Development of the Diencephalon in the Rat

II. CORRELATION OF THE EMBRYONIC DEVELOPMENT OF THE HYPOTHALAMUS WITH THE TIME OF ORIGIN OF ITS NEURONS

JOSEPH ALTMAN AND SHIRLEY A. BAYER
Laboratory of Developmental Neurobiology, Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907

ABSTRACT The development of the nuclei of the hypothalamus was examined in normal and X-irradiated embryos from day 13 (E13) to the day before birth (E22). The diencephalic neuroepithelium was subdivided into three lobes (dorsal, medial, and ventral) and two lobules (superior and inferior). The hypothalamus is derived from the ventral lobe and the inferior lobule. The ventral neuroepithelial lobe generates the neurons of most of the early arising hypothalamic structures, including those of the lateral tier nuclei associated with the medial forebrain bundle, and the heterogeneous intermediate tier nuclei. A specialized neuroepithelial region lining the diamond shaped ventricle produces the early neurohypophysial magnocellular neurons; the neurons of the paraventricular nucleus remain at this site, whereas the neurons of the supraoptic nucleus could be traced migrating laterally. The neurons of the late arising hypophysiotropic area of the posterior hypothalamus are derived from components of the inferior neuroepithelial lobule: the dorsomedial and ventromedial nuclei apparently from a shared matrix in the main portion of the inferior lobule; the tuberomammillary-arcuate complex from its posteroventral recess. The triple-decked and sequentially produced components of the mammillary system may arise from separate neuroepithelial sites. The autoradiographic results of the previous study (Altman and Bayer, '78a) showed that the structural and functional heterogeneity of the mature hypothalamus is paralleled by cytogenetic heterochronicity; the present embryonic observations indicate that many of the distinguishable components of the hypothalamus arise from a mosaic of heterogeneous neuroepithelial sites.

Since the pioneering studies of His (1893, '04) the development of the diencephalon has been investigated by many workers, for its intrinsic interest and as an aid for the rational parcellation of this complex region of the brain. His claimed that he could distinguish between alar and basal plates in the diencephalon, and proposed that the hypothalamus is derived from the basal plate, the rest of the diencephalon from the alar plate. Herrick ('10) subdivided the diencephalon into four *horizontal* zones (metathalamus, dorsal thalamus, subthalamus and hypothalamus) on the basis of the presence of three sulci in the neuroepithelium of the third ventricle of amphibian embryos, the sulcus dorsalis, medius and ventralis. Herrick's schema was widely accepted because it appears applicable to the developmental pattern of the diencephalon in practically all vertebrates, including man (Gilbert, '35; Cooper, '50; Kuhlenbeck, '54; Kahle, '56; Coggeshall, '64; Christ, '69). The hypothalamus has been subdivided on the basis of adult cytoarchitecture and connections (Nauta and Haymaker, '69) into three *sagittal* zones, the lateral, medial and periventricular. The lateral zone includes the lateral preoptic and hypothalamic areas that are traversed by the medial forebrain bundle; the medial zone includes the medial preoptic, anterior hypothalamic, ventromedial, dorsomedial, and premammillary nuclei; the periventricular zone consists of several nuclei bordering the third ventricle, stretching from the

preoptic periventricular area rostrally to the arcuate nucleus caudally.

The embryology of the mammalian hypothalamus in general and of the rat hypothalamus in particular have been neglected subjects. Brief general descriptions are provided by Rose ('42), Luyendijk ('44), Auer ('51), Ströer ('56), and Coggeshall ('64); others have been concerned with the development of the median eminence and pituitary gland (Glydon, '57; Rinne and Kiváló, '65; Campbell, '66; Jost et al., '70; Fink and Smith, '71; Daikoku et al., '71; Halász et al., '72; Monroe and Paull, '74). Some histochemical observations were made on the development of the endocrine hypothalamus by Schachenmayr ('67), Hyyppä ('69) and Pilgrim ('67, '74). Our purpose was to correlate the embryonic development of the hypothalamus of the rat with the autoradiographic data described in the previous paper (Altman and Bayer, '78a). The development of the specialized linings of the hypothalamic third ventricle will be described in the succeeding paper (Altman and Bayer, '78b).

MATERIALS AND METHODS

Dated, sperm-positive Purdue-Wistar female rats were used. The day of sperm positivity was counted as embryonic day 1 (E1). Fetuses from control females were undisturbed prior to their removal while those from the experimental females were exposed to a single dose of 200R X-ray from a Maxitron 300 kV unit six hours earlier. Fetuses from one or more control and experimental females were removed at daily intervals between days E13-E22 and immersed in Bouin's fluid for 24 hours. A total of 261 fetuses were prepared and examined.

The majority of these fetuses or dissected brains were embedded in paraffin and sectioned in the sagittal, coronal and horizontal planes at 6 μm. All sections were saved in fetuses aged E13-E14; every fifth section in fetuses aged E15-E16; every tenth section in fetuses aged E17-E22. Alternate sections were stained with cresyl violet and hematoxylin-eosin. A few fetuses were embedded in methacrylate and cut on a Sorvall Porter-Blum JB-4 microtome at 3 μm; these were stained similarly.

The most symmetrically cut and best preserved sagittal, coronal and horizontal sections of the diencephalon were examined microscopically and photographed at selected intervals. The prints were aligned and attached to each other to form long folding strips. This arrangement allowed us to compare the structure of the hypothalamus in different planes of sectioning within the same ages and across different ages in both normal and experimental embryos.

RESULTS

(a) *Overview of diencephalic development*

In an attempt to relate the embryonic development of the hypothalamus to the rest of the diencephalon an overview is presented first of the ontogeny of the whole region. To aid the identification of presumptive or developing regions, use has been made here of autoradiographic data that will be documented in detail in later publications of this series.

The embryonic development of the diencephalon of the rat from day E13 to E18 is illustrated in figure 1. The dorsal, medial and ventral sulci (Herrick, '10) are recognizable in midcoronal sections on day E13 (fig. 1A). As an alternative description, the *trilobed* diencephalon of this stage of development may be subdivided into *dorsal, middle* and *ventral lobes.* A mantle layer (differentiating zones) is not clearly discernible at this age, except perhaps incipiently at the boundary of the middle and ventral lobes. In anterior sections a small dorsal protuberance is present in some embryos. This protuberance is seen in all day E14 embryos, together with a more caudally situated ventral outpouching (fig. 1B). These two neuroepithelial regions will be referred to as the *superior* and *inferior lobules.* On day E14 differentiating zones are seen laterally, flanking the middle lobe and the upper aspect of the ventral lobe. These are the earliest differentiating cells of the diencephalon, representing the formative zona incerta and the lateral hypothalamic area, respectively.

Major changes take place on day E15 and the diencephalon assumes, in coronal sections, a *flask-shape* (fig. 1C). The ventricle constricts along the dorsal and middle lobes and they are flanked by differentiating zones. In the latter region the first pronounced nuclear condensation is seen laterally; on the basis of autoradiographic data the region is tentatively identified with the settling cells of the zona incerta (whose cells form predominantly on day E14). Lesser ventricular constriction occurs along the ventral lobe, where there is an expanding differentiating zone ventrolaterally. The dorsoventral dimensions of the dorsal and ventral lobes have grown with respect to the middle lobe. Finally, differentiating zones

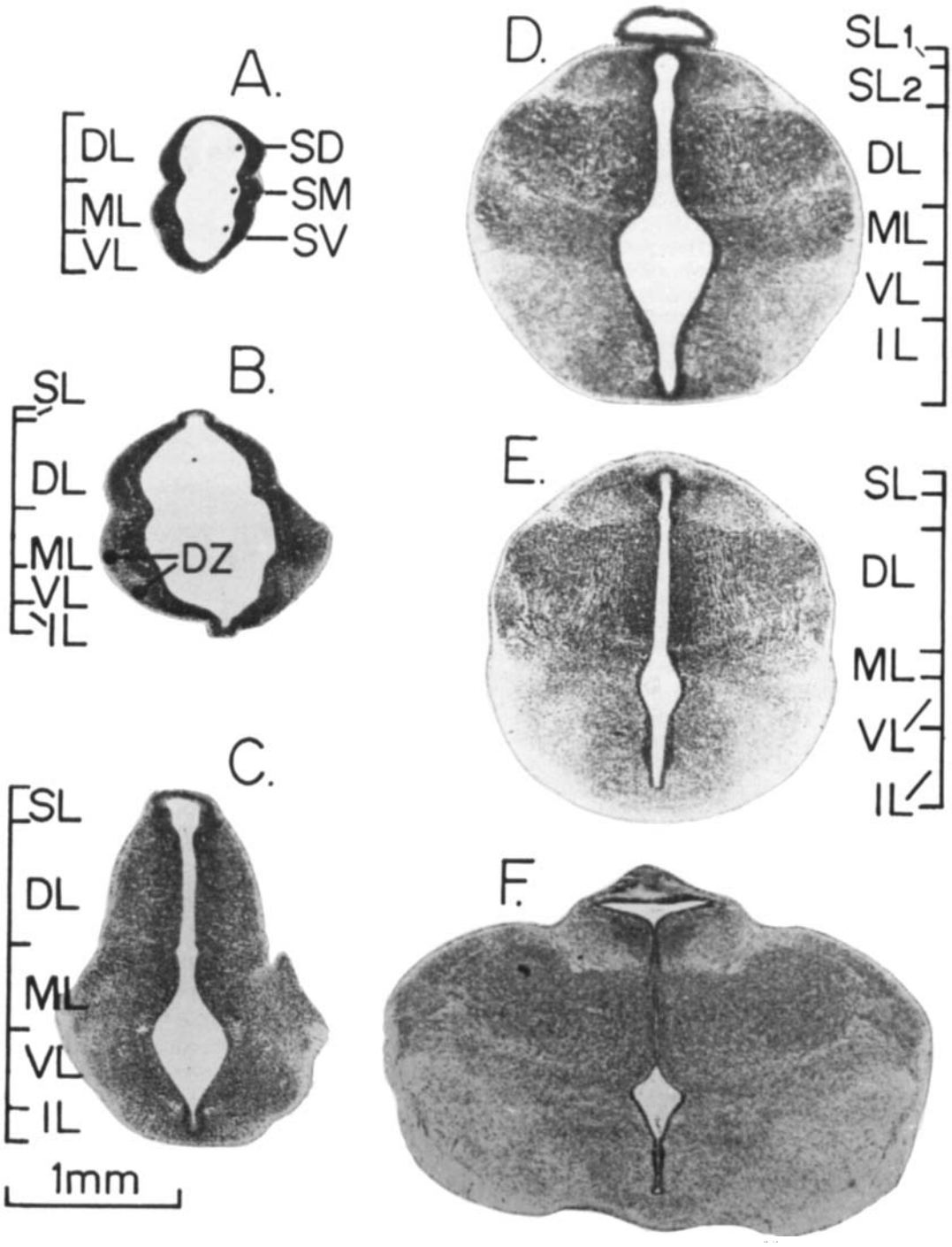

Fig. 1 Midcoronal sections through the diencephalon at the following embryonic ages: E13 (A), E14 (B), E15 (C), E16 (D), E17 (E), and E18 (F). Note the changes in the relative size of the neuroepithelial subdivisions, reflecting chronological differences in the generation of cells of different subdivisions of the diencephalon. *Abbreviations:* DL, dorsal neuroepithelial lobe; DZ, differentiating zone; IL, inferior neuroepithelial lobule; ML, middle neuroepithelial lobe; SD, sulcus dorsalis; SL, superior neuroepithelial lobule (with 2 sublobules); SM, sulcus medius; SV, sulcus ventralis; VL, ventral lobe.

begin to appear along the slightly grown superior and inferior lobules.

On day E16 the diencephalon becomes *disc-shaped* (fig. 1D). This is accounted for by the outflow of a large population of differentiating cells from the dorsal lobe neuroepithelium; on the basis of autoradiographic evidence we identify these cells as the neurons of the thalamic sensory relay nuclei (which form predominantly on day E15). The superior lobule lengthens during this period and becomes subdivided into two sublobules. The ventral lobule is now flanked by nuclear condensation, which may be identified with the lateral hypothalamus, and the inferior lobule has grown in depth and is surrounded by differentiating cells.

On day E17 (fig. 1E) nuclear condensation appears at the level of the superior lobule, signalling the settling of neurons of the limbic thalamus (which form predominantly on day E16). The lumen of the third ventricle begins to constrict also at the level of the ventral lobe such that it forms a continuous line with the inferior lobule. At this age only three prominent neuroepithelial sites remain: the *dorsal sublobule* of the superior lobule; an active small region at the boundary of the dissolving neuroepithelia of the dorsal and ventral lobes; and the inferior lobule. Evidence will be presented in this and the succeeding paper (Altman and Bayer, '78b) that these regions represent the sites of the latest forming, hypophysiotropic diencephalon. On day E18 (fig. 1F) the midcoronal diencephalon begins to assume its terminal *pumpkin-shape*.

(b) *Early development of the hypothalamic anlage*

In terms of the foregoing description, the hypothalamus is derived from the two basal subdivisions of the embryonic third ventricle, the ventral lobe and the inferior lobule. In horizontal sections cut at the base of the diencephalon in E13 and E14 embryos the lumen of the ventral lobe resembles a flying bird (fig. 2A). The "beak" is beneath the anterior neuropore and the wings spread posterolaterally to form the optic recess. In more dorsally cut sections the ventricle assumes the shape of a cross, the anterior arm constituting the foramen of Monro (fig. 2B). In E13 embryos a differentiating zone begins to appear behind the optic recess; this is easiest to see in irradiated embryos in which unaffected cells surround the medial zone of pyknotic cells (fig. 3A). The

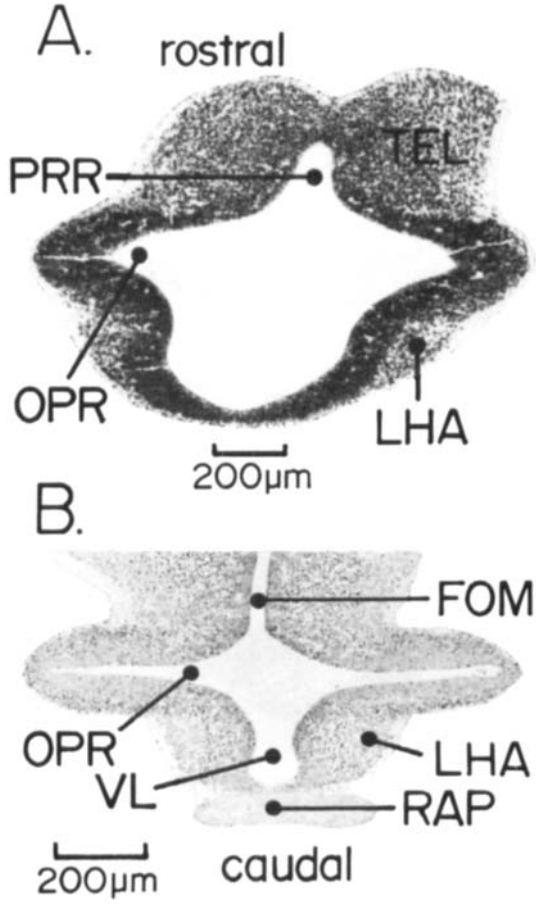

Fig. 2 Horizontal sections through the hypothalamus of a normal E14 embryo (A), and of an X-irradiated E14 embryo (B) at a somewhat more dorsal level than in A. (Shown at higher magnification in fig. 3B). *Abbreviations:* FOM, foramen of Monro; LHA, lateral hypothalamic area; OPR, optic recess of the third ventricle; PRR, preoptic recess; RAP, Rathke's pouch (hypophysial rudiment); VL, ventral neuroepithelial lobe; TEL, telencephalon. A, hematoxylin-eosin; B, cresyl violet.

radioresistant differentiating elements are identified as the early forming neurons of the lateral hypothalamic area (Altman and Bayer, '78a: figs. 6, 7) and they seem to be flanked by the early fibers of the medial forebrain bundle (fig. 3A). These two structures are better developed by day E14 (fig. 3B) by which time the bulk of the neurons of the lateral hypothalamic area have formed.

In day E15 embryos the mammillary recess of the inferior lobule has become an active neuroepithelial site (figs. 4B,C). A small nuclear condensation is recognizable behind the

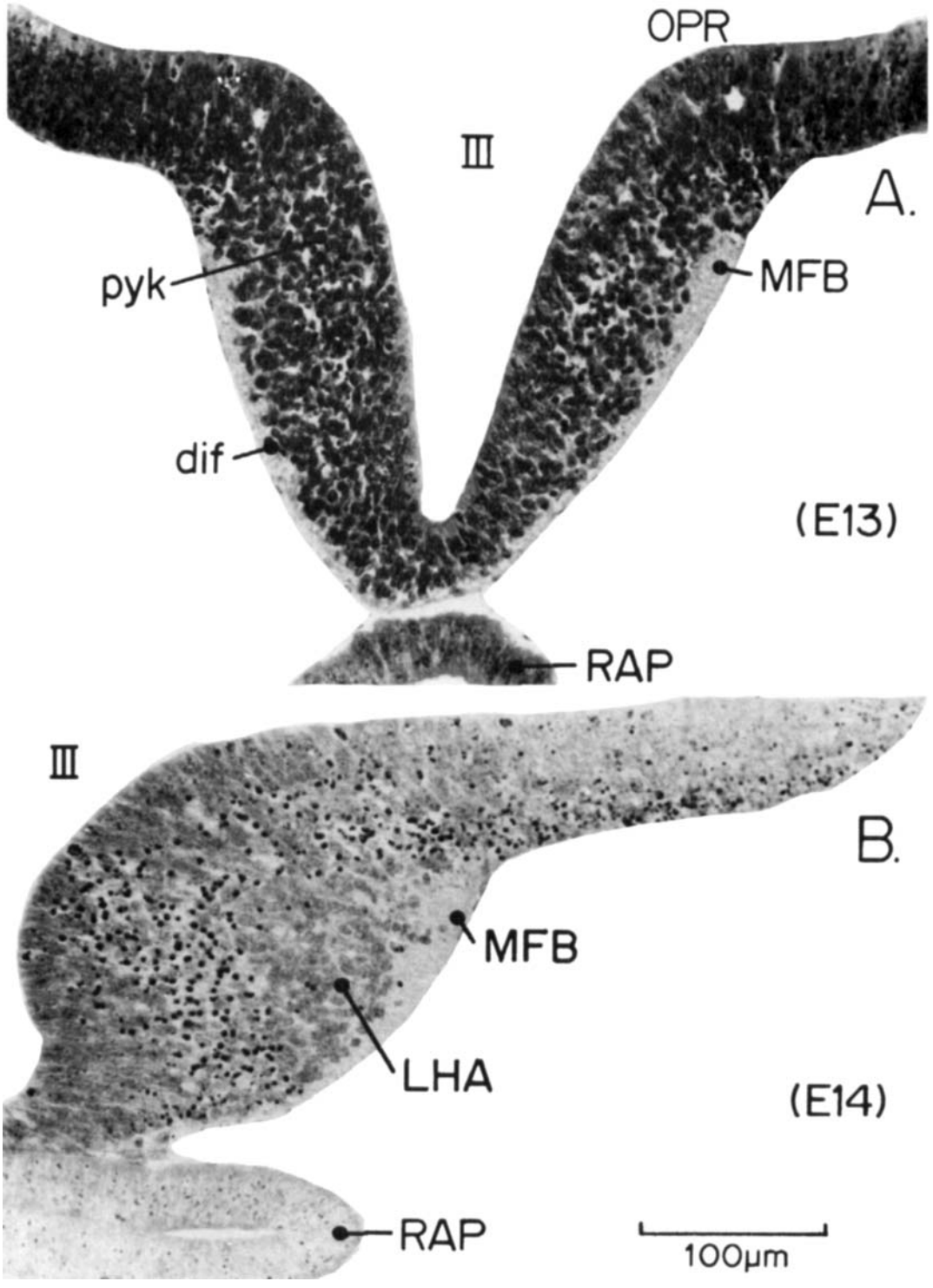

Fig. 3 Horizontal section through the ventral neuroepithelial lobe in X-irradiated day E13 (A) and E14 (B) embryos. There is a small zone of radioresistant, differentiating cells behind the optic recess (OPR) on day E13 and it increases by day E14. This region represents the lateral hypothalamic area (LHA); it is surrounded laterally by the presumed medial forebrain bundle (MFB). *Abbreviations:* dif, differentiating cells; pyk, pyknotic cells; RAP, Rathke's pouch. A, hematoxylin-eosin; B, cresyl violet.

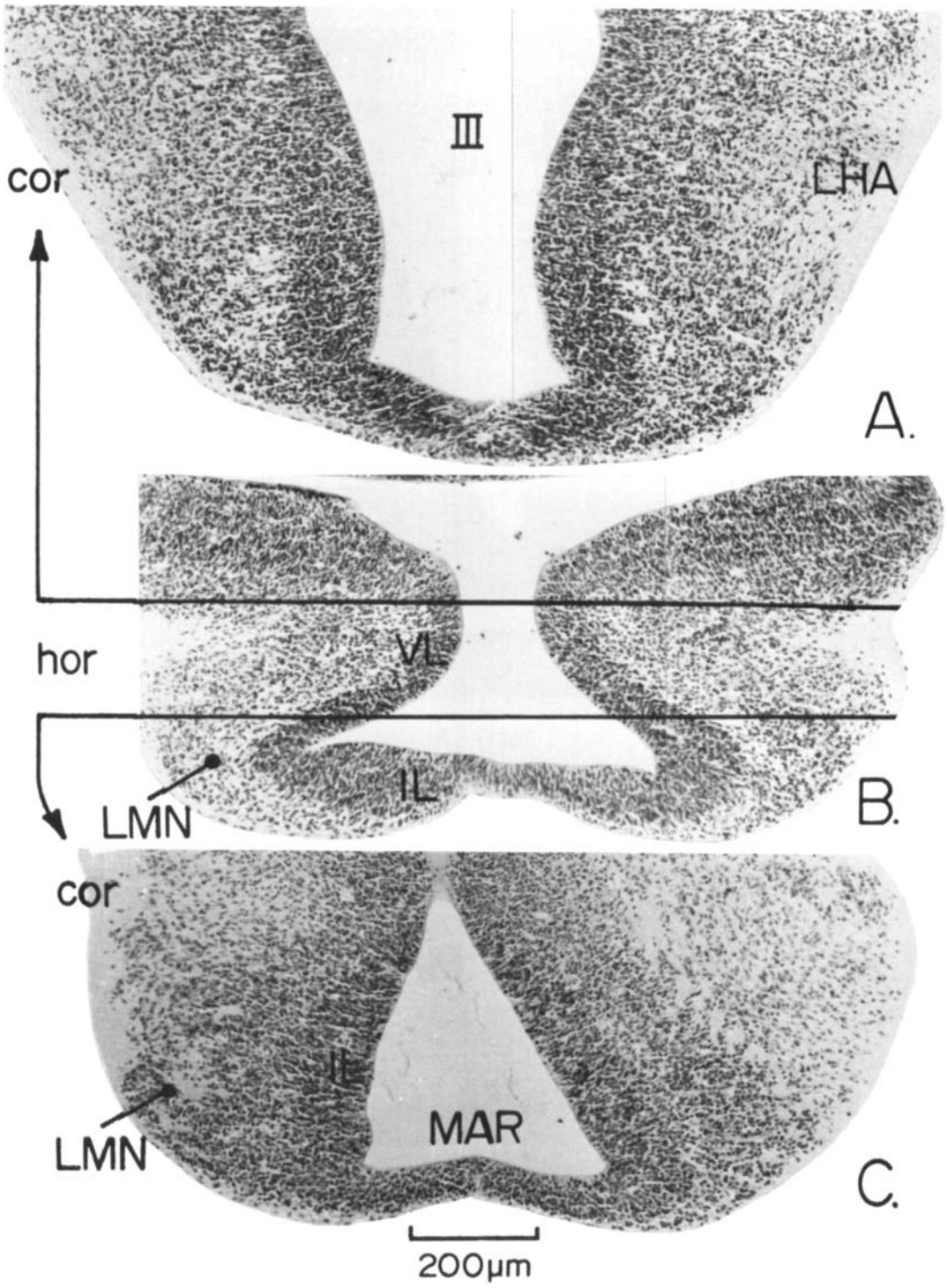

Fig. 4 Day E15 normal embryos. A and C, coronal (cor) sections at the anterior and posterior levels indicated in the horizontal (hor) section in B. The lateral hypothalamic area (LHA) appears to derive from the ventral neuroepithelial lobe (VL), the lateral mammillary nucleus (LMN) from the inferior lobule (IL), possibly before the formation of the mammillary recess (MAR).

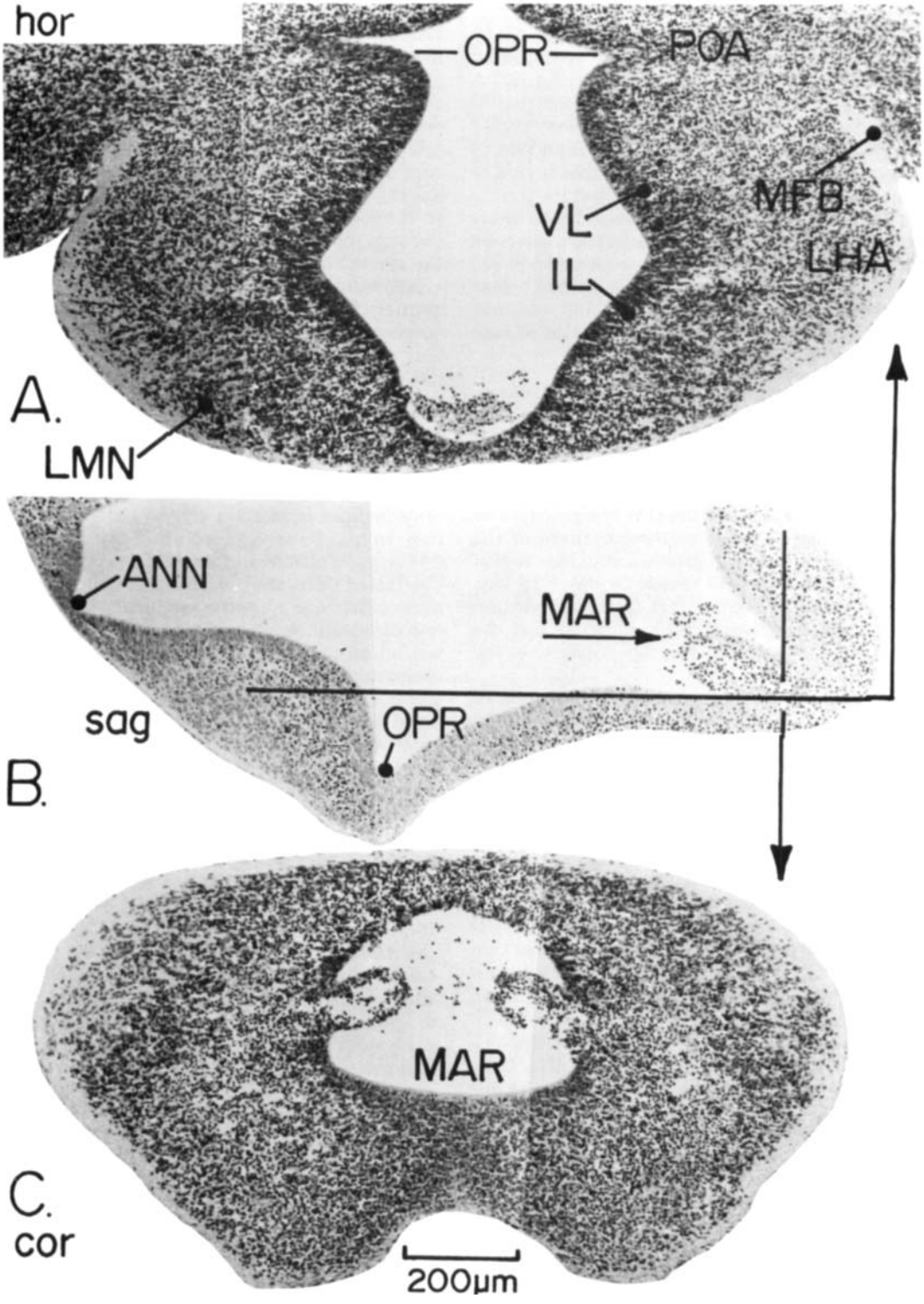

Fig. 5 Day E15 X-irradiated embryos. A, horizontal (hor) section and C, coronal (cor) section at the approximate levels indicated in the sagittal (sag) section in B. Note the consistent phenomenon of restricted neuroepithelial collapse after X-irradiation in a portion of the mammillary recess (MAR). *Abbreviations:* ANN, anterior neuropore; IL, inferior neuroepithelial lobule; LHA, lateral hypothalamic area; MFB, medial forebrain bundle; OPR, optic recess; POA, preoptic area; VL, ventral neuroepithelial lobe.

lateral hypothalamic area; this is identified as the lateral mammillary nucleus (figs. 4B,C) since the bulk of its neurons are acquired by day E15 (Altman and Bayer, '78a: fig. 9). A striking phenomenon is the neuroepithelial collapse of a portion of the mammillary recess in day E14 and in E15 rats (fig. 5) and up to day E16 (fig. 12). Apparently in this region of the mammillary recess all the juxtaventricular elements are radiosensitive and cells characterized by higher radioresistance, such as glioblasts and ependymoblasts (Altman et al., '68) have not yet formed. It is not clear whether this circumscribed region outlines the mammillary or the tubero-arcuate anlage of the posterior hypothalamus.

(c) *Development of specific hypothalamic structures*

Suprachiasmatic nucleus. The neurons of the suprachiasmatic nucleus form between days E14-E17 (Altman and Bayer, '78a: fig. 5); as a discrete structure it is recognizable by day E17 (fig. 6). The neuroepithelium of the third ventricle is still prominent in this region on E17 but begins to recede by day E18 (fig. 6B). By day E17 optic tract fibers have begun to decussate in appreciable numbers but the chiasma is situated at this age posterior to the nucleus (fig. 7B).

Median preoptic nucleus. The neurons of this nucleus form on days E13-E16 (Altman and Bayer, 78a: fig. 5); by day E17 they are beginning to scatter and may become paler (fig. 6A) suggesting the onset of differentiation. The median preoptic nucleus is situated adjacent to the neuroepithelium at the base of the lateral ventricles at the level of the foramen of Monro, not the third ventricle. This observation, together with the autoradiographic evidence of a ventral-to-dorsal gradient (Altman and Bayer, '78a: fig. 11) suggest that this nucleus is of telencephalic derivation and should not be considered a hypothalamic structure.

Supraoptic nucleus. Neurons of the supraoptic nucleus form on days E13-E15 (Altman and Bayer, '78a: fig. 5). However, the nucleus cannot be recognized in embryonic material during this period. The first cells may begin to gather on day E16 laterally near the arriving fibers of the optic tract (fig. 7A); they are not seen with certainty until day E17 (figs. 7B, 8A) and cell accumulation continues thereafter (figs. 7C, 8B). The cells of the supraoptic nucleus can be traced migrating by way of a "stream" of spindle-shaped cells (figs. 7B,C, 8) from the region of the paraventricular nucleus around the "diamond-shaped" region of the third ventricle at the junction of the ventral and medial lobes of the diencephalic neuroepithelium. The delay between cell formation and the appearance of the supraoptic nucleus is apparently due to cell migration, which seems to require about two to three days. The settling of the neurons of the supraoptic nucleus roughly coincides with the arrival of the optic tract fibers.

Internuclear magnocellular neurons. These neurons form at about the same time as the magnocellular neurons of the supraoptic and paraventricular nuclei. In embryonic material they were first recognized in clumps between the latter two nuclei on day E17 (fig. 9).

Paraventricular nucleus. The large neurons of the paraventricular nucleus form on days E13-E15 (Altman and Bayer, '78a: fig. 6). The cells should be present around the neuroepithelium soon after their production but they cannot be recognized until day E16 (fig. 7A) or more clearly until day E17 (fig. 7B). By the latter date and on day E18 (fig. 8) the paraventricular neurons are larger than surrounding cells, suggesting that their differentiation is advancing. According to the autoradiographic evidence the parvicellular elements of this nucleus are still forming at this time. But gradually the neuroepithelium is regressing in this region and after the presumed production of the neurons of the supraoptic, internuclear and paraventricular systems it becomes transformed into the specialized "convoluted ependyma" of the third ventricle (Altman and Bayer, '78b).

Anterior nucleus. This nucleus acquires its neurons by day E16 (Altman and Bayer, '78a: fig. 6); it can be identified with its differentiating cells by day E17 (fig. 7B).

Anterobasal nucleus. Our autoradiographic data suggested that the earlier forming anterior portion of the arcuate nucleus of general description may constitute another structure than the later forming posterior region which is composed of smaller cells and is contiguous with the tanycyte lined ventricle (Altman and Bayer, '78a: figs. 6-8). We referred to this region as the anterobasal nucleus. In midcoronal sections the anterobasal nucleus is delineated in day E17 embryos as a crescent shaped structure beneath the floor of the third ventricle, being composed of early differentiating pale cells (fig. 10). The nucleus is presumably

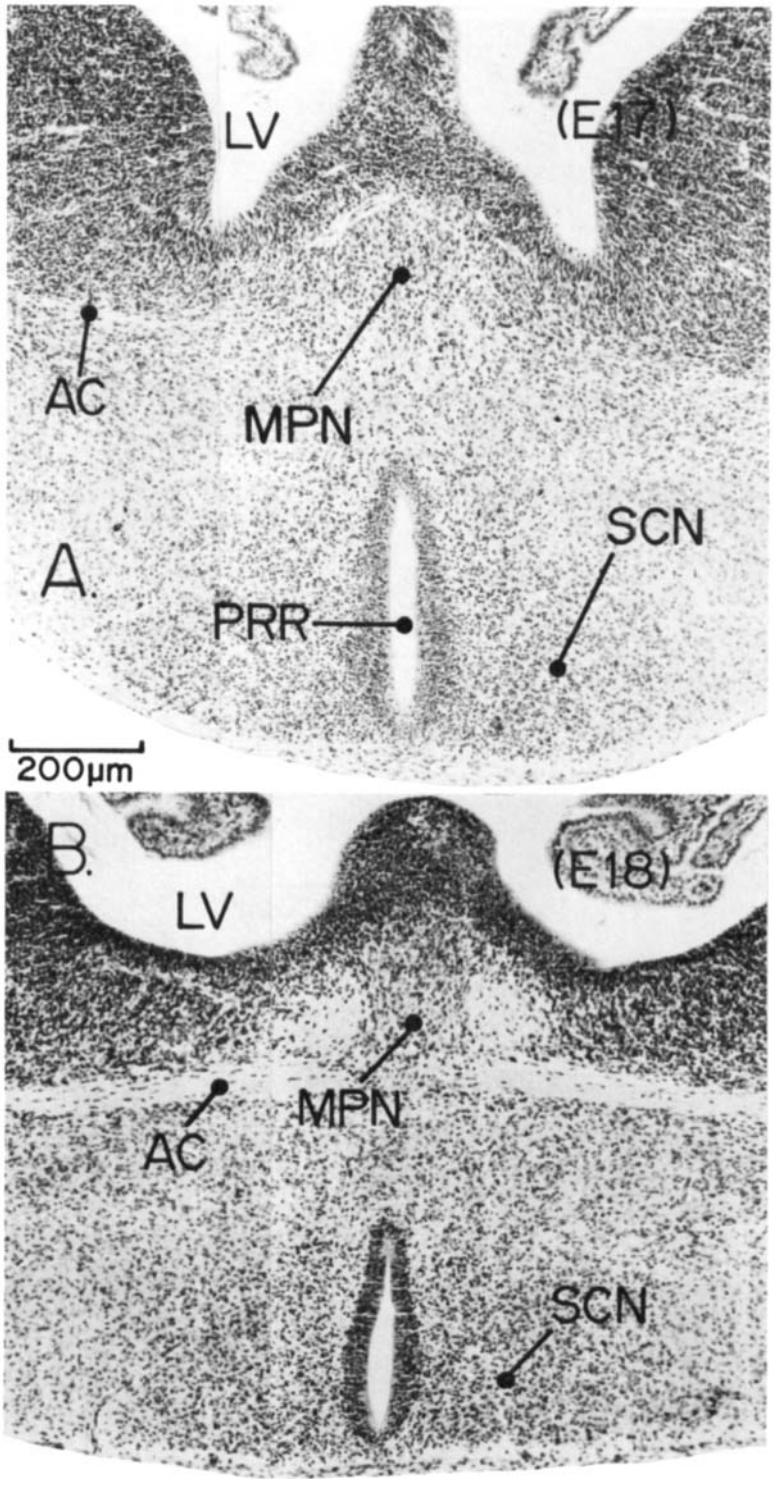

Fig. 6 Coronal sections through the level of the suprachiasmatic nucleus (SCN) and the median preoptic nucleus (MPN) in a day E17 (A) and E18 (B) embryo. The anterior commissure (AC) is recognizable by day E17 and is crossing the midline by day E18. The median preoptic nucleus is presumed to be derived from the neuroepithelium of the lateral ventricles (LV), the suprachiasmatic nucleus from the preoptic recess (PRR) of the third ventricle.

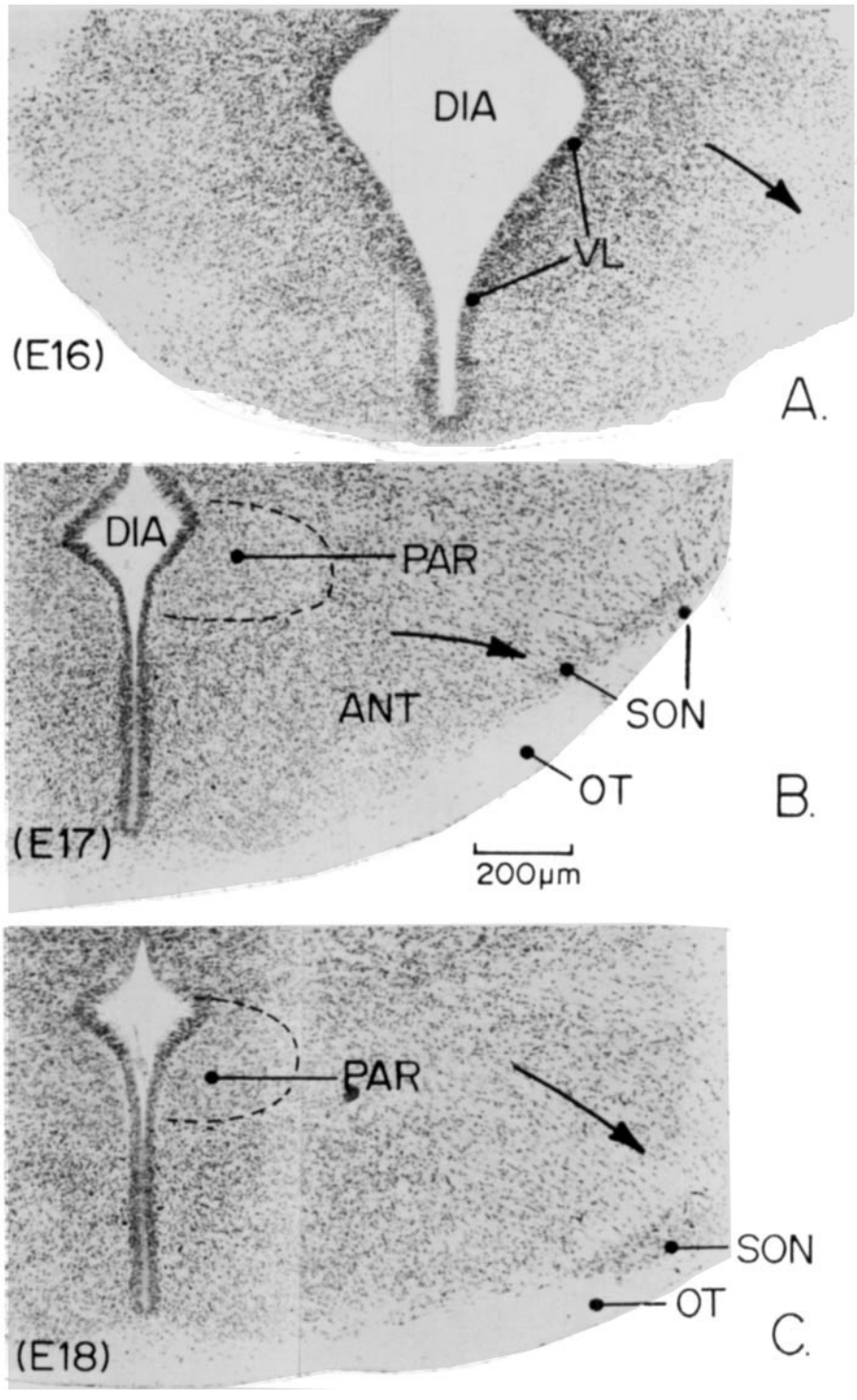

Fig. 7 Coronal sections through the diamond-shaped region (DIA), the derivative of Herrick's sulcus ventralis, in day E16 (A), E17 (B), and E18 embryos. The paraventricular (PAR) and supraoptic (SON) nuclei may be present by day E16 but are not certainly recognizable until day E17. The anterior nucleus (ANT) is maturing by day E17. Arrows show the apparent migratory route of spindle-shaped cells from the region of the paraventricular nucleus to the optic tract (OT). The extent of the still active ventral neuroepithelial lobe (VL) on day E16 is indicated in A.

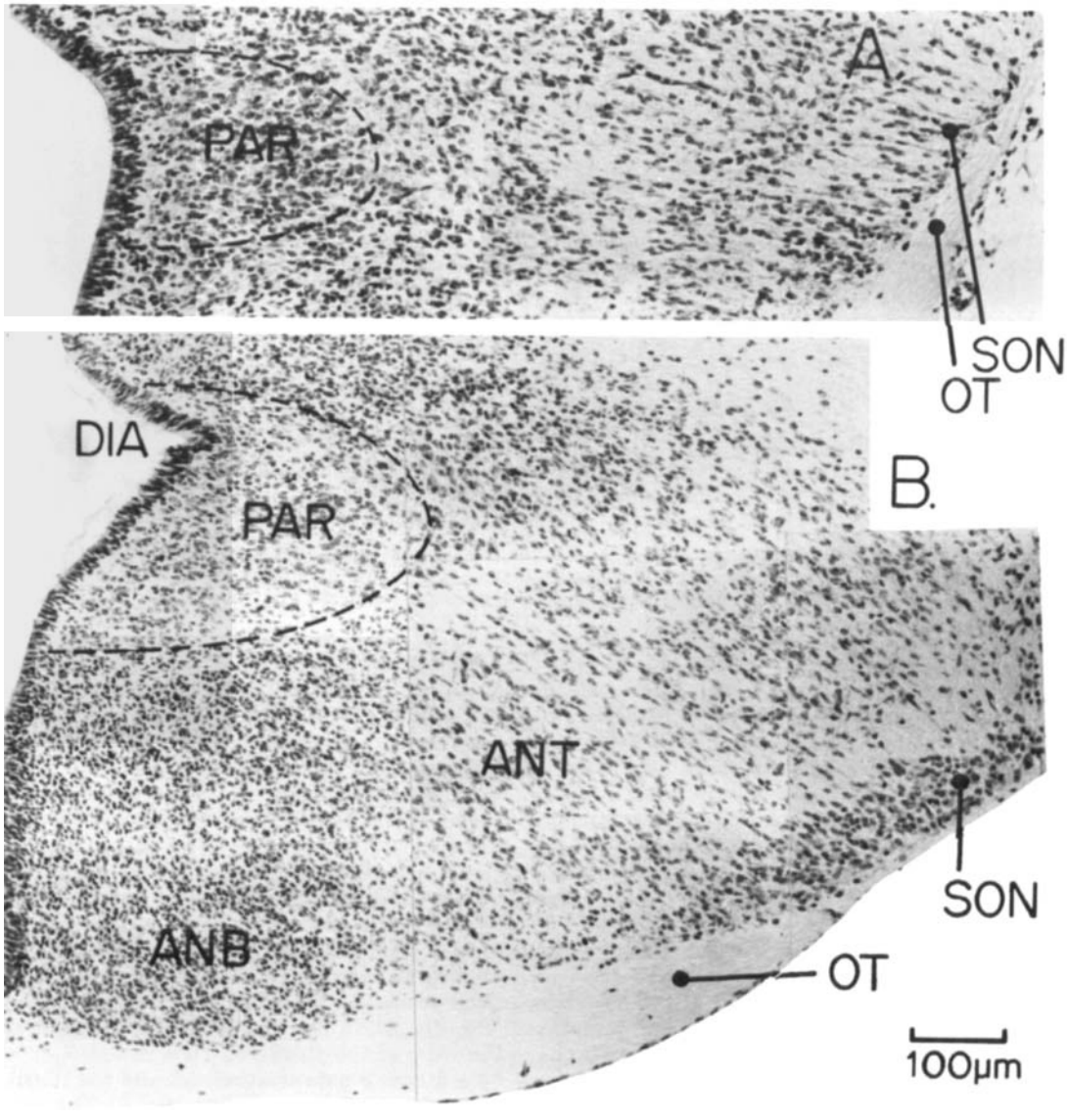

Fig. 8 Coronal sections through the level of the third ventricle diamond (DIA) in day E17 (A) and E18 (B) embryos. The spindle-shaped, apparently migrating cells can be traced from the region of the paraventricular nucleus (PAR) to the supraoptic nucleus (SON) where they accumulate adjacent to the optic tract (OT). The differentiating cells medially and ventrally may be the anterior continuation of the anterobasal nucleus (ANB) shown also in figure 10.

arising from the overlying neuroepithelium which is diminishing in width at this rostral level by day E18.

Ventromedial and dorsomedial nuclei. In day E17 embryos, in coronal sections at the level of the anterobasal nucleus the neuroepithelium is still thick between the floor and the diamond-shaped portion of the third ventricle (fig. 10A). It is this region of the ventricle that becomes transformed into the "laminated epithelium" (Altman and Bayer, '78b) and which, according to our autoradiographic studies (gradient pattern), may be the source of the ventromedial and dor-

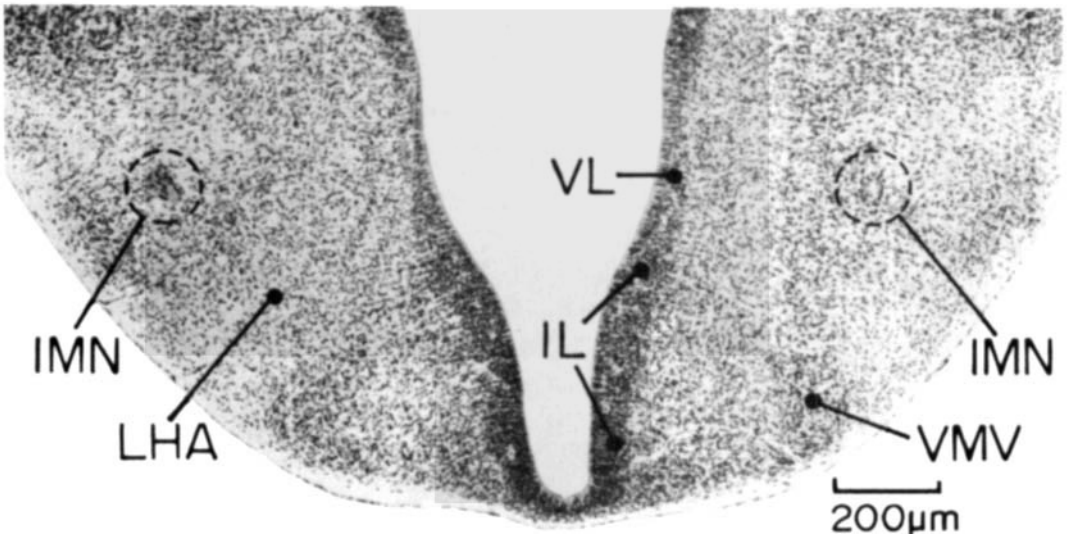

Fig. 9 Clusters of cells, presumably representing the internuclear magnocellular neurons (IMN) are seen in a coronal section from a day E17 rat. Beneath the lateral hypothalamic area (LHA) the differentiation of the ventromedial nucleus, pars ventralis (VMV) may have begun. IL, inferior lobule; VL, ventral lobe.

somedial nuclei of the hypothalamus (Altman and Bayer, '78a: figs. 17, 18). The assumed active proliferation of germinal cells in this region on day E17 is reconcilable with the autoradiographic evidence that neurons are still added on this day to the dorsal part of the ventromedial nucleus and to the dorsomedial nucleus (Altman and Bayer, '78a: fig. 7). On day E18 the thickness of this neuroepithelial region is diminishing and the ventricular walls begin to fuse.

Premammillary nuclei. The neuroepithelium surrounding the wall and the base of the mammillary recess of the third ventricle (derivative of the inferior lobule) remains active longer than most of the more rostrally situated parts of the ventricle. However, in the anterior aspect of this region two nuclear condensations with differentiating neurons may be identified by day E17 (fig. 11). It is likely that these are the ventral and dorsal premammillary nuclei that form on days E13-E15 (Altman and Bayer, '78a: fig. 8). It is uncertain whether or not these early forming nuclei arise from the adjacent neuroepithelium of the mammillary recess or from the overlying ventral neuroepithelial lobe.

Arcuate and tuberomammillary nuclei. In adult rats, ventral and posteroventral to the "laminated epithelial" ventricle there is another specialized ventricular region, characterized by horizontally oriented, spindle-shaped cells with arcing processes that can be traced to the base of the hypothalamus overlying the median eminence and to the tuberomammillary nucleus laterally (work in progress). These cells are the tanycytes, and this region of the floor of the third ventricle is surrounded by the arcuate, or infundibular, nucleus. According to our autoradiographic evidence this is the latest forming region of the hypothalamus: the arcuate nucleus neurons form on days E16-E19 (and beyond) the associated large neurons of the tuberomammillary nucleus on days E15-E18 (Altman and Bayer, '78a: fig. 8). In day E16 fetuses this region of the floor of the third ventricle is surrounded by a massive neuroepithelium and the entire region is radiosensitive (figs. 12A,B). The neuroepithelium remains apparently active on the next day (fig. 13A) and day E18, but begins to decline thereafter. The neurons of the tuberomammillary nucleus may begin to migrate laterally on day E17 (fig. 13C).

Mammillary nuclei. The neurons of the lateral mammillary nucleus form on days E12-E15 (Altman and Bayer, '78a: fig. 9) and it can be recognized in its position in day E15 (fig. 4) and E16 embryos (figs. 12B,C). Most of the cells of the nucleus are radioresistant by day E16. Among the fiber tracts of the mammillary complex, the principal mammillary

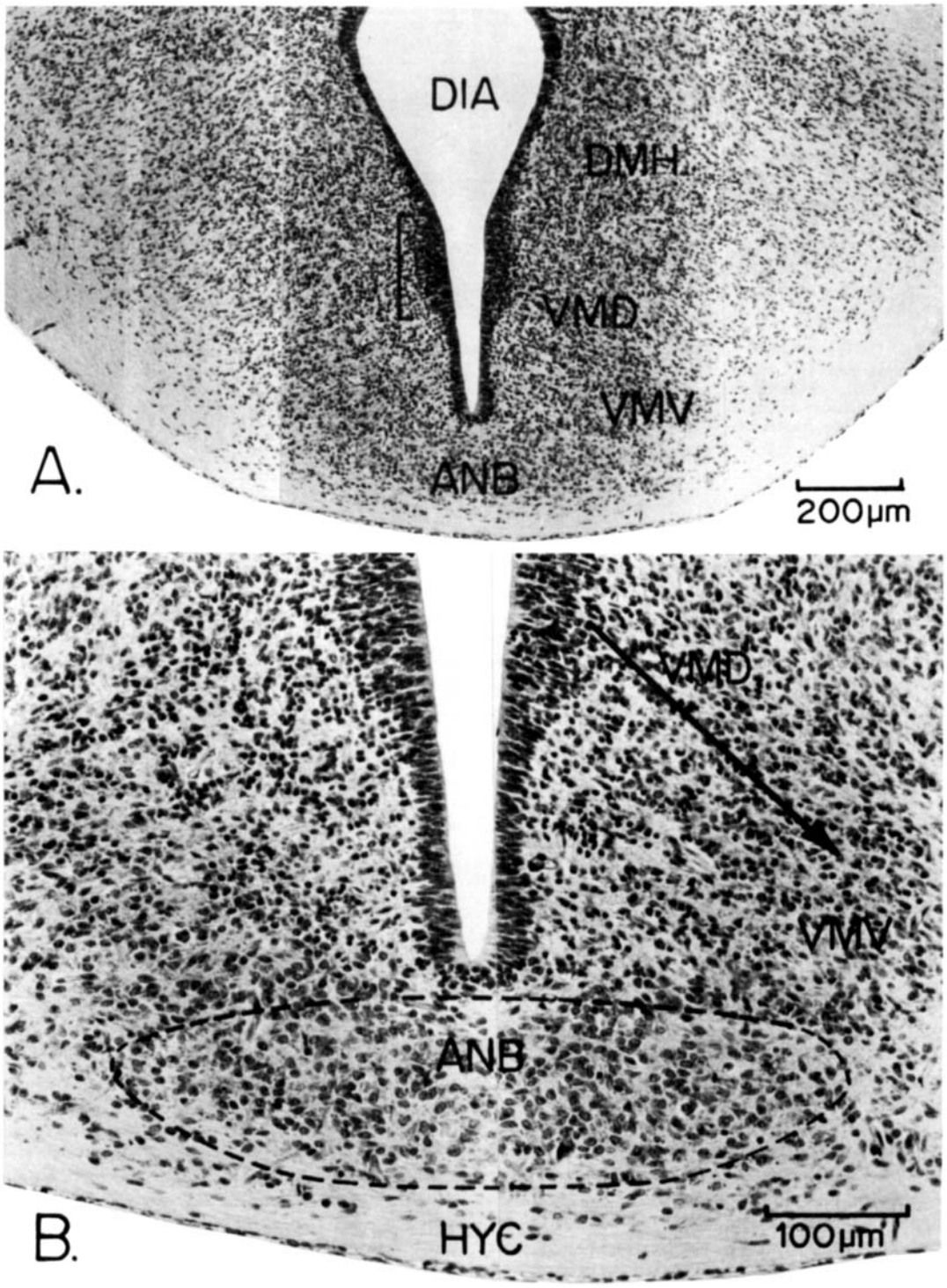

Fig. 10 A coronal section (A and B) through the dorsomedial nucleus (DMH) and ventromedial nucleus, pars ventralis (VMV) and pars dorsalis (VMD). The derivation of these nuclei from a single, discrete neuroepithelial site (bracket in A) is indicated. At this age this neuroepithelial mosaic appears more active than the dorsally situated diamond region (DIA) or the ventrally situated region that may have generated the earlier forming anterobasal nucleus (ANB) above the hypothalamic commissure (HYC). IL, inferior lobule, VL, ventral lobe.

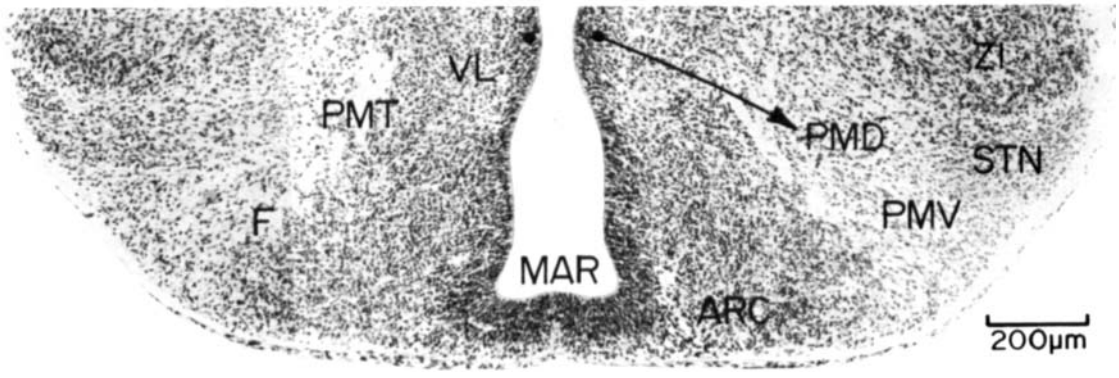

Fig. 11 Coronal section at the transition level of the ventral neuroepithelial lobe (VL) and the mammillary recess (MAR) in a day E17 embryo. Identifiable structures are the premammillary nucleus, pars ventralis (PMV) and pars dorsalis (PMD), zona incerta (ZI), subthalamic nucleus (STN), fornix (F), and the principal mammillary tract (PMT). The neuroepithelium of the mammillary recess producing the arcuate nucleus (ARC) neurons is still active. Arrow points to the possible neuroepithelial source of the premammillary nuclei on earlier days.

tract is recognizable by this time (fig. 12B). There is no evidence of the onset of differentiation of neurons of the other mammillary nuclei. The mammillary recess of the third ventricle is surrounded by a massive neuroepithelium and it, in turn, by a large body of primitive cells that occupies almost the entire base of the posterior diencephalon (fig. 12C). The primitive developmental state of the mammillary body is indicated by the collapse of the neuroepithelium in day E16 embryos after X-irradiation (figs. 12B,C; compare with fig. 5).

In day E17 fetuses the cells of the medial mammillary nucleus (which form mostly on day E15; Altman and Bayer, '78a: fig. 9) have apparently started to differentiate (fig. 13C). The cells appear to derive from the adjacent neuroepithelium of the mammillary recess. The neurons of the supramammillary nucleus (which form predominantly on day E16; Altman and Bayer, '78a: fig. 9) may also have started to differentiate. However, these cells appear to be derived from the anteriorly and dorsally situated portion of the third ventricle not the mammillary recess (fig. 13C). Such a heterogeneous derivation of the mammillary nuclei was suggested by the complex triple-decked gradients described in the previous paper (Altman and Bayer, '78a). The neuroepithelium of the mammillary recess remains prominent on day E17 (fig. 13C). These proliferative cells may be the source of the neurons of the central and ventral portions of the principal mammillary nucleus that form on days E16-E18 (Altman and Bayer, '78a: fig. 9).

DISCUSSION

Site of origin of hypothalamic nuclei

In this study we have not dealt with the development of the rostral preoptic region. Components of this region may have separate origins, either the preoptic recess of the third ventricle or the inferior horn of the lateral ventricle. A prerequisite of any attempt to determine the neuroepithelial origin of this region is a detailed examination of the development of the rostral aspect of the basal telencephalon, a task that remains to be accomplished. Relevant is our observation that the median preoptic nucleus, which we assumed initially to be of diencephalic derivation is likely to arise from the neuroepithelium of the lateral ventricles. Accordingly, the present autoradiographic and embryological investigations are restricted to the hypothalamic nuclei from the coronal level of the suprachiasmatic nucleus and paraventricular nucleus caudally.

Most recent descriptions of the developing diencephalon utilize Herrick's ('10) three landmarks, the dorsal, medial and ventral sulci. These sulci are quite pronounced in lower vertebrates (Christ, '69: figs. 2-1). They are less pronounced, though recognizable, in mammals during early development and become transformed thereafter. We have proposed a related description that centers on the subdivisions of the corpus of the neu-

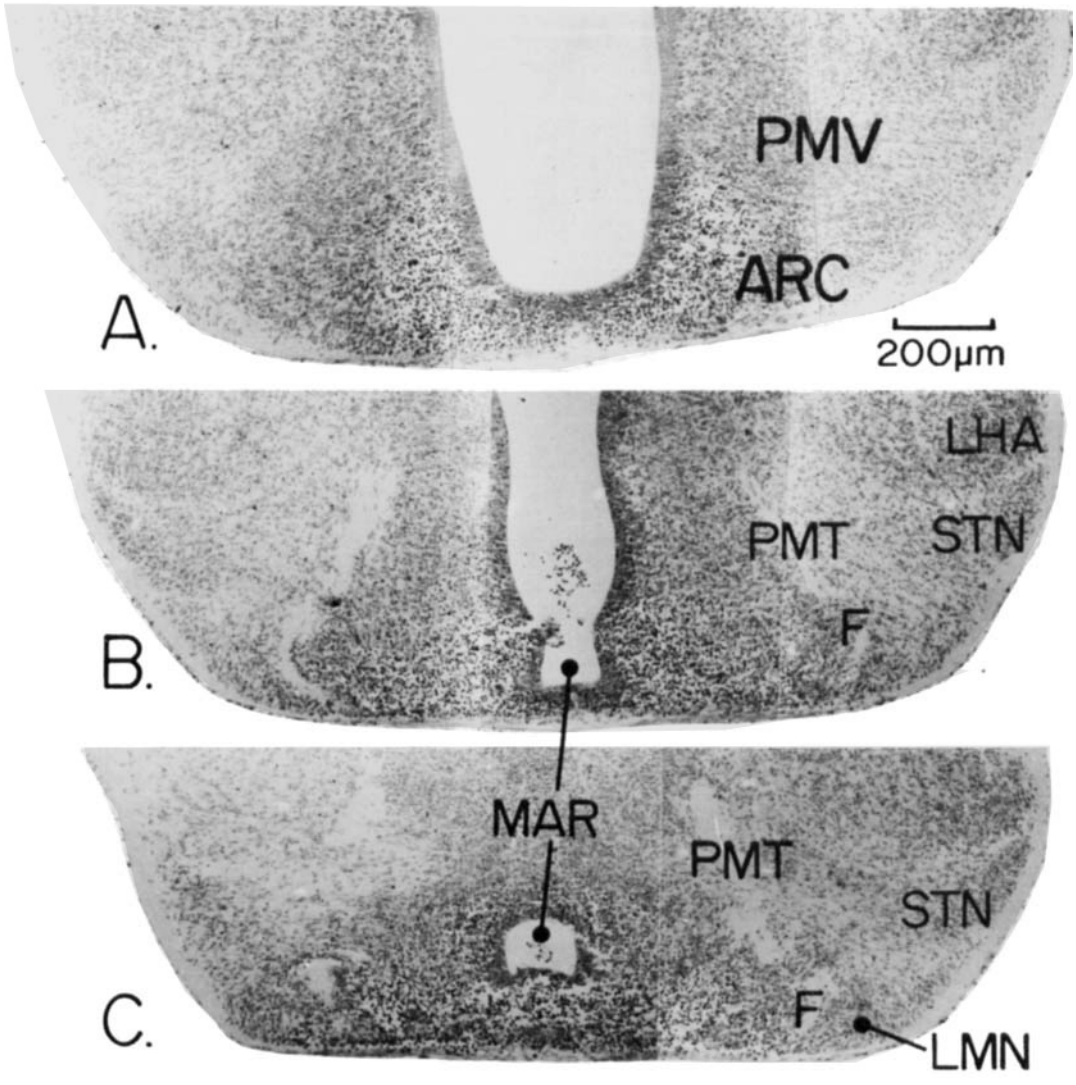

Fig. 12 Coronal sections (A-C, rostral to caudal) through the caudal hypothalamus of a day E16 X-irradiated embryo. Few pyknotic cells are present in the differentiating lateral mammillary nucleus (LMN), subthalamic nucleus (STN), lateral hypothalamic area (LHA), and premammillary nucleus, pars ventralis (PMV). Pyknotic cells predominate in the arcuate nucleus (ARC) and dead cells spill into the mammillary recess (MAR). The principal mammillary tract (PMT) and fornix (F) are recognizable at this early age.

roepithelium: the early dorsal, medial, and ventral lobes, and the superior and inferior lobules that develop soon after the onset of differentiation of the earliest forming diencephalic neurons. The ventral lobule is the source of most hypothalamic nuclei but the nuclei of the "parvicellular" (Szentágothai, '64), hypophysiotropic (Halász, '72), or the adenohypophysial endocrine hypothalamus appear to derive from the inferior lobule. This is a unique ventricular region and its lining becomes modified considerably during postnatal development (Altman and Bayer, '78b). Evidence is also available (work in progress) that the superior lobule is the source of certain diencephalic structures implicated in endocrine functions.

The three-lobed organization of the dien-

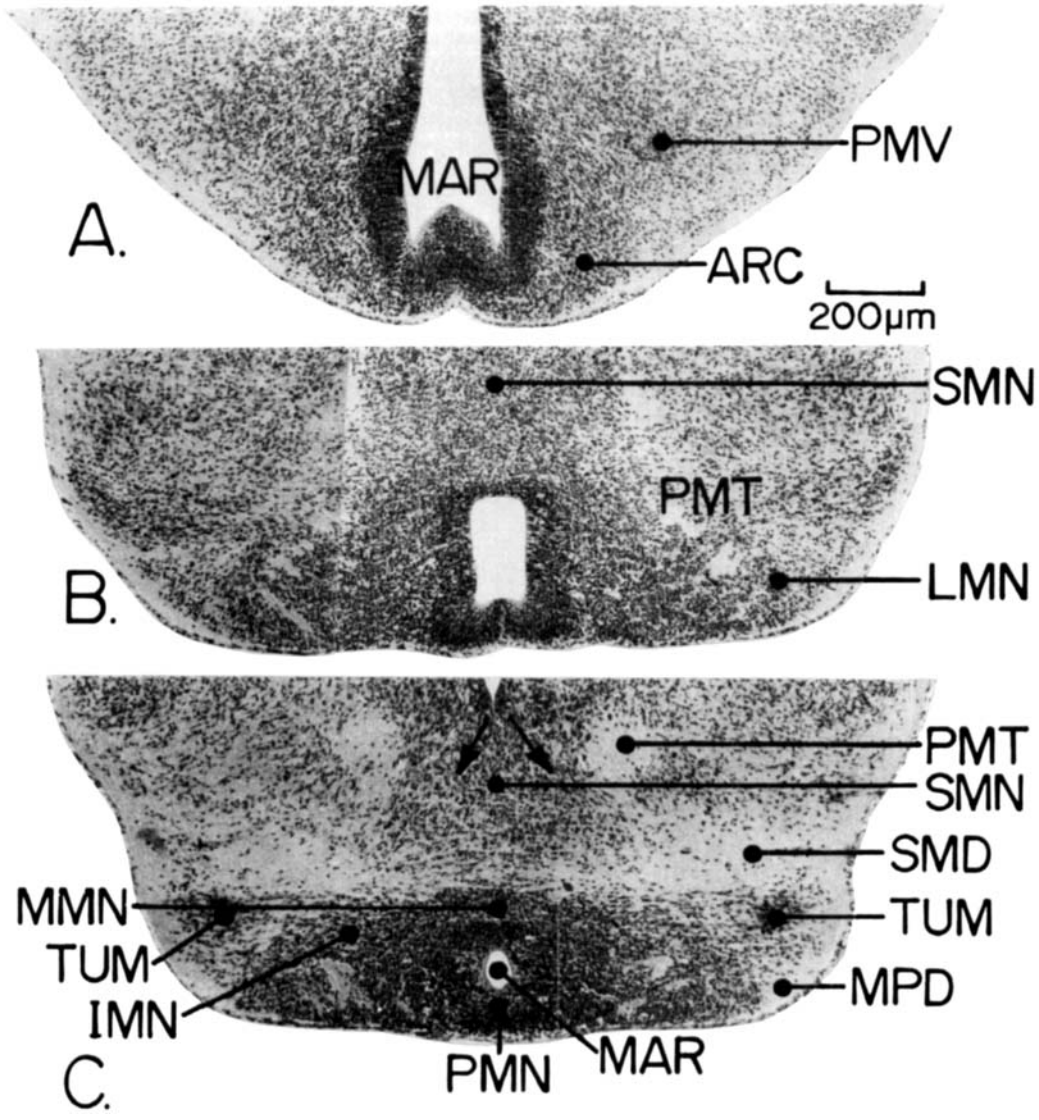

Fig. 13 Coronal sections (A-C, rostral to caudal) through the caudal hypothalamus of a day E17 normal embryo. The neuroepithelium rostral (A) and dorsal (C) to the mammillary recess (MAR) is presumed to generate the arcuate nucleus (ARC) and the supramammillary nucleus (SMN; arrows). The mammillary recess neuroepithelium may be the source the differentiating medial mammillary nucleus (MMN) and intermediate mammillary nucleus (IMN). The site of origin of the apparently migrating cells of the tuberomammillary nucleus (TUM) is not evident. Recognizable fiber tracts are the principal mammillary tract (PMT), the supramammillary decussation (SMD) and the mammillary peduncle (MPD). LMN, lateral mammillary nucleus; PMV, premammillary nucleus, pars ventralis.

cephalon is best seen on day E13, the five-lobed organization on day E14. Thereafter the structure of the neuroepithelium becomes modified and the body of the diencephalon undergoes modifications through the appearance of differentiating zones and nuclear condensations. The growth of the diencephalon is directed both ectally and endally: as the ventricular lumen becomes filled it becomes shallower, and the body of the diencephalon begins to bulge outward, at different rates in different regions, such that its shape changes

from flask, disc, to pumpkin shape. The shape of the lumen of the third ventricle changes drastically from day to day as does the thickness of the neuroepithelium; the result is a changing mosaic pattern that is particularly striking between days E15 and E17 (fig. 1). Evidently there are considerable regional differences in the chronological patterns of cell production. These neuroepithelial changes together with the changes in the corpus of the diencephalon, and combined with the autoradiographic dating of neuron origin, were of great aid in inferring the site of neurons of different nuclei. Difficulties were created by the migration of some cell groups over long distances and the modifications produced in settling patterns and the structure of the diencephalon by afferent and efferent fiber tracts. The attempt to designate each neuroepithelial mosaic as the presumptive production site of a specific diencephalic nucleus must await the detailed examination of the ontogeny of the rest of the diencephalon (work in progress). What does emerge from these studies is that the heterogeneity of the diencephalon in general and of the hypothalamus in particular is paralleled by the heterogeneity of the neuroepithelial matrix from which they arise. Presumably the specification of many of the structural and functional characteristics of neurons of different nuclei occurs at their production site prior to morphological differentiation.

Shared site of production of neurons of a dispersed nuclear system

We could trace the site of origin of the three early forming magnocellular structures of the hypothalamus, the paraventricular and supraoptic nuclei, and the internuclear magnocellular clusters, to the diamond-shaped neuroepithelium of the third ventricle at the boundary of the medial and ventral lobules (fig. 1E), or the derivative of Herrick's sulcus ventralis. Several early investigators of this neurohypophysial secretory system hypothesized that the paraventricular nucleus synthesizes primarily oxytocin and the supraoptic nucleus primarily vasopressin (Olivecrona, '57; Lederis, '61; Weyl-Sokol and Valtin, '67). But more recent studies with new techniques suggest that both nuclei secrete oxytocin and vasopressin (and neurophysins) by specialized cells (Swaab et al., '75; Defendini and Zimmerman, '78). If these two nuclei are composed of similar neurons (there is little information about the properties of the internuclear neurons) our observation of their common origin, and of their relative isochronicity (Altman and Bayer, '78a: fig. 23) would indicate that neurons with similar properties may arise at the same locus even if they are destined to settle at different sites. By implication these neurons would be specified in terms of their chemical characteristics at their generation site prior to differentiation.

A shared site of cell production was also suggested for the dorsomedial and ventromedial nuclei. The site is easy to recognize as a regional thickening of the neuroepithelium (figs. 1E, 10A,B) in day E17 embryos, when the last set of neurons are being generated for these nuclei (Altman and Bayer, '78a: fig. 7). The indication that this particular locus may be the source of these neurons came from labelling gradients. We noted that the dorsomedial and ventromedial nuclei, which in coronal sections appear as outspread wings with their fulcrum at the ventricle, have their latest forming neurons situated near the ventricle at this very site. Interestingly, this is also the region where the ventricular lining acquires a unique composition postnatally (Altman and Bayer, '78b), what we have referred to as the "laminated epithelial" wall (Altman and Bayer, '78a: figs. 17, 18). But aside from this ontogenetic suggestion of shared origins, these two nuclei have not been associated in the past. Some investigators have suggested that the ventromedial nucleus may be involved in the secretion of releasing factors that influence growth hormone (Frohman and Bernardis, '68) and thyroid hormone (Flament-Durand and Desclin, '68) secretion. But neither the ventromedial nucleus nor the dorsomedial nucleus is generally considered part of the hypophysiotropic area (Halász, '72). Traditionally the ventromedial nucleus (Hetherington and Ranson, '40), together with the lateral hypothalamus (Anand and Brobeck, '51), has been considered a regulatory system of feeding behavior (reviewed by Stevenson, '69). Ventromedial lesions produce hyperphagic obesity, lateral hypothalamic lesions aphagia. But more recent evidence suggests that the medial band may be broader than hitherto considered, extending rostrally to the paraventricular nucleus (Gold et al., '77). Whether or not the dorsomedial nucleus is part of this medial band is not clear from the available evidence. Many investigators have stressed the importance of connections be-

tween the medial and lateral bands in the regulation of feeding and drinking (Sclafani and Grossman, '69; Albert and Storlien, '69; Paxinos and Bindra, '72; Sclafani et al., '73). If the two bands do constitute a single regulatory system, it may be of some interest that the facilitatory component (the lateral hypothalamic area) acquires its cells before, and presumably matures earlier, than the inhibitory component (the ventromedial nucleus). Several recent studies have referred to the possible involvement of periventricular elements and the cerebrospinal fluid in the regulation of feeding and drinking (Epstein, '73); the laminated epithelial ventricle that we related to the ventromedial and dorsomedial nuclei could constitute such a link.

Sequential generation of nuclear components of a complex system

In the case of the supraoptic and paraventricular nuclei two structurally and functionally separated structures (together with the internuclear neurons) were apparently derived from a single production site with minimal time differences between them. In other structures we found that subdivisions of a single complex arose at markedly different times, possibly from different sites. The best example of this is the mammillary body; another may be the tuberomammillary-arcuate complex.

There is at present no general agreement on the subdivisions of the rat mammillary body except for a distinction between the lateral nucleus and what is often referred to globally as the medial nucleus. The medial mammillary nucleus was subdivided by Powell and Cowan ('54) into a larger, oval-shaped pars posterior and the vertically oriented pars medialis and pars lateralis. They also referred to (but did not illustrate in their diagrams) a pars medianus; the location of this subdivision was later illustrated by Guillery ('57). In his atlas, de Groot ('59) distinguished three mammillary nuclei, the lateral, medial and posterior; whereas Christ ('69) lists separately the median nucleus in addition to the lateral and medial nuclei. Our autoradiographic data suggested that in the midcoronal portion of the mammillary complex there is, superimposed upon these vertically oriented subdivisions, a triple-decked horizontal organization. This consists of two late forming bands, the superior band (the supramammillary nucleus) and the inferior band (the *principal mammillary nucleus)* and interposed between them is an earlier forming band consisting of three nuclei: the earliest forming large celled lateral nucleus; the early forming intermediate-size celled *medial nucleus* and small celled *intermediate nucleus.* Posteriorly the triple-decked pattern is gradually reduced to a single horizontal division, the principal mammillary nucleus, pars posterior (which corresponds to the posterior nucleus or the medial nucleus, pars posterior of other writers). Anteriorly, there is a late forming median body, which might be a separate *median nucleus* or part of either the supramammillary nucleus or the principal mammillary nucleus, pars centralis.

The correct subdivision of the mammillary complex is of great importance for studies that deal with the regional origin of mammillary efferents (fibers of the mammillothalamic and mammillotegmental tracts) and the regional distribution of mammillary afferents (fibers of the fornix and mammillary peduncle). Are specific mammillary nuclei sources of specific afferent bundles destined to reach specific thalamic and tegmental nuclei, and do different mammillary nuclei receive afferents from discrete descending and ascending sources? The question was posed and answered affirmatively in several studies using the retrograde cell degeneration technique (Powell and Cowan, '54), the Nauta technique (Guillery, '56, '57), and amino acid autoradiography (Cruce, '75, '77). But in general these studies showed considerable overlap in mammillary projection to the different thalamic (anteromedial, anterodorsal and anteroventral) and tegmental nuclei (dorsal tegmental and ventral tegmental), possibly because of an inappropriate subdivision of the mammillary body. Supporting evidence for a horizontal organization of the midcoronal and posterior aspect of the mammillary body comes from recent studies concerned with the subicular origin of the fornix. Meibach and Siegel ('75) examined the distribution of axon terminalis of the fornix following injection of a labelled amino acid; their illustration shows a distribution of the label in a horizontal strip stretching between the lateral mammillary nuclei (see their figure 1). A similar pattern was illustrated by Swanson and Cowan ('77: fig. 11), and in another study by Meibach and Siegel ('77: figs. 5, 7, 11). In the latter investigation, Meibach and Siegel suggest two projections from the subicular region to the mammillary body, one by way of the fornix and the

other by way of the medial corticohypothalamic tract, and both appear to be distributed horizontally (see their fig. 12). However, we do not have much evidence at present of the horizontal origin of efferents of the mammillary body except of a specific hippocampal projection from the supramammillary nucleus (Pasquier and Reinoso-Suarez, '76). Future examination of such a horizontal organization might be aided by a combination of anterograde and retrograde tracer studies with ^{3}H-thymidine autoradiography.

Two other nuclei that may constitute a single functional system is the tuberomammillary nucleus and the caudal arcuate nucleus, or infundibular nucleus (Broadwell and Bleier, '76). Presumably both are derived from the inferior lobule at the anterior margin of the mammillary recess and both may be directly related to the tanycyte-lined floor of the caudal third ventricle of the mature animal (Altman and Bayer, '78b). The possible relation of these contiguous (Altman and Bayer, '78a: fig. 20) lateral and medial structures was suggested by their uniquely late generation time (Altman and Bayer, '78a: fig. 8). If the tuberomammillary large neurons are indeed related to the arcuate nucleus, and therefore to the hypophysiotropic area (Halász, '72), then the designation of the latter as a "parvicellular" (Szentágothai, '64) endocrine system will require qualifications. The arcuate nucleus and the tuberomammillary nucleus may have a similar relationship to one another as the parvicellular and magnocellular portions of the paraventricular nucleus. Interestingly, both are contiguous with specialized ventricular linings that develop postnatally at the same site where the neurons were generated prenatally. The development of these ventricular linings, together with a third one that bears a similar relationship to the ventromedial and dorsomedial nuclei, will be described in the succeeding paper (Altman and Bayer, '78b).

Summary comments

These autoradiographic and embryological studies indicate that the hypothalamus is composed of several zones of sequentially acquired neuronal systems. Two major chronological gradients were discerned: in the anterior hypothalamus a topologically distorted lateral-to-medial gradient, and in the posterior hypothalamus, specifically in its caudal portion, a horizontally oriented gradient that was partially combined with a lateral-to-medial gradient. The very early forming lateral structures (lateral preoptic and hypothalamic areas, lateral mammillary nucleus) appear to be associated with large fiber tracts (the medial forebrain bundle, fornix, principal mammillary tract) that are themselves formed relatively early during development. Most of the late and latest forming periventricular structures (dorsomedial, ventromedial, periventricular, and arcuate nuclei) may be related to specialized ventricular linings that develop perinatally and are implicated directly in endocrine regulations. (The paraventricular, internuclear and supraoptic nuclei constitute a transhypothalamic system defying this stratification.) Finally, there is a heterogeneous topologically distorted intermediate zone (medial preoptic area; anterior, posterior and premammillary nuclei) which conceivably constitutes a complex interposed belt between the lateral and periventricular systems. According to recent studies, elements of this heterogeneous system, the medial preoptic area (Köves and Réthelyi, '76; Conrad and Pfaff, '76a) and the anterior hypothalamic nucleus (Conrad and Pfaff, '76b) have widespread connections with several or most hypothalamic nuclei and also with some extrahypothalamic structures.

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