

# Selective Antagonism at Dopamine D<sub>3</sub> Receptors Prevents Nicotine-Triggered Relapse to Nicotine-Seeking Behavior

Michela Andreoli<sup>1</sup>, Michela Tessari<sup>1</sup>, Maria Pilla<sup>1</sup>, Enzo Valerio<sup>1</sup>, Jim J Hagan<sup>1</sup>, and Christian A Heidbreder<sup>\*,1</sup>

<sup>1</sup>Center of Excellence for Drug Discovery in Psychiatry, GlaxoSmithKline Pharmaceuticals, Via A. Fleming 4, 37135 Verona, Italy

Drugs of abuse, including, nicotine have been shown to enhance brain reward functions in the mesocortico-limbic dopamine (DA) system in general, and the nucleus accumbens in particular. The latter occupies a prominent position in the ventral striatum and expresses a high density of DA D<sub>3</sub> receptors. As such, the present study aimed at investigating the effect of the selective D<sub>3</sub> receptor antagonist SB-277011-A on both the stable maintenance of intravenous nicotine self-administration and nicotine-triggered relapse to nicotine-seeking behavior in the rat. SB-277011-A (3–10 mg/kg i.p.) significantly reduced reinstatement of nicotine-seeking behavior without affecting nicotine self-administration *per se*. These results suggest that DA D<sub>3</sub> receptors are involved in the reinstatement of nicotine-seeking behavior independently of any interaction with the primary reinforcing effects of nicotine itself. These findings point toward the potential use of selective DA D<sub>3</sub> receptor antagonists for the pharmacotherapeutic management of relapse to drug-seeking behaviors.

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## INTRODUCTION

Nicotine and the vast majority of abused drugs, including cocaine, amphetamine, morphine, ethanol, phencyclidine, and  $\Delta^9$ -tetrahydrocannabinol have the essential commonality of enhancing basal neuronal firing and/or basal neurotransmitter release in the mesolimbic dopamine (DA) system (Di Chiara and Imperato, 1988; Koob and Le Moal, 1998). Specifically, the neurobiological mechanisms underlying dependence on drugs of abuse appear to be associated with a sustained increase in the release of DA into the extracellular space of the nucleus accumbens (NAc) (Berridge and Robinson, 1998; Di Chiara, 1995). Thus, activation of DA neurotransmission in the NAc seems to be a key component underlying the reward and/or incentive motivation produced by drugs of abuse (Berridge and Robinson, 1998; Wise and Rompre, 1989).

Although the role of DA D<sub>1</sub> and D<sub>2</sub> receptors in mediating the addictive liability of drugs has been fairly well characterized (see for example Caine *et al*, 2002; Haile and Kosten, 2001; Hummel and Unterwald, 2002), a growing body of evidence has recently strengthened the likelihood

that the DA D<sub>3</sub> receptor is significantly involved in mechanisms of drug dependence and abuse. For example, the density of DA D<sub>3</sub> receptors is elevated one- to three-fold in the NAc and ventromedial subregions of the caudate-putamen in the brains of cocaine-overdose fatalities compared with both age-matched, drug-free control subjects and cocaine-overdose victims presenting preterminal excited delirium (Mash and Staley, 1999; Staley and Mash, 1996). Furthermore, the expression of DA D<sub>3</sub> receptor mRNA in the human NAc is increased six-fold in cocaine-overdose victims (Segal *et al*, 1997). Recent studies also showed a selective increase in the DA D<sub>3</sub> receptor binding and D<sub>3</sub> receptor mRNA in the shell of the NAc of nicotine-sensitized rats without any significant changes in the expression of DA D<sub>1</sub> and D<sub>2</sub> receptors (Le Foll *et al*, 2003). These findings are in line with the distribution of DA receptors, which does not follow a homogeneous pattern throughout the NAc. Contrary to DA D<sub>1</sub> and D<sub>2</sub> receptors, DA D<sub>3</sub> receptors are expressed preferentially in medium-sized spiny neurons of the rostral and ventromedial shell of the NAc and in granule cells of the island of Calleja major (ICjM), regions in which the D<sub>2</sub> receptors are scarcely expressed (Bouthenet *et al*, 1991; Diaz *et al*, 1995; Sokoloff *et al*, 1990). The distribution of the D<sub>3</sub> receptor in the human brain also appears to follow a rather similar pattern to that observed in the rat brain (Gurevich and Joyce, 1999; Hall *et al*, 1996; Landwehrmeyer *et al*, 1993; Murray *et al*, 1994; Shafer and Levant, 1998; Suzuki *et al*, 1998).

The precise role of the D<sub>3</sub> receptor in drug dependence processes has, however, been significantly hampered by the lack of pharmacological tools showing significant selectivity

\*Correspondence: Dr CA Heidbreder, Department of Drug Dependence & Behavioral Neurochemistry, Center of Excellence for Drug Discovery in Psychiatry, GlaxoSmithKline Pharmaceuticals, Via A. Fleming 4, 37135 Verona, Italy, Tel: +39 045 921 9769, Fax: +39 045 921 8047, E-mail: Christian\_A\_Heidbreder@gsk.com  
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for DA D<sub>3</sub> over D<sub>2</sub> receptors. For example, most compounds developed so far have a 10- to 30-fold selectivity for DA D<sub>3</sub> over D<sub>2</sub> receptors *in vivo* (Audinot *et al*, 1998; Sautel *et al*, 1995; Waters *et al*, 1994). In addition, the partial agonist BP 897 is also a potent and selective antagonist at both DA D<sub>3</sub> and D<sub>2</sub> receptors (Wood *et al*, 2000). Altogether these findings emphasize the need for selective tools to investigate the role of the DA D<sub>3</sub> receptor in drug dependence. In contrast with most DA D<sub>3</sub> antagonists developed so far, the recent DA D<sub>3</sub> receptor antagonist SB-277011-A (*trans*-N-[4-[2-(6-cyano-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl]cyclohexyl]-4-quinolininecarboxamide) shows high affinity and 100-fold selectivity for D<sub>3</sub> over D<sub>2</sub> receptors and 66 other receptors, enzymes, and ion channels (Reavill *et al*, 2000; Stemp *et al*, 2000). The selective DA D<sub>3</sub> antagonist SB-277011-A has been shown to be efficacious against cocaine-seeking behavior and cocaine-induced conditioned hyperactivity, but does not seem to play a role in responding directly reinforced by cocaine (Di Ciano *et al*, 2003; Le Foll *et al*, 2002). Furthermore, SB-277011-A blocks cocaine-induced enhancement of electrical brain stimulation reward, attenuates both the development and expression of cocaine-induced place preference, and decreases cocaine-triggered relapse to cocaine-seeking behavior in a dose-dependent manner (Vorel *et al*, 2002). Accordingly, the present experiments were undertaken to assess the effect of acute systemic antagonism at DA D<sub>3</sub> receptors by using SB-277011-A on both the stable maintenance of intravenous nicotine self-administration and nicotine-triggered relapse to nicotine-seeking behavior.

## MATERIALS AND METHODS

### Animals

Male Wistar rats (Charles River, Germany) were individually housed in a temperature-controlled environment with lights on from 06.00 to 18.00 h. During the experiments, water was continuously available and animals were maintained at a constant body weight of 240–260 g (85% of their *ad libitum* body weight). All experiments were pre-reviewed and consented by a local animal care committee in complete accordance with the guidelines of the 'Principles of laboratory animal care' (NIH publication No. 86–23, revised 1985) as well as with the regulations of the Italian laws.

### Apparatus

Behavioral testing (self-administration sessions and reinstatement phase) was conducted in 16 operant chambers (Coulbourn Instruments, Leigh Valley, USA) encased in sound-insulated cubicles, equipped with a ventilation fan (Ugo Basile, Comerio, Italy) that also screened external noise. Each chamber was equipped with two levers on the front panel, located 12.5 cm apart and 2 cm above the grid floor. A food magazine was situated in an opening in the panel between the two levers, 1 cm above the floor. A 2-W white light was located 26 cm above the food magazine and was activated throughout the entire session. Presses on the right lever (RLP or 'active lever pressing'), corresponding to the fixed-ratio values required by the schedules of reinforcement, produced the delivery of 45 mg food pellets

(Bioserv, Frenchtown, NJ, USA) or the activation of the infusion pump, except during extinction and reinstatement conditions. Delivery of reinforcement was signaled by the 1-s illumination of a 4-W white stimulus light located in the same hole of the food magazine and by 1-s sounding of a Sonalert device (2.9 kHz, 80 dB). Nicotine solution was administered via an infusion pump (Model A-99Z, Razel Scientific Instruments Inc., CT, USA) at a volume of 0.022 ml over a 1-s period. Presses on the left lever (LLP) did not have any consequence. Both lever presses and reinforcement deliveries were recorded. Data acquisition and schedule parameters were controlled by Med-PC software (Med Associates Inc., Georgia, GA) running on two Compaq microcomputers interfaced with the chambers via interface modules (Med Associates Inc.).

### Experimental Procedures

**Training to lever-press for food reinforcement.** Following a 24-h deprivation period, rats were trained to press the right lever for food as a reinforcer. The procedure was initiated under a fixed-ratio (FR-1) schedule of reinforcement with a time-out period of 60-s (TO 60s). Each FR-1 session lasted until rats had received 100 pellets or was terminated following a maximal 2-h period. Rats were then gradually trained up to a final FR-2 schedule with a TO 60s over a 2-h period. Each FR-2 session was terminated after 2 h. Rats underwent surgery once they reached the criterion by which the ratio between right lever presses and number of reinforcement was less than or equal to 4. This number represents a measure of the selectivity of rats' responding, so that the smaller the ratio, the higher the level of responding specifically directed toward obtaining food reinforcement.

**Surgery.** Rats were anesthetized with pentobarbital sodium (50 mg/kg; Siegfried Zofingen Switzerland) 1 ml/kg i.p. (5-min pretreatment with atropine sulfate 0.5 mg/kg I.P.; Sigma, St Louis, MO, USA). They were then implanted with a silastic catheter in the right jugular vein. The free end of the catheter was connected to a modified C313G cannula assembly (Plastic One, VA). The resulting unit was mounted onto the skull with dental acrylic cement and fixed via tissue adhesive (3M Animal Care Products, St Paul, MN, USA).

Animals received one bolus injection of 0.250 ml of an antibiotic suspension of terramycin (Terramicina long-acting, Pfizer Inc., NY, USA) for a 72-h protection. Animals were then injected i.v. with 0.1 ml of a solution containing 4 UI/ml heparin (Liquemin, Roche S.p.A., Milano, Italy). This treatment was repeated twice a day for 5 days after surgery (recovery period).

**Nicotine self-administration training.** After the recovery period, rats were trained to nicotine self-administration on an FR-1 schedule of reinforcement. During this schedule each active lever press produced an infusion of nicotine (0.03 mg/kg/infusion) together with the activation of two conditioned stimuli, a light in the food magazine and a tone. Each infusion was followed by a 60s TO period during which responses were recorded, but had no scheduled consequences. Each session lasted until rats received 25

infusions of nicotine, or was terminated after a maximum of 3 h. If the animal met the criterion of 25 infusions within the end of the daily session, the FR value was increased to FR-2 the following day. For the FR-2 schedule of reinforcement, each session was terminated after a maximum of 2 h. This procedure lasted until rats reached at least 12 infusions of nicotine over the 2-h session for at least 3 consecutive days. The animals were then shifted to an FR-2 schedule over 1-h sessions. Responding was considered stable when the average inter-reinforcement time (IRT) did not vary more than 20% between three consecutive sessions. Stability criteria for responding to drugs of abuse such as cocaine are typically referred to as variability within parameters (eg IRT, number of active lever presses) comprised between 10 and 20% (Deroche-Gamonet *et al*, 2002; Goeders and Clampitt, 2002; Highfield *et al*, 2002). In contrast, criteria of responding stability for nicotine are generally fixed between 15 and 30% variability for at least three consecutive sessions (Corrigall *et al*, 2002; Paterson and Markou, 2002; Shoaib and Stolerman, 1999) because rats self-administering nicotine show higher variability in the pattern of responding compared with those obtaining cocaine. In our experimental conditions, the rate of responding was stable after about 3 weeks of training.

**Reinstatement phase.** Nicotine-seeking behavior was investigated 24 h after the last nicotine self-administration session. Rats showing stable nicotine self-administration were exposed to a multiple 'relapse schedule' consisting of two components. The first component was a 30-min phase during which animals were allowed to lever press under an FR-1 schedule. This instrumental response did not produce an infusion of nicotine as during training, but only the conditioned stimuli previously paired to nicotine self-administration. This stage of the test session was termed the 'CS component'. At the end of the CS component, a noncontingent s.c. injection of nicotine (0.15 mg/kg) was delivered to rats and nicotine-paired lever presses were measured during the following 90-min phase. Lever pressing had no consequences. This second stage of the test session was termed the 'priming component' (Chiamulera *et al*, 1998). This model was designed to mimic the human situation of short-term, overnight withdrawal, when nicotine-seeking might be at its highest since the abstinence period allows for resensitization of nicotinic receptors, which in turn may result in increased positive reinforcement combined with withdrawal symptoms because of cholinergic hyperactivity. Experimental data confirm that the first cigarette of the day has enhanced reinforcing effects (Fagerstrom and Schneider, 1989). The exposure to nicotine-associated stimuli is also relevant to the human situation and gives a measure of short time extinction in the self-administration environment with the contingent presentation of conditioned stimuli. Unpublished experiments have shown that over the 30 min of the CS component there is a decrease in lever pressing compared with the preceding self-administration session, but this decrease is usually more profound if the CS presentations are removed. Moreover, this phase of the test gives a measure of nicotine seeking that is not dependent on the pharmacological and

rate-altering effects of nicotine itself. After the nicotine priming, responding on the active lever did not lead to any infusion or CS presentations and represents a measure of nicotine seeking in the presence of a passive exposure to nicotine, which is also close to the human situation. The within-session extinction-reinstatement procedure has been previously used in other laboratories (see for example Schenk and Partridge, 1999; Self *et al*, 1996, 1998), with two critical differences: (i) The extinction phase was immediately preceded by a self-administration session, thus adding confounding elements (see above) to the measure of behavior. (ii) The role of cues in maintaining responding was never measured, despite growing evidence that conditioned reinforcement may play a more important role in maintaining and inducing relapse to nicotine seeking for most drugs of abuse (Balfour *et al*, 2000; Caggiula *et al*, 2001).

### Experimental Design

In a first series of experiments, the effect of SB-277011-A (0, 3, 10 mg/kg i.p.) was investigated against stable maintenance of nicotine self-administration *per se*. Once the rate of responding was stable according to the criteria described above, the animals were randomly treated with SB-277011-A according to a Latin Square Design. The following parameters were measured for each animal in each experimental group ( $n=8$ /group): the number of infusions/h, the active and inactive lever presses/h, and the IRT.

In a separate series of experiments, the effect of SB-277011-A (0, 3, 10 mg/kg i.p.) was assessed against nicotine-triggered relapse to nicotine-seeking behavior 24 h after the last nicotine self-administration session (see methodology above). The following parameters were measured for each animal in each experimental group ( $n=8$ /group): the number of active and inactive lever presses/h and the latency (s) to responding during both the cue and priming components of the drug-seeking test.

### Drugs

Nicotine bitartrate (Sigma, St Louis, MO) used during self-administration sessions was dissolved in heparinized saline and the pH was adjusted to 7.4 with NaOH. Nicotine unit doses were expressed as mg of free base/kg of body weight/infusion. Nicotine bitartrate (Sigma, St Louis, MO) used as priming during the reinstatement phase was dissolved in physiological saline and the pH adjusted to 7.4 with NaOH. The nicotine dose of 0.15 mg/kg was expressed as mg of free base and given to rats subcutaneously in a volume of 1 ml/kg.

SB-277011-A (GlaxoSmithKline Pharmaceuticals, Harlow, UK) was dissolved in 10% hydroxypropyl- $\beta$ -cyclodextrine (Sigma, St Louis, MO, USA) and administered in a volume of 1 ml/kg i.p. All solutions except nicotine bitartrate used for self-administration sessions were prepared immediately prior to use. Doses of SB-277011-A were chosen based on pharmacokinetic (Austin *et al*, 2001; Reavill *et al*, 2000) and electrophysiological (Ashby *et al*, 2000) characteristics as well as behavioural properties reported in previous studies (Di Ciano *et al*, 2003; Le Foll *et al*, 2002; Reavill *et al*, 2000; Vorel *et al*, 2002).

## Statistical Analyses

Self-administration data were analyzed by using one-way analyses of variance (ANOVA) with repeated measurements. The differences between individual means were assessed with the *post hoc* Dunnett's test. Statistical significance was set at a probability level of  $P < 0.05$  for all tests.

## RESULTS

### Effect of SB-277011-A (3, 10 mg/kg i.p.) on Stable Maintenance of Nicotine Self-Administration

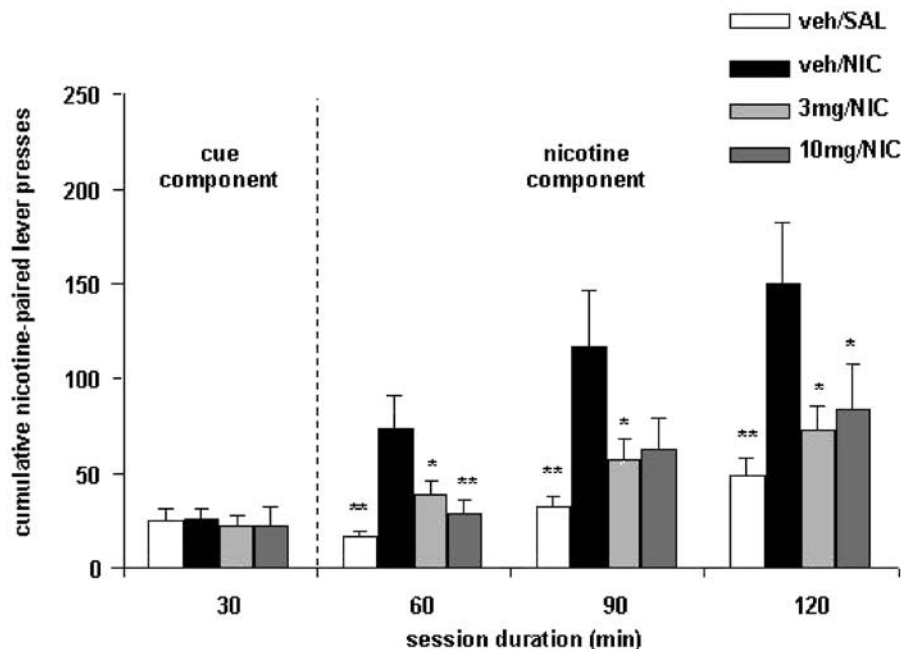
Rats trained for i.v. nicotine infusion acquired a robust self-administration behavior. SB-277011-A (0, 3, or 10 mg/kg i.p.) ( $n = 8$  rats/group) was administered 30 min prior to the beginning of the nicotine self-administration session. Each rat received all treatments spaced at least 2 days apart. SB-277011-A did not affect the number of infusions/60 min or the number of RLP/60 min compared with the vehicle group ( $F_{[2;14]} = 0.9$ ,  $P = 0.4$  and  $F_{[2;14]} = 0.4$ ,  $P = 0.7$ , respectively) at any dose tested. Furthermore, SB-277011-A failed to modify significantly both the number of LLP/60 min as well as the IRT ( $F_{[2;14]} = 0.1$ ,  $P = 0.9$  and  $F_{[2;14]} = 0.7$ ,  $P = 0.5$ ).

### Effect of SB-277011-A (3, 10 mg/kg i.p.) on Nicotine-Triggered Relapse to Nicotine Self-Administration

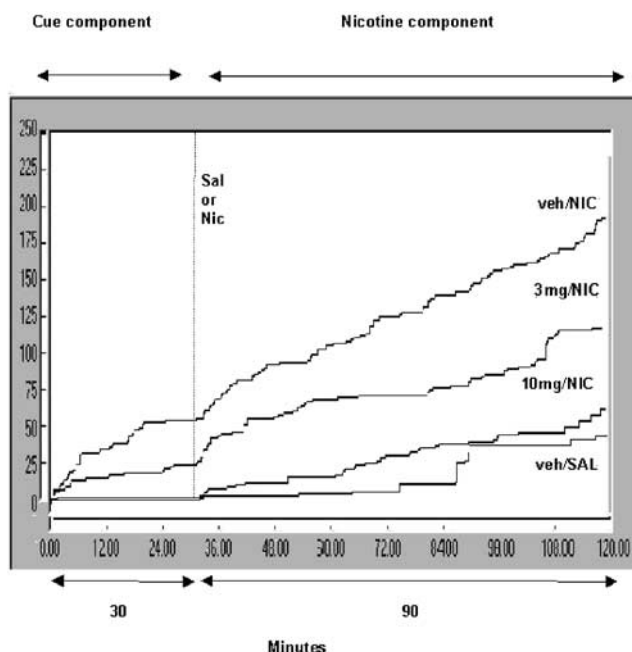
SB-277011-A (0, 3, 10 mg/kg i.p.) ( $n = 8$  rats/group) was administered 30 min before starting the relapse session. Responding was measured throughout the session up to 120 min (Figure 1). SB-277011-A (3, 10 mg/kg i.p.) did not significantly change responding during the first 30-min cue

component ( $F_{[3;21]} = 0.3$ ,  $P = 0.8$ ). Furthermore, SB-277011-A failed to affect inactive lever presses during the cue component of the relapse session ( $F_{[3;21]} = 0.6$ ,  $P = 0.6$ ). In contrast, the acute systemic administration of nicotine (0.15 mg/kg s.c.) produced a significant increase in nicotine-paired lever presses in the vehicle/nicotine group compared with the vehicle/saline control group. Furthermore, SB-277011-A produced a significant reduction in nicotine-paired lever presses compared with the vehicle/nicotine group. A one-way ANOVA with repeated measurements over time revealed a significant difference in nicotine-paired lever presses ( $F_{[3;21]} = 5.2$ ,  $P < 0.01$ ). The *post hoc* Dunnett's test confirmed significant effects of both doses of SB-277011-A ( $P < 0.05$ ) compared with responding in the vehicle/nicotine group (Figure 1). Finally, no significant changes in the number of inactive lever presses were seen during the priming component ( $F_{[3;21]} = 1.5$ ,  $P = 0.2$ ).

Latency to receive a conditioned stimulus presentation and a first self-administered nicotine infusion provides a measurement of the motivation for these reinforcers. As such, the latency to responding during both cue and priming components was evaluated. The overall ANOVA failed to yield significant main effects. The average latency (s) to responding during the cue component for the vehicle/saline, vehicle/nicotine, and SB-277011-A (3, 10 mg/kg)/nicotine groups was  $110.25 \pm 54.92$ ,  $81 \pm 23.06$ ,  $292.13 \pm 215.71$ , and  $218.13 \pm 163.50$  (mean  $\pm$  SEM,  $n = 8$  rats/group), respectively ( $F_{[3;21]} = 0.5$ ,  $P = 0.7$ ). The average latency (s) to responding during the priming component for the vehicle/saline, vehicle/nicotine, and SB-277011-A (3, 10 mg/kg)/nicotine groups was  $430.0 \pm 212.26$ ,  $163.63 \pm 55.09$ ,  $170.13 \pm 58.94$ , and  $406.63 \pm 198.27$  (mean  $\pm$  SEM,  $n = 8$  rats/group), respectively ( $F_{[3;21]} = 1.01$ ,  $P = 0.4$ ).



**Figure 1** Effect of SB-277011-A (0, 3, 10 mg/kg i.p.) on nicotine-triggered relapse to nicotine self-administration. Cumulative nicotine-paired lever presses were measured during the 'cue' and 'nicotine' components of responding, respectively. SB-277011-A was given 30 min prior to starting the relapse session. Nicotine (0.15 mg/kg s.c.) or saline (1 ml/kg s.c.) priming was given at the end of the 'cue component'. Treatment groups as follows: veh/SAL (vehicle/saline), veh/NIC (vehicle/nicotine), 3mg/NIC (SB-277011-A 3 mg/kg/nicotine), 10mg/NIC (SB-277011-A 10 mg/kg/nicotine).  $P < 0.05$ ;  $P < 0.01$ . Dunnett's test vs veh/NIC.



**Figure 2** Cumulative response records from a representative rat during cue- and nicotine-triggered relapse to nicotine-seeking behavior. SB-277011-A (3 and 10 mg/kg i.p.) or vehicle (veh) pretreatment was given 30 min prior to the beginning of the relapse session. Nicotine (0.15 mg/kg s.c.) (Nic) or saline (Sal) priming was given at the end of the cue component. The cumulative response graphs illustrate that SB-277011-A produced a dose-dependent decrease in responding during the nicotine priming reinstatement session.

Finally, analysis of individual response patterns from representative rats revealed that increasing doses of SB-277011-A produced a decrease in response rates during the nicotine priming session (Figure 2).

## DISCUSSION

The present findings suggest that selective antagonism at DA D<sub>3</sub> receptors by using the high-affinity antagonist SB-277011-A attenuated significantly nicotine-triggered reinstatement of nicotine-seeking behavior without affecting stable maintenance of nicotine self-administration *per se*. These results corroborate recent findings showing that SB-277011-A in the same dose range can block significantly cocaine-induced enhancement of brain stimulation reward, the development and expression of cocaine-induced conditioned place preference, and cocaine-triggered relapse to cocaine-seeking behavior (Vorel *et al.*, 2002).

The available evidence shows that there is no clear consensus regarding the exact role of DA D<sub>1</sub> or D<sub>2</sub> agonists and antagonists in both cocaine- and nicotine-seeking behaviors. Nonselective DA D<sub>2</sub>/D<sub>3</sub> agonists have been reported to mimic the effects of cocaine in drug discrimination (Spealman, 1996) and in cocaine self-administration paradigms (Parsons *et al.*, 1996). Furthermore, the potency of such agents was shown to correlate significantly with their *in vitro* affinity and functional activity on DA D<sub>3</sub>, but not D<sub>2</sub> receptors (Caine *et al.*, 1997; Spealman, 1996). In the case of drug priming-induced relapse to cocaine seeking,

evidence suggests that blockade of D<sub>2</sub>-like receptors by haloperidol (Ettenberg, 1990) or blockade of DA D<sub>1</sub>-like receptors by SCH 23390 (Norman *et al.*, 1999) attenuates cocaine seeking. Furthermore, activation of D<sub>1</sub>-like receptors by direct agonists can inhibit cocaine seeking (Self *et al.*, 1996, 2000). Recent studies also report that the DA D<sub>1</sub> antagonist SCH 23390 and the full agonist SKF 81297, but not the partial agonist SKF 38393, can attenuate cue-induced reinstatement of cocaine seeking (Alleweireldt *et al.*, 2002). In addition, the DA D<sub>1</sub>-like agonists SKF 81297 and SKF 82958 attenuate priming-induced reinstatement of cocaine seeking in the Squirrel monkey in contrast with the D<sub>2</sub> agonist R(-)-propylnorapomorphine hydrochloride (NPA) and the D<sub>3</sub>-preferring agonists PD 128,907 and 7-OH-DPAT, which failed to alter cocaine-triggered reinstatement of cocaine-seeking behavior (Khroyan *et al.*, 2000).

In the case of nicotine, DA antagonists at both D<sub>1</sub> and D<sub>2</sub> receptors have been shown to produce a dose-dependent extinction-like intrasession reduction of responding for nicotine self-administration (Corrigall and Coen, 1991a, b). In addition, haloperidol reduces the number of both nicotine containing and denicotinized cigarettes, thus suggesting the involvement of non-nicotinic mechanisms (Brauer *et al.*, 2001). However, haloperidol has also been shown to increase smoking behavior in both schizophrenic patients (McEvoy *et al.*, 1995) and nonpsychiatric subjects (Caskey *et al.*, 1999, 2002; Dawe *et al.*, 1995). Interestingly, DA agonist treatment can reduce responding for nicotine self-administration. For example, the DA D<sub>2</sub> agonist bromocriptine seems to produce a longer latency to smoke and a longer inter-cigarette interval, a finding that is compatible with the hypothesis that bromocriptine can decrease smoking and nicotine intake (Caskey *et al.*, 2002; Jarvik *et al.*, 2000; Murphy *et al.*, 2002). The recent study by Caskey *et al.* (2002), which directly compared haloperidol and bromocriptine in a double-blind study, is the only within-subjects drug reversal design confirming that blockade of DA D<sub>2</sub> receptors can increase, whereas activation of D<sub>2</sub> receptors can decrease smoking behavior and nicotine intake in the same subjects. However, it is well known that both nausea and vomiting are common side effects of bromocriptine, a fact further supported by the observation that increased levels of nausea were related to decreased levels of smoking with higher doses of bromocriptine (Jarvik *et al.*, 2000). Thus, the relative contributions of DA activity that are related to nausea or not remain to be fully assessed by future studies.

The present study supports the contention that selective blockade at DA D<sub>3</sub> receptors does not affect nicotine self-administration *per se*, but rather selectively diminishes nicotine-triggered reinstatement of nicotine-seeking behavior. One may argue that the effects observed in the present study are partly mediated through an action of SB-277011-A at DA D<sub>2</sub> receptors. This, however, is unlikely for the following reasons. First, in contrast with DA D<sub>2</sub> antagonists, SB-277011-A does not produce any significant effect on both spontaneous locomotion and either amphetamine- or phencyclidine-induced hyperactivity (Reavill *et al.*, 2000). It has been previously shown that the D<sub>3</sub>-preferring antagonist U-99194A can stimulate locomotor activity and potentiates both apomorphine- and amphetamine-induced hyperactivity (Waters *et al.*, 1993). This compound, however, has only

a 10-fold selectivity for DA D<sub>3</sub> over D<sub>2</sub> receptors (Audinot *et al*, 1998; Waters *et al*, 1993). Similarly, *l*-nafadotride can increase locomotor activity, but again this compound is only 10-fold selective for D<sub>3</sub> over D<sub>2</sub> receptors (Audinot *et al*, 1998; Sautel *et al*, 1995). In addition, the locomotor-stimulating effects of both *l*-nafadotride and U-99194A have been observed in DA D<sub>3</sub> knockout mice, indicating that the stimulant properties of both compounds are unrelated to their D<sub>3</sub> receptor blocking properties (Xu *et al*, 1999). Thus, these findings together with the majority of studies using DA D<sub>3</sub> knockout mice support the contention that, contrary to DA D<sub>2</sub> receptors, D<sub>3</sub> receptors do not play an inhibitory role in locomotor activity in rodents. Second, in contrast with DA D<sub>2</sub> receptor antagonists, SB-277011-A was shown not to be cataleptogenic at doses up to 78.8 mg/kg (Reavill *et al*, 2000). Third, again in contrast with DA D<sub>2</sub> receptor antagonists, SB-277011-A failed to increase serum prolactin levels (hyperprolactinemia) (Reavill *et al*, 2000). Fourth, SB-277011-A reversed the DA D<sub>3</sub>-preferring agonist quinolorane-induced decrease in extracellular DA levels in the NAc (Reavill *et al*, 2000). In contrast, the effects of quinolorane in the dorsal striatum were not reversed even by doses of SB-277011-A up to 93 mg/kg, thus further reflecting regional differences in DA D<sub>3</sub> receptor regulation of DA outflow. Fifth, lateral (right or left) shifts along the pulse frequency axis in the rate-frequency curve paradigm of the intracranial self-stimulation behavior has been considered a selective reward measure, whereas vertical shifts typically provide information about motor/performance capacity. It has been shown that DA D<sub>2</sub> receptor antagonists typically produce a right shift in the rate-frequency curve (Miliaressis *et al*, 1986a,b; Stellar *et al*, 1983). Furthermore, the effectiveness of DA receptor antagonists to produce right shifts is correlated with their relative effectiveness in displacing DA D<sub>2</sub> binding (Gallistel and Davis, 1983). If SB-277011-A would have any functionally significant DA D<sub>2</sub> receptor antagonist properties, a right shift in the rate-frequency curve of brain stimulation reward would be observed in response to treatment with SB-277011-A alone. Rather, SB-277011-A produced a left shift in the same paradigm, thus further supporting the DA D<sub>3</sub> antagonist properties of the effect (Vorel *et al*, 2002). Sixth, SB-277011-A did not produce conditioned place aversion (Vorel *et al*, 2002) in contrast with both the DA D<sub>3</sub> agonists 7-OH-DPAT and PD-128907 and the partial D<sub>3</sub> agonist BP-897 (Gyertyan and Gal, 2003). Altogether these results strengthen the suggestion that SB-277011-A diminishes nicotine-seeking behavior without altering the primary reinforcing effects of nicotine itself through a main action at DA D<sub>3</sub> receptors.

It has been recently shown that BP 897 can reduce cocaine cue-induced hyperactivity (Le Foll *et al*, 2002) as well as cue-controlled cocaine-seeking behavior independently of any interaction with the reinforcing properties of cocaine itself (Pilla *et al*, 1999). In addition, BP 897 can attenuate the discriminative stimulus properties of both cocaine and amphetamine without being self-administered on its own (Beardsley *et al*, 2001). A similar profile has been observed with SB-277011-A (Di Ciano *et al*, 2003; Le Foll *et al*, 2002; Vorel *et al*, 2002). Recently, BP 897 has been shown to lack agonist activity and to have competitive antagonist properties at DA D<sub>3</sub> receptors (Wood *et al*, 2000) in a real-time

Cytosensor microphysiometry paradigm, which measures changes in extracellular acidification rates as a determinant of changes in cellular metabolic activity (Smart and Wood, 2000). These results thus raise the possibility that it is, in fact, the antagonist property of BP 897 that mediates its effects on cocaine-seeking behavior, further supporting the therapeutic utility of selective competitive antagonists at DA D<sub>3</sub> receptors. Alternatively, one may suggest that SB-277011-A and BP-897 are acting through different mechanisms in different brain regions: one as an agonist and the other as a competitive antagonist. This hypothesis should be assessed by future studies. Also, the question of whether D<sub>3</sub> receptor-mediated effects of quinolorane on DA outflow in the NAc are mediated via terminal autoreceptors or postsynaptic receptors is unclear and warrants further investigations.

One of the key remaining questions is related to the precise sites of action of DA D<sub>3</sub> receptor ligands in regulating drug-seeking behavior. Increasing evidence converges onto the mesocortico-limbic DA system as a key player in the reinstatement of psychostimulant-seeking behavior triggered by drug priming. For example, direct infusion of DA into the NAc can reinstate cocaine seeking (Cornish and Kalivas, 2000), whereas microinfusions of the DA D<sub>2</sub> antagonist fluphenazine into the medial prefrontal cortex (mPFC) can significantly attenuate cocaine-induced reinstatement (McFarland and Kalivas, 2001). Interestingly, direct microinfusion of the DA D<sub>2</sub> antagonist haloperidol into the mPFC can decrease local DA levels whereas its systemic administration stimulates the percentage of burst firing and spikes per burst of VTA DA neurons antidromically identified from the mPFC (Gessa *et al*, 2000). In contrast, SB-277011-A does not modify basal DA neurotransmission in the NAc, but can reverse quinolorane-induced decreases in extracellular DA levels in the same brain area (Reavill *et al*, 2000). We have recently shown that the acute systemic administration of SB-277011-A can increase extracellular DA levels in the rat anterior cingulate cortex (ACg) (Lacroix *et al*, 2003). Interestingly, the projections from the ACg terminate more medially and extend further ventrally to include the core of the NAc (Ding *et al*, 2001). It has been shown that lesions of the ACg produce persistent responding for cocaine when responding is no longer under the control of contingent presentations of cocaine-associated cues (Weissenborn *et al*, 1997). Furthermore, lesions of the core subregion of the NAc impair the acquisition of cocaine seeking under a second-order schedule (Ito *et al*, 2001). The question of whether and how the effects of SB-277011-A on DA in the ACg are related to reinstatement of drug-seeking behaviors warrants further investigations.

Priming injections of cocaine can increase extracellular levels of DA in both the NAc (Neisewander *et al*, 1996) and amygdala (Tran-Nguyen *et al*, 1998) and can increase Fos protein expression in the VTA as well as caudate-putamen, central and lateral nuclei of the amygdala, and ACg (Neisewander *et al*, 2000). In addition, priming injections of *D*-amphetamine produce increases in DA chronoamperometric signals in the NAc (Di Ciano *et al*, 2001). These results point again towards DA neurotransmission in the NAc as an important factor in drug priming-induced reinstatement of drug-seeking behavior. In addition to the

NAC and ACg, the basolateral amygdala (BLA) has also been shown to play a key role in conditioned reinforcement (Everitt *et al*, 1999) and cue-controlled cocaine seeking (Whitelaw *et al*, 1996). Thus, the ACg along with the BLA both contribute to associative mechanisms controlling cocaine seeking. These findings are also in line with functional neuroimaging studies (Childress *et al*, 1999; Grant *et al*, 1996; O'Brien *et al*, 1998). Finally, another site of interest is the ICjM that shows extensive expression of the DA D<sub>3</sub> receptor (Diaz *et al*, 2000; Sokoloff *et al*, 1990). Although few studies have investigated the potential role of ICjM in brain-reward mechanisms, two pieces of evidence are worth mentioning. One study (Nakajima and Patterson, 1997) claimed that DA D<sub>2</sub>, but not D<sub>3</sub> or D<sub>4</sub>, receptors in the NAC, ICjM, and VTA are critically involved in the rewarding effects of intracranial self-stimulation behavior. However, although these authors used convincing drugs for targeting the DA D<sub>2</sub> receptor, they used (+)-UH232, which has only 10- to 20-fold selectivity for D<sub>3</sub> over D<sub>2</sub> receptors, to study the potential role of DA D<sub>3</sub> receptors. Thus, these effects can be explained by D<sub>2</sub>-mediated rather than D<sub>3</sub>-mediated mechanisms and are consistent with the results obtained with raclopride and haloperidol. Furthermore, these observations with (+)-UH232 are not in line with those of Kling-Petersen *et al* (1994). The second study involved 2-deoxyglucose autoradiographic measurements during withdrawal from cocaine self-administration (Hammer *et al*, 1993). In these studies, metabolic activity was reduced during withdrawal from cocaine in the NAC, olfactory tubercle, ICjM, piriform cortex, ACg, rostral caudate-putamen, entopeduncular nucleus, lateral hypothalamus, as well as somatosensory, auditory, and motor cortices. Furthermore, the results suggested that the longer the withdrawal period (72 vs 6 h after binge exposure), the more severe the effect on metabolic rate for glucose. Again, the potential role of the DA D<sub>3</sub> receptor in the ICjM against drug priming-induced reinstatement of drug-seeking behavior remains to be fully investigated.

## CONCLUSIONS

The results of the present study show that DA D<sub>3</sub> receptors play an important role in nicotine-triggered relapse to nicotine-seeking behavior without affecting stable maintenance of nicotine self-administration. Together with a growing body of evidence in the literature, the present findings also suggest that the neuronal processes mediating drug-induced reinstatement may be different, at least in part, from those involved in the primary reinforcing effects of the drug. Thus, efficacy in reducing drug self-administration *per se* is not necessarily predictive of efficacy in preventing relapse triggered by drug priming, cues or stress. These results point towards selective modulation of DA D<sub>3</sub> receptors as a valuable pharmacotherapeutic strategy for the management of biobehavioral dysregulations associated with nicotine dependence and addiction. Obviously, the precise site of action of SB-277011-A is still unclear and the assessment of the central sites of action of DA D<sub>3</sub> antagonists will be an important goal of future studies.

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