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

CHAPTER 14

Development of the Motor Areas

The motor cortex, the primary but not the sole source of pyramidal efferents, has been delineated in rodents as the projection area of the ventrolateral thalamic nucleus (Caviness and Frost, 1980; Frost and Caviness, 1980; Herkenham, 1980; Donoghue and Parham, 1983; Sharp and Gonzalez, 1986; Carvell and Simons, 1987; O'Donoghue et al., 1987; Williams and Faull, 1987; Sawyer et al., 1989). The ventrolateral nucleus represents the cerebellar relay area in the thalamus (Donoghue et al., 1979; reviewed in Faull and Mehler, 1985; O'Donoghue et al., 1987; Asanuma et al., 1988). In the lissencephalic cerebral cortex of the rat, the motor cortex is situated in the frontal pole and continues posteriorly in the dorsomedial neocortex. Zilles (1985) describes three subdivisions: FR1, FR2, and FR3. The cortical surface occupied by FR1–FR3 was subdivided by Krieg (1946a) into anterolateral area 8 (partly corresponding to FR3), anterior area 10 (corresponding to anterior FR2 and FR1), and dorsal area 4 (corresponding to posterior FR2 and FR1). Two additional areas, FL (the forelimb area) and HL (the hindlimb area) overlap with the primary somatosensory cortex and correspond most closely to the medial part of Krieg's (1946a) area 3. The forelimb and hindlimb areas are unique because they get specific thalamic input from two nuclei: (1) the ventrolateral nucleus, which makes FL and HL part of the motor cortex, and (2) the lateral part of the ventrobasal complex, which makes FL and HL part of the somatosensory cortex (Caviness and Frost, 1980; Frost and Caviness, 1980; Faull and Mehler, 1985; Sharp and Gonzalez, 1986; Carvell and Simons, 1987; O'Donoghue et al., 1987; Williams and Faull, 1987). Ventrolateral and ventrobasal axons end mainly in layer IV and the lower part of layer III (Killackey and Ebner, 1973; Jacobson and Trojanowski, 1975; Frost and Caviness, 1980; Herkenham, 1980; Donoghue and Ebner, 1981).

Although Marin-Padilla (1970) showed a radial gradient of cell differentiation in the motor cortex using the Golgi stain, no detailed reports on neurogenetic gradients in the motor cortex are available with [^{3}H]thymidine autoradiography. Miller (1988) combined [^{3}H]thymidine pulse labeling with retrograde transport of horseradish peroxidase to determine the time of origin of the corticospinal neurons in rats and found evidence of a combined transverse and longitudinal gradient within that population.

The present study, using comprehensive [^{3}H]thymidine autoradiographic labeling, analyzed neurogenesis in layers II-VI in six anatomically matched sections (drawings, Fig. 14–7), ranging from levels A10.2 anteriorly to A4.8 posteriorly (Pellegrino et al., 1979). The low magnification photomicrographs in Figure 14–1 show the motor areas at level A9.4 (Fig. 14–1A) and level A4.8 (Fig. 14–1B). FR1 and FR2 extend throughout the entire rostrocaudal extent of the analyzed region. FR2 is the most medial area bordering the cingulate cortex and part of the anterior retrosplenial areas, while FR1 is always lateral to FR2. Area FR3 is found only from levels A10.2 through A9.4 and forms the lateral border of the motor areas (Fig. 14–1A), adjacent to the most anterior part of PAR1. From levels A8.8 through A6.0, FL forms the lateral border of the motor cortex, lying adjacent to PAR1. HL is

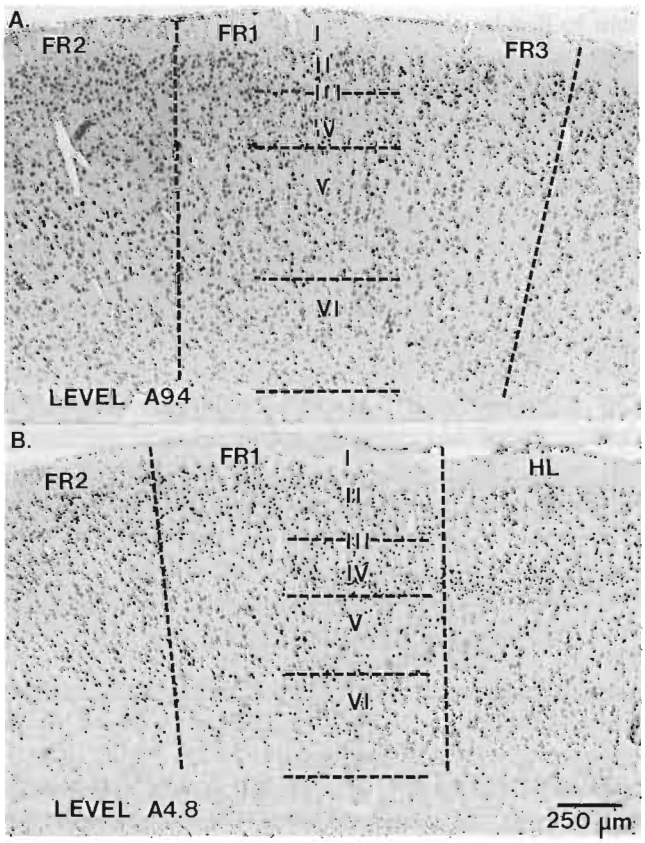

FIG. 14–1. A and **B** are low-magnification views of the motor cortex in the brain of a rat exposed to [^{3}H]thymidine on E17 and E18 and killed on P60 (6 μm paraffin sections, hematoxylin/eosin stain). Four motor areas are located in the dorsal cerebral wall at levels A9.4 **(A)** and A4.8 **(B)** (Pellegrino et al., 1979); dorsal is at the top, medial is to the left. FR2, FR1, and FR3 are present anteriorly **(A),** while FR2, FR1, and HL are found posteriorly **(B).** Area FL (not shown) is similar to area HL and is found just lateral to FR1 from levels A8.8 to A6.0 (Zilles, 1985). Roman numerals separated by dashed lines indicate how the cortex was subdivided into layers for the cell counts.

sandwiched between FL and FR1 at level A6.0; at level A4.8 (Fig. 14–1B), HL is adjacent to PAR1. Because they are part of primary somatosensory cortex, both FL (not illustrated) and HL (Figs. 14–1B and 14–3B) have a dense population of granule cells in layer IV.

Neurons were counted separately in layers VI and V. The photomicrographs in Figs. 14–2 and 14–3 show that layer VI pyramidal cells are smaller and have a higher packing density than pyramidal cells in layer V, where one often sees large neurons scattered among smaller neurons resembling those found in layer VI. Due to the differences in size and packing density, the dividing line between layers VI and V is evident even in thin 6 μm sections (*dashed lines,* Fig. 14–1). The transition between layers V and IV is also clear in FR1–FR3 since the large pyramidal cells typically seen in V are absent in IV. There is also a slight increase in packing density, and a few scattered granule cells are interspersed among the small pyramidal cells in layer IV. In HL and FL, the prominent accumulation of layer IV granule cells (Fig. 14–3B) delineates the lower border of layer III. However, the separation of layers IV, III, and II proved difficult in FR1, FR2, and FR3 (Figs. 14–2A, 14–2B, and 14–3A). In order to be consistent throughout the motor areas, neurons were counted in lower (IV/III) and upper (III/II) halves of the superficial layers (*dashed lines,* Fig. 14–1).

14.1 THE RADIAL NEUROGENETIC GRADIENT

The radial neurogenetic gradient found throughout the rest of the neocortex is also prominent in the motor areas. Nearly all superficial neurons (IV–II) are labeled throughout anterior and posterior parts of the motor cortex (Figs. 14–2 and 14–3) in an animal exposed to [^{3}H]thymidine on E17 and E18 and killed on P60, while most neurons in layer VI and many in layer V are unlabeled (Fig. 14–2). For quantification of the radial neurogenetic gradient, the cell counts in each layer of all the motor areas were combined (Fig. 14–4). Neurons in layer VI have the earliest peak (E16), and 35% are generated earlier than neurons in layers V–II. Neurons in upper layer III and II have the latest peak (E19), and 36% are generated later than those in lower layer III and layers IV–VI. The intervening layers have gradual shifts in peaks of neurogenesis between the two extremes. The magnitude of the radial gradient can be appreciated by comparing the proportion of neurons generated in different layers before and

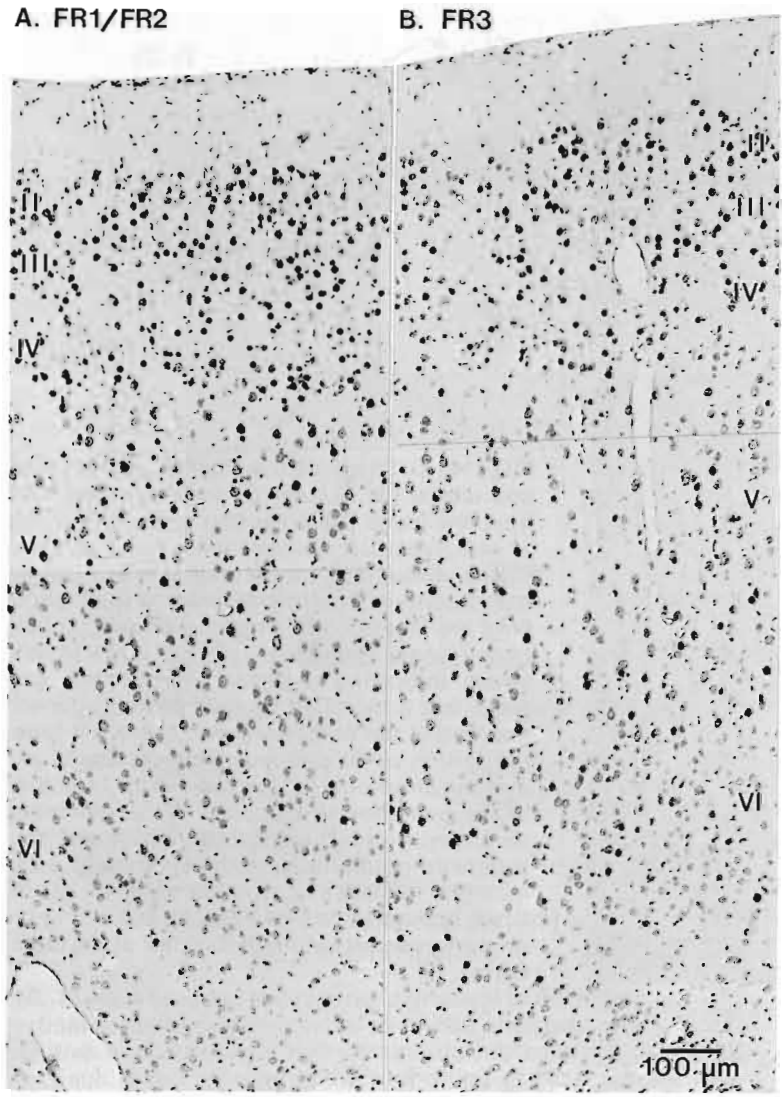

FIG. 14–2. Strips of the medial (**A,** FR1/FR2) and lateral (**B,** FR3) motor cortex at level A9.4 (Pellegrino et al., 1979) from the same section as shown in Fig. 14–1A. The placement of the vertical dashed lines in Fig. 14–1A shows where the higher-magnification photographs were taken. Roman numerals indicate the cortical layers (II–VI). There are generally more labeled neurons in the medial **(A)** than in the lateral **(B)** primary area. In both areas, neurons in layer VI are mostly unlabeled, while more layer V cells are labeled medially **(A)** than laterally **(B).** Neurons in layers II–IV are mostly labeled throughout both strips. These 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 lateral to younger medial neurogenetic gradient between FR3 and FR1/FR2.

after selected embryonic ages (E15, E17, and E19, *vertical dashed lines,* Fig. 14–4). By E17 for example, neurogenesis in layer VI is nearly completed (>85%), while neurogenesis in layer III–II has only begun (<15%). Even between adjacent layers, the neurogenetic gradients are considerable. 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), F values range from 531.45 to 169.4, df = 1, all $P < 0.0001$. In layers VI and V, 65% of the neurons are generated concurrently; in IV/lower III and upper III/II, 53% of the neurons are generated concurrently; while the lowest proportion of convergence (46%) is between layer IV/lower III and layer V (*stippled areas,* Fig. 14–4). That pattern is similar to those found in the visual (Chapter 11), auditory (Chapter 12), and somatosensory (Chapter 13) areas. There appears to be a consistent "temporal gap" between the deep layers (VI–V) and the superficial layers (IV–II).

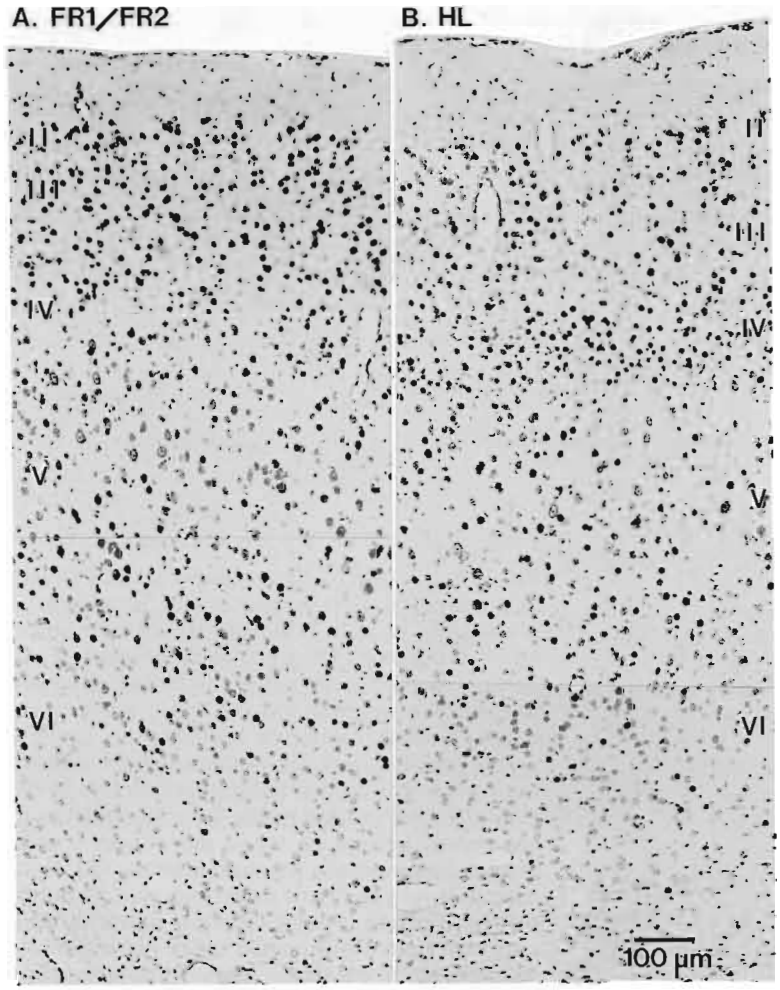

FIG. 14–3. Strips of the medial (**A,** FR1/FR2) and lateral (**B,** HL) motor cortex at level A4.8 from the same brain section as shown in Fig. 14–1B (*vertical dashed lines* indicate areas where photographs were taken). In both areas, the majority of superficial neurons throughout IV–II are labeled, while roughly equal numbers of neurons are labeled in layers VI and V in both strips, indicating a radial neurogenetic gradient, but a modified transverse neurogenetic gradient at the posterior level. There is a lower proportion of labeled neurons in layers VI and V of the anterior strips shown in Fig. 14–2 than in the posterior strips shown here, indicating an anterior (older) to posterior (younger) neurogenetic gradient. Note the prominent accumulation of layer IV granule cells in HL **(B)** when compared to FR1/FR2 **(A),** indicating the primary sensory nature of this part of the motor cortex.

14.2 THE TRANSVERSE NEUROGENETIC GRADIENT

There is a pronounced transverse neurogenetic gradient between anterior (level A9.4, Fig. 14–5A) and posterior (level A4.8, Fig. 14–5B) motor areas in both the deep (VI–V) and superficial (IV–II) layers, but in opposite directions.

Anteriorly, both layers VI and V have prominent lateral (older) to medial (younger) neurogenetic gradients (*arrow pointing left* in Fig. 14–5A). The gradient in the deep layers is visible as proportionally fewer layer V neurons are labeled in FR3 (Fig. 14–2B) when compared to FR1/FR2 (Fig. 14–2A). Neuron production in layer VI (lowest set of graphs, Fig. 14–5A) peaks on E15 in FR3 and on E16 in FR2 ($P < 0.0001$, sign test; $F = 14.53$, $df = 1$, $P < 0.0003$, SAS GLM procedure). Layer V (center set of graphs, Fig. 14–5A) has a pronounced peak of neuron production on E16 in FR3, while E16 and E17 are the peak days in FR2 ($P < 0.007$, sign test; $F = 32.17$, $df = 1$, $P < 0.0001$, SAS GLM procedure). The data for the deep layers of FR1 at level A9.4 is intermediate between FR3 and FR2 and are not shown. In contrast, the posterior motor cortex (A4.8, Fig. 14–5B) has no gradients between FR2, FR1, and HL (all sign test comparisons, $P > 0.05$); layer VI neurons originate mainly on E16 and layer V neurons mainly on E16 and E17. Consistent with that finding, both layers VI and V have about the same proportion of labeled neurons in FR1/FR2 and HL (Fig. 14–3). Thus, only the deep layers of the anterior motor areas, not the superficial layers, show the prominent transverse neurogenetic gradient seen throughout the neocortex (Chapter 3).

Because the superficial layers have no transverse neurogenetic gradients anteriorly (level A9.4), the upper set of graphs (Fig. 14–5A) show combined data. Layers IV/III arise mainly on E17–E18, layers III/II

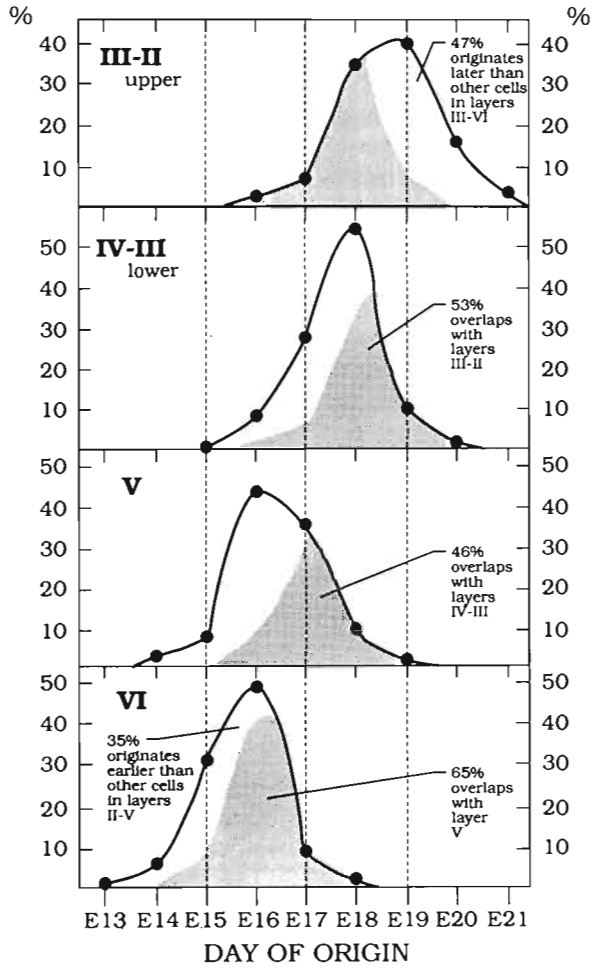

FIG. 14–4. The radial neurogenetic gradient in the motor cortical areas of rats that survived to P60 after two consecutive exposures to [^{3}H]thymidine during embryonic life. Each graph represents the proportion of cells generated from E13 to E21 in separate layers (II/upper III—top graph to VI—bottom graph) based on data combined from all areas of the motor cortex that were analyzed. Vertical hatched lines indicate relative amounts of cells that have been generated before and after E15 (*left line*), E17 (*center line*), and E19 (*right line*). Proceeding from layer VI to upper layer III/II, the peaks of neurogenesis shift from early (E16 in VI) to late (E19 in upper III/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/lower III (46% indicated in the second graph from the bottom).

on E18–E20 simultaneously in FR3, FR1, and FR2 (all sign test comparisons, $P > 0.05$). In contrast posteriorly (level A4.8), both layers IV/III and III/II have significant older/medial to younger/lateral neurogenetic gradients (*arrow pointing right,* Fig. 14–5B), in the opposite direction to the global transverse neurogenetic gradient in the neocortex. The gradient in layers III/II is robust ($P < 0.0001$, sign test; $F = 41.45$, $df = 1$, $P < 0.0001$, SAS GLM procedure). The peak day of neuron production occurs on E18 in medial FR2 and the amount of neurogenesis steadily declines thereafter, while the neurogenetic peak occurs on E19 in lateral HL (top set of graphs, Fig. 14–5B). The gradient in layers IV/III is slight (middle set of graphs, Fig. 14–5B); more IV/III neurons are generated on or before E18 in medial FR2 (89%) than in lateral HL

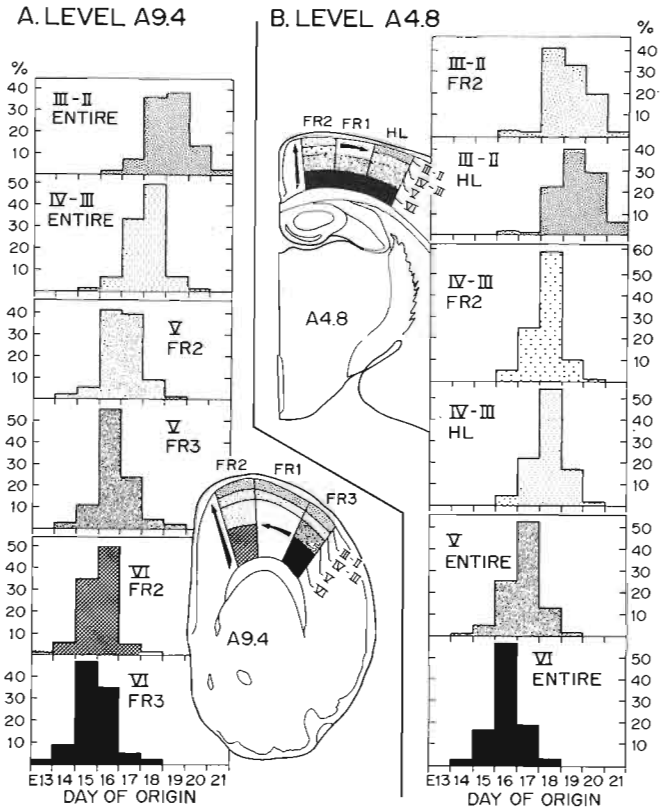

FIG. 14–5. A laminar analysis of neurogenesis in lateral versus medial locations in the motor cortex at level A9.4 (**A,** areas FR1–FR3) and at level A4.8 (**B,** areas FR1–FR2 and HL). Bar graphs are the proportion of neurons originating during single embryonic days. Data are based on counts in the brains of P60 rats that were exposed to [^{3}H]thymidine on 2 consecutive embryonic days. A radial neurogenetic gradient is found both anteriorly and posteriorly, but there are different patterns with respect to the transverse neurogenetic gradient. The deep layers (VI and V) have a prominent lateral (older) to medial (younger) neurogenetic gradient anteriorly (four bottom graphs in **A**), while deep neurons originate simultaneously in lateral and medial locations posteriorly (two bottom graphs in **B**). The superficial layers originate simultaneously anteriorly (two top graphs in **A**), but have a reversed medial (older) to lateral (younger) neurogenetic gradient posteriorly (four top graphs in **B**).

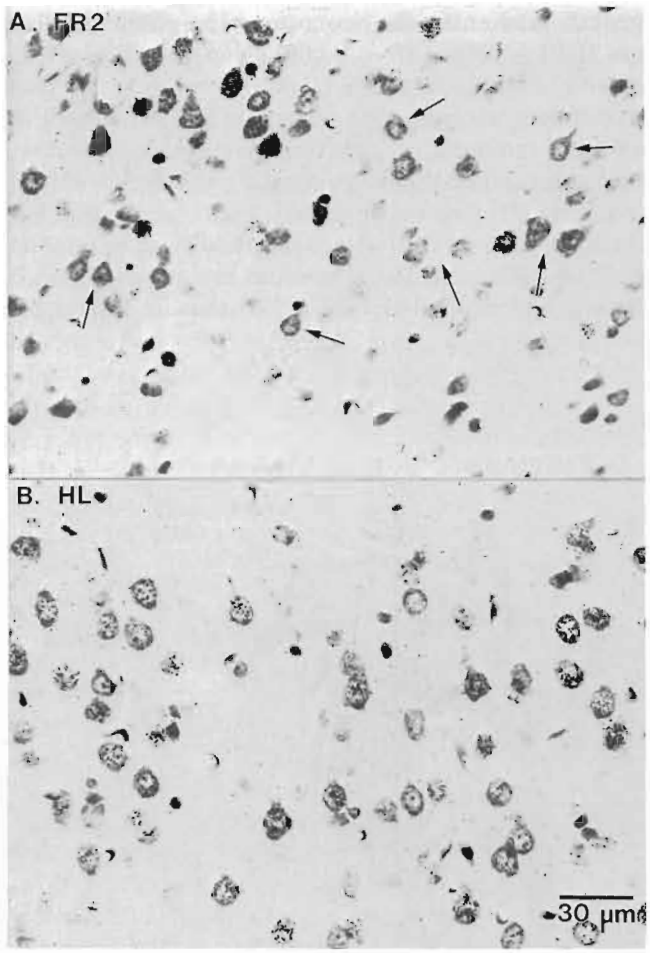

FIG. 14–6. An illustration of the reversal in the lateral (older) to medial (younger) transverse neurogenetic gradient in the superficial layers of the somato-motor cortex in the brain of a rat exposed to [^{3}H]thymidine on E19 and E20 and killed on P60. There are several groups of unlabeled neurons in medial FR2 (arrows in **A**), few or none in lateral HL **(B).** That pattern is found in all animals of the E19–E20 injection group and indicates that more superficial neurons are born later in the primary sensory area (in this case, HL) than in the adjacent medial nonsensory area.

(81%), while neurogenesis on and after E19 is greater in HL (19%) than in FR2 (11%). Since nearly every animal in the E18 + E19 group and all animals in the E19 + E20 group showed this trend, there are significant differences ($P < 0.003$, sign test; F = 13.4, df = 1, $P < 0.0003$, SAS GLM procedure). Figure 14–6 illustrates the gradient in the brain of a rat exposed to [^{3}H]thymidine on E19 and E20 and killed on P60. In FR2, many layer III/II neurons are unlabeled (*arrows*, Fig. 14–6A), while most layer III/II neurons are labeled in HL (Fig. 14–6B), indicating a later time of origin for the HL neurons. That is reminiscent of the "sandwich" gradient seen between primary and secondary parts of the visual cortex (Chapter 11).

14.3 LONGITUDINAL NEUROGENETIC GRADIENTS

The longitudinal neurogenetic gradient is found within all layers of the motor cortex, but its magnitude varies between layers. The data for anterior (A10.2) and posterior (A4.8) sections are shown to demonstrate the main features of the longitudinal gradient (Fig. 14–7). Layers VI and V have a robust anterior-to-posterior neurogenetic gradient (two bottom graphs, Fig. 14–7). Approximately 59% of the layer VI neurons are generated on or before E15 at level A10.2, but only 22% are generated during the same period at level A4.8 ($P < 0.0001$, sign test; F = 97.5, df = 1, $P < 0.0001$,

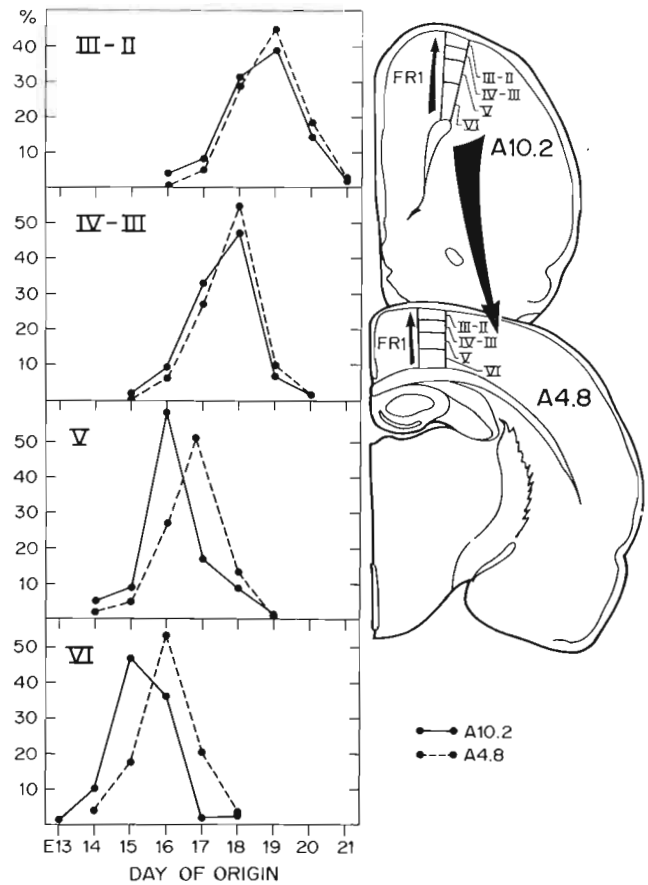

FIG. 14–7. The time of origin of neurons in layers II–VI anterior (level A10.2) and posterior (level A4.8) parts of area FR1. Data are based on counts in the brains of P60 rats that were exposed to [^{3}H]thymidine on 2 consecutive days of embryonic life. Line graphs are the proportion of neurons originating during single embryonic days; *solid lines*, anterior cell neurogenesis; *dashed lines*, posterior cell neurogenesis. In both anterior and posterior sections, there is a prominent deep (older) to superficial (younger) neurogenetic gradient between layers (*small arrows* in drawings of each level). In layers V and VI, neurogenesis at anterior levels precedes neurogenesis at posterior levels (*large arrow* between sections), while the superficial neurons (layers IV–II) are generated nearly simultaneously at both levels.

SAS GLM procedure), a divergence of 37% between the two sites. Approximately 72% of layer V neurons are generated on or before E16 anteriorly, but only 34% posteriorly ($P < 0.0001$, sign test; F = 62.33, df = 1, $P < 0.0001$, SAS GLM procedure), a divergence of 39% between the two sites. The strong anterior-to-posterior gradient in layer V can be seen by comparing the smaller proportion of neurons labeled in anterior FR1/FR2 (Fig. 14–2A) with the higher proportion of labeled neurons in posterior FR1/FR2 (Fig. 14–3A). In contrast, the superficial layers show progressively less-pronounced longitudinal gradients (top two graphs, Fig. 14–7). In layers IV–III, 45% of the neurons are generated by E17 anteriorly, 34% posteriorly ($P < 0.012$, sign test; F = 7.31, df = 1, $P = 0.0093$, SAS GLM procedure), a divergence of 11% between the two locations. In layers III–II, 44% of the neurons at level A10.2 are generated by E18, 36% at level A4.8 ($P < 0.003$, sign test; F = 6.48, df = 1, $P = 0.0139$, SAS GLM procedure), a divergence of only 9% between the anterior and posterior sites.

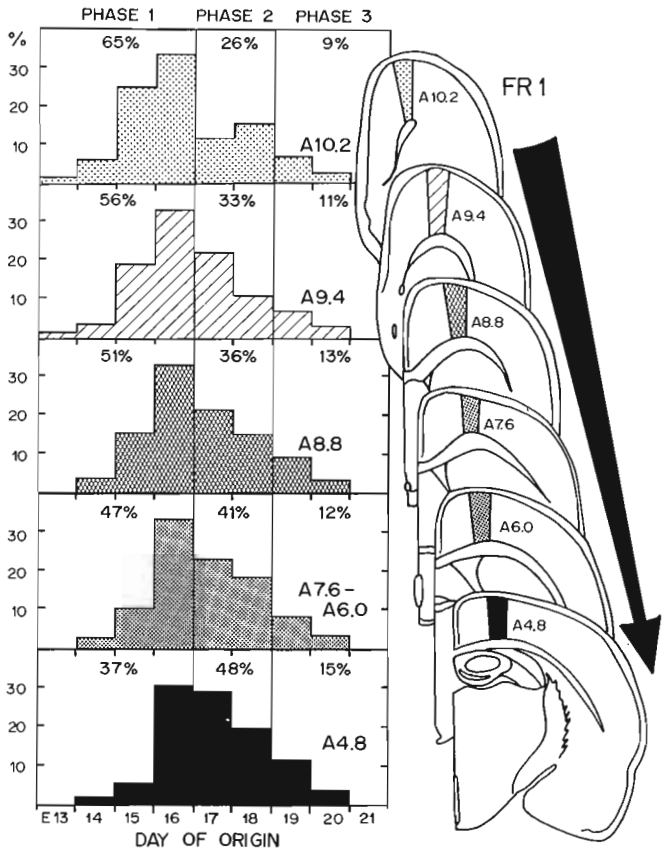

FIG. 14–8. An overview of the anterior (older) to posterior (younger) neurogenetic gradient between levels A10.2 (most anterior) and A4.8 (most posterior) of area FR1 in the motor cortex. Data are based on counts in the brains of P60 rats that were exposed to [^{3}H]thymidine on 2 consecutive days of embryonic life. Bar graphs are the proportions of neurons originating during single embryonic days in cell counts combined for layers II–VI in radial "strips" of cortex. The data for each level (or combined levels in the graph second from bottom) are grouped into three phases (1 is early, 3 is late); the percentages are the proportions of neurons that are generated during each phase. There is a stepwise anterior (older) to posterior (younger) neurogenetic gradient between levels (*large arrow*).

To show the stepwise nature of the anteroposterior gradient between the analyzed levels, the cell counts for each layer (VI–II) in FR1 were combined for each level so that the data represent the time of origin of all neurons in radial strips of cortex (drawings, Fig. 14–8). The sign test indicated that neurons at levels A7.6 and A6.0 are generated simultaneously ($P > 0.05$) so these data were combined. The strip at level A10.2 (top graph, Fig. 14–8) is generated significantly earlier than that at A9.4 ($P < 0.002$, sign test), A9.4 earlier than A8.8 ($P < 0.0001$, sign test), A8.8 earlier than A7.6–A6.0 ($P < 0.0001$, sign test), and A7.6–A6.0 earlier than A4.8 ($P < 0.0001$, sign test).

The graphs in Fig. 14–8 are broken up into three phases of neurogenesis. Phase 1 (up to E16) is the period during which most neurons of layer VI and some neurons of anterior layer V are generated. Note that there is a progressive decrease in the proportion of neurons generated from level A10.2 (65%) to level A4.8 (37%), a divergence of 28%. That difference reflects the strong anteroposterior gradient in the generation of layers VI and V neurons. Phase 2 (E17–E18) shows the opposite neurogenetic pattern. Progressively more neurons are generated posteriorly (48%) than anteriorly (26%), a spread of 22%. That difference is mainly due to the strong anteroposterior gradient within layer V, since few layer V neurons are generated at level A10.2, many at level A4.8 (Fig. 14–7). During Phase 3 (on and after E19), there is only a slight difference in neuron production from anterior (9%) to posterior (15%) levels. The decreased divergence (only 6%) reflects the weak anteroposterior neurogenetic gradient in the superficial layers (Fig. 14–7).

While Figs. 14–6 and 14–7 show data only from FR1, it is important to note that there is also a longitudinal neurogenetic gradient within FR2 and between FL (older) and HL (younger). Relevant to the latter, there is also a maturation gradient between HL and FL in terms of cytoarchitectonic specialization in layer IV (Zhang and Cooper, 1990; Cooper, personal communication) and physiological response properties (McCandlish et al., 1990).

14.4 CORRELATIONS BETWEEN NEUROGENETIC GRADIENTS IN LAYER V WITH OUTGROWTH OF THE CORTICOSPINAL TRACT

The corticospinal tract has been the most intensely studied efferent pathway from the neocortex, not only

in adults but also during development. The complete timetable of outgrowth has been described using anterograde axonal transport of horseradish peroxidase and/or tritiated amino acids (Martin et al., 1980; Schreyer and Jones, 1982; Cabana and Martin, 1985, 1986). These techniques cannot distinguish between layers VI and V, which means that some of the labeled axons could be from the earlier-generated layer VI neurons. However, layer VI axons should not extend much farther than the diencephalon since the thalamus is their chief target (Wise and Jones, 1977; Foster et al., 1981). In correlation with the early generation of layer VI neurons (Figs. 14–4, 14–5, and 14–7) and its proximity to the cortex, the thalamus is the first subcortical structure to receive cortical efferents (Cabana and Martin, 1986). In rats (Schreyer and Jones, 1982), corticofugal axons reach thalamic levels of the internal capsule by E17.5. Axons from later-generated layer V neurons reach the pontine nuclei by E19.5 and the caudal limit of the medulla by E20.5 (Schreyer and Jones, 1982). Work in the opossum (Martin et al., 1980) indicates that corticospinal axons pause in two stations, the cerebral peduncle and the pyramidal decussation, during their otherwise steady growth toward the spinal cord. Comparing timetables for layer V neurogenesis (Figs. 14–5 and 14–7) and timetables of axonal arrival in brainstem subcortical centers indicates that layer V axonogenesis begins very shortly after neurogenesis, either while the neurons are sojourning in sb2 in the intermediate zone (Chapter 7) or as they begin migrating toward the cortical plate. Precocious axonogenesis of cortical output neurons has been suggested in other developmental studies (Stensaas, 1967d; Shoukimas and Hinds, 1978; Wise et al., 1979b; Martin et al., 1980; Reh and Kalil, 1981; Berry, 1982; Schreyer and Jones, 1982), and the best evidence comes from an experimental study by Jensen and Killackey (1984). After exposing embryonic rats to low-level x-rays on or after E17 to spare most of the layer V neurons in the motor cortex, they found that, in spite of the ectopic position of layer V cell bodies beneath the cortical white matter, layer V axons extended into the spinal cord. Our neurogenetic timetables support Jensen and Killackey's (1984) conclusion that the initial projections of corticospinal neurons are determined before the pyramidal cells settle in the cortical gray matter.

In rats (Hicks and D'Amato, 1975; Donatelle, 1977; Schreyer and Jones, 1982, 1988a; Stanfield et al., 1982; Leong, 1983; Bates and Killackey, 1984; Uozmi et al., 1988; Joosten et al., 1989), hamsters (Reh and Kalil, 1981; Kalil, 1985), and opossums (Martin et al., 1980; Cabana and Martin, 1985), the growth of the corticospinal tract into the spinal cord occurs entirely during the postnatal period. On P1 in rats, corticospinal axons cross in the pyramidal decussation and extend into the dorsal columns of the upper cervical cord, then they continue to grow caudally, reaching the lumbar level in approximately one week (Donatelle, 1977; Schreyer and Jones, 1982; Joosten et al., 1987, 1989). Before the pioneer axons arrive, there is a tiered array of vimentin immunoreactive glial processes present in the region that will be occupied by the descending corticospinal tract (Joosten and Gribnau, 1989; Joosten et al., 1987); this glial matrix may be a continuation of the "roof plate channels" that precede the growth of ascending axons during development of the dorsal columns (Altman and Bayer, 1984). That area in the spinal cord may also be similar to the extracellular channels seen above and below the cortical plate before the arrival of early corticopetal fibers (Bayer and Altman, 1990; Chapter 5). After about 2 weeks, the rat corticospinal tract reaches the most caudal levels of the spinal cord (Donatelle, 1977; Schreyer and Jones, 1982, 1988b; Leong, 1983; Bates and Killackey, 1984; Joosten et al., 1987; Uozumi et al., 1988) and acquires the topographic order seen in adults. An interesting finding in rats is that the population of corticospinal projecting neurons extends through widespread areas in the neocortex of neonates then shrinks during the first few postnatal weeks as enduring contacts are established in the spinal cord (D'Amato and Hicks, 1980; Stanfield et al., 1982; Leong, 1983; Bates and Killackey, 1984; Crandall et al., 1985; Schreyer and Jones, 1988a; Uozumi et al., 1988; Joosten and Van Eden, 1989). The "pruning" of the projection population is accomplished by loss of axon collaterals rather than by death of the projecting neurons (Stanfield et al., 1982). The rat pyramidal tract loses many of its axons during the time that the corticospinal projection field shrinks (Schreyer and Jones, 1988b; Gorgels et al., 1989; Gorgels, 1990).

Whatever the extent of disorder in the primitive corticospinal tract, the lasting synaptic relationships between cortical source neurons and spinal targets in the mature corticospinal tract correlate with timetables of neurogenesis in the neocortex and the spinal cord (diagrammed in Fig. 14–9). The physiologically defined cortical motor map of the rat body (Hall and Lindholm, 1974) was confirmed to be the source of the layer V projection to spinal levels by retrograde transport of horseradish peroxidase (Wise and Jones, 1977; Wise et al., 1979a; Ullan and Arteida, 1981; Leong, 1983; Uozumi et al., 1988). When the topographic maps provided by these studies are considered in conjunction with the neurogenetic data, it becomes evident that the corticospinal projection neurons in layer V are organized in conformity with the transverse (Fig. 14–5A) and longitudinal (Fig. 14–7) neurogenetic gradients in the neocortex, and their axons terminate in a pattern in conformity with the anterior (older) to posterior (younger) neurogenetic gradient in the spinal cord (Alt-

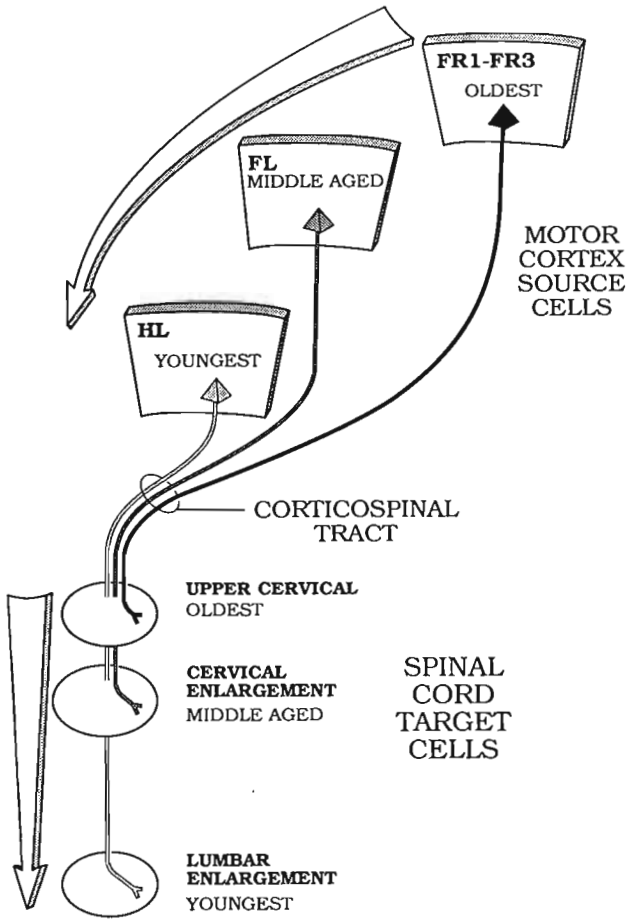

FIG. 14–9. A diagrammatic representation of the neurogenetic gradients and anatomical connections between the layer V output neurons of the corticospinal tract and their terminations in the spinal cord. The large arrows represent directions in neurogenetic gradients and point to areas of younger neurons in each structure. Younger neurons are located posterior and medial in the motor cortex, posterior in the spinal cord. There is an exact age match between cortical source neurons and spinal cord target neurons. The oldest neurons (*solid black*) in the anterior motor cortex project to the oldest neurons in the upper cervical spinal cord. "Middle aged" neurons (*dark stipple*) in area FL project to "middle aged" neurons in the cervical enlargement, while the youngest cells (*light stipple*) in area FL project to the youngest neurons in the lumbar enlargement.

man and Bayer, 1984). The overall pattern is for cortical source neurons and spinal cord target neurons to be exactly age matched. The oldest anterior and lateral neurons in FR1–FR3 (face area) project to the oldest target neurons in spinal trigeminal centers in upper cervical levels. "Middle-aged" neurons situated slightly more posteriorly and medially in FL (forelimb area) project to "middle-aged" target neurons in the cervical enlargement. The youngest neurons situated most posteriorly in HL (hindlimb area) project to the youngest target neurons in the lumbar enlargement. A similar topographic arrangement of projection neurons has been found in the motor cortex of the guinea pig (Rapisarda et al., 1985) and the hamster (Kalil, 1985).

Our neurogenetic data indicate that the corticospinal axon collaterals retained in adults is related to the birth dates of cortical motor neurons. Given the evidence for extensive remodeling in the developing corticospinal tract, what is taking place during the selective elimination of some axon collaterals? Some clues emerge when one examines the original population of corticospinal neurons. In neonate rats, layer V neurons in the occipital cortex send axons into the spinal cord that grow as far caudally as the fifth thoracic segment (Stanfield et al., 1982; Joosten et al., 1987); this projection does not exist in adults. When occipital axons are anterogradely labeled with horseradish peroxidase during the first postnatal week, they are found only in the corticospinal fiber bundle at the base of the dorsal columns in the spinal cord and regress later without ever penetrating the gray matter of the spinal cord (Joosten et al., 1987). In contrast, anterograde labeling in motor cortex indicates that the axons proceed from the spinal cord white matter after a pause, grow into the spinal gray (Joosten et al., 1987), and establish lasting synaptic contacts in the intermediate horn (laminae V, VI, and VII of Rexed; Valverde, 1966). The microenvironment in the intermediate horn may be attractive to invasion by appropriate axon collaterals from motor cortex but repulsive to invasion by inappropriate axon collaterals from occipital cortex. Perhaps as neurons are being generated according to specific timetables, there is a consequent temporal coding of cell recognition markers that appear during a critical period on the axons and/or on the target neurons, and this leads to the retention of some collaterals and the elimination of others. Such a chemoattractant hypothesis has been proposed to explain the ingrowth of corticospinal collaterals into the pons (Heffner et al., 1990).

14.5 CORRELATIONS BETWEEN NEUROGENETIC GRADIENTS AND THALAMOCORTICAL PROJECTIONS

14.5.1 The Ventrolateral Nucleus

Although there are only a few studies in rats of topographic connections between the thalamic ventrolateral nucleus (VL) and its chief target, the motor cortex, there is general agreement as to the topographic pattern of input. The facial motor relay units projecting to anterior motor cortex are located in a dorsomedial strip in VL, while hindlimb motor units projecting to posterior motor cortex are located in the lateral and dorsal parts of VL (Donoghue et al., 1979; Donoghue

and Parham, 1983; Faull and Mehler, 1985; Williams and Faull, 1987). Neurogenetic gradients in VL are such that dorsal and lateral neurons are older than medial and ventral neurons (Altman and Bayer, 1989a). The neurogenetic gradients in the motor cortex and VL are matched as follows: older neurons in lateral VL project to younger target neurons in the posterior and medial limb areas, while younger neurons in medial VL project to older target neurons in the anterior and lateral face areas, an exact chronological reversal between the birthdays of source cells and target cells. As we have seen in the visual (Chapter 11), auditory (Chapter 12), and somatosensory (Chapter 13) areas, thalamic axon length is positively correlated with age: older thalamic neurons have longer axons than younger ones. With regard to the trajectory of VL axons to the motor cortex, those terminating laterally or anteriorly will be shorter than those terminating medially and posteriorly because all axons enter the cortex ventrolaterally through the internal capsule. The same relationship holds for projections of the lateral part of the ventrobasal complex to the limb areas.

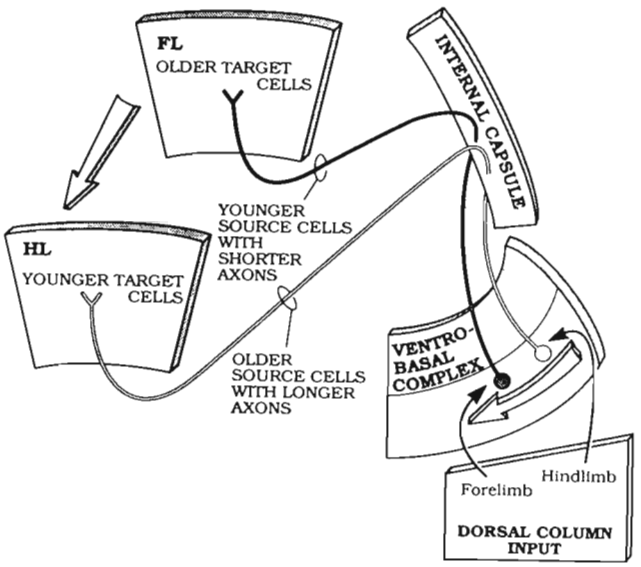

FIG. 14–10. A diagrammatic representation of the neurogenetic gradients and anatomical connections between the lateral part of the ventrobasal complex and the primary somatosensory cortex (FL and HL). The large arrows point to areas of younger neurons in each structure. There is an exact reversal of ages in source and target neurons that may be related to axonal length. Older source neurons in the lateral ventrobasal complex receive input from the gracile nucleus relaying information from the hindlimb. These neurons have longer axons (*light stipple*) that terminate in the posteromedial HL that contains younger target neurons. The converse is true for the younger source neurons in the medial ventrobasal complex. These neurons get input from the cuneate nucleus relaying information from the forelimb and pass that on via shorter axons (*dark stipple*) to older target neurons in FL.

14.5.2 The Ventrobasal Nucleus

Afferents conveying tactile and pressure information from the hindlimb travel in the spinal dorsal columns to the gracile nucleus (reviewed in Tracey, 1985; Chimelli and Scaravilli, 1987; Cliffer and Giesler, 1989); the same information from the forelimb terminates in the cuneate nucleus (Basbaum and Hand, 1973; reviewed in Tracey, 1985; Cliffer and Giesler, 1989). These axons cross the midline and terminate topographically in the lateral part of the ventrobasal complex (VBl), gracile fibers anteriorly and laterally, cuneate fibers posteriorly and medially (Lund and Webster, 1967; Angel and Clarke, 1975; Feldman and Kruger, 1980). Nociceptive input from the limbs travel to the thalamus in the spinothalamic tract and terminate in the same topographic relationship (Lund and Webster, 1967; Pechanski et al., 1983, 1984), most probably on the same neurons in VBl (Ma et al., 1987). The neurogenetic gradients in VBl (Altman and Bayer, 1989a) are such that lateral neurons originate earlier than medial neurons, thus hindlimb input is relayed to older neurons, forelimb input is relayed to younger neurons (diagrammed in Fig. 14–10).

Evidence of discrete relays for each limb through the thalamus comes from physiological maps of the cortex generated by stimulation of the periphery (Welker, 1971, 1976; Chapin and Lin, 1984) and by mapping the cortical areas affected by peripheral lesions (Dawson and Killackey, 1987; Warren et al., 1989). Direct studies of thalamic projection from VBl to the cortex suggest topographic input from the most lateral parts to area HL and from slightly more medial parts to area FL (Saporta and Kruger, 1977; Sharp and Gonzalez, 1986; Carvell and Simons, 1987; O'Donoghue et al., 1987). When the anatomical and physiological studies are considered with the data on neurogenetic gradients in the thalamus and cortex, again an exact reversal of the birthdays between thalamic source neurons and cortical target neurons can be related to the length of thalamic axons (diagrammed in Fig. 14–10). Older thalamic neurons in lateral VBl project longer axons to younger neurons in posterior and dorsal cortical area HL. Younger thalamic neurons in medial VBl project shorter axons to older neurons in anterior and dorsolateral area FL. Because it takes more time for thalamic axons to reach distant cortical targets posteriorly and dorsally than nearer cortical targets anteriorly and laterally (Martin et al., 1987), the earlier generation of neurons with long axons gives them a headstart to reach their target. We will pursue that possibility in Chapter 16.

14.6 IMPLICATIONS OF THE REVERSAL OF THE TRANSVERSE GRADIENT IN THE SUPERFICIAL LAYERS

Since FL and HL are situated lateral to FR1/FR2 and are also part of the primary somatosensory cortex, we have the opportunity to directly compare neurogenesis between lateral sensorimotor and medial "pure" motor areas. Neurogenetic gradients in the deep layers (VI–V) follow the expected lateral (older) to medial (younger) pattern (Fig. 14–5A), similar to that seen throughout the entire neocortex (Chapter 3). However, the superficial layers in the laterally situated HL (Fig. 14–5B) and FL (data are not shown) show an unexpected deviation from the global transverse gradient by having younger neurons than those in the medially situated FR1 and FR2. That deviation may be related to the sensory character of FL and HL, since we showed a similar deviation from the global transverse gradient in the visual cortex (Chapter 11). Taken together, these data indicate that primary sensory areas are neurogenetically tagged by containing a high concentration of unusually young neurons in the superficial layers.

The primary sensory cortical areas have developmental peculiarities other than deviations in neurogenetic gradients. Several studies show that the primary somatosensory cortex is neurochemically tagged with the transient expression of cholinergic markers. Acetylcholinesterase (ACHE) staining is heavy in the barrel subfields during the first 2 postnatal weeks in rats and mice (Kristt and Waldman, 1981, 1982; Robertson, 1987). At the same time, the barrel clusters in layer IV show binding of the muscarinic radioligand [^{3}H] 3-quinuclidinylbenzilate (Kristt and Kasper, 1983), and the nicotinic receptor, alpha-bungarotoxin (Fuchs, 1989). Three lines of evidence indicate that the transient cholinergic activity coincides with the ingrowth of specific thalamic afferents. First, the thalamic sensory relay nuclei are intensely stained for ACHE during the first postnatal week (Robertson, 1987). Second, destruction of vibrissae at birth alters the ACHE staining patterns in the cortex (Kristt and Waldman, 1981). Third, ventrobasal thalamic lesions eliminate the ACHE staining in layer IV (Kristt and Waldman, 1981). It has been suggested that transient cholinergic activity is associated with the establishment of permanent synaptic contacts between the thalamic sensory relay nuclei and their cortical targets (Robertson, 1987). The unusually late neurogenesis of the superficial layers in the primary sensory areas and the transient expression of cholinergic activity limited to primary sensory areas may be coincidental. However, a slight delay in the generation of superficial neurons only in primary sensory areas could be a temporal marker for thalamic axons relaying primary sensory input to display a neurochemical marker, transient cholinergic activity.