nature vs nurture: Is it the brain microenvironment or genetic predetermination that tells migrating young neurons where to go and what to become? A considerable degree of plasticity in the neuron's response to local cues explains some of the findings described in this multi-author review. However genetic restrictions are also suggested, and it is probably the unique proportion of the combination of both that shapes the different regions of the CNS.

As this century comes to a close – just as at the end of last century – developmental neurobiology is living a period of tremendous excitement. Ever since Ramón y Cajal showed that neurons were independent circuit elements, the genesis of these cells has remained the focus of much of our attention. Cajal recognized the relevance of this problem and described in extraordinary detail the transformation of the VZ postmitotic young neuron (Cajal's apolar neuroblast) into a migrating young neuron (bipolar neuroblast) and its differentiation into a mature neuron [11]. Today we can better explain where neurons come from and how committed they are. We know about some of the tracts that steer their migration and regulate their targeting. The understanding of these processes brings us closer to explaining CNS assembly and how brain function comes about.

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Principles of neural cell migration

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Summary. A basic property of immature neurons is their ability to change position from the place of their final mitotic division in proliferative centers of the developing brain to the specific positions they will occupy in a given structure of the adult nervous system. Proper acquisition of neuron position, attained through the process of active migration, ultimately affects a cell's morphology, synaptic connectivity and function. Although various classes of neurons may use different molecular cues to guide their migration to distant structures, a surface-mediated interaction between neighboring cells is considered essential for all types of migration. Disturbance of this cell-cell interaction may be important in several congenital and/or acquired brain abnormalities. The present article considers the basic mechanisms and principles of neuronal cell migration in the mammalian central nervous system.

Key words. Mammalian nervous system; neuron migration; neuron position; molecular cues; cell-cell interaction; radial migration; gliophilic cells; neurophilic cells; biphilic cells.

Immature nerve cells possess a remarkable capacity to move before they assume their final position and establish permanent synaptic relationships. In fact, the great majority of neurons in the developing vertebrate nervous system are generated in sites that are significantly different from those in which they reside in adult brain. The intervening process, termed migration, denotes the displacement of a neuronal cell body from its last cell division in the proliferative zone to its final destination in the mature brain [40, 41]. In many laminated structures of the mammalian brain, such as the cerebral or the cerebellar cortex, later generated neurons must pass by the early generated ones. Therefore, neuronal cell migration may be considered a 'biological necessity' in that it enables

communication between early and late forming neurons at the critical developmental stages, before they make their synaptic connections[48].

Migration of neurons is a distinct cellular phenomenon. In the vertebrate central nervous system migration of neurons differs in several respects from the movements of neural crest cells in the peripheral nervous system. Perhaps the most significant difference is that neural crest cells in the peripheral nervous system undergo mitotic division during migration[26], while in the central nervous system movement is initiated only after completion of the last cell division[65]. Neuronal cell migration should also be distinguished clearly from the movement of growth cones during the extension of axons and dendrites that usually occurs at later developmental stages. Although, in principle, all movements of cells and their processes may appear similar, and many basic cellular mechanisms are probably shared, there are also considerable differences which have to be considered, and from which we can learn.

Displacement of immature neurons is particularly prominent in the large mammalian brain and occurs for the most part prenatally, following final mitotic division of the neuronal cell progenitors. The length of the migratory pathway may range from less than 100 µm in the small rodent brain to several thousand µm in some areas of the human cerebrum, although the size of migratory neurons is approximately the same in most mammalian species. The migration involves dislocation of postmitotic cells from the other elements in the proliferative zone and active displacement of undifferentiated neurons in relation to the surrounding cellular milieu[39,41]. This movement of neurons proceeds along specific pathways, occurs according to a well-defined time schedule, and stops at precisely defined locations.

In terms of orientation and directionality of cell movement, migration can be classified into two basic axes:

1) *radial:* proceeding from the ventricular to the pial surface. The basic columnar organization of many brain structures in the mammalian central nervous system reflects this form of cell movement and is visible particularly during early stages of development.

2) *tangential:* running predominantly parallel to the brain surface. This migration mode allows passage of neurons from one brain compartment into another, and occurs often at later developmental stages.

Several lines of evidence from both in vivo and in vitro analysis indicate that contact interaction between migrating neurons and the surfaces of neighboring cells plays a crucial role in selecting migratory substrate, and in the orientation, displacement and stopping of neurons. With regard to pathway selections, migrating neurons fall into three major categories[48]:

1) *gliophilic* cells which selectively follow elongated glial fibers and pass by neurons or neuronal cell processes that may be lying within their trajectory.

2) *neurophilic* cells which preferentially follow neuronal, particularly axonal cell surfaces, and pass glial cell shafts even when they are traversing their migratory pathway.

3) *biphilic* cells which display temporal or regional affinities towards either glial or neuronal surfaces at different phases of their differentiation or have two classes of neurites that have different surface affinities.

Gliophilic migration

Migration along radial glial fibers is the prevalent mode of neuronal cell movement in the developing mammalian brain. It is particularly prominent during formation of the larger laminar structures such as the neocortex and hippocampus; but occurs also, to a lesser degree, in the developing diencephalon, brain stem and spinal cord[8,33,39,41,45]. The relationship between migrating neurons and elongated glial fibers, which serve as a substrate for their movement, may be best illustrated in the large monkey cerebrum at relatively late stages of development (fig. 1). In this, as in the other mammalian species, all neocortical neurons are produced in the proliferative zone situated near the lateral cerebral ventricle[44]. Following their last division, postmitotic cells break off contact with the neighboring proliferative cells at the ventricular surface and are set free for a journey that in the parts of the occipital lobe in monkey fetus may be several thousand µm long[41]. The enormous length of the migratory pathway of cortical neurons in the large primate cerebrum is often not appreciated and figures 1 and 2 are intended to illustrate this point in the monkey cerebrum at midgestation. In the human fetus, the migratory pathway is even longer although the size of migrating neurons is about the same in primates and rodents. Thus, the leading process, cell soma and a trailing process have in combined length only a fraction of the distance that cells must eventually traverse. Fascicles of elongated radial glial fibers, which span the entire thickness of the cerebral wall, may be at late developmental stages several millimeters in length (figs 1 B and 2 B). This observation led to my doubt that radial glial cells can divide two times a day and withdraw their long fibers during each cell cycle as was the prevailing view two decades ago[42].

Radial glial cells can be considered as a form of primitive astrocyte. They share several cytoplasmic molecules with the adult form of astrocytes such as glial acidic fibrillary protein (GAFP) and vimentin[28,29], but they also contain several other class-specific molecules that are expressed only during developmental stages[6,21]. Tritiated thymidine autoradiographic analysis of fetuses sacrificed at short intervals following injection showed that many radial glial cells in the rhesus monkey stop transiently to divide for up to two months[60]. Since their endfeet during this period remain attached to both the ventricular and pial surfaces, they increase in length several fold during the amitotic period and thus provide a continuous path

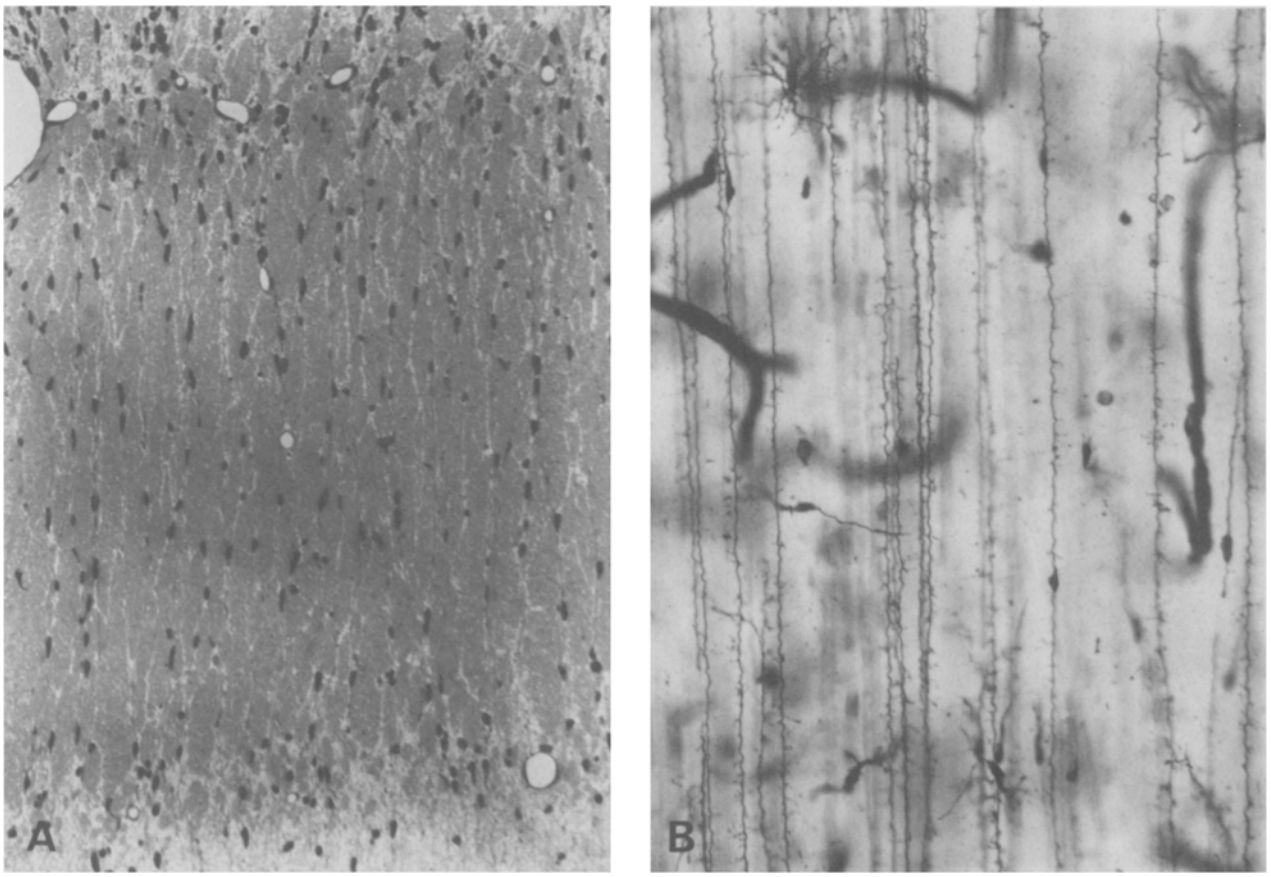

Figure 1. *A* Photomicrograph of the toluidine blue stained section across the intermediate zone in the monkey cerebral wall at midgestation. Note cohorts of migrating neurons deployed in the separate streams running from the proliferative ventricular zone situated 1000 μm below to the cortical plate 2000 μm above. *B* Golgi impregnated section at the corresponding level of the fetal cerebral wall exposes elongated shafts of radial glial fibers and an occasional bipolar migratory neuron.

between the site of a cell's origin and the site of its final positions[39,41]. This mechanism of physical continuity may be particularly important in gyrencephalic brains, including the large human cerebral hemispheres where there is a considerable shift in the position of the cortical plate during gyral formation (fig. 2B). The stability of radial glial cells in the fetus enables several generations of neurons to reach their appropriate areal and laminar position in the developing cortical plate.

Radial glial cells are, in most mammals so far studied, only a transient population of cells, typical of the fetal glial cells identified and illustrated beautifully in Golgi preparations by Retzius[56] and Ramon y Cajal[55]. In the primate cerebrum, including human, they form a separate cell line from the beginning of corticogenesis[6,28,29]. In most regions of the mammalian brain, radial fibers detach from the pial surface and terminate at various distances from the ventricular surfaces[61]. A schematic diagram of their shape, origin and of morphogenetic transformation into astrocytes is illustrated in figure 2A. In certain brain areas, radial glial cells may differentiate into specialized non-neuronal cells as Bergmann glia, tanycyte, septal glia or Müller cells[47]. In some vertebrates species, radial glial cells persist even in cerebrum and cerebellum throughout their life spans[55]. In the adult canary, for example, there is a rich remnant of radial glial fibers which permits migration of neurons that are generated throughout the life span in this species[1,2].

Electron microscopic analysis shows that during their migration to the cerebral cortex, neurons are apposed invariably to neighboring glial fibers (fig. 3). The leading process of migrating neurons display a complex and variable form with several terminal branches in contact with the single or several radial glial fibers arranged in a form of fascicle. Careful three-dimensional reconstruction from serial micrographs reveals that these branches are being actively extended and withdrawn to allow migrating cells to advance beyond the potentially obstructing axonal tracts that lie in its path and traverse the crotch between its two leading processes (fig. 4B). The strong affinity between neuronal and glial cell membrane surfaces was implied originally by the failure of migrating neurons to follow any of a myriad of differently oriented cellular processes of different origin that they encounter during their journey to the cortex[39,41]. However, occa-

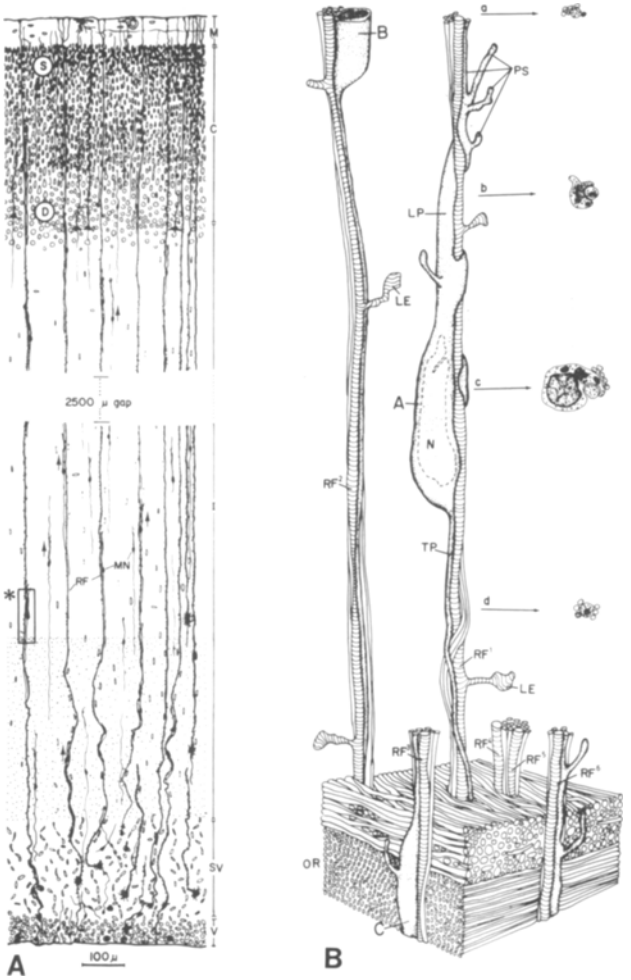

Figure 2. *A* Camera lucida drawing of the occipital cerebral wall of the monkey fetus at mid-gestation. Composite illustration is derived from Golgi impregnated section (black profiles) and from adjacent section counterstained with toluidine blue (outline of cell nuclei). The middle 2000 μm of the intermediate zone, similar in structure to the sectors drawn, is omitted. The rectangle marked with an asterisk shows the approximate position of cell reconstruction in *B*. Abbreviations: C = cortical plate; I = intermediate zone; M = molecular layer; MN = migrating neuron; RF = radial fiber; SV = subventricular zone; V = ventricular zone. *B* Three-dimensional reconstruction of migrating neurons, based on serial electron micrographs made at the level of the intermediate zone indicated by the rectangle in *A*. The lower portion of the diagram contains parallel fibers of the optic radiation (OR) and the remainder is occupied by a more disposed fiber system. Except at the lower portion of the figure, most of these fibers are deleted from the diagram to expose the radial fibers (striped vertical shafts RF_{1-6}) and their relations to the migrating cells A, B, and C. The soma of migrating cell A, with its nucleus (N) and voluminous leading process (LP), is situated within the reconstructed space, except for the terminal part of the attenuated trailing process and the tip of the vertical ascending pseudopodium. Cross sections of cell A in relation to the several vertical fibers in the fascicle are drawn at levels a through d at the right side of the figure. The perikaryon of cell B is cut off at the top of the reconstructed space, whereas the leading process of cell C is shown just penetrating between fibers of the optic radiation (OR) on its way across the intermediate zone. LE indicates lamellate expansions; PS indicates pseudopodia. (From Rakic[41]).

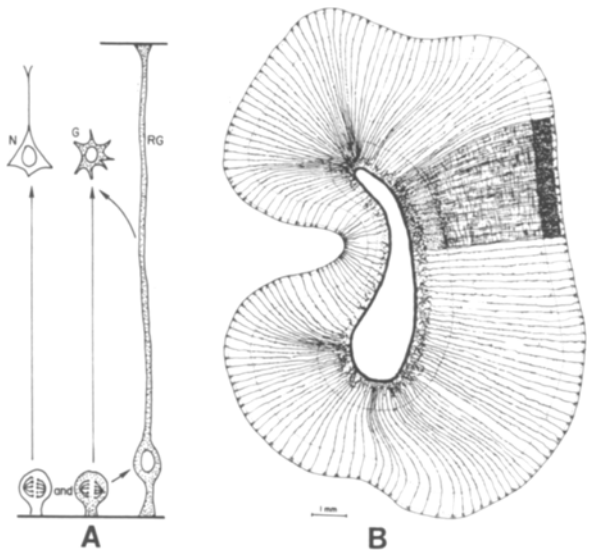

Figure 3. *A* The concept of morphogenetic transformation of radial glial cells into astrocytes based on Golgi, electron microscopic, and immunocytochemical analyses. GFAP-negative and GFAP-positive (stippled) dividing cells coexist throughout the period of neurogenesis. Initially, the GFAP-positive cells produce only radial glial cells (RG); but later, either directly or indirectly (arrows), they generate astrocytes (G) and various specialized astrocytic-like cells. All neurons (N) but perhaps also some nonneuronal cells originate from GFAP-negative precursors. (From Rakic[46]). *B* Camera lucida drawing of a coronal section of a Golgi-impregnated occipital cerebral lobe in a 97-day-old monkey fetus. Although the incipient calcarine fissure appears at the medial surface, typical sulci and gyri are still absent. The radial glial cell somas are situated close to the cerebral ventricle and their elongated fibers extend to the 3–5-mm distant pial surfaces where they divide into several branches, each terminating with an endfoot. (From Rakic[41]).

sional migrating neurons may transfer from one fascicle to another (fig. 4), indicating that adhesive molecules are not specified for a single glial fiber or single fascicle[53]. Most neurons nevertheless follow the set of radial glial fibers with which they come in contact near the cerebral ventricle. The reason why most postmitotic neurons remain attached to the same glial fascicle throughout their journey may be explained by quantitative rather than qualitative forces[46] and by local modulation which enables cell communication in two directions[10]. It is likely that a certain number of adhesion molecules must be present on adjacent glial fibers to draw a migrating neuron away from the glial fiber with which it already has formed strong attachment. Thus, by providing a surface substrate for migration, radial glial fascicles form a scaffolding that could impose constraints for lateral movement and preserve the neighbor relationship established in the proliferative zone. Another function of this neuron-glial interaction is the facilitation of cell migration through the complex assembly of closely packed cells and processes that compose the developing cerebral wall. After the migration of cortical neurons has been completed, the glial scaffolding in the telencephalon disappears as some radial glial cells degenerate, while others reenter the mitotic cycle and transform into astrocytes[41, 60, 61].

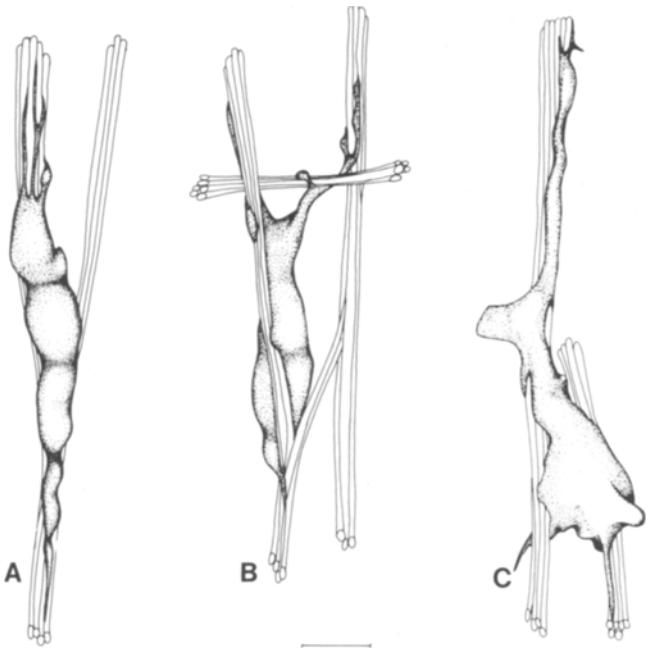

Figure 4. Computer-aided reconstructions of the three migrating neurons selected to show their relation to more than one radial glial fascicle in the developing cerebral wall of the rhesus monkey. Some of the glial fibers which lie in contiguity with the migrating cells were also traced and reconstructed but most of the neighboring cells and processes were omitted. Calibration bar = 5 µm. (From Rakic et al. [53]).

Neurophilic migration

The gliophilic mode of migration is not the only type of cell surface-mediated mechanism underlaying neuronal movement in the central nervous system. This simple fact is often neglected, as research in the past two decades has focused on the analysis of neuron-glial cell-cell interactions. However, certain classes of neurons in various brain subdivisions migrate in a direction that does not coincide with the radial glial scaffolding. In fact, such cells often pass by the nearby glial fibers without forming any permanent attachment. Instead, this class of postmitotic neuronal cell seems to adhere preferentially to the surface of nearby axons; hence, the term neurophilic migration appears appropriate [48].

Neurons forming several brain stem nuclei (e.g., gray nucleus of the pons or inferior olive of the medulla oblongata) are instructive examples of neurophilic migration [37, 48]. In these structures, neurons generated in the proliferative centers, situated at the lateral edge of the rhombencephalon, migrate tangentially over the surface of the brain stem to settle at the ventral portions of the pons and medulla oblongata. As in the telencephalon, postmitotic neurons assume a bipolar shape and have similar ultrastructural characteristics [48]. However, unlike postmitotic neurons moving to the cerebral cortex, this class of cells migrate closely apposed to the neuronal surface provided by fiber tracts that run parallel to the brain surface. While moving, they avoid radial glial fibers that are oriented perpendicularly to their trajectory (fig. 5). It may be significant that migrating neurons are attached to specific axonal tracts and do not follow axons of the corticospinal and corticobulbar system that runs through the same space in a rostrocaudal direction (a_2 in fig. 5). This finding suggests the selectivity of neurophilic adhesion that may differentiate between specific neuronal pathways. The capacity to make such a distinction may be essential since other examples of neurophilic migration in the brain stem proceeds along axonal pathways destined for the trigeminal motor nuclei [53]. Taken together, these findings suggest that, unlike in the glio-

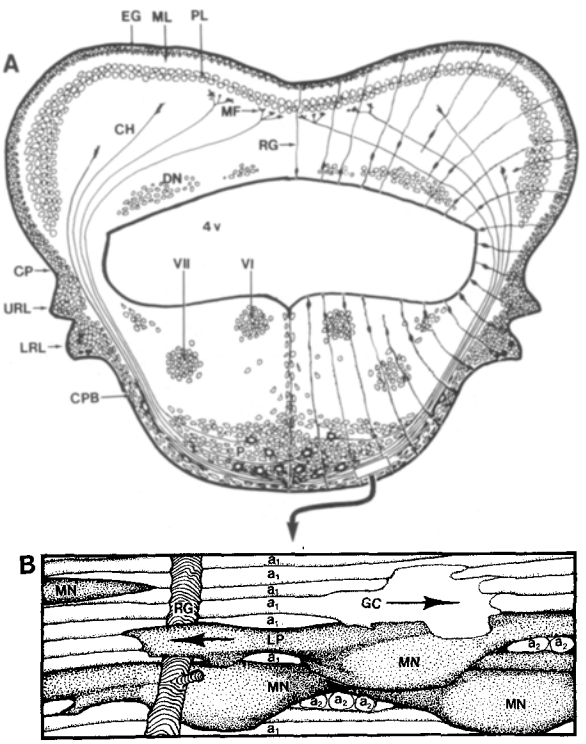

Figure 5. *A* Semischematic drawing of a transverse section of the cerebellar hemispheres (CH) and pons (P) of a 55-day-old monkey embryo. By this age, cranial nerve nuclei (e.g. VI, VII) as well as Purkinje cells (PL) and most of deep cerebellar neurons (DN) have completed their genesis near the fourth ventricle (4v) and completed their migration along radial glial fibers (RG) to final positions. However, cells generated later in the lower rhombic lip (LRL) migrate via the corpus pontobulbare (CPB) along the brain stem surface, perpendicular to the orientation of radial glial fibers, and accumulate at the bottom of the pons (P). These cells form the pontine gray nuclei, which eventually project to the opposite cerebellar hemisphere beneath the Purkinje cell layer (PL) as mossy fiber terminals (MF). Note that germinative cells originating in the upper rhombic lip (URL) simultaneously form the external granular layer (EG) that coats the external surface of the cerebellum. Progeny of cells from the external granular layer migrate inward after their last division, across the molecular layer (ML), and eventually contact 'waiting' mossy fiber terminals. *B* Enlargement of the area encompassed by the rectangle in *A* displays at higher magnification the cytological organization of the corpus pontobulbare. Note that cohorts of migratory neurons (MN) and their leading processes (LP) move along axons originating from previously generated pontine neurons (a_1) that project to the contralateral hemisphere; they pass by radial glial fibers (RG) or axons of the corticospinal and corticobulbar system that run in a rostrocaudal direction (a_2). Simultaneously, the growth cones (GC) of the most recently generated neurons situated in the contralateral pontine neurons move in the opposite direction, toward the cerebellar peduncle (CP in *A*). (From Rakic [48]).

philic system, there must be more than one set of neurophilic adhesion molecules.

It may be noteworthy that, unlike gliophilic migration, which proceeds radially or perpendicularly to the pial surface, neurophilic migration usually takes up a tangential direction, oriented parallel to the pia. Furthermore, in most cases so far reported, tangential migration occurs closer to the outer (pial) than to the inner (ventricular) surface. Finally, tangential migration has been observed mostly in places where neurons transfer across regional or segmental boundaries or even where they move from one side of the midline to another. In some cases, neurons that originate outside the central nervous system migrate to the brain following axonal pathways. For example, cells from the olfactory pit appear to migrate and enter the central nervous system along the vomero-nasal nervus terminals[63,68]. A dramatic and unique example of cell migration that crosses regional boundaries within the central nervous system is the displacement of neurons from the telencephalon to the diencephalon during formation of the pulvinar in the human fetal thalamus. In this case, neurons generated in the mitotically active ganglionic eminence of the telencephalon migrate via the corpus ganglio-thalamicum to the diencephalon[43,65]. It is likely that another set of adhesion molecules, in this case perhaps homotypic and neurophilic, provides for both recognition and attraction between apposing neuronal surfaces. Again, the decision to move along neuronal rather than glial surfaces may be a summary result of a differential quantitative contribution of distinct adhesion molecules; e.g. the relative number of neurophilic and gliophilic molecules distributed on the surface of the postmitotic cell and its immediate neighbors.

Biphilic migration

The cerebellar granule cell is a maverick among migrating neurons and, perhaps, may be classified best into the biphilic category. In this complex case, the granule cell undergoes an extraordinary morphogenetic transformation during which it extends two classes of neurites[40,55]. A single descending neurite emanating from the cell soma is clearly gliophilic as it follows exclusively Bergmann glial fibers (the cerebellar equivalent to radial glial fibers in the telencephalon). In contrast, their two horizontal processes grow preferentially along axons belonging to previously generated granule cells (parallel fibers of the molecular layer) and, therefore, behave as neurophilic[48]. These two processes, as viewed by the freeze fracture method, have different membrane properties that are morphologically distinct[15] and presumably have a different molecular composition that allows recognition and attraction between the glial and neural surface. This observation poses a still unsolved cell-biological problem of maintenance of biochemical specificity. What are the cytoplasmic organelles that direct these two types of surface molecules differentially in the two classes of simultaneously growing neurites of the same cell?

The morphologenetic transformation and translocation of the granule cell is best described by a 'four-dimensional' diagram which, in addition to the three spatial parameters, provides also the time dimension[40,42]. As presented in figure 6, shortly after its final cell division in the external granular layer, a newly generated granule cell takes a position in the deep part of the external granular layer, contacting a nearby Bergmann glial fiber (cell 1 in fig. 6). It then transiently assumes a bipolar shape (cell 2) by emitting two horizontal cytoplasmic processes that run parallel to pia, in the longitudinal plane of the cerebellar folium, at a right angle to the growing Purkinje dendritic tree. These horizontal processes extend exclusively along the surface of parallel (axonal) fibers. Next, the granule cell becomes tripolar by forming a third, vertical cytoplasmic process, which elongates in close apposition to the shaft of Bergmann glial

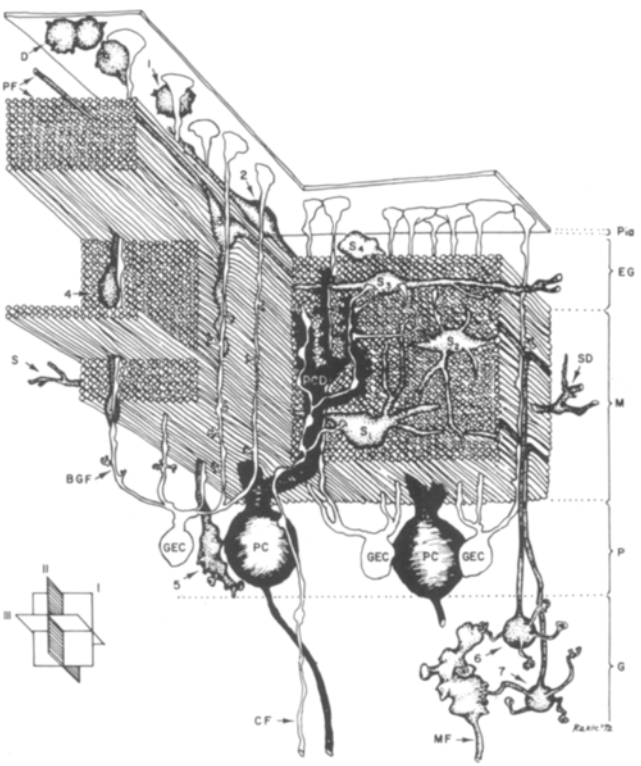

Figure 6. 'Four-dimensional' (time and space) reconstruction of the developing cerebellar cortex in the rhesus monkey. The geometric figure in the lower left corner indicates the orientation of the planes: I, transverse to the folium (sagittal); II, longitudinal to the folium; III, parallel to the pial surface. The thicknesses of the layers are drawn in their approximately true proportions for the 138-day-old monkey fetus, but the diameters of the cellular elements, particularly the parallel fibers, are exaggerated to make the reconstruction more explicit. Abbreviations: BGF, Bergmann glial fiber; CF, climbing fiber; D, dividing external granule cell; EG, external granule layer; GEC, Golgi epithelial cell (Bergmann glia); G, granular layer; M, molecular layer; MF, mossy fibers; P, Purkinje layer; PC, Purkinje cell; PCD, Purkinje cell dendrite; PF, parallel fiber; S_{1-4}, stellate cells; ST, stellate cell dendrite. A description of the temporal and spatial transformations of the postmitotic granule cells (designated with numbers 1–7) and stellate cells (S), as well as other details, are given in Rakic[40,42].

cells (cell 3). Attraction between the leading process of granule cell and the surface of glial cell has been originally implied from electron microscopic analysis[40] and is elegantly demonstrated in tissue culture of dissociated embryonic cerebellar cells[19]. When the descending process reaches the appropriate length, the nuclear part of the granule cell becomes translated within its volume. This poses another unsolved cell-biological problem; what is the mechanism that propels the cell nucleus and the surrounding cytoplasm to move always to the descending neurite. As a result of this nuclear translocation, the entire soma passes across the complex and synaptically interconnected molecular layer[40]. Several granule cells in succession follow the same Bergmann glial guide, as described above for the gliophylic migration of neurons to the developing cerebral cortex.

Change in position and shape of cerebellar granule cells provides an example of the role that neuronal cell migration has in the development of the cellular and synaptic architecture of a given brain structure. For example, the horizontal and vertical segments of the cell trailing behind the nuclear region become the parallel fiber and vertical shaft, respectively, of the T-shaped granule cell axon that form synapses upon Purkinje dendrites. When granule cells reach Bergmann cell somas at the level of the Purkinje cell layer, they detach from their guiding fiber and take up final positions in the internal granule layer where they form synapses, mostly with fibers from the brain stem (fig. 6, cells 5, 6, and 7). The depth of each parallel fiber in the molecular layer reflects the time of initial differentiation of its granule cell soma from round to bipolar. The deepest parallel fibers derive from the first granule cells to migrate, and those closest to the pial surface from the last, like the archeological strata of a buried civilization[40]. Generation of the shape of granule cell is, therefore, a multistep process which requires surface cues and reorganization of the macromolecules in the cytoplasm and on the plasma membrane. Examination of the sequence of these cellular and molecular events reveals how they determine indirectly cell position and the pattern of synaptic organization of the cerebellar cortex.

Molecular mechanism of neuronal cell migration

In the past decade, it has become evident that morphoregulatory molecules that bind a cell surface to the neighboring cells, or to the substrate, control several basic developmental events[11]. Most of these molecules are glycoproteins and are operationally called cell or substrate adhesion molecules. Selective displacement of migrating neurons along preferred surfaces, as described above, can be also best explained by the presence of surface affinities mediated by several classes of such adhesion molecules[46]. According to this model, membrane components with specific adhesion properties undergo local exo-endocytic recycling at the tip of the leading process of migrating neuron. During its expansion, the membrane bound cytoplasmic process extends preferentially along the surface with the highest binding affinity, which in the central nervous system, can be either glial or neuronal[48]. Although our understanding of selective affinities is still incomplete, separate interactions may be mediated by several sets of molecules derived from several functionally distinct adhesion component families. The first group may comprise adhesive membrane components which mediate and maintain the coherence of neural structures, and may be analogous in function to the classical junctional components identified in peripheral epithelial tissues[11]. The second group of adhesive membrane components may participate in the more dynamic process of cell recognition and selective cell migratory behaviors observed only during neural migration[38,48].

The initial model of differentiated surface-mediated adhesion was designed to account for both displacement of cell soma as well as selection of the appropriate cell migratory pathway[41,46,48]. Different lines of evidence support this model, including the ultrastructural characteristics of migrating cells, their pattern of surface growth, rate of cell displacement, and the consequences of genetic and experimental alteration of cell movement[5,20,50]. Several candidate molecules have been isolated and their effect tested on adhesion and cell movement in vitro[9,12,16,19,38,58]. Presently, the working hypothesis is that the adhesion must depend on heterotypic molecules that have different, but complimentary, binding sites on migrating neurons and on their substrates. So far candidate molecules have been identified and isolated for neuronal cells; however, their counterpart on the glial surface so far has not been found. Recent in vitro studies indicate that the molecules responsible for recognition between neurons and glial cells may not be region specific; as, for example, in tissue culture both cerebellar and hippocampal granule cells can migrate along radial glial fibers derived from either structure[16]. Because adhesion molecules may be present on the cell surface in a small quantity and only during restricted, short ontogenetic periods, their isolation has been difficult. In fact, it is still not definitely determined whether the molecules are fixed integral or peripheral cell surface components, or spread as gradients in the extracellular matrix (e.g. Liesi[30]). Thus, although much new information is already emerging, many aspects of the complex cell interaction during their displacement is still unclear. However, molecular mechanisms of neuronal migration is the focus of intensive research in several laboratories, and one can expect considerable progress in the next few years.

Radial unit hypothesis and concept of protomap

Analysis of proliferative and migratory behavior of neurons provided the basis for the *radial unit model* of cortical development during ontogeny and evolution[49]. In

this model, the proliferative zone at the surface of the cerebral ventricle is regarded as a two-dimensional mosaic or protomap that consists of *proliferative units* which contain several clones of dividing cells. Each proliferative unit produces postmitotic cells that move to the cortex along a common glial fascicle. This mechanism preserves as nearly as possible the neighbor relationship of cells that share a common site of origin. After entering at the cortex, migrating neurons pass by earlier arrived neurons and form radial *ontogenetic columns*. Thus, the protomap of radial units may help to establish a genetic blueprint for a species-specific cytoarchitectonic map of the cerebral cortex.

It was proposed that one set of controlling genes provide general instructions for individual and species-specific changes in the proto-map of the ventricular zone while another set of genes control cell production in the proliferative units[49]. These regulatory genes may arrest the cell cycle and trigger transcription of cell class-specific molecules as well as adhesion molecules that lead them to the cortical plate. According to the radial unit hypothesis, the surface area of each cytoarchitectonic region of the cerebral cortex in each species and individual depends on the number of contributing proliferative units. In contrast, the thickness of the cortex in each region depends on the number of cell divisions generated within the units. In support of this developmental model, experimental and neuropathological data indicate that each step (formation of proliferative units, formation of ontogenetic columns, and formation of cytoarchitectonic areas) can be separately affected by genetic defects or by extrinsic factors[49,50].

Not everyone agrees. It has been suggested that all cortical neurons are equipotential and that their phenotype is determined exclusively by the type of afferents they eventually receive [7,25,36]. However, several lines of evidence suggest that at least some aspect of neuronal differentiation into specific phenotypes begins prior to their entrance into the cortical plate and may proceed to a considerable extent independently of the input[5,22,34,64]. This is a conceptually and practically important point since, if all cortical cells are equipotential, there would be no need for their elaborate and position-dependent migration into specific areal or laminar positions. Early cell class-specific signs of phenotypic differentiation that is initiated prior to or during migration have been observed in many neuronal systems using a variety of molecular markers and position-specific antigenes[23,24,31,59,63,68].

It should be emphasized that the concept of protomap does not negate the significant role of afferents in the formation of cytoarchitectonic areas. The prefix proto- denotes a provisional and modifiable nature of such a map. Our recent experiments in monkey fetuses, as well as the pattern of cortical malformations reported in animals and humans, clearly show that the final number of ontogenetic columns devoted to each area and, therefore, the size of cytoarchitectonic areas, can be modified by extrinsic factors[25,49–51]. Thus, genetic alteration or lesion of a distant but synaptically related structure, or reduced input to the cortex occurring at the critical developmental period, may exert structurally and functionally significant changes that provide the setting for a new neural relationship, the outcome of which is the formation of unique cytoarchitectonic areas. Therefore, according to our model, the protomap provides the basic genetic program for a species specific pattern of cytoarchitectonic map whereas extrinsic factors influence the formation of individual variations within the constraints of the protomap[51]. Recent advances in neurobiological techniques including retrovirus gene transfer, hetereotypic transplantation and fine ablations during the critical stages of embryogenesis may enable testing of the radial unit hypothesis and will eventually provide a deeper insight into genetic and epigenetic regulation of cortical parcellation[34,49,50].

Disorders of neuronal migration

Understanding of the molecular and cellular mechanisms of neuronal movement is not only of theoretical but also of substantial biomedical importance. The process of neuronal cell migration is highly sensitive to various physical (e.g. ionizing radiation, heat), chemical (e.g. toxins, various drugs, excessive use of alcohol), and biological (some viruses) agents as well as to genetic mutations. As a result, defective neuronal migration is considered as the major cause of both gross brain malformations and more subtle abnormalities, such as abnormal neuronal positioning or altered patterns and sequence of synaptic circuits[3,4,50,66]. An instructive example may be provided by the consequences of radiation on human fetuses in utero which occurred during the Hiroshima and Nagasaki atomic explosions. Postmortem examination of the cerebrum of adult individuals who had been irradiated as fetuses during midgestation (10–17 weeks) showed a curtailment of neuronal migration that resulted in massive heterotopia, attenuation of superficial cortical layers and a reduction in callosal and cortico-cortical connections[50,62]. These are exceptional, highly dramatic cases. The most frequent migratory abnormalities in humans may be revealed by the presence of solitary ectopic neurons that have failed to reach their targets. Small foci of migratory defects have been implicated in genetic disorders such as developmental dyslexia[14]. More severe malformations, including microencephaly (small brain), schizencephaly (split brain), lissencephaly (smooth cerebrum, without convolutions), macrogyria (large convolutions) and polymicrogyria (small convolutions), may be in full or in part a consequence of defective migration and abnormal settling of nerve cells[3,4,13,66].

As a consequence of the lack of knowledge about the genetic and environmental causes of most cortical malformations, their classification was traditionally based on the cortical morphology at the time of autopsy[66]. On

the basis of the radial unit hypothesis, some cortical malformations that are partially or fully caused by defective neuronal migration can be classified into two basic categories[49, 50]. The first category comprises malformations in which the number of radial units in the cortex is reduced while the number of neurons within each ontogenetic column remains relatively normal. It can be expected that defects which belong to this category result from a reduction in the number of proliferative units at the time they are being formed – in humans, within the first 6 weeks of gestation, before the onset of corticogenesis. Once proliferative units in the ventricular zone are established, albeit in fewer numbers, each unit can produce a normal, or even greater than normal, number of neurons which become crowded in a cerebral vesicle of smaller size. It could be expected that the cortex would have a smaller surface area in spite of normal or enlarged thickness and massive neuronal ectopias. Some of these features are observed in certain severe human malformations, such as lissencephaly and pachygyria. However, we suspect that similar mechanisms may cause more subtle defects that are difficult to detect without proper quantification.

The second category consists of malformations in which the initial formation of radial units is not affected while the number of neurons within ontogenetic columns is reduced. The defect in this category should begin after the first six fetal weeks when the normal complement of proliferative units has already been established. Such malformations can be caused by interference with cell proliferation via intrinsic (genetic) or extrinsic factors (irradiation, drugs, or viral infections). Diminished production of neurons in the proliferative units results in fewer neurons within ontogenetic columns and the cortex is, therefore, thinner. The number of neurons in each ontogenetic column within the cortex could be affected either by cell death or by failure of migration. In the latter case, some neurons may survive in ectopic positions within the white matter. All of these abnormalities can be observed in several types of malformations, including so-called polymicrogyric brain[4, 66]. However, the majority of cortical malformations are a mixture of both categories, and the proposed classification refers only to the basic cellular mechanism involved in their pathogenesis. It is clear that further research is needed to gain deeper insight into the genetic and molecular mechanisms underlying normal and abnormal neuronal migration if we are to understand the making of our brain.

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