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^{3}H-Thymidine-radiographic Studies of Neurogenesis in the Rat Olfactory Bulb*

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Summary. Neurogenesis in the rat olfactory bulb was examined with ^{3}H-thymidine-radiography. For the animals in the prenatal groups, the initial ^{3}H-thymidine exposures were separated by 24 h; they were the offspring of pregnant females given two injections on consecutive embryonic (E) days (E12–E13, E13–E14, . . . E21–E22). For the animals in the postnatal (P) groups, the initial ^{3}H-thymidine injections were separated by 48 h, each group receiving either four (P0–P3, P2–P4, . . . P6–P9) or two (P8–P9, P10–P11, . . . P20–P21) consecutive daily injections. On P60, the percentage of labeled cells and the proportion of cells added during either 24 h or 48 h periods were quantified at several anatomical levels for each neuronal population in the main olfactory bulb (mitral cells, tufted cells, granule cells, interneurons in the external plexiform layer, periglomerular granule cells) and accessory olfactory bulb (output neurons, granule cells, periglomerular granule cells). The total time span of neurogenesis extends from E12 to beyond P20. Output neurons are prenatally generated over 5–9 day periods (with most neurogenesis occurring over 2–4 days) in a strict sequential order beginning with the accessory bulb output neurons (E13–E14) and ending with the interstitial tufted cells lying between the glomeruli in the main bulb (E20–E22). These data are correlated with the main and accessory bulb projection fields in the amygdala and with the chronology of amygdala neurogenesis. With the exception of the granule cells in the accessory bulb (88% generated between E15–E22), the rest of the interneuronal populations are generated postnatally and nearly simultaneously. While most neurons (75–80%) originate during the first three weeks of life, all interneuronal populations, including accessory bulb granule cells, show some neurogenesis beyond P20. Injections of ^{3}H-thymidine in juvenile and adult rats indicates neurogenesis up to P60 in the accessory bulb and up to P180 in the main bulb, especially in the main bulb granule cell population. There is circumstantial evidence for turnover of main bulb granule cells during adult life.

Key words: Main olfactory bulb – Accessory olfactory bulb – Neurogenetic patterns – ^{3}H-thymidine-radiography

Introduction

To contribute to a better understanding of the intricate anatomical interconnections which weld various brain structures together to form a system, a series of developmental studies of each structure of the limbic system was prepared using ^{3}H-thymidine-radiography (Bayer 1979a, 1980a, b) with the progressively delayed comprehensive labeling technique (Bayer and Altman 1974). This method provides an accurate estimate of the proportion of neurons originating on each day of embryonic life and on blocks of two to four days of early postnatal life. The rationale for initiating this research was to see whether or not the chronology of neurogenesis within and between structures could be related to their patterns of anatomical connections. Several correlations between neurogenetic timetables and anatomical interconnections have been found in the septal and hippocampal regions (Bayer 1980a). This report on olfactory bulb neurogenesis is the first in a series to study each structure of the olfactory system. Evidence will be provided that the sequence of neurogenesis in the olfactory bulb can be related to the pattern of amygdaloid innervation and neuro-

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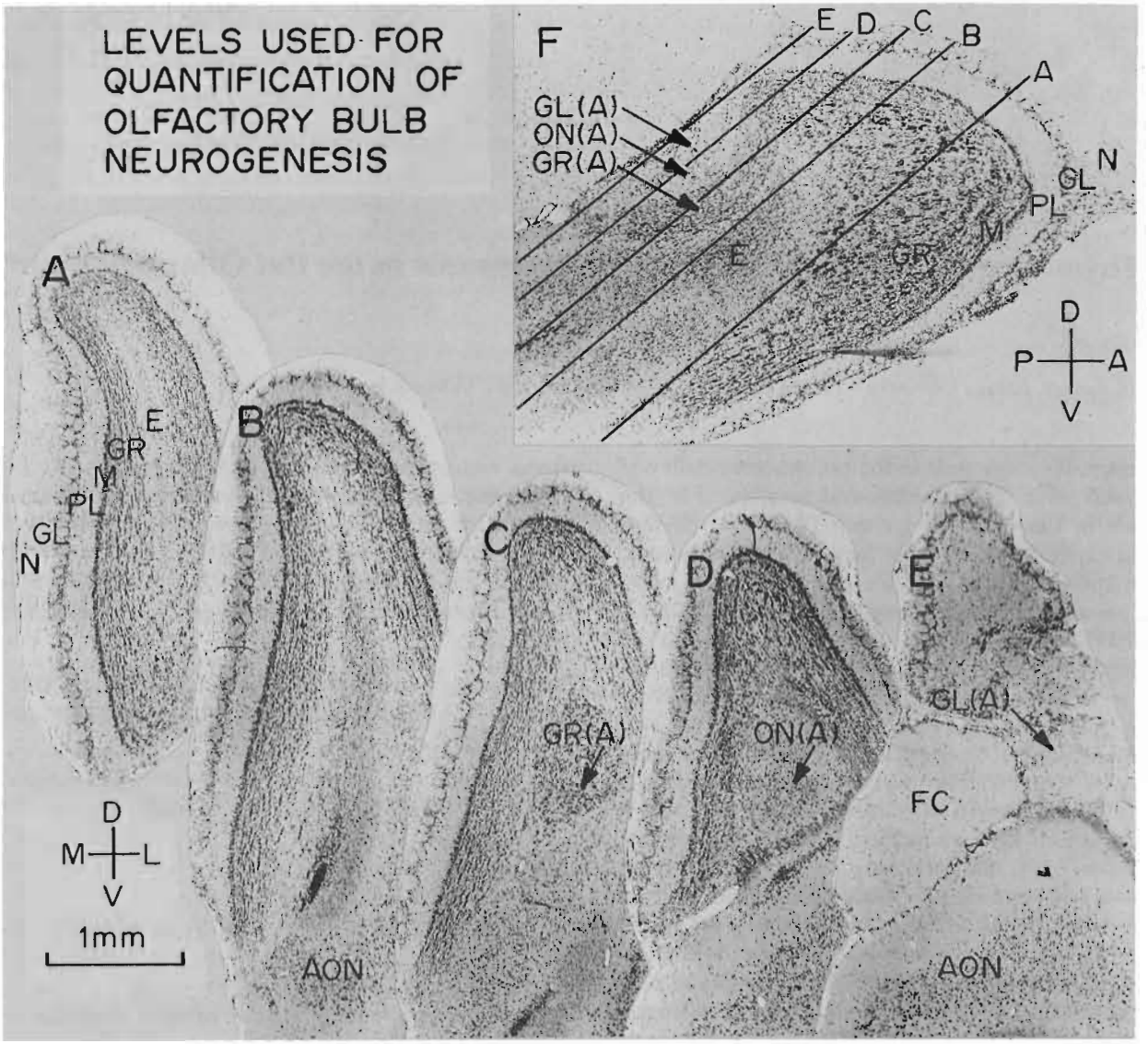

Fig. 1A–F. Low magnification views of the sections used for quantification of olfactory bulb neurogenesis. **A–E** are coronal sections cut according to the stereotaxic angle of Pellegrino et al. (1979). The approximate plane of each coronal section is indicated in the sagittal section, **F** (paraffin, 6μm, hematoxylin and eosin). Abbreviations: Main olfactory bulb: *N* olfactory nerve layer; *GL* glomerular layer; *PL* external plexiform layer; *M* mitral cell layer; *GR* granule cell layer; *E* ependymal layer. Accessory olfactory bulb: *GR(A)* granular layer; *ON(A)* output neuron layer; *GL(A)* glomerular layer; *AON* anterior olfactory nucleus; *FC* frontal cortex; *D* dorsal; *V* ventral; *M* medial; *L* lateral; *A* anterior; *P* posterior

genesis (Bayer 1980b). Possibly, the precise order of neurogenesis within and between brain structures is a prerequisite for the establishment of appropriate anatomical connections.

To date, there have only been a few ^{3}H-thymidine-radiographic studies of olfactory bulb neurogenesis, each using the single injection technique. Hinds (1967) described both pre- and postnatal development in the mouse, and Altman (1969) discussed the pattern of postnatal and juvenile acquisition of granule cells in the main bulb of the rat. None of these studies provided quantification of the proportion of neurons originating over 24–48 h periods; the total amount of postnatal neurogenesis, which is prominent in most interneuron populations in the olfactory bulb, has never been quantified. The aim of this report is to provide a complete and accurate picture of olfactory bulb neurogenesis so

Table 1. Neurogenesis of the output neurons in the accessory olfactory bulb

Injection group	N	% Labeled cells (mean ± SD)	Embryonic day	% Cells originating[a]
E12–E13	7	(A) 99.14 ± 0.38	12	8.39 (A–B)
E13–E14	8	(B) 90.75 ± 5.65	13	61.15 (B–C)
E14–E15	5	(C) 29.6 + 4.77	14	20.77 (C–D)
E15–E16	6	(D) 8.83 ± 2.32	15	5.83 (D–E)
E16–E17	12	(E) 3.0 ± 1.65	16	3.0 (E–F)
E17–E18	9	(F) 0	17	0

[a] These data are graphically represented in Fig. 3

that reliable correlations between chronologies of neurogenesis and patterns of anatomical interconnections can be made.

Methods

Since neurogenesis in the rat olfactory bulb extends will beyond the day of birth, both prenatal and postnatal developmental series were used. All series contained groups of Purdue-Wistar rats given successive daily (between 9 and 11 a.m.) subcutaneous injections of ^{3}H-thymidine (Schwarz-Mann; specific activity 6.0 CimM; 5μCi/g, b.wt.); multiple injections were given to insure comprehensive cell labeling. The prenatal developmental series contained ten groups, the offspring of pregnant females given two successive daily injections progressively delayed by one day between groups (E12–E13, E13–E14, . . . E21–E22). The day of sperm positivity was embryonic day one (E1); two or more pregnant females were injected for each group. Normally, births occur on E23, which is also designated as postnatal day zero (P0). The postnatal developmental series had 11 groups of rat pups, each group containing males from at least two litters (N = 6/litter). The pups were given either four (P0–P3, P2–P4, . . .P6–P9) or two (P8–P9, P10–P11, . . . P20–P21) consecutive daily injections.

All animals were perfused through the heart with 10% neutral formalin on P60. The brains were kept for 24 h in Bouin's fixative, then transferred to 10% neutral formalin until they were embedded in paraffin. The brains of at least five to six animals from each group were blocked coronally according to the stereotaxic angle of the Pellegrino et al. (1979) atlas. Every 15th section (6 μm) through the olfactory bulb was placed on a microscopic slide. Slides were dipped in 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 at five levels throughout the anteroposterior extent of the olfactory bulb (Fig. 1A–E). The proportion of labeled cells was determined microscopically at 500 × with the aid of an ocular grid. All cells with reduced silver grains overlying the nucleus in densities above background levels were considered labeled; 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 labeling procedure (Bayer and Altman 1974), and is described in detail elsewhere (Bayer 1980b). Briefly, a progressive drop in the proportion of labeled neurons from a maximal level (> 95%) in a specific population indicates that the precursor cells are actively producing neurons; when all neurons are unlabeled, all population members have originated. By analyzing the rate of decline in labeled neurons, one can determine the proportion of neurons originating over blocks of days (or single days) during development. Table 1 shows the data and calculations for the output neurons of accessory olfactory bulb.

For many late-originating populations of interneurons in the olfactory bulb, the multiple injections given were insufficient to label between 95–100% of the cells. Consequently, an additional experiment was performed to see what proportion originated postnatally. Six litters of male Purdue-Wistar rat pups (N = 6/litter) were injected with ^{3}H-thymidine on P0–P3 as described above. In order to alleviate the label dilution problem, one animal from each litter was given four additional injections on P10, P20, P30, and P40 and was killed on P60. One animal from the five remaining members of each litter was killed on P10, P20, P30, P40 and P60. All the brains were processed as described above, except that serial sections were prepared in the sagittal plane. The change in the proportion of labeled cells was analyzed as a function of survival time after injection. At P60, the effect of adding extra injections during the later postnatal and juvenile periods was also assessed.

Most olfactory bulb interneuron populations show some neurons still remain labeled in the P20–P21 injection group. Consequently, an experiment was performed to see if neurons could be labeled during juvenile and adult life. Groups of Purdue-Wistar male rats were given four consecutive daily injections of ^{3}H-thymidine on P30–P33 (N = 9), P60–P63 (N = 5), P120–P123 (N = 6) and P180–P183 (N = 4). Three animals from the P30–P33 injection group were killed on P60; all remaining animals were killed on P200. The brains were prepared in the same way as in the preceding experiment. The proportion of labeled neurons in each interneuron population in the olfactory bulb was determined, and the effects of both a change in survival time (P30–P33 group) and in injection time were assessed.

Trends in cell labeling were analyzed in individual animals with the sign test (Conover 1971); the rationale for the use of this statistic is provided elsewhere (Bayer 1980b). A one-way analysis of variance was applied to the data of several groups; the *T*-test was used when only two groups were compared.

Results

The Output Neurons

Accessory Olfactory Bulb. Cajal (1955) does not call the output neurons of the accessory olfactory bulb mitral cells since Golgi impregnations do not reveal true mitral cell structure. Figure 2B gives a high magnification view of these cells in a coronal section which passes tangentially through the layer (similar to level D, Fig. 1). Large, medium, and small sized neurons are present; only large and medium sized cells were considered output neurons. The autoradiogram in Fig. 2B is from an animal exposed to ^{3}H-thymidine on E15–E16. Most of the output neurons are unlabeled indicating that the population has nearly completed neurogenesis by this time. The proportion of labeled output neurons was quantified separately in medial and lateral areas (0.085 mm^2) at level D (Fig. 1). The sign test showed no differences between these areas so these data are combined in Fig. 3. Some neurogenesis occurs as early as E12, but

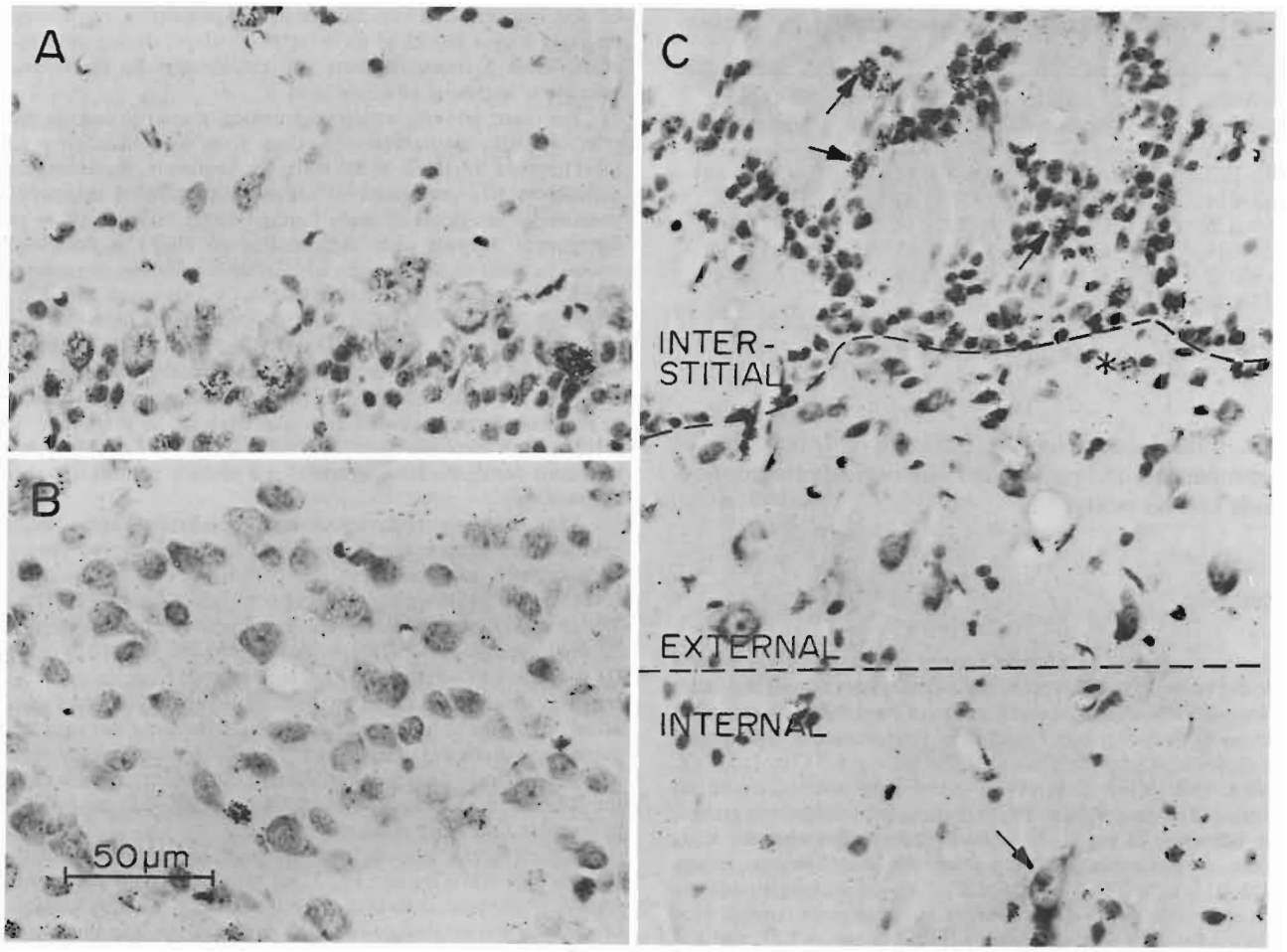

Fig. 2A–C. Autoradiograms from an animal exposed to ^{3}H-thymidine on E15–E16. **A** Mitral cells in the main bulb; **B** output neurons in the accessory bulb; **C** main bulb tufted cells divided into internal, external, and interstitial layers after Cajal (1955). *Arrows* in **C** indicate labeled interstitial tufted cells and an unlabeled internal tufted cell; *asterisk* in **C** points out a labeled external tufted cell (paraffin, 6 μm, hematoxylin and eosin)

E13 is the peak day of cell production when 60% of the population originates.

Main Olfactory Bulb

1. Mitral Cells. Figure 2A shows a high magnification view of the mitral cells in an animal exposed to ^{3}H-thymidine on E15–E16. Many of the large and medium sized cells, both considered to be mitral cells by Cajal (1955), are labeled indicating that most of the population originates on or after E15, much later than the output neurons in the accessory olfactory bulb. The proportion of labeled cells was separately quantified in medial and lateral parts of the main bulb at levels A and B in Fig. 1. The sign test showed no differences between these areas so the data are combined in Fig. 3. Only a small amount of neurogenesis occurs on E12–E14; over 80% of the population originates on E15 and E16, and neurogenesis is complete on E18. The output neurons of the accessory bulb originate significantly earlier ($p < 0.001$, sign test) than those of the main bulb.

2. Tufted Cells. Since Cajal (1955) described axons from tufted cells projecting beyond the limits of the olfactory bulb, they are also considered as output neurons. In agreement with Cajal's terminology, the large cells in the inner half of the external plexiform layer were called internal tufted cells, those lying in the outer half were called external tufted cells, and those scattered between the glomeruli were called interstitial tufted cells. In this study tufted cells were recognized by their relatively larger size than surrounding interneurons and glia. There is a size gradient between the various types of tufted cells:

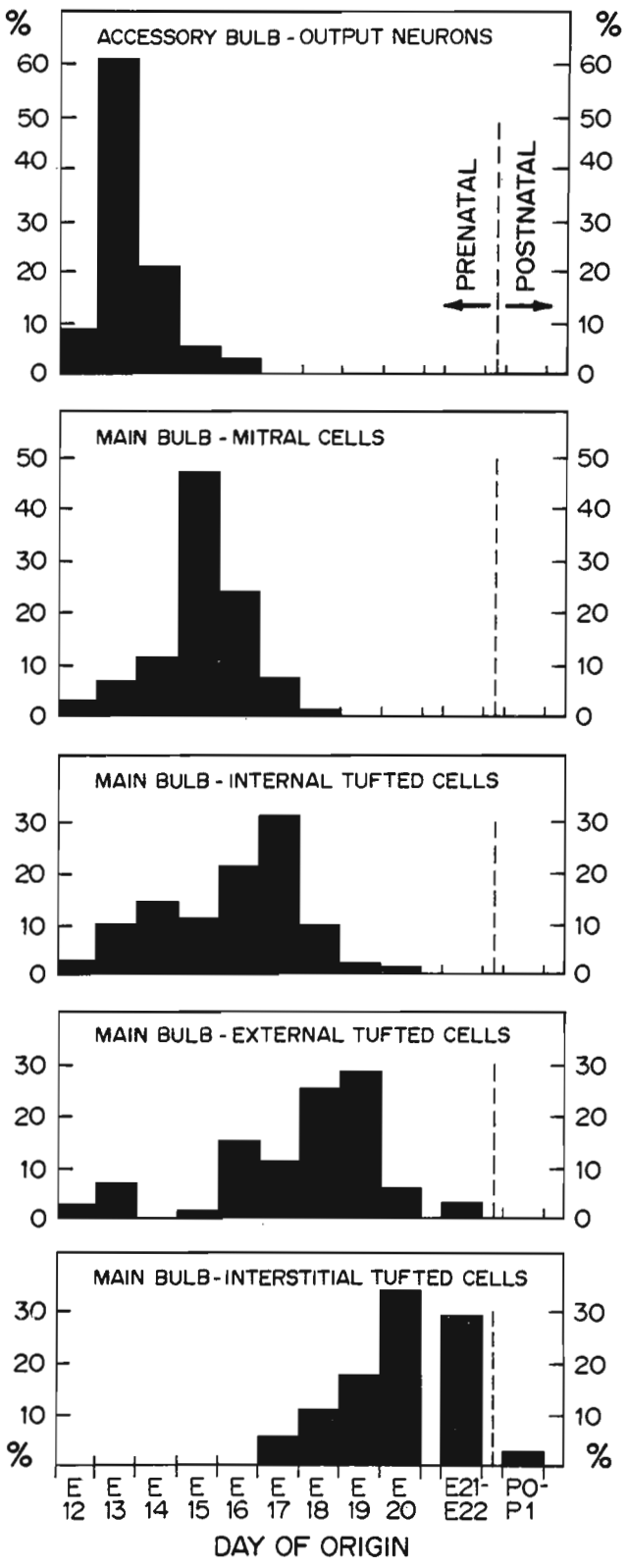

Fig. 3. Neurogenesis of the output neurons in both main and accessory parts of the olfactory bulb. *Dashed line* indicates the time of birth. Note the strong sequential gradient between the populations

internal are largest and external are smallest. These size differences are evident in Fig. 2C which is an autoradiogram from an animal exposed to ^{3}H-thymidine on E20–E21. There is only one external tufted cell labeled (asterisk, Fig. 2C), while several interstitial tufted cells are labeled (arrows, Fig. 2C) indicating a neurogenetic gradient between tufted cells in different locations. The proportion of labeled cells was separately quantified for each group of tufted cells in anterior and posterior sections (levels A and B, Fig. 1). The sign test showed no differences along the anteroposterior dimensions so the data were combined for each group in the lower three graphs in Fig. 3. The sign test showed that there is a strong deep to superficial gradient between the three tufted cell populations ($p < 0.0001$, all comparisons). Most of the internal tufted cells have originated by E18, before the peak neurogenetic period (E18 and E19) for the external tufted cells. Still later, between E20–E22, approximately 60% of the interstitial cells originate. The sign test also showed that the tufted cells originate significantly later ($p < 0.0001$) than the mitral cells, continuing the deep to superficial gradient throughout all output neurons in the main bulb.

The Interneurons

Accessory Olfactory Bulb

1. Periglomerular Granule Cells. Coronal sections passing through the glomerular layer show only faintly circular masses of fiber bundles sparsely surrounded by small cells; the proportion of labeled cells was counted in level E (Fig. 1). The peak labeling that could be obtained with two prenatal injections was approximately 90% in the E17–E18 group. From then on, the proportion of labeled cells gradually declines. Neurogenesis of the periglomerular granule cells occurs much later than that of the deep granule cells in the accessory bulb ($p < 0.0001$, sign test; Fig. 4), and approximately 68% of the cells originate postnatally, the majority arising before P16. On P20, slightly over 1% of the neurons (possibly glia) are still labeled.

2. Granule Cells. The granule cells of the accessory olfactory bulb lie in a thick crescent-shaped lamina (Fig. 1F) just beneath the layer of output neurons. Coronal sections which pass tangentially through the layer show it to be a circular mass embedded between the medial and lateral limbs of the granular layer of the main bulb (Fig. 1C). The proportion of labeled cells was separately quantified in unit areas (0.04 mm^2) in four quadrants (dorsal, ventral, medial,

lateral) at level C (Fig. 1). The sign test showed no differences between the dorsal and medial parts and between the ventral and lateral parts so the respective sets of data were combined. Neuron origin in the dorsomedial part is slightly (approximately 5%) ahead of that in the ventromedial part ($p < 0.004$, sign test). Since the two sets of data are very similar, only that for the ventromedial part is illustrated in Fig. 4. There is a prominent amount of prenatal neurogenesis on E17–E19 and only between 10–13% of the neurons originate over a protracted postnatal period. Between P0–P13, 7–10% originates. From P14 through P20 approximately 2–3% of the neurons remain labeled, indicating an even later origin.

Main Olfactory Bulb

1. Periglomerular Granule Cells. The proportion of labeled cells was determined in four unit lengths (0.184 mm) medially and laterally at levels A and B (Fig. 1). At each location, superficial and deep halves of the glomerular layer were separately quantified. The sign test showed no differences between medial and lateral parts or between anterior and posterior locations. In all cases, deep cells originated slightly earlier ($p < 0.0001$) than superficial cells and the data were combined into superficial and deep sets. Due to similarities in pattern, only data for neurogenesis of the deep periglomerular granule cells are illustrated in Fig. 4. Approximately 76% of the deep cells can be labeled in the P0–P3 injection group. Two consecutive daily prenatal injections are insufficient to give 100% cell labeling. During the first postnatal week, approximately 56% of the neurons are generated; 17.5% are added during the second week, and less than 1% are added during the third week. On P20, about 3% of the neurons (glia?) remain labeled.

2. External Plexiform Interneurons. Besides the cell bodies of the tufted cells, the external plexiform layer also contains cell bodies of smaller neurons which Cajal (1955) stated were short axon cells of "uncertain" structure. The proportion of labeled cells was determined in four adjacent unit lengths (0.184 mm) medially and laterally at levels A and B (Fig. 1). At each level, superficial and deep parts were quantified separately. The sign test showed no differences between medial and lateral parts or between anterior and posterior locations. In all cases, superficial cells originated slightly earlier ($p < 0.001$) than deep cells, and the data were combined into two sets. Due to similarities in pattern, only data for neurogenesis in the deep external plexiform interneurons are illustrated in Fig. 4. Approximately 83% of the neurons are labeled in the P0–P3 injection group. Two consecutive daily prenatal injections are insufficient to give 100% cell labeling. Most neurons are generated on or before P8 with a prominent peak on P4–P5 (75.5%). Between P10–P20, only 6% are generated; on P20, approximately 2% of the neurons, possibly glial cells, remain labeled.

3. Granule Cells. The proportion of labeled cells was determined in six different locations at levels A and B (Fig. 1). Superficial granule cells in the mitral cell layer were counted in four unit lengths (0.184 mm) medially, dorsally, and laterally; deep granule cells beneath the internal plexiform layer were counted in unit areas (0.04 mm^2) in the same locations. The sign test showed no differences between medial, dorsal, and lateral locations or between anterior and posterior levels, but in all cases, superficial cells tended to originate slightly earlier than deep cells ($p < 0.0001$), and the data were combined into superficial and deep sets. Due to similarities in pattern, only the data for neurogenesis of the deep granule cells are illustrated in Fig. 4. Approximately 75% of the cells can be labeled in the P0–P3 group, but there is additional evidence (to be discussed below) that a larger proportion of the cells arise postnatally. Approximately 41% originates during the first postnatal week, 23% during the second week, and 7% during the third week. On P20, nearly 7% remains labeled indicating a prominent level of very late neurogenesis.

The Level of Postnatal Neurogenesis in the Interneurons of the Main Olfactory Bulb

Since none of the interneuronal cell populations in the main olfactory bulb could be maximally labeled by any injection group, an additional experiment was performed to see whether the proportion of each population which originates postnatally was accurately determined in the data of Fig. 4. In this experiment, all animals were injected on P0–P3, and some were given additional injections during the later postnatal and juvenile periods to retard the effect of label dilution (see Methods). At P60, the proportion of labeled cells in either unit areas (0.04 mm^2) or unit lengths (0.184 mm) was compared between the group receiving four injections with the group receiving additional injections for each population of interneurons in the main bulb. The extra injections label significantly more granule cells but not any other cell type. This means that label dilution is a problem in the granule cell population of the main bulb; instead of 25%, only between 9% (deep) and 17% (superficial) originate prenatally.

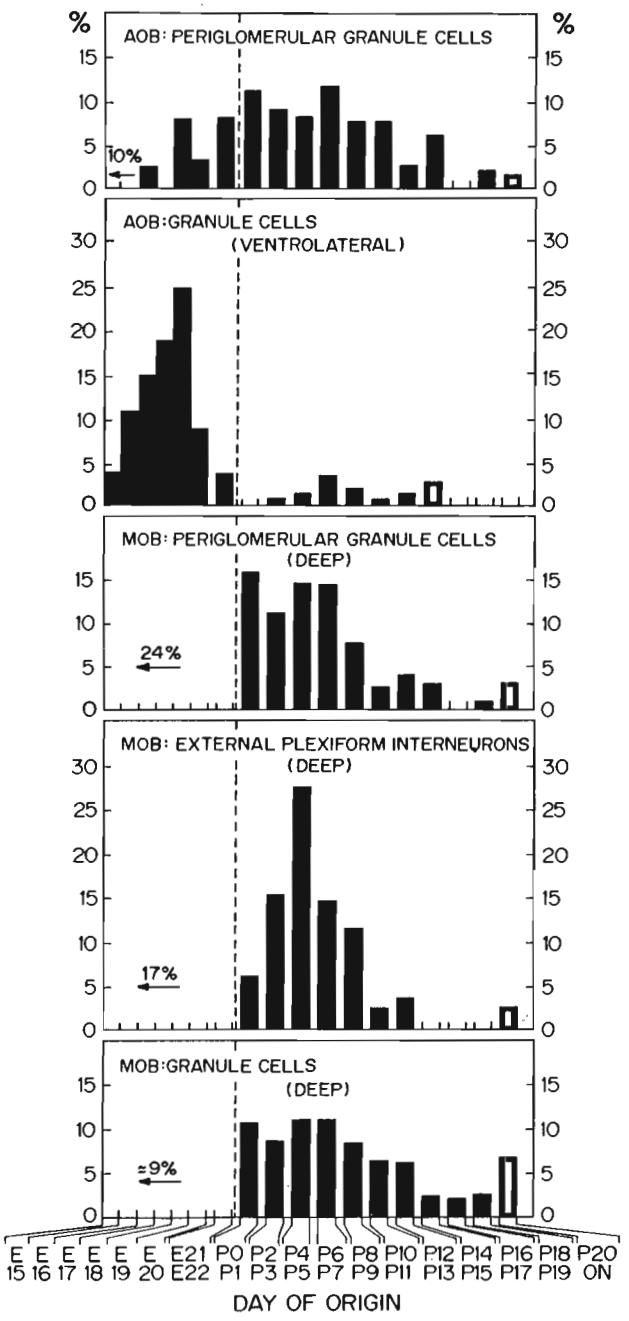

Fig. 4. Neurogenesis of the interneurons in both main and accessory parts of the olfactory bulb. *Dashed line* indicates the time of birth. Most populations (except the accessory bulb periglomerular granule cells) have weak intrinsic neurogenetic gradients. Since neurogenetic patterns are very similar, data for only one part of each population is illustrated. Note the prominent postnatal neurogenesis for all populations except the accessory bulb granule cells, and the persistent neurogenesis on the last day of observation (*open bars*)

To see if a decrease in survival time would result in a maximal level of cell labeling (similar to the extra injection group) in the main bulb granule cells, some

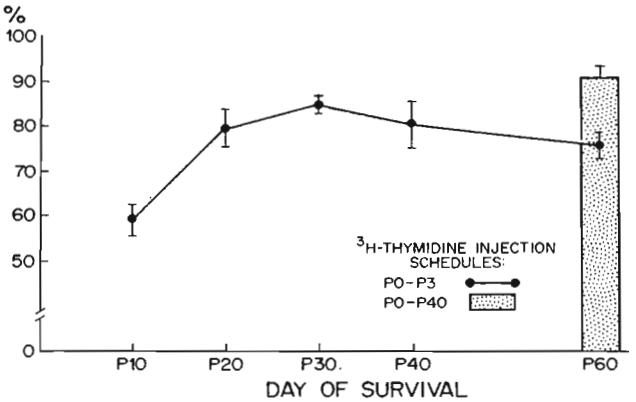

Fig. 5. The proportion of main olfactory bulb granule cells (deep to the internal plexiform layer) labeled after four consecutive injections on P0–P3. Line graph indicates proportional changes in increasing survival time up to P60; points are means with standard deviations. Bar graph indicates the effect of an additional four injections (to make a total of eight) on P10, P20, P30, and P40

animals were killed at various times before P60 (see Methods). The proportion of labeled cells was determined in either unit areas (0.04 mm^2, deep granule cells) or unit lengths (0.184 mm, superficial granule cells). The results for the deep granule cells are given in Fig. 5. The analysis of variance showed significant changes in the proportion of labeled cells with respect to survivial time ($F = 37.62$, $p < 0.00001$). There is a significant proportional increase between P10 and P30 ($p < 0.01$, Scheffé test) reflecting the progressive differentiation of mature granule cells from precursors still labeled by the P0–P3 injections. Between P30 and P60 there is a significant drop ($p < 0.01$, Scheffé test) in the proportion of labeled cells as the precursors (most of which are no longer labeled by the P0–P3 injection) continue to differentiate into mature granule cells. Data for the superficial granule cells is similar in pattern, but there is a more shallow peak at P30.

Interneuronal Neurogenesis in the Olfactory Bulb During Juvenile and Adult Life

The persistent neurogenesis beyond P20 was further quantified in a series of animals given injections of ^{3}H-thymidine during juvenile and adult life (see Methods). The proportion of labeled cells was determined in several unit areas (0.04 mm^2); no distinction was made between superficial or deep neurons in any population. While care was taken to eliminate glial cells from the counts, it should also be noted that small interneuronal nuclei are difficult to distinguish from those of astrocytes in paraffin sections and the data may be somewhat contaminated. In all inter-

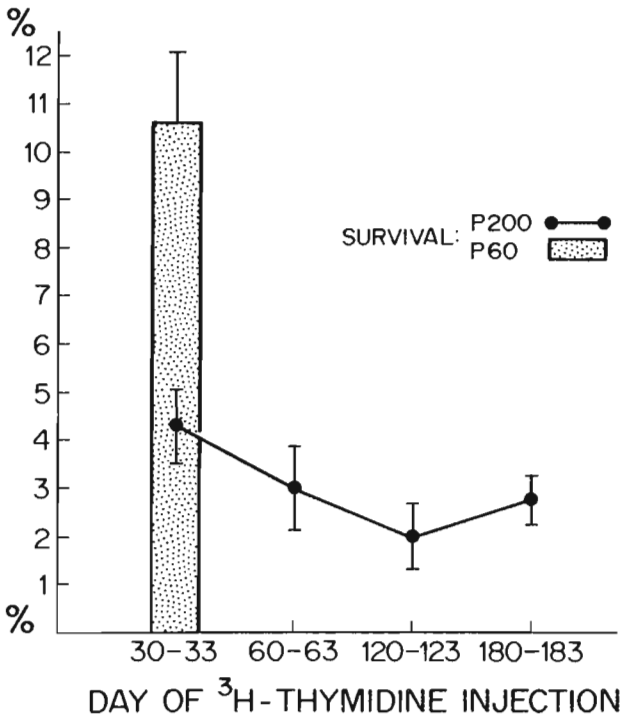

Fig. 6. The proportion of granule cells in the main olfactory bulb labeled with four consecutive ^{3}H-thymidine injections during juvenile (P30–P33) and adult (P60 on) life. Line graph shows the proportion as determined at a P200 survival date. Bar graph shows a higher proportion of labeled cells is attained in the P30–P33 group if survival time is decreased to P60. All points are means with standard deviations

neuron populations, there is some evidence for neurogenesis extending up to P60, although the cells labeled in the accessory bulb are extremely sparse and may be glia. In the main bulb, neurogenesis continues up to at least P180 in all populations. Since the main bulb granule cells illustrate these trends most prominently, their data are shown in Fig. 6. First, there is a significant decrease in the proportion of labeled cells in injection schedules beyond the P30–P33 group ($F = 10.87$, $p < 0.0003$). Second, there is a significant decrease in the proportion of labeled cells if the survival time in the P30–P33 group is extended from P60 to P200 ($T = 8.72$, $p < 0.0001$, two-tailed probability).

Discussion

The Sequential Pattern of Neurogenesis in the Olfactory Bulb

One of the strongest patterns of neurogenesis in the rat olfactory bulb is that neurons are generated in

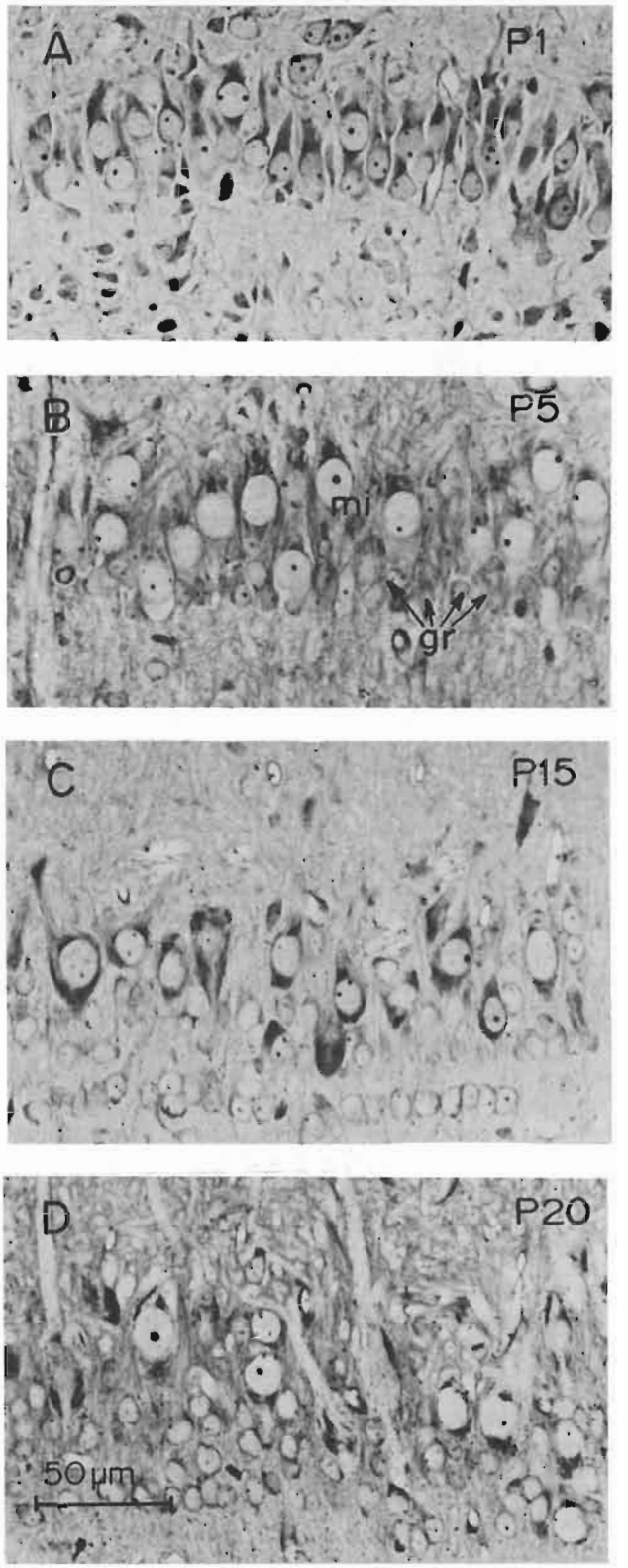

Fig. 7A–D. The mitral cell layer from P1 (**A**) to P20 (**D**). On P1 there are only mitral cells in the layer; with increasing time, granule cells (*gr*) eventually become more numerous than mitral cells (*mi*) (methacrylate, 3 µm, toluidine blue)

strict sequential order between and within the accessory and main portions of the bulb, as previously noted by Hinds (1967) in mice. Generally, the accessory olfactory bulb leads the main olfactory bulb in the generation of neurons of a particular class. The output neurons of the accessory bulb have nearly completely originated before the peak time of origin for the mitral cells (Fig. 3). Accessory bulb interneurons are generated earlier than main bulb interneurons (Fig. 4). Within the accessory bulb, output neurons are first to be generated, granule cells are next, and finally the periglomerular granule cells are produced (Figs. 3–4). Within the main bulb, the output neurons are generated in strict sequential order from the first mitral cells to the last interstitial tufted cells (Fig. 3). Only after the output neurons arise, do the various main bulb interneurons originate nearly simultaneously (Fig. 4). The sequential neurogenetic pattern is reflected in the appearance of output neurons and interneurons in the main bulb mitral cell layer. At the time of birth, there are prominent numbers of mitral cells present, and few interneurons (Fig. 7A); with increasing time, interneurons become much more numerous and eventually outnumber the mitral cells (Fig. 7B–D).

The total time for producing olfactory neurons spans from E12 to P20 and on into the adult period. This means that precursor cells of olfactory neurons must also be present over the same time span. The early output neurons of the accessory olfactory bulb and the main bulb mitral cells originate before the morphological appearance of the olfactory bulb. These neurons are probably generated by the neuroepithelium in the basal telencephalon, then migrate forward, possibly in response to the developing olfactory nerve fibers (Humphrey 1966), to form the olfactory bulb. By the time that most of the interneurons are generated, the neuroepithelium along the basal telencephalon is changing into the primitive ependyma and is no longer producing neurons (Bayer 1979b). On the other hand, the subependymal layer is prominent during late prenatal and postnatal development and is the most likely source of olfactory interneurons (Altman 1969). Thus, there is a sequential production of precursor cell types as well as neuronal cell types in the olfactory bulb, a pattern noted earlier by Hinds (1967).

Correlations Between Neurogenesis in the Olfactory Bulb and its Anatomical Connections

The sequential order of neurogenesis between the output neurons of the accessory olfactory bulb and the main bulb mitral cells (arrow, Fig. 8) sets the stage for a sequential growth of their axons into target areas in the telencephalon. The corticomedial amygdala is one of the most well-documented targets of the olfactory projection system (LeGros Clark and Meyer 1947; Powell et al. 1965; White 1965; Girgis and Goldby 1967; Heimer 1968; Price 1973). The main and accessory parts of the bulb project to nonoverlapping parts of the amygdala. The main bulb terminates in the nucleus of the lateral olfactory tract, anterior cortical, and posterolateral cortical nuclei (Scalia and Winans 1975; Broadwell 1975; Skeen and Hall 1977; Turner et al. 1978). The accessory bulb terminates in the nucleus of the accessory olfactory tract, medial, and posteromedial cortical nuclei (Scalia and Winans 1975; Broadwell 1975; Devor 1976; Skeen and Hall 1977). These anatomical relationships, along with the sequential growth of the olfactory fibers, are diagrammed in Fig. 8. Within the amygdaloid target zones, neurons are generated in a strong anterior to posterior gradient (Bayer 1980b) such that at any cross-sectional level, the part of the amygdala receiving accessory bulb input begins to originate slightly ahead of the part getting main bulb input (arrows, Fig. 8). For example, the anterior part of the medial nucleus is slightly earlier than the anterior cortical nucleus (Bayer 1980b). These combined gradients may be set up so that the olfactory target neurons in the amygdala are in a receptive state when the olfactory fibers begin to arrive. For example, anterior medial nucleus neurons may be ready to receive fibers from the accessory bulb earlier than neurons in the anterior cortical nucleus which get input from the main bulb. Schwob and Price (1978) found accessory bulb fibers reach the anterior amygdala before main bulb fibers. Thus, the temporal sequencing of neuron origin may be a necessary prerequisite for the establishment of proper anatomical connections between the olfactory bulb and the amygdala. Correlations also exist between neurogenetic patterns and topographic anatomical connections in the septohippocampal system (Bayer 1980a), and in the auditory system (Altman and Bayer 1981).

Neurogenetic gradients within a population of olfactory neurons are either nonexistent (e.g., main bulb mitral cells) or weak (e.g., superficial to deep gradient in the main bulb granule cells). Similarly the olfactory projections to the telencephalon are basically non-topographic. For example, mitral and tufted cells scattered throughout the bulb will project to similar areas in the piriform cortex and olfactory tubercle (Scott et al. 1980). In contrast, there is a large body of evidence for specificity in the projection of the olfactory nerve fibers into the glomerular

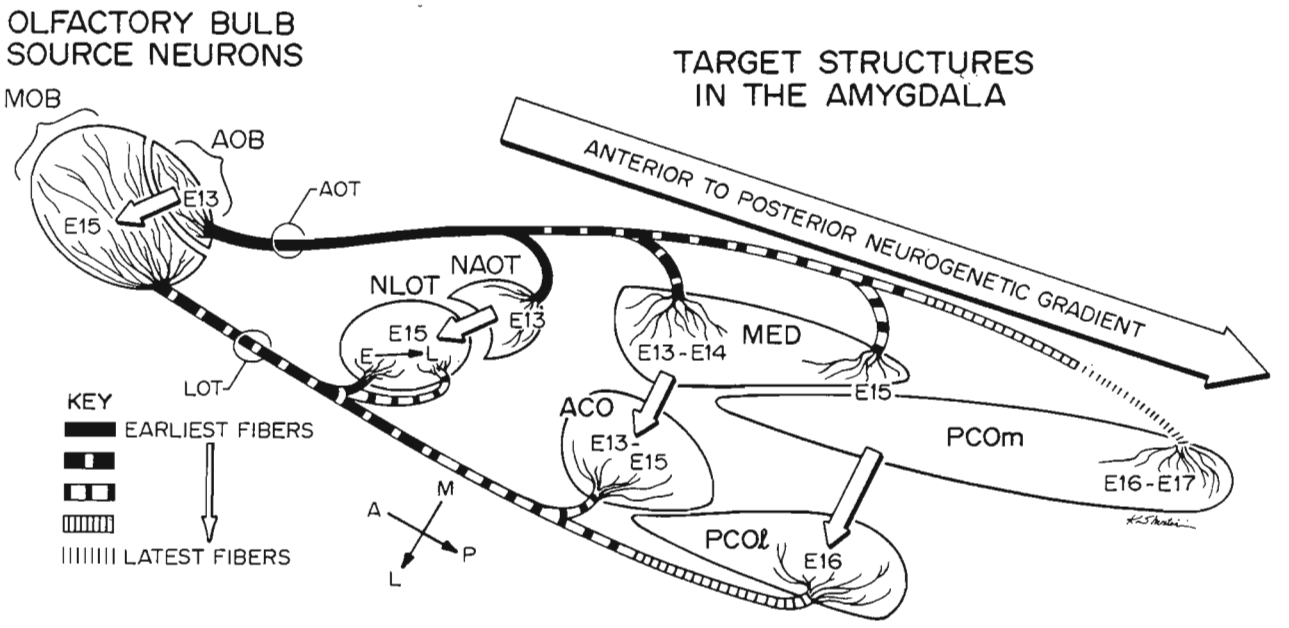

Fig. 8. Gradients of neurogenesis (*arrows*) in the olfactory bulb correlated with gradients of neurogenesis in amygdaloid target structures. Embryonic days indicated are peak days of neurogenesis. Sequential neurogenesis between accessory (*AOB*) and main (*MOB*) bulb output neurons sets up a sequential growth of fibers (*solid to dashed lines*) in the accessory olfactory tract (*AOT*) and lateral olfactory tract (*LOT*) toward target areas in the telencephalon (Schwob and Price 1978); the *AOT* and *LOT* have been separated for diagrammatic purposes. Within the amygdala, neurons in the parts receiving accessory bulb input (*NAOT* nucleus of the accessory olfactory tract; *MED* medial nucleus; *PCOm* posteromedial cortical nucleus) are generated slightly earlier than neurons in the parts receiving main bulb input (*NLOT* nucleus of the lateral olfactory tract; *ACO* anterior cortical nucleus; *PCOl* posterolateral cortical nucleus). Throughout both target zones, anterior parts, which should receive fibers first, are generated before posterior parts

layer (Adrian 1950; Leveteau and MacLoed 1966; Pinching and Døving 1974; Land and Sheperd 1974; Sharp et al. 1975; Skeen 1977; Jourdan et al. 1980). However, there is no strict point to point topography between the olfactory epithelium and the olfactory bulb. Adjacent olfactory sensory neurons project to non-adjacent glomeruli (Land and Shepherd 1974). Various specific odors will result in equally specific spatial patterns of 2-deoxyglucose uptake in the glomerular layer, but glomeruli in the same area of the bulb may be sensitive to different odors (Skeen 1977). With this type of anatomical interrelationship, it is not surprising that periglomerular granule cells surrounding anterior, posterior, medial, and lateral glomeruli originate simultaneously.

Persistent Neurogenesis During the Juvenile and Adult Periods in Olfactory Bulb Interneurons

The interneuron populations in the main bulb are unique when compared with most other neuronal populations in the central nervous system. First, these are the only populations (excepting the granule cells of the accessory bulb) so far examined which do not show a maximal level of cell labeling (95–100%) with the multiple injections of ^{3}H-thymidine. Second, they all have some neurogenesis during the adult period especially prominent in the granule cells of the main bulb as Kaplan and Hinds (1977) have observed. This latter feature is also a characteristic of the dentate granule cells in the hippocampus (Altman 1963; Kaplan and Hinds 1977; Bayer 1982; Bayer et al. 1982). With increasing age at injection, the proportion of labeled neurons decreases when the populations are examined at P200 (Fig. 7). This may indicate that the adult level of neurogenesis declines with age. Since the later injection groups also have a shorter survival period, an equally plausible explanation is that fewer neurons have time to differentiate from the pool of labeled precursor cells in the later injection groups. However, the data also show that increasing the survival time in the P30–P33 injection group from P60 to P200 results in a decrease in the proportion of labeled neurons. Altman (1969) noted a similar pattern. Since neurons are added for several weeks after the P30–P33 injections, the precursors will eventually dilute their supply of ^{3}H-thymidine by repeated cell divisions to undetectable levels and will then generate unlabeled new neurons, thus bringing down the proportion of labeled cells.

In the hippocampal dentate granule cell population, the neurons generated during the adult period accumulate and substantially increase the total number (Bayer 1982; Bayer et al. 1982). In contrast, the total number of main olfactory bulb granule cells does not significantly change between one month and one year of age (Roselli-Austin and Altman 1979). These data would indicate, then, that the rate of granule cell production in the main olfactory bulb during adult life may be balanced by an equal rate of granule cell death. There is some histological evidence for cell death since pyknotic fragments are often observed, especially within the granular layer (Bayer, unpublished). Cell turnover and regeneration are shown by neurons both in the olfactory epithelium (Monti-Graziadei and Graziadei 1979; Moulton 1974) and vomeronasal epithelium (Barber and Raisman 1978), and this phenomenon may be also present in the olfactory bulb. Thus, prolonged neurogenesis in the olfactory bulb may be more indicative of cell turnover in a numerically stable population rather than cell accumulation in a growing population.

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References

Adrian ED (1950) Sensory discrimination. Br Med Bull 6: 330–333

Altman J (1963) Autoradiographic study of cell proliferation in the brains of rats and cats. Anat Rec 145: 573–592

Altman J (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 Neurol 137: 433–458

Altman J, Bayer SA (1981) Time of origin of neurons of the rat inferior colliculus and the relations between cytogenesis and tonotopic order in the auditory pathway. Exp Brain Res 42: 411–423

Barber PC, Raisman G (1978) Cell division in the vomeronasal organ of the adult mouse. Brain Res 141: 57–66

Bayer SA (1979a) The development of the septal region in the rat. I. Neurogenesis examined with ^{3}H-thymidine autoradiography. J Comp Neurol 183: 89–106

Bayer SA (1979b) The development of the septal region in the rat. II. Morphogenesis in normal and X-irradiated embryos. J Comp Neurol 183: 107–120

Bayer SA (1980a) The development of the hippocampal region in the rat. I. Neurogenesis examined with ^{3}H-thymidine autoradiography. J Comp Neurol 190: 87–114

Bayer SA (1980b) Quantitative ^{3}H-thymidine radiographic analyses of neurogenesis in the rat amygdala. J Comp Neurol 194: 845–875

Bayer SA (1982) Changes in the total number of dentate granule cells in juvenile and adult rats: A correlated volumetric and ^{3}H-thymidine autoradiogrphic study. Exp Brain Res 46: 315–323

Bayer SA, Altman J (1974) Hippocampal development in the rat: Cytogenesis and morphogenesis examined with autoradiography and low-level X-irradiation. J Comp Neurol 158: 55–80

Bayer SA, Yackel JW, Puri PS (1982) Neurons in the rat dentate gyrus granular layer substantially increase during juvenile and adult life. Science 216: 890–892

Broadwell RD (1975) Olfactory relationships of the telencephalon and diencephalon in the rabbit. I. An autoradiographic study of the efferent connections of the main and accessory olfactory bulbs. J Comp Neurol 163: 329–346

Cajal SR (1955) Studies on the cerebral cortex [Transl.: Kraft LM]. Year Book Publ., Chicago

Conover WJ (1971) Practical Nonparametric Statistics. Wiley, New York

Devor M (1976) Fiber trajectories of olfactory bulb efferents in the hamster. J Comp Neurol 166: 31–48

Girgis M, Goldby F (1967) Secondary olfactory connections and the anterior commissure in the coypu (*Myocastor coypus*). J Anat 101: 33–44

Heimer L (1968) Synaptic distribution of centripetal and centrifugal nerve fibers in the olfactory system of the rat. An experimental anatomical study. J Anat 103: 413–432

Hinds JW (1967) Autoradiographic study of histogenesis in the mouse olfactory bulb. I. Time of origin of neurons and neuroglia. J Comp Neurol 134: 287–304

Humphrey T (1966) Correlations between the development of the hippocampal formation and the differentiation of the olfactory bulbs. Ala J Med Sci 3: 235–269

Jourdan F, Duveau A, Astic L, Holley A (1980) Spatial distribution of (^{14}C) 2-deoxyglucose uptake in the olfactory bulbs of rats stimulated with two different odours. Brain Res 188: 139–154

Kaplan MS, Hinds JW (1977) Neurogenesis in the adult rat: Electron microscopic analysis of light radioautographs. Science 197: 1092–1094

LeGros Clark WE, Meyer M (1947) Terminal Connexions of the olfactory tract in the rabbit. Brain 70: 304–328

Leveteau J, MacLoed P (1966) Olfactory discriminations in the rabbit olfactory glomerulus. Science 153: 175–176

Monti-Graziadei GA, Graziadei PPC (1979) Studies on neuronal plasticity and regeneration in the olfactory system. Morphologic and functional characteristics of the olfactory sensory neuron. In: Meisami E, Brazier MAB (eds) Neural growth and differentiation. Raven Press, New York, pp 373–396

Moulton DG (1974) Dynamics of cell populations in the olfactory epithelium. Ann NY Acad Sci 237: 52–61

Olmos J de, Hardy H, Heimer L (1978) The afferent connections of the main and the accessory olfactory bulb formations in the rat: An experimental HRP-study. J Comp Neurol 181: 213–244

Pellegrino LJ, Pellegrino AS, Cushman AJ (1979) A stereotaxic atlas of the rat brain, second edition. Plenum Press, New York

Pinching AJ, Døving KB (1974) Selective degeneration in the rat olfactory bulb following exposure to different odours. Brain Res 82: 195–204

Powell TPS, Cowan WM, Raisman G (1965) The central olfactory connexions. J Anat 99: 791–813

Price JL (1973) An autoradiographic study of complementary laminar patterns of termination of afferent fibers to the olfactory cortex. J Comp Neurol 150: 87–108

Roselli-Austin L, Altman J (1979) The postnatal development of the main olfactory bulb of the rat. J Dev Physiol 1: 295–313

Scalia F, Winans SS (1975) The differential projections of the olfactory bulb and accessory olfactory bulb in mammals. J Comp Neurol 161: 31–56

Schwob JE, Price JL (1978) The cortical projection of the olfactory bulb: Development in fetal and neonatal rats correlated with quantitative variations in adult rats. Brain Res 151: 369–374

Scott JW, McBride RL, Schneider SP (1980) The organization of projections from the olfactory bulb to the piriform cortex and olfactory tubercle in the rat. J Comp Neurol 194: 519–534

Sharp FR, Kauer JS, Shepherd GM (1975) Local sites of activity-related glucose metabolism in rat olfactory bulb during olfactory stimulation. Brain Res 98: 596–600

Skeen LC, (1977) Odor-induced patterns of deoxyglucose consumption in the olfactory bulb of the tree shrew *Tupaia glis*. Brain Res 124: 147–153

Skeen LC, Hall WC (1977) Efferent projections of the main and the accessory olfactory bulb in the tree shrew *Tupaia glis*. J Comp Neurol 172: 1–36

Turner BH, Gupta KC, Mishkin M (1978) The locus and cytoarchitecture of the projection areas of the olfactory bulb in *Macaca mulata*. J Comp Neurol 177: 381–396

White LE Jr (1965) Olfactory bulb projections of the rat. Anat Rec 152: 465–480

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