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Acute and Chronic Fluoxetine Treatment Decreases the Sensitivity of Rats to Rewarding Brain Stimulation

KIRBY LEE AND CONAN KORNETSKY

Boston University School of Medicine, Departments of Pharmacology and Psychiatry, Boston, MA 02118

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LEE, K. P. AND C. KORNETSKY. *Acute and chronic fluoxetine treatment decreases the sensitivity of rats to rewarding brain stimulation.* PHARMACOL BIOCHEM BEHAV **60**(2) 539–544, 1998.—The effects of fluoxetine on rewarding brain stimulation were determined in eight Wistar rats using a rate-independent discrete-trial threshold measure. Rats were implanted with bipolar, stainless steel electrodes either into the ventral tegmental area (VTA) or medial forebrain bundle (MFB). Acute administration of fluoxetine significantly raised the reward threshold (decreased sensitivity) at doses of 2.5, 5.0, 10.0, and 20.0 mg/kg, IP, without altering latency of response. There were no significant differences between VTA and MFB groups. To determine the effects of chronic treatment, daily injections of 5.0 mg/kg fluoxetine were administered to rats for 21 days. Chronic treatment of fluoxetine continued to significantly elevate reward thresholds with no evidence of tolerance. The results of these experiments suggest that fluoxetine does not possess abuse potential and that serotonin produces an inhibitory effect on the mesolimbic dopaminergic reward system. Furthermore, these results suggest that the antidepressant effects of fluoxetine are not the direct result of excitation of brain reward systems, at least in the same manner as abused substances, for example, cocaine. © 1998 Elsevier Science Inc.

Serotonin Reward ICSS Dopamine Self-stimulation Substance abuse

FLUOXETINE (Prozac®) is currently the most widely prescribed drug for the treatment of depression in addition to its recent approval for use in treating obsessive–compulsive disorder and bulimia nervosa (51). Despite its well-known action as a selective serotonin (5-hydroxytryptamine, 5-HT) reuptake inhibitor (52), it is not clear whether the mechanism by which fluoxetine produces its antidepressant effects in the brain is a direct result of enhanced serotonergic neurotransmission or an interaction with other neurotransmitters. A large body of evidence supports an interaction between serotonin and dopamine. For example, it is well established that the mesolimbic dopamine system is the major neural substrate involved in rewarding behaviors that include intracranial electrical self-stimulation (ICSS), drug self-administration, feeding, and sexual behavior (8,10,25,26,49). These behaviors as well as powerful reinforcing drugs of abuse such as cocaine, heroin, and amphetamine enhance dopaminergic transmission from the ventral tegmental area (VTA) to the nucleus accumbens (NACC) (25,26). Drugs that positively affect this pathway either directly or indirectly, via modulation of other neurotransmitters, may also have abuse potential. Indeed, cases of abuse and misuse of fluoxetine by experienced substance abusers have recently been reported (16,47).

Previous investigations examining the interaction of 5-HT on the mesolimbic DA system employing ICSS have resulted in a complex picture. Acute administration of fluoxetine (5,24), the 5-HT precursor 5-hydroxytryptophan (5-HTP) (2), results in decreases in rates of responding to rewarding brain stimulation, suggesting an inhibitory role for serotonin. Likewise, Fletcher and colleagues (12) reported a lowering of ICSS thresholds after producing an inhibition in 5-HT cell firing via injections of the 5-HT_{1A} agonist 8-OH-DPAT into the median raphe nucleus. However, other studies using fluoxetine have resulted in no change (1,34), or increased rates of responding following direct perfusion of 5-HT into the brains of rats self-stimulating (43) or the administration of 5-HT agonists to the 5-HT_{1A} autoreceptor, which causes an inhibition of 5-HT cell firing (37). Yet those studies, which measure the

Requests for reprints should be addressed to Conan Kornetsky, Ph.D., Boston University School of Medicine, 80 East Concord Street, L-602, Boston, MA 02118.

rate of response as the dependent variable, may have confounded results due to the inhibitory function of 5-HT on locomotor activity and possibly operant responding in rodents (15). For example, systemic injections of fluoxetine and 5-HTP (44) or the local injection of 5-HT agonists directly into the NACC (42) decrease rat locomotor activity. Along this same line of reasoning, destruction of 5-HT neurons by the selective neurotoxin *p*-chloroamphetamine increase rat locomotor activity (36). In fact, 5-HT and various 5-HT agonists have been shown to attenuate the hyperlocomotor activity produced by amphetamine (4,20,32). To minimize the possible role of nonspecific effects of decreased motor activity, a rate-independent threshold determination for rewarding brain stimulation (27) was used to examine the effects of acute and chronic administration of fluoxetine.

The purpose of this experiment was to determine whether fluoxetine increases the sensitivity of rats to rewarding brain stimulation. Such a finding would suggest that the antidepressant effects of fluoxetine are not mediated by excitation of the brain reward system. In addition, because the antidepressant effects of fluoxetine usually occur 2–4 weeks after continued treatment in humans, and because previous studies examining chronic antidepressant treatment on ICSS revealed changes in responding corresponding to enhanced reward (9,35,48), the effects of chronic fluoxetine treatment (21 days) on brain stimulation reward was determined.

METHOD

Subjects and Surgical Procedure

Eight male Wistar rats (Charles River Laboratories, Inc., Wilmington, MA) weighing 300–400 g were anesthetized with pentobarbital (Nembutal®, 50 mg/kg) and chloral hydrate (160 mg/kg). Atropine (250 μg/rat) was given prophylactically to control secretions. Rats were stereotaxically implanted with bipolar stainless steel electrodes either into the lateral hypothalamic region of the medial forebrain bundle (MFB-LH, $n = 2$) or ventral tegmental area (VTA, $n = 6$). Electrodes 0.13 mm in diameter were implanted into the MFB-LH (4.0 mm posterior to bregma, 3.1 mm lateral from the midline suture, and 8.5 mm ventral to the skull surface at a 12° angle), whereas electrodes 0.2 mm in diameter were used to overcome increased resistance of brain tissue and to aid in the probability of implantation of the electrode tip at the level of the VTA (2.7 mm posterior to bregma, 0.7 mm lateral from the midline suture, and 8.8 mm ventral to the skull surface tilted upwards at an angle of 8° with respect to the horizontal). The electrodes were placed through small burr holes in the skull and attached permanently to the surface with surgical screws and cranioplast dental cement. Following surgery animals were administered 0.05 cm^3 Gentimicin® intramuscularly. Behavioral testing began approximately 1 week postoperative recovery. Animals were maintained on a 12 L:12 D cycle, tested during the light cycle, individually housed in stainless steel cages and had access to food and water ad lib.

Training and Testing Procedure

Animals were trained and tested in a plastic chamber (23 × 23 × 38 cm) with a wheel manipulandum (15 × 7.5 cm) mounted on one wall of the test chamber inside a sound-attenuating chamber (63 × 44 × 58 cm, MED Associates, St. Albans, VT). A biphasic symmetrical square-wave pulse was delivered by a constant current stimulator (MED Associates). Each stimulus consisted of a 500-ms train with a pulse width of 0.2 ms and a delay of 0.2 ms between the positive and negative pulses at a frequency of 160 Hz. Thresholds were determined by a rate-independent, discrete trial procedure involving the use of discrete trials systematically presented over a range of stimulus intensities (27). Presentation of a noncontingent stimulus (S1) signaled the availability of an identical contingent stimulus (S2) of the same intensity. Immediate delivery of S2 occurred when the wheel was rotated one-quarter of a turn within 7.5 s after the onset of S1. Current intensities were varied according to a modification of the psychophysical method of limits. Stimuli were presented in an alternating descending and ascending series with a step size of 3 μA and with five trials of each intensity in each series. The threshold value for each series was defined as the midpoint in microamperes between the level where the subject responded three or more times out of the five stimulus presentations (a plus score) and the level where less than three responses (a minus score) were made. The animal's estimated current threshold for each test session was the mean of the series' thresholds. Using this method, it has been clearly demonstrated that the obtained thresholds are independent of any motor effects (33).

Animals required approximately 10 1-h training sessions to learn the task and approximately five additional sessions for establishment of a stable threshold level. During an experimental session, the reward threshold was determined twice, consisting of a warm up preinjection and then postinjection session. No more than one experimental session was run per day. Criteria for a stable baseline consisted of individual thresholds not varying by more than 10% for 3 consecutive days. Drug challenges were given when criteria were met.

Drugs and Drug Treatment

Fluoxetine (Sigma, St. Louis, MO) was dissolved in 30–40°C distilled water and administered intraperitoneally in a volume of 1 ml/kg of body weight. Based upon the pharmacokinetics of fluoxetine (3,17,31,41), all injections of fluoxetine or the vehicle control of distilled water were administered 1 h prior to the test session no more often than once a week. Once a stable threshold was established, vehicle control injections were administered first, followed by a random sequence of doses of fluoxetine. In most cases, a specific dose was given to each animal only once. In the few cases where a dose was repeated, the average for the two treatments was used as the datum.

Statistical Analysis

For each animal, the dependent measure consisted of the threshold values calculated for the postinjection sessions. These values were transformed to standard scores (z-scores) based on the mean and standard deviation for all of the three consecutive baseline days prior to each challenge. A z-score of 11.96 described the 95% confidence level for individual animals. Paired t-tests were performed using z-scores to compare the acute and chronic effects of fluoxetine to vehicle. A two-way repeated-measures ANOVA on two factors was performed using z-scores to compare the values of thresholds obtained at weekly intervals during the chronic fluoxetine treatment.

Comparison of VTA and MFB electrode placement sites were conducted on the basis of mean baseline threshold values and mean z-score values of the doses tested for each group. Analysis was carried out using the nonparametric Mann–Whitney U-test.

Experiment 1

The acute effects of fluoxetine (1.25–20.0 mg/kg, IP) on the threshold for rewarding brain stimulation were determined as described above in eight Wistar rats.

Experiment 2

The chronic effects of fluoxetine on the threshold for rewarding brain stimulation was determined in five of the animals used in Experiment 1. The same BSR protocol and drug preparation were used as described above. Chronic administration consisted of 5.0 mg/kg fluoxetine injected IP once daily for 21 days. Thresholds were measured five days each week (Monday–Friday). To determine if tolerance to the acute administration of fluoxetine developed, animals were administered fluoxetine 1 h prior to the test session on Mondays, Wednesdays, and Fridays (pretest group). To examine the chronic effects of fluoxetine not influenced by the acute injection, vehicle was administered 1 h prior to the test session on Tuesdays and Thursdays, and fluoxetine was administered 5 min following the test session (posttest group).

Histology

At the completion of the experiments, animals were killed with an overdose of either pentobarbital or halothane. The brains were subsequently removed from the skull and immediately immersed in methyl butane at −35°C for 10 min. The brains were then stored at −86°C until ready to cut in a cryostat. Sections (20 μm) were cut, placed on slides, and stained with thionin. Mounted brain sections were examined under a light microscope to determine the placement of the electrode tips as verified by the atlas of Paxinos and Watson (40).

RESULTS

There were no significant differences in mean baseline thresholds or z-score values of the doses tested between VTA and MFB groups ($p = 0.21$, Mann–Whitney U-test).

Experiment 1

The mean across animals of the mean threshold for each animal across all 3-day baseline sessions was 71.5 μA. The mean of the individual standard deviations used to compute z-scores was 3.7 μA. As shown in Fig. 1, fluoxetine treatment resulted in a dose-dependent significant raising of the reward threshold. Seven of the eight original rats completed the experiment; one animal died prior to obtaining a vehicle control and, thus, was excluded. One rat was not tested at the dose of 1.25 mg/kg ($n = 6$), while another rat was not tested at the dose of 2.50 mg/kg ($n = 6$) due to loss of the electrode cap.

Treatment with fluoxetine frequently resulted in marked decreases in animals' locomotor and exploratory activity. At the highest dose of 20.0 mg/kg, a decrease in food intake and general lethargy was prominent in all of the animals following administration of fluoxetine. Despite this observed effect of fluoxetine, the latency to respond was unaffected. Figure 2 depicts the stability of latency to respond at threshold at each dose of fluoxetine. The mean of the mean latency to respond for each animal for all 3-day baseline sessions was 2.16 s. The mean of the individual standard deviations used to compute z-scores was 1.39 s. The available response time was 7.5 s. Also, there was no increase in intertrial responses at any dose of fluoxetine. Such an increase would indicate late correct responses. All animals were under stimulus control during drug test sessions.

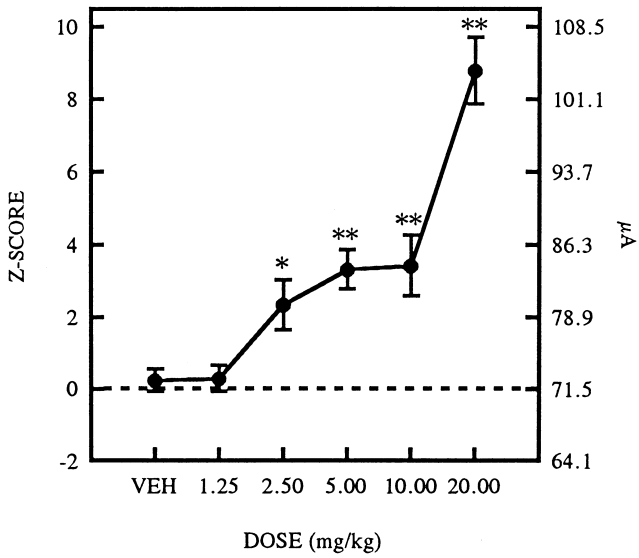

FIG. 1. Mean ± SEM z-score changes from baseline BSR thresholds as a function of the dose of fluoxetine. The right side of the figure gives the corresponding μA values. *$p < 0.05$ compared to vehicle control (z-score = 0), paired t-test analysis; *$p < 0.01$ compared to vehicle control (z-score = 0), paired t-test analysis.

Experiment 2

Figure 3 shows the mean ± SEM effects of 5.0 mg/kg fluoxetine administered intraperitoneally, daily for 3 weeks. The solid bars represent the weekly mean of the Monday, Wednesday, and Friday determination of the 5.0 mg/kg pretest dose. The nonsolid bars represent the weekly mean thresholds obtained on Tuesday and Thursday when daily fluoxetine treatment was not given until 5 min after completion

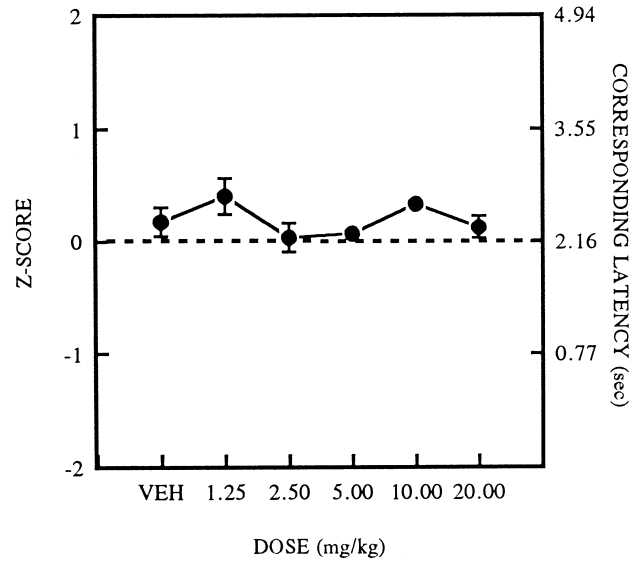

FIG. 2. Mean ± SEM z-score changes in latency of response at BSR thresholds as a function of dose of fluoxetine. A z-score ± 1.96 indicates the 95% confidence limits for individual subjects.

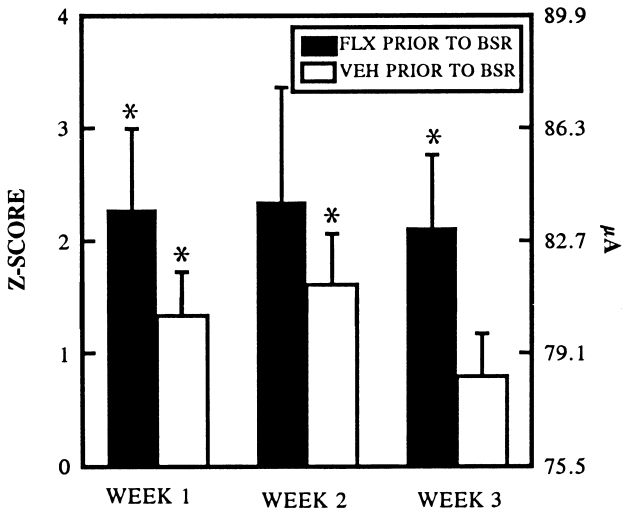

FIG. 3. Represented are the mean z-score changes from baseline in rats administered 5.0 mg/kg fluoxetine (FLX) daily for 21 days. On Monday, Wednesday, and Friday of each week, the daily dose of FLX was administered 1 h prior to threshold determinations (black bars). On Tuesday and Thursday of each week, rats were administered vehicle injections of distilled water 1 h prior to threshold determinations (white bars) and then the daily dose of FLX was administered 5 min after completion of testing. The right side of the figure gives the corresponding mA values. *$p < 0.05$ compared to baseline week (z-score 5 0), paired t-test analysis.

of the threshold determination. Due to a slight elevation in baseline thresholds following the acute study, a new baseline threshold was calculated based on a week of testing prior to chronic fluoxetine treatment. The mean across animals of the mean threshold for each animal across the 1-week baseline session was 7.55 μA. The mean of the individual standard deviations used to compute z-scores was 3.6 μA. Chronic treatment with fluoxetine continued to significantly raise thresholds (paired t-test, $p < 0.05$) in the pretest group at weeks 1 and 3 and the posttest group at weeks 1 and 2. It should be noted that the p level for both the pre- and posttest groups at weeks 2 and 3, respectively, was $p > .05 < 0.1$. A two-way repeated measures analysis of variance test on two factors revealed no significant differences or an interaction between each week of elevated levels of BSR thresholds during chronic administration in pre- and posttest groups.

All animals exhibited normal behavior through the duration of the 21-day chronic drug treatment. Food intake remained stable and there was no evidence of lethargy. Three animals received continued administration of fluoxetine 5.0 mg/kg beyond the 21 day chronic drug treatment. Two of these animals exhibited decreased food intake, lethargy, and diarrhea at days 22 and 24 of chronic drug treatment. At this point treatment was discontinued.

Histology

Histological verification of electrode placements was completed in seven of the eight animals used in these studies. For the remaining animal, it was not possible to verify electrode placement due to deterioration of the brain as a result of unexpected death over a weekend. However, there was no difference in this animal's ability to self-stimulate and obtain a stable baseline threshold. Therefore, all data was included in the study. For animals with implants into the VTA, all electrodes were found in the rostral aspect of the VTA. The electrode tip was lateral to the VTA for animal #3. Electrode tips were ventral to the VTA for animals #1 and #5. For animals with implants into the MFB, all electrode tips were found in the central aspect of the lateral hypothalamus. The electrode tip was located in the center of the MFB for animal #7 and medial to the MFB for animal #8.

DISCUSSION

The results clearly indicate that fluoxetine raises the threshold for rewarding brain stimulation, a finding that is in agreement with decreases in response rates previously reported by Katz and Carroll (24) and Cazala (5), suggesting that serotonin exerts an inhibitory influence on rewarding brain stimulation. Although a study by Matthews and colleagues (34), employing a similar procedure, report no significant effects of fluoxetine ($p = 0.07$) in rats, they did observe an elevation of reward thresholds. The differences in these results could be attributed to the strain of animals used and the time of pretreatment with fluoxetine. In the current study, Wistar rats were administered fluoxetine 60 min prior to behavioral testing, whereas Matthews and colleagues (34) administered fluoxetine to Sprague–Dawley rats 90 min prior to behavioral testing. In addition, the discrepant results reported by Andreev (1), in which fluoxetine failed to produce changes in rate of responding in rats, may be due to their procedure of testing the animals 4 h after administration of fluoxetine. Through microdialysis it has been shown that acute intraperitoneal injections of fluoxetine significantly increase extracellular levels of serotonin in the nucleus accumbens (17) and the striatum and hippocampus (31) of rats for approximately 2 h. Based on these studies and the pharmacological profile of fluoxetine and its active metabolite norfluoxetine, we tested our animals 1 h after administration of intraperitoneal injections of fluoxetine for a period of 45 to 90 min.

During the chronic regimen, there were significant elevations in reward thresholds after vehicle administration at weeks 1 and 2 (see Fig. 3). This effect is most likely due to the pharmacokinetics of fluoxetine and its active metabolite norfluoxetine (3) reaching steady-state plasma levels. Furthermore, our statistical analysis in Fig. 3 sums the mean across animals of the mean threshold for each animal during those 2 days of the week (Tuesdays and Thursdays) in which vehicle was administered prior to behavioral testing.

A strong positive correlation exists between elevated levels of dopamine and enhanced central reward mechanisms of the mesolimbic dopamine system that originates in cells of the ventral tegmental area (VTA) and project to the nucleus accumbens (NACC), (8,25,26). It has been proposed that fluoxetine's mechanism of antidepressant action may involve an interaction with the mesolimbic dopamine system implicated in reward. For instance, chronic treatment of 5.0 mg/kg fluoxetine for 8 weeks results in an upregulation of dopamine receptors in the mesolimbic forebrains of rats (19). Moreover, chronic studies of antidepressants support the hypothesis that these drugs may share a final common pathway involving enhancement of the mesolimbic dopamine system to produce their therapeutic effects. For example, chronic but not acute treatment with the tricyclic antidepressant desipramine increases rates of responding (9,35) or lowers thresholds for rewarding brain stimulation in rats (48). However, our results of continued, significant elevation of threshold following chronic administration of fluoxetine are in direct contrast with these

experiments involving desipramine. This discrepancy may be due to desipramine's nonspecific blockade of monoamine reuptake having a much higher selectivity for the norepinepherine transporter than for the serotonin reuptake site (45,50), suggesting that enhanced catecholaminergic transmission may be responsible for the rewarding effects of desipramine.

Similarly, Muscat and colleagues (38) observed a reversal of stress-induced anhedonia by fluoxetine and other antidepressants, suggesting positive modulation of the mesolimbic dopamine system. Because our rats were well acclimated to the BSR procedure and showed no evidence of stress behavior, for example, diarrhea, piloerection, and that fluoxetine continued to raise the BSR threshold even with chronic administration suggests that the antidepressant effects of fluoxetine are not the result of direct excitation on central dopaminergic reward systems.

Microdialysis studies examining extracellular levels of serotonin and dopamine in the mesolimbic system have provided some insight into the neurochemical changes occurring in the brain following treatment with fluoxetine, however, with discrepant results. Although it has been shown that infusion of serotonin into the VTA (18) or NACC (39) increases extracellular dopamine release in the NACC, acute and chronic systemic administration of fluoxetine increases extracellular levels of serotonin throughout the brain (6,13,17, 31,41) without significantly changing extracellular levels of dopamine (17,41). Whereas the former neurochemical experiments support the hypothesis that fluoxetine's action on the dopamine system may account for its antidepressant effects, our results are in agreement with the latter. In the present study, acute fluoxetine challenges administered chronically continued to significantly raise brain stimulation reward thresholds during the first and third weeks of chronic administration. It has been shown that acute and chronic administration of fluoxetine increases extracellular levels of serotonin and decreases extracellular levels of DA and its metabolites homovanillic acid and 3,4-dihydroxyphenylacetic acid in the NACC at a dose of 10.0 mg/kg per day (6,14,22), which further support our results. Although no statistically significant change was observed during the second week due to larger variance between animals, the average z-score value for that week was the highest of all 3 weeks. This potentiation in elevation of BSR thresholds during week 2 is probably due to the increase in steady-state plasma concentrations of fluoxetine and its metabolite, norfluoxetine. Microdialysis techniques have demonstrated that fluoxetine reaches a steady-state level in the brains of rats following approximately 14 days of administration of 10 mg/kg per day of this drug (14).

It is well established that drugs that lower brain stimulation reward thresholds are highly abused, whereas drugs for which there is no abuse potential either have no effect or raise the threshold for rewarding brain stimulation (8,23,25–30). Despite previous reports of fluoxetine abuse in patients of whom all had histories of substance abuse (16,47), the findings strongly suggest that fluoxetine does not possess abuse potential similar to other drugs of abuse such as cocaine (7), heroin (21), or methamphetamine (46). It is possible that these patients who abused fluoxetine may have undergone some neurochemical adaptations from previous substance abuse experiences resulting in a predisposition and/or conditioned behavior to abuse fluoxetine.

In conclusion, the present study indicates that serotonin decreases the sensitivity of the rat to rewarding brain stimulation that is consistent with an inhibitory effect on the mesolimbic dopamine system (2,5,6,11,14,22,24). Recently, Fletcher and colleagues (11) demonstrated that injection of serotonin directly into the nucleus accumbens reduces *d*-amphetamine potentiation of responding for a conditioned reward, which lends additional support to our conclusion. Furthermore, these results suggests that fluoxetine does not possess abuse potential similar to that of drugs of abuse including cocaine, heroin, and amphetamine. Similarly, the persistent raising of BSR thresholds following chronic administration of fluoxetine indicates that the antidepressant effects of fluoxetine are not associated with excitation of central reward mechanisms.

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