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Quantitative Autoradiographic Localization of the D_1 and D_2 Subtypes of Dopamine Receptors in Rat Brain

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The distribution of D_1 and D_2 receptors was studied in coronal sections of rat brain, using quantitative autoradiography. D_1 receptors were labeled with 1.8 nM ^{3}H-SKF-83566 (a brominated analog of ^{3}H-SCH-23390), while D_2 receptors were labeled with 1.0 nM ^{3}H-spiroperidol (^{3}H-SPD). The binding of both ligands to sections from brain and from a homogenate of caudate putamen (CPu mash) reached equilibrium within 80 min at 37°C. CPu mash provided a virtually unlimited number of homogeneous sections that contained a high density of both D_1 and D_2 receptors. Sections of CPu mash were used in competition studies that confirmed that the specific binding of ^{3}H-SKF-83566 was selective for D_1 receptors, and that the binding of ^{3}H-SPD was selective for D_2 receptors. Scatchard analysis of equilibrium binding of the 2 ligands in the CPu in horizontal sections of rat brain revealed K_d values of 1.1 ± 0.07 nM for ^{3}H-SKF-83566 and 0.7 ± 0.09 nM for ^{3}H-SPD. Studies of the distribution of D_1 and D_2 receptors were carried out in coronal sections of brains from 5 rats. D_1 receptors were found throughout the forebrain and were present in greater density than were D_2 receptors in all regions examined except the olfactory nerve layer. In the CPu, nucleus accumbens, and olfactory tubercle, the densities of D_1 and D_2 receptors were, respectively, approximately 2500 and 600-800 fmol/mg protein. In the substantia nigra, the density of D_1 receptors was approximately 2500 fmol/mg protein in both the compacta and the reticulata, but the density of D_2 receptors was 230 fmol/mg protein in the compacta and 70 fmol/mg protein in the reticulata. The ventral tegmental area contained only 90 fmol/mg protein of D_1 receptors, and D_2 receptors were undetectable. The entopeduncular nucleus, zona incerta, and region of the ventral internal capsule had densities of D_1 receptors of 550-950 fmol/mg protein and D_2 receptor densities of less than 100 fmol/mg protein. Densities of D_1 and D_2 receptors were, respectively, 2700 and 900 fmol/mg protein in the choroid plexus. Knowledge of the differences in the relative distributions of D_1 and D_2 receptors in various brain regions may increase our understanding of the functions of brain dopaminergic systems and may aid in the development of new therapeutic approaches for neuropsychiatric disorders.

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Abnormalities of dopaminergic systems in the brain have been implicated in disorders of thought and movement. Kebabian and Calne (1979) classified subtypes of dopamine receptors according to whether or not they were linked to activation of adenylate cyclase. Thus, stimulation of D_1 receptors activates adenylate cyclase and stimulation of D_2 receptors either inhibits or does not stimulate the activity of this enzyme (Kebabian et al., 1984).

Based on the utility of various classes of drugs in the treatment of human illnesses, the inference has been drawn that D_2 receptors play a more significant role in the pharmacotherapy of psychosis and movement disorders than do D_1 receptors (Creese et al., 1983; Seeman, 1977). Many neuroleptics interact with several different classes of receptors, including D_1 and D_2 dopamine, 5-HT-2 serotonin, and α-adrenergic receptors, but the strongest correlation between clinical dose of antipsychotic drug and *in vitro* potency at a receptor has been observed for D_2 receptors (Creese, 1983; Seeman, 1981). Furthermore, ergot derivatives used in the treatment of Parkinson's disease, including bromocriptine, lisuride, and pergolide, are potent agonists at D_2 receptors, but weak antagonists or partial agonists at D_1 receptors (Calne, 1982).

As recently as 1982 there was said to be no function known for the D_1 receptor (Laduron, 1982). However, the recent availability of a D_1-selective antagonist, SCH-23390 (Christensen et al., 1984; Iorio et al., 1983), as well as a D_1-selective agonist, SKF-38393 (Molloy and Waddington, 1984; O'Boyle and Waddington, 1984), has made it possible to carry out functional studies designed to determine the role of the D_1 receptor in the CNS. The results of these studies suggest complex interactions of D_1 and D_2 receptors in behaviors formerly thought to be mediated solely by D_2 receptors. For example, catalepsy, a behavior usually thought to be mediated by inhibition of D_2 receptors, was induced when rats were given SCH-23390 (Meller et al., 1985). Moreover, catalepsy induced by this D_1-selective antagonist could be inhibited in a dose-dependent manner by several D_2-selective agonists (Meller et al., 1985). SCH-23390 also blocked amphetamine-induced hyperlocomotion and apomorphine-induced stereotypy, behaviors thought to be inhibited by D_2 receptors (Mailman et al., 1984). Systemic administration of SCH-23390 apparently produces these effects directly, since amphetamine-induced hyperlocomotion is blocked by intraventricular injection of SCH-23390 (Mailman et al., 1984) and systemically administered SCH-23390 protects D_1, but not D_2, receptors from inactivation by the neurotoxin *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) (Meller et al., 1985). Furthermore, SCH-23390 does not cause hyperprolactinemia or prevent apomorphine-induced emesis (Iorio et al., 1983). Breese and Mueller (1985) suggested that D_1 receptors modulate the function of D_2 receptors through a mechanism dependent upon functionally intact catecholaminergic neurons. This conclusion was based on the loss of the ability of SCH-23390 to

antagonize the increase in locomotor activity produced by LY-171555 (a D_2-selective agonist) after treatment of rats with the neurotoxin 6-hydroxydopamine.

The technique of quantitative autoradiography has been used to determine the anatomic localization of receptors (Palacios et al., 1981a; Penney et al., 1981; Quirion et al., 1981; Rainbow et al., 1982; for a review, see Gehlert et al., 1984) and their subtypes (for a review, see Pazos et al., 1984). Using this technique, the distribution of D_2 receptors in rat brain has been studied with the radioligand ^{3}H-spiroperidol (^{3}H-SPD) (e.g., Neve et al., 1984; Palacios et al., 1981b). As expected on the basis of results of studies of binding in homogenates, high densities of D_2 receptors were found in the basal ganglia and in limbic regions such as the caudate putamen (CPu), nucleus accumbens, olfactory tubercle, and substantia nigra. In addition, a rostrocaudal gradient of decreasing D_2 receptor density has been described in the CPu (Altar et al., 1985).

Studies using the radioligand ^{3}H-SCH-23390 have established the specificity of this ligand for D_1 receptors (Billard et al., 1984; Itoh et al., 1984). Dawson et al. (1985) reported a method for the use of ^{3}H-SCH-23390 in quantitative autoradiographic studies of the D_1 receptor. Although insufficient amounts of ^{3}H-SCH-23390 were available for this study, sufficient quantities of ^{3}H-SKF-*R*-83566 (^{3}H-SKF-83566), a benzazepine differing from SCH-23390 only in the substitution of bromine for chlorine in the 7 position, were available. Flaim et al. (1986) characterized this compound as a D_1-selective antagonist, the binding of which was stereoselective, saturable, and reversible, with a K_d of 1.1 nM in homogenates of rat striatum.

In view of the apparently complex interactions between activation of D_1 and D_2 receptors, we carried out experiments to compare the densities of these receptors in adjacent tissue sections using quantitative autoradiography to help elucidate the gross anatomic basis of such interactions.

Materials and Methods

Chemicals and radioligands

^{3}H-SKF-*R*-83566 (85 Ci/mmol) was provided by Smith Kline and French Laboratories (Philadelphia, PA). ^{3}H-SPD (77–100 Ci/mmol) was purchased from Amersham (Arlington Heights, IL). Domperidone, ketanserin, pipamperone, and sulpiride (Janssen Pharmaceutica, Piscataway, NJ); fluphenazine (E. R. Squibb, Princeton, NJ); and SCH-23390 (Schering, Bloomfield, NJ) were provided by the manufacturers; propranolol and (+)- and (−)-butaclamol were purchased from Research Biochemicals (Wayland, MA).

Tissue preparation

Male Sprague-Dawley rats, weighing approximately 200 gm (Charles River Breeding), were decapitated and the brains rapidly removed and chilled. Tissues were sectioned at a thickness of 32 μm in a cryostat, thaw-mounted onto gelatin-coated slides, stored at −70°C, and thawed and dried at room temperature immediately before use (Rainbow et al., 1984). For studies that required multiple identical tissue sections, caudate-putamen mash (CPu mash) was prepared from freshly dissected striata which were blended by 5 strokes in a Dounce homogenizer kept on ice. This tissue was frozen in a plastic syringe barrel, then removed and treated in a manner similar to that for brains. For studies to determine the appropriate conditions for incubation and washing of sections, the brain was blocked and serial coronal sections that included the CPu were cut and assigned in a counterbalanced pattern, so as to eliminate gradient bias in receptor density. For saturation binding studies, the approximately 70 horizontal sections per brain that included the CPu were assigned in the following pattern: 4 for assay of D_1 receptors (3 total and 1 nonspecific) and 6 for assay of D_2 receptors (3 total and 3 nonspecific) for each of the 7 concentrations of the appropriate radioligand. For studies of the distribution of receptors, sequential coronal sections were assigned in the following pattern: 4 for study of D_1 receptors (2 total and 2 nonspecific), 4 for study of D_2 receptors, with the next 8 sequential sections discarded. This pattern yielded a total of 34–36 levels for study from each brain, with levels separated by 500 μm.

Assays

Labeling of D_1 receptors was carried out in buffer containing 50 mM Tris, 10 mM $MgSO_4$, 2 mM EDTA, 154 mM NaCl, and 10 mg/liter BSA, with pH adjusted to 7.4 at room temperature. For competition experiments, the usual concentration of ^{3}H-SKF-83566 was 0.15–0.25 nM. For saturation experiments, 0.05–11.8 nM ^{3}H-SKF-83566 was used, and for studies of the distribution of receptors, 1.8 nM ^{3}H-SKF-83566 was used. Nonspecific binding was defined as binding that was not inhibited by 2 μM (+)-butaclamol. In equilibrium studies, slides were incubated in either glass Coplin jars or plastic slide mailers at 37°C for 80 min. They were washed in buffer at 4°C for 20 min, with gentle up-and-down agitation every 5 min. Sections of brain or CPu mash were processed for scintillation counting by wiping the tissue off the slide with a glass-fiber filter and placing it in a glass vial. Incubation buffer was sampled for the determination of equilibrium ligand concentration after the slides were removed. Samples of ^{3}H-SKF-83566, a hydrophilic compound, on filters and in incubation fluid were counted in 3.9 ml of a toluene-based fluor containing detergent (Budget-Solve; Research Products, Mount Prospect, IL) with a counting efficiency of 40%. Brain sections were processed for autoradiography by dipping in distilled water to remove buffer salts, then drying at 60°C.

Labeling of D_2 receptors was carried out in a similar fashion, with the following exceptions. The buffer contained 50 mM Tris, 154 mM NaCl, and 10 mg/liter BSA, adjusted to pH 7.4 at room temperature. The range of concentrations of ^{3}H-SPD used for competition experiments was 0.2–0.5 nM, for saturation experiments 0.05–5.0 nM, and for mapping 1.0 nM. The slides were labeled at 24°C for 80 min and washed at 4°C for 80 min, with a change of buffer every 20 min. Nonspecific binding was defined as binding inhibited by 100 μM sulpiride. Sections were processed for scintillation counting as described above, except that the fluor for this lipophilic compound consisted of 3 gm/liter of 2a70 (Research Products) in toluene. Incubation fluid was sampled and counted as described above.

Autoradiograms

Labeled sections were apposed to LKB Ultrofilm (LKB Instruments, Gaithersburg, MD) for 1 week (^{3}H-SKF-83566) or 2 weeks (^{3}H-SPD) at room temperature. The film was developed using Kodak GBX developer. Tissue sections were stained with cresyl violet, and these were used in conjunction with the atlas of Paxinos and Watson (1982) to identify structures. The optical density of various brain regions on coronal autoradiograms was measured at an anatomical resolution of 100 μm. The mean optical density readings from autoradiograms were converted by an Atari microcomputer into the amount of radioligand bound/mg protein, using a standard reference curve. The value for "nonspecific binding" (i.e., the binding of ^{3}H-SKF-83566 or ^{3}H-SPD not displaced by 2 μM (+)-butaclamol or 100 μM sulpiride, respectively) was subtracted from that for "total" to produce the value for "specific binding" in each region.

The standard curve for tritium was developed as described by Rainbow et al. (1984), using fresh postmortem human caudate and putamen. Varying amounts of ^{3}H-SPD were mixed with aliquots of a homogenate, which were then degassed by centrifugation and frozen in syringe barrels. Sections, 32 μm thick, were cut, thaw-mounted to slides, apposed to LKB Ultrofilm, and exposed with studies of D_1 or D_2 receptors for 1 or 2 weeks, respectively. Aliquots were also taken for scintillation counting and the determination of protein by the method of Bradford (1976). Optical density readings were taken and assigned the appropriate values for nCi/mg protein on the basis of the scintillation counts and protein assay. A plot of the natural log of the relative optical density versus the natural log of the radioactivity/mg protein was linear for the range of standards used (13–263 nCi/mg protein), corresponding to 150–3100 fmol ligand/mg protein for ^{3}H-SKF-83566 (specific activity = 85 Ci/mmol) and 130–2630 fmol ligand/mg protein for ^{3}H-SPD (specific activity = 100 Ci/mmol).

Data analysis

Saturation isotherms were transformed using the method of Scatchard (1949), and estimates of the K_d and B_{max} were obtained using unweighted linear-regression analysis of the transformed data. Competition curves

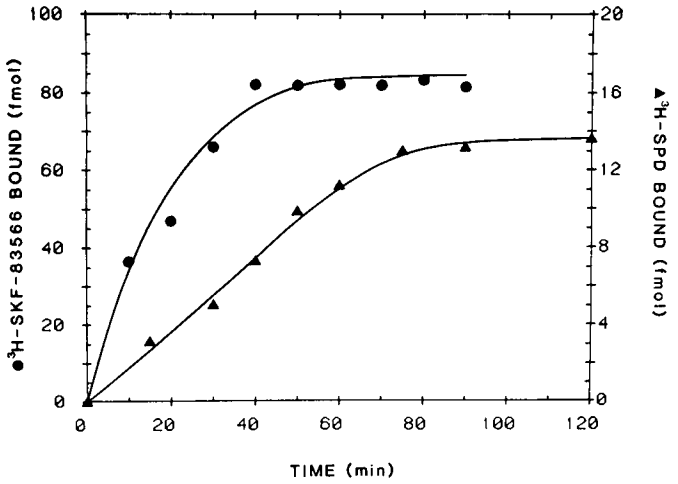


Figure 1. Time course of binding of radioligands. Coronal rat brain sections, 32 μm thick, through the head of the CPu were incubated with 0.4 nM ^{3}H-SKF-83566 at 37°C (●, *left axis*) or 0.3 nM ^{3}H-SPD at 24°C (▲, *right axis*). At the times indicated, sections were removed and washed, as described in Materials and Methods. Glass-fiber filters were used to wipe the sections off the slides, and the radioactivity present was determined by scintillation spectroscopy. Specific binding, defined with 2 μM (+)-butaclamol (^{3}H-SKF-83566) or 100 μM sulpiride (^{3}H-SPD), is shown.

were initially modeled using the following equation:

$$B_L = \frac{B}{1 + (L/IC_{50})^n} + NS$$

In this equation, B_L is the amount of radioligand bound to tissue, B is the total number of binding sites labeled in the absence of competing ligand, L is the concentration of competing ligand, IC_{50} is the concentration of competing ligand that inhibits 50% of total specific binding, n is the Hill coefficient, and NS is the amount of radioligand not specifically bound to receptors. NS was constrained to the value of nonspecific binding determined experimentally. The curve-fitting was done by nonlinear least-squares regression analysis using the mathematical modeling program NEWFITSITES, available on the NIH-sponsored PROPHET system (McGonigle et al., 1984).

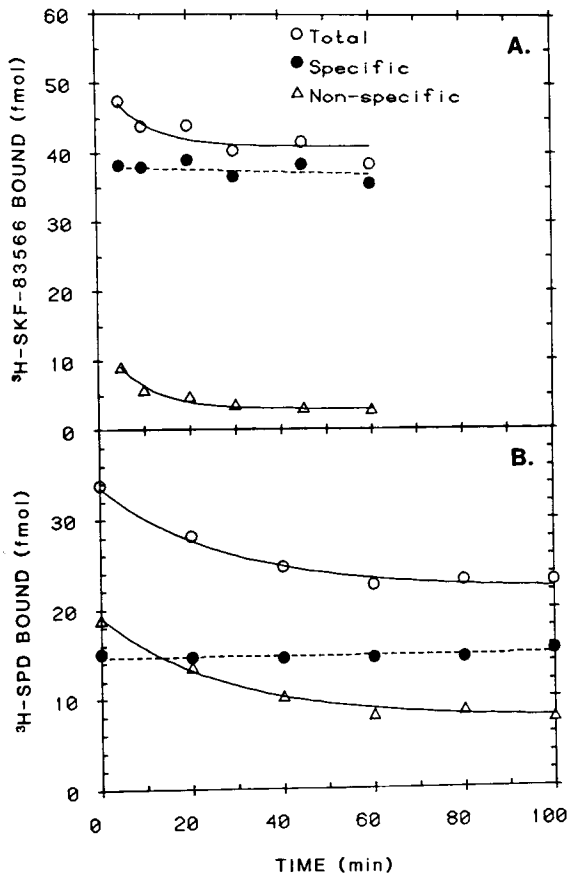


Figure 2. Determination of optimal wash times. Sections were incubated for 80 min with the appropriate ligand. Specific binding was defined as the difference between total binding and nonspecific binding. Coronal rat brain sections through the CPu at equilibrium were immersed in buffer at 4°C, with gentle up-and-down agitation every 5 min, and a change of buffer every 20 min. At the times indicated, sections were wiped off the slides with glass-fiber filters and the radioactivity present was determined by scintillation spectroscopy. *A*, 0.4 nM ^{3}H-SKF-83566 ± 2 μM (+)-butaclamol, *B*, 0.3 nM ^{3}H-SPD ± 100 μM sulpiride.

Results

Incubation conditions

The time course of the binding of ^{3}H-SKF-83566 to both tissue sections and sections of CPu mash was investigated. In these experiments, the tissues were wiped off the slides with glass-fiber filters at the end of the assay, and the total radioactivity associated with the sample was determined. At 22°C, more than 80 min were required to reach equilibrium (data not shown). Equilibrium was reached within 40 min and binding was stable for 110 min at 37°C when tissue sections were incubated with 0.4 nM ^{3}H-SKF-83566 (Fig. 1). Nonspecific binding of ^{3}H-SKF-83566 was reduced by washing slides in drug-free incubation buffer at 4°C. In these experiments, holders containing slides were lowered into containers of cold buffer and raised and lowered every 5 min (to disrupt any stagnant surface layer of buffer), with changes of buffer every 20 min. Nonspecific binding was minimal for ^{3}H-SKF-83566 after 20 min (Fig. 2*A*). Time to equilibrium, stability, and optimal wash time were similar for mash sections (data not shown). Adsorption of ^{3}H-SKF-83566 by plastic containers was reduced to 5% or less by the addition of 0.1 mg/liter BSA. The presence of albumin did not alter binding of the ligand to the tissue sections (data not shown).

Conditions for the binding of ^{3}H-SPD to tissue and mash sections were determined in a similar manner. Equilibrium was attained within 80 min at 24°C, and binding remained stable for 45 min at this temperature (Fig. 1). The optimal wash time was found to be 80 min, with a change of buffer every 20 min (Fig. 2*B*).

Pharmacological properties

To determine the IC_{50} value for the inhibition of binding of ^{3}H-SKF-83566 by a given drug, as many as 60 identical tissue slices were required. Since the density of D_2 receptors in the CPu varies in the rostrocaudal axis (Altar et al., 1985), the use of a pooled CPu mash was investigated. The densities of D_1 and D_2 receptors on sections from a single preparation of CPu mash proved to be very uniform, and therefore filter wipes of CPu mash sections were used for pharmacological characterization studies. The IC_{50} values obtained from competition experiments were converted to K_i values by the method of Cheng and Prusoff (1973).

The ability of various drugs to inhibit specific binding of ^{3}H-SKF-83566 in sections of CPu mash did not vary significantly from those reported by Flaim et al. (1986) in striatal homogenates. SCH-23390 (K_d = 0.770 ± 0.033 nM) and (+)-butaclamol (16.0 ± 1.20 nM) were the most potent drugs in inhibiting the binding of ^{3}H-SKF-83566 (Fig. 3*A*, Table 1). The binding of butaclamol demonstrated stereospecificity, as the (−)-isomer of butaclamol had a K_d approximately 3 orders of magnitude higher

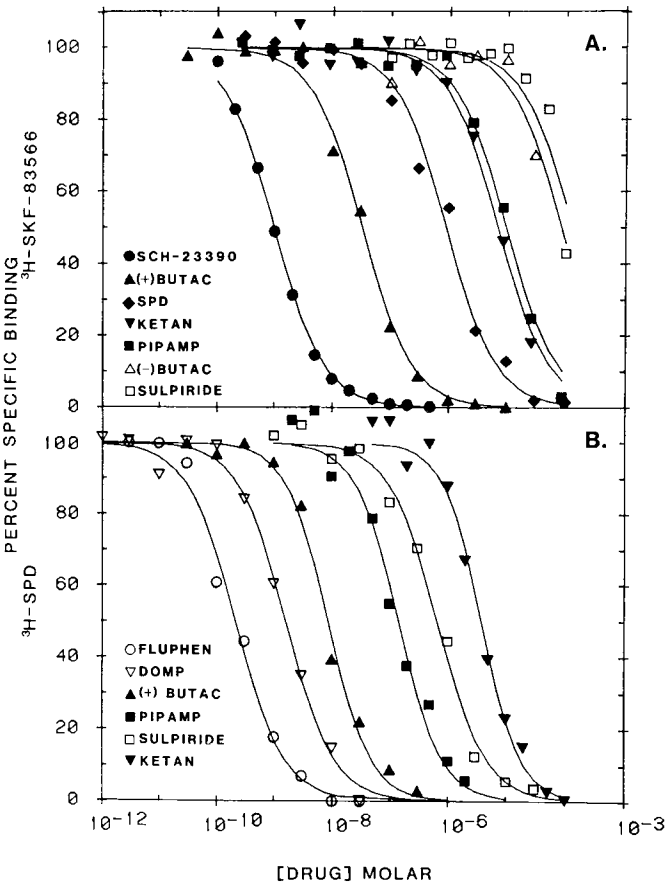


Figure 3. Inhibition of binding of ^{3}H-SKF-83566 or ^{3}H-SPD to D_1 or D_2 receptors. Sections of CPu mash were incubated with 0.15–0.25 nM ^{3}H-SKF-83566 (*A*) or 0.2–0.3 nM ^{3}H-SPD (*B*) under standard conditions. The section was wiped off the slide with a glass-fiber filter, and the radioactivity determined by scintillation spectroscopy. Nonspecific binding was defined as binding that was not inhibited by 2 μM (+)-butaclamol (^{3}H-SKF-83566) or 100 μM sulpiride (^{3}H-SPD). Each *point* was determined in triplicate; mean K_i values are given in Table 2.

than the active (+)-isomer. The D_2-selective antagonists SPD and sulpiride were not potent in displacing the binding of ^{3}H-SKF-83566, yielding K_i values of 0.90 ± 0.14 μM and 69.0 ± 4.0 μM, respectively. Inhibition of the binding of ^{3}H-SKF-83566 by the 5-HT-2 antagonists ketanserin and pipamperone was investigated at both low and high concentrations of ligand to determine whether 5-HT-2 sites were being labeled at the higher concentration used for studies of the distribution of D_1 receptors. At neither 0.16 nor 1.6 nM was there any indication of binding to 2 sites for either drug. Means of the K_i values determined were 2.20 ± 0.52 μM for ketanserin and 4.80 ± 1.10 μM for pipamperone.

The pharmacological specificity of the binding of ^{3}H-SPD was also investigated. Fluphenazine (K_i = 0.140 ± 0.012 nM), (+)-butaclamol (6.80 ± 0.45 nM), and domperidone (0.970 ± 0.127 nM) were potent inhibitors of the binding of ^{3}H-SPD, and the K_i value for sulpiride (160 ± 40 nM) was consistent with binding to a D_2 site (Fig. 3*B*, Table 1). The 5-HT-2 antagonists ketanserin (2.70 ± 0.15 μM) and pipamperone (0.97 ± 0.55 nM) were not potent in inhibiting specific binding of ^{3}H-SPD. The binding of ^{3}H-SPD was stereospecifically inhibited by the (+)- and (−)-isomers of butaclamol. Studies of the inhibition of the binding of ^{3}H-SPD by sulpiride and ketanserin were also carried out with horizontal sections of rat brain. The K_i values determined by densitometric measurement of the binding in the CPu were in good agreement with the values determined using filter wipes of CPu mash sections for both competing ligands (data not shown).

Comparison of D_1 and D_2 receptors in the CPu

Scatchard analysis of saturation binding data was carried out in horizontal sections of rat brains. The CPu is present in a sufficient number of sections from a single brain to allow the use of 7 concentrations of each ligand, and horizontal sectioning avoids the complexities caused by the rostrocaudal gradient in the densities of both D_1 and D_2 receptors (Joyce et al., 1985). In these autoradiographic studies, the fact that the CPu varies in size and shape in the dorsoventral axis does not cause problems (as it would in studies using wipes of tissue sections for scintillation counting) because the densitometric analysis can be confined to the CPu. Four brains were sectioned in this manner, and alternate sections were allocated for determination of the densities of D_1 and D_2 receptors. The "tail" of the CPu [corresponding to the area caudal to coronal plate 16 of Paxinos and Watson (1982)] was not included in the densitometric analysis. The B_{max} for D_1 receptors was determined to be 1900 ± 81 fmol/mg protein, and the K_d for ^{3}H-SKF-83566 was 1.10 ± 0.066 nM (Fig. 4*A*). The B_{max} for D_2 receptors was determined to be 900 ± 132 fmol/mg protein, and the K_d for ^{3}H-SPD was 0.720 ± 0.091 nM (Fig. 4B). Scatchard analysis of saturation binding data was also carried out using filter wipes of CPu mash sections. The K_d values for both ^{3}H-SKF-83566 and ^{3}H-SPD were in good agreement with the values determined by densitometric analysis of horizontal brain sections (data not shown).

Comparison of D_1 and D_2 receptors in various brain regions in the same rats

Figure 5 shows autoradiograms from 1 of the 5 brains studied for 9 out of the 34 levels examined. Total binding of the D_1-selective ligand ^{3}H-SKF-83566 at a concentration of 1.80 nM is shown in Figure 5*a*, and binding in the presence of 2 μM (+)-butaclamol is shown in Figure 5*b*. Similarly, total binding of the D_2-selective ligand ^{3}H-SPD at a concentration of 1.0 nM is shown in Figure 5*c*, and binding in the presence of 100 μM sulpiride is shown in Figure 5*d*. Although ^{3}H-SPD is known to label both 5-HT-2 and spirodecanone sites (Palacios et al., 1981b), the use of the highly selective D_2 antagonist sulpiride to define nonspecific binding ensures that the specific binding includes only D_2 receptors (Zahniser and Dubocovich, 1983).

For regions that are not present in enough sections from a single brain to permit saturation binding assays, the total density (B_{max}) of D_1 or D_2 receptors cannot be determined by Scatchard analysis. Brain sections used for quantitative autoradiographic localization of D_1 and D_2 receptors were incubated in concentrations of either ^{3}H-SKF-83566 or ^{3}H-SPD chosen to optimize specific binding and development time, so the measured density represents the number of receptors to which the ligand is bound at that particular (nonsaturating) concentration of ligand. This does not provide a measure of the maximum density of receptors, as would be determined by Scatchard analysis of saturation binding data. However, if the assumption is made that the K_d value of a receptor for a particular ligand is uniform throughout the brain, then the densities determined by quantitative autoradiography can be corrected according to the following equation: $B_{max} = B(L + K_d)/L$, where B_{max} is the calculated maximum density of receptors, L is the concentration of radioligand, K_d is the dissociation constant for the ligand determined by Scatchard analysis of data obtained in the CPu in sections of brain, and B is the amount of radioligand specifically bound at L. Data calculated in this manner are shown in columns 2 and 4 of Table 2, and the resultant ratio of D_1 to D_2 receptors is shown in column 5. Where 0 values were obtained for the density of D_2 receptors, the ratio of the densities of D_1 to D_2 is indeterminate.

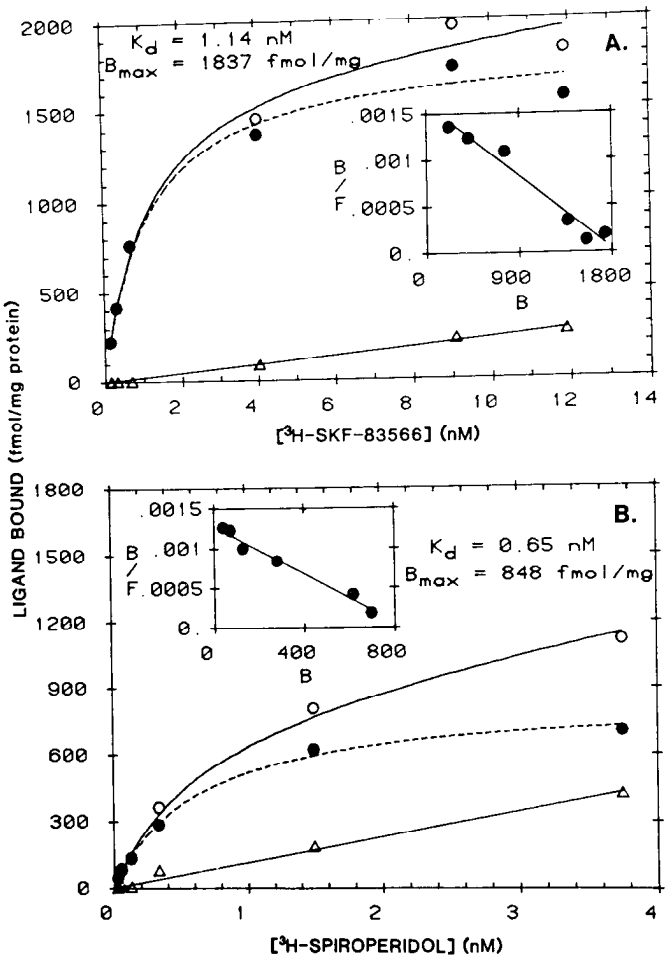


Figure 4. Densitometric analysis of saturation binding in the CPu in sections of rat brain. Sequential, horizontal 32-μm sections of brain, including the CPu, were assigned in a counterbalanced pattern for incubation with 7 concentrations of either ^{3}H-SKF-83566 ± (+)-butaclamol (*A*) or ^{3}H-SPD ± 100 μM sulpiride (*B*), for determination of the densities of D_1 or D_2 receptors. Assays were carried out in triplicate. The specific binding (●), defined as the difference between total (○) and nonspecific (△), was saturable. *Insets* show Scatchard transformations of the saturation data. $n = 4$; representative curves are shown.

Both D_1 and D_2 receptors were found throughout the brain, with the former outnumbering the latter in all but 1 region measured, the olfactory nerve layer. Brain regions identified as part of the olfactory apparatus, from olfactory bulb to olfactory nuclei and tubercle to primary olfactory and entorhinal cortices, had high to moderate densities of D_1 and D_2 receptors, generally in a ratio of 2–4:1. All areas of cerebral cortex revealed low to moderate densities of D_1 and D_2 receptors, with increased densities of D_1 receptors in layer 6, particularly in the cingulate cortex and in regions of cortex ventral to the rhinal fissure. In some regions, the density of D_2 receptors could not be distinguished from 0 because of the significant binding of ^{3}H-SPD to structures other than D_2 receptors in these regions. These nonspecific binding sites include 5-HT-2 receptors (Leysen et al., 1978) and spirodecanone sites (Palacios et al., 1981b).

The basal ganglia have the highest concentrations of dopamine receptors in the brain nuclei. In the nucleus accumbens and the olfactory tubercle, the calculated density of D_1 receptors was approximately 2500 fmol/mg protein, and the ratios of D_1 to D_2 receptors were, respectively, 3:1 and 5:1. In the entire CPu (Paxinos and Watson levels 10–18) the average densities of D_1 and D_2 receptors were, respectively, 2408 ± 116 and 784 ± 60 fmol/mg protein. The densities of both subtypes declined in a rostrocaudal direction (Fig. 6, *A* and *D*). Analysis of the densities of receptors in the medial and lateral portions of the head of the CPu (Paxinos and Watson levels 10–16) revealed not only a higher density of both subtypes of dopamine receptors in the latter, but also different rostrocaudal gradients. The density of D_1 receptors declined in the rostrocaudal direction by approximately 25% in the medial portion. In contrast, the density of D_1 receptors remained stable in the lateral compartment in these sections (Fig. 6, *B* and *C*). The density of D_2 receptors also declined by approximately 25% in the medial compartment, but increased by approximately 55% in the lateral compartment (Fig. 6, *E* and *F*).

Table 1. Affinities of D_1 and D_2 receptors for antagonists

Drug	Dissociation constant, K_i (nM) ^{3}H-SKF-83566	^{3}H-SPD
SCH-23390	0.77 ± 0.33 (2)	3100 ± 880 (3)
(+)-Butaclamol	16 ± 1.2 (2)	6.8 ± 0.45 (2)
(−)-Butaclamol	50,000 ± 6600 (2)	>10,000 (3)
Spiroperidol	900 ± 141 (2)	ND
Fluphenazine	ND	0.14 ± 0.012 (3)
Domperidone	ND	0.97 ± 0.127 (3)
Sulpiride	69,000 ± 4450 (2)	160 ± 40 (3)
Ketanserin	2200 ± 520 (3)	2700 ± 150 (5)
Pipamperone	4800 ± 1100 (2)	970 ± 550 (3)

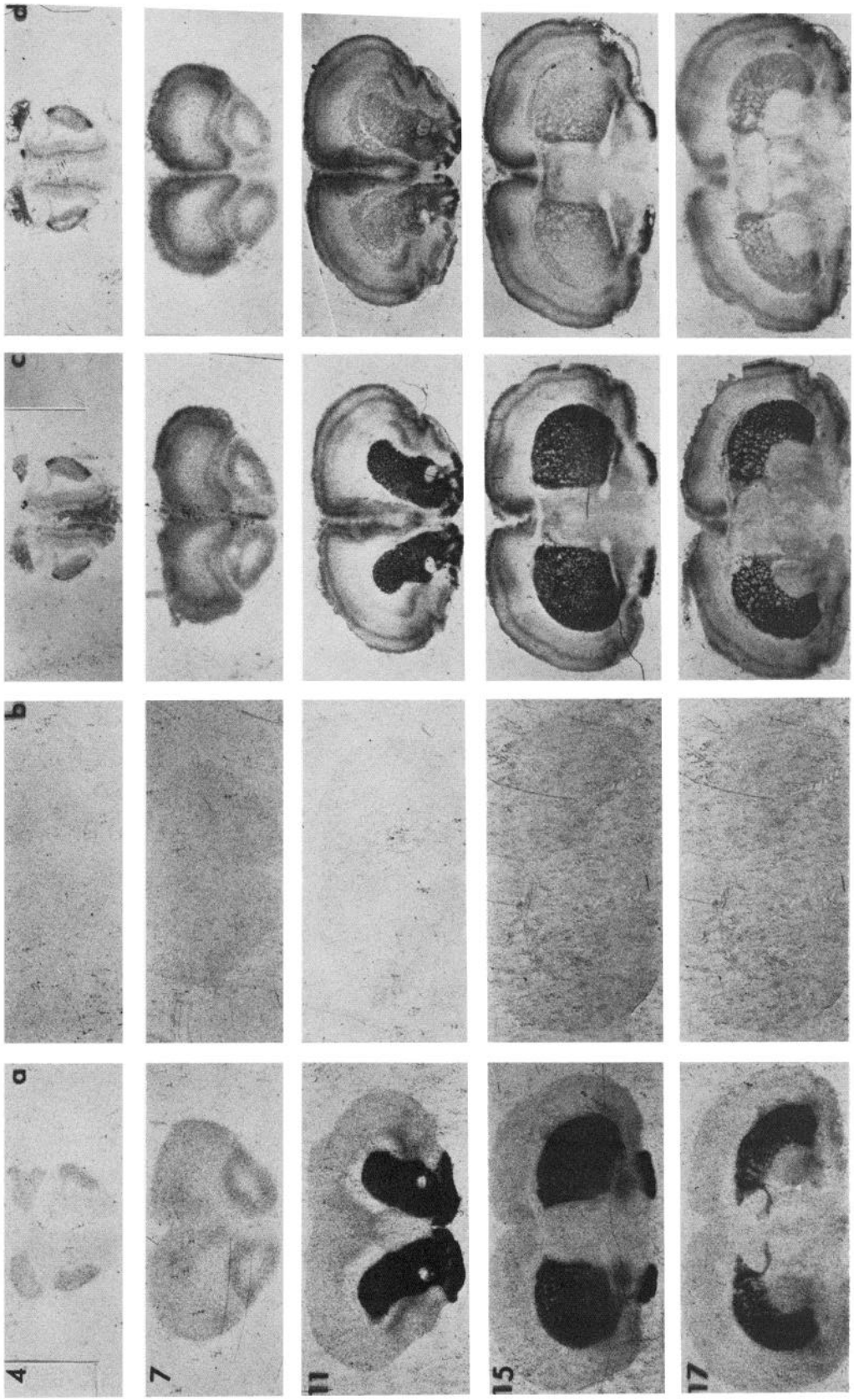
Sections of CPu mash were incubated with either 0.15–0.25 nM ^{3}H-SKF-83566 or 0.2–0.3 nM ^{3}H-SPD and 12–18 concentrations of each competing drug. The tissue was wiped off the slide with a glass-fiber filter, and the radioactivity present was determined by scintillation counting. IC_{50} values were determined using the NEWFITSITES program (PROPHET; Fig. 2), and K_i values were calculated using the Cheng and Prusoff (1973) equation $K_i = IC_{50}/(1 + L^*/K_d^*)$, where L^* = concentration of the radioligand and $K_d^* = K_d$ of the radioligand for the receptor. The results shown are mean ± SEM for triplicate determinations in the number of independent experiments shown in parentheses. ND, not determined.

In contrast, the density of D_1 receptors remained stable in the lateral compartment in these sections (Fig. 6, *B* and *C*). The density of D_2 receptors also declined by approximately 25% in the medial compartment, but increased by approximately 55% in the lateral compartment (Fig. 6, *E* and *F*).

The density of receptors in the ventral pallidum and the region around the ventral internal capsule encompassing the zona incerta and entopeduncular nucleus was moderate to high. A great preponderance of the receptors in this region were D_1 receptors (D_1:D_2 = 8:1 to 18:1). Several nuclei of the amygdala contained moderate densities of dopamine receptors. In particular, the basolateral and medial nuclei had a density of D_1 receptors of 400–500 fmol/mg protein, with a ratio of D_1 to D_2 receptors of 8:1, while the central nucleus had a similar density of D_1 receptors, but a ratio of D_1 to D_2 receptors of 2:1. In the hippocampus, the density of both D_1 and D_2 receptors was low, with a ratio of D_1 to D_2 receptors of 1:1 to 2:1.

In the substantia nigra, a very high density of D_1 receptors—approximately 2500 fmol/mg protein—was observed in both the compacta and the reticulata, whereas the density of D_2 receptors was approximately 230 and 70 fmol/mg protein, respectively, yielding respective ratios of D_1 to D_2 receptors of 11:1 and 36:1. In the ventral tegmental nucleus, the density of D_1 receptors was an order of magnitude lower than in the substantia nigra, while D_2 receptors were essentially undetectable. The density of D_1 receptors in the subthalamus was moderately high (700 fmol/mg protein), but the density of D_2 receptors could not be distinguished from background.

Areas of sensory cortex, including the temporal auditory and striate cortex, revealed low levels of D_1 receptors and levels of D_2 receptors that could not be distinguished from background. The superficial layers of the superior colliculus, which processes visual information, displayed low to moderate densities of D_1 receptors and low levels of D_2 receptors. Labeling in the brain stem and cerebellum revealed low densities of D_1 receptors and very low densities of D_2 receptors.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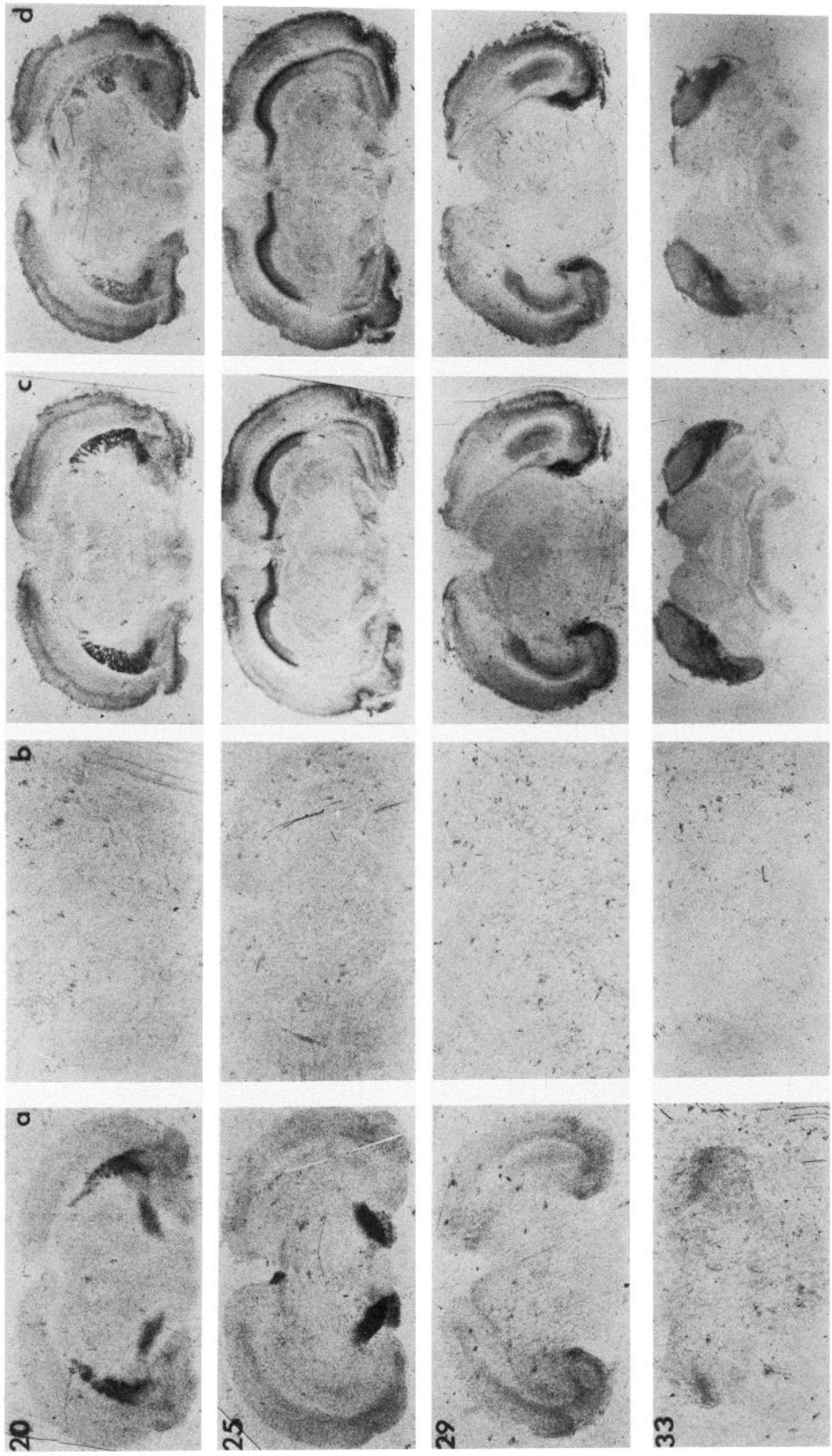

Figure 5. Autoradiograms of ^{3}H-SKF-83566 (D_1) and ^{3}H-SPD (D_2) binding at 9 selected coronal levels. Groups of 4 serial 32 μm coronal slices from 5 rats were alternately assigned to incubation with either 1.8 nM ^{3}H-SKF-83566 (*a, b*) or 1.0 nM ^{3}H-SPD (*c, d*), as described in Materials and Methods. Two sections from each group were incubated with each of the radioligands (total = columns *a* and *c*), and 2 were incubated with each radioligand plus either 2 μM (+)-butaclamol (*b*) or 100 μM sulpiride (*d*) to define nonspecific binding. After incubation, sections were washed, as described in Materials and Methods, dried briefly at 60°C, and apposed to LKB Ultrofilm for 1 (^{3}H-SKF-83566) or 2 (^{3}H-SPD) weeks. The film was developed and the autoradiograms analyzed to determine the density of receptors (Table 2). Sections from 1 representative brain are shown. The *numbers* in the upper left corner indicate the corresponding plates in the atlas of Paxinos and Watson (1982).

Table 2. Densities of D_1 and D_2 receptors in areas of rat brain

Structures	D_1 receptors (fmol/mg protein)				D_2 receptors (fmol/mg protein)				D_1/D_2
	Bound (mean ± SEM)		B_{max} (mean ± SEM)		Bound (mean ± SEM)		B_{max} (mean ± SEM)		
Int. granular layer, olf. bulb	113	24	182	39	41	21	71	36	3
Olfactory nerve layer	102	10	164	16	301	39	517	67	0.3
Anterior olfactory nuclei	276	15	445	25	57	17	97	29	5
Primary olfactory ctx.	244	16	393	25	84	18	144	31	3
Frontal, parietal ctx. 1	96	7	155	11	0	32	0	55	ID
Frontal, parietal ctx. 2–5	96	7	155	11	39	8	67	13	2
Frontal, parietal ctx. 6	153	16	246	26	39	8	67	13	4
Cingulate ctx. 6	180	22	289	35	39	8	67	13	4
Nucleus accumbens	1546	66	2491	106	427	68	733	116	3
Olfactory tubercle	1589	70	2559	113	328	35	562	61	5
Claustrum	470	18	757	30	33	46	57	80	13
Endopiriform nucleus	473	14	763	22	12	24	21	41	37
Caudate-putamen, total	1495	72	2408	116	457	35	784	60	3
Caudate-putamen, medial head	1696	68	2733	109	535	42	917	73	3
Caudate-putamen, lateral head	1870	47	3013	76	830	66	1424	113	2
Septal nuclei	111	19	178	31	39	16	66	28	3
Ventral pallidum	667	59	1074	95	70	19	119	32	9
Globus pallidus	288	30	464	49	83	18	142	30	3
Lateral preoptic area	118	8	190	14	58	11	100	19	2
Amygdala, basolateral	327	6	527	9	38	21	65	35	8
Amygdala, central	297	23	478	36	152	40	260	68	2
Amygdala, medial	226	29	364	47	26	32	44	54	8
Thalamus	75	15	121	23	22	7	38	11	3
Hypothalamus	112	8	180	13	36	2	62	3	3
Suprachiasmatic nucleus	353	28	569	46	62	7	106	12	5
Zona incerta	354	150	571	242	40	8	68	14	8
Entopeduncular nucleus	590	73	951	117	30	27	51	46	18
Hippocampus, total	52	8	84	13	56	44	95	76	1
Hippocampus, pyriform nucleus	52	8	84	13	60	23	103	40	1
Hippocampus, dentate gyrus	92	14	147	23	30	7	51	12	3
Subthalamus	443	91	714	147	0	16	0	28	ID
Substantia nigra, compacta	1608	104	2590	168	136	16	232	28	11
Substantia nigra, reticulata	1503	124	2421	201	39	9	67	15	36
Ventral tegmental area	57	6	91	10	4	5	7	9	13
Entorhinal ctx. 1–5	215	31	346	50	69	20	118	35	3
Entorhinal ctx. 6	336	21	541	33	69	20	118	35	5
Subiculum	252	27	406	44	17	12	30	20	14
Temporal auditory ctx.	137	25	222	41	26	3	45	4	5
Striate ctx. 1	91	12	147	20	62	27	107	46	1
Striate ctx. 2–5	91	12	147	20	21	12	36	20	4
Striate ctx. 6	147	22	237	36	21	12	36	20	7
Superior colliculus	189	20	305	32	66	22	113	38	3
Brain stem	49	19	79	30	25	9	43	15	2
Cerebellum	70	19	113	31	31	11	53	20	2
Choroid plexus, lateral ventricle	1711	111	2757	178	520	112	893	192	3
Choroid plexus, 3 + 4 ventricles	574	54	925	87	35	7	59	11	16

Serial 32 μm coronal rat brain sections at 34–36 levels for each of 5 rat brains were incubated with either 1.8 nM ^{3}H-SKF-83566 ± 2 μM (+)-butaclamol (D_1) or 1.0 nM ^{3}H-SPD ± 100 μM sulpiride (D_2), as described in Materials and Methods. Representative autoradiograms are shown in Figure 5; analysis was carried out as described in Materials and Methods. The density of D_1 or D_2 receptors specifically labeled under these conditions is indicated as Bound; this value has been used to calculate the density of receptors (B_{max}) using the equation B_{max} = Bound($L + K_d$)/L, where L = the radioligand concentration. The K_d values used were those determined by Scatchard analysis of the autoradiographically analyzed binding of ^{3}H-SKF-83566 or ^{3}H-SPD to CPu in sections of brains (see Fig. 4). The ratio of the densities of D_1 to D_2 receptors has been calculated using the values for B_{max}. Where zero values have been obtained for the density of D_2 receptors, the value of D_1/D_2 is indeterminate (ID). The density of receptors in structures appearing in more than 1 section per brain is given as a nonweighted average of the densities obtained in each section. The "head" of the CPu, in which densities were measured in both medial and lateral compartments (see Fig. 6), was defined as the CPu appearing in plates 10–16 of Paxinos and Watson (1982), whereas the "total" CPu is represented in plates 10–18 of Paxinos and Watson. Abbreviations used: Int., internal; olf., olfactory; ctx., cortex layer.

The choroid plexus in the lateral ventricles had densities of both D_1 and D_2 receptors as high as those found in the CPu, with a similar ratio of 3:1. Labeling in the choroid plexus in the third and fourth ventricles was less intense for both receptors.

Discussion

Until the advent of the D_1-selective benzazepine antagonists SCH-23390 and SKF-83566, it was not possible to map the distribution of D_1 receptors by quantitative autoradiography. A preliminary publication by Dawson et al. (1985) reported the feasibility of using ^{3}H-SCH-23390 for this purpose. This report describes a method for the use of ^{3}H-SKF-83566, which differs from ^{3}H-SCH-23390 in having a bromine instead of a chlorine in the 7 position, to label D_1 receptors. Although previous reports of the pharmacological specificity of the binding of ^{3}H-SKF-83566 in peripheral (Ohlstein and Berkowitz, 1985) and central (Hyttel, 1983) tissues suggested that this ligand also binds to 5-HT-2 receptors with high affinity, this was not observed, even with concentrations of ^{3}H-SKF-83566 as high as 1.6 nM. These experiments were carried out in CPu mash sections, in which binding to 5-HT-2 receptors would have been readily evident because the CPu is rich in 5-HT-2 receptors (Hamblin et al., 1984).

To set up an assay for quantitative autoradiography, it is necessary not only to determine the optimal incubation and wash conditions for the brain sections, but also to establish the pharmacological parameters of such binding. The heterogeneity of the receptor sites, even within a single brain region such as the CPu, is well known (Joyce et al., 1985). The use of brain sections from several animals is complicated by the variability in density of a given class of receptors. For example, the coefficient of variation is approximately 25% for D_2 receptors in the CPu of rats (Leff et al., 1984; S. J. Boyson, P. McGonigle, and P. B. Molinoff, unpublished observations) and humans (Seeman et al., 1984). Thus, it is difficult, if not impossible, to obtain the up to 60 identical tissue slices required for use in competition studies with a single ligand. For these reasons, the use of sections of CPu mash for determination of the kinetic and pharmacological parameters of radioligand binding was investigated. Results of kinetic studies were similar in sections of brain and CPu mash, confirming the legitimacy of using the latter preparation in preliminary studies with radioligands. Complications arising from varying densities of receptors along rostrocaudal or mediolateral gradients in different brain regions and among different animals were thus overcome. For the determination of these parameters, only the total amount of radioactivity specifically bound to the tissue needs to be measured. Because the volume of the homogenate sections is constant, these sections can be analyzed quickly by wiping them off the slide with a glass-fiber filter and counting in scintillation fluid. For studies of receptors that have a high density only in discrete regions of the brain and relatively high nonspecific binding elsewhere, the use of a homogenate composed only of those regions of high receptor density lowers the proportion of nonspecific binding and improves the signal-to-noise ratio, compared to a homogenate of whole brain.

The main purpose of this study was 2-fold: to provide a detailed map of the distribution of D_1 and D_2 receptors in rat brain, and to determine the ratios of D_1 to D_2 receptors in various brain regions in the same rats. Comparison of the densities of receptors occupied by the radioligands at nonsaturating concentrations would not provide equivalent numbers for determination of the ratio of D_1 to D_2 receptors, and many regions were too small to be included on enough sections from a single brain for Scatchard analysis of the 2 classes of receptors. The approach taken was to calculate the density of receptors in each region by dividing the density of receptors bound by the fractional occupancy ($L/L + K_d$). This calculation was based on the assumption that the affinity of the receptor for the ligand was similar in all regions studied. For these calculations, the K_d values were those obtained in Scatchard analysis of quantitative autoradiographic studies of the CPu in brain sections. The use of a corrected B_{max} makes it possible to compare the densities of D_1 and D_2 receptors in the same brain regions, which was a principal goal of this study. These calculated B_{max} values, however, do not correct for the differential quenching of the β-emission of ^{3}H by gray and white matter. Thus, the density of receptors would be underestimated in white matter regions as compared to standards composed primarily of gray matter, such as CPu (although the comparison of the densities of D_1 and D_2 receptors in the same region would be unaffected by this factor). Some methods for dealing with this problem have recently been suggested by Kuhar and Unnerstall (1985).

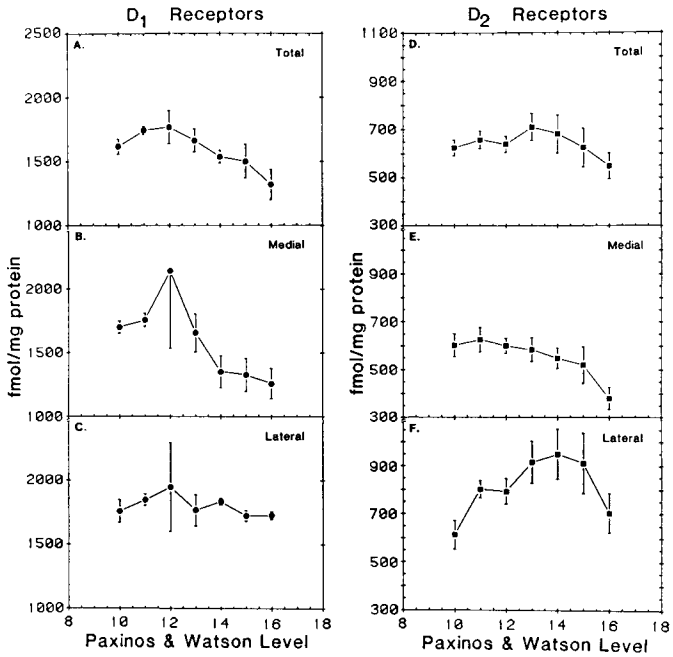

Figure 6. Rostrocaudal D_1 and D_2 receptor gradients within the CPu. Autoradiograms were generated and analyzed, as described in Table 2. The "head" (*Total*) of the CPu was arbitrarily divided into *medial* and *lateral* compartments by a *vertical line* placed midway between the medial and lateral borders of the CPu. Independent readings were taken from the entire head of the CPu and its medial and lateral compartments in successive autoradiograms corresponding to levels 10–18 in the atlas of Paxinos and Watson (1982). The density of receptors shown is the mean ± SEM of the B_{max}, calculated as described in Table 2.

Sulpiride, rather than (+)-butaclamol, was chosen to define the specific binding of ^{3}H-SPD on the basis of its superior specificity for the D_2 receptor (Zahniser and Dubocovich, 1983). Concentrations of (+)-butaclamol routinely used to define specific binding have been shown to displace ^{3}H-SPD from 5-HT-2 receptors (Grigoriadis and Seeman, 1985; Hamblin et al., 1984). In contrast, 100 μM sulpiride does not displace ^{3}H-SPD from 5-HT-2 receptors (Grigoriadis and Seeman, 1985). Measurement of the binding of ^{3}H-SPD in the presence of high concentrations of ketanserin has been used as a means of circumventing the lack of specificity of butaclamol (Altar et al., 1985; Hamblin et al., 1984). Two drawbacks to this approach are that (1) the concentrations of ketanserin used for this purpose inhibit the binding of ^{3}H-SPD to a small percentage of D_2 receptors, and (2) the choice of concentration for ketanserin relies on the ability to produce accurate and detailed inhibition curves, which is difficult to do when using autoradiographic techniques. Although ^{3}H-SPD also labels spirodecanone sites in specific re-

gions of the brain, sulpiride does not displace ^{3}H-SPD from these sites (Palacios et al., 1981b). Thus, the use of sulpiride alone to define specific binding provides the most accurate measure of the density of D_2 receptors labeled by ^{3}H-SPD.

Previous reports of the localization of dopamine receptors in the rat brain have, for the most part, dealt with the D_2 subtype (e.g., Klemm et al., 1979; Palacios et al., 1981b), particularly in the basal ganglia, in response to various lesions (e.g., Murrin et al., 1979; Neve et al., 1984). A comprehensive table of densities of D_2 receptors in various brain regions has not previously appeared. Previous reports of the localization of D_1 receptors have either been preliminary, with qualitative data provided from studies with the antagonist ^{3}H-SCH-23390 (Dawson et al., 1985) or the agonist ^{3}H-SKF-38393 (Scatton and DuBois, 1985), or confined to a single brain region (Fuxe et al., 1983). The present report presents detailed data on the densities of both D_1 and D_2 receptors studied in adjacent sections from the same rats. The use of the calculated B_{max}, rather than the density of receptors bound at a given radioligand concentration, allows for quantitative comparison of the densities of these receptor subtypes.

The present study demonstrates the presence of D_1 receptors throughout the forebrain, although it has not been demonstrated that there are dopaminergic projections throughout the forebrain. The entire neocortex has low levels of D_1 receptors, with twice the density in layer 6 as in layers 1–5.

The finding that D_1 receptors are highest in density in the CPu, nucleus accumbens, olfactory tubercle, and substantia nigra is in accordance with the results of previous studies of the distribution of dopamine-stimulated adenylate cyclase (Bockaert et al., 1976). In the first 3 structures, the density of D_1 receptors is approximately 2–3 times that of D_2 receptors, as is shown in studies carried out with homogenates (Huff and Molinoff, 1984).

In the substantia nigra, there is a remarkable divergence in the densities of the dopamine receptor subtypes. In the pars compacta, in which the cell bodies of the A9 neurons of the nigrostriatal pathway reside, the density of D_1 receptors (approximately 2600 fmol/mg protein) is as high as in the striatum, and the density of D_2 receptors (230 fmol/mg protein) is approximately 30% of that in the striatum, yielding a ratio of D_1 to D_2 receptors of 11:1. In the pars reticulata of the substantia nigra, in which the terminals of the strionigral projections are found, but which does not contain dopaminergic cell bodies, the density of D_1 receptors is similar to that in the compacta, but the density of D_2 receptors is very low, yielding a ratio of D_1 to D_2 receptors of 36:1. These ratios are consistent with the hypothesis that D_2 receptors reside primarily on the dopamine-containing cell bodies and that D_1 receptors reside primarily on the strionigral terminals. Studies of lesions of the substantia nigra and striatum provide support for this hypothesis. Thus, the density of D_2 receptors declines in the substantia nigra after administration of the neurotoxin 6-hydroxydopamine (Murrin et al., 1979; Quik et al., 1979), but dopamine-stimulated cyclase (mediated by D_1 receptors) does not (Gale et al., 1977; Quik et al., 1979; Saavedra et al., 1978). Moreover, dopamine-stimulated cyclase was reduced by 50–85% following destruction of the strionigral projection by injection of kainic acid into the striatum (Gale et al., 1977; Quik et al., 1979). More recently, Lemos et al. (1984) studied the dopamine-regulated phosphoprotein DARPP-32—which has been associated with D_1 receptors—in the mesencephalon following various lesions. After kainic acid was injected into the striatum to destroy the strionigral fibers, there was a 48% decrease in DARPP-32 in the substantia nigra, whereas when 6-hydroxydopamine was injected into the substantia nigra to destroy the nigral neurons themselves, there was no decrease in the amount of DARPP-32 within the substantia nigra.

The difference in the densities of both subtypes of dopamine receptor in the ventral tegmental area, as compared to the densities in the substantia nigra compacta, is striking. In the ventral tegmental area, the origin of the A10, mesolimbic, dopaminergic pathways, the density of D_1 receptors is 25 times lower than in the substantia nigra, and D_2 receptors are undetectable. This marked difference may provide an important clue to the differential biologic characteristics—such as response to chronic administration of neuroleptics (Chiodo and Bunney, 1983; White and Wang, 1983) or degeneration following administration of the neurotoxin MPTP (Bowden et al., 1985)—observed in studies of the A9 and A10 neurons. The low densities of D_1 and D_2 receptors could indicate that regulation of feedback to A10 neurons is not mediated locally by either subtype of dopamine receptor.

In the striatum, there is also a differential distribution of D_1 and D_2 receptors. The lateral-to-medial and rostrocaudal increasing density gradients of D_2 receptors in the striatum that have been described previously (Altar et al., 1985; Joyce et al., 1985) were confirmed in this study. The distribution of D_1 receptors did not show a similar lateral-to-medial gradient, although there was a suggestion of a modest, increasing ventromedial-to-dorsolateral gradient of D_1 receptors within the striatum. The rostrocaudal decline was less for D_1 than for D_2 receptors.

The presence of high densities of D_1 (but not D_2) receptors in the region of the ventral internal capsule, as well as moderate densities in the globus pallidus and ventral pallidum—areas which may not be terminal fields for dopaminergic innervation—raises the question of whether these might be receptors in transit within axons. That these regions are the same ones noted by Fallon and Moore (1978) and Beckstead et al. (1979) in their studies of the projections of mesencephalic dopaminergic neurons suggests that they could be autoreceptors within ascending axons. On the other hand, results of pharmacological studies *in vivo* and *in vitro* favor the view that "the autoreceptor" is of the D_2 subtype (Roth, 1984). It is more likely that these D_1 receptors are either in transit in descending axons of the strionigral pathway or are postsynaptic receptors in previously unrecognized terminal fields. The latter possibility is supported by the fact that Ouimet et al. (1984) also found DARPP-32, a dopamine receptor-regulated phosphoprotein, in the pallidum and ventral internal capsule. The presence of DARPP-32 in these regions may mean that the D_1 receptors are functionally coupled to intracellular effector mechanisms. Alternatively, it is possible that the D_1 receptors and DARPP-32 are being transported within axons to some other site.

In addition to the somatosensory, temporal-auditory, and striate areas of the neocortex, other areas of the brain concerned with processing sensory information also reveal modest to moderately high densities of D_1 receptors. Two extrastriate visual processing areas that have output to motor areas, the claustrum (Carey and Neal, 1985) and superior colliculus, contain mostly D_1 receptors. The finding that various nuclei of the amygdala, thought to be involved in cross-modal sensory processing (Murray and Mishkin, 1985) as well as limbic functions, show a heterogeneous pattern of levels, as well as ratios, of D_1 and D_2 receptors suggests that, in these areas, these receptors may be involved in a discrete fashion in modulating certain functions of the structure. The thalamus, however, as well as sensory brain stem areas other than the superior colliculus, showed very low levels of both subtypes of dopamine receptor.

It is likely, based on their distribution, that D_1 receptors are involved at many stages in the processing of olfactory stimuli. The density of D_1 receptors increases abruptly below the rhinal fissure, and they are present in varying amounts in the anterior olfactory nuclei, the primary olfactory cortex, and the entorhinal cortex, as well as in the limbic processing areas. This is also the

system, however, that includes the olfactory nerve layer, the only region in which a higher density of D_2 than D_1 receptors was observed.

The finding of very high densities of D_1 receptors and moderately high densities of D_2 receptors in the choroid plexus raises the possibility that these receptors may lie on blood vessels and modulate blood flow or secretion of cerebrospinal fluid. If so, agonists or antagonists at these receptors could be useful in altering cerebrospinal fluid dynamics for therapy in disorders such as hydrocephalus and benign intracranial hypertension. The presence of D_1 receptors on small blood vessels could also account for the low levels of D_1 receptors found in areas such as the thalamus, hippocampus, and upper layers of the cortex. This possibility cannot be evaluated with the present level of resolution of quantitative autoradiography.

These studies of the distribution of D_1 and D_2 receptors in the rat brain can facilitate the formulation of hypotheses about their roles in the normal function of the brain. Further studies in normal and pathologic human brain, as well as clinical studies using agonists and antagonists selective for either the D_1 or D_2 receptor, should enable us to test these and other hypotheses.

References

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

Beckstead, R. M., V. B. Domesick, and W. J. Nauta (1979) Efferent connections of the substantia nigra and ventral tegmental area in the rat. Brain Res. *175:* 191–217.

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.

Bockaert, J., J. Premont, J. Glowinski, A. M. Thierry, and J. P. Tassin (1976) Topographical distribution of dopaminergic innervation and of dopaminergic receptors in the rat striatum. II. Distribution and characteristics of dopamine adenylate cyclase—Interaction of *d*-LSD with dopaminergic receptors. Brain Res. *107:* 303–315.

Bowden, D. M., M. F. Dubach, R. Snyder, S. Askari, and D. C. German (1985) Three-dimensional computer analysis of MPTP-induced aminergic cell loss in the monkey. Soc. Neurosci. Abstr. *11:* 166.

Bradford, M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. *72:* 248–254.

Breese, G. R., and R. A. Mueller (1985) SCH-23390 antagonism of a D-2 dopamine agonist depends upon catecholaminergic neurons. Eur. J. Pharmacol. *113:* 109–114.

Calne, D. B. (1982) Dopamine receptors in movement disorders. In *Movement Disorders,* C. D. Marsden and S. Fahn, eds., pp. 348–355, Butterworth, Boston.

Carey, R. G., and T. L. Neal (1985) The rat claustrum: Afferent and efferent connections with the visual cortex. Brain Res. *329:* 185–193.

Cheng, Y.-C., and W. H. Prusoff (1973) Relationship between the inhibition constant (K_I) and the concentration of inhibitor which causes 50 per cent inhibition (I_{50}) of an enzymatic reaction. Biochem. Pharmacol. *22:* 3099–3108.

Chiodo, L. A., and B. S. Bunney (1983) Typical and atypical neuroleptics: Differential effects of chronic administration on the activity of A9 and A10 midbrain dopaminergic neurons. J. Neurosci. *3:* 1607–1619.

Christensen, A. V., J. Arnt, J. Hyttel, J.-J. Larsen, and O. Svendsen (1984) Pharmacological effects of a specific dopamine D-1 antagonist SCH 23390 in comparison with neuroleptics. Life Sci. *34:* 1529–1540.

Creese, I. (1983) Receptor interactions of neuroleptics. In *Neuroleptics: Neurochemical, Behavioral and Clinical Perspective,* J. T. Coyle and S. J. Enna, eds., pp. 183–222, Raven, New York.

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.

Dawson, T. M., D. R. Gehlert, H. I. Yamamura, A. Barnett, and J. K. Wamsley (1985) D-1 dopamine receptors in the rat brain: Autoradiographic localization using (^{3}H)SCH 23390. Eur. J. Pharmacol. *108:* 323–325.

Fallon, J. H., and R. Y. Moore (1978) Catecholamine innervation of the basal forebrain. IV. Topography of the dopamine projection to the basal forebrain and neostriatum. J. Comp. Neurol. *180:* 545–580.

Flaim, K. E., G. W. Gessner, S. T. Crooke, J. R. Heys, and J. Weinstock (1986) Regulation of agonist and antagonist binding to striatal D-1 dopamine receptors: Studies using the selective D-1 antagonist [^{3}H]SK&F R-83566. Life Sci. *38:* 2087–2096.

Fuxe, K., L. F. Agnati, F. Benfenati, K. Andersson, M. Camurri, and M. Zoli (1983) Evidence for the existence of a dopamine receptor of the D-1 type in the rat median eminence. Neurosci. Lett. *43:* 185–190.

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

Gehlert, D. R., H. I. Yamamura, and J. K. Wamsley (1984) Use of autoradiographic techniques for the localization of neurotransmitter receptors in brain and periphery: Recent applications. In *Neurotransmitter Receptors: Mechanisms of Action and Regulation,* S. Kito, T. Segawa, K. Kuriyama, H. I. Yamamura, and R. W. Olsen, eds. Adv. Exp. Med. Biol. *175:* 255–270.

Grigoriadis, D., and P. Seeman (1985) Complete conversion of brain D_2 dopamine receptors from the high- to the low-affinity state for dopamine agonists, using sodium ions and guanine nucleotide. J. Neurochem. *44:* 1925–1935.

Hamblin, M. W., S. E. Leff, and I. Creese (1984) Interactions of agonists with D-2 dopamine receptors: Evidence for a single receptor population existing in multiple agonist affinity-states in rat striatal membranes. Biochem. Pharmacol. *33:* 877–887.

Huff, R. M., and P. B. Molinoff (1984) Quantitative assay of dopamine receptor subtypes. In *Dopamine Receptor Agonists,* G. Poste and S. T. Cooke, eds., pp. 65–86, Plenum, New York.

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

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.

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.

Joyce, J. N., S. K. Loeschen, and J. F. Marshall (1985) Dopamine D-2 receptors in rat caudate-putamen: The lateral to medial gradient does not correspond to dopaminergic innervation. Brain Res. *338:* 209–218.

Kebabian, J. W., and D. B. Calne (1979) Multiple receptors for dopamine. Nature *277:* 93–96.

Kebabian, J. W., M. Beaulieu, and Y. Itoh (1984) Pharmacological and biochemical evidence for the existence of two categories of dopamine receptor. Can. J. Neurol. Sci. *11:* 114–117.

Klemm, N., L. C. Murrin, and M. J. Kuhar (1979) Neuroleptic and dopamine receptors: Autoradiographic localization of (^{3}H)spiperone in rat brain. Brain Res. *169:* 1–9.

Kuhar, M. J., and J. R. Unnerstall (1985) Quantitative receptor mapping by autoradiography: Some current technical problems. Trends Neurosci. *1985:* 49–53.

Laduron, P. (1982) Brain dopamine receptor: Multiple binding sites or physiological receptor site. In *Advances in Dopamine Research,* M. Kohsaka, ed., pp. 71–76, Pergamon, Oxford, UK.

Leff, S. E., R. Gariano, and I. Creese (1984) Dopamine receptor turnover rates in rat striatum are age-dependent. Proc. Natl. Acad. Sci. USA *81:* 3910–3914.

Lemos, J. R., C. Barberis, J. P. Tassin, and J. Bockaert (1984) Topographical distributions of 32K and 48K cAMP-regulated phosphoproteins: Relationships to dopamine and serotonin innervations in striatum, substantia nigra and cerebral cortex. Brain Res. *323:* 47–54.

Leysen, J. E., C. J. Niemegeers, J. P. Tollenaere, and P. M. Laduron (1978) Serotonergic component of neuroleptic receptors. Nature *272:* 168–171.

Mailman, R. B., D. W. Schulz, M. H. Lewis, L. Staples, H. Rollema, and D. L. DeHaven (1984) SCH-23390: A selective D_1 dopamine antagonist with potent D_2 behavioral actions. Eur. J. Pharmacol. *101:* 159–160.

McGonigle, P., R. M. Huff, and P. B. Molinoff (1984) A comprehen-

sive method for the quantitative determination of dopamine receptor subtypes. Ann. NY Acad. Sci. *430:* 77–90.

Meller, E., S. Kuga, A. J. Friedhoff, and M. Goldstein (1985) Selective D_2 dopamine receptor agonists prevent catalepsy induced by SCH 23390, a selective D_1 antagonist. Life Sci. *36:* 1857–1864.

Molloy, A. G., and J. L. Waddington (1984) Dopaminergic behaviour stereospecifically promoted by the D_1 agonist *R*-SK & F 38393 and selectively blocked by the D_1 antagonist SCH 23390. Psychopharmacology *82:* 409–410.

Murray, E. A., and M. Mishkin (1985) Amygdalectomy impairs crossmodal association in monkeys. Science *228:* 604–606.

Murrin, L. C., K. Gale, and M. J. Kuhar (1979) Autoradiographic localization of neuroleptic and dopamine receptors in the caudate-putamen and substantia nigra: Effects of lesions. Eur. J. Pharmacol. *60:* 229–235.

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.

O'Boyle, K. M., and J. L. Waddington (1984) Selective and stereospecific interactions of R-SK & F 38393 with (^{3}H)piflutixol but not (^{3}H)spiperone binding to striatal D_1 and D_2 dopamine receptors: Comparisons with SCH 23390. Eur. J. Pharmacol. *98:* 433–436.

Ohlstein, E. H., and B. A. Berkowitz (1985) SCH 23390 and SK & F 83566 are antagonists at vascular dopamine and serotonin receptors. Eur. J. Pharmacol. *108:* 205–208.

Ouimet, C. C., P. E. Miller, H. C. Hemmings, Jr., S. I. Walaas, and P. Greengard (1984) DARPP-32, a dopamine- and adenosine 3′:5′-monophosphate-regulated phosphoprotein enriched in dopamine-innervated brain regions. III. Immunocytochemical localization. J. Neurosci. *4:* 111–124.

Palacios, J. M., D. L. Niehoff, and M. J. Kuhar (1981a) Receptor autoradiography with tritium-sensitive film: Potential for computerized densitometry. Neurosci. Lett. *25:* 101–105.

Palacios, J. M., D. L. Niehoff, and M. J. Kuhar (1981b) (^{3}H) Spiperone binding sites in brain: Autoradiographic localization of multiple receptors. Brain Res. *213:* 227–289.

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

Pazos, A., R. Cortes, and J. M. Palacios (1984) Quantitative receptor autoradiography: Application to the characterization of multiple receptor subtypes. J. Recept. Res. *4:* 645–656.

Penney, J. B., Jr., H. S. Pan, A. B. Young, K. A. Frey, and G. W. Dauth (1981) Quantitative autoradiography of muscimol receptors. Science *214:* 1036–1038.

Quik, M., P. C. Emson, and E. Joyce (1979) Dissociation between the presynaptic dopamine-sensitive adenylate cyclase and (^{3}H) spiperone binding sites in rat substantia nigra. Brain Res. *167:* 355–365.

Quirion, R., R. P. Hammer, Jr., M. Herkenham, and C. B. Pert (1981) Phencyclidine (angel dust) σ "opiate" receptor: Visualization by tritium-sensitive film. Proc. Natl. Acad. Sci. USA *78:* 5881–5885.

Rainbow, T. C., W. V. Bleisch, A. Biegon, and B. S. McEwen (1982) Quantitative densitometry of neurotransmitter receptors. J. Neurosci. Methods *5:* 127–138.

Rainbow, T. C., A. Biegon, and D. J. Berck (1984) Quantitative receptor autoradiography with tritium-labeled ligands: Comparison of biochemical and densitometric measurements. J. Neurosci. Methods *11:* 231–241.

Roth, R. H. (1984) CNS dopamine autoreceptors: Distribution, pharmacology, and function. In *Presynaptic Modulation of Postsynaptic Receptors in Mental Diseases,* A. I. Salama, ed. Ann. NY Acad. Sci. *430:* 27–53.

Saavedra, J. M., P. E. Setler, and J. W. Kebabian (1978) Biochemical changes accompanying unilateral 6-hydroxydopamine lesions in the rat substantia nigra. Brain Res. *151:* 339–352.

Scatchard, G. (1949) The attractions of proteins for small molecules and ions. Ann. NY Acad. Sci. *51:* 660–672.

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.

Seeman, P. (1977) Anti-schizophrenic drugs—Membrane receptor sites of action. Biochem. Pharmacol. *26:* 1741–1748.

Seeman, P. (1981) Brain dopamine receptors. Pharmacol. Rev. *32:* 229–313.

Seeman, P., C. Ulpian, C. Bergeron, P. Riederer, K. Jellinger, E. Gabriel, G. P. Reynolds, and W. W. Tourtellotte (1984) Bimodal distribution of dopamine receptor densities in brains of schizophrenics. Science *225:* 728–731.

White, F. J., and R. Y. Wang (1983) Differential effects of classical and atypical antipsychotic drugs on A9 and A10 dopamine neurons. Science *221:* 1054–1057.

Zahniser, N. R., and M. L. Dubocovich (1983) Comparison of dopamine receptor sites labeled by (^{3}H)-*S*-sulpiride and (^{3}H)-spiperone in striatum. J. Pharmacol. Exp. Ther. *227:* 592–599.