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Pharmacological Actions of NGB 2904, a Selective Dopamine D_3 Receptor Antagonist, in Animal Models of Drug Addiction

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ABSTRACT

As a continuation of our work with SB-277011A, we have examined the effects of another highly elective dopamine (DA) D_3 receptor antagonist, N-(4-[4-{2,3-dichlorophenyl}-1-piperazinyl]butyl)-2-fluorenylcarboxamide (NGB 2904), in animal models of addiction. Our results indicate that by systemic administration, NGB 2904 inhibits intravenous cocaine self-administration maintained under a progressive-ratio (PR) reinforcement schedule, cocaine- or cocaine cue–induced reinstatement of cocaine-seeking behavior, and cocaine- or other addictive drug-enhanced brain stimulation reward (BSR). The action of NGB 2904 on PR cocaine self-administration was long-lasting (1–2 days) after a single injection, supporting its potential use in treatment of cocaine addiction. The effects of NGB 2904 in the BSR paradigm were dose-dependent for both NGB 2904 and cocaine; that is, only lower doses of NGB 2904 were effective, and their putative antiaddiction effect could be overcome by increasing the doses of cocaine or other addictive drugs. A dopamine-dependent mechanism is proposed to explain the effects of NGB 2904 on cocaine's actions in these animal models of drug addiction. The data reviewed in this paper suggest that NGB 2904 or other D_3-selective antagonists may have potential in controlling motivation for drug-taking behavior or relapse to drug-seeking behavior, but may have a limited role in antagonizing the acute rewarding effects produced by cocaine or other addictive drugs. In addition, NGB 2904 may also act as a useful tool to study the role of D_3 receptors in drug addiction.

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INTRODUCTION

Drug abuse is a serious health problem in many areas of the world, yet there are no widely effective medications available to treat its underlying pathology or its clinical manifestations. Preclinical studies suggest that the mesolimbic dopamine (DA) system is critically involved in drug reward and relapse (Wise 1996a, 1996b; Pierce and Kumaresan 2006). This system originates from the DA neurons in the ventral tegmental area (VTA) in the midbrain and projects to the nucleus accumbens (NAc), prefrontal cortex (PFC), and amygdala in the forebrain (Wise 2005; Kalivas and Volkow 2005). The DA hypothesis of addiction is based primarily on findings that almost all addictive drugs, such as cocaine, amphetamine, opiates, nicotine, marijuana, or ethanol increase extracellular DA in the NAc (Wise 1996b; Bardo 1998), and are self-administered by animals either intravenously or locally into DA brain loci, an effect that is blocked by selective lesions of DA terminals or pharmacological blockade of DA receptors in the NAc (Gardner 2000; Bressan and Crippa 2005). In addition, electrical stimulation of brain DA loci maintains brain stimulation reward, which is enhanced by drugs of abuse and robustly attenuated by DA receptor antagonists (Wise 1996a; Kornetsky 2004). Based on these findings, development of new medications for treatment of drug addiction has focused on manipulation of DA transmission in the reward circuitry of the brain. There are several useful pharmacological strategies to manipulate brain DA transmission: one is to modulate brain DA transporters, and another is to modulate brain DA receptors (for review see Rothman and Glowa 1995; Drago et al. 1998; Platt et al. 2002; Heidbreder et al. 2005). In the present review article, we focus on the pharmacological actions of NGB 2904, a novel DA D_3 receptor-selective antagonist, in preclinical animal models of drug addiction.

PHARMACOLOGY

D_3 Receptor Antagonists as Promising Treatments for Drug Addiction

To date, there are five DA receptor subtypes cloned and identified in the brain. These are classified into D_1-like (D_1, D_5) and D_2-like (D_2, D_3, D_4) receptor families, based upon sequence homology, intracellular signal transduction mechanisms, and pharmacological properties (Girault and Greengard 2004). Current attention in antiaddiction drug development has focused primarily on the D_1, D_2, and D_3 DA receptor subtypes. It is well documented that both D_1 and D_2 receptors are involved in drug reward and addiction (Rothman and Glowa 1995; Platt et al. 2002). In animals, D_1 or D_2 receptor antagonists inhibit cocaine's rewarding effects, as assessed by drug self-administration, conditioned place preference, and brain stimulation reward. However, such effects are mediated primarily at doses that also inhibit natural food reward or locomotor behaviors (Platt et al. 2002; Gorelick et al. 2004; Wise 2006). In humans, D_1 antagonists have been reported to have either no effect on cocaine craving in cocaine addicts or to produce increased cocaine self-administration and enhanced subjective ratings of cocaine's rewarding effects (Haney et al. 2001). Although some evidence indicates that D_2 antagonists attenuate cocaine's acute euphorigenic effects (Rothman and Glowa 1995; Newton et al. 2001; Platt et al. 2002), they are often dysphorigenic by themselves and produce significant abnormal movements (Rothman and Glowa 1995; Platt et al. 2002; O'Brien 2003). Thus, D_1

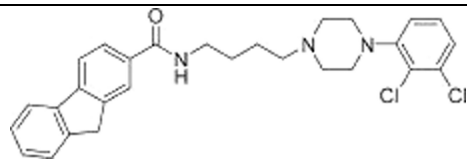
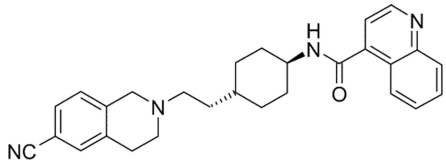
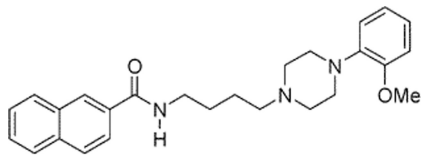
or D_2 receptor antagonists are no longer considered promising for treatment of cocaine addiction.

In contrast to D_1/D_2 receptor antagonists, DA D_3 receptor antagonists appear highly promising in attenuating the actions produced by cocaine or other addictive drugs in preclinical animal studies. DA D_3 receptors have several unique properties as compared with other DA receptor subtypes. (1) D_3 receptors have a unique anatomic distribution. They are preferentially localized in the mesolimbic DA system (Diaz et al. 2000). The greatest densities of D_3 receptor in the rat's brain are found primarily in limbic brain areas, such as the NAc, islands of Calleja, and olfactory tubercle (Stanwood et al. 2000). Transcripts for the D_3 receptor are located in those mesencephalic areas rich in DA cell bodies (see reviews by Heidbreder et al. 2005; Sokoloff et al. 2006**)**. This restricted neuroanatomic localization suggests an important role for D_3 receptors in drug reward and addiction (Sokoloff et al. 2001, 2006), a suggestion supported by the finding that D_3 mRNA and receptors are increased following exposure to addictive drugs in both animals and humans (Staley and Mash 1996; Neisewander et al. 2004). (2) D_3 receptors have the highest affinity for endogenous DA of all known receptors (Levant 1997; Sokoloff et al. 2001), suggesting a predominant role for D_3 receptors in the normal functioning of the mesolimbic DA system. (3) Pharmacological evidence demonstrates that D_3 receptors play an important role in emotional, motivational, and reinforcement functions, including the reinforcement produced by addictive drugs (Caine and Koob 1993; Duaux et al. 1998; Narita et al. 2003; Sokoloff et al. 2006). Growing evidence demonstrates that blockade of D_3 receptors by D_3 receptor antagonists or partial agonists consistently inhibits the actions of cocaine or other addictive drugs in the majority of animal models of drug addiction (for more comprehensive reviews see Heidbreder et al. 2005; Le Foll et al. 2005). These data support the potential use of D_3 receptor antagonists or partial agonists in the clinical treatment of drug addiction. Finally, (4) the net effect of D_3 receptor antagonism is a slight enhancement of DA tone in the mesolimbic DA system (Heidbreder et al. 2005), which may normalize the hypofunctional status of the mesolimbic DA system after chronic cocaine administration (Kuhar and Pilotte 1996; Gardner 1999, 2005), or relieve craving, depression, or dysphoria in human cocaine addicts after abstinence (Volkow et al. 1999, 2006). Although all this evidence suggests the potential use of D_3 receptor antagonists in the treatment of cocaine addiction, research has been hampered by lack of highly potent, selective, and systemically effective D_3 receptor antagonists in both rodents and primates.

BP-897: a Non-Selective Partial D_3 Receptor Agonist

BP-897 (N-[4-(4-(2-methoxyphenyl)piperazinyl)butyl]-2-naphthamide) is the first D_3-selective partial agonist to be developed (Pilla et al. 1999). It has modest (60–70-fold) selectivity for human D_3 *versus* human D_2 receptors, and similar (60–70-fold) selectivity over other receptors, such as α_1-, α_2-, and 5-hydroxytryptamine 1A (5-HT_{1A}) receptors (Tables 1 and 2) (Pilla et al. 1999). A series of studies has assessed the efficacy of BP-897 in animal models of drug addiction (see reviews by Garcia-Ladona and Cox 2003; Heidbreder et al. 2005). Briefly, it has been reported that BP-897 produces a significant dose-dependent reduction in cocaine self-administration under second-order reinforcement, cocaine- or cocaine-associated cue-induced reinstatement of cocaine-seeking behavior, cocaine-induced conditioned place preference, and cocaine's discriminative

TABLE 1. Chemical Structures of NGB 2904, SB-277011A, and BP-897

Ligand name	Chemical structure
NGB 2904	
SB-277011A	
BP-897	

stimulus properties as assessed in the drug discrimination paradigm. In addition, BP-897 also inhibits cocaine or nicotine cue-induced increases in locomotion and behavioral sensitization in mice or rats (Le Foll et al. 2005). These data support the potential use of BP-897 in treatment of cocaine or nicotine addiction (Garcia-Ladona and Cox 2003; Heidbreder et al. 2005; Le Foll et al. 2005). However, enthusiasm for BP-897 has been stifled by the finding that BP-897 also displays properties of a D_2 receptor antagonist (Heidbreder et al. 2005). For example, BP-897 produces a significant aversive-like effect, as assessed in the brain stimulation reward (BSR) and conditioned place preference/aversion paradigms (Duarte et al. 2003; Gyertyán and Gál 2003). Similar to the D_2 receptor antagonist haloperidol, BP-897 also produces a compensatory increase in cocaine self-administration under fixed-ratio (FR1) reinforcement (Gál and Gyertyán 2003), and inhibits quinpirole (a D_2/D_3 agonist)–induced inhibition of DA neuronal firing in the substantia nigra (Wicke and Garcia-Ladona 2001). Recent studies using microphysiometry show that BP-897 behaves as a full antagonist at both DA D_2 ($pK_b = 8.05$) and D_3 ($pK_b = 9.43$) receptors, and displays

TABLE 2. Binding affinity (K_i, nM) of NGB 2904, SB-277011A, and BP-897 at D_2-like receptor subtypes

Compound	D_2	D_3	D_2/D_3-Ratio	Reference
BP-897	61 ± 0.2	0.92 ± 0.2	66	Pilla et al. 1999
SB-277011A	2820	10.7	263	Reavill et al. 2000
	1050	11.2	93	Reavill et al. 2000
				Newman et al. (2005)
NGB 2904	217 ± 12	1.4 ± 0.6	155	Yuan et al. 1998
	911 ± 190	1.1 ± 0.2	830	Newman et al. 2003
	112 ± 22	2.0 ± 0.4	56	Grundt et al. 2005
	698 ± 120	2.7 ± 0.6	259	Newman et al. 2005

antagonistic effects in a [^{35}S]-GTPγS binding assay in cells expressing human D_3 receptors (Wood et al. 2000). In addition, BP-897 shows moderate affinity for α_1- and α_2-adrenergic receptors ($K_i = 60$ and 83 nM, respectively), 5-HT_{1A} ($K_i = 84$ nM), and DA D_2 receptors ($K_i = 61$ nM) (Pilla et al. 1999; Campiani et al. 2003). Taken together, these data suggest that: (1) BP-897 may act as a D_3 partial agonist (Pilla et al. 1999) or a D_2/D_3 antagonist (Wood et al. 2000; Wicke and Garcia-Ladona 2001); (2) the anti-cocaine/nicotine effects of BP-897 may be mediated by its antagonism of D_3 receptors (Heidbreder et al. 2005); and (3) antagonism at DA D_2 and other receptors by BP-897 suggests the potential for unwanted side effects and/or toxicities, as observed with other D_2 antagonists (Rothman and Glowa 1995; Platt et al. 2002).

SB-277011A: a Full D_3 Receptor Antagonist with Poor Bioavailability in Primates

SB-277011A (*trans-N*-[4-[2-(6-cyano-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl]cyclohexyl]-4-quinolinecarboxamide) is the most well-characterized DA D_3 receptor antagonist to date. SB-277011A has high affinity for the human cloned DA D_3 receptor, and the ratio of *in vitro* D_2/D_3 affinity of SB-277011A in human and rat is 263 and 96, respectively (Reavill et al. 2000; Newman et al. 2005). SB-277011A has a 100-fold selectivity or better over 66 other receptors, enzymes, and ion channels (Reavill et al. 2000). Recent research has confirmed this selectivity profile by screening 26 additional transmembrane receptors, 16 ion channels, and 64 kinases (see review by Heidbreder et al. 2005). Thus, SB-277011A has better than 100-fold selectivity for the D_3 receptor *versus* approximately 180 other central nervous system targets tested to date.

We and others have previously demonstrated that SB-277011A attenuates: (1) brain reward-enhancing effects produced by cocaine, nicotine, or methamphetamine; (2) cocaine- or heroin-induced conditioned place preference; (3) cocaine self-administration under progressive ratio or high-cost/low-payoff fixed ratio (FR) reinforcement schedules; (4) cocaine-seeking behavior under second-order reinforcement conditions; (5) cocaine-, nicotine-, cocaine cue- or stress-triggered relapse to cocaine-seeking behavior as assessed by the reinstatement model; (6) oral ethanol intake; and (7) relapse to ethanol-seeking behavior. These data suggest that SB-277011A is widely effective at antagonizing actions produced by cocaine and many other addictive drugs (see comprehensive review by Heidbreder et al. 2005). However, further development of SB-277011A has been halted by GlaxoSmithKline Pharmaceuticals due to unexpectedly poor bioavailability ($\sim$2%) and a very short half-life ($\sim$20 min) in primates (Stemp et al. 2000; Austin et al. 2001; Remington and Kapur 2001). Therefore, development of other D_3-selective antagonists with higher bioavailability and promising pharmacotherapeutic profiles is required (Newman et al. 2005; Boeckler and Gmeiner 2006).

NGB 2904: Another Highly Selective DA D_3 Receptor Antagonist

Receptor profile of NGB 2904

NGB 2904 (N-(4-[4-{2,3-dichlorophenyl}-1-piperazinyl]butyl)-2-fluorenylcarboxamide) is another highly selective D_3 receptor antagonist (Table 1), which demonstrates

high binding affinities at the primate and rat D_3 receptor (Table 2) (Yuan et al. 1998; Robarge et al. 2001). NGB 2904 was initially reported to have >150-fold selectivity for primate D_3 over primate D_2 receptors, and >800-fold selectivity for rat D_3 *versus* rat D_2 receptors (Yuan et al. 1998; Newman et al. 2003). In addition, it was found to have >5000-fold selectivity over D_1, D_4, and D_5 receptors, 200- to 600-fold selectivity over α_1, $5HT_2$ receptors, and >1000-fold selectivity *versus* other targets in a 60-receptor Panlabs screen (Yuan et al. 1998). These *in vitro* profiles of NGB 2904 suggest it to be a promising D_3 antagonist.

Pharmacokinetics of NGB 2904

In a preliminary pharmacokinetic study in rats, NGB 2904 showed a moderate distribution volume and high blood clearance (74% of rat liver blood flow) after a single intravenous injection of 0.5 mg/kg. NGB 2904 readily penetrates the rat brain with a steady-state brain/plasma ratio of 1.7:1 (for SB-277011A, 3.6:1), but absolute brain levels appear to be lower (62 ng/g) than those obtained with SB-277011A (147.3 ng/g) under the same experimental conditions (Reavill et al. 2000; Heidbreder, personal communication). The precise bioavailability and half-life of NGB 2904 remain to be determined, but *in vivo* studies demonstrate that its behavioral and neurochemical effects last for at least 2–4 h after a single dose (0.1–10 mg/kg, i.p.) in rats.

Locomotor Behavioral Effects of NGB 2904

In our own studies, NGB 2904 did not produce sedative-like effects or catalepsy within the dose range of 0.1–10 mg/kg (i.p.) in rats (Xi et al. unpublished data). It also failed to show proconvulsant activity or locomotion inhibition at doses under 10 mg/kg (i.p.) in rats (Xi et al. unpublished data). However, other studies demonstrate that NGB 2904 produced an increase in basal or amphetamine-stimulated locomotion at very low doses ($\leq$1.0 mg/kg, s.c.) (Pritchard et al. 2007), or an inhibition in locomotion at very high doses (ID_{50}, 138 mg/kg) in mice (Newman et al. 2005).

Effects of NGB 2904 on Cocaine Self-Administration

Drug self-administration is one of the most reliable animal models to evaluate drug reinforcement. In this model, laboratory animals have an intravenous catheter surgically inserted and are then allowed to self-administer the addictive drug by pressing a wall-mounted lever in the animals' test cages. The two most commonly used self-administration paradigms use FR or PR schedules of drug reinforcement. In the FR paradigm, a drug infusion follows after a fixed number of responses by the animal, for example, after every one (FR1) or two (FR2) lever presses. In the PR reinforcement paradigm, a progressively increasing workload (e.g., lever pressing) is imposed upon the animal in order to receive one drug administration. Eventually, a point is reached at which the animal stops responding. This is termed the PR "break-point" and is considered a measure of rewarding efficacy or strength (Richardson and Roberts 1996).

Our initial research with NGB 2904 was to determine whether NGB 2904 inhibited cocaine self-administration under low FR reinforcement. We found that systemic

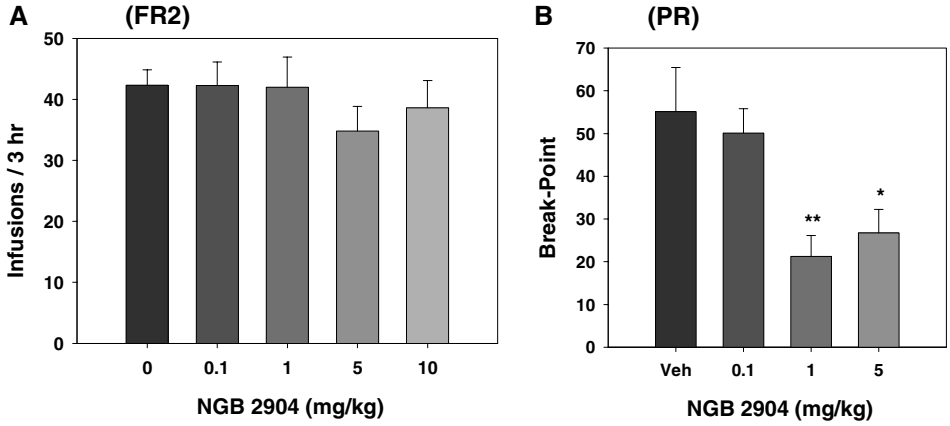


FIG. 1 Effects of NGB 2904 (0.1–10 mg/kg, i.p.) on cocaine self-administration under fixed-ratio 2 (FR2) and progressive-ratio (PR) reinforcement schedules in rats. $^{*}P < 0.05$, $^{**}P < 0.01$, when compared with the vehicle (Veh) treatment group.

administration of NGB 2904 (0.1, 1, 5, 10 mg/kg, i.p.) had no effect on stable maintenance of cocaine self-administration under an FR2 reinforcement schedule in rats (Fig. 1A) (Xi et al. 2006). This finding is consistent with previous studies demonstrating that neither BP-897 nor SB-277011A affects cocaine self-administration under low FR reinforcement conditions (Vorel et al. 2002; Di Ciano et al. 2003; Gál and Gyertyán 2003; Xi et al. 2005). Conceptually congruent with these findings, Martelle et al. (2007) recently reported that intravenous administration of NGB 2904 (1.0–5.6 mg/kg) for 5 consecutive days failed to produce a statistically significant reduction in cocaine or food self-administration under a second-order reinforcement schedule in three rhesus monkeys tested.

In contrast to this ineffectiveness observed under low FR or second-order schedules, the same doses of NGB 2904 (1–5 mg/kg, i.p.) significantly lowered the break-point for cocaine self-administration under PR reinforcement conditions (Fig. 1B), an effect that lasted for about 1–2 days after a single NGB 2904 administration (Xi et al. 2006). Such a long-lasting effect has been proposed to be related to NGB 2904's relatively high lipophilicity, leading it to be sequestered and then released from fatty tissues *in vivo* (Newman et al. 2005). Conceptually consistent with this finding, our previous study showed that SB-277011A also dose-dependently inhibited PR cocaine self-administration (Xi et al. 2005). In contrast to NGB 2904's prolonged effect, the effect produced by SB-277011A lasted for a much shorter time (<24 h).

There are two possible explanations for the different effects of NGB 2904 on FR and PR cocaine self-administration. First, animals may compensate for NGB 2904's action by increasing their drug intake or their self-administration rate under low FR conditions. We consider this possibility to be unlikely, as we did not observe a compensatory increase in cocaine intake or in cocaine self-administration pattern in our study. Second, the cumulative cocaine doses under low FR reinforcement are much higher than those under PR reinforcement. Thus, the high cumulative doses of cocaine may induce such large increases in DA so as to block the effectiveness of NGB 2904 or SB-277011A on cocaine's actions, as the large amount of DA may: (1) inhibit NGB 2904 binding to D_3 receptors, or (2) activate

other DA receptors (see further discussion below). This DA hypothesis may also explain why PR reinforcement is more sensitive than FR reinforcement in evaluating the extent of a drug's rewarding efficacy (Richardson and Roberts 1996; Rowlett 2000). In addition, the PR self-administration paradigm is also believed to measure motivation for taking addictive drugs, based upon the assumption that high reward (such as that produced by high cocaine doses) stimulates high motivation and high-effort behavior (Richardson and Roberts 1996; Stafford et al. 1998; Rowlett 2000). Thus, NGB 2904's antagonism of PR cocaine self-administration suggests that NGB 2904 attenuates cocaine's rewarding and incentive motivational properties. Finally, when NGB 2904 was substituted for cocaine under an FR reinforcement schedule, it did not maintain cocaine self-administration behavior, similar to SB-277011A's failure to support self-administration. These data suggest that NGB 2904 itself has no obvious abuse liability.

Effect of NGB 2904 on Reinstatement of Drug-Seeking Behavior in Rats

Drug addiction is characterized by high rates of relapse to drug use. The reinstatement procedure is widely used as an animal model of relapse in humans (Shalev et al. 2002). In the reinstatement model, rats are implanted with intravenous catheters and are allowed to self-administer an addictive drug until stable drug taking is achieved. Then, vehicle is substituted for the addictive drug. Since the animals are no longer rewarded by the addictive drug, they stop ("extinguish") the drug-taking behavior. Then, the experimenter determines what kinds of stimuli will "trigger" the animal to relapse—to go back to the drug-seeking behavior that previously resulted in intravenous infusions of the addictive drug. Three triggers cause relapse to drug-seeking behavior in this "reinstatement" animal model: (1) re-exposure to the drug previously used, (2) re-exposure to environmental cues (sights, sounds, smells) that were previously associated with the drug-taking behavior, or (3) exposure to mild stress. The face validity of the reinstatement model rests upon the fact that these are precisely the triggers that provoke relapse to drug-taking behavior in humans (O'Brien and Gardner 2005). We have used such "reinstatement" models to study the effects of NGB 2904 and other D_3 antagonists on reinstatement of drug-seeking behavior triggered by cocaine or cocaine-associated cues.

NGB 2904 inhibits cocaine-triggered reinstatement

Using the cocaine-triggered reinstatement model, we found that a single noncontingent intravenous injection of 10 mg/kg cocaine produces robust reinstatement of extinguished operant behavior previously reinforced by intravenous cocaine injections. Pretreatment with NGB 2904 (0.1, 1, 5 mg/kg, i.p.) significantly attenuated cocaine-triggered reinstatement of cocaine-seeking behavior. This effect was observed at doses that failed to reduce sucrose-induced reinstatement of sucrose-seeking behavior (Figs. 2A–C). These data suggest that NGB 2904 selectively inhibits addictive drug-induced, but not natural reward-induced reinstatement of reward-seeking behavior, and that such an effect is not caused by a generalized suppression of operant behavior (Xi et al. 2006). This finding is consistent with our previous findings with SB-277011A (Vorel et al. 2002).

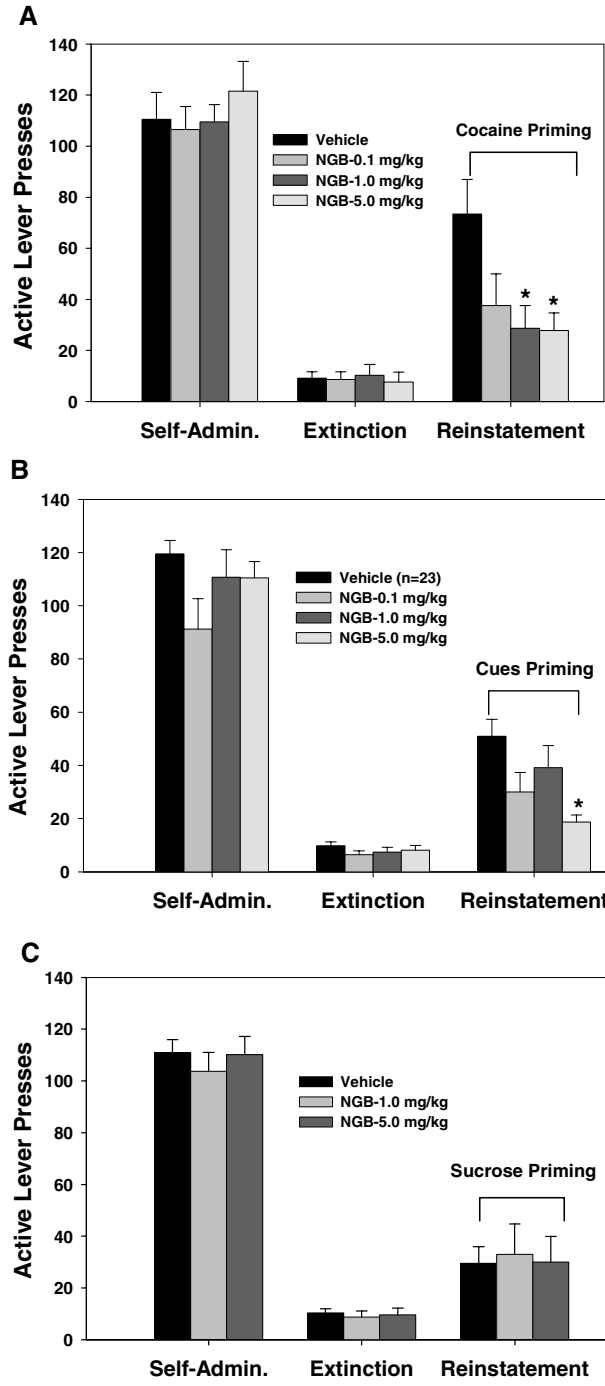


FIG. 2 Effects of NGB 2904 (0.1–5 mg/kg, i.p.) on reinstatement of reward-seeking behavior triggered by 10 mg/kg cocaine (Panel A), cocaine-associated cues (Panel B) or sucrose (Panel C). $^{*}P < 0.05$, when compared with the vehicle treatment group.

NGB 2904 inhibits cocaine cue-induced reinstatement

Further, we also observed the effects of NGB 2904 on reinstatement of cocaine-seeking behavior induced by re-introduction of cocaine-associated cues (light plus tone). We found that NGB 2904 (0.1, 1.0, 5.0 mg/kg, i.p.) dose-dependently inhibited cocaine cue-induced reinstatement of cocaine-seeking behavior in rats after 10–14 days of extinction from previous cocaine self-administration (Fig. 2B). Again, this is consistent with our previous findings with SB-277011A or BP-897 (Xi et al. 2004; Gilbert et al. 2005).

Effects of NGB 2904 on Cocaine's Discriminative Effects

Drug discrimination assesses the degree to which the subjective effects of one drug resemble the subjective effects of another drug (O'Brien and Gardner 2005). The animal is trained to make one response when drugged (e.g., with cocaine) and another when given an inactive vehicle, in order to receive a reward (e.g., food). The animal is then tested under the influence of a test compound, and the percentage of responses on the "drug lever" *versus* the "vehicle lever" yields a measure of how drug-like or vehicle-like the test compound is perceived to be by the animal. The drug discrimination procedure can also be used in antiaddiction drug development to evaluate whether a putative therapeutic agent enhances or diminishes the overall subjective state induced by the addictive drug (e.g., cocaine).

In contrast to the findings in the self-administration and reinstatement paradigms (described above), a recent study demonstrates that NGB 2904, at doses of 0.1–3 mg/kg (i.m.), does not alter the subjective effects of cocaine in three rhesus monkeys tested (Martelle et al. 2007). Since only three monkeys were tested within a narrow dose range of NGB 2904 in this study, more studies are needed with more animals and higher doses of NGB 2904.

Effects of NGB 2904 on Addictive Drug-Enhanced Brain Stimulation Reward

The electrical BSR model assesses the degree of drug-induced enhancement of brain reward in animals trained to respond to electrical stimulation of specific brain-reward loci, such as the VTA, medial forebrain bundle, and NAc (Wise 1996a; Kornetsky 2004). To assess the degree of drug-induced enhancement of brain reward, several quantitative electrophysiological techniques have been developed that allow reliable measurement of brain-reward thresholds. One such technique, the "rate-frequency curve-shift" paradigm, allows assessment of the animal's response-rate as a function of a range of electrical impulses delivered to a given reward site in the brain. Addictive drugs produce highly characteristic leftward shifts in these functions, indicating summation or synergism between the reward provided by the electrical stimulation and the drug-induced reward (Wise 1996a; Bauco and Wise 1997). This paradigm is, therefore, useful in the search for compounds with potential antiaddictive therapeutic properties and, conversely, to screen compounds for reward-enhancing properties, which might be predictive of intrinsic addictive potential (Gardner 2005).

NGB 2904 inhibits cocaine-enhanced BSR

We have observed the effects of NGB 2904 on cocaine-induced enhancement of BSR in male Long-Evans rats (Xi et al. 2006). Rats were trained to lever press for BSR of the medial forebrain bundle at the level of the lateral hypothalamus, and tested on the rate-frequency curve-shift brain-reward paradigm. Cocaine (1–10 mg/kg, i.p.) dose-dependently shifted BSR curves to the left (i.e., decreased stimulation thresholds for brain reward), indicating summation or synergism between the reward provided by the electrical brain stimulation and the cocaine-induced reward (Bauco and Wise 1997). Pretreatment with NGB 2904 (0.1, 1 mg/kg, i.p.) significantly inhibited the enhanced BSR produced by 2 mg/kg cocaine, while the higher dose of NGB 2904 (5 mg/kg, i.p.) appeared to have no statistically significant effect on cocaine-enhanced BSR (Fig. 3A). The same dose range of NGB 2904 had no effect on the enhanced BSR produced by 10 mg/kg cocaine (Xi et al. 2006).

As noted previously, NGB 2904 (1, 5 mg/kg) significantly inhibited 10 mg/kg cocaine-induced reinstatement (∼50%). Yet NGB 2904 did not affect 10 mg/kg cocaine-enhanced BSR. Such disparate effects could be related to difference in DA response to cocaine alone or to cocaine plus electrical brain stimulation. It has been reported that there is significant attenuation of cocaine-induced increases in NAc DA in rats undergoing extinction or reinstatement testing (Neisewander et al. 1996; Mateo et al. 2005). In contrast, electrical BSR *per se* appears to enhance NAc DA (You et al. 2001). Thus, it is possible that cocaine plus

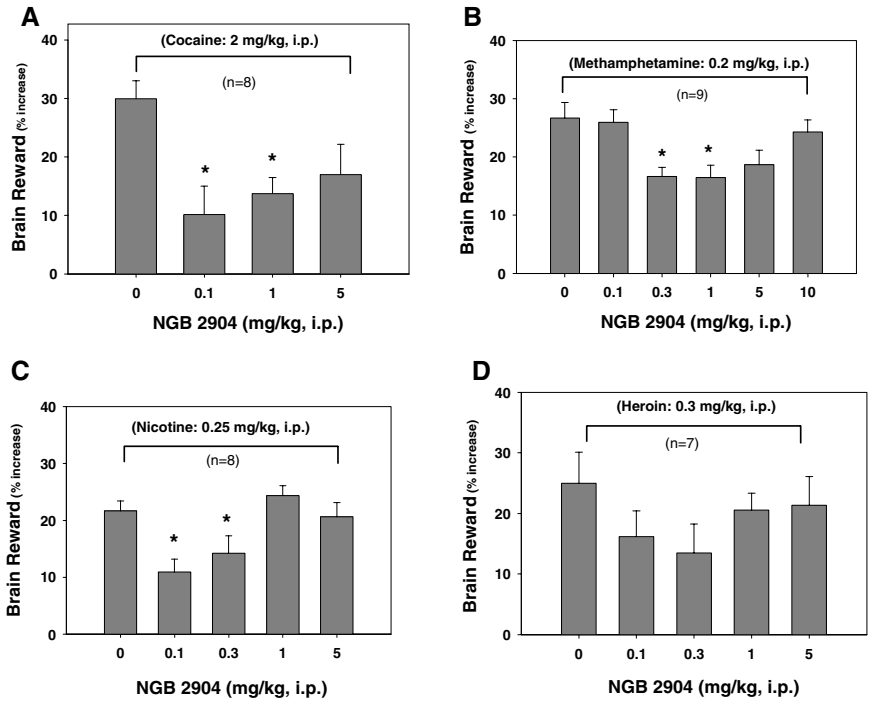


FIG. 3 Effects of NGB 2904 on brain reward-enhancing effects produced by cocaine (Panel A), methamphetamine (Panel B), nicotine (Panel C), or heroin (Panel D). $^{*}P < 0.05$, when compared with the vehicle (i.e., 0 mg/kg NGB 2904) treatment group.

electrical BSR may produce an additive or synergistic effect on extracellular DA, thereby allowing NGB 2904 to produce a significant inhibition of cocaine-triggered reinstatement, but not of cocaine-enhanced BSR.

NGB 2904 inhibits methamphetamine-enhanced BSR

To determine whether NGB 2904 produces a generalized effect on other addictive drugs, we observed the effects of NGB 2904 on methamphetamine (METH)–enhanced BSR under the same experimental conditions. METH is a more potent psychostimulant than cocaine. The strong rewarding effects of METH have been thought to be mediated by elevation of extracellular DA in the NAc, predominantly by reversal of DA transporters that mediate cytoplasmic DA release (Riddle et al. 2006). Similar to cocaine, METH (0.1–0.6 mg/kg, i.p.) produced a significant and dose-dependent enhancement of BSR in rats, manifested as a decrease in BSR threshold. Pretreatment with NGB 2904, within the dose range of 0.3–1.0 mg/kg (i.p.), significantly inhibited 0.2 mg/kg METH-enhanced BSR (Fig. 3B). However, when the NGB 2904 doses were increased to 5 or 10 mg/kg (i.p.), NGB 2904 lost its inhibitory effects on METH-enhanced BSR (Gardner et al. 2007). Thus, as against cocaine, NGB 2904 appears to lose its effect against METH outside a relatively narrow dose-effect "window." Congruently, at the same doses NGB 2904 did not inhibit BSR enhanced by high dose (0.5 mg/kg, i.p.) of METH (unpublished data).

NGB 2904 inhibits nicotine-enhanced BSR

We further examined the effects of NGB 2904 on nicotine-enhanced BSR. Systemic administration of nicotine (0.25–0.5 mg/kg, i.p.) reliably shifted BSR curves to the left in a dose-dependent manner, lowering brain-reward thresholds by 20–40%. By systemic administration, NGB 2904 (0.1–0.5 mg/kg, i.p.) significantly inhibited (by 40–50%) nicotine (0.25 mg/kg)–enhanced electrical BSR. Again, at higher doses (1–5 mg/kg, i.p.) NGB 2904 had no effect on nicotine-induced enhancement of BSR (Fig. 3C). At the same doses, NGB 2904 also failed to inhibit high-dose nicotine (0.5 mg/kg)–enhanced BSR (unpublished data). This is consistent with our previous finding with SB-277011A (Pak et al. 2006).

NGB 2904 modestly inhibits heroin-enhanced BSR

Finally, we observed the effects of the same doses of NGB 2904 on heroin-enhanced BSR. Acute administration of heroin (0.2–1.0 mg/kg, i.p.) produced a significant and dose-dependent enhancement of electrical BSR (unpublished data). Pretreatment with NGB 2904 (0.1–5 mg/kg, i.p.) produced only a modest inhibition of heroin-enhanced BSR with maximal effect occurring at 0.3 mg/kg NGB 2904 (Fig. 3D). This maximal inhibition was only marginally statistically significant ($P = 0.065$). Enlarging the sample size may allow the observed effect to become more robust. Once again, NGB 2904's effects appear to be maximal within a relatively narrow dose-effect "window" (Fig. 3D).

NGB 2904 itself has no effect on BSR

Very importantly, within the dose ranges tested, NGB 2904 has no significant effect by itself on electrical BSR. Although NGB 2904 by itself produces a slight enhancement of

brain-reward function, the effect is modest and not statistically significant (Xi et al. 2006), similar to our previous finding with SB-277011A (Vorel et al. 2002). These data suggest that selective D_3 receptor antagonism does not produce the inhibition of brain reward that has been the death knell for many previous compounds under development for treatment of addiction (O'Brien and Gardner 2005).

Together, these BSR data demonstrate that systemic administration of NGB 2904 consistently inhibited the brain reward-enhancing effects produced by cocaine, METH, nicotine, or heroin (to a lesser extent) within a relatively narrow dose range of NGB 2904. Furthermore, this effect is surmountable by increased doses of cocaine or other addictive drugs. Although the mechanisms underlying such actions remain to be further determined (see below), these findings suggest that DA D_3 receptors play an important role in mediating addictive drug-enhanced brain reward.

Effects of NGB 2904 on Cocaine-Enhanced DA in the Nucleus Accumbens

One of the most striking findings in the above BSR experiments is that NGB 2904 displays a fairly obvious "dose-effect window" of protection against addictive drug-enhanced BSR. The precise mechanisms underlying this "dose-effect window" are unclear. Given that DA D_3 receptors are distributed on both presynaptic and postsynaptic cells (see reviews by Joyce and Millan 2005; Sokoloff et al. 2006), we hypothesized that antagonism of postsynaptic D_3 receptors may block postsynaptic DA effects, thereby producing the observed putative antiaddiction effects. Alternatively, blockade of presynaptic D_3 receptors may augment DA release via a disinhibition mechanism, with the enhanced synaptic DA subsequently competing for postsynaptic D_3 binding sites and/or activating other DA receptor subtypes. To test these possibilities, we carried out *in vivo* microdialysis experiments to observe the effects of NGB 2904 (and SB-277011A) on basal and cocaine-enhanced NAc extracellular DA. We found that systemic administration of NGB 2904 or SB-277011A dose-dependently enhanced cocaine (10 mg/kg)–induced increases in NAc DA (Fig. 4). In contrast, NGB 2904 alone appeared to have no significant effect on basal levels of NAc DA (data not shown). The mechanisms underlying such differential effects of NGB 2904 on basal and cocaine-enhanced DA are unclear. The effect could be related to differential DA tone on presynaptic D_3 receptors in the absence or presence of cocaine, that is, DA tone being low in the absence of stimulation or cocaine, but increased after cocaine administration. Thus, blockade of D_3 receptors might lead to an enhancement of cocaine-induced increases in extracellular DA. Such an increase in DA produced by either NGB 2904 or higher doses of cocaine might then attenuate NGB 2904's anti-cocaine actions by competing with NGB 2904's binding to postsynaptic D_3 receptors and/or by activating other DA receptors. This DA enhancement may explain not only the specific "dose-window" effects, but also the ineffectiveness of NGB 2904 on the action produced by higher doses of cocaine or other addictive drugs as described previously. In addition, it might also explain why NGB 2904 (and also SB-277011A) selectively inhibits intravenous cocaine self-administration under PR, but not under FR, reinforcement conditions, since accumulative doses of cocaine under PR conditions are significantly lower than those under FR conditions. We should mention that, in addition to the increase in extracellular DA, preliminary data also show that NGB 2904 or SB-277011A significantly alter extracellular γ-amino butyric acid (GABA) and

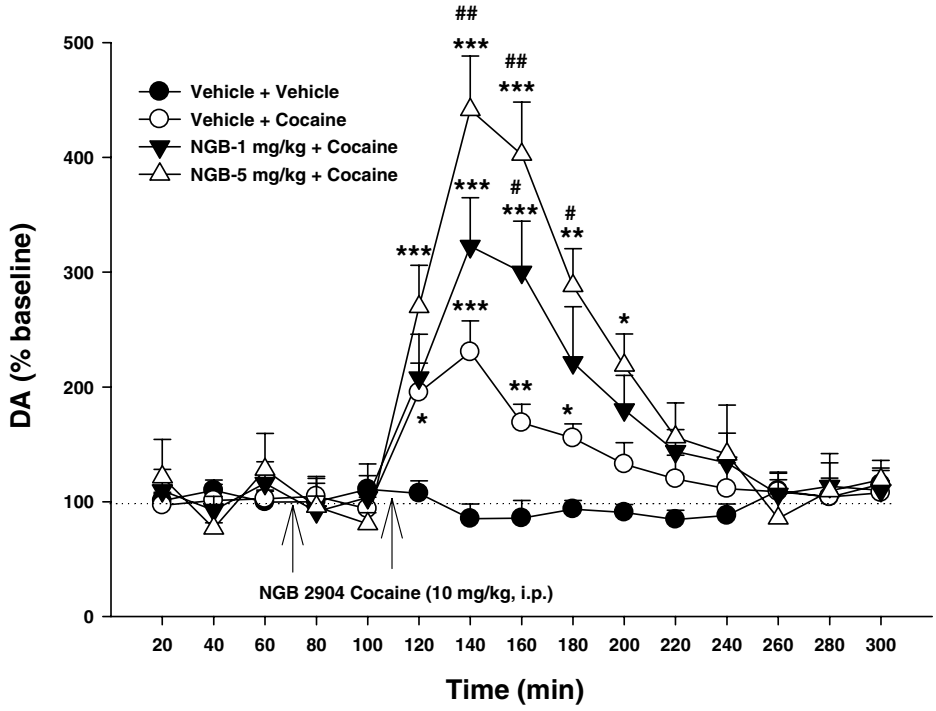


FIG. 4 Effects of NGB 2904 pretreatment on cocaine-induced increases in extracellular DA in the NAc. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, when compared with baselines before 10 mg/kg cocaine administration. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, when compared with the "Vehicle + Cocaine" treatment group.

glutamate levels in the NAc (Xi et al. unpublished data), which may also contribute to the putative therapeutic and/or drug dose effects of D_3 antagonism described previously.

D_3 Receptor-Mediated Effects of NGB 2904

Previous studies have shown that D_1- or D_2-preferring antagonists also inhibit cocaine self-administration, cocaine-enhanced BSR, and cocaine-triggered reinstatement of drug-seeking behavior (for reviews see Platt et al. 2002; Kapur and Mamo 2003; Gorelick et al. 2004). This raises the issue of whether the presently observed attenuating effects of NGB 2904 on such drug-taking or drug-seeking behaviors might be attributable to D_1 or D_2, rather than to D_3, receptor-selective antagonism. We believe this to be unlikely, because (1) multiple lines of *in vitro* evidence indicate that NGB 2904 is a highly selective D_3 receptor antagonist (Yuan et al. 1998; Robarge et al. 2001; Newman et al. 2003; Newman et al. 2005); (2) in the BSR paradigm, D_1- and D_2-preferring antagonists inhibit BSR, in a manner opposite to the brain-reward enhancement produced by addictive drugs, while the selective D_3 receptor antagonists NGB 2904 or SB-277011A do not significantly alter electrical BSR (see review by Heidbreder et al. 2005); (3) NGB 2904 does not significantly alter locomotor activity within the dose range tested (Newman et al. 2005), again unlike

D_1 or D_2 receptor antagonists. Moreover, at very low doses (26 μg–1.0 mg/kg, s.c.), NGB 2904 appears to increase spontaneous and amphetamine-stimulated locomotion in wild-type mice, but has no effect in D_3 receptor-knockout mice (Pritchard et al. 2007), suggesting that NGB 2094's effects are mediated by blocking D_3 receptors; and finally, (4) NGB 2904 inhibits D_3 receptor-mediated yawning in primates. Quinpirole is a D_3-preferring D_2/D_3 receptor agonist (Kurashima et al. 1995). Systemic administration of quinpirole produces dose-dependent yawning in rats and monkeys (Kostrzewa et al. 1993; Kurashima et al. 1995; Collins et al. 2005; Martelle et al. 2007). Collins et al. (2005) examined yawning induced by several D_2/D_3 agonists and found that the ascending limb of the quinpirole dose–response curve is mediated by activation of D_3 receptors, while the descending limb is mediated by activation of D_2 receptors. At the peak of the quinpirole dose–response curve, D_3-, but not D_2-, selective antagonists significantly reduced quinpirole-induced yawning (Collins et al. 2005). Such findings suggest that D_2/D_3 receptor agonist-induced yawning is mediated by D_3, rather than D_2, receptors. Using this *in vivo* behavioral method, Martelle et al. (2007) recently reported that NGB 2904 (3.0–5.6 mg/kg, i.m., administered 15, 30, or 120 min before quinpirole) significantly inhibited quinpirole-induced yawning in rhesus monkeys. A similar inhibitory effect was also observed with N(4-(4-(2,3–dichlorophenyl) piperazin-l-yl) butyl)-4(pyridine-2-yl) benzamide (CJB090), a novel D_3-selective partial agonist (Martelle et al. 2007). These findings lend additional support to the premise that NGB 2904 is selective for D_3 receptors *in vivo*.

SUMMARY

The effects of NGB 2904 in several animal models relating to drug addiction are summarized in Table 3. Together, these findings indicate that NGB 2904 inhibits: (1) intravenous PR cocaine self-administration; (2) cocaine- or cocaine cue-induced reinstatement of drug-seeking behaviors; and (3) cocaine-, METH, nicotine-, or heroin-enhanced BSR. Given that NGB 2904 is a highly selective D_3 receptor antagonist, as assessed both *in vitro* and *in vivo*, we believe that NGB 2904's protective effects against cocaine's addictive actions in the above preclinical models are most likely mediated by selective antagonism of D_3 receptors. Furthermore, NGB 2904 neither produces a dysphorigenic rightward shift in the BSR curve nor substitutes for cocaine in maintenance of self-administration behavior, suggesting that NGB 2904 itself has no addictive liability. Since the PR self-administration and reinstatement of drug-seeking paradigms presumably measure subjects' motivations for drug-taking or drug-seeking behavior after abstinence, the efficacy of NGB 2904 against PR cocaine self-administration and against cocaine- or cocaine cue-induced reinstatement suggests a clinical therapeutic potential for NGB 2904 or other D_3 receptor antagonists in the treatment of cocaine addiction, perhaps specifically for relapse to drug-seeking behavior. Finally, NGB 2904 also displays an obvious "dose-effect window" of effectiveness against acute cocaine or other addictive drugs at relatively low doses, and a general ineffectiveness against high doses of cocaine or other addictive drugs as assessed by BSR or FR self-administration. These data suggest that NGB 2904 or other D_3 antagonists may have limited potential against acute drug-induced reward. On the basis of *in vivo* brain microdialysis findings, a DA enhancement hypothesis is proposed to explain such dose-specific effects (Fig. 5). Further research to develop more effective D_3 receptor antagonists as potential

TABLE 3. Effects of NGB 2904 in experimental animals

Behavioral paradigm	Doses	Main finding	Reference
Locomotor behavior in rats	0.1–5 mg/kg i.p.	No effect	Xi et al. unpublished
Locomotor behavior in mice	≤1.0 mg/kg, s.c.	Increase	Pritchard et al. 2007
	>100 mg/kg i.p.	Inhibition	Newman et al. 2005
Brain stimulation reward (BSR) itself	0.1–5 mg/kg, i.p.	No effect	Xi et al. 2006
Cocaine-enhanced BSR in rats	0.1–1.0 mg/kg i.p.	Inhibition	Xi et al. 2006
Methamphetamine-enhanced BSR in rats	0.3–1.0 mg/kg i.p.	Inhibition	Gardner et al. 2007
Nicotine-enhanced BSR in rats	0.1–0.5 mg/kg i.p.	Inhibition	Xi et al. unpublished
Heroin-enhanced BSR in rats	0.3–5 mg/kg i.p.	Marginal inhibition	Xi et al. unpublished
Cocaine self-administration (FR-2) in rats	0.1–10 mg/kg i.p.	No effect	Xi et al. 2006
Cocaine self-administration (second-order) in monkeys	1–5 mg/kg i.m.	No effect	Martelle et al. 2007
Cocaine self-administration (PR) in rats	0.1–5 mg/kg i.p.	Inhibition	Xi et al. 2006
Cocaine discrimination in monkeys	1–3 mg/kg i.m	No effect	Martelle et al. 2007
Cocaine-triggered relapse to cocaine seeking in rats	0.1–5 mg/kg i.p.	Inhibition	Xi et al. 2006
Cue-triggered relapse to cocaine seeking in rats	0.1–5 mg/kg i.p.	Inhibition	Gilbert et al. 2005
Sucrose-triggered relapse to sucrose seeking in rats	1–5 mg/kg, i.p.	No effect	Xi et al. 2006
Quinpirole-induced yawning in monkeys	3–5.6 mg/kg i.p.	Inhibition	Martelle et al. 2007
Cocaine-enhanced NAc DA in rats	1–5 mg/kg i.p.	Increase	Xi et al. unpublished

therapeutic agents for the treatment of drug addiction appears to be both warranted and promising.

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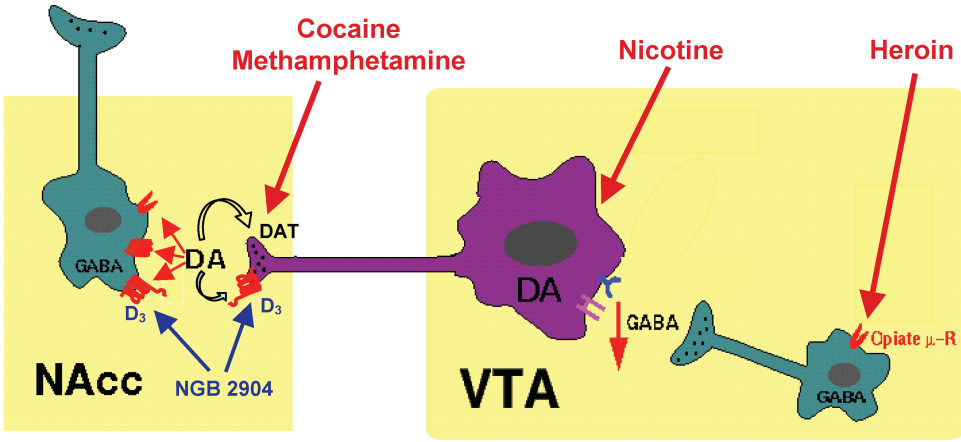

FIG. 5 Dopamine (DA) hypothesis of drug reward and NGB 2904's actions. The mesolimbic DA system originates from the ventral tegmental area (VTA) in the midbrain and projects predominantly to the nucleus accumbens (NAc) in the forebrain. Almost all addictive drugs activate VTA DA neurons and/or increase extracellular DA levels in the NAc via distinct receptor and cellular mechanisms. DA D3 receptors are located on both presynaptic terminals and postsynaptic (GABAergic) neurons. Antagonism of postsynaptic D_3 receptors by NGB 2904 may block D_3 receptor-mediated addictive effects, while blockade of presynaptic D_3 receptors by NGB 2904 may augment cocaine-enhanced NAc DA (via a disinhibition mechanism), which may subsequently attenuate NGB 2904's therapeutic actions by inhibiting NGB 2904's binding to postsynaptic D_3 receptors and/or by activating other DA receptors.

National Institute on Drug Abuse, according to the methods described in Yuan et al. 1998. We thank Dr. Amy Hauck Newman for supplying compounds and for constructive criticisms regarding this manuscript.

REFERENCES

Austin NE, Baldwin SJ, Cutler L, Deeks N, Kelly PJ, Nash M, Shardlow CE, Stemp G, Thewlis K, Ayrton A, et al. (2001) Pharmacokinetics of the novel, high affinity and selective dopamine D_3 receptor antagonist SB-277011 in rat, dog and monkey: *In vitro/in vivo* correlation and the role of aldehyde oxidase. *Xenobiotica 31:*677-686.

Bardo MT (1998) Neuropharmacological mechanisms of drug reward: Beyond dopamine in the nucleus accumbens. *Crit Rev Neurobiol 12:*37-67.

Bauco P, Wise RA (1997) Synergistic effects of cocaine with lateral hypothalamic brain stimulation reward: Lack of tolerance or sensitization. *J Pharmacol Exp Ther 283:*1160-1167.

Boeckler F, Gmeiner P (2006) The structural evolution of dopamine D_3 receptor ligands: Structure-activity relationships and selected neuropharmacological aspects. *Pharmacol Ther 112:*281-333.

Bressan RA, Crippa JA (2005) The role of dopamine in reward and pleasure behaviour—review of data from preclinical research. *Acta Psychiatr Scand 111*(suppl 437)*427*:14-21.

Caine SB, Koob GF (1993) Modulation of cocaine self-administration in the rat through D-3 dopamine receptors. *Science 260:*1814-1816.

Campiani G, Butini S, Trotta F, Fattorusso C, Catalanotti B, Aiello F, Gemma S, Nacci V, Novellino E, Stark JA, et al. (2003) Synthesis and pharmacological evaluation of potent and highly selective D_3 receptor ligands: Inihibition of cocaine-seeking behavior and the role of dopamine D_3/D_2 receptors. *J Med Chem 46:*3822-3839.

Collins GT, Witkin JM, Newman AH, Svensson KA, Grundt P, Cao J, Woods JH (2005) Dopamine agonist-induced yawning in rats: A dopamine D_3 receptor-mediated behavior. *J Pharmacol Exp Ther 314:*310-319.

Di Ciano P, Underwood RJ, Hagan JJ, Everitt BJ (2003) Attenuation of cue-controlled cocaine-seeking by a selective D_3 dopamine receptor antagonist SB-277011-A. *Neuropsychopharmacology 28:*329-338.

Diaz J, Pilon C, Le Foll B, Gros C, Triller A, Schwartz J-C, Sokoloff P (2000) Dopamine D_3 receptors expressed by all mesencephalic dopamine neurons. *J Neurosci 20:*8677-8684.

Drago J, Padungchaichot P, Accili D, Fuchs S (1998) Dopamine receptors and dopamine transporter in brain function and addictive behaviors: Insights from targeted mouse mutants. *Dev Neurosci 20:*188-203.

Duarte C, Lefebvre C, Chaperon F, Hamon M, Thiebot M-H (2003) Effects of a dopamine D_3 receptor ligand, BP897, on acquisition and expression of food-, morphine and cocaine-induced conditioned place preference, and food-seeking behavior in rats. *Neuropsychopharmacology 28:*1903-1915.

Duaux E, Gorwood P, Griffon N, Bourdel M-C, Sautel F, Sokoloff P, Schwartz J-C, Ades J, Lôo H, Poirier M-F (1998) Homozygosity at the dopamine D_3 receptor gene is associated with opiate dependence. *Mol Psychiatry 3:*333-336.

Gál K, Gyertyán I (2003) Targeting the dopamine D(3) receptor cannot influence continuous reinforcement cocaine self-administration in rats. *Brain Res Bull 6:*595-601.

Garcia-Ladona FJ, Cox BF (2003) BP 897, a selective dopamine D_3 receptor ligand with therapeutic potential for the treatment of cocaine-addiction. *CNS Drug Rev 9:*141-158.

Gardner EL (1999) The neurobiology and genetics of addiction: Implications of "reward deficiency syndrome" for therapeutic strategies in chemical dependency. In Elster J (Ed), *Addiction: Entries and Exits.* New York: Russell Sage Foundation, pp 57-119.

Gardner EL (2000) What we have learned about addiction from animal models of drug self-administration. *Am J Addict 9:*285-313.

Gardner EL (2005) Brain reward mechanisms. In Lowinson JH, Ruiz P, Millman RB, Langrod JG (Eds), *Substance Abuse: A Comprehensive Textbook*, 4th ed. Philadelphia: Lippincott Williams & Wilkins, pp 48-97.

Gardner EL, Spiller K, Xi ZX, Peng XQ, Li X, Dillon C, Ashby CR Jr, Heidbreder CA (2007) The putative dopamine D_3 receptor antagonists SB-277011A, NGB2904, S33138 or BP897 attenuate methamphetamine-enhanced brain stimulation reward in rats. College on Problems of Drug Dependence, Abstract.

Gilbert JG, Newman AH, Gardner EL, Ashby CR Jr, Heidbreder CA, Pak AC, Peng X-Q, Xi Z-X (2005) Acute administration of SB-277011A, NGB 2904, or BP 897 inhibits cocaine cue-induced reinstatement of drug-seeking behavior in rats: Role of dopamine D_3 receptors. *Synapse 57:*17-28.

Girault J-A, Greengard P (2004) The neurobiology of dopamine signaling. *Arch Neurol 61:*641-644.

Gorelick DA, Gardner EL, Xi Z-X (2004) Agents in development for the management of cocaine abuse. *Drugs 64:*1547-1573.

Grundt P, Carlson EE, Cao J, Bennett CJ, McElveen E, Taylor M, Luedtke RR, Newman AH (2005) Novel heterocyclic trans olefin analogues of *N*-{4-[4-(2,3-dichlorophenyl)piperazin-1-yl]butyl}arylcarboxamides as selective probes with high affinity for the dopamine D_3 receptor. *J Med Chem 48:*839-848.

Gyertyán I, Gál K (2003) Dopamine D_3 receptor ligands show place conditioning effect but do not influence cocaine-induced place preference. *Neuroreport 14:*93-98.

Haney M, Ward AS, Foltin RW, Fischman MW (2001) Effects of ecopipam, a selective dopamine D1 antagonist, on smoked cocaine self-administration by humans. *Psychopharmacology 155:*330-337.

Heidbreder CA, Gardner EL, Xi Z-X, Thanos PK, Mugnaini M, Hagan JJ, Ashby CR Jr. (2005) The role of central dopamine D_3 receptors in drug addiction: A review of pharmacological evidence. *Brain Res Brain Res Rev 49:*77-105.

Joyce JN, Millan MJ (2005) Dopamine D_3 receptor antagonists as therapeutic agents. *Drug Discov Today 10:*917-925.

Kalivas PW, Volkow ND (2005) The neural basis of addiction: A pathology of motivation and choice. *Am J Psychiatry 162:*1403-1413.

Kapur S, Mamo D (2003) Half a century of antipsychotics and still a central role for dopamine D_2 receptors. *Prog Neuropsychopharmacol Biol Psychiatry 27:*1081-1090.

Kornetsky C (2004) Brain-stimulation reward, morphine-induced oral stereotypy, and sensitization: Implications for abuse. *Neurosci Biobehav Rev 27:*777-786.

Kostrzewa RM, Brus R, Rykaczewska M, Plech A (1993) Low-dose quinpirole ontogenically sensitizes to quinpirole-induced yawning in rats. *Pharmacol Biochem Behav 44:*487-489.

Kuhar MJ, Pilotte NS (1996) Neurochemical changes in cocaine withdrawal. *Trends Pharmacol Sci 17:*260-264.

Kurashima M, Yamada K, Nagashima M, Shirakawa K, Furukawa T (1995) Effects of putative dopamine D_3

receptor agonists, 7-OH-DPAT, and quinpirole, on yawning, stereotypy, and body temperature in rats. *Pharmacol Biochem Behav 52:*503-508.

Le Foll B, Goldberg SR, Sokoloff P (2005) The dopamine D_3 receptor and drug dependence: Effects on reward or beyond? *Neuropharmacology 49:*525-541.

Levant B (1997) The D_3 dopamine receptor: Neurobiology and potential clinical relevance. *Pharmacol Rev 49:*231-252.

Martelle JL, Claytor R, Ross JT, Reboussin BA, Newman AH, Nader MA (2007) Effects of two novel D_3-selective compounds, NGB 2904 and CJB 090, on the reinforcing and discriminative stimulus effects of cocaine in rhesus monkeys. *J Pharmacol Exp Ther* [Epub ahead of print].

Mateo Y, Lack CM, Morgan D, Roberts DCS, Jones SR (2005) Reduced dopamine terminal function and insensitivity to cocaine following cocaine binge self-administration and deprivation. *Neuropsychopharmacology 30:*1455-1463.

Narita M, Mizuo K, Mizoguchi H, Sakata M, Narita M, Tseng LF, Suzuki T (2003) Molecular evidence for the functional role of dopamine D_3 receptor in the morphine-induced rewarding effect and hyperlocomotion. *J Neurosci 23:*1006-1012.

Neisewander JL, Fuchs RA, Tran-Nguyen LTL, Weber SM, Coffey GP, Joyce JN (2004) Increases in dopamine D_3 receptor binding in rats receiving a cocaine challenge at various time points after cocaine self-administration: Implications for cocaine-seeking behavior. *Neuropsychopharmacology 29:*1479-1487.

Neisewander JL, O'Dell LE, Tran-Nguyen LTL, Castañeda E, Fuchs RA (1996) Dopamine overflow in the nucleus accumbens during extinction and reinstatement of cocaine self-administration behavior. *Neuropsychopharmacology 15:*506-514.

Newman AH, Cao J, Bennett CJ, Robarge MJ, Freeman RA, Luedtke RR (2003) *N*-{4-[4-(2,3-dichlorophenyl)piperazin-1-yl]butyl, butenyl and butynyl}arylcarboxamides as novel dopamine D_3 receptor antagonists. *Bioorg Med Chem Lett 13:*2179-2183.

Newman AH, Grundt P, Nader MA (2005) Dopamine D_3 receptor partial agonists and antagonists as potential drug abuse therapeutic agents. *J Med Chem 48:*3663-3679.

Newton TF, Ling W, Kalechstein AD, Uslaner J, Tervo K (2001) Risperidone pre-treatment reduces the euphoric effects of experimentally administered cocaine. *Psychiatry Res 102:*227-233.

O'Brien CP (2003) Research advances in the understanding and treatment of addiction. *Am J Addict 12*(Suppl 2):S36-S47.

O'Brien CP, Gardner EL (2005) Critical assessment of how to study addiction and its treatment: Human and non-human animal models. *Pharmacol Ther 108:*18-58.

Pak AC, Ashby CR Jr, Heidbreder CA, Pilla M, Gilbert J, Xi Z-X, Gardner EL (2006) The selective dopamine D_3 receptor antagonist SB-277011A reduces nicotine-enhanced brain reward and nicotine-paired environmental cue functions. *Int J Neuropsychopharmacol 9:*585-602.

Pierce RC, Kumaresan V (2006) The mesolimbic dopamine system: The final common pathway for the reinforcing effect of drugs of abuse? *Neurosci Biobehav Rev 30:*215-238.

Pilla M, Perachon S, Sautel F, Garrido F, Mann A, Wermuth CG, Schwartz J-C, Everitt BJ, Sokoloff P (1999) Selective inhibition of cocaine-seeking behaviour by a partial dopamine D_3 receptor agonist. *Nature 400:*371-375.

Platt DM, Rowlett JK, Spealman RD (2002) Behavioral effects of cocaine and dopaminergic strategies for preclinical medication development. *Psychopharmacology 163:*265-282.

Pritchard LM, Newman AH, McNamara RK, Logue AD, Taylor B, Welge JA, Xu M, Zhang J, Richtand NM (2007) The dopamine D_3 receptor antagonist NGB 2904 increases spontaneous and amphetamine-stimulated locomotion. *Pharmacol. Biochem. Behav*. in press.

Reavill C, Taylor SG, Wood MD, Ashmeade T, Austin NE, Avenell KY, Boyfield I, Branch CL, Cilia J, Coldwell MC, et al. (2000) Pharmacological actions of a novel, high-affinity, and selective human dopamine D_3 receptor antagonist, SB-277011-A. *J Pharmacol Exp Ther 294:*1154-1165.

Remington G, Kapur S (2001) SB-277011 GlaxoSmithKline. *Curr Opin Investig Drugs 2:*946-949.

Richardson NR, Roberts DCS (1996) Progressive ratio schedules in drug self-administration studies in rats: A method to evaluate reinforcing efficacy. *J Neurosci Methods 66:*1-11.

Riddle EL, Fleckenstein AE, Hanson GR (2006) Mechanisms of methamphetamine-induced dopaminergic neurotoxicity. *AAPS J 8:*E413-E418.

Robarge MJ, Husbands SM, Kieltyka A, Brodbeck R, Thurkauf A, Newman AH (2001) Design and synthesis of [(2,3-dichlorophenyl)piperazin-1-yl]alkylfluorenylcarboxamides as novel ligands selective for the dopamine D_3 receptor subtype. *J Med Chem 44:*3175-3186.

Rothman RB, Glowa JR. (1995) A review of the effects of dopaminergic agents on humans, animals, and drug-seeking behavior, and its implications for medication development. Focus on GBR 12909. *Mol Neurobiol 11:*1-19.

Rowlett JK (2000) A labor-supply analysis of cocaine self-administration under progressive-ratio schedules: Antecedents, methodologies, and perspectives. *Psychopharmacology 153:*1-16.

Shalev U, Grimm JW, Shaham Y (2002) Neurobiology of relapse to heroin and cocaine seeking: A review. *Pharmacol Rev 54:*1-42.

Sokoloff P, Diaz J, Le Foll B, Guillin O, Leriche L, Bezard E, Gross C (2006) The dopamine D_3 receptor: A therapeutic target for the treatment of neuropsychiatric disorders. *CNS Neurol Disord Drug Targets 5:*25-43.

Sokoloff P, Le Foll B, Perachon S, Bordet R, Ridray S, Schwartz J-C (2001) The dopamine D_3 receptor and drug addiction. *Neurotox Res 3:*433-441.

Stafford D, LeSage MG, Glowa JR (1998) Progressive-ratio schedules of drug delivery in the analysis of drug self-administration: A review. *Psychopharmacology 139:*169-184.

Staley JK, Mash DC (1996) Adaptive increase in D_3 dopamine receptors in the brain reward circuits of human cocaine fatalities. *J Neurosci 16:*6100-6106.

Stanwood GD, Artymyshyn RP, Kung M-P, Kung HF, Lucki I, McGonigle P (2000) Quantitative autoradiographic mapping of rat brain dopamine D_3 binding with [^{125}I]7-OH-PIPAT: Evidence for the presence of D_3 receptors on dopaminergic and nondopaminergic cell bodies and terminals. *J Pharmacol Exp Ther 295:*1223-1231.

Stemp G, Ashmeade T, Branch CL, Hadley MS, Hunter AJ, Johnson CN, Nash DJ, Thewlis KM, Vong AKK, Austin NE, et al. (2000) Design and synthesis of *trans-N*-[4-[2-(6-cyano-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl]cyclohexyl]-4-quinolinecarboxamide (SB-277011): A potent and selective dopamine D_3 receptor antagonist with high oral bioavailability and CNS penetration in the rat. *J Med Chem 43:*1878-1885.

Volkow ND, Fowler JS, Wang G-J (1999) Imaging studies on the role of dopamine in cocaine reinforcement and addiction in humans. *J Psychopharmacol 13:*337-345.

Volkow ND, Wang G-J, Telang F, Fowler JS, Logan J, Childress A-R, Jayne M, Ma Y, Wong C (2006) Cocaine cues and dopamine in dorsal striatum: Mechanism of craving in cocaine addiction. *J Neurosci 26:*6583-6588.

Vorel SR, Ashby CR Jr, Paul M, Liu X, Hayes R, Hagan JJ, Middlemiss DN, Stemp G, Gardner EL (2002) Dopamine D_3 receptor antagonism inhibits cocaine-seeking and cocaine-enhanced brain reward in rats. *J Neurosci 22:*9595-9603.

Wicke K, Garcia-Ladona J (2001) The dopamine D_3 receptor partial agonist, BP-897, is an antagonist at human dopamine D_3 receptors and at rat somatodendritic dopamine D_3 receptors. *Eur J Pharmacol 424:*85-90.

Wise RA (1996a) Addictive drugs and brain stimulation reward. *Annu Rev Neurosci 19:*319-340.

Wise RA (1996b) Neurobiology of addiction. *Curr Opin Neurobiol 6:*243-251.

Wise RA (2005) Forebrain substrates of reward and motivation. *J Comp Neurol 493:*115-121.

Wise RA (2006) Role of brain dopamine in food reward and reinforcement. *Phil Trans R Soc Lond B Biol Sci 361:*1149-1158.

Wood MD, Boyfield I, Nash DJ, Jewitt FR, Avenell KY, Riley G (2000) Evidence for antagonist activity of the human dopamine D_3 receptor partial agonist, BP 897, at human dopamine D_3 receptor. *Eur J Pharmacol 407:*47-51.

Xi Z-X, Gilbert J, Campos AC, Kline N, Ashby CR Jr, Hagan JJ, Heidbreder CA, Gardner EL (2004) Blockade of mesolimbic dopamine D_3 receptors inhibits stress-induced reinstatement of cocaine-seeking in rats. *Psychopharmacology 176:*57-65.

Xi Z-X, Gilbert JG, Pak AC, Ashby CR Jr, Heidbreder CA, Gardner EL (2005) Selective dopamine D_3 receptor antagonism by SB-277011A attenuates cocaine reinforcement as assessed by progressive-ratio and variable-cost–variable-payoff fixed-ratio cocaine self-administration in rats. *Eur J Neurosci 21:*3427-3438.

Xi Z-X, Newman AH, Gilbert JG, Pak AC, Peng X-Q, Ashby CR Jr, Gitajn L, Gardner EL (2006) The novel dopamine D_3 receptor antagonist NGB 2904 inhibits cocaine's rewarding effects and cocaine-induced reinstatement of drug-seeking behavior in rats. *Neuropsychopharmacology 31:*1393-1405.

You Z-B, Chen Y-Q, Wise RA (2001) Dopamine and glutamate release in the nucleus accumbens and ventral tegmental area of rat following lateral hypothalamic self-stimulation. *Neuroscience 107:*629-639.

Yuan J, Chen X, Brodbeck R, Primus R, Braun J, Wasley JWF, Thurkauf A (1998) NGB 2904 and NGB 2849: Two highly selective dopamine D3 receptor antagonists. *Bioorg Med Chem Lett* 8:2715-2718.