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

CHAPTER 8

Quantitative Studies of the Nuclear Area and Orientation in the Developing Neocortex

A prominent feature of the developing cortex is the vertical orientation of cells in the neuroepithelium and the cortical plate. In Nissl-stained material the cell nuclei in these layers are spindle-shaped (VZ, CP, Figs. 8–1 and 8–2). Golgi as well as electron microscopic studies have established that these cells have radial processes emanating from vertically oriented cell bodies (Hinds and Ruffet, 1971; Stensaas, 1967a, 1967b, 1967c, 1967d). In contrast, Golgi and electron microscopy have established that many cells in the subventricular and intermediate zones have horizontally oriented processes and cell bodies (Stensaas, 1967a, 1967b, 1967d; Shoukimas and Hinds, 1978; Valverde et al., 1989). However, when we compared sagittal and coronal sections of the dorsomedial neocortex, we observed systematic differences in the appearance of the cells in the subventricular and intermediate zones that depended on the plane of the section. In the coronal plane, the cells were larger and appeared to be horizontally oriented (SV and IZ, Fig. 8–1). In the sagittal plane, the cells were smaller and appeared to be vertically oriented (SV and IZ, Fig. 8–2). That difference in cell orientation has not been previously described in the developing neocortex. Consequently, a computer-aided analysis of the area and orientation of cortical cells in the sagittal versus the coronal planes was undertaken, along with a quantitative analysis of differences in cell packing density. Some of these data are also presented in a journal article (Bayer et al., 1991a). We used methacrylate-embedded normal rat embryonic brains prepared as described in Appendix 1. The ventricular zone, subventricular zone, intermediate zone, upper intermediate zone, subplate, and cortical plate were analyzed according to the quantitative procedures described in Appendix 5.

Because of the poor delineation of the perikaryon in Nissl-stained material, we used the shape of the nucleus (fusiform to round) and its orientation (horizontal, oblique, or vertical) to indicate the orientation of the cell body. That is justified by the observations made with electron microscopy of the developing neocortex (Stensaas and Stensaas, 1968; Hinds and Ruffet, 1971) and the developing cerebellum (Altman, 1972, 1975a) that show that the cell bodies of germinal cells and young neurons contain a prominent nucleus and a thin perikaryal rim of cytoplasm. For example, in the developing cerebellum (Altman, 1972, 1975a), the nuclei of young granule cells are horizontally oriented beneath the external germinal layer when their axons grow horizontally from either end of the cell body to form a parallel fiber (see Fig. 12, Altman, 1972). Later when the cells sprout a leading process directed toward

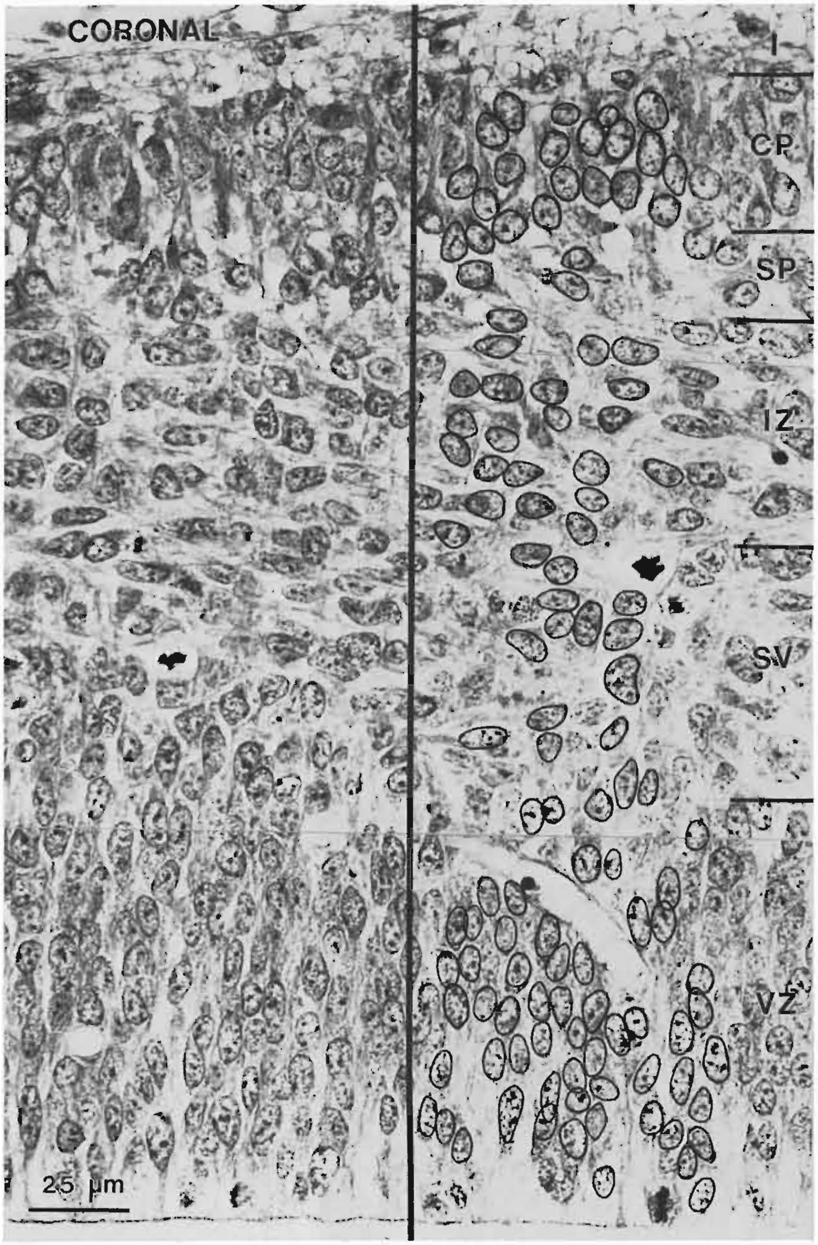

FIG. 8–1. A strip of coronally sectioned (3 μm, methacrylate, toluidine blue stain) dorsomedial neocortex in an E17 rat embryonic brain extending perpendicularly through the ventricular zone (VZ), subventricular zone (SV), intermediate zone (IZ), the indistinct subplate (SP), and a thin cortical plate (CP). Beneath the pial surface, there is a cell sparse layer I (I). The heavy line indicates the vertical meridian oriented perpendicularly to the pial (*top*) and ventricular (*bottom*) surfaces. The outlined profiles demarcate some of the cell nuclei in each layer.

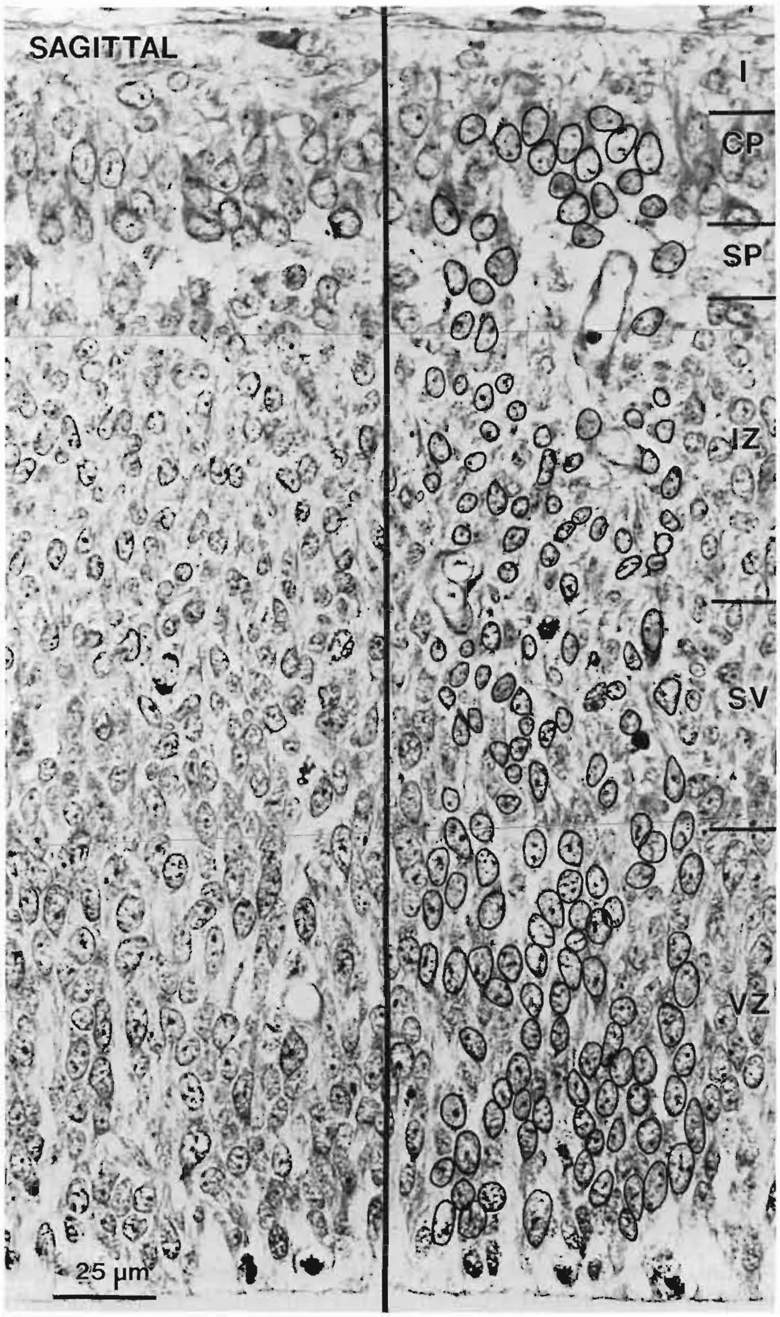

FIG. 8–2. The same as in Fig. 8–1 in the sagittally sectioned dorsomedial neocortex in an E17 rat embryonic brain.

the granular layer, the nuclei become vertically oriented (see Figs. 18 and 20, Altman, 1972). In the developing neocortex, vertical nuclear orientation has also been used as supporting evidence for radial cell migration (see Figs. 1–6 and 1–7; Berry et al., 1964; Morest, 1970; Rakic, 1972). With the exception of the early maturing Cajal-Retzius and subplate cells, Golgi studies of the developing neocortex indicate that the primitive cells in the ventricular zone, those migrating in the intermediate zone, and those that have just settled in the cortical plate have small perikarya oriented to reflect the directional growth of their few processes (usually only two) emanating from either end of the cell body (see Fig. 1–8; Stensaas, 1967a, 1967b, 1967c, 1967d). Accordingly, we consider here the orientation of the nuclei in primitive young neurons to indicate the overall orientation of the cell body.

8.1 CELL PACKING DENSITY

Cell packing density was measured in order to provide an overview of the developmental changes in the space taken up by the growing cell body (the visible nucleus and perikaryon) and the growing cell processes and incoming axons. Each layer shows a different pattern

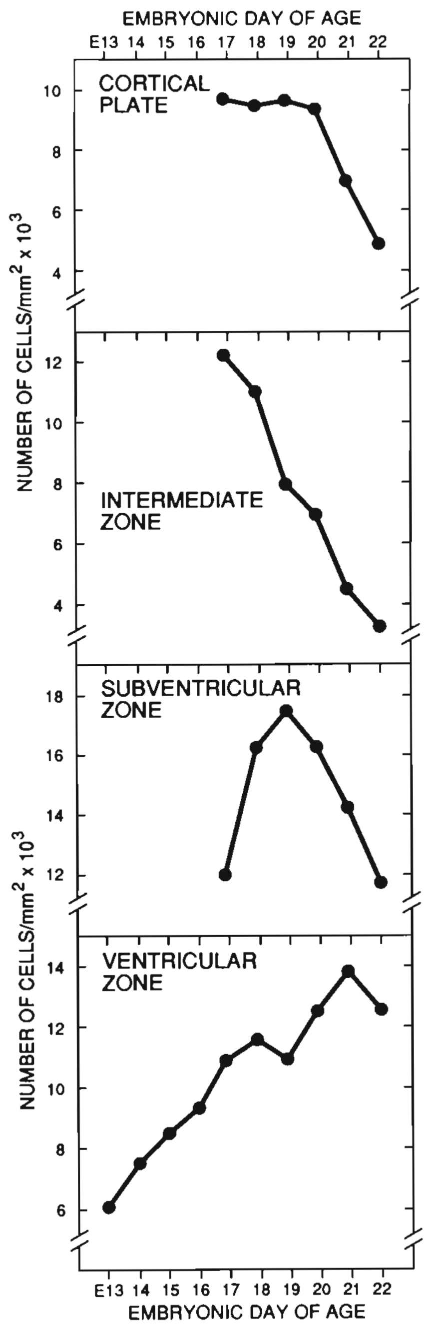

FIG. 8–3. Cell packing density in various layers of the developing neocortex between E13 to E22 (ventricular zone) and between E17 and E22 (subventricular and intermediate zones, cortical plate). Cells were counted in unit areas of the sagittally sectioned dorsomedial neocortex, and the number of cells per square millimeter (Y axis) were computed from the raw data.

of change during the observed time period. These can be related to the changes observed in the nuclear area that will be described later.

In the neuroepithelium or ventricular zone (*bottom graph,* Fig. 8–3) packing density increases from approximately 6,000 cells/mm^2 to nearly 14,000 cells/mm^2 on E21. The increase is steady between E13 and E17, reaches a plateau between E17 and E19, then there is another spurt on E20. The one-way analysis of variance indicated significant change with increasing age (F = 21.91; df = 9, 32; $P < 0.0001$). Tests on the means indicated that the age groups between E13 and E16 have significantly lower packing densities than the age groups between E20 and E22.

In the subventricular zone (second graph from bottom, Fig. 8–3), packing density ranges between 12,000–18,000 cells/mm^2. There is a symmetrical rise (E17–E18) and fall (E20–E22) around peak packing density on E19. The one-way analysis of variance indicated highly significant age changes (F = 348.37; df 9, 32; $P < 0.0001$). Tests on the means indicated that the E18–E20 age groups have significantly more densely packed cells than either the E17 or the E22 age groups.

Packing density in the intermediate zone (*second graph from the top,* Fig. 8–3) continually decreases from a high of approximately 12,000 cells/mm^2 on E17 to a low value of less than 4,000 cells/mm^2 on E22. (For these data, the upper intermediate zone was not analyzed separately.) Again, the one-way analysis of variance indicated highly significant age changes (F = 301.75; df = 9, 32; $P < 0.0001$). Tests on the means showed that age groups E17–E18 are highest in cell packing density, groups E19–E20 are intermediate, and groups E21–E22 are lowest.

A yet different pattern of age-related change is seen in the cortical plate (*top graph,* Fig. 8–3). The packing density remains constant from E17 to E20, then drops rapidly thereafter. The one-way analysis of variance indicated significant age changes (F = 248.97; df = 9, 32; $P < 0.0001$); tests on the means showed no differences between E17 and E20, a significant drop in packing density on E21, and another significant drop on E22.

8.2 NUCLEAR AREA AND ORIENTATION IN CELL BODIES OF THE VENTRICULAR ZONE, SUBPLATE, AND CORTICAL PLATE

When coronal sections and sagittal sections (Figs. 8–1 and 8–2) are examined together, there are no obvious differences in the area of cell nuclei in the ventricular zone, the subplate, or the cortical plate. In both planes, the cell nuclei are predominantly vertically oriented in the cortical plate and the ventricular zone and are variably oriented in the subplate. Statistical tests using a two-way analysis of variance (SAS GLM procedure) confirmed the qualitative observations that nuclear areas and orientations are similar in the two planes;

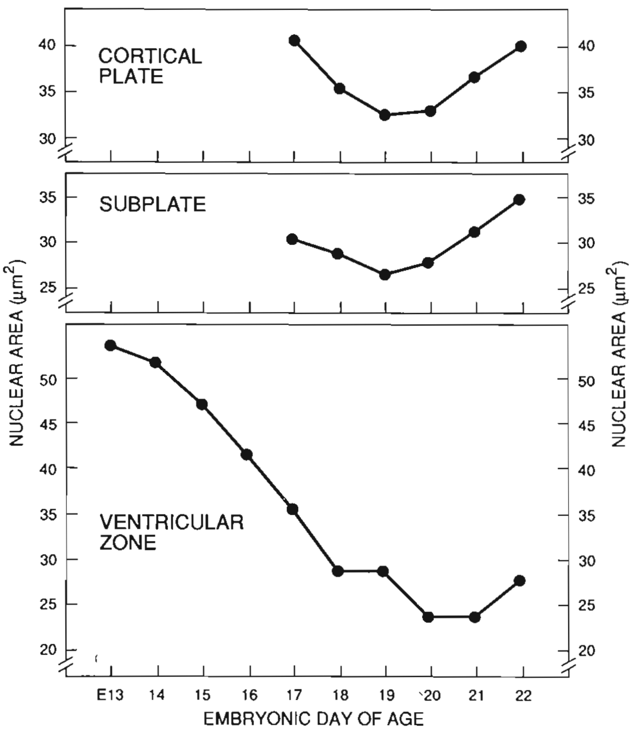

FIG. 8–4. The nuclear area in the ventricular zone from E13 to E22, and in the subplate and cortical plate from E17 to E22 in computer-determined measurements of sections (3 μm, methacrylate) of the dorsomedial neocortex. Data were combined for both sagittal and coronal planes, since there were no statistical differences between the two.

consequently, the data were combined (Figs. 8–4 and 8–5). A one-way analysis of variance (SAS GLM procedure) was used to analyze age changes in the combined data for each layer. Tukey's Studentized range and the Ryan-Einot-Gabrial-Welsch Multiple F test (REGWF) were used to test for differences between means at an overall alpha level of 0.05.

8.2.1 The Ventricular Zone

In the neuroepithelium, the nuclear area decreases steadily from a high of 54 μm^2 on E13 to 34 μm^2 on E18 and E19 (*bottom graph,* Fig. 8–4). The nuclear area drops to 24 μm^2 on E20 and E21, then increases to 28 μm^2 on E22. The analysis of variance indicated significant age effects ($F = 60.24$; df = 8, 26; $P < 0.0001$). Tests on the means indicated that age groups E13–E15 have significantly larger nuclei than those on E16; there is another significant drop in the nuclear area between E16 and E17; but the areas do not significantly change between E18 and E22. These changes are illustrated in Fig. 8–6. On E13 (Fig. 8–6A), when the ventricular zone is the sole component of the neocortical primordium, the cells have large nuclei (outlined) suspended in thick cytoplasmic cylinders; at this stage of development packing density is low (Fig. 8–3). By E20 (Fig. 8–6B), the ventricular zone cells contain smaller nuclei (outlined) suspended in thinner cytoplasmic cylinders, and packing density is high (Fig. 8–3).

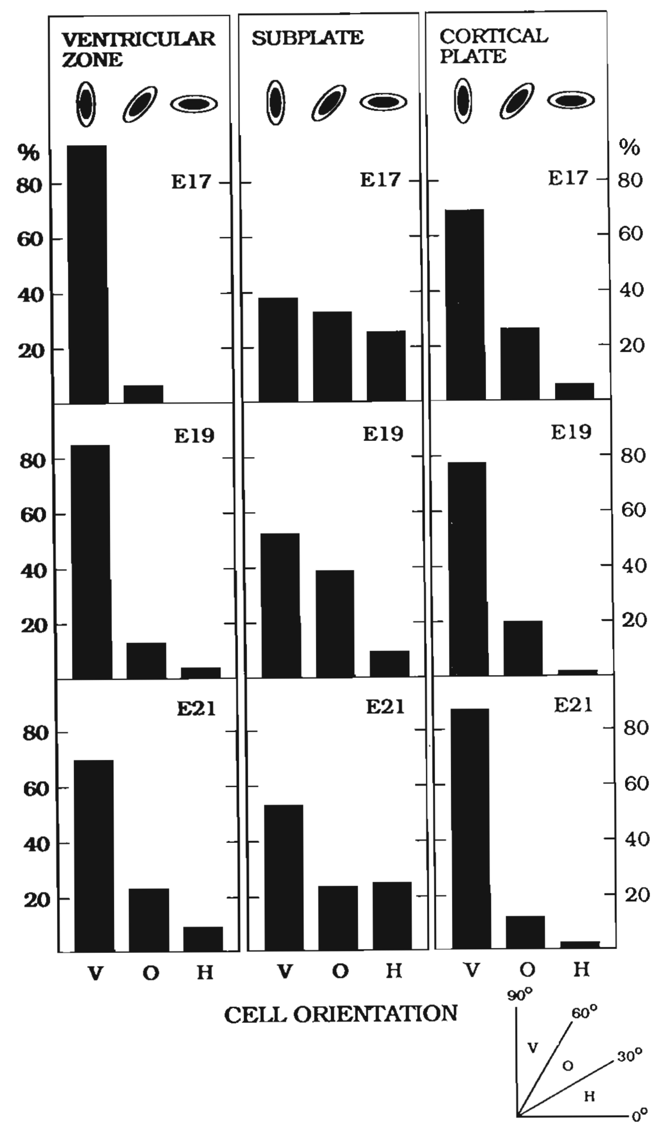

FIG. 8–5. Nuclear orientation in the ventricular zone, subplate, and cortical plate on E17, E19, and E21. The data are based on computer-determined measurements of sections (3 μm, methacrylate) of the dorsomedial neocortex. Data were combined for both sagittal and coronal planes, since there were no statistical differences between the two. Histograms indicate the proportion of cells with vertical nuclei (V, *left bars*), oblique nuclei (0, *center bars*), and horizontal nuclei (H, *right bars*). The ovals shown in the E17 graphs represent the orientation of the cell body, while the line drawing in the lower right shows the limits for each group in degrees of rotation above the horizontal axis (0°): horizontal group from 0° to 29.9°, oblique group from 30° to 59.5°, vertical group from 60° to 90°.

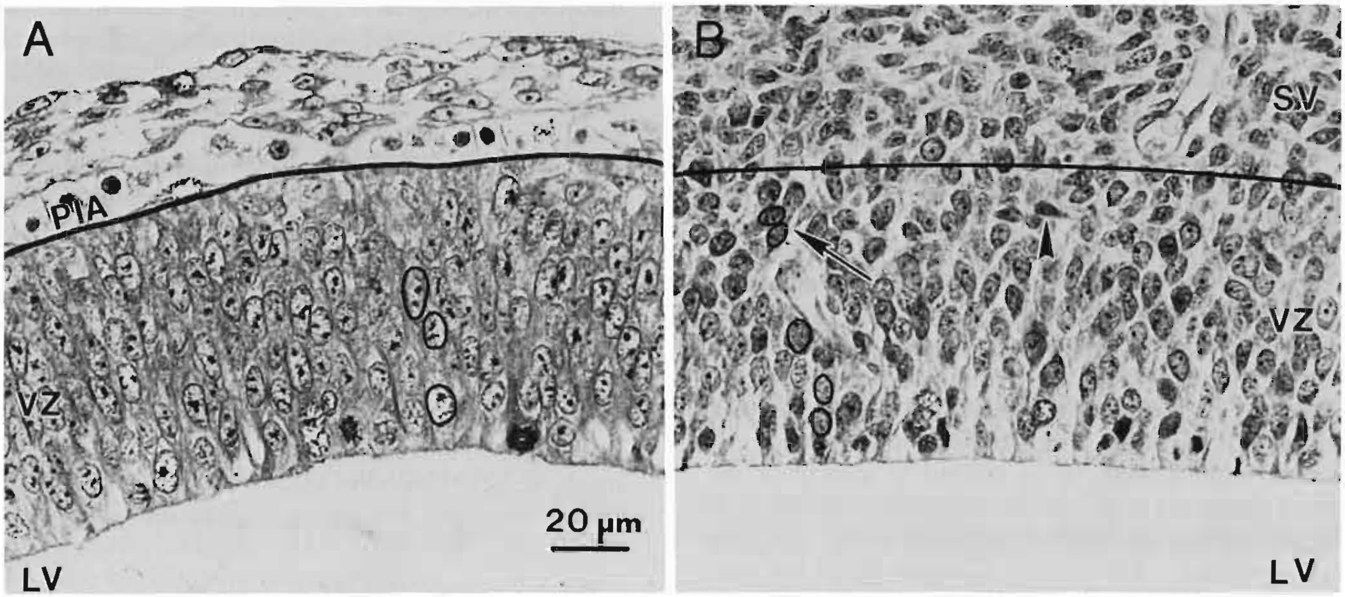

FIG. 8–6. The ventricular zone (VZ) in the coronally sectioned developing neocortex of rat embryos on E13 **(A)** and E20 **(B)** (3 μm, methacrylate, toluidine blue stain). On E13, the VZ is the sole component of the cerebral wall reaching to the pial surface (*solid line*). The VZ cells contain large nuclei that are strictly vertically oriented (*outlined*). On E20, the VZ has reduced in depth and is bordered superficially by the subventricular zone (SV). The VZ cells have smaller nuclei (*outlined*), more are packed into the same space, and some have rotated to oblique (*arrow*) and horizontal (*arrowhead*) orientations.

Orientation of the cell bodies is predominantly vertical in the ventricular zone at all ages (*left column of graphs,* Fig. 8–5). But there is a significant shift with increasing age from more (over 90% on E17) to fewer (approximately 70% on E21) vertically oriented cell bodies ($F = 11.87$; $df = 5, 18$; $P < 0.0001$). The orientation shift can be seen by comparing Figs. 8–6A and 8–6B; on E13, nearly all cell bodies have vertically oriented nuclei (*outlined profiles* in A), but, by E20, some cell bodies have oblique nuclei (*arrow* in B) or horizontal nuclei (*arrowhead* in B).

Comments

The changes in cell packing density, nuclear area, and nuclear orientation in the ventricular zone can be related to the neuroepithelial transformations that occur during the generation of cortical neurons (Chapter 4). Maximal neurogenesis occurs from E14 through E17, as cells in the primordial plexiform layer (future layer I and the subplate) and the large number of deep cells in layers VI and V are generated throughout all areas of the cortex (Chapter 3). During this period, most of the cells in the ventricular zone are probably generating neurons. These neuronal precursors have low packing density (Fig. 8–3), and the cell bodies have large nuclei (Figs. 8–4 and 8–6A) that are vertically oriented (Fig. 8–5). Between E18 and E21, progressively fewer neurons and more glia are produced each day. During that time, cells in the ventricular zone become more densely packed (Fig. 8–3), the cell bodies have small nuclei (Figs. 8–4 and 8–6B), and more cells are obliquely and horizontally oriented (Figs. 8–5 and 8–6B).

8.2.2 The Subplate and the Cortical Plate

Subplate

There are slight but significant increases in the nuclear area from the lowest value on E19 (26.5 μm^2) to the highest value on E22 (35 μm^2) (center graph, Fig. 8–4; one-way analysis of variance, $F = 3.24$, $df = 5, 18$, $P = 0.0294$). The cell bodies have nuclei with a wide range of orientations at all age groups in the subplate (middle column of graphs, Fig. 8–5); the one-way analysis of variance indicated no significant change with age.

Cortical Plate

The nuclear area (*top graph,* Fig. 8–4), is significantly higher on E17 and E22 (approximately 40.5 μm^2) than it is on E19 and E20 (between 32–33 μm^2; one-way analysis of variance, $F = 8.04$; $df = 5, 18$, $P = 0.004$). The right column of graphs in Fig. 8–5 shows that most cell bodies in the cortical plate have vertically oriented nuclei at all age groups with no significant changes over time. But there is a trend from fewer vertically oriented cell bodies on E17 (less than 70%) to more by E21 (close to 90%).

Comment

The predominant vertical orientation of cells in the cortical plate and variable orientation of cells in the subplate have been observed in Golgi studies by Cajal (1911), Stensaas (1967c), and Marin-Padilla (1971). The radial orientation of cells in the cortical plate has been postulated by these investigators, most recently by Marin-Padilla (1988), to be related to growth of the apical dendrites that first become anchored close to the pial surface and grow lengthwise as the cell body is "suspended" from above (Chapter 6). In the subplate, the dendrites grow into a horizontal fiber plexus, and the cell body takes on a polymorphic shape.

The mean nuclear areas in the cortical plate and subplate, show "dips" in the center of the observation period (Fig. 8–4). In the subplate, the decrease could be related to the presence of smaller cells migrating through toward the cortical plate. In the cortical plate, the larger mean area on E17 may be related to the hypothesis that the first occupants of the cortical plate are actually subplate cells (Chapter 5). Subplate cells begin to differentiate early (Meller et al., 1969; Molliver and van der Loos, 1970; Molliver et al., 1973; König et al., 1975; Kristt and Molliver, 1976; Rickmann et al., 1977; König and Marty, 1981; Zheng et al., 1990), an event associated with an increased cell size. The average cell area drops in the cortical plate after E17 as smaller, more immature cells move in and the larger subplate cells move out. The increasing cell area (Fig. 8–4) and the sharply decreasing packing density (Fig. 8–3) in the cortical plate on E21 and E22 can be related to the onset of growth associated with cell differentiation of layers VI–II neurons that will be completed after birth.

8.3 PLANAR DIFFERENCES IN THE AREA AND ORIENTATION OF CELL NUCLEI IN THE SUBVENTRICULAR AND INTERMEDIATE ZONES

In E17 embryos, a qualitative examination of the subventricular and intermediate zones shows that nuclei with larger profiles predominate in the coronal plane (SV and IZ, Fig. 8–1), and those with smaller profiles predominate in the sagittal plane (SV and IZ, Fig. 8–2). The cell bodies also show definite orientation differences between the two planes; most cells have horizontally oriented nuclei in coronal sections (Fig. 8–1), but these same cells appear to have vertically oriented nuclei in sagittal sections (Fig. 8–2). Both the area and orientation data for the subventricular, intermediate, and upper intermediate zones were shown to have significant planar differences using a two-way analysis of variance [SAS, ANOVA (Analysis of Variance Procedure)], and therefore data for each of these layers is shown separately (Figs. 8–7 and 8–8).

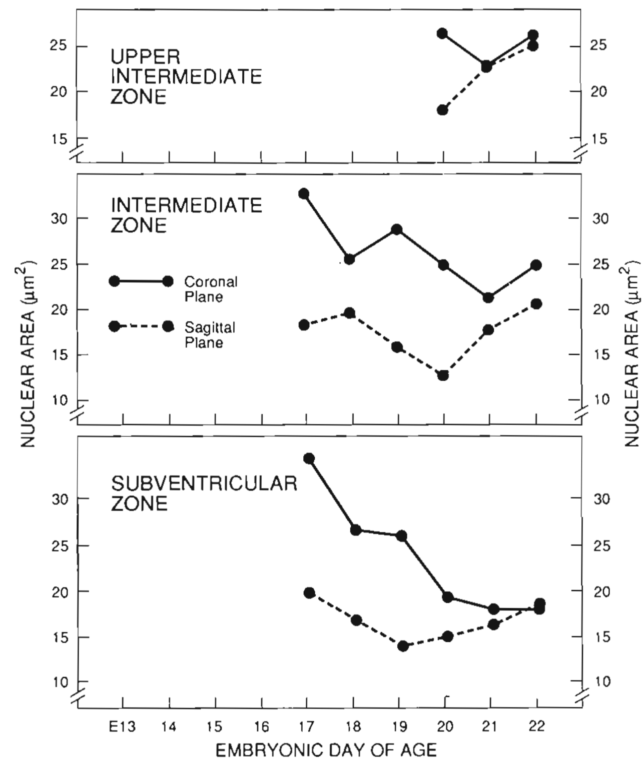

FIG. 8–7. Computer-determined measurements of the nuclear area in the subventricular, intermediate, and upper intermediate zones of the embryonic dorsomedial neocortex sectioned (3 μm, methacrylate) in the coronal (*solid lines*) and sagittal (*dashed lines*) planes from E17 to E22. In each layer, nuclear area is larger in the coronal plane than it is in the sagittal plane either during the entire period of observation (intermediate zone), or initially (E17–E19, subventricular zone; E20, upper intermediate zone).

8.3.1 Subventricular Zone

Nuclear area (*bottom graph*, Fig. 8–7), ranges between 34 μm^2 (coronal plane, E17) to 14 μm^2 (sagittal plane, E19). The two-way analysis of variance indicated a significant plane effect ($F = 47.99$; df = 1, 12; $P < 0.0001$), best shown from E17 through E19, when nuclei in the coronal plane are considerably larger than they are in the sagittal plane. There is also a significant plane by age interaction ($F = 5.65$; df = 5, 12; $P < 0.0066$) indicated by the continual drop in the nuclear area in the coronal plane from the high value on E17; by E22, the means of both planes are nearly the same (approximately 18 μm^2). Statistical tests on the nuclear orientation data (left column of graphs, Fig. 8–8) indicated a highly significant plane effect ($F = 129.49$; df = 1, 12; $P < 0.0001$). That is best seen on E17 when close to 60% of the cells in the coronal plane have horizontally oriented nuclei, while nearly 80% of the cells in the sagittal plane have vertically oriented nuclei. There is also a significant plane by age interaction ($F = 11.04$; df = 5, 12; $P = 0.0004$) best shown by the dramatic decrease in the proportion of cells in the sagittal plane with vertically oriented nuclei. It drops to less than 40% on E21. In contrast, the coronal plane always shows a high proportion (between 50–60%) of cells with horizontally oriented nuclei.

8.3.2 Intermediate Zone

The nuclear area in the intermediate zone is consistently larger in the coronal plane than it is in the sagittal plane (*center graph,* Fig. 8–7; two-way analysis of variance, $F = 63.6$; df = 1, 12; $P < 0.0001$). For example, mean nuclear areas range from 32.5 μm^2 (E17) to 21 μm^2 (E21) in the coronal plane but only from 20 μm^2 (E22) to 12.5 μm^2 (E20) in the sagittal plane. With regard to orientation (*center column of graphs,* Fig. 8–8), between 75–90% of the cells in the coronal plane have either horizontal or oblique nuclei, while cells with vertical nuclei predominate (65–80%) in the sagittal plane (two-way analysis of variance, $F = 168.1$; df = 1, 12; $P < 0.0001$).

8.3.3 Upper Intermediate Zone

The upper part of the intermediate zone was quantified separately from E20 on because, in contrast to the

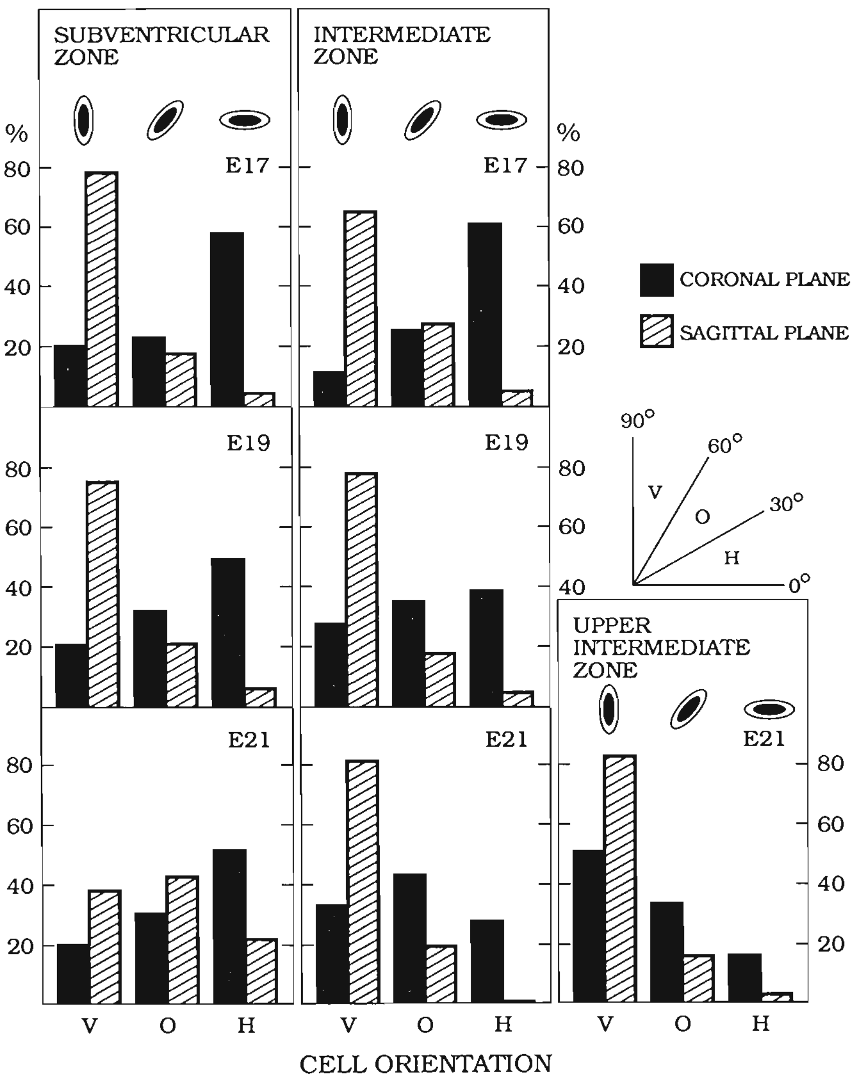

FIG. 8–8. Nuclear orientation in the subventricular and intermediate zones on E17, E19, and E21 and in the upper intermediate zone on E21. The data are based on computer-determined measurements of sections (3 μm, methacrylate) of the dorsomedial neocortex in the coronal plane (*black bars*) or the sagittal plane (*striped bars*). Histograms indicate the proportion of cells with vertical nuclei (V, *left bars*), oblique nuclei (O, *center bars*), and horizontal nuclei (H, *right bars*). The ovals shown in the top graphs represent the orientation of the cell body, while the line drawing in the legend shows the limits for each group in degrees of rotation above the horizontal axis (0°): horizontal group from 0° to 29.9°, oblique group from 30° to 59.9°, vertical group from 60° to 90°.

more densely packed cells in the lower intermediate zone, it contains sparsely scattered cells amid thick fiber bundles. The nuclear area (*top graph*, Fig. 8–7) remains nearly constant in the coronal plane (ranging from 26 to 23 μm^2), while nuclear area in the sagittal plane continually increases from 18 μm^2 on E20 to 25 μm^2 on E22. The two-way analysis of variance showed a significant plane effect ($F = 8.71$; df = 1, 6; $P < 0.0256$) due to the differences between the coronal and sagittal planes on E20. There is also a significant plane by age interaction ($F = 7.3$; df = 2, 6; $P < 0.0247$) due to the continual increase in nuclear area in the sagittal plane. Nuclear orientation is predominantly vertical in both planes (*right graph*, Fig. 8–8), yet the two-way analysis of variance indicated a significant plane effect ($F = 29.05$, df = 1, $P < 0.0017$). On E21 for example, there are more cells with vertically oriented nuclei in the sagittal plane (83%) than in the coronal plane (52%).

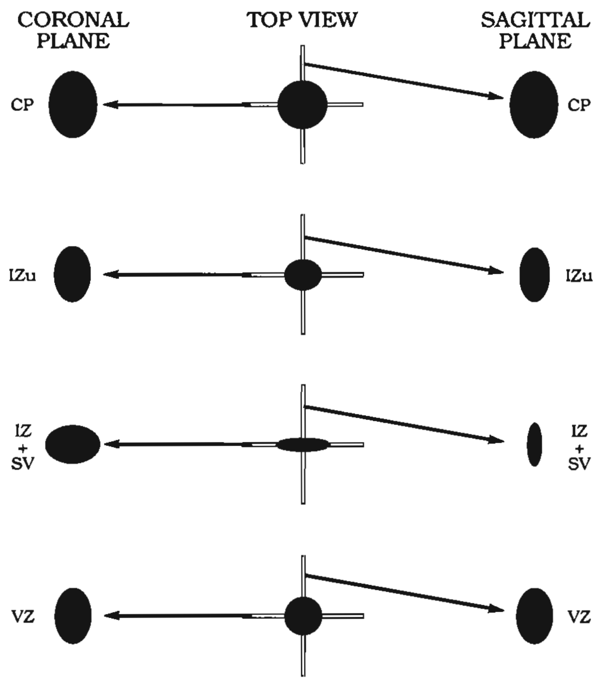

FIG. 8–9. A schematic of the shapes and orientations of cell bodies (*black circles and ellipses*) in the embryonic rat neocortex. The crossed lines in the center column (*top view*) represent the perpendicular arrangement between the sagittal plane (*vertical lines, cell body shapes in right column*) and the coronal plane (*horizontal lines, cell body shapes in left column*). In the ventricular zone (VZ) and cortical plate (CP), cell bodies are symmetrical vertically oriented ellipses in both planes. Cell bodies in the subventricular and intermediate zones (IZ + SV) are asymmetrical and shift orientations in the two planes. They appear as flattened ellipses in the top view and as horizontally oriented broad ellipses in the coronal plane. Due to the flattening, the narrow ellipses in the sagittal plane appear to run vertically and give the false impression that most cells are vertically oriented. Cells in the upper intermediate zone (IZu) are also asymmetrical, slightly flattened ellipses in the top view, broad ellipses in the coronal plane, and slightly narrower ellipses in the sagittal plane; but now the predominant nuclear orientation is vertical in both planes since these cells are radially migrating into the cortical plate.

8.3.4 Summary and Data Interpretation

Figure 8–9 summarizes the planar differences in the subventricular and intermediate zones (IZ + SV and IZu) along with the lack of planar differences in the ventricular zone (VZ) and the cortical plate (CP). The center column (*top view*) shows the perpendicular arrangement (*outlined cross*) of the coronal (*horizontal line*) and sagittal (*vertical line*) planes cutting through the cell bodies. The shapes of the coronally sectioned cell bodies are shown in the left column, while the shapes of the sagittally sectioned cell bodies are shown in the right column. The proliferating cells in the ventricular zone have the same shape and orientation when they are cut in the sagittal and coronal planes; the same can be said for the cell bodies in the cortical plate (compare Figs. 8–1 and 8–2, and data in Figs. 8–4 and 8–5). Consequently, the cell bodies in both the VZ and the CP are represented as vertically oriented, symmetrical ellipses (Fig. 8–9). However, the cell bodies in the subventricular and lower intermediate zones (IZ + SV, Fig. 8–9) have a different shape and orientation in the sagittal versus the coronal planes. On E17 for example, the coronal plane contains cross sectional areas close to twice as large as those in the sagittal plane (*two lower graphs*, Fig. 8–7) and contains cell bodies that are oriented either obliquely or horizontally, in contrast to the cell bodies in the sagittal plane that appear to be vertically oriented (Fig. 8–8). We interpret these data to indicate that the cell bodies in IZ + SV are flattened horizontal ellipses cut parallel to their long axes in coronal sections but cut perpendicular to their long axes in sagittal sections. In the sagittal plane, the narrow ellipses seen in the IZ + SV give the false impression of vertical orientation. In the upper intermediate zone (IZu, Fig. 8–9), the cell bodies are still flattened ellipses but less so than in the IZ + SV because the cross sectional areas are less divergent between the coronal and sagittal planes (*top graph*, Fig. 8–7). In both planes, the cell bodies in IZu are vertically oriented (Fig. 8–8); we interpret that to indicate that, after the cells have turned horizontally in the IZ + SV, they again turn vertically to resume their migration into the cortical plate.

8.3.5 The Significance of Cell Orientation

Since the majority of cells in the subventricular and intermediate zones are young neurons migrating to the cortical plate, their changing orientation may provide clues regarding the directional growth of their axons and gross cell movements. Our sequential survival [^{3}H]thymidine autoradiographic studies indicate that young neurons moving out of the ventricular zone sojourn for at least 24 hours in cell-specific bands in the subventricular and intermediate zones before resuming their migration toward the cortical plate (Chapter 7; Altman and Bayer, 1990b). The data in this chapter indicate that, after leaving the ventricular zone (where cells are vertically oriented), many of the putative young neurons turn horizontally while they are sojourning. Several studies have reported horizontally oriented cells in the intermediate and subventricular zones and have suggested that that is associated with axonal growth tangential to the surface of the neuroepithelium (Stensaas, 1967a, 1967b, 1967d; Stensaas and Stensaas, 1968; Derer, 1974; Shoukimas and Hinds, 1978; Wolff, 1978; Valverde et al., 1989). The direction of axonal growth is similar to the trajectory taken by ingrowing subcortical afferents (Stensaas, 1967d), mainly those from the thalamus (Caviness and Frost, 1980; Frost and Caviness, 1980; Rösner et al., 1988). We show in the following chapter that many of the horizontally oriented cells are actively migrating toward the dorsolateral and ventrolateral cortex in the lateral cortical stream, a prominent component of the intermediate zone in the anterior three-fourths of the developing neocortex.

Decreasing cell areas and less prominent horizontal cell orientation are features of the subventricular and intermediate zones between E17 and E21/E22 (Figs. 8–7 and 8–8). We propose that those changes are related to the circumstance that progressively fewer neurons and more immature glial cells occupy these layers as neurogenesis declines with increasing age. Our [^{3}H]thymidine long-survival autoradiographic studies (Chapters 3 and 11–15) indicate that by E20 only a small proportion of the total population of neurons remains to be generated (those in upper layer III and layer II). Moreover, short-survival [^{3}H]thymidine autoradiograms indicate that the later stages of neocortical development are characterized by an increasing number of locally multiplying cells, presumably glial cells (Chapter 4, Altman and Bayer, 1990b). The densely packed small cells in the subventricular zone and those scattered sparsely throughout the intermediate zone from E19 through E22 have more variable orientations (some vertical, many oblique, some horizontal) and contribute to the decreasing mean nuclear areas (Fig. 8–7) and the proportional reductions of nuclei in the horizontal group (Fig. 8–8).