The Development of the Septal Region in the Rat

II. MORPHOGENESIS IN NORMAL AND X-IRRADIATED EMBRYOS

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ABSTRACT Morphogenesis of the septal region was examined in normal rat embryos from embryonic day (E) 10 to E22. The greater part of the septal region is postulated to form from two separate anlagen which can be clearly distinguished in the telencephalon by E13 and E14. One lies in the anterior ventromedial wall and presumably forms the nucleus of the diagonal band, medial, lateral, and triangular septal nuclei. The other lies in the posterior ventrolateral ridge and presumably forms the bed nuclei of the stria terminalis and the anterior commissure. On E15, the early differentiating cells in these anlagen fuse in the same region where the anterior commissure will cross on E17. With later embryonic development, differentiating cells of the strial bed nucleus accumulate rostral and caudal to the fused area. The same pattern is found in the medial and triangular septal nuclei and in the nucleus of the diagonal band. The differentiating cells of the lateral septal nucleus accumulate dorsal and lateral to the medial and triangular septal nuclei. On E16 and E17, a prominent subependymal zone develops in the anterior septal region and presumably gives rise to the nucleus accumbens.

A quantitative analysis was made of three cell zones (neuroepithelium, subependymal zone, differentiating cell zone) at coronal levels through the developing nucleus accumbens and the nucleus of the diagonal band (anterior level) and the medial and lateral septal nuclei (middle and posterior levels). At all levels, the area of the neuroepithelium continually declines, that of the differentiating cell zone continually increases, and that of the subependymal zone shows a rise and decline. On a proportional basis, both the neuroepithelium and subependymal zone occupy significantly more area anteriorly than posteriorly, while the differentiating cell zone shows the reverse gradient. To accurately locate regions of primitive mitotic and migratory cells within the zones at each level, the number of cells surviving a single exposure to 200 R X-rays in embryonic brains (E15-E22) were compared with controls. Each zone responded differently to X-ray insult. The radiosensitivity of the neuroepithelium decreases significantly after E19; the subependymal zone is highly radiosensitive throughout; the differentiating cell zone is radioresistant throughout. The significance of these findings is discussed in the light of the autoradiographic determination of the time of formation of septal neurons (Bayer, '79).

Little is known about the embryonic development of the septal region. It has been only briefly described in a few earlier studies of the developing telencephalon in several vertebrates (Johnston, '13, '23) and in man (Hines, '22; Macchi, '51). This may be because neuroembryological studies are difficult to interpret when they are based on descriptions of normal material alone; cell groups accumulating within the embryonic brain often bear little resemblance to their adult appearance and location. Since a given nuclear group cannot be seen until after neuron formation has substantially begun and the young neuroblasts leave the germinal zone to differentiate, knowing the time when neuronal populations

are formed can help "predict" the appearance of a differentiating cell group. The first paper of the series (Bayer, '79) gave a detailed chronology of the gradients of neurogenesis in the rat septal region based on ^{3}H-thymidine autoradiography. The present study applies that information to the interpretation of morphogenetic changes in the rat embryonic septal region. However, cells lying outside the neuroepithelium may not only be differentiating, but may also be either migrating to another location or establishing a secondary germinal source of neurons and/or glia. Low-level X-irradiation (200 R) has been used to locate primitive migratory and mitotic cells in the developing nervous system (Altman et al., '68; Altman and Nicholson, '71; Bayer and Altman, '74). If animals are allowed to survive for only a short time after exposure (6 hours), the pyknotic fragments of the cells destroyed by the irradiation are still present and their positions indicate regions of cell proliferation and/or migration. In this study X-irradiation is used to quantify the degree of immaturity of various cell layers throughout a major portion of the developing septal region.

MATERIALS AND METHODS

Embryos from Purdue-Wistar pregnant females were used; the day of sperm-positivity was day one of embryonic life or E1. To accurately locate regions of immature cells in the embryonic brain, two or more pregnant females were exposed to a single dose of 200 R X-rays from a GE Maxitron unit (300 kVp; half value layer, 2.4 mm copper) between 9 and 10:30 A.M. on each embryonic day from E10 through E22. The embryos were removed six hours after the exposure. At the same time, other groups of embryos at the same ages were removed from undisturbed pregnant females. Either the entire embryo (E10-E12) or the head (E13-E22) was kept in Bouin's fixative for 24 hours, then transferred to 10% neutral formalin. The brains of embryos from E16 through E22 were dissected from the head with the aid of an American Optical stereomicroscope before they, as well as the younger embryos, were embedded in paraffin. Serial sections (6 μm) were prepared in the three planes. One set of sections was stained with cresyl-violet, the other with hematoxylin and eosin. Table 1 gives a complete list of the brains available for analysis.

To qualitatively analyze the development of the septum three dimensionally in both normal and experimental groups, a series of photomicrographs were prepared of representative brains from E13-E22 in the coronal, sagittal, and horizontal planes. The photographs were taken at regular intervals and were mounted serially in strips. The individual strips were used to simultaneously view morphological changes within the septum at various levels for each age. Two or more ages were simultaneously compared at homologous levels when several strips were used.

TABLE 1

Brains available for analysis

Age	Controls			X-ray		
	Coronal	Horizontal	Sagittal	Coronal	Horizontal	Sagittal
E10	11			17		
E11	8			29		
E12	3			9		
E13	3	1	2	5	3	3
E14	3	2	1	4	4	2
E15	6	1	2	2	5	5
E16	6	2	1	5	2	2
E17	8	3	3	6	4	1
E18	4	1	1	6	2	2
E19	6	1	2	6	2	2
E20	6	2	2	6	3	3
E21	8	2	3	5	2	0
E22	6	5	5	6	4	0

Developmental changes within the septal region were quantitatively analyzed from E15 through E22. Homologous sections at anterior, intermediate, and posterior levels through the septal region were selected for each normal and experimental brain cut in the coronal plane (table 1). A strip of tissue (25 μm wide), extending from ventricular to pial surfaces (see dashed lines in fig. 3), was first divided into zones (neuroepithelium, NE; subependymal zone, SE; differentiating cell zone, DC). The relative area occupied by each zone was determined for each strip, and the number of cells lying at least 50% within the strip was counted separately for each zone. Strips from experimental brains were subdivided in the same way, and surviving (non-pyknotic) cells were counted (figs. 4-7). Age and treatment differences in cell density were examined with an analysis of variance; the Scheffé test was used to locate significant differences between group means. Proportional differences between anterior, intermediate, and posterior levels in each zone were statistically analyzed on paired samples from individual animals by the sign test (Conover, '71).

RESULTS

The position of the septal anlagen in the forebrain

The embryonic head contains a neural plate on E11; by E12 the prosencephalon forms, and the telencephalic vesicles are first observed on E13. The septal anlagen can be clearly recognized by E14. Figure 1 shows selected sections in three planes from E13 (A, B, C) and E14 (D, E, F) embryos. By comparing anatomical changes between these ages, both the sources of the septal anlagen and the settling patterns of septal neurons can be more easily understood.

Large ventricular areas are a prominent feature of the early forebrain. The third ventricle (III) represents the most cephalic extension of the neural tube. A wing-like evagination from its rostral border on E13 forms the lateral ventricle (LV: fig. 1B), which continues expanding on E14 (fig. 1E). The neuroepithelium (NE), tightly packed spindle-shaped cells oriented perpendicular to the ventricular surface, is the sole component of the brain wall on E13. Several accumulations of differentiating cells (DC), loosely packed and randomly oriented, are beneath the pia on E14. The telencephalon (tel) appears as a semicircular anterior pouch in midline sagittal sections (figs. 1A,D). Its dorsal edge is connected to the thalamus (th) by the choroid plexus (CP), which invaginates into the brain ventricles on E14. The ventral telencephalon is separated from the hypothalamus (hyp) by the anterior neuropore (AN). More laterally, the neuropore is continuous with the rostral wall of the optic recess. Rathke's pouch (RP) has already evaginated from the roof of the oropharynx by E13 to lie beneath the hypothalamus. In a ventrally-located horizontal section on E13 (fig. 1B, plane of section indicated in A), the telencephalic wall continues straight across the midline; at a similar level on E14 (fig. 1E, plane of section indicated in D), anterior growth of the telencephalon produces a medial wall. Horizontal sections also show a prominent ridge of neuroepithelium in the lateral telencephalon. By E14, the ridge extends further medially around a large differentiating cell zone. In contrast to the sharp separation between hypothalamic and telencephalic walls medially by the anterior neuropore, their lateral walls are continuous. The coronal sections (figs. 1C,F; planes of sections indicated in A and D) cut through the anterior wall of the evaginated telencephalon, which slopes away from the midline on E13. By E14 the entire medial wall is parallel to the midline, presumably due to telencephalic expansion. Differentiating cells are located basally on E14, extending from the lower half of the medial wall (along the floor) and ending in a thick zone along the basolateral edge.

On E14 the single anlage of the medial, lateral, and triangular septal nuclei and the nucleus of the diagonal band is along the anterior ventromedial wall of the telencephalon (dotted circles in fig. 1), in agreement with the observations of Johnston ('13), Hines ('22), and Macchi ('51). The lateral telencephalic ridge is called the anlage of the globus pallidus, caudate-putamen nuclei, and the amygdala in earlier studies (Johnston, '13, '23; Hines, '22; Humphrey, '72). The anlage of the bed nucleus of the stria terminalis (squares in fig. 1) is also located along the rostral edge of this ridge, in agreement with Johnston's ('23) earlier observation. As a result of neuroepithelial growth along both the ventrolateral ridge and in the expanding telencephalic vesicles, the two anlagen come into juxtaposition on either side of the lateral ventricle. Since ^{3}H-thymidine autoradiography indicates only a small proportion of cells originate in the septal region by E14 (Bayer, '79), the regions adjacent to these anlagen probably contain few differentiating cells destined for the adult septal region.

Development of the septal region nuclei

Midline and lateral nuclear groups

By E15, both horizontal (fig. 2) and coronal (fig. 3) sections contain a prominent zone of differentiating cells adjacent to the neuroepithelium lining the medial wall of the lateral ventricle. These cells are probably destined for the adult septal region, since many neurons in both the medial septal nucleus and the caudal nucleus of the diagonal band originate around E15 (Bayer, '79). From E17 on, the nucleus of the diagonal band (DB) can be recognized as a group of cells curving along the ventromedial edge of the brain in both anterior and intermediate coronal sections (figs. 3D,E,G,H,J,K). In both coronal and horizontal sections, the cells of the medial (MS) and lateral (LS) septal nuclei simply accumulate adjacent to the neuroepithelium. The large neurons characteristic of the adult medial septal nucleus are not distinguishable from the smaller neurons of the

lateral septal nucleus during embryonic life. The neuroepithelium forming the rostral wall of the foramen of Monro, the presumed source of the triangular septal nucleus, is adjacent to a very small group of differentiating cells on E15 (fig. 2B: ts). By E17, the differentiating cells (TS) are more definite and lie near early fornix fibers (fx). On E19, the lateral and medial portions of the triangular nucleus are distinct, and a raphe forms across the midline.

Throughout the septal region midline nuclear group, the zone of differentiating cells lengthens rostrocaudally between E15 and E17 (compare figs. 2A,B with C,D) and changes little in this dimension by E19. The differentiating cell zone becomes much wider with increasing age, reflecting a strong mediolateral formation gradient. Germinal matrices are thicker at anterior and intermediate coronal levels than at the posterior level. This indicates a caudorostral gradient of formation.

Ventrolateral nuclear group

On E15, the differentiating cells adjacent to the rostral tip of the ventrolateral telencephalic ridge fuse with those near the medial wall of the lateral ventricle (fig. 2A). A posterior coronal section (fig. 3C) shows the zone of fusion along the inferior horn of the lateral ventricle on the medial side of the ventrolateral ridge. At this stage, probably few differentiating cells are destined for the bed nucleus of the stria terminalis. By E17, the enlarged differentiating cell zone contains neurons which most likely will come to lie in the bed nucleus of the stria terminalis. Early fibers of the anterior commissure (ac: figs. 2C, 3F) are lateral to them, and some fibers may cross the midline. The anterior commissure definitely crosses the midline on E18, and becomes more prominent on E19 (fig. 2E). In horizontal sections of E19 brains, differentiating cells of the strial bed nucleus expand rostrally into the septum along the ventral lateral septal nucleus (fig. 2E) and posterodorsally along the internal capsule to lie in a wedge-shaped area between the thalamus and striatum (fig. 2F). In posterior coronal sections, differentiating cells medial to anterior commissural fibers accumulate from E17-E21 (BST; figs. 3F,I,L). The intermediate coronal levels from E19-E21 may contain some cells of the rostral bed nucleus of the stria terminalis, but they may also be caudal cells of the nucleus accumbens.

Anterior coronal levels from E17-E21 (figs. 3D,G,J) show the anlage of the nucleus accumbens and its early differentiating cells. This nucleus is a direct continuation of the rostral bed nucleus of the stria terminalis. There is a prominent subependymal zone (SE) in this area, containing mitotically active and tightly packed cells oriented randomly. The subependymal zone is smaller caudally where the strial bed nucleus arises, and becomes prominent late in septal region development (E17 to the time of birth) after the majority of the strial bed nucleus neurons are formed but simultaneous with the active period of neurogenesis in the nucleus accumbens (Bayer, '79). Some differentiating cells (VNA) lie beneath the subependymal zone on E21 (fig. 3J). The outlined dotted areas in both coronal and horizontal sections on E19 indicate a caudal extension of the subependymal zone, probably beyond the nucleus accumbens anlage. This germinal zone may be a source of late-forming neurons elsewhere, since over 90% of the neu-

Abbreviations

ADNA, anlage of the dorsal nucleus accumbens
ANA, anlage of the nucleus accumbens
ac, early anterior commissure
AC, anterior commissure
AH, anterior continuation of the hippocampus
AN, anterior neuropore
bst, early bed nucleus of the stria terminalis
BST, bed nucleus of the stria terminalis
CC, corpus callosum
cp, early choroid plexus
CP, choroid plexus
db, early diagonal band nucleus
DB, diagonal band nucleus
DC, differentiating cell zone
FM, foramen of Monro
fx, early fornix
FX, fornix
hc, early hippocampal commissure
HC, hippocampal commissure
hyp, early hypothalamus
IC, internal capsule
LV, lateral ventricle
LS, lateral septal nucleus
MS, medial septal nucleus
NE, neuroepithelium
RP, Rathke's pouch
SE, subependymal zone
SM, stria medullaris
ts, early triangular septal nucleus
TS, triangular septal nucleus
tel, early telencephalon
th, early thalamus
VNA, ventral nucleus accumbens
III, third ventricle

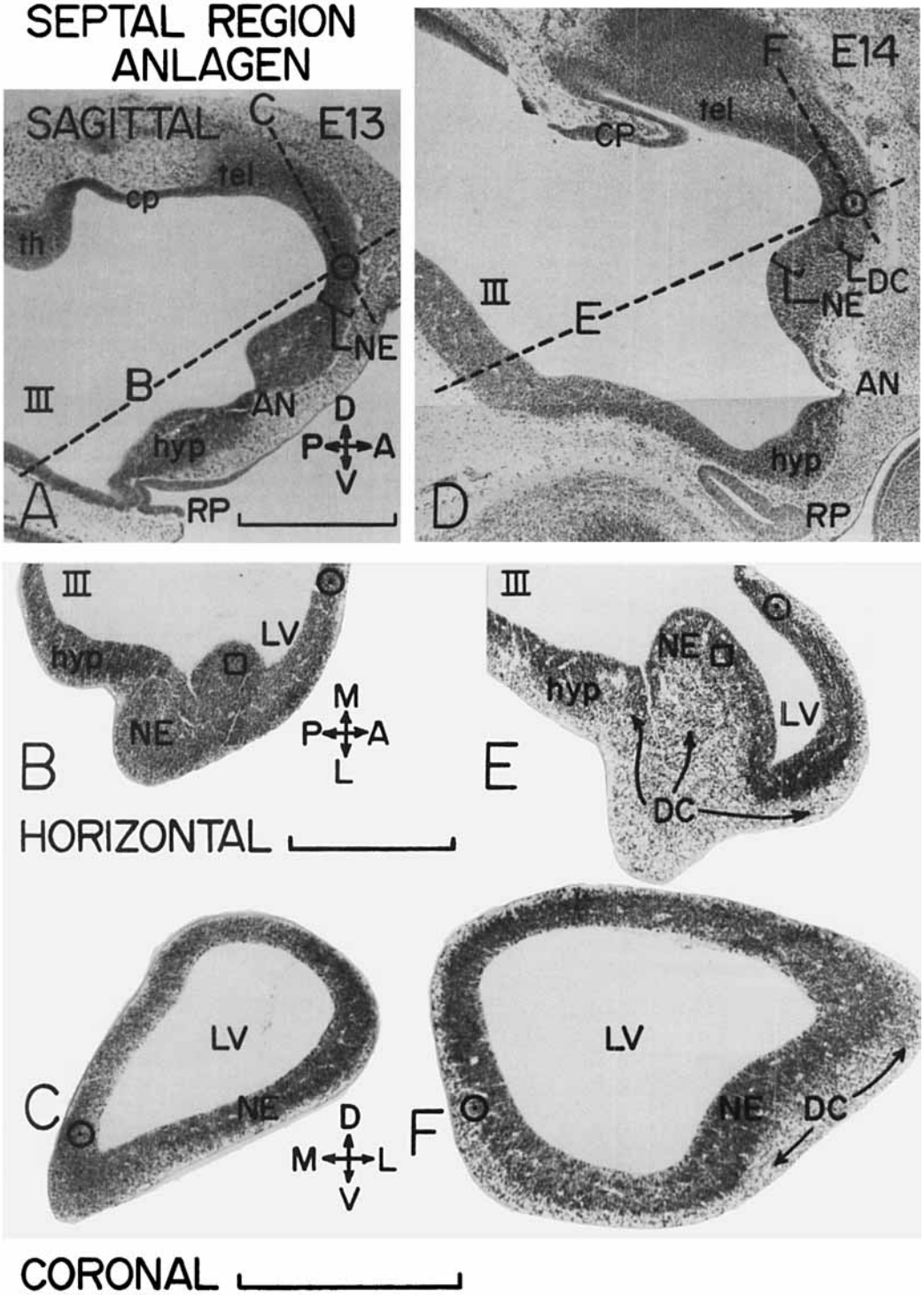


Fig. 1 Brains of E13 (A, B, C) and E14 (D, E, F) fetuses in sagittal (A, D), horizontal (B, E), and coronal (C, F) planes. Directional arrows near E13 sections give orientation: A, anterior; P, posterior; M, medial; L, lateral; D, dorsal; V, ventral. Dotted circle in each section indicates presumptive anlage of nucleus of the diagonal band, medial, lateral, and triangular septal nuclei. Square in horizontal sections indicates the presumptive anlage of the bed nucleus of the stria terminalis. Further description in text. Hematoxylin-eosin. Bars, 0.5 mm.

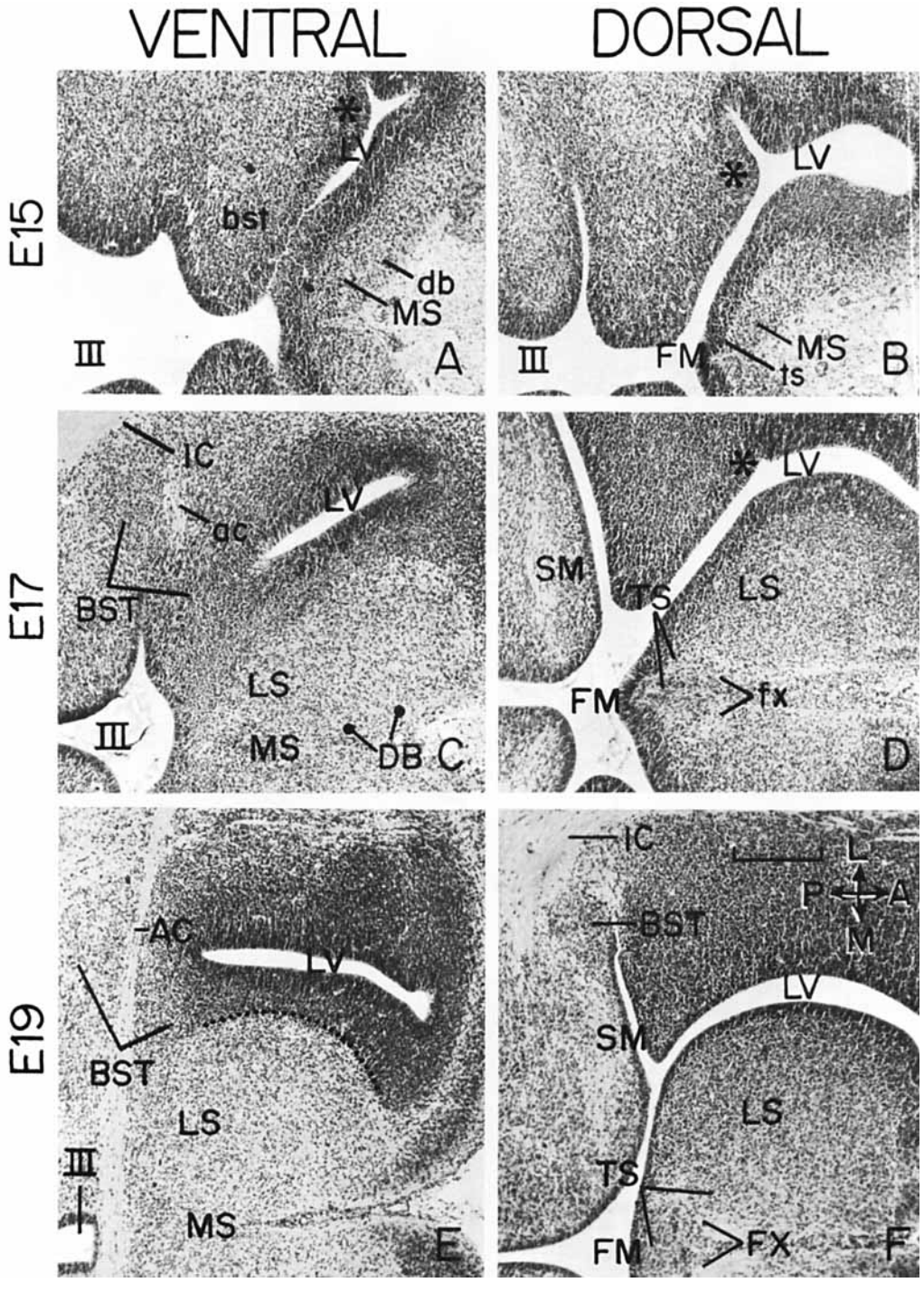


Fig. 2 Homologous horizontal sections from fetal brains: E15 (A, B), E17 (C, D), and E19 (E, F) through developing septal region at ventral (A, C, E) and dorsal (B, D, F) levels. Asterisk in A, B, and D indicates part of the ventrolateral telencephalic ridge also shown in coronal sections (fig. 3). Directional arrows in F give orientation for all sections. Hematoxylin-eosin. Bar, 0.25 mm.

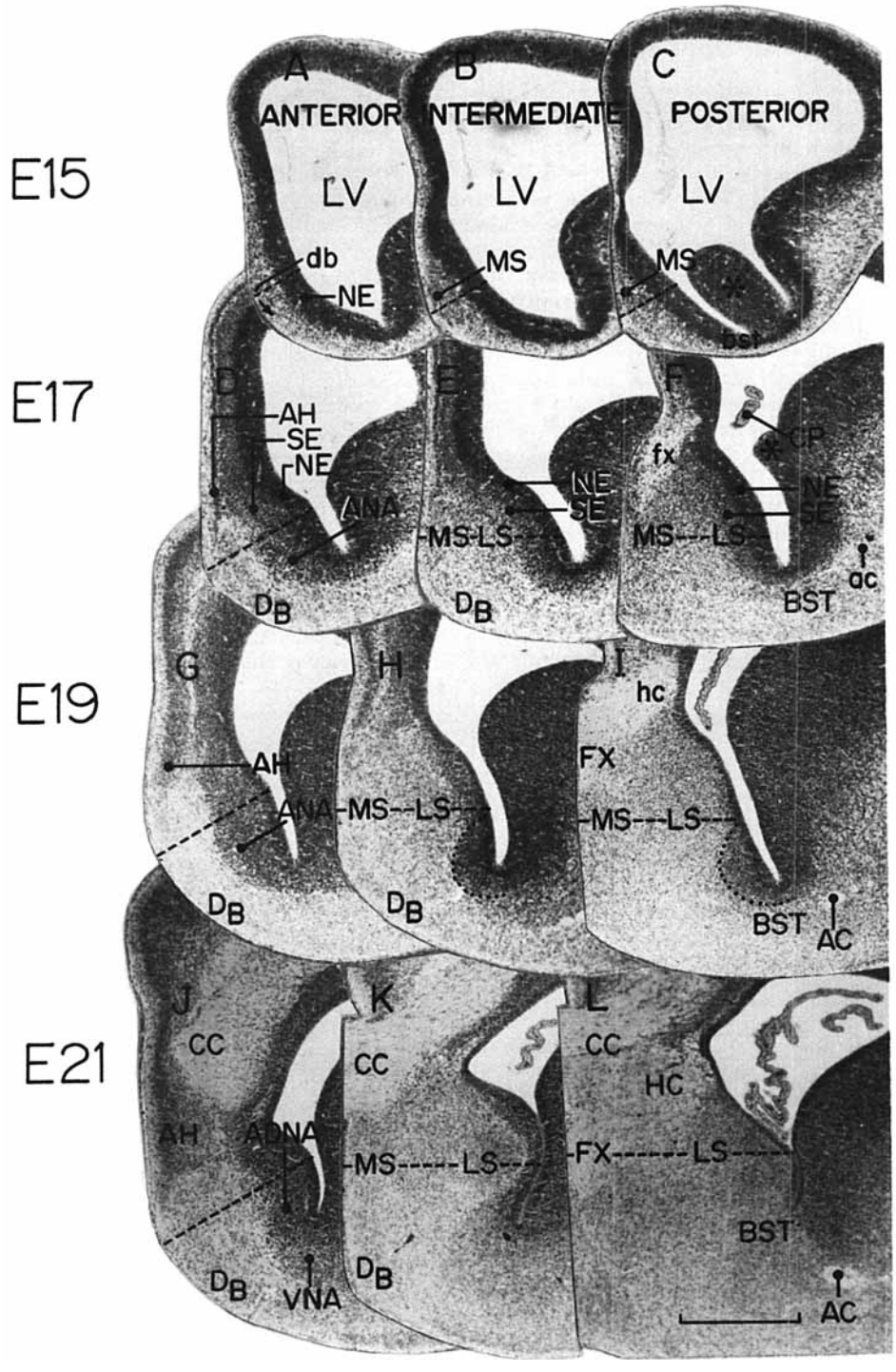


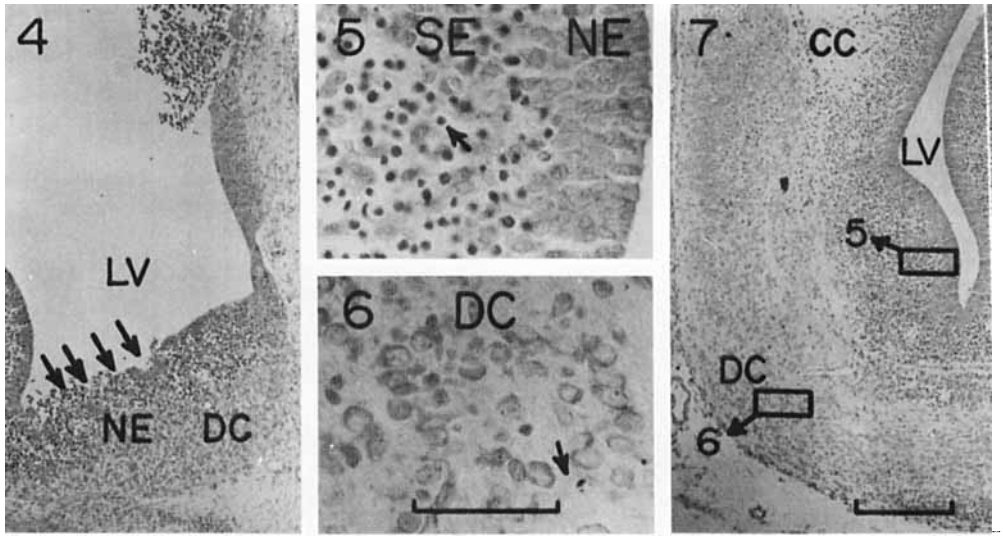
Fig. 3 Homologous coronal sections from fetal brains: E15 (A-C), E17 (D-F), E19 (G-I), and E21 (J-L) through precommissural septal region at anterior (A, D, G, J), intermediate (B, E, H, K), and posterior (C, F, I, L) levels. Top of section is dorsal. Midline is along left margin. Asterisk in C and F indicates part of the ventrolateral telencephalic ridge also shown in horizontal sections (fig. 2). Dashed lines in each section indicate location of cell strips quantified in figures 8-10. Hematoxylin-eosin. Bar, 0.5 mm.

rons in the septal region have formed by E19 (Bayer, '79).

Quantitative analysis

Areas of more mature cells within both the neuroepithelium and subependymal zone, or conversely, pockets of immature cells within the differentiating cell zone, are indistinguishable in normal material. Since mitotic and primitive migratory cells in the immature rat brain are killed by a relatively low X-ray exposure (200 R) that spares differentiating cells (Altman et al., '68; Altman and Nicholson, '71; Bayer and Altman, '74), X-irradiation was used to locate more accurately regions of immature cells in the septum. Figures 4-7 show the embryonic septal region on E15 and E21 six hours after a single exposure to 200 R X-irradiation. Small dark particles, the pyknotic remains of cells killed by the X-rays, are scattered throughout the neuroepithelium on E15 (fig. 4) and spilled into the ventricle (grouped arrows in fig. 4). On E21 (fig. 7), pyknotic fragments are scattered throughout the subependymal zone, but few are present in the neuroepithelium (fig. 5). On both E15 and E21, few pyknotic fragments are noted in the zone of differentiating cells.

The degree of maturity of each zone was quantified by counting the number of cells surviving X-irradiation exposure in the three coronal levels shown in figure 3 (MATERIALS AND METHODS). Coronal sections of the precommissural septal region were chosen, because the developing medial and lateral septal nuclei (intermediate and posterior levels, fig. 3) and the nucleus of the diagonal band and nucleus accumbens (anterior level, fig. 3) occupied the entire medial wall of the brain from the lateral ventricle to the midline. Few fiber tracts develop within these areas to change the position of the differentiating cells. The anterior level section was the first to show the anterior continuation of the hippocampus, which extended ventrally into a widening differentiating cell zone. The middle level section was the first one after the disappearance of the anterior continuation of the hippocampus within the medial septal region. The posterior level was the middle section of the group of sections which contained fibers of the fornix. Since the above distinguishing fea-



Figs. 4-7 Developing septal region in rat brains exposed to one dose of 200 R X-rays six hours before fixation. Cresyl violet. Bars, 0.5 mm (figs. 4, 7) and 50 μm (figs. 5, 6).

4 E15 fetal brain. Grouped arrows indicate regions where pyknotic fragments are spilled into lateral ventricle.

5 Subependymal (SE) and neuroepithelial (NE) cell layers on E21 taken from area designated, figure 7. Arrow indicates a pyknotic fragment in subependymal zone, where these were numerous. The neuroepithelium has no pyknotic material.

6 Differentiating cell zone (DC) on E21 taken from area designated, figure 7. Arrow indicates a pyknotic fragment.

7 E21 fetal brain showing locations, figures 5, 6.

tures of selection are not present on E15 and indistinct on E16, sections at these ages were the first, middle, and last of the series of sections through the thickened differentiating cell zone along the ventromedial brain wall, which was also attached to the dorsomedial brain wall (sections A-C, fig. 3). The developing bed nucleus of the stria terminalis is difficult to quantify because of the indistinct ventral boundary with the basal telencephalon (figs. 3F,I,L); similarly, penetrating fibers of both the fornix and hippocampal commissure make quantitative analysis of the development of the triangular septal nucleus difficult.

The relative areas taken up by each zone during development are shown in the A sections of figures 8-10. At each level, the area of the neuroepithelium decreases as the area of the differentiating cells increases. Each level also shows a transitory subependymal zone, much more prominent anteriorly, that first increases and then declines. The areal changes of each zone during development were statistically analyzed on paired samples from individual animals in the sign test (Conover, '71). Although the relative area occupied by the neuroepithelium appears quite similar from anterior through posterior levels, there is a highly significant trend for the neuroepithelium to occupy proportionally more area anteriorly (all comparisons, $p < 0.00001$). The subependymal zone follows the same pattern as the neuroepithelium; proportionally more area is occupied anteriorly (all comparisons, $p < 0.00001$). The differentiating cell zone shows the reverse gradient; a significantly larger proportional area is present posteriorly (all comparisons, $p < 0.00001$).

The effects of X-irradiation in the three cell zones are shown in the B, C, and D sections of figures 8-10. Table 2 lists the F ratios from an analysis of variance between control and experimental groups; the Scheffé test was used to locate significant differences between group means. X-irradiation has similar effects from anterior through posterior levels, but each cell zone responds differently to X-ray insult. There is a significant interaction between age and the effect of X-irradiation in the neuroepithelium (table 2; figs. 8-10B). X-irradiation significantly ($p < 0.01$) reduces cells per unit area up to E19 throughout; from E20-E22, there are no significant differences in packing density between the control and X-irradiated groups at the anterior and posterior levels. The X-irradiated group remained significantly ($p < 0.01$) below controls at the middle level, but the bar graph shows the same trend as at the other levels; a smaller proportion of cells are removed on E22 (28%) as compared to E15 (60%). In the control group, packing density remains the same with increasing age at both anterior and middle levels, but drops significantly ($p < 0.01$) between E16 and E22 posteriorly. The experimental groups show a significant increase ($p < 0.05$) in packing density from E15 to E20 at all levels, reflecting the reduced effect of X-irradiation. In contrast to the neuroepithelium, the subependymal zone (figs. 8-10C) is always radiosensitive, as indicated by the X-ray main effect F ratios listed in table 2. The X-irradiated groups are significantly ($p < 0.01$) below controls throughout. The bar graphs show that radiosensitivity slightly increases later, with a peak proportion (approximately 80%) of cells killed on E20 at all levels. There are no significant changes in packing density. Differentiating cells in the middle and posterior levels (D: figs. 9, 10) are not radiosensitive during development, (see X-ray main effect F ratios in table 2), while those at the anterior level (D: fig. 8) show a significant early X-ray effect (between E16-E18, $p < 0.05$). Packing density decreases with age in the control group (all levels, between E16 and E22, $p < 0.05$). Although the irradiated groups follow the same tendency, it is not significant.

DISCUSSION

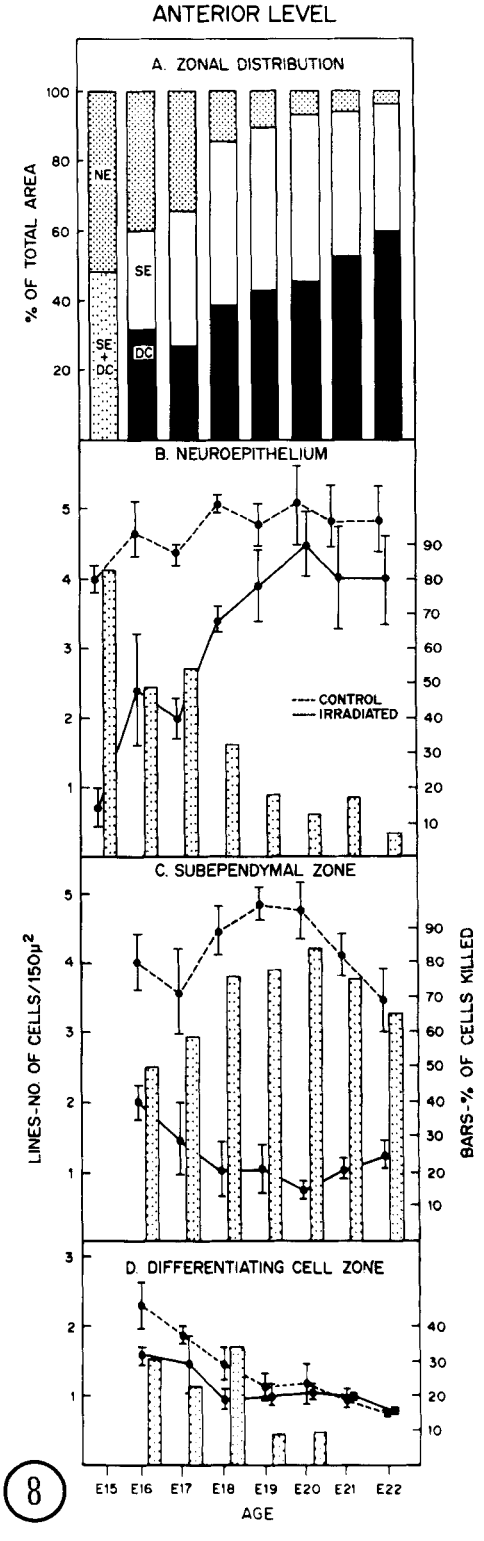
The pattern of cell accumulation in the septal region

The two anlagen that will form the septal region lie at some distance from each other within the E13 telencephalon. That of the midline nuclear group and the lateral septal nucleus lies anteriorly within the ventromedial wall; that of the bed nucleus of the stria terminalis is found more posteriorly along the medial wall of a prominent ridge of neuroepithelium extending longitudinally along the floor of the telencephalon. The identification of each of these anlagen is in agreement with earlier embryonic studies of the developing telencephalon (Johnston, '13, '23; Hines, '22; Macchi, '51). By E14 the two anlagen are closer together and on E15, the areas are beginning to coalesce. This union sets the stage for the development of the septal region nuclei where, during the rest of embryonic life and in the adult, there are indis-

tinct boundaries (in 6-μm sections) between the bed nucleus of the stria terminalis and the medial and lateral septal nuclei.

The region where the differentiating cell zones from each anlage first intermingle is interesting from two standpoints. First, the earliest-forming neurons in both the medial septal nucleus and the bed nucleus of the stria terminalis are also located in this area (Bayer, '79). Second, the fusion occurs approximately two days before (E15) and in the same area as the crossing of the anterior commissure. By E17, a few fibers of the anterior commissure either approach or cross the midline. Thus, the early forming neurons may set up a "tissue bridge" for the commissure. Johnston ('23) observed a zone of tissue across the midline in embryos before the crossing of the anterior commissure.

In both the midline nuclear group and bed nucleus of the stria terminalis, the zone of differentiating cells expands both rostrally and caudally from the early-forming zone of coalescence. The more caudal differentiating cells also accumulate dorsally along the thalamus (see figs. 2D,F: TS, BST). On the other hand, the differentiating cells in the lateral septal nucleus accumulate mediolaterally along the length of the medial and triangular septal nuclei. Finally, on E21 some differentiating cells of the ventral nucleus accumbens accumulate beneath the subependymal zone; the more dorsal differentiating cells cannot be seen until after birth. These patterns of cell accumulation correspond to the formation gradients found in the respective septal region



Figs. 8-10 A, The zonal distribution of neuroepithelium (NE), subependymal (SE), and differentiating cell (DC) zones along strips of tissue through developing septal region (dashed lines, fig. 3). Areal data was combined for both control and irradiated groups. B, Line graphs indicate neuroepithelial packing density in control (dashed line) and irradiated groups (solid line). Each mean with standard deviation is based on control and irradiated brains cut in coronal plane (see table 1 for number in each group). Bars indicate percentage of cells killed by X-rays as determined below:

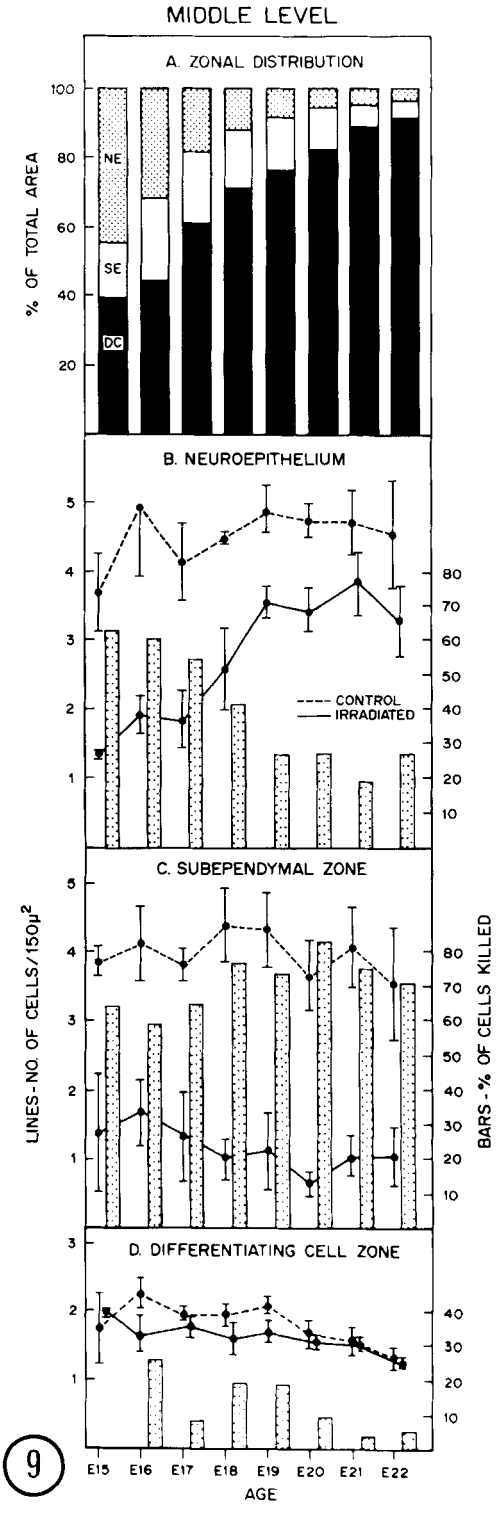
$$\frac{\text{cells}/150\ \mu\text{m}^2\ \text{(controls)} - \text{cells}/150\ \mu\text{m}^2\ \text{(irradiated)}}{\text{cells}/150\ \mu\text{m}^2\ \text{(controls)}} \times 100$$

C, as in B for subependymal zone. D, as in B for differentiating cell zone.

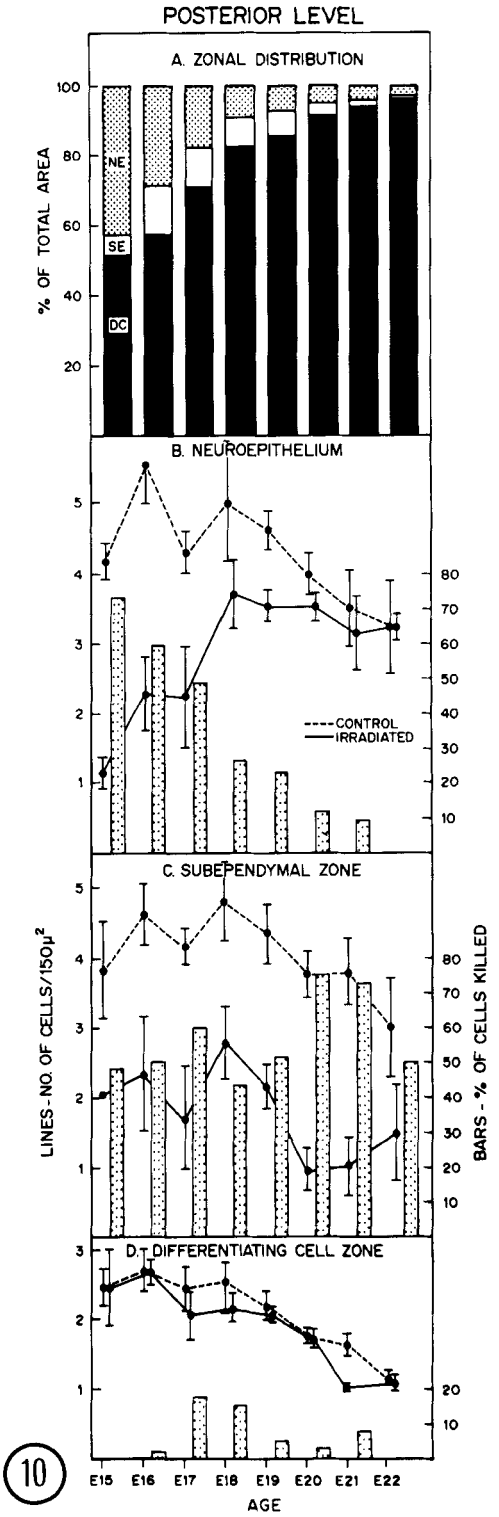
8 Anterior level sections (dashed lines—A, D, G, and J: fig. 3). Subependymal and differentiating cell zones cannot be separated on E15.

9 Middle level sections (dashed lines—B, E, H, and K: fig. 3).

10 Posterior level sections (dashed lines—C, F, I, and L: fig. 3).


MIDDLE LEVEL
A. ZONAL DISTRIBUTION
% OF TOTAL AREA
NE
SE
DC
B. NEUROEPITHELIUM
CONTROL
IRRADIATED
C. SUBEPENDYMAL ZONE
LINES - NO. OF CELLS/150μ²
BARS - % OF CELLS KILLED
D. DIFFERENTIATING CELL ZONE
E15 E16 E17 E18 E19 E20 E21 E22
AGE

9


POSTERIOR LEVEL
A. ZONAL DISTRIBUTION
% OF TOTAL AREA
NE
SE
DC
B. NEUROEPITHELIUM
CONTROL
IRRADIATED
C. SUBEPENDYMAL ZONE
LINES - NO. OF CELLS/150μ²
BARS - % OF CELLS KILLED
D. DIFFERENTIATING CELL ZONE
E15 E16 E17 E18 E19 E20 E21 E22
AGE

10

TABLE 2

F ratios from an analysis of variance between control and X-irradiated groups [1]

	Degrees of freedom	Main effects		Interaction
		Age effect	X-irradiation effect	
Neuroepithelium				
Anterior	7,1	10.98 [2]	62.44 [2]	6.55 [2]
Middle	7,1	12.13 [2]	238.17 [2]	5.35 [2]
Posterior	7,1	9.26 [2]	107.48 [2]	13.78 [2]
Subependymal zone				
Anterior	6,1	2.00	276.93 [2]	2.34
Middle	7,1	0.699	183.98 [2]	1.4
Posterior	7,1	1.405	55.15 [2]	0.357
Differentiating cell zone				
Anterior	6,1	41.5 [3]	18.95 [2]	4.51 [3]
Middle	7,1	2.44 [2]	0.785	1.04
Posterior	7,1	6.23 [2]	0.006	0.976

[1] Data of the line graphs, figures 8-10.
[2] $p < 0.001$.
[3] $p < 0.05$.

nuclei with ^{3}H-thymidine autoradiography (Bayer, '79).

Implications of the quantitative analysis

The neuroepithelium

The quantitative analysis of the septal region (exclusive of the bed nucleus of the stria terminalis and the triangular nucleus) also supports the pattern of neurogenesis observed with ^{3}H-thymidine autoradiography in this region. At all levels, the radioresistance of the neuroepithelium rises sharply between E18 and E19, and by E20 is close to control levels (figs. 8-10B). This also corresponds to the time when the last neurons (nucleus accumbens excepted) are formed in the septal region (Bayer, '79). The neuroepithelium surrounding the lateral ventricle after E20 remains a germinal matrix, as indicated by the presence of mitotic figures and the incorporation of ^{3}H-thymidine in a short-survival autoradiography study (Altman et al., '68). Most likely, the germinal cells are producing a different product. During the autoradiographic study of septal neurogenesis (Bayer, '79), it was noted that the ependymal cells lining the lateral ventricle continued to be labelled up to the time of birth in some regions. Altman and his coworkers (Altman et al., '68) found ependymal cells labelled postnatally in the anterior septal region. Thus from E20 on, the neuroepithelium might be more correctly referred to as the "primitive ependyma." Since the ependymal cells do not migrate, but remain in situ, the cells producing them are a stationary germinal zone. Such zones are much more radioresistant, and few cells are killed by 200 R X-rays (the data of figures 8-10B, and Altman et al., '68). The neuroepithelium, then, gives rise to three cell types (possibly overlapping in the time of their production). First, it produces neurons (those in the medial and lateral septal nuclei at the middle and posterior levels, those of the nucleus of the diagonal band at the anterior level). Early in development (up to E15), the pyknotic fragments of the septal neuroepithelium are spilled into the ventricle after irradiation (fig. 4). This may correspond to a time when the neuroepithelium is producing only a neuronal product. Second, it produces subependymal cells; third, the ependymal cells.

The subependymal zone

There is one neuronal group in the septal region which is probably not directly produced by a neuroepithelium. The peak time of neurogenesis in the nucleus accumbens (E19-E21, Bayer, '79) occurs after the neuroepithelium changes into the primitive ependyma but simultaneous with the prominent increase in the subependymal zone at the anterior level; differentiating cells appear below this zone on E21. These observations implicate the anterior subependymal zone as the source of the nucleus accumbens. The subependymal zone merits further consideration, since it is also present (although small) at both middle and posterior levels (figs. 9, 10A). Immature neurons destined for the medial and lateral septal nuclei may resemble primitive mitotic cells as they move out from the neuroepithelium,

thereby contributing to the tissue that was classified as "subependymal." However, three observations support the conclusion that there is a true subependymal germinal matrix at middle and posterior levels. First, mitotic figures are commonly seen here up to the time of birth. Second, the zone is always highly radiosensitive (figs. 9, 10C). Third, most of the neurons in the lateral septal nucleus have already formed by E19 (Bayer, '79). This indicates that the subependymal zone at middle and posterior levels is producing cells which will settle outside the septum. As mentioned above, the zone is large in the region of the nucleus accumbens; more importantly, it remains large all the way into the olfactory bulb. Altman ('69) observed that the granule cells of the olfactory bulb arise from the subependymal zone lining the lateral ventricle, and the area described in this study is probably part of that larger germinal matrix.

The differentiating cell zone is always radioresistant (figs. 8-10D) except early in development at the anterior level. The diagonal band neurons may leave the neuroepithelium as primitive migratory cells, still radiosensitive, and do not begin to differentiate until around E18. A Golgi-Cox study comparing the times of neuronal differentiation in various areas of the septal region is in preparation. The accumulation of differentiating cells at both middle and posterior levels reflects the mediolateral gradient of cell acquisition observed here with ^{3}H-thymidine autoradiography. The rostrocaudal differences between middle and posterior levels are more apparent than real, since the larger subependymal zone at the middle level decreases the relative area taken up by the differentiating cells.

Concluding remarks

From E17 on, the cortex immediately dorsal to the nucleus of the diagonal band and the medial, lateral and triangular septal nuclei begins to differentiate into the anterior continuation of the hippocampus, which can be followed posteriorly into the developing hippocampal region. Also on E17, a small but definite fornix bundle can be seen between the two areas at posterior levels through the septum (fig. 3F), but the fibers cannot be followed further back into the hippocampal region (Bayer and Altman, '74). The large neurons of the medial septal and diagonal band nuclei provide hippocampal afferents (Swanson and Cowan, '77; Meibach and Siegel, '77a,b; and others). They also complete their formation (medial septal on E15, diagonal band on E17; Bayer, '79) during the peak of neurogenesis in the subiculum (E16-E17; Bayer,'76) and before the peak in the CA fields of Ammon's horn (E18-E20; Bayer, '76). These observations indicate that the early fornix is probably composed of septal fibers growing toward the hippocampal region. The fornix bundle continues to enlarge throughout later embryonic development (figs. 3I,L), presumably due to fibers from the subiculum and Ammon's horn growing into the septum. The late neurogenesis in the lateral septal nucleus, which receives the bulk of the hippocampal input (Swanson and Cowan, '76, '77; Meibach and Siegel, '77a,b), may be timed so that the cells begin to differentiate as the hippocampal afferents arrive—an interesting possibility that awaits clarification with the Golgi-Cox technique (Bayer, in preparation).

No doubt, developmental corollaries also exist between the amygdala and bed nucleus of the stria terminalis. These structures presumably arise from the same embryonic source (Johnston, '23; Humphrey, '72), and are extensively interconnected anatomically (de Olmos and Ingram, '72; Krettek and Price, '78). The development of the olfactory system, a massive functional component of the rat forebrain, must be closely related to development of the septal region, since some of its germinal matrix lies within the region and gives rise to part of it, the nucleus accumbens. Thus, the septum appears to be a nodal point in telencephalic development—where hippocampal, amygdaloid, and olfactory germinal matrices meet.

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