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Development of the Preoptic Area: Time and Site of Origin, Migratory Routes, and Settling Patterns of Its Neurons

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

ABSTRACT

Neurogenesis and morphogenesis in the rat preoptic area were examined with [^{3}H]thymidine autoradiography. For neurogenesis, the experimental animals were the offspring of pregnant females given an injection of [^{3}H]thymidine on two consecutive gestational days. Nine groups were exposed to [^{3}H]thymidine on embryonic days E13–E14, E14–E15, E21–E22, respectively. On postnatal day P5, the percentage of labeled cells and the proportion of cells originating during 24-hr periods were quantified at four anteroposterior levels in the preoptic area. Throughout most of the preoptic area there is a lateral to medial neurogenetic gradient. Neurons originate between E12–E15 in the lateral preoptic area, between E13–E16 in the medial preoptic area, between E14–E17 in the medial preoptic nucleus, and between E15–E18 in the periventricular nucleus. These structures also have intrinsic dorsal to ventral neurogenetic gradients. There are two atypical structures: (1) the sexually dimorphic nucleus originates exceptionally late (E15–E19) and is located more lateral to the ventricle than older neurons; (2) in the median preoptic nucleus, where older neurons (E13–E14) are located closer to the third ventricle than younger neurons (E14–E17).

For an autoradiographic study of morphogenesis, pregnant females were given a single injection of [^{3}H] thymidine during gestation, and their embryos were removed either two hrs later (short survival) or in successive 24-hr periods (sequential survival). Short-survival autoradiography was used to locate the putative neuroepithelial sources of preoptic nuclei, and sequential survival autoradiography was used to trace the migratory waves of young neurons and their final settling locations. The preoptic neuroepithelium is located anterior to and in the front wall of the optic recess. The neuroepithelium lining the third ventricle is postulated to contain a mosaic of spatiotemporally defined neuroepithelial zones, each containing precursor cells for a specific structure. The neuroepithelial zones and the migratory waves originating from them are illustrated. Throughout most of the preoptic area, neurons migrate predominantly laterally. The older neurons in the lateral preoptic area migrate earlier and settle adjacent to the telencephalon. Younger neurons migrate in successively later waves and accumulate medially. The sexually dimorphic neurons are exceptional since they migrate past older cells to settle in the core of the medial preoptic nucleus. The median preoptic nucleus originates from a midline neuroepithelial zone that is continuous with the neuroepithelium in the midline basal telencephalon, and is, therefore, considered to represent a transitional area between the telencephalon and the diencephalon.

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Address reprint requests to Shirley A. Bayer, Department of Biology, Indiana-Purdue University, 1125 East 38th Street, Indianapolis, IN 46223.

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There has been considerable interest recently in the preoptic area because of the growing evidence that it is critically involved in reproductive endocrine regulation (Clemens et al., '76; Barry et al., '74; Witken et al., '82; Gibson et al., '84), the maintenance of male and female sexual behavior (Lisk, '62, '67; Christensen and Clemens, '74) and maternal behavior (Numan, '74). However, the results of some physiological and behavioral studies have not supported this localization. For example, the presence of gonadotropin-releasing hormone in cell bodies of the preoptic area has been questioned (Baker et al., '75; Merchenthaler et al., '80), and female sexual behavior has been reported to be unaffected by preoptic area lesions (Law and Meagher, '58; Singer, '68; Powers and Valenstein, '72). In addition, the role of the preoptic area in ovulation is controversial; some studies indicate a major role (Halasz, '69; Barraclough, '73; Gray et al., '78; Wiegand et al., '80), whereas another (Clemens et al., '76) finds the critically important ovulatory control area in the anterior hypothalamus. Support for the sexual function of the preoptic area has come from several anatomical studies that show sexual dimorphism in this region in rodents (Raisman and Field, '73; Greenough et al., '77; Gorski et al., '79, '80; Andersen, '82; Hammer, '84) and humans (Swaab and Fliers, '85). But even this has been disputed since Bleier et al. ('79, '82) localized the sexually dimorphic areas in the anterior hypothalamus rather than in the preoptic area.

The preoptic area has been a source of controversy in the neuroanatomical literature as well. Krieg ('32) states that it does not strictly belong to the hypothalamus. In fact, some of the components assigned to the preoptic area, such as the magnocellular preoptic nucleus (Loo, '31; Humphrey, '36) and the preoptic continuation of the bed nucleus of the stria terminalis (Gurdjian, '27) were recently shown to be part of the basal telencephalon (Bayer, '79a,b, '85, '87; Altman and Bayer, '86). On the other hand, many components assigned to the preoptic area have been described as anterior continuations of the hypothalamus (Gurdjian, '27; Loo, '31; Krieg, '32; Humphrey, '36; Young, '36), or part of the anterior hypothalamus (Bleier et al., '82; Bleier and Byne, '85). In view of these controversies, it was felt that a detailed study of the development of the preoptic area may aid in a better understanding of its anatomical organization.

Several morphogenetic studies have dealt with preoptic area development in relation to either the ontogeny of the diencephalon (Rose, '42; Ströer, '56; Hyyppä, '69; Keyser,

Abbreviations

AC	anterior commissure
[AC]	channel for early fibers of the anterior commissure
AH	anterior hypothalamus
ah	anterior hypothalamic neuroepithelium
ANT	anterior
at	anterior thalamic neuroepithelium
AT	anterior thalamus
BST	anterior bed nucleus of the stria terminalis
BSTl	posterior bed nucleus of the stria terminalis (lateral)
BSTm	posterior bed nucleus of the stria terminalis (medial)
bstp	posterior strial bed nucleus neuroepithelium
BT	basal telencephalon
BTA	anterolateral basal telencephalic ridge
BTP	posteromedial basal telencephalic ridge
cgm	chiasmal glial matrix
DLS	dorsal lateral septal nucleus
DT	dorsal thalamus
FM	foramen of Monro
FX	fornix
ibf	interbasal telencephalic fissure
IH	inferior horn of the lateral ventricle
ir	infundibular recess of the third ventricle
LH	lateral hypothalamus
LV	lateral ventricle
L1	most anterior level used for quantification
L2	anterior intermediate level used for quantification
L3	posterior intermediate level used for quantification
L4	most posterior level used for quantification
mh	medial horn of the lateral ventricle
mMP	medial preoptic nucleus (migrating cells)
mMPdl	medial preoptic nucleus (dorsolateral migrating cells)
mMPvm	medial preoptic nucleus (ventromedial migrating cells)
mn	median preoptic nucleus neuroepithelium
MNd	median preoptic nucleus (dorsal)
MNv	median preoptic nucleus (ventral)
mp	medial preoptic nucleus neuroepithelium
mPAL	anterior lateral preoptic area (migrating cells)
mPALd	anterior lateral preoptic area (dorsal migrating cells)
mPALv	anterior lateral preoptic area (ventral migrating cells)
mPAM	anterior medial preoptic area (migrating cells)
mPAMd	anterior medial preoptic area (dorsal migrating cells)
mPAMv	anterior medial preoptic area (ventral migrating cells)
MPdl	medial preoptic nucleus (dorsolateral)
mPPL	posterior lateral preoptic area (migrating cells)
mPPLv	posterior lateral preoptic area (ventral migrating cells)
mPPM	posterior medial preoptic area (migrating cells)
mPPMd	posterior medial preoptic area (dorsal migrating cells)
mPPMv	posterior medial preoptic area (ventral migrating cells)
mPSl	lateral superior preoptic area (migrating cells)
mPV	periventricular nucleus (migrating cells)
MPvm	medial preoptic nucleus (ventromedial)
mr	mammillary recess of the third ventricle
MS	medial septal nucleus
NC	neocortex
nc	neocortex neuroepithelium
OB	olfactory bulb
OC	optic chiasma
ON	optic nerve
or	optic recess of the third ventricle
PALd	anterior lateral preoptic area (dorsal)
PALv	anterior lateral preoptic area (ventral)
PAMd	anterior medial preoptic area (dorsal)
PAMv	anterior medial preoptic area (ventral)
PIM	intermediate preoptic area
pn	preoptic neuroepithelium
POST	posterior
PPD	posterodorsal part of the medial preoptic area
PPLv	posterior lateral preoptic area (ventral)
PPMd	posterior medial preoptic area (dorsal)
PPMv	posterior medial preoptic area (ventral)
PPV	posteroventral part of the medial preoptic area
pr	preoptic recess of the third ventricle
PS	superior preoptic area
PSl	superior preoptic area (lateral)
PVh	periventricular nucleus (horizontal limb)
RP	Rathke's pouch (anterior pituitary)
SDN	sexually dimorphic nucleus
sdn	sexually dimorphic nucleus neuroepithelium
se	septal neuroepithelium
SEl	lateral septum
SEm	medial septum
st	neuroepithelium/subependymal layer generating neostriatum
TA	transition area between MP and AH
tdv	telodiencephalic fissure (ventral part)
vIII	third ventricle
VLS	ventral lateral septal nucleus

TABLE 1. Neurogenesis in the Ventral Lateral Preoptic Area

Injection group	N	% Labeled cells (mean ± S.D.)	Day of origin	% Cells originating*
			E12	9.67 (100–A)
E13–E14	8	(A) 90.33 ± 3.28	E13	28.17 (A–B)
E14–E15	7	(B) 62.16 ± 7.34	E14	39.11 (B–C)
E15–E16	7	(C) 23.05 ± 5.12	E15	8.95 (C–D)
E16–E17	7	(D) 14.10 ± 3.30	E16	6.09 (D–E)
E17–E18	7	(E) 8.01 ± 4.38	E17	2.84 (E–F)
E18–E19	6	(F) 5.16 ± 1.86	E18	3.35 (F–G)
E19–E20	7	(G) 1.82 ± 0.54	E19	1.82 (G–H)
E20–E21	6	(H) 0.00 ± 0.00	E20	0.00

*Bottom graph, Figure 6.

'79) or the entire basal forebrain (Lammers et al., '80). All of these studies agree that the preoptic area arises from the anterior third ventricle neuroepithelium. A few long-survival [^{3}H]thymidine autoradiographic studies of neuron origin with the pulse-labeling technique have considered the preoptic area. Ifft ('72) studied preoptic neurogenesis in connection with the hypothalamus; Creps ('74) described preoptic neurogenesis in connection with the septum, olfactory tubercle and bed nucleus of the stria terminalis; Jacobson and Gorski ('81) described neurogenesis in the sexually dimorphic nucleus. In our study with the comprehensive labeling technique (Altman and Bayer, '78a), the quantification of neurogenesis was limited to the posterior preoptic area since the focus was on hypothalamic neurogenesis.

Our more recent study of embryonic and fetal hypothalamic development, using short- and sequential-survival autoradiography after pulse labeling with [^{3}H]thymidine (Altman and Bayer, '86), established that preoptic neurons originate from the neuroepithelium surrounding the preoptic recess and anterodorsal wall of the optic recess of the third ventricle, whereas the anterior hypothalamus originates from the neuroepithelium that extends from the posterodorsal wall of the optic recess to the infundibular recess. However, several nuclear components in the medial preoptic area of adults could be delineated in fetal rats, and the relationship of the median preoptic nucleus the superior preoptic area was not resolved (Altman and Bayer, '86).

The aim of this study is to provide a detailed study of the early developments of the entire preoptic area. The timetables of neurogenesis within and between the major cell groups of the preoptic area are quantified by using long-survival, comprehensive [^{3}H]thymidine autoradiography. These analyses are correlated with an extensive study of the embryonic development of the preoptic area. By using short survival (2 hrs) after a single injection of [^{3}H]thymidine to delineate active neuroepithelial zones, an attempt will be made to identify the neuroepithelial sources of discrete preoptic structures. By using sequential survival (one to several days) after a pulse [^{3}H]thymidine label, young neurons will be traced from the germinal zones to their final destinations. Finally, these observations will be related to both the functional and anatomical literature on the preoptic area.

MATERIALS AND METHODS

Long-survival [^{3}H]thymidine autoradiography

The experimental animals were the offspring of Purdue-Wistar timed-pregnant rats. The day the females were sperm positive was designated embryonic day one (E1). Normally, births occur on E23, which is also designated as postnatal day zero (P0). Two or more pregnant females were given 2 subcutaneous injections of [^{3}H]thymidine (Schwarz-Mann; sp. act. 6.0 Ci/mM; 5 μCi/g body wt) to insure comprehensive cell labeling. The injections (given between 9 and 11 a.m.) to an individual animal were separated by 24 hr. The onset of the [^{3}H]thymidine injections was progressively delayed by 1 day between groups (E13–E14, E14–E15, . . . E21–E22). All animals were perfused through the heart with 10% neutral formalin on P5. The brains in the intact skulls were kept for 24 hr in Bouin's fixative, then were transferred to 10% neutral formalin. The heads were decalcified for 3 hrs in a chelating agent containing dilute HCl (Scientific Products #D1209-1). At least 6 animals from each injection group (including males and females) were blocked coronally so that the entire diencephalon was kept in one piece. All blocks were embedded in paraffin. Every 15th section (6 μm) through the preoptic area was saved. Slides were coated with Kodak NTB-3 emulsion, exposed for 12 weeks, developed in Kodak D-19, and poststained with hematoxylin and eosin.

Sections were selected for quantitative analysis at 4 anteroposterior levels (see Figs. 2, 3). Cells were counted microscopically at X312.5 in unit areas set off by an ocular grid (0.085 mm^2). For quantification, all neurons within a designated area were assigned to one of two groups, labeled or unlabeled. Cells with silver grains overlying the nucleus in densities above background levels 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 a modification of the progressively delayed comprehensive labeling procedure (Bayer and Altman, '74) and is described in detail elsewhere (Bayer and Altman, '87). Briefly, a progressive drop in the proportion of labeled neurons from a maximal level (> 95%) in a specific population indicates that the precursor cells are producing nonmitotic neurons. By analyzing the rate of decline in labeled neurons, one can determine the proportion of neurons originating over blocks of days (or single days) during development. As an illustration of the procedure, Table 1 shows the data and calculations for the ventral lateral preoptic area at levels 1 to 4.

Throughout the quantitative analysis, it was noted that trends in cell labeling within animals were very consistent. However, variability between animals in an injection group were large enough to mask this trend. Therefore, cell labeling patterns were analyzed with the sign test (Conover, '71), a nonparametric test designed for this type of data. The sign test determines the consistency of sequential neu-

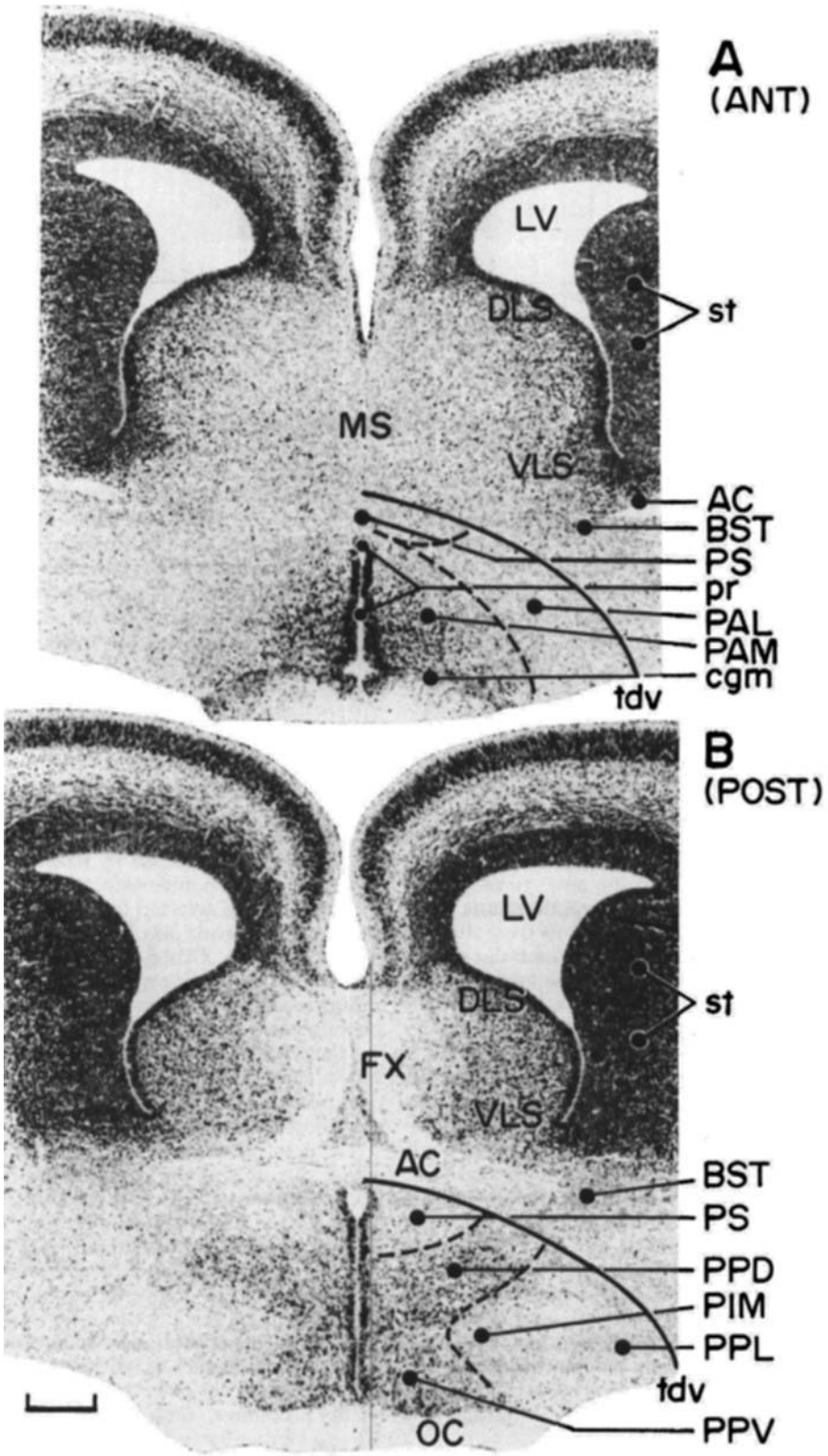

Fig.1. Autoradiograms of coronal sections through anterior (A) and posterior (B) parts of the preoptic area in the brain of an animal exposed to [^{3}H]thymidine on E16 and sacrificed on E19. The solid line delineates the preoptic area from the surrounding basal telencephalon. The dashed lines indicate subdivisions within the preoptic area based on our recent study of hypothalamic embryonic development (Altman and Bayer, '86). (6-μm paraffin sections, hematoxylin, scale = 0.25 mm)

ron production between paired locations 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*). The graphs throughout this report show the variable group data rather than the more consistent trends within individual animals. Consequently, some of the statistically significant neurogenetic gradients (between different parts of the periventricular nucleus, for example) are not conspicuous.

Short- and sequential-survival [^{3}H]thymidine autoradiography

The experimental animals were the embryos from Purdue-Wistar timed-pregnant rats given a single subcutaneous injection of [^{3}H] thymidine (Schwarz-Mann; sp. act. 6.0 Ci/mM; 5 μCi/g body wt) between 9 and 11 a.m. The day of sperm positivity is designated as gestation (embryonic) day E1. Several dams were injected for each day between E13 and E21. Survival times in each injection group varied from 2 hrs (short-survival series) to several days (sequential-survival series). For example, one dam in the E15 injection group was sacrificed 2 hrs after the injection, another 1 day later on E16, another 2 days later on E17, and so on until the last dam was sacrificed on E22. All groups were treated as the E15 group. The dams were anesthetized with pentobarbital before the embryos were removed and sacrificed by immersion in Bouin's fixative. After 24 hrs, the embryos were transferred to 10% neutral formalin until the time of embedding in either paraffin or methacrylate. The blocks were serially sectioned (every 10th saved) at 6 μm (paraffin) or at 3 μm (methacrylate) in the coronal, sagittal, and horizontal planes. For [^{3}H]thymidine autoradiography, the slides were coated with Kodak NTB-3 emulsion, exposed for 6 weeks, developed in Kodak D-19, and poststained with hematoxylin and eosin.

RESULTS

Neurogenetic gradients in long-survival autoradiograms

Methodological considerations and anatomical remarks. In the P5 long-survival series, the brains were left in the skull to eliminate possible damage to the basal forebrain during dissection. The decalcification procedure used did not deleteriously affect histological preservation. By P5, all of the cell groups in the basal forebrain have settled in their final locations; in fact, most nuclei are more distinct at P5 than in adults. This is so because the cells are smaller and their packing density is greater (the effect is the same as looking at much thicker sections of adult brains).

The selection of anatomical levels for quantification was based on our recent study of hypothalamic development (Altman and Bayer, '86) where the preoptic area was briefly described. Figure 1 shows coronal sections through anterior and posterior parts of the preoptic area in the brain of an animal sacrificed on E19 after exposure to [^{3}H]thymidine on E16. The initial subdivisions for the cell counts of the P5 animals are indicated by dashed lines. However, it was found that more nuclear groups could be distinguished at P5 than in fetal rats. Consequently, the final subdivision of the postnatal preoptic area differs from that reported in the embryonic study: (1) the medial preoptic nucleus could be separated from the medial preoptic area, (2) the sexually dimorphic nucleus, not evident prenatally, was found at P5, and (3) because the pattern of cell origin in the intermediate preoptic area (PIM) is the same as in the ventral part of the anterior medial preoptic area (PAM), the two were combined. In addition, the cell groupings in the superior preoptic area were redefined in two ways: (1) the median preoptic nucleus could be defined in the midline, and (2) the pattern of cell origin in the medial superior preoptic area (PS) was found to be the same as the dorsal part of the medial preoptic area (PAM), and the two were combined.

Neurogenesis was quantitatively assessed at four coronal levels from anterior (L1) to posterior (L4); L1–L2 represent the anterior preoptic area, L3–L4 the posterior preoptic area (abbreviated as PA and PP, respectively). Figure 2A shows L1 in an animal exposed to [^{3}H]thymidine on E15 and E16. Boundaries between the lateral and medial preoptic areas are indicated by dashed lines. The subcommissural part of the anterior bed nucleus of the stria terminalis (BST; henceforth referred to as the strial bed nucleus) invaginates the dorsal portion of the lateral preoptic area. The ventral lateral septal nucleus (VLS) forms a roof over the lateral (PAL) and medial (PAM) preoptic areas. The boundary between the septum and preoptic area is easily distinguished in the E15 + E16 injection group, since the VLS contains more labeled cells than the dorsal parts of the preoptic area. Both the lateral and medial preoptic areas contain diffusely arranged neurons. The lateral preoptic area was divided into dorsal (PALd) and ventral (PALv) parts for quantification; the medial preoptic area was divided in a similar fashion (PAMd, PAMv). More definite neuronal groupings at this level are the median preoptic nucleus (MN) in the midline above the lumen of the third ventricle and the medial preoptic nucleus (MP). The median preoptic nucleus extends dorsally into the ventral part of the medial septal nucleus (MS), and counts were made in dorsal (MNd) and ventral (MNv) parts. The medial preoptic nucleus is located adjacent to the third ventricle ependyma and contains more densely packed neurons than those in the surrounding medial preoptic area; it was subdivided into dorsolateral (MPdl) and ventromedial (MPvm) parts for quantification.

At L2 (Fig. 2B), the preoptic area contains the same structures that were represented at L1, but a few changes can be seen. The anterior strial bed nucleus is further shortening the dorsoventral extent of the lateral preoptic area. The anterior commissure (AC) cuts the median preoptic nucleus into a dorsal part overlying the commissure and a ventral part extending to the dorsal tip of the third ventricle. The medial preoptic nucleus, still adjacent to the third ventricle ependyma, is larger and more prominent.

At L3 (Fig. 3A) some notable changes take place in the preoptic area. The median preoptic nucleus is no longer present. The medial part of the posterior strial bed nucleus (BSTm) has completely cut off the dorsal part of the posterolateral preoptic area; therefore, only the ventral part (PPLv) was quantified. A triangular area, just medial to the BST in the dorsal preoptic area, was quantified as the lateral superior preoptic area (PS1). The medial preoptic area was quantified in dorsal (PPMd) and ventral (PPMv) parts. The medial preoptic nucleus contains a dense cluster of neurons in its core, the sexually dimorphic nucleus (SDN). The vertical limb of the periventricular nucleus (PVv) lines the

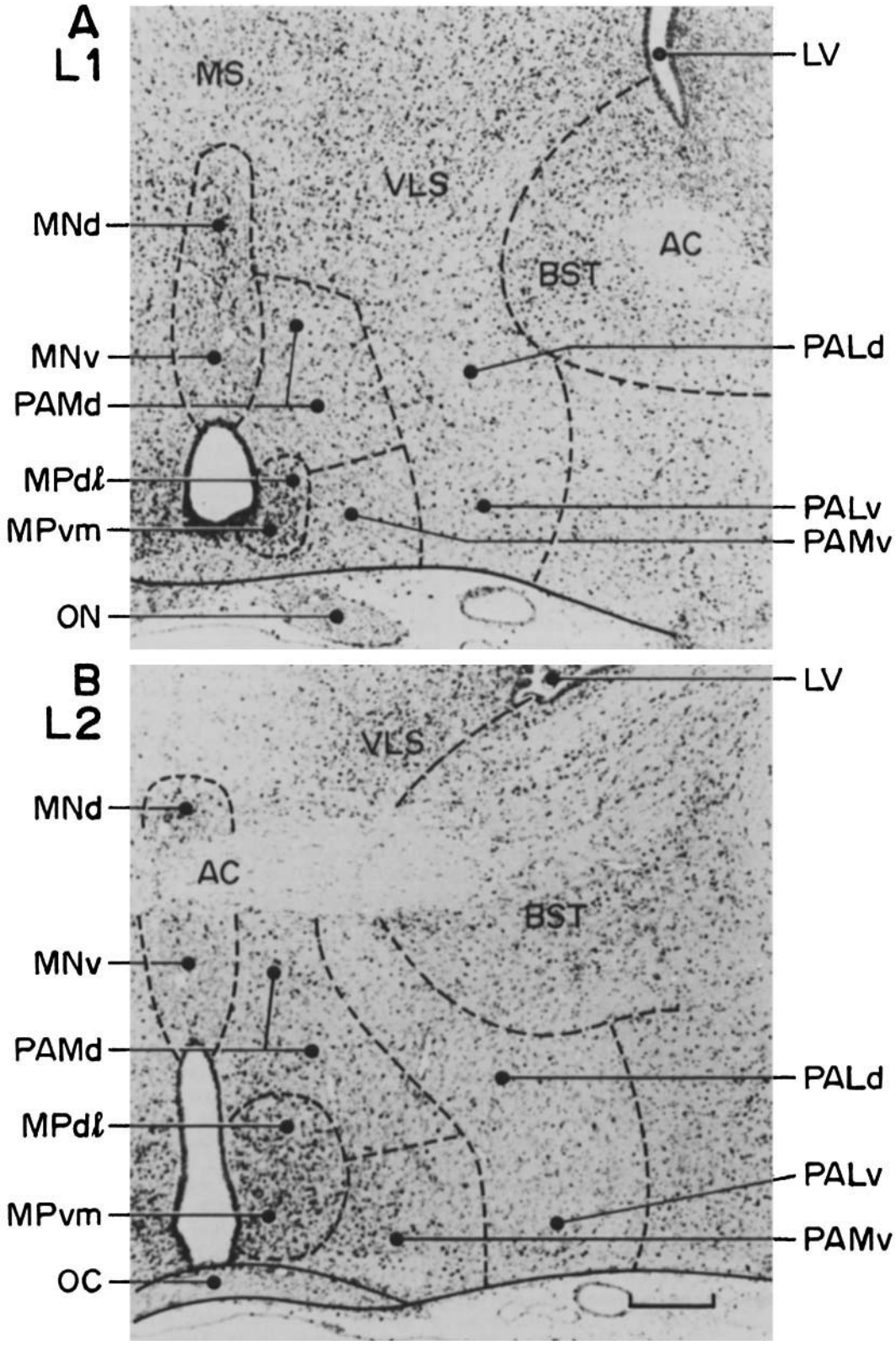

Fig. 2. Autoradiograms of the coronally sectioned anterior preoptic area in the brain of an animal exposed to [^{3}H]thymidine on E15+E16 and sacrificed on P5. These sections are representative of those selected for quantification in the long-survival P5 series of thymidine autoradiograms. L1 (A) represents the most anterior level used for quantification; L2 (B) is approximately 90–180 μm posterior to L1. Dashed lines surround major structures in the preoptic area and basal telencephalon. Solid circles in labeled preoptic area structures represent the centers of the areas that were used for cell counts at higher magnification. The solid line indicates the ventral border of the brain. (6-μm paraffin sections, hematoxylin, scale = 0.25 mm)

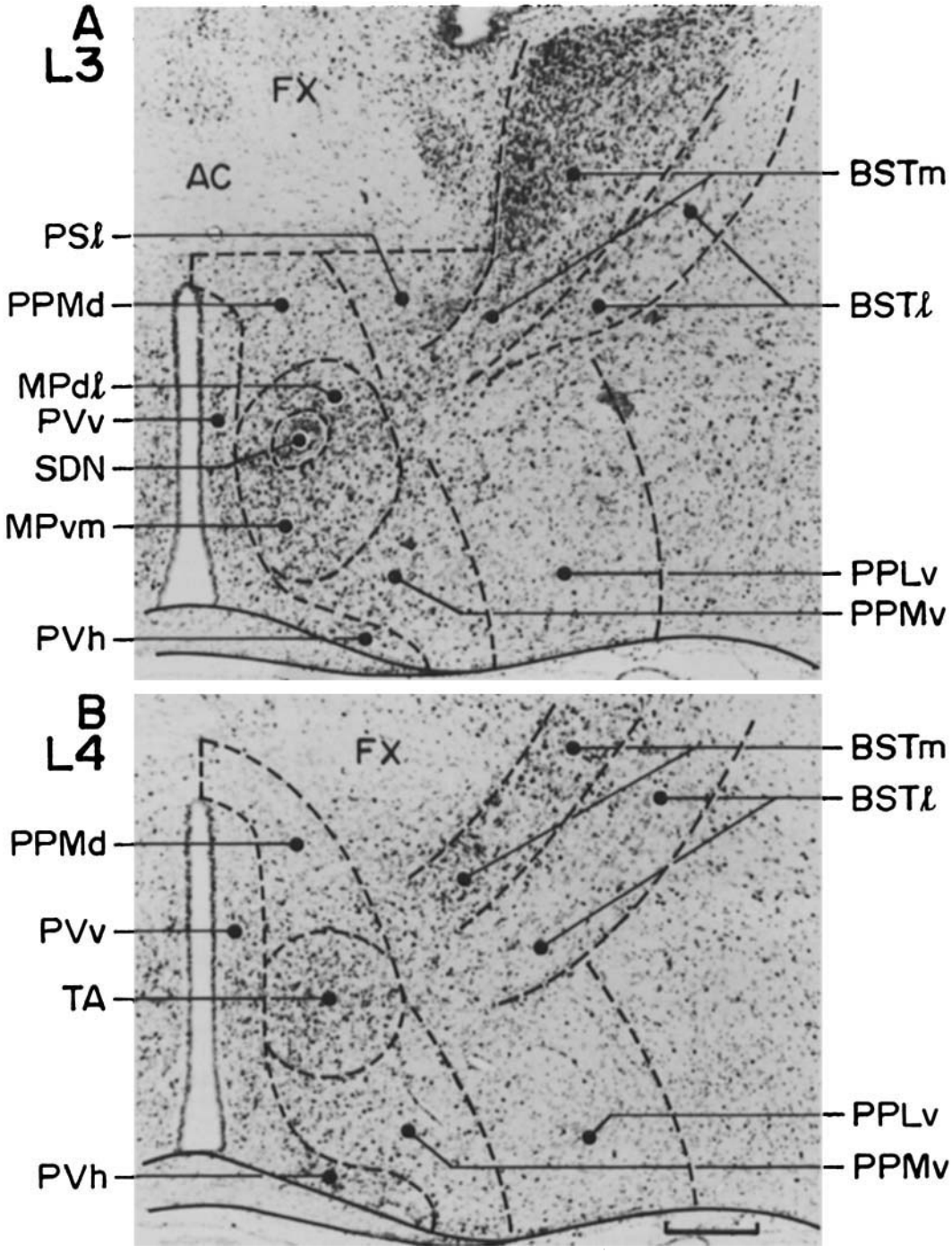

Fig. 3. The same as in Figure 2 for the posterior preoptic area showing the levels quantified for L3 (**A**) and L4 (**B**). (6-μm paraffin sections, hematoxylin, scale = 0.25 mm)

third ventricle and extends horizontally (PVh) above the most anterior fibers in the optic chiasma, displacing the medial preoptic nucleus laterally.

At L4 (Fig. 3B), the posterior part of the strial bed nucleus has enlarged, and both medial (BSTm) and lateral (BSTl) areas cut into more of the dorsal lateral preoptic area. The columns of the fornix (FX) extend to the medial part of the BST. The sexually dimorphic nucleus is no longer distinct, and the area is filled with scattered neurons, a transition area (TA), which may be a posterior continuation of the medial preoptic nucleus. The periventricular nucleus is still prominent, showing dorsal and horizontal limbs.

Overall view of the lateral to medial neurogenetic gradient. The time of origin of neurons throughout the two anterior levels of the preoptic area is presented in Figure 4, where data are combined for dorsal and ventral parts of the lateral preoptic area, the medial preoptic area, and the medial preoptic nucleus. Neurons in the lateral preoptic area are generated predominantly on E12 through E15, significantly before the majority of neurons in the medial

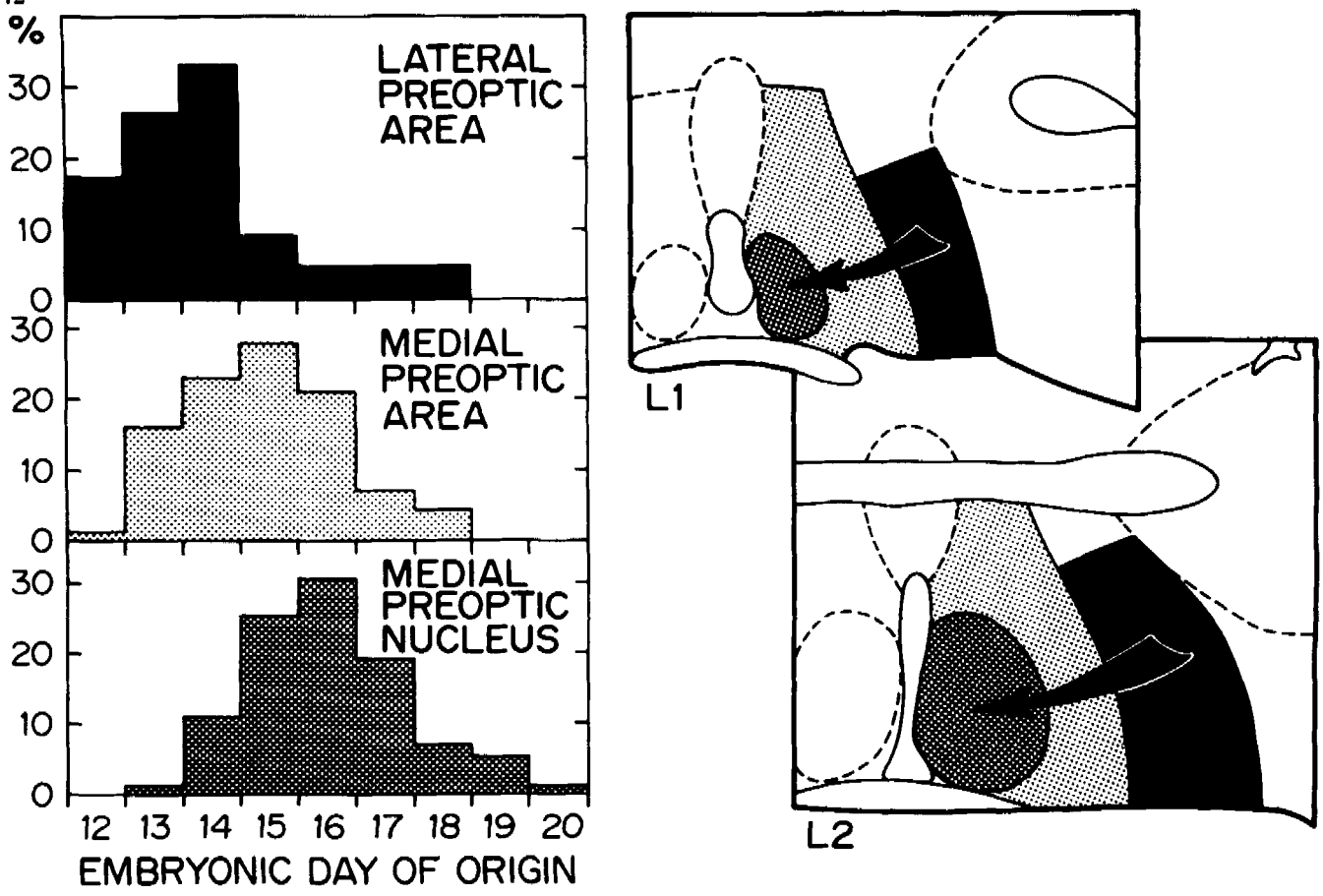

Fig. 4. Neurogenesis in the anterior preoptic area. The bar graphs indicate the proportion of neurons originating during single embryonic days. Cells were counted in the areas shaded in the drawings. Counts of several smaller areas were combined to give an overall view of the lateral to medial neurogenetic gradient (indicated by the arrow in the drawings).

preoptic area ($P < .0001$); medial preoptic area neurons originate between E13 and E16. Neurons in the medial preoptic nucleus are generated mainly between E15–E17, significantly later ($P < .0001$) than most neurons in the medial preoptic area.

The lateral preoptic area. A qualitative examination of the lateral preoptic area (Fig. 5) in an animal exposed to [^{3}H]thymidine on E14 and E15 shows that the proportion of labeled neurons increases from dorsal (PALd, Fig. 5A) to ventral (PALv, Fig. 5C), indicating an intrinsic neurogenetic gradient. The quantitative data show that throughout the lateral preoptic area, neurons are generated predominantly on E12 through E14 (Fig. 6). The sign test indicated no significant differences along the rostrocaudal plane in the lateral preoptic area (all $P > 0.05$) and the graphs show combined data. The dorsal part contains 22% of its population originating on E12, and on the average, neurons here are generated significantly earlier than neurons in the ventral part ($P < 0.0001$).

At L3, a triangular shaped lateral superior preoptic area (PSl, Fig. 3A) lies medial to the posterior bed nucleus (BSTm). Neurogenesis in PSl was compared to that in the dorsal medial preoptic area at L3 (Fig. 7). The lateral neurons originate predominantly on E13 and E14, earlier than those located medially ($P < .0001$). The pattern of neurogenesis is similar to that found in the ventral part of the lateral preoptic area (bottom graph, Fig. 6; all $P > .05$), but it occurs significantly later ($P < .0001$) than neurogenesis in the dorsal part of the anterior lateral preoptic area (top graph, Fig. 6).

The medial preoptic area. Autoradiograms of the medial preoptic area (Fig. 8) in an animal exposed to [^{3}H]thymidine on E15 and E16 show that the proportion of labeled neurons is lowest in the most posterior dorsal area (Fig. 8A), intermediate in the anterior dorsal area (Fig. 8B), and highest in the ventral area (Fig. 8C). These trends indicate combined posterior (older) to anterior (younger) and dorsal (older) to ventral (younger) neurogenetic gradients. Within the dorsal part of the medial preoptic area, the sign test indicated simultaneous neurogenesis (all $P > .05$) at L1–L2 and at L3–L4. Consequently, Figure 9 shows combined data for anterior (middle graph) and posterior (top graph) levels. More than half (61%) of the cells in the posterodorsal medial preoptic area originate on or before E14, whereas anterodorsal cells are evenly generated between E13 and E16 ($P < .041$). The sign test indicated no significant differences (all $P > .05$) between any parts of the ventral medial preoptic area along the rostrocaudal plane, and the bottom graph in Figure 9 combines data for all four levels. The neurons in the ventral population have a strong peak of neurogenesis on E15 (44% originate in a single day), significantly later than the dorsal cells ($P < .0001$). These three patterns of neurogenesis suggest that, rather than having a single population of neurons extending throughout the

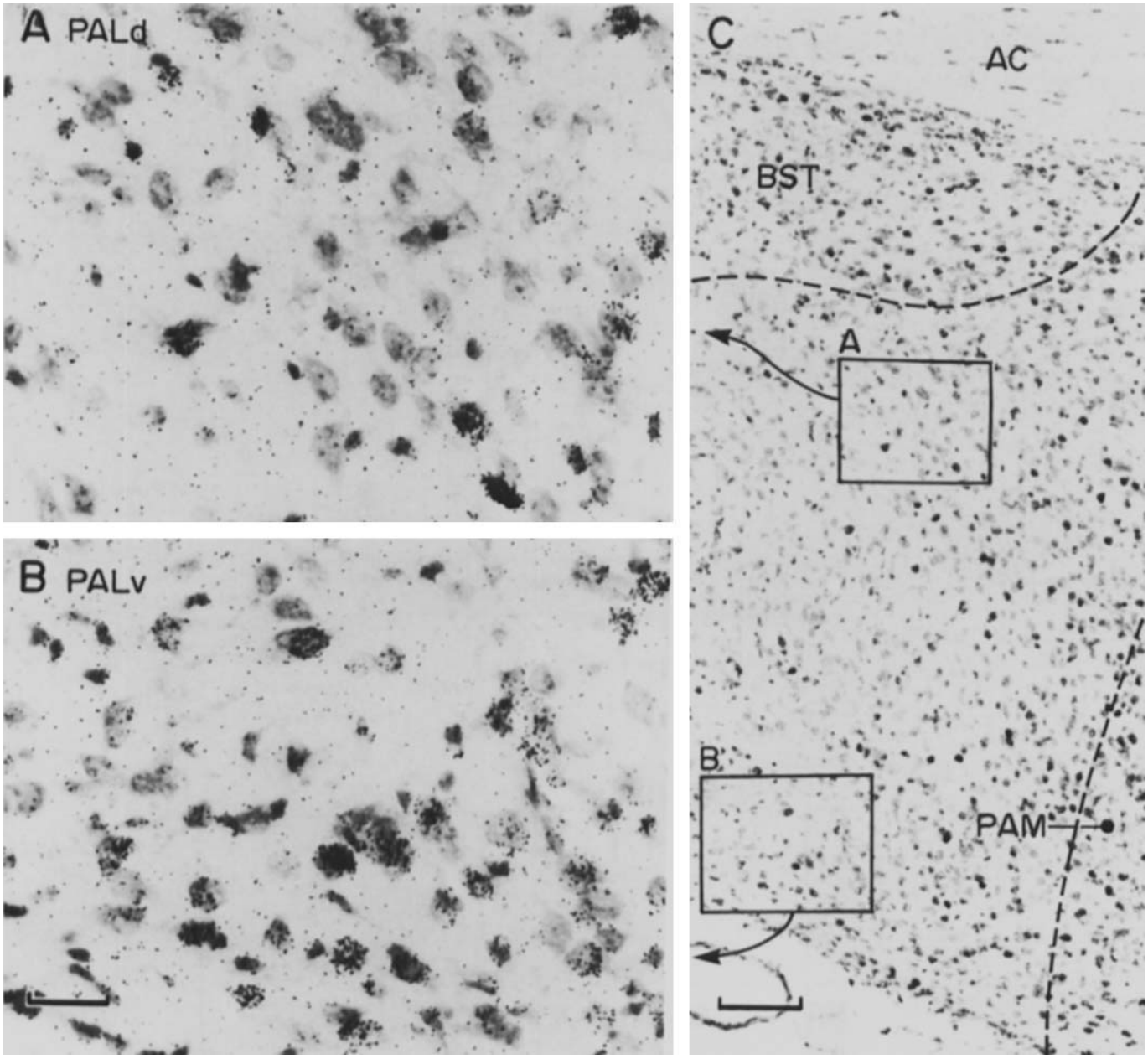

Fig. 5. Autoradiograms of the dorsal (A) and ventral (B) lateral preoptic area at at L2 in the brain of an animal exposed to [^{3}H]thymidine on E14+E15 and sacrificed on P5 (6-μm paraffin section, hematoxylin, scale = 0.25 mm). The low magnification view in C (scale = 0.1 mm) indicates the locations of the areas in A and B.

entire medial preoptic area, the posterodorsal, anterodorsal, and ventral areas represent three different populations. populations.

The medial preoptic nucleus. One of the strongest neurogenetic gradients in the entire preoptic area is found in the medial preoptic nucleus. An autoradiogram of the medial preoptic nucleus at L2 (Fig. 10) in an animal exposed to [^{3}H]thymidine on E16 and E17 shows that the dorsolateral part of the nucleus (MPdl) contains many fewer labeled cells than the ventromedial part (MPvm). The sign test indicated that there were no differences along the rostrocaudal extent of the medial preoptic nucleus at L1–L3 (all $P > .05$), so the graphs in Figure 11 show combined data. Neurons in the dorsolateral part have a pronounced neurogenetic peak on E15 and originate significantly earlier ($P < .0001$) than those in the ventromedial part, which are generated mainly on E16 and E17. The sexually dimorphic nucleus is embedded in the medial preoptic nucleus at L3; its development is extensively discussed below.

A biphasic pattern of neurogenesis is found at L4 (bottom graph, Fig. 11) in an area where the medial preoptic nucleus would be located if it extended to this level. There is a peak of neuron production on E15, few neurons are generated on E16, and approximately 50% of the population is generated on E17 and E18. There is no evidence of the strong dorsolateral to ventromedial gradient seen through-

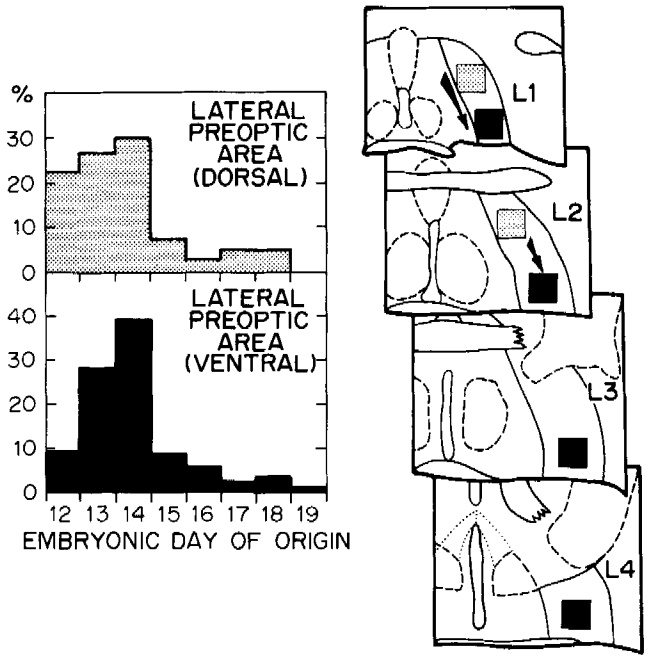

Fig. 6. Neurogenesis in the lateral preoptic area. The bar graphs indicate the proportion of neurons originating during single embryonic days. Cells were counted in the shaded areas in the drawings. There is a dorsal (older) to ventral (younger) neurogenetic gradient indicated by the arrows.

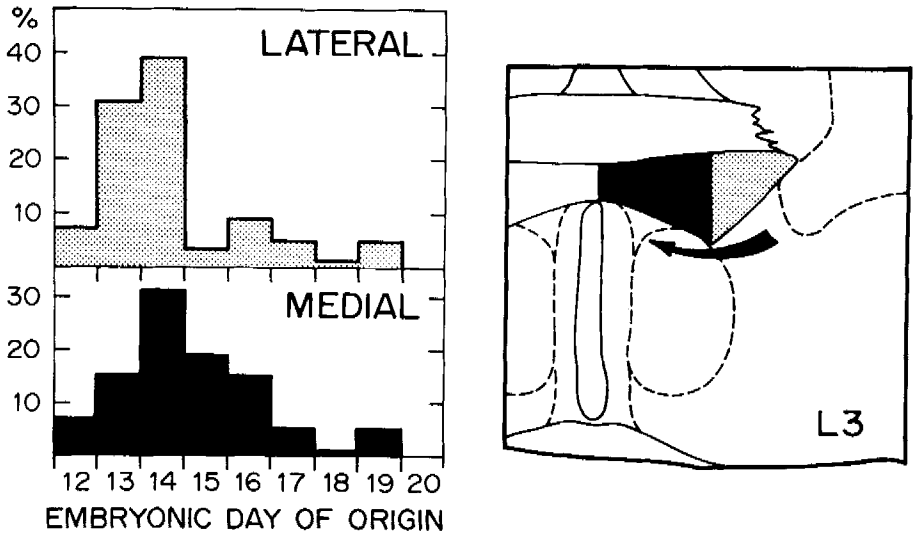

Fig. 7. Neurogenesis in the superior preoptic area (stippled) compared with that in the dorsal medial preoptic area (solid). The bar graphs indicate the proportion of neurons originating during single embryonic days. Cells were counted in the areas shaded in the drawings. There is a lateral to medial gradient of neurogenesis (arrow).

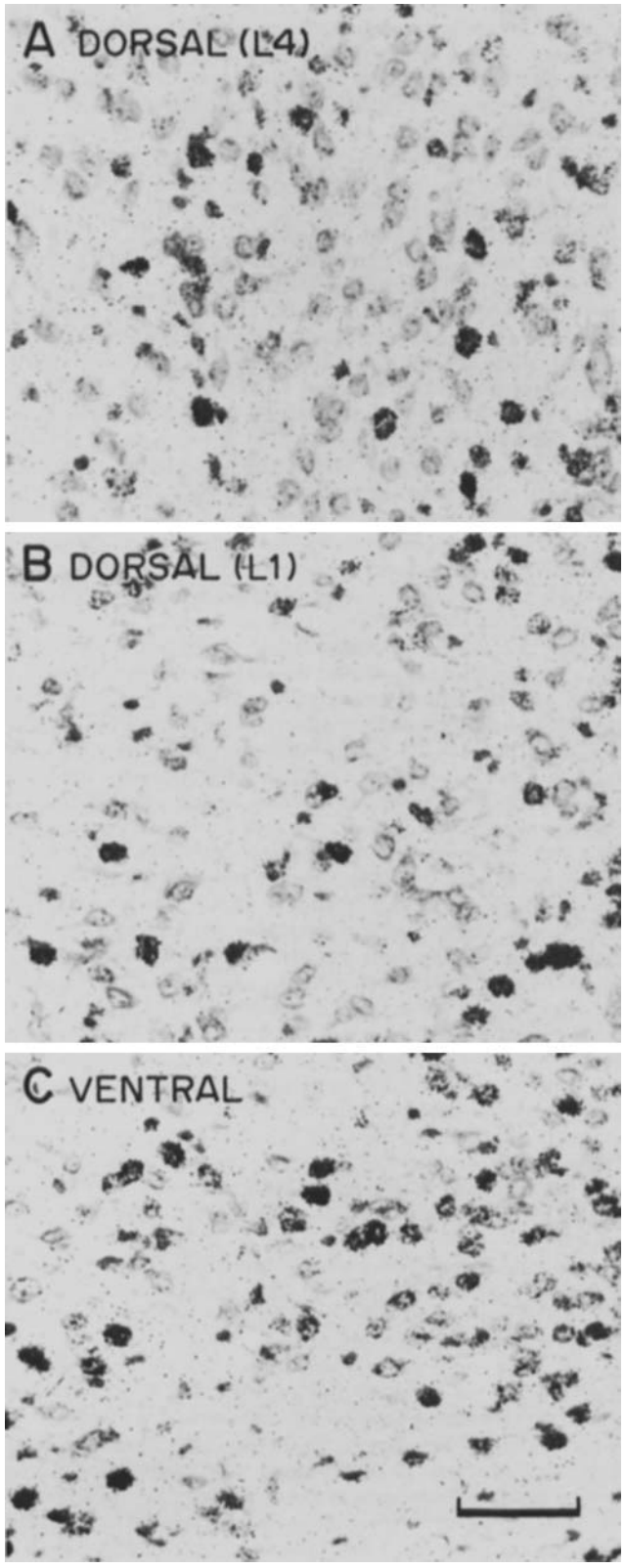

Fig. 8. A series of autoradiograms of the medial preoptic area in the brain of an animal exposed to [^{3}H]thymidine on E15+E16 and sacrificed on P5. (6-μm paraffin sections, hematoxylin, scale = 0.1 mm)

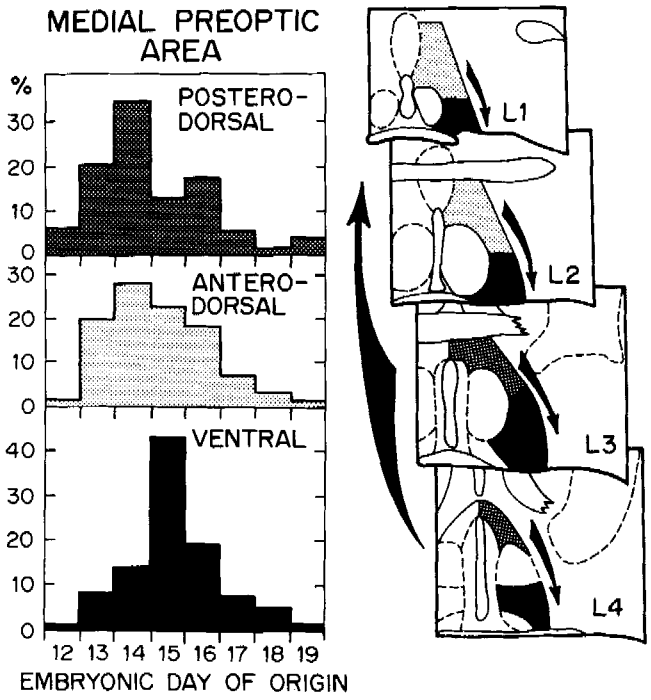

Fig. 9. Neurogenesis in the medial preoptic area. The bar graphs indicate the proportion of neurons originating during single embryonic days. Cells were counted in the shaded areas in the drawings. There is a dorsal to ventral neurogenetic gradient throughout (arrow in each drawing) and a caudal to rostral gradient in the dorsal medial preoptic area.

out the medial preoptic nucleus at L1–L3. This biphasic pattern suggests that two different neuronal populations are intermingled. The cells generated on E15 may be related to the dorsolateral part of the medial preoptic nucleus since they are generated simultaneously. The cells generated on E17 and E18 may belong to a "shell" of neurons related to the sexually dimorphic nucleus. Possibly, these neurons are in a transition area extending to the anterior hypothalamus.

The periventricular nucleus. The periventricular nucleus (PVv and PVh) is definite only in the posterior preoptic area (Fig. 3). The sign test indicated that neurogenesis is simultaneous along the rostrocaudal plane (all $P > .05$) so the graphs in Figure 12 show combined data. Approximately 41% of the neurons in the vertical limb originate on or *before* E16, whereas 74% of the neurons in the horizontal limb originate on or *after* E17 ($P < .0001$).

Delineation of the preoptic neuroepithelium and early cell movements

Choice of materials. Low magnification sagittal sections of the embryonic forebrain, cut parallel to the rapidly expanding anterior-posterior axis, were used to locate the preoptic neuroepithelium in relation to surrounding diencephalic and telencephalic structures. Since young preoptic neurons migrate predominantly lateral to their neuroepithelial sources, higher magnification coronal sections, cut parallel to the migratory route, were found particularly useful in tracing migratory pathways and identifying settling areas. The 24-hr survival period proved to be ideal for tracking early cell movements from active neuroepithelial sites, since heavily labeled cells (produced the day before) are beginning to migrate and are seen as "wave fronts" of young neurons just outside the germinal matrix. These

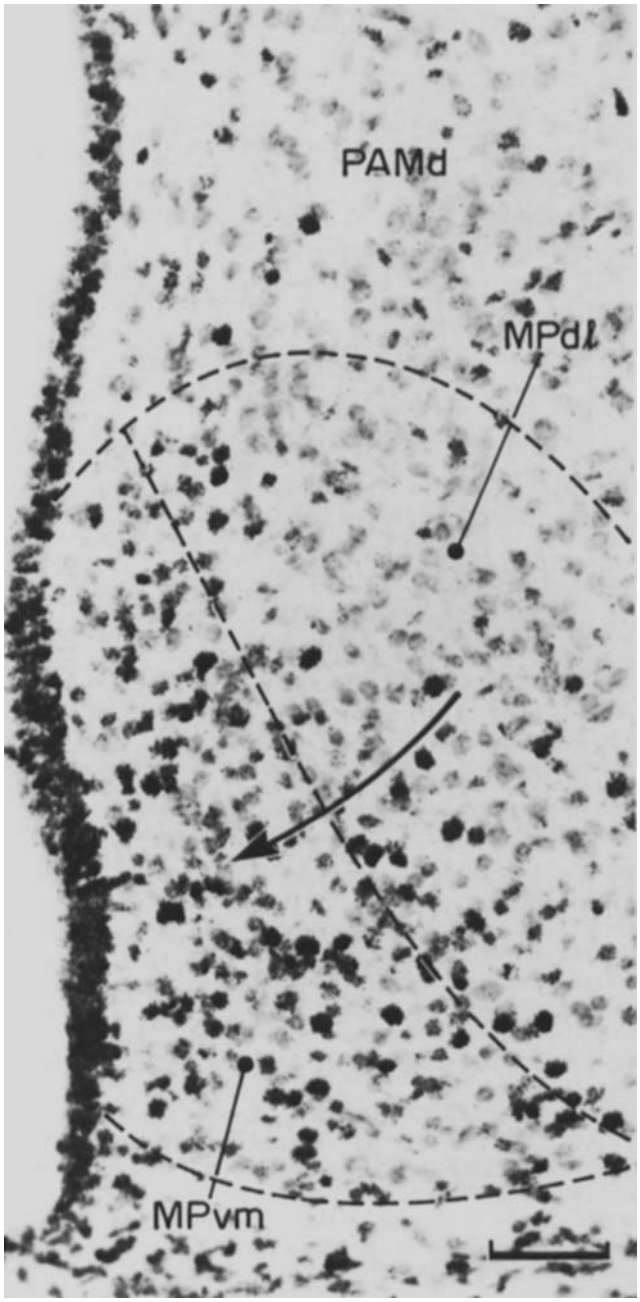

Fig. 10. The medial preoptic nucleus at L2 in the brain of an animal exposed to [^{3}H]thymidine on E16+E17 and sacrificed on P5. The arrow indicates the strong dorsolateral to ventromedial neurogenetic gradient. (6-μm paraffin section, hematoxylin, scale = 0.5 mm)

cohorts of isochronic neurons (24 hrs old) are adjacent to laterally placed unlabeled cells that were generated earlier. In much of the preoptic area, the lateral (older) to medial (younger) pattern of cell accumulation found at these early stages is retained as a neurogenetic gradient in adults.

E14 to E15 series. Figure 13 is a series of sagittal sections from an animal killed on E15, 24 hrs after exposure to a single [^{3}H]thymidine injection on E14. In the midline (Fig. 13D), the optic recess (or) separates the preoptic neuroepithelium (pn) from the neuroepithelium (ah) that generates the anterior hypothalamus (AH). The preoptic neuroepithelium forms the anterodorsal wall of the optic recess and fuses with the midline septal neuroepithelium (se) in the region where the anterior commissure will decussate on E17. In more lateral sections (Fig. 13A–C), the preoptic neuroepithelium retains its position as the anterodorsal wall of the optic recess. A "wave front" of heavily labeled cells (young neurons generated on E14) is migrating away from the germinal matrix (mPAL/mPPL, Fig. 13A–B). These young neurons are destined for the lateral preoptic area and will accumulate adjacent to older (unlabeled) neurons (PAL/PPL, Fig. 13A). The floor of the telencephalon contains two ridges, an anterior (BTA, located laterally in coronal sections) and a posterior (BTP, located medially in coronal sections). The foramen of Monro (FM, Fig. 13B–C) passes just behind the posteromedial basal telencephalic ridge and becomes continuous with the third ventricle (vIII, Fig. 13B). Just lateral to the foramen of Monro, there is a deep cleft in the lateral ventricle, the medial horn (mh, Fig. 13A). The data presented in a companion paper (Bayer, '87) indicate that the neuroepithelium in the base of the cleft and along its lateral wall gives rise to the posterior part of the bed nucleus of the stria terminalis (bstp, Fig. 13A), which extends into the preoptic area.

Figure 14 shows coronal sections through anterior (Fig. 14A), intermediate (Fig. 14B), and posterior (Fig. 14C) parts of the preoptic area in an animal exposed to [^{3}H]thymidine on E14 and sacrificed 24 hrs later (E15). The preoptic neuroepithelium has a vertical limb (the preoptic recess, pr) and a horizontal limb (the optic recess, or); both limbs are active. This is to be expected since the neurogenetic data indicate that most of the neurons in the medial preoptic area and virtually all of the cells in the medial nuclei are still to be generated. Since the majority of the neurons in the lateral preoptic area have already been produced (Figs. 4, 6), the neuroepithelium found on E15 is not likely to contain many stem cells generating lateral preoptic neurons. The preoptic neuroepithelium has a thick core (asterisk, Fig. 14B–C) flanked by thinner dorsal and lateral parts. On the basis of the neurogenetic data, it is postulated that the thin dorsal flank is the neuroepithelial source (triangle, Fig. 14B–C) of the earlier generated dorsal medial preoptic area, whereas the somewhat thicker lateral flank is the source (square, Fig. 14B–C) of the later generated ventral medial preoptic area (Fig. 9). The thick core of germinal cells is presumed to be generating precursor cells that will later give rise to the medial preoptic and periventricular nuclei.

Migrating heavily labeled neurons produced on E14 (cell groups preceded with an "m" in Fig. 14) are located just outside of the neuroepithelium. The neurogenetic data indicate that E14 is a peak day for neurogenesis in both dorsal and ventral parts of the lateral preoptic area (Fig. 6) and dorsal parts of the medial preoptic area (Fig. 9). Consequently, the heavily labeled young neurons are postulated to be migrating into these areas. The most dorsal group of migrating neurons is presumably destined for the dorsal lateral preoptic area anteriorly (mPALd, Fig. 14B) and for the lateral superior preoptic area posteriorly (mPSl, Fig. 14C). The most ventral and lateral group of labeled cells is postulated to be young neurons migrating dorsally into the ventral lateral preoptic area (mPALv/mPPLv, Fig. 14B–C). The heavily labeled cells located just above the thick neuroepithelial core are presumably neurons headed for dorsal

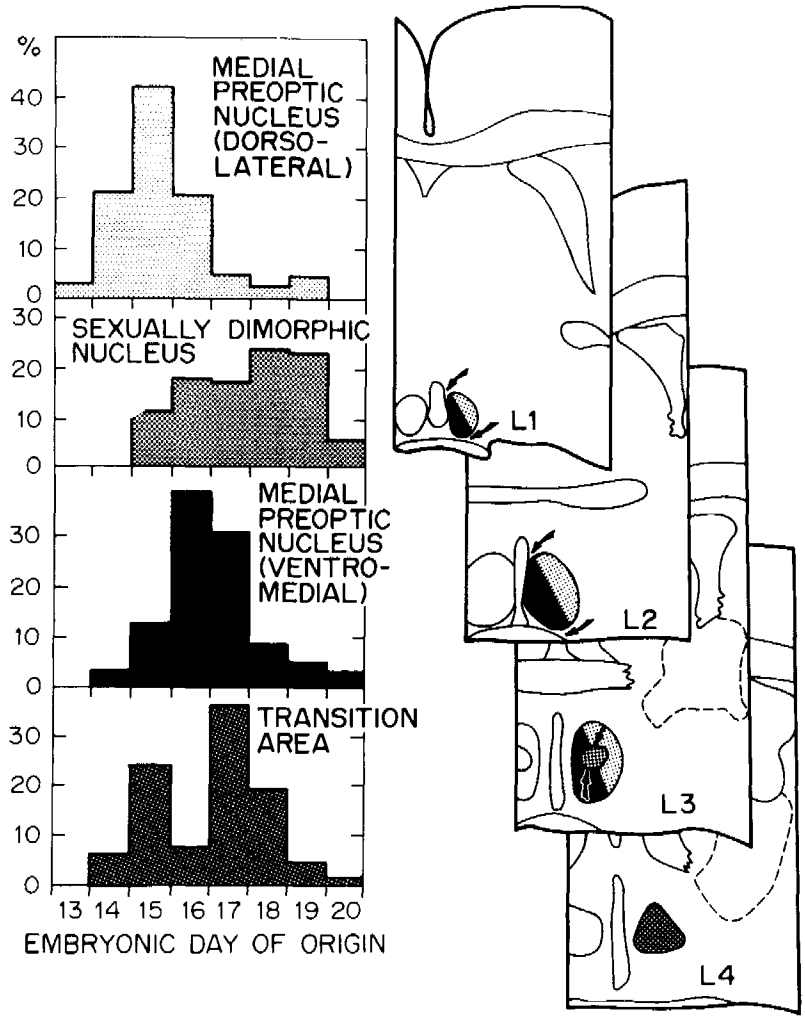

Fig. 11. Neurogenesis in the medial preoptic nucleus and related structures. The bar graphs indicate the proportion of neurons originating during single embryonic days. Cells were counted in the shaded areas in the drawings. The dorsolateral (light stipple, top graph) to ventromedial (solid, third graph from top) neurogenetic gradient within the medial preoptic nucleus is indicated by the small arrows in the drawings of L1 and L2. Both parts of the medial preoptic nucleus originate earlier than the sexually dimorphic nucleus (medium stipple, second graph from top) as indicated by the arrows at L3. The area at L4 (darkest stipple, bottom graph) has a biphasic pattern.

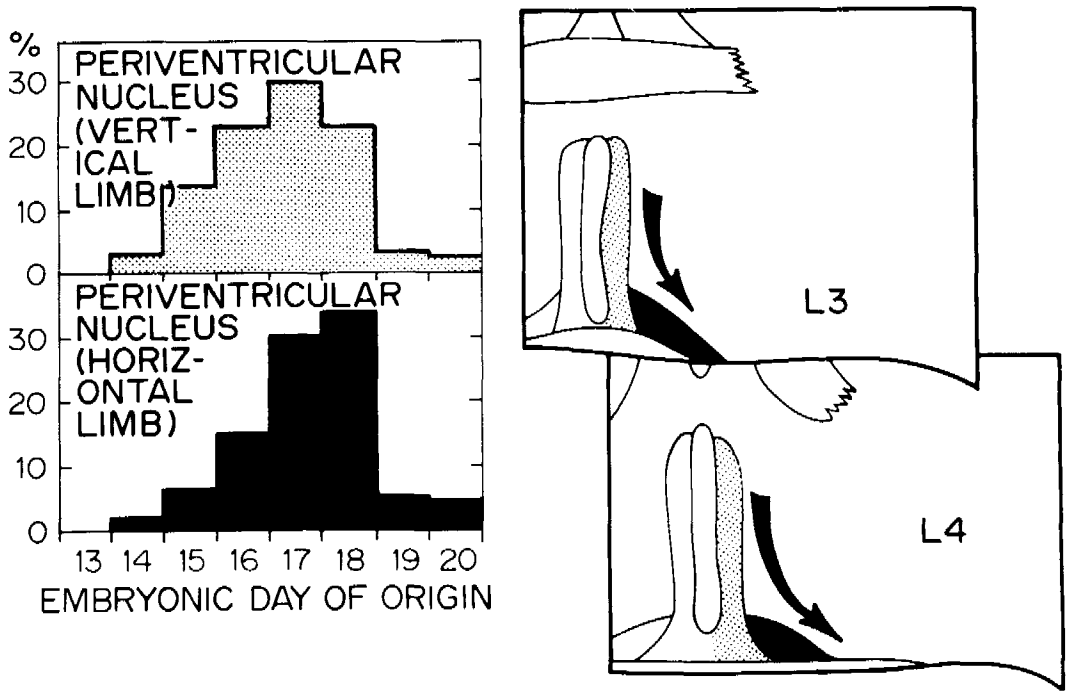

Fig. 12. Neurogenesis in the periventricular nucleus. The bar graphs indicate the proportion of neurons originating during single embryonic days. Cells were counted in the shaded areas in the drawings. There is a dorsal (stripple, top graph) to ventral (solid, bottom graph) neurogenetic gradient (arrows in drawings).

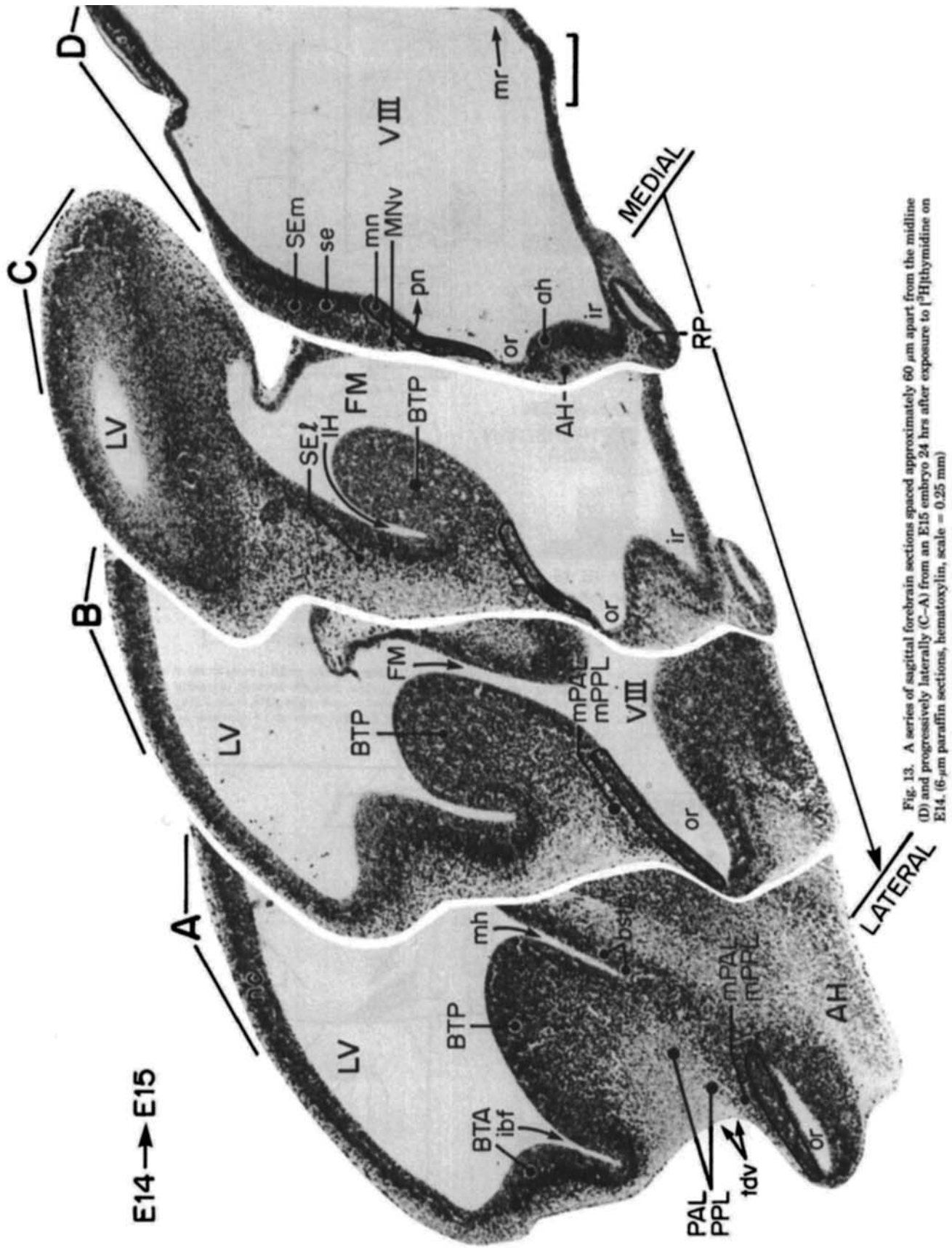

Fig. 13. A series of sagittal forebrain sections spaced approximately 60 μm apart from the midline (D) and progressively laterally (C–A) from an E15 embryo 24 hrs after exposure to [^{3}H]thymidine on E14. (6-μm paraffin sections, hematoxylin, scale = 0.25 mm)

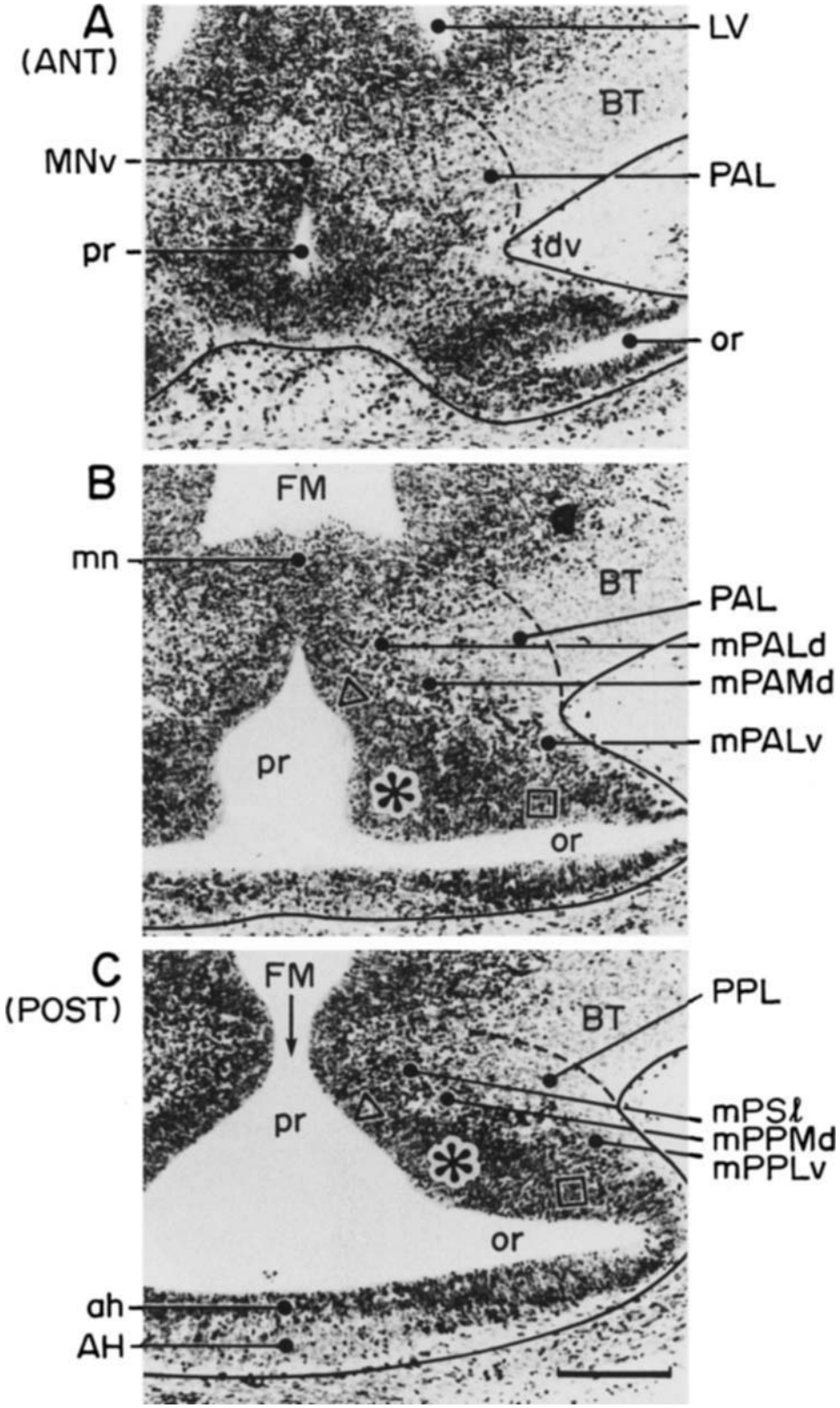

Fig. 14. A series of coronal sections through the anterior (A), intermediate (B), and posterior (C) preoptic area in an E15 embryonic brain 24 hrs after exposure to [^{3}H]thymidine on E14. (6-μm paraffin sections, hematoxylin, scale = 0.2 mm)

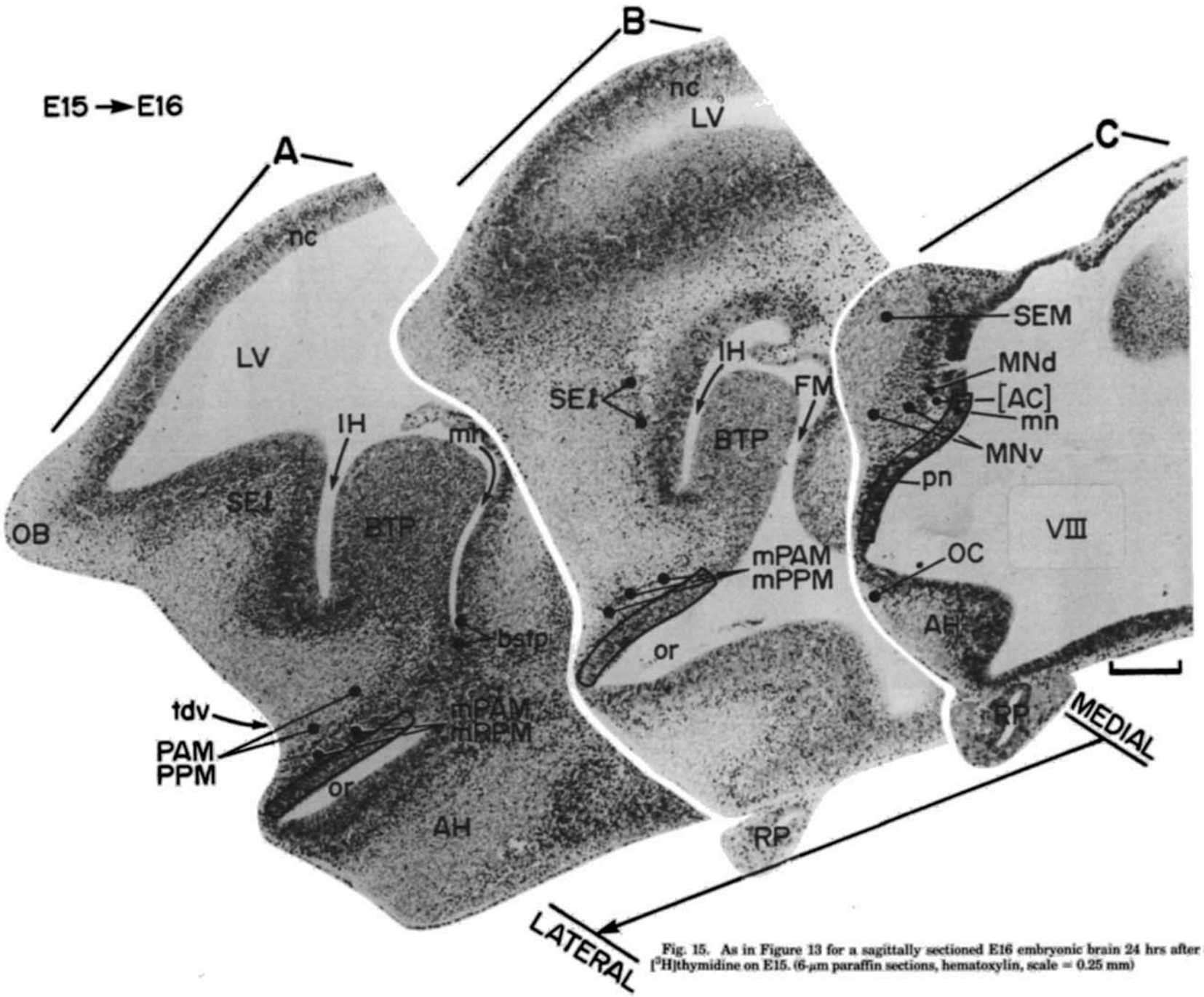

Fig. 15. As in Figure 13 for a sagittally sectioned E16 embryonic brain 24 hrs after exposure to [^{3}H]thymidine on E15. (6-μm paraffin sections, hematoxylin, scale = 0.25 mm)

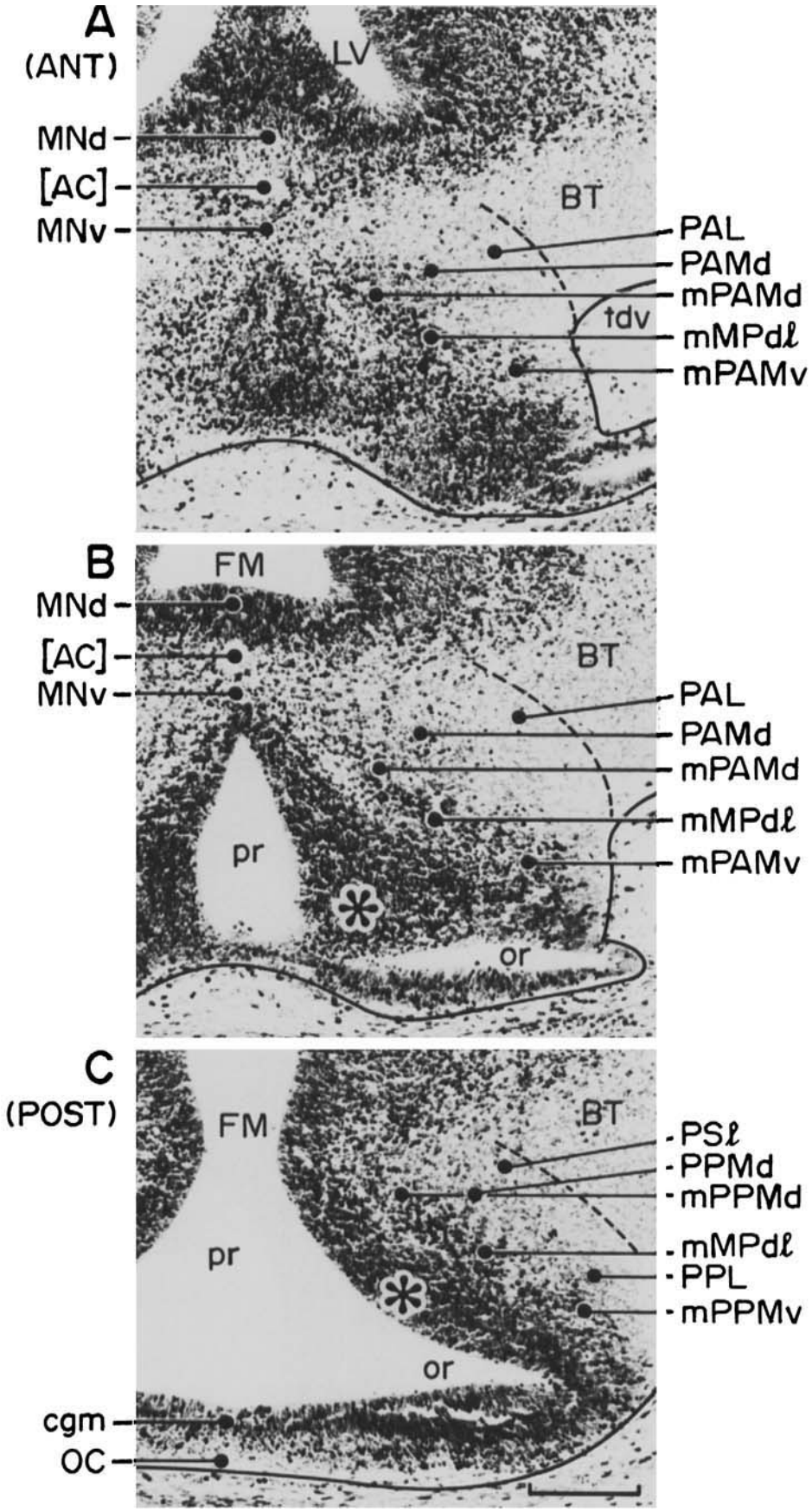

Fig. 16. As in Figure 14 for a coronally sectioned E16 embryonic brain 24 hrs after exposure to [^{3}H]thymidine on E15. (6-μm paraffin sections, hematoxylin, scale = 0.2 mm)

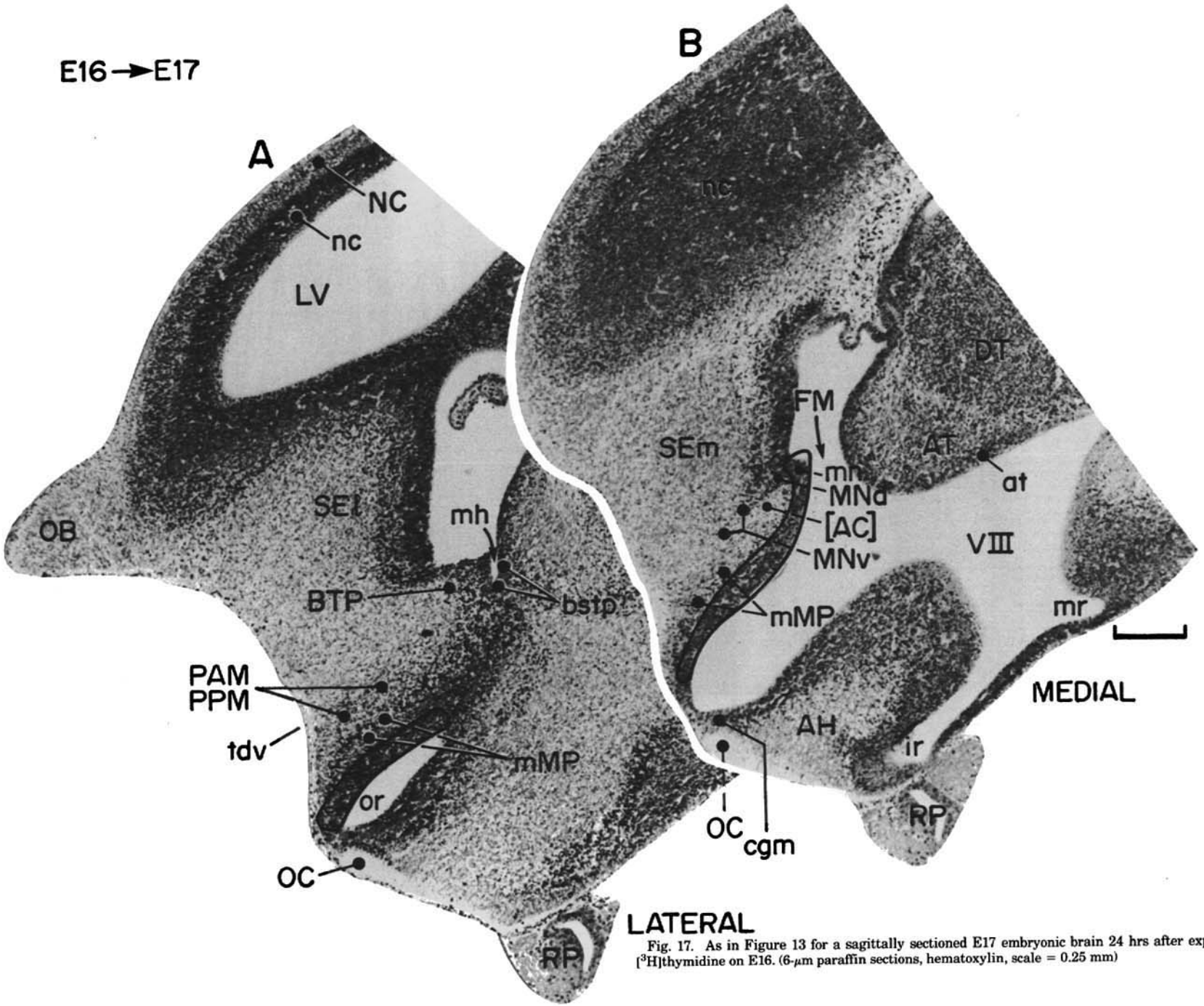

Fig. 17. As in Figure 13 for a sagittally sectioned E17 embryonic brain 24 hrs after exposure to [^{3}H]thymidine on E16. (6-μm paraffin sections, hematoxylin, scale = 0.25 mm)

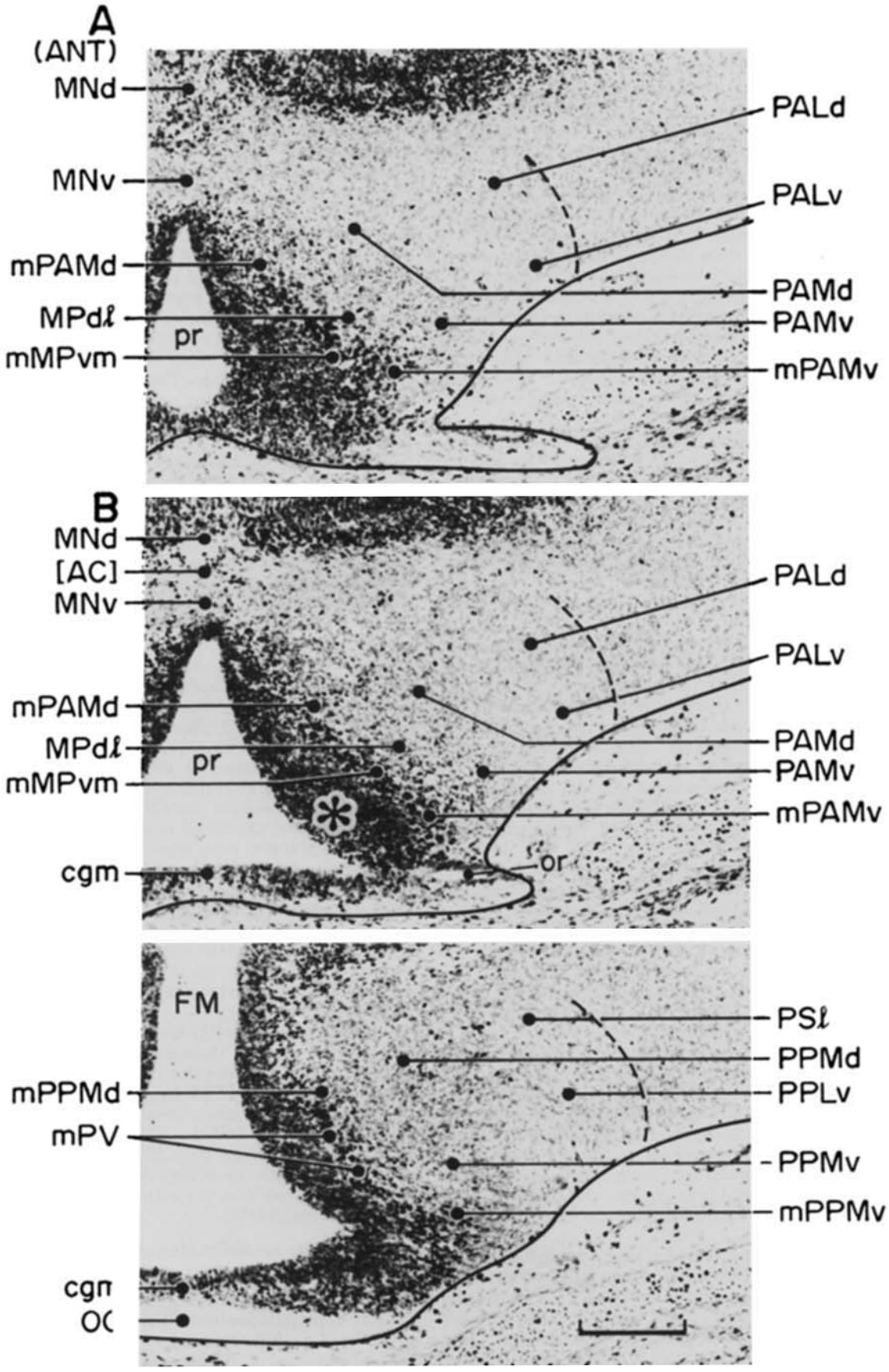

Fig. 18. As in Figure 14 for a coronally sectioned E17 embryonic brain 24 hrs after exposure to [^{3}H]thymidine on E16. (6-μm paraffin sections, hematoxylin, scale = 0.2 mm)

parts of the medial preoptic area (mPAMd/mPPMd, Fig. 14B–C). The unlabeled neurons separated from the basal telencephalon (BT in Fig. 14) by a dashed line extending to the ventral telodiencephalic fissure (tdv, Fig. 14A) are presumed to be cells in the lateral preoptic area (PAL and PPL) that were generated on or before E13.

E15 to E16 series. Figure 15 is a series of sagittal sections from the brain of an animal sacrificed on E16, 24 hrs after exposure to a single [^{3}H]thymidine injection on E15. The first fibers of the optic chiasma (OC) lie in the midline (Fig. 15C) at the base of the anterior hypothalamus (AH), and the optic tract runs along the floor of the diencephalon to posterior levels. The preoptic neuroepithelium (pn) has lengthened along the anteroposterior axis compared to its extent on E15 (Fig. 13). More laterally (Fig. 15A–B), the optic recess (or) narrows to a horizontal slit in the anterior basal diencephalon. Unlabeled medial preoptic neurons (PAM/PPM, Fig. 15A) are located dorsal to the wave front of migrating labeled neurons (mPAM/mPPM, Fig. 15A–B) generated on E15 and destined to reside in the ventral part of the medial preoptic area. The foramen of Monro (FM) and the medial horn of the lateral ventricle (mh) form the posterior boundaries of the posteromedial basal telencephalic ridge (BTP, Fig. 15A–B). The neuroepithelium at the base of the medial horn is actively generating neurons destined for the posterior strial bed nucleus (bstp). The sagittal sections shown in Figure 15 do not extend to the lateral preoptic area.

Figure 16 shows anteroposterior coronal sections through the preoptic area in an animal exposed to [^{3}H]thymidine on E15 and sacrificed on E16. The neuroepithelium lining the third ventricle still has thinner vertical (pr) and horizontal (or) limbs, flanking a prominent active core (asterisk, Fig. 16B–C). The neurogenetic data indicate that the core neuroepithelium is generating neurons for the ventromedial part of the medial preoptic nucleus (Fig. 11) and the entire periventricular nucleus (Fig. 12) since both of these structures originate mainly on and after E16. The germinal cells along the ventral wall of the optic recess (Fig. 16C) are generating glial cells (cgm) for the optic chiasma (OC) and optic nerve. Heavily labeled migrating cells generated on E15 (preceded with an "m" in Fig. 16) are seen outside of the neuroepithelium. The neurogenetic data indicate robust neuron production on E15 in the anterodorsal and ventral parts of the medial preoptic area (Fig. 9) and dorsolateral parts of the medial preoptic nucleus (Fig. 11); consequently, the heavily labeled cells are presumed to be migrating into these structures. The dorsalmost group of migrating cells consists most likely of neurons heading for the dorsal medial preoptic area (mPAMd, Fig. 16A–B); those in the center are destined for the dorsolateral medial preoptic nucleus (mMPdl, Fig. 16A–B), and those ventrolaterally for the ventral medial preoptic area (mPAMv, Fig. 16A–B; mPPMv, Fig. 16C). These neurons accumulate ventral and medial to older (unlabeled) neurons generated on or before E14 in the dorsal medial preoptic area (PAMd/PPMd), the lateral preoptic area (PAL/PPL), and the lateral superior preoptic area (PSl).

E16 to E17 series. Sagittal sections from the forebrain of an animal exposed to [^{3}H]thymidine on E16 and sacrificed one day later on E17 are shown in Figure 17. A large optic chiasma (OC) is seen just beneath the optic recess (or) near the midline (Fig. 17B). The optic recess is less prominent laterally (Fig. 17A) than it was on E16 (Fig. 15). Migrating cells heavily labeled with an E16 injection (mMP) form a new "wave front" just outside of the preoptic neuroepithelium (pn). The neurogenetic data indicate that most of these neurons will settle in medial nuclei (Figs. 11, 12). The unlabeled cells located above the migrating neurons were generated on or before E15 and have settled in the medial preoptic area (PAM/PPM). In the lateral section (Fig. 17A), the posteromedial telencephalic ridge (BTP) is receding, the medial horn (mh) is more shallow, and the posterior strial bed nucleus neuroepithelium (bstp) is thinner since fewer neurons are being produced on day E17.

Figure 18 shows coronal sections of the preoptic area in the brain of an animal exposed to [^{3}H]thymidine on E16 and sacrificed 24 hrs later on E17. The preoptic recess (pr) is now more prominent than the optic recess (a remnant is labeled "or," Fig. 18B) and the neuroepithelium has receded toward the midline. The neuroepithelium is becoming exhausted in the dorsal parts of the preoptic recess, but remains thick and active ventrally and in the shortened horizontal limb. The neurogenetic data indicate that E17 is a prominent day for neurogenesis in the ventromedial part of the medial preoptic nucleus and the transition area (Fig. 11) and throughout the periventricular nucleus (Fig. 12). Consequently, the persisting ventral neuroepithelium is postulated to be generating cells destined for these structures. Heavily labeled neurons generated on E16 are migrating (preceded with an "m" in Figure 18) just outside of the neuroepithelium. The neurogenetic data indicate that E16 is a declining day for cell production in the medial preoptic area (Fig. 9) and the dorsolateral medial preoptic nucleus (top graph, Fig. 11), but a robust day for cell production in the ventromedial part of the medial preoptic nucleus (Fig. 11) and in the vertical limb of the periventricular nucleus (Fig. 12). Therefore, the migrating cells are presumably destined for these structures. In anterior (Fig. 18A) and intermediate (Fig. 18B) levels, the most dorsomedial migrating neurons (mPAMd) and the most ventrolateral migrating neurons (mPAMv) may represent the few cells destined for the dorsal and ventral parts of the anterior medial preoptic area, whereas the majority of the migrating cells in the center (mMPvm) may settle in the medial preoptic nucleus, mainly in the ventromedial part. At the posterior level (Fig. 18C), the most dorsomedial mPPMd) and ventrolateral (mPPMv) migrating cells will presumably reside in the posterior medial preoptic area, whereas the smaller group of migrating cells in the center are headed for the periventricular nucleus (mPV), mainly the vertical limb.

The preoptic area on E17 has a prominent accumulation of unlabeled neurons throughout its entire rostrocaudal extent (Fig. 18A–C). The older neurons in the lateral preoptic area (PALd, PALv, PPLv) have been pushed farther from the ventricle as neurons accumulate in the medial preoptic area (PAMd, PAMv, PPMd, PPMv). At anterior (Fig. 18A) and intermediate levels (Fig. 18B), some of the unlabeled cells closest to the wave front of labeled migrating cells probably comprise the dorsolateral parts of the medial preoptic nucleus (MPdl).

The sexually dimorphic nucleus

Neurogenetic gradients seen in long-survival autoradiograms at P5. The autoradiograms in Figure 19 are of the sexually dimorphic nucleus (SDN) from a female (Fig. 19A) and male (Fig. 19B) exposed to [^{3}H]thymidine on E17 and E18 and sacrificed on P5. The majority of the SDN neurons are labeled and stand out against the background of unla-

TABLE 2. Male vs. Female Neurogenesis in the SDN

Inj. group	Males #	Males % labeled	Females #	Females % labeled	Statistics
E16+E17	299	86.76	297	88.82	
	300	86.77	301	83.54	
			302	89.92	
			303	95.47	U = 10 (P = 1.0)
E17+E18	331	51.85	330	83.22	
	332	86.41	401	73.25	
	333	73.99			
	334	71.75			
	406	52.82			U = 3 (P = 0.29)
E19+E20	335	15.83	336	43.43	
	342	29.99	338	20.51	
	344	27.02	339	37.16	U = 2 (P = 0.11)

beled cells in the dorsolateral medial preoptic nucleus, most of which have been generated by E17. Sexual dimorphism is already visible at P5; females tend to have a tighter cluster of neurons in SDN than males.

Quantitative demonstration of the conspicuously late neurogenesis in SDN is shown in Figure 11. Neurogenesis begins on E15 and approximately 53% of the cells are generated on or after E18, significantly later ($P < .0001$) than neurons in both parts of the medial preoptic nucleus. There is a "concentric" neurogenetic gradient within SDN: The oldest neurons are present in an outer shell; the youngest neurons are in the core. Some of the neurons that make up the shell of SDN may be present at L4 and may be partly responsible for the late wave of neurogenesis in the transition area (bottom graph, Fig. 11). Since the injection groups contained both males and females, the data for each group were analyzed with the Mann-Whitney U test (Siegel, '56) to see if there were any significant differences between the sexes. The results listed in Table 2 indicate that neurogenesis in the SDN is simultaneous for both males and females.

In animals exposed to [^{3}H]thymidine on E17+E18, the medial part of the posterior strial bed nucleus (BSTm) is conspicuously labeled, and one can follow its extension into the posterior lateral preoptic area (Fig. 20B) where it appears to continue into the SDN (Fig. 20A). Since both SDN and BSTm contain late-forming neurons, neurogenesis was compared between the two nuclei (Fig. 21) to see if there is a common germinal source. Similar to the neurogenetic gradients seen in the long survival autoradiograms at P60 (companion paper, Bayer, '87), the peak time for neurogenesis is E14 in the lateral bed nucleus (bottom graph, Fig. 21), whereas most of the medial neurons are generated onE15–E17 (middle graph, Fig. 21; $P < .0001$). Neurogenesis in the SDN is still high on E18 and E19, whereas it is declining in both parts of the BST ($P < .0001$, sign test). This difference in time of origin between the two nuclei is visible in animals that are exposed to [^{3}H]thymidine on E19+E20 (Fig. 22). Only a few scattered neurons are labeled in the medial BST, while many cells in the core of the SDN are heavily labeled.

Embryonic development in short and sequential autoradiograms. Figure 23 shows a series of autoradiograms of the posterior preoptic area in animals exposed to [^{3}H]thymidine on E18 and sacrificed 2 hrs later (Fig. 23A) or in successive 24-hr intervals after E18 (Fig. 23B–E) up to the day before birth. The E18 injection group contains heavily labeled SDN neurons, since 50% originate on or after this day (Figs. 11, 21). The only other preoptic area neurons labeled by this injection group are ventral and lateral cells in the periventricular nucleus (Fig. 12). The posterior preoptic neuroepithelium on E18 is active in the ventral part of the third ventricle. The neuroepithelium that presumably generates the SDN is indicated in Figure 23A. By E19, the lumen of the ventricle narrows considerably (Fig. 23B), the neuroepithelium is being transformed into the primitive ependyma, and heavily labeled cells are seen just lateral to its borders. The heavily labeled cells migrate farther away from the ventricle on E20 (Fig. 23C), and the ventricular lumen has become a narrow slit with a slight ventral dilation. On E21, some of the neurons arrive in the region where the SDN is presumed to be forming (arrow, Fig. 23D). More neurons settle here on E22, and the cluster of heavily labeled cells is just beginning to take on the typical appearance of the SDN (Fig. 23E). However, even at this late stage (birth occurs on the next day), the nucleus is not distinct and can only be discerned with the aid of [^{3}H]thymidine labeling in late injection groups.

The median preoptic nucleus

Neurogenetic gradients seen in long-survival autoradiograms at P5. The E15+E16 autoradiograms distinguish ventral and dorsal parts of the median preoptic nucleus (Fig. 24). At L1 (Fig. 24B), few neurons are labeled in the ventral part, many are labeled in the dorsal part, and almost all are labeled above the anterior commissure at L2 (Fig. 24A). The ventral (older) to dorsal (younger) neurogenetic gradient in the median preoptic nucleus is one of the most pronounced gradients seen in the preoptic area. This gradient is atypical since, in contrast to the rest of the preoptic area, older neurons are located *nearest* to the ventricle, whereas younger neurons are *farther* away. These gradients are quantified in Figure 25. The sign test indicated that the ventral parts of the nucleus originate simultaneously (all $P > .05$) at L1–L2 so these data are combined (bottom graph, Fig. 25). The ventral neurons originate early (72% on E13 and E14) significantly before ($P < .0001$) cells in the dorsal part of the nucleus. It is important to note that despite their midline location, the ventral neurons in the median preoptic nucleus originate simultaneously with many neurons in the lateral preoptic area (all $P > .05$), and significantly earlier than neurons throughout the medial preoptic area and medial preoptic nucleus (both comparisons, $P < .0001$). Within the dorsal part, cells at L1 are generated with a peak on E15, whereas more neurogenesis occurs on and after E16 at L2 ($P < .0001$).

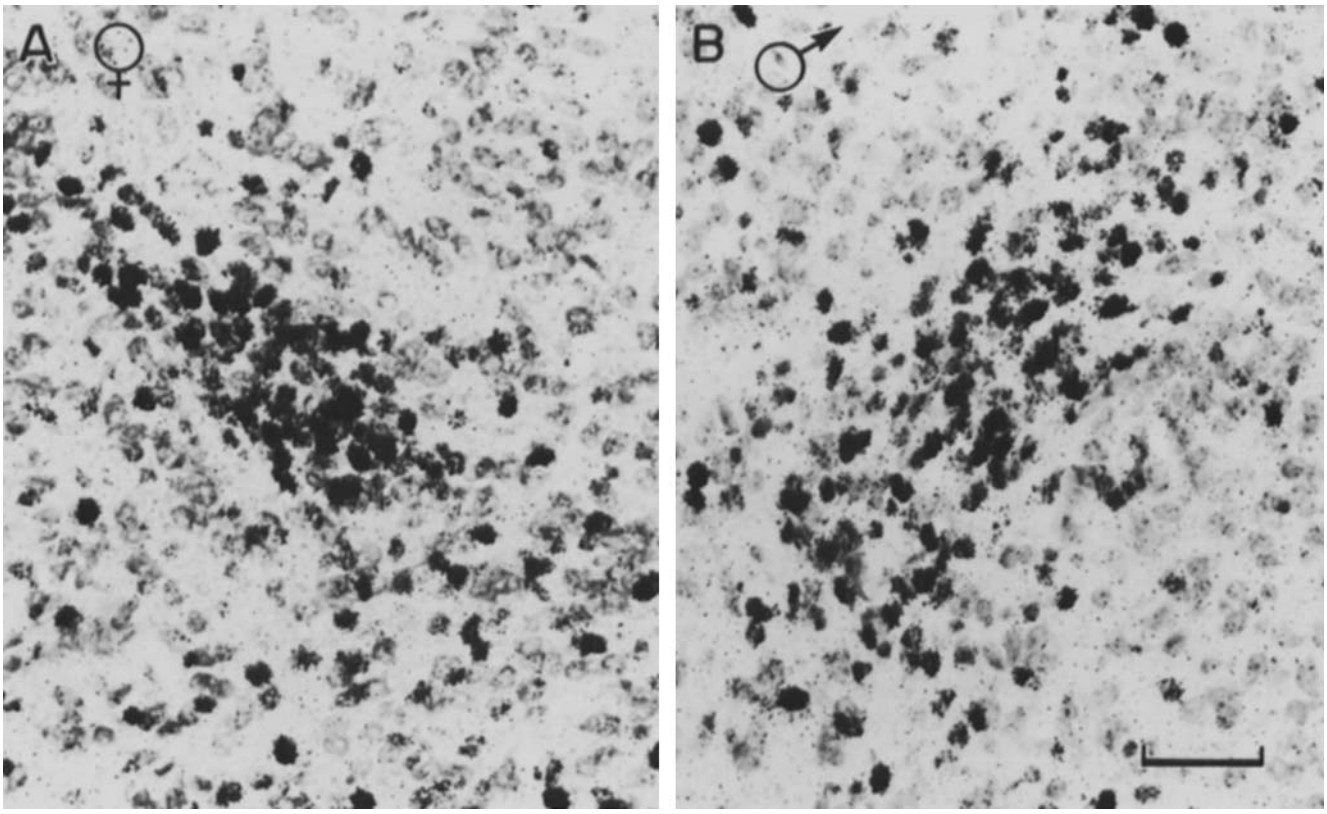

Fig. 19. The sexually dimorphic nucleus at P5 in a female (**A**) and male (**B**) exposed to [^{3}H]thymidine on E17+E18. (6-μm paraffin sections, hematoxylin, scale = 0.5 mm)

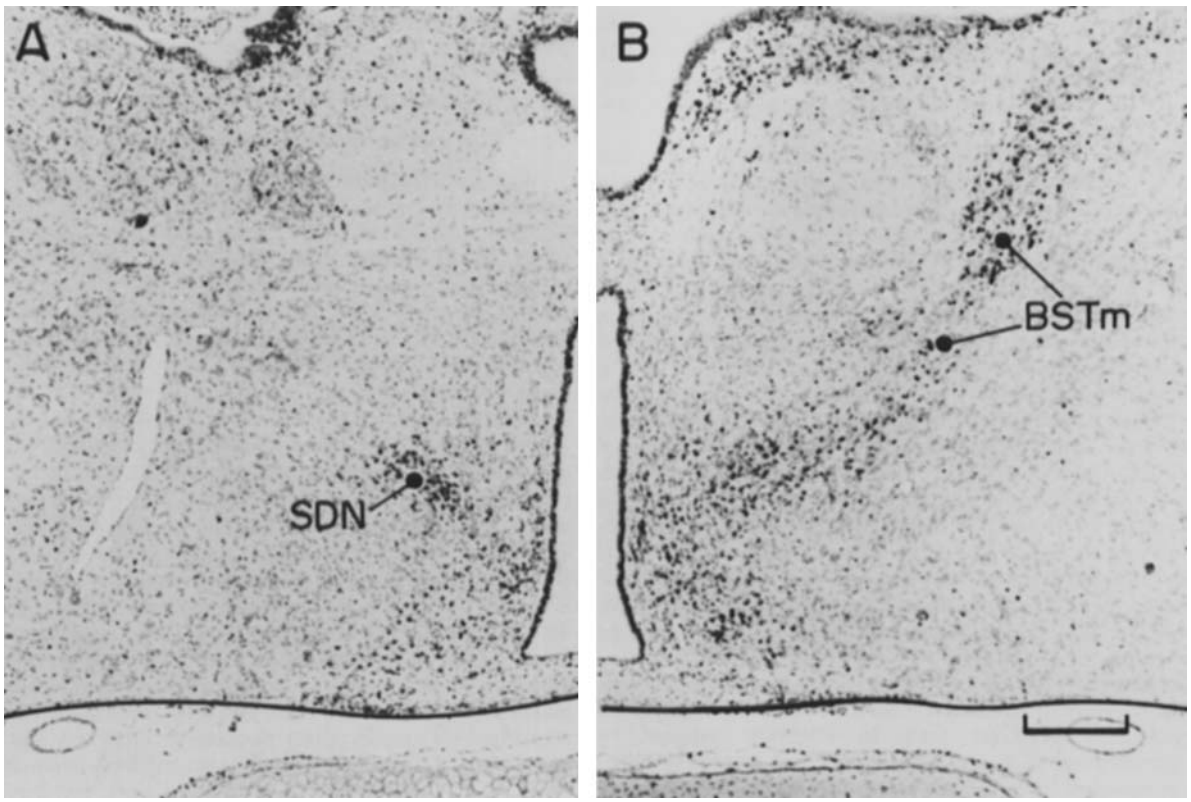

Fig. 20. Autoradiograms showing the continuum of labeled cells between the medial strial bed nucleus (**B**, BSTm) and the preoptic area in an animal exposed to [^{3}H]thymidine on E17+E18 and sacrificed on P5. The stream of labeled cells continues slightly anteriorly (**A**) where the sexually dimorphic nucleus (SDN) is located. The ventral border of the brain is indicated with a solid line. (6-μm paraffin sections, hematoxylin, scale = 0.25 mm)

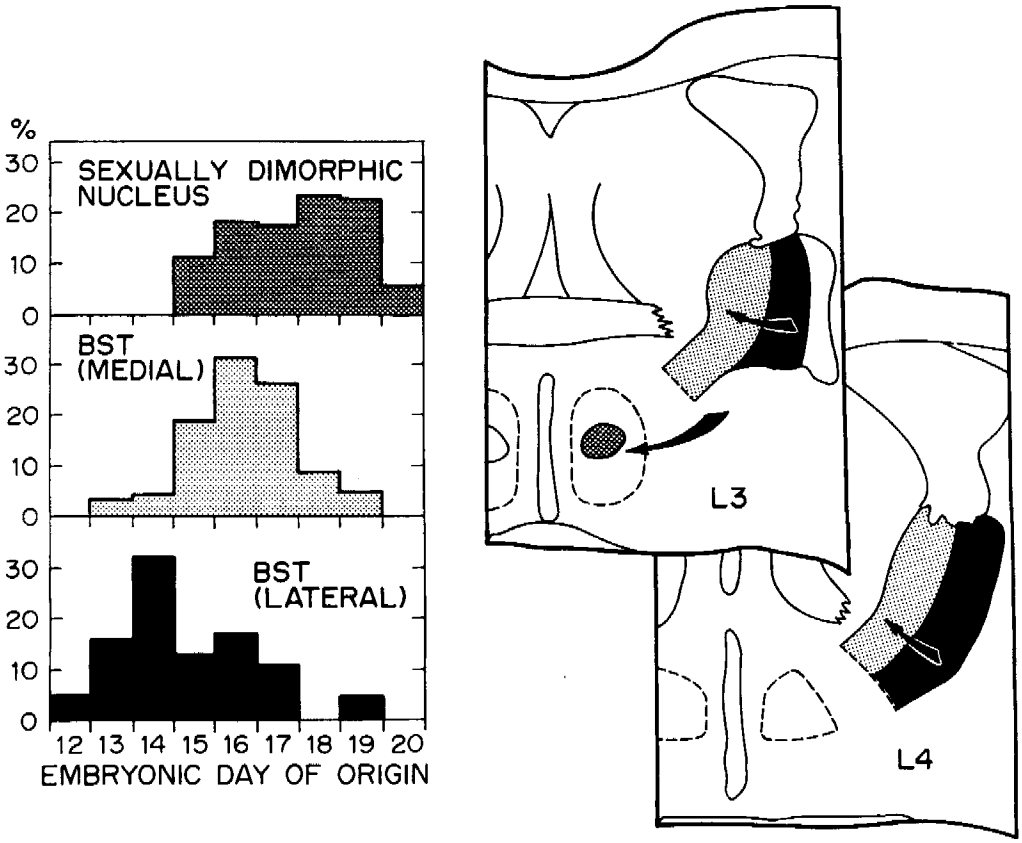

Fig. 21. Neurogenesis in the strial bed nucleus compared with that in the sexually dimorphic nucleus. The bar graphs indicate the proportion of neurons originating during single embryonic days. Shaded areas in the drawings indicate regions where cells were counted. Arrows within the strial bed nucleus indicate the lateral to medial neurogenetic gradient. Both parts of the strial bed nucleus originate significantly earlier than the sexually dimorphic nucleus (larger arrow at L3).

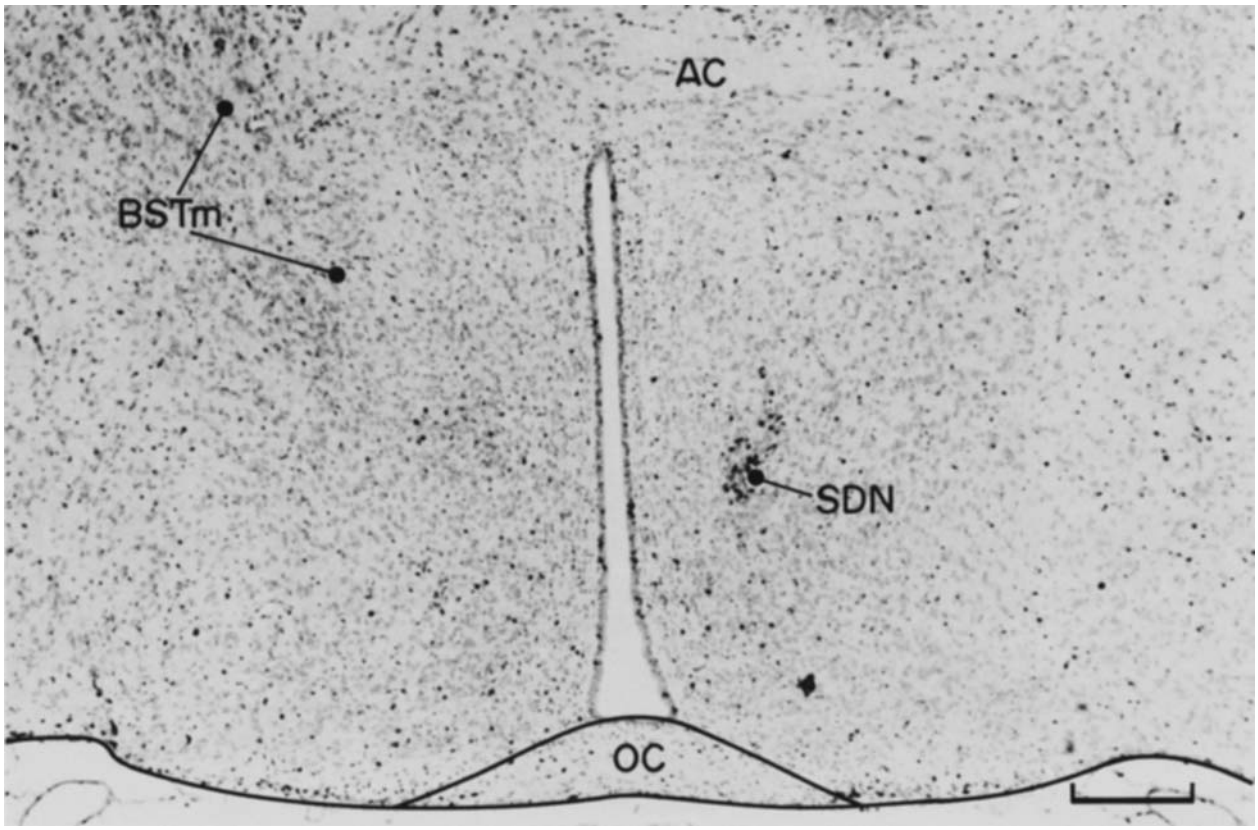

Fig. 22. An obliquely sectioned coronal autoradiogram that shows the BSTm on the left and the SDN on the right in the brain of an animal exposed to [^{3}H]thymidine on E19+E20 and sacrificed on P5. Only the SDN contains a fair amount of labeled cells. (6-μm paraffin section, hematoxylin, scale = 0.25 mm)

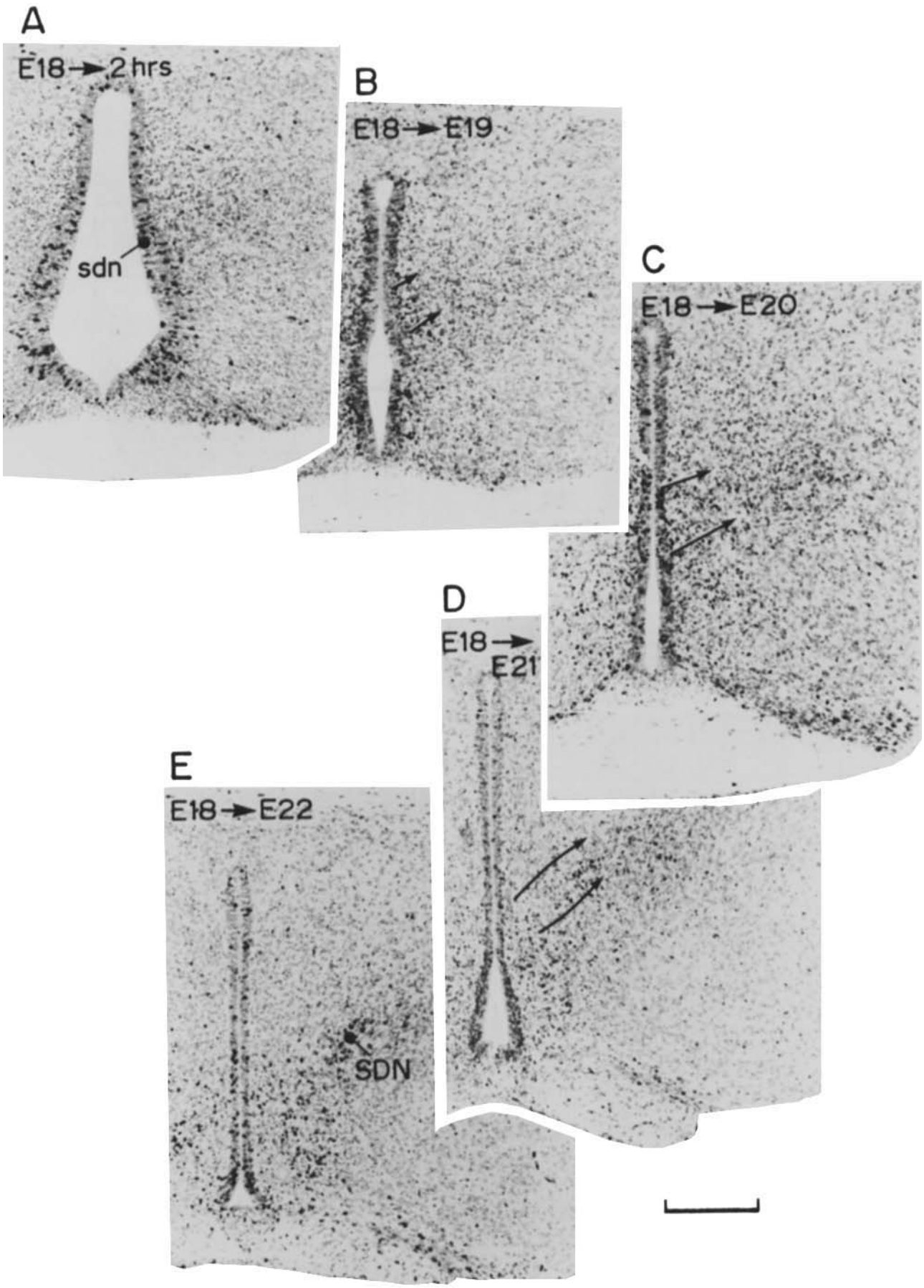

Fig. 23. A series of short- (A) and sequential-survival (B–E) autoradiograms of the posterior preoptic area from embryonic brains exposed to [^{3}H]thymidine on E18. The putative neuroepithelial source of the sexually dimorphic nucleus is indicated in A. Arrows in B, C, and D indicate the migratory pathway taken by young neurons presumably destined to settle in SDN. The nucleus is first seen on E22 (E). (6-μm paraffin sections, hematoxylin, scale = 0.2 mm)

Embryonic development seen in short and sequential autoradiograms. The neuroepithelial source of the median preoptic nucleus is best seen in midline sagittal sections. The median preoptic neuroepithelium (mn, Figs. 13D, 15C, 17B) is continuous with the neuroepithelium (se, Fig. 13D) generating the medial septum (SEm, Figs. 13D, 15C, 17B). Neurons heavily labeled with an E14 [^{3}H]thymidine injection are migrating away from this neuroepithelium on E15 (Fig. 13D). At higher magnification (not shown) the labeled neurons accumulate among unlabeled cells that originated on E13; presumably, these are neurons in the ventral portion of the median preoptic nucleus (MNv). On E16 (Fig. 15C), there is an empty area possibly containing glial guidance channels (Silver et al., '82) in the site where the anterior commissure ([AC]) will decussate. Ventral to the decussation, unlabeled (older) neurons have accumulated in the presumptive ventral median preoptic nucleus (MNv). Cells heavily labeled by an E15 [^{3}H]thymidine injection are migrating just in front of the decussation area and are presumed to be younger dorsal cells in the median preoptic nucleus (MNd, Fig. 15C). A large collection of medial septal neurons (SEm, Fig. 15C) has settled just above the median preoptic nucleus. On E17, the medial sagittal section (Fig. 17B) is slightly lateral to the midline and grazes the lateral edge of the median preoptic nucleus; cells heavily labeled on E16 (MNd, Fig. 17B) are still accumulating above and in front of the anterior commissure.

The ventral to dorsal gradient can be clearly seen in serial coronal sections through the median preoptic nucleus primordium in an animal exposed to [^{3}H]thymidine on E16 and sacrificed on E17 (Figs. 16, 26). The unlabeled cells in the midline above the preoptic recess of the third ventricle (Fig. 26A,B) are destined to become ventral median preoptic neurons (MNv). Extending dorsally from these unlabeled cells is a line of labeled neurons, presumably the future dorsal median preoptic nucleus (MNd). The ventral part of the nucleus becomes small at the front edge of the decussation of the anterior commissure (Fig. 26C), and the heavily labeled cells in the midline above the commissure are being cut off from the ventral, subcommissural cells. Farther back in the commissural decussation (Fig. 26D), heavily labeled cells (generated on E16) are moving away from the neuroepithelium above the commissure (mn), which remains active, presumably producing the youngest median preoptic neurons in the dorsal posterior part of the nucleus.

DISCUSSION

Developmental patterns in the typical preoptic areas and nuclei

The predominant neurogenetic gradients seen in the preoptic area are lateral (older) to medial (younger) and dorsal (older) to ventral (younger), reflecting an "outside-in" pattern (Angevine, '65) with respect to the neuroepithelium lining the third ventricle. The lateral to medial gradient is prominent and has been reported in all [^{3}H]thymidine autoradiographic studies of preoptic area development (Ifft, '72; Creps, '74; Altman and Bayer, '78a). The dorsal to ventral gradient was observed by Ifft ('72) and Creps ('74) in the periventricular nucleus and by Altman and Bayer ('86) in the lateral preoptic area. Similar gradients are reported here in the medial preoptic area and

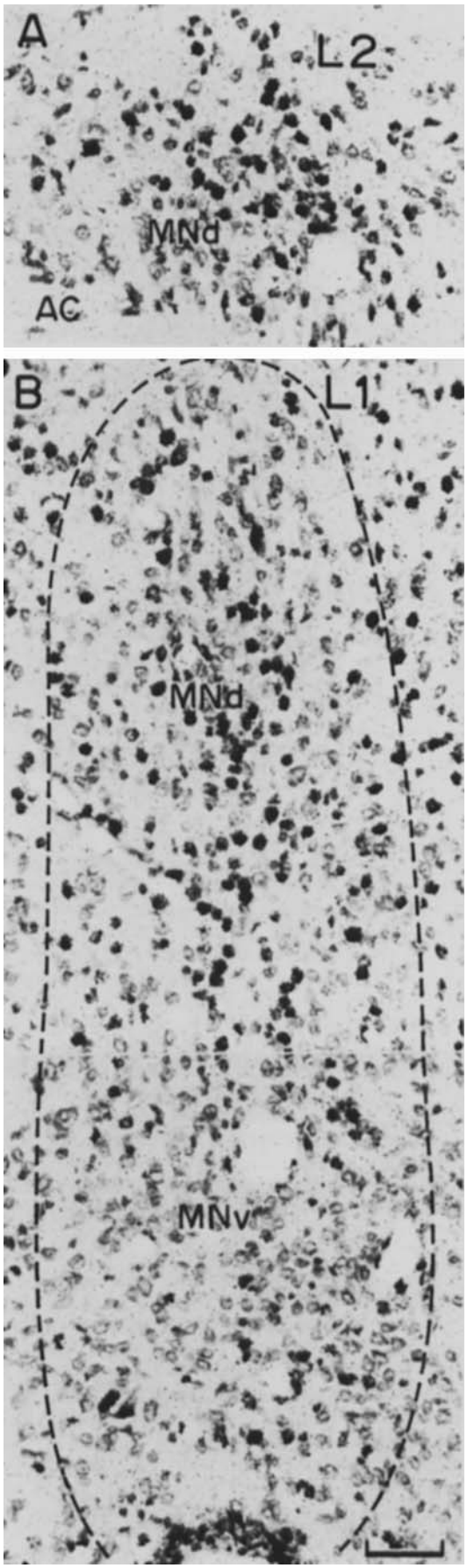

Fig. 24. Autoradiograms of the median preoptic nucleus at L1 (B) and L2 (A) in the brain of an animal exposed to [^{3}H]thymidine on E15+E16 and sacrificed on P5. (6-μm paraffin sections, hematoxylin, scale = 0.05 mm)

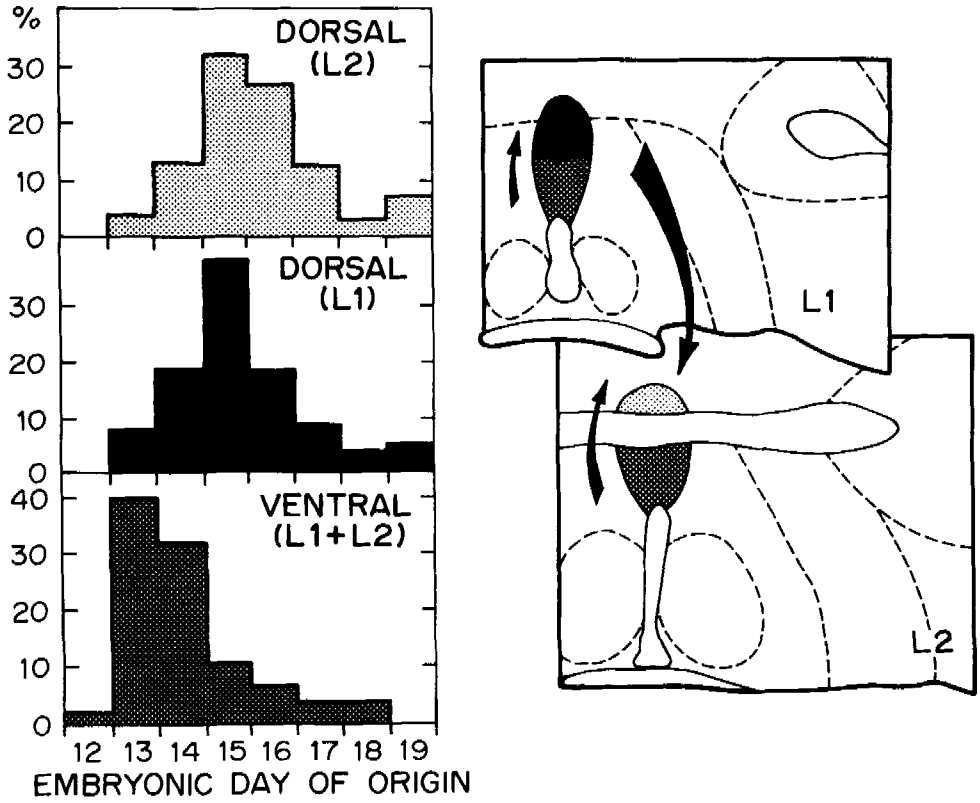

Fig. 25. Neurogenesis in the median preoptic nucleus. The bar graphs indicate the proportion of neurons originating during single embryonic days. Cells were counted in the shaded areas in the drawings. Vertically directed arrows in each drawing indicate a ventral to dorsal neurogenetic gradient. There is also a rostral to caudal neurogenetic gradient (large arrow between drawings) in the dorsal part of the nucleus (two top graphs).

the medial preoptic nucleus. Only the sexually dimorphic and median preoptic nuclei deviate from these gradients, and their atypical developmental patterns give clues to the unique nature of these two nuclei (discussed in detail below).

Armed with the comprehensive, quantitative picture of preoptic neurogenesis provided by the long-survival P5 autoradiograms, the analysis of complex morphogenetic events can be "unraveled." For example, active areas in the neuroepithelium on specific embryonic days can be associated with the generation of adult populations having coincidental peaks of neurogenesis. By timing [^{3}H]thymidine injections to heavily labeled cells generated on specific days, the sequential-survival autoradiograms (Figs. 13–18, 23) can be used to identify the consecutive waves of migrating neurons. Throughout this discussion, the hypothetical construct of a *neuroepithelial zone* is used, which is defined as a group of stem cells (or cell line) that produces neurons for a specific population (Altman and Bayer, '80; Bayer and Altman, '87). Our aim will be to show that the preoptic neuroepithelium contains a spatiotemporal "mosaic" where the stem cells of specific populations are located in segregated (or partially overlapping) zones that become active in a precise temporal sequence. These relationships are diagrammed in Figure 27, which correlates neuronal migratory waves with the position of putative preoptic neuroepithelial zones (central drawing) and the final settling places of neuronal populations throughout the anterior (upper drawings) and posterior (lower drawings) preoptic area.

The *first migratory wave* consists of the oldest neurons (generated E12–E14) in the lateral and superior preoptic areas (heavy stipple in upper and lower drawings, Fig. 27). At this time, the preoptic neuroepithelium is characterized by a thick core and thinner dorsal and lateral flanks. The majority of the lateral preoptic neurons are presumably generated by cells in the flanks (large arrows in Wave 1, Fig. 27) since these areas are the first to decline and have already receded on E15 (Fig. 14) when lateral preoptic neurogenesis is essentially complete (Figs. 4, 6). Consequently, the lateral preoptic neuroepithelial zones (heavy stipple in central drawing, Fig. 27) are represented in the far dorsal and far lateral parts of the preoptic neuroepithelial "sheet." The possibility that some lateral preoptic neurons are generated by precursors in more central areas cannot be ruled out (small arrows in wave 1, Fig. 27). However, the centrally located preoptic neuroepithelium is postulated to be generating predominantly stem cells that will produce neurons for later migratory waves.

The first wave of migratory cells in the preoptic area settles rostral to, and becomes contiguous with, the early-generated lateral hypothalamic area posteriorly (Altman and Bayer, '78a, '86). It is interesting to note that the neuroepithelial zone generating the lateral hypothalamus was postulated to be in the dorsal part of the hypothalamic primordium (Altman and Bayer, '86), similar to the dorsomedial zone postulated in this study to generate the dorsal lateral preoptic area neurons. These developmental patterns confirm descriptive anatomical studies in adults that the lateral preoptic and lateral hypothalamic areas are contiguous, and both contain cells interspersed with fibers of the medial forebrain bundle (Gurdjian, '27; Loo, '31;

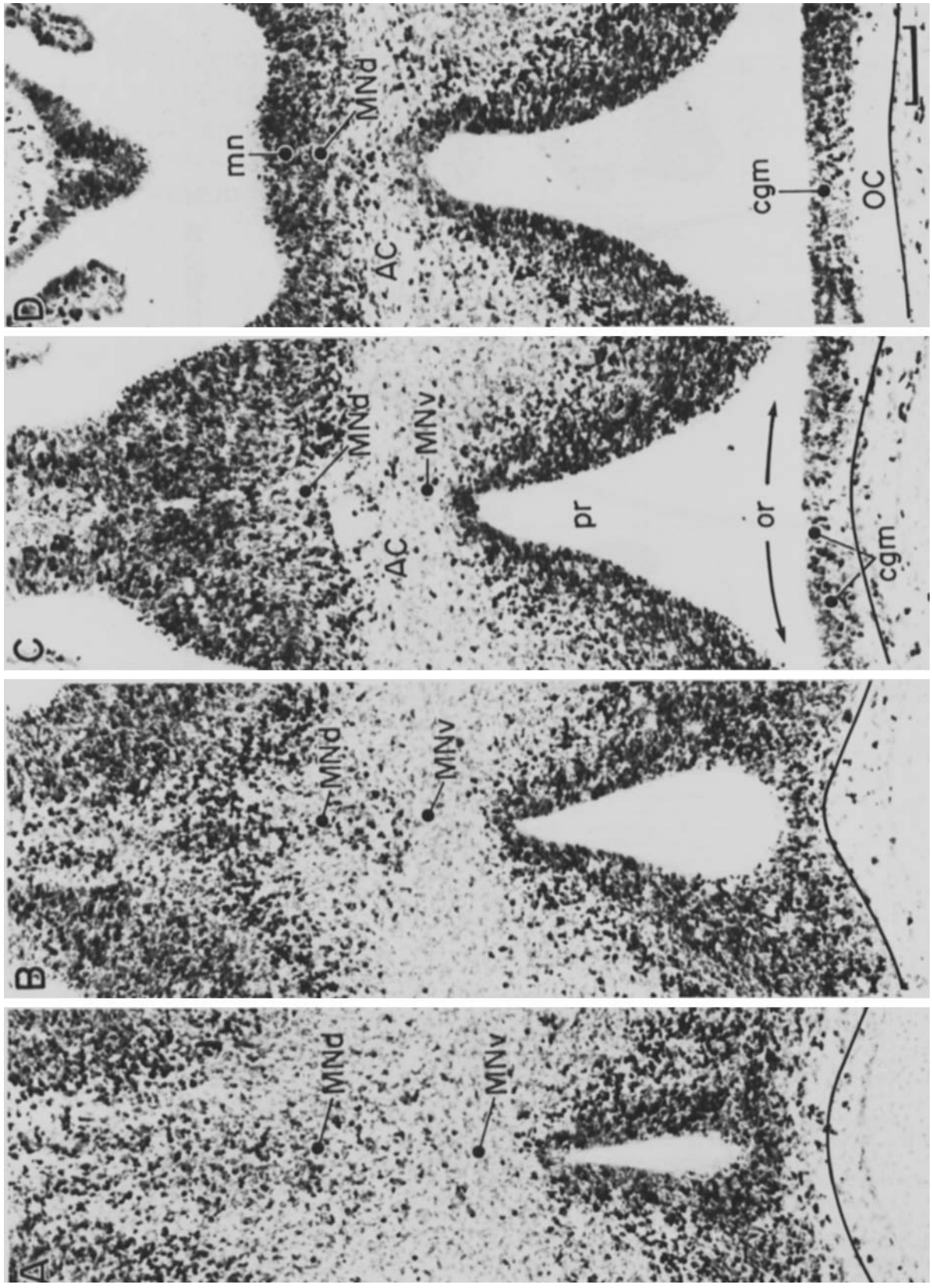

Fig. 26. A series of anterior (A) and progressively more posterior (B–D) autoradiograms of the median preoptic nucleus primordium from a coronally sectioned E17 brain 24 hrs after exposure to [^{3}H]thymidine on E16. (6-μm paraffin sections, hematoxylin, scale = 0.1 mm)

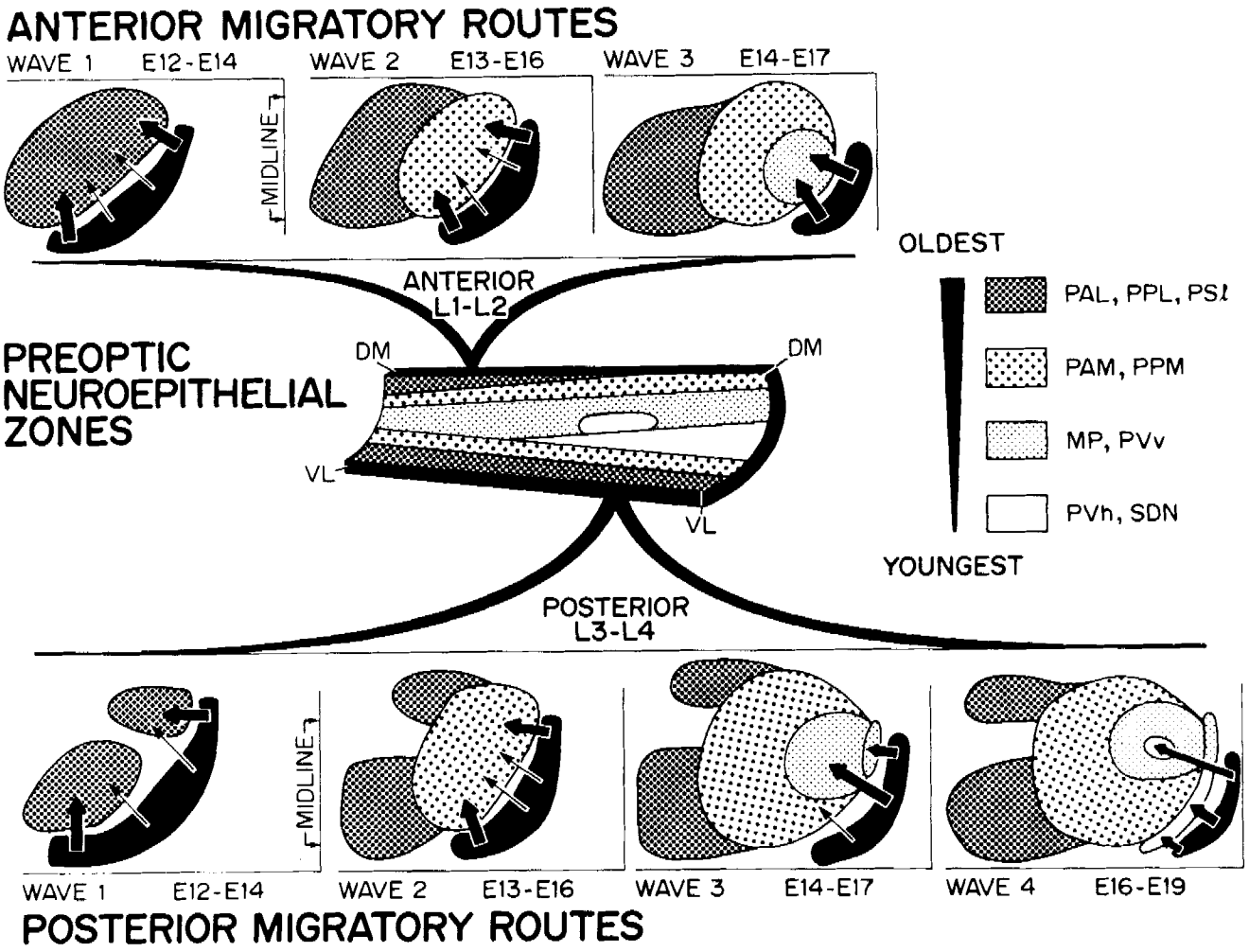

Fig. 27. A summary of preoptic area neurogenetic gradients and migratory routes from the neuroepithelium (solid crescent-shaped structures in upper and lower drawings) correlated with a hypothetical parcellation of the preoptic neuroepithelium (represented as a curved sheet in the central drawing). The preoptic neuroepithelium is postulated to contain *neuroepithelial zones*, a heterogeneous mosaic of stem cells producing neurons for specific preoptic area populations. During development, the first active neuroepithelial zones are located dorsomedially and ventrolaterally (to produce waves 1 and 2). Neurons migrate radially, accumulating in the lateral and medial preoptic areas, in an "outside-in" gradient with respect to the third ventricle. At later stages, progressively more central neuroepithelial zones become active and produce younger neurons that will accumulate closer to the ventricle in the medial preoptic, periventricular, and sexually dimorphic nuclei.

Krieg, '32; Humphrey, '36; Young, '36; Bleier and Byne, '85).

The *second wave of migratory cells* constitutes the medial preoptic area neurons (generated E13–E16, medium stipple, Fig. 27). On E15 (Fig. 14) and E16 (Fig. 16), the preoptic neuroepithelium is still characterized by a thick core and thinner dorsal and lateral flanks, but it is confined to a more central core on E17 (Fig. 18). The flank areas are presumably generating the majority of medial preoptic neurons (large arrows in wave 2, Fig. 27) since the disappearance of the flanks on E17 coincides with the decline of neurogenesis in the medial preoptic area (Fig. 9). Consequently, the neuroepithelial zones postulated to give rise to dorsal and ventral medial preoptic area neurons (medium stipple in central drawing, Fig. 27) are located in the receding dorsomedial and ventrolateral walls of the preoptic and optic recesses, respectively, between (or partially overlapping with) the neuroepithelial zones giving rise to the lateral preoptic area. Centrally placed germinal zones may also be the source of some medial preoptic area neurons (small arrows in wave 2, Fig. 27), but the majority of the core stem cells are probably proliferating locally and will generate neurons for the third and fourth migratory waves.

In the first two waves of cell migration, dorsomedial and ventrolateral zones are active concurrently. However, throughout the lateral and medial preoptic areas, there is a tendency for dorsal cells to be generated slightly earlier than ventral cells (Figs. 6, 9). This is postulated to be the result of a short lag in activity so that the ventrolateral zones generating the ventral parts of the medial and lateral preoptic areas begin to produce neurons slightly later (and continue longer) than their dorsomedial partners.

In the anterior preoptic area, the *third migratory wave* is the last and consists of cells (generated E14–E17, fine stipple, Fig. 27) that will settle densely in a structure variably named the medial preoptic nucleus (Gurdjian, '27; Bleier and Byne, '85), the preoptic nucleus (Krieg, '32), or the principal preoptic nucleus (Loo, '31; Humphrey, '36; Young, '36). On E15 (Fig. 14B) and E16 (Fig. 16B), the most centrally placed neuroepithelium in the anterior preoptic area

has a prominent evagination into the third ventricle, which declines by E17 (Fig. 18B). The central evagination is the presumed source of the medial preoptic nucleus (large arrows in anterior wave 3, Fig. 27), since its dramatic reduction by E17 coincides with the completion of neurogenesis in the dorsolateral part and the declining neurogenesis in the ventromedial part of the nucleus (Fig. 11). The neuroepithelial zone producing this third wave is postulated to be located within the zones generating the medial preoptic area (lightest shading in central drawing, Fig. 27). These neurons settle "outside-in" so that the oldest dorsolateral cells are farthest from the ventricle and the youngest ventromedial cells are adjacent to the ependyma. It is interesting to note that, correlated with early vs. late neurogenesis, there are neurochemical and neuroanatomical differences between dorsolateral and ventromedial parts of the medial preoptic nucleus. The older lateral part of the nucleus gets a more dense serotonin innervation than the younger medial part (Simerly et al., '84). Older lateral cells are more likely to contain vasoactive intestinal polypeptide, whereas younger medial cells are found with somatostatin, substance P, and L-enkephalin (Simerly et al., '86).

In the posterior preoptic area, the *third and fourth migratory waves* contain the young neurons that form a "butterfly"-shaped extension in the medial preoptic area (Fig. 1B). Humphrey ('36) noted that the posterior medial (principal) preoptic nucleus was characterized by a concavity surrounding neurons similar to the interstitial cells in the lateral and medial preoptic areas; no doubt this is the same structure we called the preoptic intermediate area (PIM, Fig. 1B) in fetal rats (Altman and Bayer, '86). The neuroepithelial zones generating the third and fourth waves are situated between the zones generating the medial preoptic area. The neuroepithelial zone generating the third wave (fine stipple in central drawing, Fig. 27) is active between E14 and E17, producing neurons destined to settle in the medial preoptic nucleus, transition area, and the dorsal part of the vertical limb of the periventricular nucleus. The trajectory of the third wave neurons is predominantly dorsolateral and forms the upper wing of the butterfly pattern of younger neurons seen in fetal rats during early development (Fig. 1B; Altman and Bayer, '86). The neuroepithelial zone generating the fourth wave (clear area in central drawing, Fig. 27) is active between E16–E19 and produces the youngest neurons in the preoptic area. These cells will settle in ventral parts of the vertical limb and throughout the horizontal limb of the periventricular nucleus and in the sexually dimorphic nucleus (discussed below). The trajectory of the periventricular neurons in the fourth wave is predominantly radial as younger neurons stay close to the vertical and horizontal limbs of the third ventricle, undercutting the older neurons in the ventral part of the medial preoptic area. These young neurons form the lower wing of the butterfly pattern seen in fetal rats (Fig. 1B; Altman and Bayer, '86).

The sexually dimorphic nucleus

Neurogenesis in the sexually dimorphic nucleus is conspicuously delayed, since it is the only structure in the preoptic area with substantial neurogenesis as late as E19 (Figs. 11, 22). These data confirm the observations of Jacobson and Gorski ('81) that neurons in the rat sexually dimorphic nucleus could be heavily labeled up to E18 (the last day for their [^{3}H] thymidine injections). Late neurogenesis of sexually dimorphic neurons may be related to the recent findings that they receive a massive projection (Simerly and Swanson, '86) from the youngest neurons in the encapsulated part of the strial bed nucleus (Bayer, '87). It is well documented that the posterior strial bed nucleus receives direct input from the accessory olfactory bulb (White '65; Scalia and Winans, '75; Broadwell, '75; Skeen and Hall, '77; Davis et al., '78). The vomeronasal epithelium and the accessory olfactory system are known to be crucial sensory inputs regulating sexual behavior (Estes, '73; Whitten and Champlin, '73; Powers and Winans, '75; Winans and Powers, '77). It is probable that there is only one synapse (in the strial bed nucleus) between the sexually dimorphic nucleus in the preoptic area and a sensory input relevant to sexual function.

Since both the posterior strial bed nucleus and the sexually dimorphic nuclei contain conspicuously late originating neurons, we entertained the hypothesis that the youngest neurons in both nuclei have a common origin. This hypothesis was rejected when a comparison between times of neurogenesis in the two nuclei (Fig. 21) showed that the youngest bed nucleus neurons originate earlier than many of the sexually dimorphic neurons, and no cells could be traced migrating into the sexually dimorphic nucleus from the neighborhood of the posterior strial bed nucleus. Instead, we found that a cluster of heavily labeled late-generated cells actively migrate away from a neuroepithelial zone in the third ventricular preoptic neuroepithelium past older cells in the periventricular and medial preoptic nuclei to settle in a dorsolateral position (Fig. 23). Creps ('74) noted that part of the medial preoptic nucleus in the mouse showed an "inside-out" neurogenetic gradient with respect to the periventricular nucleus; possibly that observation is related to the late neurogenesis of the sexually dimorphic nucleus.

We could not confirm Jacobson and Gorski's ('81) observation that the sexually dimorphic nucleus originates earlier in females than in males. In our material, there were no statistically significant differences between the sexes (Table 2). Jacobson and Gorski ('81) allowed their animals to survive into the juvenile period, whereas our animals survived only until P5. The discrepancy between these two observations might be resolved if the late-produced neurons selectively die in females after P5. Several lines of experimental evidence show that dimorphism occurs perinatally and postnatally rather than prenatally. Neither Hyyppä ('69) nor Jacobson et al. ('80) saw any sexual dimorphism in preoptic area development prenatally, during the time when all sexually dimorphic neurons are generated. However, Jacobson et al. ('80) found sexual differences as early as postnatal day one, and we found them to be obvious in our P5 series of long-survival autoradiograms. Exposing females to androgens during early postnatal development can abolish the female pattern and establish the male pattern of synaptic terminations from the stria terminalis (and other sources) in the lateral preoptic area (Field and Sherlock, '75). Gibson et al. ('84) found that preoptic area tissue from fetal male donors transplanted into the preoptic areas of female mice with a genetic hypogonadal disorder was just as effective in restoring fertility as was tissue from female donor fetuses. Thus, sexual dimorphism between males and females may depend more on epigenetic factors (the differential endocrine environment in early life) than on neurogenetic differences between the sexes.

The median preoptic nucleus

Neurogenesis in the median preoptic nucleus is unique for two reasons: (1) in spite of its location in the midline, where younger preoptic area neurons are found, neuroge-

nesis is simultaneous with older lateral peroptic area neurons, and (2) it is the only structure in the preoptic area where the oldest neurons settle closest to the third ventricle and the youngest neurons are located farther away. The ventral to dorsal neurogenetic gradient was illustrated in our earlier neurogenetic study of the hypothalamus (Altman and Bayer, '78a). This gradient also correlates with differences in anatomical projections to the preoptic area and hypothalamus. The dorsal part of the median preoptic nucleus projects to the lateral preoptic area, the intermediate part projects to the supraoptic nucleus, and the ventral part projects to the arcuate and medial ventromedial hypothalamic nuclei (Swanson, '76).

With these unique neurogenetic features, it is not surprising that the neuroepithelial zone generating the median preoptic nucleus lies outside of the third ventricle as it is seen in coronal sections (Figs. 14, 16, 18). In midline sagittal sections (Figs. 13D, 15C, 17B), the median preoptic neuroepithelium is continuous with the midline basal telencephalic neuroepithelium. The apical surfaces of the median preoptic neuroepithelial cells are facing dorsally, toward the foramen of Monro and the midline basal telencephalon (Figs. 14B, 16B, 26D). Germinal zones in the rest of the preoptic area either face medially (toward the preoptic recess) or ventrally (toward the optic recess) and neuronal migration is lateral and dorsal. In agreement with the findings of our earlier study on hypothalamic morphogenesis (Altman and Bayer, '78b), neurons migrate radially from the median preoptic neuroepithelium so that the oldest cells lie in front and above the most rostral extent of the preoptic recess. Younger cells pile up dorsal to the older cells, closer to the germinal source, and around the anterior commissure.

The descriptive anatomical literature on the preoptic area regards the median preoptic nucleus as continuous with the periventricular nucleus (Loo, '31; Humphrey, '36; Young, '36; Bleier et al., '82; Bleier and Byne, '85), and an older (dorsal) to younger (ventral) neurogenetic gradient found in the preoptic periventricular region is presumed to be developmental evidence of continuity between the two nuclei (Ifft, '72; Creps, '74). Neither the developmental patterns reported here nor the adult morphology support these observations: (1) the periventricular nucleus is generated by a neuroepithelial zone lining the walls of the preoptic and optic recesses and has no structural continuity with the zone generating the median preoptic nucleus, (2) the neurogenetic gradients within the two nuclei are divergent rather than continuous--ventral (older) to dorsal (younger) in the median preoptic; dorsal (older) to ventral (younger)in the periventricular. Finally, (3) we could not see evidence of structural continuity between the median preoptic and periventricular nuclei in our material. The median preoptic nucleus is only found anteriorly (Fig. 2), where the periventricular nucleus is absent.

The question remains as to whether or not the median preoptic nucleus should still be considered part of the preoptic area since it does not come from the third ventricle. Indeed, we speculated that it was telencephalic in our earlier study of hypothalamic neurogenesis (Altman and Bayer, '78a). However, that classification also has its problems especially when one considers the placement of the anterior commissural decussation. The anterior commissure is believed to cross the midline at the interface between the telencephalon and diencephalon (Altman and Bayer, '78a,b). If that is true, then the part of the nucleus below the commissure is "diencephalic," whereas the part of the nucleus above the commissure is "telencephalic." Apparently, the median preoptic neuroepithelium is truly transitional and serves as a bridge between the telencephalon and diencephalon.

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