

The Development of the Septal Region in the Rat

I. NEUROGENESIS EXAMINED WITH ^3H -THYMIDINE AUTORADIOGRAPHY

SHIRLEY A. BAYER

*Laboratory of Developmental Neurobiology, Department of Biological Sciences,
Purdue University, West Lafayette, Indiana 47907*

ABSTRACT Neurogenesis in the rat septal region was examined with ^3H -thymidine autoradiography. The rats in the prenatal groups were the offspring of pregnant females given two injections of ^3H -thymidine on consecutive days in an overlapping series: embryonic day (E) 13 + E14, E14 + E15, . . . E21 + E22. The rats in the postnatal groups were injected in a nonoverlapping series: the day of birth and postnatal day (P) 1, P2 + P3, P3 + P4. 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 midline nuclear group (nucleus of the diagonal band, medial and triangular septal nuclei), the lateral septal nucleus, and the ventrolateral nuclear group (nucleus accumbens, bed nuclei of the stria terminalis and the anterior commissure). The neurons within each nuclear group form in significantly different waves, those of the midline group forming between E13-E17, the lateral septal nucleus between E15-E19, the bed nuclei of the stria terminalis and anterior commissure between E14-E18, the nucleus accumbens between E17-P2. All nuclei and nuclear groups show characteristic gradients of formation. Both the midline nuclear group and the bed nucleus of the stria terminalis (including the commissural bed nucleus) have their earliest forming neurons lying near the crossing of the anterior commissure; younger neurons are located both rostrally and caudally with the youngest neurons lying in the most rostral extension of the diagonal band nucleus and the strial bed nucleus. The lateral septal nucleus forms along a strong mediolateral gradient throughout its length after neurogenesis is almost complete in the midline nuclear group. Throughout the length of the nucleus accumbens, the oldest neurons are located ventrally while progressively younger cells are found dorsally beneath the inferior horn of the lateral ventricle.

The septal region forms the subcallosal anteromedial wall of the telencephalon. Cajal ('11) was one of the first to examine this area and described three nuclei: medial nucleus, triangular nucleus, and the "external or principal nucleus" (the lateral septal nucleus). The boundaries of the septal region were expanded by Johnston ('13, '23) to include the entire ventromedial telencephalic wall. The nuclei that Cajal described were collectively named the "hippocampal primordium" (Johnston, '13); anteriorly and ventrally, the primordium was intimately related to both the lateral parolfactory nucleus (nucleus accumbens) and the medial parolfactory nucleus (diagonal band of Broca). In a later

study (Johnston, '23), the bed nucleus of the stria terminalis was added as a caudal continuation of the nucleus accumbens. Subsequent anatomical descriptions (Gurdjian, '25; Young, '36; Fox, '40; Lauer, '45) generally agreed on the major nuclei contained in the septal region: the medial, lateral, and triangular septal nuclei; the diagonal band of Broca; the bed nucleus of the stria terminalis. The nucleus accumbens was also included but always with the qualification that it may be simply a medial extension of the caudate nucleus.

The septal region is richly interconnected with other areas of the telencephalon (hippocampal region, amygdaloid region, limbic

neocortex) and diencephalon (periventricular hypothalamus, mammillary body, anterior thalamic nuclei) generally referred to as the limbic system. Since Papez ('37) proposed it and MacLean ('52) elaborated it as the anatomical substrate of emotion, the limbic system has received much attention from anatomists, physiologists, and psychologists. Several recent anatomical studies in the rat (Swanson and Cowan, '76, '77; Meibach and Siegel, '77a,b) have shown that the septum is an important component of limbic circuits and has extensive reciprocal connections with the hippocampal region. To contribute to a better understanding of the intricate anatomical organization of the limbic system, a detailed analysis of rat limbic system development was undertaken.

The technique of ^3H -thymidine autoradiography has revealed a regular and sequential production of neurons in all brain structures thus far examined (for example, hippocampus: Angevine, '65; Altman, '66; Hine and Das, '74; Bayer and Altman, '74, and others). The progressively delayed comprehensive labelling method, an improved ^3H -thymidine autoradiographic procedure which significantly increases the accuracy in timing the onset, proportion of daily acquisition, and the cessation of neurogenesis, will be used throughout the study of limbic system ontogeny. The aim of the entire study is to see what possible role the precise chronology of neuron origin between and within limbic system structures could play in the determination of anatomical linkages. The first two reports of the series deal with septal development. This paper describes the sequence of neurogenesis utilizing ^3H -thymidine autoradiography. The following paper (Bayer, '79) correlates the autoradiographic findings with embryological development utilizing the additional technique of low-level X-irradiation.

A detailed autoradiographic study of septal neurogenesis in the mouse (Creps, '74) reported several gradients of cell formation. The few papers on septal development in the rat have dealt mainly with the nucleus accumbens (Das and Altman, '70; Swanson and Cowan, '75; Lawson et al., '77). This study expands the observations of the previous reports by extensively quantifying the amount of neurogenesis taking place during each day of formation. The development of several anatomical levels within each of the following nuclei is described; medial, lateral, and triangular

nuclei; the nucleus accumbens; the interstitial nuclei of the diagonal band of Broca, the stria terminalis, and the anterior commissure. The septo-hippocampal nucleus, a caudal projection of cells into the medial septum from the anterior extension of the hippocampus, will be described with hippocampal region development.

MATERIALS AND METHODS

The prenatal developmental series contained nine groups of Purdue-Wistar rats, the offspring of pregnant female rats given two consecutive daily subcutaneous injections of ^3H -thymidine (Schwarz/Mann; specific activity 6.0 C/mM; 5 μCi /gram body weight). The multiple injections, given to insure comprehensive cell labelling, were progressively delayed by 24 hours in an overlapping series: E13 + E14, E14 + E15, . . . E21 + E22. Usually, two or more pregnant females were injected for each group. The postnatal developmental series contained three groups of pups, each receiving two consecutive daily injections in a non-overlapping series: P0 + P1, P2 + P3, P4 + P5. All injections were given between 9 and 11 A.M. The day of sperm-positivity was E1; the day of birth was P0. Normally, the rats in the Purdue-Wistar colony are born on the afternoon of E23.

All animals were transcardially perfused with 10% neutral formalin at 60 days of age. 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 of the prenatal groups were prepared for analysis. The postnatal groups contained two males each. Anatomically matched sections (6 μm , every fifteenth section was saved) were cut in the coronal plane. The slides were coated with Kodak NTB-3 emulsion, exposed for 12 weeks, developed in Kodak D-19, and post-stained with hematoxylin and eosin.

Anatomically-matched sections were selected for each of the structures listed in table 1, and the proportion of labelled cells was determined microscopically at $312.5\times$ or $500\times$ 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 com-

TABLE 1

Anatomical levels¹ used for sampling septal region nuclei

Structure	9.4	9.2	9.0	8.8	8.6	8.4	8.2	8.0	7.8	7.6	7.4	7.2	7.0	6.8	6.6	6.4
Midline nuclear group																
Triangular (TSM, TSL)														X	X	X
Medial (MS)							X		X		X					
Diagonal band (DB)		X		X												
Lateral septal nucleus																
Dorsal-medial ridge (MR)									X		X		X ²	X ²		X ²
Mediolateral ridge (MLR)									X		X		X ²	X ²		X ²
Lateral ridge (LR)									X		X		X ²	X ²		X ²
Ventral-medial ridge (MR)									X		X		X ²			
Mediolateral ridge (MLR)									X		X		X ²			
Lateral ridge (LR)									X		X		X ²			
Ventrolateral nuclear group																
Nucleus accumbens (NA)	X		X		X											
Bed nucleus of the stria terminalis (BST)																
Precommissural								X	X	X						
Postcommissural											X	X	X	X	X	X
Bed nucleus of the anterior commissure (BAC)												X	X			

¹ Pellegrino and Cushman ('67).² These areas are also called the septofimbrial nucleus by some (Young, '36; Fox, '40).

prehensive labelling procedure described by Bayer and Altman ('74). The method is based on the assumption that ³H-thymidine will only be incorporated by mitotic neuronal precursors, not by post-mitotic 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 septal region 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 percentage of labelled neurons within a specific population declines, reflecting the change 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).

The statistical analysis of the data involved two steps. First, the time of origin of neurons in different levels within each nucleus were statistically analyzed by means of the sign test (Conover, '71). This test utilizes paired samples from individual animals so that the

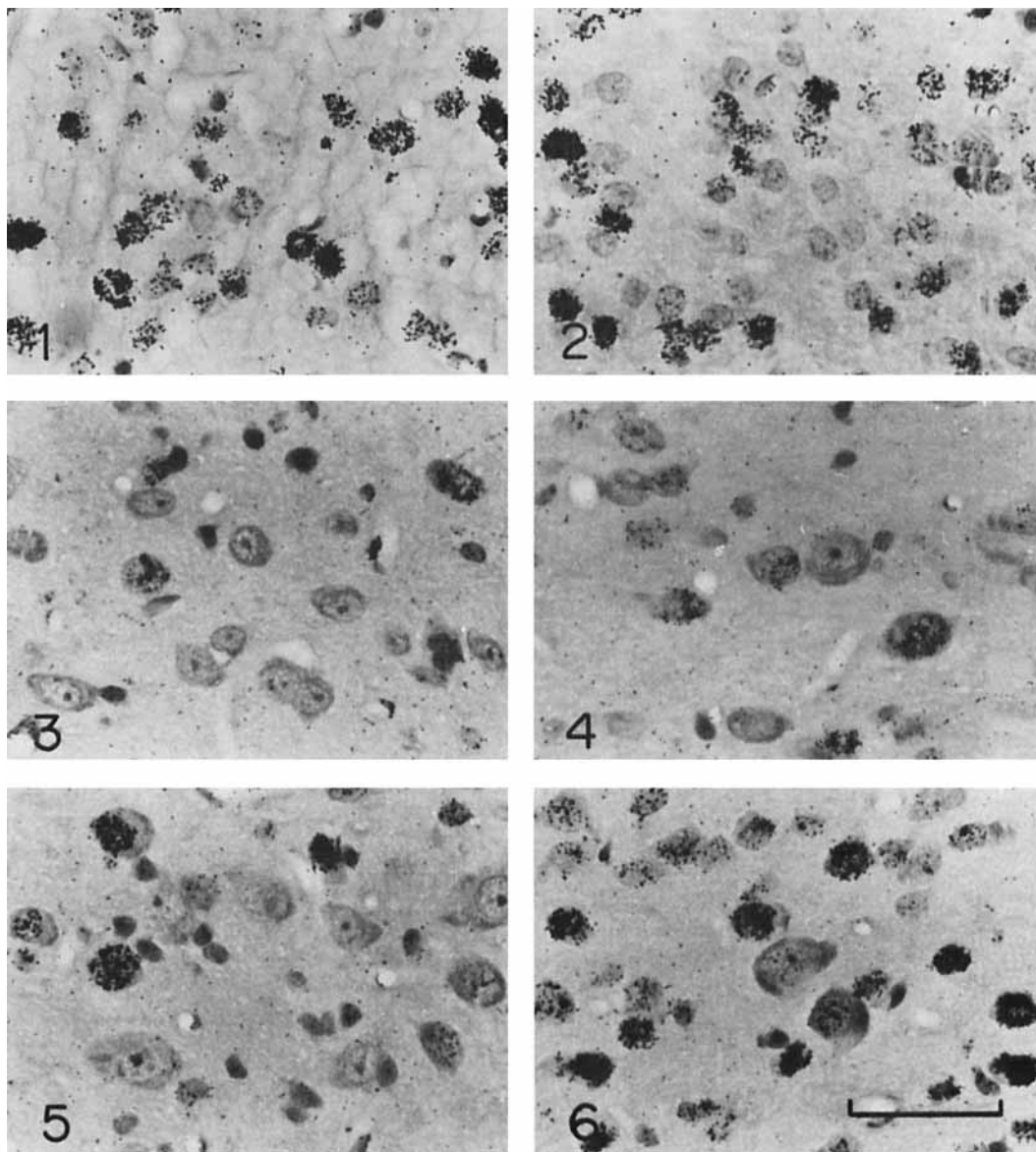
gradients of neurogenesis are not obscured by the variability between animals in a specific injection group. Any levels within a nucleus that were not significantly different were combined. Second, the significantly different levels within each nucleus were then compared, also by means of the sign test, to other septal region nuclei.

RESULTS

The midline nuclear group

Figures 1-6 show typical neurons in three septal region nuclei either bridging or lying parallel to the midline (triangular, figs. 1, 2; medial, figs. 3, 4; diagonal band, figs. 5, 6). The lateral septal nucleus also reaches the midline, and will be considered in the following section. All photographs are from the same animal exposed to ³H-thymidine on E15 + E16, an active period of neurogenesis in these nuclei. A comparison of the numbers of labelled and nonlabelled neurons in each area give qualitative evidence for the gradients of formation existing within and between each of these three nuclei.

The *triangular nucleus* is composed of small neurons (figs. 1, 2) located lateral to the columns of the fornix and stria medullaris at levels A6.6 and A6.8 and interspersed with fibers of the hippocampal commissure at level A6.4 (see drawings in fig. 7A). It is only at



Figs. 1-6 Autoradiograms of cells in the midline nuclear group from an animal exposed to ^3H -thymidine on E15 + E16. Note that the proportion of labelled cells decreases from figures 1-3, then increases in figures 4-6. For further explanation, see text. Hematoxylin-eosin. Bar, 50 μm .

- 1 Small triangular nucleus cells in midline, level A6.4.
- 2 Triangular nucleus cells lateral to columns of fornix, level A6.8.
- 3 Medium-sized and larger medial nucleus cells, midline, level A7.4.
- 4 Medial nucleus cells, midline, level A8.2.
- 5 Diagonal band nucleus cells, vertical limb, A8.8.
- 6 Diagonal band nucleus cells, vertical limb, level A9.2.

level A6.4 that this nucleus bridges the midline. Figure 1 shows that almost all neurons located in the midline can be labelled by exposure to ^3H -thymidine on E15 + E16, while many of the neurons located lateral to the columns of the fornix (fig. 2) are not labelled. These findings indicate a lateromedial gradient of formation. The laterally placed neurons from levels A6.8 to A6.4 have the same formation time ($p > 0.05$), and their data were pooled (fig. 7A). Neurons located in the midline at level A6.4 arise significantly later (fig. 7A) than those of the laterally located areas (A6.8, $p = 0.0176$; A6.6, $p = 0.0446$; A6.4, $p = 0.0367$). This nucleus has a very short period of neurogenesis; the majority of its cells originate on E15 at all levels (figs. 8A,B). Before the peak on E15, the lateral locations accumulate more neurons than the midline area on E13 and E14, while 25% of the midline neurons remain to be formed on E16.

The *medial nucleus* contains widely scattered large and medium-sized neurons (figs. 3, 4). At all levels, this nucleus bridges the midline forming a "protoplasmic commissure" (Cajal, '11). Figure 3 shows that few neurons located in the midline at level A7.4 could be labelled after exposure to ^3H -thymidine on E15 + E16, while several neurons are labelled at level A8.2 (fig. 4). These findings indicate a caudorostral gradient of growth. Figure 7B shows that each level of the medial nucleus has a different time of formation. The neurons at level A7.4 arise significantly earlier than those at level A7.8 ($p = 0.0085$) and at level A8.2 ($p = 0.0004$); the neurons at level A7.8 form significantly earlier than those at level A8.2 ($p = 0.0007$). At all levels, the peak time for neurogenesis occurs on E15 (figs. 8C-E). Level A7.4 accumulates more neurons before the peak (fig. 8C), while levels A7.8 and A8.2 are still adding a few neurons to their populations after E15 (figs. 8D,E).

The *diagonal band nucleus* has similar cells to those in the medial nucleus, with conspicuous large neurons (figs. 5, 6). The nucleus remains bilateral throughout its antero-posterior extent. In the vertical limb of the nucleus, an exposure to ^3H -thymidine on E15 + E16 labels several neurons at level A8.8 (fig. 5) and almost all neurons at level A9.2 (fig. 6), thus indicating a caudorostral gradient of neurogenesis. For the data in figure 7C, the percentages of labelled cells were determined in the vertical limb, midpoint, and horizontal limb at each level. There were no

significant differences between these areas (all $p > 0.05$), and the data were pooled for each level. The neurons at level A8.8 originate significantly earlier than those at level A9.2 ($p < 0.00001$). Unlike the other midline nuclear areas, neuronal production in this nucleus does not show a strong peak on any day of formation (figs. 8F,G); rather, the formation takes place evenly over a three (A9.2) or four (A8.8) day period.

Table 2 lists the clusters of neurons in the midline group which had simultaneous times of formation ($p > 0.05$). The formation of neurons in the midline nuclear group radiates out in both rostral and caudal directions from an early center at level A7.4 (fig. 7C; group 1, table 2). Each succeeding group contains neurons further from this early-forming center (groups 2, 3, 4, table 2). However, neurogenesis does not occur symmetrically around the center; the rostral diagonal band arises significantly later than the midline neurons of the triangular septal nucleus ($p = 0.032$).

The lateral septal nucleus

The lateral septal nucleus is the largest component of the septum, extending throughout its rostrocaudal length. The caudal part is sometimes called the septofimbrial nucleus (table 1). This nucleus forms a thick dorsolateral cap over the medial nucleus, a thinner cap over the triangular nucleus (inset in fig. 10). It extends from the midline to the lateral ventricle and is covered by the corpus callosum. The medium to small-sized neurons do not cross the midline. There are no obvious cytological differences between neurons in different areas, except that the cells near the

TABLE 2
Midline nuclear group

Levels	Clusters of simultaneous formation				
	Early		Intermediate		Late
	1	2	3	4	5
Caudal					
TSM A6.4				X	
TSL A6.4-6.8			X		
MS A7.4	X				
MS A7.8		X			
MS A8.2			X	X	
DB A8.8			X	X	
DB A9.2					X
Rostral					

Abbreviations

AC, Anterior commissure	OC, Optic chiasm
CC, Corpus callosum	OT, Optic tract
Fi, Fimbria	SF, Subfornical organ
FX, Fornix	SM, Stria medullaris
IC, Large island of Calleja	ST, Stria terminalis
LV, Lateral ventricle	VIII, Third ventricle

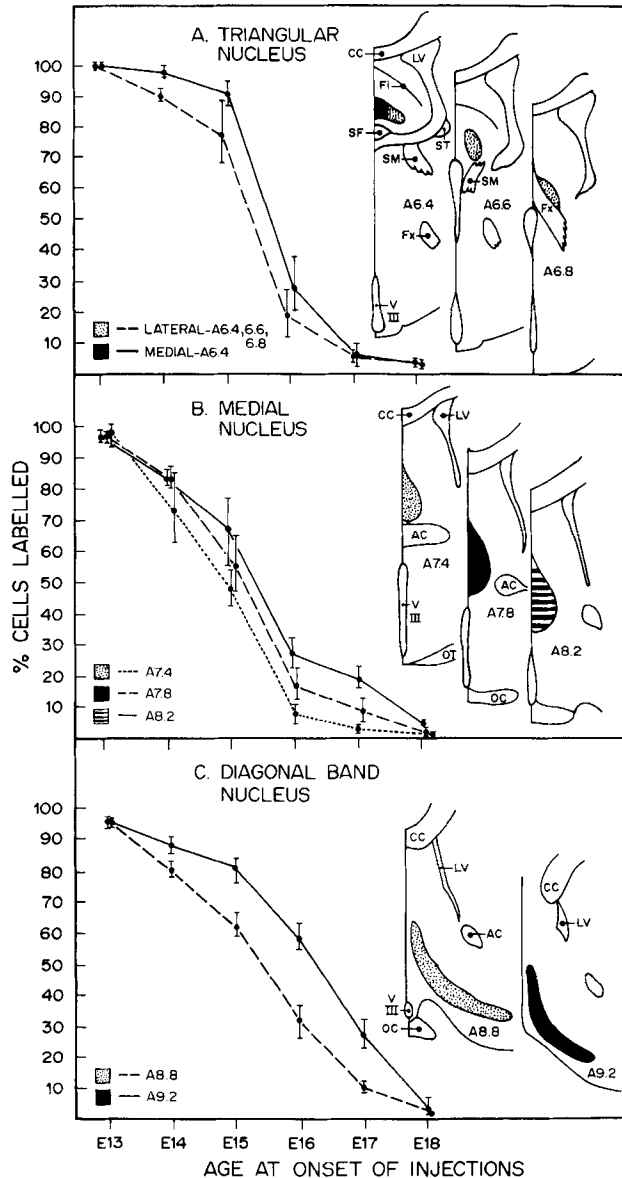


Fig. 7 Decline in percentage of labelled cells during development within each nucleus of the midline group. Each mean with standard deviation is from a group of approximately six animals. Shaded areas in drawings indicate regions where cells were counted.

- A Triangular nucleus; percentage of labelled cells declines significantly later medially, level A6.4.
 B Medial nucleus; all levels are significantly different in time of cell labelling decline.
 C Diagonal band nucleus; labelled cells persist significantly longer anteriorly (A9.2).

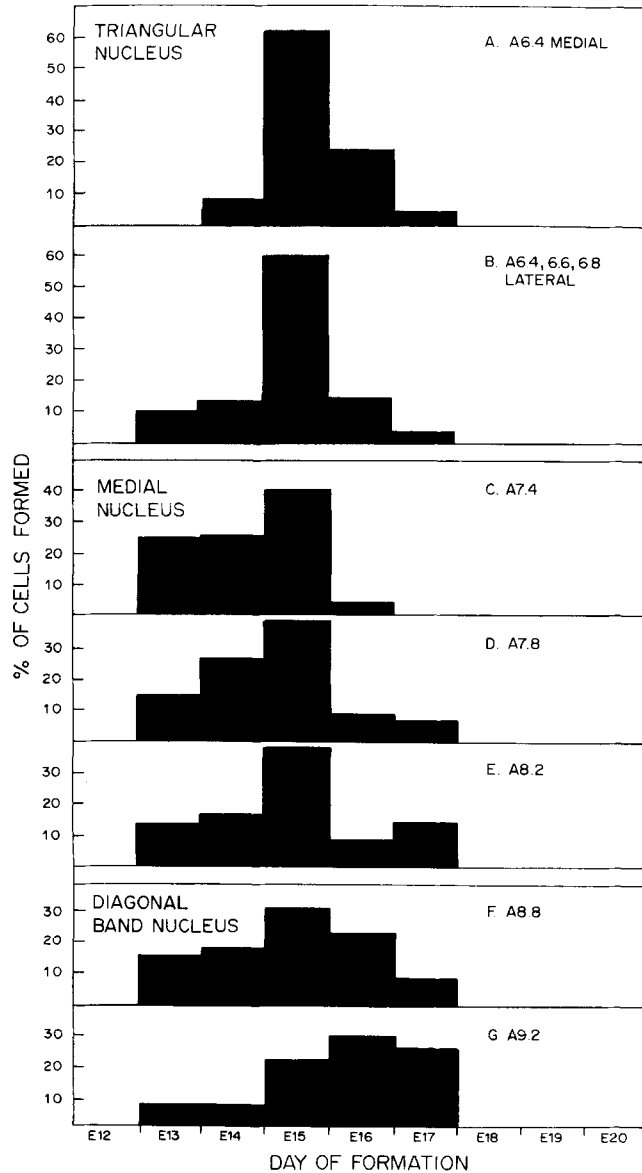


Fig. 8 Proportion of cells formed each day within midline group nuclei, equals daily decline in percentage of labelled cells in figure 7. For example, consider calculation of percentage of lateral triangular nucleus neurons (B) formed on E15: 77% cells labelled on A.M. of E15 + E16 minus 18% cells labelled on A.M. of E16 + E17 (dashed line in fig. 7A) equals 59% cells formed during E15.

A Triangular nucleus from midline at level A6.4. Graph derived from data shown in solid line of figure 7A.

B Lateral triangular nucleus at levels indicated. Graph corresponds to data presented in dashed line in figure 7A.

C Medial nucleus at level A7.4 as derived from data in dotted line of figure 7B.

D Medial nucleus at level A7.8, as derived from data in dashed line in figure 7B.

E Medial nucleus at level A8.2, as derived from data in solid line of figure 7B.

F Caudal diagonal band (level A8.8) as derived from data in dashed line in figure 7C.

G Rostral diagonal band (level A9.2) as derived from data in solid line in figure 7C.

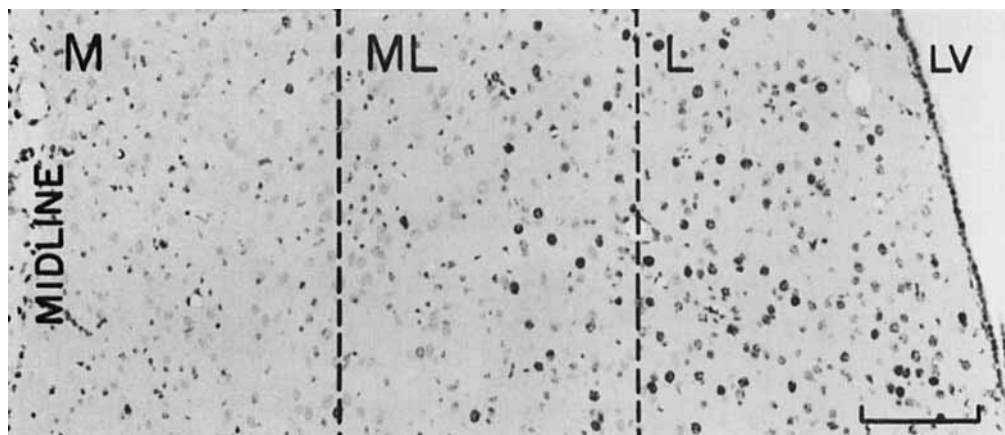


Fig. 9 Lateral septal nucleus at level A7.8 from an animal injected on E16 + E17. Note increase in number of labelled cells as the lateral ventricle (LV) is approached. Dashed lines subdivide areas where percentage of labelled cells were separately determined. M, medial; ML, mediolateral; L, lateral. Hematoxylin-eosin. Bar, 100 μ m.

lateral ventricle in the ventral portion (levels A7.0-A7.8) are slightly smaller and more densely packed.

Figure 9 shows an autoradiogram of a typical strip of cells in the lateral septal nucleus from an animal exposed to ^3H -thymidine on E16 + E17. The relative number of labelled cells increases sharply as the lateral ventricle is approached. In order to quantify this strong mediolateral gradient and also to compare rostrocaudal differences in neurogenesis, strips extending from the midline to the lateral ventricle were divided approximately into thirds (dotted lines in fig. 9) at each location listed in table 1. The percentage of labelled cells was separately calculated for the medial (M in fig. 9), mediolateral (ML in fig. 9), and lateral (L in fig. 9) parts. An analysis of the data from each of these locations showed no consistent differences between rostral and caudal strips. There was a strong mediolateral gradient for each strip, with the medial portion forming earliest, followed by the mediolateral portion, and finally by the lateral portion (all levels and comparisons, $p \leq 0.035$). The lateral nucleus could now be visualized as composed of simultaneously forming "ridges" extending throughout the depth of the nucleus from A6.4 rostrally through A7.8 (see drawings in fig. 10). Data from the medial area in all strips were pooled to form the medial ridge; the mediolateral areas were pooled to form the mediolateral ridge, and the lateral areas were pooled to form the lateral ridge (figs. 10, 11).

Figure 11 shows that each ridge of the nucleus has a short formation time of two to three days, especially the lateral ridge, with almost half the neurons forming on E17. The medial septal nucleus and the lateral parts of the triangular nucleus contain cells originating significantly earlier ($p \leq 0.0003$) than the medial ridge of the lateral septal nucleus; triangular nucleus midline neurons are simultaneous with the medial ridge.

The ventrolateral nuclear group

The ventrolateral nuclear group contains the bed nucleus of the stria terminalis, the bed nucleus of the anterior commissure, and the nucleus accumbens. Cajal ('11) does not include these nuclei in the septal region, but several later descriptive anatomical studies do so (Gurdjian, '25; Young, '36; Fox, '40; Lauer, '45).

The *bed nucleus of the stria terminalis* is the largest of the group, extending rostrally from a wedge-shaped area between the dorsal thalamus and the internal capsule to lie above and slightly medial to the interbulbar extension of the anterior commissure (see insets in figs. 15A,B). The neurons are medium-sized and uniform in appearance throughout the length of the nucleus (figs. 12-14); they are quite similar to the neurons of the nucleus accumbens (figs. 19, 20). The packing density is considerably higher in the more caudal part (fig. 12) as compared to more rostral levels. Figures 12-14 are from the same animal exposed to ^3H -

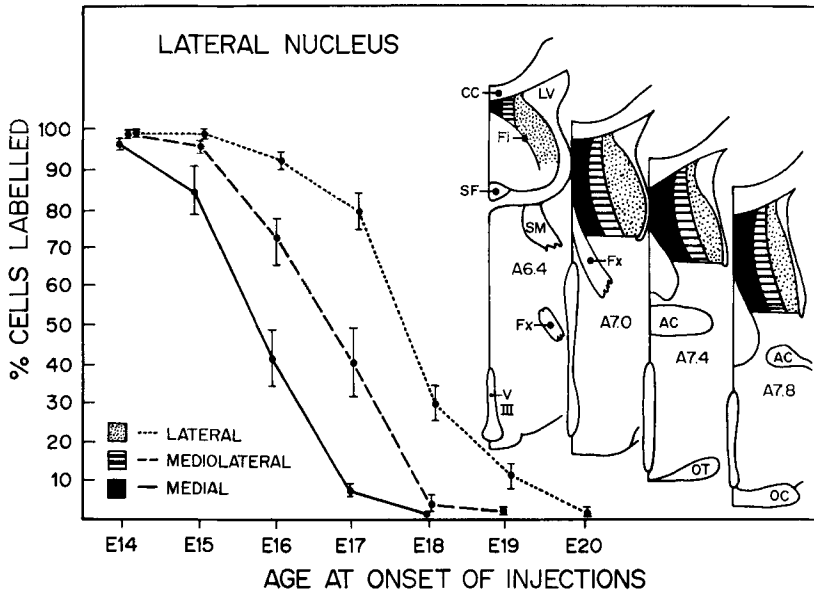


Fig. 10 Decline in percentage of labelled cells during lateral nucleus development. Shaded areas in drawings indicate regions where cells were counted. Each mean with standard deviation is from a group of approximately six animals. All rostrocaudal levels originate simultaneously. Solid line represents combined data from all medial areas throughout the rostrocaudal extent; dashed line, combined mediolateral areas; dotted line, combined lateral areas.

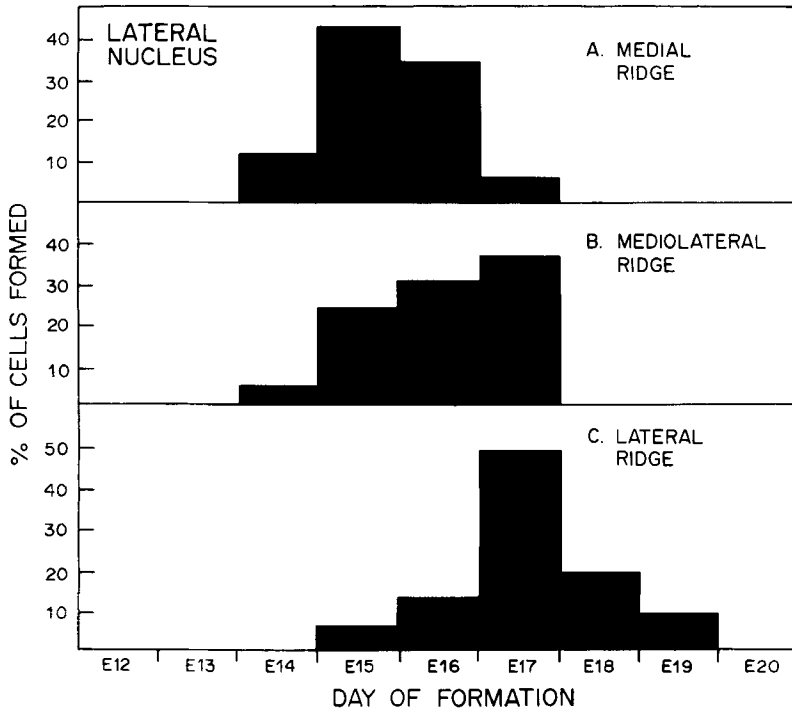


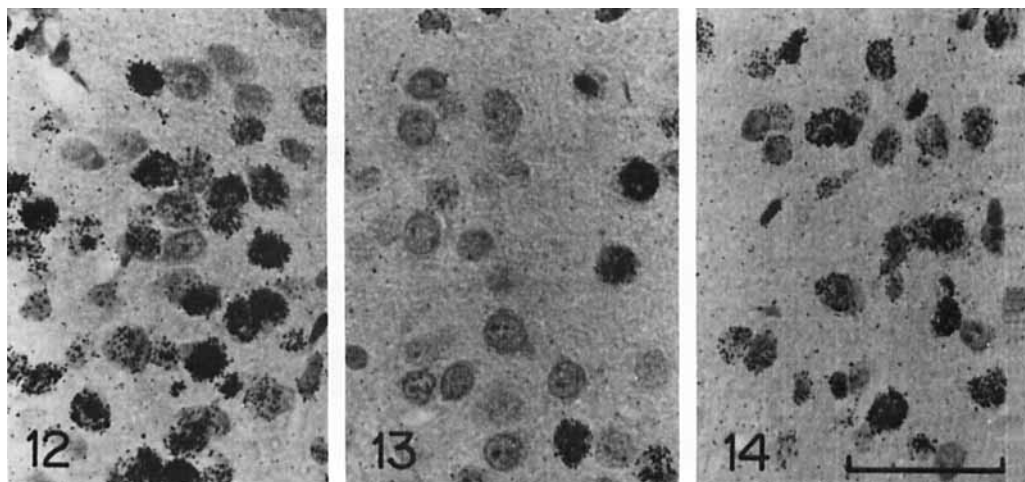
Fig. 11 Proportion of lateral nucleus neurons formed each day.

A Medial ridge, derived from data shown in solid line, figure 10.

B Mediolateral ridge, derived from data shown in dashed line, figure 10.

C Lateral ridge, derived from data shown in dotted line, figure 10.

From E15 through E17, there are successive waves of neurogenesis progressing from the medial ridge through the mediolateral ridge to the lateral ridge.



Figs. 12-14: Autoradiograms of bed nucleus of the stria terminalis neurons from an animal exposed to ^3H -thymidine on E16 + E17. Note the lower proportion of labelled cells in figure 13 as compared to figures 12 and 14. Hematoxylin-eosin. Bar, 50 μm .

- 12 Level A6.4. Packing density is much higher here than at rostral levels.
- 13 Level A7.4.
- 14 Level A8.0.

thymidine on E16 + E17. Many cells are labelled at level A6.4 (fig. 12), few are labelled at level A7.4 (fig. 13), and all cells are labelled at level A8.0 (fig. 14). This indicates a gradient of formation radiating both rostrally and caudally from an early-forming center. To quantify this double gradient, the nucleus was divided into pre- and postcommissural parts at level A7.4. There is a rostrocaudal gradient in the postcommissural part (figs. 15A, 16A,B). The neurons at levels A7.4 through A7.0 originate significantly before those from levels A6.4 through A6.8 (all levels and comparisons, $p \leq 0.0485$). The precommissural part has a caudorostral gradient (figs. 15B, 16C-E) with level A7.6 arising significantly before level A7.8 ($p = 0.0012$) and level A8.0 ($p < 0.00001$); level A7.8 is also significantly earlier than level A8.0 ($p = 0.0023$). Figure 16 shows that the majority of the neurons form on E15 and E16 for all areas except level A8.0, where a substantial amount of neurogenesis is still occurring on E17.

The *bed nucleus of the anterior commissure* is the smallest of the septal region nuclei, lying in a wedge-shaped area between the anterior commissure and the descending columns of the postcommissural fornix (figs. 17A,B). The cells are very small, quite similar to those in the triangular septal nucleus (figs. 1, 2) and are very densely packed. The time of

formation is rapid, occurring almost entirely on E15 and E16 (figs. 18A,B). This is the only nucleus in the septal region which does not show a formation gradient.

The *nucleus accumbens* is bordered posteriorly by the bed nucleus of the stria terminalis and anteriorly by the anterior olfactory nucleus; laterally, it blends with the caudate nucleus; medially it is adjacent to the large island of Calleja (see inset in fig. 21). The small to medium-sized neurons (figs. 19, 20) are similar to those of the bed nucleus of the stria terminalis and the caudate nucleus. One of the most distinguishing features of this nucleus is its late time of formation, which extends into the early postnatal period. In an animal exposed to ^3H -thymidine on E21 + E22, many of the cells are labelled in the dorsal part of the nucleus (fig. 19) while few cells are labelled in the ventral part (fig. 20). This indicates a ventrodorsal gradient of formation. To quantify this gradient as well as compare times for neurogenesis at rostral vs. caudal levels, the percentage of labelled cells was separately determined in dorsal and ventral parts at each of the levels listed in table 1. There were no consistent differences between rostral and caudal levels. At each level, the ventral part was significantly earlier than the dorsal part (all levels and comparisons, $p < 0.00001$). Figure 21 shows the pooled data

from both dorsal and ventral samples at all rostrocaudal levels. Unlike any other septal region nucleus, the cells accumulate over a long period of time (approximately a week) with only a small percentage (<30%) of the population arising on a single day. The amount of postnatal neurogenesis is small (10%) and is almost entirely limited to the dorsal part of the nucleus (figs. 21, 22).

Table 3 lists the clusters of neurons in the ventrolateral group which have simultaneous times of formation ($p > 0.05$). The formation of neurons radiates out in both rostral and caudal directions from an early center between levels A7.0-A7.6 (group 1). Caudal levels (A6.4-A6.8) are simultaneous with level A7.8 (group 2). The most rostral neurons in the bed nucleus of the stria terminalis are forming later than those at caudal levels (group 3) but arise significantly ($p < 0.00001$) earlier than the ventral neurons of the nucleus accumbens (group 4). As in the midline nuclear group, neurogenesis is asymmetrical around the early-forming center; rostral parts originate significantly later than the caudal parts.

DISCUSSION

The daily chronology of septal neurogenesis reported here shows that cell production is both rapid within a single area (most neurons in a given area are formed over 2-3 days) and sequential between several related areas. The evidence obtained here indicates a shorter time of cell acquisition than has heretofore been indicated by the single injection technique (Crepes, '74; Swanson and Cowan, '75; Lawson et al., '77). Still, the formation times of the neuronal populations presented here are conservative because they are based on animals which have within-group variability in individual development possibly by as much as 12 hours. The use of paired samples from individual animals comparing neurogenesis in one area of the septum to another (the sign test) reveals highly consistent patterns of development in all animals. The biological significance of the sequentially ordered production of neurons is not known. However, the time for neuronal production appears to be rigidly set and not modified by experimental manipulation; such as, the removal of precursors by low-level X-irradiation (Bayer and Altman, '75a,b).

Although the formation pattern within a nuclear group is regular, the question remains

as to how these groups are related to each other. These relationships are best illustrated in a schematic diagram (fig. 23) showing how neurons accumulate in the septal region during development. Each nuclear group is composed of segments of significantly different forming neuronal populations arranged along either the rostrocaudal axis (midline nuclear group, bed nucleus of the stria terminalis), the mediolateral axis (lateral septal nucleus), or the dorsoventral axis (nucleus accumbens). When 50% of a population of neurons in a given locale has formed (as indicated by less than 50% cell labelling), the segment representing that area is illustrated. As the percentage of formed cells goes up, the amount of shading increases. By following the diagram from E15-E22, one can see how each nuclear group of the septal region "grows" along its axis of formation. Pairs of nuclear groups within the septal region will now be discussed to see what can be gained by studying their parallel, sequential, or unique formation patterns.

Parallel development of the midline nuclear group and the bed nucleus of the stria terminalis

Both the midline nuclear group and the bed nucleus of the stria terminalis can be described as cylinders of cells extending rostrocaudally through the basal forebrain. One lies along the midline and the other along the inferior horn of the lateral ventricle. A surprising finding of this study is that, although the neurons in the bed nucleus of the stria terminalis form slightly later, the developmental pattern in the two groups is

TABLE 3
Ventrolateral nuclear group

Levels	Clusters of simultaneous formation				
	Early	Intermediate		Late	
	1	2	3	4	5
Caudal					
BST A6.4-6.8		X			
BAC A7.0-7.2	X	X			
BST A7.0-7.4	X				
BST A7.6	X				
BST A7.8		X			
BST A8.0			X		
↓					
Rostral					
NA ventral				X	
NA dorsal					X

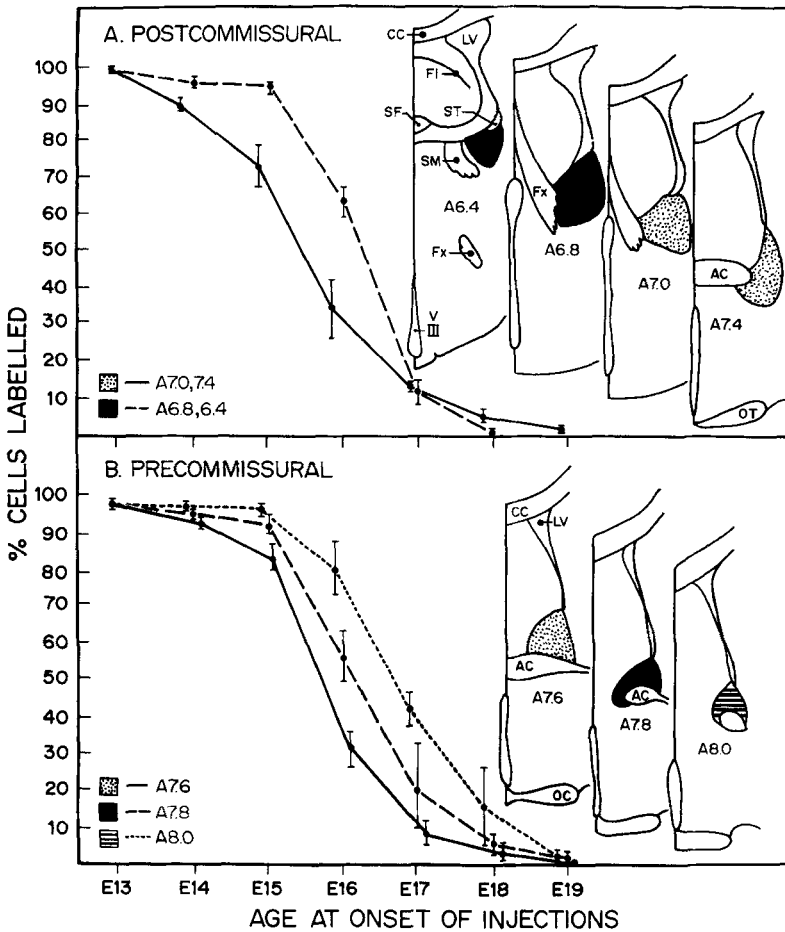
BED NUCLEUS OF THE
STRIA TERMINALIS

Fig. 15 Decline in percentage of labelled cells from postcommissural (A) and precommissural (B) parts of the bed nucleus of the stria terminalis. Shaded areas in drawings indicate regions where cells were counted. Each mean with standard deviation is from a group of approximately six animals. In the postcommissural (A) part, cells at caudal levels (dashed line) arise significantly later than those of rostral levels (solid line). The precommissural (B) part shows a reverse gradient with significantly later forming cells located rostrally.

almost exactly the same. First, the neurons located in the region of the crossing of the anterior commissure are formed earliest; second, sequentially younger neurons are deposited both rostrally and caudally around the early-forming center; third, the youngest neurons are located in the most rostral areas (compare the two groups in fig. 23). The significance of this parallel development is not known, but evidence will be provided in the next paper (Bayer, '79) that the early-forming centers in each group can be seen fusing in the embry-

onic rat brain before the anterior commissure crosses the midline; this earlier fusion may provide a "bridge" for the commissure.

The gradients in the midline nuclear group were seen by Creps ('74) in the mouse; her data also suggested a similar gradient for the strial bed nucleus. Lawson et al. ('77) concluded that there was no caudorostral gradient in the rat medial and triangular nuclei, but this was based on an incomplete developmental series (E14 and E16 were missing).

The bed nucleus of the anterior commissure

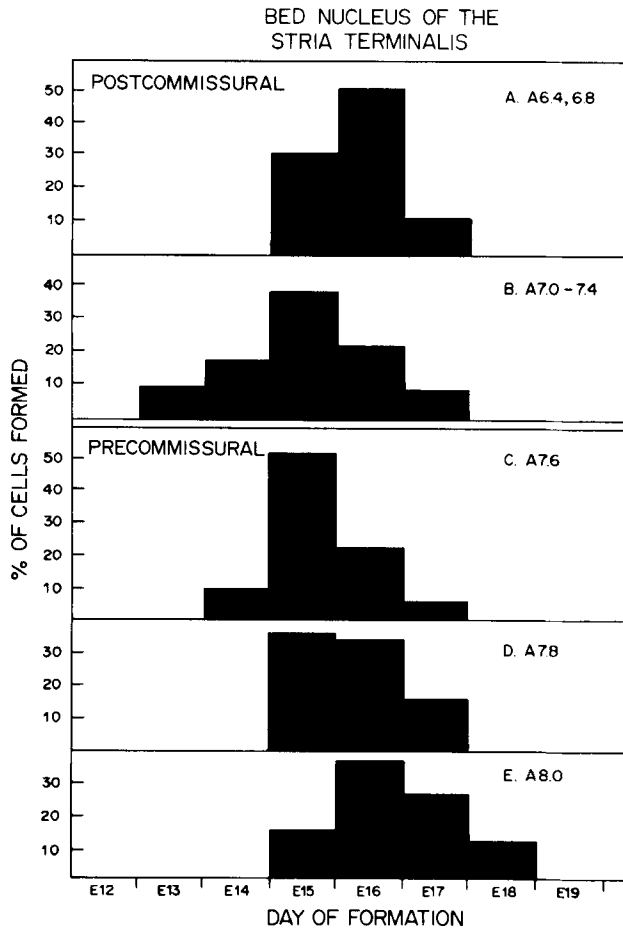


Fig. 16 Proportion of cells formed each day at different levels of the bed nucleus of the stria terminalis.

- A Cell formation at levels A6.4 - 6.8, derived from dashed line (fig. 15A).
- B Cell formation at levels A7.0, 7.2, 7.4, derived from solid line (fig. 15A).
- C Cell formation at level A7.6, derived from solid line (fig. 15B).
- D Cell formation at level A7.8, derived from dashed line (fig. 15B).
- E Cell formation at level A8.0, derived from dotted line (fig. 15B).

lies within the larger boundaries of the bed nucleus of the stria terminalis and fits into the formation gradients of the latter (table 3). Most likely, this nucleus is a specialized group of neurons within the strial bed nucleus, but the similarity in appearance to the neurons of the triangular septal nucleus may also indicate a common origin. This nucleus was briefly described by Gurdjian ('25) in the rat and has not received much attention. Information on the anatomical connections of this nucleus would shed further light on its relationships to other areas of the septal region.

Sequential development of the midline nuclear group and the lateral septal nucleus

The lateral septal nucleus forms a dorso-lateral cap over the medial and triangular septal nuclei and develops sequential to them. Neurogenesis in the medial and triangular nuclei is essentially complete on E15; by this same time, only a few cells in the medial ridge of the lateral nucleus have been produced (compare figs. 8 and 11). During E16 and E17, most of the neurons in the lateral nucleus arise along a strong mediolateral gradient

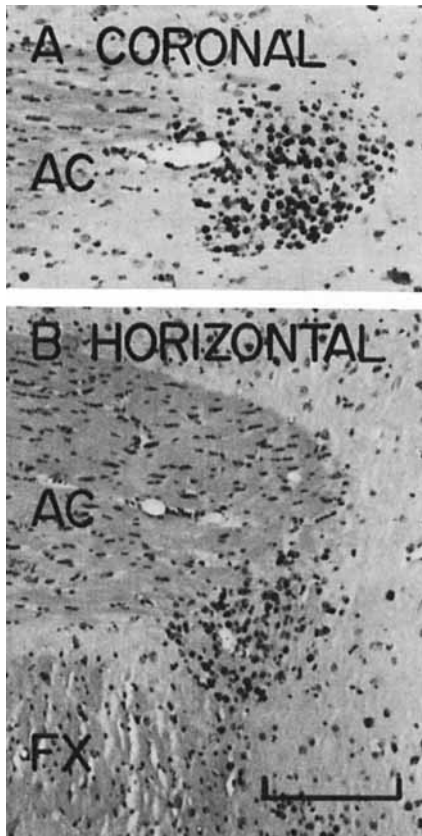


Fig. 17 The bed nucleus of the anterior commissure in both coronal (A) and horizontal (B) planes from an animal exposed to ^3H -thymidine on E15 + E16. The cells occupy a wedge-shaped area between the anterior commissure (AC) and the postcommissural columns of the fornix (FX). The neurons are tightly packed and are surrounded by a cell-sparse zone, which makes the nuclear boundary very distinct. Hematoxylin-eosin. Bar, 200 μm .

(fig. 23). This was observed by Creps ('74) in the mouse and Lawson et al. ('77) in the rat. Creps ('74) also observed that the rostral part of the lateral nucleus in the mouse was earlier forming. Since the data of figures 10 and 11 did not include the most rostral part, a strip at level A8.2 was analyzed in the same manner as described above. The labelling pattern was the same as for more caudal strips; the differences may be species-specific between rats and mice. The gradient of formation between the midline and lateral septal neurons shows the classic "outside-in" pattern described by Angevine ('65). The neuroepithelial source of these nuclei lies along the medial wall of the lateral ventricle and can be easily located in

the embryonic rat brain (Bayer, '79); younger neurons are sequentially deposited more laterally as their perikarya shift away from the receding neuroepithelium.

The unique formation pattern of the nucleus accumbens

Although the neurons of the nucleus accumbens are very similar to those of the bed nucleus of the stria terminalis (compare figs. 12-14 with figs. 19, 20) and no morphological boundaries separate the two, there are several differences between the formation patterns of the two nuclei which do not warrant classifying the nucleus accumbens as a rostral extension of the bed nucleus of the stria terminalis. In fact, neurogenesis in the nucleus accumbens is unique when compared with the septal region as a whole.

First, neurogenesis in the nucleus accumbens occurs much later than in any other septal region nuclear area (fig. 23). This late formation (taking place a few days before birth and extending into the early postnatal period) was initially observed in the rat by Das and Altman ('70) and was subsequently confirmed in both the mouse (Creps, '74) and rat (Lawson et al., '77). A contradictory finding in the rat reported neurogenesis to be complete by E17 (Swanson and Cowan, '75). On the contrary, E17 is the first day of cell formation when only 10% of the population originates in the ventral region (fig. 22B). At the same time, neurogenesis in the rostral part of the bed nucleus of the stria terminalis is declining on E17 and finishes on E18 (fig. 16E). This large difference in formation time is greater than the normal caudorostral gradient changes seen between adjacent levels in other regions of the bed nucleus of the stria terminalis (figs. 16C-E). Second, the nucleus accumbens does not have a caudorostral gradient of formation as does the precommissural segment of the bed nucleus of the stria terminalis (fig. 23). Third, neurogenesis in all areas so far examined in the septal region, including the bed nucleus of the stria terminalis, occurs over a two to three day period, while that in the nucleus accumbens is spread out over six to seven days. This longer formation time is more typical of neurons arising from a secondary germinal matrix (Altman, '66, '69; Bayer and Altman, '74). These differences suggest, and the next paper (Bayer, '79) will show, that the nucleus accumbens arises from a germinal matrix that is both active later and has a dif-

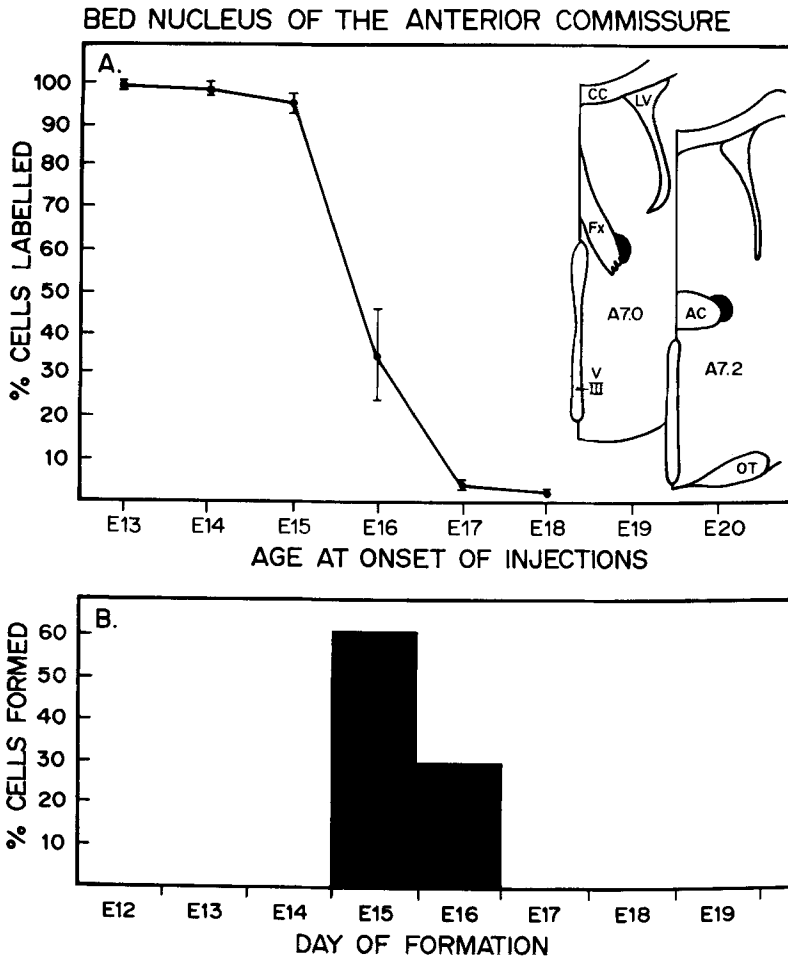
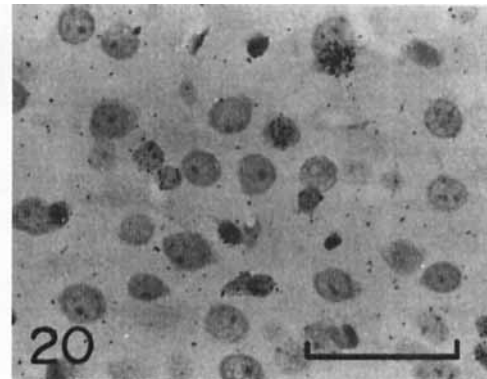
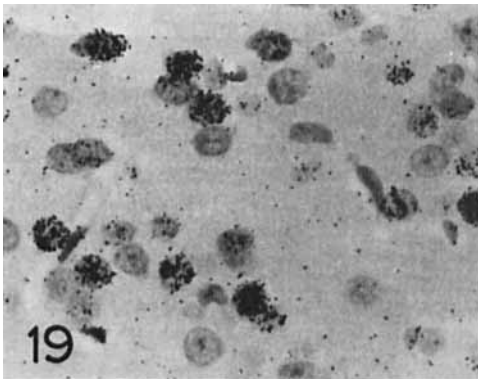


Fig. 18A Decline in the percentage of labelled cells during formation of the bed nucleus of the anterior commissure. Shaded areas in drawings indicate regions where cells were counted. Each mean with standard deviation is based on a group of approximately six animals.

B Proportion of cells added daily, derived from A. Almost all cells (>90%) form on E15 and E16. The few cells (<2%) formed on each of the other days are not shown.



Figs. 19, 20 Autoradiograms of nucleus accumbens neurons from an animal exposed to ^3H -thymidine on E21 + E22. Hematoxylin-eosin. Bar, 50 μm .

19 Neurons from dorsal area; these cells lie closer to the inferior horn of the lateral ventricle. Note that the proportion of labelled cells is greater than in figure 20.

20 Neurons from ventral area; the cells closely resemble those of the bed nucleus of the stria terminalis (figs. 12-14).

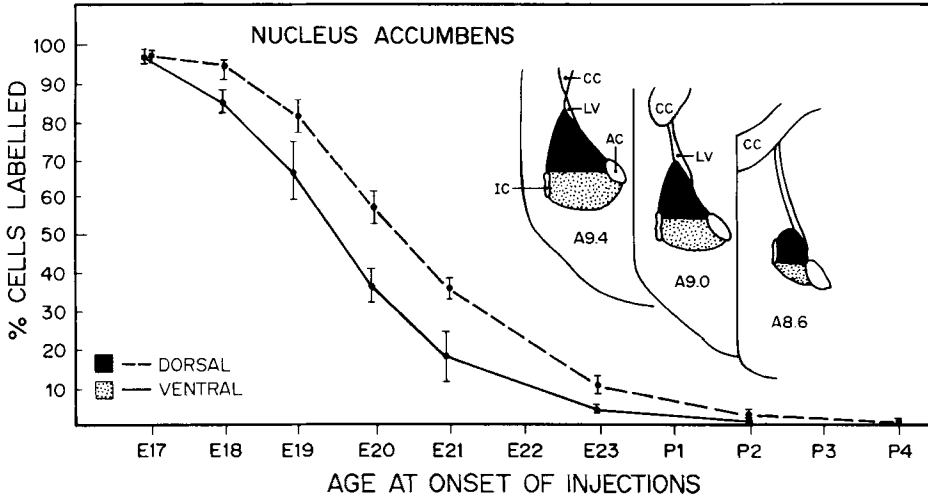


Fig. 21 Decline in percentage of labelled cells during formation of the nucleus accumbens. Shaded areas in drawings indicate regions where cells were counted. Each mean with standard deviation is based on a group of approximately six animals. There are no rostrocaudal differences; data represented in solid line are based on pooled counts from all ventral areas; dashed line, pooled data from dorsal areas.

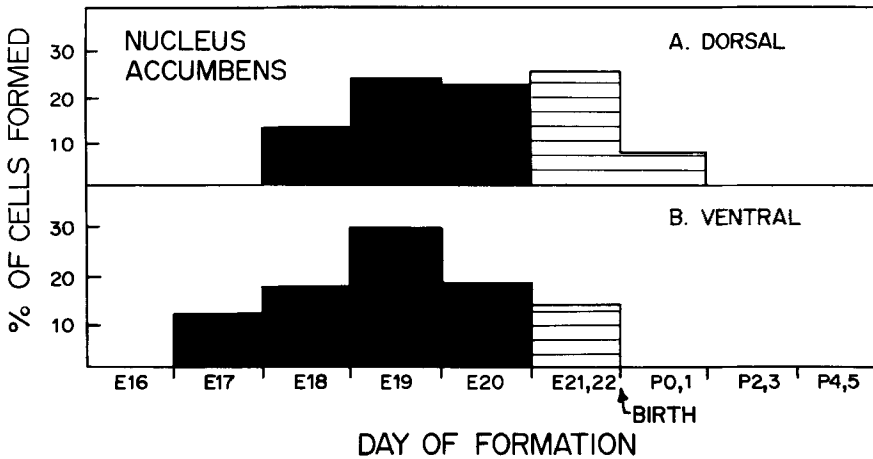


Fig. 22 Proportion of cells formed during each day of development in the nucleus accumbens, derived from figure 21.

A Dorsal area (derived from dashed line).

B Ventral area (derived from solid line); lined bars represent cell formation on a two-day period. Contrary to other septal region nuclei, neurogenesis occurs for almost a week.

GRADIENTS OF CYTOGENESIS IN THE SEPTAL REGION

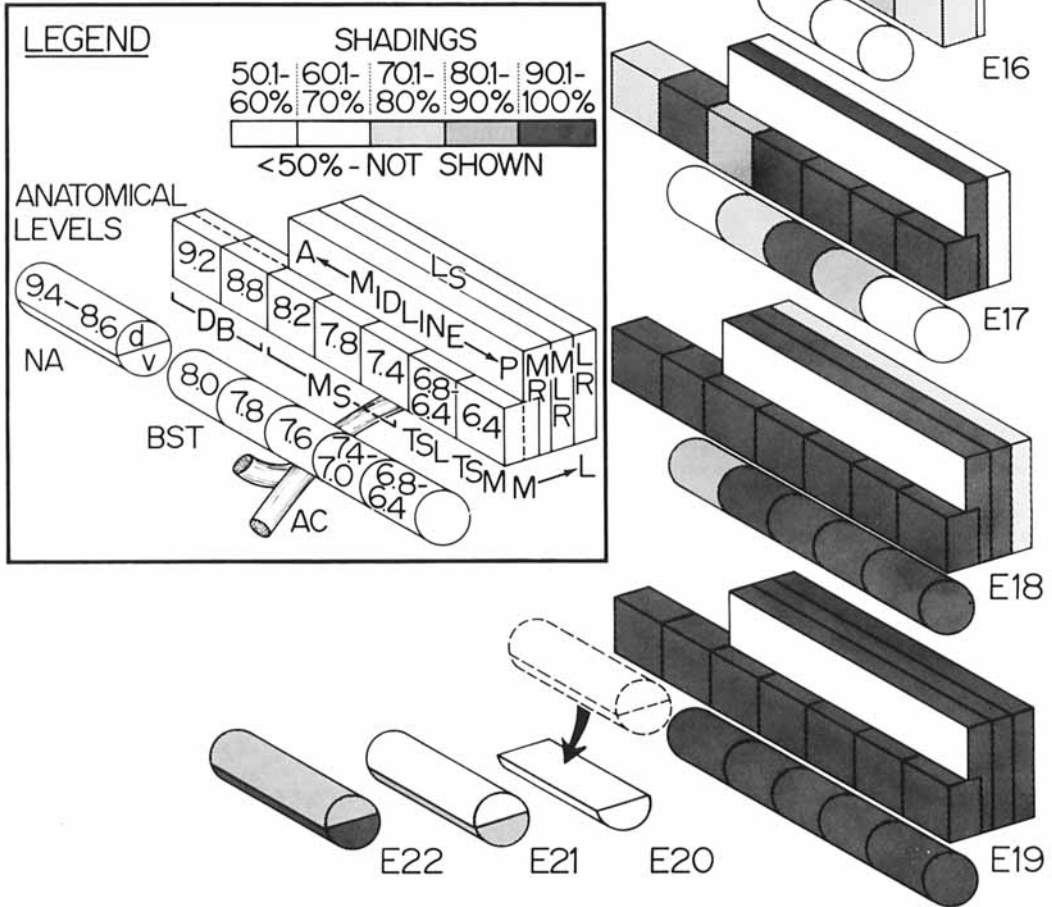


Fig. 23 A schematic diagram showing the gradients of cytogenesis observed in the septal region. The legend gives anatomical locations within the diagram: DB, diagonal band; MS, medial septal nucleus; TSL, lateral neurons of triangular septal nucleus; TSM, midline neurons of triangular septal nucleus; LS, lateral septal nucleus; MR, medial ridge of lateral septal nucleus; MLR, mediolateral ridge; LR, lateral ridge; NA, nucleus accumbens; d, dorsal nucleus accumbens; v, ventral nucleus accumbens; BST, bed nucleus of the stria terminalis; AC, anterior commissure; A, anterior; P, posterior; M, medial; L, lateral. The numbers within segments refer to A levels from Pellegrino and Cushman ('67) atlas. Shading density indicates accumulation of formed neurons by A.M. of embryonic day indicated. Areas which contained less than 50% neurons formed are not shown. By following the diagram from E15 through E22, one can see each group of septal region nuclei "grow" along a gradient. For further details, see text.

ferent direction of growth than the germinal zone giving rise to the bed nucleus of the stria terminalis.

ACKNOWLEDGMENTS

The author wishes to thank Joseph Altman for his advice and encouragement, George McCabe for statistical consultation, Sharon Evander for the histological preparation, and Mary Ward for typing the manuscript. This research was supported by the National Science Foundation, Grant BNS77-12622.

LITERATURE CITED

- Altman, J. 1966 Autoradiographic and histological studies of postnatal neurogenesis. II. A longitudinal investigation of the kinetics, migration and transformation of cells incorporating tritiated thymidine in infant rats, with special reference to postnatal neurogenesis in some brain regions. *J. Comp. Neur.*, 128: 431-474.
- 1969 Autoradiographic and histological studies of postnatal neurogenesis. IV. Cell proliferation and migration in the anterior forebrain, with special reference to persisting neurogenesis in the olfactory bulb. *J. Comp. Neur.*, 137: 433-458.
- Angevine, J. B. 1965 Time of neuronal origin in the hippocampal region. *Exp. Neurol. (Suppl.)*, 2: 1-70.
- Bayer, S. A. 1979 The development of the septal region in the rat. II. Morphogenesis in normal and X-irradiated embryos. *J. Comp. Neur.*, 183: 107-120.
- Bayer, S. A., and J. Altman 1974 Hippocampal development in the rat: Cytogenesis and morphogenesis examined with autoradiography and low-level X-irradiation. *J. Comp. Neur.*, 158: 55-80.
- 1975a Radiation-induced interference with postnatal hippocampal cytogenesis in rats and its long-term effects on the acquisition of neurons and glia. *J. Comp. Neur.*, 163: 1-20.
- 1975b The effects of X-irradiation on the postnatally-forming granule cell populations in the olfactory bulb, hippocampus, and cerebellum of the rat. *Exp. Neurol.*, 48: 167-174.
- Cajal, S. R. 1911 *Histologie du Système Nerveux de l'Homme et des Vertébrés*. Vol. II. Instituto Ramón y Cajal, Madrid.
- Conover, W. J. 1971 *Practical Nonparametric Statistics*. John Wiley, New York.
- Creps, E. S. 1974 Time of neuron origin in preoptic and septal areas of the mouse: An autoradiographic study. *J. Comp. Neur.*, 157: 161-244.
- Das, G. D., and J. Altman 1970 Postnatal neurogenesis in the caudate nucleus and nucleus accumbens septi in the rat. *Brain Res.*, 21: 122-127.
- Fox, C. A. 1940 Certain basal telencephalic centers in the cat. *J. Comp. Neur.*, 72: 1-62.
- Gurdjian, E. S. 1925 Olfactory connections in the albino rat, with special reference to the stria medullaris and the anterior commissure. *J. Comp. Neur.*, 38: 128-163.
- Hine, R. J., and G. D. Das 1974 Neuroembryogenesis in the hippocampal formation of the rat: An autoradiographic study. *Z. Anat. Entwickl.-Gesch.*, 144: 173-186.
- Johnston, J. B. 1913 The morphology of the septum, hippocampus, and pallial commissures in reptiles and mammals. *J. Comp. Neur.*, 23: 371-478.
- 1923 Further contributions to the study of the evolution of the forebrain. *J. Comp. Neur.*, 35: 337-481.
- Lauer, E. W. 1945 The nuclear pattern and fiber connections of certain basal telencephalic centers in the marmoset. *J. Comp. Neur.*, 82: 215-254.
- Lawson, S. N., M. K. May and T. H. Williams 1977 Prenatal neurogenesis in the septal region of the rat. *Brain Res.*, 129: 147-151.
- MacLean, P. D. 1952 Some psychiatric implications of physiological studies on frontotemporal portion of limbic system (visceral brain). *EEG. Clin. Neurophysiol.*, 4: 407-418.
- Meibach, R. C., and A. Siegel 1977a Efferent connections of the septal area in the rat: An analysis using retrograde and anterograde transport methods. *Brain Res.*, 119: 1-20.
- 1977b Efferent connections of the hippocampal formation in the rat. *Brain Res.*, 124: 197-224.
- Papez, J. W. 1937 A proposed mechanism of emotion. *Arch. Neurol. Psych.*, 38: 725-744.
- Pellegrino, L. J., and A. J. Cushman 1967 *A Stereotaxic Atlas of the Rat Brain*. Appleton-Century-Crofts, New York.
- Swanson, L. W., and W. M. Cowan 1975 A note on the connections and development of the nucleus accumbens. *Brain Res.*, 92: 324-330.
- 1976 Autoradiographic studies of the development and connections of the septal area in the rat. In: *The Septal Nuclei*. J. W. DeFrance, ed. Plenum, New York, pp. 37-64.
- 1977 An autoradiographic study of the organization of the efferent connections of the hippocampal formation in the rat. *J. Comp. Neur.*, 172: 49-84.
- Young, M. W. 1936 The nuclear pattern and fiber connections of the noncortical centers of the telencephalon of the rabbit (*Lepus cuniculus*). *J. Comp. Neur.*, 65: 295-401.