THE JOURNAL OF COMPARATIVE NEUROLOGY 262:446-463 (1987)

Comparative Distribution of Dopamine D-1 and D-2 Receptors in the Basal Ganglia of Turtles, Pigeons, Rats, Cats, and Monkeys

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ABSTRACT

The distribution and density of dopamine D-1 and D-2 receptors were studied in the basal ganglia of adult turtles, pigeons, rats, cats, and monkeys. Dopamine receptors were measured *in vitro* by quantitative autoradiography in alternate sections processed for D-1 and D-2 receptor subtypes and compared to adjacent sections stained for acetylcholinesterase (AChE) activity. [^{3}H]-SCH 23390 and [^{3}H]-spiroperidol were used to label the D-1 and D-2 dopamine receptor subtypes, respectively.

The anatomic distribution of both D-1 and D-2 receptors in the basal ganglia was remarkably similar across all species examined. Whereas the absolute number of D-1 and D-2 receptors in the basal ganglia varied between species, the percentage of D-1 and D-2 receptors in a region was quite similar among species.

The pattern of binding to the D-1 and D-2 receptor varied among the different species. The adult turtles, pigeons, and rats demonstrated nonpatchy D-1 and D-2 receptor binding in the striatum and pallidum. The adult cat and monkey caudate nucleus and putamen demonstrated mildly heterogeneous receptor binding in a pattern that differed from that seen with AChE staining, but did occasionally demonstrate similar patterns of the D-1 and D-2 receptor subtypes. The immature cat striatum was characterized by heterogeneous D-1 receptor binding that corresponded to heterogeneous AChE rich patches, whereas D-2 receptor binding was homogeneous.

Heterogeneous binding was seen in other basal ganglia structures including the nucleus accumbens, olfactory tubercle, and substantia nigra pars compacta and reticulata. Complementary D-1 and D-2 receptor binding patterns were seen in the pallidum and substantia nigra of the mammals.

The results of this study indicate that both D-1 and D-2 dopamine receptors are present in the basal ganglia of five different vertebrates. A common feature of dopamine receptors in the basal ganglia is their heterogeneity in distribution and density. The heterogeneity of dopamine receptors has similarities to and differences from the distribution of presynaptic dopamine and other neurotransmitter markers of the basal ganglia.

Key words: ontogeny, acetylcholinesterase staining, striosome

Accepted March 5, 1987.

The basal ganglia are phylogenetically old regions of the central nervous system found in diverse groups of vertebrates,[1] including reptiles, birds, and mammals (Reiner et al., '84; Parent, '86). A major ascending afferent pathway connects the mesencephalic substantia nigra with more rostral telencephalic regions including the basal ganglia. Dopamine, the major neurotransmitter of this pathway, is released in the basal ganglia to interact with two types of dopamine receptors, designated D-1 and D-2.

Interest in the dopaminergic innervation of the basal ganglia has been extensive since the introduction of the monoamine histofluorescence technique in the 1960s (Dalström and Fuxe, '64; Anden et al., '64). This technique allowed the detailed definition of the anatomic distribution of dopamine (DA) inputs into the basal ganglia and other regions of the cerebrum. Monoamine histofluorescence demonstrates a pattern in the striatum of birds and reptiles that is similar to that seen in mammals (Reiner et al., '84). It would not be surprising if the distributions and densities of dopamine receptor subtypes were also similar.

Recent anatomical studies using a variety of techniques have demonstrated that some structures of the basal ganglia are not homogeneous, as was once thought (Graybiel and Ragsdale, '78; Goldman-Rakic, '81). Different neurotransmitter systems have been shown to have a heterogeneous distribution in the striatum of both rats and higher mammals (Herkenham and Pert, '81; Graybiel et al., '81a,b; Gerfen, '84). Dopamine histofluorescence studies have shown that islands of strong fluorescence exist against a background of light fluorescence in the developing brain of mammals (Graybiel, '84). This pattern changes, becoming homogeneous in the adult, but can be pharmacologically changed to a heterogeneous pattern with the use of enzyme inhibitors (Olson et al., '72). The DA islands have been shown to be congruent with markers for the cholinergic system; AChE staining and cholinergic receptor autoradiography (Nastuk and Graybiel, '85). A variety of other neurotransmitter markers have subsequently been studied in relation to the patterns seen for DA and AChE staining. In some cases, there have been very good correspondences, including the opiate system (Graybiel et al., '81a). The pattern is different, however, with markers for substance P and somatostatin, in which there is a variable or poor correspondence to AChE staining (Graybiel et al., '81a). In many of these studies, markers of presynaptic activity, such as a neurotransmitter or a synthesizing enzyme, have been used.

In a few cases, postsynaptic receptors have been studied (Herkenham and Pert, '81; Nastuk and Graybiel, '85). Since mismatches between a neurotransmitter and its receptor have been reported for different systems in various areas of the nervous system (Herkenham and McLean, '86; Kuhar et al., '86), it is important to compare pre- and postsynaptic markers for each neurotransmitter system before the functional significance of a heterogeneity can be known. In the case of the DA system, most receptor studies have focused on the rat brain, where no "patchy" heterogeneity has been found, although a different type of heterogeneity has been reported. Gradients in D-2 receptor binding from rostral to caudal and medial to lateral have been reported in rats, but may be controversial (Joyce et al., '85; Nock et al., '86). In some cases, the receptor gradient does not match dopamine levels (Joyce et al., '85). The relationships of receptor gradients to the "patchy" heterogeneities seen with dopamine histofluorescence and opiate receptors are not known.

The dopamine receptor has been divided into two subtypes, designated D-1 and D-2 (Stoof and Kebabian, '84). Although other dopamine receptor subtypes have been reported, they are thought to represent different affinity states for the D-1 and D-2 receptors. The distribution of the D-2 receptor has been studied in detail in the rat, but few studies have addressed possible binding heterogeneities in higher mammals. The D-1 receptor has not been studied in detail because of the lack of a selective compound for binding studies. The recent introduction of [^{3}H]-SCH 23390 has made it possible to study the autoradiographic distribution of the D-1 receptor (Dawson et al., '85,'86).

In this study, a direct comparison of the density and pattern of D-1 and D-2 receptors is made in the basal ganglia of a variety of vertebrates. The pattern of binding is compared to a well-established marker of striatal compartments, AChE staining. The pattern of receptor binding is also compared in immature and adult cats to study the changes that occur during development. The pattern of dopamine receptor binding is discussed in relation to previously observed patterns of presynaptic dopamine markers in the basal ganglia.

METHODS

Brains were obtained from one adult turtle (*Pseudemys scripta*), one adult white Carneaux pigeon (*Columba livia*), twenty adult male Sprague-Dawley albino rats, and four kittens (*Felis domesticus*) aged postnatal day 0 (P-0) to postnatal day 7 (P-7) by rapid decapitation. Brains were obtained from three adult male cats (*Felis domesticus*) and four adult male cynomolgus monkeys (*Macaca fasicularis*) following an overdose of sodium pentobarbital. Brains from all animals, except the monkeys, were frozen by using crushed dry ice and mounted on tissue pedestals with Lipshaw embedding matrix. Monkey blocks were frozen by slow immersion into liquid isopentane at −80°C. Brains were stored at −70°C until sectioned. Brains were warmed to −10 to −20°C, and coronal sections of 20 or 30 μM were cut on a Lipshaw cryostat microtome and were then thaw-mounted onto gelatin-coated (subbed) slides. The slides were dehydrated on a warming plate at 30°C and then stored at −20°C until used in assays.

Both the D-1 and D-2 receptor assays were carried out in a 25 mM Tris-HCl buffer (pH 7.5) containing 100 mM NaCl, 1 mM $MgCl_2$, 1 μM pargyline, and 0.001% ascorbate. Slides used for receptor autoradiography were warmed to room temperature for 1 hour, then incubated with tritiated ligand at room temperature, given a 1 × 10 minute wash in

[1]Whereas there is still no generally accepted definition of the basal ganglia, most anatomists agree that the caudate nucleus, putamen, and pallidum (medial and lateral globus pallidus) are the major components. For the purpose of this paper, we also include the claustrum, and substantia nigra pars compacta (SNC) and pars reticulata (SNR) as part of the BG. Different names have been applied to the different pallidal segments in mammals. We generally use the designation of medial globus pallidus (MGP) to refer to the entopeduncular nucleus (EP) of the rat and cat, and lateral globus pallidus (LGP) to refer to the globus pallidus (GP) of rat and cat. The terminology for homologous brain regions differs among the various groups of vertebrates described in this paper. In addition, controversy persists whether some regions are homologous between birds, reptiles, and mammals. For our purposes, we follow the homologues discussed by Reiner and Carraway ('87).

buffer at 4°C and dipped in distilled water for 3 seconds. Slides were dried with a stream of cool air. Dried slides were placed in an x-ray cassette with ^{14}C plastic standards previously calibrated with ^{3}H brain paste sections (Penney et al., '81; Pan et al., '83; Penney and Pan, '86) and exposed to LKB Ultrofilm ^{3}H at 4°C for 10 to 21 days. The LKB Ultrofilm ^{3}H was developed in Kodak D19 for 3 minutes at room temperature and fixed in Kodak rapid fix for 3.5 minutes.

The D-1 assay used [^{3}H]-SCH 23390 to label the receptor. Kinetic, competition, and saturation experiments were performed with 20-μM thick sections from the rat striatum. Kinetic studies were performed for the D-1 receptor at a [^{3}H]-SCH 23390 concentration of 0.2 nM, in which the association time varied from 5 to 180 minutes and the dissociation time varied from 15 to 300 minutes by using the method of infinite dilution. Saturation studies were performed with concentrations of [^{3}H]-SCH 23390 from 0.05 to 5.0 nM. Competition and distribution studies were performed at a [^{3}H]-SCH 23390 concentration equal to the K_D(0.57 nM). Specific binding was determined by subtracting the amount bound in the presence of 1 μM cis-flupentixol (nonspecific) from the total amount bound. Competition studies were carried out using conditions described except that varying concentrations of unlabeled drug were included in the assay.

The D-2 receptor assay used [^{3}H]-spiroperidol as previously described (Richfield et al., '86,'87), except for slight modifications. The buffer was changed to that described above and the incubation time was 120 minutes. Specific binding was determined by subtracting the nonspecific binding in the presence of 10 μM dopamine from the total amount bound.

All binding data were determined directly from film densities in regions of interest. Films were analyzed as previously described (Richfield et al., '86; Penney et al., '81). The rat striatum was used to determine the binding parameters for both the D-1 and D-2 assays. Twenty readings from each area were averaged to determine the amount of ^{3}H label bound per mg of protein. In regions where the binding was heterogeneous, measurements were made for the average, high, and low values as determined by visual inspection.

Binding data from Scatchard curves in four rats, and distribution studies in four rats and the four monkeys were analyzed for right-left differences by t-test. If there was no right/left difference, the right and left values were averaged and are reported as a single value. Only one hemisphere from the kittens and cats was used for autoradiography, so right-left comparisons were not made.

Adjacent sections from the kitten, cat, and monkey brains were stained for AChE by the method of Geneser-Jensen and Blackstad ('71). Ethopropazine-HCl (0.2 mM) was added to the incubation medium to block pseudocholinesterase activity. Incubation time for the kittens varied from 60 to 90 minutes, for the adult cat and monkey from 30 to 45 minutes.

Adjacent sections from the monkeys and cats were stained with cresyl violet to verify anatomic areas. The atlases of Powers and Reiner ('80), Karten and Hodos ('67), Paxinos and Watson ('82), Snider and Lee ('81), and Snider and Niemer ('61) were used to identify structures in the brains of turtles, pigeons, rats, cats, and monkeys, respectively.

[^{3}H]-SCH 23390 (specific activity 74 to 85 Ci/mmol) and [^{3}H]-spiroperidol (76 Ci/mmol) were obtained from Amersham (Arlington Heights, IL). Mianserin was obtained from Organon (Oss, The Netherlands), dopamine and apomorphine from Sigma (St. Louis, MO), SKF 38393 from Smith, Kline and French Laboratories (Philadelphia, PA), LY 171555 from Lilly Research Laboratories (Indianapolis, IN), cis-flupentixol from Dr. John Hyttel of H. Lundbeck and Co. (Copenhagen, Denmark) and sulpiride from Ph. Delagrange (Paris, France).

RESULTS

D-1 and D-2 dopamine receptor assays

Kinetic studies were performed by using [^{3}H]-SCH 23390 to determine appropriate incubation and postincubation wash times (Fig. 1). The association rate constant (k_{+1}) was 0.0048 $\pm$ 0.0005 min^{-1} nM^{-1} as determined by pseudo first-order kinetics (Bylund, '80). The dissociation rate constant (k_{-1}) was 0.0022 $\pm$ 0.0001 min^{-1}. The equilibrium dissociation constant (K_D) as determined by the kinetic relationship (k_{-1}/k_{+1}) was 0.46 $\pm$ 0.04 nM, which is in close agreement with the value obtained by Scatchard analysis (0.57 nM). Equilibrium occurred by 150 minutes, and one 10-minute postincubation wash was adequate to provide a high degree of specific binding. Specific binding was 98–99% at a [^{3}H]-SCH 23390 concentration of 0.55 nM in the rat striatum. Specific binding fell to 78% of total binding at a [^{3}H]-SCH 23390 concentration of 5 nM (Fig. 2).

Saturation studies with [^{3}H]-SCH 23390 indicate that binding in the rat striatum was specific and saturable, and that nonspecific binding increased dramatically at concentrations of 2.5 nM and greater. Scatchard analysis produced a K_D value of 0.57 $\pm$ 0.05 nM, a maximal number of binding sites (B_{max}) of 2.85 $\pm$ 0.50 pmol/mg protein and a Hill coefficient (n_H) of 0.95 $\pm$ 0.01 (Fig. 3). There was no difference between right and left striatal values for the K_D or B_{max}.

Abbreviations

ACC	nucleus accumbens
AChE	acetylcholinesterase
ave	average
B_{max}	maximum number of binding sites
CAUD	caudate nucleus
CLAUS	claustrum
DA	dopamine
dor	dorsal
EP	entopeduncular nucleus
GP	globus pallidus
INP	nucleus intrapeduncularis
k_{-1}	dissociation rate constant
k_{+1}	association rate constant
K_D	equilibrium dissociation constant
lat	lateral
LGP	lateral globus pallidus
LPO	lobus parolfactorius
med	medial
MGP	medial globus pallidus
n_H	Hill coefficient
NST	bed nucleus of the stria terminalis
OT	olfactory tubercle
PA	paleostriatum augmentatum
P-0	postnatal day 0
P-7	postnatal day seven
PP	paleostriatum primitivum
PUT	putamen
SN	substantia nigra
SNC	substantia nigra pars compacta
SNR	substantia nigra pars reticulata
STR	striatum
ven	ventral
VP	ventral pallidum
VTA	ventral tegmental area

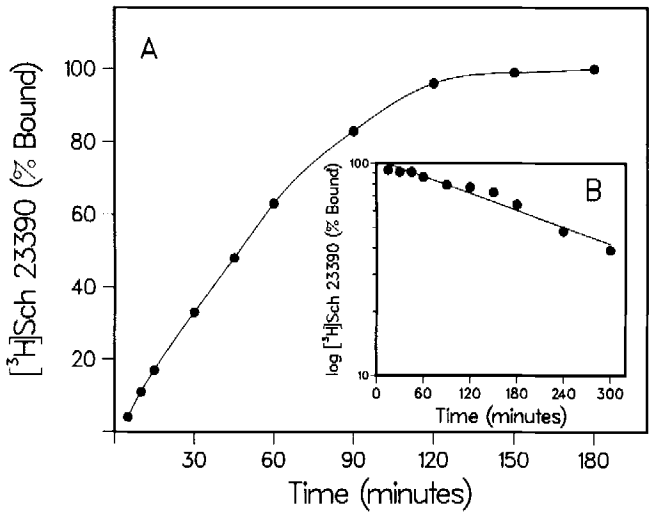

Fig. 1. Kinetic curves of [^{3}H]-SCH 23390 binding in rat striatum. D-1 receptor autoradiography was performed using [^{3}H]-SCH 23390 (0.2 nM) as described in the text. Binding was determined using quantitative autoradiography. Curves are representative of a single experiment and data points represent specific binding. Kinetic constants reported are the mean and standard deviation for three separate experiments. **A.** Association curve. The association rate constant (k_{+1}) is 0.0048 ± 0.0005 min^{-1} nM^{-1} as determined by pseudo first-order kinetics. Equilibrium for other experiments occurs by 150 minutes. **B.** Dissociation curve. The dissociation rate constant (k_{-1}) is 0.0022 ± 0.0001 min^{-1}.

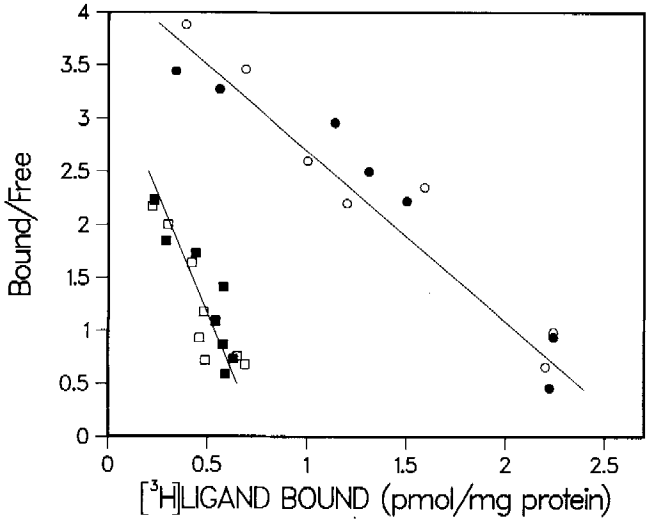

Fig. 3. Scatchard plot of [^{3}H]-SCH 23390 and [^{3}H]-spiroperidol binding in the rat striatum. Saturation experiments were performed using [^{3}H]-SCH 23390 (0.05 to 5.0 nM) to label the D-1 receptor (circles) and [^{3}H]-spiroperidol (0.05 to 2.5 nM) in the presence of 0.1 μM mianserin to label the D-2 receptor (squares) as described in the text. Curves are representative of a single experiment through the midstriatum. Right (open symbols) and left (filled symbols) striata were analyzed independently in four animals. No significant differences between sides were found for the K_D or B_{max} for either receptor subtype. The D-1 receptor has a K_D of 0.57 ± 0.05 nM and B_{max} of 2.85 ± 0.50 pmol/mg protein, whereas the D-2 receptor has a K_D of 0.25 ± 0.04 and B_{max} of 0.86 ± 0.09 pmol/mg protein.

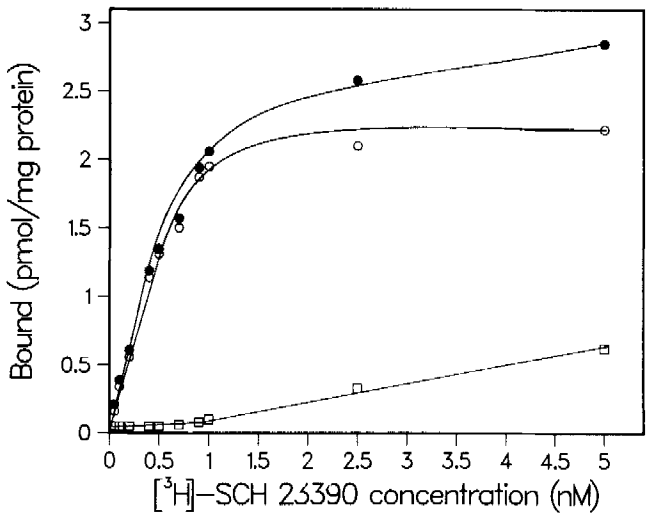

Fig. 2. Saturation curve of [^{3}H]-SCH 23390 binding in rat striatum. D-1 receptor autoradiography was performed using [^{3}H]-SCH 23390 (0.05 to 5.0 nM) as described in the text. Total binding (filled circle) represents binding in the presence of [^{3}H]-SCH 23390 alone, specific binding (open circle) represents the amount of [^{3}H]-SCH 23390 bound with nonspecific binding subtracted, and nonspecific binding (open square) represents binding in the presence of both [^{3}H]-SCH 23390 and 1 μM cis-flupentixol. Nonspecific binding is very low at [^{3}H]-SCH 23390 concentrations below 1 nM, but increased at concentrations greater than 1 nM, suggesting the ligand may bind to an additional site at higher concentrations. All experiments, except for saturation experiments, were performed at concentrations at or below 0.55 nM to ensure binding to only the D-1 receptor.

Competition studies using a variety of D-1 and D-2 selective compounds indicated that binding was selective for the D-1 receptor, in agreement with previous reports (Dawson et al., '86; Billard et al., '84; Hess et al., '86). Inhibitory constants (K_I) and Hill coefficients (n_H) were determined for the following compounds: SKF 38393 37 ± 6 nM and 0.94 ± 0.03, cis-flupentixol 78 ± 55 nM and 0.98 ± 0.06, apomorphine 600 ± 8 nM and 1.09 ± 0.4, mianserin 1850 ± 840 nM and 0.95 ± 0.05, dopamine 1990 ± 310 nM and 0.93 ± 0.19, sulpiride > 100,000 nM and LY 171555 > 100,000 nM.

Scatchard analysis of [^{3}H]-spiroperidol binding revealed a K_D of 0.25 ± 0.04 nM, a B_{max} of 0.86 ± 0.09 pmol/mg protein and a n_H of 1.02 ± 0.02. There were no significant right/left differences in any of these values. The specificity of this assay has been previously documented (Richfield et al., '86a; Altar et al., '85; Neve et al., '84). Specific binding for the D-2 receptor represented 85% of the total binding in the rat striatum at a concentration equal to the K_D.

The total number of dopamine receptors in the midportion of the rat striatum, as determined by adding the B_{max} values for the D-1 and D-2 receptors, was 3.71 pmol/mg protein. The percentages of D-1 and D-2 receptors, were 77% and 23%, respectively. The percentages of receptor subtypes obtained with single concentration incubations at the K_D values was 75% and 25% for D-1 and D-2 receptors in the same region, indicating that accurate percentages of dopamine receptor subtypes can be determined in various regions by using adjacent sections from the same animal.

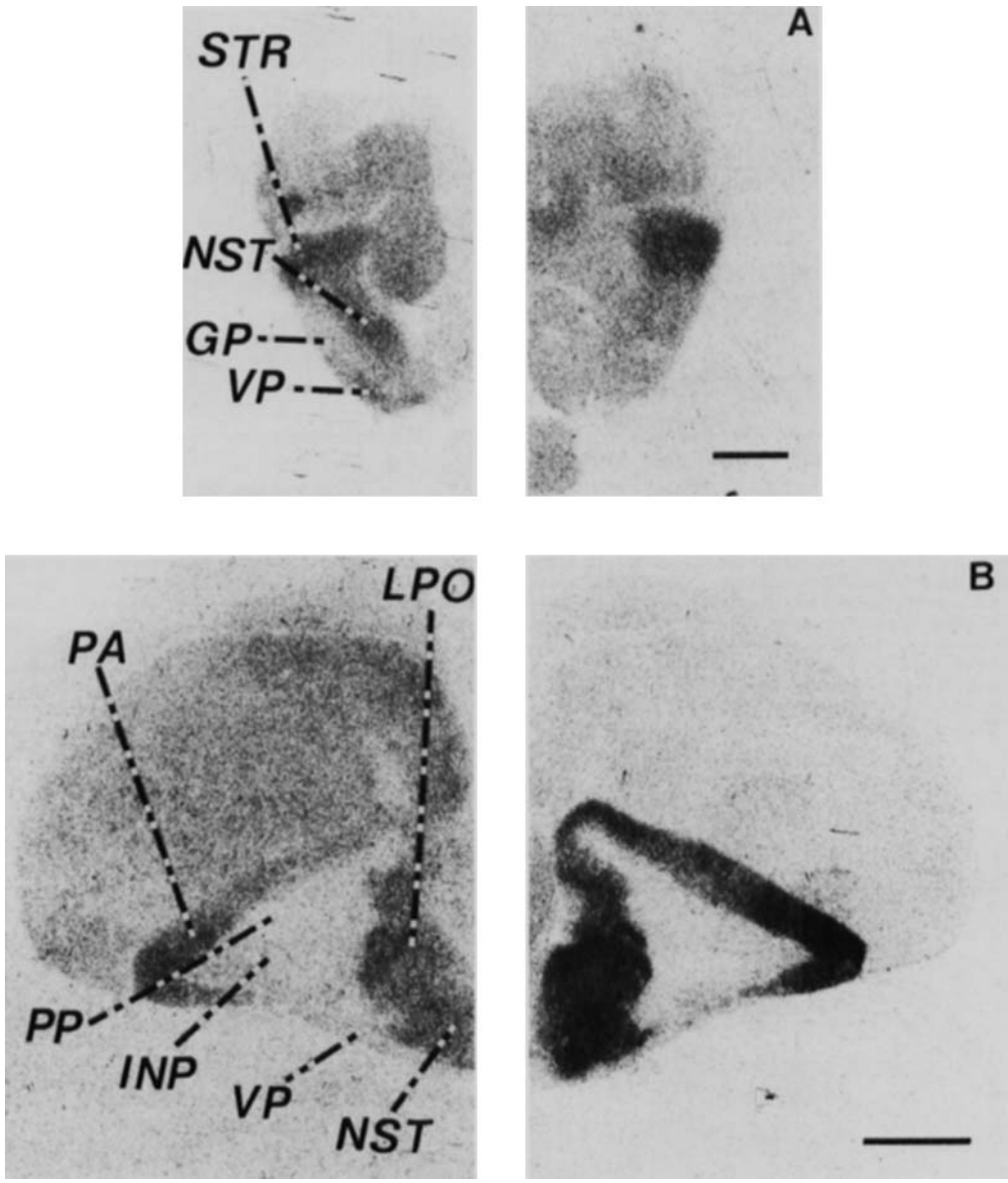

Fig. 4. Dopamine receptor binding in portions of the turtle and pigeon basal ganglia. D-1 and D-2 dopamine receptor autoradiography were performed through regions of the BG as described in the text. Adjacent sections were incubated with tritiated ligand at their respective K_D. Sections processed for D-1 receptor autoradiography were exposed to LKB Ultrofilm-^{3}H for 10 to 14 days; those processed for D-2 receptor autoradiography were exposed for 14 to 21 days. The response of Ultrofilm-^{3}H to tritium is nonlinear (Pan et al., '83). For these reasons, direct comparison of film density or photographic density between receptor subtypes in adjacent sections cannot be made visually. Valid comparisons can be made using quantitative autoradiography as reported in the text. **A.** Turtle dopamine receptor montage. Dopamine receptor autoradiograms through adjacent sections of turtle forebrain (D-1 left, D-2 right). **B.** Pigeon dopamine receptor montage. Dopamine receptor autoradiograms through adjacent sections of pigeon forebrain (D-1 left, D-2 right). Scale bar = 2 mm.

Dopamine receptor distribution in the turtle and pigeon

Dopamine receptors are present in the basal ganglia of both the turtle and pigeon (Fig. 4). D-1 dopamine receptors are present in the turtle striatum (0.19 pmol/mg protein), bed nucleus of the stria terminalis (0.18 pmol/mg protein), globus pallidus (0.08 pmol/mg protein), ventral pallidum (0.06 pmol/mg protein), and substantia nigra (0.16 pmol/mg protein). D-2 dopamine receptors are present in the turtle striatum (0.22 pmol/mg protein), bed nucleus of the stria terminalis (0.03 pmol/mg protein), and globus pallidus (0.05 pmol/mg protein).

The pigeon brain contained D-1 receptors in the paleostriatum augmentum (0.19 pmol/mg protein), lobus parolfactorius (0.20 pmol/mg protein), paleostriatum primitivum (0.01 pmol/mg protein), nucleus intrapeduncularis (0.01 pmol/mg protein), ventral pallidum (0.01 pmol/mg protein), bed nucleus of the stria terminalis (0.21 pmol/mg protein) and nucleus tegmenti pedunculopontis, pars compacta (0.04 pmol/mg protein). D-2 dopamine receptors are also present in the pigeon paleostriatum augmentatum (0.32 pmol/mg

TABLE 1. Summary of Rat Dopamine Receptor Distribution

Region	Receptor subtype: D-1 Amount bound (pmol/mg protein ± SD)[a]	D-1 Amount bound relative to midstriatum (%)[b]	D-1 Amount bound relative to total dopamine receptors (%)[c]	D-2 Amount bound (pmol/mg protein ± SD)[a]	D-2 Amount bound relative to midstriatum (%)[b]	D-2 Amount bound relative to total dopamine receptors (%)[c]
Dorsal striatum						
Rostral	1.56 ± 0.07	102	74	0.56 ± 0.02	108	26
Mid	1.53 ± 0.05	100	75	0.52 ± 0.06	100	25
Caudal	1.36 ± 0.08	89	74	0.48 ± 0.06	92	26
Dorsal	1.25 ± 0.13	82	74	0.43 ± 0.06	83	26
Ventral	1.60 ± 0.01	105	73	0.60 ± 0.07	115	27
Nucleus accumbens						
Rostral	1.63 ± 0.05	107	82	0.36 ± 0.01	69	18
Caudal	0.90 ± 0.08	59	76	0.29 ± 0.02	56	24
Olfactory tubercle						
Rostral	1.58 ± 0.06	103	72	0.60 ± 0.06	107	28
Caudal	1.64 ± 0.08	107	78	0.46 ± 0.09	82	22
Globus pallidus	0.21 ± 0.04	14	84	0.04 ± 0.01	8	16
Entopeduncular nucleus	0.56 ± 0.09	37	100	ND	0	0
Ventral pallidum	0.65 ± 0.17	42	87	0.10 ± 0.03	19	13
Substantia nigra-compacta						
Average	1.51 ± 0.10	99	92	0.14 ± 0.03	27	8
Medial	1.68 ± 0.11	110	90	0.19 ± 0.05	37	10
Lateral	1.26 ± 0.18	82	94	0.08 ± 0.03	15	6
Substantia nigra-reticulata						
Average	1.40 ± 0.13	92	93	0.10 ± 0.02	19	7
Medial	1.67 ± 0.20	109	92	0.14 ± 0.03	27	8
Lateral	1.19 ± 0.12	78	94	0.07 ± 0.03	13	6
Claustrum	0.40 ± 0.09	26	100	ND	0	0

[a]Values represent the average specific binding from four animals ± standard deviation. Sections were incubated at concentrations of tritiated ligand equal to their respective K_D.
[b]The amount bound relative to midstriatum is the amount of receptor binding in a region expressed as a percentage relative to the amount present in the midstriatum.
[c]The amount bound relative to the total number of dopamine receptors is the amount of the dopamine receptor subtype present in a particular region expressed as a percentage of the total number of D-1 and D-2 receptors present in that region.
ND = none detected.

protein), lobus parolfactorius (0.33 pmol/mg protein), paleostriatum primitivum (0.02 pmol/mg protein), nucleus intrapeduncularis (0.02 pmol/mg protein), ventral pallidum (0.02 pmol/mg protein), bed nucleus of the stria terminalis (0.42 pmol/mg protein) and nucleus tegmenti pedunculopartis, pars compacta (0.04 pmol/mg protein). The lateral portion of the paleostriatum augmentatum (PA) contained more D-1 and D-2 receptors than the medial portion. No "patchy" heterogeneities were observed.

Dopamine receptor distribution in rats

Dopamine receptors are present in all basal ganglia nuclei of the rat (Table 1). Neither the D-1 nor D-2 receptor demonstrated any "patchy" heterogeneities in the dorsal striatum (Fig. 5). The n. accumbens did have areas of higher and lower binding compared to the average for both the D-1 and D-2 receptor, whereas the olfactory tubercle had clear discontinuities in D-2 receptor binding. Both the D-1 and D-2 receptors did demonstrate two other types of heterogeneities, gradients and districts. The striatum had a decreasing receptor gradient from rostral to caudal on the order of 13–14% for both subtypes. The caudal striatum had an increasing gradient from dorsal to ventral on the order of 22 to 28%. The rostral and midstriatum did not have dorsal to ventral gradients. No medial to lateral gradient was seen at any level for either receptor subtype. There was a district of slightly increased binding in the dorsomedial portion of the striatum for both the D-1 and D-2 receptor, which appeared to be distinct from the remainder of the striatum along its rostral to caudal extent. This area, along with the ventral portion of the caudal striatum, were the only regions of the striatum devoid of traversing white matter bundles. A decreasing rostral to caudal gradient was also seen in two structures that had no significant white matter tracts coursing through them, the nucleus accumbens and olfactory tubercle.

Districts of heterogenous binding were prominent in the endopenduncular nucleus (EP). The EP had increased binding along the margins and in the medial portion of the nucleus for the D-1 receptor. The SNR and SNC had increased receptor binding in the medial portion of the nucleus for both subtypes. The claustrum had a homogeneous pattern of D-1 receptor binding, without detectable D-2 receptor binding. The density of the D-1 receptor was quite similar among BG structures except for the pallidum and claustrum, which had 14 to 40% of the number of receptors compared to the midstriatal density. The D-2 receptor density varied more dramatically among basal ganglia structures; varying from undetectable in EP and claustrum, to 20 to 27% in SN, and 8–20% in globus pallidus (GP) and ventral pallidum (VP).

Dopamine receptor distribution in neonatal and adult cats

Dopamine receptors were present in the basal ganglia of neonatal and adult cats as previously reported (Richfield et

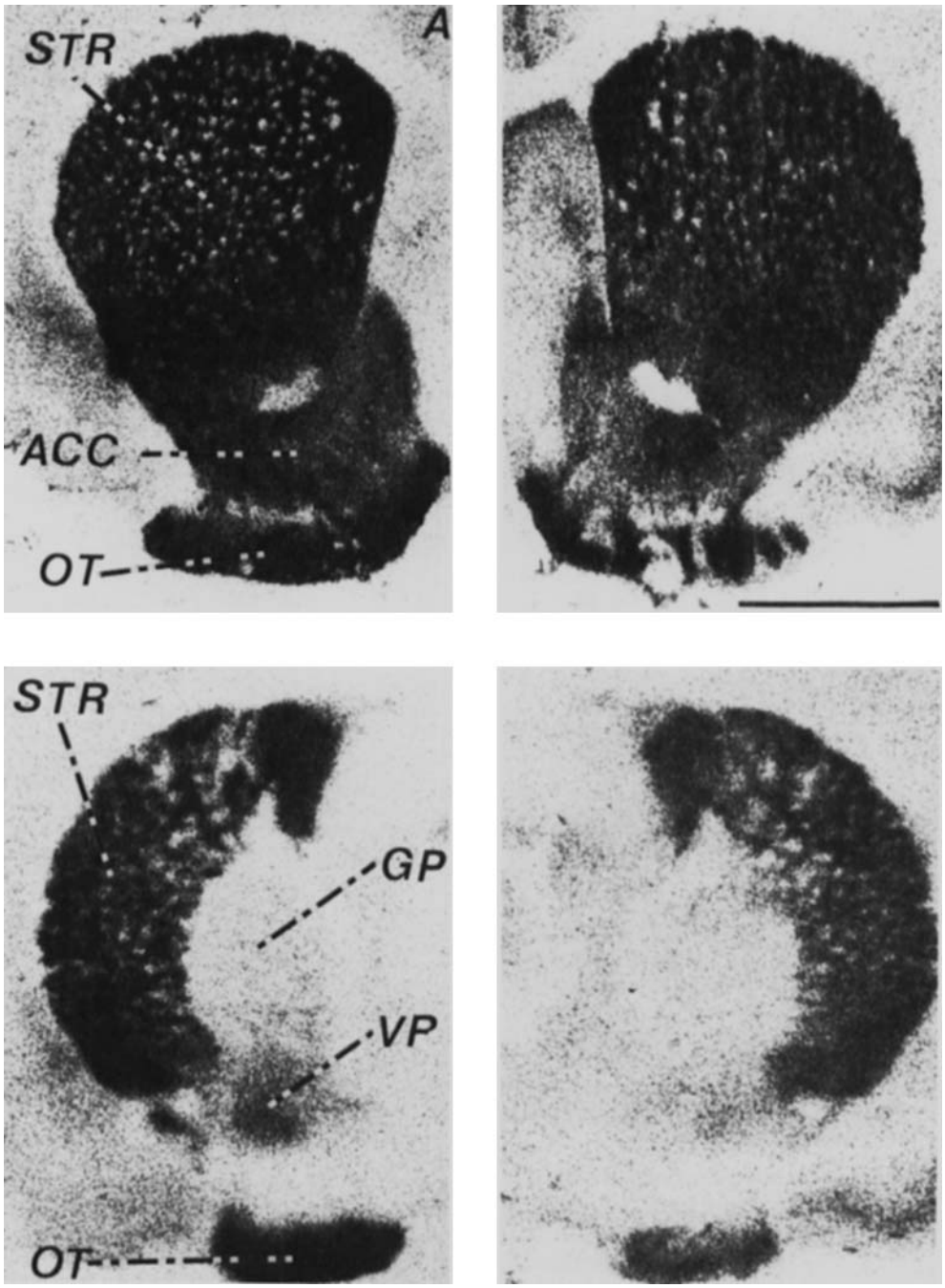

Fig. 5. Dopamine receptor montage in rat BG. Adjacent sections through various regions of the rat BG were processed for dopamine receptor autoradiography as described in the text and Figure 4. Receptor densities are reported in Table 1. **A.** Sections through striatum and pallidum. Dopamine receptor densities (D-1 left, D-2 right) are present in the striatum and pallidum of the rat. **B.** (On facing page.) Sections through entopeduncular nucleus and substantia nigra. Dopamine receptors (D-1 left, D-2 right) are heterogeneous in these structures. The entopeduncular nucleus contains only D-1 receptors, which are more prominent around the periphery and medial portions of the nucleus. The substantia nigra contains both D-1 and D-2 receptors in the pars reticulata and compacta with decreasing gradients from medial to lateral. Scale bar = 2 mm.

al., '87). The number of D-1 and D-2 receptors was less than that seen in the rat (Table 2). The pattern of receptor distribution within the caudate, putamen, and nucleus accumbens was studied in detail in relationship to the pattern of AChE staining in both neonatal (Fig. 6) and adult cats (Fig. 7). The D-1 receptor was very heterogeneous in the P-0 kitten, becoming more homogeneous with maturity at P-7 and in the adult. The areas of increased D-1 receptor density in neonatal cats were seen on a diffuse background of lesser D-1 receptor binding. Areas of increased D-1 receptor density frequently corresponded to patches of increased AChE staining, having the same size, shape, contour, and

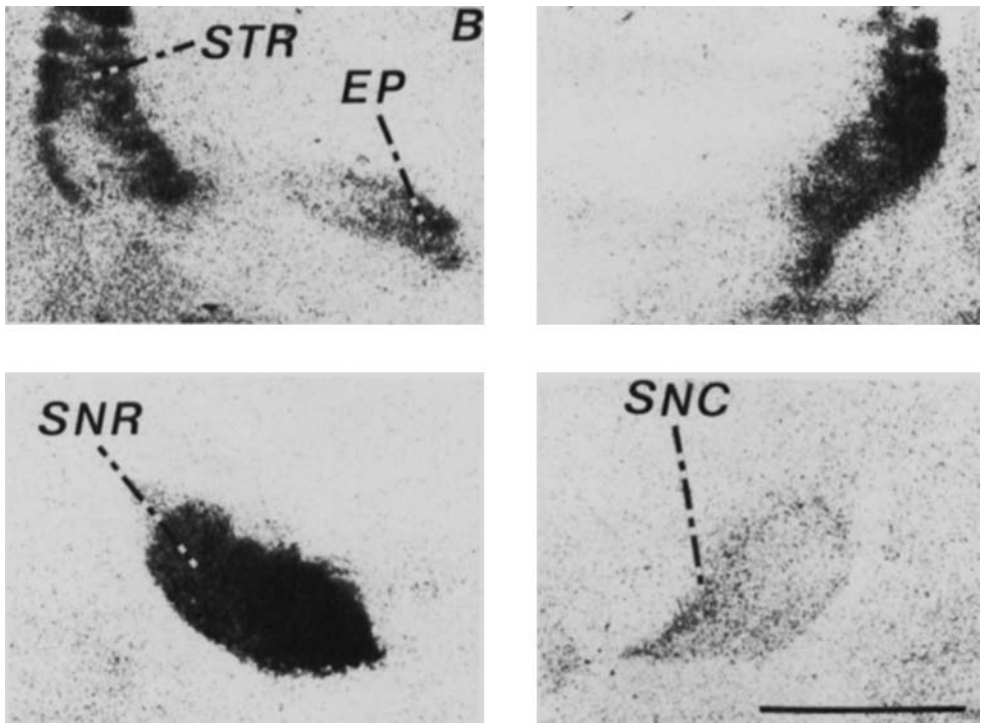

Figure 5 continued

distribution. The areas of increased D-1 receptor binding were seen most frequently in the caudate nuclei, but were also seen in the putamen, nucleus accumbens, and olfactory tubercle. The pattern of D-2 receptor binding was remarkable for its local homogeneity in all BG structures of the neonatal kitten, becoming mildly heterogeneous in the adult BG. There was a decreasing gradient from dorsal to ventral for both the D-1 and D-2 receptor density in the caudate nucleus of the P-0 kitten, which decreased at age P-7 and disappeared in adults. No medial to lateral gradient for either receptor subtype was seen at any age. This was in contrast to the pattern seen with AChE staining in which there were decreasing gradients from lateral to medial and ventral to dorsal in the P-0 kitten, which was less marked in the P-7 kitten and absent in the adult cat.

The adult cat basal ganglia displayed a different appearance in receptor binding. Both the D-1 and D-2 receptor were more homogeneous when compared to the marked heterogeneity seen with AChE staining. However, local variations in the number of both D-1 and D-2 receptors on the order of 10–20% above or below the average binding were seen. The areas of variable density rarely corresponded to areas of low AChE staining (striosome). The areas of increased receptor binding had variable sizes from 0.8 to 2.0 mm in length, variable shapes from linear to oval or round, and irregular borders. In some sections, the areas of increased binding appeared to stretch from the medial to lateral aspect of the caudate nucleus. This appearance was different from the appearance of the striosomes, which were often tubular, linear, or angled, 0.3 to 0.5 mm in length, and had sharp contours. The striosomes were most prominent in areas of lower homogeneous receptor binding. Conversely, the areas of increased receptor binding were more prominent in the matrix or area with homogeneous AChE staining. The areas of increased receptor binding could be traced on serial sections for up to 140 to 200 μM. On many occasions both D-1 and D-2 receptor binding were increased in the same pattern in adjacent sections. In other areas only one of the receptor subtypes was increased. Attempts were made to avoid artifactually finding heterogeneous binding by comparing serially adjacent sections (Fig. 8). Most autoradiographic sections were run in duplicate or triplicate and readings were made in areas verified on adjacent sections when possible. Areas of heterogeneity were also present in the putamen, nucleus accumbens, and olfactory tubercle.

The globus pallidus had a reticulated or lacelike pattern of binding for the D-2 receptor, with no detectable D-1 receptor binding. In the EP and SNR, D-1 receptor binding was high, whereas D-2 receptor binding (Fig. 9) was absent. The SNC contained both D-1 and D-2 receptors. The substantia nigra had a decreasing receptor gradient from medial to lateral for both D-1 and D-2 receptors. A district of increased D-1 and D-2 receptor binding was seen in the dorsomedial aspect of the nucleus accumbens.

Dopamine receptor distribution in monkey

Dopamine receptors were present in all nuclei of the adult monkey basal ganglia (Fig. 10), but in amounts less than for the rat and cat (Table 3). There were no right/left differences in the number of receptors for any of the structures examined. There was no medial to lateral or dorsal to ventral gradient for either receptor subtype in the caudate nuclei or putamen. There was a rostral to caudal gradient for both receptor subtypes, which was greater for the D-1 receptor. The D-1 receptor decreased by 9% in the body and 17% in the tail of the caudate nuclei, compared to the head of the nucleus, whereas the caudal portion of the putamen decreased by 29% when compared to the rostral putamen. The D-2 receptor, however, increased by 15% in the body

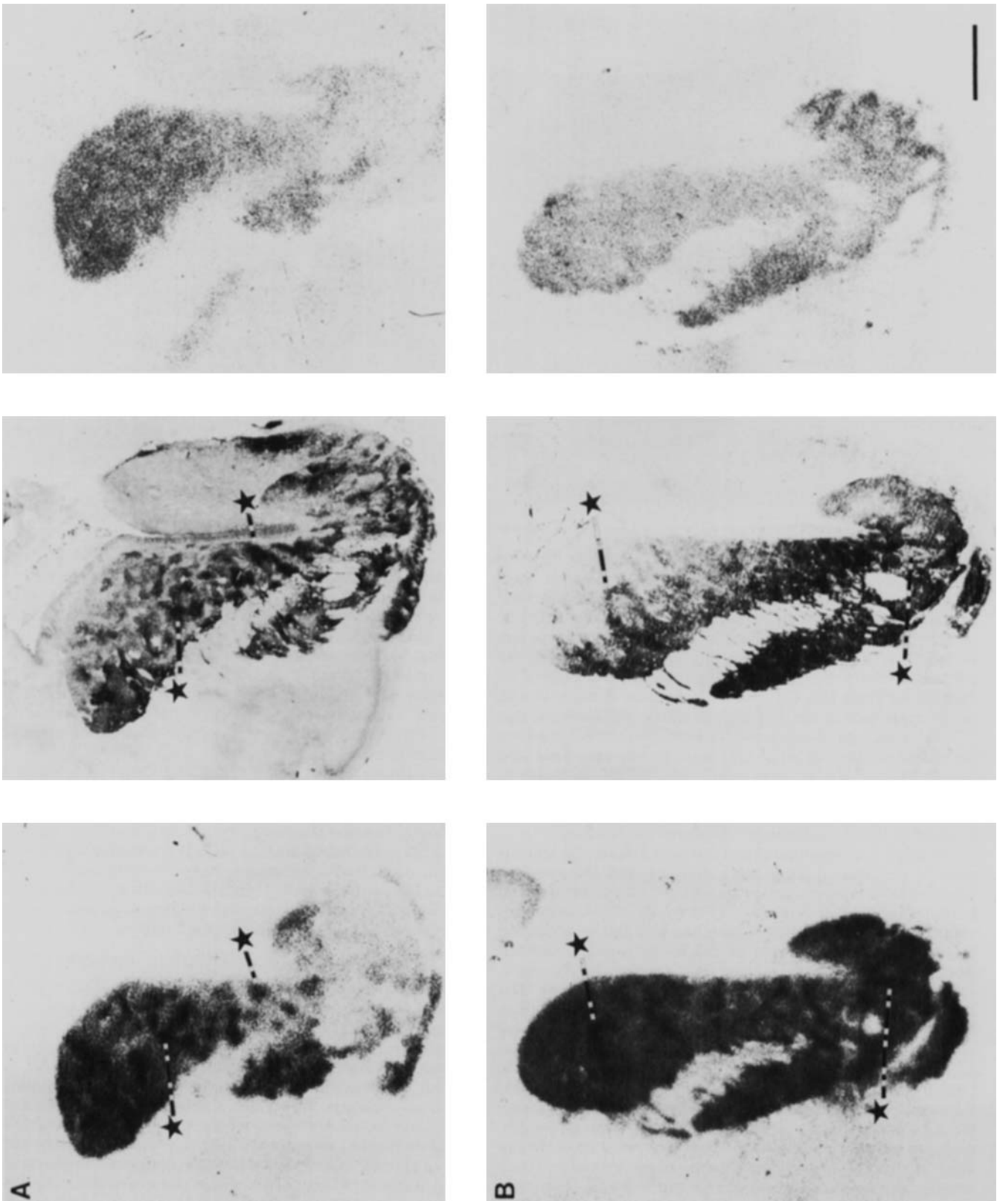
A
B

TABLE 2. Summary of Adult Cat Dopamine Receptor Distribution

	Receptor subtypes					
	D-1			D-2		
	Amount bound (pmol/mg protein ± SD)[a]	Amount bound relative to rostral caudate (%)[b]	Amount bound relative to total dopamine receptors (%)[c]	Amount bound (pmol/mg) protein ± SD)[a]	Amount bound relative to rostral caudate (%)[b]	Amount bound relative to total dopamine receptors (%)[c]
Caudate (rostral)						
Average[d]	1.26 ± 0.13	100	72	0.50 ± 0.05	100	28
High[e]	1.39 ± 0.17	110	68	0.64 ± 0.13	128	32
Low[e]	1.06 ± 0.21	84	76	0.33 ± 0.03	66	24
Caudate (caudal)	0.98 ± 0.11	78	68	0.47 ± 0.09	94	32
Putamen (rostral)						
Average	1.04 ± 0.17	83	66	0.53 ± 0.07	106	34
High	1.21 ± 0.15	96	68	0.57 ± 0.08	114	32
Low	0.89 ± 0.11	71	71	0.36 ± 0.02	72	29
Putamen (caudal)	0.68 ± 0.34	54	68	0.32 ± 0.04	64	32
Nucleus accumbens (rostral)						
Average	0.71 ± 0.29	56	62	0.43 ± 0.10	86	38
High	1.00 ± 0.12	79	64	0.57 ± 0.07	114	36
Low	0.67 ± 0.13	53	66	0.35 ± 0.02	70	34
Nucleus accumbens (caudal)	0.57 ± 0.16	45	68	0.27 ± 0.02	54	32
Olfactory tubercle						
Average	0.84 ± 0.12	67	72	0.32 ± 0.10	64	28
High	1.06 ± 0.15	84	73	0.40 ± 0.08	80	27
Low	0.63 ± 0.04	50	78	0.18 ± 0.09	36	22
Globus pallidus	ND	0	0	0.22 ± 0.01	44	100
Entopenduncular nucleus	0.82 ± 0.05	65	100	ND	0	0
Substantia nigra-compacta						
Average	0.72 ± 0.01	57	81	0.17 ± 0.01	34	19
Medial	0.81 ± 0.10	64	79	0.21 ± 0.02	42	21
Lateral	0.45 ± 0.23	36	87	0.07 ± 0.04	14	13
Substantia nigra-reticulata						
Average	0.72 ± 0.08	57	100	ND	0	0
Medial	0.80 ± 0.08	63	100	ND	0	0
Lateral	0.62 ± 0.24	49	100	ND	0	0
Claustrum	0.30 ± 0.04	24	86	0.05 ± 0.02	10	14

[a]Values represent the average of measures from four animals ± standard deviation. Sections were incubated at concentrations of ligand equal to their respective K_D.
[b]The amount bound relative to the head of the caudate nucleus is the amount of receptor binding present in a region expressed as a percentage relative to the average amount present in the head of the caudate.
[c]The amount bound relative to the total number of dopamine receptors is the amount of dopamine receptor subtype present in a particular region expressed as a percentage of the total number of D-1 and D-2 receptors present in that region.
[d]The average amount of binding present in a region represents the amount of binding present ignoring any heterogeneity of binding.
[e]The high and low amounts of binding represent the amount of binding present in a region visually selected for areas of increased or decreased binding relative to the average amount present.
ND = none detected.

and tail of the caudate compared to the head and the caudal putamen increased by 8% compared to the rostral putamen. The head of the caudate, rostral putamen, and n. accumbens displayed minimal amounts of heterogeneous binding. The pattern of binding for both the D-1 and D-2 receptors did not match the "patchy" heterogeneity seen with AChE staining. Most areas of increased receptor density appeared to be present in the matrix region as seen by AChE staining. Areas of increased or decreased binding were on the order of 10–20% and were of variable size and shape. The caudal portions of the caudate nucleus and putamen were much more homogeneous than the rostral portions.

The pallidum demonstrated dopamine receptor binding in a pattern similar to that of the rat and cat. Medial GP (EP in the rat and cat) had a moderate number of D-1 receptors in a pattern of higher binding around the periphery, but lower amounts in the central portion of the nucleus and no detectable D-2 receptors. Lateral GP (GP in the rat and cat) had nearly equivalent but low numbers of D-1 and D-2 receptors without any heterogeneity to the binding. The substantia nigra pars compacta contained only D-2 receptors, whereas the pars reticulata contained only D-1 receptors in decreasing gradients from both medial to lat-

Fig. 6. Dopamine receptor autoradiography and AChE staining in kitten BG. Dopamine receptor autoradiography was performed in adjacent sections in P-0 and P-7 kittens as described in the text and Figure 4. Adjacent sections were also processed for AChE staining. **A.** Sections through portions of the BG of a P-0 kitten. D-1 dopamine receptors (left) are quite heterogeneous with areas of increased receptor density matching areas of increased AChE staining (middle). The D-2 receptor distribution is quite homogeneous (left). Dopamine receptor gradients are present, with higher levels present dorsally. AChE gradients are also present, with higher levels laterally. **B.** Sections through portions of BG of a P-7 kitten. A similar pattern of binding and staining is seen in the P-7 kitten, except that the gradients are decreased, and the intensity of the AChE staining in the matrix is increased. Sections are in the same order as in A. Scale bar = 2 mm. Asterisks indicate areas of AChE rich patches and increased D-1 receptor binding.

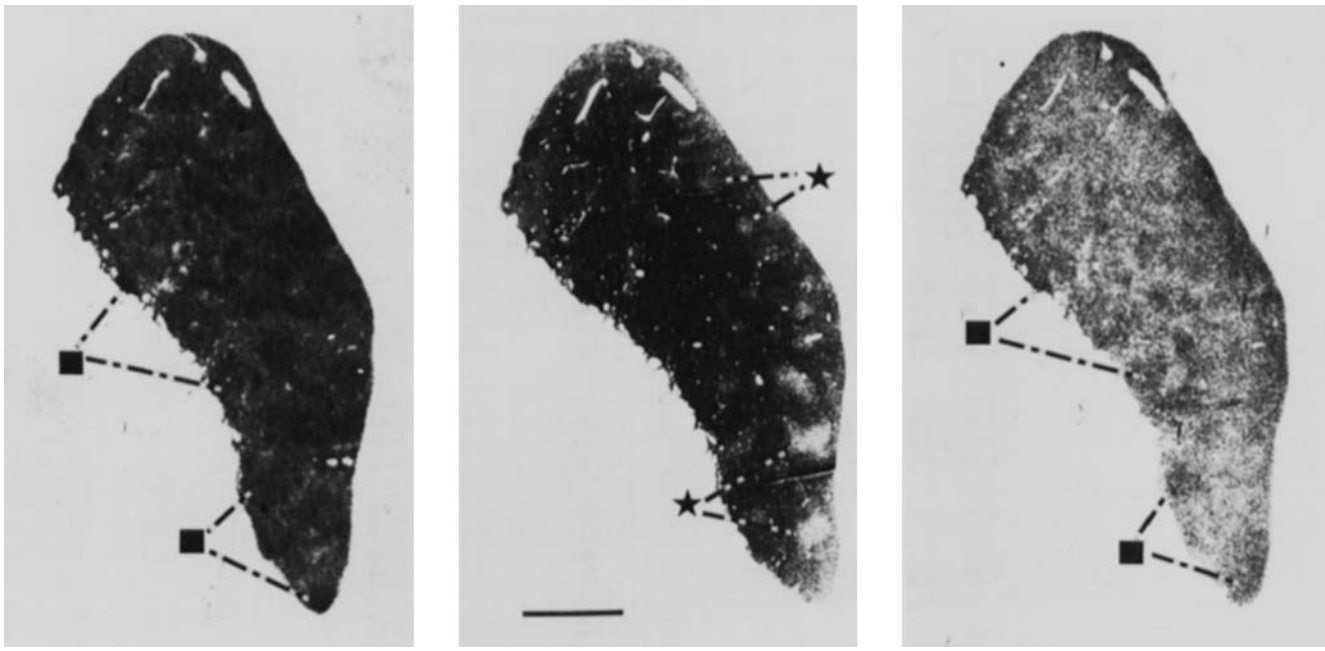

Fig. 7. Dopamine receptor autoradiography and AChE staining in the cat caudate nucleus. Dopamine receptor autoradiography was performed in adjacent sections as described in the text and Figure 4. Adjacent sections were processed for AChE staining. The distributions of both D-1 (left) and D-2 (right) receptors are heterogeneous in fashions that are different from that seen with AChE staining (middle). Some areas of striatum have increased levels of both D-1 and D-2 receptors; some areas are increased in only one subtype. The AChE staining reveals several striosomes that have no clear counterpart in the pattern of receptor binding. Scale bar = 2 mm. Asterisks indicate striosomes with low AChE staining, and filled squares indicate areas of increased D-1 and D-2 receptor binding.

eral and ventral to dorsal. The SNR D-1 receptors also decreased from ventral to dorsal.

DISCUSSION

Phylogenetic conservation of dopamine receptors

The conservation of neuronal pathways and neurotransmitters among amniotes has been established (Reiner et al., '84). In this study, we found that the dorsal striatum, consisting of caudate nucleus and putamen, contained the greatest number of both D-1 and D-2 receptors in the mammals. The homologues of the dorsal striatum in the turtle (PA) and pigeon (PP and PA) also contained measurable amounts of D-1 and D-2 dopamine receptors. There were lesser amounts in the paleostriatum (globus pallidus) of all vertebrates examined. The ventral striatum (nucleus accumbens and olfactory tubercle) and claustrum also contained measurable amounts of D-1 and D-2 receptors in the mammals. In mammals, there were twice as many D-1 receptors as D-2 receptors in the striatum, whereas D-2 receptors exceeded D-1 receptors in the turtle and pigeon striatal homologues. This difference in density of dopamine receptor subtypes suggests that in different species these structures may react differently to dopamine. The mammals also have a high percentage of D-1 receptors in the claustrum and substantia nigra pars reticulata. Although asymmetries in D-2 receptor binding in the striatum of male and female rats have been reported (Drew et al., '86), in this study no asymmetries were found in any structures examined in the rat or monkey for either receptor subtype.

Mammalian differences in dopamine receptors

There were differences in the distribution and density of both D-1 and D-2 receptors among the mammals studied. The density of D-1 and D-2 receptors was highest in the rat, followed by the cat, then the monkey. When various basal ganglia structures were compared among the mammals, the cat contained from 45 to 86% and the monkey contained from 32 to 63% of the total number of dopamine receptors compared to the rat. This rank order for species was true for all structures of the basal ganglia examined. This pattern of decreasing total number of dopamine receptors among higher mammals is similar to the pattern of decreasing cerebral metabolic rates also seen with higher mammals (Sokoloff, '84). One may speculate as to whether the increased receptor numbers result in a higher metabolic demand on a neuron.

The mammals displayed differing densities of D-1 and D-2 receptors in the paleostriatum and substantia nigra. The MGP contained moderate amounts of the D-1 receptor, varying from 24 to 65% of the number seen in the caudate nucleus without detectable D-2 receptor binding. The LGP contained far fewer numbers of D-1 receptors varying from none to 14% of the number seen in the caudate nucleus. However, low amounts of D-2 receptors were also present in the LGP. Thus, the pallidal subdivisions may process DA inputs differently. The SN also differed among the mammals in the distribution of dopamine receptors. The SNC of all mammals contained D-2 receptors, but only the rat and cat also had D-1 receptors in the SNC. The SNR of all mammals contained D-1 receptors, but only the rat also contained D-2 receptors in the SNR. These results suggest that mammals differ in their processing of information in these portions of the basal ganglia.

Heterogeneities in dopamine receptor binding

The mammals also differed in the local distribution of dopamine receptors within a region. Three types of heterogeneities were observed in the pattern of dopamine receptor

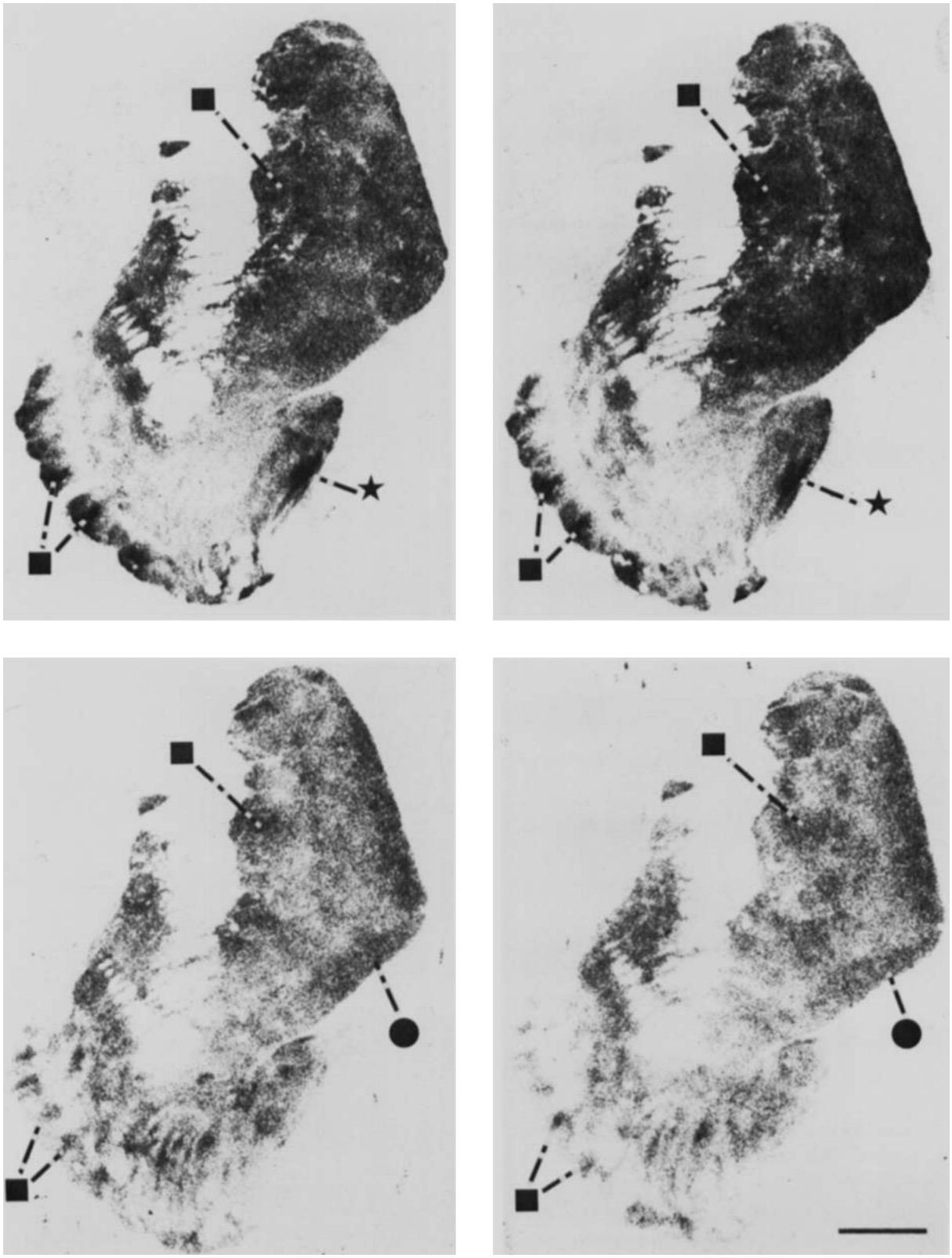

Fig. 8. Dopamine receptor autoradiography in adjacent sections of the cat brain. Four adjacent sections were processed for either D-1 (upper) or D-2 (lower) dopamine receptor autoradiography. Matching of areas with increased D-1 or D-2 receptors can be seen on adjacent sections processed for the same receptor subtype. In addition, some areas with increased amounts of both D-1 and D-2 receptors can be seen to match. There are also some areas where increased receptor subtypes do not match. Areas of heterogeneity can be seen in the caudate nucleus, putamen, nucleus accumbens, and olfactory tubercle. Scale bar = 2 mm. Filled squares indicate areas of increased D-1 and D-2 receptor binding in adjacent sections. Asterisk indicates an area with increased D-1, but not D-2 receptor binding. Filled circle indicates an area of increased D-2, but not D-1 receptor binding.

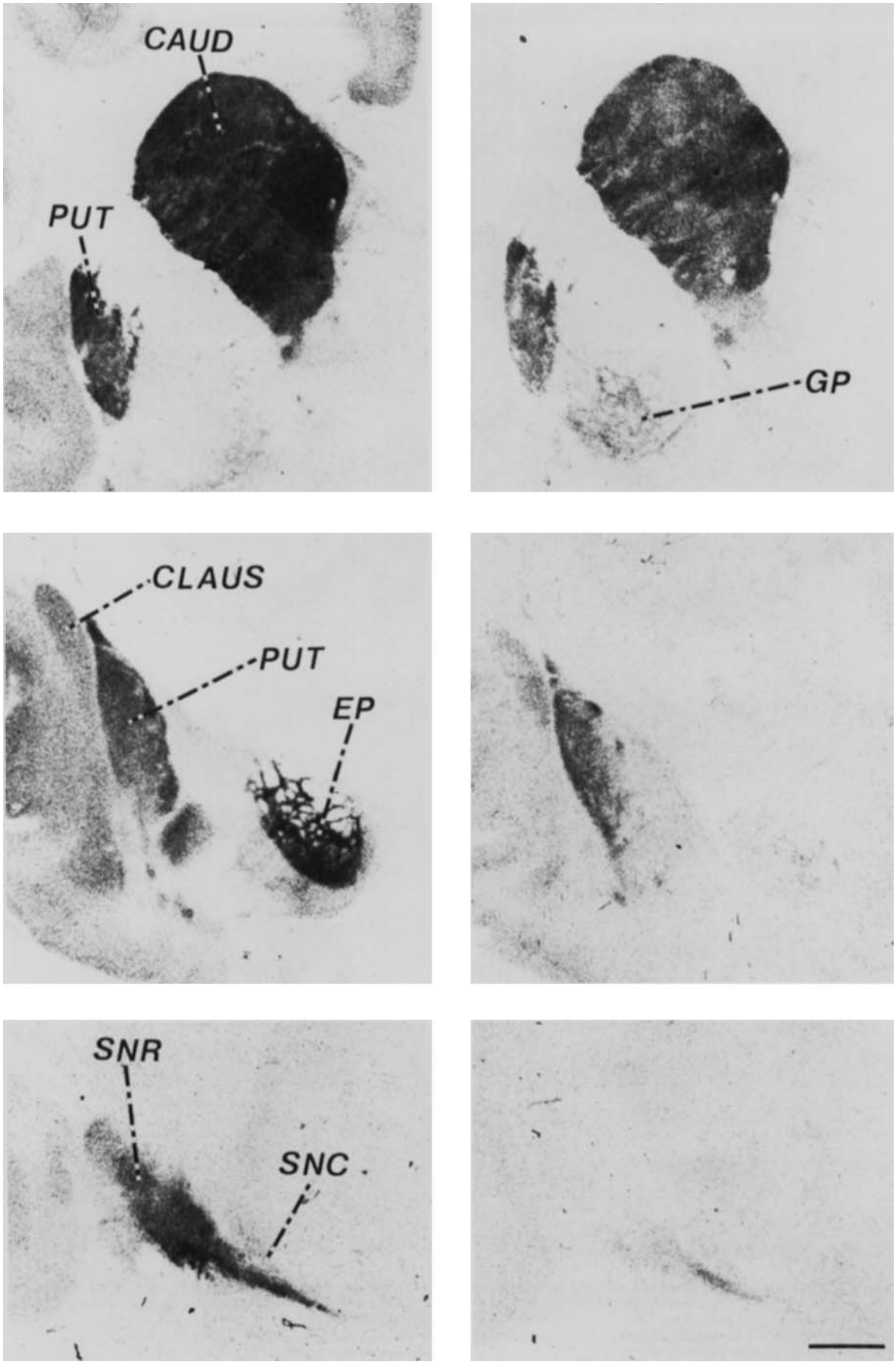

Figure 9

TABLE 3. Summary of Monkey Dopamine Receptor Distribution

	Receptor subtypes					
	D-1			D-2		
	Amount bound (pmol/mg protein ± SD)[a]	Amount bound relative to midstriatum (%)[b]	Amount bound relative to total dopamine receptors (%)[c]	Amount bound (pmol/mg) protein ± SD)[a]	Amount bound relative to head of caudate (%)[b]	Amount bound relative to total dopamine receptors (%)[c]
Caudate (head)						
Average[d]	0.98 ± 0.06	100	72	0.39 ± 0.07	100	18
High[e]	1.12 ± 0.06	114	65	0.60 ± 0.17	154	35
Low[e]	0.76 ± 0.07	78	70	0.32 ± 0.06	82	30
Caudate (body)	0.89 ± 0.09	92	66	0.45 ± 0.07	115	34
Caudate (tail)	0.81 ± 0.10	83	64	0.45 ± 0.11	115	36
Putamen (rostral)						
Average	0.90 ± 0.09	92	71	0.36 ± 0.02	92	29
High	0.96 ± 0.08	98	63	0.56 ± 0.13	114	37
Low	0.73 ± 0.10	74	70	0.32 ± 0.03	82	30
Putamen (caudal)	0.64 ± 0.04	65	62	0.39 ± 0.04	100	38
Nucleus accumbens						
Average	0.58 ± 0.04	59	74	0.20 ± 0.04	51	26
High	0.65 + 0.04	66	63	0.38 ± 0.03	97	37
Low	0.52 ± 0.02	53	78	0.15 ± 0.03	38	22
Lat. globus pallidus	0.04 ± 0.01	4	57	0.03 + 0.01	8	43
Med. globus pallidus	0.24 ± 0.01	24	100	ND	0	0
Substantia nigra-compacta						
Average	ND	0	0	0.15 ± 0.05	38	100
Substantia nigra-reticulata						
Average	0.44 ± 0.07	45	100	ND	0	0
Medial	0.51 ± 0.06	52	100	ND	0	0
Lateral	0.35 ± 0.03	36	100	ND	0	0
Ventral	0.53 ± 0.04	54	100	ND	0	0
Dorsal	0.39 ± 0.02	40	100	ND	0	0
Claustrum	0.19 ± 0.06	19	83	0.04 ± 0.03	10	17

[a]Values represent the average of measures from four animals ± standard deviation. Sections were incubated at concentrations of ligand equal to their respective K_D.
[b]The amount bound relative to the head of the caudate nucleus is the amount of receptor binding present in a region expressed as a percentage relative to the average amount present in the head of the caudate.
[c]The amount bound relative to the total number of dopamine receptors is the amount of dopamine receptor subtype present in a particular region expressed as a percentage of the total number of D-1 and D-2 receptors present in that region.
[d]The average amount of binding present in a region represents the amount of binding present ignoring any heterogeneity of binding.
[e]The high and low amounts of binding represent the amount of binding present in a region visually selected for areas of increased or decreased binding relative to the average amount present.
ND = none detected.

binding in the basal ganglia of mammals. Gradients, defined as an increase or decrease in receptor density or marker intensity following one axis, have been described for various basal ganglia markers in the past (Bockaert et al., '76; Tassin et al., '76; Versteeg et al., '76). Patches are scattered areas of increased or decreased marker within a region or background (matrix) of homogeneous marker. These have also been well described in mammalian basal ganglia (Graybiel, '81a,b; Graybiel et al., '84; Gerfen, '85,'86). A district is defined as a portion of a region that has a unique pattern of binding or labeling that distinguishes it from the remainder of the region, but may not be distinguishable on routine histologic staining. These have also been described in portions of the basal ganglia (Graybiel et al., '81a). The types of dopamine receptor heterogeneities varied among the mammals examined, regions of the basal ganglia, and receptor subtype.

Considerable interest has been generated regarding the patchy heterogeneities seen in mammals, especially cats, monkeys, and humans. Patches are seen in the prenatal brain that persist in adulthood, but may change in their neurochemical attributes. This type of heterogeneity is classically detected by using AChE staining, but has been noted with other markers (Graybiel and Ragsdale, '78). Dopamine also demonstrates heterogeneity in prenatal brains where it is localized to "islands" or patches of increased amounts surrounded by a matrix or background of lesser amounts (Olson et al., '72; Graybiel, '84). The matrix area increases in dopamine content with maturity until striatal dopamine becomes homogeneous in adulthood. The distribution patterns of the D-1 and D-2 receptors in adults were distinctly different from this pattern and from each other. Whereas the D-1 receptor had patches of increased receptor binding that corresponded to patches of increased AChE staining during development, the changes in the receptor with maturity differed from those of AChE staining and dopamine markers. In the dorsal striatum, AChE staining and dopamine histofluorescence in the matrix increased with maturation; the D-1 receptor density de-

Fig. 9. Dopamine receptor autoradiography in portions of the cat BG. Dopamine receptor autoradiography was performed in regions of the cat BG, including globus pallidus (upper), entopeduncular nucleus (middle), and substantia nigra (lower). Dopamine D-1 receptors (left) are present in the EP, SNC, and SNR; D-2 receptors are present in GP and SNC. The SN has a decreasing medial to lateral gradient as is seen in the rat. Scale bar = 2 mm.

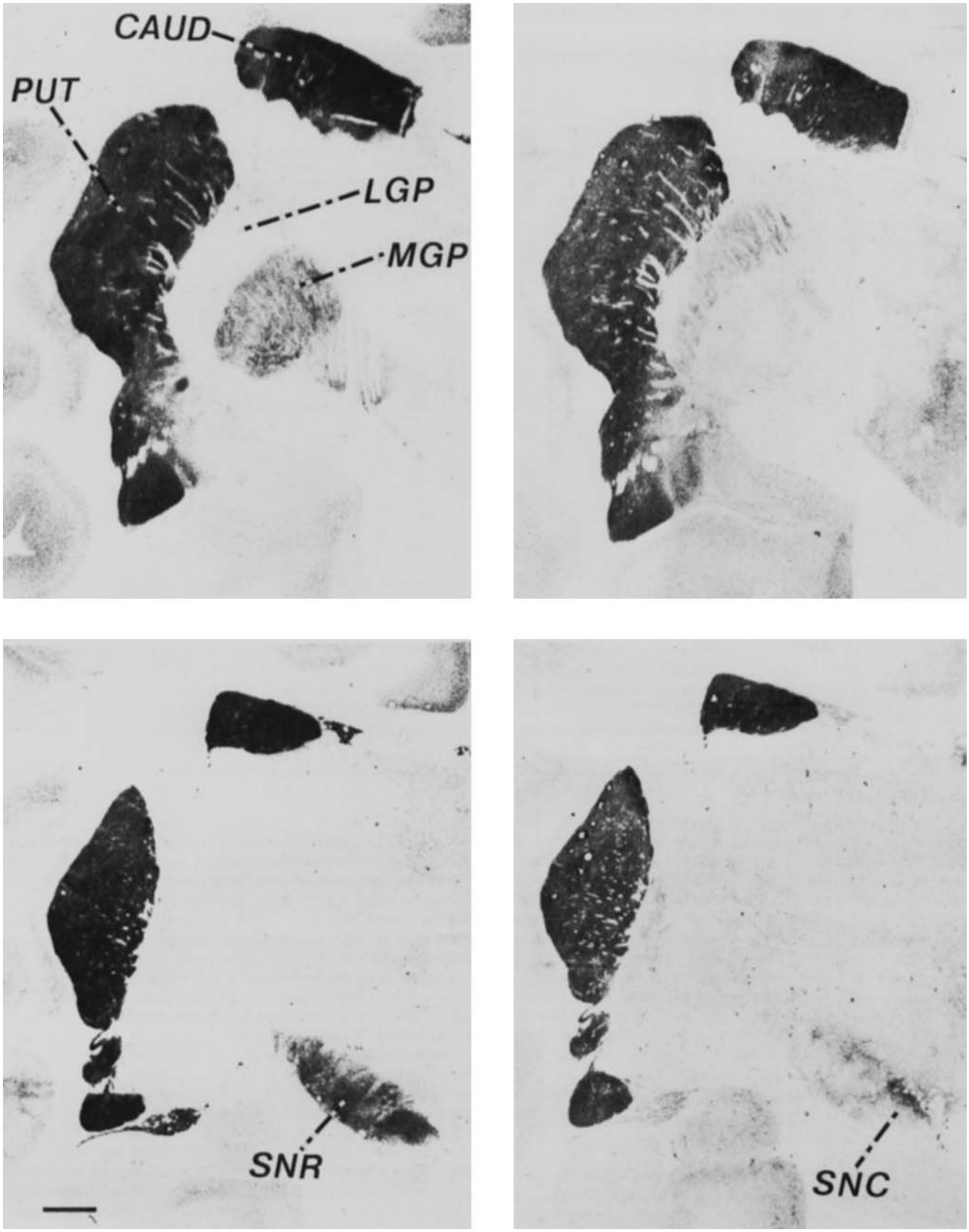

Fig. 10. Dopamine receptor autoradiography of the monkey BG. Dopamine receptor autoradiography was performed in adjacent sections from regions of the monkey BG. Dopamine D-1 receptors (left) are present in caudate nucleus, putamen, LGP, MGP, and SNR; D-2 receptors (right) are present in caudate nucleus, putamen, LGP, and SNC. Heterogeneities can be seen in the striatum and SN. Scale bar = 2 mm.

creases with maturation (Richfield et al., '87). Whereas part of this decrease in receptor density may be an artifact attributable to increasing lipid content in the striatum, it is remarkable how many receptors were present at this early age. The D-1 receptor in the adult was heterogeneous, but the pattern was unlike AChE staining and dopamine histofluorescence.

The D-2 receptor was quite homogeneous in the neonate, unlike the D-1 receptor, AChE staining, and dopamine histofluorescence. The D-2 receptor changed little in the average amount of binding with maturation in the dorsal striatum, but did become heterogeneous. The pattern of adult D-2 receptor heterogeneity did not match the adult pattern of AChE staining or dopamine histofluorescence, but did partially match the D-1 receptor pattern in that some areas show increased or decreased levels of both receptor subtypes. There were also mismatches between dopamine receptors, dopamine levels, and AChE staining in their respective gradients. These results suggest that complex developmental changes may occur independently among dopamine, dopamine receptor subtypes, and other neurochemical markers in the basal ganglia.

The adult cat demonstrated heterogeneous binding for both D-1 and D-2 receptors. Since AChE staining and immunocytochemistry are nonquantitative, it was not possible to relate the magnitude of heterogeneity in receptor binding to other measures of heterogeneity. Receptor heterogeneities were most frequently seen in the caudate nucleus, but were also seen in the n. accumbens and olfactory tubercle. They were seldom seen in the putamen. This regional pattern of heterogeneity is very similar to the distribution seen with other markers. Since the total area of the matrix region identified by AChE staining exceeded the area of the striosomes, it appeared that receptor binding areas with increased density were more commonly seen in the matrix region. These receptor changes may also correspond to the mosaic architecture of the somatic sensory-recipient sector of the cat's striatum present within the extrastriosomal matrix (Malach and Graybiel, '86).

The distribution of dopamine receptors most clearly fits with that described for substance P immunoreactivity. The distribution of substance P has been described as having areas of increased and decreased staining, which are "often thin, elongated, and tend to make a filigree pattern," and in general do not have an exact correspondence to AChE staining or enkephalin immunoreactivity (Graybiel et al., '81a). Whether dopamine receptor heterogeneities match substance P immunoreactivity heterogeneities remains to be proven, but together these studies suggest an additional compartmentation of the caudate nucleus separate from the AChE-opiate compartmentation.

The heterogeneous distribution of dopamine receptors in the caudate and putamen also resembled the terminal field projections from various areas of cortex as described by Selmon and Goldman-Rakic ('86). They reported that corticostriatal projections from association cortices terminate in longitudinal domains having restricted medial-lateral sectors. They speculated that these terminal fields would produce spatially distinct centers of excitation.

Quantification of receptor density with autoradiography suffers from the potential error of weak β particle absorption by lipids (Kuhar et al., '86; Herkenham and Sokoloff, '84; Geary et al., '85). This effect is most prominent in areas with variable or large amounts of white matter relative to the amount of white matter used in the brain tissue to calibrate standards. Thus, absolute values cannot be determined without other modifications of this technique, such as lipid extraction (Herkenham and Sokoloff, '84; Geary et al., '85) or the use of ^{14}C or ^{125}I labeled ligands (Kuhar et al., '86). The variable density of white matter fibers also affects the quantification of any neurochemical marker that is determined on the basis of tissue weight or amount of protein. Thus, in structures with regionally variable amounts of lipid or protein, only general trends in measurements can be made. With this in mind, in rats the rostral to caudal decrease in receptor density paralleled the decrease seen in dopamine levels (Bockaert et al., '76; Tassin et al., '76). The cat had a similar gradient in the caudate and putamen. The monkey, however, showed a minimal or no rostral to caudal change in the D-2 receptor, and a slight decrease in the D-1 receptor.

Cellular localization of dopamine receptors

The cellular localization of dopamine receptor subtypes in different portions of the BG remains controversial. Dopamine D-2 receptors are thought to be present on cell bodies in the SNC and to be presynaptic and postsynaptic in the striatum (Moore and Bloom, '78; Creese et al., '83; Stoof and Kebabian, '84). Recent studies suggest that the D-2 receptor may be only postsynaptic in the rat striatum (Trugman et al., '86; Joyce et al., '86). The D-1 receptor has been found to be present on postsynaptic cells in the striatum and on presynaptic terminals in the SNR (Dubois et al., '86). The current study did not specifically address the cellular location of dopamine receptor subtypes. However, the finding that D-1 receptors in substantia nigra decreased after striatal lesions suggests that D-1 receptors may be associated with specific striatal projections (Altar et al., '86; Wamsley et al., '86). In this regard, it will be of interest to compare the distribution of D-1 receptors with that of substance P. High levels of substance P immunoreactivity have been found in the medial GP, but not lateral GP of humans (Graybiel, '84), and in the SNR of rats with a decreasing medial to lateral gradient (Dr. Anton Reiner, personal communication). This pattern is similar to the pattern seen with D-1 receptor binding. Striatal axons to GP, EP, and SN have been shown to come from separate cell populations (Beckstead and Cruz, '86). If D-1 receptors are presynaptic on substance P afferents to MGP and SNR, it would be interesting to speculate that the D-2 receptor might be presynaptic on enkephalinergic afferents to LGP.

Different types of heterogeneities were seen in all three of the mammals studied, were seen for both the D-1 and D-2 receptor, and were seen in most regions of the basal ganglia examined, including caudate nucleus, putamen, n. accumbens, olfactory tubercle, pallidum, and substantia nigra. The frequency and diversity with which they were seen suggests that heterogeneous processing of information is common with the dopamine system within the basal ganglia. This is in contrast to the pattern seen in other regions of the cerebrum, which are much more homogeneous (E.K. Richfield, unpublished observation).

ACKNOWLEDGMENTS

We would like to thank Dr. Anton Reiner for providing the turtle and pigeon sections, as well as helpful anatomical discussions. We would also like to thank Dr. George Dauth for providing monkey tissue sections; Darrel Debowey for photographic assistance; and Genell Fries for secretarial assistance. This work was supported in part by the Tourette Syndrome Association (Bayside, NY) and USPHS grant NS 19613.

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