Bayer, SA and J Altman (1991) NEOCORTICAL DEVELOPMENT Raven Press.

CHAPTER 15

Development of the Limbic Cortical Areas

As early as 1664, Thomas Willis wrote that the cortex on the borders of the cerebral hemispheres had unique anatomical features resembling a "hem" or "limbus." In his 1878 paper, Broca called that area the "grande lobe limbique" (reviewed in White, 1965). In rats as well as in man, the limbic lobe is a continuous cortical band encircling the neocortex. In this chapter, we use the term *limbic cortex* to include parts of Broca's limbic lobe that have been traditionally considered neocortex by some (Papez, 1937; MacLean, 1952; Isaacson, 1974), and variously called *allocortex, peripaleocortex, periarchicortex,* and *proisocortex* by specialists in cytoarchitectonics (Abbie 1940, 1942; Sanides, 1969). The major components of the lateral limbic cortex are the insular, perirhinal, and gustatory areas above and in the rhinal sulcus. The major components of the medial limbic cortex are the dorsal peduncular, infralimbic, cingulate, and retrosplenial areas in the medial wall. The two join in the orbital areas below the frontal pole.

In 1937, Papez (1937) proposed that a group of interconnected telencephalic and diencephalic structures mediates emotional behavior. The cingulate and retrosplenial areas in the medial wall of the cerebral cortex were important parts of the "Papez circuit." Some years later, MacLean (1952) popularized Papez' hypothesis and named the interconnected cortical and subcortical structures the *limbic system*. Since then, anatomical and functional links in the limbic system in general and the medial limbic cortex in particular have been widely studied in relation to behavior (Isaacson, 1974) and support has been provided for Papez' original hypothesis. The participation of the lateral limbic cortex in other circuits involved with emotional behavior was emphasized by Livingston and Escobar (1971). Indeed, the limbic cortex has unique anatomical connections when compared to the neocortex. For example, the mediodorsal thalamic nucleus projects exclusively to the limbic cortex (Leonard, 1972; Krettek and Price, 1974, 1977; Siegel et al., 1977; Divac et al., 1978; Beckstead, 1979; Sarter and Markowitsch, 1983; Groenewegen, 1988), and dopamine innervation is more extensive in both the medial and lateral limbic areas than in the neocortex (Descarries et al., 1987; Kalsbeek et al., 1988).

In contrast to the wealth of functional and anatomical literature dealing with the limbic cortex, there are only a few [^{3}H]thymidine autoradiographic studies of neurogenesis in the medial limbic cortex, all based on pulse labeling after single injections. Fernandez (1969) questioned whether times of neuron origin in the cingulate cortex in rabbits were linked with those in the

anterior thalamic nuclei; he concluded that developmental patterns between the two structures appeared to be unrelated. In a detailed series of papers, Richter and Kranz (1978, 1979a, 1979b, 1979c, 1980) investigated the kinetics of cell proliferation and cell migration in the cingulate gyrus in rats and found that ventral superficial cells have earlier birthdays than dorsal superficial cells, but they did not link that pattern to other developmental events in the cortex. Although some of the lateral limbic areas have been quantitatively studied (Bayer, 1986), a complete neurogenetic timetable has only recently been presented (Bayer, 1990a, 1980b).

The main goal of this chapter is to present quantitative timetables of neurogenesis in the lateral and medial limbic areas and in the orbital cortex. The major findings are that the limbic cortex has neurogenetic gradients in both the transverse and longitudinal directions that differ from the global gradients found in the neocortex. We also show that the neurogenetic gradients found in the limbic cortex correlate with the pattern of its thalamic innervation.

15.1 NEUROGENETIC GRADIENTS IN THE LATERAL LIMBIC AREAS

The insular cortex forms the dorsal border of the piriform cortex. Zilles (1985) describes two areas anteriorly, the ventral agranular insular (AIV, Fig. 15–1) in the rhinal sulcus and the dorsal agranular insular (AID, Fig. 15–1) just above AIV in the lateral cortical wall. The insular cortex continues posteriorly in the rhinal sulcus as area AIP (posterior agranular insular area). AIV and AIP correspond to Krieg's (1946a, 1946b) area 13, while AID corresponds to a more circumscribed part of Krieg's area 14. Krieg (1946b) describes three cortical layers throughout the insular cortex: an outer layer of small granular type cells, an intermediate layer of pyramidal cells, and a deep layer with horizontally flattened cells. All areas of the insular cortex are situated above the claustrum, which makes this area homologous to the human insular cortex (Krieg, 1946b). The gustatory cortex (GU, Zilles, 1985, Fig. 15–1) is defined by its reciprocal connections with the taste area in the medial part of the thalamic ventromedial nucleus (Wolf, 1968; Leonard, 1969; Reep and Winans, 1982a, 1982b; Saper, 1982; Shipley and Geinisman, 1984; Kosar et al., 1986; Cechetto and Saper, 1987). GU forms the dorsal border of AID and is incorporated into Krieg's (1946a, 1946b) area 14. The cellular layers are more distinct in GU, and a sparse but definite layer IV can be distinguished, similar to that seen in the secondary somatasensory area (Zilles and Wree, 1985). Posterior to the insular cortex, the perirhinal cortex (PR, Zilles and Wree, 1985; area 35, Krieg 1946a, 1946b) lies in the rhinal

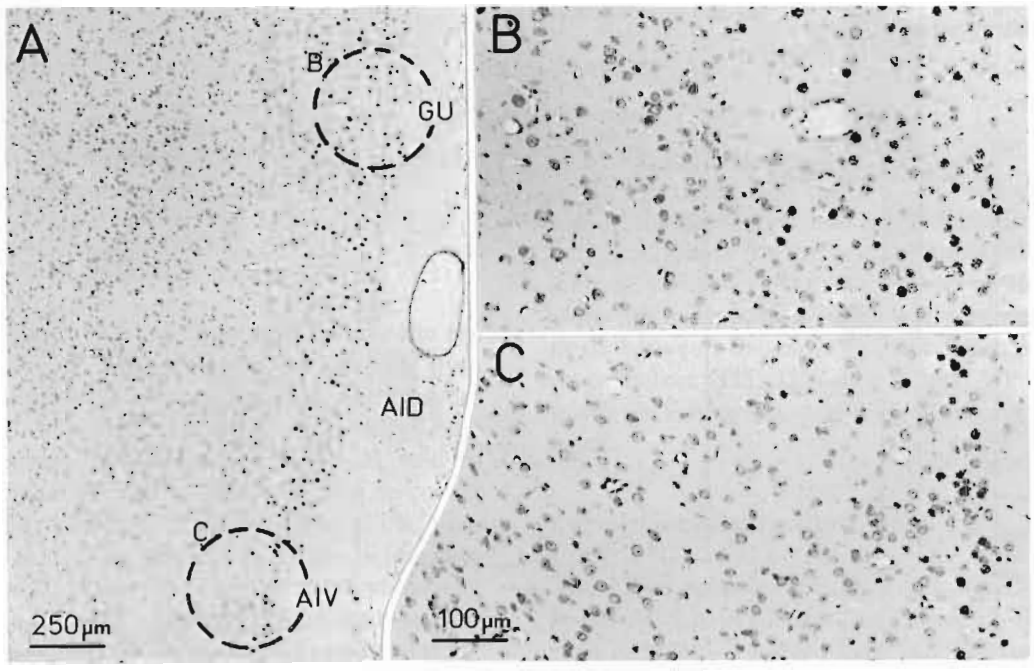

FIG. 15–1. Photomicrographs of the lateral cortical wall at level A8.2 in an animal exposed to [^{3}H]thymidine on E18 and E19 and killed on P60. **(A)** low-magnification view showing the increase in the depth of labeled cells progressing from the ventral agranular insular cortex (AIV) through the dorsal agranular insular cortex (AID) and into the gustatory cortex (GU). The areas encircled with dashed lines are shown in higher magnification in **B** and **C.** (6 µm paraffin section, hematoxylin/eosin stain.) From Bayer (1990a).

sulcus just below the auditory cortex and above the entorhinal cortex. The cortical layers are thinner, and the horizontally oriented cells in layer VI are especially prominent. For quantitative analysis, coronal sections were selected at nine anteroposterior levels (A9.2 to A1.2; drawings, Fig. 15–3).

15.1.1 Radial and Transverse Neurogenetic Gradients

When [^{3}H]thymidine injections are given on E18 and E19, cell labeling patterns in the lateral cortical wall at level A8.2 (Fig. 15–1) indicate that only a thin band of superficial cells are labeled in AIV in the rhinal sulcus (Fig. 15–1C), while the dorsally located GU (Fig. 15–1B) has a considerably thicker band of superficial labeled cells. These photomicrographs illustrate the two neurogenetic gradients seen in all other neocortical areas: (1) a radial gradient (deep cells are older than superficial cells) and (2) a transverse gradient (ventral cells are older than dorsal cells). Since the transverse gradient is found throughout the entire rostrocaudal expanse of AIV, AID, and GU, only the data for level A8.2 (drawing, Fig. 15–2) are illustrated. In layers II–IV (right column of graphs, Fig. 15–2), neurogenesis occurs earlier in AIV than in AID ($P < 0.0001$) and earlier in AID than in GU ($P < 0.0001$). When AIV and GU are directly compared, 59% of the neurons originate on or before E16 in AIV but only 26% of these neurons originate during this time in GU. In layer V (left column of graphs, Fig. 15–2), neurogenesis occurs earlier in AIV than in AID ($P < 0.0001$) and earlier in AID than in GU ($P < 0.0001$). Again, there are sharp differences between AIV and GU. For example, 75% of the layer V neurons originate on or before E15 in AIV, while only 47% of these neurons originate during the same time period in GU. Layer VI neurogenesis occurs mainly on E14 (16%) and E15 (65%) simultaneously in GU, AID, and AIV (all comparisons $P > 0.05$; data are not shown). In the deep parts of AIV and AID, fewer neurons are generated on E14 and more on E15 than would be expected by the pattern seen in GU. Possibly some of the oldest deep neurons are missing in AID and AIV. That circumstance is most likely due to the presence of the claustrum, which lies beneath the deep neurons in AIV and AID. Claustral neurons originate mainly on E15 and E16 (in preparation).

15.1.2 Unique Longitudinal Neurogenetic Gradients

Unlike the neocortex, where there is an older-anterior to younger-posterior longitudinal gradient in nearly all areas, the lateral limbic cortex shows either a sandwich pattern, where older neurons are flanked anteriorly

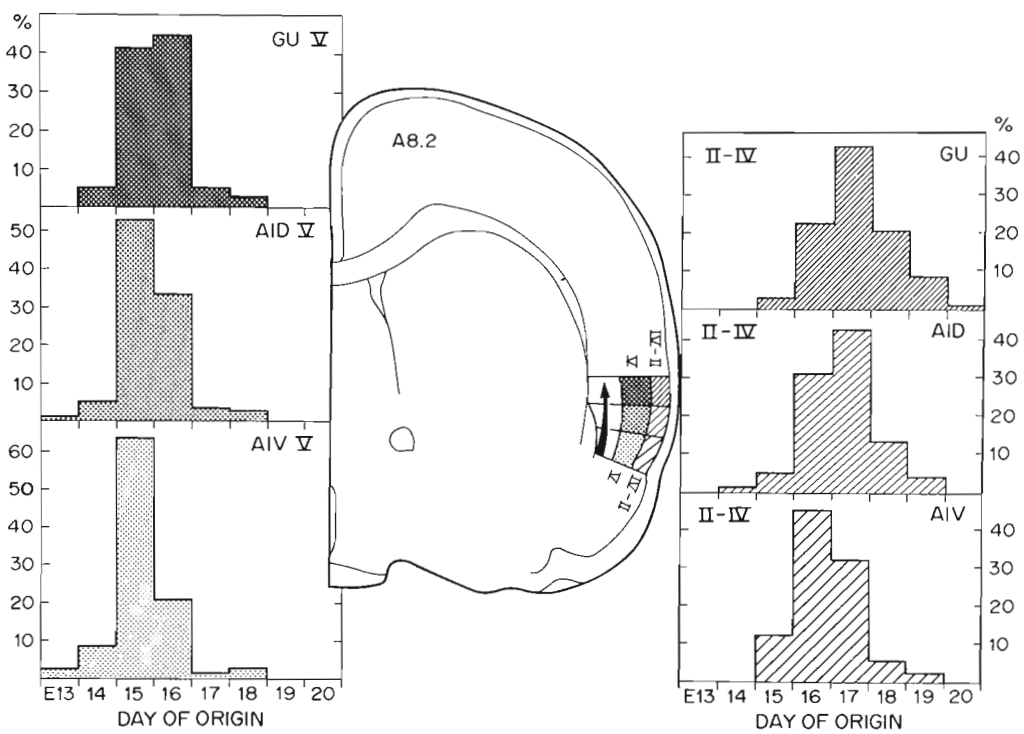

FIG. 15–2. Neurogenesis in layer V (left column of graphs) and layers IV–II (right column of graphs) in the ventrolateral cortical wall at level A8.2. All bar graphs are the proportion of neurons that are generated in a single day of embryonic life. There is a ventral (older) to dorsal (younger) neurogenetic gradient (*arrow* in drawing) between areas in all layers. From Bayer (1990a).

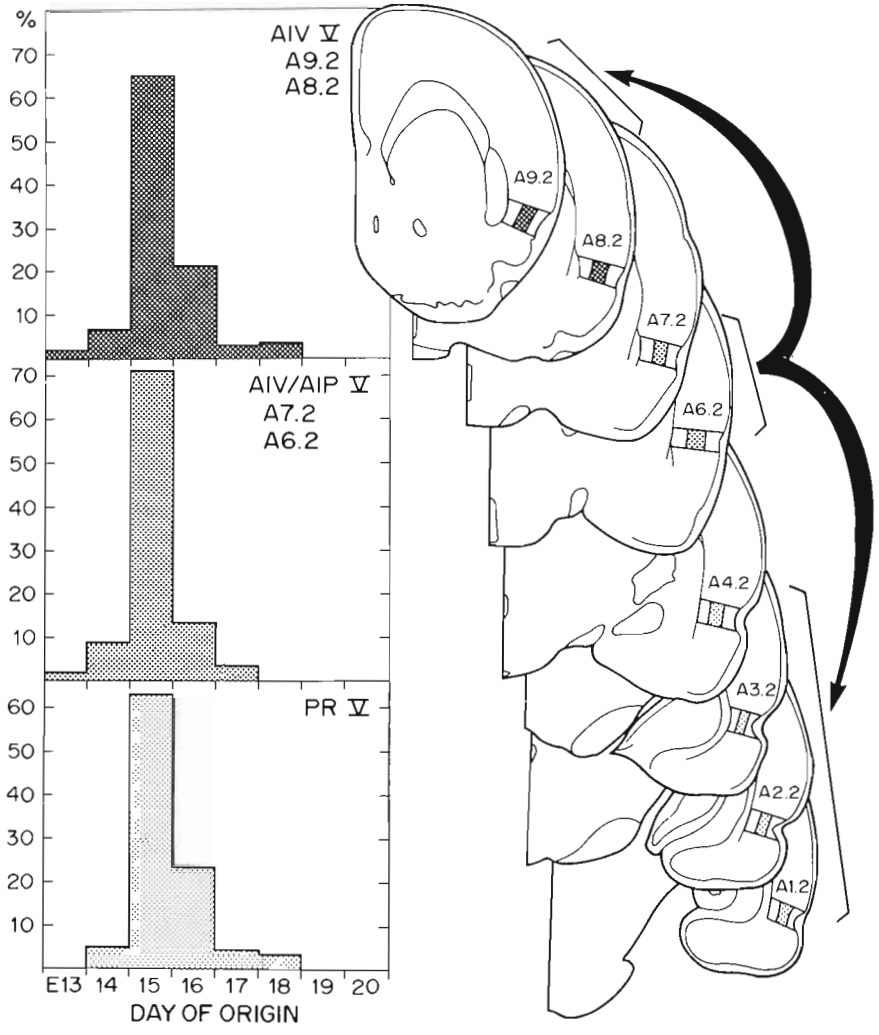

FIG. 15–3. The time of origin of layer V neurons in the rhinal sulcus between levels A9.2 (*top drawing*) and A1.2 (*bottom drawing*). Bar graphs represent the proportion of neurons originating on single days during embryonic life. The oldest neurons in A7.2–A6.2 are sandwiched by younger neurons anteriorly and posteriorly (*large bifurcating arrow* in drawings). From Bayer (1990a).

and posteriorly by younger neurons, or a posterior (older) to anterior (younger) gradient.

Cortex in the Rhinal Sulcus

The cortex in the rhinal sulcus extends nearly the entire length of the cerebral hemispheres (drawings, Fig. 15–3). Beginning with the anterior end, there is the ventral agranular insular area (AIV, A9.2–A7.2), the posterior agranular insular area (AIP, A6.2), and finally the perirhinal area (PR, A4.2–A1.2). The cortex at level A5.2 is transitional between AIP and PR. Posterior to level A1.2, the entorhinal cortex is found in the rhinal sulcus. All layers have the same pattern of neurogenesis in the anteroposterior plane, and the data in Fig. 15–3 show only those of layer V as an example. Neurons in layer V are generated in a sandwich gradient such that the older neurons are found in posterior AIV and in AIP (middle graph, Fig. 15–3); these neurons originate significantly earlier than those in anterior AIV (top graph, Fig. 15–3; $P < 0.002$, sign test) or in PR (bottom graph, Fig. 15–3; $P < 0.0001$, sign test). The sandwich pattern was found in 20 of 21 rats in the E16 + E17 and E17 + E18 injection groups, which accounts for the high levels of statistical significance. However, the magnitude of the differences were low with only a 10–14% divergence in neurogenesis between the older core and the younger edges: on or before E15, 83% of the AIV/AIP neurons, 73% of the AIV neurons, and 69% of the PR neurons originate.

Dorsal Agranular Insular Cortex

The dorsal agranular insular cortex (AID) extends from levels A9.2 to A7.2 (drawings, Fig. 15–4A). Only neurons in layer VI have a significant posterior (older) to anterior (younger) neurogenetic gradient ($P < 0.0001$; two bottom graphs, Fig. 15–4A). Nearly 23% of the layer VI neurons at levels A8.2–A7.2 originate on or before E14, while only about 11% of these neurons originate during this same period at A9.2. There are

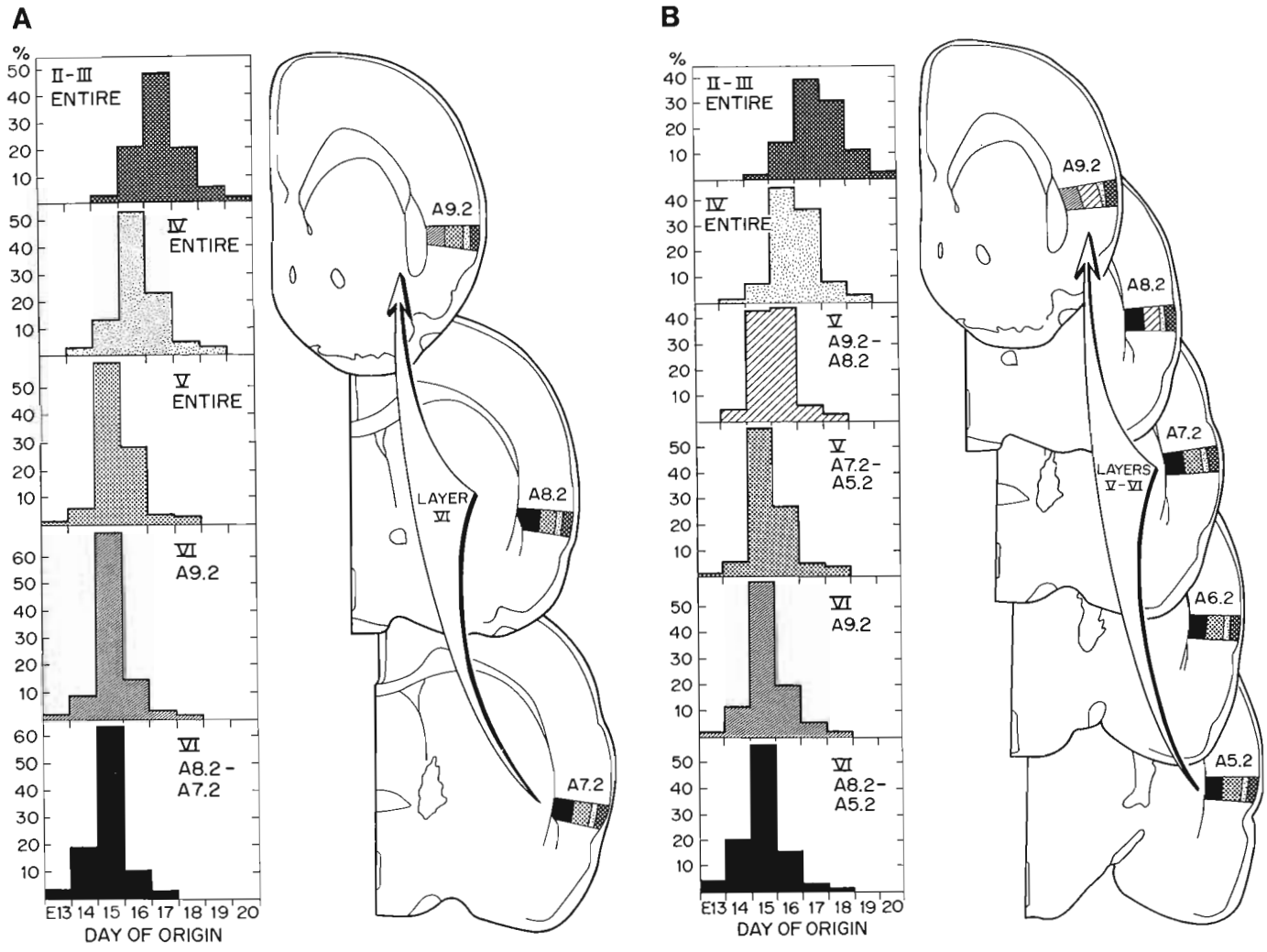

FIG. 15–4. Neurogenesis in the dorsal agranular insular cortex **(A)** and in the gustatory cortex **(B)**. All graphs represent the proportion of neurons originating during single days of embryonic life. In both cortical areas, deep neurons have a posterior (older) to anterior (younger) neurogenetic gradient, matching with similar gradients in the cortex below them in the rhinal sulcus and in the anterior primary olfactory cortex. From Bayer (1990a).

no gradients in the anteroposterior plane in layers V, IV, and II–III (all $P > 0.05$) and the data are combined in the three top graphs of Figure 15–4A. There is a highly significant deep (older) to superficial (younger) neurogenetic gradient between the layers (all comparisons, $P < 0.0001$). Layer VI has peak neurogenesis on E15, layer V on E15–E16, layer IV on E16–E17, and layers II–III on E16–E18.

Gustatory Cortex

The gustatory cortex (GU) extends from A9.2 through A5.2 (drawings, Fig. 15–4B). Both layers VI and V have neurogenetic gradients in the anteroposterior plane. Neurons in layer VI at levels A8.2–A5.2 (bottom graph, Fig. 15–4B) originate significantly earlier than those at level A9.2 (5th graph from top, Fig. 15–4B; $P < 0.0001$). These differences are best seen in the proportion of neurogenesis that occurs on or before E14 (A8.2–A5.2, 24%; A9.2, 13%). Neurons in layer V originate significantly earlier at levels A7.2–A5.2 than those at levels A9.2–A8.2 ($P < 0.002$). Neurogenetic differences in layer V are best seen in the proportion of neurons that are generated on or after E16 (A7.2–A5.2, 36%; A9.2–A8.2, 53%). Since neurons in layers IV and III–II originate simultaneously at all levels (all $P > 0.05$), the data are combined. There is a highly significant deep (older) to superficial (younger) neurogenetic gradient between the layers (all comparisons, $P < 0.0001$). Layer VI has peak neurogenesis on E15, layer V on E15–E16, layer IV on E16–E17, and layers II–III on E17–E18.

15.2 NEUROGENETIC GRADIENTS IN THE MEDIAL LIMBIC AREAS

The data presented here show that there are three neurogenetic gradients in the medial limbic cortex. (1) A radial gradient is found throughout the entire medial limbic cortex and has the same characteristics as that seen in all neocortical areas: deep neurons are older than superficial neurons. These data confirm earlier reports of that gradient in the medial limbic cortex of the rabbit (Fernandez, 1969) and rat (Richter and Kranz, 1978, 1979a, 1979b, 1979c, 1980). (2) The transverse gradient in the medial limbic cortex is in the opposite direction to that found in the neocortex. The pattern found in the neocortex would predict that the farther a cortical area is from the rhinal sulcus, the younger it will be; therefore, the most ventral medial limbic areas should contain the youngest neurons. However, the ventral medial limbic areas contain older neurons than the dorsal areas. That gradient was first reported by Richter and Kranz (1979b, 1979c). Thus, the medial limbic cortex is not a continuation (at least in terms of its ontogenetic pattern) of the somatic neocortex. That feature can be linked to phylogeny of the cerebral cortex (Sanides, 1969), and will be more fully discussed in Chapter 16. (3) The longitudinal gradient in the medial limbic cortex is the same as in the neocortex, anterior neurons are older than posterior neurons. As we will point out below, the gradient between the superficial neurons correlates with anatomical connections from the anterior thalamic nuclear complex.

15.2.1 The Dorsal Peduncular and Infralimbic Areas

Zilles (1985) delineates two cortical areas in the ventral medial cortical wall anterior to the genu of the corpus callosum. The dorsal peduncular cortex (DP, Fig. 15–5) lies just dorsal to the tenia tecta (TT, Fig. 15–5) in the anterior medial wall and is distinguished by a thickening of layer I and a thinner superficial layer (S, Fig. 15–5) than the adjacent infralimbic area (IL, Fig. 15–5), also called the prelimbic area. Krieg (1946a, 1946b) includes both the DP and IL in area 25; he notes that layer VI contains "numerous flattened granular cells." The parallel orientation of the deep neurons (D, Fig. 15–5) to the underlying white matter (WM, Fig. 15–5) is in sharp contrast to the radial (perpendicular) orientation of the superficial neurons (on the right of the dashed line, Fig. 15–5). When [^{3}H]thymidine injections are given on E17 and E18, only the superficial neurons are labeled throughout both cortical areas (Fig. 15–5), indicating that deep neurons are older than superficial neurons. In addition, there are proportion-

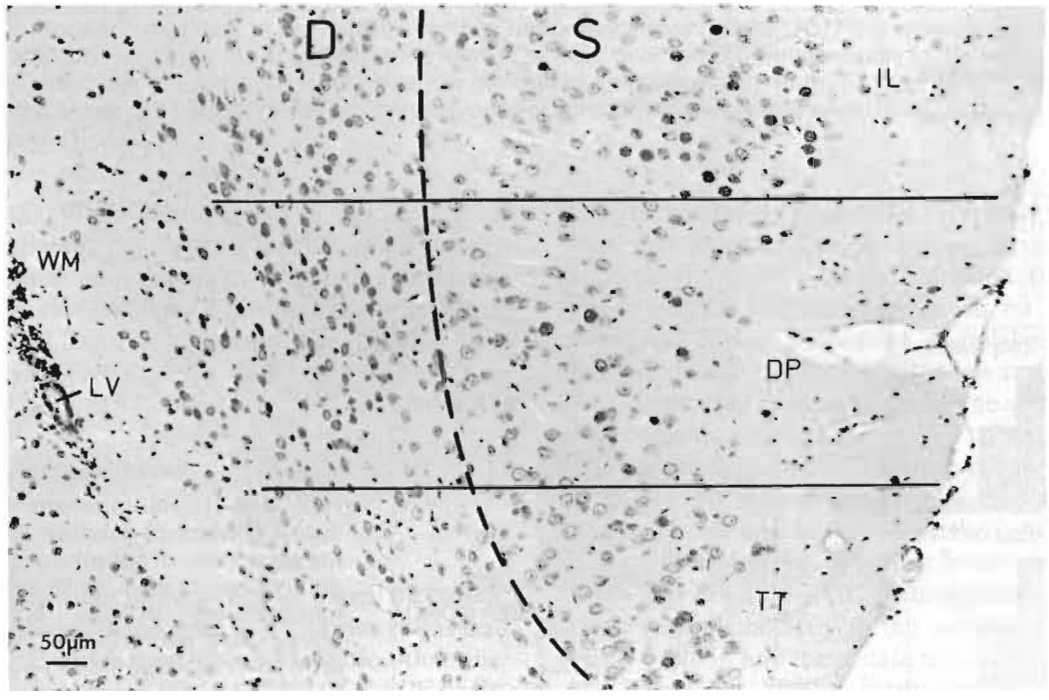

FIG. 15–5. The dorsal part of the tenia tecta (TT), the dorsal peduncular cortex (DP), and ventral part of the infralimbic area (IL) at the most anterior extent of the lateral ventricle (LV) in an animal exposed to [^{3}H]thymidine on E17–E18 and killed on P60. Solid lines are boundaries between areas; dashed line separates deep (dp) and superficial (SU) neurons. Deep neurons are unlabeled in all areas, while many superficial neurons are still labeled, especially in IL. (6 μm paraffin section, hematoxylin/eosin stain.) From Bayer (1990b).

ally more labeled superficial neurons in IL than in DP, indicating that ventral areas are older than dorsal areas.

To quantify the time of origin in DP, neurons were counted in superficial and deep halves at levels A10.6 and A9.8 (*shaded areas* in drawings, Fig. 15–6A). Since the sign test indicated that neurogenesis was simultaneous at both levels ($P > 0.05$), the data illustrated in Fig. 15–6A were combined for both levels. There is a pronounced deep (older) to superficial (younger) neurogenetic gradient ($P < 0.0001$). That same gradient is present in every area of the medial limbic cortex.

To quantify the time of neuron origin in IL, the area was divided into superficial and deep halves at levels A10.6 and A9.8 (drawings, Fig. 15–6B). The sign test indicated two neurogenetic gradients. (1) Deep neurons originate earlier than superficial neurons ($P < 0.0001$) at both levels. The deep neurons have peak neurogenesis on E15 and E16, while the superficial neurons originate between E16 and E18. (2) Neurons at level A10.6 originate slightly earlier than neurons at level A9.8. For example, more neurons in the anterior deep areas (top right graph, Fig. 15–6B) are generated on E15, while more neurons are generated on E16 in the posterior deep areas (bottom right graph, Fig. 15–

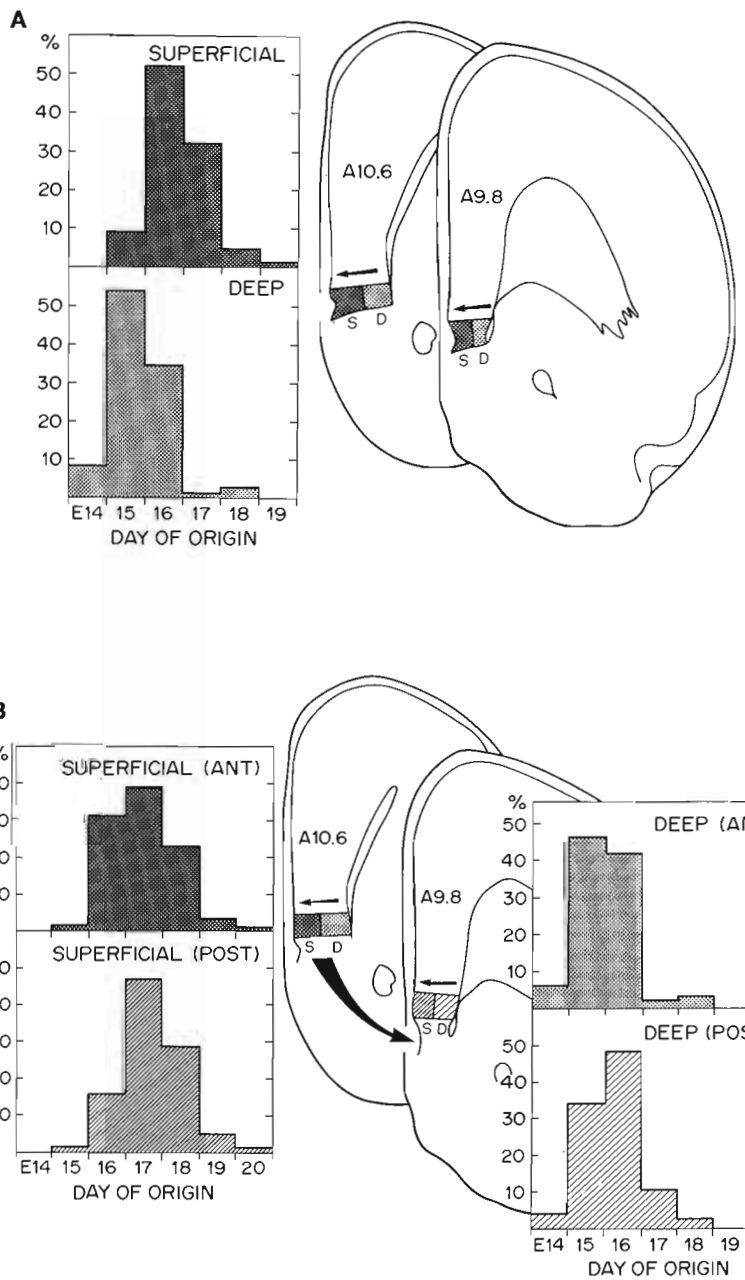

FIG. 15–6. (A) Neurogenetic timetable in the dorsal peduncular cortex. Bar graphs are the proportion of neurons originating on single embryonic days. Cells are generated simultaneously in the anterior-posterior plane. Deep neurons are generated mainly on E15–E16, superficial neurons on E16–E17. **(B)** Time of neuron origin in the infralimbic area. Bar graphs are the proportion of neurons generated on single embryonic days. There are both deep (older) to superficial (younger) and anterior (older) to posterior (younger) neurogenetic gradients. From Bayer (1990b).

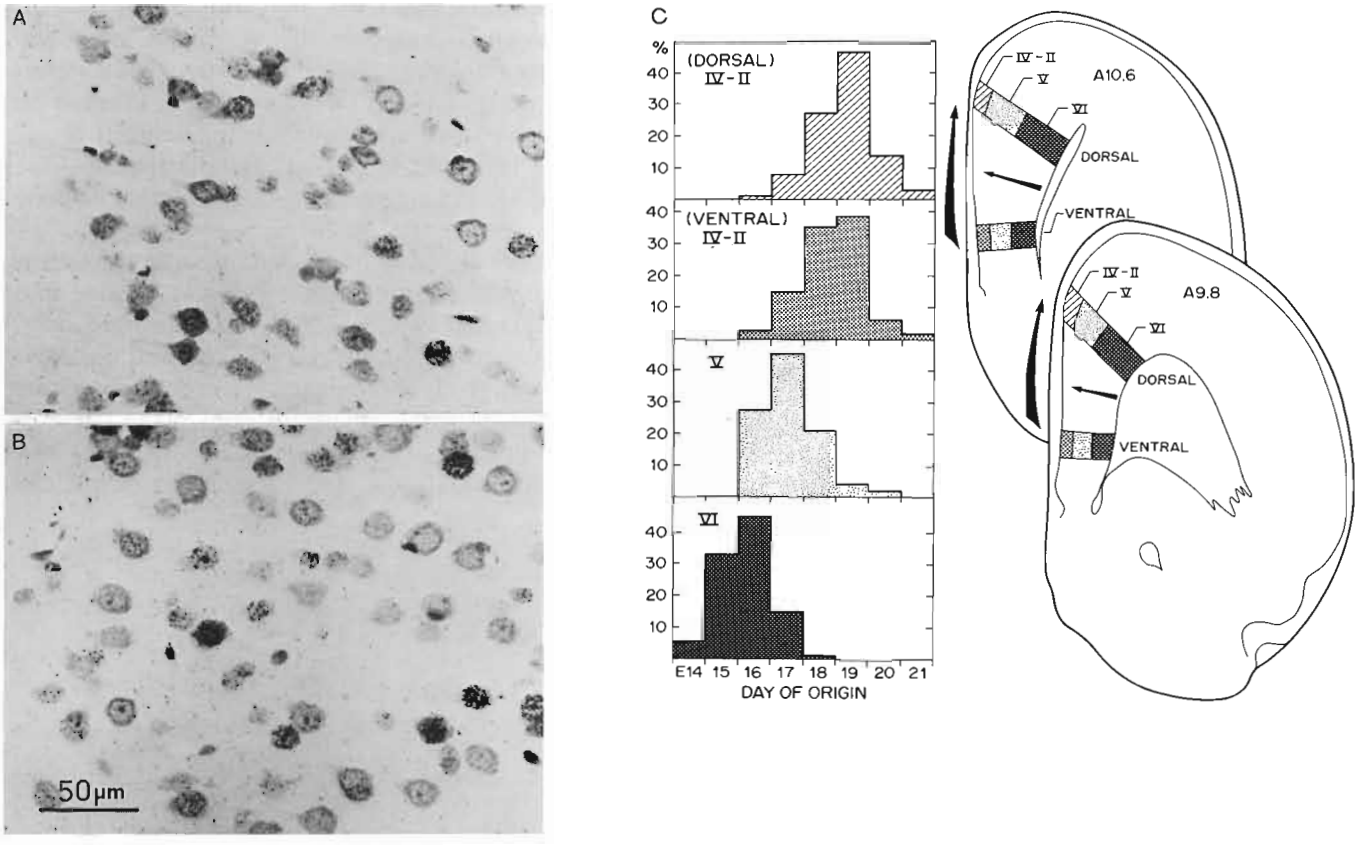

FIG. 15–7. A and **B** are photomicrographs of superficial neurons in areas CG1 **(A)** and CG3 **(B)** in an animal exposed to [^{3}H]thymidine on E18–E19 and killed on P60. CG3 **(B)** has a higher proportion of unlabeled neurons than CG1 **(A)** indicating a ventral (older) to dorsal (younger) neurogenetic gradient. (6 μm paraffin section, hematoxylin/eosin stain.) **(C)** Time of neuron origin in the anterior cingulate areas. Neurons in all layers are generated simultaneously in the anterior-posterior plane. Layers VI–V neurons are generated simultaneously in dorsal and ventral locations. There is a ventral (older) to dorsal (younger) gradient between the superficial layers. From Bayer (1990b).

6B; $P < 0.038$). When neurogenesis in the superficial layers is compared, the anterior level (top left graph, Fig. 15–6B) has more neurons originating on E16, while the posterior level has more neurons originating on E17 and E18 (bottom left graph, Fig. 15–6B; $P = 0.0002$).

15.2.2 The Cingulate Areas

CG1/CG3: The Anterior Cingulate Cortex

Zilles (1985) describes two areas of the cingulate cortex that extend anterior to the genu of the corpus callosum. The dorsal CG1 has better laminar definition than the ventral CG3 (drawings, Fig. 15–7C). CG1 is Krieg's (1946a, 1946b) area 24, while CG3 is his area 32. In the rat, the areas designated as layers V and VI in the drawings of Figure 15–7C are continuations of the same layers in the neocortex. A granular layer IV is greatly reduced (Krieg 1946a, 1946b; Zilles, 1985), and the remaining superficial layers are much thinner than in neocortex (Krieg, 1946a). In this study, the superficial layers were not divided (IV–II). When [^{3}H]thymidine injections are given on E18 and E19, there is a higher proportion of unlabeled superficial neurons in CG3 (Fig. 15–7B) than in CG1 (Fig. 15–7A), indicating a ventral (older) to dorsal (younger) neurogenetic gradient.

To quantify the time of neuron origin in the anterior cingulate cortex, neurons were counted in dorsal (CG1) and ventral (CG3) strips through layers VI, V, and combined layers IV–II at levels A10.6 and A9.8 (drawings, Fig. 15–7C). Neurogenesis in each layer occurs simultaneously in the anterior/posterior plane (all $P > 0.05$), and these data are combined in Fig. 15–7C. There is a highly significant deep (older) to superficial (younger) neurogenetic gradient between layers (all comparisons, $P < 0.0001$); neurons in VI are generated mainly on E15–E16, in V on E16–E18, in IV–II on E18–E19. Supporting the qualitative observations (Fig. 15–7A,B), the superficial neurons in CG3

originate earlier than those in CG1 (two top graphs, Fig. 15–7C). Since all 19 rats showed the same labeling pattern in the E18 + E19, E19 + E20, and E20 + E21 injection groups, the sign test indicated that the trend is highly significant ($P < 0.0001$). The magnitude of the divergence between populations is approximately 16%: on or before E18, 53% of the superficial CG3 neurons are generated (second graph from top, Fig. 15–7C) but only 37% of the superficial CG1 neurons are generated (top graph, Fig. 15–7C).

CG1/CG2: The Posterior Cingulate Cortex

Zilles (1985) divides the supracallosal cingulate cortex into two areas: dorsal CG1, Krieg's (1946a, 1946b) area 24, and ventral CG2, Krieg's (1946a, 1946b) area 23. The histological characteristics of the posterior cingulate cortex are very similar to those of the anterior cingulate cortex. When [^{3}H]thymidine is injected on E19 and E20, there are fewer labeled neurons in the superficial layers of CG2 (Fig. 15–8B) than in CG1 (Fig. 15–8A), indicating a ventral (older) to dorsal (younger) neurogenetic gradient.

To quantify the time of neuron origin in the supracallosal cingulate cortex, that area was divided into dorsal (CG1) and ventral (CG2) strips. In each strip, neurons were counted in layers VI, V, and the combined layers IV–II. Neurogenesis in each layer occurs simultaneously in the anteroposterior plane (all $P > 0.05$), and the data are combined in Fig. 15–8C. There is a highly significant deep (older) to superficial

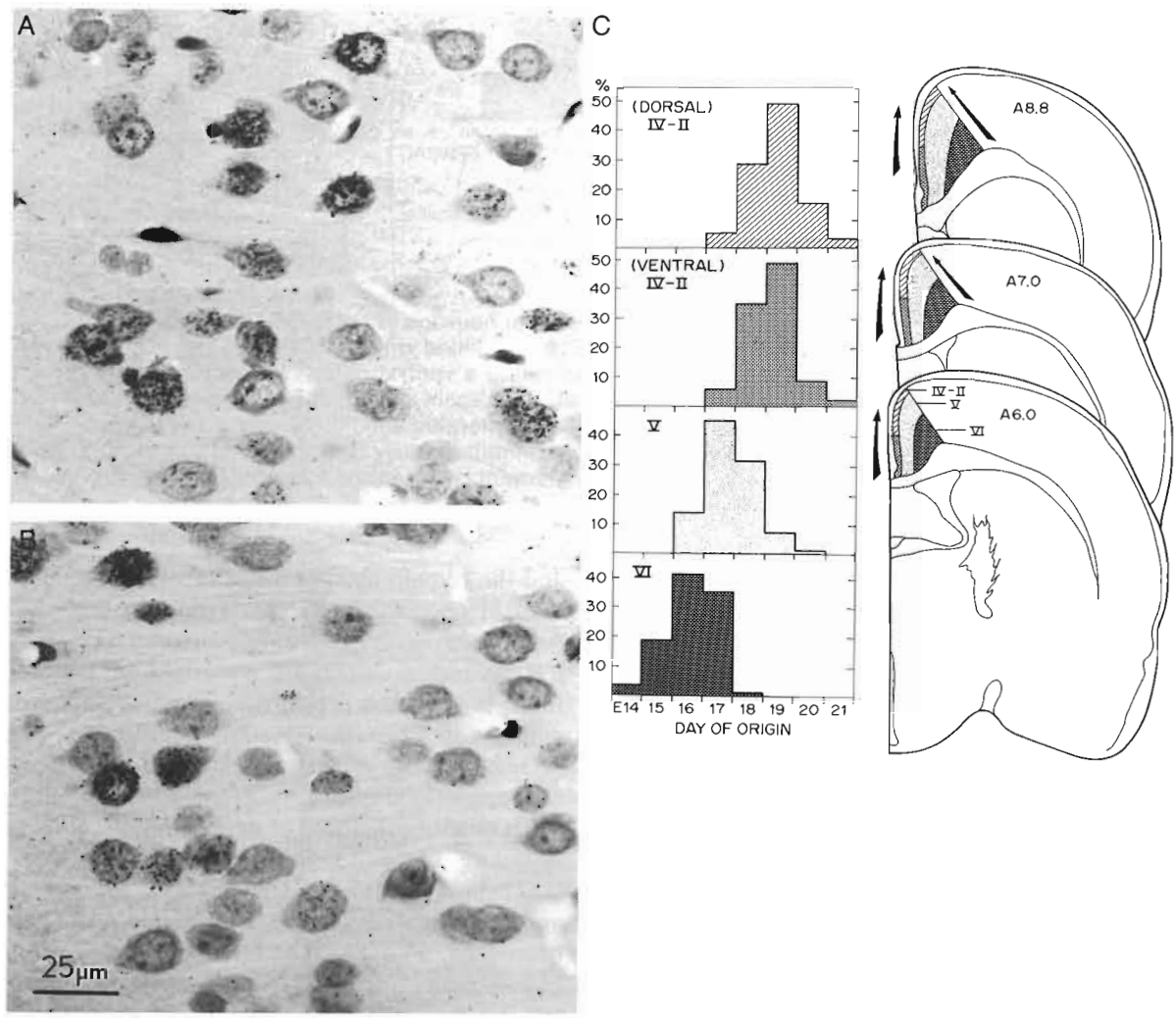

FIG. 15–8. **A** and **B** are photomicrographs of the superficial neurons in the posterior cingulate cortex in areas CG1 **(A)** and CG2 **(B)** in an animal exposed to [^{3}H]thymidine on E19–E20 and killed on P60. Area CG1 **(A)** has a higher proportion of labeled neurons than area CG2 **(B)**. (6 μm paraffin section, hematoxylin/eosin stain.) **(C)** Time of neuron origin in the posterior cingulate areas. Neurons in all layers are generated simultaneously in the anterior-posterior plane. Layers VI–V are generated simultaneously within each level, while the superficial layers have a ventral (older) to dorsal (younger) neurogenetic gradient. From Bayer (1990b).

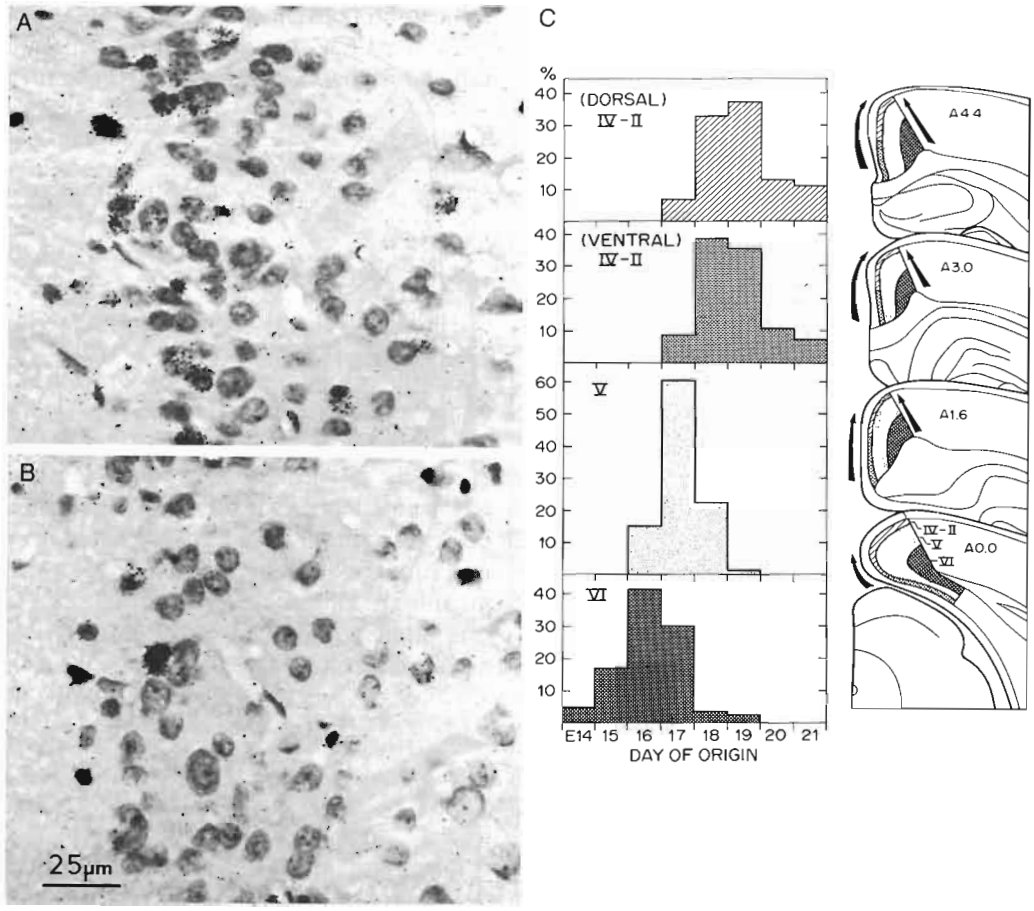

FIG. 15–9. A and **B** are photomicrographs of the superficial cells in the retrosplenial cortex in areas RSA **(A)** and RSG **(B)** in an animal exposed to [^{3}H]thymidine on E20–E21 and killed on P60. Area RSA **(A)** has a higher proportion of labeled cells than area RSG **(B)**. (6 μm paraffin section, hematoxylin/eosin stain.) **(C)** Time of neuron origin in the retrosplenial areas. Neurons in all layers are generated simultaneously in the anteroposterior plane. Layers VI–V are generated simultaneously within each level, while the superficial layers have a ventral (older) to dorsal (younger) neurogenetic gradient. From Bayer (1990b).

(younger) neurogenetic gradient between layers (all comparisons, $P < 0.0001$); neurons in VI are generated mainly on E15–E17, in V on E17–E18, in IV–II on E18–E19. As in the anterior cingulate cortex, the superficial neurons in CG2 originate slightly but significantly ($P < 0.001$) earlier (59% generated on or after E19; second graph from top, Fig. 15–8C) than those in the same layers in CG1 (67% generated on or after E19; top graph, Fig. 15–8C).

15.2.3 The Retrosplenial Area

Throughout its entire extent, the retrosplenial cortex is characterized by densely packed superficial granule cells (Fig. 15–9A,B), which are considered to make up layers IV–II in this study. Zilles (1985) divides the retrosplenial cortex into dorsal agranular (RSA) and ventral granular (RSG) areas, corresponding to Krieg's (1946a, 1946b) areas 29c and 29d, respectively. Both retrosplenial areas are granular (Krieg, 1946a) as can be seen in the photomicrographs in Figs. 15–9A and B. When [^{3}H]thymidine is injected on E20 and E21, there are fewer labeled neurons in RSA (Fig. 15–9A) than in RSG (Fig. 15–9B), indicating that some ventral neurons have earlier birthdays than dorsal neurons.

For quantification, the retrosplenial cortex was divided into dorsal (RSA) and ventral (RSG) strips. In each strip, neurons were counted in layers VI, V, and the combined layers IV–II. Neurogenesis occurs simultaneously in the anteroposterior plane (all $P > 0.05$), and the data are combined in Fig. 15–9C. There is a highly significant deep (older) to superficial (younger) neurogenetic gradient between layers (all comparisons, $P < 0.0001$); neurons in layer VI are generated mainly on E16–E17, in layer V on E17–E18, and in layers IV–II on E18–E19. In addition, the superficial neurons in RSG originate slightly but signif-

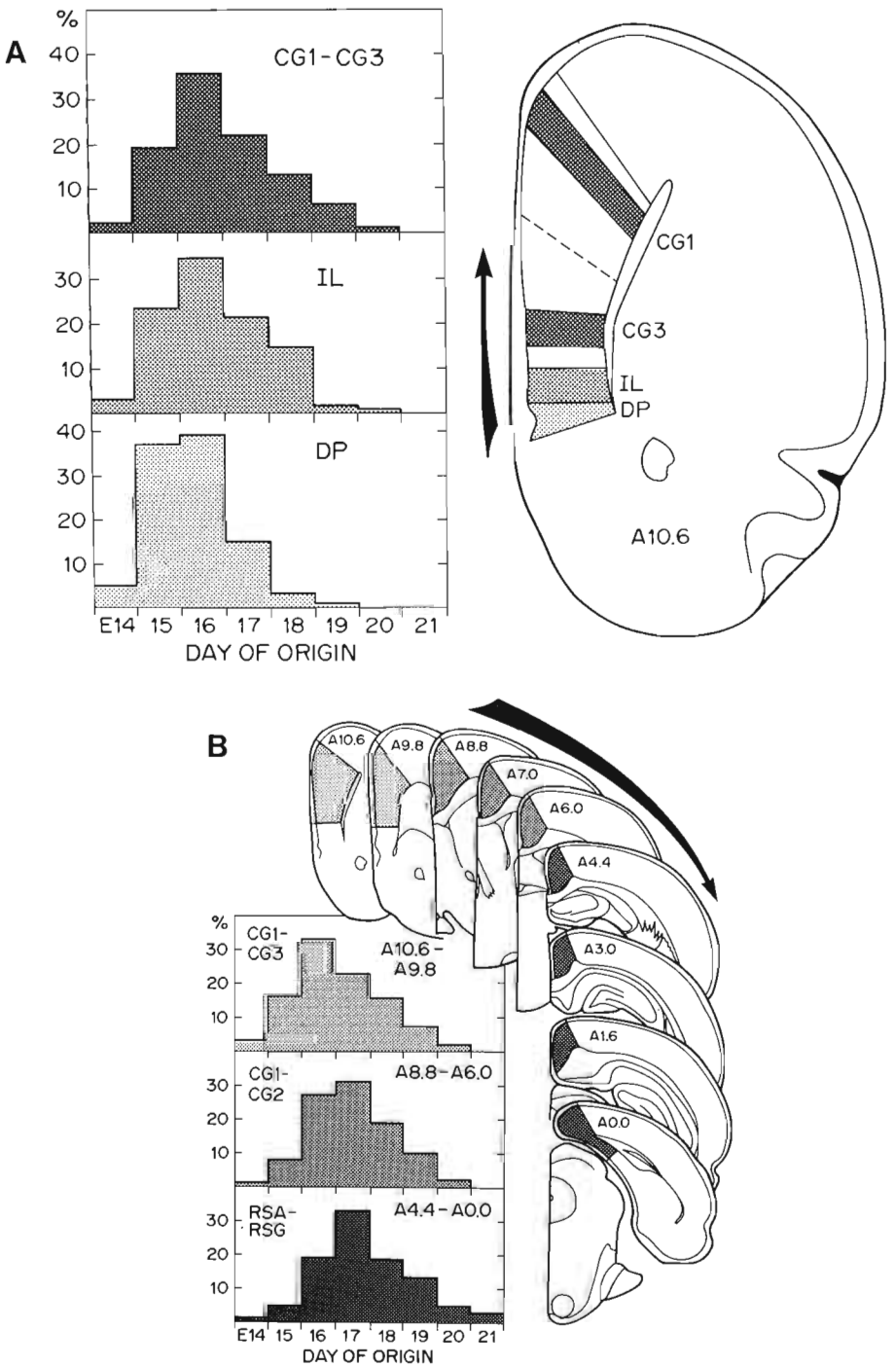

FIG. 15–10. (A) Time of origin in the anterior medial wall at level A10.6. Data from all layers have been combined in each strip to facilitate comparisons between areas. Neurogenesis is more prominent earlier in the dorsal peduncular cortex (DP) and is progressively later in the infralimbic (IL) and cingulate (CG3-ventral, CG1-dorsal) areas. **(B)** Time of origin of neurons in layers VI–II in the medial limbic cortex in the anteroposterior plane. Data for CG1 and CG3 are combined for the anterior cingulate cortex (top graph), CG1 and CG2 for the posterior cingulate cortex (center graph) and RSA and RSG for the retrosplenial cortex (bottom graph). There is an overall anterior (older) to posterior (younger) neurogenetic gradient: more neurons are generated in the anterior cingulate cortex on or before E16 than in the retrosplenial cortex. From Bayer (1990b).

icantly earlier ($P < 0.0001$; 53% generated on or after E19; second graph from top, Fig. 15–9C) than those in the same layers in RSA (61% generated on or after E19; top graph, Fig. 15–9C).

15.2.4 Gradients between Areas

Ventral to Dorsal Gradient

Anterior to the corpus callosum, the medial cortical wall lengthens. At level A10.6, four different areas are stacked from ventral to dorsal: the dorsal peduncular cortex, infralimbic cortex, CG3, and CG1 (differently shaded areas, Fig. 15–10A). To compare neurogenesis between these areas, counts of neurons in all layers were combined for each of the four cortical areas. The patterns of neurogenesis were nearly identical in CG3 and CG1, and the data were combined (top graph, Fig. 15–10A). The sign test indicated a highly significant ventral (older) to dorsal (younger) neurogenetic gradient between DP and IL ($P < 0.0001$) and between IL and CG1/CG3 ($P < 0.0001$).

Anterior to Posterior Gradient

In order to compare neurogenetic gradients throughout the entire rostrocaudal extent of the medial cortical wall, all data were pooled in the anterior cingulate (CG1/CG3, top graph, Fig. 15–10B), the posterior cingulate (CG1–CG2, middle graph, Fig. 15–10B), and the retrosplenial areas (RSA–RSG, bottom graph, Fig. 15–10B). The sign test indicated a strong anterior (older) to posterior (younger) neurogenetic gradient between the anterior and posterior cingulate areas ($P < 0.0001$) and between the posterior cingulate and retrosplenial areas ($P < 0.0001$). The data in Figs. 15–7C, 15–8C, and 15–9C indicate an anteroposterior neurogenetic gradient in the superficial layers (IV–II). These neurons are simultaneously generated in all areas up to and including E19. However, the retrosplenial areas have more labeled neurons than either the posterior cingulate areas or the anterior cingulate areas in every animal in the E20 + E21 and E21 + E22 injection groups. The overall anterior-to-posterior gradient would not be predicted by the deep layers (VI–V), since the data in Figs. 15–7C, 15–8C, and 15–9C show similar times of origin. What is different is the number of deep neurons when compared to the number of superficial neurons. The deep cell layers are more thick and make up the largest proportion of the anterior cingulate areas, a slightly lower proportion of the posterior cingulate areas, and the lowest proportion of the retrosplenial areas. Conversely, the densely packed granule cells in the superficial layers (II–IV) of the retrosplenial areas make up a substantial proportion of the total number of neurons there. When the cell counts are combined for all layers, the overall effect is that more neurons originate early in the anterior cingulate areas than in the posterior retrosplenial areas.

15.3 NEUROGENETIC GRADIENTS IN THE ORBITAL CORTEX

Neurogenesis starts especially early in the most ventral part of the medial limbic cortex and in the most ventral part of the lateral limbic cortex. To see if there is an anterior continuation between the oldest medial and lateral parts of the limbic cortex, a series of sagittally sectioned brains were used to quantify neurogenesis in the cortex tucked under the frontal pole and lying dorsal to the olfactory peduncle. Krettek and Price (1977) called this area the orbital cortex, a name also used by Zilles (1985). In sagittal sections 1.5 mm lateral to the midline (Pellegrino et al., 1979), the orbital cortex sharply curves into the olfactory peduncle without a break. Just as for the medial and lateral limbic cortical areas, there is a tendency for cell layers to become thinner and less definite with increasing distance from the somatic neocortex. Counts were made in layers VI, V, and IV–II in two strips of orbital cortex, one just dorsal to the sharp curve into the olfactory peduncle and the other just beneath the frontal pole. The data combined for all lamine in each strip are shown in Table 15–1 and indicate that neurons in the cortex located nearer to the olfactory peduncle are generated earlier (34% have accumulated on or before the morn-

TABLE 15–1. *Neurogenesis in the ventral and dorsal orbital cortex*[a]

	Ventral orbital	Dorsal orbital
Day of origin	% Neurons originating	% Neurons originating
E13	1.38	0.31
E14	4.31	4.86
E15	28.54	19.43
E16	34.67 (34.23%)	30.47 (24.59%)
E17	17.40	28.41
E18	9.65	8.80
E19	3.39	7.31
E20	0.65	0.42

[a] Neurons generated during each embryonic day in strips (combined layers II–VI) of the orbital cortex beneath the frontal pole. Cell counts were done in sagittally sectioned brains of animals exposed to [^{3}H]thymidine on 2 consecutive days of embryonic development and killed on P60. These data were calculated as described in Appendix 2. The numbers in parentheses are the proportions of neurons that had already originated by the morning of E16. The ventral orbital cortex is nearer to the olfactory peduncle and accumulates neurons earlier than either the dorsal orbital cortex ($P < 0.002$) or the frontal pole ($P < 0.038$). From Bayer (1990a).

ing of E16) than in the cortex nearer to the frontal pole (25% have accumulated on or before the morning of E16). Neurons in the frontal pole neocortex are generated later (Chapter 3). Thus, the orbital cortex appears to be the anterior contact area between the medial and lateral limbic rim around the neocortex.

15.4 CORRELATIONS BETWEEN NEUROGENETIC GRADIENTS AND ANATOMICAL CONNECTIONS IN THE LIMBIC CORTEX

15.4.1 Connections of the Anterior Thalamic Nuclear Complex with the Medial Limbic Cortex

A massive fiber projection to the medial limbic cortex comes from the anterior thalamic nuclei (Rose and Woolsey, 1948; Cowan and Powell, 1954; Domesick, 1970, 1972; Divac et al., 1978; Robertson and Kaitz, 1981; Vogt et al., 1981; Finch et al., 1984a; Thompson and Robertson, 1987; Horikawa et al., 1988). The thalamic axons course anteriorly through the medial striatum in the internal capsule then curve over the genu of the corpus callosum to course posteriorly in the cingulum bundle (Rose and Woolsey, 1948; Cowan and Powell, 1954; Domesick, 1970, 1972). There is a topographic relationship between source neurons and target areas such that the anterodorsal nucleus projects primarily to the most posterior retrosplenial and presubicular areas, the anteroventral nucleus primarily to retrosplenial areas, and the anteromedial nucleus primarily to supracallosal cingulate areas (Rose and Woolsey, 1948; Cowan and Powell, 1954; Domesick, 1972; Robertson and Kaitz, 1981; Thompson and Robertson, 1987). Early anatomical studies (Rose and Woolsey, 1948; Cowan and Powell, 1954) indicated that the anteromedial nucleus also projects to the pregenual limbic cortex; later studies found that this part of the medial limbic cortex gets input primarily from the thalamic mediodorsal nucleus (Leonard, 1969, 1972; Domesick, 1972; Robertson and Kaitz, 1981; Vogt et al., 1981; Sarter and Markowitsch, 1983; Finch et al., 1984a; Groenewegen, 1988).

Three lines of evidence indicate that neurogenetic gradients in the anterior thalamic nuclei and medial limbic cortex correlate with the pattern of anatomical interconnections. These relationships are diagrammed in Fig. 15–11. First, the work of Thompson and Robertson (1987) and Horikawa et al. (1988), shows that thalamic anteroventral and anteromedial axons are segregated in the medial limbic cortex anteroposteriorly and ventrodorsally forming a quadrant on the medial cortical wall (see trajectories of axonal projections, Fig. 15–11). Second, neurogenetic gradients in the thalamic anteroventral and anteromedial nuclei also form a quadrant (*arrows* in thalamus, Fig. 15–11) where older neurons are situated dorsolaterally, progressively younger neurons ventromedially (Altman and Bayer, 1988b). Third, both the anteroposterior and ventrodorsal neurogenetic gradients found in the supracallosal cingulate and retrosplenial cortical areas (*arrows* in cortex, Fig. 15–11) provide the third quadrant based on the data in this chapter. Following the diagram in Fig. 15–11, we give two examples that show how the three quadrants are linked. First, older neurons in dorsal AV terminate in RSA of the retrosplenial cortex (Fig. 6, Thompson and Robertson, 1987); RSA contains younger target neurons in the superficial layers (approximately 23% originate on and after E20, Fig. 15–9C) where most of the thalamic axons terminate (Domesick, 1972; Robertson and Kaitz, 1981; Vogt et al., 1981). Second, younger neurons in the medial part of AM terminate in CG2 of the supracallosal cingulate cortex (Fig. 4, Thompson and Robertson, 1987); CG2 contains older target neurons in the superficial layers (only 10% are generated on and after E20, Fig. 15–8C).

As we have seen throughout the neocortex, anatomical connections with the thalamus show a reverse age matching with neurogenetic gradients: axons from older source neurons terminate in areas containing younger target neurons. The distance between source neurons and target areas is an important consideration in medial limbic cortical connections. Older neurons in AV have longer axons to farther targets, while younger neurons in AM have shorter axons to nearer targets (compare axon lengths in Fig. 15–11). Appropriate anatomical tracing studies in the immature brain are needed to test the hypothesis that the time of neurogenesis is related to the length that the axon must grow to reach its target. Possibly the longest axons from the oldest dorsolateral AV neurons (*heavy solid lines*, Fig. 15–11) will arrive late at the most distant target, the agranular retrosplenial area, where neurogenesis is late. It follows that the shortest axons from the youngest AM neurons (*thin dashed lines*, Fig. 15–11) will arrive earlier at the nearest target, CG2, where neurogenesis is early. A similar situation is found in the hippocampus, where areas getting heavy thalamic input have unusually delayed neurogenesis (Bayer, 1980a) that may be related to the late arrival of thalamic axons.

15.4.2 Thalamocortical Connections of the Gustatory Cortex

Travers et al. (1987) reviewed the literature on the entire taste pathway. Primary taste afferents terminate topographically in the nucleus of the solitary tract (an-

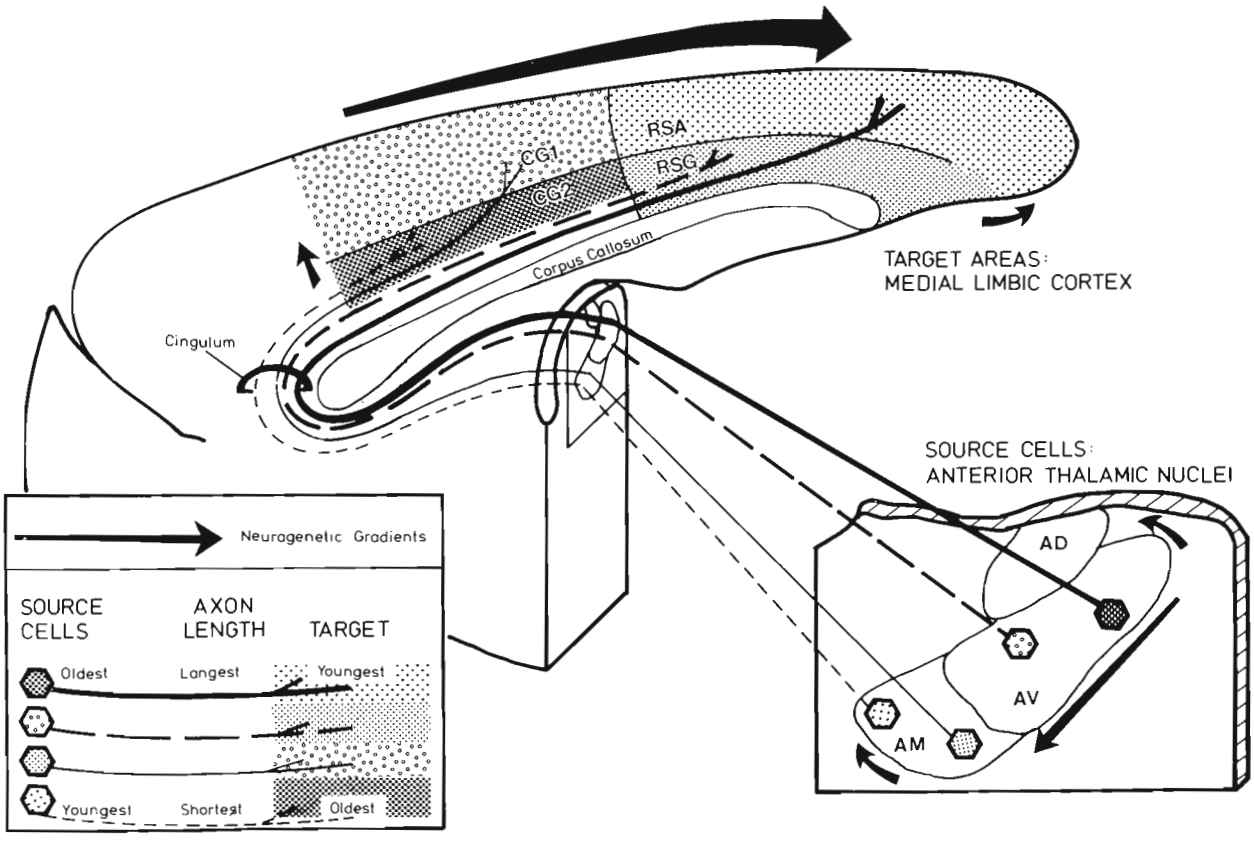

FIG. 15–11. A diagrammatic representation of the hypothesis that the dorsolateral to ventromedial neurogenetic gradients in the anteroventral and anteromedial thalamic nuclei (AV and AM) are related to the lengths that axons grow to reach their respective cortical targets. The anteroventral to posterodorsal neurogenetic gradients in posterior cingulate (CG1 and CG2) and retrosplenial (RSA and RSG) areas may be linked to times at which thalamic axons arrive in specific target zones. Older source neurons in AV have longer axons (late arriving?) that terminate in RSA where younger neurons predominate. Younger source neurons in AM have shorter axons (earlier arriving?) that terminate in CG2 where older neurons predominate. From Bayer (1990b).

terior oral cavity to anterior part, posterior oral cavity to posterior part), which in turn projects topographically to the parabrachial nuclei (input from the anterior tongue maps ventrally, the remainder of the oral cavity maps dorsally). The parabrachial nucleus relays taste input to the ventroposteromedial thalamic nucleus (VPm), which is in the most medial part of the ventrobasal complex. Using the Nauta technique, Wolf (1968) found that lesions of the VPm produce debris of degenerating fibers in the insular cortical areas and in the inferior parietal cortex just dorsal to it (the gustatory cortex). That projection has been confirmed with anterograde and retrograde tracing techniques (Gerfen and Clavier, 1979; Reep and Winans, 1982a; Saper, 1982; Guldin and Markowitsch, 1983; Kosar et al., 1986). Just as in other primary sensory areas of cortex, so also in the dorsal insular and gustatory areas there are transient cholinergic receptors during the first few weeks of postnatal life (Fuchs, 1989).

Three lines of evidence show that interconnections between VPm and its cortical projection area (AI and GU) follow the same reverse age matching that we have seen in all of the specific thalamic projections. (1) By combining physiological mapping with anterograde transport of [^{3}H] amino acids, Kosar et al. (1986) found that the most medial VPm neurons project ventrally to the dorsal agranular insular area, while progressively more lateral VPm neurons project dorsally to the gustatory area. (2) Throughout the thalamic ventrobasal complex, older neurons are lateral, younger neurons are medial, with the youngest neurons settling in medial VPm (Fig. 4, Altman and Bayer, 1989a). (3) There is a transverse neurogenetic gradient between older dorsal agranular insular and younger gustatory

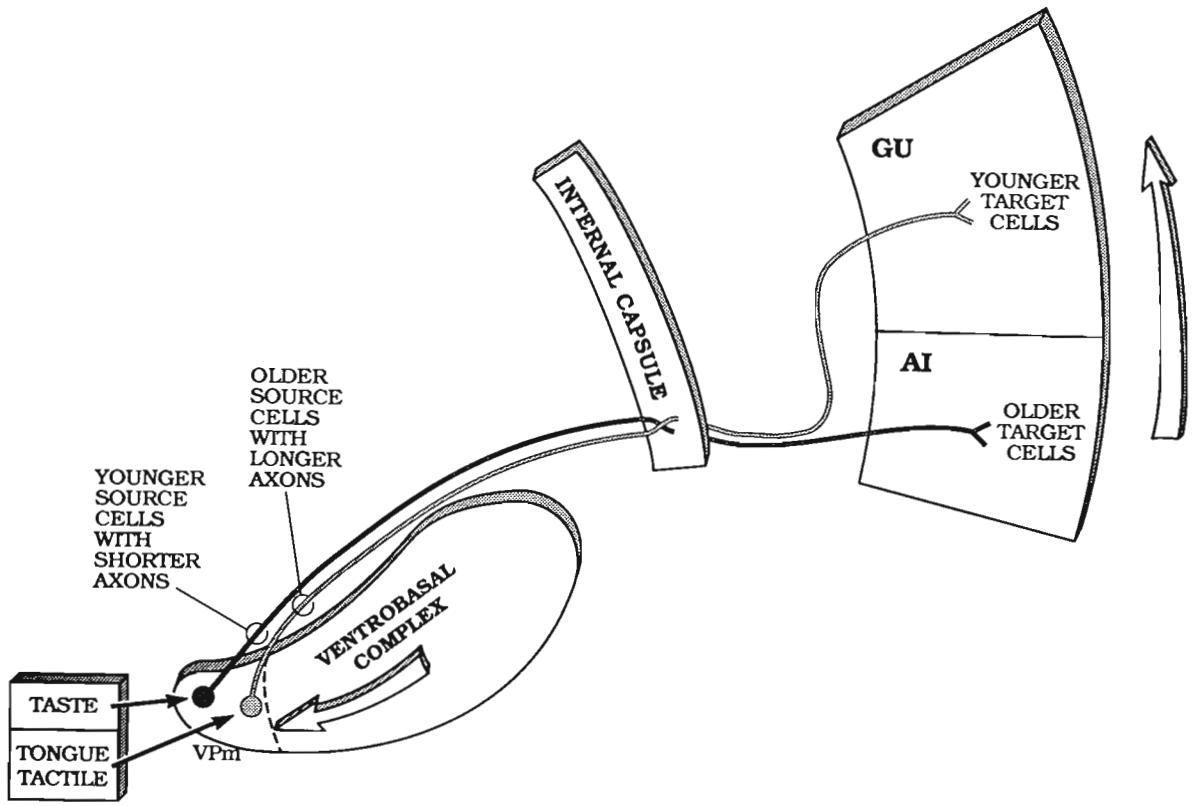

FIG. 15–12. A diagrammatic representation of the neurogenetic gradients and anatomical connections between the ventroposteromedial nucleus (VPm) in the thalamic ventrobasal complex and the gustatory (GU) and agranular insular (AI) areas. Taste input is relayed by medial VPm neurons, while tactile input from the tongue is relayed by lateral VPm neurons (Kosar et al., 1986). The large arrows point to areas containing younger neurons in each structure. There is an exact reversal of ages in thalamic source neurons and cortical target neurons that may be related to axonal length. Older neurons in lateral VPm have longer axons (*lightly stippled*) terminating on younger neurons in GU. The converse is true for the younger neurons (axons *darkly stippled*) in medial VPm that project to older neurons in AI.

areas (right column of graphs, Fig. 15–2). Figure 15–12 shows how the data from each of the three sources are matched. Younger neurons in medial parts of VPm have *shorter* axons (*dark stipple*, Fig. 15–12) that relay taste information to older neurons in the dorsal agranular insular area. Older neurons in lateral parts of VPm have *longer* axons (*light stipple*, Fig. 15–12) that relay tactile information from the tongue to younger neurons in the gustatory cortex. The reverse matching of neurogenetic gradients and anatomical connections will be related to the delayed arrival of laterally migrating neocortical neurons (Chapter 9) in the superficial layers of lateral and ventrolateral parts of the cortex (Chapter 16).