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CHAPTER 16

Theoretical Issues

16.1 THE GERMINAL SOURCE OF CORTICAL NEURONS AND GLIA

16.1.1 Germinal Cells in the Neuroepithelium

It is an unresolved issue whether the stem cells in the cortical germinal matrix are all pluripotential elements giving rise to a variety of cell types or are distinctly different cell lines that are the sources of neurons, glia, and ependymal cells. Although [^{3}H]thymidine autoradiography cannot provide a definite answer, the circumstantial evidence we have presented links different precursor cells to specific cellular progeny. Our hypothesis, based on the data we have presented in Chapter 4, is diagrammed in Fig. 16–1.

As we noted in Section 4.1.1, the majority of neuroepithelial cells have radially aligned mitotic cleavage planes located in a periventricular position (at the edge of the lateral ventricle). These mitotic figures are shown with vertically arrayed chromosomes during metaphase and anaphase in Fig. 16–1. In contrast, a few mitotic cells in periventricular positions, and the majority in paraventricular and subventricular positions, have variably oriented cleavage planes. These mitotic figures are shown with horizontal-to-oblique arrays of chromosomes in Fig. 16–1. This pattern in the developing rat cortex is in agreement with Smart and McSherry's (1982) observations in the mouse telencephalon (see their Fig. 1). They interpreted the progressive shift of mitotic cells away from the ventricle as a means to relieve spatial congestion at the ventricular surface, described as the "ventricular choke" hypothesis. We offer another interpretation that different cleavage planes and positions of the cells undergoing mitosis distinguish at least two populations of stem cells, one generating neurons and the other generating nonneuronal cells, particularly various types of glia.

Since short-survival [^{3}H]thymidine autoradiography indicates that the most active period of cortical neurogenesis coincides with segregated synthetic and mitotic zones in the neuroepithelium, we infer that the cells undergoing interkinetic nuclear migration (Sauer, 1935) are the stem cells of neurons. Accordingly, we propose that the radially cleaving mitotic cells that divide in a periventricular position are the committed precursors of neurons (*large dotted mitotic figures*, Fig. 16–1). In order to continue interkinetic nuclear migration, the daughter cells have to remain anchored to the ventriclar surface by a terminal bar (Sauer, 1935; Hinds and Ruffet, 1971). The orientation of the radially cleaving mitotic figures favors division of the terminal bar so that each daughter cell has a part, thereby assuring continued attachment to the ventricular lumen. Indeed, Hinds and Ruffet (1971) reported that radially cleaving cells in the early cerebral vesicle never lose their attachment to the ventricular surface.

Our observations in sequential survival [^{3}H]

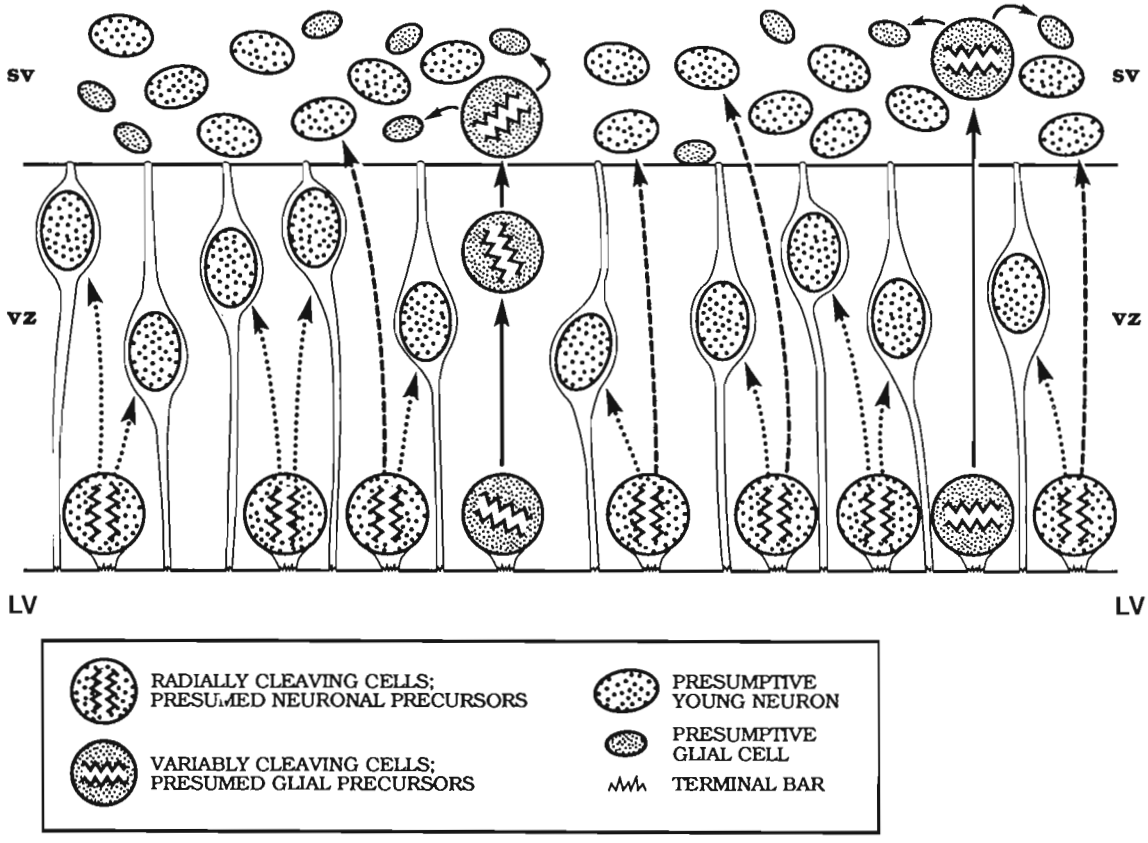

FIG. 16–1. Hypothesis of the different fate of radially cleaving cells and variably cleaving cells in the ventricular zone (vz). The radially cleaving cells with their terminal bars retained at the lumen are postulated to give rise (*dotted arrows*) to columnar daughter cells that will themselves undergo interkinetic nuclear migration. In contrast, the few variably cleaving periventricular cells lose their anchor at the lumen and their daughter cells henceforth divide in a paraventricular position or in the subventricular zone (*solid arrows* in sv). The radially cleaving cells are presumed to be precursors of neurons, while the variably cleaving cells, the precursors of neuroglia and ependymal cells.

thymidine autoradiography on E12 and E13 indicate that many neuronal precursors are rapidly turning over and are probably adding to the number of neuronal precursors. During that period, both daughter cells remain in the neuroepithelium to undergo interkinetic nuclear migration and continue to proliferate (*dotted arrows* pointing to cells with large dotted nuclei, Fig. 16–1). The neurogenetic timetables in Chapter 3 indicate that most neurons are produced between E14 and E18. During that period, one or both daughter cells may lose their attachment to the ventriclar lining and move outward (*dashed arrows*). These are the presumptive young postmitotic neurons (*large dotted ellipses,* Fig. 16–1).

The second part of our hypothesis is that the randomly cleaving mitotic cells dividing in periventricular, paraventricular, and subventricular positions, which are not undergoing interkinetic nuclear migration, are glial precursors (*small dotted mitotic figures,* Fig. 16–1). A variable cleavage plane is not favorable to the equal division of the terminal bar between the two daughter cells; one or both may lose their anchors at the lumen and, as a result, no longer return there to divide. The progeny of the variably cleaving cells may move upward and divide in a paraventricular position or, after the subventricular zone has formed, in a subventricular position (*solid arrows,* Fig. 16–1) to produce presumptive glial cells (*small dotted ellipses,* Fig. 16–1). At a later stage of development, cells dividing some distance from the germinal matrix become "locally multiplying" glia that progressively diffuse throughout the cortex (Chapter 4, Figs. 4–6 to 4–8). In animals that were injected with [^{3}H]thymidine on four successive days beginning with birth, nearly every glial cell in layers I through VII is labeled on P10, suggesting that most glial cells in the cortical gray matter arise from this locally multiplying pool of precursor cells (unpublished observations).

16.1.2 Germinal Cells in the Subventricular Zone

Since few proliferating cells are found outside the neuroepithelium from E14 to E17, there is little doubt that most of the neurons generated in the cortex during that time come from the neuroepithelium. However, there is some ambiguity from E18 through E21 because neurogenesis continues when the neuroepithelium is shrinking and the subventricular zone is expanding. That allows for the possibility that the late generated neurons in the superficial layers (IV–II) are produced in the subventricular zone (Smart and McSherry, 1982). In spite of that, we believe that the neuroepithelium is the sole source of all cortical neurons (including those of layers IV–II) and that the mitotic cells of the subventricular zone are sources of glia. We support that claim with two arguments.

First, there is a mismatch between proliferative dynamics in the subventricular zone and the time of origin of cells in layers IV–II. As shown in Chapter 4, proliferating cells in the subventricular zone are very sparse on E17 (Fig. 4–5A), begin to increase on E18 (Fig. 4–5B), reach a peak on E19 and E20 (Figs. 4–5C and 4–6), and are still quite numerous on E21 (Fig. 4–7). In contrast, the peak times for cell generation in layers IV–II occurs earlier, mainly on E17 laterally and E18 dorsally and medially. If the subventricular zone is the primary source of layer IV–II neurons, its peak of proliferation should be on E17–E18 rather than on E19–E20, and there should be a sharp decline on E21. Second, we have provided evidence that the subventricular zone is the site of a large complement of horizontally oriented young neurons that sojourn in the first inferior band (Chapters 7 and 8). Accordingly, we propose that the mitotic cells of the subventricular zone are glial cells that proliferate as satellites of the young neurons sojourning in this layer. These glial stem cells may accompany the migrating neurons and/or may be one of the sources of the locally multiplying glial population that becomes prominent after neurogenesis declines.

16.2 THE ORIGIN OF CORTICAL HETEROGENEITY

16.2.1 The Epigenetic View

Using conventional staining techniques, the neuroepithelium appears more or less homogeneous in the entire neocortex, suggesting a stock of equipotential progenitor cells. The fact that the cortical plate itself appears initially uniform in composition may have reinforced the idea that its neurons remain uncommitted for some time after they have settled in the cortex, forming a *tabula rasa*. Such observations have lent support to the epigenetic hypothesis, according to which the structural and functional heterogeneity of the different cortical areas is due to some morphogenetic influence exerted on plastic cortical plate neurons by other brain structures. As a recent exponent of this view, Creutzfeldt argued that "individual neurons of the neocortex, having a common ontogenetic history, may develop certain rather specific morphologic aspects due to circumstantial situations," among which he lists "the quantity of its thalamic, cortical, and maybe other inputs" (Creutzfeldt, 1977, p. 516). Our [^{3}H]thymidine autoradiographic evidence regarding neurogenetic gradients supports the idea that cortical neurons have a common ontogenetic history because these gradients are global in nature rather than specific for each cortical area. The only evidence of heterogeneity in cortical neurogenetic patterns is the violation of the transverse gradient by layers III and II in the primary somatosensory and primary visual areas (summarized in Chapter 17, Section 17.3).

Another line of argument for the epigenetic hypothesis comes from the study of somatotopic maps in the rodent brain. In both mice (Woolsey and van der Loos, 1970) and rats (Welker and Woolsey, 1974), the somatotopic representation of the whiskers on the face, the "barrels," can be seen in Nissl-stained normal material. van der Loos and Dörfl (1978) studied the modification of the barrels after experimental manipulations of the periphery. When some of the whiskers were destroyed at birth, there was a corresponding alteration in the same cortical area that would contain the barrels for the missing whiskers. Extra whiskers appeared in some strains of mice and the aberrations in the periphery were faithfully mapped onto the cortical surface. Because of that evidence, the authors concluded that the cortical cytoarchitectonic mosaic is "enslaved" to the periphery, rather than emanating from an intrinsic source. Similar studies in rats (Belford and Killackey, 1979; Killackey and Belford, 1979; Waters et al., 1990) and mice (Woolsey et al., 1979; Andrés, 1985; Cooper and Steindler, 1989) have confirmed the general conclusion that brain maps of the whiskers can be altered by peripheral manipulations during a critical period after birth. Alterations have also been found in the cortical representation of the limbs after removal of the forelimb (Donoghue and Sanes, 1987). The overall conclusion one might draw from these studies is that somatotopic maps in the cortex are imposed by extrinsic sources, ultimately by the primary sensory neurons in the periphery but immediately by the transfer of these maps to the cortex by the thalamic relay nuclei.

Still another line of evidence supporting the epigenetic view comes from transplant studies in rats (Stan-

field and O'Leary, 1985; O'Leary and Stanfield, 1989). When pieces of the embryonic occipital cortex (E17–E18) are transplanted heterotopically into the motor cortex of newborns, the layer V neurons in the transplant reflect the characteristics of the recipient cortical area rather than the donor cortical area. In the normal mature neocortex, layer V neurons in the occipital region do not send an axon into the pyramidal tract. However, when occipital cortex is transplanted into motor cortex, neurons in the transplant project long axons into the pyramidal tract. The conclusion drawn from these studies is that the targets of cortical projection areas are not determined by intrinsic (genetic) neuroepithelial differences, but rather by regionally specified influences (epigenetic).

16.2.2 The Preformationist View

Although conventional staining techniques fall short in revealing true heterogeneity in the cortical neuroepithelium, immunocytochemistry shows that some cells in the germinal matrix are distinguished by markers for glial cells while others lack such markers (Levitt and Rakic, 1980; Levitt et al., 1981, 1983; Misson et al., 1988a, 1988b). Radial glia can also be distinguished by antibodies against peptides produced in protooncogene expression (Johnston and van der Kooy, 1989). The changing labeling patterns in the neuroepithelium after [^{3}H]thymidine injections and the altered radiosensitivity of neuroepithelial cells after exposure to x-ray (Chapters 4 and 10) are additional lines of evidence for heterogeneity. Consequently, it seems unlikely that the neocortical neuroepithelium is a tabula rasa in the strict sense. But the question remains whether the cytoarchitectonic differences in the distribution of neurons is determined before or after the young neurons leave the cortical neuroepithelium.

The immunocytochemical studies coupled with the hypothesis of migration of cortical neurons on radial glia fibers (Rakic, 1972) have led to the formulation of an opposing preformationist view of cortical development. That view has been presented in its most radical form by Rakic (1988) who postulates that the cortical neuroepithelium is composed of an array of discrete "proliferative units," that already constitute a "proto-map" of the distinctive structural components of the mature cortex. In Rakic's view, the orderly arrangement of the radial glia links the neuroepithelial proto-map to the cortical plate so that the sequentially produced offspring of committed progenitor cells migrate on the same set of radial fibers to a specific locus in the cortical plate, forming an "ontogenetic column." If this is correct, the cortical neuroepithelium represents a preformed *columnar mosaic*.

The best currently available direct experimental method to test the columnar mosaic hypothesis is to use retroviral vectors as lineage markers for neocortical neurons. Since Rakic (1988) postulates that the neurons in each ontogenetic column are generated by a restricted number of progenitors (polyclonal), a clone of neurons resulting from one of these progenitors should form columns in the cortical gray matter. The few studies that have been done so far using retroviral vectors generally agree that one progenitor can give rise to neurons in more than one layer in the neocortex (Luskin et al., 1988; Price and Thurlow, 1988; Walsh and Cepko, 1988). But there is controversy about whether the clonally related cells form columns. Luskin et al. (1988) injected the retroviruses during an early stage of neocortical development in mice (approximately at the time that layer VI neurons were just beginning to be generated) and found that a single clone will contain relatives from layers II to VI. However, the clones appeared to be columnar in only one plane and were offset by up to 100 μm after a 90° rotation. Price and Thurlow (1988) injected the retroviruses at a later stage of cortical development in rats (during the end of layer VI–V neurogenesis) and found that clones consisted of either cells from the deep layers (V–VI) or cells from the superficial layers (IV–II), but cells in the deep layers were never clonally related to cells in the superficial layers. Moreover, neurons in a clone tended to be widely scattered in the mediolateral (up to 200 μm) as well as in the anteroposterior (>100 μm) planes; the authors concluded that there was no evidence of columnar arrangement. Similarly, Walsh and Cepko (1988) and Austin and Cepko (1990) found that neuronal clones were not distributed in a radial pattern in the cortex, and often cells in the same clone were separated from 100 μm to 500 μm. Although the retroviral technique has given evidence of strict columnar clones in the retina (Turner and Cepko, 1987) and in the chick optic tectum (Gray et al., 1988), clones in the neocortex apparently do not form columns.

Lineage studies using aggregation chimeras produced during the blastocyst stage from histochemically detectable strains of mice (Crandall and Herrup, 1990; Fishell et al., 1990) can also be used to test the ontogenetic column hypothesis. Again, these data do not support the hypothesis because results from both studies indicated that the genotype distribution in the superficial layers is statistically different from the genotype distribution in the deep layers. According to the ontogenetic column hypothesis, all neurons in a column of cortex originate from a common pool of progenitors, and there should be no statistical difference in the genotype distribution between neurons in the deep and the superficial layers.

16.2.3 A Synthetic View

Our data indicate that the cortical neuroepithelium is not a strict columnar mosaic nor is the developing cortex a strict tabula rasa. Rather, the evidence suggests that a rough-grained genetic determination in the cortical neuroepithelium, *regional and laminar specificity,* underlies the epigenetic expression of a fine-grained cytoarchitectural and columnar specialization. A diagram using layer V as an example illustrates what we mean by regional and laminar specificity (Fig. 16–2). In the mature cortex (Fig. 16–2A), layer V cells are more abundant in the motor cortex (MOC) than in the visual cortex (VC). In the developing cortex (Fig. 16–2B), many layer V neurons are generated on E17 in the cortical neuroepithelium (ne), whether they are bound for the motor area (Chapter 14) or for the visual area (Chapter 11). By E18, the putative layer V neurons generated the day before express two kinds of specificity in the cortical transitional field. (1) *Laminar specificity* is shown as these cells sojourn in the second superior band (*dark ellipses*[1] in sb2, Fig. 16–2B) rather than in the first inferior band (not shown), where concurrently generated layer IV neurons sojourn (Chapter 7, Figs. 7–1B and 7–3B). (2) Regional specificity is shown as far more putative layer V neurons accumulate in anterior sb2, beneath the presumptive motor cortex, than in posterior sb2, beneath the presumptive visual cortex (Chapter 7, Figs. 7–16, 7–17, and 7–18). Because these specializations are expressed within 1 day after the layer V neurons are generated, it follows that the fate of these neurons was sealed by the time they left the cortical neuroepithelium. This suggests that regional and laminar specificity are intrinsic to the neuroepithelium. On E19, the layer V neurons (*narrow ellipses,* Fig. 16–2B) leave sb2, migrate through the upper intermediate zone, and enter the cortical plate. Many of these neurons will settle in the superficial part of the dorsomedial cortical plate by E20 (*dark ellipses* at the surface of the CP, Fig. 16–2B).

The observations presented in Chapters 4 and 10 (summarized in Chapter 17) support the conclusion that the neuroepithelium is heterogeneous and is successively transformed as cortical development progresses. However, there are currently no techniques available to visualize the progenitors of a specific class of neurons in the neuroepithelium. Nevertheless, we may speculate about the organization that must exist in the neuroepithelium. To account for laminar specificity, we postulate that there is a genetically programmed group of progenitors of layer V neurons on

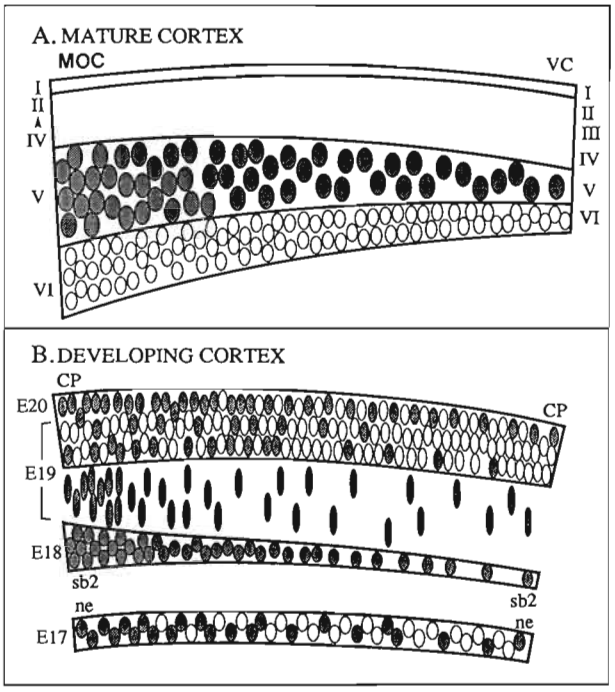

FIG. 16–2. Diagram showing how the hypothetical regional and laminar specificity that exists during development of layer V neurons is related to cytoarchitectonic features in adults. In the mature cortex **(A)**, the anterior motor cortex (MOC) has a larger population of layer V neurons (*stippled ellipses*) than does the posterior visual cortex (VC). In the developing cortex **(B)** it is postulated that on E17, the cortical neuroepithelium (ne) has regional specificity and contains a larger number of layer V progenitors (*stippled ovals*) anteriorly (*left*) than posteriorly (*right*). By E18, the young neurons that will settle in layer V are postulated to show laminar specificity by sojourning in sb2 (*stippled ovals*) in the intermediate zone rather than in another sojourn zone (the first inferior band in the subventricular zone, not shown). But more layer V neurons accumulate in anterior sb2 than in posterior sb2, reflecting the regional specificity that existed in the neuroepithelium on E17. On E19, the layer V neurons migrate through the upper intermediate zone (*narrow stippled ellipses*) and enter the lower parts of the cortical plate (*stippled ovals,* CP), again more migrate anteriorly than posteriorly. Finally, by E20, many of the layer V neurons generated on E17 settle as a superficial band in the cortical plate, but more neurons accumulate in the anterior cortical plate than in the posterior cortical plate.

E17 in the cortical neuroepithelium (*dark ellipses* in ne, Fig. 16–2B).[2] To account for regional specificity, we postulate that there is a genetically programmed anterior (many) to posterior (few) gradient in the distribution of layer V progenitors on E17 in the cortical neuroepithelium. We stress that the gradient is only a global predictor of the future differential distribution

[1] The cells in sb2 are represented in vertical orientation because this is a diagram of a sagittal section through the dorsomedial cortex. If we were representing a series of coronal sections going from anterior to posterior, the cells would be shown as horizontally oriented (see Chapter 8).

[2] That does not imply that these progenitors can produce only layer V cells, because either their ancestors or their descendants might give rise to neurons destined for other layers on other days.

of layer V neurons in the cortical plate, not a strict columnar mosaic of their exact positions in relation to the topographic distribution of motor neurons representing different body parts.

It is important here to consider that once the young neurons enter the cortical plate, they remain locked in a specific column. Consequently, the direction of migration after the young neurons leave the sojourn zones is a critical factor in the specification of the cytoarchitectonic and columnar heterogeneity in the adult cortical gray. Although we are representing only the dorsomedial cortex in Fig. 16–2B, where the neurons migrate in a predominantly radial direction, some of the putative layer V cells that sojourn in anterior sb2 will migrate laterally before they enter the cortical plate (Chapter 9). That must be so, because the lateral somatosensory cortex is deprived of an underlying neuroepithelium when the lateral ventricle progressively shrinks in a dorsomedial direction while the basal ganglia expand. The question that we cannot resolve on the basis of the available evidence is whether it is genetic programming or epigenetic interaction that determines which young neurons will follow a radial course to migrate dorsally and which will join the lateral cortical stream and become distributed in the lateral cortex. Genetic programming is suggested by the synchronization of the production of neurons in the thalamic neuroepithelium (Fig. 16–4C) and in the cortical neuroepithelium (Fig. 16–4B) in such a way that thalamic axons arrive in the intermediate zone of the lateral and dorsal cortices at different times in relation to the different arrival time of young neurons in these two regions. However, epigenetic influences may also be exerted by the incoming thalamic fibers as the young cortical neurons migrate through the upper intermediate zone prior to settling in their columnar positions in the cortical plate.

The general thrust of the above arguments is that the cortical neuroepithelium contains genetic programs for producing young neurons that are committed to settle in a specific layer of the neocortex; that is *laminar specificity*. In the case of layer V, there is support for the hypothesis that global gradients in the numbers of specified progenitor cells predispose a *regional specificity*. That is, more progenitors of layer V neurons are distributed anteriorly where the motor cortex forms than posteriorly where the visual cortex forms. Regional specificity is a forerunner of cytoarchitectonic diversity in the cortex, but it only contains a background canvas for the intricate anatomical and functional specialization that will follow. The details are painted in by the complex array of extrinsic afferent fibers and the network of commissural and associational connections. As studies with heterotypic neocortical transplants (Stanfield and O'Leary, 1985; O'Leary and Stanfield, 1989) indicate, epigenetic influences probably also determine the final target of the layer V axons from motor versus visual regions of the neocortex.

Afferents from specific thalamic relay nuclei are the prime candidates to exert a powerful epigenetic influence on cytoarchitectonic differentiation of the neocortex. In the mature cortex, the specificity of input from the thalamic relay nuclei to discrete areas has repeatedly been confirmed in the literature on thalamocortical connections (Brysch et al., 1990; Darian-Smith et al., 1990). It was shown recently (Whishaw and Kolb, 1989) that extensive lesions of the cortical surface in neonates does not result in a reorganization of the thalamic afferent input to the few remaining intact areas. Rather, the normal thalamic connections are maintained, and cells degenerate only in those thalamic nuclei that have lost their cortical targets. In the developing cortex, the afferents from the thalamic relay nuclei are already topographically arranged in the intermediate zone before the fibers penetrate the cortical plate (Dawson and Killackey, 1985; Darian-Smith et al., 1990). It is important to note that the parcellation of the cortical areas by the thalamic afferents occurs before the thalamus has its full complement of input from sensory relays in the brainstem and spinal cord (Killackey, 1985). Consequently, the thalamus itself must contain a great deal of preprogrammed specificity in its projection to the cortex.

In this context it is important to note that, in contrast to the cerebral cortex, neuroepithelial mosaicism is a hallmark of the embryonic thalamus (Altman and Bayer, 1988a). The thalamic neuroepithelium contains lobules, evaginations and invaginations of mitotically active areas, that appear at specific times during thalamic development. Short- and sequential-survival [^{3}H]thymidine autoradiography have enabled us to follow cell migration from these spatially defined germinal centers in the neuroepithelium to specific thalamic nuclei. Given these developmental characteristics, the thalamus could possess genetically specified properties to exert an inductive or epigenetic influence on the cortex. The idea that ontogenetic patterns in the thalamus and cortex are linked has also been put forth by Brysch et al. (1990). Some support for the epigenetic influence exerted by the thalamus comes from a recent report of the functional re-specification of the auditory cortex following experimentally induced visual input from the dorsal lateral geniculate nucleus following complete destruction of the medial geniculate body (Sur, et al., 1988).

16.3 NEUROGENETIC GRADIENTS IN RELATION TO THALAMIC CONNECTIONS

When the pattern of thalamocortical connections is superimposed on the pattern of neurogenetic gradients

in both the thalamus and cortex, there is an exact reversal of age-matching between thalamic source neurons and cortical target neurons so that older thalamic neurons project to younger cortical neurons. The reversed age-matching is found in every specific thalamic relay nucleus (reviewed in Chapters 11 to 15). In an attempt to explain this feature of cortical development, we may consider four factors: (1) the neurogenetic gradients in thalamic source neurons, (2) the neurogenetic gradients in cortical target neurons, (3) the length of the thalamic axons, and (4) the differential time of arrival of neurons in the dorsal versus the lateral cortical plate. The first three factors are summarized in Fig. 16–3 and the fourth is explained in Fig. 16–4.

The ventral nuclear complex of the thalamus contains the lateral ventrobasal nucleus (VBL), the medial ventrobasal nucleus (VBM, also called the *arcuate subdivision*), and the ventromedial nucleus (VM), also called the *ventroposteromedial nucleus* (VPm). VBL relays dorsal column input to the limb areas (FL and HL; Lund and Webster, 1967; Angel and Clarke, 1975; Feldman and Kruger, 1980; Pechanski et al., 1983, 1984). VBM relays trigeminal input from the vibrissae and other parts of the face to the primary somatosensory cortex (PAR1; Killackey, 1973; Killackey and Leshin, 1975; Saporta and Kruger, 1977; Jensen and Killackey, 1987; Chmielowska et al., 1989). VM relays gustatory input to the dorsal agranular insular area (AID; Gerfen and Clavier, 1979; Reep and Winans, 1982a; Saper, 1982; Guldin and Markowitsch, 1983; Kosar et al., 1986) and tactile input from the tongue to the gustatory cortex (GU; Kosar et al., 1986). Altogether, the five target areas (HL, FL, PAR1, GU, and AID) are contiguous and take up a large part of the lateral and dorsal cortex in the rat brain (*shaded areas* in cortex, Fig. 16–3).

On the basis of both neurogenetic gradients and anatomical connections, each thalamic nucleus can be further divided resulting in a total of six subdivisions (numbered areas in VB complex, Fig. 16–3). With the exception of 2 and 3, numerical order of the subdivisions refers to the chronological order of neurogenesis (Altman and Bayer, 1989a). There is a lateral (older) to medial (younger) neurogenetic gradient throughout the ventral nuclear complex, and there is an additional ventral (older) to dorsal (younger) gradient between VBL and VBM.

The oldest neurons are in the lateral part of VBL (subdivision 1), which relay sensory information from the hindlimb to area HL in the sensorimotor cortex (Lund and Webster, 1967; Angel and Clarke, 1975; Feldman and Kruger, 1980; Pechanski et al., 1983, 1984). Area HL is situated most posteriorly and medially in the cortical projection area of the ventral nuclear complex and, according to the transverse and longitudinal gradients in the cortex, contains the youngest neurons. Since HL is located farthest from the internal capsule (the entry point for thalamic axons into the cortex), the oldest neurons in the ventral nuclear complex also have the longest thalamocortical axons.

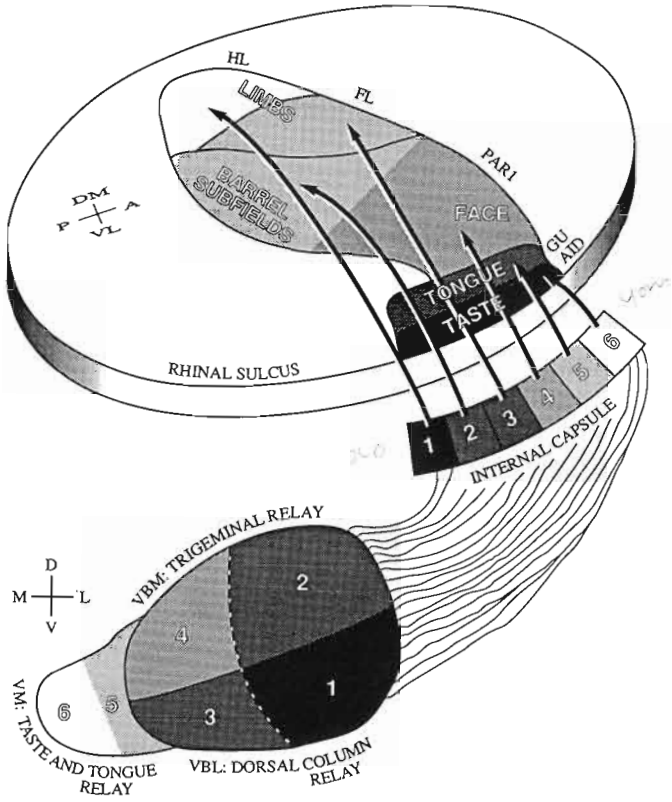

FIG. 16–3. A diagram of the projections from the thalamic ventrobasal complex to the cerebral cortex. Shaded areas in both the thalamus and the cortex represent areas where the oldest neurons are situated (*solid black*) and where the youngest neurons are situated (*clear*); neurons of intermediate age are shown in graded shadings, with older neurons in darker areas, younger neurons in lighter areas. Numbered subdivisions in the ventrobasal complex refer to populations of neurons that have different times of neurogenesis: 1 is oldest (*solid black*), 6 is youngest (*clear*); an exception is that subdivisions 2 and 3 have simultaneous times of origin. In all cases, there is an exact chronological reversal between the ages of thalamic source neurons and cortical target neurons that correlates with the length of the thalamic axons (as measured from their entry points into the cortex through the internal capsule). The oldest source neurons in the lateral ventrobasal nucleus (VBL) relay hindlimb information from subdivision 1 via the longest axons to cortical area HL that contains the youngest target neurons. The youngest source neurons in the ventromedial nucleus (VM) relay taste information from subdivision 6 via the shortest axons to cortical area AID, which contains the oldest target neurons. Source neurons and target neurons in between these two extremes are also reverse age matched, and older thalamic neurons have longer axons than the younger ones.

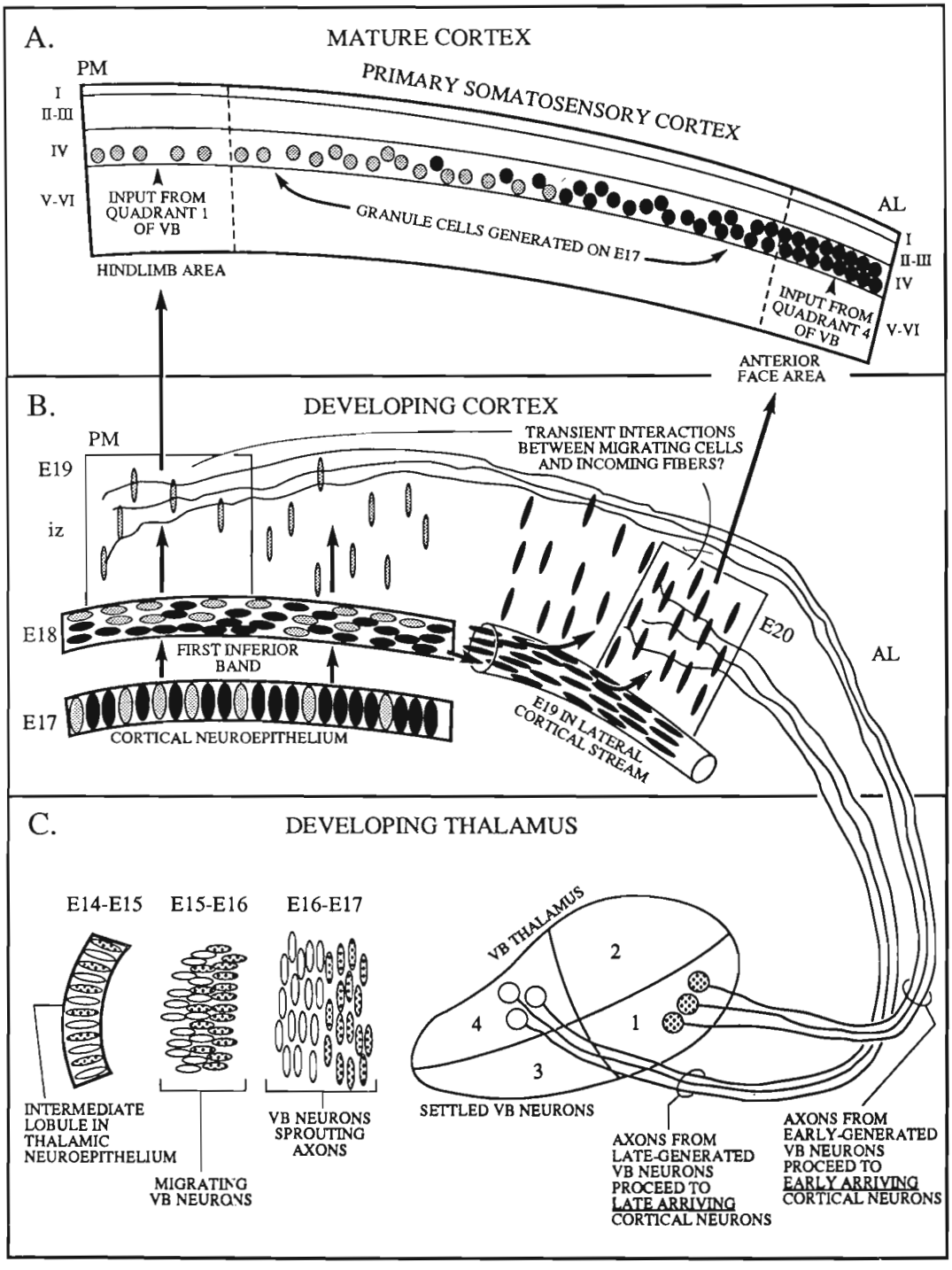

FIG. 16–4. Diagrammatic representation of the distribution of layer IV granule cells in the primary somatosensory cortex that are generated on E17 in the mature **(A)** and developing **(B)** cortex correlated with events in the developing thalamus **(C).** Since the mature cortex **(A)** contains more layer IV neurons that are generated on E17 in the anterolateral (AL) face area than in the posteromedial (PM) hindlimb area, it is postulated that the developing cortex **(B)** on E17 contains more progenitors of layer IV neurons in the cortical neuroepithelium that are destined to settle in anterior and lateral areas (*black ovals*) than those destined to settle in posterior and medial areas (*light stippled ovals*). On E18, all of the layer IV neurons are postulated to sojourn in the first inferior band (ib1), where they turn horizontally. Because their destinations in the cortical plate are directly above ib1, the neurons bound for posterior and medial areas migrate radially (*stippled narrow ellipses and vertical arrows*) from ib1 into the upper intermediate zone (iz) already on E19; some penetrate the cortical plate (not shown). In contrast, the neurons bound

Because of the combined ventral-to-dorsal and lateral-to-medial gradients in VBL and VBM, subdivisions 2 and 3 are nearly simultaneous in their generation (Altman and Bayer, 1989a). However, they are radically different in terms of somatotopic information conveyed to the cortex. Lateral VBM relays input from the whisker pad on the face to the posteromedial barrel subfields in PAR1 (Killackey, 1973; Killackey and Leshin, 1975; Saporta and Kruger, 1977; Jensen and Killackey, 1987; Chmielowska et al., 1989), while medial VBL relays input from the forelimb to area FL (Lund and Webster, 1967; Angel and Clarke, 1975; Feldman and Kruger, 1980; Pechanski et al., 1983, 1984). In the neocortex, the posteromedial barrel subfields and FL contain concurrently generated neurons. The thalamic axons reaching both areas are about the same length because those terminating in the posteromedial barrel subfields extend more posteriorly, while those terminating in area FL extend more medially.

Subdivision 4 in the medial part of VBM contains younger neurons that relay sensory information to the older neurons in the anterolateral face area in PAR1. Since that area is much closer to the internal capsule, the axons from medial VBM are shorter than those projecting from other parts of VBL and VBM.

Still younger neurons at the border between VM, VBM, and VBL (subdivision 5) relay tongue tactile information to still older neurons in GU (Kosar et al., 1986); those axons are short because of the proximity of GU to the internal capsule. Finally, the youngest neurons in the medial part of VM relay gustatory input to the oldest neurons in AID on the dorsal bank of the rhinal sulcus (Gerfen and Clavier, 1979; Reep and Winans, 1982a; Saper, 1982; Guldin and Markowitsch, 1983; Kosar et al., 1986); these have the shortest axons because AID is just at the level of the incoming internal capsule.

Obviously, the time of neuron origin is not linked to spatial representation of the body surface either in the thalamus or the cortex because the posteromedial barrel subfield and the forelimb are represented in partially contiguous and concurrently generated regions. But the time of neuron origin is always correlated with the distance between thalamic source neurons and cortical target neurons within a relay system. The question that remains is how the order of thalamic neurogenesis is relatable to the rule that older thalamic neurons with long axons terminate in cortical areas with young neurons and vice versa? That can be clarified by the different time of arrival of cogenerated cortical neurons in the dorsal and lateral cortex.

To illustrate this point, we will consider the distribution of the granule cells in layer IV that are generated on E17 throughout the primary somatosensory cortex (PAR1, FL, and HL). These neurons are the chief targets of the incoming VB complex thalamic afferents (Killackey, 1973; Killackey and Leshin, 1975; Saporta and Kruger, 1977; Jensen and Killackey, 1987; Chmielowska et al., 1989). In the mature cortex (Fig. 16–4A), more layer IV neurons generated on E17 reside in anterolateral (AL) parts (*dark circles*) than in the posteromedial (PM) parts (*light circles*). That gradient is best seen by comparing the number of layer IV neurons represented in the anterior face area and in the hindlimb area.

From that distribution, it follows that the cortical neuroepithelium on E17 (*bottom left,* Fig. 16–4B) should have a higher proportion of precursors for neurons in anterolateral layer IV (*dark ovals*) than in posteromedial layer IV (*light ovals*). On E18, the one-day old layer IV neurons, irrespective of their final destinations in the cortical plate, migrate out of the neuroepithelium (*arrows*), rotate horizontally, and sojourn in the first inferior band (ib1). Many leave ib1 on E19, but via two different migratory routes, (1) radial only, and (2) lateral first and radial later. (1) The layer IV neurons destined to settle in the posteromedial hindlimb area arise in the cortical neuroepithelium directly below their destinations in the cortical plate. Because of that, these neurons migrate only radially from ib1 for a relatively short distance in the upper intermediate zone (*light ellipses,* Fig. 16–4B), and many penetrate the lower parts of the cortical plate by E19, only 2 days after their birthdays. (2) In contrast, the layer IV neurons destined to settle in the anterolateral face area arise in the same dorsally situated cortical neuroepithelium (Chapters 2 and 9, color Figs. 2 to 7), but in order to reach their relatively distant destinations in the cortical plate, these layer IV neurons have to mi-

for anterior and lateral areas delay their entry into the upper intermediate zone and the cortical plate. Because their destinations in the cortical plate are lateral to ib1, on E19 these neurons are migrating laterally in the lateral cortical stream (*black narrow ellipses and horizontal arrow*) and do not enter the upper intermediate zone until E20 (*curved arrows*). In the developing thalamus **(C),** it is postulated that events are synchronized so that the axons of the oldest ventrobasal neurons (VB) that will settle in quadrant 1 start growing earlier and manage to reach the posteromedial intermediate zone just at the time when their target neurons are migrating through to the cortical plate on E19. Conversely, the axons of younger VB neurons that will settle in quadrant 4 start growing later but manage to reach the anterolateral intermediate zone just at the time when their target neurons are migrating through to the cortical plate on E20.

grate in the lateral cortical stream during E19 (*dark ellipses,* Fig. 16–4B). By E20, they leave the stream (*curved arrows on right,* Fig. 16–4B) and migrate radially through the intermediate zone to penetrate the lower parts of the cortical plate, 3 days after their birthdays. The shorter distance traveled by young neurons that settle posteromedially and the longer distance traveled by the young neurons that settle anterolaterally results in a reversed medial-to-lateral gradient in time of arrival.

When the generation of layer IV neurons peaks in the anterolateral primary somatosensory cortex (E17), development is well under way in the thalamus (Fig. 16–4C). Neurons in quadrant 1 of VB (the hindlimb relay) are generated on E14 (40%) and E15 (60%) in the intermediate lobule of the thalamic neuroepithelium (*stippled ovals,* Fig. 16–4C). Neurons in quadrant 4 (the anterior face relay) are generated mainly on E15 (close to 90%) in the same neuroepithelial lobule (*clear ovals,* Fig. 16–4C). The first day after their generation (E15–E16), the young neurons move out in a radial direction to form a wave front (migrating VB neurons, Fig. 16–4C) just outside of the neuroepithelium. In the next 24 hours (E16–E17), the neurons rotate vertically and begin to sprout axons (Altman and Bayer, 1989a). During all of these movements the oldest cells form the leading edge, suggesting that a neuron generated on E14 is migrating radially by E15 and is sprouting an axon by E16.

By E17 in rats, thalamic axons are already in the internal capsule, and some have reached the border of the developing cortex (Lund and Mustari, 1977; Altman and Bayer, 1989a; Catalano and Killackey, 1990; Molnár and Blakemore, 1990).[3] We postulate that the first thalamic axons in the cortex continue to grow posteromedially, proceeding to the area where the early-arriving layer IV cortical neurons are migrating through the intermediate zone on E19 (left rectangle in iz, Fig. 16–4B). The axons from later-generated thalamic neurons reach the anterolateral cortex later and proceed to areas where the later-arriving cortical neurons are migrating through the intermediate zone on E20 (right rectangle in iz, Fig. 16–4B). Recent evidence in rats indicates that, indeed, thalamocortical axons are in the intermediate zone at these times (Catalano and Killackey, 1990; Erzurumlu and Jhavari, 1990), and the postulated interaction could occur. By day E18 (not illustrated), more layer IV neurons destined to settle in posteromedial areas are generated in the cortical neuroepithelium, and more VB thalamic axons arrive from quadrant 1 on E20 to interact with the larger contingent of migrating layer IV neurons in the posteromedial intermediate zone.

If our interpretation is correct, it suggests a situation where cell proliferation is so timed in two distantly located germinal systems (the cortical neuroepithelium and the thalamic neuroepithelium) that, when the time comes for their products (the cortical neurons and the thalamic afferents) to establish an intimate relationship, *they meet and interact at the right place at the right time.* This is reminiscent of what the philosopher Leibniz, in a different context and with a somewhat different meaning, called "pre-established harmony" (Russell, 1945). Cell proliferation in presumably two autonomously developing germinal systems (the thalamic and the cortical neuroepithelia) is guided by synchronized clocks in such a way that after their progeny have undergone various developmental transformations, the axons of one can interact with the cells of the other. There is evidence for such synchronization between migrating neurons and incoming axons in other brain systems, as we have experimentally shown in the cerebellar cortex (Altman, 1973). The descent of granule cells from the external germinal layer to the granular layer is synchronized with the arrival of mossy fibers. If the descent of granule cells is delayed by x-irradiation (which interrupts the production of granule cells in the external germinal layer), the mossy fibers proceed to grow into the molecular layer and arrest the late descending granule cells.

However, the above hypothesis needs to be qualified by a unique situation in the developing cortex. For unknown reasons, the cortical neurons continue to migrate toward the cortical plate, while the VB thalamic afferents remain in the subplate and intermediate zone. Earlier studies (Wise and Jones, 1978; Dawson and Killackey, 1985) indicated that the wait in the subplate could be up to a week, but more recent evidence in rats (Catalano and Killackey, 1990; Erzurumlu and Jhavari, 1990; Reinoso and O'Leary, 1990) indicates that the pause is only a few days. It has been proposed that subplate neurons provide temporary targets for the incoming thalamic afferents (Rakic, 1983; Crandall and Caviness, 1984; Shatz and Luskin, 1986; Chun et al., 1987; Friauf et al., 1990; Kostovic and Rakic, 1990). We offer an additional point to the hypothesis stated above that the synchronized neurogenesis in thalamus and cortex insures that cortical neurons are migrating through the intermediate zone just at the same time that the thalamic afferents destined to contact them are penetrating the cortex. The synchrony allows for transient interactions that may be critical to setting the stage for the later development of appropriate permanent connections.

[3] Additional studies of cat (Wise et al., 1977) and mouse (Crandall and Caviness, 1984) embryonic neocortex, and postnatal but extremely immature opossum brain (Martin et al., 1987) indicate that thalamocortical afferents arrive very early during development of the primary somatosensory cortex.

16.4 THE PLACE OF THE NEOCORTEX IN CORTICAL EVOLUTION

Phylogenetic relationships within the cerebral cortex were often the subjects of contradicting opinions and confusing terminology in the classical neuroanatomical literature (Abbie, 1940, 1942; Herrick, 1948; Sanides, 1969). None of the hypotheses has found general acceptance, and interest in the topic of evolutionary origin has waned in recent years. Since ontogenetic patterns can provide clues about phylogenetic history, a comprehensive developmental study of the cerebral cortex should shed new light on these old controversies. With data presented on discrete areas of the neocortex (Chapters 11–14), the limbic cortex (Chapter 15), previous work on the hippocampal region (Bayer and Altman, 1974; Bayer, 1980a), and the primary olfactory cortex (Bayer, 1986), there is a complete quantitative developmental analysis of the entire telencephalic cortical mantle. These studies show that three major cortical systems can be distinguished, each with its own characteristic neurogenetic timetables and gradients.

16.4.1 Reversal of Neocortical Gradients in the Medial Limbic Cortex

One of the most prominent neurogenetic gradients found in the neocortex is that older neurons are situated ventrolaterally and younger neurons dorsomedially (Chapter 3). If the medial limbic cortex were a direct continuation of the neocortex, one would expect to see the youngest cortical areas in the most ventral parts of the medial limbic cortex. But exactly the opposite is found. Throughout the medial limbic cortex, superficial cells have a ventral (older) to dorsal (younger) gradient (Richter and Kranz 1979a, 1979b; Bayer, 1990a). Subplate incorporation into the medial limbic cortex sets up a similar gradient between the deep cells at anterior levels, and sequential survival thymidine autoradiograms show that the ventral medial limbic cortex has fewer labeled cells throughout all layers than does the neocortex (Bayer, 1990a). When one looks at the anterior third of the hemisphere, ventral limbic cortical areas in the medial wall (Fig. 15–6), beneath the frontal pole (Table 1, Chapter 15), and in the lateral wall (Fig. 15–2) are always older than the nearest area of neocortex (*solid arrows*, Fig. 16–5). As we will point out in the following discussion, that developmental shift is just one among others to signal that the limbic cortex and neocortex are "cousins" rather than "siblings."

16.4.2 Neurogenetic Gradients within Different Cortices in Relation to Phylogenetic Hypotheses

Cytoarchitectonic observations have shown that the cortex bordering the hippocampus (periarchicortex) and the piriform areas (peripaleocortex) have a simpler laminar organization than the neocortex (Abbie, 1940, 1942; Sanides, 1969). Such findings were the basis of a hypothesis that the neocortex has a dual phylogenetic origin and evolved in concentric arcs over the piriform cortex laterally and over the hippocampus medially. In contrast, Herrick's (1948) observations suggested that the neocortex has a unitary phylogenetic origin in the "dorsal cortex" or "general sensory cortex" of amphibians and reptiles. That area is situated between the forerunner of the hippocampus medially and the forerunner of the piriform cortex laterally. Those interpretations were expanded by studies in a variety of reptilian brains (Kruger, 1969; Riss et al., 1969). Our ontogenetic data suggest the following phylogenetic relationships in the mammalian cortex.

The major neurogenetic gradients throughout the cortex are represented as variously highlighted arrows in the diagram in Fig. 16–5. An analysis of all neurogenetic gradients (some of which could not be shown in Fig. 16–5) indicates that there are three ontogenetically distinct cortical systems in mammals: (1) the paleo/archicortex, (2) the limbic cortex, and (3) the neocortex. A summary of the similarities and differences between the systems is diagrammed in Fig. 16–6.

An ontogenetic link between the paleo/archicortex and the limbic cortex is shown by their sharing sandwich neurogenetic gradients: older areas are bordered anteriorly and posteriorly by younger areas. In contrast, the neocortical system diverges by showing open neurogenetic gradients, where older areas are either lateral or anterior and younger areas are either medial or posterior (Chapter 3). The limbic cortex has two foci of older neurons, one laterally (AIP, Fig. 16–5), another medially (anterior CG, IL, and DP in Fig. 16–5). Younger cells are in areas anterior and posterior to these foci (*arrows*, Fig. 16–5). In the paleo/archicortical system, there is a single focus of older cells in the posterior primary olfactory cortex (PPO, Bayer, 1986). Neurons are born progressively later in the anterior primary olfactory cortex (APO) the closer they are to the olfactory bulb (Bayer, 1986). Similarly, cells are born later as their positions shift away from PPO through lateral and medial entorhinal areas (ECL and ECM) toward the hippocampus (HP) (Bayer, 1980a).

It is noteworthy that the sandwich gradients in the paleo/archicortex and the limbic cortex "line up" according to age in the ventrodorsal plane. That is best seen in the lateral cortical wall. The focus of oldest

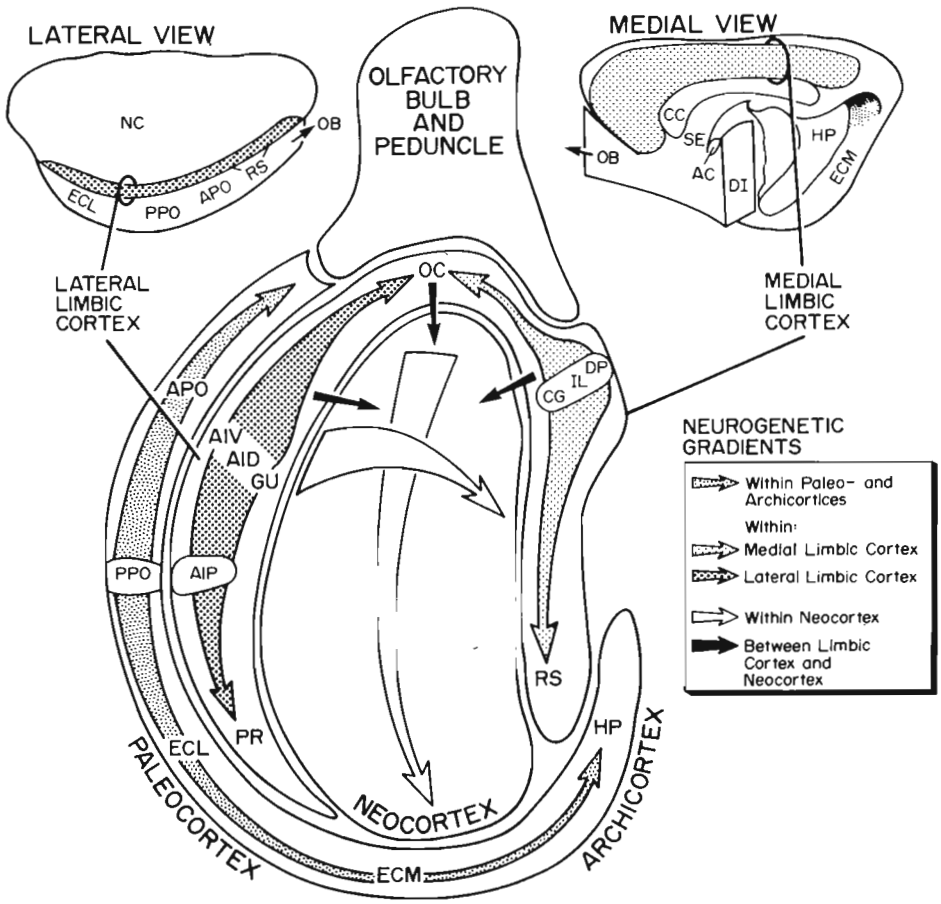

FIG. 16–5. The rat cerebral cortex realistically drawn from the lateral and medial aspects (small drawings in upper right and upper left), and a diagrammatic view of the entire cortical mantle where the top and all sides are represented in one plane (large middle drawing). The neocortex is completely surrounded by two overlapping belts. The outer belt (in reality, most ventral) is continuous anteriorly with the olfactory peduncle and contains *paleocortex* below the rhinal sulcus and in the posterior wall (anterior, APO and posterior, PPO primary olfactory cortices; lateral, ECL and medial, ECM entorhinal cortices). The paleocortex continues without a break into the *archicortex* (hippocampus, HP) that extends beneath the corpus callosum in the medial wall. An inner belt (in reality, more dorsal) is open posteriorly and contains the lateral limbic cortex in and above the rhinal sulcus (perirhinal, PR; insular-AIP, AIV, and AID; gustatory, GU), the medial limbic cortex in front of and above the corpus callosum (dorsal peduncular, DP; infralimbic, IL; cingulate, CG; retrosplenial, RS). Both limbic cortices join in the orbital cortex (OC) below the frontal pole. Directions in neurogenetic gradients are represented by arrows pointing from areas containing older cells to areas containing younger cells, and are drawn according to data presented here for the lateral limbic cortex and elsewhere for the other cortical areas. Each major cortical subdivision has characteristic arrows (see legend). Note that foci of "old" cells are found in the limbic cortical belt and in the paleo-archicortical belt but not in the neocortex. In addition, the ventral parts of the limbic cortical belt in the anterior third of the hemisphere are older than the nearest sector of neocortex (*solid black arrows*).

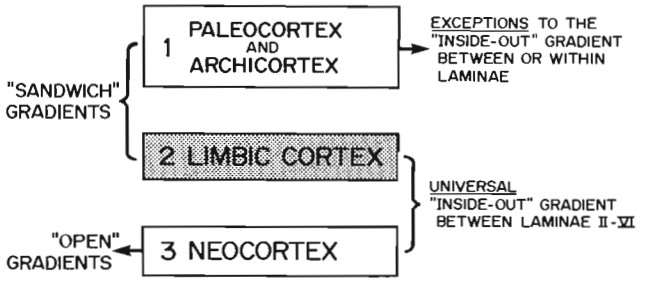

FIG. 16–6. Summary of the convergence and divergence of neurogenetic gradients between cortical ontogenetic systems. It is suggested that systems 1 and 3 evolved from separate phylogenetic sources because of divergence in ontogenetic patterns, while the limbic cortex (system 2) evolved from dual phylogenetic sources because of convergence in ontogenetic patterns with the paleo-archicortical system ("sandwich" neurogenetic gradients are shared) and the neocortical system ("inside-out" neurogenetic gradients are shared).

neurons in the PPO is just beneath the AIP. In AIV, AIP, and GU, deep neurons have a posterior (older) to anterior (younger) neurogenetic gradient (Chapter 15, Figs. 15–3 and 15–4) matching a similar gradient in the segment of paleocortex beneath them. The youngest parts of the anterior primary olfactory cortex are closest to the anterior focus of young neurons in the limbic cortex just above the olfactory peduncle (ventral and dorsal orbital areas, Table 15–1). Behind AIP and PPO, younger neurons in the perirhinal cortex (PR) are above younger neurons in the lateral entorhinal cortex (ECL).

An ontogenetic link between the neocortex and the limbic cortex is shown by their sharing neurogenetic gradients in the radial direction (not shown in Fig. 16–5). Without exception, neurons in the deep layers originate earlier than cells in the superficial layers. That is the inside-out gradient first seen by Angevine and Sidman (1961) and confirmed in every subsequent thymidine autoradiographic study of neocortical development. In contrast, the paleocortex and parts of the archicortex have exceptions to that gradient.[4] The split between the limbic cortex and the paleoarchicortex on the inside-out gradient may be related to the embryonic cortical plate. The ventrolateral extreme of the cortical plate differentiates into the lateral limbic cortex. The piriform cortex appears to differentiate at least partially from the lateral ganglionic eminence in the basal telencephalon rather than from the cortical plate (Altman and Bayer, in preparation). The cortical plate on the medial wall is highly modified in the region of the developing hippocampus, and the dentate gyrus has a different developmental history (Bayer, 1980b).

A summary of the *ontogenetic* features of the different cortical systems (Fig. 16–6) suggest a simpler *phylogenetic* scheme where the link between systems is centered in the limbic cortex. Directions in neurogenetic gradients indicate that the paleo/archicorticex (System 1) and the neocortex (System 3) are most divergent. We postulate that these two ontogenetic systems have unique phylogenetic sources. In support of Herrick's (1948) suggestion, a single phylogenetic primordium probably gives rise to the neocortex, since it is the only cortex with open neurogenetic gradients. Although they are traditionally considered to evolve from separate primordia (Abbie, 1940, 1942; Herrick, 1948; Sanides, 1969), the striking sequential nature of neurogenesis throughout the combined paleocortex and archicortex (System 1) is the basis for our suggestion that these structures constitute a single ontogenetic system and may have a single phylogenetic source. However, there is strong ontogenetic evidence for a dual phylogenetic origin of the limbic cortex. We suggest that one source is in the paleo/archicortex and the other is in the neocortex.

[4] The three most prominent exceptions are: (1) Layer II cells in the entorhinal cortex originate earlier than cells in layer III (Bayer, 1980a). (2) Superficial layer II cells in the primary olfactory cortex originate earlier than deep layer II cells (Bayer, 1986). (3) Cells in the dentate granular layer of the hippocampus have a prominent outside-in gradient (Bayer and Altman, 1974; Bayer, 1980a).