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DAILY MALE EXPOSURE ATTENUATES ESTROUS CYCLE DISRUPTION BY FLUOXETINE

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Abstract

Fluoxetine (Prozac®) produces sexual dysfunction in a substantial number of patients. In the few animal studies designed to address this sexual dysfunction in females, data have been inconsistent. Some investigators report that the drug disrupts sexual behavior without affecting the estrous cycle while we have reported robust effects of fluoxetine on the estrous cycle. The current studies were designed to initiate examination of procedural differences that may account for these contradictory outcomes. In the first experiment, intact, regularly cycling female rats were injected daily for 10 days with 10 mg/kg fluoxetine (intraperitoneally) or vehicle. Male-exposed, fluoxetine- or vehicle-treated rats were housed in a room with males and placed for 5 min/day into a male's cage. Other fluoxetine-treated females were housed in a room separate from males. In the second experiment, this protocol was repeated for 20 days and an additional group of females were exposed to male bedding for 5 min/day. Without male exposure, fluoxetine rapidly disrupted vaginal estrus and sexual receptivity so that approximately 50% of the rats failed to show vaginal estrus during the first 5 days; and the majority of the rats had a blocked cycle by 10 days of treatment. With male exposure, these reproductive effects were attenuated. The majority of rats cycled normally during the first 5 days of treatment and more than half cycled throughout the experiment. Loss of behavioral receptivity occurred even when normal estrous cyclicity was present. Although exposure to the male's bedding may have delayed the onset of estrous cycle disruption, five min daily exposure to a male's bedding did not prevent the disruptive effects of fluoxetine. These findings are consistent with evidence that fluoxetine's effect on female sexual dysfunction may result, in part, from the drugs' disruption of the hypothalamic-pituitary-gonadal axis. However, the data also evidence dissociation between the effects of fluoxetine on vaginal and behavioral estrus. These findings may also explain why different laboratories have reported the presence or absence of estrous cycle disturbances following daily treatment with fluoxetine.

Keywords

SSRIs; intact rats; sexual behavior; pheromones; progesterone; estrus

1.0 INTRODUCTION

Selective serotonin reuptake inhibitors (SSRIs), such as fluoxetine (Prozac®), are effective in the treatment of depression [32,66] but produce sexual dysfunction in a substantial number of patients [12,19,38,39,55,59]. Although the high prevalence of SSRI-induced sexual

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dysfunction in females is well recognized, the responsible mechanisms have not been identified; but a fluoxetine-induced disturbance of the hypothalamic-pituitary-gonadal (HPG) system has been implicated [25,51,58,63,64]. In contrast to a plethora of animal studies directed toward SSRI-induced sexual dysfunction in males [25], in fewer reports has the female been emphasized [16,35,47,51,60,63,65]; and in even fewer have both vaginal and behavioral estrus been examined after daily treatment with fluoxetine [35,63].

Fluoxetine rapidly increases extracellular serotonin (5-HT) by blocking the serotonin transporter (SERT) [18,48,61] and later via desensitization of 5-HT_{1A} somatodendritic autoreceptors in the dorsal raphe and 5-HT_{1B} terminal autoreceptors in some brain areas [24, 44]. Disruptive effects of 5-HT on female reproduction are well recognized [37,50,56,67]. In addition, several of the effects of fluoxetine (in particular, reduced food intake [3,4,6,23] and activation of the hypothalamic-pituitary-adrenal (HPA) axis [54,68]) might be anticipated to substantially alter the neuroendocrine system and thereby influence the female's reproductive cycle [2,7,26,27,29,53,69].

However, in a report by Matuszczyk *et al.* [35], sexual behavior and vaginal cyclicity of female rats were examined during 3 weeks of treatment with 10 mg/kg fluoxetine [35]. Fluoxetine reduced sexual behavior, but had no disruptive effect on the female's vaginal cycle. This observation was consistent with other reports that estrous cyclicity is unaffected by chronic exposure to fluoxetine [23,65]. More recently, Uphouse *et al.* [63] reported a rapid disruption of vaginal estrus in Fischer rats following initiation of daily treatment with 10 mg/kg fluoxetine [63]. More than 50% of fluoxetine-treated females failed to demonstrate vaginal proestrus/estrus within the first 5 days of treatment and 100% showed an absence of proestrous/estrous smears during the next 5 days of treatment. Consistent with their diestrous-like vaginal smears and elongated cycles, fluoxetine-treated rats had significantly lower serum levels of progesterone than their vehicle-treated counterparts [63]. This was a significant observation since it had been generally assumed that fluoxetine-induced female sexual dysfunction occurred independent of drug-induced modification of the HPG axis controlling estrous cyclicity. According to the NTP-CERHR Expert Panel Report in 2004, "The data in female rats are sufficient to qualitatively demonstrate that fluoxetine treatment with 10 mg/kg bw/day by s.c. or i.p injection results in altered estrous behavior and sexual receptivity, but has no effect on estrous cycle length" [25].

Since concurrent vaginal and behavioral estrus have been examined in only two reports [35, 63], it is important to identify explanations for the contrasting findings. In the following manuscript, we investigate one such potentially important difference between the two studies. Matuszczyk *et al.* [35] tested fluoxetine-treated females for sexual receptivity on a daily basis. In the study by Uphouse *et al.*, females were housed in a room separate from the males, and females were either exposed to males only on their predicted day of proestrus and/or on a final testing day after approximately 15 days of fluoxetine treatment [63]. Olfactory cues, specifically from the male's urine, can have a major influence on reproductive function in a variety of mammalian species [40–42,52]. The presence of the male or just the male's urine can initiate estrous cycling in aged rats or in rats with anovulatory persistent vaginal estrus [40]. Therefore, it is possible that, by daily exposing females to a male, as reported by Matuszczyk *et al.* [35], vaginal changes in fluoxetine-treated females were attenuated. In the following experiment, it was hypothesized that daily exposure of intact, regularly cycling female rats to sexually active males would attenuate the effects of fluoxetine on female rat estrous cyclicity. In addition, a group of females, housed separate from the male but exposed for 5 min/day to male bedding, was included.

2.0 MATERIALS AND METHODS

2.1 Materials

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

2.2 Animals and housing conditions

Adult female Fischer (CDF-344) rats were purchased from Charles River Laboratories (Wilmington, MA) and housed 2–3 per cage in polycarbonate cages (18 × 9.5 × 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.

2.3 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.

2.3.1 Vaginal Smearing—After a two-week acclimation to the facility, rats were individually housed with ad lib. access to food and water and their estrous cycles were monitored daily for at least two complete estrous cycles as previously described [63]. Smears containing clusters of nucleated cells and some cornified epithelial cells, but an absence of leucocytes, were considered to be proestrous smears. The presence of predominantly cornified cells with some nucleated cells, but no leucocytes, were designated as estrous smears. Leucocytes, present alone or with few nucleated or cornified cells, were considered to be diestrous smears. Only females with regular 4 to 5 day estrous cycles were used in the experiment.

2.3.2 Experimental groups—Experimental procedures took place between 1 and 4 pm, during the dark portion of the light/dark cycle. Visibility was facilitated by red lighting. Females were divided into matched groups on the basis of their vaginal smear history. Two separate experiments were conducted.

The first experiment was conducted in two stages. In the first stage, 16 rats were divided into 3 groups: fluoxetine without male exposure (n = 5), fluoxetine with male exposure (n = 6), and vehicle (distilled/deionized water) with male exposure (n = 5). Vaginal smears, body weight, and food intake were monitored daily as previously described [63] and the experiment continued for 10 days (or 2 estrous cycles) of treatment. In the second stage of the experiment, only the two groups of male-exposed females were used and rats were treated daily with fluoxetine (n = 5) or vehicle (n = 6). Vaginal smears and body weight (but not food intake) were monitored daily and the experiment continued for 15 days (or three estrous cycles). When appropriate (e.g. smear data for the first 10 days of treatment), data from the two stages were combined for analysis.

In the second experiment, 43 females were divided into 6 treatment conditions. Twenty-two females were injected daily with water and 21 were injected daily with fluoxetine. Within each subchronic treatment, females were assigned to (a) housing outside the male's housing room (n = 7 and 8, respectively for fluoxetine and water), (b) housing outside the males' area but with 5 min exposure daily to a male's bedding (n = 7 and 7, respectively for fluoxetine and water), or (c) housing in the same room as the males' housing area with five min daily exposure to a male in his home cage (n = 7 and 6, respectively for fluoxetine and water).

2.3.3 Drug treatment and behavioral testing

2.3.3.1 Experiment 1: For male exposure conditions, females were relocated to a housing area where males were maintained. Beginning on the day of diestrus 1 (day after a fully cornified vaginal smear), females were injected intraperitoneally (ip) each day with 10 mg/kg fluoxetine or the distilled/deionized water vehicle (1 ml/kg). Each day, females were placed into the home cage of a sexually active male for 5 min. Thereafter, females were weighed, smeared, given their daily injection, and returned to their home cage in the male's housing area. Because of the brief exposure time, sexual behavior, per se, was not tested; but the female's behavior while she was in the male's cage was observed and recorded. The female was qualitatively labeled as sexually receptive or nonreceptive. Females that exhibited resistive behaviors and/or rejection of the male were labeled nonreceptive. Females were labeled as sexually receptive (generally coincident with a proestrous smear) if they exhibited hopping and darting or if they showed a lordosis reflex (with or without an actual mount by the male).

A third group of fluoxetine-treated females without male exposure, as reported by Uphouse *et al.* [63], was included as a positive control in the first stage of the study. These rats were also treated daily with 10 mg/kg fluoxetine as described earlier [63], but remained in their original housing area and were never exposed to males.

2.3.3.2 Experiment 2: In the second experiment, male exposure treatment and housing away from the male were performed as described for experiment 1. An additional, male bedding, condition was added. For this condition, females were housed in a third housing room, away from male exposure. Each day, the males' cages were changed and the cages, containing soiled bedding, were immediately transferred to this third room in which the experimental females were housed. Each female was placed into a male's soiled cage for 5 min each day. The female was then returned to her home cage and the males' cages were removed.

2.4 Statistical procedures

For the first experiment, body weight and food intake data were analyzed by repeated measures ANOVA with day of treatment as the repeated factor and type of treatment as the independent factor. Comparisons of individual means were with Tukey's test and differences in vaginal estrus or behavioral receptivity were analyzed with Chi-Square procedures [72]. In the second experiment, body weights were compared by two-factor repeated measures ANOVA. Vaginal cyclicity for the entire 20-day treatment period was analyzed by Life Table Analysis followed by pair-wise comparisons with the Wilcoxin (Gehan) Statistic. Data for each of the 5-day post treatment intervals were then compared by Chi Square Procedures, using Fisher's Exact Test, as the measure of statistical significance. Alpha was 0.05.

3.0 RESULTS

3.1 Experiment 1: Estrous cyclicity and behavior

In the first experiment, fluoxetine-treated females were either exposed daily to males or were housed in an area separate from the males. Consistent with previous findings [63], when females were housed in an area separate from sexually active males, daily fluoxetine treatment disrupted the estrous cycle (See Table 1). Only 40% (2/5) of the rats showed vaginal estrus during the first 5 days of treatment and all rats evidenced blocked estrous cycles by the 10th day of treatment. Fluoxetine-treated rats had vaginal smears typical of diestrus (e.g. presence of leucocytes but not nucleated/cornified cells).

In contrast, housing in the same room as the males plus 5 min daily exposure to a male attenuated the effects of fluoxetine on vaginal cyclicity. Ninety-one percent (10/11) of fluoxetine-treated females showed vaginal estrus during the first 5 days of treatment and 70%

(7/10) of these showed evidence of behavioral receptivity. During the second 5 days of the experiment, 54% (6/11) exhibited a second vaginal estrus and each of these 6 rats also evidenced behavioral receptivity. For the 5 rats that were observed for a third cycle (e.g. days 11–15 of treatment), 3/5 showed vaginal estrus and 2/5 showed behavioral receptivity. Rats without male exposure were not tested for behavioral receptivity.

Vehicle controls, exposed daily to the male, showed normal vaginal cyclicity throughout the experiment. Out of the 11 control rats, 10 showed sexual receptivity during their first proestrus and 72% (8/11) exhibited sexual receptivity during their second proestrus. All of the 5 control rats that were observed for a third cycle showed both vaginal estrus and behavioral receptivity.

There were significant effects of treatment on vaginal cyclicity (respectively for first and second 5 days, Chi square = 10.28 and 15.30, $df = 2$, $P \leq 0.05$). For the first 5 days, this was due to the effects of fluoxetine treatment without the male. When only male-exposed females were compared, fluoxetine and vehicle-treated females did not differ in either vaginal (Chi square = 1.04, $df = 1$, $P \geq 0.05$) or behavioral estrus (Chi square = 2.34, $df = 1$, $P > .05$). During the second 5 days of the experiment, even with the male exposure, relative to the vehicle control, significantly fewer fluoxetine-treated females had a second proestrous/estrous smear (Chi square = 6.47, $df = 1$, $P \leq .05$); but since 3 of the 11 control rats also failed to be sexually receptive, sexual receptivity did not differ between the two groups (Chi square respectively = 0.78, $df = 1$, $P > .05$).

3.2 Experiment 2: Estrous cyclicity and behavior

In the second study, the subchronic treatment was extended for 20 days to include a fourth estrous cycle interval. In addition, the effects of 5 min exposure to male bedding (in a room outside the male housing area) was included. Water-treated controls, included for all the housing conditions, exhibited normal estrous cyclicity throughout the experiment (Table 2)

However, all fluoxetine-treated females did not cycle normally during the 20 days of treatment (Figure 1). Consistent with the first experiment, housing in the male's housing area plus 5 min exposure to the male reduced the effect of fluoxetine on estrous cyclicity. The presence of the male's bedding also slightly reduced the effect of fluoxetine but not as robustly as the presence of the male.

Similar to findings in the first experiment, 100% of females with male exposure showed a normal estrous cycle during the first 5 days of treatment. With the exception of two rats, such cyclicity continued throughout the experiment. In contrast, only a single fluoxetine-treated rat, without male exposure, cycled normally through the entire 20 days. Fluoxetine-treated females with male bedding resembled fluoxetine-treated rats with males during the first 5 days of the experiment. Thereafter, 6/8 (75%) of the females had at least one disrupted estrous cycle.

When cyclicity across all 20 days (4 cycle intervals) was compared, there was a significant effect of treatment condition (Life Table, Overall Wilcoxin Gehan Statistic = 53.04, $df = 5$, $P \leq .0001$) with all fluoxetine-treated groups differing significantly from their comparable vehicle-treated controls (all Wilcoxin ≥ 4.0 , $P \leq .05$). There were no differences among the vehicle-treated groups (all $P > .05$) but significant effects of type of exposure were present for fluoxetine-treated females (Life Table, Overall Wilcoxin Gehan Statistic = 10.07, $df = 2$, $P \leq .007$). Fluoxetine plus male females differed significantly from both fluoxetine without male (Wilcoxin = 9.47, $df = 1$, $P \leq .003$) and fluoxetine with bedding (Wilcoxin = 4.14, $df = 1$, $P \leq .05$).

When the three fluoxetine-treated groups were compared within each of the time periods, the greatest differences were seen during the first two time periods after initiation of treatment.

Fluoxetine with male and fluoxetine without male groups differed significantly during the first and second five-day intervals (Chi Square = 5.60 and 4.66, respectively; $df = 1$, $P \leq .05$) but not thereafter. Although rats with male bedding, alone, were never significantly different from those given fluoxetine without male, the difference during the first 5 days of the cycle only narrowly escaped statistical significance (Chi Square = 3.34, $df = 1$, $P = .067$). Pair-wise comparisons (within individual 5-day intervals) of fluoxetine plus male and fluoxetine plus bedding also failed to show statistical significance.

In spite of continued estrous cyclicity, females treated with fluoxetine and exposed daily to a male, did not always show normal sexual behavior (Table 3). Similar to findings in the first experiment, fluoxetine-treated rats showed normal sexual behavior during the first 5 days of treatment, but by the second cycle, sexual receptivity had declined even though most rats showed normal vaginal cyclicity. By the third cycle interval (e.g. days 11–15), only one fluoxetine-treated rat was sexually receptive so fluoxetine-treated rats were significantly different from the water control (Fischer Exact Test, $P \leq 0.02$). By the fourth cycle, 50% of rats with proestrous smears were sexually receptive.

3.3 Body weight and food intake

While the presence of the male appeared to attenuate the effects of fluoxetine on estrous cyclicity, there were no differences between fluoxetine-treated rats in their loss of body weight after fluoxetine. In the first experiment (Figure 2), there was a significant effect of treatment ($F_{2, 24} = 5.19$, $P \leq .02$), day of the experiment ($F_{10, 240} = 14.62$, $P \leq .0001$) and their interaction ($F_{20, 240} = 8.17$, $P \leq .0001$) that resulted from the effects of fluoxetine. By day 2 or 3 and thereafter, respectively, body weights of both groups of fluoxetine-treated rats were significantly less than the vehicle control ($q_{240,3} \geq 3.31$, $P \leq .05$) and their own starting weight ($q_{240,10} \geq 4.47$, $P \leq 0.05$). Similar effects were present in the second experiment (Figure 3). Significant treatment effects were present for the type of subchronic treatment ($F_{1,37} = 9.02$, $P \leq 0.005$), the effect of time ($F_{20,740} = 23.87$, $P \leq 0.0001$), and the interaction between time and subchronic treatment ($F_{20,740} = 72.04$, $P \leq 0.0001$). No other effects were significant.

Food intake was measured daily only in the first stage of the first experiment. Food intake for these rats is shown in Figure 4. As observed by others, fluoxetine significantly reduced food intake. There were significant effects of treatment ($F_{2,13} = 54.5$, $P \leq 0.001$), time after treatment ($F_{10,130} = 17.4$, $P \leq 0.0001$) and their interaction ($F_{20,130} = 3.38$, $P \leq 0.0001$). Both groups of fluoxetine-treated rats reduced food intake; fluoxetine-treated rats with male exposure ate slightly (but significantly) more than their fluoxetine-treated, without male, counterparts (Tukey's $q_{130,3} \geq 3.35$, $P < .05$).

4.0 DISCUSSION

These experiments were designed to test the hypothesis that daily exposure to a sexually active male would attenuate effects of fluoxetine on estrous cyclicity and sexual behavior. In the first experiment, the fluoxetine-treated females were exposed daily for 10 to 15 days to a sexually active male. In the second experiment, the treatment duration was extended to 20 days and an additional group, exposed daily to the male's bedding, was included. Collectively, these findings lead to the suggestion that male exposure reduces the deleterious reproductive effects of fluoxetine. Limited (5 min/day) exposure to the male bedding slightly delayed the effect of fluoxetine but not as robustly as daily exposure to the male.

These findings reinforce our recent observations that chronic fluoxetine leads to disruption of both vaginal and behavioral estrus in Fischer female rats. In rodents, sexual behavior is intimately linked to physiological readiness to mate [36,57]. A proper sequence of hormonal priming with estrogen and progesterone is essential if behavioral receptivity is to occur [57].

In the naturally cycling rat, release of follicle stimulating hormone (FSH) and luteinizing hormone (LH) from the pituitary and consequent follicular development in the ovary leads to secretion of estrogen. Estrogen action in the CNS is essential for later initiation of sexual behavior [49] and also stimulates proliferation of the uterine and vaginal tissue [5]. Vaginal responses to estrogen (observed within 24 to 48 hr of estrogen treatment) are evidenced by increases in the proportion of nucleated and cornified cells present in the vaginal smear. Similarly, behavioral responses require the priming effect of estrogen 24 to 48 hr earlier [36, 46].

Positive feedback from follicular estrogen leads to a preovulatory surge of LH and consequent ovulation [36,57]. However, in the naturally cycling rat, a preovulatory surge of progesterone (probably from the adrenal) synergizes with estrogen to facilitate both the preovulatory LH surge [30,31] and sexual receptivity [36,57]. Progesterone influences female sexual behavior both through interaction with nuclear progesterone receptors and through conversion to the progesterone metabolite, 3 α -hydroxysteroid-5 α -pregnan-20-one (3 α -5 α -THP, allopregnanolone) [15]. Within the medial basal hypothalamus, progesterone's interaction with its nuclear receptor may be sufficient to facilitate female sexual behavior while, in the midbrain, 3 α -5 α -THP may effectively influence sexual behavior ([1,13,17]).

Rehavi *et al.* [51] reported that 5-HT reuptake blockers, injected for 3 weeks, decreased serum estradiol levels and speculated that this might reflect effects on the HPG axis [51]. However, it has also been suggested that fluoxetine might reduce serum estrogen by enhancing peripheral metabolism of the steroid [62]. We previously reported that serum progesterone, but not estrogen, was decreased in female rats that had been treated for approximately 15 days with 10 mg/kg fluoxetine [63]. In addition, fluoxetine's reported reduction of midbrain 3 α -5 α -THP [16] may contribute to the drug's effect on female sexual behavior. Regardless of the mechanisms involved, fluoxetine appears to negatively impact the sequence of hormonal events regulating female reproduction. Significant to this possibility is evidence that fluoxetine reduces LH in blood serum [50]. However, as shown in the present experiments, the disruptive effects of fluoxetine can be delayed and/or attenuated with daily exposure to a sexually active male.

The presence of the male (or just the male's urine) is able to initiate estrous cyclicity in a variety of mammalian species [8,52] including rats [40–42]. In intact rats, this phenomena has been most thoroughly studied in aged or estrogenized females where the male's urine, via vomeronasal stimulation, leads to a cascade of events culminating in release of gonadotropin releasing hormone (GnRH) [43]. Significant to the present study was an increase in serum progesterone seen within 2 hr of exposure to the male's urine and which was abolished in adrenalectomized females [43].

In the current study, although there was a tendency for exposure to the male's bedding to delay effects of fluoxetine, bedding exposure was not as effective as exposure to a male. However, since females exposed to males were also housed in the same room with males, olfactory stimuli were present 24 h/day. Thus, it may have been the 24 h exposure to such odor rather than just the 5 min interaction with the male that differentiated this group from the group exposed only to male bedding for 5 min/day. Interestingly, however, mating stimuli have been reported to increase progesterone metabolites in brain [14] so it is not inconceivable that mating stimuli, per se, were responsible for attenuating reproductive effects of fluoxetine. Further studies will be required to differentiate these possibilities. Nevertheless, the current studies allow the conclusion that the presence of the male via either olfactory or mating stimuli can reduce the sexual dysfunction initiated by treatment with fluoxetine.

We previously suggested that the anorectic effect of fluoxetine and the consequent loss of body weight might account, in part, for fluoxetine's disruption of vaginal and behavioral estrus [63]. Without male exposure, there was a small, but significantly greater, effect of fluoxetine on food intake. However, the effects of fluoxetine on body weight were independent of whether or not the female was exposed to the male. In spite of a decline in body weight that was comparable to females not exposed to the male, females with male exposure showed normal vaginal cyclicity, at least during the first 5 days of the experiment. Thus, while the effects of fluoxetine on eating and energy expenditure probably contribute to the drug's disruption of reproductive function, additional factors also appear to be important. Nevertheless, since, with continued fluoxetine treatment, there was a decline in the number of rats showing normal vaginal cyclicity, a contribution of the reduced food intake/body weight should not be ruled out. Adipose tissue is a recognized site of steroid metabolism [28]. Early during fluoxetine treatment, it is possible that the body weight loss by females could be overcome by male exposure; but as body weight further declined, effects of the male were not sufficient to compensate for loss of adipose tissue. However, females appeared to reinitiate estrous cyclicity after 16 to 20 days of fluoxetine treatment even though body weight continued to be low [63].

It is worth noting that vaginal estrus was more sensitive to the rescuing action of male exposure than was behavioral receptivity (e.g. some females showed no evidence of sexual receptivity in spite of vaginal estrus). This is not surprising since uterine and vaginal epithelium may be more sensitive to estrogen than is the CNS. Moreover, with the relatively low levels of estrogen in intact females, behavioral receptivity [57], but not vaginal estrus [5], requires a preovulatory increase in progesterone. Furthermore, since progesterone's effect on behavior includes a contribution from both progesterone and its metabolites, it is tempting to speculate that fluoxetine's reduction of 3α - 5α -THP may contribute to the dissociation between behavioral and vaginal estrus. In support of this possibility, Frye *et al.* [16] reported that a fluoxetine-induced decrease in lordosis behavior could be reversed by infusion of allopregnanolone into the ventral tegmental area of the brain.

There are additional reasons to suspect that fluoxetine's effect on sexual behavior might dissociate from effects on vaginal cyclicity. In addition to fluoxetine's modulation of extracellular serotonin, fluoxetine also antagonizes 5-HT_{2C} and 5-HT₃ receptors [9–11,22,45], both of which have been positively implicated in the control of female rat sexual behavior [33,34,70,71]. Finally, fluoxetine enhances the conversion of progesterone to a variety of metabolites other than 3α - 5α -THP. In particular, the progesterone metabolite, 3α -hydroxy-4-pregnen-20-one (3α HP), also increased by fluoxetine [20], specifically and selectively blocks FSH release [21] and would, therefore, be expected to inhibit reproductive cyclicity. Consequently, fluoxetine may interrupt the sequence of hormonal events that are crucial for the ovulatory and behavioral response and may also alter the balance between intracellular hormone receptor mediated events and those resulting from neurosteroid events within the brain.

In summary, daily treatment with fluoxetine disrupts vaginal estrus in Fischer inbred rats. Such disruption is rapid, occurring in approximately 50% of the rats within a single cycle. Daily exposure of treated females to a sexually active male reduces and/or delays the onset of this disruption. It is hypothesized that fluoxetine disrupts the sequence of hormonal events that lead to ovulation and sexual receptivity in the rat. Daily exposure to a male, possibly via activation of the vomeronasal organ, initially compensates for the effects of fluoxetine. With longer exposure, however, such compensation may be insufficient. Moreover, since bedding alone was less effective than male exposure, additional stimuli from the male may contribute to attenuation of fluoxetine's effects. These data strengthen previous suggestions for a disruptive effect of fluoxetine on the HPG axis, hormonal events, and/or neurosteroidogenesis and should

lead to further experiments to evaluate the contribution of these neuroendocrine disturbances to female sexual dysfunction.

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Figure 1. Effects of fluoxetine and housing condition on vaginal estrus

Data are the percentage of rats showing vaginal estrus after initiation of daily treatment with 10 mg/kg fluoxetine. Rats were housed without males, in an area with males, and exposed for 5 min/day to a sexually active male, or housed without males but exposed for 5 min/day to bedding from the male's cage. Data are grouped into 5-day blocks to encompass 4 consecutive periods of regular estrous cycles. Asterisks indicate significant differences from fluoxetine-treated females housed without male and without exposure to the male's bedding.

Figure 2. Body weights of chronic fluoxetine-treated (with or without male exposure) and vehicle control rats

Shown are the mean $\pm$ S.E. body weights for female rats treated daily with 10 mg/kg fluoxetine or vehicle ($n = 11$ /group). After initiation of treatment, rats were exposed daily for 5 min to a sexually active male. Also shown is the body weight for 5 additional rats that received daily treatment with fluoxetine but were not exposed to the males. Day 0 indicates body weight the day before the first injection; Day 1 is the first day of injection. The single asterisk indicates the first day on which body weight of fluoxetine-treated rats was significantly less than their starting weight. Double asterisks indicate the first day where fluoxetine-treated rats significantly differ from the vehicle control.

Figure 3. Body weights of females under different housing conditions

Shown are the mean $\pm$ S.E. body weights for female rats treated daily with 10 mg/kg fluoxetine or vehicle. Rats were housed without males, in an area with males, and exposed for 5 min/day to a sexually active male, or housed without males but exposed for 5 min/day to bedding from the male's cage. Data in Figure 3A are for vehicle-treated rats; in 3B are data for fluoxetine-treated rats. Day 0 indicates body weight the day before the first injection; Day 1 is the first day of injection. The single asterisk indicates the first day on which body weight of all fluoxetine-treated rats was significantly less than their appropriate control.

Figure 4. Food intake of rats during the first stage of the experiment

Shown are the mean $\pm$ S.E. food intake for female rats treated daily with 10 mg/kg fluoxetine ($n = 6$) or vehicle ($n = 5$) and exposed daily to a sexually active male. Also shown is the food intake for 5 rats that received daily treatment with fluoxetine but were not exposed to the males. Day 0 indicates food intake the day before the first injection; Day 1 is the first day of injection. Single asterisks indicate days where groups differed from the vehicle control. The double asterisk indicates significant difference between fluoxetine-treated rats with and without males.

TABLE 1
NUMBER OF FEMALE SHOWING VAGINAL ESTRUS OR BEHAVIORAL RECEPTIVITY

Treatment	Days 1–5		Days 6–10		Days 11–15	
	Vaginal Estrus	Behavioral Receptivity	Vaginal Estrus	Behavioral Receptivity	Vaginal Estrus	Behavioral Receptivity
Vehicle with male	11/11 (100%)	10/11 (90.9%)	11/11 (100%)	8/11 (72.7%)	5/5 (100%)	5/5 (100%)
Fluoxetine with male	10/11 (90.9%)	7/11 (63.6%)	6/11 (54.5%)	6/11 (54.5%)	3/5 (60%)	2/5 (40%)
Fluoxetine without male	2/5 (40%)	NT	0/5 (0%)	NT	NT	NT

Regularly cycling female rats were injected daily with 10 mg/kg fluoxetine or vehicle. Vaginal cyclicity and sexual receptivity were monitored daily as described in the methods. Data were grouped into blocks of 5 days after initiation of injections. In the table are shown the number (and percentage) of females showing either vaginal estrus or behavioral receptivity during each 5-day block. NT indicates that group was not tested. The denominator indicates the number of females tested; the numerator indicates the number of animals showing vaginal estrus or behavioral receptivity. Percentages are shown in parentheses.

TABLE 2
PROPORTION OF WATER-TREATED FEMALES SHOWING VAGINAL ESTRUS

	DAYS 1–5	DAYS 6–10	DAYS 11–15	DAYS 16–20
TREATMENT	Vaginal Estrus	Vaginal Estrus	Vaginal Estrus	Vaginal Estrus
Vehicle with male	6/6 (100%)	6/6 (100%)	6/6 (100%)	6/6 (100%)
Vehicle without male	8/8 (100%)	8/8 (100%)	8/8 (100%)	8/8 (100%)
Vehicle with bedding	7/7 (100%)	7/7 (100%)	7/7 (100%)	7/7 (100%)

Regularly cycling female rats were injected daily with 1 ml/kg of distilled/deionized water. Vaginal cyclicity and sexual receptivity were monitored daily as described in the methods. Data were grouped into blocks of 5 days after initiation of injections. In the table are shown the number (and percentage) of females showing vaginal estrus during each 5-day block. The denominator indicates the number of females in each group; the numerator indicates the number of animals showing vaginal estrus. Percentages are shown in parentheses.

TABLE 3
EFFECT OF FLUOXETINE ON BEHAVIOR IN THE MALE'S CAGE

Days of Treatment	BEHAVIOR	VEHICLE WITH MALE (%)	FLUOXETINE WITH MALE (%)
DAYS 1–5	Estrous Smear	6/6 (100%)	7/7 (100%)
	Receptive	6/6 (100%)	7/7 (100%)
	Proceptive	6/6 (100%)	7/7 (100%)
	Resistive	0/6 (0%)	0/7 (0%)
DAYS 6–10	Estrous Smear	6/6 (100%)	5/7 (71.4%)
	Receptive	6/6 (100%)	2/5 (40%)
	Proceptive	6/6 (100%)	2/5 (20%)
	Resistive	0/6 (0%)	3/5 (60%)
DAYS 11–15	Estrous Smear	6/6 (100%)	5/7 (71.4%)
	Receptive	6/6 (100%)	1/5 (20%)*
	Proceptive	6/6 (100%)	1/5 (20%)*
	Resistive	0/6 (0%)	4/5 (80%)*
DAYS 16–20	Estrous Smear	6/6 (100%)	6/7 (85.7%)
	Receptive	6/6 (100%)	3/6 (50%)
	Proceptive	6/6 (100%)	1/6 (16.6%)
	Resistive	0/6 (0%)	2/6 (33.3%)

Regularly cycling female rats were injected daily with 10 mg/kg fluoxetine or vehicle and exposed for 5 min/day to a sexual active male. Vaginal cyclicity and sexual receptivity were monitored daily as described in the methods. Data were grouped into blocks of 5 days after initiation of injections. In the table are shown the number (and percentage) of females showing either vaginal estrus (estrous smear) or behavioral receptivity during each 5-day block. The denominator indicates the number of females tested. For behavioral parameters, the denominator refers to the number of females with proestrous smears during that 5-day block. The numerator indicates the number of animals showing vaginal estrus or behavioral receptivity. Percentages are shown in parentheses.

* $P \leq .05$ Fisher's Exact Text