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"Specification of Cerebral Cortical Areas"

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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

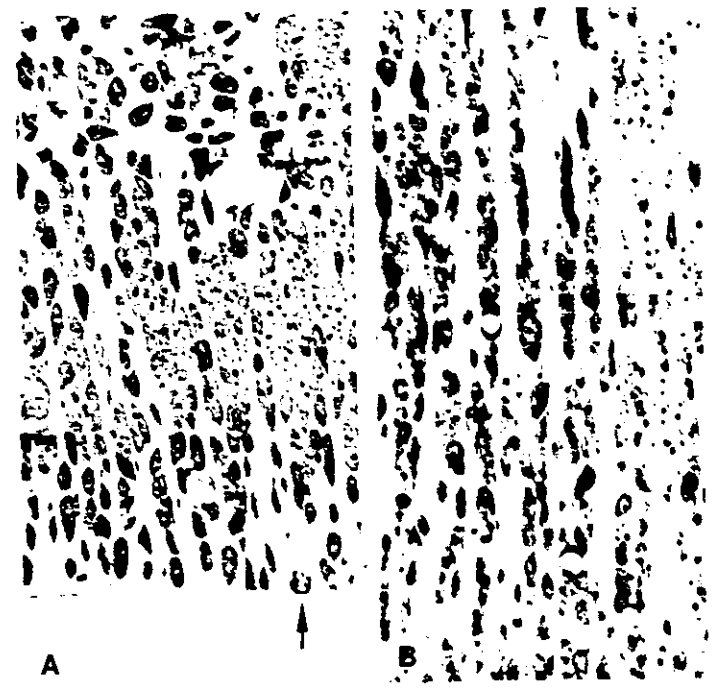

Fig. 1. (**A**) Photomicrograph of an array of proliferative units within the ventricular zone of the occipital lobe in a 91-day-old monkey embryo. Most mitotic figures are located directly at the ventricular surface (arrow), although at this age some can be found in the subventricular zone (crossed 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

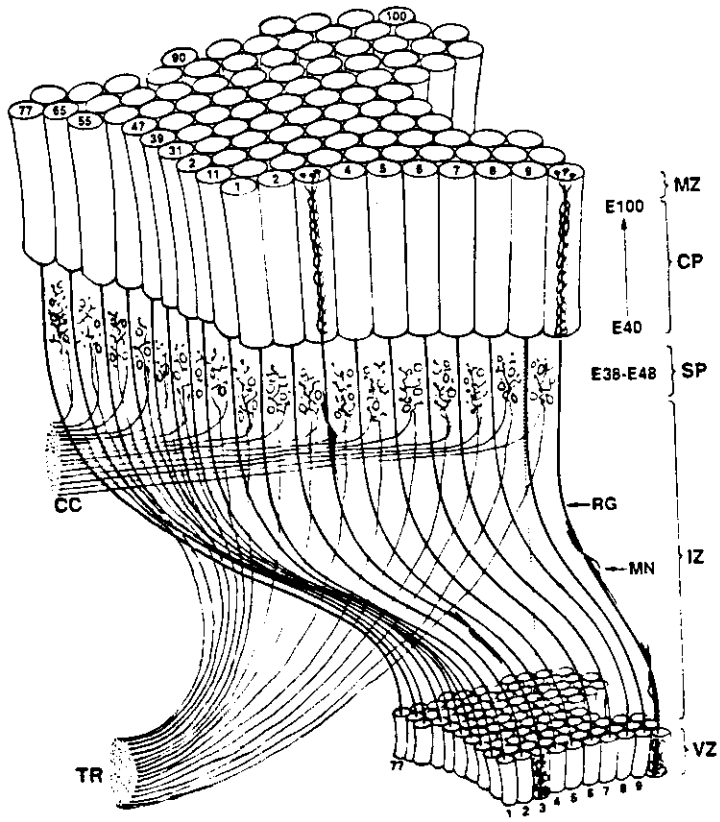

Fig. 2. The relation between a small patch of the proliferative, ventricular zone (VZ) and its corresponding area within the cortical plate (CP) in the developing cerebrum. Although the cerebral surface in primates expands and shifts during prenatal development, ontogenetic columns (outlined by cylinders) 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*).

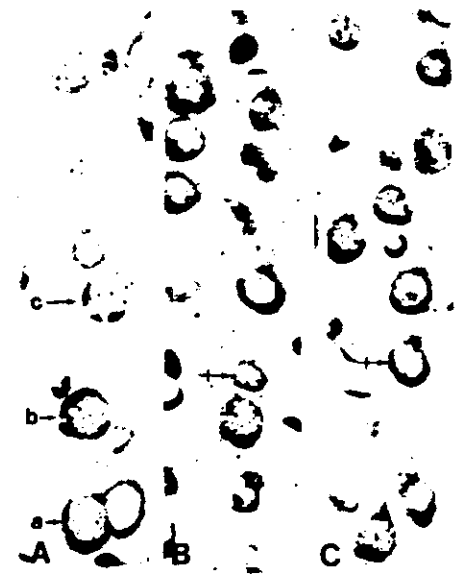

Fig. 3. Photomicrograph of the three autoradiograms showing neurons in the cortex of an adult monkey that was exposed to [^{3}H]thymidine at E70. (**A**) The most intensely radioactive neuron (a) lies deeper in the cortex than the two progressively less labeled, more superficially situated neurons (b 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-

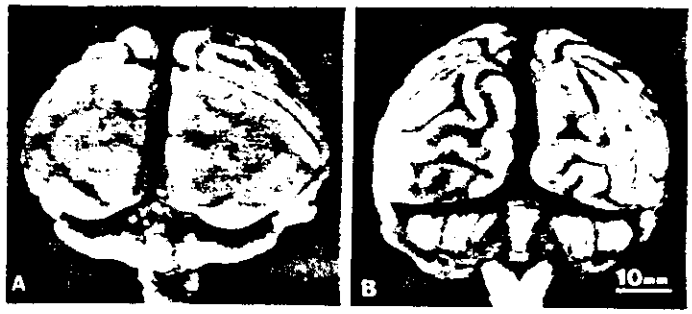

Fig. 4. Posterior view of the cerebrum in (**A**) a normal 3-year-old monkey and (**B**) an age-matched animal that underwent bilateral enucleation at E60. The development of a new pattern of sulci and gyri is seen in the normally smooth lateral surface of the occipital lobe.

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

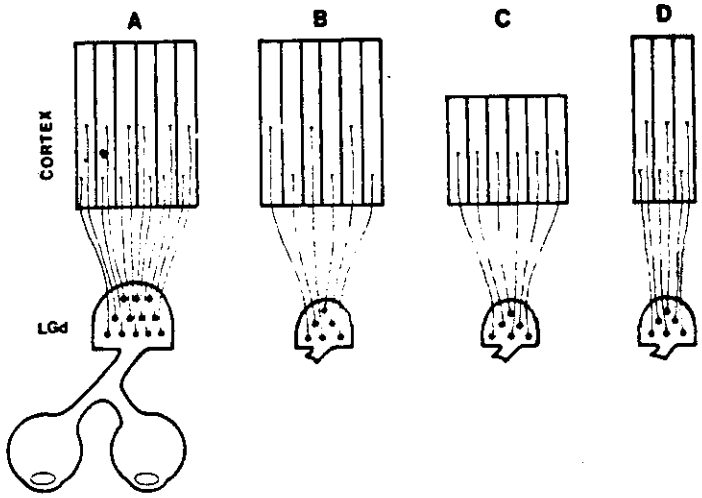

Fig. 5. Schematic outline of the possible consequences of diminished input from the dorsal lateral geniculate nucleus (LGd) to the visual cortex in adults binocularly enucleated at early embryonic stages. The normal numerical relation between geniculocortical projections and ontogenetic columns illustrated 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

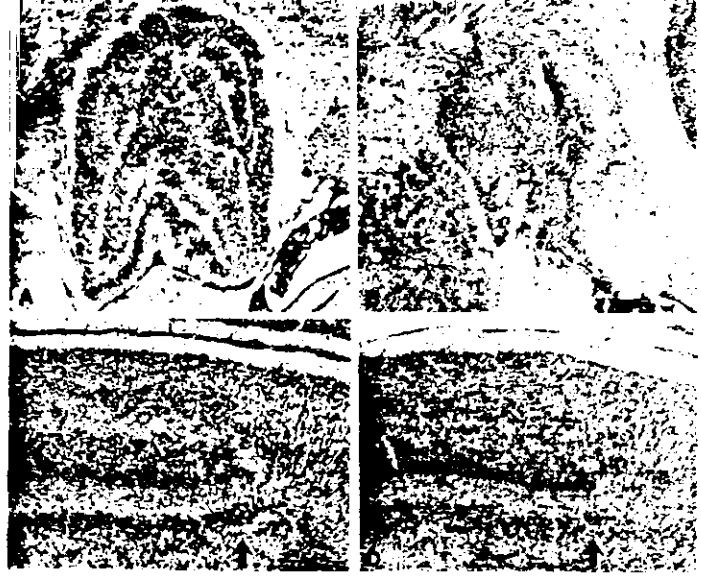

Fig. 6. Photomicrograph of the lateral geniculate nucleus in (**A**) a normal rhesus monkey and (**B**) its grossly diminished counterpart in the age-matched monkey subjected to binocular enucleation around E60. The border between the striate cortex (left) and extrastriate cortex (right) in (**C**) a 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.

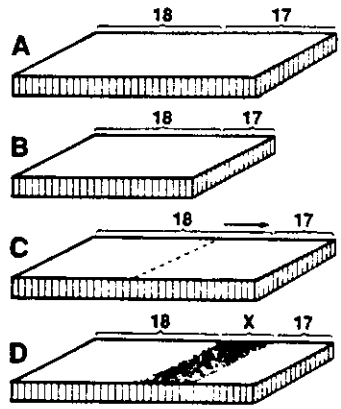

Fig. 7. Schematic representation of the possible modes of decrease in the size of area 17 caused by experimental reduction of thalamic input. (**A**) Relation between areas 17 and 18 in a normal animal; (**B**) differential cell death; (**C**) encroachment of adjacent area 18 into the territory of area 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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