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ORIGINAL INVESTIGATION

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Effects of fluoxetine and *d*-fenfluramine on cocaine-seeking behavior in rats

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Abstract *Rationale:* Serotonin (5-HT) systems may play a role in modulating cocaine-seeking behavior. *Objectives:* The present study examined the effects of acute administration of the 5-HT reuptake inhibitor (SRI) fluoxetine, and the SRI/releaser *d*-fenfluramine, on reinstatement of extinguished cocaine-seeking behavior elicited by either response-contingent presentations of cocaine-paired cues or cocaine priming. *Methods:* Separate groups of rats that had been trained to press a lever for a cocaine reinforcer (0.75 mg/kg per 0.1 ml, IV) with a light/tone stimulus complex paired with each infusion underwent daily extinction sessions during which responding had no scheduled consequences (i.e. neither cocaine nor the stimulus complex was available). Subsequently, the effects of fluoxetine (0–10.0 mg/kg, IP) on extinction and cue reinstatement of extinguished cocaine-seeking behavior were examined, as well as the effects of *d*-fenfluramine (0–3.0 mg/kg, IP) on cue reinstatement. Additionally, dose-dependent effects of fluoxetine (0–10.0 mg/kg, IP) and *d*-fenfluramine (0–1.0 mg/kg, IP) on cocaine-primed (0–15.0 mg/kg, IP) reinstatement of extinguished cocaine-seeking behavior were examined. *Results:* Fluoxetine dose-dependently attenuated cocaine-seeking behavior during extinction. Both fluoxetine and *d*-fenfluramine dose-dependently attenuated cue-reinstated cocaine-seeking behavior. In contrast, neither drug reliably altered cocaine-seeking behavior reinstated by cocaine priming. *Conclusions:* These findings suggest that 5-HT indirect agonists effectively attenuate cocaine-seeking behavior elicited by cocaine-associated stimuli, but are much less effective in attenuating cocaine-seeking behavior elicited by cocaine priming.

Keywords Serotonin · Conditioned reinforcement · Extinction · Reinstatement · Incentive motivation

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Introduction

Cocaine and cocaine-associated stimuli can elicit craving, contributing to relapse and maintenance of cocaine abuse (Jaffe et al. 1989; Wallace 1989; Rohsenow et al. 1991; Ehrman et al. 1992). This phenomenon can be studied in animals using the extinction-reinstatement model (de Wit and Stewart 1981; Markou et al. 1993; Fuchs et al. 1998). In this model, animals that have been trained to press a lever for cocaine reinforcement subsequently undergo extinction during which responses have no scheduled consequences, and are then tested for reinstatement of extinguished cocaine-seeking behavior (i.e. operant responding in the absence of cocaine reinforcement) elicited by cocaine-conditioned stimuli or cocaine priming. Reinstatement of cocaine-seeking behavior is believed to measure the degree of incentive motivation for cocaine elicited by these stimuli (Stewart et al. 1983; Robinson and Berridge 1993).

Studies examining the effectiveness of 5-HT indirect agonist treatment of cocaine abuse and craving have yielded mixed results. In single-blind studies, chronic treatment with the 5-HT reuptake inhibitor, fluoxetine, was found to reduce self-reported cocaine use and craving (Pollack and Rosenbaum 1991; Batki et al. 1993); however, these effects were not replicated in double-blind studies (Grabowski et al. 1995; Batki et al. 1996). In addition, Buydens-Branchey et al. (1998) observed that the 5-HT reuptake inhibitor/releaser fenfluramine reduced cocaine craving in dependent individuals in a double-blind study.

Preclinical studies have demonstrated that acute fluoxetine administration decreases cocaine self-administration (Carroll et al. 1990; Richardson and Roberts 1991; Peltier and Schenk 1993), which may be due to changes in cocaine reinforcement and/or incentive motivation to seek cocaine. Indeed, chronic administration of fluoxetine decreases the rewarding effects of cocaine as measured by changes in brain self-stimulation reward thresholds (Lee and Kornetsky 1998). Furthermore, chronic fluoxetine administration decreases cocaine-seeking behavior fol-

lowing exposure to a cocaine self-administration environment, but not following a cocaine priming injection (Baker et al. 2001). Paradoxically, other studies have shown that 5-HT depletion also decreases cocaine-seeking behavior following exposure to a cocaine self-administration environment (Tran-Nguyen et al. 1999, 2001). Thus, 5-HT may play a modulatory role in cocaine-seeking behavior in which either increases or decreases in 5-HT neurotransmission may attenuate the behavior, although further research is needed to address this issue.

The present study examined the effects of acute fluoxetine or *d*-fenfluramine administration on reinstatement of cocaine-seeking behavior by cocaine priming or cocaine-paired stimuli. Effects of fluoxetine on responding during initial extinction training were also examined. Based on previous findings from our laboratory (Baker et al. 2001), we predicted that acute administration of the 5-HT indirect agonists would decrease cocaine-seeking behavior elicited by cocaine-paired stimuli, but not that elicited by cocaine priming.

Materials and methods

General methods

Animals and surgery

Male Sprague-Dawley rats weighing 250–325 g at the start of the experiments were individually housed in a climate-controlled colony room with a 12-h reversed light/dark cycle (lights off at 0630 hours). Housing facilities and care of the animals were in accordance with the conditions set forth in the "Guide for the Care and Use of Laboratory Animals" (Institute of Laboratory Animal Resources on Life Sciences, National Research Council 1996).

Animals were handled at least 6 days prior to surgery. Implantation of intravenous catheters was performed under anesthesia with sodium pentobarbital (50 mg/kg, IP; Sigma Chemical Co., St Louis, Mo., USA) administered 5 min after pretreatment with atropine sulfate (10 mg/kg, IP; Sigma). Surgical procedures were performed as described by Neisewander et al. (2000). Catheter patency was maintained by daily flushing with a solution of 0.1 ml bacteriostatic saline containing heparin sodium (10 IU/ml; Elkins-Sinn Inc., Cherry Hill, N.J., USA), streptokinase (0.67 mg/ml; Astra USA, Inc., Westerborough, Mass., USA), and ticarcillin disodium (66.7 mg/ml; SmithKline Beecham Pharmaceuticals, Philadelphia, Pa., USA). Proper catheter functioning was verified periodically throughout the experiments by intravenous administration of 0.03–0.05 ml methohexital sodium (16.6 mg/kg; Jones Pharma Inc., St Louis, Mo., USA), a dose sufficient to briefly anesthetize the animal only when administered IV. The rats were allowed at least 5 days for recovery before the initiation of self-administration training.

Drugs

Fluoxetine hydrochloride (Eli Lilly & Co., Indianapolis, Ind., USA) and *d*-fenfluramine (Sigma) were dissolved in distilled water and bacteriostatic saline, respectively, and administered intraperitoneally (IP) at a volume of 1 ml/kg. Cocaine hydrochloride was obtained from the Research Technology Branch of the National Institute on Drug Abuse (Rockville, Md., USA) and was dissolved in bacteriostatic saline, filtered through a 0.2 μm filter, and administered intravenously (IV) for self-administration or IP for cocaine reinstatement.

Self-administration training

Cocaine self-administration training took place in operant conditioning chambers (28×10×20 cm; Med Associates, St Albans, Vt., USA) equipped with an active and an inactive lever, a cue light 4 cm above the active lever, a tone generator (500 Hz, 10 dB above ground noise) and a house light located opposite the levers. Each operant conditioning chamber was housed within a larger ventilated sound-attenuating chamber. Infusion pumps (Med Associates) were connected to liquid swivels (Instech, Plymouth Meeting, Pa., USA) located above the chambers. The swivels were fastened to the catheters via polyethylene 20 tubing encased inside a metal spring leash (Plastics One, Roanoke, Va., USA).

Cocaine self-administration training consisted of 19–31 daily, 2-h sessions during which rats were trained to press the active lever to obtain a cocaine infusion (0.75 mg/kg per 0.1 ml, IV). Training sessions took place during the animals' dark cycle between 0700 and 1800 hours. To facilitate acquisition of cocaine self-administration (Carroll et al. 1981), animals were initially restricted to approximately 12 g food/day beginning 2 days prior to training. They remained food restricted until they met a criterion of seven infusions per hour on a fixed-ratio (FR) 1 schedule of cocaine reinforcement for 3 days. All animals reached this criterion within 13 days of training and were then given access to food ad libitum in the home cage throughout the remainder of the experiment. They remained on an FR1 schedule until meeting a criterion of seven infusions per hour on unrestricted food access or for a maximum of 10 days of training, after which they progressed to a variable-ratio (VR) 2, and then a VR5 schedule of reinforcement. All rats received a minimum of five sessions on each schedule. Schedule completions on the active lever resulted in simultaneous activation of the house light, cue light and tone generator, followed 1 s later by activation of the infusion pump. The 0.1 ml cocaine infusion was delivered over a 6-s period, after which the cue light, tone and pump were inactivated simultaneously. The house light remained activated for a 20-s timeout period, during which lever presses had no scheduled consequences. Responses on the inactive lever had no scheduled consequences. No cocaine priming infusions were given during training.

Extinction training

Extinction training either began the day after self-administration training or continued the day after extinction testing (see below) was completed. Extinction training consisted of 1-h exposures to the self-administration environment across 20–24 consecutive days. During this time, active and inactive lever presses were recorded but had no scheduled consequences (i.e. did not activate the cocaine infusion pump or cocaine-paired stimuli).

Experiment 1: effects of fluoxetine on extinction and cocaine-primed reinstatement of cocaine-seeking behavior

The day following the last self-administration training session, rats were tested for the effects of fluoxetine on cocaine-seeking behavior under extinction conditions in which responding had no scheduled consequences. Animals were assigned to groups (n=7–8), counterbalanced for cocaine intake during self-administration, that received 0.0, 1.0, 3.0 or 10.0 mg/kg, IP fluoxetine 60 min prior to being placed into the operant conditioning chambers for a 1-h test session.

After 21 extinction training sessions, the effects of fluoxetine on reinstatement of extinguished cocaine-seeking behavior by various cocaine priming doses were examined. Rats received vehicle and fluoxetine (3.0 mg/kg, IP) 60 min prior to testing on 2 respective days in a counterbalanced order with six extinction training

sessions between test days. The extinction sessions between tests allowed for clearance of fluoxetine and its metabolite, norfluoxetine (Wong et al. 1993), and also allowed response rates to return to pre-test baseline levels. The 3.0 mg/kg dose of fluoxetine was chosen because chronic administration of this dose attenuates cocaine-seeking behavior during extinction (Baker et al. 2001). Doses of cocaine prime (0.0, 3.75, 7.5, or 15 mg/kg, IP; n=7–8) were administered to separate groups of animals counterbalanced for both cocaine intake and fluoxetine dose during the extinction test. The rats received their priming injections and were immediately placed into the chambers for a 1-h test during which responses had no scheduled consequences.

Experiment 2: effects of fluoxetine on cue reinstatement of extinguished cocaine-seeking behavior

After 21 extinction training sessions, rats were assigned to groups (n=8–9) counterbalanced for cocaine intake that received 0.0, 1.0, 3.0, or 10.0 mg/kg, IP fluoxetine 60 min prior to being placed into the operant conditioning chambers for a 2-h cue reinstatement test session. During testing, responses on the active lever were reinforced by presentation of the cocaine-paired stimulus complex (lights, tone and pump motor) using an FR1 schedule. The FR1 schedule was used in place of the VR5 training schedule because responding for conditioned stimuli is typically less robust than responding for cocaine (unpublished observation). Two rats assigned to the high dose of fluoxetine failed to make a response and were therefore eliminated because they were never exposed to the cocaine-paired stimulus complex (final n=7–9).

Experiment 3: effects of *d*-fenfluramine on cue and cocaine-primed reinstatement of extinguished cocaine-seeking behavior

After 21 extinction training sessions, rats were assigned to groups (n=9) that received 0.0, 0.3, 1.0, or 3.0 mg/kg, IP *d*-fenfluramine counterbalanced for cocaine intake. *d*-Fenfluramine was administered 15 min prior to placing the animals into the operant conditioning chambers for a 2-h cue reinstatement test session conducted in the same manner as in experiment 2. Three rats assigned to the high dose of *d*-fenfluramine failed to obtain a stimulus presentation and were therefore eliminated from this portion of the study (final n=6–9).

Animals received four additional 1-h extinction sessions before examining the effects of *d*-fenfluramine on cocaine-primed reinstatement of cocaine-seeking behavior. Rats received saline and *d*-fenfluramine (1.0 mg/kg, IP) 15 min prior to testing on 2 separate days, respectively, in a counterbalanced order with one extinction training session intervening the test days. The 1.0 mg/kg dose of *d*-fenfluramine was chosen based on the results from the cue reinstatement tests demonstrating that this dose attenuates cocaine-seeking behavior. Doses of cocaine prime (0.0, 3.75, 7.5, or 15.0 mg/kg, IP; n=7–9) were administered immediately before the 1-h test period to separate groups of animals counterbalanced for cocaine intake. Responses had no scheduled consequences.

Experiment 4: effects of a high dose of fluoxetine on cocaine-primed reinstatement of extinguished cocaine-seeking behavior

After 21 extinction training sessions, rats received saline and fluoxetine (10.0 mg/kg, IP) administration 60 min prior to testing on 2 separate days, respectively, in a counterbalanced order with six extinction training sessions between test days. Doses of cocaine prime (0.0 or 15.0 mg/kg, IP; n=9) were administered to separate groups of animals counterbalanced for previous drug history. The rats received their priming injections and were immediately placed into the operant conditioning chambers for a 1-h test session. Responses had no scheduled consequences.

Experiment 5: effects of fluoxetine on cocaine-primed reinstatement of extinguished cocaine-seeking behavior in rats repeatedly tested across cocaine priming doses

After 22–24 extinction sessions, rats were assigned to groups (n=6–8) counterbalanced for cocaine intake that received 0.0, 1.0, 3.0, or 10.0 mg/kg, IP fluoxetine 1 h prior to each of four tests for cocaine-reinstated cocaine-seeking behavior. The rats also received a cocaine priming injection (0.0, 3.75, 7.5, or 15.0 mg/kg, IP) immediately prior to placement into the operant conditioning chambers for each of the 1-h test sessions, with doses administered in ascending order across tests. The tests were separated by 4–6 days during which animals received extinction sessions. Responses had no scheduled consequences during extinction or testing.

Experiment 6: effects of *d*-fenfluramine on cocaine-primed reinstatement of extinguished cocaine-seeking behavior in rats repeatedly tested across cocaine priming doses

After 22–23 extinction sessions, rats were assigned to groups (n=6–7) counterbalanced for cocaine intake that received either 0.0 or 1.0 mg/kg, IP *d*-fenfluramine 15 min prior to each of four tests for cocaine-reinstated cocaine-seeking behavior conducted identically to those described above in experiment 5.

Statistical analysis

Cocaine intake, responses during extinction testing, and response latency were analyzed using a one-way ANOVA. Responses during the first h of cue reinstatement testing were also analyzed using a one-way ANOVA. Only responses during the first hour of testing were analyzed because the animals exhibited a marked decrease in responding during the second hour. In addition, responses per hour during the drug-free extinction session immediately prior to a given test was used as the baseline and planned paired-sample *t*-tests were conducted to compare responses during a given test session to the respective extinction baseline in order to assess cue reinstatement of responding. To simplify presentation and analysis of the data from cocaine reinstatement test sessions, differences in responding during the test session versus extinction baseline were calculated and analyzed using mixed factor ANOVAs. For experiments 1, 3, and 4, dose of 5-HT indirect agonist was a within-subjects factor and cocaine priming dose was a between-subjects factor. For experiments 5 and 6, cocaine priming dose was a within-subjects factor and dose of the 5-HT indirect agonist was a between-subjects factor. Post-hoc comparisons were conducted using Fisher LSD tests. Trend analyses were also performed when the data varied systematically by dose.

Results

All descriptive statistics given below are presented as mean±SEM and all significant effects are reported. Responding on the inactive lever was negligible (data not shown).

Self-administration training

Response rates under the VR5 schedule during the last 5 days of self-administration training in experiments 1–6 averaged 81.39±4.85, 70.83±4.95, 67.93±3.52, 56.53±6.11, 74.30±3.41, and 87.87±9.74 lever presses/h, respectively. The total number of cocaine infusions delivered during self-administration in experiments 1–6

averaged 508.83±19.17, 482.59±20.20, 451.66±16.59, 487.50±26.03, 557.21±20.81, and 580.63±42.55, respectively.

Extinction training

Responses per hour on the drug-free extinction training day immediately preceding reinstatement test days was used as a baseline. Baseline response rates for cue reinstatement testing averaged 12.48±1.72, and 9.76±1.15 lever presses/h for experiments 2 and 3, respectively. Baseline response rates for cocaine-primed reinstatement testing after 5-HT indirect agonist administration averaged 10.90±1.78, 7.00±1.19, and 13.12±2.60, while baseline response rates for testing after vehicle administration averaged 9.48±1.14, 6.56±1.39, and 10.03±2.50 lever presses/h for experiments 1, 3, and 4, respectively. Baseline response rates for each of the four cocaine-primed reinstatement tests averaged 7.04±0.73, 5.21±0.67, 4.93±0.80, and 3.96±0.71 lever presses/h for experiment 5 and 5.32±0.94, 4.32±0.79, 4.37±0.81, and 5.11±0.93 lever presses/h for experiment 6.

Effects of fluoxetine on cocaine-seeking behavior during extinction testing

Figure 1 illustrates the effects of fluoxetine on responding during the initial extinction session of rats from experiment 1. Analysis revealed an effect of dose on responding on the active lever [$F(3,24)=4.69$, $P<0.01$], and post hoc analyses demonstrated that responding was decreased at the 3.0 and 10.0 mg/kg doses of fluoxetine relative to the 0.0 mg/kg dose ($P<0.05$). A linear trend for dose ($P<0.005$) further demonstrated a dose-dependent decrease in cocaine-seeking behavior. Fluoxetine did not affect latency to first response (data not shown).

Effects of fluoxetine and *d*-fenfluramine on cue-reinstated cocaine-seeking behavior

Figure 2 illustrates the effects of fluoxetine and *d*-fenfluramine on responding during cue reinstatement of cocaine-seeking behavior of rats from experiments 2 and 3. Fluoxetine dose-dependently decreased cocaine-seeking behavior during cue reinstatement testing [$F(3,28)=5.798$, $P<0.005$; see Fig. 2A]. Post hoc analyses demonstrated that responding was decreased at 10.0 mg/kg relative to the 0.0 mg/kg dose ($P<0.05$), and there was also a strong trend toward a decrease at 3 mg/kg ($P<0.06$). Reinstatement of responding relative to baseline occurred only at the 0.0 mg/kg ($P<0.05$) and 1.0 mg/kg ($P<0.005$) doses of fluoxetine. Latency to respond was also affected by dose of fluoxetine [$F(3,28)=4.617$, $P<0.01$; see Fig. 2B]. Post hoc analyses revealed a significant increase in latency to first response at the 10.0 mg/kg dose relative to the 0.0 mg/kg dose of fluoxetine ($P<0.005$).

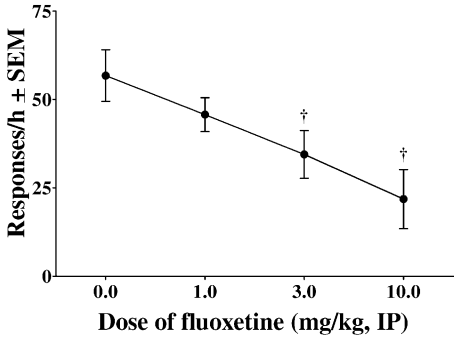

Fig. 1 Effects of fluoxetine on cocaine-seeking behavior during extinction testing expressed as mean responses per hour±SEM (n=7–8). *Daggers* represent a significant difference from 0.0 mg/kg dose (Fischer LSD test, $P<0.05$)

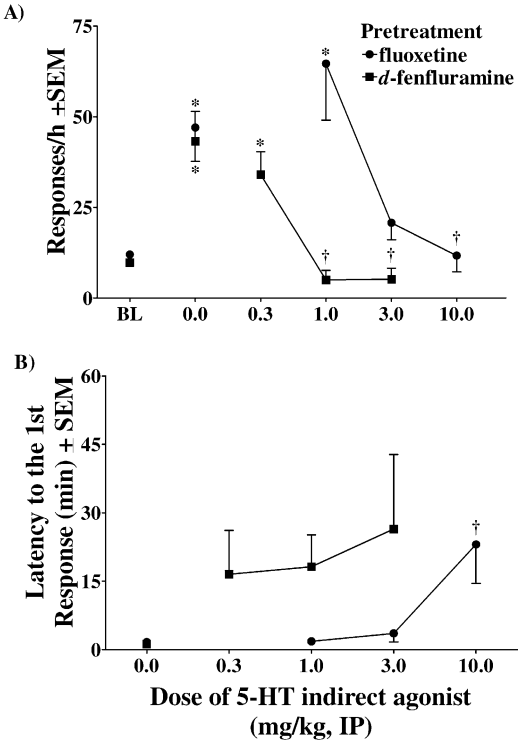

Fig. 2 Effects of fluoxetine and *d*-fenfluramine on mean responses per hour±SEM (**A**) and response latency in min±SEM (**B**) in rats tested for reinstatement of cocaine-seeking behavior by response-contingent cue presentations (n=6–9). Baseline (*BL*) rates of responding were obtained during the extinction session the day prior to testing. *Asterisks* represent a significant difference from baseline (planned t-test, $P<0.05$). *Daggers* represent a significant difference from 0.0 mg/kg dose (Fischer LSD test, $P<0.05$)

d-Fenfluramine also dose-dependently decreased cocaine-seeking behavior during cue reinstatement testing [$F(3,29)=10.786$, $P<0.001$; see Fig. 2A]. Post hoc analyses demonstrated that responding was decreased at the 1.0 and 3.0 mg/kg doses of *d*-fenfluramine relative to the 0.0 mg/kg dose ($P<0.001$). Reinstatement of responding relative to baseline occurred only at the 0.0 mg/kg

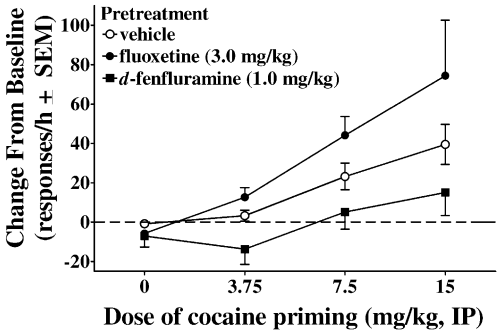

Fig. 3 Effects of fluoxetine and *d*-fenfluramine on reinstatement of cocaine-seeking behavior by a given dose of cocaine prime (n=7–9) in experiments 1 and 3, respectively. Cocaine-seeking behavior is expressed as the mean change in responses relative to baseline±SEM (see Results for baseline values)

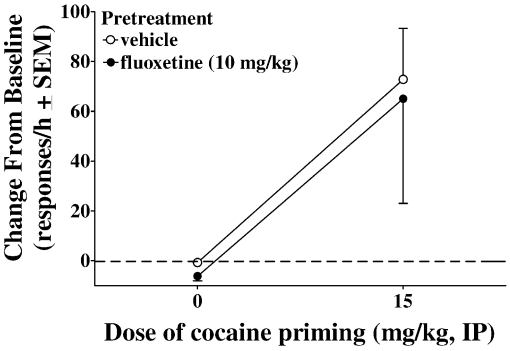

Fig. 4 Effects of a high dose of fluoxetine (10 mg/kg, IP) on responding in rats tested for reinstatement by a 15 mg/kg, IP cocaine prime (n=9). Cocaine-seeking behavior is expressed as the mean change in responses relative to baseline±SEM (see Results for baseline values)

(P<0.005) and 0.1 mg/kg (P<0.01) doses of *d*-fenfluramine. *d*-Fenfluramine did not significantly alter latency to respond (see Fig. 2B).

Figure 3 illustrates the effects of fluoxetine and *d*-fenfluramine on responding during cocaine reinstatement tests relative to extinction baselines of rats from experiments 1 and 3. Responding by the control groups in these experiments did not differ and are combined to simplify presentation; however, all analyses are based on the control data from the respective experiment. Analysis from experiment 1 revealed a dose-dependent increase in cocaine-primed responding [F(3,25)=9.797, P<0.001], and post-hoc analyses indicated an increase in responding at the 7.5 and 15.0 mg/kg doses of cocaine prime relative to the 0.0 mg/kg prime (P<0.05). There was no main effect of fluoxetine nor prime by fluoxetine interaction. Analysis from experiment 3 also revealed that cocaine priming dose-dependently increased responding [F(3,29)=5.138, P<0.01], and post-hoc analyses indicated an increase in responding at the 7.5 and 15.0 mg/kg doses of cocaine prime relative to the 0.0 mg/kg prime (P<0.05). In addition, there was a main effect of *d*-fenfluramine on responding during cocaine reinstatement [F(1,29)=5.075, P<0.05; see Fig. 3], but no prime by *d*-fenfluramine interaction, indicating a decrease in responding relative to vehicle regardless of dose of cocaine prime.

Since some animals tested at the highest cocaine priming dose in experiment 1 exhibited an increase in responding after fluoxetine pretreatment relative to vehicle pretreatment, experiment 4 investigated the effects of a higher dose of fluoxetine on reinstatement by the 15.0 mg/kg cocaine priming dose (see Fig. 4). Analysis revealed a main effect of cocaine prime [F(1,16)=14.327, P<0.005], indicating significant reinstatement of cocaine-seeking behavior. However, there was no effect of fluoxetine or prime by fluoxetine interaction.

Effects of fluoxetine and *d*-fenfluramine on reinstatement of cocaine-seeking behavior across doses of cocaine prime

Figure 5A illustrates the effects of fluoxetine on reinstatement of cocaine-seeking behavior across doses of cocaine prime relative to extinction baseline of rats from experiments 5. The analysis revealed a main effect of cocaine priming dose [F(3,72)=8.567, P<0.001]. Post-hoc analyses indicated an increase in responding at the 3.75, 7.5 and 15.0 mg/kg doses of cocaine prime relative to the 0.0 mg/kg prime (P<0.05). There was no main effect of fluoxetine nor prime by fluoxetine interaction. Trend analyses revealed a significant linear trend in animals pretreated with 0.0 mg/kg fluoxetine (P<0.05), further indicating the dose-dependent increase in cocaine-seeking behavior across cocaine doses in controls. The only other significant trend among the groups was a quadratic trend in animals pretreated with 10.0 mg/kg fluoxetine (P<0.05), indicating this dose of fluoxetine produced an inverted U-shaped cocaine reinstatement dose-effect curve in contrast to the linear increase across priming doses observed in controls (see Fig. 5A). This finding suggests that reinstatement at the highest priming dose (15 mg/kg) was attenuated by the highest dose of fluoxetine (10 mg/kg).

Figure 5B illustrates the effects of *d*-fenfluramine on reinstatement of cocaine-seeking behavior across doses of cocaine prime relative to extinction baseline of rats from experiment 6. The analysis revealed a main effect of cocaine priming dose [F(3,48)=6.308, P<0.001; see Fig. 5B]. Post-hoc analyses indicated an increase in responding at the 7.5 and 15.0 mg/kg doses of cocaine prime relative to the 0.0 mg/kg prime (P<0.05). There was no main effect of *d*-fenfluramine, prime by *d*-fenfluramine interaction, or significant trend.

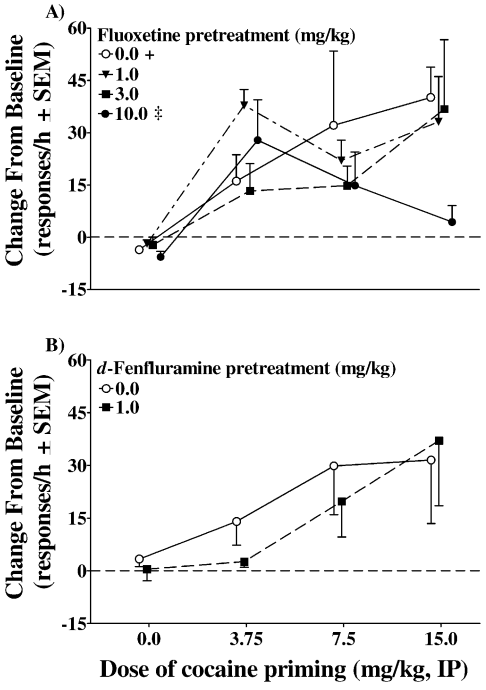

Fig. 5 Effects of fluoxetine (**A**) and *d*-fenfluramine (**B**) on reinstatement of cocaine-seeking behavior in animals repeatedly tested across a range of cocaine priming doses (n=6–8) in experiments 5 and 6, respectively. Cocaine-seeking behavior is expressed as the mean change in responses relative to baseline±SEM (see Results for baseline values). *Plus signs* represent a significant linear trend for rats treated with the 0.0 mg/kg dose of fluoxetine (P<0.05). *Double daggers* represent a significant quadratic trend for rats treated with the 10.0 mg/kg dose of fluoxetine (P<0.05)

Discussion

The 5-HT indirect agonists fluoxetine and *d*-fenfluramine both dose-dependently attenuated reinstatement of cocaine-seeking behavior by response-contingent presentations of the cocaine-paired stimulus complex. Furthermore, fluoxetine dose-dependently decreased cocaine-seeking behavior on the first day of extinction testing in the cocaine self-administration environment. Cocaine-seeking behavior during cue reinstatement tests and on the first day of extinction is motivated, at least in part, by the incentive motivational effects of cocaine-conditioned reinforcers and environmental stimuli, respectively. Thus, one explanation for the attenuating effects of the 5-HT indirect agonists on cocaine-seeking behavior during cue reinstatement and extinction is a decrease in the incentive motivational effects of cocaine-associated stimuli. This idea is consistent with previous research reporting that treatment with *d*-fenfluramine decreases responding for a water-conditioned reinforcer (Fletcher 1995). The decrease in responding for a conditioned reinforcer may be mediated via enhancement of 5-HT neurotransmission produced by the indirect agonist. Consistent with this idea, reduction of 5-HT neurotransmission potentiates responding for a conditioned reinforcer associated with food under the influence of intra-accumbens *d*-amphetamine (Fletcher et al. 1999). However, opposing results have also been reported in which depleting 5-HT decreased responding for a cocaine-conditioned reinforcer (Tran-Nguyen et al. 1999, 2001). Together with the present findings, these results suggest that either a decrease or increase in 5-HT neurotransmission may attenuate responding for a cocaine-conditioned reinforcer.

It seems unlikely that the attenuation of cocaine-seeking behavior during extinction and cue reinstatement was due to motor disruption. For instance, pretreatment with 3.0 mg/kg fluoxetine prior to cue reinstatement testing did not alter response latency, suggesting that the lack of reinstatement of responding at that dose was not due to impaired ability to lever press. However, the high dose of fluoxetine (10 mg/kg, IP) attenuated cue-reinstated responding and increased response latency, which could be due to a possible motor impairment and/or a decrease in motivation. The finding that the 10 mg/kg fluoxetine dose failed to alter response latency in animals tested on the first day of extinction suggests that even the high dose of fluoxetine did not impair ability to lever press. Furthermore, the effects of *d*-fenfluramine on response latency varied across doses but did not correspond to the dose-dependent attenuation of cue-reinstated cocaine-seeking behavior. Therefore, the overall findings suggest that the 5-HT indirect agonist effects on cocaine-seeking behavior were not simply due to motor impairment.

In contrast to the effects of the 5-HT indirect agonists on cue reinstatement, the agonists did not reliably alter cocaine-primed reinstatement of cocaine-seeking behavior. Results from experiment 3 demonstrated that *d*-fenfluramine decreased cocaine-seeking behavior regardless of priming dose. This pattern suggests that *d*-fenfluramine may have produced a non-specific decrease in motivation rather than a specific decrease in the motivational effects of cocaine priming. Indeed, others have suggested that *d*-fenfluramine reduces motivated behavior in general (Amit et al. 1991). It is also possible that some of the cocaine-seeking behavior observed during cocaine reinstatement testing is motivated by the presence of cocaine-associated environmental stimuli, and *d*-fenfluramine may attenuate the motivational effects of these stimuli resulting in the downward shift of the reinstatement dose-effect curve. In any case, the *d*-fenfluramine effect was not replicated in experiment 6. There are two likely reasons for this discrepancy. First, baseline responding was lower in vehicle-pretreated controls in experiment 6 relative to experiment 3, resulting in a floor effect for detecting attenuated responding. Second, animals were repeatedly tested across doses in experiment 6 resulting in a lower level of responding at the highest cocaine priming dose in vehicle-pretreated controls relative to responding observed in this group after a single test at the 15 mg/kg priming dose (e.g. vehicle-pretreated controls in experi-

ment 3 versus experiment 6), making it more difficult to detect a *d*-fenfluramine-induced decrease in responding.

The highest dose of fluoxetine appeared to decrease cocaine-seeking behavior at the highest cocaine priming dose in experiment 5; however, the same dose combination failed to alter responding in experiment 4. There are three possible factors contributing to this discrepancy. First, it is possible that repeated fluoxetine administration, as given in experiment 5 but not experiment 4, is necessary for fluoxetine to effectively attenuate cocaine-reinstated cocaine-seeking behavior. Second, animals in experiment 5 had experience with extinction in a cocaine-primed state prior to testing at the above dose combination, whereas animals in experiment 4 did not. Thus, it is possible that subtle effects of fluoxetine on motivation or motor behavior only manifest in animals that have weakened motivation to seek cocaine due to previous experience with extinction. Finally, in the present study as well as previous research from our laboratory (Baker et al. 2001), we observed individual differences in cocaine-reinstated cocaine-seeking behavior among animals pretreated with fluoxetine. In experiment 4, for example, three out of the nine rats in the fluoxetine-pretreated group failed to reinstate, having response rates lower than their baseline, while one rat in this group exhibited more than twice the response rate of the highest responding vehicle-pretreated rat. Thus, individual differences in response to fluoxetine may also contribute to inconsistent effects across our experiments.

Although our findings thus far suggest that 5-HT indirect agonists do not reliably alter cocaine-reinstated cocaine-seeking behavior, the observations that under some circumstances agonist-treated animals respond differently from vehicle-treated animals suggest there may be complex interactions between cocaine prime and 5-HT agonists that may modulate motivation for cocaine. Indeed, the role of 5-HT in motivation is likely complex given that depletion or enhancement of extracellular 5-HT can decrease cocaine-seeking behavior in the presence of cocaine cues (present findings; Tran-Nguyen et al. 1999, 2001; Baker et al. 2001). Furthermore, 5-HT depletion can enhance sensitivity to reinstatement of cocaine-seeking behavior by cocaine priming (Tran-Nguyen et al. 2001). The understanding of interactions between 5-HT indirect agonists and cocaine is further complicated by additional pharmacological actions of the 5-HT agonists besides their ability to block 5-HT transporters. For instance, fluoxetine and its active metabolite, norfluoxetine, are potent inhibitors of metabolizing enzyme, cytochrome P450 2D6 (2D6) (Stevens and Wrighton 1993), which is involved in the metabolism of psychostimulants (Tyndale et al. 1991). Thus, fluoxetine-induced 2D6 inhibition may prolong actions of cocaine and enhance stimulant-induced behavioral effects (Sills et al. 1999, 2000). Additionally, fluoxetine and *d*-fenfluramine produce direct although opposing actions at 5-HT_{2C} receptors. Fluoxetine functions as a 5-HT_{2C} receptor antagonist (Ni and Miledi 1997), whereas *d*-fenfluramine and its metabolite may function as agonists at this receptor (Curzon et al. 1997; Vickers et al. 1999; Rothman and Baumann 2002). Additionally, 5-HT_{2C} receptors have been implicated in modulating levels of dopamine (DA), with receptor antagonists enhancing and agonists suppressing the mesocorticolimbic DA systems (McMahon et al. 2001; Di Matteo et al. 2002; Filip and Cunningham 2002). The DA system is thought to play a role in the reinstatement of cocaine-primed cocaine-seeking behavior (Weissenborn et al. 1996; Khroyan et al. 2000; Alleweireldt et al. 2002). Furthermore, Grottick et al. (2000) showed that stimulation of 5-HT_{2C} receptors suppressed not only food-maintained behavior, but also cocaine-reinforced behavior and cocaine-primed reinstatement of responding. Thus, the multiple pharmacological actions of the 5-HT indirect agonists present several possible mechanisms by which these drugs may produce effects on cocaine-seeking behavior, thereby increasing the potential for variation between individual rats.

Clearly, further research is needed to understand the role of 5-HT systems in cocaine-seeking behavior. The use of drugs selective for receptor subtypes will be helpful in this regard. Indeed, Schenk (2000) demonstrated that administration of the 5-HT_{1A} antagonist, WAY 100635, attenuated cocaine-primed reinstatement of cocaine-seeking behavior, while ritanserin, a 5-HT_2 receptor antagonist, was ineffective in rats. Preliminary data from our laboratory have also demonstrated differential roles of 5-HT receptor subtypes in cocaine-seeking behavior reinstated by cocaine-associated stimuli versus cocaine priming (Burmeister et al. 2002). Specifically, pretreatment with the 5-HT_{1A} antagonist, WAY 100635, attenuates cocaine-primed reinstatement, but not cue reinstatement, whereas the opposite pattern was observed with pretreatment with the 5-HT_2 antagonist, ketanserin. In addition, the 5-HT_{1B} receptor subtype is involved in responding for a water-conditioned reinforcer (Fletcher and Korth 1999a, 1999b), suggesting a possible role of this receptor subtype in modulating responding for a cocaine-conditioned reinforcer. Clinical studies have shown administration of the 5-HT_2 antagonist, ritanserin, to be ineffective in reducing craving for, and self-reported use of, cocaine (Ehrman et al. 1996; Johnson et al. 1997). However, the administration of the 5-HT_2 antagonist, amperozide, reduces cocaine intake in rats (McMillen et al. 1993). Additional research is needed to understand further the role of 5-HT receptor subtypes in cocaine intake and motivation for cocaine.

In conclusion, the results from this study suggest that 5-HT indirect agonists effectively decrease cocaine-seeking behavior elicited by cocaine cues, but fail to reliably alter cocaine-seeking behavior by cocaine priming. This may have important implications for developing and evaluating treatments for cocaine dependence. Specifically, 5-HT indirect agonists may be ineffective in attenuating the incentive motivational effects of cocaine itself, and therefore may be ineffective in promoting abstinence once an individual has taken cocaine. Nevertheless, these drugs may aid in attenuating

incentive motivation elicited by cocaine cues, and therefore may be helpful in promoting abstinence while individuals remain in a cocaine-free state.

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