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Horizontal Compartmentation in the Germinal Matrices and Intermediate Zone of the Embryonic Rat Cerebral Cortex

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Cellular compartmentation was studied in the germinal matrices and the intermediate zone of the cerebral cortex of rat embryos that survived for 1 or more days after injection with [^{3}H]thymidine. In contrast to the vertical compartmentation seen in the neuroepithelium with short-survival thymidine autoradiography, sequential-survival autoradiography revealed a horizontal compartmentation both in the germinal matrices and the intermediate zone. In the neuroepithelium of embryos that survived for 24 h, the differentially labeled cells form two distinct horizontal bands. The band overlapping with the mitotic zone is composed of heavily labeled cells, whereas the band overlapping with the synthetic zone is composed of lightly labeled cells. This indicates that there are two proliferative cell populations within the neuroepithelium, one turning over fast and the other more slowly. In the cortical intermediate zone of the same embryos several horizontal bands are present. Of these, the dispositions of two bands of heavily labeled cells—the superior band and the inferior band—were followed for several days. The superior band is apparently composed of glial cells that disperse in the direction of the internal capsule and the corpus callosum. In contrast, the inferior band (which overlaps with the subventricular zone where many cells are horizontally oriented) is apparently composed of sojourning young neurons. The cells of the inferior band resume their migration toward the cortical plate after a pause of 1–2 days. These observations call for a reappraisal of the view that young cortical neurons follow a direct radial path to the cortical plate. © 1990 Academic Press, Inc.

INTRODUCTION

The autoradiographic observations reported in the preceding paper (3) were based primarily on an examination of embryos that survived for *2 h after labeling with* [^3H]*thymidine* (short-survival autoradiography). We focused there on cellular heterogeneity in the germinal matrices of the cerebral cortex with special reference to the vertical (or columnar) organization of the neuroepithelium in young embryos. In this paper we make use primarily of observations made in embryos that survived for *one to several days after labeling with* [^3H]*thymidine* (sequential-survival autoradiography) and focus on the horizontal compartmentation of cells with different labeling patterns. This horizontal stratification is evident not only in the germinal matrices of the cortex but also in the intermediate zone.

The intermediate zone (5) is a transient component of the developing cerebral cortex situated above the germinal matrices and beneath the formative cortical gray matter, the cortical plate, and the cortical subplate. It is an extremely heterogeneous field, consisting of migrating young neurons, dispersing glial cells, and a welter of afferent and efferent fibers and glial processes. As yet we have little understanding of the developmental transformations taking place in the transient intermediate zone and of their functional significance. This paper seeks a reappraisal of the implicit current conceptualization of the intermediate zone as a static framework in which young neurons steadfastly migrate on a radial path to the cortex. We provide thymidine radiographic evidence that the intermediate zone contains several cellular compartments, manifested as transient bands with different labeling patterns, where cells of different ages (in terms of time after leaving the germinal matrix) and of different types (in terms of their ultimate disposition) pause for a while prior to their migration or dispersal.

MATERIALS AND METHODS

Animals. Purdue–Wistar rats bred in our laboratory were used. Adult females were paired with males in the evening and vaginal smears were taken the next morning. Females found sperm-positive were isolated in maternity cages and were considered to have reached Gestational Day 1. Dams ranging in gestational ages between Days 12 and 21 were injected with [^{3}H]thymidine at daily intervals. An uninjected group of pregnant females was the source of control embryos.

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Injection of [^3H]thymidine. The dams were injected subcutaneously with a single dose (5 μCi/g body wt) of [^{3}H]thymidine (Schwarz-Mann, sp act, 6.0 Ci/mmol) between 9:00 and 11:00 AM. Survival time after injection was varied in each injection group from 2 h (short-survival) to several days after injection (sequential-survival).

Histological and autoradiographic procedures. At the designated times the dams were anesthetized and the embryos or fetuses were removed and immediately killed by immersion in Bouin's fluid. After 24 h, the specimens were transferred for storage in 10% neutral formalin. The specimens were blocked coronally, sagittally, or horizontally and embedded in either paraffin or methacrylate. The paraffin blocks were serially sectioned at 6 μm and the methacrylate blocks at 3 μm, and every tenth section was saved. Successive sections were either stained with cresyl violet and hematoxylin–eosin or were first prepared for autoradiography. The latter were coated with Kodak NTB-3 nuclear emulsion, exposed for 12 or 18 weeks with a desiccant, developed in Kodak D-19, and stained with hematoxylin–eosin.

Materials available for analysis. The short-survival thymidine radiograms were drawn from a collection consisting of 94 paraffin- or methacrylate-embedded embryos ranging in age from Embryonic Day 12 (E12)[1] to Day E21. The collection of sequential-survival thymidine radiograms consists of 254 paraffin- and methacrylate-embedded specimens. In addition, use was made of relevant materials drawn for our large histological collection of normal developing brains.

RESULTS

Horizontal Compartmentation in the Cortical Neuroepithelium

Radiograms from E18 and E19 rats killed 24 h after labeling with [^{3}H]thymidine (Figs. 1B and 2B) show that the cortical neuroepithelium is divisible into a thin lower band composed mainly of heavily labeled cells and a thicker upper band composed mostly of lightly labeled cells. The lower band of heavily labeled cells overlaps with the mitotic zone (compare mz in Figs. 1A and 1B and in 2A and 2B), whereas the upper band of lightly labeled cells overlaps with the synthetic zone (sz in the same figures). The most parsimonious interpretation of this horizontal neuroepithelial compartmentation is that the heavily labeled cells near the lumen represent slower cycling cells which are still either in the G2 (post-synthetic, premitotic) phase or in the process of undergoing mitosis and contain therefore a high concentration of radioactive DNA. In contrast, the lightly labeled cells in the upper part of the neuroepithelium represent faster cycling cells that have returned to the synthetic zone and are either diluting or have diluted their complement of radioactive DNA in the new cycle of chromosomal duplication. The presence of two sharply segregated, dichotomous cell populations, one turning over slowly and the other fast, indicates that there are at least two stem cell populations within the neuroepithelium.

Horizontal Compartmentation in the Transitional Intermediate Zone

In rat embryos that survived for 24 h after the administration of [^{3}H]thymidine, the differentially labeled (or unlabeled) cells that have moved into the intermediate zone are segregated in separate horizontal bands. In rats labeled on Day E16 and killed on Day E17 (Figs. 3A and 3B), there are three bands in the early maturing lateral aspect of the cortex. The band beneath the cortical plate (CP), referred to as the superior band (sb), consists of heavily labeled cells. Beneath it is a band composed mostly of lightly labeled cells; this is the middle band (mb). The third band, called the inferior band (ib), consists predominantly of heavily labeled cells.

The horizontal compartmentation of differentially labeled cells is illustrated at higher magnification in relation to the subventricular zone and the intermediate zone in radiograms from rats labeled on Day E17 or Day E18 and killed 24 h after injection (Figs. 1 and 2). In embryos that received [^{3}H]thymidine on Day E17 and were killed on Day E18 (Fig. 1B) there are three bands throughout the entire ventrolateral (earlier maturing) and dorsomedial (later maturing) expanse of the developing cortex. The inferior band overlaps with the subventricular zone (compare ib in Fig. 1B with sv in Fig. 1A; see also ib in Fig. 2B and sv in Fig. 2A). Examination of radiograms from rats of the same age labeled 2 hr before sacrifice shows that the concentration of heavily labeled cells in the subventricular zone is quite low (sv in Fig. 1A). This implies a relatively low rate of local cell proliferation. Therefore, the high concentration of *heavily* labeled cells in the subventricular zone of Day E18 rats that were flash-labeled with [^{3}H]thymidine 24 h earlier, indicates that these cells were labeled while still in the neuroepithelium and became subsequently translocated into the subventricular zone. Our material does not allow us to specify the exact time when this translocation took place but the heavy labeling indicates that most of the cells have moved into the subventricular zone some time earlier (because they have undergone fewer divisions) than the lightly labeled cells which set-

[1] Abbreviations used: bg, basal ganglia neuroepithelium; BG, basal ganglia; BT, basal telencephalon; CAL, corpus callosum; CF, callosal fibers; ch2, channel 2; CP, cortical plate; dg, dentate gyrus; E, embryonic; HI, hippocampal formation; I, layer I (marginal layer); ib, inferior band; IC, internal capsule; ICh, head of internal capsule; iz, intermediate zone; lv, lateral ventricle; mb, middle band; ML, marginal layer (layer I); mn, migrating neurons; mz, mitotic zone; ne, neuroepithelium; ro, rostral; sb, superior band; SE, septum; SP, subplate; sv, subventricular zone; sz, synthetic zone; TH, thalamus; WH, white matter.

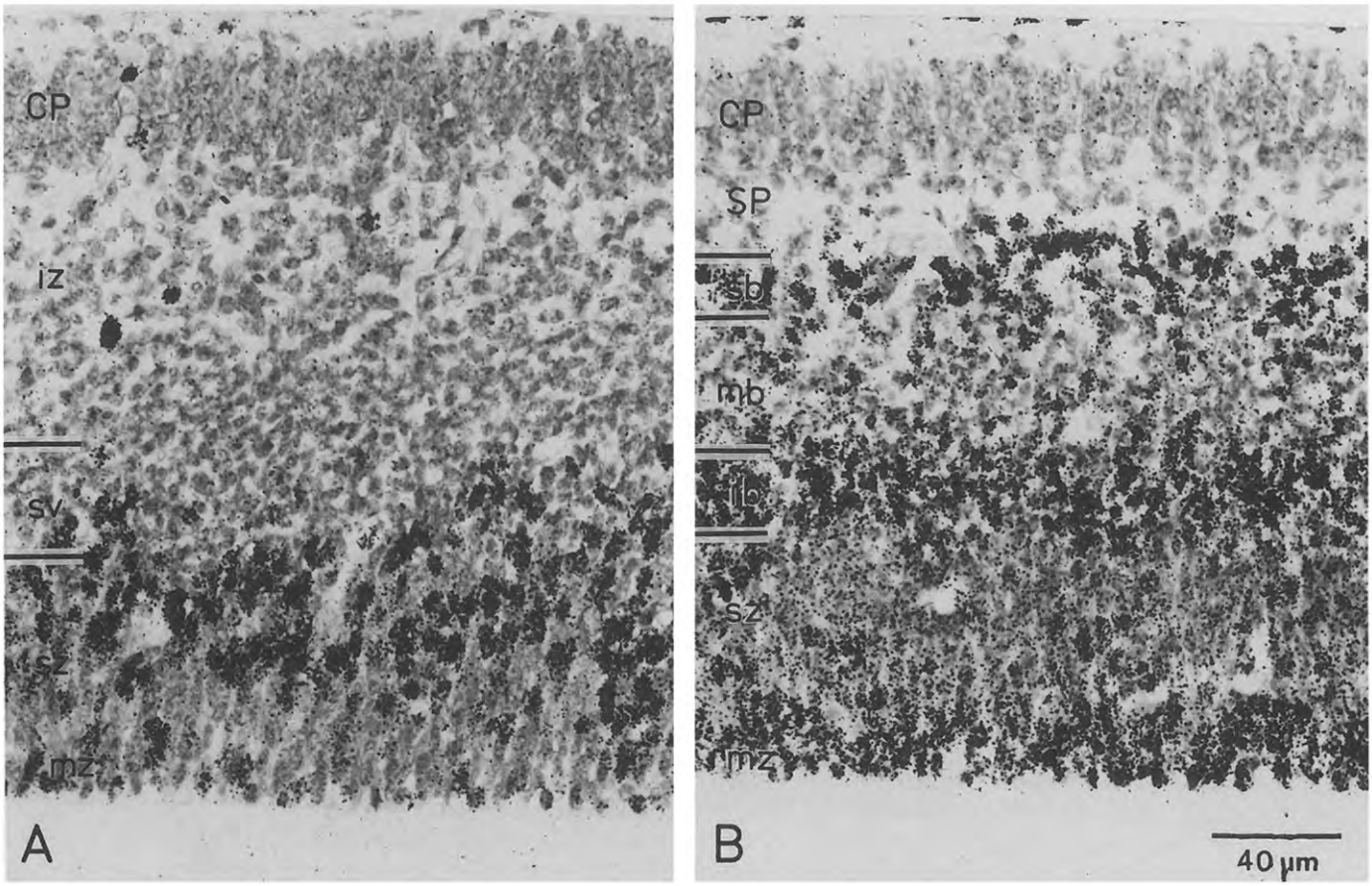

FIG. 1. Thymidine radiograms of the cerebral cortex from a rat labeled with [^{3}H]thymidine on Day E18 and killed 2 h later (A) and an embryo of the same age which received the radiochemical 24 h earlier (B). Paraffin; hematoxylin–eosin.

tle farther out in the middle band. We conclude that two cell populations coexist within the subventricular zone: (a) a small proportion of locally multiplying cells and (b) a larger complement of postmitotic cells that *pause* in this zone after leaving the neuroepithelium.

In embryos that received [^{3}H]thymidine on Day E18 and were killed on Day E19 (Fig. 2B), there are four horizontal bands in the transitional zone, the three described above (ib, mb, sb) and another band beneath the subplate containing a high proportion of vertically oriented unlabeled cells. We postulate that the latter represent "old" radially migrating neurons (mn), i.e., neurons that were formed before the injection on the morning of Day E18. These cells traverse a layer of horizontally oriented fibers in the upper portion of the intermediate zone (iz in Fig. 2A) *en route* to the cortical plate. The cortical plate itself is devoid of labeled neurons 24 h after injection with [^{3}H]thymidine (CP in Fig. 2B; see also CP in Fig. 1B).

Topographic Distribution of the Inferior Band and the Superior Band

A clue about the identity of the heavily labeled cells segregated in the inferior band and the superior band is provided by their different topographic distribution, as illustrated in low-power sagittal thymidine radiograms from a rat embryo labeled on Day E17 and killed on Day E18 (Fig. 4). In the rostral (ro) direction, the inferior band (ib) is aligned parallel to the lateral ventricle (lv) and is more extensive medially than laterally (compare vertical arrow in the midsagittal section in Fig. 4A and in the parasagittal section in Fig. 4B). In the posterior direction, the inferior band follows the ventral horn of the lateral ventricle and extends without interruption into the hippocampal formation (HI in Figs. 4A and 4B). We will present evidence elsewhere (Bayer and Altman, work in progress) that the heavily labeled cells in the hippocampal formation are young pyramidal neurons destined to migrate into Ammon's horn after a sojourn of several days. By inference, the component of the inferior band within the developing neocortex could also be composed of pyramidal neurons that sojourn here before migrating into the cortical plate.

The superior band of heavily labeled cells (sb in Figs. 4A and 4B) has a different distribution. In a cadual direction, the concentration of these cells diminishes in the transition area between the cerebral cortex and the hippocampal formation, and the band is not present in the hippocampus itself. In the rostral direction, the superior band can be followed through the formative white

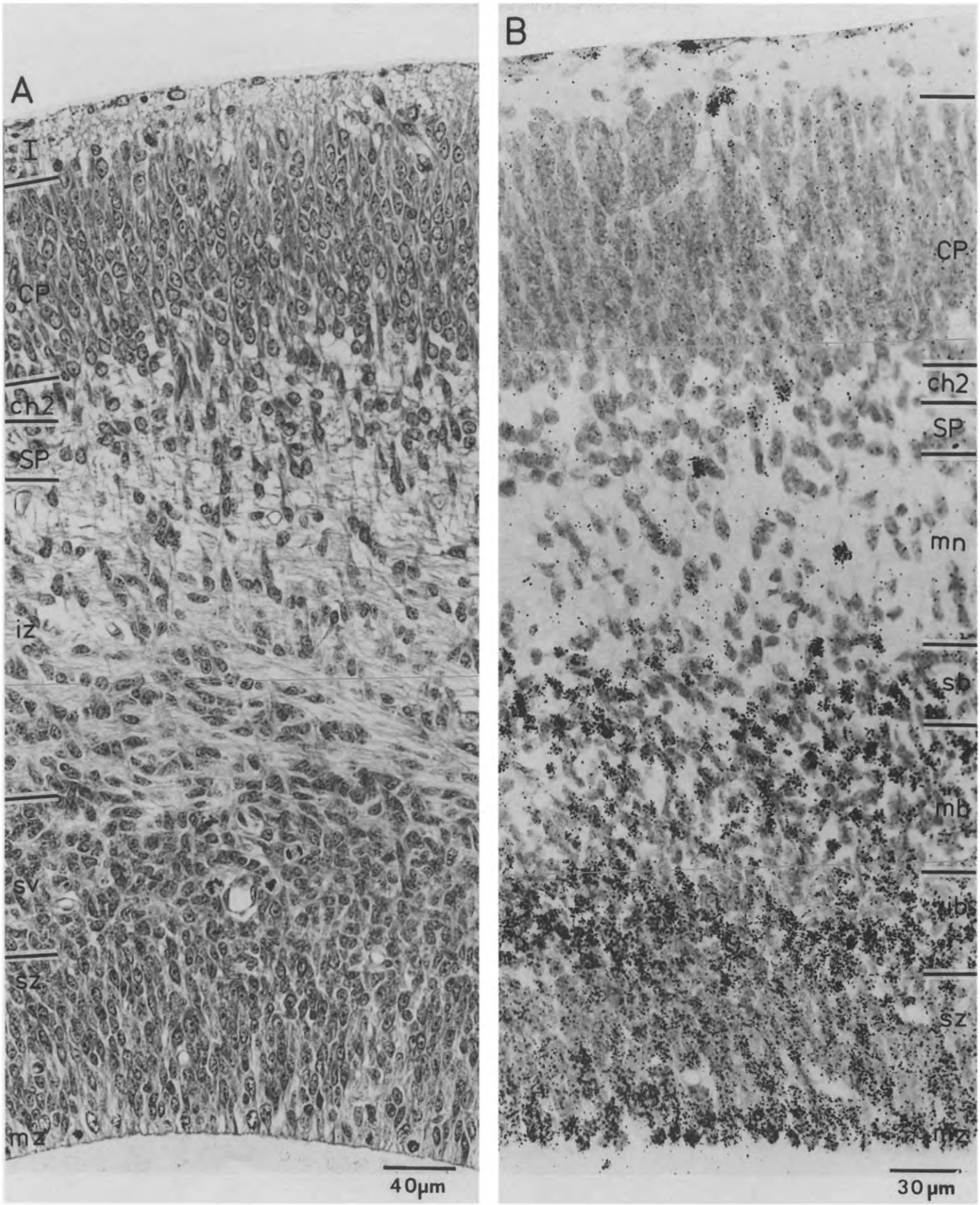

FIG. 2. (A) Nonradiographic section through the cerebral cortex of a Day E19 rat. (B) Matched thymidine radiogram from a rat labeled on Day E18 and killed on Day E19. (A) Methacrylate; (B) paraffin. Hematoxylin–eosin.

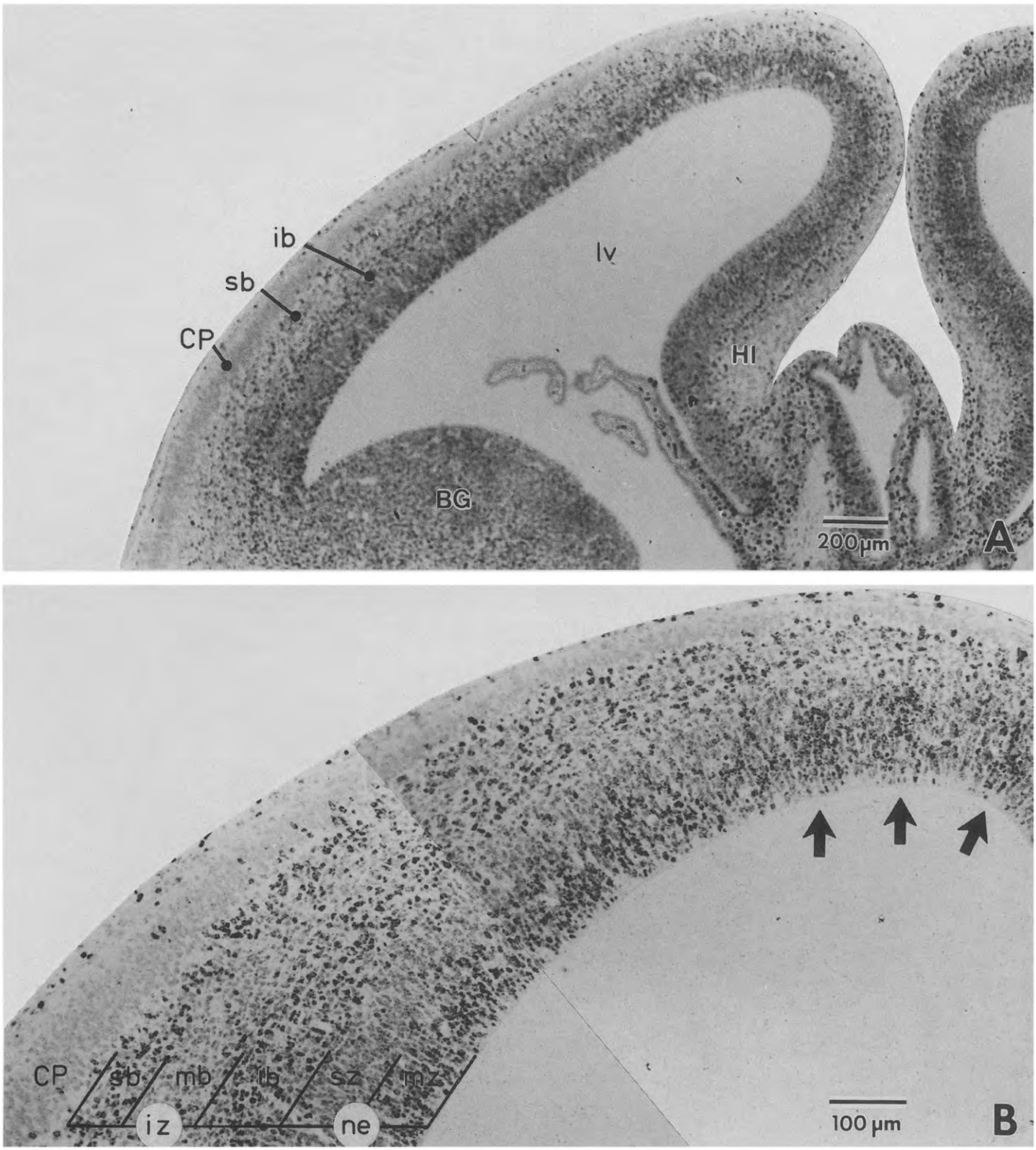

FIG. 3. (A) Low-power coronal thymidine radiogram from a rat labeled on Day E16 and killed on Day E17. (B) Horizontal thymidine radiogram from another rat labeled on Day E16 and killed on Day E17 at higher magnification. (See Discussion for the meaning of the arrows.) Methacrylate; hematoxylin–eosin.

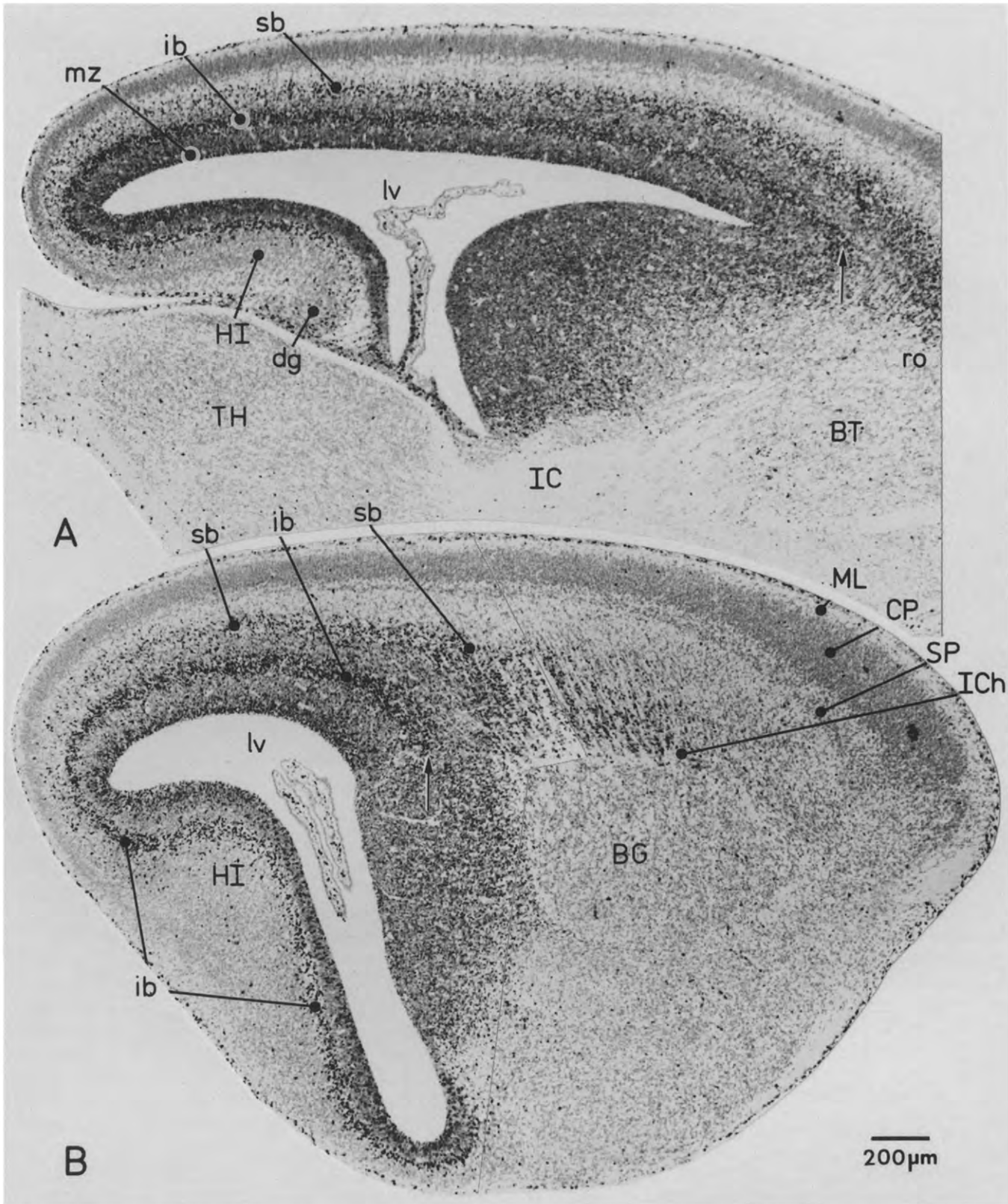

FIG. 4. Low-power midsagittal (A) and parasagittal (B) radiograms of the cerebral hemisphere from a rat labeled with [^{3}H]thymidine on Day E17 and killed on Day E18. Arrows point to the dorsal extent of the inferior band (ib). Paraffin; hematoxylin–eosin.

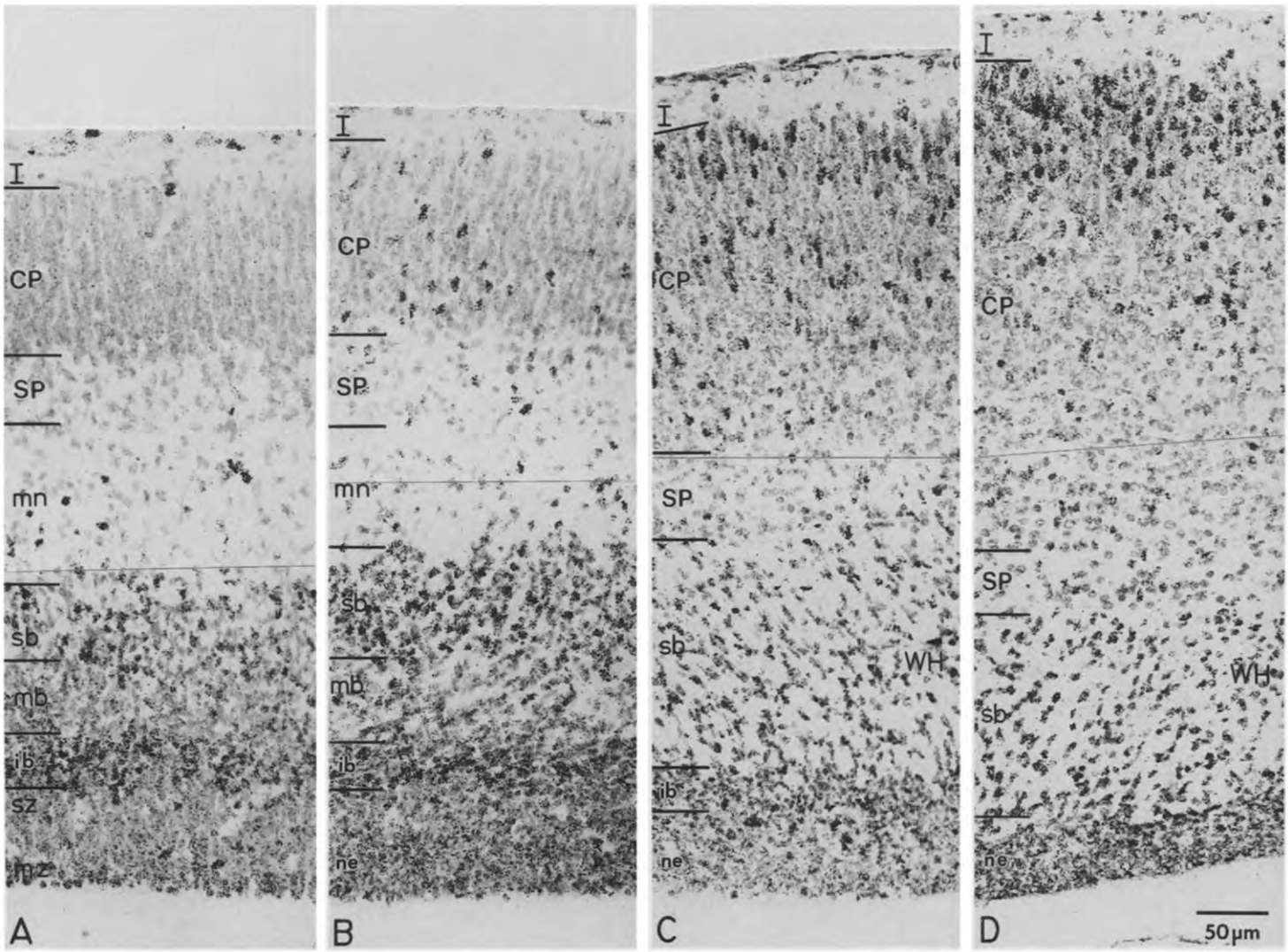

FIG. 5. Thymidine radiograms of the cerebral cortex from rats labeled on Day E18 and killed on Days E19 (A), E20 (B), E21 (C), and E22 (D). Paraffin; hematoxylin–eosin.

matter to the head of the internal capsule (ICh in Fig. 4B) above the basal ganglia (BG). This distribution suggests that the superior band consists of a population of glial cells associated with the cortical white matter and fibers of the cortical radiation.

The Disposition of the Heavily Labeled Cells of the Inferior and Superior Bands Traced in Sequential Radiograms

The fate of the heavily labeled cells of the inferior and superior bands is illustrated in sequential-survival radiograms from rats labeled with [^{3}H]thymidine on Day E18 and killed 1, 2, 3, and 4 days after injection (Fig. 5). One day after injection, the inferior band is quite prominent with its heavily labeled cells (ib in Fig. 5A), but 2 days after injection (ib in Fig. 5B), it becomes narrower and contains fewer labeled cells. At the same time, heavily labeled cells migrate through the intermediate zone and many of them penetrate the future cortical gray matter (SP and CP in Fig. 5B). Three days after injection, the inferior band has shrunken further and it contains mostly lightly labeled (later generated) cells (ib in Fig. 5C). By this time the bulk of the heavily labeled cells are situated in the upper portion of the expanding cortical plate; although some scattered heavily labeled cells are still seen in its lower part. Four days after injection (Fig. 5D), the inferior band is no longer identifiable above the shrunken neuroepithelium (ne). A few labeled cells are still migrating through the cortical plate but most of them have settled in the upper layers.

The changes occurring during this period in the superior band are different. Two days after injection on Day E18, the superior band (sb in Fig. 5B) has become broader than it was on the previous day (sb in Fig. 5A). Although there are some heavily labeled cells present in the expanding superior band (which could be migratory

elements), many lightly labeled cells are also seen. The expansion of the superior band continues 3 days after injection (sb in Fig. 5C). Its cells, which are now coextensive with the formative white matter (WH), appear to have become smaller. (The reduction in nuclear size might explain the high concentration of radioactive label in these cells in spite of their presumed continuing proliferation.) Four days after injection, when the inferior band is no longer identifiable, the superior band and the white matter (sb and WH in Fig. 5D) have become contiguous with the shrinking neuroepithelium. If this interpretation of the transpiring morphogenetic events is correct, the inference is justified that the labeled cells of the superior band consist predominantly of glial cells.

Dispersal of Labeled Glial Cells through the White Matter and the Corpus Callosum

The precursors of neurons and glia and their young progeny cannot be distinguished in the formative cerebral cortex without reliable cytological or immunochemical markers. However, there are two fibrous regions in the developing cortex where there are no migrating neurons and where, therefore, all the cells encountered are likely to be glial cells: one is the white matter at the head of the internal capsule overlying the basal ganglia, and the other is the corpus callosum. Since the corpus callosum fibers cross the midline on Day E20, we illustrate the dispersal of glial cells in these two large fibrous regions on this day.

In thymidine radiograms from rat fetuses injected with [^{3}H]thymidine on Day E20 and killed 2 h later there is a fair concentration of heavily labeled cells throughout the neuroepithelium and the subventricular zone (ne and sv in Fig. 6A). The pattern of labeling in the germinal matrix is different from that seen in short-survival radiograms in younger embryos (Fig. 1A). In the middorsal portion of the cerebral cortex there are scattered locally multiplying cells above the germinal matrix in a cell-rich zone, representing the fibers of the corpus callosum, and in the cortical white matter proper (CF and WH in Fig. 6A). Medially, the decussating fibers of the corpus callosum (CAL in Fig. 6A) contain only a few cells and they are not labeled. Laterally, the head of the internal capsule (ICh in Fig. 6A) contains many cells and some of them are labeled, suggesting a low level of local multiplication.

The site of origin of cells in the head of the internal capsule and the corpus callosum is deduced from the pattern of labeling seen in Day E20 rats that were labeled with [^{3}H]thymidine 2 days earlier (Fig. 6B). Adjacent to the germinal matrix there are two foci distinguished by high concentrations of heavily labeled cells, one laterally (short arrow on the right in Fig. 6B) and another in the midportion of the formative cortex (short arrow on the left in Fig. 6B). The labeled cells originating laterally form an arch over the entire surface of the basal ganglia (ICh, enclosed by two long arrows on the right in Fig. 6B). These cells may also penetrate the internal capsule fibers in the basal ganglia (BG), but this is uncertain because many of the labeled cells in the basal ganglia may be neurons that are generated by the active germinal matrix of this structure itself (bg). The labeled cells originating in the midcortical focus can be traced in the medial direction along the fibers of the corpus callosum (CF, enclosed in long arrows on the left in Fig. 6B). There may be another glial focus present at the site of the decussating corpus callosum (asterisk in Fig. 6B). The labeled cells, which are preferentially localized near the surface, may serve as a guidepost or conduit for the crossing fibers. The invasion of the corpus callosum by these labeled glial cells is illustrated in a parasagittal section through the corpus callosum in a rat labeled on Day E18 and killed 3 days later (Fig. 7).

DISCUSSION

In autoradiograms from rats labeled with [^{3}H]-thymidine on Days E16, E17, and E18 and killed 1 day after injection, there are alternating horizontal bands of heavily and lightly labeled cells (and in the E18 group also a band of unlabeled cells) in the germinal matrices and the intermediate zone of the developing rat cerebral cortex. This stratification is not evident in material prepared with conventional Nissl stains without thymidine radiography. Of these strata, easiest to analyze are the bands composed of heavily labeled cells, and we have sought to follow their fate with sequential-survival radiography.

Cellular Compartmentation in the Neuroepithelium

In rat embryos that survived for 24 h after injection there are two horizontal bands in the neuroepithelium. There is a band of heavily labeled cells in the synthetic zone (the DNA of these cells contains a high concentration of the administered [^{3}H]thymidine), and a band of lightly labeled cells in the mitotic zone (which have diluted their radioactive DNA). This segregation indicates that the cortical neuroepithelium is composed of a fast cycling and a slow cycling stem cell population. This observation raises a general question and a specific one. The general question concerns the fate of these two cell types. Unfortunately, we will not be able to answer this question until cytochemical markers become available which reliably distinguish the precursors of different cell types, such as neurons and glia, in the rat neuroepithelium. But there is a more specific question that we can begin to deal with. This is the problem of how the *horizontal* compartmentation of neuroepithelial cells that we describe in this paper is related to the *vertical* compartmentation of neuroepithelial cells that we have described in the preceding paper (3).

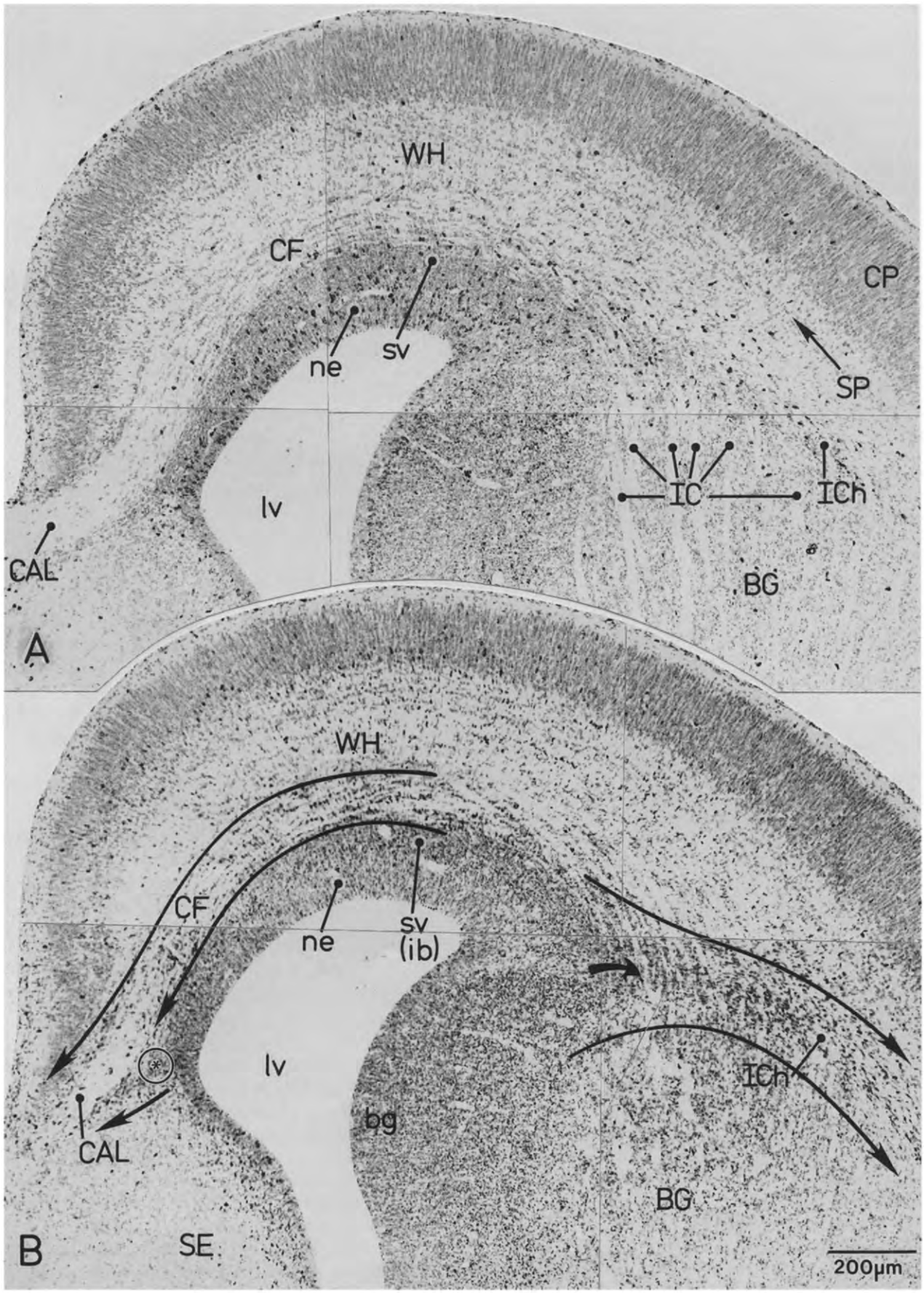

FIG. 6. Coronal radiograms of the cerebral cortex from Day E20 rats that were labeled 2 h (A) and 2 days before sacrifice. In B, small arrows mark glial foci and long arrows enclose presumed dispersing glial cells. Paraffin; hematoxylin–eosin.

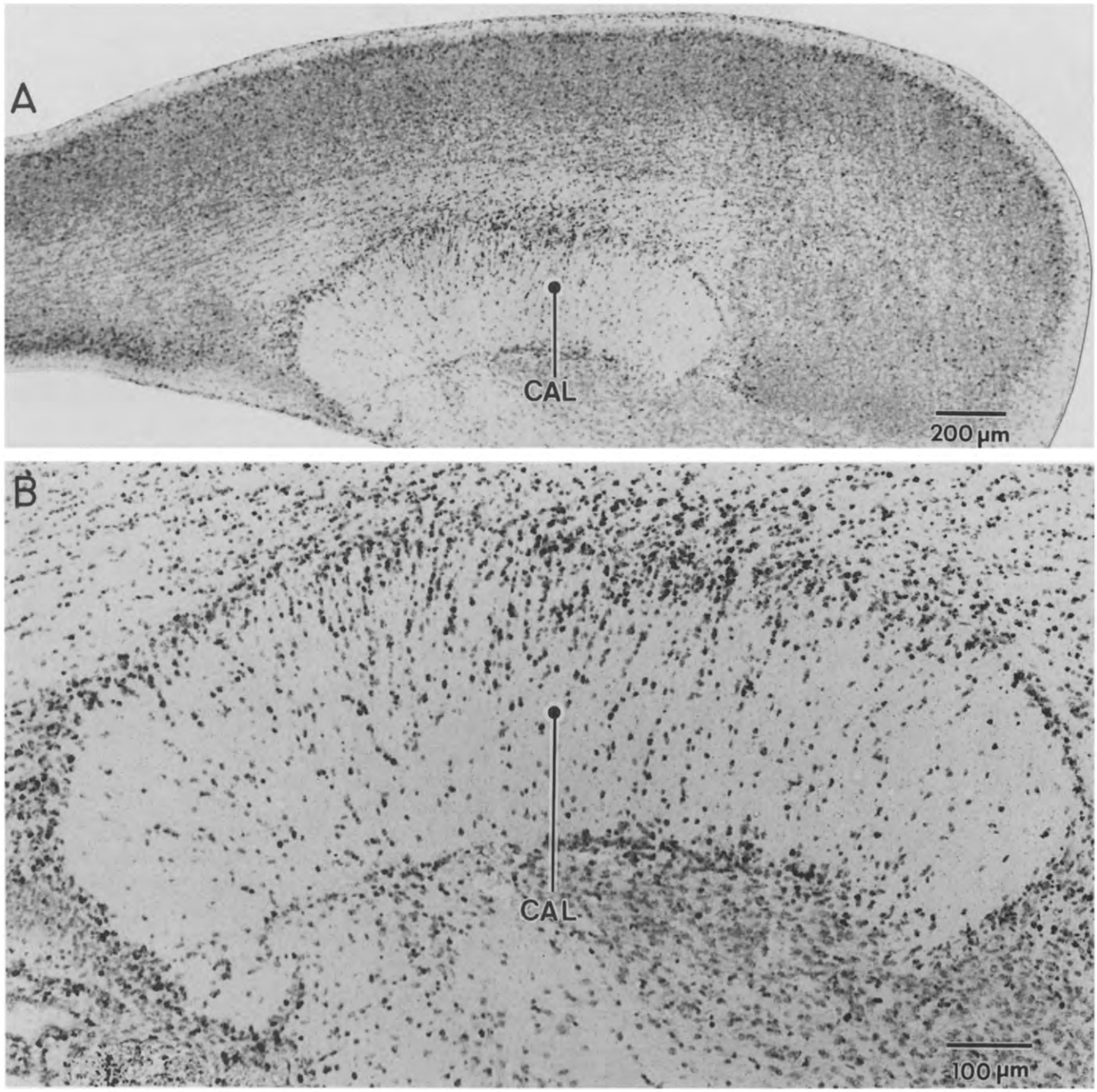

FIG. 7. (A) Parasagittal thymidine radiogram of the cerebral cortex from a rat labeled on Day E18 and killed on Day E21. (B) The corpus callosum at higher magnification. Paraffin; hematoxylin–eosin.

One difference between these two observations is that the columnar organization reported in the preceding paper was seen 2 h after injection with [^{3}H]thymidine, whereas the horizontal stratification we report here is seen 24 hr after injection. The second difference is that our preceding report of columnar neuroepithelial compartmentation was based on observations in young embryos (E12–E16), whereas the horizontal lamination reported here concerns older embryos (E17–E20). Work in progress suggests that the crucial factor in these two results is not the difference in *survival time after injection* but rather the difference in *embryonic age at the time of injection.* Preliminary observations indicate that the horizontal neuroepithelial stratification found in older embryos (E16–E18) 24 hr after injection with [^{3}H]-thymidine, as reported in this study, is not present in younger embryos (E14 or E15) following the same survival time. Instead, younger embryos display a vertical neuroepithelial compartmentation after 24 h survival, similar to the vertical microsegmentation seen 2 h after

injection (3). This, in fact, is suggested by the observation in this study of a vertical compartmentation (arrows in Fig. 3) in the late developing dorsomedial region of the cortex in rats injected on Day E16 and killed on Day E17. In the latter region, the development of the cortical plate is in an incipient stage and a clear horizontal stratification is absent in the intermediate zone. Contrariwise, preliminary observations indicate that the vertical microsegmentation which is evident in younger embryos (E12–E16) 2 h after [^{3}H]thymidine administration (3) is not present in older embryos (E17+) that survived for the same period. Indeed, the alternating radiosensitive and radioresistant columns seen in younger embryos following X-irradiation are not present in older embryos (3). Our tentative interpretation of these findings is that the neuroepithelium of younger embryos, which displays a vertical segmentation in thymidine radiograms, and the neuroepithelium of older embryos, which displays a horizontal stratification, are composed of different stem cell populations. If this is correct, there are not two *age-independent* types of stem cells present in the cortical neuroepithelium but at least four *age-dependent* types, i.e., two earlier populations of stem cells that are vertically compartmentalized and two later populations that are horizontally compartmentalized. We are in the process of further investigating this possibility.

Cellular Compartmentation in the Intermediate Zone

The two heavily labeled bands of the intermediate zone (the inferior and superior bands) are composed of early differentiating (older) cells that have undergone few divisions (possibly only one) following injection 24 h earlier. In contrast, the lightly labeled cells forming the middle band represent a later differentiating (younger) population which have undergone more cell divisions before leaving the neuroepithelium. We have not undertaken the difficult task of following the fate of the lightly labeled cells of the middle band but did obtain indirect evidence that the heavily labeled *inferior band* consists of *young neurons* that have paused at the base of the intermediate zone prior to their migration to the cortex, whereas the heavily labeled *superior band* consists of *glial cells* that have migrated out farther and later become dispersed through the fiber tracts of the cerebral hemispheres.

The accumulation and temporary sojourn of putative young neurons in the inferior band is difficult to reconcile with the prevailing view that the radially oriented cells of the neuroepithelium follow a direct radial path to the cortical plate where, indeed, the bulk of the differentiating neurons display a similar radial orientation. An earlier hypothesis was that the radial migration of neuroblasts from the neuroepithelium to the cortical plate is based on nuclear translocation within the elongated cytoplasm which, from the earliest stage of neuroepithelial development, anchors a cell at the base and apex of the presumptive cortex (4). The more widely held current view is that the radial migration of young neurons is aided by surface contact with preformed radial glial fibers (9). Neither of these theories satisfactorily accounts for the presence, both in the subventricular zone and in the intermediate zone, of a high proportion of cells that are either horizontally oriented or have a stellate shape (Fig. 2A). The apparent rotation of cells in the cortical intermediate zone has been repeatedly described in Nissl-stained (7, 8) and Golgi-impregnated (2, 6, 12) sections from different species. In addition, Golgi studies indicate that the horizontally oriented cells have horizontally oriented processes. According to Stensaas (12), horizontal cells predominate in the lower intermediate zone and he interprets their processes as efferent fibers that grow in the direction of the basal ganglia. The outgrowth of these axons apparently commences before their cell bodies have become again reoriented vertically in the upper intermediate zone as they resume their ascent to the cortical plate.

Without necessarily negating the hypothesis that the migration of neurons is aided by radial glial processes, our autoradiographic evidence establishes that some (possibly all) neurons interrupt their radial migration in the inferior band for 1–2 days (Figs. 5A and 5B). The Golgi evidence suggests one function for this temporary pause, i.e., the horizontal rotation of the cell body in relation to the lateral outgrowth of its axon in the direction of the internal capsule. In this respect the inferior band bears some resemblance to the premigratory zone of the cerebellar cortex where horizontally oriented granule cells sprout a portion of their axons (the parallel fibers) before they descend into the granular layer (1, 10). A similar pause by young neurons has been observed in the hippocampus (Bayer and Altman, in preparation).

The Glial Cells of the Corpus Callosum and of the Internal Capsule

In contrast to the shrinkage of the inferior band, the superior band expands for several days after injection with [^{3}H]thymidine and eventually becomes coextensive with the white matter (Fig. 5). The fact that, within the white matter, the superior band extends laterally to the head of the internal capsule but it is not traceable, as is the inferior band, to the hippocampus (Fig. 4B), was the basis of our inference that it is composed primarily of glial cells related to the afferent and efferent fiber tracts of the neocortex. As neurogenesis declines, glial proliferation becomes the dominant event tracked with thymidine radiography. In rat fetuses labeled on Day E18 and killed on Day E20 we were able to distinguish two foci of

presumptive proliferating glial cells along the wall of the lateral ventricle, one dispersing laterally in the direction of the basal ganglia and the other medially in the direction of the decussating corpus callosum (Fig. 6). Further work is needed to determine the disposition of the fairly large population of labeled cells aggregating above the basal ganglia where corticofugal fibers form the striated bundles of the internal capsule. We tentatively assume that at least some of these are glial cells that will disperse into the internal capsule. The disposition of the medially dispersing cells is easier to follow. In Day 20 fetuses, there are fewer glial cells in the corpus callosum medially than dorsolaterally (Fig. 6A). Short-survival thymidine radiograms of Day E20 rats indicate that most of the multiplying cells in this region are located in the germinal matrix since there are only a few locally multiplying (i.e., labeled) cells within the corpus callosum. However, in Day E20 rats that received [^{3}H]thymidine 2 days earlier, a high proportion of the cells in the corpus callosum are heavily labeled. This suggests that the majority of glial cells are formed in the germinal matrix, penetrate the corpus callosum dorsolaterally, and then disperse medially. There seems to be a separate glial focus in the germinal matrix near the crossing site of the corpus callosum; the cells generated at this site may form the "bridge" or "sling" previously described by Silver *et al.* (11) that guides or aids the decussating callosal fibers. Taken together, these observations suggest that the large fiber tracts of the developing neocortex, the internal capsule, and the corpus callosum are supplied by *separate foci* of proliferating glial cells and/or their precursors.

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REFERENCES

1. ALTMAN, J. 1972. Postnatal development of the cerebellar cortex in the rat. I. The external germinal layer and the transitional molecular layer. *J. Comp. Neurol.* **145:** 353–398.
2. ALTMAN, J., AND S. A. BAYER. 1988. The development of neurons. Page 1–26 in E. Meisami and P. S. Timiras, Eds., *Handbook of Human Growth and Developmental Biology,* Vol. 1, Part A. CRC Press, Boca Raton, FL.
3. ALTMAN, J., AND S. A. BAYER. 1990. Vertical compartmentation and cellular transformations in the germinal matrices of the embryonic rat cerebral cortex. *Exp. Neurol.* **107:** 23–35.
4. BERRY, M., AND A. W. ROGERS. 1965. The migration of neuroblasts in the developing cerebral cortex. *J. Anat.* **99:** 691–709.
5. BOULDER COMMITTEE. 1970. Embryonic vertebrate central nervous system: revised terminology. *Anat. Rec.* **166:** 257–262.
6. DERER, P. 1974. Histogenése du néocortex du rat albinos durant la période foetale et néonatale. *J. Hirnforsch.* **15:** 49–74.
7. HATAÏ, S. 1902. Observations on the developing neurones of the cerebral cortex of foetal cats. *J. Comp. Neurol.* **12:** 199–204.
8. PATON, S. 1900. The histogenesis of the cellular elements of the cerebral cortex. *Johns Hopkins Hosp. Rep.* **9:** 709–741.
9. RAKIC, P. 1971. Guidance of neurons migrating to the fetal monkey neocortex. *Brain Res.* **33:** 471–476.
10. RAMÓN Y CAJAL, S. 1911. *Histologie du Système Nerveux de l'Homme et des Vertébrés* (2 Volumes). Maloine, Paris.
11. SILVER, J., S. E. LORENZ, D. WAHLSTEN, AND J. COUGHLIN. 1982. Axonal guidance during development of the great cerebral commissures: Descriptive and experimental studies, in vivo, on the role of preformed glial pathways. *J. Comp. Neurol.* **210:** 10–29.
12. STENSAAS, L. J. 1967. The development of hippocampal and dorsolateral pallial regions of the cerebral hemisphere in fetal rabbits. II. Twenty millimeter stage, neuroblast morphology. *J. Comp. Neurol.* **129:** 71–84.