Quantitative Autoradiography of Major Neurotransmitter Receptors in the Monkey Striate and Extrastriate Cortex

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In vitro autoradiography was used to determine the binding properties and distribution of 9 major neurotransmitter receptors and their subtypes in the striate (area 17 of Brodmann) and extrastriate (areas 18 and 19) cortex of 1 infant and 3 adult rhesus monkeys. Differences in total labeling and nonspecific labeling, as well as K_d and B_{max} values, were determined for all cortical layers and sublayers in both cytoarchitectonic areas by Scatchard analysis of autoradiograms. Area 17 differed from area 18 in the laminar pattern and density of virtually every ligand examined, i.e., ³H-clonidine, ³H-prazosin, ¹²⁵I-iodopindolol, ³H-quinuclidinyl benzilate, ³H-5-hydroxytryptamine, ³H-ketanserin, ³H-muscimol, ³H-flunitrazepam, and ³H-spiperone. K_d and B_{max} values for each ligand were remarkably consistent across the 3 adult monkeys analyzed quantitatively. Particularly dramatic contrasts were observed with clonidine, 5-hydroxytryptamine, and ketanserin, which have high affinity for α_2 -adrenergic, 5-HT₁, and 5-HT₂-receptors, respectively. The differences in distribution of these ligands, especially clonidine and 5-hydroxytryptamine, correlated well with specific laminae and hence exhibited distinctly different patterns in areas 17 and 18. Other ligands, such as flunitrazepam and quinuclidinyl benzilate that bind to GABAergic and cholinergic receptors, were visually less discriminating both among layers and between regions. However, layer for layer, the B_{max} values for quinuclidinyl benzilate were higher in area 17 than 18, indicating that subtle differences between areas may be revealed only by quantitative measures. Some ligands were particularly dense in layer I (iodopindolol in areas 17 and 18; 5-hydroxytryptamine in area 18), and others subdivided cortical layers that are otherwise cytoarchitectonically uniform (e.g., flunitrazepam and clonidine in layer VI of area 17), indicating that areal differences in ligand binding are not a simple read-out of cell-packing density but most likely reflect a genuine difference related to the neurotransmitters of intrinsic and extrinsic afferents in each area. The presence of binding sites in every layer of both areas for all ligands examined indicates that their distribution across laminae is quantitative and not all-or-none. No layer contained less than 50% of binding sites present in any other layer. These find-

ings reveal that visual cortical areas differ in density and lamination of neurotransmitter receptors and presumably in their sensitivity to circulating levels of endogenous neurotransmitters and pharmacologically active compounds.

Modern neuroanatomical and electron microscopic techniques have provided unparalleled information about the intrinsic circuitry and input-output relationships of the neocortex and, together with biochemistry, histochemistry and immunocytochemistry, have enabled precise localization of major neurotransmitters and neuroactive peptides in this structure (Bjorklund et al., 1978; Brown et al., 1979; Somogyi et al., 1981; Parnavelas and McDonald, 1983; Levitt et al., 1984; Peters and Jones, 1984, 1985; Jones and Peters, 1985; Morrison and Foote, 1986). In contrast, our knowledge about the distribution of neurotransmitter receptors and their relative concentrations in various areas of the neocortex until recently has been less detailed and less comprehensive. However, newly developed *in vitro* binding autoradiographic techniques (Young and Kuhar, 1979; Palacios et al., 1981b; Penney et al., 1981; Quirion et al., 1981; Rainbow et al., 1982; Unnerstall et al., 1984a, b; Wamsley and Palacios, 1984; Kuhar et al., 1986) now permit anatomical localization and quantification of a large number of neurotransmitter receptors at the level of cortical laminae and sublaminae.

As part of an ongoing investigation of the molecular, cellular, and functional organization of the primate neocortex, we have examined the binding pattern of 9 radioactive neurotransmitter ligands in the primary visual cortex, Brodmann's area 17, and in the extrastriate cortical area 18, which borders area 17. The ligands were chosen on the basis of their high affinity, specificity, and adaptability to the *in vitro* binding procedure. Cortical area 17 and its border with area 18 were selected for the first detailed quantitative analysis because these areas are anatomically and physiologically distinct. The 6 basic layers of area 17, for example, can be further divided into several well-defined sublayers (Brodmann, 1905), each having characteristic cellular and synaptic density (O'Kusky and Colonnier, 1982), input and output relationships (Lund et al., 1975; Van Essen, 1986), and local neuronal circuitry (Gilbert and Wiesel, 1981; Lund, 1981; Rockland and Pandya, 1981; Somogyi et al., 1981; Rockland and Lund, 1983). Qualitative and quantitative differences in ligand binding are to be expected in various regions and layers of the cortex in view of the marked regional and laminar differences in the distribution of catecholamines (Bjorklund et al., 1978; Brown et al., 1979; McDonald et al., 1982; Morrison et al., 1982; Levitt et al., 1984; Morrison and Foote, 1986), ACh (Hedreen et al., 1984; Mesulam et al., 1984), and other neurotransmitters (Parnavelas and McDonald, 1983; Hendry et al., 1987).

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However, uniformity in ligand binding among regions and layers of the cortex is of comparable interest in deciphering its neurochemical interactions.

A considerable amount of data are already available on various receptors in the cortex of several species (Wise and Herkenham, 1982; Shaw et al., 1984; Pazos and Palacios, 1985; Wamsley, 1985; Hoyer et al., 1986a, b; Palacios et al., 1986; Shaw and Cynader, 1986; Kritzer et al., 1987). However, most of the previous research has not been focused on differences between cortical regions, and there exists as yet no quantitative data comparing distributions of multiple ligands in primates. In addition, the few studies available on the human cortex have not examined visual areas in detail, either qualitatively or quantitatively (e.g., Hoyer et al., 1986a, b). The rhesus monkey neocortex, particularly the visual system, is well suited for areal and laminar analysis because of the high degree of functional and anatomical specialization of its cytoarchitectonic subdivisions, and findings about receptor distribution in the primate order could have particular functional, developmental, and therapeutic implications for humans.

Materials and Methods

Tissue preparation. The brains of 3 adult (1 male, 2 females) and one 2-month-old male monkey (*Macaca mulatta*) were processed for the present study. Tissue from each animal was sectioned and assayed independently within 8–12 months of each other. The monkeys were anesthetized with Na-pentobarbital (40 mg/kg) and perfused with ice-cold PBS (pH 7.4, 1.25 liters) followed by 0.1% paraformaldehyde containing increasing concentrations of sucrose in buffered saline: 500 ml 0% sucrose, 500 ml 5% sucrose, 1 liter 10% sucrose, 500 ml 15% sucrose, 1 liter 20% sucrose. This light fixation allowed brain tissue to be sectioned more easily without affecting binding parameters (neither kinetic constants nor pharmacological profiles were affected; Bates and Gallagher, unpublished comparison of ligand binding properties of fixed and unfixed tissue sections from adult rhesus monkey cortex; Young and Kuhar, 1980a–c; Kuhar and Unnerstall, 1982). The brains were rapidly removed, blocked, and immersed in isopentane at -30°C for approximately 5 min before storing at -80°C until use. For autoradiography, 20 μm sections were processed according to individual assay procedures. In each case, tissue sections were cut not more than 2 weeks prior to assay, mounted on acid-cleaned chrom alum-subbed slides, and stored at -80°C .

Binding assays. The 9 ligands selected for analysis in this study have been tested for both their affinity and specificity in numerous studies during the past few years, and further evidence of specificity is presented under Results. For all assays, slide-mounted tissue sections were incubated in increasing concentrations of radioligands. In some assays, preincubations were run to allow metabolism of endogenous ligands prior to incubation with labeled compounds. Parallel tissue sections were incubated with excess cold-displacing drugs to allow determination of nonspecific binding. For the most part, our methods follow established procedures (listed in Table 1). In all cases, association and dissociation times, and saturation kinetics were preliminarily defined in monkey tissue sections to optimize assay procedures for our laboratory conditions. We occasionally introduced minor modifications based on updated information from the literature or from our own results. Thus, cold prazosin (an α_1 -adrenergic antagonist) was used in the ^3H -ketanserin assays of the last 2 animals based on evidence from a number of laboratories (Leyssen et al., 1982; Smith et al., 1984; Lakoski and Aghajanian, 1985; Pazos et al., 1985) that some non-5-HT-displaceable binding occurs to α_1 -adrenoceptors. The iodinated ligands I^{125} -iodopindolol, a β -adrenergic antagonist (Rainbow et al., 1984b), and I^{125} -HEAT, an α_1 -adrenergic antagonist (2-[B-(4-hydroxyphenyl)-ethyl-amino-methyl] tetralone; Glossmann et al., 1981), were also used for comparison with tritiated compounds. Although absolute quantification was not carried out due to lack of reliable I^{125} standards, the binding profiles of iodinated ligands were compared with ^3H for relative binding distribution across layers in order to determine if white matter attenuation of lower energy beta emissions (^3H) was significant (Herkenham and Sokoloff, 1984; Rainbow et al., 1984a; Unnerstall et al., 1984b; Lidow et al., 1987).

This procedure did not affect the amount of specific binding of the ligand but increased the total level of radioactivity. Both defatting and overlay of tissue onto radioactive standards were used to estimate tritium quenching (Lidow et al., 1987). Only one sublayer (IVB) of area 17 in the visual cortex and the subcortical white matter was found to be significantly affected by such procedures. Except for the white matter, areas 18 and 19 were not affected. A correction factor was calculated from the 2 quenching experiments, and estimates of corrected values are shown as open columns in B_{max} histograms for this single cortical sublayer and adjacent white matter (Figs. 4 and 5).

Following assay, sections were rinsed and dried under a stream of cool dry air. They were kept in slide boxes with dessicant and stored overnight at -80°C . Sections were then placed in X-ray film cassettes with brain paste or commercial standards (^3H -microscales, Amersham) against tritium-sensitive Ultrofilm (LKB, Inc., Gaithersburg, MD) at 4°C for varying exposure periods as listed in Table 1. Following film exposure, tissue sections were stained with cresyl violet and cover-slipped.

Quantitative densitometry. The intensity of receptor binding in tissue areas and layers was assessed using a computer-imaging system consisting of a DAGE-MTI series 68 video camera and video signal digitizing circuitry interfaced with a Digital VT100 computer. This system allows comparison of densities of exposed tritium-sensitive Ultrofilm (LKB, Gaithersburg, MD) with known labeled standards (^3H -microscales, Amersham, Chicago). In order to convert density measurements to molar amounts of bound ligand, standards were calibrated against tritium brain paste standards prepared using the radioligands employed in these studies and converted to milligram tissue equivalents (Rainbow et al., 1984a). In addition, comparison with optical density readings obtained from a photo print wheel allowed us to restrict our estimates to ranges below film saturation (below 0.80D).

The method allows overlay of cresyl violet-stained tissue sections (the same ones used to generate the autoradiograms) on the film image in real time in order to make histological identification of specific cortical cell layers for each autoradiogram (Fig. 1). A background subtraction program allowed correction for possible nonuniform illumination of the film, and a Nikon macro zoom lens magnified the autoradiographic image to permit density measurements to be restricted to single cortical layers. Individual densitometric values were taken as the mean of 6 digitizations. In addition, each film with appropriate standards was read in at least 2 different computer sessions (≥ 2 times). Values obtained across the different reading sessions were within $\pm 2\%$, indicating the stability of the measurement system. Multiple densitometric readings were taken from at least 3 consecutive brain sections at each ligand concentration (5 concentrations of ligand per analysis). Values for nonspecific binding at each concentration were determined in adjacent sections and subtracted from the mean total bound. These values were analyzed by a computerized nonlinear curve-fitting program designed for analysis of ligand binding isotherms (Lundon-1, Lundon Software, 1984). B_{max} and K_d values from resulting analyses were obtained for each animal and the mean value determined across animals (2–3 adults) for each layer and sublayer that could be identified cytoarchitecturally (Fig. 2). In cases where Scatchard-Rosenthal analysis failed to define a single site ($R < 0.85$), data are shown as relative distributions of specific binding, averaged across animals for a single ligand concentration. K_d values were calculated from optical density measurements of tritium-sensitive Ultrofilm exposed to labeled tissue sections. Comparisons were made across cortical layers, across areas, and across methods, i.e., values obtained in autoradiograms were compared to those obtained by liquid scintillation counting of whole-brain sections assayed in parallel for each of the binding assays described. The pattern of receptor distribution in the infant monkey was qualitatively similar to that in the adult monkeys. However, since the receptors may not have been fully expressed in the immature neurons of the infant monkey, the quantitative data and all illustrations in this article are based exclusively on the adult cases.

Results

Each radioligand is distributed in a characteristic pattern in both the striate (area 17) and extrastriate areas (18 and 19) of the visual cortex. The pattern of distribution of each ligand in each area is described below, and quantitative data are presented for the 2–3 most completely analyzed adult animals. Fluctuations in K_d values calculated for each ligand by Scatchard analysis

Figure 1. Photographs of video images taken from the TV monitor to illustrate quantitative densitometry. The first column (A–J) shows a digitized computer-generated image of the primary visual cortex (area 17) obtained from cresyl violet-stained sections. The second column (B–K) shows Ultrafilm exposed to the radioactivity of these sections incubated with ^3H -clonidine (B), ^3H -5-hydroxytryptamine (E), ^3H -ketanserin (H), and ^3H -

from densitometric measurements on a layer-by-layer basis in areas 17 and 18 were within the range of affinities reported for various ligands in membrane homogenates (Table 2). Moreover, there were no statistically significant differences in K_d values between layers or areas. Furthermore, white matter labeling was either at or slightly above background for each ligand; however, correcting for white matter quenching did increase binding in this region for a few ligands (see open bars on histograms in Figs. 4 and 5). Although the intensity of labeling was somewhat variable from case to case, the basic pattern of film density was remarkably similar in all 4 cases. Differences between the total labeling, nonspecific labeling, and $B_{\max}$ values are discussed only in instances where a significant discrepancy in relative intensity between ligand-incubated and control sections was encountered.

³H-clonidine

This ligand and its para-amino analog (Young and Kuhar, 1980a, b) have been used extensively to map α_2 -adrenoceptors in the rodent brain. Our data show that in the primate visual cortex, tritiated clonidine binding was characterized by a single site of high affinity with saturability and appropriate pharmacology associated with α_2 -adrenergic adrenoceptors (oxymetazoline > NE > phenylephrine). An example of saturation binding and the resulting linear ($R = 0.96$) Scatchard plot for a single sublayer (IVC-beta, area 17) are shown in Figure 2. Such analysis is representative of analyses in other layers and for other ligands as described below.

Sections incubated with ³H-clonidine (Figs. 1B, 3A) displayed the most elaborate layering pattern in the striate cortex, clearly revealing each cortical layer and sublayer that is recognized with classical Nissl stain in this cytoarchitectonic area (Fig. 1A). The most prominent feature of the distribution in autoradiograms was the presence of 4 dark bands situated in layers I, IVA, IVC-beta, and the upper stratum of layer VI. Intermediate level binding was localized in layers II, III, IVC-alpha, and the lower stratum of VI, while 2 low-density bands were situated in layers IVB and V.

Quantitative densitometric analysis confirmed visual analysis but also pointed up instructive differences. For example, as evident from histograms in Figure 4A, $B_{\max}$ values for the densely labeled layers IVC-beta and VI were greater than the more lightly labeled layers IVC-alpha and V. The distribution and intensity of labeling were similar in all 3 adult specimens except in layer I, which was much more densely labeled in one animal (maximum total binding of 103 vs 32 and 24 fmol/mg tissue for 2 additional adult cases). The control autoradiogram specimen from this animal shows that a considerable amount of the radioactivity localized in layer I was due to nonspecific binding (compare Figs. 1B and 1C). This difference is clearly depicted in the calculations of specific $B_{\max}$ values, which reveal that the dark band in layer I is indeed nonspecific (Fig. 4A). However, the other 3 dark bands in the cortex correspond to the highest concentrations of specific binding of clonidine in all 3 adults,

³H-Clonidine Binding IVC_β Area 17

Figure 2. A, Saturation binding of ³H-clonidine to layer IVC-beta of the calcarine fissure in area 17 of the occipital cortex in a representative monkey. Consecutive brain sections were labeled as described in the text with various concentrations of ³H-clonidine and apposed to LKB Ultrofilm for 60 d to generate autoradiograms. Nonspecific binding was defined as the labeling in the presence of 100 nM NE. Densitometric readings were taken in at least 3 consecutive brain sections at each concentration of ligand with or without NE and converted into molar levels of ³H-clonidine by reference to tritium microscaler (Amersham-Searle) and ³H-clonidine brain-mash standards as described in the text. B, Scatchard plot of the specific binding of ³H-clonidine in this representative monkey is shown yielding a single site with $B_{\max}$ of 54 ± 3 ($\pm$ SEM) fmol/mg tissue and a dissociation constant (K_d) of 1.5 ± 0.2 nM as computed by a nonlinear curve-fitting program (Lundon Software Inc.). Similar $B_{\max}$ data from 2 adult animals were averaged and are displayed in Figure 4A.

flunitrazepam (K). The third column (C-L) shows the Ultrofilm exposed to the adjacent section incubated by the appropriate displacing drug as control. Each sublayer and the subjacent white matter are first identified in cresyl violet-stained sections and selected regions delineated by a rectangle. Then the section is removed from the camera, and films from the receptor and its displacing agent are put in register with the image of the cresyl violet section that is retained on the screen. Following alignment, the cresyl violet image is removed and the computer generates a readout of film density, expressed as $B_{\max}$ and K_d values, as described in the text.

Table 1. Summary of ligands and procedures

Ligand	Conc. (nM)	Site labeled	Blank	Protocol	Buffer	Time	Temp.	Exposure time
³ H-Clonidine (SA 49.8 Ci/mM)	0.5, 1.5, 2.5, 3.5, 4.5	NE α_2	NE (100 μ M)	Pre-incuba- tion	0.17 M Tris-HCl 5 mM EDTA (pH 7.6)	30 min	RT	4 months
				Incubation	0.17 M Tris-HCl 1 mM MnCl ₂ 0.001% ascorbic acid (pH 7.6)	60 min	RT	
				Rinse	0.17 M Tris-HCl (pH 7.0)	2 $\times$ 5 min	4°C	
Prazosin (19.8 Ci/mM)	0.2, 0.4, 0.6, 0.8, 1.0	NE α_1	Phenylephrine (100 μ M)	Pre-incuba- tion	0.17 M Tris-HCl (pH 7.1)	30 min	4°C	
				Incubation	Same	70 min	4°C	2 months
				Rinse	Same	2 $\times$ 5 min	4°C	
¹²⁵ I-Iodopindolol (2200 Ci/mM)	10, 20, 50, 100, 150	NE β	Isoproterenol (500 μ M) Metoprolol (β_1 0.17, 0.9, 2 μ M)	Incubation	0.17 M Tris-HCl 10 mM MgCl ₂ 20 μ M phentolamine (pH 7.7)	30 min	RT	18 hrs
			Zinterol (β_2 0.03, 0.5, 1 μ M)	Rinse	0.17 M Tris-HCl 20 μ M phentolamine (pH 7.0)	2 $\times$ 5 min	4°C	
QNB (39.4 Ci/mM)	0.15, 0.3, 0.6, 1.2, 2.4	ACh mus- carinic	Atropine (1 μ M)	Incubation	0.17 M Tris-HCl (pH 7.4)	120 min	RT	14 days
				Rinse	Same	2 $\times$ 5 min	4°C	
5-HT (24.3 Ci/mM)	1, 2, 4, 8, 16	5-HT ₁	5-HT (1 μ M)	Incubation	0.17 M Tris-HCl 0.4 mM CaCl ₂ 10 μ M pargyline 1 μ M fluoxetine 0.1% ascorbic acid (pH 7.7)	60 min	RT	2 months
				Rinse	Same (pH 7.1)	5 $\times$ 60 sec	4°C	
Ketanserin (77 Ci/mM)	0.5, 1, 2, 3, 4	5-HT ₂	Methysergide (10 μ M)	Pre-incuba- tion	50 mM Tris-Hcl 0.1 μ M prazosin (pH 7.7)	30 min	RT	
				Incubation	Same	20 min	RT	3 weeks
				Rinse	Same (pH 7.1)	5 $\times$ 60 sec	4°C	
Flunitrazepam (92.3 Ci/mM)	1, 2, 4, 8, 16	Benzodiazc- pine	Clonazepam (1 μ M)	Prc-incuba- tion	0.17 M Tris-HCl (pH 7.4)	10 min	4°C	
				Incubation	Same	40 min	4°C	2 weeks
				Rinse	Same	5 $\times$ 20 sec	4°C	
Muscimol (11.5 Ci/mM)	2.5, 5, 7.5, 10, 20	GABA _A	GABA (10 mM)	Pre-incuba- tion	50 mM Tris-citrate (pH 7.1)	20 min	4°C	
				Incubation	Same	40 min	4°C	6 weeks
				Rinse	Same	2 $\times$ 15 sec	4°C	
Spiperone (22.7 Ci/mM)	0.4, 0.7, 1.2, 2.4, 6.6	DA	(+)-Butaclamol	Incubation	0.17 M Tris-HCl 120 mM NaCl 5 mM KCl 2 mM CaCl ₂ 1 mM MgCl ₂ 0.001% ascorbic acid (pH 7.7)	30 min	RT	2 months
				Rinse	0.17 M Tris-HCl (pH 7.1)	5 $\times$ 60 sec.	4°C	

SA, specific activity; RT, room temperature.

Figure 3. *A*, Laminar distribution of ^3H -clonidine binding in the striate cortex of the adult rhesus monkey. Video image of the autoradiogram shows densely labeled layers IVA, IVC-beta, and VI. *B*, Distribution of the same ligand at the border between areas 17 and 18.

as well as in the infant monkey. B_{max} values show that binding in IVB exceeds the binding in layer V, especially after correcting for tissue quenching of tritium. Our data indicate that specific binding of ligands is more reproducible than the total binding. Further, the observed discrepancy indicates that the data from a single case may give spurious results. In general, nonspecific binding and variability in film density due to artifacts occur most often in layer I.

Examination of the autoradiograms at low magnification reveals that the distribution of ^3H -clonidine in the extrastriate cortex is substantially different than in the striate cortex both qualitatively and quantitatively (Fig. 3*B*). First, the density of binding sites (B_{max}) in area 18 was about one-half that of area 17 (Fig. 4*B*). In addition, all the dark bands that characterize area 17 were absent in area 18, and only a single, broad, intermediate density band corresponding to layers II–IV was visible (Figs. 4, *A*, *B*; 10; 11). Thus, the intricate, distinct, and contrasting laminar pattern that characterizes area 17 was absent from area 18 in autoradiograms (Fig. 3*B*) of total labeling and in the B_{max} data (Fig. 4, *A*, *B*). Since the same coronal sections across the occipital lobe contained both areas 17 and 18, the

Table 2. K_d values ($\pm$ SD) for binding of ligands across cortical layers in areas 17 and 18 of 2–3 monkeys assayed independently

Ligand	K_d (nm) ^a	
	Area 17	Area 18
^3H -Clonidine	1.6 ± 0.6	1.5 ± 0.6
^3H -Prazosin	0.6 ± 0.4	0.4 ± 0.3
^{125}I -Iodopindolol	0.1 ± 0.1	0.1 ± 0.1
^3H -QNB	0.4 ± 0.1	0.3 ± 0.1
^3H -5-HT	2.4 ± 0.4	1.7 ± 0.8
^3H -Ketanserin	2.9 ± 2.4	2.5 ± 0.9
^3H -Flunitrazepam	2.5 ± 0.3	2.4 ± 0.2
^3H -Muscimol	1.8 ± 1.2	1.7 ± 0.6
^3H -Spiperone	3.1 ± 1.6	2.1 ± 2.0

Apparent affinities (K_d 's) were determined by Scatchard analysis of densitometric readings from autoradiograms generated by equilibrium saturation binding to visual cortical slices.

^a ANOVA comparisons of K_d values within areas 17 and 18 showed no statistically significant differences in apparent affinity between cortical layers. In contrast, ANOVA comparisons of B_{max} data between cortical layers did reveal significant differences (Figs. 4 and 5).

Figure 4. $B_{\max}$ and specific binding data computed for areas 17 and 18 of ligands by layer. Layer IVB of area 17 was found to be significantly affected by white matter attenuation of tritium emissions. Therefore, a correction factor (Lidow et al., 1987) has been introduced, indicated by *open columns* added to the observed density. *A* and *B*, ^3H -clonidine, average ($\pm$ SEM) of 2 adult cases. *C* and *D*, ^3H -prazosin showing relative distribution of label averaged for 2 adult cases when sections were incubated at a single (0.6 nM) concentration of ligand. *E* and *F*, ^{125}I -iodopindolol representing relative distribution of specific binding averaged for 2 adult animals when sections were incubated at a single (0.1 nM) concentration of ligand. *G* and *H*, ^3H -QNB $B_{\max}$ values averaged over 3 adult cases (infant also showed similar binding profile and density of sites).

differences between them could not be due to processing conditions, which were identical for each area.

As a result of the changes in binding distribution, the borderline between areas 17 and 18 was abrupt and visually striking in autoradiograms (Fig. 3*B*). In contrast, the more rostral border between areas 18 and 19 could not be detected in the autora-

diagrams, indicating that this ligand does not delineate subdivisions between subareas of the extrastriate cortex. The white matter subjacent to both the striate and extrastriate cytoarchitectonic areas appeared only lightly labeled, but quenching correction indicates significant white matter binding (20 fmol/mg tissue) for this ligand (Figs. 3; 4, *A*, *B*).

Figure 5. $B_{\max}$, specific binding data computed for areas 17 and 18 of ligands analyzed by layer. As in Figure 4, layer IVB of area 17 has been corrected for tissue quenching. *A* and *B*, ^3H -5-HT $B_{\max}$ averaged for 3 adult cases ($\pm$ SEM). *C* and *D*, Specific ^3H -ketanserin binding at a single (2.5 nM) concentration averaged for 2 adult cases ($\pm$ SEM). *E* and *F*, ^3H -flunitrazepam $B_{\max}$ values averaged for 2 adult cases ($\pm$ SEM). *G* and *H*, Specific ^3H -muscimol binding at a single (20 nM) concentration averaged for 2 adult cases. *I* and *J*, ^3H -spiperone $B_{\max}$ values for 2 adult cases ($\pm$ SEM).

³H-prazosin

Films exposed to the sections incubated with prazosin, which binds to α_1 -adrenergic receptors, displayed a considerably less intricate layering pattern than those exposed to sections incubated with clonidine. The α_1 subtype of the noradrenergic (NE) receptor is thought to mediate many of the postsynaptic actions of NE in the brain (Rogawski and Aghajanian, 1980) and prazosin, the α_1 -antagonist, has been shown to selectively block any actions at this receptor (e.g., Menkes et al., 1981). ³H-prazosin has been used in autoradiographic procedures to label α_1 -receptors (Dashwood, 1983; Rainbow and Biegon, 1983). In the present study, in addition to tritiated prazosin, we also employed ¹²⁵I-HEAT (Glossmann et al., 1981; Jones et al., 1983) in selected sections in order to circumvent white matter attenuation of tritium emissions (Herkenham and Sokoloff, 1984; Rainbow et al., 1984a; Unnerstall et al., 1984b). Although the ¹²⁵I-HEAT autoradiograms were not examined quantitatively, the overall binding profile was similar to the pattern of binding observed using tritiated prazosin, indicating that the pattern of binding with ³H-prazosin (described below) could not be attributed solely to white matter attenuation.

Films of sections incubated with ³H-prazosin consistently contained 2 broad bands of intermediate intensity situated in layers I, IVC-alpha, and IVC-beta through V, and a thin, weakly labeled stripe corresponding to sublayers IVA and IVB. The remainder of the cortex was moderately and uniformly labeled (Fig. 6A). This pattern, which was confirmed by the quantitative measurements of specific binding in each layer (Fig. 4C), was present throughout the striate cortex, including the calcarine fissure. However, correction for white matter attenuation of tritium did reveal the binding density of sublayer IVB to be equivalent to adjacent sublayer IVC. Labeling was quite different in area 18 (Fig. 6B). In this cytoarchitectonic field, the light intensity band in layer IV was lost and, except for the higher binding in layer I and particularly upper layer II, the rest of the cortex was essentially uniformly labeled (Figs. 4D, 6B). Although the borderline between areas 17 and 18 was less pronounced in autoradiograms for prazosin than for clonidine, it was readily detectable (Fig. 6B). Likewise, the border between areas 18 and 19 was not discernible. White matter subjacent to both striate and extrastriate areas was slightly above the background level, but the intensity of label was much less than that in any layer of either cortical area (approximately 2 fmol/mg tissue).

¹²⁵I-iodopindolol (IPN)

To localize the β -adrenergic receptor, we used the iodinated antagonist iodopindolol (Rainbow et al., 1984b). Beta-receptor subtypes have been distinguished pharmacologically (β_1 and β_2) using selective agonists and antagonists (Minneman et al., 1979; Rainbow et al., 1984b). Previous membrane and autoradiographic studies demonstrate predominantly β_1 binding (85–90%) in the visual cortex of rodents (McDonald et al., 1982; Rainbow et al., 1984b). The quantitative autoradiographic techniques used in the present study do not discriminate the specific subtype components of β -adrenergic binding. However, preliminary results from our laboratory using β_1 -selective metoprolol and β_2 -selective zinterol as displacing agents (Minneman et al., 1979) suggest that β_1 sites predominate in the primate visual cortex as well (data not shown).

In general, our results indicate that binding of IPN was generally low in both striate and extrastriate areas (Fig. 4, E, F). Labeling in area 17 was uniquely low in layer IVC-alpha and IVC-beta for this ligand, giving the appearance of denser bands on either side—that is, denser for layers IVA, IVB and V. The highest labeling for this ligand was found in layers II and III (Fig. 6C). Examination of the quantitative data indicates that the supragranular layers were slightly more radioactive than the infragranular layers (Fig. 4E). Comparison of the total binding illustrated in Figure 6 and histograms in Figure 4F shows that the dense band in layer IVA is due to nonspecific labeling. This was confirmed in 2 specimens incubated with IPN and provides another example of nonspecific binding that was corrected by subtraction of the dense stripe obtained in sections incubated by displacing drugs.

Areas 18 and 19 showed more uniform binding than area 17, with a gradual decrease in labeling from layer I through VI (Figs. 4F, 6C). For comparison, the specific binding profile for the tritiated binding antagonist, ³H-dihydroalprenolol (Palacios and Kuhar, 1980) was obtained and found to be similar to the ¹²⁵I-iodopindolol binding profile except that binding of ³H-dihydroalprenolol was significantly above background in the white matter of the occipital lobe. Since significant white matter binding was not seen with the ¹²⁵I-iodopindolol, binding to white matter appears to be a property of the dihydroalprenolol compound and not of β -adrenergic sites in general.

³H-quinuclidinyl benzilate (QNB)

Muscarinic cholinergic receptors were labeled by the ligand ³H-quinuclidinyl benzilate (³H-QNB) as employed previously in other mammalian species (Wamsley et al., 1980). While this ligand does not distinguish between the postulated muscarinic agonist subtypes (Birdsall et al., 1978), it does provide an antagonist ligand that would be expected to label all sites with the same high affinity (Wamsley et al., 1980). The potency of this antagonist at cerebral binding sites has a remarkable signal-to-noise ratio (Wamsley et al., 1981) and a short film exposure time, which allowed us to use it both to quantify muscarinic sites and to assess tissue and binding site preservation in each individual case before processing for additional ligands.

Quantitative analysis of autoradiograms reveals that QNB binds considerably more intensely than the adrenergic ligands (Fig. 4G). Furthermore, the distinction between layers appears more pronounced with QNB in both cytoarchitectonic fields (Fig. 6, D, E). In area 17, layers I, IVC-alpha, and VI are the least radioactive, while layers II–III stand out as the most intensely labeled (Figs. 4G, 6D). Intermediate levels of radioactivity were present over layers IVA, IVC-beta, and V. The main difference between areas 17 and 18 was that in the former, there were basically only 2 bands, one dark band in the supragranular layers with a peak intensity in II (300 fmol/mg tissue) and another lighter one (250 fmol/mg tissue) in the infragranular layers (Figs. 4G, 6E). The subgranular dark band stopped at the 17/18 border, although the transition was more gradual than observed in most other ligands (Figs. 4H, 6E). Area 18 had only one dark band (300 fmol/mg tissue) in layer II. The dark band gradually decreased toward VI (Figs. 4H, 6E). Both striate and extrastriate visual areas were uniformly labeled throughout, and the intensity of labeled cortex stood in contrast to the absence of any significant binding in the subcortical white matter (<30 fmol/mg tissue).

Figure 6. Video images of autoradiograms show the distribution of total radiolabeling following incubation with ligands used to map α_1 and β -adrenergic (A–C) and cholinergic (D–E) receptors. A, Distribution of ^3H -prazosin in area 17 of the calcarine fissure. B, ^3H -prazosin at the 17/18 border. C, ^{125}I -iodopindolol at the 17/18 border. D, ^3H -quinuclidinyl benzilate at the 17/18 border. Further details are in the text.

Figure 7. *A*, Low-power video image of ^3H -5-HT binding sites showing the sharp change in distribution of radioactivity at the borderline between areas 17 and 18 (arrowheads) in the adult rhesus monkey. *In vitro* binding of this radioligand provides a reliable means of identifying the size of cytoarchitectonic fields and location of their borders. *B*, Higher-power autoradiogram of the 17/18 border and lamination pattern within the calcarine fissure.

³H-5-hydroxytryptamine (serotonin)

Heterogeneity of the serotonin (5-HT) recognition sites in the brain has been demonstrated by a number of biochemical (Peroutka and Snyder, 1979) and functional studies (Rogawski and Aghajanian, 1980; Aghajanian, 1981; Glennon et al., 1984; Lucki et al., 1984). These receptor sites have been classified as 5-HT₁, labeled by tritiated 5-HT with high (nanomolar) affinity, and 5-HT₂, recognized by high-affinity binding of spiperone (Peroutka and Snyder, 1979). In the present study, we used high-affinity ³H-5-HT binding to determine the laminar distribution and quantification of 5-HT₁ sites within striate and extrastriate areas of the primate visual cortex. Recent pharmacological studies of heterogeneity within the 5-HT₁ site have provided evidence of further subtypes: 5-HT_{1a}, 5-HT_{1b}, and 5-HT_{1c} (Pazos and Palacios, 1985; Pazos et al., 1985). Although an extensive 5-HT₁ subtype characterization was not carried out in the present study in primates, we did examine the ability of the 5-HT_{1a}-selective agonist 8-hydroxy-2-(di-*n*-propylamino) tetralin (80HDPAT, 10 nM) to displace ³H-5-HT binding in both areas of the visual cortex. Our data suggest that there was displacement of ³H-5-HT binding representing 89–93% of total binding, suggesting that most of the 5-HT₁ sites were of the 5-HT_{1a} subtype. No difference in the percentage of displacement by 80HDPAT was observed between striate and extrastriate areas, nor across various laminae.

The binding of ³H-5-HT in the striate areas was among the most distinctly intensive and reliable of all ligands examined and shows a simple well-defined layering pattern (Fig. 7 and *B*_{max} histograms in Fig. 5*A*). After about 2 months exposure time for the Ultrofilm, 3 distinct slender dark bands could be seen in layers IVA, IVC-beta, and VI, with layer IVC-beta standing out as the most intensely radioactive (Figs. 1*B*, 7*B*). The remaining cortical layers were more lightly labeled, producing a highly contrasting image in the autoradiograms (Fig. 7). This profile was largely confirmed by quantitative analysis (Fig. 5*A*). Although significant IVB binding was seen, white matter attenuation prevented this sublayer from appearing heavily labeled. The trilaminar pattern of 5-HT binding changed abruptly at the border of areas 17 and 18 (Fig. 7, *A*, *B*). Because of the large and sudden drop in intense labeling, the border between the 2 areas could easily be recognized at low magnification or even when inspected by the naked eye (Fig. 7*A*). Area 18 was more uniformly and lightly labeled than area 17 (Fig. 5*B*). Radioactivity in the white matter in both areas was at background level. Low radioactivity in the white matter was not due simply to the higher absorption of beta emissions by the myelinated fibers in the subcortical white matter (Unnerstall et al., 1984b), since the 2-month-old infant, containing lesser amounts of myelin, showed an equally low density of grains in the territories below the cortical plate (40 fmol/mg tissue in both adult and infant).

³H-ketanserin (5-HT₂)

Among several candidates for mapping 5-HT receptors (Pazos et al., 1985), ³H-ketanserin has been shown to bind with high affinity to 5-HT₂ sites in the brain (Leysen et al., 1982) with little affinity for dopamine sites (Peroutka and Snyder, 1981). Spiperone was not used because it also has considerable affinity for dopamine sites (Howlett et al., 1979; Palacios et al., 1981a). 5-HT₂ receptors were identified using the specific 5-HT₂ antagonist, ³H-ketanserin (Leysen et al., 1982). However, as previously mentioned, we incorporated cold prazosin in the binding

assay to protect against α_1 -adrenergic binding. In addition, our use of methysergide as the blanking agent assured that α_1 or histamine binding was not included in specific binding to 5-HT₂ sites (Leysen et al., 1982).

Although ³H-ketanserin is used to assess only a portion of the 5-HT receptor sites, it nevertheless takes 25% less exposure time for the Ultrofilm to reach the same level of radioactivity as ³H-5-HT. The binding pattern of ketanserin in area 17 was similar to that of 5-HT (compare Figs. 7 and 8). However, careful examination shows only 2 sharply defined dark bands: one in lower layers III and IVA and the other in IVC-beta. The dark band of label that was present over layer VI in 5-HT autoradiograms was not pronounced in ketanserin-labeled sections (Fig. 5*C*, *D*). An intermediate level of binding was present in layers I, II, III, IVC-alpha, V, and VI. Layer IVB appeared lightly radioactive, but correction for tritium attenuation indicated that the binding was comparable to adjacent IV sublayers (Fig. 8*A*). This intricate binding pattern stopped abruptly at the border of area 17 (Fig. 8*B*). The 17/18 border was sharp because area 18 had a darker band in the middle of the cortex (layer III), a less intense band in layer IV, and an intermediate level of radioactivity without any distinct layering pattern in the rest of the cortex (Figs. 5, *C*, *D*; 8).

³H-flunitrazepam (Flu)

This ligand was used for assessing low-affinity GABA and benzodiazepine receptors. Since recent immunoprecipitation studies of Mohler and coworkers (Haring et al., 1985) indicate that binding sites for benzodiazepines as well as high- and low-affinity GABA sites reside on the same structural complex, it was reasonable to assume that ³H-Flu and ³H-muscimol (described below) sites should colocalize. However, there is a mismatch of these 2 ligands in the rodent cerebellum (Young and Kuhar, 1980c). In addition, ³H-muscimol fails to label deep cerebellar nuclei of the rhesus monkey despite anatomical, physiological, and biochemical evidence of GABAergic innervation (Herkenham, 1985). On the other hand, ³H-Flu binding can be demonstrated in these nuclei, consistent with the presence of the GABA/benzodiazepine complex in this region.

In our hands, ³H-Flu had a distinct bilaminar pattern throughout the entire area 17 (Fig. 9*A*). Careful quantitative analysis revealed 2 intense bands running within layer IVC and only the upper strata of layer VI (Fig. 5*E*). This was, therefore, a good example of binding that was not confined to an entire layer, but rather to only a portion of it, indicating that biochemical specificity exists even in morphologically uniform layers. An intermediate level of binding was present in layers I, II, III, and the upper moiety of VI, and only light radioactivity could be seen in layers IVB and V. Again, the apparent low binding in layer IVB is compensated by tissue quenching. After correction, the density in this layer was comparable to other sublayers (Fig. 5*E*). This pattern also changed abruptly at the 17/18 border (Fig. 9*B*). Although area 18 was also bilaminar, the distribution and intensity were different (Fig. 5*F*). In the latter field, there was one broad band situated over layers II and III, and the other was situated at the border of layers IV and V (the lower stratum of layer IV and the upper stratum of layer V), again exposing a sublaminal pattern. The remainder of the cortex was lightly and rather uniformly labeled. The white matter was devoid of significant amounts of specific binding (Fig. 9, *A*, *B*). *B*_{max} values corresponding to these binding profiles are shown in Figure 5, *E*, *F*.

Figure 8. Video image display of the laminar and areal distribution of ^3H -ketanserin labeling in area 17 (A) and at the 17/18 border (B) of the occipital lobe.

^3H -muscimol

Since the low-affinity GABA site appears to interact with benzodiazepines and mediates physiological responses to GABA (Olsen et al., 1984; Tallman and Gallager, 1985), ^3H -muscimol binding was measured primarily to determine if areas unrecognized by the benzodiazepine ligand could be detected by ^3H -muscimol. Autoradiograms of the slides incubated with this ligand displayed a dark band over layer IVC that stood out rather sharply as a thin, dark line interposed between lighter labeled sublayers IVA and IVB above and layers IVC-beta, V and VI below (Fig. 9C). Supragranular layers II and III were moderately labeled except for the lower stratum in layer IVA and layer I, which were even more lightly labeled. At the border between areas 17 and 18, the dark IVC band totally disappeared and the light density of layers I and IVA blended with the surrounding layers. As a result, area 18 had an essentially bilaminar pattern: the superficial-most layers (I–III) were represented by a single uniform band of moderate intensity, which merged gradually with the lighter radioactivity of layers IV–VI. Radioactivity of the white matter was low, even after correction for quenching. Under the incubation conditions utilized here, ^3H -muscimol apparently binds with multiple affinities; thus, no

reliable B_{max} could be obtained over the concentration range examined. However, the binding profile at a single concentration reflects high-affinity binding as shown in Figure 5, G, H.

^3H -spiperone

Radiolabeled spiperone has been utilized in a number of dopamine receptor binding studies because of its high affinity for dopamine binding sites and low levels of nonspecific binding (Leysen et al., 1978). Although there is evidence for binding to other neurotransmitter binding sites (Peroutka and Snyder, 1979), the use of specific pharmacological agents to displace various sites has been successfully employed to obtain dopamine-specific binding (Palacios et al., 1981a). In our study, binding was carried out in the presence of ketanserin to block 5-HT₂ binding; dopamine-specific binding was that binding remaining after (+)-butaclamol. In area 17, ^3H -spiperone binds most highly to layer IVC-beta; moderately to layers I, II, III, IVA, IVC-alpha, and V; and least to layers IVB and VI (Fig. 9D). No significant binding to white matter was observed, even after correction for quenching. This layering pattern, confirmed by quantitative analysis (Fig. 5E), disappeared at the 17/18 border. Examination of density indicates that area 18 does not display any marked

Figure 9. Video image of the areal and laminar distribution of ^3H -flunitrazepam in area 17 (*A*) and at the 17/18 border (*B*); ^3H -muscimol (*C*) and ^3H -spiperone (*D*) in area 17 of the visual cortex of the adult rhesus monkey (see text).

lamination; rather, it appeared to have maximal binding in the middle layers and to decrease in a continuous gradient above and below these layers without evidence of a strong preference for any layer or sublayer (Fig. 5, *I, J*). The apparent affinities (K_d)

were most variable for this ligand (example: $K_d = 1.1$ for layer IVB, $K_d = 4.2$ for layer VI). However, such changes could not be systematically correlated with B_{max} differences between layers and may represent inherent variability for this ligand binding

assay. Variation in apparent affinity for ^3H -spiperone has been previously reported in the rodent (Altar et al., 1985).

Discussion

Laminar and areal specificity

Even cursory examination of the pattern of binding of the 9 ligands used in this study demonstrates remarkable differential distribution in the primate cortex. The differences in distribution and intensity are so distinct and reliable from animal to animal that the chemoarchitecture of the neocortex, at least for primary versus secondary visual areas, may rival the discrimination of cortical areas by the classical cyto- and myeloarchitectural methods studied extensively at the turn of the century. Some ligands have a preferential affinity for the cell-rich layers; others are more concentrated over strata abundant in fibers (neuropil), but none are exclusively present in one or another cytological compartment in the same or both cortical areas examined. For example, in area 17, spiperone binding is among the densest in layer I, which is cell-poor, and low in layer VI, which is cell-rich (Figs. 10 and 11). In contrast, in area 18 it displayed low affinity for cell-poor layer I and high affinity for cell-rich layer III (Fig. 5, *I, J*). Likewise, within the cell-rich layers, the intensity of binding does not correspond to relative cell densities. QNB, for example, is equally distributed in layers V and IVC-beta of area 17, although they have unequal cell density. In contrast, this same ligand is differentially distributed over layers II and III of the area 18, which have similar cell density and fiber content.

Correspondence and noncorrespondence in the distribution of various ligands within the 2 cytoarchitectonic areas also offer insight into the relationship to the cellular organization of these areas. Our results reveal that 2 ligands may be overlapping in one area and differentially distributed in the other. For example, α_1 - and β -adrenergic receptors are distributed over identical layers in area 18 (Fig. 11, *C, D*), while they occupy different layers in area 17 (Fig. 10, *C, D*). This finding indicates that the 2 subtypes of adrenergic receptor are contained in different cellular compartments that share common territories in area 18 but are distributed in different territories within area 17. Thus, correspondence of 2 ligands in one area does not necessarily indicate their presence on the same cellular elements.

Distribution of some ligands allows further dissection of cytoarchitectonically uniform layers into biochemically distinct sublayers. For example, Flu subdivides layer VI into 2 horizontal zones that are not discernible in Nissl-stained sections but contains neurons that project to 2 different synaptic targets as shown by retrograde transport of HRP (Lund et al., 1975). Some ligands clearly display a distinct and intricate horizontal pattern of distribution with a sharp transition between layers, while others have a broader and more uniform distribution, showing only a slight, graded distribution in certain layers. Thus, analysis of receptor lamination reveals molecular heterogeneity that cannot be detected by classical cytoarchitectonic criteria.

Finally, within a given cytoarchitectonic area all receptors seemed to be distributed uniformly across the tangential plane of the cortex. We could not discern any alternating patches of higher and lower densities that would reflect foci of high concentrations of GAD-positive terminals (Hendrickson et al., 1981) or periodicity of corticocortical connections between areas 17 and 18 (Rockland and Pandya, 1981; Livingstone and Hubel, 1984).

These findings collectively indicate that the distribution of

ligands must correlate with some cellular properties other than a simple division of the cortex into cell- and neuropil-rich compartments, and the recorded differences cannot depend on differential absorptions of beta particles among these 2 principle cellular compartments. Rather, our findings indicate that binding of ligands differentiates between various areas, probably on the basis of their connectivity (see below). Similar, although perhaps less dramatic, differences in ligand binding can be expected for other cytoarchitectonic areas of the cortex. Indeed, areal specificity has already been found in the distribution of specific serotonergic agonists in the primate frontal lobe (Goldman-Rakic et al., 1987).

Correspondence with pattern of connectivity

It is of considerable theoretical and practical interest to relate neurotransmitter laminar distribution to the termination of various afferent systems and/or to the distribution of neural classes that project to specific targets within or outside the cortex. Although this approach appears to be obvious and highly promising, it is constrained by the presence of multiple and often overlapping inputs from various sources to the same layers. Further, as evident from our study, receptors can be localized at present only to a given cortical layer and occasionally to its sublayer. Since most neurons extend their dendrites and axons beyond the boundaries of a given layer, it is not possible to pinpoint neuronal types involved in transmitter receptor interaction. Additionally, because of our still incomplete knowledge of the exact site of termination of afferents and, in particular, of their relative proportion in a single layer, correlation with specific afferent systems, even for well-established receptors, provides only interesting hints but no definitive conclusions. Finally, our ignorance of the preferential distribution of these receptors in the pre- or postsynaptic elements makes any conclusion about their cellular localization rather illusive. Nevertheless, although perfect transmitter-receptor matches are not, at present, possible, the findings of their correspondence or noncorrespondence provide useful leads for future functional and anatomical analyses.

The most intensively labeled bands of ^3H -clonidine in area 17 (Fig. 11*E*) correspond best to the termination of input from the parvocellular layers of the lateral geniculate nucleus in layers IVA, IVC-beta, and the upper moiety of VI (A_1 in Fig. 11*D*). Layer IVC-alpha and the lower moiety of layer III, which receive input from the magnocellular layers of the lateral geniculate nucleus (A_2 in Fig. 11*D*) have a moderate but regularly lower intensity of binding. These 2 classes of thalamic inputs, both originating from the lateral geniculate nucleus, are known to have different physiological properties (Hubel and Wiesel, 1977) and have different biochemical compositions, as shown by the distribution of cell class-specific antigens (Hendry et al., 1984). The preferential binding of the α_2 -adrenergic receptor by ^3H -clonidine in the layers that receive predominately thalamic input is further substantiated by the distribution of this ligand in the adjacent extrastriate visual cortex. In area 18, clonidine has a substantially different distribution, but, again, its localization coincides remarkably well with input from the pulvinar, which projects predominantly to layers I and III. In this respect, the distribution of clonidine in areas 17 and 18 is very similar to that of the cholecystokinin (CCK) receptor that has also been localized to thalamocortical targets in areas 17 and 18, as well as in other cortical areas (Kritzer et al., 1987). Receptors for another monoamine, 5-HT, also seem to be localized to major

Figure 10. Semidiagrammatic representation of layer-specific distribution of 8 radioligands in the striate cortex (area 17) of the adult rhesus monkey. A, Distribution of neurons as revealed in cresyl violet-stained sections in which subdivisions into cortical layers are designated according to Brodmann (1905). Columns B–J display density of *in vitro* binding of radioligands and corresponding receptors, abbreviated in the squares below each column. This diagram is based on the density of grains inspected on the TV monitor in our computer-aided visual imaging system and photographic documentation after subtraction of nonspecific binding (e.g., Fig. 11). Therefore, the intensity corresponds well with the densitometric data obtained from the computer-aided image system, as displayed in Figures 4 and 5, except for layer IVB and white matter, which were affected by attenuation of tritium emission. CV, cresyl violet; CLON, clonidine; PZ, prazosin; IPN, iodopindolol; QNB, quinuclidinyl benzilate; KET, ketamine; FLU, flunitrazepam; MUS, muscimol; SP, spiperone; BEN, benzodiazepine.

Figure 11. Semidiagrammatic representation of 8 radioligands in the prestriate visual cortex (area 18). The method of making this diagram and the abbreviations for ligands and neurotransmitter receptors are the same as in Figure 10, except that quenching was not observed in any layer in area 18.

Figure 12. Composite diagram of the laminar organization of the primary visual cortex of the rhesus monkey integrating observations derived by several techniques (modified from Rakic and Goldman-Rakic, 1982). *A*, Cytoarchitectonic appearance of the primary visual cortex (area 17) in Nissl stain displays horizontal cell stratification into layers and sublayers. *B*, Appearance of visual cortex neurons in Golgi impregnations and afferent axonal plexules in sublayers stained by the reduced silver method. *C*, Simplified diagram of the origin and termination of major neuronal circuits within the cortex. It is evident that stellate cells of sublayers IVC-beta (S_4) and IVA (S_1) project predominantly to layer III, where they synapse either directly upon dendrites of efferent pyramidal cells (P_1 and $P_{2,2,4}$) or indirectly through another local circuit neuron (small pyramidal cell P_2). The majority of stellate cells of sublayer IVC-alpha (e.g., S_3) probably contact efferent pyramids (P_3) within layer V. Some stellate cells of layer IV-beta (S_2) contact nearby neurons within the same layer but also project to adjacent visual association cortex (E_2). However, most visual efferents are formed by large pyramids (P_{3-5}) of layer V, which project to the superior colliculus (E_3); pyramids of upper layers VI (P_4), which project to the parvocellular moiety of the lateral geniculate nucleus (E_4); and pyramids of lower VI (P_5), which project to the magnocellular moiety (E_5). *D*, Relative position of the terminal field originating from 5 major afferent systems: A_1 (from the parvocellular moiety of LGd), A_2 (from the magnocellular moiety of LGd), A_3 (from the superior temporal cortex), A_4 (from the inferior pulvinar), and A_5 (from area 18). *E*, Distribution of the α_2 -adrenergic receptor as exposed by ³H-clonidine binding in area 17.

terminal targets of axons originating in the LGd (compare Fig. 10F with A_1 in Fig. 12D). Thalamic input to layer IV in the monkey is mostly excitatory (Mitzdorf and Singer, 1979), and localization of these 2 monoamine receptors seems to be strategically placed.

Detailed examination of the autoradiograms indicates that, although some ligands can be correlated with the presently known

subcortical inputs to a given cortical area, other ligands (notably QNB and muscimol in both areas 17 and 18) are distributed preferentially in layers that are known to have a high concentration of neurons that issue corticocortical and corticosubcortical projections (Fig. 12, C, D). Thus, QNB has a high density in layers III and V, which contain mostly neurons projecting to other cytoarchitectonic areas and to the superior colliculus (Fig.

4, G, H). On the other hand, GABA receptors in both visual areas are predominantly concentrated in layers II and III (Fig. 5, G, H) that are thought to be major sites of inhibitory action. In addition, there is a distinct dark QNB band in layer IVC-alpha in area 17 that needs physiological explanation.

Although individual ligands in one or the other cortical area may be correlated with a particular layer-specific input or output, such correlations do not hold for all ligands in both areas. Possibly the lack of correspondence reflects our still imperfect knowledge about neural circuitry between and among visual areas. However, the highly specific laminar distribution of receptors, which is independent of cell or fiber density, provides a basis for expecting tighter relationships between the pattern of binding and the pattern of connectivity to become apparent with further analysis of the synaptic architecture of the visual regions.

"Mismatch" of receptors and neurotransmitters

Recently, considerable attention has been given to the "mismatch" between levels of neurotransmitters and the density of relevant receptors in various nuclei and areas of the brain (Kuhar et al., 1986; Herkenham, 1987). The general agreement is that in the CNS, the precise "matches" between transmitters and their putative receptors are the exception rather than the rule. Striking examples of both correspondence and mismatch were uncovered in the present study. The distribution of ^3H -5-HT in our study corresponds rather well with the distribution of 5-HT defined by immunoreactivity (Morrison et al., 1982; Morrison and Foote, 1986). In harmony with the present study, 5-HT immunoreactivity is high in layer IV in area 17, while lower and more diffuse across all layers in area 18. The implication of these findings is that brain-stem monoamine pathways may converge with the thalamocortical innervation. Distribution of the α_1 -adrenergic receptor in areas 17 and 18, as determined by the distribution of prazosin, also seems to correlate, though less perfectly, with inputs from the lateral pulvinar of the posterior thalamus (compare Fig. 10C and input A₄ and A₅ in Fig. 12D).

An example of mismatch was revealed by our analysis of the α_2 -adrenergic receptor, clonidine, which is present in moderate density in layer IVC-beta of area 17 in the rhesus monkey. Immunohistochemical studies of noradrenergic-containing axons in visual cortex of another macaque species, *Macaca fascicularis*, reveal a virtual absence of such fibers in the entirety of layer IV (Morrison and Foote, 1986). If the binding sites indeed signal the receptors where adrenergic transmitters act, perhaps the apparent "mismatch" exposes a limitation of the immunocytochemical methods used to label the terminal arborizations of monoaminergic fiber systems. On the other hand, the density of receptors in layer IVC-beta may be required to compensate for low levels of transmitter available in this territory for its activation (Foote and Morrison, 1987; Herkenham 1987). In support of this idea, ^3H -citalopram binding in area 17 is particularly high in layers IVB and IVC-alpha where 5-HT₁ and 5-HT₂ binding sites are relatively low (Goldman-Rakic et al., 1987). In any event, quantitative receptor autoradiography can complement immunohistochemistry in deciphering the localization of cortical neurotransmitters. For example, immunohistochemical studies show, respectively, low and high densities of norepinephrine and 5-HT fibers in layer IV of primary visual cortex. However, our data show that norepinephrine, 5-HT, and dopamine receptors are all present in layer IVC-beta of area 17, although in different concentrations. Presumably,

then, in the striate cortex, layer IV is a target of both norepinephrine and 5-HT and not a selective target of 5-HT-containing axons.

Species-specific differences

Various cytoarchitectonic properties of the visual cortex are more differentiated in primate than in nonprimate species, and it is reasonable to expect, as evidence in humans already indicates, that the binding of ligands would reflect some of these differences (Palacios et al., 1986). The most obvious cytoarchitectonic difference relevant to the present study is the sharpness of the border between areas 17 and 18, and the complexity of cortical lamination. Another less emphasized difference between the visual cortices of primate and nonprimate species is the distribution of neurotransmitter receptors within the layers that receive input from the magnocellular and parvocellular moieties of the lateral geniculate nucleus. These 2 moieties of the lateral geniculate nucleus reflect the segregation of X- and Y-like neurons that are not separated in nonprimate mammalian species examined so far. They are, however, distinct in macaque monkeys, and they terminate in the well-delineated, separate strata of layer IV (Hubel and Wiesel, 1977). In the present study, the GABAergic ligand, muscimol, showed a clear distinction in binding intensity between the sublayers, layers IVC-alpha and IVC-beta, that receive differential input from the Y- and X-like geniculate neurons, respectively (Fig. 9). In contrast, in mammals in which these 2 classes of cells and terminals are not separated, muscimol binding in the lateral geniculate nucleus exhibits no such difference in distribution and intensity (Needler et al., 1984; Wamsley, 1985).

The similarity in receptor binding among closely related primate species is remarkable, though even here some species-specific differences have been observed. For example, the distribution of 2 of the 3 ligands (QNB, muscimol) that were examined in cynomolgus monkeys by Shaw and Cynader (1986) was qualitatively similar to that in the present study (Shaw and Cynader did not provide quantitative data). On the other hand, the binding of Flu in our material obtained from rhesus monkeys displayed an additional, distinct dark band in the upper stratum of layer VI that was not observed in cynomolgus monkeys, raising the possibility that there may be a difference in distribution of this ligand within the primate order. These findings indicate that *in vitro* autoradiographic localization of receptors may prove a useful parameter for the study of cortical homologies. It can be expected that this type of quantitative analysis will provide new insight into cortical mechanisms in primates and their possible role in a number of neurological disorders in man.

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