

CHAPTER 6

Development of the Cortical Plate

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The first neurogenetic gradient was discovered by Angevine and Sidman (1961) when they used [³H]thymidine autoradiography to show that deep cells in the cortical plate (layer VI) originate earlier than its superficial cells (layer II). In Chapters 3 and 11–15, we show that the inside out or radial neurogenetic gradient is universally present throughout the neocortex and limbic cortex. In this chapter, we focus on the morphogenetic expression of that neurogenetic gradient during development of the cortical plate. First, we present some observations on dendritic development, based on the Golgi technique, that the pattern of differentiation from the base to the surface of the cortical gray is linked to initial contact with layer I by all newly arriving neurons, confirming the studies of many previous observers (Retzius, 1893; Cajal, 1911; Stensaas 1967c; Stensaas and Stensaas, 1968; Marin-Padilla, 1971, 1983, 1988; Berry, 1982; Marin-Padilla and Marin-Padilla, 1982). Second, we present in more detail [³H]thymidine autoradiographic observations to show how cells accumulate in the cortical plate and discuss those observations by offering the hypothesis that the cortical plate contains two distinct tiers, a lower one composed of layers VI and V, and an upper one composed of layers IV–II.

6.1 THE RADIAL MORPHOGENETIC GRADIENT IN THE CORTICAL PLATE: A BRIEF GOLGI ANALYSIS

Embryos from Wistar timed pregnant rats were removed between 10 A.M. and 12 noon on days E17 to E22, and the heads were placed for 25 days in the Golgi-Cox impregnation solution, as described by

Ramon-Moliner (1970). The brains of some P1 rats were also impregnated using the same method. All material was embedded in 12% celloidin and sectioned at 100 μ m on a sliding microtome. Most brains were cut in the coronal plane. Impregnated cells were visualized with the post-alkalinization method described by Ramon-Moliner (1970). Good material was obtained for days E17, E19, E21, E22, and P1. These were analyzed qualitatively in a series of photographic montages and camera lucida drawings (Figs. 6–1 and 6–2).

In our Golgi material, translocating cells were rarely seen in the intermediate zone, but cortical plate neurons, predominantly pyramidal cells, were impregnated in large numbers. The majority of pyramidal cells on day E17 (Fig. 6–1A–C) had a single, short apical dendrite growing into the outer half of layer I. On successive days, the same pattern was repeated as new pyramidal cells arrived and settled above the earlier ones in the cortical plate. In all cases, well-impregnated pyramidal cells completely contained within the section had an apical dendrite with a terminal tuft in the superficial part of layer I. By E21 (Fig. 6–2A) and P1 (Fig. 6–2B), the apical dendritic tufts of the pyramidal cells in the cortical plate formed a dense plexus beneath the pial membrane. Throughout the fetal and early postnatal period, there were well-impregnated Cajal-Retzius cells in layer I (Fig. 6–1), always lying within the plexus of the pyramidal cell apical dendritic tufts. Low magnification photographs of the cortical plate on E21 and P1 (Fig. 6–2A, B) show pyramidal cell bodies at various levels of the cortical plate as if suspended from the “ceiling” of the neocortex (layer I). By P1, the older pyramidal cells deep in the cortical plate have thick and long apical dendrites (Fig. 6–2C, D), while the younger pyramidal cells superficial in the

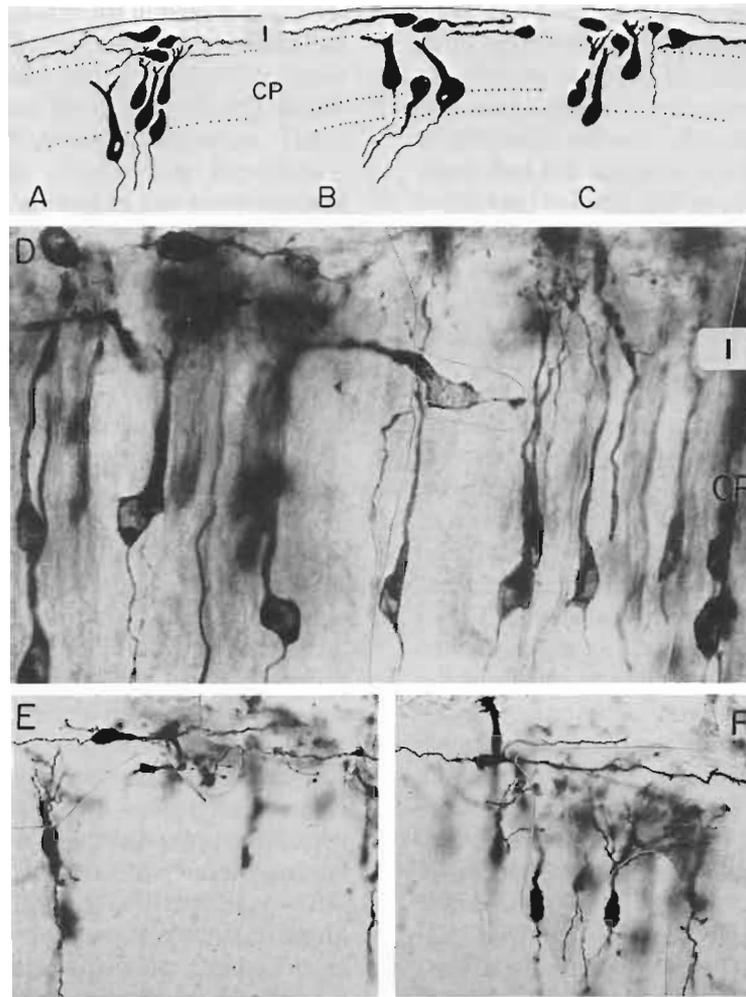


FIG. 6-1. Horizontally oriented Cajal-Retzius cells in layer I and pyramidal cells in the cortical plate (CP) in E17 (**A**, **B**, and **C**) and E21 (**D**) embryos, and in a P1 rat pup (**E** and **F**). The apical branches of pyramidal cells penetrate layer I, always growing in a superficial direction, while the axon is at the base of the cell. Basal dendrites are largely absent. (100 μm celloidin sections cut in the coronal plane, Golgi-Cox impregnation, magnification: **D**, X720, **E-F**, X288.)

cortical plate have thin and short apical dendrites (Fig. 6-1F). Only the deep pyramidal cells are beginning to grow basal dendrites at P1 (Fig. 6-2C, D).

The diagram in Fig. 6-3 shows how “inside-out” migration affects the length of the apical dendrites. The apical dendrites of the oldest cells (*solid black*, Fig. 6-3A) grow longer as the next youngest cohort of cells (*striped*, Fig. 6-3B) arrives in the cortical plate. Both sets of cells lengthen their apical dendrites as the youngest cohort of cells (*stippled*, Fig. 6-3C) arrives and grows short apical dendrites into layer I. That pattern of growth has been described in classic Golgi studies (Retzius, 1893; Cajal, 1911; Stensaas, 1967c, Sten-

saas and Stensaas, 1968; Marin-Padilla, 1971, 1983, 1988; Berry, 1982; Marin-Padilla and Marin-Padilla, 1982). Apparently, the growth of pyramidal cell apical dendrites into layer I and the inside-out settling of neurons are associated phenomena (Marin-Padilla and Marin-Padilla, 1982). Experimental verification of that hypothesis comes from the study by Pinto-Lord and Caviness (1979) in the reeler mutant mouse cortex, where the fiber plexus in layer I is placed deep within the cortical plate rather than superficially. In reelers, there is no inside-out gradient because pyramidal cells will often grow “apical” dendrites downward to contact the misplaced layer I.

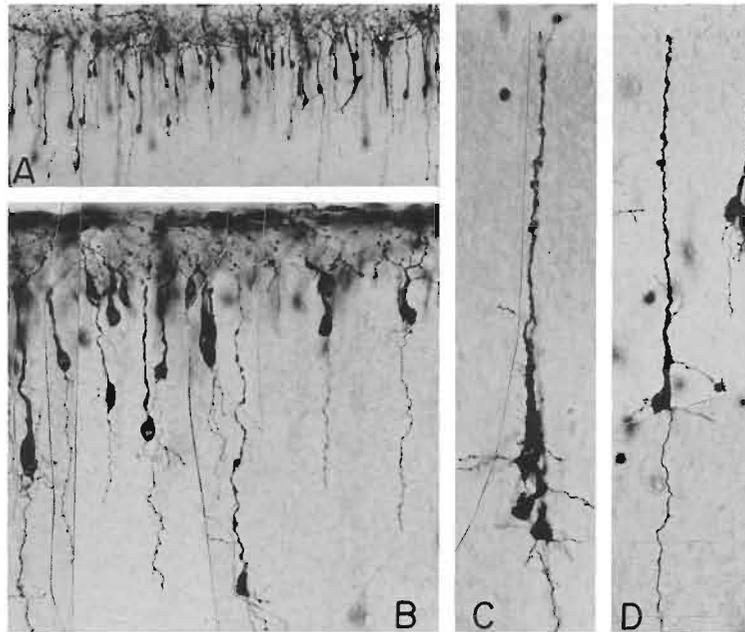


FIG. 6-2. Pyramidal cells in an E21 embryo (**A**) and in newborn rats (**B-D**). The cells are characterized by a relatively straight descending axon and an erect dendrite that penetrates layer I. Highly branched apical dendritic tufts in layer I form a dense plexus, while unbranched apical dendritic shafts connect to the pyramidal cell bodies at several depths in the cortical plate (**A** and **B**). Deep pyramidal cells have long and thick apical dendrites, but basal dendrites are only beginning to appear (**C** and **D**) at birth. (100 μm celloidin sections cut in the coronal plane, Golgi-Cox impregnation, magnification: **A**, X113, **B-D**, X288.)

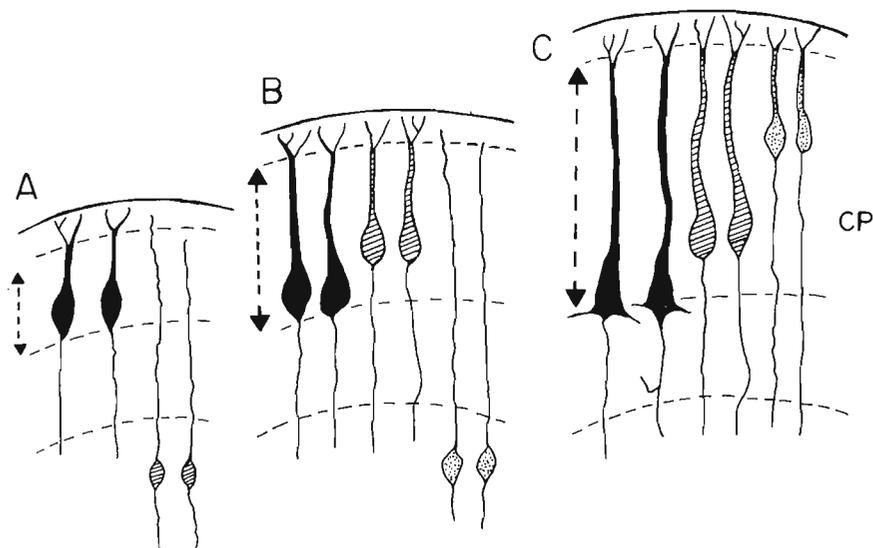


FIG. 6-3. Postulated growth pattern of the dendritic shafts of pyramidal cells. Pyramidal cells arrive sequentially in the cortical plate according to age: oldest (*black*) first, intermediate age (*striped*) next, youngest (*stippled*) last. All cell bodies first reach the surface of the cortical plate, as an apical dendrite grows upward and branches to form an apical tuft in the upper half of layer I or the molecular layer (**A**), anchoring the pyramidal cells from the top. When later arriving cells move past to reach the surface (**B** and **C**), the earlier settled cells are displaced downward, and their apical dendrites lengthen (*dashed arrows*). Basal dendrites appear at a later stage of cytodifferentiation.

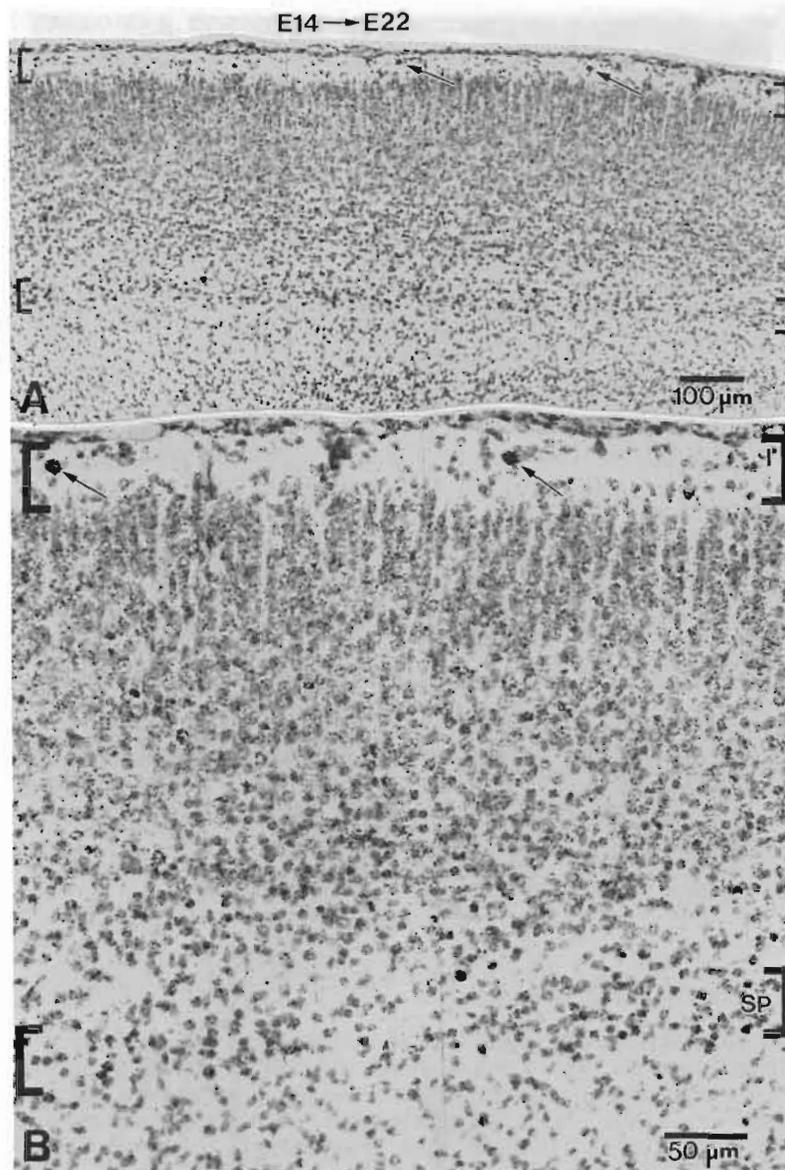


FIG. 6-4. The sagittally sectioned dorsomedial neocortex in an E22 rat that received one exposure to [^3H]thymidine on E14. All heavily labeled cells, those with dense black dots over the nucleus, are presumed to have originated on E14. Most heavily labeled cells are in layer I, where they are widely scattered, corresponding to the known distribution of the Cajal-Retzius neurons. A few heavily labeled neurons settle in the subplate, and none are in the cortical plate. (6 μm paraffin section, hematoxylin stain.)

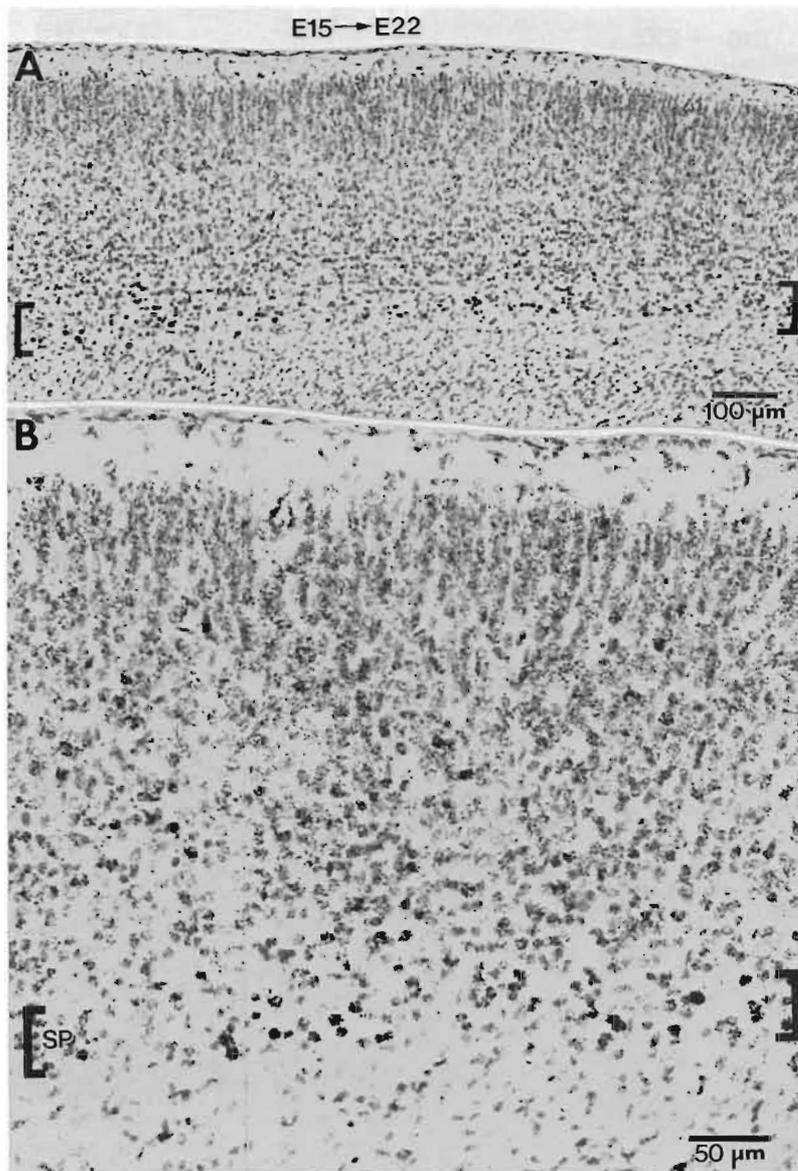


FIG. 6-5. The same as in Fig. 6-4, in an E22 embryo that received a single exposure to [³H]thymidine on E15. Heavily labeled cells are now mainly in the subplate, and a few are in the deep part of the cortical plate.

6.2 THE SETTLING OF CELLS IN THE CORTICAL PLATE: MORPHOGENETIC EVIDENCE FOR AN UNEVEN RADIAL NEUROGENETIC GRADIENT

The dorsomedial neocortex was examined in sagittal sections of E22 embryos that received a single exposure to [³H]thymidine from E14 through E21.* We tracked the changing positions of bands of heavily la-

beled cells in the cortical plate (*surrounded by brackets*, Figs. 6-4A to 6-8A). The focus is on the period between E14 to E18, because the neurons in this age group reach their destinations in the dorsomedial cortical plate by E22.

After an injection of [³H]thymidine on E14 (Fig. 6-4), heavily labeled cells settle in layer I (*arrows*) and a few in the subplate (*brackets*). That is consistent with the neurogenetic data (Chapter 3) that only Cajal-Retzius and subplate cells are generated in substantial numbers on E14 in the dorsomedial neocortex. After injections on E15 (Fig. 6-5), mostly subplate neurons

* (Details of the histological preparation are given in Appendix 2.)

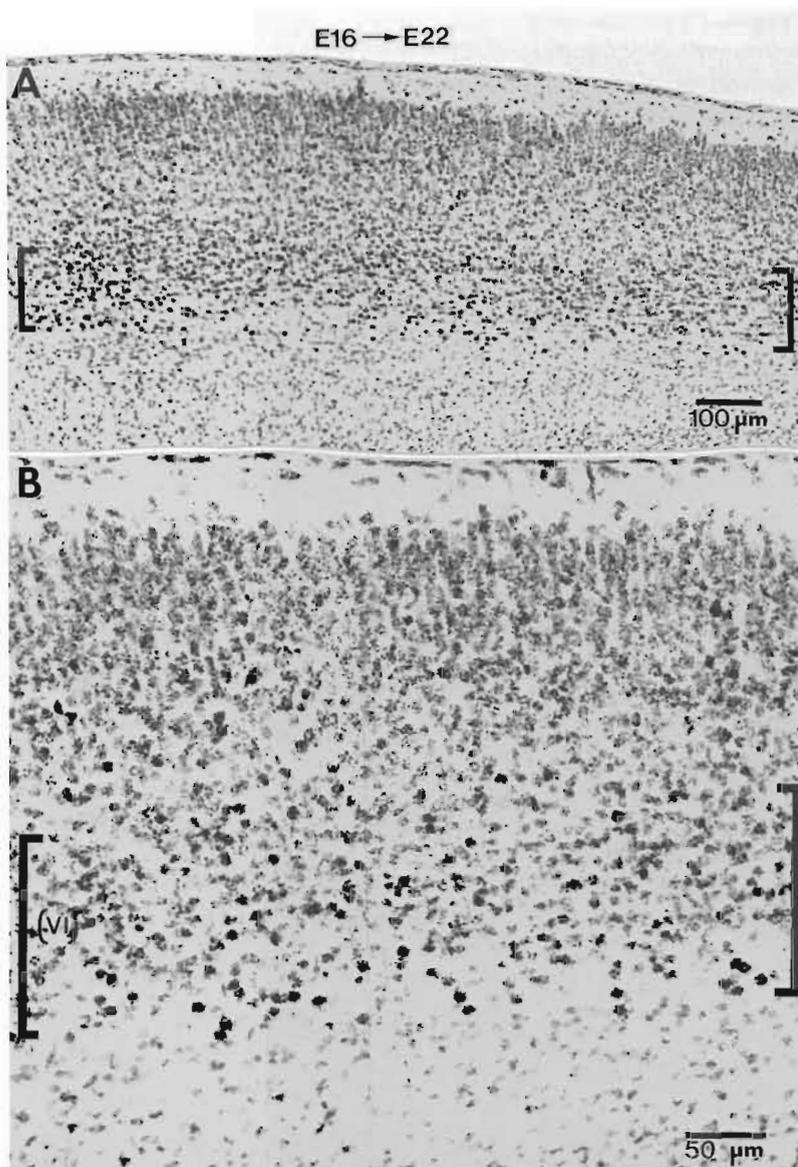


FIG. 6-6. The same as in Fig. 6-4, in an E22 embryo that received a single exposure to [^3H]thymidine on E16. Heavily labeled cells are limited to the lower third of the cortical plate, the future layer VI in the adult neocortex.

and a few scattered neurons in the deepest part of the cortical plate are heavily labeled. That pattern corresponds to the neurogenetic timetable in the dorso-medial cortex where peak neurogenesis on E15 occurs in the subplate (about 50%) and the onset of neurogenesis occurs in the future layer VI at the base of the cortical plate (about 10%). Injections on E16 (Fig. 6-6) intensely label cells throughout the lower third of the cortical plate. The neurogenetic data indicates that over 50% of the neurons in layer VI are generated on E16 in the dorsomedial cortex, so the band of heavily labeled neurons (Fig. 6-6A) outlines the part of the cortical plate that will become layer VI in the adult. Cells in the lower third of the cortical plate are less densely packed by E22 (Fig. 6-6B), indicating a more

advanced state of maturation in presumptive layer VI than in the rest of the cortical plate. Injections on E17 (Fig. 6-7) intensely label a band of neurons that reaches halfway up into the cortical plate. The neurogenetic data (Chapters 13 and 14) indicate that both layer VI and layer V neurons are generated in the dorso-medial neocortex on E17; 25-50% in anterior versus posterior parts of layer VI, 50-60% in anterior versus posterior parts of layer V. However, most of the heavily labeled neurons are still within layer VI, and only some extend into the lower part of the future layer V. Our interpretation is that even though E17 is a peak day for neurogenesis throughout layer V, many of the neurons destined to settle in the superficial part are probably generated late on E17 (during the afternoon

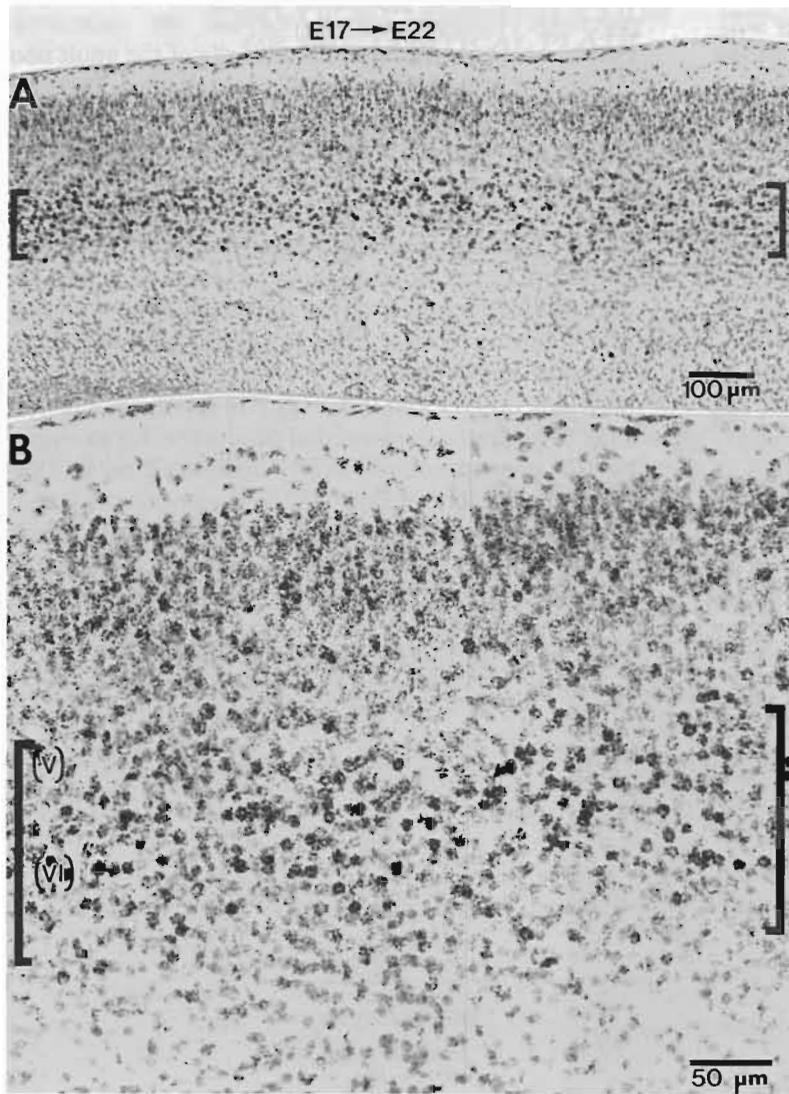


FIG. 6-7. The same as in Fig. 6-4 in an E22 embryo that received a single exposure of [^3H]thymidine on E17. The band of heavily labeled cells now reaches halfway up in the cortical plate, only slightly higher than the band resulting from the E16 injection (Fig. 6-6).

and evening) so that a single injection on the morning of E17 does not heavily label these neurons.¹ Injections on E18 (Fig. 6-8) result in a band of intensely labeled neurons in the upper one-fourth of the cortical plate, placed higher than the band resulting from the E17 injection. The neurogenetic data in the dorsomedially situated motor and visual cortical areas indicate that only a small proportion of layer V neurons are generated on E18 (10–15%), but E18 is the peak day for neurogenesis of layer IV and lower layer III (50–60%). Consequently, there are only a few scattered heavily labeled cells in the upper part of what will presumably develop into layer V, while the superficial continuous

band of intensely labeled cells is probably composed of many layer IV and some layer III neurons.

[^3H]thymidine injections on E19, E20, and E21 show that most heavily labeled cells have not yet reached their final locations in the cortical plate by E22 so these injection groups are not illustrated. When injections are given on E19, many heavily labeled neurons are still migrating through layers V and VI, and there is no continuous band of labeled neurons at the superficial border of the cortical plate. Injections on E20 label only a few neurons at the base of the cortical plate, most of the labeled cells that appear to be neurons are still either sojourning or migrating in the intermediate zone. Finally, injections on E21 produce a band of heavily labeled cells, presumably neurons generated on E21, at the base of the subventricular zone, in what we call the first inferior band (see Chapter 7), where the young neurons sojourn for approximately 24 hours before migrating into the cortical plate.

To summarize, the dorsomedial cortical plate has its

¹ All of the [^3H]thymidine injections discussed here were administered between 8:30 and 9:00 A.M. Since E17 is one of the most active days of neocortical neurogenesis, there is rapid dilution of the incorporated [^3H]thymidine label. If a cell is generated late on E17, it may not be heavily labeled by a morning injection. A [^3H]thymidine injection given on E17.5 (that would be administered between 8:30 and 9:00 P.M.) would be more likely to heavily label cells in the middle and upper parts of layer V.

full complement of neurons in layers VI and V by E22. Together, they take up the lower three-fourths of the cortical depth. Layer IV cells, mostly generated on E18, have just arrived in large numbers and occupy a superficial band. The neurons generated on E19, E20, and E21 in the dorsomedial neocortex will reside in layers II and III, with only a few destined to settle in layer IV. These supragranular cells reach their final locations perinatally and during the early postnatal period. By P5, most of the neurons are in place in the superficial layers (Fig. 3-1).

Although this evidence supports the inside-out pattern of cortical plate construction, it is apparently not constructed evenly. The sequential survival autoradiograms on E22 show that there is a wide area of overlap between the bands of heavily labeled cells resulting from injections on E16 and E17 (compare Figs. 6-6 and 6-7). The neurogenetic data in Chapters 11 through 15 indicate that many layer VI and V cells are

generated concurrently throughout the neocortex. These constitute the lower two-thirds of the adult neocortex and are often called the deep layers. The neurogenetic data on the radial gradient in all neocortical areas (Chapters 11-15) would predict a large overlap in the positions of bands of heavily labeled cells after injections on E18 through E21 because many cells in layers IV-II are generated concurrently. These constitute the upper third of the adult neocortex and are often grouped together as the superficial layers. In contrast, there is a clear morphogenetic separation between the bands of heavily labeled cells resulting from injections on E17 and those on E18 (compare Figs. 6-7 and 6-8). In the dorsomedial neocortex, for example, layer V neurogenesis is nearly completed on E17 before the peak day of layer IV neurogenesis on E18.

These patterns suggest that the cortical plate is built in two separate tiers, an upper one and a lower one (diagrammed in Fig. 6-9). After the primordial plexi-

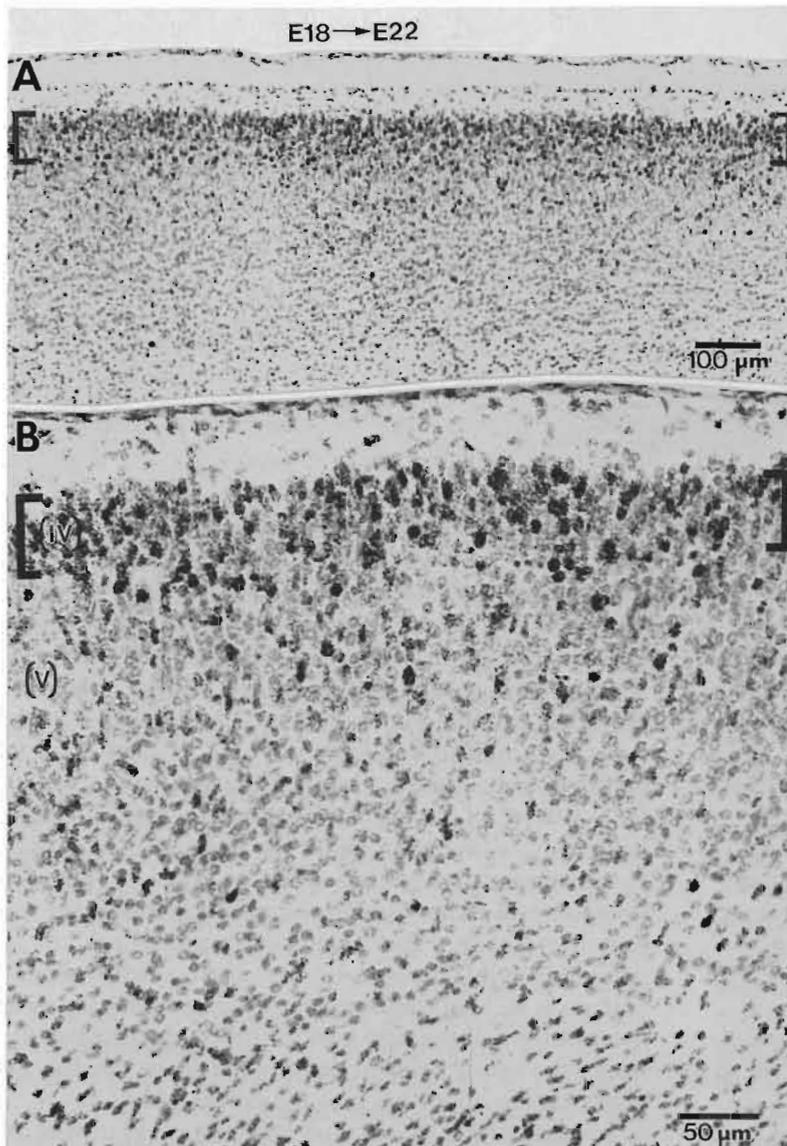
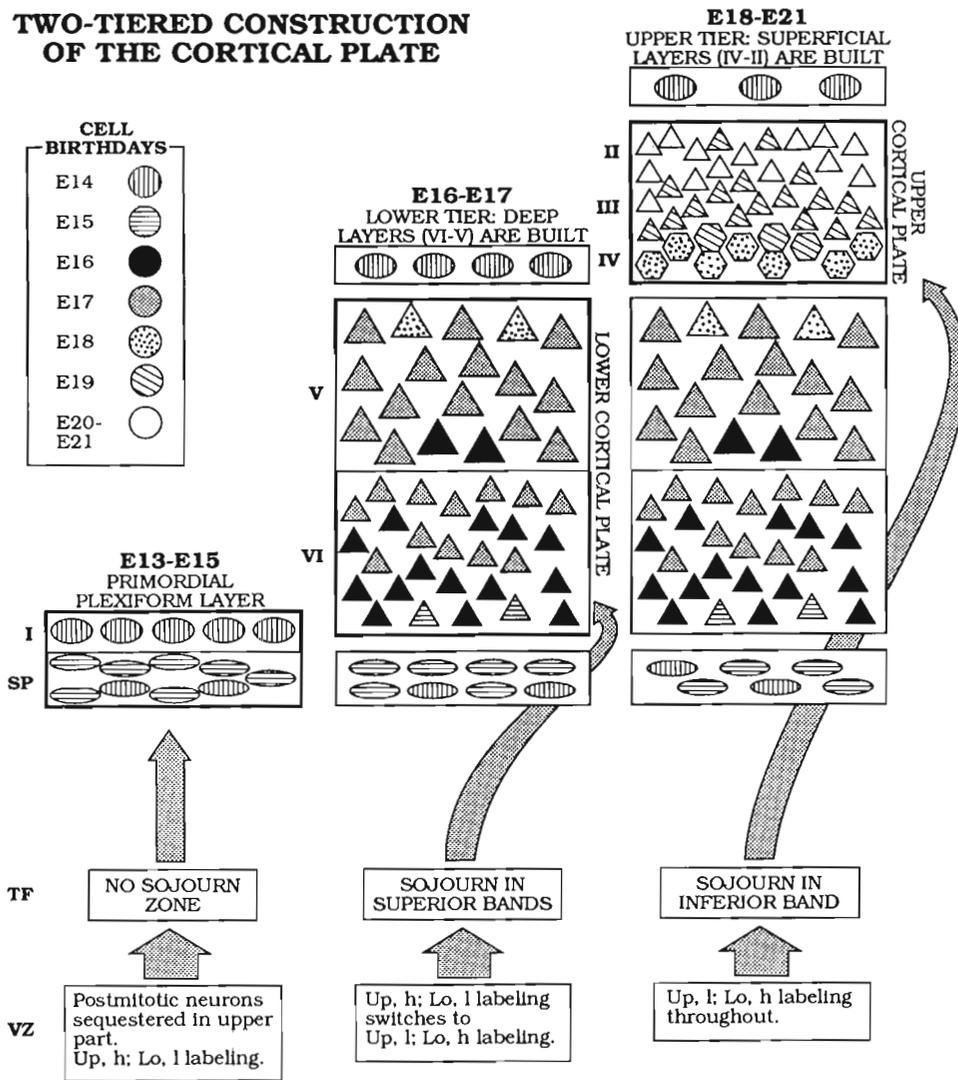


FIG. 6-8. The same as in Fig. 6-4 in an E22 embryo that received a single exposure to [3 H]thymidine on E18. The band of heavily labeled cells is now in the most superficial fourth of the cortical plate, much higher than the band resulting from the E17 injection (Fig. 6-7). The neurogenetic data (Chapters 3, 13, and 14) indicate that these neurons will occupy layers IV and lower III in the adult neocortex. There are a few heavily labeled cells scattered throughout the upper part of the future layer V.

TWO-TIERED CONSTRUCTION OF THE CORTICAL PLATE



SUCCESSIVE NEUROEPITHEAL TRANSFORMATIONS

FIG. 6-9. A diagrammatic representation of the hypothesis that the cortical plate is built in two separate tiers within the upper and lower borders of the primordial plexiform layer (layer I and the subplate). *Ellipses* represent neurons in the primordial plexiform layer, large ones are presumptive Cajal-Retzius neurons, smaller and flattened ones are presumptive subplate neurons. *Various-sized triangles and hexagons* represent pyramidal cells in the cortical plate, largest triangles are layer V neurons, medium sized triangles are those of layer VI, while the smallest triangles are those of layers II-III; *hexagons* represent the star pyramids in layer IV. The amount of shading is related to the various birthdays of the neurons. Those older (born on E14) are more darkly shaded than the youngest neurons (born on E20-21). Notice that there is little overlap in cell birthdays between the primordial plexiform layer and the lower tier of the cortical plate. Likewise, there is little overlap in cell birthdays between the upper and lower tiers of the cortical plate (based on data in Chapter 3). The sequential survival autoradiograms on E22 (Figs. 6-4 to 6-8) suggest that, although there is much overlap during building of the lower tier (layers V and VI), most of the lower tier is already built before the upper tier is started. It is interesting that successive transformations of the neuroepithelium (described in boxes representing the ventricular zone, VZ) are related to the successive generation of the primordial plexiform layer, lower tier of the cortical plate and upper tier of the cortical plate. The neurons destined to settle in each of the three parts have different sojourn characteristics in the transitional field (TF): primordial plexiform neurons do not sojourn, those in layers V and VI do so in the superior bands, those in layers IV-II do so in the first inferior band (Chapter 7).

form layer is generated between E13 and E15 (*left column*, Fig. 6–9), the lower tier of the cortical plate is assembled (*center column*, Fig. 6–9). That tier consists of the medium-sized pyramidal cells in layer VI (*represented as medium-sized triangles*) and the large pyramidal cells in layer V (*large triangles*). Cells in the deep layers arrive and settle in an inside-out gradient, but there is considerable overlap both morphogenetically and neurogenetically within the tier (notice that many layer V and VI cells have the same birthday in Fig. 6–9). The upper tier (*right column*, Fig. 6–9) consists of the star pyramidal cells in layer IV (shown as *hexagons*) and the small pyramidal cells in layers II and III (*smallest triangles*). These cells show inside-out neurogenetic and morphogenetic gradients but also have widely overlapping, concurrently generated areas (notice the number of simultaneous birthdays in the upper tier in Fig. 6–9). However, there is a temporal gap between the two tiers (few cell birthdays are simultaneous) so that the lower tier is nearly finished before the upper tier is started. In Chapters 11–14 we will show that the smallest proportion of cogeneration occurs in layers V and IV, just at the junction between the deep and the superficial layers.

The hypothesis that the cortical plate is built in two tiers is supported by cell lineage studies that indicate that different stem cells generate the lower and upper tiers. Using aggregation chimeras produced during the blastocyst stage from two histochemically detectable strains of mice, two recent studies (Crandall and Herup, 1990; Fishell et al., 1990) examined the genotypes of cells in radial columns of the neocortex in adult chimeras and found that the genotype distribution in the superficial layers was statistically different from the genotype distribution in the deep layers. Using retroviruses to detect clones (cells that originate from a common progenitor) in the embryonic rat neocortex, Price and Thurlow (1988) found that infections on E16 (our E17) produced neuronal clones with members restricted to the deep layers, and others with members restricted to the superficial layers; no clones were observed with a mixture of cells from superficial and deep layers. A similar study was done in mice (Luskin et al., 1988), but infection with the retroviruses occurred on E12/E13 (E12/E13 in mice would translate to E14/E15 in rats), possibly before divergence of progenitors for superficial and deep neurons in the dorsomedial neocortex.