Development of the Diencephalon in the Rat

IV. QUANTITATIVE STUDY OF THE TIME OF ORIGIN OF NEURONS AND THE INTERNUCLEAR CHRONOLOGICAL GRADIENTS IN THE THALAMUS

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ABSTRACT Groups of pregnant rats were injected with two successive daily doses of ^{3}H-thymidine from gestational days 13 and 14 (E13+14) until the day before birth (E21+22). With this progressively delayed comprehensive labelling procedure we determined the time of origin of neurons in the nuclei of the epithalamus, thalamus, and ventral thalamus. The zona incerta, subthalamic nucleus, reticular nucleus, posterior nucleus, and ventral lateral geniculate nucleus are composed of the earliest arising neurons (E13, or before, to E15). The neurons of the lateral habenular nucleus are produced between days E13-16. The neurons of the medial geniculate and lateral geniculate nuclei, the ventrobasal and ventrolateral complexes, and the nucleus lateralis, pars posterior, arise rapidly on days E14-15; the medial geniculate nucleus with a peak on day E14, the others with a peak on day E15. Neurons of a group of nuclei, with ill-defined boundaries medial to the sensory relay nuclei, arise apparently on days E15-16, with a peak on day E15; these may represent the intralaminar nuclei. The next group is generated on days E15-16 but with peak formation time on day E16: this includes the anteroventral, anterodorsal, anteromedial and mediodorsal nuclei. The rhomboid, reuniens and paratenial nuclei, and the paraventricular nucleus, pars anterior, arise next (E16-17). The medial habenular nucleus forms last and over a protracted period (E15-19). With their lengthy generation time the lateral and medial habenular nuclei resemble more the nuclei of the hypothalamus than the nuclei of the dorsal thalamus.

In three preceding papers of this series (Altman and Bayer, '78a,b,c) we dealt with the time and site of origin of neurons of the hypothalamus and of the cells of its specialized ventricular linings. The time of origin of cells in different regions of the hypothalamus was determined quantitatively in adult rats that were tagged with ^{3}H-thymidine from the early embryonic through the early postnatal period. Internuclear and intranuclear gradients in autoradiographic labelling patterns were noted and interpreted as directional "arrows" that point to the possible site of origin and route of dispersion of the settled cells. This information obtained in adult rats was then used, in an attempt to identify the neuroepithelial source and early differentiation of various components of the hypothalamus, in a series of normal and X-irradiated rat embryos and pups. In these continuing publications of this series we analyze the same material with respect to the development of the rest of the diencephalon. The present paper deals with the time of origin of neurons of the thalamus and adjacent structures, and internuclear gradients in this region.

There are several studies available that have used thymidine radiography [1] to study the development of the thalamus. Angevine ('70) carried out an extensive investigation of the development of the diencephalon (except

[1] In view of the many uses of autoradiography in neurobiological research it might be convenient to drop the prefix "auto" and replace it with the generic or specific name of the labelled chemical used. The term "thymidine-radiography" would imply that the study is concerned with the labelling of a proliferative system with radioactive thymidine. The term amino acid-radiography or proline-radiography might imply an axoplasmic flow study; the term cholesterol-radiography the study of lipid metabolism, and so forth.

the hypothalamus) in the mouse, and there is a similar study available in the rat by McAllister and Das ('77). Selected regions of the thalamus were examined by several investigators: the lateral geniculate nucleus in the rat (Brückner et al., '76; Lund and Mustari, '77), the anterior and midline nuclei in the rabbit (Fernández, '69; Fernández and Hermes, '73), and the dorsal lateral geniculate nucleus (Rakic, '77) in the monkey. In all these investigations a single injection of ^{3}H-thymidine was made on specific gestational days, and the time between the first appearance and the final disappearance of heavily labelled neurons in a given region was taken to reflect the time span of cell acquisition in that region. This technique has many pitfalls. Labelling intensity over the nucleus of a neuron depends on a host of factors: size of the nucleus; amount of radiochemical administered; specific activity of the radiochemical; regional cell cycle time; exposure time of tissue to the nuclear emulsion; sensitivity of the emulsion; and, last but not least, the subjective judgment involved in what is a "heavily" labelled cell. In our recent studies we have used an improved method: the progressively delayed comprehensive labelling procedure with double injections. With this technique virtually all cells in a brain region can be labelled as long as all the precursors of its neurons are still multiplying, that is, have not commenced to differentiate. (In numerous brain structures studied so far we have obtained, in one or more groups with double injections beginning on days E12+13, a cell labelling efficiency of 95-100%. The exceptions were the granule cells of the hippocampal dentate gyrus and the cerebellar cortex which form over an extended period. In the case of the postnatally forming cerebellar granule cells, four successive daily injections are used to achieve comprehensive labelling; work in progress.) As the injections are delayed at daily intervals, the date can be specified when a proportion of the cells could no longer be labelled. This proportion is considered the complement that was "born" (or ceased to multiply) on the previous day. This procedure tends to yield shorter time spans for the time of origin of neurons than estimates

Abbreviations

AD, anterodorsal nucleus
AM, anteromedial nucleus
AV, anteroventral nucleus
cc, corpus callosum
CL, central lateral nucleus
cp, cerebral peduncle
DC, dorsal central gray (midbrain)
fi, fimbria
fm, foramen of Monro
fx, fornix
HA, hippocampus, Ammon's horn
hc, hippocampal commissure
HD, hippocampus, dentate gyrus
hp, habenulopeduncular tract
HY, hypothalamus
ic, internal capsule
LA, lateral nucleus, pars anterior
LGd, lateral geniculate nucleus, pars dorsalis
LGv, lateral geniculate nucleus, pars ventralis
LH, lateral habenular nucleus
LP, lateral nucleus, pars posterior
lv, lateral ventricle
MD, mediodorsal nucleus
MG, medial geniculate nucleus
MH, medial habenular nucleus
ml, medial lemniscus
NP, nucleus of posterior commissure
ot, optic tract
PF, parafascicular nucleus
PO, posterior nucleus (thalamus)
PR, pretectal area
PT, paratenial nucleus
PV, paraventricular
PVa, paraventricular nucleus, pars anterior
PVp, paraventricular nucleus, pars posterior
RE, reuniens nucleus
RH, rhomboid nucleus
RT, reticular nucleus
sm, stria medullaris
SO, subcommissural organ
st, stria terminalis
ST, subthalamic nucleus
VC, ventral central gray (midbrain)
VB, ventrobasal complex
VE, ventrolateral complex
ZI, zona incerta
III, third ventricle

Fig. 1 Changing labelling patterns in coronal sections of the rostral thalamus in rats injected on days E14+15 (A), E15+16 (B), and E16+17 (C). There is only one region with unlabelled cells in A, representing an unidentified, very early-forming nucleus (compare with figs. 2, 3, 5). The cells in the midline region are lightly labelled in A and B due to continuing multiplication of the precursor cells of this late-forming region. In B most cells of RT are no longer labelled and the belt formed by AD, AV, AM and CL show a decrease in labelled cells. In C, representing late-neuron formation (day E16 and later), a large proportion of the neurons of the medial nuclei (PT, PV, MD and RE) are heavily labelled.

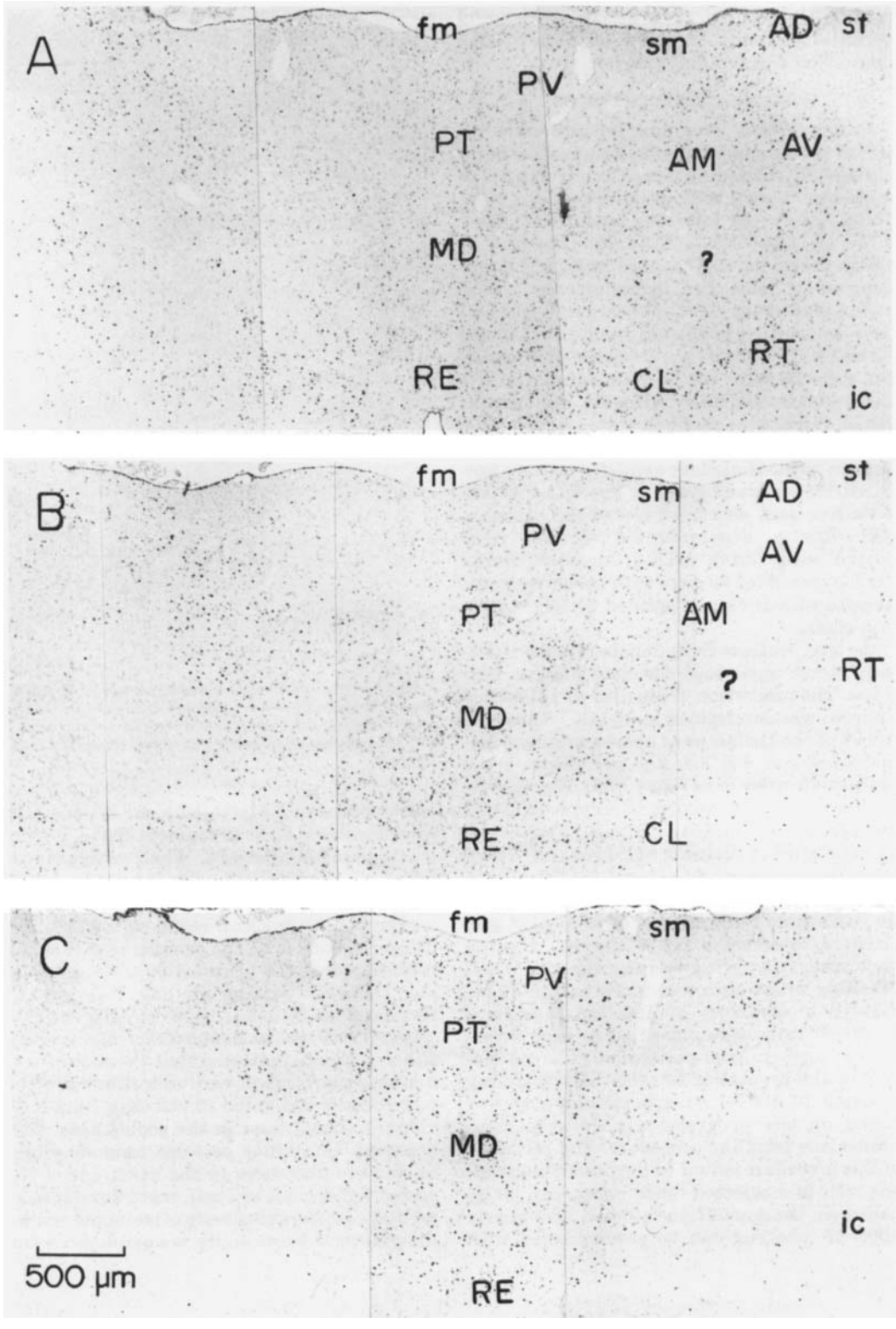
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Figure 1

made with the single injection procedure, and permits to specify the birth dates of all the neurons of a given brain region.

MATERIALS AND METHODS

Purdue-Wistar pregnant females were injected subcutaneously with 2 successive daily doses of ^{3}H-thymidine (specific activity, 6.0 c/mM; dose, 5 μc/g body weight) between 9:00-11:00 A.M. on the following gestational ages: E13+14, E14+15 ... E21+22. (The day of sperm positivity was counted as E1.) The progeny of at least two dams/injection group were killed at the constant postnatal age of 60 days by cardiac perfusion with 10% neutral formalin. The brains were embedded in paraffin, sections were cut at 6 μm serially in the three planes and every fifteenth section was saved. Successive sections were stained with cresyl violet and hematoxylin-eosin for examination without nuclear emulsion or were prepared for autoradiography. The latter procedure has been described elsewhere (Altman, '64). Briefly, deparaffinized sections were coated with Kodak NTB-3 emulsion in the dark, exposed for 90 days with a desiccant, developed with D-19, and stained with hematoxylin-eosin.

Coronal sections from 6 male rats per injection group were used for quantitative purposes. The proportion of labelled to unlabelled neurons was determined in identified areas or nuclei of the thalamus at approximately 5 levels (A6.0, 5.0, 4.2, 3.4, 2.6) according to the stereotaxic atlas of de Groot ('59). We consulted also the atlas of König and Klippel ('63) and Pellegrino and Cushman ('67) of the rat brain, and the atlas of Slotnick and Leonard ('75) of the mouse, and made several modifications. Each area or nucleus was scanned at 625 × magnification with the aid of an ocular grid oriented to traverse strips through a given structure, at a right angle to gradients of cell labelling where such was indicated. In all instances a minimum of 100 (up to several hundred) cells were classified in each structure at a given level per animal. The estimation of the proportion of cells differentiating (ceasing to divide) on a particular day was based on the progressively delayed comprehensive labelling procedure. The rationale of this procedure is that as long as virtually all the cells of a selected brain region can be labelled (in the populations studied here nearly 100% of labelling can be accomplished with

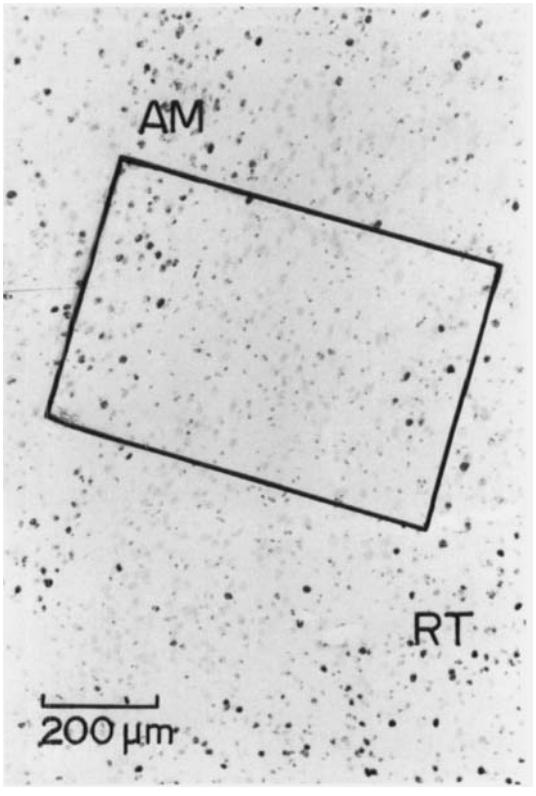

Fig. 2 Unlabelled region situated between AM medially and RT laterally in a rat injected on days E14+15. This region forming before day E14 is possibly a component of RT. The enclosed area is shown at higher magnification in figure 3.

two successive daily injections) all the cells are considered to be precursors that have not started to differentiate. When with delayed onset of injections all cells can no longer be tagged, the proportion of cells that can no longer be labelled as a result of a single day delay is taken to be the complement that differentiated on the previous day. As an example, the cells arising on day E15 are determined as follows: E15 = (15+16) − (E16+17). Previous examination of our quantitative results indicated that the sequence of neuron generation in various nuclei was often more clearly indicated in the data from individual animals than in the pooled data. This apparent variability between animals might be due to differences in the exact age of the pooled individuals or their exact developmental stage (differences were often noted within littermates). Accordingly, we employed a sta-

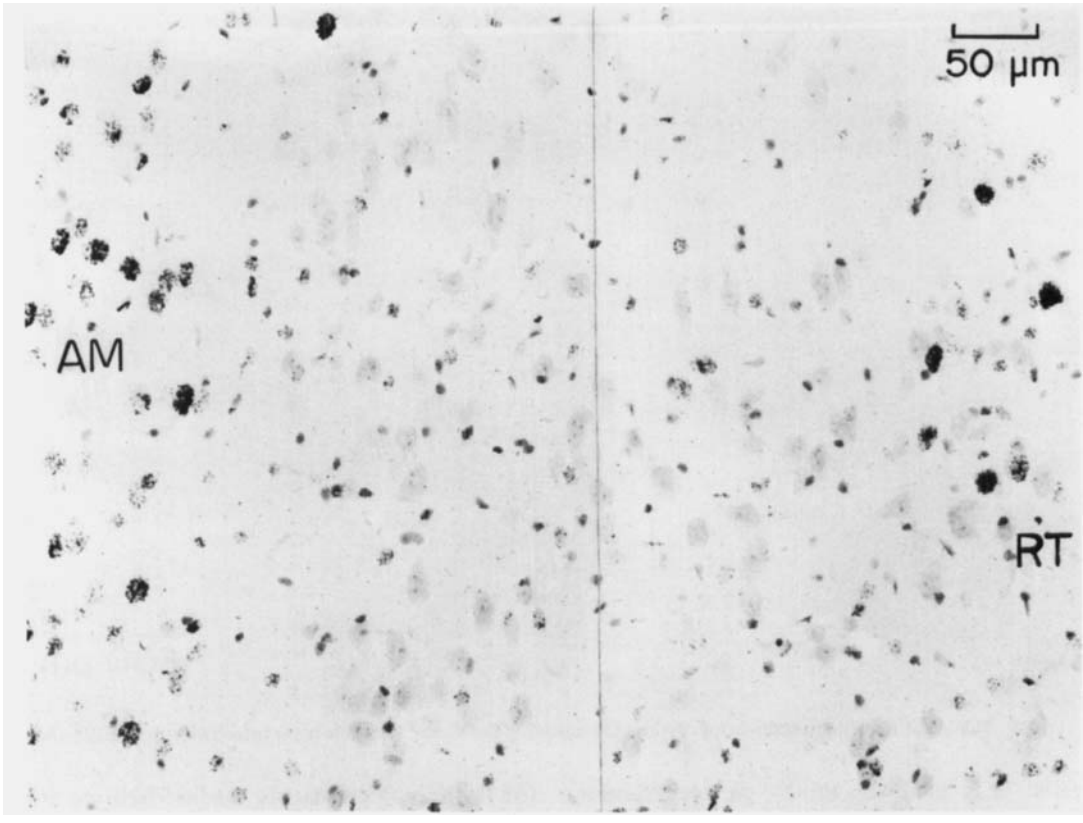

Fig. 3 The intercolated early forming region (portion of RT?) in a rat injected on days E14-15.

tistical procedure, Conover's ('71) sign test, to determine the consistency of sequential neuron production in pairs of diencephalic nuclei regardless of the chronological grouping of the individual animals. This test is based on paired comparisons (X, Y) within individual animals. The comparisons are grouped into three categories: (1) X > Y, "−" comparison; (2) X < Y, "+" comparison; (3) X = Y, "0" comparison. The zero comparisons are discarded and, depending on the total number of remaining "−" and "+" comparisons, either a binomial distribution or a normal approximation is used to calculate probabilities (p's).

The quantitative data obtained about the time of origin of different nuclear groups in the coronally sectioned series allowed us to identify *internuclear* gradients through the thalamus. As a graphic aid to visualize these gradients we attempted to construct "isochronic maps," taking into consideration the time of onset and cessation of cytogenesis.

RESULTS

1. *Rostral coronal levels*

Regional gradients

The changing regional labelling pattern in the rostral thalamus is illustrated in sequentially injected rats (fig. 1). In the autoradiogram from a rat injected on days E14+15 (fig. 1A) heavily labelled cells outline the reticular nucleus laterally. Adjacent to it medially is an unidentified structure with unlabelled cells; it is shown at higher magnification in figures 2 and 3. This appears to be the earliest forming nucleus of the anterior thalamus with its neurons arising before day E14. There follows a narrow band of heavily and lightly labelled cells. This band is composed of the anterodorsal, anteroventral and anteromedial nuclei, and a structure that we presume is the anterior extension of the nucleus centralis lateralis. Finally, there is a large, heart-shaped medial core composed of very lightly labelled

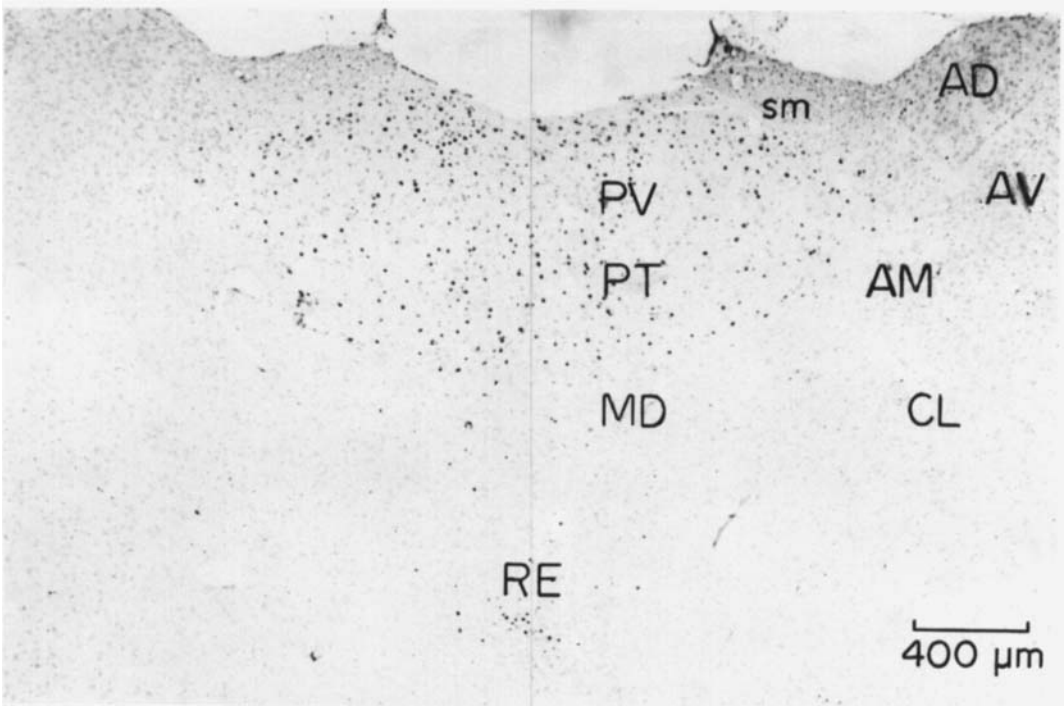

Fig. 4 The latest forming components of the midline nuclei, PV, PT, and RE, from a rat injected on days E17+18.

cells; it is made up of the paraventricular, paratenial, mediodorsal, and reuniens nuclei.

In the E15+16 rat illustrated in figure 1B, only a small proportion of the reticular nucleus neurons are labelled and none is labelled in the E16+17 rat (fig. 1C). This suggests that the neurons of the reticular nucleus are acquired by day E15; it seems to be the second structure to form rostrally in the thalamus. The neurons of the anterodorsal, anteroventral, anteromedial and central lateral nuclei are heavily labelled in the E15+16 animal (fig. 1B) and still contain many labelled cells in the E16+17 rat (fig. 1C). But cell labelling is no longer seen in the E17+18 rat illustrated in figure 4, suggesting that neurons of these nuclei form by day E16. This belt of nuclei may be the third system to arise at this level. In the E16+17 animal (fig. 1C) the medial core of the thalamus is formed of intensely labelled cells. But the neurons of the mediodorsal nucleus are no longer labelled in the E17+18 animal (fig. 4), suggesting an abrupt cessation of cytogenesis on day E16. The dorsally situated paraventricular and paratenial nuclei have many labelled cells in the E17+18 animal, as does the reuniens nucleus (fig. 4); the latter may constitute the last forming nuclei of the anterior thalamus. Few or no labelled neurons are seen in these nuclei in E18+19 animals, hence cytogenesis must come to an end at this level on day E17. The five heterochronic regions of the rostral thalamus are outlined on the low-power autoradiogram in figure 5.

Quantitative data

Group data of the proportions of neurons arising daily in nine nuclei of the rostral levels of the thalamus are summarized in figure 6. For diagrammatic purposes, we classified the nuclei at this and subsequent levels (figs. 10, 13) into six categories (fig. 6). Paired comparisons with the sign test showed that the reticular nucleus forms significantly earlier than all other nuclei at this level ($p = < 0.0001$). There were no differences between (a) the anterodorsal, anteroventral and anteromedial nuclei; (b) the rhomboid and reuniens nuclei; and (c) the paraventricular and paratenial nuclei. Each nucleus in group (a) differed significantly from each nucleus in group (c) with p levels at or below < 0.0070. The rhomboid and reuniens nuclei differed signif-

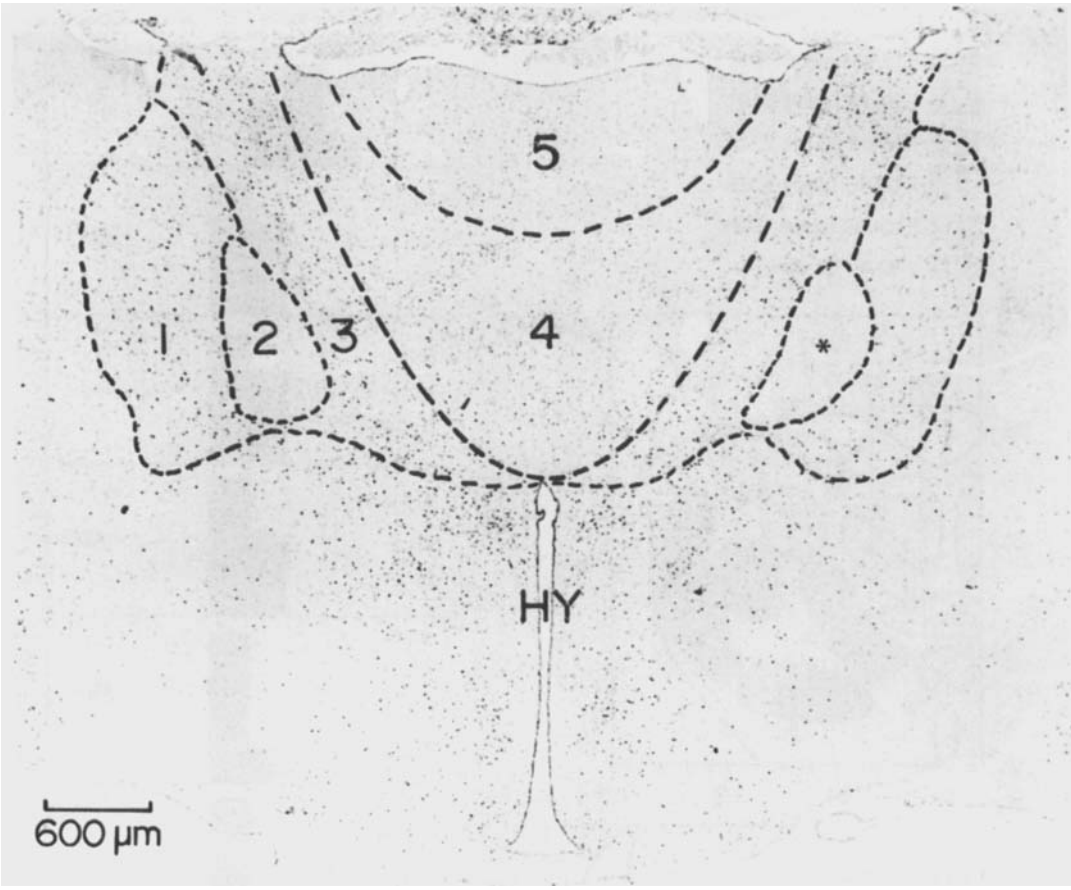

Fig. 5 Five distinguishable isochronic regions in the rostral portion of the thalamus from a rat injected on days E14+15: 1, early-forming RT (heavily labelled); 2, earliest forming intercalated region (RT?, unlabelled); 3, somewhat later-forming AV, AD, AM and CL (heavily labelled); 4, slightly later-forming MD (lightly labelled); 5, the latest forming PV and PT (very lightly labelled). Region with star shown at higher magnification in figure 2.

icantly from the mediodorsal nucleus ($p = <0.0391$ and <0.0020, respectively).

2. *Mid-coronal levels*

Regional gradients

At the level of the rostral portion of the habenular nuclei practically all nuclei are composed of heavily or lightly labelled neurons in the E14+15 rat shown in figure 7A. The exception is the zona incerta of the ventral thalamus which has many unlabelled cells. In the E15+16 rat (fig. 7B), there are few labelled cells in the zona incerta suggesting that its formation is nearly completed by day E14. It is the earliest forming structure at this level. In the E15+16 animal there is considerable reduction in labelled cells in the lateral habenular nucleus and in the lateral portion of the ventrolateral complex. Heavily labelled cells abound in the ventrolateral complex medially. In the E16+17 animal (fig. 7C) the neurons of the midline group are heavily labelled but few labelled cells are present in other nuclei. By days E17+18 the mid-dorsally situated paraventricular and medial habenular nuclei are the only structures with many labelled neurons (fig. 8), constituting the last forming nuclei at this level.

More posteriorly, at the level of the caudal portion of the habenular nuclei, the zona incerta is the only structure with a high proportion of unlabelled neurons in the E14+15 rat shown in figure 9A. In the autoradiogram from an E15+16 rat (fig. 9B) the proportion of labelled neurons declines drastically in the lateral habenular nucleus and less pronounc-

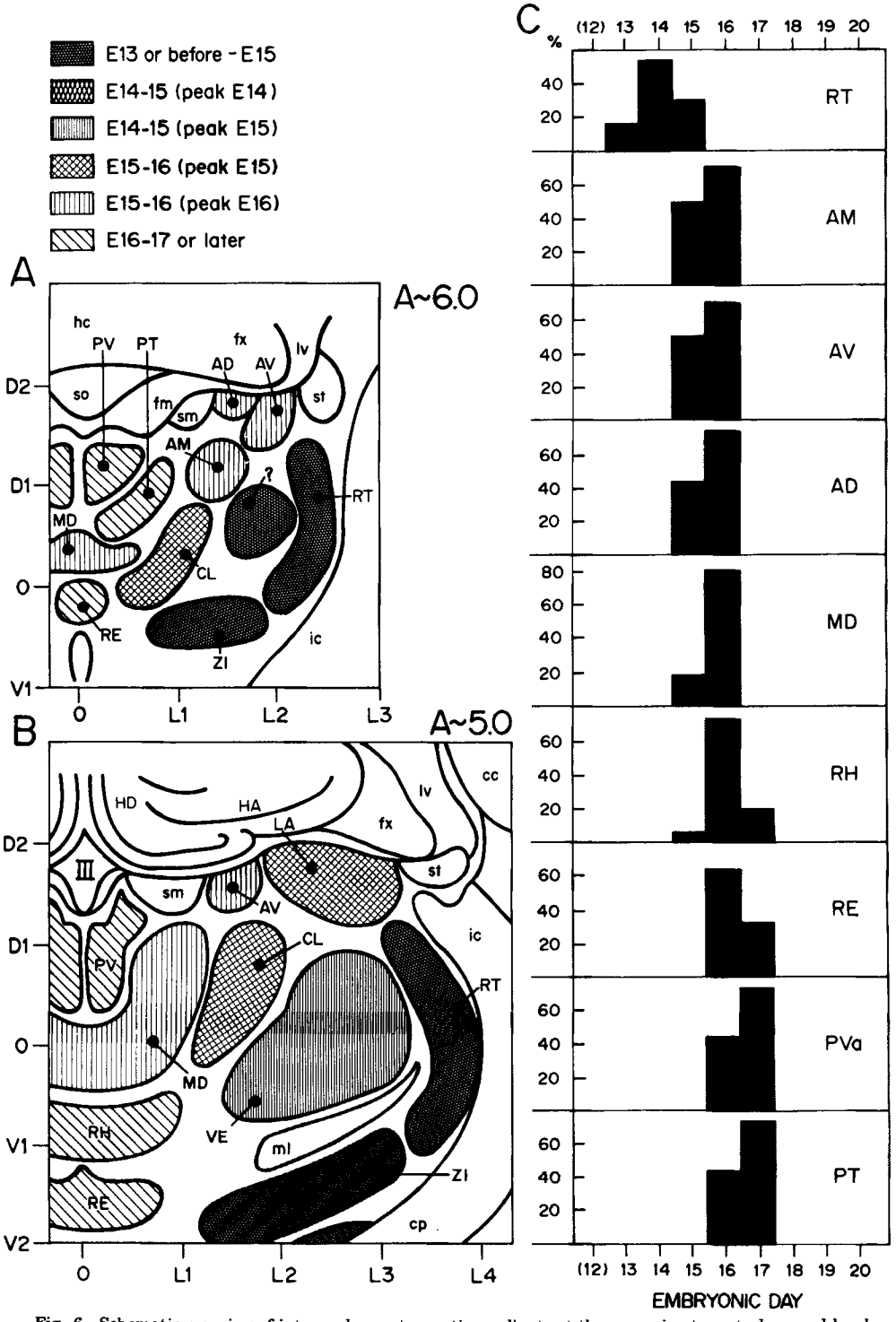

Fig. 6 Schematic mapping of internuclear cytogenetic gradients at the approximate rostral coronal levels of 6.0 (A) and 5.0 (B). The proportion of cells formed on specific days in nine rostral structures is plotted in C.

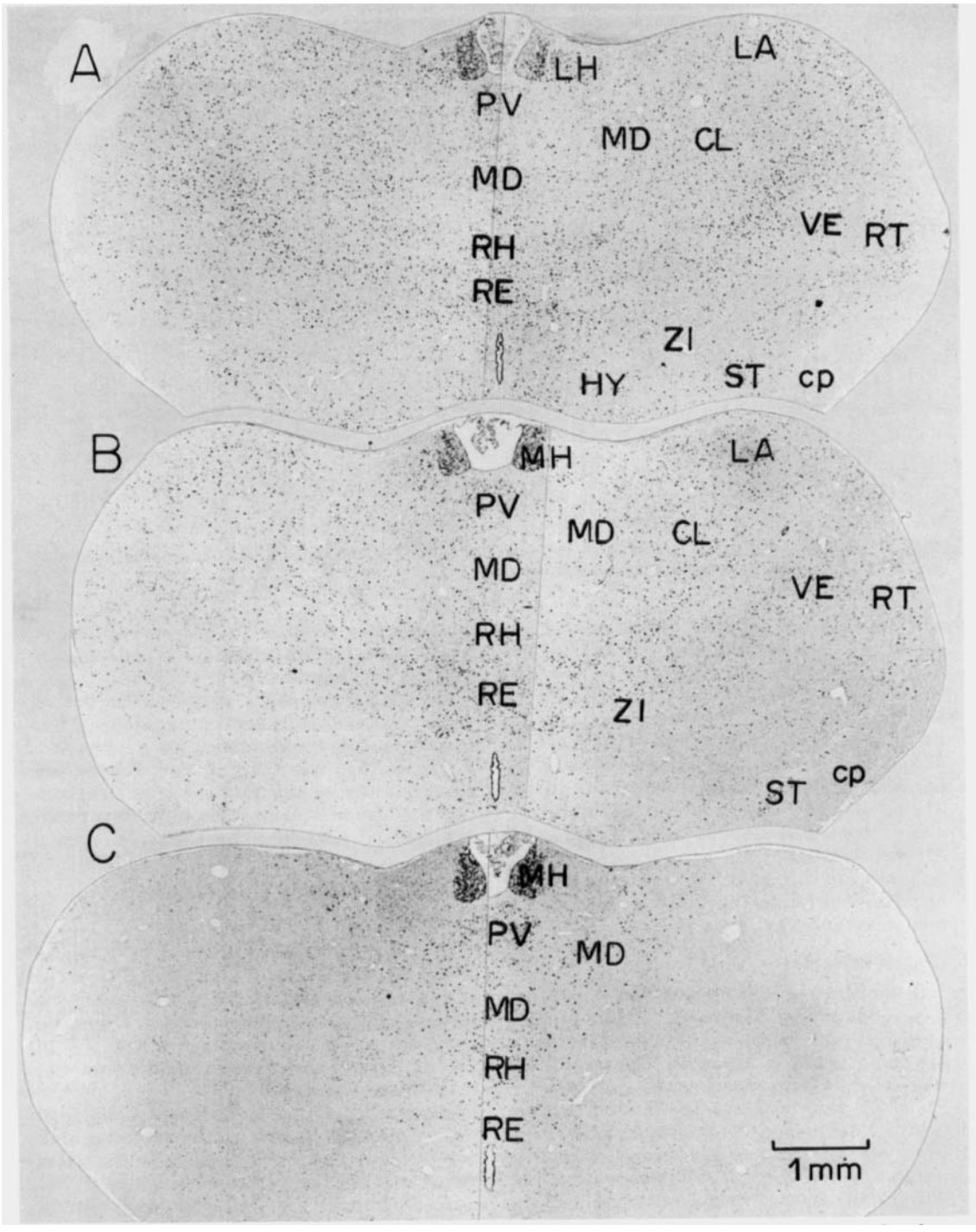

Fig. 7 Changing labelling patterns at the level of the rostral portion of the habenular nuclei in rats injected on days E14+15 (A), E15+16 (B), and E16+17 (C). In A, the only region with unlabelled cells is ZI, while lightly labelled cells are seen only in the midline nuclei (PV, MD, RH and RE). In B few of the RT and LH cells are labelled, and there may be a reduction in labelled cells in the lateral band formed by LA and VL. In C, extensive cell labelling is essentially restricted to the midline nuclei (PV, MD, RH and RE) and to MH.

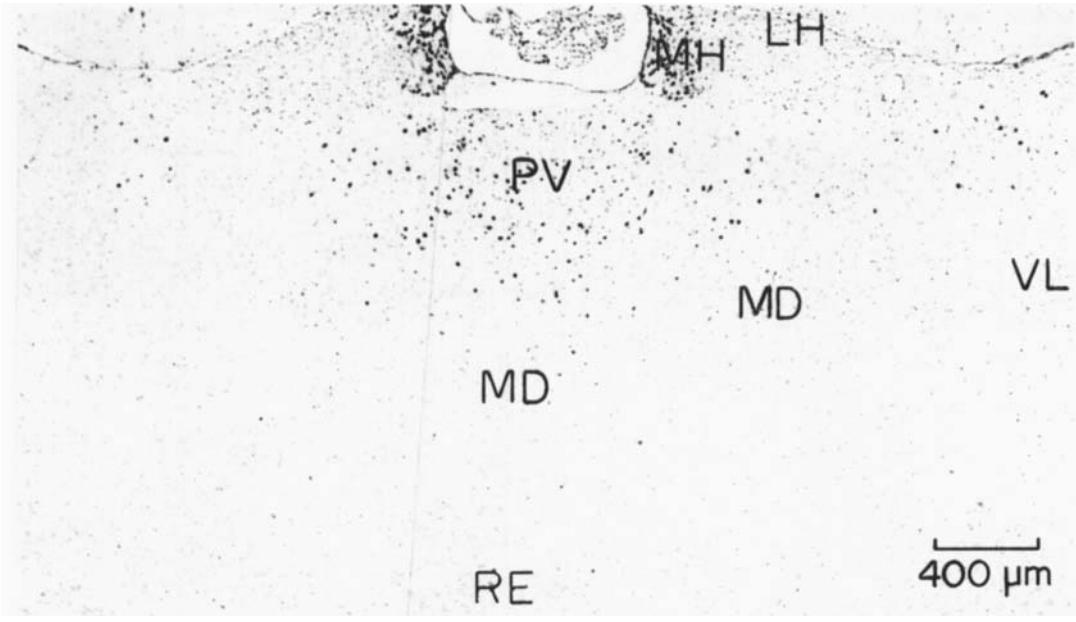

Fig. 8 At the level of the rostral habenular nucleus in this E17+18 animal neuronal labelling remains pronounced only in PV and MH. A few cells are labelled in MD and RE.

edly in the pars posterior of the lateral nucleus, the pars dorsalis and ventralis of the lateral geniculate body, and the ventrobasal complex. Labelled cells are still seen in the subthalamic nucleus. In these structures labelled cells are no longer seen in the E16+17 animal (fig. 9C), but they are abundant in the paraventricular and medial habenular nuclei. The parafascicular nucleus and the lateral nucleus have a few labelled cells. As we noted earlier, labelled cells are present in the E17+18 rat (fig. 8) in the medial habenular and paraventricular nuclei, representing the last structures that form at this level.

Quantitative data

Group data of the proportions of neurons formed daily, at midcoronal levels, in nine components of the thalamus and subthalamus are summarized in figure 10. Neurons of the zona incerta form significantly earlier ($p = <0.0002$) than neurons of the subthalamic nucleus; neurons of the lateral habenular nucleus precede those of the medial habenular nucleus ($p = <0.0001$). There was no difference in the time of origin of neurons of the ventrolateral and ventrobasal complexes. The ventrolateral complex neurons precede significantly those of the pars posterior of the lateral nucleus ($p = <0.0010$), and the parafascicular nucleus neurons those of the paraventricular nucleus neurons ($p = <0.0005$).

The long time span of neuron formation in the lateral and medial habenular nuclei differ markedly from the short time span of thalamic nuclei. For the sake of convenience, we classified the earlier forming lateral habenular nucleus with class 1 nuclei, the late forming medical habenular nucleus with class 6 nuclei.

3. *Caudal coronal levels*

Regional gradients

In the animal injected on days E14+15 shown in figure 11A practically all neurons are labelled in the caudal thalamus at the level of the subcommissural organ. There is a reduction in labelled neurons in the E15+16 rat (fig. 11B) in the pars dorsalis and pars ventralis of the lateral geniculate nucleus and the pars posterior of the lateral nucleus. Labelled cells are still abundant in the pretectal area and the paraventricular nucleus. In the latter structures labelled cells are still seen in the E16+17 animal (fig. 11C), but no longer in E17+18 animals. Apparently the latest forming midline system (fig. 8) does not reach this far caudally, unless the labelled subcommis-

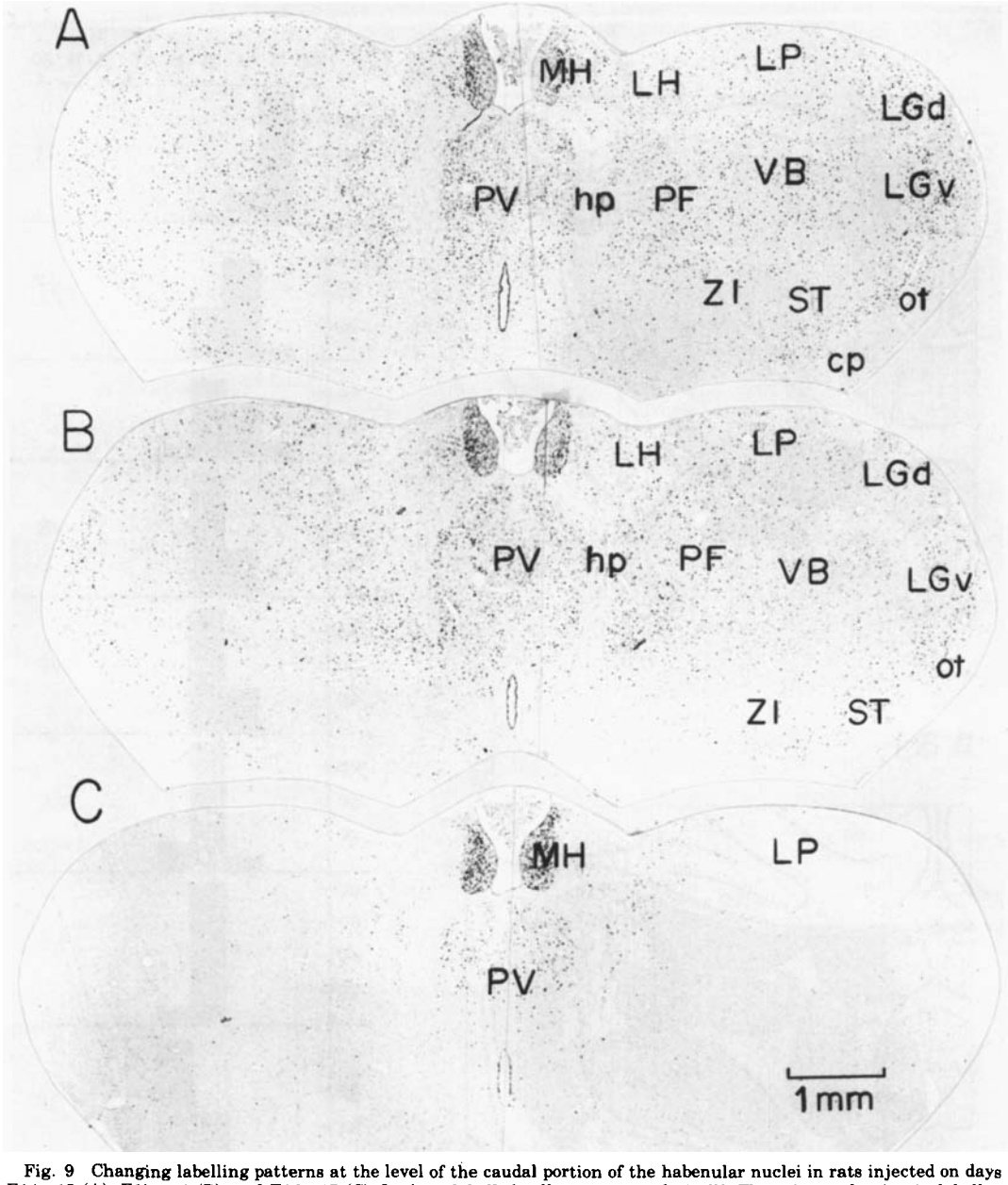

Fig. 9 Changing labelling patterns at the level of the caudal portion of the habenular nuclei in rats injected on days E14+15 (A), E15+16 (B), and E16+17 (C). In A, unlabelled cells are seen only in ZI. There is a reduction in labelled cells in most laterally situated structures in B. In C, only the dorsomedially situated MH and PV are composed predominantly of labelled neurons.

sural organ represents a portion of that system.

At the level of the superior colliculus most of the neurons of the medial geniculate body are labelled in the E14+15 rat (fig. 12A). But few neurons are labelled in the E15+16 animal except at the medial boundary of the nucleus (fig. 12B). This indicates that the medial geniculate body is the earliest arising structure in the group of thalamic relay nuclei.

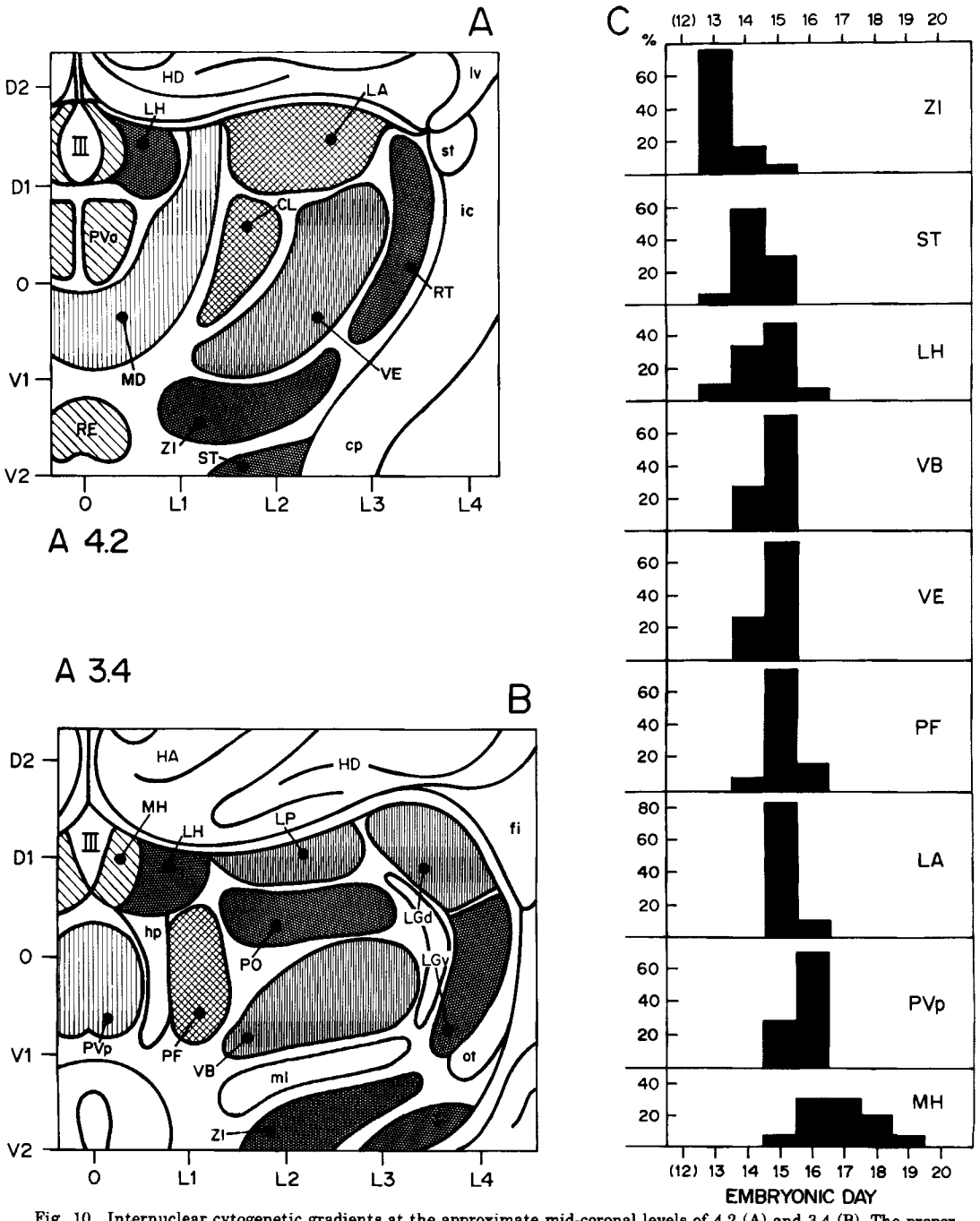

Fig. 10 Internuclear cytogenetic gradients at the approximate mid-coronal levels of 4.2 (A) and 3.4 (B). The proportion of cells formed on specific days in nine mid-coronal structures is plotted in C.

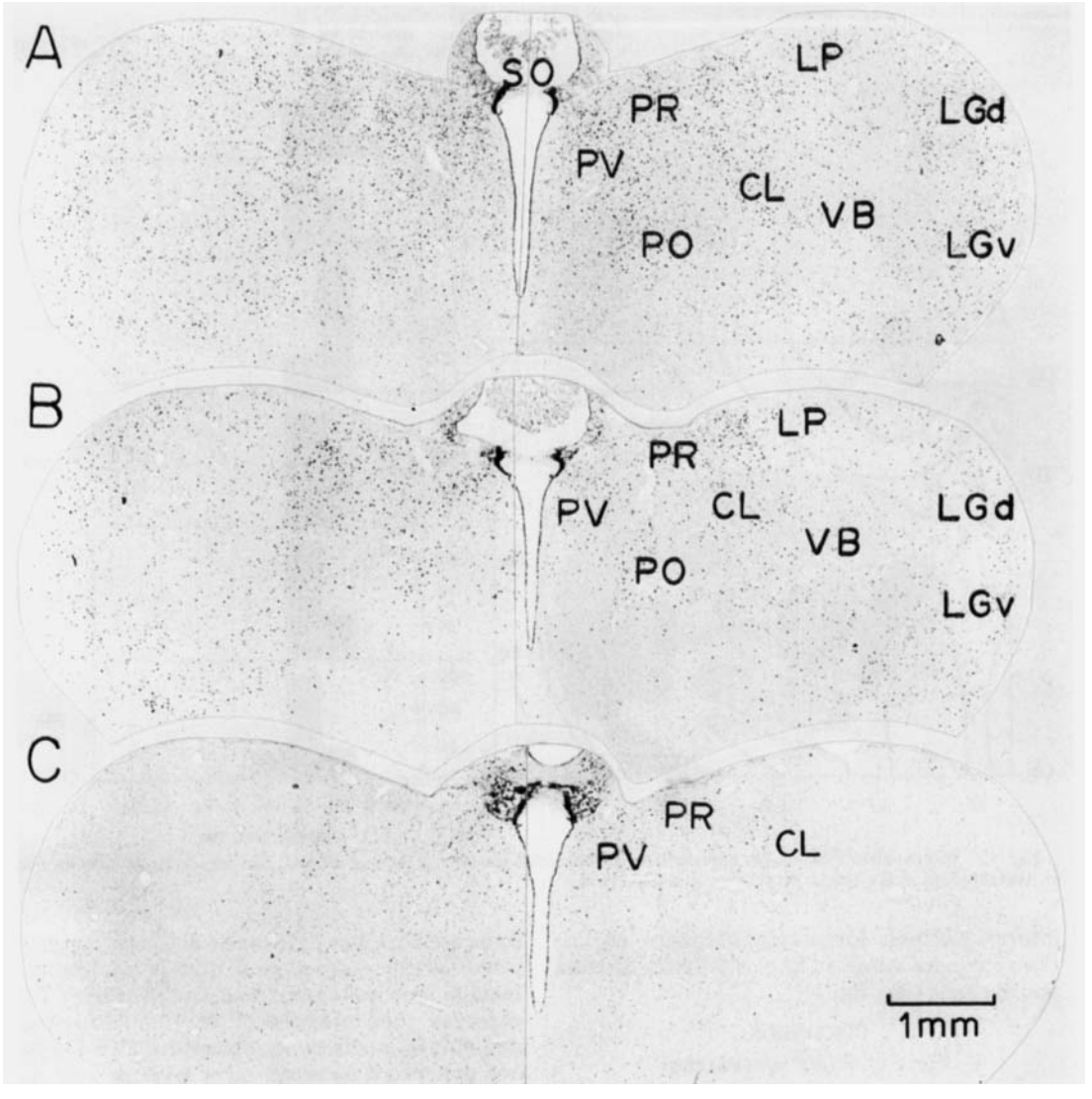

Fig. 11 Changing labelling patterns in the posterior portion of the thalamus at the level of the subcommissural organ in rats injected on days E14+15 (A), E15+16 (B), and E16+17 (C). Essentially all structures of the thalamus are composed of labelled cells in A; there is a reduction in labelled cells laterally in B, and only scattered labelled cells are seen (except in the posterior portion of the habenular nucleus and the subcommissural organ) in C. A comparison of figures 1C, 7C, 9C and 11C indicates a rostrocaudal narrowing of the late-forming medial thalamus.

Quantitative data

The earliest arising structure at these levels is the posterior nucleus (fig. 13) which antedates significantly the pars ventralis of the lateral geniculate nucleus ($p = <0.0063$). The neurons of the pars ventralis form ahead of the neurons of the pars dorsalis of the lateral geniculate body ($p = <0.0129$). The neurons of the medial geniculate body arise before those of the dorsal lateral geniculate body ($p = <0.0002$); but the latter precede the neurons of the pretectal area ($p = <0.01$). There were no significant differences between the pars posterior of the lateral nucleus and either the dorsal lateral geniculate nucleus or the pretectum. However, the pars posterior of the

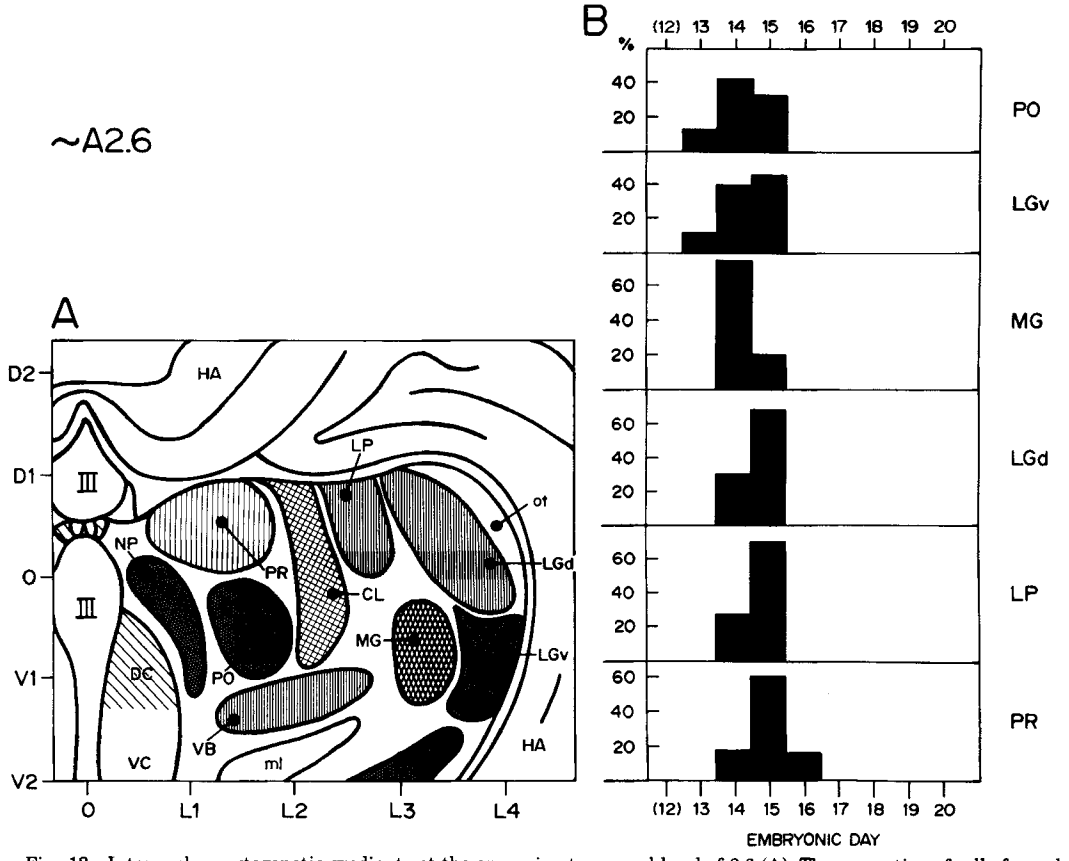

Fig. 12 Internuclear cytogenetic gradients at the approximate coronal level of 2.6 (A). The porportion of cells formed on specific days in six caudal structures is plotted in B.

lateral nucleus forms significantly earlier than the pars anterior ($p = <0.0002$; Altman and Bayer, '79a: fig. 1).

DISCUSSION

Time of origin of neurons

The progressively delayed comprehensive labelling procedure allowed us to specify quantitatively the proportion of neurons formed on specific days in the major nuclei of the epithalamus, thalamus and subthalamic region. This has not been possible with the single injection procedure used in earlier investigations that relied on the daily number or proportion of heavily labelled cells (which rarely, if ever, account for the total cell population of a nucleus) as indicators of the imminent cessation of cell proliferation (onset of differentiation). The estimates made with this improved technique differ from some published datings but, surprisingly, in other instances there was little or no discrepancy with previous estimates of peak days of neuron formation. For instance, Lund and Mustari ('77) reported that neurons of the dorsal lateral geniculate nucleus in Sprague-Dawley rats are generated between days E12-14. Our results in another albino strain (Purdue-Wistar), however, showed that neuron differentiation is only begining on day E14 and that nearly 70% of the neurons of this nucleus form on day E15. Our numerical results in this case are close to the estimates of McAllister and Das ('77) who reported days E14-16 as the time span, and day E15 as the peak time for the origin of dorsal lateral geniculate neurons. But in the case of some other nuclei, there are discrepancies between our results and the datings of McAllister and Das, who used a single injection on successive days. For instance, they reported day E15 as maximal labelling date for the zona incerta, reticular nucleus

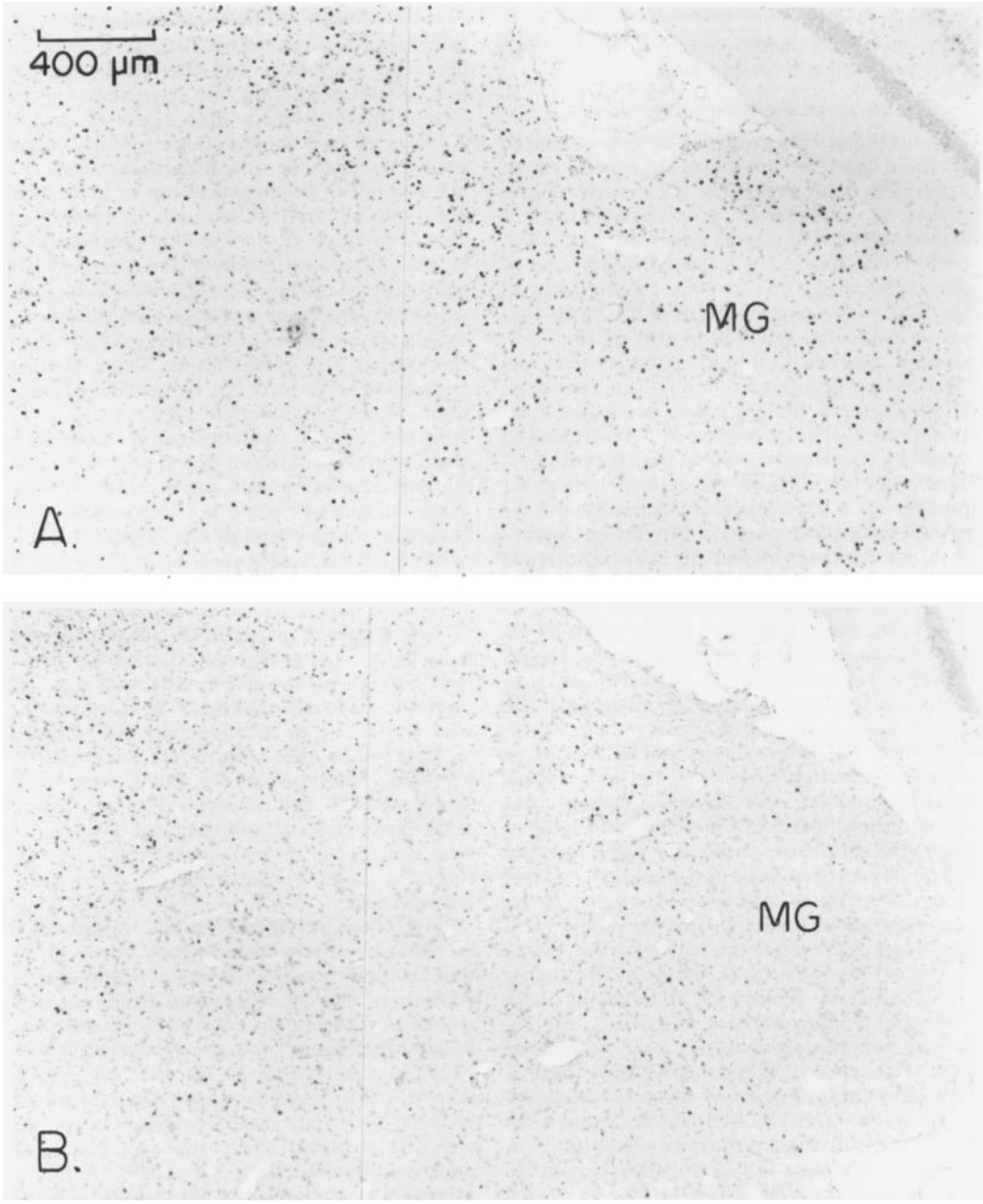

Fig. 13 Labelling patterns at the level of the superior colliculus. Most cells are labelled in MG in the rat injected on days E14+15 (A), but only the medial strip of MG is labelled in the rat injected on days E15+16 (B).

and subthalamic nucleus, whereas, we get day E13 as the peak formation time for the zona incerta (with 80% of the neurons differentiating), and day E14 for the reticular nucleus (50%) and subthalamic nucleus (60%). According to our data, these ventral thalamic nuclei are not isochronic with any of the nuclei of the dorsal thalamus, for instance, the dorsal later-

al geniculate nucleus, the ventrolateral complex, and ventrobasal complex, whose peak formation time *is* on day E15.

Gradients in the time of neuron origin

In his pioneering study of the time of neuron origin in the diencephalon of the mouse, Angevine ('70) described three chronological gradients: a ventrodorsal, a caudorostral and a lateromedial. The latter gradient was interpreted to be due to the lateral displacement of earlier forming cells by cells arising later in the midline neuroepithelium of the third ventricle. Angevine referred to this as the "outside-in" pattern and contrasted it with the "inside-out" gradient noted in the neocortex (Angevine and Sidman, '61). Angevine mentioned as a clear example of a lateromedial gradient the pattern seen in the epithalamus, where the neurons of the lateral habenular nucleus arise largely before the neurons of the medial habenular nucleus. The lateral-to-medial internuclear gradient in the epithalamus of the rat was also clearly indicated in our material both qualitatively (fig. 9) and quantitatively (fig. 10). As we shall show in the subsequent paper of this series (Altman and Bayer, '79a), a lateral-to-medial *intra*nuclear gradient is also present within the lateral and the medial habenular nuclei. However, an examination of our illustrations and quantitative data show that unequivocal lateral-to-medial, ventral-to-dorsal, and caudal-to-rostral gradients do not exist in the dorsal and ventral thalamus. Granted, there are early-forming lateral structures (reticular nucleus), ventral structures (zona incerta) and caudal structures (posterior nucleus). But the early forming lateral habenular nucleus with respect to the later forming lateral portion of the mediodorsal nucleus, represents an inversion of a lateral-to-medial gradient (fig. 10A); the reuniens and rhomboid nuclei with respect to the middle portion of the mediodorsal nucleus represents an inversion of the ventral-to-dorsal gradient (fig. 6B) and the pretectal area with respect to lateral nucleus, pars posterior, is an example of an inversion of the caudal-to-rostral gradient (figs. 10, 12).

Earlier investigators tended to look for universal gradients in the central nervous system as a reflection of some fundamental principle of development. Our effort has been, instead, to examine the specific gradients of different regions, or their absence, as a means of obtaining clues about their site of origin, route of dispersal or migration, and structural transformations after settling. We do this by starting out with Angevine's idea that in nuclear structures, such as the diencephalon, earlier forming neurons are displaced from the vicinity of the neuroepithelium by later forming neurons. In the simplest instance, this should produce an "outside-in" gradient, the oldest cells being farthest and the youngest cells nearest to their site of production. If the cells were produced medially, this should produce a simple lateral-to-medial gradient, as is the case in the epithalamus. Using this approach we infer that the two nuclear components of the epithalamus arise in two waves from the superior neuroepithelial lobule of the third ventricle (Altman and Bayer, '79b). But we may also infer that, for instance, the mediodorsal nucleus could not have arisen from the same neuroepithelial site because it is laterally situated with respect to, but forms later than, the lateral habenular nucleus. But we need not expect a lateral-to-medial gradient in all diencephalic structures; either because some may arise in regions other than the medial portion of the third ventricle, e.g., the transitional regions between the third and lateral ventricles, or because they may migrate in from other sites. Moreover, the farther a structure settles from its site of origin, the more afferent and efferent fiber tracts traverse in its vicinity, and the more neighbors it has, each with its own growth pattern, the more its linear gradient will be distorted or transformed.

With these problems in mind, we may have to accomplish two tasks before we could undertake to determine the site of origin of components of the hypothalamus. First, we have to reduce the multiplicity of structures to a smaller number of "cytogenetic systems" with common ancestral sites. Second, we have to identify the corresponding "neuroepithelial mosaics" in embryonic material as we attempted in the case of the hypothalamus (Altman and Bayer, '78b). We shall attempt to present a classification of cytogenetic systems in the next paper (Altman and Bayer, '79a) in which we will present data about intranuclear gradients in various components of the suprahypothalamic diencephalon. The attempt to relate these cytogenetic systems to neuroepi-

thelial sites identified in embryonic material will be described in the subsequent paper (Altman and Bayer, '79b).

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