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NEUROGENESIS IN THE ANTERIOR OLFACTORY NUCLEUS AND ITS ASSOCIATED TRANSITION AREAS IN THE RAT BRAIN

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Abstract—Neurogenesis in the rat olfactory peduncle was examined with [^{3}H]thymidine autoradiography. Animals in the prenatal groups were the offspring of pregnant females given an injection of [^{3}H]-thymidine on two consecutive gestation days. Nine groups of embryos were exposed to [^{3}H]thymidine on embryonic days (E) E13–E14, E14–E15, . . . E21–E22, respectively. One group of postnatal animals was given four consecutive injections of [^{3}H]thymidine on postnatal days (P) P0–P3. On P60, the percentage of labeled cells and the proportion of cells originating during either 24 or 48 hr periods were quantified at seven anatomical levels through both the anterior olfactory nucleus and the transition areas. A caudal (older) to rostral (younger) neurogenetic gradient is found both within and between structures in the olfactory peduncle. Neurons in the dorsal, lateral, and ventral–lateral transition areas are generated mainly between E14 and E19, those in the anterior olfactory nucleus mainly between E15 and E21. Only 3–4% of the neurons in the most anterior pars lateralis and pars dorsalis originate after birth. All parts of the anterior olfactory nucleus show a strong superficial (older) to deep (younger) neurogenetic gradient (the 'outside-in' pattern). In contrast, neurons in the ventral–lateral transition area and in the dorsal transition area originate in a deep to superficial neurogenetic gradient (the 'inside-out' pattern), suggesting that these areas are, in reality, primary olfactory cortex. The lateral transition area is truly 'transitional', showing no neurogenetic gradient along the superficial–deep plane. The medial transition area originates between E15 and E19 in a center (older) to edge (younger) 'sandwich' neurogenetic gradient along the rostrocaudal plane, a pattern apparently unrelated to neurogenetic gradients in other olfactory peduncle structures. These data suggest that characteristic patterns of neurogenesis, namely the 'inside-out' vs the 'outside-in' gradients, permit the assignment of different structures to cortical vs ganglionic cytoarchitectonic components of the olfactory relay system.

Key words: Anterior olfactory nucleus, Olfactory peduncle, Neurogenesis, [^{3}H]thymidine autoradiography.

The anterior olfactory nucleus is one of the major central olfactory processing stations, receiving heavy input from both mitral and tufted cells in the ipsilateral olfactory bulb.[9,14,19,20,22,31,34,36–38,42–44] In return it projects back to the ipsilateral bulb and is the only source of contralateral projections to the opposite olfactory bulb.[1,10,13,15–17,21,29,30,32,39,41,42] Toward the posterior parts of the anterior olfactory nucleus, the relatively undifferentiated gray matter takes on a more complex cytoarchitectonic pattern, forming transition areas[16,23] to the primary olfactory cortex. The transition areas also get olfactory bulb input as part of the major projection extending to the primary olfactory cortex. There has been only one previous [^{3}H]thymidine autoradiographic study of neurogenesis in the anterior olfactory nucleus of the mouse,[12] based on the pulse labeling technique using single injections of [^{3}H]thymidine. The present study is based on a comprehensive labeling method using multiple injections of [^{3}H]thymidine.[8] This technique allows an accurate delineation of both the onset and cessation of neurogenesis as well as the determination of the proportion of neurons that originate during single days of embryonic life. The data to be reported here indicate that shifts in cytoarchitectonic patterns between the anterior olfactory nucleus and the transition areas is associated with a shift in neurogenetic gradients. The following paper[7] will present additional evidence that the transition areas are anterior extensions of the primary olfactory cortex into the olfactory peduncle.

Abbreviations: AC, anterior commissure; AOB, accessory olfactory bulb; D, pars dorsalis, anterior olfactory nucleus; DOR, dorsal; DT, dorsal transition area; d, deep cells in layer II; E, pars externa, anterior olfactory nucleus; FC, frontal cortex; L, pars lateralis, anterior olfactory nucleus; LAT, lateral; LOT, lateral olfactory tract; LT, lateral transition area; M, pars medialis, anterior olfactory nucleus; MED, medial; MT, medial transition area; OT, olfactory tubercle; OV, olfactory ventricle; P, pars posterior, anterior olfactory nucleus; POC, primary olfactory cortex; s, superficial cells in layer II; V, pars ventralis, anterior olfactory nucleus; VEN, ventral; VLT, ventral lateral transition area; I, layer I (outer plexiform layer); II, layer II (cell layer in anterior olfactory nucleus, superficial cell layer in transition areas); III, layer III (deep cell layer in transition areas).

EXPERIMENTAL PROCEDURES

Since neurogenesis in the rat olfactory peduncle extends beyond 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.) s.c. injections of [^{3}H]thymidine (Schwarz-Mann; sp. act. 6.0 Ci/mM; 5 μCi/g body wt) to insure comprehensive cell labeling. The prenatal developmental series contained nine groups, the offspring of pregnant females given two successive daily injections progressively delayed by 1 day between groups (E13–E14, E14–E15, . . . E21–E22). Two or more pregnant females made up each injection group. The day the females were sperm positive was designated embryonic day one (E1). Normally, births occur on E23, which is also designated as postnatal day zero (P0). The postnatal developmental series had two groups of rat pups, each group containing males from at least two litters. The pups were given four (P0–P3, P2–P5) consecutive daily injections. All animals were perfused through the heart with 10% neutral formalin on P60. The brains were kept for 24 hr in Bouin's fixative, then were transferred to 10% neutral formalin until they were embedded in paraffin. The brains of at least six animals from each injection group were blocked coronally according to the stereotaxic angle of the Pellegrino *et al.*[35] atlas. Every 15th section (6 μm) through the olfactory peduncle was saved. Slides were dipped in Kodak NTB-3 emulsion, exposed for 12 weeks, developed in Kodak D-19, and post-stained with hematoxylin and eosin.

Sections were selected for quantitative analysis at seven anatomically matched levels[35] throughout the anteroposterior extent of the olfactory peduncle (Fig. 1). Cells were counted microscopically at × 312.5 in unit areas set off by an ocular grid (0.085 mm^2) or in strips (0.29 mm wide). For quantification, all neurons within a designated area were assigned to one of two groups, labeled or nonlabeled. Cells with reduced silver grains overlying the nucleus in densities above background levels were considered labeled; obvious endothelial and glial cells were excluded. The proportion of labeled cells (% labeled cells/total cells) was then calculated from these data.

The determination of the proportion of cells arising (ceasing to divide) on a particular day utilized a modification of the progressively delayed comprehensive labeling procedure,[8] and is described in detail elsewhere.[5,6] Briefly, a progressive drop in the proportion of labeled neurons from a maximal level (>95%) in a specific population indicates that the precursor cells are producing nonmitotic neurons. By analyzing the rate of decline in labeled neurons, one can determine the proportion of neurons originating over blocks of days (or single days) during development. Table 1 shows the data and calculations for the dorsolateral part of the pars externa at L4.

Throughout the quantitative analysis, it was noted that trends in cell labeling within animals were very consistent. For example, in the dorsolateral pars externa (Fig. 2), the percentage of labeled cells at L3 tended to be slightly lower than the percentage of labeled cells at L2. However, variability between animals in an injection group were large enough to mask this trend. Consequently, cell labeling patterns were analyzed with the sign test.[11] The sign test determines the consistency of sequential neuron production between paired locations within individual animals. The comparisons are grouped into three categories: (1) X > Y, '−' comparison; (2) X < Y, '+' comparison; (3) X = Y, 'O' comparison. The zero comparisons are discarded and, depending on the total number of remaining '+' and '−' comparisons, either a binomial distribution or a normal approximation is used to calculate probabilities (*P*). The graphs throughout this report show the more variable group data rather than consistent trends in data from individual animals. Consequently, some of the statistically significant neurogenetic gradients (L2 and L3, Fig. 2) are not conspicuous in the group data.

RESULTS

The olfactory peduncle in the rat brain is a thick stalk of gray matter forming a bridge between the olfactory bulb and the basal forebrain. It has two principal components: (1) the anterior olfactory nucleus and (2) the transition areas between the anterior olfactory nucleus proper and the primary olfactory cortex. Representative sections through the levels (L) used for quantification of the time of origin of neurons throughout the olfactory peduncle are shown in Fig. 1. L1–L4 are anterior to the coronal sections shown in the Pellegrino *et al.*[35] atlas; L5 corresponds to atlas level

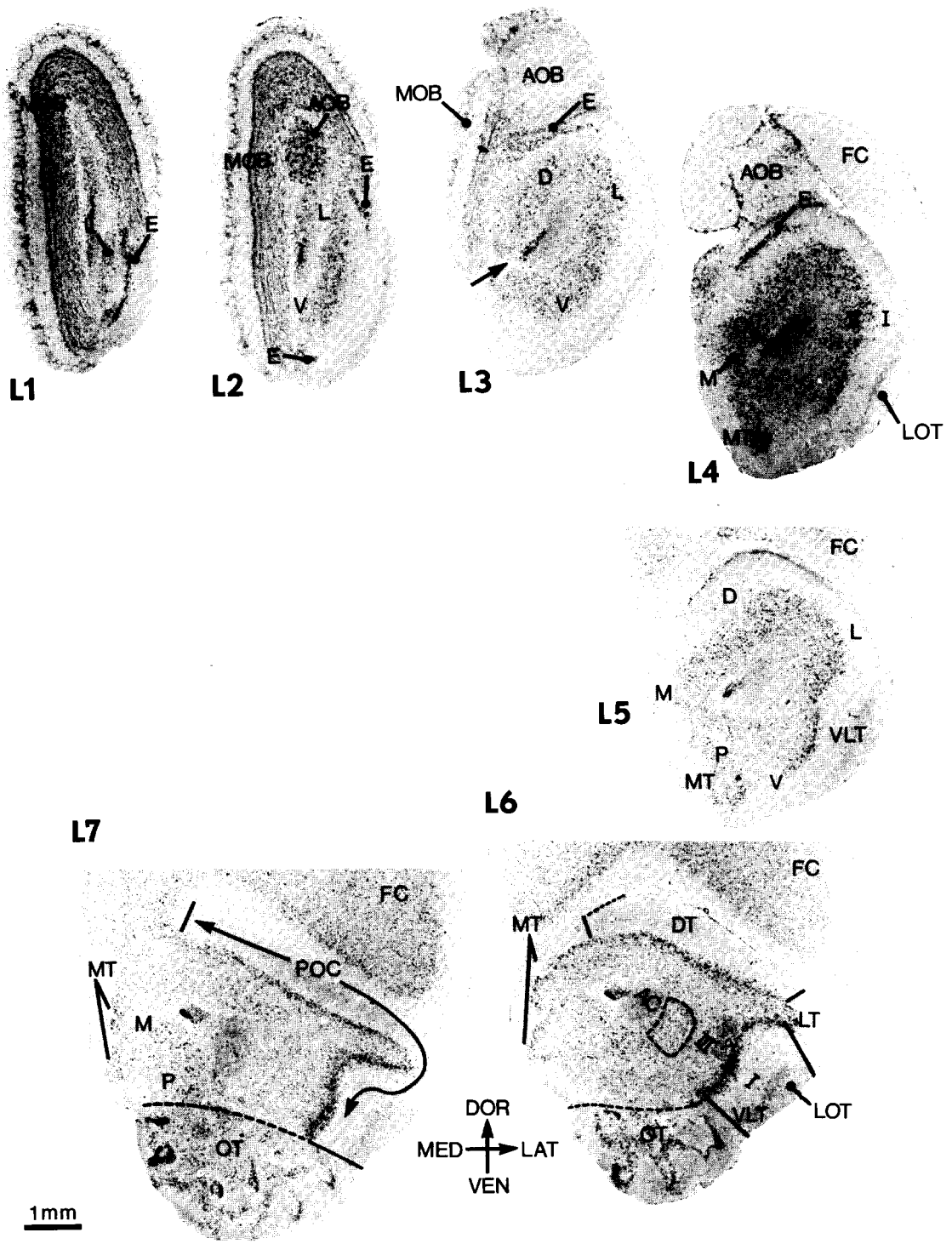

Fig. 1. Representative coronal sections of seven levels of the rat olfactory peduncle used for quantification of the time of neuron origin. The sections are cut according to the Pellegrino *et al.*[35] stereotaxic atlas. The plane of the sections is angled so that within a section the dorsal part is anterior to the ventral part (paraffin, 6 μm; hematoxylin and eosin).

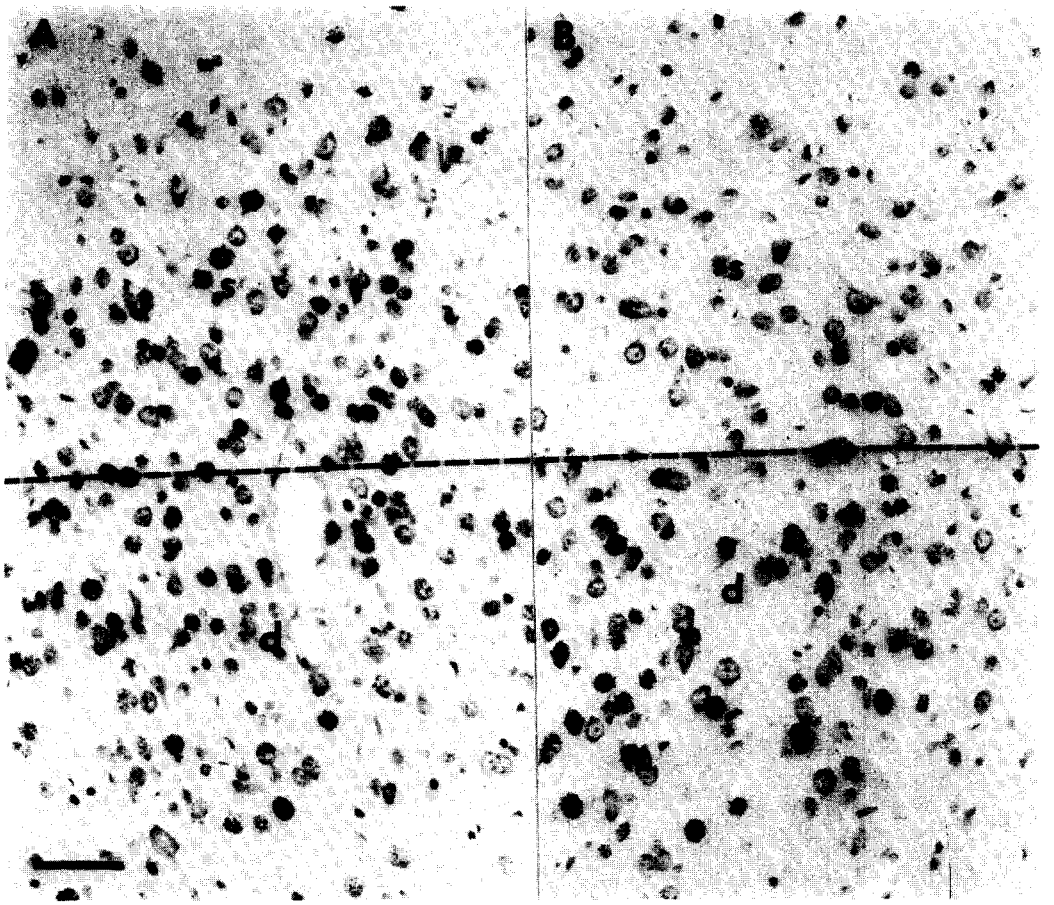

Fig. 4. Autoradiograms of the pars ventralis at L2 (A) and L5 (B) in an animal exposed to [^{3}H]thymidine on E17+E18 and killed on P60 (6 μm paraffin sections, hematoxylin and eosin, scale = 0.05 mm). Notice that the proportion of labeled cells is higher in (A) than in (B). In (B), superficial cells (s) tend to be unlabeled, while deep cells (d) are still labeled.

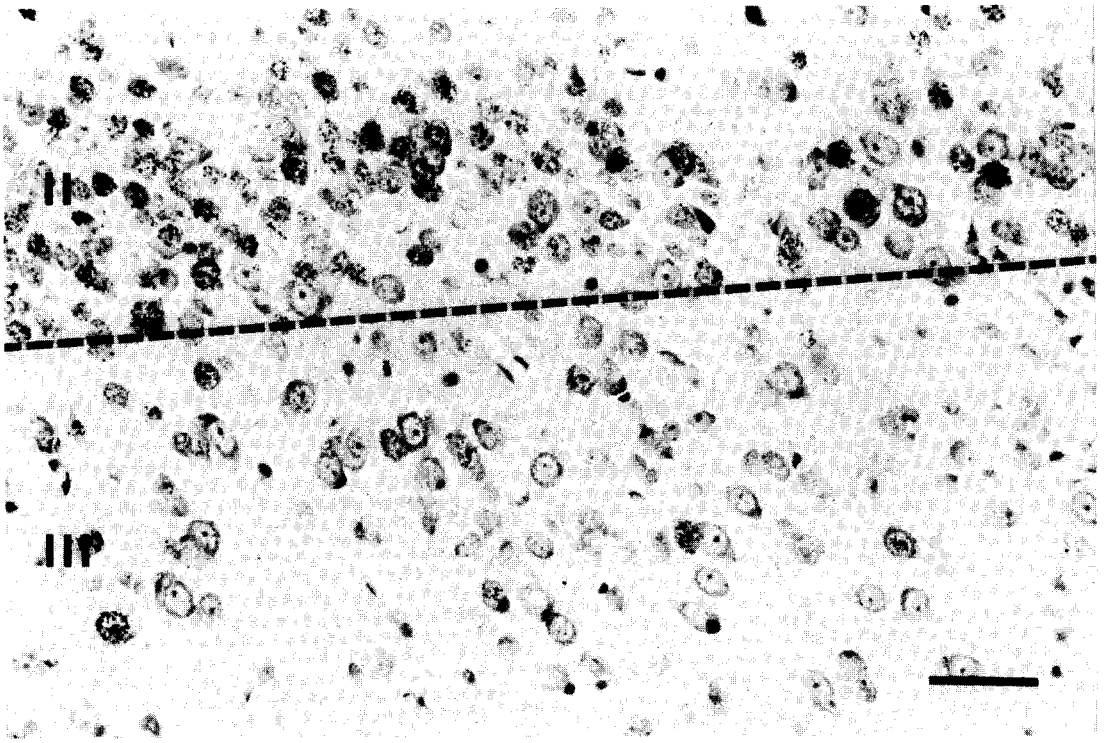

Fig. 11. An autoradiogram of the ventral–lateral transition area at L6 in an animal exposed to [^{3}H]thymidine on E16+E17 and killed on P60 (6 μm paraffin section, hematoxylin and eosin, scale bar = 0.05 mm). Layer II cells (above dashed line) have a higher proportion of label than do the deeper neurons in layer III (below dashed line).

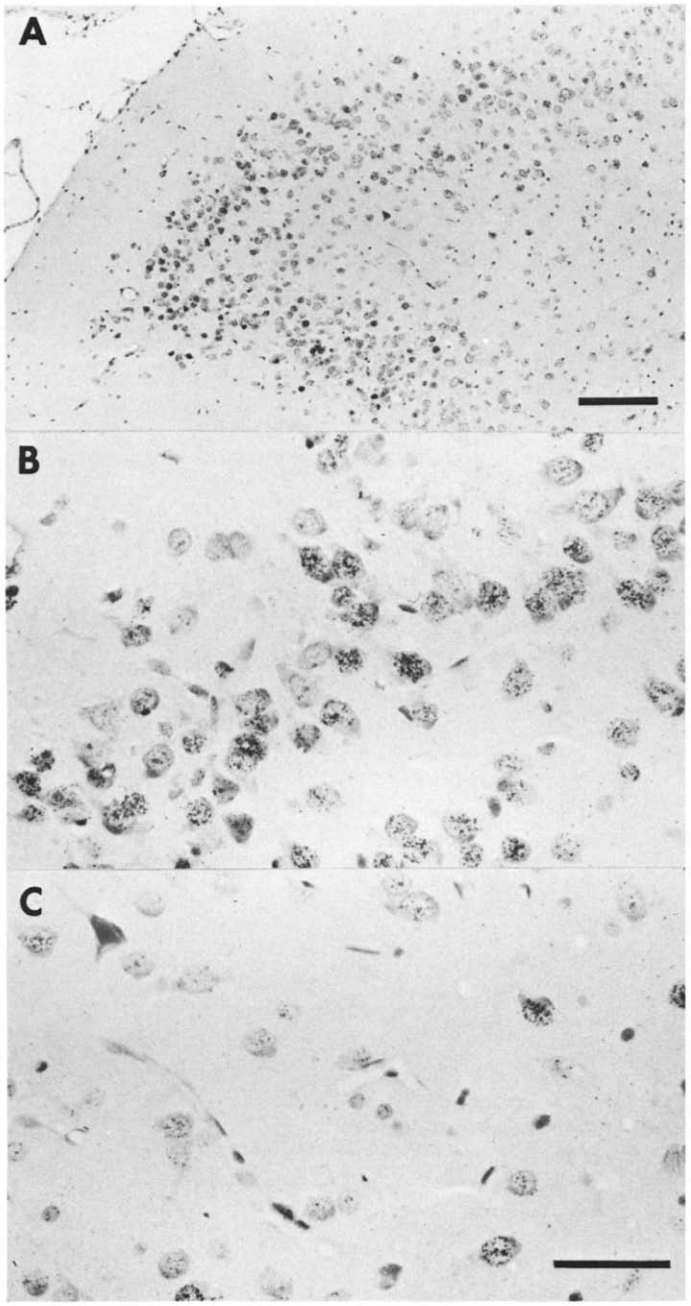

Fig. 14. Autoradiograms of the lateral transition area at L6 in an animal exposed to [^{3}H]thymidine on E16+E17 and killed on P60 (6 μm paraffin sections, hematoxylin and eosin). The low magnification view in (A) (scale bar = 0.15 mm) shows that this area is cytoarchitectonically similar to the ventral–lateral transition area (compare with Fig. 11), with more densely packed neurons in layer II (B) than in layer III (C). Scale bar for (C) and (D) = 0.05 mm. The proportion of labeled cells in superficial (B) and deep layers (C) are similar.

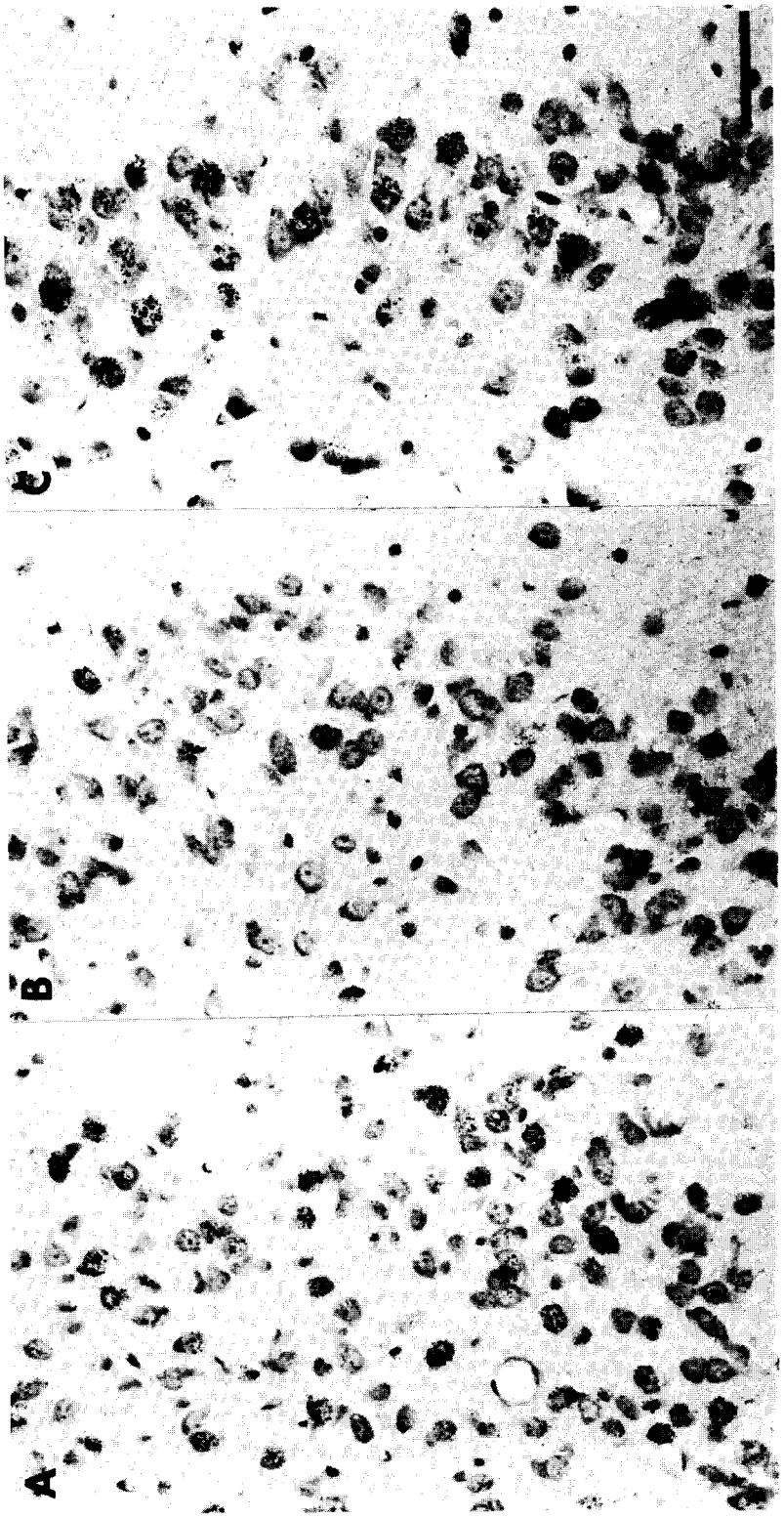

Fig. 16. Autoradiograms of the ventral part of the medial transition area at L4 (A), L5 (B), and L7 (C) in an animal exposed to [^{3}H]thymidine on E18+E19 and killed on P60 (6 μm paraffin sections, hematoxylin and eosin, scale = 0.05 mm). Notice that the proportion of labeled neurons is lower in (B) than in either (C) or (A).

Table 1. Neurogenesis of the dorsolateral pars externa (level 4)

Injection group	*N*	% Labeled cells*	Day of origin	% Cells originating†
E15–E16	6	(A) 99.3 ± 1.21	E15	8.3 (A–B)
E16–E17	12	(B) 91.0 ± 2.52	E16	42.33 (B–C)
E17–E18	9	(C) 48.67 ± 9.99	E17	30.84 (C–D)
E18–E19	6	(D) 17.83 ± 5.67	E18	11.5 (D–E)
E19–E20	6	(E) 6.33 ± 3.61	E19	3.62 (E–F)
E20–E21	7	(F) 2.71 ± 2.06	E20	2.71

* $\bar{X} \pm$ S.D.

† Graphed in Fig. 2.

The data for the dorsolateral pars externa at L4 are given as an example of how the data are derived for presentation in the bar graphs used throughout the figures in this paper. *N* refers to the number of animals analyzed in each *injection group*. The same number of animals is used to collect data for all graphs throughout this paper. The *% labeled cells* is the group mean and S.D. for the raw data (counts of the % of labeled cells to total cells) for each injection group. The S.D.s are typical of the variability seen throughout the data collection. The *% cells originating* column lists the data that are presented in the bar graph (Fig. 2, bottom). To get the height of the second bar, for example, the proportion of labeled cells in injection group E16–E17 (entry B, column 3) is subtracted from the % labeled cells in injection group E15–E16 (entry A, column 3) to get the proportion of cells originating during the day on E15 (8.3%).

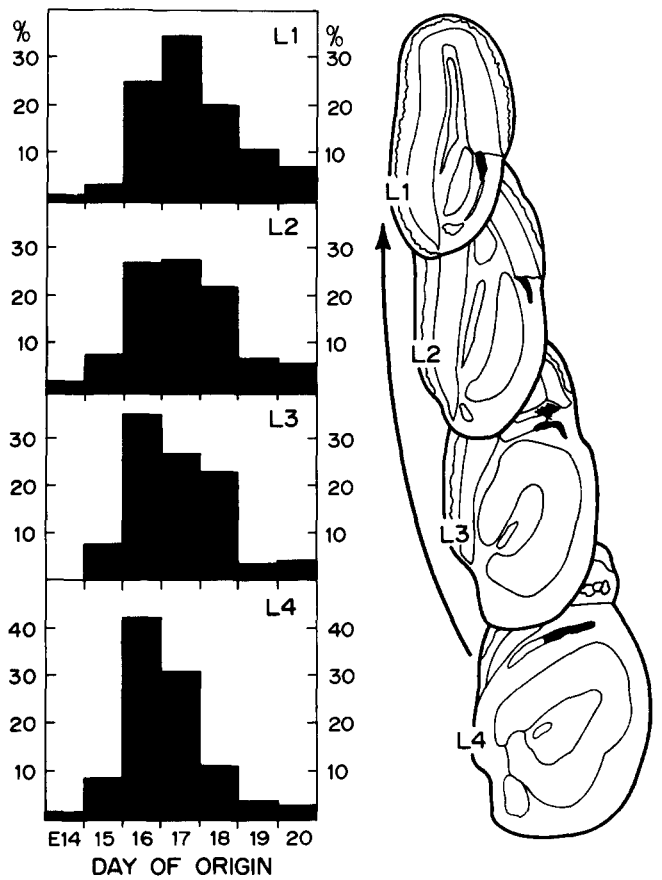

Fig. 2. Time of origin of neurons in the pars externa. Cells were counted in the areas indicated in black in the drawings. The drawing of L4 in this and all following figures does not include the small area of the anterior pole of the frontal cortex (FC) as pictured in Fig. 1. The bar graphs are the proportion of cells originating over single embryonic days. Table 1 indicates how the data were calculated for the bottom graph (L4). There is a prominent caudal to rostral neurogenetic gradient indicated by the arrow next to the drawings and by the gradual shift in neurogenetic peaks from earlier to later times at progressively more anterior levels.

A11.8, L6 to A11.4, and L7 to A11.2. The time of neuron origin throughout the olfactory peduncle is treated herein in two large data groups, the first dealing with the anterior olfactory nucleus and the second with the transition areas. The terminology of de Olmos *et al.*[16] is followed throughout.

Time of neuron origin in the anterior olfactory nucleus

One of the most prominent features of the olfactory peduncle is the anterior olfactory nucleus, which was first thoroughly described in the opossum by Herrick.[24] In the rat, the anterior olfactory nucleus appears rostrally as a swelling along the ventrolateral surface of the olfactory peduncle. It is surrounded dorsally, medially and anteriorly by the olfactory bulb (L1, Fig. 1). As the olfactory bulb recedes, more of the nucleus appears along the lateral wall (L2, Fig. 1) until it completely encircles the olfactory extension of the lateral ventricle and the interbulbar part of the anterior commissure (L2, L3, Fig. 1). The six principal subdivisions of the anterior olfactory nucleus proper are the pars externa, pars dorsalis, pars lateralis, pars ventralis, pars medialis and pars posterior. The pars externa is unique in that it is embedded in the outer plexiform layer (I indicated in L4, Fig. 1). The other subdivisions form a thick 'gray ring'[23] of pyramidal-like cells[45] (layer II indicated in L4, Fig. 1). Just as the lateral part of the ring extends farther rostrally, the medial part extends farther caudally (L6, L7, Fig. 1). Various subdivisions making up the ring are named purely on the basis of their topographic relationships to the olfactory ventricle and anterior commissure, rather than on any cytoarchitectonic differences. The basic anatomical features of the anterior olfactory nucleus in the rat brain are similar to the features described by other anatomists in the opossum,[23] Caenolestes (a small South American marsupial),[33] bat,[24] mole,[27] guinea pig,[26,28] and hamster.[39]

Pars externa. At L1 (Fig. 1) densely packed medium- to small-sized neurons in the pars externa form a lateral superficial band of cells, whose ends extend posteriorly as dorsolateral and ventrolateral clumps. Still further posteriorly, only the dorsolateral clump remains. The most posterior extent of the pars externa is a thin band of mediolaterally oriented cells subjacent to the fibers running ventral to the accessory olfactory bulb (L4, Fig. 1). With the exception of its most posterior part, the pars externa is in close proximity to the granule cells of the main olfactory bulb.

Figure 2 shows the data for the time of origin of the dorsolateral portion of the pars externa. A few older neurons are generated at all levels on E14 and E15, but from E16 on, a definite caudal to rostral gradient of neurogenesis is evident. The sign test indicates that neurons at L4 begin to originate significantly earlier than those at more anterior levels ($P<0.0001$). On the average, neurons at L3 are generated earlier than those at L2 ($P<0.002$); those at L2 are earlier than those at L1 ($P<0.0001$). The neurogenetic gradient is more prominent when L1 and L4 are compared directly: approximately 51% of the neuronal population at L4 is generated on or before E16, while 72% of the population at L1 is generated on or after E17. There is a tendency for the dorsolateral subdivision of the pars externa to contain older neurons than those present in other subdivisions. At L2, the dorsolateral cells begin to originate significantly ($P<0.0001$) ahead of the neurons in the ventromedial clump (Fig. 3A); 64% of the dorsolateral cells are generated on or before E17, while 48% of the ventromedial cells are generated on or after E18. At L4, the dorsolateral cells are significantly older ($P<0.0001$) than the dorsomedial cells (Fig. 3B); approximately 66% of the dorsomedial cells are generated on or after E17.

Pars ventralis. The pars ventralis extends throughout most of the length of the anterior olfactory nucleus proper (L2–L6, Fig. 1). For this study, it was designated as the ventral part of the layer II cells in the anterior olfactory nucleus proper (drawings, Fig. 5A). The pars ventralis sits dorsal to the olfactory tubercle at L6 and is replaced by the deep layers of the olfactory tubercle at L7. Figure 4 shows autoradiograms from the pars ventralis in an animal exposed to [^{3}H]thymidine on E17+E18. There is a tendency, especially noticeable at the more posterior level, for the superficial cells to be largely unlabeled (s, Fig. 4B), while deep cells are mostly labeled (d, Fig. 4B).

The time of origin of the pars ventralis is quantified in Fig. 5. Cells were counted in a ventral strip running throughout the depths of layer II between L2 and L6 (drawings, Fig. 5A). The sign test shows significant trends in the times of origin along the rostrocaudal plane. On the average,

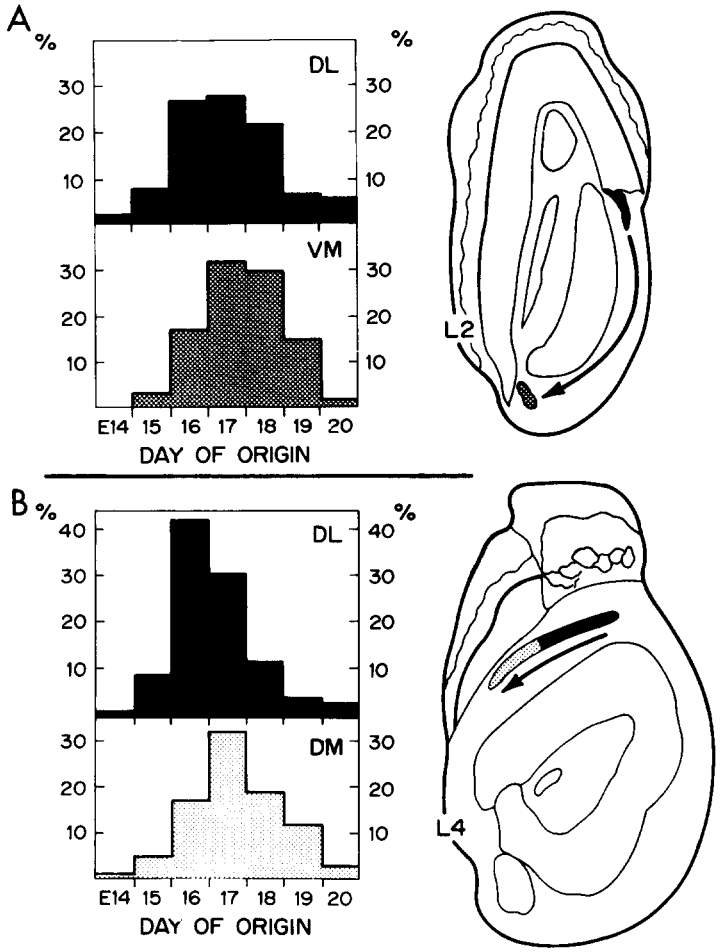

Fig. 3. Time of origin of neurons in dorsolateral vs medial parts of the pars externa. Cells were counted in the shaded areas indicated in the drawings. Bar graphs are the proportion of neurons originating over single embryonic days. (A) At L2, the dorsolateral part of the pars externa originates earlier (more neurogenesis on E16, less on E18) than the ventromedial part, indicated by the arrow in the drawing. (B) At L4, the dorsolateral part originates earlier (peak on E16) than the dorsomedial part (peak on E17) indicated by the arrow in the drawing.

neurons at L6 begin to be generated earlier than those at L5, those at L5 significantly before those at L3, those at L3 significantly before those at L2 (all levels and comparisons, $P<0.0001$). The gradient is quite strong: the bulk of neurogenesis at L6 occurs between E14 and E16 (81% of the cells generated), while 50% of the cells at L2 are generated 48 hr later between E19 and E20. At each of the levels shown in Fig. 5A, the strips were further subdivided into superficial and deep halves. Figure 5B shows the data for the subdivisions made at L2; other levels have similar data (not illustrated). The sign test shows that neurons in the superficial part of the strip are significantly ($P<0.0001$) older (81% generated on or before E19) than neurons in the deep part (42% generated on or after E20).

Pars lateralis. The pars lateralis is first visible at L1, becomes more prominent at L2 and extends back to L5 (Fig. 1). At L6, the pars lateralis is replaced by the lateral transition area. To determine the time of origin throughout the rostrocaudal extent of the pars lateralis, cells were counted in laterally placed strips running throughout the depth of layer II (drawings, Fig. 6A). The sign test shows that neurons located at L5 are significantly older than the neurons at L3, while those at L3 are significantly older than those at L2 ($P<0.0001$; all levels and comparisons). As in the pars ventralis, the gradient is prominent: approximately 69% of the neurons are generated between E16 and E18 at L5, while 66% of the neurons are generated between E19 and P0 at L2. At each of the levels shown in Fig. 6A, the strips were further subdivided into superficial and deep halves. Figure 6B shows the data for the subdivision made at L2; other levels have

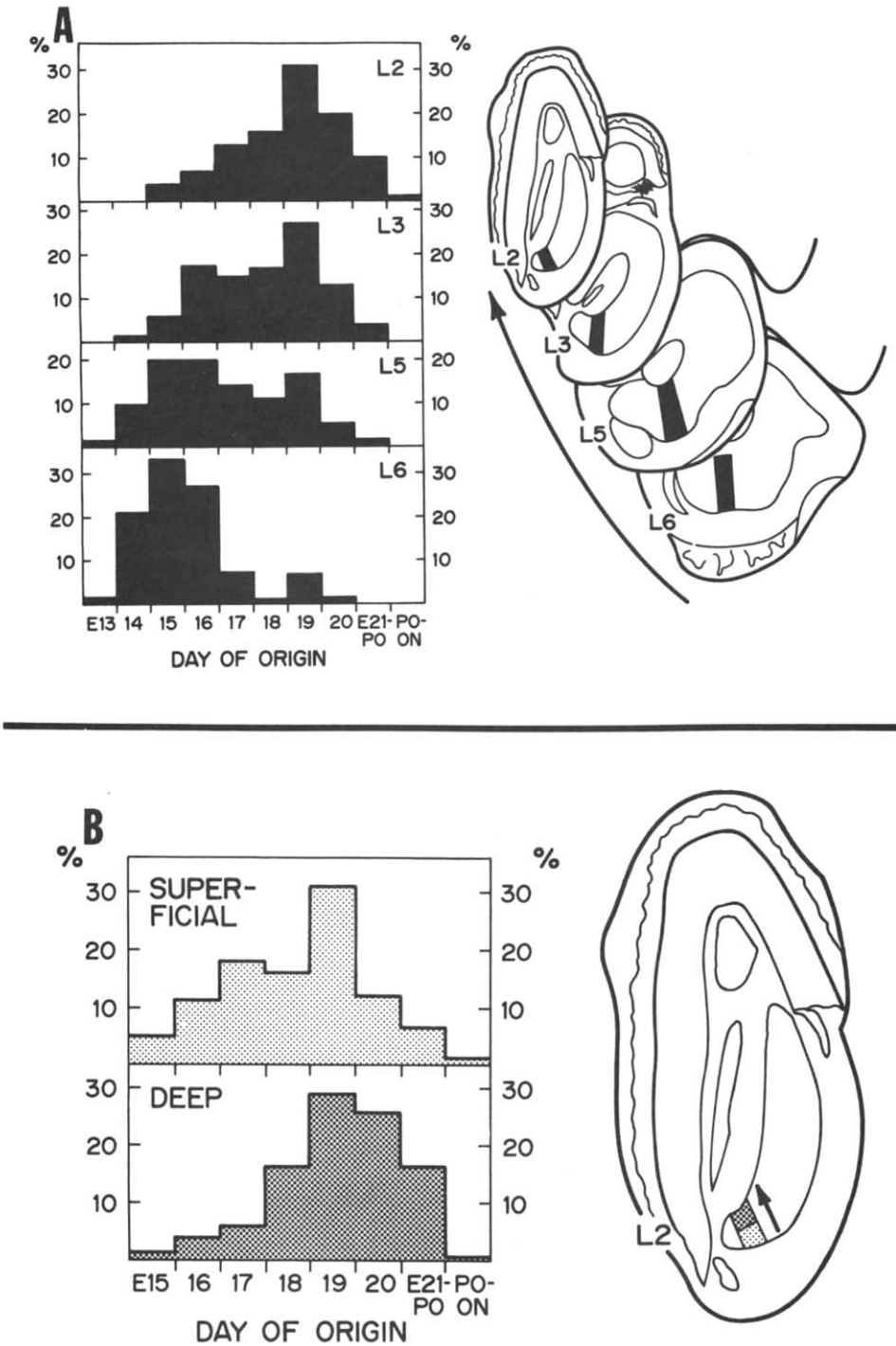

Fig. 5. Time of origin of neurons in the pars ventralis. The bar graphs are the proportion of neurons originating over 24 hr during embryonic life, or during 48 hr in the perinatal period. (A) Neurogenesis throughout the rostrocaudal extent. All neurons were counted within the black strips shown in the drawings. The bar graphs show a gradual shift in the peak of neurogenesis from early to later times at progressively more anterior levels. (B) Neurogenesis along the superficial deep plane at L2. Counts of neurons in the superficial half (light shading) and deep half (dark shading) were kept separate. On the average, superficial cells originate earlier than deep cells.

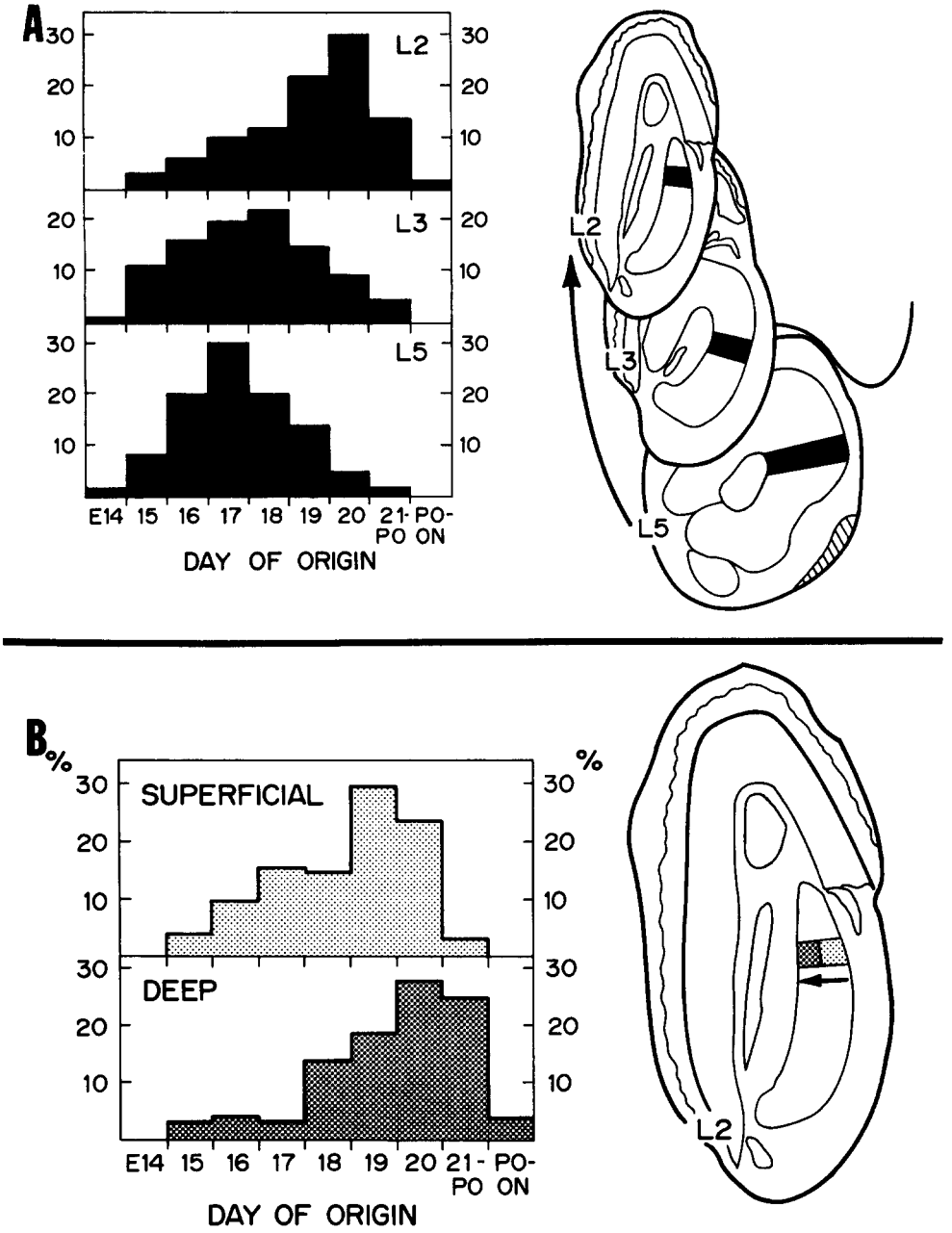

Fig. 6. Time of origin of the neurons in the pars lateralis. The bar graphs are the proportion of neurons originating over 24 hr during embryonic life or over 48 hr during the perinatal period. (A) Neurogenesis throughout the rostrocaudal extent. Neurons were counted in the black strips shown in the drawings. The bar graphs show a gradual shift in the peaks of neurogenesis from early to later times at progressively more anterior levels. (B) Neurogenesis along the superficial-deep plane at L2. Counts of neurons in the superficial half (light shading) and the deep half (dark shading) were kept separate. On the average, superficial cells tend to originate earlier than deep cells.

similar data (not illustrated). The sign test shows that neurons in the superficial part of the strip are significantly older (73% generated on or before E19) than neurons in the deep part of the strip (57% generated on or after E20).

Some larger deep cells lie just beneath layer II of the pars lateralis. Often these cells are embedded within the lateral fibers of the anterior commissure. Further posteriorly (L6) they expand into a prominent clump of cells surrounding the lateral and ventral parts of the anterior commissure. Due to their small numbers at L3 and L5 (usually <25 cells/section), these cells were not

counted. It was noted that the large deep cells tend to originate earlier than the deep cells of layer II, nearly simultaneously with the oldest superficial cells.

Pars dorsalis. The pars dorsalis is first seen at L3 (Fig. 1) as a large dorsomedial expansion of layer II continuous with the pars lateralis, but separated from the pars ventralis by a cell sparse zone (arrow in L3, Fig. 1). It extends posteriorly only as far as L5. At L6, the dorsal part of the olfactory peduncle is taken up by the dorsal transition area. The cytoarchitectonic specializations in the pars dorsalis, such as clumps of larger cells embedded in layer II, found in the rabbit[46] and cat[18] are not found in the rat.

To quantify the time of origin of neurons along the dorsomedial extent of the olfactory peduncle, cells were counted in medial and dorsolateral strips through layer II at L3 and L5. The medial strips (M, Figs 7 and 8) were initially considered to be pars medialis, while dorsolateral strips (L, Figs 7 and 8) were assigned to the pars dorsalis. However, the sign test shows that neurogenesis occurs in exactly the same pattern in both strips along the rostrocaudal and superficial–deep planes. Therefore, the entire area enclosed by the strips was designated pars dorsalis, and all graphs in Figs 7 and 8 represent combined data. Throughout the pars dorsalis, caudal neurons begin to originate significantly earlier (L5: 52% generated on or before E17; Fig. 7, bottom) than rostral neurons (L3: 82% generated on or after E18; Fig. 7, top) showing a prominent gradient of neurogenesis ($P<0.0001$). At L3 (Fig. 8A), neurogenesis covers a broad time span between E17 and P0. In sharp contrast to all other parts of the anterior olfactory nucleus, at this level there is no significant difference ($P>0.05$) between times of neuron origin along the superficial–deep plane. At L5 (Fig. 8B) however, neurons in the superficial parts of the pars dorsalis are generated significantly earlier (68% on or before E17) than the deep cells (65% on or after E18) showing the characteristic superficial to deep neurogenetic gradient ($P<0.009$).

Pars medialis. The pars medialis first appears at L4 as a small group of cells filling up the cell-sparse zone seen at L3 between the pars ventralis and the pars dorsalis (arrow in L3, Fig. 1C). It becomes larger at L5 and extends posteriorly to L7 (Fig. 1). The time of origin of neurons throughout the rostrocaudal extent of the pars medialis is shown in Fig. 9A. Cells were counted in strips through layer II at L5– L7 (drawings, Fig. 9A). As with all other parts of the anterior olfactory nucleus, there is a prominent caudal to rostral neurogenetic gradient ($P<0.0001$). The neurons at L7 are generated mainly on E15–E17 (78% of the population) while those at L5 are generated mainly on E18–E19 (65% of the population). For each of the levels shown in Fig. 9A, the strips were further subdivided into superficial and deep halves. The drawing in Fig. 9B shows the subdivision of the strip at L5. Other levels show similar data which are not illustrated. As in

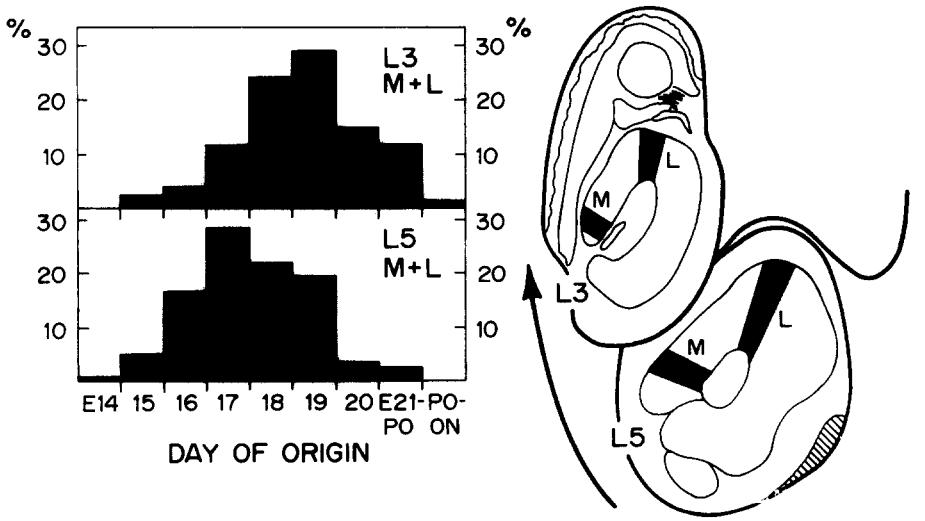

Fig. 7. Time of origin of neurons in the rostrocaudal extent of the pars dorsalis. Neurons were counted in the black strips indicated in the drawings. The bar graphs represent the proportion of neurons originating during 24 hr in embryonic life, or during 48 hr in the perinatal period. There was no difference in times for neurogenesis between medial (M) and lateral (L) strips so the data are combined. Neurogenesis peaks earlier posteriorly (L5) than anteriorly (L3).

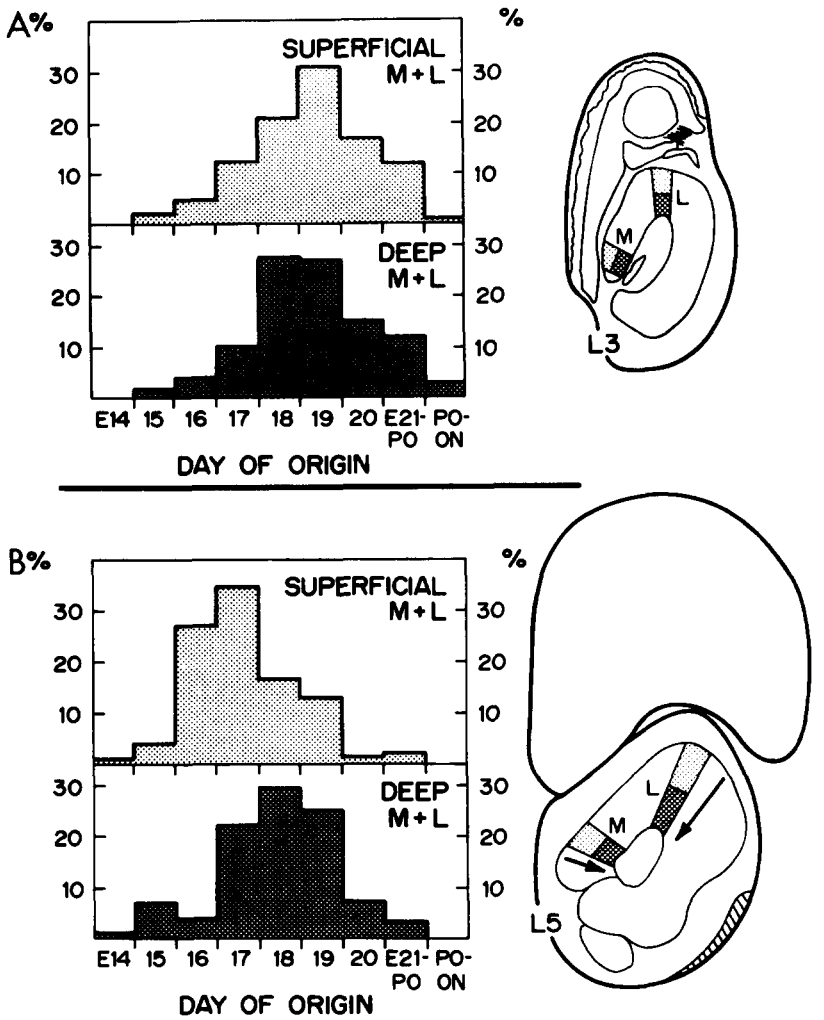

Fig. 8. Neurogenesis along the superficial–deep plane throughout the pars dorsalis. Counts of neurons in the superficial half (light shading) and deep half (dark shading) were kept separate. The bar graphs represent the proportion of neurons originating during 24 hr in embryonic life or 48 hr in the perinatal period. There was no difference between medial (M) and lateral (L) strips so the data are combined. At L3 (A), superficial and deep cells originate simultaneously, while at L4 (B) superficial cells tend to be older than deep cells (arrows in drawings).

all other parts of the anterior olfactory nucleus, there is a significant superficial to deep neurogenetic gradient ($P<0.0001$). Although the peak time of neurogenesis is between E18 and E19 for both superficial and deep cells at L5, a substantial 37% of the superficial cells but only 18% of the deep cells are generated on or before E17.

Pars posterior. The rostrocaudal extent of the pars posterior is very similar to that of the pars medialis, except that it lies slightly more ventrolateral. It comes in at L5 and extends to L7 (Fig. 1). Neurons at the more caudal level are slightly larger than more rostral neurons, confirming the observations of several previous studies.[16,18,23,28,33] Since the pattern of neurogenesis in the pars posterior is very similar to that of the pars medialis, the data are not illustrated. Neurons in the pars posterior are generated in combined caudal to rostral and superficial to deep neurogenetic gradients ($P<0.0001$). On the average, neurons in the pars posterior originate slightly earlier than those in the pars medialis (Fig. 10).

Comparison of neurogenetic patterns between different subdivisions of the anterior olfactory nucleus

At each level through the anterior olfactory nucleus proper, the sign test was also used to determine if there were any neurogenetic gradients between various subdivisions present at a

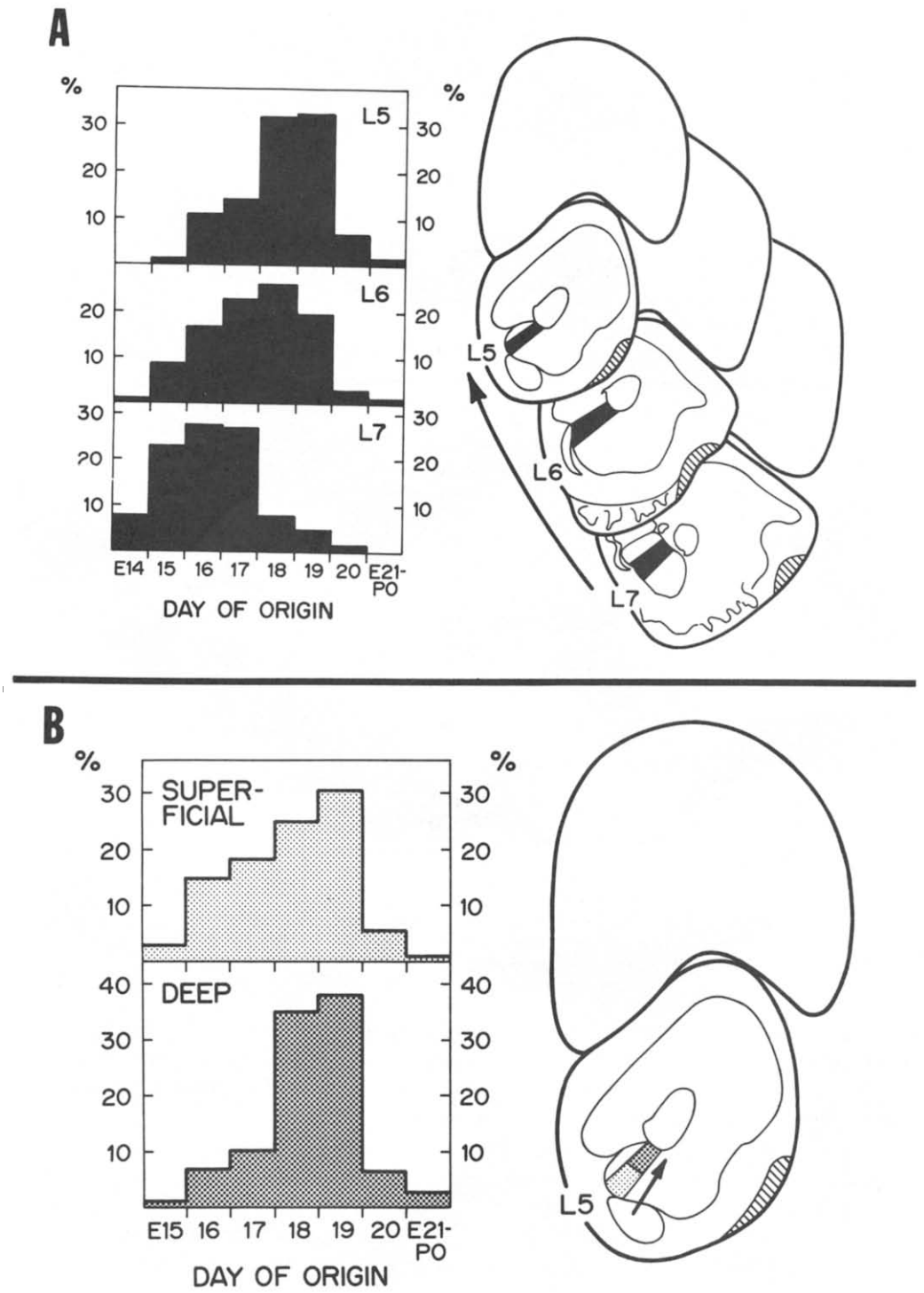

Fig. 9. Time of origin of neurons in the pars medialis. The bar graphs show the proportion of neurons originating over single days of embryonic life. (A) Neurogenesis throughout the rostrocaudal extent. All neurons were counted within the black strips shown in the drawings. There is an earlier to later shift in the peaks of neurogenesis at progressively more anterior levels. (B) Neurogenesis along the superficial–deep plane at L5. Counts of neurons in the superficial half (light shading) and deep half (dark shading) were done separately. On the average, superficial cells originate earlier than deep cells.

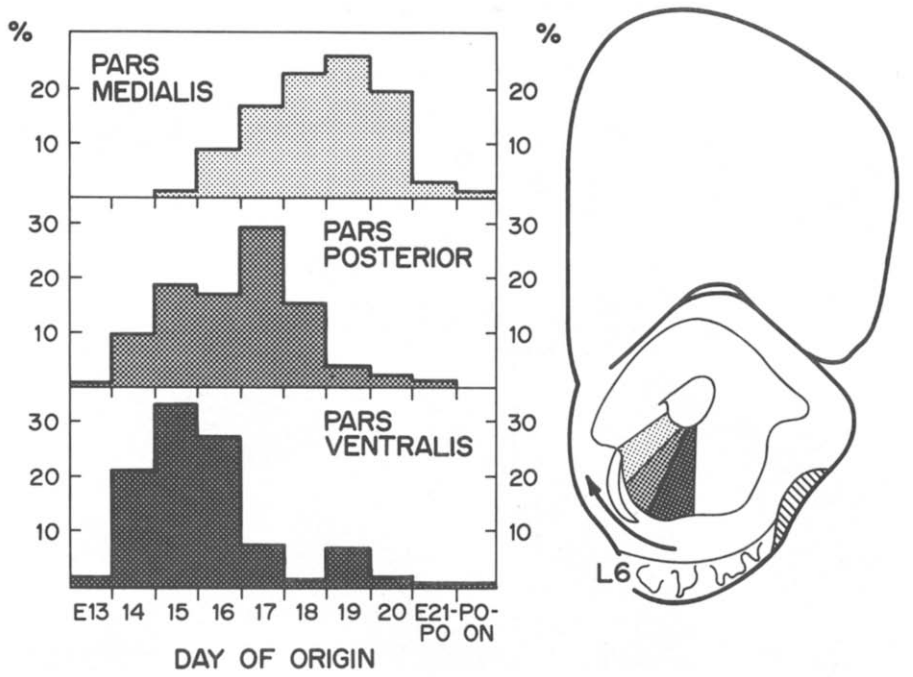

Fig. 10. Time of origin of neurons in the ventral tier of the anterior olfactory nucleus at L6. The bar graphs show a shift in neurogenetic peaks from earlier to later times, indicating a lateral to medial gradient of neurogenesis.

single level. Two of the subdivisions of the anterior olfactory nucleus are noted for their different times of origin, the pars externa and the pars medialis. The pars externa continues the superficial to deep neurogenetic gradient seen in other parts of the anterior olfactory nucleus. For example, the pars externa at L2, embedded in layer I, originates significantly earlier ($P<0.0001$) than the superficial cells in layer II of both the pars ventralis and the pars lateralis (Figs 3A, B, 5B and 6B).

At all levels which contained the pars medialis along with other subdivisions (L5 and L6), the pars medialis originated significantly later ($P<0.0001$), as shown in Fig. 10 for L6. Neurons in the pars ventralis originate significantly earlier than those in the pars posterior, while the pars medialis is generated still later (all levels and comparisons, $P<0.0001$). Thus the ventral parts of the anterior olfactory nucleus (to a lesser degree, the dorsal parts) show a generalized lateral to medial neurogenetic gradient.

The results were mixed between the pars lateralis, pars ventralis and pars dorsalis. For example, the pars ventralis was earlier than the pars lateralis at L2 ($P<0.002$) and L5 ($P<0.0001$), but it was significantly later than the pars lateralis at L3 ($P<0.002$). The pars lateralis was significantly earlier ($P<0.0001$) than the pars dorsalis at L3, but not at L5.

Time of neuron origin in the transition areas

Middle and caudal sections of the olfactory peduncle contain regions with cytoarchitectonic features not found in the anterior olfactory nucleus proper. These posterior specializations were first described by Herrick[23] and are called transition areas by de Olmos *et al.*[16] The dorsal, lateral, and ventral–lateral transition areas have three layers: an outer plexiform layer I, a thin tightly packed zone of pyramidal cells[45] in layer II, and less densely packed pyramidal and polymorph cells in layer III (these layers are indicated in L6, Fig. 1). These three transition areas appear similar to the primary olfactory cortex on the dorsal and lateral walls of the olfactory peduncle at L7. On the medial side of the olfactory peduncle, clusters of more densely packed superficial cells can be delineated from the underlying pars medialis (L4–L7, Fig. 1). This is called the medial transition area in the terminology of de Olmos *et al.*[16] Other names for this area are the anterior continuation of the hippocampus and the tenia tecta.[18,23,24,33]

Ventral–lateral transition area. The ventral–lateral transition area is first noticeable at L5 as a slightly invaginated region along the ventrolateral olfactory peduncle inserted between the pars lateralis and pars ventralis (Fig. 1). The lateral olfactory tract fibers collect in a prominent ovoid bundle in its superficial layer. It is characterized by a trilaminar structure typical of the transition areas. At L6, the layering is more sharp. The autoradiogram in Fig. 11 shows that deep cells in layer III are mostly unlabeled while superficial cells in more sparse layer IIa and more densely packed layer IIb are mostly labeled, indicating a deep to superficial neurogenetic gradient. This is the opposite pattern to what has been seen in the anterior olfactory nucleus proper.

To quantify neurogenesis of the ventral–lateral transition area at L5 (Fig. 12A) and L6 (Fig. 12B), cells were counted in a strip running through layer II (no distinction was made between IIa and IIb) and layer III. At L5 the sign test shows a weak but significant ($P<0.03$) tendency for

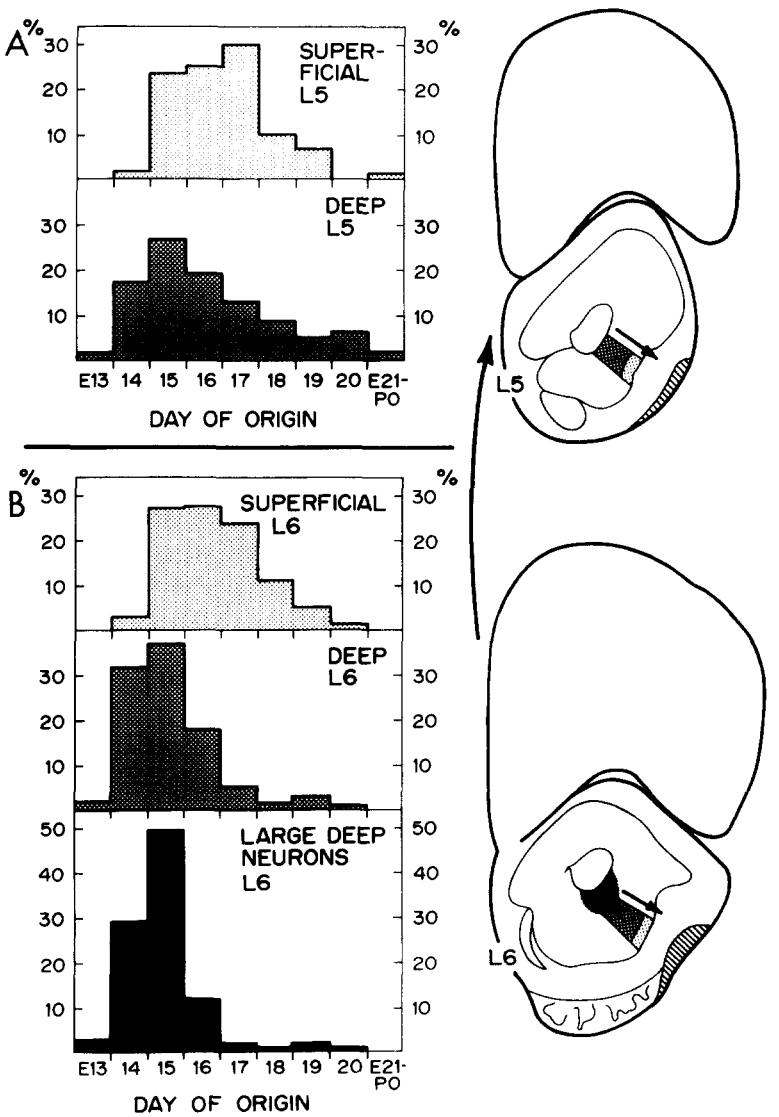

Fig. 12. Neurogenesis in the ventral–lateral transition area throughout its rostrocaudal extent. All cells were counted in the strips shaded in the drawings, keeping the counts of layers II and III separate. At L6 (B), large deep neurons near the anterior commissure (surrounded by the dotted line in Fig. 1) were counted in the area indicated in black. The bar graphs represent the time of neuron origin over single embryonic days at both L5 (A) and L6 (B). Superficial cells originate later than deep cells throughout (arrows in drawings), while cells at L5 (A) tend to originate later than cells at L6 (B, arrow between drawings) in a combined deep to superficial and caudal to rostral neurogenetic gradient. At L6 (B) deep neurons in layer III (middle graph) originate simultaneously with neurons adjacent to the anterior commissure (bottom graph).

deeper cells to be older (66% originate on or before E16) than superficial cells (51% originate on or before E16). At L6, cells in layer III are generated earlier (89% between E13 and E16) than cells in layer II (58% on or before E16) in a stronger ($P<0.0001$) deep to superficial neurogenetic gradient. At L6, the time of origin of a prominent cluster of medium- to large-sized deep neurons just ventrolateral to the anterior commissure was quantified separately (the area outlined in dots in L6, Fig. 1). These large deep neurons are generated simultaneously with the cells in layer III; both groups have prominent peaks of neurogenesis on E14 and E15 (bottom two graphs, Fig. 12B). The ventral–lateral transition area also has a caudal to rostral neurogenetic gradient. The cells in layer III at L6 originate significantly earlier than those at L5 ($P<0.0001$); the cells in layer II have a less strong but similar neurogenetic gradient between L5 and L6 ($P<0.007$).

Dorsal transition area. The dorsal transition area is present only at L6 (Fig. 1), extending across the top of the olfactory peduncle replacing the more anterior pars dorsalis. At L7 it blends imperceptibly with the primary olfactory cortex along the dorsal part of the olfactory peduncle. The time of origin of neurons in the dorsal transition area is shown in Fig. 13. Cells in layers II and III were counted separately in medial (M, Fig. 13) and lateral (L, Fig. 13) strips. The sign test shows no significant differences along the mediolateral plane so the data represented in the bar graphs of Fig. 13 are combined. Deep cells are generated significantly earlier than superficial cells ($P<0.0001$) reproducing the pattern seen in the ventral–lateral transition area. The neurogenetic gradient is especially sharp: approximately 65% of the deep cells originate on or before E15 while 93% of the superficial cells are generated on or after E16.

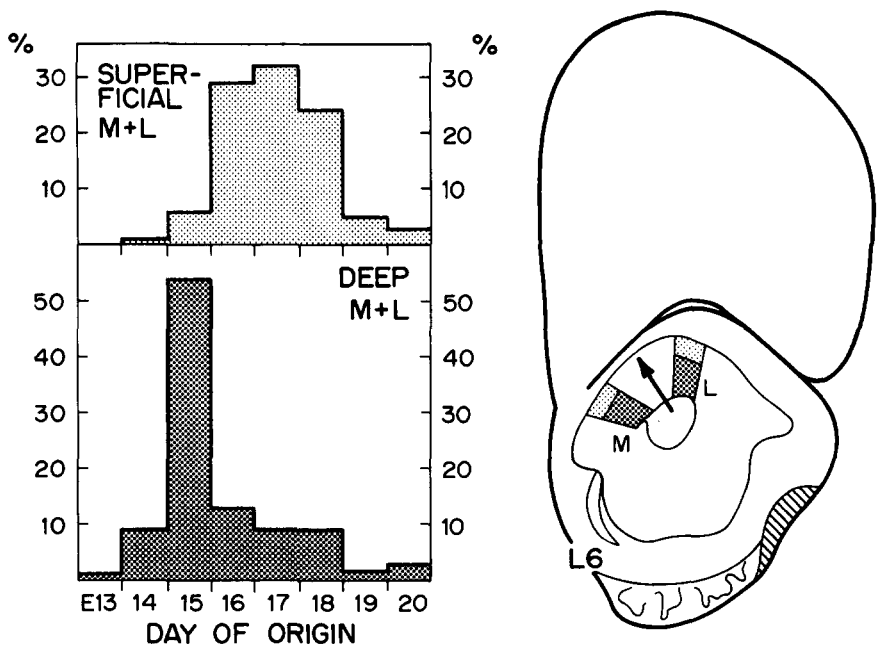

Fig. 13. Neurogenesis in the dorsal transition area. Cells were counted separately in layer II (superficial) and layer III (deep) in both medial (M) and lateral (L) strips. The bar graphs represent the proportion of neurons originating during single embryonic days. Neurons originate simultaneously medially and laterally so the data are combined. There is a strong deep to superficial neurogenetic gradient (arrow in drawing) indicated by the much later time for neurogenesis in the superficial cells when compared with the deep cells.

Lateral transition area. The lateral transition area is seen only at L6 (Figs 1 and 14A), forming a hairpin-sharp curve in the lateral wall of the olfactory peduncle. At L7 it blends imperceptibly with the primary olfactory cortex along the lateral part of the olfactory peduncle. Both superficial (Fig. 14B) and deep layers (Fig. 14C) contain approximately the same proportion of labeled and unlabeled cells. To quantify neurogenesis in the lateral transition area, cells in layers II and III were counted separately (drawing, Fig. 15). The sign test shows no significant differences between times of neuron origin along the superficial–deep plane ($P<0.05$). The absence of a

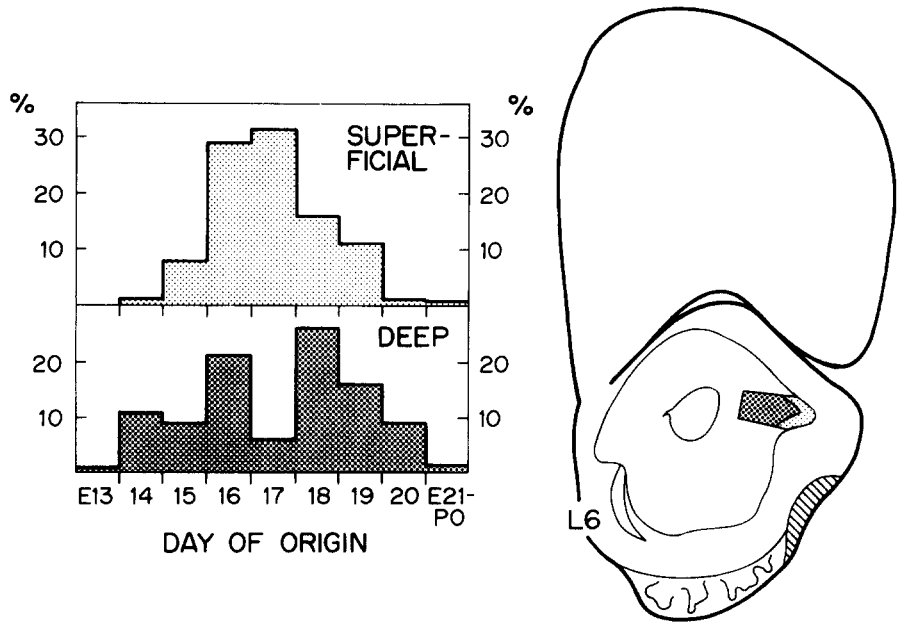

Fig. 15. Time of origin of neurons in the lateral transition area. Superficial and deep cells were counted separately as indicated by the strip shown in the drawings. The bar graphs represent the proportion of neurons that have originated during single embryonic days. Superficial and deep cells originate simultaneously, but the deep cells have an unusual biphasic pattern: neurogenetic peaks on E16 and E18 with only a small amount of cell production on E17.

neurogenetic gradient is due to the unusual pattern of neurogenesis in the deep cells. The superficial cells have the more common monophasic pattern of neurogenesis with a peak on E16–E17. On the other hand, the deep cells in the lateral transition area have a biphasic pattern of neurogenesis. Approximately 41% of the deep cells originate between E13 and E16, only 6% are generated on E17, and 63% are generated on or after E18. On the average, the older group of deep cells originates before the superficial cells, while the younger group of deep cells originates after the superficial cells.

Medial transition area. This area is considered last because its neurogenetic gradients make it a unique component of the olfactory peduncle. The medial transition area, also called the ventral tenia tecta by Price and coworkers,[21,29] is considered to be part of the anterior hippocampal rudiment.[18,23,24,33] The medial transition area is first seen as a ventromedial clump of superficial cells at L4 (Fig. 1). Slightly more posteriorly at L5, the medial transition area is composed of a loop of densely packed peripheral cells surrounding a core of sparsely packed cells. At L6 and L7, a dorsal clump of superficial cells appears above the ventral part of the medial transition area.

Throughout the medial transition area, the proportion of labeled cells is higher anteriorly (Fig. 16A) and posteriorly (Fig. 16C) than in the intervening part (Fig. 16B). To quantify neurogenesis in the medial transition area, all cells were counted in the areas indicated in the drawings (Fig. 17); no distinction was made between central and peripheral cells. The sign test indicates that the neurons at L6 begin to be generated significantly earlier than the neurons at either L4 or L7 ($P<0.0001$). The neurogenetic gradient is quite prominent: approximately 86% of the cells originate on or before E17 at L6, while 75 and 74% originate on or after E17 at L4 and L7, respectively. This is the only part of the olfactory peduncle which shows the older center to younger edge 'sandwich' type gradient. For dorsal and ventral parts of the medial transition area at L7 (Fig. 18), the sign test shows that dorsal superficial cells are generated significantly earlier than ventral superficial cells ($P<0.0001$). Cells deep to the dorsal part of the medial transition area (bottom graph, Fig. 18) are generated primarily on E15–E16, much earlier than the superficial cells ($P<0.0001$).

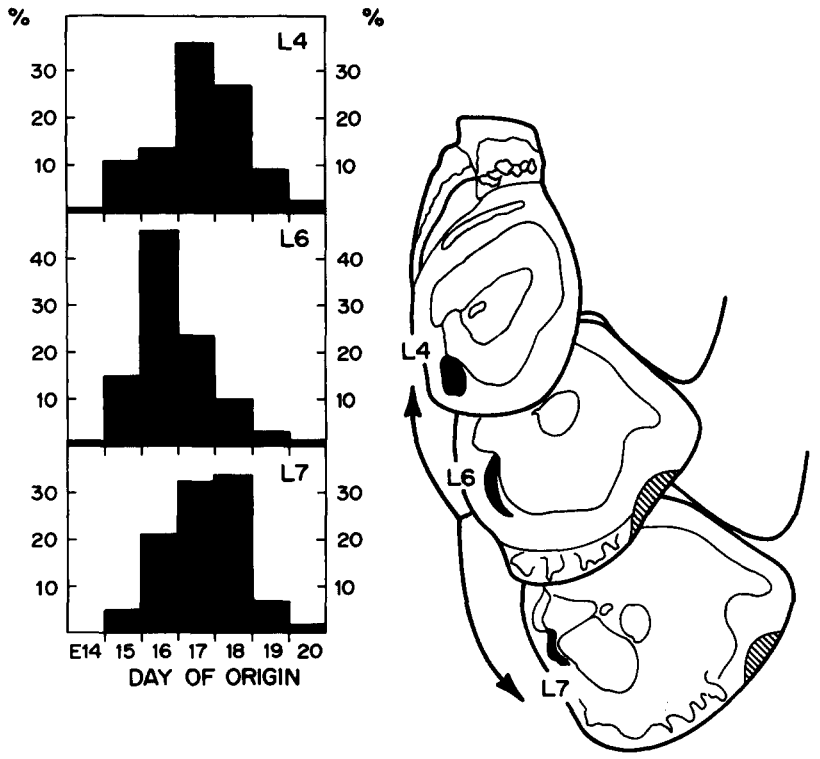

Fig. 17. Neurogenesis in the ventral medial transition area throughout its rostrocaudal extent. All cells were counted in the areas shown in black in the drawings. The bar graphs represent the proportion of neurons originating during single embryonic days. Neurogenesis occurs slightly earlier (more on E16) at L6, than at either L4 or L7 (more on E17–E18).

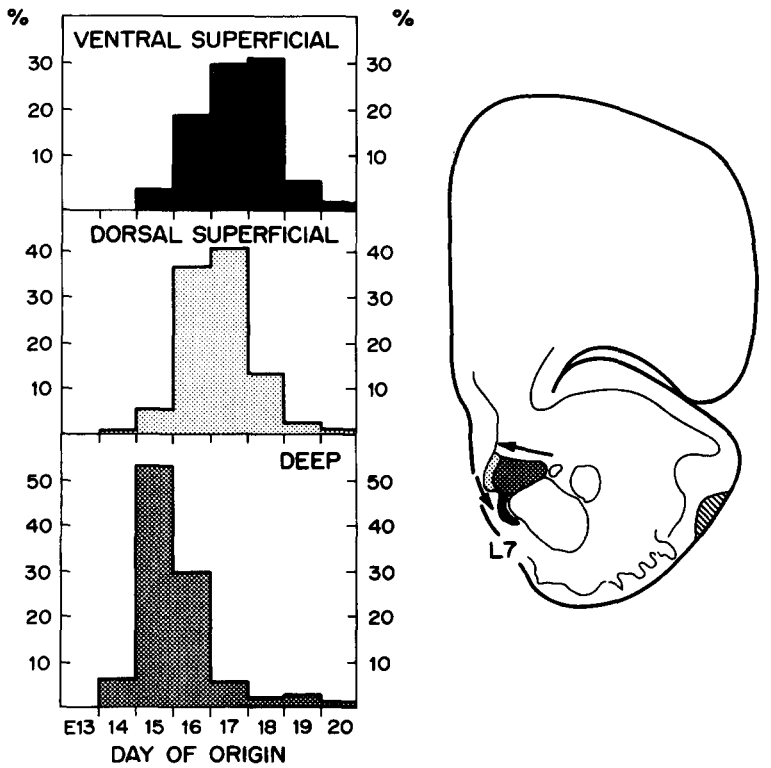

Fig. 18. Time of origin of neurons in the posterior medial transition area where superficial cells are found in dorsal (middle graph) and ventral (top graph) parts. All cells were counted in the shaded areas shown in the drawing. The bar graphs indicate the proportion of neurons originating during single embryonic days. Deep cells originate earlier (peak on E15) than superficial cells, while dorsal superficial cells originate earlier than ventral superficial cells.

DISCUSSION

The gradients of neurogenesis reported here do not agree with the observations of Creps[12] in a [^{3}H]thymidine autoradiographic study of the mouse anterior olfactory nucleus. Creps found no differences in neuron origin along either the superficial–deep or rostrocaudal planes. Since the two species are closely related, the discrepancies may be due to different methods. Creps used pulse labeling with single injections of [^{3}H]thymidine, while this paper is based on a comprehensive labeling method using multiple [^{3}H]thymidine injections. The comprehensive labeling method allows subtle neurogenetic gradients to be distinguished. The major neurogenetic gradients seen in the olfactory peduncle are summarized in Fig. 19.

Superficial–deep gradients

In Fig. 19 the ventral–lateral and dorsal transition areas show darker shading in the interior and lighter shading on the periphery indicating a deep (older) to superficial (younger) gradient (refer also to the data in Figs 12 and 13). This is the typical neurogenetic gradient seen throughout the telencephalic cortical areas, which Angevine[4] termed the 'inside-out' pattern. The next paper[7] will show that the primary olfactory cortex located posterior to the transition areas also has the cortical neurogenetic gradient. These data suggest that the ventral–lateral and dorsal transition areas are anterior extensions of the primary olfactory cortex. In contrast, the segments representing the anterior olfactory nucleus in Fig. 19 have darker exterior shading and lighter interior shading indicating a superficial (older) to deep (younger) neurogenetic gradient, the 'outside-in' pattern of Angevine[4] (refer to Figs 5B, 6B, 8B and 9B). This type of neurogenetic gradient is also found in the corticomedial amygdaloid nuclei,[6] another region which receives primary olfactory input. On the basis of its neurogenetic gradients, the anterior olfactory nucleus is a 'ganglionic' (nuclear) structure, in agreement with Herrick's[23] classification.

The lateral transition area is a true 'transition' area. It is cytoarchitectonically the same as the adjacent transition areas and primary olfactory cortex (Fig. 14), yet it does not have the typical cortical neurogenetic gradient (Figs 15 and 19). The deep cells originate in an unusual biphasic pattern. Such a pattern indicates that neurons from two different populations are intermingled within the same location. Possibly the older group of deep cells are related to deep cortical neurons, since their neurogenesis begins before the superficial cells. The younger set of deep cells may be related to deep neurons in the pars lateralis of the anterior olfactory nucleus, since they originate later (on the average) than the superficial cells.

Rostrocaudal gradients

While gradients along the superficial–deep plane differentiate the anterior olfactory nucleus from the ventral–lateral, lateral and dorsal transition areas, a caudal to rostral gradient is shared by all these structures. For example, the ventral–lateral transition area shows darker shading at L6 than at L5 (Fig. 19), just as do the segments of the anterior olfactory nucleus between these two levels. Thus, while some parts of the olfactory peduncle differentiate into cortical structures, and other parts differentiate into ganglionic (nuclear) structures, developmental events in both parts are linked in the rostrocaudal direction.

The ventral part of the medial transition area (tenia tecta) is the only structure in the olfactory peduncle which does not have a caudal to rostral neurogenetic gradient. Instead, its center is older than either end (Fig. 17). This prominent 'sandwich' gradient along the rostrocaudal plane throws neurogenetic timing in the medial transition area out of synchrony with the adjacent pars medialis. For example, at L6 neurons in the medial transition area are older (62% generated between E13 and E16) than superficial cells in the pars medialis (40% generated between E13 and E16); at L7 there is the reverse pattern with younger medial transition area neurons and older pars medialis neurons. These data indicate that neurogenesis in the medial transition area has a different developmental history than other structures in the olfactory peduncle. Descriptive anatomical studies[18,23,24,33] mention that the specializations along the medial part of the anterior olfactory nucleus form part of the hippocampal rudiment. Neurogenesis in the more posterior part of the rudiment (ventral tenia tecta near the medial septal region and in the indusium griseum) occurs mainly on E17 in a caudal to rostral gradient.[5] The medial transition area of the present

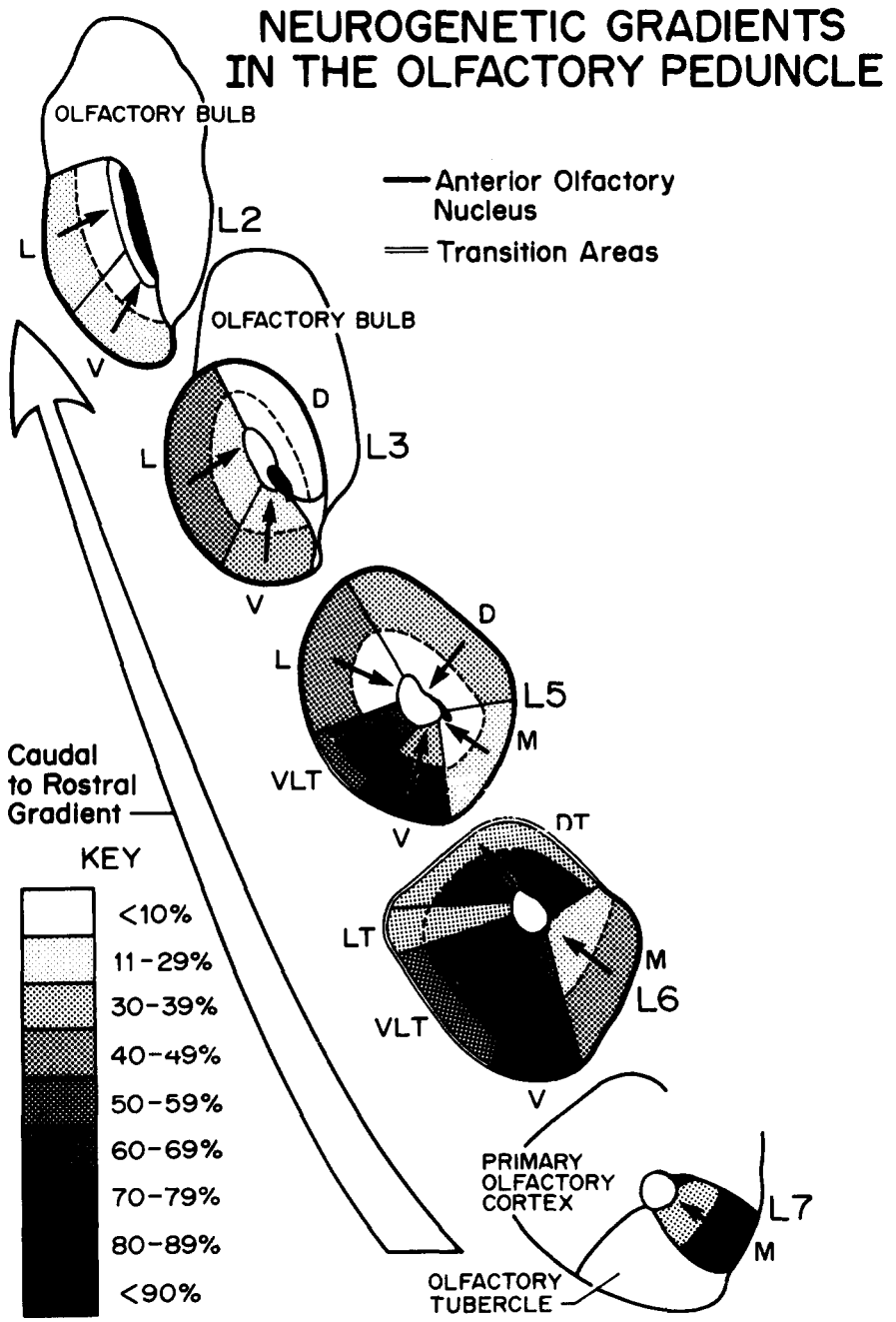

Fig. 19. Summary of the neurogenetic gradients in the olfactory peduncle. The amount of shading represents the proportion of cells which have accumulated by the morning of E17 (generated between E13 and E16). All arrows represent neurogenetic gradients. The wedge-shaped segments represent subdivisions of either the anterior olfactory nucleus or the transition areas arranged around a core fiber tract, the anterior commissure. The pars externa and medial transition area are not shown. Neurogenetic gradients along the superficial–deep plane differentiate between structures. Notice that all segments of the anterior olfactory nucleus show a superficial to deep gradient (arrows point toward core) while the ventral–lateral and dorsal transition areas have a gradient in the opposite direction (arrows point away from core); the lateral transition area has no gradient. In contrast, the caudal to rostral gradient (large outlined arrow) is shared by all structures.

report (as described by de Olmos *et al.*[16]) lies more anteriorly and ventrally, and neurogenesis occurs slightly earlier on both E16 and E17. Work is in progress analyzing the migratory patterns along the medial part of the anterior olfactory nucleus to see if the medial transition area comes from different neuroepithelial sources than those generating either the anterior olfactory nucleus or the hippocampal rudiment.

Neuroepithelial sources in the olfactory peduncle

The many shifts in neurogenetic gradients found in the olfactory peduncle suggests that multiple neuroepithelial sources generate the neurons located here. The neuroepithelium surrounding the third ventricle can be divided into discrete 'zones' each producing neurons destined for particular diencephalic nuclei.[2,3] The same may be true for the neuroepithelium surrounding the olfactory ventricle. The neurogenetic patterns reported here predict that at least three different neuroepithelial zones are present in the embryonic olfactory peduncle. Zone 1 produces the anterior olfactory nucleus. Zone 2 and its subdivisions[7] produce both the rostral primary olfactory cortex and the ventral–lateral and dorsal transition areas. Finally zone 3 produces the medial transition areas, perhaps in close association with the neuroepithelium producing the hippocampal rudiment, as Humphrey[25] has suggested. Zones 2 and 3 may cooperate to produce different parts of the lateral transition area. Since the structures produced by zones 2 and 3 share a common neurogenetic gradient along the rostrocaudal plane, their respective neuroepithelial sources may be lined up end to end along the olfactory ventricle. Schwob and Price[40] recently reported that maturation of the anterior olfactory nucleus occurs in a caudal to rostral direction, in line with its neurogenetic gradients. An embryonic developmental analysis of the entire olfactory peduncle is underway in our laboratory (Bayer, in preparation) to compare neurogenetic gradients with morphogenetic patterns.

Correlations between neurogenetic gradients and anatomical connections

The anterior olfactory nucleus is the source of a strong centrifugal projection to the olfactory bulb.[1,10,13,15–17,21,29,30,32,39,41,42] Schwob and Price[40] injected horseradish perixodase (HRP) into the olfactory bulb of the rat during the early postnatal period to follow the development of the centrifugal projections from the anterior olfactory nucleus. There is a remarkable correlation between their observations and the pattern of neurogenetic gradients reported here. In the pars lateralis for example, superficially positioned neurons in caudal parts are the first to be retrogradely labeled by HRP injections in the olfactory bulb. These are the oldest cells in the pars lateralis. Later HRP injections retrogradely label progressively deeper and more rostral neurons. These are younger cells. Schwob and Price[40] also found that the contralateral projection from the pars externa develops early, and this is in line with its earlier origin in the superficial to deep neurogenetic gradient. Schoenfeld and Macrides[39] found that various components of the pars externa in the hamster are topographically related to the contralateral olfactory bulb; their parcellation corresponds closely to the subdivisions proposed here on the basis of different times of origin. The data presented by Alheid *et al.*[1] indicated that the numbers of ipsilaterally vs bilaterally projecting neurons in the anterior olfactory nucleus of the rat varies along the rostrocaudal axis; this observation may be related to the caudal to rostral neurogenetic gradient. Since the primary olfactory cortex contains similar neurogenetic gradients to those found in the olfactory peduncle, a thorough discussion of other correlations between neurogenetic gradients and patterns of anatomical connections within the olfactory system follows in the companion paper.[7]

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