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Differential Effects of Intra-Accumbens Sulpiride on Cocaine-Induced Locomotion and Conditioned Place Preference[1]

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ABSTRACT

The effects of intra-accumbens sulpiride on conditioned place preference and locomotion produced by i.v. cocaine were investigated. Every other day during conditioning, rats received infusions of sulpiride (0–0.4 μg) into the nucleus accumbens (NAc) or caudate-putamen. Fifteen min later, they were placed into a distinct compartment and injected with saline or cocaine (4.2 mg/kg, i.v.). On the alternate days, rats received sham intracranial injections and were placed into a different compartment. Locomotion and stereotypies were assessed after the first and last injection, and conditioned place preference was assessed 24 hr after the last conditioning day. After behavioral testing, receptors occupied by sulpiride were quantified by injecting rats intracranially with their respective dose of sulpiride, followed by a systemic injection of the irreversible antagonist N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ). Receptors protected from EEDQ induced inactivation by sulpiride were revealed on autoradiograms of sections labeled with ^{3}H-sulpiride. Sulpiride did not alter cocaine-conditioned place preference or cocaine-induced stereotypies. However, the two lowest doses of intra-accumbens sulpiride attenuated cocaine-induced locomotion and occupied >42% of the sulpiride binding sites in the NAc, and the highest dose completely reversed cocaine-induced locomotion and occupied >96% of the sulpiride binding sites in the NAc. Intra-caudate sulpiride also attenuated cocaine-induced locomotion without occupying a significant number of binding sites in the NAc. These findings suggest that D2-like receptors in the NAc and anterior medial caudate-putamen are involved in cocaine-induced locomotion, but not cocaine-conditioned place preference.

DA neurotransmission in the NAc is thought to mediate the rewarding properties of cocaine (Roberts *et al.*, 1977, 1980; Robledo *et al.*, 1992). 6-OHDA lesions of dopaminergic neurons in the NAc disrupt cocaine self-administration (Roberts *et al.*, 1977; Roberts *et al.*, 1980). Also, blockade of either D1-like or D2-like receptors by administration of selective antagonists directly into the NAc increases the rate of cocaine self-administration in a manner consistent with decreasing the dose of cocaine (Phillips *et al.*, 1983; Robledo *et al.*, 1992). However, cocaine infused directly into the NAc fails to support self-administration (Goeders and Smith, 1983).

CPP is another paradigm that has been used to measure the rewarding effects of drugs, including cocaine (for review see Hoffman, 1989). This paradigm assesses the rewarding effects of drugs indirectly by measuring the incentive motivational properties of environmental stimuli that have become associated with the drug through classical conditioning. If the drug produces rewarding effects, drug-associated stimuli acquire positive incentive motivational properties and elicit approach from the animal. Thus, the rewarding effects of the drug are reflected as an increase in the amount of time animals spend in a drug-associated environment relative to a neutral environment. CPP produced by i.p. administration of cocaine is not altered by 6-OHDA lesions of the NAc nor by systemic administration of D2-selective antagonists (Spyraki *et al.*, 1982; Cervo and Samanin, 1995). In contrast, CPP produced by either i.v. or intraventricular administration of cocaine is reversed by D2-selective antagonists (Spyraki *et al.*, 1987; Morency and Beninger, 1987). These findings suggest that cocaine-CPP produced by the latter routes of administration is more dependent on DA neurotransmission through D2-like receptors relative to that produced by i.p. administration. However, the role of the NAc in cocaine-CPP is equivocal in light of the finding that intra-accumbens administration of cocaine does not produce CPP, even at doses that produce conditioned activity (Hemby *et al.*, 1992). Because intra-accumbens administration of cocaine fails to

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ABBREVIATIONS: CPP, conditioned place preference; NAc, nucleus accumbens; dopamine, DA; EEDQ, N-ethoxycarbonyl-2-ethoxy-1, 2-dihydroquinoline; CPu, caudate putamen; 6-OHDA, 6-hydroxydopamine; BSA, bovine serum albumin; Fmol, femptamoles; ANOVA, analysis of variance.

support self-administration or to produce CPP, it is possible that the local anesthetic effects of cocaine disrupt processes involved in reward when administered directly into the brain.

Locomotor activity and stereotypic behaviors produced by direct and indirect DA agonists, including cocaine, are thought to be mediated by the mesolimbic and nigrostriatal pathways, respectively. 6-OHDA lesions of the NAc and CPu disrupt psychomotor stimulant-induced locomotion and stereotypic behaviors, respectively (Kelly and Iversen, 1976; Kelly *et al.*, 1975). Furthermore, locomotor activity is more prevalent after intra-accumbens cocaine administration, whereas stereotypies are more prevalent after intra-caudate administration (Delfs *et al.*, 1990). After acute administration of cocaine, D2-selective antagonists reverse cocaine-induced locomotion when administered into the NAc (Neisewander *et al.*, 1995), and reverse cocaine-induced stereotypies when administered into the CPu (Karler *et al.*, 1995).

The present study examined the hypothesis that blockade of D2-like receptors in the NAc would reverse both cocaine-induced locomotion and CPP. To examine this hypothesis, cocaine-induced behaviors were measured in animals pretreated with intra-accumbens administration of the D2/D3 antagonist sulpiride. An inherent problem with studies using intracranial drug infusions is the inability to identify the receptor population(s) occupied by the drug (for review see Wise and Hoffman, 1992). Several control measures were used in this study to determine if the behavioral changes produced by intra-accumbens sulpiride were due to blockade of D2-like receptors in the NAc. First, stereotypies that are thought to be mediated by regions of the CPu distal to the NAc (Arnt, 1985; Karler *et al.*, 1995) were also measured. Changes in cocaine-induced stereotypies after intra-accumbens administration of sulpiride would suggest that sulpiride had diffused to a distal region of the CPu. Second, anatomical controls were run in a subsequent experiment that examined the effects of sulpiride administration into a region of the CPu dorsal to the intra-accumbens infusion site on cocaine-induced behaviors. Finally, our study used a new technique to estimate receptor occupancy by intracranially administered sulpiride (Neisewander *et al.*, 1995). Briefly, the non-selective irreversible antagonist EEDQ was administered systemically 15 min after intra-accumbens administration of sulpiride. The population of receptors protected by sulpiride from EEDQ-induced inactivation was then analyzed using quantitative autoradiography.

Methods

Drugs. Cocaine hydrochloride (Sigma Chemical Co., St. Louis, MO) was dissolved in saline and injected i.v. at a volume of 1 ml/kg. EEDQ (Sigma) was dissolved in 60% ethanol and 40% saline, and injected i.p. at a volume of 1 ml/kg. (-)Sulpiride (Research Biochemicals Inc., Natick, MA) was dissolved in saline and injected intracranially at a volume of 0.5 μl/side. ^{3}H-Sulpiride (New England Nuclear Corp., Boston, MA) and (+) butaclamol (Research Biochemicals Inc., Natick, MA) were used in the radioligand binding assays.

Animals. Male Sprague-Dawley rats (Charles River Labs, Wilmington, MA) weighing 275 ± 25 g at the start of the experiment were housed individually and maintained on a 12-hr light/dark cycle. They were handled for 4 days before surgery.

Surgery. Rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) in combination with atropine sulfate (10 mg/kg, i.p.). Catheters were implanted into the jugular vein and exited through an incision across the head as described by Depoortere *et al.* (1993). The animals were then placed into a stereotaxic instrument. The connective tissue was retracted exposing the skull. Guide cannulae (23 gauge) were implanted bilaterally into either the NAc or the CPu using the following coordinates, respectively: 2.2 mm AP and ± 1.6 mm ML from bregma and -6.6 mm DV from the surface of the skull; 2.2 mm AP and ± 1.6 mm ML from bregma and -4.6 mm DV from the surface of the skull. These coordinates were derived using the Paxinos and Watson (1986) atlas. Small screws were drilled into the skull approximately 5 mm anterior and posterior to the guide cannulae to provide an anchor for dental acrylic used to secure the guide cannulae and the metal end of the catheter to the skull. Throughout the study, catheters were flushed daily with 0.1 ml of a solution of heparinized saline (30 U/ml), streptokinase (4 mg/ml) and ticarcillin disodium (400 mg/ml) to maintain patency.

Apparatus. The apparatus consisted of rectangular Plexiglas chambers divided into two 36 × 24 × 30 cm compartments. One compartment had citrus scented bedding beneath a wire mesh floor and all but the front wall were white. The other compartment had cedar scented bedding beneath a bar grid floor and all but the front wall were black. The front wall of the apparatus was transparent to allow direct observation of the animals' behaviors. The wall dividing the two compartments, perpendicular to the front wall, was a removable partition. On the CPP test day, this partition was replaced by a similar partition containing an opening in the center (8 × 12 cm high) which allowed the animals free access to both compartments simultaneously. Previous experiments from our laboratory have demonstrated that rats show equal preference for the two compartments (Khroyan *et al.*, 1995; O'Dell *et al.*, 1996). Each compartment had two sets of photodetectors and light sources mounted to the front and back walls such that the emitted beams were 25 cm apart and 4 cm above the floor. A computer-automated relay system recorded crosses, defined as the number of times the photobeams were interrupted consecutively by the animals moving from one end of the compartment to the other.

Experimental design and procedure. Animals were randomly assigned to receive bilateral i.c. infusions of one of four doses of sulpiride (0–0.4 μg/0.5 μl/side) into the NAc followed 15 min later by an i.v. injection of saline or cocaine (4.2 mg/kg). This dose was selected based on previous research indicating that it reliably produces both CPP and stimulant behaviors (O'Dell *et al.*, 1996). The experimental design resulted in the following groups: saline i.c./saline i.v. (n = 10), saline i.c./cocaine i.v. (n = 8), 0.025 μg sulpiride i.c./cocaine i.v. (n = 11), 0.1 μg sulpiride i.c./cocaine i.v. (n = 9), 0.4 μg sulpiride i.c./saline i.v. (n = 9) and 0.4 μg sulpiride i.c./cocaine i.v. (n = 11). In a subsequent experiment, animals were randomly assigned to receive bilateral infusions of 0 or 0.4 μg/0.5 μl/side sulpiride into the CPu followed 15 min later by an i.v. injection of saline or cocaine (4.2 mg/kg). The experimental design resulted in the following groups: saline i.c./saline i.v. (n = 10), saline i.c./cocaine i.v.(n = 7), 0.4 μg sulpiride i.c./saline i.v. (n = 8), and 0.4 μg sulpiride i.c./cocaine i.v. (n = 8).

Each conditioning trial took place over a 2-day period. On one day, animals received bilateral infusions of their assigned dose of saline or sulpiride. The injection cannulae (30 gauge) were connected via PE20 tubing to 10-μl Hamilton syringes placed into an infusion pump. They were then inserted to a depth 1 mm beyond the guide cannulae. One min later, the pump was activated and administered 0.5 μl over 3 min and 10 sec. The injection cannulae were left in place for 1 min following the infusion. Fifteen min later, the animals were placed into a compartment and immediately received an injection of saline or cocaine (4.2 mg/kg, i.v.). The animals were then confined to that compartment for 30 min. On the alternate day of each trial, the animals received a sham intracranial injection using an identical procedure as described above, except that the injection cannulae

were disconnected from the infusion pump. This procedure was used to minimize tissue damage from repeated intracranial injections. Fifteen min after the sham injections, the animals were placed into the alternate compartment for 30 min without receiving an i.v. injection. Animals did not receive i.v. saline injections prior to placement into the alternate compartment in order to minimize the risk of infection. This conditioning procedure was repeated over 6 consecutive days for a total of three conditioning trials. The compartment paired with the i.v. injections and the order of placement into the compartments were counterbalanced across groups. After the first and last administration of cocaine, stereotypies were measured by an observer unaware of the animals' previous treatment. The observer recorded the presence of headbobbing, and directed sniffing of the floor, walls or ceiling every 10 sec for 30 min. Locomotor activity was measured daily as the number of crosses detected by the automated photocell system.

The day after the last conditioning trial, animals were tested for CPP. The solid partition was removed from the apparatus and replaced with a partition containing an opening that allowed the rats free access to both compartments. All animals were placed into the black compartment, such that half began in their injection-paired compartment and half began in their noninjection compartment. The amount of time spent in each compartment was then measured for 15 min by an observer who was unaware of the animals' previous treatment. Entry into a compartment was defined as the animals' two front paws touching the floor of that compartment. CPP was defined as a significant increase in the amount of time spent in the injection-paired compartment relative to the noninjection compartment.

Receptor occupancy. Forty-eight to 72 hr after the test for CPP, animals were intracranially injected with their assigned dose of saline or sulpiride. Fifteen min later, all animals were injected with the irreversible antagonist EEDQ (10 mg/kg, i.p.), except half of the animals from the two control groups that had received i.c. saline were injected with saline i.p.. EEDQ was administered 15 min after sulpiride so that protection from inactivation would begin at the same time as behavioral testing began after sulpiride infusions during conditioning. All of the animals were killed 90 min later by decapitation. The 90-min period was used to ensure enough time to allow EEDQ to inactivate unoccupied receptors. The brains were rapidly removed and frozen in -20°C isopentane. They were then stored at -70°C and were later sectioned in the coronal plane at a thickness of 20 μm at -14°C. Sections containing both the NAc and the CPu were taken at four levels including 2.2, 1.7, 1.2 and 0.7 mm anterior to bregma. The sections were thaw-mounted onto gelatin-coated slides. They were then desiccated under a vacuum for 2 to 3 hr at 4°C and for 8 hr at -20°C, and then stored at -70°C until assayed.

Before the radioligand binding assay, the sections were thawed and dried at room temperature for 20 min. Duplicate sections were preincubated two times for 5 min each at 4°C in a buffer containing 50 mM Tris HCl (pH of 7.7 at room temperature). The sections were then labeled with 15 nM ^{3}H-sulpiride in the presence or absence of 1 μM (+) butaclamol in a buffer containing 50 mM Tris, 120 mM NaCl and 10 mg/liter BSA (pH of 7.5 at room temperature). Incubation occurred for 60 min at 22°C, and the reaction was terminated by two 5-min washes in ice-cold buffer. After 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 placed into light-proof cassettes and exposed to tritium-sensitive film for 6 wk (Amersham Corp., Arlington Heights, IL). A slide containing plastic tritium standards that were calibrated using mash sections of rat brain was also apposed to each piece of film (Artymyshyn *et al.*, 1990). The film was processed in Kodak GBX developer and fixer. The autoradiograms were analyzed with the Macintosh-based IMAGE software package. Optical density was converted into nCi/mg of radioligand bound using a standard curve as a reference. ^{3}H-Sulpiride binding in the entire intact region of the lateral CPu, medial CPu, NAc core and NAc shell was measured.

Verification of cannula placement and catheter patency. Cannula placements were estimated during tissue sectioning by drawing the cannula tracts at each plate of the Paxinos and Watson atlas (1986) within 2.7- to 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 figure 1. To verify catheter patency, rats were administered sodium brevital (0.17 mg/0.1 ml, i.v.) 24 hr before conditioning and again after completion of behavioral testing. This dose of brevital is only potent enough to produce anesthesia when administered i.v.. Only animals anesthetized by the brevital were included in the study.

Statistical analyses. Locomotor activity (*i.e.*, crosses) and time-sampled observations of sniffing were analyzed using ANOVA with drug treatment as the between group measure and injection as a repeated measure. Time-sampled observations of headbobbing were analyzed using nonparametric Kruskal-Wallis ANOVA because some groups failed to exhibit any headbobbing. Separate one-predictor regression analyses were used to determine the correlation between the change in cocaine-induced locomotion and the occupancy of ^{3}H-sulpiride binding sites in the NAc core, NAc shell, medial CPu and lateral CPu. CPP data were analyzed using nonparametric Wilcoxon signed-ranks tests comparing the amount of time spent in the injection-paired compartment and the noninjection compartment because these measures are not orthogonal. Fmol ^{3}H-sulpiride bound/mg protein was analyzed using separate one-way ANOVA for each brain region at each level with drug treatment as a between group measure. Significant parametric ANOVA were analyzed further using Fisher LSD tests. Significant nonparametric ANOVA were analyzed further using Mann-Whitney *U* tests for between subjects comparisons and Wilcoxon signed-rank tests for within subjects comparisons. Significant one-predictor correlations were analyzed further by including each predictor that was significantly correlated with the change in locomotion in a multiple regression analysis.

The percentage of receptors occupied by sulpiride administered i.c. was estimated using the ratio of binding from the following treatments groups (*i.e.*, group designations represent i.c. administration/systemic administration): (sulpiride/EEDQ − vehicle/EEDQ)/(vehicle/vehicle − vehicle/EEDQ).

Binding in the vehicle/EEDQ group represents the population of receptors not inactivated by EEDQ. This value was subtracted from

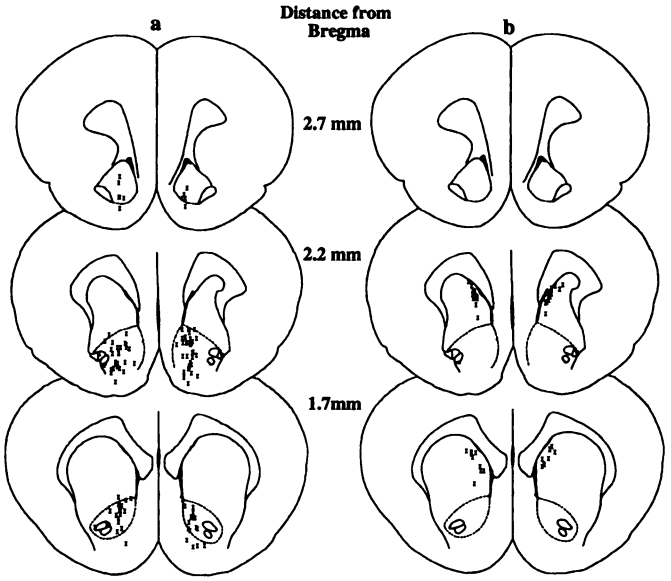

Fig. 1. Approximate position of cannula tips, represented by X, for animals receiving intra-accumbens infusions (a) or intra-caudate infusions (b). The drawings were adapted from illustrations in the Paxinos and Watson (1986) atlas.

binding in the sulpiride/EEDQ groups and the vehicle/vehicle group, such that the value in the numerator represents receptors protected from EEDQ inactivation by the intra-accumbens sulpiride, and the value in the denominator represents the total amount of receptors available for protection from EEDQ. The ratio, therefore, provides an estimate of the percentage of receptors occupied by the antagonist.

Results

Locomotion. The effects of intra-accumbens administration of sulpiride on cocaine-induced locomotion are illustrated in figure 2a. Cocaine produced an increase in locomotion that was dose-dependently reversed by intra-accumbens sulpiride. The overall ANOVA revealed a significant main effect of treatment [$F(5,52)=25.9$, $P < .0001$]. Pair-wise comparisons indicated that animals treated with cocaine alone exhibited more crosses relative to saline controls (Fisher LSD test, $P < .05$). The two lowest doses of sulpiride (0.025 and 0.1 μg/0.5 μl/side) attenuated cocaine-induced locomotion. Animals treated with these doses before cocaine administration exhibited fewer crosses than animals treated with cocaine alone, yet they exhibited more crosses than saline controls (Fisher LSD test, $P < .05$). The highest dose of sulpiride (0.4 μg/0.5 μl/side), however, completely reversed cocaine-induced locomotion. Animals treated with this dose of sulpiride did not differ from saline controls. There was no significant difference in the number of crosses exhibited by animals treated with intra-accumbens sulpiride and i.v. saline as compared to the saline controls. There was also a significant main effect of injection day [$F(2,104)=11.2$, $P < .0001$], but no significant interaction between dose and injection day was obtained. This finding indicates that animals exhibited fewer crosses after the third injection relative to the first injection, regardless of treatment.

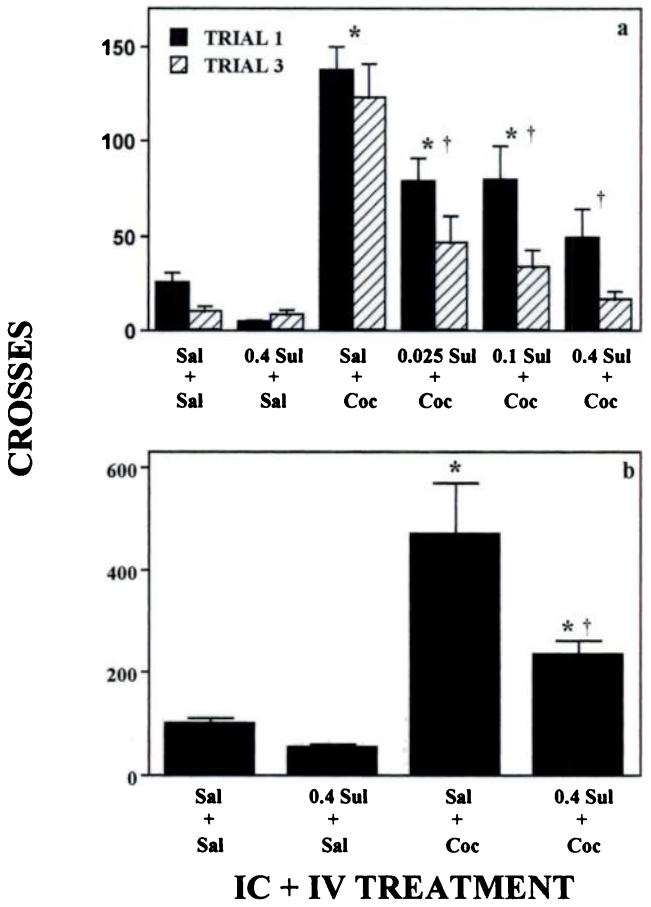

Fig. 2. Locomotor activity crosses (±S.E.M.) after injections 1 and 3 of saline or cocaine (4.2 mg/kg, i.v.) in animals pretreated with bilateral infusions of varying doses of intra-accumbens sulpiride (a) and locomotor activity crosses summed across injections 1 to 3 of saline or cocaine in animals pretreated with bilateral infusions of varying doses of intra-caudate sulpiride (b). Asterisks represent a significant difference from animals treated with saline alone, $P < .05$, Fisher LSD test. Daggers represent a significant difference from animals treated with cocaine alone, $P < .05$, Fisher LSD test.

The effects of intra-caudate administration of sulpiride on cocaine-induced locomotion are illustrated in figure 2b. Cocaine produced an increase in locomotion that was attenuated by intra-caudate sulpiride. The ANOVA revealed a significant interaction between the i.c. treatment and i.v. treatment [$F(1,21)=5.1$, $P < .04$]. Pair-wise comparisons indicated that animals treated with cocaine alone exhibited more crosses relative to saline controls (Fisher LSD test, $P < .05$). Animals treated with 0.4 μg/0.5 μl/side sulpiride before cocaine administration exhibited fewer crosses than animals treated with cocaine alone, yet they exhibited more crosses than saline controls (Fisher LSD test, $P < .05$). There was no significant difference in the number of crosses exhibited by animals treated with intra-accumbens sulpiride and i.v. saline as compared to the saline controls. There was also no significant effect of injection day or an interaction between dose and injection day.

One-predictor regression analyses revealed that the decrease in cocaine-induced locomotion was significantly correlated with the occupancy of ^{3}H-sulpiride binding sites in the NAc core ($r^2(30) = 0.41$, $P < .01$), NAc shell ($r^2(30) = 0.3$, $P < .01$), and the medial CPu ($r^2(30) = 0.21$, $P < .01$), data not shown. A multiple regression analysis revealed that the occupancy of ^{3}H-sulpiride binding sites in the NAc core and shell ($r^2(29) = 0.41$, $P < .01$) failed to account for any additional variance in cocaine-induced locomotion than that accounted for by the occupancy of ^{3}H-sulpiride binding sites in the NAc core alone. Also, the occupancy of ^{3}H-sulpiride binding sites in the NAc core and medial CPu ($r^2(29) = 0.44$, $P < .01$) failed to account for any additional variance in cocaine-induced locomotion than the occupancy of ^{3}H-sulpiride binding sites in the NAc core accounted for alone (*i.e.*, < 2%).

Headbobbing. The effects of intra-accumbens administration of sulpiride on cocaine-induced headbobbing are illustrated in figure 3a. Cocaine produced an increase in headbobbing that was not altered by intra-accumbens sulpiride. The Kruskal-Wallis ANOVA revealed a significant effect of treatment after the first [$H(5) = 21.8$, $P < .0006$] and third i.v. injection [$H(5) = 28.3$, $P < .0001$]. None of the animals treated with i.v. saline exhibited headbobbing. Animals treated with cocaine exhibited an increase in headbobbing relative to saline controls after both the first and third i.v. injection regardless of pretreatment with sulpiride (Mann-Whitney U, $P < .001$). Animals treated with cocaine alone, as well as animals treated with cocaine and 0.025 μg sulpiride, exhibited sensitization of headbobbing reflected as an increase in the amount of headbobbing after the third injection relative to the first injection (Wilcoxon signed-rank test, $P < .05$). However, animals treated with the 0.1 and 0.4 μg/0.5 μl/side doses of sulpiride did not exhibit sensitization of headbobbing. This lack of sensitized headbobbing is likely

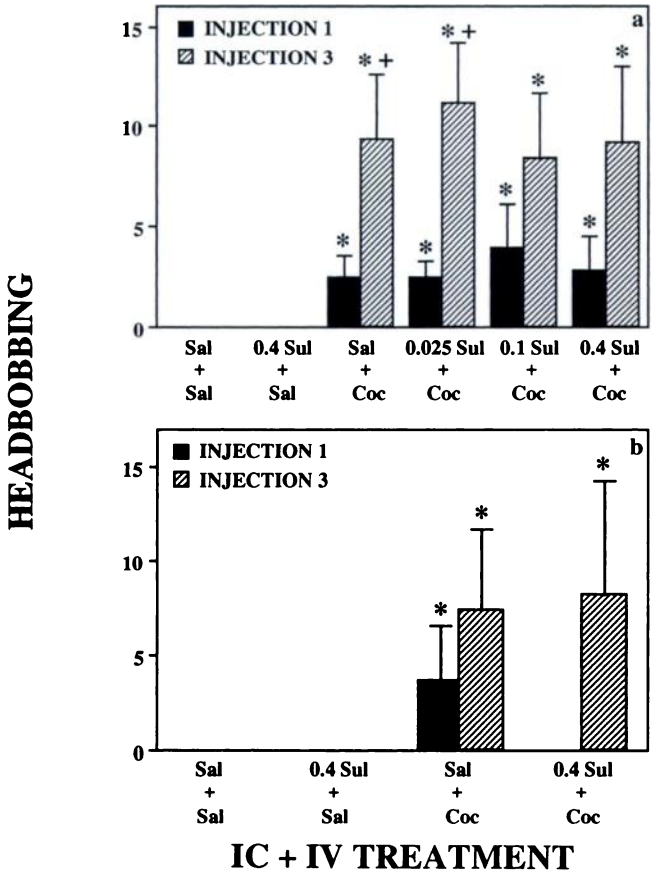

Fig. 3. Time-sampled observations of headbobbing (±S.E.M.) after injections 1 and 3 of saline or cocaine in animals pretreated with bilateral infusions of varying doses of intra-accumbens (a) or intra-caudate (b) sulpiride. Asterisks represent a significant difference from animals treated with saline alone, $P < .05$, Mann Whitney U test. Plusses represent a significant difference in the amount of time-sampled head-bobbing after the third i.v. injection relative to the first i.v. injection, $P < .05$, Wilcoxon signed-rank test.

due to the greater variability after the first i.v. injection in these groups relative to the cocaine controls, thereby decreasing the sensitivity for detecting sensitization of headbobbing. This explanation is supported by the finding that there was no significant difference in the amount of headbobbing between these groups and the cocaine controls following the third i.v. injection (Wilcoxon signed-rank test, $P < .05$).

The effects of intra-caudate administration of sulpiride on cocaine-induced headbobbing are illustrated in figure 3b. Cocaine produced an increase in headbobbing that was not altered by intra-caudate sulpiride after the third i.v. injection. The Kruskal-Wallis ANOVA revealed a significant main effect of treatment after the first [$H(3) = 8.33$, $P < .04$] and third i.v. injection [$H(3) = 11.1$, $P < .01$]. None of the animals treated with i.v. saline exhibited any headbobbing. Animals treated with cocaine alone exhibited more time-sampled headbobbing relative to saline controls after both the first and third i.v. injection. Animals treated with intra-caudate sulpiride and i.v. cocaine exhibited more time-sampled headbobbing relative to saline controls after the third i.v. injection only (Mann-Whitney U, $P < .05$). Both cocaine-treated groups exhibited a trend toward sensitization of headbobbing after the third injection relative to the first injection, although this difference was not significant.

Sniffing. The effects of intra-accumbens administration of sulpiride on cocaine-induced sniffing are illustrated in figure 4a. Cocaine produced an increase in sniffing that was not altered by intra-accumbens sulpiride. The overall ANOVA revealed a significant main effect of treatment [$F(5,52) = 12.9$, $P < .0001$]. Animals treated with cocaine exhibited a significant increase in sniffing relative to saline controls, regardless of pretreatment with intra-accumbens sulpiride (Fisher LSD test, $P < .05$). Animals treated with intra-accumbens sulpiride and i.v. saline did not differ in the amount of sniffing relative to saline controls. There was no significant effect of injection day, nor was there a significant interaction between dose and injection day, indicating that sniffing was not altered by repeated administration of cocaine (data not shown).

The effects of intra-caudate administration of sulpiride on cocaine-induced sniffing are illustrated in figure 4b. Animals treated with cocaine exhibited a significant increase in sniffing relative to saline controls, regardless of pretreatment with intra-caudate sulpiride. The ANOVA revealed a significant effect of i.v. treatment [$F(1,21) = 26.5$, $P < .0001$] but no significant effect of i.c. treatment. Animals treated with intra-caudate sulpiride and i.v. saline did not differ in the amount of sniffing relative to saline controls. There was no

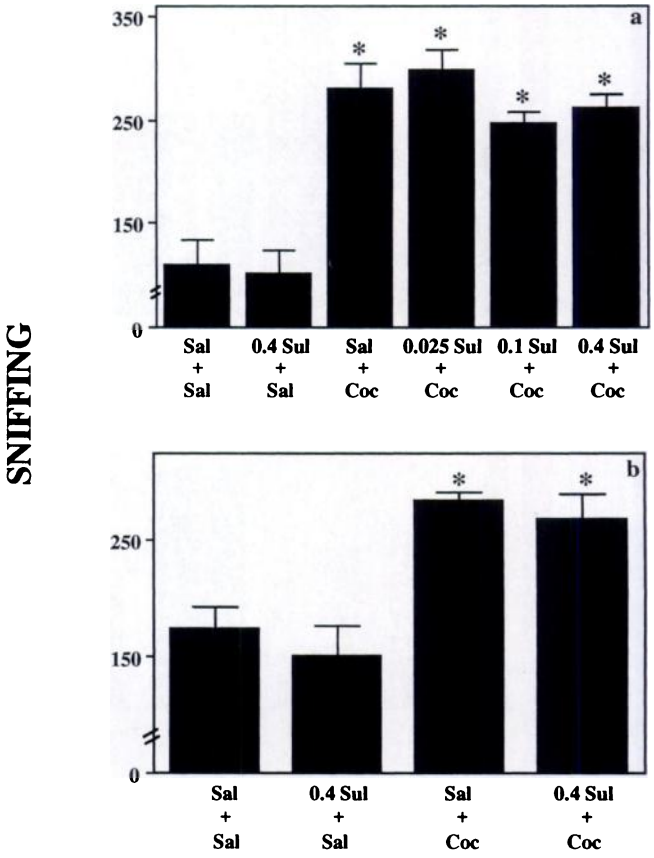

Fig. 4. Time-sampled observations of sniffing (±S.E.M.) totaled across injections 1 and 3 of saline or cocaine administration in animals pretreated with bilateral infusions of varying doses of intra-accumbens (a) or intra-caudate (b) sulpiride. Asterisks represent a significant difference from animals treated with saline alone, $P < .05$, Fisher LSD test.

significant effect of injection day, nor was there a significant interaction between dose and injection day, indicating that sniffing was not altered by repeated administration of cocaine (data not shown).

Place conditioning. The amount of time spent in the injection compartment relative to the noninjection compartment on the CPP test day by animals pretreated with intra-accumbens or intra-caudate sulpiride during conditioning is illustrated in figure 5, a and b, respectively. Animals conditioned with i.v. saline, regardless of intracranial pretreatment with saline or sulpiride, did not exhibit place preference because there was no significant difference in the amount of time spent in the two compartments. Thus, our data indicate that i.v. injections of saline do not alter place preference in controls. Conversely, all animals conditioned with i.v. cocaine, regardless of intracranial pretreatment with saline or sulpiride, exhibited place preference that was evident as an increase in the amount of time spent in the injection compartment relative to the noninjection compartment (Wilcoxon sign-rank test, $P < .05$).

Receptors occupied by intracranial sulpiride. Representative autoradiograms illustrating the pattern of receptor occupancy after intracranial infusions of sulpiride are shown in figure 6. Binding data from the vehicle/vehicle and vehicle/EEDQ groups from the two experiments were combined to obtain the most accurate estimate of control values. The mean fmol ^{3}H-sulpiride bound/mg protein (±S.E.M.) in each brain region and the estimated percentage of receptors occupied by each dose of intra-accumbens sulpiride is reported in table 1. All groups exhibited significantly more binding relative to the vehicle/EEDQ group in the NAc core and shell (Fisher LSD test, $P < .05$). There was a dose-dependent increase in the number of ^{3}H-sulpiride binding sites occupied by intra-accumbens sulpiride in several of the regions examined. The 0.025-μg dose of sulpiride occupied 42 to 85% of the binding sites in the NAc and 4 to 43% of the binding sites in the CPu. The 0.1-μg dose of sulpiride occupied 53 to 111% of the binding sites in the NAc and 26 to 81% of the binding sites in the CPu. Finally, the 0.4-μg dose of sulpiride occupied 96 to 111% of the binding sites in the NAc and 15 to 106% of the binding sites in the CPu. The 0.4-μg dose of sulpiride occupied a similar number of binding sites in animals receiving saline (data not shown) as those receiving cocaine, because there was no significant difference between these groups. These data suggest that three administrations of cocaine over a 6-day period is not sufficient to alter ^{3}H-sulpiride binding in the regions analyzed.

The mean fmol ^{3}H-sulpiride bound/mg protein (±S.E.M.) and the percentage of receptors occupied by intra-caudate sulpiride in each brain region is reported in table 2. Both sulpiride-treated groups exhibited significantly more binding in the medial CPu relative to the vehicle/EEDQ group (Fisher LSD test, $P < .05$). However, these groups did not exhibit more binding in the NAc core or shell relative to the vehicle/EEDQ group. The 0.4-μg dose of sulpiride occupied 49 to 112% of the medial CPu binding sites, 5 to 75% of the lateral CPu binding sites and 0 to 26% of the NAc binding sites. There was no significant difference in binding sites occupied by the 0.4-μg dose of sulpiride in animals conditioned with saline *versus* cocaine, again indicating that previous cocaine administration did not alter ^{3}H-sulpiride binding.

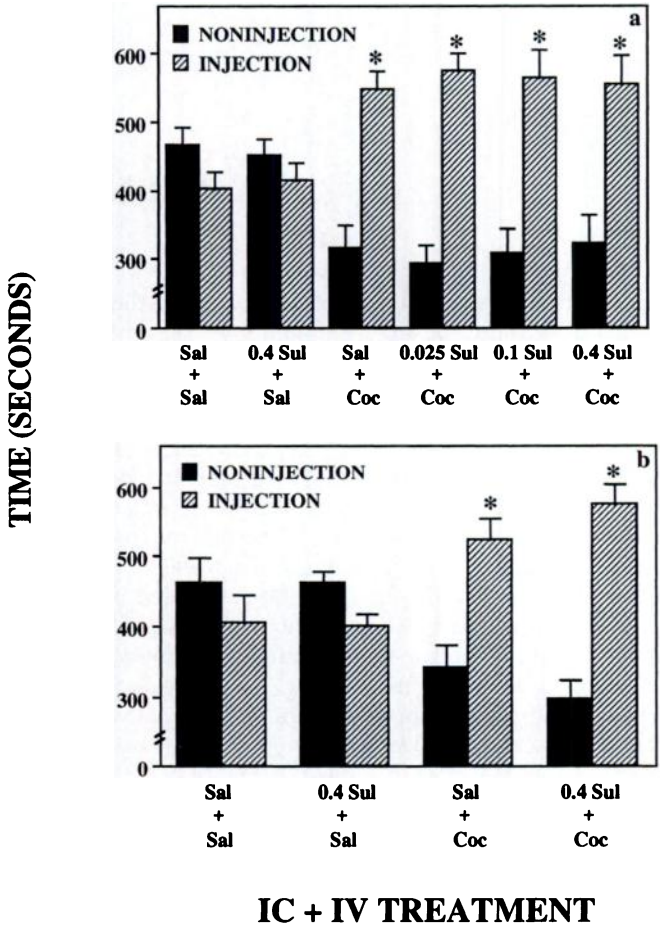

Fig. 5. Time spent (±S.E.M.) in the noninjection compartment (black bars) and the injection compartment (hatched bars) during the test for CPP in animals conditioned with bilateral infusions of varying doses of intra-accumbens (a) or intra-caudate (b) sulpiride. Asterisks represent a significant difference in the amount of time spent in the injection compartment relative to the noninjection compartment, $P < .05$, Wilcoxon signed-rank test.

Discussion

Our results indicate that D2-like receptors in the NAc and anterior medial CPu are involved in cocaine-induced locomotion. Intra-accumbens administration of the two lowest doses of sulpiride attenuated cocaine-induced locomotion and occupied >42% of the ^{3}H-sulpiride binding sites in the NAc. Intra-accumbens administration of the highest dose of sulpiride completely reversed cocaine-induced locomotion and occupied >96% of the ^{3}H-sulpiride binding sites in the NAc. The decrease in cocaine-induced locomotion was highly correlated with occupancy of ^{3}H-sulpiride binding sites in both the NAc core and shell. However, occupancy of ^{3}H-sulpiride binding sites in the NAc shell accounted for only 0.6% of the variance in cocaine-induced locomotion not already accounted for by occupancy of ^{3}H-sulpiride binding sites in the NAc core. The dose of intra-accumbens sulpiride that reversed cocaine-induced locomotion also occupied a significant number of ^{3}H-sulpiride binding sites in the medial CPu. Furthermore, the decrease in cocaine-induced locomotion was also correlated with the occupancy of ^{3}H-sulpiride binding sites in the medial CPu. However, occupancy of ^{3}H-sulpiride binding sites in the medial CPu accounts for only

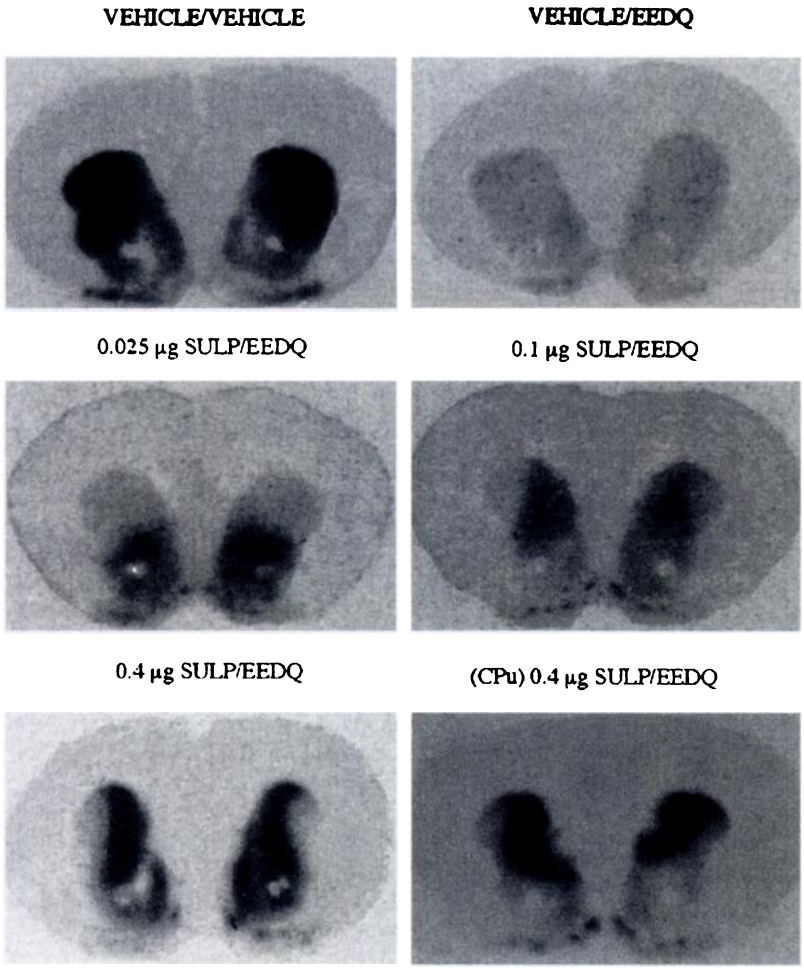

Fig. 6. Representative autoradiograms of sections taken 1.7 mm anterior to Bregma and labeled with 15 nM ^{3}H-sulpiride. Labels indicate intracranial treatment/systemic treatment before the animal was killed. The darkened regions represent the population of binding sites occupied by the sulpiride administered intracranially.

3% of the variance in cocaine-induced locomotion not already accounted for by the occupancy of ^{3}H-sulpiride binding sites in the NAc core. Although causation cannot be inferred from correlational statistics, these results suggest that blockade of D2-like receptors in the NAc core is sufficient to reverse cocaine-induced locomotion. These findings are consistent with those of Maldonado-Irizarry and Kelley (1995) indicating that excitotoxic lesions of the NAc core, and not of the NAc shell, alter motor activity in rats. Intra-caudate administration of sulpiride attenuated cocaine-induced locomotion without occupying a significant number of ^{3}H-sulpiride binding sites in the NAc (*i.e.*, <26%). The latter finding suggests that D2-like receptors in the anterior medial CPu may also play a role in cocaine-induced locomotion.

The 0.4-μg dose of sulpiride occupied a similar number of ^{3}H-sulpiride binding sites in the CPu after intra-caudate administration and after intra-accumbens administration. This finding is surprising because one would expect greater occupancy in the CPu after infusion of sulpiride directly into this area relative to infusion into a neighboring brain region. The autoradiograms revealed that sulpiride diffused dorsally along the cannulae tract and occupied sites along the border between the CPu and the ventricles. Most of the infusion sites into the NAc were located more distal to the ventricles than the infusion sites into the CPu. Thus, it is possible that a greater concentration of sulpiride diffused into the lateral ventricles after infusion into the CPu relative to infusion into the NAc. If this is the case, then intra-caudate administration of sulpiride may have attenuated cocaine-induced locomotion by blocking D2-like receptors in distal brain regions. It seems unlikely, however, that diffusion of an amount sufficient to occupy a functionally significant population of D2-like receptors in distal brain regions occurred in our study given the relatively low dose of sulpiride used (0.4 μg). Also, the autoradiograms revealed that sulpiride administered into the CPu did not occupy a significant amount of ^{3}H-sulpiride binding sites in posterior regions of the NAc that border the lateral ventricles. Furthermore, cocaine-induced stereotypies, which are thought to be mediated by the ventrolateral CPu (Arnt, 1985; Karler *et al.*, 1995), were not reliably altered by intra-caudate administration of sulpiride.

Sensitization of locomotion was not observed after repeated administration of cocaine. In fact, consistent with our previous research, a decrease locomotion was observed after repeated administrations of cocaine (O'Dell *et al.*, 1996). Because a decrease in locomotion was also evident in saline controls, it appears the decrease was likely due to habitation to the CPP compartment rather than tolerance to the locomotor effects of cocaine. The only behavior that was sensitized after repeated administration of cocaine was headbobbing; however, this was not reliably observed in cocaine-treated animals. The reason sensitization of cocaine-induced headbobbing was not reliably observed may be due to the low number of cocaine injections (*i.e.*, a total of three in our study) because sensitized headbobbing has been reliably observed after six injections of cocaine (O'Dell *et al.*, 1996).

TABLE 1

Fmol ^{3}H-sulpiride bound/mg protein (±SEM) and estimated % receptors occupied by intra-accumbens administration of sulpiride

Region and Distance from Bregma (mm)	NAc Administration/Systemic Administration							
	Vehicle/vehicle $N = 14$	Vehicle/EEDQ $N = 17$	0.025 μg Sulp/EEDQ $N = 7$	% receptors occupied[a]	0.1 μg Sulp/EEDQ $N = 6$	% receptors occupied[a]	0.4 μg Sulp/EEDQ $N = 11$	% receptors occupied[a]
NAc core								
2.2	503 ± 45[b]	174 ± 31[c]	410 ± 42[b]	72	484 ± 51[b]	94	503 ± 35[b]	99
1.7	439 ± 37[b]	123 ± 32[c]	390 ± 50[b]	85	474 ± 44[b]	111	454 ± 36[b]	105
1.2	416 ± 39[b]	86 ± 19[c]	272 ± 63[bc]	56	340 ± 62[b]	77	403 ± 42[bc]	96
0.5	335 ± 46[b]	39 ± 12[c]	162 ± 63[c]	42	195 ± 31[b]	53	250 ± 44[b]	71
NAc shell								
1.7	425 ± 36[b]	143 ± 33[c]	361 ± 45[b]	77	391 ± 44[b]	88	421 ± 21[b]	99
1.2	372 ± 26[b]	95 ± 19[c]	272 ± 63[b]	64	340 ± 63[b]	88	403 ± 43[bd]	111
Medial CPu								
2.2	614 ± 50[b]	121 ± 24[c]	291 ± 87[bc]	34	520 ± 45[bd]	81	643 ± 57[bd]	106
1.7	689 ± 51[b]	126 ± 31[c]	265 ± 75[c]	25	497 ± 51[bd]	66	543 ± 80[bd]	74
1.2	644 ± 49[b]	96 ± 20[c]	331 ± 108[bc]	43	420 ± 52[bc]	59	378 ± 56[bc]	51
0.5	502 ± 57[b]	52 ± 15[c]	211 ± 60[c]	35	368 ± 44[b]	70	285 ± 51[bc]	52
Lateral CPu								
2.2	656 ± 53[b]	94 ± 20[c]	172 ± 41[c]	14	459 ± 42[b–d]	65	442 ± 51[b–d]	62
1.7	816 ± 60[b]	91 ± 26[c]	123 ± 34[c]	4	348 ± 34[b–d]	35	363 ± 74[b–d]	38
1.2	922 ± 70[b]	82 ± 20[c]	193 ± 86[c]	12	304 ± 52[c]	26	219 ± 51[c]	15
0.5	747 ± 87[b]	56 ± 19[c]	154 ± 55[c]	14	255 ± 69[c]	28	174 ± 34[c]	17

[a] The ratio of (sulpiride/EEDQ − vehicle/EEDQ)/(vehicle/vehicle − vehicle/EEDQ) × 100.
[b] Represents a significant difference from the vehicle/EEDQ group, Fisher LSD test, $P < .05$.
[c] Represents a significant difference from the vehicle/vehicle group, Fisher LSD test, $P < .05$.
[d] Represents a significant difference from the 0.025 sulpiride/EEDQ group, Fisher LSD test, $P < .05$.

In contrast to the effects of intra-accumbens sulpiride on locomotion, this treatment did not alter cocaine-CPP even at a dose that completely reversed cocaine-induced locomotion and occupied >96% of the ^{3}H-sulpiride binding sites in both the NAc core and shell. Thus, the lack of an effect of intra-accumbens sulpiride on cocaine-CPP is not likely due to insufficient dosage. These findings suggest that stimulation of D2-like receptors in the NAc is not necessary for cocaine-CPP. However, the data do not preclude the possibility that these receptors may play a role in this behavior. Indeed, White *et al.* (1991) demonstrated that stimulation of D2-like receptors in the NAc by intra-accumbens administration of a D2-selective agonist is sufficient to produce CPP.

Self-administration experiments have demonstrated that intra-accumbens infusion of D2-selective antagonists alters the rate of cocaine self-administration in a manner consistent with reward reduction (Phillips *et al.*, 1983; Robledo *et al.*, 1992). Although these findings appear to conflict with ours, there are differences between the two paradigms used that may account for this discrepancy. The self-administration paradigm is sensitive to detecting an attenuation of the rewarding effects of cocaine after blockade of DA receptors as an increase in the rate of responding similar to decreasing the dose of cocaine. Conversely, the CPP paradigm may be relatively insensitive to detecting an attenuation, because low doses of cocaine that are presumably less rewarding produce the same degree of CPP as high doses that are presumably more rewarding (Nomikos and Spyraki, 1988; Bardo *et al.*, 1995; O'Dell *et al.*, 1996). However, an advantage of the CPP paradigm is that it can detect a complete reversal of the rewarding effects of cocaine since the animal is tested in a nondrugged state. Conversely, it is difficult to interpret a complete reversal of cocaine self-administration by DA antagonists due to the confounding effects of these drugs on motor activity (Rolls *et al.*, 1974; Fibiger *et al.*, 1976; Fowler *et al.*, 1976; Caine and Koob, 1994; Salamone *et al.*, 1994). For instance, Phillips *et al.* (1983) reported a lack of responding for cocaine after intra-accumbens administration of a high dose of spiroperidol; however, the pattern of responding did not resemble extinction. Thus, rate-increasing effects in self-administration experiments can indicate that a receptor system is involved in the rewarding effects of cocaine, but the necessity of the receptor system demonstrated as a complete reversal of self-administration is confounded

TABLE 2

Fmol ^{3}H-sulpiride bound/mg protein (±SEM) and estimated % receptors occupied by intra-caudate administration of sulpiride

Region and Distance from Bregma (mm)	CPu Administration/Systemic Administration			
	0.4 μg Sulp/EEDQ (saline controls)	% receptors occupied[a]	0.4 μg Sulp/EEDQ (cocaine-conditioned)	% receptors occupied[a]
NAc core				
2.2	260 ± 57[b]	26	223 ± 67[b]	14
1.7	181 ± 49[b]	18	206 ± 41[b]	26
1.2	67 ± 25[b]	0	120 ± 46[b]	8
0.5	33 ± 14[b]	3	113 ± 15[b]	25
NAc shell				
1.7	188 ± 42[b]	16	203 ± 34	21
1.2	78 ± 17[b]	0	132 ± 36[b]	13
Medial CPu				
2.2	674 ± 88[c]	112	471 ± 76[c]	70
1.7	633 ± 137[c]	90	581 ± 102[c]	80
1.2	428 ± 56[bc]	51	413 ± 78[bc]	49
0.5	315 ± 51[bc]	58	353 ± 51[c]	67
Lateral CPu				
2.2	514 ± 151[c]	75	434 ± 91[c]	60
1.7	286 ± 76[bc]	27	383 ± 64[bc]	40
1.2	140 ± 28[c]	6	246 ± 55[b]	18
0.5	92 ± 34[c]	5	233 ± 39[b]	26

[a] The ratio of (sulpiride/EEDQ − vehicle/EEDQ)/(vehicle/vehicle − vehicle/EEDQ) × 100.
[b] Represents a significant difference from the vehicle/vehicle group (see table 1), Fisher LSD test, $P < .05$.
[c] Represents a significant difference from the vehicle/EEDQ group (see table 1), Fisher LSD test, $P < .05$.

by motor impairing effects of DA antagonists. Finally, recent theories suggest that different processes may contribute to drug-seeking behavior (for reviews see Robinson and Berridge, 1993; Markou *et al.*, 1993; DiChiara, 1995). The relative contribution of these processes, and hence the neural mechanisms involved, may be different for the two paradigms. If this is the case, then the role of D2-like receptors in CPP and self-administration may differ.

It is also difficult to compare results from localization studies regardless of the paradigms used due to differences in reporting histological information. The EEDQ technique used in our study offers the advantages of allowing one to visualize the spread of an intracranially administered drug and to estimate the percentage of receptors occupied by the drug. 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 sulpiride given *in vivo*. Estimates of receptor occupancy were nearly 100% in some cases, suggesting that competitive displacement of sulpiride by EEDQ was negligible. This finding is not surprising because other studies have suggested that EEDQ has only moderate affinity for DA receptors (Meller *et al.*, 1985; Saller *et al.*, 1989; Burger and Martin-Iverson, 1994). Furthermore, previous research has demonstrated that EEDQ decreases B_{max} values for 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 be interpreted as absolute measures of occupancy. For instance, extracellular DA was higher during behavioral testing when animals had received cocaine relative to the *in vivo* occupancy test when animals received sulpiride alone. Because DA may compete with sulpiride for binding to D2-like receptors, occupancy values may be overestimated. However, administering cocaine during the *in vivo* occupancy test would result in *dopamine-induced* protection of binding sites from EEDQ-induced inactivation. These sites would then be labeled with ^{3}H-sulpiride and the occupancy estimates would remain high. Displacement of sulpiride by extracellular DA from D_2 receptors is likely very low, and perhaps negligible, for two reasons. First, the concentration of sulpiride was considerably higher than dopamine. Dialysis studies estimate basal concentrations of DA in the NAc to be 5 to 50 nM (for review see Kiyatkin, 1995) whereas the concentrations of sulpiride in our study were 1.4 to 2.3 mM. Furthermore, sulpiride has a higher affinity for D2 receptors (*i.e.*, K_d = 3.2 nM; Jastrow *et al.*, 1984) relative to DA (*i.e.*, $K_d = 10^3$ nM; Civelli *et al.*, 1991). In any case, this source of error does not vary systematically across sulpiride dosage groups because they received the same dose of cocaine. Therefore, relative differences in occupancy are informative.

Although our results suggest that D2-like receptors in the NAc are not necessary for cocaine-CPP, the findings that systemic administration of a D2-selective antagonist reverses cocaine-CPP and alters cocaine self-administration suggest that D2-like receptors in other brain regions are necessary for the rewarding effects of cocaine (Woolverton, 1986; Morency and Beninger, 1987; Spyraki *et al.*, 1987; Britton *et al.*, 1991; Hubner and Moreton, 1991; Caine and Koob, 1994). Indeed, the involvement of D2-like receptors in the medial prefrontal cortex has been demonstrated for cocaine-self administration (Goeders and Smith, 1983, 1986). Several other brain regions, including the ventral tegmental area (Mogenson *et al.*, 1979; Roberts and Koob, 1982), ventral pallidum (Hubner and Koob, 1990; Hiroi and White, 1993; Robledo and Koob; 1993) and amygdala (Hiroi and White, 1991; Brown and Fibiger; 1993; McGregor and Roberts, 1993; Wilson *et al.*, 1994) have also been shown to be involved in the rewarding effects of psychomotor stimulants. Future research is needed to determine if blockade of D2-like receptors in these brain regions is sufficient to reverse the rewarding effects of cocaine. It is possible, however, that parallel processes contribute to cocaine-CPP such that blockade of D2-like receptors in several regions may be necessary to completely reverse this behavior.

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