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MODEST EFFECTS OF REPEATED FLUOXETINE ON ESTROUS CYCLICITY AND SEXUAL BEHAVIOR IN SPRAGUE DAWLEY FEMALE RATS

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Abstract

In an earlier study, we reported that daily fluoxetine treatment (10 mg/kg/day) rapidly disrupted estrous cyclicity and sexual receptivity in adult, regularly cycling Fischer rats. The current study was designed to investigate if comparable fluoxetine treatment would similarly affect intact, regularly cycling Sprague Dawley rats. In the first experiment, fluoxetine was injected for 24 days. After 11–14 days of daily fluoxetine treatment, 40% of the rats showed a transient disturbance of the estrous cycle with elimination of sexual receptivity. In these affected rats, reduced sexual receptivity generally preceded disruption of vaginal cyclicity. In a second experiment, a shorter exposure was used to attempt to dissociate effects of fluoxetine on behavior and estrous cyclicity. Nine days of fluoxetine treatment eliminated sexual receptivity and proceptivity (hops/darts) in 40% and 46%, respectively, of rats without altering the estrous cycle. Female rats then received a 10th fluoxetine injection 30 min prior to assessment of sexual motivation (measured with the male preference paradigm). There was no effect of fluoxetine on male preference, but fluoxetine significantly reduced the number of crossings and seconds of grooming during preference testing. Therefore, effects of fluoxetine on estrous cyclicity and behavior of Sprague Dawley female rats were smaller and required longer to develop than previously reported in Fischer female rats. These findings reinforce a probable relationship between fluoxetine's effect on sexual activity and neuroendocrine disturbances and illustrate the importance of strain selection in attempting to model human disease.

Keywords

SSRI; sexual motivation; lordosis; rat strain; food intake; body weight

1.0 Introduction

Selective serotonin reuptake inhibitors (SSRIs), such as fluoxetine, are frequently prescribed for a variety of mood and eating disorders, such as anxiety, depression, anorexia, bulimia, and premenstrual dysphoria [14,33,43,55]. Although highly effective for the treatment of depression, fluoxetine has been reported to produce sexual side effects in 30–60% of the female patient population [2,4,15,20,33,38]. While several suggestions have been made [11,16–18,

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51,56,57], the mechanisms responsible for SSRI-induced sexual dysfunction, especially in the female population, remain unclear.

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 [23,34]. This is surprising since fluoxetine is a potent anorectic [8,12] and estrous cyclicity can be readily disrupted by food restriction [25,63]. Therefore, we previously evaluated the relationship between fluoxetine treatment and food intake on body weight and estrous cyclicity [60]. In that experiment with Fischer female rats, food intake was dramatically suppressed in the initial 24 hr of daily treatment with 10 mg/kg fluoxetine [60]. Both estrous cyclicity and sexual receptivity were eliminated in 63% of Fischer female rats after the first five days of treatment and in 100% of the females after ten days of treatment. Pair-feeding of a vehicle-treated control group led to comparable declines in estrous cyclicity and behavior [60].

Our findings that fluoxetine disrupted the estrous cycle contrasted with earlier reports. In the best known such report, Wistar female rats that were treated daily with 10 mg/kg of fluoxetine showed reduced sexual receptivity and sexual motivation, but there was no evidence that the estrous cycle had been disrupted [34]. Subsequently, Heisler et al. [22] and van de Kar et al. [62] reported that daily fluoxetine treatment did not disrupt estrous cyclicity. To explore potential explanations for these contrasting findings, Sarkar et al. [53] compared the effect of fluoxetine in female rats, exposed daily to males, to that in female rats that were isolated from males. Distinct from the experiment by Uphouse et al. [60], Matuszczyk et al. [34] had housed fluoxetine-treated female rats in the presence of males and had tested female rats daily with a sexually active males. Because olfactory cues from the male's urine can alter female reproductive function in a variety of mammalian species [39–41,48], it was hypothesized that male exposure might reduce the estrous cycle disruption during fluoxetine treatment. In support of this hypothesis, Sarkar et al. [53] reported that fluoxetine's effect on vaginal estrus of Fischer rats was attenuated by daily exposure to a male. However, this attenuation was evidenced as a delay in the onset rather than a complete elimination of the estrous cycle disruption. Therefore, in the following experiment, we considered the possibility that there was a strain difference in the effect of fluoxetine on estrous cyclicity.

Sprague Dawley female rats were selected since Van de Kar et al. [62] previously reported that daily treatment with 10 mg/kg fluoxetine had no effect on estrous cyclicity in this rat strain. In addition, Sprague Dawley rats are a prominent strain used in the study of neuropharmacology. Furthermore, Sprague Dawley rats have a heavier body weight than Fischer rats [54], and Tropp and Markus [58] recently reported that Fischer female rats were especially vulnerable to disruption of estrous cyclicity following mild food deprivation while the same food restriction had no effect on the estrous cycle of Sprague Dawley female rats. Furthermore, early during treatment, fluoxetine increases corticotropin releasing hormone [19,28] which can reduce food intake and disrupt estrous cyclicity and Sprague Dawley rats are less "stress responsive" than Fischer rats [13,26] and respond differently to serotonergic and antidepressant compounds [7,42,61].

In the following experiment, we examined the effects of daily fluoxetine treatment in Sprague Dawley female rats. Housing and treatment conditions were identical to those previously reported to eliminate vaginal cyclicity and sexual receptivity in Fischer female rats [60]. Sexual receptivity and sexual motivation were also examined since sexual side effects of fluoxetine had been reported to occur without an estrous cycle disruption [34]. Only modest effects of fluoxetine were seen in the absence of an estrous cycle disturbance. These findings, therefore, lend support to the suggestion that a neuroendocrine disturbance may contribute to effects of SSRIs on female sexual behavior and point to a possible strain difference in the effect of

fluoxetine on estrous cyclicity. In particular, Fischer female rats may be the preferred strain for the study of SSRI-induced disruption of reproductive function.

2.0 Results

2.1 Experiment 1

Prior to initiation of daily injection with water or fluoxetine, Sprague Dawley female rats showed both four and five-day estrous cycles, which were equally distributed across the two treatment conditions. The mean $\pm$ S.E. cycle length prior to treatment was 4.40 ± 0.10 and 4.40 ± 0.11 days for fluoxetine and vehicle treatments, respectively (Table 1). In the vehicle control group, preinjection cycle length was maintained after initiation of the daily injections [(mean $\pm$ S.E. cycle length was 4.20 ± 0.07 days (median = 4.00, range = 4.00 to 5.00)]. In contrast, 6 of the 15 (40%) fluoxetine-treated rats showed elongated cycles at least once during the experiment so that median cycle lengths of fluoxetine- and water-treated rats were significantly different (Mann-Whitney $U = 161.5$, $df = 14$ and 14 , $P \leq 0.05$; Table 1 and Figure 1).

Similarly, fluoxetine treatment reduced sexual receptivity in only a portion of the Sprague Dawley female rats (Figure 2). The relatively low receptivity throughout the experiment resulted exclusively from the same six rats that showed disturbance of the estrous cycle; each of these rats failed to show sexual receptivity during at least one five-day period and the absence of sexual receptivity occurred prior to occurrence of an elongated vaginal cycle. However, with the exception of these six rats, the remaining fluoxetine-treated rats showed normal vaginal cyclicity and were sexually receptive during each proestrus. Nevertheless, the proportion of fluoxetine-treated rats showing sexual receptivity was never significantly different from expectation ($P > 0.05$). For the six fluoxetine-treated rats that had disturbance of both vaginal estrus and behavior, the disruption was transient, so that when rats were tested on the last proestrous day prior to termination of the experiment, both water and fluoxetine-treated rats showed equivalent sexual behavior (see Table 2). Neither lordosis frequency nor lordosis quality was significantly different (both $t < 1.0$, $df = 23$, $P > 0.05$).

Fluoxetine significantly reduced body weight (Figure 3A) and food intake (Figure 3B) of Sprague Dawley female rats. Body weights of fluoxetine-treated rats were significantly less than their starting weight from day 4 through day 20 of treatment (Tukey's $q_{728,27} \geq 5.20$, $P \leq 0.05$). Thereafter, their body weights began to approach that of their pretreatment starting weight. In contrast, vehicle-treated rats gained weight during the experiment. With the exception of day 12 of treatment, after the 8th day of the experiment, the body weight of vehicle-treated rats was significantly greater than their starting weight (Tukey's $q_{728,27} \geq 2.77$, $P \leq 0.05$). Thus, even though body weights of the fluoxetine-treated rats returned to their starting levels, their body weight remained significantly lower than that of their vehicle controls (all $P = 0.05$). As a result, there was a significant effect of treatment ($F_{1,28} = 15.92$, $P \leq 0.0005$), duration of treatment ($F_{26,728} = 11.39$, $P \leq 0.0001$) and the interaction between type of treatment and duration ($F_{26,728} = 12.02$, $P \leq 0.0001$).

A significant effect of fluoxetine on food intake (Figure 3B) (ANOVA for treatment, $F_{1,28} = 57.04$, $P \leq 0.0001$) was most evident during the first ten days of treatment (all Tukey's $q_{644,2} \geq 2.77$, $P \leq 0.05$) so there was a significant effect of day of treatment ($F_{23,644} = 10.29$, $P \leq 0.0001$) as well as a day by treatment interaction ($F_{23,644} = 6.45$, $P \leq 0.0001$). Food intake of fluoxetine-treated rats was significantly less than the vehicle control from day 1 through day 10 of treatment.

2.2 Experiment 2

The second experiment was designed to determine if there was any evidence for a fluoxetine-induced disturbance of sexual activity in the absence of an estrous cycle disturbance. During the 10 days of treatment, estrous cyclicity did not differ between fluoxetine and vehicle-treated rats ($P > 0.05$; Table 3). All rats showed proestrous smears on the days predicted from their pretreatment cycle pattern. However, in spite of normal cyclicity, 5/15 fluoxetine-treated rats showed neither lordosis behavior nor proceptivity and one rat refused to allow the male to mount (see Table 4) and 7/15 of fluoxetine-treated rats showed resistive behavior. However, every rat that did show lordosis behavior had an L/M ratio of 1.0 and all but one of these also exhibited hopping and darting behavior. A single control rat was resistive, refused to let the male mount, and showed no proceptivity. All other controls had L/M ratios of 1.0. Nevertheless, there were no significant effects of treatment on the various behaviors (all $P > 0.05$). Although fluoxetine-treated rats received slightly fewer mounts during the 10 min test interval, there were no significant differences between the two groups.

After testing for sexual receptivity, rats received their daily injection with fluoxetine or vehicle and 30 min later were tested in the partner preference paradigm. Due to a technical problem, one vehicle- and 3 fluoxetine-treated rats were not videotaped and were not included in the partner preference analysis. Data for the remaining rats are shown in Table 5. There was no significant difference in the male preference score ($t < 1.0$, $df = 15$, $P > 0.05$) or total social time ($t = 1.55$, $df = 15$, $P > 0.05$) between the fluoxetine- and water-treated rats.

Fluoxetine may have influenced the female's activity and possibly anxiety or fear associated with the procedure since the drug significantly reduced the number of center crossings and seconds of grooming, respectively, during the partner-preference test (respectively, $t = 2.42$ and 2.35 , $df = 15$, $P \leq 0.05$).

Consistent with the first experiment, there was a significant effect of treatment ($F_{1,19} = 14.40$, $P \leq 0.001$), day of the treatment ($F_{10,190} = 3.68$, $P \leq 0.0001$) and their interaction ($F_{10,190} = 7.02$, $P \leq 0.0001$) on body weight (daily data not shown). The mean body weight after 10 days of treatment was significantly less in fluoxetine-treated (286.66 ± 1.2) relative to the vehicle-treated (248.62 ± 2.0) rats.

3.0 Discussion

A major objective of the current experiment was to determine if repeated fluoxetine treatment would block the estrous cycle in regularly cycling, intact Sprague Dawley female rats, similar to that reported in Fischer rats [60]. Unlike the SSRI's effect in Fischer rats, fluoxetine had only minor effects on vaginal estrus of Sprague Dawley female rats, consistent with the report by Van de Kar, et al. [62]. However, the estrous cycle was elongated in 6 of the 15 (40%) rats following 11–15 days of fluoxetine treatment and these same 6 rats failed to exhibit sexual receptivity prior to the vaginal disruption. In contrast to Fischer rats, the cycle disruption in Sprague Dawley female rats was transient and was evidenced primarily as elongation, rather than cessation, of estrous cyclicity. Thus, the disruption of the estrous cycle appeared to be less severe and required longer treatment time in the Sprague Dawley female rats compared to that seen in Fischer female rats [60].

In the second experiment, we took advantage of two observations from the first experiment: (1) every Sprague Dawley female cycled normally for the first two estrous cycles during fluoxetine treatment; and (2) when sexual behavior was disturbed, the behavioral disruption preceded an elongated vaginal cycle. The second experiment was, therefore, designed to evaluate effects of sexual receptivity and motivation under conditions where the female's estrous cycle was not disrupted. Forty percent of fluoxetine-treated females failed to show

sexual receptivity in spite of a normal proestrous vaginal smear. This finding is consistent with a prior report that sexual behavior and motivation can be reduced by fluoxetine even if the estrous cycle is not disrupted [34].

That acute treatment with fluoxetine can reduce lordosis behavior in hormonally primed, ovariectomized rats [17,52] is further evidence that fluoxetine's effects on behavior and estrous cyclicity can be dissociated. Nevertheless, in intact, regularly cycling female rats, the proportion of females in which this dissociation occurs is relatively small. At the time of the 2004 NTP-CERHR Expert Panel Report, when it was concluded that 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" [23], there were only three published reports (one with Sprague Dawley rats) in which fluoxetine effects on estrous cyclicity had been examined. Since that time, four replications of the effects of fluoxetine on estrous cyclicity with Fischer rats [52, 60] and the current experiments with Sprague Dawley rats have been completed. From these collective findings, a reconsideration of the conclusions of the Panel is warranted. Specifically, fluoxetine does disrupt estrous cyclicity, but there appears to be individual/strain differences in the probability that such a disruption will occur. It is particularly interesting that the proportion of Sprague Dawley female rats affected by fluoxetine is not inconsistent with the reported incidence of sexual dysfunction in the human population [10,20]. That a smaller proportion of the outbred Sprague Dawley strain (current experiment) than the inbred Fischer strain (100% affected) [53,60] show sexual dysfunction after fluoxetine may indicate the existence of a genetic predisposition to occurrence of SSRI-induced sexual dysfunction in females. A consideration of published differences between Fischer and Sprague Dawley rats may provide clues to the underlying mechanisms responsible for fluoxetine-induced sexual dysfunction.

Since fluoxetine increases extracellular serotonin (5-HT) [21,30,44] and elevation of 5-HT has a negative influence on reproductive cyclicity and behavior [36,59], it is tempting to speculate that Fischer/Sprague Dawley differences in 5-HT activity may account for their different vulnerability to estrous cycle disruption. Relative to Sprague Dawley rats, the Fischer strain has been reported to have higher serotonergic activity in the hippocampus [5,6] but, interestingly, this difference is reversed in the hypothalamus [50]. Fischer rats also have more mRNA for the serotonin transporter in the raphe [7] so it is not unreasonable that Fischer and Sprague Dawley female rats differ in their response to a serotonergic drug and consequent disruption of the neuroendocrine axis. Three weeks of fluoxetine treatment has been reported to reduce serum estrogen [47], serum luteinizing hormone [46] and serum progesterone levels [60] in rats, but no strain comparisons have been made.

Fluoxetine is well known to activate the hypothalamic-pituitary-adrenal (HPA) axis [19,28], most likely through its elevation of hypothalamic 5-HT [37,45]; corticotropin releasing hormone (CRH) decreases reproductive functioning probably via reduced gonadotropin releasing hormone release from hypothalamic neurons [29,49]; and Fischer and Sprague Dawley rats differ in activation of the HPA axis [13,26]. Chronic central CRH infusion has been reported to decrease food intake and body weight in rats [49], and increased hypothalamic 5-HT levels have been reported to cause carbohydrate aversion, reduce feeding and increase energy expenditure [27,31,32,35]. Therefore, intrinsic differences in CRH and/or response to fluoxetine may contribute to differential vulnerability for development of sexual dysfunction.

In the current experiment, fluoxetine treatment led to a significant decline in food intake and body weight in Sprague Dawley rats. In a post hoc comparison of the effect of fluoxetine on food intake in the current experiment (Sprague Dawley rats) and that reported by Uphouse et al. for Fischer female rats [60], there were significant main effects of treatment and strain (all

$P \leq 0.0001$) but not their interaction. Food intake normalized after 10 days of fluoxetine treatment in Sprague Dawley females (current experiment) while, in Fischer rats, food intake was suppressed for at least 16 days [60]. The loss in body weight was also slightly less robust (females maintained approximately 95% of starting weight throughout) in the Sprague Dawley rats (current study) compared to that seen earlier in Fischer female rats (maintaining approximately 90% of body weight) [60]. Similar to findings for food intake, post hoc statistical comparison of the two experiments led to significant treatment and strain ($P \leq 0.0001$) effects but not their interaction. However, since Sprague Dawley female rats also have a heavier starting body weight compared to Fischer female rats, Sprague Dawley rats may be able to maintain estrous cyclicity in spite of the decline in body weight and food intake during fluoxetine treatment.

In addition to the study of body weight, food intake, estrous cyclicity and sexual receptivity, we also examined the effects of fluoxetine on male preference (an established model for assessment of sexual motivation [1,9]). After 10 days of fluoxetine (and 30 min after the daily treatment), there were no differences between fluoxetine- and vehicle-treated females in male preference. Similarly, Sarkar et al. [52] found no effect of fluoxetine on male preference in ovariectomized, Fischer rats after hormonal priming with estrogen and progesterone. These findings differ from that of Matuszczyk et al. [34] who reported decrements in sexual motivation after only 7 days of fluoxetine in Wistar rats. The explanation for these different findings is currently unclear.

Overall, these findings allow the suggestion that Sprague Dawley female rats may be less sensitive to the effects of fluoxetine on estrous cycle disruption compared to Fischer rats. Fluoxetine reduced food intake and body weight in Sprague Dawley rats while producing only modest effects on estrous cyclicity and sexual behavior, but such effects did occur. Thus, these findings reinforce the hypothesis that disruption of estrous cyclicity (and a presumed neuroendocrine disturbance) contributes to SSRI-induced sexual dysfunction and reinforce the importance of including multiple strains in the study of neurally active compounds. A major problem with any animal model for the study of human disease is that the effects of pharmacological intervention may differ in individuals with and without the disease. Fischer rats, with their hyperfunctional HPA axis and greater propensity for development of “depressive-like” behaviors [24], may be a better strain than the more commonly used Sprague Dawley strain for study of SSRI-induced sexual dysfunction.

4.0 Experimental Procedures

4.1 Materials

Fluoxetine hydrochloride was purchased from Sigma/Aldrich Chemical Company (St. Louis, MO). Rat food (Harlan Teklad Rodent Diet) was purchased from Harlan Teklad (Madison, WI). Suture materials were purchased from Butler Company (Arlington, TX). All other supplies came from Fischer Scientific (Houston, TX).

4.2 Animal and Housing Conditions

Adult female Sprague Dawley rats were purchased from Charles River Laboratories (Wilmington, MA). After arrival, rats were housed 2–3 per cage in polycarbonate cages ($45.72 \times 24.13 \times 21.59$ cm) with food and water available *ad libitum*. The animal facility rooms were maintained at 25°C and 55% humidity. Rats were housed in a 12 hr-12 hr light/dark cycle with lights off at 12 noon. After a two-week acclimation to the animal facility, rats were housed with *ad libitum* access to food and water, and their vaginal smears were monitored daily for at least two complete estrous cycles as previously described [60]. The relative proportion of nucleated cells, cornified cells, or leukocytes in the vaginal smear were used to determine the

stage of the cycle. Smears with clusters of nucleated cells with some cornified epithelial cells but an absence of leukocytes were designated as proestrous smears. The presence of predominantly cornified cells with some nucleated cells, but no leukocytes, were designated as estrous smear. Leukocytes, present alone or with few nucleated cells and cornified cells, were designated as diestrous smears.

4.3 Experiment 1

4.3.1 Housing and treatments—Rats were individually housed for two weeks prior to initiation of injections. Thereafter, they were injected daily with 10 mg/kg fluoxetine (in distilled water) or the water vehicle (1 ml/kg). Injections began on the day of diestrus 1 (1 day after a fully cornified smear) and continued for 24 days. Body weight, vaginal smears, and food intake were monitored daily between 1 and 4 pm. Each day, a premeasured quantity of food was placed in the food bin and the amount remaining was determined the following day. The difference was taken as the rat's 24 hr food intake.

4.3.2 Measurement of sexual behavior—Sexual receptivity was measured on the predicted day of proestrus. Testing of female sexual behavior took place between 1:00 and 4:00 pm as previously described [60]. Females to be tested were moved from their home cage and were placed in a covered black cage to avoid light exposure; females then were moved to the testing room where the males were housed. Because we did not want to disturb the female's cycle, the male was not allowed to complete mating. Instead, the presence or absence of a lordosis reflex in response to the male's first mount was determined and females were rated as either receptive or nonreceptive.

On the final day of predicted proestrus, near the termination of the experiment, the female was left in the male's cage for 10 mounts or a maximum of 10 min. On this final test, sexual receptivity was quantified as the lordosis to mount (L/M) ratio (number of lordosis responses divided by the number of mounts by the male) as previously described [60]. The experiment was then terminated.

4.4 Experiment 2

4.4.1 Treatment of animals and measurement of sexual receptivity—Female, Sprague Dawley rats were housed 2 per cage and their vaginal smears were monitored as described in experiment 1. Thereafter, rats were injected daily with 10 mg/kg fluoxetine or water for 9 days. Body weight and vaginal smears were monitored daily between 10 and 12 am. The second day the female exhibited a proestrous smear after initiation of drug or vehicle treatment, the female did not receive the daily injection that morning but was transported to the male's housing area between 1 and 4 pm. Sexual behavior was monitored for 10 mounts by the male or for a maximum of 10 min. L/M ratios and lordosis quality were monitored as previously described [60]. Proceptive behavior (measured as the number of hops and darts) [3] was also recorded. Immediately after testing for sexual receptivity, females were injected with 10 mg/kg fluoxetine or vehicle and transported to a separate room for measurement of partner preference. After partner preference testing, females were returned to their home cage but received no further injections. Vaginal smears continued to be monitored for five to six days after discontinuation of daily injections.

4.4.2 Partner-Preference Testing—Thirty min after the fluoxetine or vehicle injection, partner preference was monitored for 10 min in a clear plexiglass box (91.44 × 31.75 × 31.12 cm) as previously described [52]. At the ends of the apparatus were two compartments (each 16.51 cm 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 12.7, 14.48, 6.35, 14.48, and 12.7 cm. The female was placed into the center (6.35 cm center area) of the apparatus and allowed to move freely throughout the apparatus for 10 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 12.7 cm 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 6.35 cm center area. Each videotape was scored by two 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.

4.5 Data analysis

The L/M ratio, the presence or absence of receptivity or proceptivity, and resistive behavior were compared by the non-parametric Mann-Whitney U test or Fischer's exact test. Body weight and food intake data were analyzed by repeated measures ANOVA followed by Tukey's test for comparison of individual means. The total number of crossings, seconds of grooming, TWM, TST, and male preference scores were compared by Student's t-test. Statistical tests were performed with SuperANOVA (v1.11), StatView 4.5 from Abacus Concepts, or SPSS 15.0 for Windows. The statistical reference was Zar [64].

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Figure 1. Percentage of female Sprague Dawley rats showing a proestrous smear after daily fluoxetine treatment

Intact, regularly cycling Sprague Dawley female rats were injected with 10 mg/kg fluoxetine (1 ml/kg) or an equivalent volume of distilled water beginning at diestrus 1 and continuing for 24 days. Data are the percentage of rats showing a proestrous smear in each of the treatment groups. Data were grouped into 5-days intervals for presentation. Control animals showed regular cyclicity throughout the experiment. In contrast, 6 out of 15 fluoxetine-treated rats showed an elongated cycle at least once during the experiment.

Figure 2. Percentage of female Sprague Dawley rats showing sexual receptivity during daily fluoxetine treatment

Data are for the same fluoxetine-treated females shown in Figure 1. Data are the percentage of females that showed sexual receptivity coincident with a proestrous smear. N's for each of the 5-day intervals after injection with fluoxetine are 12, 12, 10, 12, and 12, respectively, but do not necessarily include the same set of animals during each 5-day interval.

Figure 3. Body weight and food intake of female Sprague Dawley rats during daily treatment with fluoxetine

Intact, regularly cycling female rats were injected daily with 10 mg/kg fluoxetine (1 ml/kg) or an equivalent volume of distilled/deionized water. Day 0 indicates body weight the day before the first injection; day 1 is the first day of injection. Body weight is shown in 3A and food intake is shown in 3B. Data are the mean $\pm$ S.E. body weight or food intake (in grams) for 15 fluoxetine and 15 control rats for each indicated day. In 3B, errors were so small that they are not always visible on the graph. On day 23–24, data are for 12 fluoxetine and 13 control rats. Since body weight was measured prior to injection, body weight is shown for one day more than is shown for food intake. The single asterisk indicates days on which there was a significant

decrease in body weight or food intake in the fluoxetine-treated rats relative to their starting weight.

TABLE 1

Cycle Lengths of Female Rats after Treatment with Fluoxetine or Vehicle for 24 Days.

Treatment	N	Pretreatment Mean Cycle Length $\pm$ S.E.	During Treatment Mean Cycle Length $\pm$ S.E.	During Treatment Median Cycle Length (range)
Vehicle (water 1 ml/kg)	15	4.40 $\pm$ 0.11	4.20 $\pm$ 0.07	4.00 (4.00–5.00)
Fluoxetine (10 mg/kg)	15	4.40 $\pm$ 0.10	5.20 $\pm$ 0.46	4.30 (4.00–9.30)*

Data are the estrous cycle lengths for water- and fluoxetine-treated rats before and during treatment with fluoxetine or vehicle. The single asterisk indicates significant difference between the median values for fluoxetine and vehicle-treated rats (Mann-Whitney U).

TABLE 2

Lordosis Behavior of Female Rats after Repeated Treatment with Fluoxetine

Treatment	N	Lordosis/Mount Ratio (Mean $\pm$ S.E.)	Lordosis Quality (Mean $\pm$ S.E.)
Vehicle (1 ml/kg)	13	0.86 $\pm$ 0.08	2.98 $\pm$ 0.08
Fluoxetine (10 mg/kg)	12	0.86 $\pm$ 0.06	2.97 $\pm$ 0.02

Data are the lordosis/mount ratios and lordosis quality for rats tested on proestrous of the final week (after 20–23 days of treatment) prior to completion of the experiment. Rats that failed to exhibit a proestrous smear during the last five days of the experiment were not tested. There was no significant difference in lordosis behavior between the two groups.

TABLE 3

Cycle Lengths of Female Rats after Treatment with Fluoxetine or Vehicle for Ten Days

Treatment	N	Pretreatment Mean Cycle Length $\pm$ S.E.	During Treatment Mean Cycle Length $\pm$ S.E.	Post-Treatment Mean Cycle Length $\pm$ S.E.
Vehicle (water 1 ml/kg)	6	4.55 $\pm$ 0.24	4.55 $\pm$ 0.24	5.62 $\pm$ 0.9
Fluoxetine (10 mg/kg)	15	4.33 $\pm$ 0.11	4.45 $\pm$ 0.22	5.27 $\pm$ 0.30

Data are the average estrous cycle lengths for 6 water and 15 fluoxetine-treated rats before injections, during 10 days of fluoxetine or vehicle treatment, and for 6 days after discontinuation of injections.

TABLE 4

Sexual Behavior in Female Rats Treated Subchronically with Fluoxetine

BEHAVIOR	WATER (Mean± S.E.)	FLUOXETINE (Mean ± S.E.)
N	6	15
Number (%) Allowing Mounts	5 (83.3%)	14 (93.3%)
Mean ± S.E. Number of Mounts	8.3 ±4.0	6.2 ± 3.9
Number (%) Showing Lordosis	5 (83.3%)	9 (60.0%)
Number (%) Showing Proceptivity	5 (83.3%)	8 (53.3%)
Number (%) Resistive	1 (16.6%)	7 (46.6%)

Data are for rats tested with a sexually active male for 10 min prior to the 10th daily injection with vehicle or fluoxetine.

TABLE 5**Male Preference Testing after Fluoxetine Treatment**

BEHAVIOR	WATER (Mean $\pm$ S.E.)	FLUOXETINE (Mean $\pm$ S.E.)
N	5	12
Time with Male	290.2 $\pm$ 73.0	398.5 $\pm$ 39.9
Time with Female	151.9 $\pm$ 83.0	110.5 $\pm$ 37.4
Social Time	442.1 $\pm$ 53.2	509.1 $\pm$ 17.8
Male Preference Score	0.67 $\pm$ 0.13	0.78 $\pm$ 0.07
Crossing	20.7 $\pm$ 7.7	7.8 $\pm$ 1.47*
Grooming	73.1 $\pm$ 24.0	28.3 $\pm$ 7.46*

Data represent seconds of behavior during 10 min in the male preference apparatus. Data were monitored 30 min after the 10th daily injection with water or 10 mg/kg fluoxetine.

* indicates significant difference between the means (Student's t-test, $P \leq 0.05$).