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SUBCHRONIC TREATMENT WITH FLUOXETINE ATTENUATES EFFECTS OF ACUTE FLUOXETINE ON FEMALE RAT SEXUAL BEHAVIOR

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Abstract

These experiments were designed to evaluate the hypothesis that fluoxetine-induced sexual dysfunction in female rats derived from disruption of neuroendocrine events that normally facilitate sexual behavior. If so, exogenous hormonal priming to ovariectomized rats should eliminate fluoxetine's effect. Ovariectomized rats were subchronically treated with 10 mg/kg fluoxetine or distilled/deionized water vehicle for 9 consecutive days. On the 8th day of treatment, rats were primed with 10 µg estradiol benzoate followed 48 hr later with 500 µg progesterone. In a pretest for sexual behavior on the 10th day, there was no difference between subchronic treatments. Sexual receptivity was again monitored 30 min after injection on the 10th day (acute treatment) with distilled/deionized water, 10 mg/kg fluoxetine or 20 mg/kg fluoxetine. Thereafter, the female's behavior was monitored for 20 min in a male preference procedure. After the acute treatment in rats subchronically treated with water, fluoxetine (10 or 20 mg/kg) significantly reduced both lordosis frequency and quality and reduced (but not significantly) time spent with the male. In rats subchronically treated with fluoxetine, the lordosis-inhibiting effect of an acute injection with fluoxetine was significantly attenuated relative to that of the subchronically water-treated rats. In contrast to expectation, subchronic treatment with fluoxetine increased, rather than reduced, the relative time females spent near the male. Activity, as measured by center crossings, and grooming were also reduced by subchronic treatment with fluoxetine.

Keywords

SSRI; sexual motivation; lordosis; subchronic

1.0 Introduction

Fluoxetine (Prozac®) is frequently prescribed for depression as well as for a variety of other disorders such as premenstrual dysphoria, anxiety, anorexia and bulimia which are more prevalent in females than in males [4,19,22,33]. A reported undesirable side effect of fluoxetine, as well as other selective serotonin reuptake inhibitors (SSRIs), is a high incidence of sexual dysfunction [8,15,28]. Although such sexual dysfunction occurs in both genders, more experimental emphasis has been placed on identifying the responsible mechanisms in males than in females [17]. Yet, as much as 32% of female patients may experience SSRI-

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induced sexual dysfunction [32]. The mechanisms responsible for such drug-induced sexual dysfunction in females have been difficult to identify, perhaps due to the complexity of the female reproductive cycle and the number of neural and endocrine loops involved [2,9]. Nevertheless, most investigators have assumed that female sexual dysfunction occurs independent of a primary disturbance of the neuroendocrine events that normally contribute to sexual motivation and satisfaction [17]. However, we reported recently that daily treatment with 10 mg/kg fluoxetine disrupted both the female rat's estrous cycle (as monitored by the female's vaginal smear) and sexual behavior [40]. The onset of the estrous cycle disturbance was rapid, occurring in approximately 50% of rats during the first cycle. Every rat failed to cycle during the second predicted estrous cycle. However, as fluoxetine treatment continued, females began to resume both estrous cyclicity and sexual receptivity [40]. Therefore, these findings question the assumption that fluoxetine-induced sexual dysfunction occurs independent of a disturbance of the neuroendocrine axis.

Female rat sexual behavior is tightly linked to ovulation and the synchronous timing for both events is controlled by the hypothalamic-pituitary-gonadal (HPG) axis with estrogen as the ultimate conductor [26,34]. Unlike primates, normally cycling female rats show sexual receptivity only during the proestrous (ovulatory) portion of the reproductive cycle. Removal of the ovaries leads to immediate cessation of sexual behavior which can be restored by treatment with estrogen and progesterone [26]. If sufficiently high doses of estrogen are used, estrogen can facilitate female rat sexual receptivity in the absence of progesterone [34]. However, progesterone reduces the amount of estrogen needed and is required for the complete repertoire of rodent sexual behavior [34]. Changes in the appearance of the vaginal smear reflect the cyclic changes in estrogen and progesterone and are often used to monitor the female's hormonal cycle. Consequently, fluoxetine's ability to disrupt the female rat's estrous cycle is likely to reflect an effect of the SSRI on the neuroendocrine axis which could account, at least in part, for reported inhibitory effects on female sexual behavior.

If fluoxetine inhibits female rat sexual behavior by blocking the occurrence of the appropriate hormonal events, then exogenous restoration of these hormones in an ovariectomized female should reduce effects of fluoxetine on sexual behavior. Although fluoxetine has been previously studied in ovariectomized females primed with estrogen and/or progesterone, the results have been equivocal [10,25]. In one report, ovariectomized rats were treated with 10 mg/kg fluoxetine for as long as 42 days [25]. At weekly intervals, females were hormonally primed with estrogen and progesterone and tested for sexual receptivity (measured by the lordosis reflex) and sexual motivation (measured by the percentage of time females spent in the vicinity of a male). Effects on sexual receptivity were small and did not develop until approximately 28 days of treatment. In contrast, fluoxetine reduced the percentage of time the female spent with the male after 7 days of treatment but not thereafter. The transient and inconsistent relationship between these two aspects of female sexual behavior was not discussed. In a second report, an acute treatment with 20 mg/kg fluoxetine robustly inhibited lordosis behavior while considerably smaller effects were seen after 15 days of treatment with 10 mg/kg fluoxetine [10]. However, in the latter case, sexual behavior was not tested until 2 days after the final fluoxetine injection. Thus, there is an insufficient database to determine if fluoxetine's disruption of sexual behavior in female rats can be overcome by exogenous hormonal stimulation.

The onset and duration of SSRI-induced sexual dysfunction in human females has not been clearly defined. Some investigators report improvement after chronic fluoxetine treatment while others report continued sexual dysfunction [17]. When the female rat's estrous cycle was monitored, we found resumption of both vaginal cyclicity and behavioral receptivity after 15 to 16 days of fluoxetine treatment [40]. Such synchronous resumption of both behavioral and vaginal cyclicity is particularly interesting. Fluoxetine is well known for its inhibition of

serotonin (5-HT) reuptake [12,14] and drugs that elevate extracellular 5-HT are known to inhibit female rat sexual behavior [27,38], presumably via activation of postsynaptic 5-HT_{1A} receptors in the ventromedial nucleus of the hypothalamus [38]. Therefore, an acute injection of fluoxetine would be expected to inhibit sexual receptivity via increased activation of these 5-HT_{1A} receptors. However, with repeated agonist activation (as occurs in response to repeated fluoxetine treatment), hypothalamic 5-HT_{1A} receptors may become desensitized. Seven to ten days of treatment with fluoxetine was reported to reduce the ability of a 5-HT_{1A} receptor agonist to activate the hypothalamic-pituitary-adrenal axis [23,31,41]. Consequently, it was hypothesized that, relative to the effects of a single injection of the drug, prior fluoxetine treatment would attenuate the drug's effect on sexual behavior. In the following experiment, 9 days treatment with fluoxetine was used to simulate conditions that, in the intact female, blocked the estrous cycle in 100% of the treated females [40].

2.0 Results

2.1 Effects of fluoxetine on sexual receptivity in the pretest

Prior to their daily injection, on the 10th day of the experiment, females were pretested for sexual behavior (10 mounts with a sexually active male) to assess the effect of the subchronic treatments on sexual receptivity. This pretest was used to evaluate the effects of the subchronic treatment 24 h after the last injection and prior to any drug treatment on the 10th day. Two rats (both given subchronic fluoxetine) could not be tested because the males refused to mount them. These two rats were excluded from the analyses. For all other rats, both groups showed high levels of lordosis responding. One hundred percent of the water-treated female had a maximum L/M ratio of 1.0 in the pretest while only 72.4% (21/29) of the fluoxetine-treated females showed the maximum L/M ratio (Fischer Exact Test, $P \leq .03$). Nevertheless, L/M ratios of all the fluoxetine-treated rats (even without a maximum L/M ratio) were still high. Three of these fluoxetine-treated rats had L/M ratios of 0.8 and five had L/M ratios of 0.9. There was a modest difference in median L/M ratios between subchronic treatments primarily due to the absence of variability in the water-treated rats (See Table 1; for L/M and quality, respectively, Mann-Whitney U, $df = 28$ and 33 , $U = 3.24$ and 4.97 , $P \leq 0.002$ and 0.001). Similarly, lordosis quality of most fluoxetine-treated rats ranged from 2.1–2.5. Only a single fluoxetine-treated rat had a quality score below 2.0. Therefore, the majority of L/M ratios and lordosis quality scores 24 h after subchronic treatment with fluoxetine were well within the scores typically found with normally cycling proestrous rats or with ovariectomized rats, hormonally primed with estrogen and progesterone. Furthermore, proceptive behavior, as indicated by the presence of hopping and darting behavior, was comparable between rats treated subchronically with water (73.5%) or fluoxetine (86.2%) (Fischer's Exact test, $P > .05$).

2.2 Effects of fluoxetine on sexual receptivity following the 10th day injection

After the pretest for sexual receptivity, rats were injected with water, 10 mg/kg fluoxetine or 20 mg/kg fluoxetine. Thirty min later, sexual behavior was monitored for 10 mounts from a sexually active male (Figure 1). This test was used to evaluate the acute effect of fluoxetine (as assessed by the behavior of rats previously subchronically treated with water) compared to the effects of an injection of fluoxetine in rats subchronically treated with fluoxetine. Subchronic treatment with fluoxetine reduced the effect of an acute fluoxetine injection on lordosis behavior (Figure 1A). There were significant effects of the subchronic treatment ($F_{1,57} = 6.06$, $P \leq .02$), type of acute injection ($F_{2,57} = 43.47$, $P \leq .0001$), and their interaction ($F_{2,57} = 8.37$, $P \leq .0006$). In rats subchronically treated with water, both 10 and 20 mg/kg of fluoxetine significantly reduced L/M ratios (Tukey's, $q_{57,6} = 5.21$ and 13.24 , respectively for 10 and 20 mg/kg, $P \leq 0.05$). In rats subchronically treated with fluoxetine, acute treatment with either dose of fluoxetine also produced a decline in lordosis behavior compared to the water-water control (Tukey's $q_{57,6} = 4.16$ and 6.77 , respectively for the 10 and 20 mg/kg dose, $P \leq$

0.05), but only the dose of 20 mg/kg fluoxetine significantly reduced L/M ratios compared to the fluoxetine-water rats (Tukey's $q_{57,6} = 5.31$, $P \leq 0.05$). The acute injection of 20 mg/kg fluoxetine was significantly less effective in reducing L/M ratios in rats subchronically treated with fluoxetine compared to rats treated subchronically with water ($q_{57,6} = 6.47$, $P \leq .05$).

Lordosis quality is shown in Figure 1B. Eight rats (all subchronically treated with water and injected on the 10th day with 20 mg/kg fluoxetine) had L/M ratios of zero after injection so no lordosis quality score could be assessed. For all the remaining rats, there was not a significant effect of the subchronic treatment ($P > 0.05$), but there was a significant effect of the acute injection ($F_{2,49} = 14.27$, $P \leq .0001$) and a significant interaction between type of subchronic treatment and the acute injection ($F_{2,49} = 6.48$, $P \leq .005$). Relative to the water-water control, an acute injection of 20 mg/kg fluoxetine reduced lordosis quality independent of subchronic treatment (Tukey's $q_{49,6} \geq 7.2$, $P \leq .05$). Only in rats subchronically treated with water was 10 mg/kg fluoxetine significantly different (Tukey's $q_{49,6} = 5.9$, $P \leq .05$). In rats subchronically treated with fluoxetine, the acute injection of water slightly (but not significantly) reduced lordosis quality relative to the water-water control. Consequently, compared to the fluoxetine-water control group, lordosis quality was not significantly altered by the acute treatment with fluoxetine ($P > .05$).

Prior to the injection on the 10th day, a majority of rats showed evidence of proceptivity during the sexual behavior testing (See Table 2). However, proceptivity was reduced in all rats after the acute injection. Independent of the acute treatment, a decline in proceptivity was present in all rats subchronically treated with fluoxetine (Fisher's Exact Test, all $P \leq .05$). In rats subchronically treated with water, only the acute treatment of 20 mg/kg significantly reduced proceptivity (Fisher's Exact Test, $P \leq .05$).

2.3 Partner preference

Nineteen rats subchronically treated with water and 21 rats subchronically treated with fluoxetine were tested for partner preference. Of these, one rat treated subchronically with fluoxetine and then injected with 20 mg/kg fluoxetine never moved away from its initial placement in the center of the apparatus and was eliminated from the analysis. For the remaining rats, the relative amount of time spent with the male (TWM/TST) is shown in Figure 2. In rats subchronically treated with water, there was a tendency for the acute fluoxetine to reduce TWM/TST. However, there were no significant effects of the acute treatment or its interaction ($P > .05$). There was, however, a significant effect of the type of subchronic treatment on TWM/TST ($F_{1,33} = 4.76$, $P \leq .05$). Independent of the acute treatment, females treated subchronically with fluoxetine spent more time overall with the male than did females treated subchronically with water. Total social time (e.g. time spent with either stimulus animal) was not affected by either subchronic or acute treatments (all $P > 0.05$; data not shown).

Both subchronic and acute treatment with fluoxetine appeared to reduce overall activity as indicated by the number of times females crossed the center of the test apparatus (Figure 3A). There were significant effects of both subchronic ($F_{1,34} = 13.93$, $P \leq .0001$) and acute ($F_{2,34} = 11.01$, $P \leq .0002$) treatments, but not their interaction ($P > .05$), on number of crossings. Across all acute conditions, rats treated subchronically with fluoxetine showed fewer crossings than rats subchronically treated with water. Within acute treatments, 20 mg/kg fluoxetine produced the greatest decrements. A similar effect was seen for grooming (Figure 3B) with significant effects for the subchronic treatment ($F_{1,34} = 8.25$, $P \leq .008$) and acute treatment ($F_{2,34} = 6.06$, $P < .006$), but not their interaction. The higher dose of fluoxetine also produced the greatest reduction in grooming. The amount of grooming and number of crossings were significantly correlated with each other ($r = .535$, $P \leq .0008$) but neither measure significantly correlated with the TWM/TST. However, the number of crossings did correlate significantly with the L/

M ratio after acute injection ($r = .440, P \leq .005$) and this L/M ratio was slightly, but significantly, correlated with TWM/TST ($r = .342, P \leq 0.04$).

2.4 Body weight

Consistent with findings in intact females [40], subchronic treatment with 10 mg/kg fluoxetine reduced body weight (see Figure 4). There were significant effects of the subchronic treatment ($F_{1,63} = 22.8, P \leq .0001$), day of treatment ($F_{9,567} = 47.1, P \leq .0001$) and their interaction ($F_{9,567} = 64.8, P \leq .0001$). Body weight was significantly reduced 24 hr after the first injection with fluoxetine and remained reduced thereafter (Tukey's $q_{567,2} \geq 2.42, P \leq .05$).

3.0 Discussion

Based on our findings in intact females [40], we addressed two separate questions in an ovariectomized, hormonally primed model. We had reported that the effects of subchronic treatment with fluoxetine on sexual behavior in the intact female were tightly linked to the drug's effect on vaginal cyclicity. Since the female rat's sexual receptivity is dependent on appropriate hormonal priming, we hypothesized that the effect of the drug on sexual behavior would be diminished (or absent) if hormonal priming could be controlled. We further hypothesized that subchronic fluoxetine would actually reduce, rather than enhance, acute effects of fluoxetine on sexual behavior.

In general, these findings are consistent with our prior suggestion that subchronic fluoxetine-induced sexual dysfunction may result from a disruption of the neuroendocrine axis [40]. After subchronic (9 days) treatment with 10 mg/kg fluoxetine and following estrogen plus progesterone priming of ovariectomized rats, neither lordosis frequency (e.g. L/M ratio) nor lordosis quality were substantially disrupted. Thus, exogenous hormonal priming-elicited facilitation of sexual behavior occurred in spite of subchronic fluoxetine treatment. However, when these same rats were administered an acute injection of 10 or 20 mg/kg fluoxetine, some reduction in sexual behavior did occur, but only at the higher dose of the drug. In contrast, in rats subchronically treated with water, acute treatment with either 10 or 20 mg/kg fluoxetine reduced L/M ratios and lordosis quality.

Inhibitory effects of an acute treatment with fluoxetine on sexual behavior are consistent with prior reports on lordosis behavior in female rats [10] and lateral displacement in female hamsters [11]. Fluoxetine rapidly increases extracellular 5-HT in a variety of brain areas including the hypothalamus [21,24,29,36]. Thus, acute effects of fluoxetine are consistent with 5-HT's ability to inhibit female rat sexual behavior [27,38]. Since 5-HT's lordosis inhibiting effect resides, in part, from the neurotransmitter's activation of 5-HT_{1A} receptors [39], it is tempting to speculate that fluoxetine acutely reduces lordosis behavior via enhanced activation of 5-HT_{1A} receptors. The reduced effectiveness of the acute treatment with fluoxetine in rats subchronically treated with fluoxetine is consistent with such a possibility and with prior findings that seven to ten days of fluoxetine treatment reduced the effect of a 5-HT_{1A} receptor agonist on activation of the hypothalamic-pituitary-adrenal (HPA) axis [31,41]. The role of 5-HT_{1A} receptors in SSRI-induced female sexual dysfunction has not yet been examined. However, Sukoff Rizzo et al. reported that 5-HT_{1A} receptor antagonists reversed the effect of repeated fluoxetine treatment on non-contact penile erections in male rats [35]. Regardless of the responsible mechanism, the current findings lead to the suggestion that subchronic treatment with fluoxetine reduces negative effects of fluoxetine on female rat sexual behavior. When the hormonal condition was exogenously manipulated, subchronic fluoxetine had only minor effects on female sexual behavior. Only the acute administration of 20 mg/kg dose of fluoxetine was effective in reducing the L/M ratio in subchronically fluoxetine-treated rats. However, both acute and subchronic fluoxetine may have contributed to a reduction in the female's proceptivity.

It is important to note that the weight reduction after fluoxetine treatment was roughly comparable to that which was observed in intact females. We previously reported that pair-feeding rats with fluoxetine-treated rats mimicked the effects of fluoxetine on estrous cyclicity and speculated that fluoxetine's disruption of energy balance may have been a contributing factor to the reproductive dysfunction [40]. It is, therefore, important to note that hormonal priming effectively facilitated lordosis behavior in spite of a significant decline in body weight.

The relatively robust effect of an acute injection of fluoxetine on sexual receptivity was not mirrored by an equally robust decrease in the percentage of time spent by females with a male. In rats subchronically treated with water, the percentage of time spent with the male was reduced by acute treatment with fluoxetine, but this was not significantly different from the acute water injection. Unexpectedly, in comparison to rats subchronically treated with water, relative time with the male actually increased in rats treated subchronically with fluoxetine. The male preference test is a useful procedure for evaluating the effects of treatments on sexual motivation of female rats [3], so that the current findings might be interpreted as evidence that subchronic fluoxetine does not reduce the female's sexual motivation. However, in the current experiment, to avoid differential adaptation in rats receiving subchronic fluoxetine or water, we did not pre-adapt test females to the male preference testing apparatus. Such adaptation over several days is a routine procedure to reduce any novelty-induced fear or anxiety that might be associated with a new environment. That such novelty-induced anxiety may have been present during the testing was indicated by the presence of a substantial amount of self-grooming by the water-water controls. Stress-induced activation of the HPA axis or treatment with corticotropin releasing hormone increases self-grooming [6,7,37]. Therefore, the current findings may have reflected a reduction by fluoxetine of the female's anxiogenic response to the testing environment. If so, the lower amount of grooming by females subchronically treated with fluoxetine is consistent with the reported anxiolytic effects of fluoxetine [16,33]. Consistent with the greater amount of grooming in rats subchronically treated with water (even when given an acute treatment with fluoxetine), most investigators report fluoxetine-induced reductions in anxiety only after several days of fluoxetine treatment and not after acute treatment [1,5]. A single treatment with fluoxetine is thought to increase, not decrease, the HPA axis [13,31] so that an increase in grooming after an acute fluoxetine treatment might have been expected. Such a fluoxetine-induced increase in self-grooming has been previously reported [1]. Therefore, the relative lower amount of grooming by females that received an acute 20 mg/kg injection of fluoxetine was unexpected.

However, subchronic fluoxetine reduced activity as measured by the number of times the test female crossed the center area. This apparent decrease in mobility induced by fluoxetine is in contrast to a reported increase in locomotor activity in gerbils [30] but is consistent with a recent report in mice [20]. Therefore, the effect of subchronic fluoxetine on the relative time spent with the male may have been confounded by a reduced tendency to explore the test apparatus and an increased tendency to remain stationary in a single position. For the subchronically treated rats, this was reflected in the large variability of the male preference ratio. However, since neither grooming nor crossings were correlated with TWM/TST, a fluoxetine-induced increase in time spent with the male cannot be ruled out.

In summary, the current findings lead to the suggestion that effects of subchronic fluoxetine on female sexual dysfunction may result from a disruption of the neuroendocrine axis while acute effects may not. In hormonally primed, ovariectomized rats that had never received prior fluoxetine, an acute treatment with fluoxetine inhibited lordosis behavior. Subchronic treatment with fluoxetine reduced this inhibition leading to the suggestion that adaptive responses to fluoxetine offered protection against SSRI-induced sexual disruption. Moreover, estrogen-progesterone priming for sexual receptivity remained effective in rats subchronically treated with fluoxetine. When the male preference ratio was assessed as a measure of the

female's sexual motivation, modest, but not significant, decrements in the male preference ratio were seen following acute fluoxetine. In contrast, subchronic treatment with fluoxetine actually increased the male preference ratio. However, locomotor and anxiolytic effects of the drug, evident in the preference apparatus, may have interfered with accurate assessment of the male preference ratio. Further studies will be needed to differentiate these effects of fluoxetine from the SSRI's effect on female sexual motivation.

Our findings allow the suggestion that SSRI-induced sexual dysfunction might be expected to lessen, rather than increase, with increased duration of drug treatment. Although this possibility has been mentioned in the human literature [18,28], the results are difficult to interpret since few investigators have assessed the presence or degree of sexual dysfunction prior to SSRI treatment. In addition, these findings allow the suggestion that SSRI-induced human female sexual dysfunction could be less prevalent in females taking contraceptives. Unfortunately, such data have yet to be generated. In fact, in only a few studies has the possibility of a neuroendocrine disturbance even been addressed. Such studies clearly need to be conducted. Finally, when the current findings are coupled with findings in the intact female, the exclusion of a neuroendocrine explanation for SSRI-induced female sexual dysfunction appears to have been premature.

4.0 Experimental Procedures

4.1 Materials

Fluoxetine hydrochloride was purchased from Sigma/Aldrich Chemical Company (St. Louis, MO). Estradiol benzoate and progesterone and all other supplies came from Fischer Scientific (Houston, TX).

4.2 Animal and housing conditions

Adult female Fischer rats were purchased from Charles River Laboratories (Wilmington, MA) and housed initially 3 per cage in polycarbonate cages ($18 \times 9.5 \times 8.5$ in) with food and water available ad lib. Rats were housed in rooms maintained at 25°C and 55% humidity and with a 12 hr-12 hr light/dark cycle with lights off at 12 noon. After a two-week adaptation to the facility, rats were anaesthetized with AErrane® and ovariectomized as previously described [42].

4.3 Treatment of animals

All procedures were in accordance with the NIH Guide for the Care and Use of Animals in Research and were approved by the Institutional Animal Care and Use Committee at Texas Woman's University.

4.3.1 Hormonal treatment, drug administration, and behavioral testing—Two weeks after ovariectomy, rats were individually housed. Four to five days later, daily injections commenced. Rats were injected intraperitoneally (i.p.) daily with fluoxetine (10 mg/kg) ($N = 34$) or vehicle (distilled water) ($N = 31$) for the next 9 days. Body weight was measured each day immediately prior to injection. On the 8th day of fluoxetine or vehicle treatment, rats received an injection of estradiol benzoate (10 μg) in the morning. Forty-eight hour later (10th day of fluoxetine or vehicle), rats were injected with 500 μg progesterone. Estradiol benzoate (10 μg) and progesterone (500 μg) were dissolved in sesame seed oil and injections were given subcutaneously in a volume of 0.1 ml per rat. Behavioral testing began 4 to 6 hrs after the progesterone injection. Each female was placed into the home cage of a sexually active male until the male had achieved 10 mounts. After this first test (pretest), rats were injected i.p. with vehicle (distilled/deionized water, 1 ml/kg), 10 mg/kg fluoxetine or 20 mg/kg fluoxetine. Thirty min later, rats were tested again for sexual receptivity.

Following the test for sexual receptivity, females were transferred to a testing room containing the partner preference apparatus. Females were placed into the test apparatus for 20 min. Behavior was videotaped for later analysis.

4.3.2 Sexual behavior testing—Sexual behavior was measured as previously described [42]. Briefly, females were placed into the home cage of a sexually active male until the male had accomplished 10 mounts. Sexual receptivity was quantified as the lordosis to mount (L/M) ratio (number of times the female exhibited a lordosis reflex in response to the male's mount) and lordosis quality. Females were scored as proceptive if they showed at least one hop/dart event during testing, but the behavior was not quantified. Lordosis quality (relative degree of arching of the back) was measured on a score of 1 to 4 as previously described [42].

4.3.3 Partner preference testing—Partner preference was monitored for 20 min in a clear plexiglass box (36 × 12.5 × 12.25 in). At the ends of the apparatus were two compartments (each 6.5 in long) separated from the main area by hardware cloth. An ovariectomized female was housed at one end and a sexually active male was housed at the opposite end. These stimulus animals were placed in the area behind the hardware cloth and were physically inaccessible to the test animal. The positioning of the stimulus animals was counterbalanced across tests.

The compartment accessible to the test female was divided into five distinct areas by markings on the floor of the apparatus. From left to right, these areas measured 5.0, 5.7, 2.5, 5.7 and 5.0 in. The female was placed into the center (2.5 in center area) of the apparatus and allowed to move freely throughout the apparatus for 20 min. The test female's behavior was videotaped for later analysis.

When all four of the test female's feet were in one of the lateral most 5 in areas, adjacent to either the stimulus male or stimulus female, the test female was scored as spending time near that stimulus animal. Seconds spent near the male (TWM) or the female (TWF) were summed as the test female's total social time (TST). TWM divided by TST was used as the index of male preference. In addition, the number of times the female crossed the center of the apparatus and the total time the female spent in self-grooming were recorded. A female was scored as crossing the center area when all four feet had traversed the 2.5 in center area.

Each videotape was scored by two different individuals. The individual scores were compared by Pearson's rho ($r \geq 0.92$ for all measures) and data from the two individuals were averaged prior to statistical analysis.

4.4 Statistical procedures

Body weight data were analyzed by repeated measures ANOVA with type of subchronic treatment as the independent factor and day of treatment as the repeated factor. Because control rats had no variability in their pretest L/M ratios, the Mann-Whitney test was used to compare pretest L/M ratios and lordosis quality. Proportional data were compared with Fisher's Exact Test. Post-test L/M ratios and lordosis quality were compared by two factor ANOVA with subchronic treatment and acute treatment conditions as the independent factors. Number of crossings, seconds of grooming, TWM, TST, and male preference scores were compared by two-factor ANOVA. Post-hoc comparisons were with Tukey's or Dunnett's tests. Correlations between variables were computed using Pearson's procedures. Statistical tests were performed using Superanova (v 1.11) or StatView 4.5 from Abacus Concepts or SPSS 11 for Mac OS X from SPSS Inc. The statistical reference was Zar [43] and alpha was set at 0.05.

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Literature references

1. Bagdy G, Graf M, Anheuer ZE, Modos EA, Kantor S. Anxiety-like effects induced by acute fluoxetine, sertraline or m-CPP treatment are reversed by pretreatment with the 5-HT_{2C} receptor antagonist SB-242084 but not the 5-HT_{1A} receptor antagonist WAY-100635. *Int J Neuropsychopharmacol* 2001;4:399–408. [PubMed: 11806866]
2. Basson R. The complexities of female sexual arousal disorder: potential role of pharmacotherapy. *World J Urol* 2002;20:119–26. [PubMed: 12107543]
3. Clark AS, Kelton MC, Guarraci FA, Clyons EQ. Hormonal status and test condition, but not sexual experience, modulate partner preference in female rats. *Horm Behav* 2004;45:314–23. [PubMed: 15109905]
4. Dimmock PW, Wyatt KM, Jones PW, O'Brien PM. Efficacy of selective serotonin-reuptake inhibitors in premenstrual syndrome: a systematic review. *Lancet* 2000;356:1131–6. [PubMed: 11030291]
5. Drapier D, Bentue-Ferrer D, Laviolle B, Millet B, Allain H, Bourin M, Reymann JM. Effects of acute fluoxetine, paroxetine and desipramine on rats tested on the elevated plus-maze. *Behav Brain Res* 2007;176:202–9. [PubMed: 17095104]
6. Dunn AJ, Berridge CW. Physiological and behavioral responses to corticotropin-releasing factor administration: is CRF a mediator of anxiety or stress responses. *Brain Res Brain Res Rev* 1990;15:71–100. [PubMed: 1980834]
7. Dunn AJ, Berridge CW, Lai YI, Yachabach TL. CRF-induced excessive grooming behavior in rats and mice. *Peptides* 1987;8:841–4. [PubMed: 3501576]
8. Fourcroy JL. Female sexual dysfunction: potential for pharmacotherapy. *Drugs* 2003;63:1445–57. [PubMed: 12834363]
9. Frohlich PF, Meston CM. Evidence that serotonin affects female sexual functioning via peripheral mechanisms. *Physiol Behav* 2000;71:383–93. [PubMed: 11150571]
10. Frye CA, Petralia SM, Rhodes ME, Stein B. Fluoxetine may influence lordosis of rats through effects on midbrain 3 alpha,5 alpha-THP concentrations. *Ann N Y Acad Sci* 2003;1007:37–41. [PubMed: 14993038]
11. Frye CA, Rhodes ME. Zaprinast, a phosphodiesterase 5 inhibitor, overcomes sexual dysfunction produced by fluoxetine, a selective serotonin reuptake inhibitor in hamsters. *Neuropsychopharmacology* 2003;28:310–6. [PubMed: 12589384]
12. Fuller RW, Wong DT, Robertson DW. Fluoxetine, a selective inhibitor of serotonin uptake. *Med Res Rev* 1991;11:17–34. [PubMed: 1994152]
13. Gibbs DM, Vale W. Effect of the serotonin reuptake inhibitor fluoxetine on corticotropin-releasing factor and vasopressin secretion into hypophysial portal blood. *Brain Res* 1983;280:176–9. [PubMed: 6317143]
14. Gobert A, Rivet JM, Cistarelli L, Millan MJ. Potentiation of the fluoxetine-induced increase in dialysate levels of serotonin (5-HT) in the frontal cortex of freely moving rats by combined blockade of 5-HT_{1A} and 5-HT_{1B} receptors with WAY 100,635 and GR 127,935. *J Neurochem* 1997;68:1159–63. [PubMed: 9048762]
15. Gregorian RS, Golden KA, Bahce A, Goodman C, Kwong WJ, Khan ZM. Antidepressant-induced sexual dysfunction. *Ann Pharmacother* 2002;36:1577–89. [PubMed: 12243609]
16. Hedges DW, Brown BL, Shwalb DA, Godfrey K, Larcher AM. The efficacy of selective serotonin reuptake inhibitors in adult social anxiety disorder: a meta-analysis of double-blind, placebo-controlled trials. *J Psychopharmacol* 2007;21:102–11. [PubMed: 16714326]
17. Hines RN, Adams J, Buck GM, Faber W, Holson JF, Jacobson SW, Keszler M, McMartin K, Segreaves RT, Singer LT, Sipes IG, Williams PL. NTP-CERHR Expert Panel Report on the reproductive and developmental toxicity of fluoxetine. *Birth Defects Res B Dev Reprod Toxicol* 2004;71:193–280. [PubMed: 15334524]

18. Kennedy SH, Fulton KA, Bagby RM, Greene AL, Cohen NL, Rafi-Tari S. Sexual function during bupropion or paroxetine treatment of major depressive disorder. *Can J Psychiatry* 2006;51:234–42. [PubMed: 16629348]
19. Kessler RC, McGonagle KA, Swartz M, Blazer DG, Nelson CB. Sex and depression in the National Comorbidity Survey. I: Lifetime prevalence, chronicity and recurrence. *J Affect Disord* 1993;29:85–96. [PubMed: 8300981]
20. Kliethermes CL, Crabbe JC. Pharmacological and genetic influences on hole-board behaviors in mice. *Pharmacol Biochem Behav* 2006;85:57–65. [PubMed: 16949140]
21. Koch S, Perry KW, Nelson DL, Conway RG, Threlkeld PG, Bymaster FP. R-fluoxetine increases extracellular DA, NE, as well as 5-HT in rat prefrontal cortex and hypothalamus: an in vivo microdialysis and receptor binding study. *Neuropsychopharmacology* 2002;27:949–59. [PubMed: 12464452]
22. Kornstein SG, Schatzberg AF, Yonkers KA, Thase ME, Keitner GI, Ryan CE, Schlager D. Gender differences in presentation of chronic major depression. *Psychopharmacol Bull* 1995;31:711–8. [PubMed: 8851644]
23. Li Q, Muma NA, van de Kar LD. Chronic fluoxetine induces a gradual desensitization of 5-HT_{1A} receptors: reductions in hypothalamic and midbrain Gi and G(o) proteins and in neuroendocrine responses to a 5-HT_{1A} agonist. *J Pharmacol Exp Ther* 1996;279:1035–42. [PubMed: 8930214]
24. Malagie I, Trillat AC, Jacquot C, Gardier AM. Effects of acute fluoxetine on extracellular serotonin levels in the raphe: an in vivo microdialysis study. *Eur J Pharmacol* 1995;286:213–7. [PubMed: 8605960]
25. Matuszczyk JV, Larsson K, Eriksson E. Subchronic administration of fluoxetine impairs estrous behavior in intact female rats. *Neuropsychopharmacology* 1998;19:492–8. [PubMed: 9803425]
26. McEwen BS, Jones KJ, Pfaff DW. Hormonal control of sexual behavior in the female rat: molecular, cellular and neurochemical studies. *Biol Reprod* 1987;36:37–45. [PubMed: 2436675]
27. Mendelson SD. A review and reevaluation of the role of serotonin in the modulation of lordosis behavior in the female rat. *Neurosci Biobehav Rev* 1992;16:309–50. [PubMed: 1528523]
28. Montgomery SA, Baldwin DS, Riley A. Antidepressant medications: a review of the evidence for drug-induced sexual dysfunction. *J Affect Disord* 2002;69:119–40. [PubMed: 12103459]
29. Perry KW, Fuller RW. Effect of fluoxetine on serotonin and dopamine concentration in microdialysis fluid from rat striatum. *Life Sci* 1992;50:1683–90. [PubMed: 1375306]
30. Prinssen EP, Ballard TM, Kolb Y, Nicolas LB. The effects of serotonin reuptake inhibitors on locomotor activity in gerbils. *Pharmacol Biochem Behav* 2006;85:44–9. [PubMed: 16920181]
31. Raap DK, Evans S, Garcia F, Li Q, Muma NA, Wolf WA, Battaglia G, Van De Kar LD. Daily injections of fluoxetine induce dose-dependent desensitization of hypothalamic 5-HT_{1A} receptors: reductions in neuroendocrine responses to 8-OH-DPAT and in levels of G_z and G_i proteins. *J Pharmacol Exp Ther* 1999;288:98–106. [PubMed: 9862759]
32. Shen WW, Hsu JH. Female sexual side effects associated with selective serotonin reuptake inhibitors: a descriptive clinical study of 33 patients. *Int J Psychiatry Med* 1995;25:239–48. [PubMed: 8567191]
33. Simon NM, Zalta AK, Worthington JJ 3rd, Hoge EA, Christian KM, Stevens JC, Pollack MH. Preliminary support for gender differences in response to fluoxetine for generalized anxiety disorder. *Depress Anxiety* 2006;23:373–6. [PubMed: 17068858]
34. Sodersten, P. Estradiol-progesterone interactions in the reproductive behavior of female rats. In: Ganten, D.; Pfaff, D., editors. *Current Topics in Neuroendocrinology: Actions of Progesterone on the Brain*. Springer-Verlag; New York: 1981. p. 141-174.
35. Sukoff Rizzo SJ, Schechter LE, Rosenzweig-Lipson S. 5-HT_{1A} receptor antagonism reverses and prevents fluoxetine-induced sexual dysfunction in rats: clinical implications. *Eur Neuropsychopharmacol* 2006;16:S345–346.
36. Tao R, Fray A, Aspley S, Brammer R, Heal D, Auerbach S. Effects on serotonin in rat hypothalamus of D-fenfluramine, aminorex, phentermine and fluoxetine. *Eur J Pharmacol* 2002;445:69–81. [PubMed: 12065196]
37. Temel Y, Helmy A, Pinnock S, Herbert J. Effect of serotonin depletion on the neuronal, endocrine and behavioural responses to corticotropin-releasing factor in the rat. *Neurosci Lett* 2003;338:139–42. [PubMed: 12566172]

38. Uphouse L. Female gonadal hormones, serotonin, and sexual receptivity. *Brain Res Rev* 2000;33:242–57. [PubMed: 11011068]
39. Uphouse L, Caldarola-Pastuszka M, Montanez S. Intracerebral actions of the 5-HT_{1A} agonists, 8-OH-DPAT and buspirone and of the 5-HT_{1A} partial agonist/antagonist, NAN-190, on female sexual behavior. *Neuropharmacology* 1992;31:969–81. [PubMed: 1436391]
40. Uphouse L, Hensler JG, Sarkar J, Grossie B. Fluoxetine disrupts food intake and estrous cyclicity in Fischer female rats. *Brain Res* 2006;1072:79–90. [PubMed: 16423328]
41. Van de Kar LD, Raap DK, Battaglia G, Muma NA, Garcia F, DonCarlos LL. Treatment of cycling female rats with fluoxetine induces desensitization of hypothalamic 5-HT(1A) receptors with no change in 5-HT(2A) receptors. *Neuropharmacology* 2002;43:45–54. [PubMed: 12213258]
42. White S, Uphouse L. Estrogen and progesterone dose-dependently reduce disruptive effects of restraint on lordosis behavior. *Horm Behav* 2004;45:201–8. [PubMed: 15047015]
43. Zar, J. *Biostatistical Analysis*. 4. Prentice-Hall; Englewood Cliffs, NJ: 1999.

Figure 1. Fluoxetine effects on lordosis frequency

Ovariectomized rats were subchronically treated with 10 mg/kg fluoxetine or the water vehicle. After a pretest for sexual receptivity, rats, hormonally primed with 10 μ g estradiol benzoate and 500 μ g progesterone, were injected with water, 10 mg/kg fluoxetine or 20 mg/kg fluoxetine. Sexual behavior testing occurred 30 min later. In 1A, data are the mean $\pm$ S.E. L/M ratios. N's per treatment were 12, 7, and 15, respectively, for rats subchronically treated with water and injected with water, 10 mg/kg or 20 mg/kg fluoxetine. N's per treatment of rats subchronically treated with 10 mg/kg fluoxetine were 7, 14 and 8. In 1B, data are the mean $\pm$ S.E. lordosis quality. N's per treatment were 12, 7, and 7, respectively, for rats subchronically treated with water and injected with water, 10 mg/kg or 20 mg/kg fluoxetine. N's per treatment of rat

subchronically treated with 10 mg/kg fluoxetine were 7, 14 and 8. Single asterisks indicate a significant difference from the water-water control. The # symbol indicates a significant difference from the fluoxetine-water control. The double asterisk indicates a significant reduction in the effect of the acute fluoxetine treatment in rats previously injected with fluoxetine.

Figure 2. Fluoxetine effects in male preference testing

Ovariectomized rats were treated as described for Figure 1. Male preference testing took place immediately after lordosis testing. Data are the mean $\pm$ S.E. male preference ratios. N's per treatment for rats subchronically treated with water and injected with water, 10 mg/kg fluoxetine or 20 mg/kg fluoxetine were 6, 5, and 8, respectively. For rats subchronically treated with 10 mg/kg fluoxetine, N's were 5, 8, and 7.

Figure 3. Fluoxetine effects on crossings and grooming

Ovariectomized rats were treated as described for Figure 1. Male preference testing took place immediately after lordosis testing. Data are the mean $\pm$ S.E. number of times the female crossed the midline of the apparatus (3A) and seconds of grooming (3B). N's per treatment for rats subchronically treated with water and injected with water, 10 mg/kg fluoxetine or 20 mg/kg fluoxetine were 6, 5, and 8, respectively. For rats subchronically treated with 10 mg/kg fluoxetine, N's were 5, 8, and 7.

Figure 4. Fluoxetine effects on body weight

Data are the mean $\pm$ S.E. body weights for 31 rats subchronically injected with water and 34 rats subchronically treated with 10 mg/kg fluoxetine. Rats were ovariectomized 2 weeks before initiation of the experiment. Day 1 in the figure indicates body weight immediately prior to the first injection. The asterisk indicates the first day on which body weights of fluoxetine-treated rats were significantly lower than the water controls.

TABLE 1**LORDOSIS BEHAVIOR AFTER SUBCHRONIC TREATMENT WITH VEHICLE OR FLUOXETINE**

SUBCHRONIC TREATMENT	N	L/M RATIO MEDIAN (RANGE)	LORDOSIS QUALITY MEDIAN (RANGE)
Vehicle	34	1.0 (1.0-1.0)	3.0 (2.3-3.0)
Fluoxetine	29	1.0 (0.8-1.0)	2.55 (1.8-3.0)

TABLE 2
PERCENTAGE OF FEMALE SHOWING PROCEPTIVE BEHAVIOR

SUBCHRONIC TREATMENT	ACUTE TREATMENT	N	Percentage showing proceptivity (pretest)	Percentage showing proceptivity (after acute treatment)
Vehicle	Water	12	75.0	41.6
Vehicle	10 mg/kg fluoxetine	7	85.7	42.8
Vehicle	20 mg/kg fluoxetine	15	73.3	6.6 [*]
Fluoxetine	Water	7	100	28.5 [*]
Fluoxetine	10 mg/kg fluoxetine	14	85.7	7.1 [*]
Fluoxetine	20 mg/kg fluoxetine	8	75.0	0 [*]

^{*} $P \leq .05$ by Fisher's Exact Test