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Development of the Precerebellar Nuclei in the Rat: II. The Intramural Olivary Migratory Stream and the Neurogenetic Organization of the Inferior Olive

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 labeled on days E13 and E14, and killed at daily intervals thereafter, were analyzed to trace the migratory route and settling pattern of neurons of the inferior olive. Long-survival thymidine radiograms from perinatal rats injected on day E14 were used to subdivide the inferior olivary complex on the basis of neurogentic criteria.

The inferior olivary neurons originate on days E13 and E14 in the primary precerebellar neuroepithelium. The olivary neurons labeled on day E14 (the late generated components) translocate into the inferior olivary premigratory zone on day E15. On day E16 these cells join the olivary migratory stream, which follows an intramural circumferential path between the gray and white matters of the medulla. By day E17 the olivary migratory stream is reduced to a small band near the corpus of the inferior olive, which has been settled by this time by neurons generated on day E13. As a result, the unlabeled cells are situated on day E17 dorsomedially and the labeled cells ventrolaterally. The regional segregation of neurons forming subdivisions of the inferior olive begins on day E18, and by day E19 the major subdivisions are all recognizable.

In thymidine radiograms from perinatal rats injected on day E14, four neurogenetic components can be distinguished in the inferior olive, those composed: (1) of unlabeled cells (generated on day E13), (2) of predominantly unlabeled cells, (3) of predominantly labeled cells (generated on day E14), and (4) of labeled cells. By combining these neurogenetic differences with the morphological features of the inferior olivary complex, we propose a modification of the currently accepted classification. The four major divisions of the inferior olive are the successively produced posterodorsal olive, anterolateral (principal) olive, posteroventral olive, and anteroventral olive. The location and configuration of these divisions are illustrated in relation to the traditional classification both in the coronal and the sagittal plane.

Key words: cell migration, neurogenesis, thymidine autoradiography

According to His (1891), the neurons of the human inferior olive originate in the rhombic lip (*Rautenlippe*) and migrate ventromedially during the second and third months of embryonic life by way of the olivary stream (*Olivenstreifen*). His had clearly recognized a dense concentration of neuroblasts in the rhombic lip dorsolaterally, and also ventromedially in a stream adjacent to the formative inferior olive. He inferred that the two were interconnected by a *helleres Mittelstück* (a lighter midportion; His, 1891) consisting of bands of migrating cells. The brief descriptions by His and single illustration (His, 1891, Fig. 2) suggest that he recognized only as a source of olivary neurons the migra-

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tory stream that courses within the medullary parenchyma near its surface. It was Essick ('12) who, in his study of the development of the basal pontine gray in humans, clearly distinguished between a parenchymal and superficial migration of the rhombencephalon. Essick concluded that " . . . we have in the rhombic lip or 'Rautenlippe' of His a common ancestor for the olive, pontine nuclei and arcuate nuclei — the nuclei pontis being formed by a migration through a restricted pathway, the corpus ponto-bulbare; the nuclei arcuati along with part of the olive by a superficial migration over the ventral surface of the medulla" (Essick, '12, p. 54). In his illustrations, Essick calls the subpial, superficial stream the "olivo-arcuate migration." A reconciliation of His's view, who derived the neurons of the inferior olive from a parenchymal migration, and Essick's view, who derived them from a superficial migration, was proposed by Harkmark ('54). On the basis of observations and experiments in the chick, Harkmark concluded that both the "deep" and "superficial" migrations contribute neurons to the inferior olive, specifically " . . . that the deep cell strand in the chick gives rise to the dorsal lamella, the superficial strands to the ventral lamella of the olive" (Harkmark, '54, p. 167). Relating his findings to Kooy's ('17) homologization of the avian dorsal lamella with the accessory olives and the ventral lamella with the principal olive, Harkmark suggested that " . . . it would be interesting to investigate whether there is any evidence in mammals that the accessory olives develop from the deep cell strands and the principal olive from the peripheral ones" (Harkmark, '54, p. 167).

His, Essick, and Harkmark have all agreed that the neurons of the inferior olive derive from the rhombic lip dorsolaterally. In fact, Harkmark has obtained experimental evidence casting some doubt on this in that he found that even after extensive lesions of the rhombic lip, portions of the pons and inferior olive showed little hypoplasia. Harkmark tried to explain this by postulating cell migration from the contrateral side. In a more recent study, Ellenberger et al. ('69) cast doubt on the existence of a rhombic lip in the rat and proposed that the "marginal" and "submarginal" migrations of the inferior olive derive from the alar plate of the rhombencephalon, and not only from its lateral but also medial parts. Ellenberger and his associates were the first to use thymidine radiography to determine the time of origin of inferior olivary neurons. They concluded that olivary neurons in the rat are produced between days E14 and E15, predominantly on day E14. They proposed that the earlier generated neurons settle in the medial accessory, dorsal accessory, and principal nuclei, and the later generated neurons are limited to the ventral portion of the medial accessory olive. In our study of olivary neurogenesis, using quantitative long-survival thymidine radiography (Altman and Bayer, '78), we concluded that the production of inferior olivary neurons comes to an end before the morning of day E15, with over 70% of the cells being produced on day E13 or earlier, and less than 30% on day E14.

In the present study, utilizing sequential-survival and long-survival thymidine radiography, we offer conclusive evidence that the superficial (subpial) rhombencephalic migratory stream cannot be a source of inferior olivary neurons because the cells of this stream are labeled with ^{3}H-thymidine on day E15 whereas the production of olivary neurons ends on day E14. This lends support to His's view that the olivary neurons are derived from the deep (parenchymal) *Olivenstreifen*. However, the source of this migratory stream cannot be the rhombic lip as His proposed, because this begins to form on day E15, after the generation of olivary neurons. (The lower rhombic lip is, in fact, a source of auditory neurons not of precerebellar neurons; Altman and Bayer, '87.) The neurons of the inferior olive will be shown to derive from a region medial to the future rhombic lip from the ventral primary neuroepithelium of the rhombencephalon.

The regional segregation of early and late generated olivary neurons is used in this study to propose a new, neurogenetically based subdivision of the inferior olivary complex. The subdivision of the inferior olive currently in use conforms largely to the scheme proposed by Kooy ('17). Kooy made an extensive comparative study of the vertebrate inferior olive, examining 23 species of fish, 20 species of birds, and 29 species of mammals. Significantly, his approach was an "anthropomorphic" one, trying to fit his comparative findings into a pre-established classification based on previous anatomical studies in humans. As he wrote: "The olive in Man is generally divided into three parts: a medial and a dorsal one, called accessory olives, and a larger one between these two: the principal olive . . . I think I have succeeded in finding these parts in all orders of Mammals . . . I have tried to avoid the use of new names

Abbreviations

AD	anterodorsal olive
AL	anterolateral (principal) olive
ALi	anterolateral olive, inner lamella
ALm	anterolateral olive, middle lamella
ALo	anterolateral olive, outer lamella
AP	area postrema
AV	anteroventral olive
ca	caudal
ce	cerebellar primary neuroepithelium
cp	primordium of the choroid plexus
CP	choroid plexus
DA	dorsal accessory olive (Gwyn et al.)
DC	dorsal cap of olive
DM	dorsomedial cell column (Gwyn et al.)
GR	nucleus gracilis
IO	inferior olive
iom	inferior olivary intramural migratory stream
iop	inferior olivary premigratory zone
LR	lateral reticular nucleus
MA	medial accessory olive (Gwyn et al.)
me	medial
ME	medulla
ml	mediolateral
pcp	primary precerebellar neuroepithelium
pcsr	rostral primary precerebellar neuroepithelium
PD	posterodorsal olive
pes	posterior extramural migratory stream
PH	nucleus prepositus hypoglossi
PO	pontine region
PR	principal olive (Gwyn et al.)
PV	posteroventral olive
PVl	posteroventral olive, lateral subdivision
PVm	posteroventral olive, medial subdivision
RA	raphe nuclei
RL	nucleus of Roller
ro	rostral
SL	solitary nucleus
tc	tela choroidea
v4	fourth ventricle
VL	ventrolateral outgrowth (Gwyn et al.)
VS	sensory nucleus of the trigeminal nerve
XII	hypoglossal nucleus

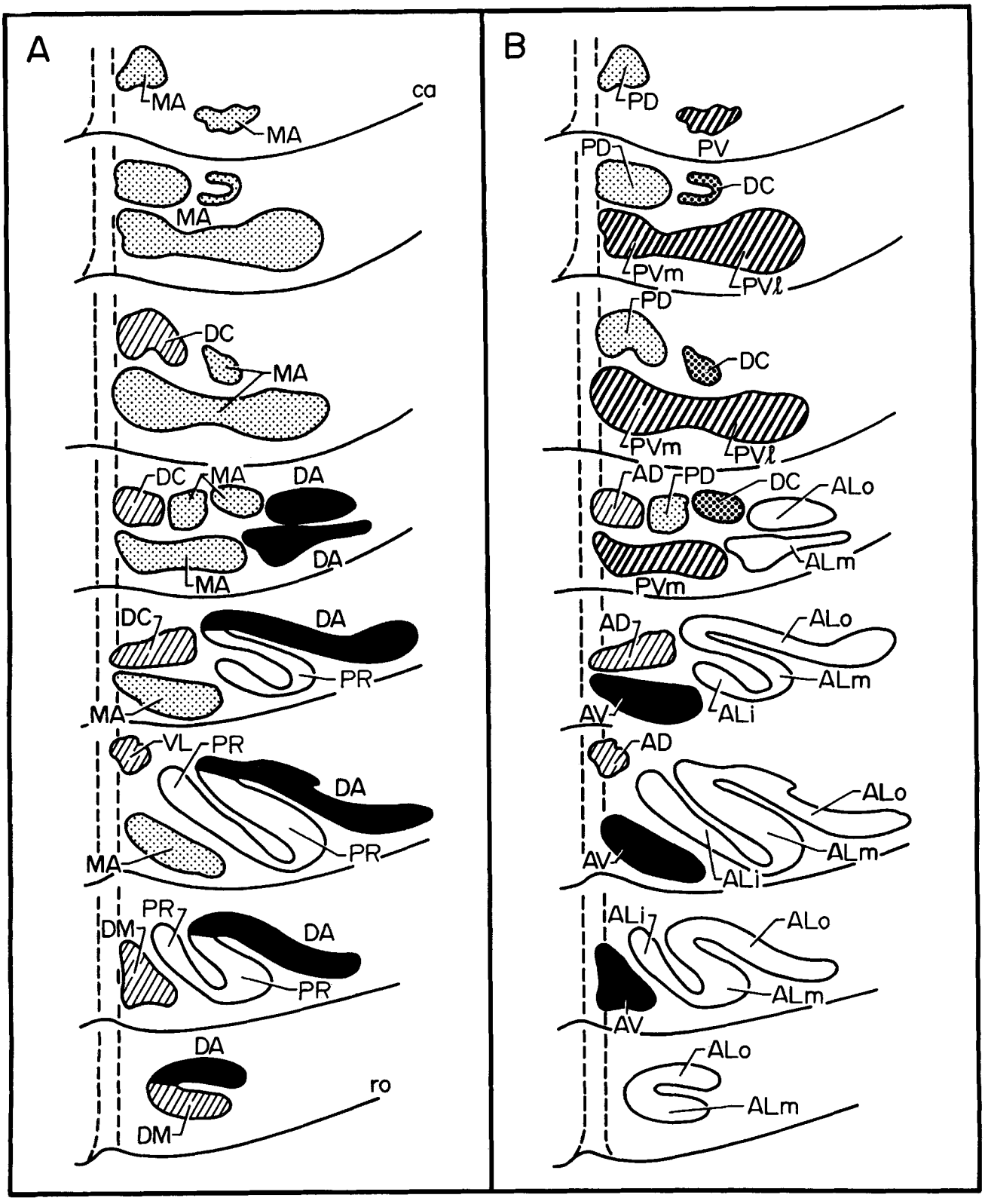

Fig. 1. Tracings of the inferior olive from coronal thymidine radiograms from caudal (ca) to rostral (ro) in a rat injected with ^{3}H-thymidine on day E14 and killed on day E22. A: Subdivision of components of the inferior olive, based on the scheme in adult rats of Gwyn et al ('77). B: Our subdivision based on neurogenetic criteria (for details, see Fig. 8B).

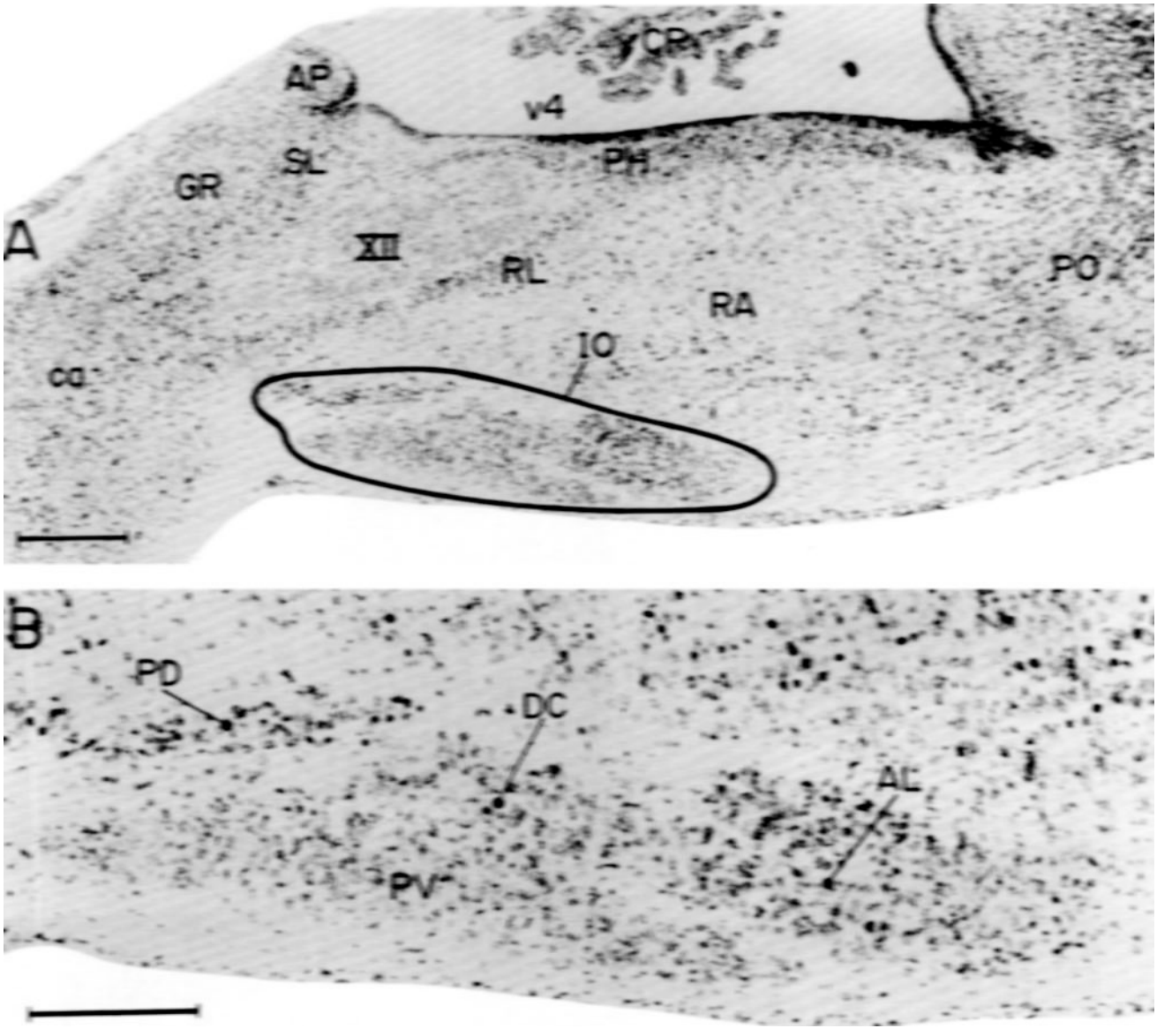

Fig. 2. **A:** Low power, parasagittal radiogram from a rat injected with ^{3}H-thymidine on day E13 and killed on day E20. **B:** The inferior olive, with its subdivisions, at higher magnification. Paraffin. Scales: A, 300 μm; B, 200 μm.

. . ." (Kooy, '17, pp. 264–265). As Kooy showed, a principal olive is not recognizable in fish and birds, and what appears to be the homologue of the human principal olive in most mammals (primates excluded) is not larger or more prominent, but in fact smaller, than the accessory olives. Kooy, therefore, used the term *ventro-lateral* olive interchangeably for the principal olive of lower mammals. But more unfortunate than this misleading terminology was Kooy's classification. Presumably because he assumed that the principal olive of all mammals should have the same U-shaped configuration as it has in humans, he assigned the dorsal lamella found in many mammals (although continuous with the other lamellae of the principal olive) to the dorsal accessory olive (Fig. 1A). As we note below, Kooy's classification is difficult to reconcile with the available neurogenetic evidence and, accordingly, we attempt a subdivision of the rat inferior olive based on thymidine radiographic observations.

MATERIALS AND METHODS

The material used in this study was identical with that described in detail in the preceding paper of this series (Altman and Bayer, '87). Special use was made of sequential-survival radiograms from rats labeled with ^{3}H- thymidine on days E13, E14, and E15, and killed thereafter at daily intervals up to day E22.

RESULTS

Cytogenetic organization of the inferior olive

Kooy's ('17) extensive survey of the inferior olive included the mouse but not the rat. This gap was filled by Gwyn et al. ('77), who published a detailed description of the rat

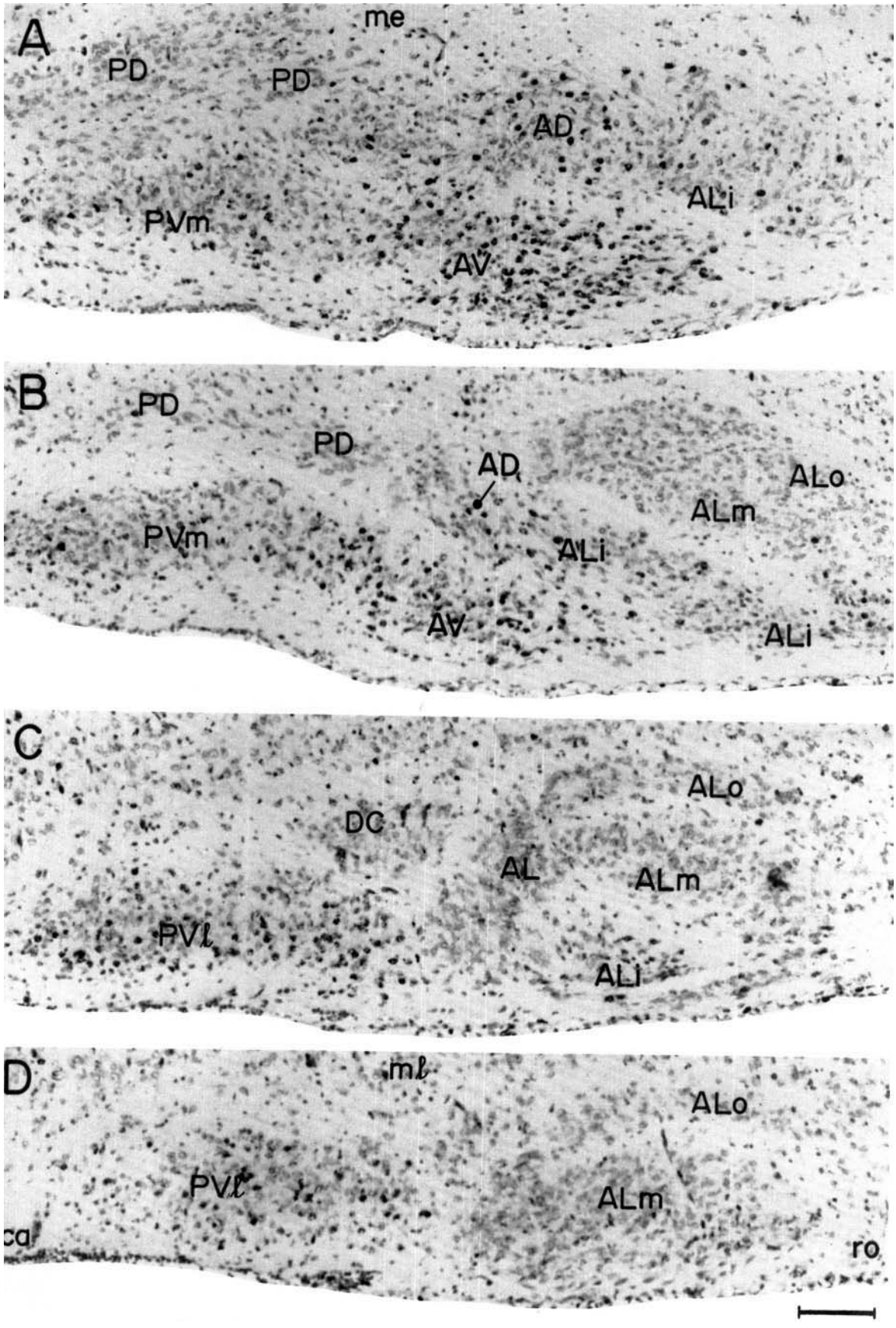

Fig. 3. Parasagittal radiograms, from medial (A) to mediolateral (D) from a rat labeled with ^{3}H-thymidine on day E14 and killed on day E22. Paraffin. Scale: 100 μm.

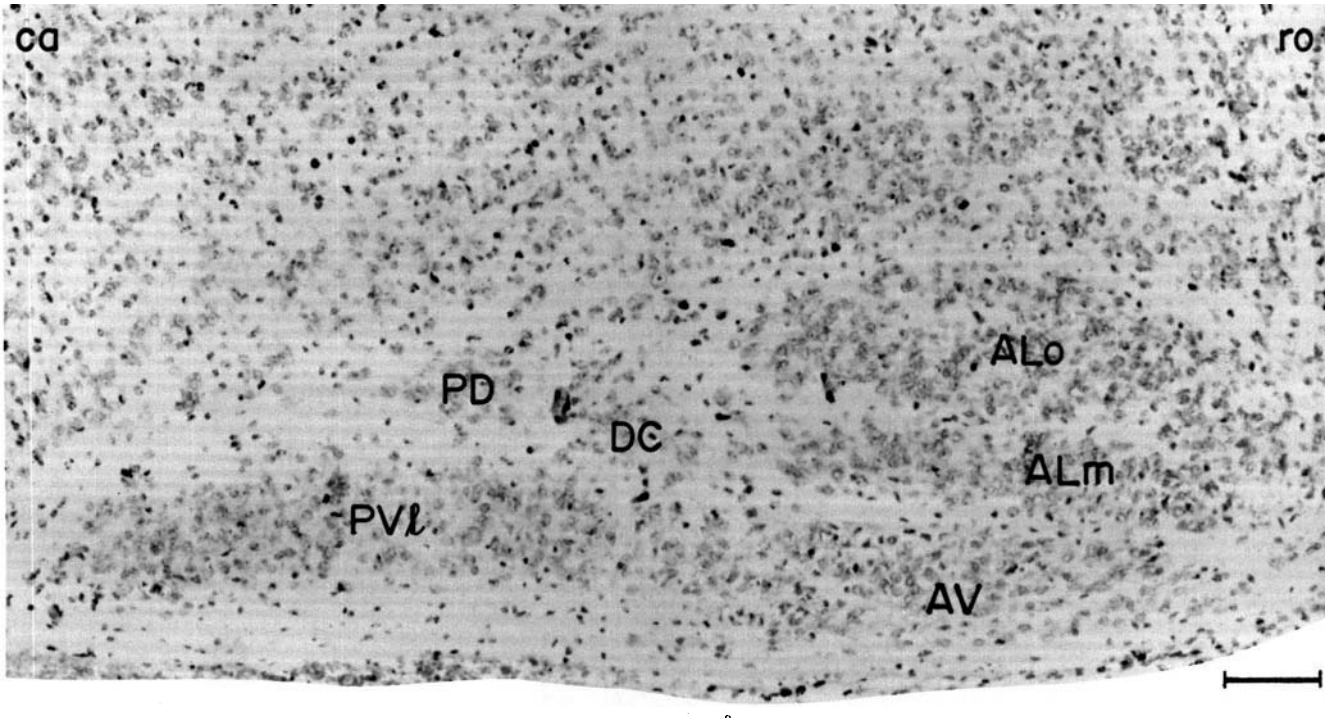

Fig. 4. Parasagittal radiogram from a rat labeled with ^{3}H-thymidine on day E15 and killed on day E22. Paraffin. Scale: 100 μm.

inferior olive, following closely Kooy's scheme. Figure 1A represents an attempt to transcribe the scheme of Gwyn et al., based on adult rats, to a series of coronal tracings from caudal to rostral in a perinatal (day E22) rat. Because it is difficult to present our ontogenetic observation within the framework of Gwyn's scheme, Figure 1B shows an alternate classification based on developmental observations. Much of this section is concerned with the evidence for the neurogenetic scheme presented in Figure 1B.

In rats injected on day E13 and killed perinatally, virtually all the neurons of the inferior olive are labeled, some heavily, others lightly (Fig. 2A,B). Four components may be distinguished at this level: (1) a heavily labeled (early differentiating) posterodorsal division (PD), (2) a mostly heavily-labeled, larger anterolateral division (AL), (3) a lightly labeled dorsal region with some heavily labeled cells, the dorsal cap (DC), and (4) an extensive, lightly labeled (late differentiating) posteroventral division (PV).

Figure 3 shows a series of sagittal sections from medial (Fig.3A) to lateral (Fig. 3D) from a rat labeled on day E14 and killed on day E22. Caudally and mediodorsally (Fig. 3A,B), the neurons of the posterodorsal division (PD) are no longer labeled. Rostrally (Fig. 3A–C), neurons in the outer (ALo) and middle (ALm) lamellae of the anterolateral (principal) division are, likewise, unlabeled, but many are still labeled in the inner lamella (Ali). The neurons of the dorsal cap (DC in Fig. 3C) are mostly unlabeled, except a few ventrally. Caudally and ventrally there is an admixture of heavily labeled and unlabeled cells in the posteroventral division (PVm in Fig. 3A,B and PV1 in Fig. 3C,D). Two additional regions (not seen in the section in Fig. 2B) are the medially located anterodorsal division (AD in Fig. 3A,B), with an admixture of heavily labeled and unlabeled cells, and the predominantly heavily labeled, medially situated anteroventral division (AV in Fig. 3A,B). The latter is composed of the latest generated neurons of the inferior olive.

In rats labeled on day E15 ands killed on day E22 (Fig. 4), the neurons of the inferior olive are no longer labeled, indicating that olivary neurogenesis ceases before the morning of day E15.

The labeling pattern of the rat inferior olivary complex is illustrated in coronal sections, from caudal to rostral, in radiograms from a rat tagged on day E14 and killed on day E22 (Fig. 5,6). (The levels chosen closely approximate those illustrated in Fig. 1B.) The unlabeled posterodorsal division (PD) is delineated from the posteroventral division (PV) not only by the latter having an admixture of labeled and unlabeled cells, but also by an actual discontinuity between them (arrow in Fig. 5B,C). Within the extensive posteroventral division there is apparently a neurogenetic gradient from medial to lateral, the medial subdivision (PVm) having few labeled cells, the lateral subdivision (PV1) many more. The possibility that these are two separate structures is suggested by the different appearance of their maturing neurons at higher magnification (Fig. 7A). The discontinuity between the early-produced posterodorsal division and the later-produced anterodorsal division (AD and PD in Fig. 5C) is not clear. In contrast to the apparent medial-to-lateral neurogenetic gradient in the posteroventral division, the gradient in the anterolateral division (principal olive) appears to be a modified lateral-to-medial one: the neurons in the outer and medial lamellae (ALo and ALm in Figs. 5D, 6A–C) are typically unlabeled, whereas most of them are labeled in the inner lamella (ALi in Figs. 6B,C, 7B). The highest proportion of labeled neurons is seen in the anteroventral division (AV in Figs. 6A–C, 7B). A sum-

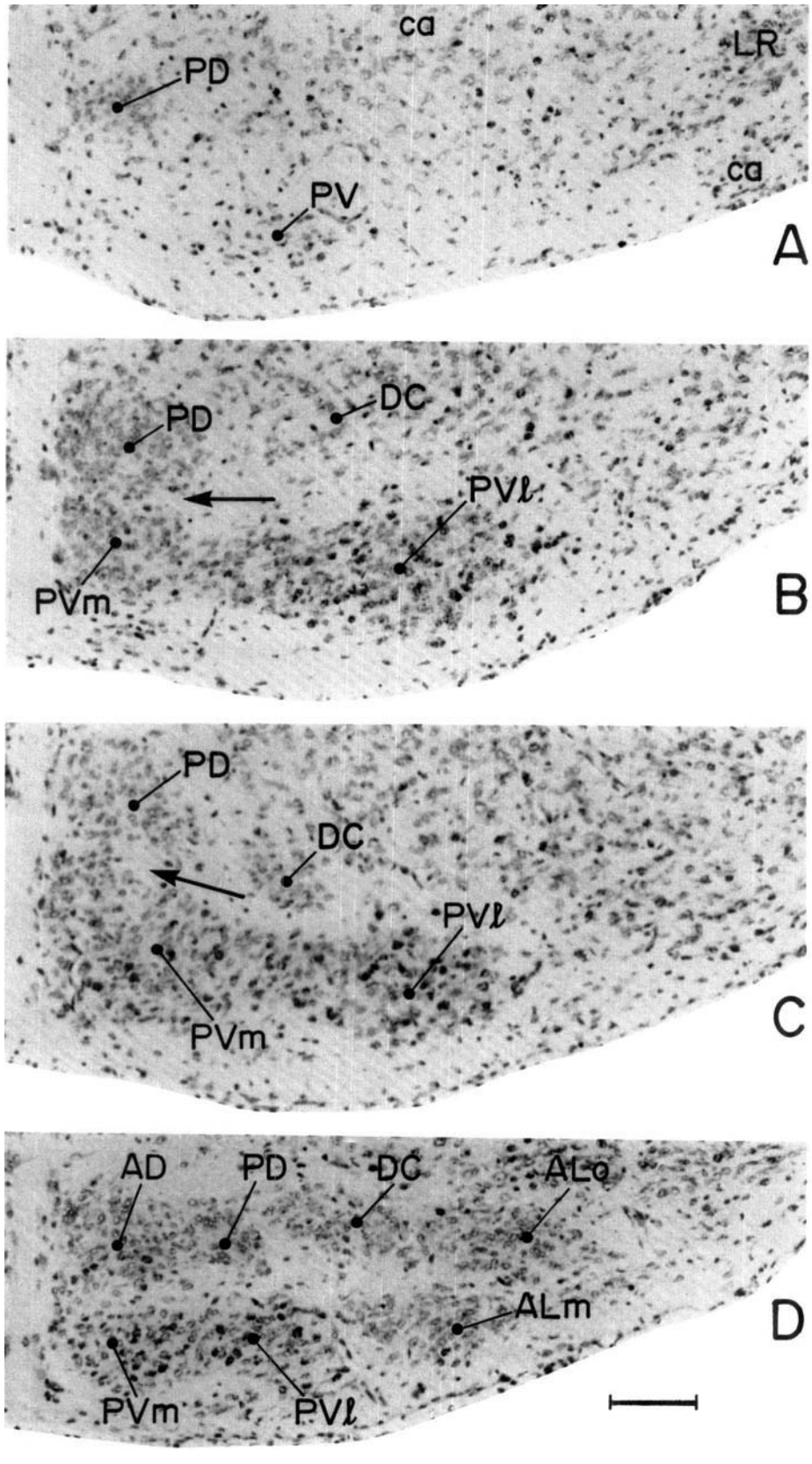

Fig. 5. Coronal radiograms, from caudal to rostral, from a rat labeled with ^{3}H-thymidine on day E14 and killed on day E22. Paraffin. Scale: 100 μm.

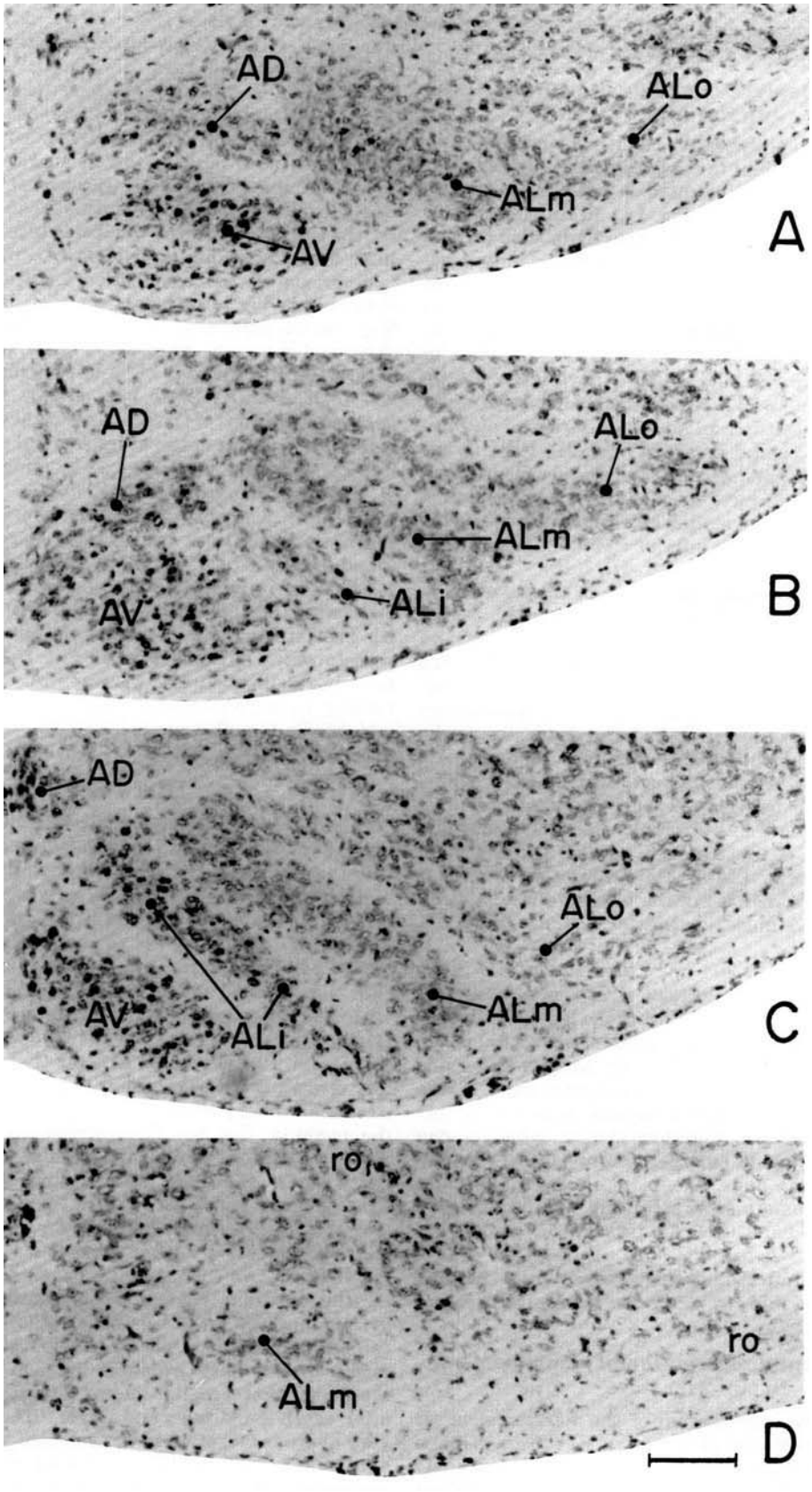

Fig. 6. Continuation of the series shown in Figure 5.

mary of the four labeling patterns (nearly all labeled, mostly labeled, mostly unlabeled, unlabeled) is presented diagrammatically in sagittal and coronal planes in Figure 8A,B.

Site of origin and migratory route of neurons of the inferior olive

The labeling of the proliferative cells of the primary precerebellar neuroepithelium in rats injected on day E14 and killed 2 hours later was illustrated in the previous paper (Altman and Bayer, '87: pcp in Fig. 1A). In rats injected on day E14 and killed 1 day later, the heavily labeled precursor cells are translocated into the inferior olivary premigratory zone (iop in Figs. 9A, 10A; compare with Altman and Bayer, '86, Fig. 3) and a few have joined the trailing portion of the inferior olivary migratory stream (iom in Figs. 9A, 10A) composed of unlabeled (earlier generated) neurons. In rats injected on day E14 and killed 2 days later, a zone largely free of labeled cells is seen between the primary precerebellar neuroepithelium and the inferior olivary migratory stream (horizontal arrow in Figs. 9B, 10B). In the inferior olivary migratory stream, the labeled cells predominate and some of these have reached the caudal portion of the inferior olive. Two days after labeling of the precursor cells, the anterior part of the inferior olive is still composed mostly of unlabeled cells (IO in Figs. 9B, 10B).

The intramural (parenchymal) course of the inferior olivary migratory stream is illustrated in a coronal section from a rat labeled on day E14 and killed 2 days later (iom in Fig. 11A). The route is a circumferential one beneath the medullary gray matter. By this time the stream is approaching the site of the previously settled unlabeled (earlier generated) olivary neurons. In these day E16 rats the posterior extramural (superficial) migratory stream (pes) is small and very few of its cells have reached the midline. The location of the inferior olivary migratory stream in relation to the inferior olive in rats labeled on day E14 and killed on day E16 is illustrated in coronal sections from caudal to rostral (Fig. 12A–D). The migratory stream is limited to the caudal portion of the inferior olive, confirming the observation made in sagittal sections (Figs. 9B, 10B).

In rats injected on day E14 and killed on day E17 (Figs. 11B, 13), the intramural migratory stream is reduced to a remnant near its termination. Most of the labeled cells now occupy a position ventral to the earlier settled unlabeled cells. This initial segregation of olivary neurons generated before day E14 (the unlabeled cells) and after day E14 (the labeled cells) is also illustrated in sagittal sections (Fig. 14). The posterior extramural migratory stream, which on day E16 is just beginning to emerge posteroventrally (pes in Fig. 9B), has become quite prominent by day E17 (pes in Fig. 14). Its cells remain separated from the inferior olive by a broad white band (Fig. 14). Instead of joining the inferior olive, as it has hitherto been assumed, the cells of the posterior extramural migratory stream cross the midline of the medulla midway along the rostrocaudal extent of the olive (Fig. 13B,C).

The developmental segregation of components of the inferior olive

The neurogenetic organization of the inferior olive, as seen in perinatal rats injected on day E14 (Figs. 3–8), is becoming partially recognizable in rats injected on day E14 and killed on day E18 (Figs. 15–16). Caudally, the earliest produced posterodorsal division forms a discrete component (PD in Figs. 15A,B, 16A,B) as does the medial portion of the posteroventral division (PVm in the same sections). However, some of the late produced neurons of the lateral portion of the posteroventral division (PVl in Fig. 15C and arrow in Fig. 16A,B) have yet to settle. Rostrally, many of the late generated cells of the anteroventral division (AV in Fig. 15A,B) have settled medially, but some of the cells are still in a lateral position (arrow in Fig. 16C). The inner lamella of the anterolateral (principal) division, with its labeled cells, seems to be identifiable (ALi in Fig. 16C,D), but the segregation of the unlabeled cells of the middle and outer lamellae is not yet evident (AL in Figs. 15, 16C,D). Comparison of the organizational pattern of the inferior olive on this day with the pattern seen on the previous day (Fig. 14) suggests that the early generated posterodorsal olive, and the early components of the posteroventral and anterolateral (principal) olive have already settled by day E17 (PD?, PVm? AL? in Fig. 14A,B). However, the late generated neurons of the posteroventral olive and the latest generated neurons of the anteroventral olive (PV? and AV? in Fig. 14B) have yet to reach the midportion of the olive on this day (compare Figs. 14A, 15A).

The structural maturation of the inferior olivary complex continues on the next day. In rats injected on day E14 and killed on day E19 (Figs. 17,18), the dorsal cap (DC in Fig. 17C) can be recognized with some certainty and, with less assurance, the anterodorsal division (AD in Figs. 17C, 18A–C). The latest-produced cells of the lateral portion of the posteroventral division (PV1 in Fig. 17A,B) and of the anteroventral division (AV in Fig. 18A–C) are also settled. Finally, in the anterolateral (principal) division, the inner and middle lamellae are recognizable, and less clearly the outer lamella (Fig. 18A–C).

DISCUSSION

This study confirms our earlier result that the neurons of the rat inferior olive are generated on days E13 and E14. Administration of ^{3}H- thymidine beginning on the morning of day E15 no longer labels olivary neurons. Because the cells of the posterior extramural migratory stream, what Essick ('12) has called the olivo-arcuate migration, Harkmark ('54) the superficial migration, and Ellenberger et al. ('69) the submarginal migration, are consistently labeled with injections made on day E15, it follows that this stream is not composed of migrating olivary cells. In line with His's (1891) view, we were able to show that the olivary migratory stream is a parenchymal or intramural one, coursing circumferentially between the white and gray matters of the medulla. But our observations do not support His's inference that the source of this migration is the rhombic lip. We have shown in the preceding paper of this series that the upper and lower rhombic lips are part of the rhombencephalic neuroepithelium that form far-laterally around the lateral recess of the fourth ventricle (Altman and Bayer, '87, Figs. 6,7). Of these two components, the upper rhombic lip (the germinal trigone) is contributing cells to the cerebellum, and the lower lip to the auditory system. The precerebellar component of the rhombencephalon is situated medial to the lower rhombic lip, and in this region the secondary precerebellar neuroepithelium begins to form after day E15 (Altman and Bayer, '87, Fig. 1B,2). The olivary neurons originate in the primary precerebellar neuroepithelium and we have followed the course of their intramural migration in sequential radiograms in rats labeled with ^{3}H-thymidine on day E14.

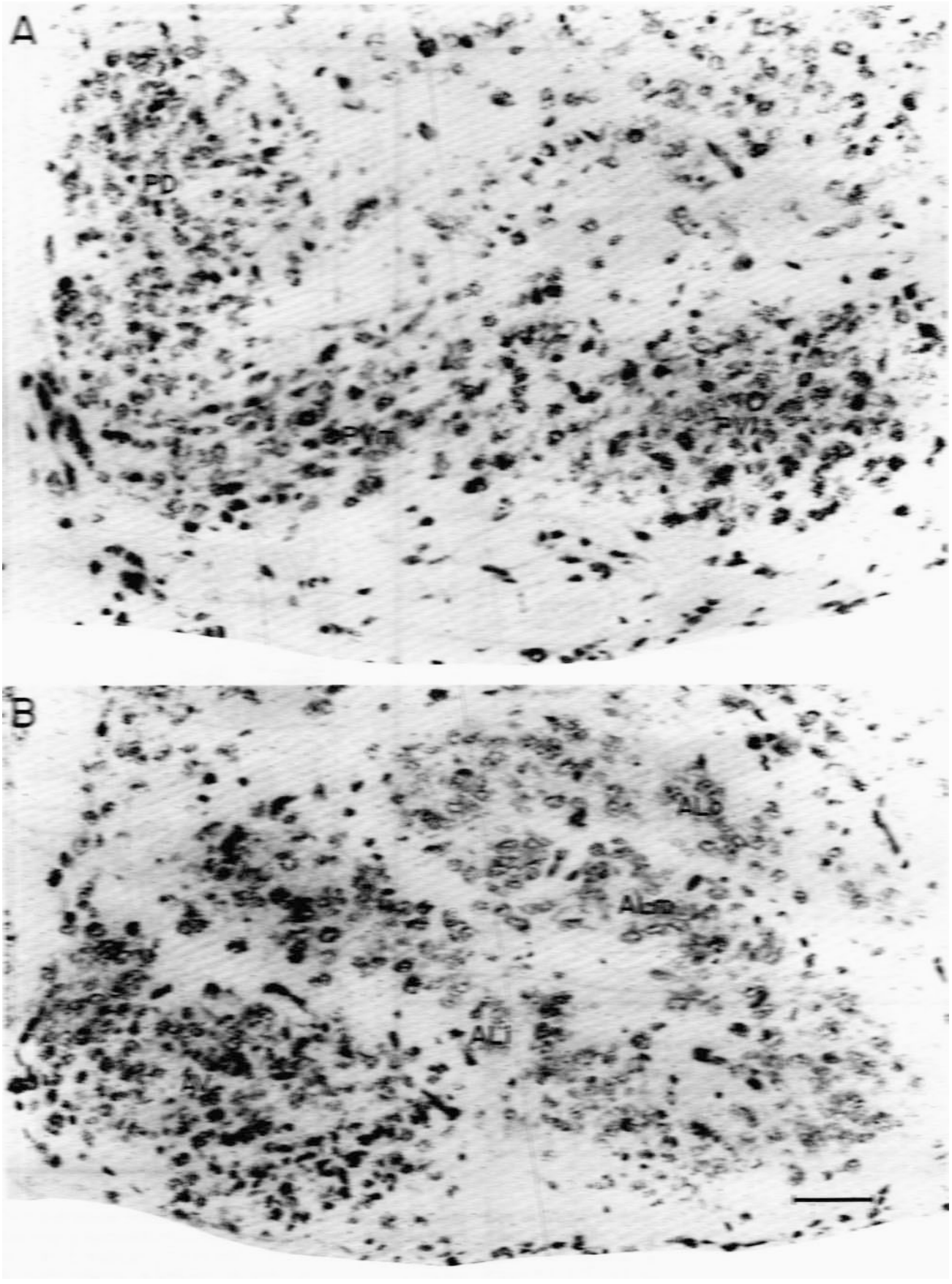

Fig. 7. Coronal radiograms of the inferior olive at caudal (A) and rostral (B) levels from a rat labeled with ^{3}H-thymidine on day E14 and killed on day E22. Paraffin. Scale: 50 μm.

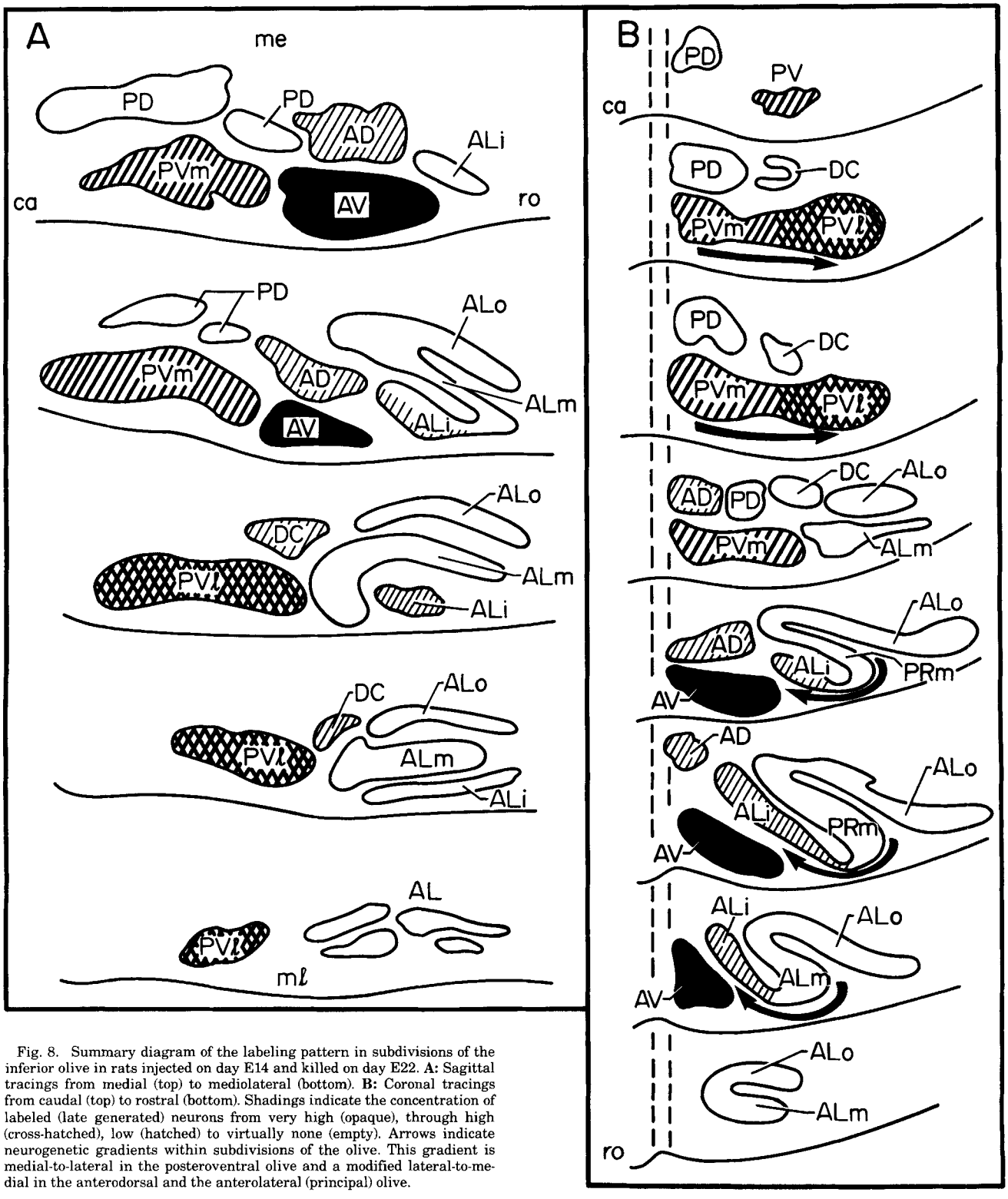

Fig. 8. Summary diagram of the labeling pattern in subdivisions of the inferior olive in rats injected on day E14 and killed on day E22. **A:** Sagittal tracings from medial (top) to mediolateral (bottom). **B:** Coronal tracings from caudal (top) to rostral (bottom). Shadings indicate the concentration of labeled (late generated) neurons from very high (opaque), through high (cross-hatched), low (hatched) to virtually none (empty). Arrows indicate neurogenetic gradients within subdivisions of the olive. This gradient is medial-to-lateral in the posteroventral olive and a modified lateral-to-medial in the anterodorsal and the anterolateral (principal) olive.

In the present examination of the settling patterns and neurogenetic organization of the inferior olive, we have relied heavily on sequential and long-survival (fetal) thymidine radiograms from rats labeled on day E14. We could distinguish in this group, first, regions of the olive composed of unlabeled cells (neurons generated before day E14) from regions composed of labeled cells (neurons generated on day E14). Because in other olivary regions there was either a neurogenetic gradient present or an admixture of labeled and unlabeled cells, we could also distinguish, al-

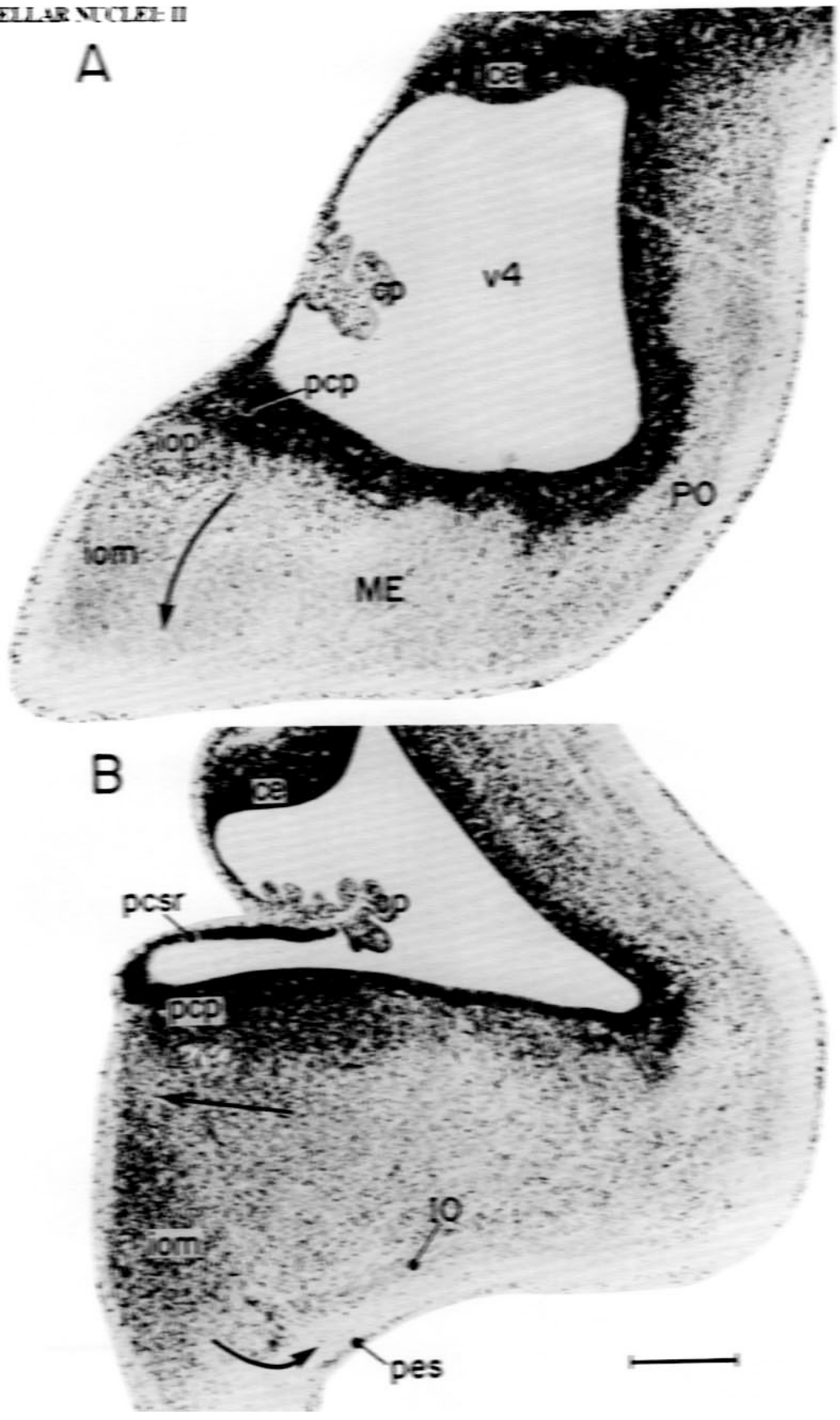

Fig. 9. Parasagittal radiograms from a rat labeled with ^{3}H-thymidine on day E14 and killed on day E15 (A), and another injected on the same day and killed on day E16 (B). Curved arrows indicate the intramural course of young neurons from the inferior olivary premigratory zone (iop), through the inferior olivary migratory stream (iom) to the inferior olive (IO). Paraffin. Scale: 300 μm.

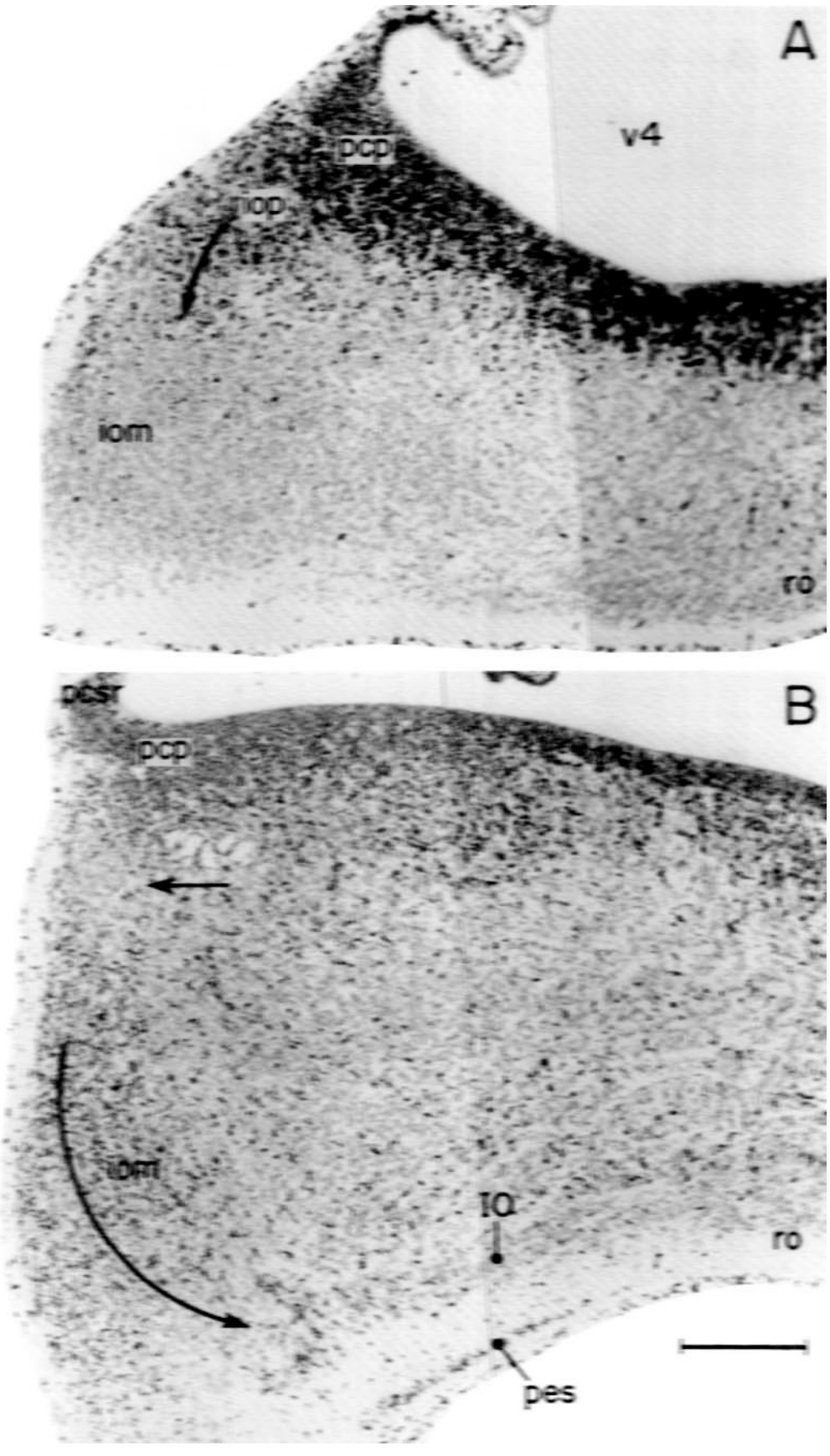

Fig. 10. The inferior olivary premigratory zone (iop) and migratory stream (iom) at higher magnification in rats injected on day E14 and killed on days E15 (A) and E16 (B). Paraffin. Scale: 200 μm.

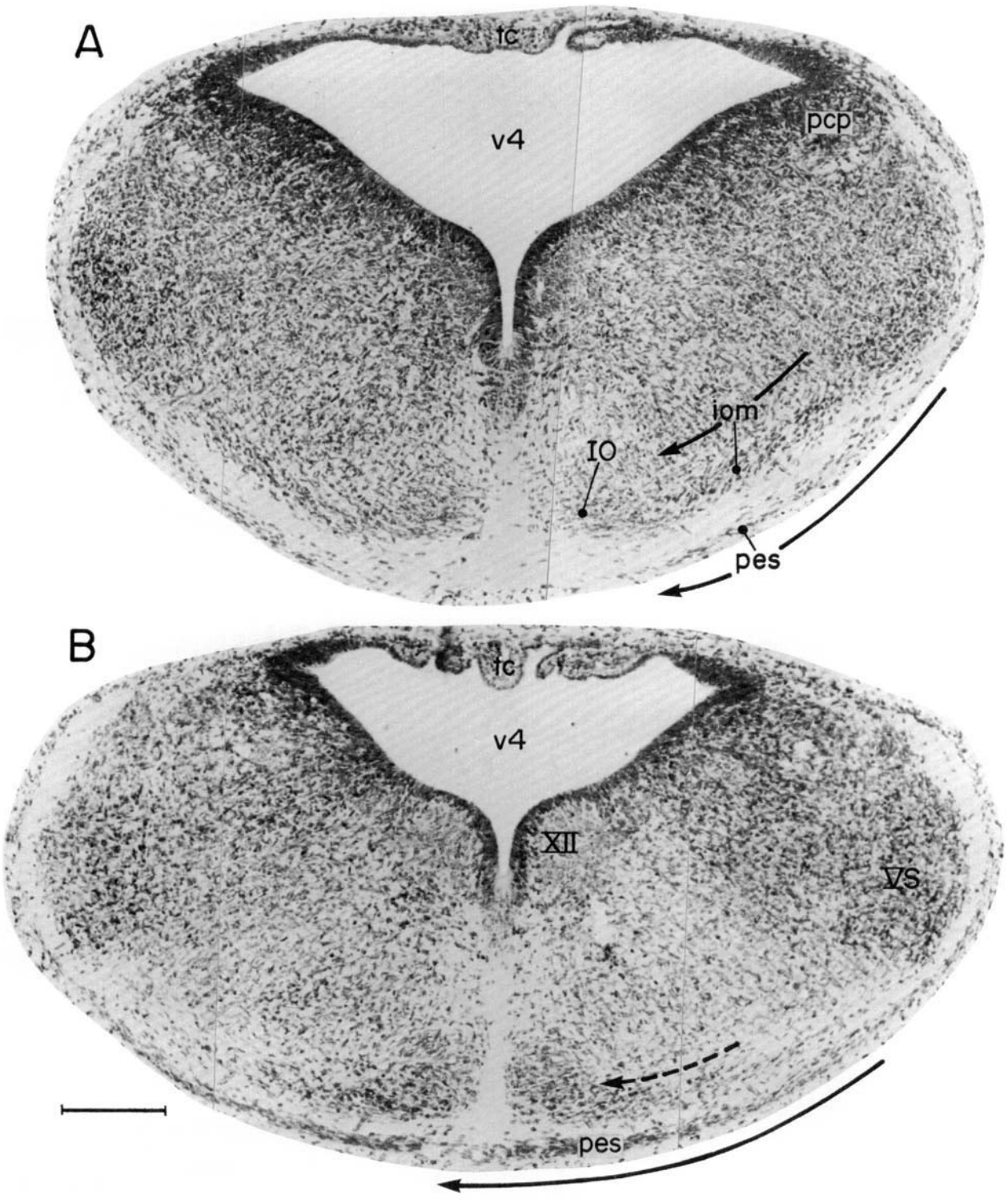

Fig. 11. Matched coronal radiograms from rats that were labeled with ^{3}H-thymidine on day E14 and killed on days E16 (**A**) and E17 (**B**). The labeled cells of the intramural olivary migratory stream (iom) have reached the vicinity of the inferior olive (IO) by day E16 and some have apparently settled. By day E17 the straggling cells of olivary stream are limited to the vicinity of the inferior olive (arrow in **B**). Some of the labeled cells of the posterior extramural migratory stream are approaching the midline beneath the olive on day E16 (pes and arrow in **A**) and reach it, and possibly cross to the opposite side, by day E17 (pes and arrow in **B**). Paraffin. Scale: 200 μm.

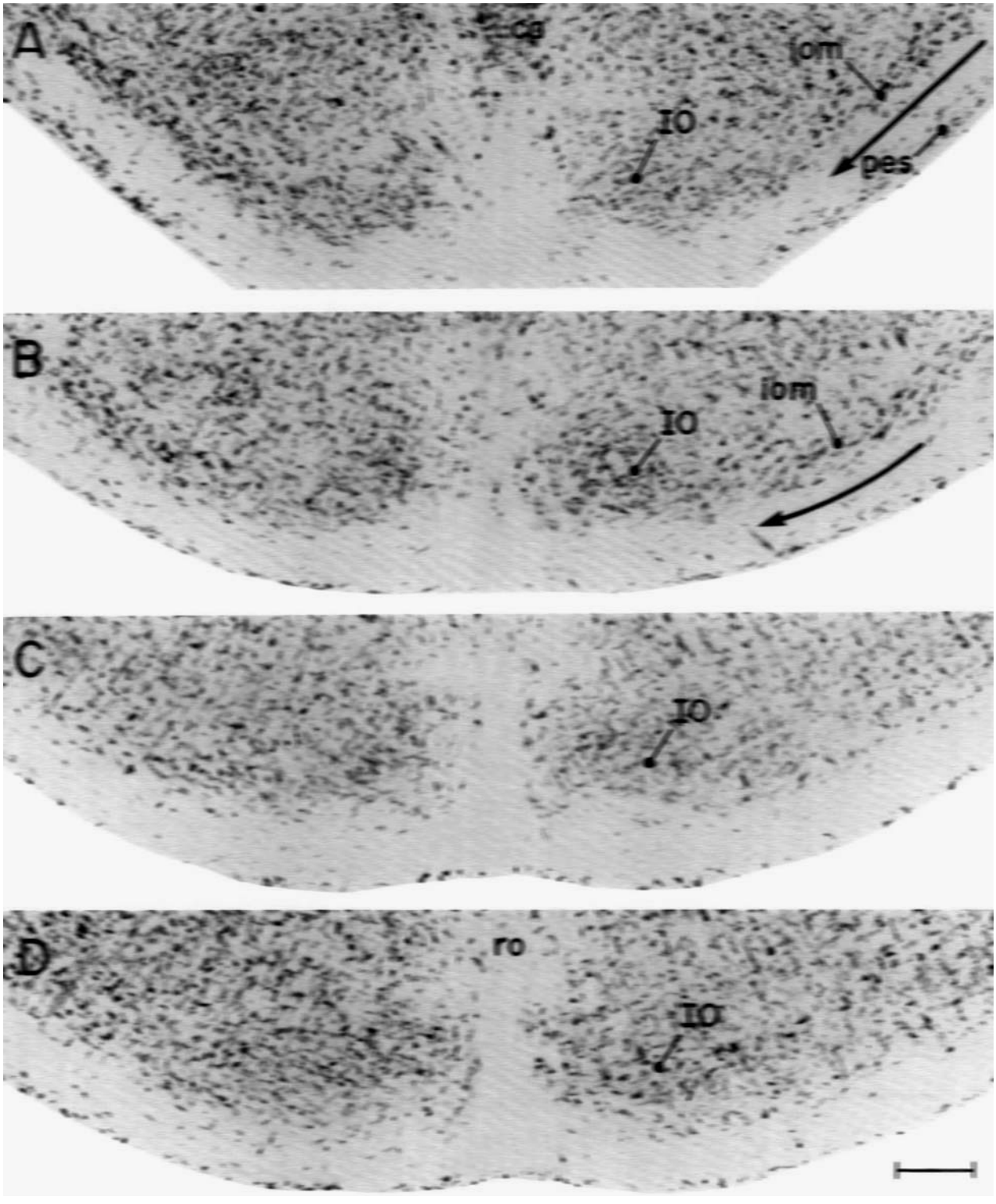

Fig. 12. Coronal radiograms from caudal (A) to rostral (D) through the formative inferior olive from a rat labeled on day E14 and killed on day E16. The labeled cells of the inferior olivary migratory stream (iom and arrow) are localized caudally (A and B). Only a few pioneering cells of the posterior extramural migratory stream (pes in A) are detectable at this age. Paraffin. Scale: 100 μm.

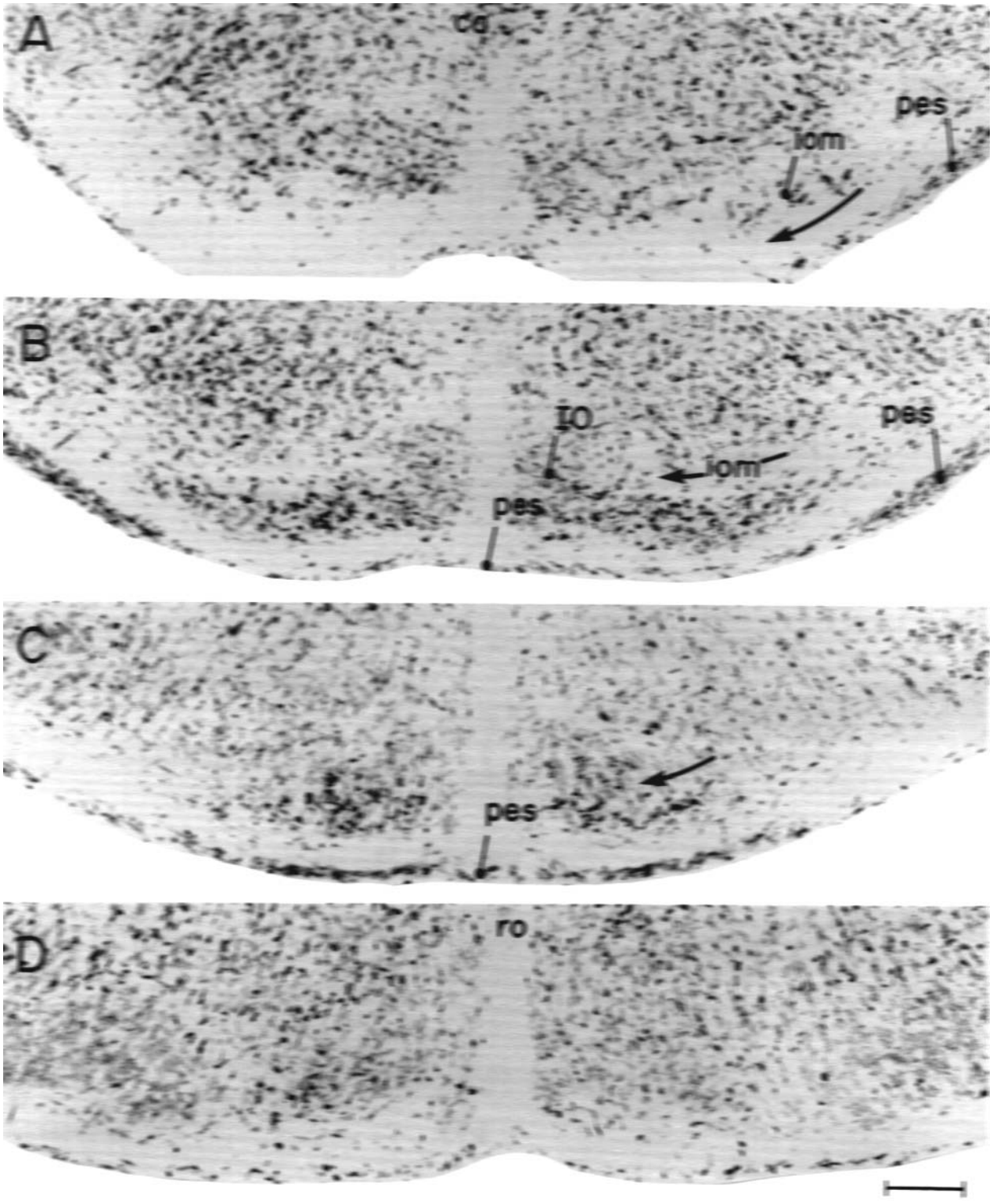

Fig. 13. Coronal radiograms from caudal (A) to rostral (D) from a rat labeled on day E14 and killed on day E17. The intramural olivary migratory stream (iom and arrow) is limited to the vicinity of the olive. The posterior extramural migratory stream has greatly increased in volume caudally (pes in A and B) and is seen crossing the midline in the midportion of the olive (B and C). There is no continuity between the extramural stream and the inferior olive. Paraffin. Scale: 100 μm.

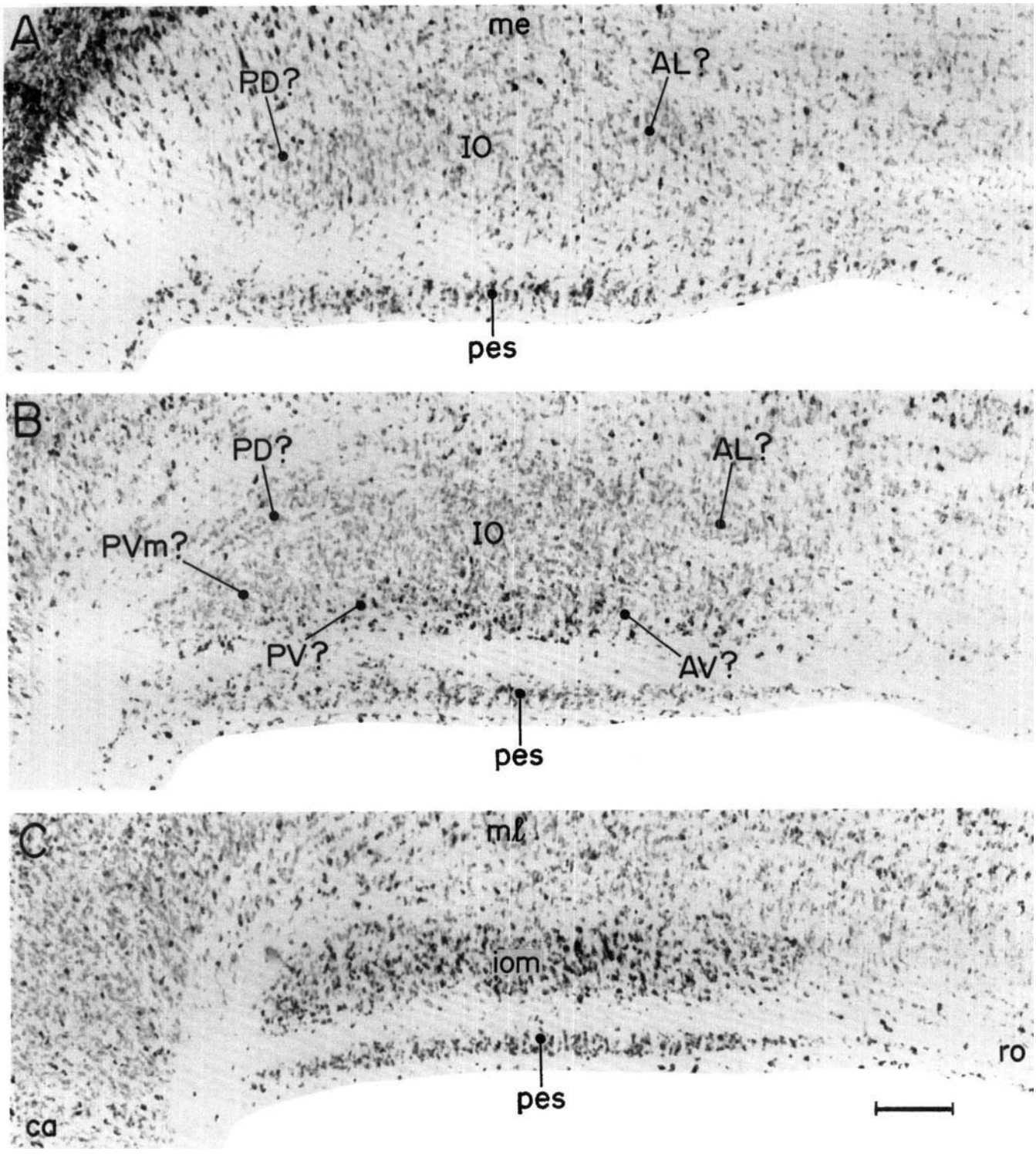

Fig. 14. Parasagittal radiograms from medial (A) to mediolateral (C) from a rat labeled with ^{3}H-thymidine on day E14 and killed on day E18. Medially (A) the inferior olive (IO) is devoid of labeled cells. More laterally (B) the labeled cells are concentrated ventrally. Still more laterally (C) the plane passes through the intramural migratory stream (iom). The posterior extramural migratory stream is quite thick caudally and tapers off rostrally. This stream is separated from the inferior olive by a layer of white matter. Paraffin. Scale: 100 μm.

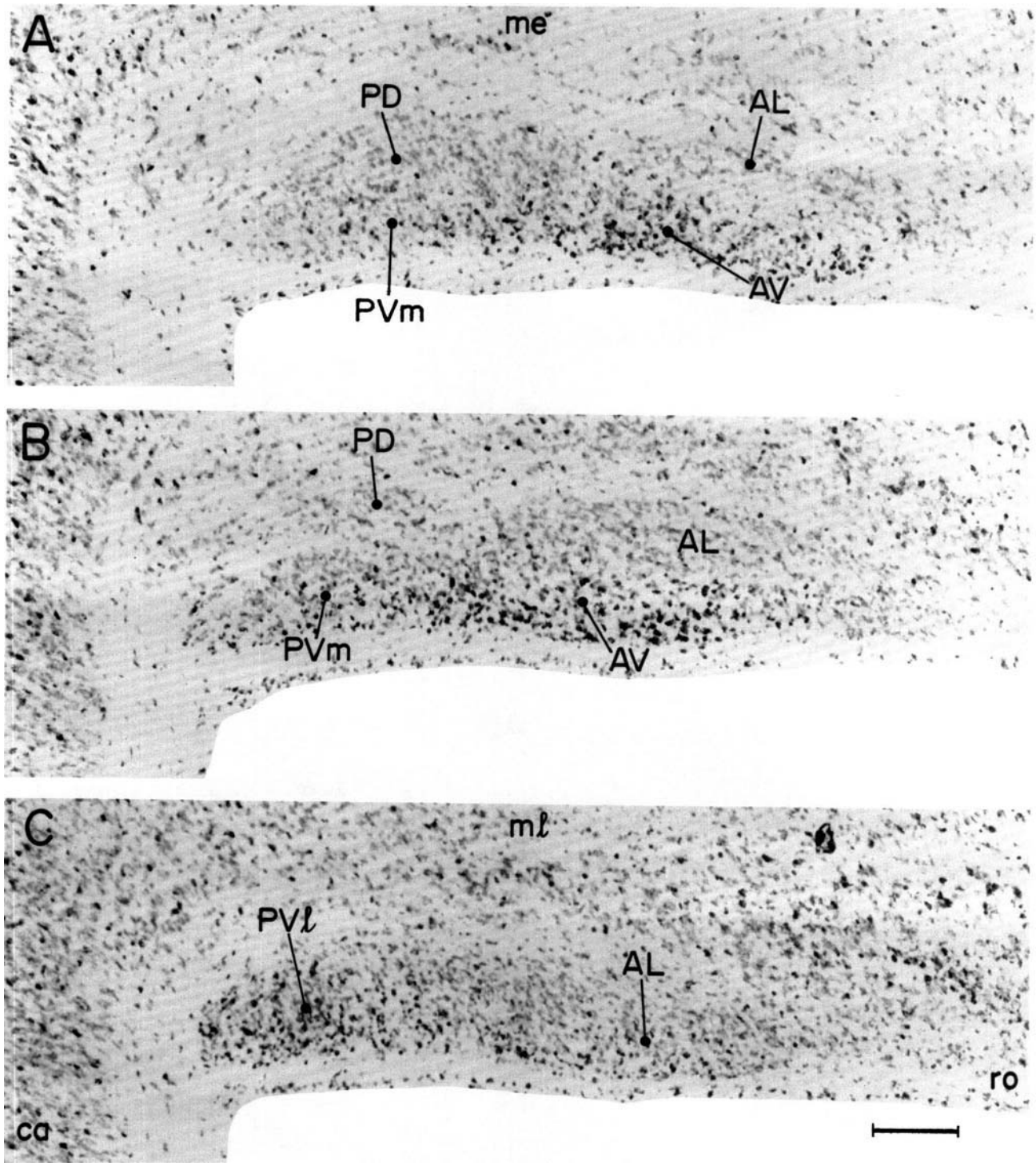

Fig. 15. Parasagittal radiograms from medial (A) to lateral (C) from a rat labeled with ^{3}H-thymidine on day E14 and killed on day E18. Several of the neurogenetic subdivisions of the inferior olive are recognizable. These include the early generated (unlabeled) posterodorsal (PD) and anterolateral or principal (AL) olive, and the late generated posteroventral (PVm and PV1) and anteroventral (AV) olives. Paraffin. Scale: 100 μm.

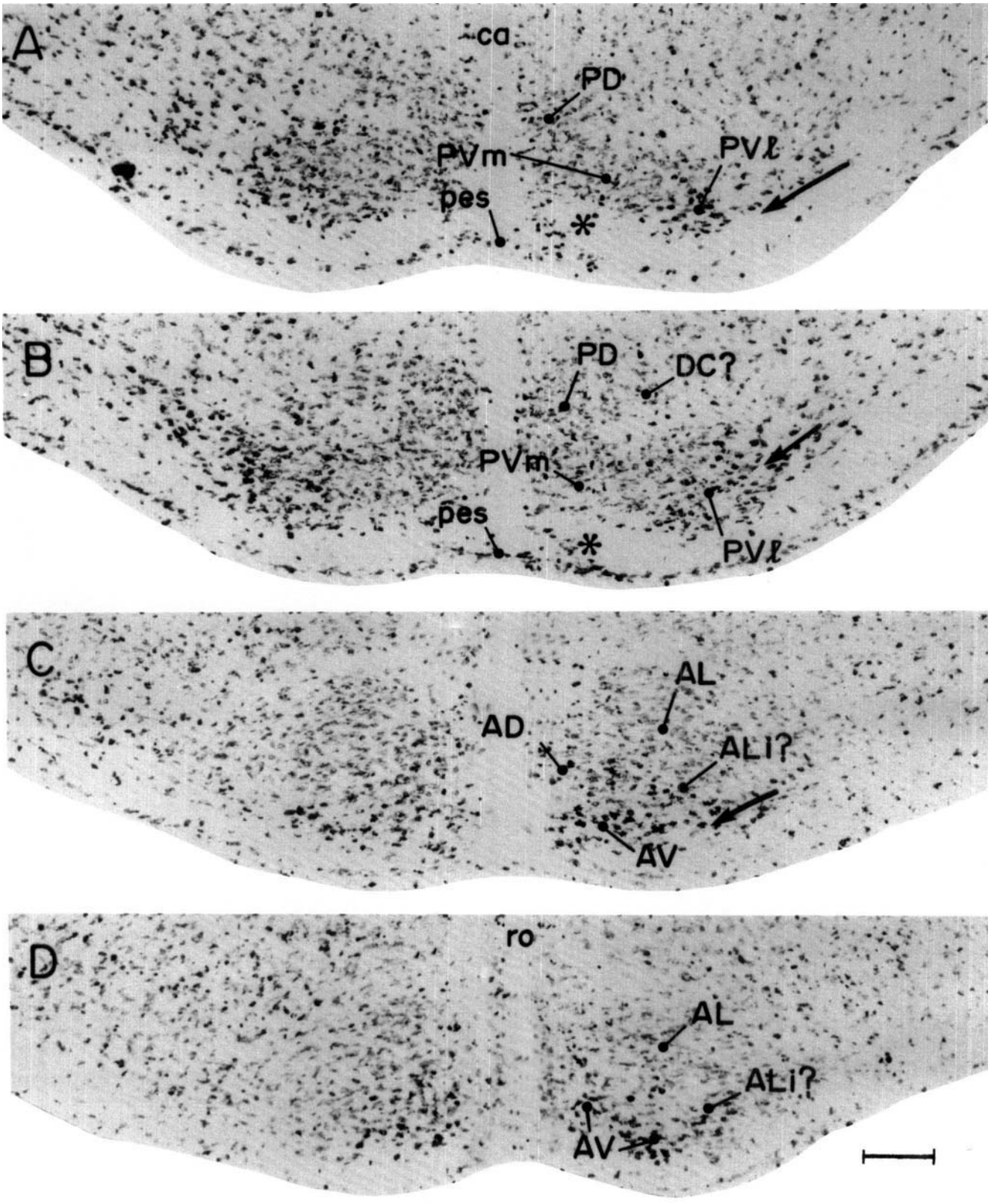

Fig. 16. Coronal radiograms of the inferior olive from rostral (A) to caudal (D) from a rat labeled with ^{3}H-thymidine on day E14 and killed on day E18. A few straggling cells of the olivary intramural migratory stream (arrow in A and B) are still seen. The segregation of the lamellae of the anterolateral (principal) olive is still not evident. The posterior extramural migratory stream (pes in A and B) is reduced and a few of its cells may approximate the inferior olive (asterisk in A and B). Paraffin. Scale: 100 μm.

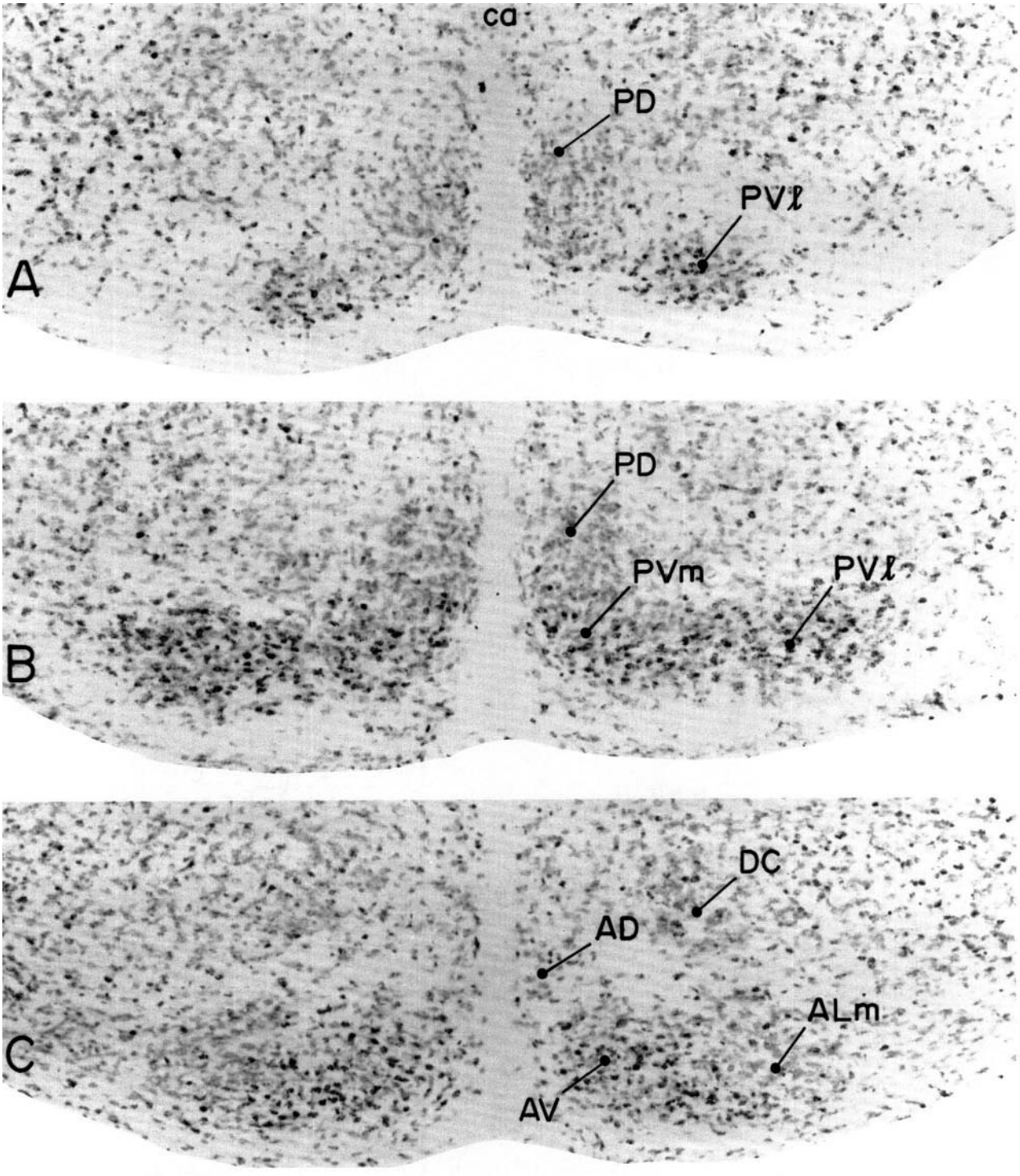

Fig. 17. Coronal radiograms from caudal (A) and rostralward (C) from a rat labeled on day E14 and killed on day E19. All the subdivisions of the inferior olive, including the anterodorsal olive (AD) and the dorsal cap (DC), are now recognizable. Paraffin. Scale: 100 μm.

though with lesser assurance, regions composed predominantly of labeled cells or predominantly of unlabeled cells. This fourfold distinction, coupled with the morphological features of the inferior olivary complex, was used for a revised neurogenetic classification of the olive, as shown in Figures 1B and 8A,B. The two major differences between our classification and that of Gwyn et al. ('77), which is an application of Kooy's ('17) system to the rat, are the following. First, the neurogenetic evidence does not support the existence of a separate dorsal accessory olive (DA in Fig.

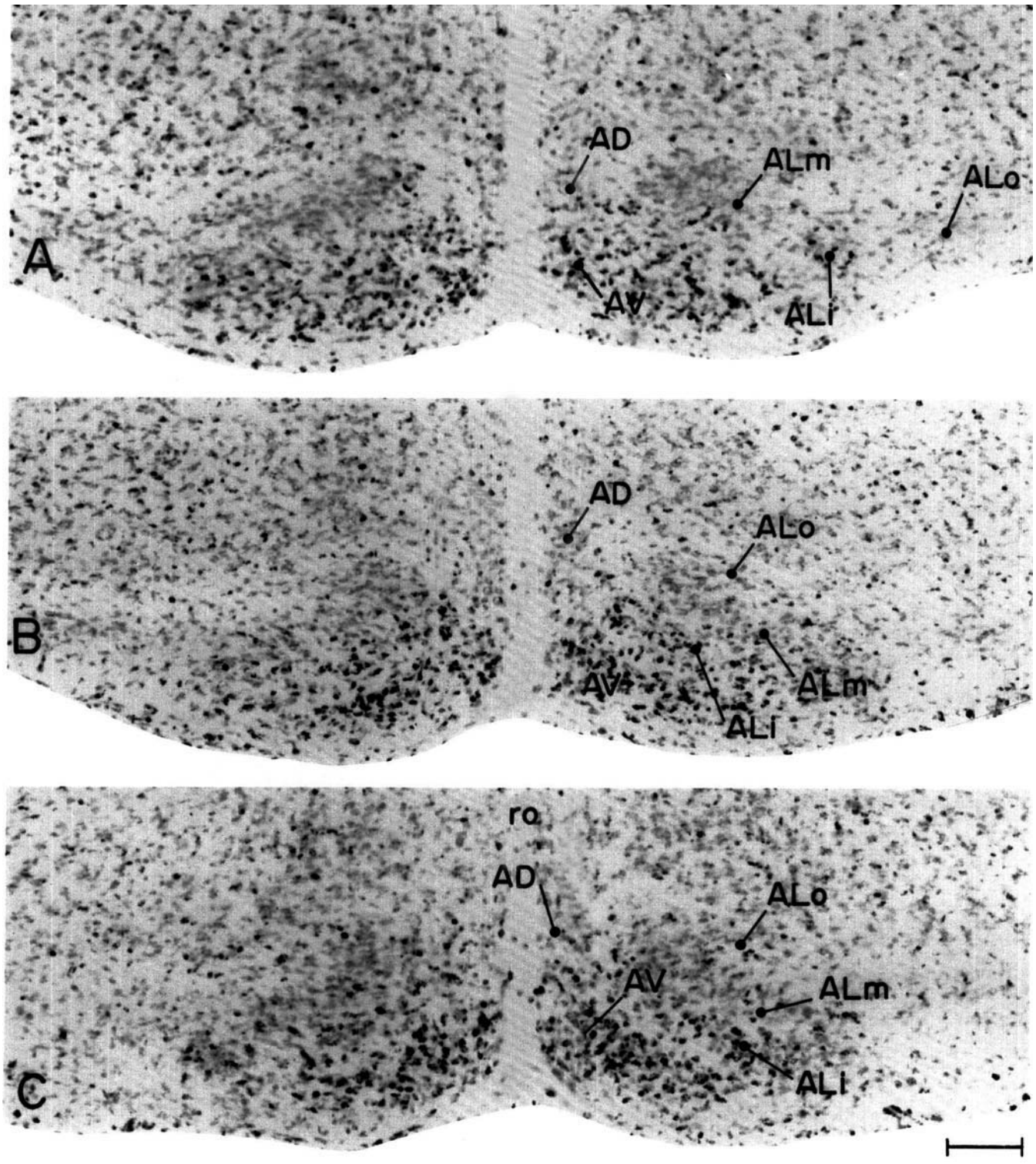

Fig. 18. Continuation of the series shown in Figure 17, from the midportion of the inferior olive (A) to its caudal extent (C). In addition to the anterodorsal olive (AD), the lamellae of the anterolateral (principal) olive become now distinguishable. Paraffin. Scale 100 μm.

1A). This region is viewed rather as part of the anterolateral (principal) olive, its outer lamella (ALo in Figs. 1B, 8). This outer lamella is structurally contiguous with the middle lamella of the principal olive (ALm) and, like the latter, is composed of early-generated neurons. Second, the extensive medial accessory olive of Gwyn et al. ('77) (MA in Fig. 1A) is divided into three neurogenetically different components: the earliest generated posterodorsal division (PD), the later generated posteroventral division (PV) (with possibly two parts, a medial and a lateral; PVm, PV1), and the latest generated anteroventral division (AV).

There is no evidence that neurons destined to form the different divisions of the inferior olive travel by different routes, as it was suggested by Harkmark ('54). We were able to follow the circumferential route of olivary neurons, all of which first gather in the caudal half of the future olive and then disperse rostrally. On day E17 (Fig. 13) only two zones could be distinguished with certainty, the settled, early generated neurons dorsomedially and the settling, late generated neurons ventrolaterally. By the next day (Fig. 15) at least four of the components of the inferior olive could be clearly discerned by their labeling pattern and regional segregation. The pattern identified on day E18 allowed us to infer the onset of olivary organization on the previous day, as shown in Figure 14. By day E19 (Figs. 17,18) the structural organization of the olive resembles in essentials the adult pattern.

The division of the inferior olivary complex proposed by us does not have the merit of being simpler than the currently accepted classification. Nor is it self-evident that a morphological classification based on a neurogenetic principle is necessarily superior to another based on a different (avowedly comparative) criterion. The relative merits of anatomic classifications can only be judged by their usefulness in research and promotion of understanding. The appropriate subdivision of the inferior olive is of great importance in relation to ongoing efforts by neuroanatomists to unravel its pattern of topographic projection to the cerebellum (reviewed by Brodal and Kawamura, '80; Gould, '80) and the elusive logic of this pattern. Because of the great variability in the morphological subdivisions of both the cerebellum and the inferior olive in different species, and because the neurogenetic approach adopted here is currently applicable only to the rat (Ellenberger et al., '69; Marchand and Poirier, '82), we shall limit this discussion to olivocerebellar relations in the rat.

The organization of olivocerebellar projection in the rat has recently been studied by several investigators (Brown, '80; Eisenman, '81; Furber and Watson, '83; Campbell and Armstrong, '83; Sotelo et al., '84; Wharton and Payne, '85) with axoplasmic tracer techniques. All reports agree that the pattern is a topographical one. But the detailed results are difficult to compare with one another mainly because the injection sites and diffusion of the tracers vary from study to study. But some agreement can be discerned and these lend some support for the classification we are proposing. Brown ('80), who used the HRP technique, summarizes her results to the effect that the "vermis has been shown to receive projections basically from the accessory nuclei, while the lateral hemisphere receives projections only from the principal olive" (Brown, '80, p. 273). However, in one of her case reports in which the injection is limited to the medial part of crus I and crus II in the intermediate region of the cerebellar cortex, she illustrates labeled cells distributed through all three lamellae of the anterolateral division, including the outer lamella, which she describes as the dorsal accessory olive (Brown, '80, Fig. 1B,C). The same pattern of labeling of all three lamellae is reported by Furber and Watson ('83, Fig. 2) following an injection far-laterally in crus II. HRP labeling of the intermediate hemisphere, again including crus II, is similarly illustrated by Sotelo et al. ('84, Figs. 27, 28) to backfill cells in all three lamellae. The work of Campbell and Armstrong ('83), using leucine autoradiography, suggests that the outer lamella of the anterolateral division sends fibers to the far-lateral cerebellar cortex and the middle and inner lamellae to the intermediate cortex (see their summary Fig. 9). The conclusion of Brown ('80) that the vermis receives projections mainly from the accessory nuclei is modified by Sotelo and his associates, who write that the "caudal half of the medial accessory olive projects mainly to the vermis of the posterior lobe, whereas its rostral half projects to the flocculus, paraflocculus and the intermediate cortex" (Sotelo et al., '84, p. 177). Significantly the caudal and rostral halves of the traditional medial accessory olive (MA in Fig. 1B) with dissimilar projections are also dissimilar neurogenetically. We have designated the earlier-generated region as the posteroventral olive (PVm and PV1) and the latest-generated region as the anteroventral division of the olive (AV).

Evidently, much more research is needed to establish the exact pattern of olivary projection to the cerebellum in the rat, and the utility of our neurogenetic classification will have to be directly assessed. By combining thymidine radiography with HRP (the one labeling the nucleus and marking its time of origin, and other labeling the cytoplasm and revealing its projection), it should be possible to determine quantitatively whether olivary neurons generated at different times and settling in different regions establish connections with different areas of the cerebellar cortex.

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