**Neuropsychopharmacology (2003) 28,** 1150–1159

**www.neuropsychopharmacology.org**

# Cocaine-Seeking Behavior in Response to Drug-Associated Stimuli in Rats: Involvement of $D_3$ and $D_2$ Dopamine Receptors

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Previous studies employed a second-order schedule paradigm maintained by cocaine reinforcement to show that BP897, a dopamine $D_3$ partial agonist, selectively modulated drug-seeking behavior. We investigated its effect on drug-seeking behavior induced by presentation of stimuli associated with and predictive of cocaine availability after a period of extinction and in the absence of any further cocaine. Male rats were trained to associate discriminative stimuli ($S^D$) with the availability of intravenous (i.v.) 0.25 mg/0.1 ml/infusion cocaine ($S^{D+}$) or no-reward ($S^{D-}$) saline solution. Each infusion of cocaine or saline was followed by a response-cue signaling 20-s time-out (TO). After meeting the self-administration training criterion rats were placed on extinction conditions during which i.v. solutions and $S^D$s were withheld. Every other 3 days on which rats met the extinction criterion, reinstatement tests were conducted, presenting the $S^{D+}$ or $S^{D-}$ noncontingently together with a contingent presentation of cocaine- or saline-cues signaling 20-s TO. Regardless of the order of presentation or the nature of the stimuli (auditory or visual), cocaine-associated but not saline-associated stimuli reinstated responding on the previously active lever. Presentation of cocaine-associated stimuli induced lasting drug-seeking behavior for at least eight test sessions. BP897 (1.0 mg/kg i.p.) significantly attenuated this behavior. Since it has been reported that BP897 can interact with a panel of different receptors with high affinity, we evaluated the effects of 7-OH-DPAT, an agonist to $D_3$ receptors, raclopride, a preferential antagonist to $D_2$ receptors, and WAY 100,635, an antagonist at 5-$HT_{1A}$ receptors, on drug-seeking behavior. 7-OH-DPAT (0.1–3.0 mg/kg i.p.) had biphasic effects on reinstatement induced by the cocaine-associated cues, low dosages reducing and high dosages increasing the impact of cocaine-associated stimuli on rats' behavior. Raclopride (0.1, 0.3 mg/kg s.c.) completely prevented drug-seeking behavior induced by the reintroduction of cocaine-associated stimuli. WAY 100,635 (0.1–1.0 mg/kg s.c.) had no effect on this behavior. These results, while confirming that the partial agonist at the $D_3$ receptors, BP897, might be a useful medication, also suggest a role of $D_2$ receptors in cue-induced cocaine-seeking behavior.

*Neuropsychopharmacology* (2003) **28,** 1150–1159, advance online publication, 2 April 2003; doi:10.1038/sj.npp.1300169

**Keywords:** reinstatement; relapse; dopamine; cocaine; BP897; 7-OH-DPAT; raclopride; WAY 100,635; rat

## INTRODUCTION

Drug craving, defined as 'the desire to experience the effect(s) of a previously experienced psychoactive substance', is one of the most important factors causing relapse to drug abuse (Gawin and Kleber, 1986; Rohsenow *et al*, 1990; Gawin, 1991). In human addicts and in laboratory animals, drug-seeking behavior can arise from drug withdrawal (Gawin and Kleber, 1986), a state in which extracellular dopamine (DA) is reduced in the limbic areas of the central nervous system (Kuhar and Pilotte, 1996). Drug craving can also develop after a 'priming dose' of the same or a different drug of abuse (drug 'prime') (Stewart, 1983; Jaffe *et al*, 1989) or as a consequence of exposure to stimuli previously associated with consumption of the drug of abuse ('cue-induced craving') (Childress *et al*, 1988; Ehrman *et al*, 1992). In these conditions, extracellular DA is increased in the limbic areas of the central nervous system (Fontana *et al*, 1993; Kiyatkin, 1993; Di Ciano *et al*, 1998; Ito *et al*, 2000).

Since cocaine craving can arise from states involving either hypo- or hyperfunction of the central DA system, it seemed reasonable to develop therapeutic agents to act on both dysfunctions. DA dysfunctions such as those described during cocaine relapse might, for example, be controlled by compounds acting as partial agonists at DA receptors (Pulvirenti and Koob, 1994; Childress and O'Brien, 2000). Since they bind to DA receptors with high affinity but low intrinsic activity, their effects on DA neurotransmission may depend, at least in part, on the concentration of the

This manuscript is dedicated to the memory of Dr Rosario Samanin.

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Received 02 August 2002; revised 15 January 2003; accepted 16 January 2003

Online publication: 22 January 2003 at http://www.acnp.org/citations/Npp012203468

endogenous neurotransmitter (Hoyer and Boddeke, 1993). In the presence of low levels of DA they could act as agonists, potentially compensating the dopaminergic deficit, as during early cocaine abstinence. By contrast, when there is a transient increase of endogenous DA release (like during 'drug prime' or cue-induced craving) a partial agonist would have an antagonist-like effect, thus potentially reducing cue-induced craving (Pulvirenti and Koob, 1994; Childress and O'Brien, 2000).

Agents with affinity for $D_3$ receptors could be particularly interesting in this respect. DA $D_3$ receptor levels are highest in limbic regions such as the ventral striatum in rats and humans (Sokoloff *et al*, 1990; Murray *et al*, 1994), where cocaine and cocaine-associated stimuli interact with DA transmission (Whitelaw *et al*, 1996; Di Ciano *et al*, 1998; Ito *et al*, 2000; Weiss *et al*, 2000). Recent evidence suggests that 1-(4-(2-naphthoylamino)butyl)-4-(2-methoxyphenyl)-1A-piperazine HCl, (BP897) a partial agonist at $D_3$ receptors, reduces drug-seeking behavior maintained by cocaine in a second-order schedule in rats, without any intrinsic rewarding activity (Pilla *et al*, 1999). Together with the findings that BP897 also attenuates cocaine- and D-amphetamine-induced conditioned locomotion (Le Foll *et al*, 2002; Aujla *et al*, 2002), this suggests that the $D_3$ partial agonist attenuates the behavioral effects of cocaine- or D-amphetamine-paired stimuli.

We further investigated the effects of BP897 on drug-seeking behavior induced by the presentation of stimuli associated with and predictive of cocaine availability after a period of extinction and in the absence of any further cocaine (Weiss *et al*, 2000). The results indicate that BP897 affects cocaine-associated stimuli-induced drug-seeking behavior in rats. Since BP897 interacts with a panel of different receptors with high affinity, its *in vivo* activity could not solely be attributed to a $D_3$ mediated response (Cussac *et al*, 2000). We therefore also looked at whether 7-hydroxy-*N*,*N*-di-*n*-propyl-2-aminotetraline (7-OH-DPAT), an agonist to $D_3$ receptors (Levesque *et al*, 1992), raclopride, a preferential antagonist to $D_2$ receptors (Sokoloff *et al*, 1990), and WAY 100,635, an antagonist at 5-$HT_{1A}$ receptors (Fletcher *et al*, 1996), influenced drug-seeking behavior. The results are discussed in the light of binding profiles, comparing the compounds' affinity for receptors other than $D_3$ subtypes.

## MATERIALS AND METHODS

### Animals

Male Sprague–Dawley CD-COBS rats (Charles River, Italy) were used, weighing 250–275 g at the beginning of the experiments. They were housed individually at constant room temperature (21 $\pm$ 1°C) and relative humidity (60%) under an inverted light/dark schedule (light 8.00 pm–8.00 am) with food and water *ad libitum*. Animals were allowed to adapt to laboratory conditions for at least 2 weeks and were handled for 5 min a day during this period.

### Animal Care

Procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national (D.L. no. 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, December 12, 1987; Guide for the Care and Use of Laboratory Animals, US National Research Council, 1996).

### Apparatus

Animals were trained and tested using standard rodent operant test chambers (ENV-007, MED Associates Inc., St Albans, VT) constructed from heavy-duty aluminum except for clear polycarbonate back, door and top, and equipped with two retractable levers. In half the chambers the right-hand lever was designated as the active lever, in the others the left-hand one. Approximately 15 g pressure was required to depress the lever and close the switch. The chambers had 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 produced by a white noise generator (made in-house) or a 7-kHz, 70-dB intermittent tone generated by a tone source (made in-house), both presented through an 80-ohm speaker fitted in the center of the back panel. Intravenous (i.v.) infusions were administered by a syringe pump (PHM-100, MED Associates Inc., St Albans, VT) located inside the sound-attenuating cubicles. The experimental chamber was installed inside a sound-attenuating chamber, with an exhaust fan mounted on one side. 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, recording the results of each experiment on files on the hard disk.

### Experimental Procedures

All training and testing was conducted during the dark phase of the light/dark cycle at approximately the same time each day, and each rat was always exposed to the same chamber. To facilitate acquisition of cocaine self-administration the rats were trained to press a lever for food pellets on a fixed ratio 1 (FR1) schedule. During this time, the animals were placed on a restricted diet (15 g/day rat chow, Altromin MT, Rieper, Bolzano, Italy). The first session consisted of 30 min of noncontingent delivery of one 45-mg food pellet (Noyes improved formula A/I, Sandown Scientific, Esher, Surrey, UK) every 30 s. In addition, each lever press delivered one food pellet. From the second 30-min session food pellets were available on a continuous reinforcement schedule. During these sessions only the active lever was available. Animals received a minimum of three 30-min food-training sessions in which they earned at least 100 pellets. As soon as this behavior was mastered, rats returned to an unrestricted diet and the chronic jugular catheter was implanted.

### Chronic Jugular Catheter

Catheters were made in-house using guide cannulae (C313G 5UP, Plastic One Inc., Roanoke, VA), silicon tubing

(0.30 × 0.60 and 0.64 × 1.19 mm i.d. × o.d., Degania Silicone Ltd, 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 anesthetized with equithesin 3.0 ml/kg intraperitoneally (i.p.) and a silastic catheter, sterilized in 70% alcohol, was implanted in the right jugular vein. During the 5-day recovery period, rats received a daily subcutaneous (s.c.) injection of 10 mg/kg of gentamicin sulfate (Gist-Brocades, Caserta, Italy). Catheters were kept patent by daily i.v. infusions of 0.1 ml heparinised (30 U/ml) sterile saline before and after each self-administration session. Periodically during the self-administration period, 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 controlateral jugular vein.

### Cocaine Self-Administration and Conditioning

Rats were trained according to the experimental procedure described by Weiss *et al* (2000) with some modifications. The experimental procedure involved training the rats to self-administer i.v. cocaine while simultaneously establishing discriminative stimuli associated with, and predictive of, cocaine availability or nonavailability. Rats were trained to self-inject i.v. cocaine (MacFarlan-Smith, Edinburg, UK), 0.25 mg/0.1 ml/infusion, under an FR1 for 2-h/day. These sessions started with an extension of the active lever and concurrent presentation of a white noise (20 dB above background) that lasted throughout the session and served as the discriminative stimulus ($S^{D+}$) for cocaine availability. After each infusion, the lever remained inactive for 20 s to prevent accidental overdosing. This time-out (TO) period was signaled by the cue light above the active lever coming on.

From day 3 of training, sessions started with an extension of both levers and concurrent presentation of $S^{D+}$. During these sessions pressure on the active lever produced a cocaine infusion followed by a signaled 20-s TO period. On days 5 and 6, the self-administration procedure was changed from a single 2-h session to two 1-h sessions daily separated by a 1-h period in which rats were returned to their home cage.

From day 7 a third daily 1-h session was introduced during which the cocaine solution was replaced with saline. These sessions began with an 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 nonavailability ($S^{D-}$). Each response on the active lever produced an infusion of 0.1 ml of saline and presentation of an intermittent tone (7 kHz, 70 dB) as a TO cue for 20 s, during which time the lever remained inactivate.

Thus, from day 8 rats were placed on a 'discrimination learning' regimen that comprised three daily 1-h sessions, separated by 1-h resting in the home cage, when either cocaine or saline was available as the only infusion solution, in an unpredictable sequence. Each training day included one saline and two cocaine sessions presented in random order. During this phase, the $S^{D+}$ and $S^{D-}$ continued to be present throughout the drug and saline sessions, and cocaine and saline infusions were always followed by the signaled 20-s TO period. The $S^{D}$'s were not turned off during the TO periods. Responses on the inactive lever (when presented) were recorded but had no programmed consequences.

This training was conducted daily until cocaine-reinforced responding stabilized ($\pm$ 10% over 3 consecutive training days) and the rats almost stopped responding during saline sessions ($\leqslant$5 responses during each of the three successive sessions).

### Extinction

Beginning 1 day after meeting the self-administration training criterion each rat was placed on extinction conditions until the end of the experiment. Sessions began by extension of both levers with no presentation of $S^{D}$'s. 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 over 3 consecutive days was met.

### Reinstatement

Reinstatement tests began 1 day after individual animals met the extinction criterion. Tests lasted 1 h and involved exposing the rats 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 previous active lever were followed by activation of the syringe pump motor and a 20-s signaled TO period during which time the levers remained inactive. After the initial reinstatement test session rats were placed on extinction conditions again until retested. Test sessions for each animal were separated by at least 3 days during which rates of responding under extinction conditions remained at the criterion. To control for order effects, different drug doses and the vehicles were administered in a random sequence across the reinstatement test sessions.

### Cocaine-Associated Cues Induced Drug-Seeking Behavior

In the first experiment, two groups of eight rats were randomly assigned to be initially tested with cocaine- (group 1) or saline- (group 2) associated stimuli. To verify the behavioral selectivity of the cues, animals were retested after at least 3 days in which they meet the extinction criterion, with reversed cue conditions.

In a second experiment, in an attempt to demonstrate that it was not the 'nature' of the stimuli but its incentive salience that drove the animals' behavior during the reinstatement test, two groups of rats ($n = 8$) underwent discriminative training with inverted $S^{D}$'s. Thus the $S^{D+}$ consisted of continuous illumination of the self-adminis-

tration chamber's house light, and the $S^{D-}$ consisted of white noise 20 dB above the environment noise. Stimuli during TO were also inverted: during the cocaine sessions TO was signaled by an intermittent tone (7 kHz, 70 dB), while during the saline sessions it was signaled by illumination of the light above the lever. Like in the previous experiments, rats were randomly assigned to be initially tested with cocaine- (group 1) and saline- (group 2) associated cues and after 3 days of extinction criterion they were retested with reversed cue conditions.

In a third experiment, to see whether the rats could be tested more than once, we evaluated their resistance to extinction of response reinstatement under the cocaine-associated stimuli presentation. Eight rats received the self-administration training as described in the first experiment followed by the extinction phase. As soon as they meet the criterion, they were initially tested for reinstatement in the presence of saline-associated stimuli and then repeatedly tested with cocaine-associated cues. Each reinstatement test was separated by at least 3 days of extinction in which the number of lever presses returned to $\leqslant 5$.

### Effects of Drug Treatment on Cue-Induced Reinstatement of Drug-Seeking Behavior

We evaluated the effects of 0.1, 0.3, and 1.0 mg/kg BP897 (Laboratoire Bioprojet, Paris, France) on reintroduction of cocaine-associated cue-induced drug-seeking behavior in a group of eight rats. The compound, dissolved in 2 ml of sterile saline, or vehicle, was given i.p. 30 min before testing (Pilla *et al*, 1999). All the experimental procedures were identical to the first experiment, except that the four cocaine-associated cue sessions and the saline-associated cues sessions were arranged in a random sequence.

To verify whether the marked activity of BP897 on $D_2$ and 5-$HT_{1A}$ receptors contributed to cue-induced cocaine-seeking behavior, we evaluated the influence of 7-OH-DPAT, raclopride, and WAY 100,635 on cue-induced drug-seeking behavior. Three groups of seven rats received cocaine self-administration training and extinction and were given i.p. 0.1, 0.3, 1.0, and 3.0 mg/kg 7-OH-DPAT; s.c. 0.03, 0.1, and 0.3 mg/kg raclopride; and s.c. 0.1, 0.3, and 1 mg/kg WAY 100,635, dissolved in 2 ml sterile saline, or vehicle, respectively, 15 min (Millan *et al*, 2000), 30 min (Weissenborn *et al*, 1996), and 40 min (Cervo *et al*, 2000) before testing. Each test session was separated by at least three extinction sessions in which rats remained at the criterion. The different doses of the compounds and vehicle were administered in a counterbalanced order.

### Statistical Analysis

Data are the mean $\pm$ SEM number of active and inactive lever presses during the self-administration, extinction, and reinstatement phases. First of all, in each experiment the number of cocaine infusions earned in the two separate 1-h sessions were analyzed by mixed factorial ANOVA or one-way ANOVA for repeated measurements. Since there were no differences in the first and second hours of cocaine self-administration, these data were pooled for statistical analysis. The number of lever presses during the last 3 days of extinction, before or between the different reinstatement test sessions, also did not differ because they had to meet the extinction criterion, so they too were pooled.

Differences between the mean numbers of the last three cocaine and saline self-administration sessions, the preceding three extinction sessions, and the reinstatement sessions were analyzed by mixed factorial ANOVA or one-way ANOVA for repeated measurements. Whenever a significant effect was found, *post hoc* comparisons were done with the Newman–Keuls test.

## RESULTS

### Cocaine Self-Administration and Conditioning

In all the experiments, the separate groups of rats developed stable cocaine self-administration and the number of lever presses for saline gradually decreased (mean $\pm$ SEM number of days required to meet the training criterion ranged from $16.3 \pm 1.7$ to $19.3 \pm 1.1$). There were no differences during the final 3 days of training or between the first and second daily hours of self-administration. During this phase responding on the inactive lever was minimal in all groups.

### Extinction

There was no increase in the number of active lever presses during the first days of extinction, but the number then gradually decreased and rats meet the extinction criterion after an average of $16.9 \pm 2.7$ sessions. The mean $\pm$ SEM number of days to extinction was similar between groups, ranging from $16.7 \pm 1.0$ to $21.8 \pm 3.6$. In this stage, responding on the inactive lever was negligible.

### Reinstatement

Reintroduction of the cocaine-associated cues led to immediate recovery of responding that was significantly higher than after introduction of the saline-associated stimuli and the three preceding extinction sessions (both $P<0.01$, Newman–Keuls). The overall behavioral output after presentation of cocaine-associated cues was similar to during cocaine self-administration and significantly different from saline self-administration ($P<0.01$, Newman–Keuls). The number of lever presses during the presentation of saline-associated stimuli did not differ from the three preceding extinction sessions. Since they had to meet the extinction criterion, lever presses during the extinction sessions preceding the reintroduction of the cocaine- and saline-associated stimuli did not differ between groups. These data are presented in Figure 1 but not in the other figures.

Figure 1 shows the responses on the active and inactive levers during self-administration training (last three sessions), extinction (last three sessions before the reinstatement tests), and reinstatement responses after reversal and reintroduction of the stimuli associated with and predictive of cocaine availability or nonavailability. Figure 1a shows the responses of two groups of rats in which the cocaine $S^{D+}$ was white noise and TO was signaled by illumination of the light on the lever, and saline $S^{D-}$ involved illumination

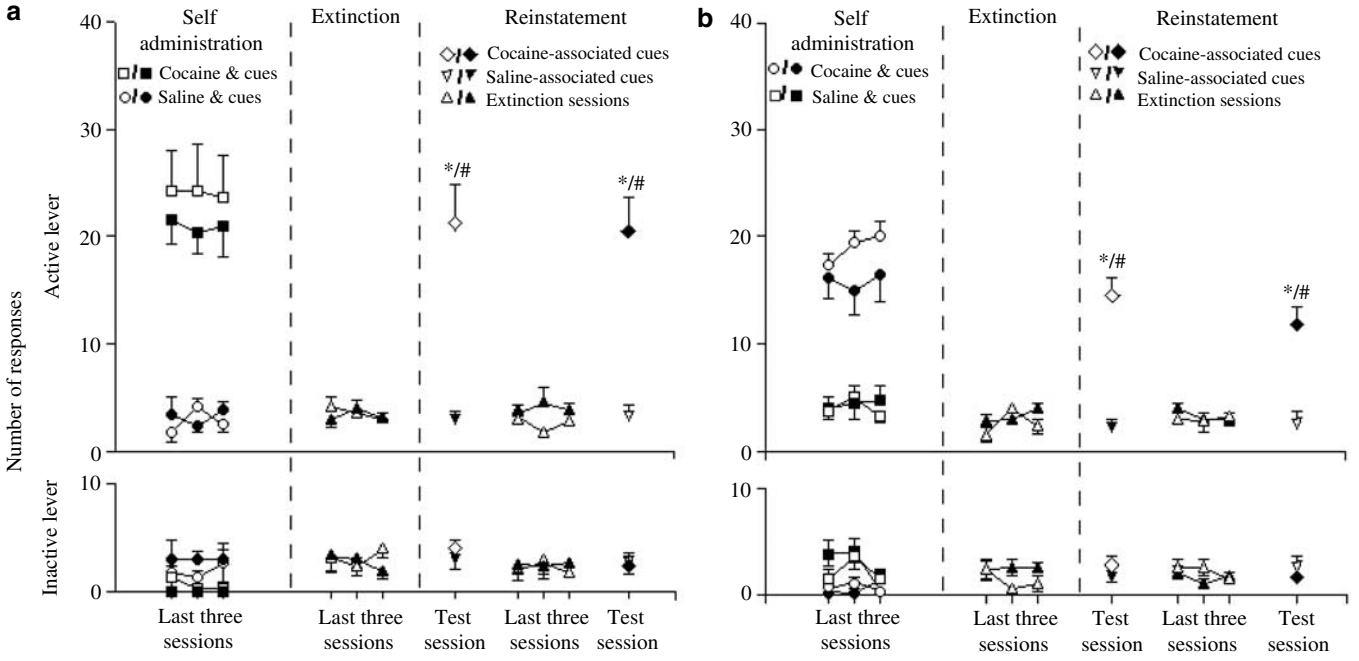


**Figure 1** Responses on the active and inactive levers during the self-administration training, extinction, and reinstatement sessions after reversal of the stimuli associated with and predictive of cocaine and saline availability. Two groups of rats (open or closed symbols) are shown, in which the order of the presentation of cocaine- and saline-associated stimuli were inverted (mean ± SEM of eight rats). (a) Cocaine-associated stimulus was white noise 20 dB over background, and the saline no-reward stimulus was house light on; (b) cocaine- and saline-associated stimuli were reversed. Data were analyzed by mixed factorial ANOVA followed by Newman–Keuls *post hoc* comparison. $P<0.01$ *vs* number of responses during the preceding extinction sessions, Newman–Keuls test. *$P<0.01$ *vs* number of responses during the no-rewarding stimuli presentation, Newman–Keuls test.

of the house light, with TO signaled by an intermittent sound. Figure 1b illustrates the number of lever presses when the association of stimuli was inverted. Mixed factorial one-way ANOVA found a significant interaction group × test sessions ($F_{int}(5,60)=20.9$, $P<0.01$). The dependence of responding or nonresponding on the cues was indicated by a significant difference in the mean number of responses between groups during the initial reinstatement phase and after reversal of the stimuli ($P<0.05$, Newman–Keuls test). Moreover, the presentation of saline-associated cues did not alter the number of lever presses, and responding was negligible on the inactive lever under both cocaine- and saline-associated stimuli.

In the experiment in which the stimuli associated with cocaine and saline were inverted, independently of the 'nature' of the stimuli only the cocaine-associated cues reinstated the drug-seeking behavior above the saline-associated cues reintroduction and extinction levels ($P<0.05$, Newman–Keuls test) on the active lever ($F(5,60)=19.1$, $P<0.01$, mixed factorial ANOVA) but not on the inactive lever (Figure 1b).

Responses on the active and inactive lever across repeated reinstatement sessions under saline- and cocaine-associated stimuli are shown in Figure 2. In this experiment too responding on the active lever remained at the extinction levels (data not shown) during testing with saline-associated cues, whereas the cocaine-associated cues led to strong recovery of responding. When these stimuli were repeatedly presented they modified the number of presses on the active lever ($F(29,173)=12.9$, $P<0.01$, one-way ANOVA for repeated measurement) but not on the inactive one. *Post hoc* comparison by the Newman–Keuls test indicated that the presentation of the cocaine-associated cues revived responding during the first eight tests, with increasingly more lever presses than with the saline-associated stimuli

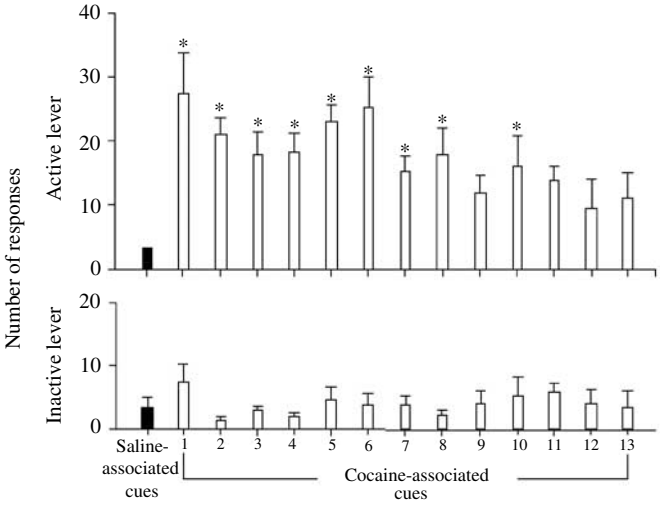


**Figure 2** Responses on the active and inactive lever (mean ± SEM of eight rats) during reinstatement sessions under saline- and repeated cocaine-associated stimuli presentation (see Materials and methods for further details). Data were analyzed by one-way ANOVA for repeated measures followed by Newman–Keuls *post hoc* comparison. *$P<0.05$, different from the no-rewarding stimuli presentation and the three preceding extinction sessions (data not shown, see Results for details), Newman–Keuls test.

($P<0.05$) and more than during the three extinction sessions preceding each cocaine-associated stimuli presentation (data not shown, $P<0.01$). In test 10 but not test nine, and in tests 11–13 cocaine-associated cues still increased the number of lever presses ($P<0.05$ compared to the saline-associated stimuli presentation and to preceding extinction days, Newman–Keuls test).

### Effect of BP897 on Cue-Induced Reinstatement of Drug-Seeking Behavior

Figure 3 shows the effects of BP897 on reinstatement induced by reintroduction of the stimuli associated with cocaine compared with saline nonreinforced. Reintroduction increased the number of presses on the active lever ($P<0.01$ *vs* saline-associated cues and *vs* the three preceding extinction days, data not shown, Newman–Keuls test) but not on the inactive one. BP897 pretreatment significantly modified rats' behavior after cocaine-associated stimuli ($F(11,95)=18.4$, $P<0.01$, one-way ANOVA for repeated measurement). *Post hoc* comparisons by the Newman–Keuls test showed that 1.0 mg/kg, but not 0.1 or 0.3 mg/kg BP897 significantly reduced the number of active lever responses induced by reintroduction of cocaine-associated stimuli ($P<0.01$ *vs* vehicle-treated group), which was no longer different from the saline-associated cues.

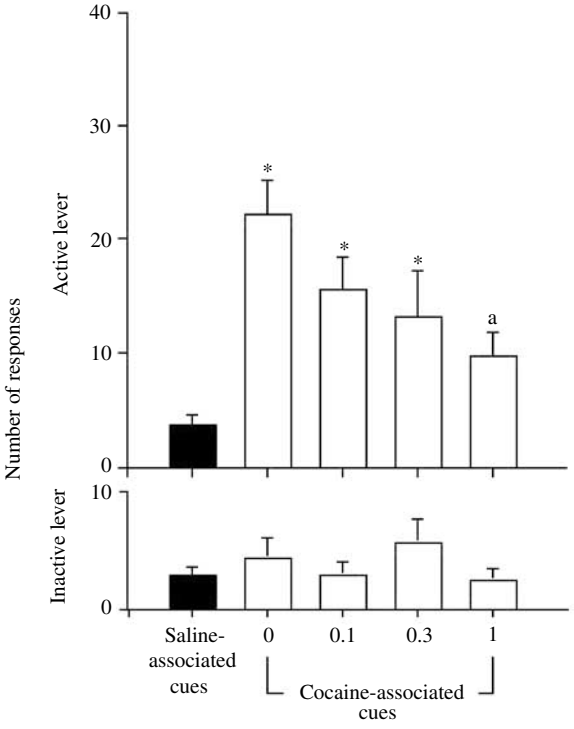


**Figure 3** Effects of BP897 on the number of presses on the active and inactive levers on reinstatement of cocaine-associated stimuli. For comparison, the figure also shows the average number of lever presses in the presence of the stimuli associated with no reward. Histograms present the mean ± SEM numbers of presses on active and inactive levers by eight rats. BP897, dissolved in sterile saline, or vehicle was given i.p. 30 min before testing. *$P<0.05$, different from the no-reward stimuli and the three preceding extinction sessions (data not shown, see Results for details), Neuman–Keuls test. [a]$P<0.05$, different from the vehicle + cocaine-associated stimuli, Newman–Keuls test.

### Effects of 7-OH-DPAT, Raclopride, and WAY 100,635 on Cue-Induced Reinstatement of Drug-Seeking Behavior

Figure 4a shows the effects of 7-OH-DPAT on cocaine-seeking behavior induced by the reintroduction of cues. Cocaine-associated stimuli increased the number of presses ($F(13,97)=11.6$, $P<0.01$, one-way ANOVA for repeated measurements, $P<0.01$ *vs* saline-associated cues reintroduction and *vs* the preceding extinction sessions, data not shown, Newman–Keuls test). *Pos hoc* comparisons (Newman–Keuls test) indicated that 7-OH-DPAT 0.1 and 0.3 mg/kg completely antagonized the effect of presentation of the cocaine-associated stimuli ($P<0.05$ *vs* vehicle-treated rats) while 1.0 and 3.0 mg/kg increased the number of lever presses after reintroduction of cocaine-associated cues ($P<0.05$ *vs* vehicle-treated rats). Cocaine- and saline-associated reintroduction and 7-OH-DPAT at all doses had no effect on the number of inactive lever presses.

Figure 4b shows the effects of raclopride on reinstatement under stimuli associated with cocaine or saline reintroduction. A significant increase in the number of active lever responses followed the reintroduction of cocaine-associated cues ($P<0.01$ *vs* saline-associated stimuli as well as the preceding extinction days, data not shown, Newman–Keuls), but there was no effect on the inactive lever. Raclopride dose dependently reduced the number of active lever responses induced by cocaine-associated stimuli ($F(11,71)=10.5$, $P<0.01$, one-way ANOVA for repeated measurements) after 0.1 and 0.3 mg/kg, but not 0.03 mg/kg, when they were no longer different from the saline-associated cues or the preceding extinction sessions (data not shown).

Figure 4c shows the effects of WAY 100,635 on cocaine-seeking behavior induced by reintroduction of the cues. Cocaine-associated cues increased the number of lever presses ($F(11,71)=11.7$, $P<0.01$, one-way ANOVA for repeated measurements, $P<0.01$ *vs* saline-associated stimuli reintroduction and *vs* the preceding extinction sessions, data not shown, Newman–Keuls test), which was not modified by any dose of WAY 100,635. Re-introduction of cocaine- or saline-associated stimuli, and WAY 100,635, had no effect on the number of inactive lever presses.

## DISCUSSION

The results of these experiments, together with previous findings from Weiss and co-workers (Weiss *et al*, 2000, 2001; Ciccocioppo *et al*, 2001), indicate that independent of their nature (light or sound), the discriminative stimuli associated with and predictive of the possibility of self-administering cocaine can elicit reliable cocaine-seeking behavior in the absence of further drug availability. Moreover, the noncontingent presentation of cocaine-associated cues after 3 days in which rats remained on a stable extinction criterion induced drug-seeking behavior lasting at least eight test sessions. These behavioral effects cannot be attributed to nonspecific arousal or spontaneous recovery since responding on the inactive lever remained negligible and, more importantly, responding in the presence of stimuli associated with no reward remained at the extinction level in all the test sessions.

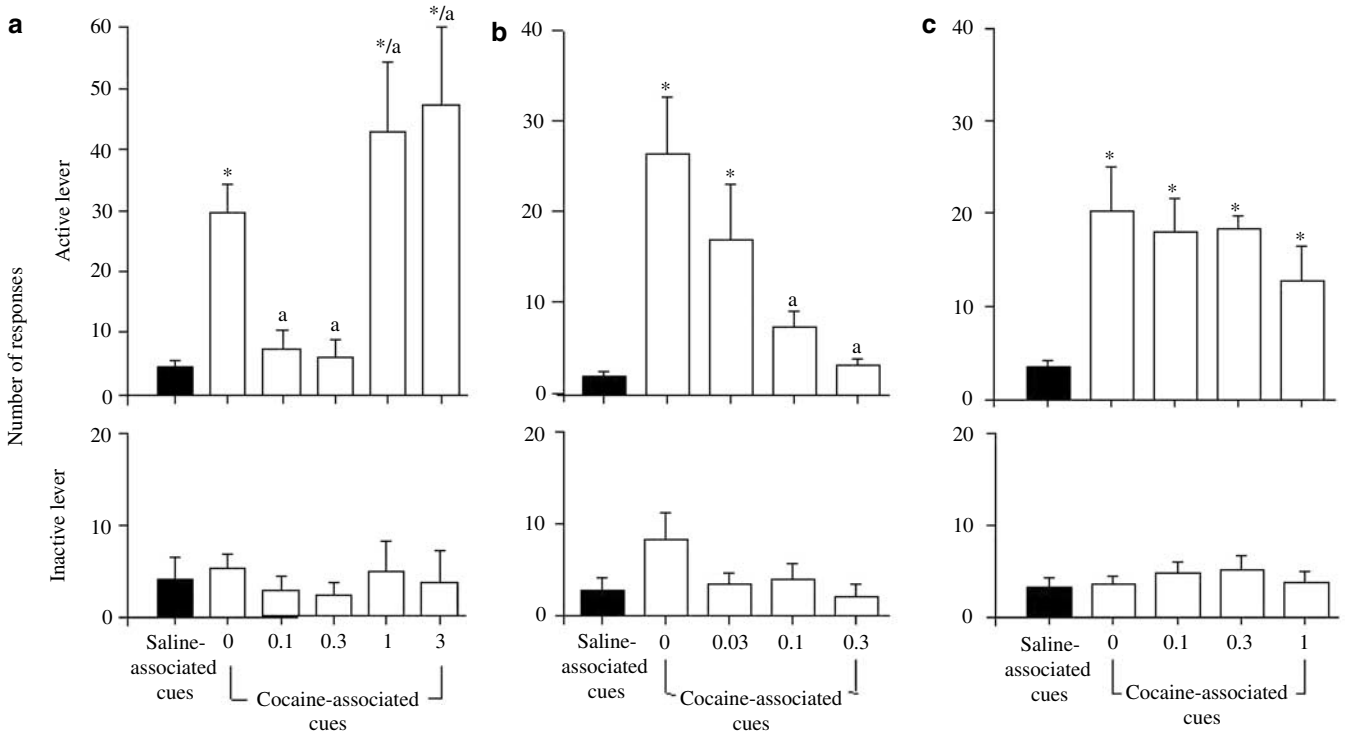


**Figure 4** Effects of (a) 7-OH-DPAT, (b) raclopride, and (c) WAY 100,635 on the number of presses on the active and inactive levers after reintroduction of cocaine-associated stimuli. For comparison the figure also shows the average number of lever presses with the no-reward stimuli. Histograms present the mean ± SEM of presses on active and inactive levers by seven rats. 7-OH-DPAT dissolved in sterile saline, or vehicle, was given i.p. 15 min before testing. Raclopride, dissolved in sterile saline, or vehicle, was given s.c. 30 min before testing. WAY 100,635, dissolved in sterile saline, or vehicle, was given s.c. 40 min before testing. *$P<0.05$, different from the no-reward stimuli presentation and the three preceding extinction sessions (data not shown, see Results for details), Neuman–Keuls test. [a]$P<0.05$, different from vehicle + cocaine-associated stimuli presentation, Neuman–Keuls test.

The high resistance to extinction observed in this procedure is very different from that seen with the contingent presentation of conditioned stimuli (de Wit and Stewart, 1981; Fuchs *et al*, 1998) or with reintroduction of contextual discriminative stimuli (Alleweireldt *et al*, 2001) associated with cocaine self-administration. This may be attributable to the complex stimuli associated with cocaine during the self-administration training in our procedure. In fact, during training the rats were not only exposed noncontingently to the cocaine $S^{D+}$ but responses on the active lever also resulted in presentation of a response cue whose purpose was to signal the 20-s TO period (acting as conditioned stimulus). Thus, during the reinstatement phase the $S^{D+}$ reintroduction may have primarily facilitated the start of responding and the response-contingent TO stimulus, acting as a conditioned reinforcer, may have maintained subsequent drug-seeking behavior. However, studies are already in progress in our laboratory to address this important issue.

Systemic administration of BP897, a $D_3$ receptor partial agonist (Pilla *et al*, 1999), significantly attenuated drug-seeking behavior induced by reintroduction of cocaine-associated cues. This effect could not be attributed to drug-induced disruption of behavior since responses on the inactive levers were not affected. However, it cannot be ruled out that the low rate of responding on the inactive lever prevented us detecting some subtle motor impairment. It also seems unlikely that BP897 substituted for cocaine's effects, reducing the motivating actions of cocaine-associated stimuli, since at doses reducing drug-seeking behavior BP897 did not substitute for cocaine in the self-administration paradigm (Pilla *et al*, 1999). Thus, the effect of the $D_3$ partial agonist seems to be specific on rats' behavior elicited by cocaine-associated cues but not on cocaine primary reinforcing properties (Pilla *et al*, 1999). This selective activity on the behavioral consequences of exposure to cocaine-associated stimuli may be consistent with dissociable neural mechanisms underlying responding with conditioned reinforcement and responding for cocaine itself (Meil and See, 1997; Whitelaw *et al*, 1996; Everitt and Wolf, 2002).

The mechanism underlying this effect is unknown. BP897 is a $D_3$ partial agonist both *in vitro* and *in vivo* (Pilla *et al*, 1999), but has also been reported to be a $D_3$ antagonist *in vitro* (Wood *et al*, 2000; Wicke and Garcia-Ladona, 2001). Since reintroduction of cocaine-associated stimuli significantly increased DA release in the nucleus accumbens and amygdala (Weiss *et al*, 2000), and considering the brain distribution of these receptors (Levesque *et al*, 1992; Levant, 1998), one possibility is that BP897 acts by blocking $D_3$ receptors in one or both of these brain regions. That antagonistic activity at $D_3$ receptors may be useful in controlling cocaine-seeking behavior induced by the reintroduction of cocaine-associated stimuli was suggested by Weiss *et al* (2001) using nafadotride. This was confirmed by the fact that SB-277011, a selective $D_3$ receptor

antagonist (Reavill *et al*, 2000), reduced seeking behavior maintained by cocaine in a second-order schedule (Everitt *et al*, 2001).

Another possibility is that BP897, acting as a $D_3$ receptor agonist, reduces the activity of dopaminergic cells in the ventral tegmental area (Diaz *et al*, 2000; Mirenowicz and Schultz, 1996) that project to forebrain targets. This idea is based on our and previous findings (Fuchs *et al*, 2002) that 7-OH-DPAT, an agonist at $D_3$ receptors (Levesque *et al*, 1992; Levant, 1997), had biphasic effects on the reinstatement induced by cocaine-associated stimuli, low dosages reducing and high dosages increasing its impact on rats' behavior. A possible explanation for this two-directional effect is that 7-OH-DPAT may preferentially stimulate $D_3$ autoreceptors, preventing the increase of extracellular DA release consequent to the presentation of cocaine-associated stimuli. Indeed, *in vivo* low doses of 7-OH-DPAT reduce DA extracellular release in the dorsal and ventral striatum (Gilbert *et al*, 1995; Gainetdinov *et al*, 1996) and in the frontal cortex (Dekeyne *et al*, 2001). Alternatively, the biphasic profile of 7-OH-DPAT, which shows $D_3/D_2$ selectivity similar to BP897 (Levant, 1997), may be because at low doses it shares $D_3$ partial agonist activity (Newman-Tancredi *et al*, 1999; Malmberg *et al*, 1998; Wicke and Garcia-Ladona, 2001) while at higher doses it behaves as a $D_2$ agonist (Levesque *et al*, 1992; Levant *et al*, 1996); in this it differs from BP897 which could act as an antagonist on $D_2$ receptors (Pilla *et al*, 1999), possibly reducing cocaine-associated stimuli-induced drug-seeking behavior. This interpretation would agree with the suggestion that high doses of 7-OH-DPAT (3–10 mg/kg i.p.) may trigger cocaine-seeking behavior after a very short period of extinction (Self *et al*, 1996). Further investigation will clarify whether this interpretation also accounts for the efficacy of PD 128,907, a $D_3$ agonist, in reducing drug-seeking behavior induced by cocaine-associated cues (Weiss *et al*, 2001).

It has been suggested that BP897, while showing a certain selectivity towards $D_3$ over $D_2$ receptors, is also a potent antagonist at h$\alpha_{1A}$- and, to a lesser extent, h$\alpha_{2A}$-adrenoceptors, and has partial agonist properties at serotonin h5-$HT_{1A}$ receptors (Cussac *et al*, 2000).

That $D_2$ receptors may also be involved in drug-seeking behavior induced by cocaine-associated cues is demonstrated by our finding that raclopride, a preferential antagonist to $D_2$ receptors (Sokoloff *et al*, 1990; Levant, 1997), completely prevented drug-seeking behavior induced by the reintroduction of cocaine-associated cues, with no effect on the number of inactive lever presses. Raclopride's effect on drug-seeking behavior might not be confused with a rate-altering effect since this drug dose dependently reduced responding induced by a light cue conditioned to cocaine, leaving intact the behavior maintained by a conditioned stimulus associated with food reinforcement (Weissenborn *et al*, 1996). Moreover, raclopride, at the same doses reducing drug-seeking behavior, did not modify the primary reinforcing properties of cocaine, at least when it was self-administered under a FR5 (Caine and Koob, 1994). Thus, the antagonistic activity at $D_2$ receptors shared by BP897 may have contributed to the antirelapse activity *in vivo*. However, this is not necessarily true. DA $D_2$ and $D_3$ receptors have a quite different distribution in the brain (Bouthenet *et al*, 1991; Levant, 1998; Camps *et al*, 1990; Richfield *et al*, 1987). So drugs acting on $D_3$ or $D_2$ receptors might both reduce cued reinstatement but through different neural sites of action. The central sites of action of these dopaminergic effects on reinstatement of cocaine seeking will be the subject of future investigations.

No information is available on the role of 5-$HT_{1A}$ receptors in cocaine-seeking behavior induced by drug-associated stimuli or on how cues affect extracellular 5-HT levels. However, WAY 100,635, a selective 5-$HT_{1A}$ antagonist (Fletcher *et al*, 1996), attenuated cocaine-induced reinstatement of extinguished drug-taking behavior in a dose-dependent manner (Schenk, 2000). The fact that in our experimental conditions WAY 100,635 given at the dosages used by Schenk did not modify cocaine-seeking behavior induced by the reintroduction of cocaine-associated stimuli makes it unlikely that BP897 modifies rats' behavior by acting through these receptors. These results, although only preliminary, suggest a different involvement of serotonin neurotransmission in cocaine-seeking behavior induced by a priming drug injection or by reintroduction of cocaine-associated stimuli.

On the topic of $\alpha_1$- and $\alpha_2$-adrenoceptors in drug-seeking behavior elicited by cocaine-associated stimuli there is still no information, but studies with selective compounds will address this important question.

In conclusion, together with previous findings (Weiss *et al*, 2000, 2001; Ciccocioppo *et al*, 2001), our experiments suggest that presentation of stimuli associated with and predictive of cocaine availability induced a reliable and enduring cocaine-seeking behavior in the absence of any further drug availability. While confirming that partial agonists at the $D_3$, such as BP897, could be useful as medication, these results also suggest that DA $D_2$ receptors are involved in the behavior elicited by cocaine-associated stimuli.

## ACKNOWLEDGMENTS

Fruitful discussions with Dr Friedbert Weiss, Department of Neuropharmacology, Scripps Insitute, La Jolla, CA, USA, and Professor Vincenzo Crunelli, School of Bioscience, Cardiff University, Cardiff, Wales, UK, are gratefully acknowledged.

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