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CHAPTER 1

Early Cortical Development

A Brief Historical Review

1.1 ABOUT THE NEUROEPITHELIUM IN GENERAL

1.1.1 Brief Note on Terminology

The bulk of the cellular elements of the vertebrate central nervous system originates in a sheet of germinal cells that lines the embryonic ventricular system. In young embryos, that sheet extends from the caudal tip of the neural tube to the rostral cap of the telencephalon. The germinal matrix is traceable to stem cells that successively form the neuroectoderm, the neural plate, the neural groove, and, after fusion of the latter, the neural tube. In the older literature, the lining of the embryonic ventricles was called the *ependymal layer,* but that term is rarely used today because the ependyma contains differentiated cells lining the ventricles in the mature nervous system, whereas the ventricular lining of the embryonic nervous system is composed of undifferentiated germinal cells. Two currently used terms for the germinal matrix are the *neuroepithelium* (Langman et al., 1966) and the *ventricular zone* (Boulder Committee, 1970). Just as the terms *neuro*ectoderm, *neural* plate, and *neural* tube are used, the term *neuro*epithelium refers to the progenitors that will give rise to a variety of cells specific to the nervous system, i.e., neurons, neuroglia, tanycytes, as well as a smaller population of ependymal cells. The term *neuroepithelium* draws attention to the uniqueness and great significance of the germinal matrix in the ontogeny of the central nervous system and is more descriptive than the neutral term *ventricular zone*. However, because of the wide acceptance of the latter we shall use both terms interchangeably as synonyms.

1.1.2 Some Properties of the Neuroepithelium

The neuroepithelium is composed of intensely staining, columnar cells oriented perpendicular (radial) to the ventricular surface. Mitotic cells lining the ventricular lumen are an exception in that they tend to be round and lightly stained. The neuroepithelium was originally believed to be a stratified matrix. His (1889) described two cell types in it, those that he called *Keimzellen* (germinal cells), which he believed were a source of neurons, and *Epithelzellen* (spongioblasts), which he thought were the source of neuroglia cells. Schaper (1897) disputed His's identification of the Keimzellen as a distinct type of stem cell and argued that they were epithelial cells in the process of division. Schaper also postulated that the cells of the neuroepithelium were "indifferent" elements that would give rise to both neurons and neuroglia.

Schaper's view obtained partial support in a study by Sauer (1935). Sauer noted that the germinal cells of the neural tube were anchored by thin cytoplasmic processes to the inner and outer surfaces of the neuroepithelium. Sauer hypothesized that the nuclei of neuroepithelial cells underwent a to-and-fro movement within the cytoplasm during the cell generation cycle (Fig. 1–1). Sauer referred to this phenomenon as *interkinetic nuclear migration,* and he described the neuroepithelium as a *pseudostratified epithelium* rather than a truly stratified one. With reference to the dispute between His and Schaper, Sauer concluded that there was only one cell type in the pseudostratified neuroepithelium: "The spongioblasts are interkinetic stages, and the germinal cells the mitotic stages, of the same cell" (Sauer, 1935; p. 397).

Sauer's hypothesis of interkinetic nuclear migration was later confirmed in studies using autoradiography (Sidman et al., 1959; Sauer and Walker, 1961; and others), transmission electron microscopy (e.g., Hinds and Ruffett, 1971), and scanning electron microscopy (e.g., Seymour and Berry, 1975; see Fig. 1–2). When the nuclei of proliferative cells are tagged with [^{3}H]thymidine (a radiochemical that selectively labels the DNA of duplicating chromosomes) the translocation of cell nuclei within the neuroepithelium can be followed during the cell generation cycle. In embryos killed within 1–2 hours after injection, the nuclei that have just incorporated thymidine into their chromosomal DNA (the synthetic phase) aggregate some distance from the lumen in a band called the *synthetic zone*. In embryos killed several hours after tagging with [^{3}H]thymidine, the labeled nuclei tend to be located near the lumen in the mitotic zone. Although the

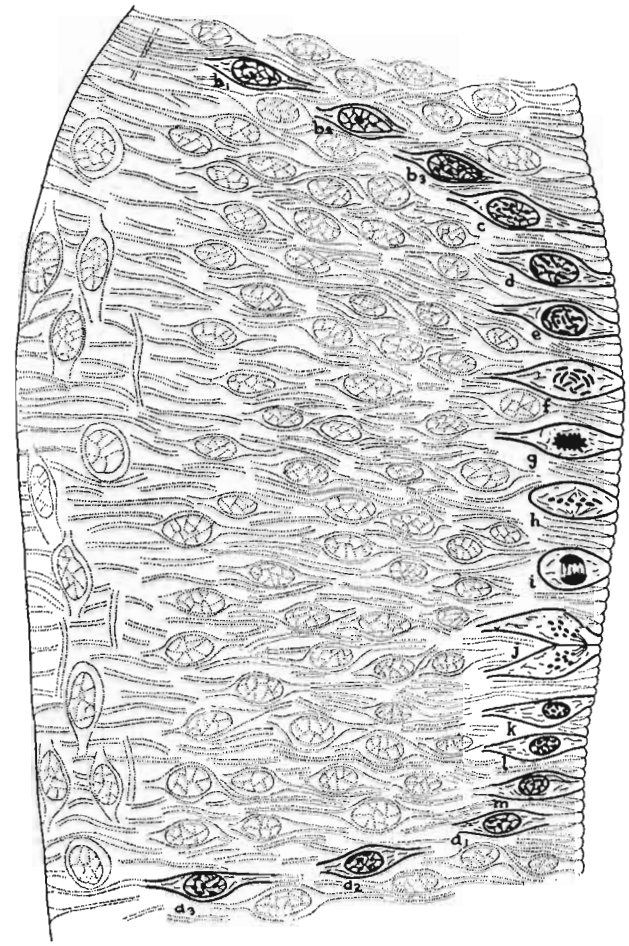

FIG. 1–1. Sauer's (1935) diagram of interkinetic nuclear migration in the neural tube of a young pig embryo. Successive letters from b1 to d3 indicate the to-and-fro vertical movement of a single proliferative cell and of one of its progeny.

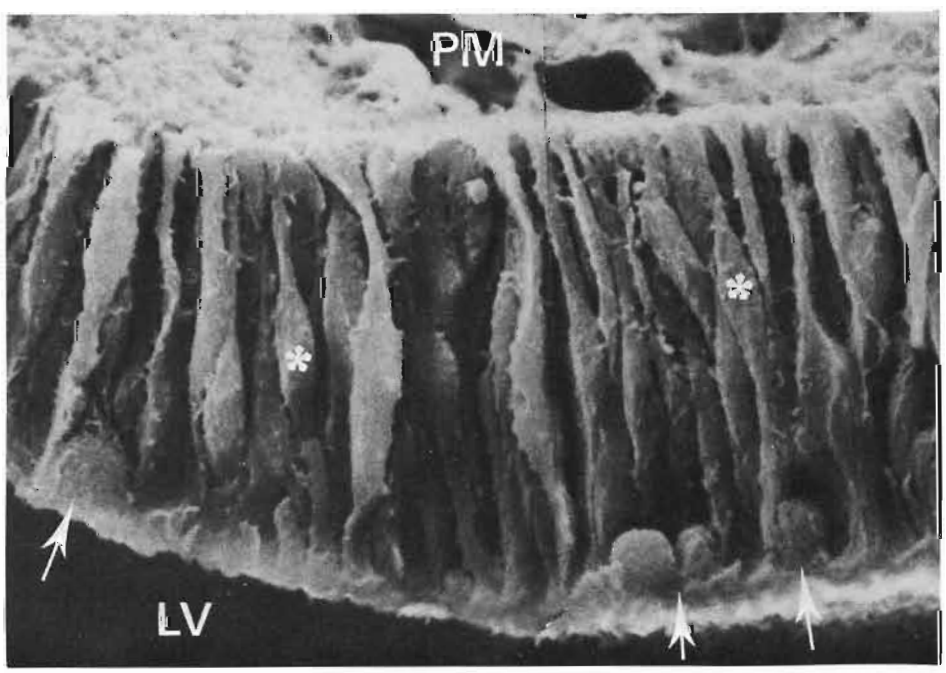

FIG. 1–2. Scanning electron micrograph of the telencephalic neuroepithelium from an E12 rat embryo. The stretching of cell bodies from the wall of the lateral ventricle (LV) to the primitive pia mater (PM) is evident. Asterisks indicate the bulging region of cells where the nuclei are presumably situated. The round cells without processes (*arrows*) are believed to be those undergoing mitotic division near the lumen. From Seymour and Berry, 1975.

validity of Sauer's hypothesis of interkinetic nuclear migration is not in doubt, new research (to be described in Chapter 4) suggests that the neuroepithelium in the cerebral cortex is not a pseudostratified germinal matrix at all stages of development but one in which cells with different kinetic properties occupy different compartments.

1.2 THE GERMINAL MATRIX OF THE CEREBRAL CORTEX

Most of the early observations about the structural organization of the neuroepithelium were made in the caudal neural tube, the primordium of the spinal cord. Although the neuroepithelium of the cerebral cortex is in many respects similar to that of the neural tube, there are considerable differences in the morphogenesis of the spinal cord and the cerebral cortex that warrant a consideration of possible differences in the organization of the neuroepithelium itself in these two neural systems. Indeed, it has been recognized for some time that the germinal matrix of the cerebral cortex is somewhat different from that of the neural tube in that it has two components: a portion near the ventricle, what Kershman (1938) called the ependymal zone, and another part some distance from the ventricle, Kershman's subependymal zone. The subependymal zone, now called the *subventricular zone** (Boulder Committee, 1970), is situated outside the columnar neuroepithelium. The subventricular zone is considered a component of the germinal matrix because it contains mitotic cells (Kershman, 1938; S, Fig. 1–3) and also cells that can be labeled with [^{3}H]thymidine (Smart, 1961; Altman, 1966; Hinds, 1968). The proliferating cells of the subventricular zone evidently do not move to the lumen, i.e., undergo interkinetic nuclear migration. The subventricular zone is easily distinguished from the neuroepithelium by the variable orientation of its cells (Fig. 1–3).

1.3 THE INTERMEDIATE ZONE OF THE CEREBRAL CORTEX

As the development of the cortex progresses, a new zone containing variably oriented, lightly staining cells and a rich matrix of fibers begins to form above the germinal matrix. This is what Kershman (1938) called the intermediary zone (I, Fig. 1–3) and Stensaas (1967a, 1967b, 1967d) the intermediate lamina; its widely used current name is the *intermediate zone* (Boulder Committee, 1970). The intermediate zone, unlike the subventricular zone, contains few (if any)

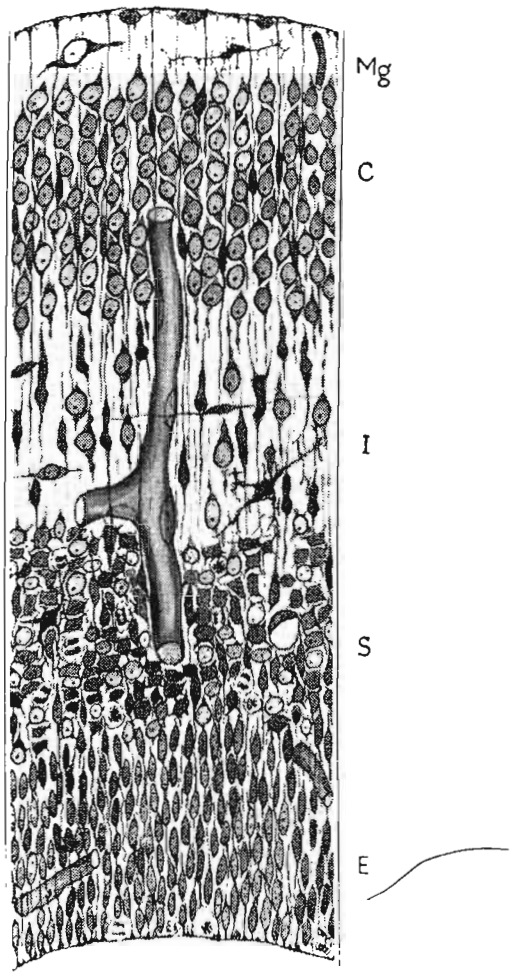

FIG. 1–3. Kershman's (1938) diagram of the developing cortex of an 11-week human embryo. C, cortical zone (cortical plate); E, ependymal zone (neuroepithelium); I, intermediate zone; Mg, marginal zone (layer I); S, subependymal zone (subventricular zone). Silver carbonate method.

mitotic cells. Stensaas distinguished between an upper and lower portion of the intermediate zone; the former containing many horizontally oriented cells, the latter mostly vertically (radially) oriented cells. It is generally assumed that a fair proportion of the cells of the intermediate zone are neurons migrating to the cortical plate. There are two stages in the embryonic development of the intermediate zone: first, it increases in depth, then it begins to shrink and is eventually replaced by the cortical white matter.

1.4 THE SETTLING OF NEURONS IN THE CORTICAL GRAY

1.4.1 The Cortical Plate

The cortical zone (C, Fig. 1–3), now generally called the *cortical plate,* forms above the intermediate zone

* The perpetuation of the prefix "sub" in this context is unfortunate; the term supraventricular would be more descriptive of the topography of this layer.

and beneath the subpial cell-sparse zone, the marginal layer (Mg, Fig. 1–3); the latter is also called the marginal zone and, more commonly, layer I. The cortical plate is composed of densely packed, radially oriented cells, the majority of which are presumed to be settling neurons. As development proceeds, the thickness of the cortical plate progressively increases. At a later stage of development the cortical plate neurons begin to differentiate, cell packing density decreases, and the horizontal cellular and fibrous layers of the cortical gray matter develop.

1.4.2 Chronoarchitectonics of the Cortical Gray

One notion regarding the histogenesis of the different layers of the cerebral cortex has been that, as successive waves of neurons leave the neuroepithelium, the later generated neurons displace the earlier generated ones with the result that the oldest neurons form the superficial layers and the younger neurons successively form the deeper layers. This idea may have been supported by the fact that cells residing in layer I, the Cajal-Retzius cells (the superficial horizontal cells in Figs. 1–3 and 1–4; to be dealt with below), are the earliest differentiating cortical neurons. But, in fact, Cajal (1911), referring to his own work and the work done by other investigators in the 1880s and 1890s, has clearly stated that the deep pyramidal cells differentiate before the superficial pyramidal cells. As he wrote in 1904: "The neurogenetic investigations of Vignal, ourselves, Retzius, Kölliker, Stefanowska, etc., agree on an essential point, namely that the morphological

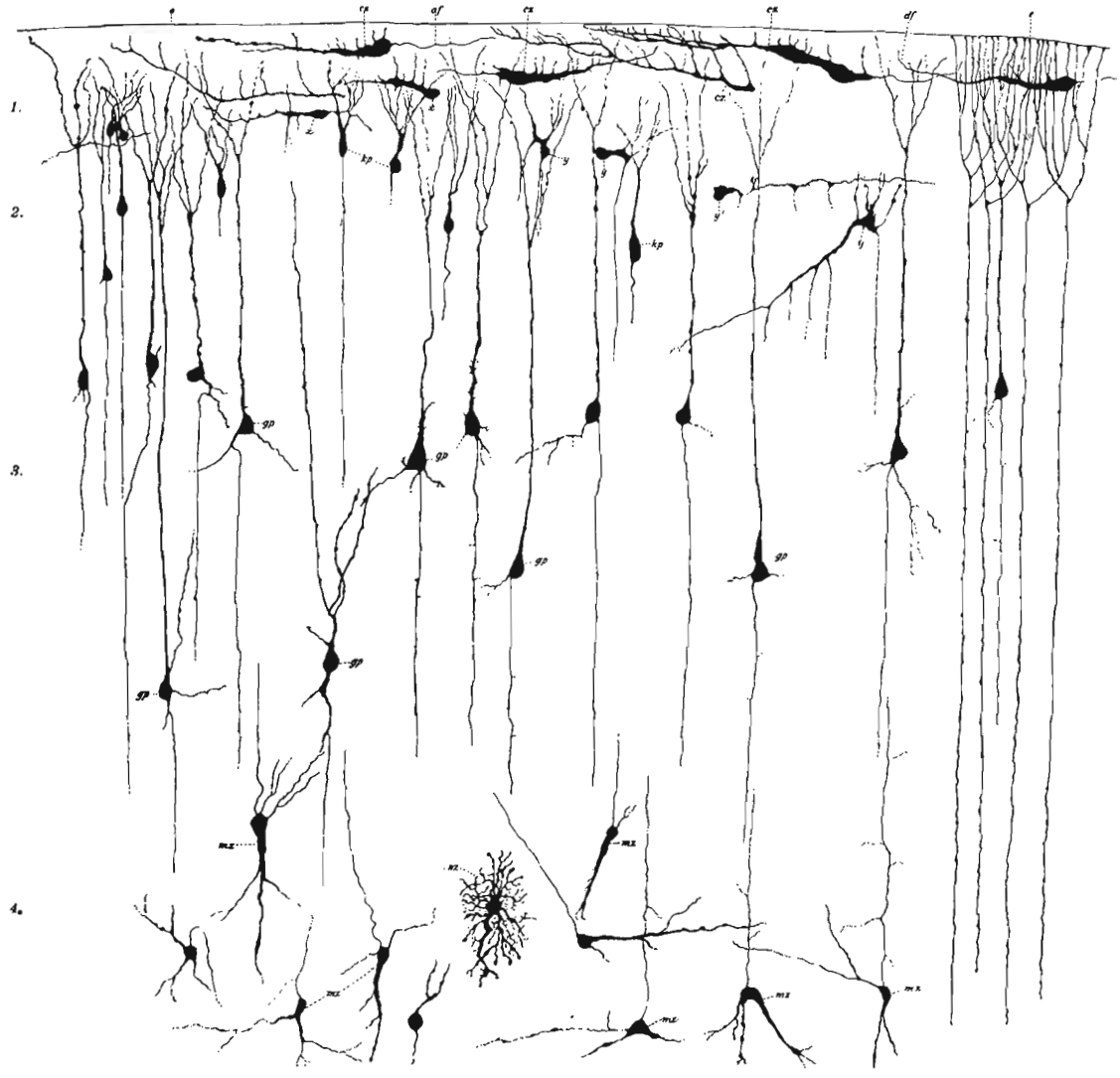

FIG. 1–4. Retzius's (1893) drawing of Golgi-stained neurons and glia cells in the cerebral cortex of a 14-cm-long dog fetus. Retzius designates the marginal zone as layer 1, the cortical plate as 2 and 3, and the subplate as layer 4. cz, Cajal cells (Cajal-Retzius cells); gp, large pyramidal cells; kp, small pyramidal cells; mz, cells with superficially directed axons; nz, neuroglia cells. (Other letters refer to the processes of Cajal-Retzius cells.)

differentiation of the pyramids is initiated in the deepest zones . . . progressing subsequently toward the superficial or small pyramids to which the neurons of latest development belong. At the moment of birth, the giant pyramids are the most advanced in morphology and intraprotoplasmic differentiation." (DeFelipe and Jones, 1988; p. 455).

Significantly, these early observations dealt with the question of neuronal differentiation in the cerebral cortex not with neurogenesis and the problem of cell migration. It was the early [^{3}H]thymidine autoradiographic work of Angevine and Sidman (1961) that established that the neurons destined to occupy the depth of the cortex are generated first and the subsequently generated waves of neurons bypass the earlier generated ones by active migration and settle above them. This "inside-out" migratory and settling pattern of cortical neurons was subsequently confirmed in the rat (Berry and Rogers, 1965; Hicks and D'Amato, 1968; Bisconte and Marty, 1975), hamster (Shimada and Langman, 1970), monkey (Rakic, 1974), cat (Luskin and Shatz, 1985a), and ferret (Jackson et al., 1989). But, as noted below, there is an exception to this generalization in that the neurons settling in the most superficial layer of the cortex (layer I) and in its deepest layer (layer VII, or the subplate) are generated ahead of those settling in layers VI–II of the cortical plate proper.

1.4.3 The Primordial Plexiform Layer

Two cortical layers, the marginal zone (layer I) and the cortical subplate (often called layer VIb or VII in the adult), have morphogenetic properties that distinguish them from the cortical plate. As we noted, layer I (Mg, Figs. 1–3 and 1, Fig. 1–4) is a subpial, cell-sparse zone in which the horizontally oriented Cajal-Retzius cells of the developing cerebral cortex are embedded. The Cajal-Retzius cells begin to differentiate before the cortical plate has begun to form (Cajal, 1890; Retzius, 1893; Cajal, 1911). The subplate, identified as layer IV in Fig. 1–4 taken from Retzius (1893) and as layer VII in Fig. 1–5 taken from Marin-Padilla (1978), is a cell-rich layer beneath the cortical plate where polymorph cells predominate. Early observations with the Golgi technique by Retzius (1893) suggested that subplate neurons, particularly those with ascending axons, also differentiate quite early during cortical development.

Because the initial [^{3}H]thymidine autoradiographic observations of Angevine and Sidman (1961) were concerned only with the inside-out neurogenetic gradient between cells in the cortical plate, the possibility that the most superficial neurons of the cortex were the oldest was not considered. It was the Golgi studies of Marin-Padilla (1971, 1972, 1978, 1983; Marin-Padilla and Marin-Padilla, 1982) that continued to emphasize the early differentiation of Cajal-Retzius cells and subplate cells, challenging the conclusion that the inside-out neurogenetic gradient between neurons in the cortical plate represents the whole picture of laminar development. Subsequent [^{3}H]thymidine autoradiographic studies (König et al., 1975, 1977; Rickmann et al., 1977; Raedler and Raedler, 1978; Wolff, 1978; Kostovic and Rakic, 1980; König and Marty, 1981; Caviness, 1982; Luskin and Shatz, 1985a; Chun et al., 1987) supported Marin-Padilla's developmental observations that the oldest cortical neurons are the Cajal-Retzius cells and the subplate cells.

Marin-Padilla (1971, 1978) proposed that the neurons of layer I and the subplate, together with early cortical afferents, constitute a morphogenetic framework, the primordial plexiform layer (I and VII, Fig. 1–5), inside which the later-produced neurons of the cortical plate (CP) accumulate. The terminology adopted by Marin-Padilla is based on the hypothesis that the early-dif-

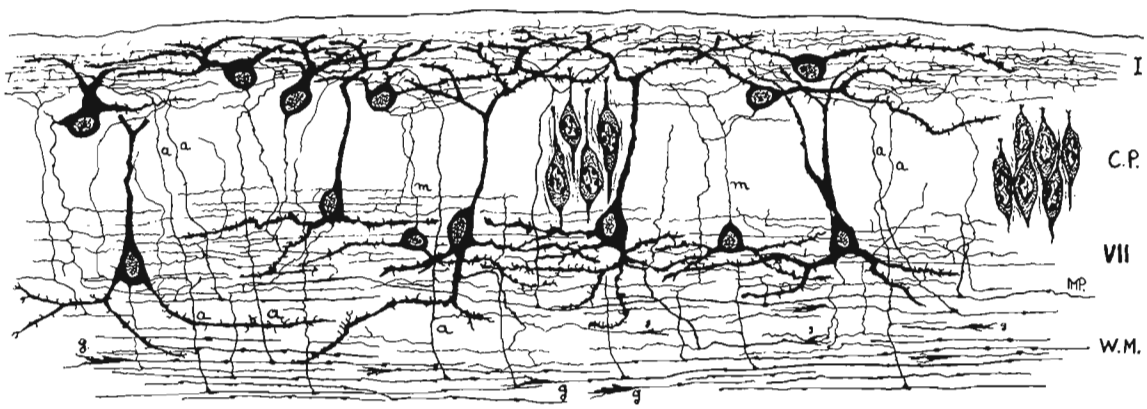

FIG. 1–5. Composite figure of camera lucida drawings from Golgi preparations of the cat cerebral cortex on E25. I, layer I; C.P., cortical plate; VII, layer VII (subplate); W.M., white matter. Smaller letters refer to cell processes. From Marin-Padilla, 1978.

ferentiating neurons of layer I and the subplate constitute a phylogenetically ancient (reptilian) cortical system. According to this view, the phylogenetically younger cortical plate neurons, which evolve and become elaborated into a complex laminar structure only in mammals, splits the primordial plexiform layer into a superficial component (layer I, or marginal zone) and a deep component (layer VII, or subplate).

Marin-Padilla's ontogenetic hypothesis is supported by many observations. First, the pioneer cell populations of the primordial plexiform layer differentiate early and have the first mature synaptic contacts (Stensaas, 1967c; Meller et al., 1968, 1969; Molliver and van der Loos, 1970; Molliver et al., 1973; König et al., 1975; Kristt and Molliver, 1976; Raedler and Sievers, 1976; Rickmann et al., 1977; König and Marty, 1981; Zheng et al., 1990). Second, layer I and the subplate are the targets of the earliest distributed monoaminergic fibers to the cerebral cortex (Seiger and Olson, 1973; Molliver and Kristt, 1975; Schlumpf et al., 1980; Caviness and Korde, 1981; Lidov and Molliver, 1982; Crandall and Caviness, 1984; Fujimiya et al., 1986; Mulligan and Tork, 1987; Kalsbeek et al., 1988). Third, thalamic afferents are primarily confined to either layer I or to the subplate during early cortical development (Rakic, 1983; Crandall and Caviness, 1984; Shatz and Luskin, 1986; Chun et al., 1987) and only grow into the cortical plate later (mainly postnatally in rats). These lines of evidence suggest that the Cajal-Retzius cells and the subplate cells play an organizing role during early development of the cortex.

1.4.4 Migration of Cortical Neurons

Most observers have assumed that the radially oriented cells of the neuroepithelium follow a direct radial path to the cortical plate where, indeed, the young neurons show a similar radial orientation. An earlier hypothesis, proposed by Berry and Rogers (1965) and Morest (1970), was that the radial migration of young neurons to the cortical plate is based on nuclear translocation within the elongated cytoplasmic processes that anchor many cells at the base and apex of the presumptive cortex (Fig. 1–6). A more widely accepted current view is Rakic's theory (1972) that the radial migration of young neurons is aided by surface contact with preformed radial fibers of glial cells that traverse the embryonic cerebral cortex (Fig. 1–7). Neither of these theories satisfactorily accounts for the presence, both in the subventricular zone and in the intermediate zone, of a high proportion of cells that are either horizontally oriented or have a stellate shape (Figs. 1–3 and 1–8). At least some of the cells must, therefore, undergo rotation in the intermediate zone after leaving the neuroepithelium. The apparent rotation of cells in the cortical intermediate zone has been repeatedly described in Nissl-stained (Paton, 1900; Hataï, 1902) and Golgi-impregnated (Stensaas, 1967a, 1967b, 1967d; Derer, 1974) sections in different species. In addition, Golgi studies indicate that the horizontally oriented cells have horizontally oriented processes. According to Stensaas (1967b, 1967d),

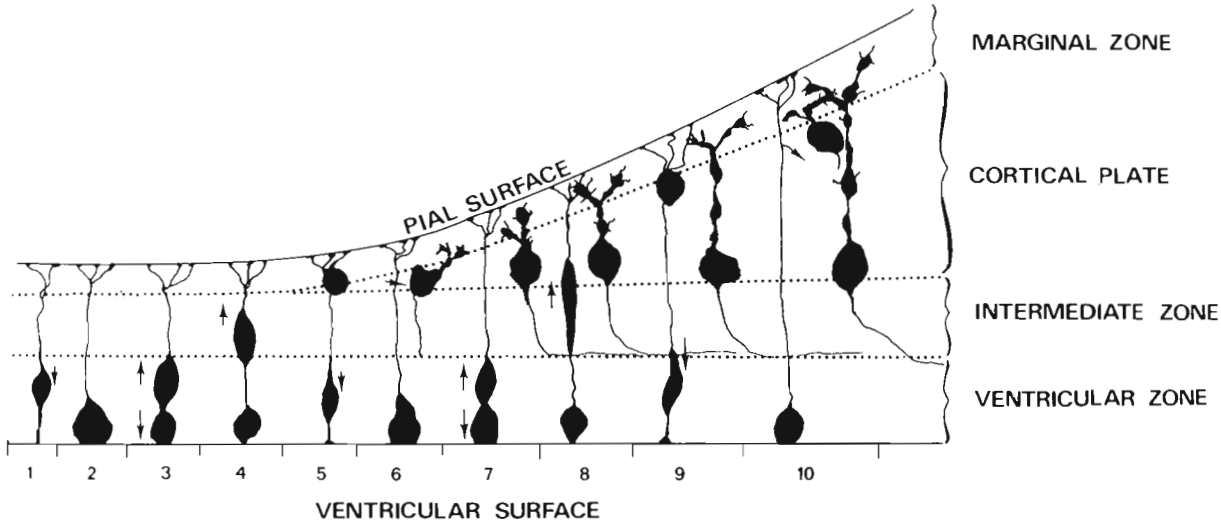

FIG. 1–6. Diagrammatic representation of a hypothesis of the production and migration of neurons (*arrows*) in the cerebral cortex, according to Berry and Rogers (1965) and Morest (1970). From Berry, 1974.

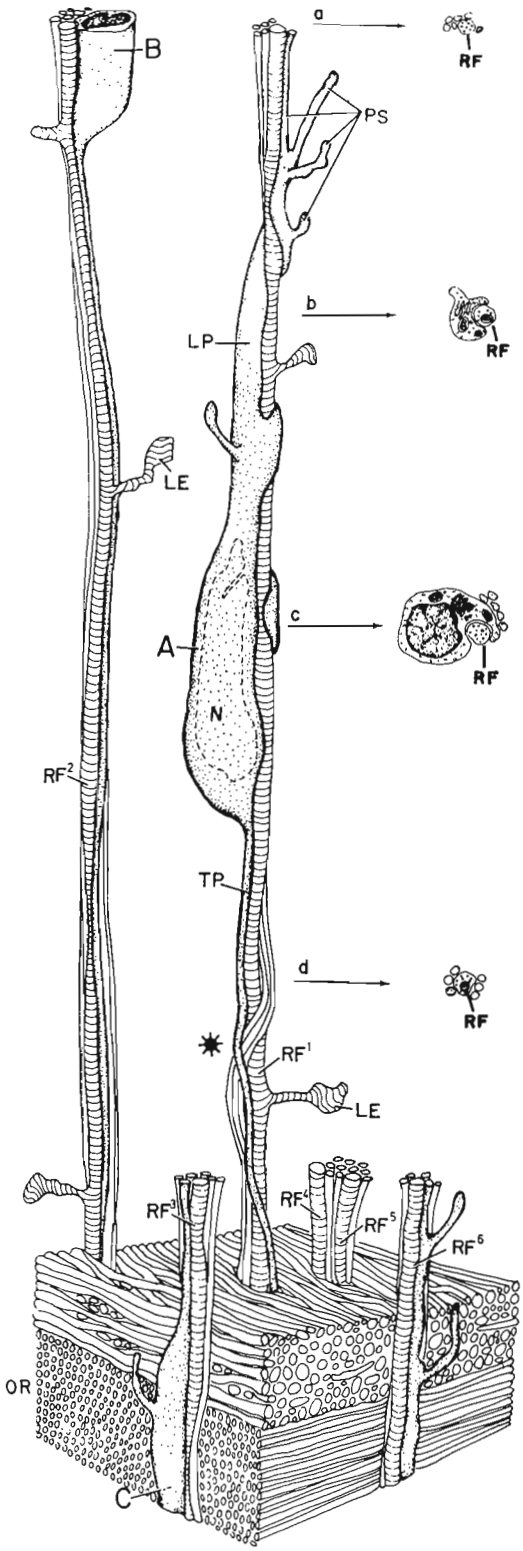

FIG. 1–7. A semidiagrammatic reconstruction from Rakic (1972) showing that migrating young neurons (stippled cell bodies and processes labeled A, B, and C) are closely apposed to radial glia fibers (striped processes labeled RF^1, RF^2, and RF^3) in the intermediate zone of the fetal monkey neocortex. Other radial fibers (RF^4 to RF^6) are not reconstructed to show attached young neurons. The profiles labeled a to d on the right show how RF^1 and cell A appear in cross sections at the levels of the arrows. (OR, fibers in the optic radiation; other labels refer to cell processes.) These reconstructions prompted Rakic (1972) to propose that young neurons are guided to the cortical plate by migrating on radial glia fibers.

horizontal cells predominate in the lower intermediate zone (lower intermediate lamina, Fig. 1–8) and he interprets their processes as efferent fibers that grow in the direction of the basal ganglia. The outgrowth of these axons apparently commences before their cell bodies have again become reoriented vertically in the upper intermediate zone (upper intermediate lamina, Fig. 1–8) to resume their ascent to the cortical plate.

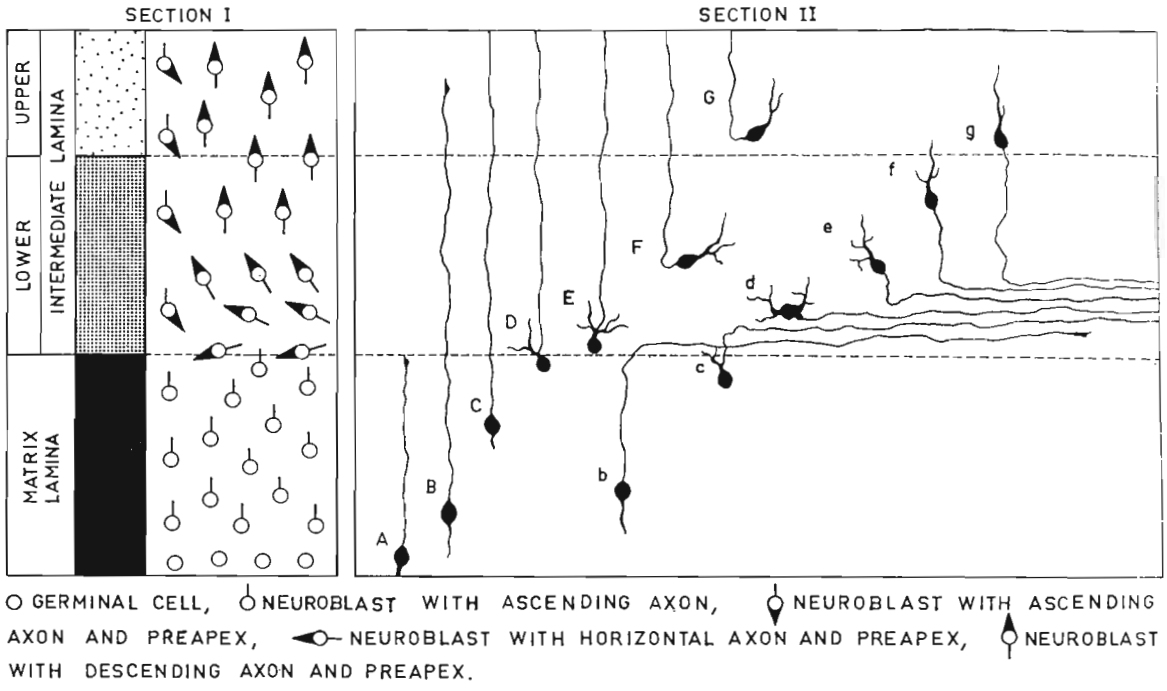

FIG. 1–8. Stensaas's (1967b) schematic representation of changes in the orientation of neuroblasts (young neurons) as they ascend from the matrix lamina (neuroepithelium), through the lower and upper layers of the intermediate lamina (intermediate zone), to the cortical plate (not shown).

1.4.5 SUMMARY

This brief historical review suggests that there are three stages, or life cycles, in the embryonic development of the cellular elements of the cerebral cortex, and there are three corresponding morphogenetic fields where these cellular transformations take place. The first stage is the growth of a large pool of proliferative precursor cells of neurons and glia. This takes place in the germinal matrix (primarily the neuroepithelium and, to a lesser extent, the subventricular zone) of the cortical primordium. The second stage is the translocation of differentiating cells into the intermediate zone. It is apparently here that the migrating young neurons begin to sprout their axons before they proceed toward the surface of the cortex. The third stage is the settling of neurons in the future cerebral cortex, composed of the primordial plexiform layer and the cortical plate. It is at the latter sites that the final differentiation of neurons, including their dendritic development and synaptic organization, takes place. Although new observations described in this book indicate that the development of the cortex is a far more complex process, the tripartite organization of the developing cortex into a germinal matrix, an intermediate staging field, and a final settling area appears to be justified.