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USE OF AN OPERANT PARADIGM FOR THE STUDY OF ANTIDEPRESSANT-INDUCED SEXUAL DYSFUNCTION

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Abstract

These studies were designed to develop a paradigm for detection of antidepressant-induced sexual dysfunction in female rats. Ovariectomized, Fischer rats were conditioned to nose poke to open a guillotine door to gain access to a sexually active male. To develop the procedure, we examined the acquisition and stability of the response with a 15 s fixed interval, compared rats treated with 10 µg estradiol benzoate and 500 µg progesterone to those that received only estradiol benzoate, and performed a preliminary analysis of effects of 5, 10 and 15 mg/kg fluoxetine. We then more fully evaluated the effects of 5 mg/kg fluoxetine. Fluoxetine reduced sexual motivation as assessed by the number of nose pokes, number of nose poke episodes, and by the latency to approach the male. In addition, changes in the female's sexual motivation were examined before and after ejaculation during the final conditioning trials. The number of nose pokes was reduced and the latency to initiate a new nose poke episode was increased following ejaculation. The robustness of the antidepressant-induced decline in sexual motivation is in marked contrast to findings with several other animal models for sexual dysfunction and illustrates the usefulness of the operant procedure.

Keywords

sexual motivation; fluoxetine; SSRI; hypoactive sexual desire; operant; chamber; nose poke response; female; rat

Introduction

Depression is a world-wide problem affecting approximately 121 million people (Warden et al. 2007). Currently available antidepressants vary in purported mechanism of action, but most lead to some degree of sexual dysfunction (Gregorian et al. 2002; Montgomery et al. 2002; Schweitzer et al. 2009; Segraves 2007). Women are 2–3 times more likely to develop depression (Gorman 2006; Hildebrandt et al. 2003; Shifren et al. 2008) and antidepressant-emergent sexual dysfunction is more severe in women than in men (Clayton et al. 2007;

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Frohlich and Meston 2000; Segraves 2007; Shen and Hsu 1995) and is often responsible for patient noncompliance in continuing treatment (Clayton et al. 2007; Clayton 2002; Nurnberg et al. 2008; Segraves 2007). However, few effective options are available for treating antidepressant-induced sexual dysfunction.

In spite of the high prevalence of sexual dysfunction following antidepressant treatment, there are surprisingly few studies with animal models with which effective therapeutic interventions might be identified. In humans, female sexual behavior can be broadly grouped into low desire/arousal, orgasmic difficulties or dyspareunia (Segraves 2007; Shifren et al. 2008) with low sexual desire and/arousal (libido) being the most common complaints following antidepressant treatment (Clayton et al. 2007; Segraves 2007; Shifren et al. 2008).

Unlike humans, female rodent sexual activity is dependent on gonadal hormones (McEwen and Parsons 1982; Pfaff 1970; Sodersten 1981) and includes multiple behaviors that are generally categorized as appetitive (grooming, excitement, motor activation), precopulatory (proceptivity, solicitation), and consummatory (receptivity, lordosis) (Pfaus et al. 2003). Solicitation/appetitive behaviors allow the female to obtain proximity to the male (interest/arousal). Proceptive behaviors engage the male's attention on the female (interest/arousal). Receptive behavior includes the actual act of copulation (lordosis; genital stimulation). Defensive/resistive/avoidance behaviors (reminiscent of aversion to sexual activity) terminate the mating sequence and/or reduce the frequency of mounts and intromissions by the male.

Selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine are associated with the greatest prevalence of sexual dysfunction in humans (Montgomery et al. 2002; Outhoff 2010) and have been the most investigated in animal models (Uphouse 2014). However, in a majority of such studies, only the consummatory response (e.g. lordosis behavior) has been examined. This is unfortunate given the predominant complaints regarding sexual desire/arousal in humans, which may be better regarded as decrements in appetitive and precopulatory systems. Although animal models of female sexual motivation have been developed that are effective in differentiating hormonally-primed from nonhormonally-primed ovariectomized rats (Clark et al. 2004; Erskine 1989; Pfaus et al. 1999), such models have been surprisingly insensitive to the effects of antidepressants such as SSRIs (Sarkar et al. 2008; Uphouse 2014).

Operant approaches are typically considered to be a standard approach to measuring appetitive and motivational behavior, but they have received little emphasis in the area of female sexual motivation (Agmo 2014; Matthews et al. 1997). However, Matthews and colleagues (1997) required female rats to perform a nose poke response to gain access to a male where sexual activity could occur. With this approach, the authors were able to demonstrate that sexual activity was reinforcing. More recently, Cummings and Becker (2012) described a variation of this procedure for investigating female sexual motivation. In the paradigm used by Cummings and Becker (2012), females were trained to make nose pokes to open a door to gain access to a sexually active male. When ovariectomized females were hormonally primed with estradiol benzoate and progesterone and tested with a fixed

interval (15 s) schedule, they exhibited more nose pokes during the 15 s interval than did ovariectomized females without hormonal priming. With this paradigm, the authors showed that appetitive sexual behavior, as measured by the operant nose poke, was sensitive to hormonal priming. Thus, the paradigm holds promise for investigating a broader range of manipulations that impact sexual motivation. In this preliminary report, we have developed a similar paradigm and used it to evaluate the effect of the SSRI, fluoxetine. It was hypothesized that fluoxetine would reduce the female's sexual motivation to access the male and that this would be shown by fewer nose pokes and by an increasing latency to approach the male.

Methods

All procedures were in accordance with PHS guidelines and were approved by the IACUC at Texas Woman's University and the IACUC at the University of North Texas.

Subjects

Eighteen female Sasco Fischer rats and 18 male Sprague-Dawley rats were used in the experiments. Rats were purchased from Charles River Laboratories (Wilmington, MA). Males were between 100 and 150 days of age when their conditioning was initiated. Females were approximately 80 days of age when initiated into the study.

Apparatus—The apparatus was a 41 cm high $\times$ 55 cm wide $\times$ 30.5 cm deep operant chamber divided into two compartments (41 cm high $\times$ 22 cm wide $\times$ 30.5 cm deep or 41 cm high $\times$ 33 cm wide $\times$ 30.5 cm deep) by a guillotine door (see Figure 1). The female was initially placed in the smaller compartment (female compartment) where she could make the operant response; the male was tethered in the larger compartment (male compartment). The smaller compartment was equipped with two nose poke detectors to measure operant, or goal-directed, behavior; each detector was located inside the 4-cm nose poke hole, the center of which was 5 cm above the chamber floor. The centers of the holes were spaced 10 cm apart. Infrared photoemitter-detector pairs were placed across the middle of the hole; a nose poke was defined as any break in the beam that lasted at least 30 ms. Each hole could be lit via a yellow LED located 10 cm inside the hole. Only one hole was active during the study; responses on the inactive hole were used to assess general exploratory behavior.

The smaller compartment was separated from the larger compartment via a Plexiglas guillotine door., which could be raised by a DC gear motor and custom-built pulley system; the time to raise or lower the door required approximately 500 ms. When open, the entrance way measured 8 cm wide and 10 cm high and provided access between the two compartments. A female rat introduced into the smaller compartment could open the door to gain access to the male by successful nose pokes on the active hole. A sexually active male rat was tethered in the larger compartment that served as the goal box. The default state of the door was closed when the female was introduced to the chamber. Nose pokes that satisfied the response requirements would open the guillotine door, allowing the female to pass between compartments. The location of the female could be tracked by sets of infrared detectors located on either side of the door. Due to their tethers, male rats could not cross into the operant side of the chamber. The tether was a custom-made plastic harness that

connected the male to the ceiling via a flexible 11 cm steel chain leash. Programming of experimental events and data collections were accomplished by a data acquisition card (USB-6009, National Instruments, Austin, TX, USA) and custom software written in Labview™ (National Instruments, Austin, TX, USA).

Surgical procedures and treatments: Sasco Fischer female rats, obtained from Charles River Laboratories (Wilmington, MA), were ovariectomized under AErrane® anesthesia 2–4 weeks after their arrival to the facility, as previously described (White and Uphouse 2004). Two weeks later, females were hormonally primed with 10 µg estradiol benzoate and 48 h later with 500 µg progesterone for training in the operant paradigm. Hormones were administered s.c. in a volume of 0.1 ml/rat. Fluoxetine was dissolved in deionized water and was administered i.p. in a volume of 1.0 ml/kg, except for the 15 mg/kg dose which was administered in a volume of 1.5 ml/kg.

Training of females in the operant chamber—Hormonally-primed, ovariectomized rats were conditioned to nose poke in order to open a guillotine door to gain access to a sexually active male. In the shaping process, the investigator manually opened the door when the female's investigation of the chamber led her to the vicinity of the active nose poke hole. Females rapidly learned the relationship between the door and compartment entry; the female would try to open the guillotine door by nosing and pawing and, when unsuccessful, she would direct her attention toward the areas around the door. The door was opened as she oriented toward and moved toward the nose poke hole. During successive shaping intervals, the investigator required the female to come closer and closer to the hole until she initiated the nose poke response on her own. The complete procedure for training females involved: (a) week 1: condition female to chamber with free access to the male, i.e. the door was always raised and nose pokes produced no programmed consequences; (b) weeks 2 and 3: hand-shape the female to make one nose poke to open the door to gain access to the male in the male's chamber; (c) weeks 4 and 5: hand-shape the female to make an increasing number of nose pokes to gain access to the male; (d) weeks 6 and 7: introduction of a response-initiated interval where the female's first nose poke began a 15-s delay; the first nose poke at the end of the delay opened the door to gain access to the male's compartment. One or two additional weeks of training were given if necessary until females made a minimum of 3 nose pokes per episode, but this was only necessary during the procedure development phase. Training was never more frequent than weekly and generally occurred once per week on the same day of the week. During the shaping to produce a single nose poke response to open the door to the male, females experienced an average of 7.4 ± 0.11 (range of 3–15) shaping trials per session. During shaping to a 15-s delay, the females averaged 6.0 ± 0.75 episodes (range of 3–10) per session. If a female failed to open the door during test days, she was given a maximum score of 300. Similarly, if the female opened the door but did not cross to the male's chamber, she was given a score of 300 s.

The female was always free to return to the operant compartment. If the female re-entered the smaller operant compartment, the door was closed behind her and she could reopen the door by completing the response requirement again. With the exception of the first session (which lasted 30 min), training sessions continued for 15 min. A male was always present

during training and the female was paired with the same male for all training. All training was with 10 µg estradiol benzoate and 500 µg progesterone priming.

There was no requirement that the male/female pair copulate during training but only that the female enter the male's chamber and interact with the male. The session was terminated when the female re-entered the female's chamber within 30 s of the end of the timed session. Nevertheless, females usually did receive an ejaculation during the conditioning trials. To prevent negative conditioning of the male, if the male failed to ejaculate with the experimental female by the time the session had ended, the male was left in the chamber with a non-experimental, estradiol benzoate and progesterone primed female until ejaculation occurred. In this case, the chamber door was closed and the non-experimental female remained in the male's chamber.

Conditioning of males: Sexually experienced males were adapted to the tethering process through successive stages of (a) adaptation to a collar; (b) adaptation to a leash; and (c) adaptation to being tethered in the corner of the apparatus. The collar consisted of a small plastic harness constructed from a plastic cable tie that was placed around the male's neck and secured. One to two days of training (depending on the male) were required before the male easily allowed attachment of the collar. Once the male accepted the collar, he was allowed to mate, collared but not tethered, with a non-experimental, receptive female. This mating occurred in a Plexiglas box separate from, but similar to, that of the experimental chamber. If the male readily mated, he was moved to the next phase of his adaptation. First the male's collar was attached to an 11 cm chain with a small swivel hook on either end. One end of the chain was attached to the collar and the other end was attached to a hook in the upper right hand corner of the Plexiglas training box. The male was left alone in the apparatus for approximately 5 min while he became accustomed to being tethered. Thereafter, a non-experimental, receptive female was introduced to the apparatus and the male was allowed to mate. The introduction of the female calmed the male but was not always accompanied by mating. If mating did not occur within 15 min, the tether was removed and the male was allowed to mate until he ejaculated. This tethering process continued until the male (tethered to the corner hook) readily initiated mating upon introduction of the female. Mating was allowed to continue until the male ejaculated. At least two successive such ejaculation days while the male was tethered were required before he was moved to the actual experimental chamber for one additional day of adaptation with ejaculation.

Procedural development: Seven ovariectomized rats were used for development of the procedures. During conditioning, rats were always hormonally primed with estradiol benzoate and progesterone. After they were trained to produce the nose poke response, we initiated training with a 15-s delay between the first nose poke and the nose poke that would open the door to the male. After conditioning as described above, rats were then given a two-week washout period. The following week, the same rats were hormonally primed with 10 µg estradiol benzoate, only, and were tested. One week later, a pilot examination of the effects of fluoxetine was conducted in rats hormonally primed with estradiol benzoate and progesterone. Two rats received 15 mg/kg fluoxetine; two rats received 10 mg/kg fluoxetine;

and 3 received 5 mg/kg fluoxetine. A before-and-after paradigm was used with females given a 5 min test and then injected with fluoxetine. Testing occurred 30 min after the fluoxetine injection.

Effects of 5 mg/kg fluoxetine—Eleven rats were used in this experiment. After females reached a stable level of performance during testing with a 15-s delay, a within-subjects design was used with eight females examined before and after fluoxetine treatment. On the day of testing, females that were hormonally primed with 10 µg estradiol benzoate followed 48 h later with 500 µg progesterone were tested for 5 min in the chamber and their behavior was recorded. After the 5 min, eight females were removed and injected with 5 mg/kg fluoxetine. Thirty min later, females were returned to the chamber for an additional 5 min test. Data were compared before and after fluoxetine treatment on the following parameters: (1) number of nose pokes per 15-s episode; (2) number of episodes; (3) total number of nose pokes per 5-min interval; and (4) time to enter the male's compartment after the door opened. If the female failed to open the door or enter the male's chamber, she was given a score of 300 s for each parameter.

Three animals were tested before and after a water injection to determine if the injection, alone, affected the behavior. Females were tested for 5 min and then injected with deionized water. Thirty min later, females were returned to the chamber for a 5 min post-test as for the fluoxetine treatment.

Effects of ejaculation during conditioning of rats used for the 5 mg/kg fluoxetine study:

Data during training sessions were evaluated for (1) the number of nose pokes performed to open the door, (2) the time to initiate a nose poke episode, and (3) the latency to cross to the male's chamber after the door had opened. Although not originally planned in the experimental design, we examined these parameters from final training sessions to compare the female's behavior before and after ejaculation. One rat received two ejaculations in the same session and both were included in the analysis. Data were compared for the episodes before and after ejaculation.

Drugs and materials

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

Statistical Analysis

A session was defined as a day of testing. In most cases, the following parameters were assessed within sessions: (a) time to open = time to open the guillotine door; (b) time to cross = time to enter the male's chamber after the door had been opened; (c) episodes = number of sequences of entry into the male's chamber; (d) average number of nose pokes = average number of nose pokes per episode; (e) total nose pokes = number of nose pokes summed across episodes. In the case of the evaluation of the data for ejaculation effects,

time to initiate the next nose poke (time to begin nose poking after returning to the male's chamber) was also measured.

Parametric data were analyzed by t-test for matched pairs (before and after fluoxetine or before and after water). Because most fluoxetine-treated females did not leave the female's chamber during the 5 min interval, data for time to leave the chamber after the door had opened were compared by nonparametric Wilcoxon procedures (Zar 2010).

Results

Procedure development

After the initial shaping, as indicated by their rapidity to open the guillotine door, rats varied in the degree to which they had successfully acquired the task. Two rats rapidly opened the door while five rats were slower to do so (see Figure 2). During additional sessions, behavior for all rats improved so that they were opening the door within approximately the first minute after placement into the chamber. By these final sessions, females made a majority of their nose pokes on the active rather than the inactive nose poke hole. The average number nose pokes per session in the inactive hole was 0.66 ± 0.4 (range of 0–2).

After training, rats were then given a two-week rest period to eliminate hormones before testing with just 10 μ g estradiol benzoate priming. Because training of females was on a staggered schedule, two females were still undergoing conditioning during this 2-week wash out period so that only 5 rats were included in the comparison. When compared to their behavior with estradiol benzoate and progesterone priming (Figure 3), rats primed only with estradiol benzoate took a significantly longer time to open the door ($t = 2.84$, $p = 0.025$) and were slower to enter the male's chamber. However, because of the performance variability, differences in time to cross to the male's chamber approached but did not reach statistical significance ($t = 2.09$, $p = 0.07$).

The following week, after all rats had completed their conditioning, rats were examined before and after injection with 5 ($n = 3$), 10 ($n = 2$) or 15 ($n = 2$) mg/kg fluoxetine (see Figure 4). When the data from 10 and 15 mg/kg fluoxetine were combined and compared to the 5 mg/kg dose, there was a clear dose-dependent effect of treatment on the time to open the door ($t = 7.19$, $p = 0.001$) but not on the time to cross. From this pilot study, 5 mg/kg fluoxetine was selected for further study.

Effects of 5 mg/kg fluoxetine

Bad on the variability females took to achieve a stable performance level, an additional conditioning trial was included in the shaping phase to assure that behavior during the 15-sec delay trials was successful (Table 1). Thereafter, 8 rats were tested before and after 5 mg/kg fluoxetine and 3 rats were tested before and after a water injection. Since the behavior of Fischer females has been shown to be affected by an i.p. injection (Uphouse et al. 2005), an independent group of rats was used for testing with the water injection.

Fluoxetine reduced female rat sexual motivation as assessed by number of nose pokes per episode, number of nose poke episodes, total number of nose pokes (nose pokes/episode

times number of episodes), and by latency to cross to the male (Figure 5 and Table 2). The average number of nose pokes per episode that females made during the 15-s delay interval was reduced by approximately 50% following fluoxetine treatment. As the number of episodes was reduced, the total number of nose pokes during the 5-min test interval was also reduced by fluoxetine. As expected from earlier studies showing effects of fluoxetine on time spent with a sexually active male in a pacing paradigm (Adams et al. 2012), fluoxetine also increased the female's latency to enter the male's chamber even after the door had opened. Six of the 8 females failed to ever enter the male's chamber after fluoxetine. Although there was no obvious evidence that females treated with the low dose of 5 mg/kg fluoxetine had any motor disturbance, we directly tested this possibility by manually moving the female to the male's chamber at the end of the 5 min test. Every female rapidly (within 5 s) left the male's chamber so we are confident the female's lack of entry into the male's chamber was not a result of any motor disturbance.

Data for rats injected with water are shown in Table 3. Unlike the fluoxetine treatment, the female's behavior was not changed by the water injection (all $p > 0.05$).

In a final analysis of the training data, we examined the female's behavior during final training sessions for the episode before and the episode after an ejaculation had taken place. Data before and after ejaculation are shown in Table 4. After the male's ejaculation, females took longer to initiate the next episode of nose pokes and exhibited a fewer number of nose pokes during the 15-sec delay interval. The most robust change was in the female's latency to initiate a new nose poke episode.

Discussion

To our knowledge, this is the first report of an attempt to quantitatively evaluate antidepressant-induced decrements in female rat sexual motivation with an operant model. Attempts to demonstrate antidepressant-induced decrements in female sexual motivation in animal models have been surprisingly disappointing. The current findings allow the suggestion that the nose poke paradigm initially described by Cummings and Becker (2012) offers a promising approach for the study of antidepressant-emergent sexual dysfunction.

During development of the paradigm, variability in female performance was initially large, but after further training, all females were successfully performing the nose poke response to open the door to the male. We first compared rats treated with estradiol benzoate and progesterone versus treatment only with estradiol benzoate. This comparison was important in validating the operant model in the Fischer inbred strain. A dose of 10 μ g estradiol benzoate, alone, leads to high levels of lordosis behavior in Fischer females. However, proceptivity (generally viewed as evidence of the female's motivation to mate) does not typically occur without the progesterone priming. As rats given only estradiol benzoate priming evidenced lower responses indicative of sexual motivation (e.g. time to open the door and time to enter the male's chamber after the door had opened), the paradigm was able to successfully differentiate the two types of hormonal priming. This differentiation is consistent with the hormonal conditions required for proceptivity in the Fischer inbred rat.

When different doses of fluoxetine were examined in a preliminary dose response analysis, motivational behaviors were reduced by all doses of fluoxetine. From these preliminary data, we chose 5 mg/kg fluoxetine as a dose with which to expand these preliminary studies.

In general, the current evidence with fluoxetine is consistent with studies in which fluoxetine has been shown to reduce female rat lordosis behavior and proceptivity (Miriyala et al. 2011; Sarkar et al. 2008; Ventura-Aquino and Fernandez-Guasti 2013). The findings are also consistent with prior studies in which fluoxetine was reported to reduce the female's exit from a compartment to interact with a sexually active male (Adams et al. 2012) and with one report for a decrease in preference for the male in the partner preference paradigm (Matuszczyk et al. 1998). However, in a more recent study, when fluoxetine was used in the partner preference model of sexual motivation, no effect of fluoxetine was seen (Sarkar et al. 2008). In fact, in the majority of studies with SSRI antidepressants, there has been little evidence that the more traditional animal models of sexual motivation discriminate between antidepressant-treated and control rats (Kaspersen and Agmo 2012; Snoeren et al. 2011a,b). For example, in our laboratory, we used the partner preference paradigm to examine hormonally primed, ovariectomized Fischer females given a single or multiple treatments with fluoxetine (Sarkar et al. 2008). The female's preference for spending time near the male incentive was not reduced by fluoxetine even under conditions where the antidepressant significantly reduced lordosis behavior. Thus, the current paradigm may be more suitable for detection of SSRI-induced sexual motivational deficits than the partner preference paradigm.

There may, therefore, be advantages to the current paradigm over the more commonly used animal models of female rat sexual motivation. Most important, the paradigm allowed a robust differentiation between fluoxetine-treated and control rats at a relatively low dose of fluoxetine that has been shown to have only minor effects on locomotor or consummatory (e.g. lordosis) behavior in Fischer rats (Miriyala et al. 2011). In addition, the paradigm offers the opportunity to obtain trial-by-trial behavioral assessment of the female's sexual motivation so that training effects could also be examined. Although it was not an original objective of the current project, we quickly observed that the female's behavior in training after ejaculation was different from that before ejaculation. When we examined behavioral parameters before and after ejaculation, the number of nose pokes and the time to initiate a nose poke declined after ejaculation. Such an apparent ejaculation-induced decline in the female's sexual motivation is consistent with studies using the pacing paradigm where females can control entry and exit from the male (Erskine 1989; Paredes and Vazquez 1999). This could also be an excellent paradigm for studying effects of longer-term antidepressant treatment. Moreover, if sufficient time were allowed for drug metabolism, the current paradigm could allow the same female to be subjected to multiple pharmaceutical treatments and/or a dose response effect within type of treatment. After the female acquired a stable response rate, the female's behavior remained relatively consistent even with as much as two weeks between testing.

Limitations of the paradigm include the long-term training that is required for females to acquire the nose poke response and to associate it with the ability to access the male.

However, this limitation may be offset by the potential for repeated testing within the same animal.

In summary, the operant conditioning paradigm shows considerable promise as a tool for studying female sexual motivation in general and for the study of SSRI-induced sexual dysfunction in particular. In spite of their potential for producing sexual dysfunction in humans, an SSRI-induced reduction in female sexual motivation has been difficult to demonstrate in animal models. Therefore, the robust effect of fluoxetine in the current paradigm is unique. Future studies will be required to determine if these robust outcomes can be extended to other SSRIs, such as citalopram/paroxetine, and if the paradigm is equally effective for other rat strains. Additionally, while emphasis in the current paper was on female sexual motivation, it has not escaped our attention that the paradigm could also be useful for studying male sexual dysfunction.

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Figure 1. Description of the conditioning apparatus

The front of the apparatus is shown in A with arrows indicating the active and inactive nose poke holes, the motor controlling opening of the door and the system controller. A close-up of the guillotine door separating the two chambers is shown in B with arrows indicating the Plexiglas door and the infrared detectors.

Figure 2. Behavior with 15sec fixed interval delay

Data are the mean - S.E. time to open the door during conditioning trials with the 15 sec fixed interval delay. Data are grouped to show the rats that reached the criterion during initial shaping ($n = 2$, HIGH) and those that required additional experience ($n = 5$, LOW). On the basis of these results, additional shaping trials were introduced to the methodology.

Figure 3. Comparison of behavior of rats after estradiol benzoate and progesterone or estradiol benzoate, only, hormonal priming

Data are the mean $\pm$ S.E. for time to open the door and time to cross to the male's chamber after hormonal priming with 10 μ g estradiol benzoate and 500 μ g progesterone (EP) or priming only with estradiol benzoate (EO). $n = 5$ /treatment. The asterisk indicates a significant difference between EO and EP rats on the respective parameter.

Figure 4. Preliminary dose response for fluoxetine

Data are the mean $\pm$ S.E. for time to open the door and time to cross to the male's chamber before and after treatment with 5 ($n = 3$), 10 ($n = 2$) or 15 ($n = 2$) mg/kg fluoxetine. If an animal failed to open the door, the female was given a maximum score of 300 s. Similarly, if the female opened the door but did not cross to the male's chamber, she was given a score of 300 s. The asterisk indicates a significant dose-dependent effect for time to open the door.

Figure 5. Effects of 5 mg/kg fluoxetine

Data are the mean $\pm$ S.E. for time to the number of nose pokes/episode and number of episodes before and after treatment with 5 mg/kg fluoxetine ($n = 8$). The asterisk indicates a significant difference before and after fluoxetine ($p < 0.02$).

TABLE 1**BEHAVIOR DURING LAST 3 TRAINING SESSIONS**

	N	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.
PARAMETER		SESSION 1	SESSION 2	SESSION 3
Time to open	11	24.90 (2.1)	25.78 (2.0)	19.25 (0.5)
Time to cross	11	7.20 (0.3)	8.90 (0.4)	10.50 (1.1)
Number of nose pokes/episode	11	4.80 (0.6)	4.90 (0.6)	5.30 (0.4)
Number of episodes	11	6.00 (0.7)	6.60 (0.7)	5.30 (0.6)

TABLE 2**EFFECTS OF FLUOXETINE INJECTION**

TIME	N	BEFORE FLUOXETINE	AFTER FLUOXETINE	STATISTICS
PARAMETER		Mean $\pm$ S.E.	Mean $\pm$ S.E.	
Time to open		22.00 $\pm$ 2.32	55.80 $\pm$ 24.6	p > 0.05
Time to cross	8	32.80 $\pm$ 9.33	247.80 $\pm$ 34.12	Wilcoxon test, p 0.012

TABLE 3**EFFECTS OF WATER INJECTION**

TIME	N	BEFORE WATER	AFTER WATER	STATISTICS
PARAMETER		Mean $\pm$ S.E.	Mean $\pm$ S.E.	
Time to open		19.80 $\pm$ 1.55	36.29 $\pm$ 19.25	p > 0.05
Time to cross	3	35.50 $\pm$ 10.70	50.36 $\pm$ 20.08	Wilcoxon test, p = 0.59
Number of nose pokes/episode	3	4.17 $\pm$ 0.60	3.33 $\pm$ 0.88	t = 1.1, p = 0.37
Number of episodes	3	3.66 $\pm$ 0.66	2.00 $\pm$ 0.577	t = 0.55, p = 0.63

TABLE 4**EFFECTS OF EJACULATION**

TIME	N	BEFORE EJACULATION	AFTER EJACULATION	STATISTICS
PARAMETER		Mean $\pm$ S.E.	Mean $\pm$ S.E.	
Time to open		43.24 $\pm$ 7.10	103.57 $\pm$ 22.93	t =2.49, p = 0.04
Time to cross	9	11.99 $\pm$ 3.06	18.80 $\pm$ 6.13	t = 1.33, p = 0.22
Number of nose pokes/episode	9	5.55 $\pm$ 0.29	3.44 $\pm$ 0.37	t = 3.92, p = 0.004
Time to initiate the next episode	9	14.66 $\pm$ 2.72	64.57 $\pm$ 16.13	t = 3.38, p = 0.01