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Behavioral and Neurochemical Changes in the Dopaminergic System After Repeated Cocaine Administration

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Abstract

In order to determine whether repeated cocaine administration produced persistent changes in dopamine (DA) receptor binding and release consistent with behavioral sensitization, rats were treated with either cocaine (25 mg/kg ip) or saline twice daily for 14 consecutive days followed by a 3-d withdrawal period. The DA transporter site was assayed using [^{3}H]GBR 12935, whereas D_1 and D_2 sites were assayed using [^{3}H]SCH 23390 and [^{3}H]spiperone, respectively. The density (B_{max}) of the DA transporter binding sites in the ST of the cocaine-treated group increased significantly ($p < 0.05$) over controls 3 d after the last injection, whereas the density of striatal D_1 and D_2 binding sites remained unchanged. The DA transporter in the nucleus accumbens (NA) was also studied with [^{3}H]GBR 12935 and was unchanged following drug treatment. D_1 and D_2 binding parameters for the NA were not determined in this study. Furthermore, cocaine administration did not affect the affinities (K_d) of the radioligands used to label the transporter, D_1, or D_2 sites in any of the studies performed. In addition, striatal DA release was measured using in vivo microdialysis in anesthetized rats. Linear regression analysis on maximal decreases in DA release after apomorphine (0.02, 0.2, and 2.0 mg/kg sc) injection showed no difference in the functional capacity of the ST to modulate DA transmission between control and treated groups. Moreover, animals pretreated with cocaine showed a significant ($p < 0.01$) decrease in locomotor activity (LA) after a presynaptic, autoregulating dose of apomorphine (0.03 mg/kg sc) was given. These results suggest that the effects seen after repeated exposure to cocaine may be regulated, in part, by changes in striatal DA transporter binding site densities and not necessarily by DA-releasing mechanisms or D_1 and D_2 receptor modification.

Index Entries: Cocaine; dopamine; hypersensitivity; behavior.

Abbreviations: DA, dopamine; NA, nucleus accumbens; ST, striatum; LA, locomotor activity; STB, stereotypic behavior.

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Introduction

Cocaine's reinforcing properties are attributed to the blockade of dopamine's (DA) high-affinity, sodium-dependent, transporter protein (Iversen, 1973; Horn, 1978; Ritz et al., 1987; Kuhar et al., 1990; Izenwasser and Cox, 1990). The behavioral and neurochemical alterations associated with repeated cocaine exposure are not completely understood. Most of cocaine's effects, including behavioral sensitization, euphoria, and craving, are thought to be mediated by the mesolimbic and nigrostriatal DAergic pathways (Ritz et al., 1987; Johanson and Fischman, 1989). As a result, much attention has been given to neurochemical and receptor changes in DA systems as a consequence of repeated exposure.

Repeated exposure to cocaine, as well as other psychomotor stimulants, result in an increased behavioral response to subsequent drug challenge (Mayfield et al., 1992). This is referred to as "sensitization" or "reverse tolerance" (Peris et al., 1990). This behavioral sensitization may play a role in the evolution and development of cocaine-induced paranoid psychosis, like those reported for amphetamine (Snyder, 1973; Robinson and Becker, 1986; Yi and Johnson, 1990). Both LA and STB succumb to this phenomenon. Various researchers have shown this behavioral phenomenon over a wide range of experimental paradigms (e.g., doses). This phenomenon can be detected days, weeks, and even months after repeated drug administration has been discontinued (Peris et al., 1990; Zeigler et al., 1991; Striplin and Kalivas, 1992). Alterations in functional DA release, DA receptors, and DA transporters have all been implicated in explaining this behavioral response (Peris et al., 1990).

Since the compensatory changes in receptor binding characteristics and tissue function is crucial in the development of therapeutic agents used to treat cocaine addiction, the purpose of this study was to examine the nature of the changes in behavior, DA transporter binding, D_1 and D_2 DA receptor binding, and DA release using the same injection–withdrawal paradigm.

Methods

Animals

Male Sprague-Dawley rats (Harlan-Sprague-Dawley, Indianapolis, IN) weighing between 125 and 215 g were used in these experiments. Animals were adapted to controlled environmental conditions for at least 1 wk. Temperature was maintained at 21 ± 1°C in a room with a 12-h light–dark cycle. Food (Purina Rat Chow, Ralston-Purina Co., St. Louis, MO) and water were provided ad libitum.

Chemicals and Drugs

[^{3}H]GBR 12935, [^{3}H]spiperone, and [^{3}H]SCH 233990 were purchased from New England Nuclear (Boston, MA). Butaclamol and apomorphine came from Research Biochemicals Inc. (Natick, MA). All other drugs and chemicals used in these experiments were purchased from Sigma Chemical Co. (St. Louis, MO).

Drug Administration

Rats were divided into two groups. Treated animals received 25 mg/kg (ip) cocaine hydrochloride twice daily for 14 consecutive days. Control animals were given an equivalent volume of saline twice daily. Injections during this time period were carried out in the animal's home cage. All subsequent studies were done 3 d after last injection.

Behavioral Studies

All challenge doses given 3 d after last injection for behavioral studies were done outside the animal's home cage. Animals were acclimated to their respective cages at least 1 h prior to any injection.

Locomotor Activity

After 14 d of drug treatment followed by a 3-d withdrawal period, animals from both groups were injected with a challenge dose of either cocaine (15 mg/kg ip) or saline and placed individually in electronically monitored activity chambers. LA was automatically recorded every 5 min for 1 h. Counts were recorded each time the animal disrupted a beam of light. Based on preliminary work in our laboratory, 15 mg/kg ip cocaine caused optimal LA responses. The four groups monitored were saline-saline (n = 6), saline-cocaine (n = 4), cocaine-saline (n = 6), and cocaine-cocaine (n = 6).

In a second behavioral study, both groups were injected with a challenge dose of either apomorphine (0.03 mg/kg sc) or saline and were monitored for LA as previously described. Based on preliminary work in our laboratory, 0.03 mg/kg sc apomorphine produced maximal decreases in LA. The four groups in this study were saline-saline (n = 6), saline-apomorphine (n = 6), cocaine-saline (n = 4), and cocaine-apomorphine (n = 6).

Stereotyped Behavior

Animals from both groups were injected with a challenge dose of either cocaine (25 mg/kg ip) or saline and placed individually in Plexiglas™ cages. STB was measured for 30 min. The intensity of stereotypy was assessed every 5 min according to the scoring system of Naylor and Costall (1971): 0, animals same as saline-treated animals; 1, discontinuous sniffing, constant exploratory activity; 2, continuous sniffing and small head movements, periodic exploratory activity; 3, continuous sniffing and small head movements, discontinuous biting, gnawing, and licking, brief periods of LA; 4, continuous gnawing, biting and licking, no exploratory activity. The cumulative stereotypic rating for each animal was determined as the summation of each 5-min score for 30 min. The four groups monitored were saline-saline (n = 6), saline-cocaine (n = 7), cocaine-saline (n = 7), and cocaine-cocaine (n = 5).

Binding Assays

Rats used in binding studies were sacrificed by decapitation. Brains were rapidly removed over ice, and the striata and NA were dissected, frozen over dry ice, and stored at –70°C until day of assay.

[^{3}H]SCH 23390 Binding

D_1 receptors were evaluated using previously established procedures (Billard et al., 1984; Schulz et al., 1985). Briefly, three striatal tissue pools (3–4 animals/pool) for both treated and control groups were homogenized in 100 vol (w/v) of ice-cold Tris-HCl buffer (50 m*M*, pH 7.4 at 25°C). The homogenate was centrifuged at 20,000*g* for 10 min at 4°C. The supernatant was discarded, and the pellet was centrifuged as described above in fresh buffer. The final pellet was resuspended in 50 m*M* Tris-HCl buffer, pH 7.4, containing 120 m*M* NaCl, 5 m*M* KCl, 2 m*M* $CaCl_2$, and 1 m*M* $MgCl_2$ to yield a concentration of 3 mg/0.7 mL. A small aliquot was taken for protein determination. Binding was initiated by the addition of 700-µL aliquots of homogenized membranes to assay tubes containing 100 µL of 50 m*M* Tris-HCl buffer (with 1 m*M* EDTA and 0.1% ascorbic acid), 100 µL of increasing concentrations of [^{3}H]SCH 23390 (ranging from 0.02–20 n*M*), and 100 µL of ketanserin (1 µ*M*). Incubation time was 15 min at 37°C. Parallel incubations in the presence of 10 µ*M* SCH 23390 defined specific binding. Final assay volume was 1 mL.

[^{3}H]Spiperone Binding

D_2 receptors were assayed using the method of Pugsley et al. (1989). Briefly, three striatal tissue pools (6 animals/pool) for both treated and control groups were homogenized in 20 vol (w/v) of ice-cold Tris-HCl buffer (50 m*M*, pH 7.4 at 20°C) containing 5 m*M* KCl, 1.5 m*M* $CaCl_2 \cdot 2H_2O$, 4 m*M* $MgCl_2 \cdot 6H_2O$, and 1 m*M* EDTA. The homogenate was centrifuged at 45,000*g* for 10 min at 4°C. The supernatant was discarded, and the pellet was resuspended as described above in the same buffer, except pH was 7.4 at 37°C. The homogenate was incu-

bated for 10 min at 37°C and then set on ice for 45 min. Afterward, the homogenate was centrifuged again as described above. The resulting pellet was then suspended in the original buffer for a concentration of 4 mg/0.7 mL. A small aliquot was taken for protein determination. Binding was initiated by the addition of 700-μL aliquots of homogenized membranes to assay tubes containing 100 μL of the initial 50 m*M* Tris-HCl buffer (with 0.1% ascorbic acid), 100 μL of increasing concentrations of [^{3}H]spiperone (ranging from 0.08–2.0 n*M*), and 100 μL of ketanserin (1 μ*M*). Incubation time was 30 min at 37°C. Parallel incubations in the presence of butaclamol (1 m*M*) defined specific binding. Final assay volume was 1 mL.

[^{3}H]GBR 12935 Binding

The DA transporter for both striatal and NA tissue was assayed using the procedure of Akunne et al. (1992). Briefly, three tissue pools (6 animals/pool) for both treated and control groups were homogenized (Polytron, setting 6, 30 s, Brinkmann) in 15 vol of ice-cold $NaPO_4$ buffer (55.2 m*M*, pH 7.4). The homogenate was centrifuged (Sorvall RC-2B centrifuge, SS-34 rotor) at 45,000*g* for 10 min at 4°C. The supernatant was discarded, and the resulting pellet was resuspended in the same volume of buffer, moderately vortexed, and centrifuged as above. The final pellet was suspended in 1.5 mL of buffer, and a 0.5-mL aliquot was taken for protein determination. The remaining tissue was brought to a 25-mL vol. Binding was initiated by the addition of 200-μL aliquots of homogenized membranes to assay tubes containing 25 μL of 55.2 m*M* $NaPO_4$ buffer and 25 μL of increasing concentrations of [^{3}H]GBR 12935 (ranging from 0.03–100 n*M*). Incubation time was 2 h at 25°C. Parallel incubations in the presence of 10 μ*M* GBR 12909 defined specific binding. Final assay volume was 250 μL.

All incubations were done in triplicate and terminated by filtration under reduced vacuum pressure over Whatman GF/B glass fiber filters (presoaked in 0.5% polyethylenimine for the DA transporter assay) using a Brandell Cell Harvester (M-48R) (Brandell, Gaithersburg, MD). Filters were rinsed three times each with 5 mL of the appropriate ice-cold buffer. Filters containing the membrane bound with the radioligand were placed in counting vials prefilled with 8 mL of Ready Gel scintillation liquid (Beckman, Brea, CA). Radioactivity was determined by a Beckman LS-9800 Liquid Scintillation Spectrometry (Beckman) at an efficiency of 50%. Protein content was determined according to the procedure of Bradford (1976).

In Vivo Microdialysis

Procedures for in vivo microdialysis were based on the methods of Ungerstedt (1984). By the end of the injection paradigm, the weights of all rats used in this experiment fell in the recommended range needed for accurate placement of probes using specific stereotaxic coordinates (Paxinos and Watson, 1986). Briefly, animals were anesthetized with urethane (1500 mg/kg ip) and mounted in a stereotaxic device. The skull was exposed, and a 3-mm hole was made using a 2.5-mm drill bit. After removing the dura, a dialysis probe (4-mm membrane length; BAS, West Lafayette; IN) was implanted into the left striatum according to the coordinates relative to Bregma (A 0.7, L 2.8, and D 7.0). Probes were continuously perfused (2 μL/min) with an artificial CSF (147 m*M* NaCl, 4 m*M* KCl, 3 m*M* $CaCl_2$; pH 7.0) and the body temperature of each animal was maintained at 37°C. Samples were collected every 20 min until a stable DA baseline was established as determined by HPLC-EC detection using a C-18 reverse-phase column (3 × 100 mm, BAS). Mobile phase consisted of: 100 m*M* chloroacetic acid, 0.164 m*M* SOS, 0.113 m*M* EDTA, and 247 m*M* THF). Animals from both saline- and cocaine-treated groups were then given a specific dose of apomorphine (0.02, 0.2, 2.0 mg/kg sc), and the maximal percent decreases in the level of dopamine were recorded. A dose–response curve (n = 3–4/dose) was determined for both control (saline-apomorphine) and treated (cocaine-apomorphine) animals.

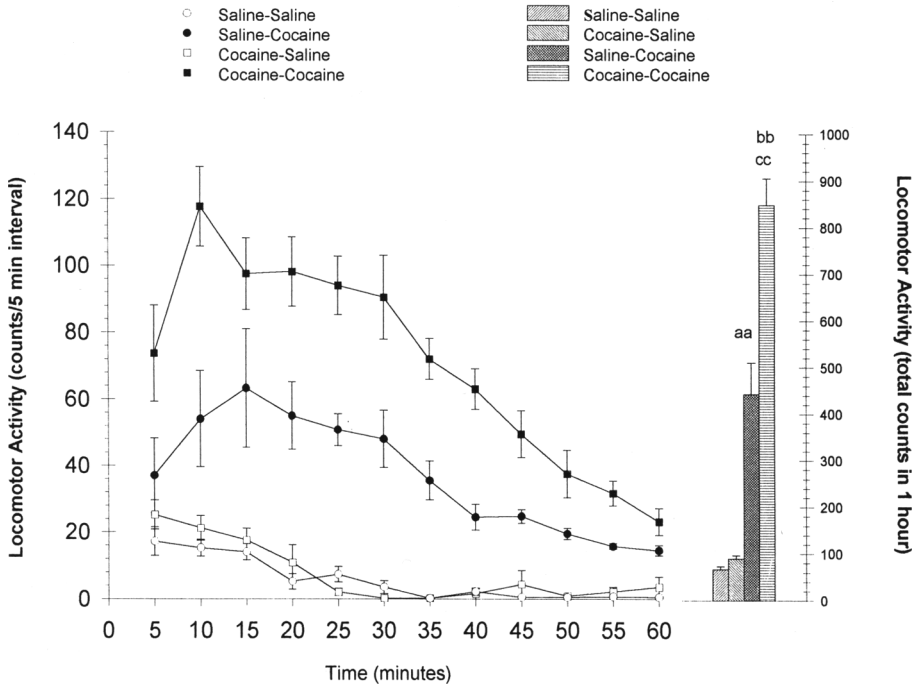

Fig. 1. Effect of repeated cocaine administration on cocaine-induced locomotor activity in the rat. Animals were treated with either cocaine (25 mg/kg ip) or saline twice daily for 14 consecutive days followed by a 3-d withdrawal period. Animals were then given a challenge dose of either cocaine (15 mg/kg ip) or saline and monitored every 5 min for 1 h. Those groups included saline-saline, saline-cocaine, cocaine-saline, and cocaine-cocaine. Mean values ± SE (n = 4–6/group) are shown. $^{aa}p < 0.01$ as compared to saline-saline; $^{bb}p < 0.01$ as compared to cocaine-saline; $^{cc}p < 0.01$ as compared to saline-cocaine.

Analysis of Data

Data from locomotor activity experiments were analyzed using one-way analysis of variance (ANOVA) in conjunction with Newman-Keuls test, whereas STB was analyzed using the Wilcoxon scores procedure (SAS/STAT, 1988). Both tests were performed at a significance of 0.05.

The saturation curves for specifically bound radioligands were transformed using the LUNDON (Lundeen and Gordon, 1986) radioligand binding analysis software package. All saturation isotherms were best fit by a single site model, and final derivation of radioligand affinity (K_d) and the density of binding sites (B_{max}) was made. Statistical differences between groups were tested by one-way ANOVA at a significance of 0.05.

Data from in vivo microdialysis were also analyzed using one-way ANOVA at a significance of 0.05. Bonferonni's multiple comparisons test was used for the separation of means (SAS/STAT, 1988). Regression analysis was used to determine the best linear fit and to test if slopes were significantly different from each other.

Results

Behavior

Cocaine (15 mg/kg ip) administered to animals pretreated with saline for 14 consecutive days resulted in significant ($p < 0.01$) increases in LA when compared to the saline-treated group given a challenge of saline (Fig. 1). There

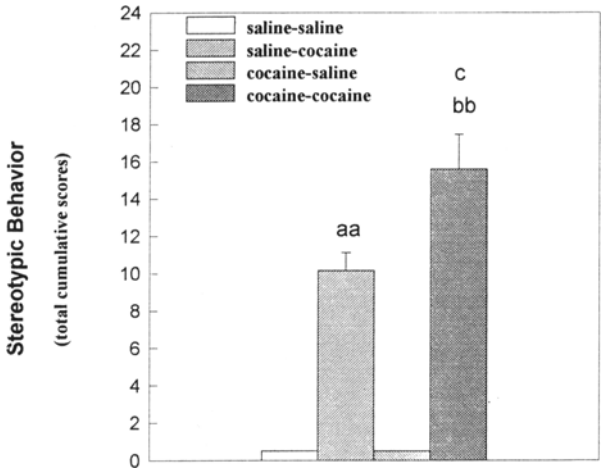

Fig. 2. Effect of repeated cocaine administration on cocaine-induced stereotypic behavior in the rat. Animals were treated with either cocaine (25 mg/kg ip) or saline twice daily for 14 consecutive days followed by a 3-d withdrawal period. Animals were then given a challenge dose of either cocaine (25 mg/kg ip) or saline and monitored every 5 min for 30 min. Those groups included saline-saline, saline-cocaine, cocaine-saline, and cocaine-cocaine. Mean values ± SE (n = 5–7/group) are shown. $^{aa}p < 0.01$ as compared to saline-saline; $^{bb}p < 0.01$ as compared to cocaine-saline; $^{c}p < 0.05$ as compared to saline-cocaine.

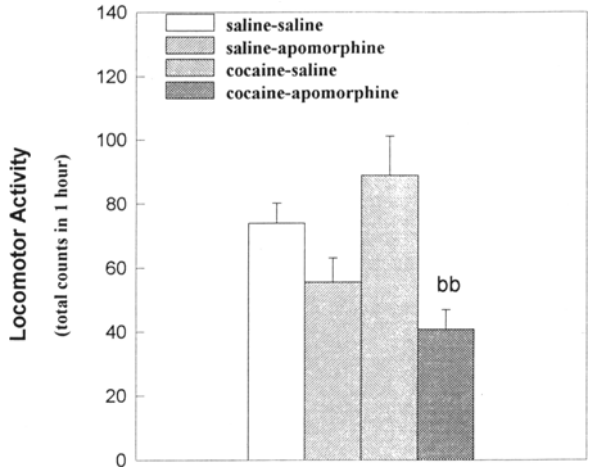

Fig. 3. Effect of repeated cocaine administration on apomorphine-induced decreases in locomotor activity in the rat. Animals were treated with either cocaine (25 mg/kg ip) or saline twice daily for 14 consecutive days followed by a 3-d withdrawal period. Animals were then given a challenge of a presynaptic dose of apomorphine (0.03 mg/kg sc) or saline and monitored every 5 min for 1 h. The groups include saline-saline, saline-apomorphine, cocaine-saline, and cocaine-apomorphine. Mean values ± SE (n = 4–6/group) are shown. $^{bb}p < 0.01$ as compared to cocaine-saline.

was more than a fourfold increase in LA. It can also be noticed that the increase in this group was gradual with a peak of activity occurring at 15 min. When animals pretreated with cocaine for 14 d were challenged with another dose of cocaine (15 mg/kg ip), there was a significant ($p < 0.01$) increase in LA when compared to its control (Fig. 1). This increase was immediate—reaching a peak in 10 min. This rise in LA maintained its high level during the 60-min assay period. The activity of this group was more than eightfold compared to its control group.

The effect of a challenge dose of cocaine (25 mg/kg ip) on animals pretreated with either cocaine or saline for 14 d on STB is presented in Fig. 2. As can be seen from the figure, cocaine administration resulted in a significant ($p < 0.01$) increase in STB for both groups. The increase in stereotypy in animals pretreated with saline, however, was almost half what was observed in the cocaine-cocaine-treated group.

The results in Figs. 1 and 2 clearly show that cocaine pretreatment resulted in animal "sensitization" or "reverse tolerance." In both behavioral parameters, prior exposure to cocaine resulted in a twofold increase when the data were compared to saline-pretreated animals.

Figure 3 shows data regarding apomorphine administration (0.03 mg/kg sc) in cocaine- or saline-pretreated animals. The data clearly show that apomorphine was able to ($p < 0.01$) reduce LA in cocaine-pretreated animals significantly with no significant effects in the saline controls.

D_1 and D_2 Receptor Binding Sites

In order to investigate the mechanism of action of cocaine sensitization, D_1 and D_2 bind-

Table 1
Effect of Repeated Cocaine Administration on the Binding of [^{3}H]GBR 12935 to Recognition Sites Associated with the Striatal and Nucleus Accumbens DA Transporter

	[^{3}H]GBR 12935 binding sites			
	Striatum		Nucleus accumbens	
	Saline	Cocaine	Saline	Cocaine
B_{max} (fmol/mg protein)	10,198 ± 659	16,106 ± 1544*	8357 ± 2001	9495 ± 1344
K_d (n*M*)	2.70 ± 0.60	4.10 ± 1.00	1.80 ± 1.00	2.4 ± 0.30

Mean values ± SE (*$p < 0.05$).

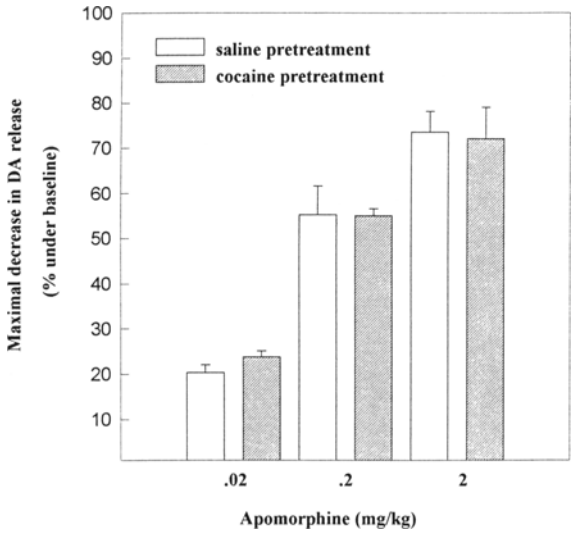

Fig. 4. Effect of repeated cocaine administration on apomorphine-induced decreases in striatal DA release. Animals were treated with either cocaine (25 mg/kg ip) or saline twice daily for 14 consecutive days followed by a 3-d withdrawal period. Anesthetized animals were then given varying doses of apomorphine (0.02, 0.2, and 2.0 mg/kg sc) or saline (n = 3–4/dose), and monitored until the maximal percent decrease in the level of DA was recorded. The dose–response curve for the two groups was generated using regression analysis to determine a best-fit line. No significant ($p < 0.05$) differences in the slopes were found.

ing was measured in striatal tissue. Alterations in the binding sites might contribute to the sensitization phenomenon to cocaine. As can be seen, however, there were no significant changes in the B_{max} of D_1 nor D_2 binding sites in the rat ST 3 d after the last injection of cocaine. Furthermore, cocaine treatment did not alter the K_d of [^{3}H]SCH 23390 for the D_1 receptor or [^{3}H]spiperone for the D_2 receptor in this injection paradigm (Table 1).

In Vivo Microdialysis

Possible changes in DA release may offer some explanation for the enhanced behavioral effects seen as a result of repeated cocaine exposure. On the contrary, however, the dose–response curves generated for the two groups from varying doses of apomorphine showed no changes in the maximal percent decrease in DA overflow as depicted by the two regression lines with almost identical slopes (Fig. 4).

DA Transporter Binding Sites

The primary site of action for cocaine is the DA transporter. Repeated cocaine administration may alter its binding characteristics and, thus, affect its response to subsequent injections to cocaine. Animals treated repeatedly with cocaine showed a significant ($p < 0.05$) increase in B_{max} for the DA transporter binding site in the ST, whereas no changes in the NA were observed. K_d values did not change in any of the areas observed (Table 2).

Discussion

In the present study, cocaine administration given twice daily for 14 consecutive days

Table 2
Effect of Repeated Cocaine Administration on the Binding of [^{3}H]Spiperone and [^{3}H]SCH 23390 to Recognition Sites Associated with Striatal D_2 and D_1 Receptors, Respectively

	Striatum			
	[^{3}H]Spiperone		[^{3}H]SCH 23390	
	Saline	Cocaine	Saline	Cocaine
B_{max} (fmol/mg protein)	813 ± 53	1002 ± 14	1318 ± 119	1126 ± 105
K_d (n*M*)	0.07 ± 0.008	0.088 ± 0.015	0.546 ± 0.03	0.555 ± 0.03

Mean values ± SE (*$p < 0.05$).

resulted in behavioral sensitization for both LA and STB in animals given a challenge dose of cocaine 3 d after last injection. The density of striatal DA transporter binding sites was increased in pretreated rats, whereas no changes were found in the nucleus accumbens. Repeated cocaine administration also left striatal D_1 and D_2 binding sites unchanged as well as in vivo striatal DA release. Apomorphine-induced decreases in LA were evident in subjects previously exposed to cocaine. These findings show that repeated exposure to cocaine resulted in limited compensatory changes in the DA system, specifically in striatal tissue.

LA and STB are thought to be mediated by the mesolimbic and nigrostriatal dopminergic pathways, respectively (Johanson and Fischman, 1989). Possible changes that could contribute to sensitization of these behaviors include upregulation of postsynaptic DA receptors (Zeigler et al., 1991; Laruelle et al., 1992), enhanced DA release (Akimoto et al., 1989), subsensitivity of autoreceptors (Yi and Johnson, 1990), and changes in transporter function or number (Izenwasser and Cox, 1990; Peris et al., 1990; Farfel et al., 1992). Research in support of these findings, however, is equivocal (Zahniser et al., 1988). Steketee et al. (1991) and Striplin and Kalivas (1992) have investigated the possible role for G proteins in behavioral sensitization. They suggested that the action of psychostimulants on DA perikarya may be more instrumental in the development of sensitization than terminal fields. The uncoupling of G-protein-dependent inhibitory mechanisms in the A10 region, for example, may lead to a hyperactivation of DA transmission.

In addition to possible neurochemical changes, some degree of sensitization could be explained by environmental conditioning (Kalivas et al., 1988; Pert et al., 1990). Although this factor cannot be dismissed, it is unlikely that conditioning plays a major role in the behavioral changes produced by repeated cocaine in the present experiment. The conditioning cues associated with the injection procedure, which was carried out in the home cage, are unlikely, because the response to saline in the activity chambers located outside the home environment was equivalent between rats receiving repeated saline and repeated cocaine.

In the present study, there were no significant changes in the B_{max} of D_1 or D_2 receptor sites 3 d after the last injection. Although there was a 23% increase in B_{max} for the D_2 site in the cocaine-treated group, this increase was not significant. The upregulation of either of these receptors or the increased affinity for the radioligand could have offered an explanation for the enhanced behavioral response described. Other studies on D_1 and D_2 receptor binding as a consequence of repeated cocaine exposure have been done with equivocal results. D_1 and D_2 binding site densities have been shown to increase, decrease, or remain

unchanged (Kleven et al., 1990; Zeigler et al., 1991; Farfel et al., 1992). These differences are probably the result of species variations, dosage, injection schedule, withdrawal period, and binding assay conditions. For instance, Kleven et al. (1990) found a decrease in B_{max} for D_1 in the ST immediately after the last injection that was sustained for at least 2 wk. They also used a once-a-day injection paradigm delivering a relatively low to moderate dose (10–20 mg/kg) of cocaine. Our study, on the other hand, utilized a twice-a-day injection paradigm delivering a total of 50 mg/kg/d for 14 consecutive days. This difference in dose and injection schedule may result in different compensatory changes in neuronal systems. This phenomenon has been seen in a more dramatic way in behavioral studies of continuous vs intermittent drug exposure (Zeigler et al., 1991). Time after last injection (or withdrawal period) is also a crucial variable when measuring behavioral and neurochemical changes. In the same study by Kleven et al. (1990), for example, they showed a significant decrease in B_{max} for striatal D_2 20 min after the last injection, but no difference from controls 2 wk later. This suggests that changes in receptor density after drug exposure are transient and dependent on the time after the last injection.

The persistent effects of cocaine, however, may be mediated downstream from the DA receptor to the signal-transduction mechanisms. As mentioned previously, researchers are already beginning to look at the G-regulatory proteins, the catalytic subunit of adenylate cyclase, and other components that are responsible for exerting D_1- and D_2-mediated effects (Steketee et al., 1991; Mayfield et al., 1992; Striplin and Kalivas, 1992).

Presynaptic D_2 receptors or autoreceptors have been suggested to be impulse-regulating, synthesis-modulating, or release-modulating (Bannon and Roth, 1983). D_2 release-modulating autoreceptors appear to be very sensitive to the concentration of synaptic DA (Dwoskin and Zahniser, 1986). The nonspecific DAergic agonist, apomorphine, given in increasing doses yielded a dose-dependent decrease in DA release. This decrease in the maximal percent of DA released was similar in both control and treated groups, suggesting no change in the autoreceptor-releasing mechanism. It is important, however, to be cautious in drawing conclusions about these types of data. A composite of many neuronal processes in the ST—including DA—are involved in an in vivo study of this type. It is possible that some alterations in the DA system may be masked by more predominant changes elsewhere. In addition, the effects of anesthesia must be considered. Although urethane is thought to be a suitable general anesthetic for studying neural function because of its ability to produce a plane of anesthesia without affecting neurotransmission in various subcortical areas in the central nervous system, its composite effects cannot be ignored.

The behavioral study performed showed a decrease in LA in the cocaine-treated group after receiving an autoreceptor-stimulating dose of apomorphine. This effect was not seen in the saline controls. Autoreceptor activation prompted a decrease in the release of endogenous DA, thus resulting in a significant decrease in locomotor response. The differences in the behavioral response between the two groups are not consistent with the in vivo dialysis results. The net outcome of this decreased locomotor response may be related to numerous systems that act in concert with DA, such as the cholinergic system (Claye and Soliman, 1990).

Other studies performed in vitro (Dwoskin et al., 1988; Yi and Johnson, 1990) have shown both supersensitivity and subsensitivity of striatal D_2 autoreceptors after chronic cocaine treatment. Subsequent studies (manuscript in preparation) in our laboratory, however, have shown that under the same experimental paradigm used in the present study, amphetamine-induced release of DA in striatal slices was similar in both saline- and cocaine-treated animals. Resting DA release, on the other hand, was higher in the cocaine-treated group and may offer, at least in part, an explanation for the sensitization of behavior observed in the pres-

ent study. These in vitro results lend support to the present in vivo microdialysis findings.

Cocaine indirectly increases the concentration of DA in the synapse by blocking its reuptake. The increase in DA transporter binding sites found in the ST may be a homeostatic mechanism to decrease transmission of the postsynaptic cellular signal by regulating the level of DA in the synapse. The greater number of transporter proteins available provide for greater effectiveness in removing synaptic DA. On the other hand, it may be expected that this increase could account for cocaine's more pronounced behavioral effects, as seen in this study. Although there have been reports of decreases (Farfel et al., 1992) or no changes (Allard et al., 1990; Kula and Baldessarini, 1991) in DA transporter binding after repeated cocaine administration, Cheng et al. (1993) found significant increases in RTI-55-labeled DA uptake sites in rat striatal tissue 24 h after a 14-day injection (7.5 and 15 mg/kg bid) paradigm. The similarities in species, injection schedule, and relatively short drug-free periods may contribute to our similar findings. The results that are in disagreement with the present study may be owing to a variety of experimental differences. Farfel et al. (1992) showed a decrease in the B_{max} of treated monkeys 2 wk after the last injection of cocaine. The discrepancy between these two studies may be owing to a difference in species or length of the drug-free period. Kula and Baldessarini (1991), on the other hand, found no changes in B_{max} after cocaine treatment. They used relatively low doses of cocaine in their experiment with a less frequent injection schedule. Allard et al. (1990) also reported that [^{3}H]GBR 12935 binding in striatal tissue was unchanged after repeated cocaine exposure compared to controls.

It has also been suggested that the transporter protein in both the ST and NA may not only be differentially affected by cocaine and other psychoactive agents, but that their structure is different (Lew et al., 1991). They showed in their study that using a radioiodinated diphenylpiperazine derivative, the specific photoaffinity-labeled band with a rat striatal preparation was slightly lower in molecular weight than that observed with rat NA (mol wt 72,400 and 76,800 respectively) (Lew et al., 1991). These findings suggest the possibility of DA transporter heterogeneity (Graham and Langer, 1992). One interesting finding in this study was the lack of change in the density of the DA transporter in the NA after repeated cocaine administration. An increase in this transporter protein could have easily offered an explanation for the robust increase in LA mentioned earlier. Subsequent studies in our laboratory (manuscript in preparation) have revealed that under the same injection paradigm, a significant change occurred in [^{3}H]DA uptake in vitro in the NA, but not in the ST. Compared to controls, the uptake of [^{3}H]DA in NA slices from animals pretreated with cocaine was significantly reduced. This implies an increase in the amount of available DA for synaptic activity and can offer an explanation for the sensitization of LA seen in this study. These data are also supported by a similar study done by Izenwasser and Cox (1990) that also showed a reduction in [^{3}H]DA uptake in vitro in rat NA, but not ST 24 h after 3 d of cocaine injections. In addition, Sharpe et al. (1991) suggested that the medial shell of the NA should be more sensitive to the effects of cocaine than the caudate nucleus. This may explain why cocaine elicits first locomotor stimulation followed by stereotypy in the rat (Robinson and Becker, 1986).

In summary, the present study indicates that repeated cocaine injections caused an increase in the densities of [^{3}H]GBR 12935-labeled DA transporter binding sites in rat striatal tissue 3 d after the last injection of cocaine, whereas no changes were seen in the NA. Although robust behavioral changes were observed for both LMA and STB in cocaine-treated rats, it is clear from this study that the neurochemical changes that may contribute to this sensitization phenomenon are different and region-specific. Receptor density changes may not always be a reliable indicator of behavioral changes

seen in this type of study. Functional data (e.g., [^{3}H]DA uptake), as mentioned earlier, will always need to follow binding results. This is also true for D_1 and D_2. The characterization of the DA transporter and other receptor systems will offer us more insight into its role in cocaine's reinforcing and behavioral properties for the development of therapeutic agents.

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