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Development of the Rat Thalamus: IV. The Intermediate Lobule of the Thalamic Neuroepithelium, and the Time and Site of Origin and Settling Pattern of Neurons of the Ventral Nuclear Complex

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ABSTRACT

Short-survival, sequential, and long-survival thymidine radiograms of rat embryos, fetuses, and young pups were analyzed in order to examine the time of origin, settling pattern, migratory route, and site of origin of neurons of the ventral nuclear complex of the thalamus. Quantitative examination of long-survival radiograms established that the bulk of the neurons of the ventral nuclear complex are generated between days E14 and E16 but with statistically significant differences between its three nuclei. The ventrobasal nucleus is the oldest component (97% of the cells are generated on days E14 and E15); the ventrolateral nucleus is next (82% of the cells are generated on days E14 and E15); and the ventromedial nucleus is last (51% of the cells are generated on days E14 and E15). In addition to this caudal-to-rostral (from the ventrobasal nucleus to the ventrolateral nucleus) and lateral-to-medial (from the ventrobasal nucleus to the ventromedial nucleus) *inter*nuclear gradients, there are lateral-to-medial and ventral-to-dorsal *intra*nuclear neurogenetic gradients within the ventrobasal and ventrolateral nuclei.

Qualitative examination of short and sequential survival thymidine radiograms indicate that the neurons of the ventral nuclear complex originate in the unique intermediate thalamic neuroepithelial lobule, which is distinguished from the rest of the thalamic neuroepithelium by the presence of a mitotically active secondary neuroepithelial matrix. Two sublobules can be distinguished in the intermediate lobule during the early stages of thalamic development. On the basis of their location and chronological pattern of cell production and differentiation, it is inferred that the neurons of the ventrobasal nucleus originate in the earlier differentiating, posteroventrally situated *inverted* sublobule, and the neurons of the ventrolateral nucleus are produced in the later differentiating, anterodorsally situated *everted* sublobule. The neurons of the ventromedial nucleus appear to originate from the intermediate neuroepithelial lobule after its two sublobules are no longer distinguishable.

The heavily labeled neurons generated soon after injection on day E15 form a wave front that translocates in a lateral direction at a steady rate of 215 μm/day. Examination of methacrylate-embedded materials showed that, in day E15 rats the actively migrating cells are spindle-shaped, with their long

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axis oriented horizontally. The far-laterally situated differentiating cells (the oldest neurons) become vertically oriented by day E16. Associated with this change in polarity, vertically oriented fibers appear among the cells. These fibers can be traced to the internal capsule, which, on this day, remains within the confines of the diencephalon. By day E17 the vertically oriented cells greatly increase in number. Concurrently, the bulk of the internal capsule increases and the growing fibers penetrate the telencephalon from its basal aspect. The passive translocation of the cells of the ventral nuclear complex continues in a lateral direction for several days.

Key words: neuroembryology, ventrobasal nucleus, ventrolateral nucleus, ventromedial nucleus, thymidine autoradiography

This work is a continuation of a series of studies in which we examine the hypothesis that the morphologically distinguishable sublobules (eversions and inversions) of the thalamic neuroepithelium of the embryonic rat are a source of neurons of different thalamic nuclei. In the first study (Altman and Bayer, '88a), we described the progressive fractionation of the thalamic neuroepithelium during the early embryonic development of the rat forebrain. On day E13 the thalamic neuroepithelium is divisible into two parts, what we have called the rostral and caudal thalamic *lobes*. By day E14 both the rostral and caudal lobes have become partitioned into smaller units referred to as neuroepithelial *lobules*. By day E15 the neuroepithelial lobules have become further fractionated into still smaller units, the neuroepithelial *sublobules*. The sublobules derived from the rostral lobe, and their fate, were described in two previous articles (Altman and Bayer, '88b,c). The partitioning caudal lobe produces the intermediate and posterior thalamic lobules. The derivatives of the posterior lobule are the subject of the next two studies in this series (Altman and Bayer, '89b,c). The present work is concerned with the derivatives of the intermediate thalamic lobule. In the rat, this lobule is the largest single component of the thalamic neuroepithelium, and by day E14 it can be distinguished from all the other components of the thalamic neuroepithelium by a secondary germinal matrix, the subependymal layer. We have previously proposed that the intermediate lobule is the germinal source of the thalamic ventral nuclear complex (Altman and Bayer, '88a), and here we seek to provide support for that hypothesis.

The ventral nuclear complex serves as the principal thalamic relay station in the somesthetic and somatomotor pathways to the cerebral cortex. On the basis of its connectivities and functions (for recent reviews, see Faull and Mehler, '85; Jones, '85), three components are distinguished within this complex, the ventrolateral (VL), ventrobasal or ventral posterior (VB), and ventromedial (VM) nuclei. The VL reaches farthest anteriorly and does not extend far beyond the midportion of the ventral nuclear complex posteriorly. It is the major target of afferents from the cerebellum. The VB, which is the principal thalamic relay station of the somesthetic pathway to the cerebral cortex, occupies over one-half of the posterior part of the ventral nuclear complex in the rat. The fibers of the trigeminal lemniscus, which relay somatosensory information from the head, face, and mouth region, terminate in the medial subnucleus of the VB, and the medial lemniscal fibers, which relay information from the trunk, limbs, and tail, terminate in the lateral subnucleus. The VM is the smallest of the three nuclei of this complex and it has the most heterogeneous connections, including afferents from the substantia nigra and gustatory afferents.

Neurogenesis in the ventral nuclear complex has been investigated in the mouse by Angevine ('70) who observed a caudal-to-rostral internuclear gradient and also a lateral-to-medial gradient. In the rat, McAllister and Das ('77), using the flash labeling procedure, reported that the majority of neurons in the posterior VB is generated on days E15, but rostrally neurogenesis extends to day E16, confirming the caudal-to-rostral internuclear gradient. In our study with the cumulative labeling procedure (see Fig. 10C in Altman and Bayer, '78), we found that the neurons of the ventral nuclear complex are generated on day E14 and E15, with a peak on the latter day.

MATERIALS AND METHODS

The materials examined in this study were identical with those described in detail in the first article of this series (Altman and Bayer, '88a). We made particular use of four collections. (1) Short-survival radiograms were used to locate the site of origin of neurons of the ventral nuclear complex. This series consists of 94 paraffin- or methacrylate-embedded embryos whose mothers were injected with a single dose of ^{3}H-thymidine on successive days, extending from day E12 to E21, and were killed 2 hours after injection. The number of specimens examined in the relevant injection groups ranged from 6 to 12. (2) Sequential-survival radiograms were examined to trace the migratory paths of neurons. This series consists of 254 paraffin- or methacrylate-embedded embryos or fetuses whose mothers were injected between days E12 and E21. Within each injection group, the embryos or fetuses were killed at daily intervals after the injection either up to day E22 or, in the case of late fetal injections, up to postnatal day 5 (P5). A minimum of 6 specimens were examined in every relevant survival group. (3) The long-survival series consists of 44 paraffin-embedded brains of P5 rats whose mothers were injected with two successive daily doses of ^{3}H-thymidine, with a single-day delay between the groups, on gestational days E13+E14, E14+E15, E15+E16, E16+E17, E17+E18, and E18+E19. The data from 6–8 pups in every injection group were used for the quantification of the birth dates of neurons in subdivisions of the ventral nuclear complex, with special reference to internuclear and intranuclear neurogenetic gradients. (4) Selected specimens from a collection of serially sectioned, methacrylate embedded embryonic brains were

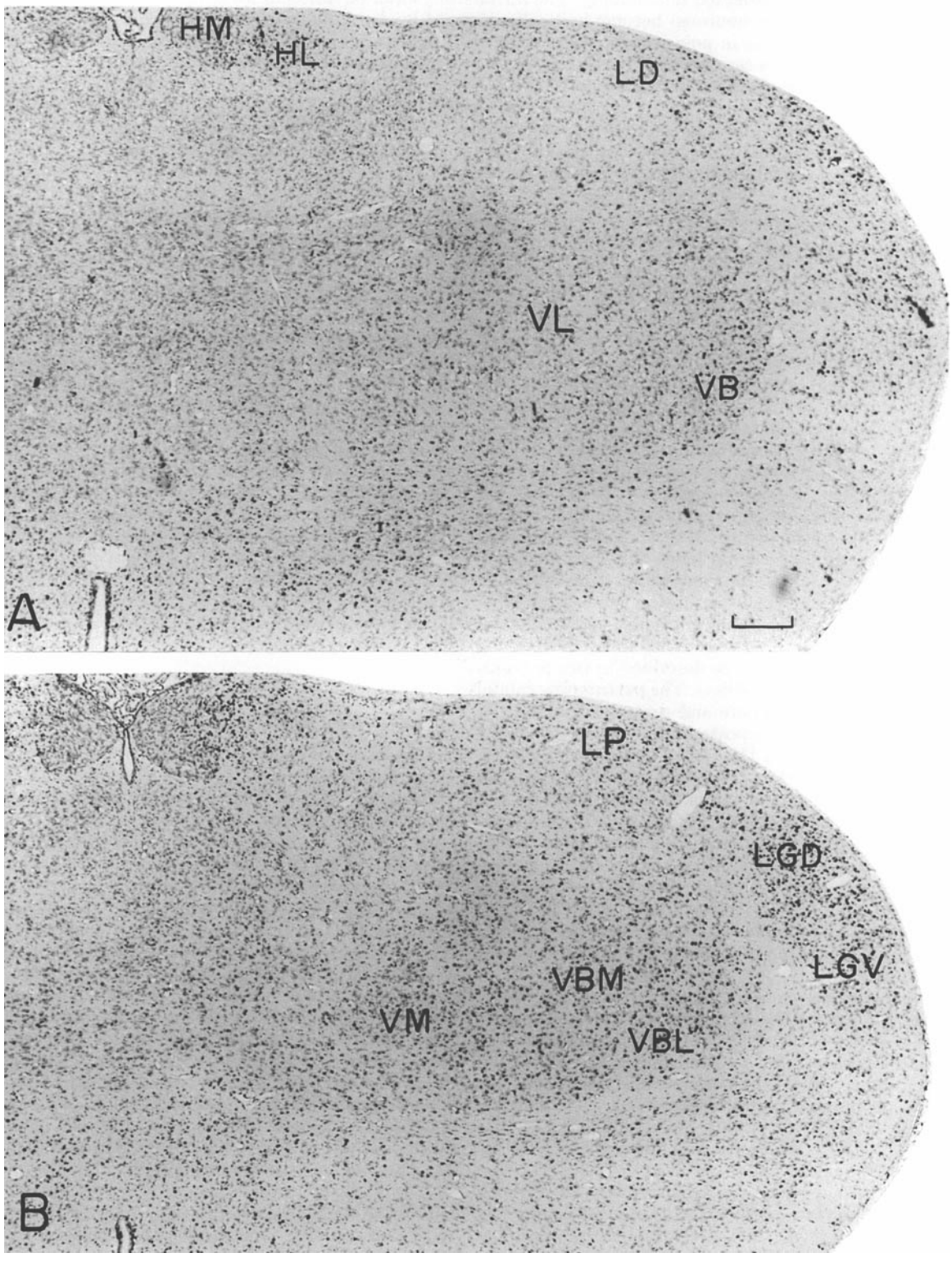

Fig. 1. Coronal thymidine radiograms, at midrostral (**A**) and midcaudal (**B**) levels of the thalamus, from a P5 rat labeled on days E14+E15. Paraffin, hematoxylin and eosin (H&E). Scale: 200 μm.

qualitatively evaluated to assess changes in cell shape and orientation, and to assess the growth of fibers, on successive days of thalamic development.

For the identification of the three thalamic nuclei of the ventral nuclear complex, we relied primarily on Faull and Mehler's ('85) atlas of the rat thalamus. We also consulted other sources, e.g., Emmers ('65), Lund and Webster ('67), Faull and Carman ('78), Herkenham ('79), Haroian et al. ('81), and Jones ('85). There is some discrepancy in nomenclature (many prefer VP to VB and designate a part of VL as VA), but, more disturbingly, there is considerable discrepancy in the delineation of the VL. We have opted to follow the delineation of the VL in two experimental studies that have dealt with cerebellofugal projections in the rat, i.e., that of Faull and Carman ('78; see their Fig. 12) and Haroian et al. ('81; see their Figs. 16–18). The latter delineation appeared congruent with some distinguishing cytological features in our material. However, because we are not in a position to resolve the existing conflict in the literature, we provide outlines of the areas where the respective cell counts were made (see Figs. 5–7).

The proportion of labeled and unlabeled cells in the whole ventral nuclear complex was quantified at 6 equidistant coronal levels from rostral to caudal (L1–L6). The VB, which extends from level 2 to 6 (see Fig. 5B), was divided into a lateral part (VBL) and a medial part (VBM), and both the VBL and VBM were further divided into external (ex) and internal (in) halves. The VL, which extends from level 1 to 3 (see L1–L3 in Fig. 6B) was divided into four quadrants (Q1–Q2 ventrally and Q3–Q4 dorsally). The small ventromedial nucleus, which extends from level 1 to level 5, was not divided (see Fig. 7). In these areas cells were counted microscopically at ×312.5 in unit areas set off by an ocular grid (0.085 mm^2). All cells within a designated area were dichotomized as labeled or unlabeled. Cells with silver grains overlying the nucleus in densities above background level were considered labeled; obvious endothelial and glial cells were excluded. The proportion of labeled cells (% labeled cells/total cells) was then calculated from these data.

The determination of the proportion of cells arising (ceasing to divide) on a particular day utilized the progressively delayed comprehensive labeling procedure, described in detail elsewhere (Bayer and Altman, '87). Briefly, consequent to daily delays in the onset of injection of ^{3}H-thymidine, a progressive reduction in the proportion of labeled cells (from a maximal level (>95%) in a given population) is taken to indicate that the precursor cells have become nonmitotic neurons. By analyzing the daily rate of decline in labeled cells, we determine the proportion of neurons originating on a particular day during development. For the statistical evaluation of internuclear and intranuclear gradients, we have used the nonparametric sign test (Conover, '71). The sign test determines the consistency of sequential neuron production between paired locations within individual animals. This statistic is used because experience has shown that even though there is variability in neurogenesis between animals of nominally the same gestational ages, the chronological sequence of neurogenesis among different brain regions is very consistent within animals. The graphs throughout this series of studies show the more variable group data rather than the consistent trends within individual animals.

Abbreviations

Abbreviations in capital letters refer to mature brain structures; capital letters followed by m refer to the putative migratory streams of brain structures; letters in lower case refer to the putative cell lines of particular brain structures in the neuroepithelium.

AQ	aqueduct
at	anterior thalamic neuroepithelial lobule
atp	anterior transitional protuberance
c	caudal
ex	external half of ventrobasal nucleus
FM	foramen of Monro
hb	habenular neuroepithelium
HL	lateral habenular nucleus
HM	medial habenular nucleus
IC	inferior colliculus
in	internal half of ventrobasal nucleus
it	intermediate thalamic neuroepithelial lobule
its	intermediate thalamic subependymal layer
LD	lateral dorsal nucleus
lgd	dorsal lateral geniculate neuroepithelium
LGD	dorsal lateral geniculate nucleus
LGDm	dorsal lateral geniculate migratory stream
LGV	ventral lateral geniculate nucleus
LP	lateral posterior nucleus
mg	medial geniculate neuroepithelium
MG	medial geniculate nucleus
MI	massa intermedia
mz	migratory zone
ne	neuroepithelium
PC	posterior commissure
pin	pineal rudiment
pt	posterior thalamic neuroepithelial lobule
r	rostral
rp	reticular protuberance
rt	reticular thalamic neuroepithelial lobule
RT	reticular thalamus
RTm	reticular thalamic migratory stream
sl	sulcus limitans
STM	stria medullaris
sz	synthetic zone
TL	telencephalon
vb	ventrobasal neuroepithelium
VB	ventrobasal nucleus
VBL	lateral ventrobasal nucleus
VBm	ventrobasal migratory stream
VBM	medial ventrobasal nucleus
vl	ventrolateral neuroepithelium
VL	ventrolateral nucleus
VLm	ventrolateral migratory stream
vm	ventromedial neuroepithelium
VM	ventromedial nucleus
VMm	ventromedial migratory stream
v3d	third ventricle, dorsal (thalamic)

RESULTS

Time of origin of neurons of the ventral nuclear complex

Qualitative observations. The labeling pattern of components of the ventral nuclear complex is illustrated at midrostral and midcaudal levels in day P5 rats that were cumulatively labeled with ^{3}H-thymidine beginning on days E14, E15, or E16 (Figs. 1–3). In the rat labeled on days E14+E15, the cells of the ventrolateral nucleus (VL in Fig. 1A) and of the ventromedial nucleus (VM in Fig. 1B) tend to be lightly labeled, but the ventrobasal nucleus (VB in Fig. 1A) and its lateral and medial subdivisions (VBL and VBM in Fig. 1B) contain many heavily labeled cells. In the rat labeled on days E15+E16, many of the cells of both the VL (Fig. 2A) and of the VB (VB in Fig. 2A and VBM and VBL in Fig. 2B) are heavily labeled and so are also the cells of the VM (Fig. 2B). In the rat labeled on days E16+E17, neither

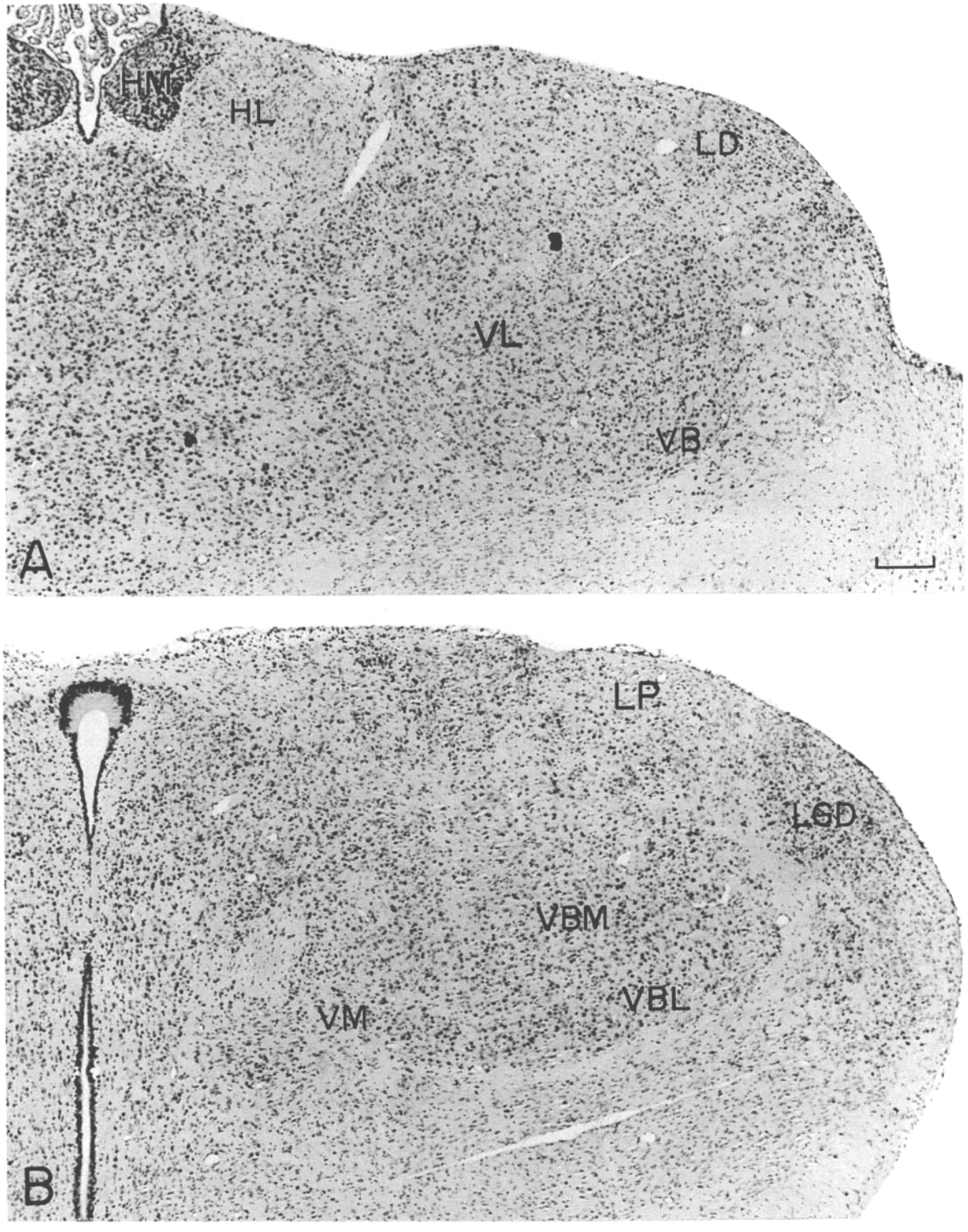

Fig. 2. Coronal thymidine radiograms, at midrostral (**A**) and midcaudal (**B**) levels of the thalamus, from a P5 rat labeled on days E15+E16. Paraffin, H&E. Scale: 200 μm.

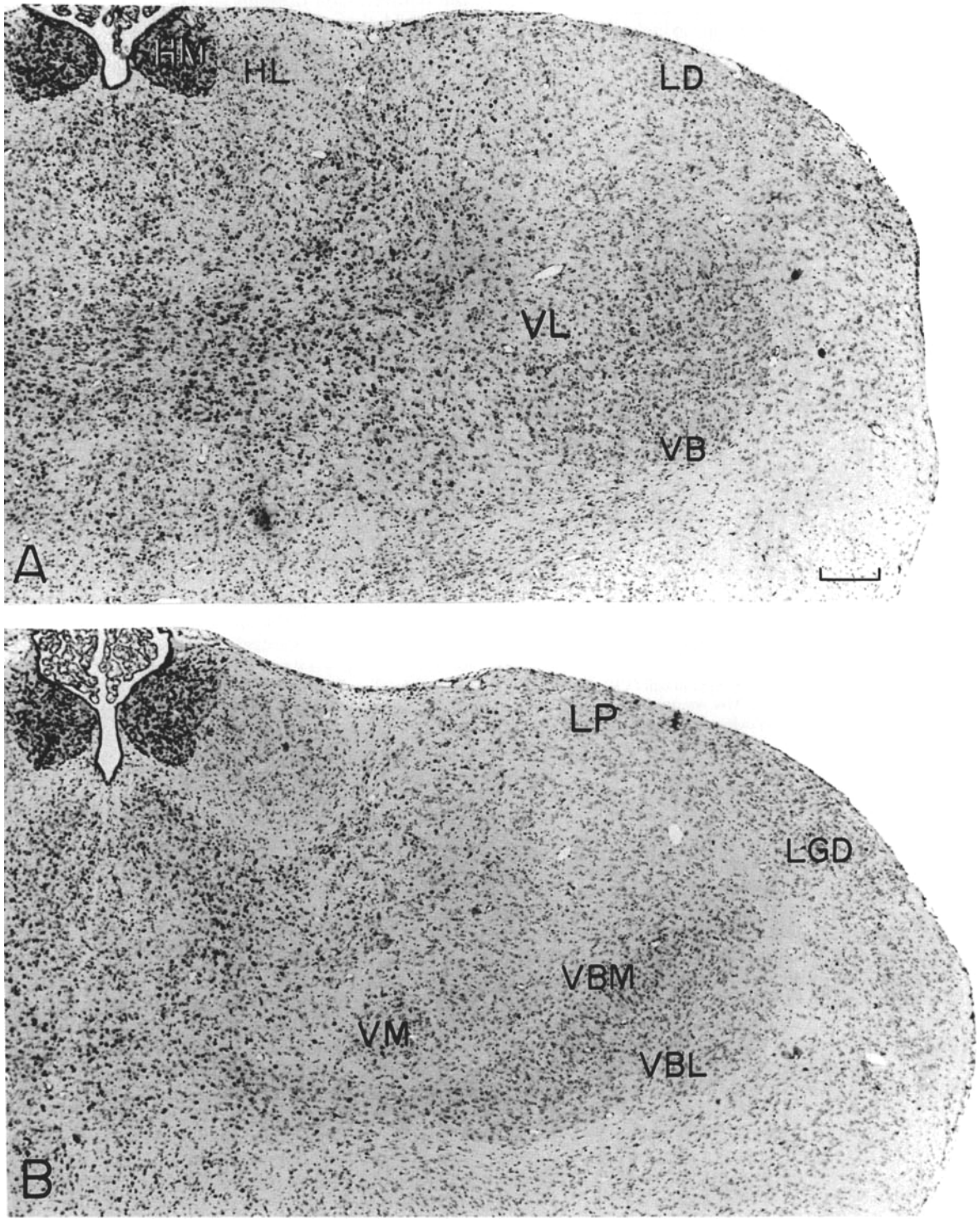

Fig. 3. Coronal radiograms, at midrostral (A) and midcaudal (B) levels of the thalamus, from a P5 rat labeled on days E16+E17. Paraffin, H&E. Scale: 200 μm.

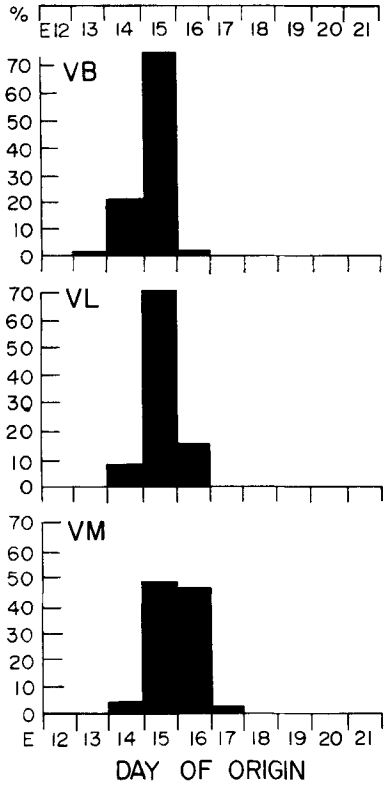

Fig. 4. Time of neuron origin in the ventrobasal (VB), ventrolateral (VL), and ventromedial (VM) nuclei. The results indicate a caudal-to-rostral and a lateral-to-medial internuclear neurogenetic gradient.

the cells of the VL (Fig. 3A) nor those of the VB (VB in Fig. 3A and VBM and VBL in Fig. 3B) are labeled any longer, but some heavily labeled cells are still present in the VM (Fig. 3B). These observations suggest that the bulk of the neurons of the ventral nuclear complex are generated between days E14 and E16 with a combined caudal-to-rostral and lateral-to-medial internuclear neurogenetic gradient (neurogenesis in the VB antedating somewhat that in the VL, and the VM lagging behind the other two).

Quantitative determination of internuclear gradients. To provide an overview of internuclear gradients within the ventral nuclear complex, the quantitative data were combined for each of the three nuclei (Fig. 4). In the VB, 22% of the cells originate on day E14 and 75% on day E15. In the VL, only 9% of the cells originate on day E14, 73% on day E15, and 17% on day E16. In the VM, 5% of the cells are generated on day E14, 47% on day E15, 46% on day E16, and 2% on day E17. The statistical evaluation of the results indicated a highly significant internuclear neurogenetic gradient between VB and VL ($p < 0.0001$), and lateral-to-medial gradients between VL and VM ($p < 0.0001$) and VB and VM ($p < 0.001$).

Intranuclear gradients in the ventrobasal nucleus. There were no statistically significant neurogenetic differences between the four quadrants of VB along the rostrocaudal plane (L2 to L6 in Fig. 5B); the data were therefore combined (Fig. 5A). The combined data showed that the neurogenetic differences were significant *between* the external halves of the earlier generated VBL and later generated VBM (right column in Fig. 5A; $p < 0.0001$) and *between* the internal halves of these subnuclei (left column in Fig. 5A; $p < 0.001$). *Within* VBL (two lower graphs in Fig. 5A), the neurons of the external half (ex) of the subnucleus are generated somewhat ahead of the neurons of the internal half (in); this difference is statistically significant ($p < 0.003$). *Within* VBM (two upper graphs in Fig. 5A), a similar outside-in gradient is indicated, but this difference was not significant. These results indicate that there is both a ventral-to-dorsal and lateral-to-medial intranuclear neurogenetic gradient within the VB.

Intranuclear gradients in the ventrolateral nucleus. There were no significant differences in the birth dates of neurons along the rostrocaudal axis (L1 to L3 in Fig. 6B) in the four quadrants of the VL; the data were therefore combined (Fig. 6A). The neurogenetic pattern within the VL was similar to that obtained in the VB. There are lateral-to-medial gradients between Q1 and Q2 ($p < 0.002$) and Q3 and Q4 ($p < 0.0001$), and ventral-to-dorsal gradients between Q1 and Q3 ($p < 0.002$) and Q2 and Q4 ($p < 0.015$). The overall trend is for the oldest neurons to be located in Q1, where 13% of the neurons are generated on day E14 and 80% on day E15. The youngest neurons are located in Q4, where 61% of the cells are generated on day E15 and 32% on day E16.

Intranuclear gradients in the ventromedial nucleus. Because of the small size of the VM, possible lateral-to-medial and ventral-to-dorsal gradients were not examined. There is a pronounced caudal-to-rostral gradient in the VM (Fig. 7A); caudally (L5), over 82% of the neurons are generated on day E15; at intermediate levels (L2–4), 50% of the neurons are generated on day E16; and rostrally (L1), the peak production of neurons (over 75%) has shifted to day E16. All the differences were statistically significant ($p < 0.0001$). These results suggest that the VM, which is unique in having a pronounced caudal-to-rostral neurogenetic gradient, originates from a different neuroepithelial source than the VB and VL (which do not have such a gradient).

Site of origin of neurons of the ventral nuclear complex

The intermediate neuroepithelial lobule on day E14. In the first of this series we identified a unique feature of the intermediate thalamic neuroepithelial lobule, the presence of a secondary proliferative zone some distance from the lumen of the ventricle (see Figs. 5, 6 in Altman and Bayer, '88a). The intermediate lobule, representing a relatively large ventricular eversion on day E14, is shown in relation to the rest of the thalamic neuroepithelium in a low-power thymidine radiogram from a rat labeled on the morning of day E14 and killed 2 hours later (Fig. 8A). Here, but not elsewhere in the thalamic neuroepithelium (as seen at higher magnification in Fig. 8B), there are labeled cells not only in the synthetic and mitotic zones of the neuroepithelium (sz and mz) but also in the subependymal layer (its). But is the region designated as the subependymal layer truly a germinal zone? We had to entertain the possibility that the labeled cells are postmitotic migratory elements that have left the neuroepithelium during the 2-hour period that has elapsed between the administration of ^{3}H-thymidine and the death of the embryos. To answer this question we have examined the distribution of mitotic cells in the

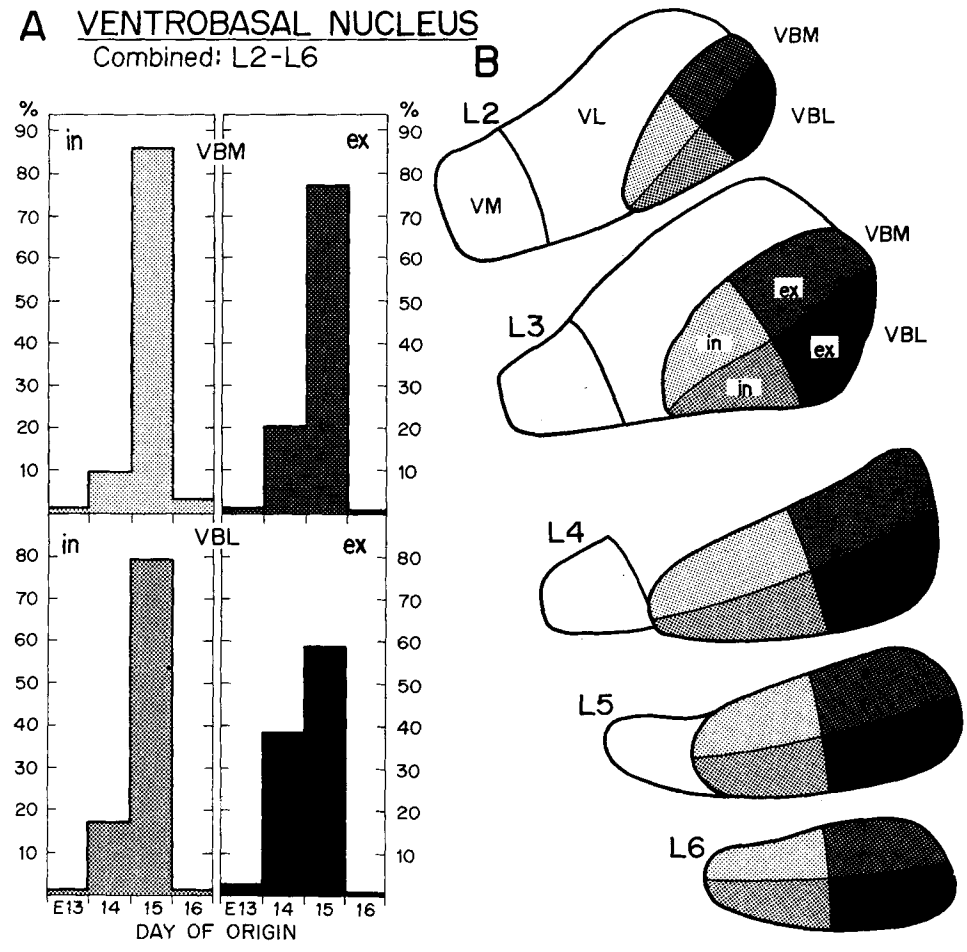

Fig. 5. Time of neuron origin in the internal and external halves (in, ex) of the medial (arcuate) subnucleus of the VB (VBM) and in the internal and external halves of the lateral subnucleus of the VB (VBL). There are two gradients present: from the VBL to the VBM (essentially ventral-to-dorsal) and an outside-in gradient within each subnucleus.

intermediate lobule in nonradiographic methacrylate sections of rat embryos from day E14 onward.

Observations made in this material (Fig. 9) showed that in contrast to other regions of the thalamic neuroepithelium, in which mitotic cells are limited to the ventricular boundary, the intermediate thalamic lobule contains a fair number of mitotic cells on day E14 far from the lumen (vertical arrows in Fig. 9B). Importantly, mitotic cells at this age are rare in the subependymal layer itself (its) but are numerous in a region that corresponds to the core of the synthetic zone of the neuroepithelium (compare Figs. 8B, 9B). Beginning on day E15 (as described below; see Figs. 12, 13), mitotic cells occur in abundance in the subependymal layer, establishing that this zone is truly a secondary proliferative matrix.

The intermediate neuroepithelial lobule on day E15. The intermediate neuroepithelial lobule is illustrated in serial coronal thymidine radiograms, from rostral to caudal, from rats labeled on day E15 and killed 2 hours later (Figs. 10, 11). By this age, the intermediate lobule, as demarcated by the presence of a subependymal layer (its), has become partitioned into two sublobules, a dorsal eversion (empty circle) and a ventral inversion (solid circle). The dorsal everted sublobule, which we have described previously as the putative VL neuroepithelium (Altman and Bayer, '88a), begins rostrally (vl in Fig. 10A) above the reticular protuberance (rp) and can be traced some distance caudally (vl in Figs. 10B–F, 11A–D). The ventral everted sublobule, the putative VB neuroepithelium, emerges farther caudally above the reticular protuberance (vb in Figs. 10D–F). Still farther caudally, where the putative VL neuroepithelium is no longer recognizable, the putative VB neuroepithelium assumes a flattened shape (Figs. 11E,F).

The inference that the more anterodorsally situated neuroepithelial eversion is the putative source of neurons of the VL, and the more posteroventrally situated neuroepithelial inversion is the source of neurons of the VB, is based on the following considerations: (1) the location of the two structures (one more anterodorsal, the other more posteroventral), (2) their different neurogenetic patterns, and (3) the width of the unlabeled differentiating zone surrounding the two neuroepithelial sublobules in short-survival radiograms of day E15 rats. First, as we illustrated in P5 rats, the dorsally situated VL begins at level 1 anteriorly and extends

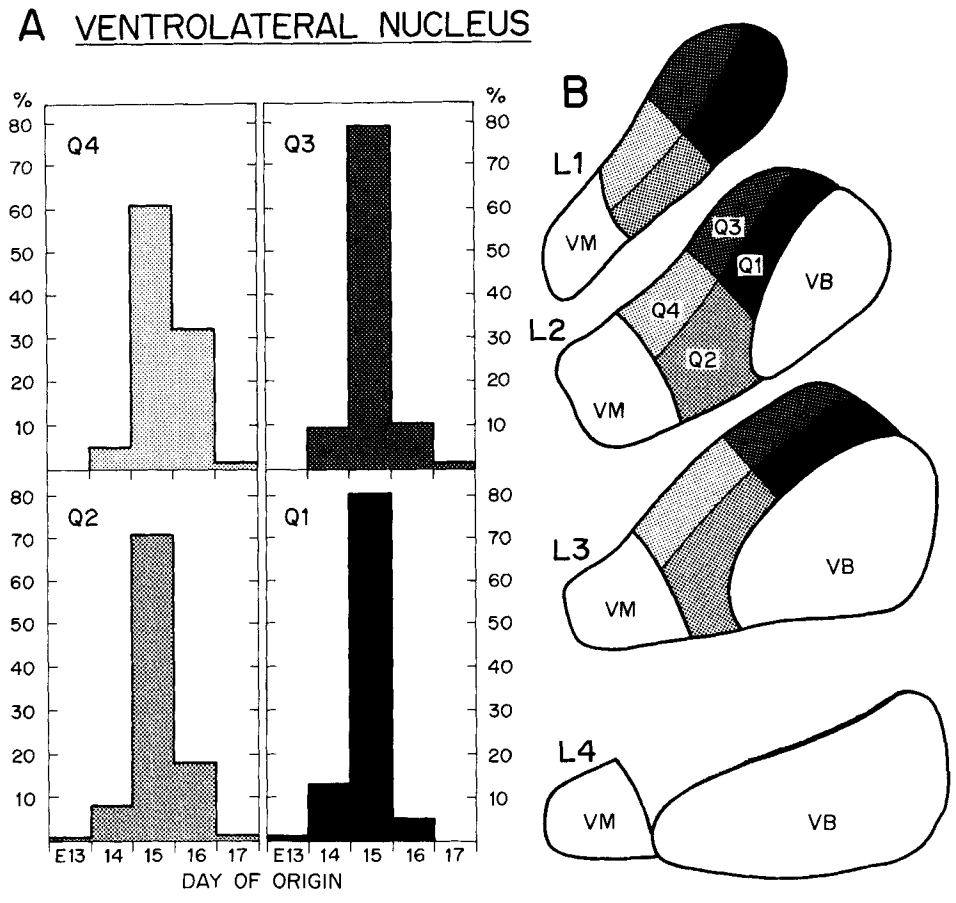

Fig. 6. Time of origin of neurons in the four quadrants (Q1–Q4) of the ventrolateral nucleus. There is a lateral-to-medial and a ventral-to-dorsal neurogenetic gradient in this nucleus.

only to level 3 (Fig. 6B), whereas the ventrally situated VB (Fig. 5B) begins at level 2 and extends as far posteriorly as level 6. Thus in relation to one another the VL is situated anterodorsally and the VB posteroventrally. Second, in the VL less than 10% of the neurons are generated on day E14, whereas in the VB over 20% of the neurons are generated on this day (Fig. 4). Third, paralleling these distinguishing features, the unlabeled differentiating zone (composed of cells generated before day E15) is very small in E15 rats at anterior levels, where only a dorsal eversion is present above the reticular protuberance (vl in Figs. 10A–C); this matches the paucity of neurons generated on day E14 in the anterodorsally situated VL (Fig. 4). In contrast, the unlabeled differentiating zone is much larger caudally, where the ventral inversion becomes the predominant component of the intermediate lobule (vb in Figs. 11D–F); this matches the higher proportion of neurons generated in the posteroventrally situated VB on day E14 (Fig. 4).

The cytological organization of the intermediate neuroepithelial lobule on day E15 is illustrated in coronal sections in Figures 12 and 13. A distinguishing feature of this lobule, as we noted earlier, is the presence of mitotic cells not only along the lumen of the ventricle but in two additional locations, the interior of the neuroepithelium (ne and downward arrows in Fig. 12) and in the subependymal layer (its and upward arrows). Mitotic cells are rare or absent in the latter two locations in other components of the thalamic neuroepithelium. At the less-developed anterior and midcoronal levels (Fig. 13A) of the intermediate lobule, the densely packed cells of the neuroepithelium and the loosely packed cells of the subependymal layer tend to be spindle-shaped, with their long axis oriented in the mediolateral plane. The same was observed at these levels in horizontal sections (not shown). However, at more developed posterior levels (Fig. 13B), there is an additional layer laterally. Here the long axis of the cells is less regularly aligned and at least some of them, particularly those situated far-laterally, have become oriented in the dorsoventral plane. This layer is considered to be composed of the earliest migrating and settling neurons of the ventrobasal nucleus (VBm and VB in Fig. 13B).

The intermediate neuroepithelial lobule on day E16. The number of labeled cells remains high in the intermediate neuroepithelial lobule on day E16, but there is an evident reduction in the thickness of the labeled region from rostral (Fig. 14A) to caudal (Fig. 14C). Over its entire extent, the intermediate lobule can be distinguished from the rest of the thalamic neuroepithelium by the presence of a subependymal layer (its in Fig. 14). However, the distinction between its two components, the dorsal eversion and the ventral inversion, is barely visible rostrally (Fig. 14A) and is no longer evident caudally (Fig. 14B,C). Because in the latest generated component of VL (Q4 in Fig. 6A), 32%

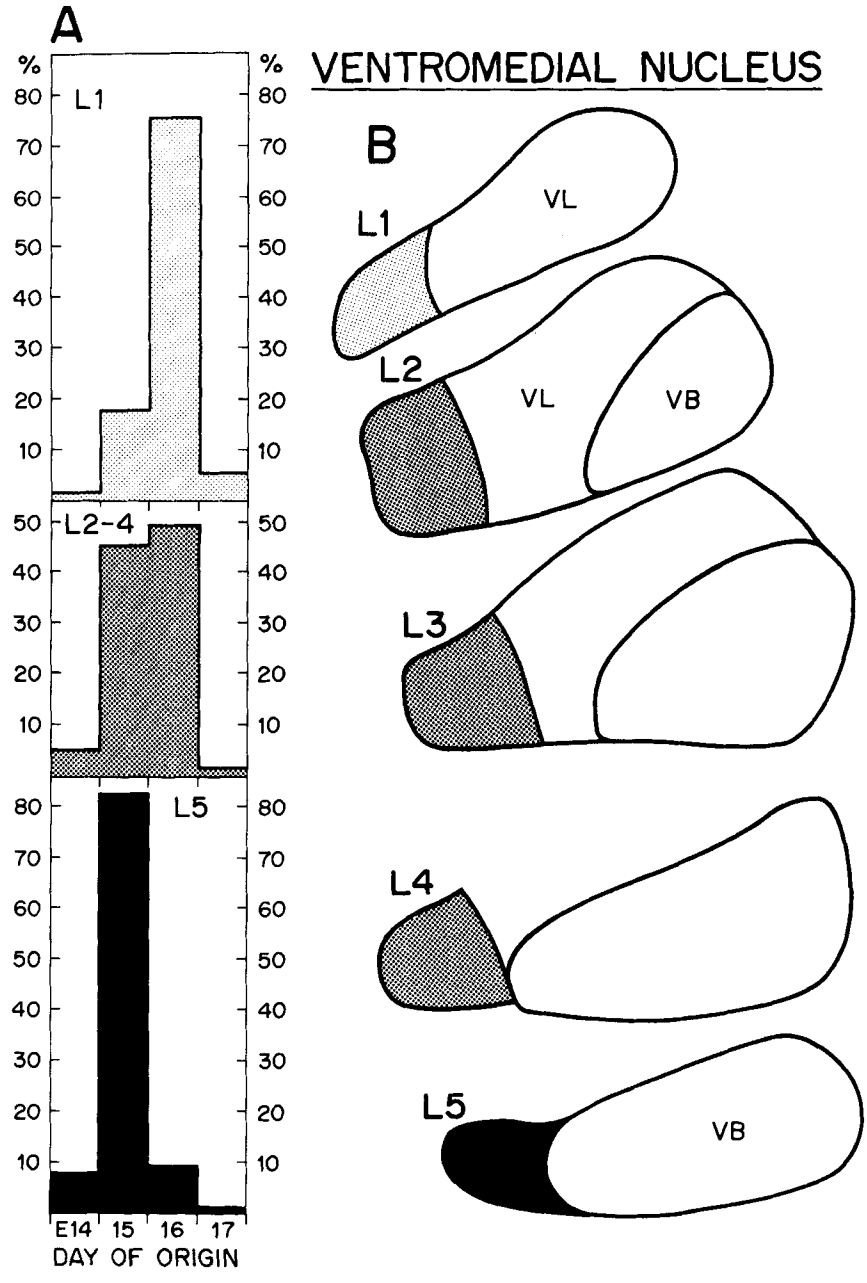

Fig. 7. Time of origin of neurons in the ventromedial nucleus. There is a caudal-to-rostral (from L5 to L1) neurogenetic gradient in this nucleus.

of the cells originate on day E16, we retain the designation for the dorsal eversion as the putative neuroepithelium of the VL (vl in Fig. 14A). In contrast, neurogenesis has virtually ended by day E16 in the VB (Fig. 5). Therefore, the mitotically active ventral inversion of the intermediate lobule, and its continuation caudally, can no longer be considered a source of VB neurons. Instead, it is designated as the putative neuroepithelium of the VM (vm in Fig. 14A–C), a structure which, at rostral levels, receives about 75% of its neurons on day E16 (L1 in Fig. 7A).

The intermediate thalamic neuroepithelial lobule on day E17. The intermediate thalamic neuroepithelium remains mitotically active on day E17 rostrally (Fig. 15A,B), but few labeled cells are present caudally (Fig. 15C). Insofar as only a negligible proportion of cells of the VL is produced on day E17 (Fig. 6), we assume that the still abundant proliferative cells rostrally produce neurons for medially situated, late-generated thalamic structures (work in progress). The VM does receive a small complement of neurons generated on day E17 (Fig. 7); therefore, it is possible that some of the labeled cells ventrally represent precursors of these late elements (vm? in Figs. 15A,B).

There is a drastic reduction in the width of the neuroepithelium of the intermediate thalamic lobule, as seen in non-

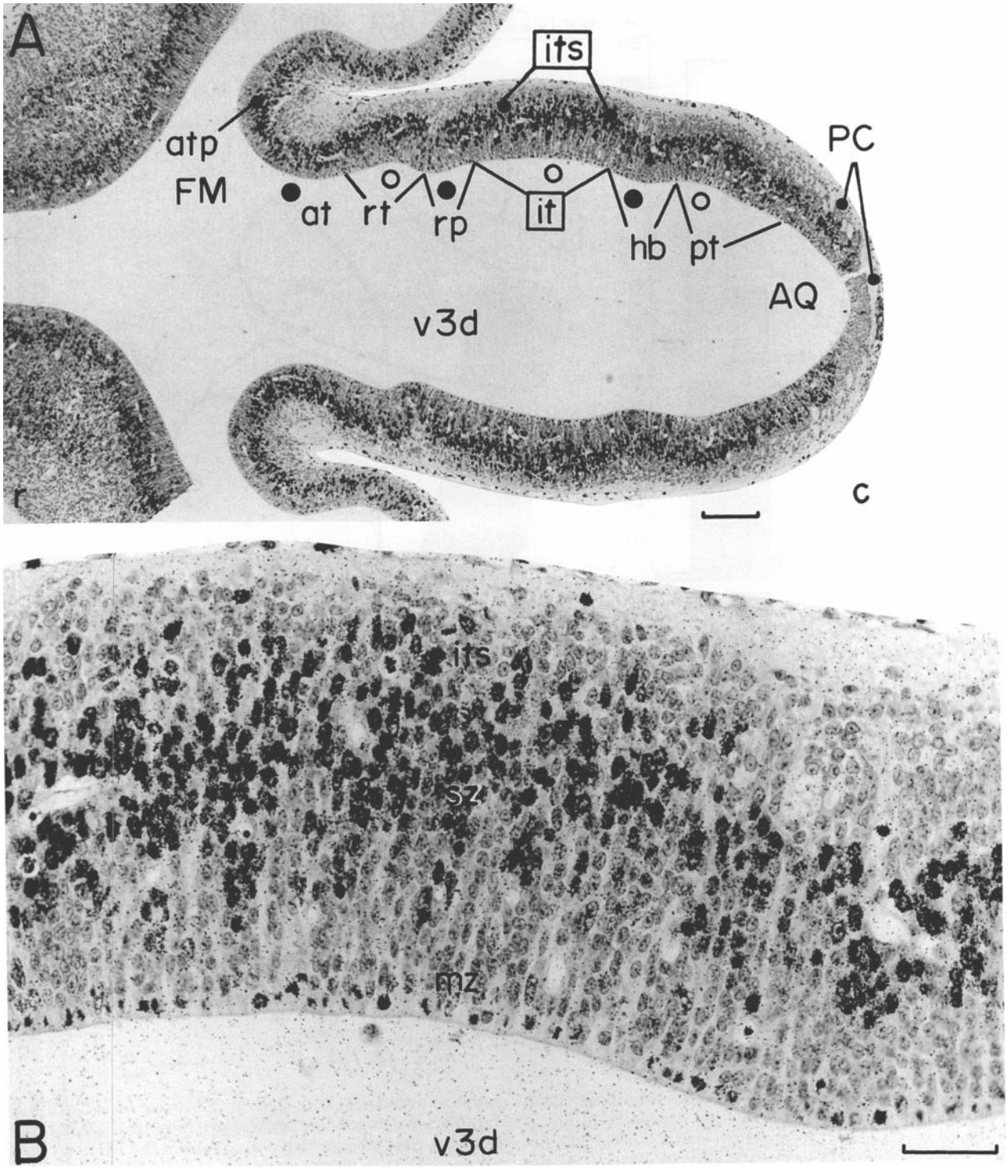

Fig. 8. **A.** Low-power horizontal radiogram of the thalamic neuroepithelium from a rat labeled on day E14 and killed 2 hours later. **B.** The region of the intermediate thalamic lobule (it) at higher magnification. Methacrylate, cresyl violet (CV). In this and all the subsequent figures, open circles indicate neuroepithelial eversions (concavities) and solid circles indicate inversions (convexities). Scales: A, 200 μm; B, 50 μm.

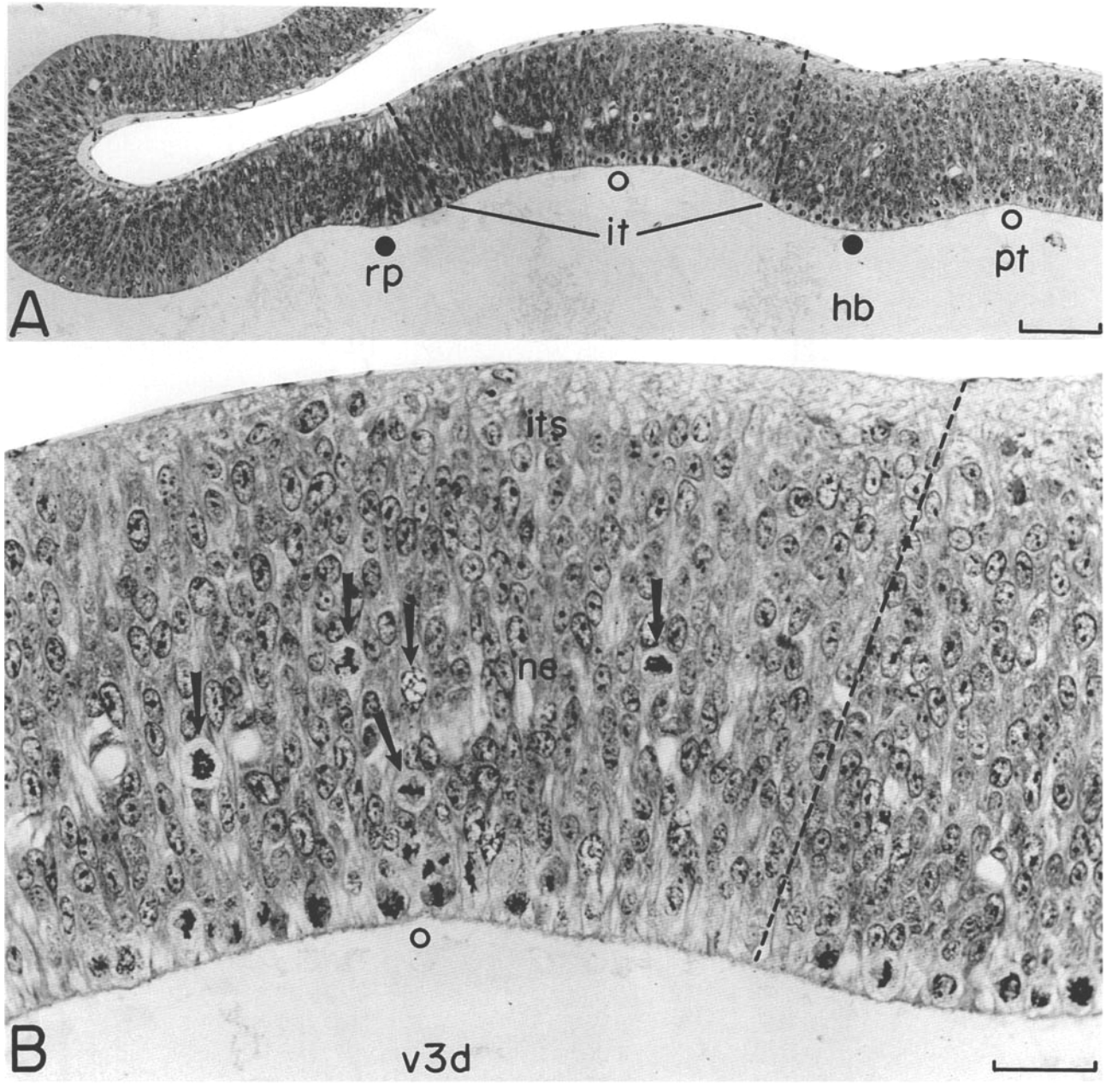

Fig. 9. A horizontal methacrylate section, at low (**A**) and high (**B**) magnifications, to show the abundance of mitotic cells (arrows) in the intermediate thalamic lobule some distance from the lumen. CV. Scales: A, 100 μm; B, 30 μm.

autoradiographic methacrylate-embedded sections, from day E16 (ne in Fig. 16A) to day E17 (ne in Fig. 16B). A fair number of mitotic cells are seen on day E16 both in the neuroepithelium (horizontal arrows in Fig. 16A) and the subependymal layer (vertical arrows). By day E17 the neuroepithelium contains few mitotic cells (none are seen in Fig. 16B), but many are still present in the subependymal layer (vertical arrows).

Migration of neurons of the ventral nuclear complex

The speed of migration. The migration of neurons from the germinal matrix of the intermediate neuroepithelial lobule is illustrated in matched sequential radiograms from rats labeled with ^{3}H-thymidine on day E15 and killed on days E16 (Fig. 17B), E17 (Fig. 17C), E18 (Fig. 18A), and

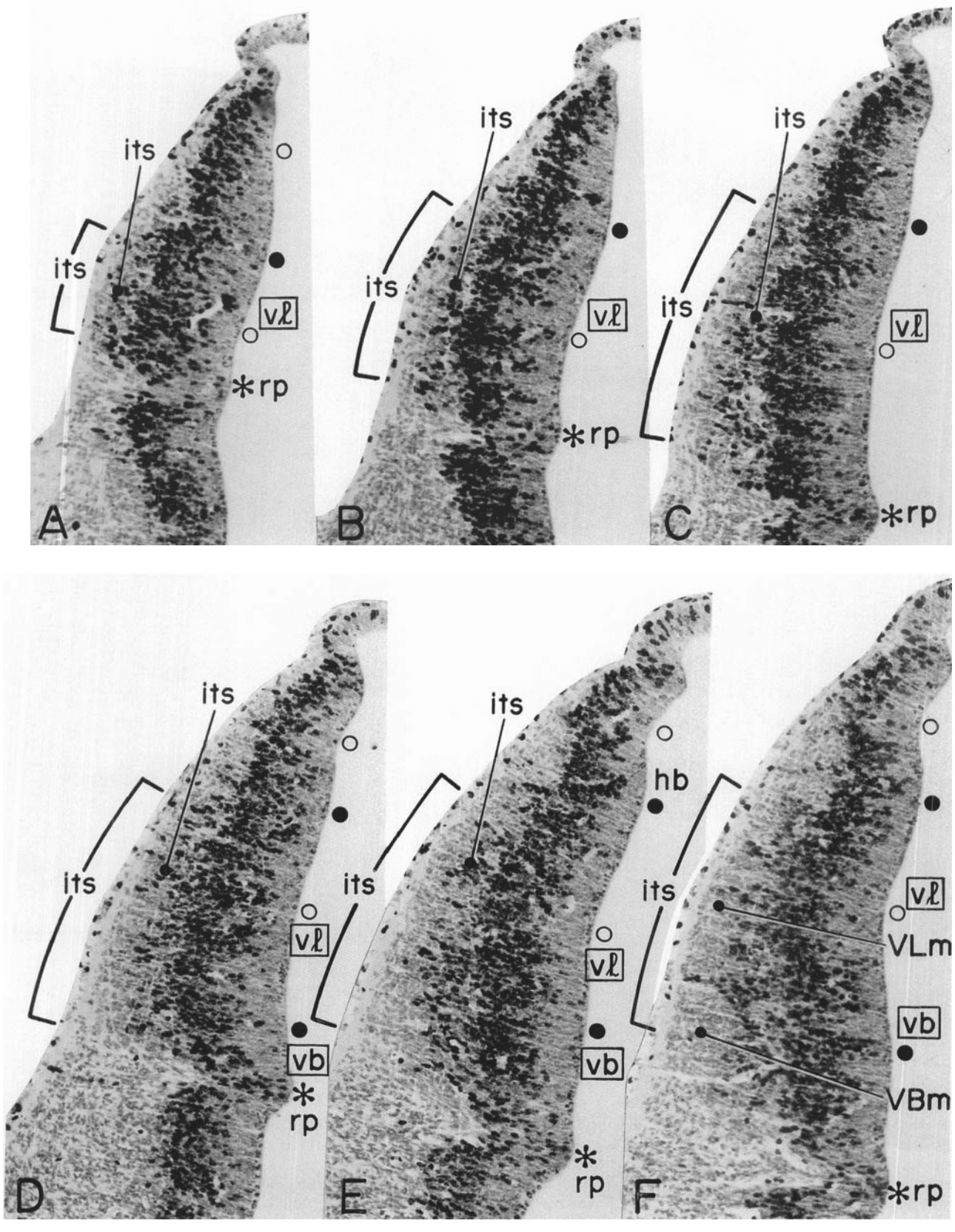

Fig. 10. Coronal radiograms of the thalamus containing the intermediate thalamic neuroepithelial lobule and its subependymal layer (its and brackets), from rostral (**A**) to caudal (**F**), from a rat labeled on day E15 and killed 2 hours later. Paraffin, H&E. Scale: 100 μm.

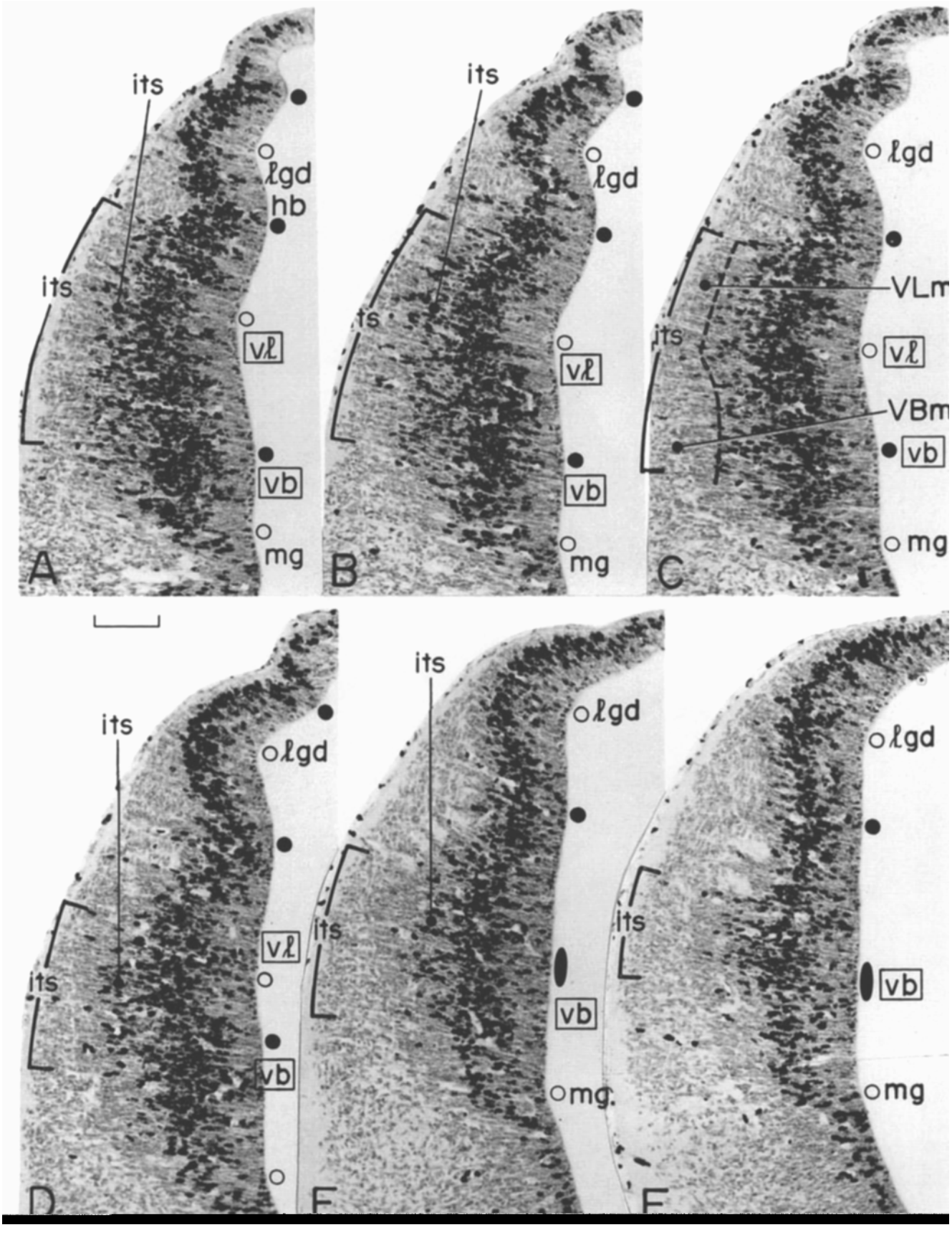

Fig. 11. Continuation of the series shown in Figure 10. Paraffin, H&E. Scale: 100 μm.

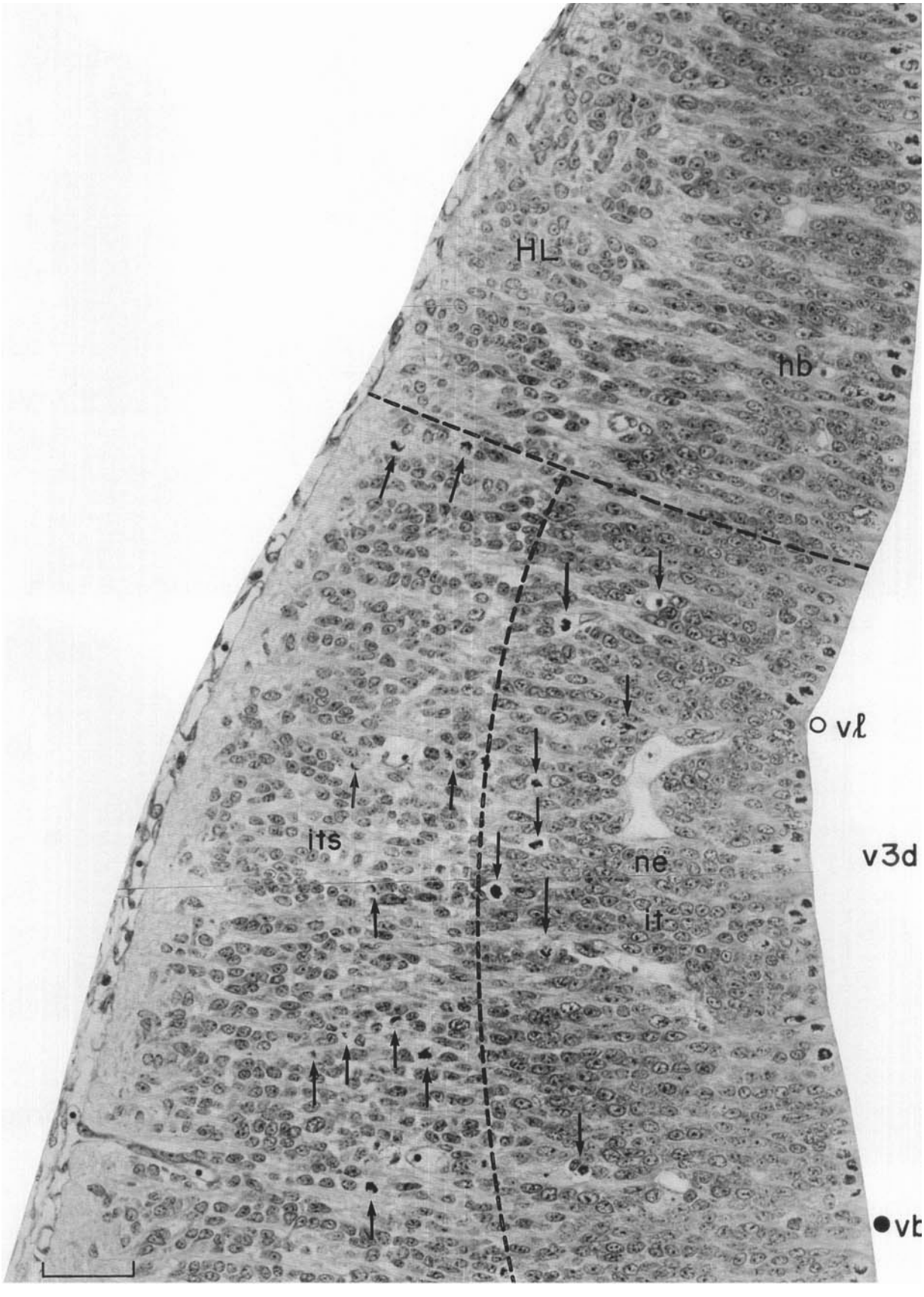

Figure 12

E20 (Fig. 18B). In an attempt to estimate the speed of migration of those neurons that were presumably generated soon after injection on the morning of day E15 (the heavily labeled cells that show little label dilution), we have used the following procedure. First we measured in day E15 rats the distance of the core of the proliferative neuroepithelium of the intermediate lobule (vl, vm, and thin vertical line at the bottom of Fig. 17A) from the midline of the thalamus (thick vertical line bisecting the third ventricle at its base in Fig. 17A). We have chosen this position as the starting point of those cells leaving the neuroepithelium on day E15, rather than the location of the neuroepithelium on the succeeding days, because, due to the progressive shrinkage of the third ventricle, the neuroepithelium is correspondingly displaced medially. Then we measured the distance between this starting point (indicated by the vertical line at a fixed distance from the midaxis of the third ventricle) and the location of the wavefront of heavily labeled cells on succeeding days (vertical arrows in Figs. 17B,C, 18A,B). The results indicate a steady rate of migration of 215 μm/day through days E15 and E20.

Regional chronological differences in cell migration and in the settling of neurons. The heavily labeled cells seen in rats injected on day E15 and killed on the succeeding days do not represent the true leading edge of migrating neurons from the putative sublobules of the VL and VB. Ahead of the heavily labeled cells is a wavefront of unlabeled cells that were generated before injection on the morning of day E15. We designate the unlabeled wavefront as component 1 of the outflow (e.g., VBm1 in Figs. 17, 18), the heavily labeled wave behind it as component 2 (e.g., VBm2 in Figs. 17, 18), and the next, less heavily labeled wave as component 3 (e.g., see VLm3 in Fig. 20). The unlabeled wavefront widens appreciably from rostral (Fig. 19A) to caudal (Fig. 19F) and paralleling this caudal-to-rostral gradient in cell differentiation, the width of the neuroepithelium is narrower caudally (ne and broken line in Fig. 20B) than rostrally (Fig. 20A).

The hypothetical identification of the zone of unlabeled migratory cells of the intermediate thalamic lobule as either the VB or the VL migration is based on our quantitative results (Fig. 4), which show that 20% of the neurons of the posteroventrally situated VB, but only 9% of the neurons of the anterodorsally situated VL, are generated on day E14. In rats injected on day E15 and killed on day E16, the small unlabeled wavefront of cells is limited at rostral levels to the ventral portion of the intermediate lobule; we assume that this is the most rostral portion of the VB migration (VBm1 in Figs. 19A,B, 20A). The unlabeled ventral wavefront expands progressively in the caudal direction (VBm1 in Figs. 19C–F, 20B). At rostral levels dorsally the neuroepithelium is flanked exclusively by labeled cells (that is, those generated after the morning of day E15); this we designate as the putative VL migration (VLm in Figs. 19A,B, VLm2 in Fig. 20A). More caudally there is a small dorsal unlabeled migratory zone (VLm1 in Figs. 19C,D, 20A,B).

The caudal-to-rostral gradient in the labeling pattern of cells leaving the intermediate thalamic neuroepithelial lobule is evident in rats labeled on day E15 and killed 2 days later (Fig. 21). In the animal illustrated, the proportion of unlabeled cells is smaller than in the animal killed 1 day after injection (Fig. 19), presumably because this embryo was less advanced developmentally at the time of injection. The third ventricle has shrunken considerably rostrally and the former components of the VL and VB sublobules are no longer distinguished by an eversion and inversion, respectively (Fig. 21A–C). But the former neuroepithelium of the VB is clearly delineated caudally by a ventricular inversion from the former putative neuroepithelial sources of the dorsal lateral geniculate nucleus (dorsal eversion and LGDm in Fig. 21D) and medial geniculate nucleus (ventral eversion, flanked by MG). The VM migration (VMm in Fig. 21C,D) is distinguished by a striated pattern produced by vertically oriented fibers. Finally, in rats injected on day E15 and killed on day E18, both the neurons of the VL (VL in Fig. 22A,B, and VL(1), VL(2), and VL(3) in Fig. 23) and the neurons of the VB (VB in Figs. 22C,D) appear to have settled in their final locations.

Outgrowth of efferents of the ventral nuclear complex

The internal capsule is first identifiable on day E16. On the morning of this day its fibers are confined to the diencephalon (IC in Figs. 14A, 20A,B, and, at higher magnification in Fig. 24A). By day E17 the bulk of the internal capsule increases greatly and its fibers begin to penetrate the telencephalon (TL) from its medial aspect (IC in Figs. 17C, 21B, and at higher magnification, Fig. 24B).

The emergence of the internal capsule is associated with a change in the orientation of the main axis of migrating cells of the intermediate lobule from horizontal to vertical. On day E15, before the emergence of the internal capsule (Figs. 10, 11), the cells flanking (and presumably just leaving) the germinal matrix at rostral levels (its in Fig. 13A) tend to be horizontally oriented, as are the fibers associated with them. Only at developmentally more advanced caudal levels is there a thin lateral band where some of the cells are vertically oriented (VB in Fig. 13B). By day E16 this lateral band of vertically oriented cells is present along the entire length of the ventral nuclear complex (thick vertical arrows in Figs. 24A, 25A). These vertically oriented cells are associated with similarly oriented fibers (Figs. 25A, 26A) that can be traced to the internal capsule (thin vertical arrows in Figs. 24A, 25A). By day E17 the population of vertically oriented cells has greatly increased (thick vertical arrows in Figs. 24B, 25B), at the expense of horizontally oriented cells (thick horizontal arrows in Figs. 24, 25), and there is an associated increase in vertically oriented fibers (Figs. 25B, 26B). The latter can be traced to the expanded internal capsule (thin vertical arrows in Figs. 24B, 25B). The change in the orientation of the cells leaving the intermediate thalamic neuroepithelium suggests that they are undergoing two concurrent transformations, their active migration has come to an end and they begin to sprout efferents that grow downward to join the internal capsule en route to the cerebral cortex. By day E18 (not illustrated) only a few cells remain in the ventral nuclear complex with a vertical orientation, other cells are haphazardly oriented, and the majority have assumed a roundish shape.

Fig. 12. Coronal methacrylate section from the midportion of the intermediate lobule of a day E15 rat. Horizontal broken line is the approximate boundary between the intermediate lobule and the putative neuroepithelium of the habenular nuclei (hb) and the lateral habenular nucleus (HL). Vertical broken line is the approximate boundary between the neuroepithelium (ne) and the subependymal layer (its) of the intermediate lobule. Notice the numerous mitotic figures (arrows). Scale: 50 μm.

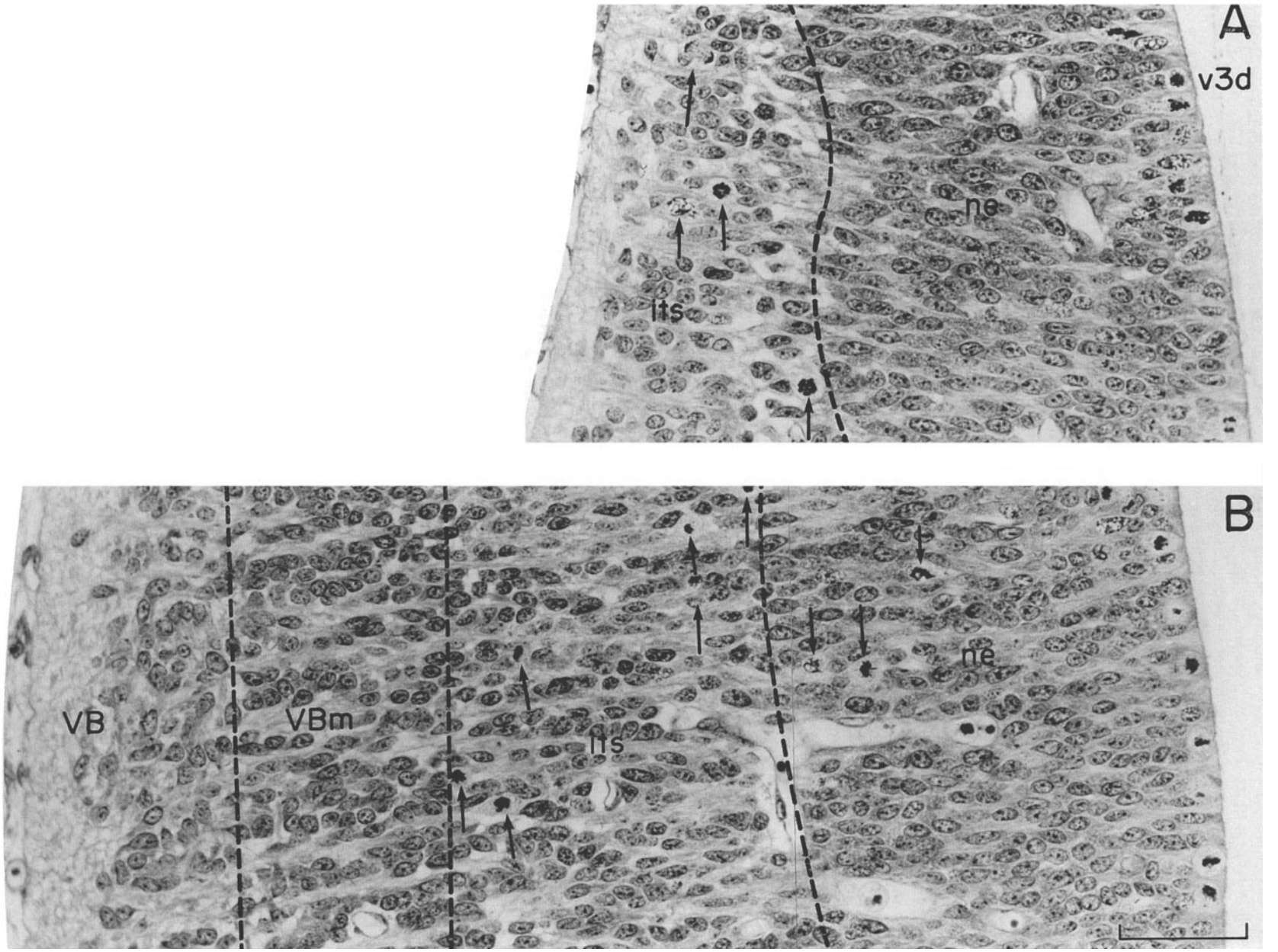

Figure 13

DISCUSSION

This article is concerned with a description of the fate of the early generated cells of the intermediate thalamic neuroepithelial lobule. In the first of this series (Altman and Bayer, '88a), we traced the progressive partitioning of the thalamic neuroepithelium (1) into a rostral and a caudal lobe, (2) the caudal lobe into an intermediate and posterior lobule, and (3) the intermediate lobule into two sublobules, a more anterodorsally situated neuroepithelial eversion and a more posteroventrally situated neuroepithelial inversion. We postulated that (1) the everted anterodorsal sublobule generates the neurons of the VL, (2) the inverted posteroventral sublobule is the source of neurons of the VB, whereas (3) the neurons of the VM are generated in the intermediate lobule after the separation of the two sublobules is no longer visible. The present study provides support for this hypothesis.

Nature and significance of the subependymal layer of the intermediate lobule neuroepithelium

We were able to distinguish, in short-survival thymidine radiograms, the intermediate thalamic lobule from other components of the thalamic neuroepithelium by the presence of labeled cells not only within the neuroepithelium but also in the subependymal layer. But, as we noted, this thymidine radiographic evidence does not unequivocally prove the existence of a secondary germinal matrix for the following reason. Because the embryos used for short-survival radiography survived for 2 hours after the administration of ^{3}H-thymidine, it is conceivable that the labeled cells located outside the neuroepithelium are not mitotic elements but have migrated there after they have undergone division within the neuroepithelium. If this were true the uniqueness of the intermediate lobule would still stand, because such a pattern is not seen in other regions of the thalamic neuroepithelium, but the interpretation of the presence of a germinal subependymal layer would not be justified.

Our observations in nonradiographic methacrylate-embedded sections definitively established that the intermediate lobule is flanked laterally by a genuine subependymal layer and they also indicated how this secondary germinal matrix might arise. Mitotic cells abound in the intermediate lobule not only near the lumen of the ventricle (as in the rest of the thalamic neuroepithelium) but in two other locations: (1) the core of the neuroepithelium and (2) some distance lateral to it (Figs. 12, 13, 16). In the core of the neuroepithelium, mitotic cells are present as early as day E14 (Fig. 9B), and in the subependymal layer they become abundant between days E15 (Figs. 12, 13) and E17 (Fig. 16B). The best interpretation of these observations is that those cells that are destined to move into the subependymal layer do not translocate medially to the lumen to undergo mitosis there (the phenomenon known as interkinetic nuclear migration) but divide in and around the synthetic zone, and their daughter cells then move laterally where, forming the subependymal layer, they undergo further divisions. In thymidine radiograms the proportion of labeled cells is at no time as high in the subependymal layer as it is in the synthetic zone of the neuroepithelium (Figs. 8B, 10, 11, 14, 15). But this does not necessarily imply a low level of cell proliferation in the secondary germinal matrix proper because a large proportion of the cells in the subependymal layer must be postmitotic elements that are migrating into the formative thalamus.

The significance of the presence of a secondary germinal matrix in the intermediate thalamic lobule is not clear. Secondary germinal matrices have been known to exist in the embryonic nervous system of mammals for some time and two types have been distinguished: (1) a superficially situated subpial matrix, of which the best example is the external germinal layer of the cerebellum, and (2) a subependymal (or subventricular) matrix, which is found in particular in the formative cerebral cortex. There is ample evidence that the external germinal layer of the cerebellar cortex gives rise to short-axoned microneurons, the basket, stellate, and granule cells (reviewed in Altman, '82). It is tempting to speculate that the derivatives of secondary germinal matrices in other parts of the brain are also destined to produce short-axoned neurons. The implications of this, with reference to the ventral nuclear complex, are, first, that the subependymal germinal cells give rise to the short-axoned neurons of the ventral nuclear complex, and, second, that only this region of the thalamus contains short-axoned neurons because a subependymal layer is not seen elsewhere in the thalamic neuroepithelium. But neither of these expectations is sufficiently supported by the available evidence. First, although short-axoned neurons have been described in the ventral nuclear complex in different mammalian species (Ramón y Cajal, '11; Scheibel and Scheibel, '66; Tömböl, '67; Yen et al., '85), their existence in the rat has been questioned or denied (Saporta and Kruger, '77; Spacek and Lieberman, '74; McAllister and Wells, '81; Ralston, '83; Williams and Faull, '87). Second, there is some evidence for the existence of interneurons in other relay nuclei of the thalamus, for instance, the dorsal lateral geniculate nucleus (reviewed by Sherman and Koch, '86), the putative neuroepithelial source of which is devoid of a subependymal layer (Altman and Bayer, '89c).

Identification of components of the intermediate lobule

In the introductory work of this series (Altman and Bayer, '88a), we proposed three spatiotemporal criteria for the identification of a particular neuroepithelial region (like a sublobule or a distinct patch) as the putative source of neurons of a specific brain structure: (1) a good match between the times of high mitotic activity in the neuroepithelial region and neurogenesis in the brain structure, which it is supposed to supply with neurons, (2) the shortest and most straightforward route between the location of the neuroepithelial region and the presumed target structure, and (3) the successful tracing of migratory neurons from the putative source to their destination. Our identifications of the everted anterodorsal sublobule as the putative source of neurons of the VL, and of the inverted posteroventral sublobule as the putative source of neurons of the VB, partially

Fig. 13. Coronal methacrylate sections from the rostral (**A**) and caudal (**B**) portion of the intermediate lobule of a day E15 rat. Broken line in A separates the neuroepithelium (ne) and the subependymal layer (its). Additional broken lines in B indicate the boundaries between the ventrobasal migration (VBm) and the settling cells of the ventrobasal nucleus (VB). Arrows point to mitotic cells. CV. Scale: 50 μm.

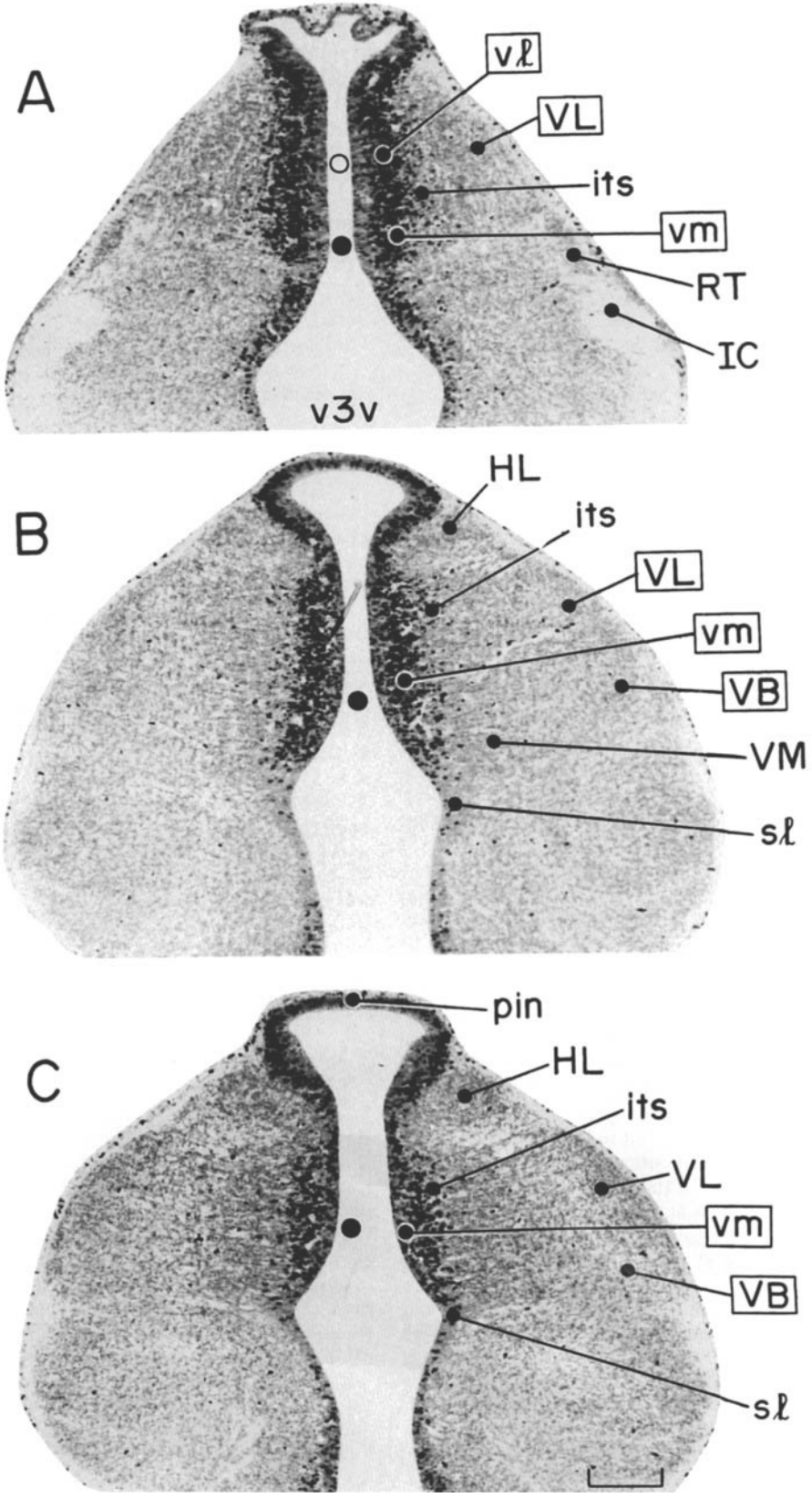

Fig. 14. Coronal sections of the thalamus, from rostral (A) to caudal (C), from a rat labeled on day E16 and killed 2 hours later. Paraffin, H&E. Scale: 200 μm.

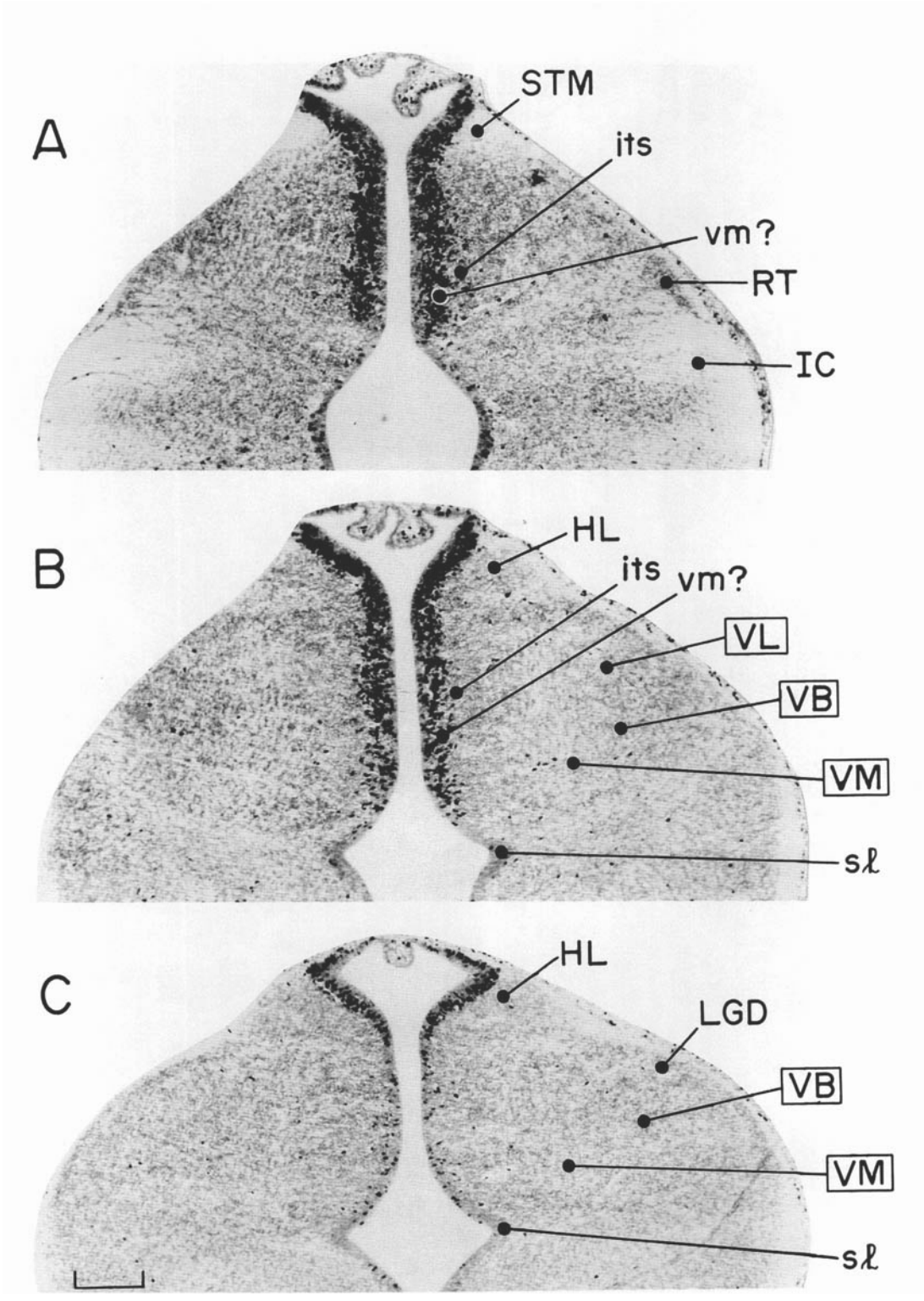

Fig. 15. Coronal sections of the thalamus, from rostral (A) to caudal (C), from a rat labeled on day E17 and killed 2 hours later. Paraffin, H&E. Scale: 200 μm.

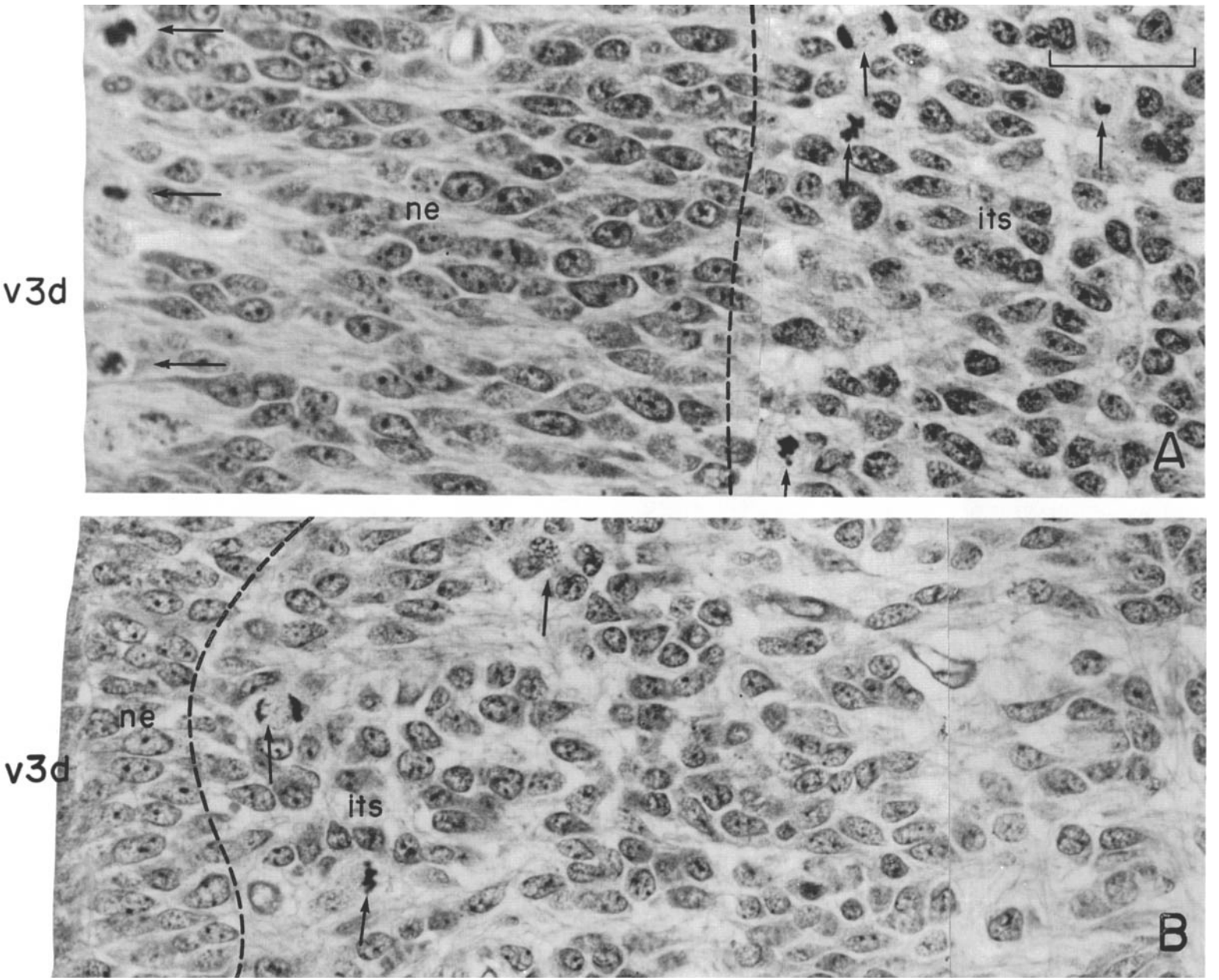

Fig. 16. Structure of the neuroepithelium (ne) and the subependymal layer (its) in coronal sections of the intermediate thalamic lobule on day E16 (**A**) and day E17 (**B**). Horizontal arrows point to neuroepithelial mitotic cells near the lumen. Vertical arrows point to mitotic cells in the subependymal layer. Methacrylate, CV. Scale: 30 μm.

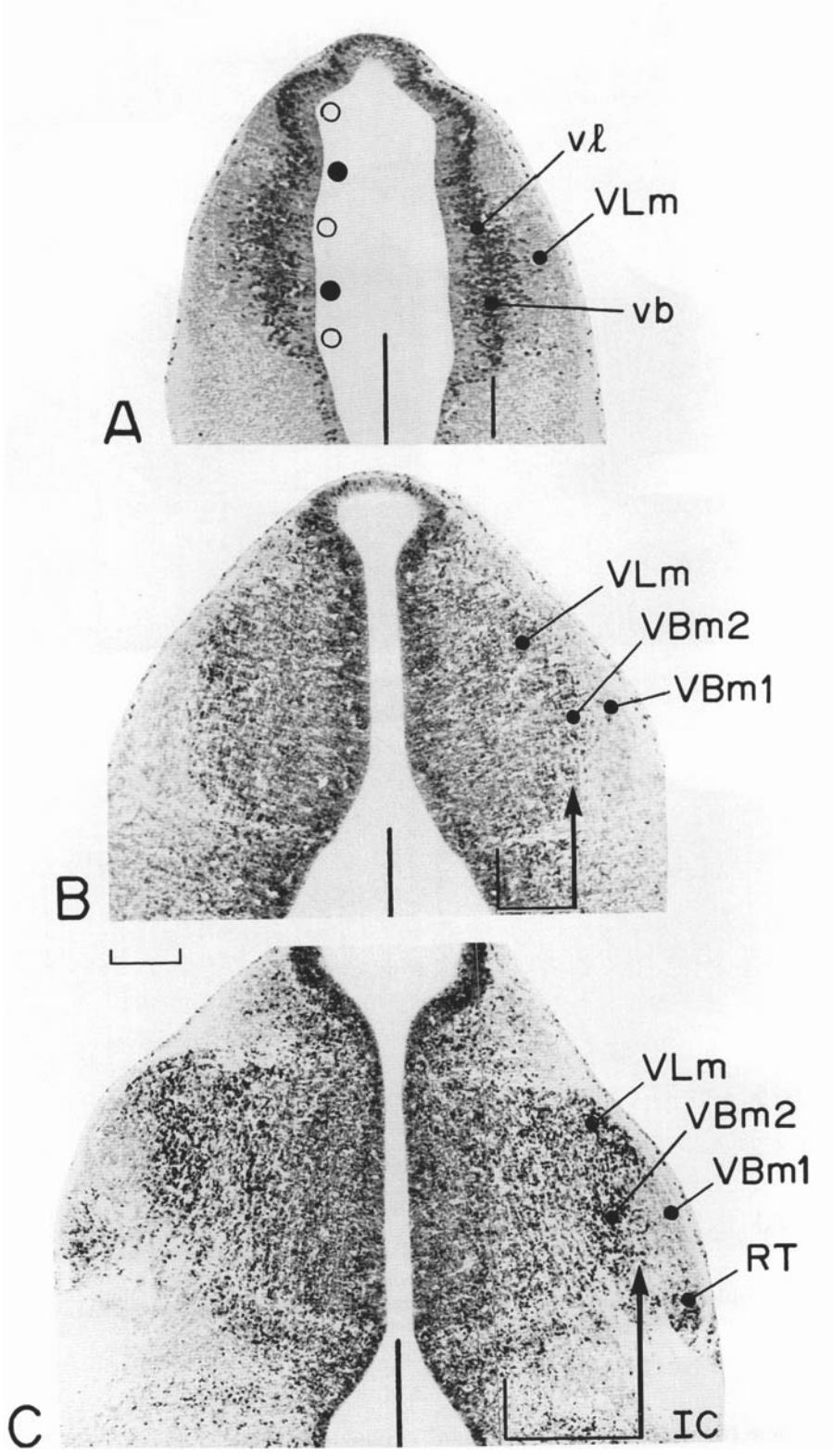

Fig. 17. Coronal radiograms from the midportion of the thalamus from rats injected on day E15 and killed 2 hours (**A**), 1 day (**B**), and 2 days (**C**) after labeling. Heavy vertical line in center delineates the midline of the thalamus. The second (thin) vertical line to the right indicates in all figures the location of the core of the neuroepithelium on day E15. Vertical arrow points to the point reached by the heavily labeled cells (those generated on day E15) on successive days. Bottom horizontal line is a measure of the distance traversed by the heavily labeled cells. Paraffin, H&E. Scale: 200 μm.

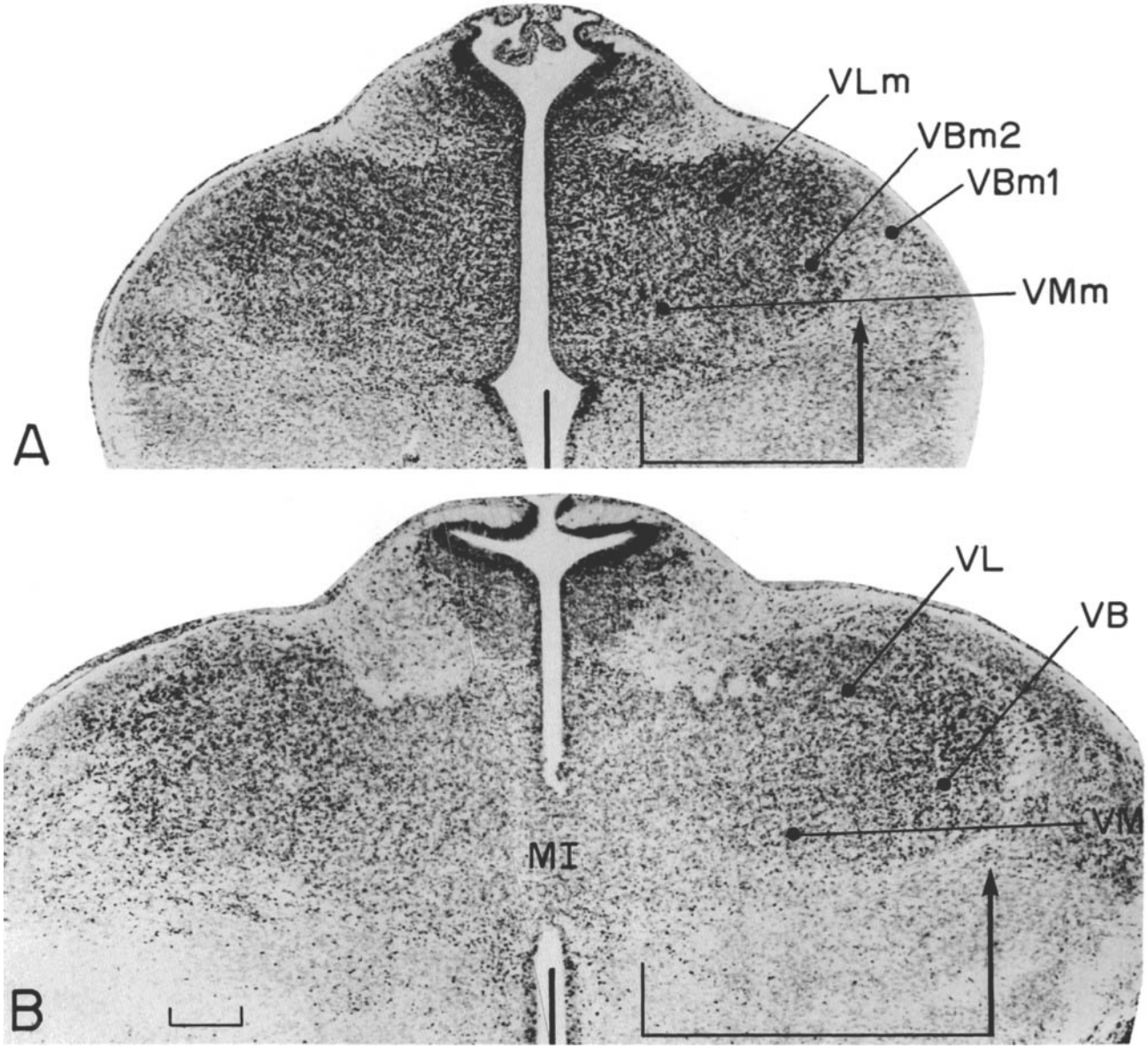

Fig. 18. Continuation of the series in Figure 17. Radiograms from rats injected on day E15 and killed 3 days (**A**) and 5 days (**B**) after labeling. Paraffin, H&E. Scale: 200 μm.

satisfy these criteria. These two neuroepithelial sublobules are most prominent on day E15, coincident with the peak time of neurogenesis in both VL and VB (Fig. 4). There are also some spatiotemporal clues that distinguish the two sublobules from one another. In short-survival radiograms of day E15 rats, there are fewer unlabeled cells anteriorly, where the everted sublobule is the only component of the intermediate lobule (vl in Fig. 10A–C), than caudally, where the inverted sublobule predominates (vb in Figs. 10D,E). And in the midportion of the intermediate lobule, where both sublobules are present, there are fewer unlabeled cells dorsally than ventrally (vl and vb in Figs. 10D–F, 11A–D). This pattern matches the much smaller proportion of neurons generated on day E14 in the anterodorsally situated VL

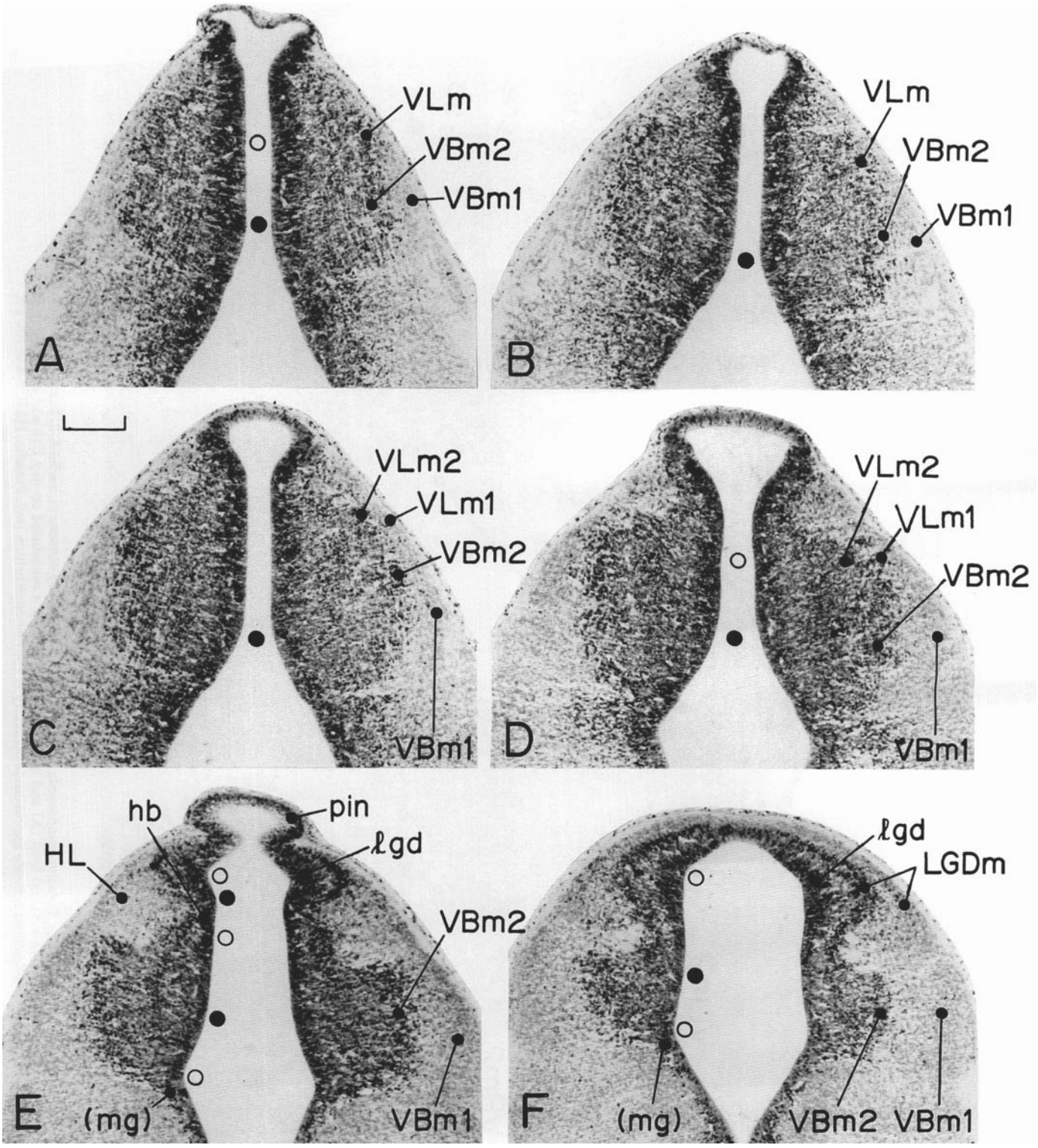

Fig. 19. Coronal radiograms, from rostral (A) to caudal (F), from a rat labeled on day E15 and killed 1 day later. Paraffin, H&E. Scale: 200 μm.

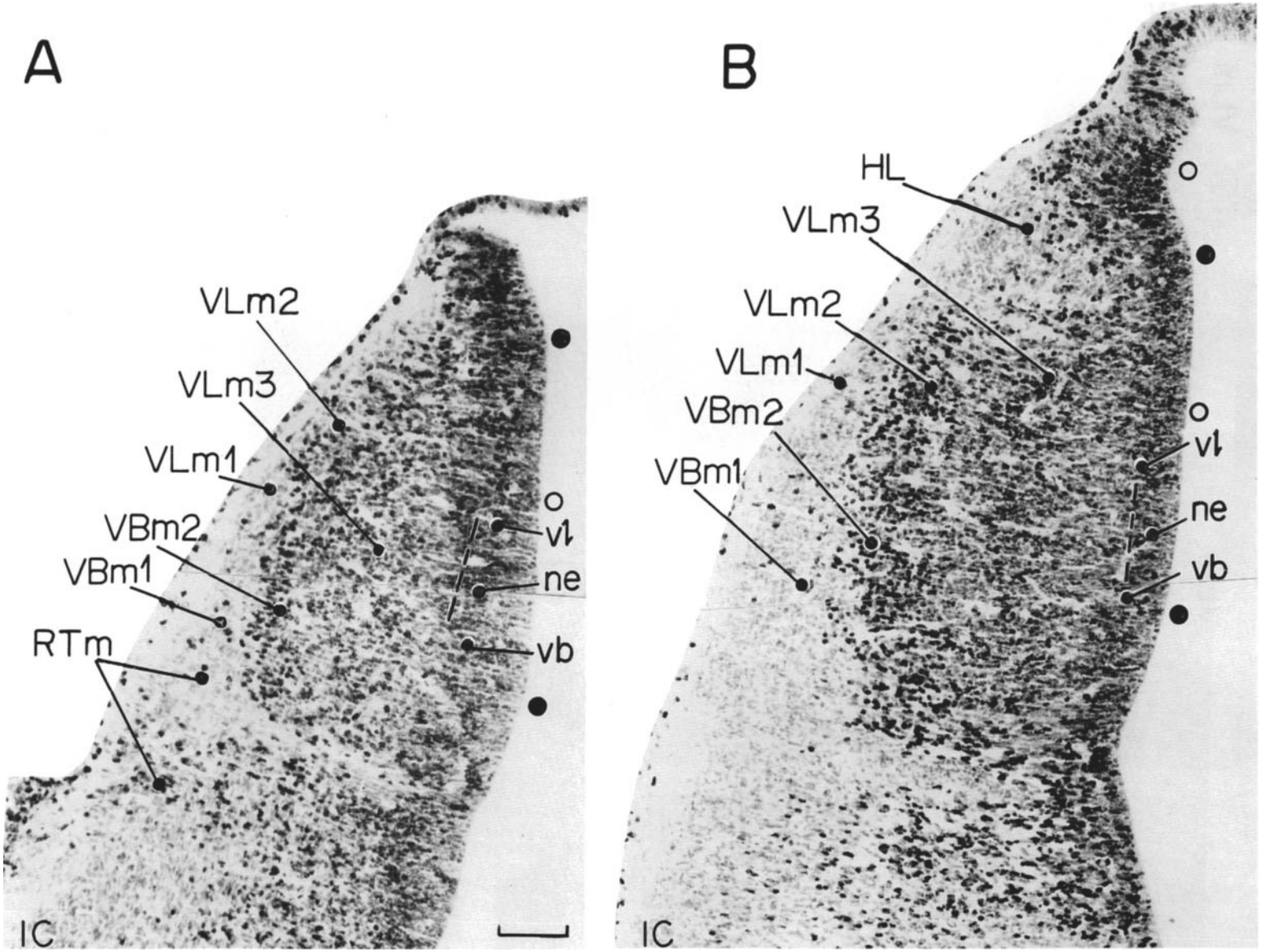

Fig. 20. Putative components of the VB and VL migratory streams (1, earliest, 3, latest) in coronal sections, at a rostral level (**A**) and more caudally (**B**), from a rat labeled on day E15 and killed 1 day later. Broken lines indicate approximate boundary of the neuroepithelium (ne). Paraffin, H&E. Scale: 100 μm.

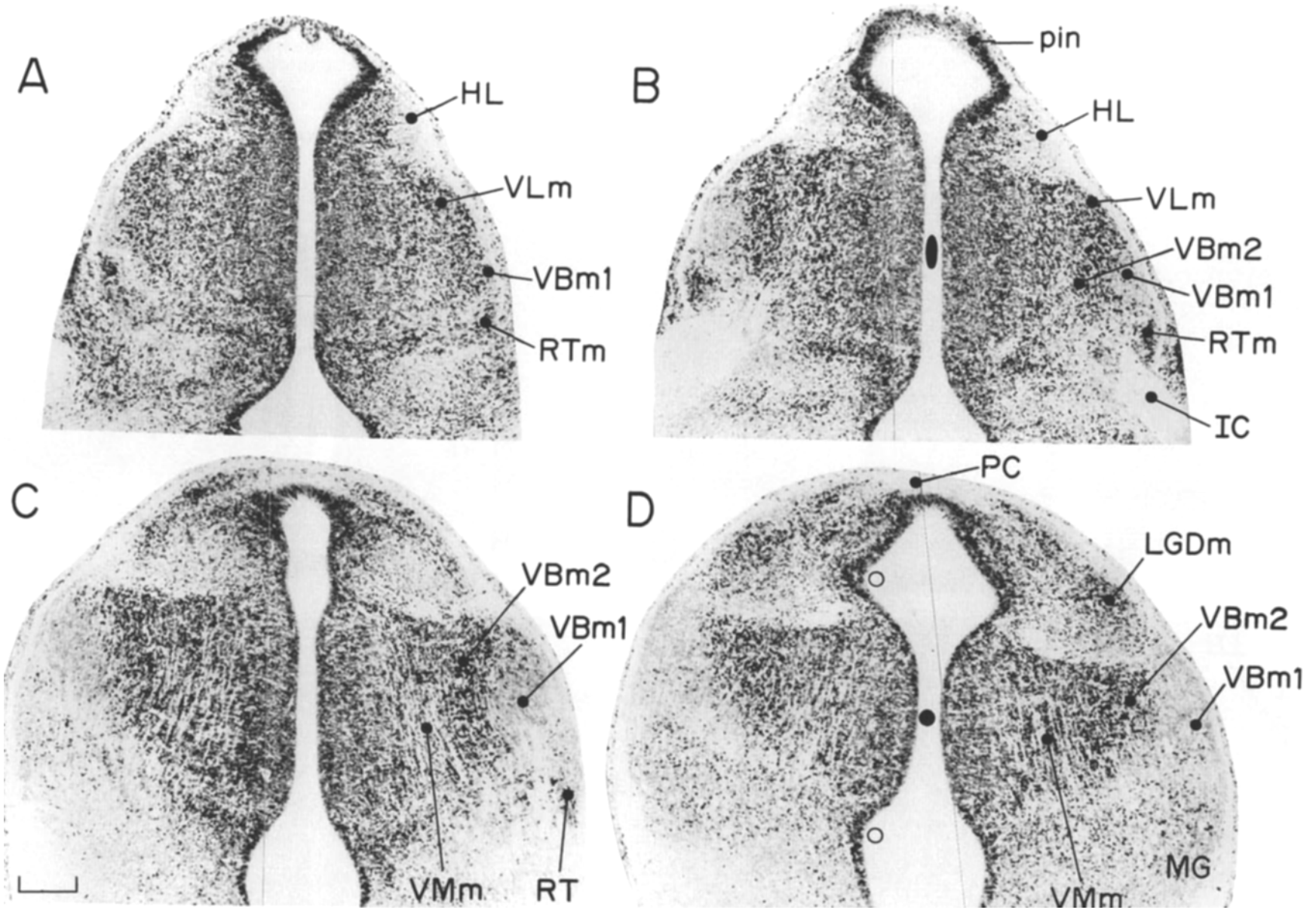

Fig. 21. Coronal radiograms, from rostral (**A**) to caudal (**D**), from a rat labeled on day E15 and killed 2 days later. Paraffin, H&E. Scale: 200 μm.

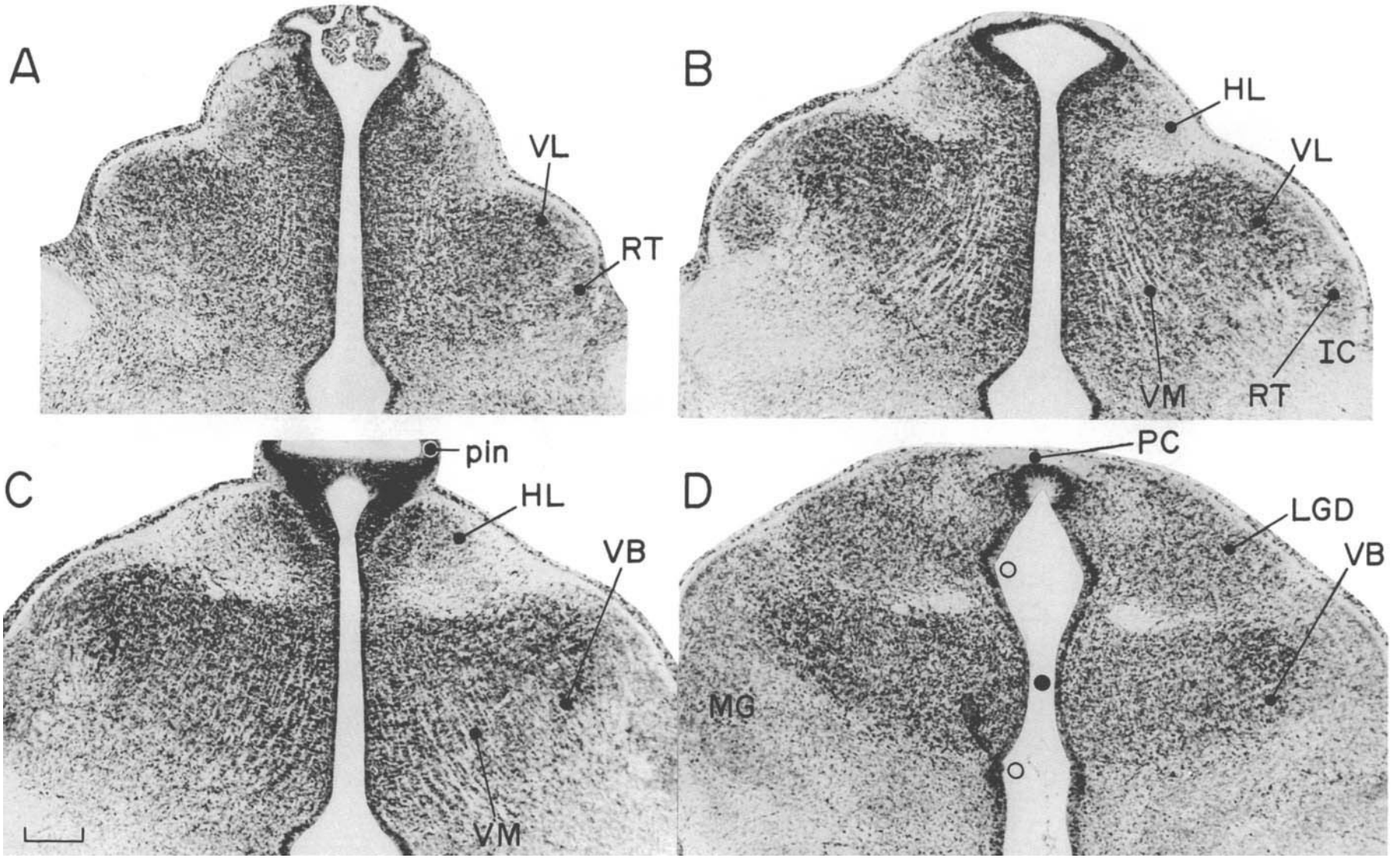

Fig. 22. Coronal radiograms, from rostral (**A**) to caudal (**D**), from a rat labeled on day E15 and killed on day E18. Paraffin, H&E. Scale: 200 μm.

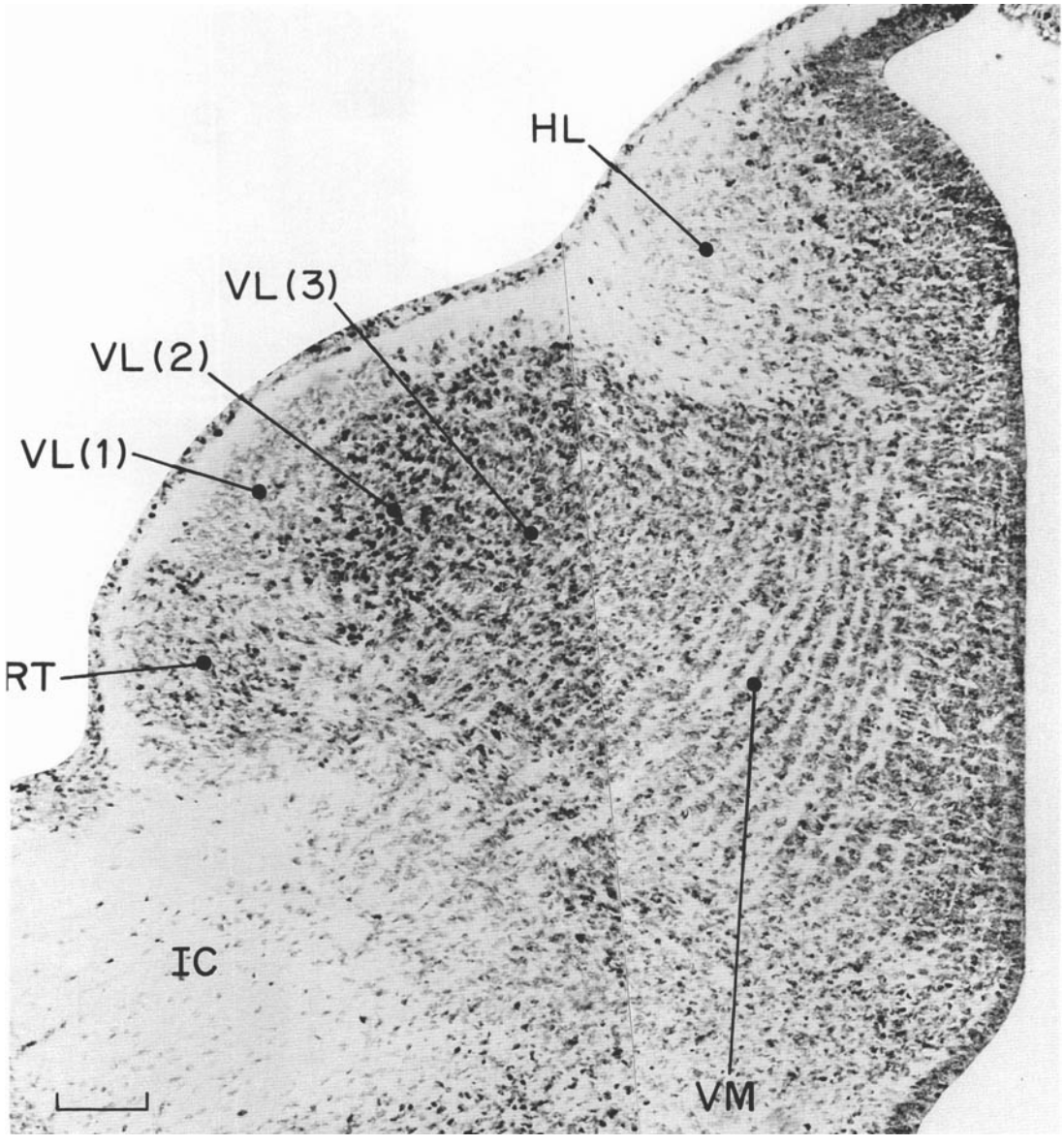

Fig. 23. Coronal radiogram from a rat labeled on day E15 and killed on day E18. Paraffin, H&E. Scale: 100 μm.

than in the posteroventrally situated VB (Fig. 4). This pattern persists in sequential radiograms of rats labeled on day E15 and killed on the subsequent days (Figs. 17–22). Although we have not been able to clearly distinguish two separate migratory paths to the formative VL and VB (the best separation is seen in Fig. 19A,D), the distinction between the two (the VLm dorsally and the VBm ventrally) is suggested not only by the different proportions of heavily labeled and unlabeled cells in the dorsal and ventral components of the migration but by the difference in the width of the differentiating thalamus (narrow dorsally and broad ventrally; Figs. 17B,C, 19A–D, 20).

Cell migration, changes in cell orientation, and the outgrowth of efferents

The cells of the intermediate lobule neuroepithelium are spindle-shaped and their long axis is oriented in the horizontal plane, perpendicular to the ventricular wall (ne in Figs. 12, 13). This shape and orientation are common features of cells of the pseudostratified neuroepithelium throughout the embryonic nervous system. The majority of the cells of the subependymal layer, which presumably contain not only cells undergoing division but also postmitotic migratory cells, are packed less tightly and have somewhat

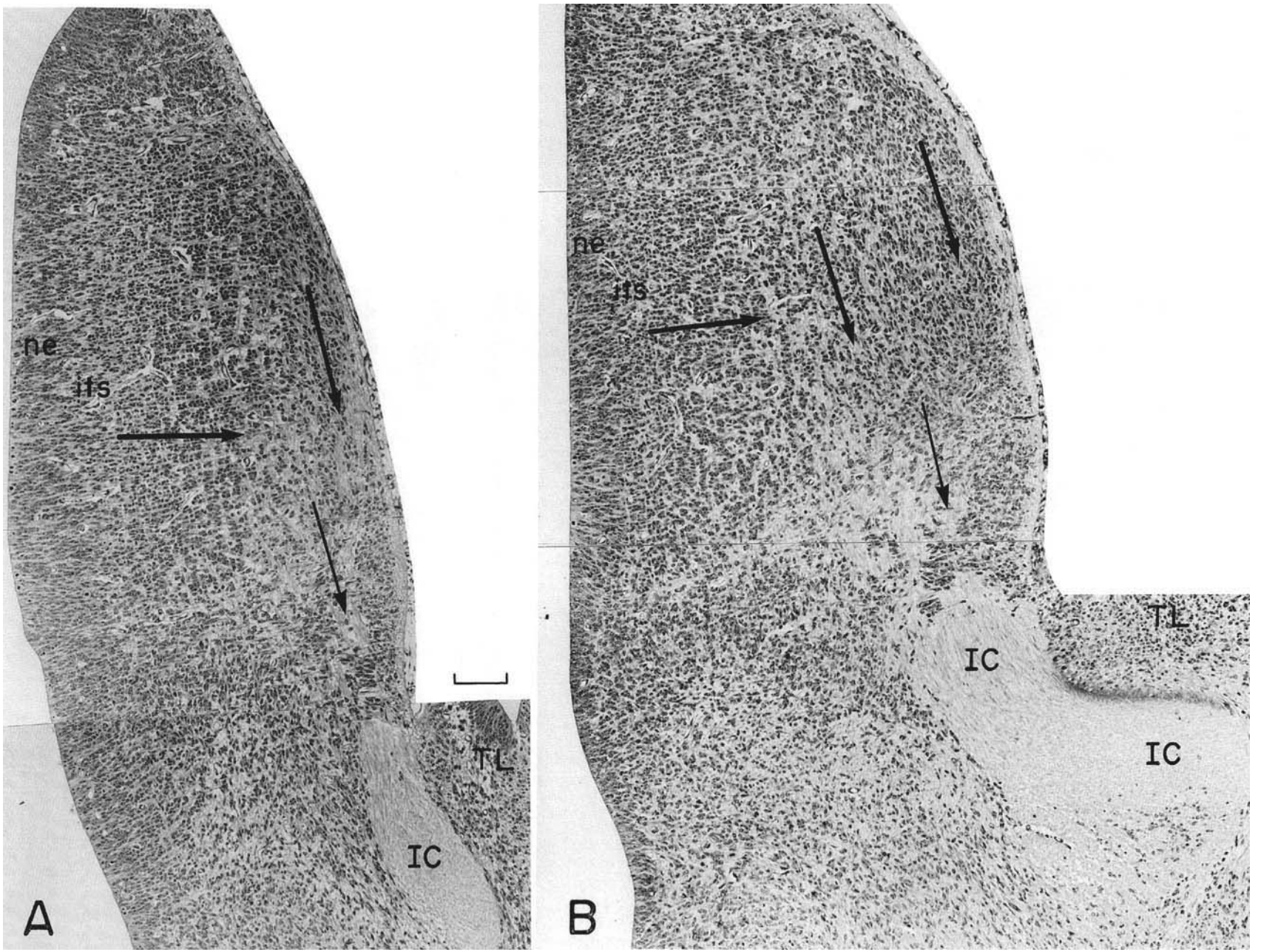

Fig. 24. Anterior coronal methacrylate sections through the developing ventral nuclear complex on day E16 (**A**) and day E17 (**B**). The thick horizontal arrow highlights the zone of horizontally oriented cells adjacent to the subependymal layer (its). The thick vertical arrows point to the zone of vertically oriented cells farther laterally. The thin vertical arrow is aligned with the descending fibers that join the internal capsule (IC). CV. Scale: 100 μm.

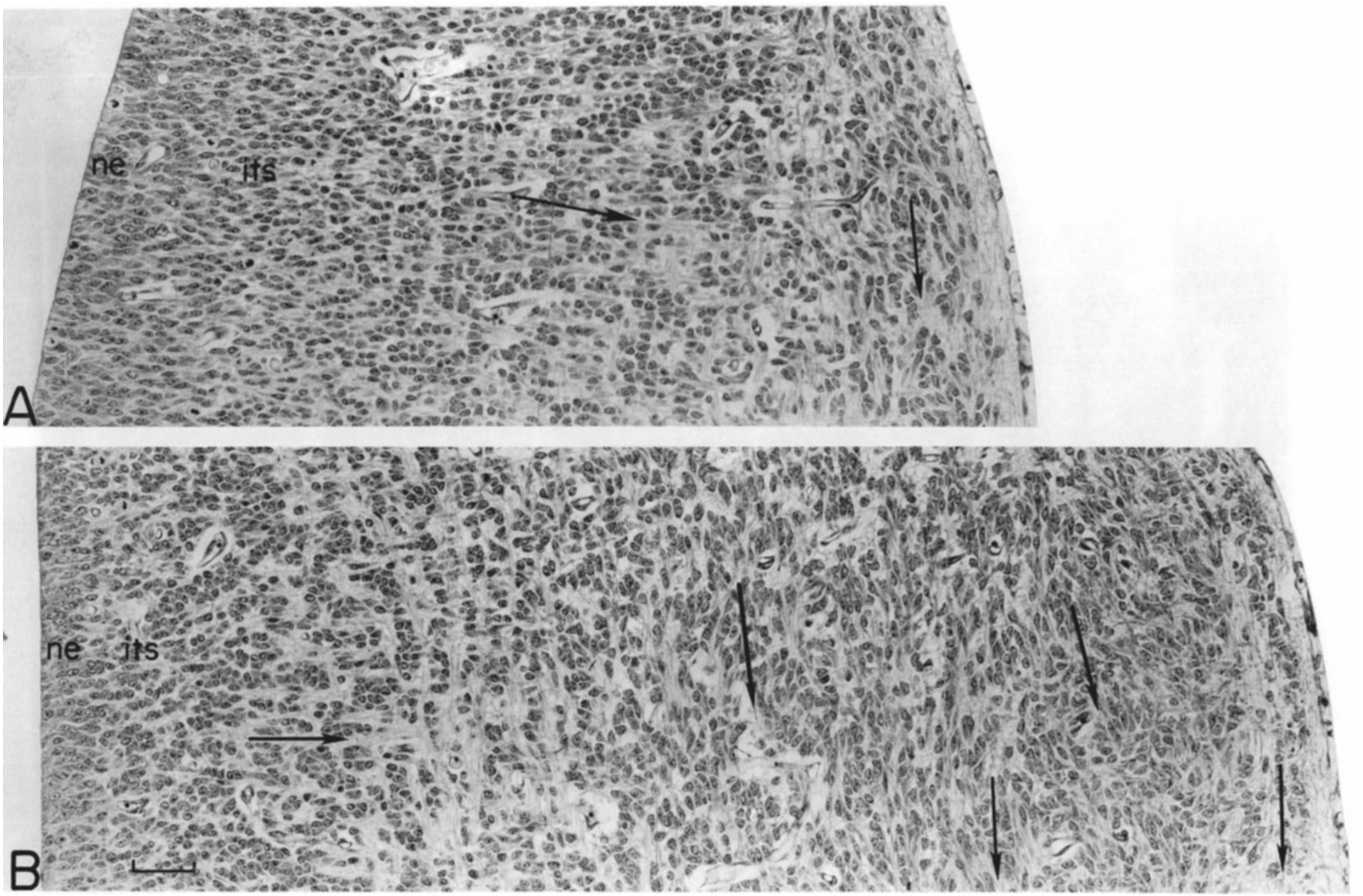

Fig. 25. The cellular structure of the intermediate thalamic lobule at higher magnification on day E16 (**A**) and E17 (**B**). Thick horizontal and vertical arrows, and thin downward directed arrow, are used as in Figure 24. Methacrylate, CV. Scale: 50 μm.

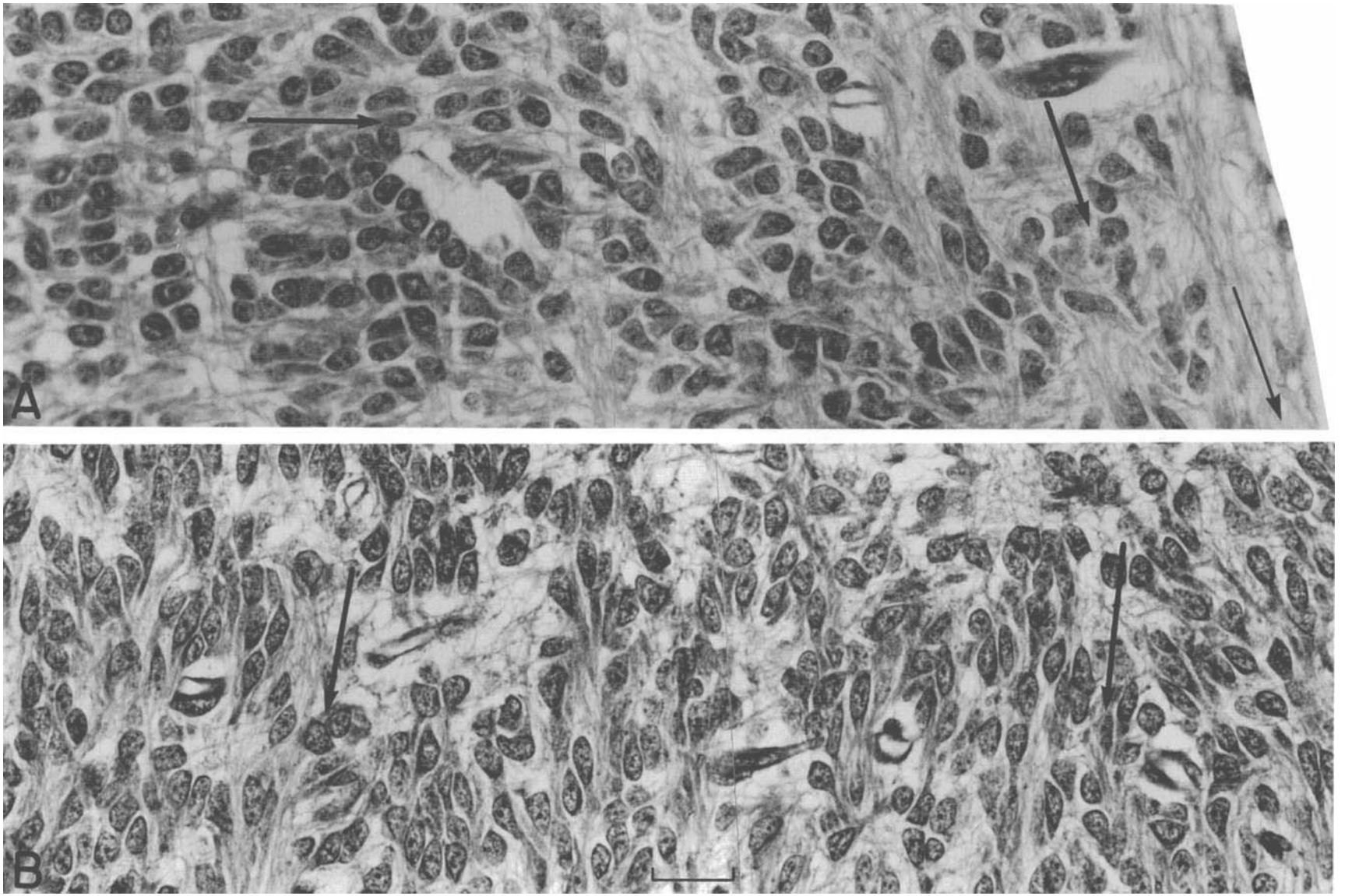

Fig. 26. The lateral aspect of the developing ventral nuclear complex in days E16 (**A**) and E17 (**B**) rats at higher magnification. Arrows are used as in Figure 24. Methacrylate, CV. Scale: 20 μm.

smaller nuclei than the neuroepithelial cells, but a fair proportion of these, too, are horizontally oriented (its in Figs. 12, 13). Horizontally oriented cells predominate also in the migratory zone adjacent to the subependymal layer in day E16 rats (VBm in Fig. 13B, heavy horizontal arrows in Figs. 24A, 25A, 26A). But farther laterally, and extending to the wall of the diencephalon, there is a narrow band composed of vertically oriented cells (VB in Fig. 13B, heavy vertical arrows in Figs. 24A, 25A, 26A). The latter are associated with some vertically oriented fibers (thin vertical arrows in Fig. 26A) that can be traced to the formative internal capsule (IC in Fig. 24A). The band of vertically oriented cells expands greatly by day E17 (heavy vertical arrows in Figs. 24B, 25B, 26B), at the expense of the more medially situated horizontally oriented cells, and there is an associated increase in the bulk of the internal capsule whose fibers have begun to penetrate the telencephalon (TL) at its base (IC in Fig. 24B).

Historically, it was Ramón y Cajal ('11) who called attention to the importance of bipolar cells and their orientation in the developing nervous system in relation to axonogenesis. In our recent study of the early development of the rat spinal cord (Altman and Bayer, '84), we have documented developmental changes in the orientation of the differentiating ventral horn cells (1) from round to horizontal bipolar just before the outgrowth of motor fibers, (2) to vertical bipolar at the onset of dendrogenesis, and (3) to multipolar at later stages of dendritic development (see summary Fig. 25 in Altman and Bayer, '84). Similarly, we could distinguish the earlier-generated, contralaterally projecting interneurons (Cajal's commissural cells) from the later-generated ipsilaterally projecting interneurons by the polarity of these young neurons (see summary Figs. 124–125 in Altman and Bayer, '84). Fibers could be traced from the microzones of the spinal cord consisting of horizontal bipolar cells into the lateral funiculus, and from the vertical bipolar cells into the contralateral ventral funiculus. Such considerations give some credence to our interpretation that the change in the polarity of the differentiating cells from horizontal to vertical occurs when they begin to sprout fibers that grow ventrally to form the internal capsule. These are undoubtedly thalamocortical axons because we could trace the progressive growth of the internal capsule toward the cerebral cortex on the subsequent days (not illustrated).

Examination of sequential thymidine radiograms from rats injected with ^{3}H-thymidine on day E15 and killed at daily intervals thereafter has established that the heavily labeled cells, those that are considered to have left the neuroepithelium soon after the injection, migrate radially at a steady speed of 215 μm/day (Figs. 17, 18). This steady pace is somewhat surprising in view of the observation that in the course of their migration the cells change their orientation and begin to sprout ventrally oriented fibers. It seems unlikely that the vertically oriented cells, which are engaged in the extrusion of axons are, at the same time, actively migrating in a lateral direction. The best interpretation of these observations is that after the cells have become vertically polarized and begin to sprout axons, they are *passively* displaced in a lateral direction by the new waves of cells actively migrating from the neuroepithelium and settling more medially (work in progress).

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