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Basal ganglia and cerebral cortical distribution of dopamine D_1- and D_2-receptors in neonatal and adult cat brain

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Quantitative receptor autoradiography was performed on neonatal and adult cat brains. Serial sections through the basal ganglia were assayed for D_1- and D_2-dopamine receptors and acetylcholinesterase (AChE) staining. The neonatal basal ganglia revealed patches of increased D_1-receptor density that frequently overlapped with patches of increased AChE staining, while the D_2-receptor distribution was more homogeneous. The adult basal ganglia revealed a mild amount of heterogeneity for both the D_1- and D_2-receptors, varying from 10 to 25%, with little correspondence to the marked heterogeneity seen with AChE staining. A distinct laminar distribution of the D_1-receptor, without significant D_2 binding, was seen in the cerebral cortex.

The dopamine system has projections to both the basal ganglia and cerebral cortex in a variety of species including rat, cat and primates [13]. The basal ganglia projection is heterogeneous during prenatal and early postnatal development, but becomes homogeneous with maturity as seen with dopamine histofluorescence or tyrosine hydroxylase immunohistochemistry [7, 9]. This apparent adult homogeneity can be modified by pretreatment of the animal with 2-methyl-paratyrosine [15], indicating that the adult dopamine system may have an underlying biochemical or physiological heterogeneity not observed under normal conditions. The basal ganglia of cats and primates is heterogeneous during both development and maturity for a variety of other neurotransmitter systems including acetylcholine [6, 14], enkephalin [8], and substance P [5, 8]. The role of postsynaptic dopamine receptors in basal ganglia heterogeneity is not entirely known.

Dopamine D_2-receptors have been well studied autoradiographically in rat basal ganglia [1, 10, 11, 16, 17]. They have been found to be homogeneous during development (E.K.R., unpublished observations) and at maturity [17], but adults may have gradients in receptor density across the striatum [1, 10, 11]. The D_1-receptor has also

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been recently studied autoradiographically in the rat [3], but heterogeneity and gradients were not described. Dopamine receptors have not been studied in detail in developing or adult cat in comparison to other histochemical markers of basal ganglia heterogeneity.

The presence of dopamine receptors in areas outside of the basal ganglia remains controversial. D_2-receptors have been reported in areas of both known and unsuspected dopamine innervation [12]. Difficulty in studying the D_2-receptor has been due to the low number of receptors present and the high degree of non-dopaminergic and non-specific binding seen with some ligands [2, 19]. The D_1-receptor has not been studied in detail outside the basal ganglia because of the lack of a selective agent for labelling. The recent introduction of [^{3}H]SCH 23390 provides a highly selective ligand for studying the D_1-receptor autoradiographically.

Four kittens aged postnatal day 0 (P-0) to postnatal day 7 (P-7) were killed by decapitation and their brains rapidly removed. Two adult male cats were killed by an overdose of pentobarbital and their brains rapidly removed. The cerebral hemispheres were divided into right and left halves. The blocks were frozen using crushed dry ice and stored at $-70°C$ until sectioned. Twenty or 30-μm thick cryostat sections were thaw-mounted onto subbed microscope slides and stored at $-20°C$ until used in assays. Serial adjacent sections were processed separately for D_1- and D_2-receptors and acetylcholinesterase (AChE) staining. The receptor incubation and postwash buffer for both ligands was 25 mM Tris-HCl (pH 7.5), 100 mM $NaCl_2$, 1 mM $MgCl_2$, 1 μM pargyline and 0.001% ascorbate. Mounted sections were allowed to warm to room temperature for 1 h, then placed directly into incubation medium with tritiated ligand for 120 min at room temperature, followed by a 10-min wash at 4°C, and a 3-s dip in distilled water. Slides were dried using a stream of cool air. D_2 autoradiography employed [^{3}H]spiroperidol (76 Ci/mmol, Amersham, Arlington Hts., IL 60005) at concentrations of 250 pM and 125 pM, which are the K_d and 1/2 K_d, respectively. The D_2 assay contained 100 nM mianserin to block out binding to the serotonin (5-HT_2) receptor [17]. The D_1 assay used [^{3}H]SCH 23390 (85 Ci/mmol, Amersham, Arlington Hts., IL 60005) at concentrations of 550 pM and 275 pM, the K_d and 1/2 K_d respectively. *cis*-Flupenthixol (1 μM) and dopamine (25 μM) were used as blanks for D_1 and D_2 binding, respectively. Specific binding was determined by subtracting the amount present in blanks from the total amount bound. Slides were exposed to LKB Ultrofilm ^{3}H for 14–21 days and developed in Kodak D-19 for 3 min. Quantitative densitometry was performed as previously described [17]. An average of 20 readings were taken per region of interest from 3 separate sections. Densitometric readings from basal ganglia were compared to regions of AChE heterogeneity by visual inspection. AChE staining was performed according to the method of Geneser-Jensen and Blackstad [4]. Ethopropazine HCl (0.2 mM) was added to the incubation medium to block the activity of pseudocholinesterase. Incubation times for kitten sections varied from 60 to 90 min and adult sections varied from 30 to 60 min.

The density of D_1- and D_2-receptors in the basal ganglia were slightly higher in the neonatal kittens than in the adult cats (Table I). The relative percentage of D_1-

TABLE I

SUMMARY OF DOPAMINE RECEPTOR BINDING

The values represent the average of measures from two animals ± S.D. for a particular structure. The % total dopamine receptors is the percentage of D_1- or D_2-receptors for that anatomical region at each age. Hot areas were regions of increased receptor binding that were visually distinct from the surrounding density of binding (low areas). n.e., not examined; n.d., none detected. *Receptors were measured at the K_d values as described in the text.

Receptor	Structure	P-0 kitten		Adult cat	
		Amount bound (pmol/mg protein)	% Total dopamine receptors	Amount bound (pmol/mg protein)	% Total dopamine receptors
D_1*	Caudate	1.47 ± 0.03	70%	1.08 ± 0.27	72%
	Hot areas	1.56 ± 0.04		1.22 ± 0.25	
	Low areas	1.30 ± 0.18		1.06 ± 0.13	
	Putamen	1.52 ± 0.09	76%	0.96 ± 0.03	65%
	Nucleus accumbens	0.94 ± 0.41	70%	0.57 ± 0.25	62%
	Olfactory tubercle	1.31 ± 0.15	89%	0.88 ± 0.04	72%
	Claustrum	n.d.		n.d.	
	Substantia nigra	n.e.		0.86 ± 0.01	
D_2*	Caudate	0.64 ± 0.16	30%	0.43 ± 0.01	28%
	Hot areas	n.d.		0.53 ± 0.06	
	Low areas	n.d.		0.42 ± 0.08	
	Putamen	0.47 ± 0.12	24%	0.51 ± 0.04	35%
	Nucleus accumbens	0.41 ± 0.07	30%	0.35 ± 0.11	38%
	Olfactory tubercle	0.17 ± 0.02	11%	0.35 ± 0.12	28%
	Claustrum	0.24 ± 0.06	100%	0.05 ± 0.02	100%
	Substantia nigra	n.e.		0.06 ± 0.02	

and D_2-receptors in different structures was well preserved between the neonatal and adult brains. The D_1-receptor was approximately 70% of the total number of dopamine receptors in all structures except for the claustrum, which contained only D_2-receptors.

In the adult cat the basal ganglia appeared more homogeneous for both the D_1- and D_2-receptors compared to the marked heterogeneity seen with AChE staining (see Fig. 1). Variations in the number of both D_1- and D_2-receptors on the order of 10–25% were seen in the cat, but rarely corresponded to areas of low AChE staining (striosomes). The striosomes tended to be 0.3–0.5 mm across with tubular shapes and sharp contours. The size, shape and contour of dopamine receptor 'hot areas' were different from that seen with AChE staining. Areas of increased D_1- and D_2-receptors tended to be larger in size, 0.8–1.0 mm across, with less distinct boundaries and either long narrow shapes or large round shapes. Areas of increased D_1- and D_2-receptor density often overlapped each other in serial sections. The areas of increased receptor binding tended to be more prominent in the matrix (non-striosomal) area of AChE staining.

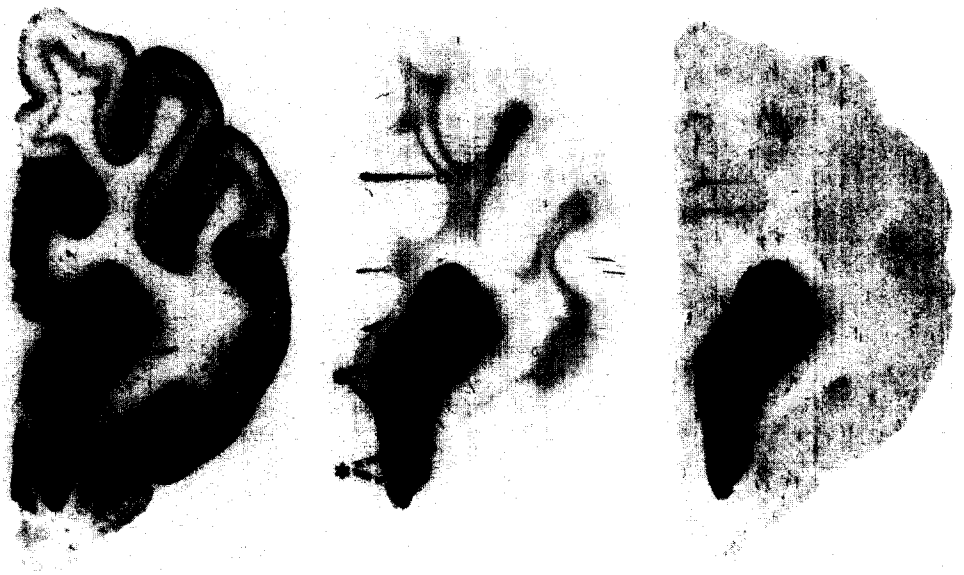

Fig. 1. Adult cat dopamine receptor autoradiograms and AChE staining. Dopamine D_1- (left) and D_2 (right)-receptor autoradiography and AChE staining (middle) were performed as described in the text on alternate sections. Both the adult D_1 and D_2 dopamine receptors demonstrate some areas of slightly increased receptor binding in areas that do not correspond to areas of low AChE staining (indicated by *). The cortical laminar distribution of the D_1-receptor can be seen in the D_1 autoradiogram. Scale bar in middle figure represents 3 mm.

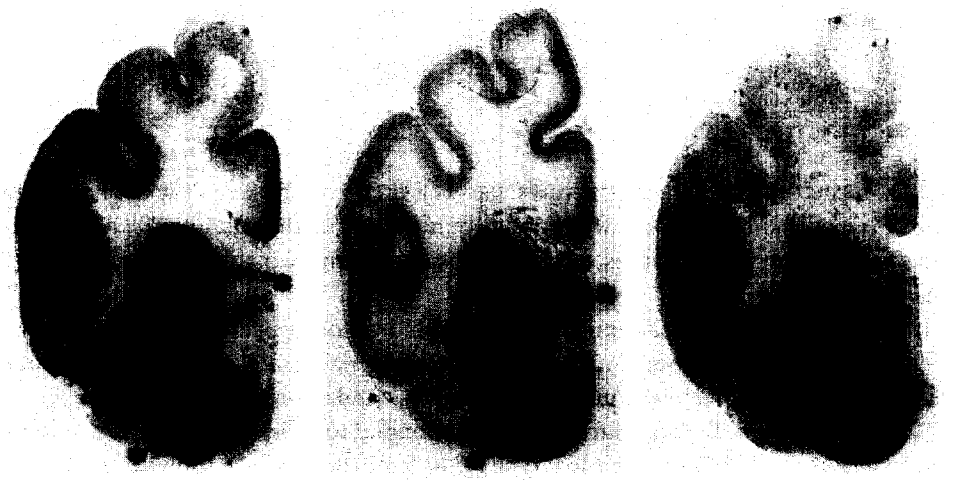

Fig. 2. Neonatal (P-0) cat dopamine receptor autoradiograms and AChE staining. Dopamine D_1 (left) and D_2 (right) autoradiography and AChE staining (middle) were performed as described in the text on alternate sections in P-0 kittens. The kitten demonstrated areas of increased D_1-receptor density (indicated by *) that correspond to areas of increased AChE staining (indicated by *), while the D_2-receptor density is more homogeneous. The neonatal kittens also demonstrate laminar cortical binding to the D_1-receptor, but not the D_2-receptor. Scale bar in middle figure represents 3 mm.

The D_1-receptor was quite heterogeneous in the P-0 and P-2 kittens, becoming more homogeneous with maturity. Areas of increased D_1-receptors appeared as patches that frequently corresponded to patches of increased AChE staining (Fig. 2). The size, shape and contour of D_1-receptor patches were very similar to the patches seen with AChE staining. Patches of increased D_1 binding were most prominent in the caudate nucleus, but also seen in the putamen, nucleus accumbens and olfactory tubercle. Unlike the D_1-receptor, the D_2-receptor in adjacent sections appeared more homogeneous at all ages. There was a dorsal (higher) to ventral (lower) gradient of D_1- and D_2-receptors present in developing kitten, that decreased with aging and disappeared in maturity. There was no medial to lateral gradient of dopamine receptors seen at any age. These receptor gradients were in contrast to the AChE staining gradient, which was higher in lateral and ventral areas than in medial and dorsal areas in neonatal brains.

The adult cerebral cortex demonstrated high numbers of D_1-receptors in a laminar pattern that varied from one gyrus to another. This is in contrast to the D_2-receptor, which did not demonstrate any appreciable specific binding in the cerebral cortex. The D_1-receptor was present in cortical layers 1, 2 and 6, with the number of receptors varying between, and occasionally within gyri. The neonatal brains also demonstrated D_1-receptor cortical binding in a laminar pattern that was different than that seen in the adult and varied between gyri.

While the D_1- and D_2-receptors are known to have different pharmacologic profiles and intracellular biochemical effects [18], these results indicate that the D_1- and D_2-receptors also have unique developmental and anatomical distribution patterns in the cat. The D_1-receptor appears to have a developmental pattern similar to dopamine histofluorescence and tyrosine hydroxylase immunohistochemistry with an early heterogeneous pattern that is later obscured in maturity [7, 9]. This is in contrast to the D_2-receptor which is homogeneous during development, but becomes mildly heterogeneous with maturity. Neither receptor shows the marked degree of heterogeneity seen with AChE staining in the adult cat. However, all regions with AChE heterogeneity show dopamine receptor heterogeneity including caudate nucleus, putamen, nucleus accumbens and olfactory tubercle. The regions of dopamine receptor heterogeneity may represent a different compartmental interaction in the basal ganglia independent of striosomes. Substance P immunoreactivity has been shown to be complicated with both light-on-dark and dark-on-light patterns having indistinct borders [8]. These zones were also 'thin and elongated and tended to make a filigree pattern across the section' [8]. Areas of poor match between substance P immunoreactivity and cholinesterase staining were found [8]. This description is similar to the pattern seen with dopamine receptor autoradiography. A poor correlation has also been found between somatostatin staining and AChE staining [8]. Other neurochemical markers will need to be compared to areas of dopamine receptor heterogeneity to determine compartmental interactions.

Although D_2-cortical receptors have been reported to be present in all regions of neocortex in rat [12], these sites were not seen in cat cerebral cortex. This may be a species difference in receptors. It may also be due to a very low number of receptors in cat cerebral cortex. The lowest number of D_2-receptors found in this study was in the claustrum with 0.05 pmol/mg protein representing 11% of the number of D_2-

receptors seen in caudate nucleus. Values less than this were not detected. In contrast, a marked laminar distribution of D_1-receptors was seen in all areas of cerebral cortex. These findings indicate that the D_1-receptor may play a functionally greater role in cerebral cortex function than previously suspected. The D_1-receptor in cortex may be involved in the symptoms of cognitive loss seen in some patients with Parkinson's disease or behavioral abnormalities found in patients with schizophrenia.

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