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Synchronized overproduction of neurotransmitter receptors in diverse regions of the primate cerebral cortex

(receptor autoradiography/monoaminergic receptors/cholinergic receptors/GABAergic receptors/postnatal development)

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ABSTRACT **A remarkable diversity of neurotransmitter receptors develops concurrently in disparate areas of the primate cerebral cortex. The density of dopaminergic, adrenergic, serotonergic, cholinergic, and GABAergic receptors (where GABA is γ-aminobutyric acid) in rhesus monkey reaches a maximum level between 2 and 4 months of age and then declines gradually to adult levels in all layers of sensory, motor, and association regions. The synchronized development of neurotransmitter receptors in diverse layers and regions of the neocortex occurs *pari passu* with synaptogenesis, demonstrating unusual coordination of biochemical and structural maturation and supporting the hypothesis that the entire cerebral cortex matures as an integrated network, rather than as a system-by-system cascade.**

The mechanisms underlying division of the cerebral cortex into distinct cytological, anatomical, and functional regions are poorly understood (1–7). During most of this century, it has been generally accepted that various cortical regions develop sequentially, with the primary sensory areas differentiating before motor areas, and the association areas differentiating last. This view, based on the intensity of cell staining, pattern of distribution of myelin (8, 9), the emergence of various enzymes (10, 11), and differences in metabolic activity (12), has had a major influence on the interpretation of physiological and psychological data (13–15). Recently, however, a number of findings in developing nonhuman primates have begun to challenge such a strictly hierarchical model of cortical ontogeny. For example, the schedules of neurogenesis for the somatosensory, visual, motor, and prefrontal cortices overlap with relatively small variation in the time of onset and termination of cell division among these regions (16, 17). Moreover, the regional variation in cell production that does exist, is not related to cytoarchitectonic borders or hierarchical functional schemes but rather fits a smooth spatio-temporal centrifugal gradient centered in the cardinal axis of the cerebral vesicle. In addition, the columnar organization of connections in the dorsoventral and orbital prefrontal association and motor regions is expressed as early as or earlier than similar organizational features in sensory cortices (18–22). Finally, the production, peak in density, and phase of elimination of synapses occur concurrently in the sensory, motor, limbic, and association areas (23). In the present article, we report that the postnatal development of a diverse set of neurotransmitter receptors is also synchronized in widespread cortical areas that subserve sensory, motor, and associative function.

MATERIALS AND METHODS

In vitro binding autoradiography was used to examine quantitatively the postnatal development of dopaminergic, serotonergic, adrenergic, muscarinic cholinergic, and GABAergic receptors (where GABA is γ-aminobutyric acid) in the prefrontal association, primary motor, somatosensory, and visual areas of rhesus monkeys. At least two animals were examined at birth and at 1, 2, 4, 8, 12, 36, and 60 months of age. The nine receptor sites were labeled using radioligand binding assays and densitometric procedures as described (24, 25). D_1-dopaminergic receptors were labeled with [^{3}H]SCH23390 [(*R*)-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1*H*-3-benzazepin-7-ol]; D_2-dopaminergic receptors were labeled with [^{3}H]raclopride; 5-HT_1-serotonergic receptors (where 5-HT is serotonin) were labeled with [^{3}H]hydroxytryptamine creatine sulfate; 5-HT_2-serotonergic receptors were labeled with [^{3}H]ketanserin; α_1-adrenergic receptors were labeled with [^{3}H]prazosin; α_2-adrenergic receptors were labeled with [^{3}H]clonidine; β-adrenergic receptors were labeled with ^{125}I-labeled pindolol; M_1 muscarinic receptors were labeled with [^{3}H]pirenzepine; high-affinity $GABA_A$ receptors were labeled with [^{3}H]muscimol; benzodiazepine receptors were labeled with [^{3}H]flunitrazepam.

The autoradiograms were analyzed with a computer imaging system that allows the overlay of the digitized images of cresyl violet-stained sections and the corresponding autoradiograms on the computer screen to facilitate histological identification of specific layers on the autoradiographic images. The computer compares the optical densities of the film images with those of ^{3}H or ^{125}I standards (Amersham) that were apposed to the film along with the tissue sections and converts the optical densities of 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 are linearly related to tissue radioactivity on ^{3}H-sensitive Ultrofilm (24).

The maximum number of specific receptor sites (B_{max}) and their affinity (expressed as steady-state dissociation constant, K_d) for each radioligand were calculated with the nonlinear curve-fitting computer programs KINETIC/EDBA/LIGAND/LOWRY from Elsevier-Biosoft (Cambridge, U.K.). The analyses were based on concentrations of radioactive ligands specifically bound to tissue labeled with five concentrations of free ligands in incubating solutions. The number of data points employed for quantative analysis of saturation binding is the minimal number of points that allows the accurate estimation of B_{max} and K_d values for a one-site receptor model (24, 25).

RESULTS AND DISCUSSION

At birth, B_{max} values for every receptor examined were lower than those found in the 5-year-old adult animals. During the first 2 months of life, the receptor density of each subtype

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Abbreviation: GABA, γ-aminobutyric acid.

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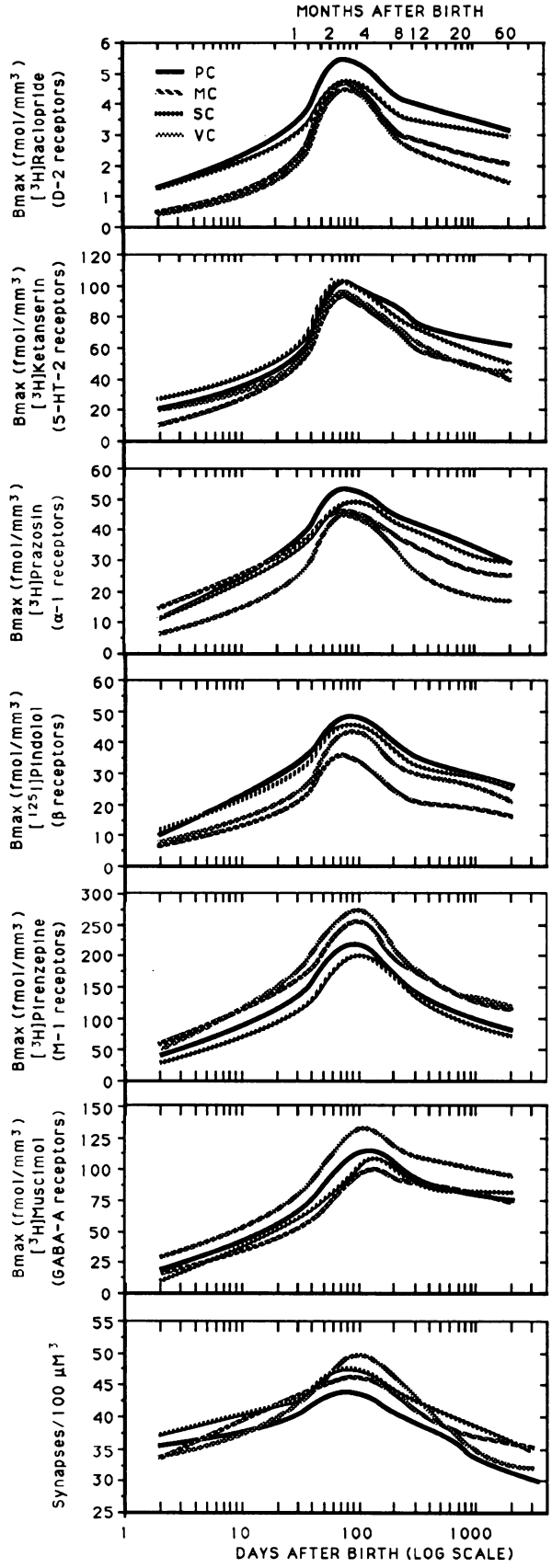

FIG. 1. Developmental changes in the overall (across all layers) density of the specific binding of radioligands labeling representative selection of neurotransmitter receptor subtypes in the prefrontal (PC), primary motor (MC), somatosensory (SC), and primary visual cortical (VC) regions. The figure also includes developmental changes in synaptic density in the same regions (adapted from ref. 23). For receptor densities the curves were obtained by locally weighted least squares fit with 50% of smoothing (KALEIDA GRAPH, Synergy Software, Reading, PA) based on mean B_{max} values obtained from the measurements of the entire cortical thickness in at least two animals at birth and at 1, 2, 4, 8, 12, 36, and 60 months of age. Age is presented in postnatal days on a logarithmic scale. The original data on synaptogenesis by Rakic *et al.* (23) was presented as the number of synapses per unit area of neuropile. We recalculated the data per volume of tissue, which did not alter the timing of events and allowed us to compare the developmental course of cortical synapses with that of neurotransmitter receptors obtained in the present study. The curves representing synaptogenesis were fitted in the same manner as those for neurotransmitter receptors.

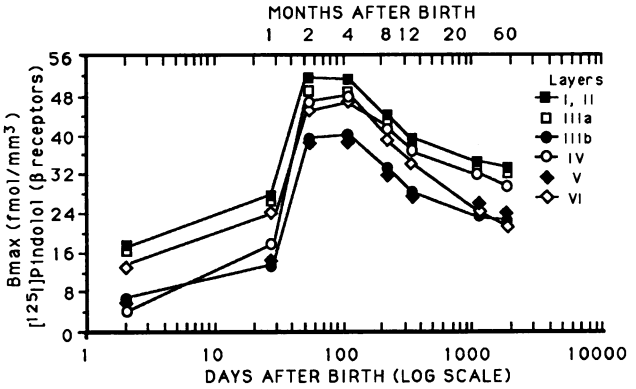

FIG. 2. Developmental changes in the β-adrenergic-specific binding of ^{125}I-labeled pindolol in different layers of the monkey somatosensory cortex. Each data point represents the mean B_{max} value obtained from at least two animals. The connecting lines were drawn for layers I, II, IIIb, IV, and VI. Age is presented in postnatal days on a logarithmic scale. Note that the developmental increase and following decrease in binding density takes place in all cortical layers simultaneously. However, different layers may exhibit different magnitude of change. Thus, at birth the highest density of ^{125}I-labeled pindolol binding was observed in layers I, II, IIIa, and VI. Within the first 2 postnatal months, the amplitude of increase of binding density was much higher in layer IV than in other layers and at the end of this developmental period, the highest density of binding was seen in this layer along with layers I, II, IIIa, and VI. After 4 months, the decrease in binding density was higher in layer VI than in other layers. These data suggest that the differences in the amplitudes of temporarily synchronized changes in receptor density between different layers are the cause of developmental changes in the laminar profile of receptors illustrated in Fig. 3. Age is plotted on a logarithmic scale as in Fig. 1.

increased to levels that were up to two times higher than in the adults (Fig. 1). From 2 to about 4 months, receptor density achieved a relatively steady state but then began a gradual decline that tapered off around the time of puberty (3 years of age). The three cardinal phases of developmental change—a rapid increase, a short plateau, and a long decline in receptor density—occurred virtually synchronously in the primary visual, somatosensory, primary motor, and prefrontal association cortices (Fig. 1). Furthermore, in these functionally diverse areas, the overproduction of neurotransmitter receptors took place contemporaneously in all layers (Fig. 2). The only observed region-specific and ligand-specific difference was in the absolute amplitudes of increase and decrease in receptor density, which also varied from layer to layer (Fig. 1). For example, the increase in density of α_1 receptors was higher in the prefrontal cortex than in the motor cortex (Fig. 1). Although the phase of decline in receptor density occurred simultaneously for all receptors, the density of monoaminergic and cholinergic receptors decreased significantly more than that of GABAergic sites (Fig. 1). Finally, the extent of overproduction and subsequent decline in the density of β-adrenergic receptors varied across

different layers (Fig. 2). Although the differences in magnitude of fluctuation among receptors produced a changing pattern of relative laminar composition at different ages (e.g., Fig. 3), the time course of developmental change remained highly synchronized in all layers (Fig. 2).

We have not found any significant developmental changes in the apparent affinity (K_d) for the majority of the ligands used in this study (Table 1). This may indicate that, in primate cerebral cortex, the kinetic properties of neurotransmitter receptors are already developed by birth. The only exception was [^{3}H]muscimol, which autoradiographically labels high-affinity $GABA_A$ sites. This ligand displays a lower affinity in newborn animals than at other ages (Table 1). Based on recent information about subunit heterogeneity (26) and its effect on affinity for various GABA/benzodiazepine ligands, it is possible that this represents an important developmentally regulated difference.

An excess of neurotransmitter receptors followed by elimination has been reported for selected cortical areas in rodents and cats (27–34), but synchronous overproduction of several neurotransmitter receptors in anatomically and functionally diverse areas of the neocortex is unexpected. We attribute the observed changes in the density of neurotransmitter receptors to an initial overproduction followed by a decrease of binding sites during infancy and adolescence. They cannot be explained simply by dilution due to changes in cortical volume. In fact, the phase of increase in the density of neurotransmitter receptors occurs in parallel with an increase in cortical volume (35). Furthermore, the most significant decrease in receptor binding occurs during the period when the cortex stops growing or shows only a slight increase in overall volume (36) and, therefore, the decrease can confidently be attributed to attrition.

In contrast to the synchronous development of receptors, the concentration and rate of synthesis of neurotransmitters appear to have a regionally dependent developmental time-

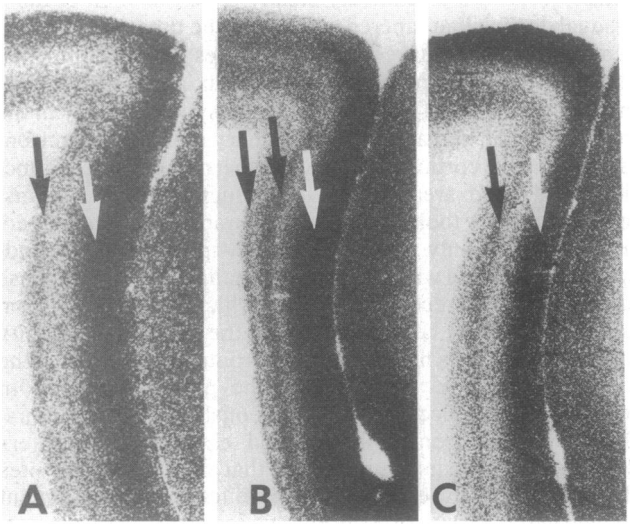

FIG. 3. Developmental changes in the laminar distribution of the β-adrenergic-specific binding of ^{125}I-labeled pindolol in monkey somatosensory cortex. (*A*) Newborn animal. (*B*) Two-month-old animal. (*C*) Three-year-old animal. These autoradiograms can be used to compare the distribution of the radioligand at various ages but not for accurate representation of the relative densities of binding between ages. The cortical laminas with the highest receptor densities at each age are indicated with arrows. Note that at birth, the highest density of ^{125}I-labeled pindolol binding is in the superficial layers I, II, and IIIa and the deep layer VI. At 2 months of age, in addition to these layers, the highest density of binding is also seen in the middle layer IV. Finally, in adult animals the highest density of ^{125}I-labeled pindolol binding is seen only in the superficial layers I, II, and IIIa and in the middle layer IV.

Table 1. Steady-state dissociation constants (K_d) for selected radioligands in prefrontal, motor, and visual cortex of developing monkeys

Ligand	Age(s), months	K_d, nM: Prefrontal cortex	Motor cortex	Visual cortex
[^{3}H]Raclopride	0–60	3.8 ± 1.6	3.2 ± 1.4	3.3 ± 1.0
[^{3}H]Ketanserin	0–60	2.8 ± 1.2	2.7 ± 0.9	3.4 ± 1.1
[^{3}H]Prazosin	0–60	3.6 ± 0.9	3.1 ± 1.3	4.0 ± 1.6
^{125}I-labeled pindolol	0–60	0.18 ± 0.08	0.2 ± 0.07	0.21 ± 0.08
[^{3}H]Pirenzepine	0–60	4.6 ± 1.2	3.9 ± 2.1	4.4 ± 1.9
[^{3}H]Muscimol	Newborn	14.5 ± 3.7	15.2 ± 3.0	14.9 ± 3.3
	1	8.8 ± 2.2	9.1 ± 2.7	10.6 ± 4.1
	2	1.7 ± 0.7	2.4 ± 0.9	1.9 ± 0.8
	4	1.1 ± 0.4	2.2 ± 1.1	1.8 ± 0.8
	12	8.1 ± 2.3	9.9 ± 1.5	9.0 ± 3.6
	60	9.6 ± 3.1	10.1 ± 2.1	11.2 ± 2.3

There were no postnatal changes in K_d values of [^{3}H]raclopride, [^{3}H]ketanserine, [^{3}H]prazosin, ^{125}I-labeled pindolol, and [^{3}H]pirenzepine. In contrast, K_d values for [^{3}H]muscimol displayed developmental changes.

table (37, 38). On the other hand, the period of overproduction and elimination of receptors corresponds rather closely to the course of synaptogenesis assessed by quantitative electron microscopy in the cortex of the same species (refs. 23 and 39 and Fig. 1), suggesting the possibility that synaptic density and the number of available receptors may be interdependent. However, it is difficult to interpret the present finding in the context of specific neuronal circuits. For example, elimination of synapses involves mostly asymmetrical contacts situated on dendritic spines (23, 39), whereas most receptors (acetylcholinergic, GABAergic, and dopaminergic) examined in this study are involved in symmetrical synapses, at least in the adult monkey cortex (40–42). It is conceivable that, during the peak period of receptor overproduction, a larger proportion of receptors is present outside local synaptic sites as seems to be the case at the neuromuscular junction (43). We often observed that the laminar distribution of receptors during the peak period was less sharp than at other times. However, much more information is needed about the nature of these events before their relationship can be understood at the cellular level.

It should be emphasized that the present findings do not negate the hierarchical mode of structural and functional maturation of the brain that clearly progresses from subcortical structures to the cortex, which differentiates last. Yet, whereas the hierarchical principle of development is the rule within any given system, the various levels of maturity of different systems emerge in the cortex concurrently. The present data and our previous finding of synchronous overproduction of cortical synapses (23) suggest that the entire gamut of cortical circuitry may be orchestrated by a common diffusible factor or a synchronous activation of regulatory genes. A search for these factors would be rewarded by insights into the origin of the regional diversity for which the cortex is appreciated. If the final pattern of synaptic organization is the result of competition between various inputs, including thalamocortical, corticocortical, callosal, and local synaptic connections, then maximum competition is assured by simultaneous interaction of all inputs.

The lack of sequential development of sensory, motor, and associative cortical circuitry calls for reexamination of the traditional step-wise concept of cortical maturation from sensory to motor to association (23, 39). The hypothesis of a sequential development of cerebral cortical areas stems in part from Fletchsig's observation (8) that in the human infant brain the white matter subjacent to association areas of the

parietotemporal and frontal lobes is less intensely stained with myelin-specific dyes, such as hematoxylin, than the areas subjacent to the visual and sensorimotor cortex. It should be emphasized that areas with less myelin in infancy also contain less myelin in the adult brain. For example, the principal sulcus of the prefrontal cortex in adult macaques is poorly myelinated (44) and callosal fibers connecting the association areas of the frontal lobes in adult monkeys contain up to 20% more unmyelinated fibers than callosal connections of the somatomotor areas (45). Paradoxically, the pyramidal tract, carrying the cortico-spinal output system of the motor cortex, myelinates last—a fact usually overlooked by proponents of the strict sequential model (8, 9). The argument is further weakened by the finding that neuronal activity associated with both simple reflexes and complex behavior can occur before the formation of myelin (46, 47). Thus, the preeminence of myelin staining as a criterion for rating the level of functional competence is not well-justified.

The psychological evidence in favor of step-wise sequential development seems to be equally tenuous. Piaget's original idea (48) that the child goes through a sensory stage, during which it cannot form associations, has been embraced by many developmental psychologists (e.g., refs. 13–15). However, some of the ingenious tests used by Piaget (48) could not distinguish clearly between the infants' level of motor coordination and conceptual competence and it is now agreed that the child develops conceptual ability earlier than had been thought (49). Simultaneous maturation of sensory, motor, and association areas is also indicated by the concurrent increase and decrease in synaptic density (19) and metabolic activity (50) of functionally diverse areas in human infants. Therefore, the idea of synchronized development of the cerebral cortex is appealing from both a neurobiological and psychological point of view, as it emphasizes the integrated nature of behavior and the fact that few functions of the organism, however simple, are carried out by a single part of the cortex in isolation from other synaptically related areas (51).

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