Bayer, SA and J Altman (1991) NEOCORTICAL DEVELOPMENT Raven Press.

CHAPTER 3

Overview of Global Neurogenetic Gradients in the Neocortex and Limbic Cortex

In Chapter 2, we described *morphogenetic* gradients of differentiation in the cortex, i.e., the ventrolateral-to-dorsomedial and anterior-to-posterior gradients in the growth of the intermediate zone and the cortical plate. Besides gradients of growth and differentiation, there are also *neurogenetic* gradients: in most (although not all) brain regions, neurons are spatially distributed in an orderly fashion according to their times of origin. Neurogenetic gradients can be described in directional terms, such as lateral-to-medial, rostral-to-caudal, outside-in, inside-out. Using long-survival [^{3}H]thymidine autoradiography, the neurogenetic gradients found between major areas in the neocortex and the lateral and medial limbic areas are described in this chapter. A detailed account of the methods used is given in Appendix 2. Briefly, proliferating neuronal progenitors are labeled during the embryonic period, and the animals survive until after the labeled neurons have settled in their final locations. Since neurogenetic gradients are part of the organization of the mature brain, there is a temporal foundation to the spatial relationships that exist within and between brain structures, a *chronoarchitectonic* map. In this chapter, we produce a global chronoarchitectonic map emphasizing the neurogenetic gradients between cortical areas. Detailed maps of neurogenetic gradients within each cortical area are given in Part Three (Chapters 11–15).

The chronoarchitectonics of a mature brain region are the outcome of at least three developmental events: (1) when the postmitotic young neurons are generated and leave the neuroepithelium; (2) what paths they follow to reach their destinations; and (3) what kinds of transformations they undergo during maturation. If one knows the timing for the first event (neurogenesis) in each of the major cortical areas, then inferences can be made about the identity of the primitive embryonic cells taking part in the second and third events. Therefore, the neurogenetic data presented here will used in Part Two (Chapters 4–10), where we directly examine the course of embryonic cortical morphogenesis. We will be able to identify the immature neurons sojourning in the subventricular and intermediate zones and follow their migrations to the cortical plate. With a fair degree of precision, we will also be able to determine when cells destined for each cortical layer arrive and settle in the cortical plate.

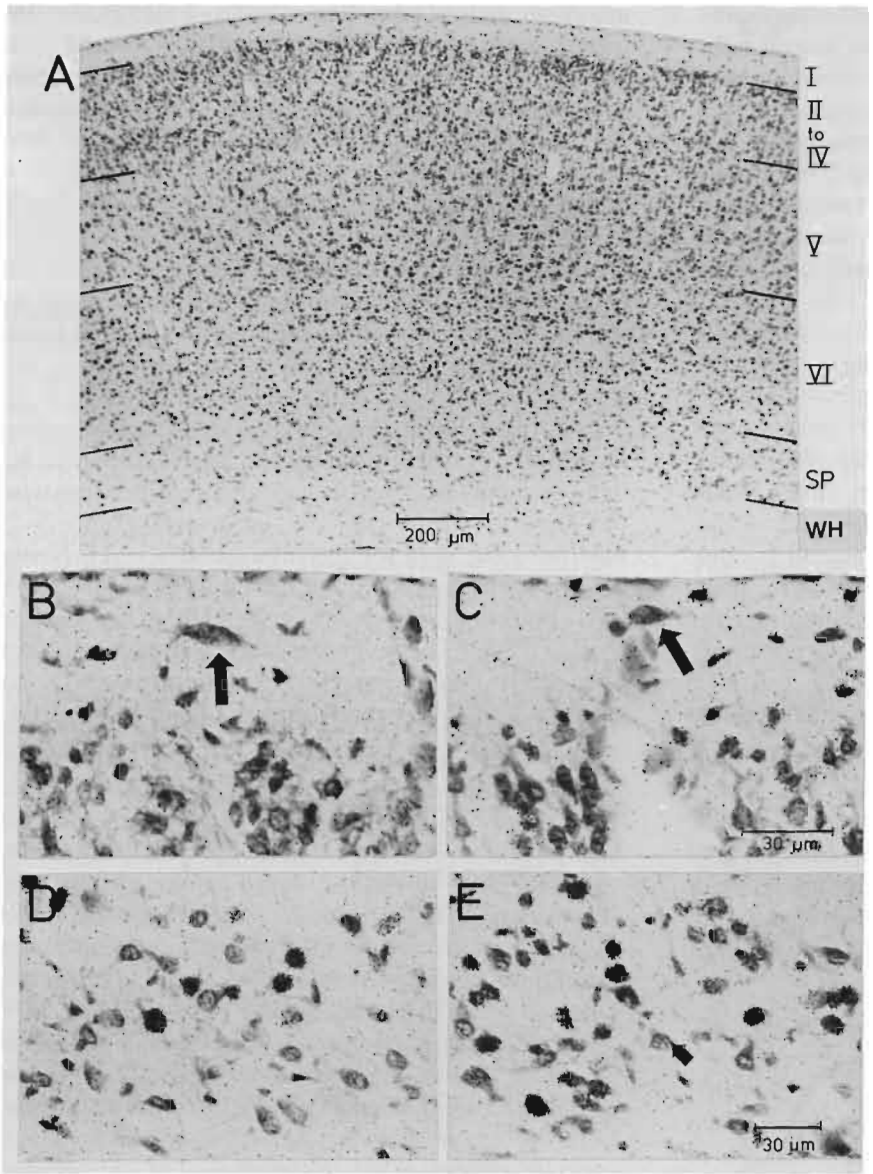

FIG. 3–1. The dorsal neocortex in an animal exposed to [^{3}H]thymidine on E15 + E16 and killed on P5. **(A)** Low magnification view showing all the layers of the neocortex from the most superficial (I) to the deep white matter (WH). The subplate (SP), also called layer VII, forms a separate layer beneath VI. (6 µm paraffin section, hematoxylin stain.) **(B, C)** High-magnification views of the large horizontal cells, presumptive Cajal-Retzius cells (*arrows*), in layer I. Labeled cells have black specks on the nucleus that are produced when radioactive decay reduces silver grains in the emulsion layer overlying the section. Both Cajal-Retzius cells are unlabeled when injections begin on E15. **(D, E)** High magnification views of the polymorphic cells in ventrolateral **(D)** and dorsomedial **(E)** areas in the subplate. Many subplate cells are labeled when injections begin on E15, indicating that they originate later than the Cajal-Retzius cells. In addition, the proportion of labeled subplate cells is greater dorsomedially **(E)** than ventrolaterally **(D)**. From Bayer and Altman, 1990.

3.1 NEUROGENETIC GRADIENTS IN LAYER I AND THE SUBPLATE

Since the work of Cajal (1911) and Retzius (1893), it has been known that the pioneer cell populations of the neocortex are the Cajal-Retzius cells in layer I and the polymorph cells in the deepest layer, variably called layer VIb, VII or, more recently, the subplate. We quantified the time of origin of neurons in layer I and the subplate using the [^{3}H]thymidine autoradiographic comprehensive labeling method (Bayer and Altman, 1974; Appendix 2), and showed that the cells of layer I and the subplate have a neurogenetic gradient exactly opposite to that found within the cortical plate. The experimental animals were exposed to [^{3}H]thymidine on two consecutive days during embryonic life and were killed on P5.[1] Anterior, intermediate, and posterior levels (*drawings*, Fig. 3–2A) were chosen for analysis and large horizontal cells (Figs. 3–1B, C) were counted in the outer half of layer I from the rhinal sulcus to the cingulate gyrus (*superficial*

[1] Three circumstances make it more appropriate to use animals killed on P5 rather than on P60. (1) At P5 layer VII, or the subplate (SP, Figs. 3–1 and 3–7), forms a distinct layer embedded in and just above the white matter underlying layer VI in the neocortical areas. (2) Also at that age, each coronal section (6 µm) of the neocortex contains approximately 20–40 large horizontal cells in layer I (I, Fig. 3–1A). These are presumed to be Cajal-Retzius cells (Fig. 3–1B, C). (3) In single 6 µm sections of P60 brains, the number of large horizontal cells in layer I and the cells in the subplate have been reduced by the enormous growth of the neocortex (Marin-Padilla, 1971; Marin-Padilla and Marin-Padilla, 1982) and possibly also by cell death (Sas and Sanides, 1970; Bradford et al., 1977).

shaded area in drawings, Fig. 3–2A). Due to the scarcity of large cells per section, medial and lateral subdivisions were not made. In the same sections, counts were done in three to four areas of the subplate (Figs. 3–1D, E) from lateral to medial. The sign test (Conover, 1971) and the repeated measures analysis of variance (SAS, general linear models procedure) were used to analyze the data. (A complete discussion of the rationale for the use of statistical tests is given in Appendix 3.)

Neurogenetic Gradients Between Layer I and the Subplate

Cajal-Retzius and subplate neurons originate simultaneously in anterior and posterior sites; there is simultaneous neurogenesis medially and laterally in the subplate when the entire neurogenetic period is considered (all $P > 0.05$, sign test). Consequently, the data in Fig. 3–2A are combined across both anterior/posterior and medial/lateral subdivisions. The presumptive Cajal-Retzius cells originate mainly on E14 (63.5%) significantly earlier than subplate cells, which are generated on both E14 (41%) and E15 (45%) ($P < 0.0001$, sign test; $F = 48.33$, $df = 8$, $P < 0.0001$, repeated measures analysis of variance). That is typical of *noncortical* neurogenetic gradients: superficial cells are older than deep cells (*arrows,* Fig. 3–2A).

Neurogenetic Gradients within the Subplate

Qualitative observations in the subplate indicated that equal proportions of cells were labeled throughout medial and lateral parts in all except one injection group. Animals exposed to [^{3}H]thymidine on E15 + E16 showed fewer labeled cells ventrolaterally (Fig. 3–1D) than dorsomedially (Fig. 3–1E). That labeling pattern indicates a brief but pronounced lateral (older) to medial (younger) neurogenetic gradient at the time when the majority of the cells are generated. Figure 3–2B illustrates the data for the intermediate level. Neurogenesis in the subplate underlying the gustatory cortex (GU, bottom graph) peaks on E14 (53%) and declines by E15 (28%). The reverse pattern is shown in the subplate cells underlying the motor cortex (MO, top graph) where 24% originate on E14 and 51% on E15.

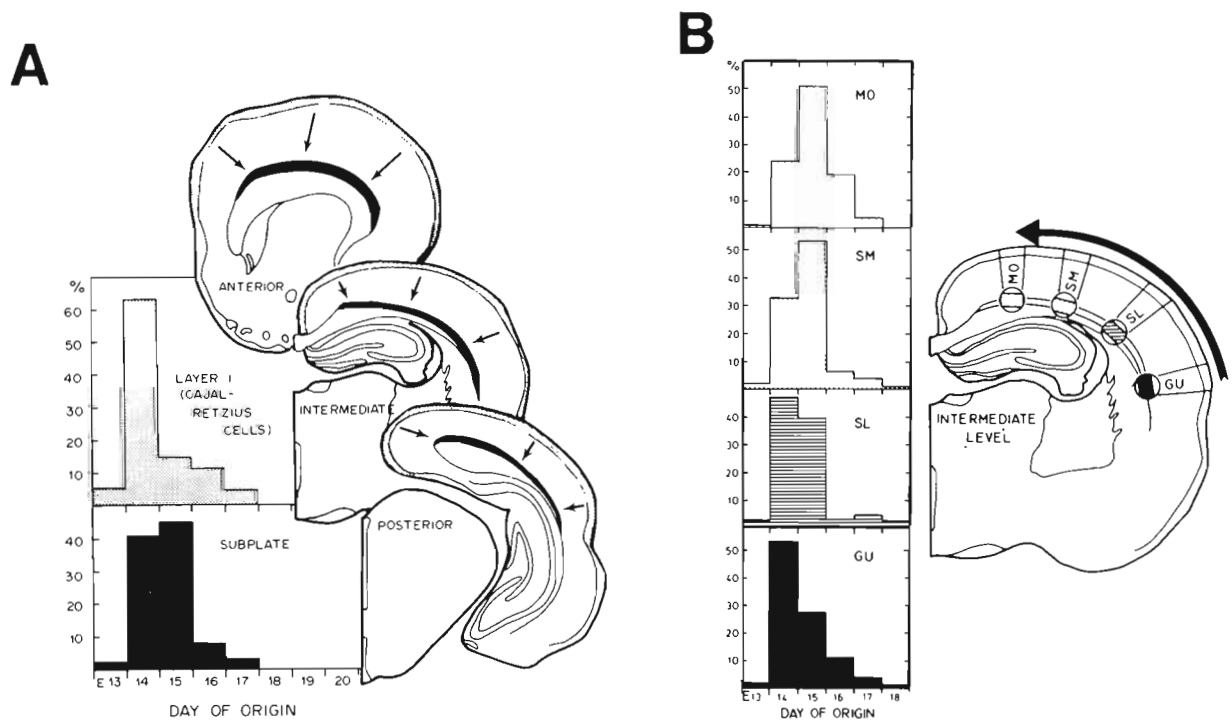

FIG. 3–2. (A) Neurogenesis of the presumptive Cajal-Retzius cells in layer I (*top graph*) and the polymorphic cells in the subplate (*bottom graph*). Bar graphs are the proportion of cells originating on a single embryonic day. Most of the Cajal-Retzius cells originate on E14, while a substantial number of subplate cells are still generated on E15, indicating an older superficial-to-younger deep neurogenetic gradient between the two populations (*arrows in drawings*). **(B)** Neurogenesis of the polymorphic cells in the subplate underlying the neocortex in four separate locations: gustatory cortex (GU, *bottom graph*), lateral and medial parts of the somatosensory cortex (SL and SM, respectively, *two center graphs*), and motor cortex (MO, *top graph*). Most subplate cells originate on E14 and E15, but there is a gradual shift in peak production from E14 in the ventrolateral subplate (GU) to E15 in the dorsomedial subplate (MO). From Bayer and Altman, 1990.

The neurogenetic patterns in intervening subplate areas beneath lateral and medial parts of the somatosensory cortex (SL and SM, respectively) show gradual lateral-early to medial-late shifts in the gradient. These developmental differences are significant ($P < 0.0001$, sign test on E15 + E16 only; $F = 27.37$, $df = 1, P < 0.0001$, repeated measures analysis of variance).

Summary

The early neurogenesis of the Cajal-Retzius and subplate neurons reported here confirms previous [^{3}H]thymidine autoradiographic studies (König et al., 1977; Rickmann et al., 1977; Raedler and Raedler, 1978; Wolff, 1978; Kostovic and Rakic, 1980; König and Marty, 1981; Caviness, 1982; Luskin and Shatz, 1985b; Chun et al., 1987). Although two of these studies (König et al., 1977; Luskin and Shatz, 1985a) provided evidence that subplate neurons are generated later than Cajal-Retzius cells, most studies stressed the early cogeneration of these neurons. What is emphasized here and in the report by Raedler and Raedler (1978) is that the cells in the primordial plexiform layer have an outside-in neurogenetic gradient (older superficial to younger deep), that is opposite to the inside-out neurogenetic gradient in the cortical plate (younger superficial to older deep) (Angevine and Sidman, 1961). The shift in neurogenetic gradients between neurons in the primordial plexiform layer and the cortical plate provide support for Marin-Padilla's hypothesis (1971, 1978) that the mammalian neocortex has two distinct phylogenetic and ontogenetic components (see Chapter 1). Not only are the Cajal-Retzius and subplate neurons the first to originate and differentiate (the "reptilian" component), but they also have a different neurogenetic gradient than the cells in the cortical plate (the "mammalian" component).

3.2 NEUROGENETIC GRADIENTS IN LAYERS VI–II

In the layers of the cortex that develop from the cortical plate (layers VI–II), there are three global neurogenetic gradients: a radial one from deep to superficial, a transverse one from ventrolateral to dorsomedial, and a longitudinal one from anterior to posterior. These global gradients have also been found in the opossum cortex (Sanderson and Weller, 1990). While the radial gradient is universal in all cortical areas, there is considerable variability within cortical areas in the magnitude of the longitudinal and transverse gradients, a topic that will be discussed in Part Three (Chapters 11–15).

The Radial Gradient

Deep neurons close to the white matter originate earlier than superficial neurons close to layer I. This inside-out gradient, first reported by Angevine and Sidman (1961), has been confirmed in every subsequent [^{3}H]thymidine autoradiographic study of neocortical neurogenesis (Fernandez, 1969; Shimada, 1970; Shimada and Langman, 1970; Butler and Caley, 1972; Caviness and Sidman, 1973; Fernandez and Bravo, 1974; Rakic, 1974, 1977, 1978, 1982; Brückner et al., 1976; Smart and Smart, 1977, 1982; Wolff et al., 1978; Richter and Kranz, 1979b, 1980; Caviness, 1982; Todd and Smart, 1982; Luskin and Shatz, 1985a; Miller, 1986, 1988; Cavanagh and Parnavelas, 1988; Jackson et al., 1989).

The Transverse Gradient

Ventrolateral neurons are older than dorsomedial neurons in every layer. The transverse gradient has been found in several species, namely rats (Raedler and Raedler, 1978; Raedler et al., 1980), mice (Shimada, 1970; Smart and Smart, 1977, 1982; Todd and Smart, 1982), and in the visual cortex of cats (Luskin and Shatz, 1985a) and ferrets (Jackson et al., 1989). However, the medial limbic cortex diverges from that gradient (Richter and Kranz, 1979b, 1980), indicating that limbic and neocortex are unique in spite of their contiguity.

The Longitudinal Gradient

Anterior neurons are older than posterior neurons in every layer. The longitudinal gradient has been found in the ferret visual cortex (Jackson et al., 1989) and between output cells of the corticospinal tract (Miller, 1988). Marin-Padilla (1988) showed an anterior-to-posterior gradient of cell differentiation using the Golgi stain. Here again, the limbic cortex diverges by having gradients in exactly the opposite direction (older posterior to younger anterior) in some areas (Bayer, 1990a; Chapter 15).

3.2.1 The Radial and Longitudinal Neurogenetic Gradients

To provide an overview of the radial and longitudinal gradients in the anterior/posterior plane, we used sagittally sectioned brains of rats killed on postnatal day 60 after two consecutive daily exposures to [^{3}H]thymidine during embryonic life. Sections approximately 1.5 mm lateral to the midline (L1.5, Pellegrino et al., 1979) were selected. At that level, all

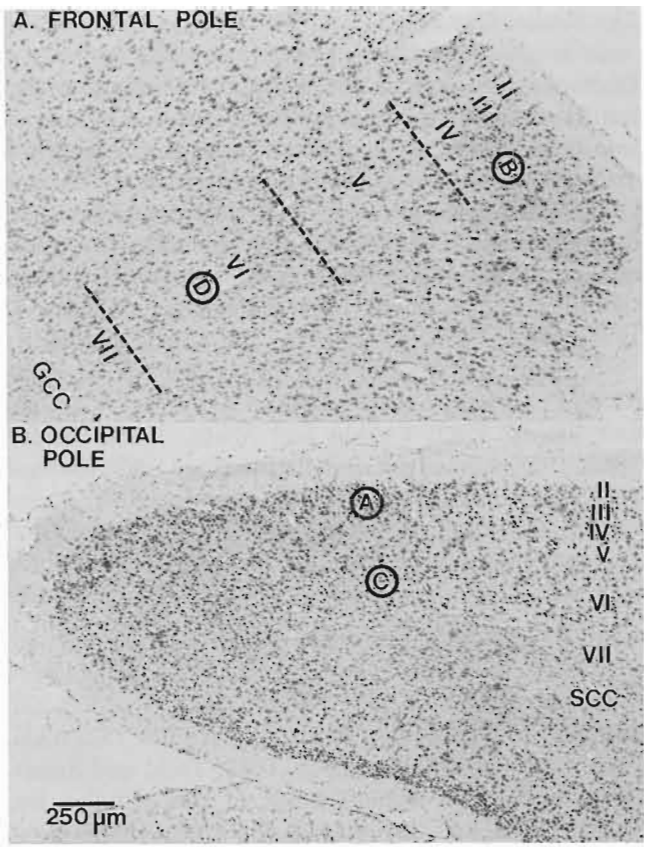

FIG. 3–3. The frontal **(A)** and occipital **(B)** poles in a sagittally sectioned brain 1.5 mm lateral to the midline. This rat was exposed to [^{3}H]thymidine on E17 + E18 and killed on P60 (6 μm paraffin section, hematoxylin and eosin stain). For each photograph, dorsal is toward the top and posterior is to the left. The genu (GCC in A) and splenium (SCC in B) of the corpus callosum underly each pole, respectively. Roman numerals indicate the cortical layers. *Dashed lines* in **A** show dividing lines between the deep layers (V–VII) for the cell counts. *Circled letters* **(A–D)** indicate areas of the high magnification photographs in Fig. 3–4.

layers of the dorsomedial cortex (Fig. 3–3) lie perpendicular to the transverse plane and parallel to the anterior/posterior plane and are therefore appropriate for comparing gradients in neurogenesis between layers both radially and longitudinally. At L1.5, the motor cortex lies in the frontal pole (Fig. 3–3A) and the medial visual cortex in the occipital pole (Fig. 3–3B).[2]

Radial Gradient

The photomicrographs in Fig. 3–4 show examples of the labeling patterns seen in an animal injected with [^{3}H]thymidine on E17 and E18 and killed on P60.

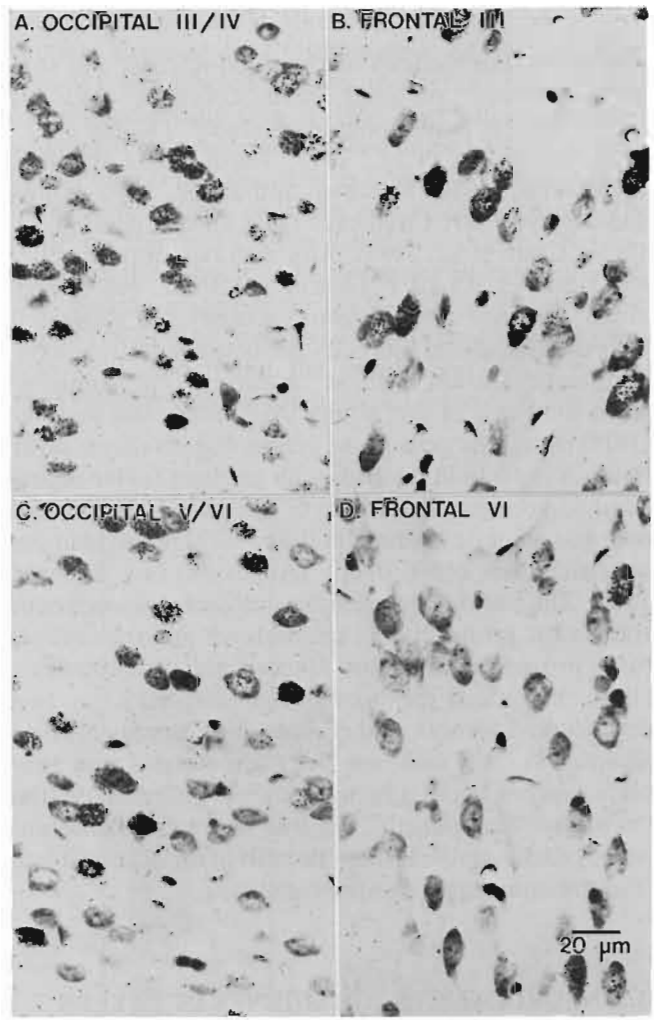

FIG. 3–4. [^{3}H]thymidine labeling patterns in the occipital (**A** and **C**) and frontal (**B** and **D**) poles produced by injections on E17 + E18. The areas of each photograph are indicated by the circled letters in Fig. 3–3. Note that very few labeled cells are found in layer VI in the frontal pole **(D),** a mixture of labeled and unlabeled cells are in layers V and VI of the occipital pole **(C),** while the superficial cells in layers III and IV are predominantly labeled in both poles (**A** and **B**). Those patterns indicate two neurogenetic gradients: (1) older deep to younger superficial and (2) older anterior (frontal) to younger posterior (occipital). Most cells originating before E17 (those unlabeled) are found in the anterior deep layers, some in the posterior deep layers; while cells originating on or after E17 (those labeled) are found mainly in superficial and posterior locations.

[2] Two circumstances make analysis of neurogenesis in each pole especially suitable for study of the radial and longitudinal gradients. (1) Since the frontal and occipital poles are separated by a longitudinal distance greater than that between other parts of the neocortex, the most robust expression of a neurogenetic gradient in that plane is likely to be found. (2) Unlike other parts of the mature cortex, at L1.5 there is an exceptionally good representation of cells in the subplate, or layer VII. These cells tend to accumulate in the cingulum bundle that courses over the genu (GCC, Fig. 3–3A) and splenium (SCC, Fig. 3–3B) of the corpus callosum and are much more sparse in other cortical areas. Due to that circumstance, we were able to quantify the time of origin in seven highly cellular cortical layers (*dashed lines,* Fig. 3–3A) giving us a more complete analysis of the radial gradient.

Nearly all superficial cells are labeled in the occipital pole (A) and most are labeled in the frontal pole (B) while the deep layers in both poles (C and D) have unlabeled cells. The graphs in Fig. 3–5 summarize the radial neurogenetic gradient in the motor and visual areas: layer VII neurons (subplate) originate mainly between E14 and E16, layer VI neurons between E15 and E17, layer V neurons between E16 and E18, layer IV neurons between E17 and E19, and layers II–III neurons between E17 and E20. These differences are highly significant (all comparisons: $P < 0.0001$, sign test; F values ranged from 246.46 to 82.32, df = 1, $P < 0.0001$, repeated measures analysis of variance). Indeed, every comparison of the radial gradient between adjacent layers throughout the entire limbic cortex and neocortex is significant at the $P < 0.0001$ level.

Even though the majority of neurons are generated in 2–3 days, the data in Fig. 3–5 indicate that the total time span for neurogenesis in a particular cortical layer usually takes 4 or 5 days. That is in contrast to the thalamus, where 80–90% of the neurons in individual thalamic nuclei are often generated in a single day (Alt-

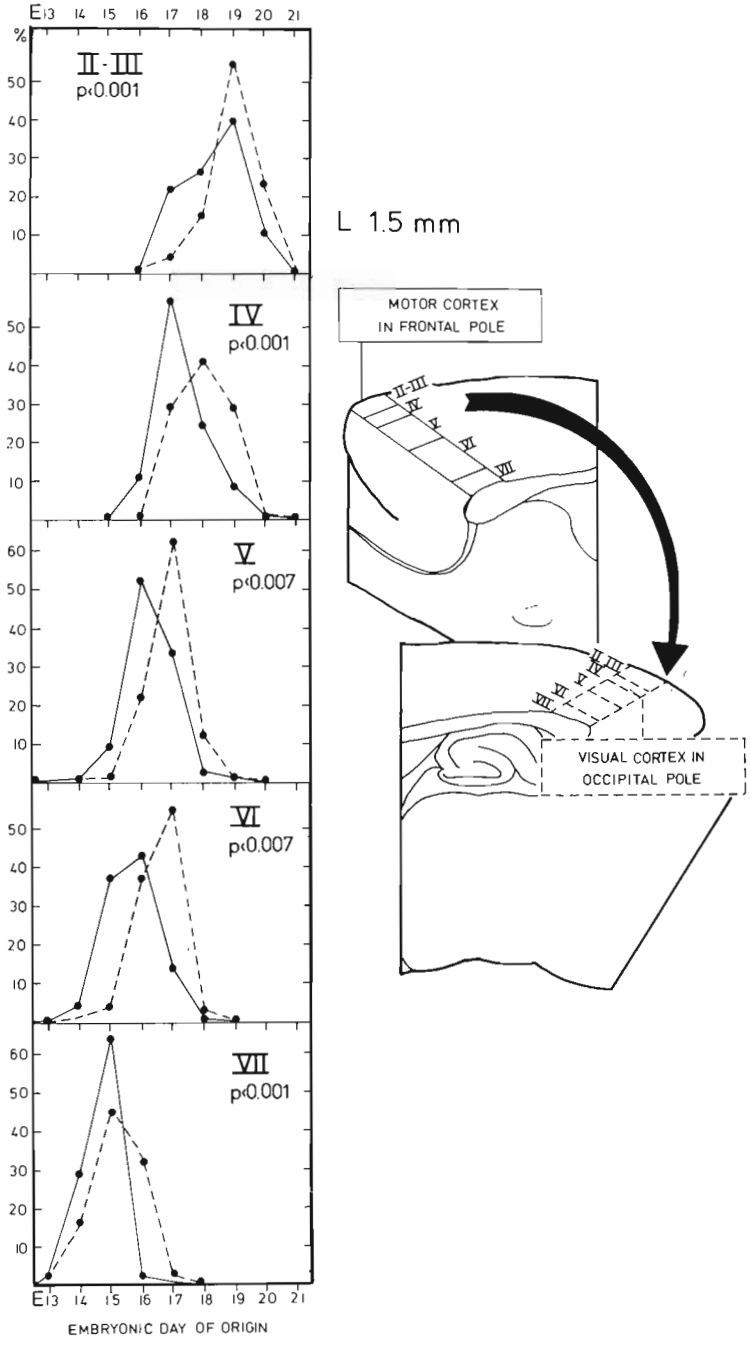

FIG. 3–5. A laminar analysis of daily cell production in the frontal and occipital neocortical poles (*solid and broken lines, respectively*). The section (broken into two parts in drawing) is 1.5 mm lateral to the midline, a level comparable to a longitudinal cut through the dorsomedial neocortex in coronal sections. The animals survived to P60 after being exposed to [^{3}H]thymidine on two consecutive embryonic days (E13 + E14, E14 + E15 . . . E21 + E22). Roman numerals in the drawings and in the graphs refer to the cortical layers. The radial neurogenetic gradient is expressed in the following pattern: the closer a layer is to the white matter (VII is deepest and closest, *bottom graph*), the earlier its neurons are produced (peak on E15); neurogenesis is latest in the layers closest to layer I (II–III, *top graph;* peak on E19). The longitudinal neurogenetic gradient (*arrow between frontal and occipital poles in drawing*) is expressed in the systematic later shift in the time of origin of neurons in the occipital pole when compared to the frontal pole. The alpha level in each graph refers to the significance of the longitudinal gradient.

man and Bayer, 1988a). Individual thalamic nuclei contain homogeneous neuronal populations (Ma et al., 1987). The protracted cortical neurogenesis may be related to heterogeneity of neuronal populations within a given cortical layer, distinguished by their dendritic and axonal arborizations using the Golgi technique (Parnavelas and McDonald, 1983; Peters and Jones, 1984; Valverde, 1986; Valverde and Facal-Valverde, 1986) and by their different neurochemical characteristics demonstrated with immunocytochemistry (Ribak, 1978; McDonald et al., 1982a, 1982b, 1982c; Wolff et al., 1984; Conti et al., 1987; Eadie et al., 1987; Escalpez et al., 1987; Meinecke and Peters, 1987; Dori et al., 1989; Satoh and Suzuki, 1990).

By combining [^{3}H]thymidine autoradiography and immunocytochemistry, it has been shown that both pyramidal cells and nonpyramidal cells containing gamma-amino butyric acid (GABA) have the inside-out neurogenetic gradient (Miller, 1985), as do the nonpyramidal neurons containing the peptides somatostatin (Cavanagh and Parnavelas, 1988) and vasoactive intestinal polypeptide (VIP) (Cavanagh and Parnavelas, 1989)). Perhaps the spatially overlapping neurochemical subsets of cells lengthens the total timespan of neuron production in each cortical layer. Using the pulse labeling with [^{3}H]thymidine, Miller (1985) compared times of neuron origin of GABA neurons with non-GABA cells and concluded that the two populations are generated concurrently. However, Fairen et al. (1986), also using pulse labeling with [^{3}H]thymidine, found evidence that GABA cells are slightly younger than neighboring non-GABA cells. Studies combining the more sensitive comprehensive labeling of [^{3}H]thymidine with neurotransmitter immunocytochemistry may reveal subtle differences in the birthdays of neuronal subpopulations in each layer.

Longitudinal Gradient

The photomicrographs in Fig. 3–4 illustrate that the majority of anterior layer VI cells (D) are unlabeled, indicating generation before E17, while many of the cells in posterior layers VI and V (C) are intensely labeled, indicating generation on or after E17. When that pattern is quantitatively analyzed (Fig. 3–5), it is seen that anterior cells originate significantly earlier than posterior cells in every layer ($P < 0.007$ to $P < 0.001$, sign test; all $P < 0.0001$, repeated measures analysis of variance). Anterior cells in layers VI, V, and IV–III (three middle graphs, Fig. 3–5) have a peak time of origin 1 day before posterior cells. Anterior and posterior cells in layer VII (*bottom graph,* Fig. 3–5)[3] and layers II–III (*top graph,* Fig. 3–5) share peak times of origin, but more anterior cells are generated before the peak, while more posterior cells are generated after the peak. Data not illustrated indicate that there is also a longitudinal neurogenetic gradient between the anterior (somatosensory area) and posterior (lateral visual area) parts of dorsolateral cortical wall in each layer ($P < 0.037$ to $P < 0.0001$, sign test) and between anterior (gustatory area) and posterior (auditory area) parts of the ventrolateral cortical wall ($P < 0.038$ to $P < 0.0001$, sign test).

3.2.2 The Transverse Neurogenetic Gradient

The transverse neurogenetic gradient was analyzed in animals exposed to [^{3}H]thymidine on 2 consecutive days during embryonic life and killed on P5.[4] The five frontal levels chosen for analysis were equally spaced between the genu (anterior section, L1) and the splenium (posterior section, L5) of the corpus callosum (Fig. 3–6). Frontal sections overlying the corpus callosum were selected because these are cut perpendicular to the longitudinal axis of the cerebral cortex, and none of the cortical layers are cut tangentially. Cells were counted separately in layer VI, layer V, and the

[3] These data seem to contradict the fact that we did not find a longitudinal gradient in the subplate (layer VII) in animals that were killed on P5 (Fig. 3–2A). Those data were collected in coronal sections, where the distance between the most anterior level and the most posterior level was much shorter. In sagittal sections, the distance between measurable anterior and posterior sites is greater. There probably is a longitudinal gradient in the subplate on P5, but it is not observable in coronal sections. Note also that there is considerable neurogenesis on E16 in posterior layer VII. The P5 data indicate that most subplate neurogenesis is finished on E15. That is consistent with a more anterior sampling of the population.

[4] Two reasons prompted our choice of animals surviving to P5 rather than to P60. (1) The P5 survival brains were sectioned coronally exactly perpendicular to the longitudinal axis of the cerebral hemispheres so that ventrolateral and dorsomedial cortical areas in the same section are the same distance from the frontal pole. Since our P60 survival brains were cut in the coronal plane according to the angle in the Pellegrino et al. atlas (1979) (dorsal parts of the section are more anterior than ventral parts), the comparisons of neurogenesis between distant cortical areas in the same section (e.g., ventrolateral auditory cortex and dorsomedial visual cortex) were compromised by variations in the distance from the frontal pole. (2) At P5 the cells are smaller and the packing density within a 6 μm section is higher. That makes cytoarchitectonic differences between cortical areas obvious even in low magnification sections. For example the sections shown in Fig. 3–6 indicate a clear increase in cell density in layer IV of the somatosensory area (PAR) compared to motor (FR) and gustatory (GU) areas.

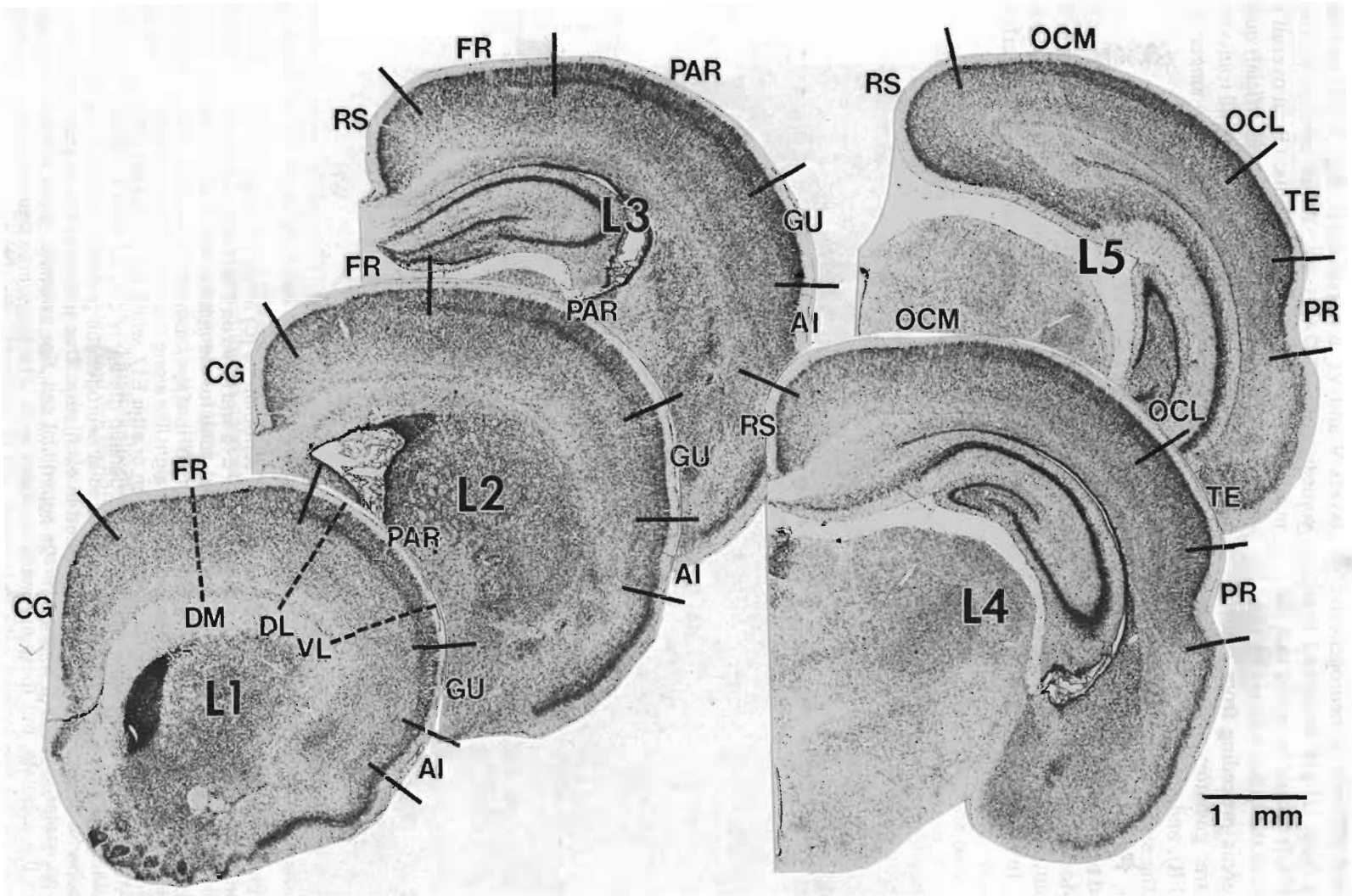

FIG. 3–6. The five coronal levels of the P5 rat brain chosen for the analysis of [^{3}H]thymidine autoradiograms. Level one (L1) is most anterior, L5 is most posterior. In each photograph, medial is to the left, dorsal is at the top, lateral is at the right, and ventral is at the bottom (6 μm paraffin sections, hematoxylin stain). The letters separated by straight lines along the edges of each section refer to the cortical areas as described by Zilles (1985). Note that cytoarchitectonic differences can be seen between areas. For example, a dense band of granule cells is found in the superficial parts of the somatosensory cortex (PAR in L1, L2, and L3) and a slightly less dense band is seen in the center of the visual cortex (between OCL and OCM in L4 and L5). The *vertical dashed lines* in L1 indicate the strips of cortex shown at higher magnification in Fig. 3–7.

combined layers II–IV in ventrolateral-to-dorsomedial cortical areas in each of the five sections (Fig. 3–7 shows layer boundaries in L1). Essentially, levels 1–5 showed the same global patterns of neurogenesis; consequently, only data from L1 are described here (Figs. 3–8, 3–9, and 3–10). It should be noted that the agranular insular area lies in the rhinal sulcus (AI), and the remaining cortical areas proceding from ventrolateral to dorsomedial are: gustatory (GU), somatosensory (PAR), motor (FR), and cingulate (CG). High magnification views of the strips of cortex at L1 (*indicated by dashed lines,* Fig. 3–6) are shown in Fig. 3–7 in an animal exposed to [^{3}H]thymidine on E17 and E18 and killed on P5. Most of the cells in layers IV–II are labeled throughout, indicating that these cells are generated after E17. In layers V and VI however, there are regional differences: most of the cells in layers V and VI are unlabeled ventrolaterally (Fig. 3–7C), while progressively more labeled cells are seen within layers V and VI in dorsolateral (Fig. 3–7B) and dorsomedial (Fig. 3–7A) areas. These labeling patterns indicate two neurogenetic gradients: (1) an overall radial gradient (older deep to younger superficial) quantified in the sagittal series, and (2) an overall transverse neurogenetic gradient (older lateral to younger medial).

Neurogenesis of Layer VI

Cells in layer VI lie adjacent to the white matter in the medial limbic (CG) and lateral limbic (AI) regions but are separated from the white matter by the underlying

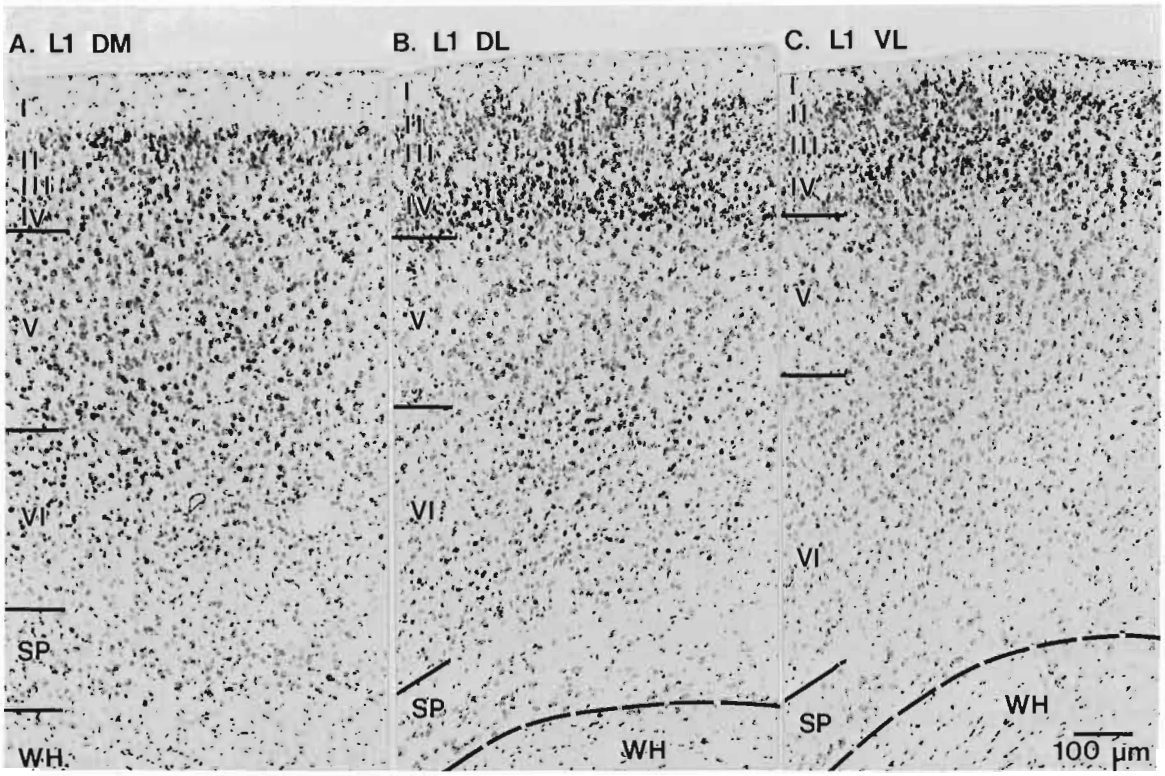

FIG. 3–7. Strips of dorsomedial **(A),** dorsolateral **(B),** and ventrolateral **(C)** areas of cortex in the L1 level from an animal exposed to [^{3}H]thymidine on E17 + E18 and killed on P5. Roman numerals separated by lines indicate how the cortical layers were divided for the cell counts. The *dashed lines* in **B** and **C** follow the curvature of the white matter (WH) at the base of the subplate (SP, also called layer VII). In **A,** the white matter extends in a straight line along the base of the subplate. The labeled cells (solid black to dark nuclei) originated on or after E17, while the unlabeled cells (pale or clear nuclei) originated before E17. Two neurogenetic gradients can be seen by comparing the labeling patterns in the photographs. The radial neurogenetic gradient is shown by having more superficial cells than deep cells labeled in each strip. That indicates the average deep cell has an earlier birthday than the average superficial cell. For example, there are no labeled cells in the subplate, but there are many in layers II–IV. The transverse neurogenetic gradient is shown by the few labeled cells in layers V and VI ventrolaterally **(C),** more labeled cells in those layers dorsolaterally **(B),** and even more labeled cells in those layers dorsomedially **(A).**

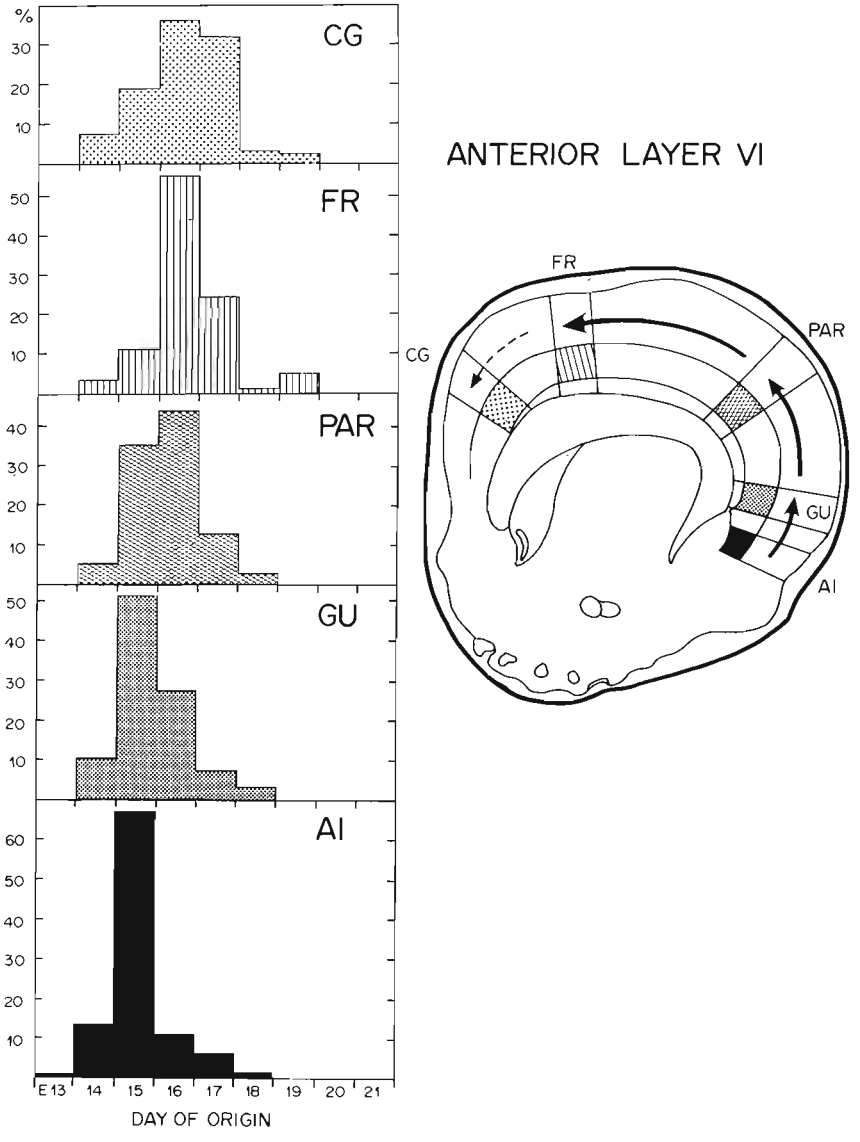

FIG. 3–8. The proportions of layer VI neurons generated at daily intervals in agranular insular (AI), gustatory (GU), somatosensory (PAR), motor (FR), and cingulate (CG) areas in the anterior neocortex of P5 animals that received two consecutive daily exposures to [^{3}H]thymidine during embryonic life. There are significant ventrolateral (older) to dorsomedial (younger) neurogenetic gradients between areas in the lateral and dorsal walls (*solid arrows in drawing*); neurogenesis peaks on E15 in AI (*bottom graph*), on E16 in FR (*second graph from top*). However, the ventromedial CG (*top graph*) has a higher proportion of cells born on E14 and E15 than the immediately lateral motor area (FR, *second graph from top*), weakening the gradient (*broken arrow in drawing*). That indicates that the medial limbic cortex has different neurogenetic patterns than the neocortex.

subplate in the gustatory (GU), somatosensory (PAR), and motor (FR) areas. In areas where the subplate is separate, subplate cells were not included in the quantification of layer VI. Throughout the rat cerebral cortex, layer VI is a prominent component, taking up approximately one-third of the total depth of the gray matter.

The oldest layer VI neurons are located in the agranular insular area in the rhinal sulcus, and cell birthdays are progressively delayed as one proceeds in the dorsomedial direction. Neurogenesis peaks on E15 in ventrolateral areas (*two bottom graphs,* Fig. 3–8), occurs about equally on E15 and E16 in the dorsolateral parietal area (somatosensory), and shifts to E16 and E17 in the dorsomedial areas (FR and CG, *two top graphs,* Fig. 3–8). The transverse gradient is strong between adjacent areas in the lateral and dorsal walls (*solid arrows in drawing,* Fig. 3–8; $P < 0.003$ to $P < 0.0001$, sign test; $P > 0.0001$, repeated measures analysis of variance). Comparisons between distant areas highlight the magnitude of the transverse neurogenetic gradient even more dramatically; about 81% of the cells originate on or before E15 in the agranular insular area, while only 15% of these cells are born in the motor area during the same time period (compare graphs AI and FR, Fig. 3–8). All levels analyzed gave the same result. In the posterior section, for example, approximately 86% of the layer VI cells originate on or before

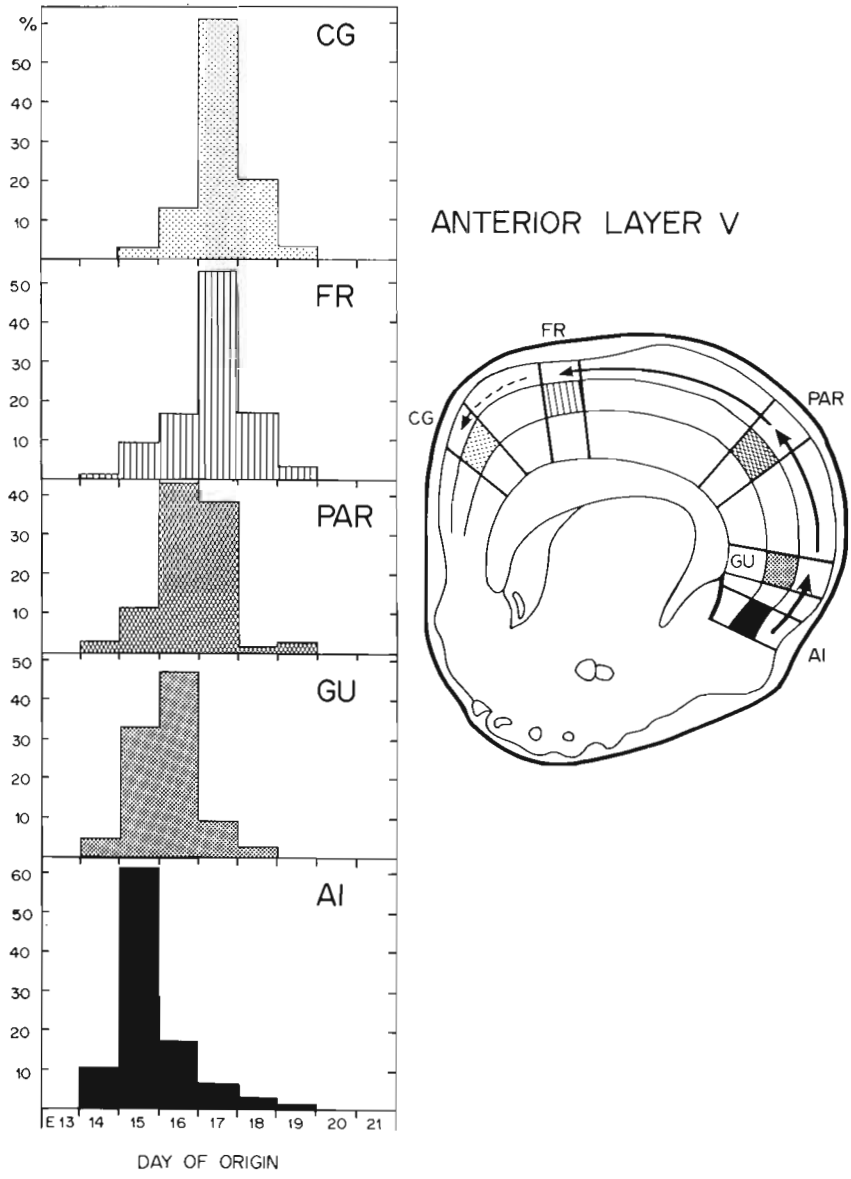

FIG. 3–9. The proportions of layer V neurons generated at daily intervals in the anterior neocortex in P5 animals after two consecutive daily exposures to [^{3}H]thymidine during embryonic life. Abbreviations as in Fig. 3–8. Throughout layer V, significant ventrolateral (older) to dorsomedial (younger) neurogenetic gradients exist between areas in the lateral and dorsal walls (*solid arrows in drawing*); for example, there is a prominent neurogenetic peak on E15 in AI (*bottom graph*) and on E17 in FR (*second graph from top*). However, the neurogenetic pattern in the CG (*top graph*) is nearly the same as that in FR, indicating again that the medial limbic cortex does not fully follow the transverse neocortical neurogenetic gradient (*broken arrow in drawing*).

E15 in the perirhinal area in the rhinal sulcus but only 8% originate during this time in the dorsomedial visual area.

The medial limbic area shows a less-pronounced ventrolateral (older) to dorsomedial (younger) neurogenetic gradient. The sign test indicates a relatively low probability ($P < 0.044$) that the motor cortex in the frontal region (FR) is older than the cingulate area (CG) in the medial wall (*broken arrow in drawing*, Fig. 3–8), while the repeated measures analysis of variance indicates no significant differences between the two areas. That is due to the circumstance that the cingulate area (*top graph*, Fig. 3–8) contains a high proportion of older cells in layer VI: 26% are generated on E14 and E15 in the cingulate area compared with 15% in the adjacent motor cortex. The motor area "catches up" on E16 and has fewer neurons produced on and after E17 than the cingulate area. The same situation is found in the posterior section between the medial visual and agranular retrosplenial areas, where patterns of neurogenesis between the two areas are not significantly different. We will later present evidence (Chapter 5) that the unusually older cells in layer VI originate at the same time as those in the subplate and will propose the hypothesis that the subplate "infiltrates" layer VI in the limbic areas.

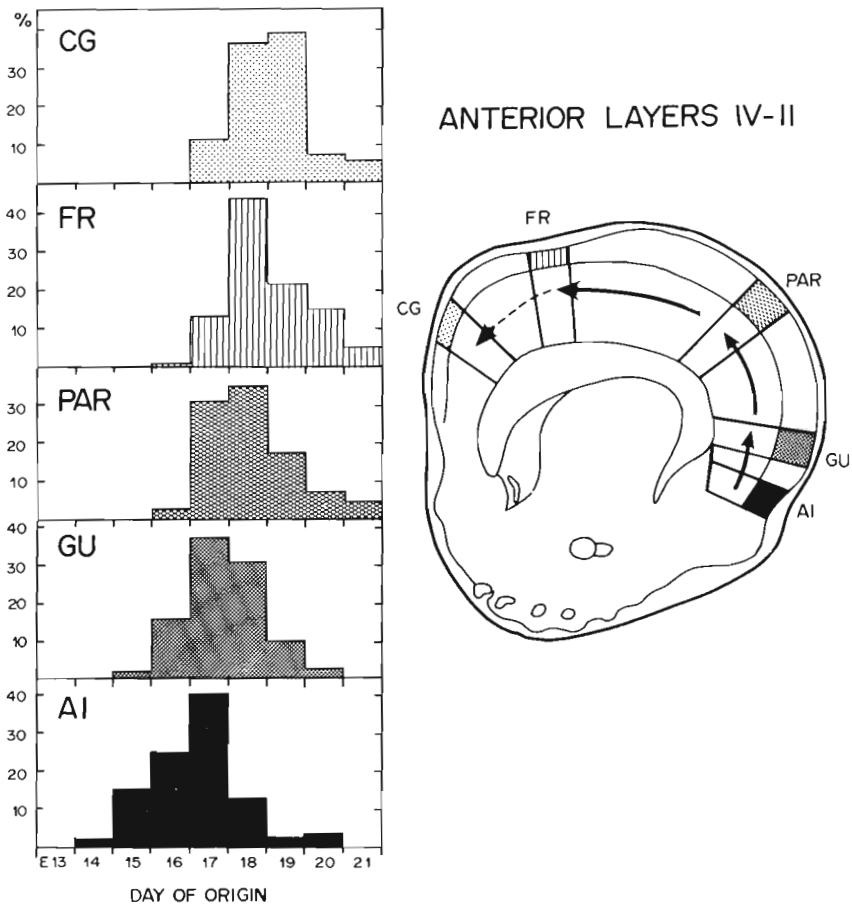

FIG. 3–10. The proportions of neurons in combined layers IV–II generated at daily intervals in the anterior neocortex in P5 animals after two consecutive daily exposures to [^{3}H]thymidine during embryonic life. Abbreviations as in Fig. 3–8. Throughout layers IV, III, and II, significant ventrolateral (older) to dorsomedial (younger) neurogenetic gradients are found between areas in the lateral and dorsal walls (*solid arrows in drawing*); for example, peak neurogenesis occurs on E17 in AI (*bottom graph*) and on E18 in FR (*second graph from top*). However, there is a break in the pattern between FR and CG (*top graph*) since more cells are born on E20 and E21 in FR than in CG. This pattern in the medial limbic cortex reverses the transverse neurogenetic gradient seen throughout the lateral and dorsal neocortex.

Neurogenesis of Layer V

Layer V, like layer VI, is a prominent part of the rat neocortex, taking up approximately the middle third of the total cortical depth. Layer V neurons were counted in the same strips used for layer VI neurons. The oldest neurons are located in the agranular insular area and progressively younger neurons are located dorsally and medially ($P < 0.0001$, sign test and repeated measures analysis of variance). Neurogenesis peaks in the ventrolateral wall on E15 (AI, *bottom graph,* Fig. 3–9), on E17 in the dorsomedial motor (FR) and cingulate (CG) areas (*two top graphs,* Fig. 3–9). The differences between distant areas are quite large: nearly 90% of the layer V cells originate on or before E16 in the agranular insular area, but only 27% of the cells in the motor area are generated during the same time (compare graphs AI and FR, Fig. 3–9). The posterior section shows the same dramatic differences: 91% of the layer V cells originate on or before E16 in the perirhinal cortex, but only 19% are generated in the medial visual cortex during that time. In contrast, neurogenesis in the motor area (FR) is only slightly earlier ($P < 0.002$, sign test) than in the cingulate area (CG). The neurogenetic gradient is weakened because more layer V cells are generated on E15 in the cingulate area (61%) than in the motor area (53%), a trend contrary to the expected direction. That is another indication that the limbic cortex has a different developmental pattern than the neocortex. In the posterior section, the transverse gradient also weakens at the junction between the medial visual area and the agranular retrosplenial area.

Neurogenesis of Layers II, III, and IV

The rat neocortex does not have prominent superficial layers. In P5 brains, these three layers together constitute only the upper third of cortical depth. Consequently, cells were counted in the combined superficial layers (IV–II, see Fig. 3–7) in the same areas used for counting layers VI and V. By P60, cell maturation and differentiation afford a better separation of the superficial layers, especially in the sensory areas, and these are separately quantified in the chapters dealing with the intrinsic neurogenetic gradients in each cortical area (Chapters 11–15).

The oldest layer II–IV neurons are located in the agranular insular area, progressively younger neurons are situated dorsally and medially ($P < 0.0001$, sign test and repeated measures analysis of variance). Neurogenesis is prominent in the ventrolateral wall between E15 and E18 (AI and GU, *two bottom graphs,* Fig. 3–10), on and after E18 in the dorsomedial motor (FR) and cingulate (CG) areas (*two top graphs,* Fig. 3–10). The differences between distant areas are particularly pronounced: nearly 82% of the layer IV–II neurons originate on or before E17 in the agranular insular area but only 14% of the neurons in the motor area originate during the same time (compare graphs AI and FR, Fig. 3–10). The posterior section shows similar dramatic differences: 78% of the superficial cells in the perirhinal area are generated on or before E17 while only 11.5% of these cells are generated in the medial visual cortex during that time. However, neurogenesis in the motor (FR) and cingulate (CG) areas have different patterns in layers IV–II neurogenesis, especially on and after E19. The transverse neurogenetic gradient is weakened (sign test, $P < 0.042$; repeated measures analysis of variance, $P < 0.0171$) because more cells are generated on E19, fewer on E20–E21 in the cingulate area than in the motor area. The same pattern is also found between the medial visual area and the agranular retrosplenial area in the most posterior section. The superficial layers throughout the medial limbic cortex have a ventral (older) to dorsal (younger) neurogenetic gradient (Bayer, 1990b; Richter and Kranz, 1979b, 1980; Chapter 15) that sets these areas off from the developmental patterns seen in the neocortex.

3.3 LINKING THE TRANSVERSE AND LONGITUDINAL GRADIENTS TO AFFERENT FIBER GROWTH INTO THE DEVELOPING NEOCORTEX

We postulate that the neurogenetic gradients that exist between different cortical areas are important in the establishment of cortical circuitry. The evidence for this comes from the correlation between these gradients and the order in which different cortical areas become innervated during development.

The major afferents to the cerebral cortex are the nonspecific inputs from the basal telencephalon, the locus coeruleus, the raphe nuclei, the substantia nigra, and the intralaminar thalamic nuclei. Specific afferents come from the relay nuclei in the thalamus. One of the striking features of neocortical development is that afferent fibers arrive early, typically before most neurons in the superficial layers have been generated and concurrently with the peaks of neurogenesis in layers V and VI. Furthermore, the afferent fibers, in spite of coming from various regions of the neuraxis, penetrate the developing cortex in the same areas and follow similar lines of growth in the neocortical primordium. The diagrams in Figure 3–11 illustrate that the global longitudinal and transverse neurogenetic gradients (A) can be related to the pattern of fiber ingrowth from each of the major afferent systems (B–D). We will also show that the noradrenergic and serotonergic innervations (Fig. 3–11D) have a striking correlation with the divergent neurogenetic gradients between the limbic and neocortical parts of the cerebrum.

3.3.1 Afferents from the Basal Telencephalon

The neurons of the magnocellular basal telencephalic nuclei are among the oldest in the forebrain, originating in a prominent caudal (older) to rostral (younger) neurogenetic gradient (Bayer, 1985). The neurons that project to the cortex are cholinergic (Semba et al., 1989), and their axons account for approximately 70% of the total acetylcholine present in the adult cortex (Johnston et al., 1981; Hartgraves et al., 1982) while a sparse population of intrinsic bipolar cholinergic neurons (Levey et al., 1984) accounts for the remaining 30%. In the adult rat, magnocellular basal telencephalic afferents project not only to limbic cortical areas (Divac, 1975, 1979; Kelly and Moore, 1978; Lamour et al., 1984; Wolff et al., 1984) but also project topographically to the neocortex (Bigl et al., 1982; Lamour et al., 1982; McKinney et al., 1983; Price and Stern, 1983; Saper, 1984; Carey and Rieck, 1987). Neurogenetic gradients (Bayer, 1985) and cortical projections (Saper, 1984) of the magnocellular basal telencephalic nuclei can be related to the transverse gradient of cortical neurogenesis in the following way: older neurons in caudal and ventral parts of the basal telencephalon project to older lateral cortical areas, while younger neurons in the rostral and dorsal basal telencephalon project to younger dorsomedial cortical areas.

There are divergent data concerning the development of basal telencephalic afferents to the cortex. Using immunocytochemical analysis of choline acetyltransferase in the fetal and the postnatal periods, axons from magnocellular basal telencephalic neurons do not become immunoreactive until the second postnatal week (Parnavelas et al., 1988; Dori and Parnavelas, 1989), even though magnocellular basal telencephalic axons can be labeled by injections of horseradish peroxidase into cortex at birth (Dinopoulos et al., 1989). That suggests that basal forebrain afferents are among the latest to mature in the neocortex. Exactly the opposite suggestion comes from studying

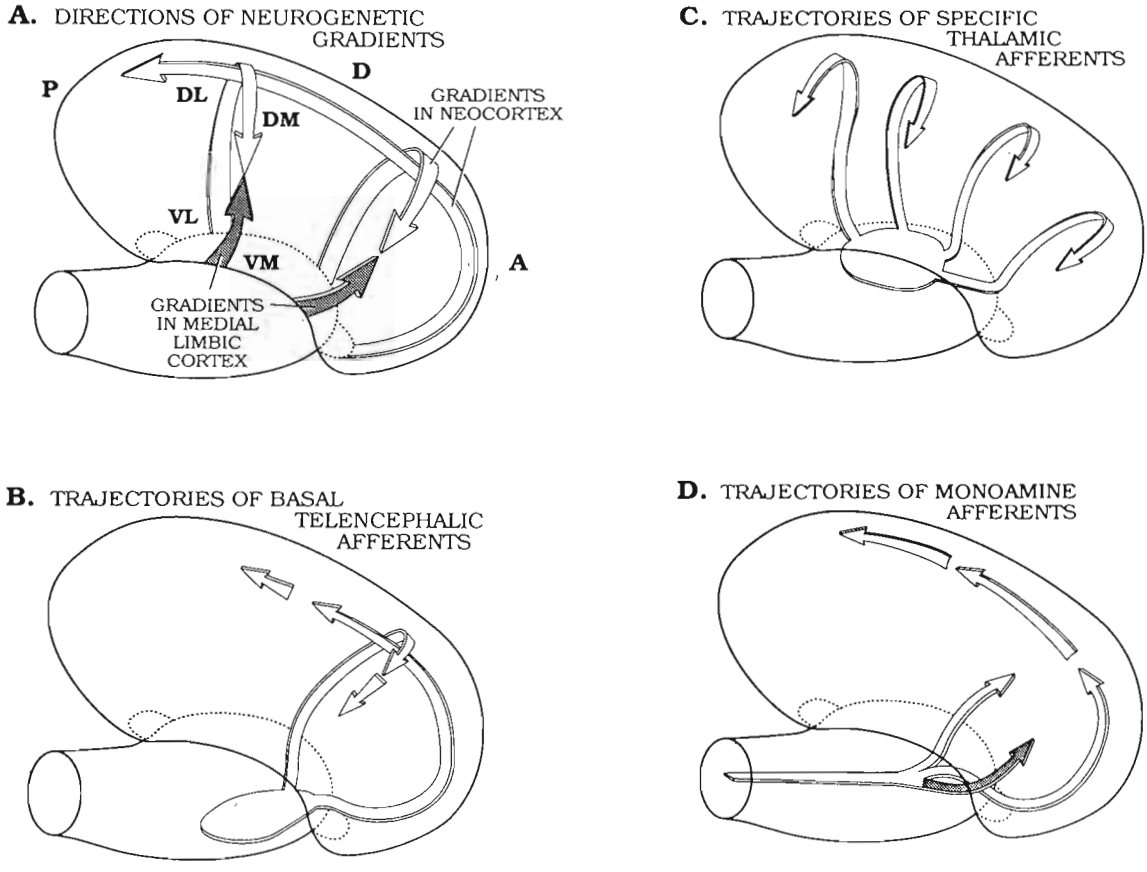

FIG. 3–11. **A** to **D** are diagrams showing the directions of neurogenetic gradients **(A)** and the trajectories of growing axons from the basal telencephalon **(B),** from specific sensory relay nuclei in the thalamus **(C),** and from brainstem monoamine centers **(D).** In **A,** directional labels are shown that apply to all the diagrams: A, anterior; D, dorsal; DL, dorsolateral; DM, dorsomedial; P, posterior; VL, ventrolateral; VM, ventromedial. The *clear arrows* in **A** indicate two neocortical neurogenetic gradients and point to areas that contain younger neurons dorsomedially (the transverse gradient) and posteriorly (the longitudinal gradient). The *stippled arrows* in **A** indicate the ventral (older) to dorsal (younger) neurogenetic gradient in the medial limbic cortex that reverses the direction of the transverse neocortical gradient. In **B, C,** and **D,** *arrows* indicate the trajectories of axonal growth. The axons first enter the developing cortex in the ventrolateral (*clear arrows* in **B, C,** and **D**), anterior (*clear arrows* in **B** and **D**), and ventromedial (*stippled arrow* in **D**) walls, where in all cases the older neurons are located. The axons grow dorsomedially in neocortex (*clear arrows* in **B, C,** and **D**), posteriorly in neocortex (*clear arrows* in **B** and **D**), and dorsally in medial limbic cortex (*stippled arrow* in **D**), toward the sites where the younger neurons are located.

the basal forebrain afferents with another marker: receptors for nerve growth factor (NGF).[5] NGF receptor-rich fibers penetrate the primordial plexiform layer of the neocortex as early as E15, and Koh and Loy (1989) suggest that these axons are from the magnocellular basal telencephalic neurons. Given the early birthdays of those neurons (Bayer, 1985) and their proximity to the cortex, the basal telencephalon may indeed be among the first extrinsic afferents to reach neocortex. But their delayed expression of choline aceytltransferase is intriguing. Oddly enough, the specific thalamocortical sensory relay afferents transiently display cholinergic activity during the first postnatal week (Kristt, 1985; Robertson, 1987). Possibly, the permanent expression of cholinergic activity by the basal forebrain afferents is timed to occur after the specific thalamocortical projections are no longer cholinergic.

[5] The survival of neurons in the basal telencephalic magnocellular nuclei is linked to the early presence of NGF (Barde, 1989). Very soon after their generation (as early as E13), the somatic membranes of caudal neurons in the globus pallidus contain receptors for NGF, while rostral neurons show the NGF receptor slightly later (Koh and Loy, 1989), following the caudal to rostral neurogenetic gradient of magnocellular basal telencephalic neurons (Bayer, 1985).

The pattern of growth (Koh and Loy, 1989) of NGF receptor-rich fibers is remarkable when compared to the pattern of neocortical neurogenesis. The fibers enter through the anterolateral edges of the cortex, then grow dorsally, medially, and posteriorly to cover the entire cortex (Fig. 3–11B) just along the lines of the global transverse and longitudinal neurogenetic gradients. That means that neurogenetically older areas of cortex would be the first to receive afferent fibers, while neurogenetically younger areas of cortex would be the last to receive afferent fibers.

3.3.2 Afferents from the Thalamus

Thalamic afferents arrive on E16/E17 in the lateral neocortex (Lund and Mustari, 1977; Wise and Jones, 1978; Catalano and Killackey, 1990; Molnár and Blakemore, 1990), and line up in the subplate under the earlier arriving afferents from the basal forebrain (Koh and Loy, 1989). Until very recently these afferents were thought to remain in the subplate during the embryonic stage and grow into the cortical plate postnatally (Rakic, 1977; Rickmann et al., 1977; Crandall and Caviness, 1984; Shatz et al., 1988). However, recent evidence in rats using crystals of the carbocyanine dye, DiI, shows that the thalamocortical afferents invade the deep part of the cortical plate on E19/E20 (Catalano and Killackey, 1990; Erzurumlu and Jhaveri, 1990; Reinoso and O'Leary, 1990). While more detailed discussions of the interconnections between the thalamus and cortex will be dealt with in the chapters on intrinsic neurogenetic gradients (Chapters 11–15), it is appropriate to mention here that all specific thalamocortical afferents that enter the neocortex via the internal capsule follow a pronounced lateral-to-medial trajectory of growth (Caviness and Frost, 1980; Frost and Caviness, 1980; Fig. 3–11C), correlating with the prominent transverse neurogenetic gradient seen in Figs. 3–8, 3–9, and 3–10.

3.3.3 Afferents from the Substantia Nigra and Ventral Tegmental Area

The substantia nigra and ventral tegmental area, catecholamine cell groups A9 and A10 of Dahlstrom and Fuxe (1964), are the primary sources of dopamine axons in the neocortex (Leonard, 1969; Carter and Fibiger, 1977; Divac et al., 1978; Fallon and Moore, 1978; Fallon et al., 1978; Beckstead et al., 1979; Gerfen and Clavier, 1979; Greatrex and Phillipson, 1982; Loughlin and Fallon, 1984; Kalsbeek et al., 1987, 1989). In adults, dopamine fibers are limited to layer VIb (VII) in most neocortical areas but are distributed throughout all layers in the medial and lateral limbic areas (Descarries et al., 1987). The D1 dopamine receptor has the same distribution pattern (Richfield et al., 1989). The visual cortex is exceptional because, along with limbic cortex, there is evidence for a specific dopamine innervation from the ventral tegmental area to nearly all layers of anterior medial secondary area (OC2M; Phillipson et al., 1987).

From a developmental perspective, dopamine and other monoamine neurons are remarkable because the cell bodies and axons express neurotransmitter very soon after neurogenesis (Golden, 1972; Olson and Sieger, 1972; Olson et al., 1972). That has facilitated the study of the sources and migratory pathways of dopamine neurons during the embryonic period. Long-survival [^{3}H]thymidine autoradiographic studies indicate that the substantia nigra (SN) neurons originate between E13–E15 (Altman and Bayer, 1981), while short- and sequential-survival radiographic studies locate the neuroepithelial source of SN neurons in the ventroposterior diencephalon (Golden, 1972; Specht et al., 1981a, 1981b; Voorn et al., 1988). Using immunocytochemical staining for tyrosine hydroxylase (Specht et al., 1981a, 1981b) or dopamine (Voorn et al., 1988), the posterior migration of SN neurons has been followed from their germinal source to their settling site in the floor of the brain at the crest of the mesencephalic flexure. Dopamine fibers reach the anterolateral neocortex as early as E15 (Kalsbeek et al., 1988). Subsequent growth proceeds in the dorsomedial direction (frontal cortex is reached by E16–E17) and in the posterior direction (Kalsbeek et al., 1988; Parnavelas et al., 1988) along the same lines as the transverse and longitudinal neurogenetic gradients. Older areas of cortex are reached first by the incoming fibers, younger areas last. The pattern of growth is similar to that taken by basal telencephalic axons. Like most other cortical afferents, dopamine axons remain in the subplate and layer I before birth, and only penetrate the cortical plate at birth in limbic (Kalsbeek et al., 1988) and visual (Parnavelas et al., 1988) areas.

3.3.4 Afferents from the Locus Coeruleus

The locus coeruleus (LC), catecholamine group A6 of Dahlstrom and Fuxe (1964), is the source of noradrenergic axons that innervate the cerebral cortex (Swanson and Hartman, 1975; Jones and Moore, 1977; Divac et al., 1978; Fallon et al., 1978; Divac, 1979; Room et al., 1981; Saper, 1982; reviewed in Lindvall and Björklund, 1983; Finch et al., 1984b). Locus coeruleus axons enter the basal forebrain in the medial forebrain bundle, then one group joins the medial septal/diagonal band complex to curve dorsally and enter the cortex over the genu of the corpus callosum, while another group courses through the ventral pallidum between the striatum and the olfactory tubercle and en-

ters the ventrolateral cortex (Swanson and Hartman, 1975). Noradrenergic innervation of neocortex has a fairly consistent density throughout all cortical areas, with little regional specification (Parnavelas et al., 1988). Locus coeruleus neurons originate very early (Altman and Bayer, 1980c), and their cell bodies express noradrenalin soon after their generation (Olson and Sieger, 1972). Although there is little spatial segregation between locus coeruleus neurons that project to different brain structures (Lindvall and Björklund, 1983), there appears to be a chronoarchitectonic subdivision in the nucleus. By combining [^{3}H]thymidine autoradiography with retrograde transport of horseradish peroxidase, Steindler and Trosko (1989) showed that only the oldest locus coeruleus neurons, presumably those with the longest axons, project to the cerebral cortex.

In spite of the great distance between the locus coeruleus and the cerebral cortex, noradrenergic axons reach the ventral edge of the entire cortical primordium as early as E17 (Levitt and Moore, 1979; Verney et al., 1984). It is remarkable that the medial frontal cortex, lateral frontal cortex, and cortex at the base of the frontal pole are innervated simultaneously (Fig. 3–11D; Levitt and Moore, 1979; Molliver, 1982; Verney et al., 1984). The subsequent growth of noradrenergic axons is primarily directed posteriorly. That trajectory of growth follows the longitudinal (anterior to posterior) neurogenetic gradient found throughout the neocortex, except that the early innervation of the medial wall violates the transverse neurogenetic gradient (Verney et al., 1984).

The neurogenetic data presented in Figs. 3–8, 3–9, and 3–10 indicate that the cortical medial wall contains older cells in layer VI, and cells that are older than expected in layers V and IV–II. Chapter 15 will show that the infralimbic cortex in the ventral medial wall is one of the oldest cortical areas, and superficial cells (IV–II) have a ventral (older) to dorsal (younger) neurogenetic gradient throughout the entire extent of the medial limbic cortex which is the reverse of the transverse gradient seen throughout the rest of the cortex (Fig. 3–11A; Bayer, 1990b). Thus, the ingrowth of noradrenergic axons does correlate with patterns of neurogenesis so that older cortical areas are the first to be innervated by noradrenergic axons, and younger cortical areas receive noradrenergic axons later. Another remarkable feature of noradrenergic axons is their cortical innervation pattern. Like other cortical afferents, they first arborize above and below the cortical plate in layer I and the subplate. But unlike most cortical afferents, they penetrate the cortical plate during the embryonic period (Levitt and Moore, 1979; Molliver, 1982; Verney et al., 1984; Parnavelas et al., 1988). During the first postnatal week, approximately 20–30% of the synapses in the cortical plate contain monoamines and are most likely to originate from the locus coeruleus projection (Molliver and Kristt, 1975; Kristt, 1985). As the noradrenergic fibers are maturing, the axons make characteristic loops through layers II–IV that course in a sinusoid fashion from medial to lateral (Papadopoulos et al., 1989); close to 90% of these terminals will form conventional synapses, most frequently on dendritic spines.

3.3.5 Afferents from the Raphe Nuclei

The raphe nuclei are the sources of serotonin input to the cerebral cortex (Conrad et al., 1974; Divac, 1979; Gerfen and Clavier, 1979; Saper, 1982; Finch et al., 1984b; Kalsbeek et al., 1987, 1989; Condes-Lara et al., 1989). The dorsal raphe nucleus projects to lateral limbic cortex (Azimitia and Segal, 1978; Deacon et al., 1983), while the median raphe nucleus projects to the medial limbic cortex and adjoining medial cortical areas (Azimitia and Segal, 1978). Serotonin uptake in these areas is highest in layer I, with an additional peak in the deeper layers (Audet et al., 1989), while the serotonin S2 receptor is most prominent in layers V–VI (Altar et al., 1985).

[^{3}H]thymidine dating studies indicate that raphe neurons originate mainly on days E12 and E13 (Altman and Bayer, 1980a, 1980b) and are among the first monoamine neurons to show detectable fluorescence with the Falck-Hillarp method (Olson and Sieger, 1972). Serotonin afferents closely follow the pattern of the ingrowing noradrenergic fibers from the locus coeruleus and grow into the cortex along with dopamine fibers from the ventral tegmental area and the substantia nigra (Kalsbeek et al., 1987, 1989). A few serotonergic axons can be seen in the medial, lateral, and anterior edges of the neocortical primordium on E17, but the majority of the fibers do not arrive until E19 (Lidov and Molliver, 1982; Molliver, 1982; Parnavelas et al., 1988). For the next 3–4 days, the axons grow posteriorly in layer I and the subplate (Lidov and Molliver, 1982; Molliver, 1982; Parnavelas et al., 1988). That trajectory of growth correlates with the global longitudinal gradient found throughout the neocortex. The early innervation of medial cortex (Fig. 3–11D) correlates with the earlier neurogenesis of the medial limbic cortical areas when compared to dorsomedial neocortex (Fig. 3–11A). Again, the overall principle is obeyed: earlier generated cortical areas are the first to be contacted by serotonergic afferents, younger areas are contacted later. At birth, the axons are still dense in layer I and the subplate and penetrate the cortical plate between P5 and P7, with the adult pattern of innervation being reached by P12 (Fujimiya et al., 1986).