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The selective dopamine D_3 receptor antagonist SB-277011A reduces nicotine-enhanced brain reward and nicotine-paired environmental cue functions

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Abstract

Increasing evidence suggests that enhanced dopamine (DA) neurotransmission in the nucleus accumbens (NAc) may play a role in mediating the reward and reinforcement produced by addictive drugs and in the attentional processing of drug-associated environmental cues. The meso-accumbens DA system is selectively enriched with DA D_3 receptors, a DA receptor subtype increasingly implicated in reward-related brain and behavioural processes. From a variety of evidence, it has been suggested that selective DA D_3 receptor antagonism may be a useful pharmacotherapeutic approach for treating addiction. The present experiments tested the efficacy of SB-277011A, a selective DA D_3 receptor antagonist, in rat models of nicotine-enhanced electrical brain-stimulation reward (BSR), nicotine-induced conditioned locomotor activity (LMA), and nicotine-induced conditioned place preference (CPP). Nicotine was given subcutaneously within the dose range of 0.25–0.6 mg/kg (nicotine-free base). SB-277011A, given intraperitoneally within the dose range of 1–12 mg/kg, dose-dependently reduced nicotine-enhanced BSR, nicotine-induced conditioned LMA, and nicotine-induced CPP. The results suggest that selective D_3 receptor antagonism constitutes a new and promising pharmacotherapeutic approach to the treatment of nicotine dependence.

Keywords

Brain-stimulation reward; conditioned locomotor activity; conditioned place preference; dopamine D_3 receptors; nicotine

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Introduction

Nicotine, like other addictive drugs such as amphetamines, cannabinoids, cocaine, ethanol, and opiates, enhances brain reward functions in the central meso-accumbens dopamine (DA) brain reward system (for reviews, see Clarke, 1990; Di Chiara, 2000; Gardner, 2005; Wise and Gardner, 2002). This nicotine-enhanced meso-accumbens brain reward is mediated by increased DA in the reward-related nucleus accumbens (NAc) (e.g. Imperato et al., 1986; Pontieri et al., 1996; Yoshida et al., 1993). The enhanced NAc DA is important to nicotine's addictive effect (Di Chiara, 2000; Gardner, 2005; Pontieri et al., 1996; Wise and Gardner, 2002), and appears to result from increased meso-accumbens DA neuronal firing (Calabresi et al., 1989; Mereu et al., 1987; Nomikos et al., 2000). This most likely occurs via a site of action within the ventral tegmental area (VTA) (Corrigall et al., 1994; Nomikos et al., 2000; Wise and Gardner, 2002; Yeomans and Baptista, 1997), and may involve both D_1 (Harrison et al., 2002) and D_2 (Huston-Lyons et al., 1993) receptor mechanisms. Congruently, lesioning the DA-containing neurons that project from the VTA to the NAc through the meso-accumbens system attenuates nicotine self-administration in animals (Corrigall et al., 1992, 1994; Di Chiara, 2000; Stolerman and Shoaib, 1991). In humans, pharmacologically induced DA blockade produces a compensatory increase in cigarette smoking and nicotine intake (Caskey et al., 1999; 2002) while administration of a DA agonist produces a compensatory decrease in cigarette smoking and nicotine intake (Caskey et al., 2002; Jarvik et al., 2000), suggesting that nicotine self-administration is mediated by DA mechanisms in the same manner as other addictive drugs (e.g. de Wit and Wise, 1977; Gardner, 2000, 2005; Ikemoto and Wise, 2004; Wise and Rompré, 1989; Wise et al., 1995a,b; Yokel and Wise, 1975, 1976).

Considerable evidence indicates that D_1 and D_2 receptors play significant roles in drug-induced reward and incentive motivation (e.g. Baker et al., 1998; de Wit and Wise, 1977; Ettenberg et al., 1982; Gardner, 2005; Ikemoto et al., 1997; Nakajima, 1989; Nakajima et al., 1993; Spyraki et al., 1987; Wise and Gardner, 2002; Wise and Rompré, 1989). Recently, however, the DA D_3 receptor has gained attention with respect to the meso-accumbens VTA–NAc DA system, drug-induced reward, motivation and relapse, and the behavioural manifestations of addiction (for reviews, see Heidbreder et al., 2004, 2005; Joyce and Millan, 2005; Newman et al., 2005). This has led to the suggestion that the DA D_3 receptor may be a useful therapeutic target for anti-addiction medications (Caine and Koob, 1993, 1995; Caine et al., 1997; Heidbreder and Hagan, 2005; Levant, 1997; for reviews, see Heidbreder et al., 2004, 2005; Joyce and Millan, 2005; Le Foll et al., 2005a; Newman et al., 2005).

To date, studies of the involvement of DA D_3 receptors in the neurobiology of drug addiction have been limited, due to a lack of suitably selective D_3 compounds. Recently, however trans-N-[4-[2-(6-cyano-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl]cyclo-hexyl]-4-quinolinecarboxamide (SB-277011A), a highly selective and potent D_3 receptor antagonist with good brain-penetrant properties in rodents was synthesized (Reavill et al., 2000; Stemp et al., 2000). Recent research with SB-277011A suggests that selective DA D_3 antagonism appears to have a promising anti-addiction profile against cocaine, heroin, and ethanol in animal models (for reviews, see Heidbreder et al., 2004, 2005). With respect to nicotine, recent studies have shown that nicotine-induced behavioural sensitization (Le Foll et al., 2003a) and nicotine-induced conditioned locomotion (Le Foll et al., 2003b) are both associated with significant *increases* in DA D_3 receptor binding and mRNA levels in the NAc, without significant alteration in D_1 or D_2 receptor mRNA. The same studies also showed that nicotine cue-conditioned locomotor activity (LMA) is attenuated both by the D_3 receptor antagonist SB-277011A and by BP-897, a partial D_3 receptor agonist with D_3 antagonist properties (Wicke and Garcia-Ladona, 2001; Wood et al., 2000). In addition, D_3

receptor ligands have recently been shown to block nicotine-induced conditioned place preference (CPP) (Le Foll et al., 2005b), and we have recently shown that SB-277011A blocks nicotine-induced relapse to nicotine-seeking behaviour (Andreoli et al., 2003b). We take such findings to suggest that highly selective DA D_3 receptor antagonists may prove useful as anti-nicotine addiction pharmacotherapeutic agents. Therefore, in the present series of experiments, we further investigated the effects of several doses of SB-277011A on nicotine-enhanced brain stimulation reward (BSR), nicotine-associated cue-conditioned LMA, and the expression of nicotine-induced CPP.

Materials and methods

Animals

For the nicotine-enhanced BSR experiments, male Long–Evans rats (Charles River Laboratories, Raleigh, NC, USA) experimentally naive at the start of experiments, and weighing 300–325 g at time of surgery, were used. They were housed individually in a climate-controlled environment with food and water freely available with the exception of the time spent each day in the test chambers. All BSR experiments were approved by the Animal Care and Use Committee of the National Institute on Drug Abuse of the U.S. National Institutes of Health, and were carried out in compliance with applicable United States federal and Maryland state laws and regulations. For the cue-conditioned LMA experiments, male Wistar rats (Charles River Deutschland, Sulzfeld, Germany) experimentally naive at the start of experiments, and weighing 250–360 g at the start of drug-cue pairings, were used. They were housed individually in a climate-controlled environment with food and water freely available with the exception of the time spent each day in the test chambers. All cue-conditioned LMA experiments were conducted under a Project License obtained according to Italian law regulating animal experimentation (Article 7, Legislative Decree 116, 27 January 1992), which acknowledges European Directive 86/609/EEC for the protection of animals used for experimental and other scientific purposes. For the nicotine-induced CPP experiments, male Sprague–Dawley rats (Taconic Farms, Germantown, NY, USA) experimentally naive at the start of experiments, and weighing 200–210 g at the start of drug-cue pairings, were used. They were housed two per cage in a climate-controlled environment with food and water freely available with the exception of the time spent each day in the test chambers. All cue-conditioned CPP experiments were approved by the Animal Care and Use Committee of St John's University, and were carried out in compliance with applicable United States federal and New York state laws and regulations. All experiments – whether conducted in Italy, New York, or Maryland – were carried out in accordance with the principles of laboratory animal care stipulated in the Guide for the Care and Use of Laboratory Animals (National Academy of Sciences, 1996).

Drugs and chemicals

(−)Nicotine (+)bitartrate salt was used in all experiments (Sigma-Aldrich Corporation, St Louis, MO, USA) and was dissolved in sterile physiological saline and the pH adjusted to 7.4 with NaOH. The doses of nicotine are expressed as free base. SB-277011A was provided by GlaxoSmithKline Pharmaceuticals (Verona, Italy, and Harlow, Essex, UK) and was placed into solution using 25% (for the BSR experiments), 10% (for the LMA experiments), or 20% (for the main CPP experiment testing SB-277011A's effect on nicotine-induced CPP; see below) 2-hydroxypropyl-β-cyclodextrin (for the BSR and CPP experiments, obtained from Fluka Division of Sigma-Aldrich Corporation, St Gallen, Switzerland; for the LMA experiments, obtained from Wacker-Chemie GmbH, München, Germany). In two additional CPP experiments carried out to determine whether SB-277011A by itself

produces appetitive or aversive effects (see below), SB-277011A was placed into solution using 2% w/v methylcellulose.

Behavioural methods and testing

BSR (nicotine-enhanced brain-stimulation reward) experiments

Surgery: Under an anaesthetic mixture of sodium pentobarbital and chloral hydrate, each rat was surgically implanted, using standard aseptic stereo-taxic technique, with a unilateral monopolar stainless-steel stimulating electrode (Plastics One, Roanoke, VA, USA) aimed at the medial forebrain bundle at the level of the lateral hypothalamus. The target implant stereotaxic coordinates were, from bregma, AP −2.5 mm, ML +1.7 and DV −8.4 mm, using the rat brain atlas of Paxinos and Watson (1998). The top of the electrode and the electrode connector (to which the wires from the brain stimulator connected via a quick-connect electrical mini-plug) were cemented to the skull with acrylic resin cement. A wire wrapped around a jeweller's screw implanted in the skull and connected to a mini-pin in the electrical connector at the top of the electrode was used to accommodate return electrical current. The skin was drawn up around the acrylic mound and sutured, prophylactic antibiotics and post-surgical analgesics given, and each animal was kept warm and under observation until all anesthesia effects were gone. Rats were given 5 d to recover fully from surgery, under daily veterinary supervision, before experiments were started.

Apparatus: All training and testing occurred in standard operant chambers, each containing a retractable wall-mounted lever and a cue light immediately above the lever (MED Associates, Georgia, VT, USA). The operant chambers were enclosed in ventilated, sound-attenuating cabinets. Depression of the lever activated a stimulator programmed to deliver trains of 0.1 ms cathodal pulses, each pulse-train having 500 ms duration.

Procedure: Animals (n=14) were placed once daily into the operant chambers, connected to the brain stimulator via their skull-mounted electrical connectors, and trained using an electrical BSR procedure slightly modified from studies that we have previously described (Lepore et al., 1996; Vorel et al., 2002). To find an animal's optimal stimulation amperage, the animals were placed in the apparatus for 1 h per day and allowed to 'auto-shape' (i.e. learn by themselves) lever-pressing, on a FR-1 reinforcement schedule, for BSR at 300 μA and 124 Hz. The amperage was then increased gradually until the animal produced moderate rates of responding (45–60 responses/30 s). Our experience was that 300 μA was optimal for most animals; in no case did the amperage need to be increased to >350 μA to achieve the desired behavioural output of 45–60 responses/30 s. Once this training was complete, the rats were then transferred to a rate frequency procedure (Campbell et al., 1985) in which animals were allowed to lever press, on a FR-1 reinforcement schedule (which continued for the duration of experimentation), for a series of 16 different pulse frequencies, ranging from 141 to 25 Hz, presented to the animals in descending order. Each pulse frequency was preceded by a 500 ms priming stimulation to 'inform' the animal as to the frequency of the immediately following BSR, and then the rat was allowed to lever press for BSR during a 30-s period. Each pulse frequency was offered for two 30-s periods (time bins) before descending to the next frequency. We have, therefore, labelled such sweeps of pulse frequencies from 141 to 25 Hz as 'twinned time-bin sweeps'. When one such 'twinned time-bin sweep' of pulse frequencies from 141 to 25 Hz was completed, the procedure was repeated – what we call a 'double-sweep'. This double-sweep procedure then repeated itself twice more, for a total of three double-sweeps per day. The first double-sweep of each day was a 'warm-up' session and discarded from the data. The second double-sweep was taken as the baseline BSR session while the third was taken as the test BSR session. The four response rate measures at each stimulation frequency of the baseline BSR session were averaged, as were the four of the test BSR session, yielding an average baseline and an

average test lever press rate at each frequency for the baseline BSR and test BSR sessions. Reward threshold, θ_0(the frequency at which the animal failed to respond for rewarding stimulation), was then determined for the baseline BSR session and the test BSR session. This was done by mathematically fitting each rate-frequency curve (one for the baseline BSR session; one for the test BSR session) by iterative computer programs derived from the Gauss – Newton algorithm for nonlinear regression to three different sigmoidal curve-fitting mathematical growth models that appear to accurately fit rate-frequency electrical brain reward functions (Coulombe and Miliaressis, 1987): the Gompertz model ($\gamma' = a e^{-e(b-cX)}$), the logistic model ($\gamma' = a/[1+e^{(b-cX)}]$), and the Weibull function ($\gamma' = a[1-e^{-(bX)^C}]$). From each curve-fitting model, a θ_0 solution was obtained. The three solutions for θ_0 were averaged to produce a mean θ_0 for each rate-frequency BSR function generated by a given animal over a given descending series of pulse frequencies. When mean θ_0 was stable (three successive days with each θ_0 value within 10% of overall mean θ_0), animals were injected, between the baseline and test BSR session with subcutaneous (s.c.) nicotine (0.25 mg/kg) and intra-peritoneal (i.p.) SB-277011A (0, 3, 6, or 12 mg/kg). SB-277011A or its vehicle was given 30 min, and nicotine 30 s before test sessions, 30 min being the time interval between the baseline and test BSR sessions (during which interval the animals remained in their operant test chambers). The sequence of the SB-277011A doses was counterbalanced.

A second group of animals (n=9) was also run in the BSR paradigm, and tested at a higher dose of nicotine (0.5 mg/kg s.c.). These animals were run using an inter-day, rather than an intra-day, testing protocol, with baseline and test BSR sessions on separate days. In this group, when mean θ_0 was stable (5 successive days with each θ_0 value within 10% of overall mean θ_0), animals were given, on the same day, a 'warm-up' BSR session followed by a baseline BSR session. Then, when daily BSR sessions were again stable (3 more successive days with each θ_0 value within 10% of overall mean θ_0), animals were given, on the same day, a warm-up BSR session followed by a test BSR session. Successive warm-up and baseline BSR sessions then followed, with at least 3 days during which each θ_0 value fell within 10% of overall mean θ_0 being required before the next warm-up and test BSR session was run. Nicotine (0.5 mg/kg s.c.) and SB-277011A (0, 3, 6, or 12 mg/ kg i.p.) were administered 30 s and 30 min, respectively, before test sessions. Since warm-up BSR results were discarded (by definition, see above), drug effects on BSR were computed by comparing test BSR sessions against the immediately previous baseline BSR sessions. The sequence of the SB-277011A doses was counterbalanced.

Histology: Upon completion of experiments, animals were killed by anaesthetic overdose (sodium pentobarbital). The rats were then decapitated and their brains removed and stored in 10% buffered formalin solution. Brains were transferred to a 20% sucrose solution at least 24 h before sectioning. Coronal sections (50 μm thick) were made on a vibratome and stained with Cresyl Violet. Reconstructions of electrode tracks were made using a microscope and the rat brain atlas of Paxinos and Watson (1998).

Statistical analysis: Shifts in mean θ_0 values produced by nicotine, nicotine+vehicle, or nicotine+ SB-277011A were analysed using Student's t test where appropriate and also by one-way analysis of variance (ANOVA) for repeated measures followed by multiple comparison testing using the Newman– Keuls post-hoc test (Kirk, 1982; Winer, 1962). Minimally acceptable statistical significance was set at a probability level of $p<0.05$ for all tests.

LMA (nicotine cue-conditioned locomotor activity) experiments

Apparatus: Locomotor activity was recorded by 16 computerized AccuScan Versamax units (AccuScan Instruments Inc., Columbus, OH, USA). Each unit consisted of a clear

Plexiglas cage (40 cm wide × 40 cm deep × 32 cm high) equipped with 32 infrared light beam sensors located 1.3 cm above the floor. Photo beam interruptions were monitored in 5-min periods (time bins) from each monitor and were analysed by AccuScan Analyzer computer software.

Procedure: The AccuScan Versamax activity monitoring units described immediately above were also used as cue-conditioning chambers following acute nicotine or saline administration. The conditioning phase consisted of 30-min conditioning (cue-pairing) sessions. There were four groups of animals (n=16 per group). Groups 1 and 2 were the *saline*- and *nicotine-paired* groups. Each rat in these two groups was injected with saline in the home cage and 3 h later with either saline (1 ml/kg s.c.) or nicotine (0.5 mg/kg s.c. in a volume of 1 ml/kg) and then immediately placed into a locomotor activity chamber (drug-paired environment) for 30 min. Groups 3 and 4 were control, unconditioned animals. Each rat in these two groups was injected with either saline (1 ml/kg s.c.) or nicotine (0.5 mg/kg s.c. in a volume of 1 ml/kg) in its home cage, and then 3 h later was injected with saline and immediately placed into a locomotor activity chamber (drug-*un*paired environment) for 30 min. Groups 3 and 4 were, therefore, the *saline*- and *nicotine-unpaired* groups. This procedure was performed once a day for 4 d. The test phase occurred on the following day (day 5), and was conducted identically to the conditioning phase days, except that each animal was given an injection of saline and then immediately placed into a locomotor activity chamber. Thus, during the test phase, locomotor activity was induced only by the saline- or nicotine-associated environmental cues of the locomotor activity chambers. To assess the effects of D_3 receptor antagonism on nicotine-cue-induced locomotor activity, on the test day animals were divided into two pharmacological treatment groups: 0 mg/kg i.p. SB-277011A (i.e. the β-cyclodextrin vehicle) or 10 mg/ kg i.p. SB-277011A (n=8 per group). In a further experiment, an additional 36 animals were trained as a *nicotine-paired* group only, as described above. On the test day these animals were divided into three pharmacological treatment groups: 0 (i.e. vehicle), 1, or 3 mg/kg i.p. SB-277011A. All pharmacological treatments were given 30 min prior to LMA testing.

Statistical analysis: Raw LMA data counts were converted to Area Under the Curve for the first 20 min of testing (AUC 0–20 min), using Simpson's Rule for AUC calculation

$$[(\text{MaxTime} - \text{MinTime})/3 * n] \times [\gamma 1 + 4 * (\gamma 2 + \gamma 4 + \ldots) + 2 * (\gamma 3 + \gamma 5 + \ldots) + \gamma n],$$

where n represents the total number of time bins and γ represents the absolute value of each time bin in sequential order (see Epperson, 2001 for the theory and derivation of AUC calculations). Simpson's Rule for AUC calculation was chosen over the Trapezoidal Rule for AUC calculation because it yields a more accurate numerical integration (Epperson, 2001). The data were then analysed by ANOVA followed by multiple comparison testing (Kirk, 1982; Winer, 1962). The effects of SB-277011A on LMA were analysed by three-way ANOVA with main factors of environment (paired vs. unpaired), conditioning drug (saline vs. nicotine), and treatment (vehicle or SB-277011A, 10 mg/kg), taking into account the following interactions: (1) drug × environment; (2) drug × treatment; (3) environment × treatment, and (4) drug × environment × treatment. Differences within each interaction were further analysed by performing planned comparisons using the 'a priori t test' (Kirk, 1982, pp. 95–97).

For the effects of SB-277011A (0, 1, 3 mg/kg i.p.) on the nicotine-paired group only, a one-way ANOVA was performed. Minimally acceptable statistical significance was set at a probability level of p<0.05 for all tests.

CPP (nicotine-induced conditioned place preference) experiments

Apparatus: An automated, three-chambered, Plexiglas CPP apparatus was used as previously described (Dewey et al., 1999; Horan et al., 2000), with modifications. The two pairing chambers of the apparatus were identical in dimensions (25 × 14 × 36 cm) and were separated by removable Plexiglas guillotine doors. The pairing chambers were composed of distinct visual and tactile cues. The walls of one of the pairing chambers were white with cage bedding on the floor and the walls of the second chamber consisted of alternating white and black boxes (1.2 × 1.8 cm) in a chessboard pattern and a Plexiglas floor. The two pairing chambers were separated by a third, connecting tunnel (7.5 × 7.5 × 36 cm). This third connecting tunnel was equipped with the same distinct visual and tactile cues as the two pairing chambers; the half of the connecting tunnel adjacent to the white-walled pairing chamber was similarly white-walled, while the half of the connecting tunnel adjacent to the chessboard-patterned wall was similarly chessboard patterned, and so forth. Thus, while strictly speaking a third chamber existed, a third set of visual and tactile cues did not.

Procedure: CPP expression was assessed as previously described (Horan et al., 2000; Vorel et al., 2002). Expression studies with acute SB-277011A or vehicle were divided into four phases: acclimation, handling, conditioning, and testing. The animals were not exposed to the chambers prior to the start of the pairings. During days 1–3, animals were acclimated to the animal facility. During handling (days 4–6), animals were transported to the laboratory and handled for 5 min each. During conditioning (days 7–22), animals were exposed to once-daily conditioning sessions. For each conditioning session, animals (n=40) were injected with nicotine (0.6 mg/kg s.c. in a volume of 1 ml/kg) or vehicle (1 ml/kg s.c.) and then immediately confined for 30 min in an appropriate cue-specific chamber. During conditioning, nicotine was always paired with one cue-specific environment, and vehicle was paired with the other; nicotine or vehicle exposure (and appropriate environmental pairing) alternated from day to day. This was done over a 16-d period, i.e. animals were given eight pairings with nicotine and there was a 24-h separation between exposure to vehicle and nicotine. The animals in each group were randomly assigned to a 2 × 2 factorial design with one factor being the pairing chamber and the other factor being the order of conditioning. In this counterbalanced procedure, the animals were randomly assigned to one of the two pairing chambers, so that half of the subjects received the drug in one compartment (white walls, with bedding on the floor) and the other half in the other compartment (alternating black and white squares on the walls, with a smooth chamber floor). This procedure resulted in the animals receiving equal exposure to the two compartments and due to random assignment, controlled for side preference. An additional group of 10 animals was paired with vehicle in both chambers of the apparatus during the conditioning phase.

On the test day (day 23), the 40 animals that had been paired in the CPP apparatus alternating with nicotine and vehicle during the conditioning phase were randomly divided into four groups of 10 and received either SB-277011A (1, 3, or 10 mg/kg i.p.) or vehicle in the home cage 30 min before they were placed in the CPP apparatus. Then, the guillotine doors were removed and the animals were allowed to move freely within the apparatus for 15 min. The amount of time spent in each chamber was determined using an automated timing system. Each of the pairing chambers contained infrared micro-beams (two per chamber) that were wired to an automated timer. When an animal entered a chamber, the beam was broken and a timer began recording. Once the animal left the chamber, the timer stopped. In addition, the circuitry had an internal timer that shut off after 15 min. The third small connecting chamber was so small that it was not possible for an animal to stay totally within it. Thus, the sum of the times spent in the two pairing chambers was always found to equal 15 min. On the test day, the 10 animals that had been paired in the CPP apparatus

alternatingly with vehicle and vehicle during the conditioning phase were tested in identical manner to the 40 animals that had been paired in the CPP apparatus alternatingly with nicotine and vehicle during the conditioning phase.

To test for the possibility that SB-277011A alone might produce place preference or aversion, an additional CPP experiment was carried out with four separate groups of new animals (n=10 per group). In this additional experiment, injections of vehicle (1 ml/kg 2% w/v methylcellulose solution) or 1, 3, or 10 mg/kg SB-277011A were paired with one of the distinctive CPP cue chambers *during CPP conditioning*. These animals received four pairings of distinctive cue chamber and the appropriate dose of SB-277011A or vehicle, with each pairing separated by 24 h. On the test day, these animals received an i.p. injection of vehicle in the home cage 30 min before being placed into the CPP apparatus. Time spent in each cue-distinctive chamber was recorded. In all other methodological respects, this additional CPP experiment was carried out as detailed above for the main CPP experiment.

To test for the possibility that SB-277011A alone might produce behavioural effects on the CPP *test day*, a second additional CPP experiment was carried out with four separate groups of new animals (n=10 per group). In this second additional CPP experiment, the effects *on CPP expression* of vehicle or 1, 3, or 10 mg/kg of SB-277011A *administered on the test day* were assessed. For each CPP conditioning session, animals were injected with vehicle (1 ml/kg s.c. of deionized distilled water) and then immediately confined for 30 min in one of the cue-specific chambers. Twenty-four hours later, the animals were paired with vehicle in the other cue-specific chamber. This pairing cycle was repeated eight times over a 16-d period. Thus all animals received vehicle injection in both chambers. On the test day, animals received SB-277011A (1, 3, or 10 mg/kg i.p.) or vehicle in the home cage 30 min before being placed in the CPP apparatus. Time spent in each cue-distinctive chamber was recorded. In all other methodological respects, this additional CPP experiment was carried out as detailed above for the main CPP experiment.

Statistical analysis: Data were analysed using Student's t test where appropriate (orthogonal observations, independent samples) (Ferguson, 1981) and also by one-way ANOVA (Kirk, 1982; Winer, 1962) followed by multiple comparison testing using the Newman– Keuls test (Kirk, 1982; Winer, 1962). Specifically, the times spent in each chamber by the different experimental groups of animals (i.e. vehicle/vehicle paired during training vs. vehicle/nicotine paired during training; vehicle/nicotine paired during training and SB-277011A 0 mg/kg on test day, vehicle/ nicotine paired during training and SB-277011A 1 mg/kg on test day, vehicle/nicotine paired during training and SB-277011A 3 mg/kg on test day, vehicle/nicotine paired during training and SB-277011A 10 mg/kg on test day) on test day (i.e. CPP expression) were analysed using the above-specified statistical tests. To analyse the data generated in the first additional CPP experiment (to study the possibility that SB-277011A alone might produce appetitive or aversive effects when paired with cue-distinctive chambers *during CPP conditioning*), ANOVA was used. To analyse the data generated in the second additional CPP experiment (to study the possibility that SB-277011A might by itself produce behavioural effects *during CPP expression* on the test day), ANOVA was used. In addition, to test even further for the possibility that SB-277011A might, on the test day, produce some form of 'negative contrast' between the positive CPP expected to be observed in the nicotine-paired compartment and the behaviour *actually observed* in the nicotine-paired compartment, the time spent in the nicotine-paired compartment by SB-277011A–treated rats was compared to the 7.5 min theoretical CPP 'indifference level' for each of the four tested groups of rats (vehicle, 1 mg/kg SB-277011A, 3 mg/kg SB-277011A, 10 mg/kg SB-277011A) using 'one-sample t tests' (Rosner, 2006). Minimally acceptable statistical significance was set at $p<0.05$ for all tests.

Results

BSR (nicotine-enhanced brain-stimulation reward) experiments

Histological verification of electrode implant locations revealed that all electrode tips were within 0.5 mm of the desired target locus in the brain, and (congruently) no animals had to be excluded from the experiment because of failure to reach satisfactory BSR levels and stability.

As shown in Figure 1, nicotine at a dose of 0.25 mg/kg significantly enhanced brain reward (i.e. lowered electrical BSR thresholds) compared to baseline (Figure 1a) (t=9.96, d.f.=13, p<0.001), and pretreatment with SB-277011A (3, 6, or 12 mg/kg) dose-dependently attenuated nicotine-enhanced BSR (Figure 1b). This effect of SB-277011A was confirmed by one-way ANOVA, which revealed a statistically significant SB-277011A main treatment effect ($F_{3,39}$= 18.6, p<0.001). Follow-up individual pairwise comparisons using the Newman–Keuls post-hoc test confirmed that SB-277011A (3, 6, or 12 mg/kg i.p.) given prior to nicotine on the test day produced a statistically significant dose-dependent inhibition of nicotine-enhanced BSR relative to 0 mg/kg SB-277011A (Figure 1b).

Figure 2 shows that nicotine at a dose of 0.5 mg/kg significantly enhanced brain reward (i.e. lowered electrical BSR thresholds) compared to baseline (Figure 2a) (t=9.35, d.f.=8, p<0.001), and that pretreatment with SB-277011A (3, 6, 12 mg/kg) attenuated nicotine-enhanced BSR (Figure 2b). This effect of SB-277011A was confirmed by one-way ANOVA, which revealed a statistically significant SB-277011A main treatment effect ($F_{3,24}$=4.5, p<0.025). Follow-up individual pairwise comparisons using the Newman–Keuls post-hoc test showed that SB-277011A given prior to nicotine on the test day produced a statistically significant inhibition of nicotine-enhanced BSR, but only at a dose of 12 mg/kg SB-277011A (Figure 2b).

LMA (nicotine cue-conditioned locomotor activity) experiments

Effect of SB-277011A on LMA—Rats that had received injections of nicotine in the locomotor activity chambers during conditioning sessions showed enhanced locomotor response, measured by horizontal activity counts, with respect to all other groups (Figure 3). To determine the role of each of the conditioning groups in the overall effects reported above, AUC 0–20 min data were analysed as detailed above (see ‘Behavioural methods and testing’).

SB-277011A decreased nicotine cue-induced conditioned LMA (Figure 3). An ANOVA with main factors of environment, conditioning drug, and treatment revealed significant main effects of conditioning drug ($F_{1,54}$ = 16.22, p<0.001) and treatment ($F_{1,54}$ = 21.19, p<0.001), but no significant effect of environment: ($F_{1,54}$ = 1.85, p=n.s.). Although the ANOVA showed no significant environment × conditioning drug interaction ($F_{1,54}$ = 2.38, p = n.s.), a planned comparison showed that, in vehicle-treated animals, there was a statistically significant difference between the nicotine-paired and the nicotine-unpaired groups (p<0.05) as well as between the nicotine-paired and the saline-paired groups (p<0.001). There was no significant difference between the nicotine-unpaired and the saline-unpaired groups (p = 0.08) or between the saline-paired and saline-unpaired groups (p = 0.37). The ANOVA also showed no significant environment × conditioning drug × treatment interaction (F_{154} = 0.45, p=n.s.), but again, a planned comparison showed that pretreatment with SB-277011A (10 mg/kg) in the nicotine-paired group significantly reduced locomotor activity vs. the nicotine-paired group pretreated with vehicle (p<0.0001). Thus, treatment with SB-277011A abolished the difference between the nicotine-paired and the saline-paired groups, reducing an impressive degree of statistically significant difference

(*p*< 0.001) to statistical insignificance (*p* = 0.26). Treatment with SB-277011A also produced a slight but significant effect in the unpaired groups (nicotine-unpaired vehicle-pretreated vs. nicotine-unpaired SB-277011A–pretreated, *p* = 0.04).

When lower doses of SB-277011A (1, 3 mg/kg i.p.) were tested for their effects on nicotine cue-conditioned LMA in a separate experiment, SB-277011A had no effect on cue-conditioned LMA (data not shown). Statistically, this was confirmed by an ANOVA of the AUC of horizontal activity counts ($F_{2,33}$=1.2, *p*=n.s.).

CPP (nicotine-induced conditioned place preference) experiments

In the animals given vehicle/vehicle exposure (i.e. vehicle in both CPP compartments) on the training days, testing for CPP expression after exposure to vehicle on the test day produced no place preference (7.4 ± 0.3 min vs. 7.6 ± 0.3 min spent in the two CPP compartments respectively). This confirms that the CPP procedure used here was *non*-biased (i.e. no a-priori preferences among the animals for either chamber of the CPP apparatus *prior* to CPP training, and no a posteriori preferences for either chamber *after* vehicle exposure in *both* CPP compartments on the CPP training days). The unbiased CPP procedure is generally considered to be the preferred methodology, as the biased CPP procedure can yield false-positive results due to ceiling effects (Cunningham et al., 2003) or unconditioned motivational responses to the initially non-preferred environment (e.g. anxiety) (Tzschentke, 1998). As shown in Figure 4a, nicotine (0.6 mg/kg s.c.) produced a significant CPP (9.7 ± 0.4 min in the nicotine-paired cue-distinctive compartment) compared to vehicle (7.4 ± 0.3 min in the vehicle-paired cue-distinctive compartment), measured as CPP expression on the test day. This was confirmed by Student's *t* test for the difference between the means (of time spent in the nicotine-paired CPP chamber on the test day) of the vehicle/ vehicle-paired group given vehicle on the test day vs. the vehicle/nicotine-paired group given vehicle on the test day (*t*=4.44, d.f.=18, *p*<0.001) (Figure 4a). Thus, the dose of nicotine used in the present experiments produced a statistically significant CPP.

In addition, and referring directly to the effect of D_3 receptor antagonism on nicotine-induced CPP, SB-277011A dose-dependently attenuated this statistically significant expression of nicotine-induced CPP (Figure 4b). Statistically this effect of SB-277011A was confirmed by one-way ANOVA over the four groups of animals shown in Figure 4b, which revealed a statistically significant main effect over all four groups ($F_{3,36}$=18.3, *p*<0.0001). Follow-up individual pairwise group comparisons using the Newman– Keuls test showed that the vehicle/nicotine paired groups given SB-277011A (1, 3, or 10 mg/kg i.p.) on the test day *each differed significantly* from the vehicle/ nicotine paired group given vehicle (i.e. 0 mg/kg SB-277011A) on the test day (*p*<0.01 for all three pairwise comparisons). Newman–Keuls tests also revealed that the vehicle/nicotine paired group given SB-277011A at 1 mg/kg i.p. on the test day *differed significantly* from the vehicle/nicotine paired group given SB-277011A at 10 mg/kg i.p. on the test day (*p*<0.05) (Figure 4b). Thus, SB-277011A at all doses tested produced a statistically significant inhibition of nicotine-induced CPP, but SB-277011A at 10 mg/kg produced a *significantly greater* inhibition of nicotine-induced CPP than did SB-277011A at 1 mg/kg, indicating that SB-277011A's attenuation of nicotine-induced CPP showed a significant dose-dependency.

Finally, SB-277011A by itself appeared not to produce aversion, 'negative contrast', or indeed any negative behavioural effects at all. In the first additional CPP experiment carried out to test this possibility (vehicle or SB-277011A given during *CPP conditioning*), the effects of vehicle or 1, 3, or 10 mg/kg SB-277011A on CPP acquisition in animals given vehicle on the test day were assessed. CPP expression on test day in these animals is shown in Table 1. Animals given SB-277011A in a cue-distinct environment developed neither a preference nor an aversion to that environment. Statistical analysis by ANOVA of the data

shown in Table 1 indicate that repeated pairing of SB-277011A with a given cue-distinctive chamber of the CPP apparatus did not significantly alter the amount of time spent, on test day, in the SB-277011A–paired chambers compared to the vehicle-paired chamber ($F_{3,34}$=1.88, p=0.15). In the second additional CPP experiment carried out to test this possibility (vehicle or SB-277011A given during *CPP expression*), the effects of vehicle or 1, 3, or 10 mg/kg SB-277011A on CPP expression in animals given vehicle on conditioning days were assessed. CPP expression on test day in these animals is shown in Table 2. Animals given SB-277011A 30 min prior to CPP expression-testing on the test day displayed no alterations in their behaviour. Statistical analysis by ANOVA of the data shown in Table 2 indicate that SB-277011A produced no statistically significant alterations in behaviour on the test day ($F_{3,36}$=0.34, p=0.8). This was further confirmed by the results of the one-sample t tests (Rosner, 2006) carried out on times spent in the nicotine-paired compartment by SB-277011A–treated rats on the test day as compared to the 7.5 min theoretical CPP 'indifference level' for each of the four tested groups of rats (vehicle, 1 mg/kg SB-277011A, 3 mg/kg SB-277011A, 10 mg/kg SB-277011A). From those one-sample t tests, it was clear that SB-277011A did *not* produce any negative behavioural effect on the test day (Vehicle group: t=0.26, d.f.=9, p=n.s.; 1 mg/kg SB-277011A group: t=1.19, d.f.=9, p=n.s.; 3 mg/ kg SB-277011A group: t=0.85, d.f.=9, p=n.s.; 10 mg/ kg SB-277011A group: t=0.17, d.f.=9, p=n.s.).

Discussion

The present experiments demonstrate that pre-treatment with the potent and selective D_3 receptor antagonist SB-277011A significantly reduces nicotine's effects on BSR and significantly attenuates nicotine-associated environmental-cue effects on LMA and CPP. The observed effects of SB-277011A support the hypothesis that D_3 receptors play an important role in mediating nicotine-induced enhancement of brain reward and nicotine-associated cue-induced incentive motivational effects.

The present finding that nicotine significantly enhances the rewarding efficacy of BSR is consistent with previous reports (e.g. Bauco and Wise, 1994; Bozarth et al., 1998; Huston-Lyons and Kornetsky, 1992; Ivanová and Greenshaw, 1997; Panagis et al., 2000; Wise et al., 1992, 1998). Some of those previous reports have gone further than merely demonstrating that nicotine enhances BSR, and have implicated DA mechanisms in such enhancement. Thus, nicotine's enhancement of BSR has been shown to be attenuated by DA receptor antagonists (Huston-Lyons et al., 1993; Ivanová and Greenshaw, 1997) and nicotinic receptors expressed on DA neurons have been shown to be essential to nicotine's augmentation of BSR (Wise et al., 1998). However, no previous work has implicated D_3 receptor mechanisms in nicotine's effects on brain-reward substrates. Thus, the present findings with SB-277011A constitute the first demonstration that DA D_3 receptor mechanisms are involved in nicotine's effects on brain reward and that selective D_3 receptor antagonism attenuates nicotine-enhanced BSR.

The present finding that nicotine-associated environmental cues come to elicit LMA is consistent with previous findings (Bevins et al., 2001; Bevins and Palmatier, 2003; Le Foll et al., 2003b; Palmatier and Bevins, 2002; Palmatier et al., 2003; Walter and Kuschinsky, 1989). In some of those previous studies, it was reported that the expression of nicotine-paired cue-induced LMA was blocked by DA receptor antagonists (Bevins et al., 2001), by the D_3 receptor partial agonist BP-897 (Le Foll et al., 2003b), and by SB-277011A (Le Foll et al., 2003b). However, in those previous studies, the effects of the various DA-active compounds were not assessed in appropriate *unpaired* control groups (i.e. animals that received vehicle or nicotine in their home cages rather than in the cue-specific locomotor activity chambers). Thus, it is not established that the pharmacological effects reported in

those studies were due to specific action on nicotine-*paired* cue-induced LMA, since pharmacological effects on nicotine-*unpaired* cue-induced LMA were not tested. The present data show that SB-277011A–induced D_3 receptor antagonism appears to robustly attenuate nicotine-paired cue-induced LMA ($p<0.0001$), although SB-277011A treatment also slightly but significantly affected locomotor activity in the unpaired group ($p=0.04$).

The present finding that nicotine produces a robust CPP in male rats is consistent with previous findings from both our laboratory (Ashby et al., 2002; Dewey et al., 1999; Horan et al., 1997, 2001) and other groups (for review, see Le Foll and Goldberg, 2005). Interestingly, most previous reports of nicotine-induced CPP have used the biased CPP methodology, rather than the unbiased procedure used in the present experiments (for review, see Le Foll and Goldberg, 2005). The present findings suggest that nicotine-induced CPP is not dependent upon the biased methodology. The present findings also show that SB-277011A dose-dependently attenuated the expression of nicotine-induced CPP. In previous studies, it has been reported that the D_3 receptor partial agonist BP-897 and the putative D_3 receptor antagonist ST-198 significantly attenuated nicotine-induced CPP (Le Foll et al., 2005b). However, it should be noted that BP-897 alone has been reported to produce conditioned place *aversion* (Duarte et al., 2003; Gyertyán and Ga´l, 2003) and, congruently, to *inhibit* electrical BSR, an aversive-like effect (Campos et al., 2004). Aversive-like effects produced by BP-897 could easily confound the interpretation of experiments (e.g. Le Foll et al., 2005b) in which BP-897 was used as a putative D_3 receptor antagonist. In contrast, SB-277011A in the present experiments did not by itself produce a significant place preference or aversion, and SB-277011A administered on the test day following repeated vehicle/vehicle pairings on acquisition days did not produce any significant alteration in the rats' behaviour. Also, BP-897 has been shown to have affinity for neurotransmitter receptors other than D_3 (e.g. D_2, a_1 adrenergic, a_2 adrenergic, 5-HT_{1A}, 5-HT_{2A}) (Cussac et al., 2000; Heidbreder et al., 2005; Pilla et al., 1999; Xi et al., 2005), and data obtained using cloned human receptors show that BP-897 has potent partial agonist effects at 5-HT_{1A} receptors and potent antagonist effects at a_1 and a_2 receptors (Cussac et al., 2000). Thus, action at one or more of those sites may be responsible for BP-897's effects in the CPP paradigm. ST-198 may also possess D_2 antagonist action, as (like BP-897) it significantly attenuates L-dopa-induced dyskinesias in monkeys (Bézard et al., 2003). Therefore, the present findings with SB-277011A appear to constitute the first clear demonstration that selective DA D_3 receptor antagonism attenuates nicotine-induced CPP.

Non-selective D_1- or D_2-like receptor antagonists inhibit addictive-drug-induced enhancement of brain reward (e.g. Nakajima, 1989; Ranaldi and Wise, 2001; Wise, 1994; Wise and Bozarth, 1985; Wise and Rompré, 1989) as well as addictive-drug-associated cue-induced behavioural effects (e.g. Cervo et al., 2003; Ciccocioppo et al., 2001; Weiss et al., 2000). Such findings raise the issue of whether the presently observed inhibitory effects of SB-277011A on nicotine-enhanced BSR and nicotine-associated cue-induced LMA and CPP might be attributable to D_1 or D_2 receptor-selective antagonism rather than D_3 receptor-selective antagonism. However, accumulating evidence does not support this notion: (1) SB-277011A is a highly potent and highly selective D_3 receptor antagonist with 80- to 100-fold selectivity for D_3 over other DA receptors; high affinity for the human ($pK_i=7.95$) and rat ($pK_i=7.97$) cloned DA D_3 receptor; and 100-fold selectivity over 66 other receptors, enzymes, ion channels, and transporters in the central nervous system (Reavill et al., 2000; Stemp et al., 2000); (2) the effects of SB-277011A in various animal models relating to addiction are significantly different from those produced by D_1- or D_2-preferring antagonists (Heidbreder et al., 2005); for example, D_1 and D_2 antagonists significantly elevate electrical BSR thresholds (Baldo et al., 1999; Harrison et al., 2002; Hunt et al., 1994; Nakajima and O'Regan, 1991; Panagis and Spyraki, 1996; Stein, 1962; Stein and Ray, 1960), while SB-277011A does not alter BSR thresholds (Vorel et al., 2002); similarly, D_1- and D_2-

preferring antagonists produce *aversion* in the CPP paradigm (e.g. Shippenberg and Herz, 1988; for review, see Tzschentke, 1998), while SB-277011A produces neither reward nor aversion in the CPP paradigm (Gyertyán and Gál, 2003; Vorel et al., 2002; Xi et al., 2005); (3) SB-277011A does not significantly alter locomotor activity (Reavill et al., 2000; Xi et al., 2005), whereas locomotor inhibition is a classic property of D_2 antagonists; (4) at doses of many-fold those used in the present experiments, SB-277011A does not block quinelorane-induced decreases in DA in the dorsal striatum where a high density of D_2 receptors are located, and (5) SB-277011A does not significantly alter prolactin levels (Reavill et al., 2000), whereas alteration of prolactin levels is a classic property of D_2 antagonists. SB-277011A, but not D_2 antagonists, significantly increases acetylcholine levels in the rat frontal cortex (Lacroix et al., 2003).

If the presently observed inhibitory effects of SB-277011A on nicotine-enhanced BSR and on nicotine-associated cue-induced LMA and CPP cannot be attributed to D_1 or D_2 antagonism, might they be attributable to *non-specific* behavioural inhibition? Again, the evidence seems against such a possibility. SB-277011A, within the dose range used in the present experiments, has been demonstrated to have no effect on locomotor activity or on food- or sucrose-taking behaviour (Heidbreder et al., 2005; Reavill et al., 2000; Vorel et al., 2002) and to *not* induce sedation or catalepsy (Vorel et al., 2002; Xi et al., 2005). Since expression of nicotine-associated cue-induced LMA or CPP may be presumed to involve encoding and storage of cue-induced associations, plus memory retrieval of such cue-induced associations, the possibility arises that SB-277011A's inhibition of cue-induced LMA or CPP might be mediated by interference with general aspects of memory storage and retrieval. However, this again seems unlikely, as SB-277011A reverses scopolamine-induced memory deficits as assessed by a three-choice-point water labyrinth test (Laszy et al., 2005) and significantly increases extracellular levels of acetylcholine in the anterior cingulate cortex (Lacroix et al., 2003). Both of these effects would be expected to improve rather than to interfere with memory. Indeed, a comprehensive review of the scientific literature shows that SB-277011A appears to be singularly devoid of nonspecific behavioural effects (Heidbreder et al., 2005), and it is, therefore, unlikely that SB-277011A's effects in the present study were due to non-specific effects.

The present findings with nicotine should be understood within the context of accumulating evidence that D_3 receptor antagonism may be a useful addition to the pharmacotherapeutic armamentarium against addiction. We and others have shown that SB-277011A attenuates: (1) cocaine-enhanced electrical BSR (Vorel et al., 2002); (2) acquisition and expression of cocaine-seeking under second-order reinforcement (Di Ciano et al., 2003); (3) cocaine- or heroin-induced CPP (Ashby et al., 2003; Vorel et al., 2002); (4) cocaine self-administration under progressive ratio and variable-cost/variable-payoff fixed-ratio reinforcement (Gilbert et al., 2003; Xi et al., 2005); (5) cocaine- or nicotine-triggered relapse to drug-seeking behaviour as assessed by the reinstatement model (Vorel et al., 2002; Andreoli et al., 2003b); (6) stress-triggered relapse to cocaine-seeking behaviour as assessed by the reinstatement model (Xi et al., 2004); (7) drug-associated environmental cue-triggered relapse to cocaine-seeking behaviour as assessed by the reinstatement model (Gilbert et al., 2005); (8) oral ethanol intake (Andreoli et al., 2003a; Thanos et al., 2005); and (9) relapse to ethanol-seeking behaviour in animals extinguished from ethanol-taking behaviour (Marcon et al., 2003). Consistent with such findings, the present study shows that SB-277011A, within the same dose range as in those prior experiments, inhibits nicotine-enhanced BSR and two forms of nicotine-induced Pavlovian cue-association learning (LMA, CPP) that may underlie nicotine-associated cue-induced drug-seeking behaviour. It is of note that SB-277011A *by itself*, within the same dose range, has no effect on BSR (Vorel et al., 2002), does not produce preference or aversion in the CPP paradigm (present data), and does

not sustain intravenous self-administration behaviour in rats (Xi et al., 2005). Thus, it may be inferred to lack addictive potential.

In conclusion, the present study demonstrates that selective blockade of DA D_3 receptors by SB-277011A inhibits nicotine-enhanced BSR and nicotine-paired cue-induced LMA and CPP. Overall, the present findings suggest that DA D_3 receptors play an important role in the neural and behavioural substrates of nicotine addiction, and that selective DA D_3 receptor antagonists merit further investigation as anti-nicotine pharmacotherapies, especially as current therapeutic interventions for smoking cessation are inadequate (Vaszar et al., 2002).

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Statement of Interest

Dr Heidbreder and Dr Pilla are employees of GlaxoSmithKline Pharmaceuticals, the developer of SB-277011A. Dr Ashby and Dr Gardner have been recipients of research contract and research grant support from GlaxoSmithKline Pharmaceuticals.

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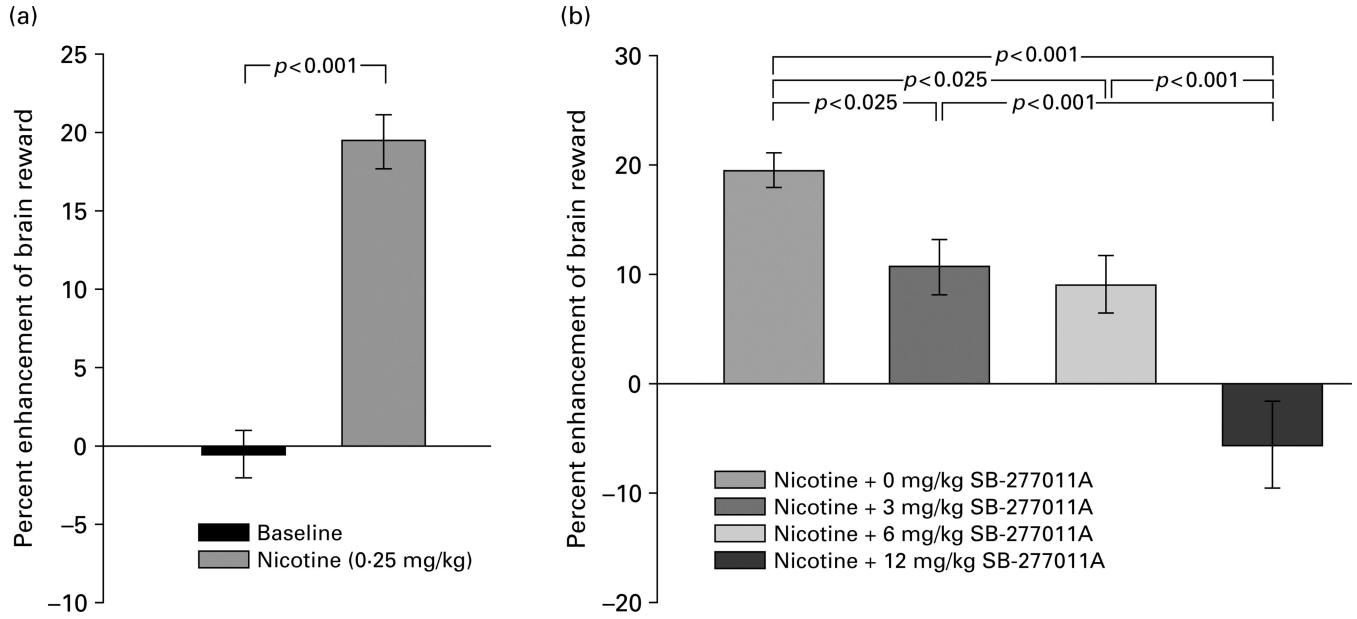

Figure 1.

Effect of nicotine (0.25 mg/kg s.c.) and various doses of the DA D_3 receptor antagonist SB-277011A (0, 3, 6, 12 mg/ kg i.p.) on electrical brain reward thresholds (θ_0) in laboratory rats. Changes in brain reward threshold are plotted as percent enhancement or inhibition of brain reward ± SEM. Statistical significance levels for specific inter-treatment comparisons, wherein nicotine is compared to baseline by the paired Student's *t* test (a) and other inter-treatment comparisons are made by post-ANOVA Newman–Keuls tests (b), are as shown directly on the figure.

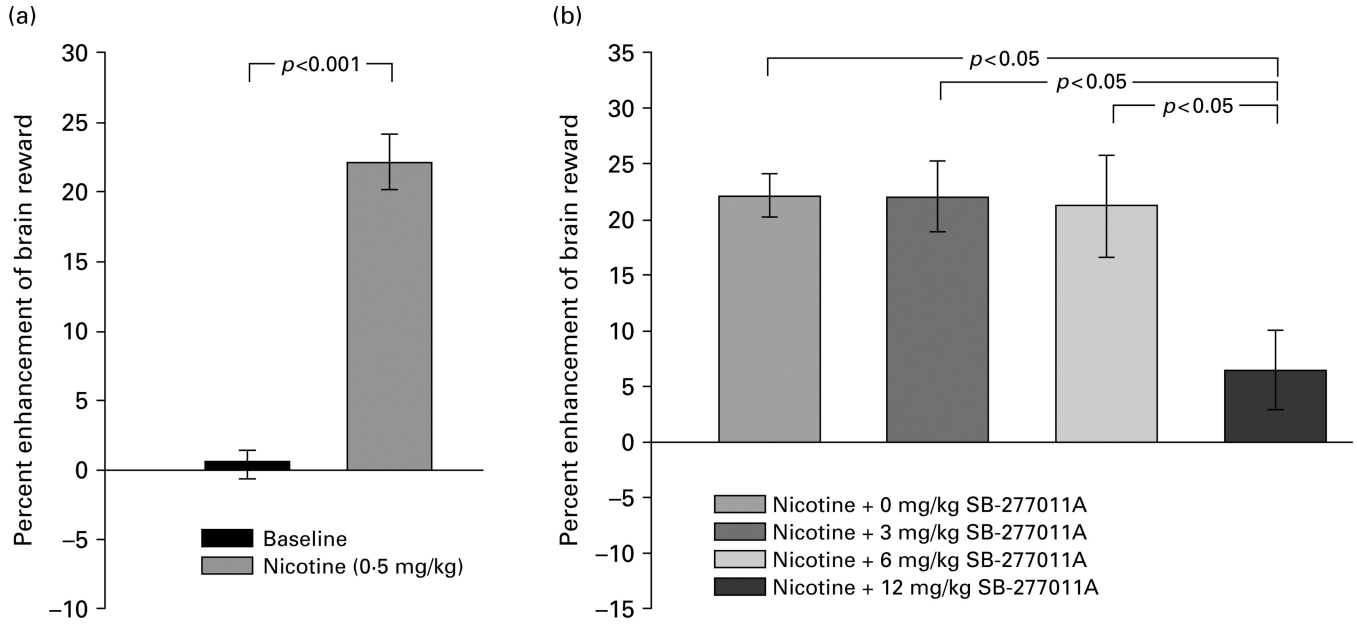

Figure 2.

Effect of nicotine (0.5 mg/kg s.c.) and various doses of the DA D_3 receptor antagonist SB-277011A (0, 3, 6, 12 mg/kg i.p.) on electrical brain reward thresholds (θ_0) in laboratory rats. Changes in brain reward threshold are plotted as percent enhancement or inhibition brain reward ± SEM. Statistical significance levels for specific inter-treatment comparisons, wherein nicotine is compared to baseline by the paired Student's *t* test (a) and other inter-treatment comparisons are made by post-ANOVA Newman–Keuls tests (b), are as shown directly on the figure.

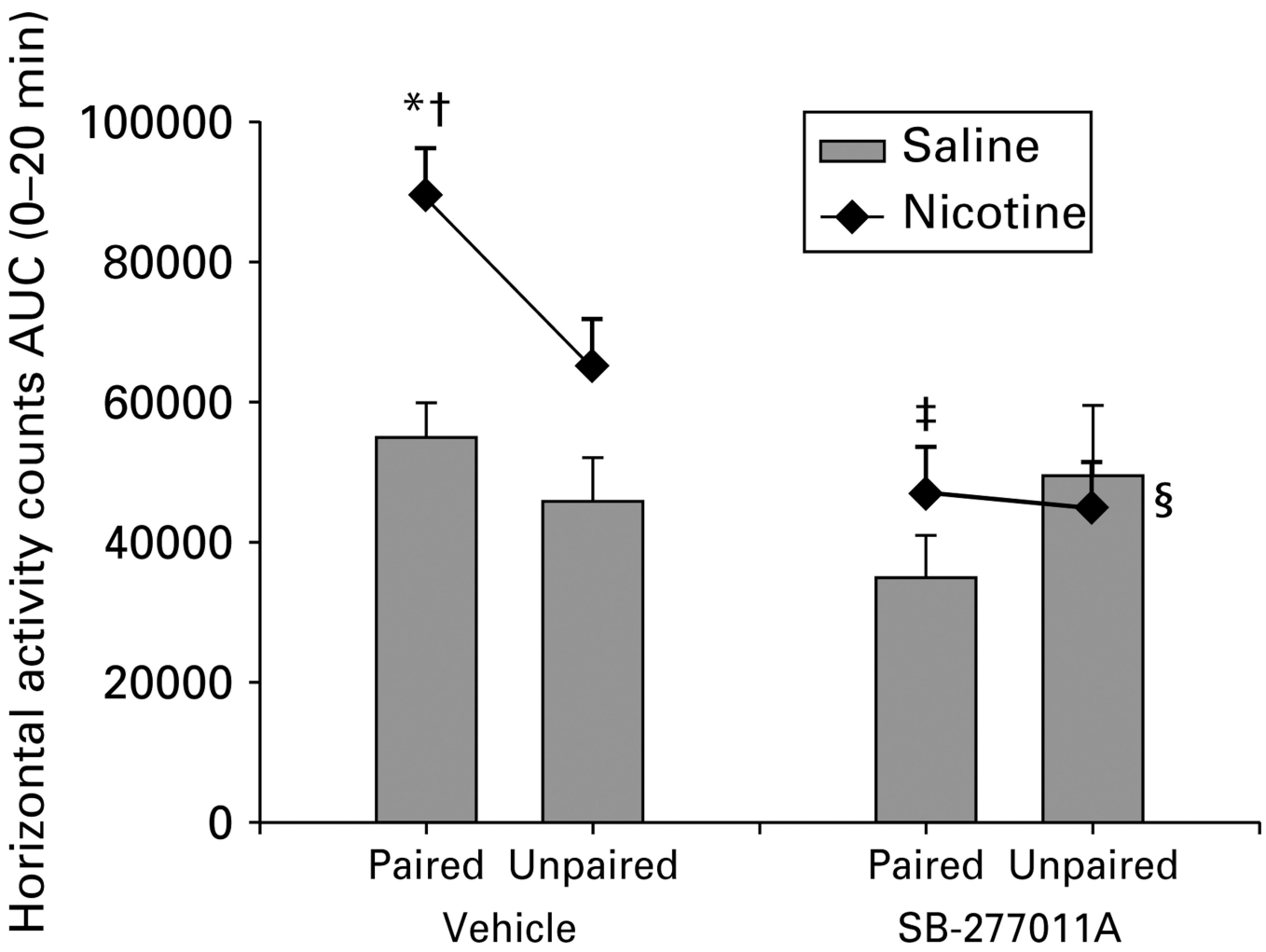

Figure 3.

Nicotine (0.5 mg/kg s.c.) cue-conditioned locomotion in laboratory rats, shown as the area under the curve (AUC - see text) of horizontal activity counts for the first 20 min of locomotion in the four groups of animals treated with vehicle or with SB-277011A (10 mg/kg i.p.). Rats that received nicotine in the cue-specific test environment for 4 consecutive days (nicotine-paired) showed enhanced locomotor response compared to rats that received saline in the test environment (saline-paired), while there was no difference between the nicotine and the saline unpaired groups. SB-277011A produced an attenuation of nicotine-associated cue-induced locomotor activity and slightly reduced the nicotine unpaired response. * $p < 0.05$ vs. nicotine unpaired; † $p < 0.001$ vs. vehicle saline paired; ‡ $p < 0.0001$ vs. vehicle nicotine paired; § $p = 0.04$ vs. vehicle nicotine unpaired. Data are expressed as means $\pm_{S.E.M.}$.

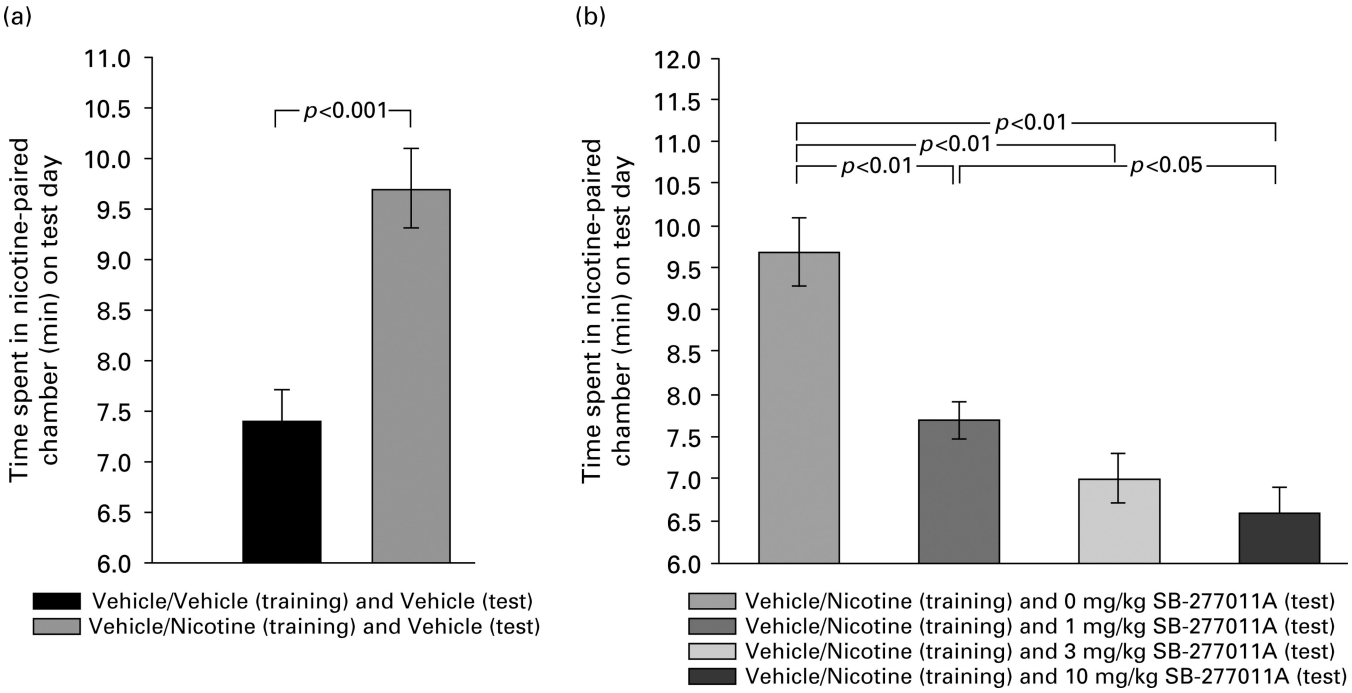

Figure 4.

Effect of nicotine (0.6 mg/kg s.c.) and various doses of the DA D_3 receptor antagonist SB-277011A (0, 1, 3, 10 mg/kg i.p.) on the expression of conditioned place preference in laboratory rats. Times spent in the nicotine-paired chamber on test day are shown as means ± SEM. Statistical significance levels for specific inter-group comparisons, as determined by Student's *t* test for independent samples (a) and by post-ANOVA Newman–Keuls tests (b), are as shown directly on the figure (also see text).

Table 1

Place conditioning response to SB-277011A (SB) in male Sprague–Dawley rats

Treatment pairings	Treatment given on the test day	Time spent in the chambers (min)		No. of crossings
		Paired	Unpaired	
Vehicle/SB, 1 mg/kg	Vehicle[a]	8.3±1.4[b]	6.7±1.4	28.0±3.2
Vehicle/SB, 3 mg/kg	Vehicle	9.4±1.0	5.6±1.0	29.9±1.8
Vehicle/SB, 10 mg/kg	Vehicle	9.8±0.9	5.2±0.9	32.8±3.5
Vehicle/vehicle	Vehicle	7.5±0.4	7.5±0.4	27.0±2.6

[a]Vehicle was 1 ml/kg of a 2% methylcellulose solution (w/v).

[b]Each value represents the mean number of minutes spent in each chamber ± S.E.M. A total of 9–10 rats were examined for each treatment pairing. All animals received four pairings with the appropriate dose of SB/vehicle with each pairing being separated by a 24-h period. On the test day, animals received vehicle 30 min before being placed into the CPP apparatus.

Table 2

Effect of vehicle or SB-277011A (SB) on expression of the conditioned place preference response to vehicle in male Sprague–Dawley rats

		Time spent in chambers (min)	
Treatment pairings	**Treatment given on the test day**	**Vehicle chamber 1**	**Vehicle chamber 2**
Vehicle/vehicle	Vehicle[a]	7.6 ± 0.3[b]	7.4 ± 0.3
Vehicle/vehicle	SB, 1 mg/kg	7.8 ± 0.3	7.2 ± 0.3
Vehicle/vehicle	SB, 3 mg/kg	7.8 ± 0.3	7.2 ± 0.3
Vehicle/vehicle	SB, 10 mg/kg	7.5 ± 0.3	7.5 ± 0.3

[a]The vehicle used during treatment pairings was 1 ml/kg s.c. of distilled water. The vehicle used on the test day was 1 ml/kg i.p. of 2% methylcellulose.

[b]Each value represents the mean number of minutes spent in each chamber ± S.E.M. A total of 10 rats were examined for each treatment pairing. On the test day, animals received either 1 ml/kg i.p. vehicle (2 % methylcellulose) or SB-277011A (1, 3 or 10 mg/kg i.p.) 30 min before being placed into the CPP apparatus. The ‘Time spent in chambers’ represents the times spent, on the test day, in the two different chambers that were each paired with vehicle on the CPP acquisition days (‘Treatment pairings’).