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Overlap of Dopaminergic, Adrenergic, and Serotoninergic Receptors and Complementarity of Their Subtypes in Primate Prefrontal Cortex

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Quantitative *in vitro* autoradiography was used to determine and compare the areal and laminar distribution of the major dopaminergic, adrenergic, and serotonergic neurotransmitter receptors in 4 cytoarchitectonic regions of the prefrontal cortex (Walker's areas 12, 46, 9, and 25) in adult rhesus monkeys. The selective ligands, ^{3}H-SCH-23390, ^{3}H-raclopride, ^{3}H-prazosin, and ^{3}H-clonidine were used to label the D_1 and D_2 dopamine receptor subtypes and the α_1- and α_2-adrenergic receptors, respectively, while ^{125}I-iodopindolol was used to detect β-adrenergic receptors. The radioligands, ^{3}H-5-hydroxytryptamine and ^{3}H-ketanserin labeled, respectively, the 5-HT_1 and 5-HT_2 receptors. Densitometry was performed on all cortical layers and sublayers for each of the 7 ligands to allow quantitative as well as qualitative comparison among them in each cytoarchitectonic area.

Although each monoamine receptor was distributed in a distinctive laminar-specific pattern that was remarkably similar from area to area, there was considerable overlap among the dopaminergic, adrenergic, and serotoninergic receptors, while subtypes of the same receptor class tended to have complementary laminar profiles and different concentrations. Thus, the D_1 dopamine, the α_1- and α_2-adrenergic, and the 5-HT_1 receptors were present in highest relative concentration in superficial layers I, II, and IIIa (the "S" group). In contrast, the β_1- and β_2-adrenergic subtypes and the 5-HT_2 receptor had their highest concentrations in the intermediate layers, IIIb and IV (the "I" group), while the D_2 receptor was distinguished by relatively high concentrations in the deep layer V compared to all other layers (the "D" class). Consequently, clear laminar differences were observed in the D_1 vs D_2 dopaminergic, the α- vs β-adrenergic, and the 5-HT_1 vs 5-HT_2 serotoninergic receptor subtypes in all 4 areas examined.

The anatomical overlap of different monoaminergic receptors in the same cortical strata suggests that there may be families of receptors linked by localization on common targets, while the complementary laminar distribution of the D_1 vs D_2, the 5-HT_1 vs 5-HT_2 and the α- vs β-adrenergic receptors raises the possibility that different subtypes within a given class may have distinctive actions in cortex by virtue of their localization on different cells or possibly different portions of the same cell. Understanding the anatomical arrangement of receptors within the cortical layers may aid in the analysis of monoaminergic modulation of higher cortical function.

The prefrontal cortex of primates is widely acknowledged to play an essential role in the regulation of behavior by goals, ideas, and expectations (for review, see Goldman-Rakic, 1987). Modern neuroanatomical tracing techniques have provided unparalleled information about the input–output relationships of this area and, together with fluorescence histochemical, autoradiographic, and immunohistochemical studies, have enabled precise localization of its major neurotransmitters and neuroactive peptides (Levitt et al., 1984; Berger et al., 1986, 1988; Lewis et al., 1986, 1987, 1988; Schwartz and Goldman-Rakic, 1988). The distribution of tyrosine hydroxylase (TH), the rate-limiting enzyme in catecholamine biosynthesis is denser in the dorsomedial and ventrolateral prefrontal areas compared with the principal sulcal cortex (Lewis et al., 1987; Berger et al., 1988). Further, the TH-positive arborizations in these areas have a bilaminar distribution with particularly high concentrations in layer I (Berger et al., 1988; Lewis et al., 1988). Noradrenergic projections to prefrontal areas also have a bilaminar distribution (Levitt et al., 1984), with highest concentrations in the infragranular layers (Morrison et al., 1982; Lewis et al., 1986; Lewis and Morrison, 1989). In contrast, serotoninergic fibers are more concentrated in layer IV and in deep layer III than in other layers (Lewis et al., 1985, 1986). The laminar profiles of some of the monoamine transmitters may be distinctive for prefrontal cortex since the distribution of TH-immunoreactive fibers (Lewis et al., 1986, 1987, 1988) and of labeling due to high-affinity uptake of ^{3}H-dopamine in the prefrontal cortex (Berger et al., 1986, 1988) differ both qualitatively and quantitatively from that in the cingulate gyrus or motor cortex (Berger et al., 1986, 1988; Lewis et al., 1986, 1987).

In contrast to the relatively broad store of information on the monoaminergic innervation in the macaque prefrontal cortex, knowledge about the monoamine receptors which are the targets of these modulatory neurotransmitters is more limited. Detailed information on this subject would be useful in specifying more precisely the postsynaptic targets of various classes of brainstem projections to this part of the primate cortex, as well as for providing much needed information about the potential neural sites of action of psychoactive drugs, including the neuroleptic medications. Accordingly, the present study employed *in vitro* autoradiographic techniques (Kuhar et al., 1986; Palacios et al., 1981) and quantitative densitometry to examine the

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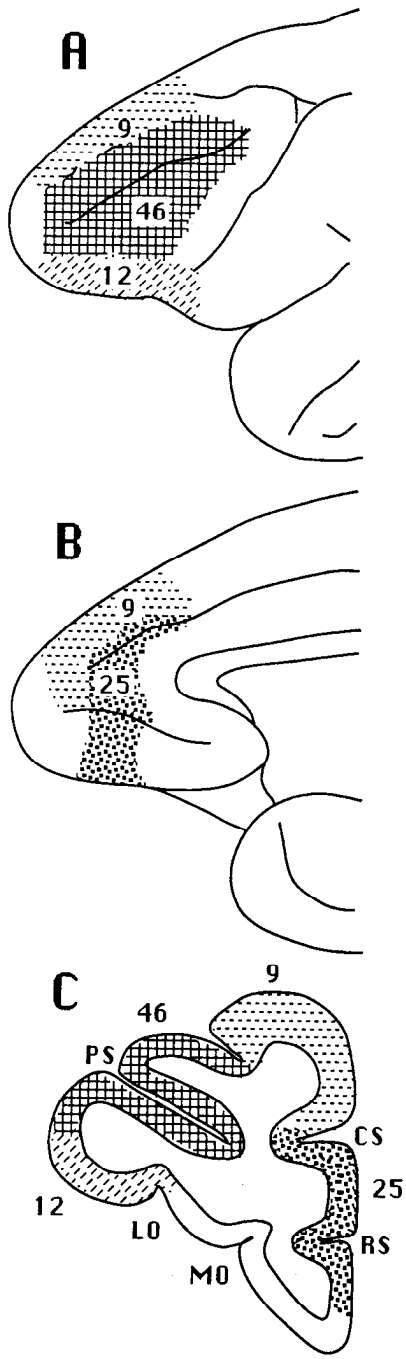

Figure 1. Schematic drawing showing the location of Walker's area 9, 46, 12, and 25 examined in the present study. *PS,* principal sulcus; *LO,* lateral orbital sulcus; *MO,* medial orbital sulcus; *AS,* arcuate sulcus; *CS,* cingulate sulcus; *RS,* rostral sulcus. Cytoarchitectonic areas are numbered according to Walker (1940).

distribution of 7 major monoamine receptor subtypes in 4 distinct prefrontal areas of rhesus monkeys. While α- and β-adrenergic-specific and 5-HT_1 and 5-HT_2 serotonin-specific ligands and their appropriate binding conditions have been known for some time (see Materials and Methods), the recent development of the dopamine-specific ligands, ^{3}H-SCH23390 and ^{3}H-raclopride make it possible to identify the D_1 and D_2 receptors without ambiguity for the first time. Finally, comparison of dopaminergic, adrenergic, and serotonergic receptors in the

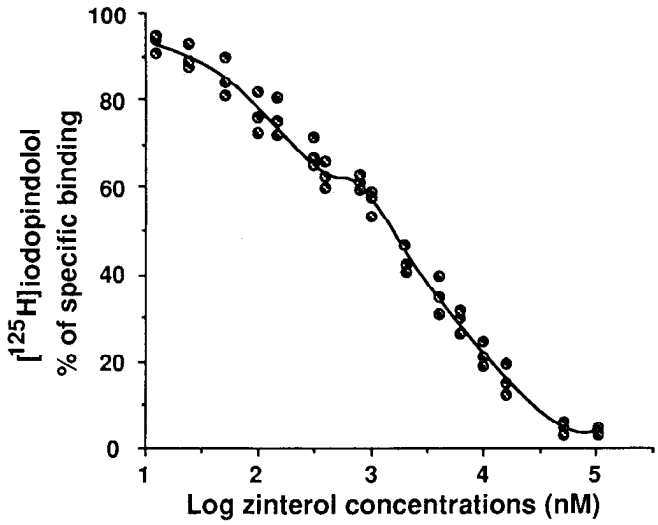

Figure 2. Inhibition of ^{125}I-iodopindolol binding with the β_2-selective agonist, zinterol in layer II of area 9 in one of the monkeys. The line is the computer-generated best fit to a 2-site model. In this case, β_1 receptors (low-affinity sites; K_i = 2.0 μm) constituted 66.3% and β_2 receptors (high-affinity sites; K_i = 0.04 μm) constituted 33.7% of the entire population of β-adrenergic receptors.

very same cytoarchitectonic areas of the same animals provides an opportunity for a comprehensive view of monoamine receptor allocation in prefrontal cortex.

Materials and Methods

Tissue preparation. Data on receptor binding were obtained from 4 adult (2 females, 2 males) rhesus monkeys (*Macaca mulatta*). Prefrontal cortex from each animal was sectioned at 20 μm and assayed independently within 1 or 2 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 1 liter of 0.1% paraformaldehyde containing increasing concentrations of sucrose: 500 ml 0% sucrose; 500 ml 5% sucrose; 1 liter 10% sucrose; 500 ml 15% sucrose; 1 liter 20% sucrose. We have determined that this fixation protocol enhances tissue preservation without measurably decreasing binding or altering kinetic constants. The brains were rapidly removed, blocked, and immersed in isopentane at −30°C for approximately 5 min before storing at −40°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-coated slides, and stored at −80°C until the time of assay.

Binding assays. The comparative analysis of the monoamine receptors in this study was confined to well-characterized ligands which recognize monoaminergic receptor subtypes and, if possible, label the entire subtype. As the binding procedures used have been described previously in Rakic et al. (1988) and Lidow et al. (1989a), they are summarized for each ligand only briefly here and in Table 1. Dopamine D_1 receptors were labeled with the antagonist, ^{3}H-SCH23390 (Faim et al., 1985; Boyson et al., 1986; Dawson et al., 1986, 1987), and D_2 receptors were labeled with the antagonist ^{3}H-raclopride (Kohler and Radesater, 1986; Lidow et al., 1989d). The ^{3}H-SCH23390 binding assay was carried out in the presence of mianserin to prevent its binding to 5-HT_2 (Bischoff et al., 1986) and 5-HT_{1c} sites (Nicklaus et al., 1988).

α_1-Adrenergic receptors were labeled with the antagonist ^{3}H-prazosin (Rainbow and Biegon, 1983; Rakic et al., 1988); high-affinity α_2-adrenergic receptors were labeled with the partial agonist, ^{3}H-clonidine (Young and Kuhar, 1980; Rakic et al., 1988), and the β-adrenergic receptors were labeled with the antagonist ^{125}I-iodopindolol (Rainbow et al., 1984; Aoki et al., 1986; Reznikoff et al., 1986; Rakic et al., 1988). In the case of ^{125}I-iodopindolol, the use of isoproteronol as the blanking agent assured that 5-HT_1 binding was not included in specific binding (Rainbow et al., 1984). The relative percentage of β_1 and β_2 receptors was determined in experiments in which the ability of various concentrations of

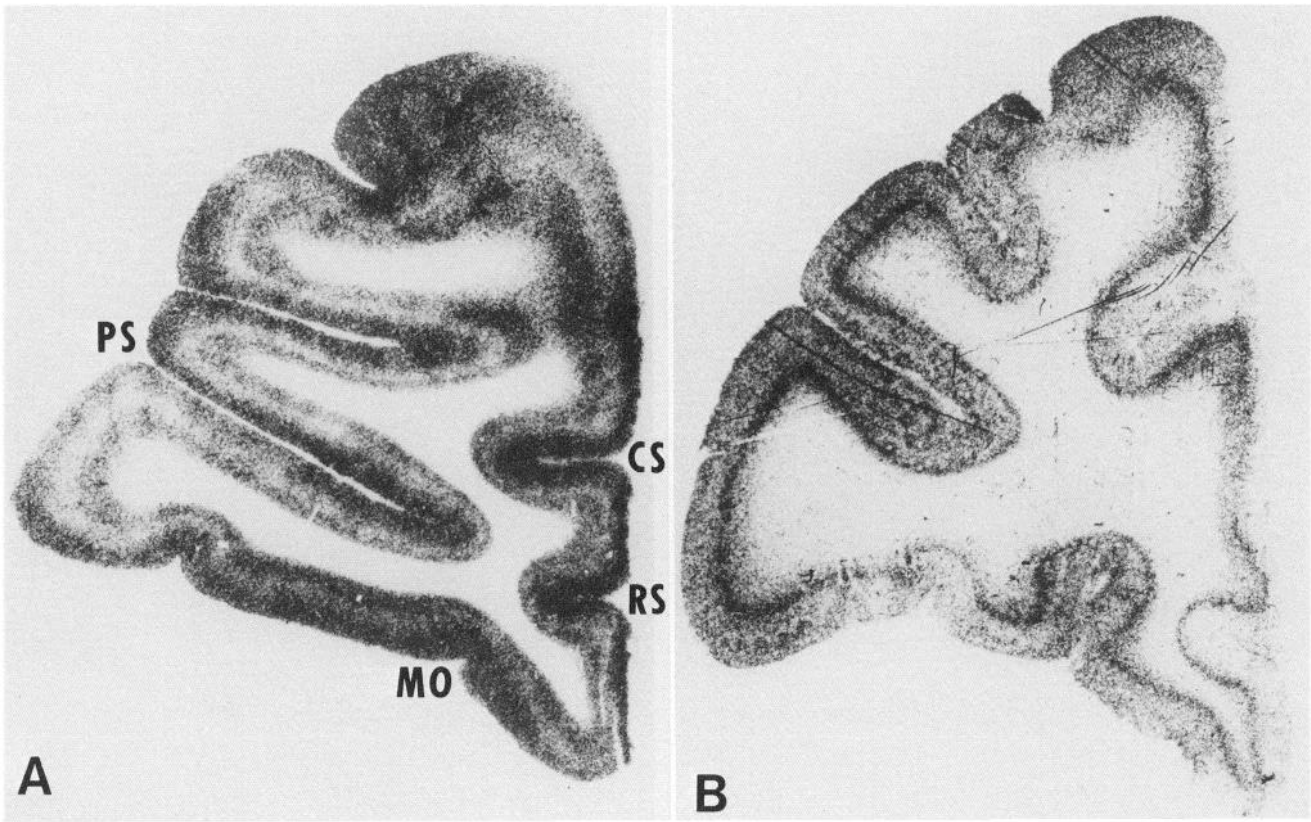

Figure 3. Autoradiographs of coronal sections cut through the prefrontal cortex. *A*, Labeling with ^{3}H-SCH23390 in the presence of mianserin. *B*, Labeling with ^{3}H-raclopride. Abbreviations as in Figure 1.

the β_2 selective agonist zinterol (Minneman et al., 1979) to inhibit the specific binding of ^{125}I-iodopindolol was measured. For this purpose, tissue sections were incubated for 70 min with 150 pM ^{125}I-iodopindolol in the presence of 10 nM–100 mM zinterol. GTP, 100 mM, was added to the incubation buffer (Table 1) in order to reduce agonist-specific negative cooperativity.

Serotonin 5-HT-1 receptors were labeled with the agonist ^{3}H-5-HT (Pazos and Palacios, 1985; Hoyer et al., 1986; Lidow et al., 1989c), and 5-HT$_2$ receptors were labeled with the antagonist ^{3}H-ketanserin (Pazos et al., 1985; Hoyer et al., 1986; Lidow et al., 1989c). The incubation buffer for ^{3}H-5HT binding contained fluoxetine to prevent ^{3}H-5-HT binding to serotonin uptake sites (Fuller, 1985) and pargyline to prevent the oxidation of the ligand by monoamine oxidase present in the tissue (Fuller, 1985). Incubation of tissue sections with ^{3}H-ketanserin was conducted in the presence of prazosin to prevent its binding to α_1 receptors (Leysen et al., 1982). In addition, our use of methysergide as the blank assured that the ligand did not bind to α_1, histamine (Leysen et al., 1982), or a unique ketanserin site associated with dopamine nerve terminals (Leysen et al., 1987).

The binding assays of ^{3}H-raclopride, ^{3}H-prazosin, ^{3}H-clonidine, and ^{3}H-ketanserin included preincubation to eliminate endogenous ligands (see Table 1).

Quantitative densitometry. The density of receptor binding in cortical areas and layers was assessed using an image-analysis system consisting of a DAGE-MTI series 68 video camera and video signal digitizing circuitry interfaced with a Digital VT100 computer. This computer-imaging system allows the overlay on a TV monitor of the digitized images of cresyl violet-stained sections and corresponding autoradiograms in order to histologically identify the autoradiographic image of each cortical layer and sublayer. The system is also capable of subtraction of the film images of sections representing nondisplaceable, hence nonspecific, binding from film images of adjacent sections with total binding, thus allowing direct on-screen observation of the images representing specific binding. Optical densities were converted to concentration of labeled compounds per tissue wet weight for each cortical layer. The optical densities for all autoradiographs produced on Ultrofilm in this study were between 0.08 and 0.80 (diffuse optical density). In this range, they are linearly related to variations in tissue radioactivity (Geary et al., 1985).

The distribution of radioligands was examined in 4 subdivisions of prefrontal cortex following the cytoarchitectonic map of Walker (1940): the inferior frontal gyrus or convexity that extends from the lateral orbital sulcus to beneath the principal sulcus (Walker's area 12); the banks and depths of the principal sulcus (area 46); the dorsomedial cortex lying between the principal sulcus and longitudinal fissure (area 9); and the cortex of the medial wall anterior to the genu of the corpus callosum (area 25) (Fig. 1). Comparable regions were sampled from each animal, and the cytoarchitectonic area from which the reading was taken was confirmed by analysis of Nissl-stained sections. Sections in which the plane of section through the cortex was tangential rather than perpendicular were excluded from analysis.

Statistical analysis. Analysis of saturation binding was performed using the nonlinear curve-fitting computer programs BINKIN2 and FITFUNCTION, which were accessed through the NIH-sponsored PROPHET computer network. The analysis was based on ligand-specific binding obtained with 5 concentrations of free ligand in incubating solutions. We have previously established that 5 concentrations is the minimum number needed to obtain a relatively accurate estimate of B_{max} and K_d for a 1-site receptor model (Lidow et al., 1989c). Twelve replications of total binding (3 replicates/animal) and 4 replications of blanks (1/animal) were measured for each concentration of free ligand. B_{max} and K_d values obtained from different layers of each neocortical area of each animal were compared with the GT2 modification of Gabriel (1978). The latter is a conservative *a posteriori* multiple-comparison method based on analysis of variance that allowed us to compare each layer with each other layer within and between cortical areas. Using this method, 95% confidence intervals were calculated for each K_d or B_{max}, and these values were plotted graphically on histograms in order to provide a comprehensive visual display of all possible comparisons within and between areas. K_d or B_{max} values with overlapping confidence intervals are statistically identical; K_d or B_{max} values in which intervals do not overlap are considered statistically different. Plots of these com-

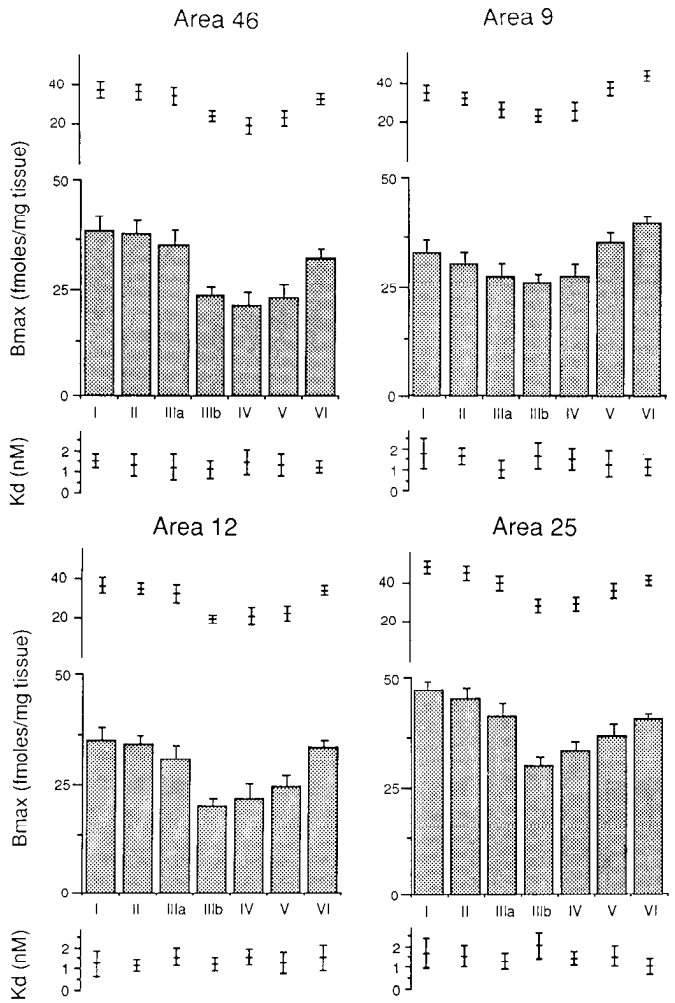

Figure 4. Histograms representing the layer-by-layer distribution of D_1-specific ^{3}H-SCH22390 labeling in Walker's areas 46, 9, 12, and 25. Error bars represent means ± SEM. Note that layers I, II, and IIIa, as well as V and VI, contain the high concentrations of the ligand in all areas, while layers IIIb and IV are characterized by a relatively low density of labeling. The Gabriel coefficients for B_{max} values are displayed at the *top* of each histogram; those for K_d values are presented at the *bottom* of each. In this and all subsequent histograms, K_d or B_{max} value with overlapping confidence intervals do not differ significantly; only those with nonoverlapping intervals are statistically significant.

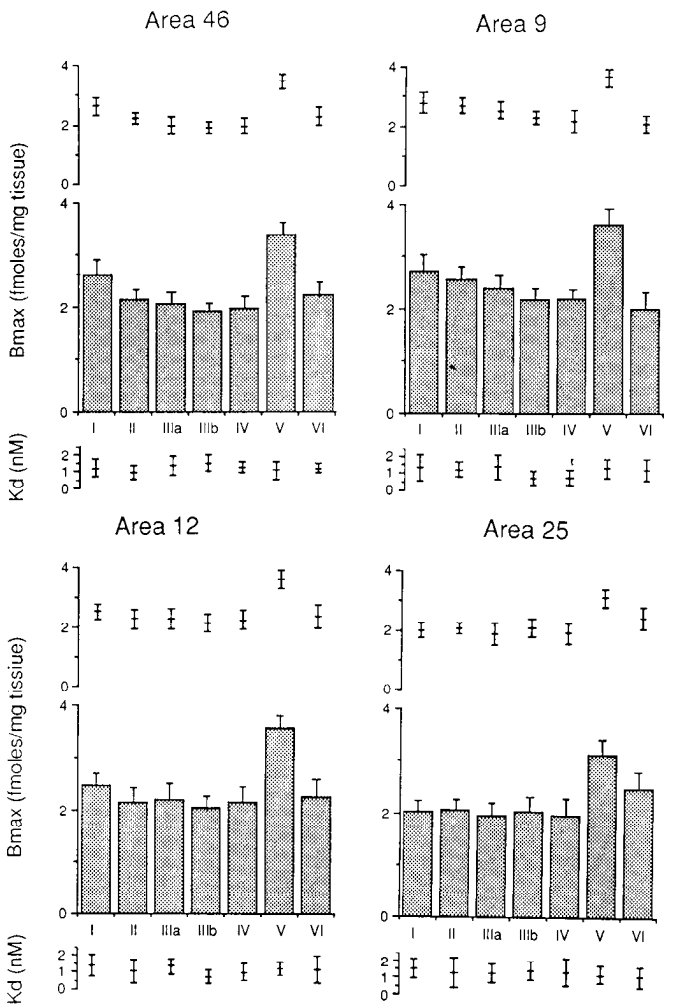

Figure 5. Histograms representing the laminar profile of D_2-specific ^{3}H-raclopride binding in the 4 prefrontal areas. In contrast to Figure 3, the highest concentrations of D_2 receptors are found in layer V. Conventions as in Figure 4.

parison intervals are provided in the histograms of this report (Figs. 4, 5, 7, 8, 10, 11, 13, and 14) to facilitate comparison of variation in K_d or B_{max} within each cortical area as well as to identify the layers for which these variations are statistically significant.

Analysis of the inhibition of β-adrenergic binding of ^{125}I-iodopindolol by the β-2 specific agonist, zinterol, were performed using the FITCOMP program, which is a part of the PROPHET computer network. Sixteen concentrations of displacing ligand were used in this particular experiment. A typical inhibition curve is presented in Figure 2. The F test, which compares the sum of the squares of the residuals, showed a statistically significant improvement of fit from a 1-site to a 2-site model ($p < 0.05$). FITCOMP provided percentages of low-affinity β_1 and high-affinity β_2 receptor sites and IC_{50} values for these sites. These percentages and the B_{max} of ^{125}I-iodopindolol for the entire population of β receptors were used to calculate the concentrations of β_1 and β_2 receptor sites. The K_i's for each receptor subtype were calculated by the method of Cheng and Prusoff (1973). The data provided by FITCOMP cannot be used to calculate standard errors of the mean or comparison intervals for K_i's.

Results

Each radioligand had a characteristic laminar distribution in the prefrontal cortex with little variation over the 4 cytoarchitectonic areas examined, and the K_ds for each ligand were statistically equivalent in all layers of all 4 areas (see Figs. 4, 5, 7, 8, 10, 11, 13, and 14). Also, nonspecific binding was low and in no case exceeded 25% of total binding. The specific labeling in areas 12, 46, 9, and 25 is described below both qualitatively and quantitatively.

Dopamine D_1 receptors (^{3}H-SCH23390). In the presence of mianserin, the density of ^{3}H-SCH23390 binding sites in the 6 layers of the cortex ranged from 23 fmol/mg tissue to 47 fmol/mg tissue. ^{3}H-SCH23990-specific binding was present in every layer. However, the labeling was greatest in superficial layers I, II, and IIIa, only somewhat less dense in deep layers V and/or VI and least in middle layers IIIb and IV. Also, this ligand was one of the few examined that had as high or higher density in layer VI compared to layer V. Little regional variation in binding density was observed in this pattern (Figs. 3*A*, 4), but area 25 contained the highest density of binding sites, particularly in the superficial layers.

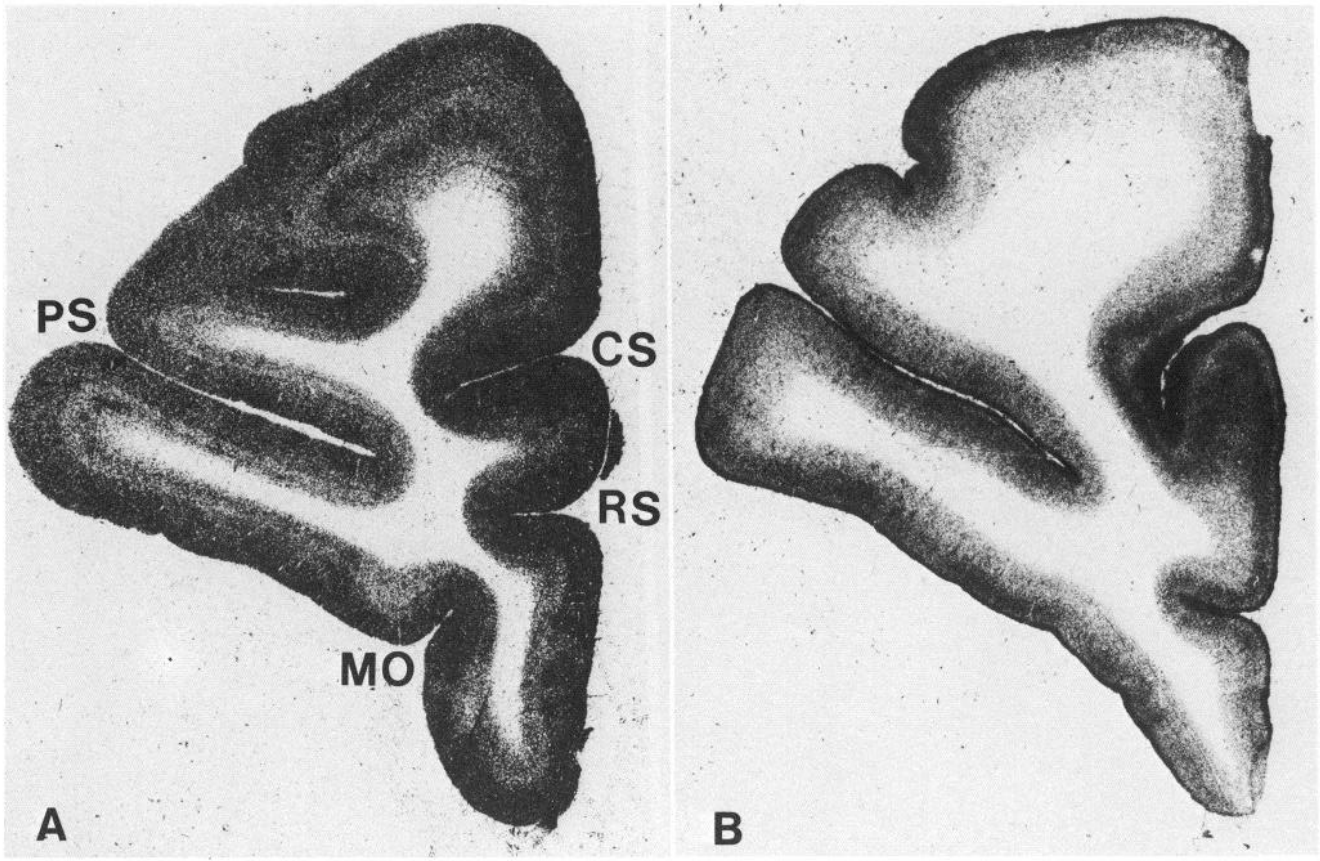

Figure 6. Autoradiographs of prefrontal sections labeled with ^{3}H-prazosin (*A*) and ^{3}H-clonidine (*B*). The complementary nature of the binding patterns in *A* and *B* are evident. Abbreviations as in Figure 1.

Dopamine D_2 receptors (^{3}H-raclopride). The presence of D_2 receptors in cerebral cortex has been questioned (for review, see DeKeyser et al., 1988a; Lidow et al., 1989d) and, indeed the B_{max} values for D_2 specific binding of ^{3}H-raclopride were extremely low, ranging from approximately 2 to 3.5 fmol/mg tissue across the different layers. Nevertheless, a clear and distinctive pattern of labeling resulted: D_2 specific binding was consistently most concentrated in layer V, while the binding in all other layers was lower (Figs. 3*B*, 5). This pattern of stratification, which was amazingly uniform in all 4 prefrontal regions, was unlike that observed with any other ligand.

α_1-Adrenergic receptors (^{3}H-prazosin). The density of α_1-specific binding of ^{3}H-prazosin sites in the cortical layers varied from about 50 to 120 fmol/gm tissue. In all 4 prefrontal areas, α_1-specific binding showed a descending pattern of concentration from layer I through layer IV and then a modest increase again in layers V and VI (areas 46 and 12) or mainly in layer V (areas 9 and 25) (Figs. 6*A*, 7). This pattern was similar to that observed with the D_1-specific ligand, although the concentration of α_1 binding sites in the deeper layers was lower relative to that in superficial strata. Area 25 generally contained the highest absolute binding, which was especially apparent in the supragranular layers.

α_2-Adrenergic receptors (^{3}H-clonidine). The α_2-specific binding of ^{3}H-clonidine ranged from 22 to 62 fmol/mg tissue (Figs. 6*B*, 8). Unlike the α_1 subtype, α_2 receptors generally showed a monotonic pattern of decreasing concentration from layer I through layer VI with the density in layer I being more than twice that in layer VI in all 4 prefrontal areas examined. Area 25 was the only area in which there was any deviation from this pattern. In this area, layer II binding was less than that of both adjacent layers and hence disrupted the descending concentration gradient across the layers. However, both the density and pattern of α_2-specific labeling was remarkably similar among areas 9, 46, and 12.

β-Adrenergic receptors (^{125}I-iodopindolol). Norepinephrine β receptors, as measured by ^{125}I-iodopindolol binding, had extremely low concentrations, ranging from 16 to 25 fmol/mg tissue. Although present in all layers, this labeling was denser in layers IIIa and IIIb than in deeper or more superficial layers in all areas (Figs. 9, 10). This pattern was particularly characteristic of the β_1 subtype which had somewhat higher concentrations than the β_2 subtype (Figs. 11, 12). The β_1 receptor also appeared to be less evenly distributed across the laminae than its β_2 counterpart.

5-HT_1 receptor (^{3}H-5-HT). The concentration of specific binding of ^{3}H-5-HT in the prefrontal cortex was among the highest of all radioligands examined and had a distinctive layering pattern in which the highest density was found in the superficial layers, I, II, and IIIa, the lowest in the intermediate layers IIIb and IV and an intermediate level of binding defined the deepest layers V and VI (Figs. 13, 14). The absolute density of binding was remarkably similar from region to region, including area 25. For example, the B_{max} values for layers I, II, and IIIa were approximately 150 fmol/mg in all 4 areas of cortex examined and half that (75 fmol/mg) in layers IIIb and IV in all 4 areas.

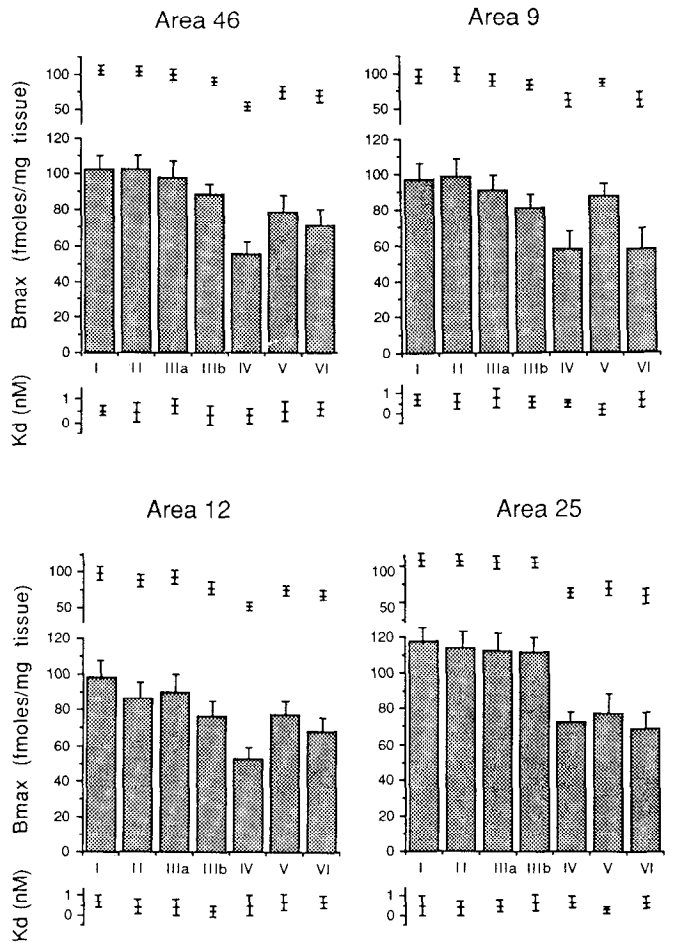

Figure 7. Histograms of layer-by-layer labeling of α_1-specific ^{3}H-prazosin binding. Note that the ligand is most concentrated in the superficial laminae and is least concentrated in layer IV in all 4 areas. Conventions as in Figure 4.

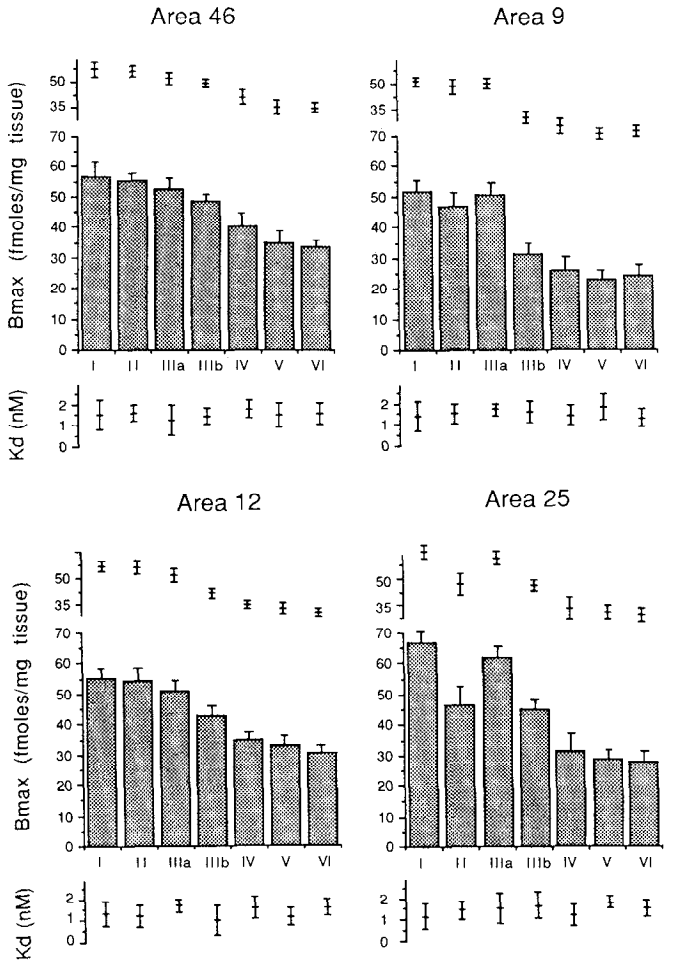

Figure 8. Histograms of α_2-specific ^{3}H-clonidine binding, again showing the highest concentrations to be located in the upper layers, I, II, and IIIa in all areas. Conventions as in Figure 4.

5-HT$_2$ receptors (^{3}H-ketanserin). In the presence of prazosin (Table 1), the density of ^{3}H-ketanserin binding ranged from 25 to 90 fmol/mg tissue and the laminar pattern of binding was virtually identical in all 4 prefrontal subdivisions. The highest concentrations were found in layers IIIa, IIIb, and IV, with lower values in the surrounding layers (Figs. 13, 15).

Discussion

The present analysis of 7 major monoaminergic receptor sites in the primate prefrontal cortex has revealed several findings that may have implications for understanding neurochemical interactions in normal and diseased primate cerebral cortex. First, there is considerable overlap in the location of several dopaminergic, adrenergic, and serotoninergic receptors in the prefrontal cortex. One grouping, which will be referred to as the "S" group, was formed by the D_1, α_1, α_2, and $5HT_1$ subtypes that were most concentrated in the superficial layers, while the β_1, β_2, 5-HT$_2$ receptors (the "I" group) had their highest density in the intermediate layers. The D_2 dopamine receptor subtype (the "D" class) alone was most concentrated in a deep layer (layer V) and evenly distributed across all others. The D_1 receptor could arguably belong to this category as D_1-specific binding was as high or higher in layer VI than in superficial strata in areas 9 and 12.

An unexpected related finding is that the peak concentrations of the different subtypes within a given neurotransmitter category were in some degree complementary. Clear differences characterized the disposition of the D_1 versus D_2 dopaminergic, the α- versus β-adrenergic, and the 5-HT$_1$ versus the 5-HT$_2$ serotonergic receptors. Finally, a striking feature of the present results is the degree of similarity among different prefrontal subregions in the laminar distribution and density of each monoaminergic ligand examined. A common profile across areas was found for all monoamine receptors examined, although some receptors were more concentrated in area 25 compared with the other prefrontal regions. Each of these findings will be discussed in greater detail below.

Overlapping laminar distribution of receptor subtypes

A consistent result in the present study is the preferential localization and high concentration of the D_1 dopaminergic receptor, and α_1- and α_2-adrenergic receptors, and the 5-HT$_1$ serotoninergic receptor in superficial laminae I, II, and IIIa in all areas studied. Similar findings have been obtained in other areas of the cortical mantle (Rakic et al., 1988; Lidow et al., 1989a). It is tempting to speculate that the convergence of different receptors in common cortical strata reflects an association with common cortical elements such as the spine-laden, and hence

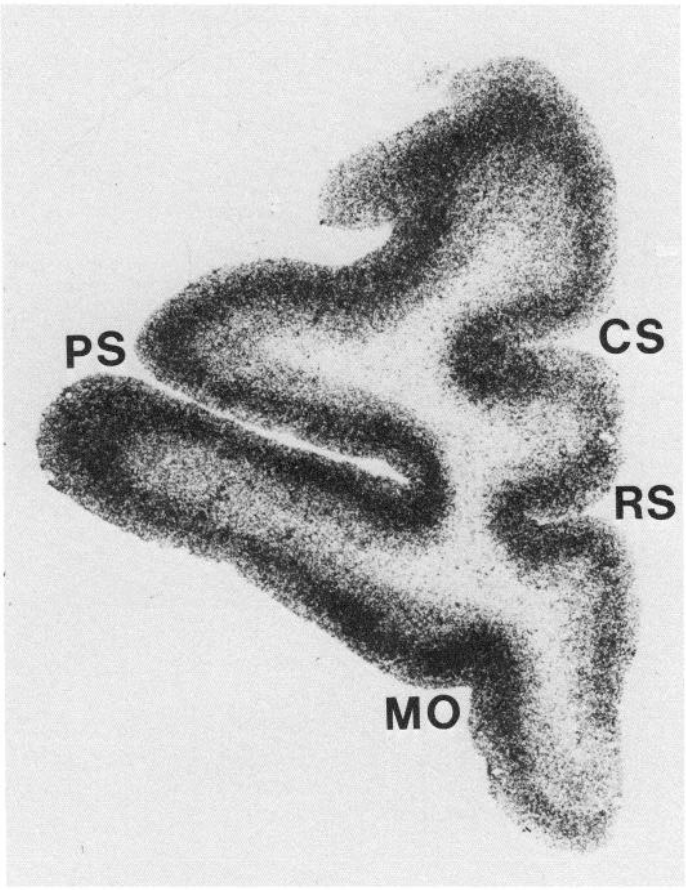

Figure 9. Autoradiograph of ^{125}I-iodopindolol binding in the prefrontal cortex. Abbreviations as in Figure 1.

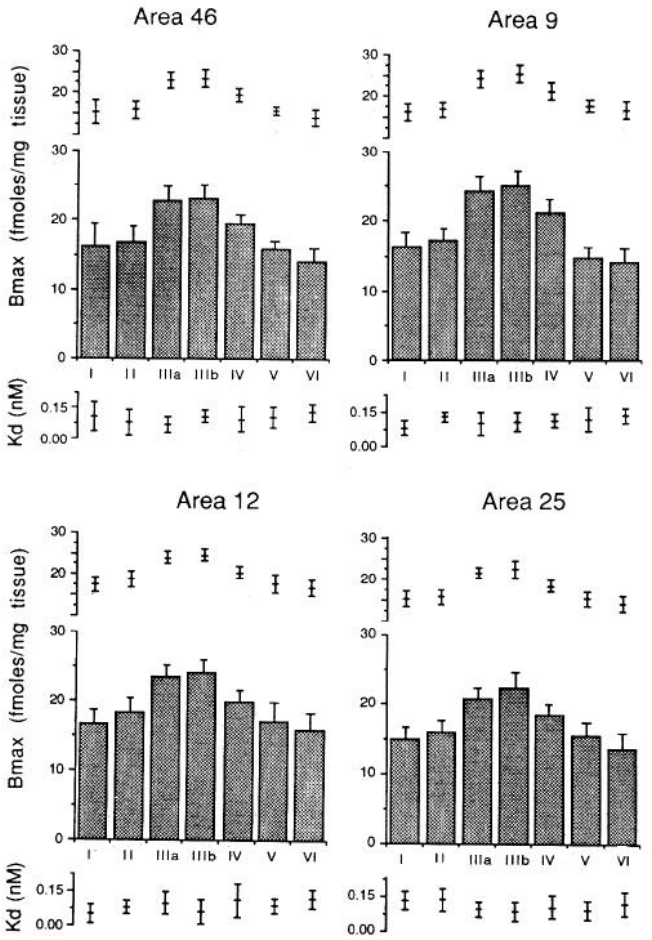

Figure 10. Histogram of β-specific ^{125}I-iodopindolol binding in the various layers of the 4 prefrontal areas. Binding is most concentrated in the intermediate layers in all areas. Conventions as in Figure 4.

synapse-dense, apical dendrites of layer III and V corticocortical neurons, whose dendrites ascend vertically through the cortical layers to the upper strata. Spines are most concentrated on these apical dendrites (Globus and Scheibel, 1966, 1967; Feldman and Dowd, 1975; Parnavelas et al., 1977), and, in general, there is an exponential increase in the mean spine density with increasing distance from the cell body along the apical dendritic shaft (Valverde, 1967; see Feldman, 1984, for review). It may be relevant that the spineheads of pyramidal neurons are a prominent target of DA- or TH-positive boutons in the superficial layers of the macaque prefrontal cortex (Goldman-Rakic et al., 1989). The superficial cortical layers in prefrontal cortex are a prime location for integrative functions as they receive extensive callosal and ipsilateral corticocortical projections (Goldman-Rakic and Schwartz, 1982).

The β-adrenergic and 5-HT$_2$ receptors were also clustered in the same layers, in this case, the intermediate thalamorecipient layers of the cortex. It is possible that these receptors may be associated predominantly with the granule cell or local circuit neurons in layer IV and/or with pyramidal neurons in layers IIIa&b that are the main targets of mediodorsal thalamic (Giguère and Goldman-Rakic, 1988) and corticocortical (Schwartz and Goldman-Rakic, 1984) afferents in primate prefrontal cortex. In addition, the I receptors could also be associated with the smooth and/or spinous surfaces of the proximal dendrites, cell soma, and basilar dendrites of the very same pyramidal neurons that send their distal processes to the superficial layers.

The unique distribution of the D_2 receptor in layer V raises the possibility of an association with the corticostriatal and corticotectal neurons that reside in this layer. We should note, however, that because the D_2 receptor density is so low, every other receptor examined in this study has a concentration in layer V higher than that of D_2, even though each is more concentrated in superficial or intermediate layers. The considerable convergence of monoaminergic receptors in the infragranular layers places them all in position to influence, to one degree or another, the output and feedback functions thought to be localized in these layers. The further dissection of the functional contribution of monoamine receptors in cortex should be aided by detailed knowledge of their precise location within the cortical mantel.

Complementary distribution of receptor subtypes

A remarkable finding in the present study is the complementary distribution of subtypes *within* different neurotransmitter classifications. Thus, while D_1 receptors were concentrated in the superficial and to a lesser degree, the deep strata, D_2 receptor sites were most prominent in layer V. Likewise, α- and β-adrenergic receptor subtypes had their highest concentration in different layers: the α-adrenergic receptors in superficial strata (layers I, II, and IIIa); the β-adrenergic receptors in intermediate strata (IIIb and IV). Finally, the 5-HT$_1$ and 5-HT$_2$ receptors were

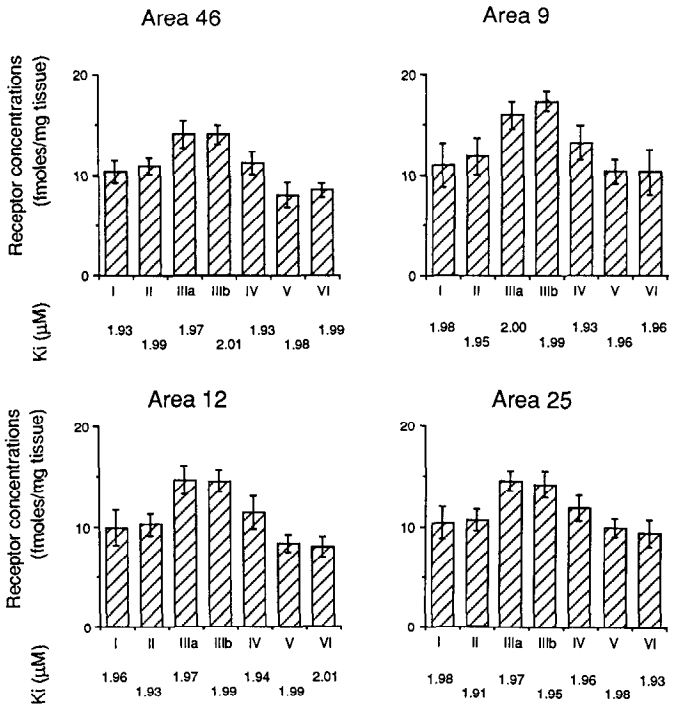

Figure 11. Histograms representing the distributions of β_1 receptors in prefrontal areas obtained as described in the text. Conventions as in previous histograms.

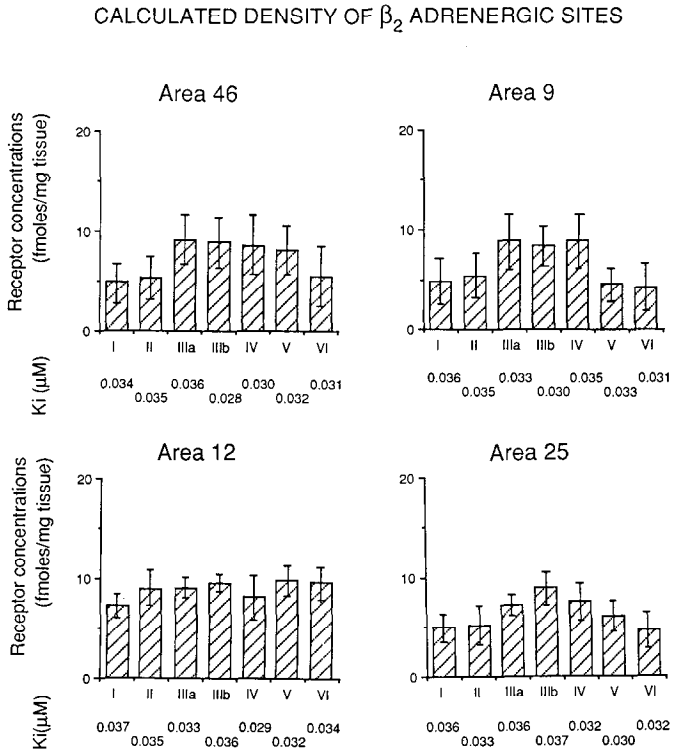

Figure 12. Histograms of β_2 receptors in prefrontal cortex. Conventions as in previous histograms.

concentrated in the superficial and intermediate layers, respectively. Our quantitative analysis permitted the further characterization that for each "pair" of receptor subtypes, one subtype was always more concentrated than the other; the more concentrated was in the superficial layers and was invariably more concentrated even in the deeper layer of peak concentration of its counterpart. This cannot be due to differential subtraction since in all cases, different ligands were used to define subtype specific binding. The consistency of this pattern in all 3 neurotransmitter classes—dopaminergic, adrenergic, and serotoninergic—is notable. One clear functional implication is that the different receptor subtypes are related to different neuronal compartments, either on the same cell (e.g., soma and/or dendrites vs spines) or to different cells (e.g., projection vs. interneuron). Different target structures may, in turn, be related to dual sources of neurotransmitter innervation, as has been described for 5-HT, for example (Wilson et al., 1989). Our finding that dopamine-immunopositive boutons are widely distributed on the spines, dendritic shafts, *and* soma of the same pyramidal neuron (Goldman-Rakic et al., 1989) is compatible with a heterogeneous distribution of receptor subtypes at different sites along the neuron and its processes.

Dopaminergic receptors in prefrontal cortex

Among the neurotransmitter receptors examined in the present study, dopamine receptors are of special interest in relation to the sites of action of neuroleptic medications in the CNS. The recent availability of SCH23390 (Hyttel, 1982; Iorio et al., 1983) has made it possible to selectively label D_1 receptors, and using this compound in the present study we have found that D_1 receptor sites are particularly prominent in layers I, II, III (upper), V, and VI; only layers IV and lower layer III had relatively few D_1 receptors. The widespread and denser distribution of D_1 receptors relative to that of the D_2 receptors in the primate prefrontal cortex is interesting in light of our recent finding that SCH23390 injected into area 46 of rhesus monkeys produced an increased latency and decreased accuracy of response in delayed-response performance, whereas D_2 antagonists were without effect in this region (Sawaguchi and Goldman-Rakic, 1989). However, in the rat, blockade of the D_2 receptor, but not of the D_1 receptor, antagonizes dopamine's inhibitory effects on the spontaneous activity of prefrontal neurons (Sesack and Bunney, 1988). The role of the dopamine receptors in cortical function appears to be quite complicated and may even be different in anesthetized and awake behaving animals or on spontaneous neural activity and integrated behavior; species differences also need to be considered.

The high concentration of D_1-specific ^{3}H-SCH23390 binding in superficial layers of the macaque cortex found in the present study in fact differs from studies in other species. Thus, studies in rat prefrontal cortex show a high density of binding in layer V (Boyson et al., 1986; Dawson et al., 1986), layers V and VI (Richfield et al., 1989), or layers IV, V, and VI (Dawson et al., 1988). It is likely that the discrepancies in laminar profiles between the studies in rodents reflect different degrees of blocking serotoninergic binding. ^{3}H-SCH23390 binds to both 5-HT_2 and 5-HT_{1C} sites in addition to D_1 receptors, and failure to block these additional sites significantly alters the distribution of binding by this ligand (M. S. Lidow, personal communication). Nevertheless, none of the binding patterns observed in rat matches the bilaminar distribution of ^{3}H-SCH23390 binding found in the present study of monkey prefrontal cortex, even when all serotonergic sites are effectively blocked, as they were in one of these studies (Richfield et al., 1989). Thus, species

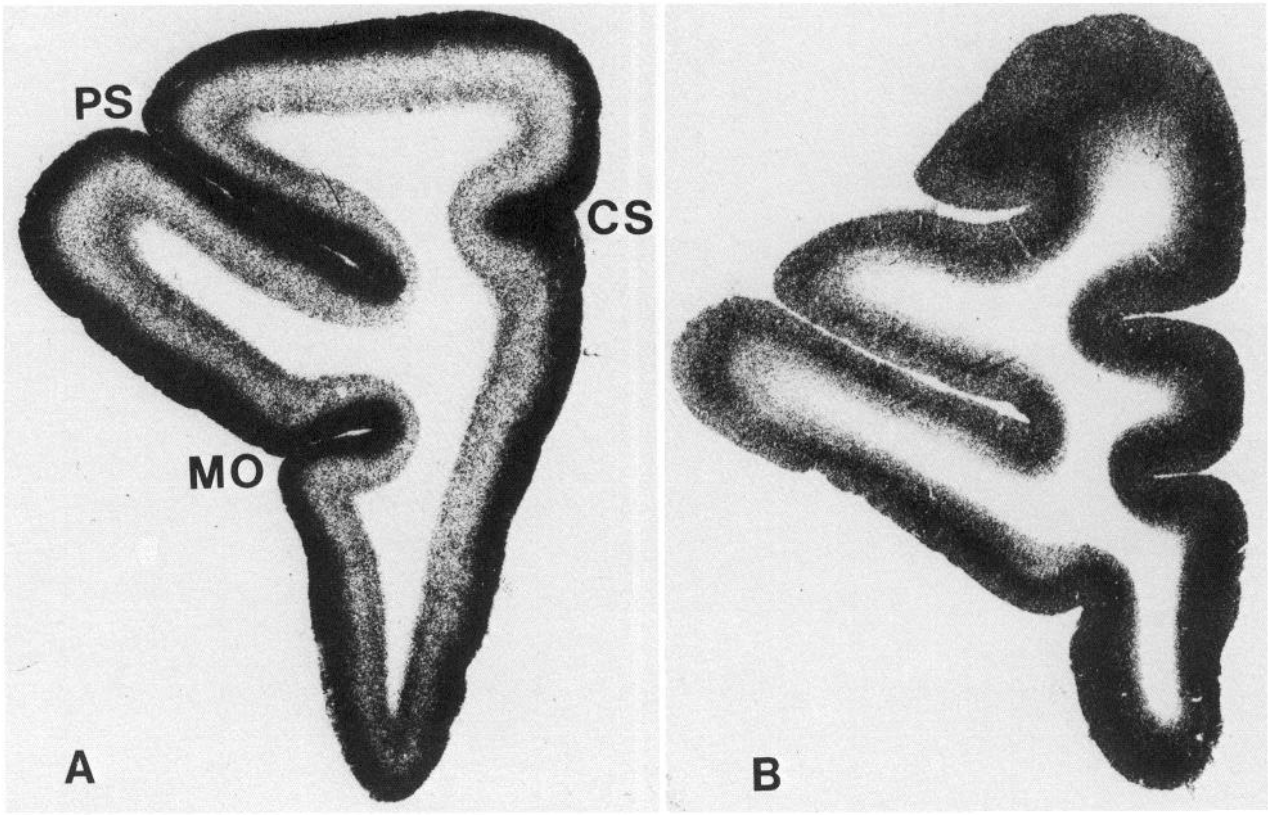

Figure 13. Autoradiographs representing ^{3}H-5-HT (*A*) and ^{3}H-ketanserin in the presence of prazosin (*B*) binding in prefrontal areas. Note complementary pattern of binding of the 2 receptor subtypes in all prefrontal areas shown. Abbreviations as in Figure 1.

differences cannot be ruled out, particularly as a dense dopamine innervation of superficial layers in prefrontal cortex appears to be a primate specialization (Berger et al., 1988); such differences in dopamine input could explain the high concentration of D_1 receptors in the superficial layers found in this study in monkey compared with comparable analyses in the rat.

^{3}H-SCH23390 binding in cat prefrontal cortex is bilaminar as in the monkey, despite very high (up to 75%) nonspecific binding (Richfield et al., 1989). However, Dawson et al. (1987) reported that the highest density of ^{3}H-SCH23390 D_1-specific binding (determined by using pifluxol as blank) in Brodmann's area 9 of human prefrontal cortex was in sublayer Va, and no evidence of the bilaminar pattern characteristic for monkey prefrontal cortex was observed. Thus, a number of different results has been obtained in several studies of prefrontal cortex in rat, cat, monkey, and human. These differences may reflect species differences, particularly between rodent and primate, as discussed above, but a large measure of the variability across studies is likely due to the binding assays used, and specifically to the use of nonspecific ligands.

D_2-binding sites are also present in all layers of the 4 prefrontal areas examined. This bears emphasis because the very existence of D_2 receptors in any cortical area, including prefrontal cortex, has been questioned (see Fallon and Loughlin, 1987, for recent review). The literature ranges from reports of the complete absence of cortical D_2 receptors in cortex (e.g., Altar et al., 1985; Bouthenet et al., 1985; Gehlert et al., 1985; Dubois et al., 1986; Kohler and Radesater, 1986; Charuchinda et al., 1987; DeKeyser et al., 1988) to positive findings (Carboni et al., 1985; Liskowsi and Potter, 1985; Martres et al., 1985; Boyson et al., 1986; Camus et al., 1986; Bouthenet et al., 1985; Palacios and Pazos, 1987; Stefanini et al., 1987). In the present study of primates, we consistently found a low density of D_2 receptors in the prefrontal cortex, and similarly low concentrations were observed in our recent homogenate binding studies with the same D_2-specific antagonist (Lidow et al., 1989d). These findings concur with an earlier study of rodent cortex using the D_2-specific ligand 3H-iodosulpiride (Martres et al., 1985), and together with it, they provide strong evidence that the difficulty in detecting D_2 receptors in the cortex is due to their low concentration combined with the use of nonspecific ligands.

The preferential localization of D_2 receptors in layer V of primate prefrontal cortex is in agreement with a study in the rat by Martres et al. (1985). However, the relatively homogeneous cortical distribution of D_2 sites reported in rat (Boyson, 1986; Richfield et al., 1989) and cat (Richfield et al., 1989) may be due to the assay conditions used. The use of ^{3}H-spiperone in the presence of ketaserin (mianserin) using (−)sulpiride or dopamine as blanks creates very high nonspecific binding which can reach 90% of the total binding (Richfield et al., 1989), with attendant problems in evaluating binding data. The high concentration of D_2 receptors in layers I and II of human cortex found with ^{3}H-CV205-502 and ^{3}H-spiperone in the presence of ketanserin (Camps et al., 1989), with (+)butaclomol as a blank, also does not conform to our findings. Again, the D_2 specificity of this labeling pattern can be questioned because of the binding of both radiolabeled compounds to α-adrenergic and 5-HT_{1A} sites, in addition to the D_2 sites (Lidow et al., 1989d). Also, as

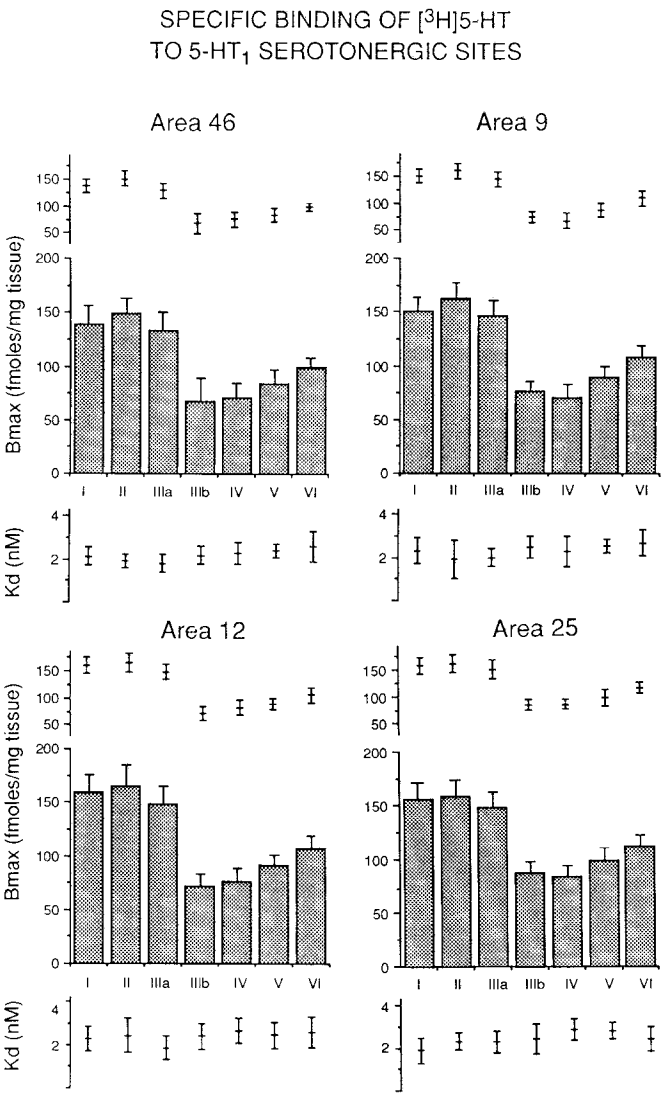

Figure 14. Histograms of 4 prefrontal areas showing the concentration of 5-HT-1 specific binding ^{3}H-5-HT in the various layers. Note the high concentrations in layers I, II, and IIIa in all areas. Conventions as in Figure 4.

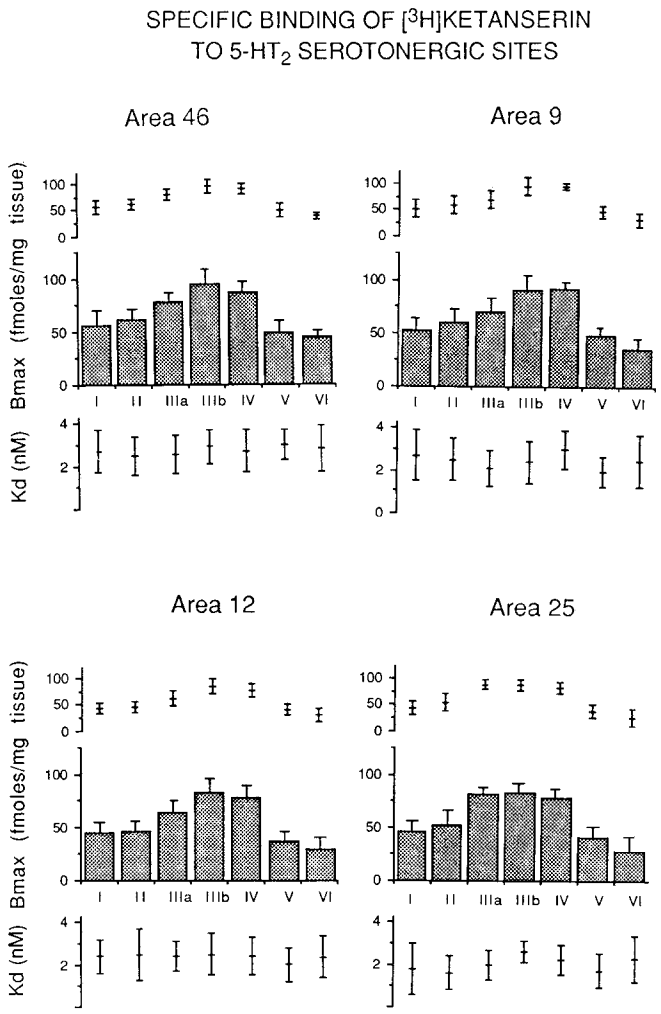

Figure 15. Laminar histograms of 5-HT$_2$-specific ^{3}H-ketanserin binding in the 4 prefrontal areas show the highest density present in the intermediate layers in all 4 areas. Conventions as in Figure 4.

^{3}H-CV205-502 is an agonist, the specificity of its binding is strongly dependent on assay conditions (Camus et al., 1986).

Adrenergic and serotoninergic binding sites

The distribution of adrenergic receptors also appears to exhibit species differences. For example, the highest density of α_1 receptors in rat prefrontal cortex defined with ^{125}I-HEAT (Jones et al., 1985) is found in layers III and IV, while in monkey they are highest in the superficial strata but are abundant in all layers except IV. The distributions of β-adrenergic receptors in monkey and rat prefrontal cortex found with ^{125}I-iodopindolol also do not correspond. Thus, the rat has a homogenous distribution of β sites across all cortical layers (Rainbow et al., 1984), while in monkey layers III and IV have a higher receptor density than other layers.

Our data on 5-HT$_1$-specific binding of ^{3}H-5-HT in monkey are, however, in accord with findings in human prefrontal cortex (Biegon et al., 1986; Pazos et al., 1987a). In all cases, the highest binding is found in the most superficial layers. Again, however, there is a difference with rat prefrontal cortex, where ^{3}H-5-HT binding is highest in layer V rather than in superficial layers (Pazos and Palacios, 1985), a finding reminiscent of the D_1 receptor comparison between rat and monkey described above. In contrast, the highest density 5-HT$_2$-specific binding of ^{3}H-ketanserin is consistently found in the middle cortical layers in monkey, human (Pazos et al., 1987b) and rat (Pazos et al., 1985).

Relationship to monoamine innervation

The specific receptor patterns revealed in different cortical areas generally appear to correspond closely to the monoaminergic innervation of the cortex, although it is not possible from the present results to determine whether the various receptors are located presynaptically on the afferent terminals or postsynaptically on cortical neurons. TH immunohistochemistry (Lewis et al., 1988), dopamine immunohistochemistry (Goldman-Rakic et al., 1989; Williams et al., 1989), and autoradiography of ^{3}H-dopamine uptake (Berger et al., 1986, 1988) all reveal a basic bilaminar pattern of fibers with a high density of fibers in superficial strata and another band in layers V/VI in several areas of prefrontal cortex. The bilamination of the dopamine innervation thus corresponds to our observations on the relatively high concentrations of D_1-binding sites in the superficial layers and D_1 and D_2 sites in the deep layers of prefrontal areas as

Table 1. Protocols of saturation autoradiographic assays

Ligand	Site labeled	Conc. (nM)	Blank (μM)	Protocol	Buffer	Time	Temp.	Exposure time
[^{3}H]SCH23390	dopamine D_1	1–10	SKF83566 (1)	incubation	0.05M Tris-HCl (pH 7.4), 120mM NaCl, 5mM KCl, 2mM $CaCl_2$, 1mM $MgCl_2$, 1.0μM mianserin, 0.05M Tris-HCl (pH 7.4)	90 min	room t°	3 month
				rinse		2 × 10 min	4°C	
[^{3}H]raclopride	dopamine D_2	0.3–3.0	(+)butaclomol (1)	preincubation	50 mM Tris-HCl (pH 7.4), 150mM NaCl, 50mM Tris-HCl (pH 7.4), 150mM NaCl, 5mM KCl, 2mM $CaCl_2$, 1mM $MgCl_2$, 0.1% ascorbic acid 50mM Tris-HCl (pH 7.4)	20 min	room t°	8 month
				incubation		45 min	room t°	
				rinse		6 × 1 min	4°C	
[^{3}H]prazosin	norepinephrine α_1	0.2–1.0	phenylephrine (100)	preincubation	0.17M Tris-HCl (pH 7.1)	30 min	4°C	3.5 month
				incubation	same buffer	70 min	4°C	
				rinse	same buffer	2 × 5 min	4°C	
[^{3}H]clonidine	norepinephrine α_2	0.5–4.0	norepinephrine (100)	preincubation	0.17M Tris-HCl (pH 7.6), 5mM EDTA, 0.17M Tris-HCl (pH 7.6), 1mM $MnCl_2$ 0.1% ascorbic acid 0.17M Tris-HCl (pH 7.6)	30 min	room t°	3.5 month
				incubation		60 min	room t°	
				rinse		2 × 5 min	4°C	
[^{125}I]iodopindolol	norepinephrine β	0.025–0.200	isoproterenol (100)	incubation	0.02M Tris-HCl (pH 7.4), 100 μM phentolamine 0.02M Tris-HCl (pH 7.4)	70 min	room t°	2 d
				rinse		3 × 20 min	4°C	
[^{3}H]5-HT	serotonin 5-HT_1	2–32	serotonin (10)	incubation	0.17M Tris-HCl (pH 7.7), 0.4mM $CaCl_2$, 0.1% ascorbic acid, 1μM fluoxetine 10μM pargyline 0.17M Tris-HCl (pH 7.7)	60 min	room t°	2.5 month
				rinse		5 × 1.0 min	4°C	
[^{3}H]ketanserin	serotonin 5-HT_2	0.5–3.5	methysergide (10)	preincubation	0.05M tris-HCl (pH 7.7), 0.1 μM prazosin	30 min	room t°	1 month
				incubation	same buffer 0.05M Tris-HCl (pH 7.7)	20 min	room t°	
				rinse		5 × 0.4 min	4°C	

contrasted with the intermediate laminae. Yet the correspondence between dopamine afferents and their putative postsynaptic targets is far from precise. For example, dopamine innervation is reported to be densest in deep layer I (Berger et al., 1988; Lewis et al., 1988), whereas no marked sublamination is evident in our autoradiograms, although D_1 binding tends to be slightly higher in layer I than in II and IIIb. Neither could we find a tight relationship between the overall concentration of dopaminergic binding sites and the overall density of the dopamine innervation revealed by TH immunohistochemistry in the cytoarchitectonically defined subdivisions of the prefrontal cortex. Thus, Lewis et al. (1988) found the highest concentration of TH-immunoreactive fibers in Walker's area 9, while the lowest concentration of these fibers was found in area 46, with intermediate concentrations in areas 12 and 25. However, with few exceptions, the B_{max} values for D_1 and D_2 receptor binding in each layer were remarkably similar in all 4 areas.

The noradrenergic innervation of prefrontal cortex, like the dopaminergic innervation, is also reported to be bilaminar (Levitt et al., 1984; Lewis et al., 1988), with bands of fibers in superficial and deep strata and, according to a recent immunohistochemical analysis of dopamine-β-hydroxylase (DBH) staining, more concentrated in the deeper, especially layer V, than superficial cortical layers (Lewis and Morrison, 1989). Consequently, α-adrenergic receptors are dense in superficial layers where the norepinephrine input is weaker, although the moderate density of α_1 receptors present in the deeper layers does correspond to the preferential adrenergic innervation in this layer. The discrepancy between DBH immunohistochemistry and clonidine receptor autoradiography is interesting in light of findings both in rat (U'Pritchard et al., 1979) and monkey (Arnsten and Goldman-Rakic, 1985), indicating that most of the α_2 receptors in prefrontal cortex are postsynaptic.

The 5-HT input to the prefrontal cortex has variously been described as relatively uniform across layers (Lewis et al., 1986, 1988; Berger et al., 1988), somewhat more concentrated in layer IV (Lewis et al., 1985), or slightly more dense in infragranular layers (Lewis et al., 1985). The present findings on 5-HT receptors clearly demonstrate the necessity of specifying the particular receptor subtype that is being "matched" or "mismatched" with afferent input. For example, the 5-HT innervation of prefrontal cortex revealed by tritiated citalopram binding is relatively uniform across layers and corresponds neither to the 5-HT_1 nor the 5-HT_2 subtypes alone but matches their combined complementary distributions (Lidow et al., 1989c). Furthermore, the 2 receptor subtypes may correlate with recent immunohistochemical evidence of dual serotonergic systems innervating the cortex—one originating in the dorsal raphe and innervating the deeper layers of the cortex and the other originating in the medium raphe and terminating in layers I and II (Wilson et al., 1989).

"Prefrontal" patterns of ligand binding

The common laminar profile of most monoamine receptors among the different prefrontal areas, although probably not strictly a function of the similar cytoarchitecture across these areas, is nevertheless in some sense a "signature" of the granular prefrontal cortex. For instance, β-adrenergic receptors are most concentrated in the superficial layers in the somatosensory areas, whereas in prefrontal areas they have a preferential distribution in the intermediate layers. Likewise, α_2 receptors are densest in layer III of areas 1 and 2 and not in the superficial laminae as they are in prefrontal cortex (Lidow et al., 1989a). In addition, the muscarinic M_1 receptor is distributed differently in prefrontal cortex than in many other cortical areas (Lidow et al., 1989b). Thus, in general, the concept of a "prefrontal" archetype for rhesus monkeys seems valid and in accord with other regional differences that have previously been documented in primary and secondary visual areas (Kritzer et al., 1987; Rakic et al., 1988), as well as in primary motor and somatosensory cortices of rhesus monkeys (Lidow et al., 1989a).

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