

CHAPTER 9

Cell Migration in the Developing Neocortex

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An intensely studied phenomenon of neocortical development is the migration of cells to the cerebral cortex. That interest was prompted by Angevine and Sidman's (1961) [³H]thymidine autoradiographic demonstration of an inside-out neurogenetic gradient in the neocortex: the earliest-produced neurons settle in the depth of the cortex adjacent to the white matter, while the later-produced neurons settle progressively more superficially. By necessity, younger neurons must migrate past older ones to reach their destinations. Since both the precursor cells in the ventricular zone (the neuroepithelium) and neurons in the cortical plate (the primordium of the cortical gray) are radially oriented, it is not surprising that two hypotheses of neocortical cell migration proposed that young neurons migrate from the ventricular zone to the cortical plate by following a strict radial path. Berry and co-workers (Berry et al., 1964; Berry and Rogers, 1965) and Morest (1970) suggested that the nuclei of young neurons are translocated from the ventricular zone to the cortical plate within long, external radial processes that extend to the pia (see Fig. 1–6). Rakic (1972, 1978, 1982, 1988) observed that radial glia had long, external processes reaching the pia, while young migrating neurons had rather short processes that were closely ap-

posed to radial glia fibers, and he postulated that the glial fibers guide the young neurons to their destinations in the cortical plate (see Fig. 1–7).

However, earlier observations and more recent empirical evidence have cast doubt on the hypothesis that simple radial migration can account for all aspects of the translocation of cells from the ventricular zone to the cortical plate. It has been known for some time that horizontally oriented cells abound in the subventricular and intermediate zones of the cortex. Such horizontal cells have been visualized with the Golgi technique (Stensaas, 1967b, 1967d; Derer, 1974; Valverde et al., 1989), electron microscopy (Shoukimas and Hinds, 1978), histochemical marking after retroviral infection (Walsh and Cepko, 1988; Austin and Cepko, 1990), and quantitative Nissl studies (Chapter 8). Even before the radial glia hypothesis was proposed, Hicks and D'Amato (1968) tracked the migration of neocortical neurons with [³H]thymidine autoradiography and reported that the young neurons migrate via "complex paths . . . leaving the proliferative zones to follow along the curving corticopetal fibers, only to leave them as they arrived at the cortical plate" (p. 626).

In this chapter we show with sequential-survival

[³H]thymidine autoradiography that many of the young neurons in the subventricular and lower intermediate zones of the dorsal embryonic neocortex are actively migrating to the lateral and ventrolateral cortex in a prominent stream that skirts the border between the striatum and the neocortex. The impetus to search for laterally migrating cells was the visualization of the embryonic neocortex in three-dimensional computer reconstructions. The images in Chapter 2 (Color Figs. 2–5) and others that we include here show a progressive shrinkage of the anterior cortical ventricular zone from ventrolateral to dorsomedial. Since we knew from [³H]thymidine dating studies (Chapters 3 and 11–15) that neurons settling in layers IV–II of the lateral and ventrolateral cortex continue to be generated for some time after ventricular shrinkage has begun, we surmised that the neurons settling in the ventrolateral and lateral parts of the anterior cortex must be generated more medially and, therefore, could not reach their destinations by simple radial migration. Our examination of sequential-survival [³H]thymidine autoradiograms not only reveals the existence of a distinct stream of laterally migrating cells, which we call the *lateral cortical stream*, but also shows that there is a progressive delay in the time of arrival of synchronously generated neocortical neurons in the cortical plate laterally and ventrolaterally when compared to the dorsal part of the cortical plate. We also present preliminary observations showing that the neocortical ventricular zone generates neurons that will settle outside of the neocortex in the basal telencephalon.

9.1 CHANGING SPATIAL RELATIONSHIP BETWEEN THE VENTRICULAR ZONE AND THE CORTICAL PLATE

Color Fig. 6* is a medial view of the Skandha-generated images that reconstruct the ventricular zone (VZ, white) and the cortical plate (CP, green) on E16 (*upper left*), E17 (*lower left*), E19 (*upper right*), and E21 (*lower right*). (Details of the computer reconstruction technique are given in Appendix 4.) The spatial relationships between the two layers has been faithfully maintained by making the subventricular and intermediate zones transparent (0% opacity). Illumination has been set so that the dorsomedial (DM) edges of the VZ and the CP are brightest, and the most intense shadows are cast on the inferior dorsal surfaces.

The images in Color Fig. 6 show that the spatial relationship between the cortical plate and the ventricular zone changes during development. From its inception on E16 (*upper left*), the ventrolateral edge of the cortical plate (CPvl) extends farther down (0.2

mm) than the ventrolateral edge (vl) of the ventricular zone (VZ). By E17 (*lower left*), the ventrolateral edge of the ventricular zone shrinks farther dorsomedially (0.42 mm) from the ventrolateral edge of the thin cortical plate. The distance between the ventrolateral edges of the two layers progressively increases on E19 (0.76 mm, *upper right*) and E21 (0.99 mm, *lower right*). These changes are primarily the result of growth of the basal ganglia (not reconstructed; Chapter 2, Figs. 2–8 to 2–10). The ventricular zone narrows even more dramatically anteriorly, where the basal ganglia are large, and widens posteriorly, where the basal ganglia are smaller. Throughout most of the cortex, the cortical plate extends farther ventrolaterally than does the ventricular zone. It is only in a narrow strip of the most posterior cortex that the cortical plate and the ventricular zone have the same span.

Color Fig. 7 shows the same images rotated +90° in the Y axis so that the posterior edges of the ventricular zone (VZp) and the cortical plate (CPp) are facing the observer. The images are also rotated –20° in the X axis so that the frontal poles are tipped down, the occipital poles up; this allows visualization of the widening gap between the cortical plate and the ventricular zone (filled by the subventricular and intermediate zones). The narrowing of the anterior ventricular zone (VZa) becomes progressively more prominent between E16 (*upper left*) and E21 (*lower right*). On E16, the posterior edge of the cortical plate does not extend as far back as the posterior edge of the ventricular zone (refer to Chapter 2, Fig. 2–13), but from E17 (*lower right*) on, the posterior edges of the ventricular zone and the cortical plate (connected with black lines) have the same span.

Taken together, the images in Color Figs. 6 and 7 show the dramatic medial shrinkage of the ventricular zone in the anterior cortex. As the basal ganglia grow from their primordia in the ventrolateral telencephalon, the neocortical ventricular zone is displaced dorsomedially. Since this happens from the earliest appearance of the cortical plate and during the most active period of cortical neurogenesis, many young neocortical neurons must be generated from 0.2 to 1.0 mm medial to their settling sites. This warrants the conclusion that migrating neurons cannot reach the lateral and ventrolateral parts of the cortical plate by following a strict radial path.

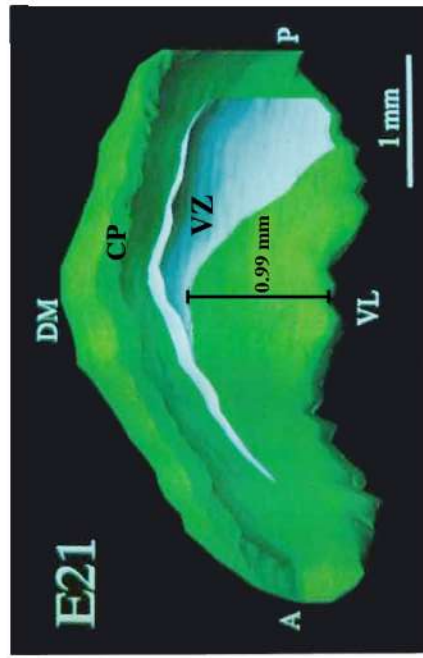
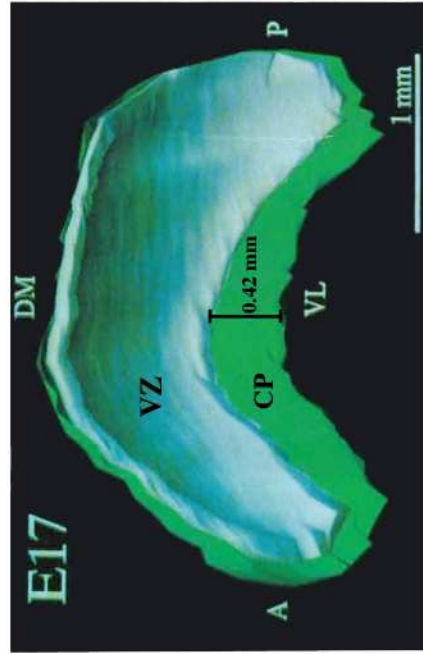
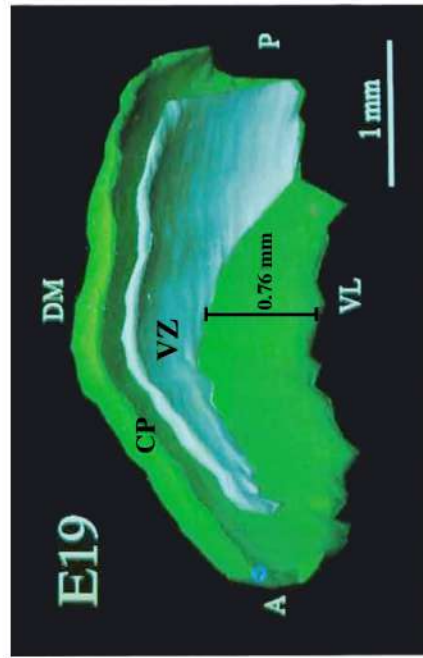
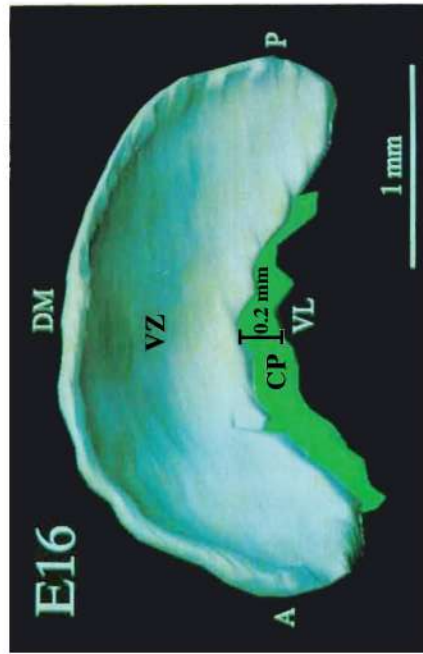
9.2 TRACKING CELL MIGRATION IN THYMIDINE AUTORADIOGRAMS

9.2.1 Choice of Injection Groups for Observation

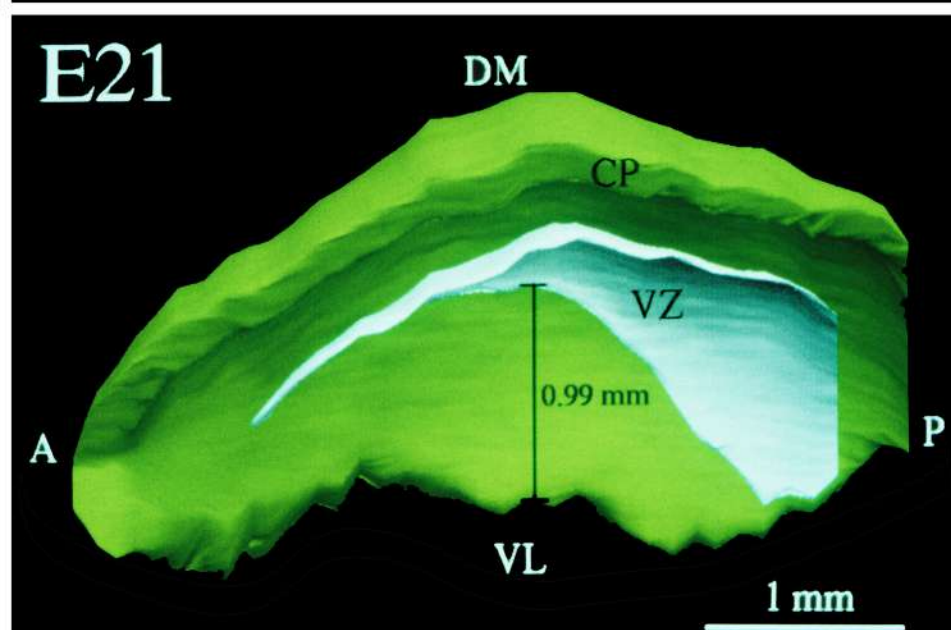
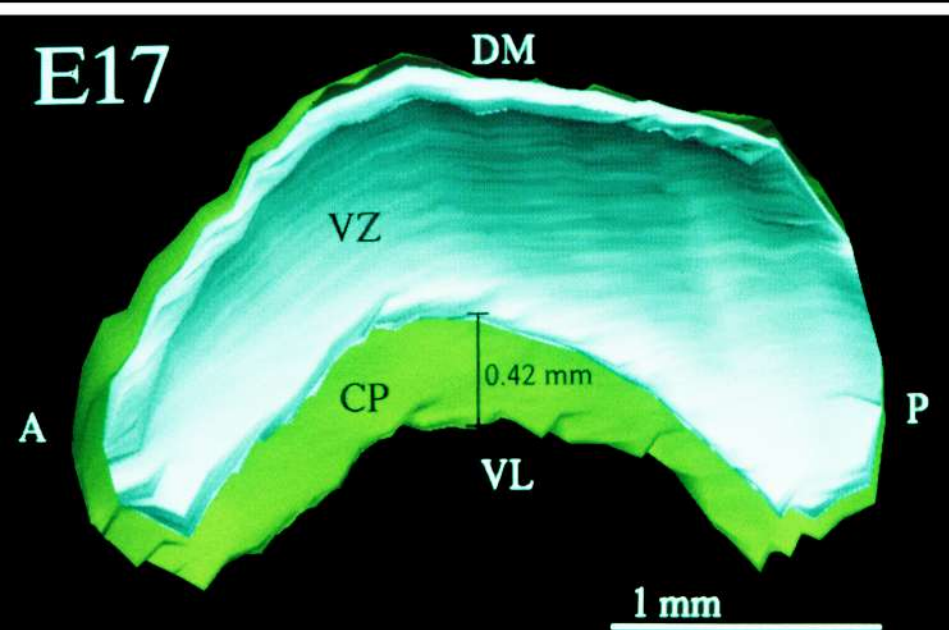
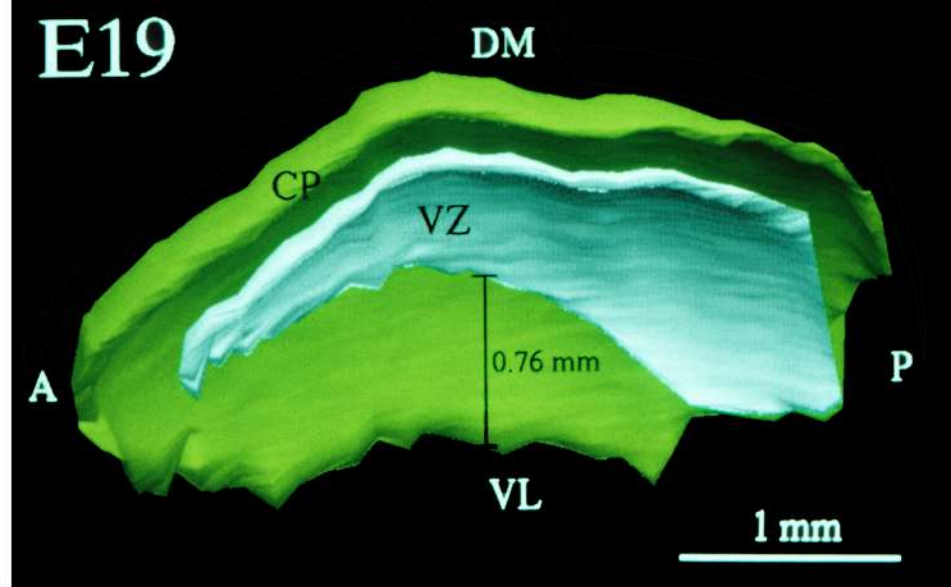
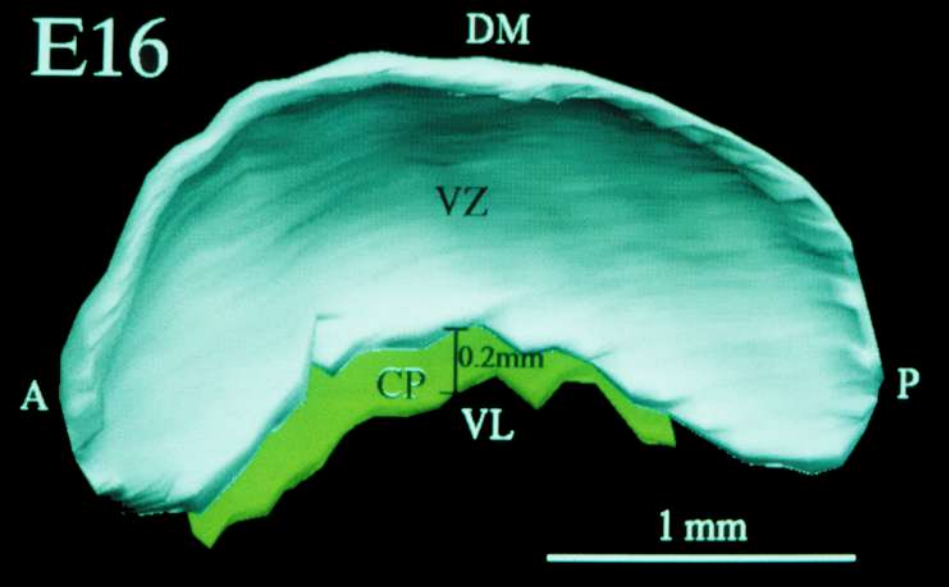
Since cells in the superficial layers of the neocortex are generated later than deep cells (Chapters 3 and 11–

* All color figures appear in the color plates following page 64.

Note that Color Figures 6 and 7 are on the next five pages.



COLOR FIG. 6. Medial views of computer-generated (Skandha) three-dimensional reconstructions of ventricular zone (white) and cortical plate (green) in the embryonic neocortex from E16 (upper left) to E21 (lower right). For all photographs, anterior (A) is left, posterior (P) is right, dorsal is at the top, and ventral is at the bottom. The space between the dorsomedial (DM) cortical plate and the dorsomedial ventricular zone that widens between E17 and E21 is taken up by the subventricular and intermediate zones that are made transparent. Numbers written into the images indicate the distance between the ventrolateral edges of the cortical plate and the ventricular zone.



E16

DM

P

A

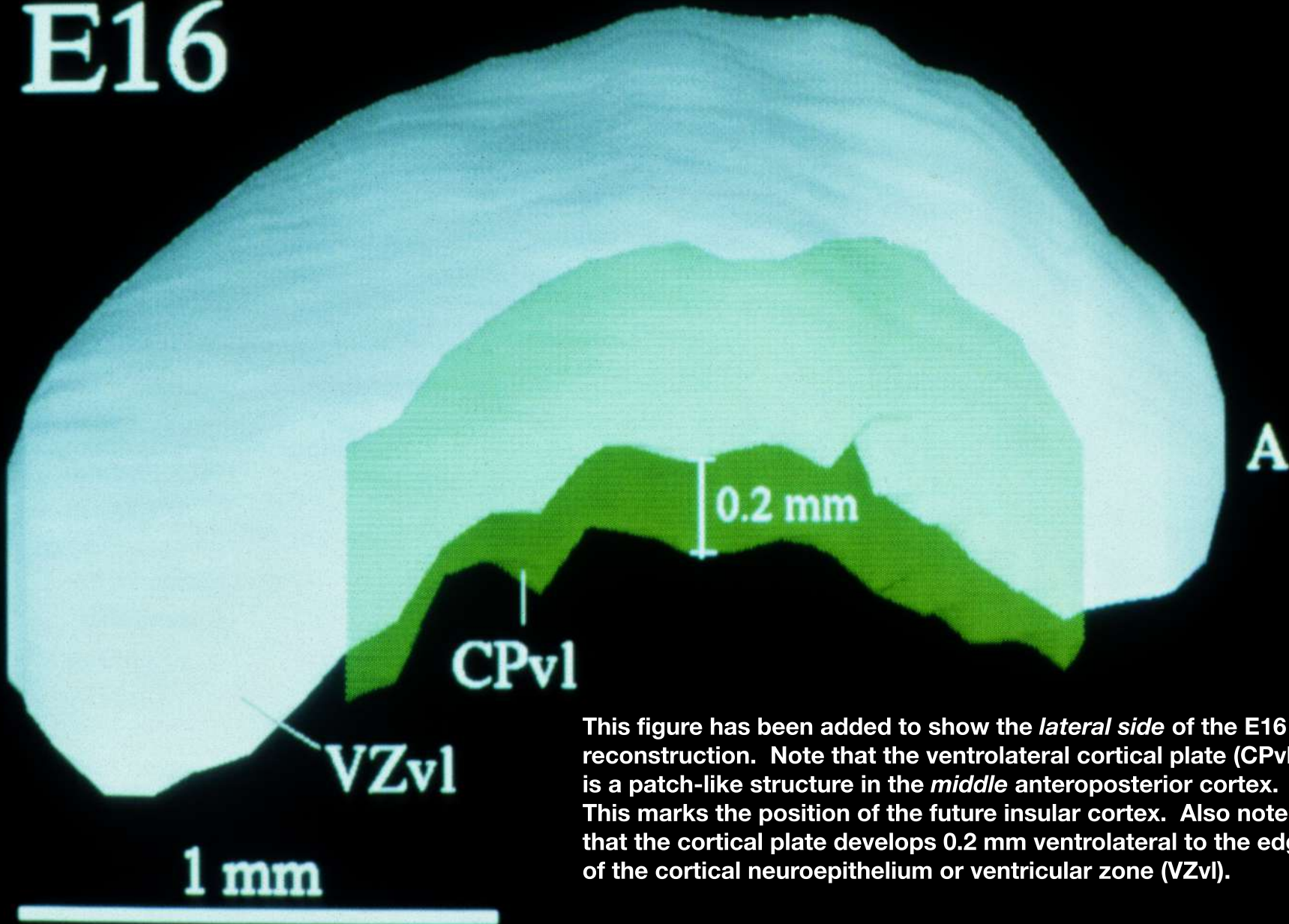
0.2 mm

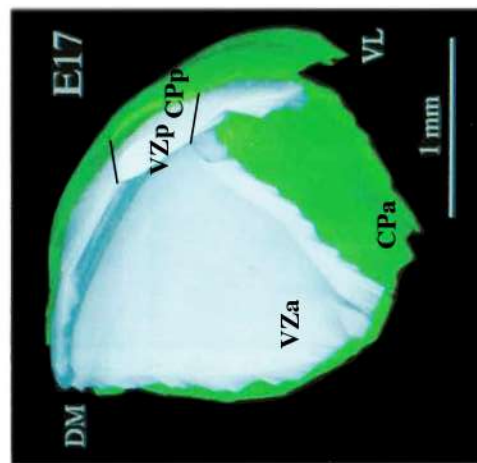
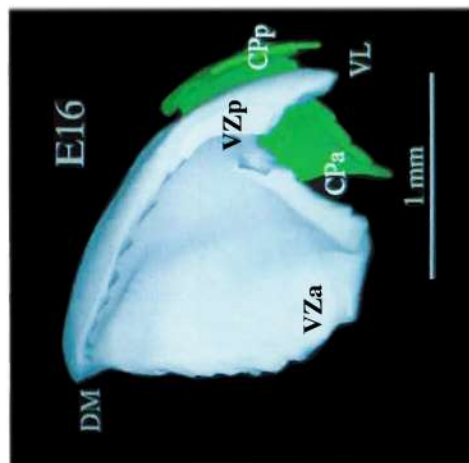
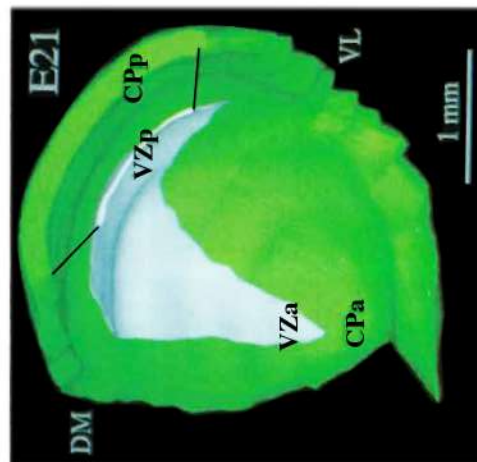
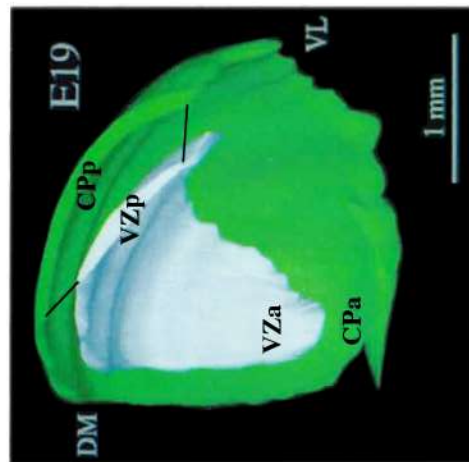
CPvl

VZvl

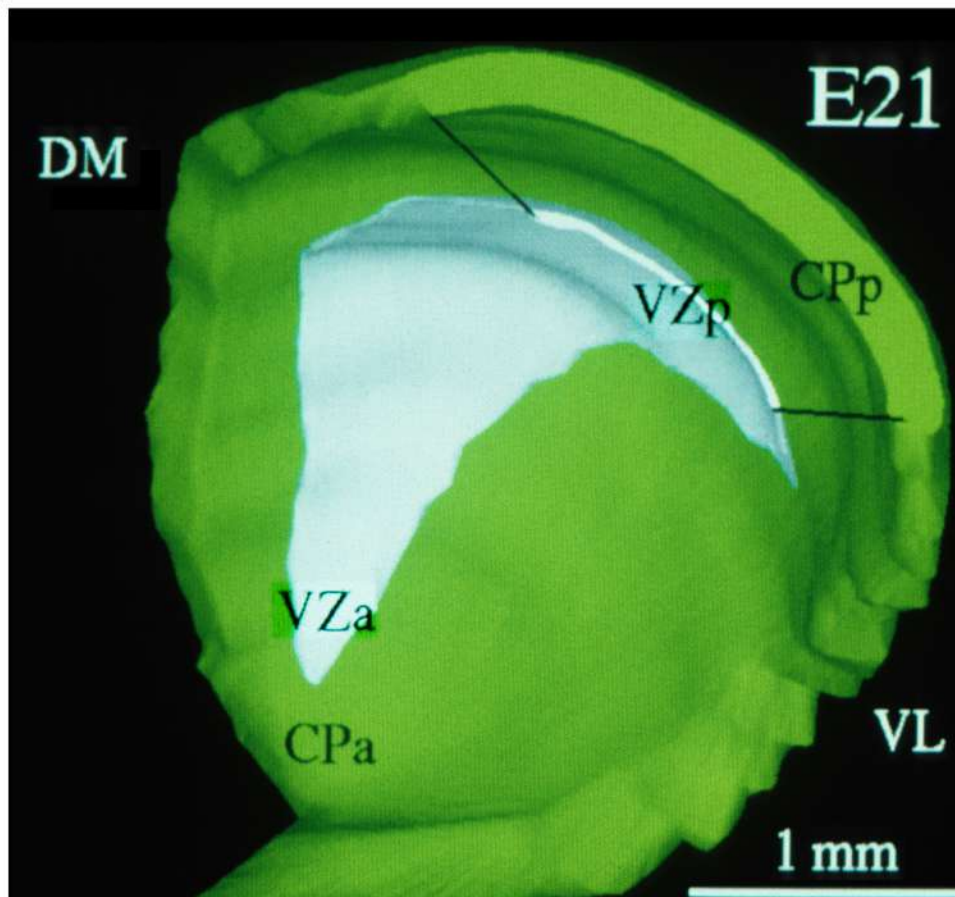
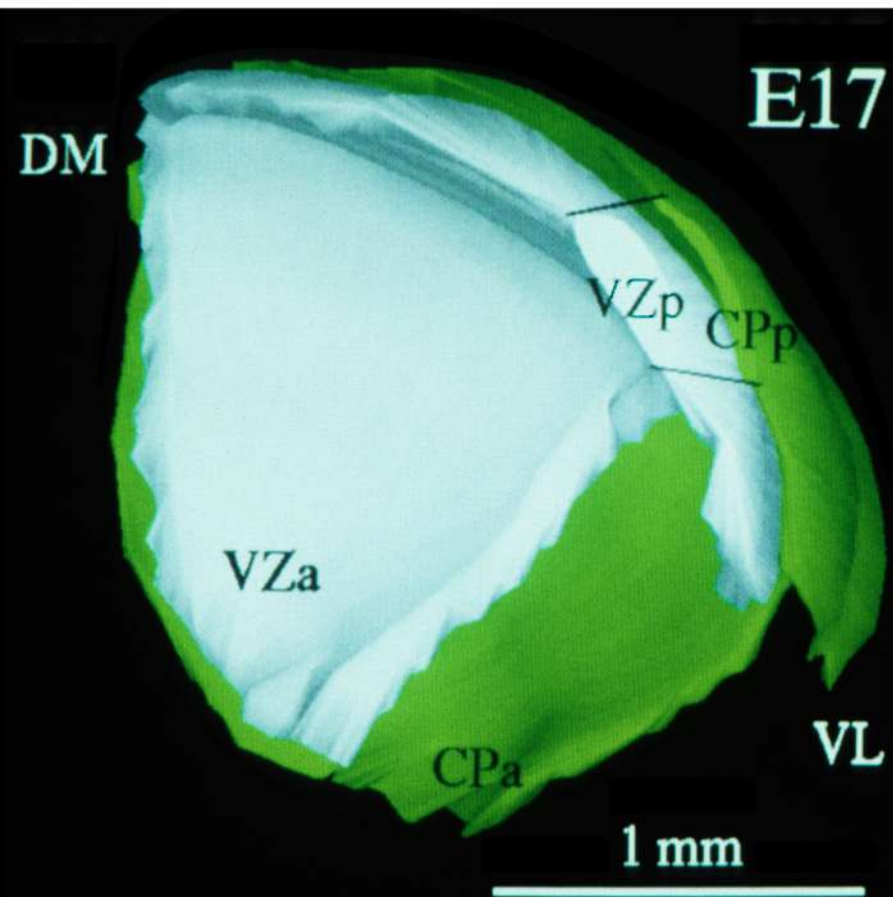
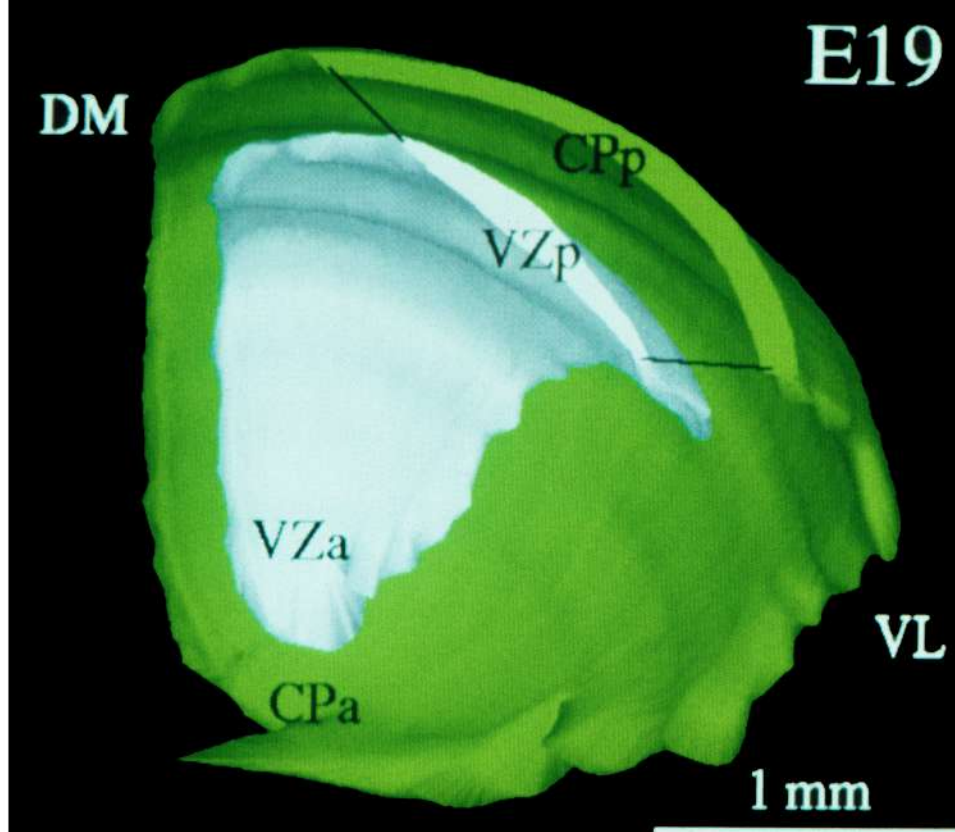
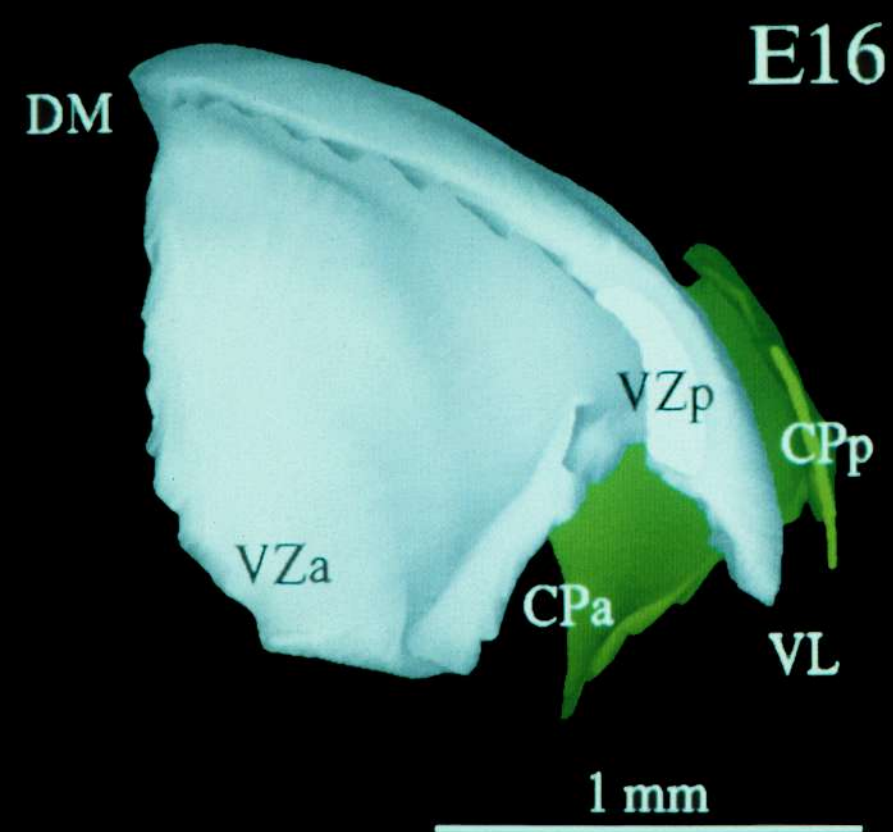
1 mm

This figure has been added to show the *lateral side* of the E16 reconstruction. Note that the ventrolateral cortical plate (CPvl) is a patch-like structure in the *middle* anteroposterior cortex. This marks the position of the future insular cortex. Also note that the cortical plate develops 0.2 mm ventrolateral to the edge of the cortical neuroepithelium or ventricular zone (VZvl).





COLOR FIG. 7. The same images in Color Fig. 6 rotated $+90^\circ$ in the Y axis and -20° in the X axis so that the observer is viewing the ventricular zone (white wedge) and the cortical plate (green shell) from their posterior edges (VZp and CPp) and is looking slightly downward toward the frontal pole (medial is right, lateral is left, dorsal is top, ventral is bottom). The coextensive span of the posterior cortical plate and ventricular zone is readily apparent (connected by black lines), while the prominent narrowing of the anterior ventricular zone (VZa) can be seen as one looks toward the frontal pole. The anterior cortical plate (CPa) is large and tucks back and under on E19 and E21 to form the primordium of the future orbital cortex overlying the olfactory peduncle.



15) the superficial cells in the lateral cortex are more likely to have lateral migratory paths. Consequently, our aim was to find an injection group that would maximally label young neurons migrating to the superficial layers of the lateral and ventrolateral cortical plate. Table 9-1 shows the peak days of neurogenesis in each one of the superficial layers (IV-II) in laterally and ventrolaterally situated cortical areas (summarized from Chapters 11-15). E17 is the peak day for layer IV neurogenesis in most of these areas; E18 is the peak for layer III, E19 for layer II. The days when the last neurons are generated in layer II are also listed in Table 9-1 (last column) and range from E19 (secondary auditory cortex) to E21 (primary somatosensory cortex). Since E17 is also the time when many layer V cells are generated in the laterally situated primary somatosensory cortex, a maximal number of heavily labeled young neurons migrating to the lateral cortical plate

should be seen in daily intervals after an E17 injection. A few observations will also be presented from the E18 injection group because many neurons destined to settle in the lateral cortex are also generated on that day.

9.2.2 Cell Migration in the Lateral Cortical Stream and the Delayed Settling of Neurons in the Lateral Cortical Plate

Following a single injection of [^3H]thymidine on E17, the locations of intensely labeled cells are shown in the anterior neocortical primordium in animals killed from E18 through E21 (Figs. 9-1 and 9-2). On E18 (Fig. 9-1A), one day after injection, there are as yet no heavily labeled cells in the cortical plate. Instead, young neurons generated on E17 are concentrated in two bands: (1) the first inferior band (ib1) in the deepest part of the subventricular zone and (2) the second su-

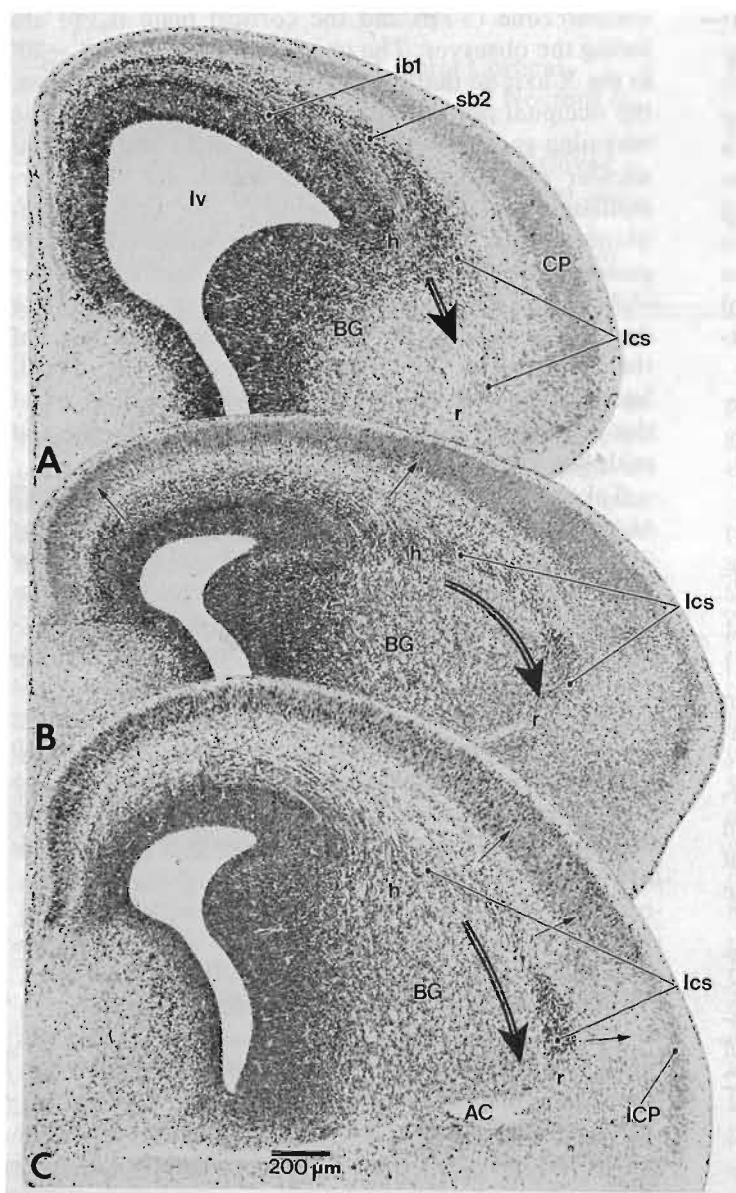


FIG. 9-1. Coronal sections of the anterior neocortical primordium in rat embryonic brains at daily intervals after a single injection of [^3H]thymidine on E17. The black bands or dots represent heavily labeled cells. One day after injection on E18 (**A**), the cortical plate (CP) contains no labeled cells. Heavily labeled cells arrive in the dorsal CP 2 days after injection on E19 (**B**) and arrive in the lateral CP 3 days after injection on E20 (**C**), but have not yet reached the insular cortical plate (ICP). Between E18 (**A**) and E20 (**C**) heavily labeled cells also migrate into the head (h) of the lateral cortical stream (lcs) and eventually fill the reservoir (r). (3 μm methacrylate sections, hematoxylin stain.)

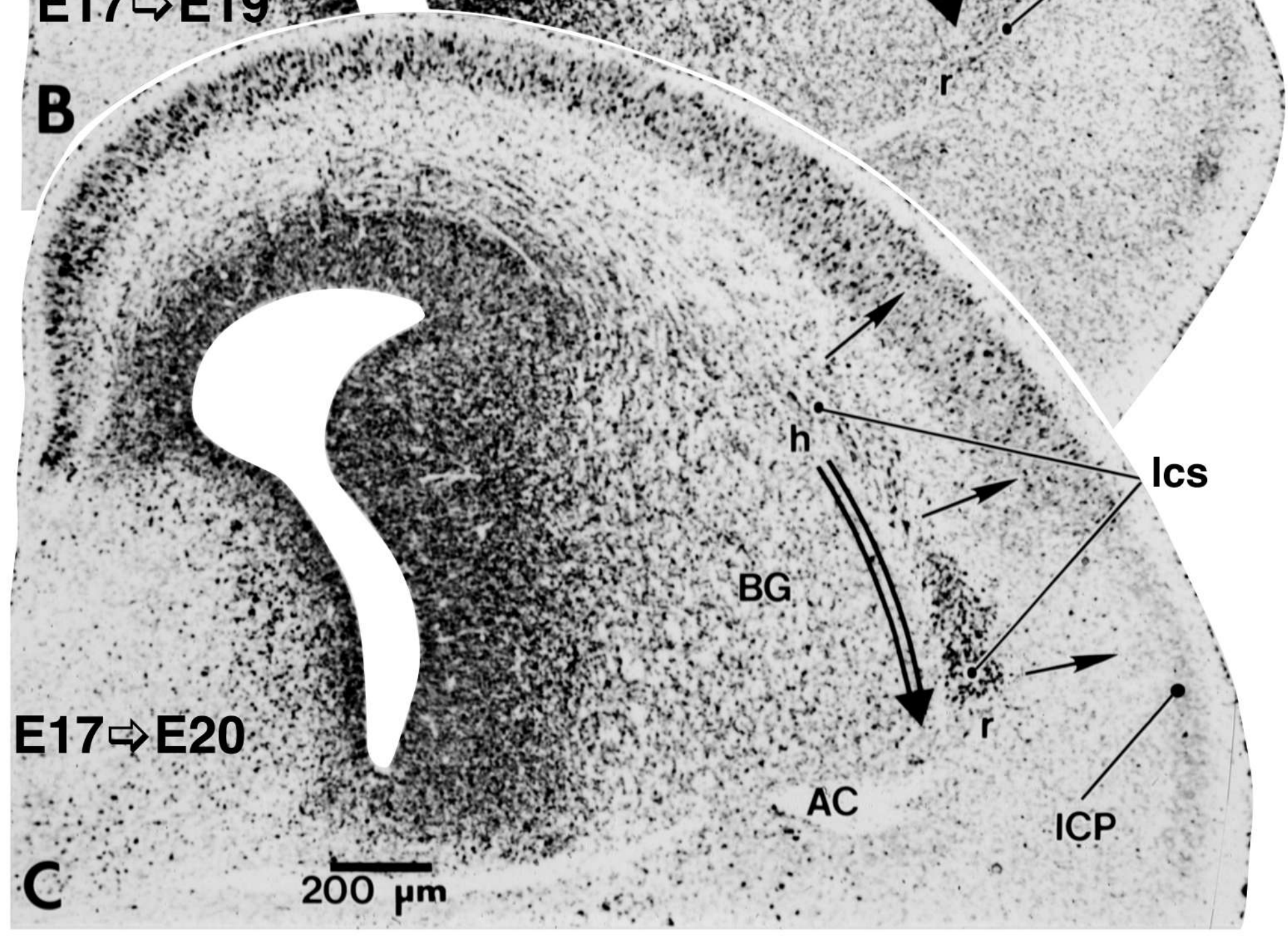
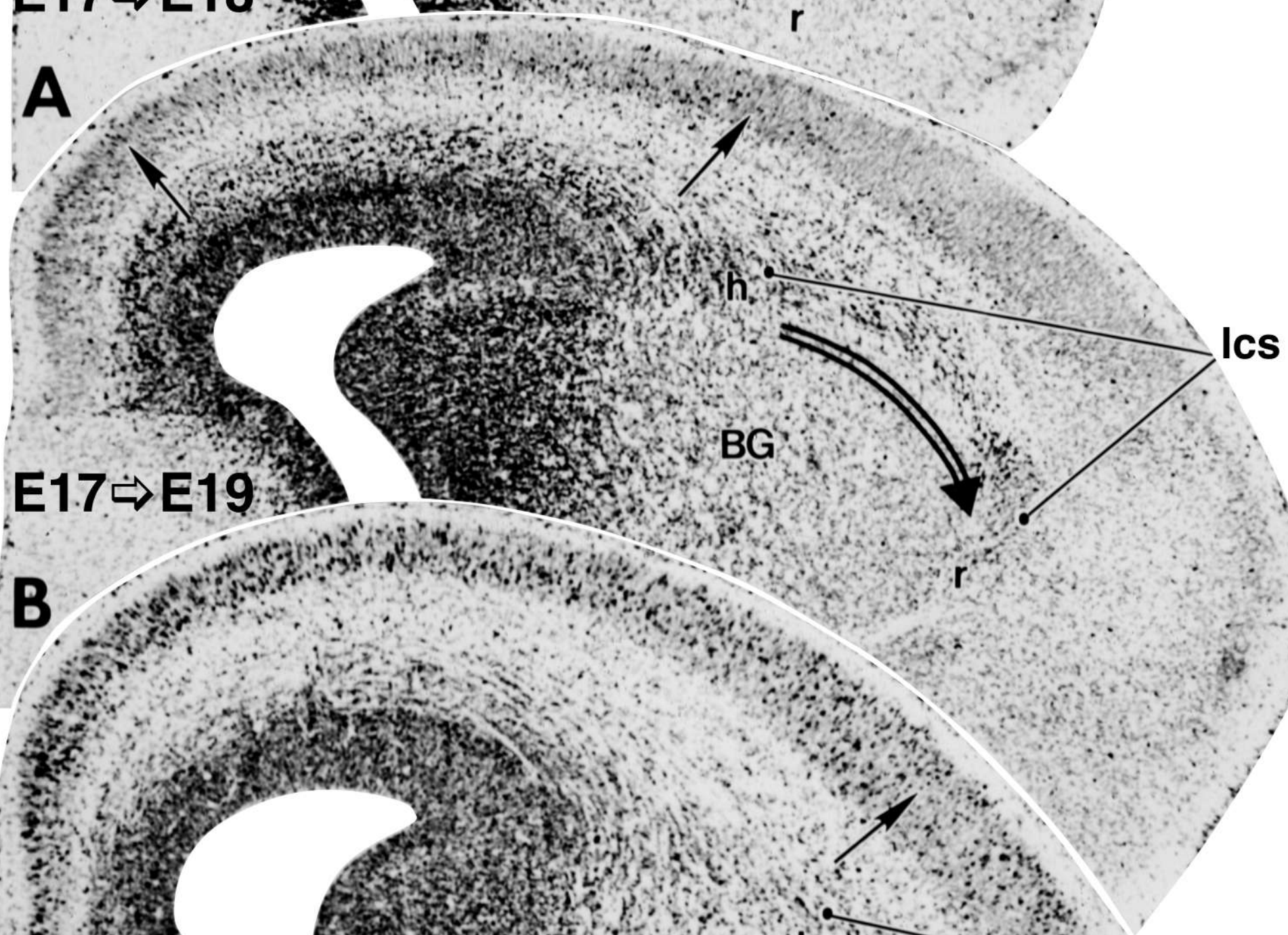
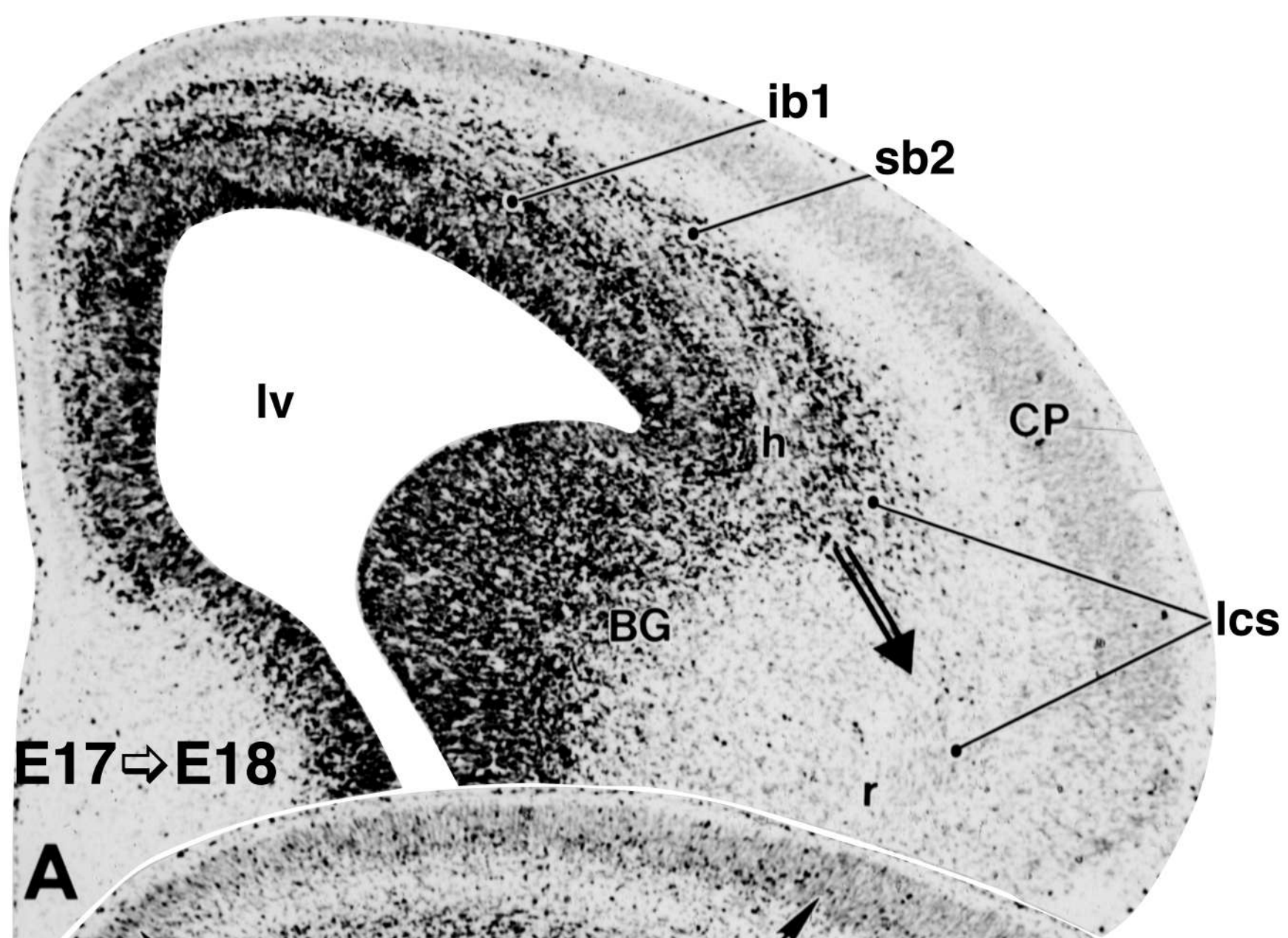


TABLE 9-1. Peak days of neurogenesis of the superficial cells (LIV-LII) in lateral and ventrolateral cortical areas^a

Cortical Area	Layer IV	Layer III	Layer II	Last cells layer II
TE1 A3.4	E17	E18	E18-E19	E20
TE3 A3.4	E17	E17	E18	E19
PAR1 A5.4	E17-E18	E18	E18-E19	E20-E21
PAR2 A5.4	E17	E17-E18	E18	E20
PAR1 A7.4	E17-E18	E18-E19	E19	E20-E21
PAR2 A7.4	E17	E17-E18	E19	E20
AI A9.2-				
A7.2	E16	E17	E18	E19
GU A9.2-				
A5.2	E16-E17	E17-E18	E19-E20	E20

^a These data are from Chapters 11-15. *Peak* is defined as the day on which the highest proportion of cells is generated in the entire span of neurogenesis for each layer. Note that many of the superficial cells are generated at times when the ventricular zone no longer extends directly beneath the respective cortical areas. Auditory areas: TE1, primary, TE3, secondary; Somato-sensory areas: PAR1, primary, PAR2, secondary; Agranular Insular area, AI; Gustatory area, GU. The numbers (A3.4-A9.2) refer to levels of coronal sections in the Pellegrino *et al.* (1979) stereotaxic atlas of the rat brain.

terior band (sb2) in the center of the intermediate zone (Chapter 7). Heavily labeled cells are also densely distributed in what we call the *head* (h) of the lateral cortical stream (lcs). The head spans the lateral edges of ib1 and sb2. On this day, the lateral cortical stream contains unlabeled cells (those generated before E17) migrating (*double arrow*) along the lateral border of

the basal ganglia (BG). There is also a dense accumulation of unlabeled cells in what we call the *reservoir* (r) at the base of the lateral cortical stream.

By E19 (Fig. 9-1B), 2 days after injection, the first neurons generated on E17 reach the dorsal neocortical plate (*single arrows*), but none have yet penetrated the lateral cortical plate. Bands of heavily labeled cells are no longer seen in the subventricular and intermediate zones, but a concentrated group of heavily labeled cells, presumably young neurons generated on E17, is now in the head (h) and in the lateral cortical stream (lcs) as it arches around (*double arrow*) the border of the basal ganglia (BG). Cells in the reservoir (r) are still mainly unlabeled, except for a few dorsal ones.

By E20 (Fig. 9-1C), 3 days after their generation, the heavily labeled neurons first arrive in the lateral cortical plate (*top two single arrows*). Many heavily labeled cells are still in the intermediate zone beneath the thin portion of the cortical plate that will presumably differentiate into the insular area (ICP, *lowest single arrow*). By now, the head (h) of the lateral cortical stream contains only lightly labeled cells. The many heavily labeled cells that were in the head on E19 are migrating in the lateral cortical stream (lcs) and are now distributed throughout the reservoir (r).

On E21 (Fig. 9-2), 4 days after their generation, an oblique coronal section of the anterior neocortical primordium shows that the heavily labeled cells (presum-

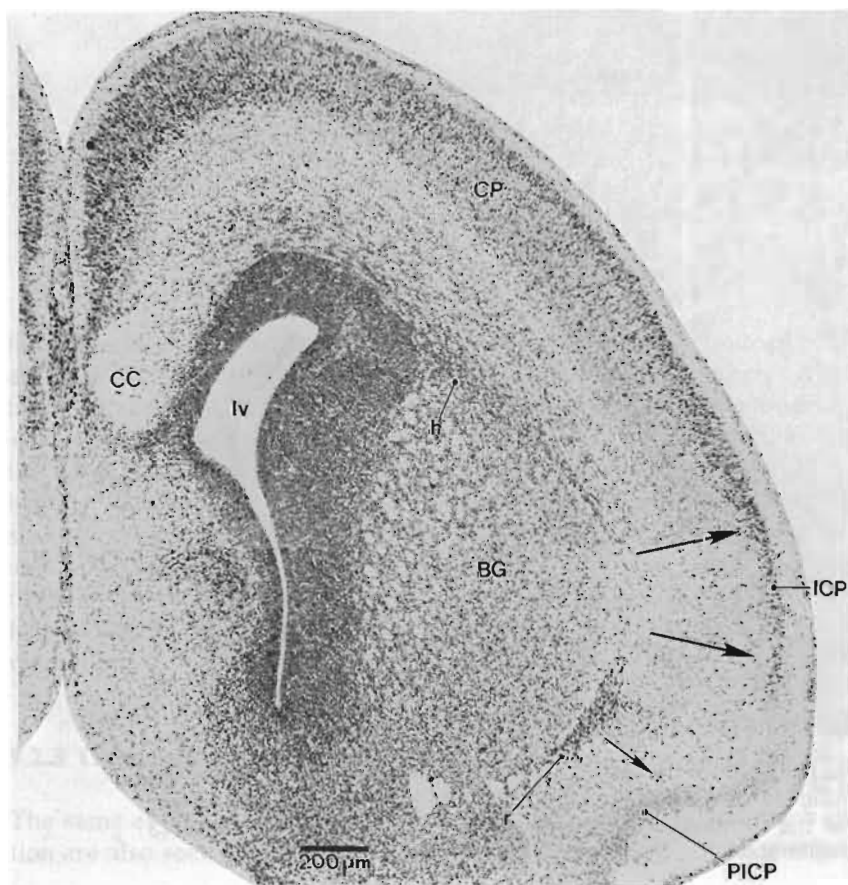


FIG. 9-2. An oblique coronal section of the anterior neocortex in an E21 rat embryonic brain that was exposed to a single injection of [³H]thymidine on E17. Four days after injection, heavily labeled cells have now penetrated the insular cortical plate (ICP) and are widely distributed in the reservoir (r). Some cells appear to be migrating from the reservoir into the piriform cortical plate (PICP). (3 μm methacrylate section, hematoxylin stain.)

E17⇒E21

CP

CC

lv

h

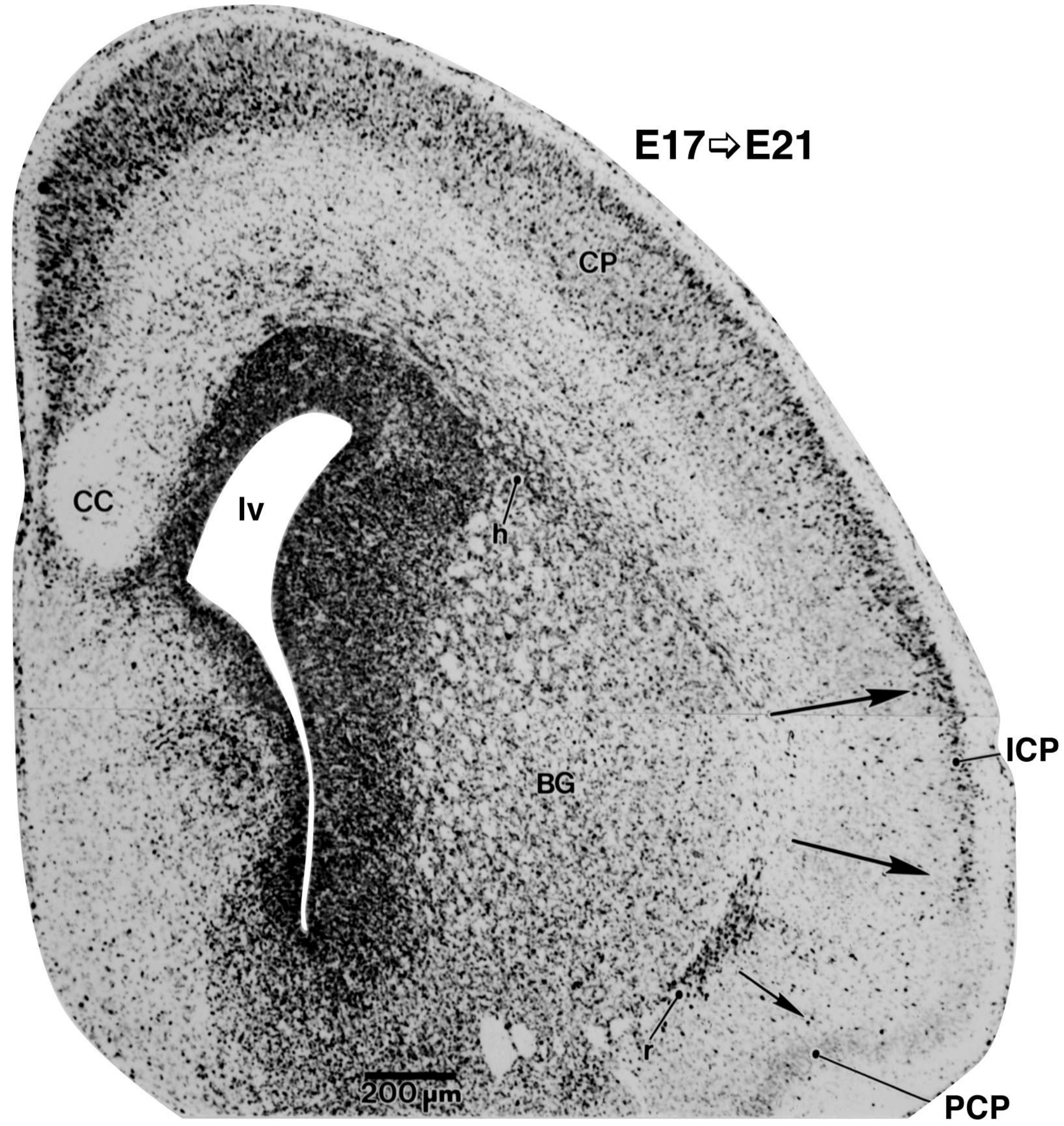
BG

ICP

r

200 μ m

PCP



ably E17 birthdays) begin to penetrate the thin insular cortical plate (ICP, *two top arrows*). By this time, there are no heavily labeled cells in the lateral cortical stream (now indistinct), but many are still in the reservoir (r). Some of these cells appear to be migrating into the deep layers of the piriform cortex primordium (PICP).

9.2.3 High Magnification Views of the Delayed Time of Arrival of Neurons in the Lateral and Ventrolateral Cortical Plate

By E19, 2 days after [^3H]thymidine injection, heavily labeled neurons are distributed throughout the dorsal cortical plate (Fig. 9-3A). Some have already reached the superficial border, others are still scattered in the upper (izu) and lower (izl) parts of the intermediate zone. In the lateral cortical plate (Fig. 9-3B), most of the labeled cells are still in the intermediate zone but a few are crossing through the subplate and are reach-

ing the deep border of the cortical plate. By E20, 3 days after [^3H]thymidine injection, heavily labeled neurons are distributed throughout the lateral cortical plate (Fig. 9-4A). However, farther ventrolaterally in the insular cortical plate (ICP, Fig. 9-4B), most of the heavily labeled cells are still in the lateral cortical stream (lcs, Fig. 9-4B), a few of them are migrating radially (*arrow*) through the upper intermediate zone. Those cells will not reach the superficial parts of the insular cortical plate until E21 (Fig. 9-2).

9.2.4 High Magnification Views of Migration in the Lateral Cortical Stream and the Accumulation of Cells in the Reservoir

On E18 (Fig. 9-5), cells heavily labeled by a single [^3H]thymidine injection on E17 are concentrated in the head (h) of the lateral cortical stream (*outlined by dashed lines*). Some heavily labeled cells are sparsely scattered in the upper part of the lateral cortical stream

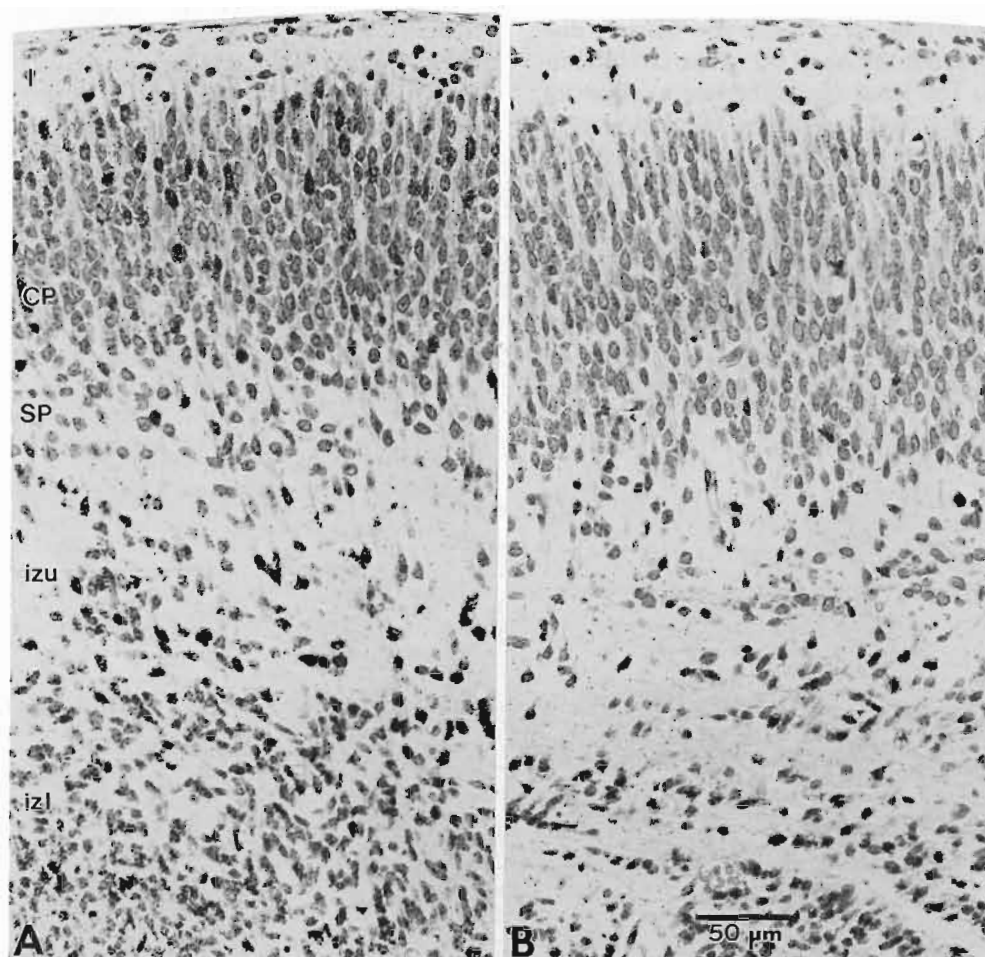
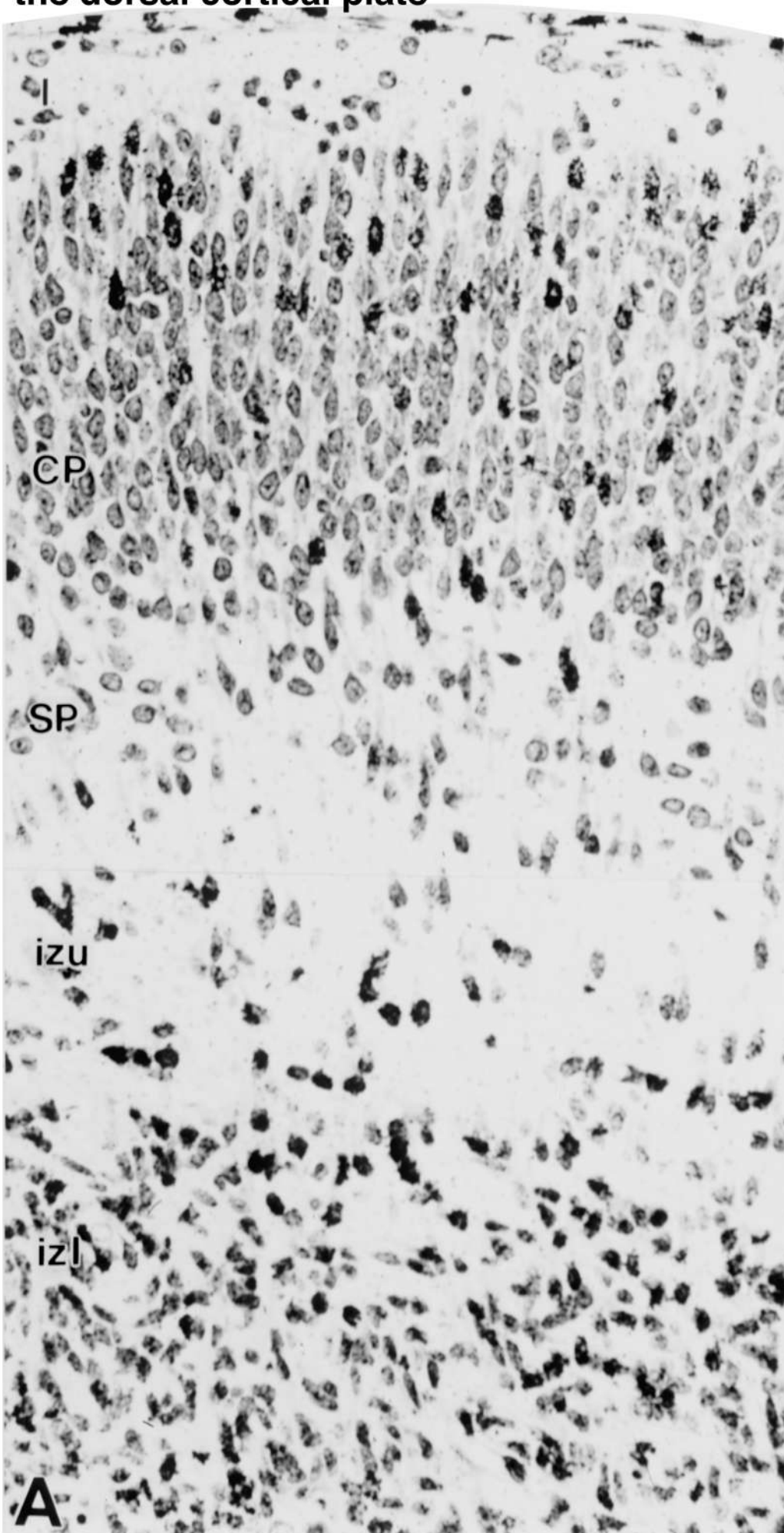


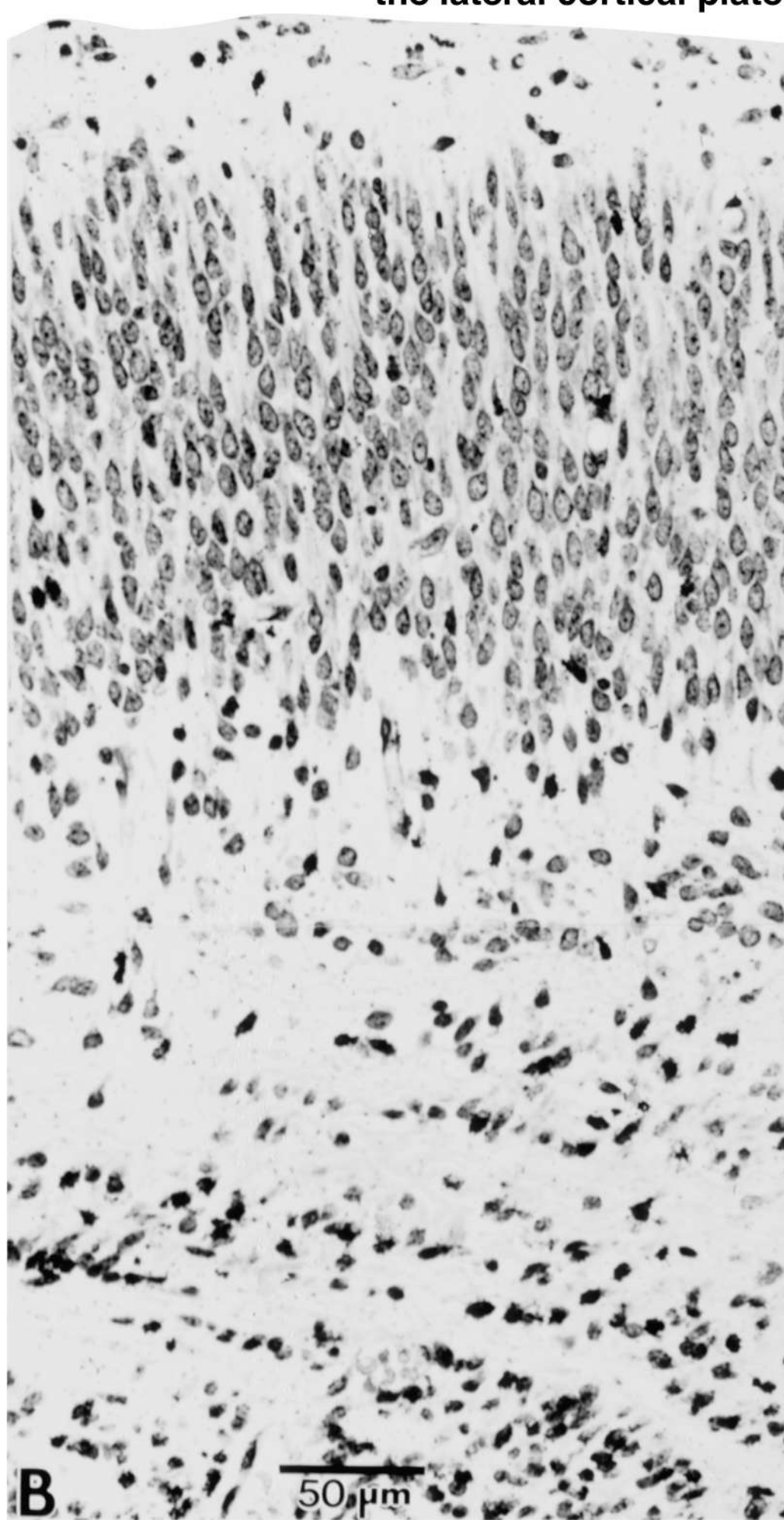
FIG. 9-3. The dorsal (A) and lateral (B) parts of the cortical plate (CP) in a rat embryo exposed to a single injection of [^3H]thymidine on E17 and killed on E19. Intensely labeled cells have reached the most superficial part of the CP in A, but are just reaching its deepest part in B. (3 μm methacrylate sections, hematoxylin stain.)

E17⇒E19

**Labeled cells are throughout
the dorsal cortical plate**



**Labeled cells are mainly in the deep part of
the lateral cortical plate**



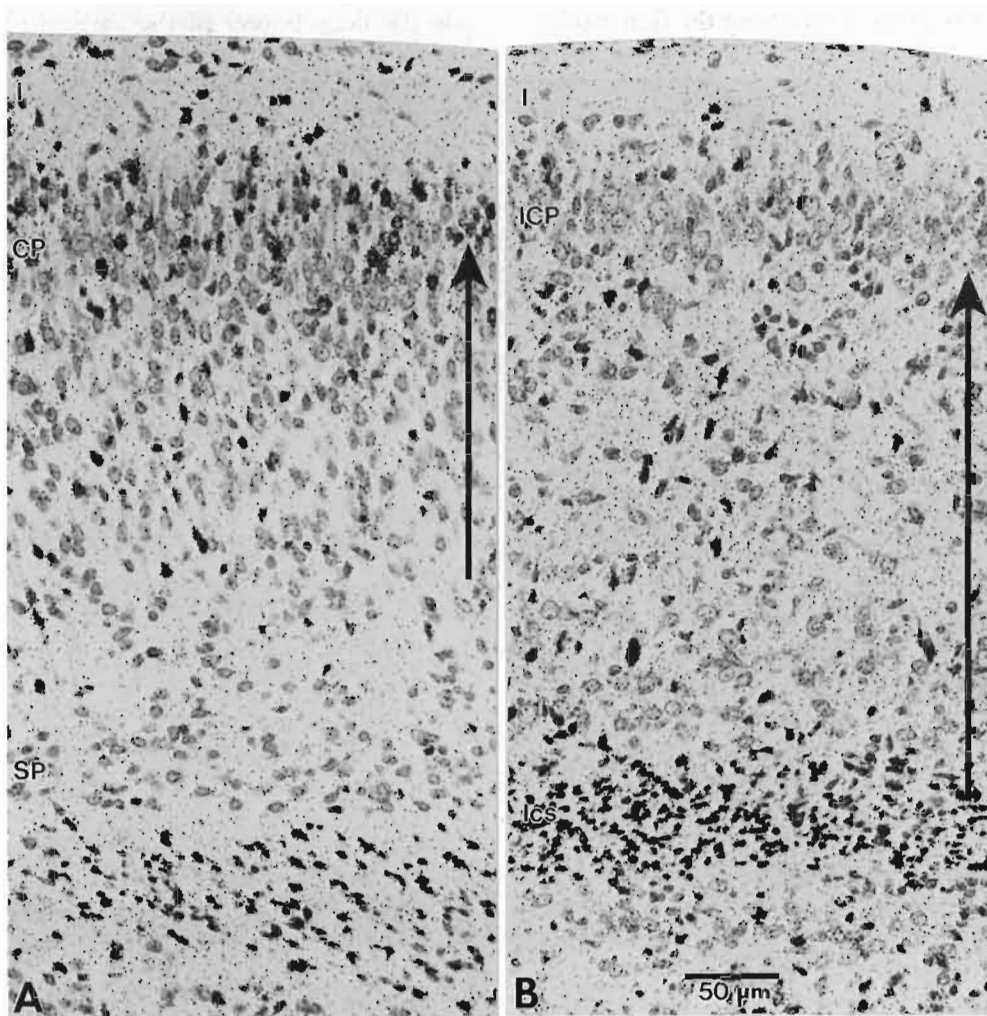


FIG. 9-4. The lateral cortical plate (CP in **A**) and ventrolateral insular cortical plate (ICP in **B**) in a rat embryo exposed to a single injection of [^3H]thymidine on E17 and killed on E20. Intensely labeled cells have reached the most superficial part of the CP in **A** but are just reaching its deepest part in **B** and many are still in the lateral cortical stream (lcs). (3 μm methacrylate sections, hematoxylin stain.)

but none have yet reached its lower part or the reservoir (r). By E19 (Fig. 9-6A), the heavily labeled cells have left the head, are migrating in the lateral cortical stream (*large double arrow*), and are beginning to enter the dorsal part of the reservoir. On E20 (Fig. 9-6B), heavily labeled cells are leaving the lateral cortical stream and the reservoir (*single arrows*). Many of them are in the lateral cortical plate; others are migrating toward the ventrolateral cortical plate. However, heavily labeled cells are still abundant throughout the reservoir.

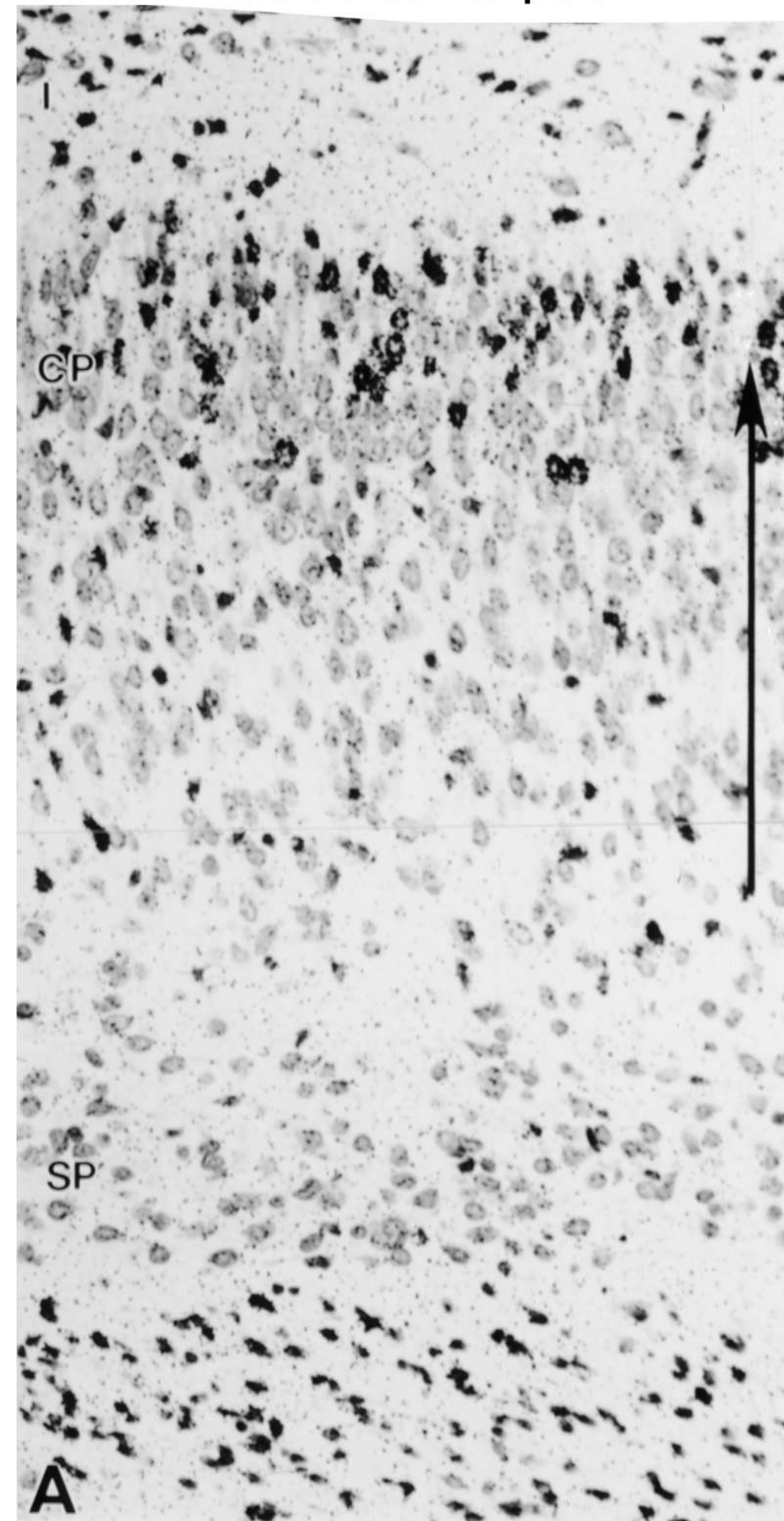
9.2.5 Observations in the E18 Injection Group

The same events that are seen following an E17 injection are also seen in sequential-survival [^3H]thymidine

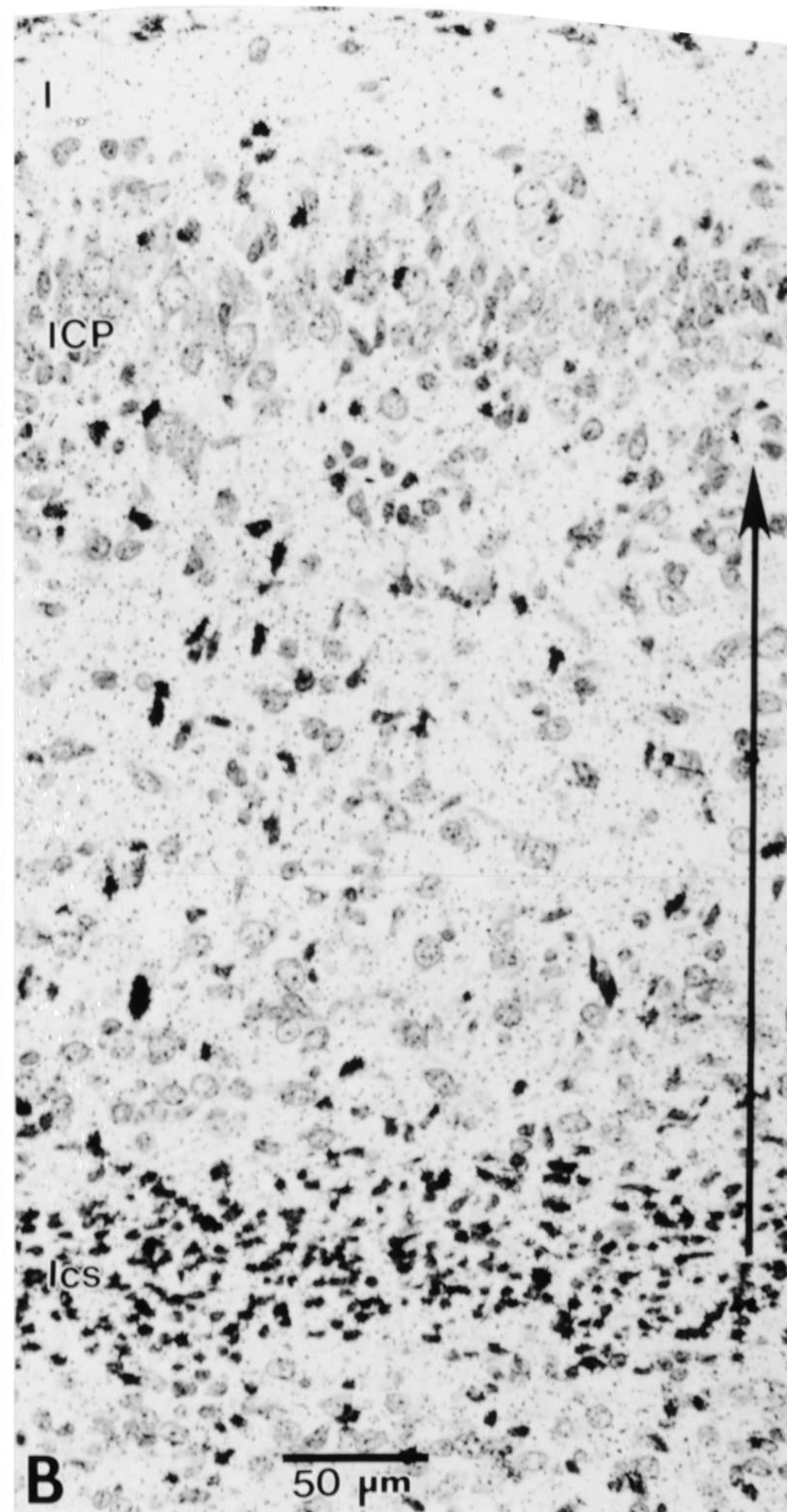
autoradiograms after an E18 injection. Figure 9-7 shows that the heavily labeled cells, presumably generated on E18, have penetrated the dorsal cortical plate by E20; that is shown at higher magnification in Figure 9-8A. However, the lateral cortical plate (CP) and the insular cortical plate (ICP) are devoid of labeled cells (Fig. 9-7). The high-magnification view of the lateral cortical plate in Figure 9-8B shows a few scattered labeled cells, but these are presumed to be locally multiplying glial cells and endothelial cells, not neurons. The labeled neurons are still in the lateral cortical stream (*large curved arrow*, Figs. 9-7 and 9-8B). In subsequent survival times (not shown), the neurons generated on E18 were found to have the same delay in arrival as those generated on E17. It takes 3 days to reach the lateral cortical plate and 4 days to reach the insular cortical plate.

E17⇒E20

**Labeled cells are superficially
located in the lateral cortical plate**



**Labeled cells are mainly in the deep part of
the insular cortical plate**



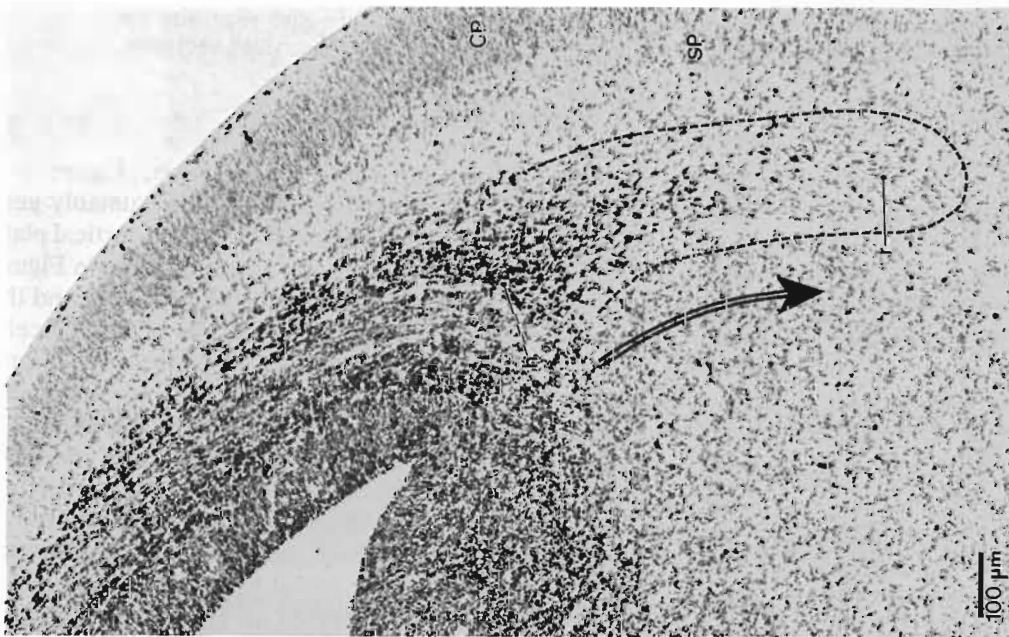


FIG. 9-5. The lateral cortical stream (outlined) including the head (h) and the upper part of the reservoir (r) in a rat embryonic brain exposed to a single injection of [3 H]thymidine on E17 and killed on E18. Heavily labeled cells are in the head and are beginning their migration into the lateral cortical stream but have not yet reached the reservoir. (3 μ m methacrylate section, hematoxylin stain.)

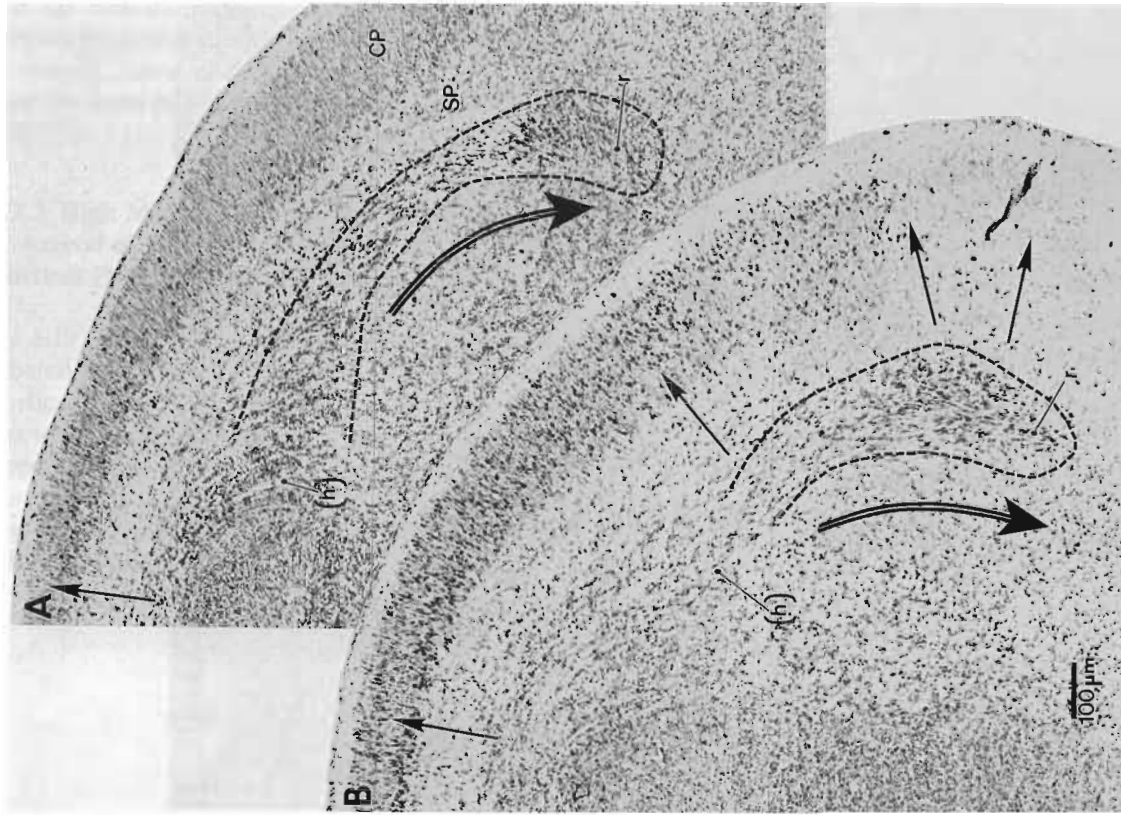


FIG. 9-6. The lateral cortical stream and reservoir (outlined) in rat embryonic brains exposed to a single injection of [3 H]thymidine on E17 and killed on E19 (A) and E20 (B). Heavily labeled cells are in the dorsal part of the reservoir by E19 and are throughout its extent by E20. Heavily labeled cells leave both the stream and the reservoir (lateral arrows in B) to penetrate the lateral cortical plate and move toward the insular cortical plate. (3 μ m methacrylate sections, hematoxylin stain.)

The lateral cortical stream (outlined)
in an E17⇒E18 rat embryo.

Note the lack of labeled
cells in the reservoir (r).

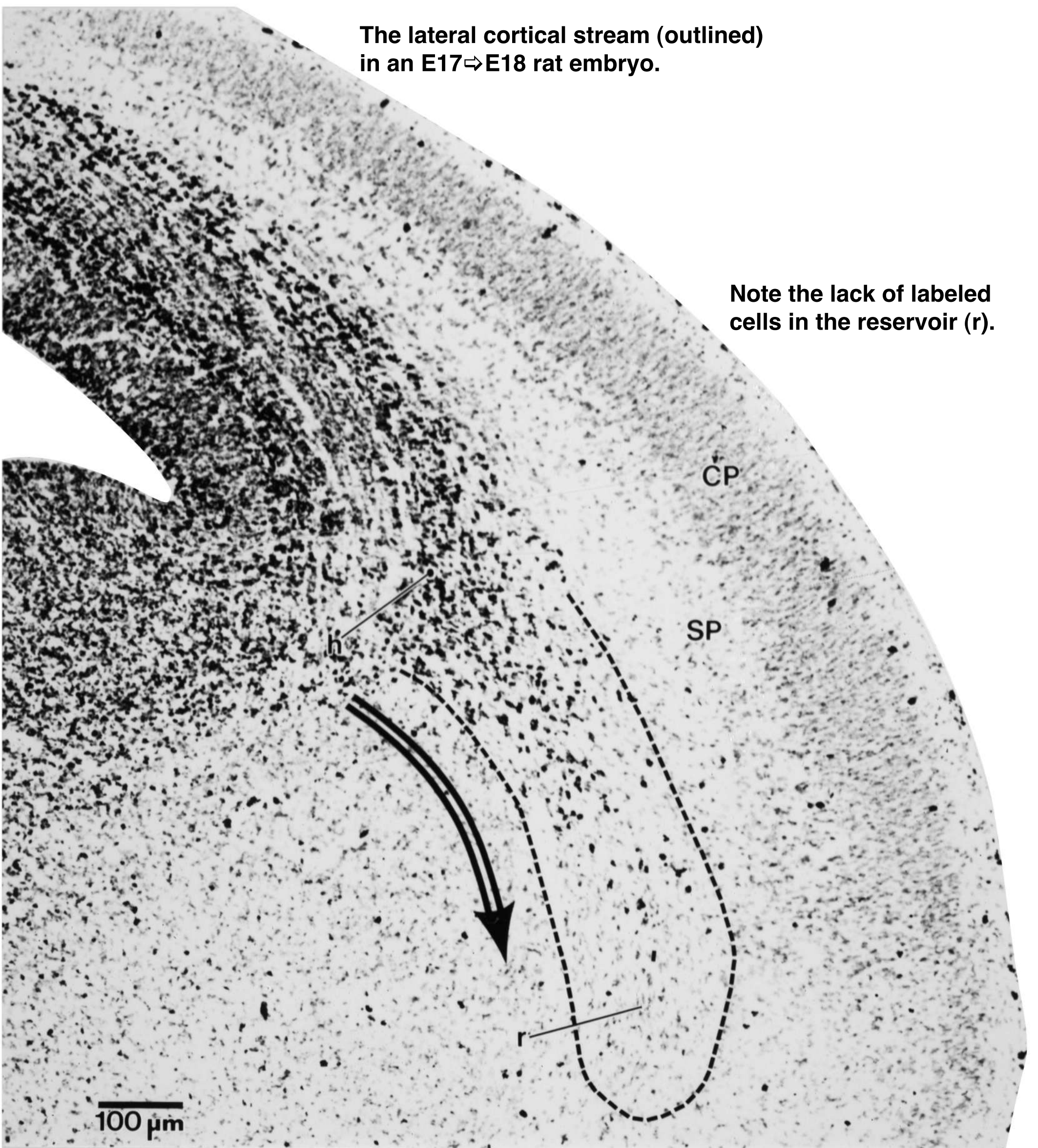
CP

SP

h

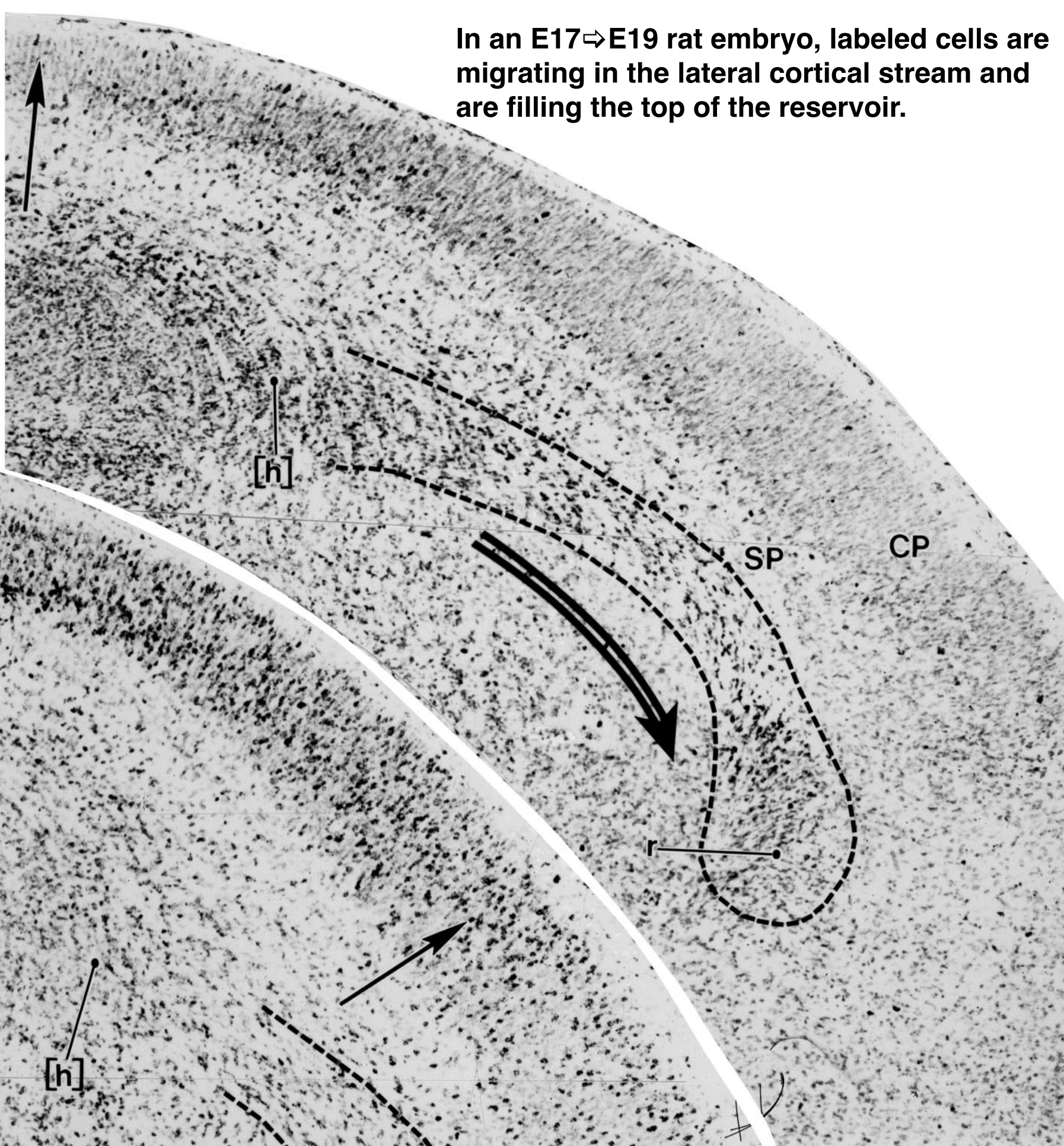
r

100 μm

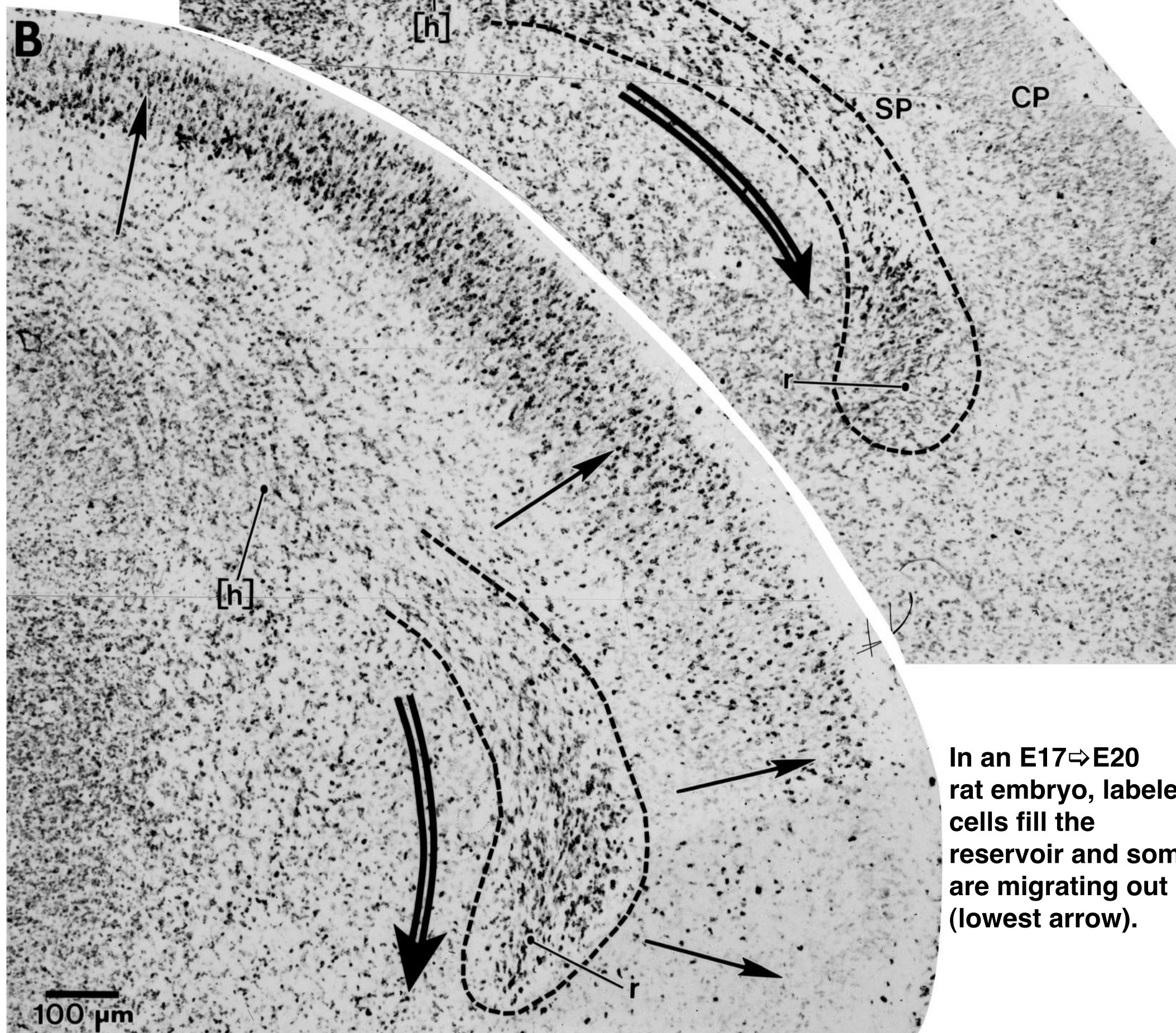


A

In an E17⇒E19 rat embryo, labeled cells are migrating in the lateral cortical stream and are filling the top of the reservoir.

**B**

In an E17⇒E20 rat embryo, labeled cells fill the reservoir and some are migrating out (lowest arrow).



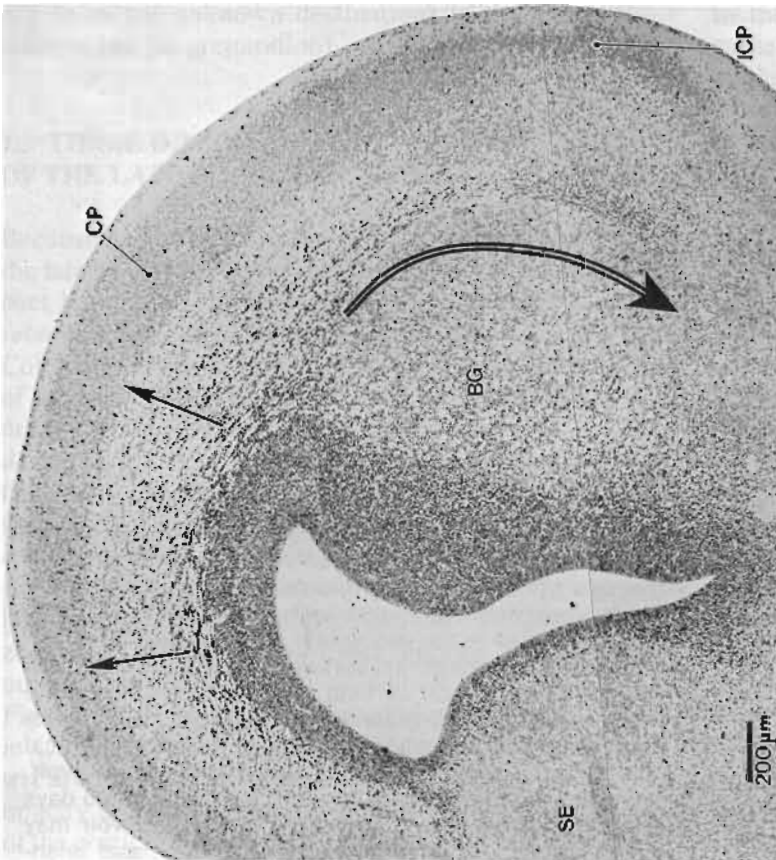


FIG. 9-7. The anterior neocortex in a rat embryo that was exposed to a single injection of [^3H]thymidine on E18 and was killed on E20. Two days after injection, heavily labeled cells have penetrated the dorsal cortical plate (CP) and ventrolateral insular cortical plate (ICP). Heavily labeled cells are migrating into the lateral cortical stream (*large curved arrow*) around the basal ganglia (BG). (3 μm methacrylate section, hematoxylin stain.)

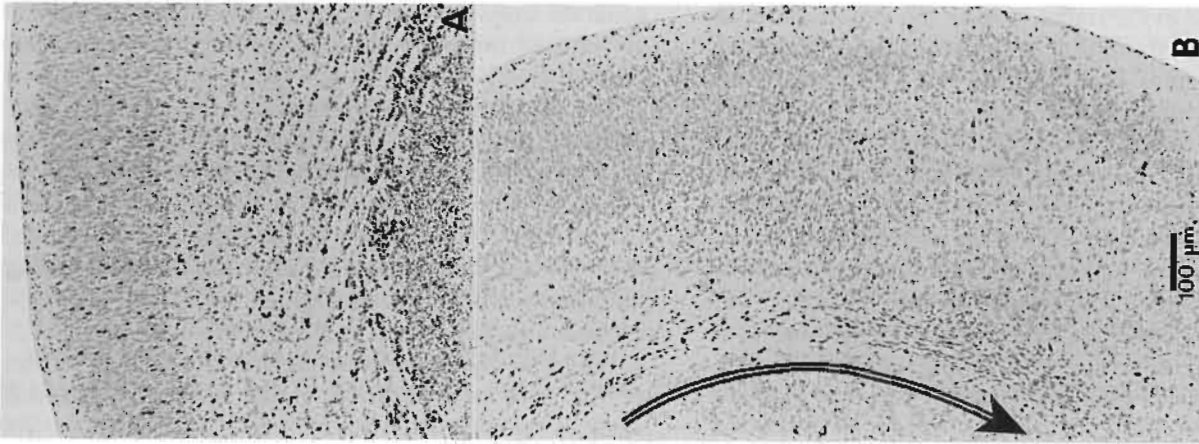


FIG. 9-8. High-magnification views of the dorsal cortical plate (A) and ventrolateral cortical plate (B) from the same section shown in Fig. 9-7. Heavily labeled cells (young neurons) are in the cortical plate dorsally, but laterally they are still in the lateral cortical stream (*large curved arrow* in B). The sparsely scattered labeled cells in the cortical plate in B are most likely locally multiplying glial cells.

E18⇒E20

Note the prominent tangential migration in the lateral cortical stream (large curved arrow).

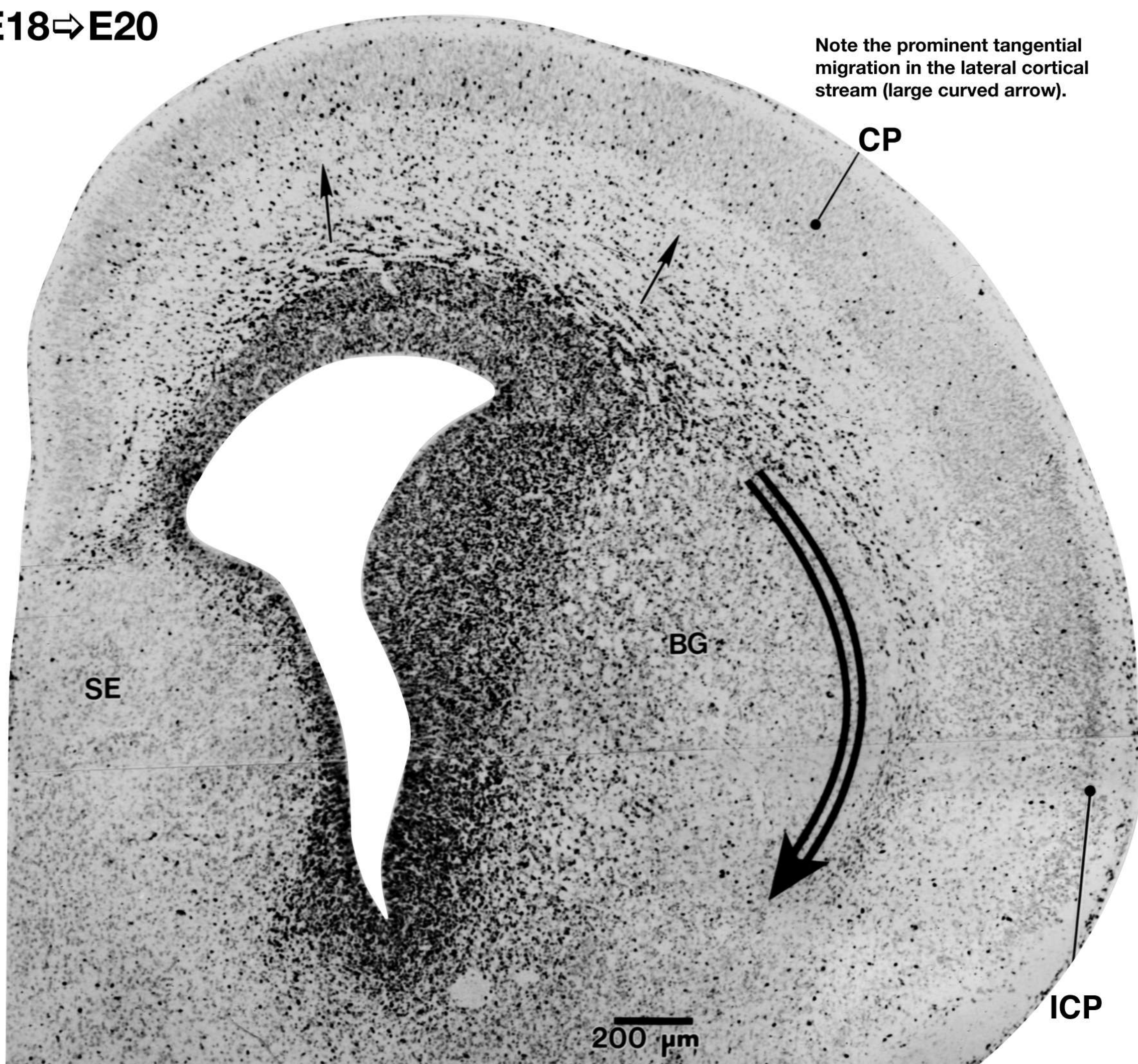
CP

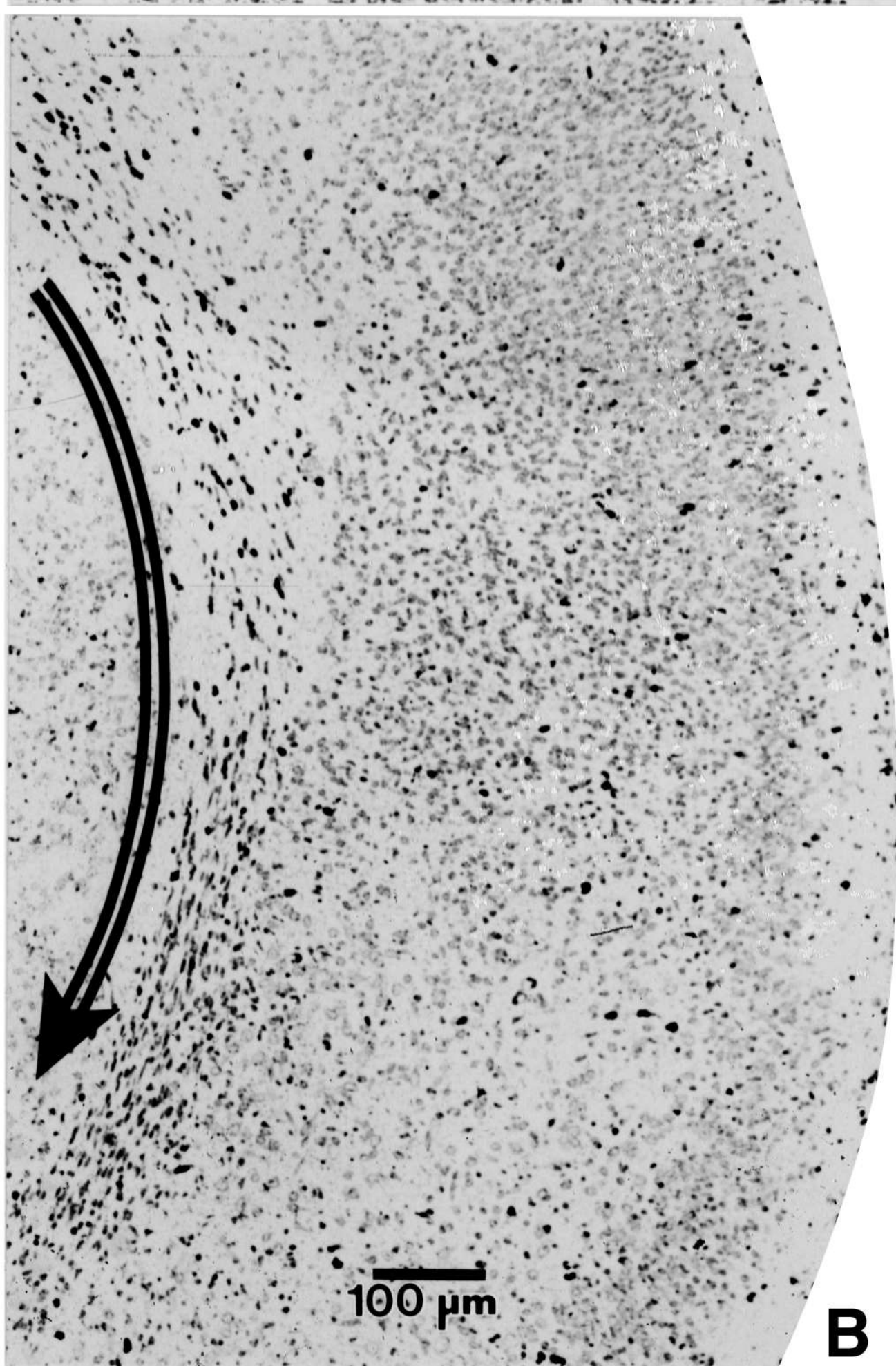
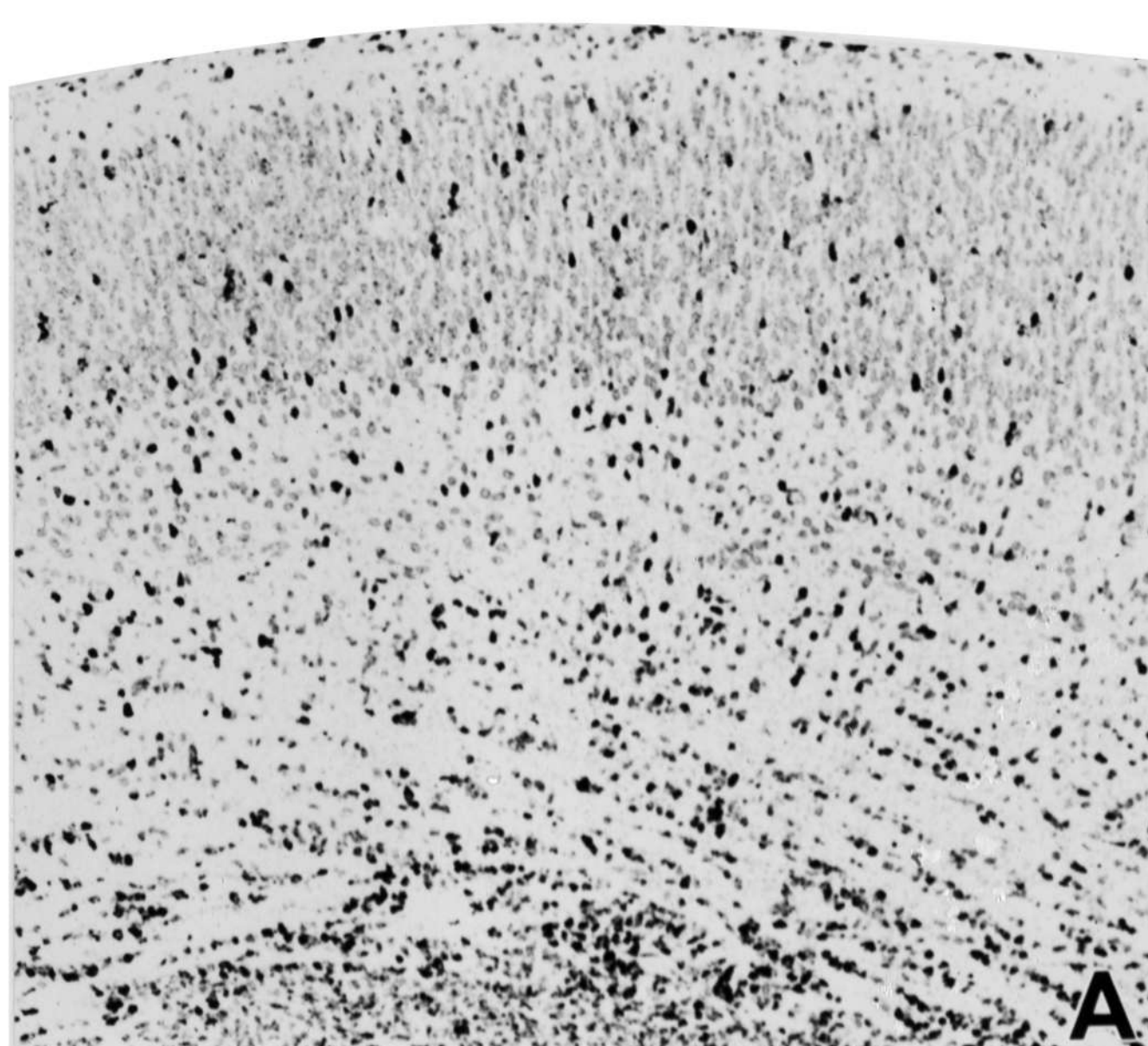
BG

SE

ICP

200 μ m





9.2.6 Summary and Comments on the Thymidine Autoradiographic Data

The findings in sequential-survival [^3H]thymidine autoradiograms (summarized in Fig. 9-9) show two migratory paths: (1) a radial one to the dorsal cortical plate and (2) a lateral one to the lateral and ventrolateral cortical plate. The latter path was inferred by observing sequential shifts in the positions of heavily labeled cells in the lateral cortical stream (Figs. 9-1 and 9-2). A radial path in the dorsal cortex and a lateral path in the lateral cortex have also been inferred after tracking the dispersal of clonally related cells in the developing mouse cerebral cortex after retroviral infection (Austin and Cepko, 1990). Depending on the migratory path and the distance traveled, there is a progressive delay in the time of arrival of neurons in the cortical plate. Neurons that migrate only radially arrive in 2 days in the dorsomedial neocortex (shortest distance). Those that migrate laterally arrive in 3 days in the lateral neocortex (longer distance) and in 4 days in the ventrolateral neocortex (longest distance). A surprising finding is that the neocortical ventricular zone generates cells that settle (5 or more days later)

outside of the neocortex, in the piriform cortex and in other sites in the basal telencephalon (question mark, Fig. 9-9). In fact, sequential-survival [^3H]thymidine autoradiograms after a pulse label on E15 indicates that the ventricular zone, which heretofore was thought to generate only neocortical neurons, is a major source of neurons throughout the piriform cortex (in preparation).

The possibility that neurons generated in the neocortical ventricular zone migrate outside of the neocortex can be inferred from a recent study of the ontogeny of cholinergic neurons in the mouse (Schambra et al., 1989) where young cholinergic neurons appear to be leaving the lateral cortical stream and penetrating the basal telencephalon (see Figs. 6-1D, 7-1C and 7-2E and F in Schambra et al., 1989).

We have shown earlier (Altman, 1966) that a portion of what we now describe as the head of the lateral cortical stream (a cluster of primitive cells near the lateral edge of the lateral ventricle above the striatum) contains proliferative cells in the adult rat cortex. These cells were interpreted to be locally multiplying glia. From this finding we infer that heavily labeled glia as well as neurons coexist in the head of the lateral

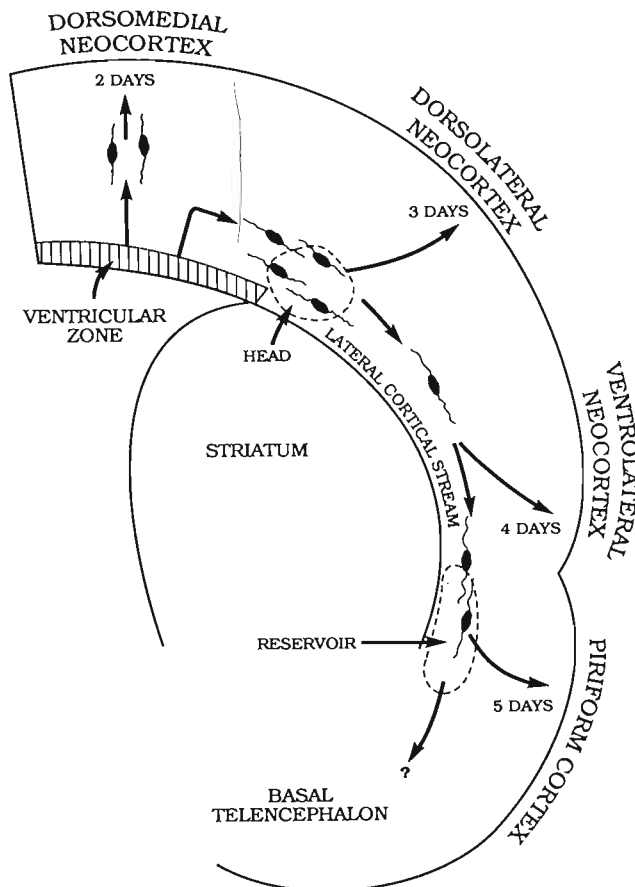
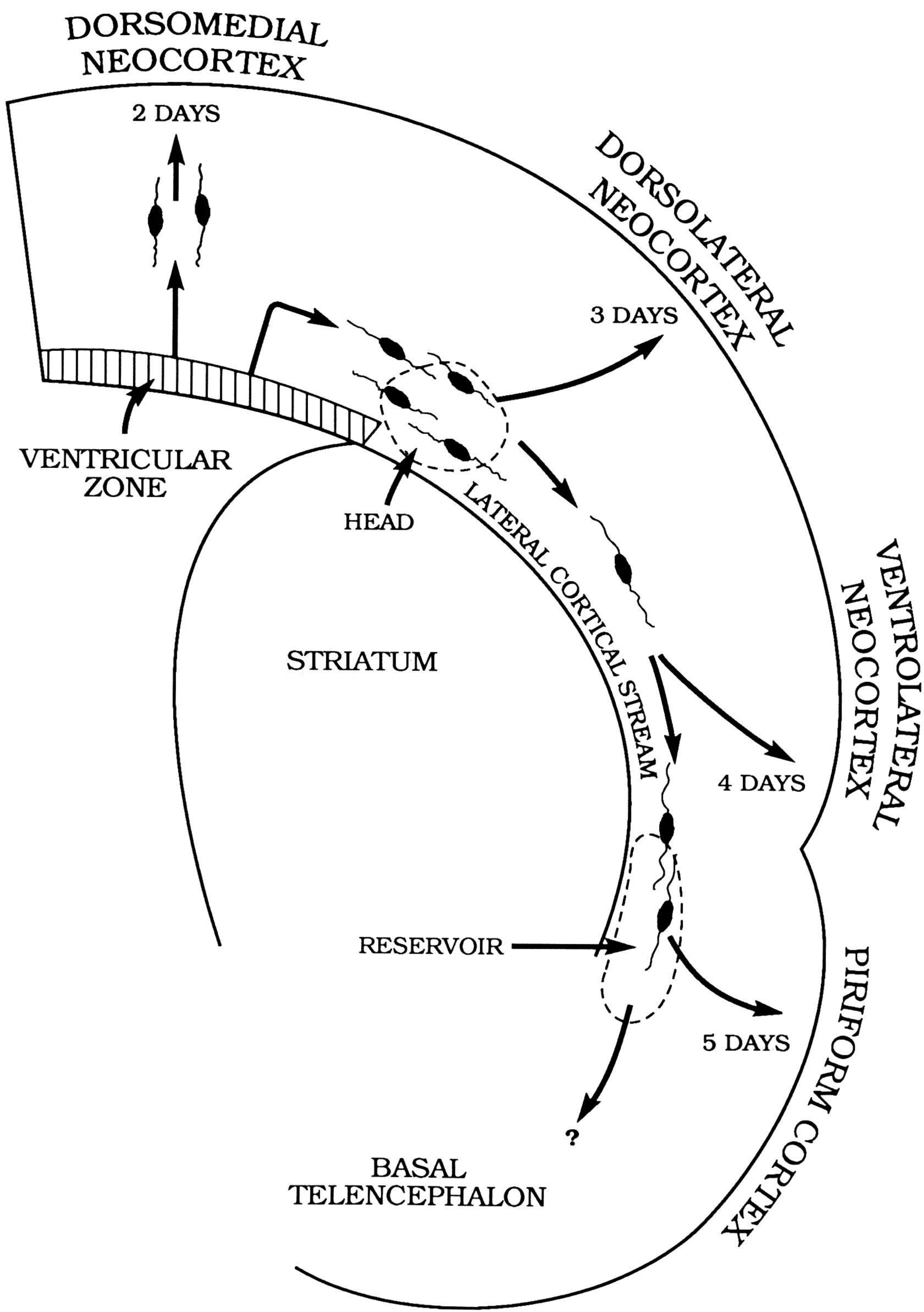


FIG. 9-9. A diagram of the major points made in this chapter regarding cell migration in the anterior and middle parts of the developing neocortex, where the striatum forms a prominent eminence in the lateral ventricle. Neurons generated in the ventricular zone (*striped layer*) migrate radially to the dorsal cortical plate in 2 days, migrate laterally to the lateral cortical plate in 3 days and to the ventrolateral cortical plate in 4 days. Some cells generated in the ventricular zone migrate in the lateral cortical stream for up to 4 days and accumulate in the reservoir. From there, some migrate into the piriform cortex 5 days after their generation. Other cells in the reservoir may move into as yet unidentified areas in the basal telencephalon (?) 5 or more days later.



cortical stream in the embryonic cortex. Since all heavily labeled cells move out of the head (Figs. 9–1, 9–5, and 9–6), it is quite likely that from the beginning of its appearance, the lateral cortical stream contains not only young migrating neurons but also glial cells that accompany the neurons. We have shown that the cortical germinal matrix becomes transformed from being a source of both neurons and glia to one that produces only nonneuronal cells after cortical neurogenesis is completed (Chapter 4). The lateral migratory stream could be understood as a pathway for both neurons and glia in embryonic brains, but only as a pathway for glia in adult brains. Since glia retain their proliferative capacity, the remnants of the head of the lateral cortical stream become pockets of locally multiplying cells in the adult brain.

The reservoir is large during late embryonic stages (E19 to E21, Figs. 9–1B, 9–1C, and 9–2), but it shrinks considerably after birth (unpublished observations). In adults, the few cells that remain in the reservoir constitute the most anterolateral intercalated mass in the amygdala (Bayer, 1980c). The reservoir tends to retain heavily labeled cells for several days after birth. For example, the cells labeled on E17 and E18 constitute 65% of the total cells in the reservoir in animals surviving to P5 but only 34% of the total in animals surviving to P60, indicating that many of these cells move out to as yet unknown destinations in the basal telencephalon (in preparation).

9.3 THREE-DIMENSIONAL RECONSTRUCTIONS OF THE LATERAL CORTICAL STREAM

Because [³H]thymidine autoradiograms showed that the lateral cortical stream (LCS) was a relatively compact bundle in the lower intermediate zone, that was reconstructed (Color Fig. 8) in the E19 brain shown in Color Figs. 6 and 7. Every tenth section (100 μ m apart) of the neocortex was reexamined and an outline was drawn around the head, body, and reservoir of the densely packed spindle-shaped cells streaming out of the lateral edge of the neocortical germinal matrix and skirting the border of the basal ganglia; these three components were reconstructed together as the lateral cortical stream (LCS, blue, Color Fig. 8). A single outline delineated the ventricular and subventricular zones (VZ + SV, white, Color Fig. 8), and another outline delineated the cortical plate (CP, green, Color Fig. 8). All three structures are shown in a medial view in Color Fig. 8A. The cortical plate and germinal matrix are shown at full opacity; the LCS is shown at 50% opacity in order to see the shorter ventrolateral edge of the cortical plate behind the LCS. Note that the LCS disappears as soon as the ventrolateral edges of the

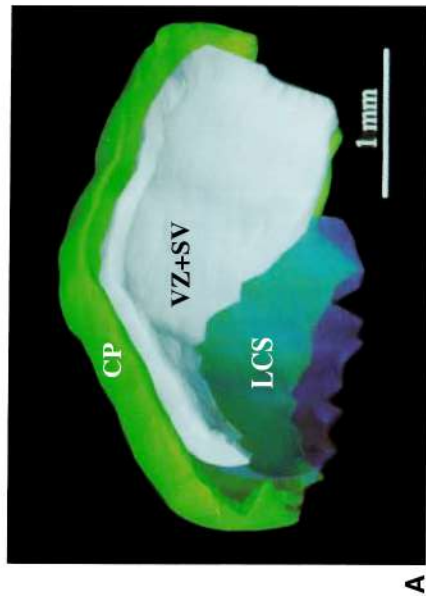
germinal matrix and the cortical plate become coextensive in the posterior cortex. All three structures are shown in a front view in Color Fig. 8B. The cortical plate is shown at 50% opacity and appears as a veil draped over the front edges of the germinal matrix (VZ + SV) and the LCS. (The cleft in the frontal pole is where the medial wall was cut away in the reconstruction.) In order to better view the front edges of the germinal matrix and the LCS, Color Fig. 8C is a front view with the anterior third of the cortical plate stripped away, making the swollen head and reservoir of the LCS more readily visible. The close apposition of the head of the LCS and the lateral edge of the anterior germinal matrix is easily seen. Color Fig. 8D shows the structures rotated -35° in the Y axis so that the ventrolateral edges of the cortical plate and the LCS are more easily seen. The cortical plate is shown at 50% opacity so that the LCS is visible beneath it. Notice that the LCS extends farther ventrolaterally than does the cortical plate.

9.4 CELL ORIENTATION IN THE INTERMEDIATE ZONE BENEATH ANTERIOR AND POSTERIOR PARTS OF THE DORSAL NEOCORTEX

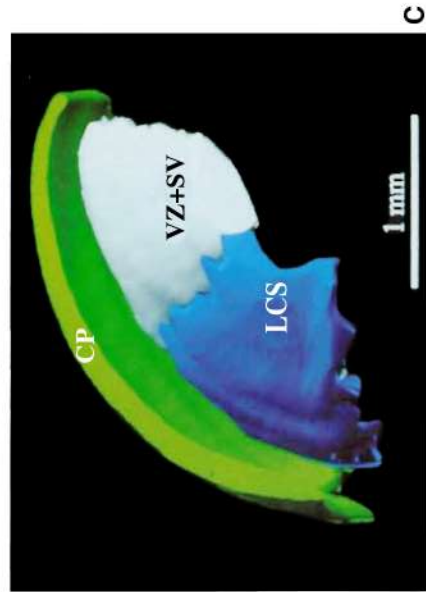
In the sequential survival autoradiograms described earlier, the lateral cortical stream is most prominent in the anterior three-fourths of the neocortical primordium where the basal ganglia occupy a large part of the lateral telencephalon. In more posterior coronal sections, the lateral cortical stream becomes progressively less noticeable and disappears as soon as the sections are behind the basal ganglia. As that change is taking place, the cortical ventricular zone becomes broader, extending farther beneath the lateral cortical wall. In the most posterior sections, the ventricular zone and the cortical plate have the same mediolateral span (Color Fig. 7). Heavily labeled cells arrive simultaneously at both dorsal and lateral sites in cortical sections behind the basal ganglia (not shown), indicating that lateral cell migration probably does not occur in the most posterior neocortical primordium. In Chapter 8 and in Bayer et al., 1991a, we reported that horizontally oriented nuclei predominate in the anterior intermediate zone of the dorsal neocortex where the ventricular zone has shifted dorsomedially and where cells are migrating laterally. Would cells with horizontally oriented nuclei predominate in a posterior level of the dorsal neocortex where the ventricular zone has not shifted dorsomedially and where cells are not migrating laterally?

To answer that question, we quantitatively investigated whether nuclear orientation was different in the

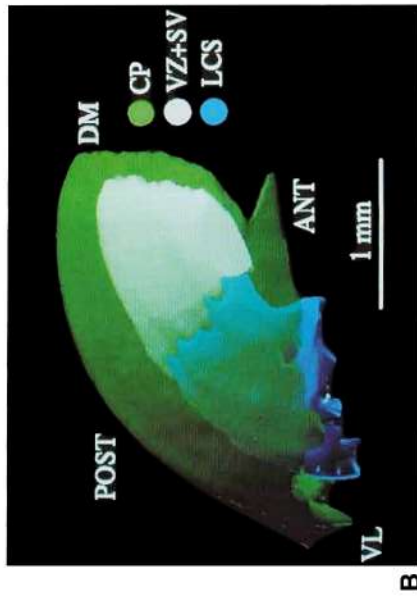
Color Figure 8 is on the next page.



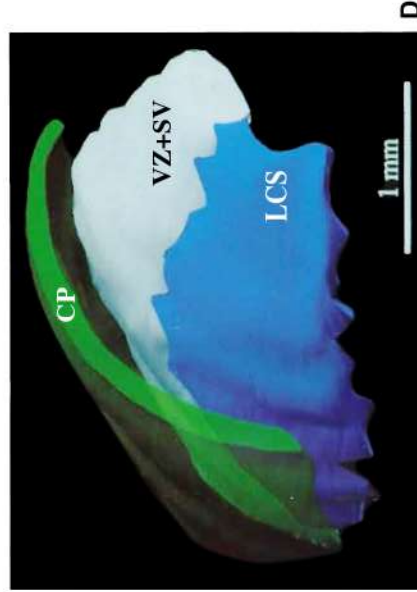
A



C

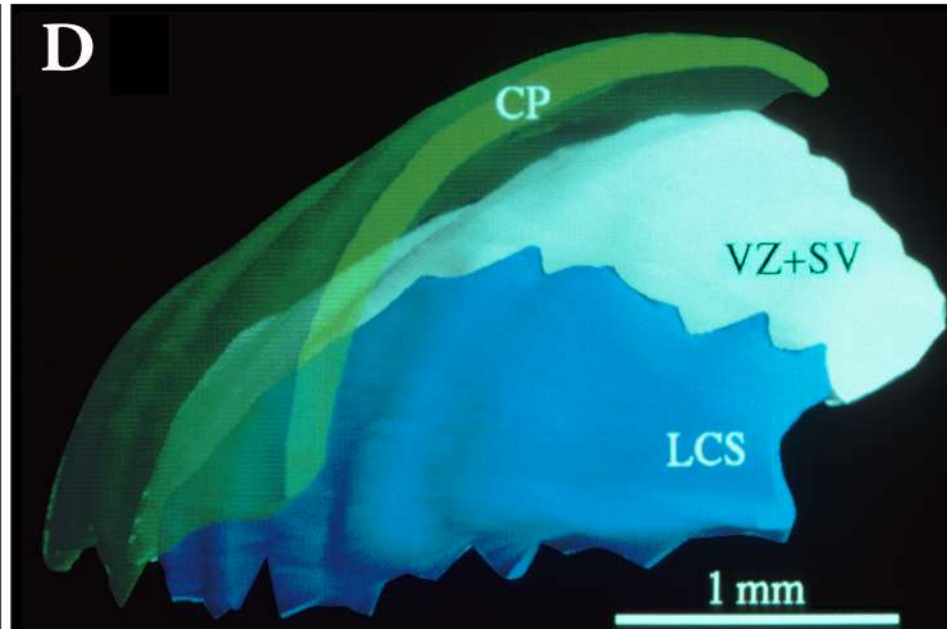
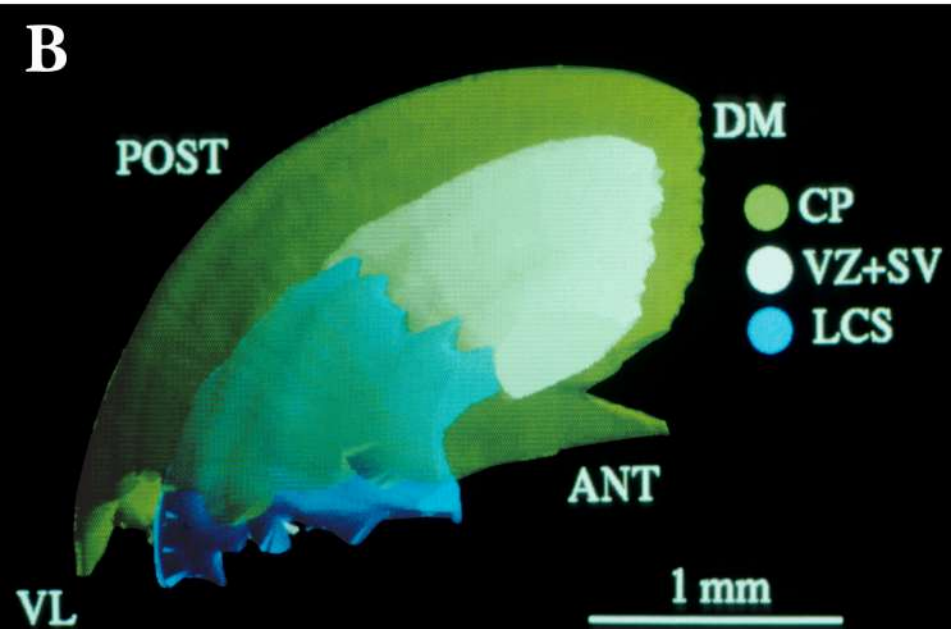
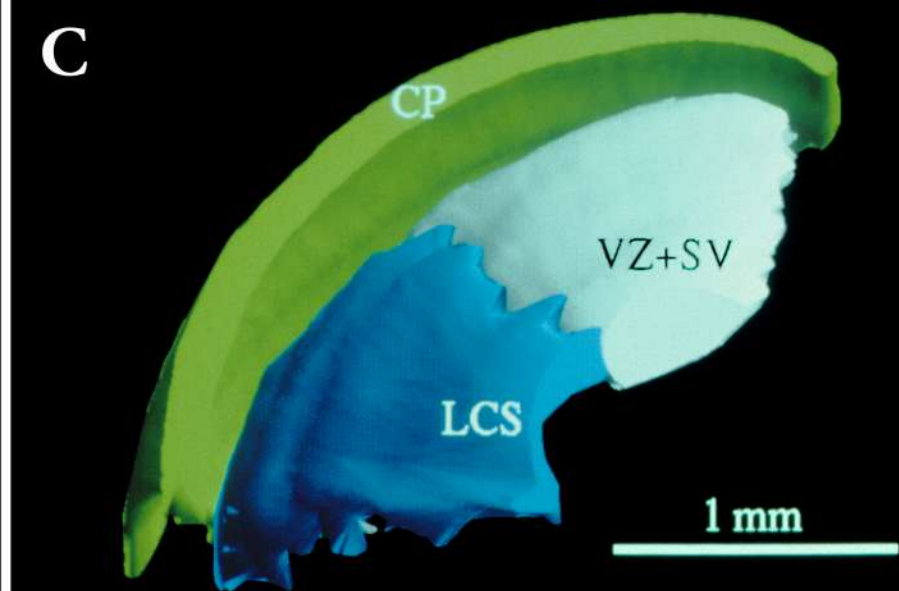
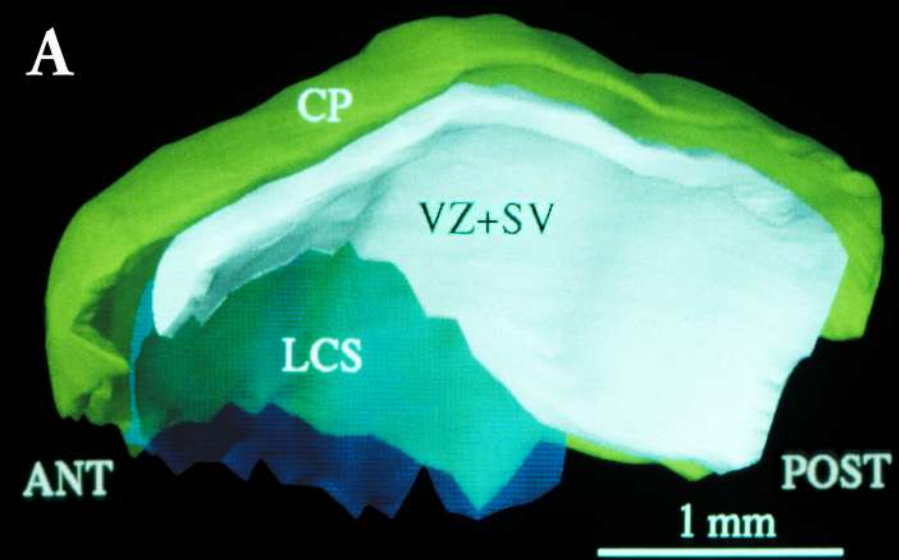


B



D

COLOR FIG. 8. Skandha-generated images of the cortical plate (CP, green), the lateral cortical stream (LCS, blue), and the germinal matrix (including ventricular and subventricular zones, VZ+SV, white) in the E19 brain that was reconstructed in Color Figs. 6 and 7. **A** is a medial view with the LCS shown at 50% opacity so that the CP can be seen behind it. **B** is a front view (Y axis rotation -90°) with the CP shown at 50% opacity so that the VZ+SV and the LCS can be seen beneath the CP. **C** is also a front view with the anterior third of the CP stripped away. **D** is a side view (-35° Y axis rotation from the view in **C**) with the CP shown at 50% opacity. Note that the LCS extends below the ventrolateral edge of the CP, and cannot be detected posteriorly where the CP and VZ+SV are coextensive.



intermediate zone of the anterior versus the posterior dorsal neocortex (drawings, Fig. 9–10; see Appendix 5). The anterior section examined was at the decussation of the anterior commissure, while the posterior section examined was the one where the hippocampal primordium extended as far down as the ventrolateral edge of the cortical plate. Only the anterior section contained a medially displaced ventricular zone. The data of three age groups (*bar graphs*, Fig. 9–10) were selected to show the orientations of cell nuclei in the intermediate zone at early (E17), middle (E19), and late (E21) stages. On E17 (*left column of graphs*, Fig. 9–10), the anterior intermediate zone has a predominance of cells with horizontally oriented nuclei (approximately 63%), while the posterior intermediate zone has only 31% of its cells with horizontally oriented nuclei, more (36%) are vertically oriented. On E19 (*center column of graphs*, Fig. 9–10), cells with vertically oriented nuclei predominate in the posterior intermediate zone (54%), while the anterior intermediate zone still contains more cells with horizontally oriented (38%)

than vertically oriented (28%) nuclei. By E21 (*right column of graphs*, Fig. 9–10), the cells at both anterior and posterior levels have a broad distribution of nuclear orientations. The repeated measures analysis of variance indicated that the anterior and posterior strips have significantly different means ($F = 30.68$; $df = 1, 6$; $P < 0.0015$), best shown by the data on E17 and E19. There is also a significant interaction between means and age ($F = 23.22$; $df = 5, 6$; $P < 0.0007$) best shown by the shift to similarities in the data at anterior and posterior sites on E21.

To summarize, cells with horizontally oriented nuclei are more common in the intermediate zone of the anterior dorsal neocortex (where the ventricular zone does not extend laterally) than in the posterior dorsal neocortex (where the ventricular zone does extend laterally). These findings indicate that many of the horizontally oriented cells in the anterior developing neocortex must be actively migrating laterally to parts of the cortical plate devoid of an underlying ventricular zone.

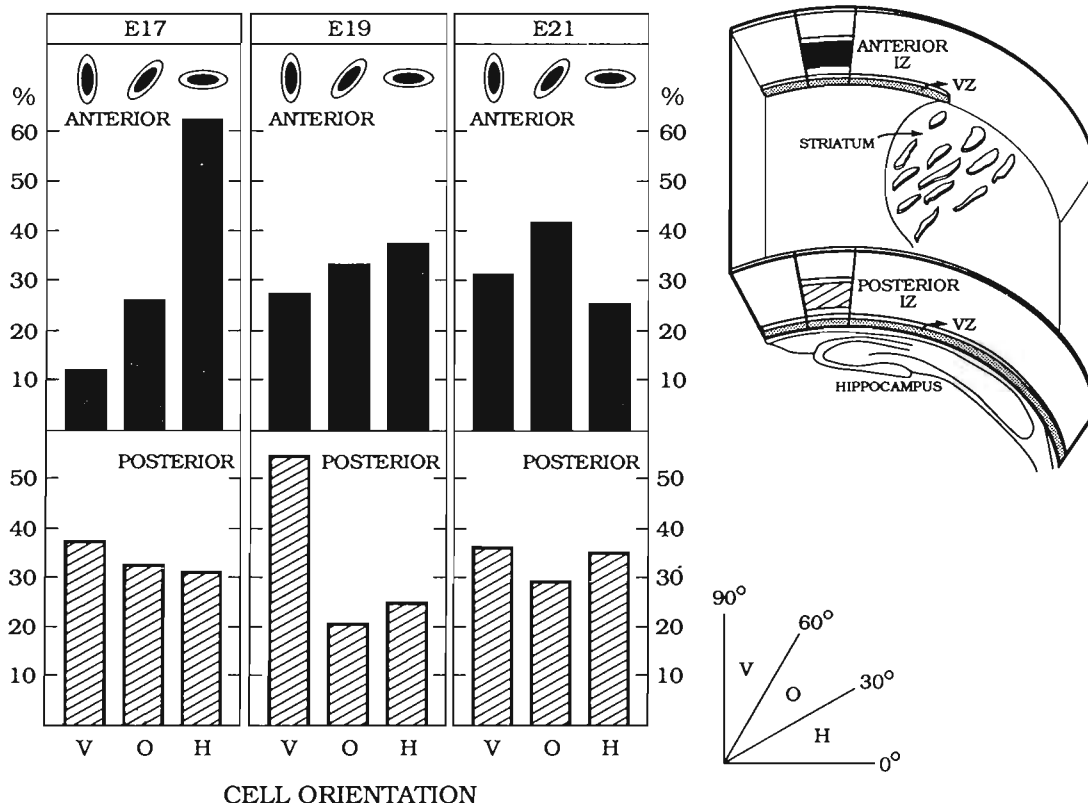
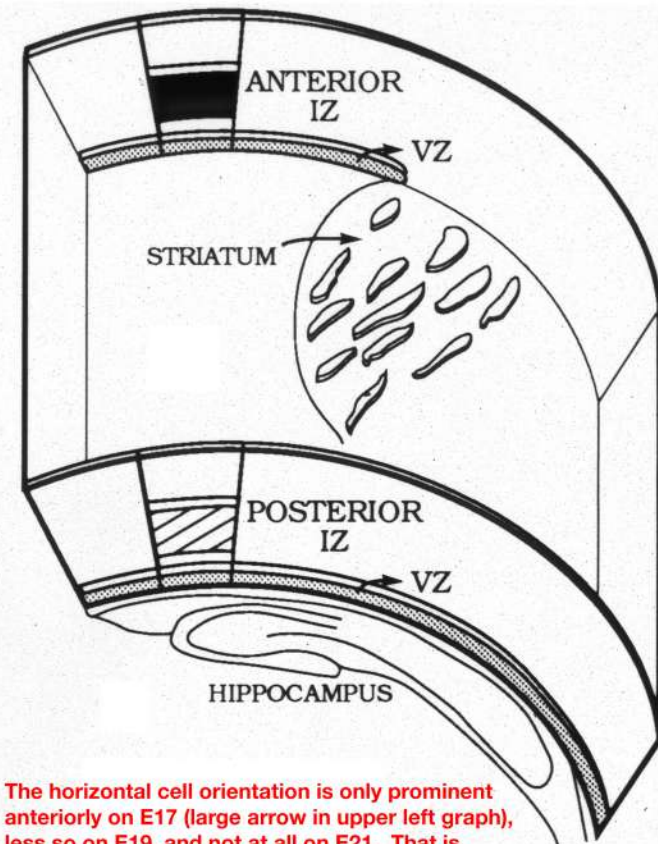
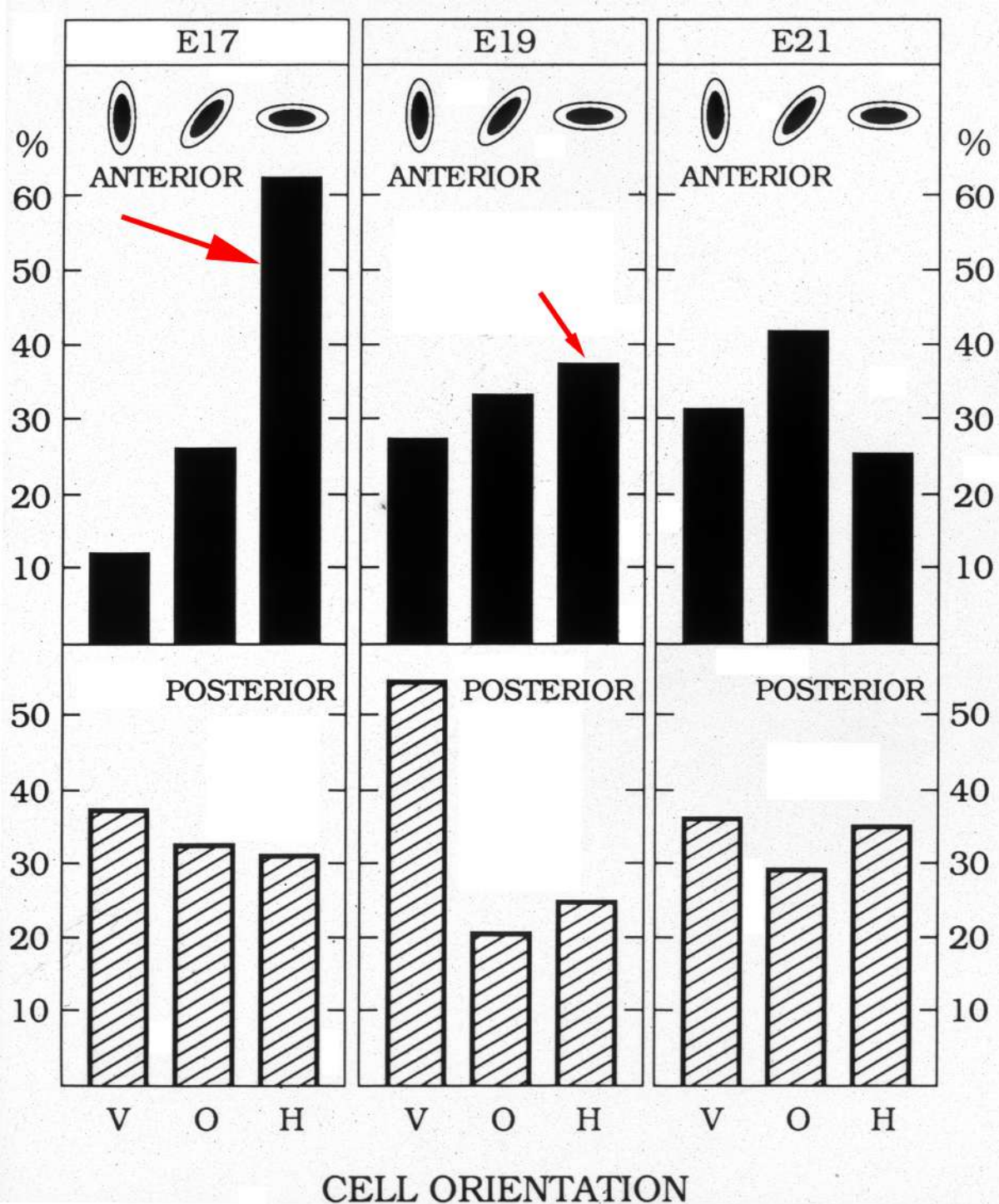
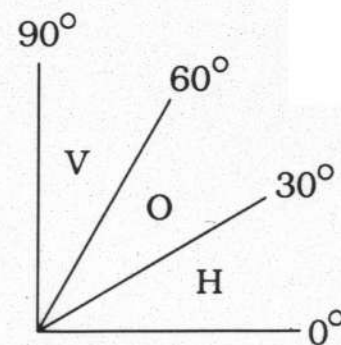


FIG. 9–10. Orientation of nuclei in the intermediate zone on E17, E19, and E21. The data are based on computer-determined measurements of coronal sections (3 μ m, methacrylate) of the dorsal neocortex at an anterior level (*black bars, upper drawing*) or at a posterior level (*striped bars, lower drawing*). Histograms indicate the proportion of cells with vertical nuclei (V, *left bars*), oblique nuclei (O, *center bars*), and horizontal nuclei (H, *right bars*). The ovals shown in the top graphs of each age represent the orientation of the cell bodies, while the line drawing in the legend shows the limits for each group in degrees of rotation above the horizontal axis (0°): horizontal group, 0°–29.9°, oblique group, 30°–59.9°, vertical group, 60°–90°.



The horizontal cell orientation is only prominent anteriorly on E17 (large arrow in upper left graph), less so on E19, and not at all on E21. That is where much of the tangential migration occurs in the developing neocortex because the striatum bulges into the floor of the lateral ventricle and deprives the anterolateral neocortex from an underlying neuroepithelium (ventricular zone).



9.5 RELATING THESE DATA TO OTHER HYPOTHESES OF NEOCORTICAL CELL MIGRATION

For close to 20 years, the literature on cell migration in the neocortex has been dominated by the radial glia hypothesis proposed by Rakic (1972, 1978, 1982, 1988). The basic assumptions are that radial glia link discrete sites in the ventricular zone to specific columns in the cortical plate, and young neurons are guided to their exact destinations in the cortical plate by migrating on radial glia fibers (Rakic, 1988). Indeed, those neurons that settle in the dorsal cortical plate follow a radial path (Figs. 9-1B, 9-3A, 9-7, and 9-8A). But the same sections show that neurons settling in the lateral and ventrolateral cortical plate follow a lateral path rather than a radial one (Figs. 9-1 to 9-8).

Most studies concerned with the radial glia fiber system have examined the dorsal neocortex where a ventricular zone is always present (Meller and Tetzlaff, 1975; Pinto-Lord et al., 1982; Goffinet, 1984; Gadisseux and Evrard, 1985; Misson et al. 1988a; Gadisseux et al., 1989). However, two studies (Misson et al., 1988b; Edwards et al., 1990) used RC1 and RC2, monoclonal antibody markers for radial glia, to examine the lateral and ventrolateral neocortex in mice. They found a dense immunoreactive band in the same position as the lateral cortical stream that we describe here. The low magnification photographs in these publications do not allow us to distinguish between glial cells with short processes (which we postulate migrate with neurons) and true radial glia, which have cell bodies in the ventricular zone and long processes extending to the pial surface. A Golgi study by Smart and Sturrock (1979) in the mouse telencephalon presented evidence of a circumferential fiber band around the basal ganglia extending from the head of the LCS to the reservoir. The band consists of scattered perikarya with shorter and longer processes, described by Smart and Sturrock as "ependymogial cells." Of particular interest is the photograph (Smart and Sturrock, 1979: Fig. 7) of a mouse telencephalon on E16 (approximately corresponding to E18/E19 in rats), which shows

that the band in the LCS has two components: a larger set that radiates outward to the lateral and ventrolateral cortical plate and a smaller one that extends into the reservoir. (Preliminary examination of our Golgi material in E18-E20 rats confirm that observation.)

Obviously, the original radial glia hypothesis requires some modification. First, the cells migrating laterally follow a circumferential rather than a radial path. Second, the presumed glial cells in the LCS, as visualized with the Golgi technique (Smart and Sturrock, 1979), do not have the exact topographic features of radial glial cells with perikarya located in the germinal matrix. Further research with immunohistochemical techniques that can distinguish between glial cells and migrating neurons are needed to clarify the identity of the cells with shorter and longer processes.

Lateral migration of neocortical neurons has been suggested by others. Hicks and D'Amato (1968) proposed that the cells migrate along the incoming thalamocortical axons. The Golgi studies of Valverde et al., (1989) prompted the authors to surmise that the horizontal orientation of cells in the lower intermediate zone could be lateral migration. After infecting ventricular zone cells with a histochemically detectable retrovirus Walsh and Cepko (1988) noted that clusters of marked cells, presumably clones, appear to be migrating tangentially to the surface of the ventricular zone. More recently, Austin and Cepko (1990) tracked clones of cells produced after retroviral infection in the developing mouse neocortex and essentially have come to similar conclusions about the lateral migration of cortical neurons that we described. (Bayer et al., 1991b).

It is intriguing that the migrating neurons leave the lateral cortical stream at several points and rotate radially to enter the cortical plate at specific loci. Since thalamocortical fibers are growing into the cortex in the vicinity of the lateral cortical stream, there is ample opportunity for interaction between them and the laterally migrating neurons, as suggested by Hicks and D'Amato (1968). Thalamocortical fibers may determine where and when a particular neuron will leave the stream and migrate radially into the cortex (see Chapter 16).