Sensitization to the Rewarding Effects of the Specific Dopamine Uptake Inhibitor GBR12783

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ABSTRACT

The conditioned place preference (CPP) induced by increasing doses (1.25–40 mg/kg) of cocaine or the specific dopamine uptake inhibitor GBR12783 was investigated in rats previously treated with cocaine (10 or 20 mg/kg), GBR12783 (10 mg/kg) or morphine (10 mg/kg) for 15 days. In solvent-pretreated rats, cocaine- and GBR12783-induced CPPs were biphasic, with the highest scores observed at 20 mg/kg. Prior exposure to GBR12783 sensitized the rats to the rewarding effects of low doses of either GBR12783 or cocaine. Pretreatment with cocaine 20 mg/kg, but not 10 mg/kg, sensitized the rats to its own rewarding effects. Furthermore, it was less efficient than GBR12783 in sensitizing the animals to the rewarding effects of both drugs. These data confirm the major role of dopamine uptake inhibition in the sensitization process. On the other hand, the magnitude of CPP induced by a high dose of both drugs (20 mg/kg) was decreased after pretreatment with either GBR12783 or cocaine, reaching the lower scores observed at 40 mg/kg. This decrease was unrelated to altered anxiety level but was associated with sensitization to stereotypies. Morphine pretreatment modified neither the CPP induced by high doses of cocaine or GBR12783 nor cocaine- or GBR12783-induced stereotypies. However, prior exposure to morphine sensitized the rats to the rewarding effects of cocaine (2.5 mg/kg) but not to those of GBR12783, suggesting that other mechanisms working in concert with dopamine may facilitate the rewarding effect of cocaine without affecting that of GBR12783.

Drugs of abuse such as cocaine, morphine and amphetamine share several behavioral and rewarding properties (Kalivas and Stewart, 1991; Koob, 1992; Robinson and Ber- ridge, 1993). Repeated exposures to these drugs produce a locomotor sensitization that results in a potentiated response to a challenge dose of the drug (Post and Rose, 1976; Robin- son and Becker, 1986; Kalivas and Duffy, 1987; Kalivas and Stewart, 1991). Cross-sensitization between the locomotor-activating effects of cocaine, morphine or amphetamine has also been reported (Vezina and Stewart, 1990; Kalivas and Stewart, 1991; Cunningham et al., 1997).

Until recently, sensitization and cross-sensitization to the rewarding effects of these drugs have received less attention. This issue deserves further investigation in view of the pos- tulated role of the sensitization process in the development of addiction (Robinson and Berridge, 1993). Few results have yet been reported. The acquisition of cocaine or amphetamine self-administration is facilitated after pre-exposure to these drugs (Horger et al., 1990; Piazza et al., 1990). Furthermore, amphetamine has been reported to sensitize rats to the rewarding effects of cocaine (Horger et al., 1992). Morphine potentiates the action of amphetamine on brain stimulation reward (Bespalov and Zvartau, 1995) or on the conditioned reinforcement paradigm (Cunningham and Kelley, 1992). Repeated exposure to amphetamine, morphine or cocaine was found to enhance the drug-induced rewarding effects as measured by CPP. Cross-sensitization between morphine and amphetamine was obtained, and an increased sensitivity to cocaine was also observed after chronic treatment with mor- phine or amphetamine (Lett, 1989; Shippenberg and Heid- breder, 1995; Shippenberg et al., 1996). In this last proce- dure, the drug is paired with distinctive environmental stimuli, and a preference for this drug-paired environment in a drug-free state is considered to be a measure of reward (Hoffman, 1989).

Although the neural substrates underlying behavioral sen- sitization to these drugs remain unclear, they cause similar changes in the mesolimbic dopaminergic system, arising from the VTA and projecting to the ventral striatum. In fact, acutely, they increase extracellular DA levels preferentially within the ventral striatum (Di Chiara and Imperato, 1988). Altered neurochemical and electrophysiological responsive- ness of this system has been reported after repeated exposure to cocaine, morphine or amphetamine. Indeed, these drugs produce greater levels of extracellular DA in the ventral

**ABBREVIATIONS:** CPP, conditioned place preference; VTA, ventral tegmental area; DA, dopamine; NE, norepinephrine; 5-HT, serotonin; GBR12783, 1-[2-(diphenylmethoxy)ethyl]-4-(3-phenyl-2-(propenyl)-piperazine; DMSO, dimethyl sulfoxide; ANOVA, analysis of variance.
striatum in response to a challenge injection after their repeated administration (see reviews: Kalivas and Stewart, 1991; Robinson and Berridge, 1993).

Cocaine prevents the removal of DA, NE and 5-HT from the synaptic cleft, thus potentiating monoaminergic neurotransmissions. The activating and reinforcing effects of cocaine have been linked to the inhibition of neuronal DA uptake (Reith et al., 1986; Ritz et al., 1987; Kalivas and Stewart, 1991; Woolverton and Johnson, 1992). However, at doses that block DA uptake, cocaine also inhibits reuptake of NE and 5-HT (Hadfield and Nugent, 1983; Reith et al., 1986). Therefore, these monoaminergic systems could modulate any of cocaine’s behavioral effects. In contrast to cocaine, GBR12783, a piperazine derivative initially described by Van Der Zee et al. (1980), is a highly selective and potent DA uptake inhibitor, devoid of DA-releasing effect (Bonnet and Costentin, 1986; Vaugeois et al., 1992). Like cocaine, GBR12783 increases locomotor activity, and its repeated administration induces a sensitization to its locomotor effects (Duterte-Boucher et al., 1990; Boulay et al., 1996). Moreover, GBR12783 produces CPP (Le Pen et al., 1996).

The aim of the present study was to assess the effect of a cocaine pre-exposure on the CPP induced by a large dose range of cocaine and to evaluate the specific role of DA uptake inhibition in sensitization to the rewarding effects. For this purpose, we have studied the effect of GBR12783 on the CPP induced by GBR12783. Furthermore, we have investigated whether a cross-sensitization may occur between cocaine and GBR12783. In order to further test the hypothesis of a common substrate in drug abuse, we have studied the influence of morphine pre-exposure on cocaine- and GBR12783-induced CPP.

Materials and Methods

Animals and treatments. Male Sprague-Dawley rats (200 g at the beginning of the experiment) purchased from Charles River (St. Aubin lès Elbeuf, France) were maintained on a 12-hr light/dark cycle (light on between 7:00 A.M. and 7:00 P.M.) at constant temperature with laboratory chow and water ad libitum. The experiments were carried out between 9:00 A.M. and 5:00 P.M. For chronic treatments, animals were injected once daily for 15 days. They were treated with cocaine (10 or 20 mg/kg), morphine (10 mg/kg), GBR12783 (10 mg/kg) or their solvents (saline or 5% DMSO in distilled water). Drug solutions were freshly prepared. Cocaine hydrochloride and morphine hydrochloride were obtained from “la Coopération Pharmaceutique Française” (Melun, France); they were dissolved in saline. GBR12783 [1-(2-diethylaminoethyl)-4-(3-phenyl-2-(propenyl)-piperazine)] was synthesized in the laboratory of Pr. Robba (Caen, France); GBR12783 was solubilized in DMSO and diluted in distilled water (final concentration of DMSO, 5%). All drugs were injected i.p. in a volume of 5 ml/kg. Doses always refer to the free bases.

Conditioned place preference. The apparatus consisted of an enclosure (L = 80 cm, W = 25 cm and H = 35 cm) divided into two main compartments (L = 32 cm, W = 25 cm) separated by a small, “neutral” compartment (15 × 15 cm). Two openings to the two main compartments (12 × 16 cm) could be occluded by sliding doors. The neutral compartment had grey floor and walls. One of the main compartments had a black floor, the wall in front of the door was black and the others were white. The other compartment had a black floor and walls that were stripped vertically black and white. The experiment consisted of three distinct phases: preconditioning, conditioning and postconditioning.

One day after the end of chronic treatments, the preconditioning phase was carried out over 3 consecutive days. Rats were placed in the neutral compartment, and the sliding doors were removed to allow free access to the entire apparatus for 15 min. On the third day, the amount of time spent in each compartment was monitored by an image analysis system (see below) to assess unconditioned preference. Rats showing a strong unconditioned preference (>700 sec for the neutral compartment or >500 sec for one of the main compartments and <100 sec for the other) were excluded. None to four rats per experiment (~60 rats) were excluded for this reason.

Place preference conditioning began 2 days later and was conducted by using an unbiased procedure. Subjects were assigned to a treatment group to receive either cocaine or GBR12783 (1.25–40 mg/kg). For this purpose, rats were counterbalanced according to their initial preferences, so that in each treatment group, half of the rats received the drug in the preferred compartment and half in the least-preferred one. Immediately after drug injection, the animals were confined to the appropriate compartment for 30 min. On the next day, they were injected with vehicle and confined to the other compartment. Each rat received two drug pairings (on days 1 and 3) and two vehicle pairings (on days 2 and 4).

The postconditioning test was conducted 1 day after the last conditioning session. Subjects were allowed free access to the apparatus for 15 min, and the amount of time spent in each compartment was monitored.

Elevated plus-maze test. In rats previously tested for CPP with the lowest dose of either cocaine or GBR12783, the anxiety level was evaluated with the elevated plus maze 14 days after cessation of chronic treatment with either cocaine or GBR12783. The apparatus consisted of a wooden Greek cross placed 52 cm above the floor. The four arms were 50 cm long and 9.5 cm wide. Two opposite arms were surrounded by walls (30 cm high) (closed arms), whereas the other two were devoid of enclosing walls (open arms). The whole device was painted black and the room was brightly illuminated. Thirty minutes after a challenge injection of GBR12783 (20 mg/kg), cocaine (20 mg/kg) or solvent, the rat was placed at the center of this maze, with its head facing a closed arm. The number of entries, the time spent and the distance traveled in open and closed arms were recorded for a 5-min test period by using an image analysis system (described later). The values obtained were converted into percentages of data on open arms relative to the total data on both open and closed arms. The plus maze was wiped clean after each animal testing.

Quantification of stereotyped behavior. GBR12783- and cocaine-induced stereotypies were evaluated in rats chronically treated with either cocaine (20 mg/kg), GBR12783 (10 mg/kg) or morphine (10 mg/kg). Six days after the end of chronic treatments, a period that corresponded to the first conditioning trial during CPP, animals received a challenge injection of cocaine (20 mg/kg) or GBR12783 (20 mg/kg). Immediately after the challenge injection, rats were introduced into individual cages (L = 20 cm, W = 16 cm and H = 25 cm), and drug-induced stereotyped behavior (e.g., sniffing, head and limb movements) were rated. Furthermore, stereotyped behaviors (sniffing, head and limb movements and biting/gnawing) induced by an acute injection of the highest dose (40 mg/kg) of GBR12783 or cocaine was measured in two additional groups of solvent-pretreated rats. The following 5-point rating scale, according to Paulson et al. (1991), was used: (0) normal activity; (1) mild, discontinuous stereotyped behavior; (2) moderate, discontinuous stereotyped behavior; (3) moderate, continuous stereotyped behavior and (4) intense, continuous stereotyped behavior directed at one place.

Image analysis system. The image analysis system (VideoTrack 512, Viewpoint, Lyon, France) consisted of video cameras positioned above the apparatus, a video interface and a microcomputer. It converted the video input signals into binary images so that each animal corresponded to a white spot against a black background. During experimentation, the movements of these spots were recorded and translated into the time (sec) spent in each compartment by the center of gravity of a spot. Virtual windows on a computer
screen corresponded to different areas of the experimental apparatus (three compartments for the CPP test; the center area and the two arms for the elevated plus maze).

Statistical analysis. In the CPP test, the scores (means ± S.E.M.) are expressed as the change in time spent in the drug-paired compartment, before and after conditioning. A paired Student’s t test was used to determine whether an individual dose produced significant place conditioning. A significantly greater amount of time spent on the drug-paired side on the postconditioning test compared with the preconditioning phase was defined as a CPP. CPP data were analyzed by a two-way ANOVA, with pretreatment and conditioning dose as main factors. One-way ANOVA followed by Fisher’s least significant difference test were subsequently performed to study dose-response curves for each pre-exposure condition. For each CPP conditioning dose, a one-way ANOVA followed by Dunnett’s test was used to determine the effects of chronic treatments on drug-induced CPP in solvent-pretreated animals.

In the elevated plus-maze test, means ± S.E.M. of data were compared by a two-way ANOVA, with pretreatment and treatment as factors. A Fisher’s least significant difference test was used for multiple comparisons.

Because stereotyped behavior rankings are measured on an ordinal scale only, they were analyzed by using nonparametric statistics, with the Kruskal-Wallis test followed by a Wilcoxon test for multiple comparisons.

Results

Effects of repeated administrations of cocaine and GBR12783 on cocaine-induced CPP. Figure 1 presents the CPP produced by increasing doses of cocaine in animals that had previously received a once-daily injection of solvent or cocaine (20 mg/kg) or GBR12783 (10 mg/kg) for 15 days. Two-way ANOVA revealed a main conditioning dose effect \[F(5,202) = 5.35; P < .001\]. This effect varied according to the pretreatment, as reflected by a significant pretreatment × conditioning-dose interaction \[F(10,202) = 3.27; P = .001\].

In solvent-pretreated rats, cocaine induced a CPP at each tested dose. This CPP differed by cocaine test dose \[F(5,90) = 11.12; P < .001\]. Cocaine induced a biphasic effect on CPP with a dose-dependent increase in the strength of CPP from 1.25 to 20 mg/kg. Doses of 1.25, 2.5 and 5 mg/kg differed from the dose of 10 mg/kg \(P < .01\) and \(P < .001\) and \(P < .05\), respectively). The effect of highly potent doses of 10 and 20 mg/kg was followed by a relative decrease at 40 mg/kg \(P < .01\) and \(P < .001\), respectively).

After repeated administration of cocaine (20 mg/kg), conditioning doses of 2.5 to 20 mg/kg cocaine produced a significant CPP, with no overall difference between groups \[F(5,51) = 1.75; P > .05\]. The pretreatment appeared to induce a leftward shift in the cocaine dose-response curve. In fact, a CPP of higher magnitude for the conditioning dose of 2.5 mg/kg \(P < .05\) and of weaker magnitude for the conditioning dose of 20 mg/kg \(P < .01\) was observed compared with the solvent-pretreated group. In contrast, a 15-day pretreatment with cocaine at 10 mg/kg did not modify the CPP induced by these doses of cocaine (data not shown).

In the GBR12783-pretreated group, all cocaine conditioning doses, except 40 mg/kg, produced a significant CPP with no significant dose-effect relationship \[F(5,61) = 0.35\]. Prior exposure to GBR12783 (10 mg/kg) induced a CPP of higher magnitude compared with that in the solvent-pretreated group for the conditioning doses of 1.25 and 2.5 mg/kg \(P < .05\). Furthermore, after chronic GBR12783 treatment, the cocaine conditioning dose of 20 mg/kg elicited a CPP of weaker magnitude \(P < .05\).

Effects of repeated administrations of GBR12783 and cocaine on GBR12783-induced CPP. The CPP produced by increasing doses of GBR12783 in rats previously treated once daily with solvent, cocaine (20 mg/kg) or GBR12783 (10 mg/kg) for 15 days is shown in figure 2. There was a significant difference between conditioning doses \[F(5,210) = 3.34; P < .01\] and a pretreatment × conditioning-dose interaction \[F(10,210) = 2.08; P < .05\].

In solvent-pretreated rats, each tested dose of GBR12783 elicited a CPP. A one-way ANOVA revealed an overall difference between doses \[F(5,96) = 4.9; P < .001\]. Whereas effects elicited by doses from 1.25 to 10 mg/kg did not differ, a stronger amplitude of CPP was observed at 20 mg/kg \(P < .01\) followed by a decrease at 40 mg/kg \(P < .001\).

In rats pretreated with GBR12783 (10 mg/kg), conditioning doses of GBR12783 from 1.25 to 40 mg/kg produced a significant CPP with no difference between groups \[F(5,61) = 1.26\]. Furthermore, after this pretreatment, the lowest doses of GBR12783 elicited a CPP of higher magnitude than that observed in vehicle-pretreated rats; this difference was statistically significant for the conditioning dose of 2.5 mg/kg \(P < .05\). A CPP of weaker magnitude compared with that in solvent-treated rats was produced at the conditioning dose of 20 mg/kg \(P < .05\).

After preexposure to cocaine (20 mg/kg), a significant CPP was observed at all conditioning doses of GBR12783, except 1.25 mg/kg, with no difference between groups \[F(5,53) = \]
1.22]. This pretreatment resulted in a trend for an increase in the magnitude of CPP induced by GBR12783 at the conditioning dose of 2.5 mg/kg and a decrease for the conditioning dose of 20 mg/kg.

Effects of repeated administration of morphine on cocaine- and GBR12783-induced CPP. The CPP produced by increasing doses of cocaine was measured in animals that had previously received a once-daily injection of saline or morphine (10 mg/kg i.p.) for 15 days (fig. 3, upper part). The ANOVA revealed a significant conditioning-dose effect \( F(5,161) = 6.93; P < .001 \) and a main pretreatment effect \( F(1,161) = 5.14; P < .05 \) but lack of a pretreatment \( \times \) dose interaction.

As described above (fig. 1) in saline-pretreated rats, cocaine induced a CPP at each tested dose. This CPP differed by cocaine test dose \( F(5,82) = 3.08; P < .05 \), with the highest magnitude observed up to 20 mg/kg. After chronic treatment with morphine (10 mg/kg), a one-way ANOVA revealed an overall difference between groups \( F(5,79) = 5.7; P < .001 \). The cocaine conditioning dose of 1.25 mg/kg was ineffective, whereas those of 2.5 to 40 mg/kg produced a significant CPP (\( P < .001 \)). A CPP of higher magnitude was observed for the conditioning dose of 2.5 mg/kg in morphine-pretreated rats compared with solvent-pretreated rats (\( P < .001 \)).

The CPP produced by increasing doses of GBR12783 in rats that had previously received a once-daily injection of saline or morphine (10 mg/kg i.p.) for 15 days is shown in fig. 3, lower part. This CPP differed by GBR12783 test dose \( F(5,162) = 5.48; P < .001 \).

In saline-pretreated rats, conditioning doses of 2.5 to 40 mg/kg of GBR12783 produced a significant CPP with an overall difference between doses \( F(5,82) = 4.18; P < .01 \), with the maximal amplitude observed at the conditioning dose of 20 mg/kg. In rats pretreated with morphine (10 mg/kg), conditioning doses of GBR12783 from 2.5 to 40 mg/kg produced a significant CPP with no difference between groups \( F(5,80) = 2.01 \). Prior exposure to morphine (10 mg/kg) did not modify the magnitude of the GBR12783-induced CPP in comparison with that induced in saline-pretreated rats [two-way ANOVA, no pretreatment effect, \( F(1,162) = 0.03; P > .05 \)]. In particular, CPP scores induced by low conditioning doses of GBR12783 (1.25 and 2.5 mg/kg) represent cumulative data from three experiments, each of which indicates that morphine pretreatment did not influence GBR12783 rewarding effects.
Effects of a challenge dose of cocaine or GBR12783 on the elevated plus maze, 14 days after cessation of a 15-day treatment with these drugs. Fourteen days after cessation of a 15-day treatment with cocaine (20 mg/kg), GBR12783 (10 mg/kg) or their respective solvents, rats received a challenge dose of solvent or cocaine (20 mg/kg) or GBR12783 (20 mg/kg). They were tested 30 min later on the elevated plus-maze apparatus. In saline-pretreated rats, the acute injection of cocaine significantly increased the percentages of entries and time spent on open arms in comparison with a saline injection (fig. 4).

Similar increases were observed in rats pretreated with cocaine, as reflected by the F values of the cocaine main effect \(F(1,31) = 11.43, P < .01\) for percent of entries; \(F(1,31) = 12.16, P < .001\) for percent of time spent] and the absence of a pretreatment × treatment interaction \(P > .05\) for each parameter studied. Equivalent data were obtained when the percentages of distance traveled on open arms were considered (percent in saline-saline, 0.17 ± 0.13; saline-cocaine, 16.79 ± 5.43; cocaine-saline, 3.47 ± 2.13 and cocaine-cocaine, 20.72 ± 6.95). Furthermore, in rats injected acutely with solvent, pretreatment with cocaine did not modify the anxiety level \(P > .05\) for each parameter).

Similarly, a challenge dose of GBR12783 (20 mg/kg) significantly increased the percent of entries and the time spent on open arms in comparison with a solvent injection in both solvent- and GBR12783-pretreated animals \(\text{GBR12783 main effects: } F(1,32) = 18.58, P < .001\) for percent of entries; \(F(1,32) = 20.19, P < .001\) for percent of time spent; absence of a pretreatment × treatment interaction for each parameter studied \(P > .05\). In the same way, the effect of an acute injection of GBR12783 on the percent of distance traveled on open arms was not modified after chronic treatment (percent in solvent-saline, 4.34 ± 1.93; solvent-drug, 30.20 ± 10.12; drug-saline, 1.41 ± 0.61 and drug-drug, 36.03 ± 9.66). GBR12783 pretreatment did not alter the anxiety state \(P > .05\).

Effects of a challenge dose of cocaine or GBR12783 on stereotyped behaviors after a 15-day treatment with cocaine, GBR12783 or morphine. Rats were chronically treated with either cocaine (20 mg/kg), GBR12783 (10 mg/kg), morphine (10 mg/kg) or saline for 15 days. Six days after discontinuation of chronic treatments, stereotyped behavior was rated immediately after a challenge injection of cocaine (20 mg/kg) or GBR12783 (20 mg/kg) for two consecutive 15-min periods (table 1).

In cocaine- as well as GBR12783-pretreated rats, scores of stereotyped behaviors elicited by cocaine were significantly higher than those observed in both saline- and morphine-pretreated rats. In contrast, prior exposure to morphine did not modify cocaine-induced stereotyped behavior. Similarly, sensitization and cross-sensitization between cocaine and GBR12783 were obtained, whereas morphine pretreatment remained ineffective when stereotypies induced by GBR12783 were considered.

In saline-pretreated rats challenged with the highest dose of cocaine or GBR12783 tested for CPP (40 mg/kg), both drugs produced stereotypies (table 2). The onset of stereotyped behavior was slightly delayed and the intensity progressively developed so that the maximal effect was observed from the 15th min post injection. Stereotypies induced by cocaine were restricted to sniffing and head and limb movements, whereas GBR12783 also elicited biting/gnawing (nine of 13 rats). After cocaine injection, two rats were excluded from analysis, one because of convulsion and the other because of obvious dizziness.

Discussion

The present study demonstrates that prior exposure to either cocaine or the specific DA uptake inhibitor GBR12783...
results in an enhancement of the conditioned rewarding effects of low doses of cocaine, i.e., sensitization. Furthermore, cocaine appears somewhat less efficient than GBR12783 itself in sensitizing rats to the rewarding effects of GBR12783. Sensitization to the rewarding effects of cocaine was observed after chronic treatment with morphine, whereas this pretreatment did not modify the magnitude of GBR12783-induced CPP.

After two conditioning sessions, solvent-pretreated animals exhibited a marked place preference in response to doses of cocaine ranging from 1.25 to 40 mg/kg, as previously reported (Le Pen et al., 1996). It was interesting to study the effects of different pretreatments on the CPP produced by this large panel of cocaine doses. A 15-day cocaine treatment (20 mg/kg) appeared to induce a leftward shift in the dose-response curve when compared with the effects obtained in solvent-pretreated rats. The finding that a higher magnitude of cocaine-induced CPP was observed at 2.5 mg/kg confirms and extends a previous report that the CPP produced after chronic cocaine (20 mg/kg) but not 10 mg/kg was required to intensify the cocaine-induced CPP at 2.5 mg/kg and to slightly enhance the cocaine-induced CPP at 10 mg/kg, but not at 20 mg/kg (Lett, 1989).

Similarly, cocaine doses that failed to produce place conditioning after two conditioning sessions in saline-pretreated rats became effective after cocaine pre-exposure (Shippenberg and Heidbreder, 1995). In this last study, pretreatment with cocaine at 10 mg/kg was quite effective in inducing sensitization, contrasting with our own findings. However, it should be pointed out that Shippenberg and Heidbreder failed to observe a CPP with cocaine at a dose up to 10 mg/kg in saline-pretreated animals. These discrepancies probably result from differences in experimental conditions.

In order to further assess the role of DA uptake inhibition in the acute reinforcing effect of cocaine and in the sensitization process, we have studied the effect of selective blockade of this uptake system by GBR12783. The latter produced a significant CPP, with the highest magnitude observed at a dose of 20 mg/kg (Le Pen et al., 1996; vide infra). Such findings demonstrate that, like cocaine, GBR12783 can function as a rewarding stimulus in drug-naive animals. In the present study, GBR12783 was shown to produce a sensitization to its own rewarding effects. Furthermore, sensitization to the rewarding effects of low doses of cocaine was observed after GBR12783 pretreatment. A slight increase in the magnitude of CPP induced by GBR12783 was observed after cocaine pretreatment (20 mg/kg). The findings that prior exposure to GBR12783, a pure DA uptake inhibitor, enhanced its own behavioral response and that cross-sensitization to the rewarding effects occurred between cocaine and GBR12783 add to increasing evidence suggesting that DA uptake inhibition contributes to and is even sufficient for the development and expression of sensitization to the rewarding effects of cocaine.

We have previously shown in drug-naive rats that despite an equivalent locomotor stimulation, cocaine induced the highest magnitude of CPP at a lower dose than GBR12783 (Le Pen et al., 1996). However, a 15-day exposure with GBR12783 at 10 mg/kg was sufficient to produce sensitization, whereas pretreatment with a higher dose of cocaine (20 mg/kg, but not 10 mg/kg) was required to intensify the cocaine-induced CPP at 2.5 mg/kg and to slightly enhance the rewarding effects of 2.5 mg/kg GBR12783. Furthermore, cocaine pretreatment (20 mg/kg) completely failed to increase the CPP induced by the lowest dose of either cocaine or GBR12783 (1.25 mg/kg). In contrast, sensitization and a trend for sensitization to this low dose of cocaine and GBR12783, respectively, were observed after chronic treatment with GBR12783 (10 mg/kg). Because GBR12783 is a selective and potent inhibitor of DA uptake, our data indicate that although the acute reinforcing effect of cocaine appears higher than the GBR12783 effect, selective blockade of the DA uptake complex could be especially involved in the sensitization process. On the other hand, the ability of GBR12783 to induce sensitization may be linked to its long duration of action compared with that of cocaine. We have observed that behavioral activation elicited by acute injection of 10 mg/kg GBR12783 persisted for ~3 hr in rats (G. Le Pen and D. Duterte-Boucher, unpublished observation). Thus, it is possible that repeated administration of doses of GBR12783 lower than those of cocaine results in specific

### Table 1

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<tr>
<th>Pretreatment</th>
<th>Stereotypy scores</th>
<th>Average cumulative rating (0–30 min)</th>
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<tr>
<td></td>
<td>0–15 min</td>
<td>15–30 min</td>
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<tr>
<td>Saline</td>
<td>0.69 ± 0.11</td>
<td>0.27 ± 0.07</td>
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<tr>
<td>Cocaine (20 mg/kg)</td>
<td>2.36 ± 0.24*</td>
<td>3.5 ± 0.21a</td>
</tr>
<tr>
<td>GBR12783 (40 mg/kg)</td>
<td>2.69 ± 0.12b</td>
<td>3.50 ± 0.22a</td>
</tr>
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Means ± S.E.M. of 11 to 13 rats per group.
* P < .05, ** P < .01, compared with the saline-pretreated group.
† P < .01, ‡ P < .001, compared with the GBR12783-pretreated group.

### Table 2

<table>
<thead>
<tr>
<th>Challenge</th>
<th>Stereotypy scores</th>
<th>Average cumulative rating (0–30 min)</th>
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<tr>
<td></td>
<td>0–15 min</td>
<td>15–30 min</td>
</tr>
<tr>
<td>Saline</td>
<td>1.74 ± 0.2</td>
<td>3.25 ± 0.25*</td>
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<tr>
<td>Cocaine (20 mg/kg)</td>
<td>3.63 ± 0.13**</td>
<td>3.35 ± 0.21*</td>
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<tr>
<td>GBR12783 (10 mg/kg)</td>
<td>6.88 ± 0.36**</td>
<td>6.46 ± 0.43*</td>
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<tr>
<td>Morphine (10 mg/kg)</td>
<td>7.46 ± 0.15**</td>
<td>7.21 ± 0.47*</td>
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Means ± S.E.M. of nine rats per group.
* P < .05, ** P < .01, compared with the saline-pretreated group.
† P < .01, ‡ P < .001, compared with the GBR12783-pretreated group.
inhibition of DA uptake that lasts long enough to induce sensitization.

In solvent-pretreated animals as in drug-naive animals (Le Pen et al., 1996), the highest magnitude of CPP was obtained at 20 mg/kg for either cocaine or GBR12783; then the scores were reduced at 40 mg/kg. In cocaine- and GBR12783-pretreated rats, the CPP induced by 20 mg/kg of either cocaine or GBR12783 declined when compared with that in solvent-pretreated rats, so that the scores approached those obtained in control rats for a cocaine or GBR12783 test dose of 40 mg/kg. This reduction in the magnitude of CPP after high doses of both drugs could result from an increase in toxic/aversive effects conflicting with rewarding properties. High doses of different drugs of abuse have been shown to induce aversive effects that may affect the extent of the place preference they induce. For instance, cocaine or GBR12909 increases arterial blood pressure, heart rate and core temperature (Tella, 1996; Anshah et al., 1996) that likely represent a sickness experience. Neither tolerance nor sensitization develops to these effects after repeated i.p. cocaine administration (Anshah et al., 1996).

Among aversive effects, anxiogenic properties have been described after acute administration of cocaine and GBR12783 in rats or mice, when different paradigms were used (Rogerio and Takahashi, 1992; Yang et al., 1992; Simon et al., 1993, 1994). Furthermore, chronic cocaine administration can induce anxiety-like behavior in rats (Yang et al., 1992). Therefore, the reduction of CPP could result from a sensitization to the anxiogenic effects. However, neither cocaine nor GBR12783 induced anxiogenic effects as evaluated with the elevated plus maze, and actually both drugs seemed to elicit anxiolytic effects after their acute administration. In accordance with our data, a decrease in agoraphobia, as shown by an increased central ambulatory behavior in an open field, was previously reported after acute cocaine administration (Broderick, 1992). Furthermore, the repeated administration of cocaine or GBR12783 did not modify the anxiolytic-like responses. These results do not support the hypothesis that anxiogenic effects and their sensitization may affect the magnitude of CPP observed with high doses of cocaine or GBR12783.

Stereotyped behavior may also affect the strength of CPP. Actually, the highest dose of cocaine and GBR12783 tested for CPP (40 mg/kg) produced stereotyped behaviors in saline-pretreated rats. Furthermore, after cocaine and GBR12783 pretreatments, sensitization and cross-sensitization to the stereotyped behaviors have been demonstrated in rats challenged with a high dose (20 mg/kg) of cocaine or GBR12783. A similar sensitization has been observed over the course of chronic treatment with these drugs at the 10 mg/kg dose (not shown). High stereotypy frequencies have been associated with a sickness experience, a pathological state or "poor welfare" (see Mason, 1991 for review), with an increase in the aversive component of a drug’s action reducing the reward value of the drug experiment (Wall et al., 1990). More importantly, stereotypies have been linked with lowered awareness of external events or restricted attention (Robbins and Sahakian, 1981; Mason, 1991 for review). Therefore, compulsive stereotyped behavior restricted to the same location after high doses of both drugs may disrupt the ability of rats to associate the drug with environmental cues, thus leading to a reduced CPP. In support of this hypothesis, prior exposure to morphine did not modify the CPP induced by high conditioning doses of cocaine or GBR12783. Likewise, repeated administrations of morphine did not enhance stereotyped behavior induced by a high dose of cocaine as well as of GBR12783.

Our data have been interpreted as a shift to the left of the CPP dose-response curves after prior exposure to cocaine and GBR12783. However, no evidence of enhanced CPP was observed at intermediate conditioning doses of cocaine or GBR12783. This failure does in fact strengthen the idea developed above that the CPP scores must be considered, at each tested dose, as the net result of negative and positive effects. In this respect, it is well known that GBR12783- or cocaine-induced stereotypies progressively develop with increasing doses (Duterte-Boucher et al., 1990; Mason, 1991), and with repetition of the treatment, the animal becomes sensitized to the drug. Therefore, a progressive concurrent increase in stereotyped behavior may affect the strength of the CPP induced by intermediate doses of cocaine or GBR12783, resulting in an apparent lack of sensitization to the drug's rewarding effects. In accordance with this hypothesis, prior exposure to morphine, which has no incidence on stimulant-induced stereotyped behavior, led to the highest cocaine CPP magnitude from the low dose of cocaine (2.5 mg/kg), and this magnitude was maintained up to higher doses. Taken as a whole, the present pattern of results suggests that an optimally effective conditioning dose is required for the expression of sensitization to stimulant rewarding effects. Thus, lower doses would be suboptimal and higher doses would become ineffective, probably as a result of associated negative effects. This explanation is in agreement with other authors who have reported similar biphasic behavioral effects after acute injection of stimulants such as pipradol and amphetamine (White and Hiroi, 1992) and that may be extended to sensitization.

Morphine, via its action at mu receptors, inhibits (γ-aminobutyric acid) GABA neurons present in the VTA, leading to increased firing of midbrain DA neurons. Thus, it indirectly enhances the extracellular DA levels in the nucleus accumbens (Di Chiara and Imperato, 1988). Its repeated administration leads to sensitization to its locomotor effects as well as its reinforcing effects associated with changes in mesolimbic dopaminergic transmission (Lett, 1989; Kalivas and Stewart, 1991; Gaiardi et al., 1991; Spanagel, 1995; Shippenberg et al., 1996). Recent biochemical data show that chronic exposure to opiates or cocaine induces similar alterations in the cyclic AMP second-messenger system in the nucleus accumbens (see review of Nestler, 1992). Our findings that prior morphine exposure sensitizes rats to the rewarding effect of cocaine extend those of previous reports (Lett, 1989; Shippenberg and Heidbreder, 1995). These data strengthen the hypothesis of a common substrate that underlies the rewarding effects of drug of abuse. In this respect, it is therefore surprising that prior exposure to morphine did not modify the GBR12783-induced CPP, suggesting that chronic administration of morphine may have induced adaptive changes that facilitate cocaine's effect without affecting GBR12783's effect.

Recent findings have shown that the endogenous opioid systems modulate sensitization to the motivational and stimulant effects of morphine as well as those of cocaine (see reviews, Spanagel, 1995; Hurd, 1996). Therefore, chronic
treatment with morphine may have induced similar disruption of the opioid systems, leading to cross-sensitization with cocaine. Nonetheless, on the basis of few available data, cocaine and GBR derivatives were roughly similar in their effects on nigrostriatal opioid systems (see review, Hurd, 1996), suggesting that neuroadaptation in opioid systems induced by morphine might have affected the behavioral responses to GBR12783 as well as to cocaine.

In contrast to GBR12783, which specifically inhibits DA uptake, cocaine concurrently inhibits 5-HT and NE uptake. These additional properties of cocaine may modulate its reinforcing effects (for references, Kleven and Koek, 1997; Walsh and Cunningham, 1997). A variety of evidence indicates that the noradrenergic neurons of the locus ceruleus is important in the brain mechanisms of opiate abuse (see review, Nestler, 1992). Morphine modulates synaptic NE concentrations in the projection fields (hippocampus and cortex) (Matsumoto et al., 1994; Simonato, 1996). Moreover, it is likely that brain 5-HT may also be implicated in opioid dependence, although few studies have addressed this question. Drugs that increase 5-HT neurotransmission attenuate the withdrawal-induced hyperactivity of locus ceruleus neunrons (Akaoka and Aston-Jones, 1993). Morphine acts in the area of the dorsal raphe nucleus to enhance 5-HT release in specific forebrain sites (Tao and Auerbach, 1995). Furthermore, serotonergic activation enhances morphine-induced DA release (for review, Grant, 1995), indicating that a modulation of the dopaminergic system by serotonergic neurons may be involved in morphine reward. Therefore, during the CPP procedure, acute blockade of 5-HT or NE uptake by cocaine, not shared by GBR12783, may reveal disruption in the balance between neurotransmitters after chronic exposure to morphine.

Recently, apart from dopaminergic neurons and GABAergic interneurons, a subset of non-DA VTA neurons that express D_{2}, 5-HT_{1A} and mu receptors has been shown to be inhibited by DA as well as 5-HT and opioids (Cameron et al., 1997). By interacting with the DA and 5-HT transporter, cocaine may inhibit these neurons through an effect on both D_{2} and 5-HT_{1A} receptors. A similar inhibitory effect may be obtained with opioids, directly mediated by opioid receptors. The characteristics of these “tertiary” cells show a potential convergence of the action of cocaine and opioids.

It is noteworthy that morphine and cocaine, which cross-sensitize, both possess a high abuse liability, whereas GBR derivatives could have a lower addictive potential (Woźniacki and Glowa, 1996; Le Pen et al., 1996; Tella et al., 1996). Therefore, activation of DA transmission might be a necessary step for inducing reward, but interaction with other neurotransmissions or additional properties of drugs of abuse may be responsible for their especially high abuse liability. This hypothesis emphasizes the need for further work to address more precisely which adaptive changes underlie cross-sensitization between morphine and cocaine.

In summary, our study shows, with the CPP paradigm, that prior exposure to the selective DA uptake inhibitor GBR12783 induces sensitization to the rewarding effects of low doses of either cocaine or GBR12783 or cocaine. Furthermore, pre-treatment with cocaine sensitizes rats to its own rewarding effects but appears less efficient than GBR12783 to sensitize to the rewarding effects of GBR12783. These results indicate that DA uptake inhibition plays a major role in the sensitization process. On the other hand, the fact that cross-sensitization is observed between morphine and cocaine, but not between morphine and GBR12783, may reflect the complexity of cocaine’s actions and warrants further investigation.

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