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A novel cytoarchitectonic area induced experimentally within the primate visual cortex

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ABSTRACT **The cerebral cortex is divisible into a number of cytoarchitectonic areas, but developmental mechanisms that regulate their number and size remain unknown. Here we provide evidence that reducing the population of selected thalamic fibers projecting into the primary visual cortex (area 17) of monkeys during midgestation induces the formation of a novel cytoarchitectonic area situated along the border of and embedded within area 17. This region, termed area X, differs cytoarchitectonically from both area 17 and the adjacent secondary visual cortex (area 18). We propose that an aberrant combination of thalamic and cortical connections acting on a portion of prospective area 17 deprived of its normal thalamic input may result in formation of a hybrid cortex. Our results support the protomap hypothesis of cortical parcellation and suggest how during evolution new cytoarchitectonic regions may arise by cell–cell interactions that depend on a unique combination of intrinsic properties of cortical neurons and afferent fibers.**

The developmental mechanisms underlying the parcellation of cerebral neocortex into structurally and functionally distinct areas is an intensely investigated topic in neuroscience (1–7). This problem is central to our understanding of brain evolution, human cognitive capacity, and cortical plasticity (8). The major issue is whether, before receiving input from the thalamus, all immature cortical neurons are pluripotential or whether they already have a certain level of phenotypic or area-specific commitment. The primary visual cortex (area 17 or striate cortex) of primates is well-suited for addressing this issue because it receives a unique thalamic input from the lateral geniculate nucleus (LGN) and is clearly differentiated from the adjacent prestriate cortex (area 18), which receives thalamic input mainly from the pulvinar, the adjacent nucleus in the thalamus. Recent experiments in rhesus monkey embryos suggest that the developing cerebral wall contains a protomap of cortical parcellation but that subsequent maturation into species-specific cytoarchitectonic areas requires contact interactions with appropriate afferent axonal systems (9). According to the protomap hypothesis, both intrinsic and extrinsic genetic signals are essential for species-specific cortical organization (9, 10).

To assess the relative roles of intrinsic and extrinsic factors in the maturation of cerebral cortical areas, we removed some thalamic afferents by bilateral enucleation performed in embryonic monkeys. In postnatal animals deprived of any retinal input from midgestation, the LGN has fewer neurons and consequently area 17 in the cortex receives a smaller thalamic input. As a result, area 17 occupies a smaller total surface area; yet, remarkably, it retains normal cytoarchitectonic characteristics (9, 11). Rakic (9) suggested that this reduction in visual cortex could be due to the transformation of a fraction of the normal volume of area 17 into a "hybrid" cortex that acquires unusual cytoarchitectonic features. Theoretically, such a partial transformation could be induced if neurons normally destined to become part of area 17 received afferent connections appropriate for other cortical areas. According to this hypothesis, cortical neurons must possess some intrinsic properties that can be modified during development, but only to a limited degree. Here we provide direct evidence that prenatal manipulation of geniculocortical axons can result in the transformation of parts of area 17 into cortical regions with cytological characteristics that are similar to, yet clearly distinguishable from, both area 17 and the adjacent secondary visual cortex, area 18. This experimentally induced, cytoarchitectonically unique cortical area, referred to here as area X, is located at the border between normal areas 17 and 18 and as islands surrounded by an otherwise cytologically normal area 17.

MATERIALS AND METHODS

The study was carried out on two experimental and two age-matched control monkeys (*Macaca mulatta*). Pregnant females were subjected to hysterotomies under halothane/oxygen endotracheal anesthesia, and bilateral retinal ablations of fetuses were performed *in utero* at embryonic day 81 (E81) and E90 using the surgical procedures described (12). The healthy offspring were delivered at term (E165) and underwent euthanasia under deep anesthesia at 3 months and 3 years of age, respectively. The brains were fixed by intracardial perfusion with mixed aldehydes. The blocks containing occipital lobe and thalamus were embedded in celloidin and cut serially in 35-μm-thick sections. Every 10th section was stained with cresyl violet and mounted on slides. The cytoarchitectonic areas of the occipital lobe were reconstructed from serial sections by using a drawing tube. The surface area was corrected for dehydration shrinkage but not for global curvature. The density and number of neurons were determined by video-enhanced differential interference contrast optics and a computer-aided three-dimensional counting procedure. This method allows for morphometric estimates that are independent of section thickness and thus do not require subsequent correction for section thickness or cell size (13).

RESULTS

In both of the experimental animals, as anticipated from the previous study (9), the LGN was noticeably smaller and had considerably fewer neurons than in age-matched controls (Table 1). The number of LGN neurons was 800,000 in the E81 sample and 1,100,000 in the E90 sample. This compares with 1,300,000–1,400,000 in respective age-matched controls and in adult rhesus monkeys (14). In animals enucleated at

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Abbreviations: LGN, lateral geniculate nucleus; E81, etc., embryonic day 81, etc.

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Table 1. Surface area and neuron number comparisons of E81 and E90 bienucleates with corresponding age-matched controls

	3 months		3 years	
	E81	Control	E90	Control
Visual cortex				
Surface area, mm^2				
Area 17	566	629	661	654
Area X	73	—	13	—
Areas 17 and X	639	629	674	654
Neuron number $\times 10^{-6}$				
Area 17	247	288	302	310
Area X	24	—	4	—
Areas 17 and X	271	288	306	310
LGN				
Neuron number $\times 10^{-6}$	0.8	1.3	1.1	1.4

The number of neurons was determined by video-enhanced differential interference contrast optics and a computer-aided, three-dimensional counting procedure (8) that is independent of section thickness and, thus, does not require correction for section thickness or cell size; accuracy is within 5% with this method.

earlier embryonic ages, the LGN contains only ≈500,000 neurons (9), indicating a correlation between the number of geniculate neurons that survive to maturity and the time of ablation. Furthermore, as in the early enucleations, the LGNs of both experimental samples were devoid of layers.

The surface of the occipital lobe displayed abnormal gyral configurations in both experimental animals but the changes were less pronounced than in those after enucleations performed at earlier stages (9). In Nissl-stained preparations, the outer border of area 17 was clear and sharp (Fig. 1*A*). The total cortical thickness and individual laminar thicknesses, the basic cytoarchitectonic pattern, and the cell packing density in area 17 were within normal limits (Table 2). The surface area and volume of area 17 in the E90 sample were comparable to normal and in the E81 sample were ≈10% smaller than in the age-matched control (Table 1). This stands in marked contrast to the dramatic decrease in the volume of cortex observed after enucleation at earlier embryonic ages (9). There is good evidence that an age-related decrease in cortical volume is directly dependent on the number of remaining neurons in the LGN (9).

In both enucleated animals there was a band situated along part of the border between areas 17 and 18, with distinct cytoarchitectonic characteristics—area X (Fig. 1*A*). In addition, the E81 sample had one large and two small regions of area X that were entirely surrounded by a cytoarchitectonically normal area 17 (Figs. 1*B* and 2). In this case, the summation of the surfaces of area X was 70 mm^2, compared with 550 mm^2 for area 17 (Table 1). The E90 sample also displayed an island of area X situated in the lateral operculum. It had a surface area of 13 mm^2 and was surrounded by 661 mm^2 of cytoarchitectonically normal area 17 (Table 1).

We initially thought that area X might comprise segments of area 18 displaced into area 17. However, area X is, in several important respects, different from both areas 17 and 18. To appreciate the observed changes, we should recognize that these two cytoarchitectonic areas in primate cerebrum have very distinct characteristics (Fig. 3). For example, area 17 is visibly thicker than area 18. There are also well-pronounced laminar differences in cell size, cell packing densities, and the pattern of their constellation. Most notably, layer IV in area 17 can be divided into four distinct sublayers—IVA, IVB, IVCα, and IVCβ—while area 18 has a relatively thin single-tiered layer IV (15). Likewise, layer VI in area 17 is divisible into sublayers VIA and VIB, while in area 18 this layer is cytologically uniform. The most obvious

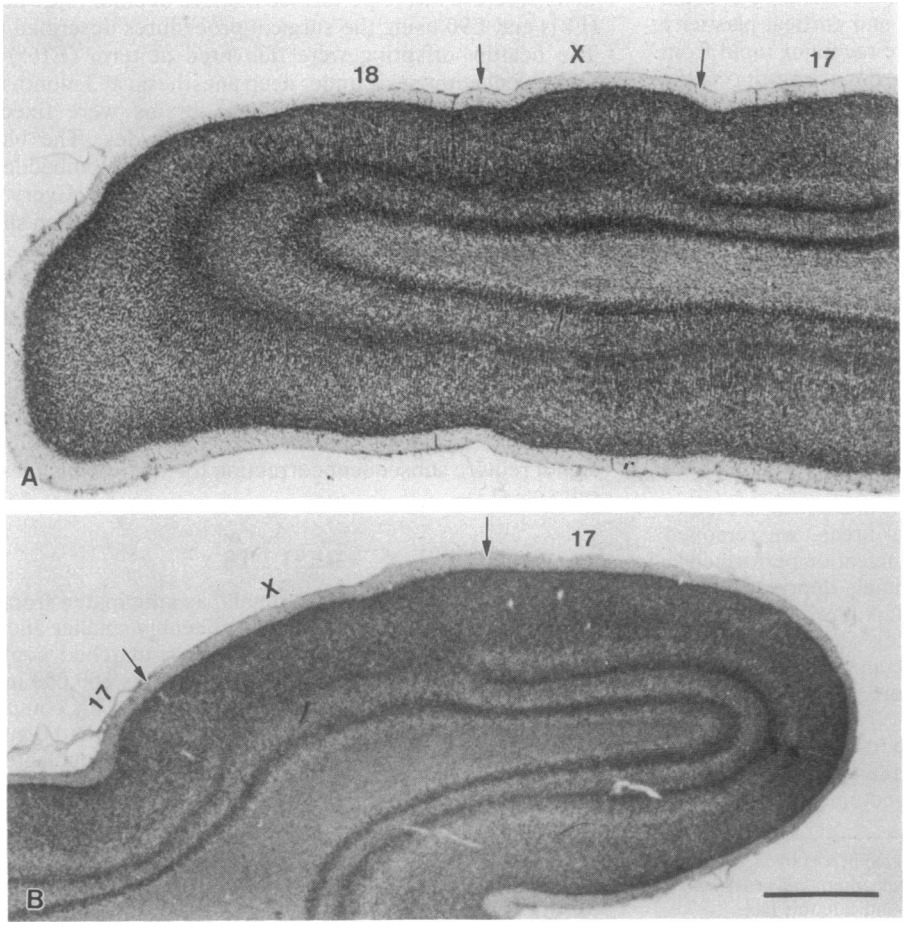

FIG. 1. (*A*) Coronal section across the lateral surface of the occipital lobe in a 3-month-old monkey bilaterally enucleated on E81. The brain was embedded in celloidin and cut in gapless 35-μm-thick serial sections. Every 10th section was stained with cresyl violet. Note the abrupt changes in cytoarchitectonic patterns (arrows) that delineate area 17, area 18, and a strip of area X interposed between them. (*B*) An island of area X inserted within the normal-looking area 17 of the lateral operculum. See reconstruction from serial sections in Fig. 2. (Bar = 1.0 mm.)

Table 2. Comparison of neuronal densities and thicknesses of visual cortex laminae in area 17 (A17), area X (AX) and area 18 (A18) of 3-month-old rhesus monkey bienucleated at E81

Layer	Measurement	A17 (C)	A17 (E81)	AX (E81)	A18 (E81)	A18 (C)
1	Neuron density	44 ± 5	49 ± 6	39 ± 6	27 ± 4	31 ± 7
	Layer thickness	100 ± 20	110 ± 10	110 ± 20	110 ± 10	110 ± 20
2 and 3	Neuron density	300 ± 31	318 ± 41	287 ± 27	273 ± 25	265 ± 22
	Layer thickness	340 ± 30	330 ± 40	480 ± 40	450 ± 40	470 ± 30
4	Neuron density	544 ± 31	531 ± 24	281 ± 52	406 ± 46	423 ± 33
	Layer thickness	450 ± 20	430 ± 20	310 ± 30	220 ± 10	210 ± 20
5	Neuron density	349 ± 24	371 ± 31	321 ± 22	285 ± 34	277 ± 25
	Layer thickness	130 ± 20	120 ± 20	120 ± 20	160 ± 20	150 ± 20
6	Neuron density	444 ± 32	408 ± 38	423 ± 27	322 ± 33	302 ± 27
	Layer thickness	140 ± 20	130 ± 20	130 ± 20	170 ± 30	180 ± 20
Overall	Neuron density	395 ± 24	389 ± 29	281 ± 22	294 ± 24	279 ± 25
	Cortical thickness	1160 ± 40	1120 ± 40	1150 ± 60	1110 ± 70	1120 ± 50

Measurements for area 17 and area 18 in a 3-month-old control (C) are also provided for comparison. Neuronal densities are measured in thousands of neurons per mm^3 and laminar thicknesses are measured in μm and were determined as described in Table 1. The 95% confidence intervals are also provided.

difference between area X and adjacent areas 17 and 18 is in the thicknesses and composition of its cortical layers (Fig. 3 and Table 2). Area X is missing three of four sublayers that are characteristic of layer IV in area 17. First, sublayer IVB (the cell-sparse layer that includes the line of Gennari) is not present. Second the cell-dense sublayer IVA is also missing (Figs. 1 and 3*B*). Instead, sublayer IVCβ—well-pronounced in the portion that has a normal appearance of area 17—seems to be in continuity with the entire nonlaminated layer IV in area X (Fig. 1). Layers I, II, and III in area X are slightly thicker than in either area 17 or 18 in the same specimen (Fig. 3). However, infragranular layers V and VI are thinner in area X than in area 18 and resemble closely the pattern observed in area 17. For example, layer VI has two distinct strata that are characteristic for normal area 17.

The cytometric data reveal more clearly the changes in area X compared with areas 17 and 18. For example, the number of neurons per radial probe in area X is lower than in area 17. Although overall cell-packing density is closer to that of area 18, the distribution of cells across layers differs significantly (Table 2). For example, layer IV in area X is less dense than the corresponding layer in area 18 (Fig. 3*C*), while layer V is more densely packed. Furthermore, area X has a distinct sublayer VIB, which is normally present in area 17 but absent in area 18 (Fig. 3*C*). Finally, area X has larger pyramidal neurons in layers II and III, which contributes to

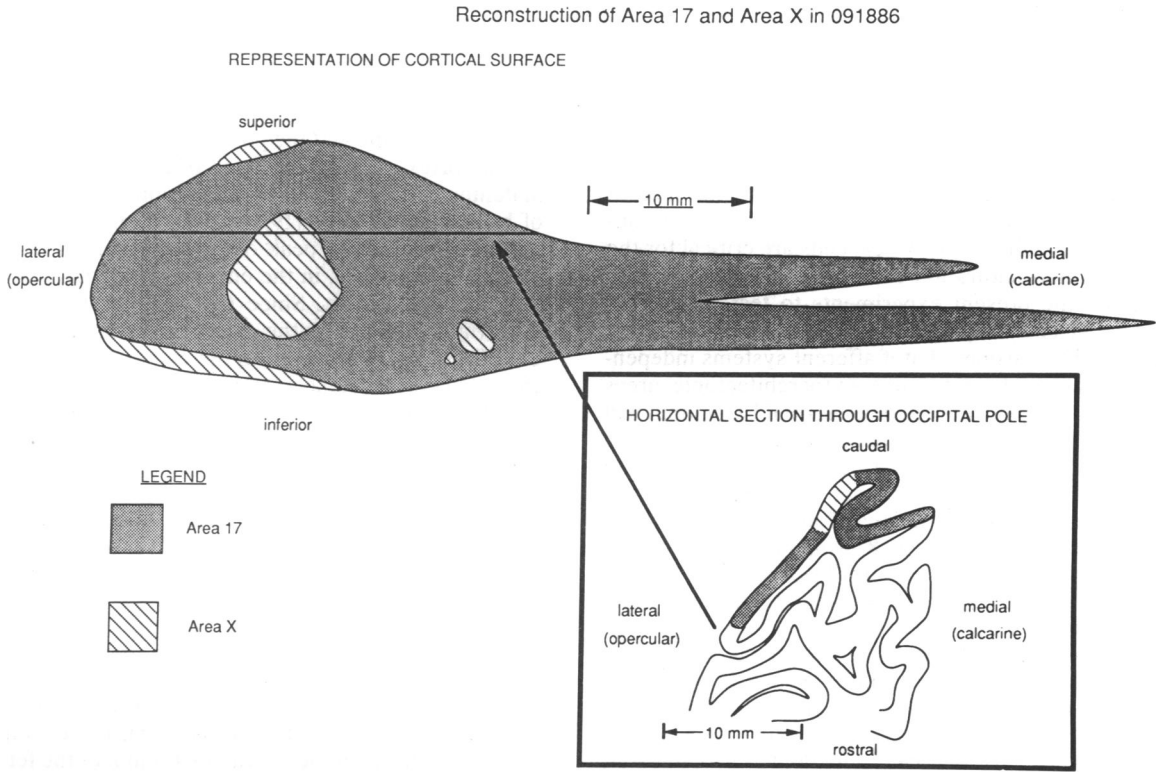

FIG. 2. Computer-aided reconstruction of the visual cortex from serial sections of the occipital lobe in the 3-month-old monkey operated on at E81. The extent of area 17 and the border to the adjacent cytoarchitectonic areas were outlined with a drawing tube. The shaded surface indicates the extent of area 17; the hatched surface represents the extent of area X. The horizontal line across the reconstructed area denotes the position of the section outlined at the lower magnification in the rectangle and in Fig. 1*B*. In the normal monkey, the central visual field would be mapped in the lateral opercular half of area 17 (left) and the inferior visual field would be mapped onto the superior surface of area 17.

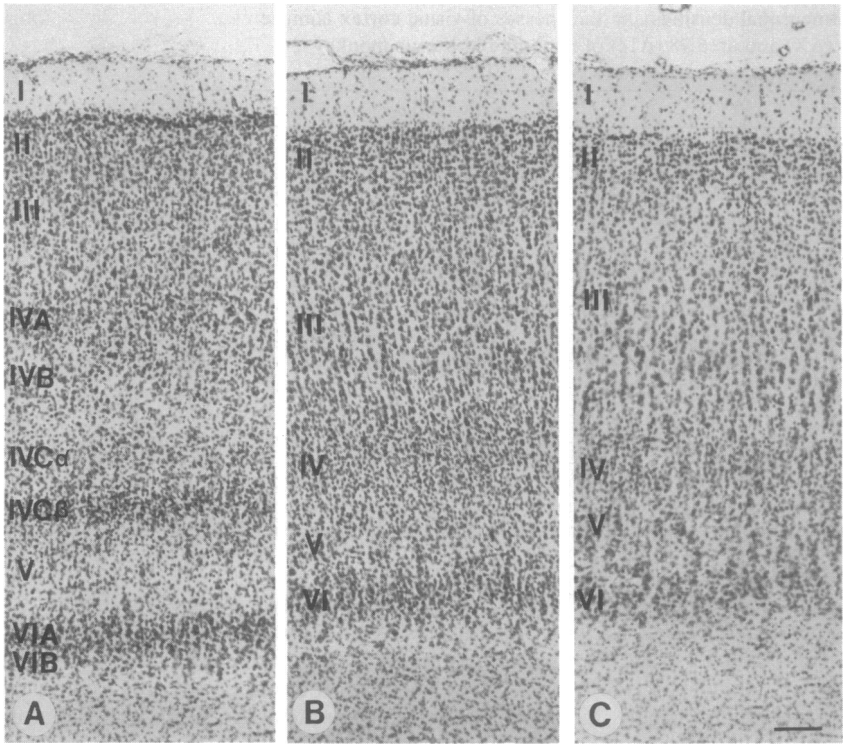

FIG. 3. Representative examples of the cytoarchitectonic pattern in area 17 (*A*), area X (*B*), and area 18 (*C*) in the 3-month-old monkey operated on at E81. Designation of areas 17 and 18 is according to Brodman (15); area X has not been previously described. Cresyl violet-stained 35-μm-thick sections. (Bar = 0.1 mm.)

a lower overall density of cells than observed in area 17 (Table 2). However, due to the increased thickness of layers II/III in area X the percentage of neurons in these layers is greater than in either area 17 or 18. Therefore, area X is characterized by cytoarchitectonic features distinct from both areas 17 and 18. Furthermore, the changes in thickness and character of the cortical laminae did not proceed in the same direction—some layers resemble area 17, and others resemble area 18. In fact, area X differs from both visual areas in more aspects than most of Brodmann's areas differ from each other in normal animals (15).

DISCUSSION

Recent experiments in fetal rhesus monkeys suggest that the developing cerebral wall contains a protomap of mature cytoarchitectonic areas (9). However, contacts between numerous afferent systems and cortical cells are critical for the subsequent normal maturation of cortical cytoarchitecture. We conducted the present experiments to test the role of specific thalamic cortical projections to formation of primary visual cortex. We reasoned that if afferent systems independently and exclusively determine cytoarchitectonic areas then presumptive area 17 devoid of its normal LGN input will receive input from adjacent thalamic nuclei and transform into adjacent area 18. If, on the other hand, there is an intrinsic protomap one would expect that interactions between a portion of presumptive area 17 and "inappropriate" input destined normally for area 18, would result in a hybrid cortex that is cytologically neither 17 nor 18. The present study demonstrates that the removal of normal thalamic input to the visual cortex during development results in the creation of a cytoarchitectonic area, which we call area X.

The timing and sequence of cellular events in the developing primate neocortex before and after bilateral retinal ablation provide insight into the possible cellular mechanisms involved in the formation of area X. Our depletion of thalamic input occurred after neurons destined for layers IV, V, and VI had been generated, but before the genesis and completion of migration of neurons destined for layers II and III (16, 17). Furthermore, cytoarchitectonic differentiation and the emergence of the border between areas 17 and 18 in normal macaques do not occur until about E100 (18). Thus, the cytoarchitectonic features of the visual cortex in normal monkeys emerge 1 or 2 weeks after the described surgical manipulation and only after neurons have attained their normal positions. Injections of [^{3}H]thymidine to label cell birthdays after bilateral enucleation in monkey fetuses demonstrate that postmitotic neurons settle in the supragranular layers of the cortex on schedule in the typical "inside-out" pattern from layer VI proximately to layer I distally (P.R., unpublished data).

We propose that area X is a hybrid cortex formed in response to an altered numerical relationship between the thalamic afferents and the population of target neurons in the visual cortex at a critical stage of development. Numerical matching between neuronal populations is a key determinant of brain development (19). Normally, the number of LGN neurons falls from a peak of 2.0 million to ≈1.4 million by E100 (14); however, in the enucleated animals, cell elimination is more severe. Since the surgical manipulation was performed just before the stage when thalamic fibers grow out of the subplate zone into the cortex (20–22), axons from the adjacent thalamic nuclei—such as the pulvinar—may invade the area vacated by the deleted population of late-forming geniculocortical afferents to the visual cortex. Although the neuronal rearrangement in the cortical plate could have occurred primarily in response to a reduction in number of incoming geniculocortical fibers (9), secondary changes in corticocortical connectivity probably also contributed to the cytoarchitectonic alterations. For example, layer IVB, which was absent in the area X of enucleates, normally contains a high number of reciprocal connections with other cortical areas (23, 24). Likewise, the more abundant neuropile in layers II and III of area X may be due to the expansion or decreased elimination of corticocortical connections that normally terminate in these layers (25). For example, supragranular laminae in the lateral operculum of the fetal monkey contain transient, supernumerary projections to the superior temporal sulcus (26), and such connections may be maintained in area X of the enucleates.

The cytoarchitectonic features of area X cannot be explained by the degeneration of a subpopulation of target

neurons since some layers actually contain more cells than the corresponding layers of area 17. Furthermore, all neurons in area X appear viable, have a normal ultrastructure, and form abundant synaptic connections (J.-P. Bourgeois and P.R., unpublished observations). The reduction in the number of geniculocortical afferents affects the size of area 17 without affecting its thickness or cell number per radial unit (9). In addition, we found that normal cytochrome oxidase distribution, density of synapses per unit volume of neuropile, and complement of receptors for the major neurotransmitters were preserved in this portion of the occipital cortex (27, 28). Importantly, the portion of the presumptive area 17, which is devoid of geniculate input but which may receive inappropriate afferents from the pulvinar, is not transformed into the normal area 18, suggesting that its constituent cells have their own intrinsic program. This finding, therefore, supports the hypothesis that the reduction of the volume of area 17 reflects the conversion of its portions into the hybrid area X due to the acquisition of new connectivity (9). While it could be argued that the hybrid cortex develops solely in response to a hybrid pattern of input, such an entirely extrinsic model fails to explain the sharp border of area X and the equally sharp borders between areas 17 and 18 in the normal cortex of the rhesus monkey.

Our findings have implications for understanding the cellular changes that contribute to the evolution of cortical parcellation. In our experiment, the numerical relationship between axonal input and neuronal targets was altered by reducing the number of thalamic afferents, thereby freeing a part of the visual cortex for alternative development and possibly unique functional characteristics. In contrast, during evolution such an imbalance in input/target relations (9, 19, 29) may arise from an enlarged target area via the increased production of neurons. For example, a mutation of early regulatory genes could enhance global or area-specific neuronal proliferation in the ventricular zone (9). According to the radial unit hypothesis, the ventricular zone consists of proliferative units that produce cohorts of cells that migrate outwardly along the same pathway to the cortex where they form ontogenetic radial columns (9). An increase in the area of the cortical mantle or its subdivisions occurs because of an increase in the number of contributing radial units, which, in turn, could create new input/target ratios and induce different synaptic relationships with subcortical structures or other cortical areas, thereby creating new cytoarchitectonic regions (29). Provided that these cellular events are both heritable and functionally advantageous, new traits would be acted upon by natural selection.

The present results may also aid in understanding the timing and pathogenesis of secondary congenital anophthalmia in humans. As in our experimental monkeys, humans with retinal degeneration before birth have a smaller primary visual cortex (30–32). However, in congenitally blind humans, the prenatal period during which retinal degeneration affects the cerebral cortex and the function of the remaining visual area is not known. Thus, a correlation between the reduction in primary visual cortex and the timing of the lesion within gestation may be precisely established in the experimental model. The present results suggest that retinal loss during a critical midgestational period may induce the development of a cortical region with different cytoarchitectonic characteristics. These findings, together with reports of a higher metabolic activity in the visual cortex in human subjects experiencing retinal loss early compared with later in life (33), suggest that the occipital cortex, if developed in the absence of retinal input, may serve nonvisual functions. The possibility that one cortical area serves as a substrate for processing attributes of an "inappropriate" modality seems likely; for example, it has been shown that rerouting input from the retina via the thalamus into the developing rodent somatosensory or auditory cortex induces nonvisual cortical areas to process elementary visual information (34, 35). Precise information on the critical periods underlying numerical changes and the developmental mechanisms for such changes will help to focus on possible genetic and environmental causes and the subsequent functional modifications in secondary anophthalmia.

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