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Migratory patterns of clonally related cells in the developing central nervous system

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Summary. Neurons and glioblasts that arise in the ventricular zone migrate to form discrete nuclei and laminae as the central nervous system develops. By stably labeling precursor cells in the ventricular zone, pathways taken by different cells within an individual clone can be described. We have used recombinant retroviruses to label precursor cells with a heritable marker, the *E. coli lacZ* gene; clones of lacZ-positive cells are later mapped histochemically. Here we review results from three regions of the chicken central nervous system – the optic tectum, spinal cord, and forebrain – and compare them with previous results from mammalian cortex and other regions of the vertebrate CNS. In particular, we consider the relationship between migratory patterns and functional organization, the existence of multiple cellular sources of migratory guidance, and the issue of whether a cell's choice of migratory pathway influences its ultimate phenotype.
Key words. Optic tectum; spinal cord; forebrain; radial glia; retrovirus; lineage.

Introduction

Structurally diverse regions of the central nervous system arise from an apparently homogeneous neuroepithelium. One of the first steps in the histogenesis of each region is the migration of cells from the neuroepithelium to their final sites of differentiation. These migrations result in the delivery of appropriate numbers and types of cells to specific sites at particular times. Differences among regions in organization and function may arise in part from

differences in migratory patterns. Furthermore, it has been suggested repeatedly that the environment cells encounter as they migrate influences the phenotype they acquire in maturity (see McConnell[41] and Price[51] for reviews). For these reasons, a fuller appreciation of migratory pathways and dynamics is likely to aid in understanding how structural and functional attributes of the nervous system arise.

The bulk of our knowledge of neural migration derives from early light microscopy, and from subsequent ultrastructural and autoradiographic investigations (reviewed in Sidman and Rakic[66]). From histological studies at the light and electron microscopic levels, it has been possible to obtain revealing views of migrating cells, and to infer some aspects of cells' migratory history from series of such images. ^{3}H-thymidine labeling studies, in which cells are labeled at their last division in the ventricular zone, have given information about when a cell is born, and have permitted calculation of the migratory rates and patterns adopted by cells born at a given time. However, this method provides information about the average behavior of large cohorts of cells born at the same time and not about the specific movements of individual cells. More recently, it has become possible to mark small cohorts of clonally related cells by labeling dividing neuroepithelial cells. All of the cells within each clone arise from a single point in the ventricular zone, although individual cells may be born and initiate migration over a protracted period. The migratory pathway taken by the individual cells may then be inferred from the arrangement of the labeled cells with respect to their point of origin.

Two current methods are applicable for marking clonal cohorts. The first calls for directly injecting single neuroepithelial cells with a fluorescent or enzymatic label[6, 22, 27, 74] (fig. 1 A). The strength of this technique is that one can choose particular cells for injection. However, access to cells is difficult in many systems. Also, the labels dilute as the cells divide and elaborate processes, so observations have to be made fairly soon after injection, which is often before cells have reached their final destinations or acquired their definitive phenotypes.

The second method, which we have used, makes use of recombinant retroviruses to label the precursors[53, 63] (fig. 1 B). Although the ability to specify the labeled precursor is lost, the label is stably expressed and allows progeny to be traced indefinitely. Furthermore, virus can be injected near, and will infect, cells too small or too deeply buried to be impaled by a microelectrode. We originally devised the retroviral method to study cell lineage, and have used it for this purpose in several tissues. However, we soon realized that the distribution of cells within a clone provided interesting information about the migratory routes that clonally related cells follow from their sites of origin to their destinations. In this paper, we review our studies of migratory patterns in three areas of the chicken nervous system: the optic tectum[11, 13, 15], the spinal cord[32, 33], and the forebrain[14]. We then compare these results to those obtained from both retroviral and conventional histological studies in other areas of the CNS. Because most of the data reviewed here were obtained with the retroviral method, we precede our discussion of migration per se with a brief description of this method.

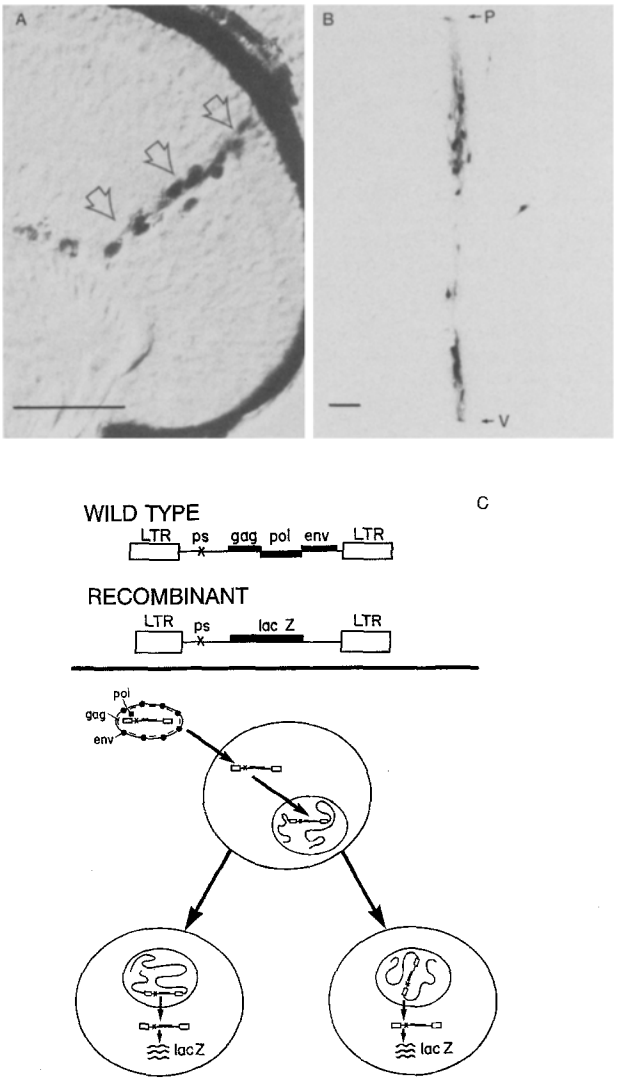

Figure 1. Labeling of clonally related cells. *A* A clone of cells from *Xenopus* retina, labeled by injecting HRP directly into a retinal precursor[22]. Bar, 50 μm. *B* A clone of lacZ-positive cells in an E8 chicken optic tectum, generated by injecting a recombinant retrovirus bearing the *lacZ* gene into the lumen of the neural tube at E3. Bar, 50 μm. *C* The retroviral method for labeling progenitors *Top*: Structural genes of the wild type virus are replaced with the *E. coli lacZ* gene to produce the recombinant retroviral genome. *Bottom*: When a recombinant retrovirus infects a dividing cell, the viral genome is reverse transcribed, integrated into the host cell's DNA, and handed down to the progeny of the cell, which can then be identified histochemically[62].

Retroviruses as heritable markers

The use of recombinant retroviruses as lineage markers exploits the fact that part of the wild-type retroviral life cycle is the incorporation of retrovirally derived DNA

into the host cell genome. Once integrated, the 'provirus' is replicated during normal cell division, and is transcribed and translated to make new retroviruses. The retroviruses used for lineage tracing differ from the wild type viruses in that genes encoding structural proteins (which are necessary for virus production) were removed, and replaced with the *E. coli lacZ* gene (which encodes a β-galactosidase that we term lacZ; fig. 1 C, top). These recombinant retroviral genomes are packaged into infectious virus particles in helper cell lines that carry the genes for the necessary structural proteins. When such a virus infects a dividing cell, its genome is integrated into host DNA and handed down to the cell's progeny (fig. 1 C, bottom). But, because the structural genes are not present in the host genome, new virions cannot be made, and the viral genome is restricted to the progeny of the originally infected cell. The *lacZ* gene can be transcribed and translated, however, and lacZ-positive cells are readily identified with a histochemical stain that turns cells blue.

To use this method in embryos, one injects small numbers of virus particles into the lumen of the neural tube where the virus has access to dividing precursor cells at the ventricular surface. One then examines embryos for the presence of lacZ-positive cells at subsequent developmental stages.

In every part of the CNS we have studied, lacZ-positive cells have been found in spatially restricted clusters at early developmental stages[11, 13, 15, 32, 33, 36]. We have several reasons to believe that these clusters are clones. First, small numbers of virions are injected into each embryo. Second, the clusters of labeled cells are often separated by large expanses of unlabeled tissue, making it unlikely that two (or more) independent viral infections give rise to each cluster. Third, both in vitro[36, 63] and in vivo[13, 71], varying the number of virus particles injected affects the number of clusters generated but not the number of cells in a cluster. If many clusters were polyclonal, changing the viral titer should affect both cluster number and cluster size. Finally, when two different retroviral markers are injected simultaneously[11, 32], all of the cells in each cluster express either one viral marker or the other. If clusters were polyclonal – i.e., arose from independent infections by more than one virion – both markers should have been expressed in some clusters. Thus, use of the retroviral marking system permits one to identify the cells that comprise a clone, and to infer their migratory history from their distribution.

Optic tectum

The avian tectum is a highly laminated structure that serves as the major center for the processing of visual information[26]. The tectum arises from the apparently homogeneous neuroepithelium of the dorsal mesencephalon. In the chick, the first neurons are born on embryonic day (E) 3 (counting the first day of incubation as E1)[31]. At about E5, a fiber layer appears superficially. As postmitotic neurons leave the ventricular zone, they migrate through this fiber layer (the intermediate zone) to their final positions in the tectal plate. There, they acquire their distinctive morphologies. As migration proceeds, the tectal plate is gradually divided into the 13 most superficial of the 15 definitive laminae; the two other laminae derive from the intermediate zone, which eventually becomes the major tectal white-matter tract (layer 14), and a thin layer of neurons that lie adjacent to the ventricle (layer 15)[9, 10, 30, 31, 60].

Lineage studies in the optic tectum show that a single precursor cell can give rise to neurons that lie in many different laminae and have different morphologies[13]. The same precursor cells that give rise to various neuronal types also give rise to at least two histochemically different kinds of astroglia[11]. Currently, there is no evidence for the existence of committed progenitors that produce individual cell types in the tectum.

Ventricular zone mixing

To learn about early events in tectal neurogenesis, we injected tecta with retrovirus at varying times during the period when neurons are born, and then examined them all at the same stage: on E8, after neurogenesis is nearly complete, but before laminae have formed. When embryos were injected with virus around Hamburger-Hamilton[17] stage 17 (E3), we found that lacZ-positive cells were arranged in radially aligned clusters, generally only one strand wide (fig. 1 C), but occasionally made up of more than one strand. (A few cells also appeared offset from the radial axis; these are discussed further, below.) Although the radial arrangement of cells was not altered by injecting virus either earlier or later in development, the width of the clone (i.e., the number of radial strands making up a clone) increased with earlier injections and decreased with later injections. At the latest stages tested (E4), clones often consisted of just a few cells arranged radial to one another in a single strand. These results indicate that between our earliest injection times (around stage 11, E2) and E3 (stage 17), horizontal displacement of cells within the plane of the ventricular zone ceased, and only vertical displacement of progeny continued to occur (see fig. 2 A). This restriction was also evident when we examined differences in clonal widths along the known tectal developmental gradient: clones from rostral and ventral areas, which were more mature when labeled, were on average narrower than caudal and medial clones[13]. The ventricular zone restriction occurs, then, before stage 17, when the first neurons are born[31]. This result is consistent with the idea that proliferative cell divisions occurring before any neurons are born populate the ventricular zone with multipotential progenitors, whereas a 'stem-cell' mode of division generates postmitotic neurons and glioblasts (fig. 2 B).

Early migration of postmitotic cells

After they are born in the ventricular zone, neurons and glioblasts migrate towards the pia to form the tectal lam-

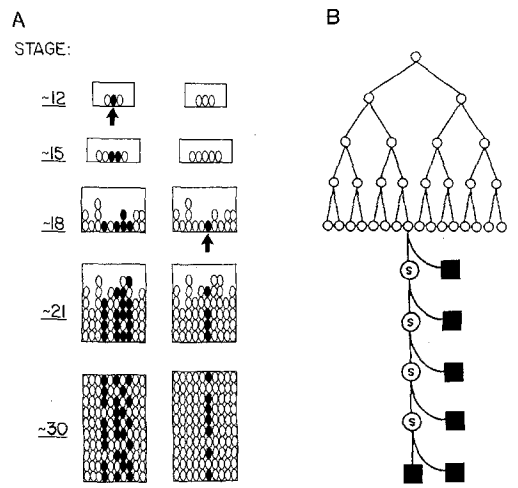

Figure 2. *A* Diagram showing how labeled cells are displaced after viral infection at particular developmentel stages. Injection on E2 (stage 12) results in wide clones with many strands. Injection on E3 (stage 18) results in narrow clones. Thus, dividing cells cease to be displaced within the plane of the ventricular zone between E2 and E3, but radial displacement continues. *B* Modes of cell division. Proliferative cell divisions (top) may correlate with the period of tangential cell displacement in the tectum, whereas 'stem-cell' divisions (bottom) may correlate with the period of radial displacement.

inae. To follow these migrations, we injected a series of embryos at stages 15–17, then sacrificed them at various stages. The migratory pathways that we observed are sketched in figure 3.

As long as the cells remain in the ventricular zone (until ca E5–6; fig. 3 B), they are arranged in strict radial arrays. Around stage 30 (E7), however, one or a few cells emerge from many radial arrays, beneath the pial surface. These cells then begin to migrate orthogonally to the radial arrays (fig. 3 C), forming queues of tangentially migrating cells. This is an oriented migration, with most cells moving laterally, but a substantial minority migrates medially. There is almost no rostrocaudal deviation from the mediolateral pathway. Most of the cells that choose the tangential pathway eventually differentiate into one neuronal subtype: the 'multipolar' cells that make up the major tectal efferent cell layer (the stratum griseum central, or layer 13). Interestingly, Domesick and Morest[9] and Puelles and Bendala[54] inferred a tangential migratory route for this cell type from Golgi studies, and Senut and Alvaredo-Mallart[65] observed lateral mixing in layer 13 in chick/quail tectal chimeras.

Only a small percentage of the cells in each clone migrates tangentially. The rest migrate radially, passing through the intermediate zone (where the tangential migration occurs), to form the tectal plate (ca E8; fig. 3 D). About a day later, however, another tangential migration occurs. Small cells migrate away from the radial arrays, again leaving their clones of origin just beneath the pial surface. In contrast to the oriented tangential migration of the multipolar efferent cells, these small cells scatter in all directions (fig. 3 E), and are likely to become astrocytes. The cells that remain in the radial arrays maintain

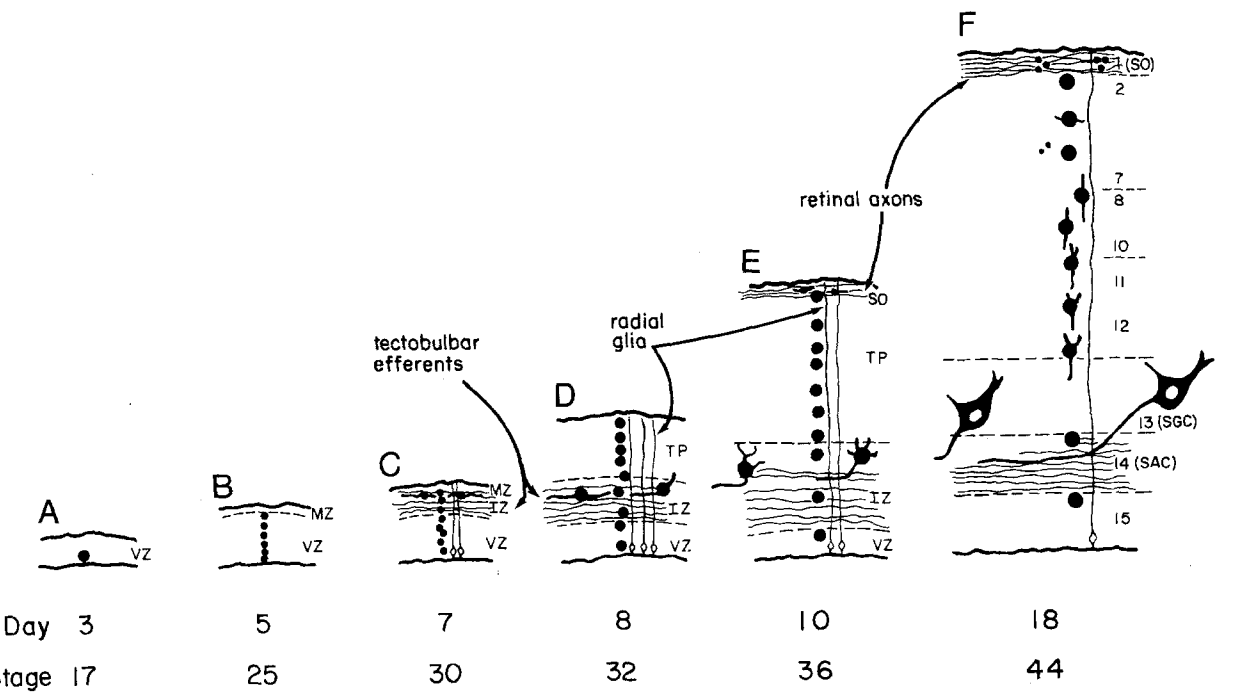

Figure 3. Migratory patterns in the optic tectum. *A* Embryos are injected with virus on E3 to infect dividing progenitors at the ventricular surface. *B* At E5, when the tectum consists of a ventricular zone (VZ) and a marginal zone (MZ), clonally related cells are arranged in strict radial arrays. *C* By E7, the MZ is thicker and cells have begun to migrate tangentially within it. *D* The majority of cells ignore the tangential pathway and migrate radially throught the fiber layer (now the intermediate zone, IZ), to form the tectal plate (TP). *E* A second tangential migration occurs about E10, when presumptive astrocytes leave the radial axis and scatter in all directions. SO, stratum opticum. *F* The radial arrangement of cells is maintained during the rest of embryogenesis.

their arrangement throughout embryogenesis, and eventually differentiate into a range of neuronal types, and into astrocytes (fig. 3F).

Thus, migration in the tectum is characterized by three migratory pathways: the radial component, which comprises the majority of cells; the oriented tangential migration, a pathway followed by a particular subtype of tectal neurons; and the scattered superficial migration of cells that we have tentatively identified as astroglia[15]. Moreover, all of these migratory routes can be taken by cells that arise at a single site from a single progenitor.

Migratory guides

The precise geometry of migration in the tectum suggested the cells were using extrinsic cues for guidance. We have been able to identify candidate guidance structures for each of the migratory components.

The radial component is, unsurprisingly, likely to be guided by radial glia. Work by Vanselow et al.[72] has shown that radial glia are present in the tectum from E8 until after hatching. Using a variety of histological and immunohistochemical techniques, we have studied the fasciculation patterns of the radial glia, and find that the glial fibers are bundled into regularly spaced palisades as they cross the intermediate zone. Simultaneous visualization of radial glia and migrating cells demonstrates a close co-localization of migrating cells with the radial glial bundles, at least within the intermediate zone[14,15].

The tangential migration of multipolar cells seems to be guided by fascicles of axons. The tangentially migrating cells travel along fasciculated bundles of fibers that course laterally in the tectum toward subtectal targets[15]. These fiber bundles are the first efferent tectal fibers, and arise from the multipolar efferent cells[12]. Thus, the migrating multipolar cells are traveling either along the axons of other multipolar cells, or are extending their own axons early, and are translocating their cell bodies through them. The fact that some cells move medially from their parent clone, although all multipolar efferent axons course laterally, suggests that the cells are migrating on other axons rather than through their own.

The factors that initiate the tangential migration of presumptive astrocytes are not known. However, this migration begins at just about the time that retinal afferents grow into the tectum and occurs in the subpial lamina that the retinal axons traverse[12,57]. Thus, this migration may be stimulated or guided by retinal axons. Enucleation experiments are in progress to test this hypothesis. Whatever the outcome, however, it is clear that cells migrating along three different pathways in the tectum depend on and differentiate among cues from three different sources.

Spinal cord

The mature spinal cord is organized very differently from the optic tectum, in that it has both laminar[5,39] and nuclear[25,40] features, but no apparent radial component to its structure or to its functional organization. Given how different these two structures ultimately become, it has been interesting to discover that there are marked similarities in the migratory paths taken by their cells early in development.

As in other parts of the central nervous system, chick spinal neurons are born in the ventricular zone and, after leaving the mitotic cycle, migrate outward. Using recombinant retroviruses to label precursors in the ventricular zone, we showed that single cells give rise to progeny in both gray and white matter and in more than one longitudinal column[32]. For example, progenitors that give rise to motoneurons in a particular motor pool or column also give rise to motoneurons in other pools and columns, to a variety of other types of neurons, to glia (both astrocytes and cells tentatively identified as oligodendrocytes), and to ependymal cells. These results demonstrate that in the spinal cord, as in the tectum, the neuroepithelial cells are multipotential even shortly before the neurons are generated.

Ventricular zone mixing

As in the tectum, tangential movement of cells within the ventricular zone of the spinal cord becomes limited by the time the first neurons are born. When animals are injected at stage 8 (E2) and examined at stages 21–27 (E4–7), clusters of clonally related cells span up to 220 μm (1 1/2 segment lengths) along the rostrocaudal axis (fig. 4A). In contrast, injection at stages 12–14 (early E3) generally results in clones with a considerably smaller dispersal[33]. Clonally related cells within the ventricular zone usually form single, discrete, planar clusters (fig. 4B) with rostrocaudal spreads of less than 40 μm. These results suggest that little mixing occurs along the rostrocaudal axis of the ventricular zone after stages 12–14, and, in particular, that cells within the ventricular zone are unlikely to migrate from one segment to another after stages 12–14. This is of interest because somatic and autonomic motoneurons appear to be endowed with knowledge of their positions along the rostrocaudal axis such that their projections map systematically onto the rostrocaudal axis of their targets[29,56]. Furthermore, whereas injection of embryos at stage 8 sometimes results in the labeling of clones that span the entire dorsoventral extent of the ventricular zone at stages 21–27 (fig. 4A), injections at stages 12–14 usually label cells that are radially arrayed within the ventricular zone, with very limited dorsoventral spreads (fig. 4B). After stage 12–14 injections, we occasionally find clones that are restricted to a narrow transverse plane but contain discrete streams of cells separated along the dorsoventral axis. Thus, the dorsoventral restriction either may not be as rigid as is the rostrocaudal restriction or may occur later and still be incomplete at stages 12–14. Just as the rostrocaudal restriction limits the dividing neuroepithelial cells to specific segments or subsegments as development pro-

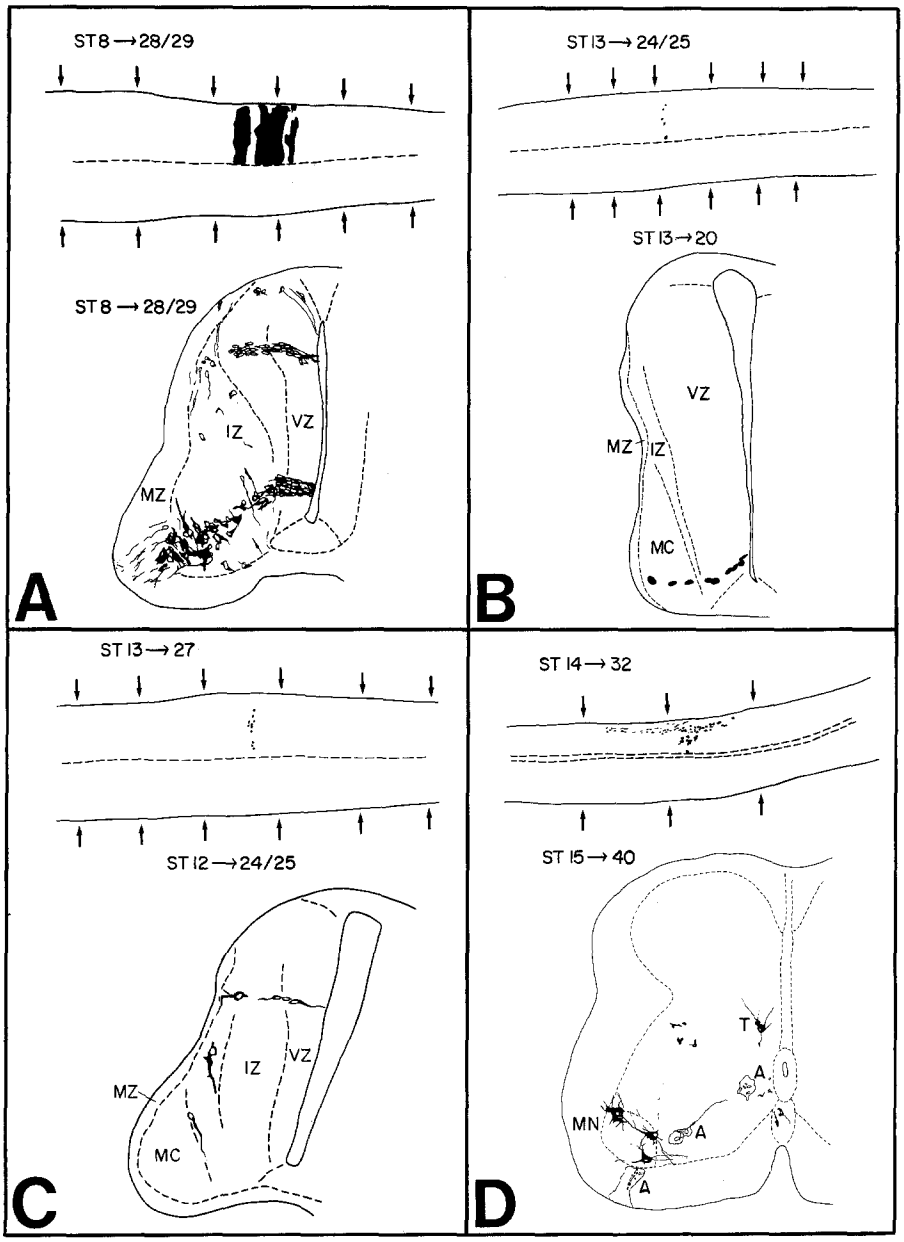

Figure 4. Migratory patterns in the spinal cord. Reconstructions of clones labeled and analyzed at the stages indicated. Top drawings in each part are longitudinal views (from dorsally in *A–C* and laterally in *D*); lower drawings are transverse views. IZ, intermediate zone; MC, motor column; MZ, marginal zone; VZ, ventricular zone *A* Early injections label large clones with wide dispersal of cells along both rostrocaudal and dorsoventral axes. *B* Later injections label smaller clones with very restricted spread along both the rostrocaudal and dorsoventral axes. Initially, cells migrate radially out of the ventricular zone. *C* Following the initial radial migration, some of the cells in a dorsal clone turn 90° and migrate ventrally along a circumferential pathway. *D* At later stages, oligodendrocytes or their precursors migrate rostrally and caudally in the white matter, resulting in a 'T'-shaped clone (top figure). The lower figure shows a clone containing 3 motoneurons (MN) a preganglionic neuron in the column of Terni (T), many astrocytes (A), and unidentified cells.

ceeds, the dorsoventral restriction may confine cells to either the ventral (basal) or dorsal (alar) parts of the spinal cord, which, in general, process separate types of information (motor and sensory, respectively). Therefore, although the neuroepithelial precursors remain multipotential with regard to cellular phenotype, they become spatially restricted to populate limited zones of the spinal cord as development proceeds.

Migrations

In the spinal cord, most neurons are born between early E3 and E8 (stages 14–34)[10, 21, 28]. With the exception of one population of cells described below, neurons in the basal part of the spinal cord are born before those in the alar cord. The first-born cells form from bipolar neuroepithelial cells that extend processes into the marginal zone, lose their ventricular attachments, and translocate

their somata into their distal processes[19,60]. Later-born cells appear to migrate radially across the medial intermediate zone to reach their final sites of differentiation[16]. As with the ventral-to-dorsal gradient of birthdates, this medial portion of the intermediate zone disappears in the ventral spinal cord before it does dorsally (E6 compared with E9)[16].

From our retroviral studies, it is clear that cells migrating out of the ventricular zone follow a radial pathway[33]. Injections of virus into the lumen of the neural tube at stages 12–14 (early E3) followed by reaction for lacZ at stages 21–29 (E4–7) produces clusters of labeled cells arranged in a linear fashion, often extending from the ventricular zone through the intermediate zone toward the external limiting membrane (fig. 4B). The mode of migration is consistent with migration along radial glia. Although radial glia are known to exist in the chick spinal cord[60,67], it is not known whether migrating neurons use them as guides, as they do in the mammalian cerebral cortex and tectum (compare references 7 and 35). Experiments are underway to determine whether this is the case. In any event, clonally related cells originating in a single site in the ventricular zone frequently migrate along a common, stereotyped radial pathway.

Following the radial migration, a secondary, nonradial migration sometimes occurs. The idea that a group of cells of dorsal origin migrates ventrally was previously postulated by Langman and Haden[28] on the basis of the disappearance of a population of early-born dorsolateral neurons. Retroviral studies provide direct support for this idea[33]. After clones of cells migrate radially away from the dorsal portion of the ventricular zone, some of the cells closest to the pia turn ventrally and follow a tangential pathway parallel to the surface of the spinal cord (fig. 4C; also see 4A). They follow a group of circumferentially coursing axons that extend ventrally to the anterior commissure and are among the first axons to form in the spinal cord[20,48,60]. Many of these axons originate in a population of very early-born, dorsolaterally located interneurons[19,28]. The ventrally streaming cells are themselves dorsolaterally located and many of them extend their own axons along the circumferential pathway, suggesting that they may be commissural interneurons. Thus, the ventrally migrating cells may be following the axons of homotypic cells; alternatively, the cells may be extending their own axons within the commissural pathway and then translocating their cell bodies through the axons. In either case, it appears that many spinal cord neurons initially migrate radially, perhaps along radial glial guides, but then turn in a nearly orthogonal direction and follow circumferentially oriented axons.

Some cells originating in the ventral portion of the spinal ventricular zone also undergo a tangential migration, but they migrate dorsally rather than ventrally[33]. This is seen less frequently than is the dorsal-to-ventral migration. Also, the dorsally migrating cells are located deeper within the intermediate zone than are those migrating ventrally. Their deeper location may merely reflect a difference in time of origin of the two groups of cells, but it could result from a more fundamental difference in mode of migration. It is possible that some of the dorsally migrating cells are preganglionic autonomic neurons, undergoing a secondary ventrolateral-to-dorsomedial migration from the ventral horn to the column of Terni[34]. A dorsal migration of cholinergic neurons in the mammalian spinal cord has also been recently postulated[2,49,50].

As a result of the combination of radial and tangential migrations, clonally related cells ultimately become distributed in transversely oriented planar arrays with very restricted rostrocaudal spreads in the gray matter. During the second week of development, however, a third type of migration occurs within the white matter. By stages 32–41, cells in the marginal zone that we have tentatively identified as oligodendrocytes[32] are found in longitudinal (i.e., rostrocaudally dispersed) arrays, parallel to the underlying axon tracts. They usually appear to originate from the planar arrays of gray and white matter cells just described, producing 'T'-shaped clones (fig. 4D). In contrast to the minimal rostrocaudal spread of the majority of cells, these streams of oligodendrocytes extend up to several segments rostrally and/or caudally. At times, several parallel streams arise from the same parent clone. These results suggest that oligodendrocytes or their precursors initially migrate from the ventricular zone to the marginal zone in a radial fashion, and then turn and follow axons in either a rostral or caudal direction.

In summary, at least three mutually orthogonal modes of migration occur in the spinal cord. Cells initially migrate radially from the ventricular zone, perhaps along radial glia. Some of these cells then turn and migrate ventrally or dorsally in the transverse plane, perhaps following an axonal substrate. Finally, oligodendrocytes or their precursors migrate rostrally or caudally along the underlying longitudinal axon tracts.

Thus, migrating cells in the spinal cord and tectum share many features. In both structures, migrating cells are initially radially organized, and radial migration may be guided by radial glia in spinal cord as it is in tectum. A tangential migration orthogonal to the radial arrays occurs in both regions, and the tangentially migrating cells travel along axon tracts. Furthermore, in both structures the axon tracts may be generated by homotypic cells and are among the earliest axon pathways to form. Finally, in both tectum and spinal cord there is a third tangential migration of cells that are believed to be glia. Although the glial type and the directions of the migrating cells differ in the two structures, both glial migrations may be stimulated by or occur along subpial tracks of long axons.

Forebrain

The chicken forebrain is largely divided into large, non-laminar cytoarchitectonic areas[3,46]. Cells arise at the ventricular zone and migrate outward, but the pattern of histogenesis is 'outside-in', i.e., the first-born cells are the most superficial[69,70]. Only the medial forebrain, a thin strip of tissue that contains the hippocampus, bears any similarity to the laminated cerebral cortex of mammals[3]; there is little known radial functional organization in the lateral areas of the forebrain. The following discussion concerns the lateral, noncortical, forebrain only.

While lineage has not been systematically studied in the forebrain, our preliminary observations indicate that neurons and astrocytes can be found in the same clones. Most forebrain neurons are stellate, and differ from one another mostly in size, density of spines, and extent of arborization[68]; therefore, they are much more difficult to classify on the basis of the morphology provided by lacZ histochemistry than are neurons in the tectum or spinal cord. Thus, it is still an open question whether single precursors give rise to many different neuronal subtypes.

As in the tectum and spinal cord, clones in the forebrain arise as discrete radial clusters[14]. The extent to which ventricular zone mixing contributes to clone shape is not yet known, but injections at early times do produce wider clones than injections at late times, indicating that mixing in the ventricular zone is likely to be restricted in a similar way in the forebrain as in tectum and spinal cord. Studies of clones in the forebrain show that although clonally related cells begin to migrate in coherent radial arrays (E8; fig. 5A), the cells eventually disperse. Thus, by E10 individual clones spread dorsoventrally (orthogonal to the radial axis) over a few hundred μm, and by E19 clonally related cells can be found scattered over > 2 mm, both rostrocaudally and dorsoventrally[14] (fig. 5B). These results are consistent with those obtained from chick/quail chimeras, in which cells from transplanted forebrain tissue mix extensively with host forebrain[1].

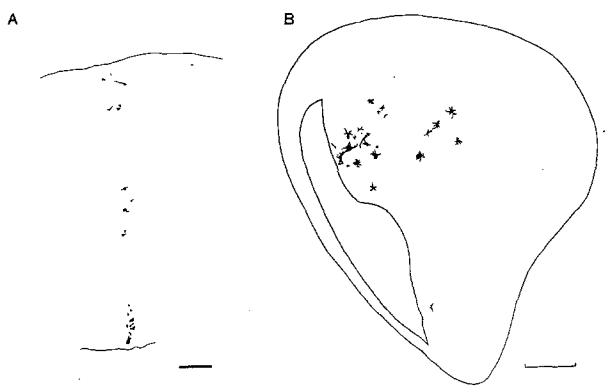

Figure 5. Clones of cells in chicken forebrain. *A* Camera lucida drawing of a typical clone from E8 telencephalon, in which clonally related cells are radially arrayed. Bar, 100 μm. *B* Camera lucida reconstruction from serial sections of a clone from an E19 telencephalon, following dispersal of cells. Bar, 1 mm.

A study of radial glial persistence in the forebrain shows that radial glia change in a way that could explain the behavior of the migrating forebrain cells. Radial glia are present at E8, crossing the forebrain from ventricle to pia, but they are not fasciculated into regular palisades as in the tectum. By E12, very few radial fibers stain with a cell-type specific antibody; instead, short, twisted fiber fragments appear. These may be deteriorating radial glia, or may represent a phase in transformation of radial glia into astrocytes (as is thought to occur in mammalian cortex; e.g., Schmechel and Rakic[64]). By E15, there is very little staining with radial glia specific antibodies in the forebrain. These results have been confirmed by placing the lipophilic marker dil[23] into the forebrain ventricle. Any fibers that extended to the ventricle would be labeled with dil, even if they were not stained by the antibody. With both of these methods, the decline in the radial glia population is evident and parallels the loss of radial organization of clonally related cells[14].

It is tempting to speculate that the disappearance of radial glia and the dispersal of neurons are related. For example, an intrinsic program that leads to the loss (or transformation) of radial glia could leave neurons without a source of radial guidance, and thus free to disperse. Alternatively, the neurons might lose contact with radial glia for reasons of their own, and the loss of neurons might cause the radial glia to die or to differentiate into astrocytes; indeed, Hatten[18] has shown that the elongate morphology of radial glia in culture depends on continued interactions with neurons. Regardless, it is clear that whatever strategy the tectum uses to keep clonally related cells radially restricted is not used in the forebrain.

Cortex

The mammalian cerebral cortex, like the optic tectum, is a laminated structure, with a pronounced radial functional organization[24]. Cells are born in the ventricular zone, and migrate outward through the intermediate zone to the cortical plate, where the cells differentiate and the six cortical laminae are formed (fig. 6). ^{3}H-thymidine studies have shown the pattern of cortical development to be inside-out (see McConnell[41] and Rakic[59] for reviews). This pattern and some recent transplantation experiments[42], raise the possibility that the birthday of neurons influences which laminae they populate and what their phenotypes will be.

Although the cerebral cortex is a telencephalic structure and the optic tectum is mesencephalic, these structures share major features of radial and laminar organization. It is therefore interesting to compare the migratory patterns that give rise to these two structures.

Recently, three groups have used the retroviral method to study lineage and migration in rodent cortex[36,52,73].

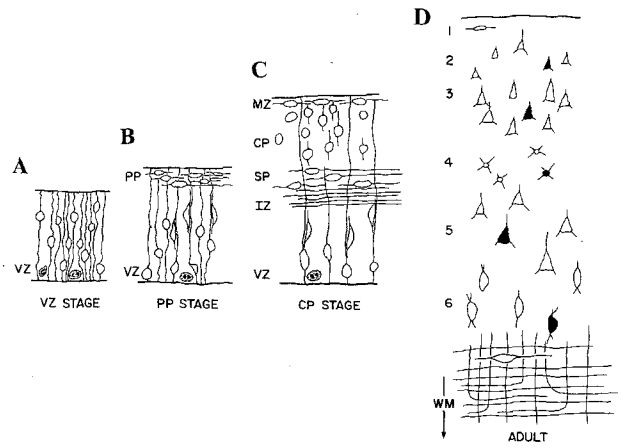

Figure 6. Schematic drawing of mammalian cerebral cortical development. *A* The cortex starts out as a pseudostratified neuroephithelium. VZ, ventricular zone. *B* Cells making up the preplate (PP) appear superficial to the ventricular zone. *C* Cells migrating outward along radial glia form the cortical plate (CP), which divides the preplate into the subplate (SP) and Cajal-Retzius cells, which populate the marginal zone (MZ). *D* The adult cortex is made up of 6 laminae containing characteristic cell types. The filled-in cells represent a typical, somewhat radial clone, as described by Luskin et al.[36]. Figure adapted from A. Pearlman et al. (in preparation).

Because this work is reviewed elsewhere in this volume, we will only mention it briefly here. Work by Luskin et al.[36] – both in vivo and in vitro – indicates that the glial and neuronal lineages have diverged in mouse cortex by E13. A similar result was obtained by Price and Thurlow[52], who found neurons and astrocytes in separate clones in rat cortex, but also found some clones that appear to contain both neurons and cells in the white matter (possibly oligodendrocytes). Clonally related neurons often reside in several different laminae, however, indicating that a single progenitor can give rise to multiple neuronal types (fig. 6).

All three groups using retrovirus to study cortical development find that there is radial migration, as suggested by classic studies, but also find some nonradial displacement of cells (fig. 6D). Luskin et al.[36] noted a predominance of radial arrangement, whereas Walsh and Cepko[73] and Price and Thurlow[52] stressed the existence of nonradial dispersal. These apparent differences partly reflect differences in emphasis, but may also result from species difference (mouse vs rat) and differences in the parts of the cortex examined. However, all three groups agree that migration is much more strongly radial in tectum than in cortex.

It is possible that the difference between the tectum and cortex is due in part to differences in radial glial organization in the two areas. The radial glial fibers in tectum are bundled into large, strikingly regular palisades that are even visible in unstained tissue using Nomarski optics. Because of the regular shape of the tectum, the glial fibers ascend almost directly radially from the ventricle to the pia[14,72]. In contrast, in the cerebral cortex, although glia are fasciculated, the glial palisades are not as well separated as in tectum, nor as regularly spaced[44]; because the fascicles are closely apposed, it may be relatively easy for migrating neurons to move from one fascicle to another. Furthermore, radial glia in many areas of the cerebral cortex take oblique pathways across the intermediate zone[43,44]; it is possible that the organization of these fascicles relative to one another is not maintained during their tangential ascent, and that clonally related cells that are migrating on two separate fascicles may thus be dispersed.

These considerations suggest the possibility that the migration of cortical cells is less radially oriented than in tectum because its radial glia are less strictly radially aligned. Another possibility is that nonradial dispersal in the cortex is similar to nonradial dispersal in the chicken forebrain, which may occur because the radial glia disappear before migration is complete. A third possibility is that nonradial dispersal is analogous to the tangential migration of multipolar neurons, described in tectum. However, tangential migration in the tectum is highly oriented, occurs in one embryonic lamina, and is restricted to a particular neuronal subtype; tangentially dispersed cells in the cortex are found in many cortical layers (which contain different neuronal phenotypes), and no highly directed tangential migration has been observed. Thus, the first two explanations of tangential dispersal in the cortex seem more likely.

However, there is another candidate for directed tangential migration in the cortex. This is a set of cells that lies outside the cortex proper: the subplate cells. These cells share many similarities with the multipolar efferent neurons of the tectum. Both are the first-born neurons in their respective areas; both are efferent cells; neither enters the cortical/tectal plate, but both lie at the most superficial aspect of the intermediate zone; both cell types are oriented tangentially early in development (fig. 6; cf. fig. 3), and later become multipolar[9,37,38,54,61]. As yet, no one has injected virus into mammalian embryos early enough to label these cells, so their actual migratory path is unknown. However, this question is of obvious interest and could be tested directly by the retroviral method.

General conclusions

Radial migration

In every tissue to which the retroviral method has been applied (tectum[13,15], forebrain[14], retina[71], cerebral cortex[36,52,73], and spinal cord[32,33]), clones of cells begin their migrations as radial arrays. Of these, the radial organization of clones is maintained in retina and tectum (figs 1 and 3). In the cortex there is some tangential spread of clonally related cells but a radial tendency persists (fig. 6). Clones in the avian forebrain and in the spinal cord eventually become quite nonradial (figs 4 and 5) although clones remain planar in the spinal cord. Thus, clonally related cells are radially organized when

they begin their migration away from the neuroepithelium, whether or not they remain radially organized in maturity. It is possible that a radially restricted ascent of cells through and out of the ventricular zone is a common feature of early CNS development, with region-specific migratory mechanisms predominating at later stages. Subsequent radial migration – e.g., in retina, tectum, and cortex – appears to correlate with a functional radial organization, although there is no reason at present to conclude that the functional and migratory patterns are causally related.

Tangential migration

In both spinal cord and tectum, there are clearly oriented streams of cells that migrate tangentially. Tangential spread of cells also occurs in mammalian cortex and avian forebrain, but it is not yet clear whether it is caused actively by directed tangential migration, or passively, by loss of radial guidance. However, several examples of oriented tangential migration have been described in other regions of the nervous system. The cells of the lateral trigeminal motor nucleus of the chick originate medially but later migrate laterally through the marginal zone[8,45]. In the medulla, cells migrate ventromedially from the rhombic lip along separate but parallel marginal and submarginal pathways[4,47]. A similar migration along a marginal pathway occurs in the pons[58]. Neurons that will populate the oculomotor nucleus migrate across the mesencephalic midline in a submarginal tangential pathway[55]. In some of these regions but not in others, the migrating cells first extend axons and then translocate their somata distally. Common to each region, however, is the migration of cells in a direction parallel to the pial surface and perpendicular to both the radial and rostrocaudal axes of the nervous system. Thus, tangential migration may be an integral part of development in many areas of the nervous system. Furthermore, finding this type of nonradial migration in the two regions we have studied most extensively suggests that further studies using recombinant retroviruses or direct injection of ventricular zone precursors may reveal that tangential migration is a more common phenomenon than previously expected. Tangential migration over long distances could serve to allow a ventricular zone to contribute cells to a distant region and/or allow multiple ventricular zones to contribute cells to the same region. Within structures like the spinal cord, tangential migration could contribute to the formation of nuclear groups. Within the tectum, the purpose of the tangential migration is not clear, perhaps because the rules by which information is processed in the deep layers are not well understood.

Migratory guides

In tectum, the evidence indicates that radial glia guide the radial migration and that axons guide the tangential migration of neurons. In spinal cord, the migrations are similar, and the guidance structures may be as well. These results fit well with an idea put forward by Rakic[58] and others that migration is accomplished by oriented 'neurophilic' and 'gliophilic' migrations along different guidance structures. Similar guided migrations occur in the cerebellum, where granule cells leave axonal guides to follow Bergman glia[58]. In all three of these tangential migrations, as well as in several enumerated above[4,45,47,55,58], migrating neurons appear to be guided at least in part by homotypic neuronal processes. At least in tectum, radial and tangential guides intermingle at crucial stages of development. This intermingling of pathways raises the question of how cells decide what path to follow. An idea consistent with current concepts of cell guidance is that neurons and glia bear different adhesive molecules that allow migrating cells to differentiate among axonal and glial pathways. A related question is whether the ultimate phenotype of cells is influenced by their choice of migratory path. Because lineage does not appear to contribute to the determination of cell type in at least some parts of the CNS[11,13,22,32,71,74], it is likely that the environment into which a cell is born and/or migrates is important in determining its phenotype. Our current aim is to perturb migration by interfering with cell-cell interactions, in order to test directly the idea that migratory path is a determinant of neuronal phenotype.

Migratory building blocks

In considering the different neural areas we have discussed here, one is struck by the large differences in their organization and functional architecture. However, in many of the structures discussed, there are striking similarities in the kinds of migratory paths that cells take, implying that a few basic migratory strategies (e.g., radial along glia, tangential along axons) may be combined in different ways to generate very different neural structures. This similarity is particularly striking in the spinal cord and tectum, in both of which cases an initial radial organization is followed by a directed tangential migration. However, in the tectum, additional radial migration results in a laminated structure superficial to the tangentially migrating multipolar layer. In the spinal cord, one set of tangentially migrating cells remains superficial, while another set migrates tangentially in a deeper fiber layer. The two structures are ultimately extremely different in form and function. These results suggest that a relatively small set of cues may be combined in different ways to orchestrate the development of very dissimilar neural structures.

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Cell lineage and cell migration in the developing cerebral cortex

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Summary. Modern techniques which trace lineages of individual progenitor cells have provided some clues about the processes that determine cell fate in the brain, and have also given us some information about migratory patterns of clonally related cells. In many parts of the central nervous system, progenitors are multipotent; single clones can contain multiple neuronal types or even mixtures of neurons and glia. In addition, one can observe a wide distribution in clone size, even when marking is done in a narrow time window. This suggests that progenitor cells may be fairly plastic and responsive to environmental signals. In the developing cortex, clonally related cells are initially grouped near each other, as in the retina and tectum. However, the subsequent migration of these cells from the ventricular zone to the cortex along glial fibers is accompanied by a progressive dispersion of clonally related neurons.
Key words. Cell lineage; cerebral cortex; neuronal development; glial development; radial glia.

The lineage relationships of neural cells can provide important information about factors influencing a cell's fate. These factors can be divided, roughly, into those that are intrinsic to the cell, on the basis of inherited patterns of gene activity, and those that reflect the cell's environment, presumably acting through intercellular signalling. Some invertebrates show highly 'determinate' patterns of cell cleavages, where identifiable cells give rise to a stereotyped set of progeny. Such patterns suggest that a cell's fate may be independent of its environment, although exceptions to this generalization are being revealed by careful experimental studies[16, 17, 92, 103]. On the other hand, patterns of cell cleavages which are highly variable and unpredictable (also called 'indeterminate') immediately suggest an important role for intercellular signals in determining cell fate, and can provide clues to help locate those signals.

The development of the cerebral cortex illustrates several problems common to the whole brain[51], as well as others that are unique. For example, the cortex contains neurons and glia, and one of the oldest questions in developmental neurobiology is how these two neural types diverge. His[34] first suggested that neurons arise from Keimzellen ('germ cells'), located along the ventricular lining of the brain, while glia derive from 'spongioblasts', or radially oriented epithelial cells. He compared these cell types to the germ cells and Sertoli cells or the testis – an analogy that explicitly implied a true genetic divergence of progenitors at an early stage. This model has influenced the design of experiments and even the terms of discussion ever since. While the 'lineage' of a cell strictly refers to its parentage and progeny, the terms 'neuronal and glial lineages' are often used more loosely, with this very usage implying that the two represent sep-