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

CHAPTER 13

Development of the Somatosensory Areas

The somatosensory area is the target of topographically organized afferents from the thalamic ventrobasal complex (Bernardo and Woolsey, 1987; Carvell and Simons, 1987; Chmielowska et al., 1989; Keller and White, 1987; Killackey, 1973; Killackey and Leshin, 1975; Saporta and Kruger, 1977; White and Keller, 1987; Wise and Jones, 1978). Because of the prominence of layer IV granule cells (Fig. 13–1), the primary somatosensory area is more conspicuous in the rat neocortex than the primary auditory and visual areas (Krieg, 1946b; Zilles and Wree, 1985). It is also the largest sensory area, taking up most of the lateral and dorsolateral cortical walls above the gustatory area.

The primary somatosensory cortex has received

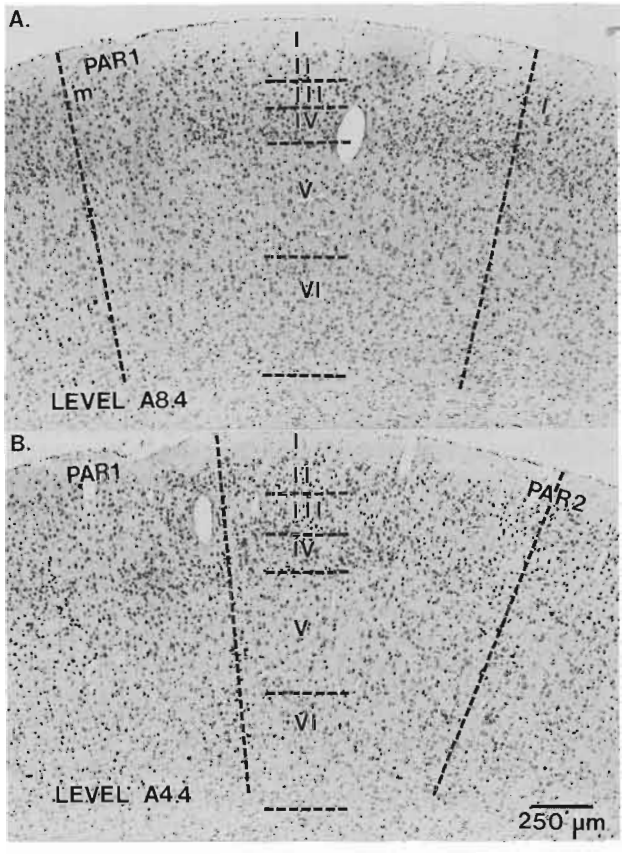

FIG. 13–1. **A** and **B** are low magnification views of the somatosensory cortex in the brain of a rat exposed to [^{3}H]thymidine on E17 and E18 and killed on P60 (6 µm paraffin sections, hematoxylin/eosin stain). Two somatosensory areas are located in the lateral, dorsolateral angle, and dorsal parts of the cerebral wall at levels A8.4 **(A)** and A4.4 **(B)** (Pellegrino et al., 1979); dorsolateral is at the top, dorsomedial is to the left. The primary sensory area (PAR1) is wide anteriorly **(A)** and narrows posteriorly **(B)** where it is flanked by the secondary area (PAR2) (Zilles, 1985). Roman numerals separated by dashed lines indicate how the cortex was subdivided into layers for the cell counts.

considerable attention in rodents due to the discovery of discrete cortical representations of individual facial vibrissae, the "barrels." The barrels are composed of clusters of granule cells in layer IV (Woolsey and van der Loos, 1970; Welker, 1971, 1976; Welker and Woolsey, 1974; Rice and van der Loos, 1977; Waite, 1977; Chapin and Lin, 1984; Rice, 1985; Rice et al., 1985; Armstrong-James and Fox, 1987; Wallace, 1987). Histochemical stains for succinic dehydrogenase (Killackey and Belford, 1979) and cytochrome oxidase (Land and Simons, 1985) indicate that the "hollows" of the barrels are regions of intense metabolic activity. An additional somatotopic body representation lies in a secondary area that is posterior and ventrolateral to the primary area (Emmers, 1965; Welker and Sinha, 1972). Since the parietal bone covers most of the somatosensory cortex (Krieg, 1946a), Zilles (Zilles, 1985; Zilles and Wree, 1985) prefers to use the physiologically neutral labels PAR1 for the primary area, or Krieg's (1946a, 1946b) areas 1–3, and PAR2 for the secondary area, or Krieg's (1946a, 1946b) area 2a. A posteromedial part of the primary somatosensory cortex blends with the motor cortex that controls the limbs (Zilles and Wree, 1985). The overlap areas are designated FL (forelimb) and HL (hindlimb) by Zilles (1985) and their neurogenesis will be discussed in Chapter 14 on the motor cortex.

In spite of the many developmental studies of the barrel subfields, only two papers have appeared on neurogenetic gradients in the somatosensory area in mice (Fairen et al., 1986; Cobas and Fairen, 1988); both demonstrated the inside-out gradient in GABA- and non-GABA-containing neurons. In our study, seven sections were chosen for quantitative analysis, from level A9.4 anteriorly to A3.4 posteriorly (Pellegrino et al., 1979). PAR1 extends through the entire anteroposterior extent and occupies a broad area of the dorsolateral cortical wall at levels A9.4 and A8.4 (Fig. 13–1A). From levels A7.4 to A4.4, area PAR1 is situated dorsomedial to area PAR2 (Fig. 13–1B). Neurons were counted separately in layers VI, V, IV, III, and II in radial strips (Figs. 13–2 and 13–3) of PAR1 located at the dorsolateral angle of the cortex (drawings, Figs. 13–6 and 13–7), and in the central part of PAR2 (drawing, Fig. 13–5). The dashed lines in Figure 13–1 indicate the separation of layers for the cell counts. The

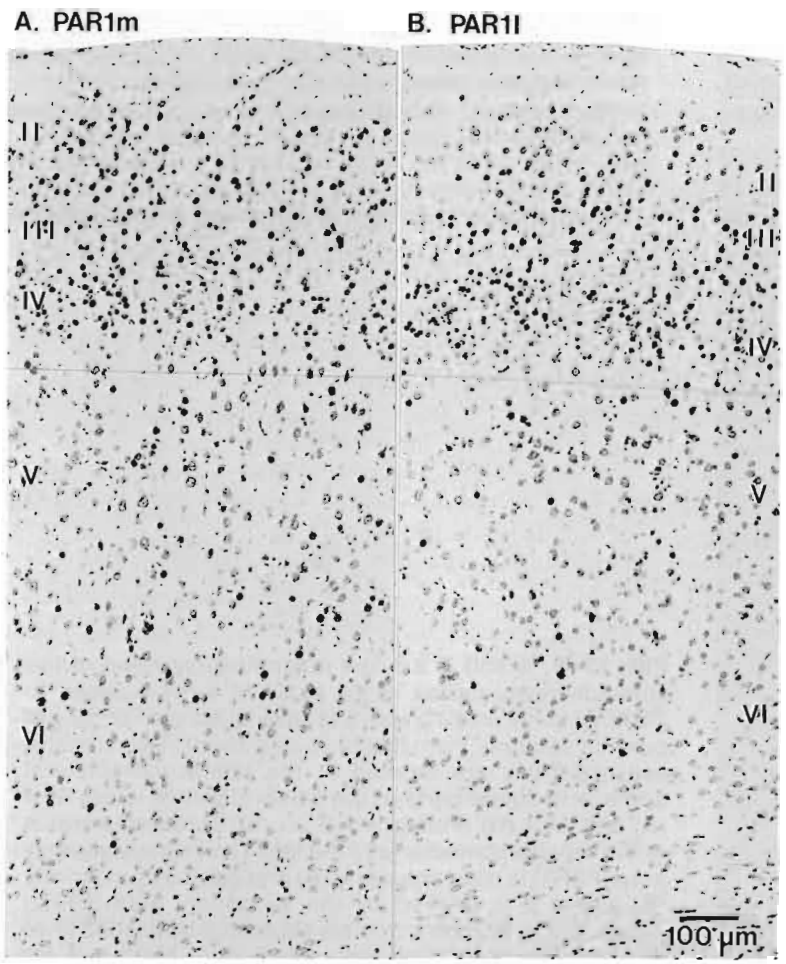

FIG. 13–2. Strips of the medial (**A,** PAR1m) and lateral (**B,** PAR1l) primary somatosensory cortex at level A8.4 (Pellegrino et al., 1979) from the same brain as shown in Fig. 13–1A. The placement of the vertical dashed lines in Fig. 13–1A shows where the higher magnification photographs were taken. Roman numerals at the sides of each photograph indicate the cortical layers (II–VI). There are generally more labeled neurons in the medial **(A)** than in the lateral **(B)** primary area. In both areas, neurons in layer VI are mostly unlabeled while more layer V neurons 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 ventrolateral to younger dorsomedial neurogenetic gradient between PAR1m and PAR1l. Note the dense accumulation of granule cells in layer IV in both strips.

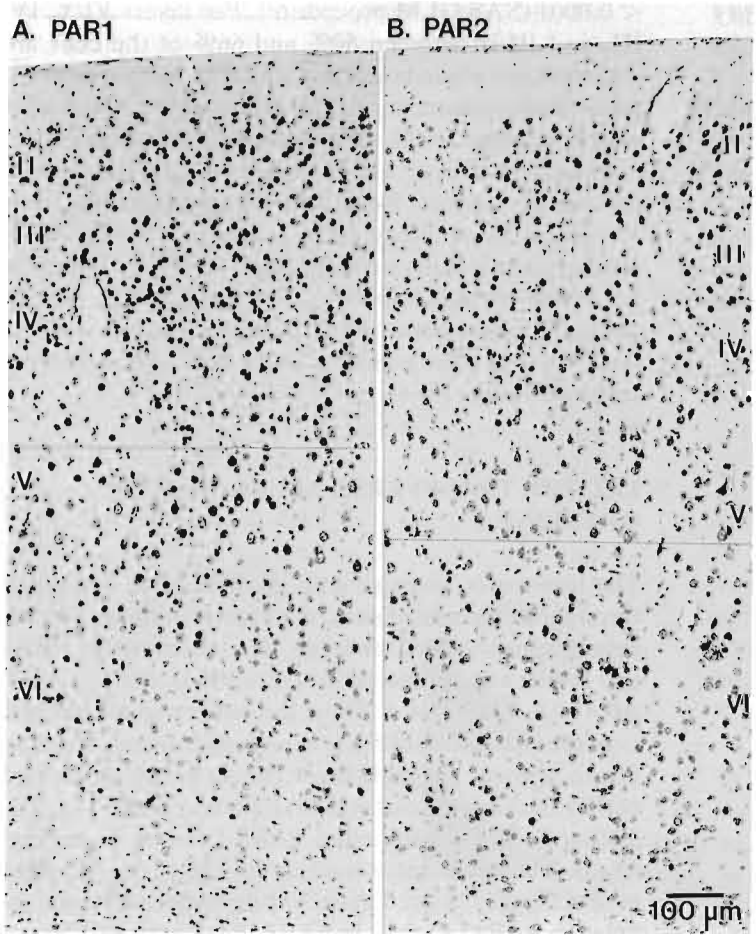

FIG. 13–3. Strips of the primary (**A,** PAR1) and secondary (**B,** PAR2) somatosensory cortex at level A4.4 from the same brain section as shown in Fig. 13–1B (vertical dashed lines indicate areas where photographs were taken). In both areas, the majority of superficial neurons throughout layers IV–II are labeled, while more neurons are labeled in layers VI and V 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. 13–2 (fewer neurons in layers VI and V are labeled) than in the posterior strips shown here, indicating an anterior (older) to posterior (younger) neurogenetic gradient. Note the sparse accumulation of layer IV granule cells in PAR2 **(B)** when compared to PAR1 **(A).**

photomicrographs in Figure 13–2 and 13–3 show the typical cytoarchitectonics of the somatosensory areas. Throughout, layers VI and V are wide and take up nearly two-thirds of the total depth. The layer VI pyramidal cells are typical of those in the visual and auditory areas, smaller and more closely packed than the layer V pyramidal cells. The difference between PAR1 and PAR2 is most clearly seen in layer IV, where densely packed granule cells are characteristic of PAR1 (Figs. 13–2 and 13–3A) but are more sparsely distributed in PAR2 (Fig. 13–3B). The lower border of layer III is easily delineated by the sharp drop in the occurrence of granule cells. Since layers II and III have medium- to small-sized pyramidal cells, they appear to be continuous in thin (6 μm) sections. Accordingly, the lower half (adjacent to IV) was considered layer III, while the upper half (adjacent to I) was considered layer II.

13.1 THE RADIAL NEUROGENETIC GRADIENT

The photomicrographs in Figs. 13–2 and 13–3, from the brain of a rat exposed to [^{3}H]thymidine on E17 and E18 and killed on P60, show the radial gradient between deep and superficial layers. Most of the neurons in layer VI and many in layer V are unlabeled, while the majority of the neurons in layers IV–II are labeled. The radial neurogenetic gradient was analyzed by combining the data of each layer (VI–II) from all the somatosensory areas (Fig. 13–4). Neurons in layer VI have the earliest peak (E15–E16), and 34% are generated earlier than neurons throughout layers V–II. Neurons in layer II have the latest peak (E19), and 39% are generated later than neurons throughout layers VI–III. The intervening layers have stepwise shifts in peaks of neurogenesis between the two extremes. The full magnitude of the radial gradient can be appreciated by comparing the proportion of neurons generated in each population before and after selected embryonic ages (E15, E17, and E19, *vertical dashed lines,* Fig. 13–4). On E17 for example, neurogenesis in layer VI is nearly completed (>90%), while neurogenesis in layer II has just begun (<10%). Even between adjacent layers, the neurogenetic gradients are substantial. The sign test indicated that all comparisons were significant ($P < 0.0001$); repeated measures analysis of variance F values ranged from 480.06 to 202.21, df = 1, all P

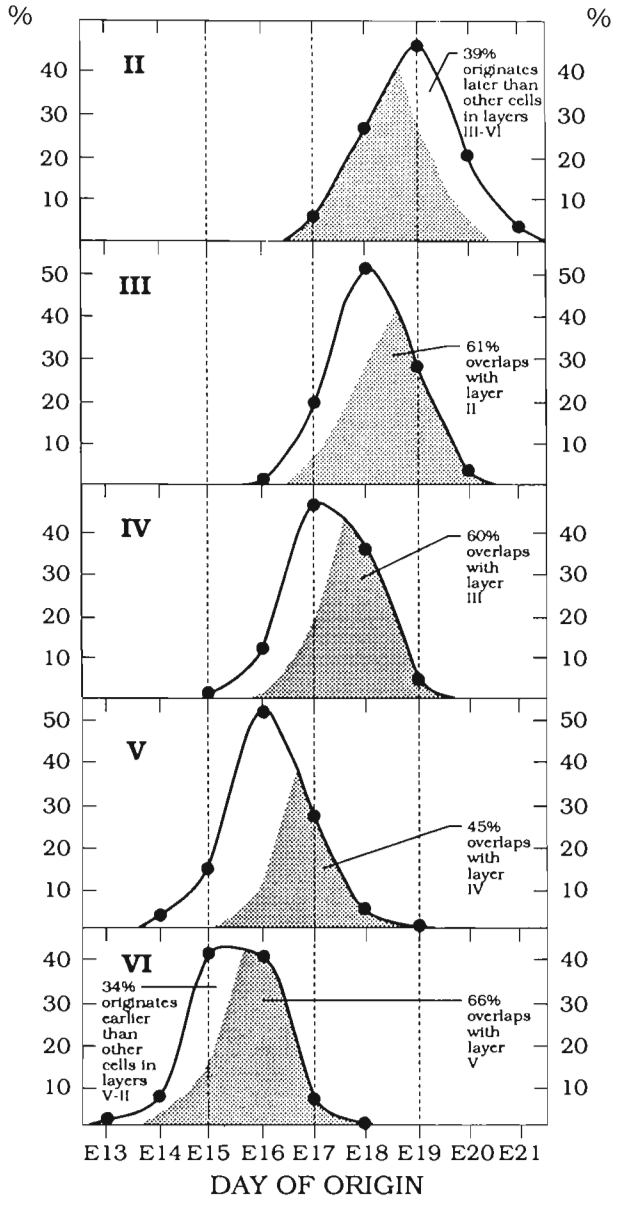

FIG. 13–4. The radial neurogenetic gradient in the somatosensory cortical areas of animals that survived to P60 after two consecutive exposures to [^{3}H]thymidine during embryonic life. Each graph represents the proportion of neurons generated from E13 to E21 in separate layers (II—top graph to VI—bottom graph) based on data combined from all areas of the somatosensory 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*). As one proceeds from layer VI to layer II the peaks of neurogenesis shift from early (E15–E16 in VI) to late (E19 in II). Stippled areas represent that portion of the population that is generated concurrently with neurons in the adjacent layer. Note that the least amount of concurrent generation occurs between layers V and IV (45% indicated in the graph second from the bottom).

< 0.0001 (SAS GLM procedure). For layers VI/V, IV/III, and III/II between 60% and 66% of the cells are cogenerated, while layers V/IV have a much lower proportion of cogeneration (45%). Throughout our studies of the sensory areas the density of the granule cell population in layer IV is positively correlated with greater divergence from layer V neurogenesis: it is lowest in the auditory areas (Chapter 12, Fig. 12–4) where granule cells are sparse in layer IV, higher in the visual areas (Chapter 11, Fig. 11–2) where granule cells are more concentrated, and highest in the somatosensory area where granule cells are most concentrated (Fig. 13–4).

13.2 THE TRANSVERSE NEUROGENETIC GRADIENT

The transverse neurogenetic gradients that are found throughout the entire neocortex are pronounced in the somatosensory cortical areas. Neurogenesis in PAR2 and PAR1 was compared in layers VI–II at levels A7.4, A6.4, A5.4, and A4.4. Since the pattern in all levels is similar, only the data for level A5.4 (*drawing,* Fig. 13–5) are shown. In each layer, neurons are generated earlier in the ventrolateral PAR2 (*solid lines,* Fig. 13–5) than in the dorsomedial PAR1 (*dashed lines,* Fig. 13–5). That gradient is evident in Figure 13–3, where proportionally more layer V pyramidal cells are labeled in PAR1 (A) than in PAR2 (B). Neurons in layers VI and II have peak generation times on different days in PAR2 and PAR1 (bottom and top graphs, Fig. 13–5). Even though the other layers have the same peak times of neurogenesis, more neurons are generated before the peak in PAR2 and after the peak in PAR1. Layer VI shows a 26% divergence in the proportion of neurons generated between ventrolateral and dorsomedial areas (60% on or before E15 in PAR2, only 34% during the same time in PAR1; $P < 0.037$, sign test; $F = 25.28$, df $= 1$, $P < 0.0001$, SAS GLM procedure). In layer V, 18% more neurons are generated on or before E15 in PAR2 than in PAR1 ($P < 0.002$, sign test; $F = 39.67$, df $= 1$, $P < 0.0001$, SAS GLM procedure). In layer IV, 22% more neurons are generated on or before E17 in PAR2 than in PAR1 ($P < 0.0001$, sign test; $F = 78.47$, df $= 1$, $P < 0.0001$, SAS GLM procedure). In layer III, 29% more neurons are generated on or before E17 in PAR2 than in PAR1 ($P < 0.0001$, sign test; $F = 68.68$, df $= 1$, $P < 0.0001$, SAS GLM procedure). In layer II, 32% more neurons are generated on or before E18 in PAR2 than in PAR1 ($P < 0.0001$, sign test; $F = 36.1$, df $= 1$, $P < 0.0001$, SAS GLM procedure). The bar graphs show the combined data for neurogenesis in all layers. Up to and including E15, PAR2 leads PAR1 in neurogenesis by 10% (19% versus 9%); nearly equal proportions of neu-

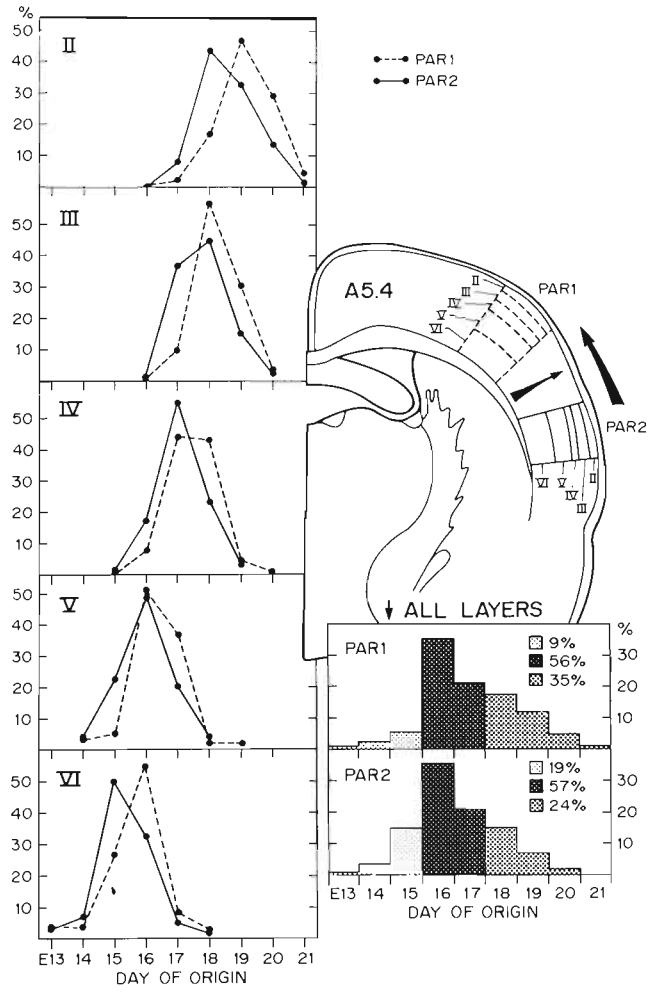

FIG. 13–5. A comparison between neurogenesis in the secondary somatosensory area (PAR2) and in the primary somatosensory area (PAR1) at level A5.4. Data are based on counts in the brains of P60 animals that were exposed to [^{3}H]thymidine on 2 consecutive days of embryonic life. Both line and bar graphs are the proportions of neurons originating during single embryonic days. Line graphs show data for individual layers, while bar graphs show data combined from cell counts in all layers. Besides the prominent deep (older) to superficial (younger) neurogenetic gradient between layers, PAR2 neurons (*solid lines*) originate slightly earlier than PAR1 neurons (*dashed lines*) within each layer.

rons are generated on E16 and E17 (57% and 56%, respectively); finally, PAR1 leads PAR2 by 11% on and after E18 (35% versus 24%).

Within PAR1, there is a ventrolateral-to-dorsomedial neurogenetic gradient, most easily seen in the widened anterior part. In the E17–E18 injection group, for example, more layer V neurons are labeled medially (Fig. 13–2A), indicating later times of origin, than laterally (Fig. 13–2B). Layer VI shows the same labeling pattern in the E16–E17 injection group, just as do the superficial layers in injection groups E18–E19 (layer IV), and E19–E20 (layers III–II).

13.3 THE LONGITUDINAL NEUROGENETIC GRADIENT

The global longitudinal neurogenetic gradient is found within all layers of the somatosensory cortex. For example, more labeled neurons are seen in layers VI and V of posterior PAR1 (Fig. 13–3A) than in anterior PAR1 (Fig. 13–2A). That gradient is illustrated by comparing anterior (A8.4) and posterior (A3.4) sections (Fig. 13–6). Approximately 55% of layer VI neu-

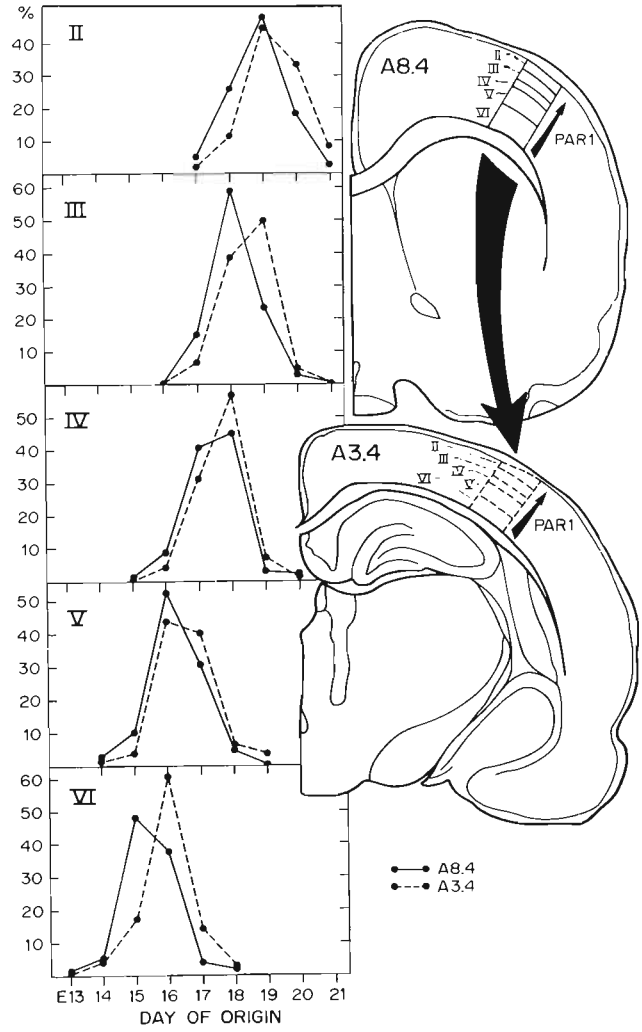

FIG. 13–6. The time of origin of neurons in layers II–VI in anterior (level A8.4) and posterior (level A3.4) parts of the primary somatosensory cortex (PAR1). Data are based on counts in the brains of P60 animals that were exposed to [^{3}H]thymidine on 2 consecutive days of embryonic life. The line graphs are the proportions 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 each layer, neurogenesis of anterior cells precedes neurogenesis of posterior cells (*large arrow* between sections).

rons are generated on or before E15 at level A8.4, while only 22% are generated during the same period at level A3.4 (bottom graphs, $P < 0.0001$, sign test; F = 66.95, df = 1, $P < 0.0001$, SAS GLM procedure), a divergence of 33% between the two sites. Neurons in layers V and IV have maximum divergences of 15% and 14% between the anterior and posterior sites ($P < 0.0001$ to $P < 0.003$, sign test; F = 52.31 to 17.24, df = 1, $P < 0.0001$, SAS GLM procedure). The divergence between the birth dates of neurons at anterior and posterior sites increases in layer III (29%; $P < 0.001$, sign test; F = 51.3, df = 1, $P < 0.0001$, SAS GLM procedure) and in layer II (21%; $P < 0.0001$, sign test; F = 101.61, df = 1, $P < 0.0001$, SAS GLM procedure).

To show the stepwise anteroposterior gradient between the analyzed levels, the cell counts for each layer (VI–II) in PAR1 were combined for each level so that the data represent birth dates of neurons in radial strips of cortex (drawings, Fig. 13–7). Since the sign test indicated that the cells at levels A7.4 through A5.4 were generated simultaneously ($P > 0.05$), these data were combined in the center graph (Fig. 13–7). Neurons in the strip of PAR1 at level A9.4 (top graph, Fig. 13–7) are generated earlier than those at A8.4 ($P < 0.0001$, sign test and SAS GLM procedure); those in A8.4 earlier than at A7.4-A5.4 ($P < 0.0001$, both tests); those in A7.4-A5.4 earlier than at A4.4 ($P < 0.0001$, both tests), and those in A4.4 earlier than at A3.4 ($P < 0.0001$, both tests). The graphs in Fig. 13–7 are divided into three periods of neurogenesis. During phase 1 (up to and including E15) when the deep neurons are generated, there is a progressive decrease in the accumulation of neurons from anterior (26%) to posterior (8%), a reflection of the strong anteroposterior gradient in the generation of layer VI neurons. Phase 2 (E16–E17) is an active period throughout all areas and the maximum difference between levels is only 7%. However, the cell type being generated varies with the level, more superficial neurons anteriorly, more deep neurons posteriorly. During Phase 3 (on and after E18), there is again a progressive shift in the amount of neurons generated, but it is now in the opposite direction since a lower proportion of neurons in the superficial layers are being generated anteriorly than posteriorly.

13.4 COMMENTS ON BARREL SPECIALIZATIONS AND NEUROGENETIC GRADIENTS

In our material, we could not analyze neurogenesis in individual barrels because these are seen only in preparations where the cortex is flattened before embedding

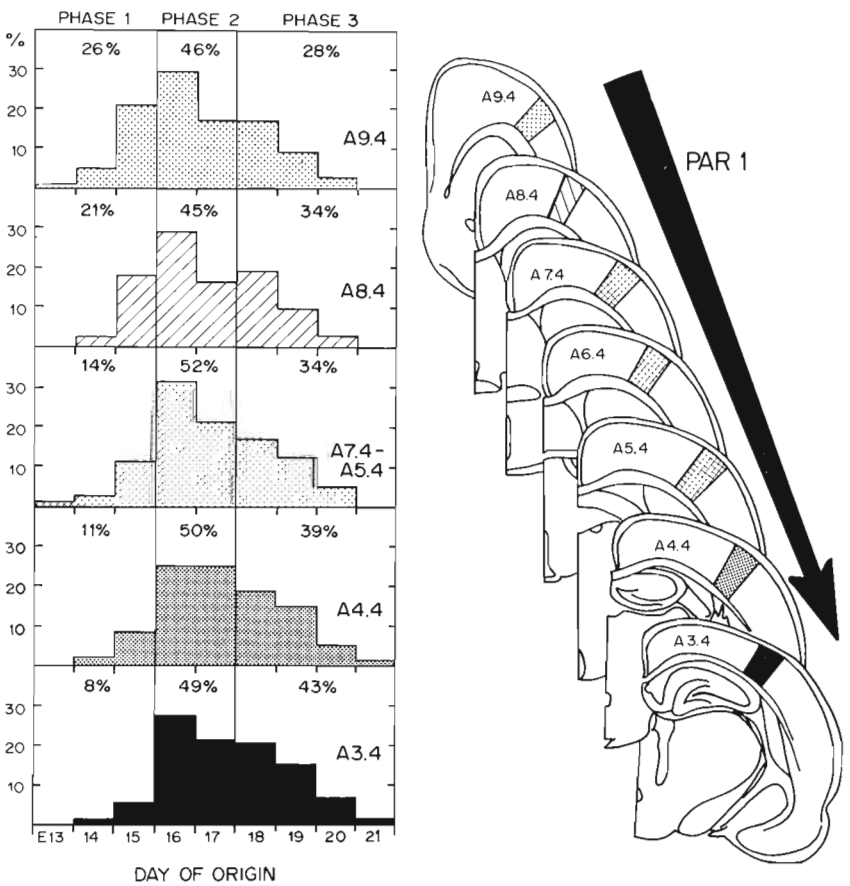

FIG. 13–7. An overview of neurogenetic gradients between levels A9.4 (most anterior) and A3.4 (most posterior) of the primary somatosensory cortex (PAR1). Data are based on counts in the brains of P60 animals that were exposed to [^{3}H]thymidine on two consecutive days during embryonic life. Bar graphs are the proportion of neurons originating during single embryonic days in cell counts combined for layers II-VI in a "strip" of cortex. The data for each level (or combined levels in the center graph) are grouped into three phases (1 is early, 3 is late); the percentages written into the graphs 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).

and is cut tangentially to the pial surface. However, the data presented earlier would predict that anterolateral barrels contain older neurons than posteromedial barrels. In this context, it is important to note that recent studies on the maturation of the rat somatosensory cortex indicate general transverse and longitudinal gradients since the anterolateral face area develops earlier than the posteromedial barrel subfield (McCandlish et al., 1989; Zhang and Cooper, 1990). However, it is possible that neurogenetic diversity may exist between cytoarchitectonic specializations because there is a pocket of early maturation in the center of the anterolateral face area rather than at its most anterolateral edge (Cooper, personal communication).

13.5 CORRELATION BETWEEN NEUROGENETIC GRADIENTS AND THALAMOCORTICAL PROJECTIONS

The somatotopic relay of information from the thalamus to the cortex ultimately depends on topographically organized input to the thalamus via relays in the brainstem from the spinal nerves (dorsal column nuclei) and cranial nerve V (trigeminal nuclei). We will concentrate on trigeminal input here; Chapter 14 will provide details of the spinal input. The pattern of development has been examined in the entire trigeminal afferent pathway in rodents (reviewed in Killackey, 1985). The sequence of development of the vibrissae on the face (rows A–E) proceeds from ocular (first to form in each row) to nasal (last to form), correlating with a strict spatiotemporal segregation of trigeminal nerve afferents to the brainstem sensory trigeminal nuclei (Arvidsson, 1982; Erzurumlu and Killackey, 1983). There are three sets of barrel representations for the vibrissae in the brainstem, one in the principal sensory nucleus of the trigeminal, another in the subnucleus interpolaris, and a third in the subnucleus caudalis (Belford and Killackey, 1979; Arvidsson, 1982; Erzurumlu and Killackey, 1983; Tracey, 1985). Each of these areas sends a topographic projection to the medial part of the ventrobasal complex (VBm), also called the *arcuate subdivision,* in the contralateral thalamus (Smith, 1973; Fukushima and Kerr, 1979; Feldman and Kruger, 1980; Peschanski, 1984; Huang, 1989). There is another somatotopic map of the whisker pad in VBm (van der Loos, 1976; Belford and Killackey, 1978, 1979; Woolsey et al., 1979; Land and Simons, 1985).

From a developmental perspective, the projection from the brainstem is organized so that input relayed from the first-forming whiskers (#1 in rows A–E) terminate in areas occupied by older neurons in dorsolateral parts of VBm, whereas input relayed from the last-forming whiskers (the highest number in each row) terminate in medially adjacent areas that are occupied by younger VBm neurons (Fig. 13–8; Waite, 1977; van der Loos, 1976; Woolsey et al., 1979; Verley and Onnen, 1981; Altman and Bayer, 1989a). There is no neurogenetic gradient in VBm along the anterior posterior plane (Altman and Bayer, 1989a), thus the information relayed from row E to posterior parts of VBm and that relayed from row A to anterior parts of VBm (Waite, 1973) contact neurons of the same age. Neurons in the VBm project heavily to the hollows of the barrel subfield in layer IV of PAR1 (Killackey, 1973; Killackey and Leshin, 1975; Saporta and Kruger, 1977; Jensen and Killackey, 1987; Chmielowska et al., 1989). Within PAR1, two-thirds of the total area con-

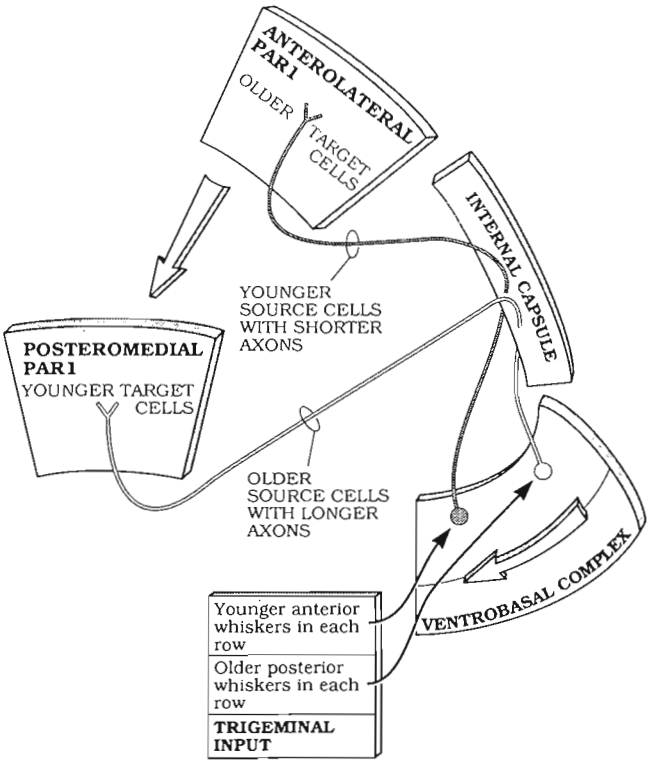

FIG. 13–8. A diagrammatic representation of the neurogenetic gradients and anatomical connections between the medial part of the ventrobasal complex (the arcuate subdivision) and the primary somatosensory cortex (PAR1). The large arrows point to areas of younger neurons in each structure. There is an 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 brainstem trigeminal nuclei that relay information from the older posterior whiskers in each row of vibrissae. These neurons have longer axons (*lightly stippled*) that terminate on younger target neurons in the posteromedial somatosensory cortex. The converse is true for the younger source neurons in the medial ventrobasal complex. They receive input from brainstem trigeminal nuclei that relay information from the younger anterior whiskers in each row of vibrissae and project via shorter axons (*darker stipple*) to older target neurons in the anterolateral somatosensory cortex.

tains the somatotopic map of the head and half of that is occupied by the posteromedial barrel subfield representing the vibrissae (Dawson and Killackey, 1987). In the cortical map, the whiskers closer to the eye in each row on the face are positioned posterior and medial, while those closer to the nose are anterior and lateral. The anterior/posterior topography (dorsal to ventral rows of vibrissae) is maintained between thalamus and cortex while the medial/lateral topography (ocular to nasal position in each row) is reversed (van der Loos, 1976; Woolsey et al., 1979; Land and Simons, 1985; Bernardo and Woolsey, 1987).

The neurogenetic gradients in PAR1 (Figs. 13–2, 13–6, and 13–7) and in VBm (Altman and Bayer, 1989a) match up with the anatomical connections in the following way. Older thalamic source neurons in lateral parts of VBm project to younger cortical target neurons in posteromedial parts of PAR1 (lightly stippled axons, Fig. 13–8), while younger thalamic source neurons in medial parts of VBm project to older cortical target neurons in anterolateral parts of PAR1 (darkly stippled axons, Fig. 13–8). As in the visual (Chapter 11) and auditory (Chapter 12) thalamocortical connections, there is an exact reversal of the neurogenetic gradients between source cells and target cells. That reversal bears a constant relationship to the relative axonal lengths of the specific thalamic afferents. VBm axons enter the ventrolateral cortex through the internal capsule, then course dorsomedially and somewhat posteriorly to reach their respective targets (Caviness and Frost, 1980; Frost and Caviness, 1980; Bernardo and Woolsey, 1987). Older target neurons in lateral cortical areas are contacted by shorter thalamic axons from younger neurons, while younger target neurons in medial cortical areas are contacted by longer thalamic axons from older neurons (diagrammed in Fig. 13–8).

We entertain the possibility that the chronological reversal between neurogenetic gradients and anatomical connections is related to the lateral migration of neurons that settle in the somatosensory cortex (Chapter 9). Layer IV neurons that settle in medial parts of the somatosensory cortex arrive in the cortical plate approximately one day earlier than those that settle in lateral parts. Just as in the visual system, thalamocortical fibers arrive early during neocortical development and remain in the subplate before growing radially to form terminals in layer IV (Wise and Jones, 1978; Friauf et al., 1990). Dawson and Killackey (1987) and Darian-Smith et al. (1990) have shown that the waiting thalamic axons already have the topographic arrangement found in adults; that is, lateral thalamic axons contact medial cortical targets and vice versa. Recent evidence in rats indicates that thalamocortical fibers arrive in the intermediate zone and subplate when the layer IV neurons are in the intermediate zone (E19–E20; Catalano and Killackey, 1990; Molnár and Blakemore, 1990; Erzurumlu and Jhaveri, 1990), and the layer IV neurons migrate through them before reaching their appropriate settling sites in the cortical plate. Perhaps the early arrival of specific thalamic relay axons in the cortex has an influence on where migrating neurons will enter the cortical plate, a topic that will be discussed in Chapter 16.