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Effects of SCH-23390 on Dopamine D1 Receptor Occupancy and Locomotion Produced by Intraaccumbens Cocaine Infusion

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ABSTRACT This study examined the effects of both systemic and intraaccumbens administration of SCH-23390 in rats on dopamine D1 receptor occupancy and on locomotor activity produced by intraaccumbens infusion of cocaine. In experiment 1, rats received SCH-23390 (0–1 mg/kg, IP) 15 minutes prior to intraaccumbens infusion of cocaine (0 or 100 µg/side). In experiment 2, rats received coinfusion of SCH-23390 (0–1 µg/side) and cocaine (0 or 100 µg/side) into the nucleus accumbens (NAc). After behavioral testing, receptors occupied by SCH-23390 were quantified by injecting animals with their respective dose of SCH-23390, followed by a systemic injection of the irreversible antagonist N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ). Receptors occupied by SCH-23390, and therefore protected from EEDQ-induced inactivation, were quantified from autoradiograms of sections labeled with ^{3}H-SCH-23390. Systemic administration of SCH-23390 dose-dependently (0.1–1.0 mg/kg) reversed cocaine-induced locomotion and occupied 72–100% of D1-like receptors in the anterior NAc. D1 receptor occupancy following systemic administration of SCH-23390 was evident as an inverted U-shaped, dose-dependent change, with the greatest occupancy observed at the intermediate dose of 0.3 mg/kg. Intraaccumbens infusion of SCH-23390 did not alter cocaine-induced locomotor activity despite occupying 40–60% of D1-like receptors in the anterior NAc core and shell. The findings that systemic, but not intraaccumbens, administration of SCH-23390 potently reversed locomotion produced by intraaccumbens cocaine infusion suggest that stimulation of D1 receptors in regions other than the NAc is involved in locomotion produced by intraaccumbens infusion of cocaine, and that stimulation of D1 receptors in the NAc is not necessary for this behavior. **Synapse 30:194–204, 1998.**

INTRODUCTION

Cocaine-induced locomotion is reversed by systemic administration of either a D1-selective or a D2-selective antagonist, indicating that stimulation of both receptor subfamilies is necessary for this behavior (Fontana et al., 1993; McCreary and Marsden, 1993; Tella, 1994; Ushijima et al., 1995). Localization studies have demonstrated that infusion of cocaine directly into the nucleus accumbens (NAc) elicits locomotor activity that is reversed by systemic administration of the nonselective dopamine antagonist cis-flupenthixol (Delfs et al., 1990; Hemby et al., 1992). Additionally, intraaccumbens administration of the D2-selective antagonists eticlopride and sulpiride (i.e., 0.1 µg/side) potently reverses locomotor activity elicited by systemic administration of cocaine, whereas only relatively high doses (i.e., 1–3 µg/side) of the D1-selective antagonist SCH-23390 decrease this behavior (Kaddis et al., 1993; McGregor and Roberts, 1993; Neisewander et al., 1995). Furthermore,

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a behaviorally ineffective dose of intraaccumbens SCH-23390 was found to occupy a similar number of D1-like receptors in both the core and shell regions of the NAc as a higher effective dose, suggesting that the effect of SCH-23390 at higher doses may be due to diffusion from the NAc to other brain regions (Neisewander et al., 1995). Thus, the role of D1-like receptors in the NAc in cocaine-induced locomotion remains unclear.

It is possible that systemically administered cocaine activates parallel neural pathways involved in locomotor activity, such that blockade of D1-like receptors in multiple regions is necessary to reverse this behavior. The present study, therefore, focused on the role of D1-like receptors in the mesolimbic dopaminergic pathway by examining the effects of both systemic and intraaccumbens infusions of SCH-23390 on locomotor activity elicited by intraaccumbens cocaine infusion. Receptor occupancy by SCH-23390 was assessed subsequently by administering the nonselective irreversible receptor antagonist N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) systemically 15 minutes after administration of SCH-23390. D1-like receptors protected by SCH-23390 from EEDQ-induced inactivation were then analyzed using quantitative autoradiography.

MATERIALS AND METHODS

Drugs and chemicals

SCH-23390 (Research Biochemicals, Inc., Natick, MA) was dissolved in bacteriostatic saline and administered intraperitoneally at a volume of 1 ml/kg or intracranially at a volume of 0.5 µl. Cocaine hydrochloride (NIDA Drug Supply System) was dissolved in saline and injected intracranially at a volume of 0.5 µl. EEDQ (Sigma, St. Louis, MO) was dissolved in ethanol and injected intraperitoneally at a volume of 1 ml/kg. ^{3}H-SCH-23390 (71.3 Ci/mmol; New England Nuclear Corp., Boston, MA) and (+)butaclamol (Research Biochemicals, Inc.) were used in the radioligand binding assays.

Animals

Male Sprague-Dawley rats weighing 250 ± 25 g were housed individually in a climate-controlled facility with a 12-hour light/dark cycle. The housing conditions and care of the animals were consistent with those specified in the Guide for the Care and Use of Laboratory Animals adopted by the National Institutes of Health. The animals were acclimated to handling for 3 days prior to surgery.

Surgery

Animals were anesthetized with pentobarbital (50 mg/kg, IP) given in combination with atropine sulfate (10 mg/kg, IP) to minimize bronchial secretions. They were then shaved across the head, and placed into a stereotaxic instrument. A midsagittal incision was made across the skull, and the connective tissue was retracted. Holes (1–2 mm) were drilled into the skull, and guide cannulae (23-gauge stainless steel) were then lowered through the holes into the NAc using the following coordinates derived from the atlas of Paxinos and Watson (1986): +2.2 mm AP and ±1.6 mm ML with respect to Bregma, and −6.6 mm DV from the surface of the skull. Screws were drilled into the skull approximately 5 mm anterior and posterior to the cannulae. The cannulae were then anchored by applying dental acrylic to the skull and screws. Wire stylets were placed into the guide cannulae to maintain patency. The rats were allowed at least 3 days to recover and were handled each recovery day.

Behavioral testing procedure

Rats were placed into clear Plexiglas cages (44 × 24 × 20 cm high) for 60 minutes. The cages had a wire-bar floor, a perforated metal lid, and two sets of photocells and light sources located 32 cm apart and 4 cm above the floor of the cage. A computer-automated relay system recorded the number of times the animals broke the two photobeams consecutively by moving from one end of the cage to the other (i.e., crosses). The presence of sniffing and stereotypic head-bobbing was also recorded every 10 seconds by an observer unaware of the animals' previous drug treatment. No head-bobbing was observed, and therefore, only sniffing data are presented in the results.

Intraaccumbens infusions

The injection cannulae (30-gauge stainless steel) were connected to gas-tight Hamilton syringes by PE-20 tubing, and the syringes were placed into an infusion pump (Harvard Apparatus, Millis, MA). A minute air bubble was made in the tubing so that its movement could be measured in order to verify that the proper amount of fluid had been infused. The rats were gently restrained and the injection cannulae were inserted to a depth 1 mm beyond the tip of the guide cannulae. The infusion began 1 minute later and took place over a 190-second period. The injection cannulae were removed 1 minute after the infusion had been completed.

Experiment 1: Effects of systemic SCH-23390 administration on behaviors produced by intraaccumbens cocaine infusion

Rats were tested a total of four times with 2 rest days intervening between tests 1 and 2, and 5 rest days intervening between tests 2–4. The first 2 test days were designed to examine the effects of SCH-23390 on locomotion following acute intraaccumbens saline or cocaine infusion. The rats received either 0 (N = 6), 0.03 (N = 9), 0.1 (N = 8), 0.3 (N = 7), or 1.0 (N = 9) mg/kg SCH-23390, IP. Fifteen minutes later, the ani-

mals received bilateral infusions of saline on one of the test days and cocaine (100 µg/0.5 µl/side) on the other test day, with the order counterbalanced across groups. Behavioral testing began immediately after the infusions. The remaining test days were designed to examine whether the behavioral effects of cocaine would be altered following repeated intraaccumbens infusion, and if so, whether administration of SCH-23390 would prevent such changes from occurring. On the third test day, animals were pretreated with their assigned dose of SCH-23390, and 15 minutes later they received bilateral infusions of cocaine (100 µg/0.5 µl/side). On the fourth test day, they received bilateral infusions of cocaine (100 µg/0.5 µl/side) without any pretreatment. Behavioral testing began immediately after the infusions.

Experiment 2: effects of coinfusion of SCH-23390 and cocaine into the NAc on behavior

Rats were tested twice, with 2 days between tests. The rats received either 0 (N = 8), 0.1 (N = 5), 0.5 (N = 6), or 1.0 (N = 5) µg/0.5 µl/side SCH-23390 coinfused with cocaine (100 µg/0.5 µl/side) on one test day and coinfused with saline on the other test day, counterbalanced for order across groups. Behavioral testing began immediately after the infusions.

Receptor occupancy

Two days after completing the behavioral tests, the animals received SCH-23390 at the same dose and route of administration as they had received during behavioral testing. Fifteen minutes later, the animals were injected with EEDQ (10 mg/kg, IP), except that half of the controls receiving intraaccumbens saline infusions were injected with vehicle. The animals were then sacrificed 90 minutes after their systemic injection of vehicle or EEDQ. The 90-minute period was used to ensure enough time to allow EEDQ to inactivate unoccupied receptors. The brains were rapidly removed and immersed in isopentane at −20°C for 2 minutes to promote uniform freezing, and then stored at −70°C. The brains were later sectioned in the coronal plane at a thickness of 20 µm at −12°C. The sections were thaw-mounted onto gelatin-coated slides and desiccated under vacuum for 2–3 hours at 4°C and then for 8 hours at −20°C. The slides were then stored at −70°C until assayed for radioligand binding.

Prior to the radioligand-binding assay, the sections were thawed and dried at room temperature for 20 minutes. Duplicate sections were incubated with 4 nM ^{3}H-SCH-23390 in the presence or absence of 1 µM (+)butaclamol in buffer containing 50 mM Tris, 10 mM $MgSO_4$, 2 mM EDTA, 154 mM NaCl, and 10 mg/l BSA (pH 7.5 at room temperature). Incubation occurred for 75 minutes at 37°C, and the assay was terminated by a 20-minute wash in ice-cold buffer. The sections were then dipped in ice-cold distilled water to remove buffer salts, and then dried at 57°C. Next, the sections were laid into lightproof X-ray cassettes and were apposed to tritium-sensitive film (Amersham, Arlington Heights, IL). A slide containing plastic tritium standards (American Radiolabelled Chemicals, Inc., St. Louis, MO) that were calibrated using mash sections of rat brain (Artymyshyn et al., 1990) was also apposed to each piece of film. Following a 7-day exposure period, the film was processed in Kodak GBX developer and fixer. The autoradiograms were analyzed with a Macintosh-based image processing system using Image software (National Institutes of Health). Optical density was converted into nCi/mg of radioligand bound using a standard curve as a reference.

Cannula placement

Cannula placements were determined during tissue sectioning by drawing the cannula tracts at each plate of the atlas of Paxinos and Watson (1986) within 2.7–0.5 mm anterior to bregma. The lowest point of the tract was estimated as the site of infusion.

Data analyses

Locomotor activity (i.e., crosses) and time-sampled observations of sniffing were analyzed using repeated-measures analyses of variance (ANOVAs) with dose of cocaine (i.e., saline vs. 100 µg/side) as a repeated measure and dose of SCH-23390 as a between-subjects factor. Significant interactions were further analyzed using Fisher LSD tests. The regions analyzed in the autoradiograms included the pole, core, and shell of the NAc, the medial and lateral caudate putamen (CPu), and the medial prefrontal cortex. The entire intact portion of each region was included in the analysis. The control group measures from each experiment were combined in order to obtain a more accurate estimate of control binding values. The binding data, expressed as mean fmol bound/mg protein, were analyzed using separate ANOVAs for each brain region, and subsequent pairwise comparisons were made using Fisher LSD tests. The percentage of receptors occupied by SCH-23390 was estimated using the ratio of binding in the following treatment groups:

$$\frac{\text{SCH-23390/EEDQ} - \text{vehicle/EEDQ}}{\text{vehicle/vehicle} - \text{vehicle/EEDQ}}.$$

Binding in the vehicle/EEDQ treatment group represents the population of receptors not inactivated by EEDQ. This value was subtracted from the binding in both the SCH-23390/EEDQ and vehicle/vehicle groups, such that the value in the numerator represents receptors protected from EEDQ by the intracranially administered antagonist, and the value in the denominator represents the total amount of receptors available for protection from EEDQ. The ratio of these measures,

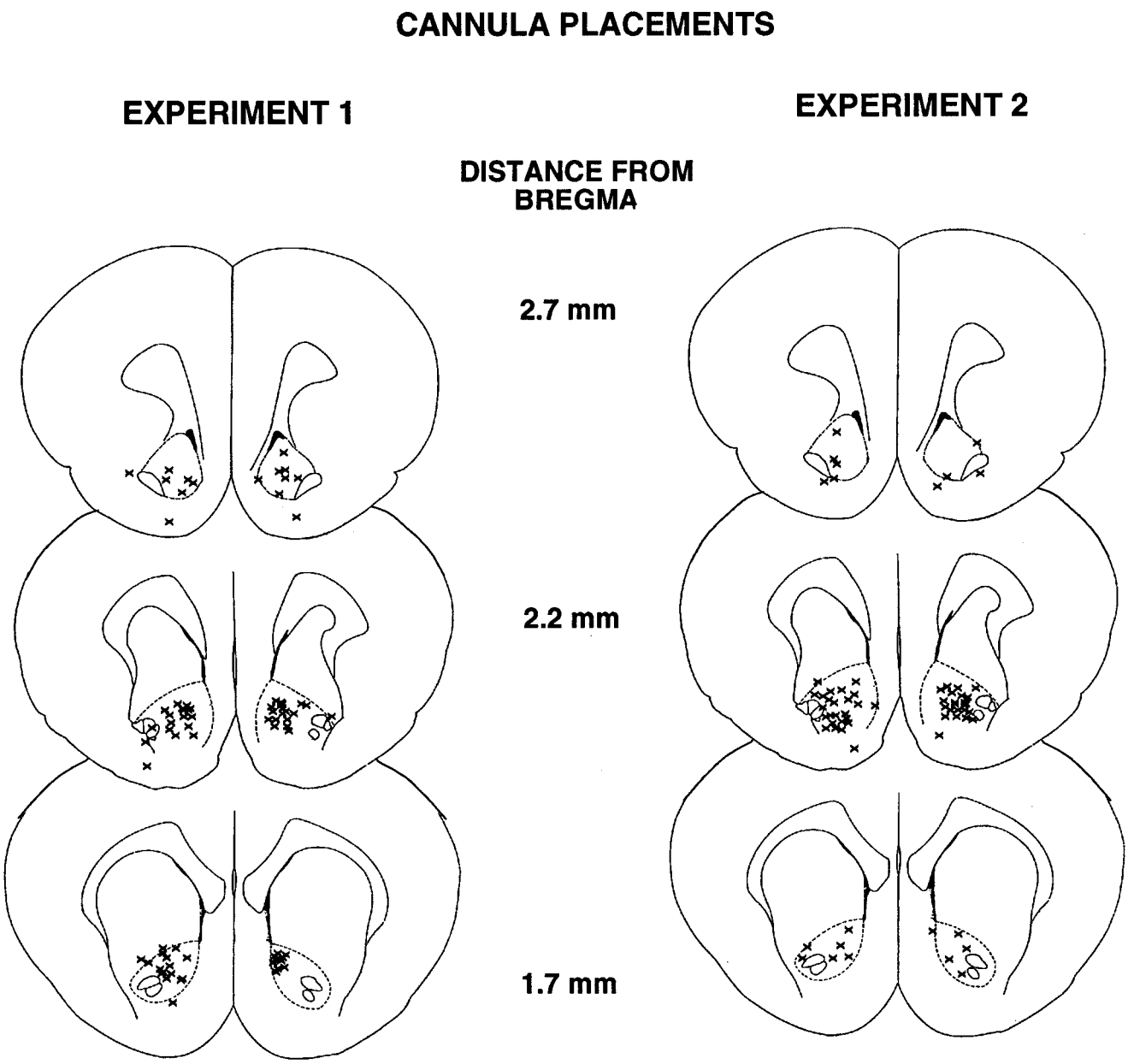

Fig. 1. Approximate positions of the tip of the injection cannulae, each represented by X, for animals receiving intraaccumbens administration of cocaine and systemic administration of SCH-23390 (experiment 1) and for animals receiving intraaccumbens coadministration of cocaine and SCH-23390 (experiment 2). Drawings were adapted from the atlas of Paxinos and Watson (1986).

therefore, provides an estimate of the percentage of receptors occupied by the antagonist.

RESULTS

Cannula placements

The approximate positions of the injection cannulae are illustrated in Figure 1. For all of the animals included in this study, the site of the drug infusions fell within 0.5 mm of the intended infusion site.

Effects of systemic administration of SCH-23390 on behavior

Intraaccumbens cocaine infusion produced an increase in locomotion that was dose-dependently reversed by SCH-23390 administered IP (Fig. 2a). The overall ANOVA revealed a significant interaction between the repeated measure (i.e., saline vs. cocaine) and SCH-23390 ($F_{4,34} = 3.50$, $P < .05$). A subsequent one-way ANOVA of crosses following intraaccumbens

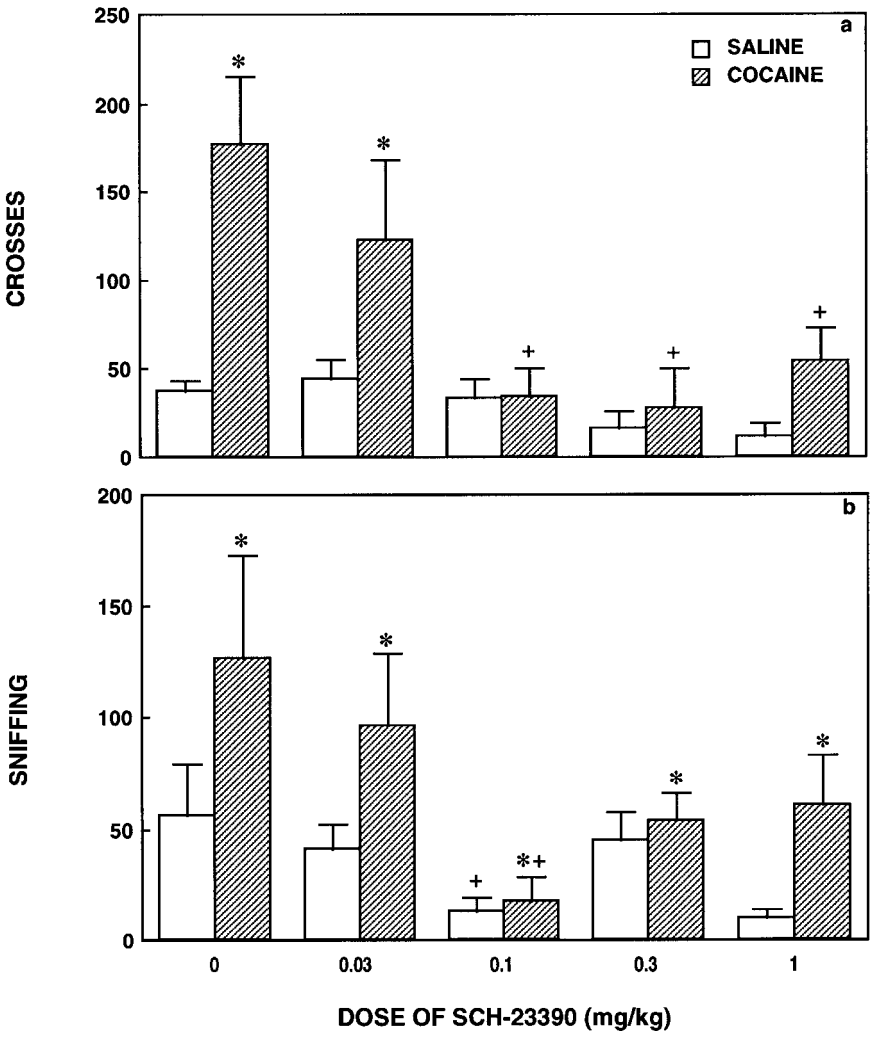

Fig. 2. Mean (±SEM) locomotor activity crosses (**a**) and time-sampled observations of sniffing (**b**) following bilateral infusion of saline or cocaine (100 µg/0.5 µg/side) into the NAc of animals pretreated with varying doses of SCH-23390 (mg/kg, IP). For locomotor activity, asterisks represent a significant increase following cocaine infusion relative to saline ($P < .05$, Fisher LSD test). +, significant decrease relative to animals pretreated with 0 mg/kg SCH-23390 ($P < .05$, Fisher LSD test). For sniffing, asterisks represent a significant ANOVA main effect collapsed across SCH-23390 dose. +, significant difference from animals receiving 0 mg/kg SCH-23390 collapsed across saline vs. cocaine infusions.

saline infusion revealed no significant differences among the groups ($F_{4,34} = 2.4$, n.s.). Thus, SCH-23390 did not alter baseline locomotor activity at any dose. In contrast, a one-way ANOVA of crosses following intraaccumbens cocaine infusion revealed a significant effect of SCH-23390 ($F_{4,34} = 3.65$, $P < .05$). Subsequent pairwise comparisons indicated that the 0.1–1.0 mg/kg doses of SCH-23390 decreased cocaine-induced locomotion relative to vehicle-pretreated controls (Fisher LSD test, $P < .05$). Furthermore, within-subjects comparisons indicated that intraaccumbens cocaine infusion produced an increase in locomotor activity relative to intraaccumbens saline infusion only in animals pretreated with either vehicle or 0.03 mg/kg SCH-23390 (Fisher LSD test, $P < .05$). Therefore, cocaine-induced locomotion was reversed by the three highest doses of SCH-23390.

Intraaccumbens cocaine infusion also produced an increase in sniffing that was dose-dependently reversed by SCH-23390 pretreatment (Fig. 2b). The overall ANOVA revealed a main effect of the repeated measure ($F_{1,34} = 8.5$, $P < .01$), a main effect of SCH-23390 ($F_{4,34} = 3.1$, $P < .05$), but no interaction ($F_{4,34} = .97$, n.s.). The main effect of the repeated measure indicated that intraaccumbens cocaine infusion increased sniffing relative to saline infusion, regardless of pretreatment with SCH-23390. However, the cocaine-induced increase in sniffing was not very robust, since within-subjects comparisons indicated that only animals pretreated with 1 mg/kg SCH-23390 exhibited a significant increase in sniffing following cocaine infusion relative to saline infusion (Fisher LSD test, $P < .05$). Further analysis of the main effect of SCH-23390 indicated that 0.1 mg/kg SCH-23390 decreased sniffing relative to vehicle-pretreated controls, regardless of infusion of saline vs. cocaine (Fisher LSD test, $P < .05$).

Locomotor activity did not change following repeated intraaccumbens infusions of cocaine (data not shown). There was no difference in the locomotor activity produced by intraaccumbens cocaine infusions in animals pretreated with vehicle across the test days ($F_{2,10} = 1.27$, n.s.). These animals exhibited a mean (±SEM) of 177 ±

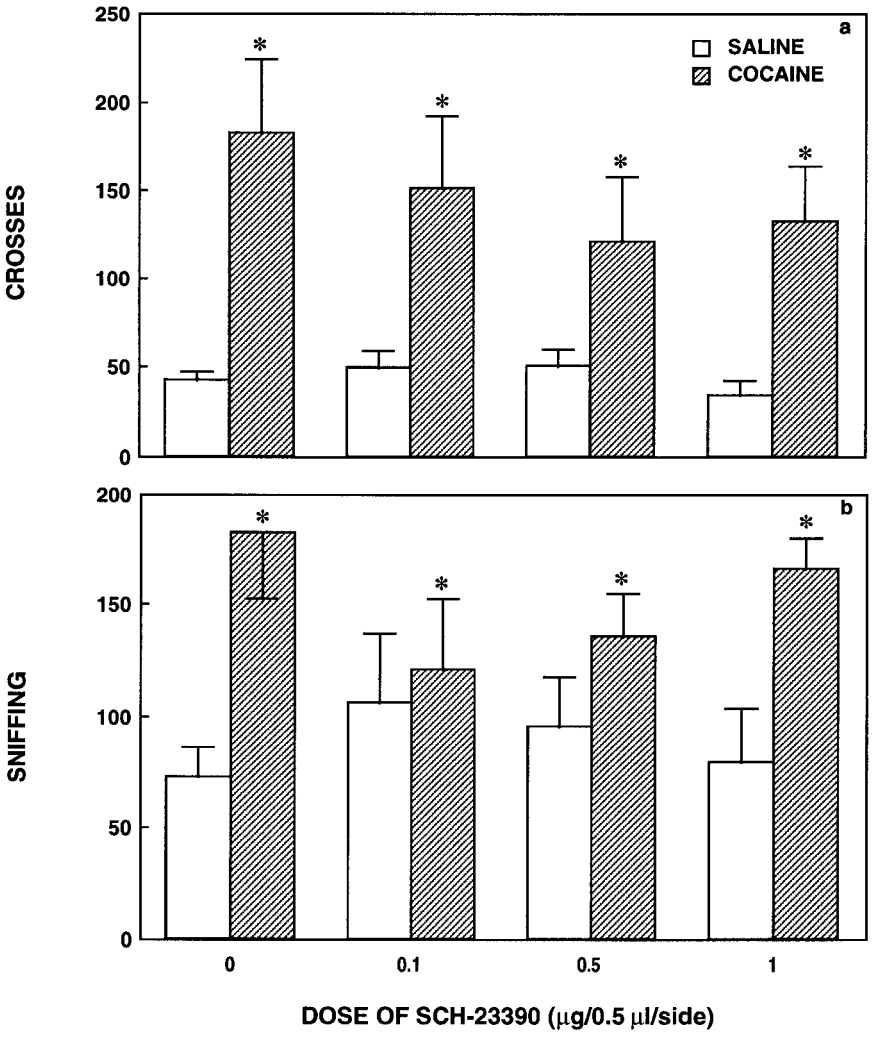

Fig. 3. Mean (±SEM) locomotor activity crosses (**a**) and time-sampled observations of sniffing (**b**) following bilateral infusion of saline or cocaine (100 μg/0.5 μg/side) with varying doses of SCH-23390 into the NAc. *Significant increase following cocaine infusion relative to saline ($P < .05$, ANOVA main effect collapsed across SCH-23390 dose).

38 crosses following the first cocaine infusion and 158 ± 31 crosses following the third infusion. Furthermore, animals pretreated with SCH-23390 prior to cocaine infusions 1 and 2 did not differ from controls in their locomotor response to the third infusion of cocaine alone ($F_{4,34} = 0.3$, n.s.).

Effects of coinfusion of SCH-23390 and cocaine on behavior

Intraaccumbens cocaine infusion produced an increase in locomotion that was not altered by coinfusion of SCH-23390 (Fig. 3a). The overall ANOVA revealed a main effect of the repeated measure ($F_{1,20} = 28.9$, $P < .0001$), but no effect of SCH-23390 ($F_{3,20} = 0.4$, n.s.), nor an interaction ($F_{3,20} = 0.6$, n.s.). Furthermore, within-subjects comparisons indicated that all groups exhibited a significant increase in locomotion following cocaine infusions relative to saline infusions (Fisher LSD test, $P < .05$). These results indicate that locomotion produced by intraaccumbens cocaine infusion was not altered by coinfusion of SCH-23390.

Intraaccumbens cocaine infusion produced an increase in sniffing that was not altered by coinfusion of SCH-23390 (Fig. 3b). The overall ANOVA revealed a main effect of the repeated measure ($F_{1,20} = 28.2$, $P < .0001$), but no effect of SCH-23390 ($F_{3,20} = 0.1$, n.s.), nor an interaction ($F_{3,20} = 2.8$, n.s.). However, the cocaine-induced increase in sniffing was not very robust, since within-subjects comparisons indicated that only animals pretreated with saline or the 1 μg/side dose of SCH-23390 exhibited a significant increase in sniffing following cocaine infusions relative to saline infusions (Fisher's LSD test, $P < .05$). These results suggest that neither baseline nor intraaccumbens cocaine-induced sniffing was altered by coinfusion of SCH-23390.

Receptors occupied by SCH-23390

The mean fmol ^{3}H-SCH-23390 bound/mg protein (±SEM), as well as estimates of the percentage of D1-like receptors occupied by systemic administration of SCH-23390, are reported in Table I. All groups

exhibited greater binding than the vehicle/EEDQ group (Fisher LSD test, $P < .05$), indicating that all doses of SCH-23390 produced significant receptor occupancy. Across doses of SCH-23390, there was a U-shaped, dose-dependent change in occupancy. The 0.3 mg/kg dose produced significantly greater occupancy than the 0.03 mg/kg dose in all regions except the medial prefrontal cortex, and produced greater occupancy than the 1.0 mg/kg dose in the NAc core (Fisher's LSD test, $P < .05$). SCH-23390 occupied more ^{3}H-SCH-23390 binding sites in the anterior regions of the brain (60–100%) relative to the posterior regions (38–72%).

The distribution of D1-like receptors protected from EEDQ-induced inactivation following intraaccumbens infusion of SCH-23390 was evident as darkened regions on the autoradiograms (e.g., see Baker et al., this issue; Neisewander et al., 1995). The mean fmol ^{3}H-SCH-23390 bound/mg protein (±SEM), as well as estimates of the percentage of D1-like receptors occupied by intraaccumbens infusion of SCH-23390, are reported in Table II. All groups exhibited significantly less binding relative to the vehicle/vehicle group in all brain regions (Fisher LSD test, $P < .05$), indicating that a significant number of ^{3}H-SCH-23390 binding sites were not occupied by the intraaccumbens infusion of SCH-23390, and therefore, were not protected from EEDQ binding. However, a dose-dependent increase in receptor occupancy by SCH-23390 was also evident as a significant increase in binding relative to the vehicle/EEDQ group. The highest dose of 1.0 µg/side produced significant occupancy of ^{3}H-SCH-23390 binding sites in all regions examined, with the greatest occupancy in the NAc core (48–60%), intermediate occupancy in the NAc shell (40–45%) and medial CPu (32–40%), and the least occupancy in the lateral CPu (21–23%). The intermediate dose of 0.5 µg/side produced significant occupancy in the NAc core (43–51%) and medial CPu (25–32%), and the lowest dose of 0.1 µg/side produced significant occupancy in the most anterior region of the NAc core (55%) only.

The accuracy of the occupancy estimates depends on the assumption that changes in binding across groups are not due to altered binding kinetics by the drug treatments, and that EEDQ does not competitively displace the antagonist in vivo. The finding that occupancy estimates were as high as 100% in some cases suggests that competitive displacement of the antagonist by EEDQ was negligible. This finding is not surprising, since other studies have suggested that EEDQ has only moderate affinity for dopamine receptors (Burger and Martin-Iversen, 1994; Meller et al., 1985; Saller et al., 1989). Furthermore, previous research has demonstrated that EEDQ decreases B_{max} values for D1-like and D2-like receptors without altering K_d values (Hess et al., 1988). It is important to note, however, that there is some inherent measurement error in the occupancy estimates, and they should not

TABLE I. fmol ^{3}H-SCH-23390 Bound/mg Protein (±SEM) and Estimated Percent Receptors Occupied by SCH-23390 Administered Systemically

Region and distance from bregma (mm)	Vehicle/ vehicle	Vehicle/ EEDQ	0.03 mg/kg SCH/EEDQ	% occupied[1]	0.1 mg/kg SCH/EEDQ	% occupied[1]	0.3 mg/kg SCH/EEDQ	% occupied[1]	1.0 mg/kg SCH/EEDQ	% occupied[1]
NAc core										
1.7	3,302 ± 207[2]	129 ± 50[3]	2,181 ± 336[2,4]	65	2,831 ± 494[2]	85	3,630 ± 186[2]	110	2,541 ± 443[2,4]	76
NAc shell										
1.7	3,629 ± 267[2]	137 ± 49[3]	2,245 ± 340[2–4]	60	2,665 ± 451[2]	72	3,576 ± 167[2]	98	2,675 ± 451[2]	73
Medial CPu										
1.7	3,659 ± 430[2]	150 ± 95[3]	2,347 ± 340[2–4]	63	2,935 ± 503[2]	79	3,645 ± 147[2]	100	2,572 ± 447[2]	69
−0.26	2,800 ± 287[2]	98 ± 27[3]	1,210 ± 167[2–4]	41	1,590 ± 254[2,3]	55	2,038 ± 130[2,3]	72	1,494 ± 276[2,3]	52
Lateral CPu										
1.7	3,845 ± 421[2]	117 ± 62[3]	2,412 ± 361[2–4]	62	3,064 ± 520[2]	79	3,780 ± 163[2]	98	2,765 ± 541[2]	71
−0.26	3,599 ± 289[2]	115 ± 29[3]	1,455 ± 219[2–4]	38	2,007 ± 322[2,3]	54	2,390 ± 130[2,3]	65	1,818 ± 339[2,3]	49
Medial prefrontal cortex										
1.7	202 ± 21[2]	12 ± 12[3]	177 ± 22[2]	87	167 ± 40[2]	82	204 ± 28[2]	101	207 ± 60[2]	103

[1]Values represent the ratio of (SCH-23390/EEDQ − vehicle/EEDQ)/(vehicle/vehicle − vehicle/EEDQ) × 100.
[2]Represents a significant difference from the vehicle/EEDQ group, Fisher LSD test, $P < .05$.
[3]Represents a significant difference from the vehicle/vehicle group, Fisher LSD test, $P < .05$.
[4]Represents a significant difference from the 0.3 mg/kg SCH 23390/EEDQ group, Fisher LSD test, $P < .05$.

TABLE II. fmol ^{3}H-SCH-23390 Bound / mg Protein (±SEM) and Estimated Percent Receptors Occupied by SCH-23390 Administered Into the NAc

Region and distance from bregma (mm)	NAc administration/systemic administration							
	Vehicle/vehicle	Vehicle/EEDQ	0.1 µg SCH/EEDQ	% occupied[1]	0.5 µg SCH/EEDQ	% occupied[1]	1.0 µg SCH/EEDQ	% occupied[1]
NAc pole/core								
2.2	3,472 ± 461[2]	280 ± 100[3]	2,045 ± 218[2,3]	55	1,900 ± 504[2,3]	51	2,202 ± 449[2,3]	60
1.7	3,373 ± 405[2]	430 ± 86[3]	1,596 ± 288[3]	40	1,755 ± 489[2,3]	45	1,947 ± 252[2,3]	52
1.2	2,876 ± 371[2]	223 ± 71[3]	783 ± 284[3]	21	1,367 ± 442[2,3]	43	1,594 ± 204[2,3]	52
0.5	2,789 ± 296[2]	228 ± 74[3]	582 ± 267[3]	14	1,138 ± 449[3]	36	1,464 ± 189[2,3]	48
NAc shell								
1.7	3,680 ± 498[2]	361 ± 68[3]	1,299 ± 267[3]	28	1,520 ± 377[3]	35	1,847 ± 292[2,3]	45
1.2	2,757 ± 423[2]	194 ± 77[3]	536 ± 110[3]	13	1,082 ± 311[3]	35	1,211 ± 212[2,3]	40
Medial CPu								
2.2	3,699 ± 381[2]	101 ± 101[3]	883 ± 184[3]	22	1,028 ± 250[2,3]	26	1,235 ± 188[2,3]	32
1.7	3,843 ± 382[2]	173 ± 113[3]	932 ± 250[3]	21	1,343 ± 278[2,3]	32	1,413 ± 230[2,3]	34
1.2	3,744 ± 360[2]	127 ± 90[3]	718 ± 281[3]	16	1,139 ± 307[2,3]	28	1,587 ± 243[2–4]	40
0.5	3,911 ± 409[2]	224 ± 82[3]	716 ± 237[3]	13	1,157 ± 258[2,3]	25	1,558 ± 128[2–4]	36
−0.26	3,070 ± 401[2]	153 ± 16[3]	187 ± 31[3]	1	401 ± 46[3]	9	748 ± 106[3]	20
Lateral CPu								
2.2	3,903 ± 450[2]	104 ± 82[3]	329 ± 52[3]	6	785 ± 157[3]	18	948 ± 191[2,3]	22
1.7	4,068 ± 428[2]	169 ± 107[3]	489 ± 106[3]	8	820 ± 99[3]	17	1,130 ± 192[2,3]	25
1.2	3,889 ± 426[2]	119 ± 71[3]	446 ± 128[3]	9	662 ± 104[3]	14	944 ± 178[2,3]	23
0.5	4,021 ± 438[2]	205 ± 67[3]	336 ± 34[3]	3	585 ± 80[3]	10	1,007 ± 134[2–4]	21
−0.26	3,569 ± 455[2]	181 ± 50[3]	212 ± 26[3]	1	509 ± 72[3]	10	789 ± 131[3]	18

[1]Values represent the ratio of (SCH-23390/EEDQ − vehicle/EEDQ)/(vehicle/vehicle − vehicle/EEDQ) × 100.
[2]Represents a significant difference from the vehicle/EEDQ group, Fisher LSD test, $P < .05$.
[3]Represents a significant difference from the vehicle/vehicle group, Fisher LSD test, $P < .05$.
[4]Represents a significant difference from the 0.1 µg SCH 23390/EEDQ group, Fisher LSD test, $P < .05$.

be interpreted as absolute measures of occupancy. For instance, extracellular DA levels were higher during behavioral testing when animals had received cocaine relative to the in vivo occupancy assay when animals received SCH-23390 alone. Since DA may compete with SCH-23390 for binding to D1-like DA receptors, occupancy values may be overestimated. However, this source of error does not vary systematically across SCH-23390 dosage groups, since each group received the same dose of cocaine. Therefore, relative differences in occupancy across SCH-23390 dosage groups are informative.

DISCUSSION

The results from experiment 1 suggest that stimulation of D1-like receptors is necessary for locomotor activity produced by intraaccumbens cocaine infusion. This behavior was reversed by systemic administration of the three highest doses of SCH-23390 (0.1–1.0 mg/kg, IP). The finding that SCH-23390 potently reverses locomotor activity produced by intraaccumbens cocaine infusion is consistent with previous research indicating that similar doses of SCH-23390 reverse the locomotion produced by systemic administration of cocaine (Fontana et al., 1993; McCreary and Marsden, 1993; Tella, 1994). Thus, stimulation of D1-like receptors appears to be necessary for locomotor activity produced either by systemic or intraaccumbens cocaine administration.

Intraaccumbens cocaine infusion also produced an increase in sniffing that was likely due to the disruption of habituation. Although sniffing behavior was not quantified in time bins, it was noted that animals receiving saline or cocaine exhibited similar amounts of sniffing early during the test period. However, animals receiving saline typically were inactive by the end of the test period, whereas animals receiving cocaine remained active, resulting in an increase in sniffing behavior. Systemic administration of SCH-23390 produced a U-shaped, dose-dependent decrease in both spontaneous sniffing and sniffing produced by intraaccumbens cocaine infusion. Only animals receiving the intermediate dose of 0.1 mg/kg SCH-23390 exhibited a decrease in sniffing relative to controls.

The overall pattern of changes in behavior suggests that cocaine remained localized in the NAc following infusion, as neither stereotypic head-bobbing nor sensitized locomotor activity following repeated intraaccumbens cocaine infusion was observed. Stereotypic head-bobbing is produced by infusion of psychomotor stimulants into the CPu (Kelley and Delfs, 1991). Thus, the lack of head-bobbing suggests that cocaine did not spread to the CPu. None of the behaviors measured were altered following repeated infusion of cocaine into the NAc. Systemic administration and localized infusion of psychomotor stimulants into the ventral tegmental area (VTA), but not the NAc, produce sensitization of locomotor activity (Hooks et al., 1992; Kalivas and Weber, 1988; Paulson and Robinson, 1991). Thus, the lack of sensitization of locomotion in the present study suggests that cocaine did not diffuse to the ventricles and spread to the VTA.

Systemic administration of SCH-23390 produced an inverted U-shaped, dose-dependent change in receptor occupancy. Specifically, the 0.3 mg/kg dose occupied more ^{3}H-SCH-23390 binding sites than the 0.03 mg/kg dose in all regions except the medial prefrontal cortex,

and occupied more ^{3}H-SCH-23390 binding sites than the 1.0 mg/kg dose in the NAc core. The overall pattern of results suggests a correspondence between receptor occupancy and reversal of cocaine-induced behavior. Specifically, the intermediate doses of SCH-23390 (0.1 and 0.3 mg/kg) occupied a greater number of ^{3}H-SCH-23390 binding sites and produced a greater attenuation of cocaine-induced locomotion and sniffing relative to the lowest (0.03 mg/kg) and highest (1.0 mg/kg) doses of SCH-23390. We previously observed a similar pattern of changes following intraaccumbens infusion of SCH-23390 in which a dose of 0.5 µg/side produced greater occupancy of ^{3}H-SCH-23390 binding sites in some brain regions relative to a dose of 3 µg/side (Neisewander et al., 1995). The reason for less occupancy at higher doses of SCH-23390 is unclear. It is possible that higher concentrations of SCH-23390 are less stable in solution and may precipitate after injection, resulting in less drug distribution relative to lower doses. Alternatively, it is possible that higher doses of SCH-23390 act nonspecifically (i.e., at a non-D1-like site) to produce an increase in dopamine in the NAc, and the extracellular dopamine then competes with SCH-23390 for binding to D1 receptors. This explanation seems unlikely, however, since SCH-23390 has a higher affinity for dopamine D1 receptors relative to dopamine (Waddington and O'Boyle, 1987). In any case, this counterintuitive pattern of receptor occupancy demonstrates the utility of obtaining receptor occupancy measures for interpreting the pharmacologic mechanisms of behavior.

In addition to dose-dependent differences in occupancy of ^{3}H-SCH-23390 binding sites, there were also differences in occupancy in anterior regions relative to posterior regions of both the NAc and CPu following systemic administration of SCH-23390. Specifically, 60–100% of ^{3}H-SCH-23390 binding sites were occupied in anterior regions of the brain, whereas 38–72% of ^{3}H-SCH-23390 binding sites were occupied in posterior regions regardless of dose administered. These region-dependent differences in receptor occupancy likely reflect differences in the distribution of SCH-23390 across brain regions. Furthermore, these results suggest that systemic administration of SCH-23390 may reverse behaviors mediated by the anterior regions of the NAc/CPu more potently than behaviors mediated by posterior regions.

The results from experiment 2 failed to support the hypothesis that stimulation of D1-like receptors *within the NAc* is necessary for locomotor activity produced by intraaccumbens cocaine infusion. Intraaccumbens infusion of SCH-23390 with cocaine failed to alter locomotor activity at any dose. The greatest receptor occupancy was observed at the highest dose of intraaccumbens SCH-23390, which occupied 40–60% of D1-like receptors in the NAc core and shell. However, effective doses of SCH-23390 administered systemically occupied 69–100% of D1-like receptors in the anterior regions of the NAc and CPu. Therefore, it is possible that intraaccumbens infusion of SCH-23390 failed to occupy enough D1-like receptors in the NAc to reverse locomotion produced by intraaccumbens cocaine infusion. However, it is important to consider differences in the distribution of receptors occupied by SCH-23390 given systemically vs. intracranially. Systemic administration of SCH-23390 occupies D1-like receptors throughout the entire region, whereas intracranial infusion of SCH-23390 occupies a significant population of receptors within 0.5 mm of the infusion site (i.e., typically 100%) and a nonsignificant population of receptors in subregions located more distally from the infusion site. Therefore, occupancy measures within an entire region following intracranial drug infusion likely underestimate occupancy in subregions affected by the intraaccumbens cocaine infusion. Previous research from our laboratory offers two other lines of evidence that argue against the idea that an insufficient number of receptors were occupied by SCH-23390 to reverse locomotion produced by intraaccumbens cocaine. First, we found that a higher dose of 3 µg/side SCH-23390 occupied a similar portion of D1-like receptors in the NAc (48–60%) as did the 1 µg/side dose of SCH-23390 used in the present study, suggesting that maximal receptor occupancy in the NAc was obtained. Furthermore, the 3 µg/side dose used previously effectively reversed locomotor activity produced by systemic administration of cocaine, but failed to occupy a greater number of D1-like receptors in the NAc than a lower, ineffective dose of 0.5 µg/side, suggesting that stimulation of D1-like receptors in the NAc is not necessary for cocaine-induced locomotion (Neisewander et al., 1995). Second, we found that locomotor activity produced by 4.2 mg/kg IV cocaine was reversed by 0.5 µg/side SCH-23390, but not by 1.0 µg/side. These doses, however, occupied a similar number (25–62%) of D1-like receptors in the NAc core and shell (Baker et al., this issue). Collectively, these findings suggest that stimulation of D1-like receptors in the NAc is not necessary for cocaine-induced locomotion.

Although the findings suggest that stimulation of D1-like receptors in the NAc is not necessary for cocaine-induced locomotion, they do not preclude the possibility that these receptors play a role in this behavior. Indeed, McGregor and Roberts (1993) demonstrated that 1 µg/side intraaccumbens SCH-23390 significantly decreased locomotor activity elicited by systemic administration of 10 mg/kg cocaine, suggesting that D1-like receptors in the NAc are involved. Intraaccumbens infusion of D1-selective agonists also increases locomotor activity (Anderson and Nielsen, 1986; Arnt, 1987; Breese et al., 1987; Dreher and Jackson, 1989; Meyer, 1993; Meyer et al., 1993), and coinfusion of D1- and D2-selective agonists into the NAc produces synergistic effects on locomotor activity (Dreher and Jackson, 1989; Essman et al., 1993; Plaznik et al.,

1989). Therefore, stimulation of D1-like receptors in the NAc may contribute to cocaine-induced locomotion, but does not appear to be necessary for this behavior.

The findings that systemic, but not intraaccumbens, administration of SCH-23390 potently reversed locomotion produced by intraaccumbens cocaine infusion strongly suggest that stimulation of D1 receptors in regions other than the NAc are involved in cocaine-induced locomotion. Indeed, D1-like receptors in the ventral pallidum, VTA, and substantia nigra pars reticulata have been shown to play a role in locomotor activity elicited by dopamine and opioid agonists (Alesdatter and Kalivas, 1993; Gong et al., 1996; LaHoste and Marshall, 1990; Robertson, 1992; Stewart and Vezina, 1989; Yurek and Hipkens, 1993, 1994). It is possible that intraaccumbens cocaine produces stimulation of D1-like receptors in these regions indirectly via a pathway activated by stimulation of a D2-like, serotonin, or norepinephrine receptor in the NAc. In particular, stimulation of D2-like receptors in the NAc may be critical for initiating cocaine-induced locomotion, since blockade of these receptors is sufficient to reverse locomotion produced by systemic administration of cocaine (Baker et al., 1996; Neisewander et al., 1995). Further research is needed to determine population(s) of D1-like receptors necessary for cocaine-induced locomotion.

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