Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 5303–5306, July 1990
Neurobiology

Hypercolumns in primate visual cortex can develop in the absence of cues from photoreceptors

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Contributed by Pasko Rakic, April 30, 1990

ABSTRACT **The visual cortex in primates consists of an array of anatomically and chemically identifiable cellular modules (hypercolumns) with distinct physiological properties. For example, layers II/III in the macaque monkey contain a regular array of cytochrome oxidase-rich blobs. Furthermore, the surrounding cytochrome oxidase-poor interblob regions have a higher density of neuropeptide Y-positive aspiny stellate cells. Neurons in the blobs are thought to mediate predominantly low spatial frequencies and color vision, while those in the interblobs appear to be engaged in pattern vision and high spatial frequency analysis. In this study we examined the role of the retina in the development of hypercolumns. A bilateral retinal ablation was performed in embryos at midgestation, before any photoreceptors had established contacts with other retinal neurons and before layers II/III of the cortex—or their synaptic connection—had been generated. We found that the cortex in operated animals had cytochrome oxidase blobs and that their size and spacing were normal. In addition, neuropeptide Y-containing neurons were preferentially distributed in the interblob region as in control animals. Our findings indicate that some basic aspects of the cyto- and chemoarchitectonic organization of the cerebral cortex, which presumably evolved for the analysis of form and color, can emerge in the absence of cues from the retinal photoreceptors that mediate these attributes of vision.**

The cerebral neocortex is composed of many distinct cytoarchitectonic areas, each of which is an aggregate of modular compartments with characteristic anatomical, chemical, and physiological properties (1–3). The mechanisms that control the development and differentiation of these compartments are not known, although they may be important for understanding the evolution of the neocortex and the biological basis of human cognition. This modular organization is perhaps best characterized in the macaque monkey primary visual (striate) cortex (4, 5). In layers II/III of this area, the cytochrome oxidase (CytOx)-containing blobs (variably called also puffs, dots, patches, or spots) are distributed in columns of cells whose spatial frequency tuning as well as chromatic and orientation-selective receptive field properties are different from cells situated in columns in the surrounding interblob regions (6–9). Blobs and interblob regions also differ in the patterns of cortical and subcortical connectivity, which may explain, in part, their distinct physiological properties (6–12). We reported recently that the density of neuropeptide Y (NPY)-containing neurons in macaque monkey is significantly higher in interblob regions than within blobs, indicating that they also differ in their respective arrays of local circuit neurons (13).

Several of the questions raised by these findings are as follows. To what extent does the development of cytochemical differences between blobs and interblob regions, which may mediate distinct aspects of visual function such as the perception of form and color, depend upon molecular or electrical cues originating in subsets of retinal photoreceptors that specialize in mediating these sensations? Would the organization of cortical cyto- and chemoarchitecture be visibly altered in the absence of the retina from early developmental stages? Would the levels of oxidative enzymes or neuroactive peptides expressed in the cortex be drastically reduced in the absence of visual input? Answering these questions could clarify the role that input from the sensory receptors at the periphery may play in specifying these cytological characteristics and in regulating differentiation of the visual cortex. By inference such an answer could also elucidate the mechanisms of specification of other regions of the cerebral cortex. We addressed these questions by analysis of the distribution of CytOx-positive blobs and the deployment of NPY-containing neurons in layers II/III of normal and age-matched prenatally enucleated rhesus monkeys.

METHODS

The study was performed on tissue from the opercular region of six adults, two full-term normal neonates (a newborn sacrificed immediately after cesarean section performed at the 165th day of gestation, and a 5-day-old infant), and two 9-month-old monkeys (*Macaca mulatta*) subjected to bilateral retinal ablation at the 81st and 120th day of gestation by a standardized procedure (14). Animals were anesthetized with an intravenous overdose of Nembutal (100 mg/kg) and perfused through the aorta with Somogyi and Takagi's fixative (15). Tissue blocks (about 8 mm × 10 mm × 12 mm) were dissected from the lateral operculum of the occipital lobe, which contains a representation of the central visual field. The blocks were placed in 30% sucrose in 0.1 M phosphate buffer (pH 7.4) until they sunk and then mounted on a freezing stage. The nearly flat shape of the opercular region allowed precise sectioning parallel to the pial surface at 40-μm intervals. Series of alternate sections were labeled histochemically for CytOx (6–13) or for NPY-like immunoreactivity by the avidin/biotin/peroxidase method (16), as described previously (13, 17).

Computer-assisted two-dimensional plots of the distribution of NPY-containing neurons were obtained from tangential sections and aligned with camera lucida drawings of the CytOx blobs in adjacent sections. In order to align precisely adjacent regions, we used the perpendicularly perforating blood vessels, which exhibit an identical pattern among sections. Immunolabeled stellate neurons in the blobs, in interblob regions, and at the interface between them were counted. The total area of layers II/III and that occupied by blobs were measured by computerized planimetry (13).

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Abbreviations: CytOx, cytochrome oxidase; NPY, neuropeptide Y.

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RESULTS

The visual cortex of both neonates had CytOx blobs in layers II/III comparable in both their distribution and size to adults (Fig. 1). However, the intensity of labeling in these animals was lower than in normal adults. As described previously, the two 9-month-old animals enucleated prenatally had normal cytoarchitectonic features of the visual cortex, including a characteristic laminar pattern in the area striata (18). Within the striate cortex of both operated animals and their age-matched controls, CytOx-positive reaction product was localized homogeneously in layers IVA, IVC_B, and VI and heterogeneously in superficial layers II/III. This normal laminar pattern of CytOx distribution was somewhat surprising since these territories are the main recipients of visual input from the retina by means of the geniculocortical projections.

Within layers II/III, we found clearly defined CytOx-positive blobs in both the experimental and age-matched control animals. Light brownish patches could be clearly distinguished from the bright yellow background of the surrounding tissue. As in normal neonates, the blobs were less intensely labeled than in normal adult monkeys (Fig. 1 *B* and *C*). The average diameter of the blobs in neonates and enucleates was about 230 μm, as determined in 2138 and 1451 cross-sections across the blobs, respectively. This size is similar to that found in our neonates, 9-month-old animals, and in normal adults (19). The distance between blobs and the number of blobs per unit area in the operculum of normal neonates and enucleates were only 1.5% and 5% less than in normal adults, respectively (Fig. 1). The pattern of blob distribution was similar in all three groups of animals. It should be emphasized that, in the present study, we have analyzed only a portion of the occipital cortex in the lateral operculum. Since the size of the blobs, and particularly the interblob distance, varies considerably throughout different regions of area 17, we could not determine the total number of blobs in either experimental or control animals. Such an undertaking would require a reconstruction of the entire striate cortex from serial tangential sections stained with CytOx.

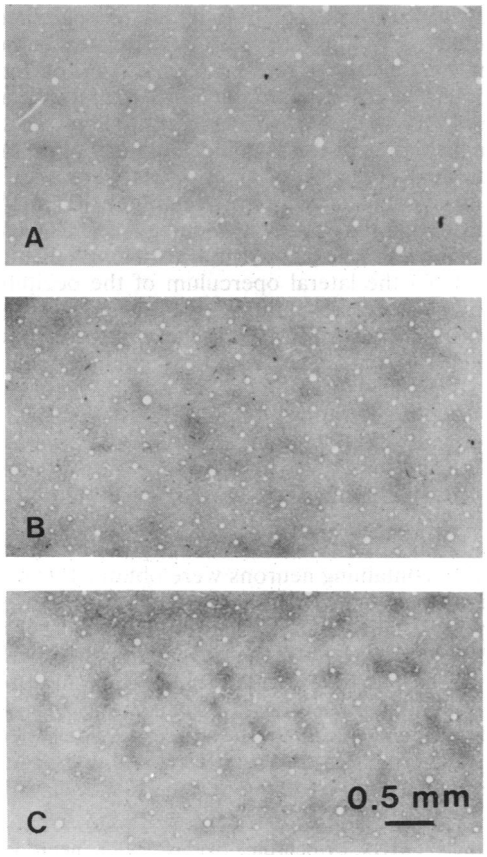

FIG. 1. Tangential sections through layers II/III of the opercular portion of the striate cortex. (*A*) Five-day-old neonate monkey. (*B*) Nine-month-old animal subjected to prenatal bilateral ocular enucleation. (*C*) Normal adult. (CytOx histochemistry.)

NPY-containing neurons were found throughout layers II/III in both normal neonates and in one of the enucleates that was examined by using an immunocytochemical method (Fig. 2). They had the morphological features of aspiny stellate neurons, which are characteristic of this region in normal adults (13, 17). Although NPY-containing neurons were found in the blobs and in the interblob regions of the normal and enucleated animals, even cursory examination revealed that the majority of these neurons were situated in the interblob regions and very few were located in the blobs (Fig. 3*A*). The difference between the actual density of immunolabeled neurons in blobs and interblob regions was confirmed by a statistical analysis using the χ^2 test (13). Our calculation included corrections for the difference in the relative volume of blobs (about 15%) and interblobs (about 85%) in each sample (13). The same calculation was performed for the interblob regions, using the density of labeled cells in blobs as the reference. The statistical analysis revealed a significant difference in the incidence of NPY-containing neurons in the blobs compared with interblob regions of neonates ($\chi^2 = 3.23$, df = 1) and of the enucleated monkey ($\chi^2 = 9.84$, df = 1) at the $P < 0.01$ level or lower (Fig. 3*B*). Thus, although fewer immunolabeled neurons should be expected in the blobs—because they occupy a smaller volume than interblob regions—the relative incidence of neurons observed is significantly less than expected on the basis of a hypothetical uniform distribution throughout layers II/III (13). There is also a striking quantitative similarity in the preferential distribution of NPY-containing stellate neurons between blobs and interblobs among neonates, the prenatally enucleated animal, and normal adults (Fig. 3*B*).

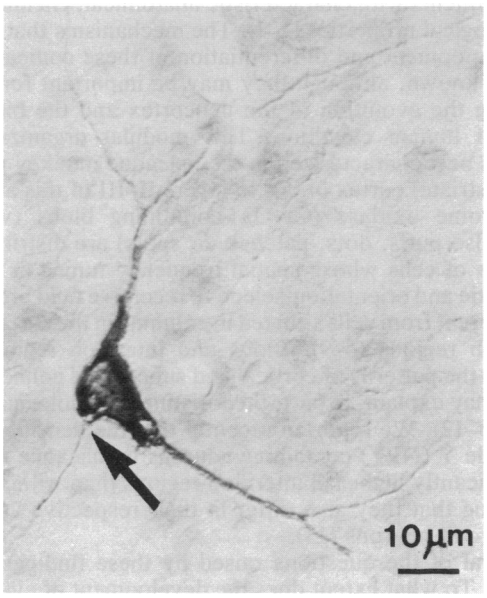

FIG. 2. NPY-containing neuron in layer III of the striate cortex of a 9-month-old animal subjected to bilateral ocular enucleation 6 weeks before birth. This nonpyramidal cell has dendrites without spines and is indistinguishable from those found in similarly labeled sections from normal adults (13, 17). The arrow indicates the origin of the axon. (Differential interference contrast optics.)

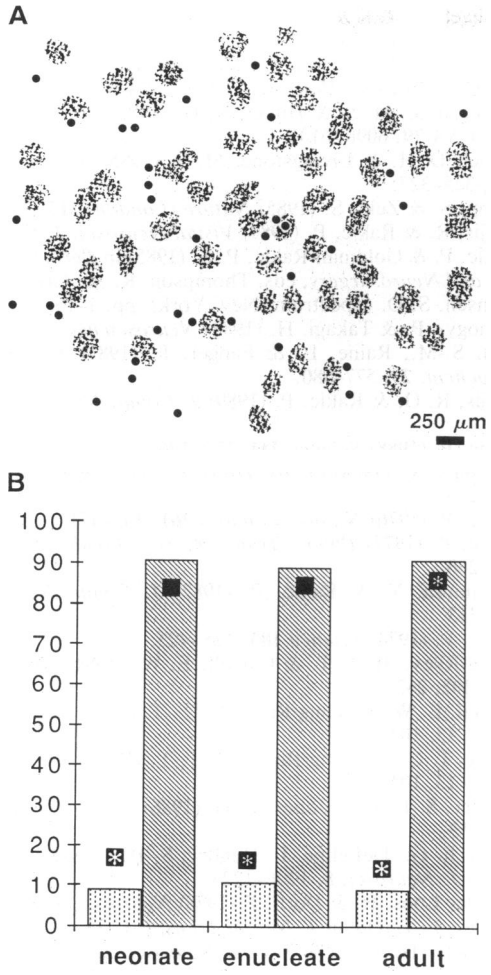

FIG. 3. (*A*) Example of the distribution of NPY-containing neurons (dots) and CytOx-rich blobs (shaded areas) in layers II/III, plotted from two adjacent sections of the striate cortex of an animal subjected to bilateral ocular enucleation prenatally. (*B*) Column chart of the distribution of NPY-containing neurons in layers II/III of a normal 5-day-old infant monkey (n = 611), a prenatally enucleated animal (n = 926), and six normal adults (n = 606). For each specimen, the left bar represents the percentage of labeled neurons found in the blobs plus half the labeled neurons found in the interface with interblob regions. The right bar indicates the percentage of neurons found in the interblobs plus half the labeled neurons in the interface with the blobs. Filled squares indicate the percentage of labeled neurons expected on the basis of the volume occupied by blobs and interblob regions. Asterisks within the squares indicate statistically significant differences (small asterisk, $P < 0.01$; large asterisk, $P < 0.001$) between the number of neurons found in each portion of layers II/III and that expected assuming uniform distribution between blobs and interblob regions (13).

DISCUSSION

CytOx blobs have been observed previously in monkey fetuses (19), suggesting that their emergence in the cortex is not triggered by visual experience. The present study shows normal size and distribution of CytOx blobs in prenatally enucleated animals, indicating that cues from retinal photoreceptors, of either molecular or electrical nature, are not essential for the development of the blobs. It is noteworthy that these animals were operated on before the axons of the lateral geniculate nucleus entered the developing cortical plate (20, 21). One embryo was enucleated at embryonic day 81, before any synaptic contacts were established between photoreceptors and bipolar interneurons of the retina (22) and before neurons destined to layers II/III of the visual cortex were generated (23). The second animal was operated on at embryonic day 120—after layers II/III were settled in the cortex, but before they developed a normal synaptic pattern (J.-P. Bourgeois and P.R., unpublished observation).

Since the rods and cones do not establish connections with bipolar cells until after the stage when we ablated the fetal retinae (22), the cerebral cortex in the enucleates developed in the absence of any contact-mediated information from the photoreceptors. However, an instructive role of the input from retinal ganglion cells to the thalamus, which could occur prior to the enucleation, cannot be excluded. Likewise, we cannot determine what role geniculocortical projections may play in the development of the CytOx blobs. Although these afferents are reduced in number in prenatal enucleates, their number has been shown to correlate closely with the surface area of the primary visual cortex (18). Thus, the timing and design of the present study allows us only to conclude that photoreceptors are not essential for the development of cortical hypercolumns.

In juvenile monkeys enucleated as fetuses, as in adult animals subjected to monocular enucleation or other forms of visual deprivation (24–28), the labeling intensity of the blobs is diminished compared with normal adults. One explanation for the low intensity of labeling in these cases is a reduction in oxidative metabolism consequent to transsynaptic deafferentation. A similar mechanism may explain the low labeling intensity of neonatal blobs, which have lacked visual stimulation before birth. However, the remarkable similarity in the size of blobs in enucleates and controls in our study contrasts with the reduction in blob size in monocularly deprived adults (24–26). One can speculate that competition between deprived and normally innervated ocular dominance columns results in shrinkage of the deprived blobs. Alternatively, the mechanisms that regulate CytOx activity may differ at various developmental stages, as postulated to explain age-related differences in the response of CytOx activity to visual deprivation in layer IV (26).

We also found that the preferential distribution of NPY-containing neurons in interblob regions occurs in the absence of visual experience (neonates) and even in the absence of cues from retinal photoreceptors (prenatal enucleates). This result implies that, in addition to the pattern of distribution of CytOx, other molecular features of the striate neurons are also, at least in part, determined intrinsically. It should be pointed out, however, that monocular enucleation and visual deprivation in adults may result in a decrease in the intensity of immunocytochemical labeling and the number of neurons containing γ-aminobutyric acid and tachykinins (27, 28). By contrast, comparable changes in immunoreactivity do not occur in NPY-containing neurons after prenatal enucleation. It is unclear whether this reflects age-related differences in the sensitivity of cortical neurons to transsynaptic deafferentation or changes in the mechanisms that regulate the expression of their neurotransmitters.

Our results in the primate visual cortex stand in contrast to some of the findings in the rodent somatosensory cortex. In rats and mice, the primary somatosensory area contains specialized cellular modules, the so-called barrels, which correspond in number and distribution to the mystacial vibrissae in the whisker pad. The barrels are thought to be induced by cues from specialized receptors in the vibrissal follicles (29, 30). According to this concept, cortical neurons are pluripotential and the developing cortical plate is a tabula rasa, devoid of an intrinsic genetic program, to develop an area-specific or species-specific modular organization (31). Our results caution against such a broad generalization. Although the difference between the tabula rasa hypothesis and our results may be due to the species and areas studied (e.g., primates do not have barrel fields and rodents do not have blobs) or in the timing of the experiments (prenatal in primates, postnatal in rodents), it seems unlikely that the

neurons of the cortical plate do not have any species-specific and area-specific intrinsic programs. Numerous observations indicate that certain basic aspects of the regional cytoarchitecture of the cerebral cortex may be specified by instructions from the embryonic protomap of the cerebral cortex contained within the ventricular zone of the cerebral vesicle, although full development of cortical areas also requires input from the thalamus and other structures (reviewed in ref. 18). The existence of the protomap hypothesis is supported by recent studies indicating that certain protein products of oncogenes, glycoconjugates, vimentin, and various types of adhesion molecules are distributed in a spatially restricted manner early in the developing vertebrate brain, including the cerebral vesicles, before input from receptors at the periphery has an opportunity to induce differentiation of postmitotic cells (32–36). The present results also indicate that the specification of certain chemoarchitectonic patterns extends beyond differences among areas, to the level of functionally distinct compartments within the visual cortex, and that photoreceptor-induced activity is not essential for this level of specification. This finding, of course, does not deny the important role of the periphery in the development and maintenance of functional circuitry, as both intrinsic and extrinsic factors appear to cooperate in building the normal adult cytoarchitectonic pattern of the cerebral cortex (18).

We are grateful to Drs. C. Gilbert, P. Goldman-Rakic, C. Michael, M. Schwartz, and R. Williams for critical comments during the preparation of this manuscript and to Ms. K. Szigeti and Mr. J. Musco for technical assistance. This work was supported by grants from the U.S. Public Health Service and the March of Dimes Birth Defects Foundation.

1. Schmitt, F. O., Worden, F. G., Adelman, G. & Dennis, S. G., eds. (1981) *The Organization of the Cerebral Cortex* (Massachusetts Inst. Technol., Cambridge, MA).
2. Jones, E. G. & Peters, A., eds. (1984) *Cerebral Cortex* (Plenum, New York), Vol. 2.
3. Rakic, P. & Singer, W., eds. (1988) *Neurobiology of Cerebral Cortex* (Wiley, New York).
4. Hubel, D. H. & Wiesel, T. N. (1977) *Philos. Trans. R. Soc. London Ser. B.* **198,** 1–59.
5. Martin, K. A. C. (1988) *Trends Neurosci.* **11,** 380–387.
6. Livingstone, M. S. & Hubel, D. H. (1984) *J. Neurosci.* **4,** 2830–2835.
7. Michael, C. R. (1987) *Soc. Neurosci. Abstr.* **13,** 2.
8. Ts'o, D. Y. & Gilbert, C. D. (1988) *J. Neurosci.* **8,** 1712–1727.
9. Silverman, M. S., Grosof, D. H., De Valois, R. L. & Elfar, S. D. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 711–715.
10. Livingstone, M. S. & Hubel, D. H. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 6098–6101.
11. Hubel, D. H. & Livingstone, M. S. (1985) *Nature (London)* **315,** 325–327.
12. Shipp, S. & Zeki, S. (1985) *Nature (London)* **315,** 322–325.
13. Kuljis, R. & Rakic, P. (1989) *Visual Neurosci.* **2,** 57–62.
14. Rakic, P. & Goldman-Rakic, P. S. (1985) in *Perinatal Neurology and Neurosurgery*, eds. Thompson, R. A., Green, J. R. & Johnsen, S. D. (Spectrum, New York), pp. 1–15.
15. Somogyi, P. & Takagi, H. (1982) *Neuroscience* **7,** 1779–1793.
16. Hsu, S.-M., Raine, L. & Fanger, F. (1981) *J. Histochem. Cytochem.* **29,** 577–580.
17. Kuljis, R. O. & Rakic, P. (1989) *J. Comp. Neurol.* **280,** 393–409.
18. Rakic, P. (1988) *Science* **241,** 170–176.
19. Horton, J. C. (1984) *Philos. Trans. R. Soc. London Ser. B* **304,** 199–253.
20. Rakic, P. (1976) *Nature (London)* **261,** 467–471.
21. Rakic, P. (1977) *Philos. Trans. R. Soc. London Ser. B* **278,** 245–260.
22. Nishimura, Y. & Rakic, P. (1987) *J. Comp. Neurol.* **262,** 290–313.
23. Rakic, P. (1974) *Science* **183,** 425–427.
24. Wong-Riley, M. T. T. & Carroll, E. W. (1984) *Nature (London)* **307,** 262.
25. Carroll, E. W. & Wong-Riley, M. T. T. (1987) *Soc. Neurosci. Abstr.* **13,** 1046.
26. Trusk, T. C. & Wong-Riley, M. T. T. (1987) *Soc. Neurosci. Abstr.* **13,** 1046.
27. Hendry, S. H. C. & Jones, E. G. (1986) *Nature (London)* **320,** 750–753.
28. Jones, E. G., DeFelipe, J., Hendry, S. H. C. & Maggio, J. E. (1988) *J. Neurosci.* **8,** 1206–1224.
29. Van der Loos, H. & Dorfl, J. (1978) *Neurosci. Lett.* **7,** 23–30.
30. Andres, F. L. (1990) *J. Neural Transpl.* **1,** 33–47.
31. Killackey, H. P. (1990) *J. Cog. Neurosci.* **2,** 1–17.
32. Cooper, N. G. F. & Steindler, D. A. (1986) *J. Comp. Neurol.* **249,** 157–168.
33. Steindler, D. A., Cooper, N. G. F., Faissner, A. & Schachner, M. (1989) *Dev. Biol.* **131,** 243–260.
34. Johnson, J. & Van der Kooy, D. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 1066–1070.
35. Lumsden, A. & Keynes, R. (1989) *Nature (London)* **337,** 424–428.
36. Schambra, U. S., Sulik, K. K., Petrusz, P. P. & Lauder, J. M. (1989) *J. Comp. Neurol.* **288,** 101–122.