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Attenuation of Cue-Controlled Cocaine-Seeking by a Selective D_3 Dopamine Receptor Antagonist SB-277011-A

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Conditioned stimuli (CS) previously paired with drugs of abuse can elicit cravings in humans, relapse to drug use, and can also reinforce drug-seeking behavior in both humans and animals, events that are believed to be subserved in part by activation of the mesolimbic dopamine system. Converging anatomical, pharmacological, and behavioral evidence implicates dopamine D_3 receptors in the mechanisms underlying cue-controlled behaviors. The purpose of the present study was therefore to investigate the effects on cocaine-seeking behavior of a novel D_3 receptor antagonist, SB-277011-A, which is 100-fold more selective for D_3 over D_2 dopamine receptors. We have established previously that second-order schedules of reinforcement provide an animal model of cue-controlled drug-seeking both prior to and after cocaine has been self-administered. SB-277011-A dose-dependently decreased cocaine-seeking maintained by a cocaine-associated conditioned reinforcer in both the first, drug-free interval and also following self-administration of cocaine. At higher doses, SB-277011-A also increased the latency to receive the first CS presentation and cocaine infusion, thereby decreasing the number of cocaine infusions self-administered under the second-order schedule of reinforcement. SB-277011-A had no effect on cocaine intake under an FR-1 schedule of reinforcement, or on responding for sucrose under a second-order schedule of reinforcement, at any dose tested. These results therefore suggest that D_3 dopamine receptors may be critically involved in cue-controlled drug-seeking behavior independently of any interaction with the reinforcing effects of cocaine itself, and may therefore provide a therapeutic target in the treatment of relapse to cocaine use induced by CSs.
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INTRODUCTION

Cocaine abuse has been described as a chronic relapsing disorder, characterized by intense drug use interspersed with periods of abstinence (Gawin and Kleber, 1986). Drug-seeking, craving and relapse are well known to be triggered by environmental stimuli that have acquired motivational salience through repeated associations with a self-administered drug, especially the psychomotor stimulants (Childress *et al*, 1999; Everitt *et al*, 2001; Robinson and Berridge, 1993; Stewart *et al*, 1984). Subsequent use of the drug both strengthens the conditioned properties of these cues and amplifies their effects on behavior (Everitt *et al*, 1999). An understanding of the factors controlling cue-controlled drug-seeking, and their interaction with a self-administered drug may facilitate the development of pharmacological treatments of addictions by targeting drug-seeking behavior elicited and maintained by these conditioned stimuli (CS).

Second-order schedules of cocaine reinforcement provide an animal model of cue-controlled drug-seeking, both before and after the self-administered drug. As such, they allow for the measurement of resumption of cocaine use following a period of drug-seeking that is maintained by the CS, and also for the measurement of the potentiative effects of further psychostimulant use on drug-seeking behavior following a relapse to cocaine use (Everitt and Robbins, 2000). This schedule provides not only a model of environmental factors controlling relapse, but also of the impact of the drug on further craving and drug-seeking following resumption of drug use after abstinence. Under the schedule we have used, cocaine is available after a fixed time has elapsed; high response rates during this interval are maintained by contingent presentations of a cocaine-paired CS which acts as a conditioned reinforcer (Arroyo *et al*, 1998; Everitt and Robbins, 2000; Goldberg *et al*, 1975; Kelleher, 1966), and perhaps is related to cocaine-induced craving in human subjects (Robinson and Berridge, 1993). Once cocaine is self-administered, responding is further potentiated by the self-administered drug, reflecting the ability of psychomotor stimulants such as cocaine to amplify the control over behavior by conditioned reinfor-

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cers (Arroyo *et al*, 1998; Di Ciano and Everitt, 2001c; Pilla *et al*, 1999).

Subjective reports of craving following exposure to drug-paired stimuli (Ehrman *et al*, 1992) have been shown in functional imaging studies to be accompanied by activation of limbic structures such as the amygdala, cingulate cortex, and ventromedial prefrontal cortex (Breiter *et al*, 1997; Childress *et al*, 1999; Grant *et al*, 1996). Projections from these areas converge on the nucleus accumbens where they interact with mesolimbic dopaminergic afferents (Groenewegen *et al*, 1999) known to be important in mediating the reinforcing properties of psychostimulants (Berridge and Robinson, 1998; Di Chiara, 1998; Koob, 1996a, b; Wise, 1996). Specifically, D_3 DA receptors are primarily localized in the nucleus accumbens and amygdala (Bouthenet *et al*, 1991; Diaz *et al*, 1994, 1995; Landwehrmeyer *et al*, 1993), areas that have been shown to be critical in behaviors controlled by the presentation of conditioned reinforcers (Cador *et al*, 1989), and therefore represent a potential candidate for pharmacological therapeutic interventions that might act to diminish conditioned control over drug-seeking and relapse.

Anatomical and neurochemical studies have supported a role for limbic-striatal interactions in the control over behavior by conditioned reinforcers. For example, presentation of a drug CS to animals can induce large conditioned increases in DA (Di Ciano *et al*, 1998; Ito *et al*, 2000), supporting the suggestion that DA in the NAcc may be involved in cue-controlled drug-seeking (Wyvell and Berridge, 2000). By comparison, the contingent presentation of a CS did not increase DA efflux in the NAcc (Ito *et al*, 2000; Di Ciano *et al*, 2001a), while glutamatergic antagonism in the NAcc attenuated drug-seeking maintained by conditioned reinforcers (Di Ciano and Everitt, 2001c), suggesting that the NAcc may participate in this behavior through activity in its limbic-cortical afferents. Moreover, the integrity of the basolateral amygdala has been shown to be important in mediating the ability of conditioned reinforcers to support instrumental behavior (Burns *et al*, 1996; Cador *et al*, 1989), including cocaine-seeking behaviour (Whitelaw *et al*, 1996). Together, these studies suggest a critical role for limbic-striatal interactions in behavior controlled by contingent CSs, such as that studied with second-order schedules of reinforcement.

Strong support for the suggestion that D_3 receptors may be targets in the treatment of addictive behaviors was provided by the finding that the D_3 receptor partial agonist BP-897 decreased cue-controlled cocaine-seeking during the predrug interval of a second-order schedule of cocaine reinforcement (Pilla *et al*, 1999). However, the mechanism by which BP-897 produced this effect remains unclear as it can act as both an agonist and antagonist at dopamine D_3 receptors (Pilla *et al*, 1999; Wicke and Garcia-Ladona, 2001; Wood *et al*, 2000). The purpose of the present experiment was therefore to investigate the effects of a novel D_3 receptor antagonist, SB-277011-A, that is 100-fold selective for D_3 over D_2 receptors (Reavill *et al*, 2000), on cocaine-seeking using a second-order schedule of reinforcement. SB-277011-A has been shown to have no effect on spontaneous or stimulant-induced or basal locomotor activities, suggesting that D_3 receptor antagonists are free of the behavioral side effects associated with nonselective DA antagonists (Reavill *et al*, 2000). In support of the hypothesis that D_3 receptors mediate cue-controlled drug-seeking, in the present study we report that SB-277011-A decreased responding under a second-order schedule of cocaine reinforcement, without effect on cocaine intake under an FR-1 schedule of reinforcement, or on responding for sucrose under a second-order schedule of reinforcement.

METHODS

Animals

In total, 39 (seven in the FR-1 study, 22 in the second-order study, 10 in the sucrose study) naive male Lister-Hooded rats weighing 280–300 g at the time of surgery (Charles River; Kent, UK) were individually housed under a reversed 1200 h light–dark cycle (lights on at 2000 h). Rats were maintained on a diet of 20 g of Purina lab chow/day, sufficient to maintain body weight and growth throughout the experiment. Water was freely available and food was given within 2 h after daily testing. Experiments were carried out between 0900 and 2000 h, 6 or 7 days a week. Experiments were conducted in accordance with the United Kingdom 1986 Animals (Scientific Procedures) Act (Project License PPL 80/1324).

Apparatus

Rats were tested in operant chambers (Med Associates; 29.5 cm × 32.5 cm × 23.5 cm). Three sides were constructed from Perspex and the fourth was made of stainless steel, on which two 4 cm wide retractable levers were secured. The two levers were 12 cm apart, and 8 cm from the grid floor. Above each lever was a cue light (2.5 W, 24 V), and a red house light (2.5 W, 24 V) was located on the opposite wall. The floor of the chamber was lined with absorbent paper and covered with a metal grid. The testing chamber was placed within a sound- and light-attenuating box, equipped with a ventilation fan that also screened external noise. Silastic tubing shielded with a metal spring extended from each animal's i.v. catheter to a liquid swivel (Stoelting, Wood Dale, IL, USA) mounted on an arm fixed outside the operant chamber. Tygon tubing extended from the swivel to a Razel infusion pump (Semat Technical Ltd, Herts, UK) located adjacent to the external chamber. The operant chamber was interfaced to the Whisker operant control system with software written in Visual C++ (Cardinal, 2000).

Surgery

Rats were anesthetized with ketamine hydrochloride (100 mg/kg i.p.; Ketaset) and xylazine (9 mg/kg i.p.; Rompun) and supplemented with ketamine as needed (~20 mg). All rats in the cocaine studies were prepared with a single catheter in the right jugular aimed at the left vena cava. Catheters consisted of a 22 g cannula attached to Silastic tubing (0.012 ID) and fixed to nylon mesh. The mesh end of the catheter was sutured subcutaneously (s.c.) on the dorsum. All surgical instruments were thoroughly sterilized prior to surgery. To prevent infection, rats were treated postsurgically with 10 mg/kg Baytril (Bayer) s.c. for 8 days. See Caine *et al* (1992).

PROCEDURE

Acquisition of cocaine self-administration under an FR-1 schedule of reinforcement. Daily experimental testing began 7–10 days after surgical procedures. On each testing day, rats were connected to the i.v. line prior to the start of the training session. During initial training, rats acquired a lever press response for cocaine (0.75 mg/kg/inf/0.1 ml/5 s) under a fixed-ratio (FR-1, time-out 20 s) schedule of reinforcement. Under this schedule, each bar press resulted in illumination of the stimulus light above the lever and retraction of both levers, followed by a 'time-out' consisting of extinction of the house light for 20 s. Following this 20 s interval, the house light was again illuminated, the stimulus light was extinguished, and the two levers were again inserted into the testing box. 'Priming' injections of cocaine were never given. Active and inactive levers were counterbalanced between the left and right sides for individual animals. Presses on the inactive lever had no programmed consequences, but were recorded to assess general levels of activity. Sessions were limited to 2 h a day. To prevent accidental overdose, rats were limited to 30 infusions a day during the first 7 days of self-administration.

Acquisition of responding under a second-order schedule. Following stable responding under an FR-1 schedule of reinforcement, 22 rats were trained under a second-order schedule of the type FRx(FRy:S), such that x number of unit schedule requirements resulted in a cocaine infusion, and y number of lever presses resulted in illumination of the stimulus light (conditioned reinforcer) for 1 s within the unit schedule (Arroyo *et al*, 1998). Therefore, under a second-order schedule, rats were presented with two visual stimuli: (1) a 1 s illumination of the stimulus light and extinguishing of the house light after y responses, and (2) a 20 s illumination of the stimulus light and extinguishing of the house light during each cocaine infusion. The schedule requirements were gradually increased, beginning with a value of y set at 1 and a value of x of 5; each lever press resulted in a 1 s CS presentation, and following five such CS presentations, rats received a cocaine infusion and associated 20 s presentation of this stimulus light. Subsequently, the response requirements increased to FR10(FR1:S), FR10(FR2:S), FR10(FR4:S), FR10(FR7:S), and FR10(FR10:S). Following stable responding under the final schedule, a fixed interval (FI) 15 min(FR10:S) second-order schedule was introduced, and rats were allowed to stabilize and establish individual patterns of responding under this schedule. Under this schedule, cocaine was available following completion of an FR of 10 responses after an overall FI 15 min had timed out; during the (FI) 15 min, each FR10 response was reinforced by presentation of the drug-paired CS. Animals were permitted five infusions of cocaine per day under this schedule, or a maximum of 2 h per daily session.

Administration of SB-277011-A. Two unique and naive groups of rats were trained to respond for cocaine such that one group received SB-277011-A following stable responding under an FR-1 schedule of reinforcement and the other received SB-277011-A following stable responding under a second-order schedule of reinforcement. It took approximately 7–10 days for rats to reach stable responding under the final schedules, which was defined as less than a 30% variation in responding for at least 2 consecutive days. After rats achieved a stable baseline level of responding under either of the schedules, a habituation infusion of the vehicle was given. Pilot studies suggested that initial injections of the large volume of vehicle disrupted responding under a second-order schedule. Therefore, prior to habituation injections, the rats tested under second-order schedules were also given weekly injections of saline in a volume of 2 ml during the acquisition of responding under this schedule. The vehicle was 10% w.v. 2-hydroxypropyl-β-cyclodextrin, and all injections were given i.p. in a volume of 5 ml/kg. After stable responding was again achieved following the habituation injections, four counterbalanced doses of SB-277011-A were given in a Latin-square design. Stable responding was achieved between treatments. The four doses were selected on the basis of preclinical data and because they produced dose-dependent changes in responding in our pilot studies; they were vehicle, 0.333, 3.333, and 10 mg/kg of SB-277011-A.

Following completion of the initial dose–response study, 20 mg/kg and 30 mg/kg were tested to define further the behavior–dose response relation of SB-277011-A. Control injections of vehicle were counterbalanced such that half the rats were given the dose of drug first, and the other were given vehicle first. Thus, there were four within-subjects orders of treatment: (1) veh-30-veh-20, (2) veh-30-20-veh, (3) 30-veh-veh-20, (4) 30-veh-20-veh. Equal numbers of rats were randomly assigned to these four orders of treatment. At least 2 days of stable responding without treatment separated each injection. All injections were given 1 h prior to the start of experimental sessions.

Responding for sucrose under a second-order schedule of reinforcement. To determine whether the effects of SB-277011-A on cue-maintained responding were selective to cocaine-seeking, a separate group of 10 rats were trained to respond for a 20% sucrose solution under an FI15 min (FR10:S) schedule of reinforcement. The training and pretreatment were the same as that described above for cocaine. Only the 3, 10, and 20 mg/kg doses were used because of their effectiveness in the cocaine-seeking procedure. Rats were permitted one sucrose reinforcer per session.

Drugs

SB-277011-A (*trans-N*-[4-[2-(6-cyano-1,2,3,4-tetrahydro-2-isoquinolinyl)ethyl]-cyclohexyl]-4-[2–3H]quinolinylcarboxamide) was obtained from GlaxoSmithKline and is 100-fold more potent as a D_3 receptor antagonist than as a D_2 receptor antagonist (Reavill *et al*, 2000). SB-277011-A was made fresh daily and dissolved in 10% w.v. 2-hydroxypropyl-β-cyclodextrin by sonicating at 35°C for approximately 30 min. 2-Hydroxypropyl-β-cyclodextrin was obtained from Sigma, dissolved in double-distilled water (ddH_2O), made fresh as needed, and stored at 5°C.

Statistical Analyses

For all sessions, the number of active and inactive lever presses were recorded. In addition, the total number

and temporal pattern of reinforcements were also recorded. Bar presses on the active levers are presented as the mean ± SEM number of responses. Data collected from sessions of responding under second-order schedules of reinforcement were analyzed using two-way repeated-measures (interval × dose) ANOVAs and planned comparisons of each dose to vehicle separately for each interval. For responding for drug under the FR-1 conditions, data were analyzed using one-way repeated-measures ANOVAs and planned comparisons of vehicle to each dose. For all analyses, the vehicle control from the initial dose–response was compared to subsequent vehicle injections and collapsed by averaging the three conditions if they were not significantly different. This single value was then used as a comparison in subsequent analyses.

Latency to first CS, first cocaine infusion, and index of curvature were analyzed with one-way repeated-measures ANOVAs on the effect of dose for the first interval only. Latency provides a measure of the motivation to respond for the reinforcer (either the conditioned reinforcer or cocaine), and index of curvature provides a measure of the degree of scalloping in responding during interval schedules of reinforcement. The degree of scalloping is a measure of the ability of the reinforcer (ie cocaine) to maintain response patterns and also reflects the value of the drug reinforcer. Typically, under FI schedules, each interval begins with a pause in reinforcing, and responses show an accelerating increase towards the end of the interval. Index of curvature was calculated in the present study based on a method developed by Fry *et al* (1960). Index of curvature was measured for the first interval only to assess drug-seeking. Number of responses, latency to first CS and US, and index of curvature for the sucrose reinforcer were analyzed separately and are presented in Table 1.

For all analyses, criteria for significance were set at $p < 0.05$. The Huynh–Feldt correction for nonsphericity was used for all within-subjects comparisons (denoted as p_{HF} throughout).

RESULTS

Effect of SB-277011-A on Responding for Cocaine Under an FR-1 Schedule of Reinforcement

Active lever. One rat was excluded from the analyses because its response rates on the inactive lever were more than 2 SDs above the group mean (outlier). The three vehicle conditions were not significantly different from each other and were therefore collapsed into one control group. Injection of six counterbalanced doses of SB-277011-A to rats prior to the self-administration of cocaine under an FR-1 schedule of reinforcement was associated with approximately 50 cocaine infusions in 2 h, a rate that was not different from that observed following injection of vehicle (Figure 1). A one-way ANOVA on the effect of dose (six levels) revealed no significant effects ($n = 6$: $F(5, 25) = 1.212$, $p_{HF} = 0.333$).

Inactive lever. Rats made on average less than 10 responses on the inactive lever during the 2 h daily cocaine self-administration sessions. There were no significant effects of counterbalanced doses of SB-277011-A on the number of responses on the inactive lever (Figure 1; $n = 6$).

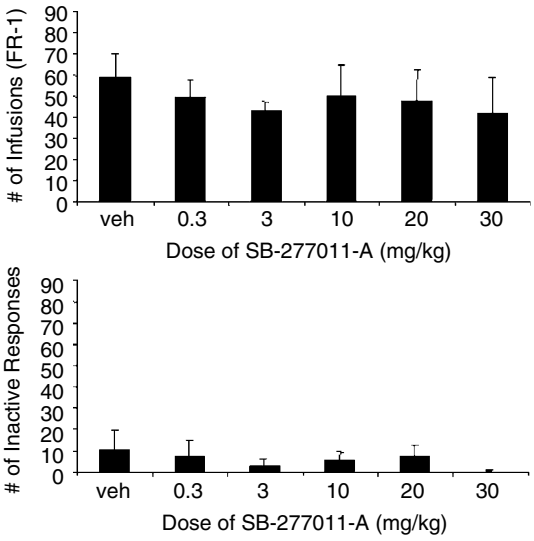

Figure 1 Effect of SB-277011-A on the number of responses on the active (top panel) and inactive (bottom panel) lever for cocaine under an FR-1 schedule of reinforcement. No significant effects were revealed ($n = 6$).

Effect of SB-277011-A on Responding for Cocaine Under a Second-Order Schedule

All 22 rats acquired responding under the FI15(FR10:S) second-order schedule for cocaine. During the course of the experiment, four rats were excluded early during the study because of illness that was not related to the self-administered cocaine or any of the treatments administered during the study. Two additional rats were excluded from the analyses due to patency failure of the catheters before completion of the final two treatment doses. Data from all three vehicle conditions were collapsed for both the active and inactive lever analyses as responses under these vehicle conditions were not significantly different from each other.

Active lever. Injection of increasing doses of SB-277011-A was associated with dose-dependent decreases in the number of responses observed during both the first and second intervals of responding for cocaine under a second-order schedule of reinforcement (Figure 2). Under control conditions following treatment with the vehicle, overall responding was approximately 165 ± 18 during the first interval and increased to approximately 218 ± 28 during the second interval following self-administered cocaine. A two-way repeated-measures interval (two levels) × dose (six levels) ANOVA revealed a significant effect of interval ($n = 16$; $F(1, 15) = 10.212$, $p_{HF} = 0.006$) and dose ($F(5, 75) = 13.251$, $p_{HF} \leqslant 0.0001$), indicating that response rates were greater in the second than the first interval, and that SB-277011-A produced dose-dependent decreases in responding in both intervals. Planned comparisons of the vehicle to each of the doses for each interval revealed that during the first interval, responding under the 10, 20, and 30 mg/kg doses was lower

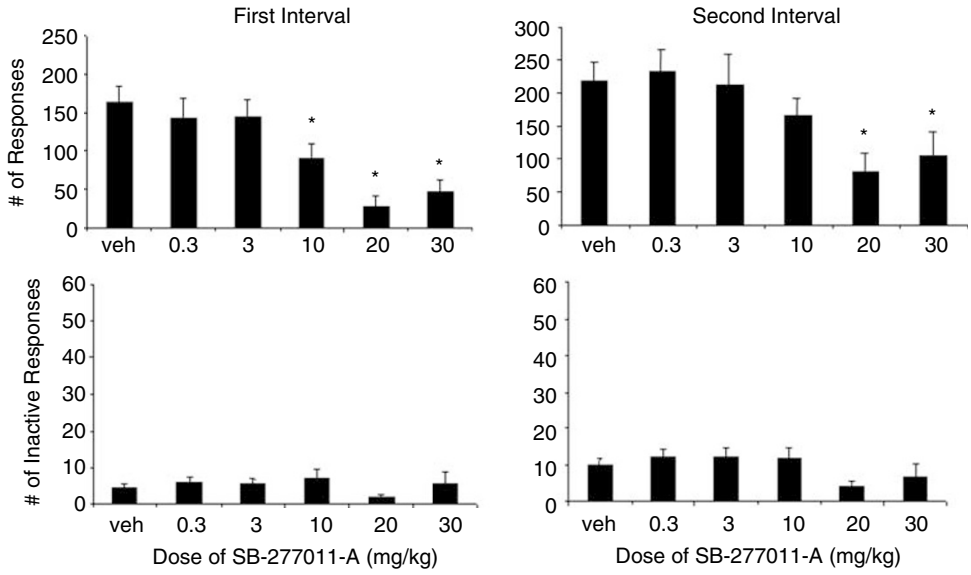

Figure 2 Effect of SB-277011-A on the mean ± SEM number of responses on the active (top panels) and inactive (bottom panels) levers during the first interval (left panels) and second interval (right panels) of responding for cocaine under a second-order schedule of reinforcement. *Signifies different from vehicle control ($n = 16$).

than those following vehicle. During the second interval, responding under the 20 and 30 mg/kg doses was significantly lower than vehicle.

Inactive lever. A constant number of presses on the inactive lever was observed following administration of six counterbalanced doses of SB-277011-A (Figure 2). ANOVA revealed an effect of interval but not of dose, indicating that responding during the second interval was significantly higher than in the first interval ($F(1, 15) = 8.441$, $p_{HF} = 0.011$; $n = 16$), but SB-277011-A had no effect on responding.

Intake. Under the second-order schedule of reinforcement used in the present study, rats were permitted a maximum of five cocaine infusions per day, or a maximal 2 h session. Pretreatment with increasing doses of SB-277011-A decreased the number of cocaine infusions self-administered during the allotted 2 h at the two highest doses tested ($n = 16$; Figure 3). A significant effect of dose was revealed for the six doses ($F(5, 75) = 11.019$, $p < 0.001$). Planned comparisons revealed that the number of cocaine infusions self-administered following the 20 and the 30 mg/kg doses of SB-277011-A was significantly lower than vehicle.

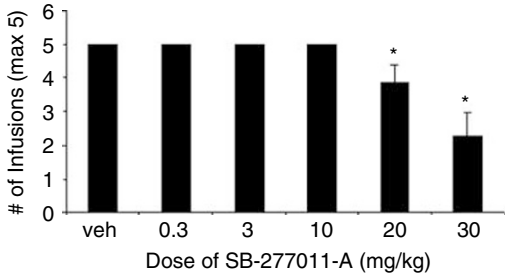

Figure 3 Effect of SB-277011-A on the number of cocaine infusions received during the 2 h session during which cocaine was available under an FI15(FR10:S) schedule of reinforcement. A total of five infusions were permitted during the sessions. All five infusions were received following pretreatment with 0, 0.3, 3, and 10 mg/kg of SB-277011-A. Dose-related decreases in intake were observed following administration of 20 and 30 mg/kg of SB-277011-A ($n = 16$; *signifies different from vehicle).

Effect of SB-277011-A on Index of Curvature During Responding for Cocaine Under a Second-Order Schedule of Reinforcement

One rat was excluded from the analyses of index of curvature because, due to computer failure, these data were not available for the 20 mg/kg dose. No significant differences were revealed for the three vehicle conditions so they were averaged and used as a single comparison for all doses. The mean index of curvature during the first interval following six doses of SB-277011-A (0.3 mg/kg: 0.23 ± 0.07; 3 mg/kg: 0.30 ± 0.03; 10 mg/kg: 0.25 ± 0.06; 20 mg/kg: 0.35 ± 0.08; 30 mg/kg: 0.27 ± 0.09) was similar to that following vehicle (0.35 ± 0.03; data not shown). No significant effects were revealed.

Effect of SB-277011-A on Latency to Respond During Responding for Cocaine Under a Second-Order Schedule of Reinforcement

Latency to receive the first CS presentation and first self-administered cocaine infusion provides a measure of the motivation for these reinforcers. For the 20 mg/kg dose, response rates were decreased to such an extent that three rats did not receive either a CS or US presentation, and for the 30 mg/kg dose of SB-277011-A eight rats did not receive either the CS or US. Therefore, for these rats, the maximal session time, 120 min, was entered as the latency to receive the first CS and US presentations. Analyses revealed no

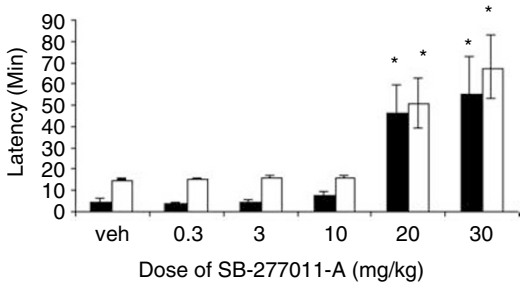

Figure 4 Effect of SB-277011-A on the latency to receive the first CS presentation (filled bars) and first cocaine infusion (open bars) during responding for cocaine under a second-order schedule of reinforcement. *Signifies different from vehicle.

significant differences between the vehicle conditions, and they were therefore collapsed and used as a single point of comparison for all doses.

Administration of six increasing doses of SB-277011-A produced increases in the latency to the first contingent presentation of the CS and the first US following administration of the 20 and 30 mg/kg dose of SB-277011-A (Figure 4). A two-way dose (six levels) × condition (two levels; latency to CS or US) ANOVA revealed a significant effect of dose ($n = 17$; $F(5, 80) = 13.927$, $p < 0.001$) and condition ($F(1, 16) = 110.11$, $p < 0.0001$), suggesting that the latency to receive the first CS and US increased with the dose of SB-277011-A administered. Planned comparisons revealed that the latency to receive the first CS and US was different from vehicle following either the 20 or 30 mg/kg doses.

Individual Response Records

Inspection of individual response patterns from representative rats revealed dose-related changes in responding during both the first and second intervals (Figure 5). Under vehicle, scalloped responding was observed, characteristic of the interval schedule used. Superimposed on these were the postreinforcement pauses typical of responding under ratio schedules, in this study reinforced by presentation of the CS. Administration of 10 mg/kg SB-277011-A prior to self-administration sessions decreased response rates during both the first and second intervals, but the latency to receive the first CS presentation was unaltered. By contrast, following 20 mg/kg of SB-277011-A the latency to receive both the first CS and US was increased, while responding was decreased to levels below those observed following 10 mg/kg of SB-277011-A. Following both the 10 and 20 mg/kg doses, scalloped responding was still evident for the cocaine infusion.

Effect of SB-277011-A on Responding for Sucrose Under a Second-Order Schedule of Reinforcement

Following administration of SB-277011-A to rats prior to responding for sucrose under an FI15(FR10:S) schedule of reinforcement, responding was similar to that observed following pretreatment with the vehicle. In addition, latencies to respond for either the CS or to obtain sucrose at the end of the first interval remained at the same level as that observed following treatment with vehicle (Table 1). One-way ANOVAs on the effect of dose revealed no significant effects.

Table 1 Effect of SB-277011-A on the Number of Responses and Latency to Receive the First CS or US Presentation During Responding for Sucrose Under an FI15(FR10:S) Schedule of Reinforcement

Dose	Vehicle	3 mg/kg	10 mg/kg	20 mg/kg
# Responses	177 ± 66	169 ± 44	250 ± 73	91 ± 31
Latency to CS	6.2 ± 0.9	6.8 ± 0.9	5.7 ± 1.5	6.9 ± 1.3
Latency to US	17.3 ± 1.2	17.1 ± 1.4	16.2 ± 0.2	19.5 ± 1.9

DISCUSSION

In these experiments the effects of a novel, highly selective dopamine D_3 receptor antagonist, SB-277011-A, on cocaine-seeking and cocaine-taking were investigated. Cocaine-seeking maintained by presentation of a cocaine-associated conditioned reinforcer was dose-dependently decreased, as indicated by marked reductions in responding under a second-order schedule of reinforcement. At higher doses, SB-277011-A also increased the latency to respond for this conditioned reinforcer and increased the latency to receive the first self-administered cocaine infusion, thereby leading to decreased drug intake. By contrast, SB-277011-A had no effect on cocaine intake under an FR-1 schedule of reinforcement, even at the highest doses tested. The selectivity of D3 receptors in mediating cue-controlled drug-seeking was further supported by the finding that SB-277011-A had no effects on responding for sucrose under a similar second-order schedule of reinforcement.

An important aspect of second-order schedules of reinforcement is that they allow cocaine-seeking maintained by the contingent presentation of cocaine-associated stimuli to be measured, free of the rate-altering effects of the drug on behavior (Arroyo *et al*, 1998). Previous studies have established the conditioned reinforcing properties of such stimuli under these schedules with the demonstration that responding under an interval schedule increased when drug-associated CSs were presented contingent on responding (Kelleher, 1966; Thomas and Stubbs, 1967). In addition, omission of the CS after training has been shown to result in a marked decrease in responding (Arroyo *et al*, 1998; Goldberg *et al*, 1981; Kelleher and Goldberg, 1977), emphasizing its importance in maintaining behavior.

As we have reported previously (Arroyo *et al*, 1998; Di Ciano and Everitt, 2001c), responding during the second interval, after cocaine had been self-administered, was increased over that measured in the first interval. This is consistent with the suggestion that stimulant drugs potentiate the control over instrumental behavior by a conditioned reinforcer (Arroyo *et al*, 1998; Cador *et al*, 1989; Pilla *et al*, 1999; Whitelaw *et al*, 1996), and thereby increase cue-controlled cocaine-seeking after relapse. Thus,

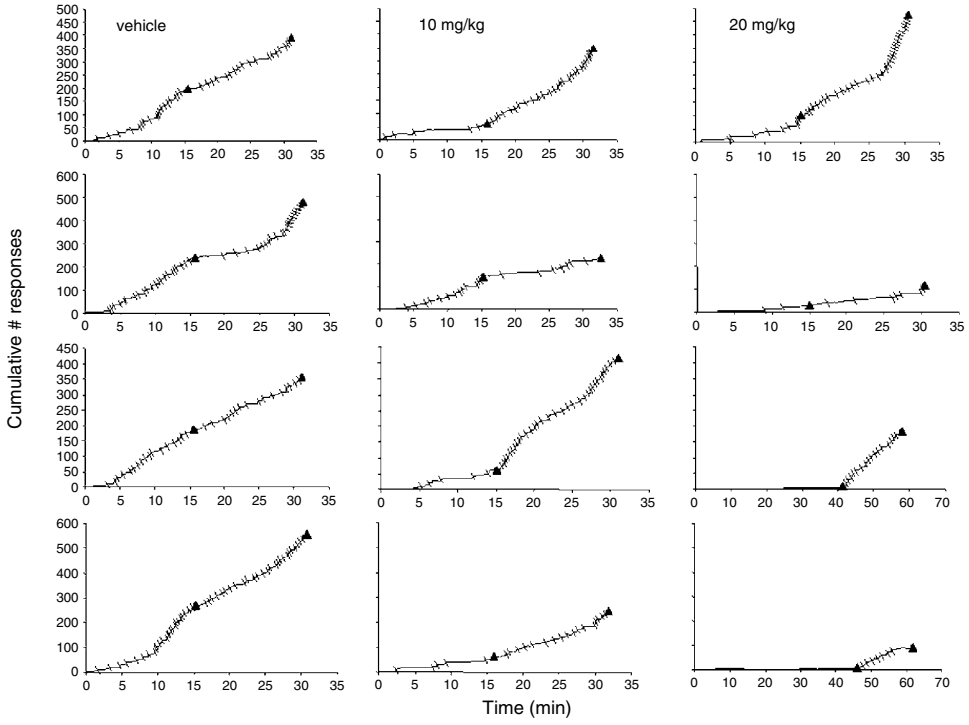

Figure 5 Individual response patterns for four representative rats (each row represents one rat) during responding for cocaine under an FI15(FR10:S) schedule of reinforcement following pretreatment with either vehicle (left column), 10 mg/kg (middle column) or 20 mg/kg (right column) of SB-277011-A. The cumulative response graphs illustrate that at the 10 mg/kg dose the response rates were decreased both before and after the first self-administered cocaine infusion (triangle), while the latency to receive the first CS presentation (diagonal deflections) was unaltered. By contrast, at the higher dose, the latency to receive both the first CS presentation and the first cocaine infusion were increased, as were the number of responses made.

the proportional decreases in responding during the first and second intervals produced by pretreatment with SB-277011-A is most parsimoniously explained by an attenuation of the impact of the conditioned reinforcing properties of the drug-paired stimulus, and not an effect on the primary reinforcing properties of cocaine.

At the higher doses tested, SB-277011-A attenuated not only responding under the second-order schedule of reinforcement, but also increased the latency to the first presentation of the contingent CS and the first cocaine infusion and thereby decreased intake of cocaine under this schedule. This increase in latency suggested that the decrease in cocaine intake under the second-order schedule was related to a decreased motivation to respond for cocaine, and not to a change in the reinforcing properties of cocaine itself. This suggestion is strengthened by the further observations that cocaine self-administration under an FR-1 schedule of reinforcement was not altered by SB-277011-A. Moreover, responding for sucrose under a second-order schedule of reinforcement was also unaltered by SB-277011-A. Thus, the effects of SB-277011-A were selective to cocaine-seeking under the control of cocaine-paired stimuli. Furthermore, baseline response rates for cocaine and sucrose were similar, so suggesting that the attenuation of drug-seeking produced by SB-277011-A was not related to a selective decrease in high-density responding (Salamone, 1996). Indeed, if SB-277011-A selectively attenuated high-density behaviors, then responding during the second interval would have been more disrupted than that during the first.

SB-277011-A has been shown to be 100-fold selective for D_3 over D_2 receptors. Indeed, administration of SB-277011-A at doses higher than those used in the present study (90 mg/kg) did not alter spontaneous locomotion or stimulant-induced locomotion, and was noncataleptic (Reavill *et al*, 2000). Thus, SB-277011-A is free of the motoric inhibition characteristic of nonselective $D_{2/3}$ receptor antagonists, and allows for the selective measurement of the effects of D_3 receptor blockade. Therefore, increased latencies, decreases in responding, and reduced intake of cocaine under a second-order schedule of cocaine reinforcement are most likely related to a behaviorally selective effect of blockade of D_3 receptors.

The present finding that SB-277011-A had no effect on cocaine self-administration under continuous reinforcement stands in contrast to findings reported for nonselective dopaminergic receptor agonists and antagonists. For example, cocaine self-administration was increased in a manner suggesting a reduction of the reinforcing effects of the drug following administration of the mixed D_2/D_3 or D_1/D_2 receptor antagonists YM-09-151-2 (Britton *et al*, 1991; Spealman, 1990), spiperone (Corrigall and Coen, 1991; Hubner and Moreton, 1991), sulpiride, metoclopramide, thioridiazine, chlorpromazine, haloperidol, pimozide, or α-flupenthixol (Roberts and Vickers, 1984). In addition, the D_2/D_3 dopamine receptor agonist 7-OH-DPAT had the opposite effect of dose-dependently decreasing cocaine self-administration (Caine and Koob, 1993) while a leftward shift in the dose–response curve confirmed that the D_2/D_3 receptor agonists 7-OH-DPAT and quinelorane potentiated

the reinforcing effects of cocaine (Parsons *et al*, 1996). The efficacy of a variety of D_2/D_3 receptor agonists to alter cocaine self-administration has been shown to be correlated with the potency of these compounds in binding to D_3 receptors *in vitro* (Caine *et al*, 1997). However, until now, lack of a selective D_3 receptor antagonist *in vivo* has precluded direct investigation of the contribution of D_3 receptors to the reinforcing effects of cocaine.

In support of the present observation, several studies have suggested instead that D_2/D_3 DA receptors may be critical in stimulus-maintained behaviors. For example, raclopride decreased cocaine-potentiated responding for a cocaine-paired stimulus (Weissenborn *et al*, 1996), while 7-OH-DPAT and quinpirole, which have D_2/D_3 receptor agonist properties, increased responding for a conditioned stimulus previously paired with cocaine (Self *et al*, 1996).

Mesolimbic DA in the nucleus accumbens has been shown to mediate the reinforcing properties of drugs of abuse (Di Chiara, 1999; Koob, 1996a, b; Wise, 1996) through interaction with its glutamatergic limbic afferents. These mechanisms are also critical in the control over behavior by CSs (Di Ciano *et al*, 2001b; Di Ciano and Everitt, 2001c), in a manner that has been shown to be dependent upon the basolateral amygdala (Cador *et al*, 1989; Whitelaw *et al*, 1996). Indeed, D_3 DA receptors are preferentially localized in the nucleus accumbens and amygdala (Bouthenet *et al*, 1991; Diaz *et al*, 1994, 1995; Landwehrmeyer *et al*, 1993), thereby suggesting that D_3 receptor antagonism may be a potential therapeutic target for the treatment of cocaine-seeking, especially when dependent on the presentation of conditioned reinforcers. In support of this hypothesis, the present study found that the selective D_3 receptor antagonist SB-277011-A decreased cue-controlled cocaine-seeking under a second-order schedule of reinforcement, without any effect on cocaine-taking (self-administration under continuous reinforcement), therefore suggesting that blockade of D_3 receptors did not directly interfere with the reinforcing properties of cocaine. Moreover, the index of curvature was not changed by administration of SB-277011-A, suggesting that the ability of cocaine to maintain response patterns was intact, consistent with the hypothesis that SB-277011-A did not influence the reinforcing properties of cocaine itself.

The neural site of action of SB-277011-A is unknown, but the candidate structures are relatively few since, in rats, the distribution of D_3 receptors is largely restricted to the postsynaptic sites in the nucleus accumbens, amygdala, and to mesolimbic DA cell bodies (Bouthenet *et al*, 1991; Diaz *et al*, 1994, 1995; Landwehrmeyer *et al*, 1993), where they have a localization suggestive of an autoreceptor function. The findings from this laboratory that both a D_3 dopamine receptor partial agonist, BP 897 (Pilla *et al*, 1999) and the full D_3 receptor antagonist SB-277011-A both decreased cocaine-seeking without effect on cocaine self-administration, suggests a common final mechanism and one that opposes conditioned activation of the DA system by a conditioned reinforcer.

It has been demonstrated that presentation of a drug-associated CS to animals can induce large conditioned increases in nucleus accumbens DA (Di Ciano *et al*, 1998; Ito *et al*, 2000), suggesting that DA in the nucleus accumbens may be involved in cue-controlled drug-seeking (Wyvell and Berridge, 2000) through interactions with limbic cortical afferents to the nucleus accumbens (Di Ciano *et al*, 2001b, c). Indeed, converging evidence implicates two dissociable amygdala-striatal subsystems in the control over behavior by either contingent or noncontingent CSs. For example, lesions of the central amygdala impaired the acquisition of a Pavlovian approach response (Parkinson *et al*, 1999), while infusion of the D_2/D_3 receptor agonist 7-OH-DPAT into this area also attenuated acquisition of Pavlovian approach (Hitchcott and Phillips, 1998). Moreover, central nucleus lesions impaired the enhancement of instrumental behavior by presentation of a Pavlovian CS (Pavlovian-instrumental behavior) (Hall *et al*, 2001a, b). Thus, the central amygdala may mediate conditioned increases in DA measured in the nucleus accumbens following the noncontingent presentation of a CS (Di Ciano *et al*, 1998; Ito *et al*, 2000), perhaps via projections to the ventral tegmental area (Parkinson *et al*, 2000).

By comparison, the basolateral amygdala may participate in the control over behavior by the contingent presentation of CSs (ie conditioned reinforcement). For example, lesions of the basolateral amygdala impaired the acquisition of a new response with conditioned reinforcement (Burns *et al*, 1993; Cador *et al*, 1989) and the acquisition of cocaine-seeking under a second-order schedule (Whitelaw *et al*, 1996). Reversible inactivation of the basolateral amygdala also blocked both the acquisition and expression of the response-maintaining properties of a CS under both second-order and reinstatement conditions (Kruzich and See, 2001; Grimm and See, 2000; Kantak *et al*, 2002). Neurochemical investigations have also revealed that the noncontingent presentation of a cocaine-paired CS to rats did not increase DA levels in the whole amygdala (Tran-Nguyen *et al*, 1998). Nevertheless, infusion of the D_2/D_3 receptor agonist 7-OH-DPAT into the basolateral amygdala potentiated responding for a conditioned reinforcer (Hitchcott *et al*, 1997; Hitchcott and Phillips, 1998), thereby implicating the basolateral amygdala in this behavior. Together, these studies suggest that the basolateral amygdala may be a critical site of action of SB-277011-A in attenuating cue-maintained responding under a second-order schedule of reinforcement. In addition, these findings also suggest that the partial agonist BP897 that has been previously shown to decrease responding for cocaine under a second-order schedule of reinforcement during the first interval only (Pilla *et al*, 1999), may do so through similar antagonistic mechanisms as SB-277011-A, but it is plausible that the partial agonist also decreases dopamine neuronal activity by a direct action on D_3 autoreceptors on ventral tegmental neurons (Diaz *et al*, 1995). Future intracerebral studies will clarify this issue.

CONCLUSIONS

Administration of a novel, highly selective D_3 DA receptor antagonist SB-277011-A decreased cue-maintained responding for cocaine under a second-order schedule of reinforcement with no effect on cocaine intake or responding for sucrose under a second-order schedule of reinforcement. Together with previous findings that high doses of SB-277011-A had no effects on spontaneous or stimulant-

induced activity, these results suggest a selective role for D_3 DA receptors in the motivational impact of CSs. These findings therefore suggest the potential therapeutic utility of D_3 DA receptor antagonists in the treatment of cue-controlled cocaine-seeking and relapse.

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