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

CHAPTER 7

Stratification in the Cortical Transitional Field

Strictly speaking, the term *cortical transitional field* refers to the layers outside of the neuroepithelium that are present during embryonic development but are absent in the adult cortex. So defined, it includes the subventricular zone and the intermediate zone. Both zones are prominent features of the embryonic neocortex. The intermediate zone in particular becomes quite large and has three major components during the peak periods of neurogenesis: (1) young neurons migrating to the cortical plate, (2) a fibrous zone of afferents growing into the cortex, and (3) young neurons migrating to destinations outside of the neocortex.[1] In this chapter, we show that neurons destined to settle in the cortical plate first accumulate in bands in the transitional field. There are separate bands for putative layer VI, layer V, and layers IV–II cells. The cells may pause or sojourn for variable periods of time in the bands, possibly to interact with afferent fibers. The observations supporting these findings were made in paraffin sections of rat embryonic brains that survived for 1 to 4 days after a single injection of [^{3}H]thymidine (sequential-survival) and in matching methacrylate sections of normal embryonic rat brains. (Methods are given in Appendices 1 and 2.) For the interpretations, we relied heavily on the neurogenetic timetables that are presented in Chapters 3 and 11–15. Some of these observations have been reported in greater detail in journal articles (Altman and Bayer, 1990a, 1990b, 1991a, 1991b, 1991c).

The picture that emerges is a complex one and the interpretations are tentative. We describe first the stratification seen in Nissl-stained methacrylate sections with the horizontal banding pattern seen in [^{3}H]thymidine autoradiograms. Since there are time-dependent changes in banding patterns that can be linked with major neurogenetic periods, the cells sojourning in the different bands are tentatively identified. Next, we follow the cells in two of the bands in an attempt to determine the type of cells that accumulate in each one. Finally, we deal with regional differences in the banding patterns in anterior versus posterior parts of the developing cortex. The band in the intermediate zone that contains putative layer V pyramidal cells is more prominent in the presumptive motor cortex than it is in the presumptive visual cortex. In contrast, the band in the subventricular zone

[1] Sequential-survival autoradiography indicates that cells generated in the neocortical germinal matrix migrate ventrolaterally into the basal telencephalon. As we briefly illustrate in Chapter 9, the cells can be followed to the piriform cortex and to the anterolateral intercalated mass of the amygdala. There are probably other destinations for these cells, and we have plans to continue these investigations in an embryonic study of basal telencephalic development.

that contains putative layer IV-II cells has a homogeneous appearance anteriorly and posteriorly; regional differences in the concentration of layer IV cells are attributed to regional differences in radial versus lateral migration of these cells (Chapter 9).

7.1 THE SEQUENTIAL APPEARANCE OF FIVE BANDS OF HEAVILY LABELED CELLS IN THE TRANSITIONAL FIELD

We analyzed autoradiograms of rat embryos at daily intervals between E17 and E22 that had received a single exposure to [^{3}H]thymidine 24 hours earlier. In these autoradiograms, heavily labeled and unlabeled cells form discrete to diffuse bands in the cortical transitional field. We first describe the heavily labeled bands as they are seen in low magnification, [^{3}H]thymidine autoradiograms. We then analyze high magnification autoradiograms from the same injection groups, matching them with sections from methacrylate-embedded normal embryos, to locate exactly the heavily labeled bands within the different layers of the cortical transitional field. During the peak periods of neurogenesis, we assume that most of the bands con-

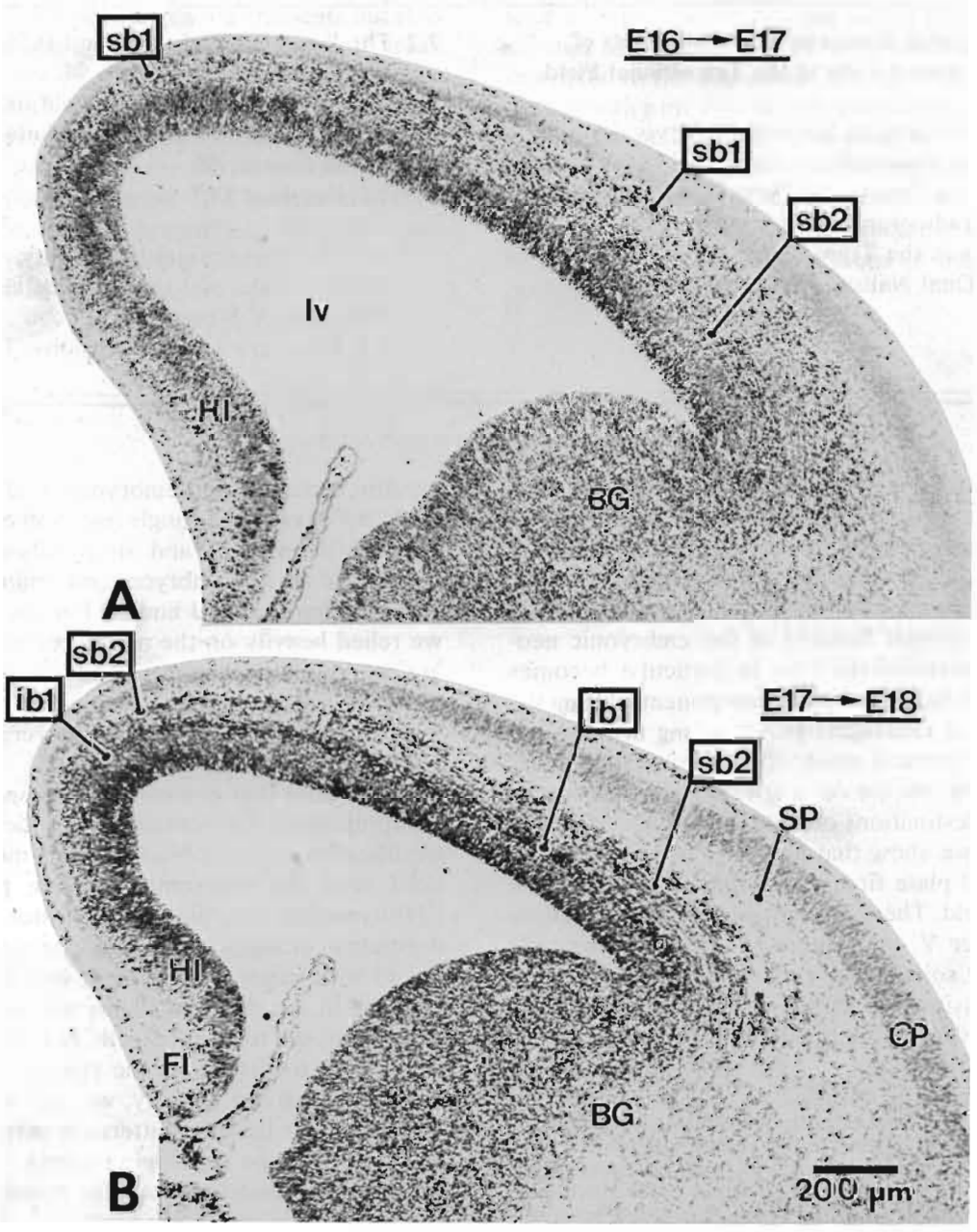
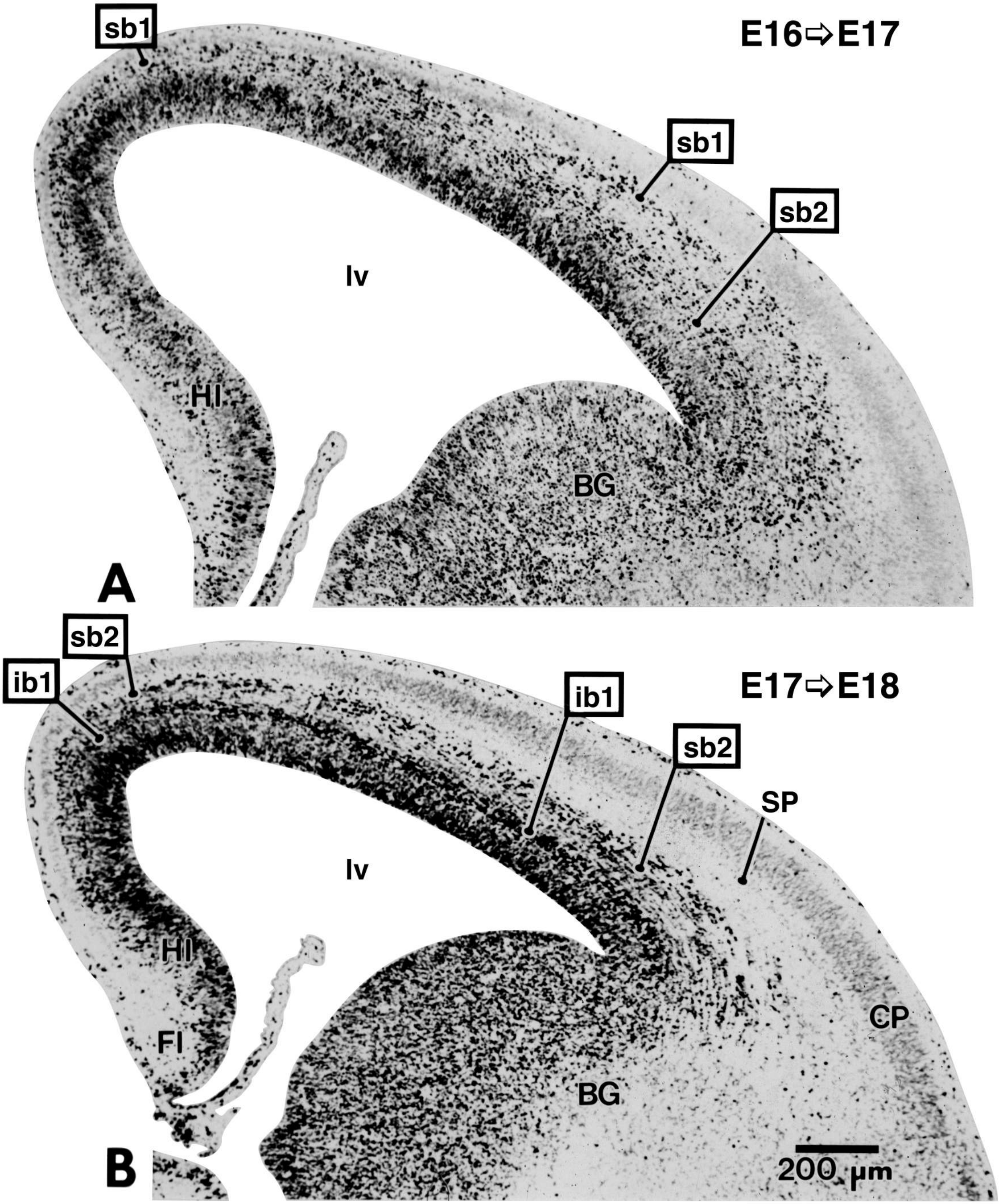


FIG. 7–1. Coronal autoradiograms of the cortex of E17 **(A)** and E18 **(B)** rats that received [^{3}H]thymidine 24 hours earlier. (3 µm methacrylate sections, hematoxylin stain.)


sb1
E16⇨E17
sb1
sb2
lv
HI
BG
A
sb2
ib1
ib1
E17⇨E18
sb2
SP
lv
HI
FI
CP
BG
B
200 µm

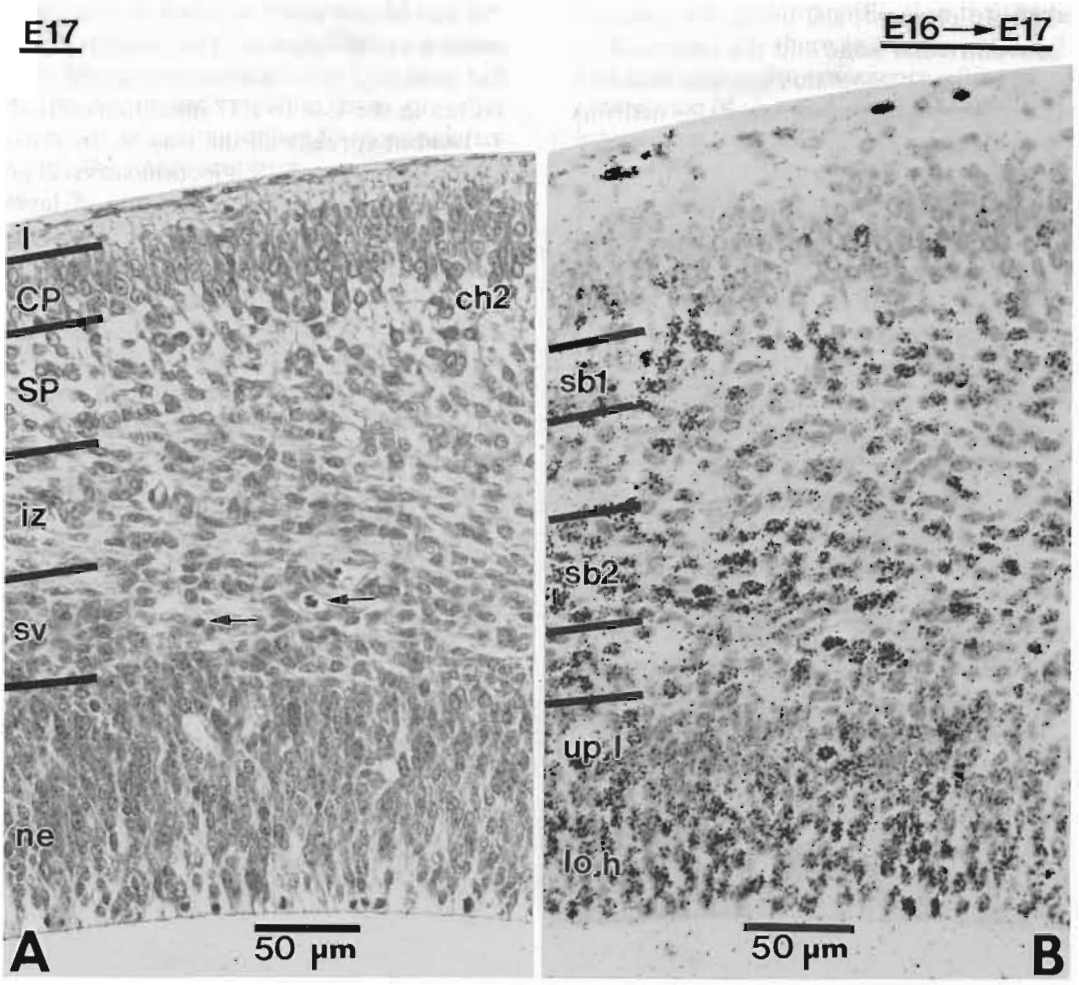


FIG. 7–2. Coronal methacrylate section (3 μm, hematoxylin stain) of the neocortex from an E17 rat **(A)** and a matched paraffin autoradiogram (6 μm section, hematoxylin stain) from a rat that received [^{3}H]thymidine on E16 and was killed on E17.

tain postmitotic neurons. Our interpretation is that the cells in the heavily labeled bands were generated within the 24-hour period after their precursors incorporated [^{3}H]thymidine. For example, if an injection is given on E17, heavily labeled cells in the transitional field 24 hours later (on E18) represent young neurons with cell birthdays on E17. We use the timetables for cortical neurogenesis (Chapters 3 and 11–15) to identify the heavily labeled cells. The cells in the unlabeled bands, are considered to be older neurons that were generated before the injection was given.

7.1.1 Observations in Sequential Survival Thymidine Autoradiograms

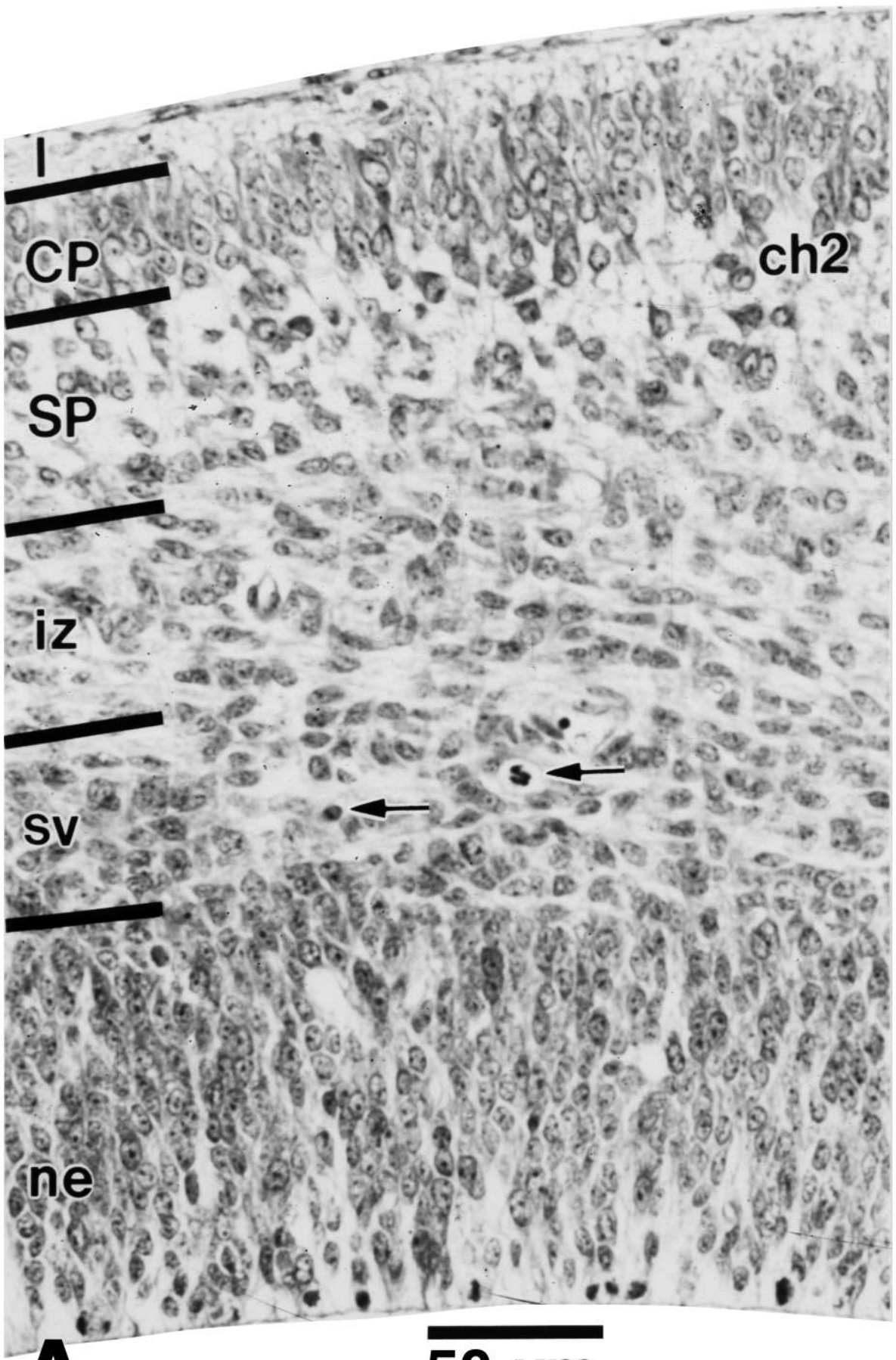
E16 to E17 Injection/Survival Group

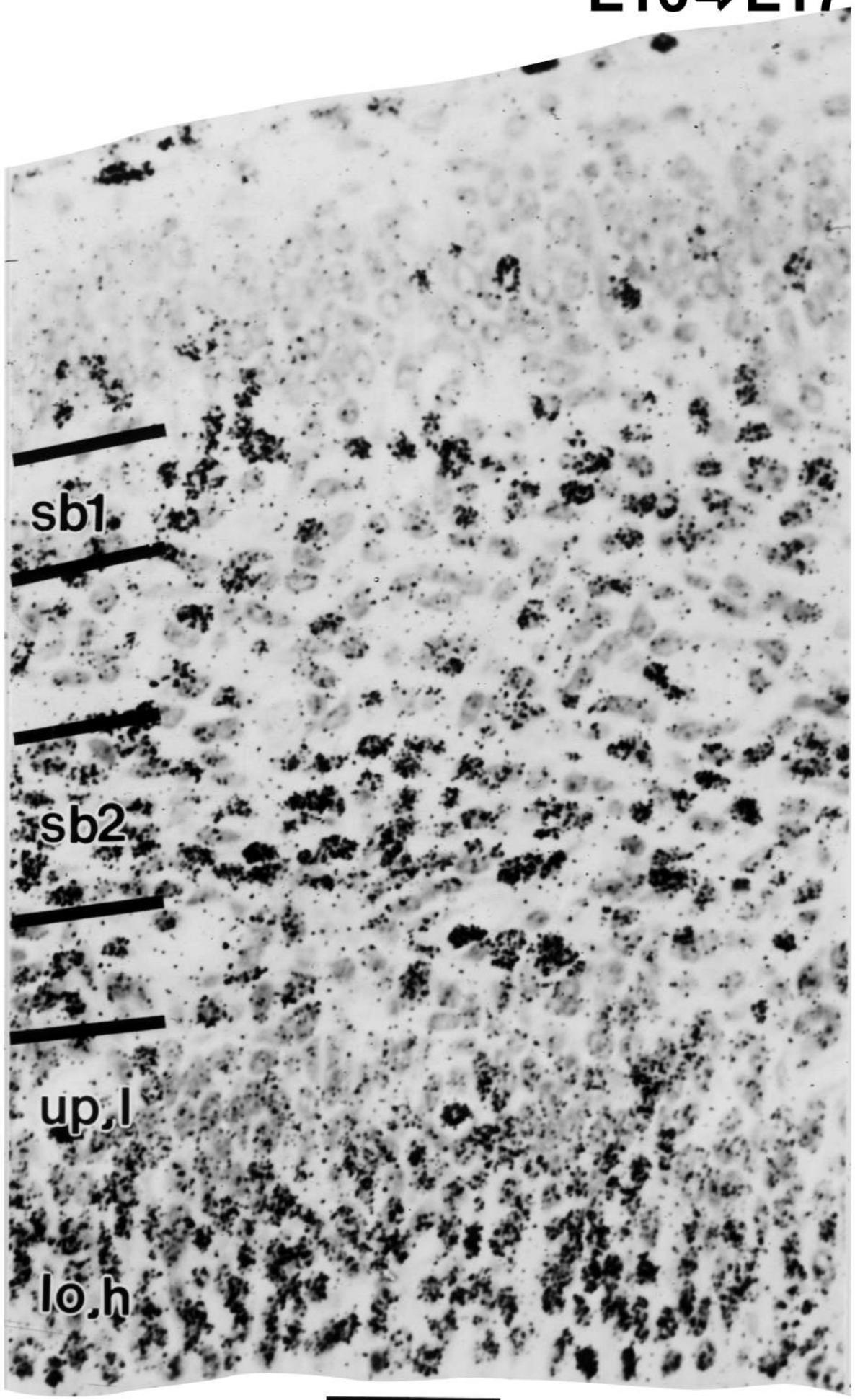
A single [^{3}H]thymidine injection on E16 in rats that survived until E17 (Figs. 7–1A and 7–2) produces two bands of heavily labeled cells in the transitional field. The upper one, the *first superior band* (sb1), spans the entire cortex from ventrolateral to dorsomedial just below the cortical plate (CP). The lower one, the *second superior band* (sb2), is limited to the earlier maturing ventrolateral and lateral parts of the neocortex. High-magnification views of the lateral cortex in a matched methacrylate-embedded normal embryo on E17 (Fig. 7–2A) and an autoradiogram of an E17 embryo injected with [^{3}H]thymidine on E16 (Fig. 7–2B) shows that most of the heavily labeled cells in sb1 are at the external edge of the intermediate zone, some are situated in a band that will become the subplate, and a few have actually penetrated the cortical plate[2] (*ar-*

[2] Notice that the ventrolateral part of the cortical plate in the low magnification picture (Fig. 7–1A) is completely clear of labeled cells. That indicates that the heavily labeled cells generated on E16 arrive earlier in the dorsal and lateral parts of the cortical plate than in the ventrolateral part of the cortical plate. In Chapter 9, we will show the same phenomenon after E17 injections and relate the delay to lateral migration of some cortical neurons.

E17

E16⇨E17





50 μm

50 μm

A

B

rows, Fig. 7–2B). In contrast, sb2 lies at the junction between the subventricular zone and the intermediate zone. We postulate that sb1 contains neurons destined to settle in cortical layer VI while sb2 contains neurons destined to settle in cortical layer V and offer the following supporting arguments.

The first superior band is first seen in the E15–E16 injection/survival group ventrolaterally (Chapter 4, sb1 in Fig. 4–9D) and spreads all the way to the dorsomedial cortex in the E16–E17 injection/survival group (sb1 in Fig. 7–1A). Since the cortical level examined here (mid-anteroposterior section) contains the presumptive motor area (medial) and somatosensory area (lateral) where layer VI neurons are produced mainly on E15 and E16 (Chapters 13 and 14; Figs. 13–4 and 14–4), we assume that sb1 contains the layer VI cells. The fact that a band is formed in the upper intermediate zone suggests that layer VI cells accumulate there for a short pause possibly to interact with the first thalamocortical afferents that arrive just at that time.

The second superior band is interpreted to be another wave of neurons. This band is limited to the earlier maturing ventrolateral and lateral parts of the neocortex in the E16 to E17 injection/survival group (Fig. 7–1A) but spreads all the way to the dorsomedial cortex in the E17 to E18 injection/survival group (Fig. 7–1B). The neurogenetic timetable of layer V neurons indicates that peak production occurs on E16 in the laterally situated somatosensory area (Chapter 13, Fig. 13–4) and on E17 in the dorsally situated hindlimb motor area (Chapter 14, Fig. 14–5B). Thus, an injection on E16 should heavily label many layer V neurons in the ventrolateral cortex, which correlates with the appearance of sb2 only ventrolaterally on E17. Our interpretation that sb2 contains layer V neurons is further supported by evidence described later where we show that sb2 is prominent anteriorly and at middle anteroposterior levels where the agranular motor cortex will form and is either reduced or absent posteriorly where the granular visual cortex will form.

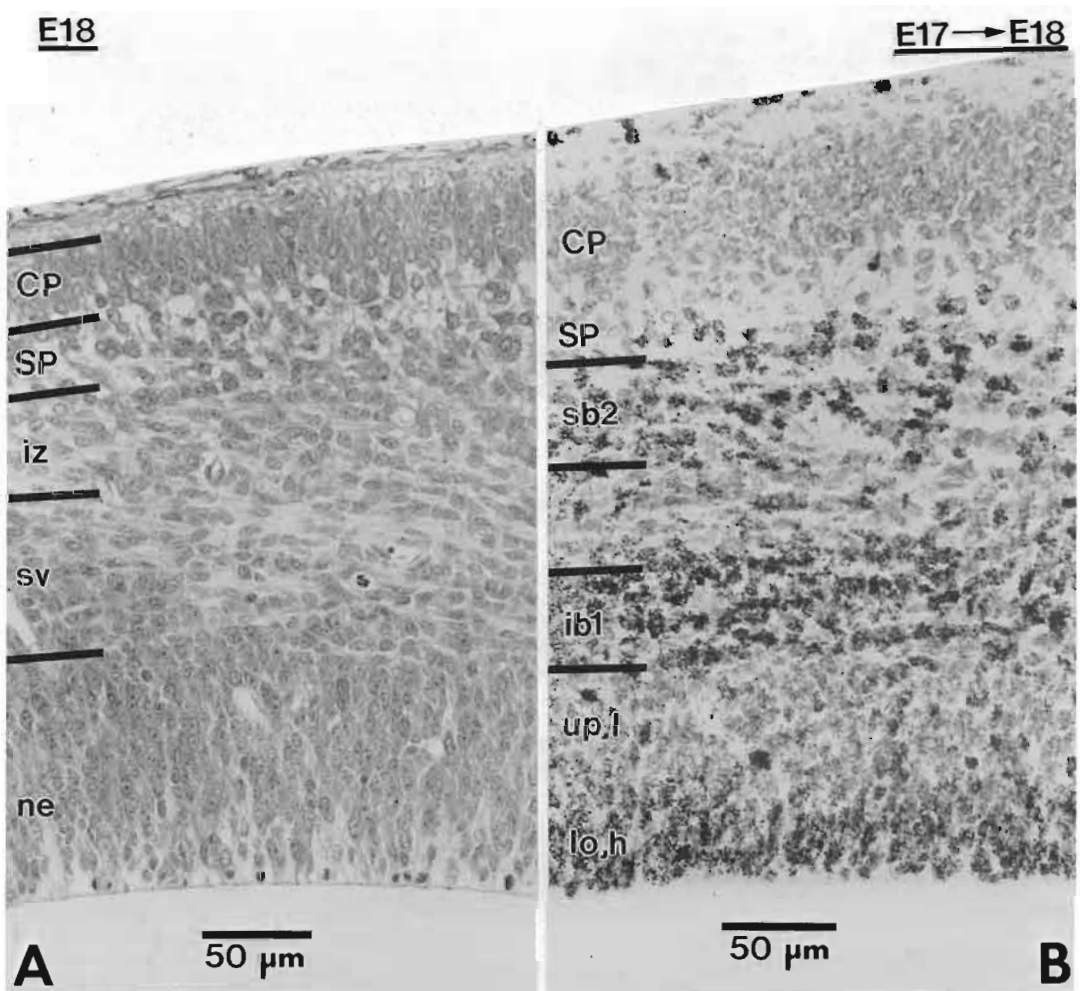


FIG. 7–3. Coronal methacrylate section (3 μm, toluidine blue stain) of the neocortex from an E18 rat **(A)** and a matched paraffin autoradiogram (6 μm paraffin section, hematoxylin stain) from a rat that received [^{3}H]thymidine on E17 and was killed on E18 **(B).**

E18

CP

SP

iz

sv

ne

A

50 μm

E17⇨E18

CP

SP

sb2

ib1

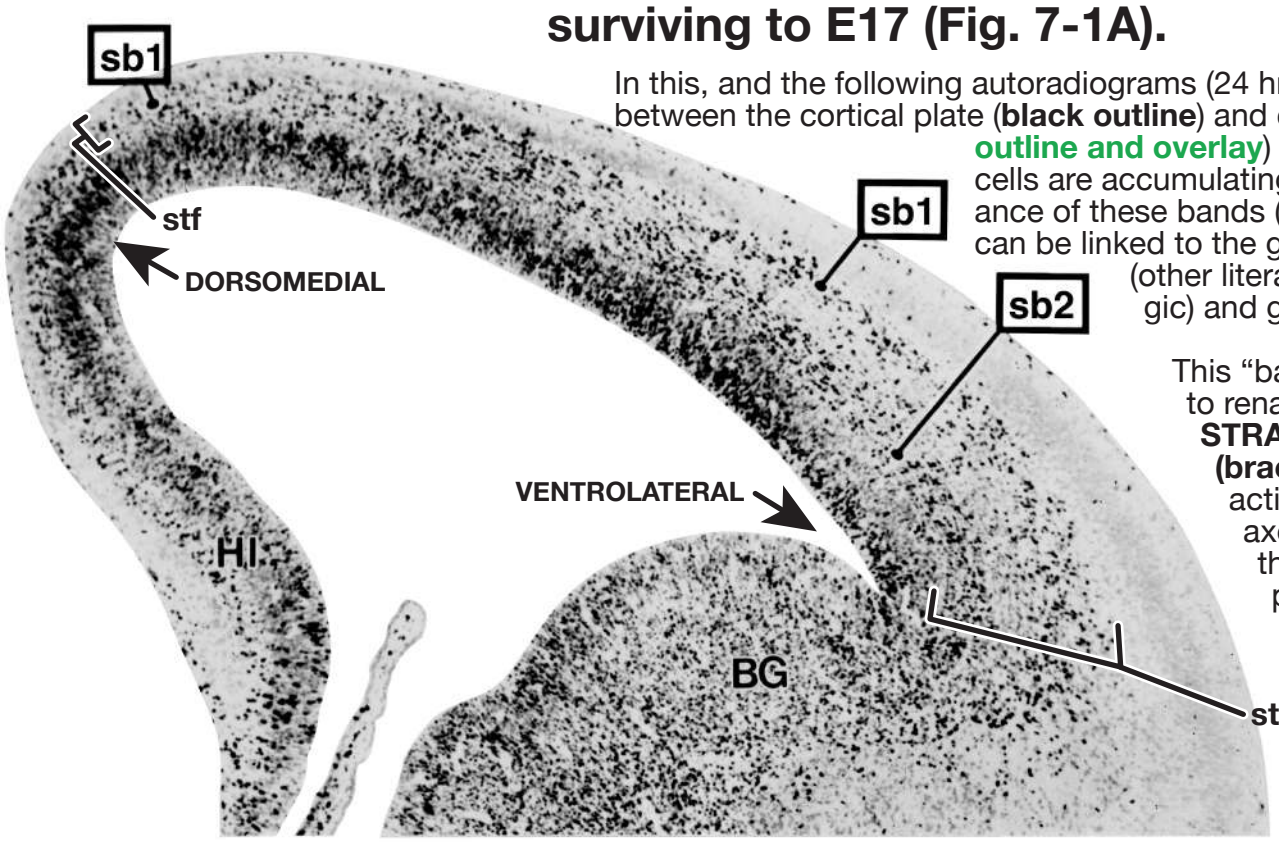
up,l

lo,h

50 μm

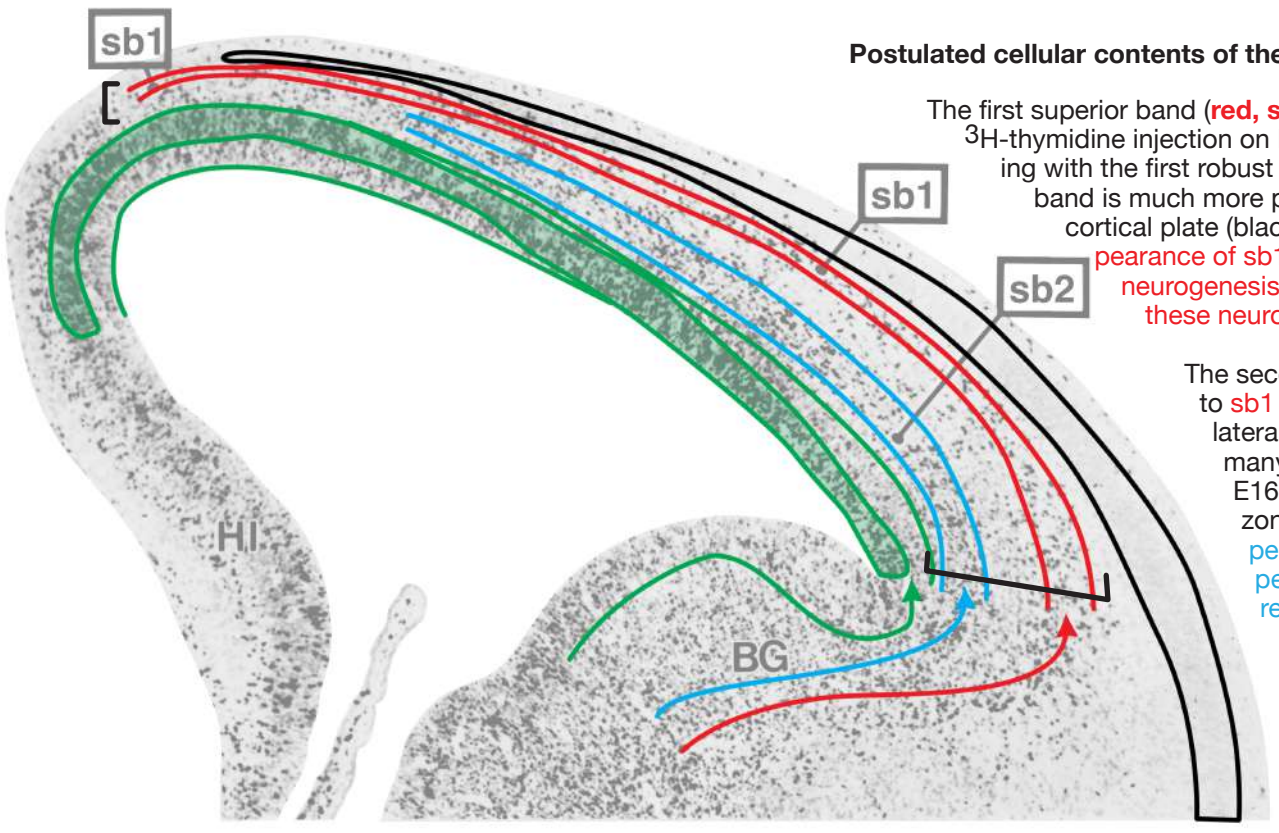
B

Interpretation of an autoradiogram from a rat exposed to ^{3}H thymidine on E16, surviving to E17 (Fig. 7-1A).



In this, and the following autoradiograms (24 hr survival), the intermediate zone between the cortical plate (**black outline**) and cortical germinal zones (**green outline and overlay**) has ***BANDS*** where heavily labeled cells are accumulating. The appearance and disappearance of these bands (only visible with autoradiography) can be linked to the generation of layer-specific neurons (other literature indicates these are glutamatergic) and glia.

This "banding" phenomenon prompted us to rename the intermediate zone the **STRATIFIED TRANSITIONAL FIELD (brackets, stf)**. We postulate that interactions between neurons, glia, outgoing axons, and incoming axons take place there that are necessary for the proper anatomical connections of the cortex to the rest of the brain.



Postulated cellular contents of the stratified transitional field (brackets):

The first superior band (**red, sb1**) first appears one day after a ^{3}H-thymidine injection on E15 (see Fig. 4-9D in Chapter 4) correlating with the first robust day for layer VI neurogenesis. That band is much more prominent on E17 just beneath the cortical plate (black outline). The appearance and disappearance of sb1 correlates closely with peaks in layer VI neurogenesis and may represent a staging site for these neurons.

The second superior band (**blue,sb2**) lies deep to sb1 just outside the ventrolateral and dorsolateral cortical germinal zones (**green**). Since many layer V neurons were generated on E16, they may accumulate in this sojourn zone on E17. The appearance and disappearance of sb2 correlates closely with peaks in layer V neurogenesis and may represent a staging site for these neurons.

Postulated contents of the cortical germinal zones (green lines and overlay):

The cortical neuroepithelium is uniformly thick on E17, while the subventricular zone is a thin ventrolateral area where superficial mitotic cells are regularly seen. After an E16 injection of ^{3}H-thymidine, heavily labeled cells (green overlay) are deep in the ventrolateral area (the later pattern seen after E17 injections) and superficial in the dorsomedial area (the early pattern seen after E13-E15 injections). This shifting labeling pattern correlates with very high peaks of neurogenesis in layers VI and V. We postulate the shifting labeling patterns in the cortical germinal zones indicate that ***progenitor cells are already specified to produce neurons in a given layer***. On E17, progenitors of Cajal Retzius and subplate neurons are ABSENT because these populations have already been completely generated. Layer VI progenitors are very sparse because most have completed their final neurogenetic divisions on E16 and their progeny is in sb1. Layer V progenitors are becoming more sparse because many of these are now undergoing their final neurogenetic divisions. On the other hand, there is a superabundance of superficial-layer (IV-II) progenitors because the final neurogenetic division of only some layer IV neurons is happening now. Most of the superficial-layer progenitors are self-replicating and increasing their numbers in either the subventricular zone or the neuroepithelium for later neurogenesis.

In this and the following figures, the colored arrows indicate migration of cells from the basal ganglia into the cortical germinal zones and stratified transitional field.

There are many unlabeled cells between sb1 and sb2 (Fig. 7–2B) with birthdays before E16. These could be some layer VI neurons generated on E15 and still sojourning in the upper intermediate zone or other unidentified neurons migrating either to more ventrolateral parts of the neocortex or into the basal telencephalon.

E17 to E18 Injection/Survival Group

A single [^{3}H]thymidine injection on E17 and survival to E18 (Fig. 7–1B) also produces two heavily labeled bands in the transitional field, one in the intermediate zone that we call the second superior band (sb2) and the other in the subventricular zone that we call the *first inferior band* (ib1). Both bands span the developing cortex from ventrolateral to dorsomedial. In the lateral cortex, the match between the methacrylate section of a normal E18 embryo (Fig. 7–3A) and an autoradiogram of an E18 embryo that was injected on E17 (Fig. 7–3B) shows that sb2 is situated in the superficial part of the intermediate zone (iz), while ib1 is situated in the deepest part of the subventricular zone (sv) just outside the neuroepithelium (ne). We propose that in the E17 to E18 injection/survival group: (1) sb1 is no longer present; (2) sb2 still contains neurons destined to settle in layer V, and (3) ib1 contains neurons destined to settle in layer IV.

The dissolution of sb1 containing the putative layer VI neurons is in line with neurogenesis of layer VI. Most layer VI neurons originate on E15 and E16 in the somatosensory and motor areas; a [^{3}H]thymidine injection on E17 will heavily label no more than 10% of the layer VI cells, and this small group may not be detectable as a discrete band in the transitional field on the next day (E18).

The superficial band of heavily labeled cells in this group is designated as sb2, the band that contains putative layer V neurons generated on E17. Between E17 and E18, sb2 is displaced upward, closer to the external margin of the intermediate zone (compare Figs. 7–2B and 7–3B) and is clearly present all the way to the dorsomedial edge of the developing cortex (compare Figs. 7–1A and B). The dorsomedial expansion is reconcilable with the evidence that the production of layer V neurons peaks on E17 in the dorsomedial hindlimb motor area (Chapter 14, Fig. 14–5B). The upward shift of sb2 could result from the great expansion of the subventricular and intermediate zones below and the disappearance of the labeled and unlabeled cells that were above sb2 on E17 (see Fig. 7–2B).

An important developmental event on E18 is the formation of the first inferior band (ib1, Fig. 7–1B). Three criteria distinguish ib1 from either sb1 or sb2: (1) the tight packing of the heavily labeled cells of ib1 are more prominent at low magnification than either sb1 or sb2 (Fig. 7–1B); (2) there is no ventrolateral-to-dorsomedial gradient in the emergence of ib1 in contrast to the emergence of sb1 and sb2; and (3) ib1 is located in the lower part of the subventricular zone (Fig. 7–3B), while sb1 and sb2 are in the intermediate zone. In view of the fact that there is a sudden rise in the production of layer IV cells on E17 in the somatosensory areas (Chapter 13, Fig. 13–4), we postulate that the first inferior band emerging in the E17–E18 injection/survival group contains sojourning neurons destined to settle in layer IV.

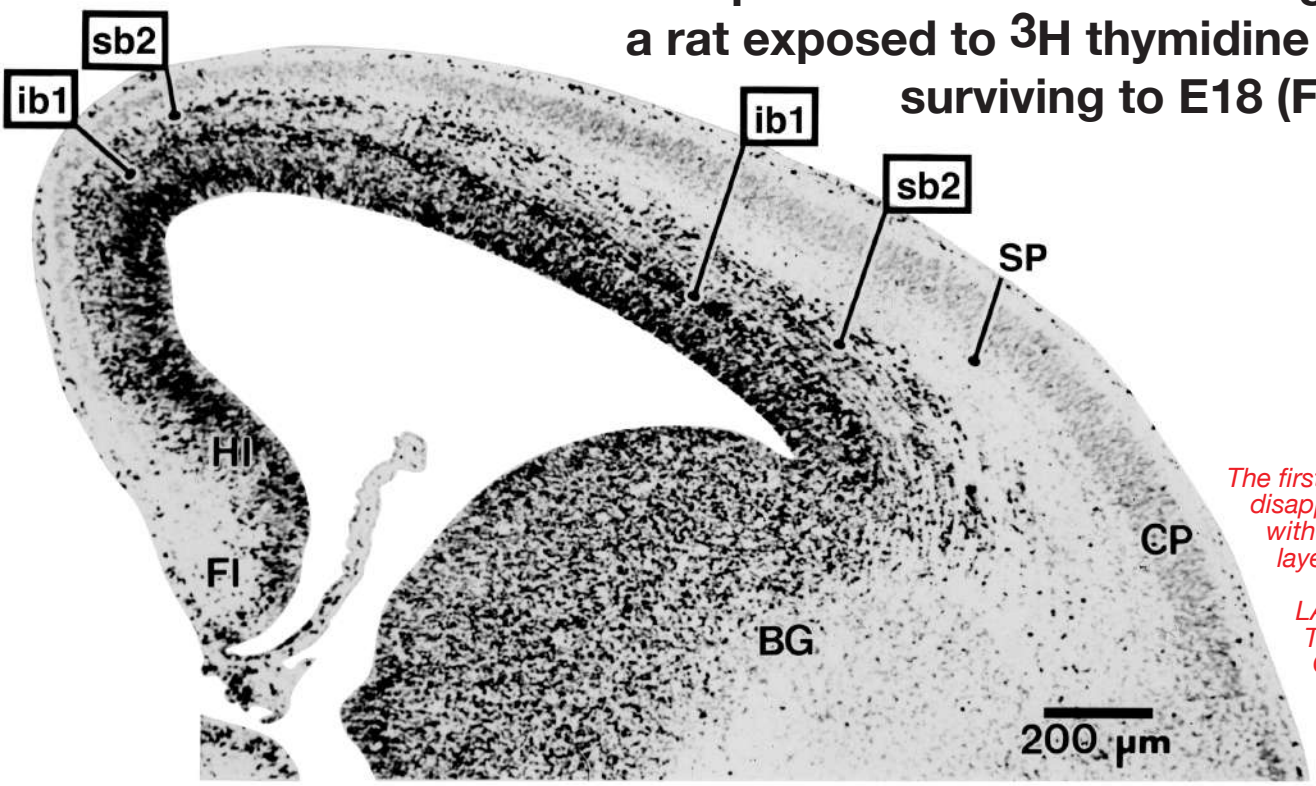
E18 to E19 Injection/Survival Group

A single [^{3}H]thymidine injection on E18 and survival to E19 (Fig. 7–4A) also produces two heavily labeled bands in the transitional field: a diffuse one in the intermediate zone that we call the *third superior band* (sb3) and another one in the lowest part of the subventricular zone, the persisting first inferior band (ib1). Both bands are limited to the lateral and dorsomedial cortex where there is an underlying neuroepithelium. The match between the methacrylate section of a normal E19 embryo (Fig. 7–5A) and an autoradiogram of an E19 embryo that was injected on E18 (Fig. 7–5B) shows that sb3 is situated in the lower intermediate zone (izl) while ib1 occupies the same location as it did in the E17–E18 injection/survival group, a band just outside of the neuroepithelium. Using the supporting arguments given below, we propose the following: (1) sb2 is no longer present; (2) ib1 still contains neurons destined to settle in the superficial layers, at this time layers IV and III; (3) sb3 is a region where glial cells accumulate.

1. Since we postulated that sb2 is the putative staging area of layer V neurons and the quantitative neurogenetic data indicate that the production of layer V neurons has been reduced to a trickle by E18 in both the somatosensory and motor areas (Chapters 13 and 14, Figs. 13–4 and 14–4), few layer V neurons should be left to accumulate in the transitional field on E19. By comparing Figures 7–3B and 7–5B, two features can be seen that distinguish sb2 from sb3 and also lead to the inference that sb2 has disappeared by E19. First, sb3 in the E18–E19 injection/survival group is more diffusely packed with heavily labeled cells than is sb2 in the E17–E18 injection/survival group. Second, cells in sb3 appear to be smaller and more variably oriented than the larger and predominantly horizontally oriented cells in sb2.

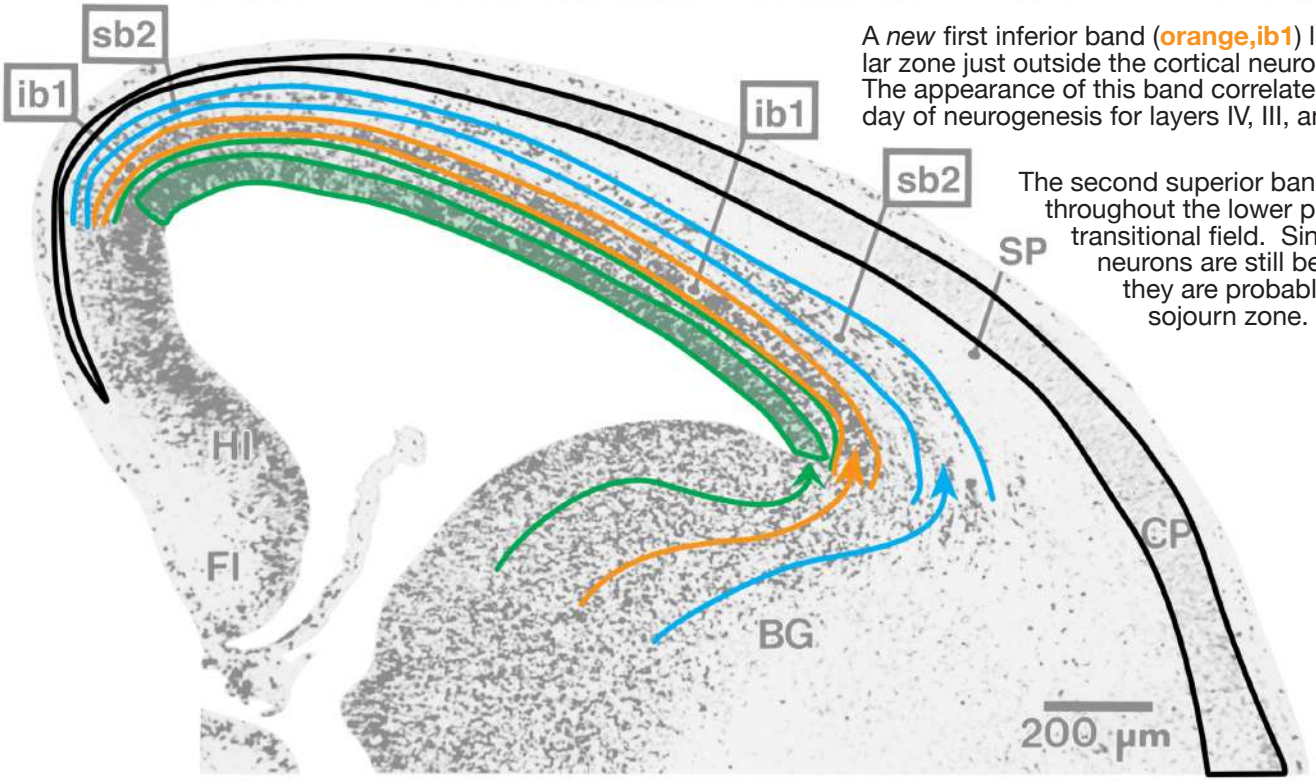
2. E18 is a day for robust production of neurons in layers IV–III in the somatosensory and motor areas (Chapters 13 and 14, Figs. 13–4 and 14–4). Since ib1 still occupies the same location on E19 as it did on E18

Interpretation of an autoradiogram from a rat exposed to ^{3}H thymidine on E17, surviving to E18 (Fig. 7-1B).



The first superior band has disappeared, correlating with the cessation of layer VI neurogenesis.

*LAYER VI PROGENITORS IN THE CORTICAL NEUROEPITHELIUM ARE NOW **ABSENT**.*



A *new* first inferior band (**orange,ib1**) lies in the subventricular zone just outside the cortical neuroepithelium (**green**). The appearance of this band correlates with the first robust day of neurogenesis for layers IV, III, and II.

The second superior band (**blue, sb2**) is present throughout the lower part of the stratified transitional field. Since many layer V neurons are still being generated on E17, they are probably accumulating in this sojourn zone.

The cortical neuroepithelium (**green**, see also Chapter 4) shows a uniform labeling pattern with deep heavily labeled cells (green overlay) and superficial lightly labeled cells. This labeling pattern correlates with waning production of deep cortical neurons (only layer V) and the increasing production of superficial neurons (IV-II). From our neurogenetic timetables acquired with long-survival autoradiography (Chapters 3, 11-15) most of the heavily labeled cells are neurons generated on E17 that will occupy layers V and IV. As layer V neurons migrate to the cortical plate (black outline), they still accumulate in sb2. As future age groups will show, ib1 will be present until layer II neurons finish their neurogenesis. As in Figure 7A, the fact that heavily labeled cells are nonrandomly distributed only 1 day after neurogenesis implies that neural progenitors in the cortical neuroepithelium and subventricular zone are already specified. **All of the remaining layer V progenitors are undergoing their final neurogenetic divisions**, while some layer IV-II progenitors are in their neurogenetic divisions (mainly those of layer IV) others (layers III-II) are still self-replicating and staying in the cortical germinal zones.

The colored arrows indicate neuronal contributions from germinal zones in the basal ganglia.

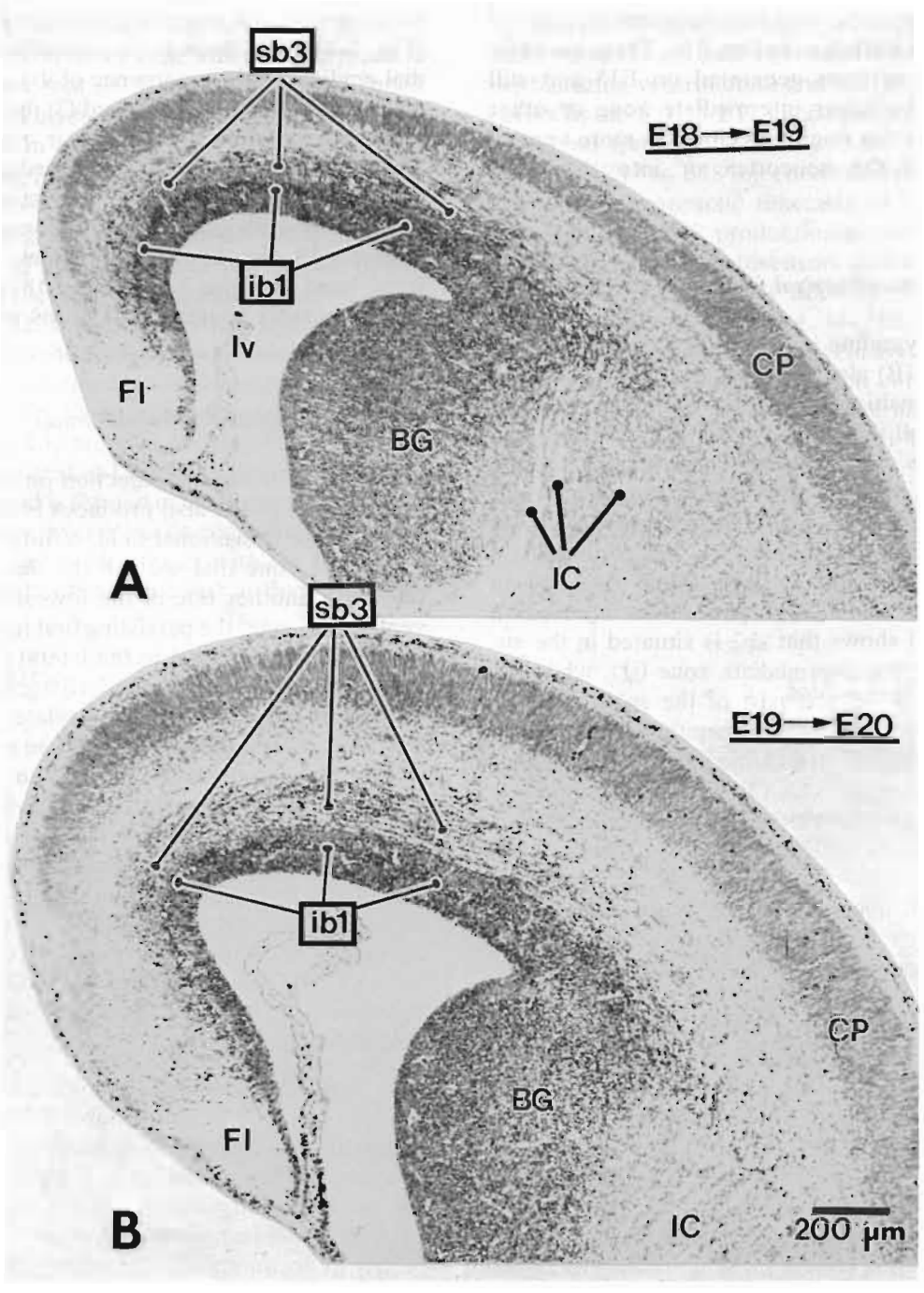
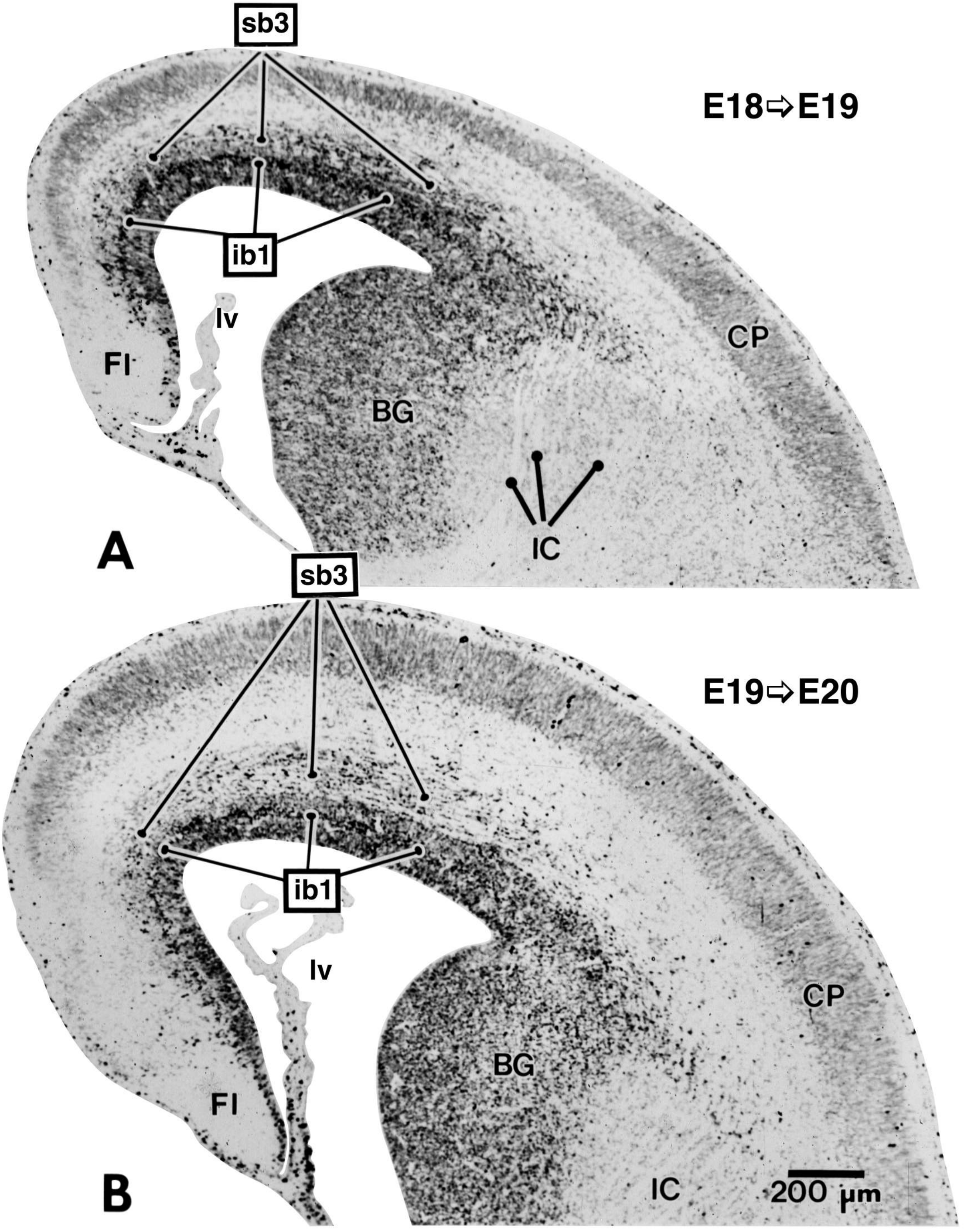


FIG. 7–4. Coronal autoradiograms of the cortex of E19 **(A)** and E20 **(B)** rats that received [^{3}H]thymidine 24 hours earlier. (6 μm paraffin sections, hematoxylin stain.)

(compare Figs. 7–3B and 7–5B), we presume that layer IV neurons continue to accumulate in ib1, as they did on the previous day and now layer III neurons join them.

3. Since sb3 cannot be linked to the production of any neuronal population, we postulate that sb3 is composed of glial cells rather than neurons.

There are some changes with regard to ib1 on E19 that deserve more comment. First, ib1 shrinks in a dorsomedial direction in parallel with the shrinking neuroepithelium (compare the extent of ib1 in Figs. 7–4A and 7–1B). The shrinkage of the neuroepithelium is also shown in the three dimensional reconstructions of the developing neocortex in Chapter 2. Second, ib1


sb3
E18⇨E19
ib1
lv
Fl
CP
BG
IC
A
sb3
E19⇨E20
ib1
lv
CP
BG
Fl
IC
200 µm
B

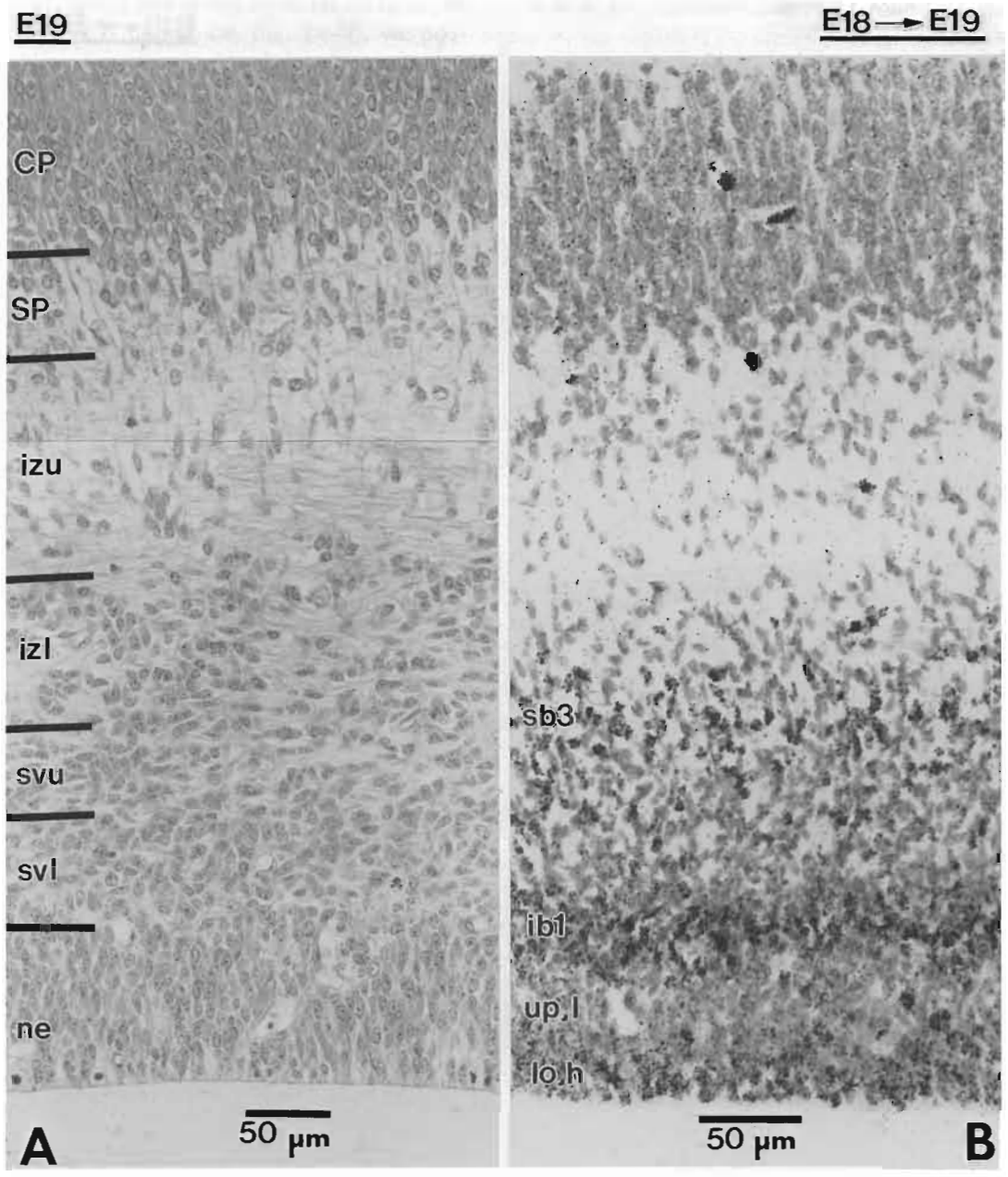


FIG. 7–5. Coronal methacrylate section (3 μm, toluidine blue stain) of the neocortex from an E19 rat **(A)** and a matched paraffin autoradiogram (6 μm section, hematoxylin stain) from a rat that received [^{3}H]thymidine on E18 and was killed on E19 **(B).**

is now covered by a densely packed layer of unlabeled cells in the upper subventricular zone (svu) in the E18–E19 injection/survival group (Fig. 7–5B), in contrast to the pattern seen in the E17–E18 group (Fig. 7–3B). The unlabeled cells were generated before E18, most probably on E17, and may be the layer IV neurons that were sojourning in ib1 the day before.

E19 to E20 Injection/Survival Group

A single [^{3}H]thymidine injection on E19 and survival to E20 (Fig. 7–4B) continues to produce two heavily labeled bands of cells in the transitional field, sb3 and ib1. The match between the methacrylate section of a

E19

CP

SP

izu

izl

svu

svl

ne

A

50 μm

E18⇨E19

sb3

ib1

up,l

lo,h

50 μm

B

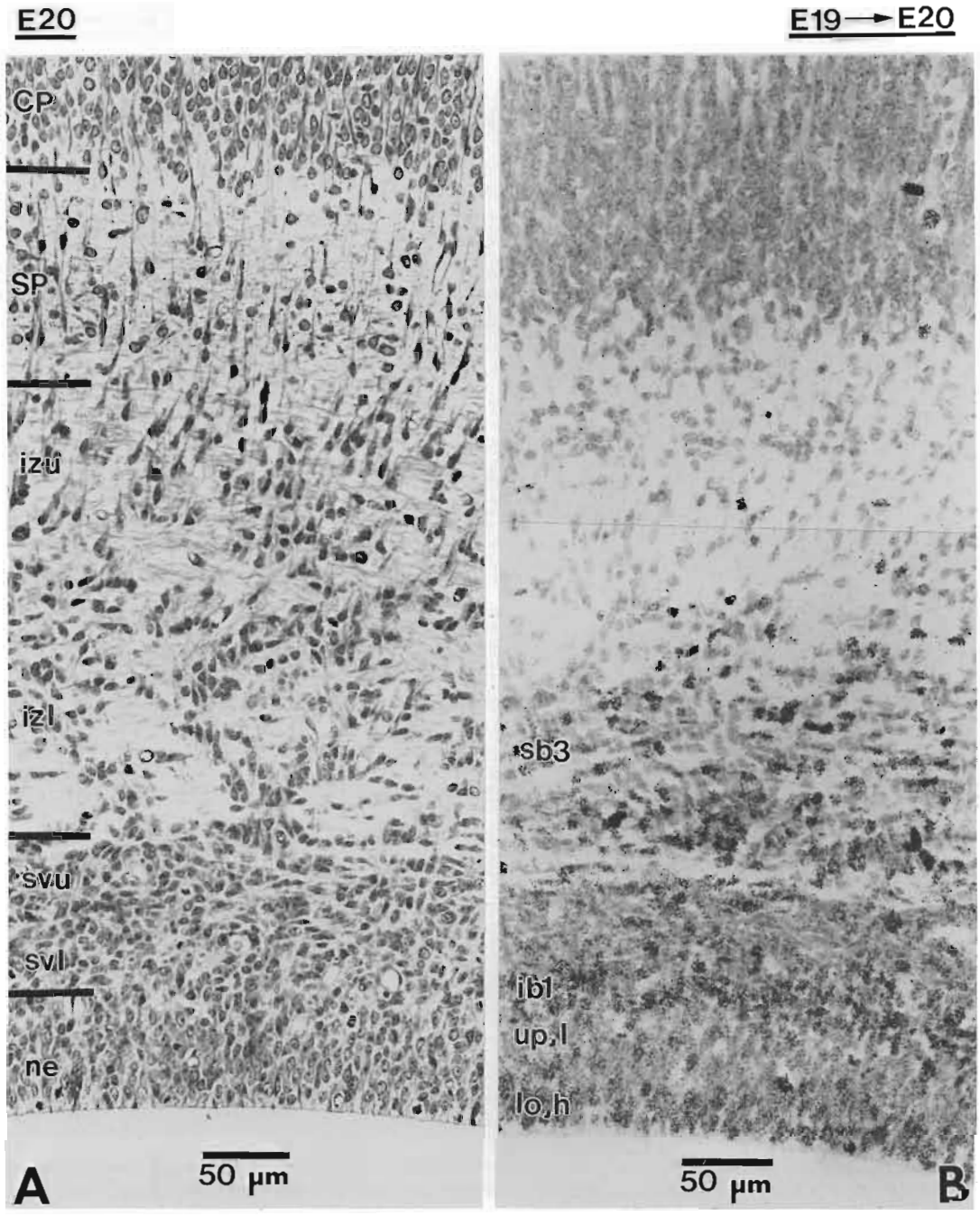


FIG. 7–6. Coronal methacrylate section (3 μm, toluidine blue stain) of the neocortex from an E20 rat **(A)** and a matched paraffin autoradiogram (6 μm section, hematoxylin stain) from a rat that received [^{3}H]thymidine on E19 and was killed on E20 **(B)**.

normal E20 embryo (Fig. 7–6A) and an autoradiogram of an E20 embryo that was injected on E19 (Fig. 7–6B) shows that sb3 and ib1 remain in the same positions as they were in the E18–E19 injection/survival group (compare with Fig. 7–5B). There is an increase in the scattered labeled cells in sb3 but it still overlaps with the lower intermediate zone; it is still postulated to contain the glial precursor cells of the future white matter. The scattered heavily labeled cells in the upper intermediate zone (izu) are presumed to be dispersing glial cells. As in the previous injection/survival group, ib1 remains adjacent to the neuroepithelium but sinks more deeply beneath the unlabeled cells that accumulate in the upper subventricular zone (svu, Fig. 7–6A). Because day E19 is the peak time of the production of layer II neurons in the somatosensory cortex

E20

E19⇨E20

CP

SP

izu

izl

svu

svl

ne

sb3

ib1

up,l

lo,h

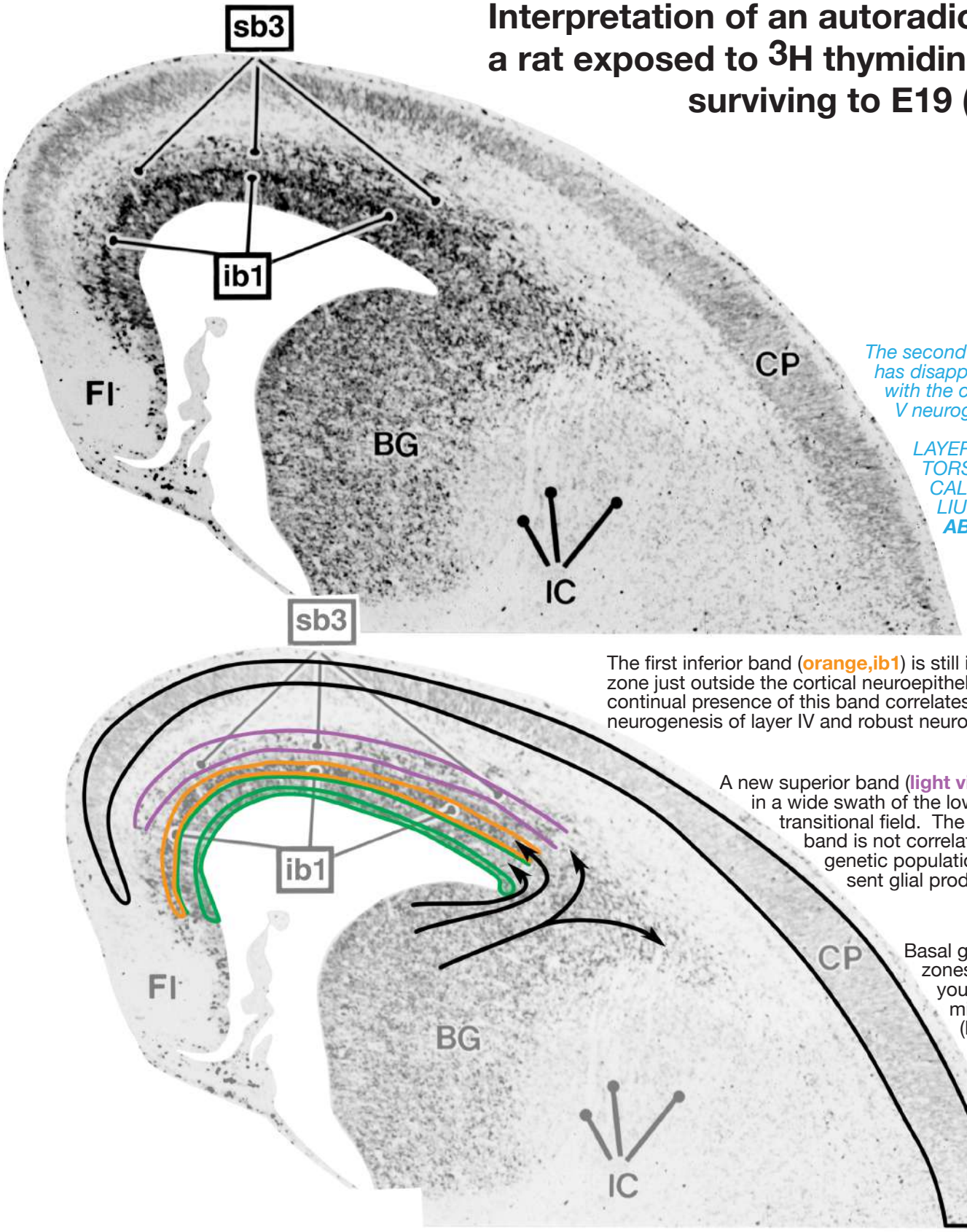
50 μm

50 μm

A

B

Interpretation of an autoradiogram from a rat exposed to ^{3}H thymidine on E18, surviving to E19 (Fig. 7-4A).



The second superior band has disappeared, correlating with the cessation of layer V neurogenesis.

*LAYER V PROGENITORS IN THE CORTICAL NEUROEPITHELIUM ARE NOW **ABSENT**.*

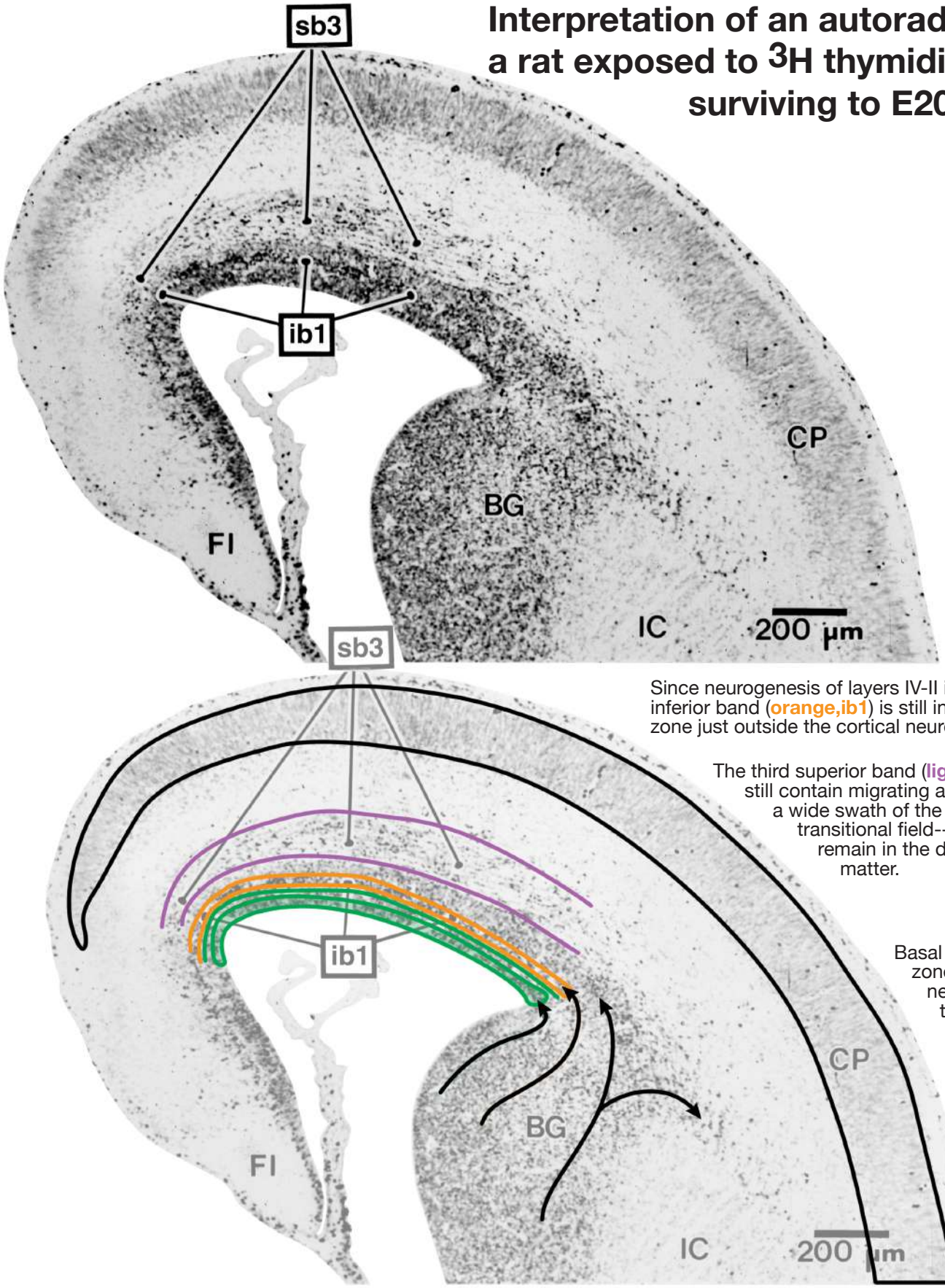
The first inferior band (**orange,ib1**) is still in the subventricular zone just outside the cortical neuroepithelium (**green**). The continual presence of this band correlates with waning neurogenesis of layer IV and robust neurogenesis of layer III.

A new superior band (**light violet, sb3**)appears in a wide swath of the lower stratified transitional field. The appearance of this band is not correlated with any neurogenetic population, and may represent glial production.

Basal ganglionic germinal zones also contribute young neurons that migrate tangentially (laterally and medially) in the cortical stratified transitional field (**black arrows**).

The shrinking cortical neuroepithelium (**green**, see also Chapter 4) continues to show a uniform labeling pattern with deep heavily labeled cells (green overlay) and superficial lightly labeled cells. This labeling pattern correlates with the generation of only superficial neurons (IV-II). From our neurogenetic timetables acquired with long-survival autoradiography (Chapters 3, 11-15) most of the heavily labeled cells in ib1 are neurons generated on E18 that will occupy layers IV and III. As in Figures 7-1A and 7-1B, the fact that heavily labeled cells are nonrandomly distributed only 1 day after exposure to the radiolabel implies that *progenitors in the cortical neuroepithelium and subventricular zone are restricting their potential to produce either glia, ependymal cells, or later developing neurons in layers III and II*.

Interpretation of an autoradiogram from a rat exposed to ^{3}H thymidine on E19, surviving to E20 (Fig. 7-4B).



Since neurogenesis of layers IV-II is continuing, the first inferior band (**orange,ib1**) is still in the subventricular zone just outside the cortical neuroepithelium (**green**).

The third superior band (**light violet, sb3**) may still contain migrating and proliferating glia in a wide swath of the lower stratified transitional field--possibly destined to remain in the deep cortical white matter.

Basal ganglionic germinal zones contribute young neurons that migrate tangentially (laterally and medially) in the cortical stratified transitional field (**black arrows**).

The cortical neuroepithelium (**green**, see also Chapter 4) continues to shrink but still shows a uniform labeling pattern of deep heavily labeled cells (green overlay) and superficial lightly labeled cells. This labeling pattern correlates with the continuing, but diminished, production of superficial neurons (III-II), more glial precursors, and more ependymal cells. From our neurogenetic timetables acquired with long-survival autoradiography (Chapters 3, 11-15) most of the heavily labeled cells in ib1 are neurons generated on E19 that will occupy layers III and II. As in the previous Figures (7-1A and B, 7-4A), the fact that heavily labeled cells are nonrandomly distributed only 1 day after exposure to the radiolabel implies that *progenitors in the cortical neuroepithelium and subventricular zone are already specified to produce either glia, ependymal cells, or neurons in layers III and II*.

(Chapter 13, Fig. 13–4) and of the layer III–II neurons in the motor cortex (Chapter 14, Fig. 14–4), we postulate that ib1 is now occupied mainly by sojourning layer III–II neurons. The unlabeled cells in the upper subventricular zone might be older neurons destined to reside in layers IV–III that have not yet resumed migration to the cortical plate.

E20 to E21 Injection/Survival Group

A single [^{3}H]thymidine injection on E20 and survival to E21 (Fig. 7–7A) produces three heavily labeled bands of cells in the transitional field, a diffuse one in the lower intermediate zone (the same as sb3 of the

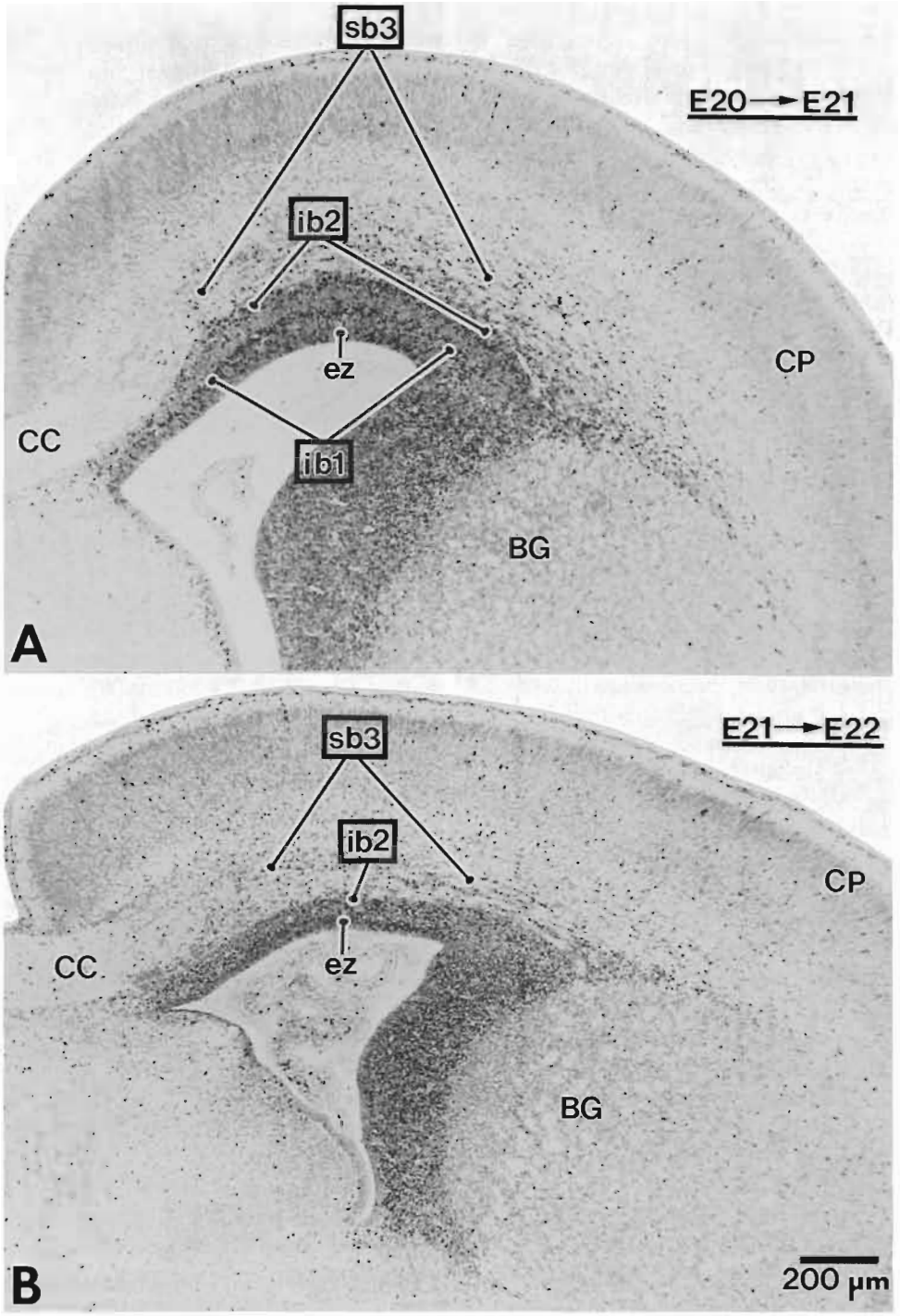
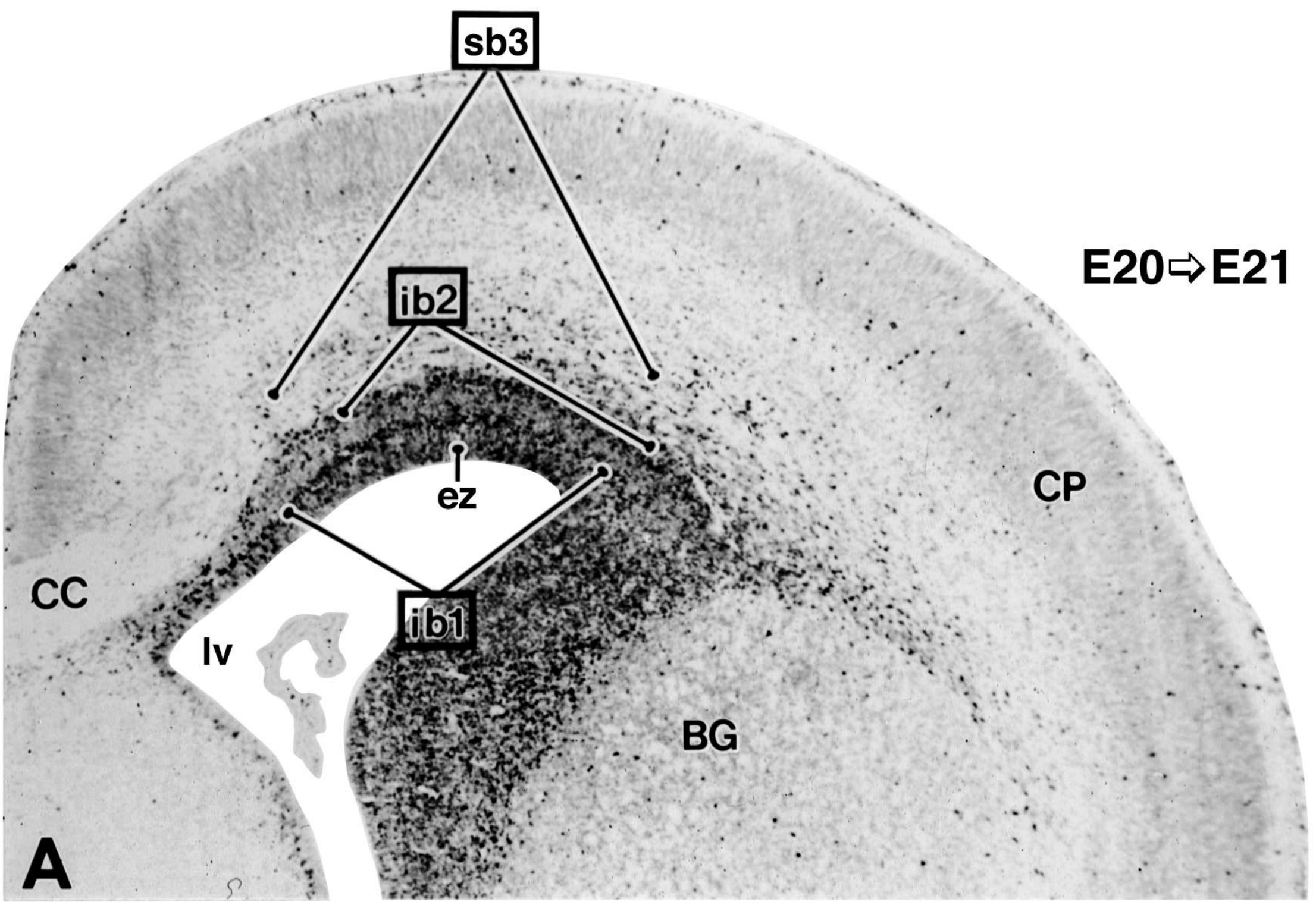
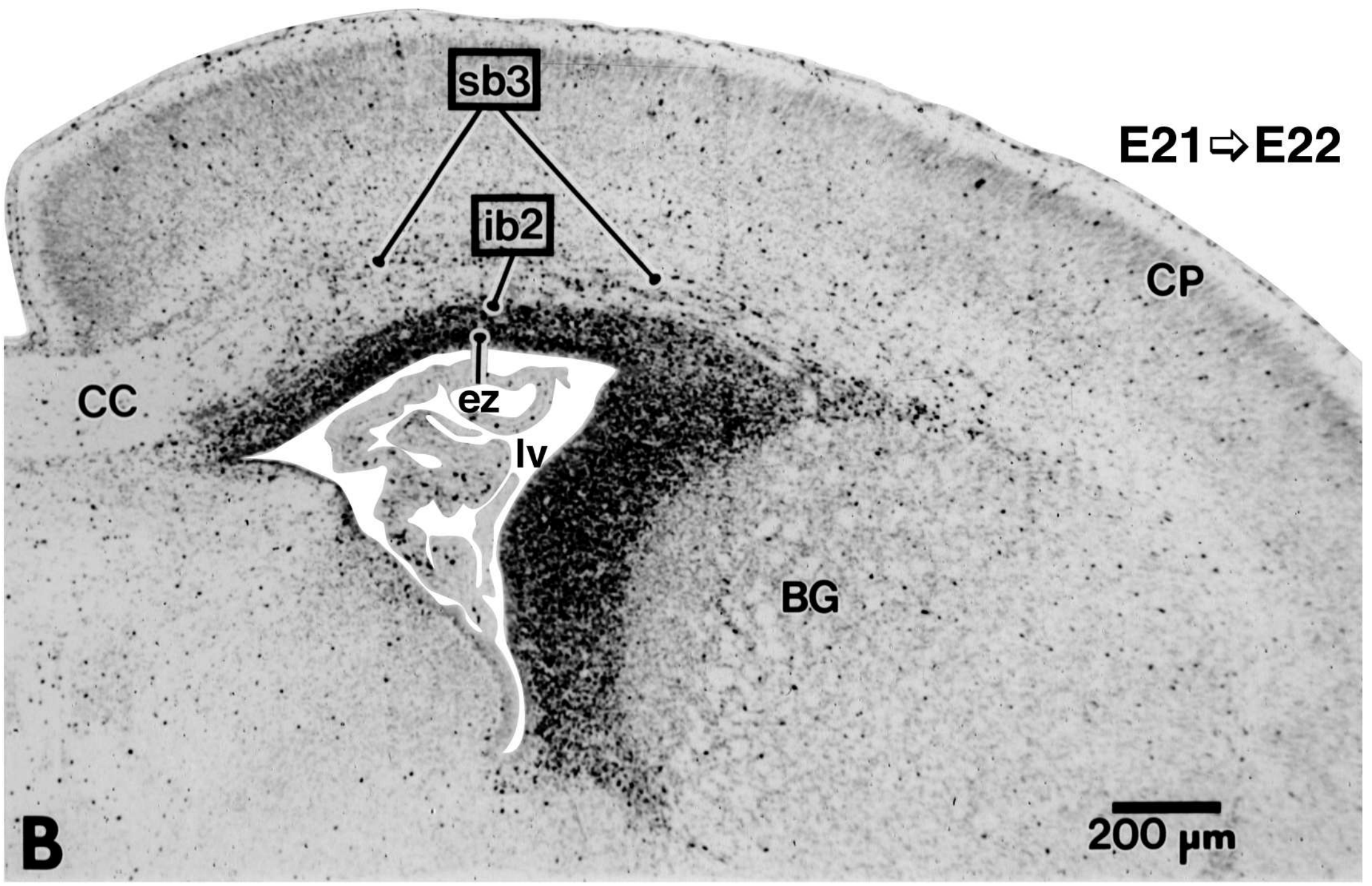


FIG. 7–7. Coronal autoradiograms of the cortex of E21 **(A)** and E22 **(B)** rats that received [^{3}H]thymidine 24 hours earlier. (6 μm paraffin sections, hematoxylin stain.)


sb3
ib2
ez
ib1
lv
CC
CP
BG
E20⇨E21
A


sb3
ib2
ez
lv
CC
CP
BG
E21⇨E22
200 µm
B

previous two groups), a concentrated band at the base of the subventricular zone (the same as ib1 of the previous three groups), and another concentrated band at the superficial border of the subventricular zone. The latter is a new band that we call the *second inferior band* (ib2). The match between the methacrylate section of a normal E21 embryo (Fig. 7–8A) and an autoradiogram of an E21 embryo that was injected on E20 (Fig. 7–8B) shows the placement of the three bands at higher magnification. The only notable change

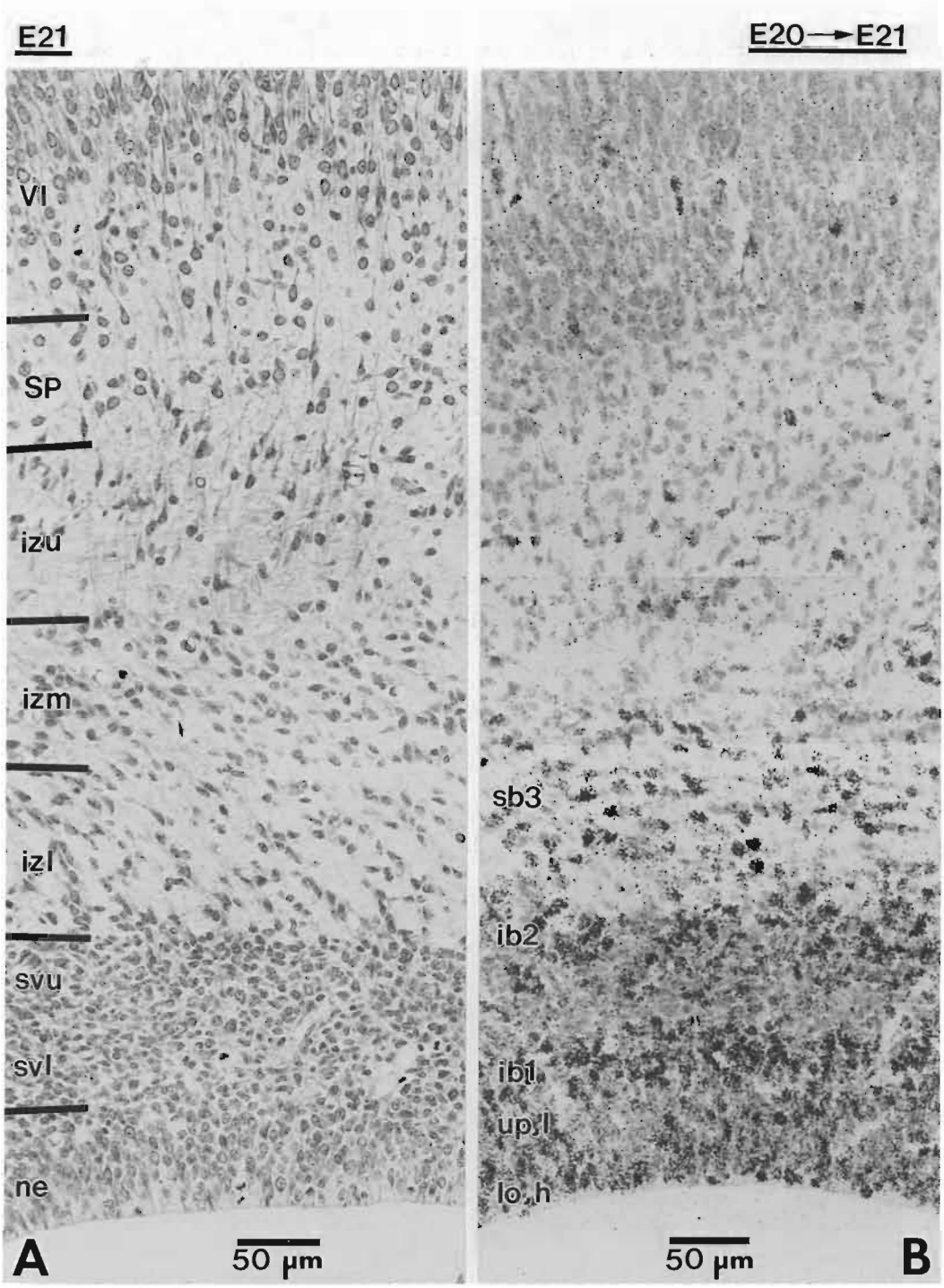


FIG. 7–8. Coronal methacrylate section (3 μm, toluidine blue stain) of the neocortex from an E21 rat **(A)** and a matched paraffin autoradiogram (6 μm section, hematoxylin stain) from a rat that received [^{3}H]thymidine on E20 and was killed on E21 **(B).**

E21

E20⇨E21

VI

SP

izu

izm

izl

svu

svl

ne

sb3

ib2

ib1

up,l

lo,h

50 μm

50 μm

A

B

in sb3 is that its cells are more diffusely scattered than they were on E20, and there is a general decrease in the packing density of cells in the intermediate zone, especially in the lower part (compare Figs. 7–6B and 7–8B). We assume that ib1 still contains neurons, namely, layer II neurons generated on E20 that are distributed superficially throughout the somatosensory and motor areas (Chapters 13 and 14, Figs. 13–4 and 14–4). However, the most notable event in this injection group is the emergence of the second inferior band (ib2) in the upper margin of the subventricular zone. We cannot link the appearance of ib2 to the neurogenesis of any neocortical population. Possibly ib2 is another band of glial cells that is supplying a different fiber tract (the corpus callosum?) than does sb3 (the deep white matter). The scattered labeled cells above sb3 in the upper intermediate zone and in the cortical plate (Fig. 7–8B) are presumed to be locally multiplying glia (see Chapter 4).

E21 to E22 Injection/Survival Group

A single [^{3}H]thymidine injection on E21 and survival to E22 (Fig. 7–7B) again produces two heavily labeled bands in the transitional field, a diffuse one in the intermediate zone (the persisting sb3) and the other in the upper subventricular zone (ib2, the same as the previous group). The match between the methacrylate section of a normal E22 embryo (Fig. 7–9A) and an autoradiogram of an E22 embryo that was injected on E21 (Fig. 7–9B) shows that cells in sb3 are even more diffusely arranged than they were in the E20 to E21 injection/survival group. However, ib2 is still fairly concentrated (compare Figs. 7–8B and 7–9B). The scattered labeled cells above sb3 in the upper intermediate zone and in the cortical plate are likely to be locally multiplying glia. The most notable change on E22 is the absence of a heavily labeled band of cells in the lower subventricular zone. We interpret that to mean that the first inferior band (ib1, which is always located in the lower subventricular zone adjacent to the neuroepithelium) is no longer present on E22. The dissolution of ib1 is reconcilable with the fact that only a negligible proportion of layer II neurons is generated on E21 that will settle in the somatosensory and motor areas (Chapters 13 and 14, Figs. 13–4 and 14–4). The persistence of a conspicuous ib2 near the surface of the subventricular zone beyond the period of neurogenesis supports the inference that this band is not composed of sojourning young neurons but of some other cell type.

7.1.2 Sojourn Zones and Their Significance

The evidence described here suggests that neurons destined for cortical layers VI–II, instead of migrating steadily through the subventricular and intermediate zones to the cortical plate, interrupt their migration to accumulate in cell-specific bands that we call *sojourn zones*. Strictly speaking, a band of heavily labeled cells should be seen in the same location for at least two observation periods to be called a sojourn zone rather than a *wave front*. We will show that cells heavily labeled by an injection of [^{3}H]thymidine on E18 appear in ib1 on E19 and may remain there until E20. However, the cells that form sb1 and sb2 are seen only the first day after a [^{3}H]thymidine injection on either E15, E16, or E17. Do sb1 and sb2 represent wavefronts of steadily migrating neurons rather than sojourn zones? The best evidence that sb1 and sb2 are cell-specific sojourn zones comes from observations made in rats labeled on E16 and killed 24 hours later (Figs. 7–1A and 7–2B) where both sb1 (the putative band of layer VI neurons) and sb2 (the putative band of layer V neurons) coexist in the lateral and ventrolateral cortex. The neurogenetic timetables indicate that E16 is a major period for the production of both layer VI and layer V neurons in somatosensory areas 1 and 2 (Chapter 13, Fig. 13–4). Notwithstanding their cogeneration, some cells (the putative layer VI neurons) migrate a long distance to form sb1 beneath the cortical plate, while other cells (the putative layer V neurons) migrate only a short distance to form sb2 in the lower intermediate zone. Evidently, these two classes of cogenerated cells do not migrate together at a steady rate but sort themselves out in cell-specific bands.

These findings provide support for the hypothesis that the cortical transitional field is the chief staging area for different classes of neocortical neurons where important morphogenetic processes are transpiring in the intermediate zone within the superior bands and in the subventricular zone within the inferior bands. The banding pattern seen with [^{3}H]thymidine autoradiography may be related to the strata of incoming fibers in the intermediate zone that have been seen with tract-tracing methods by Crandall and Caviness (1984), and to the immunoreactive bands for various cell adhesion molecules (Godfraind et al., 1988) or for neurite-associated proteins (Yamamoto et al., 1986).

7.1.3 Autoradiographic Evidence for Stratification Gradients in the Transitional Field

Considering the cortical transitional field as a whole, the first and second superior bands (sb1 and sb2) and the first inferior band (ib1) not only appear sequentially

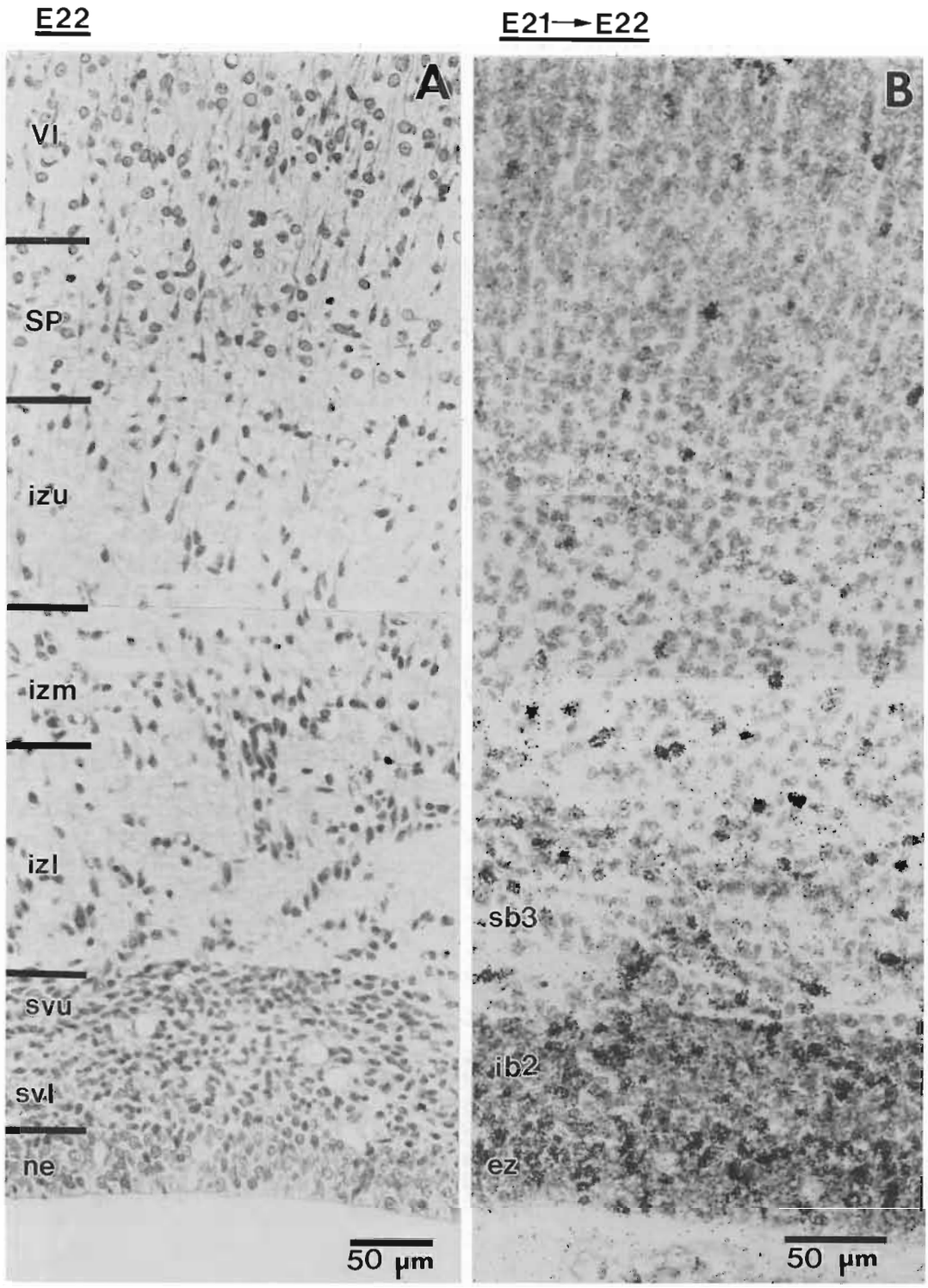


FIG. 7–9. Coronal methacrylate section (3 μm, toluidine blue stain) of the neocortex from an E22 rat **(A)** and a matched paraffin autoradiogram (6 μm section, hematoxylin stain) from a rat that received [^{3}H]thymidine on E21 and was killed on E22 **(B).**

but are stacked from top to bottom according to the age of the neurons that sojourn within them (diagrammed in Fig. 7–10). First, the oldest neurons (layer VI) sojourn on E16 and E17 in sb1 in a superficial location just below the cortical plate. Second, the next oldest neurons (layer V) sojourn on E17 and E18 in sb2 farther from the base of the cortical plate than sb1 (compare Figs. 7–1A and B). Third, successively younger neurons (layers IV, III, II) sojourn on E18 through E21 in ib1 at the external surface of the neu-

E22

E21⇨E22

A

B

VI

SP

izu

izm

izl

svu

svl

ne

sb3

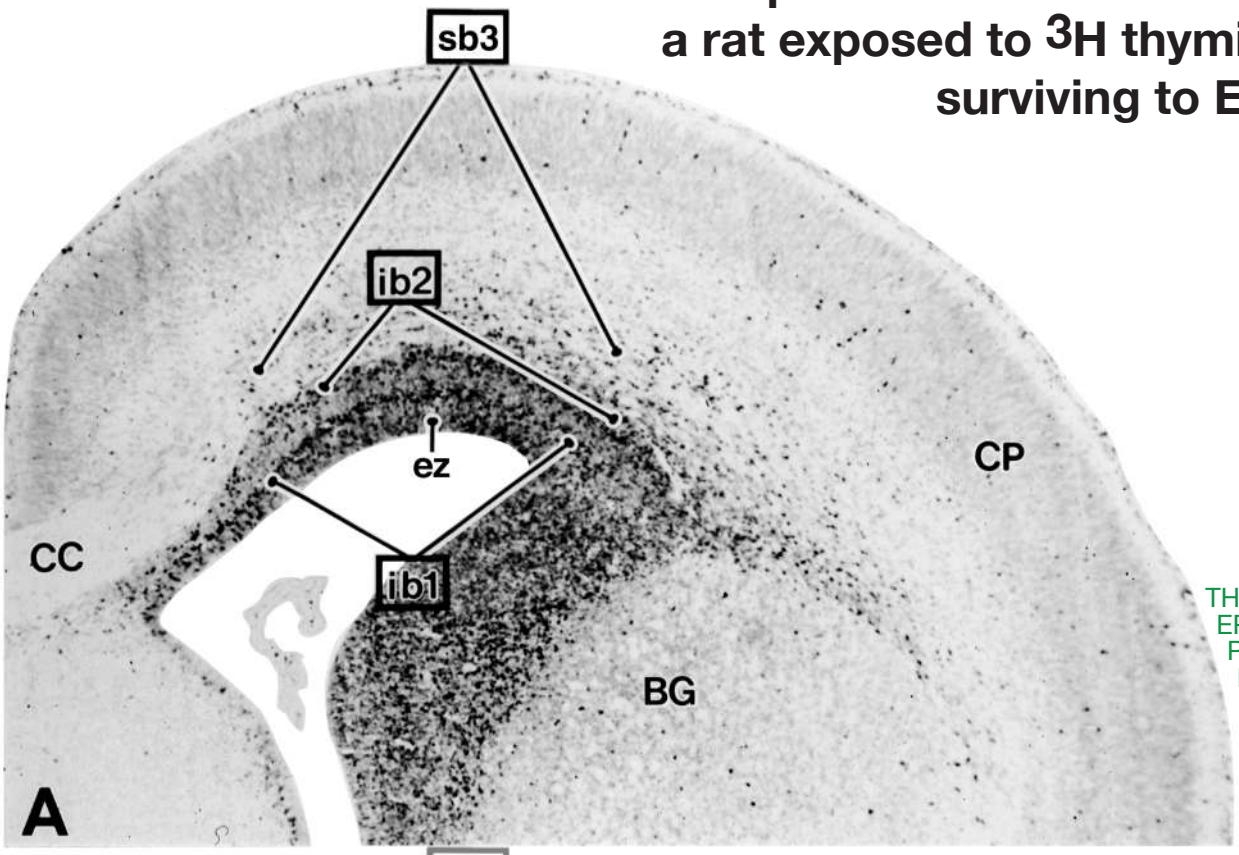
ib2

ez

50 μm

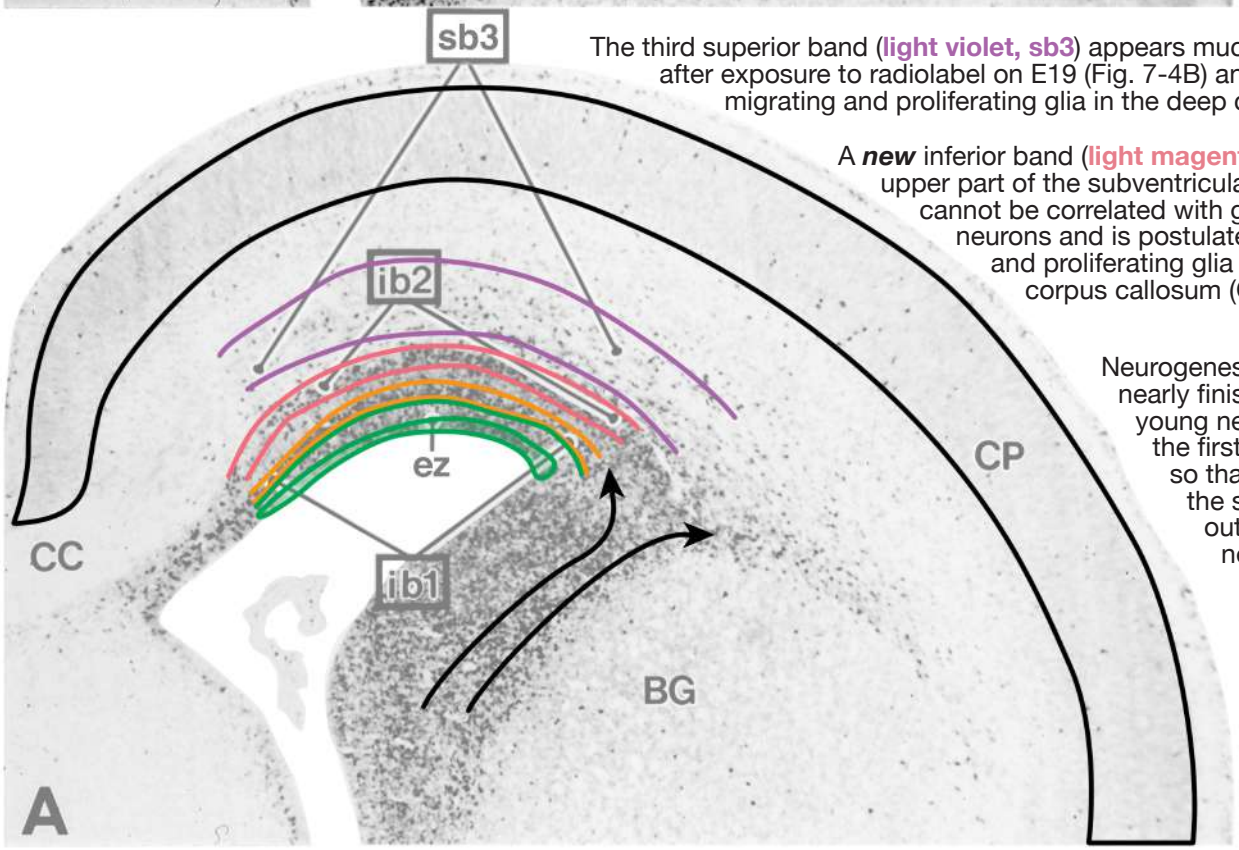
50 μm

Interpretation of an autoradiogram from a rat exposed to ^{3}H thymidine on E20, surviving to E21 (Fig. 7-7A).



THE CORTICAL NEUROEPITHELIUM IS DISAPPEARING AND TRANSFORMING ITSELF INTO AN EPENDYMAL ZONE.

THERE ARE FEW TO NO NEURAL PROGENITORS PRESENT.



The third superior band (**light violet, sb3**) appears much the same as it did after exposure to radiolabel on E19 (Fig. 7-4B) and may still contain migrating and proliferating glia in the deep cortical white matter.

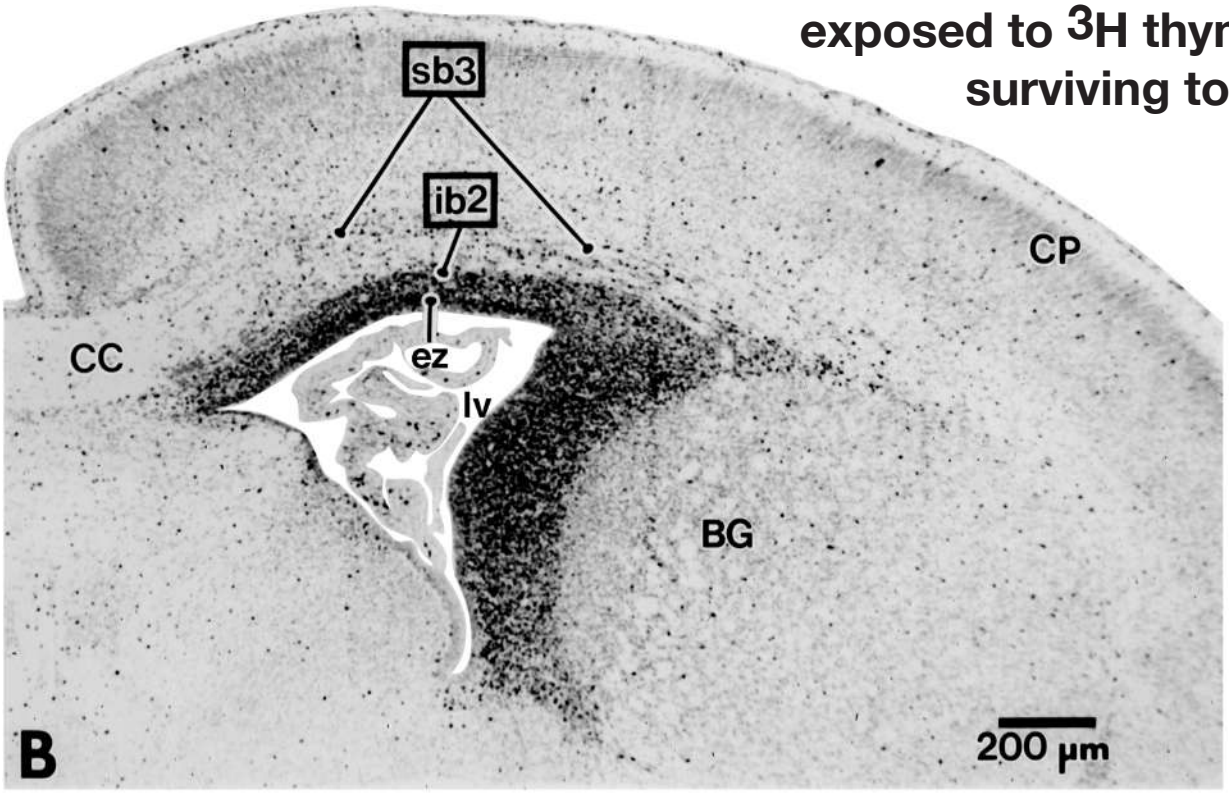
A ***new*** inferior band (**light magenta, ib2**) appears in the upper part of the subventricular zone. This band cannot be correlated with generation of cortical neurons and is postulated to contain migrating and proliferating glia that may migrate into the corpus callosum (CC).

Neurogenesis of layers III and II is nearly finished on E20, but enough young neurons have migrated into the first inferior band (**orange, ib1**) so that it is still visible on E21 in the subventricular zone just outside the shrinking cortical neuroepithelium (**green**).

Basal ganglionic germinal zones contribute young neurons that migrate tangentially (laterally and medially) in the cortical stratified transitional field (**black arrows**).

By E21, the cortical neuroepithelium (green line below ib1) has few to no remaining neuronal precursors because most have undergone their final neurogenetic divisions on E20, and the youngest cortical neurons are now located in ib1. The heavily labeled cells at the ventricular surface form an ependymal zone (ez, **green overlay**, see also Chapter 4) lining the ventricular surface. Other cells deep to the ez are probably proliferating glia, and migrating neurons. From our neurogenetic timetables acquired with long-survival autoradiography (Chapters 3, 11-15) most of the heavily labeled cells in ib1 are neurons generated on E20 that will occupy superficial parts of layer II.

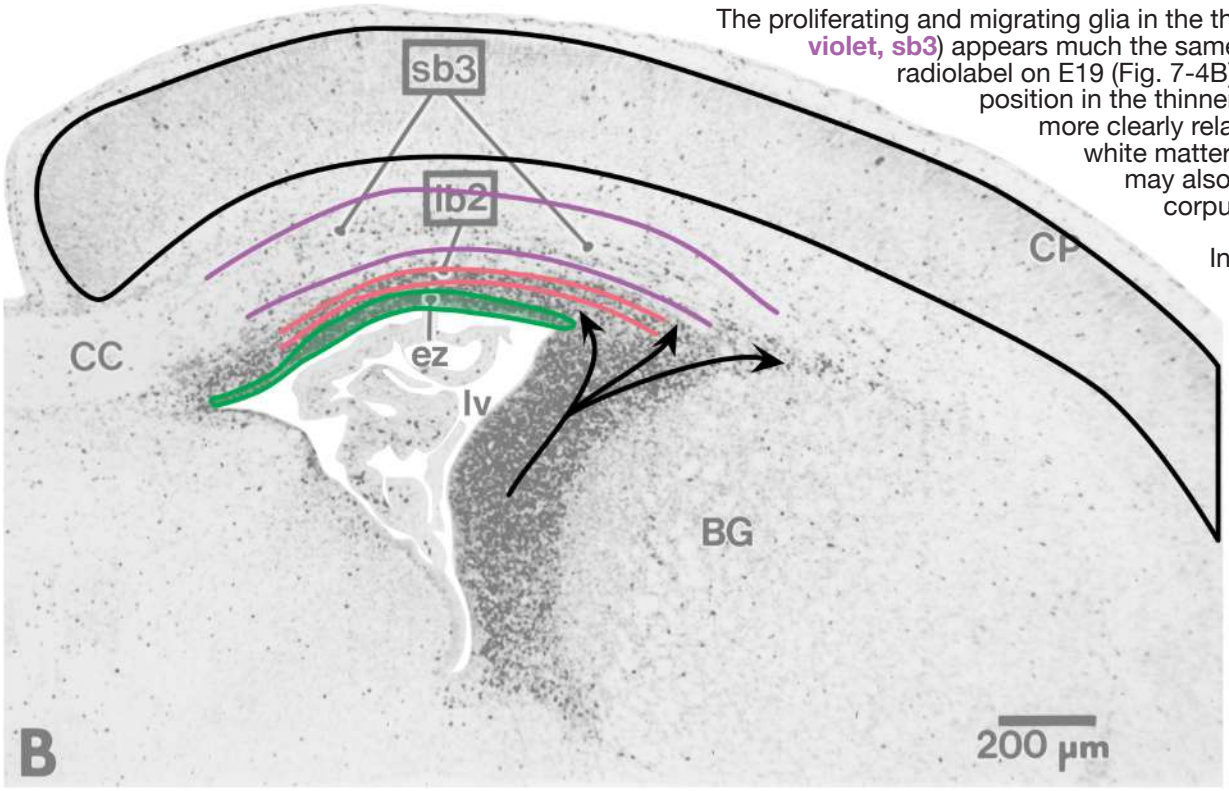
Interpretation of an autoradiogram from a rat exposed to ^{3}H thymidine on E21, surviving to E22 (Fig. 7-7B).



The first inferior band has disappeared, correlating with the cessation of layer II neurogenesis.

THE CORTICAL NEUROEPITHELIUM IS NOW COMPLETELY TRANSFORMED INTO AN EPENDYMAL ZONE.

A FEW NEURAL PROGENITORS ARE SCATTERED IN THE SUBVENTRICULAR ZONE TO CONTRIBUTE TO ADULT NEUROGENESIS.



The proliferating and migrating glia in the third superior band (**light violet, sb3**) appears much the same as it did after exposure to radiolabel on E19 (Fig. 7-4B) and E20 (Fig. 7-7A). Its position in the thinner stratified transitional field is more clearly related to the deep cortical white matter in the mature brain, but cells may also migrate from here into the corpus callosum.

Inferior band 2 (**light magenta, ib2**) is still in the upper part of the subventricular zone. Cells may migrate medially toward the corpus callosum (CC), which has few labeled cells or remain around the deep lateral callosal radiation.

Some of the youngest cortical neurons may also migrate into the neocortex from the basal ganglionic germinal zones (**black arrows**) .

By E22, the cortical neuroepithelium has been completely transformed into a primitive ependymal zone of heavily labeled cells at the ventricular surface (ez, **green overlay**, see also Chapter 4). From our neurogenetic timetables acquired with long-survival autoradiography (Chapters 3, 11-15) most of the heavily labeled cells outside the ez are NOT neurons, but proliferating and migrating glia.

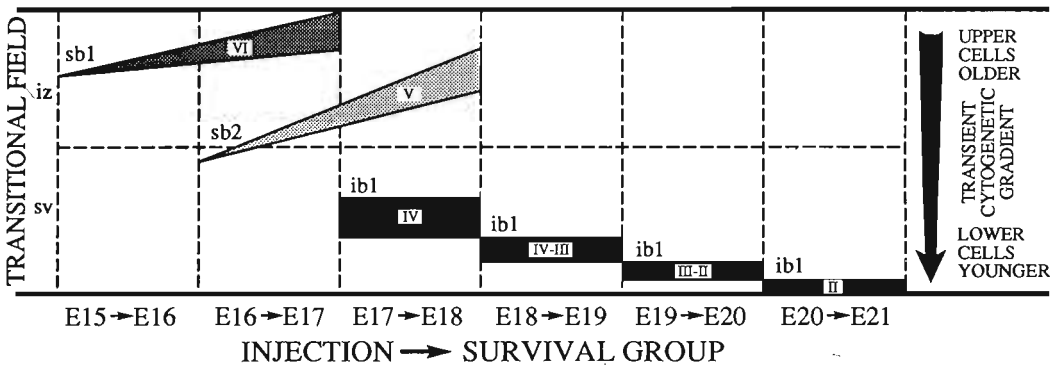


FIG. 7–10. Diagrammatic representation of the three sequentially appearing bands in the transitional field 1 day after injection with [^{3}H]thymidine. Note that the sequential appearance (sb1 first, sb2 second, and ib1 third) correlates with neurogenetic patterns of the neurons that are hypothesized to accumulate within the bands. Also note that the positions of the bands at different levels is according to an outside-in stratification gradient, where older neurons in layer VI putatively sojourn in the most superficial band (sb1) closest to the cortical plate, while the younger neurons in the superficial layers (IV-II) putatively sojourn in the deepest band (ib1) just outside of the neuroepithelium. That transient stratification gradient in the transitional field is a mirror image of the permanent stratification gradient that these cells will take in the cortical plate: layer VI will be deepest, and layer II will be most superficial.

roepithelium in the lower subventricular zone. Each new contingent of heavily labeled cells in ib1 appears below the level of those on the previous day. The general correlation that emerges from the pattern of heavily labeled bands is that earlier-generated neurons sojourn above later-generated neurons. The transient stratification gradient in the transitional field (Fig. 7–10) is the mirror image of the permanent stratification gradient in the cortical plate where older neurons settle below younger neurons (Chapter 6).

But there are two notable exceptions in the stratification gradient described above: the third superior band (sb3) and the second inferior band (ib2) appear after and above the first inferior band. We will present evidence that sb3 can be linked to glial development in the deep white matter. It is very possible that ib2 is also linked to glial development, but at this point all we can say is that ib2 cannot be linked to any neuronal group.

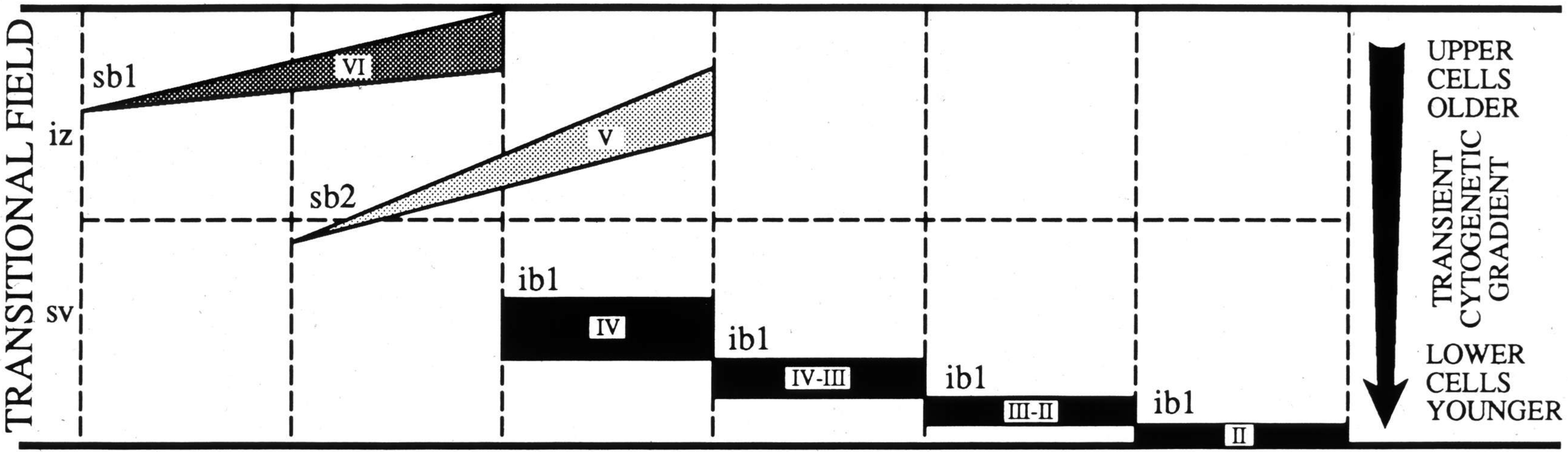
7.1.4 The Dual Nature of the Subventricular Zone

The observations presented earlier indicate that the subventricular zone is not only, and perhaps not primarily, a germinal matrix, but rather it is a premigratory staging area for postmitotic cells that sojourn in the first inferior band (ib1). However, since mitotic cells are in the subventricular zone (Chapter 4), could these be the source of the labeled cells in ib1? That seems unlikely when the density of labeled cells in ib1 is compared to the amount of cell proliferation in the subventricular zone. The ib1 is most dense and most prominent on E18, when it is filled with cells that were born on E17 (Figs. 7–1B and 7–3B), while in Chapter 4 we showed that the distribution of proliferating cells in the subventricular zone is quite sparse on E17 (see Fig. 4–5A). If these scattered proliferating cells were the sole source of the large population of labeled cells in the inferior band on E18, each precursor cell in the subventricular zone would have had to go through several divisions within the 24 hours after the injection of [^{3}H]thymidine. Since rapidly cycling cells quickly dilute the incorporated label, predominantly lightly labeled cells should accumulate in ib1. However, ib1 is packed with heavily labeled cells on E18. The only other source for these cells is the neuroepithelium. Thus, two cell populations must coexist within the subventricular zone: a small group of proliferating cells (we presume they are glial precursors) and a large group of postmitotic presumptive young neurons that accumulate there after they were generated in the neuroepithelium. The more general conclusion that we draw from this is that the neuroepithelium is likely to be the sole source of neurons in layers IV–II of the neocortex. (That hypothesis is more fully discussed in Chapter 16.)

7.2 THE FATE OF CELLS IN THE FIRST INFERIOR BAND AND THE THIRD SUPERIOR BAND

To follow the fate of the cells that sojourn in the different bands of the transitional field, we tracked the heavily labeled cells in the first inferior band (ib1) and

CELL SORTING OF NEURONS INTO SOJOURN ZONES



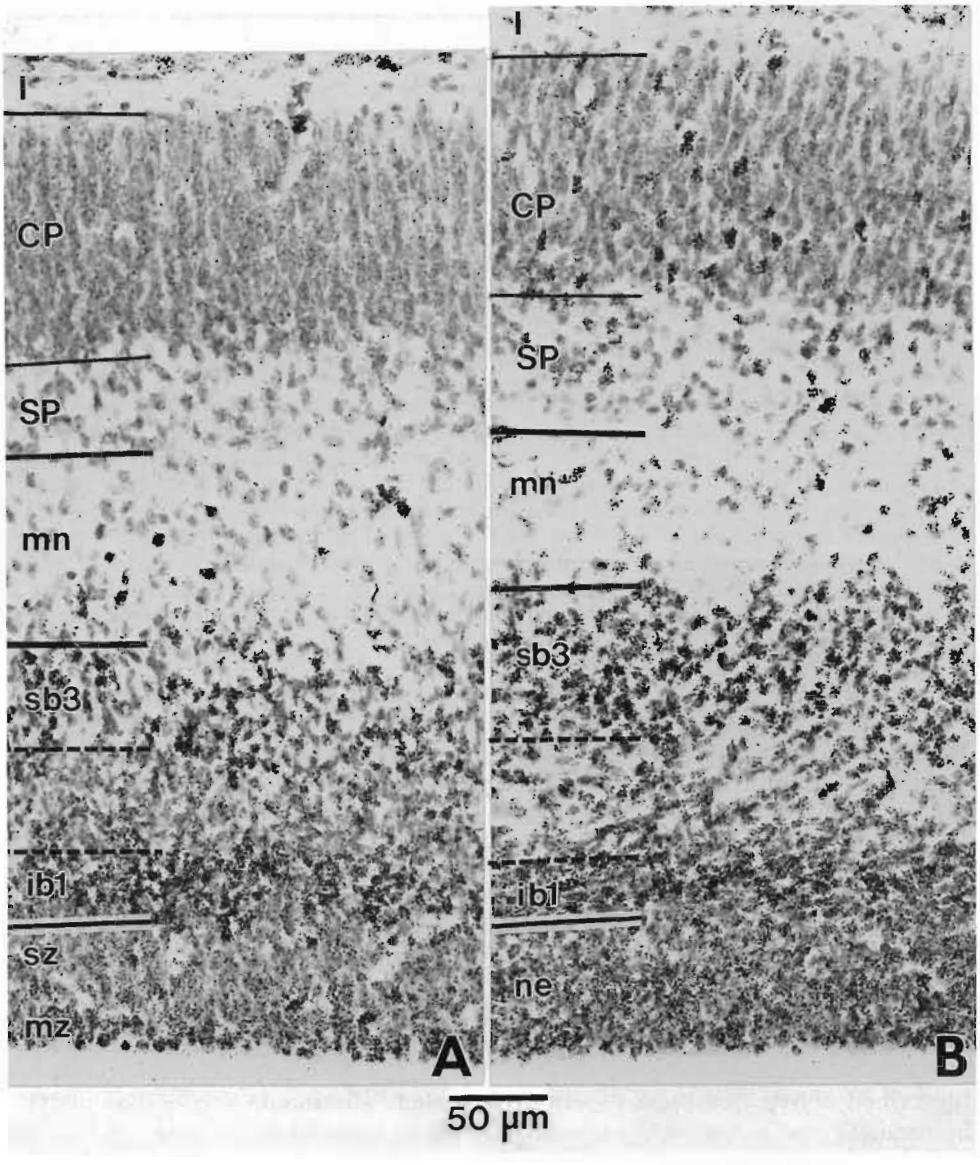


FIG. 7–11. The distribution of heavily labeled cells of the first inferior band (ib1) and the third superior band (sb3) in matched autoradiograms from rats that received [^{3}H]thymidine on E18 and were killed on E19 **(A)** and E20 **(B).** (6 μm paraffin sections, hematoxylin stain.)

the third superior band (sb3) for 4 successive days after an E18 [^{3}H]thymidine injection (Figs. 7–11 and 7–12).

The decrease in labeled cells in ib1 can be linked to the migration of labeled cells to the cortical plate. The first day after injection (Fig. 7–11A), heavily labeled cells (those generated on E18) are densely packed in ib1 at the base of the subventricular zone and are sparsely scattered in sb3 in the center of the intermediate zone. There are few labeled cells in the upper band of migrating neurons (mn, Fig. 7–11A) and, except for an occasional presumed endothelial cell, labeled cells are absent in the subplate (SP) or the cortical plate (CP). Two days after injection (Fig. 7–11B), ib1 becomes narrower and contains fewer heavily labeled cells and more that are lightly labeled. Concurrently, heavily labeled cells appear in the upper band of migrating neurons, in the subplate, and especially in the lower cortical plate. In contrast to the decrease of heavily labeled cells in ib1 between 1–2 days after [^{3}H]thymidine injection, the heavily labeled cells in sb3 increase (compare Figs. 7–11A and B). We infer that it is the heavily labeled cells of ib1 rather than those of sb3 that have penetrated the cortical plate. Three days after injection (Fig. 7–12A), ib1 contains only lightly labeled cells (presumed birthdays after E18). The bulk of heavily labeled neurons (presumed birthdays on E18) have reached the upper part of the thickening cortical plate (CP) but some are still scattered in its lower part. These observations indicate that cells generated on E18 sojourn in ib1 on E19, many are still

there on E20 (Fig. 7–11), but all are gone by E21 (Fig. 7–12A). Four days after injection (Fig. 7–12B), ib1 is no longer visible above the shrunken neuroepithelium (ne). A few heavily labeled neurons are still migrating through the cortical plate but most of them have settled in its upper layers. Indeed, by this time the cortical plate also contains some lightly labeled neurons (presumed birthdays after E18).

The labeling pattern in sb3 is compatible with the hypothesis that it contains predominantly glial cells. One day after injection (Fig. 7–11A), heavily labeled cells are sparsely scattered in sb3. Two days after injection (Fig. 7–11B), heavily labeled cells increase in sb3; the additional labeled cells are presumably young neurons migrating through this layer to the cortical plate from the underlying ib1. But sb3 also thickens relative to the previous day and contains more lightly labeled cells. Three days after injection, (Fig. 7–12A), sb3 expands in a downward direction and becomes coextensive with the white matter (WH). Heavily la-

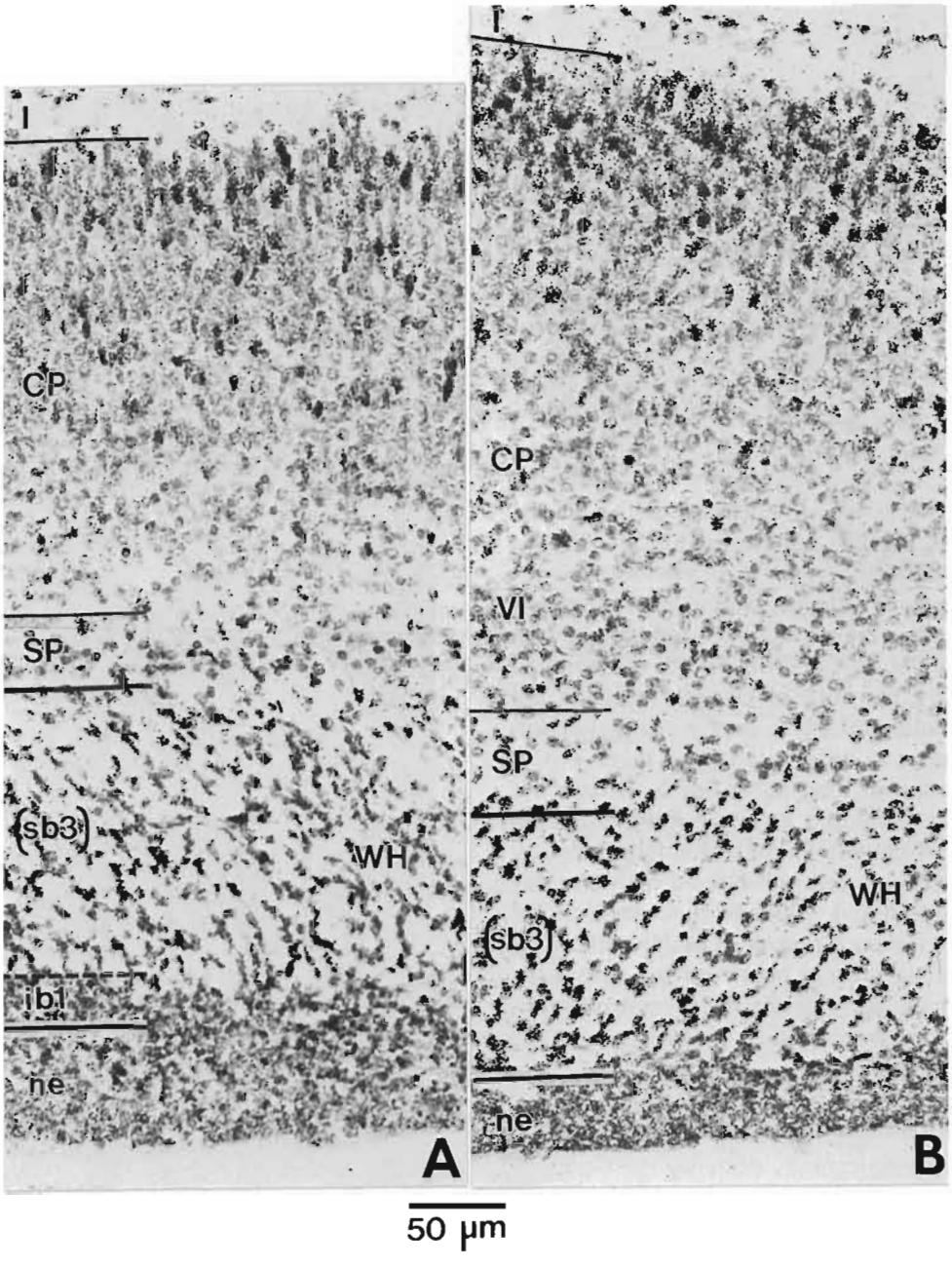


FIG. 7–12. Continuation of the series shown in Fig. 7–11. The distribution of heavily labeled cells of the first inferior band (ib1) and the third superior band (sb3) in matched autoradiograms from rats that received [^{3}H]thymidine on E18 and were killed on E21 **(A)** and E22 **(B).** (6 μm paraffin sections, hematoxylin stain.)

beled cells are still present in sb3 but the proportion of lightly labeled cells has increased. It is noteworthy that the heavily labeled cells in sb3 are generally smaller by this time than are the heavily labeled neurons in the cortical plate. Four days after injection (Fig. 7–12B), the labeled cells that constitute sb3 are still coextensive with the cortical white matter (sb3 and WH) at the base of the cortex adjacent to the shrunken neuroepithelium (ne). These observations support our interpretation that the labeled cells of sb3 contain glial cells that become associated with the fibers of the cortical white matter.

7.3 BANDS IN THE TRANSITIONAL FIELD IN RELATION TO THE DEVELOPMENT OF CYTOARCHITECTONIC DIFFERENTIATION OF THE CORTEX

Variations in the six-layered neocortex, brought about by regional differences in the concentration of different classes of neurons, have been used from the beginning of this century to distinguish different cytoarchitectonic areas in the cerebral cortex (Campbell, 1905; Brodmann, 1909; Vogt and Vogt, 1919; Economo and Koskinas, 1927). Although the validity of some of the more elaborate parcellations, like that of the Vogts, has been questioned on methodological grounds (e.g., Lashley and Clark, 1946), there is no reason to doubt the distinction between the two most markedly heterogeneous regions, the agranular (motor) and the granular (sensory) cortices. From the developmental perspective, there is the unresolved issue (a) when and where is the commitment made during cortical development for a neuron to differentiate as a layer V pyramidal cell (and sprout long axons) or as a layer IV granular cell (and develop a locally ramifying axonal plexus), and (b) when and where is the determination made that the motor cortex will have a high concentration of large pyramidal cells and the visual cortex will have a high concentration of granular cells? The observations that we present below offer a partial answer to the latter question, while the former question will be discussed in Chapter 16.

7.3.1 Regional Differences in the Second Superior Band

Support for the identification of the second superior band (sb2) in the E17–E18 injection/survival group (Fig. 7–1B) as the sojourn zone of neurons destined to settle in layer V comes from evidence for regional differences in the concentration of heavily labeled cells in this band at anterior and posterior levels of the dorsal cortex (Altman and Bayer, 1991b). At anterior levels (Figs. 7–13A, B), and in the midportion of the cortex (Figs. 7–14A, B), sb2 forms a conspicuous band of heavily labeled cells in the intermediate zone beneath the cortical plate (CP). The band arches across the cortex from ventrolateral to dorsomedial roughly in parallel with the lateral ventricle (lv). In sharp contrast, at posterior levels, little more than a few scattered heavily labeled cells represent sb2 in the intermediate zone (sb2 in brackets in Figs. 7–15A, B).

The great difference in the concentration of heavily labeled sb2 cells between the anterior and posterior poles of the cortex is shown at higher magnification in Fig. 7–16. Anteriorly (Fig. 7–16A), sb2 is composed of heavily labeled cells piled up several cells deep. Posteriorly (sb2, Fig. 7–16B), only a few scattered heavily labeled cells hint at the location of sb2 within the intermediate zone (iz). Additional samples, from roughly equidistant levels through the dorsal aspect of the developing cortex, indicate a decreasing concentration of heavily labeled sb2 cells from rostral to cau-

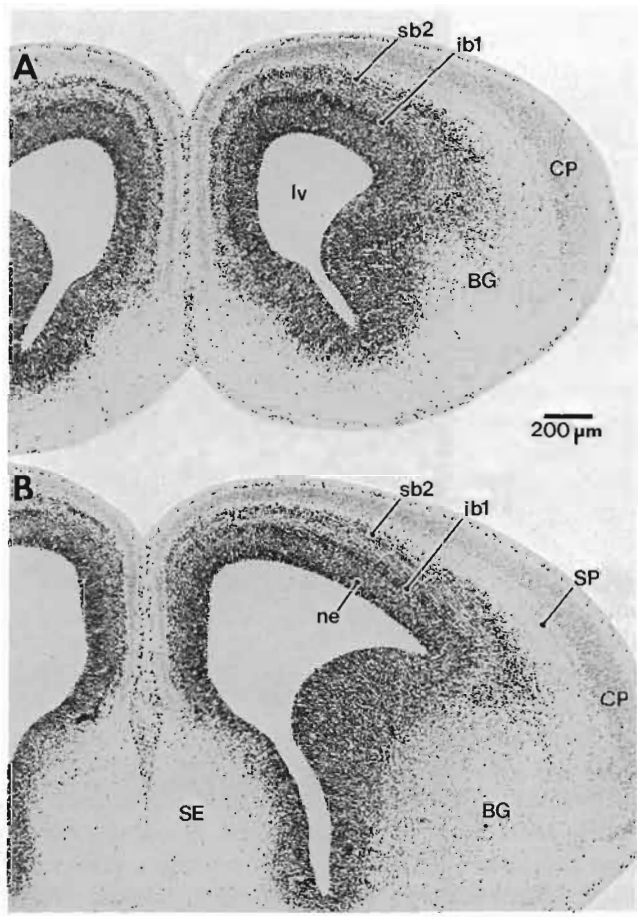


FIG. 7–13. Selected coronal autoradiograms of the cerebral cortex from rostral to caudal from a rat that received [^{3}H]thymidine on E17 and was killed on E18. **(A)** level of the frontal cortex; **(B)** level of the septum. (6 µm paraffin sections, hematoxylin stain.)

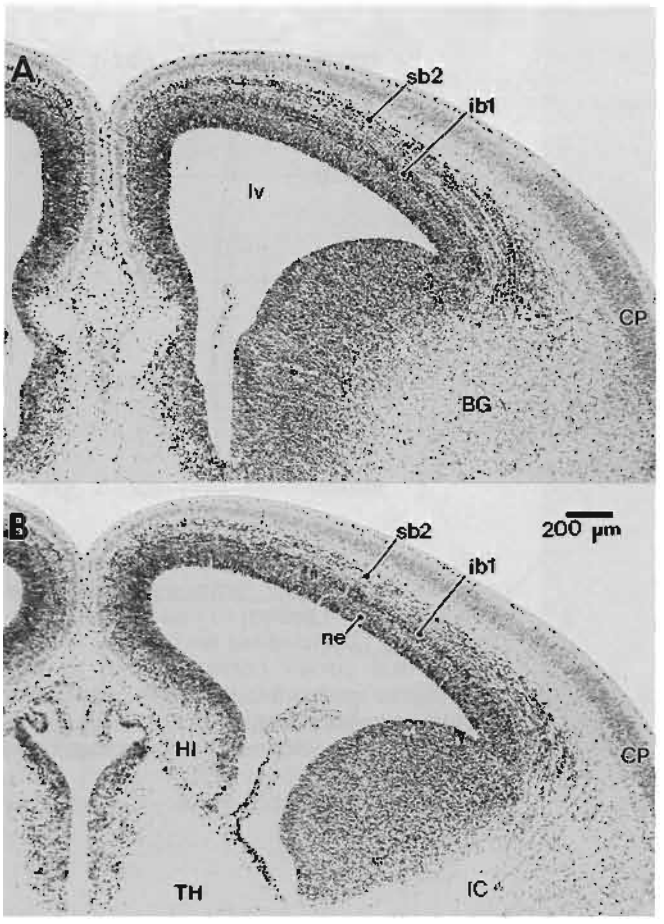


FIG. 7–14. Continuation of the series shown in Fig. 7–13. **(A)** level of the anterior thalamus; **(B)** level of the anterior hippocampus. (6 μm paraffin sections, hematoxylin stain.)

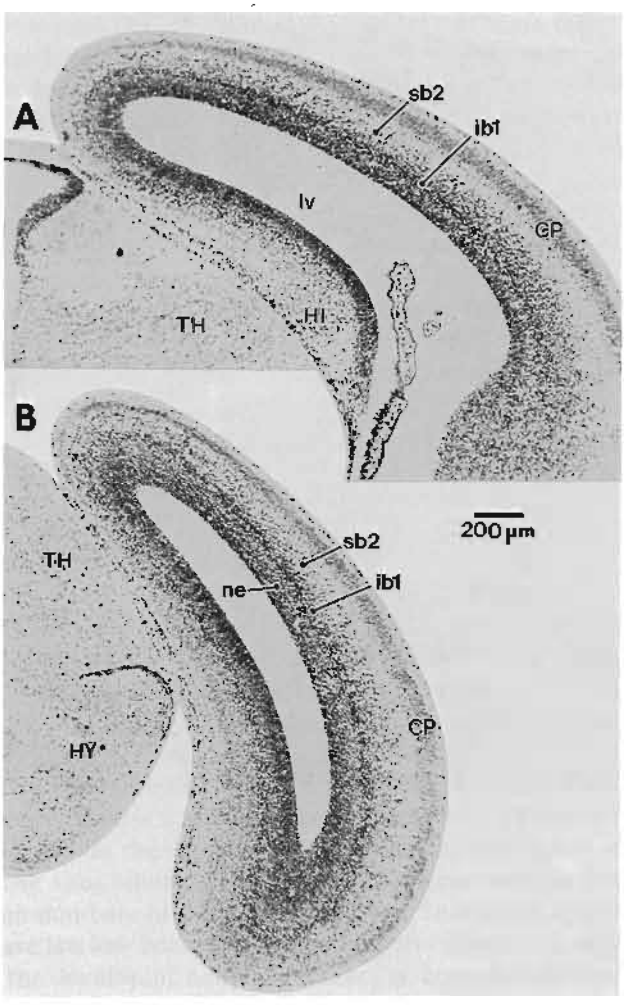


FIG. 7–15. Continuation of the series shown in Fig. 7–13 and 7–14. **(A)** level of the mid-thalamus; **(B)** level of the posterior thalamus. (6 μm paraffin sections, hematoxylin stain.)

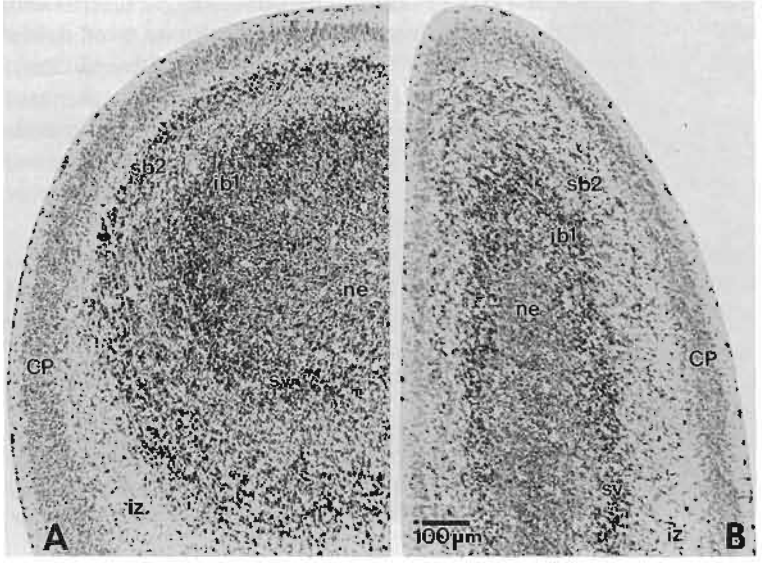


FIG. 7–16. Coronal autoradiograms of the anterior tip of the cortex **(A)** and its posterior tip **(B)** from a rat labeled on E17 and killed on E18. (6 μm paraffin sections, hematoxylin stain.)

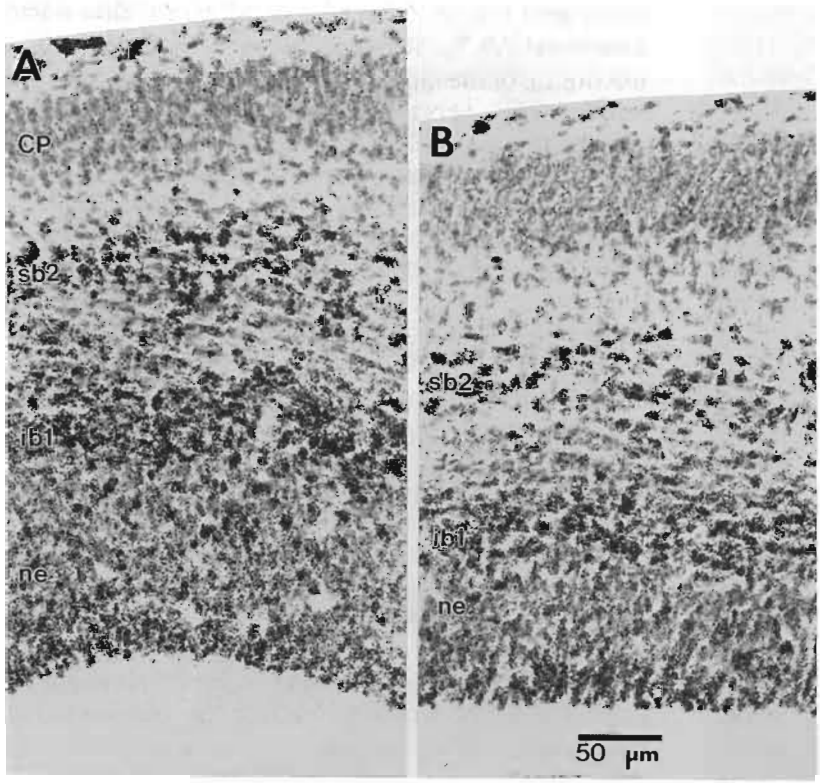


FIG. 7–17. Selected coronal autoradiograms of matched regions of the dorsal neocortex from rostral to caudal in a rat labeled on E17 and killed on E18. **(A)** level of the frontal cortex corresponding to that shown in Fig. 7–13A. **(B)** level of the midcortex corresponding to that shown in Fig. 7–14A. (6 μm paraffin sections, hematoxylin stain.)

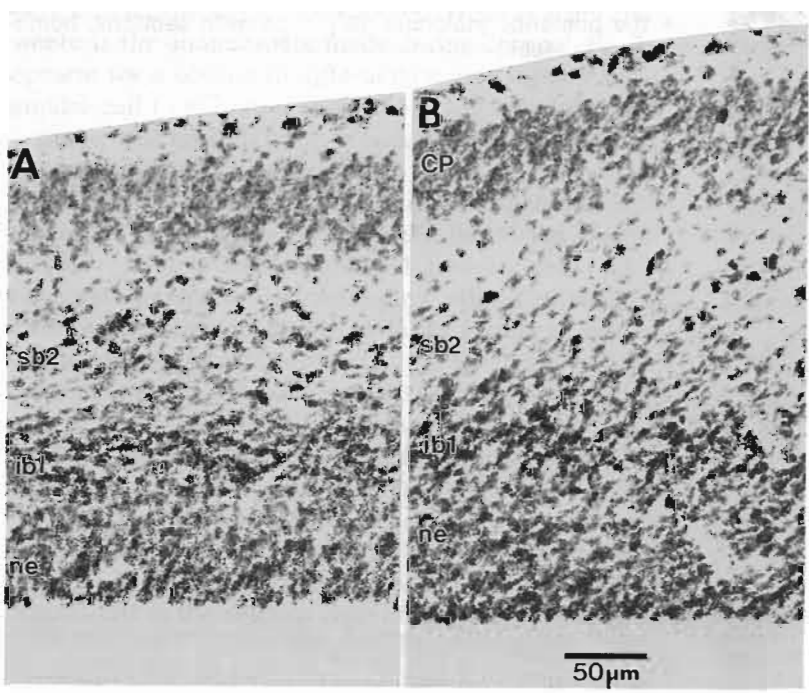


FIG. 7–18. Continuation of the series shown in Fig. 7–17. **(A)** level of the posterior cortex corresponding to that shown in Fig. 7–15A. **(B)** level of the posterior cortex corresponding to that shown in Fig. 7–15B. (6 μm paraffin sections, hematoxylin stain.)

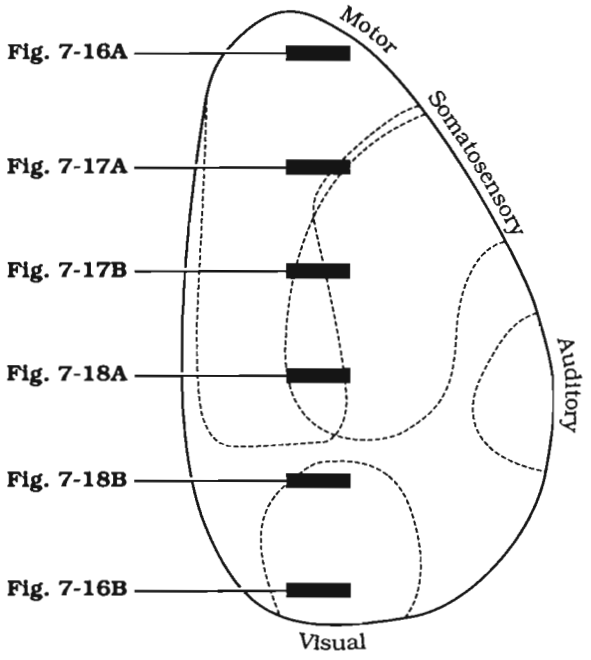


FIG. 7–19. Schematic diagram of the top view of the adult rat neocortex with its functional parcellation. The transposed approximate location of the coronal planes of the [^{3}H]thymidine autoradiograms shown in Figs. 7–16 through 7–18 are indicated.

dal (Figs. 7–17A, B and 7–18A, B).[3] The six sections of coronal radiograms illustrated in Figs. 7–16, 7–17, and 7–18 are projected upon a diagram of the functional subdivisions of the adult rat neocortex in Fig. 7–19. To the extent that the transposition of sections from an E18 brain to an adult brain is justified, the following correlations are indicated. The anterior sections shown in Figs. 7–16A and 7–17A, which have the highest concentration of heavily labeled sb2 cells, coincide with the future site of the motor cortex. The midcortical sections shown in Figs. 7–17B and 7–18A, which have an intermediate concentration of sb2 cells, coincide with the region where the motor and somatosensory areas overlap. Finally, the posterior sections shown in Figures 7–18B and 7–16B, which have the lowest concentration of sb2 cells, coincide with the visual cortex.

The high concentration of large layer V pyramidal cells anteriorly and their paucity posteriorly are illustrated in thick coronal sections from an adult rat brain in Figure 7–20. The section in Fig. 7–20A is at the level of the anterior septum and corresponds to the forelimb motor area (FL of Zilles [1985], at approximate interaural level A9.7). The section in Fig. 7–20B is at the anterior tip of the hippocampus and corresponds to the hindlimb motor area (HL of Zilles [1985], at approximate interaural level A7.2). The section in Fig. 7–20C is at the level of the midportion of the superior colliculus, and the section in Fig. 7–20D is at the level of the midportion of the inferior colliculus—both representing the visual cortex (OC1M and OC1B of Zilles [1985] at approximate levels A2.2 and A0.2, respectively).

Since there are neurogenetic gradients in the generation of neocortical neurons, an E17 injection might label more layer V neurons anteriorly than posteriorly and thereby account for the difference in the concentration of heavily labeled cells accumulating in sb2. But the neurogenetic timetables indicate that substantial proportions of layer V neurons are generated on E17 in both the hindlimb motor area (Chapter 14, Fig. 14–5B) and the visual areas (Chapter 11, Fig. 11–4). Therefore after an E17 [^{3}H]thymidine injection, the concentration of heavily labeled layer V neurons should be as high in the presumptive visual cortex as it is in the presumptive motor cortex.

We conclude that, in the E17–E18 injection/survival group, there is a high concentration of heavily labeled sb2 cells in the intermediate zone of the neocortex at those sites where layer V neurons are concentrated in high numbers in the adult neocortex. In regions where there is a low concentration of heavily labeled sb2 cells in the developing neocortex, there is, correspondingly, a paucity of layer V large pyramidal cells in the adult neocortex. That supports our hypothesis that, in rats labeled with [^{3}H]thymidine on E17 and killed on E18, sb2 is composed of sojourning pyramidal cells destined to settle in layer V.

7.3.2 The Paradoxical Uniformity of the First Inferior Band and the Differential Distribution of Layer IV Neurons

We hypothesized earlier that the first inferior band, which appears at the base of the subventricular zone 24 hours after a [^{3}H]thymidine injection on E17 (ib1, Figs. 7–1B and 7–3B), is composed of young neurons that will settle in layer IV. We postulated further that ib1 also contains many layer IV neurons after a [^{3}H]thymidine injection on E18. Since the concentration of granule cells in layer IV is highest in the sensory projection areas and lowest in the motor areas (Brodmann, 1909; Economo and Koskinas, 1927; Krieg, 1946a, 1946b; Zilles, 1985), it follows that the concentration of heavily labeled cells in ib1 should be high posteriorly and anterolaterally where the visual and

[3] Examination of additional serial sections indicated that the decreased prominence of sb2 going from anterior to posterior is not perfectly regular from level to level. But in the material available to us we were unable to detect a systematic pattern in the small regional variations in the concentration of sb2 cells.

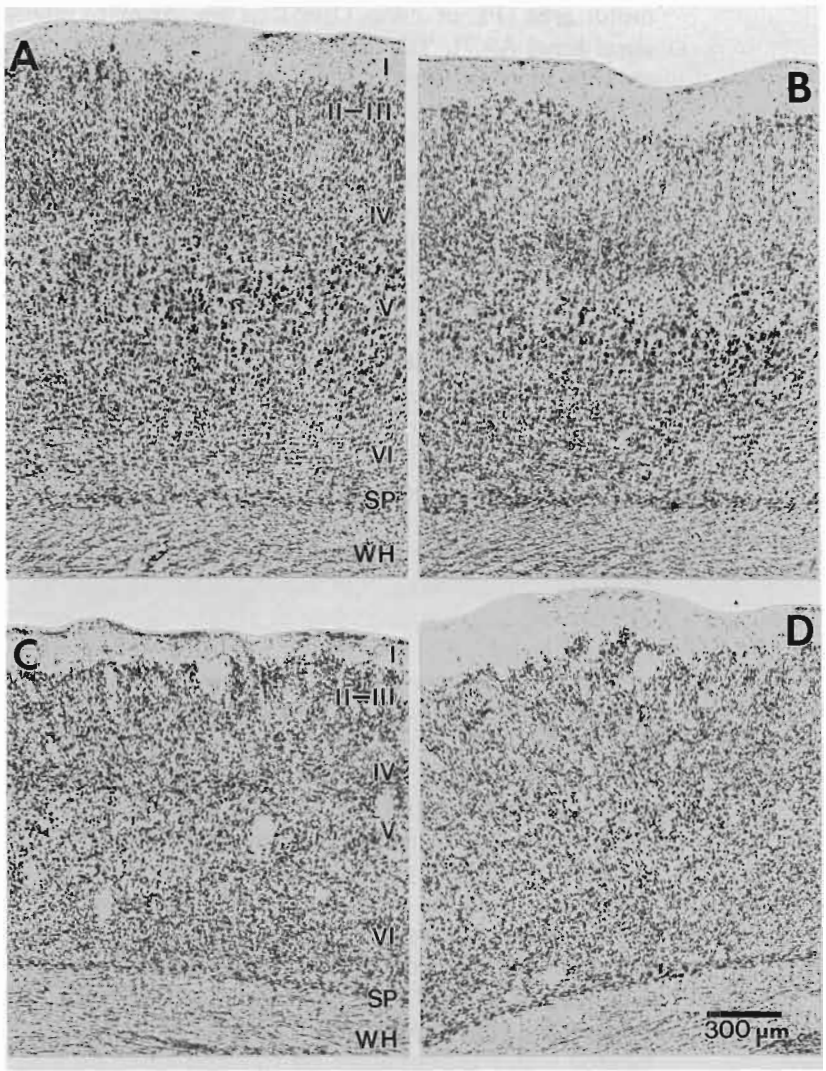


FIG. 7–20. Selected strips of the anterior and posterior dorsal neocortex from a P60 rat to show cytoarchitectonic differences. **(A)** level of anterior septum; **(B)** level of posterior septum; **(C)** level of midportion of the superior colliculus; **(D)** level of midportion of the inferior colliculus. Cryostat sections.

somatosensory areas develop and low anterodorsally where the motor area develops. Contrary to expectation, 24 hours after a [^{3}H]thymidine injection on E17, heavily labeled cells are uniformly distributed in ib1 (Figs. 7–13 to 7–15), and the same is true after a [^{3}H]thymidine injection on E18 (Fig. 7–21). How can this uniformity in labeling be linked to a heterogeneous distribution of layer IV cells in the adult cortex?

One part of the answer lies in the different spatial relationship between ib1 and the cortical plate at anterior versus posterior levels. At midanterior levels where the motor (medial) and somatosensory (lateral) areas are developing, ib1 has a restricted mediolateral span in relation to the cortical plate. Two hours after an E18 injection (Fig. 7–21A), the heavily labeled proliferating cells in the neuroepithelium (ne) stop far short (*arrow*) of the lateral tip of the cortical plate (CP). One day after an E18 injection, the heavily labeled cells sojourning in ib1 also stop far short (*arrow*) of the ventrolateral edge of the cortical plate (Fig. 7–21B).[4] Only the future motor cortex (medial), not the future somatosensory cortex (lateral) has an underlying ib1. At posterior levels where the visual cortex is developing both medially and laterally, ib1 has an expanded mediolateral span in relation to the cortical plate. There, the entire cortical plate has an underlying neuroepithelium and ib1 (*arrow* and CP, Fig. 7–21C).

It is important to note in this context that, in spite of the confined dorsomedial position of ib1 anteriorly on E19 (Fig. 7–21B), the concentration of heavily labeled cells (presumptive E18 birthdays) in the cortical plate by E22 is greater laterally than medially (compare

[4] The shrinkage of the anterior ventricular zone has been previously illustrated in the three-dimensional computer reconstructions of the developing neocortex in Chapter 2 (Color Figs. 2 to 5), and the gap between the anterolateral edges of the ventricular zone and the cortical plate is shown more clearly in the three-dimensional computer reconstructions in Chapter 9 (Color Figs. 6 and 7).

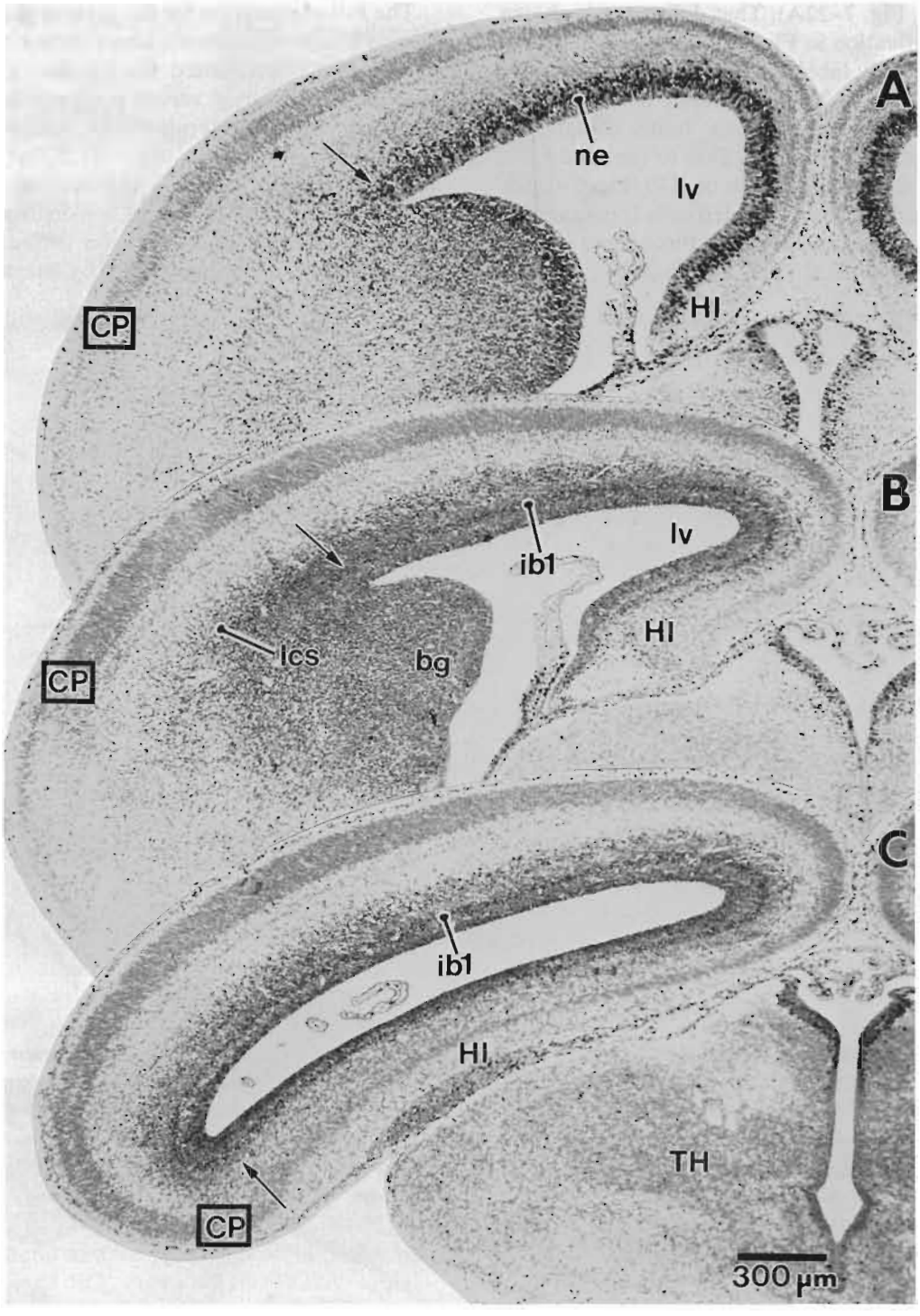


FIG. 7–21. Coronal autoradiograms, at a mid-cortical level, from a rat labeled with [^{3}H]thymidine on E18 and killed 2 hours afterwards **(A),** and a corresponding autoradiogram from a rat labeled with [^{3}H]thymidine on the same day and killed 24 hours later on E19 **(B).** Note the gap between the lateral spread of the neuroepithelium (*arrow* and ne in **A**) and ib1 (*arrow* and ib1 in **B**) in relation to the ventral lateral tip of the cortical plate (CP in box). In contrast, ib1 and the cortical plate have the same span in the posterior cortex in a rat labeled on E18 and killed on E19 **(C).** (6 μm paraffin sections, hematoxylin stain.)

SSC and MOC, Fig. 7–22A). That difference is shown at higher magnification in Fig. 7–23. There is a sparse scattering of heavily labeled cells in the future motor cortex (MOC, Fig. 7–23A) compared to the distinct band of heavily labeled cells in the future somatosensory cortex (SSC, Fig. 7–23B). Due to the broad distribution of ib1 at posterior levels on E19 (Fig. 7–21C), the concentration of heavily labeled cells (presumptive E18 birthdays) is particularly high throughout the future visual cortex on E22 (VC, Fig. 7–23C).

The full explanation for the greater concentration of layer IV cells in sensory areas rather than in motor areas can be accounted for by diverging migratory pathways at anterior versus posterior levels. The somatosensory area anterolaterally, with the largest population of layer IV cells, does not have an underlying neuroepithelium and ib1. Consequently, the cells sojourning dorsomedially in ib1 are distributed over two areas, a few by radial migration to the motor cortex directly above, and many more by lateral migration to

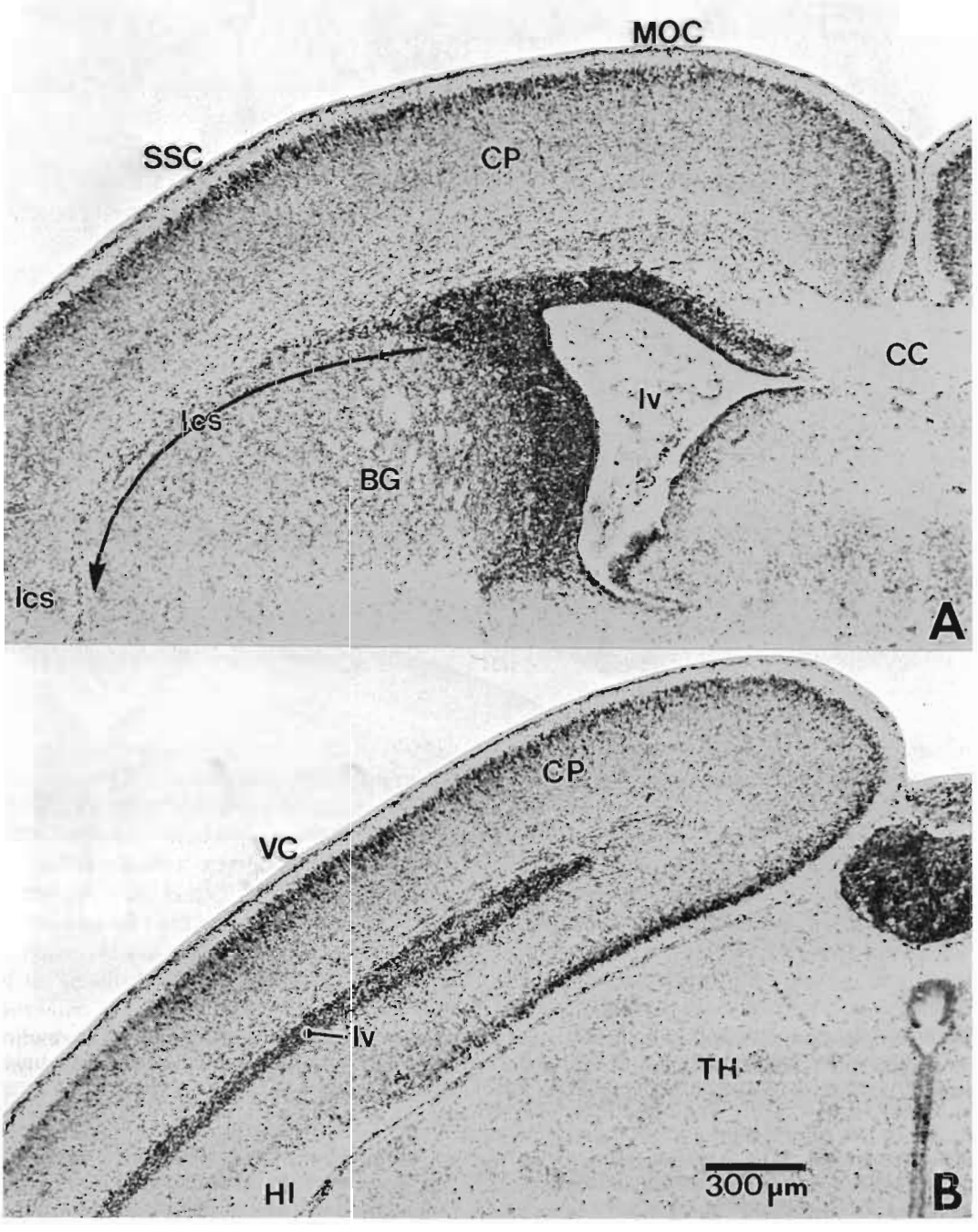


FIG. 7–22. Coronal autoradiograms from a rat labeled with [^{3}H]thymidine on E18 and killed on E22 at midcortical **(A)** and posterior **(B)** levels. *Arrow* in **A** runs beneath the lateral cortical stream (lcs) under the somatosensory cortex (SSC). (6 µm paraffin sections, hematoxylin stain.)

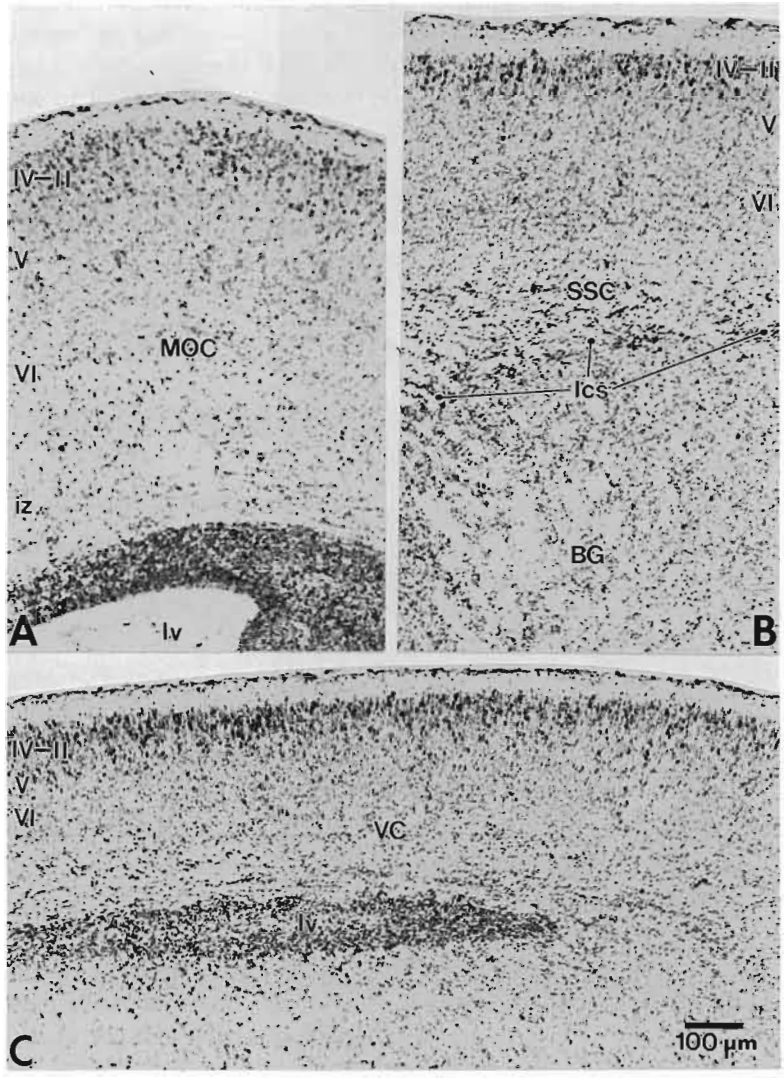


FIG. 7–23. The differential concentration of heavily labeled cells in the superficial portion (*upper tier*) of the cortical plate (IV–II) at a midcortical level dorsally **(A)** and laterally **(B),** and in the posterior cortex **(C).** (6 μm paraffin sections, hematoxylin stain.)

the somatosensory cortex. Thus, even though there is a dense accumulation of heavily labeled ib1 cells beneath the motor cortex, most of these cells will migrate laterally into the sensory areas rather than radially into the motor area. In Chapter 9 we will describe in detail how the neurons that have sojourned in ib1 use the *lateral cortical stream* to migrate around the basal ganglia into the lateral and ventrolateral parts of the cortical plate (lcs and BG, Figs. 7–21B, 7–22A, and 7–23B). Since there is an ib1 throughout the entire expanse of the posterior cortex, most of the heavily labeled cells in posterior ib1 migrate only radially and the visual cortex acquires a large population of layer IV cells.

7.3.3 Summary and Conclusions

When the observations presented here are considered as a whole, developmental events in the transitional field predict the major cytoarchitectonic divisions in the adult cortex. Regional differences in sb2, an anterior (more) to posterior (less) gradient in the concentration of heavily labeled cells, predict that the presumptive motor cortex will acquire a higher number of layer V pyramidal cells than the sensory areas. It is important to note that the regional differences in sb2 are global in nature and do not indicate a detailed map of the cytoarchitectonic diversity of the adult cortex. On the other hand, diverging cell migration pathways from ib1 to the cortical plate at anterior versus posterior levels predict that the somatosensory and visual areas will acquire a higher number of layer IV granule cells (and possibly layers III-II cells) than the motor areas. In Chapter 16, we will discuss the possibility that the gradients in sb2 and the divergent migratory pathways from ib1 could serve as a substrate for interactions with the various specific thalamic relay afferents to bring out the full expression of cytoarchitectonic diversity in the adult cortex.