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Unique Profiles of the α1-, α2-, and β-Adrenergic Receptors in the Developing Cortical Plate and Transient Embryonic Zones of the Rhesus Monkey

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Film receptor autoradiography was used to study the distribution of α1-, α2-, and β-adrenergic receptors in the occipital lobe of developing rhesus monkeys ranging in age from the 65th embryonic day (E65) to adulthood (5 years of age). The study shows that each adrenergic receptor subtype has a unique distribution in the cortical plate and transient embryonic zones of the developing cerebral wall. For example, α1 receptors are prominent throughout the proliferative ventricular and subventricular zones while α2 receptors in the same zones form three distinct bands interdigitated with three bands rich in β sites. There are also considerable temporal differences in the emergence of adrenergic receptor subtypes in specific embryonic zones. For instance, the high density of α1 sites in the germinal zones occurs only in conjunction with intensive proliferative activity. In contrast, β receptors emerge in these zones after the majority of cortical neurons have been generated. The transient embryonic zones often display higher densities of adrenergic sites than the cortical plate itself. In particular, the subplate zone subjacent to the developing visual cortex contains the highest density of α2-adrenergic sites in the cerebral wall throughout all prenatal ages studies. Finally, the regional differences in the density of β-adrenergic receptors are evident in the subplate zone underlying the prospective striate and extrastriate cortex before such differences emerge in the cortical plate. The early appearance, unique pattern of distribution, and time-dependent changes of adrenergic receptors in the transient embryonic zones suggest their involvement in the regulation of the cortical development.

[Key words: visual cortex, ventricular zone, subventricular zone, intermediate zone, marginal zone, receptor autoradiography]

Noradrenergic axons from the locus coeruleus are among the earliest to enter the developing cerebral cortex (for review, see Parnavelas et al., 1988), where they initially form synaptic connections with polymorphic cells of the marginal and subplate zones situated above and below the cortical plate (Caviness and Korde, 1981). It has been shown that most synapses in these two embryonic zones, as well as the early synapses in the cortical plate itself, are noradrenergic (Molliver and Kristt, 1975; Zecevic and Molliver, 1978; Kristt, 1979). Because of the early presence of noradrenaline in the embryonic cerebral wall, it has been suggested that this neurotransmitter may be involved in the regulation of cortical development, including cell division (Cruise et al., 1985; Slotkin et al., 1988; Nakaki et al., 1990), maturation of neurons (Slotkin et al., 1987), synthesis of nerve growth factor (Schwartz and Mishler, 1990), and synaptogenesis (Blue and Parnavelas, 1982). The physiological and biochemical effects of noradrenaline are mediated by three classes of receptors designated as α1, α2, and β (Watson and Abbot, 1992), all of which have been identified in the adult primate cerebral cortex (Rakic et al., 1988; Lidow et al., 1989a; Goldman-Rakic et al., 1990). Although there have been several studies of adrenergic receptors in the developing cortex (McDonald et al., 1982; Aoki et al., 1986; Goffinet et al., 1986; Slesinger et al., 1988; Lidow and Rakic, 1992a; Liu et al., 1992), most of them have focused on the postnatal period. Consequently, very little is known about the distribution of these sites in the fetal cerebral cortex when it contains transient embryonic zones. In order to fill this gap in our knowledge, we conducted an autoradiographic study of the adrenergic receptors in the cerebral wall of the macaque monkeys during both prenatal and postnatal developmental periods. The rhesus monkey were selected for this study because these primates have well-defined cortical cytoarchitecture, similar to that in humans (Brodmann, 1905). Furthermore, the fetal monkeys have highly developed transient ventricular, subventricular, and subplate zones (Rakic, 1975, 1977a; Kostovic and Rakic, 1990), and the normative data exist on the time of neuron origin (Rakic, 1974) and course of synaptogenesis (Rakic et al., 1986; Bourgeois and Rakic, 1993).

In this article, we report that α1-, α2-, and β-adrenergic receptors are present in high density in the transient embryonic zones of the monkey occipital lobe (areas 17 and 18 of Brodmann, 1905) and describe the complex changes in the unique laminar distribution of these sites that accompany the maturation of the monkey telencephalic wall.

A preliminary account of this study has been presented in abstract form (Lidow and Rakic, 1992b).

Materials and Methods

Tissue preparation. Data for the present report were obtained from 26 rhesus monkeys (*Macaca mulatta*) of both sexes ranging in age from embryonic day 65 (E65) to 5 years of age (Table 1). The animals were anesthetized with Na-pentobarbital (40 mg/kg) and perfused with ice-cold phosphate buffer saline (pH 7.4; 1.25 liters) followed by 0.1%

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Table 1. Age and number of monkeys examined

Developmental stage		Age	Number of animals
Formation of subplate	Formation of infragranular layers	E65	1
		E70	2
	Formation of supragranular layers; thalamic axons invade the cortex	E90	2
		E93	1
Dissolution of subplate		E107	1
		E110	1
		E115	1
		E120	1
		E124	1
		E128	1
	Beginning of segregation of LGN axons within the primary visual cortex	E141	1
		E143	1
Term at E165		P1	3
Receptor distribution and density close to those in adults		P365	3
Puberty		P1095	3
Adulthood		P1825	3

E, embryonic age from conception in days. P, postnatal age in days. The identification of developmental stages is based on work by Napier and Napier (1967), Rakic (1974, 1975, 1977a,b, 1983), Rakic et al. (1986), Kostovic and Rakic (1990), and Lidow et al. (1991a).

paraformaldehyde containing increasing concentrations of sucrose in buffered saline: 500 ml of 0% sucrose; 500 ml of 5% sucrose; 1 liter of 10% sucrose; 500 ml of 15% sucrose; and 1 liter of 20% sucrose. This fixation protocol enhances tissue preservation without measurably decreasing binding or altering kinetic constants (Rakic et al., 1988). The brains were rapidly removed, blocked, and immersed in isopentane at −30°C for 5 min before storing at −80°C. Tissue was cut into 20-μm-thick sections on a Bright Cryostat. Sections were mounted on acid-cleaned chrome alum subbed slides and kept at −80°C until the time of assay, conducted no more than 2 weeks after tissue had been sectioned.

Binding assays. The autoradiographic assays in this study were conducted with well-characterized ligands that recognize the entire class of adrenergic receptors. Since the binding procedures used have been described previously in Lidow et al. (1989a), Goldman-Rakic et al. (1990), and Lidow and Rakic (1992a), they are briefly summarized for each radioligand here and in Table 2.

The α1-adrenergic receptors were labeled with the antagonist, ^{3}H-prazosin (Rainbow and Biegon, 1983). Nonspecific binding was determined in the presence of 100 μM phenylephrine. The high-affinity α2 receptors were labeled with the partial agonist ^{125}I-clonidine (Gerhardt et al., 1990). It has been shown that ^{125}I-clonidine also can label imidazoline sites associated with mitochondrial membranes (Parini et al., 1989). To assure the α2 specificity of ^{125}I-clonidine labeling, the parallel tissue sections were labeled with this radioligand in the presence of 100 μM noradrenaline, which does not bind to imidazoline sites. In all cases, noradrenaline was able to displace more than 95% of ^{125}I-clonidine binding, indicating that, under assay conditions used in this study, the radioligand binding to nonadrenergic sites is negligible.

The β-adrenergic receptors were labeled with the antagonist ^{125}I-pin-

Table 2. Protocols of autoradiographic assays for labeling adrenergic receptors

Ligand	Site labeled	Conc. (nM)	Blank	Protocol	Buffer	Time	Temp.	Exposure time
^{3}H-prazosin	noradrenaline α1	0.2–15.0	phenylephrine 100 μM	preincubation	0.17 M Tris-HCl	30 min	4°C	3.5 months
				incubation	same buffer	70 min	4°C	
				rinse	same buffer	2 × 5 min	4°C	
^{125}I-clonidine	noradrenaline α2	0.01–4.00	noradrenaline 100 μM	preincubation	0.17 M Tris-HCl (pH 7.6), 5 mM EDTA	30 min	room temp.	2 days
				incubation	0.17 M Tris-HCl (pH 7.6), 1 mM $MnCl_2$, 0.1% ascorbic acid	60 min	room temp.	
				rinse	0.17 M Tris-HCl (pH 7.6)	2 × 5 min	4°C	
^{125}I-pindolol	noradrenaline β	0.02–2.00	isoproterenol 100 μM	incubation	0.02 M Tris-HCl (pH 7.4), 100 μM phentolamine	70 min	room temp.	2 days
				rinse	0.02 M Tris-HCl (pH 7.4)	3 × 20 min	4°C	

Table 3. Apparent affinity values for binding of adrenergic radioligands during development of the visual cortex (area 17)

	^{3}H-prazosin		^{125}I-clonidine		^{125}I-pindolol	
Age	CP	IZ	CP	IZ	CP	IZ
E65	8.39 ± 0.13	8.35 ± 0.21	9.11 ± 0.21	9.24 ± 0.12	9.77 ± 0.30	9.78 ± 0.25
E90	8.43 ± 0.37	8.41 ± 0.34	9.16 ± 0.16	9.19 ± 0.21	9.70 ± 0.21	9.81 ± 0.34
E115	8.35 ± 0.30	8.50 ± 0.36	9.20 ± 0.31	9.10 ± 0.37	9.68 ± 0.24	9.72 ± 0.22
E124	8.40 ± 0.28		9.17 ± 0.30	9.18 ± 0.29	9.74 ± 0.28	9.79 ± 0.30
E143	8.46 ± 0.23		9.10 ± 0.24		9.74 ± 0.19	
P1	8.44 ± 0.12		9.23 ± 0.30		9.68 ± 0.21	
P365	8.51 ± 0.38		9.15 ± 0.18		9.80 ± 0.25	

Since the apparent affinities of the radioligands have a log-normal distribution (Hancock et al., 1988), the data are presented as -log K_d. CP, cortical plate; IZ, intermediate zone (including subplate and ventricular zone).

dolol (Rainbow et al., 1984; Aoki et al., 1986), and nonspecific binding was determined in the presence of 100 μM isoproterenol. The use of isoproterenol as the blanking agent assured that 5-HT$_1$ binding was not included in the specific binding (Rainbow et al., 1984). The binding assays of ^{3}H-prazosin and ^{125}I-clonidine included a preincubation step to remove endogenous ligands. At least five different concentrations of each radioligand have been used in every assay. In our previous studies (Rakic et al., 1988; Lidow et al., 1989a–c, 1991b, 1992; Goldman-Rakic et al., 1990), we learned that five well-placed data points are sufficient for saturation analysis assuming a one-site receptor model. For every concentration, total and nonspecific binding was determined on three sections. All assays were repeated twice for each animal studied.

After the assay was completed, labeled sections were apposed to ^{3}H-sensitive Ultrofilm (Amersham Corp., Arlington Heights, IL). The exposure times are shown in Table 2. The autoradiograms were developed and tissue sections stained with cresyl violet for analysis of cytoarchitecture. All radioligands were obtained from New England Nuclear (Boston, MA) and the nonradioactive chemicals were purchased from Research Biochemicals (Natick, MA) and Sigma (St. Louis, MO).

Analysis of autoradiograms. Due to the difficulty in obtaining monkey fetuses, we seldom examined more that one animal at each specific age (Table 1). This precluded us from conducting a rigorous quantitative autoradiographic study. Nevertheless, we believe that our results are valid. First, we determined that the receptor affinities to individual radioligands (measured as equilibrium dissociation constant, K_d) did not differ significantly either between various cortical lamina at any single age or between cortices of different ages (Table 3). This indicates that none of the observed changes in receptor density can be related to changes in receptor affinity. Second, cortices of close ages had similar receptor distributions, and we observed a steady and systematic progression of developmental changes in receptor distributions. These observations assure us that we have obtained a rather accurate picture of the distributions of adrenergic receptors at all ages studied.

The autoradiograms were examined with an MCID image analyzer (Imaging Res. Inc., St. Catharines, Ontario, Canada), which allows the overlay of the digitized images of cresyl violet–stained sections and the corresponding autoradiograms on the computer screen in order to facilitate histological identification of specific layers on the autoradiographic images. This computer system was also used for comparison of the optical densities of the film images with those of the ^{3}H- or ^{125}I-standards (Amersham Corp., Arlington Heights, IL) that were apposed to the film along with the tissue sections. The system converts the optical densities of the autoradiograms into concentrations of labeled compounds per tissue volume. On all autoradiograms used in this study, the diffuse optical densities were between 0.08 and 0.80. In this range they were linearly related to tissue radioactivity on ^{3}H-sensitive Ultrofilm (Lidow et al., 1988). When needed, the statistical analysis of saturation binding was performed utilizing the nonlinear curve fitting computer programs KINETIC/EDBA/LIGAND/LOWRY from Elsevier-BIOSOFT Co. (Cambridge, UK). In the case of ^{3}H-prazosin, correction for developmental changes in absorption of ^{3}H-generated emissions was conducted as described earlier by Lidow and Rakic (1992a).

In the present study, we examined primary visual cortex, cytoarchitectonic area 17, and secondary visual cortex, cytoarchitectonic area 18 of Brodmann (1905). Laminar divisions of area 17 were identified according to the criteria of Lund (1973). Laminae for area 18 were defined as described by Brodmann (1905). In E65–E93 specimens, the border between areas 17 and 18 cannot be distinguished on the basis of the cytoarchitectonic criteria. Thus, for these ages, the cortical areas were identified according to position in the occipital lobe (Kostovic and Rakic, 1984).

Results

Prenatal development

Adrenergic receptors were present in the occipital lobe of the monkey cerebral cortex at all prenatal ages examined. In order to facilitate the description of the distributions of α1-, α2-, and β-adrenergic sites, we divided all the fetal specimens into five age groups (two or three specimens each) representing specific stages in cortical development.

E65 and E70. The earliest ages examined in this study correspond to the end of the period when, in the macaque monkey, all the neurons destined for infragranular layers of the visual cortex have been generated (Rakic, 1974). At this stage, the developing cerebral wall contains all the embryonic strata, including the ventricular, subventricular, intermediate, and marginal zones, as well as the cortical plate.

In the regions of prospective cytoarchitectonic areas 17 and 18, the highest densities of α1 receptors, labeled with ^{3}H-prazosin, were seen in the marginal zone, cortical plate, and throughout the proliferative ventricular and subventricular zones (Figs. 1*A*, 5, 8). The densities of α1 receptors in the incipient subplate and intermediate zones were less than 80% compared to those in the above-mentioned laminae (Figs. 1*A*, 5, 8).

In contrast to α1 sites, the highest densities of α2 receptors, labeled with ^{125}I-clonidine, were observed in the subplate and intermediate zones as well as in the marginal zone. The other laminae had at least a 60% lower densities of α2 sites (Figs. 1*B*, 6, 9). The laminar distribution of α2 receptors was similar in both prospective visual areas 17 and 18 (Figs. 1*B*, 9). The distribution of β-adrenergic receptors was dramatically different from those of either α1 or α2 sites. Thus, the high densities of β-adrenergic receptors, labeled with ^{125}I-pindolol, were observed only in the marginal zone of both prospective areas 17 and 18. Other laminae in the embryonic cerebral wall were practically devoid of these receptor sites in the E65 and E70 specimens (Figs. 1*C*, 7, 10).

E90 and E93. During this developmental period, the supragranular layers of the monkey visual cortex are being generated (Rakic, 1974, 1975). This is also the time when axons from specific thalamic nuclei begin to invade the cortical plate in this species (Rakic, 1977b, 1983).

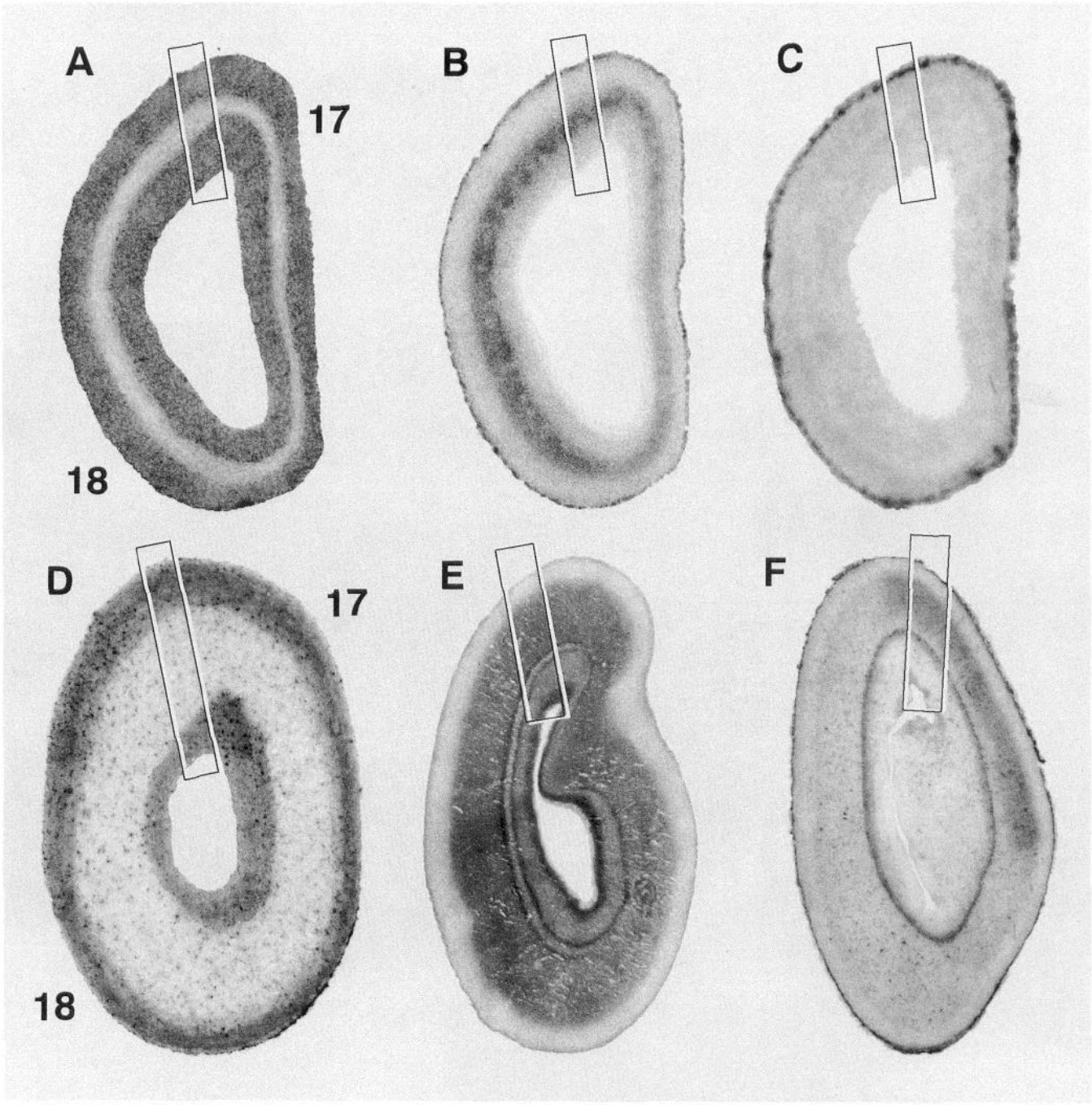

Figure 1. Typical autoradiograms of the occipital lobe at E70 (*A–C*) and E93 (*D–F*). *A* and *D*, ^{3}H-prazosin binding (α1 receptors); *B* and *E*, ^{125}I-clonidine binding (α2 receptors); *C* and *F*, ^{125}I-pindolol binding (β receptors). *17*, prospective cytoarchitectonic area 17; *18*, prospective cytoarchitectonic area 18. *Boxes* indicate areas approximately corresponding to those shown in Figures 5–7. The magnification of each image is adjusted to provide a uniform size, which makes it easier to compare receptor distribution at different developmental ages. The images are provided for comparison of binding patterns only. This and other figures are not suitable for comparison of the absolute receptor densities between ages and different receptors subtypes. These and other autoradiograms were digitized using BDS image analysis system and then printed on the RosterOps CorrectPrint 300i high-resolution printer.

Similar to the earlier ages, the α1 receptors in the E90–E93 specimens were concentrated in the marginal zone, cortical plate, and throughout the ventricular and subventricular zones in the region of both areas 17 and 18 (Figs. 1*D*, 5, 8). The subplate and intermediate zones were almost devoid of these receptor sites.

The highest densities of α2 receptors were observed in the marginal and subplate zones and near the surface of the cerebral ventricle (Figs. 1*E*, 6, 9). The cortical plate had the lowest density of α2 receptor sites, $<50\%$ compared to that in the subplate zone. The marginal zone had a slightly higher density of receptors, yet still substantially lower than in the subplate zone ($\approx 85\%$). Near the surface of the cerebral ventricle underlying the lateral expanse of the occipital lobe, the α2 receptors were distributed

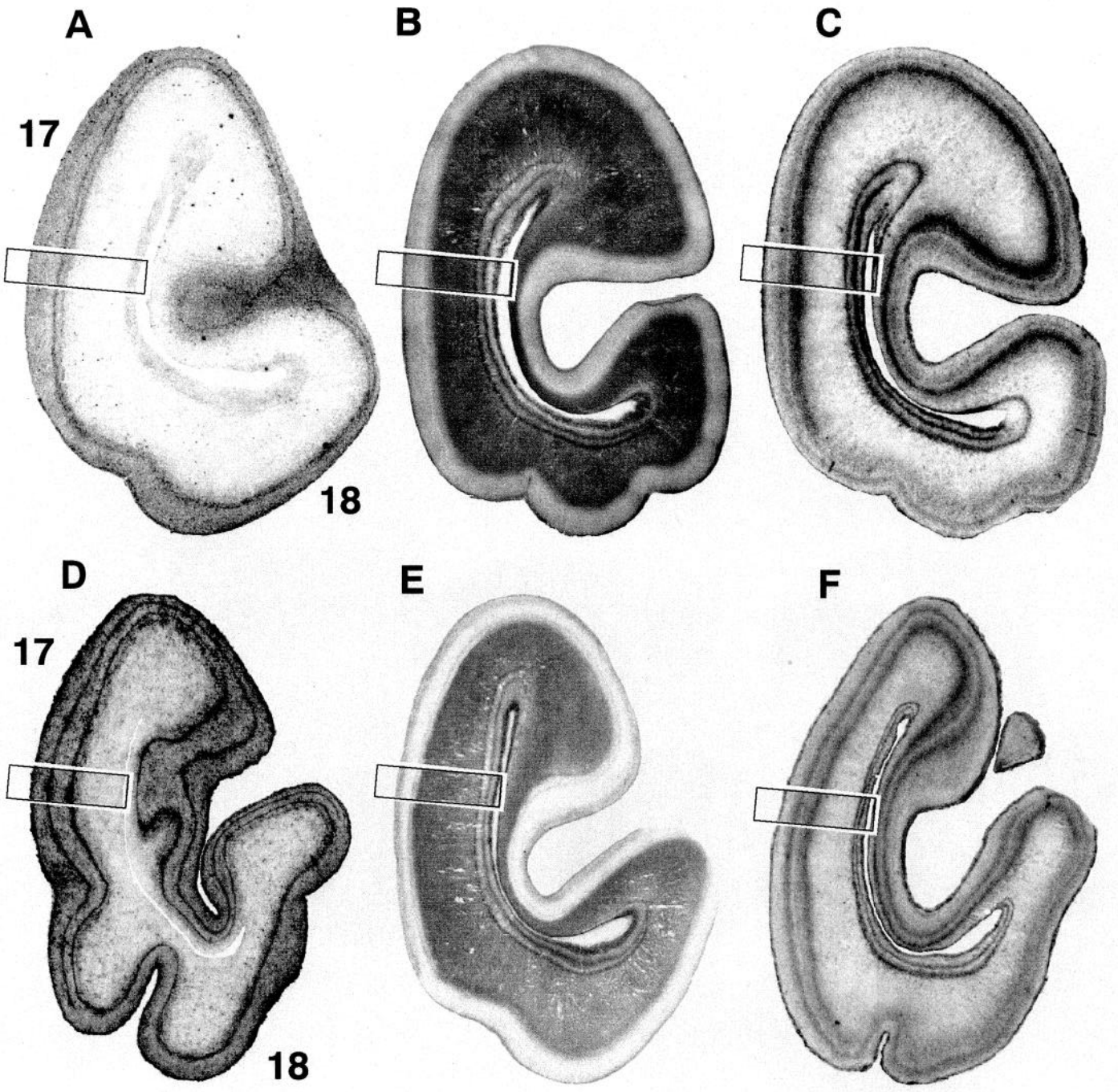

Figure 2. Typical autoradiograms of the occipital lobe at E110 (*A–C*) and E120 (*D–F*). *A* and *D*, ^{3}H-prazosin binding (α1 receptors); *B* and *E*, ^{125}I-clonidine binding (α2 receptors); *C* and *F*, ^{125}I-pindolol binding (β receptors). Conventions are as in Figure 1.

in three distinct narrow bands (Figs. 1*E*, 6, 9). The band of high receptor density closest to the ventricle occupied a cell-poor space between the ventricular and subventricular germinal zones. The second narrow band of high receptor density was located on the border between the subventricular and intermediate zones. The third band was seen slightly above the second one. The space between the second and third bands was characterized by an extremely sparse cell population. These three bands of high density of α2 receptors could be detected under both areas 17 and 18 in the lateral wall of the occipital lobe. However, the distribution of α2 receptors was not multilaminar near the ventricle in the medial part of the occipital lobe. There we observed only one high-density band that abutted with the deepest band in the lateral part of the lobe (Fig. 1*E*).

In contrast to α-adrenergic receptors, β sites in this developmental period diverged in their distribution between cortical areas 17 and 18. While the transient embryonic zones of the occipital lobe subjacent to the prospective area 18 were almost

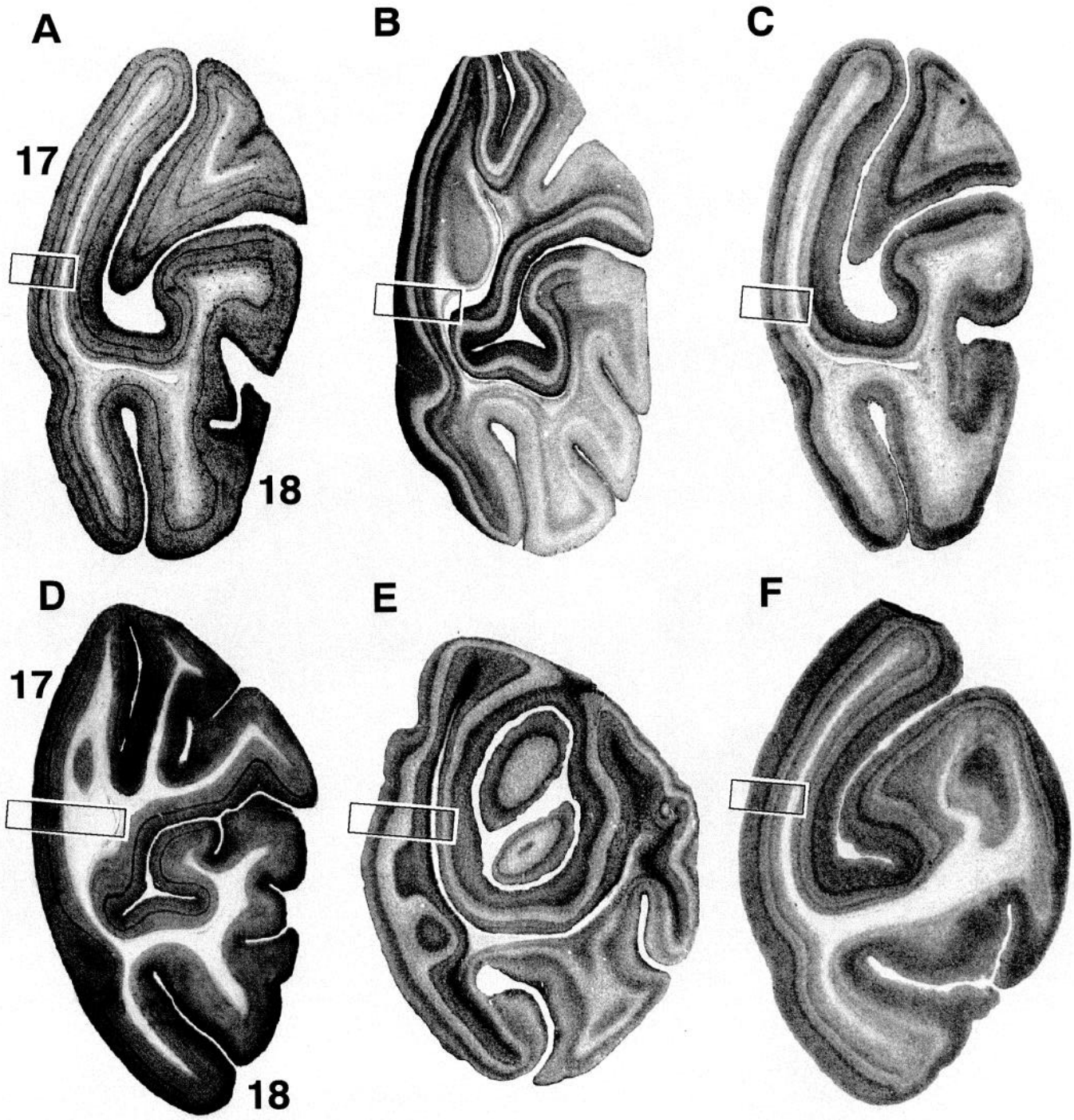

Figure 3. Typical autoradiograms of the occipital lobe at E143 (*A–C*) and P1 (*D–F*). *A* and *D*, ^{3}H-prazosin binding (α1 receptors); *B* and *E*, ^{125}I-clonidine binding (α2 receptors); *C* and *F*, ^{125}I-pindolol binding (β receptors). Conventions are as in Figure 1.

devoid of β receptors, in the region of prospective area 17, the subplate, and the adjacent half of the intermediate zone were heavily labeled by β-specific ligand (Figs. 6*B*, 7). In addition, both visual cortical areas displayed relatively high receptor densities in the marginal zone and in the part of the intermediate zone bordering the subventricular zone (Figs. 1*F*, 7, 10). The latter one corresponds to the cell-poor strip between the two more superficial bands of α2 receptors described earlier. In both E90 and E93 specimens, the cortical plate of the occipital pole was practically devoid of β receptors (Figs. 1*F*, 7, 10).

E107, E110, and E115. This developmental period corresponds to the end of the generation and the completion of migration of cortical neurons in the occipital lobe (Rakic, 1974, 1975). These fetal ages are also characterized by a steady de-

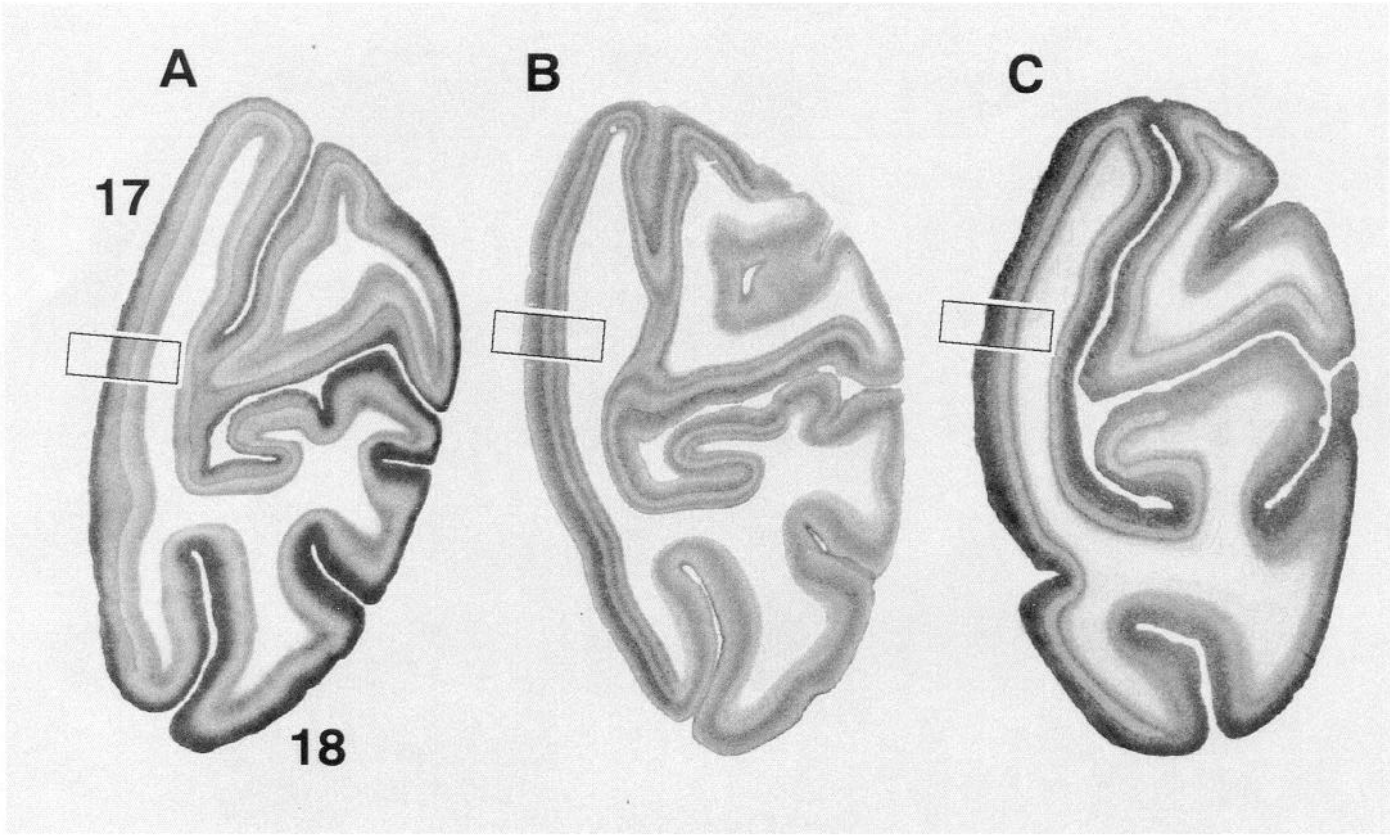

Figure 4. Typical autoradiograms of the occipital lobe of a 1-year-old monkey. *A*, ^{3}H-prazosin binding (α1 receptors); *B*, ^{125}I-clonidine binding (α2 receptors); *C*, ^{125}I-pindolol binding (β receptors). Conventions are as in Figure 1.

crease of the size of the subplate zone, as well as the dissolution of the germinal zone near the cerebral ventricle (Kostovic and Rakic, 1990).

In all three specimens of this age group, the highest densities of the α1 receptors in area 17 were observed in layers I (former marginal zone), IVcβ, and VI (Figs. 2*A*, 5, 8). In area 18, the highest densities of α1 sites were restricted to layers I and VI (Figs. 2*A*, 8). In other layers of both cortical areas, the density of receptor sites was less than 50% of that found in the high-density layers. A slight increase in the density of α1 receptor sites was also detected throughout the germinal zones surrounding the cerebral ventricle (Figs. 2*A*, 5, 8). However, their density in this region at E107–E115 was less than 50% of that calculated for the corresponding region at E65–E93.

The distribution of α2 sites was basically similar to that described for E90–E93 (Figs. 2*B*, 6, 9). We calculated that from E90 to E115, the densities of α2 sites in layer I, the subplate zone, and the three bands near the cerebral ventricle were at least 30% higher than in any lamina of the adult occipital cortex (mean $B_{max} \pm$ SEM for subplate for all animals between age E90 and E115 = 119 ± 21 fmol/mm^3).

In cortical area 17, the highest density of β receptors was observed in layer VI. Slightly lower receptor densities (80–90%) were seen in layers I, IVb, V, and the subplate zone (Figs. 2*C*, 7, 10). In area 18, the highest densities of β sites were detected in layers I, IV, VI, and the subplate zone (Figs. 2*C*, 10). In addition, a high density of β receptors, comparable to that in the subplate zone subjacent to area 17, was observed near the cerebral ventricle (Figs. 2*C*, 7, 10). Here, as in the case of α2 sites, the lateral and medial parts of the occipital lobe had different distributions of β receptors. On the lateral side, the highest density of these receptors could be observed in three thin parallel bands. The deepest band occupied the strip of high cell density situated between the ependyma and the earlier-described α2-rich lamina within the germinal zone. The second band corresponded to a dense cell population overlying this α2-labeled lamina. Finally, the third band corresponded to the deep, cell-poor part of the intermediate zone surrounded by a high density of α2 sites. On the medial side of the occipital lobe, only a single band in the proximity of the cerebral ventricle could be detected (Fig. 2 *C*).

E120, E124, and E128. This is the fetal stage of massive invasion of the geniculocortical fibers into the cortical plate of area 17 (Rakic, 1977b, 1983).

As in the previous developmental stage, area 17 displayed the highest density of α1 receptors in layers I, IVb, IVcβ, and VI (Figs. 2*D*, 5, 8). The distribution of α1 sites in area 18 was also very similar to that observed at the previous age where the highest receptor density was recorded in layers I and VI (Figs. 2*D*, 8). The subplate, intermediate, and germinal zones were practically devoid of α1 receptors at this developmental stage (Figs. 2*D*, 5, 8).

The highest densities of α2 sites were observed in layers I, II, III, and V of area 17 and layers I and VI of area 18 (Figs. 2*E*, 6, 9). In all other layers of both cortical areas, the receptor density was 50–70% of that in the above-mentioned laminae. However, the highest α2 receptor density was detected not in the cortical plate, but in the transient subplate zone (Figs. 2*E*,

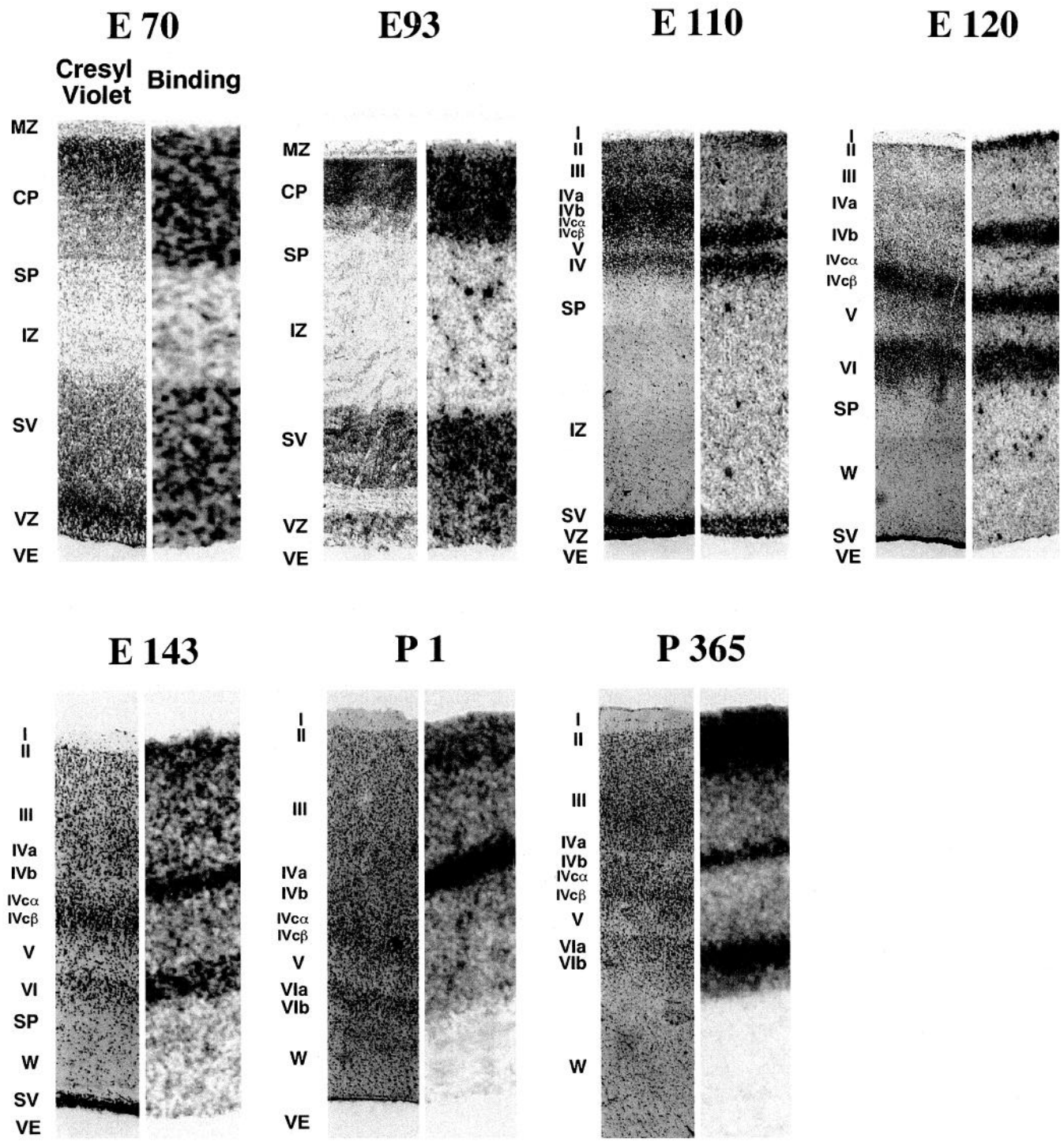

Figure 5. Laminar distribution of ^{3}H-prazosin labeling (α1 receptors) in the developing primary visual cortex (area 17). *MZ*, marginal zone future layer I; *CP*, cortical plate; *SP*, subplate zone; *IZ*, intermediate zone; *W*, white matter (former SP and IZ); *SV*, subventricular germinal zone; *VZ*, ventricular germinal zone; *VE*, ventricle. In order to make evaluation and comparison of the binding patterns easier, the magnification of each image is adjusted to fit uniform dimensions.

6, 9). In this zone, the receptor density was more than 30% higher than in any of the cortical layers. The intermediate zone was also rather rich in α2 receptors (Figs. 2*E*, 6, 9). As in the previous ages, the α2 receptors were organized in three bands near the lateral surface of the cerebral ventricle. Only one band could be observed on the medial side (Figs. 2*E*, 9).

At E120–E128, the highest density of β receptors, in area 17, was observed in layers I, IVb, and VI (Figs. 2*F*, 7, 10). The other cortical layers had receptor densities of less than 60% of those in layers I, IVb, and VI (Figs. 2*F*, 7, 10). In area 18, β receptors were concentrated in layers I, the deep half of III, and IV (Figs. 2*F*, 10). The other cortical layers and the subplate zone displayed a much lower density (50–70%) compared to the above-mentioned laminae (Figs. 2*F*, 10). A high density of β sites was also observed close to the ventricular surface (Figs. 2*B*, 7, 10). On the lateral side, these receptors form three bands similar to

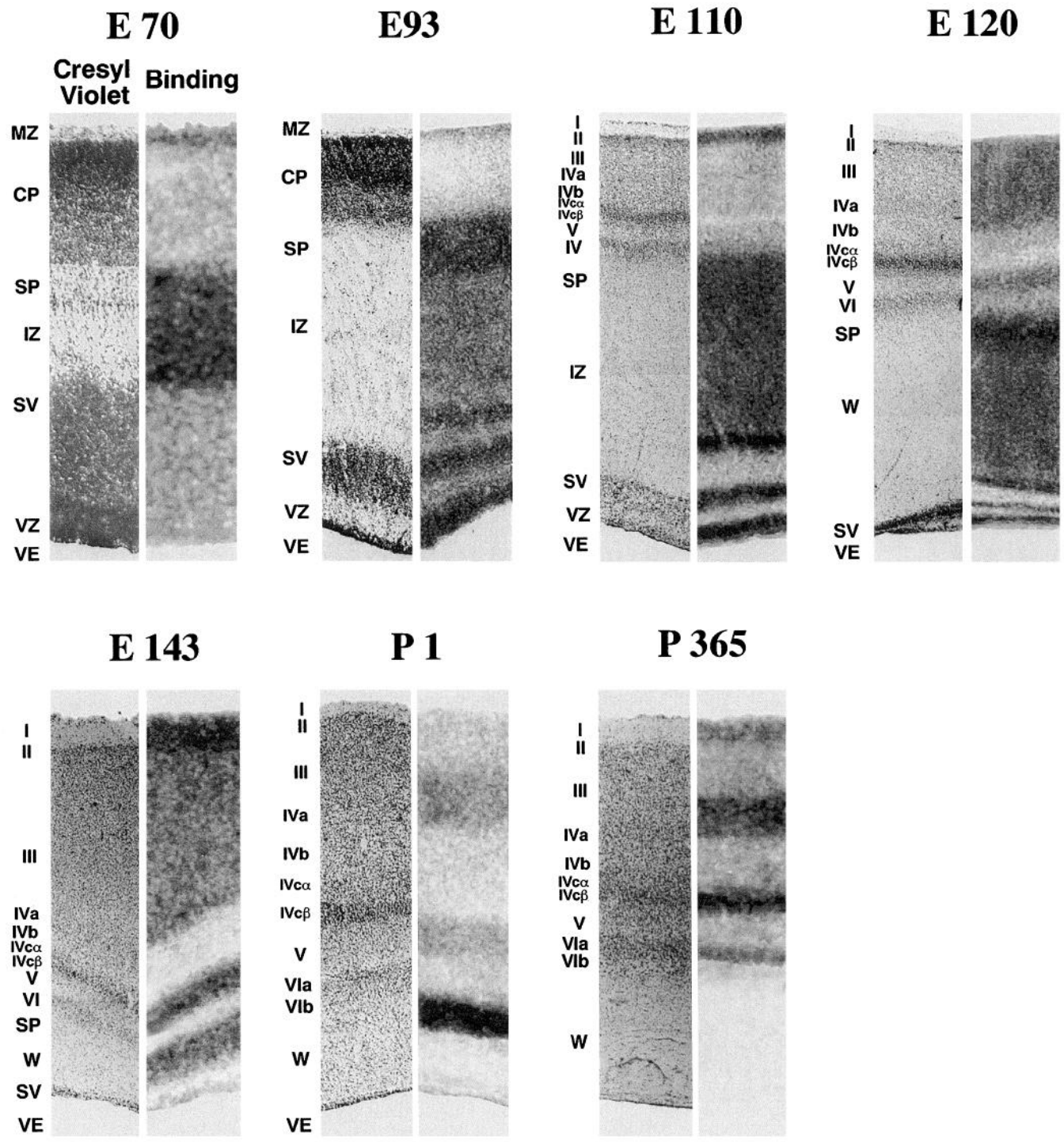

Figure 6. Laminar distribution of ^{125}I-clonidine labeling ($\alpha 2$ receptors) in the developing primary visual cortex (area 17). Conventions are as in Figure 5.

those described at E107–E115. Only one very narrow band of heavy labeling could be found in the medial wall of the occipital lobe near the ependyma (Fig. 2*B*).

E141 and E143. During this final stage of fetal development, geniculocortical fibers in area 17 began to segregate into the different sublaminae of layer IV (Rakic, 1977b, 1983).

At this age, in area 17, the highest density of $\alpha 1$ receptors could be detected in layers I, IVb, and VI (Figs. 3*A*, 5, 8). In area 18, the highest receptor densities were observed in layers I and VI (Figs. 3*A*, 8). Practically no $\alpha 1$-specific labeling was present below the cortical plate in the entire occipital lobe (Figs. 2*F*, 3).

The distribution of $\alpha 2$ receptors in the cortex and the subplate zone was very similar to that recorded in the specimens of earlier age groups (Figs. 3*B*, 6, 9). However, at E141 and E143, we found a very low density of binding in the intermediate zone, and the strip near the ventricular border was only slightly labeled (Figs. 3*B*, 6, 9).

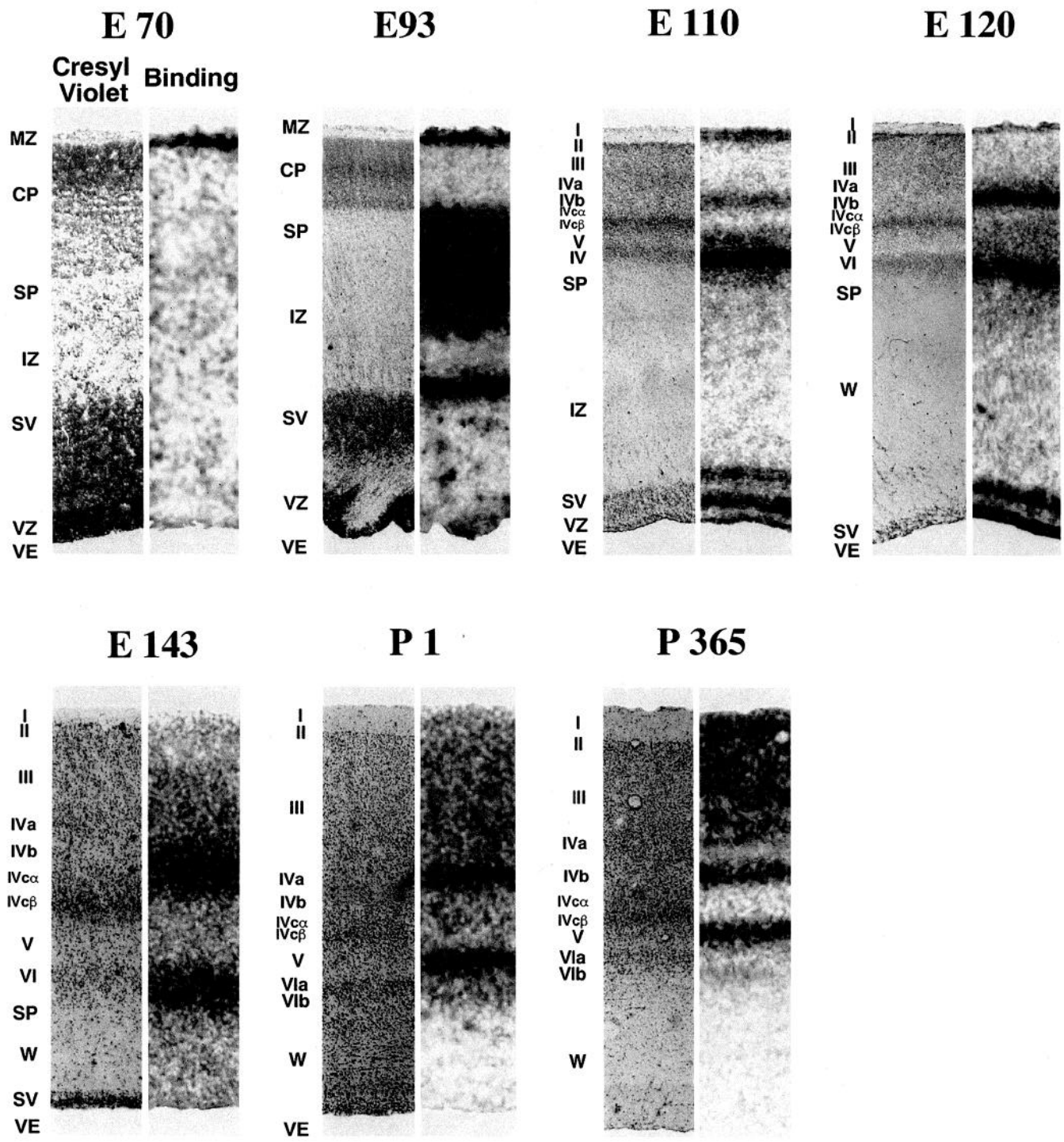

Figure 7. Laminar distribution of ^{125}I-pindolol labeling (β receptors) in the developing primary visual cortex (area 17). Conventions are as in Figure 5.

The distribution of β receptors undergoes considerable changes during this developmental period. For example, we found that in contrast to the earlier fetal ages, layer I of area 17 contained considerably fewer β-adrenergic sites. In the same area, high β receptor densities were observed in layers IVb and VI. Slightly lower receptor densities ($\approx$80%) were observed in layers III and IVa. The densities of β receptors in other cortical layers were less than 50% of those in layers IVb and VI (Figs. 3*C*, 7, 10). In area 18, the receptor distribution was identical to that at E120–E128 (Figs. 3*C*, 10). In contrast to the findings at earlier ages, we detected practically no β receptors in the remainder of the subplate zone or near the ventricular surface in the E141 and E143 specimens. (Figs. 3*C*, 7, 10).

Postnatal period

The autoradiographic analysis of the distribution of the adrenergic receptors in area 17 of the rhesus monkey during the postnatal period [from birth (P1) to adulthood (P1825)] has been

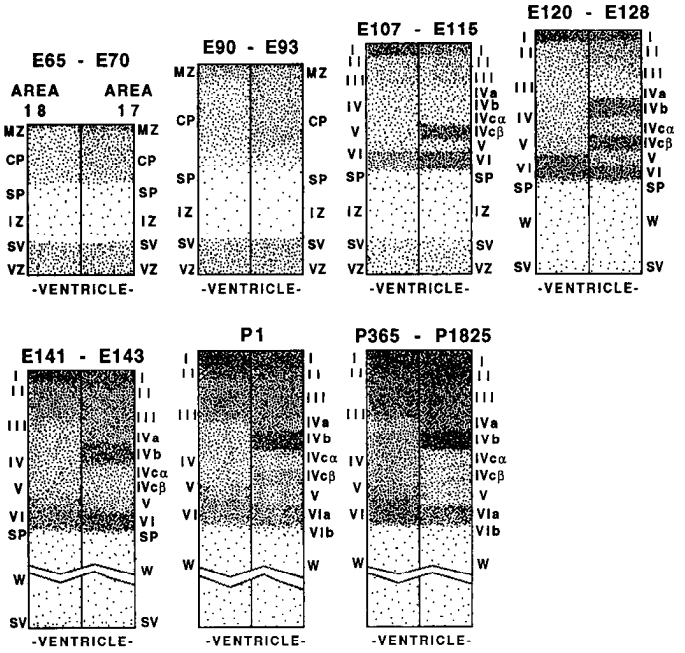

Figure 8. Semidiagrammatic representation of the distribution of $\alpha 1$ receptors in cytoarchitectonic areas 17 and 18 in the lateral part of the developing occipital lobe. Conventions are as in Figure 5.

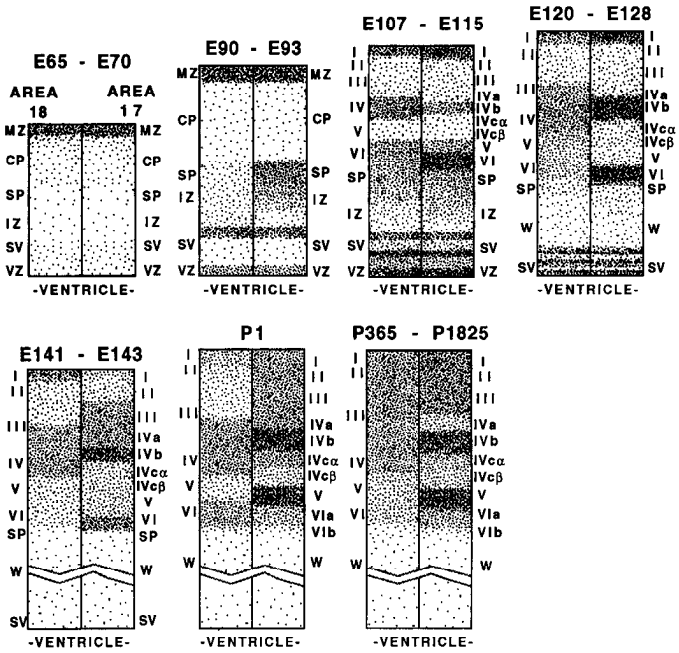

Figure 10. Semidiagrammatic representation of the distribution of β receptors in cytoarchitectonic areas 17 and 18 in the lateral part of the developing occipital lobe. Conventions are as in Figure 5.

recently described (Lidow and Rakic, 1992a). Therefore, for this area, we provide here only selected data in order to complete the picture of the development of cortical adrenergic receptors and compare their postnatal distributions with those at fetal ages. In addition, we supply here the totally new data on the distribution of adrenergic receptors in area 18 at the postnatal ages.

During infancy and adolescence, the highest density of $\alpha 1$ receptors in area 17 was detected in layer IVb (Figs. 3*D*, 4*A*, 5, 8). Among other layers, the superficial laminae, I, II, II, and IVa, contained slightly higher receptor densities than the deep laminae, IVc, V, and VI (Figs. 3*D*, 4*A*, 5, 8). In area 18, the highest receptor densities were observed in layers I, II, the upper half of III, and VI during the entire postnatal period studied (Figs. 3*D*, 4*A*, 8).

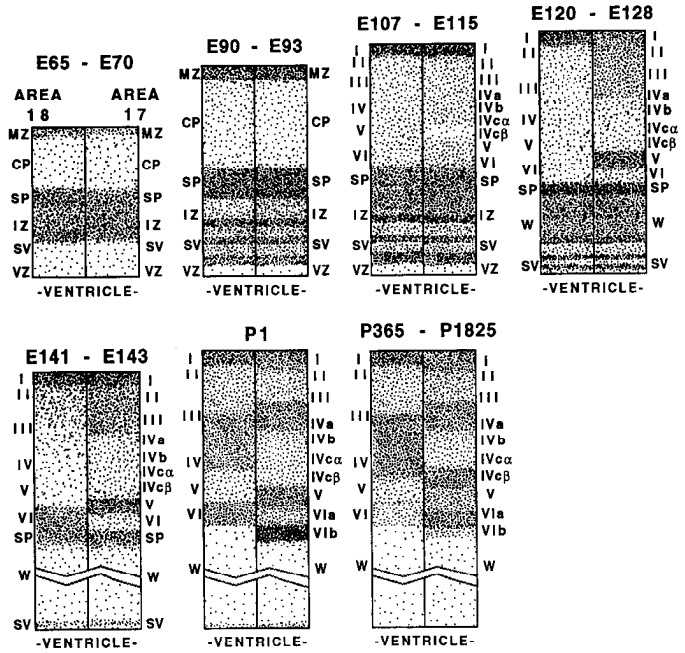

Figure 9. Semidiagrammatic representation of the distribution of $\alpha 2$ receptors in cytoarchitectonic areas 17 and 18 in the lateral part of the developing occipital lobe. Conventions are as in Figure 5.

At birth, the highest density of $\alpha 2$ receptors in area 17 was found in layer VIb, which is most likely the remnant of the fetal subplate zone. Slightly lower receptor densities were detected in layers I, the deep half of III, IVa, and V of the same area (Figs. 3*E*, 6, 9). However, this neonatal distribution changes within the first 12 months of the animal's life, and from 1 year of age to adulthood, layers I, the lower half of III, IVa, VIcβ, and VIa displayed the highest density of $\alpha 2$ sites in area 17 (Figs. 4*B*, 6, 9).

In area 18 of newborn monkeys, $\alpha 2$ receptors were concentrated in layers I, the deep half of III, IVa, and VI (Figs. 3*E*, 9). At the older ages, however, layer VI no longer expressed high receptor density (Figs. 4*B*, 9). Thus, the adult distribution of $\alpha 2$ sites in area 18 consisted of the highest receptor densities in layers I, the lower half of III, and IVa.

From birth to adulthood, the highest density of β-adrenergic receptors in area 17 was observed in layers IVb and V. Among the other cortical layers, the superficial laminae had higher receptor densities than the deep layers (Figs. 3*F*, 4*C*, 7, 10). In area 18 of the newborn animals, the highest receptor densities were found in the deep half of layer III and layers IV, and VI (Figs. 3*F*, 10). At the later ages however, layers I, II, III, and IV possessed the highest receptor density (Figs. 4*C*, 10).

Discussion

Adrenergic receptors are present in the transient embryonic zones

The fetal telencephalic wall contains several cellular layers or zones that do not have counterparts in the adult cerebrum (for

review, see Rakic, 1982, 1988). For example, proliferative cells are organized into the ventricular and subventricular zones which, in the monkey, are prominent only during the midgestational period. Likewise, the intermediate zone situated between these proliferative zones and cortical plate can be divided into several sublayers, the most prominent of which is the subplate zone located below the developing cortical plate in most mammals studied (Kostovic and Rakic, 1980, 1990; Luskin and Shatz, 1985; Chun et al., 1987; Peduzzi, 1988; Valverde and Facal-Valverde, 1988). Fibers originating from the thalamus (Rakic, 1976, 1977a), basal forebrain, and brainstem monoamine nuclei (Kostovic and Rakic, 1990) "wait" in this transitional compartment before entering the cortical plate. Until now, the studies involving biochemical maturation of these embryonic cerebral zones have concentrated largely on the receptors for neurotransmitters such as GABA or somatostatin (Gonzales et al., 1989; Cobas et al., 1991; Shaw et al., 1991; Meinecke and Rakic, 1992), which are produced intrinsically by cortical cells.

In the present study, we found that receptors to noradrenaline, which are supplied by extrinsic input, are also present in large quantities in the ventricular, subventricular, intermediate, subplate, and marginal zones at all embryonic ages examined. Moreover, the $\alpha 2$ adrenergic receptors in the subplate and intermediate zone have considerably higher density than $GABA_A$ (labeled with ^{3}H-muscimol), NMDA (^{3}H-MK801), AMPA (^{3}H-CNQX), D_1-dopaminergic (^{3}H-SCH23390), D_2-dopaminergic (^{3}H-raclopride), 5-HT_1 (^{3}H-hydroxytryptamine), 5-HT_2 (^{3}H-ketanserin), and M_1 muscarinic (^{3}H-pirenzepine) receptors at all prenatal ages studied (Lidow, and Rakic, 1992b).

The transient embryonic zones often had higher densities of adrenergic sites than the cortical plate itself. For example, throughout the prenatal ages, the subplate zone subjacent to the developing visual cortex has the highest density of $\alpha 2$ sites. Furthermore, between E65 and E115, the density of these receptor sites in the subplate zone is higher than in the adult cortex (Rakic et al., 1988; Lidow and Rakic, 1992a). Between E90 and E93, the density of β receptors is also higher in the subplate zone than in most layers of the occipital cortex. A high density of adrenergic receptors in the transient embryonic zones, particularly the ventricular and subventricular laminae, that lack adrenergic synapses (Berger et al., 1993) supports the hypothesis of a neurotrophic role of noradrenaline in the formation of the cerebral cortex. This hypothesis (reviewed in Parnavelas et al., 1985) was originally formulated on the basis of the observations that the noradrenergic fibers are among the first to arrive at the developing neocortex (Kristt, 1979; Levitt and Moore, 1979) and that the destruction of noradrenergic innervation often results in abnormal cortical development (Maeda et al., 1974; Felten et al., 1982; Brenner et al., 1985).

A significant finding of the present study is that each adrenergic receptor subtype examined has a unique distribution in the transient embryonic zones of the developing primate cerebral wall. Thus, $\alpha 1$ receptors can only be observed in the ventricular, subventricular, and marginal zones; β sites, in addition to these laminae, can also be found in the subplate zone; and $\alpha 2$ receptors occupy the ventricular, subventricular, intermediate, subplate, and marginal zones. Particularly striking in this respect is the complementarity of the distribution of $\alpha 2$ and β receptors in the germinal and the deep part of the intermediate zones observed at E107–E128. In this region, $\alpha 2$ sites concentrate in three distinct laminae. The deepest one corresponds to the cell-poor space between the ventricular and subventricular zones, and the other two can be found below and above another cell-poor strip situated in the depth of the intermediate zone. In contrast, β sites concentrate mainly below and above the $\alpha 2$-rich strip situated in the middle of the ventricular-subventricular zone as well as in the cell-poor lamina of the intermediate zone surrounded by the bands containing a high density of $\alpha 2$ receptors.

While the specific reasons for such complex laminar arrangement of adrenergic receptor subtypes is unknown, the differential distribution of adrenergic receptor subtypes in the transient embryonic zones suggests that they have different and specific roles in the regulatory activity of their neurotransmitter.

Adrenergic receptors may influence generation of cortical cells

A high density of adrenergic receptors in the germinal zones suggests that noradrenaline may be involved in regulation of mitotic activity of the progenitors that form the neuronal and glial cells destined for the cerebral cortex. This possibility is also supported by previous experiments with the alteration of noradrenaline content of the brain, which indicate that noradrenaline regulates DNA synthesis during brain development (Barochovsky and Patel, 1982; Slotkin et al., 1988).

A particularly intriguing observation is that the presence of $\alpha 1$- and β-adrenergic receptors in the cortical germinal zones coincides with specific stages of cortical development. Thus, the highest density of $\alpha 1$ sites in these zones is observed when the proliferative activity there is at its highest. The density of these sites falls dramatically at the time of decline in the rate of cell division. In contrast, β receptors begin to appear in the germinal zones only after the majority of cortical neurons have been generated. This sequence of events suggests that $\alpha 1$ sites may be involved in promotion, while β sites may participate in suppression of cell proliferation in the germinal zones of the developing cerebral cortex. This hypothesis is supported by the recent findings that in the hepatocytes and vascular smooth muscle cell cultures, stimulation of $\alpha 1$ receptors enhances cell division, while stimulation of β receptors results in the suppression of cell proliferation (Cruise et al., 1985; Nakaki et al., 1990). Thus, in the developing cerebrum, noradrenaline may both stimulate and suppress cell proliferation, depending upon the receptor subtype expressed by the germinal cells. Since both the ventricular and subventricular zones produce a variety of neuronal types (Rakic, 1988; McConnell, 1991; Parnavelas et al., 1991), noradrenaline can regulate the proportion of various types of neurons in the cerebral cortex.

Adrenergic receptors in the transient subplate zone

In the present study, we found a particularly high density of β and $\alpha 2$ adrenergic receptors in the transient subplate zone of the fetal monkey. The role of adrenergic receptors in this embryonic zone is unknown, but some possibilities are beginning to emerge. For example, it has recently been shown that β receptors are involved in stimulation of the synthesis and release of nerve growth factor (NGF) in cultured cortical astrocytes (Schwartz and Mishley, 1990). The fact that both β sites and low-affinity NGF receptors are highly concentrated in the subplate zone during the same developmental period (Allendoerfer et al., 1990; Meinecke and Rakic, 1993) suggests that β receptors may be involved in the regulation of NGF in this zone. In addition, β receptors may stimulate the activity of ornithine decarboxylase (Morris et al., 1983; Morris and Slotkin, 1985), an enzyme involved in the biosynthesis of spermidine and sper-

mine, which are essential for cell differentiation, migration, and establishment of proper connectivity (Slotkin and Bartolome, 1986).

In the present study, we did not try to distinguish between the different β receptor subtypes. In the adult animals, $\beta 1$ receptors are thought to be involved in neuronal function, while $\beta 2$ sites are mostly associated with glial cells (for review, see Fillenz, 1990). During development, however, $\beta 2$ receptors may play a much more active role. For example, this receptor subtype is involved in regulation of ornithine decarboxylase activity in developing rat brain (Morris and Slotkin, 1985).

Although in the fetal cerebrum, $\alpha 2$ receptors are the most abundant in the subplate and intermediate zones, their specific role in cortical development is obscure. The $\alpha 2$ is the only adrenergic receptor subtype that has been detected in the presynaptic elements (Fillenz, 1990) and therefore may be in a position to modulate the early, development-related activity of this neurotransmitter. However, it is unlikely that, from E70 to E93, noradrenaline acts through the classical synaptic connections, since at this fetal age noradrenergic fibers have not yet invaded the occipital lobe (Berger et al., 1993). It has been suggested that noradrenaline may be involved in regulating the timing at which afferents leave the subplate zone and enter the cortical plate (Blue and Parnavelas, 1982). The high density of $\alpha 2$ receptors in the primate subplate zone throughout the entire period of its existence makes them natural candidates for carrying out this strategic developmental function.

Adrenergic receptors and specification of cortical areas

The participation of noradrenaline in the specification of the cytoarchitectonic areas has been discussed, since it became apparent that the noradrenergic innervation in the primate has an area-specific pattern of distribution (Levitt et al., 1984; Morrison and Foote, 1986). We found that the differences in receptor distribution between areas 17 and 18 in the cortical plate are preceded by region-specific distribution within the subjacent sectors of the subplate zone. For example, no differences in the distribution of β receptors between the prospective areas 17 and 18 can be detected in the cortical plate of the specimens of E90–E93 age group. However, in the same set of specimens, a high density of β-adrenergic receptors can be observed in the subplate zone subjacent to the prospective area 17, while the subplate zone below the prospective area 18 is practically devoid of these receptor sites. This observation provides new support for the hypothesis that the areal specification of the subplate zone may lead the areal specification of the overlying cortical plate (Ghosh et al., 1990; Kostovic and Rakic, 1990; O'Leary and Borngasser, 1992). It also suggests that β-adrenergic receptors may play a role in the establishment of the primary and secondary visual cortical areas.

Adrenergic receptors and connectivity of the cerebral cortex

Adrenergic receptor subtypes display complex laminar distributions in the occipital lobe of the adult rhesus monkeys (Rakic et al., 1988). Here we report that these unique patterns characteristic for each adrenergic receptor subtype is achieved through multiple, time-dependent changes in receptor distribution within the developing cerebral wall. In order to elucidate the forces that may govern the deployment of adrenergic receptors, it is instructive to compare the distribution of these receptors with the emergence and distribution of the major afferents in the cerebral cortex.

The comparison between our data and information available in the literature revealed that the $\alpha 2$-adrenergic sites in adult monkey visual cortex are closely associated with the terminals originating in the parvocellular layers of the lateral geniculate nuclei. They tend to concentrate in the deep half of layer III and layers IVa, IV$c\beta$, and VIa of area 17, which receive input from the parvocellular layers of the lateral geniculate nuclei and are considered to be associated with the processing of color vision (Livingstone and Hubel, 1984; Jones, 1985; Lund and Yoshioka, 1991), it is possible that $\alpha 2$ receptors are also involved in this process. In addition, the $\alpha 2$ receptors of areas 17 and 18 concentrate in the layers innervated by the pulvinar, such as layer I of area 17 and layers I, the deep half of III, and IV of area 18 (Benevento and Rezak, 1976; Lund, 1988). Such a close spatial correlation between the laminar distributions of pulvinar terminals and $\alpha 2$ receptors suggests that these receptor sites may also participate in the modulation of the extrageniculate visual pathway (Jones, 1985). However, the adult distribution of $\alpha 2$ receptors in the monkey visual cortex becomes apparent only late in postnatal development, long after the final position of the thalamic terminals has been established (Rakic, 1976, 1977b, 1983). In addition, the developmental changes in the distribution of $\alpha 2$ sites in area 17 do not precisely follow the developmental changes in the distribution of terminals from the parvocellular portion of the geniculocortical projections. Finally, although the developmental changes in the distribution of $\alpha 2$ receptors in area 18 can be correlated with the changes in the pattern of the pulvinar innervation (Kostovic and Rakic, 1984), there is a lag of nearly 30 d before the receptor distribution follows that of the innervation. For example, pulvinar terminals are found predominantly in layers I, III, IV, and VI at E100 (Kostovic and Rakic, 1984), while the $\alpha 2$ receptors begin to concentrate in the same layers only between E143 and birth. By that developmental age, the pulvinar innervation of area 18 had already changed to adult distribution, with axonal terminals concentrating in layers I, III, and IV (Benevento and Rezak, 1976). Similar distribution of $\alpha 2$ receptors is achieved only by the end of the first postnatal year. It should be noted, however, that since in the present study we examined only the high-affinity $\alpha 2$ sites it is possible that a much closer correlation can be achieved when the entire population of $\alpha 2$ receptors is taken into consideration.

In contrast to the $\alpha 2$ receptors, the laminar patterns of $\alpha 1$ and β sites do not correlate well with the distribution of any specific afferents to the occipital cortex of the developing or adult monkey (Kostovic and Rakic, 1984; Lund and Yoshioka, 1991). There is also no correspondence between the laminar distribution of noradrenergic innervation of areas 17 and 18 (Foote and Morrison, 1984; Morrison and Foote, 1986) and that of adrenergic receptor subtypes (individually or combined) in the adult or developing rhesus monkeys. However, while the laminar pattern of noradrenergic innervation and receptor distribution in the visual cortex does not correspond spatially, both receptors and innervation appear in the cortical plate at about the same time. Also, both adrenergic receptors and noradrenergic innervation attain adult pattern of distributions at the time of puberty (Foote and Morrison, 1984; Berger et al., 1993). These observations suggest that while interactions with noradrenergic innervation may not determine the specific patterns of the distribution of adrenergic sites in the primate cortex, such interactions, nevertheless, may play a role in the establishment of the time course of the maturation of cortical adrenergic receptors.

The present finding of a high density of adrenergic receptors

in the transient embryonic zones of the developing cerebral wall opens up the possibility that the noradrenergic system may play multiple roles in the production and differentiation of cortical neurons. Other neurotransmitters, such as serotonin, NMDA, or GABA, have also been proposed to serve as neurotrophic factors in cortical development (Lauder, 1988; Meinecke and Rakic, 1992; Komuro and Rakic, 1993). Therefore, it is likely that several neurotransmitters participate in the regulation of cortical development, each acting at specific times and through different mechanisms. For example, while noradrenaline may influence the migration and maturation of cortical neurons through regulation of the synthesis and release of NGF (Schwartz and Mishley, 1990), serotonin may perform a similar function through the modulation of the release of S100 growth factor (Liu and Lauder, 1992).

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