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Effects of 7-OH-DPAT on cocaine-seeking behavior and on re-establishment of cocaine self-administration

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Abstract

Effects of the D2-like dopamine agonist, 7-hydroxy-*N*,*N*-di-*n*-propyl-2-aminotetralin (7-OH-DPAT), on cocaine-seeking behavior and re-establishment of cocaine self-administration were examined. Rats were trained to lever press for cocaine infusions (0.25 mg/kg iv). Some were then tested for cocaine-seeking behavior (i.e., lever presses in the absence of cocaine re-inforcement) immediately following acute 7-OH-DPAT (0.001, 0.01, 0.1, or 1.0 mg/kg sc) or saline administration. Others were tested immediately or 2–23 h following repeated daily 7-OH-DPAT (1.0 mg/kg sc) or saline administration for extinction of cocaine-seeking behavior, cocaine reinstatement of cocaine-seeking behavior, and re-establishment of cocaine self-administration following extinction. 7-OH-DPAT-induced changes in locomotion were also assessed. Cocaine-experienced animals exhibited cross-tolerance to the transient hypoactivity produced by acute 7-OH-DPAT administration. Acute administration of low doses (0.01–0.1 mg/kg) of 7-OH-DPAT attenuated cocaine-seeking behavior, whereas the highest dose (1.0 mg/kg) initially attenuated, then increased, cocaine-seeking behavior. In animals tested immediately following one of the repeated administrations, 7-OH-DPAT did not alter cocaine self-administration, but sensitized locomotion. Repeated 7-OH-DPAT administration also increased cocaine-seeking behavior when administered 0 h, but not 2 or 4 h, before cocaine priming (15 mg/kg ip) and testing. In animals tested 17–23 h following one of the repeated administrations, cocaine-seeking behavior and re-establishment of cocaine self-administration were attenuated, but maintenance of self-administration following re-establishment, cocaine reinstatement of extinguished cocaine-seeking behavior, and spontaneous locomotion were unaltered. The findings suggest that following repeated administration, 7-OH-DPAT produces a transient increase (<2 h) in incentive motivation for cocaine that is followed by a protracted decrease in incentive motivation for cocaine.
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Keywords: Self-administration; Extinction; Re-instatement; Sensitization; Incentive motivation; D3 dopamine receptors

1. Introduction

Cocaine and cocaine-associated stimuli are thought to produce incentive motivational effects that contribute to cocaine craving and relapse (Stewart et al., 1984; Robinson and Berridge, 1993). Incentive motivation for cocaine can be measured in animals as cocaine-seeking behavior using the extinction/re-instatement model (Stewart, 1983; Markou et al., 1993; Carroll and Comer, 1996). In this model, animals are typically trained to lever press for a cocaine reinforcer and are subsequently tested for extinction and reinstatement of cocaine-seeking behavior (i.e., nonreinforced lever pressing) following exposure to cocaine or cocaine-associated stimuli (De Wit and Stewart, 1981; Stewart, 1983).

Studies with D2-like agonists suggest that stimulation of D2-like receptors may modulate incentive motivation for cocaine. Consistent with this idea, acute administration of the D2-like dopamine (DA) agonist 7-hydroxy-*N*,*N*-di-*n*-propyl-2-aminotetralin (7-OH-DPAT) at high doses reinstates extinguished cocaine-seeking behavior (3 and 10 mg/kg ip) and increases cocaine re-instatement of extinguished cocaine-seeking behavior (0.3 mg/kg sc) (Self et al., 1996). However, it is unclear whether low doses of 7-OH-DPAT (i.e., $\leq$0.1 mg/kg sc) alter cocaine-seeking behavior. Low doses of 7-OH-DPAT produce some behavioral effects that are opposite to those produced by the higher doses examined previously. For instance, high doses of 7-OH-DPAT are self-

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administered, produce conditioned place preference (CPP), reinstate extinguished cocaine-seeking behavior, and increase cocaine reinstatement of extinguished cocaine-seeking behavior (Caine and Koob, 1993; Caine et al., 1999; Mallet and Beninger, 1994; Chaperon and Thiébot, 1996; Self et al., 1996). In contrast, low doses of 7-OH-DPAT fail to produce self-administration, CPP, or re-instatement of extinguished cocaine-seeking behavior (Nader and Mach, 1996; Khroyan et al., 1995; Self et al., 1996) and attenuate cocaine-CPP and decrease the rate of cocaine self-administration (Caine and Koob, 1993, 1995; Caine et al., 1999; Khroyan et al., 1999; Parsons et al., 1996). Based on these behavioral effects of low doses, it seems that low doses of 7-OH-DPAT would attenuate cocaine-seeking behavior and that targeted development of drugs that produce the low-dose behavioral profile may be useful for the treatment of cocaine dependence. On the other hand, both low and high doses of 7-OH-DPAT possess cocaine-like discriminative stimulus effects (Acri et al., 1995; Lamas et al., 1996; Spealman et al., 1999; Sinnott et al., 1999; Caine et al., 2000) and these effects may facilitate cocaine-seeking behavior (Caine et al., 2000). Thus, one goal of the present study was to examine the effects of low doses of 7-OH-DPAT on extinction of cocaine-seeking behavior.

Another goal of the present study was to examine whether the effects of 7-OH-DPAT on cocaine-seeking behavior change after repeated administration of the drug. This question is particularly important since a chronic regimen would most likely be employed for treatment of cocaine dependence. Furthermore, repeated administration of 7-OH-DPAT may have different effects than acute administration due to possible neural adaptations in systems that mediate incentive motivation for cocaine. For instance, the incentive sensitization theory suggests that enduring sensitization of mesolimbic DA systems elicited by chronic cocaine exposure may underlie compulsive drug seeking and relapse in cocaine addicts (Robinson and Berridge, 1993). Chronic continuous 7-OH-DPAT administration (14 day, 1 mg/kg/day) produces adaptations in DA systems, including a decrease in D2 receptor binding in the nucleus accumbens, ventral pallidum, and substantia nigra and an up-regulation of D3 receptors in the ventral pallidum and substantia nigra in rats (Stanwood et al., 2000). Thus, the present study examined whether repeated 7-OH-DPAT administration (1 mg/kg sc) would alter propensity for cocaine-seeking behavior.

The present study assessed the effects of acute and repeated 7-OH-DPAT administration on extinction of cocaine-seeking behavior. Effects of repeated 7-OH-DPAT administration on cocaine re-instatement of extinguished cocaine-seeking behavior and re-establishment of cocaine self-administration following extinction were also assessed. To thoroughly evaluate the effects of repeated 7-OH-DPAT administration, both its immediate and protracted (i.e., late-emerging, long-lasting) effects were examined. Protracted effects were measured 17–23 h after administration and are thought to reflect changes in incentive motivation that result from neural adaptations to the 7-OH-DPAT regimen in the absence of immediate effects of 7-OH-DPAT. Locomotor activity was also measured as a behavioral control to assess whether effects of 7-OH-DPAT on motor activity may have influenced operant measures.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats, weighing 250 ± 25 g at the start of the experiment, were housed individually in a climate-controlled colony room with a reversed 12-h light–dark cycle (lights on at 1800 h). Housing conditions were in accordance with the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources on Life Sciences, National Research Council, 1996). Animals were acclimated to handling over a 5-day period prior to the start of the experiment.

2.2. Experiment 1: Dose-dependent effects of acute 7-OH-DPAT treatment

2.2.1. Autoshaping

To expedite cocaine self-administration training, animals were first trained to press a lever for food re-inforcement (45-mg food pellets; Noyes, Lancaster, NH) across five daily sessions. Autoshaping was conducted in operant chambers ($25 \times 30 \times 27$ cm high; BRS/LVE, Laurel, MD) equipped with a response lever, a food dispenser, and a house light. After a minimum criterion of responding (≥ 10 schedule completions/30-min session) was met, the schedule demand was increased from a fixed ratio (FR) 1 to a variable ratio (VR) 2 to a VR 5 schedule of food reinforcement. Two days prior to and throughout autoshaping, animals were restricted to 15–18 g of food per day.

2.2.2. Surgery

Forty-eight hours after the last food training session, animals were deeply anesthetized with pentobarbital sodium (50 mg/kg ip; Sigma, St. Louis, MO), which was administered 5 min after an injection of atropine sulfate (10 mg/kg ip; Sigma). Jugular catheters were then implanted that exited on top of the animals' head using a method described previously (Fuchs et al., 1998). Throughout the experiment, catheters were flushed daily with 0.1 ml of a solution of bacteriostatic saline, heparin sodium (10 U/ml; Elkins-Sinn, Cherry Hill, NJ), streptokinase (0.67 mg/ml; Astra Pharmaceutical Products, Westborough, MA), and ticarcillin (66.67 mg/ml; SmithKline Beecham Pharmaceuticals, West Chester, PA). Catheter tips were sealed with a plastic cap when not in use. Catheter patency was verified periodically by administering 0.03–0.05 ml of methohexital sodium (16.6 mg/ml iv, Eli Lilly, Indianapolis, IN), which produces a rapid loss of muscle tone only when administered intravenously. Animals

were given 5 days to recover from surgery prior to self-administration training.

2.2.3. Self-administration training

Self-administration training was conducted during the animals' dark cycle, during 2-h sessions on 21 consecutive days. Animals were initially trained to press a lever for infusions of cocaine hydrochloride (0.25 mg/kg/0.1 ml iv; NIDA Drug Supply System, Research Triangle Park, NC) on an FR 1 schedule of cocaine re-inforcement. After they met a minimum criterion of responding (i.e., ≥ 10 schedule completions/any given hour/2-h session), the schedule demand was increased to a VR 2 and then to a VR 5 schedule of cocaine re-inforcement. To facilitate acquisition of self-administration, animals were restricted to 15–18 g of food per day until they met the minimum response criterion for three consecutive days. Food was available ad libitum thereafter. No priming injections of cocaine were given during training.

Training was conducted in a different set of sound-attenuated operant chambers ($20 \times 28 \times 20$ cm high; Med Associates, St. Albans, VT) equipped with an active lever, an inactive lever, a stimulus light, a house light, and a tone generator (500 Hz, 10 dB above background). Throughout training, schedule completions on the active lever resulted in inactivation of the house light and presentation of a stimulus complex consisting of a light and tone followed 1 s later by activation of the infusion pump for 6 s. Then, after a 20-s timeout period, during which all stimuli were inactivated, the house light was reilluminated. A computer-automated system recorded lever presses and controlled reinforcer delivery.

2.2.4. Cocaine-seeking behavior

Testing occurred after a 7-day abstinence period since previous research from our laboratory has demonstrated an increase in cocaine-seeking behavior during the course of abstinence (Tran-Nguyen et al., 1998). Animals were handled on days 1–6 following the last self-administration session. On day 7, animals received a sc injection of 0.001 ($n=9$), 0.01 ($n=9$), 0.1 ($n=9$), or 1.0 ($n=8$) mg/kg of (±)7-OH-DPAT hydrobromide (Research Biochemicals, Natick, MA) or 1.0 ml/kg of saline ($n=8$) and were placed into the operant chambers where cocaine-seeking behavior (i.e., nonreinforced lever presses on the active lever) was measured for 2 h. The range of 7-OH-DPAT doses selected produces the low- and high-dose behavioral profiles (0.001–0.1 and 1.0 mg/kg sc, respectively; Khroyan et al., 1995). Dose assignment was counterbalanced based on cocaine intake. During testing, the stimulus complex that had been previously paired with cocaine infusions was activated every 5 min to mimic the frequency of stimulus presentations during self-administration training. This passive mode of stimulus presentation was used to model inadvertent exposure of human cocaine abusers to cocaine-associated stimuli. Lever presses were recorded, but had no scheduled consequences.

2.3. Experiment 2: Effects of repeated 7-OH-DPAT treatment

2.3.1. Surgery and self-administration training

Surgery and self-administration training procedures were similar to those used in Experiment 1, except that animals did not undergo autoshaping prior to self-administration training. To expedite acquisition, the length of self-administration sessions was instead increased to 3 h until the minimum response criterion (i.e., ≥ 10 schedule completions/any given hour/3-h session) was met (i.e., 1–7 days). Session length was then decreased to 2 h for the remaining 21 sessions.

2.3.2. Daily 7-OH-DPAT treatment

Animals were handled on days 1–6 following the last self-administration session. Starting on day 7, animals received daily treatment with (±)7-OH-DPAT (1.0 mg/kg sc; $n=13$) or saline (1 ml/kg sc; $n=9$) for 46 days. The 1.0-mg/kg/day dose of 7-OH-DPAT has been shown to produce regulatory changes in DA receptors when administered in a 14-day regimen through osmotic minipumps (Stanwood et al., 2000). Furthermore, intermittent administration of this high dose was expected to maintain tapering blood levels of 7-OH-DPAT for extended periods each day. Assignment to treatment groups was counterbalanced based on cocaine intake. Daily treatment was administered between 1500 and 1600 h except on test days examining immediate treatment effects. Table 1 indicates the sequence of the behavioral tests that were conducted as described below.

2.3.3. Locomotor activity

Locomotor activity was measured for 1 h before and after animals received their 1st and 14th daily treatment (i.e., 7-OH-DPAT or saline) and for 5 h after animals received their 40th treatment. Locomotor activity was measured in Plexiglas chambers ($24 \times 44 \times 20$ cm high)

Table 1
Sequence of behavioral tests in Experiment 2

Treatment day	Test	Time of testing (h) following daily treatment
1	locomotor activity	−1 and 0
14	locomotor activity	17–23 and 0
15, 17, 19	extinction of cocaine-seeking behavior	17–23
20–26	re-establishment/maintenance of self-administration	17–23
27–28	maintenance of self-administration	0
36, 38	cocaine reinstatement of cocaine-seeking behavior	17–23 and 0
40	locomotor activity	0
42, 44, 46	cocaine-primed cocaine-seeking behavior	0, 2, and 4

equipped with two photodetectors and two light sources that emitted photobeams 32 cm apart and 4 cm above the floor. A computer-automated system recorded the number of times the photobeams were broken consecutively by an animal moving from one end of the chamber to the other (i.e., crosses).

2.3.4. Cocaine-seeking behavior

To examine protracted effects of the daily treatment on cocaine-seeking behavior, nonreinforced lever presses were measured for 3 h, 17–23 h after the administration of the daily treatment, on treatment days 15, 17, and 19, as described previously.

2.3.5. Re-establishment and maintenance of self-administration

To examine protracted effects of the daily treatment on re-establishment and maintenance of self-administration following extinction, animals were placed into the operant chambers on treatment days 20–26, 17–23 h after administration of their daily treatment, with cocaine available on a VR 5 schedule of reinforcement for 2 h. Immediate effects of the daily treatment on maintenance of self-administration were then examined on treatment days 27–28. Animals received their daily treatment immediately prior to placement to the operant chamber. As during self-administration training, each schedule completion resulted in both a cocaine infusion and presentation of the light–tone stimulus complex.

2.3.6. Reinstatement of cocaine-seeking behavior

On treatment days 36 and 38, cocaine reinstatement of cocaine-seeking behavior was measured with or without an injection of the animals' daily treatment administered immediately prior to testing. The order of these conditions was counterbalanced across the two test days. At the start of each of these test days, cocaine-seeking behavior was allowed to extinguish over 3 h (extinction phase). On one of these test days, animals then received a saline priming injection (1 ml/kg ip) and cocaine-seeking behavior was measured for an additional 2 h (saline phase). They then received a cocaine priming injection (15 mg/kg ip) and reinstatement of extinguished cocaine-seeking behavior was measured for 2 h (cocaine reinstatement phase). On the other test day, animals received saline priming injections (1 ml/kg sc and 1 ml/kg ip) at the start of the saline phase and received their daily treatment and a cocaine priming injection at the start of the cocaine reinstatement test phase.

Time-dependent effects of 7-OH-DPAT pretreatment on cocaine-primed cocaine-seeking behavior were further examined on treatment days 42, 44, and 46. Animals received an injection of their daily treatment and remained in their home cages for 0, 2, or 4 h. Animals then received a priming injection of cocaine (15 mg/kg ip) and were placed immediately into the self-administration environment, where reinstatement of cocaine-seeking behavior was assessed for 2 h. The order of test sessions for the three pretreatment intervals was counterbalanced. Throughout all tests for cocaine-seeking behavior, the stimulus complex that had been previously paired with cocaine infusions was activated every 5 min.

2.4. Data analysis

Cocaine infusions self-administered during the training and test sessions, total cocaine intake, response latency, cocaine-seeking behavior, and locomotion were analyzed by ANOVA with dose or group as the between-subjects factor and time (i.e., 30-min intervals), test day, or pretreatment interval as the repeated measures factor, where appropriate. Interactions were further analyzed for simple main effects, and pairwise comparisons were made using Tukey's or Fisher's LSD tests. All significant interactions and main effects are reported. When heterogeneity of variance was indicated by Maunchly's test of sphericity, the degrees of freedom were adjusted using the Greenhouse–Geisser correction factor. Lever presses on the inactive lever were not included as a dependent measure because less than 5% of responding occurred on this lever during testing. Changes in the degrees of freedom across analyses reflect the loss of a control animal in Experiment 2 due to damage to the head mounting of the catheter following treatment day 20.

3. Results

3.1. Experiment 1a: Dose-dependent effects of acute 7-OH-DPAT administration

3.1.1. Cocaine intake

Cocaine intake did not differ among the cocaine-trained groups. The mean number of cocaine infusions (±S.E.M.) self-administered by the cocaine-trained groups was 24.24 ± 2.43 infusions/session (i.e., 1 infusion/4.95 min) and the mean cocaine intake (±S.E.M.) was 2.16 ± 0.39 mg/session.

3.1.2. Cocaine-seeking behavior

Acute administration of 7-OH-DPAT produced a dose-dependent change in cocaine-seeking behavior (see Fig. 1), without altering the latency of the first response (data not shown). The overall ANOVA of cocaine-seeking behavior across time revealed a significant dose × time interaction effect [$F(6.6,62.7) = 2.48$, $P < .05$]. Responding in the group that received saline was robust initially and then declined by the third 30-min interval (Tukey's test, $P < .05$). There was no difference in responding between the groups that received 0.001 mg/kg 7-OH-DPAT and saline. In contrast, the groups that received 0.01–0.1 mg/kg of 7-OH-DPAT responded less during the first hour than the group that received saline (Tukey's test, $P < .05$). Furthermore, the group that received 1.0 mg/kg of 7-OH-DPAT responded less during the first hour (Tukey's test, $P < .05$), but

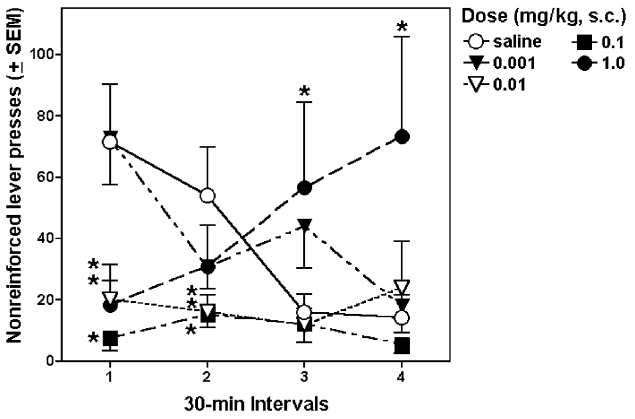

Fig. 1. Effects of acute administration of 7-OH-DPAT on cocaine-seeking behavior. Nonreinforced lever presses on the active lever (±S.E.M.) were measured for 2 h immediately after acute 7-OH-DPAT administration. * Significant difference from the group that received saline (Tukey's test, $P<.05$).

responded more during the second hour (Tukey's test, $P<.05$), than the group that received saline.

3.2. Experiment 2: Effects of repeated 7-OH-DPAT

3.2.1. Cocaine intake

Cocaine intake did not differ between the groups. The mean number of cocaine infusions self-administered (±S.E.M.) was 27.22±14.25 infusions/session (i.e., 1 infusion/4.41 min) and the mean cocaine intake (±S.E.M.) was 2.64±0.45 mg/session.

3.2.2. Effects of acute and repeated 7-OH-DPAT administration on locomotor activity

Immediately after treatment, acute administration of 1.0 mg/kg of 7-OH-DPAT produced an increase in locomotor activity that became sensitized following repeated administration (see Fig. 2A and B). The overall ANOVA of locomotion during the hour following the 1st and 14th administration of daily treatment revealed significant group [$F(1,20)=14.75$, $P<.001$] and test day [$F(1,20)=14.64$, $P<.001$] main effects and a significant test day × group interaction effect [$F(1,20)=14.85$, $P<.001$]. Collapsed across 30-min intervals, the 7-OH-DPAT-treated group exhibited more locomotor activity than the saline-treated group following both the 1st (ANOVA group simple main effect, $P<.05$) and 14th (ANOVA group simple main effect, $P<.001$) daily treatment administration. Furthermore, the 7-OH-DPAT-treated group exhibited more locomotor activity following the 14th, relative to the 1st, administration of 7-OH-DPAT (ANOVA test day simple main effect, $P<.001$).

The 40th administration of 7-OH-DPAT produced a robust increase in locomotor activity relative to saline administration (see Fig. 2C). The ANOVA of locomotor activity revealed a significant time × group interaction effect [$F(2.1,39.8)=7.35$, $P<.005$], indicating that the 7-OH-DPAT-treated group exhibited more locomotor activity than the saline-treated group during the first 3 h of testing following daily treatment administration (Tukey's test, $P<.05$).

There were no protracted effects of repeated administration of 7-OH-DPAT on locomotion when animals were tested 17–23 h following the 13th daily treatment administration (i.e., during the hour preceding the 14th daily treatment administration, see Fig. 2B). The overall ANOVA of locomotion during the hour preceding the 1st and 14th daily treatment administration revealed a significant main effect of time [$F(1,20)=109.29$, $P<.0001$] only, indicating that locomotion decreased during the second, relative to the first, 30 min of testing regardless of group or test day.

3.2.3. Cocaine-seeking behavior

In animals tested 17–23 h following daily treatment with 1.0 mg/kg of 7-OH-DPAT, 7-OH-DPAT produced a significant decrease in cocaine-seeking behavior (see Fig. 3) without altering the latency of the first response (data not shown). The overall ANOVA of responses across test days revealed a significant main effect of group [$F(1,20)=5.48$, $P<.05$], indicating that the 7-OH-DPAT-treated group responded less than the saline-treated group regardless of test day. The ANOVA also revealed a significant main effect of test day [$F(1.4,27.4)=41.84$, $P<.0001$], indicating that responding decreased across the three test days (Fisher's LSD, $P<.05$).

3.2.4. Re-establishment of cocaine self-administration

During initiation of the 7-OH-DPAT repeated-administration regimen and testing for its protracted effects on cocaine-seeking behavior, the animals had undergone a 27-day period of abstinence from cocaine self-administra-

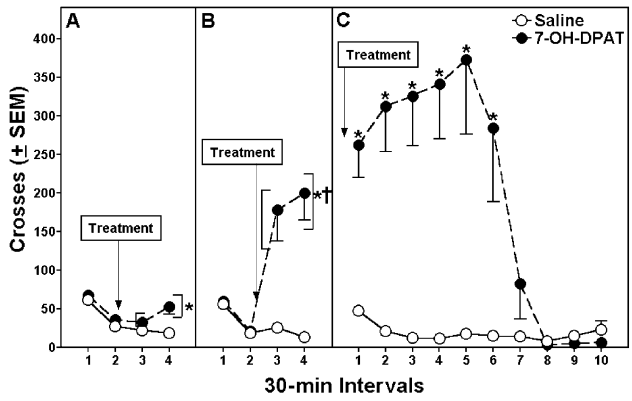

Fig. 2. Effects of acute and repeated 7-OH-DPAT administration on locomotor activity. Crosses (±S.E.M.) were measured for 1 h before and 1 h after the 1st (Panel A) and 14th administration of daily treatment (Panel B) and for 5 h after the 40th administration of daily treatment (Panel C). * Significant difference from the saline-treated group [ANOVA group simple main effect, $P<.05$ (Panel A), $P<.001$ (Panel B); Tukey's test, $P<.05$ (Panel C)]. †Significant difference from treatment day 1 (ANOVA test day simple main effect, $P<.001$).

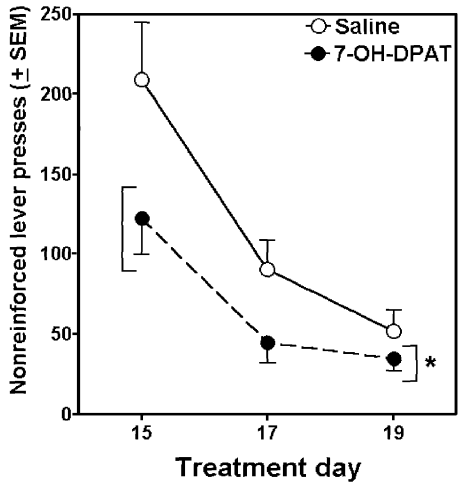

Fig. 3. Effects of repeated 7-OH-DPAT treatment on extinction of cocaine-seeking behavior assessed 17–23 h posttreatment. Nonreinforced lever presses (±S.E.M.) were measured during 2-h sessions. * Significant difference from the saline-treated group (ANOVA group main effect, $P<.05$).

tion. Re-establishment of cocaine-self-administration was then examined as the repeated-administration regimen continued. In animals tested 17–23 h following daily treatment with 1.0 mg/kg of 7-OH-DPAT, 7-OH-DPAT produced a transient decrease in re-establishment of self-administration following extinction, whereas it did not alter maintenance of self-administration in the same animals tested immediately posttreatment (see Fig. 4). The overall ANOVA of cocaine infusions 17–23 h posttreatment on days 20–26 indicated a significant main effect of test day only [$F(3.1,52.7)=4.46$, $P<.005$]; however, planned comparisons revealed that the 7-OH-DPAT-treated group self-administered less cocaine than the saline-treated group on the first and second test days (planned t test, $P<.05$).

The overall ANOVA of cocaine infusions immediately following the animals' daily treatment (i.e., 7-OH-DPAT or saline) on days 27–28, relative to days 25–26, did not reveal any significant effects. Thus, 1.0 mg/kg of 7-OH-DPAT administration immediately prior to testing did not alter cocaine self-administration in the 7-OH-DPAT-treated group. The ANOVA of cocaine intake during the self-administration test days revealed no difference in cocaine intake between the groups, indicating that overall there was no difference in cocaine history between the groups prior to reinstatement testing.

3.2.5. Reinstatement of cocaine-seeking behavior

Saline priming did not alter cocaine-seeking behavior in either group, whereas cocaine priming reinstated cocaine-seeking behavior in both groups, and this effect was enhanced by 7-OH-DPAT administration immediately prior to testing (see Fig. 5). The overall ANOVA of lever presses during extinction, saline, and cocaine reinstatement phases revealed a significant three-way group by test day × time interaction effect [$F(2.3,43.6)=2.99$, $P=.05$]. The subsequent ANOVA of lever presses during the *extinction* phase (i.e., intervals 1–6) across the two test days revealed a significant main effect of time [$F(2.8,53.5)=19.66$, $P<.0001$] only, indicating that responding decreased in both groups following the first 30 min of testing (Tukey's test, $P<.05$). The ANOVA of lever presses across the 30-min intervals immediately before and after *saline priming* revealed no significant effects, indicating that saline priming did not alter responding in either group.

The ANOVA of lever presses across the 30-min intervals immediately before and after *cocaine priming* revealed a significant main effect of time [$F(1,19)=7.33$, $P<.05$] only, indicating that responding increased during the first 30 min following, relative to the 30 min preceding, cocaine priming regardless of group or the time of daily treatment administration prior to testing (i.e., immediately versus 17–23 h prior, see Fig. 5A and B, respectively). The ANOVA of lever presses during the entire cocaine reinstatement phase (i.e., intervals 11–14) revealed a significant test day × group interaction effect [$F(1,19)=5.94$, $P<.05$] and a significant test day main effect [$F(1,19)=6.36$, $P<.05$]. Accordingly, there was no difference in responding between the groups following priming with cocaine alone. In contrast, following priming with cocaine and the animals' daily treatment (i.e., saline or 1.0 mg/kg of 7-OH-DPAT), the 7-OH-DPAT-treated group exhibited more responding than the saline-treated group (ANOVA group simple main effect, $P<.05$). Furthermore, the 7-OH-DPAT-treated group exhibited significantly more responding following priming with cocaine and 7-OH-DPAT than following priming with cocaine alone (ANOVA test day simple main effect, $P<.01$).

Further examination of the time-dependent effects of 7-OH-DPAT on cocaine-primed cocaine-seeking behavior

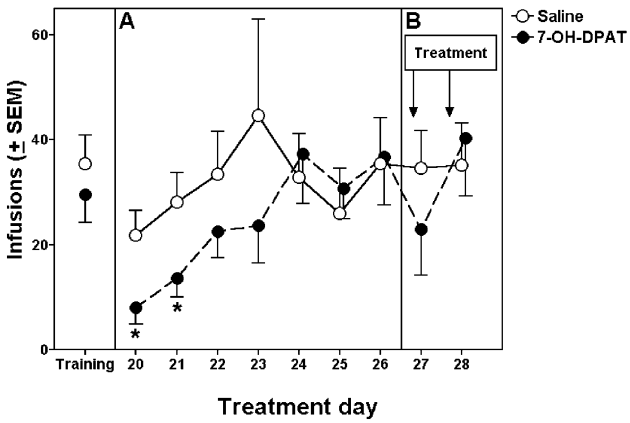

Fig. 4. Effects of repeated 7-OH-DPAT treatment on re-establishment and maintenance of cocaine self-administration (cocaine infusions±S.E.M.). Cocaine self-administration was measured during daily 2-h sessions, 17–23 h (Panel A) or immediately (Panel B) following daily treatment. The training mean represents the mean number of cocaine infusions (±S.E.M.) self-administered during the last 3 days of self-administration training. * Significant difference from the saline-treated group (planned t test, $P<.05$).

revealed that 1.0 mg/kg of 7-OH-DPAT produced an increase in cocaine-seeking behavior when it was administered immediately before cocaine priming and testing, relative to when it was administered 2 or 4 h before cocaine priming and testing (see Fig. 6). The ANOVA of lever presses revealed a significant main effect of pretreatment interval [$F(2,38) = 4.00$, $P < .05$] only, indicating that responding decreased in both groups when reinstatement testing occurred 2 or 4 h, relative to 0 h, after daily treatment administration. However, a planned one-way ANOVA of lever presses by the 7-OH-DPAT-treated group revealed a significant pretreatment interval effect [$F(2,24) = 5.18$, $P < .05$], indicating that the 7-OH-DPAT-treated group exhibited more responding at the 0 pretreatment interval, relative to the 2- and 4-h pretreatment intervals (Fisher's LSD, $P < .05$). In contrast, a one-way ANOVA of lever presses by the saline-treated group did not reveal a significant pretreatment interval effect, suggesting that the saline-treated group responded similarly regardless of pretreatment interval.

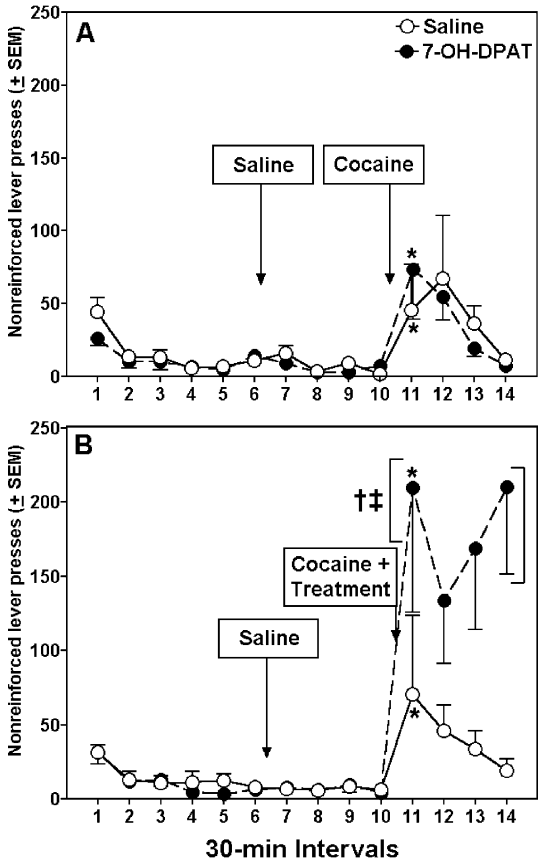

Fig. 5. Effects of repeated 7-OH-DPAT treatment on extinction, saline-reinstatement, and cocaine reinstatement of cocaine-seeking behavior. Extinction of nonreinforced responding was measured first for 3 h, 17–23 h after daily treatment on treatment days 36–38. Subsequently, saline-reinstatement was measured for 2 h following saline priming. Cocaine reinstatement was then measured without (Panel A) or with (Panel B) 7-OH-DPAT treatment administered immediately prior to cocaine priming and testing. The order of the two test days was counterbalanced. * Significant difference from the interval preceding cocaine priming (ANOVA time main effect, $P < .05$). †Significant difference from reinstatement produced by cocaine alone (ANOVA test day simple main effect, $P < .01$). ‡Significant difference from the saline-treated group (ANOVA group simple main effect, $P < .05$).

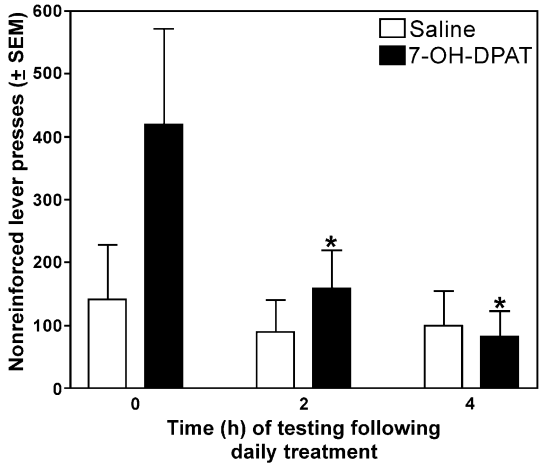

Fig. 6. Effects of 7-OH-DPAT pretreatment on cocaine-seeking behavior following cocaine priming. Animals received an injection of their daily treatment (i.e., 1.0 mg/kg of 7-OH-DPAT or saline) on treatment days 42, 44, and 46. All rats received a cocaine priming injection (15 mg/kg ip) 0, 2, or 4 h later and were placed into the chambers, where nonreinforced lever presses (±S.E.M.) were measured for 2 h. The order of test days was counterbalanced. * Significant difference from cocaine-seeking behavior measured immediately following 7-OH-DPAT administration (Fisher's LSD, $P < .05$).

4. Discussion

4.1. Effects of acute 7-OH-DPAT administration

Acute administration of 7-OH-DPAT produced a dose-dependent change in cocaine-seeking behavior (see Fig. 1). Low doses of 7-OH-DPAT (0.01–0.1 mg/kg) attenuated cocaine-seeking behavior, whereas the highest dose (1.0 mg/kg) initially attenuated, then enhanced, cocaine-seeking behavior relative to saline treatment. It is unlikely that acute administration of 7-OH-DPAT impaired operant responding, since 7-OH-DPAT did not alter response latency. Furthermore, the highest dose of 7-OH-DPAT transiently attenuated cocaine-seeking behavior but did not produce hypoactivity (see Fig. 2a, left panel). Previous studies suggest that acute administration of low doses of 7-OH-DPAT (i.e., <1.0 mg/kg sc) produce hypoactivity, and the 1.0-mg/kg dose produces hypoactivity followed by hyperactivity in cocaine-naive animals (Daly and Waddington, 1993; Khroyan et al., 1995), and repeated administration of 7-OH-DPAT produces tolerance of hypoactivity and sensitization of hyperactivity in cocaine-naive animals (Khroyan et al., 1995; Mattingly et al., 1996). Thus, it appears that cocaine experience produced cross-tolerance to the motor suppressive effects of 7-OH-DPAT in the present study. It is also possible, however, that the suppressive effects of 7-OH-DPAT were masked by a

floor effect, since the effects of 7-OH-DPAT on locomotion were examined in habituated rats. Therefore, while the decrease in cocaine-seeking behavior in the absence of an effect on response latency likely reflects a 7-OH-DPAT-induced attenuation of the incentive motivation for cocaine, it is unclear as to what extent these results were influenced by the acute effects of 7-OH-DPAT on general activity.

The increase in responding elicited by the highest dose of 7-OH-DPAT in the presence of passive stimulus presentation during the second hour of testing is consistent with an earlier report indicating that high doses of 7-OH-DPAT (0.4 and 1.0 mg/kg ip) produce an increase in responding for cocaine-paired stimulus presentations in the absence of cocaine reinforcement (Caine et al., 1999). In both studies, the increase in responding may have been produced by stimulant or cocaine-like discriminative stimulus effects of 7-OH-DPAT since previous studies indicate that these doses elicit hyperactivity (Khroyan et al., 1995) and fully substitute for cocaine (Acri et al., 1995; Lamas et al., 1996; Spealman, 1996; Sinnott et al., 1999; Caine et al., 2000), respectively. Furthermore, the increase in responding may reflect a 7-OH-DPAT-induced enhancement of incentive motivation for cocaine. However, these motivational effects are not likely specific for cocaine since similar 7-OH-DPAT dose–effect curves have been reported for responding reinforced by food or electrical brain stimulation on progressive ratio schedule of reinforcement (Depoortere et al., 1996; 1999).

Differential effects of low versus high doses of 7-OH-DPAT on cocaine-seeking behavior may be due to preferential stimulation of different populations of DA D2-like receptors. For instance, the decrease in cocaine-seeking behavior elicited by lower doses of 7-OH-DPAT may be mediated by an increase in stimulation of presynaptic DA D2-like receptors and, in turn, a decrease in extracellular DA levels (Levant et al., 1996; De Boer et al., 1997). However, a slight increase in stimulation of postsynaptic DA D3, relative to D2, receptors may also account for these findings (Khroyan et al., 1995). In contrast, the increase in cocaine-seeking behavior produced by the 1.0-mg/kg dose of 7-OH-DPAT likely resulted from increased stimulation of postsynaptic DA D2 receptors (Levant et al., 1996). Future studies will need to examine the involvement of specific DA receptor subtypes in the acute effects of 7-OH-DPAT on cocaine-seeking behavior.

4.2. Effects of repeated 7-OH-DPAT administration

In animals tested 17–23 h following daily 7-OH-DPAT treatment, 7-OH-DPAT history attenuated cocaine-seeking behavior and re-establishment of cocaine self-administration following extinction (see Figs. 3 and 4). The decrease in cocaine-seeking behavior was likely due to a 7-OH-DPAT-induced decrease in incentive motivation for cocaine rather than 7-OH-DPAT-induced behavioral suppression since 7-OH-DPAT did not alter locomotion in animals tested 17–23 h after daily treatment (see Fig. 2B). The transient 7-OH-DPAT-induced decrease in cocaine self-administration is also consistent with a decrease in incentive motivation for cocaine. Alternatively, this effect may reflect a 7-OH-DPAT-induced change in cocaine reinforcement. In any case, repeated 7-OH-DPAT treatment did not alter cocaine reinstatement of extinguished cocaine-seeking behavior when tested 17–23 h postadministration (see Fig. 5A), suggesting that there was no detectable cross-sensitization to the priming effects of cocaine. Furthermore, repeated administration of 7-OH-DPAT did not alter cocaine self-administration beyond the first two test days (see Fig. 4), suggesting that the protracted effects of 7-OH-DPAT on cocaine reinforcement and/or incentive motivation dissipate with continued cocaine self-administration.

7-OH-DPAT administered immediately prior to testing as part of the repeated-administration regimen increased cocaine reinstatement of extinguished cocaine-seeking behavior (see Fig. 5B), similar to its effects in 7-OH-DPAT-naive rats (Self et al., 1996). Repeated administration of 7-OH-DPAT also produced sensitization of its stimulant effects (see Fig. 2). However, it is unlikely that the increase in cocaine-seeking behavior was simply due to 7-OH-DPAT-induced behavioral activation since the time-course of 7-OH-DPAT-induced hyperactivity (i.e., 3 h; see Fig. 2C) did not correspond to the time-course of the 7-OH-DPAT-induced enhancement of cocaine-primed cocaine-seeking behavior (i.e., <2 h; see Fig. 6). Furthermore, 7-OH-DPAT does not show cross-sensitization to cocaine-induced locomotion (Mattingly et al., 2001). 7-OH-DPAT may have facilitated cocaine reinstatement by enhancing the discriminative stimulus effects of the cocaine primer (Lamas et al., 1996; Spealman, 1996; Caine et al., 2000) and/or by increasing transiently (<2 h) the incentive motivation for cocaine. 7-OH-DPAT administered immediately prior to testing did not alter cocaine self-administration in the present study, contrary to an earlier report by Caine et al. (1999) that had suggested that 7-OH-DPAT pretreatment (1.0 mg/kg ip) decreases self-administration of a similar dose of cocaine in a manner consistent with reward enhancement. Differences in the animals' 7-OH-DPAT history may account for the discrepancy across the studies. Prior to testing, animals received one to four administrations of 7-OH-DPAT (0.32 or 1.0 mg/kg ip) in Caine et al.'s study, while animals received 26 daily administrations of 7-OH-DPAT in the present study. Thus, long-term repeated administration of 7-OH-DPAT might have produced tolerance to the suppressive effects of 7-OH-DPAT on cocaine self-administration. Alternatively, 7-OH-DPAT may have acquired conditioned reinforcing effects in Caine et al.'s study since 7-OH-DPAT administration consistently preceded cocaine availability.

In conclusion, to the extent that cocaine-seeking behavior reflects incentive motivation for cocaine, the present findings suggest that repeated administration of 7-OH-DPAT produces a transient increase in the incentive motivational effects of cocaine that is followed by a protracted decrease

in incentive motivation cocaine. We hypothesize that the immediate enhancing effect of 7-OH-DPAT on cocaine-seeking behavior is related to its direct stimulation of D2-like receptors, whereas the protracted decrease in cocaine-seeking behavior may be due to a 7-OH-DPAT-induced reversal of cocaine-induced regulatory changes in DA D3 receptors. Although no consistent pattern of changes in DA D1 or D2 receptors has been observed across studies employing various cocaine administration regimens (Goeders and Kuhar, 1987; King et al., 1994; Lauriel et al., 1994; Neisewander et al., 1994; Claye et al., 1995; Wallace et al., 1996; Moore et al., 1998; Sousa et al., 1999), a reliable increase in DA D3 receptor binding in the ventral striatum has been found (Staley and Mash, 1996; Wallace et al., 1996; Tran-Nguyen et al., 1997). Furthermore, we have found that chronic 7-OH-DPAT administration reverses increases in DA D3 binding that occur as a result of chronic cocaine self-administration (unpublished observation). Further research is needed to examine the mechanism(s) of the immediate versus protracted effects of repeated 7-OH-DPAT administration on cocaine-seeking behavior since this information may aid in the development of new treatments that maximize anticraving effects while minimizing acute reinforcing/incentive motivational effects.

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References

Acri JB, Carter SR, Alling K, Geter-Douglas B, Dijkstra D, Wikstrom H, Katz JL, Witkin JM. Assessment of cocaine-like discriminative stimulus effects of dopamine D3 receptor ligands. Eur J Pharmacol 1995;281: R7–9.

Caine SB, Koob GF. Modulation of cocaine self-administration in the rat through D-3 dopamine receptors. Science 1993;260:1814–6.

Caine SB, Koob GF. Pretreatment with the dopamine agonist 7-OH-DPAT shifts the cocaine self-administration dose–effects function to the left under different schedules in the rat. Behav Pharmacol 1995;6: 333–47.

Caine SB, Negus SS, Mello NK, Bergman J. Effects of dopamine D1-like and D2-like agonists in rats that self-administer cocaine. J Pharmacol Exp Ther 1999;291:353–60.

Caine SB, Negus SS, Mello NK, Bergman J. Effects of dopamine D1-like and D2-like agonists in rats trained to discriminate cocaine from saline: influence of experimental history. Exp Clin Psychopharmacol 2000;8: 404–14.

Carroll ME, Comer SD. Animal models of relapse. Exp Clin Psychopharmacol 1996;4:11–8.

Chaperon F, Thiébot MH. Effects of dopaminergic D3-receptor-preferring ligands on the acquisition of place conditioning in rats. Behav Pharmacol 1996;7:105–9.

Claye LH, Akunne HC, David MD, DeMattos S, Soliman KF. Behavioral and neurochemical changes in the dopaminergic system after repeated cocaine administration. Mol Neurobiol 1995;11:55–66.

Daly SA, Waddington JL. Behavioural effects of the putative D-3 dopamine receptor agonist 7-OH-DPAT in relation to other "D-2-like" agonists. Neuropharmacology 1993;32:509–10.

De Boer P, Enrico P, Wright J, Wise LD, Timmerman W, Moor E, Dijkstra D, Wikstrom HV, Westerink BH. Characterization of the effects of dopamine D3 receptor stimulation on locomotion and striatal dopamine levels. Brain Res 1997;758:83–91.

Depoortere R, Perrault G, Sanger DJ. Behavioural effects in the rat of the putative dopamine D3 receptor agonist 7-OH-DPAT: comparison with quinpirole and apomorphine. Psychopharmacology 1996;124:231–40.

Depoortere R, Perrault G, Sanger DJ. Intracranial self-stimulation under a progressive-ratio schedule in rats: effects of strength of stimulation, D-amphetamine, 7-OH-DPAT and haloperidol. Psychopharmacology 1999;142:221–9.

De Wit H, Stewart J. Reinstatement of cocaine-reinforced responding in the rat. Psychopharmacology 1981;75:134–43.

Fuchs RA, Tran-Nguyen LTL, Specio SE, Groff RS, Neisewander JL. Predictive validity of the extinction/reinstatement model of drug craving. Psychopharmacology 1998;135:151–60.

Goeders NE, Kuhar MJ. Chronic cocaine administration induces opposite changes in dopamine receptors in the striatum and nucleus accumbens. Alcohol Drug Res 1987;7:207–16.

Khroyan TV, Baker DA, Neisewander JL. Dose-dependent effects of the D3-preferring agonist 7-OH-DPAT on motor behaviors and place conditioning. Psychopharmacology 1995;122:351–7.

Khroyan TV, Fuchs RA, Beck AM, Groff RS, Neisewander JL. Behavioral interactions produced by co-administration of 7-OH-DPAT with cocaine or apomorphine. Psychopharmacology 1999;142:383–92.

King GR, Ellinwood EH, Silvia C, Joyner CM, Xue Z, Caron MG, Lee TH. Withdrawal from continuous or intermittent cocaine administration: changes in D2 receptor function. J Pharmacol Exp Ther 1994;269: 743–9.

Lamas X, Negus SS, Nader MA, Mello NK. Effects of the putative dopamine D3 receptor agonist 7-OH-DPAT in rhesus monkeys trained to discriminate cocaine from saline. Psychopharmacology 1996;124: 306–14.

Lauriel LG, Corrigall WA, George SR. Dopamine receptor density, sensitization and mRNA levels are altered following self-administration of cocaine in rat. Brain Res 1994;634:31–40.

Levant B, Bancroft GN, Selkirk CM. In vivo occupancy of D2 receptors by 7-OH-DPAT. Synapse 1996;24:60–4.

Mallet PE, Beninger RJ. 7-OH-DPAT produces place conditioning in rats. Eur J Pharmacol 1994;261:R5–6.

Markou A, Weiss F, Gold LH, Caine SB, Schulteis G, Koob GF. Animal models of drug craving. Psychopharmacology 1993;112:163–82.

Mattingly BA, Fields SE, Langfels MS, Rowlett JK, Robinet PM, Bardo MT. Repeated 7-OH-DPAT treatments: behavioral sensitization, dopamine synthesis and subsequent sensitivity to apomorphine and cocaine. Psychopharmacology 1996;125:33–42.

Mattingly BA, Caudill A, Abel M. Differential effects of 7-OH-DPAT on the development of behavioral sensitization to apomorphine and cocaine. Pharmacol, Biochem Behav 2001;68:217–426.

Moore RJ, Vinsant SL, Nader MA, Porrino LJ, Friedman DP. Effects of cocaine self-administration on dopamine D2 receptors in rhesus monkeys. Synapse 1998;30:88–96.

Nader MA, Mach RH. Self-administration of the dopamine D3 agonist 7-OH-DPAT in rhesus monkeys is modified by prior cocaine exposure. Psychopharmacology 1996;125:13–22.

Neisewander JL, Lucki I, McGonigle P. Time-dependent changes in sensitivity to apomorphine and monoamine receptors following withdrawal from continuous cocaine administration in rats. Synapse 1994;16:1–10.

Parsons LH, Caine SB, Sokoloff P, Schwartz J-C, Koob GF, Weiss F. Neurochemical evidence that postsynaptic nucleus accumbens D3 receptor stimulation enhances cocaine reinforcement. J Neurochem 1996; 67:1078–89.

Robinson TE, Berridge KC. The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Res Rev 1993;20:247–91.

Self DW, Barnhart WJ, Lehman DA, Nestler EJ. Opposite modulation of cocaine-seeking behavior by D1- and D2-like dopamine receptor agonists. Science 1996;271:1586–9.

Sinnott RS, Mach RH, Nader MA. Dopamine D2/D3 receptors modulate cocaine's reinforcing and discriminative stimulus effects in rhesus monkeys. Drug Alcohol Depend 1999;54:97–110.

Sousa FC, Gomes PB, Macedo DS, Marinho MM, Viana GS. Early withdrawal from repeated cocaine administration upregulates muscarinic and dopaminergic D2-like receptors in rat neostriatum. Pharmacol, Biochem Behav 1999;62:15–20.

Spealman RD. Dopamine D3 receptor agonists partially reproduce the discriminative stimulus effects of cocaine in squirrel monkeys. J Pharmacol Exp Ther 1996;278:1128–37.

Spealman RD, Barrett-Larimore RL, Rowlett JK, Platt DM, Khroyan TV. Pharmacological and environmental determinants of relapse to cocaine-seeking behavior. Pharmacol, Biochem Behav 1999;64:327–36.

Staley JK, Mash DC. Adaptive increases in D3 dopamine receptors in the brain reward circuits of human cocaine fatalities. J Neurosci 1996;16: 6100–6.

Stanwood GD, Lucki I, McGonigle P. Differential regulation of dopamine D2 and D3 receptors by chronic drug treatments. J Pharmacol Exp Ther 2000;295:1232–40.

Stewart J. Conditioned and unconditioned drug effects in relapse to opiate and stimulant drug self-administration. Prog Neuro-Psychopharmacol Biol Psychiatry 1983;7:591–7.

Stewart J, de Wit H, Eikelboom R. Role of unconditioned and conditioned drug effects in the self-administration of opiates and stimulants. Psychol Rev 1984;91:251–68.

Tran-Nguyen LTL, Coffey GP, Fuchs RA, Specio SE, Joyce JN, Neisewander JL. Changes in dopamine D1 and D3 receptor subtypes during withdrawal from cocaine self-administration. Soc Neurosci Abstr 1997;22:925.

Tran-Nguyen LTL, Fuchs RA, Coffey GP, Baker DA, O'Dell LE, Neisewander JL. Time-dependent changes in cocaine-seeking behavior and extracellular dopamine levels in the amygdala during cocaine withdrawal. Neuropsychopharmacology 1998;19:49–59.

Wallace DR, Mactutus CF, Booze RM. Repeated intravenous cocaine administration: locomotor activity and dopamine D2/D3 receptors. Synapse 1996;23:152–63.