THE JOURNAL OF COMPARATIVE NEUROLOGY 257:529-552 (1987)

# Development of the Precerebellar Nuclei in the Rat: IV. The Anterior Precerebellar Extramural Migratory Stream and the Nucleus Reticularis Tegmenti Pontis and the Basal Pontine Gray

JOSEPH ALTMAN AND SHIRLEY A. BAYER
Laboratory of Developmental Neurobiology, Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907 (J.A.) and Department of Biology, Indiana University-Purdue University, Indianapolis, Indiana 46223 (S.A.B.)

ABSTRACT

Sequential thymidine radiograms from rats injected on days E16, E17, E18, and E19 and killed 2 hours after injection and at daily intervals up to day E22 were used to establish the site of origin, migratory route, and settling patterns of neurons of the nucleus reticularis tegmenti pontis and basal pontine gray. The nucleus reticularis tegmenti pontis neurons, which are produced predominantly on days E15 and E16, derive from the primary precerebellar neuroepithelium. These cells, unlike those of the lateral reticular and external cuneate nuclei, take an anteroventral subpial route, forming the anterior precerebellar extramural migratory stream. This migratory stream reaches the anterior pole of the pons by day E18. In rats injected on day E16 and killed on day E18 some of the cells that reach the pons are unlabeled, indicating that they represent the early component of neurons generated on day E15. The cells labeled on day E16 begin to settle in the pons on day E19, 3 days after their production. These cells, migrating in an orderly temporal sequence, form a posterodorsal-to-anteroventral gradient in the nucleus reticularis tegmenti pontis.

Unlike the neurons of all the other precerebellar nuclei, the basal pontine gray neurons derive from the secondary precerebellar neuroepithelium. The secondary precerebellar neuroepithelium forms on day E16 as an outgrowth of the primary precerebellar neuroepithelium, and it remains mitotically active through day E19, spanning the entire period of basal pontine gray neurogenesis. The secondary precerebellar neuroepithelium is surrounded by a horizontal layer of postmitotic cells, representing the headwaters of the anterior precerebellar extramural migratory stream. In rats injected on day E18 and killed on day E19 the cells are labeled in the proximal half of the stream around the medulla but those closer to the pons are unlabeled, indicating an orderly sequence of migration. In rats injected on day E18 and killed on day E20 the labeled cells reach the pole of the pons. In the basal pontine gray the sequentially generated neurons settle in a precise order. The neurons generated on day E16 form a small core posteriorly and the neurons generated on days E17, E18, and E19 form regular concentric rings around the core in an inside-out sequence.

Key words: cell migration, neurogenesis, pons, thymidine autoradiography

Accepted July 30, 1986.

The *basal pontine gray* is a synaptic relay station between the cerebral cortex and the cerebellum (reviewed by Brodal, '72; Allen and Tsukahara, '74). There is a somatotopic organization in this pathway from the cerebral cortex to the pons (Brodal, '68, in the cat; Brodal, '78a,b; Wiesendanger et al., '79, in the monkey; Mihailoff et al., '78, in the rat) and from the pons to the cerebellum (Hoddevik, '75; Brodal and Walberg, '77, in the cat; Brodal, '79, '82, in the monkey; Burne et al., '78; Eisenman and Noback, '80; Eisenman, '81; Mihailoff et al., '81b; Watt and Mihailoff, '83, in the rat). The basal pontine gray has been divided by Brodal and Jansen ('46) into medial, ventral, lateral, and peduncular nuclei, and this subdivision has been applied to the rat by Mihailoff et al. ('81a). Since there are neither cytological differences nor clear boundaries between these "nuclei" this subdivision is merely a matter of convenience. The pontine fibers travel in the middle cerebellar peduncle (brachium pontis) and distribute in the cerebellum as mossy fibers (Tsukahara et al., '68).

The *nucleus reticularis tegmenti pontis*, known to earlier anatomists as the pontine reticular tegmental nucleus of Bechterew, is situated dorsal to the basal pontine gray. Like the latter, this nucleus receives fibers from the cerebral cortex (Brodal and Brodal, '71, in the cat; Brodal, '80a, in the monkey) and projects to the cerebellum (Hoddevik, '78, in the cat; Brodal, '80b, in the monkey; Azizi et al., '81; Mihailoff et al., '81b, in the rat). The projection from the nucleus reticularis tegmenti pontis, like the pontocerebellar projection to the cerebellum, is topographically organized in an as-yet-not-clearly-understood, complex manner.

In a long-survival thymidine radiographic study in the rat (Altman and Bayer, '78: Fig. 3) we have established that the neurons of the nucleus reticularis tegmenti pontis are produced between days E15 and E17, with a peak of about 65% of the cells on day E16 and less than 10% on day E17. The neurons of the basal pontine gray are produced between days E16 and E19, with a peak of about 55% of the cells on day E17. There is a clear neurogenetic gradient in the basal pontine gray. The earliest-generated pontine neurons are situated in its core near the pyramidal tract, and the later-generated cells form shells around this core. Essick ('12) claimed that the pontine gray neurons originate in the rhombic lip of the lateral recess of the fourth ventricle dorsally and migrate to the pons anteroventrally by way of the "pontobulbar body." This scheme was applied by Taber Pierce ('66) to the development of the basal pontine gray and the nucleus reticularis tegmenti pontis of the mouse. In contrast, we were able to trace the basal pontine gray neurons from the medial neuroepithelial invagination, some distance from the lateral rhombic lip, by way of the superficial (extramural) pontine migratory stream (Altman and Bayer, '78: Figs. 14, 15). A few of these cells reach the anteroventral region of the pons by day E17. The cell population of the basal pontine gray grows rapidly on the subsequent days.

In the first paper in this series (Altman and Bayer, '87a), we have made a distinction between the primary precerebellar neuroepithelium and the later-emerging secondary precerebellar neuroepithelium. In the succeeding papers (Altman and Bayer, '87b,c) we have presented evidence that the primary precerebellar neuroepithelium is a source of the neurons of the inferior olive, which migrate by way of the intramural olivary migratory stream, and of the lateral and external cuneate nuclei, which migrate by way of the posterior extramural migratory stream. In this paper, we shall present evidence that the first contingent of cells migrating by way of the anterior extramural migratory stream originates in the primary precerebellar neuroepithelium. These cells settle in the nucleus reticularis tegmenti pontis. The subsequently arising secondary precerebellar neuroepithelium is the source of the basal pontine gray neurons, and these, too, migrate anteroventrally by way of the anterior extramural stream.

## MATERIALS AND METHODS

The material used in this study was identical with that described in the first paper of the series (Altman and Bayer, '87a). Special use was made of sequential radiograms from rats labeled with $^3$H-thymidine on days E16, E17, and E18 and killed thereafter at daily intervals up to day E22.

## RESULTS

### Neurogenetic gradients in the nucleus reticularis tegmenti pontis and the basal pontine gray

In rats tagged with $^3$H-thymidine on day E16 and killed on day E22 (Fig. 1) the neurons of the nucleus reticularis

*Abbreviations*

| | |
|---|---|
| aes | anterior extramural migratory stream |
| AQ | aqueduct |
| ca | caudal |
| ce | cerebellar primary neuroepithelium |
| CE | cerebellum |
| cp | primordium of the fourth ventricle choroid plexus |
| cpl | lateral choroid plexus primordium |
| cpm | medial choroid plexus primordium |
| DT | dorsal tegmental nucleus (Gudden's) |
| EC | external cuneate nucleus |
| ecm | external cuneate extramural migratory stream |
| egl | external germinal layer |
| ic | inferior collicular neuroepithelium |
| IC | inferior colliculus |
| IO | inferior olive |
| iom | inferior olivary intramural migratory stream |
| is | isthmal canal |
| IST | isthmus |
| LR | lateral reticular nucleus |
| lrl | lower rhombic lip |
| me | medullary neuroepithelium |
| ME | medulla |
| mr | medial recess of fourth ventricle |
| NRT | nucleus reticularis tegmenti pontis |
| pcp | precerebellar primary neuroepithelium |
| pcs | precerebellar secondary neuroepithelium |
| pcsc | caudal precerebellar secondary neuroepithelium |
| pcsr | rostral precerebellar secondary neuroepithelium |
| pes | posterior precerebellar secondary neuroepithelium |
| PG | basal pontine gray |
| pi | postisthmal (cerebellar) recess of the fourth ventricle |
| PO | pontine region |
| PR | prepositus nucleus |
| PY | pyramidal tract |
| ro | rostral |
| SO | superior olive |
| ST | nucleus of the solitary tract |
| TB | nucleus of the trapezoid body |
| tc | tela choroidea |
| url | upper rhombic lip |
| v4 | fourth ventricle |
| v4a | anterior fourth ventricle |
| v4p | posterior fourth ventricle |
| vm | medullary velum |
| VII | facial motor nucleus |
| VM | motor nucleus of the trigeminal |
| VS | principal sensory neurons of the trigeminal |
| VT | ventral tegmental nucleus (Gudden's) |

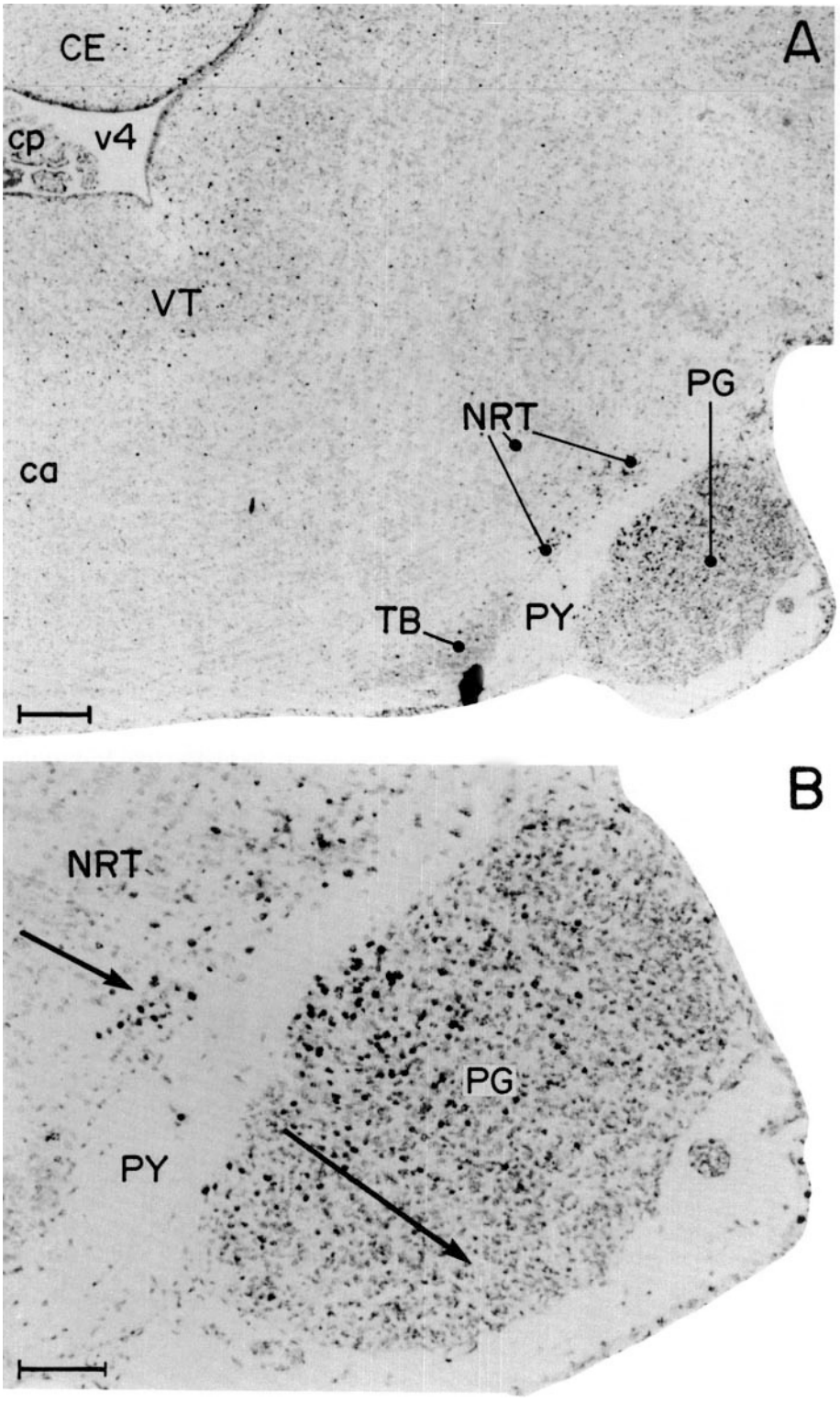


Fig. 1. **A:** Parasagittal radiogram through the basal pontine gray (PG) and nucleus reticularis tegmenti pontis (NRT) from a rat tagged with $^{3}$H-thymidine on day E16 and killed on day E22. **B:** Same at higher magnification. Arrows indicate the neurogenetic gradient from posterodorsal to anteroventral. Paraffin. Scales: A, 200 $\mu$m; B, 100 $\mu$m.

tegmenti pontis (NRT; a list of all abbreviations used and their meanings precedes Fig. 1) are no longer labeled posterodorsally, but most of those situated near the pyramidal tract (PY) anteroventrally are heavily labeled. In the basal pontine gray (PG) there is a small core of heavily labeled cells near the pyramidal tract posteromedially but in the larger surrounding region the cells show intermediate or low labeling, indicating label dilution. These observations establish, first, that the neurons of the nucleus reticularis tegmenti pontis and the basal pontine gray are generated sequentially. In the nucleus reticularis tegmenti pontis neuron production is terminating on day E16 while in the basal pontine gray it is just beginning. Second, in both structures there is a posterodorsal-to-anteroventral neurogenetic gradient. Specifically, in the nucleus reticularis tegmenti pontis the unlabeled older cells are situated posterodorsally, and in the basal pontine gray the heavily labeled older cells occupy the same relative position. Third, with respect to the pyramidal tract, the relationship is reversed: the youngest nucleus reticularis tegmenti pontis neurons and the oldest basal pontine gray neurons occupy its vicinity.

The settling pattern of the basal pontine gray neurons generated on the subsequent days is illustrated in sequential radiograms in Figures 2 and 3. In perinatal rats injected on day E17 (Figs. 2A, 3A), most of the neurons situated in the core of pontine gray are no longer labeled; these are the neurons generated on day E16. This early-generated inner region is surrounded by a shell in which most cells are heavily labeled; these are the neurons generated on day E17. Neurons in the outer half of the pontine gray are lightly labeled. In perinatal rats tagged on day E18 (Figs. 2B, 3B) there are two zones in the outer half of the pontine gray: (1) an inner zone with heavily labeled cells—these are going to have their last divisions on day E18; and (2) an outer zone with lightly labeled cells, indicating continuing cell proliferation for some time after the date of injection. In perinatal rats tagged on day E19 (Figs. 2C, 3C), which is the last day of pontine neurogenesis, the labeled cells are limited to the outer shell.

## The site of origin and migratory route of neurons of the nucleus reticularis tegmenti pontis

The primordia of the inferior colliculus and cerebellum (ic and ce in Fig. 4A) rotate posteroventrally (upper solid arrow in Fig. 4A) on day E15 and continue this rotation on day E16 (Fig. 4B). Partially as a result of this flexure, and because of the rapid shrinkage of the lumen of the fourth ventricle, the tela choroidea (tc) approaches the dorsal surface of the caudal half of the medulla (lower solid arrow in Fig. 4A). On day E16, a proliferative derivative of the shrinking primary precerebellar neuroepithelium (pcp in Fig. 4B) spreads rostrally along the base of the tela choroidea (broken arrow in Fig. 4A) to form the secondary precerebellar neuroepithelium (pcs in Fig. 4B). Since the secondary precerebellar neuroepithelium does not appear until day E16, the early-generated (day E15) cells of the nucleus reticularis tegmenti pontis must derive from the primary precerebellar neuroepithelium. The later-generated (day E16) cells could derive from the secondary precerebellar neuroepithelium but it is more reasonable to assume that all come from the same germinal source. Hence we propose that the neurons of the nucleus reticularis tegmenti pontis are produced in the primary precerebellar neuroepithelium, presumably in the region (pcp) indicated in Figure 4B in a rat labeled on day E16 and killed 2 hours later. This neuroepithelial zone is still mitotically active on day E17 (Fig. 8A) but begins to decline on day E18 (Fig. 8B) and has only a few labeled cells by day E19 (Figs. 8C, 9).

The neurons of the nucleus reticularis tegmenti pontis migrate from this posterodorsal production site anteroventrally by a subpial route on the lateral wall of the medulla and pons; this is the anterior precerebellar extramural migratory stream. Typically these cells reach the rostral tip of the pons outside their settling area on day E18 (aes in Fig. 5A). In some animals (illustrated in Altman and Bayer, '78: Fig. 11A), a few pioneering cells may already be seen in this position on day E17. Then the cells turn medially around the pole of the pons, as shown in a horizontal section in Figure 5B. In rats tagged on day E16 and killed on day E18, the anterior extramural migratory stream is composed of unlabeled and of heavily labeled cells. The former represent those neurons of the nucleus reticularis tegmenti pontis produced on day E15, the latter those generated on day E16 (and the few that may be produced on day E17). The neurons of this nucleus begin to settle on day E19 in the midportion of the pontine pole dorsally (Fig. 6). By day E20 the anterior extramural migratory stream is composed, in rats tagged with $^3$H-thymidine on day E16, of lightly labeled cells (aes in Fig. 7). These cells are pontine gray neurons (PG in Fig. 7A) that start to accumulate beneath the heavily labeled cells of the nucleus reticularis tegmenti pontis (NRT in Fig. 7C), and some of the heavily labeled cells of the pontine gray (PG in Fig. 7B).

## The site of origin and migratory route of neurons of the basal pontine gray

The secondary precerebellar neuroepithelium begins to form on day E16 (Altman and Bayer, '87a: Fig. 2A) and its caudal (pcsc) and rostral (pcsr) portions remain mitotically active through days E17 (Fig. 8A), E18 (Fig. 8B), and E19 (Fig. 8C). Since the production of neurons of the nucleus reticularis tegmenti pontis comes to an end on day E17 (Altman and Bayer, '78: Fig. 3), and since the production of neurons in the basal pontine gray follows the same time course as the mitotic activity in the secondary precerebellar neuroepithelium, we postulate that the precerebellar secondary neuroepithelium is an exclusive source of basal pontine gray neurons. The rostral portion of the secondary neuroepithelium has a tonsillike appearance (pcsr in Figs. 8, 9, 10B). It is situated beneath the primordium of the choroid plexus (cp) and above the medial recess of the fourth ventricle (mr). The causal secondary neuroepithelium (pcsc in Figs. 8, 9) is different in that it is not directly linked with the choroid plexus of the anterior fourth ventricle but forms the anterior vertical wall of the posterior fourth ventricle (v4p). However, the two portions of the secondary precerebellar neuroepithelium share two characteristics: (1) their external wall is in a subpial position (this is unlike primary neuroepithelia, and more like the external germinal layer of the cerebellum); and (2) both are surrounded by the postmitotic cells of the anterior extramural migratory stream (aes in Figs. 8C, 9).

The anterior precerebellar migratory stream (aes) begins as a horizontal sheet of postmitotic cells that move out along the entire length of the rostral secondary neuroepithelium to form a parallel sheet of unlabeled cells, as seen in short-survival thymidine radiograms in Figures 8C and

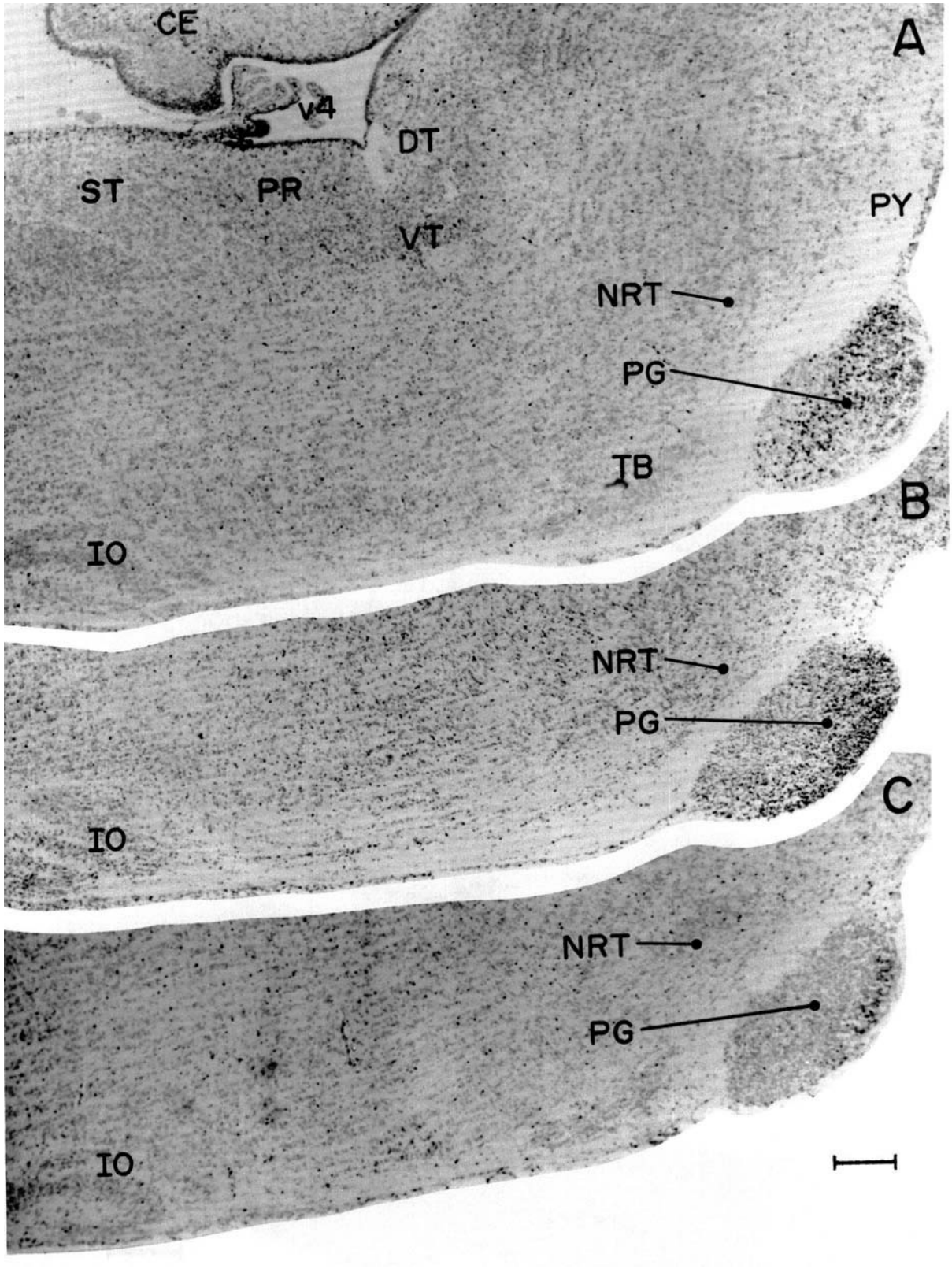


Fig. 2. Parasagittal radiograms from day E22 rats that received $^{3}$H-thymidine on days E17 (A), E18 (B), and E19 (C). Asterisk in *A*, original source of pontine gray neurons. Paraffin. Scale: 200 $\mu$m.

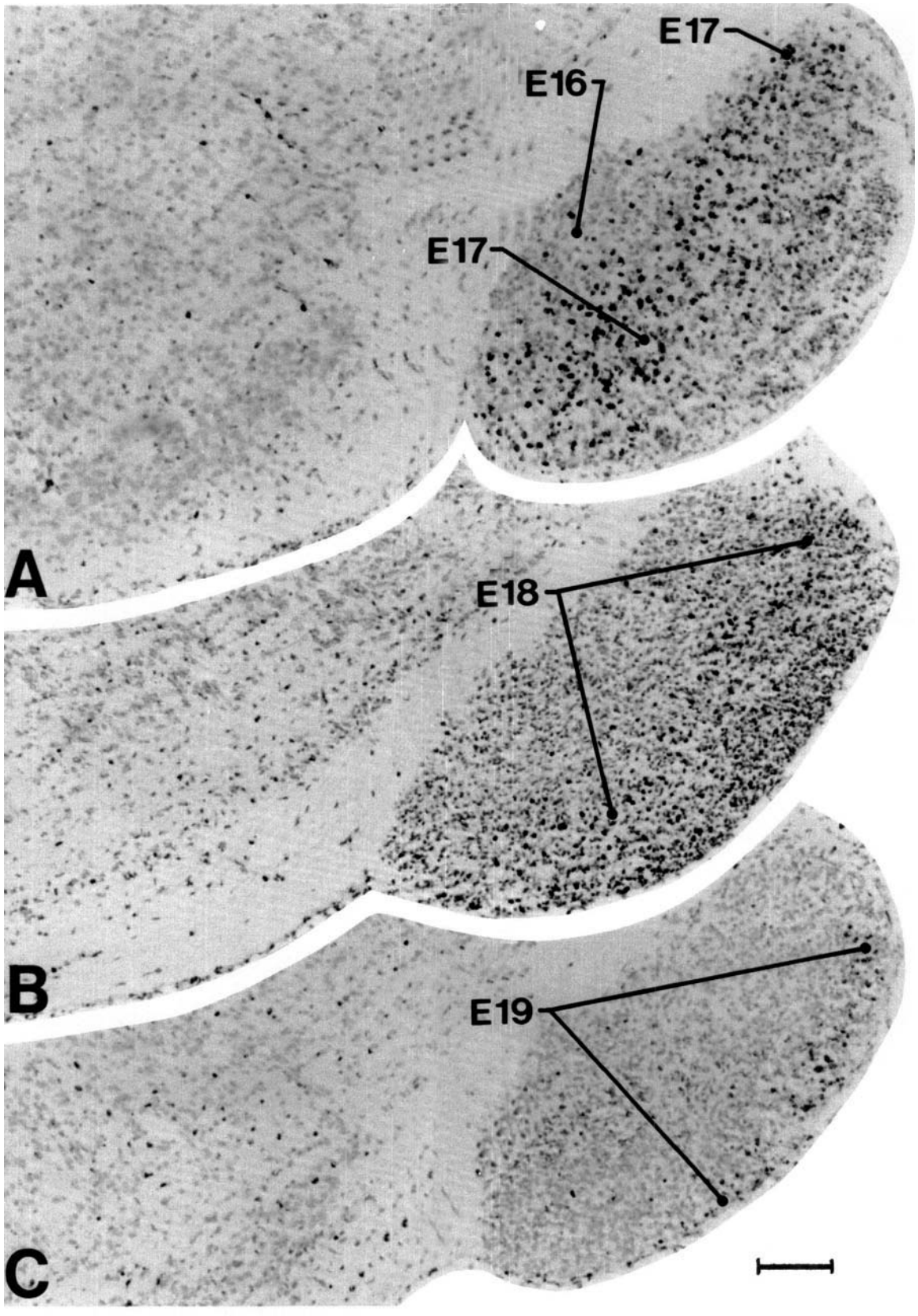


Fig. 3. Higher magnification of the basal pontine gray in day E22 rats that received $^{3}$H-thymidine on days E17 (A), E18 (B), and E19 (C). The ages indicate the inferred time of origin of neurons of the basal pontine gray. Paraffin. Scale: 100 $\mu$m.

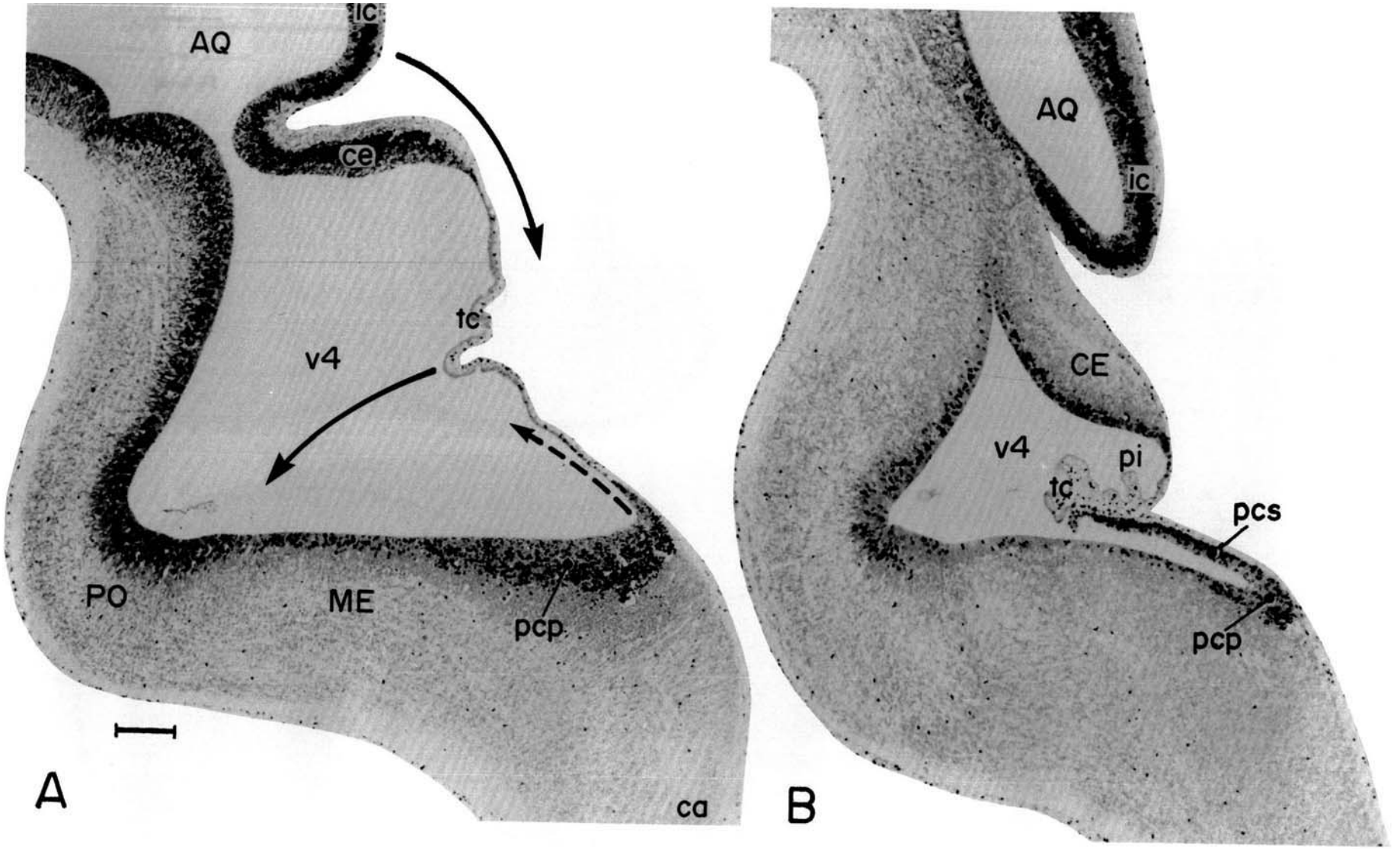


Fig. 4. Parasagittal radiograms from rats labeled with $^{3}$H-thymidine on day E15 (**A**) and day E16 (**B**) and killed after 2-hour survival. Upper solid arrow in A indicates the posteroventral flexion of the inferior collicular (ic) and cerebellar (ce) neuroepithelia between days E15 and E16. Lower solid arrow indicates the concurrent invagination of the tela choroidea (tc). Broken arrow shows the site and direction of the dispersal of the secondary precerebellar neuroepithelium (pcs in B) on the subsequent day. Paraffin. Scale: 200 $\mu$m.

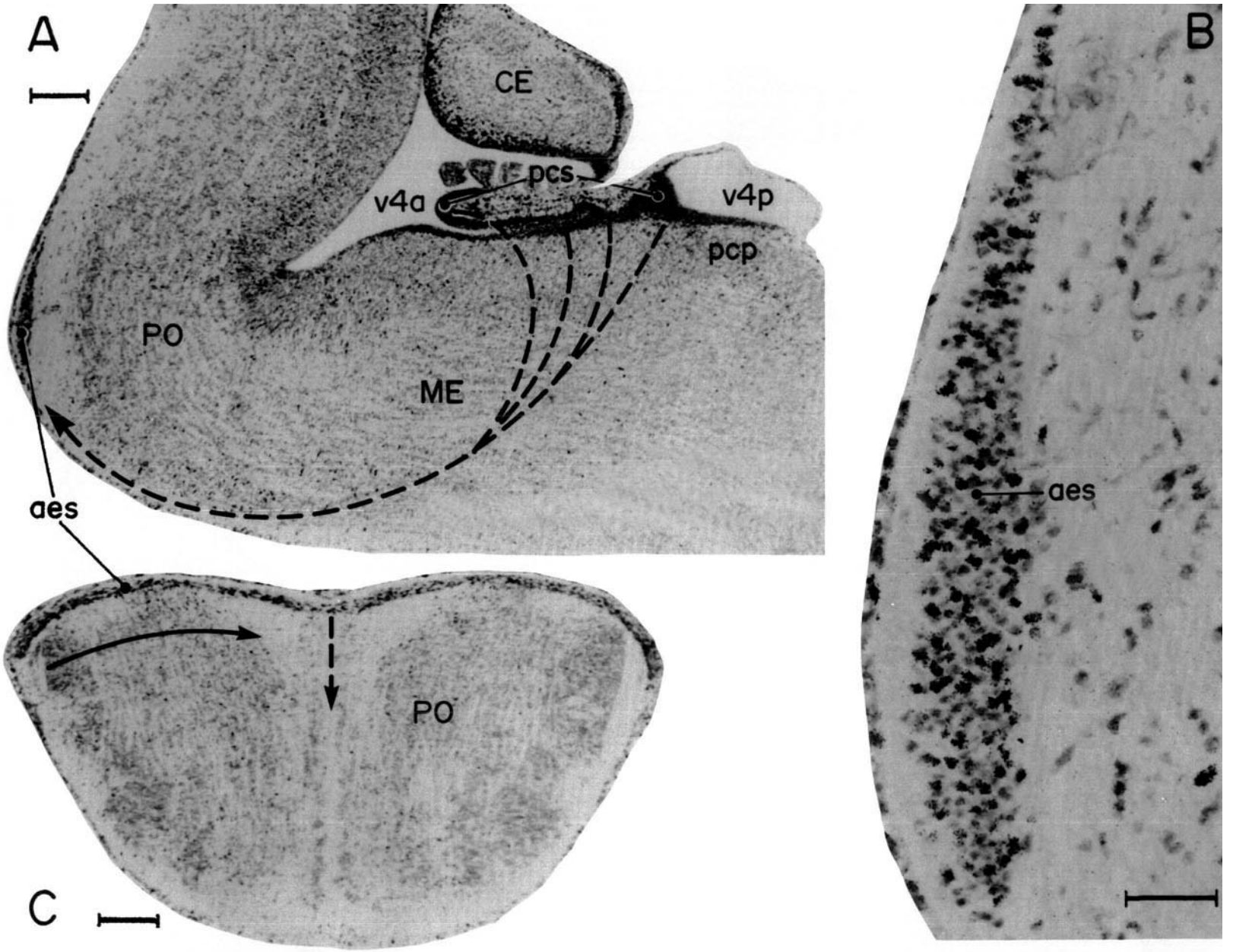


Fig. 5. **A:** Parasagittal radiogram from a rat labeled with $^{3}$H-thymidine on day E16 and killed on day E18, showing the arrival of the first contingent of cells of the anterior extramural stream (aes) at the rostral pole of the pons (PO). Broken arrow indicates the path of the stream on the lateral wall of the medulla and pons. **B:** The anterior extramural stream, with its unlabeled and heavily labeled cells at higher magnification. **C:** Horizontal radiogram from another rat injected on day E16 and killed on day E18. The solid arrow indicates the lateromedial course of the anterior extramural stream, the broken arrow the direction of settling of neurons of the nucleus reticularis tegmenti pontis on the subsequent days. Paraffin. Scales: A, 200 $\mu$m; B, 200 $\mu$m; C, 50 $\mu$m.

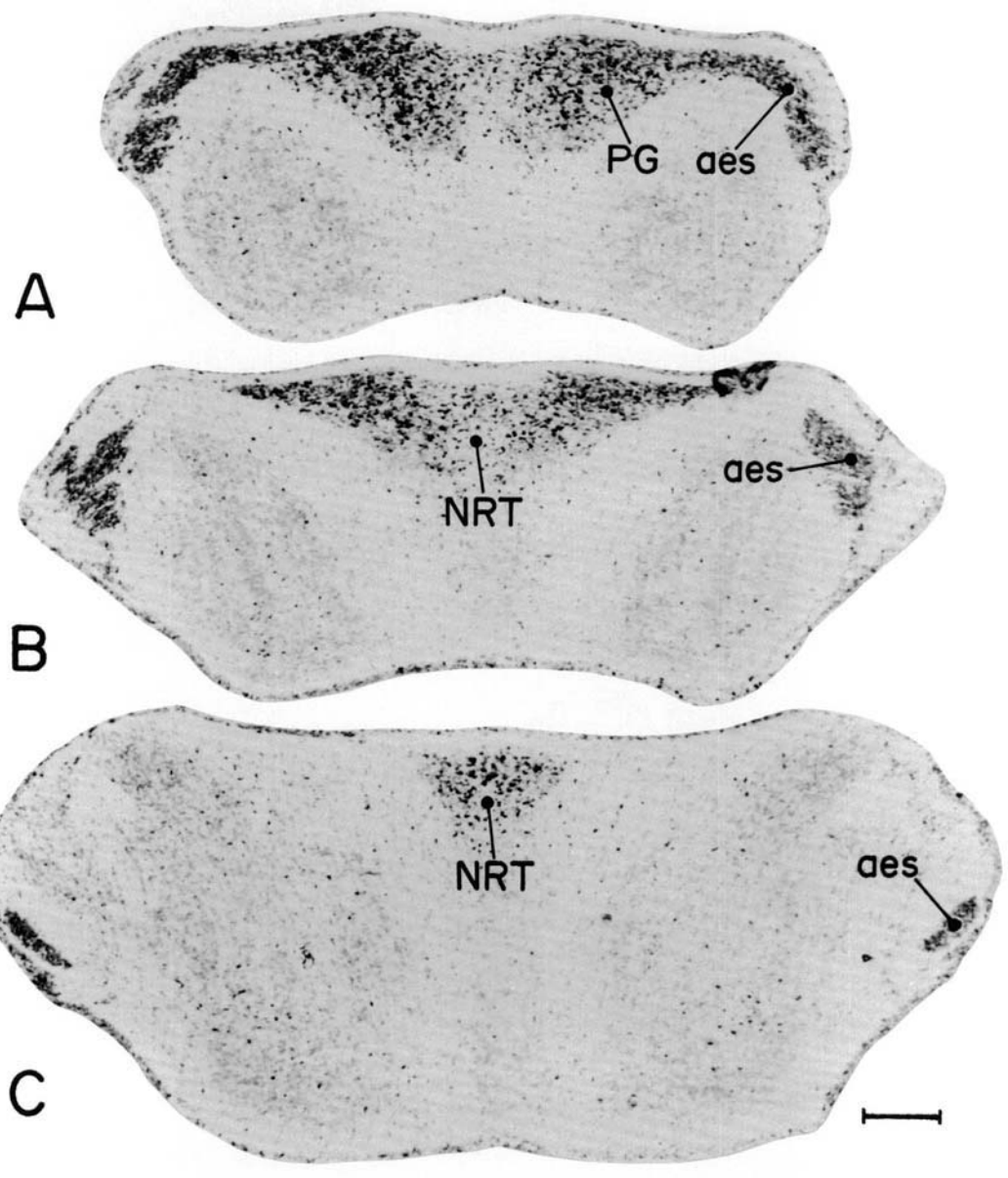


Fig. 6. Horizontal radiograms from ventral (A) to dorsal (C) to show the settling heavily labeled (and unlabeled) neurons of the nucleus reticularis tegmenti pontis (NRT) dorsomedially in a rat labeled on day E16 and killed on day E19. The ventrolaterally situated heavily labeled cells may be the first-arriving, early-generated basal pontine gray neurons (PG in A). Paraffin. Scale: 200 $\mu$m.

9. A smaller complement of these cells lines up near the caudal secondary neuroepithelium. In rats injected on day E18 and killed on day E19 (Fig. 13A) these cells are heavily labeled, thus providing evidence of their direct derivation from the rostral secondary neuroepithelium. The cells of this horizontal sheet produced laterally in the rostral secondary neuroepithelium move in a medial direction (arrow in Fig. 13A) where they are joined by cells of the caudal secondary neuroepithelium. Then the whole complement of cells takes a sharp turn and moves laterally. The subsequent course of the stream is illustrated in low-power coronal radiograms from rostral (Fig. 10A) to caudal (Fig. 11B) from a rat labeled on day E18 and killed on day E20. The stream first courses rostrally over the dorsal wall of the medulla (Figs. 10A,B, 11A); then it dips ventrally to reach the pons (Fig. 11B). The final course of the stream is shown in a horizontal section from another rat injected on day E18 and killed on day E20 (Figs. 11C, 12B). As the stream reaches the basal pole of the pons, it turns sharply medially (arrow in Figs. 11C, 12B) and the labeled cells settle in the basal pontine gray (PG) rostral to the previously generated and settled unlabeled cells. The fact that the cells are labeled along the entire length of the anterior migratory stream in this group of animals indicates that the long

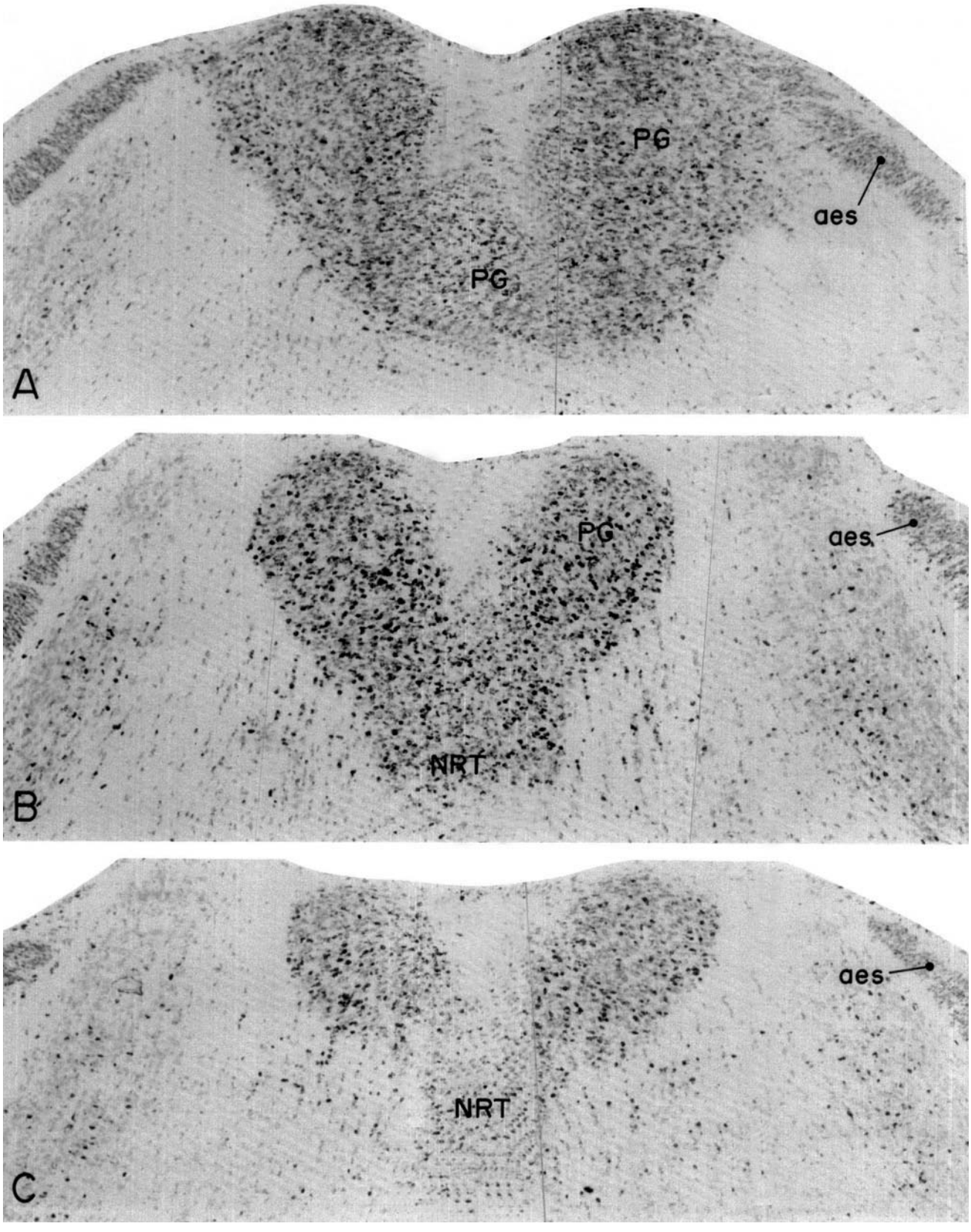


Fig. 7. Horizontal radiograms from ventral (**A**) to dorsal (**C**) from a rat labeled with $^{3}$H-thymidine on day E16 and killed on day E20. Dorsally (**C**) the nucleus reticularis tegmenti pontis (NRT) is composed of heavily labeled and unlabeled cells. Ventrally (A) the lightly labeled, late-generated cells of the basal pontine gray (PG) predominate. Between these two levels (**B**), a complement of heavily labeled cells of the pontine gray is seen. By this age, the anterior extramural stream (aes) is composed of lightly labeled cells migrating to the basal pontine gray. Paraffin. Scale: 100 $\mu$m.

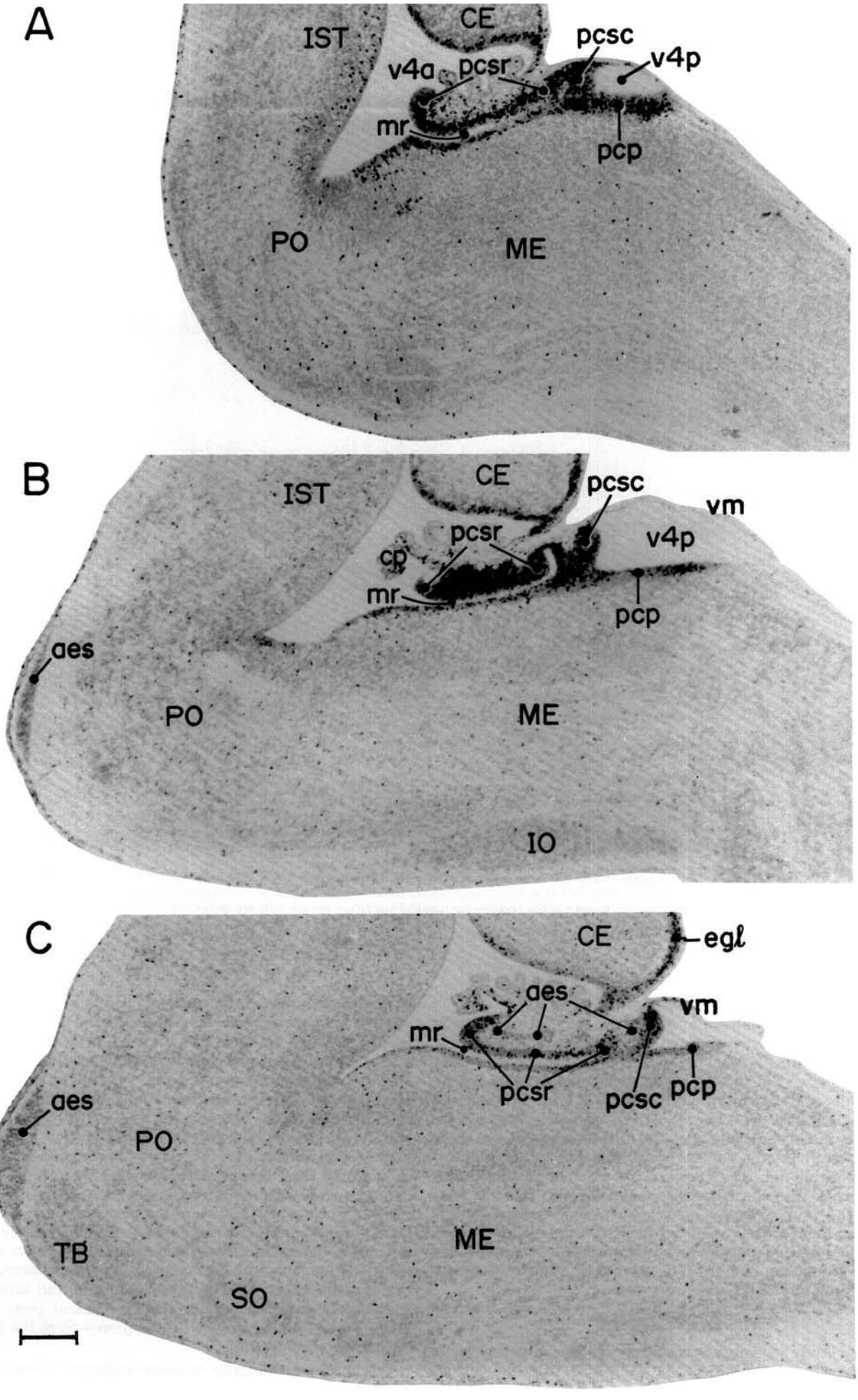


Fig. 8. Parasagittal radiograms from rats that received $^{3}H$-thymidine on days E17 (A), E18 (B), and E19 (C) and were killed 2 hours after labeling. High level of mitotic activity is indicated in the rostral (pcsr) and caudal (pcsc) portions of the secondary precerebellar neuroepithelium. Paraffin. Scale: 200 $\mu$m.

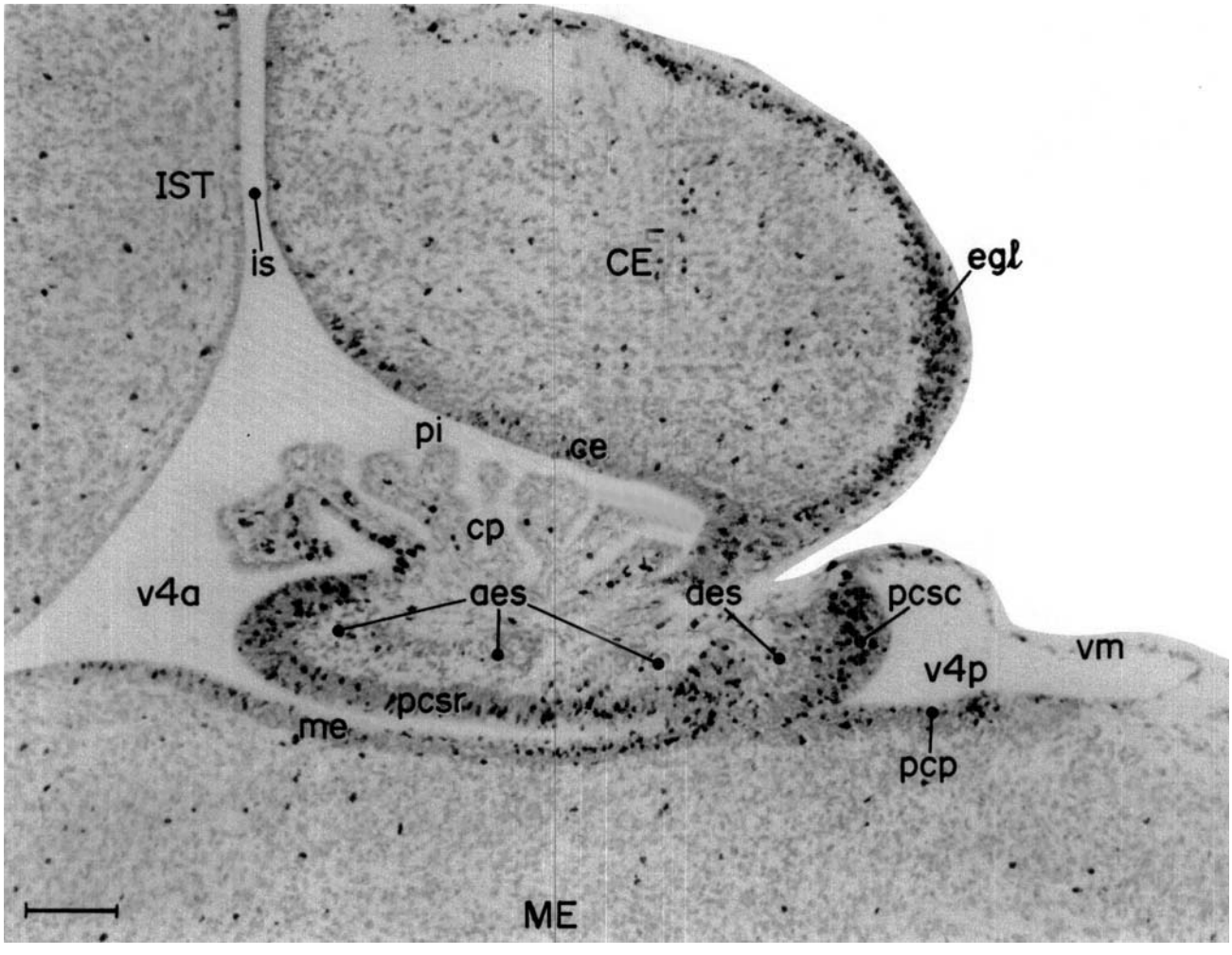


Fig. 9. Parasagittal radiogram from a rat labeled with $^{3}$H-thymidine on day E19 and killed 2 hours later (higher magnification of Fig. 8C). Note continuing mitotic activity in the rostral (pcsr) and caudal (pcsc) secondary neuroepithelia. Most of the cells of the anterior extramural migratory stream (aes) are unlabeled. Paraffin. Scale: 100 $\mu$m.

course (see Fig. 5A) is traversed within 2 days.

The speed and pattern of cell migration were analyzed in greater detail in rats injected on day E18 and killed 1 day later (Figs. 13–16). Most of the cells of the stream near their dorsal origin (Figs. 13A, 15A,B) are labeled. However, more laterally and rostrally the proportion of labeled cells gradually decreases (Fig. 13B,C). At the transition region from the medulla to the pons (Figs. 14A, 16B) only a rare labeled cell is seen. At the pontine level (Fig. 14B,C) the anterior extramural migratory stream is composed of unlabeled cells as are also the cells of the basal pontine gray. This indicates that the young basal pontine gray neurons course in this stream in an orderly sequence. Cells generated on a particular day reach about the midpoint of their path 2 days after their production and begin to enter their ventromedial settling area after 3 days.

## DISCUSSION

This study in embryos confirms our previous finding in adult rats (Altman and Bayer, '78: Fig. 3) that the neurons of the nucleus reticularis tegmenti pontis and of the basal pontine gray are generated in sequence, the former predominantly on days E15 and E16, and the latter between days E16 and E19. We provide conclusive support for our hypothesis (Altman and Bayer, '78: Fig. 14) that these neurons originate in the rhombencephalon dorsomedially, rather than laterally in the rhombic lip, as claimed by Essick ('12) and Taber Pierce ('66). The dating of the time of origin of nucleus reticularis tegmenti pontis neurons suggests that they derive from the primary neuroepithelium, as do the neurons of the lateral reticular nucleus and the external cuneate nucleus, but take a different migratory route. The latter form the posterior extramural stream and apparently terminate contralaterally (Altman and Bayer, '87c). In contrast, the nucleus reticularis tegmenti pontis neurons form the anterior extramural migratory stream and terminate ipsilaterally near the midline of the rostral pons. Upon arrival these neurons settle in a sequence from the caudal (older) to rostral (younger).

The basal pontine gray neurons originate in the second-

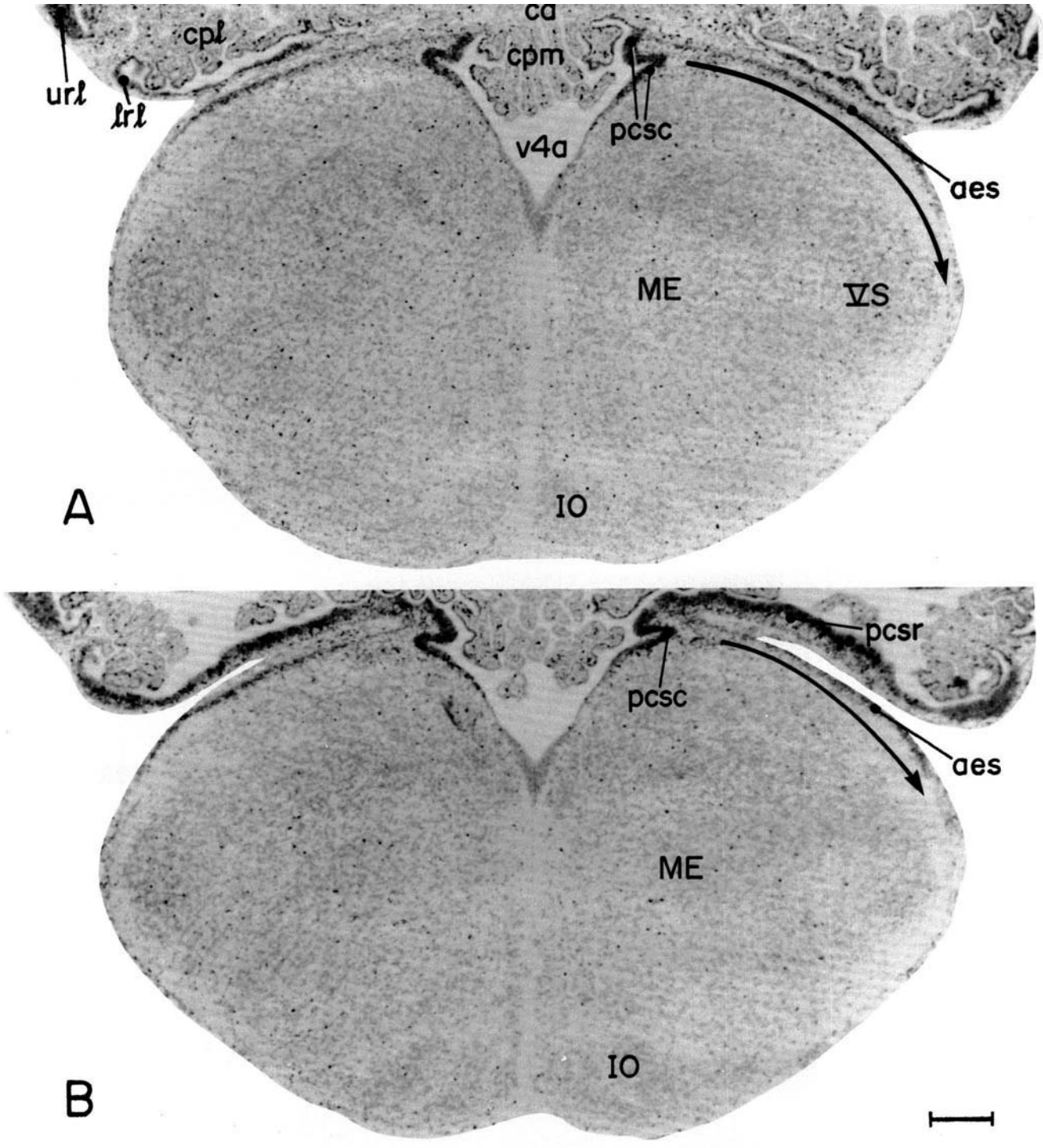


Fig. 10. Coronal radiograms from caudal (**A**) to rostral (**B**) from a rat labeled with $^{3}$H-thymidine on day E18 and killed 2 days later. Arrows show the dipping of the anterior extramural stream (aes) ventrolaterally while it shifts rostrally. Paraffin. Scale: 200 $\mu$m.

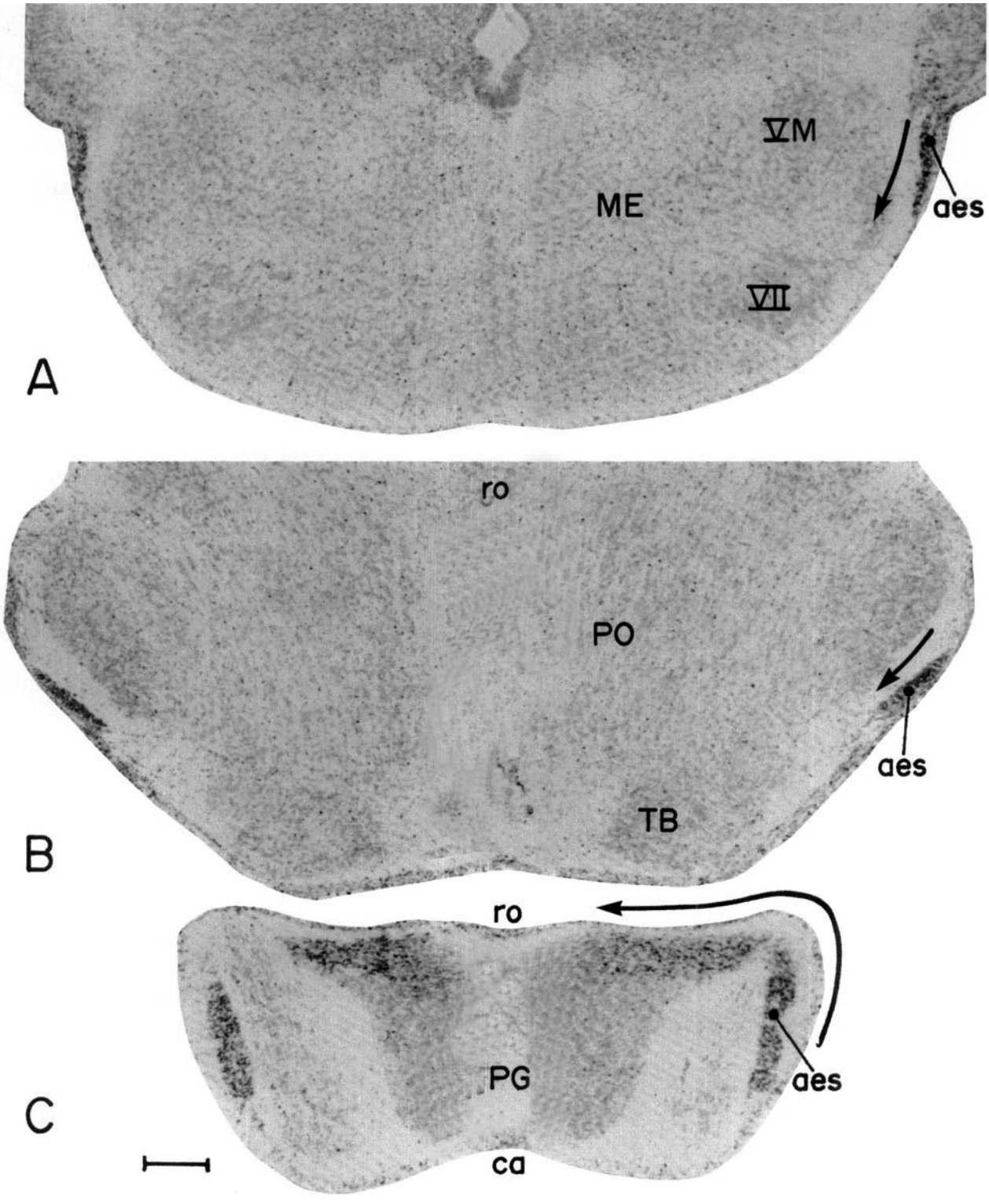


Fig. 11. A,B. Continuation of the coronal series shown in Figure 10, from caudal to rostral. C. Horizontal section from another rat labeled on day E18 and killed 2 days later, to show the sharp turn (arrow) of the anterior extramural stream (aes) at the rostral pole of the pons. The labeled cells settle in the basal pontine gray (PG) rostral to the earlier-generated, unlabeled cells. Paraffin. Scale: 200 $\mu$m.

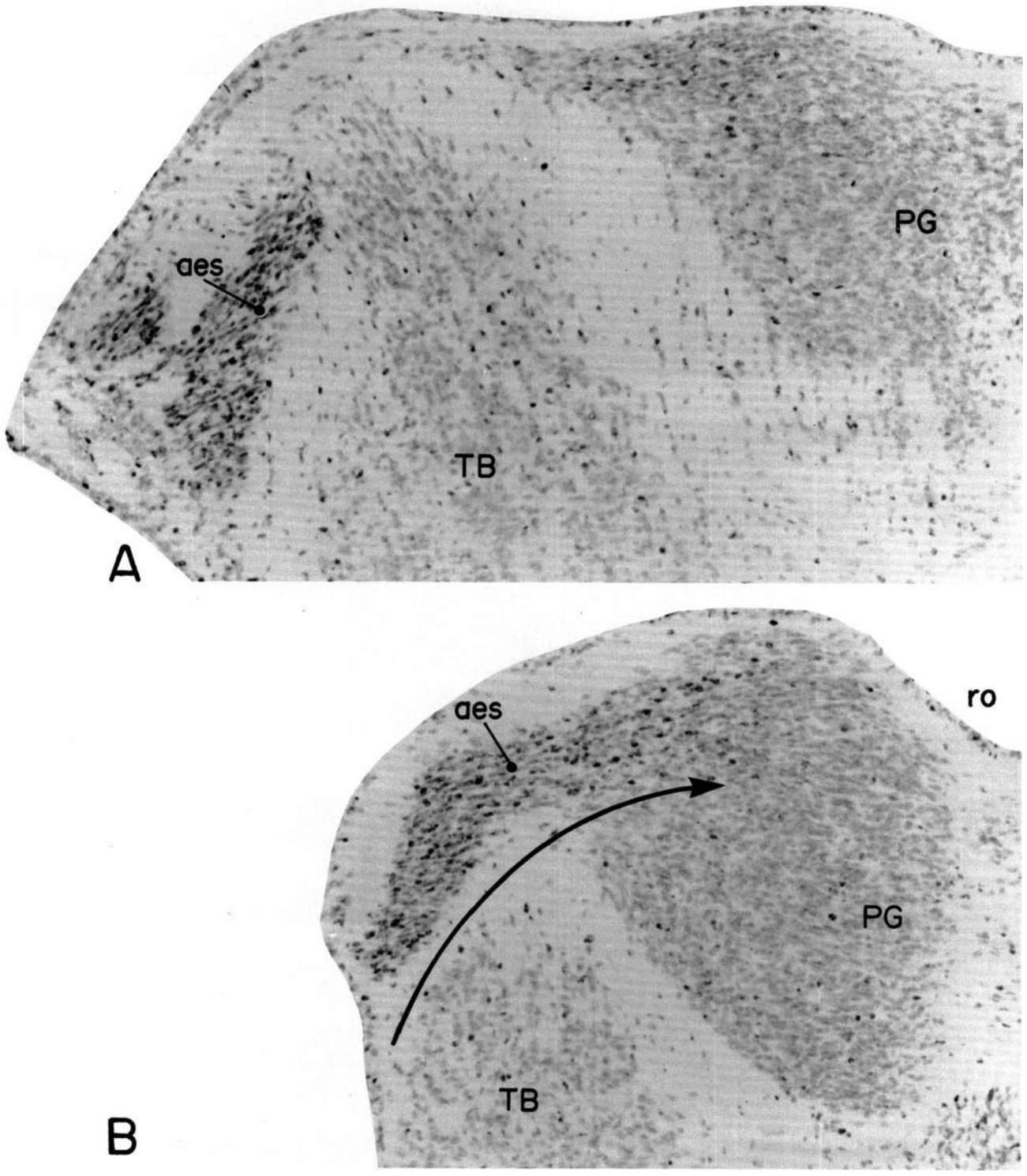


Fig. 12. Horizontal radiograms from dorsal (A) to ventral (B) through the basal pons of a rat labeled with $^{3}H$-thymidine on day E18 and killed on day E20. Arrow in B shows the final turn of the anterior extramural stream (aes) before the cells settle in the basal pontine gray (PG). Paraffin. Scale: 100 $\mu$m.

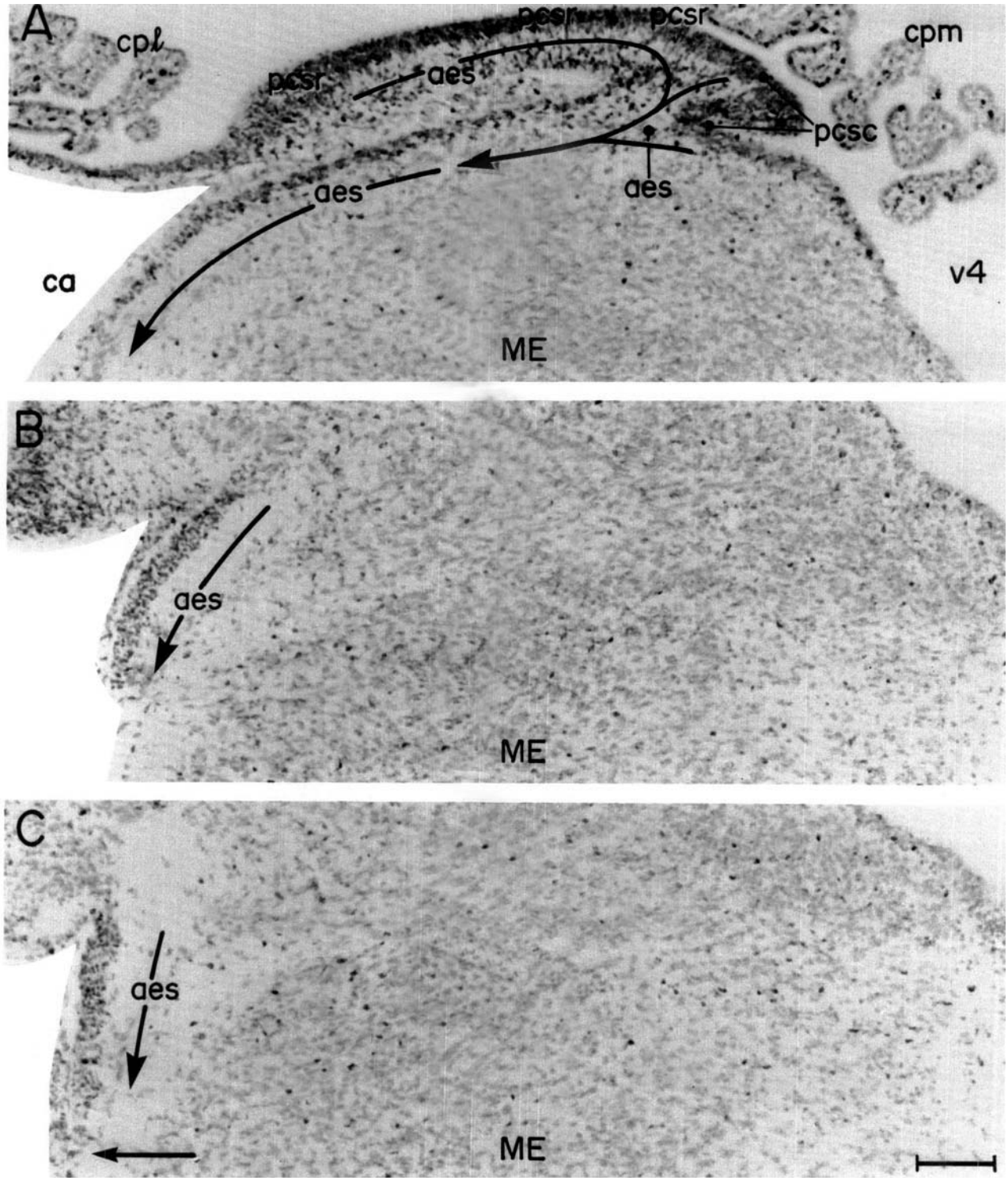


Fig. 13. Coronal radiograms from caudal (A) to rostral (C) at the level of the medulla, from a rat labeled on day E18 and killed 1 day later. Arrow in A shows the initial course of the labeled cells of the anterior extramural stream (aes) produced in the rostral (pcsr) and caudal (pcsc) portions of the secondary precerebellar neuroepithelium. Arrow in C indicates the distance covered by the cells labeled 24 hours earlier, where they catch up with unlabeled cells generated earlier. Paraffin. Scale: 100 $\mu$m.

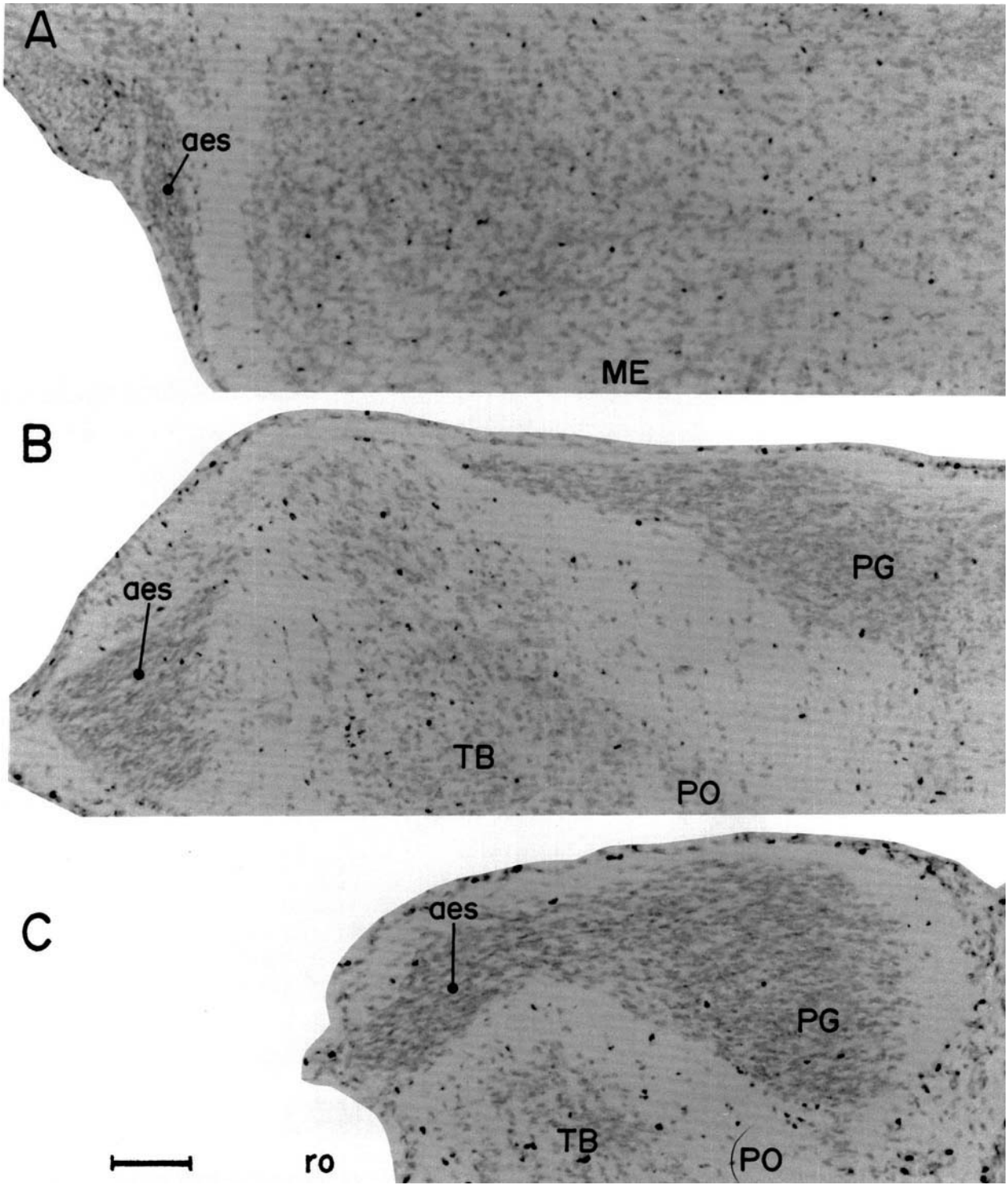


Fig. 14. Continuation of the series shown in Figure 13, from caual to rostral. At the transition level between medulla to pons (A) and at the pontine level (B,C) the extramural migratory stream (aes) is composed of unlabeled cells. Paraffin. Scale: 100 $\mu$m.

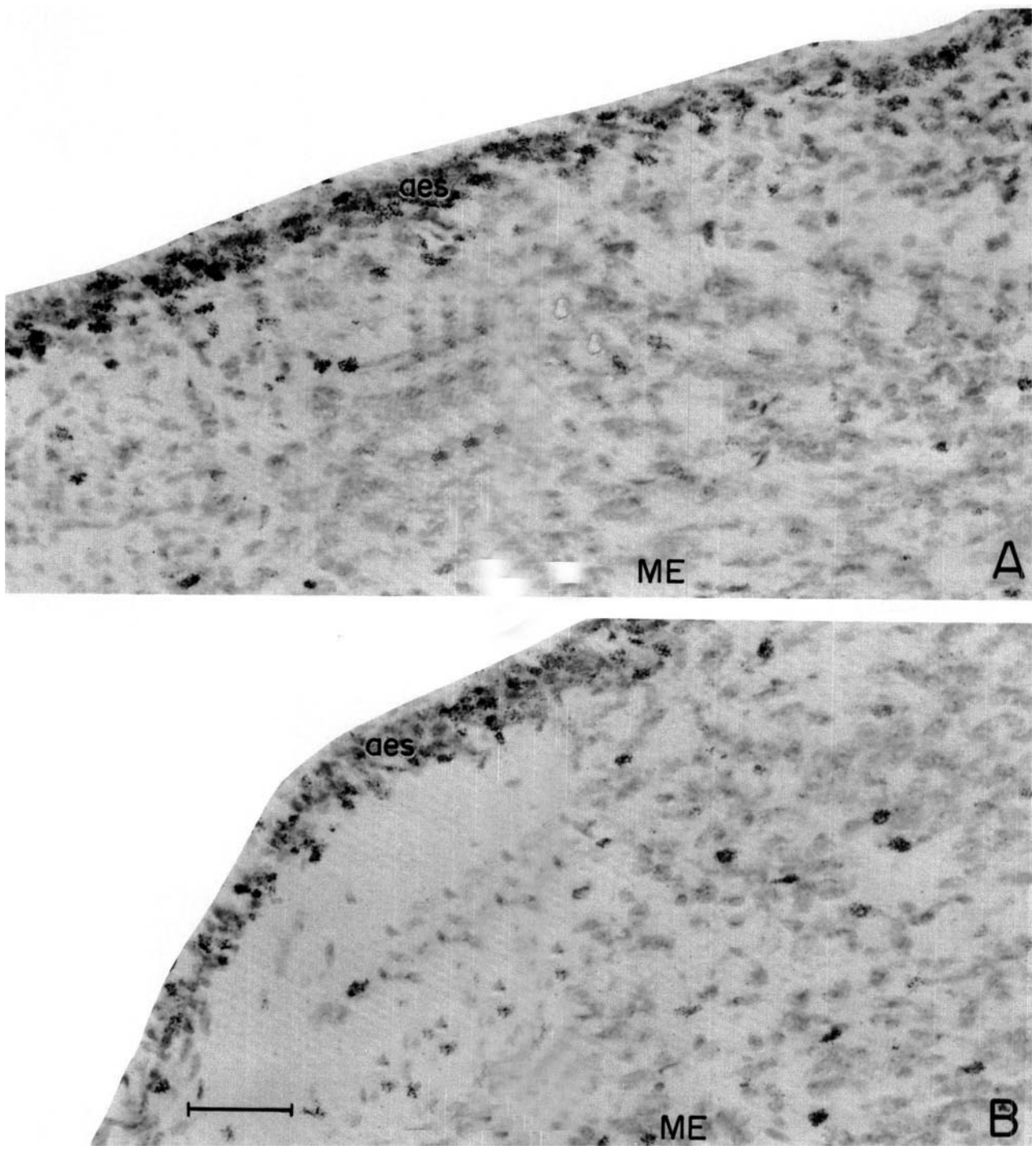


Fig. 15. Coronal radiograms from caudal (A) to rostral (B) at the level of the secondary precerebellar neuroepithelium from a rat labeled on day E18 and killed 1 day later. Practically all cells of the anterior migratory stream are labeled. Paraffin. Scale: 50 $\mu$m.

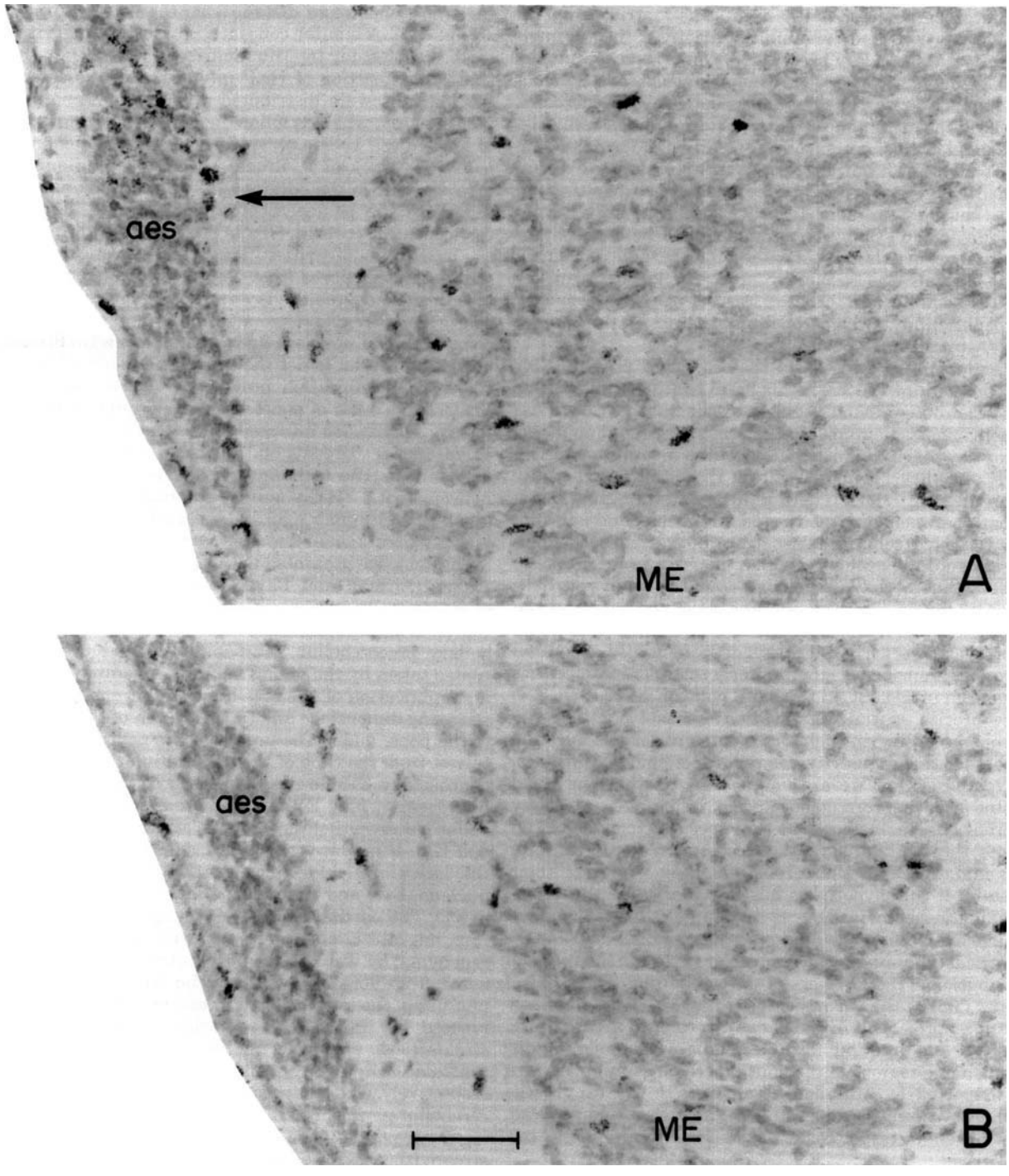


Fig. 16. Continuation of the series shown in Figure 15 from caudal (A) to rostral (B) at more anterior levels of the medulla. Arrow in A shows the approximate point in the anterior extramural stream that the cells labeled 24 hours earlier have reached. Paraffin. Scale: 50 $\mu$m.

ary precerebellar neuroepithelium. This matrix begins to form on day E16 and remains mitotically active through day E19. The secondary precerebellar neuroepithelium has two components. The rostral component spreads horizontally in an anterior direction underneath the tela choroidea and above the medial recess of the anterior fourth ventricle. The caudal component expands dorsally and forms the anterior wall of the posterior fourth ventricle. Both are distinguished from the primary precerebellar neuroepithelium (1) in that they are superficially exposed to the external environment and (2) by being surrounded by a transitory zone that forms the "headwaters" of the anterior extramural stream. The secondary precerebellar neuroepithelium resembles in these respects the secondary neuroepithelium of the cerebellum, the external germinal layer (Altman, '82).

After leaving the region of the secondary precerebellar neuroepithelium, the migrating basal pontine gray neurons follow the route previously taken by the nucleus reticularis tegmenti pontis neurons. The neurons generated on a particular day reach the rostral level of the medulla within 24 hours, and they begin to settle in the basal pontine gray region after 48 hours. There is little intermingling along the path between early-generated (unlabeled) and late-generated (labeled) cells, and the settling of the neurons follows the same orderly sequence. Thus, the precise neurogenetic gradient that we have previously found in adult rats (Altman and Bayer, '78: Fig. 10), and have confirmed in the present study in fetal rats (Figs. 2, 3) is the outcome of two factors: (1) the orderly migration of sequentially generated pontine neurons and (2) their orderly settling, in concentric shells from inside out, in the basal pontine gray.

The precise spatial organization of basal pontine gray neurons according to their birth dates raises the question of whether this regularity has any functional significance. A hypothesis that we are entertaining is that the basal pontine gray neurons generated at different times carry different genetic instructions that specify what cortical afferents they will preferentially accept synaptic contacts from and where in the cerebellum they will send their axons to. If this hypothesis has any merit, then the temporal markers provided by $^3$H-thymidine radiography about the age of a neuron should predict the regional distribution of cortical afferents in the pontine gray and/or the regional distribution of mossy fibers in the cerebellum. The study of Brodal ('68) in the cat indicated a precise, somatotopically organized projection in the pons with cortical areas of face, forelimb, and hindlimb terminating in overlapping but distinguishable regions of the pontine gray. A similar pattern was reported by Mihailoff et al. ('78) in the rat. In the monkey, Wiesendanger et al. ('79) have reported, instead, multiple small target zones in the pons from different cortical areas.

While most projection studies have not been designed to identify a concentric pattern of pontine organization, there is some evidence for such a pattern. With reference to pontocerebellar projection in the rat, Burne et al. ('78), using the HRP retrograde labeling technique with injections made in the lateral cerebellar areas, have noted a dual source of mossy fibers in the basal pontine gray—one medial and the other lateral. They went on to suggest that "The dual pontine aggregates, located approximately equidistant from the central area, appear to be arranged in concentric zones, with the anterior parts of lateral cerebellum receiving from the center and posterior lobules receiving from zones more distant from the core" (Burne et al., '78: p. 346). A similar concentric pattern is suggested in a more recent study by Mihailoff et al. ('81b). They reported that after injection of HRP into crus I of the cerebellum, labeled cells were distributed along the perimeter of the pontine gray, whereas following crus II injections the focus of labeled cell bodies occupied more central regions of the pons. The work of P. Brodal suggests a similar lamellar arrangement in the pontine gray of the monkey both with regard to the pontocerebellar (Brodal, '79, '82) and the corticopontine (Brodal, '78a,b) projection. With reference to the latter, Brodal writes: "The overall arrangement of terminal fields of fibres from various cortical regions resembles to some degree the layers of an onion; fibres from the sensorimotor region terminate in a central core while fibres from surrounding areas are added externally (Brodal, '78: p. 273). Whether or not the concentric model of basal pontine gray organization provides a clearer picture of the topographic order of pontocerebellar projection than its arbitrary subdivision into medial, ventral, lateral, and peduncular nuclei (Brodal and Jansen, '46) remains to be determined. The technique of double labeling of pontine neurons with $^3$H-thymidine autoradiography (to distinguish them by their age) and with HRP histochemistry (to identify their axonal trajectories) is well suited to this task.

The evidence presented in this paper and in the preceding papers (Altman and Bayer, '86a,b,c) is summarized diagrammatically in Figures 17 and 18. Figure 17 illustrates the structural relationship between the primary and secondary precerebellar neuroepithelia, and the migratory routes taken by the young neurons generated by them. As a simplification of the ongoing morphogenetic events, the changing dimensions and configuration of the medulla and of the pons, and of the precerebellar neuroepithelia themselves, from the time of the onset of inferior olivary neurogenesis (day E13) to the termination of basal pontine gray neurogenesis (day E19), are ignored. The primary precerebellar neuroepithelium (pcp) is active between days E13 and E16. It produces the neurons of the inferior olive (IO), the lateral reticular nucleus (LR), the external cuneate nucleus (EC), and the nucleus reticularis tegmenti pontis (NRT). The earliest-generated, young inferior olivary neurons migrate circumferentially within the medullary parenchyma, by way of the inferior olivary migratory stream (iom), from dorsal to ventral. The subsequently produced neurons of the lateral reticular and external cuneate nuclei proceed by way of the posterior extramural migratory stream (pes), cross the midline ventrally, and terminate contralaterally in a ventrolateral and dorsolateral position, respectively. The last-produced neurons of the primary precerebellar neuroepithelium, those of the nucleus reticularis tegmenti pontis, form the anterior extramural migratory stream (the narrower portion of aes in Fig. 17) and terminate anteromedially in the pons (NRT). The secondary precerebellar neuroepithelium has a rostral (pcsr) and a caudal (pscs) component and is the exclusive source of neurons of the basal pontine gray (PG). These neurons migrate also by way of the extramural migratory stream (broader portion of aes in Fig. 17).

The temporal course of precerebellar neurogenesis, the speed of cell migration along the different paths, and the onset of settling of neurons are summarized in Figure 18. The evidence for the time of origin of neurons of the inferior olive, lateral reticular nucleus, external cuneate nucleus, nucleus reticularis tegmenti pontis, and basal pontine gray

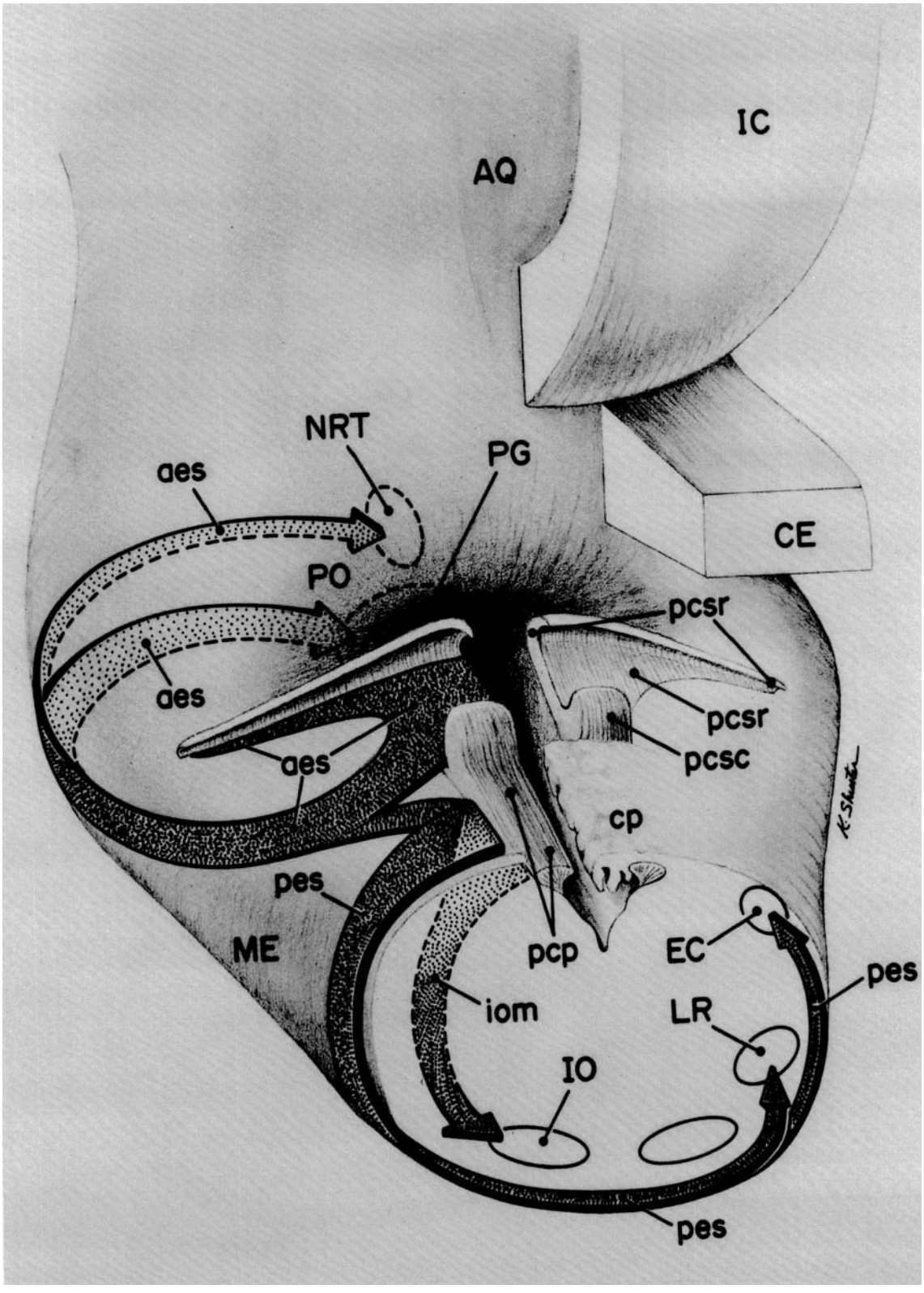


Fig. 17. Summary diagram of the structural relationship between the primary and secondary precerebellar neuroepithelia, and the migratory routes of neurons destined to settle in the different precerebellar nuclei. See text for details.

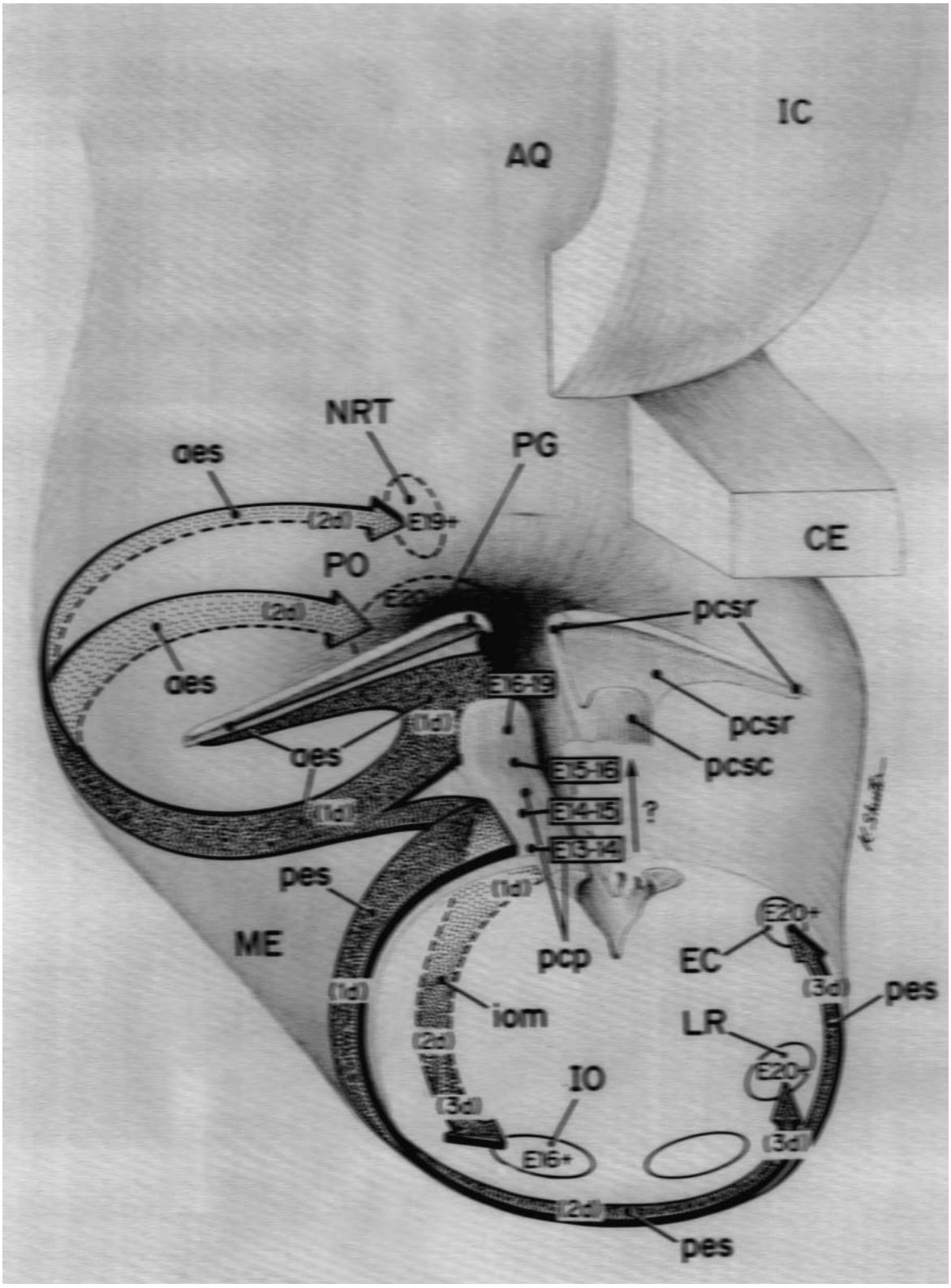


Fig. 18. The temporal course of precerebellar neurogenesis, the speed of cell migration along the different paths, and the onset of settling of neurons of the different precerebellar nuclei. See text for details.

is based on our previous long-survival radiographic studies in adults (summarized in Altman, '82). These dates have been transferred (using close approximations) to the primary and secondary precerebellar neuroepithelia that generate these neurons (dates in rectangles). The dates E13–14 refer to the generation of the inferior olivary neurons, E14–15 to the neurons of the lateral reticular and external cuneate nuclei, E15–16 to the neurons of the nucleus reticularis tegmenti pontis , and E16–19 to the neurons of the basal pontine gray. The caudal-to-rostral gradient in the generation site of the neurons derived from the primary precerebellar neuroepithelium is conjectural. We do not know whether the successively generated neurons deriving from this germinal system are generated in the same locus successively or whether there is a genuine shift in locus as mitotic activity declines in one cell line and increases in the next.

The neurons generated in the inferior olivary primordium are translocated within a day into the inferior olivary premigratory zone. (This appears to hold also for the secondary precerebellar neuroepithelium, as within 1 day after their generation the young neurons of the basal pontine gray are situated in the headwaters of the anterior extramural migratory stream.) Two days after labeling, the cells of the inferior olivary migratory stream are in a ventrolateral position, and 3 days later straggling cells are in the vicinity of the inferior olive while the bulk has penetrated it. Cell migration in the subpial, posterior extramural migratory stream appears to be faster than in the olivary parenchymal stream. Within 1 day after labeling the cells are in a lateral position, and 2 days later the labeled cells have crossed the midline. Three days after labeling these cells have reached their terminal position ventrolaterally and dorsolaterally on the opposite side, and on day E19, 4 days after their generation, the neurons of the lateral reticular nucleus and the external cuneate nucleus have penetrated the parenchyma. Since the medulla has grown appreciably during this period, the speed of cell migration is undoubtedly much faster extramurally than intramurally. This is borne out by the time course of cell migration along the anterior extramural migratory stream. Within 2 days after their generation the nucleus reticularis tegmenti pontis neurons have reached far rostrally the midline of the pons and by the next day are settling in the nucleus. The same applies also to the basal pontine gray. One day after injection the late-labeled cells are coursing in the dorsolateral portion of the medulla and 2 days later they begin to penetrate the corpus of the basal pontine gray.

In the first paper of this series (Altman and Bayer, '87a) we have presented evidence that both the cerebellum and the precerebellar nuclei derive from a unique and shared neuroepithelial system, the rhombencephalon. We have defined the rhombencephalon in a narrow sense as the two bridgeheads of the surface (alar) plate between the medulla and the isthmus where the neural groove fails to fuse. In addition, we have referred to other similarities between the cerebellar and precerebellar neuroepithelia. For instance, both have subpial formative components, represented by the external germinal layer in the cerebellum and by the extramural migratory streams in the precerebellar system. But there is at least one major difference between the two, and this is in the way they are formed: the cerebellum is assembled by a *convergent migration* of its neurons from different sources and the precerebellar nuclei are formed by a *divergent migration* of neurons from one source.

The three major cellular components of the cerebellum, the neurons of the deep nuclei, the Purkinje cells, and its microneurons (basket, stellate, and granule cells), derive from three separate germinal matrices, and each class of cells, following different routes, converges to form the unitary structure of the cerebellum. The deep neurons arise in the ventrolateral neuroepithelium of the lateral cerebellar plate and migrate medially over the surface of the primordial cerebellum (Altman and Bayer, '85). The Purkinje cells originate in the ventral portion of the cerebellar neuroepithelium and migrate by modified radial trajectories toward the surface. At the same time the deep neurons move from the surface to the depth of the cerebellum. Associated with these migrations is the formation of a secondary matrix, the external germinal layer, which disperses from caudal to rostral over the surface of the formative cerebellar cortex. Subsequently the derivatives of the external germinal layer either settle in the molecular layer or migrate downward past the Purkinje cell layer to form the granular layer (Altman, '82). In contrast, the precerebellar nuclei arise from a single neuroepithelial source and are dispersed over a large area extending from the lower medulla to the pons. Apparently subgroups of precerebellar neurons are dispatched to different loci in the medulla and pons, presumably to intercept signals transmitted along different pathways and relay them to the cerebellum.

## ACKNOWLEDGMENTS

This research program is supported by grants from the National Institutes of Health and the National Science Foundation. We are grateful for the technical assistance of William Boyle, Peggy Cleary, Gail Garrison, Julie Henderson, Mark O'Neil, and Kathy Shuster.

## LITERATURE CITED

Allen, G.I., and N. Tsukahara (1974) Cerebro-cerebellar communication systems. Physiol. Rev. *54*:957–1006.

Altman, J. (1982) Morphological development of the rat cerebellum and some of its mechanisms. In S. Palay and V. Chan-Palay (eds): The Cerebellum: New Vistas. Berlin: Springer, pp. 8–49.

Altman, J., and S.A. Bayer (1978) Prenatal development of the cerebellar system in the rat: II. Cytogenesis and histogenesis of the inferior olive, pontine gray, and the precerebellar reticular nuclei. J. Comp. Neurol. *179*:49–76.

Altman, J., and S.A. Bayer (1985) Embryonic development of the rat cerebellum. I. Delineation of the cerebellar primordium and early cell movements. J. Comp. Neurol. *231*:1–26.

Altman, J., and S.A. Bayer (1987a) Development of the precerebellar nuclei in the rat. I. The precerebellar neuroepithelium of the rhombencephalon. J. Comp. Neurol. *257*:477–485.

Altman, J., and S.A. Bayer (1987b) Development of the precerebellar nuceli in the rat. II. The intramural olivary migratory stream and the neurogenetic organization of the inferior olive. J. Comp. Neurol. *257*:486–508.

Altman, J., and S.A. Bayer (1987c) Development of the precerebellar nuclei in the rat. III. The posterior precerebellar extramural migratory stream and the lateral reticular and external cuneate nuclei. J. Comp. Neurol. *257*:509–524.

Azizi, S.A., G.A. Mihailoff, R.A. Burne, and D.J. Woodward (1981) The pontocerebellar system in the rat: An HRP study. Posterior vermis. J. Comp. Neurol. *197*:543–558.

Brodal, A. (1972) Cerebrocerebellar pathways. Anatomical data and some functional implications. Acta Neurol. Scand. [Suppl.] *48,51*:153–193.

Brodal, A., and P. Brodal (1971) The organization of the nucleus reticularis tegmenti pontis in the cat in the light of experimental anatomical studies of its cerebral cortical afferents. Exp. Brain Res. *13*:90–110.

Brodal, A., and J. Jansen (1946) The ponto-cerebellar projection in the rabbit and cat. Experimental investigations. J. Comp. Neurol. *84*:31–118.

Brodal, P. (1968) The corticopontine projection from the visual cortex in the cat. I. Demonstration of a somatotopically organized projection from the primary sensorimotor complex. Exp. Brain Res. *5*:210–234.

Brodal, P. (1978a) Principles of organization of the monkey corticopontine projection. Brain Res. *148*:214–218.

Brodal, P. (1978b) The corticopontine projection in the Rhesus monkey. Origin and principles of organization. Brain *101*:251–283.

Brodal, P. (1979) The pontocerebellar projection in the Rhesus monkey: An experimental study with retrograde axonal transport of horseradish peroxidase. Neuroscience *4*:193–208.

Brodal, P. (1980a) The cortical projection to the nucleus reticularis tegmenti pontis in the Rhesus monkey. Exp. Brain Res. *38*:19–27.

Brodal, P. (1980b) The projection from the nucleus reticularis tegmenti pontis to the cerebellum in the Rhesus monkey. Exp. Brain Res. *38*:29–36.

Brodal, P. (1982) Further observations on the cerebellar projections from the pontine nuclei and the nucleus reticularis tegmenti pontis in the Rhesus monkey. J. Comp. Neurol. *204*:44–55.

Brodal, P., and F. Walberg (1977) The pontine projection to the cerebellar anterior lobe. An experimental study in the cat with retrograde transport of horseradish peroxidase. Exp. Brain Res. *29*:233–248.

Burne, R.A., M.A. Eriksson, J.A. Saint-Cyr, and D.J. Woodward (1978) The organization of the pontine projection to lateral cerebellar areas in the rat: Dual zones in the pons. Brain Res. *139*:340–347.

Eisenman, L.M. (1981) Pontocerebellar projections to the pyramis and copula pyramidis in the rat: Evidence for a mediolateral topography. J. Comp. Neurol. *199*:77–86.

Eisenman, L.M., and C.R. Noback (1980) The ponto-cerebellar projection in the rat: Differential projections to sublobules of the uvula. Exp. Brain Res. *38*:11–17.

Essick, C.R. (1912) The development of the nuclei pontis and the nucleus arcuatus in man. Am. J. Anat. *13*:25–54.

Hoddevik, G.H. (1975) The pontocerebellar projection onto the paramedian lobule in the cat: An experimental study with the use of horseradish peroxidase as a tracer. Brain Res. *95*:291–307.

Hoddevik, G. (1978) The projection from nucleus reticularis tegmenti pontis onto the cerebellum in the cat. Anat. Embryol. (Berl.) *153*:227–242.

Mihailoff, G.A., R.A. Burne, and D.J. Woodward (1978) Projections of the sensorimotor cortex to the basilar pontine nuclei in the rat: An autoradiographic study. Brain Res. *145*:347–354.

Mihailoff, G.A., C.B. McArdle, and C.E. Adams (1981a) The cytoarchitecture, cytology, and synaptic organization of the basilar pontine nuclei in the rat. I. Nissl and Golgi studies. J. Comp. Neurol. *195*:181–201.

Mihailoff, G.A., R.A. Burne, S.A. Azizi, G. Norell, and D.J. Woodward (1981b) The pontocerebellar system in the rat: An HRP study. II. Hemispheral components. J. Comp. Neurol. *197*:559–577.

Taber Pierce, E. (1966) Histogenesis of the nuclei griseum pontis, corporis pontobulbaris and reticularis tegmenti ponti (Bechterew) in the mouse. An autoradiographic study. J. Comp. Neurol. *126*:219–239.

Tsukahara, N., H. Korn, and J. Ström (1968) Pontine relay from cerebral cortex to cerebellar cortex and nucleus interpositus. Brain Res. *10*:448–453.

Watt, C.B., and G.A. Mihailoff (1983) The cerebellopontine system in the rat. I. Autoradiographic studies. J. Comp. Neurol. *215*:312–330.

Wiesendanger, R., M. Wiesendanger, and D.G. Rüegg (1979) An anatomical investigation of the corticopontine projection in the primate (*Macaca fascicularis* and *Saimiri sciureus*). II. The projection from frontal and parietal association areas. Neuroscience *4*:747–765.