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Specification of cortical areas

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Prenatal genesis of connections subserving ocular dominance in the rhesus monkey

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In foetal monkey brain neuronal projections carrying input from the two eyes initially overlap; they segregate during the second half of gestation and become fully separated in subcortical visual centres and partially separated in the cortex three weeks before birth and thus before visual experience.

DEVELOPMENT of the binocular visual system has been a major topic of research since the demonstration more than a decade ago that neuronal function can be altered in kittens deprived of vision in one eye[1]. More recently, similar observations in the rhesus monkey[2] have underlined the relevance of this research to an understanding of mechanisms of amblyopia *ex* anopsia in children. In several respects, the development of the visual system is similar in man and monkey. For example, in both species all neurones that comprise the visual system are generated during the first two-thirds of gestation[3–5]. In both species, approximately one-half of the axons from the retinal ganglion cells of each eye are distributed at the optic chiasma to both the ipsilateral and the contralateral dorsal lateral geniculate bodies (LGd). In both man and monkey, the LGd has 6 laminae. Three laminae (1, 4 and 6) receive direct inputs from the contralateral eye and the remaining three (2, 3 and 5) from the ipsilateral eye[6].

The recently developed autoradiographic method of orthograde axoplasmic[7] and transneuronal transport[8,9] provide a simple and powerful method for exploring the structural development of the binocular visual system by tracing the distribution of radioactive label following injection of isotopes into one eye. Radioactivity transported to the appropriate laminae of the LGd and transneuronally to the primary visual cortex (area 17 of Brodmann) is distributed in the sublayers IVA and IVC over columns 350 μm wide that alternate with columns of the same width containing low grain counts[9]. The autoradiographic method thus confirms earlier more traditional anatomical and electrophysiological evidence for columnar organisation. It has also provided the first anatomical evidence for a segregated representation of each eye in the superior colliculus (SC), where fibres from the two eyes terminate in complementary, alternating territories 0.1–0.5 mm wide[10,11].

Autoradiography has also been used to determine the anatomical consequences of monocular deprivation at early postnatal ages. This method reveals a reciprocal expansion and contraction of the ocular dominance columns in layer IV of the visual cortex that are related to non-occluded and occluded eyes, respectively[12]. It has been suggested that the basic connections subserving binocular vision are determined innately, even though, as mentioned above, changes in the width of columns can be induced by an imbalance in visual experience during the first few postnatal months[12]. This interpretation, however, has not been fully accepted by some investigators who argue that visual experience rather than genetic factors may play a fundamental inductive role in establishing functional connections. (For a recent review on the subject, see ref. 13.) An analysis of the prenatal development of the circuitry involved in ocular dominance in the monkey may therefore help to resolve the issue.

Pregnant rhesus monkeys (*Maccaca mulatta*) were subjected to laparotomy and hysterotomy under halothane–oxygen anaesthesia at appropriate gestational intervals (Table 1). Equal amounts of ^{3}H-proline and ^{3}H-fucose (total 1.0–1.5 mCi) were injected into the vitreous body of one eye of each foetus. After injection, the foetuses were returned to the uterus, and the chorio-allantoic membrane, uterine and abdominal walls were sutured in layers. Fourteen days later, after an interval sufficient for concentration of tracer in LGd, SC and visual cortex by axonal and transneuronal transport, the living foetuses were removed from the uterus by a second caesarian section and perfused intracardially with a buffered paraformaldehyde–glutaraldehyde mixture. The brains were embedded in polyester wax, sectioned serially at 8 μm and processed by the standard autoradiographic method.

Retinogeniculate projections

The youngest foetus in this series was injected at E64 (embryonic day 64) by which time all LGd neurones have been generated[14]. This foetus was killed at E78, before the LGd becomes laminated[6]. Thus, this experiment was designed to determine whether retinal ganglion cell axons from each eye are segregated in the LGd before it develops laminae.

Radioactive tracer, transported in an orthograde direction from ganglion cells in the injected eye to the optic nerve, through the optic chiasma and the optic tracts, is concentrated in approximately equal amounts in both LGds. Evidently, equal numbers of crossed and uncrossed retinal fibres are already distributed to each LGd at this foetal age. Although there are areas with slightly higher or lower grain densities, segregation into separate layers is not discernible (Fig. 1*a*). Rather, grains are distributed more or

Table 1 Experimental protocol for three foetal monkeys injected at various times for autoradiography

Animal	Embryonic day (E) when injected	Embryonic day (E) when killed	Exposure time for LGd and SCsg (weeks)	Exposure time for cortex (weeks)
(1) 052775	E64	E78	2, 4	2, 4, 8, 16, 20, 24
(2) 021075	E110	E124	2, 4	2, 4, 8, 16, 20, 24
(3) 061275	E130	E144	2, 4	2, 4, 8, 16, 20, 24

Three foetal monkeys were injected once each at the specified embryonic (E) day into one eye with equal parts of ^{3}H-proline and ^{3}H-fucose. After injection, each foetus was replaced in the uterus and killed 14 d later.

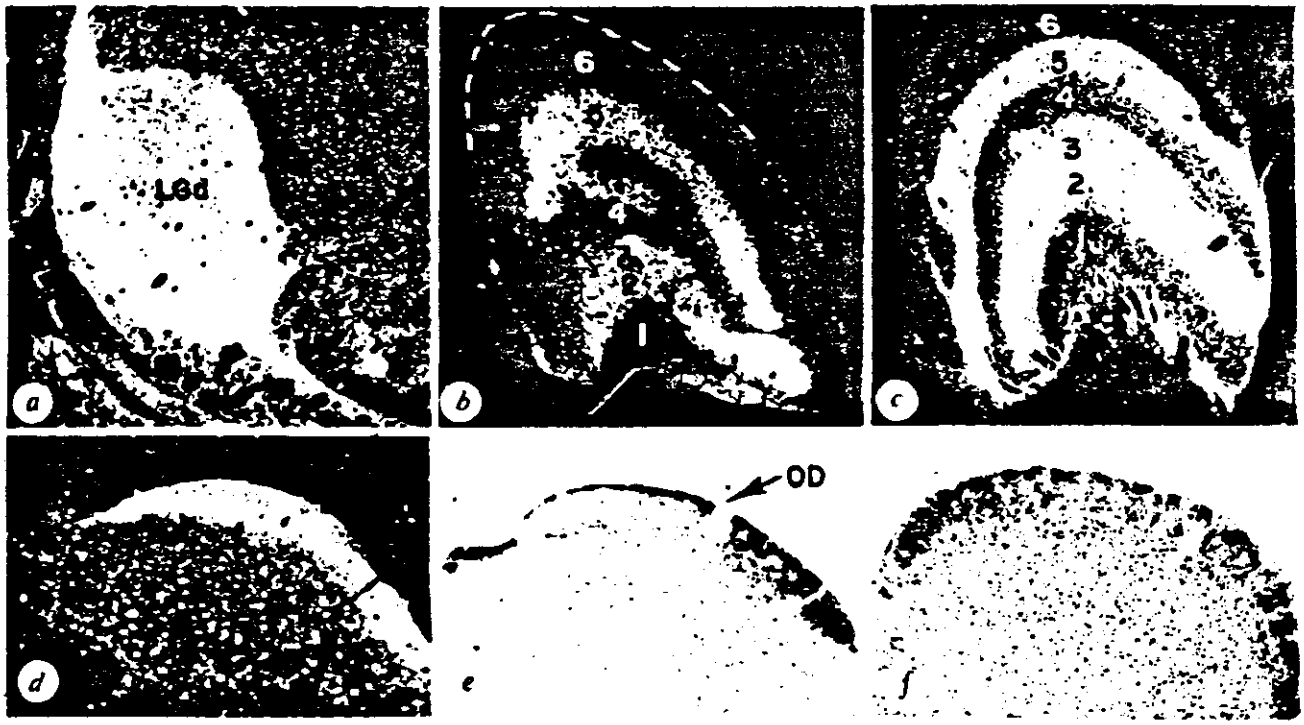

Fig. 1 Dark-field and bright-field illumination photographs of autoradiograms from foetuses exposed by hysterotomy and injected into the vitreous body of one eye with a mixture of ^{3}H-proline and ^{3}H-fucose at different embryonic (E) days. Foetuses were replaced in the uterus and killed 14 d later by a second caesarian section. All photographs are the same magnification (×20). The terms "contralateral" and "ipsilateral" are used with respect to the injected eye. *a*, Contralateral LGd in the foetus injected at E64 and killed at E78. The radioactive label does not suggest a laminar pattern. *b*, Ipsilateral LGd in the foetus injected with comparable doses of the same isotopes at E110, killed at E124. Label is concentrated over the emerging laminae 2, 3 and 5. *d*, Dark-field photograph of a coronal section of the ipsilateral superior colliculus (SC) of the foetus injected at E64 and killed at E78. Label is uniformly distributed over the entire nucleus. *e*, Bright-field photograph of a sagittal section of the contralateral SC in the foetus injected at E110 and killed at E124. Retinotectal projections are clearly segregated into ocular dominance patches indicated by alternating areas of high and low grain counts. The large, empty zone corresponds to the optic disk (OD) representation. *f*, Bright-field photograph of a coronal section of the ipsilateral SC in the foetus injected and killed at slightly later gestational ages (E130–E144). Retinotectal projections are concentrated into discrete patches of dense labelling that correspond to areas devoid of grains in the contralateral SC. The optic disk representation is not present at this level.

less diffusely throughout the entire ipsilateral and contralateral LGd, indicating the possibility that at an early stage of development (around E64) projections from both eyes basically overlap in their distribution among neurones of the LGd with no evidence of the laminar segregation characteristically seen in the adult nucleus.

The second foetus in this series was injected in one eye with a mixture of ^{3}H-proline and ^{3}H-fucose at E110, that is, at an age when the characteristic laminar pattern of the LGd is beginning to emerge[5]. Fourteen days later, at E124, when this foetus was killed, the six-layered pattern of cells of the LGd, although still somewhat irregular, is already clearly developed. The laminae 2, 3 and 5 of the LGd are found to be intensely labelled ipsilateral to the injected eye (Fig. 1*b*) whereas laminae 1, 4 and 6 are labelled in the contralateral nucleus. The laminar segregation of ipsilateral and contralateral retinal inputs to LGd therefore occurs in the interval between E64 and E110 when laminar segregation of their target cells is also achieved. In both LGd, the more ventral laminae (one contralaterally and two ipsilaterally) are more intensely labelled than the more dorsal ones.

The distribution of radioactive label in a third foetus injected and killed at slightly later gestational ages (E130–E144) is essentially the same, except that the distribution of grains over the appropriate laminae resembles closely the adult patern (Fig. 1*c*); that is ,the laminar organisation of cells in the LGd is more regular and more sharply outlined than seen in younger foetuses.

Retinotectal projections

Substantial amounts of radioactivity were transported through the brachium of the superior colliculus into the tectum in all three foetuses. In the youngest foetus, which was injected at E64 and killed at E78, the tracer is concentrated uniformly over the superficial layer of both SC with no evidence of complementary segregation of projections from the ipsilateral and contralateral eyes (Fig. 1*d*). The amount of label is noticeably greater on the contralateral side, however. Fibres from the two eyes therefore initially overlap in the SC, as well as in the LGd.

By contrast, in the foetus injected at E110 and killed at E124, the radioactive label is distributed in a pattern of alternating dense and light concentrations (Fig. 1*e*). Although not as sharply defined, these correspond closely in number and pattern to the ocular dominance 'clumps' observed in the mature monkey[11]. The alternating pattern is most evident in the posterior region of the SC where the retinal axons penetrate more deeply into the superficial grey. The width of the 'clumps' in the foetal monkey, ranging from 0.07 to 0.3 mm, is substantially smaller than that of the adult monkey (0.1–0.5 mm)[11], presumably reflecting the smaller size of the SC in the foetus. As in the adult, the pattern of grain distribution on both sides fits like pieces in a puzzle; that is, territories of the contralateral SC which contain low concentrations correspond to territories of the ipsilateral SC which contain high concentrations. In addition, a sector which contains no label in the contralateral SC represents the position of the optic disk at this foetal age (OD in Fig. 1*e*).

The oldest foetus in this study, injected at E130 and killed at E144, shows essentially the same pattern of alternating grain distribution in the SC. The contrast between heavily and lightly labelled territories is more distinct and the boundaries of these territories are defined as sharply as in the adult monkey (Fig. 1*f*).

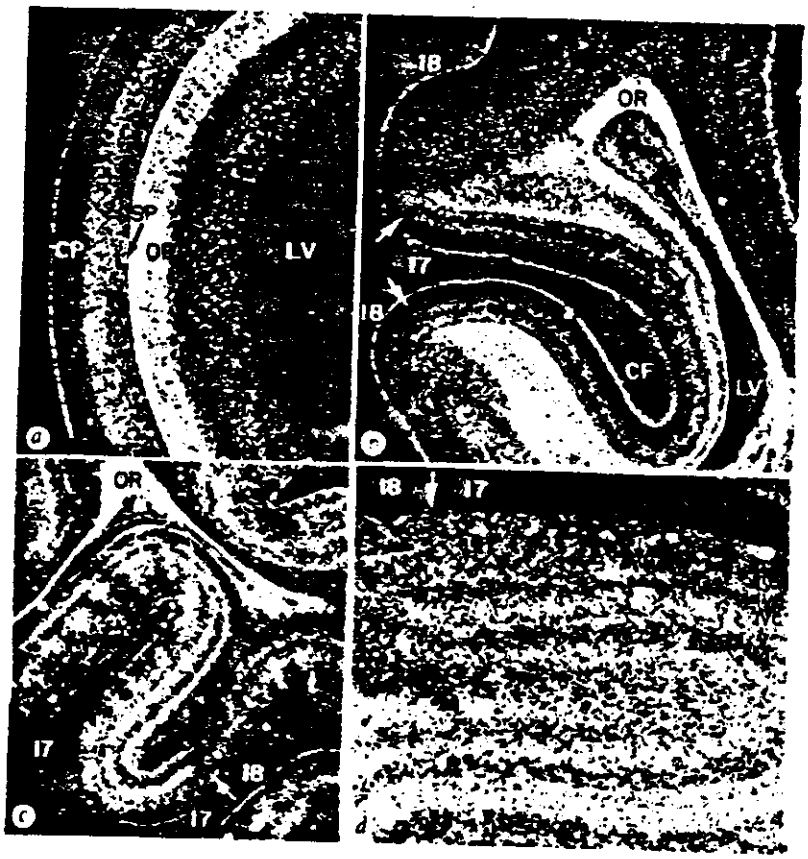

Fig. 2 Dark-field photographs of the cerebral wall and cortex at the level of the occipital lobe in the foetus injected with a mixture of ^{3}H-proline and ^{3}H-fucose at various foetal ages and killed 14 d later. The high grain background in these autoradiograms is due in part to the long exposure (Table 1) and partly to the high dose of ^{3}H-proline-^{3}H-fucose mixture that produces unspecific labelling similar to background levels found in autoradiograms of liver, kidney, spleen and other organs prepared from the same foetuses. Therefore, only a concentration of grains well above background in the autoradiograms of the cerebrum is considered to represent radioactivity in the axons of the visual pathways. *a*, A coronal cut across the cerebral wall in the foetus injected at E64 and killed at E78. The large lateral vesicle (LV) is on the right. The optic radiation (OR) is heavily labelled, but fibres stop at the subcortical zone (SP) and do not invade the developing cortical plate (CP). Approximately ×27. *b*, Occipital lobe of the foetus whose one eye was injected with the same mixture of labels at E110 and killed at E124. The optic radiation (OR) surrounds the lateral ventricle (LV) and emanates axons only to area 17 of the calcarine fissure (CF). Projections to the cortex stop sharply at the borderline between area 17 and area 18 (arrows). Some axons and/or terminals have entered the cortical plate but are distributed uniformly over layers IV and VI. Approximately ×8.7. *c*, Visual cortex of the foetus injected and killed at a slightly older age (E130–E144). More axons and/or terminals have invaded the cortex and are concentrated over layers IV and VI in area 17. ×12. *d*, Higher magnification photograph of the visual cortex demonstrates segregation of axons and/or terminals over sublayers IVA and IVC, as well as alternating regions of higher (black arrowheads) and lower grain counts. The limits of zones with high grain densities in both layers occur sharply at the borderline to area 18. ×30.

Geniculocortical projections

Radioactive label was transported transneuronally into neurones of the LGd and the geniculocortical fibres of the three foetuses as revealed by a high concentration of label of the optic radiations (OR in Fig. 2*a*). Because the amount of radioactivity transferred transneuronally is less than 3% of that transported through the primary pathway[4], autoradiographic exposures were lengthened appropriately for demonstration of geniculocortical projections (Table 1). As expected, this was done at the expense of relatively low background (Fig. 2).

The youngest foetus was injected at E64 and killed at E78, that is when only a fraction of neurones destined for layer IV of the visual cortex are generated[3], and many of these neurones which have already undergone their last cell division have not yet reached their final position and are still migrating towards the cortex through the fibre plexus of the intermediate zone[4]. The autoradiograms show that fibres from the optic radiation (OR in Fig. 2*a*) have reached the occipital lobe, but they have not entered the developing cortex in any substantial number, and most of the label is concentrated in the subcortical intermediate zone of the developing cerebral wall. There is an abrupt decline in grain density at the external border of this zone (SP in Fig. 2*a*) which is situated external to the optic radiation but internal to the developing cortex. The distribution of grains in the intermediate zone forms a continuous, uniform band without periodic variations. Thus, at this stage in the telencephalon, there is no evidence of segregation of fibres derived from the two eyes. This is not unexpected since, in the same foetus, axons of the retinal projections from the two eyes are not yet segregated in the LGd (see above).

The second foetus was injected at E110 and was killed at E124, that is, after all cortical neurones are generated and also after they have completed their migration to the cortex[4]. In this foetus, the location of LGd axons are clearly identified by a dense concentration of grains over the optic radiation (OR) in the occipital lobe (Fig. 2*b*). Numerous fibres pass from the optic radiation towards the cortex of area 17, but stop abruptly at the border of this field with area 18 (Fig. 2*b*). Although the great majority of these fibres is concentrated in the intermediate zone below the primary visual area, small numbers of them do enter the cortex, where they are uniformly distributed over layers IV and VI (Fig. 2*b*). There is, however, no horizontal bilaminar concentration of silver grains over sublayers IVA and IVC and no alternating radially aligned concentrations of grains that might indicate the incipient formation of ocular dominance columns.

In the third foetus that was injected and killed at a slightly later age (E130–E144) more grains are concentrated over layers IV and VI presumably because more geniculocortical fibres have invaded the cortex. In this specimen, a bilaminate pattern of grain concentration can be discerned over sublayers IVA and IVC in autoradiograms exposed 12 to 24 weeks (Fig. 2*c*). The boundaries between these two sublayers are not as sharp as in the adult, however. By and large, the grains over sublayers IVA and IVC at this foetal age are distributed as a continuous sheet, although there are sectors in which an alternating light and dense pattern of grain concentration seems to emerge (Fig. 2*c*). This pattern is of low contrast and, although difficult to see in photomicrographs, it is clearly present when a grain count is performed (Fig. 3). Sectors with higher counts in sublayers IVA are in register with those of IVC, and the same is true of sectors with lower counts (Figs 2*c* and 3). In all likelihood, these represent the emerging ocular dominance columns. Presumably, only the sectors, 100–150 μm wide, which contain the highest grain counts (underlined numerals in Fig. 3) and those with the lowest grain counts (numerals between outlines) correspond to territories occupied predominantly by terminals derived from the injected and uninjected eye, respectively. Therefore, the width of the sectors with high concentration of grains is not 50% of the double columns (Fig. 3) which indicates that there is

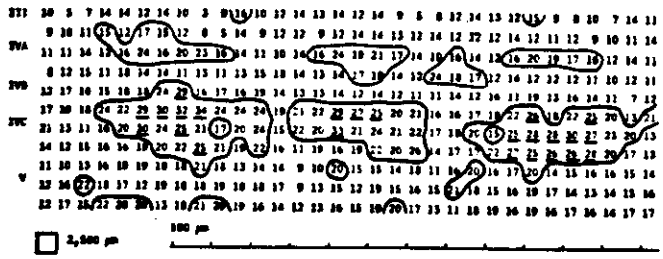

Fig. 3 Results of grain counts over a 0.8-mm² area of layers III, IVA, IVB, IVC and V of the primary visual cortex in monkey foetuses injected at E130 and killed at E144. The autoradiogram is photographed in dark-field illumination and its negative projected by a Prado (Leitz GMBH) apparatus on to grid paper in which each square corresponds to 2,500 μm² area of the cortex. Grains were counted in 352 unit areas along a 1.6-mm length of the calcarine cortex. Areas with 15 grains or more per 2,500 μm² in layer IVA are outlined by a thin line. Areas with 20 grains or more per 2,500 μm² in layer IVC are outlined by a thick line. Areas with 25 grains or more are underscored by a short, thick line. The distance between the two regions of highest grain counts that correspond to the pair of ocular dominance columns is approximately 500 μm. Note that the width of areas with higher grain counts is not half of the double column. Presumably, territories of terminals from the uninjected eye still considerably overlap the territories of injected eye and only a narrow, approximately 100–150-μm wide zone of highest and lowest grain counts may be considered to correspond predominantly to areas which receive projections derived predominantly from one eye.

still considerable overlap between the terminals derived from the two eyes. The combined width of an ipsilateral and contralateral ocular dominance column as determined by the distance between two peaks in grain concentrations is 500–600 μm. The comparable figure for the width of two columns in the mature monkey is 700–800 μm (ref. 9). The difference in width may be explained by a substantial increase in the cortical surface area between E144 and maturity.

Sequence of developmental events

The topographical interrelationship of the central connections in the adult visual system is the result of multiple, complex cellular interactions and events which occur during ontogeny. During the extended prenatal period of primates, young neurones change their position relative to one another; many of them migrate long distances to attain their final locations; and their dendritic and axonal processes differentiate and are deployed while the entire brain changes its external and internal form dramatically[4,8,14]. These cellular activities are coordinated in space and in time and may play a fundamental role in the formation of complex cytoarchitectonic patterns and point-to-point connectivity of widely separated visual centres.

These observations indicate that cellular events fundamental to the prenatal development of the visual system in the rhesus monkey may be separated into two broad phases: in the first phase, axons derived from each eye invade target structures, and their endings are distributed in an overlapping manner; in the second phase, the terminal axons derived from the two eyes become segregated from each other in their target structures. Although injections of isotopes at closer intervals and shorter survival times are needed to define the duration of each phase, the available evidence suggests that the first phase may be relatively short, even in the protracted development of the rhesus monkey, whereas the second phase may last for several weeks. Thus, in the foetus injected at E110 and killed at E124, afferents from the LGd are distributed in an overlapping manner over the entire IVth layer of the visual cortex. Even 6 weeks later, on the seventh postnatal day, the segregation into monocular columns is far from complete[12]. It is important to emphasise that although differentiation of pathways begins later in the cortex than in subcortical structures, the initial segregation of binocular connections in the cortex nevertheless begins well before birth—that is, before visual experience.

Thus the effect of monocular deprivation on the relative size of territories corresponding to each eye[17] may not necessarily involve the induced expansion of territories related to the functional eye. Rather, since domains of ocular representation overlap with each other during ontogeny (Fig. 3), it is possible that the territory of the functional eye remains arrested at a given developmental stage, while the territories of the deprived eye shrink. In this context, our developmental data support the hypothesis that alterations in the relative width of ocular dominance columns could be degradative rather than inductive.

Recently, autoradiographic studies have demonstrated that radial columnar organisation is not confined to the primary visual cortex but is a generalised characteristic of the mammalian neocortex[15,16]. For example, in the motor and prefrontal cortex of the neonatal monkey, callosal and cortico–cortical connections form well developed, sharply delineated columns whose development at this age is much more advanced than that of the ocular dominance columns of the visual cortex[16]. The precocious development of columns in the frontal lobe of the monkey adds further support to the notion that columnar organisation of the cortex may be largely specified innately. The significant alteration of monocular dominance columns by unbalanced visual stimulation during early stages of postnatal life in primates may occur because columns are not yet fully developed.

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SCIENCE

Concurrent Overproduction of Synapses in Diverse Regions of the Primate Cerebral Cortex

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Synapses develop concurrently and at identical rates in different layers of the visual, somatosensory, motor, and prefrontal areas of the primate cerebral cortex. This isochronic course of synaptogenesis in anatomically and functionally diverse regions indicates that the entire cerebral cortex develops as a whole and that the establishment of cell-to-cell communication in this structure may be orchestrated by a single genetic or humoral signal. This is in contrast to the traditional view of hierarchical development of the cortical regions and provides new insight into the maturation of cortical functions.

THE CEREBRAL CORTEX IS DIVIDED into numerous cytoarchitectonic areas that are specialized structural and functional units (*1*). Cortical differentiation is most fully expressed in the human brain and underlies the subdivision of the cortex into sensory, motor, and associative systems. Although this cortical diversity is of major conceptual and biomedical importance, the mechanisms of its development are unknown (*2, 3*). Studies based on histological and histochemical parameters such as the density and distribution of myelin (*4*), levels of various enzymes (*5*), and metabolic activity (*6*) tend to support a hierarchical model of cortical development in which primary sensory and motor areas mature before adjacent secondary areas, and the association regions differentiate last. Although this model has had a major influence on physiological and psychological studies (*7*), a number of recent findings are not entirely consistent with it. For example, neurons in the primary visual cortex begin and complete their genesis later than neurons in the adjacent secondary visual areas (*8*), and the columnar organization of connections in the prefrontal association cortex (*9*) emerges prior to that of ocular dominance columns in the primary visual cortex (*10*).

We examined the pre- and postnatal course of synaptogenesis in five areas of the monkey cerebral cortex that mediate, respectively, visual, somatosensory, motor, associative, and limbic functions. On the basis of the available literature (*3–6*), we expected that synaptogenesis would proceed in clearly segregated waves in different cortical regions, with the sensory areas achieving maturation earlier than association areas. We also predicted that synaptogenesis would exhibit laminar specificity, and perhaps follow the inside to outside pattern of cortical neurogenesis (*11*) or the sequence of ingrowth of various afferents. Contrary to these expectations, however, our results revealed a simultaneous synaptogenesis in all areas and layers examined.

Rhesus monkeys (*Macaca mulatta*) of various pre- and postnatal ages were perfused with mixed aldehydes (*12*), and 1 by 2 by 3 mm blocks were dissected from the visual, somatosensory, motor, and prefrontal cortices, and the dentate gyrus of the hippocampus, and processed for electron microscopic analysis (Fig. 1). More than 500,000 synapses were identified from 22 monkeys in a total of 25,000 electron micrographs. Twenty percent of these synapses were selected randomly for further classification on the basis of their termination (on spines, dendritic shafts, or somas) or their morphology (symmetrical or asymmetrical) in each layer (*13*). The data are expressed as density of synapses per unit area of neuropil to provide a measure that is unaffected by age-related changes in the extracellular space, by the growth of neuronal perikarya, or by the addition of glial cells, myelin sheaths, or blood vessels.

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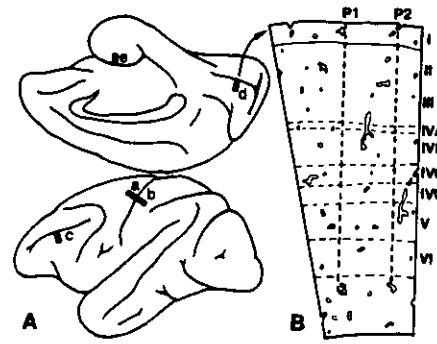

Fig. 1. (A) The lateral surface of the left cerebral hemisphere (bottom) and the medial surface, inverted (top), show the five cortical areas examined: a, motor cortex (Brodmann's area 4) in precentral gyrus; b, somatosensory cortex (area 1) in the postcentral gyrus; c, prefrontal cortex (area 9) in the upper bank of the principal sulcus; d, visual cortex (area 17) in the upper bank of the calcarine fissure; and e, molecular layer in the dentate gyrus (area 34). The blocks were postfixed in osmium, embedded in Epon-Araldite, and 600-angstrom sections were cut across the entire width of the cortex. (B) An outline of an ultrathin section across the visual cortex (d), as an example. The two vertical lines (P1 and P2) indicate the localization of two probes each yielding about 100 electron micrographs that were printed at a final magnification of ×14,000. Similar probes were prepared for other cortical areas except the dentate gyrus, where probes were taken only across the width of the molecular layer of the suprapyramidal and infrapyramidal limbs.

During the last 2 months of gestation, synaptic density increased at a rapid rate in the five cortical areas examined, reaching between 15 and 20 synapses per 100 μm^2 of neuropil by the time of birth (Fig. 2). Although this density is about the same as in sexually mature adults, it continued to increase during infancy and remained above adult levels for about the same length of time in all five areas (Fig. 2, A–E). The highest density ranged from 26 synapses per 100 μm^2 in the prefrontal cortex (Fig. 2C) to 34 synapses per 100 μm^2 in the visual cortex (Fig. 2D). Analysis of covariance revealed no significant differences among the slopes of increase in the four neocortical areas (Fig. 2, A–D), but the slope of the increase in the dentate gyrus (Fig. 2E) was lower than that of each other area ($P < 0.001$) (*14*). This lag in the rate of synaptic increase in the dentate gyrus might be because the dentate gyrus, unlike the neocortex which has a full complement of neurons before birth (*11*), acquires additional neurons during the first three postnatal months (*15*).

Synaptogenesis proceeded concurrently in all cortical layers; the density of synapses per unit area of neuropil in the relatively cell-poor layer I was not substantially different from that in neuron-rich layers II through VI in any of the five areas examined. Although the distribution of various classes of synapses differed from area to area as well as from layer to layer (*16*), the density per unit area of neuropil of all synaptic types combined was nevertheless similar in all areas and layers.

Synaptic density increased for several months after birth before beginning to decline in all layers and areas (Fig. 2). The decline occurred rapidly at first and then slowed during the second half of the first year; after this there was an even more gradual reduction throughout life (Fig. 2). The decrease in synaptic density cannot be attributed to dilution caused by an increase in cortical volume since extracellular space, neuronal somata, glial cells, and other tissue elements such as blood vessels and myelin sheaths were not included in our measurement. Furthermore, the percentage of neuropil in the cortex does not change appreciably during the period of synaptic decrease in the rhesus monkey (*17*). Finally, if the decrease in synaptic density were due to dilution, we would expect all classes of synapse to be affected similarly. However, synapses situated on dendritic spines, which make up 60 to 70 percent of the cortical synapses in the rhesus monkey, sustained the largest share of this loss. Synapses on dendritic shafts (30 to 40 percent) and cell somas (below 1 percent) contributed less to the age-related changes. In addition, the ratio between asymmetrical and symmetrical synapses changed in the prefrontal cortex from 4:1 at birth to 7:1 during the 4th month, and then again reached 4:1 at puberty. Changes in this ratio in the motor cortex were even larger—7:1 at birth, 24:1 during the 4th month, and again 7:1 in adult animals. We can conclude, therefore, that the decrease in synaptic density is achieved by elimination of synapses. Furthermore,

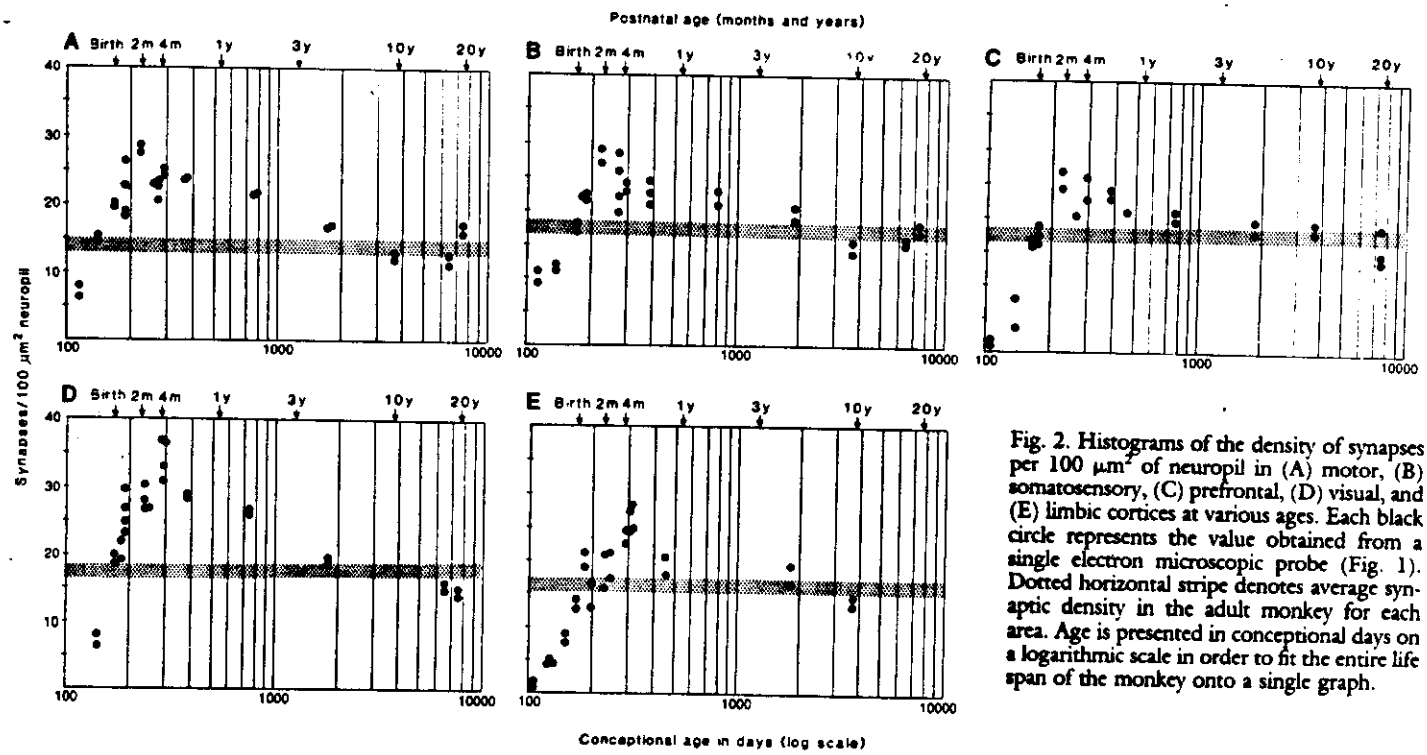

Fig. 2. Histograms of the density of synapses per 100 μm^2 of neuropil in (A) motor, (B) somatosensory, (C) prefrontal, (D) visual, and (E) limbic cortices at various ages. Each black circle represents the value obtained from a single electron microscopic probe (Fig. 1). Dotted horizontal stripe denotes average synaptic density in the adult monkey for each area. Age is presented in conceptional days on a logarithmic scale in order to fit the entire life span of the monkey onto a single graph.

synaptic density finally stabilizes at the same value of 15 to 20 synapses per 100 μm^2 of neuropil in all five regions examined (dotted stripes in Fig. 2). This value is similar to that found previously for structures of the primate brain as different as the retina and neostriatum (*18*). The value of 15 to 20 synapses per 100 μm^2 of neuropil may be structurally, metabolically, or physiologically optimal. Whether this value is species-specific remains to be determined.

Although transient overproduction of synapses could be predicted from previous observations made in various species (*19*), as well as in human cortex (*20*), our study compares the timing and magnitude of these events in different cortical layers and brain regions from the same specimens. The isochronic course of synaptogenesis in the primate cerebral cortex during infancy was unexpected because, since the time of Flechsig (*4*), the areas examined have been thought to mature anatomically, biochemically, and functionally at different rates (*3, 5–7*). It was also unexpected that synaptic density increased at identical rates in all cortical layers, since neurons of each layer are generated at different times (*8, 11*) and receive different ratios of monoaminergic, thalamic, cortico-cortical, and local synaptic connections (*21*). The simultaneous "overshoot" phase in diverse areas and layers of the cortex and the final common density achieved suggests that the cortex develops as a whole rather than regionally, and that formation of synapses throughout the entire cortical mantle may be regulated by common genetic or humoral signals. Simultaneous overproduction of synapses may be essential for competitive interactions between extrinsic afferents such as the competition that has been postulated between the projections of the two eyes during the formation of visual centers (*10, 22*). Our results, as well as other recent studies on the visual (*22*) and peripheral (*23*) nervous systems, suggest that if experience alters synaptic number during development it does so by causing selective survival of certain synapses, and not by regulating their initial formation.

Our findings contrast with the classical view of a hierarchical sequence of functional development from the sensory to motor, and finally to associative functions (*7*). However, they may help to explain certain behavioral findings that were heretofore puzzling. For example, rhesus infants as young as $2\frac{1}{2}$ months of age have the capacity to tactually discriminate texture and size differences at the level of an adult monkey (*24*). Likewise, visual tracking of small objects, visually guided reaching, and discrimination of facial features, skills indicative of visual cortical function, appear between $1\frac{1}{2}$ and 2 months (*25*); visual object discrimination performance first becomes possible at about 2 months of age (*26, 27*). Although fully independent use of the digits does not mature until between 7 and 8 months after birth, some independent finger usage begins at 2 months and is quite efficient by 4 months (*28*). Numerous other indices of adult posture and progression, as well as regression of infantile motor reflexes commonly attributed to the development of "descending" control, occur around 2 months of age (*29*). Performance on a memory task sensitive to hippocampal damage in adult rhesus monkeys is possible at 2 months and reaches mature levels at approximately 4 months of age (*27*). Delayed-response performance follows a similar ontogenetic sequence (*30*). The latter task measures cognitive functions that are mediated by the principal sulcus from which the prefrontal sample was taken in our study (*31*).

Thus, various indices of sensory, motor, limbic, and associative cortical function are all expressed between 2 and 4 months of age, a time period which coincides with excess synapse production. The attainment of these behavioral milestones within the first few postnatal months indicates that the synchronous production of a critical mass of synapses in each cortical area may be essential for their parallel emergence. However, behavioral competence continues to increase beyond the stage of excess synapses. This suggests that full functional maturation may be related to synapse elimination and acquisition of synaptic efficiency at the molecular level. Increasingly complex cortical capacities might also evolve from the accretion and storage of information and subsequent interactions among cortical areas rather than from further changes in the number of synaptic contacts.

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SCIENCE

Specification of Cerebral Cortical Areas

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How the immense population of neurons that constitute the human cerebral neocortex is generated from progenitors lining the cerebral ventricle and then distributed to appropriate layers of distinctive cytoarchitectonic areas can be explained by the radial unit hypothesis. According to this hypothesis, the ependymal layer of the embryonic cerebral ventricle consists of proliferative units that provide a proto-map of prospective cytoarchitectonic areas. The output of the proliferative units is translated via glial guides to the expanding cortex in the form of ontogenetic columns, whose final number for each area can be modified through interaction with afferent input. Data obtained through various advanced neurobiological techniques, including electron microscopy, immunocytochemistry, [^{3}H]thymidine and receptor autoradiography, retrovirus gene transfer, neural transplants, and surgical or genetic manipulation of cortical development, furnish new details about the kinetics of cell proliferation, their lineage relationships, and phenotypic expression that favor this hypothesis. The radial unit model provides a framework for understanding cerebral evolution, epigenetic regulation of the parcellation of cytoarchitectonic areas, and insight into the pathogenesis of certain cortical disorders in humans.

THE HUMAN CEREBRAL CORTEX IS PERHAPS THE MOST remarkable product of brain evolution, not only because it makes up two-thirds of the neuronal mass and contains about three-quarters of all our synapses, but also because it is the structure that most distinctively sets us apart from other species. One of the most prominent features of the cerebral cortex is its parcellation into cytoarchitectonic areas. These areas are defined as regions of the cortex with distinct cellular, biochemical, connectional, and physiological characteristics (*1*). For example, individual cytoarchitectonic areas may process specific sensory information, control precise motor activity, or be involved primarily in complex cognitive functions such as language, facial recognition, or spatial orientation. Unraveling principles governing cytoarchitectonic parcellation is of great biomedical and theoretical significance, since it holds the key to explaining the evolution of human creativity and the pathogenesis of many mental disorders.

Although in recent years considerable attention has been devoted to the specification of topographic maps within given cytoarchitectonic areas (*2*), relatively little progress has been made in understanding the formation and regulation of the size of cytoarchitectonic areas themselves. Even cursory comparison of the pattern of cytoarchitectonic maps in various species reveals several important points. First, the surface of the neocortex has expanded enormously during phylogeny (for example, the surface of the hedgehog neocortex is less than 1% of that of a macaque monkey and 0.1% of that of a human). Second, cytoarchitectonic areas do not expand equally (for example, the primary visual cortex occupies 1/5 of the neocortical surface in monkeys and only 1/30 of that in humans). Third, new functional and anatomical areas are introduced during evolution (for example, Broca's language area in man). Fourth, there are large variations in the sizes of cortical areas between individuals of the same species and between the two hemispheres in the same individual (for example, larger temporal areas on the left side in right-handed humans).

How are these inter- and intraspecies differences in cortical parcellation generated? How does the surface of areas expand so enormously during evolution while the thickness of the cortex remains relatively constant? To what extent is the number of neurons in each cytoarchitectonic area innately determined? Do environmental factors, including functional activity and experience, play a role in this process? If so, when does the susceptible period for

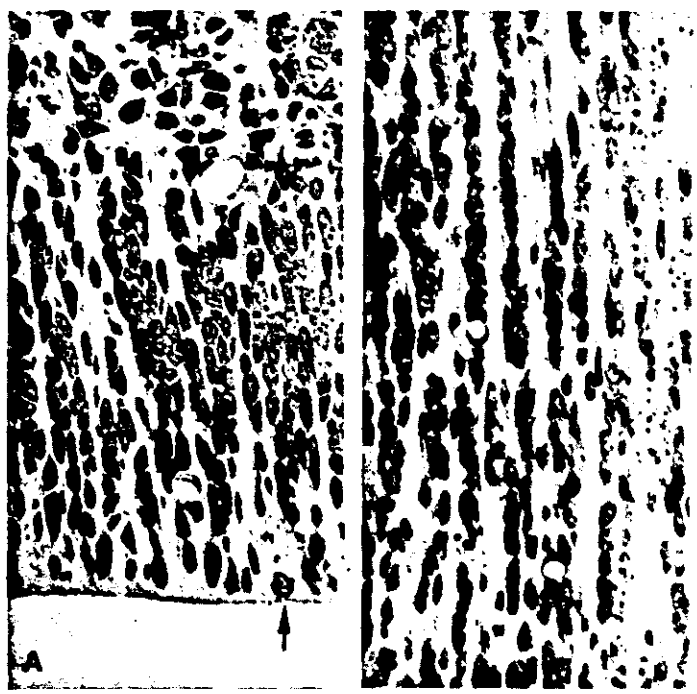

Fig. 1. (**A**) Photomicrograph of an array of proliferative units within the ventricular zone of the occipital lobe in a 91-day-old monkey embryo. Most mitotic figures are located directly at the ventricular surface (arrow), although at this age some can be found in the subventricular zone (crossed arrow). (**B**) Cortical plate in the occipital lobe of the same animal showing ontogenetic columns composed of neurons that have originated from the set of proliferative units illustrated in (A). Epon-embedded tissue, cut at 1 μm, stained with cresyl violet.

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structural and functional modifiability of cortical maps end? Considerable progress has been made in defining and answering these questions, particularly for the visual cortex. Yet, to obtain closure on some of these questions, it may be essential to take into account early events that include the site and time of origin of cortical neurons and the mechanisms that control their number, migration, and the timing of their disposition in the cortex.

This article formalizes a hypothesis of cortical development based on studies of neocortical development for which anatomical methods, immunocytochemistry, [^{3}H]thymidine and receptor autoradiography, electron microscopy, and intrauterine surgical manipulations of brain development were used in nonhuman primates. Improvements of intrauterine surgery made possible experimental manipulations of nervous system development in large mammals that were traditionally limited to avian and amphibian embryos (*3*). The large size of the fetal monkey's cerebrum and the presence of visible landmarks at its convoluted surface allow precise excision of selected structures or cortical areas, while its protracted development enables accurate timing of cellular events.

Cortical Neurons in Primates Are Generated Prenatally

Although it had been suspected for a long time that most cortical neurons in man were formed before birth, only the method of marking DNA replication with [^{3}H]thymidine provided precise data on the onset and termination of corticogenesis in primates. Examination of adult monkeys exposed to [^{3}H]thymidine during pre- and postnatal ages revealed that the genesis of all cortical neurons occurs during the middle of gestation which, in this species, lasts about 165 days (*4, 5*). Cortical neurogenesis starts around the 40th embryonic day (E40) and lasts between 1 and 2 months; in the limbic cortex (Brodmann's area 24) it stops at E70 and in the primary visual cortex (area 17) at E100. Neocortical neurons in this species are not produced during the remainder of gestation or at any time after birth (*6*). Comparative cytological analysis indicates that in humans, with a gestational period of about 265 days, the first cortical neurons are also generated around E40, but their production continues until E125 (*7*). Thus, unlike most species that have been examined, in which corticogenesis lasts until birth or even shortly afterward (*8*), primates including humans acquire their full complement of cortical neurons during the first half of gestation.

Cortical Neurons Originate Outside the Cortex

The high density of mitotic figures that can be observed at the ventricular surface in the human fetal cerebrum suggested that most cortical neurons might be produced there (*9*), but again, the direct evidence comes from [^{3}H]thymidine autoradiographic analysis. Examination of series of monkey embryos killed within 2 hours after injection of this DNA precursor revealed that all neurons destined for the neocortex are produced in the proliferative zone near the cerebral ventricle (*10*). This zone in the fetus is organized as a pseudostratified epithelium in which precursor cells divide asynchronously; their nuclei move away from the ventricular surface to synthesize DNA and move back to the surface to undergo another mitotic division (*10*). Golgi, electron microscopic, and immunocytochemical analyses revealed that neuronal and glial cell lines coexist in the ventricular zone from the onset of corticogenesis (*11, 12*). Furthermore, as illustrated in Fig. 1A, the ventricular zone is divided by glial septa into well-defined columns of precursor or stem cells termed "proliferative units" (*7*). From my own counts in the rhesus monkey, the number of stem cells in a unit is only 3 to 5 at early stages and up to 12 at later stages. [^{3}H]Thymidine autoradiographic analysis shows that, around E40, proliferative units start producing postmitotic neurons, which migrate to their prespecified areal and laminar positions in the cortex (*4*).

Neurons Migrate Along Radial Glial Guides

A massive migration of cortical neurons in primates occurs during midgestation and coincides with the rapid growth of the cerebral wall and buckling of its surface. A combination of Golgi, electron microscopic, and [^{3}H]thymidine autoradiographic analyses revealed that postmitotic neurons find their way through the intricate cellular lattice of the intermediate and subplate zones by following shafts of radial glial cells (*11*). These elongated non-neuronal cells stretch

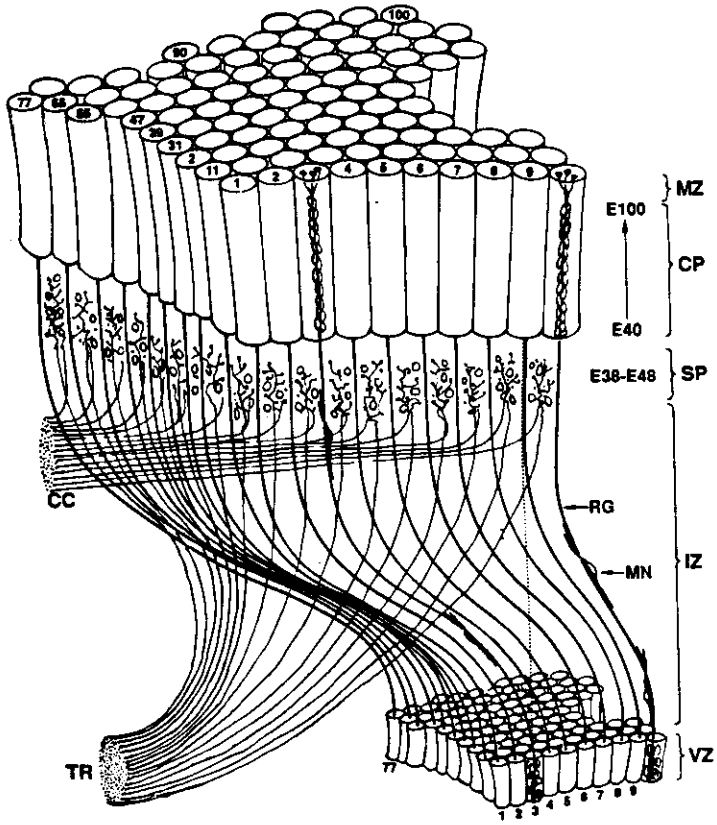

Fig. 2. The relation between a small patch of the proliferative, ventricular zone (VZ) and its corresponding area within the cortical plate (CP) in the developing cerebrum. Although the cerebral surface in primates expands and shifts during prenatal development, ontogenetic columns (outlined by cylinders) remain attached to the corresponding proliferative units by the grid of radial glial fibers. Neurons produced between E40 and E100 by a given proliferative unit migrate in succession along the same radial glial guides (RG) and stack up in reverse order of arrival within the same ontogenetic column. Each migrating neuron (MN) first traverses the intermediate zone (IZ) and then the subplate (SP), which contains interstitial cells and "waiting" afferents from the thalamic radiation (TR) and ipsilateral and contralateral cortico-cortical connections (CC). After entering the cortical plate, each neuron bypasses earlier generated neurons and settles at the interface between the CP and marginal zone (MZ). As a result, proliferative units 1 to 100 produce ontogenetic columns 1 to 100 in the same relative position to each other without a lateral mismatch (for example, between proliferative unit 3 and ontogenetic column 9, indicated by a dashed line). Thus, the specification of cytoarchitectonic areas and topographic maps depends on the spatial distribution of their ancestors in the proliferative units, whereas the laminar position and phenotype of neurons within ontogenetic columns depends on the time of their origin.

across the fetal cerebral wall from the beginning of corticogenesis but are most prominent during midgestation when many of them temporarily stop dividing (*13*). During the migratory period, cohorts of cells originating in individual proliferative units follow a radial pathway consisting of a single or multiple glial fibers. All migrating neurons may have the same binding affinity for all radial glial cells, but the spatio-temporal order of neuronal migration is preserved, since each postmitotic neuron remains in contiguity with a given radial glial fascicle (Fig. 2). Although we have evidence that an occasional neuron may translocate to nearby radial glial fascicles (*14*), they do not follow other classes of cellular processes present in the migratory zone. Thus, one pair of binding complementary molecules with gliophilic properties can account for the entire phenomenon of radial migration (*15*). The possible cellular and molecular mechanisms underlying the guidance and translocation of migrating neurons have been reviewed elsewhere, and several candidates for recognition and adhesion molecules are being tested (*16*). In this review, I focus exclusively on the relevance of glial scaffolding for the radial unit hypothesis of cortical parcellation.

Migrating neurons on their way to the cortex follow glial guides through a long and tortuous pathway across the rapidly expanding intermediate and subplate zone (Fig. 2). Afferents from the thalamus (*17*) and from other cortical areas (*18*) wait transiently in this zone, among early generated interstitial neurons (*5, 19*). The existence of the subplate zone provides an opportunity for interaction between migrating neurons and populations of ingrowing afferents prior to their arrival at the cortical plate (Fig. 2). The significance of these contacts is not fully understood, but the presence of transient synapses, neurotransmitters, and neuromodulators suggests some sort of chemical interaction (*5, 19*). Neurons traversing this zone remain attached to glial guides before entering the cortical plate to form radially oriented columns (Fig. 1B).

Proliferative Units Produce Ontogenetic Columns

My earlier [^{3}H]thymidine data showed that the genesis of cortical neurons in monkeys does not begin until E40 (*4*). Therefore, neuronal progenitors prior to that time only form other progenitors. After E40, however, progenitor cells start to produce dissimilar daughters (by asymmetrical division), one of which becomes a neuron while the other may remain as a stem cell or die. The evidence that stem cells in the proliferative units divide asymmetrically comes from a [^{3}H]thymidine autoradiographic analysis, which revealed that the number of grains present over neurons situated progressively more superficially in the cortex diminish stepwise by halves (Fig. 3A). This finding indicates that one daughter cell remained in the proliferative unit and diluted its radioactivity by subsequent divisions. Some divisions, however, must be symmetrical, since a proliferative unit, which might initially start from a single precursor, contains several cells during later stages of corticogenesis and therefore can be regarded as a polyclone. This is also evident from the pattern of [^{3}H]thymidine labeling, which shows that within the same column intensely labeled and more lightly labeled neurons (presumably from the same progenitor) can be interspersed among unlabeled neurons that originate from a different progenitor in the same proliferative unit (Fig. 3, B and C). Eventually, all postmitotic cells generated in a single proliferative unit (Fig. 1A) form a morphologically identifiable stack of neurons in the cortex (Fig. 1B) variously termed "ontogenetic" or "embryonic" columns (*7*).

The number of neurons in an ontogenetic column of the rhesus monkey ranges between 80 in the anterior cingulate cortex (area 24), where neurogenesis lasts about 1 month, to more than 120 in the primary visual cortex (area 17), where neurogenesis lasts almost 2 months (*4, 5*). However, this number can be modified by differential cell death (*20*). Within each column, earlier generated neurons occupy deeper positions and therefore those arriving later have to pass them to become situated more superficially (Fig. 2). This relation, called the "inside-out" gradient of neurogenesis, suspected by classical anatomists (*21*), has been confirmed by [^{3}H]thymidine autoradiography in a number of species (*4, 8*). The inside-out gradient is particularly sharp in primates where each daily injection of [^{3}H]thymidine labels a highly selective sample of cortical neurons (*4*).

Fig. 3. Photomicrograph of the three autoradiograms showing neurons in the cortex of an adult monkey that was exposed to [^{3}H]thymidine at E70. (**A**) The most intensely radioactive neuron (a) lies deeper in the cortex than the two progressively less labeled, more superficially situated neurons (b and c). (**B** and **C**) Unlabeled neurons (crossed arrows) may be interspersed among radioactive neurons within the same ontogenetic column. Further explanation is in the text.

Ontogenetic Columns Contain a Variety of Neuronal Phenotypes

The radial unit hypothesis predicts that each proliferative unit must produce multiple neural phenotypes, which terminate in a single ontogenetic column. Indeed, a recent study in which RNA retrovirus–mediated gene transfer was used to mark ventricular cells and all their progeny with β-galactosidase shows that labeled neurons in the mouse cortex form interrupted radial columns (*22*). This finding confirms the basic postulate of the radial unit hypothesis, including the polyclonal nature of proliferative units. Similar recombinant retrovirus studies, as well as labeling of clones with fluorescent dyes, carried out in the retina and optic tectum indicate that a single progenitor can produce more than one cell phenotype, all of which end up within the same radial ontogenetic column (*23*).

After their last division, prospective cortical neurons probably become committed to a basic cell type before reaching their final position. The evidence for this view is strong. First, neurons that remain near the cerebral ventricle as a consequence of x-irradiation of rat embryos acquire the morphology and connections expected on the basis of their time of origin (*24*). Second, ventricular cells transplanted from ferret embryos into the telencephalon of a newborn host assume cortical positions, morphological characteristics, and connections appropriate for the donor (*25*). Third, a subset of neurons destined to form the corpus callosum in the monkey fetus send their axons to the contralateral hemisphere before entering the ipsilateral cortical plate (*26*). Fourth, in the reeler mouse, a neurological mutant, in which cortical laminae are positioned inappropri-

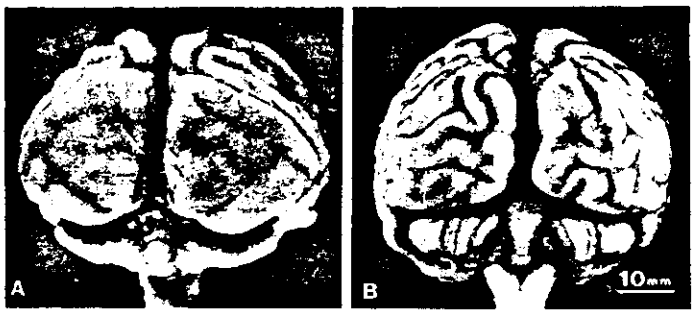

Fig. 4. Posterior view of the cerebrum in (**A**) a normal 3-year-old monkey and (**B**) an age-matched animal that underwent bilateral enucleation at E60. The development of a new pattern of sulci and gyri is seen in the normally smooth lateral surface of the occipital lobe.

ately, neurons differentiate into phenotypes corresponding to the time and place of their origin rather than their ectopic location (*27*). Finally, in the monkey embryo, the ventricular zone subjacent to areas where the density of neurons in adults is high produces more neurons over a longer period than ventricular zones underlying areas where the density is low (*5, 28*). These findings collectively indicate that the range of morphologies and patterns of synaptic contacts of cortical neurons may be specified in large measure before reaching their final positions. Even neurons that have been described as having exuberant connections during development are limited in the type and number of connections they can make (*29*). Understanding the origin of neuronal lineages within the units does not explain how they find appropriate positions in cytoarchitectonic areas and how they become an integral part of the cortical circuitry as a whole.

Cortex Is the Sum of Its Ontogenetic Columns

The radial unit hypothesis posits that each proliferative unit begets a corresponding ontogenetic column (Fig. 2). Earlier work (*5, 7*) suggested that proliferative units within the ventricular zone are a mosaic forming a proto-map of prospective cytoarchitectonic areas; that is, proliferative units within the ventricular zone generate a series of area-specific neuronal cohorts. The proto-map in this context is simply a diagrammatic prediction of what each region of the ventricular zone will produce if postmitotic neurons are constrained in their migratory behavior or otherwise prevented from randomly mixing while on their way to the cortex. Indeed, the ventricular proto-map is translated via the radial glial scaffolding to the expanding cortical plate in the form of arrays of ontogenetic columns. The number of units producing neurons for specific cytoarchitectonic areas can be expected to vary among species and individuals. From counts on samples of ventricular zones, the total number of ontogenetic columns in the macaque cerebrum is estimated to range between 15 and 20 million (*30*). In humans, this number must be ten times larger and individual variation perhaps even greater; in mice it is about 1/15 as large. Area 17 in the rhesus monkey is estimated to contain between 2.5 and 3 million ontogenetic columns.

Columnar organization of the adult cortex, which can be conceived of as a mosaic of interrelated columns or radially organized modules of neurons (*31, 32*), may be a reflection of its developmental history in keeping with the radial unit hypothesis (*7*). It was originally shown by Mountcastle (*31*) that neurons situated within a single column in the somatosensory cortex are responsive to a specific modality and receptive field of stimulation. A similar anatomical and functional columnar organization was later found in other sensory and association cortices (*33*). Projections from a given thalamic nucleus, subnucleus, or "cell cluster" that terminate within the cortex in the form of stripes may therefore be considered as innervating a series of radial columns arranged as "colonnades," each serving as a basic processing module (*34*). The relatively constant size of columnar afferent terminal fields among species with vastly different cerebral surface areas supports the idea that the cortex expands by the addition of such radial units rather than by their enlargement (*35*).

What Controls Radial Unit Number?

The remarkable expansion of the cortical surface during evolution is not associated with a corresponding increase in cortical thickness (*36*). The radial unit hypothesis provides an explanation of how this expansion could occur. As discussed earlier, proliferation in the ventricular zone initially proceeds by symmetrical division and only at later stages switches to asymmetrical division. Therefore, a single additional round of mitotic division at the stage of proliferative unit formation (for example, before E40 in the human and monkey) doubles the number of units and consequently doubles both the number of ontogenetic columns and number of cells in the cortex. In contrast, an extra round of asymmetrical division in each proliferative unit after E40 increases the number of neurons within each ontogenetic column by only one and therefore has a small effect on the thickness of the cortex.

While the size of individual cytoarchitectonic areas depends on the number of contributing proliferative units, the thickness of the cortex depends on the number of neurons produced in each unit. Therefore, determination of the number of proliferative units is a crucial step in both individual development and evolution of the cortex. We could envision that an area of the ventricular proto-map can be duplicated by an additional round of cell division within the ventricular zone during unit formation, before the stage of neuronal production. At least two sets of controlling genes, analogous to homeotic or segmentation genes (*37*), may be involved. One set could provide instruction for changes and duplication in the proto-map at the ventricular surface, thereby influencing the size of cytoarchitectonic areas. Subsequently, another set of genes could control the identity of various neuronal phenotypes produced within the proliferative units, thereby generating variations in the composition of ontogenetic columns subserving specific modalities.

Another possibility is that all neurons of the cortex are initially equipotential. In this extreme case, the cortical plate is a "tabula rasa," and there is no need for a map at the ventricular surface, since any cortical neuron could transform to any phenotype, carry out any function, and subserve any modality. Area specificity would be determined exclusively by the complement of afferents that each area receives after the completion of neuronal migration (*32, 38*). This possibility appears to be supported by the ostensible similarity in the laminar pattern of all cortical areas and certain common aspects of their synaptic circuitry (*34*). It also seems to be in harmony with reports that intra-area topographic maps can be altered by cues from the periphery (*3*) and with findings that single units with essentially normal visual receptive field properties can be recorded from the somatosensory cortex when input from the retina is experimentally rerouted to somatosensory thalamus (*39*). The tabula rasa hypothesis leaves control of cytoarchitectonic parcellation entirely to cues received from the periphery via the thalamus. However, this mechanism is not reconcilable with accumulated evidence that axonal input cannot be the sole determinant of the organization of its target structure, even if it has a considerable modulating effect. In addition to the arguments already presented [see (*24–28*)], it is worth mentioning that correct topological connections in anophthalmic

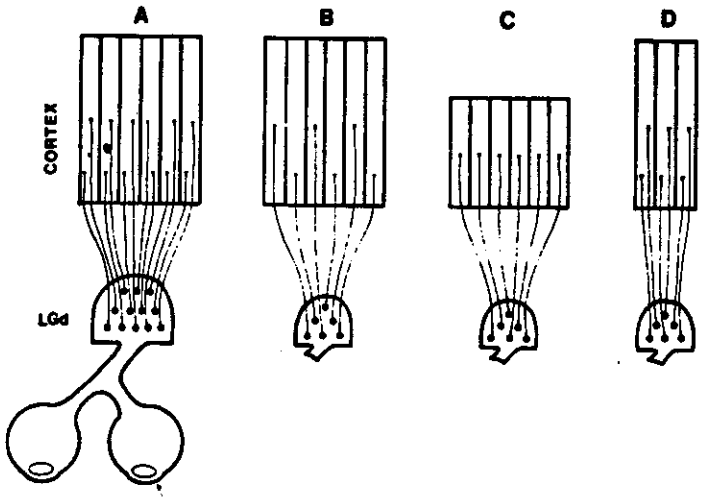

Fig. 5. Schematic outline of the possible consequences of diminished input from the dorsal lateral geniculate nucleus (LGd) to the visual cortex in adults binocularly enucleated at early embryonic stages. The normal numerical relation between geniculocortical projections and ontogenetic columns illustrated in (**A**) can be altered in the three basic ways (**B**, **C**, and **D**) that are discussed in the text.

mice (*40*) and in early enucleated animals (*41*) can form in the absence of information from the periphery. Finally, there is considerable evidence that some cue must be present in neurons of the cortical plate to attract appropriate sets of input (*42*).

The radial unit hypothesis postulates that proliferative units produce cohorts of neurons which have a certain level of area-specific competence as they move to the cortex but that the number of ontogenetic columns devoted to a given area can be further regulated by afferents from subcortical and other cortical areas. This regulation can occur early in the subplate zone (SZ in Fig. 2) where migratory neurons and "waiting" afferents destined for the cortex have a chance to interact (*5, 17, 19*) or later by influencing the rate of cell death. In either case, this model involves both genetic and epigenetic regulation of cortical parcellation, in harmony with the concepts of selective stabilization, neuronal group selection, competitive elimination, regressive events, and self-organization, which have been proposed to explain the formation of synaptic connections (*43*). All of these concepts are based on the fact that initially there is a larger number of participating neurons and synapses. The present model of cytoarchitectonic parcellation also involves cell interaction followed by elimination. This hypothesis is being tested by manipulating the number of cortical afferents from the thalamus and from other cortical areas in monkey embryos.

Manipulation of Input to the Cortex

The role of afferents in cortical parcellation was examined by altering the number of axons comprising specific thalamo-cortical systems (TR in Fig. 2) at early embryonic stages and determining the size of corresponding cortical target areas in adult animals. The finding that binocular enucleation performed around E60 reduces the number of geniculate neurons to less than one-half the number in age-matched controls (*41*) without altering any other component in the thalamo-cortical system provided the key for this approach. The occipital lobe in five early enucleates displayed dramatic and remarkably reproducible changes in the convolutions of the occipital operculum (Fig. 4). These changes did not occur when enucleation was performed in the second half of gestation. Use of antero- and retrograde transport of axonal tracers in early enucleates reveals the presence of topographically well defined reciprocal connections of the occipital lobe with the vestige of the lateral geniculate nucleus (*41*).

With respect to the radial unit model, the diminished thalamic input could theoretically produce several outcomes. One possibility is that both the number and height of the ontogenetic columns—that is, the surface and width of area 17—would remain the same as in controls (Fig. 5, A and B). A second possible outcome is reduction in cortical width because of transneuronal degeneration consequent to thalamic degeneration, with the number of columns or surface of area 17 unchanged (Fig. 5C). Either of these two outcomes would effectively eliminate the possibility that the number of thalamic axons controls the number of ontogenetic columns. However, the third possibility—namely, that the number of ontogenetic columns devoted to area 17 is reduced while the thickness of this cortex remains the same—would suggest that thalamic input can influence the size of a cytoarchitectonic area (Fig. 5D). Results from the present study clearly support the third possibility, illustrated in Fig. 5D.

Area 17 in five enucleated monkeys was well differentiated from adjacent area 18, and its thickness and characteristic laminar pattern were surprisingly normal (Fig. 6). However, the surface area and the total number of neurons in area 17 in the early enucleated subjects were less than half those in age-matched controls. What is most important is that, despite the drastically reduced number of geniculo-cortical afferents, area 17 contained the normal number of neurons per unit volume of each layer and per each radial column (*41*). We also examined the distribution of ten major neurotransmitter receptors in early enucleates and found that, in spite of some modification, ligand binding retained the basic laminar pattern characteristic of area 17 (*44*). Furthermore, cytochrome oxidase "puffs" in layers II and III, which are thought to subserve color and form vision (*45*), were segregated and maintained in the visual cortex of early binocular enucleates (*46*). Finally, synaptic density per unit of neuropil, as revealed by quantitative electron microscopy, developed within the normal range in all layers (*47*). These results indicate that the basic cytological, synaptic, and biochemical characteristics of area 17 can develop in the absence of information from the retina. However, the number of ontogenetic columns, and therefore the surface of the visual cortex, can be modified by the number of geniculo-cortical axons.

The observed reduction in the size of area 17 in enucleates could result either from the creation of fewer columns or from an increase in their elimination (Fig. 6B). However, neither possibility is likely, since enucleation was performed after all proliferative units should have been formed, and cell death restricted to entire columns of cells has never been observed. It is, therefore, possible that the total number of ontogenetic columns in the cortex of animals that were operated on remained the same and that the adjacent cytoarchitectonic area, which normally receives input mainly from the thalamic nucleus (pulvinar), expanded (Fig. 7C). This would require respecification of neurons genetically programmed to be part of area 17 to accommodate input characteristic for area 18. This mismatch may create a "hybrid" cortex that retains some characteristics of area 17 but takes on some features of area 18 as a result of receiving different input (X in Fig. 7D). Our preliminary data show an unusual pattern of receptor binding and change in synaptic composition of the region directly adjacent to area 17. This possibility is attractive because it provides a testable model of how a new type of cytoarchitectonic area can be introduced during evolution.

Regulation by thalamic input is probably only one part of a complex interactive process that occurs during parcellation of the neocortex. For example, prenatal resection of the fetal cortex, which eliminates or decreases the amount of cortico-cortical input to the

unoperated areas on both sides (*48*). Newly created cytoarchitectonic areas resulting from either thalamic or cortical lesions may have a novel pattern of connections or a redistribution of transmitters, receptors, and local synaptoarchitecture. Properties of the hybrid areas created experimentally by early manipulation of various types of afferents to the cortex need further analysis by anatomical and physiological methods, as they may hold the key for understanding both evolution of the cortex and the pathogenesis of inherited disorders of higher cortical function.

Cortical Malformations and the Radial Unit Model

The radial unit hypothesis of cortical development provides insight into the pathogenesis of some inherited and acquired cortical malformations observed in animals and man. Methodological limitations would allow even extreme changes in the relative size of cortical areas to remain undetected. However, one rare but directly relevant example is the condition of congenital anophthalmia, in which a child is born without eyes. In such individuals, area 17 has normal thickness and lamination but a greatly reduced surface (*49*). Although the cause of anophthalmia is unknown, the result is totally explicable by our findings from bilateral enucleation experiments, which indicate that this cortical abnormality is caused by a defect in eye formation during the first third of gestation. Other pertinent examples are focal malformations found in both humans and mice, in which only a segment of the cortex confined to several radial units is highly disturbed and sharply delineated from adjacent areas (*50*), an indication that only cells originating from a small part of the ventricular zone have been affected. Finally, in humans in whom the cortical auditory area in one hemisphere is larger than in the other, the corresponding thalamic auditory nucleus (medial geniculate body) is also larger (*51*). The positive correlation between the number of thalamic axons and size of the corresponding area in the cortex is consistent with the proposed hypothesis.

Because of a lack of knowledge about the etiology of most cortical malformations in humans, their classification was traditionally based on the appearance of the cortex at autopsy (*52*). The radial unit hypothesis suggests that the pathogenesis of some cortical malformations can be classified into two categories. The first category comprises malformations in which the number of radial units in the cortex is reduced while the number of neurons within each ontogenetic column remains relatively normal. It can be expected that defects in this category result from an early occurring event, which alters the number of proliferative units at the time they are being formed—in humans, within the first 6 weeks of gestation, before the onset of corticogenesis. Once proliferative units in the ventricular zone are established, albeit in fewer numbers, each unit can produce a normal or even greater number of neurons, which are destined to be ontogenetic columns but are crowded in the diminished cerebral vesicle. It could be expected that the cortex would have smaller surface area in spite of normal or enlarged thickness and massive neuronal ectopias. These features are observed in some human malformations such as lissencephaly and pachygyria (*52*).

The second category consists of malformations in which the initial formation of radial units is not affected while the number of neurons within ontogenetic columns is reduced. The defect in this category should begin after the first six fetal weeks when the normal complement of proliferative units has already been established. Such malformations can be caused by interference with cell proliferation via intrinsic (genetic) or extrinsic (irradiation or viral infections) factors. Diminished production of neurons in the proliferative units

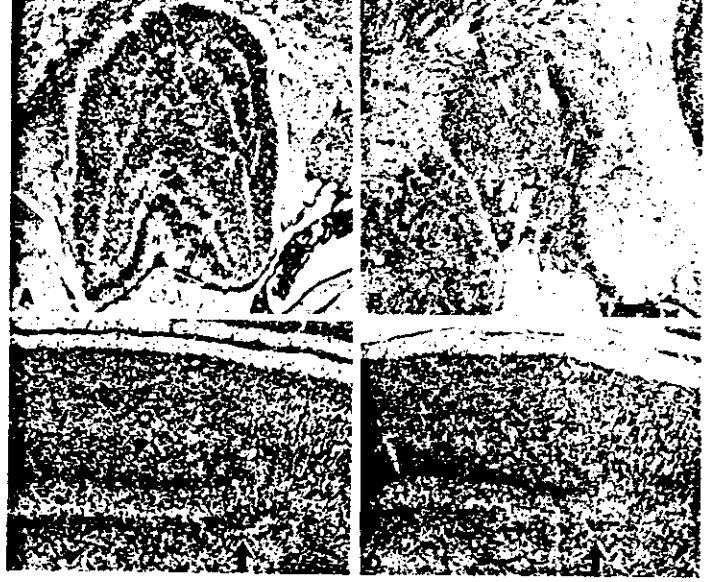

Fig. 6. Photomicrograph of the lateral geniculate nucleus in (**A**) a normal rhesus monkey and (**B**) its grossly diminished counterpart in the age-matched monkey subjected to binocular enucleation around E60. The border between the striate cortex (left) and extrastriate cortex (right) in (**C**) a normal adult monkey and (**D**) an age-matched binocular enucleate are marked with arrows. Photomicrographs (A and B) and (C and D) are reproduced at the same magnification.

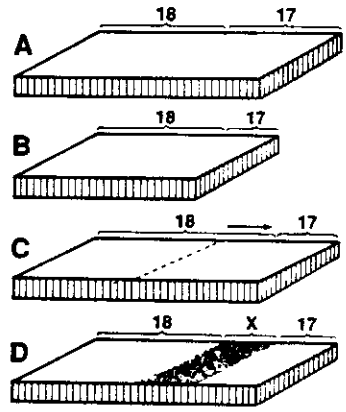

Fig. 7. Schematic representation of the possible modes of decrease in the size of area 17 caused by experimental reduction of thalamic input. (**A**) Relation between areas 17 and 18 in a normal animal; (**B**) differential cell death; (**C**) encroachment of adjacent area 18 into the territory of area 17; and (**D**) formation of an abnormal cytoarchitectonic area (X) that consists of neurons genetically destined for area 17 but which receive input characteristic for area 18. Further explanation is in the text.

results in fewer neurons within ontogenetic columns and the cortex is therefore thinner. The number of neurons in ontogenetic columns could also be affected by cell death or by a failure of their migration. In the latter case, some neurons may survive in ectopic positions within the white matter. All of this can be observed in the so-called polymicrogyric brain (*52*). It should be recognized that cortical malformations may have features of only one or the other category, but in practice, most show a mixture of both. The proposed classification suggests possible developmental mechanisms by separating defects of unit formation from defects of ontogenetic column formation.

Conclusions and Prospects

The radial unit model is a working hypothesis that postulates both genetic and epigenetic mechanisms for establishing the pattern and size of cytoarchitectonic areas during ontogenetic and phylogenetic development. According to this hypothesis, the ventricular zone consists of proliferative units that constitute a proto-map of prospec-

tive cytoarchitectonic areas. We suggest that one set of controlling genes provide general instructions for individual and species-specific changes in the proto-map. Another set of genes control cell production in the units. At later developmental stages, each proliferative unit becomes a polyclone that, mostly through asymmetrical division, produces cohorts of postmitotic cells that migrate along common radial glial guides and stack up in reverse order of arrival in the cortex. These stacks of neurons, called ontogenetic columns, become basic processing units in the adult cortex. The surface area of each cytoarchitectonic region during evolution and in each individual, therefore, depends on the number of contributing proliferative units, while the thickness of the cortex depends on the number of cell divisions within the units. In support of this concept, experimental and neuropathological data indicate that each step (formation of proliferative units, formation of ontogenetic columns, and formation of cytoarchitectonic areas) can be separately affected by genetic defects or by extrinsic factors. It can, therefore, be predicted that genetic alteration as well as mechanical, chemical, or viral lesions of distant but synaptically related structures that result in reduced input to the cortex would affect subsequent developmental events and provide the setting for new cell relationships, the net outcome of which could be the emergence of a unique cytoarchitectonic map. However, further research in this field is needed to gain deeper insight into the genetic and molecular mechanisms involved in parcellation of the normal or diseased human cerebral cortex.

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30. The number of ontogenetic columns can be counted in cresyl violet–stained sections (for example, in Fig. 1B) and their number estimated in the fetal cerebral cortex. Present estimates are limited to the midgestational period (E81, E90) since subsequent development of sulci and gyri, neuronal growth, elaboration of dendrites, arrival of afferents, formation of synapses, proliferation of glial cells, and emergence of horizontal lamination distort the initially simple radial organization. Preliminary data indicate that the monkey's cortex has about 20 million units, but many more cases have to be processed to provide statistically significant results. The ontogenetic columns counted here should not be confused with the "minicolumns" [A. J. Rockell, R. W. Hirons, T. P. S. Powell, *Brain* **103**, 221 (1980)], which are based on the number of neurons in 30-μm-wide radial strips of adult cortex. Our recent study indicates that the number of cells in minicolumns of the rhesus monkey is more variable than hitherto assumed [R. W. Williams, K. Ryder, P. Rakic, *Soc. Neurosci. Abstr.* **13**, 1044 (1987)].
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SCIENCE

Development of Visual Centers in the Primate Brain Depends on Binocular Competition Before Birth

Abstract. *Removal of one eye before birth permanently changes the cellular organization and synaptic connectivity of visual centers in the primate brain. The most notable alterations are (i) the lateral geniculate nucleus develops only two cellular layers and one interlaminar fiber band instead of the normal six layers and five bands, (ii) aberrant synaptic connections are formed between the intact eye and the geniculate neurons that have lost their normal input, and (iii) ocular dominance columns fail to develop in the visual cortex.*

It has been known for some time that monocular enucleation or sensory deprivation in the neonatal period causes functional and structural alterations in the visual system of mammals (*1*) including primates (*2*, *3*). The effect is particularly prominent in the dorsal lateral geniculate nucleus (LGd), which in most Old World primates as well as in humans consists of six horseshoe-shaped cellular layers separated by five fiber-rich interlaminar bands (Fig. 1A). Three of the layers (1, 4, and 6) receive input from the contralateral eye (Fig. 1B) and the remaining three (2, 3, and 5) from the ipsilateral eye (*4*). The LGd neurons subserving each eye project to layer IV of the visual cortex (Fig. 2D) in the form of separate and alternating ocular dominance columns (*5*). The two ventralmost layers of the LGd contain large cells and are termed "magnocellular," while the upper four layers have smaller cells and are called "parvocellular" (Fig. 1A).

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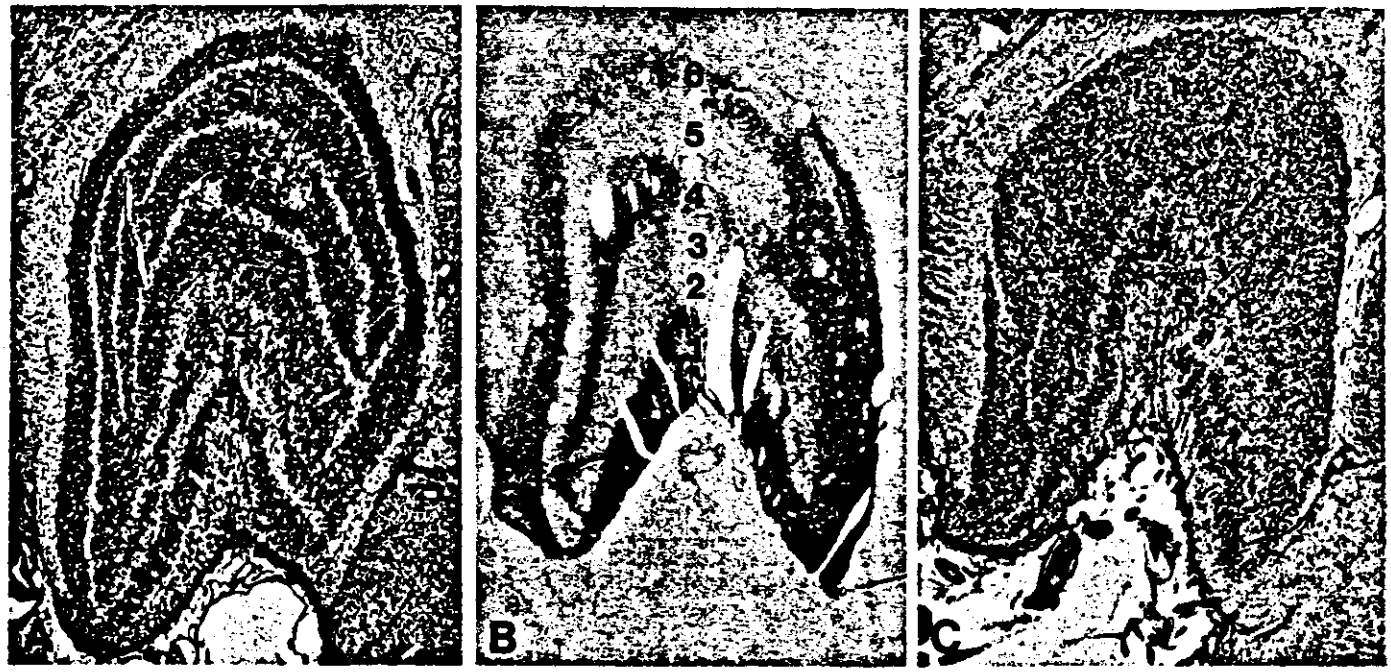

Fig. 1. (A) Nissl-stained coronal section of the lateral geniculate nucleus (LGd) in a normal adult monkey, showing six cellular layers (1 to 6) and five interlaminar bands. (B) Autoradiograph of the LGd of a normal adult monkey showing labeling of layers 1, 4, and 6 after injection of the contralateral eye with radioactive tracer. (C) Nissl-stained LGd in a monkey of the same age from which one eye was removed at the second fetal month showing the presence of the magnocellular (*m*) and parvocellular (*p*) moiety and the absence of the normal six-layered pattern.

The parvocellular and magnocellular moieties of the monkey LGd project to different sublayers of the cortex (*6*) and belong respectively to the X- and Y-like systems, which among their other functional properties are presumably related to color and noncolor vision (*7*). Although these features of LGd organization in primates are not fully understood, the developmental mechanisms and the biological significance of cell segregation into separate layers and functional subsystems have been of considerable practical and theoretical interest (*3*, *4*, *8*).

This study was initiated after pilot experiments in the monkey indicated that enucleation of one eye before birth had even more profound effects on the structure of the LGd than when performed postnatally (*9*). This can be expected, since in primates all LGd neurons are generated (*10*) and their basic connections established before birth (*11*). It should be emphasized, however, that retinogeniculate projections from the two eyes overlap before sorting out into three alternating layers in the LGd during the second half of gestation (*11*). Furthermore, the geniculocortical terminals are also initially intermixed before becoming segregated into ocular dominance columns (*3*, *11*), and the projections from magno- and parvocellular layers of the LGd are unseparated before becoming distributed into appropriate sublayers of layer IV (*11*). Thus, enucleation of one eye before birth can reveal the extent to which competition between axons originating from two eyes may influence the development of binocular or X- and Y-like neuronal systems (or both) in the absence of any visual experience.

Twelve monkeys (*Macaca mulatta*) were studied. Two animals in the second month and two in the third month of pregnancy were subjected to hysterotomy; the fetuses were temporarily removed from the uterus and, after eye enucleation, replaced in the uterus (*11*). Pregnancies were carried to full term (5½ months), when each fetus was delivered by cesarean section and allowed to develop to the ages of 2 months to 1 year. In two of these animals, a mixture of [^{3}H]proline and [^{3}H]fucose (total of 1.0 to 1.5 mCi) was injected into the vitreous body of the intact eye 14 days before they were killed. As a control, four normal fetuses corresponding in age to that at the time of eye enucleation and four postnatal monkeys corresponding in age to that at death were processed in a similar way. In each brain, a 0.5- to 1.0-mm thick coronal slice was dissected from the middle level of the LGd contralateral to the eye injection and processed for electron microscopic analysis. The remainder of each brain was processed for autoradiography (*11*).

In the two monkeys in which one eye was enucleated during the second fetal month, the LGd was located in its usual position and was normal in size and shape (Fig. 1C). Although the cell packing density may have been slightly altered, the number of neurons was not significantly diminished. The most dramatic finding was the absence of six cell layers and all but one of the interlaminar bands in the LGd (Fig. 1C). The single remaining interlaminar band was situated between the magno- and parvocellular moieties and presumably corresponds to the space between layers 2 and 3 that normally receives input from the same eye. In the two monkeys in which one eye was enucleated at the end of the third fetal month, the LGd also attained a normal position and external shape, but the dimensions of the nucleus were smaller than in the animals operated on 1 month earlier (*12*). In addition, these specimens contained some indication of layers comparable to the stage of lamination that existed at the fetal age when the surgery was performed. Thus, the ingrowth of projections from both eyes during the first half of gestation is essential for both the initiation and completion of cellular lamination in the monkey LGd. However, the segregation into magno- and parvocellular moieties of the nucleus proceeds normally in the absence of one eye.

When radioactive tracer was injected in the remaining eye of the adult monkeys that had been enucleated at fetal month 2, anterogradely transported label was distributed over the entire LGd (Fig. 2A). Thus, the neurons in the positions normally occupied by layers 1, 4, and 6, which should receive projections from the removed, contralateral eye, received

corresponding to the appropriate layers 2, 3, and 5. Furthermore, electron microscopic examination revealed that typical retinal synapses were present at positions of all presumed layers (Fig. 2, B and C). Therefore, the axons that originated in the remaining eye probably formed synapses with all LGd neurons. The functional significance of this abnormal synaptic arrangement is unknown, but the changes seem to be long-lasting since they were observed in a 1-year-old monkey lacking one eye from the early fetal age.

When one eye was removed at fetal month 3 and the remaining eye injected was also distributed rather diffusely over the LGd. In this case, however, small, elongated, less densely labeled territories were visible within the posterior pole of the nucleus in a pattern similar to that described in normal 3-month-old fetuses (*11*). Therefore, even after the process of separation of the terminals originating from two eyes has begun, it is arrested if one eye is removed during the formative period.

In the visual cortex, transneuronally transported radioactive label injected into the eye of mature monkeys that were monocularly enucleated at prenatal periods formed continuous horizontal sheets localized mainly within layer IV ocular dominance columns (Fig. 2E) that are characteristic of the normal visual cortex in this species (Fig. 2D) (*3*). The vertical segregation of the parvocellular and magnocellular moieties of the LGd input into sublayers IVA, IVCα, and IVCβ was still achieved, however (Fig. 2E).

These results indicate that the integrity of projections from the two eyes during prenatal development is necessary for the establishment of normal cell distribution and synaptic organization in the primate visual system. More specifically, competition between the two eyes is essential since neurons and their interconnections formed abnormally even

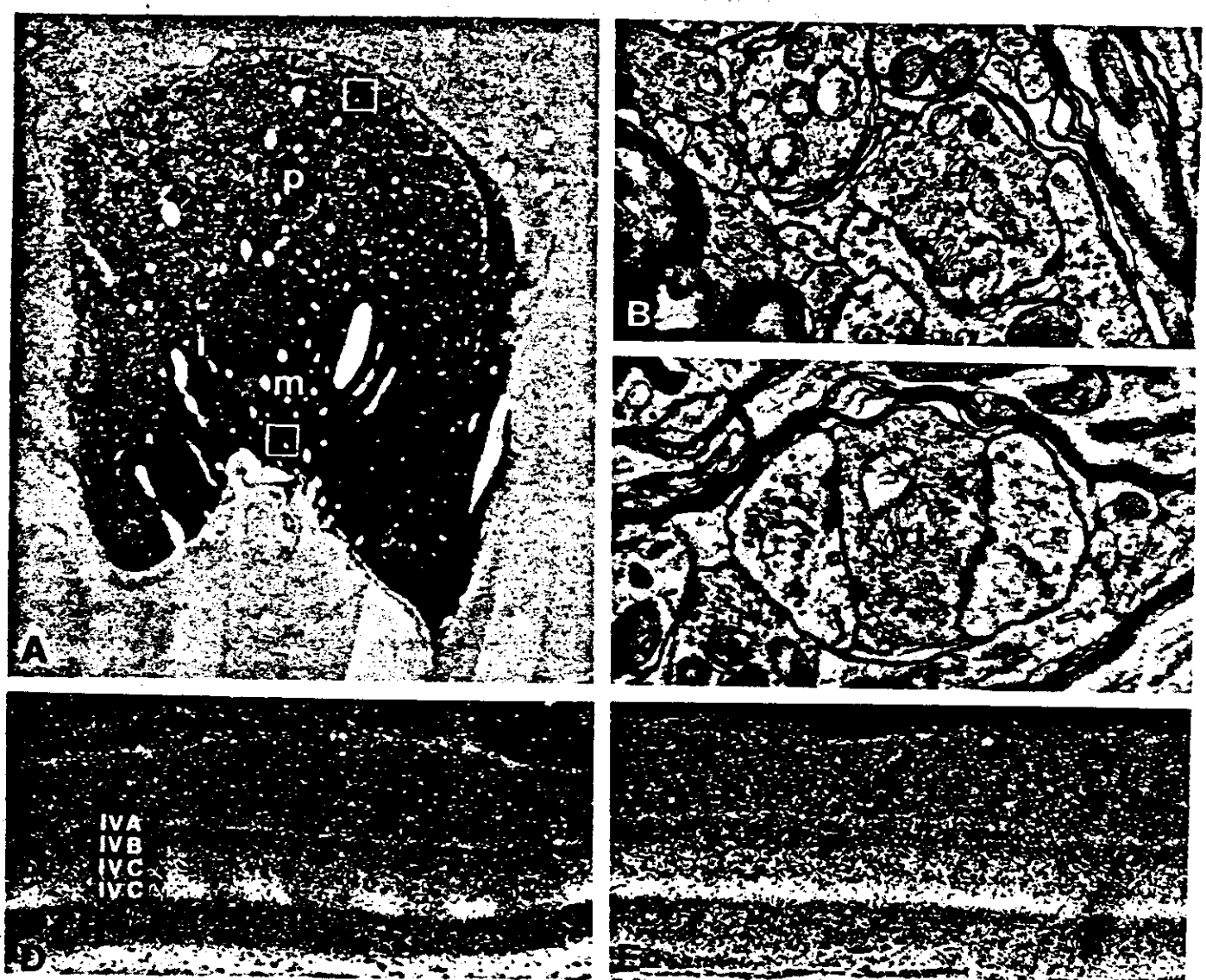

Fig. 2. (A) Autoradiograph of the LGd in a 2-month-old monkey from which one eye was removed at the second fetal month, showing the spread of radioactive retinal input over the entire nucleus. Although the magnocellular moiety (*m*) receives more dense input than the parvocellular (*p*), just as in the control monkeys (Fig. 1B), the layering pattern is not discernible. (B and C) Electron micrographs of retinal axon terminals (*rt*) in the left LGd in a monkey whose right eye was enucleated in the second fetal month. Such terminals are uniformly distributed and found even in the territories of presumptive layers 1 and 6 (indicated by rectangles in A), which normally receive input from the contralateral (removed) eye. (D) Dark-field autoradiograph of the primary visual cortex in a normal adult monkey. Transneuronally transported label is distributed in the form of alternating ocular dominance columns and over sublayers IVA, IVCα and IVCβ. (E) Autoradiogram of the primary visual cortex of a 2-month-old monkey from which one eye was removed at the second fetal month and the remaining eye injected with tracer 2 weeks before death. The label forms horizontal, uniform sheets without indication of ocular dominance columns, but segregated input into sublayers IVA, IVCα, and IVCβ is established.

though LGd neurons received morphologically normal synaptic input from the single remaining eye. This study further emphasizes that the binocular competition critical for the initial formation of the normal visual system does not require visual experience since it exercises its influence before birth. Finally, the dependence of normal development on prenatal binocular competition is selective; segregation of the LGd into magno- and parvocellular moieties and the laminar distribution of their terminals in the cerebral cortex developed normally in all experimental animals. This example of how an error in development of a single structure can alter distant but related structures in the complex primate brain may offer insight into various abnormalities of lamination or connections that occur in congenital malformations in humans.

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12. The smaller size of the LGd in the monkeys enucleated at later gestational ages may be due to neuronal atrophy, loss of neurons, or both. This effect may be related to the higher dependence of already committed, more mature LGd neurons on the proper retinal input.
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Neuronal–glial interaction during brain development

Pasko Rakic

Previous Golgi and electron-microscopic analyses in primates indicate that the interaction between radial glial cells and immature neurons may play a crucial role in the orientation, displacement and positioning of neurons within the cerebral and cerebellar cortices. Recent immunocytochemical studies using an astroglial specific marker confirm that neuronal and glial cell classes coexist in the embryonic primate brain and in addition show that they originate from two separate cell precursors. Furthermore, DNA labeling with [³H]thymidine indicates that radial glial cells do not divide during the peak of neurogenesis when their elongated fibers serve as guides for migrating neurons and provide the structural basis for compartmentalization of developing neuron tissue.

A major theme of developmental neurobiology in the last two decades has been the role of cell-to-cell interactions in neurogenesis. However, with few exceptions, most investigators have focused on the interaction among various classes of neurons perhaps because for many decades the prevailing view was that glial cells are formed only after all (or most) of the neurons destined for a given structure complete their genesis. This view has been challenged by Golgi and electron-microscopic studies[10,19,20] and recently has been refuted by the use of a glial specific cell marker[13,14]. Although it has become increasingly evident that glial cells may play an important role in neurogenesis from the earliest formative stages, many issues including the exact time of their determination, the details of glial cell lineage, the nature of their interaction with adjacent neurons, and their other possible functions during various phases of ontogenetic development, need to be clarified. Some of these issues are discussed below.

When and where do glial and neuronal cell lines diverge?

Since both neurons and the macroglial cells of the CNS are derived from the neuroepithelium of the primitive neural tube, it is obvious that they must form separate cell lines at some time during ontogenetic development. It has proven to be rather difficult, however, to determine just when and where this divergence occurs and this uncertainty has persisted for almost a century. In his classical study, published in 1889, Wilhelm His[6] proposed that the germinative epithelium of the developing cerebral wall consists of two classes of precursor cells, one neuronal (Keimzellen or 'germinal cells') and the other glial ('spongioblasts'). This view is illustrated schematically in Fig. 1A. An opposing view suggested a few years later by Schaper[20] holds that the germinal zone consists of a single precursor cell type which in time gives rise to both neurons and glial cells (Fig. 1B). This concept seems to have prevailed because His erroneously considered that the spongioblasts form a multinuclear syncytium. The cellular homogeneity of the germinative zone gained support from histological studies of Sauer[18] and later by electron microscopic and [³H]thymidine ([³H]TdR) autoradiographic analyses[1,9,10]. In addition, since studies of the time of cell origin showed few labeled glial cells in adult animals that had been exposed to [³H]TdR as embryos, some investigators suggested that glial cells are generated after all, or most, of the neurons destined for a given structure have been formed. Based on such data Fujita[3] proposed a rigid scheme for the successive generation of

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neurons and glial cells (Fig. 1C) which has been accepted in many contemporary monographs[6,10]. It has now become evident that routine light and electron microscopy cannot reveal differences between glial and neuronal cell lines at early developmental stages. Furthermore, the problem of the dilution of [^{3}H]thymidine label by subsequent divisions makes this method unsuitable for determining the time of emergence of glial cells in a given structure unless a specific experimental design is followed (see discussion in Refs 27, 28, 31).

For many years the distribution of radial glial cells in the fetal brain provided at least one line of evidence that glial and neuronal cell lines coexist during embryonic development. This transient cell class was first revealed by the Golgi method at the turn of the century[22] and was later shown by electron microscopy to be present during the middle and late stages of primate corticogenesis[19]. The somata of the radial glial cells are usually located near the ventricular surface, and their elongated processes traverse the entire width of the cerebral wall and terminate at the pial surface (Fig. 2). Since neither silver impregnation nor electron microscopy can establish the identify of such cells at early stages[20], we have recently used an antibody to glial fibrillary acidic protein (GFA) which has a high specificity for astroglial cells[4]. Using the peroxidase-antiperoxidase-immunocytochemical method, Levitt and Rakic[14] demonstrated the existence of radial glial cells during the first third of gestation in all major subdivisions of the embryonic primate brain.

Because, in the rhesus monkey, both glial cells and neurons increase in number in the course of corticogenesis[20,25] their separate precursor cells must co-exist. Indeed, the immunocytochemical method confirmed that the ventricular zone consists of at least two classes of ventricular cells[14] and that in this respect, His' concept[8] of the coexistence of independent glial and neuronal cell lines in the germinal matrix seems to be correct. Although glial cells are recognizable in the occipital lobe from the first third of gestation (47th embryonic day) we have not yet been able to determine the exact time when the two cell lines diverge, since GFA may not be present or detectable in cells at earlier stages even though these cells may already be committed to a glial lineage.

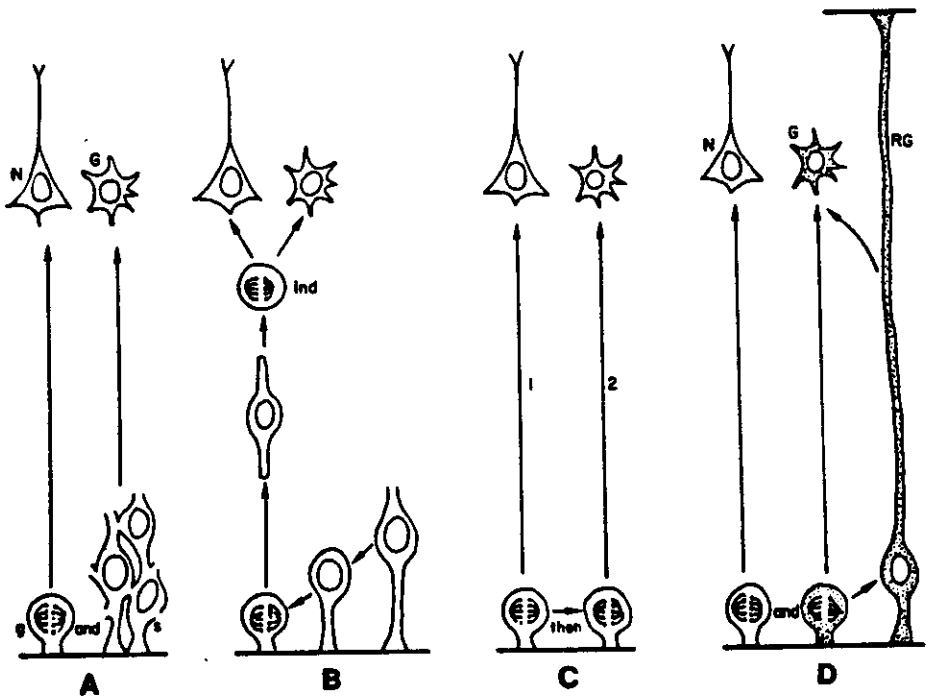

Fig. 1. Drawing that illustrates previous theories of the origin of neuronal and glial cell lines (A, B, C,) and the current scheme (D). (A) Almost a century ago His[8] distinguished two separate cell lines in the germinal matrix (or ventricular zone) lining the embryonic ventricles: the round 'germinal cells' (g), with mitotic nuclei which lie close to the ventricular surface and give rise to neurons (N), and the 'spongioblasts' (S) whose nuclei lie at various distances form the ventricle. The 'spongioblasts', which His thought formed a syncytium, give rise to glial cells (G). (B) Sauer[18] proposed that these two 'cells types' simply represented different mitotic phases of a single cell class. According to Schaper[20], cells of the ventricular zone produce indifferent cells (ind) that migrate into the intermediate (or mantle) zone where they further divide into either neurons or both neurons and glial cells. (C) The results of [^{3}H]thymidine autoradiography led Fujita[5] to suggest that the dividing cells first give rise to neurons (1); then after neurogenesis has ceased, the same dividing population begins to produce glial cells (2). (D) The localization of glial fibrillary acidic protein (GFA) by electron microscopic immunohistochemistry[19] has demonstrated that both GFA-positive (stippled) and GFA-negative mitotic cells coexist in the ventricular zone. The GFA positive cells initially produce radial glial cells (RG) and later either directly, or indirectly, astrocytes or various specialized astrocyte-like cells. (Modified from Ref. 13.)

Is the glial phenotype expressed before or after cell division?

Until recently there was little firm evidence to challenge Schaper's[20] notion that the proliferative zones produce 'indifferent cells' which become transformed into neurons or glial phenotype after migration to their final destinations. Our recent electron microscopic immunocytochemical analysis[19] again using the antibody against GFA as a specific glial cell marker demonstrates that there are at least two mitotically active precursor cell types in the embryonic proliferative zone (i.e. GFA-positive and GFA-negative cells). The localization of this antigen in cells which are synthesizing DNA and are actively dividing, indicates that proliferative cells may be committed to their lineages prior to the cessation of cell division.

Although at present it is not clear whether all or only some GFA-positive cells are eventually transformed directly into astrocytes (Fig. 1D) some of them certainly pass through the transient stage of radial glial cells[14,21,22,28]. Using [^{3}H]TdR autoradiography in a series of experiments with variable durations between injection and sacrifice, Schmechel and I have shown that many radial glial cells remain in a dormant stage for up to 2 months during midgestation in the rhesus monkey[28]. Furthermore our analysis revealed that at some point the radial glial cells re-enter the mitotic cycle and subsequently became transformed into astrocytes[21,27,28]. Although Ramon y Cajal[23] suspected that radial glial cells can divide, this was the first DNA labeling evidence that cells of this phenotype divide prior to transformation into astrocytes. Thus, some of the mysteries of glial cell origin and their fate are being unraveled but there are still many unresolved questions. For example, we do not know whether the GFA-negative cells consist exclusively of prospective neurons or of multiple cell classes, including precursors of oligodendrocytes.

Function of the transient radial glial cells

The geometric regularity and the remarkable length of the radial fibers, which may be as long as 15–20 mm in the monkey fetus, and even longer in the human fetus, suggest that the functions of these cells may be related to some form of interaction with other cells that are situated along their trajectories. Another sugges-

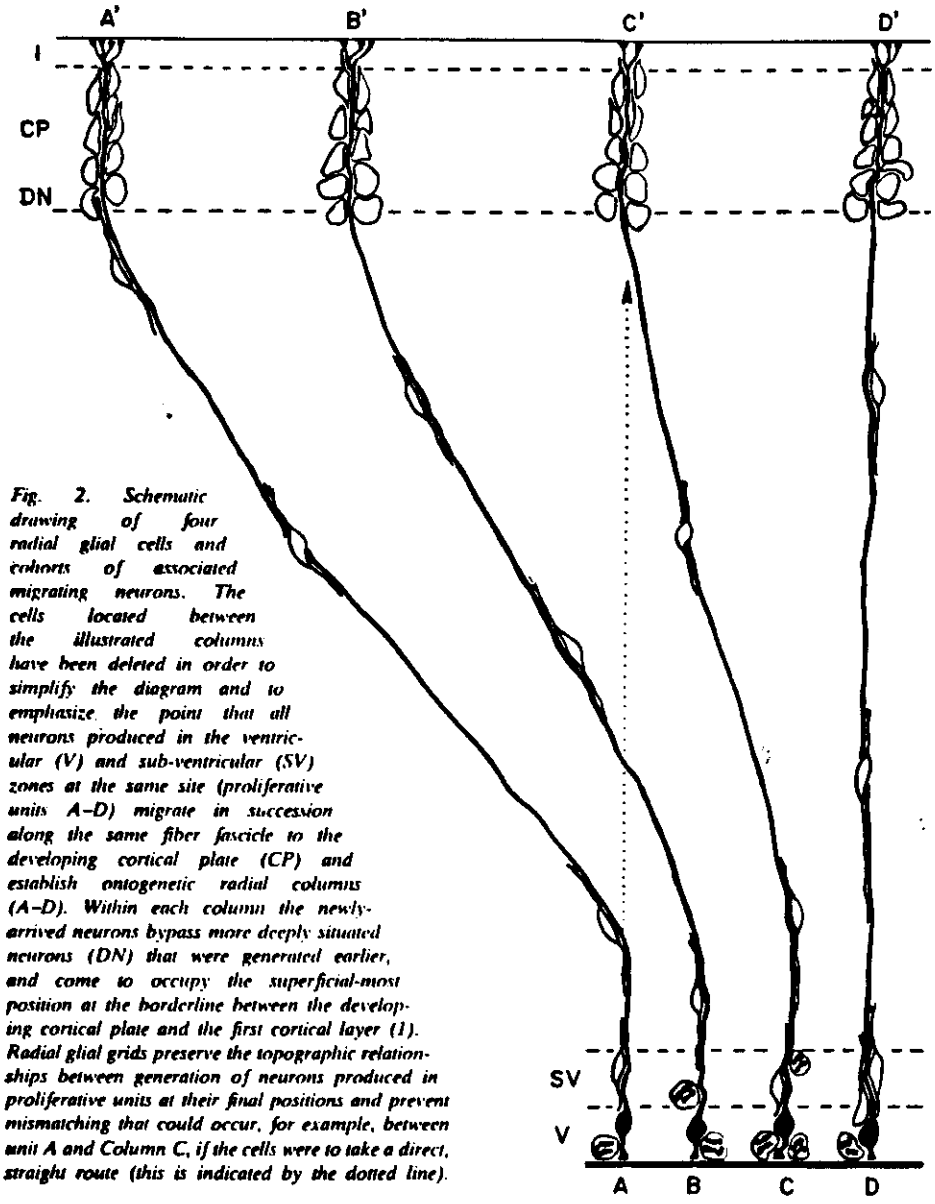

Fig. 2. Schematic drawing of four radial glial cells and cohorts of associated migrating neurons. The cells located between the illustrated columns have been deleted in order to simplify the diagram and to emphasize the point that all neurons produced in the ventricular (V) and sub-ventricular (SV) zones at the same site (proliferative units A–D) migrate in succession along the same fiber fascicle to the developing cortical plate (CP) and establish ontogenetic radial columns (A–D). Within each column the newly-arrived neurons bypass more deeply situated neurons (DN) that were generated earlier, and come to occupy the superficial-most position at the borderline between the developing cortical plate and the first cortical layer (I). Radial glial grids preserve the topographic relationships between generation of neurons produced in proliferative units at their final positions and prevent mismatching that could occur, for example, between unit A and Column C, if the cells were to take a direct, straight route (this is indicated by the dotted line).

tion was that the radial glial cells may be involved in transport processes[17]. This idea is rather attractive since it suggests that the radial glial cells transfer information between the prospective final position of neurons and the site of their origin in the proliferative zones. This information could be carried by the intracytoplasmic transport of various molecules as the radial glial cells seem to have a considerable capacity for retrograde transport from their endfeet to cell bodies[3,9]. The formation of the glial limitans membrane at the cerebral surface and around blood vessels[19,20] may provide a substrate for cerebrospinal fluid–brain and blood–brain barriers, respectively[17,20]. The trophic interactions between immature astrocytes and developing neurons that have been demonstrated in tissue culture[21] may also be extended to the radial glial cells. These cells may also influence the regulation of extracellular fluid, as has been suggested for astrocytes in the adult brain[12]. This may be especially important in developing nervous tissue where extracellular spaces seem to play a role in allowing growth or even in directing the elongation of nerve processes[11,24].

Although all these functions may be critically important, I shall only emphasize the possible role of radial glial fibers in guiding migrating neurons from their sites of origin to their final positions in the cerebral and cerebellar cortices[10,19]. This concept is based on the ultrastructural observation[10,19] that migrating neurons remain attached to neighboring glial fibers throughout their trajectories (Fig. 2). Such a neuron–glial relationship has been observed in the cerebellar and cerebral cortex of many species (e.g. see Refs 2, 7, 15 and 30), as well as in the primate hippocampal formation[16]. Although cellular relationships are somewhat more complex in the cerebellar cortex where the granule cells pass through several intricate phases of morphogenetic transformations the general principle seems to be the same[16]. As reviewed elsewhere[8] there is some indirect evidence from the study of neuroglial mutant mice which supports this hypothesis of a guiding mechanism. More recently it became evident that in addition to guiding neurons through the densely packed neuropil, the radial fibers may also ensure the faithful mapping of the ventricular surface onto the expanding and convoluted primate cerebral cortex by preventing the lateral intermixing of cells that are generated in different regions[21]. This model minimizes the amount of genetic information needed for each neuron to reach its correct locus and to establish its correct synaptic relations within the cortex.

The nature of the glia-neuron interaction

Transmission electron microscopy displays a surface apposition between the migrating neurons and radial glial fibers with intermembranous spaces of about 200–300 Å (Refs 18, 19). It is noteworthy that similar appositions exist between migrating neurons and many other adjacent cells. However, migrating neurons selectively follow the curving course of glial fibers rather than the often more regular surfaces of other cells, suggesting a considerable affinity between the membranes of these two cell classes. Although the binding affinity between neuron and glial cell surfaces may be nonspecific (e.g. all migrating neurons may have the same affinity for all radial glial cells), the spatio-temporal order of cell migration would be achieved since each postmitotic neuron becomes attached exclusively to a neighboring radial glial fiber (Fig. 2). We have evidence which suggests that migrating neurons may occasionally translocate to a nearby radial glial cell but not to other classes of cellular processes[23,25].

How can one reconcile the apparent contradiction that neurons and glia show a strong affinity for each other and at the same time permit the movement of one along the other? Among several possibilities that could account for this phenomenon one is that the membranes of two cells are fixed at any one point along their interface. The migrating cell could nevertheless move by adding new membrane components to its growing tip and the leading process would progressively extend along the radial glial fiber while the nucleus transfers to a new position within

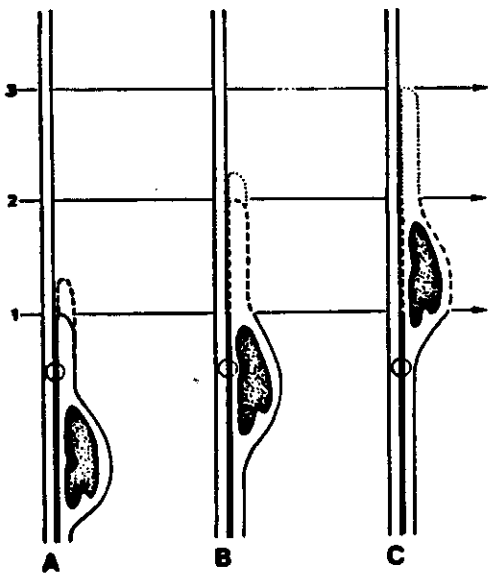

Fig. 3. Diagram showing a possible mechanism for the displacement of migratory cells along the surface of radial glial fibers (vertical shafts A–C). To traverse the distance between levels 1 and 2, new membrane may be inserted along the interface of the two cells (dashed line in A), while the nucleus moves within the cytoplasm of the leading process (B). As the leading tip grows to reach level 3 additional new membrane is inserted (dotted line in C) along the glial surface and the nucleus moves to a higher position between level 1 and 2, resulting in an overall displacement of the cell body. This model does not require movement of the neuronal surface along glial fibers, and the binding sites (circles) between the two apposing membranes may remain constant for some time.

the perikaryal cytoplasm (Fig. 3 B, C). The rate of movement of migrating neurons in the primate telencephalon varies between 2 and 5 $\mu m\ h^{-1}$, and this rate is compatible with a capacity for generation and insertion of new membrane along the leading process. Several observations give credence to such a mechanism including the finding of an increase in the surface area of migrating cells[22] and the predominant growth of neuronal processes at their tips[1]. Since glial and neuronal cell surfaces may contain binding or complementary molecules that attract each other this model can be tested. Thus, looking ahead, one can anticipate production of antiserum that would label binding sites or whose application *in vivo* would interfere with neuronal migration. These new approaches will enhance our prospects for understanding the complex cellular events that occur during the development of the mammalian brain.

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