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D-1 Dopamine Receptors in the Rat Brain: A Quantitative Autoradiographic Analysis

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The distribution of dopamine D-1 receptors has been determined in the rat brain by a quantitative *in vitro* light-microscopic autoradiographic method. The binding of [*N*-methyl-^{3}H]-SCH 23390 to slide-mounted tissue sections takes place with characteristics expected of a substance that recognizes D-1 receptors. The binding is saturable, has high affinity, and exhibits an appropriate pharmacology and stereospecificity in several discrete microscopic brain regions as determined by quantitative autoradiography. The highest density of D-1 receptors occurs in the caudate-putamen, accumbens nucleus, olfactory tubercle, and the substantia nigra pars reticulata. High concentrations of D-1 receptors were associated with the intercalated and medial nuclei of the amygdala, entopeduncular nucleus, and major island of Calleja. Furthermore, moderate to low concentrations were observed in several other structures, such as the frontal cortex, subthalamic nucleus, and several thalamic, hypothalamic, and hippocampal areas. The distribution of D-1 receptors correlates very well with projection areas of dopaminergic pathways. This technique furnishes a powerful assay for the accumulation of detailed pharmacologic and anatomical data about D-1 receptors, and the results suggest possible CNS sites of action of D-1 dopamine receptor selective compounds.

Pharmacological, biochemical, anatomical, and physiological studies have indicated that there are multiple receptors for dopamine (see for review Creese and Leff, 1982; Creese et al., 1983; Kebabian and Calne, 1979; Stoof and Kebabian, 1984). Dopamine type-1 (D-1) receptors are associated with stimulation of adenylate cyclase on agonist activation (Kebabian and Calne, 1979). Dopamine type-2 (D-2) receptors mediate either the inhibition of adenylate cyclase (Cote et al., 1981; Onali et al., 1981, 1984) or are unassociated with the enzyme (Kebabian and Calne, 1979). D-2 receptors have been extensively characterized by radioligand binding studies using ^{3}H-spiperone (Creese et al., 1977; Fields et al., 1977) and the selective D-2 antagonist ^{3}H-sulpiride (O'Connor and Brown, 1982; Theodorou et al., 1979; Woodruff and Freedman, 1981). D-1 receptors have been characterized using ^{3}H-*cis*-(Z)-flupenthixol (Huff and Molinoff, 1985; Hyttel, 1978a, b; Murrin, 1983) and ^{3}H-*cis*-(Z)-piflutixol (Hyttel, 1981), but both compounds show similar affinities for the D-1 and D-2 receptors and a high degree of nonspecific binding. As such, these two compounds are not suitable ligands for the study of D-1 receptors by autoradiography.

Recently, Iorio et al. (1983) described a putative neuroleptic, benzazepine SCH 23390 [(R)-(+)-8 chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepine-7-ol hemimaleate], and postulated that it was a selective D-1 receptor antagonist based on its potent blockade of dopamine (DA)-stimulated adenylate cyclase, its weak displacement of ^{3}H-spiperone binding, its failure to induce prolactinemia, and its selective displacement of ^{3}H-piflutixol binding from striatal slices (Hyttel, 1983). A tritiated form of SCH 23390 has been determined to bind with stereospecificity, with high affinity, and in a saturable manner to receptor sites that have been characterized as the D-1 receptors (Billard et al., 1984). Therefore, ^{3}H-SCH 23390 has been designated as the most suitable ligand for investigations attempting to characterize the D-1 receptor.

In vitro autoradiographic techniques have previously been used to localize DA receptors microscopically. Extensive studies have localized D-2 receptors using ^{3}H-spiperone (Klemm et al., 1979; Murrin and Kuhar, 1979; Palacios and Wamsley, 1984; Palacios et al., 1981a), ^{3}H-sulpiride (Gehlert and Wamsley, 1984, 1985; Jastrow et al., 1984), ^{3}H(−)-DO710 (Sokoloff et al., 1985), and ^{125}I-iodosulpride (Martres et al., 1985a, b); these investigations have demonstrated that the D-2 receptor is associated primarily with the tuberoinfundibular system, nigrostriatal system, periglomerular DA system of the olfactory complex, and, to a minor extent, the mesocortical and mesolimbic systems. Recently, we utilized ^{3}H-SCH 23390 (Dawson et al., 1985a), and Scatton and Dubois (1985) used ^{3}H-SKF 38393 to localize D-1 receptors to several mesocortical and mesolimbic system structures where the existence of DA terminals and receptors was suspected but could not be demonstrated with previously available radioligands.

In the present study, ^{3}H-SCH 23390 was evaluated for its suitability and specificity as a D-1 receptor antagonist by using the quantitative technique of *in vitro* receptor autoradiography (Unnerstall et al., 1982; see for review Kuhar, 1985b). In addition, the regional distribution and density of D-1 dopamine receptors in discrete microscopic regions of the rat brain are described.

Materials and Methods

Materials

[*N*-methyl-^{3}H]-SCH 23390 (specific activity, 72 Ci/mmol), SCH 23390 (R-enantiomer), *cis*-(Z)-piflutixol, *trans*-(E)-piflutixol were generous gifts from Schering-Plough Corp. (Bloomfield, NJ). Fluphenazine (New England Nuclear, Boston, MA), forskolin (Calbiochem-Behring Corp., La Jolla, CA), ketanserin (Janssen Pharmaceutica Inc., Piscataway, NJ), SKF 38393 (Smith Kline & French Labs, Philadelphia, PA), ADTN and triprolidine (Burroughs Wellcome Co., Research Triangle Park, NC), Naloxone (Endo Laboratories Inc., Garden City, NJ), atropine (J. T.

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Baker Chemical Co., Phillipsburg, NJ), serotonin and propranolol (Sigma Chemical Co., St. Louis, MO), diazepam (Hoffman-LaRoche, Nutley, NJ), sulpiride (S.P.A. per l'industria chemica e. Pharmaceutica, Milan, Italy), and methysergide (Sandoz Inc., E. Hanover, NJ) were either purchased or were gifts from the manufacturers.

Tissue preparation

Male Sprague-Dawley rats (150–300 gm) were purchased from Simonsen Laboratories and kept under a controlled light–dark cycle and temperature until sacrificed by intracardial perfusion (while under deep chloroform anesthesia) with 0.9% saline. The brains were rapidly dissected from the skull and frozen by slow immersion into isopentane at −80°C. Sections (10 μm thick) were cut on a Harris cryostat (−18°C) microtome (Harris Manufacturing, N. Billerica, MA) and thaw-mounted onto cold chrome-alum/gelatin-coated microscope slides. The slide-mounted tissue sections were then stored for short periods of time in a self-defrosting freezer (−20°C).

Biochemical investigations

The dissociation, association, and saturation kinetics of ^{3}H-SCH 23390 binding to slide-mounted tissue sections were studied by exposing sections of forebrain, containing primarily striatum, to various rinse times (3 sec to 20 min), incubation times (1–60 min), and incubation concentrations (0.05–10.0 nM), respectively, in 50 mM Tris HCl buffer (pH 7.4) containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, and 1 mM $MgCl_2$. A concentration of 0.3 nM of ^{3}H-SCH 23390 was used in the dissociation and association experiments, and an incubation time of 30 min (followed by two 5-min rinses in ice-cold buffer) was used in subsequent saturation experiments. Nonspecific binding was determined by incubating serial tissue sections in separate coplin jars under identical conditions, except for the additional presence of 10^{-6} M unlabeled SCH 23390. The amount of radioactivity contained in these tissue sections was quantified by wiping the tissue from the slide with Whatman GF/B glass microfiber disks. The tissue-laden filters were then placed individually in scintillation vials, scintillation cocktail was added, and the radioactivity bound to the tissue section was determined by liquid scintillation counting. Dissociation, association, and saturation curves were plotted from the data obtained.

Autoradiographic procedures

The regional distribution of D-1 receptors was determined by incubating serial sections of forebrain, brain stem, and spinal cord (cut at approximately 250 μm intervals) for 30 min at room temperature in 50 mM Tris HCl (pH 7.4) containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, and 1.0 nM ^{3}H-SCH 23390. Serial sections were also incubated in the same media, with the addition of 5×10^{-6} M fluphenazine or 10^{-6} M unlabeled SCH 23390, to produce autoradiograms representing nonspecific binding. The sections were then dipped in ice-cold buffer, followed by 2 5-min rinses in fresh buffer. The buffer salts were removed by a rapid dip in ice-cold distilled water, and the sections were dried (30 sec) by blowing cool, dry, filtered air over their surfaces.

Autoradiograms were generated by apposition of the labeled tissue sections to LKB Ultrofilm (LKB Instruments, Rockville, MD) in Wolf X-ray cassettes. After a 3 or 6 week exposure period, the films were removed and developed. The autoradiograms produced by the labeled tissue sections were examined using a Leitz Orthoplan microscope or by examining photomicrographs of the latent images on the tritium-sensitive film taken with an Orthomat camera system attached to the Leitz microscope. Anatomical areas were identified and named according to Paxinos and Watson (1982). Cresyl violet staining of the tissue sections was performed in order to verify the anatomic areas identified from the autoradiograms.

In order to ensure that the autoradiographic grains were due to ^{3}H-SCH 23390 binding to D-1 receptors, saturation and displacement assays were performed in sections of rat brain through the prefrontal cortex, striatum, and substantia nigra. The saturation studies were executed on serial tissue sections using the conditions outlined above, except that the ^{3}H-SCH 23390 concentration was varied from 0.05 to 10.0 nM. Competition studies were also carried out using the conditions outlined above, except that various concentrations of unlabeled SCH 23390 (R-enantiomer) (10^{-6}–10^{-13} M), fluphenazine (10^{-5}–10^{-11} M), SKF 38393 (10^{-4}–10^{-10} M), sulpiride (10^{-4}–10^{-8} M), methysergide (10^{-4}–10^{-10} M), ketanserin (10^{-4}–10^{-10} M), *cis*-(Z)-piflutixol (10^{-5}–10^{-11} M), or *trans*-(E)-piflutixol (10^{-5}–10^{-11} M) were added to the incubation media. Micromolar concentrations of several pharmacologically unrelated compounds (atropine, diazepam, forskolin, naloxone, nomifensine, propranolol, 5-HT, and triprolidine) were added to the incubation media in order to determine their effects on ^{3}H-SCH 23390 binding. Autoradiograms were then generated, and the autoradiographic grain densities were quantitated as described below. This provided a means for plotting saturation isotherms and displacement curves from data obtained from several microscopic regions of the rat brain. Scatchard analysis was performed on the saturation data, yielding K_d's and B_{max}'s for several areas. Competition curves, constructed from the densitometric data, were analyzed using an iterative, nonlinear, least-squares, curve-fitting computer program (Munson and Rodbard, 1980).

Quantitation

Analysis of the autoradiographic grain densities on the film (D-1 receptors) in corresponding tissue areas was accomplished using computer-assisted microdensitometric analysis. A DADS model 560 (Stahl Research, Rochester, NY) photometry system (an Oki computer interfaced with an MPV-Compact photometer attached to the Leitz microscope) was used for this purpose. Optical density values were converted into molar quantities of bound ligand by referencing a tritium-labeled polymer (Autoradiographic [^{3}H]-Microscales; Amersham Corp., Arlington Heights, IL). These standards are calibrated for the tritium concentration in gray–white matter areas according to the method of Unnerstall et al. (1982). Since beta-particles emitted by tritium have a relative low energy, tritium "quenching" tends to occur in areas of high white matter content, resulting in varying degrees of autoradiographic efficiency over a tissue section (Kuhar and Unnerstall, 1985; Rainbow et al., 1984). This leads to an underestimation of the concentration of radioactivity in areas of high white matter density (Kuhar and Unnerstall, 1985; Rainbow et al., 1984). Values for total and nonspecific ^{3}H-SCH 23390 binding were obtained for each region by averaging 5 readings over an individual area. Care was taken, when possible, to take readings from predominantly gray matter in order to minimize the underestimation of bound radioactivity. Results reported in this investigation are from at least 2 separate experiments involving tissue from 3 or more animals per experiment.

Results

Properties of ^{3}H-SCH 23390 binding to striatal brain sections

The dissociation rate of ^{3}H-SCH 23390 was evaluated first by incubating 2 sets of 6 sections for 20 min in 0.3 nM ^{3}H-SCH 23390 and then subjecting them to various rinse times. Two 5-min rinses were determined to be optimal and provided the highest specific-to-nonspecific (signal-to-noise) ratio without significant loss in specific binding (Fig. 1*A*). A rate constant for dissociation was not calculated due to the high amount of total binding remaining at 20 min.

Variation of the incubation time from 1 to 60 min (using the rinse time determined in the previous experiment) was performed to examine the association rate of ^{3}H-SCH 23390 to striatal brain sections (Fig. 1*B*). An incubation time of 30 min was determined to be optimal. The association rate constant (K_{+1}) was computed to be 0.015 min^{-1} nM^{-1} using pseudo-first-order kinetics (Weiland and Molinoff, 1981).

The binding of ^{3}H-SCH 23390 to brain sections was saturable, specific, and of a high affinity (Fig. 1*C*). Scatchard analysis of the binding indicates a dissociation constant (K_d) of 1.86 nM and a maximal number of binding sites (B_{max}) of 72.91 fmol/mg tissue (Fig. 1*D*). These values are in agreement with those reported by Billard et al. (1984) for striatal membrane preparations. A Hill plot of these data yielded a slope of 0.97, indicating that there was no cooperativity and that the binding was taking place to a single population of receptors. For routine autoradiographic studies, a concentration of 1 nM was chosen, which maintains "zone A" conditions. Using the parameters outlined above, 95–98% of ^{3}H-SCH 23390 binding to tissue sections was specific. Such low levels of nonspecific binding virtually ensure that essentially all of the visualized autoradiographic grains were due to specific ^{3}H-SCH 23390 binding.

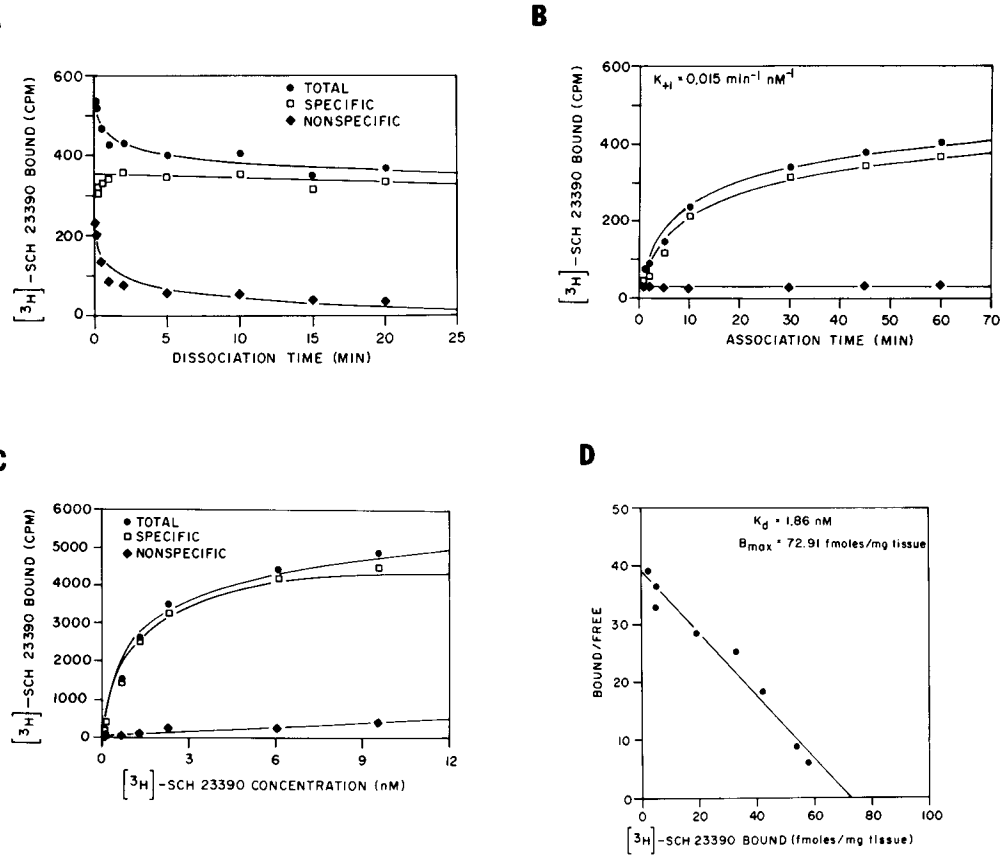

Figure 1. *A*, Dissociation analysis of ^{3}H-SCH 23390 binding to slide-mounted tissue sections. The binding procedure was performed as described in the text. After incubation for 20 min with 0.3 nM ^{3}H-SCH 23390, the incubation was stopped by infinite dilution in fresh, cold buffer for various times. These curves represent the mean of 2 separate experiments performed in triplicate. *B*, Equilibrium time course of ^{3}H-SCH 23390 binding. Binding of ^{3}H-SCH 23390 (0.3 nM) was carried out as described in the text. The incubation was stopped by immersion in fresh, cold buffer for 2 5-min rinses. These curves represent the average of 2 separate experiments performed in triplicate. The association rate constant was derived assuming pseudo-first-order kinetics. *C*, Saturation of ^{3}H-SCH 23390 binding in tissue sections. The tissue sections, in areas representing the striatum, were incubated with varying concentrations of ^{3}H-SCH 23390 (0.05–10 nM) with or without 1 μM cold SCH 23390 as described in the text. Binding of ^{3}H-SCH 23390 at each concentration was measured by liquid scintillation counting and fit to a hyperbolic curve by computer fitting. The data represent the mean of 2 separate experiments performed in triplicate. *D*, Scatchard plot of the specific binding of ^{3}H-SCH 23390 to slide-mounted tissue sections. Linear regression yielded a single component ($r = 0.98$) with a K_d (dissociation constant) of 1.86 nM and a B_{max} of 72.91 fmol/mg tissue. A Hill plot of the data yielded a slope of 0.97. Data are the average of 2 separate experiments performed in triplicate.

Quantitative autoradiographic analysis of ^{3}H-SCH 23390 binding to brain sections

To establish that the pharmacological specificity of ^{3}H-SCH 23390 binding to tissue sections was similar to the binding in striatal membrane preparations, we evaluated the displacement of ^{3}H-SCH 23390 binding in several discrete microscopic regions of the brain by using several related and unrelated compounds. K_i values for several brain areas are reported in Table 1. The most potent compound inhibiting ^{3}H-SCH 23390 binding was SCH 23390 itself, with a K_i ranging from 0.214 to 0.343 nM. *Cis*-(Z)-Piflutixol inhibits specific binding by 50% at 0.274–1.94 nM, depending on the brain area examined; this value is about 1000 times more potent than *trans*-(E)-piflutixol (Table 1). Of the other DA receptor antagonists tested, fluphenazine had a K_i ranging from 0.649 to 12.3 nM and sulpiride had a k_i of at least 10,000 nM. In contrast to the apparent single-site binding characteristics demonstrated with the DA antagonists, competition experiments performed using the selective D-1 agonist, SKF 38393, to displace ^{3}H-SCH 23390 binding was heterogeneous. The latter results were fit to a 1-site model in several regions of the brain. However, the binding was susceptible to guanine nucleotide regulation (data not shown), suggesting that a high- and low-affinity agonist state exists for the D-1 receptor. The 5-HT antagonists methysergide and ketanserin had K_i's ranging from 176 to 598 nM and from 110 to 2790 nM, respectively. Micromolar concentrations of atropine, diazepam, forskolin, naloxone, nomifensine, propranolol, 5-HT, and triproldine showed little or no effect on the binding of ^{3}H-SCH 23390 to several rat brain areas.

Saturation studies performed in several regions of the rat brain and subsequently analyzed using quantitative autoradiography demonstrated saturable, high-affinity binding of ^{3}H-SCH 23390 to many brain structures; the K_d's and B_{max}'s are reported in Table 2. A K_d of 2.9 nM and a B_{max} of 220.7 fmol/mg tissue were obtained in the caudate-putamen, and a K_d of 2.7 nM and B_{max} of 186.2 fmol/mg tissue were obtained in the nucleus accumbens. These values are in close agreement with those obtained in slide-mounted tissue wipes through areas corresponding to the striatum. Hill plots of the data gave slopes that approached unity, indicating no cooperactivity and binding to a single population of receptors.

Regional distribution of ^{3}H-SCH-23390 binding sites (D-1 receptors)

The distribution of D-1 receptors exhibited a marked regional heterogeneity. The amount of ^{3}H-SCH 23390 bound (reported

Table 1. Affinities for ^{3}H-SCH 23390 binding to rat brain areas by various compounds as determined by quantitative autoradiography

Compound	Affinity, k_i (nM)					
	Accumbens nucleus	Caudate putamen	Olfactory tubercle	Claustrum	Frontal cortex lamina VI	Substantia nigra reticulata
Dopamine antagonists						
Fluphenazine	11.2	8.91	1.89	11.2	0.649	12.3
cis-(Z)-Piflutixol	1.09	1.01	1.94	0.274	0.617	0.904
trans-(E)-Piflutixol	1210	1050	1040	85	1620	1520
Sulpiride	50,000	100,000	90,000	30,000	10,000	25,000
SCH 23390 (R-enantiomer)	0.343	0.248	0.214	0.299	0.237	0.328
Dopamine agonists						
SKF 38393	56.8	7.99	41.6	–	17.6	39.0
5-HT antagonists						
Ketanserin	2790	541	582	307	110	307
Methysergide	598	362	301	272	176	272

Relative potency of several compounds in inhibiting ^{3}H-SCH 23390 binding to discrete microscopic regions of rat brain. Serial sections of rat brain through areas corresponding to the accumbens nucleus, caudate putamen, claustrum, frontal cortex-lamina VI, olfactory tubercle, and substantia nigra reticulata were incubated under the conditions described in the text (1 nM ^{3}H-SCH 23390) with the addition of several concentrations of various drugs. Binding was quantitated by converting optical density to fmol/mg tissue by reference to a tritium-labeled polymer standard curve. Competition curves were analyzed with the ALLFIT program for 1-site binding models. K_i values were estimated from IC_{50} values using the equation $K_i = IC_{50}/(1 + [L]/K_d)$. Atropine, diazepam, forskolin, naloxone, nomifensine, propranolol, 5-HT, and triprolidine failed to displace ^{3}H-SCH 23390 in all areas examined at a concentration of 10 μM. Data reported are a representative sample from 1 experiment utilizing 3 separate animals.

as fmol/mg tissue) and the amount of binding relative to the most dense area of D-1 receptors, the caudate-putamen nucleus (expressed as a percentage of this value), are summarized in Table 3. Autoradiographic grain densities, indicating the presence of D-1 receptor binding, were ranked by arbitarily assigning *very high* densities as those being greater than 50 fmol/mg tissue, *high* being between 25 and 50 fmol/mg tissue, *low* between 5 and 10 fmol/mg tissue, and *very low* being less than 5 fmol/mg tissue. The distribution of the D-1 receptors is illustrated in the photographic atlas presented in Figures 2–5. The discussion of the distribution of D-1 receptors that follows is presented according to the brain subdivision in which they occur.

Cortex

Low to very low concentrations of D-1 receptors were found in the cortex. These sites were localized to all areas and laminae of the cerebral cortex. The anteromedial and suprarhinal systems of the prefrontal cortex contain low to very low numbers of D-1 receptors, but the amount was approximately 5 times greater than that demonstrated in the other laminae (Fig. 2). In examining the laminar distribution of ^{3}H-SCH 23390 binding sites within the neocortex, we found a differential distribution, with the most superficial layers exhibiting almost 50% fewer D-1 receptor sites than the deeper layers. The highest density of binding sites within the cortical parenchyma was localized to a continuous band that includes lamina VI and parts of lamina V. There is an increasing rostrocaudal gradient of receptor density within this band. The anterior cingulate cortex exhibited this gradation (lamina VI greater than lamina I) in receptor density, whereas within the posterior cingulate and retrosplenial cortices there was a uniform receptor distribution. The entorhinal and primary olfactory cortices contained, in general, higher concentrations of D-1 receptors than did the other cortices. The temporal cortex also exhibited a differential distribution within the laminae and contained approximately twice as many receptors as the other laminae in the neocortex (Figs. 2–5).

Basal ganglia

In the basal ganglia, marked differences in the amount of ^{3}H-SCH 23390 binding were noted. The head, body, and tail of the caudate-putamen nucleus presented very high concentrations of ^{3}H-SCH 23390 binding sites, the ventral pallidum presented moderate concentrations, and the globus pallidus had low concentrations (Figs. 3 and 4).

Septal area

Very high concentrations of D-1 receptors were noted in association with the accumbens nucleus. The dorsal part of the lateral septal nucleus had low concentrations of D-1 receptors, and the intermediate part of the lateral septal nucleus and the medial septal nucleus contained very low numbers of D-1 receptors (Fig. 3*A*).

Amygdala

As can be seen in Figure 4, *B* and *C*, the amount of binding varied widely in the amygdaloid nuclear complex, with the intercalated and ventral part of the basolateral nucleus exhibiting

Table 2. Affinities (K_d) and maximal number of binding sites (B_{max}) for several rat brain structures as determined by quantitative autoradiography

Area	K_d (nM)	B_{max} (fmol/mg tissue)
Accumbens nucleus	2.7	186.2
Caudate-putamen	2.9	220.7
Claustrum	3.7	94.4
Endopiriform nucleus	5.0	75.4
Frontoparietal cortex lamina VI	1.9	7.8
Olfactory tubercle	2.9	203.0

Dissociation constants (K_d) and maximal binding capacities (B_{max}) of ^{3}H-SCH 23390 to discrete microscopic regions of the brain were determined by performing Scatchard analysis. Saturation isotherms were generated by incubating serial sections of rat brain in varying concentrations (0.5–10.0 nM) of ^{3}H-SCH 23390. The autoradiograms were analyzed utilizing computer-assisted microdensitometry, and optical density values were subsequently converted to fmol/mg tissue. Hill plots approached unity in all areas examined. The values reported are the means of at least 2 separate experiments on groups of 3 animals.

Table 3. Regional distribution of ^{3}H-SCH 23390 binding sites (dopamine D-1 receptors) in rat brain

Area	^{3}H-SCH 23390 bound (fmol/mg tissue ± SEM)	Binding relative to the caudate-putamen (%)
Amygdala		
Basolateral nucleus (BL)	7.47 ± 0.37	8.7
Basolateral nucleus, ventral part (BLV)	19.84 ± 1.59	23.1
Central nucleus (Ce)	1.01 ± 0.26	1.2
Intercalated nucleus (I)	33.70 ± 1.88	39.3
Lateral nucleus (L)	2.36 ± 0.28	2.8
Medial nucleus (Me)	29.68 ± 1.20	34.6
Posteromedial cortical nucleus (PMCo)	12.34 ± 0.87	14.4
Basal ganglia		
Caudate putamen (CPu)	85.76 ± 2.23	100.0
Globus pallidus (GP)	6.91 ± 0.52	8.1
Ventral pallidum (VP)	19.48 ± 0.89	22.7
Brain stem and midbrain		
Central gray (CG)	1.24 ± 0.11	1.4
Dorsal raphe nucleus (DR)	1.36 ± 0.29	1.6
Interpeduncular nucleus (IP)	1.86 ± 0.10	2.2
Substantia nigra compacta (SNC)	6.55 ± 0.58	7.6
Substantia nigra lateralis (SNL)	1.94 ± 0.20	2.3
Substantia nigra reticulata (SNR)	83.15 ± 5.79	97.0
Superficial gray layer of the superior colliculus (SuG)	4.00 ± 0.21	4.7
Cortex		
Anterior cingulate (ACg)		
Laminae I–II	2.22 ± 0.27	2.6
Laminae III–IV	2.32 ± 0.08	2.7
Laminae V–VI	6.45 ± 0.46	7.5
Entorhinal (Ent)	8.11 ± 0.31	9.5
Frontal (Fr)		
Anteromedial system (AM)	6.27 ± 0.22	7.3
Lamina I	1.24 ± 0.13	1.4
Laminae II–III	1.02 ± 0.14	1.2
Lamina IV	1.95 ± 0.25	2.3
Lamina V	1.86 ± 0.07	2.2
Lamina VI	2.23 ± 0.25	2.6
Suprarhinal system (SRH)	4.85 ± 0.21	5.7
Frontoparietal (FrPa)		
Lamina I	0.49 ± 0.04	0.6
Laminae II–III	0.81 ± 0.06	0.9
Lamina IV	0.81 ± 0.02	0.9
Lamina V	1.22 ± 0.08	1.4
Lamina VI	1.09 ± 0.09	1.3
Posterior cingulate (PCg)		
Laminae I–II	1.34 ± 0.17	1.6
Laminae III–IV	1.30 ± 0.10	1.5
Laminae V–VI	1.35 ± 0.15	1.6
Primary olfactory (Po)	4.88 ± 0.17	5.7
Retrosplenial (RSpl)	0.91 ± 0.20	1.1
Striate (Str)		
Lamina I	0.63 ± 0.10	0.7
Laminae II–III	0.61 ± 0.10	0.7
Lamina IV	1.14 ± 0.09	1.3
Lamina V	1.22 ± 0.18	1.4
Lamina VI	4.10 ± 0.28	4.8
Temporal (Te)		
Lamina I	2.70 ± 0.32	3.1
Laminae II–III	2.45 ± 0.37	2.9
Lamina IV	2.30 ± 0.26	2.7
Lamina V	2.44 ± 0.52	2.8
Lamina VI	4.17 ± 0.40	4.9
Hippocampus		
Dentate gyrus, molecular layer (Mol)	2.72 ± 0.19	3.2
Field CA1 of Ammon's horn (CA1)		
Lacunosum molecular layer (LMO1)	0.56 ± 0.04	0.7
Oriens layer (Or)	0.82 ± 0.09	1.0
Field CA2 of Ammon's horn (CA2)	0.65 ± 0.09	0.8
Field CA3 of Ammon's horn (CA3)	0.96 ± 0.04	1.1
Field CA4 of Ammon's horn (CA4)	1.69 ± 0.23	2.0
Subiculum (S)	1.52 ± 0.08	1.8
Hypothalamus		
Anterior hypothalamic area (AHy)	0.62 ± 0.06	0.7
Arcuate nucleus (Arc)	0.48 ± 0.06	0.6
Posterior nucleus (Pn)	1.28 ± 0.08	1.5
Suprachiasmatic nucleus (SCh)	12.02 ± 0.27	14.0
Ventromedial nucleus (VMH)	0.89 ± 0.30	1.0
Septal area		
Accumbens nucleus (Acb)	64.20 ± 3.24	74.9
Lateral septal nucleus, intermediate part (LSI)	2.53 ± 0.17	3.0
Lateral septal nucleus, dorsal part (LSD)	8.31 ± 0.42	9.7
Medial septal nucleus (MS)	1.21 ± 0.25	1.4
Thalamus		
Anterodorsal nucleus (AD)	2.00 ± 0.11	2.3
Anteromedial nucleus (Am)	2.34 ± 0.09	2.7
Anteroventral nucleus (AV)	4.56 ± 0.05	5.3
Dorsolateral geniculate nucleus (DLG)	3.02 ± 0.29	3.5
Lateral habenular nucleus (LHb)	0.30 ± 0.01	0.3
Laterodorsal nucleus (LD)	1.52 ± 0.02	1.8
Medial geniculate nucleus (MG)	0.39 ± 0.03	0.5
Medial habenular nucleus (MHb)	0.15 ± 0.03	0.2
Mediodorsal nucleus (MD)	1.11 ± 0.20	1.3
Paraventricular nucleus (PV)	3.27 ± 0.16	3.8
Reticular nucleus (Rt)	0.35 ± 0.10	0.4
Ventrolateral nucleus (VL)	0.92 ± 0.02	1.1
Ventrolateral geniculate nucleus (VLG)	1.11 ± 0.08	1.3
Ventromedial nucleus (VM)	3.11 ± 0.07	3.6

Table 3. Continued

Area	^{3}H-SCH 23390 bound (fmol/mg tissue ± SEM)	Binding relative to the caudate-putamen (%)
Remaining forebrain areas		
Anterior olfactory nuclei (AON)	6.89 ± 0.67	8.0
Claustrum (Cl)	15.28 ± 0.92	17.8
Endopiriform nucleus (En)	10.42 ± 0.92	12.2
Entopeduncular nucleus (EP)	27.89 ± 1.96	32.5
Islands of Calleja (ICj)	2.14 ± 0.15	2.5
Islands of Calleja, major island (ICjM)	32.61 ± 1.44	38.0
Lateral ventricle (LV)	4.51 ± 0.33	5.3
Olfactory tubercle (Tu)	78.81 ± 3.52	91.9
Subthalamic nucleus (STh)	12.80 ± 0.67	14.9
Ventral tegmental area (VTA)	1.73 ± 0.27	2.0
Zona incerta (ZI)		
Medial	1.48 ± 0.20	1.7
Lateral	6.94 ± 1.12	8.1

Slide-mounted brain sections were labeled as described in the text with 1 nM ^{3}H-SCH 23390 and apposed to LKB Ultrofilm for 21 or 42 d to generate autoradiograms. Nonspecific binding was defined by labeling in the presence of 5×10^{-6} M fluphenazine and was subtracted from all readings. The data were analyzed and quantitated as described in the text and the legend to Table 1. The values reported represent means ± SEM of 3 experiments (3 animals). Furthermore, the amount of binding (expressed as a percentage) relative to the most enriched area, the caudate-putamen, is reported. At least 5 densitometric readings were taken from each brain structure.

high levels of receptors. The medial nucleus demonstrated a variation in receptor density, the most rostral aspects contained moderate concentrations, the caudal aspects contained low amounts, and the nucleus at the level of the dorsal hypothalamic area exhibited high concentrations (not shown). A small area on the lateral inferior aspect of the lateral nucleus (Fig. 5*B*) contained high concentrations of autoradiographic grains, whereas the rest of the lateral nucleus had very low numbers. The basolateral nucleus had a uniform distribution of a low amount of autoradiographic grains, whereas the central, basomedial, and anterior cortical nuclei contained very low amounts. In addition, the posteromedial cortical nucleus contained a moderate amount of D-1 receptors.

Hippocampus

There were very few ^{3}H-SCH 23390 binding sites in the hippocampal formation. However, there was a laminar distribution, in that the molecular layer of the dentate gyrus contained approximately 5 times as many receptors as the other areas (Fig. 4, *B, C*).

Hypothalamus

The suprachiasmatic nucleus exhibited a moderate concentration of D-1 receptors, whereas the other areas and nuclei of the hypothalamus contained very low numbers of D-1 receptors (Figs. 4, *A–C*; 5*A*).

Thalamus

Very low D-1 receptor densities were observed in all thalamic nuclei. The anterodorsal, anteromedial, anteroventral, dorsolateral geniculate, paraventricular, and ventromedial nuclei contained the highest concentration of D-1 receptors (Figs. 4, *A–C*; 5, *A, B*).

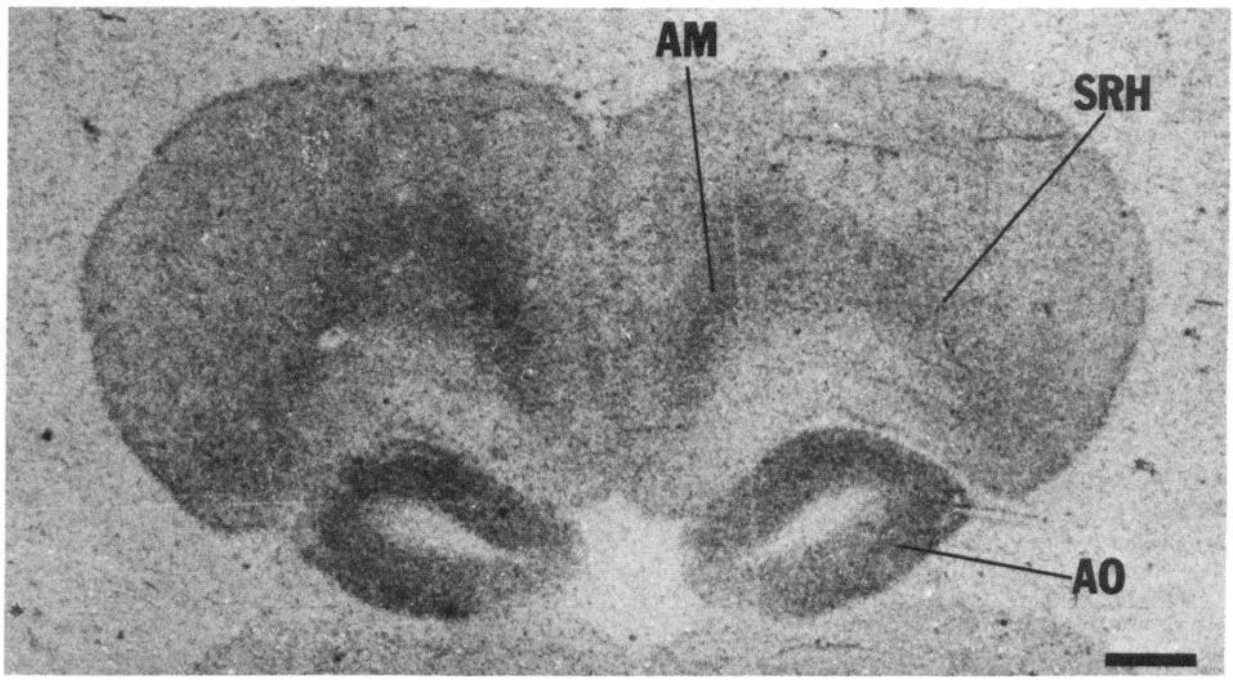

Figure 2. Localization of D-1 receptors in rat prefrontal cortex. Bright-field photomicrograph of a representative autoradiogram through a section of rat prefrontal cortex. As discussed in the text, slide-mounted tissue sections were labeled with 1 nM ^{3}H-SCH 23390. Autoradiograms were generated by apposition of labeled tissue sections to tritium-sensitive film for 6 weeks. Low concentrations of D-1 receptors are seen in the anteromedial (*AM*) and suprarhinal (*SRH*) systems of the prefrontal cortex. In addition, low concentrations of D-1 receptors are observed in the anterior olfactory nuclei (*AO*). Bar, 1 mm.

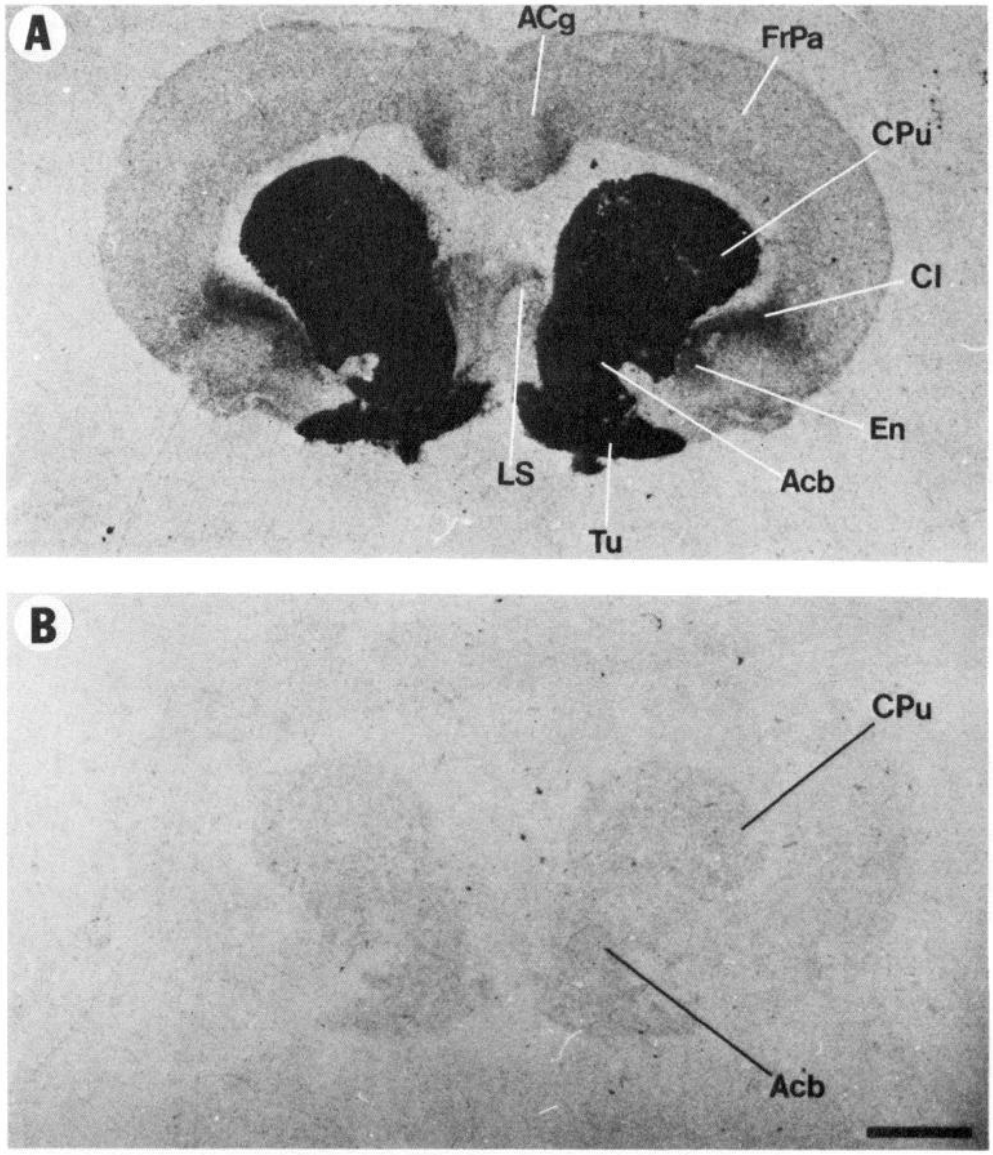

Figure 3. Specific ^{3}H-SCH 23390 binding to rat brain sections. *A,* Photomicrograph from an autoradiogram generated by apposition of labeled tissue sections (incubated with 1 nM ^{3}H-SCH 23390) to tritium-sensitive film for 3 weeks. Autoradiographic grains appear on LKB Ultrofilm as black dots against a white background. Note the very high concentration of D-1 receptors in the caudate-putamen (*CPu*), accumbens nucleus (*Acb*), and olfactory tubercle (*Tu*). Abbreviations: *ACg,* anterior cingulate cortex; *FrPa,* frontoparietal cortex, *Cl,* claustrum, *En,* endopiriform nucleus, *LS,* lateral septal nuclei. Abbreviations and structures are identified according to Paxinos and Watson (1982). *B,* Autoradiogram in this photomicrograph is from a section adjacent to the one shown in *A,* incubated with added 5×10^{-6} M fluphenazine. The low density of autoradiographic grains observed demonstrates that the majority of grains appearing in *A* are due to specific ^{3}H-SCH 23390 binding. Bar, 500 μm.

Remaining forebrain areas

Very high densities of D-1 receptors were seen in the olfactory tubercle (Figs. 3*A*; 4*A*). High concentrations were noted in the entopeduncular nucleus and the major island of Calleja. The endopiriform nucleus and the claustrum contained a moderate number of D-1 receptors, while the subthalamic nucleus and the zona incerta exhibited low to moderate concentrations of D-1 receptors. The lateral aspect of the zona incerta contained higher concentrations of D-1 receptors than the medial aspect. Of note was the relatively high to moderate binding of ^{3}H-SCH 23390 to the entopeduncular nucleus, subthalamic nucleus, and the zona incerta. Proceeding rostrocaudally, these nuclei blend in with one another, making identification difficult. However, care was taken to examine cresyl violet-stained tissue sections in order to confirm the anatomical structure reported, and the autoradiograms shown were selected to demonstrate binding to these 3 areas without significant overlap from the adjacent nuclear region (Fig. 4, *B, C*).

Of the other areas examined, the anterior olfactory nuclei, the ventral tegmental area, and the choroid plexus contained low to very low D-1 receptor concentrations. White matter structures generally contained low numbers of D-1 receptors. Binding in the pituitary was not above background binding.

Brain stem and midbrain

Representative sections were examined through several areas of the brain stem, midbrain, and spinal cord. The substantia nigra zona reticulata had the highest ^{3}H-SCH 23390 binding of any midbrain structure. There was also a differential distribution within the substantia nigra, in that the zona compacta and lateralis contained low to very low concentrations of D-1 receptors, respectively, in contrast to the very high concentrations observed in the zona reticulata (Fig. 6, *A–C*). Very low concentrations of D-1 receptors were found in the central gray, dorsal raphe nucleus, interpeduncular nucleus, and the superficial gray layer of the superior colliculus (Fig. 6, *A–C*).

Of the other areas examined (such as the cranial nerve nuclei, pontine nuclei, inferior colliculus, locus coeruleus, and nucleus tractus solitarius), binding of ^{3}H-SCH 23390 was not above background (data not shown). In addition, sections through the cerebellum (primarily the molecular cell layer), cervical, thoracic, and lumbar spinal cord exhibited a uniform distribution

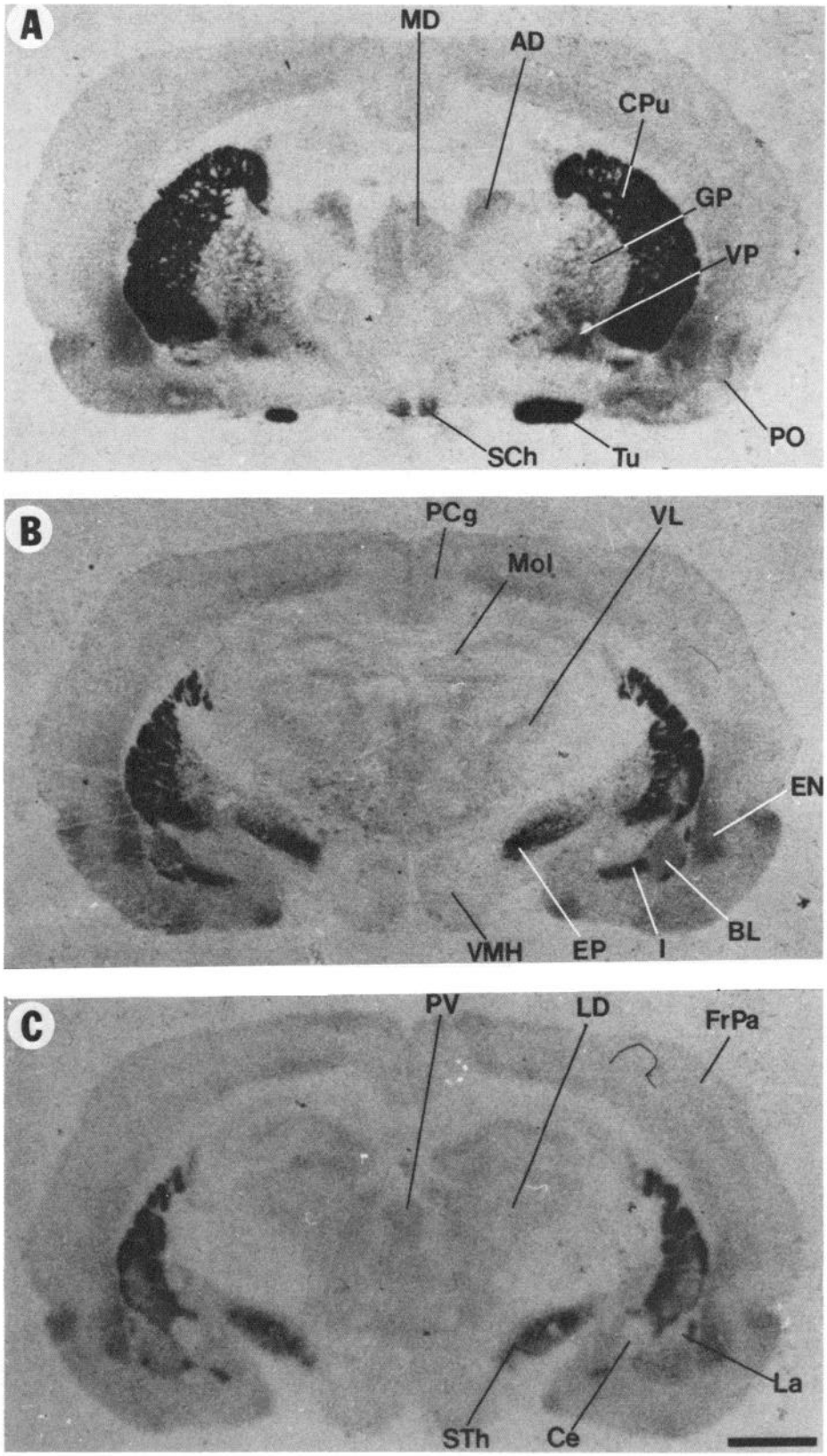

Figure 4. A–C, Regional distribution of D-1 receptors in rat forebrain sections. Photomicrographs from autoradiograms generated by apposition to tritium-sensitive film for 3 weeks. Anatomical localization of D-1 receptors were confirmed by staining the labeled tissue sections and comparing them to autoradiograms using the atlas of Paxinos and Watson (1982). Nonspecific binding, determined in the presence of 5×10^{-6} M fluphenazine, produced uniform grain densities slightly above background levels. Abbreviations as in Table 3. Bar, 500 μm.

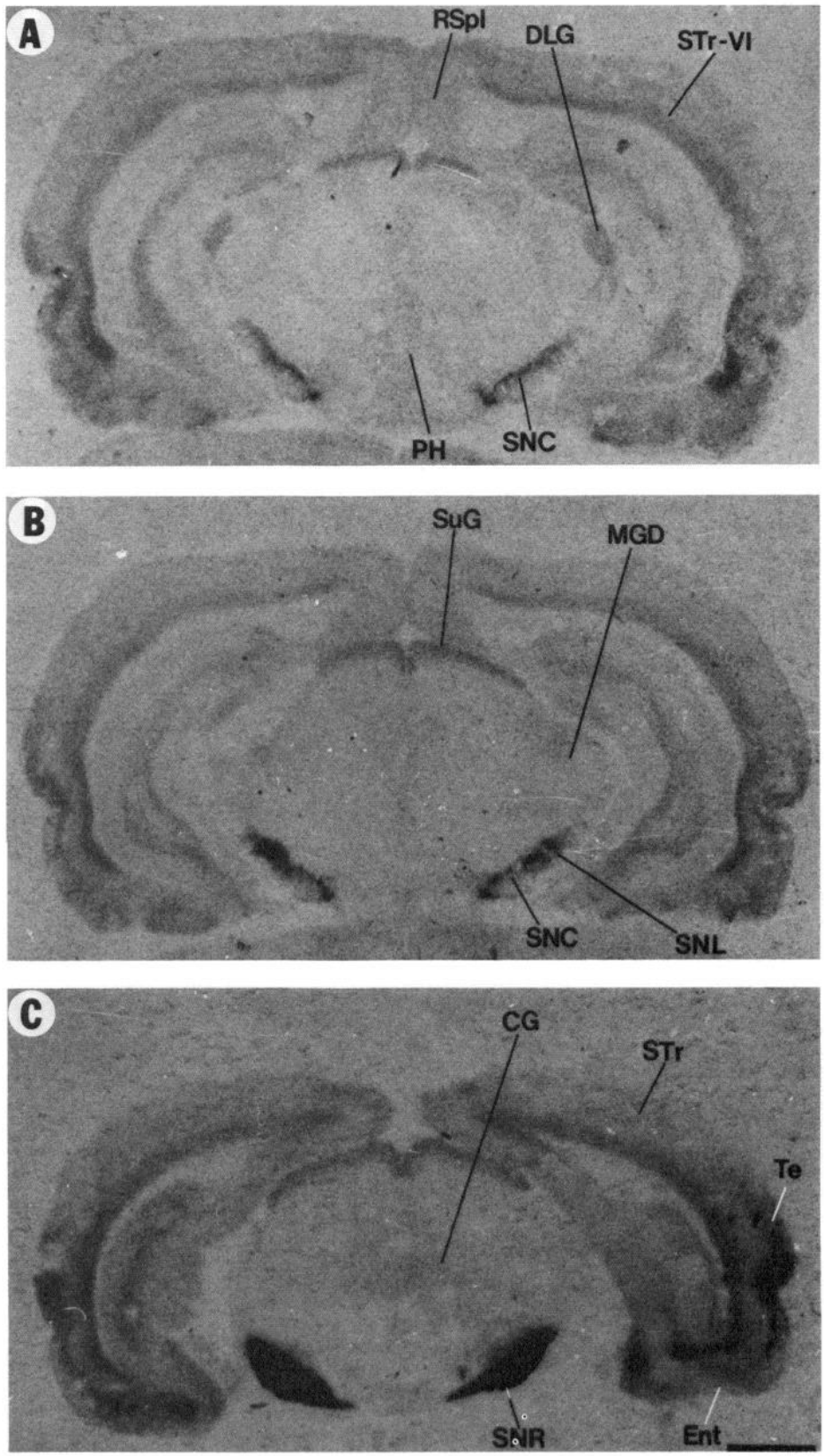

Figure 5. A–C, Regional distribution of D-1 receptors in sections of rat forebrain in a rostral–caudal gradient through the substantia nigra. Abbreviations as in Table 3. Bar, 500 μm.

of very low amounts of ^{3}H-SCH 23390 binding sites (data not shown).

Discussion

^{3}H-SCH 23390 labels DA receptors of the D-1 type (Billard et al., 1984; Dawson et al., 1985a; Schulz et al., 1985). The similar high affinity and saturable binding of ^{3}H-SCH 23390 to slide-mounted tissue sections, coupled with the potency and stereoselectivity of piflutixol isomers, indicated that the binding under the conditions employed for autoradiography involves the same sites as those seen in homogenate studies. It was possible to analyze the pharmacologic characteristics and specificity of ^{3}H-SCH 23390 binding to several microscopic regions of the brain by combining quantitative autoradiography with classic saturation and competition experiments (utilizing ^{3}H-SCH 23390 to label the D-1 receptor). The K_i values reported by Billard et al. (1984), utilizing ^{3}H-SCH 23390 to label the D-1 receptors, and the K_i values reported by Leff et al. (1985) for ^{3}H-flupenthixol binding to D-1 receptors using striatal membrane preparations, are in close agreement with those we have obtained for several brain structures by quantitative autoradiography. A comparison between the K_i values obtained in the present autoradiographic study with those obtained in membrane homogenates in an area corresponding to the caudate-putamen can be found in Table 4. In addition, Scatchard analysis of the saturation isotherms yielded K_d and B_{max} values approximately equal to those reported in brain homogenate studies (Billard et al., 1984; Schulz et al., 1985). Therefore, the results presented here provide the first detailed quantitative analysis of the distribution of D-1 receptors in the rat brain.

Physiological studies have indicated that SCH 23390 interacts potently with the 5-HT neuronal system. For example, *in vitro* studies indicate that SCH 23390 competes with 5-HT-induced constriction of isolated perfused artery preparations (Hicks et al., 1984; Ohlstein and Berkowitz, 1985), a 5-HT$_2$ receptor-mediated phenomenon, with nanomolar potency. SCH 23390 also inhibits 5-HT$_2$-sensitive ^{3}H-spiperone binding with nanomolar affinity (Christensen et al., 1984; Hicks et al., 1984; Hyttel, 1983) and 5-HT$_1$-sensitive ^{3}H-5-HT binding with micromolar affinity (Hicks et al., 1984). It is well known that spiperone is not a selective 5-HT$_2$ ligand, in that it interacts with the dopaminergic and adrenergic systems and it labels a spirodecanone site with high potency (Leysen et al., 1981; Morgan et al., 1984; Palacios et al., 1981a). Therefore, competition experiments performed with spiperone should be interpreted with caution. When using the more selective 5-HT$_2$ ligand, ketanserin (Laduron et al., 1982; Leysen et al., 1981, 1982) in competition experiments with ^{3}H-SCH 23390 binding, a K_i of 1005 nM was obtained in striatal membrane preparations (Billard et al., 1984). This is in close agreement with our results, obtained in several microscopic brain regions by quantitative autoradiography (Tables 1, 4), and thus possibly indicates a moderate interaction of ^{3}H-SCH 23390 with 5-HT$_2$ receptors. However, competition experiments utilizing ketanserin to displace 5-HT$_2$ receptor binding should also be interpreted with caution. Ketanserin labels 5-HT$_2$ receptors in certain discrete regions of the brain (such as the claustrum, endopiriform nucleus, and lamina IV of the neocortex), whereas in the basal ganglia and several other rat brain structures, including the dorsal raphe, ^{3}H-ketanserin binding is predominantly due to an interaction with the ketanserin-recognition binding site (Leysen et al., 1983; Pazos et al., 1985). Methysergide, another 5-HT antagonist utilized in these studies, has a complex pharmacological picture in that it interacts not only with 5-HT$_2$ receptors (Leysen et al., 1982), but also with dopaminergic and adrenergic receptors. Despite the inadequacies of ketanserin and methysergide as 5-HT$_2$ ligands, there is at least a 100-fold greater selectivity for the D-1 receptor versus the 5-HT$_2$ receptor as defined by ^{3}H-SCH 23390 binding to slide-mounted tissue sections. One can speculate that there may even be a greater selectivity given the complex pharmacology of ketanserin and methysergide. Support for this hypothesis can be found when comparing the anatomical distribution and density of 5-HT$_2$ and D-1 receptors, since there is a marked anatomical difference in the location of these receptors (Pazos et al., 1985). However, in structures containing a moderate to high 5-HT$_2$ receptor density (such as lamina IV of the neocortex, claustrum, endopiriform nucleus, and suprachiasmatic nucleus), there could possibly be a minimal to moderate interaction of ^{3}H-SCH 23390 binding with 5-HT$_2$ receptors. The results presented and discussed in this communication also suggest that under the conditions we employed to label dopamine D-1 receptors with ^{3}H-SCH 23390, there is a nonsignificant labeling of 5-HT$_1$ receptors. Serotonin inhibited ^{3}H-SCH 23390 binding with a K_i of 44,000 nM in striatal membrane preparations (Billard et al., 1984), and a 10 μM concentration of 5-HT failed to inhibit ^{3}H-SCH 23390 binding as determined by quantitative autoradiography.

SCH 23390 has been reported to be very potent in blocking psychopharmacological effects that are generally believed to be characteristic of D-2 receptor antagonists (Creese et al., 1983), such as amphetamine-induced locomotor behavior and certain apomorphine-induced stereotypies (Christensen et al., 1984; Iorio et al., 1983; Mailman et al., 1984). *In vitro* studies involving the inhibition of the potassium-evoked release of radiolabeled ACh, a functional model system for the D-2 receptor (Stoof and Kebabian, 1982), have demonstrated that SCH 23390 dose dependently inhibited this phenomenon (Plantje et al., 1984a, b). However, Plantje et al. (1984b) pointed out that SCH 23390 has 10,000-fold greater selectivity for D-1 versus D-2 receptors

Table 4. Comparison of the D-1 receptor affinities (K_i) of several drugs as determined by quantitative autoradiography with ^{3}H-SCH 23390 and by binding assay in striatal membrane preparations labeled with ^{3}H-flupentixol or ^{3}H-SCH 23390

Drug	Quantitative autoradiography	^{3}H-SCH 23390 binding[a]	^{3}H-Flupentixol binding[b]
Fluphenazine	11.2	11.2	2.7
cis-(Z)-Piflutixol	1.09	2.9	0.4
trans-(E)-Piflutixol	1210	95	254
Sulpiride (S)	50,000	100,000	1,051,000
SCH 23390	0.343	0.3	0.28
SKF 38393	56.8	—	3.1 (K_h) 158 (K_l)
Ketanserin	541	1005	191
Methysergide	362	217	—
Atropine	[c]	—	—
Diazepam	[c]	100,000	—
Forskolin	[c]	—	—
Naloxone	[c]	—	—
Nomifensine	[c]	—	0
Propranolol	[c]	—	10,000
Serotonin (5-HT)	[c]	44,000	—
Triprolidine	[c]	—	—

[a] Taken from Billard et al. (1984).

[b] Taken from Leff et al. (1985).

[c] A 10 μM concentration failed to displace ^{3}H-SCH 23390 from slide-mounted tissue sections as determined by quantitative autoradiography.

K_h and K_l are high- and low-affinity K_i's, respectively, for ^{3}H-flupentixol binding competition studies with SKF 38393 (Leff et al., 1985).

in their functional D-1 and D-2 receptor assay. SCH 23390 had a 100,000-fold selectivity for D-1 over D-2 receptors, as determined by comparing the IC_{50} values for the inhibition of DA-stimulated adenylate cyclase activity and ^{3}H-spiroperidol binding, respectively (Iorio et al., 1983). Under our conditions, the selective D-2 antagonist sulpiride inhibited ^{3}H-SCH 23390 binding with a K_i of greater than 10,000 nM (Table 1), indicating that ^{3}H-SCH 23390 binding is highly selective for the D-1 receptor. When comparing the anatomical distribution of D-2 receptors, as determined by autoradiography (Gehlert and Wamsley, 1984, 1985; Gehlert et al., 1986; Klemm et al., 1979; Martres et al., 1985a, b; Murrin et al., 1979; Palacios and Wamsley, 1984; Palacios et al., 1981a; Sokoloff et al., 1985), with that of the D-1 receptor, a marked regional difference in the anatomic location of the 2 dopamine subtypes is noted, further demonstrating the selectivity of SCH 23390 for the D-1 receptor. In addition to the regional differences, there are areas of significant overlap, suggesting a possible modulatory role between the 2 DA receptor systems.

Recently, Leff et al. (1985) combined computer-modeling techniques with classic competition experiments to investigate the interaction of dopaminergic agonists and antagonists with ^{3}H-antagonist-labeled D-1 receptors. They were able to show that antagonist/^{3}H-antagonist competition curves model a single D-1 receptor, whereas agonist/^{3}H-antagonist competition curves best model a high- and low-affinity agonist binding of the D-1 receptor. Preliminary observations reported in the present study (Table 1) concerning the selective D-1 agonist SKF 38393 used in competition with the binding of ^{3}H-SCH 23390 indicate that the resulting competition curve is extremely shallow for certain brain areas and that the binding is subject to guanine nucleotide regulation (data not shown). This suggests the presence of heterogeneous populations of agonist-binding states of the D-1 receptor. These sites may be differentially localized by using the quantitative technique of receptor autoradiography, as has been accomplished with the high- and low-affinity states of the D-2 receptor (Dawson et al., 1985b).

Cortical DA innervation was initially discovered by combining biochemical and lesion studies (Thierry et al., 1973a, b). Further biochemical and histochemical investigations combined with selective lesioning experiments demonstrated that DA, DA-containing fibers, and DA-containing nerve terminals are present in the deep layers of the frontal, cingulate, and entorhinal cortices (Berger et al., 1974; Lindvall and Bjorklund, 1974; Lindvall et al., 1974; Tassin et al., 1974, 1975). In addition, DA-sensitive adenylate cyclase activity was identified in the cerebral cortex (Von Hungen and Roberts, 1973), suggesting the existence of DA receptors; this was later confirmed by other investigators (Bockaert et al., 1977; Mishra et al., 1975). An extension of knowledge and a possible verification of the functional role of these DA terminals would involve the localization of DA receptors in the cerebral cortex. Murrin and Kuhar (1979) localized DA receptors *in vivo* using ^{3}H-spiperone, a D-2 antagonist, to the anterior cingulate cortex and the suprarhinal and supra-accumbal areas at the level of the forceps minor. *In vitro* studies using ^{3}H-spiperone (Palacios and Wamsley, 1984; Palacios et al., 1981a), ^{3}H-sulpiride (Gehlert and Wamsley, 1985), ^{3}H-(−)-DO710 (Sokoloff et al., 1985), and ^{125}I-iodosulpride (Martres et al., 1985a, b) confirmed the existence of D-2 receptors in the cortices. The results presented here also demonstrate the existence of D-1 receptors in the prefrontal cortex and show that their distribution (Fig. 2) closely parallels the distribution of DA nerve terminals determined by Berger et al. (1976), Lindvall and Bjorklund (1984), and Thierry et al. (1984).

Von Hungen and Roberts (1973) demonstrated the existence of a DA-sensitive adenylate cyclase, a property consistent with the presence of D-1 receptors (Kebabian and Calne, 1979), in the cerebral cortex. Later, an excellent correlation was observed between the topographical distribution of DA-sensitive adenylate cyclase activity and DA levels in various cortical areas (Bockaret et al., 1977; Tassin et al., 1978; Thierry et al., 1984). The results presented here support these observations, since the distribution of the D-1 receptors tends to parallel closely that of DA-sensitive adenylate cyclase. Bilateral electrolytic lesions of ventral mesencephalic tegmentum produce an increase in the DA-sensitive adenylate cyclase activity, indicating a postsynaptic localization for cortical D-1 receptors (Tassin et al., 1982). Of interest would be the confirmation of this finding by combining the technique of autoradiography with selective lesioning experiments.

DA innervation of the cerebral cortex has been extensively studied in the rat. The DA fibers are distributed in layers II–VI, with highest density in layers V and VI (for review, see Lindvall and Bjorklund, 1983, 1984). This same general trend was observed for D-1 receptors, with the deeper layers containing higher densities of receptors than the superficial layers. In contrast, the laminar distribution of the D-2 receptor (Martres et al., 1985b) is unique and different from that of the D-1 receptor: D-2 receptors are mainly localized to lamina V, with very small concentrations in laminae I–III and VI. DA terminals, with relatively well-defined projections, have been observed in the cortices of rat brain, except for the striate cortex (Lindvall and Bjorklund, 1983). However, evidence has been obtained for a DA projection from the A10 region to the visual cortex in the cat (Tork and Turner, 1981).

The localization of D-1 receptors described here in other brain structures also correlates well with the distribution of dopaminergic nerve terminals. Areas containing high concentrations of D-1 receptors in general have been reported to contain nerve endings from dopaminergic neurons, primarily those located in the substantia nigra, ventral tegmental area, and retrorubral nucleus (see Lindvall and Bjorklund, 1983, 1984, for review). The basal ganglia, septal nuclei, the different nuclei of the amygdaloid complex, hippocampus, subthalamic nucleus, claustrum, and entopeduncular nucleus are structures where the presence of DA, DA fibers, and DA biochemical markers has been observed (for review and references, see Lindvall and Bjorklund, 1983, 1984; Moore and Bloom, 1978). The results presented here suggest that besides being associated with nigrostriatal system, D-1 receptors are predominantly linked to the mesocortical system (see above), the mesodiencephalic system, and the incertohypothalamic system. In general, areas containing high concentrations of DA, DA fibers, or DA biochemical markers contain high concentrations of D-1 receptors. However, exceptions also occur, since a close correlation between the distribution of the receptors and the distribution of DA fibers or other markers does not exist in areas such as the central amygdaloid nucleus (Ben-Ari et al., 1975; Fallon et al., 1978; Fuxe et al., 1974), the lateral habenular nucleus (Lindvall and Stenevi, 1978), or the medial caudal aspect of the zona incerta (Bjorklund et al., 1975). The existence of discrepancies between the location of receptors and the distribution of fibers and neurotransmitters has been referred to as the "mismatch" problem (Kuhar, 1985a). The "mismatch" problem has also been found with the serotonergic system (Pazos and Palacios, 1985; Pazos et al., 1985) and the histaminergic system (Palacios et al., 1981b). Several explanations for these discrepancies have been proposed by Kuhar (1985a), e.g., the receptors and neurotransmitters are contained within 2 completely different neurons, receptor ligands often label only a subpopulation of receptors, or autoradiographic efficiency due to gray and white matter quenching may lead to artifactual regional differences in receptor localization. Also, one must keep in mind that the brain contains D-2 receptors whose distribution differs from that of D-1 receptors, and this may account for several areas where DA markers exist but the D-1 receptor does not.

White matter regions contained very low levels of ^{3}H-SCH 23390 binding. However, we may have underestimated binding to those structures with high white matter content, in that white matter has a greater self-absorption (attenuation) of beta particles than gray matter (Alexander et al., 1981). In any case, in areas of predominantly white matter, this underestimation would be at most 30% (Rainbow et al., 1984), thus making the values reported for these areas actually semiquantitative as opposed to quantitative.

The D-1 receptor has been described as a "receptor in search of a function," or more appropriately as having "no known function" (Stoof and Kebabian, 1984). However, with the development of selective antagonists and agonists for both the D-1 and D-2 receptors, the individual roles these receptor subtypes play in CNS physiology are beginning to be elucidated. For example, until recently a behavioral role for the D-1 receptor has received little attention. In fact, on the basis of direct and indirect evidence, stereotypy has been considered to be a D-2 receptor-mediated process (Seeman, 1980). However, Molloy and Waddington (1985) have provided evidence that sniffing and grooming appear to be selectively mediated through D-1 mechanisms, and D-2 mechanisms seem to modulate the expression of rearing and locomotion initiated by D-1 stimulation. Furthermore, Iorio et al. (1983) have shown that SCH 23390 prevents the motor stimulation and arousal produced by apomorphine and amphetamine, and Gessa et al. (1985) demonstrated that when SCH 23390 prevents the excitatory response to apomorphine, the existence of a population of D-2 receptors mediating sedation and sleep is disclosed. These investigations suggest that a complex interaction occurs between D-1 and D-2 receptor occupancy. The blockade of one receptor type may produce an allosteric change in the other (Gessa et al., 1985), or possible interactions between these receptors may involve a change in adenylate cyclase activity. This could lead to a modulated physiological response depending on whether the D-1 or D-2 receptor was predominantly stimulated. It also appears that each receptor subtype has distinct and separate physiological functions, although the specific effects mediated by these receptors are still being elucidated.

A separate DA retinal system is known to exist (Moore and Bloom, 1978; Lindvall and Bjorklund, 1983). Results presented here suggest a central role for D-1 receptors in control of visual activity. The suprachiasmatic nucleus, which contains moderate concentrations of D-1 receptors, receives direct retinal projections (Moore and Lenn, 1972) and is involved in circadian rhythms (Kupfermann, 1981). The superficial gray layer of the superior colliculus (Wurtz and Albano, 1980), the claustrum (LeVay and Sherk, 1981; Sanides and Buchholtz, 1979), the lateral geniculate nucleus, and the striate cortex (Gilbert and Wiesel, 1979; Toga and Collins, 1981) are all connected with the visual system and contain low concentrations of D-1 receptors.

Several neurological and psychiatric disorders such as tardive dyskinesia, Parkinson's disease, Huntington's chorea, Gilles de la Tourette's syndrome, schizophrenia, and drug-induced psychosis are involved with a disturbance of central dopaminergic systems. These observations imply that DA plays a very important role in cognitive function and integration of movement. Different areas of the cortex serve specific processing roles (see Iversen, 1983, 1984, for review)—the sensory cortex for modality-specific sensory analysis, the temporal lobe association cortex for perceptual analysis of complex sensory stimuli, and the frontal lobes serving the highest levels of neural integration. Results presented in this study indicate the existence of D-1 receptors in the cerebral cortices. This suggests that the D-1 receptor may play a direct role in cognitive function. We have recently used ^{3}H-SCH 23390 to label D-1 receptors in human cortex, and we found a low density of D-1 receptors in the superior frontal gyrus (unpublished observations). These results have interesting implications in the therapy of schizophrenia, which has been attributed to overactivity of cerebral dopaminergic mechanisms in the brain (see Rupniak et al., 1983, for review). Current antipsychotics in clinical use are, in general, potent D-2 antagonists, and long-term use is associated with the development of tardive dyskinesia, an irreversible involuntary movement disorder (for review, see DeVeaugh-Geiss, 1982). With the introduction of more selective agents for the dopamine receptor subtypes, perhaps therapy can be directed at the specific receptor alterations and reduce the severe adverse side effects in this and other disorders involving central dopaminergic systems.

Quantitative autoradiography of ^{3}H-SCH 23390 binding to rat brain sections provides a sensitive assay for the localization of D-1 receptors in well-defined highly circumscribed areas. The results of the present study demonstrate a striking correlation between D-1 receptors and terminals of dopaminergic pathways. The localization of D-1 receptors to several discrete brain structures will, we hope, help elucidate more of the physiologic functions associated with the D-1 receptor and ultimately lead to a better understanding of central dopaminergic systems.

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