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Dopamine D_3 Receptor Ligands Block Nicotine-Induced Conditioned Place Preferences through a Mechanism that does not Involve Discriminative-Stimulus or Antidepressant-Like Effects

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Environmental stimuli previously paired with drug taking appear to play a critical role in nicotine dependence. Converging anatomical, pharmacological, and behavioral evidence implicates dopamine D_3 receptors (D_3Rs) in the mechanisms underlying stimulus-controlled drug-seeking behavior. This study assessed the effects of BP 897, a D_3R partial agonist and ST 198, a D_3R antagonist, on nicotine-induced conditioned place preferences (CPPs), used as a measure of drug-seeking behavior, on food-maintained responding and on discrimination performance under a two-lever-choice nicotine discrimination procedure. BP 897 and ST 198 both blocked the expression of nicotine-induced CPP at doses selective for D_3R. They had no effect on locomotor activity in the CPP apparatus and no significant effect on nicotine discrimination performance or food-maintained responding under the discrimination procedure. Involvement of antidepressant actions in the effects of BP 897 and ST 198 on CPP is unlikely, since we found no effect of D_3R blockade with BP 897 or genetic depletion of D_3Rs in a forced swimming test, used as a behavioral test for antidepressant activity. This suggests that D_3R ligands reduce the motivational effects of nicotine by a mechanism distinct from those of nicotine replacement therapy and bupropion, the two currently used aids for smoking cessation in humans. These findings support the use of D_3R ligands as aids for smoking cessation and indicate that their effects would be selective for those rewarding or reinforcing effects of nicotine that contribute to the maintenance of tobacco-smoking behavior, without affecting subjective responses to nicotine or producing any antidepressant-like effects.
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INTRODUCTION

The dopaminergic mesolimbic system, which originates in the ventral tegmental area, and projects, notably, to the nucleus accumbens, is critically involved in the reinforcing effects of drugs of abuse. The ability to increase levels of dopamine in the nucleus accumbens is a common feature of all addictive drugs, including nicotine (Imperato *et al*, 1986; Pidoplichko *et al*, 1997), that seems implicated in their reinforcing effects (Wise and Rompre, 1989). Maintenance of and relapse to drug-seeking and drug-taking behavior can be markedly facilitated by environmental stimuli that acquire motivational salience through repeated associations with a self-administered drug (Schuster and Woods, 1968; Goldberg, 1973; Goldberg and Gardner, 1981; Goldberg *et al*, 1981; Stewart *et al*, 1984; Robinson and Berridge, 1993; Childress *et al*, 1999), and presentation of stimuli previously associated with administration of cocaine or amphetamine can elevate dopamine levels in the nucleus accumbens (Di Ciano *et al*, 1998a, b; Ito *et al*, 2000), a factor that may trigger drug-seeking behavior (Phillips *et al*, 2003). These factors may be particularly important in nicotine dependence (Caggiula *et al*, 2001), since environmental stimuli associated with nicotine administration play a critical role in sustaining intravenous nicotine self-administration behavior by animals (Caggiula *et al*, 2002a, b).

There is a growing body of evidence indicating that reactivity to drug-associated stimuli is controlled by D_3 receptors (D_3Rs) (Le Foll *et al*, 2000, 2002, 2003a). In marked contrast to D_1 and D_2 receptors, the D_3R has a restricted pattern of expression in the rat brain, with selective expression in the nucleus accumbens (Bouthenet

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et al, 1991; Diaz *et al*, 1995, 2000), a brain area strongly implicated in reinforcing effects of drugs of abuse (Koob, 1992). The density of D_3Rs is elevated in long-term cocaine abusers (Staley and Mash, 1996; Segal *et al*, 1997) and in cocaine-treated animals (Le Foll *et al*, 2002). A selective increase in D_3R binding and D_3R mRNA has also been found in the shell of the nucleus accumbens of nicotine-treated rats, without any significant changes in the expression of D_1 and D_2 receptors (Le Foll *et al*, 2003a, b).

Analysis of the role of D_3Rs in drug dependence processes has recently been facilitated by the availability of highly selective ligands. The D_3R partial agonist, BP 897, displays a 70-fold selectivity for D_3 over D_2 receptors and is able to reduce cue-induced cocaine-seeking behavior (Pilla *et al*, 1999) and conditioned hyperactivity produced by stimuli previously associated with cocaine administration (Le Foll *et al*, 2002). Similar effects (Le Foll *et al*, 2002; Di Ciano *et al*, 2003) have been found with the D_3R antagonist, SB-277011-A (trans-*N*-[4-[2-(6-cyano-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl]cyclohexyl]-4-quinolininecarboxamide), which has a high affinity for the D_3R and a 100-fold selectivity for D_3 over D_2 receptors (Reavill *et al*, 2000). These D_3 ligands also block cocaine- (Vorel *et al*, 2002; Duarte *et al*, 2003) and opiates-induced (Ashby *et al*, 2003; Francès *et al*, 2004a) conditioned place preferences (CPPs). Such findings suggest that D_3R partial agonists and antagonists may be particularly useful for decreasing the influence of conditioned-environmental stimuli on drug-seeking behavior and, thus, reducing the tendency for relapse (Le Foll *et al*, 2000).

Since environmental stimuli repeatedly associated with nicotine administration appear to be particularly important in nicotine dependence (Caggiula *et al*, 2001, 2002a, b), D_3R ligands may be clinically useful tools for reducing relapse to smoking behavior in human tobacco users (Le Foll *et al*, 2003a). In one recent study evaluating the effects of D_3R blockade on nicotine self-administration by rats, however, nicotine-associated stimuli failed to re-initiate extinguished nicotine-seeking behavior and D_3R blockade by SB-277011-A mainly decreased the re-initiation of extinguished nicotine-seeking behavior that was produced by a pre-session priming injection of nicotine (Andreoli *et al*, 2003). This would suggest that D_3R blockade primarily alters the reinforcing effects of nicotine and interoceptive discriminative-stimulus effects of nicotine that trigger re-initiation of drug-seeking behavior. Also, nicotine replacement and bupropion are the two medications most frequently used to treat nicotine dependence in humans and bupropion has clear nicotine-like discriminative-stimulus effects in rats (Wiley *et al*, 2002; Young and Glennon, 2002).

To further assess the effects of D_3R blockade on the control of nicotine-seeking behavior by conditioned-environmental stimuli, we used a CPP procedure, an animal model involving the direct control of nicotine-motivated behavior by associated environmental stimuli (Le Foll and Goldberg, 2004a, b). To assess the effects of D_3R blockade on nicotine's discriminative-stimulus and psychomotor effects, we used a classical two-lever choice drug-discrimination procedure (Colpaert, 1999). Two D_3R ligands, BP 897, a selective D_3R partial agonist and ST 198, ((E)-*N*-(4-[1,2,3,4-tetrahydroisoquinolin-2-yl]-butyl)-3-phenylacrylamide), a recently described D_3R antagonist, were evaluated with these two procedures at doses selective for the D_3R. ST 198 presents a 65-fold selectivity for the D_3 over the D_2 receptor (Bezard *et al*, 2003; Mach *et al*, 2004): inhibition constants (K_i) are 12 and 780 nM for inhibiting binding to D_3 and D_2 receptors, respectively (Bezard *et al*, 2003). ST 198 has a lower affinity for human D_1 ($K_i \approx 25\,\mu M$), $D_{4.4}$ ($K_i \approx 5\,\mu M$), and D_5 ($K_i \approx 12\,\mu M$) receptors, as well as for a variety of nondopaminergic receptors (Bezard *et al*, 2003). BP 897 and ST 198 were tested for their effects on the expression of preferences that had developed for distinctive compartments repeatedly associated with nicotine administration under the CPP procedure, and for their effects on food-maintained responding and discrimination performance under the nicotine discrimination procedure.

Finally, since two antidepressant drugs (bupropion and nortriptyline) are effective aids for smoking cessation in humans (Fiore *et al*, 2000), we considered the possibility that any effectiveness of D_3R ligands in the treatment of nicotine dependence or the reduction of nicotine-induced CPP might be related to antidepressant effects. This hypothesis is strengthened by the putative role of dopamine and D_3Rs in the regulation of mood (Maj *et al*, 1997; Willner, 1997; Lammers *et al*, 2000). Bupropion and nortriptyline, as well as other antidepressants such as imipramine, all have positive effects on performance in the forced swimming test (Porsolt *et al*, 1977; Steru *et al*, 1985; Zocchi *et al*, 2003), but D_3R ligands have not been studied with this procedure. To address the issue of potential antidepressant effects of D_3R ligands, we compared the effects of BP 897 and imipramine in the forced swimming test. In addition, D_3R-deficient mice and their wild-type littermates were used to further assess the effect of D_3R blockade by genetic deletion alone and in combination with imipramine treatment on performance in the forced swimming test.

MATERIALS AND METHODS

Rats used in this study were maintained in facilities fully accredited by the American Association for the Accreditation of Laboratory Animal Care (AAALAC), and all experimentation was conducted in accordance with the guidelines of the Institutional Care and Use Committee of the Intramural Research Program, National Institute on Drug Abuse, NIH, and the Guide for Care and Use of Laboratory Animals. All experimental procedures involving mice were in strict accordance with the guidelines of the French Ministry of Agriculture on the use and care of laboratory animals.

Drugs

Nicotine ((−)-nicotine hydrogen tartrate) and imipramine (imipramine hydrochloride) were purchased from Sigma Chemical Company (St Louis, MO, USA) and diluted in saline. pH of nicotine was adjusted to 7.0 with dilute NaOH. Nicotine was administered in a volume of 1.0 ml/kg subcutaneously (s.c.), immediately before the session for the CPP experiment and 10 min before the session for the nicotine discrimination experiments. BP 897 (gift from Bioprojet laboratory, Paris, France) and ST 198 (synthesized at Johann Wolfgang Goethe University, Frankfurt,

Germany) were dissolved in water and administered 30 min before the sessions. Imipramine was administered intraperitoneally, 30 min before the test. The pH of the ST 198 solution was adjusted to pH 6 with NaOH 0.1 N. ST 198 was administered orally by Kendall® feeding tubes. All doses of the drugs were expressed as mg free base per kg body weight.

Experiment 1: Nicotine-Induced CPPs

Subjects. Male Sprague–Dawley rats ($n = 247$) were obtained from Charles River (Wilmington, MA). They were experimentally naive at the start of the study, initially weighed 230–260 *g* and were housed in groups of two per cage. Water and lab chow were available at all times in the home cage. Experimental procedures were conducted during the light phase of a 12-h/12-h light/dark cycle (lights on at 0700 h). The rats were allowed to acclimate to the animal colony for at least 3 weeks before training and were repeatedly handled during this period. All rats were housed in a temperature- and humidity-controlled room. A total of 37 rats displaying a high initial bias in the apparatus (ie more than 600 s spent in one side of the apparatus over a 900 s period of time) were eliminated from the study. Therefore, data from 210 rats were analyzed (103 rats in the saline groups and 107 rats in nicotine groups).

Apparatus. Eight identical locomotor activity monitors (MED Associates, St Albans, VT) were enclosed in four sound-attenuation chambers (BRS/LVE, Laurel, MD). A standard two-compartment place preference insert ($42 \times 42\,cm^2$, Med Associates) was situated inside each locomotor activity monitor. The two sides of the apparatus were differentiated by their floor type (mesh *vs* bar) and a small light was added on the wall of the sound-attenuation chambers on the side of the mesh floor, to increase the difference between the two sides. Each monitor consisted of a 16×16 infrared photocell array separated by 2.5 cm. These detectors were interfaced to a computer that tabulated the time spent per side and distance traveled. Thus, the time spent in one compartment could be deducted from the time spent in the other compartment (sum of both equaled 900 s). This CPP apparatus produces only minimal bias: the rats tend to prefer the grid-mesh floor over the bar side in our apparatus (53.2 *vs* 46.8% of the total time of the pre-test session) (Le Foll and Goldberg, 2004a).

Procedure. Each CPP experiment had three phases: one pre-test, six conditioning sessions, and one post-test session. These sessions were conducted over 5 subsequent days (two sessions per day for conditioning sessions).

Pre-test: A 900-s pre-test was given to determine initial preference for the floor and illumination stimuli using a procedure identical to that used for the post-conditioning preference test. All rats were weighed just before placement in the apparatus. Animal placement (ie mesh *vs* bar side of the chamber) was counterbalanced within each subgroup. During the pre-test, rats could move freely from side to side in the place preference apparatus.

Conditioning: We used a 'biased' stimulus assignment procedure, that is, the compartment paired with nicotine was the initially nonpreferred side of the apparatus, as measured during the pre-test. In the morning, all rats received an s.c. injection of saline before being placed for 20 min in one compartment. After 4 h, rats received an injection of either 0.1 mg/kg nicotine (nicotine conditioning) or saline (saline conditioning) before being placed for 20 min in the other compartment. Recently, we have demonstrated that nicotine is able to induce CPP over a large range of doses (Le Foll and Goldberg, 2004a), but we found a maximal effect at 0.1 mg/kg and this was within the range of doses most frequently found effective in inducing CPP in rats by others (Le Foll and Goldberg, 2004a). Since, nicotine's ability to induce place preference is highly dependent on the stimulus assignment procedure used (Acquas *et al*, 1989; Calcagnetti and Schechter, 1994; Le Foll and Goldberg, 2004a), we chose a bias procedure in which the animals received nicotine administrations in the initially nonpreferred compartment of the CPP apparatus, as assessed by a preconditioning test. This bias procedure appears more suitable than unbiased procedures for studying nicotine-induced CPP, since nicotine administrations in the initially preferred compartment of the CPP apparatus were not able to induce CPP in our previous study (Le Foll and Goldberg, 2004a).

Place preference test: The CPP test was given the day after the last conditioning trial. The conditions of this test were identical to those during the pre-test session, that is, the rats could move freely from side to side in the place preference chamber. Rats were treated acutely with either saline, BP 897 (0.1, 0.3, and 1 mg/kg, $n = 14$–16) or ST 198 (3, 30, and 100 mg/kg, $n = 12$–14), 30 min before the place preference test.

Data analysis. The outcome of the CPP experiment was determined by analyzing the raw time scores in the less preferred side of apparatus. All data were subjected to a repeated measure of analysis of variance (ANOVA, pre-test and post-test) with the alpha level set at 0.05. *Post hoc* comparisons were performed with LSD *post hoc* test. We first compared the time spent in the initially less preferred side of the apparatus by rats of various groups during the pre-test. Nicotine place preferences were determined by comparing the time spent in the initially less preferred side of the apparatus (drug-paired side) by the various groups to the time spent by the saline control group. Distance traveled during the test session was also analyzed.

Experiment 2: Nicotine Discrimination

Subjects. Male Sprague–Dawley rats (Charles River, Wilmington, MA) experimentally naive at the start of the study and initially weighing 290–350 g were housed individually. The rats were allowed 7 days of free feeding after being delivered to the animal facility. Before the start of the study, rats were diet restricted (3 NIH07 biscuits/day) for 10 days and the diet restriction was maintained throughout the study to maintain the animal weight at 85% of their *ad lib* weight at the beginning of the study. Enrichments (fresh fruits and vegetables) were provided on Saturdays. Water was available *ad libitum*. All rats were housed in a temperature- and humidity-controlled room and were maintained on a 12 h light/dark cycle—the lights were on

from 0700 to 1900 h. Experiments were conducted during the light phase.

Apparatus. A total of 12 standard operant-conditioning chambers (Coulbourn Instruments, Lehigh Valley, PA) were used. Each chamber contained a white house light and two levers, separated by a recessed tray into which a pellet dispenser could deliver 45 mg food pellets (F0021, Bioserv, Frenchtown, NJ). Each press of a lever with a force of 0.4 N through 1 mm was recorded as a response and was accompanied by an audible click. The operant-conditioning chambers were controlled by microcomputers using the MED Associates MED-PC software package (MED Associates Inc., East Fairfield, VT).

Drug-discrimination procedure. Rats were trained as described previously (Yasar and Bergman, 1994; Le Foll and Goldberg, 2004b) under a discrete-trial schedule of food-pellet delivery to respond on one lever after an injection of a training dose of 0.4 mg/kg nicotine, and on the other lever after an injection of 1 ml/kg of saline vehicle ($n = 24$). Injections of nicotine or saline were given subcutaneously 10 min before the start of the session. At the start of the session, a white house light was turned on and in its presence the rats were required to make 10 consecutive responses (fixed ratio 10 schedule of food delivery) on the lever appropriate to the pre-session treatment. The completion of 10 consecutive responses on the correct lever produced delivery of a 45 mg food pellet and initiated a 45-s time-out, during which lever-press responses had no programmed consequences and the chamber was dark. Responses on the incorrect lever had no programmed consequences other than to reset the fixed-ratio requirement on the correct lever. After each time-out, the white house light was again turned on and the next trial began. Each session ended after completion of 20 fixed-ratio trials or after 30 min elapsed, whichever occurred first. Discrimination-training sessions were conducted 5 days per week under a double alternation schedule (ie DDSSDDSS, etc, D = drug; S = saline). Training continued until there were eight consecutive sessions, during which rats completed at least 90% of their responses during the session on the correct lever and no more than four responses occurred on the incorrect lever during the first trial. Test sessions with other doses and other drugs were then initiated.

During the test sessions, a range of doses of BP 897 (0.1, 0.3, 1, 3, and 10 mg/kg) and ST 198 (3, 10, 30, and 100 mg/kg) were substituted for the training dose of nicotine and were given in combination with 0.4 mg/kg nicotine. These D_3 ligands were also administered together with various doses of nicotine to assess possible shifts in the dose–response curve for nicotine discrimination (0.3 and 1 mg/kg of BP897 and 30 and 100 mg/kg of ST 198 were tested): we used a within-subjects design: the same rats received the various treatments regimen during various test sessions. Test sessions were identical to training sessions, with the exception that both levers were active and 10 consecutive responses on either one of the two levers resulted in delivery of a food pellet. Switching responding from one lever to the other lever reset the ratio requirement. In a test phase, a single alternation schedule was introduced and test sessions were usually conducted on Tuesdays and Fridays. Thus, a 2-week sequence starting on Monday was: DTSDTSTDST (T = test). In this way, test sessions occurred with equal probability after saline and drug sessions. Test sessions were conducted only if the criterion of 90% accuracy and not more than four incorrect responses during the first trial was maintained in the two preceding training sessions.

Data analysis. Two independent measures of behavior were collected in the nicotine-discrimination study: a measure of discrimination performance expressed as the percentage of nicotine-associated responses and a measure of motor performance expressed as response rate. The percentage of nicotine-associated responses during each session (training or test) reflected the percentage of the number of responses emitted on the nicotine-associated lever relative to the total number of responses emitted on both levers during a session. The percentage of nicotine-associated responses was individually calculated for each rat and then expressed as a group mean ($\pm$SEM). Nicotine-associated lever selection data were excluded from analysis if a rat emitted fewer than 10 responses during the test session or if the response rate was inferior to 0.5 responses/s. No generalization to the nicotine cue was defined as the percentage of responses on the nicotine-associated lever was 20% or lower. Response rate (responses/s) during each session was calculated by dividing the total number of responses emitted on both levers during a session by the total session length. Response rates were individually calculated for each rat and then expressed as a group mean ($\pm$SEM).

ANOVA was used to analyze experimental data from the nicotine-discrimination study. *Post hoc* analysis was performed using Dunnett's test following detection of a significant main effect (ie a significant effect of drug's dose for within group comparisons) by one-way ANOVA. Statistical analyses were performed on raw (rates of responding) or transformed (percentages of nicotine-associated lever selections) data. ED_{50} values and 95% confidence intervals (CIs) for nicotine dose–response curves after different pretreatments were calculated by linear regression using four or five points on the ascending portions of the dose–response curves. Data were considered statistically significant at $P<0.05$. Two ED_{50} values were considered statistically different if their 95% confidence limits did not overlap.

Experiment 3: Assessment of Antidepressant Actions with a Forced Swimming Test

Subjects. Experiments were conducted with either male Swiss mice ($n = 9$–11) or with D_3R-deficient mice and their wild-type littermate ($n = 12$), obtained from breeding and mating C57Bl6 × 129sv hybrid heterozygous mice bearing a mutation invalidating the D_3R gene, originally obtained from S. Fuchs (Weizmann Institute, Rehovot, Israel) (Accili *et al*, 1996). DNA was prepared from a piece of the tail (3–5 mm), using the DNAeasy tissue kit (Qiagen France, Courtaboeuf, France), and amplified with the mixture of primers GCA GTG GTC ATG CCA GTT CAC TAT CAG and CCT GTT GTG TTG AAA CCA AAG AGG AGA GG, amplifying exon 3 of the wild-type D_3R, and TGG ATG TGG AAT GTG TGC GAG and GAA ACC AAA GAG GAG AGG

GCA GGA C, amplifying the PGK cassette of the mutated gene. Agarose gel electrophoresis allowed us to detect homozygous wild-type mice (a single band at 137 bp), homozygous mutated mice (a single band at 200 bp), and heterozygous mice (two bands at 137 and 200 bp). Homozygous mutated mice and their wild-type littermates were used in the study.

Measurement of forced swimming time. All mice were grouped four per cage for 1 week prior to the experiment, on a 12 h/12 h light/dark cycle (lights on at 0700 h), with food and water available *ad libitum*. Home cages measured were 25 cm wide × 15 cm large × 13 cm high and the floor was covered by sawdust. Mice were brought to experimental rooms 2 h before the experiments. The forced swimming time was used to measure immobility and escape time in mice (for details, see Porsolt *et al*, 1977). The apparatus consisted of two glass cylinders (height: 25 cm; diameter: 10 cm), each containing 10 cm of water at 23−25°C. Two mice were placed into the cylinders for 6 min and tested at the same time. Immobility time was measured during the last 4 min of the 6-min testing period. A mouse was judged to be immobile when it floated in an upright position and made only small movements to keep its head above water. Results were converted to escape time (reflecting total time minus immobility time).

Statistical analysis. Values reported are means ± SEM. Differences between groups of Swiss mice treated with saline, imipramine (32 mg/kg i.p.) and BP 897 (1 and 2 mg/kg i.p.) were analyzed by one-way ANOVA, followed by the Dunnett's multiple comparison *post hoc* test. Differences between D_3R-deficient mice and their wild-type littermate were analyzed by two-way ANOVA, followed by LSD *post hoc* test.

RESULTS

Experiment 1: Nicotine CPPs

Times spent in the initially less preferred side of the apparatus during the pre-conditioning test by the rats from the different groups are shown in Figure 1 (open bars). There were no basal differences between groups (all $P > 0.3$). After conditioning with a dose of 0.1 mg/kg nicotine, and in agreement with our previous studies (Le Foll and Goldberg, 2004a, b), rats displayed nicotine-induced CPP: repeated measures of ANOVA indicated a significant effect of time ($F(1, 196) = 18.6$, $P < 0.0001$), no significant effect of nicotine treatment ($F(1, 196) = 0.5$, $P = 0.06$), but a significant time × nicotine treatment interaction ($F(1, 196) = 5.1$, $P = 0.03$). *Post hoc* analysis indicated that there were significant nicotine-induced CPPs in rats receiving either saline ($P < 0.0001$), BP 897 at the 0.1 mg/kg dose ($P = 0.006$), and ST 198 at the 3 mg/kg ($P = 0.01$) and 30 mg/kg ($P = 0.007$) doses, as compared to saline control animals. As stated in Materials and methods, nicotine-induced CPP is defined by comparison with the group of rats receiving saline in both compartments during conditioning sessions and treated with saline the test day. In contrast, there were no significant nicotine-induced CPPs in rats receiving BP 897 at the higher 0.3 ($P = 0.17$) and 1 mg/kg ($P = 0.053$) doses or ST 198 at the 100 mg/kg dose ($P = 0.2$) (left part of Figure 1). These compounds were also given to control, saline-pretreated animals (right part of the Figure 1), and there were no significant effects, except an increase in the time spent in the nonpreferred side of the apparatus at the 1 mg/kg dose of BP 897 ($P = 0.005$).

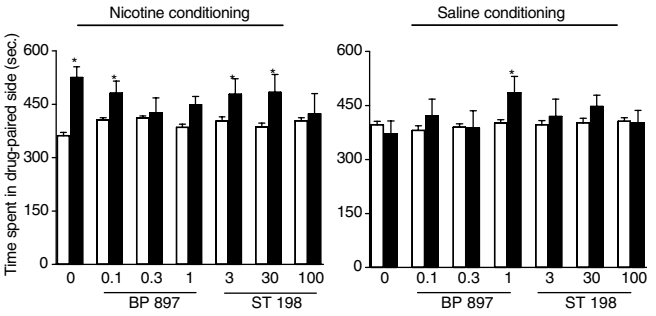

Figure 1 BP 897 (D_3R partial agonist) and ST 198 (D_3R antagonist) inhibit the expression of nicotine CPPs. The figures represent the time spent in seconds (+SEM) in the initially less-preferred side of the apparatus during pre-conditioning (open bar) and post-conditioning (closed bar) 900-s sessions. All the animals received three pairings of either nicotine (0.1 mg/kg, left panel) or saline (right panel). On the test day, animals received either saline ($n = 19$–21), BP 897 ($n = 14$–16, i.p.) or ST 198 (12–14, p.o.) 30 min after being placed into the CPP apparatus and the time spent in each chamber was automatically recorded for 900 s. *$P < 0.05$ as compared to saline-treated rats the test day.

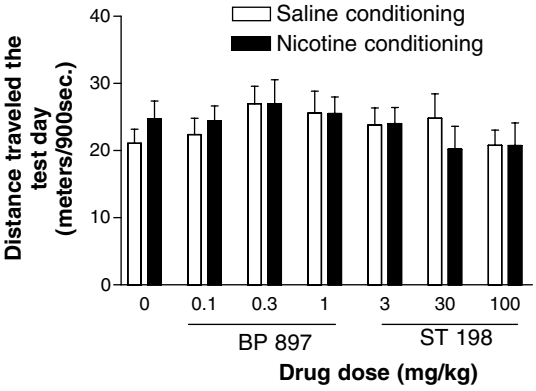

Figure 2 BP 897 (D_3R partial agonist) and ST 198 (D_3R antagonist) do not modify locomotor activty during the CPP experiment. The figure represents the distance traveled in meters (+SEM) during the 900-s test session of the CPP experiment (see Figure 1).

Locomotor activities displayed by the rats during test sessions are shown in Figure 2. Neither BP 897 nor ST 198 administration altered locomotor activity of the rats in these experiments. Repeated measures of ANOVA indicate no significant effect of nicotine conditioning ($F(1, 179) = 0.01$, $P = 0.91$), no significant effect of BP 897 or ST 198 administration ($F(6, 179) = 0.86$, $P = 0.52$), and no significant conditioning × treatment interaction ($F(6, 179) = 0.53$, $P = 0.78$).

Experiment 2: Nicotine Discrimination

Establishment of the nicotine discrimination baseline. To reach the final level of accuracy (eight consecutive sessions with at least 90% of the responses on the correct lever and no more than four incorrect responses during the first trial)

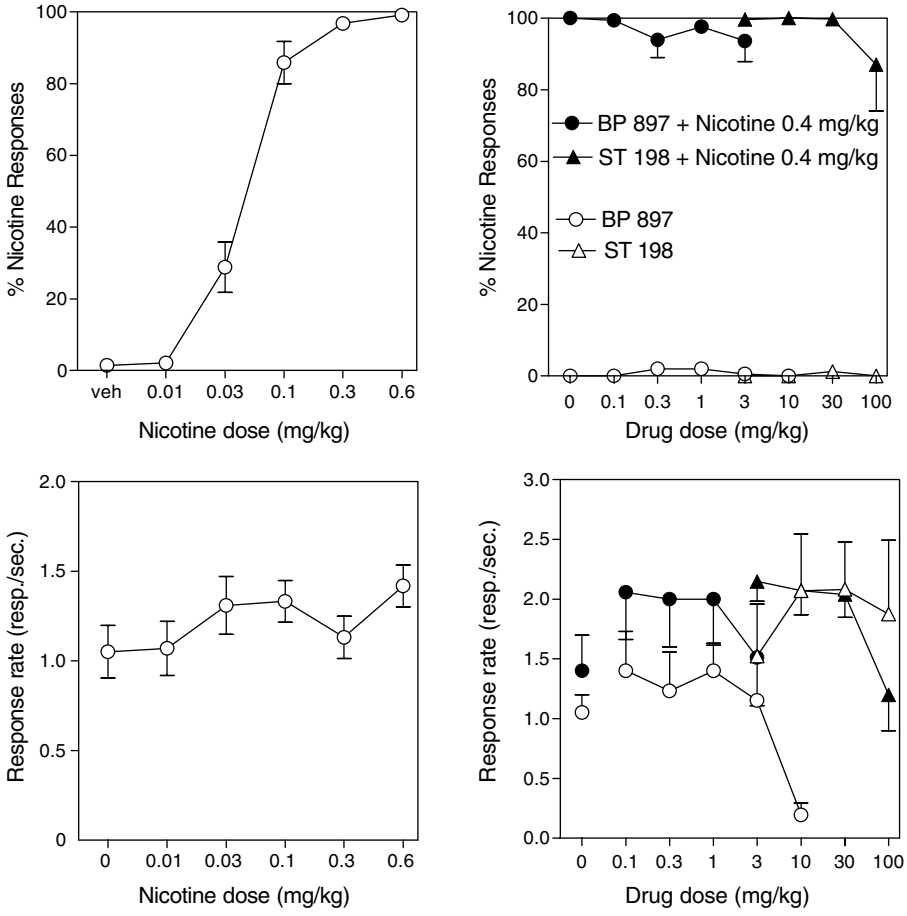

Figure 3 BP 897 (D_3R partial agonist) and ST 198 (D_3R antagonist) do not produce nicotine-like effects and do not block the discriminative-stimulus effects of 0.4 mg/kg nicotine. Left panels: Dose-effect function for the discriminative-stimulus effects of nicotine in rats ($n = 24$) trained to discriminate 0.4 mg/kg nicotine from saline. Right panels: Effects of BP 897 and ST 198 in rats trained to discriminate 0.4 mg/kg nicotine from saline. Data are means ± SEM from $n = 6$ rats. The percentage of nicotine-appropriate responding is shown as a function of dose during substitution test sessions (open symbols) and during combination test sessions when the compounds were given together with the 0.4 mg/kg training dose of nicotine (filled symbols) (upper panels). Response rates are expressed as responses per second averaged over the session (bottom panels). BP 897 disrupt the responding of the rats at doses that are not selective for the D_3R.

required 18–70 sessions with a mean value (±SEM) of 36.6 ± 3.0 sessions. Once the training criterion was reached, accuracy during maintenance training sessions remained high (95–100% responding on the appropriate lever). Rates of responding during training sessions were stable across sessions during the whole study and were slightly higher after nicotine than after saline pretreatment, as was observed in previous studies using the same 0.4 mg/kg training dose of nicotine (Shoaib *et al*, 1997; Gasior *et al*, 2002). When doses of 0.01–0.6 mg/kg nicotine were substituted for the 0.4 mg/kg training dose, there was a dose-dependent reduction in responding on the drug lever as dose decreased with responding almost exclusively on the drug lever at 0.1–0.6 mg/kg and responding almost exclusively on the saline lever at the lower dose of 0.01 mg/kg nicotine (one-way ANOVA: $F(5,138) = 92.9$, $P < 0.0001$, Figure 3, left upper panel). The nicotine dose–response curve remained stable throughout the study.

Generalization tests with BP 897 and ST 198. Figure 3 (open symbols) shows the percentage of responses made on the drug lever and overall rates of responding during sessions when different doses of nicotine (left panel), BP 897 or ST 198 (right panel) were substituted for the 0.4 mg/kg training dose of nicotine. Neither the selective D_3 partial agonist BP 897, nor the D_3 antagonist ST 198 produced nicotine-like responding on the drug lever (Figure 3, right panel and open symbols). There was a significant effect of BP 897 on the rate of responding (one-way ANOVA $F(5,48) = 2.63$, $P = 0.0.04$). *Post hoc* analysis indicated that BP 897 had no effect on responding at doses between 0.1 and 3 mg/kg (all $P > 0.53$), but significantly decreased responding at a dose of 10 mg/kg (Figure 3, $P = 0.002$), a 30-fold higher dose than that which blocked the expression of nicotine-induced CPP (0.3 mg/kg, Figure 1). ST 198 had no effect on the rates of responding at doses up to 100 mg/kg, the dose which blocked the expression of nicotine-induced CPP (one-way ANOVA $F(4,42) = 1.51$, $P = 0.21$). Due to the limited availability of ST 198, higher doses were not tested. These results indicate that the two D_3 ligands do not produce nicotine-like effects and do not disrupt behavior at doses selective for the D_3R.

Effects of BP 897 and ST 198 on the discriminative-stimulus effects of the training dose of nicotine. Figure 3 (right panel and filled symbols) shows that BP 897 and ST

198, at doses selective for the D_3R, did not block or significantly reduce the discriminative-stimulus effects of the training dose of nicotine (all $P>0.2$). BP 897 in combination with the training dose of nicotine also had no significant effect on response rates, compared to nicotine alone (one-way ANOVA $F(4,25)=0.6$, $P=0.7$), although there was a trend for increased response rates at doses of 0.1–1 mg/kg (Figure 3). When ST 198 was given in combination with the training dose of nicotine, there was a significant change in response rates (one-way ANOVA $F(4,25)=3.05$, $P=0.04$). *Post hoc* analysis indicated that the low 3 mg/kg ST 198 significantly increased response rates compared to nicotine alone ($P=0.04$); a similar trend has been noticed for ST 198 at the dose of 10 and 30 mg/kg (see Figure 3, $P=0.08$ for both), but not for the dose of 100 mg/kg ($P=0.6$). These results indicate that the two D_3 ligands do not block the discriminative-stimulus effects of 0.4 mg/kg nicotine.

Effects of D_3R selective doses of BP 897 and ST 198 on the dose–response curve for nicotine discrimination. Figure 4 shows the effects of selected doses of BP 897 and ST 198 on the dose–response curve for nicotine discrimination. ED_{50} values for drug-lever selection with 95% CIs are presented in Table 1. Neither BP 897 nor ST 198, at the highest doses selective for the D_3R, produced a significant shift of the dose–response curve for nicotine discrimination (Figure 4). This lack of effect is also indicated by overlapping 95% CIs of ED_{50} values (Table 1). One rat died after administration of ST 198 30 mg/kg. Autopsy of the animal did not reveal any cause of death.

Table 1 ED_{50} Values (95% CIs) for Percentage of Drug-Lever Selection when Nicotine was Administered Alone and with Selected Doses of BP 897 and ST198

	ED_{50} (95% CI) (mg/kg)
Nicotine alone	0.05 (0.04–0.06)
Nicotine+0.3 mg/kg BP 897	0.03 (0.02–0.05)[a]
Nicotine+1 mg/kg BP 897	0.08 (0.05–0.15)[a]
Nicotine+30 mg/kg ST 198	0.06 (0.01–0.15)[a]
Nicotine+100 mg/kg ST 198	0.08 (0.04–0.18)[a]

[a]Overlapping 95% CI compared with the dose–response curves of nicotine alone.

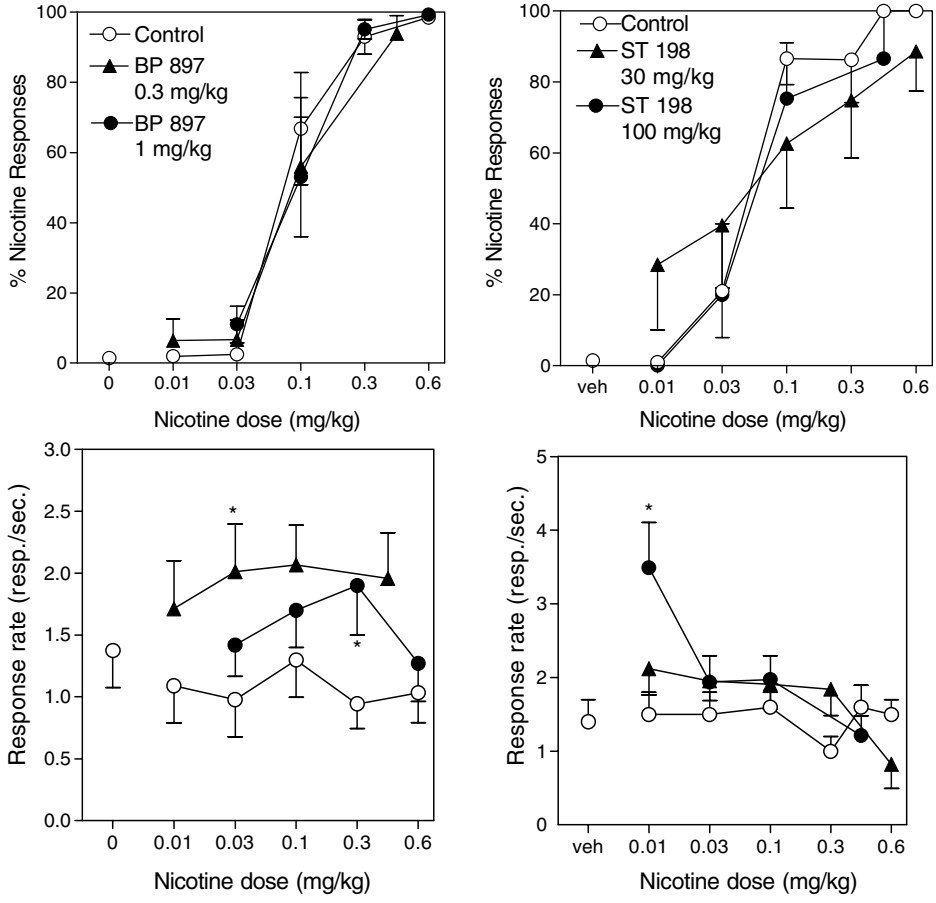

Figure 4 BP 897 (D_3R partial agonist) and ST 198 (D_3R antagonist) given acutely 30 min before the session did not modify the discrimination of nicotine (no shift of the curve); nicotine dose–response curves after pretreatment with BP 897 (left panels) and ST 198 (right panels). Data are means (±SEM) from five to six nicotine-trained rats. The percentage of responses on the lever associated with nicotine administration is shown as a function of dose (mg/kg, log scale) (upper panels). Response rates are expressed as responses per second (bottom panels). ED_{50} values with 95% CIs for these dose–response curves are given in Table 1.

BP 897 at doses of 0.3 and 1 mg/kg in combination with various doses of nicotine significantly increased response rates, compared to nicotine alone. For experiments with BP 897 at the dose of 0.3 mg/kg, two-way ANOVA revealed a significant effect of BP 897 ($F(1,50) = 5.33$, $P = 0.03$), no significant effect of nicotine dose ($F(4,50) = 1.22$, $P = 0.31$), and no significant interaction between BP 897 and the dose of nicotine ($F(4,50) = 1.21$, $P = 0.32$). For experiments with BP 897 at the dose of 1 mg/kg, two-way ANOVA revealed a significant effect of BP 897 ($F(1,50) = 5.23$, $P = 0.03$), no significant effect of nicotine dose ($F(4,50) = 0.53$, $P = 0.71$), and no significant interaction between BP 897 and the dose of nicotine ($F(4,50) = 0.77$, $P = 0.55$).

ST 198 at doses of 30 and 100 mg/kg in combination with various doses of nicotine significantly also increased response rates, compared to nicotine alone. For experiments with ST 198 at the dose of 30 mg/kg, two-way ANOVA revealed a significant effect of ST 198 ($F(1,60) = 4.62$, $P = 0.04$), no significant effect of nicotine dose ($F(5,60) = 1.16$, $P = 0.34$), and no significant interaction between ST 198 and the dose of nicotine ($F(5,60) = 1.63$, $P = 0.17$). For experiments with ST 198 at the dose of 100 mg/kg, two-way ANOVA revealed a significant effect of ST 198 ($F(1,48) = 6.54$, $P = 0.01$), a significant effect of nicotine dose ($F(4,48) = 2.59$, $P = 0.048$), and a significant interaction between ST 198 and the dose of nicotine ($F(4,48) = 3.04$, $P = 0.03$). *Post hoc* analysis revealed a significant increase of response rates in the group of rats receiving 30 mg/kg ST 198 in combination with 0.01 mg/kg nicotine.

These results indicate that the two D_3 ligands do not alter the discriminative-stimulus effects of nicotine across a large range of nicotine doses, but do tend to increase response rates of the rats when given in combination with nicotine.

Experiment 3: Assessment of Antidepressant Actions of BP 897 with a Forced Swimming Test

Figure 5a shows the time spent attempting to escape from the water-filled cylinders during the 4 min test period following administration of imipramine at a dose of 32 mg/kg or BP 897 at doses of 1 and 2 mg/kg or saline vehicle.

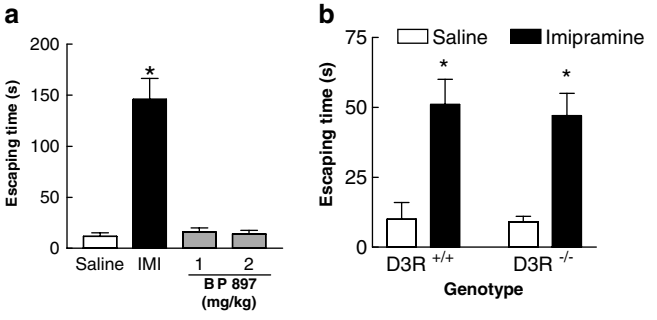

Figure 5 BP 897 administration or D_3R invalidation does not interfere with the forced swimming test. (a) Duration of mobility (escaping time) in water after injection of saline, imipramine (IMI, 32 mg/kg i.p.) or BP 897 (1 or 2 mg/kg i.p.) in Swiss male mice ($n = 9$–11). $^*P < 0.001$ *vs* saline by ANOVA. (b) Duration of mobility (escaping time) in water after injection of saline or imipramine (IMI, 32 mg/kg i.p.) in D_3R wild-type mice or in D_3R-deficient mice. No significant effect of genotype on imipramine response.

One-way ANOVA indicated a significant effect of treatment ($F(3,35) = 32.6$, $P < 0.0001$). *Post hoc* analysis indicated a significant effect of imipramine ($P < 0.0001$), but no effect of BP 897 at 1 and 2 mg/kg ($P > 0.99$).

Figure 5b shows the time spent attempting to escape from the water-filled cylinders during the 4 min test period by D_3R-deficient mice and their wild-type littermates following administration of imipramine or saline vehicle. Two-way ANOVA analysis of results indicated no effect of genotype ($F(1,44) = 0.05$, $P = 0.82$), a significant effect of imipramine ($F(1,44) = 10.88$, $P < 0.002$), and no interaction between genotype and imipramine treatment ($F(1,44) = 0.01$, $P = 0.91$). *Post hoc* analysis indicated no basal differences in escape behavior between D_3R-deficient mice and their wild-type littermates ($P = 0.94$) and a significant effect of imipramine in both $D3R^{+/+}$ and $D3R^{-/-}$ mice ($P = 0.02$ and $P = 0.03$, respectively).

DISCUSSION

In the CPP paradigm, animals are tested in a drug-free state to determine whether they prefer an environment in which they previously received nicotine as compared to an environment in which they previously received saline (Le Foll and Goldberg, 2004a). On the test day, the approach and association of the animals with the drug-paired side may be considered a measure of drug-seeking behavior. In agreement with our previous studies, a dose of 0.1 mg/kg nicotine induced significant CPP using a bias procedure, as compared to saline-conditioned animals (Le Foll and Goldberg, 2004a, b). Two D_3 selective ligands, BP 897 and ST 198, dose-dependently blocked the expression of nicotine-induced CPPs, in agreement with the proposed role of D_3Rs in reactivity to drug-associated cues. In parallel drug-discrimination studies, doses of BP 897 and ST 198, which effectively blocked the expression of nicotine-induced CPP, did not produce nicotine-like discriminative-stimulus effects when substituted for the training dose of nicotine, and did not significantly alter either the dose–response curve for nicotine discrimination or the ED_{50} values for nicotine discrimination. These findings suggest that BP 897 and ST 198 can act selectively to reduce the motivational effects of nicotine-associated stimuli.

Since pramipexole, a D_2R/D_3R agonist, is an effective treatment for depression in humans (Lattanzi *et al*, 2002; Ostow, 2002) and the antidepressants bupropion and nortriptyline have been used as aids for smoking cessation, as is proposed for BP 897 (Le Foll *et al*, 2003a), we used a forced swimming test to assess the potential anti-depressant-like effects of BP 897 that may have contributed to its blockade of expression of nicotine-induced CPP. This test is sensitive to the effects of bupropion (Cooper *et al*, 1980) and other antidepressant drugs (Porsolt *et al*, 1978). In contrast to the antidepressant, imipramine, which effectively increased performance (escape behavior) in the forced swimming test, doses of BP 897 as high as 2 mg/kg had no effect on performance in the forced swimming test. This is in agreement with the lack of efficacy of various dopaminergic ligands in this test (Renard *et al*, 2001). D_3R invalidation also had no effect on escape behavior in the forced swimming test and did not prevent the effects of

imipramine. It may be difficult to extrapolate results obtained with mice to rats, although the pharmacokinetic parameters of BP 897 do not differ in the two species (Sokoloff, unpublished results). Nevertheless, our findings suggest that D_3Rs are not involved in the acute response to antidepressants in the forced swimming test and that the blockade of the expression of nicotine-induced CPP by BP 897 and ST 198 does not involve buproprion-like antidepressant effects.

It is unlikely that blockade of the expression of nicotine-induced CPP was due to a nonspecific disruptant effect of these D_3R ligands on behavior, since, at these doses, BP 897 and ST 198 did not decrease locomotor activity in the CPP apparatus during test sessions and did not disrupt food-maintained behavior under the drug-discrimination procedure. It is also unlikely that blockade of the expression of nicotine-induced CPP by BP 897 and ST 198 in the present experiments was due to direct effects on the memory of drug-associated stimuli, since BP 897 has no effect on habituation to neutral cues in an open field environment (Le Foll *et al*, 2002). However, habituation may involve different memory processes than those needed for CPP. It is worth noting that BP 897 has no effect in the passive avoidance test, which measures reactivity to aversive stimuli and is also used as an animal model for anxiolytic effects of drugs (Le Foll *et al*, 2002). This procedure involves association of a context with an unconditioned stimulus, which is more similar to the learning involved in CPP. Nevertheless, the 1 mg/kg dose of BP 897 did increase time spent in the nonpreferred side of the apparatus, an effect that may reflect anxiolytic properties of this ligand (Rogoz *et al*, 2003). Finally, it is unlikely that a shift in the basal preference for one side of the apparatus during repeated conditioning sessions contributed to the present results with nicotine-induced CPP, since no shift was observed in saline-treated control rats. It is also unlikely that this effect is mediated through aversive properties of BP 897, since these later properties have been inconsistently found following repeated pairing of BP897 effects with a particular environment (Duarte *et al*, 2003; Gyertyan and Gal, 2003; Francès *et al*, 2004b) and in the present study BP 897 was only administered acutely during the test session.

Under some conditions, D_2 receptor blockade appears to disrupt conditioned associations between environmental stimuli and the interoceptive effects of psychoactive drugs. For example, raclopride, a preferential antagonist at D_2R/D_3Rs (Sokoloff *et al*, 1990; Levant, 1997), can block drug-seeking behavior induced by the reintroduction of cocaine-associated stimuli (Weissenborn *et al*, 1996). Such a D_2 receptor-mediated effect is, however, unlikely in the present experiments. First, BP 897 and ST 198 have a high affinity for the D_3R ($K_i = 0.92$ and 12 nM for BP 897 and ST 198, respectively) and relatively high D_2/D_3 selectivity (70 and 65 times lower affinity at the D_2 receptor for BP 897 and ST 198, respectively). Although these ligands are able to occupy D_2 receptors *in vivo*, they do so only at much higher doses than those which blocked the expression of nicotine-induced CPP in the present experiments, as shown by other *in vivo* experiments. For instance, in D_3R-deficient mice, *c-fos*-activating effects of a 1 mg/kg dose of BP 897 were noted in wild-type mice, but not in D_3 knockout mice (Pilla *et al*, 1999). The D_3R selectivity of the does of BP 897 and by ST 198 used in the present study was also demonstrated by showing that the effects of these agents are abolished in D_3R-deficient mice, but not in wild-type controls, using a behavioral model based on dizocilpine-induced locomotion, which allows the direct assessment of D_3R blockade *in vivo* (Bezard *et al*, 2003; Leriche *et al*, 2003). On the contrary, D_2 receptor occupancy is achieved by BP 897 at an ED_{50} of $\sim 15\,mg\,kg^{-1}$ and cataleptic effects of BP 897 appear with an ED_{50} of $\sim 12\,mg\,kg^{-1}$, and no effects could be detected at 1 mg/kg and lower on these paradigms, whereas these doses are active in blocking nicotine-induced CPP in our experiment. This is also illustrated by the finding that high doses of BP 897 were needed to decrease responding for food in the drug-discrimination paradigm (10 mg/kg), an effect typical of agents blocking D_2 receptors (Desai *et al*, 2003), but 10–30-fold lower doses of BP 897 or ST 198 were effective in blocking the expression of nicotine-induced CPP. This is consistent with the absence of any significant motor effects induced by administration of BP 897 or ST 198 in the present CPP experiments or in previous published studies (Pilla *et al*, 1999; Le Foll *et al*, 2002, 2003a).

The mechanism underlying the present effects of BP 897 and ST 198 on nicotine-induced CPP remains to be determined. BP 897 is a D_3 partial agonist (Pilla *et al*, 1999). As a partial agonist, BP 897 may act as an antagonist in a situation of high dopamine transmission, a feature associated with presentation of drug-associated stimuli (Di Ciano *et al*, 1998a, b; Weiss *et al*, 2000). Since, BP 897 and ST 198 had the same effect on nicotine-induced CPP, one hypothesis is that BP 897 acts like an antagonist *in vivo* in this paradigm. Another highly selective D_3R antagonist, SB-277011-A, produces effects similar to BP 897, including disruption of nicotine-triggered relapse to nicotine-seeking behavior (Andreoli *et al*, 2003), inhibition of cocaine (Le Foll *et al*, 2002) and nicotine conditioning (Le Foll *et al*, 2003a), and reduction of cue-induced cocaine-seeking behavior (Pilla *et al*, 1999; Di Ciano *et al*, 2003). Therefore, a blockade of dopamine transmission is likely to be involved. Since the D_3Rs are overexpressed in the brain of cocaine- (Le Foll *et al*, 2002) and nicotine-treated (Le Foll *et al*, 2003a, b) animals, this antagonistic activity at D_3Rs may serve to normalize dopamine transmission (Le Foll *et al*, 2003a). This blockade of the dopamine transmission may also explain the ability of SB-277011-A to block nicotine-triggered relapse to nicotine-seeking behavior, since elevation in dopamine levels in the nucleus accumbens has been implicated in the initiation of drug-seeking behavior (Phillips *et al*, 2003). Another possibility is that BP 897 reduces dopamine transmission through D_3 autoreceptors (Diaz *et al*, 2000; Le Foll *et al*, 2004) in the ventral tegmental area (Le Foll *et al*, 2002). It also seems likely that other brain structures, such as the amygdala (Le Foll *et al*, 2002) and the somatosensory cortex (Le Foll *et al*, 2002; Francès *et al*, 2004a), are involved.

In conclusion, the present findings show that administration of BP 897 and ST 198 is able to block the expression of nicotine-induced CPPs. These D_3R ligands had no significant effects on the discriminative-stimulus effects of nicotine, indicating that their effects would be selective for those rewarding or reinforcing effects of nicotine that contribute to the maintenance of tobacco-smoking

behavior, without affecting the ability to discriminate nicotine's other effects. Involvement of antidepressant actions in the present effects of BP 897 and ST 198 on CPP is unlikely, since we found no effect of D_3R blockade or genetic depletion of D_3Rs in the forced swimming test, suggesting that the mechanism by which D_3R ligands reduce the motivational effects of nicotine are distinct from those of bupropion. The present findings support and extend previous findings that D_3R partial agonists and antagonists attenuate conditioned responses to various types of drug-associated stimuli and lend support to the proposed use of these compounds as aids in smoking cessation treatment.

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