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Dopamine D_3 Receptor Antagonism Inhibits Cocaine-Seeking and Cocaine-Enhanced Brain Reward in Rats

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The dopamine D_3 receptor is preferentially localized to the mesocorticolimbic dopaminergic system and has been hypothesized to play a role in cocaine addiction. To study the involvement of the D_3 receptor in brain mechanisms and behaviors commonly assumed to be involved in the addicting properties of cocaine, the potent and selective D_3 receptor antagonist *trans*-*N*-[4-[2-(6-cyano-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl] cyclohexyl]-4-quinolininecarboxamide (SB-277011-A) was administered to laboratory rats, and the following measures were assessed: (1) cocaine-enhanced electrical brain-stimulation reward, (2) cocaine-induced conditioned place preference, and (3) cocaine-triggered reinstatement of cocaine seeking behavior. Systemic injections of SB-277011-A were found to (1) block enhancement of electrical brain stimulation reward by cocaine, (2) dose-dependently attenuate cocaine-induced conditioned place preference, and (3) dose-dependently attenuate cocaine-triggered reinstatement of cocaine seeking behavior. Thus, D_3 receptor blockade attenuates both the rewarding effects of cocaine and cocaine-induced drug-seeking behavior. These data suggest an important role for D_3 receptors in mediating the addictive properties of cocaine and suggest that blockade of dopamine D_3 receptors may constitute a new and useful target for prospective pharmacotherapies for cocaine addiction.

Key words: cocaine; addiction; dopamine; mesolimbic; mesocorticolimbic; D_3 receptor; D_3 antagonist; brain stimulation reward; BSR; self-stimulation; ICSS; conditioned place preference; CPP; self-administration; reinstatement; relapse

Aberration in brain dopamine (DA) function is hypothesized to underlie drug addiction (Wise and Rompre, 1989; Koob, 1992). Of the five major DA receptor subtypes (Gardner and Ashby, 2000), the D_3 receptor shows preferential localization in the mesocorticolimbic system (Levant, 1998; Suzuki et al., 1998), has a unique pharmacological profile (Emilien et al., 1999), and activates unique presynaptic and postsynaptic signaling systems (Emilien et al., 1999; Kuzhikandathil and Oxford, 1999) compared with D_1 and D_2 receptors. In view of its association with limbic loci, the D_3 receptor is suggested to play a role in emotional, motivational, and reinforcement functions, including the reinforcement produced by addictive drugs (Caine and Koob, 1993; Pilla et al., 1999). Some evidence suggests that D_3 receptor activation inhibits mesocorticolimbic DA function (Gilbert et al., 1995; Lejeune and Millan, 1995) and that D_3 receptor inhibition activates the mesocorticolimbic DA system (Nissbrandt et al., 1995); however, many of these studies have been confounded by a lack of selective pharmacological tools.

Decreased basal mesolimbic DA function in laboratory animals appears correlated with high vulnerability to drug seeking and drug taking (Nestler, 1993; Gardner, 1999) in animals with both genetic- and drug history-induced vulnerability (Nestler, 1993; Gardner, 1999) and in acute withdrawal from addictive drugs (Parsons et al., 1991; Weiss et al., 1992). Also, recruitment of "opponent process" neural mechanisms may occur during repeated drug taking such that mesolimbic reinforcement function is diminished, producing a decreased reward "set point" and consequent drug-seeking and drug-taking behavior (Koob et al., 1993). From such evidence, it has been postulated that mesolimbic hypo-DA function may be a fundamental substrate of addiction (Gardner, 1999). The present experiments addressed the effects of selective DA D_3 receptor antagonism in animal models relevant to addiction, experiments that to date have been hampered by lack of D_3-selective compounds. For the present experiments, a novel brain-penetrant, highly selective D_3 receptor antagonist was used, *trans*-*N*-[4-[2-(6-cyano-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl]cyclohexyl]-4-quinolininecarboxamide (SB-277011-A; Reavill et al., 2000; Stemp et al., 2000). SB-277011-A has high affinity for both human and rat D_3 receptors, with 80- to 100-fold selectivity over D_2 receptors and 66 other receptors, enzymes, and ion channels (Reavill et al., 2000). Also, SB-277011-A readily enters the brain after systemic administration (Reavill et al., 2000; Stemp et al., 2000). The compound has been shown to lack effects on spontaneous amphetamine- and phencyclidine-stimulated locomotor activity and is free from cataleptic effects even at high doses (Reavill et al., 2000).

Specifically, the present experiments in rats were undertaken to determine the effect of acute D_3 receptor antagonism on (1) the enhancement of electrical brain stimulation reward (BSR) by

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cocaine, (2) cocaine-seeking behavior as measured by cocaine-induced conditioned place preference (CPP), and (3) cocaine-seeking behavior as measured by cocaine-triggered reinstatement of operant behavior (lever pressing) previously reinforced by intravenous cocaine injections. In addition, the effect of subchronic D_3 receptor antagonism on cocaine-induced CPP was assessed. Finally, the ability of acute D_3 receptor antagonism to produce catalepsy was assessed.

MATERIALS AND METHODS

Animals

For the BSR and cocaine-triggered reinstatement experiments, male Long–Evans rats (350–475 gm at time of surgery) were used, housed individually. For the CPP and catalepsy experiments, male Sprague Dawley rats (200–225 gm at start of drug–cue or food–cue pairings) were used, housed two per cage. The use of the two different strains was because of the fact that the work was performed at two different institutions, each maintaining a different strain as the standard colony stock animal. Rats were kept on a 12 hr light/dark schedule, with food and water available *ad libitum* except for reinstatement animals during preliminary food-reinforced operant training and CPP animals in the food-paired studies. Animals were allowed to acclimate to the animal facility for 3–5 d before handling. Experiments were performed in accord with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*, in compliance with all applicable state and federal animal welfare regulations, and were reviewed by appropriate institutional animal care and use committees.

Drugs and chemicals

Cocaine hydrochloride (Sigma, Saint Louis, MO) was dissolved in saline. SB-277011-A (GlaxoSmithKline, Harlow, Essex, UK) was dissolved in 2% methylcellulose (Sigma) for CPP and catalepsy experiments and in β-cyclodextrin (Sigma) for other experiments.

Brain stimulation reward experiments

Surgery. Using standard surgical and stereotaxic technique, rats were implanted with monopolar brain stimulation electrodes (Plastics One, Roanoke, VA) in the medial forebrain bundle at the level of the lateral hypothalamus (stereotaxic coordinates: −2.56 mm posterior to Bregma, ±1.9 mm from midline, and −8.6 mm from the skull surface, according to the rat brain stereotaxic atlas of Paxinos and Watson, 1982). Correct placement was confirmed by standard postmortem histology.

Apparatus. Conventional operant chambers were used, each equipped with a retractable lever as described previously (Lepore et al., 1996). Two hundred fifty millisecond trains of 0.1 msec cathodal square wave pulses were delivered contingent on a single lever press.

Procedure. Animals were trained on a standard rate–frequency curve shift threshold-measuring BSR paradigm as described previously (Lepore et al., 1996), using a descending series of 16 different pulse frequencies ranging from 141 to 25 Hz (initial current, 200 μA, adjusted to produce moderate response rates of 45–60 responses/30 sec). The range of frequencies was tested sequentially twice. Θ_0, the frequency at which animals failed to respond for rewarding stimulation, was operationally defined as the reward threshold. Each rate–frequency BSR function generated by a given animal over a given descending series of pulse frequencies was mathematically fitted by iterative computer programs derived from the Gauss–Newton algorithm for nonlinear regression to three different sigmoid curve-fitting mathematical growth models that appear to accurately fit rate–frequency electrical brain reward functions (Coulombe and Miliaressis, 1987): the Gompertz model ($Y' = ae^{-e(b - cX)}$), the logistic model ($Y' = a/[1 + e^{(b - cX)}]$), and the Weibull function ($Y' = a[1 - e^{-(bX)^c}]$). From each curve-fitting model, a Θ_0 solution was obtained. The three solutions for Θ_0 were averaged to produce a mean Θ_0 for each rate–frequency BSR function generated by a given animal over a given descending series of pulse frequencies. When mean Θ_0 was stable (3 successive test days with each Θ_0 value within 10% of overall mean Θ_0), animals were injected with cocaine (2.0 mg/kg, i.p.), SB-277011-A (3.0, 6.0, or 12.0 mg/kg, i.p.), vehicle, or SB-277011-A followed by cocaine. SB-277011-A was given 30 min and cocaine was given 30 sec before test sessions. Shifts in mean Θ_0 values produced by vehicle, cocaine, SB-277011-A, or SB-277011-A followed by cocaine were subjected to statistical analyses.

Conditioned place preference experiments

Apparatus. An automated CPP apparatus was used as described previously (Horan et al., 2000). The conditioning chambers had distinctive visual and tactile cues, white walls with commercial rodent bedding on the floor versus white and black checkerboard walls and a smooth Plexiglas floor. The visual and tactile cues were balanced such that no side preference was exhibited before conditioning.

Procedure. CPP acquisition and expression were both assessed as described previously (Horan et al., 2000). Acquisition had four phases: acclimation, handling, conditioning, and testing. During days 1–3, animals were acclimated to the animal facility. During handling (days 4–6), animals were transported to the laboratory and handled for 5 min each. During conditioning (days 7–14), animals were exposed to once-daily conditioning sessions. For each conditioning session, animals were injected with either vehicle or SB-277011-A (0.3, 1.0, 3.0, or 10.0 mg/kg, i.p.) in the home cage, followed 30 min later by cocaine (15.0 mg/kg, i.p.) or vehicle, and then immediately confined for 30 min in an appropriate cue-specific chamber. During conditioning, cocaine was always paired with one cue-specific environment, and vehicle was paired with the other; cocaine or vehicle exposure (and appropriate environmental pairing) alternated from day to day. On the test day (day 15), animals were allowed to move freely between both cue-specific chambers for 15 min, and the amount of time spent in each was recorded.

Expression studies with acute SB-277011-A or vehicle were divided into four phases: acclimation, handling, conditioning, and testing. The acclimation and handling phases were identical to those of acquisition. The expression conditioning phase differed from that of acquisition in that, during the former, no SB-277011-A was administered. On expression test days (day 15), animals received SB-277011-A (0.3, 1.0, 3.0, or 10.0 mg/kg, i.p.) or vehicle in the home cage 30 min before they were placed in the apparatus and allowed free access to both chambers for 15 min. Expression studies with subchronic SB-277011-A or vehicle were similarly divided into acclimation, handling, conditioning, and testing. The acclimation, handling, and conditioning phases were identical to those of the acute expression studies. Then animals were given either daily injections of SB-277011-A (3.0 mg/kg, i.p.) or vehicle for 14 d and tested for CPP for 15 min on the next day, 30 min after receiving SB-277011-A or vehicle in the home cage. In a final CPP study, animals were tested for the effects of SB-277011-A (10.0 mg/kg, i.p.) or vehicle on food-induced CPP. The procedure for studying food-induced CPP was as we have described previously (Dewey et al., 1998). Briefly, the procedure was the same as that used for cocaine-induced CPP, except that the appetitive substance was Froot Loops (Kellogg Co., Battle Creek, MI), a fruit-flavored breakfast cereal that is very appealing to laboratory rats.

Self-administration and reinstatement experiments

Surgery. Using a standard surgical technique (Harms and Ojeda, 1974), rats were implanted with intravenous catheters in the external jugular vein. With daily flushing to maintain catheter patency, rats were allowed 1 week recovery before testing.

Apparatus. Conventional operant chambers were used, each equipped with two levers (one active, one not), as described previously (Vorel et al., 2001).

Procedure. This was conducted similar to published procedures (de Wit and Stewart, 1981; Vorel et al., 2001). Each press on the active lever produced an intravenous cocaine infusion (0.5 mg/kg per infusion) and a light signal. Presses on the inactive lever were counted but had no consequence. Self-administration sessions took place daily for 3 hr. After acquisition of stable self-administration (defined as three consecutive daily test sessions in which cocaine self-administration did not vary by >10%), saline was substituted for cocaine. Lever pressing was progressively extinguished. Extinction was defined as three consecutive daily test sessions with no more than 10 lever presses per session. Rats then received an injection of SB-277011-A (3.0, 6.0, or 12.0 mg/kg, i.p.) or vehicle 30 min before being placed in test chambers. After 30 min in the test chamber, a nonsignaled, noncontingent priming dose of cocaine was given intravenously at a dose of 1.0 mg/kg, sufficient to trigger robust reinstatement of the drug-seeking lever-pressing behavior (de Wit and Stewart, 1981; Vorel et al., 2001). Postpriming responses on both the active and inactive levers were counted; no responses resulted in cocaine injections. The test session lasted for 3 hr.

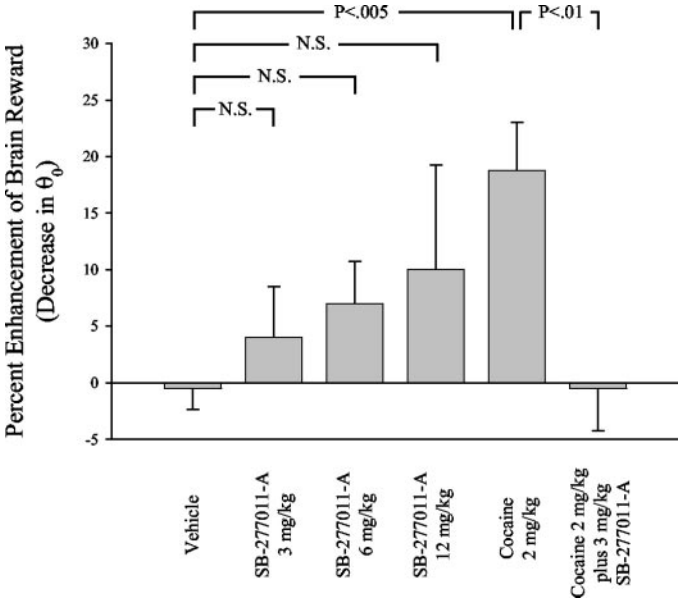

Figure 1. The dopamine D_3 receptor antagonist SB-277011-A blocks cocaine-induced enhancement of brain stimulation reward. In animals with stimulating electrodes in the medial forebrain bundle trained to extremely stable day-to-day performance of electrical brain stimulation reward, cocaine (2.0 mg/kg, i.p.) produced a robust enhancement of the brain reward as measured by shift in the Θ_0 brain reward threshold ($t = 5.61$; df = 4; $p < 0.005$). Pretreatment with SB-277011-A (3.0 mg/kg, i.p.) robustly attenuated enhancement of the brain-stimulation reward by cocaine ($t = 4.39$; $p < 0.01$).

Catalepsy experiments

Apparatus. A standard wooden catalepsy bar was used, with a diameter of 1.2 cm, mounted 10.0 cm above the floor of the test chamber, as described previously (Ferre et al., 1990).

Procedure. Animals were transported to the testing room and handled for 5 min each, on each of 3 d, to allow for acclimation. On the test day, animals were injected with vehicle, SB-277011-A (10.0 mg/kg), or haloperidol (1.0 mg/kg), and catalepsy was measured at 30 and 60 min after injection. For each catalepsy determination, the forepaws of the animal were gently placed over the bar, and the latency to move both forepaws to the floor was measured. Between determinations, animals were returned to their home cages.

Data analysis

BSR data were analyzed by one-way ANOVA with repeated measures and by Student's *t* test for paired observations (Winer, 1971; Kirk, 1982). CPP and catalepsy data were analyzed by one-way ANOVA, with *a posteriori* individual group comparisons by the Newman–Keuls test (Winer, 1971; Kirk, 1982). Cocaine-triggered reinstatement data were analyzed by one-way ANOVA, with *a posteriori* individual group comparisons by the Duncan multiple range test (Winer, 1971; Kirk, 1982).

RESULTS

Selective acute D_3 receptor antagonism blocks the enhancement of brain reward by cocaine

Overall one-way ANOVA for the entire BSR data set revealed a significant treatment effect ($F_{(5,20)} = 2.73$; $p < 0.05$). As shown in Figure 1 and as commonly reported in the literature (Kornetsky et al., 1979; Bauco and Wise, 1997), cocaine (2.0 mg/kg, i.p.) robustly enhanced medial forebrain bundle brain reward functions as evidenced by robust lowering of BSR threshold (vehicle vs cocaine, $t = 5.61$; df = 4; $p < 0.005$). As also shown in Figure 1, the D_3 antagonist SB-277011-A (3.0 mg/kg, i.p.) completely blocked the enhancing effect of cocaine on brain-reward ($t = 4.39$; $p < 0.01$). This cannot be attributed to a D_3 receptor antagonist-induced diminution of the brain reward, because SB-277011-A by itself produced no statistically significant alteration of BSR thresholds at doses as high as 12.0 mg/kg. Because a complete blockade of cocaine-enhanced BSR was obtained with SB-277011-A at 3.0 mg/kg intraperitoneally, and in view of the limited quantity of SB-277011-A available, higher SB-277011-A doses were not tested against cocaine-enhanced BSR. It is unknown whether SB-277011-A doses <3.0 mg/kg would have altered cocaine effects on BSR, because lower doses were not evaluated.

Selective acute D_3 receptor antagonism blocks acquisition of cocaine-induced CPP

As shown in Table 1 and as commonly reported in the literature (for review, see Tzschentke, 1998), cocaine (15.0 mg/kg, i.p.) produced a robust CPP. As also shown in Table 1, acute systemic administration of SB-277011-A at all doses tested, 30 min before each administration of cocaine during the CPP acquisition phase, produced a robust blockade of the acquisition of cocaine-induced CPP ($F_{(5,52)} = 4.51$; $p < 0.004$). This cannot be attributed to a D_3 antagonist-induced place aversion, because SB-277011-A by itself produced neither a significant place preference nor aversion at doses as high as 10.0 mg/kg (overall ANOVA for vehicle and all doses of SB-277011-A tested, $F_{(3,34)} = 1.88$; p = NS). Newman–Keuls *a posteriori* group comparisons revealed that each of the four doses of SB-277011-A tested (0.3, 1.0, 3.0, and 10.0 mg/kg) produced an equivalent blockade of the acquisition of cocaine-induced CPP.

Selective acute D_3 receptor antagonism blocks expression of cocaine-induced CPP

As was the case for the animals tested for acquisition of cocaine-induced CPP (Table 1), so too did the animals tested for expression of cocaine-induced CPP show a robust CPP to cocaine (Table 2), congruent with literature reports (Tzschentke, 1998). As also shown in Table 2, acute systemic administration of SB-277011-A, 30 min before behavioral testing on the CPP expression test day (and, thus, exposure to the previously cocaine-associated cues within the CPP test apparatus), produced a robust blockade of the expression of cocaine-induced CPP ($F_{(5,52)} = 4.35$; $p < 0.003$). As is the case for the SB-277011-A blockade of acquisition of cocaine-induced CPP (noted above), this blockade by SB-277011-A of the expression of cocaine-induced CPP cannot be attributed to a D_3 antagonist-induced place aversion, because, as noted above, SB-277011-A by itself produced neither a significant place preference nor aversion at doses as high as 10.0 mg/kg. Newman–Keuls *a posteriori* individual group comparisons performed on the cocaine CPP expression data revealed that the SB-277011-A protective effect against the expression of cocaine-induced CPP was seen primarily at the 1.0, 3.0, and 10.0 mg/kg doses of SB-277011-A. The 0.3 mg/kg dose of SB-277011-A produced some protective effect against expression of cocaine-induced CPP (Newman–Keuls; $p < 0.05$), but the effect was noticeably less than that produced by the 1.0, 3.0, or 10.0 mg/kg doses.

Selective subchronic D_3 receptor antagonism blocks expression of cocaine-induced CPP

As was the case for the animals tested under basal vehicle conditions in the above experiments on acute SB-277011-A attenuation of acquisition (Table 1) or expression (Table 2) of cocaine-induced CPP, so too did the animals tested under basal vehicle conditions in the experiments on subchronic SB-277011-A atten-

Table 1. Effect of SB-277011-A or vehicle on acquisition of cocaine-conditioned place preference

Pretreatment (30 min before daily place-conditioning sessions)	Drug (immediately before daily place-conditioning sessions, alternating every other day with vehicle[a])	Time spent in chambers on test day[b]	
		Drug-paired	Vehicle-paired[a]
Vehicle[c]	Vehicle[a]	7.9 ± 0.9	7.1 ± 0.9
Vehicle[c]	Cocaine[d]	10.6 ± 0.4*	4.4 ± 0.4
SB-277011-A (0.3 mg/kg)	Cocaine[d]	7.4 ± 0.8	7.6 ± 0.8
SB-277011-A (1 mg/kg)	Cocaine[d]	7.1 ± 0.6	7.9 ± 0.6
SB-277011-A (3 mg/kg)	Cocaine[d]	6.4 ± 0.6	8.6 ± 0.6
SB-277011-A (10 mg/kg)	Cocaine[d]	7.9 ± 0.9	7.1 ± 0.9

[a]Distilled water (1 ml/kg, i.p.).

[b]Each value represents mean time (minutes) spent in each chamber ± SEM on the test day. Total possible combined time in both chambers was 15 min. Ten rats were examined for each treatment pairing. During conditioning, animals were exposed to the cocaine-paired (4 daily 30 min sessions) and vehicle-paired (4 daily 30 min sessions) chambers on alternating days. Half of the cocaine-exposed animals received cocaine in one cue-distinctive environmental chamber; the other half received it in the other chamber. The vehicle/vehicle animals received vehicle in each cue-distinctive chamber, on alternating days, for a total of eight daily 30 min sessions.

[c]Methylcellulose solution (2% w/v; 1 ml/kg, i.p.).

[d]Cocaine (15 mg/kg, i.p.).

*Significantly different from times spent in the cocaine-paired chamber on the test day by animals pretreated with 0.3, 1.0, 3.0 or 10.0 mg/kg SB-277011-A 30 min before each place conditioning session, $p < 0.05$, ANOVA and Student–Newman–Keuls test.

Table 2. Effect of acute SB-277011-A or vehicle on expression of cocaine-conditioned place preference

Drug (immediately before daily place-conditioning sessions, alternating every other day with vehicle[a])	Treatment on test day (30 min before place preference expression testing)	Time spent in chambers on test day[b]	
		Drug-paired	Vehicle-paired[a]
Vehicle[a]	Vehicle[c]	7.3 ± 0.4	7.7 ± 0.4
Vehicle[a]	SB-277011-A (3 mg/kg)	7.2 ± 0.4	7.8 ± 0.4
Vehicle[a]	SB-277011-A (10 mg/kg)	7.9 ± 0.6	7.1 ± 0.6
Cocaine[d]	Vehicle[c]	10.1 ± 0.4	4.9 ± 0.4
Cocaine[d]	SB-277011-A (0.3 mg/kg)	9.3 ± 0.5	5.7 ± 0.5
Cocaine[d]	SB-277011-A (1 mg/kg)	7.3 ± 0.6*	7.7 ± 0.6
Cocaine[d]	SB-277011-A (3 mg/kg)	7.3 ± 0.7*	7.7 ± 0.7
Cocaine[d]	SB-277011-A (10 mg/kg)	8.0 ± 0.6*	7.0 ± 0.6

[a]Distilled water (1 ml/kg, i.p.).

[b]Each value represents mean time (minutes) spent in each chamber ± SEM on the test day. Total possible combined time in both chambers was 15 min. Ten rats were examined for each treatment pairing. During conditioning, animals were exposed to the cocaine-paired (4 daily 30 min sessions) and vehicle-paired (4 daily 30 min sessions) chambers on alternating days. Half of the cocaine-exposed animals received cocaine in one cue-distinctive environmental chamber; the other half received it in the other chamber. The vehicle/vehicle animals received vehicle in each cue-distinctive chamber, on alternating days, for a total of eight daily 30 min sessions. On the test day, animals received either vehicle or SB-277011-A 30 min before being placed in the CPP apparatus and tested for expression of place conditioning.

[c]Methylcellulose solution (2% w/v; 1 ml/kg, i.p.).

[d]Cocaine (15 mg/kg, i.p.).

*Significantly different from times spent in the cocaine-paired chamber on the test day by animals given cocaine/vehicle alternating place-conditioning sessions plus vehicle 30 min before place preference expression testing, $p < 0.05$, ANOVA and Student–Newman–Keuls test.

uation of expression of cocaine-induced CPP show a robust CPP to cocaine (Table 3), in agreement with the data reported above and with literature reports (Tzschentke, 1998). Subchronic daily systemic administration of 3.0 mg/kg SB-277011-A for 14 d before testing for expression of cocaine-induced CPP produced a robust blockade of the expression of cocaine-induced CPP ($F_{(1,18)} = 20.90$; $p < 0.0002$; Table 3). It should be noted that the last dose of subchronic SB-277011-A was given on the CPP expression test day (see Materials and Methods). Therefore, as is the case for the blockade of cocaine-induced CPP expression by acute SB-277011-A (noted above), this blockade of cocaine-induced CPP expression by subchronic SB-277011-A cannot be attributed to a D_3 antagonist-induced place aversion, because SB-277011-A by itself produced neither a significant place preference nor aversion at doses as high as 10.0 mg/kg.

Selective acute D_3 receptor antagonism does not block expression of food-induced CPP

As shown in Table 4 and as reported previously (Bechara and van der Kooy, 1992; Slusher et al., 2001), food produced a robust CPP (food-paired vs non-food-paired chamber, $t = 5.74$; df = 9; $p < 0.001$). Acute systemic administration of even the highest dose of SB-277011-A (10 mg/kg) 30 min before behavioral testing on the CPP expression test day (and, thus, exposure to the previously food-associated cues within the CPP test apparatus), did not block expression of the food-induced CPP (Table 4).

Selective acute D_3 receptor antagonism blocks cocaine-triggered relapse to behavior previously reinforced by intravenous cocaine injections

In the experiment to test for blockade by SB-277011-A of cocaine-triggered relapse to behavior previously reinforced by intravenous cocaine injections, intravenously catheterized rats acquired highly stable day-to-day cocaine self-administration. Substitution of vehicle for cocaine produced extinction of self-administration behavior. The extinction period varied from 10 to 18 d, consistent with our previous studies (Vorel et al., 2001). As shown in Figure 2 and as commonly reported in the literature (de Wit and Stewart, 1981; Vorel et al., 2001), after extinction, a single noncontingent intravenous injection of 1.0 mg/kg cocaine produced robust re-

Table 3. Effect of subchronic (2 weeks) SB-277011-A or vehicle on expression of cocaine-conditioned place preference

Drug (immediately before daily place-conditioning sessions, alternating every other day with vehicle[a])	Treatment given daily for 2 weeks before place preference expression testing	Treatment on test day (30 min before place preference expression testing)	Time spent in chambers on test day[b]	
			Drug-paired	Vehicle-paired[a]
Cocaine[c]	Vehicle[d]	Vehicle[d]	10.0 ± 0.8	5.0 ± 0.8
Cocaine[c]	SB-277011-A (3 mg/kg)	SB-277011-A (3 mg/kg)	5.6 ± 0.6*	9.4 ± 0.6

[a]Distilled water (1 ml/kg, i.p.).

[b]Each value represents mean time (minutes) spent in each chamber ± SEM on the test day. Total possible combined time in both chambers was 15 min. Ten rats were examined for each treatment pairing. During conditioning, animals were exposed to the cocaine-paired (4 daily 30 min sessions) and vehicle-paired (4 daily 30 min sessions) chambers on alternating days. Half of the cocaine-exposed animals received cocaine in one cue-distinctive environmental chamber; the other half received it in the other chamber. Animals then received daily injections of vehicle or SB-277011A in their home cages for 2 weeks. On the test day, animals received either vehicle or SB-277011A 30 min before being placed in the CPP apparatus and tested for expression of place conditioning.

[c]Cocaine (15 mg/kg, i.p.).

[d]Methylcellulose solution (2% w/v; 1 ml/kg, i.p.).

*Significantly different from times spent in the cocaine-paired chamber on the test day by animals given subchronic vehicle for 2 weeks plus vehicle 30 min before place preference expression testing, $p < 0.01$, ANOVA and Student–Newman–Keuls test.

Table 4. Effect of acute SB-277011-A or vehicle on expression of food-conditioned place preference

Treatment (during daily place-conditioning sessions, alternating every other day with no food)	Treatment on test day (30 min before place preference expression testing)	Time spent in chambers on test day[a]	
		Food-paired[b]	No-food-paired
No food	Vehicle[c]	7.9 ± 0.9	7.1 ± 0.9
Food[b]	Vehicle[c]	9.6 ± 0.5*	5.4 ± 0.5
Food[b]	SB-277011-A (10 mg/kg)	9.6 ± 0.4*	5.4 ± 0.4

[a]Each value represents mean time (minutes) spent in each chamber ± SEM on the test day. Total possible combined time in both chambers was 15 min. Ten rats were examined for each treatment pairing. Froot Loops, a sweetened fruit-flavored breakfast cereal that is highly appetitive to laboratory rats, was used as the food. During conditioning, animals were exposed to the food-paired (5 daily 30 min sessions) and no-food-paired (5 daily 30 min sessions) chambers on alternating days. Half of the food-exposed animals received food in one cue-distinctive environmental chamber; the other half received it in the other chamber. The no food/no food animals received no food in each cue-distinctive chamber on alternating days. On the test day, animals received either vehicle or SB-277011-A 30 min before being placed in the CPP apparatus and tested for expression of place conditioning.

[b]Froot Loops.

[c]Methylcellulose solution (2% w/v; 1 ml/kg, i.p.).

*Significantly different from time spent in the food-paired chamber on the test day by animals given no food/no food alternating place-conditioning sessions plus vehicle before place preference expression testing, $p < 0.05$, ANOVA and Student–Newman–Keuls test.

instatement of the extinguished operant behavior previously reinforced by intravenous cocaine injections, i.e., robust reinstatement of cocaine-seeking behavior ($t = 2.76$; df = 15; $p < 0.02$). Acute pretreatment with SB-277011-A produced a dose-dependent attenuation of this cocaine-triggered reinstatement of the extinguished cocaine-seeking behavior ($F_{(4,28)} = 7.9$; $p < 0.001$) (Fig. 2). *A posteriori* individual group comparisons using the Duncan multiple range test revealed that 3.0 mg/kg SB-277011-A did not block cocaine-triggered cocaine-seeking behavior, but that cocaine-triggered cocaine-seeking behavior was significantly blocked by 6.0 mg/kg SB-277011-A ($p < 0.05$) and 12.0 mg/kg SB-277011-A ($p < 0.01$; see individual group comparison significance levels in Fig. 2).

SB-277011-A by itself did not, at any dose tested (3.0, 6.0, or 12.0 mg/kg), trigger reinstatement of cocaine seeking (data not shown). Over the dose range tested, SB-277011-A did not affect responses on the inactive (non-cocaine-paired) lever (data not shown). In preliminary studies with limited numbers of animals, SB-277011-A by itself did not alter cocaine self-administration per se.

Selective acute D_3 receptor antagonism does not affect other aspects of animal behavior

In the catalepsy test, SB-277011-A was noncataleptogenic (Table 5). This agrees with our previous observations with SB-277011-A (Reavill et al., 2000; Stemp et al., 2000). In the present experiments, daily observations of animals also showed that animals given SB-277011-A continued to eat normally, maintained normal body weights, showed no altered reactivity to novel stimuli and situations, and continued to be gentle and easy to handle.

DISCUSSION

The present findings indicate a key role for DA D_3 receptors in cocaine-induced brain reward enhancement and drug seeking and are in accord with emerging pharmacological (Caine and Koob, 1993; Caine et al., 1997), human postmortem (Staley and Mash, 1996), and genetic (Duaux et al., 1998) literature implicating D_3 receptors in addiction.

Much previous work has implicated D_1 and D_2 receptors in the enhancement of BSR by cocaine (Nakajima, 1989; Nakajima et al., 1993), cocaine-induced CPP (Spyraki et al., 1987; Baker et al., 1998), and reinstatement of cocaine seeking (Self et al., 1996), but previous findings with putative D_3-selective agonists and antagonists have been variable and contradictory. Decreased cocaine self-administration was reported with the putative D_3 agonists 7-hydroxy-*N,N*-di-n-propyl-2-aminotetralin (7-OH-DPAT) (Caine and Koob, 1993; Parsons et al., 1996), quinpirole (Caine and Koob, 1993), quinelorane (Parsons et al., 1996; Caine et al., 1997), pramipexole (Caine et al., 1997), and *R*-(+)-trans-3,4,4a,10b-tetrahydro-4-propyl-2H,5H-[1]benzopyrano[4,3-b]-1,4-oxazin-9-ol (PD-128907) (Caine et al., 1997). The putative D_3 agonists *N*-[4-[4-(2-methoxyphenyl)-1-piperazinyl]butyl]naphthalene-2-carboxyamide (BP-897) and 7-OH-

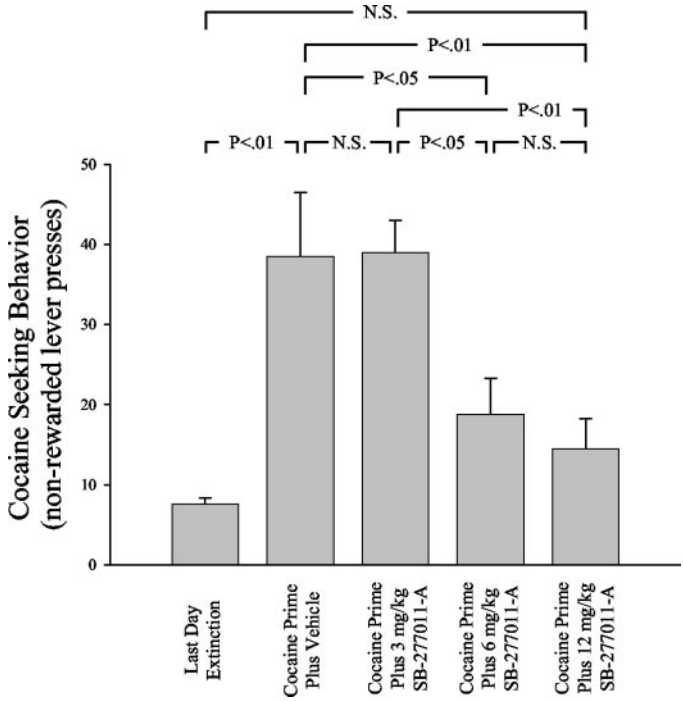

Figure 2. The dopamine D_3 receptor antagonist SB-277011-A attenuates cocaine-induced relapse to cocaine-seeking behavior. In intravenously catheterized animals trained to highly stable day-to-day cocaine self-administration, substitution of vehicle for cocaine produced extinction of self-administration behavior. The extinction period varied from 10 to 18 d. After extinction, a single noncontingent intravenous injection of 1.0 mg/kg cocaine (*Cocaine Prime*) produced robust reinstatement of the extinguished operant behavior previously reinforced by intravenous cocaine injections (i.e., cocaine seeking). Pretreatment with SB-277011-A (3.0, 6.0, or 12.0 mg/kg, i.p.) produced dose-related attenuation of the cocaine-triggered reinstatement of drug seeking ($F_{(4,28)} = 7.9$; $p < 0.001$; significance levels of *post hoc* individual group comparisons by the Duncan multiple range test are shown). The *numbers* of lever presses depicted represent post-cocaine-prime presses on the active (i.e., previously reinforced, before extinction) lever.

DPAT inhibited cocaine-seeking behavior as assessed by second-order reinforcement (Pilla et al., 1999) and CPP (Khroyan et al., 1999). 7-OH-DPAT inhibited cocaine-enhanced BSR, but contradictorily, the putative D_3 antagonist (+)-cis-5-methoxy-1-methyl-2-(di-n-propylamino)tetralin similarly inhibited and the putative D_3 antagonist 5,6-dimethoxy-2-(*N*-dipropyl)-aminoindan had no effect on cocaine-enhanced BSR (Kling-Petersen et al., 1994, 1995). Khroyan et al. (2000) found that 7-OH-DPAT and PD-128907 did not alter cocaine-triggered reinstatement.

Such previous findings are problematic, because the compounds used have poor D_3/D_2 selectivity, mixed agonist and antagonist properties, significant affinity at other brain receptors, poor brain penetration, or a combination thereof (Levant, 1997). In contrast, SB-277011-A is a potent and highly selective D_3 antagonist with 80-fold selectivity for D_3 versus D_2 receptors (Reavill et al., 2000), 80- to 100-fold selectivity over 66 other receptors, enzymes, and ion channels, and high brain penetration (Reavill et al., 2000). SB-277011-A thus satisfies the requirements for a pharmacological agent with unambiguous D_3 receptor selectivity. We find that SB-277011-A robustly attenuates the rewarding properties of cocaine as measured by BSR and the incentive motivational properties of cocaine as measured by CPP and reinstatement and therefore suggest that D_3 antagonists merit further study as addiction treatments.

Reduction in the rewarding (BSR) properties of cocaine may have therapeutic potential, especially because SB-277011-A by itself produces no dysphoric shift in brain reward. In animal models, D_2 antagonists block cocaine-enhanced BSR (Tsibulsky et al., 1995) and cocaine self-administration (de Wit and Wise, 1977; Ettenberg et al., 1982). In humans, D_2 antagonists can attenuate the euphoric effects of cocaine (Sherer et al., 1989; Newton et al., 2001), but D_2 antagonists are themselves dysphorigenic and therefore countertherapeutic (Gawin, 1991). Humans receiving D_1 and D_2 antagonists continue to use cocaine (Ohuoha et al., 1997; Grabowski et al., 2000), as do schizophrenic patients receiving high-dose neuroleptics (Schneier and Siris, 1987; Dixon et al., 1991). D_3 antagonism may prove superior in this regard. However, the attenuation of the cue incentive properties of cocaine by SB-277011 may confer even more clinical potential. Such cue incentive properties (Bindra, 1968; Robinson and Berridge, 1993) appear mediated by hippocampal and amygdaloid mechanisms (Childress et al., 1999; Kilts et al., 2001). The amygdala seems especially involved (Everitt et al., 1999), particularly in drug-enhanced stimulus–reward associations (Robledo et al., 1996; Harmer and Phillips, 1999). Amygdala lesions or inactivation impair the ability of drug-associated cues to trigger reinstatement (Meil and See, 1997; Grimm and See, 2000) and impair acquisition of cocaine seeking under second-order reinforcement (Whitelaw et al., 1996). The amygdala is relatively enriched with D_3 receptors (Gurevich and Joyce, 1999), and amygdaloid D_3 mechanisms may be involved in acquisition of drug-paired cue incentive value (Hitchcott and Phillips, 1998a,b). We speculate that our findings of attenuation of cocaine-induced cue incentive value by SB-277011-A may involve amygdaloid mechanisms regulating the incentive value of drug-associated environmental cues. Consistent with this view, SB-277011-A inhibits cocaine seeking as measured by second-order reinforcement (Everitt et al., 2001).

We believe that our findings are unlikely to have resulted from aversive effects of SB-277011-A, because neither BSR inhibition nor place aversion was seen. Equally unlikely is that our findings are attributable to interference with associative effects, because

Table 5. Catalepsy measurements after SB-277011-A or haloperidol

Group	Drug given on test day	Catalepsy at 30 min (sec)[a]	Catalepsy at 60 min (sec)[a]
1	Vehicle[b]	0.6 ± 0.2	0.7 ± 0.3
2	Haloperidol (1 mg/kg)	18.0 ± 3.1*	70.0 ± 19.6*
3	SB-277011-A (10 mg/kg)	1.7 ± 0.4	2.4 ± 0.9

[a]Each value represents mean time (seconds) spent on the catalepsy test bar ± SEM. Ten rats were examined in each drug group. The animals received vehicle, haloperidol, or SB-277011-A 30 and 60 min before being tested for catalepsy.

[b]Methylcellulose solution (2% w/v; 1 ml/kg, i.p.).

*Significantly greater than vehicle or SB-277011-A, $p < 0.01$, ANOVA and Student–Newman–Keuls test.

SB-277011-A does not affect memory in animal tests (D. N. Jones and J. J. Hagan, unpublished data on file). Motoric artifacts are also unlikely, because SB-277011-A neither alters locomotor activity nor elicits sedation or catalepsy and does not affect stimulant-induced hyperlocomotion (Reavill et al., 2000).

A number of workers (Caine and Koob, 1993; Kling-Petersen et al., 1995; Parsons et al., 1996; Caine et al., 1997; Khroyan et al., 1999; Pilla et al., 1999) have reported that D_3-selective agonists produce effects (i.e., reductions in cocaine reward and seeking) similar to our findings with SB-277011-A, a selective D_3 antagonist. However, it must be reiterated that the putative D_3 agonists used in those previous studies do not possess full D_3 agonist properties (Levant, 1997). At best, they are partial D_3 agonists or, more likely, mixed D_3 agonist/antagonists for which the antagonist properties may sometimes predominate. BP-897 is representative. Recent evidence suggests that BP-897 attenuates the discriminative stimulus effects of cocaine in monkeys (Beardsley et al., 2001) and inhibits cocaine seeking in rodents (Pilla et al., 1999; Preti, 2000). BP-897 has been considered (and used as) a D_3 partial agonist, but because the BP-897 D_3 antagonist properties may predominate at rodent somatodendritic D_3 and human D_3 receptors (Wood et al., 2000; Wicke and Garcia-Ladona, 2001), previous findings with BP-897 may actually agree with the present SB-277011-A findings. Along a similar line of argument, there are reports that D_3 receptor agonists reproduce the discriminative stimulus effects of cocaine and enhance cocaine self-administration (Caine and Koob, 1995; Spealman, 1996), although the compounds used in those studies have poor D_3/D_2 selectivity (Levant, 1997).

Human DA neurons, compared with those in rodents, contain relatively few D_3 receptors (Gurevich and Joyce, 1999). However, the D_3 receptor has by far the highest DA affinity of all known DA receptors, dramatically greater receptor occupancy than other DA receptors, and greater signaling relative to other DA receptors, all in cloned human DA receptor assay systems (Richtand et al., 2001). Thus, despite the relative paucity of D_3 receptors on human DA neurons, the D_3 receptor may figure in human diseases (Seeman, 1999; Richtand et al., 2001). Colocalization studies show that most D_3-expressing neurons in the ventral limbic forebrain, including the nucleus accumbens, also express D_1 mRNA (Schwartz et al., 1998). Thus, our findings of attenuation of cocaine seeking with a D_3 antagonist may be consistent with reports (Self et al., 1996; Khroyan et al., 2000) that D_1 agonists inhibit cocaine-seeking behavior. Unfortunately, D_1 agonists are avidly self-administered (Self and Stein, 1992), dramatically enhance brain reward (Gardner et al., 1999), facilitate DA release in reward-relevant brain loci (Tomiyama et al., 1995), and partially substitute for addictive drugs in drug discrimination (Rosenzweig-Lipson and Bergman, 1993), making them unlikely treatments for addiction. D_3 antagonists, exemplified by SB-277011-A, appear more promising.

On the basis of the present data (Table 3), the subchronic effect of SB-277011-A on CPP requires further exploration. Clearly, the issue of dose dependency needs clarification. However, the very limited quantities of SB-277011-A available precluded subchronic dosing in the present study at doses >3.0 mg/kg or for >2 weeks. Although, in Table 3, it seems that SB-277011-A may have produced aversive effects (drug-paired, 5.6; vs vehicle-paired, 9.4), this seeming difference was not statistically significant. Also, additional limited subchronic SB-277011-A dosing in the present study using BSR yielded no rightward (i.e., dysphoric) shifts in brain reward functions (data not shown).

In the present study, the minimum effective dose of SB-277011-A to attenuate cocaine-induced CPP (0.3 mg/kg) was much lower than the minimum effective dose to attenuate cocaine-triggered reinstatement (6.0 mg/kg). This difference could be dose-related in the sense that the reinstatement-triggering dose of cocaine (1.0 mg/kg, i.v.) may have been significantly suprathreshold and therefore more difficult to overcome. Cocaine doses <1.0 mg/kg will trigger reinstatement (de Wit and Stewart, 1981). Alternatively, it could be that cocaine-induced CPP is fundamentally more vulnerable to D_3 antagonism than cocaine-induced reinstatement. In this regard, the absence of SB-277011-A dose dependence in blocking cocaine-induced CPP within the dose range used in the present experiments may be relevant; dose dependence may exist at lower doses. Similarly, it may be relevant that cocaine-associated cue-induced increases in forebrain DA are substantially lower than cocaine-induced increases (Bradberry et al., 2000), and perhaps easier for D_3 antagonism to overcome.

As we suggest above, the attenuation of the cue incentive properties of cocaine by SB-277011-A may indicate a unique clinical potential. We presume that the different experimental animal paradigms used in the present experiments have unique relevance for different aspects of human cocaine addiction. BSR presumably measures the direct rewarding properties of cocaine and may come closest to modeling the cocaine-induced subjective "high." CPP presumably measures drug-seeking behavior specifically evoked by the incentive salience (Bindra, 1968; Bolles, 1972; Dickinson and Balleine, 1994) acquired by environmental cues after repeated association with cocaine. Reinstatement presumably measures drug-seeking behavior specifically evoked by re-exposure to cocaine after behavioral extinction and (perforce) pharmacological detoxification. Although all three paradigms are arguably relevant to human cocaine dependence, and although SB-277011-A did produce effects in all three paradigms, the present data suggest that selective D_3 antagonism may hold the highest promise for attenuating cue-evoked relapses to cocaine use. Notably, however, almost no other potential pharmacotherapies have been found that block cocaine-triggered reinstatement (however, see De Vries et al., 2001). This may suggest a relatively unique therapeutic utility for D_3 antagonism.

In summary, the present findings show that the novel D_3 receptor antagonist SB-277011-A attenuates the rewarding and incentive motivational properties of cocaine in rats and confirm that D_3 receptors play an important role in these processes. The present findings further suggest that potent, selective D_3 antagonists hold promise as anti-addiction pharmacotherapeutic agents.

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