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Localization of dopamine receptor subtypes occupied by intra-accumbens administration of selective antagonists that reverse cocaine-induced locomotion.

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Research report

Localization of dopamine receptor subtypes occupied by intra-accumbens antagonists that reverse cocaine-induced locomotion

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Abstract

The purpose of this study was to examine whether blockade of either dopamine D1-like or D2-like receptors by selective antagonist administration into the nucleus accumbens (NAc) is sufficient to reverse cocaine-induced locomotion, and to develop a new technique that enables the population of receptors occupied by the antagonists to be quantified. Locomotor activity was assessed in rats that had received bilateral intra-accumbens injections of the D1-selective antagonist SCH-23390 (0–3.0 μg/0.5 μl/side) or the D2/D3-selective antagonist sulpiride (0–1.0 μg/0.5 μl/side), followed 15 min later by injections of saline or cocaine (15 mg/kg, i.p.). To assess receptor occupancy by the antagonists, 105 min prior to sacrifice the rats received intra-accumbens injections of the antagonist, followed 15 min later by an injection of the non-selective irreversible antagonist, *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ; 10 mg/kg, i.p.). Receptors were labeled with [^{3}H]SCH-23390 or [^{3}H]sulpiride in sections containing the NAc, and the autoradiograms allowed quantitation of receptors occupied (i.e. protected from EEDQ) by the antagonist given in vivo. Only a dose of 3 μg/side SCH-23390 reversed cocaine-induced locomotion, whereas a dose of 0.5 μg/side did not alter cocaine-induced locomotion despite occupying the same amount of [^{3}H]SCH-23390 binding sites in the NAc. Intermediate doses of 0.1 and 0.3 μg/side sulpiride reversed cocaine-induced locomotion, and also occupied the greatest number of [^{3}H]sulpiride binding sites in the NAc. The results suggest that blockade of D2-like, but not D1-like, receptors in the NAc is sufficient to reverse cocaine-induced locomotion, and also demonstrate the importance of quantifying receptors occupied by drugs administered intracranially.

Keywords: Cocaine; Sulpiride; SCH-23390; Dopamine receptor; D1-like; D2-like; *N*-Ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline; Receptor occupancy; Locomotor activity; Stereotypy; Nucleus accumbens

1. Introduction

One of the major techniques used to investigate the neural mechanisms of drug-induced behavior is localized intracranial administration. A serious limitation of this technique, however, is the inability to verify specifically the population of receptor sites the drug is acting on since it may spread to neighboring regions. Attempts to localize drug spread include injection of radiolabeled drug, measurement of drug concentration in brain tissue, and anatomical control groups that receive drug injection into neighboring regions (see Wise and Hoffman [47] for review). These techniques, however, do not allow quantitation of the receptors occupied by the drug.

Despite the limitations of intracranial drug administration, much information regarding the neural mechanisms of cocaine-induced behaviors has been gained using this technique. Administration of cocaine directly into the nucleus accumbens (NAc) elicits locomotor activity that is reversed by co-administration of the non-selective dopamine antagonist *cis*-flupenthixol [16,21]. Administration of cocaine into the anterior dorsal and ventrolateral striatum also produces an increase in locomotion that has a delayed onset and is less robust relative to the response produced by intra-accumbens infusions. Delfs et al. [16] suggested that locomotion produced by cocaine infusion at the former two sites may have resulted from diffusion to the NAc. These findings provide strong evidence that cocaine-in-

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duced locomotion is mediated in part by dopamine in the NAc.

More recent studies have examined the role of dopamine receptor subtypes in cocaine-induced locomotion. Dopamine receptors are classified based on gene homologies into two major subfamilies referred to as D1-like and D2-like [41]. The D1-like subfamily includes D1 and D5 receptor subtypes and the D2-like subfamily includes D2, D3, and D4 receptor subtypes. The selectivity of available dopamine antagonists only distinguishes between subfamilies of receptors. Systemic administration of either a D1-selective or a D2-selective antagonist is sufficient to reverse locomotor activity produced by systemic administration of cocaine [19,27], indicating that stimulation of both receptor subfamilies is necessary for this behavior. It is possible that the specific population of D1-like and D2-like receptors necessary for cocaine-induced locomotion may be in the NAc. Indeed, intra-accumbens administration of the D2-selective antagonist eticlopride potently (i.e. 0.1 μg) reverses locomotor activity elicited by systemic administration of cocaine, whereas only relatively high doses (i.e. 1–3 μg) of the D1-selective antagonist SCH-23390 have been reported to decrease this behavior [23,28].

The purpose of the present study was to further examine whether blockade of either D1-like or D2-like receptor subtypes in the NAc is sufficient to reverse locomotor activity elicited by systemic administration of cocaine, and to develop a new technique that enables the population of dopamine receptors occupied by the antagonists to be quantified. Dose-dependent changes in baseline and cocaine-induced locomotion were assessed using intra-accumbens administration of five different doses of SCH-23390 or the D2/D3-selective antagonist sulpiride. Receptor occupancy by the antagonists was assessed subsequently by administering the non-selective irreversible antagonist *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline [20] (EEDQ) systemically 15 min after intra-accumbens administration of antagonist. The population of receptors protected by the selective antagonist from EEDQ-induced inactivation was then analyzed using quantitative autoradiography.

2. Materials and methods

2.1. Drugs and chemicals

SCH-23390 and (−)-sulpiride (Research Biochemicals Inc., Natick, MA) were dissolved in distilled water and injected intracranially at a volume of 0.5 μl. Cocaine hydrochloride was dissolved in saline and injected intraperitoneally at a volume of 1 ml/kg. 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), [^{3}H]sulpiride (68.7 Ci/mmol; New England Nuclear Corp., Boston, MA), and (+)-butaclamol (Research Biochemicals Inc., Natick, MA) were used in the radioligand binding assays.

2.2. Animals

Male Sprague–Dawley rats weighing 250 ± 25 g were housed individually in a climate-controlled facility with a 12-h light/dark cycle. They were acclimated to handling for 3 days prior to surgery.

2.3. Surgery

Animals were anesthetized with pentobarbital (50 mg/kg, i.p.) and atropine sulfate (10 mg/kg, i.p.). The head was then shaved and cleaned, and they were placed into a stereotaxic instrument. A midsagittal incision was made across the skull and the connective tissue was retracted. Holes approximately 1–2 mm in diameter were drilled into the skull and guide cannulae (23 gauge stainless steel) were then lowered through the holes into the NAc at the following coordinates: +2.2 mm AP and ±1.6 mm ML with respect to bregma, and −6.6 mm DV from the surface of the skull. Four screws were drilled into the skull approximately 5 mm anterior and posterior to the cannulae. The cannulae were secured to the skull and screws using dental acrylic cement. Approximately 12 h after surgery, wire stylets were placed into the guide cannulae to maintain patency. The rats were allowed at least 3 days to recover from surgery and were handled each recovery day.

2.4. Behavioral testing

The effects of SCH-23390 on baseline and cocaine-induced behaviors were examined first. Rats received bilateral injections of either 0 (n = 13), 0.1 (n = 8), 0.3 (n = 8), 0.5 (n = 12), 1.0 (n = 9), or 3.0 (n = 9) μg/0.5 μl/side SCH-23390 into the NAc. The injection cannulae (30 gauge stainless steel) were connected to gastight Hamilton syringes by PE-20 tubing and the syringes were placed into an infusion pump (Harvard Apparatus). 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 min later and took place over a 190-s period. The injection cannulae were removed 1 min after the infusion had been completed. Fifteen min later, half of the animals in each group received an injection of cocaine (15 mg/kg, i.p.)

and the other half received saline. Immediately after this injection, the rats were placed into clear Plexiglas cages (44 × 24 × 20 cm high) for 60 min. 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. The latter measure is referred to as crosses. The presence and intensity of head-down sniffing was also measured every 2.5 min based on a 10-s observation period. Intensity was rated on a scale of 1–3 depending on the percentage of time the animal was engaged in the behavior as follows: 0 = 0%, 1 = < 25%, 2 = 25–75%, and 3 = > 75%. The sum of these ratings yielded an overall sniffing score. Three days after the first behavioral test, the animals were injected with the same intracranial dose of SCH-23390 as given previously. Fifteen min after the intracranial injections, the animals that had received saline on test 1 were injected with cocaine and those that had received cocaine on test 1 were injected with saline. Behavior was then measured for 60 min as described above.

Two days after completing the above tests, the animals were reassigned to groups receiving bilateral injections of either 0 ($n = 13$), 0.05 ($n = 8$), 0.1 ($n = 8$), 0.3 ($n = 8$), 0.5 ($n = 13$), or 1 ($n = 8$) μg/0.5 μl/side sulpiride into the NAc. Group assignment was random, except for the constraint that there was no overlap in groups that were to be included in the receptor occupancy measures described below. The effects of intra-accumbens sulpiride on behaviors following injections of saline or cocaine (15 mg/kg, i.p.) were examined over two test days using the same procedure as described above.

2.5. Quantitation of receptors occupied by intra-accumbens antagonists

Three days after completing the behavioral tests, the animals received intracranial injections of SCH-23390 (0.5 or 3 μg/0.5 μl/side) or sulpiride (0.1, 0.3, or 0.5 μg/0.5 μl/side) at the same dose that they had received during behavioral testing. These doses were selected for receptor occupancy measures based on preliminary data using 0.5 μg/0.5 μl/side of each antagonist. These data indicated that this dose of SCH-23390 did not block cocaine-induced locomotion, and therefore, the highest dose was included for comparison. In contrast, this dose of sulpiride attenuated cocaine-induced locomotion, and therefore, lower doses were included for comparison. Fifteen min after the intracranial injections, the animals were given an injection of EEDQ (10 mg/kg, i.p.). Two additional groups of animals were injected intracranially with saline. Fifteen min later, these animals were injected with either vehicle or EEDQ (10 mg/kg, i.p.). All of the animals were sacrificed 90 min after their systemic injection of vehicle or EEDQ. The brains were removed and immersed in isopentane at −20°C for 2 min 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. Sections were taken at four levels including 2.2, 1.7, 1.2, and 0.5 mm anterior to bregma. The sections were thaw-mounted onto gelatin-coated slides, desiccated under vacuum for 2–3 h at 4°C and then for 8 h at −20°C, and 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 min. Duplicate sections from animals injected intracranially with SCH-23390 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 of 7.5 at room temperature). Incubation occurred for 75 min at 37°C, and the reaction was terminated by a 20-min wash in ice-cold buffer. Duplicate sections from animals injected intracranially with sulpiride were preincubated in 50 mM Tris (pH of 7.7 at 4°C) two times for 5 min each at 0°C. The sections were then labeled with 15 nM [^{3}H]sulpiride in the presence or absence of 1 μM (+)-butaclamol in buffer containing 50 mM Tris and 120 mM NaCl (pH of 7.7 at 4°C). Incubation occurred for 60 min at 22°C, and the reaction was terminated by two 5-min washes in ice-cold buffer. Following the wash procedures, the sections were dipped in ice-cold distilled water to remove buffer salts, and then dried at 57°C. The sections were then laid into light-proof X-ray cassettes and were apposed to tritium-sensitive film (Amersham, Arlington Heights, IL). A slide containing plastic tritium standards (American Radiolabelled Chemicals Inc.) that were calibrated using mash sections of rat brain [5] was also apposed to each piece of film. Sections labeled with [^{3}H]SCH-23390 were exposed at room temperature for 7 days and sections labeled with [^{3}H]sulpiride were exposed at room temperature for 6 weeks. The film was processed in Kodak GBX developer and fixer. The autoradiograms were analyzed with a Macintosh-based image processing system using the Image software. Optical density was converted into nCi/mg of radioligand bound using a standard curve as a reference.

2.6. Cannula placement

Cannula placements were determined during tissue sectioning by drawing the cannula tracts at each plate of the Paxinos and Watson atlas [33] within 2.7–0.5 mm anterior to bregma. The most ventral point of the tract was designated as the point of infusion and is illustrated for each subject on Fig. 1.

2.7. Data analyses

Locomotor crosses following saline vs. cocaine administration were analyzed using repeated measures ANOVAs. Significant interactions were further analyzed by one-way ANOVA tests of simple effects, and subsequent pairwise comparisons were made using Newman-Keuls tests. Scores for head-down sniffing were analyzed using non-parametric Kruskal-Wallis ANOVAs, and subsequent pairwise comparisons were made using Mann-Whitney *U*-tests for between subjects comparisons and Wilcoxon signed-ranks tests for within subjects comparisons. The regions analyzed in the autoradiograms included the core and shell of the NAc, the lateral CPu, and the medial CPu. The entire intact portion of each region was included in the analysis. The ^{3}H ligand binding data, expressed as mean fmol bound/mg protein, were analyzed using separate one-way analyses of variance for each brain region, and subsequent pairwise comparisons were made using Newman-Keuls tests. The percentage of receptors occupied by the antagonists administered into the NAc was estimated using the ratio of binding in the following treatment groups (i.e. group designations represent NAc administration/systemic administration):

$$\frac{\text{Antagonist/EEDQ} - \text{Vehicle/EEDQ}}{\text{Vehicle/Vehicle} - \text{Vehicle/EEDQ}}$$

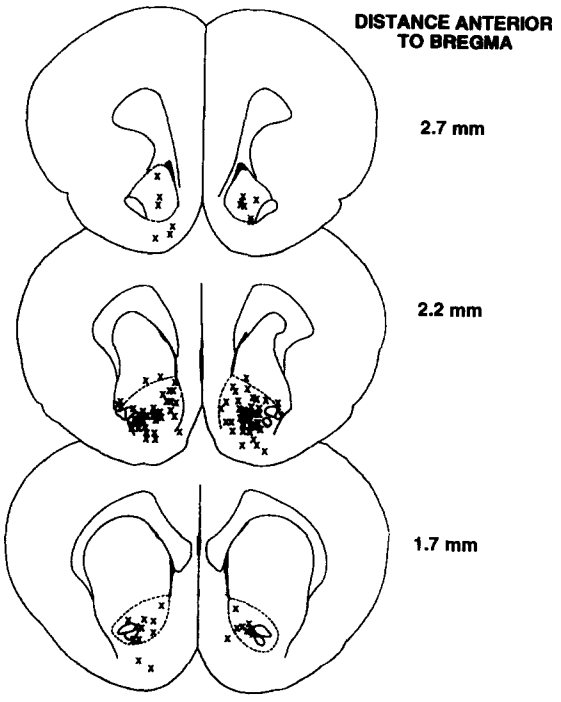

Fig. 1. Approximate position of cannula tips, represented by X's, for each animal included in this study. The drawings were adapted from illustrations in the Paxinos and Watson atlas [33].

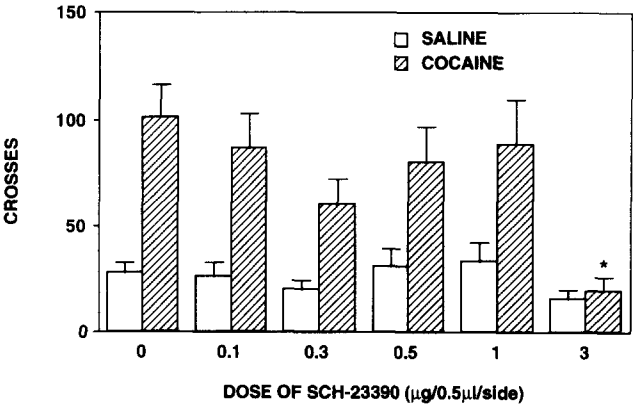

Fig. 2. Locomotor activity crosses (±S.E.M.) following saline or cocaine (15 mg/kg, i.p.) administration in animals pretreated with bilateral infusions of varying doses of SCH-23390 into the NAc. Asterisk (*) represents a significant difference from corresponding control group receiving 0 μg/0.5 μg/side SCH-23390, $P < 0.05$, Newman-Keuls test.

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

3. Results

3.1. Effects of intra-accumbens SCH-23390 on baseline and cocaine-induced behavior

The effects of intra-accumbens SCH-23390 on locomotion following saline or cocaine administration are illustrated in Fig. 2. Cocaine administration produced an increase in locomotion over baseline in all animals except those injected with the highest dose of SCH-23390 into the NAc. The overall ANOVA revealed a significant interaction between the repeated measure (i.e. crosses following saline vs. cocaine administration) and dose of SCH-23390 ($F_{5,49} = 2.26$, $P < 0.05$). A subsequent one-way ANOVA of crosses following saline administration revealed no significant differences among the groups ($F_{1,54} = 0.88$, n.s.). Thus, SCH-23390 administration into the NAc did not alter baseline locomotor activity at any dose. In contrast, a one-way ANOVA of crosses following cocaine administration revealed a significant effect of SCH-23390 dose ($F_{1,54} = 2.85$, $P < 0.05$). Subsequent pairwise comparisons indicated that only the 3 μg/side dose of SCH-23390

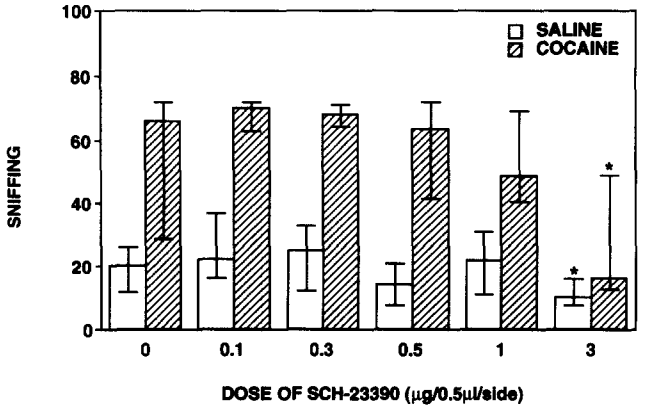

Fig. 3. Head-down sniffing scores (median with semi-interquartile ranges) following saline or cocaine (15 mg/kg, i.p.) administration in animals pretreated with bilateral infusions of varying doses of SCH-23390 into the NAc. Asterisk (*) represents a significant difference from corresponding control group receiving 0 μg/0.5 μg/side SCH-23390, $P < 0.05$, Mann-Whitney U-test.

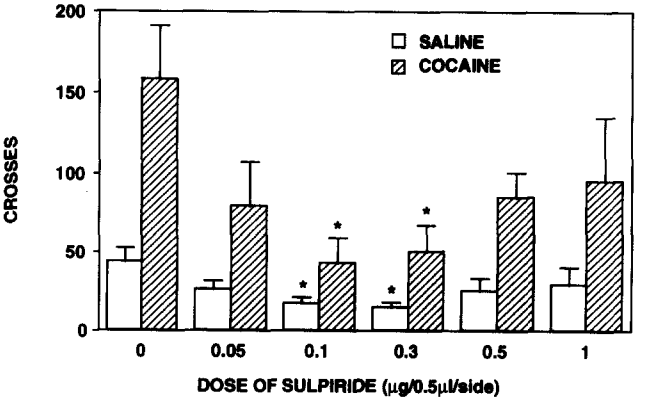

Fig. 4. Locomotor activity crosses (±S.E.M.) following saline or cocaine (15 mg/kg, i.p.) administration in animals pretreated with bilateral infusions of varying doses of sulpiride into the NAc. Asterisk (*) represents a significant difference from corresponding control group receiving 0 μg/0.5 μg/side sulpiride, $P < 0.05$, Newman-Keuls test.

decreased locomotion relative to controls (Newman-Keuls test, $P < 0.05$). Furthermore, each group exhibited a significant increase in locomotion following cocaine vs. saline administration ($P < 0.05$), except animals receiving 3 μg/side SCH-23390 into the NAc. Therefore, cocaine-induced locomotion was reversed completely by the highest dose of SCH-23390, and was not altered significantly at any other dose.

The effects of intra-accumbens SCH-23390 on head-down sniffing following saline or cocaine administration are illustrated in Fig. 3. Cocaine administration produced an increase in sniffing over baseline in all animals regardless of dose of SCH-23390 injected into the NAc (Wilcoxon signed-rank tests, $P < 0.05$). A Kruskal-Wallis ANOVA of sniffing scores revealed a significant effect of SCH-23390 dose following both saline administration ($H = 12.5$, $P < 0.05$) and cocaine administration ($H = 16.7$, $P < 0.01$). Subsequent pairwise comparisons indicated that the 3 μg/side dose of SCH-23390 decreased both baseline and cocaine-induced sniffing relative to controls (Mann-Whitney U-test, $P < 0.05$).

3.2. Effects of intra-accumbens sulpiride on baseline and cocaine-induced behavior

The effects of intra-accumbens sulpiride on locomotion following saline or cocaine administration are illustrated in Fig. 4. Cocaine administration produced an increase in locomotion over baseline that was attenuated by the two intermediate doses of intra-accumbens sulpiride. The overall ANOVA revealed significant main effects of the repeated measure (i.e. crosses following saline vs. cocaine administration; $F_{1,49} = 34.8$, $P < 0.01$) and dose of sulpiride ($F_{5,49} = 2.87$, $P < 0.05$), but no significant interaction between these variables. These findings indicate that cocaine increased locomotion relative to baseline regardless of dose of sulpiride injected into the NAc, and that sulpiride dose-dependently altered locomotion in the same manner regardless of whether the locomotion was saline- or cocaine-induced. Pairwise comparisons, however, revealed that animals injected with the two intermediate doses of sulpiride (0.1 and 0.3 μg/side) did not exhibit an increase in locomotion following cocaine relative to saline administration. Furthermore, these groups exhibited significantly fewer crosses relative to controls (Newman-Keuls test, $P < 0.05$). These findings indicate that these two doses of sulpiride decreased baseline locomotion and reversed cocaine-induced locomotion.

The effects of intra-accumbens sulpiride on baseline and cocaine-induced head-down sniffing are illustrated in Fig. 5. There were no significant differences in baseline ($H = 4.4$) or cocaine-induced ($H = 0.8$) head-down sniffing across groups. Cocaine administration

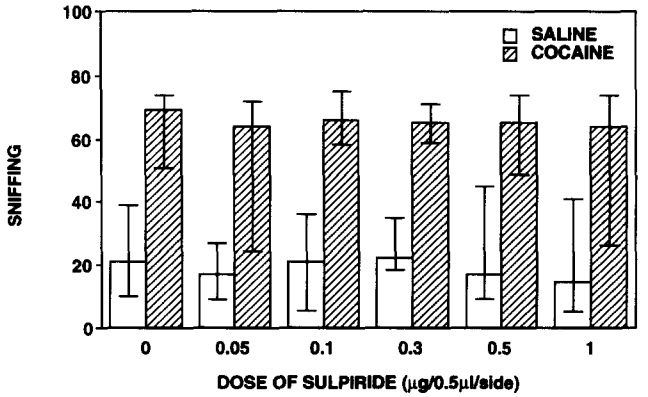

Fig. 5. Head-down sniffing scores (median with semi-interquartile ranges) following saline or cocaine (15 mg/kg, i.p.) administration in animals pretreated with bilateral infusions of varying doses of sulpiride into the NAc.

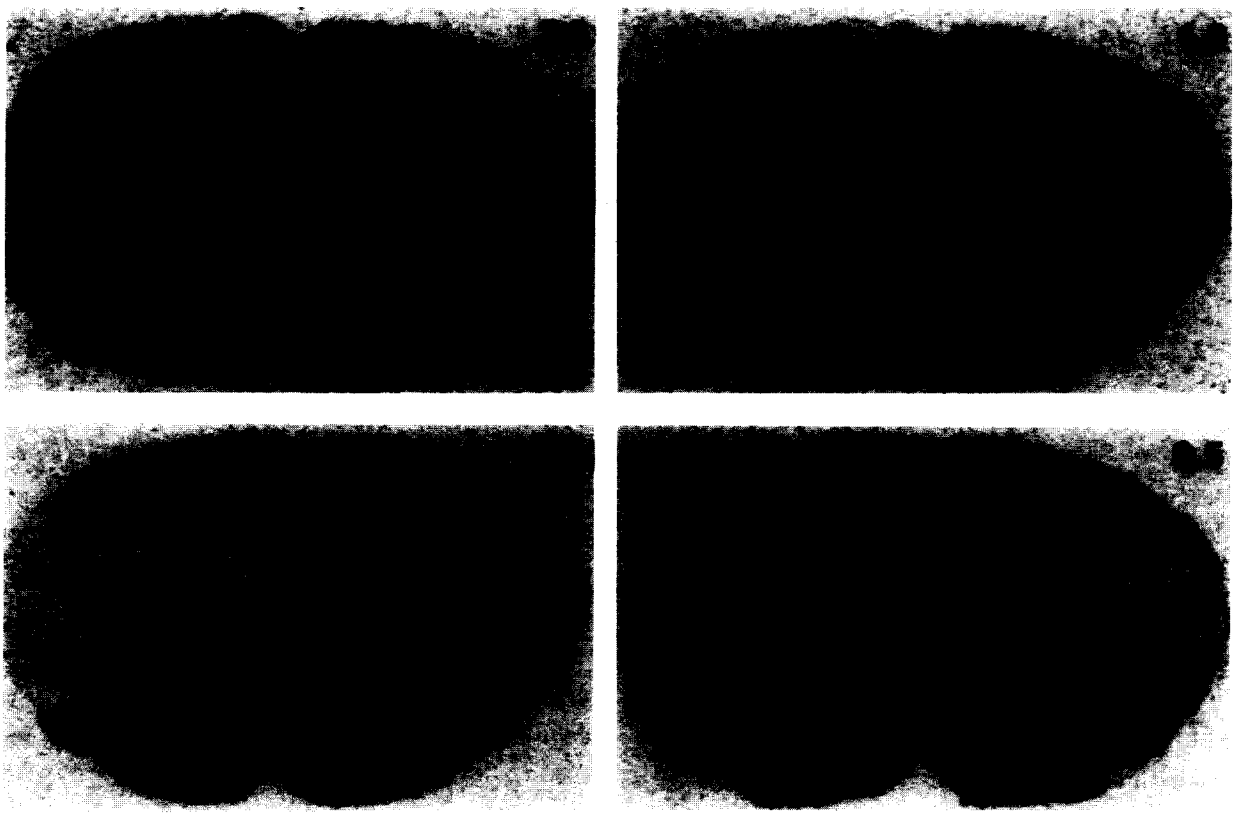

Fig. 6. Representative autoradiograms of sections labeled with 4 nM [^{3}H]SCH-23390. These sections were obtained from an animal that had received bilateral infusions of 0.5 μg/0.5 μl/side SCH-23390 into the NAc, followed by a systemic injection of EEDQ (10 mg/kg, i.p.) 15 min later. The darkened regions represent the population of binding sites occupied by the SCH-23390 administered intracranially. Numbers in the upper righthand corner indicate distance (mm) anterior with respect to bregma.

Table 1
Amount (fmol) [^{3}H]SCH-23390 bound/mg protein (±S.E.M.) and estimated percentage of receptors occupied by SCH-23390 administered into the NAc

Region and distance from bregma (mm)	NAc administration/systemic administration					
	Vehicle/ vehicle $n = 8$	Vehicle/ EEDQ $n = 5$	SCH (0.5 μg/side)/ EEDQ $n = 6$	Percent occupied [a]	SCH (3.0 μg/side)/ EEDQ $n = 6$	Percent occupied [a]
NAc core						
2.2	3612 ± 251 [b]	437 ± 135 [b]	2242 ± 421	56.8	2339 ± 282	59.9
1.7	3449 ± 265 [b]	429 ± 106 [b]	2756 ± 305 [b]	77.0	2024 ± 349	52.8
1.2	2885 ± 219 [b]	344 ± 64 [b]	1626 ± 461	50.4	1598 ± 311	49.4
0.5	1855 ± 120 [b]	180 ± 25 [b]	1045 ± 212	51.6	1183 ± 190	59.9
NAc shell						
1.7	2998 ± 290 [b]	298 ± 36 [b]	2493 ± 395 [c]	81.3	1596 ± 262	48.4
1.2	2152 ± 217 [b]	256 ± 37 [b]	1386 ± 336	59.6	1213 ± 206	50.5
Medial CPU						
2.2	3408 ± 256 [b]	257 ± 42 [b]	1289 ± 476	32.8	1758 ± 296	47.6
1.7	3683 ± 279 [b]	270 ± 32 [b]	1566 ± 461	38.0	2062 ± 272	52.5
1.2	3614 ± 295 [b]	316 ± 47 [b]	1364 ± 450	31.8	1884 ± 263	47.5
0.5	3596 ± 271 [b]	278 ± 31 [b]	1121 ± 375 [b]	25.4	1881 ± 225	48.3
Lateral CPu						
2.2	3429 ± 262 [b]	247 ± 34 [b]	1246 ± 462	31.4	1526 ± 284	40.2
1.7	3920 ± 318 [b]	271 ± 39 [b]	1043 ± 320	21.2	1603 ± 267	36.5
1.2	3827 ± 306 [b]	285 ± 51 [b]	962 ± 304	19.1	1405 ± 192	31.6
0.5	3958 ± 282 [b]	282 ± 39 [b]	1068 ± 380	21.3	1449 ± 184	31.7

[a] Values represent the ratio of (SCH-23390/EEDQ − vehicle/EEDQ)/(vehicle/vehicle − vehicle/EEDQ) × 100.
[b] Represents a significant difference from all groups, Newman-Keuls test, $P < 0.05$.
[c] Represents a significant difference from SCH-23390 3.0 μg/EEDQ group, Newman-Keuls test, $P < 0.05$.

produced a general trend toward an increase in head-down sniffing across all groups that was significant in animals injected with 0.05, 0.5, and 1.0 μg/side sulpiride into the NAc (Wilcoxon singed-rank tests, $P < 0.05$). Thus, sulpiride did not appear to alter baseline or cocaine-induced head-down sniffing.

Receptors occupied by intra-accumbens antagonists

Representative autoradiograms of sections from animals receiving 0.5 μg/side SCH-23390 or 0.5 μg/side sulpiride into the NAc are illustrated in Figs. 6 and 7, respectively. The autoradiograms illustrate the ability to visualize receptors occupied by antagonist administration in vivo using protection from EEDQ binding. The mean fmol [^{3}H]SCH-23390 bound/mg protein (±S.E.M.) in each brain region is reported in Table 1. EEDQ occupied 88–93% of [^{3}H]SCH-23390 binding sites (i.e. ratio of vehicle/EEDQ group to vehicle/vehicle group). All groups exhibited significantly less binding relative to the vehicle/vehicle group in all brain regions (Newman-Keuls test, $P < 0.05$), indicating a significant number of [^{3}H]SCH-23390 binding sites were unoccupied by SCH-23390 administered intracranially. All groups exhibited significantly more [^{3}H]SCH-23390 binding relative to the vehicle/EEDQ group in all brain regions (Newman-Keuls test, $P < 0.05$), indicating a significant number of [^{3}H]SCH-23390 binding sites were occupied by SCH-23390 administered intracranially. The 0.5 μg/side dose occupied a greater percentage of [^{3}H]SCH-23390 binding sites in the core and shell of the NAc (50–81%) relative to the CPu (19–38%). The 3.0 μg/side dose, however, occupied only a slightly greater percentage of binding sites in the NAc (48–60%) relative to the CPu (32–52%). Surprisingly, the 0.5 μg/side dose produced greater occupancy of [^{3}H]SCH-23390 binding sites in the NAc relative to the 3 μg/side dose that reached significance in regions 1.7 mm anterior to bregma (Newman-Keuls test, $P < 0.05$). The 3 μg/side dose, however, occupied more receptors in the CPu relative to the 0.5 μg/side dose (i.e. 31–52% vs. 19–38%, respectively).

Fig. 7 illustrates that sulpiride occupied a large population of [^{3}H]sulpiride binding sites in the CPu, as well as the NAc. This is further demonstrated by the mean fmol [^{3}H]sulpiride bound/mg protein (±S.E.M.) in each brain region reported in Table 2. EEDQ occupied 81–93% of [^{3}H]sulpiride binding sites (i.e. ratio of vehicle/EEDQ group to vehicle/vehicle group). All groups exhibited significantly less binding relative to

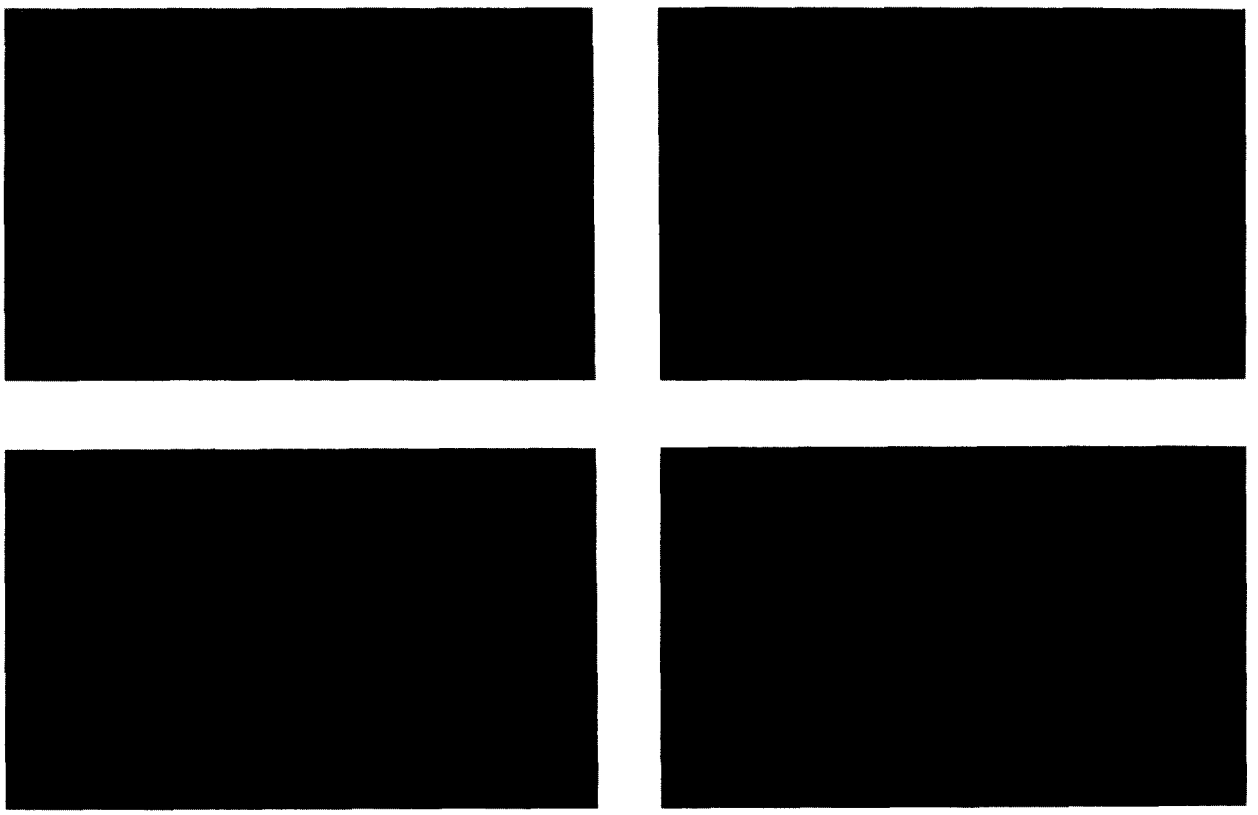

Fig. 7. Representative autoradiograms of sections labeled with 15 nM [^{3}H]sulpiride. These sections were obtained from an animal that had received bilateral infusions of 0.5 μg/0.5 μl/side sulpiride into the NAc, followed by a systemic injection of EEDQ (10 mg/kg, i.p.) 15 min later. The darkened regions represent the population of binding sites occupied by the sulpiride administered intracranially. Numbers in the upper righthand corner indicate distance (mm) anterior with respect to bregma.

Table 2
Amount (fmol) [^{3}H]sulpiride bound/mg protein (±S.E.M.) and estimated percentage of receptors occupied by sulpiride administered into the NAc

Region and distance from bregma (mm)	Nac administration/systemic administration							
	Vehicle/ vehicle $n = 8$	Vehicle/ EEDQ $n = 7$	Sulpiride (0.1 μg/side)/ EEDQ $n = 9$	Percent occupied [a]	Sulpiride (0.3 μg/side)/ EEDQ $n = 6$	Percent occupied [a]	Sulpiride (0.5 μg/side)/ EEDQ $n = 9$	Percent occupied [a]
NAc core								
2.2	649 ± 27 [b]	121 ± 20 [b]	423 ± 42 [b]	57.2	511 ± 63 [b]	73.9	364 ± 37 [b]	46.0
1.7	557 ± 35	81 ± 18 [b]	449 ± 34 [c]	77.3	490 ± 42	85.9	402 ± 48 [c]	67.4
1.2	519 ± 25	49 ± 8 [b]	361 ± 58 [c]	66.4	463 ± 43	88.1	309 ± 48 [c]	55.3
0.5	357 ± 31 [b]	27 ± 5 [b]	253 ± 46 [c]	68.5	211 ± 72 [c]	55.8	153 ± 48 [c]	38.2
NAc shell								
1.7	451 ± 39	70 ± 7 [b]	354 ± 46 [c]	74.5	372 ± 45	79.3	308 ± 47 [c]	62.5
1.2	384 ± 41	40 ± 9 [b]	316 ± 64	80.2	363 ± 41	93.9	209 ± 38 [c]	49.1
Medial CPu								
2.2	695 ± 23 [b]	82 ± 13 [b]	487 ± 50 [c]	66.1	584 ± 144 [b]	81.9	447 ± 62 [c]	59.5
1.7	686 ± 23 [b]	75 ± 9 [b]	479 ± 39 [c]	66.1	534 ± 98 [c]	75.1	459 ± 47 [c]	62.8
1.2	679 ± 21 [b]	50 ± 9 [b]	415 ± 63 [c]	58.0	496 ± 98 [c]	70.9	338 ± 54 [c,d]	45.8
0.5	636 ± 29 [b]	43 ± 5 [b]	374 ± 53 [c]	55.8	303 ± 94 [c]	43.8	180 ± 34 [b]	23.1
Lateral CPu								
2.2	705 ± 23 [b]	83 ± 13 [b]	440 ± 65 [c]	57.4	561 ± 163 [c]	76.8	314 ± 55 [c,d]	37.1
1.7	719 ± 23 [b]	66 ± 12 [b]	448 ± 54 [c]	58.5	452 ± 98 [c]	59.1	225 ± 46 [b]	24.3
1.2	769 ± 32 [b]	60 ± 16 [c]	395 ± 69 [c]	47.2	400 ± 131 [c]	47.9	173 ± 39 [c,d]	15.9
0.5	709 ± 29 [b]	61 ± 14 [c]	384 ± 63 [c]	49.8	346 ± 147 [c]	44.0	137 ± 30 [c,d]	21.1

[a] Values represent the ratio of (sulpiride/EEDQ − vehicle/EEDQ)/(vehicle/vehicle − vehicle/EEDQ) × 100.
[b] Represents a significant difference from all groups, Newman-Keuls test, $P < 0.05$.
[c] Represents a significant difference from vehicle/vehicle group, Newman-Keuls test, $P < 0.05$.
[d] Represents a significant difference from sulpiride 0.3 μg/EEDQ group, Newman-Keuls test, $P < 0.05$.
[e] Represents a significant difference from all groups except sulpiride 0.5 μg/EEDQ group, Newman-Keuls test, $P < 0.05$.

the vehicle/vehicle group in all brain regions (Newman-Keuls test, $P < 0.05$) except for binding in the anterior regions of the NAc in groups receiving the 0.1 and 0.3 μg/side doses. Thus, there was a significant number of binding sites unoccupied by sulpiride administered intracranially in all but the later cases. All groups exhibited significantly more [^{3}H]sulpiride binding relative to the vehicle/EEDQ group in all brain regions (Newman-Keuls test, $P < 0.05$) except the 0.5 μg/side dose in the posterior regions of the lateral CPu. Thus, there was a significant number of binding sites occupied by sulpiride administered intracranially in all but the latter case. A similar pattern of binding sites occupied by sulpiride across brain regions was observed in each dosage group. In general, sulpiride occupied a greater percentage of receptors in anterior regions relative to posterior regions with the exception of the NAc core, and a greater percentage of receptors in the NAc and medial CPu relative to the lateral CPu. An inverted U-shaped dose-dependent pattern of occupancy of binding sites by sulpiride was observed. In general, the 0.3 μg/side dose produced a greater occupancy of binding sites relative to both the 0.1 and 0.5 μg doses that reached significance in NAc and medial CPu 2.2 mm from bregma (Newman-Keuls test, $P < 0.05$). Furthermore, the 0.1 μg/side dose produced greater occupancy of binding sites relative to the 0.5 μg/side dose that reached significance in the NAc core 2.2 mm from bregma.

The accuracy of the estimates of occupancy depends on the assumption that changes in binding across groups were not due to altered binding kinetics by the drug treatments, and that EEDQ did not competitively displace the antagonist in vivo. To further address these concerns, a small equal-sized sample of binding was measured in a region consistently occupied by the antagonist (i.e. a visibly darkened region) in the core of the NAc at levels 2.2–1.2 mm from bregma in the 0.5 μg/side SCH-23390, 0.3 μg/side sulpiride, and respective control groups. Occupancy estimates of 94–110% relative to controls for both drugs were obtained (data not shown). These results suggest 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 [9,29,40]. Furthermore, previous research has demonstrated that EEDQ decreases B_{max} values for D1-like and D2-like receptors without altering K_d values [22]. The antagonist administered in vivo could potentially occupy binding sites at a non-saturating concentration of radioligand, and this would lead to underestimation of receptor occupancy. The finding that antagonist-treated animals exhibited the same amount of binding in the sampled regions

relative to controls suggests that this was not a problem at the concentrations of radioligand used in the present study.

4. Discussion

The results from this study do not support the hypothesis that blockade of D1 receptors in the NAc is sufficient to reverse cocaine-induced locomotor activity. Cocaine-induced locomotion was not altered by any dose of intra-accumbens SCH-23390, except the highest dose of 3 μg/side, consistent with Kaddis et al. [23]. The 3 μg/side dose of SCH-23390 did not alter baseline locomotor activity, suggesting that it produced a specific reversal of cocaine-induced locomotion. However, the results from the binding assays indicate that the ineffective dose of 0.5 μg/side occupied the same or slightly greater number of [^{3}H]SCH-23390 binding sites in both the core and shell of the NAc as the 3 μg/side dose. Therefore, the effects of the 3 μg/side dose are not likely due to blockade of D1-like receptors in the NAc.

Although the findings suggest that blockade of D1-like receptors in the NAc is not sufficient to reverse cocaine-induced locomotion, they do not preclude the possibility that these receptors play a role in this behavior. Indeed, McGregor and Roberts [28] have demonstrated that 1 μg/side intra-accumbens SCH-23390 significantly decreased locomotor activity elicited by 10 mg/kg cocaine, suggesting that D1-like receptors in the NAc are involved. It is unclear whether this dose of SCH-23390 produced a complete or partial reversal of cocaine-induced locomotion since this study did not include a control group for baseline locomotor activity. The 1 μg/side dose of SCH-23390 did not decrease cocaine-induced locomotion in the present study. This discrepancy may be due to differences in dose of cocaine. The higher dose used in the present study may have resulted in threshold stimulation of D1 receptors in other brain regions involved in cocaine-induced locomotion, and therefore, the behavior would be affected less by intra-accumbens SCH-23390. In any case, it is unclear whether the decrease in cocaine-induced locomotion by 1 μg/side dose of SCH-23390 in McGregor and Roberts' study was due to blockade of D1 receptors in the NAc since it is possible that the SCH-23390 may have spread to another region. Although the present study did not measure receptors occupied by this dose, it was demonstrated that the ineffective dose of 0.5 μg/side occupied a similar number of [^{3}H]SCH-23390 binding sites in the NAc as the effective dose of 3 μg/side. Therefore, it seems that doses greater than 0.5 μg/side may produce effects, at least in part, by blocking D1-like receptors in other brain regions. Thus, the role of D1 receptors in the NAc in cocaine-induced locomotion remains equivocal. Intra-accumbens administration of D1-selective agonists increases locomotor activity [2,4,7,12,17,30,31] and co-administration of D1- and D2-selective agonists into the NAc produces synergistic effects on locomotor activity [17,18,36]. Therefore, it seems likely that D1-like receptors in the NAc play a role in cocaine-induced locomotion.

The findings that systemic administration of SCH-23390 potently reverses cocaine-induced locomotion [19,27], yet only relatively high doses of intra-accumbens SCH-23390 ($\geq$ 1 μg/side) decrease this behavior, strongly suggest that stimulation of D1 receptors in regions other than the NAc are involved in cocaine-induced locomotion. One possible region that may play a role is the CPu. The present study found that the 3 μg/side dose which reversed cocaine-induced locomotion and sniffing occupied more [^{3}H]SCH-23390 binding sites in the CPu relative to the 0.5 μg/side dose which did not alter the behavior (i.e. 31–52% vs. 19–38%, respectively). In general, it is believed that stimulant-induced locomotor activity is mediated by mesolimbic dopamine neurons terminating in the NAc, whereas stereotypies are mediated by nigrostriatal dopamine neurons terminating the CPu [13,14,24,25]. However, due to the proximity of these pathways, it is difficult to manipulate only one of these systems using lesion or intracranial drug administration techniques, and some changes in locomotor activity following manipulations in the CPu have been reported [3,11,16,35,45]. Perhaps blockade of D1-like receptors in both the NAc and CPu is necessary to reverse locomotion produced by cocaine administered systemically. If this is the case, then the 3 μg/side dose may have reversed cocaine-induced locomotion by blocking a sufficient number of D1-like receptors in both the NAc and CPu. Consistent with this idea, the 3 μg/side dose also reduced sniffing behavior which is mediated, at least in part, by the CPu [6,10,43].

The autoradiograms of [^{3}H]SCH-23390 binding revealed that the SCH-23390 administered intracranially occupied sites along the border of the lateral ventricles. Therefore, it is likely that some SCH-23390 diffused into the ventricles and may have been transported to distal brain regions. D1-like receptors in the ventral pallidum, ventral tegmental area, and substantia nigra pars reticulata have been shown to play a role in locomotor activity elicited by dopamine and opioid agonists [1,26,37,44,48,49]. Perhaps at the higher dose of 3 μg/side, a functionally significant population of D1-like receptors in one or more of these distal brain regions may have been blocked.

In contrast to the effects of SCH-23390, intra-accumbens sulpiride potently decreased cocaine-induced locomotor activity. A complete reversal of cocaine-induced locomotion was observed at doses of 0.1–0.3

μg/side, whereas higher doses of 0.5–1.0 μg/side did not significantly alter this behavior. Since sulpiride is an antagonist at both D2 and D3 dopamine receptor subtypes, it is possible that the U-shaped dose–response curve is due to differences in the ratio of D2:D3 receptor blockade at different doses. Sulpiride has a higher affinity for D2 receptors relative to D3 receptors [42]. Thus, the effects of lower doses may be due to preferential blockade of D2 receptors, whereas higher doses may block both D2 and D3 receptors. Consistent with this idea, studies using D3-preferring agonists and antagonists have suggested that D3 receptors play an inhibitory role in locomotor activity [15,32,34,45,46], and therefore, the higher doses of sulpiride may be less effective in reversing cocaine-induced locomotion due to greater occupancy of D3 receptors. Although this reasoning seems plausible, the receptor occupancy data from the present study fail to support this explanation since occupancy of [^{3}H]sulpiride bindings sites would be expected to increase with increasing doses of sulpiride. The results, however, indicated an inverted U-shaped dose–response curve with the greatest amount of occupancy at the most behaviorally effective doses of 0.1 and 0.3 μg/side. The reason for less occupancy of binding sites at higher doses of sulpiride is unclear. One potential explanation is that high concentrations of sulpiride may be less stable in solution and may precipitate when injected into the brain, resulting in less diffusion and binding relative to lower concentrations that remain in solution. Alternatively, it is possible that dopamine release is increased with increasing doses of sulpiride due to blockade of autoreceptors, and that the increased amount of dopamine competes with sulpiride for binding sites. This explanation seems unlikely, since sulpiride has a higher affinity for dopamine receptors relative to dopamine [42]. Lastly, it is possible that the kinetics of in vivo binding of sulpiride involve negative cooperativity in which high concentrations of ligand inhibit binding. Regardless of the explanation, these data exemplify the utility of receptor occupancy measures for interpreting data obtained using intracranial drug administration.

The greatest differences in occupancy of [^{3}H]sulpiride binding sites between groups receiving the behaviorally effective doses of 0.1 and 0.3 μg/side and the group receiving the ineffective dose of 0.5 μg/side were in the NAc and the lateral regions of the CPu. However, the patterns of occupancy across dosage groups were similar across all regions, and therefore, the role of D2-like receptors in subregions of the CPu/NAc cannot be interpreted. Furthermore, the autoradiograms illustrate binding along the lateral ventricles, suggesting that some sulpiride may have been transported to distal brain regions. It seems unlikely, however, that the low doses of 0.1–0.3 μg/side would result in diffusion of an amount sufficient to occupy a functionally significant population of D2-like receptors in distal brain regions. The observation that sulpiride spread considerably from the injection site was surprising since sulpiride is a hydrophilic compound[3], and therefore, must diffuse through interstitial fluid rather than passing through cell membranes. The diffuse pattern of sulpiride binding will necessitate the use of anatomical control groups in future studies aimed at elucidating the role of D2-like receptors in anatomical subregions using intracranial administration of sulpiride. However, the findings at hand, together with previous research indicating that cocaine-induced locomotor activity is mediated by dopamine in the NAc [16,21,24] and is potently reversed by D2-like, but not D1-like, antagonists [23,28], are consistent with the hypothesis that blockade of D2-like receptors in the NAc is sufficient to reverse cocaine-induced locomotor activity.

Determining receptor occupancy offers several advantages over current histological procedures for interpreting the anatomical and pharmacologic mechanisms of drugs administered intracranially. The ability to visualize the spread of the drug allows more accurate interpretation of the anatomical region(s) affected by intracranial drug administration. In addition, the technique allows one to determine dose-dependent differences in occupancy and to correlate this measure with behavioral changes. This was particularly useful in interpreting the U-shaped dose–effect curve observed following intra-accumbens sulpiride administration, since occupancy did not increase with increasing doses as would be expected. Furthermore, comparing the degree of receptor occupancy by effective and ineffective doses may allow one to determine the threshold number of receptors in a given region needed to produce a behavioral effect. Alternatively, if there is no difference in occupancy by effective vs. ineffective doses in a given region, the findings would suggest that receptors in other regions are involved with the effective doses. For instance, in the present study, such a comparison suggested that intra-accumbens doses of SCH-23390 greater than 0.5 μg/side likely produce effects by blocking D1-like receptors in regions other than the NAc. This finding has broad implications since high doses of SCH-23390 into the NAc have also been used to characterize neural mechanisms of the reinforcing properties of cocaine [38,39]. The technique could be refined in future studies by measuring receptors in distal brain regions to determine whether drug may have been transported through the ventricular system. In addition, the actual selectivity of high doses of intracranially administered antagonist could be assessed by measuring occupancy of the alternative receptor subfamily. The receptor occupancy technique has a broad range of application since EEDQ is a

non-selective monoamine receptor antagonist and may, therefore, be used in studies localizing behavioral effects produced at other monoamine receptors.

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