The Journal of Neuroscience, January 1987, 7(1): 213–222

# Picomolar Affinity of $^{125}$I-SCH 23982 for $D_1$ Receptors in Brain Demonstrated with Digital Subtraction Autoradiography

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**Iodinated SCH 23390, $^{125}$I-SCH 23982 (DuPont-NEN), was examined using quantitative autoradiography for its potency, selectivity, and anatomical and neuronal localization of binding to the dopamine $D_1$ receptor in rat brain sections. $^{125}$I-SCH 23982 bound to $D_1$ sites in the basal ganglia with very high affinity ($K_d$ values of 55–125 pM), specificity (70–85% of binding was displaced by 5 μM *cis*-flupenthixol), and in a saturable manner ($B_{max}$ values of 65–176 fmol/mg protein). Specific $^{125}$I-SCH 23982 binding was displaced by the selective $D_1$ antagonists SCH 23390 ($IC_{50}$ = 90 pM) and *cis*-flupenthixol ($IC_{50}$ = 200 pM) and the $D_1$ agonist SKF 38393 ($IC_{50}$ = 110 nM) but not by $D_2$-selective ligands (*l*-sulpiride, LY 171555) or the $S_2$ antagonist cinanserin. Compared with $^3$H-SCH 23390, the 5- to 10-fold greater affinity for the $D_1$ site and 50-fold greater specific radioactivity of $^{125}$I-SCH 23982 makes it an excellent radioligand for labeling the $D_1$ receptor. The concentrations of $D_1$ sites were greatest in the medial substantia nigra and exceeded by over 50% the concentration of $D_1$ sites in the lateral substantia nigra, caudoputamen, nucleus accumbens, olfactory tubercle, and entopeduncular nucleus. Lower concentrations of $D_1$ sites were present in the internal capsule, dorsomedial frontal cortex, claustrum, and layer 6 of the neocortex. $D_1$ sites were absent in the ventral tegmental area. Intrastriatal injections of the axon-sparing neurotoxin, quinolinic acid, depleted by 87% and by 46–58% the concentrations of displaceable $D_1$ sites in the ipsilateral caudoputamen and medial and central pars reticulata of the substantia nigra, respectively. No $D_1$ sites were lost in the lateral substantia nigra. Destruction of up to 94% of the mesostriatal dopaminergic projection with 6-hydroxydopamine did not reduce $D_1$ binding nor, with one exception, increase striatal or nigral $D_1$ receptor concentrations. $^{125}$I-SCH 23982 selectively labels $D_1$ binding sites on striatonigral neurons with picomolar affinity, and these neurons contain the majority of $D_1$ sites in rat brain.**

The $D_1$ and $D_2$ classification of dopamine receptor subtypes in brain was initially based on the response of adenylate cyclase in rat striatal homogenates to dopaminergic ligands (Kebabian and Calne, 1979; Stoof and Kebabian, 1981). Corroboration of this $D_1$ and $D_2$ receptor dichotomy by receptor binding methods has been made possible by the development of $D_1$- and $D_2$-selective radioligands. For example, radioligand binding assays for the $D_2$ receptor (Creese and Snyder, 1975; Seeman et al., 1975; Creese et al., 1977) have widened our knowledge of $D_2$ receptors, including their regulation and function in brain (Seeman, 1980; Creese et al., 1983). Quantitative autoradiography with these $D_2$-selective compounds, especially $^3$H-spiperone (Palacios et al., 1981), has produced pharmacologically defined, quantitative maps of the $D_2$ receptor distribution in brain (Neve et al., 1984; Altar et al., 1985a, b).

Only recently has a radioligand binding assay for the $D_1$ receptor become available. Iorio and colleagues (1983) and Hyttel (1983, 1984) demonstrated that SCH 23390 [R-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepin-7-ol hemimaleate] is a selective antagonist of the stimulation by dopamine of the adenylate cyclase of rat striatal membranes. The tritiated form of SCH 23390 was subsequently shown to selectively label the $D_1$ receptor of rat striatal membranes with high affinity ($K_d$ value of 300–400 pM) (Billard et al., 1984).

Structure–activity studies by Kebabian and colleagues (Itoh et al., 1984; Sidhu and Kebabian, 1985) and by O'Boyle and Waddington (1985) have shown that the 7-Cl, but not *N*-methyl, substituent of SCH 23390 is crucial for its $D_1$-antagonist activity. Replacing the chlorine with $^{125}$I preserved in racemic form the $D_1$ selectivity and augmented the specific activity of SCH 23982 (Sidhu and Kebabian, 1985). The iodinated form of the active (+)-enantiomer of SCH 23390, R(+)-8-$^{125}$I-iodo-7-hydroxy-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepine, or $^{125}$I-SCH 23982 (Fig. 1), has since been made available from DuPont-NEN (Boston, MA). We have used $^{125}$I-SCH 23982 to label the $D_1$ receptor in brain sections and, with quantitative receptor autoradiography, to characterize the binding affinity, capacity, and pharmacological specificity of this receptor throughout the basal ganglia. Using $^{125}$I-SCH 23982, we also show for the first time the presence of virtually all brain $D_1$ binding sites on intrinsic striatal neurons and striatonigral neurons and the discrete localization of $D_1$ sites in the substantia nigra.

## Materials and Methods

*Chemical lesioning techniques.* Male albino rats (MbF:SD; obtained from Marland Farms, Hewitt, NJ), each weighing 220–240 gm, were given free access to food and water and were housed in an animal vivarium (21–24°C) maintained under a 12 hr light : dark cycle. Twelve rats received an intrastriatal injection of 70 or 125 nmol quinolinic acid (Sigma Chem. Co., St. Louis, MO) dissolved in 0.6 or 1.0 μl, respectively, of sterile saline. Quinolinic acid was injected to destroy neurons whose cell bodies are intrinsic to the striatum (Schwarcz et al., 1982, 1984). Injections were made with a syringe microburet (Micro-Metric Instruments Co., Cleveland, OH) and were delivered during 2 min via a 32 gauge stainless steel cannula that terminated in the central caudatepu-tamen [$A$ = 8.0; $L$ = 2.4; $V$ = −4.4 mm, according to the atlas of König

Received Feb. 26, 1986; revised July 11, 1986; accepted July 17, 1986.

We greatly appreciate the generous supply of $^{125}$I-SCH 23982 by Ms. Ruth Greene of DuPont-NEN Corporation.

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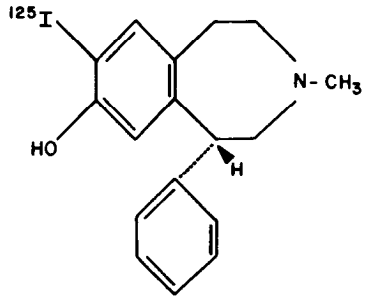


*Figure 1.* Structure of $^{125}$I-SCH 23982.

and Klippel (1967)]. The cannula was withdrawn 5 min after the end of the infusion.

In another group of 5 rats, 6 μg of the selective dopamine neurotoxin, 6-hydroxydopamine (Ungerstedt, 1968, 1971; Uretsky and Iversen, 1970; Breese and Traylor, 1971) (Sigma Chem. Co., St. Louis, MO), was injected into the substantia nigra ($A = 2.4$; $L = 1.0$; $V = 7.8$ mm) 30 min after a 15 mg/kg i.p. (5 ml/kg sterile saline) injection of the norepinephrine reuptake blocker desmethylimipramine (Merrill-Dow, Cincinnati, OH) dissolved in distilled water. The desmethylimipramine was injected to spare noradrenergic neurons from 6-hydroxydopamine toxicity by retarding the uptake of the toxin by central noradrenergic neurons (Breese and Traylor, 1971). The 6-hydroxydopamine was dissolved in 1.0 μl of sterile saline containing 0.1% ascorbic acid (Ungerstedt, 1971). The 6-hydroxydopamine was injected during 5 min via a 32 gauge cannula whose beveled tip was directed caudally. The cannula was removed 5 min after the end of the infusion.

All lesioned animals were killed by cervical decapitation 7 d after surgery. Maximal degeneration of intrinsic striatal cells (Schwarcz et al., 1982, 1984) and dopaminergic cells of the substantia nigra (Hökfelt and Ungerstedt, 1973; Altar et al., 1984a) occurs 7 days after the respective lesioning technique. The extent of dopamine denervation was assessed by the depletion of dopamine in the caudoputamen of the injected (left) hemisphere compared with the uninjected (right) caudoputamen. Dopamine was measured by gas chromatography with mass fragmentography (GC/MF) (Wood, 1982).

*Brain matrix autoradiography.* The brains of the lesioned rats were frozen in isopentane at −10 to −20°C. Consecutive 15 μm coronal or sagittal sections were cut on a cryostat (Hacker Instruments, Clifton, NJ) at −12°C. For analysis of $^{125}$I-SCH 23982 binding to the striatum of intact animals, coronal sections were cut from a block of rat forebrain located 8.7–9.5 mm anterior to the interaural plane (König and Klippel, 1967). Coronal sections that were used in the saturation or pharmacological competition experiments were cut from a "brain matrix" of 4 adjacently frozen brain hemispheres (Altar, 1987). After removal from the calvarium, each brain was rinsed in ice-cold 0.9% NaCl, blotted dry, and placed on a metal block. Coronal sections were made rostral and caudal to the region of interest with a tissue slicer blade (Thomas Scientific, Philadelphia, PA). The rostral section decreased the number of cryostat cuts required to reach the area of interest, and the caudal section provided a flat surface for mounting the brain to the cryostat chuck. To facilitate alignment of the 4 brain hemispheres in the rostrocaudal dimension and to create greater surface areas to freeze 1 hemisphere to the next, sagittal cuts were made about 2 mm from the most lateral extent of the neocortex. This cut exposed only the lateral aspect of the hippocampus and the surrounding neocortex. Each brain was bisected midsagittally. The right halves of the 4 brains were stacked next to one another, as were the left halves, and laid on the metal block with their ventral surfaces upward. The ventral surfaces of the brains were frozen with a 15 sec exposure to a cryogenic aerosol spray (Lerner Laboratories, New Haven, CT). The brain assembly, or "matrix," was flipped over and the dorsal surface was sprayed for 15 sec. The brain matrix was frozen in isopentane at −20°C, mounted on a cryostat chuck, and sectioned in the coronal dimension. Brain matrix autoradiography with a matrix of 4 hemispheres decreased the amount of cryostat cutting by 75% and decreased the work involved in all subsequent autoradiography procedures.

Sagittal views of $^{125}$I-SCH 23982 binding to the substantia nigra and striatum were obtained with tissue located 3.0–5.0 mm lateral to the midline (Paxinos and Watson, 1982). Horizontal sections were collected

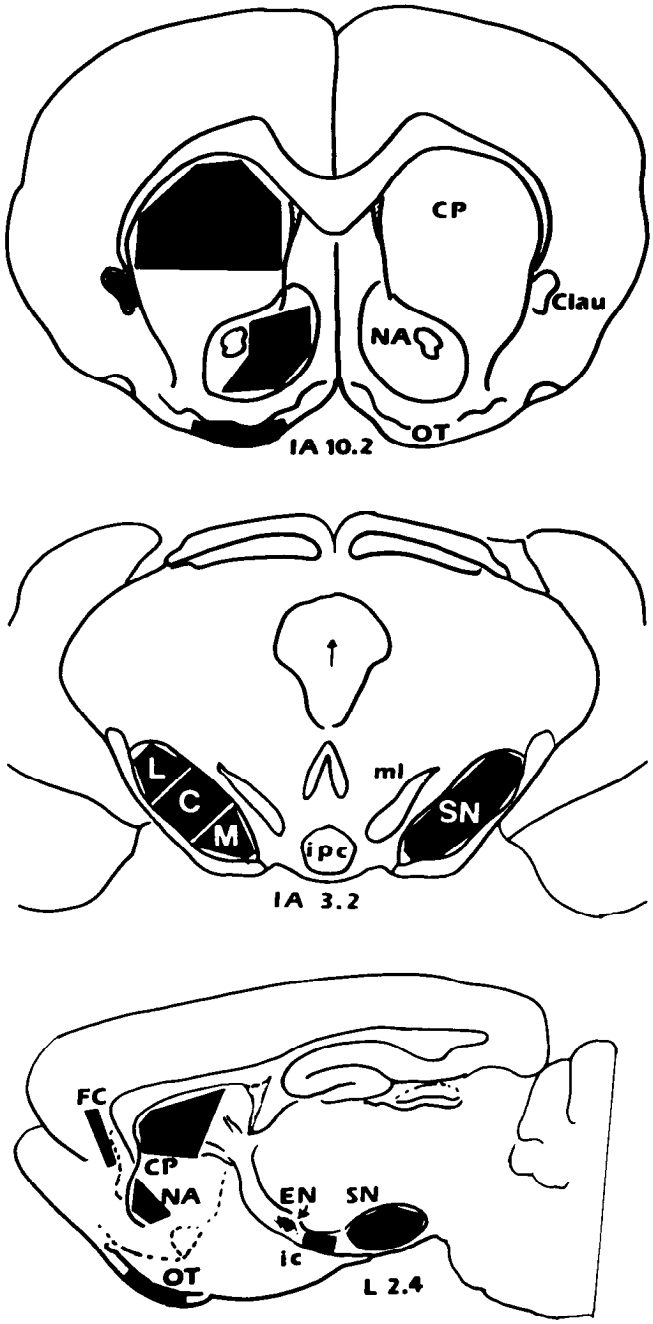


*Figure 2.* Illustrations of typical coronal and sagittal sections collected for the present studies. Anterior (*A*) and lateral (*L*) numbers are in millimeters from interaural line or midline, respectively (Paxinos and Watson, 1982). *Boxes* define regions in which $^{125}$I-SCH 23982 binding was quantified. Abbreviations: *C*, central third of the substantia nigra; *Clau*, claustrum; *CP*, caudateputamen; *EN*, entopeduncular nucleus; *FC*, layer 6 of frontal cortex; *IC*, internal capsule; *L*, lateral third of the SN; *M*, medial third of the SN; *NA*, nucleus accumbens; *SN*, substantia nigra, pars reticulata; *OT*, olfactory tubercle.

through the substantia nigra and caudoputamen. The individually cut brain sections were thawed onto gelatin-coated microscope slides and stored at −15°C in a humidified microscope slide box for 1 or 2 days (Herkenham and Pert, 1982).

*$^{125}$I-SCH 23982 binding assay.* The sections were thawed on a slide warmer, air-dried for 10 min, and laid horizontally on a ¼-in.-thick aluminum plate at 23°C. Each section was covered by 175 μl of a 50 mM, pH 7.4 (at 23°C) Tris buffer. The temperature and pH employed were very close to the optimal values for $^{3}$H-SCH 23390 binding (Schulz

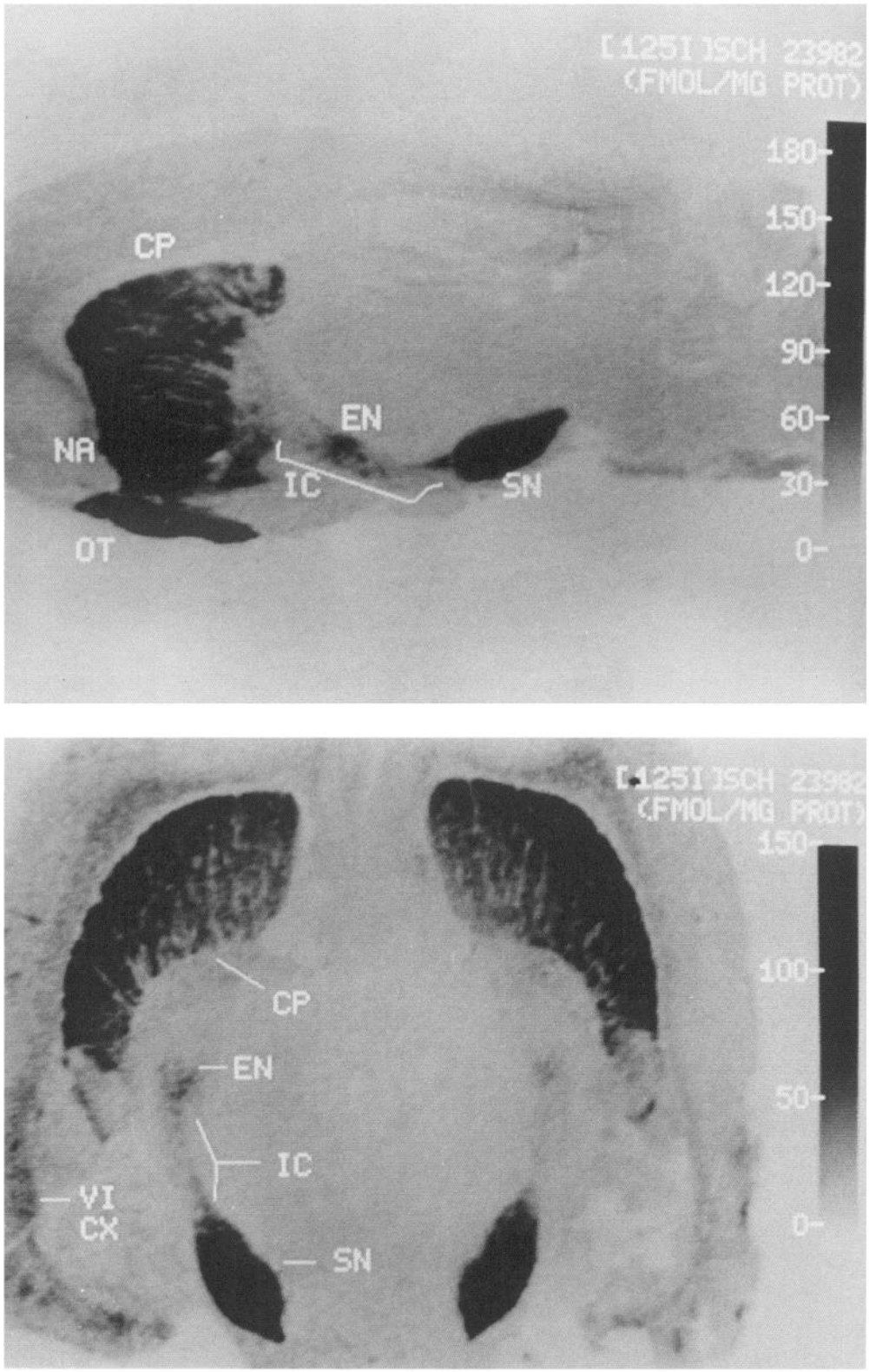


*Figure 3.* Linearized autoradiographic image of total $^{125}$I-SCH 23982 binding (70 pM) to a sagittal (*top*) or horizontal (*bottom*) section. Most $D_1$ binding in brain was found in the substantia nigra (*SN*), caudoputamen (*CP*), nucleus accumbens (*NA*), and olfactory tubercle (*OT*). Moderate binding was observed in the internal capsule (*IC*), entopeduncular nucleus (*EN*), and sixth layer of the neocortex (*VI CX*). Gray scale to the right of each figure relates the linearized image gray value to the concentration of bound $^{125}$I-SCH 23982.

et al., 1985). The buffer contained 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$ (Sidhu and Kebabian, 1985), and 2–770 pM $^{125}$I-SCH 23982 (2200 Ci/mmol) (DuPont-NEN, Boston). Nonspecific binding was determined in alternate sections with 5 μM *cis*-flupenthixol (Hyttel, 1978; Murrin, 1983).

For the displacement experiments, sections were coincubated with $^{125}$I-SCH 23982 and one of 7–12 concentrations of either SCH 23390 (Schering Corp., Bloomfield, NJ), *cis*-flupenthixol (Lundbeck, Copenhagen), SKF 38393 (Smith, Kline and French Laboratories, Philadelphia, PA), cinanserin (Janssen Pharmaceutica, Berse, Belgium), (*l*)-sulpiride (Rivizza, S. p. A., Milan), or LY 171555 (Lilly Research Labs., Indianapolis, IN). One hour later, the sections were rinsed for 2 min in ice-cold buffer pH 7.4 (at 4°C), and for 1 sec in ice-cold distilled water to remove the buffer and salts. The sections were dried within 5 min by

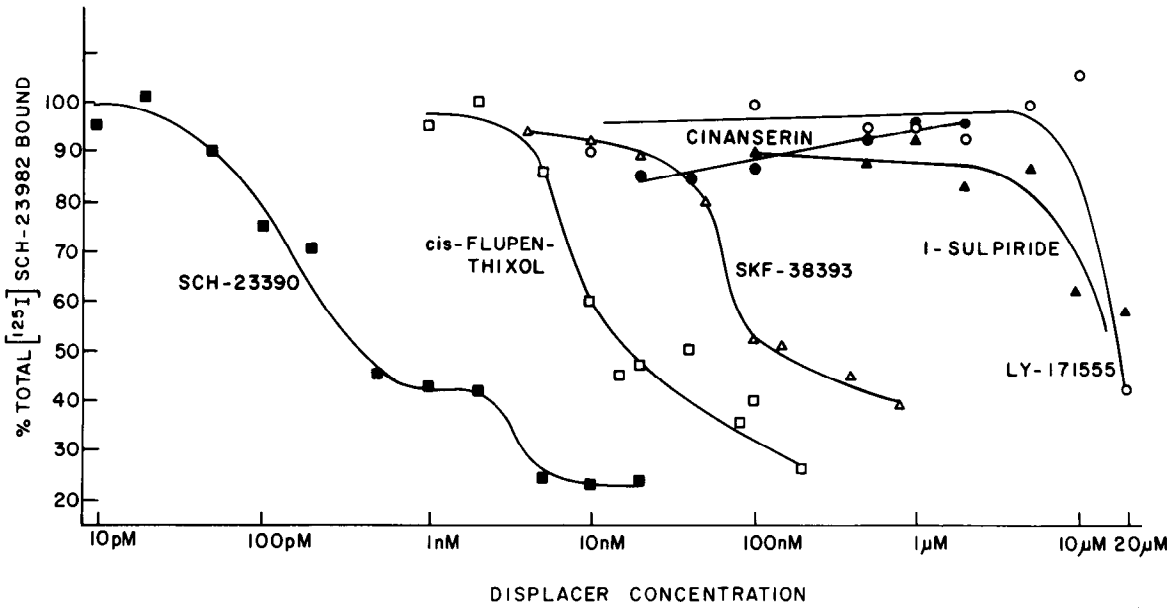


*Figure 4.* Displacement of $^{125}$I-SCH 23982 from binding sites in the caudoputamen by SCH 23390, *cis*-flupenthixol, SKF 38393, cinanserin, (*l*)-sulpiride, or LY 171555. Values are means of 4 brains and represent the percentage of binding in the absence of any displacer.

a stream of room-temperature air and sealed for 1–3 d in a desiccant-containing slide box to further dehydrate the sections.

*Autoradiography.* The slides were apposed for 18 hr to $^3$H-Ultrofilm (LKB, Bromma, Sweden) in a 3-layered, desiccant-containing autoradiography cassette. Included with each piece of film were 6 15-μm-thick standards cut from homogenized, centrifuged (30 sec × 50 × *g*), and frozen rat brain pastes (Neve et al., 1984) to which were added $^{125}$I-SCH 23982 in concentrations ranging from 39,000 to 1,200,000 dpm/mg protein, or the equivalent of 8–253 fmol SCH 23982/mg protein. The protein content of representative standards was measured by the method of Lowry et al. (1951). The equivalent SCH 23982 concentration of each standard was calculated on the basis of the specific activity of $^{125}$I-SCH 23982 used in the binding assays.

Under darkroom illumination, the autoradiographs were developed for 3½ min in Kodak D-19 and exposed to Kodak indicator stop (30 sec), Kodak fixer (4 min), cold tap water (10 min), and room-temperature air (1 hr) to dry.

*Digital subtraction autoradiography.* The amount of SCH 23982 bound in the brain regions illustrated in Figure 2 was quantified with a computer-assisted image analyzer (EyeCom II, Spatial Data Systems, Melbourne, FL). The logit-log gray value-to-concentration conversions (Snowhill and Boast, 1986) and the quantitation of iodinated ligand concentrations with the use of $^{125}$I standards (Israel et al., 1984; Rainbow et al., 1984) have been described. The SCH 23982 concentrations in the original sections were quantitatively represented in "linearized" computer images in which a new set of gray values was computed and displayed for each digitized autoradiograph. The new gray values represented $^{125}$I-SCH 23982 binding in each image as a linear function of the equivalent concentration of SCH 23982. The techniques of linearization and visualizations of specific ligand binding by digital subtraction autoradiography have been described (Altar et al., 1984b, 1985a, b). Briefly, the gray values in the digitized image can be converted to new gray values that are linearly related to $^{125}$I-SCH 23982 concentration. Areas of maximal binding are converted to the darkest (black) gray values, and areas of minimal binding are converted to the lightest (white) gray values. Importantly, all in-between gray values represent $^{125}$I-SCH

**Table 1. Affinity ($K_d$) and density ($B_{max}$) of $D_1$ sites for $^{125}$I-SCH 23982 in basal ganglia regions measured autoradiographically**

| Brain region | Kinetic value[a] | |
|---|---|---|
| | $K_d$ (pM) | $B_{max}$ (fmol/mg protein) |
| M. substantia nigra | 125 ± 26 | 176 ± 34 |
| L. substantia nigra | 91 ± 20 | 110 ± 8[b] |
| Caudoputamen | 93 ± 24 | 85 ± 16[c] |
| Nucleus accumbens | 55 ± 12 | 71 ± 16[d] |
| Olfactory tubercle | 56 ± 9 | 65 ± 4[d] |

Sections, 15 μm thick, were sequentially cut from a brain matrix consisting of 4 hemispheres and incubated in a concentration of 2–770 pM $^{125}$I-SCH 23982. Alternate sections were coincubated in 5 μM *cis*-flupenthixol to define nonspecific binding. $K_d$ and $B_{max}$ values were derived from the best fit to a parabola by iterative, nonlinear regression analysis (Bliss and James, 1966).

[a] Values are means ± SEM.

[b] $p < 0.05$ vs medial substantia nigra; Neuman-Keul's multivariate analysis.

[c] $p < 0.05$.

[d] $p < 0.01$ vs medial substantia nigra; Dunnett's *t* test.

**Table 2. Amount of $^{125}$I-SCH 23982 bound to 7 regions of sagittal sections incubated in 60 pM $^{125}$I-SCH 23982 alone (Total) or displaced by unlabeled drugs**

| Brain region | SCH 23982 concentration (fmol/mg protein) | | | |
|---|---|---|---|---|
| | | Displaced binding[a] | | |
| | Total binding | *cis*-Flu-penthixol | SKF 38393 | Cinan-serin |
| Substantia nigra | 93 ± 2[b] | 75 ± 2 | 75 ± 2 | 4 ± 2 |
| Caudoputamen | 73 ± 5 | 52 ± 4 | 51 ± 5 | 6 ± 4 |
| Nucleus accumbens | 82 ± 5 | 62 ± 5 | 61 ± 6 | 10 ± 4 |
| Olfactory tubercle | 66 ± 3 | 48 ± 6 | n.d.[c] | n.d.[c] |
| Entopeduncular nucleus | 49 ± 3 | 36 ± 8 | 35 ± 8 | 6 ± 7 |
| Internal capsule | 38 ± 3 | 15 ± 2 | 16 ± 2 | 4 ± 2 |
| Frontal cortex | 35 ± 3 | 13 ± 2 | 14 ± 3 | 7 ± 2 |

[a] Displaced binding is the difference between the total binding and that which occurred in the presence of 5 μM of the indicated displacers. No significant displacement occurred with 5 μM (*l*)-sulpiride or LY 171555.

[b] Values are means ± SEM of single determinations for each of 4 brains.

[c] Value was not determined.

**Table 3. Displacement by SCH 23390, *cis*-flupenthixol, or SKF 38393 of $^{125}$I-SCH 23982 binding to $D_1$ sites in the basal ganglia**

| | Unlabeled displacer | | | | | |
|---|---|---|---|---|---|---|
| | SCH 23390 | | *cis*-Flupenthixol | | SKF 38393 | |
| Brain region | $IC_{50}$ (pM) | nH | $IC_{50}$ (pM) | nH | $IC_{50}$ (pM) | nH |
| Caudateputamen | 190 ± 20 | 0.7 ± 0.3 | 7.6 ± 0.8 | 1.1 ± 0.9 | 78 ± 15 | 1.0 ± 0.1 |
| Nucleus accumbens | 95 ± 30 | 0.5 ± 0.1 | 4.8 ± 1.2 | 1.1 ± 0.2 | 89 ± 16 | 1.3 ± 0.4 |
| Olfactory tubercle | 142 ± 4 | 0.8 ± 0.1 | 5.1 ± 0.8 | 1.3 ± 0.1 | 73 ± 15 | 1.0 ± 0.3 |

Consecutive sections at the level of the striatal complex (Fig. 2) were incubated in 65 pM $^{125}$I-SCH 23982 in the absence or presence of one of 7–12 concentrations of unlabeled displacer. $IC_{50}$ and Hill (nH) values are expressed as means ± SEM of 4 brains.

23982 concentration in a proportional manner. This proportionality between gray value and ligand concentration allows one to visually superimpose a linearized image of total binding with an image of nonspecific binding ($^{125}$I-SCH 23982 binding to an adjacent section in the presence of 5 μM *cis*-flupenthixol) and subtract the gray values of the 2 images. The result is a quantitative image of the amount of specific $^{125}$I-SCH 23982 that was displaced by *cis*-flupenthixol. This difference image therefore shows only the $D_1$ sites throughout the brain section. Variations in the concentration of the $D_1$ binding sites are still linearly related to the gray values illustrated in the gray value scale (e.g., Fig. 5).

*Data analysis.* Average concentrations of SCH 23982 were calculated for the brain regions illustrated in Figure 2. The affinity ($K_d$) and capacity ($B_{max}$) values for the $D_1$ sites were derived from the best fit to a parabola by iterative, nonlinear regression analysis (Bliss and James, 1966). $IC_{50}$ and Hill (nH) values were calculated by logit-log analysis (Rodbard and Lewald, 1970). Differences in $^{125}$I-SCH 23982 concentrations between brain hemispheres in the lesion experiments were calculated with the paired *t* test.

## Results

As viewed in sagittal or horizontal section, the total binding of $^{125}$I-SCH 23982 was almost exclusively localized to the striatal complex (caudoputamen, nucleus accumbens, olfactory tubercle) and substantia nigra (Fig. 3). Moderate amounts of binding were present in the entopeduncular nucleus and less binding was found in the claustrum and sixth layer of the neocortex. A similar pattern of specific binding was observed in the striatal complex in coronal sections (Fig. 5) and in subtracted images of specific binding of sagittal and horizontal sections (data not shown). Notably, specific $^{125}$I-SCH 23982 binding was absent in the ventral tegmental area (data not shown, but see Fig. 5).

$^{125}$I-SCH 23982 bound with high affinity ($K_d$ values of 55–125 pM) to the striatal complex and substantia nigra (Table 1). The concentrations of binding sites were greatest in the medial substantia nigra ($B_{max}$ value of 176 fmol/mg protein), which exceeded by at least 50% the concentrations of $D_1$ sites in the lateral substantia nigra or in any region of the striatal complex ($B_{max}$ values of 65–85 fmol/mg protein).

The displacement of $^{125}$I-SCH 23982 by unlabeled compounds (5 μM each) was quantified in 7 regions of sagittal sections. $^{125}$I-SCH 23982 binding to the striatal complex and entopeduncular nucleus was displaced equally (73–86%) by *cis*-flupenthixol and by the selective $D_1$ agonist SKF 38393 (Table 2) but was not displaced by the selective $D_2$ antagonist (*l*)-sulpiride or $D_2$ agonist LY 171555 (data not shown). No binding of $^{125}$I-SCH 23982 to the striatal complex was significantly displaced with the $S_2$ antagonist cinanserin (Table 1). $^{125}$I-SCH 23982 binding to the internal capsule or frontal cortex was displaced only 37–39%, but again equally by *cis*-flupenthixol and SKF 38393 and insignificantly by cinanserin (Table 2), *l*-sulpiride, or LY 171555 (data not shown).

In coronal sections, SCH 23390 was the most potent compound tested against $^{125}$I-SCH 23982, occupying the $D_1$ site with an $IC_{50}$ value of 95–190 pM (Table 3). SCH 23390 displaced $^{125}$I-SCH 23982 in a biphasic manner from sites in the caudateputamen (Fig. 4), and consistently with Hill coefficients less than 1.0 (Table 3). *cis*-Flupenthixol displaced binding with less potency ($IC_{50}$ values of 5–8 nM) than SCH 23390 but from an apparent single population of $D_1$ sites or 2 sites with equal affinity for SCH 23982. The $D_1$ agonist SKF 38393 displaced $^{125}$I-SCH 23982 from each brain region and with Hill coefficients that approximated 1.0, but with lower potencies ($IC_{50}$ values of 73–89 nM) than either of the antagonists SCH 23390 or *cis*-flupenthixol (Table 3). (*l*)-Sulpiride and LY 171555 displaced $^{125}$I-SCH 23982 only at concentrations of 10 μM or above and cinanserin produced no displacement at concentrations as high as 2 μM (Fig. 4; Table 3).

Intrastriatal injections of 125 nmol quinolinic acid produced an 87% decrease in the concentration of $D_1$ sites in the caudateputamen and a 47% decrease in the substantia nigra (Table 4). The loss of $D_1$ binding in the caudoputamen was generally widespread at the anterior–posterior level where the injection was made but never included the nucleus accumbens or complete rostral–caudal extent of the caudoputamen. $D_1$ concentrations were decreased by 58% in the central portion of the substantia nigra and by 46% in the medial nigra but were unchanged in the lateral nigra (Table 4; see Fig. 2 for location of the nigral subregions). The marked decreases in specific $^{125}$I-SCH 23982 binding of the left caudoputamen and substantia nigra following 125 nmol quinolinic acid are illustrated in Figure 5.

Intrastriatal injections of 70 nmol quinolinic acid produced similar but smaller amounts of $D_1$ binding losses in either the caudoputamen or substantia nigra compared with the 125 nmol injections (Table 4).

The intranigral injection of 6-hydroxydopamine in 5 rats produced a 70 ± 14% (mean ± SEM) decrease in the dopamine concentration of the lesioned caudateputamen (176 ± 76 pmol/mg protein) compared to the dopamine concentration of the intact caudoputamen (587 ± 33 pmol/mg protein; $p < 0.01$, Student's *t* test). Nevertheless, the 6-hydroxydopamine injections did not decrease the concentration of $D_1$ sites in either the caudoputamen or substantia nigra (Table 4). This was the case even in 3 of these animals that showed a 90% or greater decrease in striatal dopamine levels (average lesioned hemisphere = 51 ± 6 pmol/mg protein; intact hemisphere = 619 ± 48 pmol/mg protein; $p < 0.01$, Student's *t* test). The equivalent binding of $^{125}$I-SCH 23982 in the caudoputamen and substantia nigra of the left and right hemispheres in 1 of these animals is shown in

**Table 4. $^{125}$I-SCH 23982 concentrations in substantia nigra and caudateputamen following striatal injections of quinolinc acid and nigral injections of 6-hydroxydopamine**

| Injection | Brain region | Specific $^{125}$I-SCH 23982 binding (fmol/mg protein) | | |
|---|---|---|---|---|
| | | Left hemisphere | Right hemisphere | Change (%) |
| Striatal quinolinic acid, 125 nmol | Caudoputamen | 13 ± 3[c] | 114 ± 12 | ↓87 ± 4 |
| | Substantia nigra | 70 ± 13[c] | 133 ± 22 | ↓47 ± 5 |
| | Lateral SN | 89 ± 19 | 88 ± 13 | n.c. |
| | Central SN | 49 ± 1[c] | 128 ± 21 | ↓58 ± 5 |
| | Medial SN | 88 ± 12[c] | 159 ± 16 | ↓46 ± 4 |
| Striatal quinolinic acid, 70 nmol | Caudoputamen | 33 ± 12[c] | 92 ± 13 | ↓75 ± 8 |
| | Substantia nigra | 99 ± 9[c] | 137 ± 16 | ↓27 ± 4 |
| | Lateral SN | 111 ± 10 | 119 ± 16 | n.c. |
| | Central SN | 87 ± 8[b] | 154 ± 16 | ↓41 ± 9 |
| | Medial SN | 138 ± 20 | 146 ± 20 | n.c. |
| Nigral 6-hydroxy-dopamine, 6 μg | Caudoputamen | 71 ± 17 | 82 ± 15 | n.c. |
| | Substantia nigra | 129 ± 16 | 107 ± 18 | n.c. |
| | Lateral SN | 104 ± 4[a] | 80 ± 10 | ↑37 ± 15 |
| | Central SN | 161 ± 11 | 113 ± 21 | n.c.[d] |
| | Medial SN | 146 ± 19 | 144 ± 25 | n.c.[d] |

Male rats received an intrastriatal injection of 125 or 70 nmol quinolinic acid or an intranigral injection of 6 μg 6-hydroxydopamine and were killed 7 d later. Coronal sections through the substantia nigra were incubated in 70 pM $^{125}$I-SCH 23982. Nonspecific binding was defined with 5 μM *cis*-flupenthixol. $^{125}$I-SCH 23982 concentrations were measured in the areas shown in Figure 5. Values are means ± SEM ($n$ = 6/group for quinolinic acid; 5/group for 6-hydroxydopamine).

[a] $p < 0.05$.

[b] $p < 0.02$.

[c] $p < 0.01$ vs right (intact) hemisphere; paired $t$ test.

[d] No change from right hemisphere.

Figure 5. The 6-hydroxydopamine injections were also not associated with a change in $D_1$ concentrations in the substantia nigra of the lesioned hemisphere except for a 37% increase in $D_1$ binding in the lateral portion of the substantia nigra, pars reticulata (Table 4). However, in subsequent studies using longer (3 week) survival times and larger amounts (12 μg) of 6-hydroxydopamine that resulted in >90% depletions of striatal dopamine, we found no increase in $^{125}$I-SCH 23982 concentrations in the lateral portion of the substantia nigra of the denervated hemisphere (C. A. Altar and M. R. Marien, unpublished observations). As observed here at 1 week, virtually complete destruction of the nigrostriatal dopamine projection for 3 weeks was not associated with any change in $^{125}$I-SCH 23982 binding site concentrations in the caudateputamen.

## Discussion

### *$D_1$ properties of $^{125}$I-SCH 23982*

Progress in our understanding of the $D_1$ receptor and its function in brain has been accelerated by the recent discovery of selective $D_1$ receptor agonists and antagonists. $^{125}$I-SCH 23982 is likely to further accelerate the study of the $D_1$ receptor for several reasons. As shown here, $^{125}$I-SCH 23982 displayed a 55–125 pM affinity constant for the $D_1$ site in brain sections. This affinity is 5- to 10-fold higher than that obtained with $^3$H-SCH 23390 for the $D_1$ site in striatal homogenates (Billard et al., 1984; Schulz et al., 1985). The higher affinity of $^{125}$I-SCH 23982 is probably not a result of differences in the properties of ligand binding to brain sections ahd homogenates. For example, $^{125}$I-SKF 83692, the racemate of $^{125}$I-SCH 23982, binds with a similar high affinity ($K_d$ value of 100 pM) to $D_1$ sites in striatal homogenates (Sidhu and Kebabian, 1985). Indeed, a correspondence in the affinities of a radiolabel for its binding site in homogenates and brain sections has many precedents in the literature, including $^3$H-tryptamine (Altar et al., 1985; Wood et al., 1984), $^3$H-spiperone (Creese and Snyder, 1975; Palacios et al., 1981), and $^{125}$I-LSD (Hartig et al., 1983; Nakada et al., 1984).

Another advantage of $^{125}$I-SCH 23982 is its approximately 50-fold greater specific radioactivity compared with the tritiated $D_1$-selective labels that are presently available. Autoradiography with the $D_1$ agonist $^3$H-SKF 38393 requires 3 weeks for image development (Scatton and DuBois, 1985), and a similar period would probably be required for autoradiography with monotritiated SCH 23390. In contrast, $^{125}$I-SCH 23982 autoradiographs were produced in the present study in only 18 hr. The high affinity of $^{125}$I-SCH 23982 for the $D_1$ receptor, when compared with this very high specific radioactivity, has allowed visualization of $D_1$ sites in the basal ganglia even when only a 500 fM concentration of the radioligand was used (C. A. Altar and M. R. Marien, unpublished observations).

Quantification of binding with iodinated standards prepared in our laboratory yielded $B_{max}$ values in the caudoputamen that were within an admittedly large range of $B_{max}$ values for the binding to striatal homogenates of $^3$H-SCH 23390 (347 fmol/mg protein: Schulz et al., 1985; 500 fmol/mg protein: Billard et al., 1984) and $^{125}$I-SKF 83692 (12 fmol/mg protein: Sidhu and Kebabian, 1985). A much lower affinity ($K_d$ = 8 nM) and higher capacity (1250 fmol/mg protein) of $^3$H-SKF 38393 for the $D_1$ site has been reported (Scatton and DuBois, 1985). Pharmacological displacement studies of $^{125}$I-SCH 23982 binding to sections revealed the same rank order of potency obtained by Sidhu and Kebabian (1985) using homogenates: with SCH

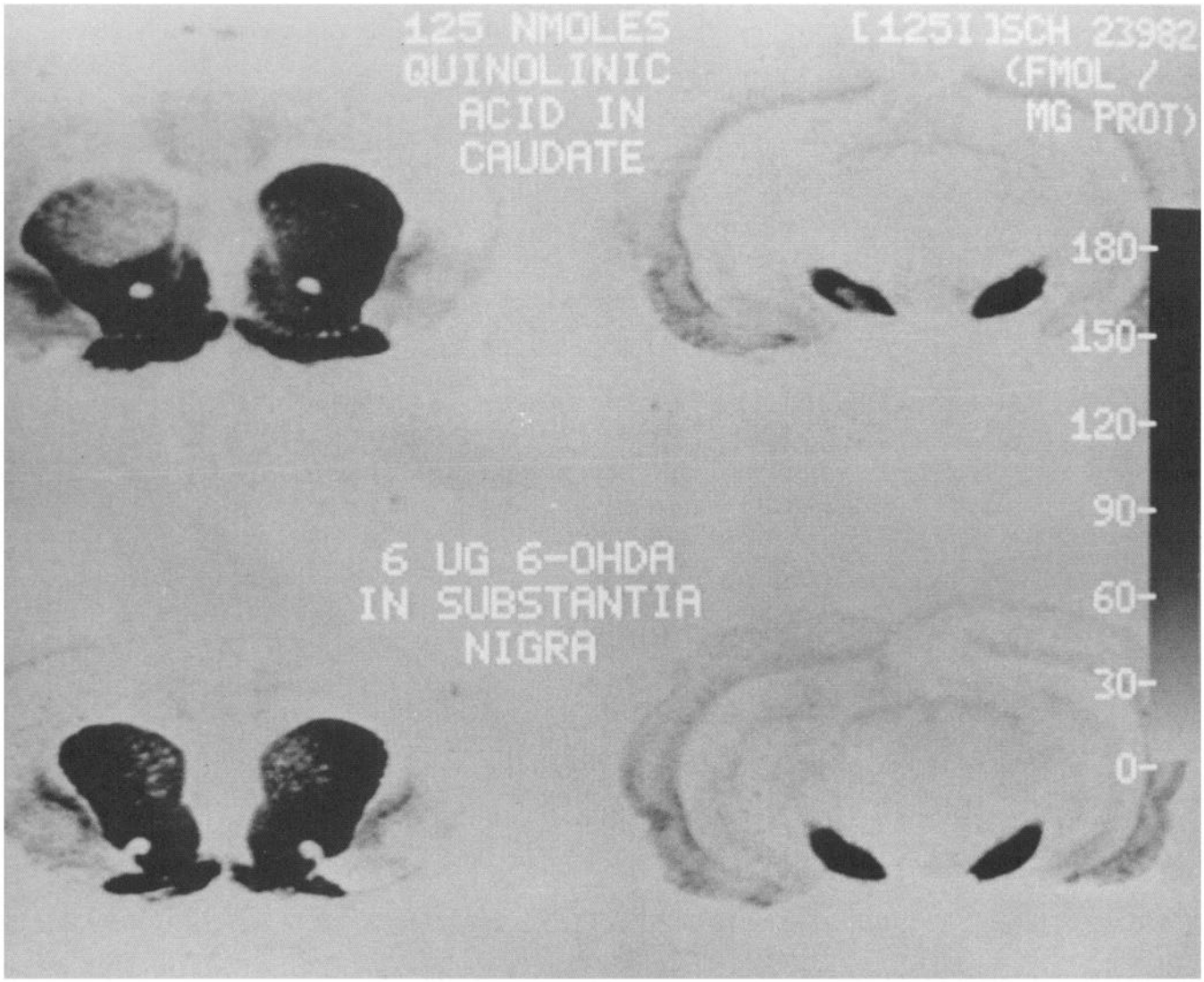


*Figure 5.* Digital subtraction autoradiography reveals *cis*-flupenthixol-displaceable $D_1$ sites on striatonigral neurons. For each panel in the figure, the superimposed and linearized images of total $^{125}$I-SCH 23982 binding and binding in the presence of 5 μM *cis*-flupenthixol of neighboring sections were subtracted. This resulted in a difference image of the displaceable $D_1$ sites. *Top,* Brain from a rat injected 1 week before sacrifice with 125 nmol quinolinic acid. Specific $D_1$ binding was markedly decreased in the ipsilateral caudateputamen and substantia nigra. *Bottom,* Brain from a rat injected 1 week before sacrifice with 6 μg of 6-hydroxydopamine that destroyed more than 90% of the nigrostriatal dopamine neurons. Specific $D_1$ binding was not decreased in the ipsilateral caudateputamen or substantia nigra.

23390 > *cis*-flupenthixol > SKF 38393 ≫ (*l*)-sulpiride = LY 171555. The last 2 compounds, a $D_2$ antagonist and $D_2$ agonist, respectively, and the $S_2$ antagonist cinanserin, were each inactive ($IC_{50}$ > 1 μM) against $^{125}$I-SCH 23982 binding. The greater $D_1$ potency of SCH 23390 and *cis*-flupenthixol compared with SKF 38393 is consistent with the 10- to 20-fold lower affinity of $^3$H-SKF 38393 for binding to the $D_1$ site (Scatton and DuBois, 1985) compared with $D_1$ antagonists (Billard et al., 1984; Sidhu and Kebabian, 1985). In summary, the binding of $^{125}$I-SCH 23982 to fresh-frozen rat brain sections and the digital quantification of the autoradiographic images reveal saturable and high-affinity binding of this ligand that is quantitatively and pharmacologically similar to $D_1$ binding to brain homogenates.

*Localization and function of $D_1$ sites on striatonigral neurons*

The quinolinic acid-induced destruction of neurons that are intrinsic to the caudateputamen resulted in a marked (up to 87%) loss of $D_1$ sites in the striatum. These sites do not necessarily reside on striatonigral neurons. However, the concomitant loss (up to 58%) of $D_1$ sites in the substantia nigra most likely resulted from the degeneration of striatonigral cells following the destruction of their perikarya (Schwarcz et al., 1982, 1984). This demonstrates that the majority of $D_1$ binding sites in the substantia nigra reside on striatonigral neurons. Using similar quinolinic acid injection parameters but more resolute sampling of the substantia nigra, we (C. A. Altar and K. Hauser, unpublished observations) have discovered that up to 87% of the $D_1$ sites within subregions of the substantia nigra, pars reticulata are lost following lesions of the striatum that destroy virtually all (~98%) striatal $D_1$ sites. The failure of 6-hydroxydopamine-induced mesostriatal dopamine neuron losses of up to 94% to decrease the concentration of $D_1$ sites in either the striatum or substantia nigra demonstrates that few, if any, $D_1$ sites are intrinsic to mesostriatal dopamine neurons.

Each of these autoradiographic observations corroborates much of what is known about the $D_1$ receptor based on changes in either adenylate cyclase or its product, cAMP. Thus, dopamine-stimulated adenylate cyclase (DAC) is present in the rat neostriatum (Kebabian et al., 1972) and substantia nigra, pars reticulata (Gale et al., 1977; Kebabian and Saavedra, 1976). These regions contained the highest concentrations of $D_1$ sites labeled with $^{125}$I-SCH 23982. The dopamine receptor–adenylate cyclase complex is not on dopaminergic cells of the substantia nigra (Kebabian and Saavedra, 1976; Nagy et al., 1978), and neither were the $D_1$ binding sites identified in the present experiments. Following destruction of mesostriatal dopaminergic neurons, the concentration of $D_1$ binding sites increased but only in the lateral substantia nigra. Following nigrostriatal lesions with kainic acid, Nagy and coworkers (1978) reported that levels of DAC per milligram of protein, but not per substantia nigra, increased in the lesioned substantia nigra. The marginal ($p < 0.05$) level of significance obtained for increases in the substantia nigra of DAC (Nagy et al., 1978) and $D_1$ concentrations (present results), and our subsequent failure to observe increases in $D_1$ concentrations in the striatum or nigra even 3 weeks after nigrostriatal denervation, indicates that $D_1$ receptors do not proliferate following dopamine denervation, at least not in the relatively rapid and robust manner demonstrable with $D_2$ autoradiography (Neve et al., 1984) or homogenate binding assays (Creese et al., 1983; Seeman et al., 1975). In contrast, DAC in the substantia nigra and striatum is markedly decreased by lesions of the striatonigral pathway (Gale et al., 1977; Spano et al., 1977), and $D_1$ sites were greatly depleted in these areas following striatal injections with quinolinic acid. Of course, some striatal $D_1$ sites may reside on neurons that do not project to the nigra, and some nigral $D_1$ sites may reside on neurons that do not originate in the striatum. Nevertheless, because most $D_1$ sites in the caudoputamen and substantia nigra were lost after striatal cell destruction, and because of the preponderance of $D_1$ sites in these 2 areas, the striatonigral pathway is probably the major $D_1$ receptor-containing pathway in rat brain. That $D_1$ binding was also observed in the internal capsule is consistent with the well-characterized passage of striatonigral fibers through this fiber bundle (Garcia-Munoz et al., 1977; Marshall and Ungerstedt, 1977). Whether $D_1$ sites in the region of the internal capsule represent receptor protein on the axonal membrane or are receptors in axonal transit is unknown.

Striatonigral neurons contain GABA (Jessell et al., 1978; Waddington and Cross, 1978; Guidotti et al., 1979), substance P (Brownstein et al., 1977; Hong et al., 1977), and dynorphin B (Vincent et al., 1982; McLean et al., 1985). Because the pattern of nigral $D_1$ binding losses following striatal lesions resembled almost exactly the topography of losses in dynorphin B or substance P concentrations (Kohno et al., 1984; McLean et al., 1985), it is possible that $D_1$ receptors are on the terminals of substance P- or dynorphin B-containing striatonigral neurons. The absence of changes in the concentration of $D_1$ sites in the lateral substantia nigra following rostral striatal lesions may have been because the substance P input to this region is derived exclusively from the most posterior caudoputamen (Kohno et al., 1984). In fact, we have recently shown that $D_1$ receptors in the lateral substantia nigra are also spared by striatal injections of quinolinic acid that produce neuronal toxicity restricted to the central region of the caudoputamen. Conversely, $D_1$ sites are lost in the lateral substantia nigra following quinolinic lesions that are restricted to the caudal caudoputamen (C. A. Altar and K. Hauser, unpublished observations).

In what way is dopamine most likely able to stimulate $D_1$ receptors in the substantia nigra? The axon terminals of the striatonigral pathway are in direct synaptic contact with dopaminergic dendrites in the pars reticulata (Wassef et al., 1981), but they may also form synaptic contacts in this brain region with serotonergic terminals (Gale, 1982) and GABAergic interneurons (Grace and Bunney, 1979, 1985). It is well established that dopamine is present in (Björklund and Lindvall, 1975) and released from (Geffen et al., 1976; Korf et al., 1976; Nieoullon et al., 1977) dendrites of the nigrostriatal pathway. The coexistence in the pars reticulata of dendritically released dopamine, DAC, and $D_1$ binding sites suggests that dendritically released dopamine interacts with $D_1$ receptors to modulate adenylate cyclase in the terminals of striatonigral neurons. The presence of $D_1$ sites in the entopeduncular nucleus suggests that $D_1$ receptors may also mediate GABAergic striatoentopeduncular neurons (Pan et al., 1985) in a similar manner.

In conclusion, $^{125}$I-SCH 23982 is a selective radioligand with picomolar affinity for the $D_1$ receptor in brain sections. Its binding to the caudoputamen, nucleus accumbens, and olfactory tubercle is displaced by $D_1$ receptor agonists (SCH 23390, *cis*-flupenthixol) and an antagonist (SKF 38393), but not by an $S_2$ antagonist (cinanserin) or by a $D_2$ agonist (LY 171555) or $D_2$ antagonist (*l*-sulpiride). $D_1$ sites are most concentrated in the medial substantia nigra but are also present in high concentration in the lateral substantia nigra, nucleus accumbens, olfactory tubercle, and caudoputamen. Lesser amounts of $D_1$ binding are present in the claustrum, sixth neocortical layer, internal capsule, and entopeduncular nucleus. The combination of chemical lesioning techniques and digital subtraction autoradiography has revealed for the first time the localization of $D_1$ binding sites to striatonigral neurons and not nigrostriatal neurons. The significance of $D_1$ receptors to the function of the striatoentopeduncular pathway or to GABAergic neurons of the striatonigral pathway has yet to be determined, as does the role of $D_1$ receptors in the regulation of substance P- or enkephalin B-containing neurons of the striatonigral projection.

## References


Altar, C. A. (1987) Future trends and methods of ligand autoradiography. In *Receptor Binding: Ligand Autoradiography,* F. Leslie, ed., Liss, New York (in press).

Altar, C. A., S. O'Neil, and J. F. Marshall (1984a) Sensorimotor impairment and elevated levels of dopamine metabolites in the neostriatum occur rapidly after intranigral injection of 6-hydroxydopamine or gamma-hydroxybutyrate in awake rats. Neuropharmacology *23:* 309–318.

Altar C. A., R. J. Walter, K. A. Neve, and J. F. Marshall (1984b) Computer-assisted video analysis of [$^3$H]spiroperidol binding autoradiographs. J. Neurosci. Methods *10:* 173–188.

Altar C. A., H. Kim, and J. F. Marshall (1985a) Computer imaging and analysis of $D_2$ or $S_2$ sites labeled by [$^3$H]spiroperidol in rat basal ganglia or neocortex. J. Pharmacol. Exp. Ther. *233:* 527–538.

Altar, C. A., S. O'Neil, R. J. Walter, and J. F. Marshall (1985b) Brain dopamine and serotonin receptor sites revealed by digital subtraction autoradiography. Science *228:* 597–600.

Altar, C. A., A. M. Wasley, and L. L. Martin (1985c) Autoradiographic localization and pharmacology of unique [$^3$H]tryptamine binding sites in rat brain. Neuroscience *17:* 263–273.

Billard, W., V. Ruperto, G. Crosby, L. C. Iorio, and A. Barnett (1984) Characterization of the binding of $^3$H-SCH 23390, a selective D-1 receptor antagonist ligand, in rat striatum. Life Sci. *35:* 1885–1893.

Björklund, A., and O. Lindvall (1975) Dopamine in dendrites of sub-

stantia nigra neurons: Suggestions for a role in dendrite terminals. Brain Res. *83:* 531–537.

Bliss, C. I., and A. T. James (1966) Fitting the rectangular hyperbola. Biometrics *22:* 573–602.

Breese, G. R., and T. D. Traylor (1971) Depletion of brain noradrenalin and dopamine by 6-hydroxydopamine. Br. J. Pharmacol. *42:* 88–99.

Brownstein, M. J., E. A. Mroz, M. L. Tappaz, and S. E. Leeman (1977) On the origin of substance P and glutamic acid decarboxylase (GAD) in the substantia nigra. Brain Res. *135:* 315–323.

Creese, I., and S. H. Snyder (1975) Dopamine receptor binding: Differentiation of agonist and antagonist states with [$^3$H]dopamine and [$^3$H]haloperidol. Life Sci. *17:* 993.

Creese, I., R. Schneider, and S. H. Snyder (1977) [$^3$H]Spiroperidol labels dopamine receptors in pituitary and brain. Eur. J. Pharmacol. *46:* 377–381.

Creese, I., D. R. Sibley, M. W. Hamblin, and S. E. Leff (1983) The classification of dopamine receptors: Relationship to radioligand binding. Annu. Rev. Neurosci. *6:* 43–71.

Gale, K. (1982) Evidence for GABA receptors on serotonergic afferent terminals in the substantia nigra and on nigral efferent projections to the caudal mesencephalon. Brain Res. *231:* 209–215.

Gale, K., A. Guidotti, and E. C. Costa (1977) Dopamine-sensitive adenylate cyclase: Location in substantia nigra. Science *195:* 503–505.

Garcia-Munoz, M., N. M. Nicolaou, I. F. Tulloch, A. K. Wright, and G. W. Arbuthnott (1977) Feedback loop of output pathway in striatonigral fibers? Nature *265:* 363–365.

Geffen, L. B., T. M. Jessel, A. C. Cuello, and L. L. Iversen (1976) Release of dopamine from dendrites in rat substantia nigra. Nature *260:* 258–260.

Grace, A. A., and B. S. Bunney (1979) Paradoxical GABA excitation of nigral dopaminergic cells: Indirect mediation through reticulata inhibitory neurons. Eur. J. Pharmacol. *59:* 211–218.

Grace, A. A., and B. S. Bunney (1985) Opposing effects of striatonigral feedback pathways on midbrain dopamine cell activity. Brain Res. *333:* 271–284.

Guidotti, A., K. Gale, A. Suria, and G. Toffano (1979) Biochemical evidence for two classes of GABA receptors in rat brain. Brain Res. *172:* 566–571.

Hartig, P., M. Kadan, A. Krohn, M. Evans, and R. Waltz (1983) [$^{125}$I]LSD: A selective, high sensitivity ligand for serotonin $5HT_2$ receptors. Soc. Neurosci. Abstr. *9:* 334.

Herkenham, J., and C. B. Pert (1982) Light microscopic localization of brain opiate receptors: A general autoradiographic method which preserves tissue quality. J. Neurosci. *2:* 1129–1149.

Hökfelt, T., and U. Ungerstedt (1973) Specificity of 6-hydroxydopamine-induced degeneration of central monoamine neurones: An electron and fluorescence microscopic study with special reference to intracerebral injection of the nigro-striatal dopamine system. Brain Res. *60:* 269–297.

Hong, J. S., H.-Y. T. Yang, G. Racagni, and E. Costa (1977) Projections of substance P containing neurons from neostriatum to substantia nigra. Brain Res. *122:* 541–544.

Hyttel, J. (1978) Effects of neuroleptics on $^3$H-haloperidol and $^3$H-cis(Z)-flupenthixol binding and on adenylate cyclase activity in vitro. Life Sci. *23:* 551–558.

Hyttel, J. (1983) SCH 23390—The first selective dopamine D-1 antagonist. Eur. J. Pharmacol. *91:* 153–154.

Hyttel, J. (1984) Functional evidence for selective dopamine D-1 receptor blockade by SCH 23390. Neuropharmacology *23:* 1395–1401.

Iorio, L. C., A. Barnett, F. H. Leitz, V. P. Houser, and C. A. Korduba (1983) SCH 23390, a potential benzazepine antipsychotic with unique interactions on dopaminergic systems. J. Pharmacol. Exp. Ther. *226:* 462–468.

Israel, A., F. M. Correra, M. Nina, and J. M. Saavedra (1984) Quantitative determination of angiotensin II binding sites in rat brain and pituitary gland by autoradiography. Brain Res. *322:* 341–345.

Itoh, Y., M. Beaulieu, and J. W. Kebabian (1984) The chemical basis for the blockade of the D-1 dopamine receptor by SCH 23390. Eur. J. Pharmacol. *100:* 119–122.

Jessell, T. M., P. C. Emson, G. Paxinos, and A. C. Cuello (1978) Topographic projections of substance P and GABA pathways in the striato- and pallidonigral system: A biochemical and immunohistochemical study. Brain Res. *152:* 487–498.

Kebabian, J. W., and D. B. Calne (1979) Multiple receptors for dopamine. Nature *277:* 93–95.

Kebabian, J., and J. M. Saavedra (1976) Dopamine-sensitive adenylate cyclase occurs in a region of substantia nigra containing dopaminergic dendrites. Science *193:* 683–685.

Kebabian, J. W., G. L. Petzold, and P. Greengard (1972) Dopamine-sensitive adenylate cyclase in caudate nucleus of rat brain and its similarity to the "dopamine receptor." Proc. Natl. Acad. Sci. USA *69:* 2145–2149.

Kohno, J., S. Shiosaka, K. Shinoda, S. Enagaki, and M. Tohyama (1984) Two distinct strio-nigral substance P pathways in the rat: An experimental immunohistochemical study. Brain Res. *308:* 309–317.

König, J. F. R., and R. A. Klippel (1967) *The Rat Brain: A Stereotaxic Atlas of the Forebrain and Lower Parts of the Brain Stem,* Krieger, New York.

Korf, J., M. Zieleman, and B. H. C. Westerink (1976) Dopamine release in substantia nigra? Nature *260:* 257–258.

Lowry, O. H., N. J. Rosenbrough, A. L. Farr, and R. J. Randall (1951) Protein measurement with the folin phenol reagent. J. Biol. Chem. *193:* 265–275.

Marshall, J. F., and U. Ungerstedt (1977) Striatal efferent fibers play a role in maintaining rotational behavior in the rat. Science *198:* 62–64.

McLean, S., M. J. Bannon, N. Zamir, and C. B. Pert (1985) Comparison of the substance P- and dynorphin-containing projections to the substantia nigra: A radioimmunocytochemical and biochemical study. Brain Res. *361:* 185–192.

Murrin, L. C. (1983) Characteristics of $^3$H-cis-flupenthixol binding in rat striatum. Life Sci. *33:* 2179–2186.

Nagy, J. I., S. R. Vincent, J. Lehmann, H. C. Fibiger, and E. G. McGeer (1978) The use of kainic acid in the localization of enzymes in the substantia nigra. Brain Res. *149:* 431–441.

Nakada, M. T., C. M. Wieczorek, and T. C. Rainbow (1984) Localization and characterization by quantitative autoradiography of [$^{125}$I]LSD binding sites in rat brain. Neurosci. Lett. *49:* 13–18.

Neve, K. A., C. A. Altar, C. A. Wong, and J. F. Marshall (1984) Quantitative analysis of [$^3$H]spiroperidol binding to rat forebrain sections: Plasticity of neostriatal dopamine receptors after nigrostriatal injury. Brain Res. *302:* 9–18.

Nieoullon, A., A. Cheramy, and J. Glowinski (1977) Release of dopamine in vivo from cat substantia nigra. Nature *266:* 375–377.

O'Boyle, K. M., and J. L. Waddington (1985) Structural determinants of selective affinity for brain D-1 dopamine receptors within a series of 1-phenyl-1H-3-benzazepine analogues of SK&F 38393 and SCH 23390. Eur. J. Pharmacol. *115:* 291–295.

Palacios, J. M., D. L. Niehoff, and M. J. Kuhar (1981) [$^3$H]-Spiperone binding sites in brain: Autoradiographic localization of multiple receptors. Brain Res. *213:* 277–289.

Pan, H. S., J. B. Penney, and A. B. Young (1985) Gamma-aminobutyric acid and benzodiazepine receptor changes induced by unilateral 6-hydroxydopamine lesions of the medial forebrain bundle. J. Neurochem. *45:* 1396–1404.

Paxinos G., and C. Watson (1982) *The Rat Brain in Stereotaxic Coordinates,* Academic, New York.

Rainbow, T. C., B. Parsons, and B. B. Wolfe (1984) Quantitative autoradiography of $\beta_1$- and $\beta_2$-adrenergic receptors in rat brain. Proc. Natl. Acad. Sci. USA *81:* 1585–1589.

Rodbard, D., and J. E. Lewald (1970) Computer analysis of radioligand assay and radioimmunoassay data. Acta Endocrinol. *147:* 79–103.

Scatton, B., and A. DuBois (1985) Autoradiographic localization of $D_1$ dopamine receptors in the rat brain with [$^3$H]SKF 38393. Eur. J. Pharmacol. *111:* 145–146.

Schulz, D. W., E. J. Stanford, S. W. Wyrick, and R. B. Mailman (1985) Binding of [$^3$H]SCH 23390 in rat brain: Regional distribution and effects of assay conditions and GTP suggest interactions at a $D_1$-like dopamine receptor. J. Neurochem. *45:* 1601–1611.

Schwarcz, R., W. O. Whetsell, and R. M. Mangano (1982) Quinolinic acid: An endogenous metabolite that produces axon-sparing lesions in rat brain. Science *219:* 316–318.

Schwarcz, R., A. C. Foster, E. D. French, W. O. Whetsell, and C. Köhler (1984) Excitotoxic models for neurodegenerative disorders. Life Sci. *35:* 19–32.

Seeman, P. (1980) Brain dopamine receptors. Pharmacol. Rev. *32:* 229–313.

Seeman, P., M. Chau-Wong, J. Tedesco, and K. Wong (1975) Brain receptors for antipsychotic drugs and dopamine: Direct binding assays. Proc. Natl. Acad. Sci. USA *72:* 4376–4380.

Sidhu, A., and J. W. Kebabian (1985) An iodinated ligand identifying the D-1 dopamine receptor. Eur. J. Pharmacol. *113:* 437–440.

Snowhill, E., and C. Boast (1986) Quantitative image analysis and its use to study variations in regional benzodiazepine receptor binding. In *Quantitative Receptor Autoradiography,* C. A. Boast, E. Snowhill, and C. A. Altar, eds., pp. 13–28, Liss, New York.

Spano, P. F., M. Trabucchi, and G. DiChiera (1977) Localization of nigral dopamine-sensitive adenylate cyclase on neurons originating from the corpus striatum. Science *196:* 1343–1345.

Stoof, J. C., and J. W. Kebabian (1981) Opposing roles for D-1 and D-2 dopamine receptors in efflux of cyclic AMP from rat neostriatum. Nature *294:* 366–368.

Ungerstedt, U. (1968) 6-Hydroxydopamine induced degeneration of central monoamine neurons. Eur. J. Pharmacol. *5:* 107–110.

Ungerstedt, U. (1971) Stereotaxic mapping of the monoamine pathways in the rat brain. Acta Physiol. Scand. Suppl. *367:* 1–48.

Uretsky, N. J., and L. L. Iversen (1970) Effects of 6-hydroxydopamine on catecholamine-containing neurons in the rat brain. J. Neurochem. *17:* 269–278.

Vincent, S., T. Hökfelt, I. Christensson, and L. Terenius (1982) Immunohistochemical evidence for a dynorphin immunoreactive striatonigral pathway. Eur. J. Pharmacol. *85:* 251–252.

Waddington, J. L., and A. J. Cross (1978) Denervation supersensitivity in the striatonigral GABA pathway. Nature *276:* 618–621.

Wassef, M., A. Berod, and C. Sotelo (1981) Dopaminergic dendrites in the pars reticulata of the rat substantia nigra and their striatal input. Combined immunocytochemical localization of tyrosine hydroxylase and anterograde degeneration. Neuroscience *6:* 2125–2139.

Wood, P. L. (1982) A mass fragmentographic assay for dopamine and its metabolites using negative chemical ionization. Biomed. Mass. Spectrosc. *9:* 302–306.

Wood, P. L., C. Pilapil, F. LaFaille, N. P. V. Nair, and R. A. Ennon (1984) Unique [$^3$H]tryptamine binding sites in rat brain: Distribution and pharmacology. Arch. Int. Pharmacodyn. Ther. *268:* 194–201.