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ARTICLE

Selective antagonism at dopamine D_3 receptors attenuates cocaine-seeking behaviour in the rat

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Abstract

Dopamine (DA) D_3 receptors have been suggested to play a role in mechanisms underlying the ability of drug-associated cues to induce drug-seeking behaviour. The present study investigated whether SB-277011-A, a selective DA D_3 receptor antagonist, modulates reinstatement of cocaine-seeking behaviour induced by cocaine-associated stimuli. The study also explored whether or not this modulation is generable to seeking behaviours associated with a nutritive reinforcer such as sucrose. Separate groups of rats were trained to associate discriminative stimuli (S^D) with the availability of cocaine or sucrose pellets vs. non-reward under a FR1 schedule of reinforcement. Each reinforced response was followed by a response-cue signalling a 20-s time-out (TO). After the self-administration training criterion was met, rats underwent extinction during which cocaine, sucrose pellets and S^Ds were withheld. Reinstatement tests, separated by 3 d during which rates of responding under extinction conditions remained at the criterion, were performed by presenting S^Ds non-contingently together with the contingent presentation of response-cues signalling a 20-s TO. Within- and between-subjects experimental designs revealed that 10 and 30 mg/kg SB-277011-A attenuated reinstatement of cocaine-seeking. SB-277011-A (10 mg/kg) did not modify conditioned reinstatement triggered by sucrose pellet-associated cues. These results, provided they can be extrapolated to abstinent human addicts, suggest the potential therapeutic use of selective DA D_3 receptor antagonists for the prevention of cue-controlled cocaine-seeking and relapse.

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Introduction

Cocaine abuse is a chronic relapsing disorder that is characterized by drug use interspersed with periods of abstinence (Gawin and Kleber, 1986). Numerous factors, including drug-associated cues, stress and re-exposure to the drug itself may trigger relapse or reinstatement of drug-seeking behaviours (O'Brien et al., 1988). Drug-seeking behaviour can be evoked solely by re-exposing abstinent or formerly detoxified subjects to environmental stimuli that have acquired motivational salience because of their repeated association with the self-administered drug (Childress et al., 1999; Ehrman et al., 1992; O'Brien et al., 1992).

Relapse to drug-seeking behaviour resulting from exposure to drug-associated stimuli has been reproduced and characterized in laboratory animals (de Wit and Stewart, 1981; Meil and See, 1996; Weiss et al., 2000). Extinction–reinstatement paradigms are currently being used to gain information on the anatomical, neurochemical, and pharmacological substrates underlying drug-seeking behaviour elicited by drug-associated cues (Ciccocioppo et al., 2001; Shaham et al., 2003; Shalev et al., 2002; Weiss et al., 2000).

It has been recently demonstrated that in the rat, presentation of discriminative stimuli predictive of cocaine availability together with conditioned stimuli associated with cocaine infusion can induce a significant conditioned increase in extracellular levels of dopamine (DA) both in the nucleus accumbens (NAc)

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and basolateral amygdala (BLA) (Di Ciano et al., 1998; Ito et al., 2000; Weiss et al., 2000), as well as activation of c-*fos* in the NAc, BLA and medial prefrontal cortex (mPFC) (Ciccocioppo et al., 2001; Neisewander et al., 2000). Neuroimaging studies in humans have also shown that cocaine cue-induced craving is associated with activation of the NAc, BLA and mPFC (Breiter et al., 1997; Childress et al., 1999; Garavan et al., 2000; Grant et al., 1996).

Although there is support for a role of DA D_1 and D_2 receptors in controlling drug-seeking behaviour evoked by cocaine-associated cues (Cervo et al., 2003; Weiss et al., 2000), a growing body of evidence suggests that DA D_3 receptors may play a significant role in the control of drug-seeking behaviour (for comprehensive reviews, see Heidbreder et al., 2004, 2005; Joyce and Millan, 2005; Newman et al., 2005). First, DA D_3 receptors show highest density in limbic regions such as the ventral striatum (Bouthenet et al., 1991; Diaz et al., 1994, 1995; Landwehrmeyer et al., 1993), brain areas that seem to play a key role in behaviours controlled by the presentation of cocaine-associated cues (Di Ciano et al., 1998; Ito et al., 2000; Weiss et al., 2000; Whitelaw et al., 1996). Second, DA D_3 receptors are up-regulated in the NAc of cocaine overdose fatalities (Mash, 1997; Segal et al., 1997; Staley and Mash, 1996), and in rats after cocaine self-administration (Neisewander et al., 2004) or cocaine cue-induced conditioned hyperlocomotion (Le Foll et al., 2002). Third, selective blockade of DA D_3 receptors by SB-277011-A (*trans-N*-[4-[2-(6-cyano-1,2,3,4-tetrahydroisoquinolin-2yl)ethyl]cyclo-hexyl]-4-quinolininecarbo-xamide), a highly potent and highly selective DA D_3 receptor antagonist (Reavill et al., 2000), attenuates the rewarding effects of cocaine as measured by intra-cranial self-stimulation and conditioned place preference (Vorel et al., 2002). Fourth, selective DA D_3 receptor blockade attenuates cocaine- or stress-triggered reinstatement of cocaine-seeking behaviour (Vorel et al., 2002; Xi et al., 2004). Furthermore, SB-277011-A reduces cocaine cue-induced drug-seeking under a second-order schedule maintained by cocaine self-administration (Di Ciano et al., 2003). Finally, recent studies show that SB-277011-A and NGB 2904, another selective DA D_3 receptor antagonist (Newman et al., 2003; Yuan et al., 1998), produce a dose-dependent inhibition of cue-induced reinstatement of cocaine-seeking behaviour (Gál and Gyertyán, 2005; Gilbert et al., 2005).

Stimuli paired contiguously with cocaine infusions in self-administering rats can reinstate responding following extinction (Gál and Gyertyán, 2005; Gilbert et al., 2005), but they often produce only weak and transient effects. The lack of robust and enduring behavioural effects of cocaine-associated cues in these and other 'reinstatement' paradigms (deWit and Stewart, 1981; Fuchs et al., 1998; Tran-Nguyen et al., 1998; Weissenborn et al., 1995) appears inconsistent with the presumed strength and persistence of motivating effects of drug cues in humans (Childress et al., 1993). An important consideration concerning the stimulus control of drug-seeking behaviour involves the role of discriminative stimuli (Weiss et al., 2000). Discriminative stimuli signal the availability of a reinforcer and thereby set the occasion to engage in behaviour that brings the organism in contact with the reinforcing substance. Indeed, a condition often associated with drug craving in humans is cognitive awareness of drug availability (Meyer and Mirin, 1979). It has been argued, therefore, that the manner in which drug-associated contextual cues attain their incentive properties is likely to involve the predictive nature of these stimuli rather than only classically conditioned stimulus-response associations (McFarland and Ettenberg, 1997). This hypothesis is supported by the results obtained in a recently developed model of extinction/reinstatement in which cocaine-associated cues induce a robust and enduring drug-seeking behaviour in abstinent rats (8–10 test sessions), as measured by the recovery of extinguished responding at a previous drug-paired lever (Weiss et al., 2000, 2001; Cervo et al., 2003). This procedure, which imposes a discriminative stimulus (S^D) predictive of drug availability to discrete cues usually paired to each drug self-infusion, significantly differs from the more common extinction/reinstatement procedure and seems to model the persistence of conditioned cue reactivity and cue-induced craving in humans (Childress et al., 1993). Using such a procedure, we have recently shown that BP 897, a non-selective DA D_3 partial agonist, reduces drug-seeking behaviour induced by presentation of drug-associated cues that are predictive of cocaine availability after a period of extinction and in the absence of cocaine (Cervo et al., 2003). However, the interpretation of the latter finding was confounded by the fact that BP 897 has moderate to high affinity for different receptors (Cussac et al., 2000; Pilla et al., 1999) and shows antagonist properties at both DA D_2 and D_3 receptors (Wicke and Garcia-Ladona, 2001; Wood et al., 2000). Furthermore, BP 897 produces conditioned place aversion in rats (Duarte et al., 2003; Gyertyán and Gál, 2003, but see Aujla and Beninger, 2005).

Accordingly, the present study assessed the efficacy of the selective DA D_3 receptor antagonist SB-277011-A in preventing drug-seeking behaviour induced by re-exposure to cocaine-associated cues. In an attempt to better characterize the specificity of the observed effects, the efficacy of SB-277011-A to modulate seeking behaviour induced by re-introduction of cues associated with a natural reward (sucrose pellets) was also assessed.

Materials and Methods

Animals

Male Sprague–Dawley CD-COBS rats (Charles River, Calco, Italy) weighing 150–175 g at the beginning of the experiments were used. They were housed individually at constant room temperature (21 ± 1 °C) and relative humidity (60%) under an inverted light/dark schedule (lights 08:00 to 20:00 hours) with food and water ad libitum. Animals were allowed to adapt to laboratory conditions for at least 2 wk and were handled for 5 min a day during this period. Procedures involving animals and their care were conducted in conformity with institutional guidelines in compliance with national (D.L. n. 116, G.U., suppl. 40, 18 Febbraio 1992, Circolare No. 8, G.U., 14 Luglio 1994) and international laws and policies (EEC Council Directive 86/609, OJL 358, 1, Dec. 12, 1987; Guide for the Care and Use of Laboratory Animals, U.S. National Research Council, 1996).

Drugs

Cocaine hydrochloride (MacFarlan Smith, Edinburgh, UK) was dissolved in sterile physiological saline once a week, aliquoted for daily use and stored at −20 °C until used. SB-277011-A (Psychiatry CEDD, GlaxoSmithKline, Verona, Italy) was dissolved immediately before use in 10% w/v of 2-hydroxypropyl-β-cyclodextrin (Sigma, Milan, Italy) and injected i.p. in a volume of 2 ml/kg.

Apparatus

Animals were trained and tested using standard rodent operant test chambers (ENV-007, MED Associates Inc., St Albans, VT, USA) constructed from heavy-duty aluminium except for clear polycarbonate back, door and top, and equipped with two retractable levers and three lights, each 2.8 W, 24 V, one in the middle back of the ceiling (the house light), and two on the front panel 6 cm above each lever. Auditory stimuli consisted of a 20-dB white noise above the background or a 7-kHz, 70-dB intermittent tone that were both presented through an 80-ohm speaker fitted in the centre of the back panel. Each experimental chamber was placed inside a sound-attenuating chamber with an exhaust fan mounted on one side. Intravenous infusions were administered by a syringe pump (PHM-100, MED Associates Inc.) located inside each sound-attenuating cubicle. Sound generator, stimulus lights, pellet dispenser and syringe pump were controlled by an IBM compatible computer with MED software, which also monitored input from the levers, and recorded data from each experiment.

Chronic jugular catheter

Catheters were made in-house using guide cannulae (C313G 5UP, Plastic One Inc., Roanoke, VA, USA), silicon tubing (0.30×0.60 and 0.64×1.19 mm i.d. × o.d., Degania Silicone Ltd, Emek Hayarden, Israel), dental cement (Paladur, Heraus Kulzer GmbH, Wehrheim/Ts., Germany) and silicon rubber (Elastosil E43, Wacker-Chemie GmbH, München, Germany) according to Caine et al. (1993), with a few modifications. Rats were anaesthetized with equithesin 3.0 ml/kg i.p. and a silastic catheter, sterilized in 70% alcohol, was implanted in the right jugular vein. During the 5-d recovery period, rats received one daily subcutaneous (s.c.) injection of 45 mg/kg ampicillin (Amplital®, Pharmacia, Nerviano, Italy). Catheters were kept patent by daily intravenous (i.v.) infusions of 0.1 ml heparinized (30 U/ml) sterile 0.9% saline before and after each self-administration session. When rats showed unusual poor acquisition of lever pressing or poor patterns of self-administration, the catheters' patency was verified by injecting 0.05 ml i.v. of a solution containing 1.25 mg/ml midazolam maleate (Roche, Basel, Switzerland) + 25 mg/ml ketamine hydrochloride (Sigma-Aldrich, Milan, Italy). Animals with patent catheters displayed clear signs of sedation within 3 s (Caine et al., 1999). Rats with clogged catheters had a new catheter implanted in the contralateral jugular vein.

Effect of SB-277011-A on seeking behaviour induced by re-introduction of cocaine-associated cues

All training and testing sessions were conducted during the dark phase of the light/dark cycle at approximately the same time every day, and each rat was always exposed to the same chamber. Self-administration and conditioning procedures were identical to those previously described (Cervo et al., 2003) with the exception that in the present study rats were not trained for food at the beginning of the experiment, but were required to immediately press the active lever for cocaine self-administration.

Rats ($n = 7$) were trained to self-administer i.v. cocaine 0.25 mg/0.1 ml/infusion, under a fixed ratio 1 (FR1) schedule for 2-h per day while simultaneously establishing discriminative stimuli associated with and predictive of cocaine availability or non-availability. Training sessions started with extension of the active and inactive levers and concurrent presentation of white noise (20 dB above background) that lasted throughout the session and served as the discriminative stimulus (S^{D+}) for cocaine availability. No limit in the number of cocaine infusions was imposed. After each infusion that lasted 6 s, the lever remained inactive for 20 s to prevent accidental overdosing. This time-out (TO) period was signalled by a cue light above the active lever.

From day 3 of training, rats were subjected to a discrimination learning regime that comprised 3 daily 1-h sessions presented in random order. Two sessions were related to 1-h cocaine-self-administration as stated above. In the third session cocaine was replaced with saline. Saline sessions began with extension of both levers and simultaneous illumination of the house light that remained on for the duration of the session and served as a discriminative stimulus for cocaine non-availability (S^{D-}). Each response on the active lever produced an infusion of 0.1 ml saline and presentation of the intermittent tone (7 kHz, 70 dB) as a TO cue for 20 s, during which time the lever remained inactive. This training was conducted daily until cocaine-reinforced responding stabilized ($\pm 10\%$ over three consecutive training days) and rats almost stopped responding during saline sessions ($\leqslant 5$ responses during each of the three successive sessions).

After meeting the self-administration training criterion each rat was placed under extinction conditions until the end of the experiment. Sessions began with extension of both levers with no presentation of S^Ds. Responses on the previously active lever resulted in activation of the syringe pump motor, but had no other programmed consequences. Responses on the inactive lever were also recorded, but had no programmed consequences. One daily 1-h extinction session was conducted until a criterion of $\leqslant 5$ responses per session on active and inactive levers separately over three consecutive days was met.

Reinstatement tests began one day after individual animals met the extinction criterion. Tests lasted 1-h during which rats were exposed to the S^{D+} or S^{D-} under conditions identical to the discrimination learning phase, except that cocaine and saline were not available. In both conditions, responses on the previously active lever were followed by activation of the syringe pump motor and a 20-s signalled TO period during which both levers remained inactive. To study the dose–response effect of SB-277011-A, each reinstatement test session was followed by a new extinction session until re-test. Test sessions for each animal were separated by at least 3 d during which rates of responding under extinction conditions remained at the relevant criterion. To control for order effects, different drug doses and the vehicle were administered in a random sequence across reinstatement test sessions.

Seeking behaviour induced by re-introduction of sucrose pellet-associated cues

In this part of the study we evaluated the efficacy of SB-277011-A to modulate seeking behaviour induced by re-introduction of stimuli associated with a nutritive reward (sucrose) in order to verify if the results obtained in the cocaine-associated cue experiment reflect a selective activity towards drug-seeking behaviour or a more general 'anhedonia' for reward. In this first experiment we evaluated the reproducibility of reinstatement induced by cues associated with sucrose pellet self-administration. The procedure used in this study was essentially the same as that described for the previous experiment except that rats were not catheterized and the reinforcer was no longer cocaine, but a 45-mg sucrose pellet (Noyes formula F, Sandown Scientific, Esher, Surrey, UK).

Thus, eight free-feeding rats were trained to press the active lever for sucrose pellets with simultaneous presentation of S^Ds associated with and predictive of pellet availability (S^{D+}) or non-availability (S^{D-}). These sessions started with extension of the active and inactive levers and concurrent presentation of appropriate S^D, the white noise predictive of sucrose pellet availability or illumination of the house light predictive of no reward, which lasted throughout the session.

Sucrose pellets were available under an FR1 schedule of reinforcement. After each reinforced response the lever remained inactive for 20 s. This TO period was signalled by a cue light above the active lever. Each session was terminated after 30 min or after 35 sucrose pellets were earned. This limit was imposed to avoid satiety and to maintain a similar number of reinforcers per session with the cocaine experiment. Non-reinforced sessions were identical to saline sessions described in the cocaine experiment with the only difference that the active lever press resulted in the triggering of the feeder motor for

0.5 s instead of the infusion pump. The duration of each session was 30 min. This training was conducted daily until sucrose pellet-reinforced responding stabilized (±10% over three consecutive training days) and rats almost stopped responding during no-reward sessions (⩽5 responses during each of the three successive sessions).

Extinction and reinstatement test sessions were identical to those described for cocaine except that the duration was decreased to 30 min. After having met the extinction criterion, rats were tested according to a within-subjects design consisting of several presentations of sucrose pellet-associated stimuli and one of no-reward-associated cues randomly presented. After the initial reinstatement test session, rats underwent extinction conditions until retested. Test sessions for each animal were separated by at least 3 d during which rates of responding under extinction conditions remained at the relevant criterion.

Effect of SB-277011-A on seeking behaviour induced by sucrose pellet-associated cues: a between-subjects experimental design

Because of the diminishing ability of cues associated with sucrose pellet self-administration to induce reinstatement, a between-subjects experimental design was used to evaluate whether SB-277011-A influenced the seeking behaviour for a natural reward. The procedure used in this experiment was identical to the one described in the previous study, except that four independent groups of eight animals were used. As soon as animals reached the extinction criterion each group was randomly assigned to tests in the presence of sucrose pellet-associated cues or no-reward-associated stimuli following pre-treatment with either SB-277011-A (10 mg/kg i.p., 30-min pre-treatment) or vehicle. The dose selected was the minimal active dose that reduced cocaine-seeking behaviour in the within-subjects experimental design (see Results section).

Effect of SB-277011-A on drug-seeking behaviour induced by cocaine-associated cues: a between-subjects experimental design

This experiment examined the effect of SB-277011-A (10 mg/kg) or vehicle on drug-seeking behaviour induced by cocaine-associated cues in a between-subjects experimental design. The procedure used in this experiment was identical to the one described in the first experiment except that four independent groups of seven rats were used. As soon as rats reached the self-administration criterion they underwent extinction sessions as described above. When the extinction criterion was met rats were randomly assigned to tests under either saline-associated cues or cocaine-associated stimuli following pre-treatment with either SB-277011-A (10 mg/kg i.p., 30 min pre-treatment) or vehicle.

Statistical analysis

All data are expressed as the mean ± S.E.M. number of active and inactive lever presses during the self-administration, extinction and reinstatement phases.

In the first and second experiments, the number of cocaine infusions or sucrose pellets earned in the two separate daily sessions were analysed by a one-way analysis of variance (ANOVA) for repeated measurements with test sessions as the main factor. Also the number of lever presses during the last 3 d of saline or no-reward self-administration and the last 3 d of extinction, before and between the different reinstatement sessions were analysed separately as above. Since there were no differences between sessions, the last 3 d for each condition were pooled for further statistical analyses. Thus, the effect of SB-277011-A on reinstatement induced by cocaine-associated cues or sucrose pellet-associated cues to revive seeking behaviour were analysed by a one-way ANOVA with repeated measurements with test sessions as the main factor. Whenever a significant effect was found, post-hoc comparisons were done using the Newman–Keuls test.

In the third and fourth experiments, the number of cocaine infusions or sucrose pellets earned in the two separate daily sessions were analysed by a mixed factorial ANOVA (with test sessions as the within-subjects factor and groups as the between-subjects factor). Since no differences were found, data from the two daily sessions were pooled for successive analyses. Thus, differences between groups in the daily mean number of lever presses during the last 3 d of cocaine or sucrose pellet, the last 3 d of saline or no-reward self-administration sessions and the preceding three extinction sessions were analysed by mixed factorial ANOVA (with test sessions as the within-subjects factor and groups as the between-subjects factor). The effects of SB-277011-A on the number of lever presses from the reinstatement sessions were analysed by two-way ANOVA (with treatment and cues presentation as the main factors). Whenever a significant effect was found, post-hoc comparisons were done using the Newman–Keuls test.

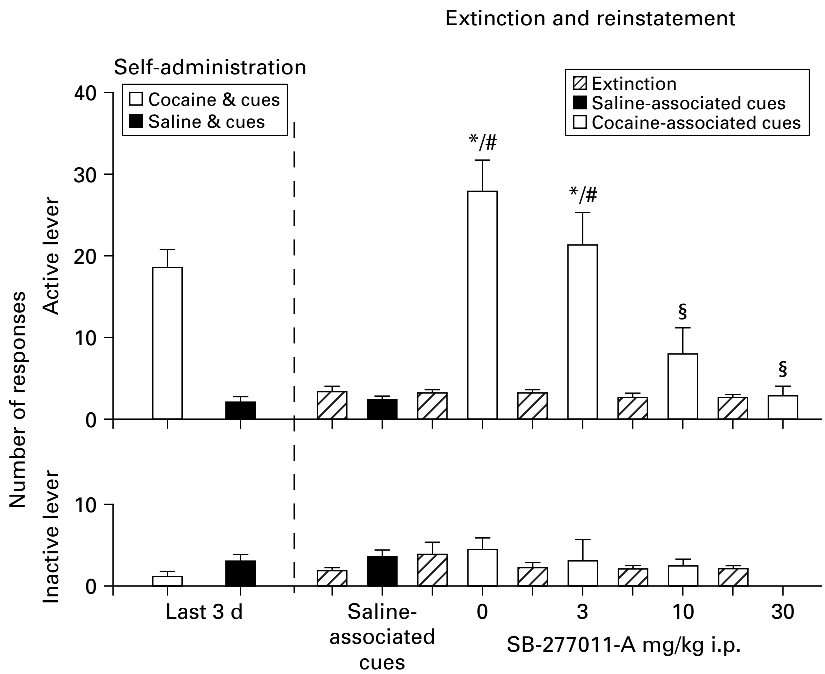

Figure 1. Effect of SB-277011-A on the number of responses on the active and inactive levers after re-introduction of cocaine-associated stimuli. Results are the mean ($n=7$) $\pm$ S.E.M. number of presses on the active and inactive levers. The number of lever presses during self-administration training (mean of last 3 d), extinction (mean of last 3 d before reinstatement sessions), and in the presence of stimuli associated with no-reward during reinstatement session is also shown. Data were analysed by one-way ANOVA for repeated measurements (with sessions as main factor) followed by Newman–Keuls post-hoc comparison. * $p<0.05$ different from the no-reward stimuli; # $p<0.01$ different from the three preceding extinction sessions; § $p<0.01$ different from the vehicle + cocaine-associated stimuli group, Newman–Keuls test.

Results

Effect of SB-277011-A on seeking behaviour induced by re-introduction of cocaine-associated cues

Figure 1 shows the responses on the active and inactive levers during self-administration training (mean of the last three sessions), extinction (mean of the last three sessions before reinstatement tests) and reinstatement phases.

Rats developed stable cocaine self-administration and the number of lever presses for saline gradually decreased (the mean $\pm$ S.E.M. number of days from the beginning of discrimination regimen required to meet the training criterion was 19.3 ± 1.1). During the last 3 d of stable self-administration, there were no significant differences between the first and second daily hours of self-administration, and responding on the inactive lever was minimal (in following statistical data AL = active lever, IL = inactive lever) [$F_{AL}(5, 30)=0.7$, $p>0.05$ and $F_{IL}(5, 30)=1.6$, $p>0.05$]. Also the number of lever presses during saline sessions did not differ [$F_{AL}(2, 12)=1.2$, $p>0.05$ and $F_{IL}(2, 12)=1.7$, $p>0.05$].

During extinction sessions the number of presses on the active lever gradually decreased and all rats met the extinction criterion after an average of 16.9 ± 2.7 sessions. At this stage responding on the inactive lever was always below the criterion and no significant differences were found during the last 3 d preceding the first reinstatement test session on the number of presses on either the active or inactive lever [$F_{AL}(2, 12)=0.9$, $p>0.05$ and $F_{IL}(2, 12)=1.1$, $p>0.05$].

Re-introduction of cocaine-associated cues led to immediate recovery of responding that was significantly higher than after introduction of saline-associated stimuli and the three preceding extinction sessions (both $p<0.01$, Newman–Keuls test). The overall behavioural output after presentation of cocaine-associated cues was similar to cocaine self-administration and significantly different from saline self-administration ($p<0.01$, Newman–Keuls test). The number of active lever presses during presentation of saline-associated stimuli did not differ from the three preceding extinction sessions. As reported in Figure 1, lever presses during extinction

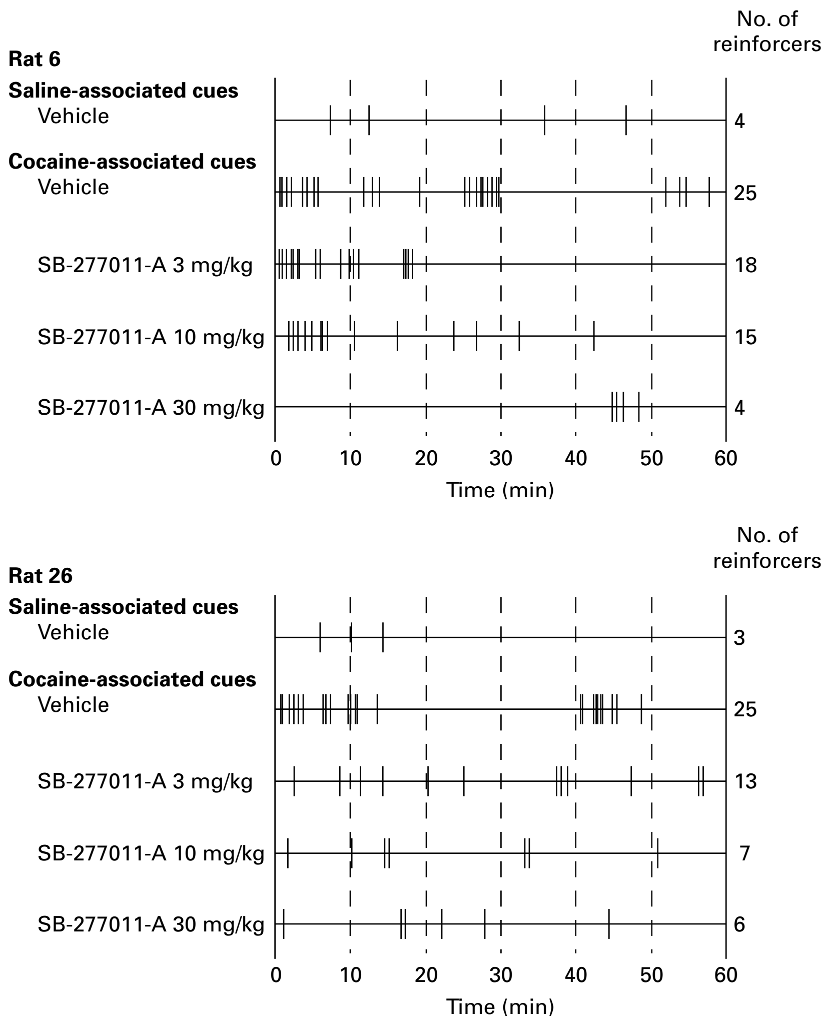

Figure 2. Effect of SB-277011-A on the frequency of active lever presses during reinstatement sessions in two representative rats. The abscissa represents time in minutes, and each mark depicts an active lever press. In vehicle-treated rats, re-introduction of cocaine-associated stimuli elicited a marked behavioural response. SB-277011-A reduced responding during the entire session in a dose-dependent manner.

sessions preceding re-introduction of cocaine- and saline-associated stimuli did not differ.

Pre-treatment with SB-277011-A (0, 3, 10, 30 mg/kg i.p.) significantly reduced the number of lever responses induced by cocaine-associated cues in a dose-dependent manner [$F_{AL}(11, 66) = 25.2$, $p < 0.01$, one-way ANOVA for repeated measurements]; these lever responses were no longer significantly different after 10 and 30 mg/kg from either the number of presses induced by saline-associated stimuli or those emitted during the three preceding extinction sessions ($p > 0.05$, Newman–Keuls test). SB-277011-A did not significantly alter the number of responses on the inactive lever [$F_{IL}(11, 66) = 1.7$, $p > 0.05$, one-way ANOVA for repeated measurements]. As shown in Figure 2, response patterns of individual animals on the active lever revealed that the frequency of response in the presence of cocaine-associated cues was greatest at the beginning of the session, but sustained responding was observed throughout the whole session. SB-277011-A reduced responding during the entire session in a dose-dependent manner.

Seeking behaviour induced by re-introduction of sucrose pellet-associated cues

In this experiment rats developed stable self-administration for sucrose pellets within 7 training

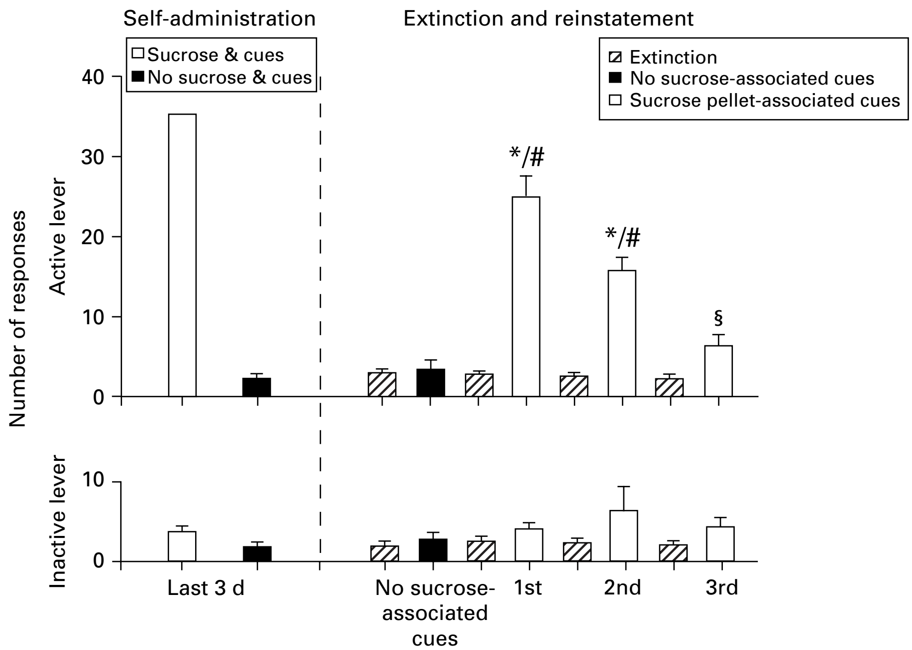

Figure 3. Seeking-behaviour induced by repeated re-introduction of sucrose pellet-associated cues. Data are the mean ($n = 8$) ± S.E.M. number of presses on the active and inactive levers. For the sake of comparison, the figure also shows the average number of lever presses during self-administration training (mean of last 3 d), extinction (mean of last 3 d before reinstatement sessions), and in the presence of stimuli associated with no-reward during the reinstatement session. Data were analysed by a one-way ANOVA for repeated measurements (with sessions as the main factor) followed by the Newman–Keuls post-hoc test. * $p < 0.01$ vs. no-rewarding stimuli; # $p < 0.01$ vs. the three preceding extinction session; § $p < 0.01$ different from the first and second re-introduction of sucrose pellet-associated stimuli, Newman–Keuls test.

days with no differences in responding during the first and second hour of sucrose pellet availability. The number of lever presses during the no-reward session dropped gradually to <5 per session in 10 training days. The mean number of days required to meet the training criterion was 16.2 ± 0.8 from the beginning of discrimination regimen. One-way ANOVA with repeated measurements found no differences either during the final 3 d between the first and second daily 30 min of self-administration [$F_{AL}(5, 35) = 0.1$, $p > 0.05$ and $F_{IL}(5, 35) = 0.5$, $p > 0.05$] or during the last three sessions of no reward [$F_{AL}(2, 14) = 0.1$, $p > 0.05$ and $F_{IL}(2, 14) = 0.5$, $p > 0.05$], thus these data were pooled for all subsequent analyses. During this training phase, responding on the inactive lever was insignificant in all experimental groups.

Rats met the extinction criterion after an average of 15.0 ± 2.6 sessions and responding on the inactive lever was always below the criterion. One-way ANOVA for repeated measurements found no significant differences during the last 3 d of extinction on the number of presses on either the active or inactive lever [$F_{AL}(2, 14) = 0.1$, $p > 0.05$ and $F_{IL}(2, 14) = 0.5$, $p > 0.05$].

As depicted in Figure 3, the efficacy of sucrose pellet-associated stimuli to reinstate seeking behaviour showed low resistance to extinction when compared with cocaine-associated stimuli (Cervo et al., 2003; but see also the result of the previous experiment in which re-introduction of cocaine-associated cues increased the number of lever presses in four test sessions). An ANOVA on the number of lever presses emitted during the three phases of the experiment (self-administration, extinction and reinstatement) yielded a significant effect of sessions on the number of active [$F(9, 63) = 102.9$, $p < 0.05$], but not on inactive [$F(9, 63) = 1.6$, $p > 0.05$] lever presses. Re-introduction of sucrose pellet-associated stimuli selectively increased the number of active lever presses during either the first or the second re-introduction session ($p < 0.05$ compared to re-introduction of no-reward-associated cues and preceding extinction days, Newman–Keuls test). A third presentation of sucrose-associated cues had no significant effect on the active number of levers presses.

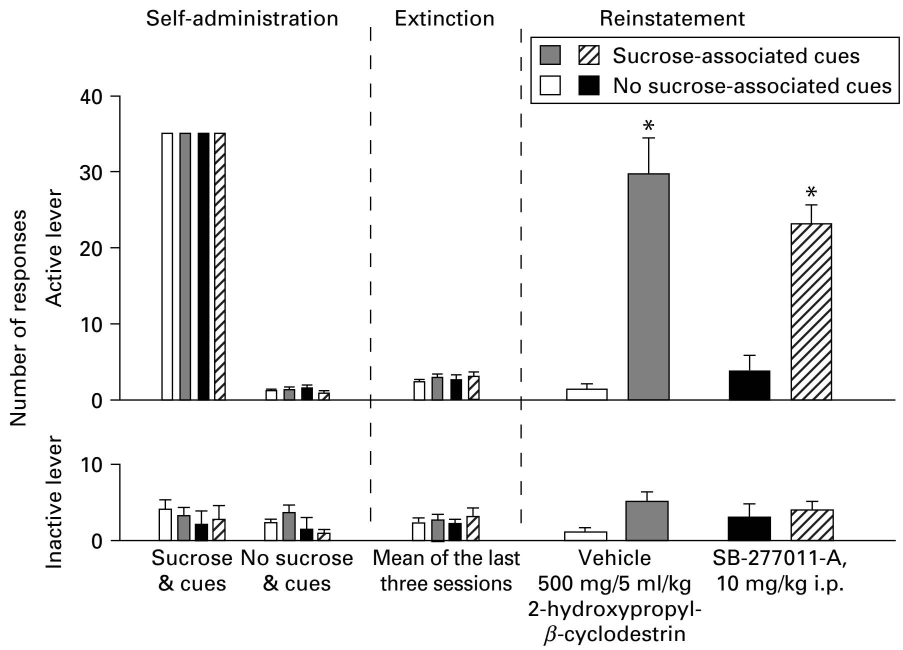

Figure 4. Effect of SB-277011-A on seeking-behaviour induced by sucrose pellet-associated cues: evaluation in a between-subjects experimental design. Results are the mean ($n=8$) ± S.E.M. number of presses on the active and inactive levers. For the sake of comparison, the figure also shows the average number of lever presses during self-administration training (mean of last 3 d), extinction (mean last 3 d before reinstatement session), and in the presence of the stimuli associated with no-reward during reinstatement session. Reinstatement data were analysed by a two-way ANOVA (with groups and stimulus presentation as the between-groups factor) followed by Newman–Keuls post-hoc comparison. * $p<0.01$ compared with respective no-sucrose-associated stimuli group, Newman–Keuls test.

Effect of SB-277011-A on seeking behaviour induced by sucrose pellet-associated cues

Figure 4 reports the effects of SB-277011-A on seeking behaviour triggered by sucrose pellet-associated cues. Mixed factorial ANOVA yielded no significant differences between the four groups of animals during the two daily sessions of sucrose pellet self-administration on either the active lever [group: $F(3, 28)=0.1$; session: $F(5, 140)=0.3$; group × session: $F(15, 140)=1.1$, all $p>0.05$] and inactive lever [group: $F(3, 28)=1.5$; session: $F(5, 140)=0.5$ and group × session: $F(15, 140)=1.0$, all $p>0.05$]. Consequently, these data were pooled for further analyses. Statistical analysis of the number of lever presses emitted during the last 3 d of self-administration, no reward and extinction yielded a significant effect of sessions on the active [$F(8, 224)=1104$, $p<0.01$], but not on the inactive [$F(8, 224)=0.8$, $p>0.05$] lever. No significant differences were found between groups on either the active [$F(3, 28)=1.4$, $P>0.05$] or the inactive [$F(3, 28)=0.5$, $p>0.05$] lever. No significant interaction group × session was found on either lever [$F_{AL}(8, 224)=1.1$, $F_{IL}(8, 224)=0.4$, $p>0.05$]. Post-hoc comparison using the Newman–Keuls test found that during the sucrose self-administration sessions rats in each experimental group pressed the active lever significantly more than during the no-reward and extinction sessions ($p<0.01$), which were not significantly different from each other ($p>0.05$).

Two-way ANOVA yielded a significant effect of stimuli presentation on the active [$F(1, 28)=68.9$, $p<0.01$], but not on the inactive lever [$F(1, 28)=0.2$, $p>0.05$]. No significant effects of treatment was found on both levers [$F_{AL}(1, 28)=1.9$, $F_{IL}(1, 28)=1.9$, all $p>0.05$] and no significant interaction between SB-277011-A and reward-associated stimuli on both the active [$F(1, 28)=2.4$, $p>0.05$] and inactive [$F(1, 28)=2.2$, $p>0.05$] levers was found. Post-hoc comparison using the Newman–Keuls test found that, irrespective of pre-treatment (10 mg SB-277011-A or vehicle), the number of active lever presses emitted by animals tested under sucrose-associated cues did not differ ($p>0.05$) and were significantly higher than the number of presses under no-reward-associated stimuli ($p<0.01$).

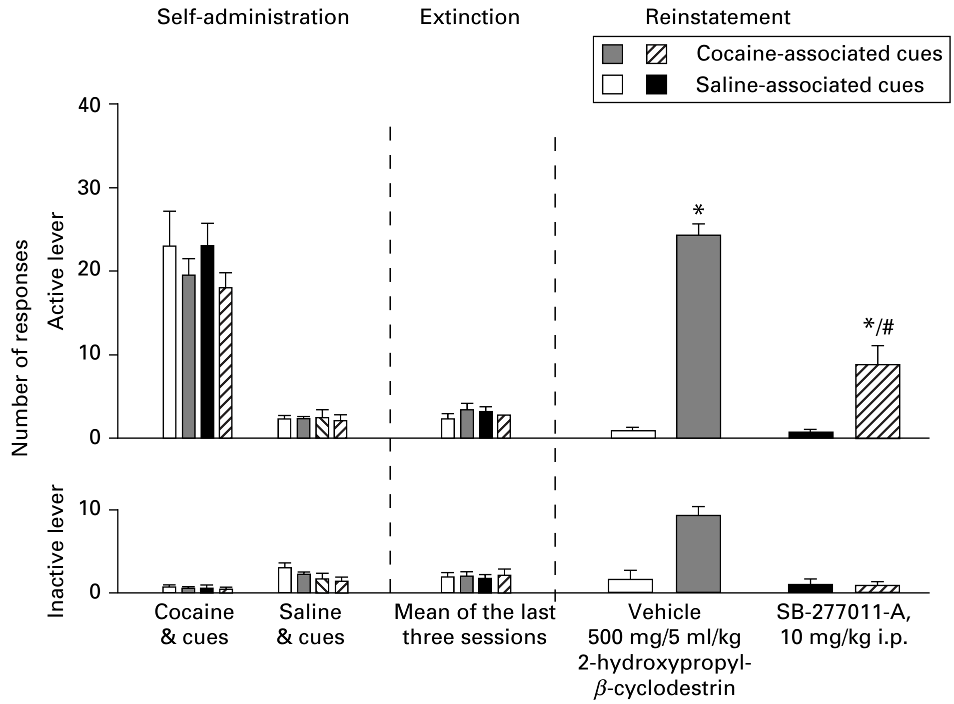

Figure 5. Effect of SB-277011-A on drug-seeking-behaviour induced by cocaine-associated cues: evaluation in a between-subjects experimental design. Results are the mean ($n=7$) ± S.E.M. number of responses on the active and inactive levers. For the sake of comparison, the figure also shows the average number of lever presses during self-administration training (mean of last 3 d), extinction (mean of last 3 d before extinction session), and in the presence of stimuli associated with no-reward during the reinstatement session. Reinstatement data were analysed by a two-way ANOVA (with groups and stimulus presentation as the between-groups factor) followed by the Newman–Keuls post-hoc test. * $p<0.01$ different from the no-reward stimuli group; # $p<0.01$ different from the vehicle + cocaine-associated stimuli group, Newman–Keuls test.

Effect of SB-277011-A on seeking-behaviour induced by cocaine-associated cues

Figure 5 depicts the effects of SB-277011-A on responding on the active and inactive levers during reinstatement sessions following re-introduction of cocaine- or saline-associated cues. The number of responses emitted during the self-administration training and extinction are also shown. No significant difference in responding across the four groups of rats was found between the first and second hour of cocaine self-administration [active lever – group: $F(3, 24)=2.0$; session: $F(5, 120)=0.3$; group × session: $F(15, 120)=1.1$; inactive lever – group: $F(3, 24)=1.3$; session: $F(5, 120)=0.5$; group × session: $F(15, 120)=0.6$, all $p>0.05$, mixed factorial ANOVA]. Accordingly, these data were pooled for successive analyses. When the number of lever presses emitted during the last 3 d of self-administration and extinction were analysed, mixed factorial ANOVA found no significant differences for groups [$F_{AL}(3, 24)=1.5$, $F_{IL}(3, 24)=0.6$, $p>0.05$]; a significant effect of sessions was found on the active [$F(8, 192)=1064$, $p<0.01$] but not on the inactive [$F(8, 192)=0.3$, $p>0.05$] lever. No interaction group × session was found on either levers [$F_{AL}(24, 192)=0.9$, $F_{IL}(24, 192)=0.3$, $p>0.05$]. A significant difference between the number of presses during cocaine self-administration compared with no-reward or extinction sessions was found on the active ($p<0.01$), but not on the inactive ($p>0.05$) lever.

A two-way ANOVA of the number of lever presses following re-introduction of cocaine-associated stimuli revealed a significant effect of cue presentation on the active [$F(1, 24)=26.4$, $p<0.01$], but not on the inactive lever [$F(1, 24)=1.5$, $p>0.05$]. A significant effect of treatment on the active [$F(1, 24)=10.7$, $p<0.01$] but not on the inactive [$F(1, 24)=1.2$, $p>0.05$] lever was also found. There was also a significant interaction between cue presentation and treatment on the number of responses on the active [$F(1, 24)=6.0$, $p<0.05$], but not on the inactive [$F(1, 24)=2.3$, $p>0.05$] lever. Post-hoc comparison using the Newman–Keuls test indicated that in rats treated with vehicle, re-introduction of cocaine-associated cues significantly increased the number of active lever presses ($p < 0.01$ compared with re-introduction of saline-associated stimuli). SB-277011-A (10 mg/kg) reduced the effect produced by re-introduction of cocaine-associated cues ($p < 0.01$ compared with the vehicle + cocaine-associated stimuli group). However, these same rats still pressed the active lever more frequently when compared with re-introduction of saline-associated cues ($p < 0.05$, Newman–Keuls test).

Discussion

The results of the present series of experiments demonstrate that the selective DA D_3 receptor antagonist SB-277011-A reduces drug-seeking behaviour induced by re-introduction of cocaine-associated cues in abstinent rats. In addition, SB-277011-A, at a dose that reduces cocaine-seeking, does not alter conditioned reinstatement of seeking behaviour triggered by cues associated with a nutritive reward (sucrose pellets). These results confirm those obtained recently using either a second-order schedule of reinforcement (Di Ciano et al., 2003), a model of cocaine cue-induced relapse to cocaine-seeking behaviour (Gál and Gyertyán, 2005; Gilbert et al., 2005; Xi et al., 2005), or another selective DA D_3 receptor antagonist (Campiani et al., 2003; Gilbert et al., 2005), and strongly suggest a selective involvement of DA D_3 receptors in drug-seeking behaviour.

Stimuli paired contiguously with cocaine infusion in self-administering rats can reinstate responding following extinction, but they often produce only weak and transient effects (de Wit and Stewart, 1981; Fuchs et al., 1998) or fail to elicit cocaine-seeking behaviour (Tran-Nguyen et al., 1998; Weissenborn et al., 1995). The high resistance to extinction that characterize this procedure (Cervo et al., 2003; Weiss et al., 2001) is very different from that seen by contingent or non-contingent presentation of drug-associated conditioned stimuli. This may be attributable to the complex stimuli associated with cocaine during the self-administration training. In our procedure, during the reinstatement phase, re-introduction of the S^{D+} may primarily facilitate the start of responding, and the response-contingent TO stimulus, acting as conditioned reinforcer, may maintain subsequent drug-seeking behaviour (Cervo et al., 2003; Weiss et al., 2000, 2001). At present, it is unknown whether SB-277011-A reduces the effect of S^{D+} or conditioned stimulus or both, and studies are currently in progress to clarify this important issue. However, the resistance to extinction of cocaine-seeking behaviour in our procedure permits the comparison of each animal's behaviour under vehicle or drug treatment. Thus, the present study confirms and extends previous findings by demonstrating that SB-277011-A reduces drug-seeking behaviour both in a within- and between-subjects experimental design.

One may argue that the attenuation of cocaine-associated cue-induced responding by SB-277011-A could be attributed to non-specific impairments in motor performance. This, however, can be reasonably ruled out for the following reasons. First, pre-treatment with SB-277011-A did not significantly alter responses on the inactive levers. Second, SB-277011-A, within the dose range tested in the present experiments, had no significant effect on either locomotor activity or food- or sucrose-taking behaviour, and is non-cataleptic (Di Ciano et al., 2003; Reavill et al., 2000). Third, SB-277011-A is a selective DA D_3 receptor antagonist with a 100-fold selectivity for DA D_3 over DA D_2 receptors and 66 other receptors, enzymes, ion channels, and transporters (Reavill et al., 2000; Stemp et al., 2000). As such, the behavioural effects of SB-277011-A are significantly different from those produced by D_2-preferring receptor antagonists, which can alter motor performance (for a comprehensive review see Heidbreder et al., 2005).

To further exclude non-specific effects on the rate of responding, and to assess whether SB-277011-A induces a state of general 'anhedonia' that would result in a reduction of seeking behaviour, we evaluated its effect on seeking behaviour induced by re-introduction of cues associated with a natural reward. Re-introduction of stimuli associated with and predictive of sucrose pellets elicited reliable seeking behaviour in the absence of further reinforcer availability. This behavioural effect cannot be attributed to non-specific arousal or spontaneous recovery since responding on the inactive lever remained negligible and responding in the presence of stimuli associated with no reward remained at the extinction level. However, in contrast with the strong resistance to extinction observed with cocaine-associated cues that lasted at least eight sessions (Cervo et al., 2003; Weiss et al., 2001), seeking behaviour induced by repeated presentation of sucrose-associated stimuli rapidly extinguished. The mechanisms behind this phenomenon are not known, but one explanation may lie in differences in the neurocircuitry mediating the effects of cocaine- vs. sucrose-associated stimuli (Baptista et al., 2004; Martin-Fardon et al., 2003). The fact that sucrose self-administration sessions lasted 30 min may account for the reduced resistance to extinction. However, this seems to be unlikely since rats always earned 35 reinforcers in association with conditioned stimuli, a number that is not dissimilar (actually higher) from the number of cocaine infusions earned during the 2-h sessions.

When SB-277011-A was studied in a between-subjects experimental design it clearly reduced drug-seeking, but not sucrose pellet-seeking behaviour. These findings are of relevance for several reasons. First, they confirm that reduction of cocaine-seeking behaviour mediated by SB-277011-A is not related to a general effect on motor performance. Second, the attenuation of drug-seeking behaviour produced by SB-277011-A is not related to a selective decrease in high-density responding since lever presses in response to re-introduction of cocaine- or sucrose-associated stimuli were similar (Salamone, 1996). Third, our data suggest that SB-277011-A does not affect rat perception. In fact, the same complex stimuli that failed to reinstate drug-seeking behaviour still induced sucrose pellet-seeking behaviour. Fourth, the lack of efficacy of SB-277011-A in altering responding to sucrose-associated stimuli also suggests that SB-277011-A does not induce 'anhedonia' since the animals still engaged in active seeking-behaviour for a highly palatable natural reward. Finally, our results suggest that SB-277011-A-induced reduction in cue-triggered reinstatement of cocaine-seeking behaviour cannot be related to interference with memory encoding and retrieval since the acute administration of SB-277011-A itself produces a significant increase in extracellular levels of acetylcholine in the anterior cingulate cortex (Lacroix et al., 2003) and reverses scopolamine-induced memory deficits (Laszy et al., 2004). Both of these effects would be expected to improve rather than to interfere with memory. The efficacy of SB-277011-A in the control of drug-seeking behaviour does not seem to be specific to cocaine, but generalizes to several drugs of abuse such as nicotine- (Andreoli et al., 2003b), alcohol- (Andreoli et al., 2003a; Thanos et al., 2005), and heroin-seeking behaviours (Ashby et al., 2003). Furthermore, SB-277011-A has also been reported to significantly attenuate stress-induced reinstatement of cocaine-seeking behaviour in male Long Evans rats (Xi et al., 2004).

Although the precise neural sites of action of SB-277011-A are still unknown, there are strong arguments supporting the possibility that SB-277011-A acts by blocking DA D_3 receptors in key brain regions. Namely, re-introduction of cocaine-associated stimuli significantly increases DA release in the NAc and BLA (Di Ciano et al., 1998; Ito et al., 2000; Weiss et al., 2000) where DA D_3 receptors are expressed (Bouthenet et al., 1991; Diaz et al., 1994, 1995; Landwehrmeyer et al., 1993). Furthermore, functional deactivation of the BLA, the core of the NAc, or the dorsomedial PFC blocks cocaine cue-induced reinstatement of drug-seeking behaviour (Di Ciano and Everitt, 2004a,b; Fuchs et al., 2004, 2005; Meil and See, 1997; See, 2002; Whitelaw et al., 1996), Further experiments are now required to delineate clearly the exact mechanisms of action of selective DA D_3 receptor antagonists. As such, studies examining the effect of direct microinfusion of SB-277011-A into areas such as the BLA, the ventral tegmental area (VTA), NAc, and subregions of the mPFC are warranted.

In conclusion, the results of the present study strongly support the potential therapeutic use of selective DA D_3 receptor antagonists for the prevention of cue-controlled cocaine-seeking and relapse.

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None.

Statement of Interest

None.

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