation, investigation time was further analyzed in two different ways. First the male investigation ratio [ratio of time spent investigating the male (TIM) relative to total investigation time (TTI)] was examined (male investigation ratio; TIM/TTI) (Table 4). There was a significant effect of the test interval ($F_{1,13} = 7.39$, $p = 0.01$) and of the test interval by treatment interaction ($F_{1,13} = 5.05$, $p = 0.04$) while the main effect of treatment was not significant ($F_{1,13} = 1.54$, $p = 0.23$). Prior to injection, there were no significant differences between the two groups. However, after injection, the male investigation ratio declined and this was considerably more substantial in fluoxetine-treated rats ($q_{13,4} = 4.94$, $p = 0.05$) so that, after injection, there was a significant difference between water and fluoxetine-treated rats in male investigation ratios ($q_{13,4} = 4.17$, $p = 0.05$).

In an additional analysis, the test female's active investigation while near either the male (TIM/TWM) or female (TIF/TWF) incentive was examined (Table 4). Females that failed to spend any time near the incentive were not included in the ANOVA. The proportion of the time near the male that test females spent in active investigation (TIM/TWM) varied with treatment ($F_{1,10} = 14.09$, $p = 0.04$) and test interval ($F_{1,10} = 15.46$, $p = 0.003$). Both effects reflected the greater decline in the ratio in rats treated with fluoxetine (for fluoxetine vs water in the posttest, $q_{10,4} = 5.69$, $p = 0.05$).

The proportion of time near the incentive female that test rats spent in active investigation also varied with time ($F_{1,12} = 32.87$, $p = 0.001$) and treatment ($F_{1,12} = 4.87$, $p = 0.05$). A significant interaction between time and treatment for the proportion of TWF that females spent in active investigation ($F_{1,12} = 12.37$, $p = 0.004$) resulted from the near absence of any active investigation when fluoxetine-treated rats were near the female ($q_{12,4} = 4.19$ for fluoxetine vs water in posttest and fluoxetine in posttest vs pretest). In both cases, fluoxetine treatment was the primary contributor to significant effects (for TIM/TWM and TIF/TWF) (for the difference between fluoxetine-treated rats in pretest and posttest, respectively, $q_{12,4} = 5.89$ and 9.29 , $p = 0.05$).

Before injection, both water-treated and fluoxetine-treated rats showed significant correlations between the TWM and active investigation of the male (for water and fluoxetine-treated rats, respectively, $\rho = 0.93$ and 0.98 , $p = 0.001$). However, after injection, a significant correlation was present for the water-treated rats ($\rho = 0.97$, $p = 0.001$) but not for the fluoxetine-treated rats ($\rho = 0.03$, $p > 0.05$).

3.1.3 Sexual behavior testing after partner preference test—After completion of the testing in the partner preference paradigm, the incentive female was removed from the apparatus and the barrier separating the incentive male from the test female was removed to allow 10 min of nonpaced mating. Unlike prior reports [16, 18, 23], effects of fluoxetine on sexual behavior were relatively small (Table 5). One rat in each of the treatment groups failed to receive any mounts during the test and was eliminated from the sexual behavioral analyses. Every water-treated rat showed L/M ratios at least as high as 0.90. In contrast, lordosis behavior after treatment with fluoxetine was variable with rats falling into two clearly distinguishable categories. Of the 7 fluoxetine-treated rats that were mounted, 4 showed little evidence of a decline in sexual behavior (mean $\pm$ S.E. = 0.97 ± 0.03) while 3 rats showed L/M ratios of 0.55 ± 0.12 leading to a significant difference between the two fluoxetine-treated groups ($t = 9.64$, $df = 5$, $p = 0.01$). Lordosis quality was slightly, but significantly, reduced by fluoxetine ($t = 4.52$, $df = 12$, $p = 0.001$). There were no significant differences in the number of mounts received by the two treatment groups. Hopping and darting behavior (proceptivity) was low in both groups and did not differ between the two treatments ($p > 0.05$).

Since the acute effects of fluoxetine on lordosis behavior differed from other reports, and since there appeared to be two distinct groups of animals in their response to fluoxetine, additional experiments were designed in an attempt to determine what factors may have been responsible for this variation. Three major variables were investigated: (1) size of the testing area (Experiment 2), (2) presence or absence of time in the arena (Experiment 3) and (3) presence or absence of a pretest before injection with fluoxetine (Experiment 3).

3.2 Effects of environment (test area) on sexual behavior after fluoxetine

A large and a small testing area were examined. The central area of the partner preference apparatus was used for the large area and the male's home cage was used for the small test area. All females were pretested in the male's cage before being injected with 15 mg/kg fluoxetine or the water vehicle. After a 40 min delay (to coincide with the sexual behavior testing times in the experiments described above), rats were tested for nonpaced sexual behavior. L/M ratios and lordosis quality are shown in Figure 3. For the L/M ratio, there was a significant effect of time ($F_{1,28} = 59.42$, $p = 0.001$), treatment ($F_{1,28} = 39.35$, $p = 0.001$), and their interaction ($F_{1,28} = 42.41$, $p = 0.001$). Test area was not a significant factor and no interactions with test area were significant (all $p > 0.05$). Regardless of the test area, fluoxetine significantly reduced L/M ratios (for large and small area, respectively, $q_{8,28} = 11.76$ and 8.41 , $p = 0.05$).

For lordosis quality, two fluoxetine-treated rats, tested in the arena, had L/M ratios of zero after injection and were excluded from the analysis of lordosis quality. For the remaining rats, the effect of fluoxetine on lordosis quality was similar to that for the L/M ratios. There was a significant effect of time ($F_{1,26} = 38.06$, $p = 0.001$), water vs fluoxetine treatment ($F_{1,26} = 22.10$, $p = 0.001$) and the interaction between time and treatment ($F_{1,26} = 21.62$, $p = 0.001$). Regardless of the test area, fluoxetine significantly reduced lordosis quality (for large and small area, respectively, $q_{8,26} = 9.38$ and 5.89 , $p = 0.05$). No other effects were significant ($p > 0.05$).

There were no significant effects of either treatment or test area on number of mounts. Average number of mounts for fluoxetine and water-treated rats in the arena and male's cage, respectively, were 13.0 ± 2.25 , 10.22 ± 2.04 , 17.57 ± 3.18 , and 15.50 ± 2.59 .

After conclusion of the 10 min nonpaced sexual behavior test, rats that had been tested in the arena were then allowed the opportunity to escape (e.g. paced mating) the mating arena into a small area (burrow) behind the wire mesh barrier. Mating activity continued for an additional 10 min. As anticipated from their lordosis behavior, fluoxetine-treated rats spent a majority of this time in the burrow, away from the male, while water-treated females spent the majority of their time in the male's vicinity. Mean $\pm$ S.E. for time spent in the burrow for water and fluoxetine-treated rats, respectively, was 229.44 ± 66.15 and 408.09 ± 63.30 and narrowly escaped statistical significance ($t = 1.94$, $df = 18$, $p = 0.068$).

3.3 Effect of pretest and arena experience on sexual behavior

In the next experiment, 4 groups of animals [(1) Pretest-preference arena exposure with male incentive behind wire mesh barrier; (2) Pretest-no arena exposure; (3) No pretest-preference arena exposure with male incentive behind wire mesh barrier; and (4) No pretest-no arena exposure] were examined. For all conditions, sexual behavior testing occurred in the testing arena 40 min after injection with fluoxetine. L/M ratios and lordosis quality are shown in Figure 4. Prior time in the arena had no effect on either L/M ratios or lordosis quality (ANOVA, all effects, $p > 0.05$). In contrast, prior experience with a pretest significantly reduced L/M ratios ($F_{1,28} = 8.79$, $p = 0.006$) and lordosis quality ($F_{1,26} = 6.25$, $p = 0.019$) after fluoxetine. There was no interaction between time in the arena and the presence or absence of a pretest but the effect on L/M ratios seemed to be more robust in females without prior arena experience (Tukey's $q_{30,4} = 3.95$, $p = 0.05$) while lordosis quality differences were more robust in rats with arena experience (Tukey's $q_{30,4} = 3.56$, $p < 0.05$).

To confirm that the L/M ratio after fluoxetine was, in fact, a fluoxetine-induced decline relative to the pretest, data from rats given a pretest were analyzed by repeated measures ANOVA (pre vs post data). There was a significant decline in both the L/M ratio and lordosis quality after fluoxetine (respectively for time factor, $F_{1,12} = 75.12$, $p = 0.001$ and $F_{1,12} = 22.38$, $p = 0.001$). Pretest L/M ratios and lordosis quality, respectively, were 0.99 ± 0.007 and 3.0 ± 0.0 . No other factors were significant.

There were no significant effects on number of mounts by the male (all $p < 0.05$). The mean $\pm$ S.E. average number of mounts for Pretest (male/ no male) and No Pretest (male/ no male), respectively, were: 11.17 ± 1.70 , 13.5 ± 2.27 , 11.00 ± 1.52 , and 11.38 ± 1.55 .

When the experiment was completed, the female was allowed the option of escaping the mating area as described above. Since there were no significant effects of prior experience in the arena, all rats were combined for analysis. All rats had received fluoxetine and rapidly escaped to the burrow; there were no significant differences between rats in the pretest or no pretest condition in time spent in the burrow. The mean $\pm$ S.E. for time in the burrow for rats

previously pretested or not pretested, respectively, was 310.66 ± 64.48 and 426.42 ± 36.55 ($t = 2.62$, $p > 0.05$).

3.4 Effects of 20 mg/kg fluoxetine with and without a pretest

Given the above findings with apparent pretest amplification of the effects of fluoxetine on lordosis behavior, an additional 18 rats received either a pretest or no pretest and were then injected with 20 mg/kg fluoxetine. With this higher dose, there was not a significant difference in L/M ratios between rats that had and had not been pretested ($t = 0.112$, $df = 16$, $p > 0.05$). Mean $\pm$ S.E. L/M ratios after fluoxetine for rats with a pretest and without a pretest, respectively, were 0.35 ± 0.09 and 0.33 ± 0.13 .

4.0 Discussion

There were four major findings in this experiment: (1) the male preference ratio was not significantly affected by fluoxetine treatment; (2) the female's active investigation of the male was reduced by fluoxetine; (3) pretesting for sexual receptivity amplified fluoxetine's inhibition of lordosis behavior; and (4) when given the opportunity to escape from the male, fluoxetine-treated females displayed escape behavior.

These findings are generally consistent with previous studies where the antidepressants, fluoxetine [18] or paroxetine [19], failed to significantly reduce the female's male preference ratio. While such findings might indicate that the partner preference paradigm is inappropriate for detection of antidepressant-induced reductions in female rat sexual motivation, such a conclusion would be premature. Effects of fluoxetine were apparent when either the time near the male or female incentive was examined and the directional change (e.g. reduced time with the male and increased time with the female) is consistent with what would be expected from a reduction in sexual motivation and would have been anticipated to lead to a decline in the male preference ratio. However, the male preference ratio is computed within individual subjects while the respective averages for TWM and TWF represent between-group differences. Fluoxetine-treated females fell into two groups with 5 of the 8 fluoxetine-treated females spending more time with the female than the male. Four of these 5 rats spent essentially no time near the male and 2 of these never crossed the center of the apparatus. As a consequence, male preference ratios for fluoxetine-treated rats ranged from 0 to 1.0. Because of this extreme variability in ratio, there was like to have been insufficient statistical power to detect a group difference. Therefore, it is questionable if the female's relative time near the sexual incentive can be used to reflect sexual motivation.

The significant increase in time near the female after fluoxetine was a surprise since this observation previously has not been noted. However, in those studies in which fluoxetine has been examined in the partner preference paradigm, time spent with the female has not received attention so we can only speculate about the generality of these findings.

Active investigation of the male did, however, reveal significant effects of fluoxetine, suggesting that the inclusion of this measurement may complement the use of the partner preference paradigm. Nevertheless, total active investigation time, as well as male investigation time, was reduced by fluoxetine treatment so it is currently unclear if this is a reflection of a decline in sexual motivation or a reduction in overall activity. We suspect that the low active investigation may have resulted from a general decline in activity after fluoxetine; when active investigation of the male was expressed as a proportion of time near the male incentive (e.g. TIM/TWM), time investigating was significantly suppressed by fluoxetine. This observation, coupled with the low number of crossings after fluoxetine, lead us to suggest that both male preference scores and active investigation of the male are confounded by the drug's effect on activity.

Such a hypoactivity effect of fluoxetine is not surprising given the recognized role of 5-HT in motor function [25] and has been reported by other investigators [26, 27]. Fluoxetine's distributional $\frac{1}{2}$ life is of the order of minutes (21.1 min from Holladay et al. [28]) and locomotor effects occur rapidly [26]. However, even though fluoxetine has a long $\frac{1}{2}$ life of at least 13 hr [26], locomotor effects begin to dissipate 30-40 min after injection [26, 29]. This is one reason that behavioral effects of fluoxetine are generally studied after at least 30 min following injection. However, given the confounding potential of locomotor effects on the measure of male preference, it may be preferable in future studies to examine the female's preference at longer intervals following treatment.

Given prior reports of fluoxetine's inhibitory effect on lordosis behavior, the relatively small effect of fluoxetine on lordosis behavior in the absence of a pretest was unexpected. A robust decline in lordosis behavior after acute or repeated treatment with fluoxetine has been repeatedly observed [16, 18, 23]. This is even more surprising since hypoactivity would be expected to also affect the female's sexual activity. In fact, we recently reported that the L/M ratio was severely disrupted immediately after fluoxetine and began to stabilize 30 min after injection as locomotor activity returned to normal [29]. The current findings would, therefore, allow the suggestion that lordosis behavior examined at least 30 min after fluoxetine is not confounded by the antidepressant's locomotor effects.

The effect of fluoxetine on lordosis behavior was enhanced when females were pretested for sexual behavior prior to the injection with fluoxetine. One possible explanation for this finding is that the association between pretesting and the fluoxetine injection led the female to develop a negative association with the male and/or mating environment which was then evidenced during the later mating test. Since an effect of the pretest occurred whether the female was later tested for the first time in the larger test arena or again in the male's cage, whatever association may have occurred, it appears to have been independent of the actual test location. Acute fluoxetine treatment is often regarded as anxiogenic [30-33] leading to elevations in both corticotropin releasing hormone and consequent corticosterone [32], both of which have been reported to reduce female rat lordosis behavior [34, 35]. Since the act of mating with an unfamiliar male can also be stressful [36], it is possible that the dual stresses of mating and fluoxetine led to a cumulative decline in the behavior that was not evident when either the pretest or fluoxetine was excluded. If so, pretesting might shift fluoxetine's dose response curve to the left so that a lower dose of fluoxetine was required to inhibit lordosis behavior. Support for this suggestion is derived from the final study where the effect of pretesting was examined after a dose of 20 mg/kg fluoxetine. With this higher dose, both groups showed inhibition of lordosis behavior and there was no difference between rats that had been pretested and those that had not been pretested.

When females were allowed the option of escaping the male, fluoxetine-treated rats uniformly left the male's compartment for an escape burrow and stayed there for the majority of the testing time. This escape behavior was monitored in 2 separate experiments (Experiments 2 and 3). Only in Experiment 2 were water-treated rats included and the group difference narrowly escaped statistical significance. However, in the third experiment, an additional 16 rats given fluoxetine showed essentially the same pattern of escape behavior. When data from the two experiments were combined post facto, effects of fluoxetine were clearly evident ($t = 2.22$, $df = 43$, $p = 0.03$). Escape behavior occurred whether or not the female had been pretested immediately before the fluoxetine injection and was independent of the female's earlier L/M ratio ($\rho = 0.19$ for L/M ratio vs time in the burrow, $p > 0.05$). Thus, even females that were showing high L/M ratios left the arena when given the option. However, further studies are needed in the paced mating paradigm (and without the prior testing activity) to determine if fluoxetine truly does reduce the female's propensity for mating. Such experiments will be important because the female's latency to return to the

male has been interpreted to reflect the degree of the female's sexual motivation [14, 37, 38]. Thus, the fact that fluoxetine-treated females tended to leave and stay away from the male might evidence a fluoxetine-induced reduction in the female's motivation for mating. However, since the escape option occurred immediately after 10 min of nonpaced mating in the arena when the female was unable to escape, it could also reflect an impact of fluoxetine on sexual satiation.

Collectively, these findings expand current attempts to model antidepressant-induced sexual dysfunction and illustrate the value of including multiple measures in the analysis. While any single measurement failed to provide clear evidence of sexual dysfunction, the collective profile clearly differentiated fluoxetine-treated from water-treated females. However, equally evident is the confounding effect of fluoxetine's suppression of activity on all measures in the partner preference paradigm. Only L/M behavior appeared to be unaffected by the low activity since there was no correlation between the number of crossings in the partner preference paradigm and the L/M ratios ($\rho = 0.32$, $p > 0.05$).

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Highlights

Fluoxetine reduced measures of female rat sexual behavior.

The male preference ratio was not affected by fluoxetine treatment, but active investigation of the male was reduced by the SSRI.

Pretesting for sexual receptivity amplified fluoxetine's inhibition of lordosis behavior.

When given the opportunity to escape from the male, fluoxetine-treated females displayed escape behavior.

Fig. 1. Male Preference ratios before and after fluoxetine

Data are the mean $\pm$ S.E. male preference ratio (TWM/TST) for hormonally-primed, ovariectomized rats. Four-six hr after progesterone injection, the test female was allowed to move freely in the apparatus for 10 min (BEFORE). She was then injected ip with either 15 mg/kg fluoxetine (FLX) or distilled water and returned to her home cage. Thirty min later, rats were again tested for 10 min in the partner preference apparatus (AFTER). Ns were 8/group. There were no significant effects of test interval or treatment ($p > 0.05$).

Fig. 2. Active investigation after fluoxetine

Active investigation times of the incentives (sexually active male and ovariectomized female) are shown for the same test females as described in Figure 1. * indicates significant difference from the 10 min before injection within treatment group; ** indicates significant difference from the water-control group in the same time interval.

Fig. 3. Size of test arena and sexual behavior after fluoxetine

Hormonally primed, ovariectomized rats were pretested for sexual behavior and immediately injected with either water or 15 mg/kg fluoxetine (FLX). Forty min after injection, sexual behavior was tested in the partner preference arena or in the home cage of a male for 10 min. Data are the mean $\pm$ S.E. L/M ratios (A) or lordosis quality (B). Ns for Arena (water/fluoxetine) and Male Cage (water/fluoxetine) were 9, 11, 6, and 7 respectively. One rat from the arena water group had no mounts and 2 rats from the arena fluoxetine group had L/M ratios of zero and were therefore excluded from the analyses. * indicates significant difference from the 10 min before injection within treatment group, ** indicates significant difference from the water-control group in the same time interval.

Fig. 4. Pretesting amplifies effects of fluoxetine

Data are the mean $\pm$ S.E. L/M ratios (A) or lordosis quality (B) for rats that did or did not receive a prior mating experience (Pretest vs No Pretest) and rats that did or did not experience 10 min of no-contact time in the arena (with male vs without male). Data are for a 10 min test 40 min after injection with 15 mg/kg fluoxetine. Ns for Pretest (male/ no male) and No Pretest (male/ no male), respectively, were 6, 8, 11, and 8. One rat had no mounts and 2 rats had L/M ratios of zero during the pretest and were therefore excluded from the analyses. There was a significant effect of pretest ($p < 0.05$) but not arena ($p > 0.05$) and there was no interaction between pretest and arena. Nevertheless, the effect of the pretest on L/M ratios was more robust for rats tested without arena experience while, for lordosis quality, effects were greater in rats with arena experience (Tukey's, $q < 0.05$). * indicates a significant main effect of the pretest condition. @ indicates significant post hoc comparisons between pretest and no pretest within the same testing area.

Table 1

Summary of Experimental Protocols

Time Relative to Injection with Fluoxetine									
TIME	-5 min	-10 min	0 min Inject	0-25 min	25 min	30 min	35 min	40 min	50 min
Experiment 1									
Fluoxetine Or Water Conditions	5 min adaptation	Partner Preference Test	15 mg/kg FLX or water	Home Cage	Partner Preference Test	NPM ^a Arena			
Experiment 2									
Male Cage Condition		Pretest	15 mg/kg FLX	Home Cage	NPM Cage				
Arena Condition		Pretest	15 mg/kg FLX	Home Cage	5 min adaptation	NPM Arena	PM ^b Arena		
Experiment 3									
Pretest-arena		Pretest	15 mg/kg FLX	Home Cage	5 min adaptation	Arena	NPM Arena		PM Arena
No pretest-arena		Home Cage	15 mg/kg FLX	Home Cage	5 min adaptation	Arena	NPM Arena		PM Arena
Pretest-cage		Pretest	15 mg/kg FLX	Home Cage	NPM Cage				
No pretest-cage		Home Cage	15 mg/kg FLX	Home Cage	NPM Cage				
Experiment 4									
Pretest		Pretest	20 mg/kg FLX	Home Cage	NPM Cage				
No Pretest		Home Cage	20 mg/kg FLX	Home Cage	NPM Cage				

^aNPM = nonpaced mating;

^bPM = paced mating

Table 2

Preference behavior before (Pre) and after (Post) injection

Treatment	TWM ^a (Mean ± S.E.)		TWF ^b (Mean ± S.E.)		TST ^c (Mean ± S.E.)	
	Pre	Post	Pre	Post	Pre	Post
H ₂ O N = 8	225.13 ± 40.44	139.25 ± 26.97	127.06 ± 27.31	73.25 ± 7.79	352.19 ± 18.09	212.5 ± 26.09
Fluoxetine N = 8	231.56* ± 41.51	81.00 ± 60.76	85.56* ± 14.14	289.19** ± 92.09	324.63 ± 42.63	370.19 ± 80.04

^aTime with Male Incentive;

^bTime with Female Incentive;

^cTotal Social Time

* Significant difference between Pre and Post, within the same treatment conditions

** Significant difference from water group in same time interval

Table 3
Effects of fluoxetine on midline crossings before (Pre) and after injection (Post)

	Number of Crossings (Mean $\pm$ S.E.)	
Treatment	Pre	Post
H₂O <i>N</i> = 8	30.43 $\pm$ 2.97	24.5 $\pm$ 3.24
Fluoxetine <i>N</i> = 8	29.56 $\pm$ 3.15 *	3.93 $\pm$ 2.71 **

* Significant difference between Pre and Post within the same treatment conditions

** Significant difference from water group in same time interval

Table 4

Active Investigation before and after fluoxetine

Treatment	Total Investigation Time (Mean $\pm$ S.E.)		Male Investigation Ratio (TIM/TTD) (Mean $\pm$ S.E.)		Ratio (TIM/TWM) (Mean $\pm$ S.E.)		Ratio (TIF/TWF) (Mean $\pm$ S.E.)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
H₂O <i>N</i> = 8	281.44 $\pm$ 17.64	144.19* $\pm$ 21.11	0.71 $\pm$ 0.06	0.67 $\pm$ 0.08	0.91 $\pm$ 0.04	0.74 $\pm$ 0.02	0.65 $\pm$ 0.08	0.50 $\pm$ 0.05
Fluoxetine <i>N</i> = 8	242.94 $\pm$ 33.67	36.81*** $\pm$ 13.19	0.73 $\pm$ 0.05	0.34*** $\pm$ 0.17	0.76 $\pm$ 0.06	0.28*** $\pm$ 0.17	0.71 $\pm$ 0.09	0.09*** $\pm$ 0.04

TIM= Time actively investigating the incentive male; TIF= Time actively investigating the incentive female; TTI= Total time investigating; TWM=Time near the incentive male; TWF=Time near the incentive female; (TIM/TTD) = Time spent investigating the male over total time investigating; (TIM/TWM) = Time spent investigating the male over time spent near the male (TWM values are from Table 1); TIF/TWF= Time spent investigating the incentive female over time spent near the incentive female (TWF values are from Table 1)

* significant difference from Pre, within treatment

*** significant difference between fluoxetine and water-treated rats, within time interval