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

CHAPTER 12

Development of the Auditory Areas

In the lissencephalic cortex of the rat, the auditory areas are located in the posterolateral cortical wall beneath the squamous portion of the temporal bone. The primary auditory area, 41 in Krieg's (1946a) classification, is the most dorsal and anterior part; Zilles et al. (1980) calls it TE1. Two secondary auditory areas (TE3, TE2) form an incomplete belt around the ventral and posterior borders of the primary area. Zilles' TE3 (anterior) and TE2 (posterior) correspond to Krieg's (1946a) areas 36 and 20. Area 20 was considered to be primary cortex by Krieg (1946a), while Zilles et al. (1980) considers that area to be secondary cortex based on electrophysiological data in the literature.

To our knowledge, there has never been a detailed [^{3}H]thymidine autoradiographic study of neurogenesis in the auditory cortex of any mammal; consequently, we provide a comprehensive analysis of areas TE1, TE2, and TE3. Five sections were chosen for quantitative analysis, ranging from Pellegrino et al's. (1979) level A3.4 anteriorly (Fig. 12–1A) to level P0.4 posteriorly (drawings, Figs. 12–5 and 12–6). Area TE1 extends longitudinally from levels A3.2 to A1.4 and is situated just dorsal (left side of the photographs in Fig. 12–1) to the secondary areas TE2 and TE3. Area TE3, extending from levels A3.2 to A2.4, lies just anterior to TE2, which extends from levels A0.4 to P0.4. Developmental patterns indicated that the secondary area at level A1.4 (Fig. 12–1B) is transitional between TE3 and TE2, and those data are not presented.

Neurons were separately counted in layers VI, V, IV, III, and II in anterior and posterior parts of TE1, TE2, and TE3. The dashed lines in Fig. 12–1 show the boundaries between the layers based on cytoarchitectonic characteristics (Figs. 12–2 and 12–3). Layers VI and V are thick, each taking up approximately one-

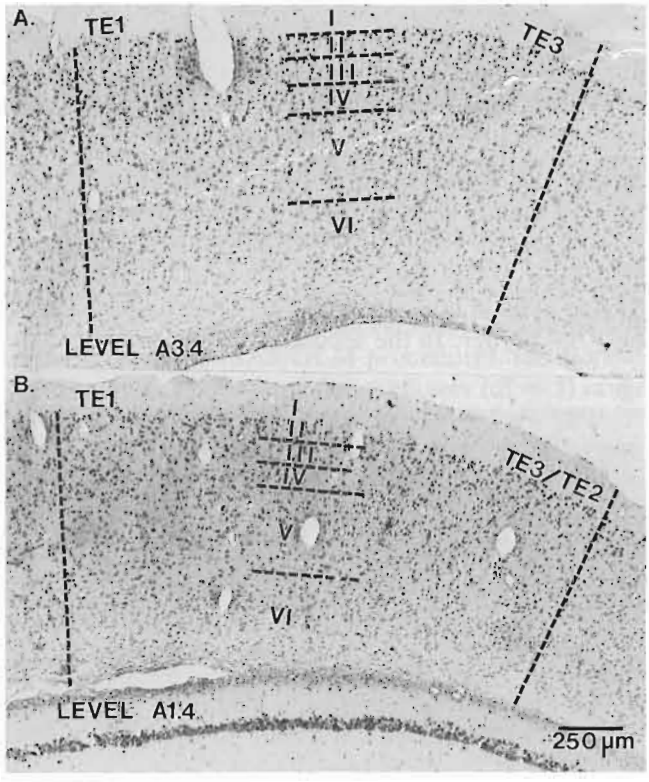

FIG. 12–1. A and **B** are low magnification views of the auditory cortex in the brain of a rat exposed to [^{3}H] thymidine on E17 and E18 and killed on P60 (6 µm paraffin section, hematoxylin/eosin stain). Three auditory areas are located in the ventrolateral cerebral wall at levels A3.4 **(A)** and A1.4 **(B)** (Pellegrino et al., 1979); lateral is at the top, dorsal is to the left. The primary sensory area (TE1) is dorsal to the secondary areas (TE3, anterior; TE2, posterior, Zilles, 1985); at level A1.4 **(B)** the secondary cortex is transitional between TE3 and TE2. Roman numerals separated by dashed lines indicate how the cortex was subdivided into layers for the cell counts.

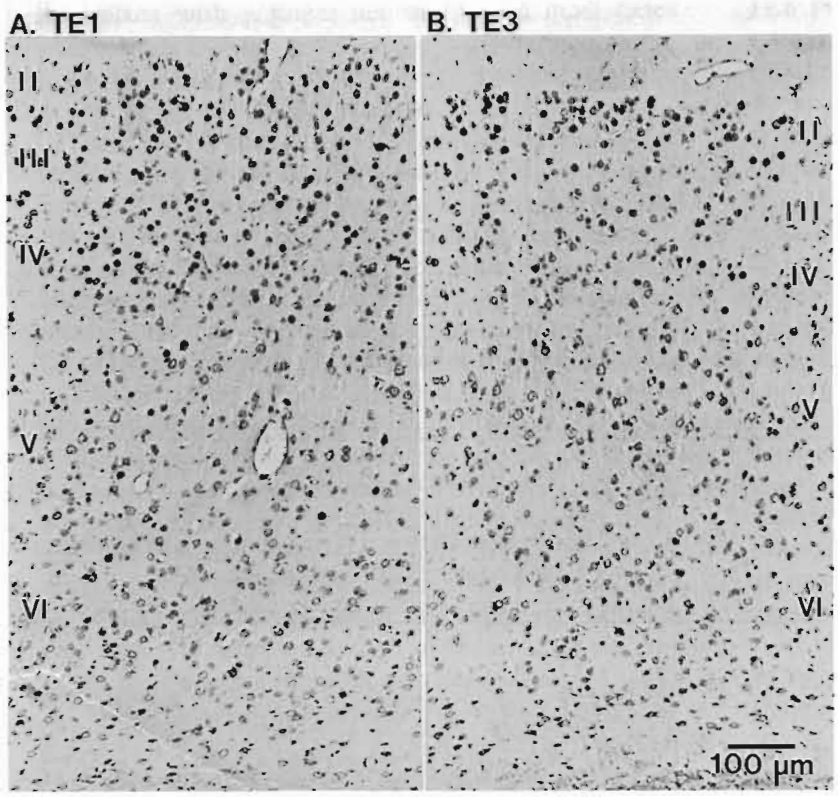

FIG. 12–2. Strips of the primary (**A,** TE1) and secondary (**B,** TE3) auditory cortex at level A3.4 (Pellegrino et al., 1979) from the same brain as shown in Fig. 12–1A. The placement of the vertical dashed lines in Fig. 12–1A shows where the higher magnification photographs were taken. Roman numerals at the sides of each photograph indicate the cortical layers (II–VI). There are generally more labeled neurons in the primary area (**A,** TE1) than in the secondary area (**B,** TE3). In both areas, deep neurons (V–VI) are mostly unlabeled, while superficial cells throughout II–IV are labeled in the primary area **(A),** throughout II–III in the secondary area **(B).** The labeling patterns indicate that both areas have a radial neurogenetic gradient with older, unlabeled deep neurons (origin before E17) and younger, labeled superficial neurons (origin on or after E17), and an older ventral to younger dorsal neurogenetic gradient between TE3 and TE1. Note the increased numbers of small neurons in layer IV of the primary area **(A)** compared to the secondary area **(B).**

third of the total cortical depth. Layer VI pyramidal cells are slightly smaller and more densely packed than those in layer V (see Figs. 12–2 and 12–3). Throughout TE1 (Figs. 12–2A and 12–3A), there is a scattering of granule cells in layer IV that facilitated delineation of the V/IV border. In the secondary areas, granule cells in layer IV are very sparse, but the pyramidal cells there are smaller and more densely packed than those in layer V (Figs. 12–2B and 12–3B). Since the borders between layers IV, III, and II were indistinct, the band of cortex between the external limit of layer V and the internal limit of layer I was divided into thirds for quan-

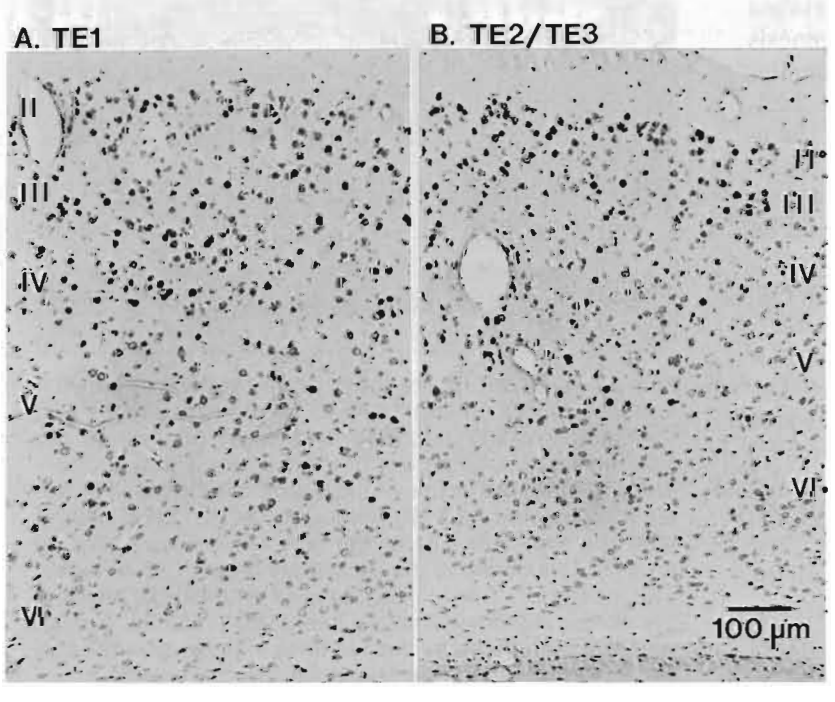

FIG. 12–3. Strips of the primary (**A,** TE1) and secondary (**B,** TE2/TE3) auditory cortex at level A1.4 from the same brain section as shown in Fig. 12–1B (*vertical dashed lines* indicate areas where photographs were taken). In both areas, the majority of superficial neurons throughout IV–II are labeled, while more neurons are labeled in layers V and VI in the primary area **(A)** than in the secondary area **(B),** indicating both radial and transverse neurogenetic gradients. There is a lower proportion of labeled neurons in the anterior strips shown in Fig. 12–2 (fewer cells in layers V and VI are labeled) than in the posterior strips shown here, indicating an anterior (older) to posterior (younger) neurogenetic gradient.

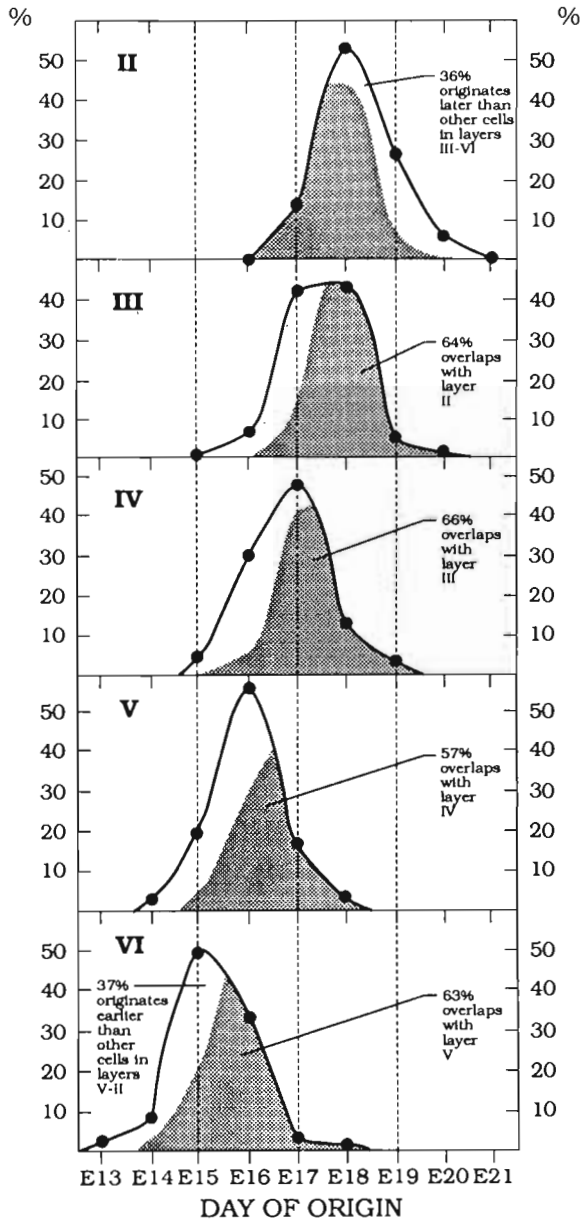

FIG. 12–4. The radial neurogenetic gradient in the auditory cortical areas of rats that survived to P60 after two consecutive exposures to [^{3}H]thymidine during embryonic life. Each graph represents the proportion of neurons generated from E13 to E21 in separate layers (II—top graph to VI—bottom graph) based on data combined from all areas of the auditory cortex that were analyzed. Vertical hatched lines indicate relative amounts of neurons that have been generated before and after E15 (left line), E17 (center line), and E19 (right line). As one proceeds from layer VI to layer II the peaks of neurogenesis shift from early (E15 in VI) to late (E18 in II). Stippled areas represent that portion that is generated concurrently with the adjacent layer. Note that the least amount of concurrent generation occurs between layers V and IV (57% indicated in the second graph from the bottom).

tification: the lower third is called layer IV; the middle third, layer III; and the upper third, layer II.

12.1 THE RADIAL NEUROGENETIC GRADIENT

The prominent radial neurogenetic gradient between deep and superficial layers is shown after [^{3}H] thymidine injections on E17 and E18 (Figs. 12–2 and 12–3). Practically all of the neurons in layer VI and many of the neurons in layer V (especially anteriorly, Fig. 12–2) are unlabeled, while the majority of neurons in layers IV–II are labeled. To analyze the radial neurogenetic gradient, the cell counts in all auditory areas were combined separately for each layer, and timetables of the birthdates of neurons were constructed (Fig. 12–4). Neurons in layer VI have the earliest peak (E15), and 37% of them are generated earlier than those throughout layers V–II. Neurons in layer II have the latest peak (E18), and 36% of them are generated later than those throughout layers VI–III. The intervening layers have progressive shifts in peak times of neurogenesis between the two extremes. The full magnitude of the radial gradient can be appreciated by comparing the proportion of neurons generated before and after selected embryonic ages (E15, E17, and E19, *vertical dashed lines,* Fig. 12–4). For example, on E17, neurogenesis in layer VI is nearly completed (>90%), while neurogenesis in layer II has just begun (<20%). Even between adjacent layers, the neurogenetic gradients are substantial. The sign test indicated that all comparisons between adjacent layers were significant ($P < 0.0001$). In the repeated measures analysis of variance (SAS GLM procedure), the F values for the means between adjacent layers (df = 1) ranged from 225 to 142, all $P < 0.0001$. For layers VI/V, IV/III, and III/II between 63% and 66% of the neurons are cogenerated, while the lowest proportion of cogeneration (57%) is between layers IV and V (*stippled areas,* Fig. 12–4); that pattern is similar to those in the visual (Chapter 11), somatosensory (Chapter 13), and motor areas (Chapter 14).

12.2 THE TRANSVERSE NEUROGENETIC GRADIENT

In all cases where neurogenesis was compared between the dorsal primary area TE1 and the ventral secondary area TE3, there was a ventral (older) to dorsal (younger) neurogenetic gradient within each layer. That gradient is shown between the auditory areas in a rat that was exposed to [^{3}H]thymidine on E17 and E18 and killed on P60 (Figs. 12–2 and 12–3). Nearly all of layer VI and V neurons and many of layer IV neurons are unlabeled ventrally (Figs. 12–2B and 12–3B) while a few layer V neurons and most of layer IV neurons are labeled dorsally (Figs. 12–2A and 12–2B).

The data in Fig. 12–5 are for level A3.2; the data for other levels (A2.4 and A1.4) are similar and are not shown. The line graphs (Fig. 12–5) indicate that neurogenesis in each layer of the secondary area (TE3, *filled circles*) is more prominent before the peak day, while neurogenesis in the primary area (TE1, *empty circles*) is more prominent after the peak day. The gradient is best shown in the supragranular layers. In layer II (top graph, Fig. 12–5), 25% more neurons are generated on or before E18 in TE3 than in TE1 ($P < 0.0001$ sign test; F = 106.46, df = 1, $P < 0.0001$, SAS GLM procedure). In layer III (second graph from top, Fig. 12–5), 23% more neurons are produced on or before E17 in TE3 than in TE1 ($P < 0.0001$, sign test; F = 43.36, df = 1, $P < 0.0001$, SAS GLM procedure). Layer V also shows a strong gradient (second graph from bottom, Fig. 12–5), where 19% more neurons originate on or before E15 in TE3 than in TE1 ($P < 0.0001$, sign test; F = 14.22, df = 1, $P = 0.0004$, SAS GLM procedure). The differences in layers VI (bottom graph, Fig. 12–5) and IV (center graph, Fig. 12–5) are slight but most of the animals showed the same trend. For layer VI, 11% more neurons are generated on or before E15 in TE3 than in TE1 ($P < 0.002$, sign test; F = 24.04, df = 1, $P = 0.0001$, SAS GLM procedure). For layer IV, 9% more cells are generated on or before E16 in TE3 than in TE1 ($P < 0.002$, sign test; F = 7.84, df = 1, $P = 0.0069$). When the data for all layers are combined (bar graphs, Fig. 12–5), the overall trend ($P < 0.0001$, sign test) is for 11% more neurons to be generated on or after E16 in TE1 (75%) than in TE3 (64%).

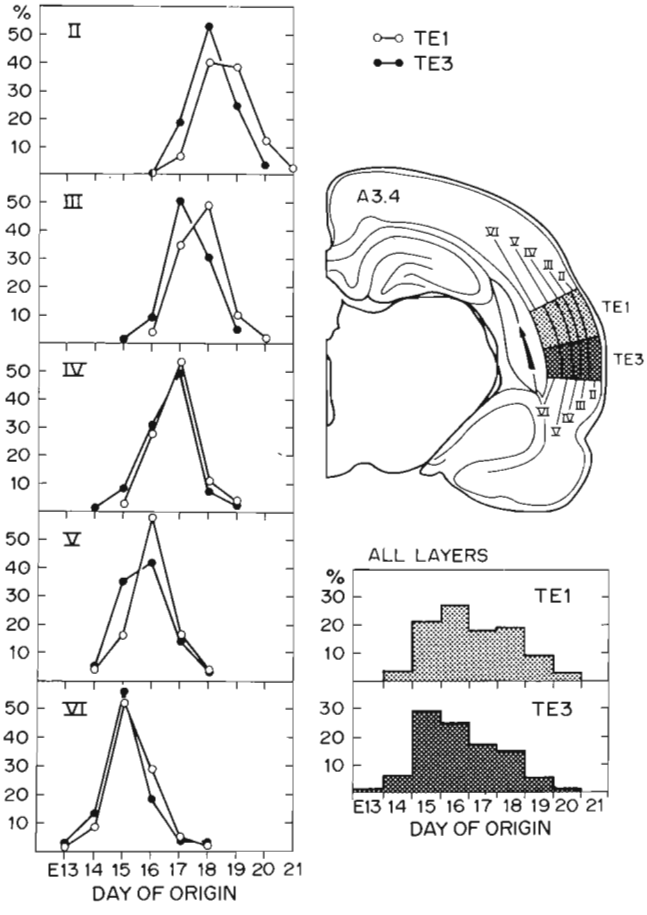

FIG. 12–5. Neurogenesis of the auditory cortex at level A3.4 in rats that survived to P60 after two consecutive exposures to [^{3}H]thymidine during embryonic life. The line graphs show proportions of neurons originating during single embryonic days in each layer of the primary (TE1, *empty circles*) and secondary (TE3, *filled circles*) areas. There is a pronounced deep (older) to superficial (younger) neurogenetic gradient between layers in both areas. In each lamina, neurogenesis in the primary area is more prominent after the peak day, while that in the secondary area is more prominent before the peak day. The bar graphs are the proportions of neurons generated on single days when the data from all laminae are combined in the primary (*top*) and secondary (*bottom*) areas.

12.3 LONGITUDINAL NEUROGENETIC GRADIENTS

12.3.1 The Primary Area

Neurogenetic gradients in the primary auditory area (TE1) fit into the global radial and longitudinal gra-

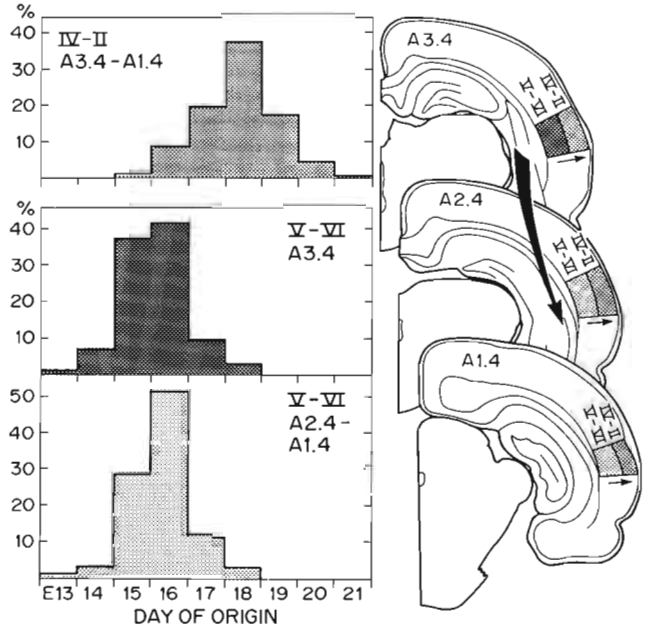

FIG. 12–6. Time of neuron origin in anterior and posterior parts of the primary auditory cortex in rats that survived to P60 after two consecutive exposures to [^{3}H] thymidine during embryonic life. The bar graphs are proportions of neurons originating during single embryonic days. The combined layers VI–V (two bottom graphs) have a significant anterior (older) to posterior (younger) neurogenetic gradient (*arrow* in drawings), while the superficial layers (IV–II, top graph) originate simultaneously in that plane.

dients in the rest of the neocortex. Since layers VI–V on the one hand, and IV–II on the other, showed similarities in longitudinal gradients, the data were combined. The sign test indicated that neurons in layers IV–II are generated simultaneously ($P > 0.05$) in the anteroposterior plane, and these data are combined in the top graph (Fig. 12–6). Neurons in the deep layers at level A3.4 are generated significantly earlier than those in the deep layers at levels A2.4 and A1.4 ($P < 0.0001$, sign test; $F = 43.63$, df = 1, $P < 0.0001$, SAS GLM procedure; arrow in drawings, Fig. 12–6). Neurogenesis in the anterior deep layers leads neurogenesis in the posterior deep layers by approximately 13%: 46% of the anterior neurons have been generated on or before E15 while only 33% of the posterior neurons are generated during the same time. The gradient in layer V is qualitatively indicated by the lower proportion of labeled neurons in TE1 anteriorly (Fig. 12–2A) than posteriorly (Fig. 12–3A).

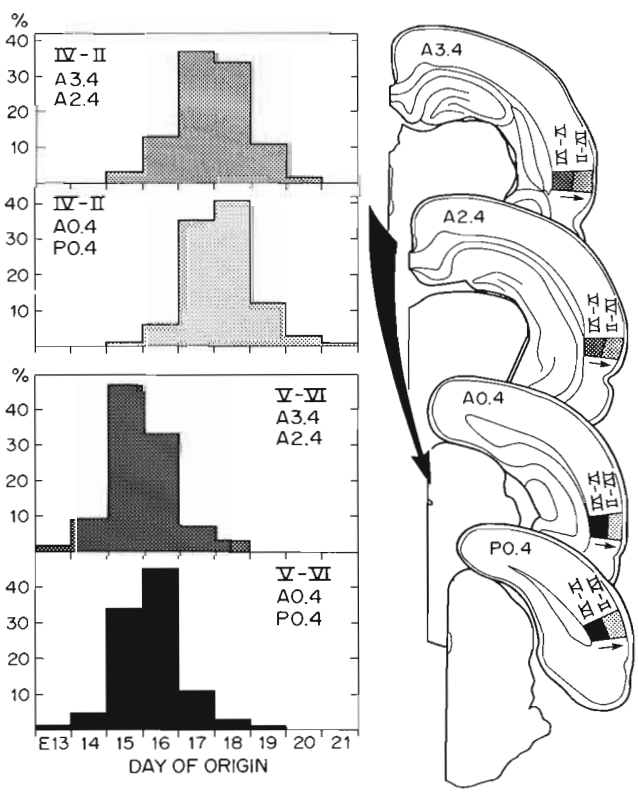

FIG. 12–7. Time of neuron origin in the secondary auditory cortex (TE3–A3.4 to A2.4 and TE2–A0.4 to P0.4) in rats that survived to P60 after two consecutive exposures to [^{3}H]thymidine during embryonic life. The bar graphs are proportions of neurons originating during single embryonic days. Both deep (VI–V) and superficial (IV–II) cells have an anterior (older) to posterior (younger) neurogenetic gradient (*arrow* in drawings).

12.3.2 The Secondary Areas

Just as in the primary auditory area, neurogenetic gradients in the secondary auditory areas (TE2 and TE3) fit into the global longitudinal gradient in the rest of the neocortex (Fig. 12–7). For simplicity, the data of the deep layers (VI–V) and superficial layers (IV–II) were combined. The longitudinal gradient is more pronounced in the secondary areas than in the primary area since both deep and superficial layers show that pattern (*arrow* between section drawings, Fig. 12–7). Neurons in either the deep or superficial layers at levels A3.4 to A2.4 (area TE3) are generated significantly earlier than those in levels A0.4 to P0.4 (area TE2). Neurogenesis in the anterior deep layers (lower set of graphs, Fig. 12–7) is 17% ahead of neurogenesis in the posterior deep layers: in TE3, 57% are generated on or before E15, in TE2 only 40% ($P < 0.0001$, sign test; $F = 84.21$, df = 1, $P < 0.0001$, SAS GLM procedure). In layers IV–II (upper set of graphs, Fig. 12–7), TE3 leads TE2 by approximately 10%: in TE3, 53% are produced on or before E17, in TE2 only 43% ($P < 0.0001$, sign test; $F = 27.82$, df = 1, $P < 0.0001$, SAS GLM procedure). In the E17–E18 injection group, only layer II has a high concentration of labeled neurons in TE3 (Fig. 12–2B) while many layer III neurons are still labeled in TE2 (Fig. 12–3B).

12.4 CORRELATIONS BETWEEN NEUROGENETIC GRADIENTS AND THALAMOCORTICAL CONNECTIONS

The auditory system in the rat has not been intensively studied. For example, the detailed tonotopic mapping of the entire auditory system carried out in primates (Fitzpatrick and Imig, 1982), cats (Merzenich et al., 1982), and gerbils (Ryan et al., 1982) has yet to be done in rats. Thalamic projections to the rat auditory cortex were demonstrated with total (Vaughan, 1983) or partial (Ryugo and Killackey, 1974) lesions of the medial geniculate body, and with retrograde transport of label to the medial geniculate body after horseradish peroxidase injections into the auditory cortex (Guldin and Markowitsch, 1983). Medial geniculate projections to layers IV and I were described in two general studies of thalamocortical connections using [^{3}H] amino acids and horseradish peroxidase (Jacobson and Trojanowski, 1975; Herkenham, 1980), while Vaughan (1983) also found a sparse input to layer VI. Faye-Lund (1985) showed that the auditory cortical areas project topographically to both the inferior colliculus and the medial geniculate body. As late as 1985 no work had yet dealt with the details of topographic input to auditory cortex from the medial geniculate body. Webster (1985) reviewed two studies that had been done

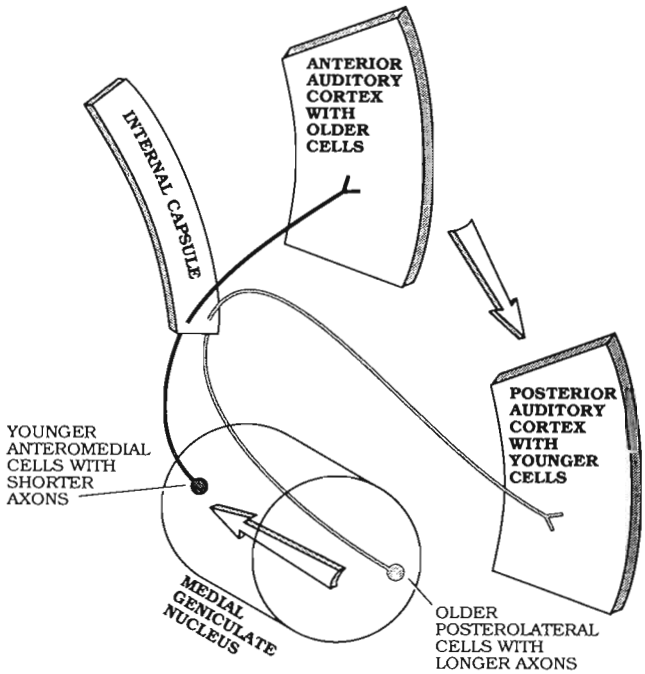

FIG. 12–8. A diagrammatic representation of the neurogenetic gradients and anatomical connections between the medial geniculate nucleus and the auditory cortex. The large arrows point to areas containing younger neurons in each structure. There is an exact reversal of ages in thalamic source neurons and cortical target neurons that may be related to axonal length. Older neurons in the posterolateral medial geniculate body have longer axons (*lightly stippled*) terminating on younger neurons in the posterior auditory cortex. The converse is true for the younger neurons (axons *darkly stippled*) in the anteromedial medial geniculate body.

earlier but were not published. Finally, two studies (Winer and Larue, 1987; Scheel, 1988) have recently appeared that dealt exclusively with medial geniculate/cortical interconnections in the rat. According to these studies, the projections are organized so that posterolateral areas of the medial geniculate project to posterior parts of auditory cortex (mainly posterior TE1 and TE2), while anterior and medial parts of the medial geniculate project to anterior parts of auditory cortex (mainly anterior TE1 and TE3). That is similar to the pattern found in the tree shrew (Oliver and Hall, 1978) but appears to be reversed in the anterior/posterior axis in the guinea pig (Redies et al., 1989).

In the rat medial geniculate nucleus, there is a combined posterolateral (older) to anteromedial (younger) gradient of neurogenesis (Fig. 12–8; Altman and Bayer, 1989b). Given the anterior (older) to posterior (younger) neurogenetic gradient in the auditory areas (Figs. 12–6 and 12–7) the two sets of gradients match up (diagrammed in Fig. 12–8) so that older posterolateral medial geniculate neurons project to younger neurons in posterior auditory areas—an exact reversal in the ages of source and target neurons. Again, there is a positive correlation between the ages of thalamic relay neurons and the lengths of their axons. Older neurons in the posterolateral medial geniculate body send longer axons (*light stipple,* Fig. 12–8) to the younger neurons in the posterior auditory cortex, while the converse is true for younger neurons in the anteromedial-medial geniculate body (*darkly stippled* axons, Fig. 12–8). We will discuss the implications of these correlations more fully in Chapter 16.