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Quantitative ^{3}H-Thymidine Radiographic Analyses of Neurogenesis in the Rat Amygdala

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ABSTRACT Neurogenesis in the rat amygdala was examined with ^{3}H-thymidine radiography. The animals were the offspring of pregnant females given two injections of ^{3}H-thymidine on consecutive days in an overlapping series: Embryonic day (E) 12 + E13, E13 + E14 . . . E21 + E22. On 60 days of age, the percentage of labelled cells and the proportion of cells added during each day of formation were determined at several anatomical levels within the following components of the amygdala: anterior amygdaloid area, bed nuclei of the lateral and accessory olfactory tracts, central, medial, anterior cortical, posterolateral cortical, posteromedial cortical, basomedial, basolateral, and lateral nuclei, the amygdalo-hippocampal area, and the intercalated masses.

All large and many small neurons originate in most nuclei between E13 and E17, those in the intercalated masses between E15 and E19, those in the amygdalo-hippocampal area between E16 and E19. The anterior amygdaloid area, intercalated masses, central, medial, posterolateral cortical, posteromedial cortical, basomedial, basolateral, and lateral nuclei have strong rostral-to-caudal intranuclear gradients. There are five additional intranuclear gradients: 1) medial to lateral in the central nucleus, anterior amygdaloid area, and anterior intercalated masses; 2) lateral to medial in the bed nucleus of the lateral olfactory tract and basolateral nucleus; 3) superficial to deep in the amygdalo-hippocampal area, posterolateral, and posteromedial cortical nuclei; 4) ventral to dorsal in the medial nucleus; and 5) dorsal to ventral between the small and large-celled parts of the lateral nucleus. Only the bed nucleus of the accessory olfactory tract and the anterior cortical nucleus do not have intranuclear gradients. Between 10 and 15% of the total cell population in most nuclei are very small neurons and/or glia which originate simultaneously between E18 and E20. This population is absent in the ventral part of the medial, anterior cortical, and anterior basomedial nuclei; these contiguous areas may form a distinct subunit in the amygdala.

In contrast to the pronounced intranuclear gradient, internuclear gradients are weak. Neurogenesis in the central nucleus and corticomedial and basolateral complexes appears to take place both concurrently and independently. There are groups of early-originating neurons in the central, medial, and basolateral nuclei located near the periphery of the amygdala. Each of these groups is surrounded by younger neurons farther within the interior. The youngest cells are in the centrally placed intercalated masses. These settling patterns suggest that cells in the amygdala arise simultaneously from more than one neuroepithelial source during morphogenesis.

The chronology of neurogenesis in the amygdala can be related to some of its anatomical connections. Rostral-to-caudal gradients in the corticomedial complex may be timed to coincide with early vs. late arrival of olfactory fibers. The subdivisions based on intranuclear gradients in the central, medial, and basolateral nuclei match the subdivisions based on patterns of anatomical connections.

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The amygdala forms the floor of the telencephalon from the olfactory tubercle to the ventral part of the hippocampal region. Johnston ('23) was one of the first to describe the anatomy of this region, which he found to be relatively constant in a variety of mammals. Additional descriptive anatomical studies in the rat (Gurdjian, '28; Brodal, '47; Krettek and Price, '78b), rabbit (Young, '36), bat (Humphrey, '36), cat (Fox, '40), monkey (Lauer, '45), and man (Crosby and Humphrey, '41) confirmed and expanded Johnston's observations. Seven areas and/or nuclei have been traditionally included in the amygdala: 1) the anterior amygdaloid area, 2) the bed nucleus of the lateral olfactory tract, and the 3) central, 4) medial, 5) cortical, 6) basal, and 7) lateral nuclei. In this study, the cortical and basal nuclei are further subdivided according to Krettek and Price ('78b).

^{3}H-thymidine radiographic studies of neurogenesis in the amygdala have been restricted to two abstracts (Sidman and Angevine, '62; McConnell, '75) in the mouse and a descriptive report in the hamster (ten Donkelaar et al., '79). This is the first report to extensively quantify the patterns of neurogenesis throughout the amygdala by utilizing the progressively delayed comprehensive labelling technique introduced by Bayer and Altman ('74). This method has been used to increase accuracy in timing the onset, proportion of daily acquisition, and cessation of neurogenesis throughout all nuclei and areas of the amygdala from embryonic day 12 to embryonic day 22 (the day before birth).

MATERIALS AND METHODS

The developmental series contained ten groups of Purdue-Wistar rats, the offspring of pregnant female rats given two consecutive daily subcutaneous injections of ^{3}H-thymidine (Schwarz-Mann; specific activity 6.0 C/mM; 5 μC/gram body weight). The multiple injections, given to insure comprehensive cell labelling, were progressively delayed by 24 hours in an overlapping series: E12 + E13, E13 + E14 . . . E21 + E22. Usually, two or more pregnant females were injected for each group. All injections were given between 9 and 11 A.M. The day of sperm positivity was E1; the day of birth was PO. Normally, the rats in the Purdue-Wistar colony are born on the afternoon of E23.

All animals were transcardially perfused with 10% neutral formalin on P60. The brains were kept for 24 hours in Bouin's fixative, then transferred to fresh 10% neutral formalin until they were embedded in paraffin. Usually, the brains of six males from each group were prepared for analysis. Serial sections (6 μm, every fifteenth section was saved) were cut in the coronal plane using the stereotaxic angle of Pellegrino et al. ('79). The slides were dipped in Kodak NTB-3 emulsion, exposed for 12 weeks, developed in Kodak D-19, and poststained with hematoxylin and eosin.

Anatomically matched sections were selected at various rostrocaudal levels through each of the amygdaloid nuclei, and the proportion of labelled cells was determined microscopically at 312.5 × or 500 × with the aid of an ocular grid. All cells which had reduced silver grains overlying the nucleus in densities above background levels were considered labelled; obvious endothelial and glial cells were excluded. The determination of the proportion of cells arising (ceasing to divide) on a particular day utilized a modification of the progressively delayed comprehensive labelling procedure described by Bayer and Altman ('74). The method is based on the assumption that ^{3}H-thymidine will only be incorporated by mitotic neuronal precursors, not by postmitotic early neurons. Within a specific population, a maximal percentage (> 95%) of labelled neurons indicates that most of the precursors are still dividing at the time of the onset of the injections, and few neurons are originating. Specific neuronal populations in the amygdala are maximally labelled after two successive daily injections at some time during embryonic development. If the onset of the injections is progressively delayed by 24 hours, injection schedules will follow when the the percentage of labelled neurons within a specific population declines, reflecting the changes of precursors into early neurons. The proportion of neurons originating each day is equal to the daily decline in the percentage of labelled neurons. For instance, the neurons originating on day E17 are determined as follows: E17 = (% neurons labelled E17 + E18) − (% neurons labelled E18 + E19). To give an example of how neurogenesis was determined throughout the amygdala, Table 1 shows the data and calculations for the nucleus of the lateral olfactory tract.

Throughout the quantitative analysis, it was noted that trends in cell labelling within animals were very consistent. For example, the percentage of labelled cells in the ventral part of the medial nucleus tended to be lower than in the dorsal part; however, variability

TABLE 1. Neurogenesis in the nucleus of the lateral olfactory tract

Injection group	N	Medial: % Labelled cells $\bar{x}$ ± S.D.	Medial: Embryonic day	Medial: % Cells originating*	Lateral: % Labelled cells $\bar{x}$ ± S.D.	Lateral: Embryonic day	Lateral: % Cells originating*
E14 + E15	6	(A) 97.8 ± .84	14	22.8 (A-B)	(F) 96.6 ± 1.95	14	38.43 (F-G)
E15 + E16	6	(B) 75 ± 7.54	15	64.36 (B-C)	(G) 58.17 ± 10.15	15	53.53 (G-H)
E16 + E17	11	(C) 10.64 ± 3.44	16	9.42 (C-D)	(H) 4.64 ± 2.16	16	3.42 (H-I)
E17 + E18	9	(D) 1.22 ± .67	17	1.22 (D-E)	(I) 1.22 ± .83	17	1.22 (I-J)
E18 + E19	6	(E) 0	18	0	(J) 0	18	0

* These data are graphically represented in Figure 4.

between animals in an injection group was large enough to mask this trend. Accordingly, a statistical procedure was used—the sign test (Conover, '71)—to determine the consistency of sequential neuron 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, "O" 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).

RESULTS

The Anterior amygdala

Anterior amygdaloid area. The anterior amygdaloid area is a diffuse collection of various-sized neurons lying lateral to the preoptic area and deep to the nucleus of the lateral olfactory tract and the anterior cortical nucleus (see drawings, Fig. 2). Anteromedially, there are scattered large cells resembling those in the horizontal limb of the diagonal band (Fig. 1A,C). Deep to the posterior part of the nucleus of the lateral olfactory tract is a cluster of large cells (Fig. 1B,D) that is separated from the remaining anterior amygdaloid area by a cell-sparse zone; Krettek and Price ('78b) called this layer III of the nucleus of the lateral olfactory tract. The autoradiograms in Figure I are from an animal exposed to ^{3}H-thymidine on E14 + E15. Many of the large cells in the anteromedial part of the anterior amygdaloid area are unlabelled (Fig. 1C), while a majority of the large cells clustered deep to the posterior nucleus of the lateral olfactory tract are heavily labelled (Fig. 1D). Throughout both anterior and posterior parts of the anterior amygdaloid area are many unlabelled small cells (open arrows, Fig. 1C).

The percentage of labelled cells in the anterior amygdaloid area was separately determined in medial and lateral zones at anterior and posterior levels of the nucleus of the lateral olfactory tract; large-to-medium sized cells clustered at the posterior nucleus of the lateral olfactory tract were separately quantified. Statistics showed that neurogenesis occurred simultaneously at anterolateral, posterolateral, and posteromedial parts (data are combined in the middle graph, Fig. 2). Neurogenesis in the anteromedial part is significantly earlier ($P < 0.00001$, top graph, Fig.2) with a definite peak on E13, when many of the large cells originate. The large cells clus-

Abbreviations

AAA	Anterior amygdaloid area	LCE	Large cells adjacent to the central nucleus (Brodal's "X")
AC	Anterior commissure	LV	Ventral part of the lateral nucleus
ACO	Anterior cortical nucleus	ME	Medial nucleus
AH	Ammon's horn	MEd	Dorsal part of the medial nucleus
AHA	Amygdalo-hippocampal area	MEv	Ventral part of the medial nucleus
BL	Basolateral nucleus	NAOT	Nucleus of the accessory olfactory tract
BM	Basomedial nucleus	NLOT	Nucleus of the lateral olfactory tract
CE	Central nucleus	OT	Optic tract
CL	Claustrum	PC	Piriform cortex
CP	Caudate-putamen complex	PCOl	Posterior cortical nucleus, lateral part
DG	Dentate gryrus	PCOm	Posterior cortical nucleus, medial part
EC	Entorhinal cortex	POA	Preoptic area
IC	Intercalated masses	ST	Stria terminalis
LAA	Large-celled cluster in the anterior amygdala	SU	Subiculum
LD	Dorsal part of the lateral nucleus		

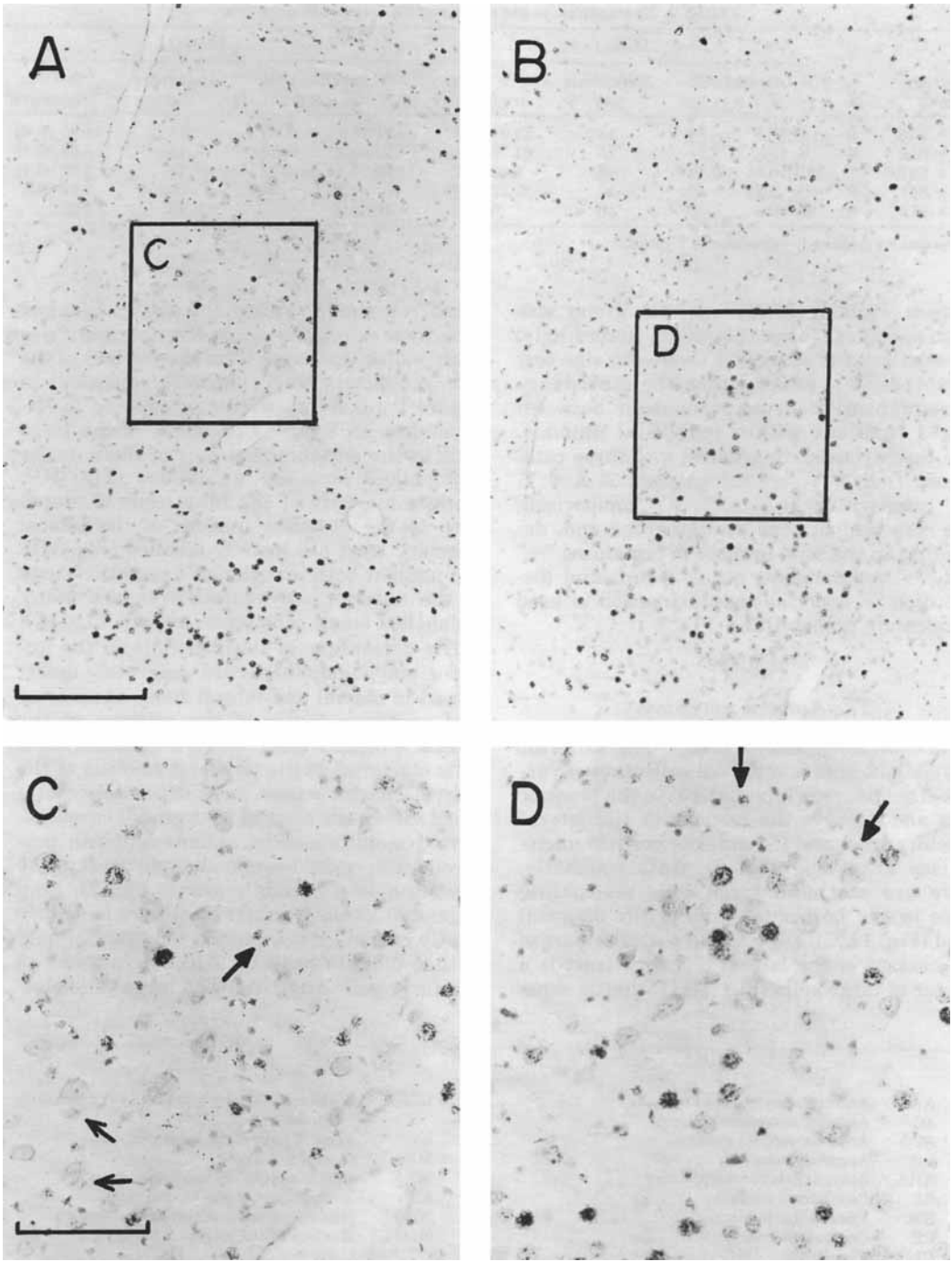


Fig. 1. The anterior amygdaloid area in an animal exposed to ^{3}H-thymidine on E14 + E15 and killed on P60 (coronal sections, 6 μm paraffin, hematoxylin-eosin, bar = 0.25 mm in A, B, 0.1 mm in C, D). Large cells in the anterior part (A) are unlabelled (enlarged in C), while those in the posterior part (B) are labelled (enlarged in D).

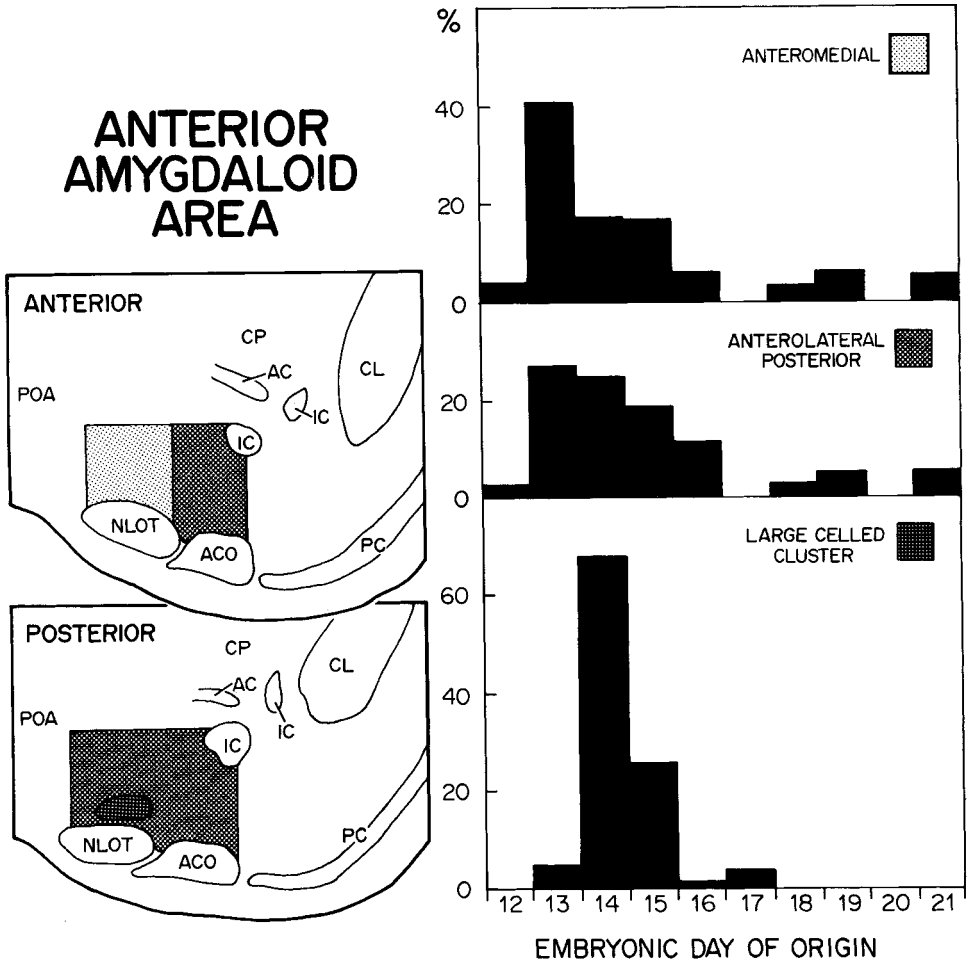


Fig. 2. Neurogenesis in the anterior amygdaloid area. Drawings show levels quantified. Bar graphs are the proportion of cells originating on embryonic days calculated according to the example given in Table 1. Neurons in the anteromedial part originate slightly before those in the anterolateral and posterior parts and in the large-celled cluster. Small cells originate late (E18–E21) throughout much of the anterior amygdaloid area.

tered deep to the posterior nucleus of the lateral olfactory tract originate significantly later ($P < 0.021$, bottom graph, Fig. 2). Throughout the anterior amygdaloid area there is a tendency for about 15–20% of the total population to originate very late in gestation (these cells were not quantified in the cluster). All of these cells are small (solid arrows, Fig. 1C,D) and resemble those in the intercalated masses.

Nuclei of the lateral and accessory olfactory tracts. The nucleus of the lateral olfactory tract is a distinct spherical cluster, except in primates (Crosby and Humphrey, '41; Lauer, '45), of densely packed medium-sized neurons, which forms the anterior ventromedial edge of the amygdala (Fig. 3A). The nucleus of the accessory olfactory tract contains diffusely packed smaller cells (Fig. 3B) posteromedial to the nucleus of the lateral olfactory tract (Young, '36; Krettek and Price, '78b; see drawings, Fig. 4). Figure 3 shows autoradiograms from an animal exposed to ^{3}H-thymidine on E15 + E16. There are more labelled cells medially than laterally in the nucleus of the lateral olfactory tract (Fig. 3A), while most of

the cells throughout the nucleus of the accessory olfactory tract are unlabelled (Fig. 3B).

The percentage of labelled cells in the nucleus of the lateral olfactory tract was separately determined in medial and lateral parts at anterior, intermediate, and posterior levels (L1–L3, Fig. 4 drawings). There were no significant differences throughout the anteroposterior extent, but at each level, neurogenesis in the lateral parts preceded ($P < 0.0002$, data are combined in the middle graph, Fig. 4) that in the medial parts (data are combined in the top graph, Fig. 4). Neurogenesis in the nucleus of the accessory olfactory tract significantly precedes that in the nucleus of the lateral olfactory tract ($P < 0.0001$) and is biphasic. The first and largest surge of neurogenesis occurs on E12 and E13 (74% of the population forms); the second occurs on E15 (17% forms).

The central nucleus

Throughout its rostrocaudal extent, the central nucleus lies dorsolateral to the medial nucleus and adjacent to the lateral nucleus in the dorsal part of the amygdala; it fuses with the caudoputamen complex dorsally. Anteriorly, the medial edge of the central nucleus blends with diffusely packed large cells similar to those in the dorsal part of the medial nucleus; Fox ('40) found these in the cat, and Brodal ('47) labelled this area "X" in the rat. These cells appear to be oriented toward the

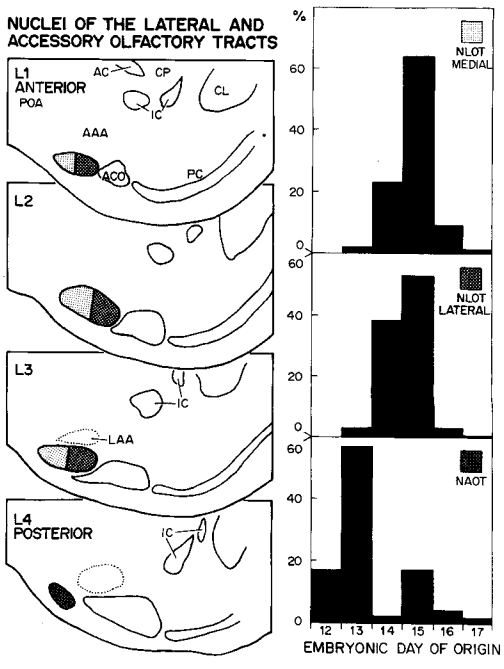


Fig. 4. Neurogenesis in the bed nuclei of the lateral (NLOT) and accessory (NAOT) olfactory tracts. Drawings show levels quantified. Bar graphs are the proportion of cells originating on embryonic days; data are listed in Table 1. Neurogenesis in the NAOT is biphasic and early, while neurogenesis in the lateral NLOT precedes that in the medial NLOT.

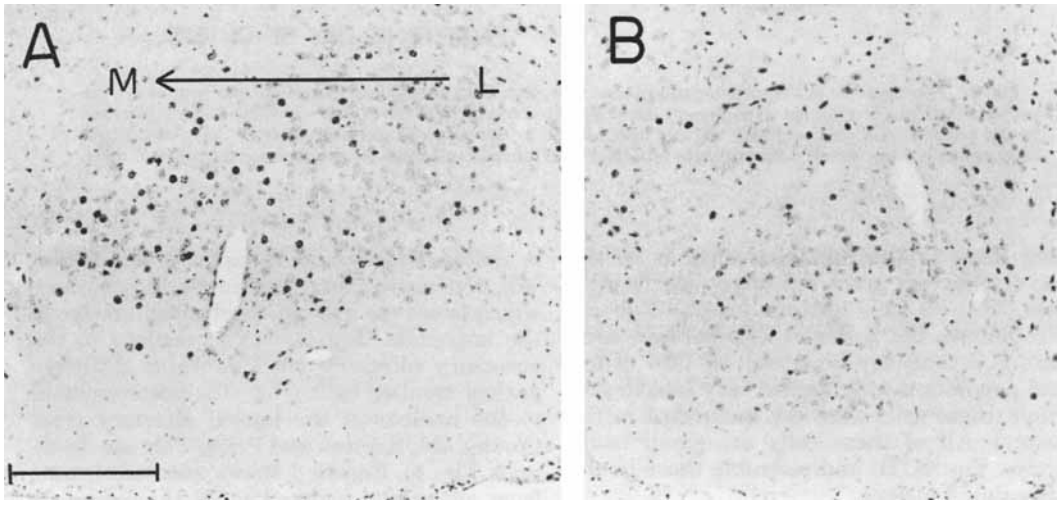


Fig. 3. The bed nuclei of the lateral (A) and accessory (B) olfactory tracts in an animal exposed to ^{3}H-thymidine on E15 + E16 and killed on P60 (coronal sections, 6 μm paraffin, hematoxylin-eosin, bar = 0.25 mm). Most cells are unlabelled in the bed nucleus of the accessory olfactory tract (B) while in the bed nucleus of the lateral olfactory tract there is a lateral-to-medial gradient of neurogenesis (arrow in A).

lateral preoptic and hypothalamic areas (Fig. 5A). Posteriorly, the central nucleus is narrower and the medial large cells are absent (Fig. 5B). The autoradiograms in Figure 5 are from an animal exposed to ^{3}H-thymidine on E15 and E16. Anteriorly, many heavily labelled cells are located in the lateral part; medially placed cells are unlabelled (Fig. 5A). Both heavily and lightly labelled cells can be found throughout the entire posterior central nucleus (Fig. 5B,C). This suggests both medial-to-lateral and anterior-to-posterior gradients.

The percentage of labelled cells was separately determined anteriorly in Brodal's "X" and in medial and lateral parts of the central nucleus. Posteriorly, the central nucleus was divided into medial, intermediate, and lateral parts (drawings, Fig. 6). Both anteriorly and posteriorly, neurogenesis in the medial parts of the central nucleus significantly precedes neurogenesis in more lateral parts (all levels and comparisons, $P < 0.001$). There is also a highly significant rostrocaudal gradient in both medial and lateral parts (all levels and comparisons, $P < 0.00001$). Similar to those in the medial part of the anterior central nucleus, the neurons in the area labelled "Brodal's 'X' " have their peak neurogenesis on E13, but they significantly lag behind ($P < 0.014$) neuron production in the medial part of the central nucleus by having more neurogenesis on and beyond E15 (Fig. 6).

The corticomedial complex

Medial nucleus and amygdalo-hippocampal area. The medial nucleus forms the medial wall of the amygdala from the anterior amygdaloid area to the ventral tip of the hippocampus. Anteriorly, the medial nucleus extends to the ventral surface of the telencephalon (Fig. 7A). More posteriorly, the ventral region is filled with neurons that appear similar to those of the medial nucleus and blend with the hippocampal region (Johnston, '23; Young, '36; Fox, '40; Brodal, '47). This area is separated from the posterodorsal part of the medial nucleus by fibers running toward the stria terminalis, and Krettek and Price ('78b) labelled it the amygdalo-hippocampal area (Fig. 7B,C). The autoradiograms in Figure 7A and B are from an animal exposed to ^{3}H-thymidine on E15 + E16. Most neurons are unlabelled in the anterior ventral part of the medial nucleus while a slightly higher proportion of neurons are labelled dorsally (Fig. 7A). The posterodorsal part of the medial nucleus has many labelled cells; in the amygdalo-hippocampal area, neurons appear to be unlabelled; actually, they are very lightly labelled (Fig. 7B). Figure 7C shows the amygdalo-hippocampal area in an animal exposed to ^{3}H-thymidine on E17 + E18; only superficial cells are unlabelled, while deep cells are heavily labelled. These patterns suggest that neurons originate much earlier in the medial nucleus than in the amygdalo-hippocampal area. Within the medial nucleus there are combined ventrodorsal and rostrocaudal gradients, while the amygdalo-hippocampal area has a superficial-to-deep gradient.

The percentage of labelled cells was separately determined in ventral and dorsal areas at anterior, intermediate, and posterior levels in the medial nucleus (drawings, Fig. 8). There were no significant differences between anterior and intermediate levels. At both of these levels, neurogenesis in ventral locations occurs mainly on E13, significantly before ($P < 0.00001$) it occurs in dorsal locations in E13–E15 (Fig. 8). In the posterodorsal area of the medial nucleus, neurogenesis occurs mainly on E15 (Fig. 8, middle graph), significantly later ($P < 0.002$) than in anterior parts of the medial nucleus. The percentage of labelled cells was separately determined in superficial and deep areas of the amygdalo-hippocampal area (bottom drawing, Fig. 8). Neurogenesis occurs mainly between E16–E18 superficially, significantly before ($P < 0.00001$) the E18–E19 peak at deeper locations (bottom two graphs, Fig. 8).

The *cortical nucleus* forms the floor of the amygdala throughout its entire rostrocaudal extent and projects caudally beneath the ventral subiculum. Although it is adjacent to the piriform cortex, the cell layers are not continuous. This large nucleus has been divided into three parts, similar to the divisions of Krettek and Price ('78b).

The *anterior cortical nucleus* is wedged between the piriform cortex and the nucleus of the lateral olfactory tract anteriorly, and between the posterolateral cortical nucleus and the medial nucleus posteriorly. It is composed of small cells similar to those in the ventral parts of the medial nucleus; a slightly higher superficial packing density is the only evidence of cortical structure. The autoradiogram in Figure 9 is from an animal exposed to ^{3}H-thymidine on E15 + E16, when a majority of the cells are unlabelled. The percentage of labelled cells was separately quantified at anterior, intermediate, and posterior levels (drawings, Fig. 9). There were no significant differences between levels and the graph in Figure 9 represents combined data. Neurogenesis occurs mainly on E13–E15 with a peak on E13.

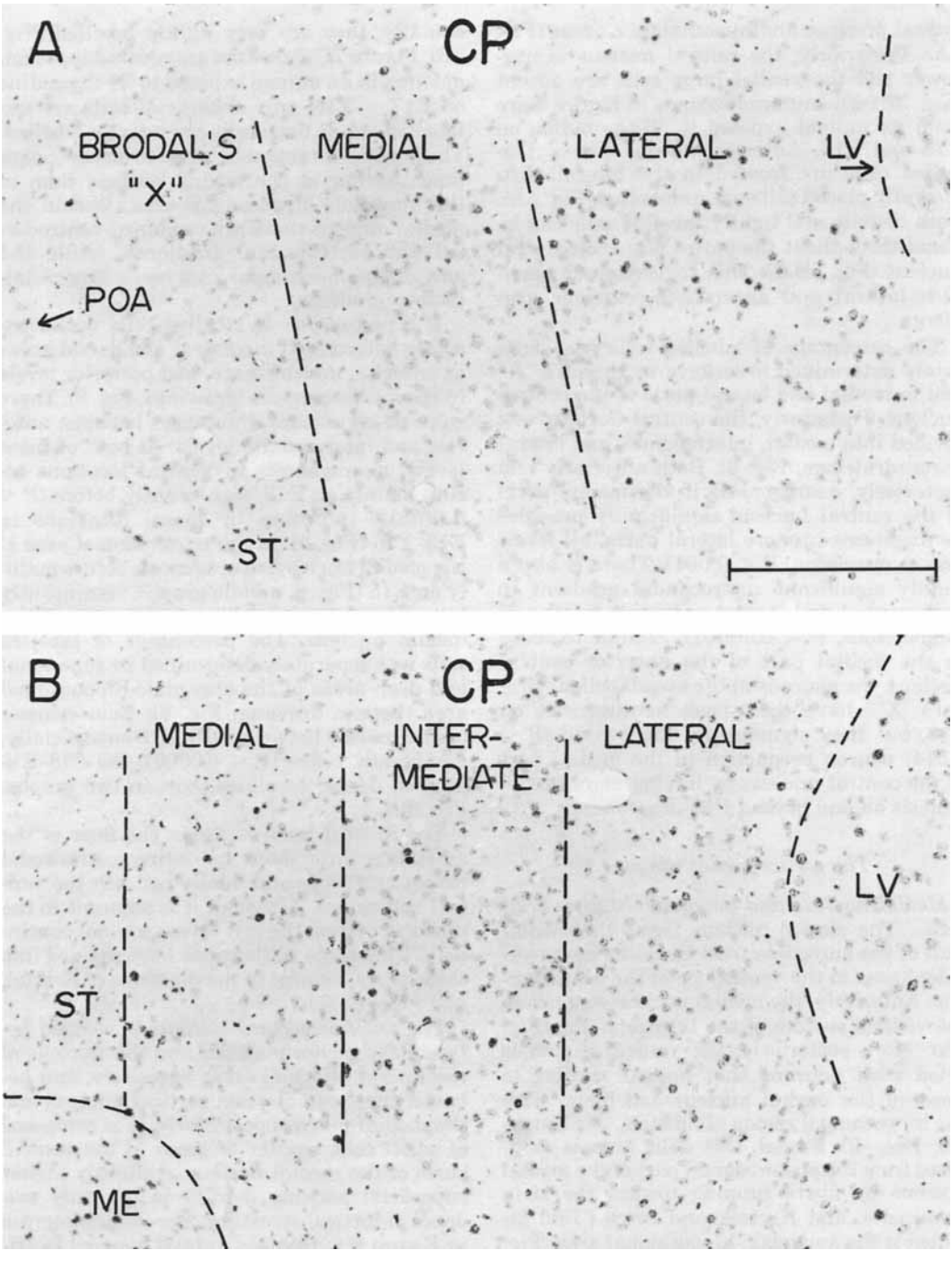


Fig. 5. The central nucleus at anterior (A) and posterior (B) levels in an animal exposed to ^{3}H-thymidine on E15 + E16 and killed on P60 (coronal sections, 6 μm paraffin, hematoxylin-eosin, bar = 0.25 mm). Anteriorly (A), most cells are unlabelled in Brodal's "X" and in the medial part of the central nucleus, while there are both unlabelled and heavily labelled cells laterally. Posteriorly (B), both heavily and lightly labelled cells (appear to be unlabelled) are scattered throughout all parts.

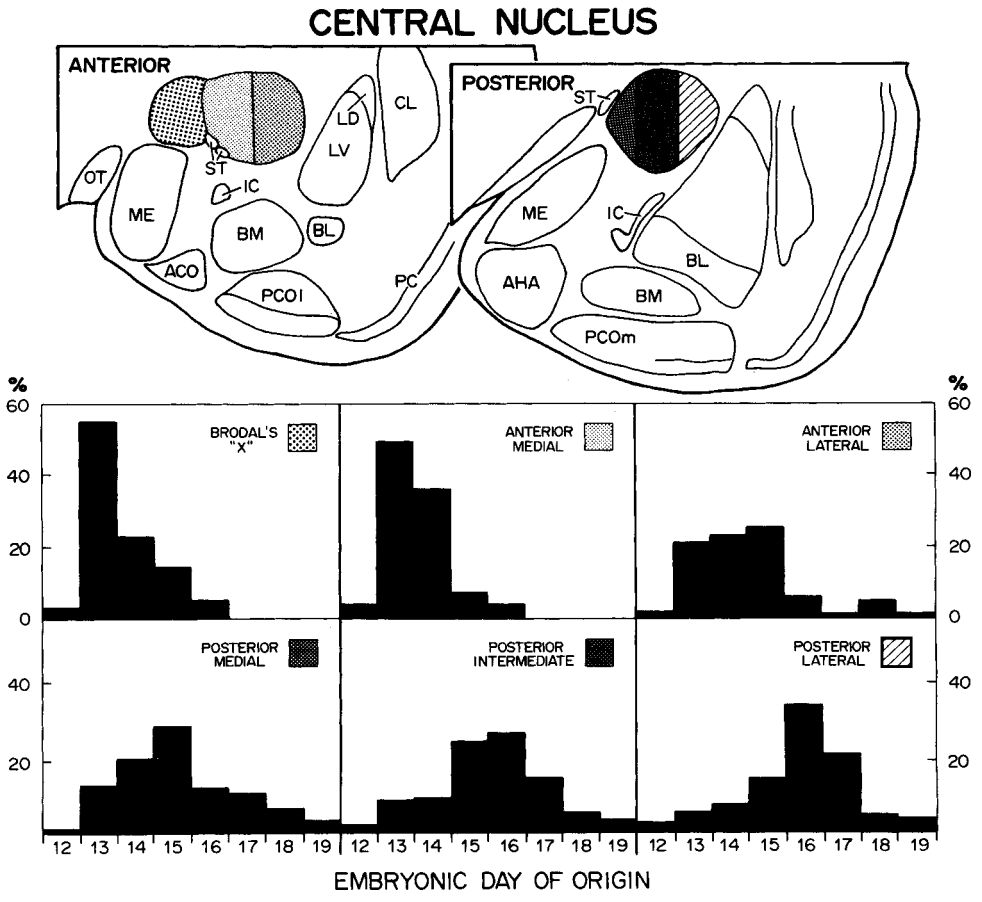


Fig. 6. Neurogenesis in the central nucleus. Drawings show levels quantified. Bar graphs are tbe proportion of cells originating on embryonic days calculated according to the example in Table 1. There is a prominent medial-to-lateral gradient at both anterior and posterior levels of the central nucleus. Also, anterior levels complete their neurogenesis earlier than posterior levels.

The *posterolateral cortical nucleus* is adjacent to the piriform cortex throughout its length; Krettek and Price ('78b) called this the periamygdaloid cortex. Anteriorly, it is bordered by the anterior cortical nucleus; posteriorly it blends with the posteromedial cortical nucleus (Fig. 10; drawings Fig. 11). There is a laminar arrangement with most of the densely packed neurons in layer II being oriented perpendicular to the surface; these superficial cells are smaller than those in the piriform cortex. Beneath layer II, the diffusely packed neurons are randomly arranged and do not form a distinct lamina (Fig. 10A,B). The autoradiograms in Figure 10A and B are from an animal exposed to ^{3}H-thymidine on E16 + E17. Anteriorly (Fig. 10A), the few labelled cells are scattered both superficially and deeply. There are more labelled cells posteriorly (Fig. 10B) and most of them are deep to layer II. These labelling patterns suggest both rostral-to-caudal and superficial-to-deep gradients.

The percentage of labelled cells was separately determined in superficial and deep areas at anterior (Ll) and posterior levels (L2, Fig. 11). Both superficial and deep cells at the anterior level originate mainly on E14 and E15, significantly before those at posterior levels were neurogenesis occurs between E15 and E17 ($P < 0.0005$; right column of graphs, Fig. 11). At level one, cells in both superficial and deep areas are generated simultaneously; at level two, superficial cells originate significantly before deep cells ($P < 0.031$).

The *posteromedial cortical nucleus* lies between the amygdalo-hippocampal area and posterolateral cortical nucleus anteriorly; it

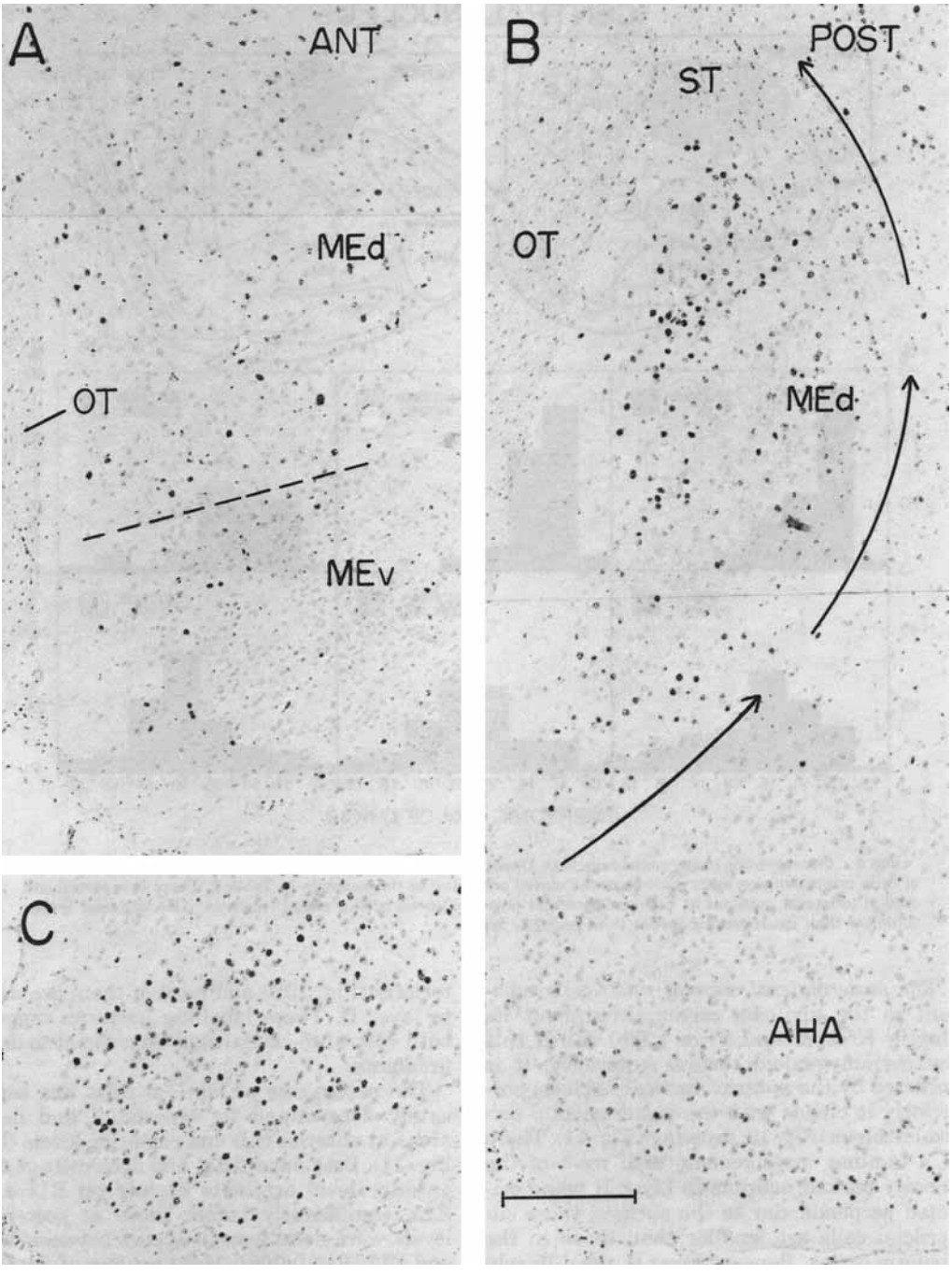


Fig. 7. The medial nucleus and amygdalo-hippocampal area at anterior (A) and posterior (B) levels in an animal exposed to ^{3}H-thymidine on E15 + E16 and killed on P60. Anteriorly (A), there are more unlabelled cells in ventral than dorsal parts of the medial nucleus (separated by dashed line). Posteriorly (B), the dorsal part of the medial nucleus has many labelled cells. The amygdalo-hippocampal area (B) has very lightly labelled cells and is separated from the medial nucleus by fibers running toward the stria terminalis (arrows). Autoradiogram in C is from an animal exposed to ^{3}H-thymidine on E17 + E18; only a few superficial cells are unlabelled (coronal sections, 6 μm paraffin, hematoxylin-eosin, bar = 0.25 mm).

extends posteriorly for some distance beneath the ventral subiculum (Fig. 10B,C; drawings, Fig. 11). Small-to-medium-sized cells are randomly scattered throughout the nucleus and there is no evidence of lamination, although there are a few larger, horizontally oriented cells in the deep areas. The autoradiogram in Figure 10B is from an animal exposed to ^{3}H-thymdine on E16 + E17; most of the unlabelled cells are superficial. The same pattern is shown in Figure 10C in an animal exposed to ^{3}H-thymidine on E17 + E18. These patterns

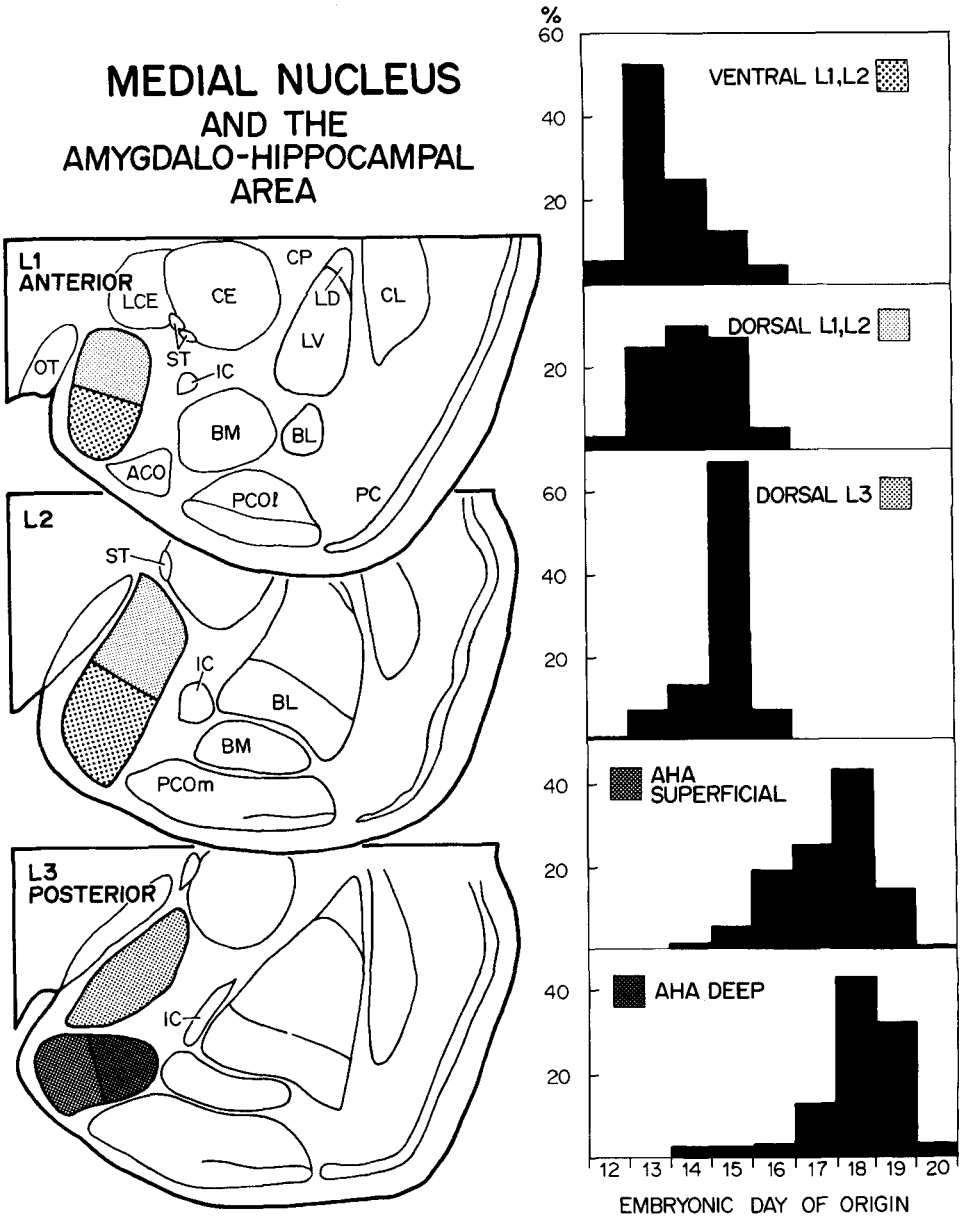
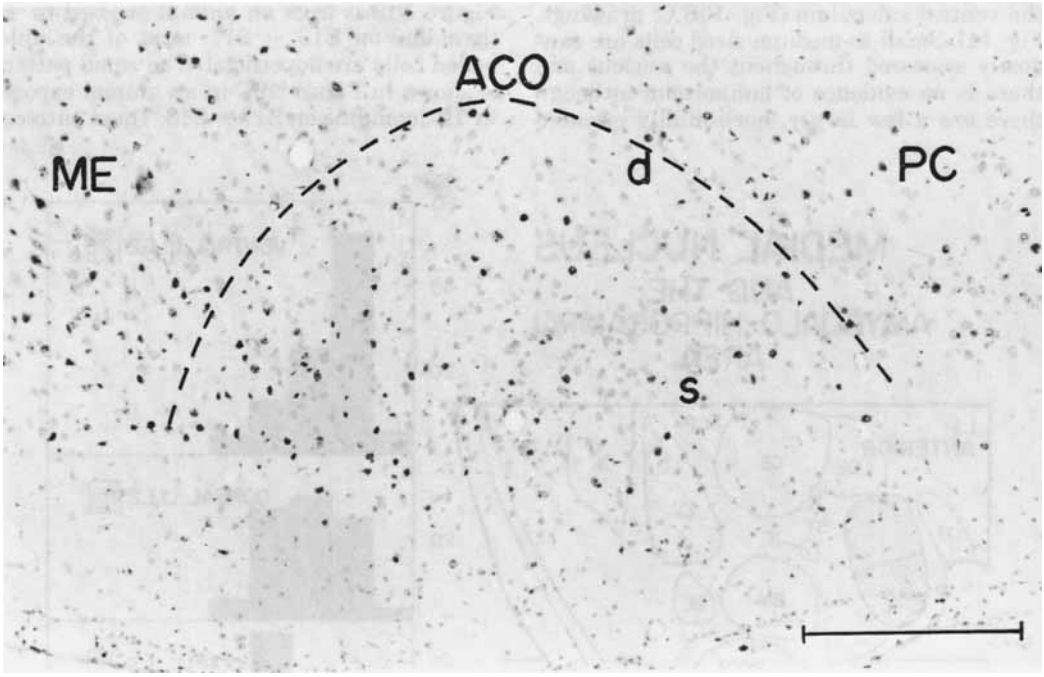


Fig. 8. Neurogenesis in the medial nucleus and the amygdalo-hippocampal area. Drawings show the levels quantified. Bar graphs are the proportion of cells originating on embryonic days calculated according to the example in Table 1. There are combined ventral-to-dorsal and anterior-to-posterior gradients in the medial nucleus, while the amygdalo-hippocampal area originates later in a superficial-to-deep gradient.



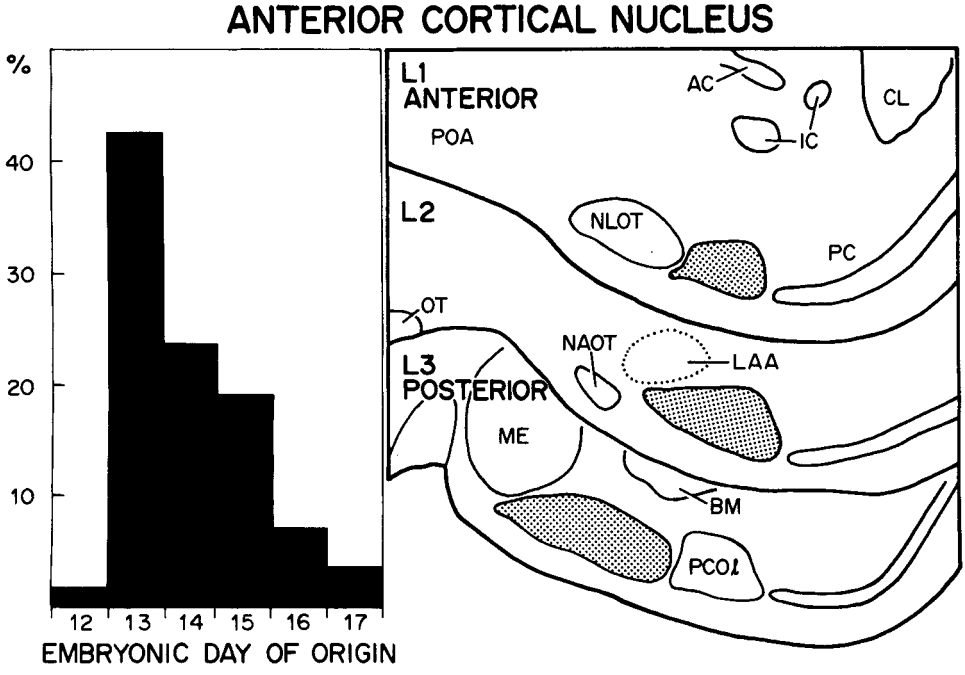


Fig. 9. Neurogenesis in the anterior cortical nucleus. The autoradiogram is from an animal exposed to ^{3}H-thymidine on E15 + E16 and killed on P60 (coronal sections, 6 μm paraffin, hematoxylin-eosin, bar = 0.25 mm). Drawings show levels quantified. Bar graph is the percentage of cells originating during embryonic days calculated according to the example in Table 1. This nucleus is unique in that it has no intranuclear neurogenetic gradients.

suggest combined rostral-to-caudal and superficial-to-deep gradients.

The percentage of labelled cells was separately determined in superficial and deep areas anteriorly (L2) and posteriorly (L3, Fig. 11). At each level, superficial cells are generated significantly earlier than deep cells ($P < 0.00001$). Anterior cells are generated mainly on E15 and E16, significantly before posterior cells, where neurons originate mainly on E16 and E17 ($P < 0.0009$; left column of graphs, Fig. 11).

Gradients within the corticomedial nuclei are summarized in Figure 12. The compartmentalized L-shaped areas represent anterior and intermediate parts of the complex; the box at the posterior level is that part of the posteromedial cortical nucleus which extends beneath the ventral subiculum. Shadings represent the proportion of neurons that have accumulated in specific locations by the *morning* of the embryonic day indicated. There are three types of intranuclear gradients. First, neurogenesis proceeds from ventral to dorsal in the medial nucleus (E14–E16, Fig. 12); second, from rostral to caudal in the dorsal part of the medial nucleus (E14–E15, Fig. 12) and in the posterlateral and posteromedial cortical nuclei (E15–E17, Fig. 12). Third, superficial neurons originate before deep ones at the intermediate posterolateral cortical nucleus, throughout the posteromedial cortical nucleus (E15–E17, Fig. 12), and in the amygdalohippocampal area (E17–E19, Fig. 12).

The earliest-originating neurons (all levels and comparisons $P < 0.00001$) in the entire corticomedial complex lie in the anteroventral medial nucleus (finished by E16, Fig. 12). The latest-originating neurons (all levels and comparisons, $P < 0.0027$) are located deep in the amygdalo-hippocampal area (finished by E20, Fig. 12). Between the generation times of the earliest and latest neurons there are two main internuclear gradients. First, in the anterior part of the complex, the early center of neurogenesis in the ventral medial nucleus is flanked by simultaneously forming slightly younger neurons in the dorsal medial nucleus (an intranuclear gradient) and in the more lateral anterior cortical nucleus (a medial-to-lateral internuclear gradient). Neurogenesis in the anterior cortical nucleus precedes ($P < 0.00001$) that in the anterior part of the anterolateral cortical nucleus, also comforming to the medial-to-lateral internuclear gradient. Second, there is an overall rostral-to-caudal gradient. Neurogenesis in each nucleus at the anterior part of the complex significantly precedes ($P < 0.003$) that in nuclei at intermediate and posterior parts. The amygdalo-hippocampal area is exceptional in that it forms significantly later than the deep cells in the posterior part of the posteromedial cortical nucleus ($P < 0.0093$). The ventral part of the medial and the anterior cortical nuclei reach the 96–10% level 2 to 3 days before other parts of the corticomedial complex. These two areas do not have a late-forming (E18–E20) population of very small neurons and/or glia similar to those scattered throughout the rest of the corticomedial complex. These late-forming cells resemble those in the anterior amygdaloid area (solid arrows, Fig. 1) and in the intercalated masses (Fig. 20).

The basolateral complex

Generally, the basolateral complex has been divided into two nuclei, the lateral and basal, which have small and large-celled divisions (Johnston, '23; Young, '36; Humphrey, '36; Fox, '40; Crosby and Humphrey, '41; Lauer, '45; Brodal, '47). Gurdjian ('28) included the large-celled part of the basal nucleus in the lateral nucleus. In this study, the basal nucleus bas been divided into a small-celled basomedial nucleus and a large-celled basolateral nucleus according to the format of Krettek and Price ('78b). Often, a basal accessory nucleus has also been described, but it is only found in some mammals (Johnston, '23) and not in the rat (Brodal, '47).

The *basomedial nucleus* occupies a central position at its anterior part, being surrounded by the central, medial, anterior cortical, posterolateral cortical, and basolateral nuclei (L1, Fig. 14). At intermediate and posterior levels it is sandwiched between the ventral border of the basolateral nucleus and the deep cells of the posterior cortical nuclei (L2, L3, Fig. 14). Anteriorly, small neurons are densely packed within a fairly well-circumscribed area; but they imperceptibly blend with the neurons in the ventral part of the medial and anterior cortical nuclei (Fig. 13A); posteriorly, the slightly larger cells are diffusely scattered and are difficult to delineate from the overlying basolateral nucleus, the underlying cortical nuclei, and the deep cells of the amygdalohippocampal area (Fig. 13B). The autoradiograms in Figure 13 are from an animal exposed to ^{3}H-thymidine on E16 + E17. Almost all cells are completely unlabelled in the anterior part (Fig. 13A) while most cells are labelled (some very lightly) in the posterior

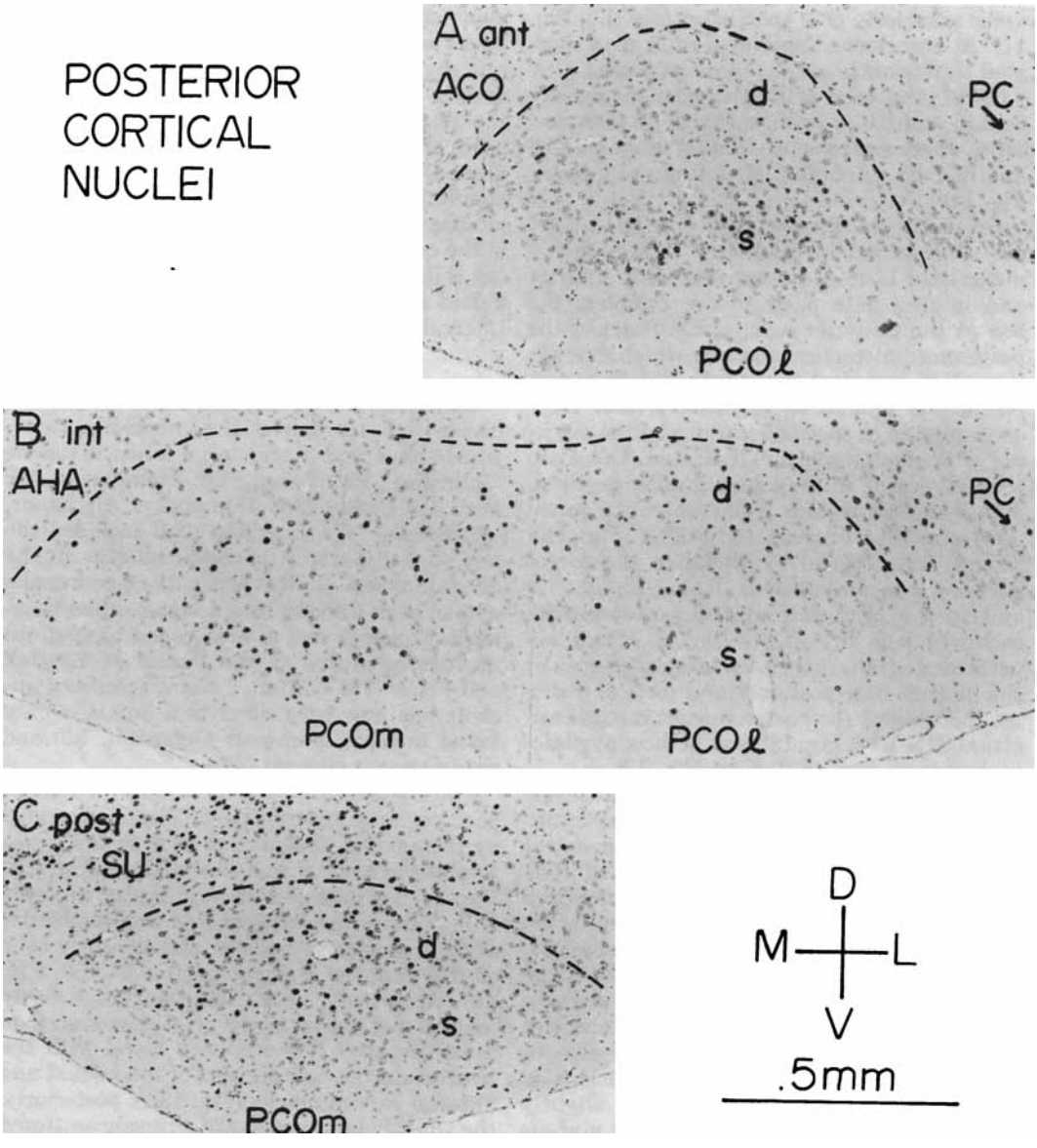


Fig. 10. The posterolateral (PCOl) and posteromedial (PCOm) cortical nuclei at anterior (A) and intermediate (B) levels in an animal exposed to ^{3}H-thymidine on E16 + E17 and killed on P60. Note that more labelled cells are located posteriorly, indicating an anterior-to-posterior neurogenetic gradient. The only unlabelled cells in B are those located more superficially, indicating a superficial-to-deep gradient. Autoradiogram in C is the posterior PCOm in an animal exposed to ^{3}H-thymidine on E17 + E18. Note all unlabelled cells are superficial (coronal sections, 6 μm paraffin, hematoxylin-eosin).

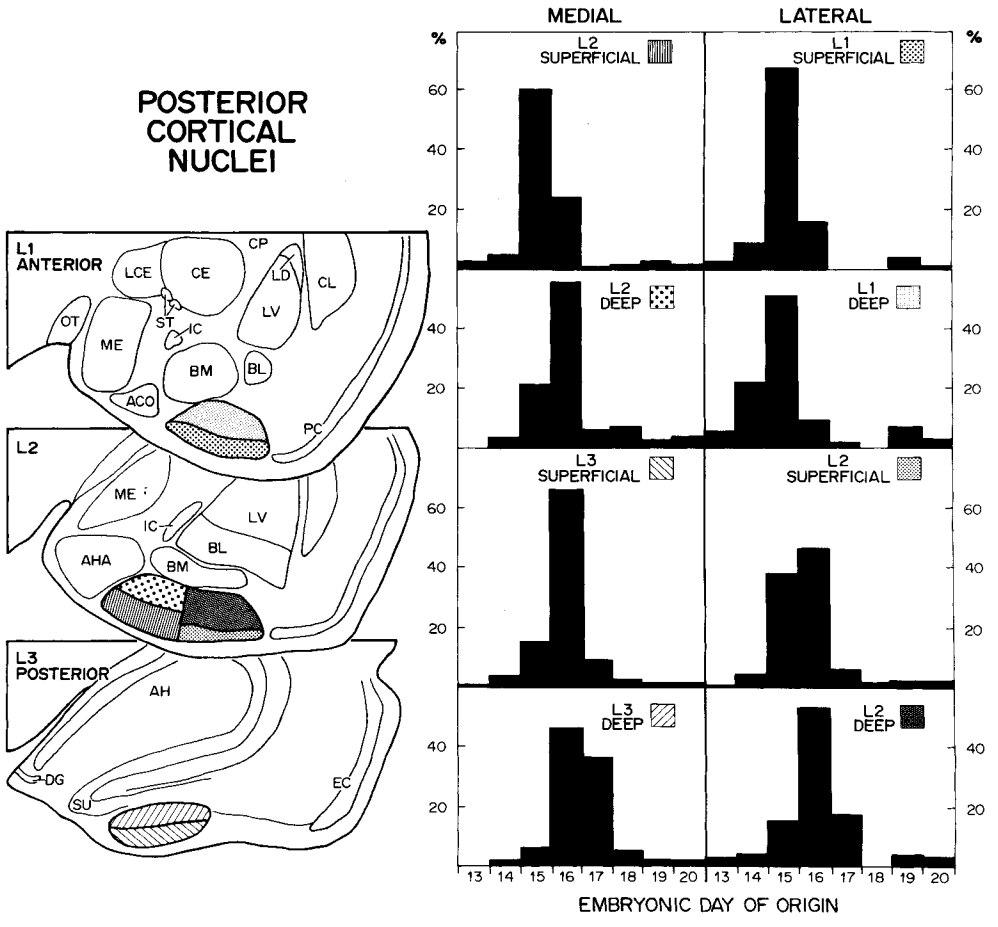
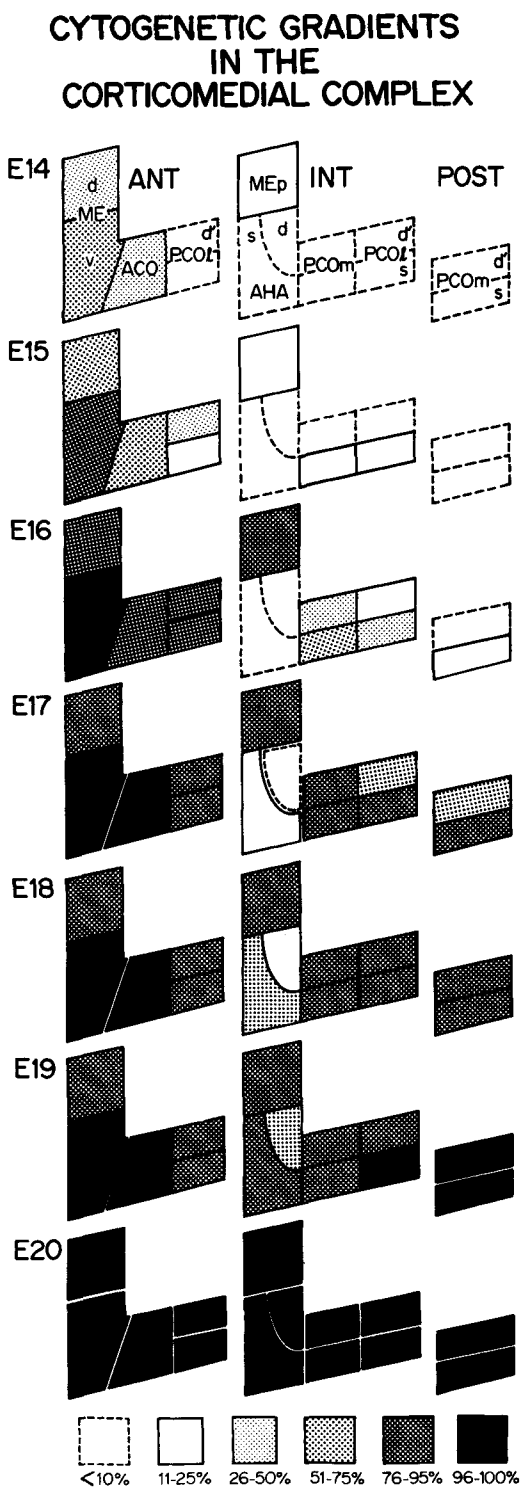


Fig. 11. Neurogenesis in the posterior cortical nuclei. Drawings indicate levels quantified. Bar graphs are the percentage of cells originating on embryonic days calculated according to the example given in Table 1. In the posterolateral cortical nucleus (lateral column graphs) there is a rostral-to-caudal gradient between L1 and L2 and a superficial-to-deep gradient at L2; there is no depth gradient at L1. In the posteromedial cortical nucleus (medial column graphs), there are superficial-to-deep gradients at both L2 and L3 and a rostral-to-caudal gradient between L2 and L3.

part (Fig. 13B), suggesting a very strong rostral-to-caudal gradient. The percentage of labelled cells was separately determined at anterior, intermediate, and posterior levels (drawings, Fig. 14). The square areas in Figure 13 show typical placement of the grid for cell counts. Neurogenesis proceeds in a significant rostral-to-caudal gradient between anterior and intermediate levels ($P < 0.00001$) and between intermediate and posterior levels ($P < 0.0215$). Peak times for neurogenesis occur on E14–E15 at L1, E15–E16 at L2, and E16–E17 at L3. Approximately 10% of the total cell population at intermediate and posterior levels is late-forming (E18–E20) small cells that are similar to those in the intercalated masses.

The *basolateral nucleus* lies beneath the lateral nucleus throughout its length. Anteriorly, it is composed of large cells which are more distinct in the medial part; posteriorly, there are smaller cells that are difficult to



distinguish from those in the basomedial nucleus (Fig. 13B). Krettek and Price ('78b) called the anterolateral part of the basolateral the ventral endopiriform nucleus. The autoradiograms in Figure 15 are from an animal exposed to ^{3}H-thymidine on E16 + E17. There are more labelled cells in medial areas (Fig. 15A) than in lateral areas (Fig. 15B). Posteriorly, many labelled cells are present throughout both medial (Fig. 15C) and lateral areas (Fig. 15D). This suggests combined rostrocaudal and lateromedial gradients of neurogenesis.

The percentage of labelled cells was separately determined in both medial and lateral locations at anterior, intermediate, and posterior levels of basolateral nucleus (drawings, Fig. 16). At each level, neurogenesis in lateral areas significantly precedes that in medial areas (all levels and comparisons, $p \leq 0.0007$). Between levels, there is a strong rostrocaudal gradient. Neurogenesis in anterolateral areas occurs mainly on E14–E15, in posterolateral areas on E15–E16 ($P < 0.0002$; right column of graphs, Fig. 16). Neurogenesis in anteromedial areas occurs mainly on E15, in posteromedial areas on E16 ($P < 0.0001$; left column of graphs, Fig. 16). Throughout the basolateral nucleus there is a tendency for approximately 10% of the population to originate between E17 and E20. These are small cells (arrows, Fig. 15) and resemble the smaller cells in the intercalated masses.

The *lateral nucleus* forms the apex of the basolateral complex and lies between the central nucleus and the claustrum throughout its length (drawings, Fig. 18). It is composed of large-to-medium-sized cells at anterior and intermediate (Fig. 17A,C) levels and, as in the basolateral nucleus, smaller cells posteriorly (Fig. 17B,D). Throughout its length (less prominent at anterior levels), there is a group of slightly smaller cells located at the dorsal tip (Fig. 17A,B), in accordance with the observations of Gurdjian ('28) and Brodal ('47). The autoradiograms in Figure 17 are from an animal exposed to ^{3}H-thymidine on E16 and E17. At the intermediate level, dorsal areas (Fig. 17A) have proportionally fewer labelled cells than do ventral areas (Fig. 17C), suggesting a dorsal-to-ventral gradient. At posterior levels, the same pattern is seen (Fig.

Fig. 12. A summary of the neurogenetic gradients in the corticomedial complex. Shadings indicate the percentage of cells which are unlabelled (have already originated) by the morning of the embryonic day indicated. See text for further discussion.

17B,D), only more labelled cells are present both ventrally and dorsally.

The percentage of labelled cells was separately determined in ventral and dorsal areas at anterior, intermediate, and posterior levels of the lateral nucleus (drawings, Fig. 18). Neurogenesis proceeds in a rostrocaudal gradient in both areas. Anteroventrally, cells are generated mainly on E15, posteroventrally on E16 ($P < 0.00001$, left column of graphs, Fig. 18); anterodorsally, cells are generated mainly on E15, posterodorsally on E15 and E16 ($P < 0.0018$, right column of graphs, Fig. 18). Neurons in dorsal areas originate significantly before those in ventral areas at the posterior ($P < 0.0007$) and intermediate ($P < 0.0414$) levels; neurogenesis is simultaneous throughout the anterior level where the dorsal part is very small. Throughout the lateral nucleus, as in the basolateral and certain parts of the basomedial nuclei, approximately 8–13% of the total cell populations forms between E18 and E20. Arrows in Figure 17 indicate the late-forming cell types.

Gradients within the basolateral complex are summarized in Figure 19. The compartmentalized triangular areas represent anterior, intermediate, and posterior levels of the basolateral and lateral nuclei. The basomedial nucleus is represented by circular shapes at each level. Shadings represent the proportion of neurons that have accumulated in specific locations by the *morning* of the embryonic day indicated. The only prominent gradients throughout the complex are intranuclear. All nuclei show a rostral-to-caudal gradient; additional gradients are lateral to medial in the basolateral (E15–E16, Fig. 19) and dorsal to ventral in the lateral (E16–E17, Fig. 19). Internuclear gradients are clearly evident only at the anterior level. Although early forming neurons are located in both the basomedial and basolateral (lateral part) nuclei (E15, Fig. 19), the basomedial nucleus is the first to finish (E16, Fig. 19; $P < 0.035$). Neurons throughout the basolateral nucleus originate significantly earlier than those in the lateral nucleus (all levels and comparisons, $P \leq 0.035$). At both intermediate and posterior levels, all areas overlap in their development to such an extent that no one nucleus as a whole (for example, both parts of the lateral nucleus) is significantly different from any other nucleus. At the intermediate level, neurons in the lateral part of the basolateral nucleus originate earliest ($P \leq 0.003$); next are those in the basolateral (medial part) and lateral (dorsal part) nuclei ($P \leq 0.041$), and last, those in the ventral part of the lateral nucleus; the basomedial nucleus is simultaneous with both basolateral (medial part) and lateral (both parts) nuclei. At the posterior level, both the basolateral (lateral part) and lateral (dorsal part) nuclei originate simultaneously and significantly earlier ($P \leq 0.008$) than the concurrently generated basolateral (medial part), lateral (ventral part), and basomedial nuclei.

The anterior basomedial nucleus reaches the 96–10% level by E16, 4 days before any other part of the basolateral complex. Even though most areas attain the 76–95% level by E17, the 96–100% level is not reached until E20 (Fig. 19). With the exception of the anterior basomedial nucleus, the basolateral complex contains a population (10–15% of the total) of very small neurons and/or glia that originate simultaneously between E18–E20.

The intercalated masses

The intercalated masses are clumps of densely packed small cells (Fig. 20), that are anteriorly associated with the temporal limb of the anterior commissure and posteriorly associated with the fibers between the central and medial nuclei and the nuclei in the basolateral complex (drawings, Fig. 21). The autoradiograms in Figure 20 are from an animal exposed to ^{3}H-thymidine on E17 and E18. The proportion of labelled cells increases as one proceeds from anteromedial (Fig. 20A), to anterolateral (Fig. 20B), to intermediate (Fig. 20C) groups; almost all cells are labelled in the posterior group (not illustrated). This pattern suggests a rostrocaudal gradient.

The percentage of labelled cells was separately determined in lateral and medial locations at two anterior levels (L1, L2, Fig. 21), and at intermediate (L3, Fig. 21) and posterior (L4, Fig. 21) levels. Most of the intercalated cells are generated between E15 and E19. Neuron origin is simultaneous in anteromedial areas (data are combined in the first graph, Fig. 21) and in anterolateral areas (data are combined in the second graph, Fig. 21). Anteromedial cells are generated significantly earlier than anterolateral cells ($P < 0.00001$). Neurogenesis also proceeds in a rostrocaudal gradient from anterior to intermediate ($P < 0.0001$) to posterior ($P < 0.00001$) levels.

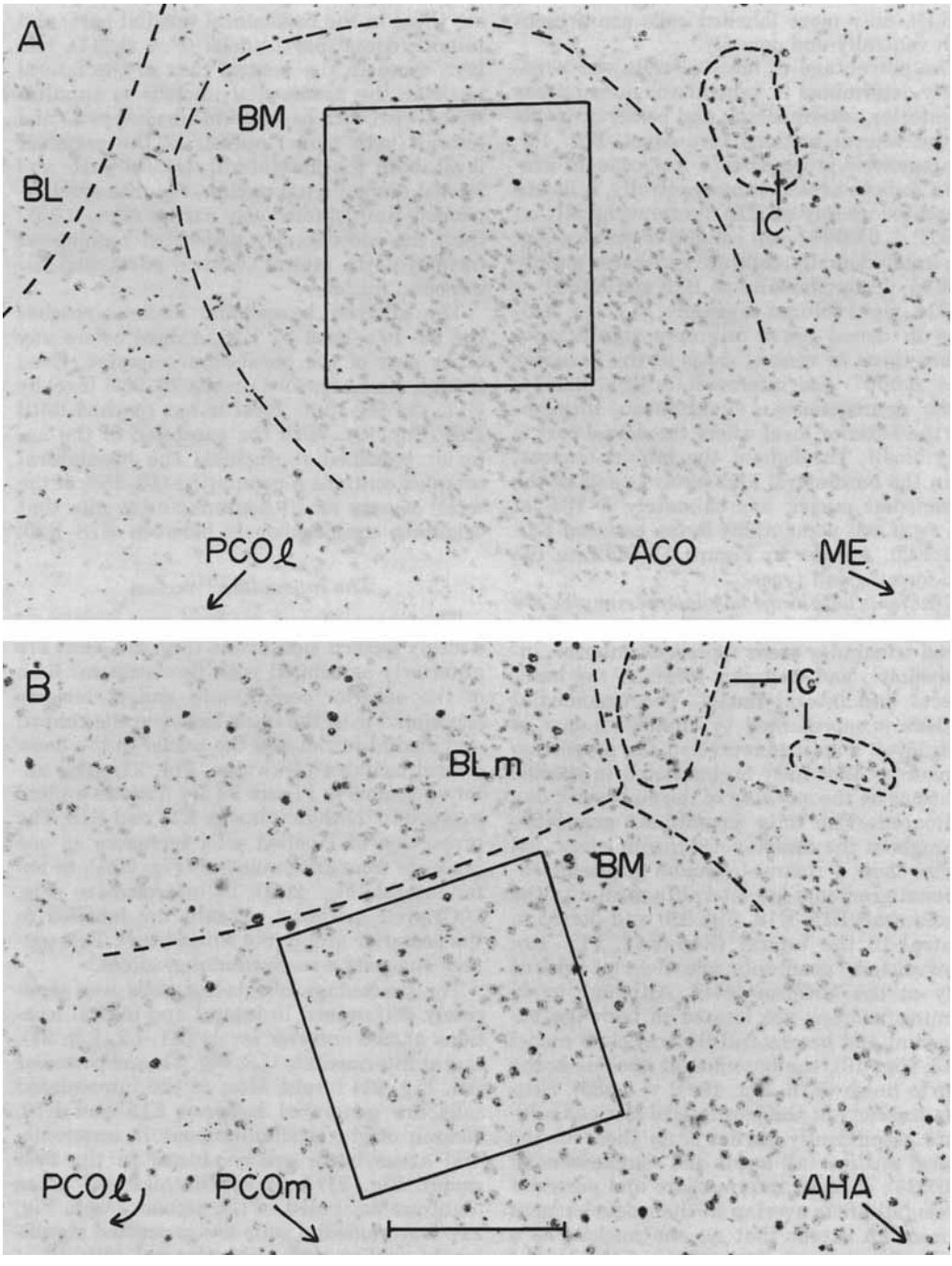


Fig. 13. The basomedial nucleus in an animal exposed to ^{3}H-thymidine on E16 + E17 and killed on P60 (coronal sections, 6 μm paraffin, hematoxylin-eosin, bar = 0.25 mm). Notice that the position of the basomedial nucleus shifts from medial to the basolateral anteriorly (A), to ventral to the basolateral posteriorly (B). Few neurons are labelled anteriorly, while most are labelled posteriorly.

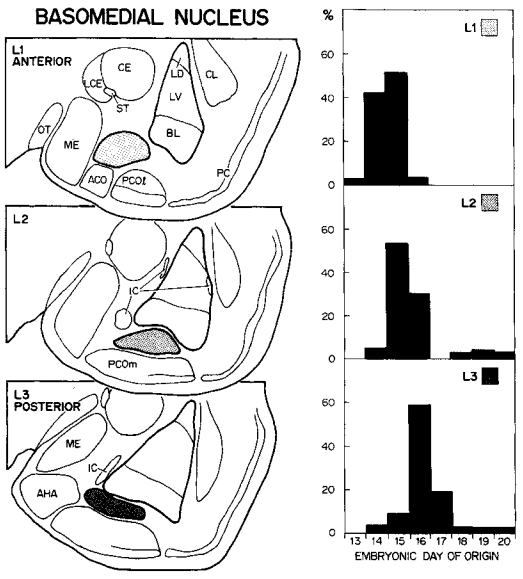


Fig. 14. Neurogenesis in the basomedial nucleus. Drawings indicate the levels quantified. Bar graphs are the percentage of cells originating on embryonic days calculated according to the example in Table 1. There is a strong rostral-to-caudal gradient of neurogenesis.

DISCUSSION

Neurogenetic patterns in the amygdala and their implications for its morphogenesis

To provide an overview of the pattern of development in the amygdala, a three-dimensional diagram of its major nuclei, along with anterior and posterior cross sections are shown in Figure 22. All arrows indicate the major gradients of neurogenesis described in this report. The numbers within nuclear areas in the cross sections are the proportion of cells which have originated (percentage of unlabelled cells) by either E15 (anterior) or E16 (posterior). Generally, the nuclei are organized into longitudinal strips, and with only a single exception (the anterior cortical nucleus), neurons are generated in a strong anterior-to-posterior gradient within each strip (large superficial arrow, Fig. 22). This generalized gradient has also been noted in the mouse (Sidman and Angevine, '62; McConnell, '75) and hamster (ten Donkelaar et al., '79). There are several additional strong intranuclear gradients—specifically, a ventral-to-dorsal gradient in the medial nucleus, medial to lateral in the central nucleus, superficial to deep in both the posterior cortical nuclei and the amygdalo-hippocampal area, and lateral to medial in the basolateral nucleus. These prominent intranuclear gradients are not matched by internuclear gradients which occur in only a few instances. There is a medial-to-lateral gradient between the medial, anterior cortical, and anterior part of the posterolateral cortical nucleus, and a ventral-to-dorsal gradient between the basolateral and lateral nucleus at the anterior level. The earlier development of the more rostral posterolateral cortical nucleus over the more caudal posteromedial cortical nucleus is interpreted to be more a reflection of the general rostrocaudal gradient rather than a true internuclear gradient. Although the bulk of neurogenesis in the basolateral complex lags behind neuron production in both the central nucleus and corticomedial complex in agreement with observations in the mouse (Sidman and Angevine, '62; McConnell, '75) and hamster (ten Donkelaar et al., '79), in the rat this is only partially true. Note that not only are there *fewer* unlabelled cells in the lateral nucleus on either E15 or E16 than in many other locations in the amygdala, but also there are *more* unlabelled cells in the lateral part of the basolateral nucleus (especially anteriorly) than in lateral parts of the central nucleus and in several locations throughout the corticomedial complex. At a given point in development, neurons are being generated in specific locations within most major nuclei.

What seems to be most striking is that there are early originating "centers" around which younger neurons are located within each major nucleus and/or nuclear complex. First, the medial part of the central nucleus is surrounded by younger neurons both anteromedially (LCE, Brodal's "X", '47) and laterally. Second, the ventral part of the medial nucleus is capped by a crescent of younger neurons in the dorsal part of the medial nucleus, the anterior basomedial nucleus and the anterior cortical nucleus. Third, the lateral part of the basolateral nucleus is flanked by later originating neurons in the lateral, medial part of the basolateral, posterolateral cortical (anterior), and basomedial (posterior) nuclei. Another notable feature is that younger neurons surrounding these early centers tend to face each other. The later originating cells in the crescent around the ventral part of the medial nucleus are adjacent to neurons in Brodal's "X", the medial part of the basolateral nucleus, and the anterior part of the posterolateral

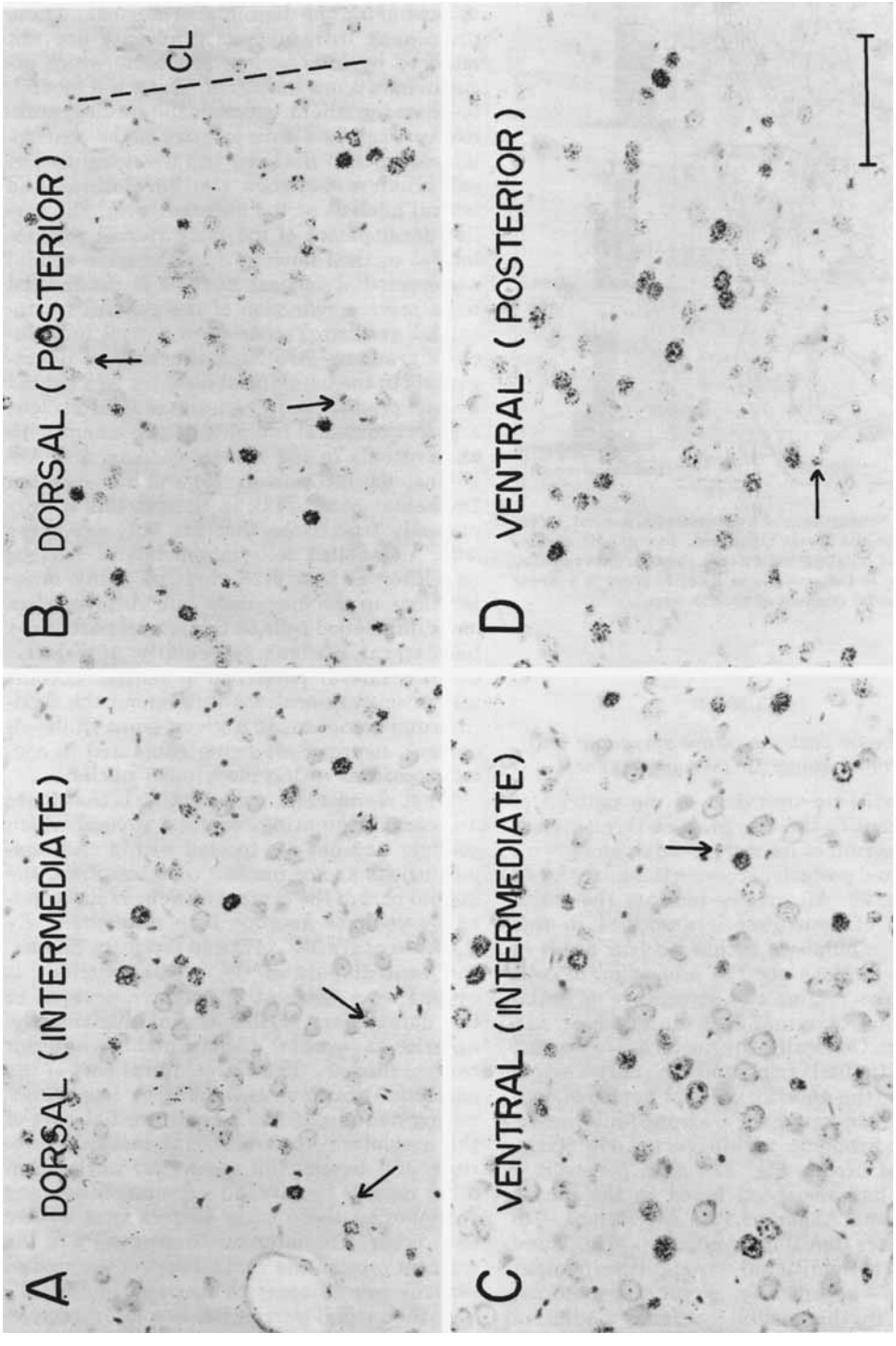


Fig. 15. The basolateral nucleus at anterior (A,B) and posterior (C,D) levels from an animal exposed to ^{3}H-thymidine on E16 + E17 and killed on P60 (coronal sections 6 μm paraffin, hematoxylin-eosin, bar = 0.1 mm). There are more cells labelled medially (A,C) than laterally at both levels, while the proportion of labelled cells is higher throughout the posterior level.

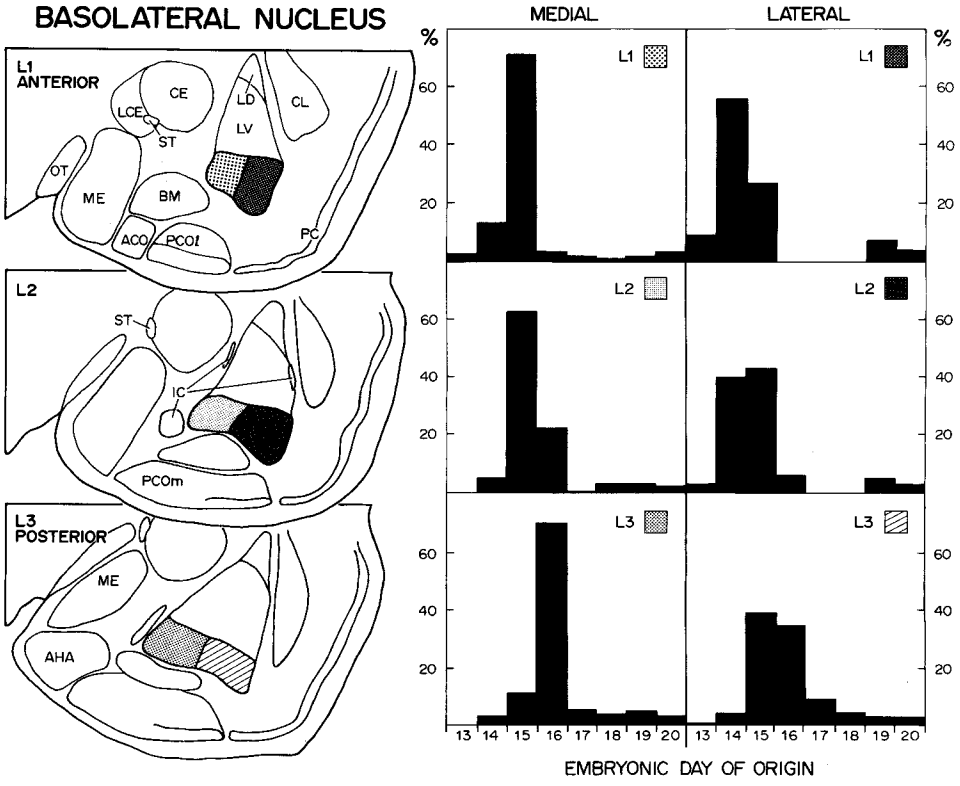


Fig. 16. Neurogenesis in the basolateral nucleus. Drawings indicate the levels quantified. Bar graphs are the percentage of cells originating during embryonic days calculated according to the example given in Table 1. There are lateral-to-medial gradients within levels and an overall rostral-to-caudal gradient between levels.

cortical nucleus, which are themselves satellites around an early forming center. The youngest neurons in the central nucleus are near those in the ventral part of the lateral nucleus, and the younger deep neurons of the posterior cortical nuclei face the late-forming cells in the posterior basomedial nucleus. Finally, the latest-forming neurons in the entire amygdala lie in the intercalated masses which occupy a centrally located "seam" between the central nucleus and corticomedial and basolateral complexes. In brief, almost all arrows seem to be pointing toward the interior (Fig. 22).

To summarize, the chronology of neurogenesis in the amygdala reported here consistently shows strong *intra*nuclear gradients (often more than one) while *inter*nuclear gradients are usually weak. Thus, the data of this study indicate that most nuclei within the amygdala develop nearly simultaneously, each according to a specific pattern. This is in marked contrast to the developmental pattern in the hippocampal region (Bayer, '80) and cerebellar cortex (Altman and Bayer, '78a), where neurons in different areas are produced sequentially. It is similar to developmental patterns in the septal region where the midline septal nuclei and the bed nucleus of the stria terminalis develop simultaneously (Bayer, '79a). In addition, throughout the hypothalamus (Altman and Bayer, '78b), thalamus (Altman and Bayer, '79a,b), mesencephalon (Altman and Bayer, in preparation), pons (Altman and Bayer, '80d), and medulla (Altman and Bayer, '80a,b,c), sequential neurogenesis occurs within a subsystem (for example, the ventrobasal complex in the thalamus) while simultaneous neurogenesis occurs between subsystems. Thus, the combined sequential

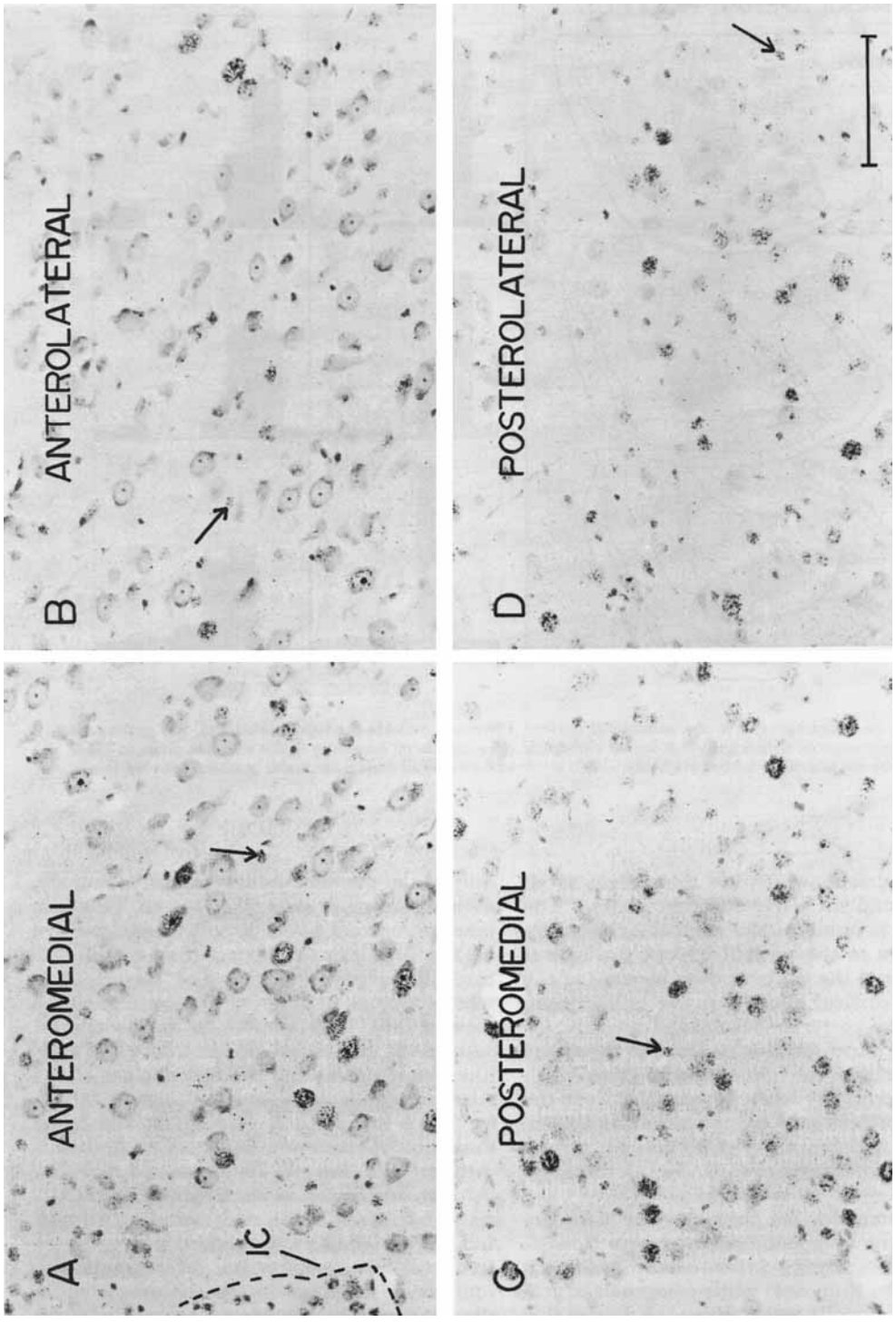


Fig. 17. The lateral nucleus at intermediate (A,C) and posterior (B,D) levels in an animal exposed to ^{3}H-thymidine on E16 + E17 and killed on P60 (coronal sections, paraffin 6 μm, hematoxylin-eosin, bar = 0.1 mm). Dorsal cells (A,B) are smaller and have a lower proportion of labelled cells than those located ventrally (C,D). Generally, more cells are labelled throughout the posterior level.

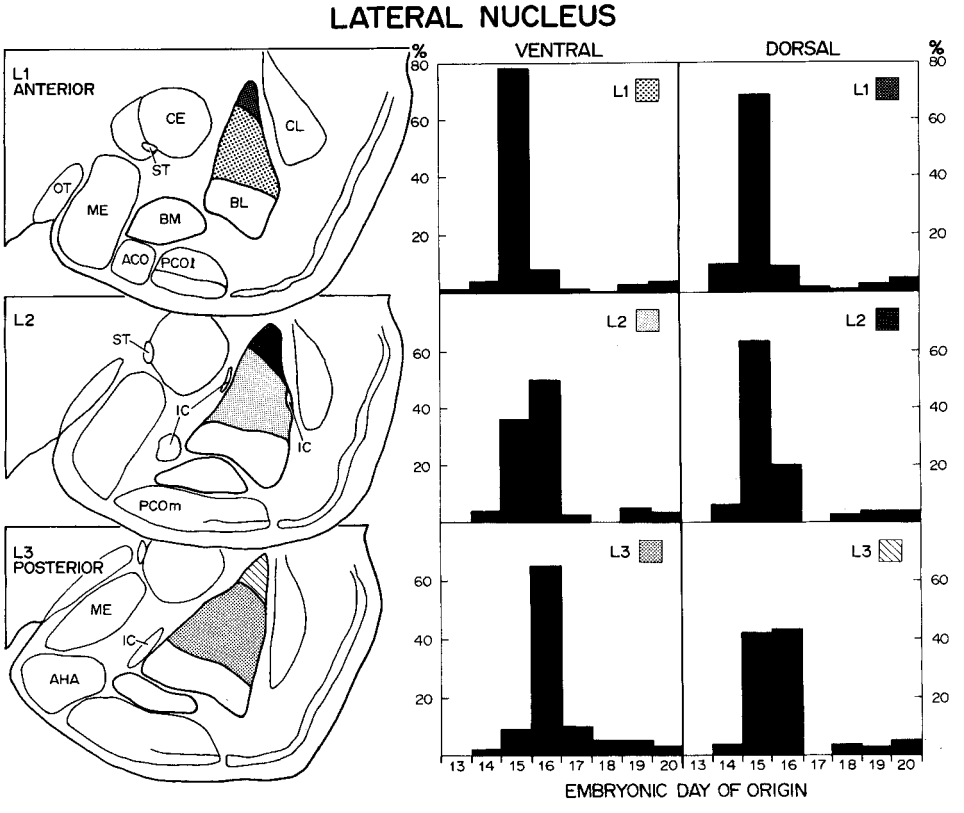
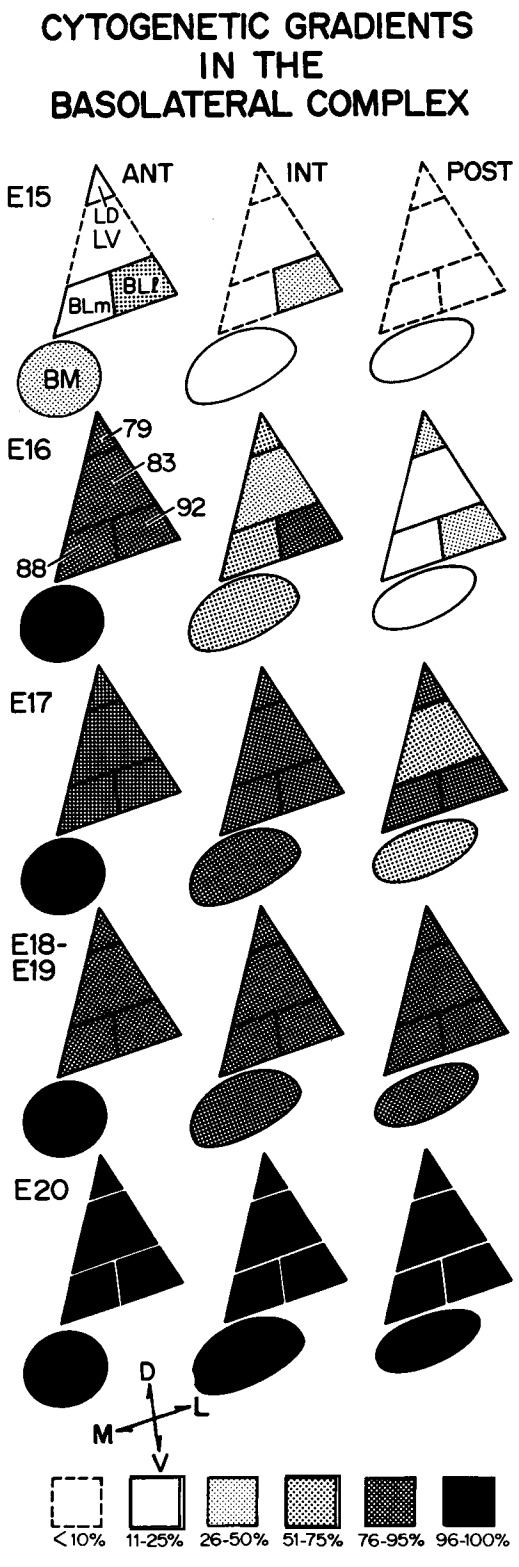


Fig. 18. Neurogenesis in the lateral nucleus. Drawings indicate the levels quantified. Bar graphs are the percentage of cells originating on embryonic days calculated according to the example given in Table 1. There are dorsal-to-ventral gradients within levels 2 and 3 and an overall rostral-to-caudal gradient between all levels.

and simultaneous timetables of neuron production appear to be a basic characteristic of ganglionic structural development.

Sequential neurogenetic gradients between groups of neurons within a structural subsystem can often be traced to a common neuroepithelial source; the term "cytogenetic zone" (Altman and Bayer, '79a,b) has been applied to such subsystems. For example, the sequentially formed medial and lateral septal nuclei are early and late products, respectively, of the lateral ventricular neuroepithelium lining the ventral medial telencephalic wall (Bayer, '79b). In the hypothalamus, the supraoptic and paraventricular nuclei originate sequentially from a specific area of neuroepithelium in the third ventricle (Altman and Bayer, '78c). Most previous embryonic studies of amygdala development locate its neuroepithelial source in the posterior basal telencephalon (Holmgren, '25; Macchi, '51; Källén, '51; Hewitt, '58; Humphrey, '68, '72). Figure 23 shows drawings of both anterior and posterior cross sections of the rat basal telencephalon on embryonic day 15 which presumably contain the primordial amygdala. Notice that anteriorly there is a larger area of differentiating cells than posteriorly, as one would predict from the strong rostral-to-caudal gradient shown in the timetable of amygdaloid neurogenesis. The neurogenetic patterns also predict that there are multiple centers in the neuroepithelium which serve as sources for the cytogenetic zones in the amygdala. For example, the corticomedial



complex may originate from more medial neuroepithelium, while the basolateral complex may originate more laterally (arrows, Fig. 23). Several morphological studies of amygdaloid embryonic development have postulated that the various nuclear complexes are derived from multiple embryonic sources (Holmgren, '25; Källén, '51, Humphrey, '68, '72); the ridges in the basal telencephalon, as shown in the anterior cross section (Fig. 23), have often been used to delimit these various sources. However, the amygdaloid primordium is intimately connected with the primordia of surrounding basal telencephalic structures. Holmgren ('25) thought that the claustrum and parts of tbe piriform cortex may be derived from the same neuroepithelial source as the basolateral complex. The caudoputamen complex and parts of the globus pallidus are probably closely associated with the source of the central nucleus (Källén, '51). Thus, in order to more accurately interpret the embryonic events not only in the amygdala, but also in the rest of the basal telencephalon, studies are now in progress to first prepare the chronology of neurogenesis in the entire basal telencephalon, including that in the olfactory bulb.

Comments on amygdaloid anatomical categories.

The neurogenetic patterns in the amygdala suggest some specific comments with regard to its anatomical categories. The gradients of neurogenesis in the *anterior amygdaloid area* are difficult to correlate with those in the amygdala proper. Even though they are more rostrally placed, most anterior amygdaloid area neurons tend to originate later than those both in the ventral part of the medial nucleus and in the medial part of the central nucleus. Thus, the anterior amygdaloid area does not participate in the strong rostral-to-caudal neurogenetic gradient so typical of the rest of the amygdala. This diffuse part of the basal telencephalon is inseparable from the lateral preoptic area (Brodal, '47), and may be more related to preoptic neurogenetic gradients.

There are three similarities between the *anterior basomedial nucleus,* the *ventral part of the medial nucleus,* and the *anterior cortical nucleus.* First, neurons in these areas appear

Fig. 19. A summary of the neurogenetic gradients in the basolateral complex. Shadings indicate the percentage of cells which are unlabelled (have already originated) by the morning of the embryonic day indicated. Numbers in the anterior level on E16 are the percentages of unlabelled cells in each area. See text for discussion.

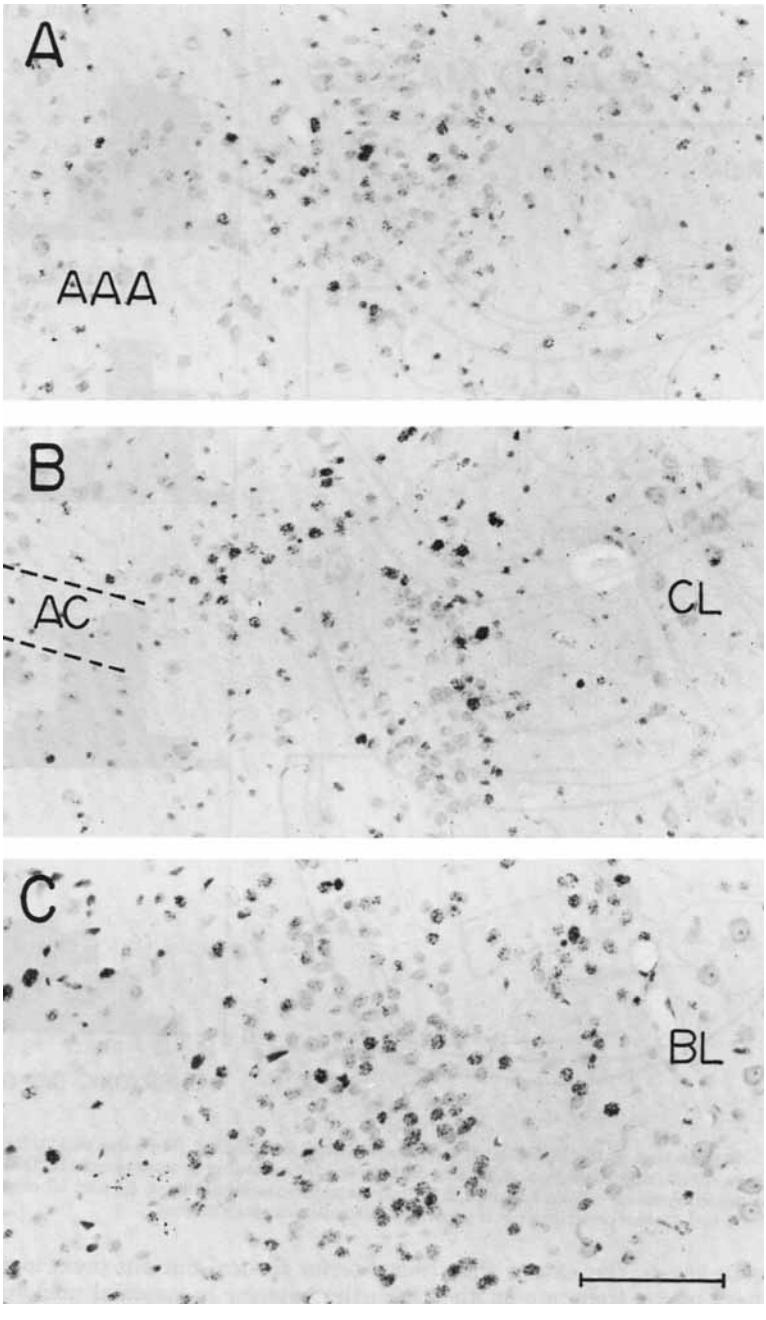


Fig. 20. The intercalcated masses in an animal exposed to ^{3}H-thymidine on E17 + E18 and killed on P60 (coronal sections, 6 μm paraffin hematoxylin-eosin, bar = 0.1 mm). There are progressively more labelled cells as one proceeds from anteromedial (A) to anterolateral (B) to more caudal levels (C).

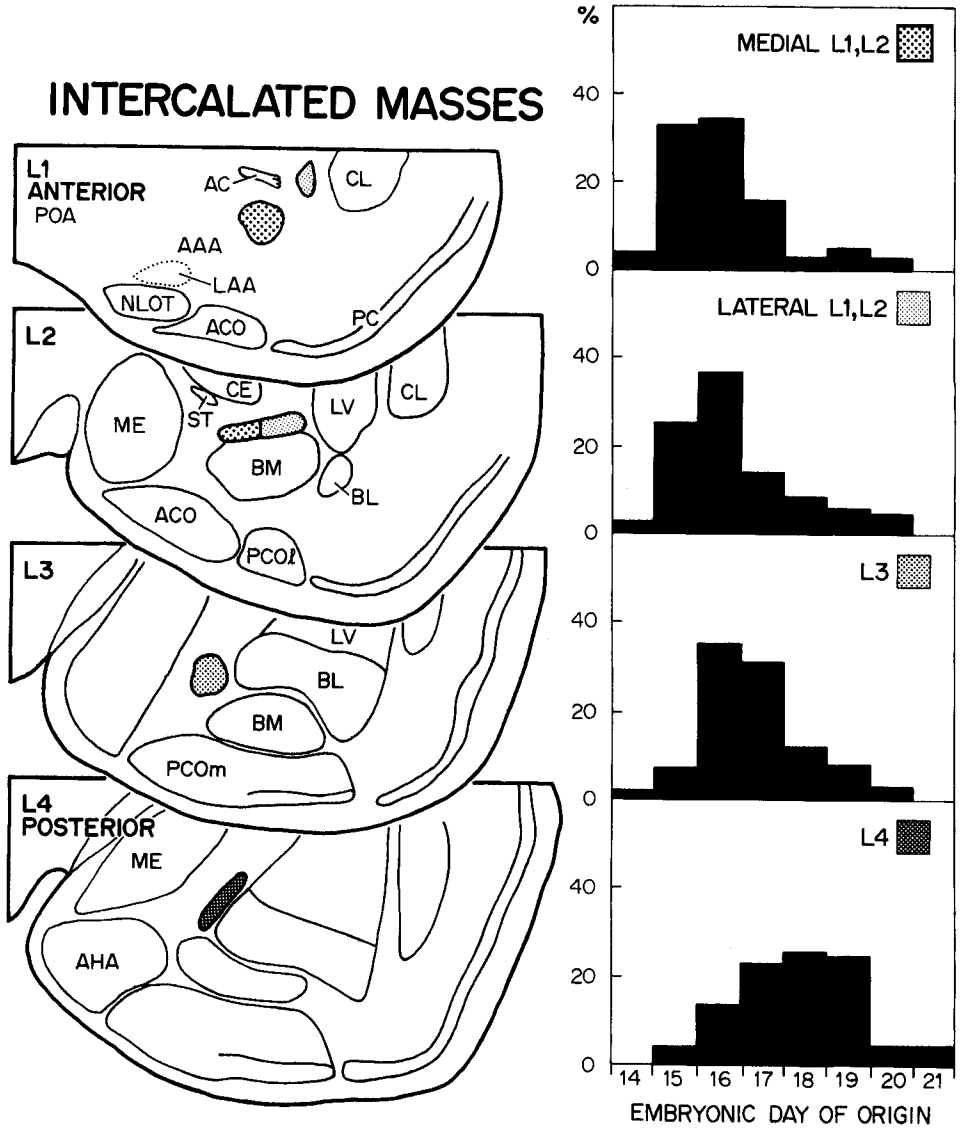


Fig. 21. Neurogenesis in the intercalated masses. Drawings indicate the levels quantified. Bar graphs are the percentage of cells originating on embryonic days calculated according to the example in Table 1. There is a medial-to-lateral gradient within L1 and L2, but no rostral-to-caudal gradient. L1 and L2 originate earlier than either L3 or L4, thus participating in an overall rostral-to-caudal gradient.

to be homogeneous, to the extent that lines delimiting these nuclei from one another are often arbitrary. Gurdjian ('28) called this collection of small cells the "ventromedial nuclear area" where distinctions could not be made between basal, medial, and anterior cortical nuclei. Second, there is a sequential neurogenetic gradient between the ventral part of the medial nucleus (earliest to originate), the anterior cortical nucleus (next to originate), and the anterior basomedial nucleus (last to originate). Third, these three structures together form a unique group in the amygdala by not having a population of late-forming small cells; notice that these structures reach the 96–100% level a few days before most other areas in the amygdala (Figs. 13 and 18). On both developmental and cytoarchetectonic

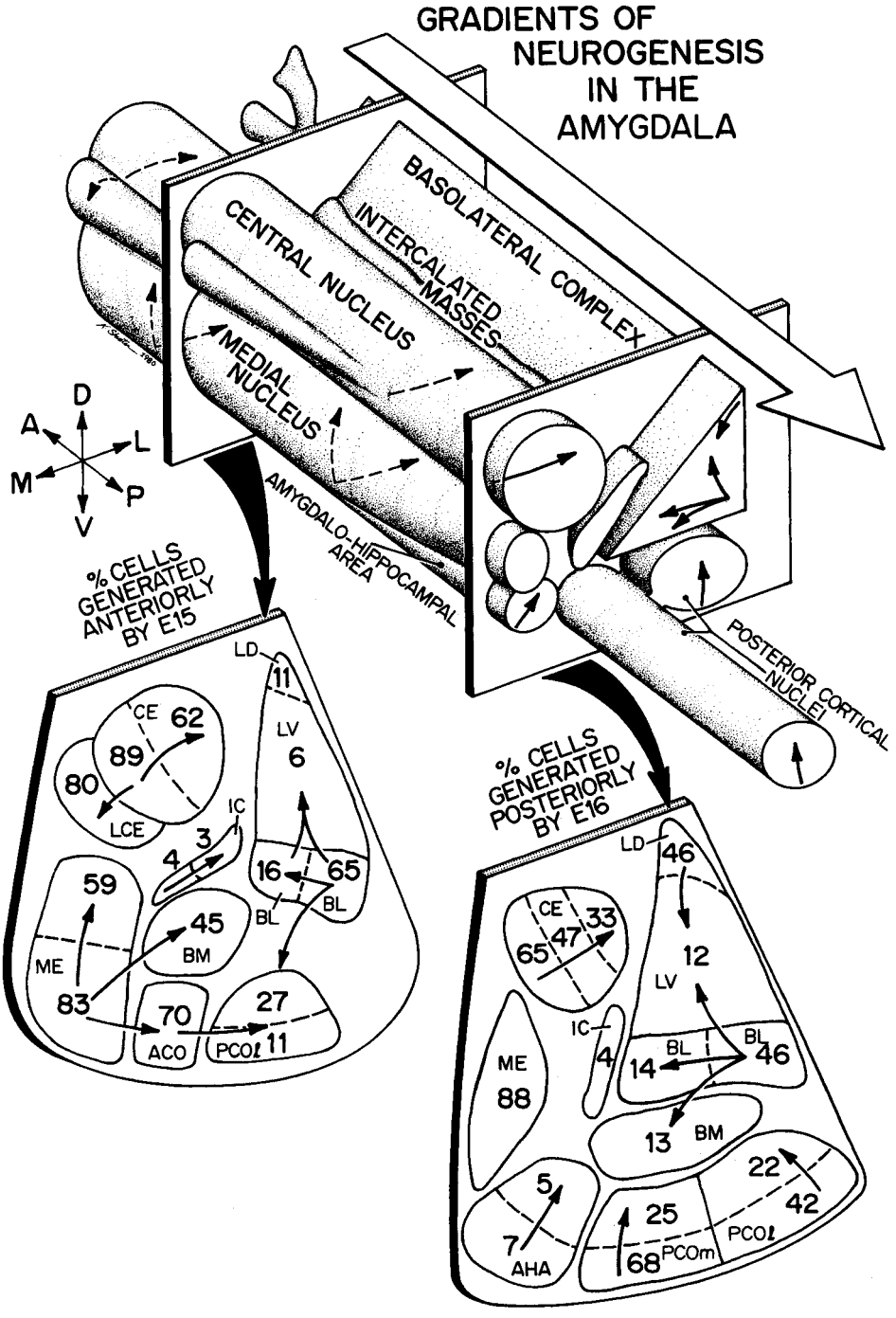


Fig. 22. A three-dimensional diagrammatic view of the amygdala with anterior and posterior cross sections. All arrows represent gradients of neurogenesis. See text for discussion.

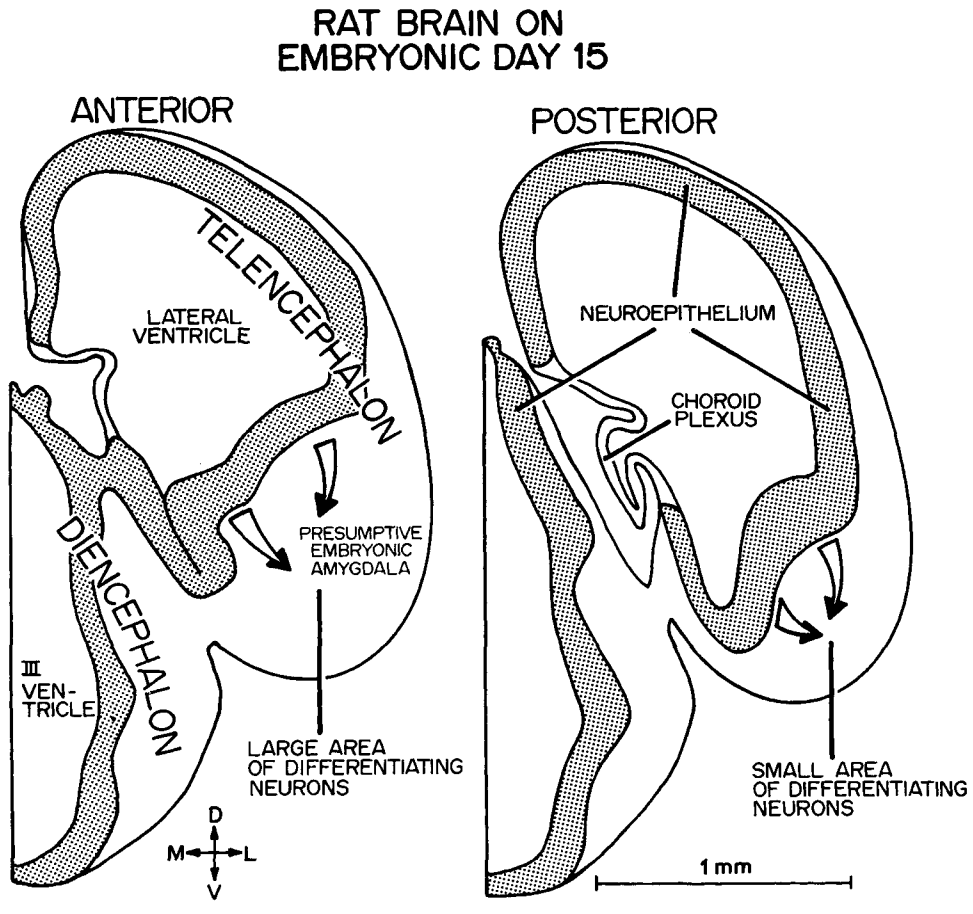


Fig. 23. Drawings (traced with the aid of a Zeiss microprojector) of the rat brain on E15 at anterior and posterior cross sections of the presumptive primordial amygdala. Note there is evidence for a rostral-to-caudal gradient of cell differentiation in accordance with the same strong neurogenetic gradient. See text for further discussion.

grounds, then, the anterior basomedial, anterior cortical, and ventral part of the medial nucleus should be considered a single entity.

The *posterolateral cortical nucleus* would seem to be more correctly referred to as a nucleus rather than as "periamygdaloid cortex" (Krettek and Price, '78b), even though it has the most distinct cortical structure in the amygdala. Typically, cerebral cortical structures have a deep-to-superficial gradient (Angevine, '65; Bayer, '80; and others), including the piriform cortex (Bayer, unpublished). In contrast, the posterolateral cortical nucleus at the anterior level has no depth gradient (similar to the anterior cortical nucleus), and at its posterior level, has a superficial-to-deep gradient (similar to the posteromedial cortical nucleus).

Neurogenesis in the *amygdalo-hippocampal area* has a span of development (E16–E19) similar to many neurons in Ammon's horn and the prosubiculum (Bayer, '80), while it has a pattern of development (superficial to deep) similar to that typically found in the amygdala. Thus, it appears to be a transition area, as Krettek and Price ('78b) have suggested, rather than a caudal part of the medial nucleus (Johnston, '23; Young, '36; Fox, '40; Brodal, '47). There is a neuroepithelial bridge between the medial amygdaloid and ventral hippocampal primordia (Johnston, '23; Bayer, unpublished), which may give rise to the

amygdalo-hippocampal area.

Correlations between anatomical connections and the patterns of neurogenesis.

The olfactory bulb has long been known to provide a prominent input to the corticomedial complex of the amygdala and the nucleus of the lateral olfactory tract (LeGros Clark and Meyer, '47; Powell et al., '65; White, '65; Girgis and Goldby, '67; Heimer, '68, '69; Price, '73). More recently, projections of the main vs. accessory olfactory bulbs were found to differentially terminate in nonoverlapping parts of the amygdala. The main bulb terminates in the nucleus of the lateral olfactory tract and anterior cortical and posterolateral cortical nuclei (Scalia and Winans, '75; Broadwell, '75; Skeen and Hall, '77; Rosene and Heimer, '77; Turner et al., '78). The accessory olfactory bulb terminates in the nucleus of the accessory olfactory tract and medial and posteromedial cortical nuclei (Winans and Scalia, '70; Scalia and Winans, '75; Broadwell, '75; Devor, '76; Skeen and Hall, '77). Note that both the main and accessory bulbs project to a rostrally placed, early originating component (anterior cortical and medial nuclei, respectively), and to a caudally placed, late-originating component (posterolateral and posteromedial cortical nuclei, respectively). The strong rostral-to-caudal gradient of neurogenesis throughout the corticomedial complex may be related to the olfactory input, since the olfactory fibers growing toward the amygdala would arrive earlier in the anterior parts (being closer to the target), than in the posterior parts. There may also be a correlation with the differential olfactory projections to, and sequential neurogenesis of, the bed nuclei of the accessory and lateral olfactory tracts. Hinds ('67) reported that the mitral cells of the accessory olfactory bulb originate before those in the main bulb in the mouse; the same pattern has been noted in the rat (Bayer, in preparation). Thus, the earlier-originating accessory olfactory bulb mitral cells project to the earlier-originating neurons in the nucleus of the accessory olfactory tract, while the later-originating main bulb mitral cells project to the later-originating neurons in the nucleus of the lateral olfactory tract.

Recently, topographic connections of various amygdaloid nuclei have been under investigation. In many cases, the subdivisions of amygdaloid nuclei based on neurogenetic gradients match their subdivisions based on patterns of anatomical connections. With respect to the combined medial-to-lateral and anterior-to-posterior neurogenetic gradients in the central nucleus, neurons in the medial vs. lateral parts of the central nucleus have different dendritic (Hall, '72) and ultrastructural (Wakefield and Hall, '74a) characteristics. There are projections from the basolateral amygdaloid nucleus (Krettek and Price, '78b), anterior hypothalamus (Wakefield and Hall, '74b; Veening, '78b), and solitary nucleus (Ricardo and Koh, '78) to the medial part of the central nucleus, while the lateral parts receives projections from the lateral amygdaloid nucleus (Krettek and Price, '78b) and parafascicular thalamic nucleus (Ottersen and Ben-Ari, '79). There are also anatomical connections of the central nucleus organized in an anteroposterior format. The piriform cortex (Veening, '78a) and dorsal raphe nucleus (Veening, '78b) project more heavily to the anterior part of the central nucleus, while the anterior central projects more heavily to the hypothalamus and central gray (Hopkins and Holstege, '78). The entorhinal cortex has been reported to project to the posterior part of the central nucleus (Veening, '78a). With regard to the ventral-to-dorsal neurogenetic gradient in the medial nucleus, the reciprocal connections between the medial nucleus and the ventromedial bypothalamic nucleus more heavily involve the dorsal part of the medial nucleus (McBride and Sutin, '77; Veening, '78b). Inputs from the paraventricular, parafascicular, and medial ventrobasal complex in the thalamus terminate primarily in the dorsal part of the medial nucleus (Veening, '78b; Ottersen and Ben-Ari, '79), while the ventral part of the medial nucleus is the main source of the projection to the dorsomedial thalamic nucleus (Siegel et al., '77). Finally, anterior and posterior parts of the basolateral nucleus have both differential times of neurogenesis and differential projections to the dorsomedial thalamic nucleus (Krettek and Price, '77) and to the nucleus accumbens (Krettek and Price, '78a). Thus, the chronology of neurogenesis in the amygdala can be matched with some patterns of anatomical connections between the amygdala and the limbic and olfactory systems. It is hoped that further studies of the chronology of neurogenesis in the olfactory system and basal telencephalon (Bayer, in preparation) and additional studies of the topographic anatomical connections of individual amygdaloid nuclei will contribute to a clearer understanding of the role the amygdala plays in the rhinencephalon.

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