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Migratory Paths of Neurons and Glia in the Embryonic Chick Spinal Cord

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To study the migration of chick spinal cord neurons, we labeled individual cells in the ventricular zone with recombinant retroviruses, then identified their progeny histochemically. First, we analyzed cell mixing in the ventricular zone. Some clones labeled at early neural tube stages spread widely along both the dorsoventral and rostrocaudal axes. However, clones labeled later were confined to narrow domains along both axes. These results imply that displacement of cells within the ventricular zone becomes progressively restricted. Second, we studied the migration of cells out of the ventricular zone by infecting embryos at a fixed stage and varying the time of analysis. At first, most clones consisted of radial arrays of cells, suggesting that the initial migration is predominantly radial. In many clones, however, neurons turned orthogonally from parental radial arrays and migrated along the path of circumferentially oriented axons. By hatching, clonally related cells in the gray matter were usually distributed in narrow transverse slabs, but some white matter glial cells had migrated longitudinally for up to several segments. We conclude that the dispersal of clonally related cells results from (1) early mixing of progenitors within the neural tube; (2) radial stacking of progeny in the ventricular zone; (3) migration of progeny from the ventricular zone in spoke-like routes; (4) circumferential migration of some neurons along axons; (5) short-distance dispersal of differentiating neurons; and (6) a late, longitudinal migration of glia through white matter tracts. Finally, we show that floor plate cells differ from other spinal cord cells in both their lineage and migration patterns.

[Key words: spinal cord, migration, chick, ventricular zone, neuroblast, retrovirus, floor plate]

Like their cortical and tectal counterparts (McConnell, 1991), cells in nonlaminated regions of the CNS originate in the ventricular zone (VZ) and then migrate to their final sites of differentiation. Our understanding of the histogenesis of such regions has been hampered by their complex geometry. Thus, the development of the spinal cord has been extensively studied, yet, with the exception of the somatic and autonomic preganglionic motoneurons (Chu-Wang et al., 1981; Thors et al., 1982; Liuzzi et al., 1985; Dorado et al., 1990; Markham and Vaughn, 1991; Prasad and Hollyday, 1991; Phelps et al., 1993), little is known about the migratory patterns that lead to its mature structure or about the mechanisms by which its neurons and glia migrate.

Recently, the study of neural migration has benefited from methods that allow small groups of cells to be labeled selectively. One effective approach has been to label and then trace sets of migrating cells that share a neurotransmitter (e.g., Schwanzel-Fukuda and Pfaff, 1989; Wray et al., 1989; Phelps et al., 1993) or axonal target (e.g., Heaton et al., 1978; Chu-Wang et al., 1981; Liuzzi et al., 1985; Bourrat and Sotelo, 1990; Markham and Vaughn, 1991; Prasad and Hollyday, 1991). A different approach has been to track cells migrating from single sites in the VZ by labeling their progenitor cells with injected tracers (e.g., Fraser et al., 1990; Stern et al., 1991; Kimmel et al., 1994) or recombinant retroviruses (e.g., Luskin et al., 1988; Gray and Sanes, 1991; Walsh and Cepko, 1992). Here, we apply the retroviral method to the chick spinal cord.

In a previous study (Leber et al., 1990), we studied cell lineage in the spinal cord. We showed that progenitor cells in the VZ are pluripotent with respect to the phenotype of cells they will produce: cells that give rise to motoneurons can also produce interneurons, glia, and ependymal cells. In contrast to their *phenotypic* diversity, however, descendants of individual progenitors appeared to be quite restricted in their *spatial* distribution within the gray matter of the spinal cord. They dispersed widely along the mediolateral axis and to a moderate degree along the dorsoventral axis, but very little along the rostrocaudal axis. As a result, clonally related cells were usually confined to planar arrays spanning the gray matter of the spinal cord. We felt that an understanding of the genesis of these arrays would provide insight into spinal cord histogenesis. We used recombinant retroviruses to insert the *Escherichia coli β-galactosidase* (*lacZ*) gene into the genome of dividing progenitor cells. We then used a histochemical reaction to detect the gene product, lacZ, in their progeny. The distribution of cells belonging to a single clone reflects both the spread of the progeny within the VZ and the movements of cells as they migrate out of the VZ to their final destinations. By varying the times of infection and analysis, we were able to study each type of movement separately.

Preliminary results have been reported (Leber and Sanes, 1990; Gray et al., 1990).

Materials and Methods

Viruses. The two retroviral vectors used in this study were used in our previous study (Leber et al., 1990) and are described in detail by Galileo

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et al. (1990). The first, LZ10, was constructed from a Rous sarcoma virus and encodes a gag-lacZ fusion protein that accumulates in the cytoplasm of the progeny of infected cells. The second, LZ12, derives from a Moloney murine virus, and encodes a protein in which lacZ is fused to a nuclear localization sequence from the SV40 T antigen. Accordingly, infection with LZ12 leads to nuclear rather than cytoplasmic localization of lacZ. Virus-producing cells were cultured, and virions were concentrated, tested for replication-competent virus (which was never found), and titered as described in Sanes et al. (1986) and Galileo et al. (1990). The titers of viral concentrates were $\sim 10^6$ active virions per ml, as determined by infection of cultured fibroblasts.

Injections. White Leghorn chicken eggs (SPAFAS, Roanoke, IL) were incubated at 37–38°C and the embryos staged according to Hamburger and Hamilton (1951). Viral concentrate (either LZ10, LZ12, or a mixture of the two) was diluted with Earle's 199 medium, mixed with Polybrene and fast green, and injected on top of the neural plate or into the lumen of the prospective spinal cord (Leber et al., 1990). After topical application of ampicillin, the eggs were sealed with tape and returned to the incubator.

Histology. Embryos were killed after a variable period of incubation. Their spinal cords were dissected free from surrounding tissues and fixed by immersion in cold 0.4% glutaraldehyde and 2.0% formaldehyde in phosphate-buffered saline (PBS; 150 mM NaCl and 15 mM Na phosphate, pH 7.3) for 1 hr. Embryos older than stage 30 were first perfused with the same fixative. After several rinses in PBS, the tissue was stained overnight for lacZ, as detailed by Galileo et al. (1990), rinsed again in PBS, and refixed in 2.0% glutaraldehyde and 2.0% formaldehyde in PBS for at least 4 hr. The tissue was cleared in glycerol, to facilitate visualization of small or deeply buried stained cells. The rostrocaudal and dorsoventral spreads of labeled cells within a cluster were measured using an ocular micrometer. Slices of spinal cord containing labeled cells were cut by hand, returned to PBS, dehydrated, embedded in Poly/Bed 812 (Polysciences, Inc.; Warrington, PA), and sectioned in the transverse plane at 20 μm. Some sections were counterstained with basic fuchsin. After mounting with immersion oil, sections were viewed with bright-field, Nomarski, or phase contrast optics. The distribution of cells in some clones were mapped using a drawing tube attached to a dissecting microscope (for whole mounts) or to a compound microscope (for sectioned material). In the latter case, several sections were superimposed to reconstruct clones.

Clonal analysis. Isolated clusters of cells were interpreted to be single clones, using criteria discussed in Leber et al. (1990). In some cases, too many infections took place to be able to distinguish clonal boundaries. Such animals or areas of spinal cord were excluded from further analysis. When mixtures of LZ10 and LZ12 were injected, cells interpreted to be clonally related on the basis of their clustering were almost always of the same phenotype (e.g., Fig. 1*A,B*), whereas clusters in regions of spinal cord excluded from analysis because of excessive infection were often of mixed phenotype. In only one case did an isolated cluster of lacZ-positive cells need to be reinterpreted as polyclonal on the basis of cellular phenotypes.

Although we are confident that the vast majority of clusters of lacZ-positive cells represent clones, it is likely that some clusters are not *entire* clones (Leber et al., 1990). In particular, clusters separated by several hundred μm were interpreted to be separate clones, but may have been parts of single clones in some cases. In these cases, we would have underestimated the extent of cell displacement in the VZ. This problem was more likely to occur after injections at early stages (see below) and, therefore, does not change our conclusion that VZ mixing becomes progressively restricted. Instead, it suggests that we may have underestimated the extent of this restriction.

Clones in the sacral spinal cord (which were sometimes quite large even following injection at late stages) were excluded from this analysis because of the sacral cord's unique development. Whereas most of the spinal cord forms when the neural plate rolls up into a neural tube, the sacral cord develops from the cavitation of a solid mass of tail bud-derived cells (Criley, 1969).

In all, about 1100 clones from 134 embryos were analyzed. Three hundred forty-three of the clones were obtained from 61 embryos killed between stages 19 and 29. An additional nine embryos killed at these stages had no labeled spinal cord clones. Another 750 labeled clones were obtained from 73 animals killed at stages 31–41. Most of this latter group were obtained in the course of a previous study (Leber et al., 1990) but were not analyzed at that time because they did not contain motoneurons.

Results

As in most other areas of the CNS, neurons in the spinal cord are generated in a pseudostratified VZ and undergo interkinetic movements as they proceed through the cell cycle. The chick neural tube begins to close at stage 8, most spinal motoneurons are born between stages 15 and 23, and most other spinal neurons are born between stages 14 and 32. Postmitotic cells migrate out of the VZ into the intermediate zone (IZ), where they complete their differentiation. Glial cells or their precursors also originate in the VZ, but their division may continue in the IZ or white matter. Ventral-to-dorsal gradients of mitosis and differentiation are generally apparent, although some dorsal and intermediate cells are generated very early (Hamburger, 1948; Fujita, 1964; Langman and Haden, 1970; Hollyday and Hamburger, 1977; McConnell and Sechrist, 1980).

In a previous study (Leber et al., 1990), we injected virus into the spinal canal at stages 11–18 and analyzed the phenotype and distribution of labeled cells after the spinal cord was relatively mature. Although we found no evidence to suggest that progenitor cells in the spinal VZ were committed to produce particular classes of neurons or glia, we were impressed with the stereotyped spatial distributions of clonally related cells. Cells in the gray matter had a far greater spread in the transverse plane than they did along the rostrocaudal axis, whereas groups of glial cells in the white matter were dispersed longitudinally. Although the distribution of labeled clonal relatives clearly reflected their prior movements, we were able to infer little about the spread of progeny in the VZ or the specific migratory routes they had followed in the IZ. We, therefore, undertook two new sets of experiments. In the first, we sought to determine the extent that the spinal neuroepithelial cells move tangentially (parallel to the ventricular surface) within the VZ. To this end, we varied the stage at which we injected virus and analyzed the distribution of labeled cells in the VZ as a function of this stage. In the second, we analyzed the migratory patterns taken by cells after leaving the VZ. For this purpose, we fixed the stage of injection and analyzed the distribution of labeled cells in the IZ at various stages. In both sets of studies, we were able to infer migratory movements from static pictures, based on the knowledge that each retrovirally marked clone originated from a single infected cell in the VZ. We describe each of these separately.

Ventricular zone mixing

If cells move tangentially through the VZ, clones labeled with early injections should become more widely dispersed within the VZ than clones labeled with later injections. To test this idea, we injected embryos at stages ranging from 8–18, and assessed the distribution of labeled cells at stages 19–29. Cells lying outside of the VZ were excluded from this analysis since their position also reflected their subsequent migration through the IZ. The fraction of clones that still contained cells in the VZ ranged from 96% (26/27) at stages 19–21 to 69% (70/101) at stages 27–29.

We first analyzed embryos injected at stages 15–18, the latest stages used in our previous study. At stages 19–29, clonally related cells within the VZ were almost always arranged in single, narrow, radially oriented arrays, one or a few cell widths in diameter. The progeny of the originally infected cell remained adjacent to one another, but as expected, interkinetic movements spread their nuclei throughout the depth of the VZ. Only a few clones (6/98; 6%) consisted of more than one adjacent

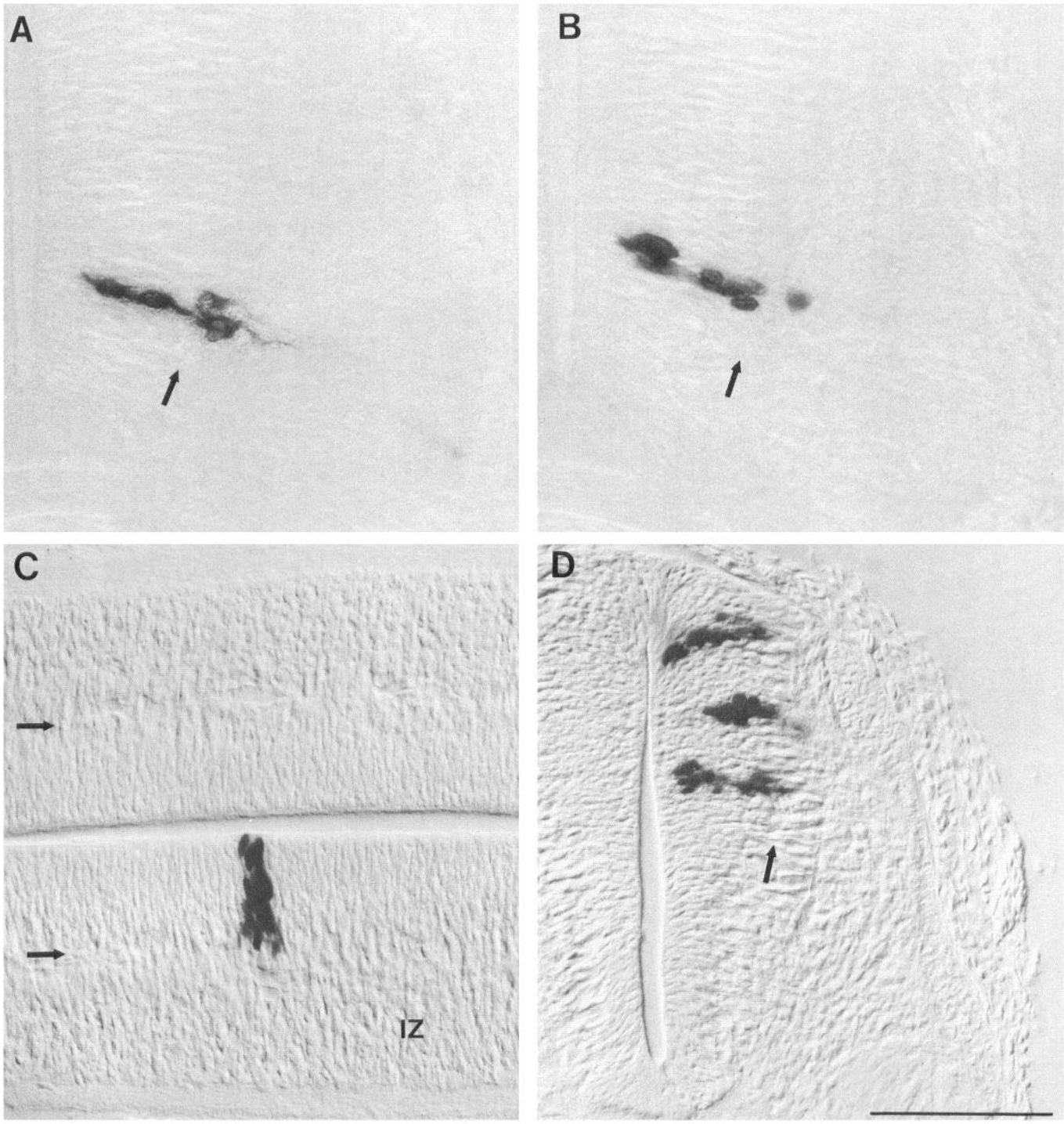

Figure 1. Clonally related cells in the VZ aggregate in narrow, radial arrays. *A* and *B*, Cross-sections through the spinal cord at stage 25 showing two labeled clones in the same embryo at stage 25. Cells in *A* show cytoplasmic staining (LZ10 virus) whereas cells in *B* show nuclear staining (LZ12 virus). The labeling of cells in each group is homogeneous. *C*, Horizontal section at stage 29 showing a clone three cells wide in the rostrocaudal dimension. Labeled cells in adjacent sections are in the intermediate zone (IZ). *D*, Cross-section through the spinal cord at stage 29 showing a clone consisting of three separate radial arrays of cells, all within the same 20 μm thick transverse section but spread over 100 μm dorsoventrally. Adjacent sections contain more medial cells in the middle strand. Virus was injected at stage 14 in *A*, *B*, *D*, and stage 12 in *C*. *Arrows* indicate the lateral edges of the VZ. Dorsal is up in all cross-sections. Scale bar, 50 μm in *A* and *B*; 100 μm in *C* and *D*.

radial strand. This radial distribution suggests that little or no tangential movement of cells occurred within the VZ between the time of viral infection and the time the animals were killed.

We next injected virus at stages 8–14, to label ancestors of the progenitors that had been labeled in the initial set of embryos. When analyzed at stages 19–29, clones again were composed of radial arrays of cells (Fig. 1), but now many consisted of two or more separate radial strands (Figs. 1*D*, 2). Clones labeled at stages 8–9, when the neural tube is closing, spanned up to 1-1/2 segments along the rostrocaudal axis (Fig. 2*D*) and

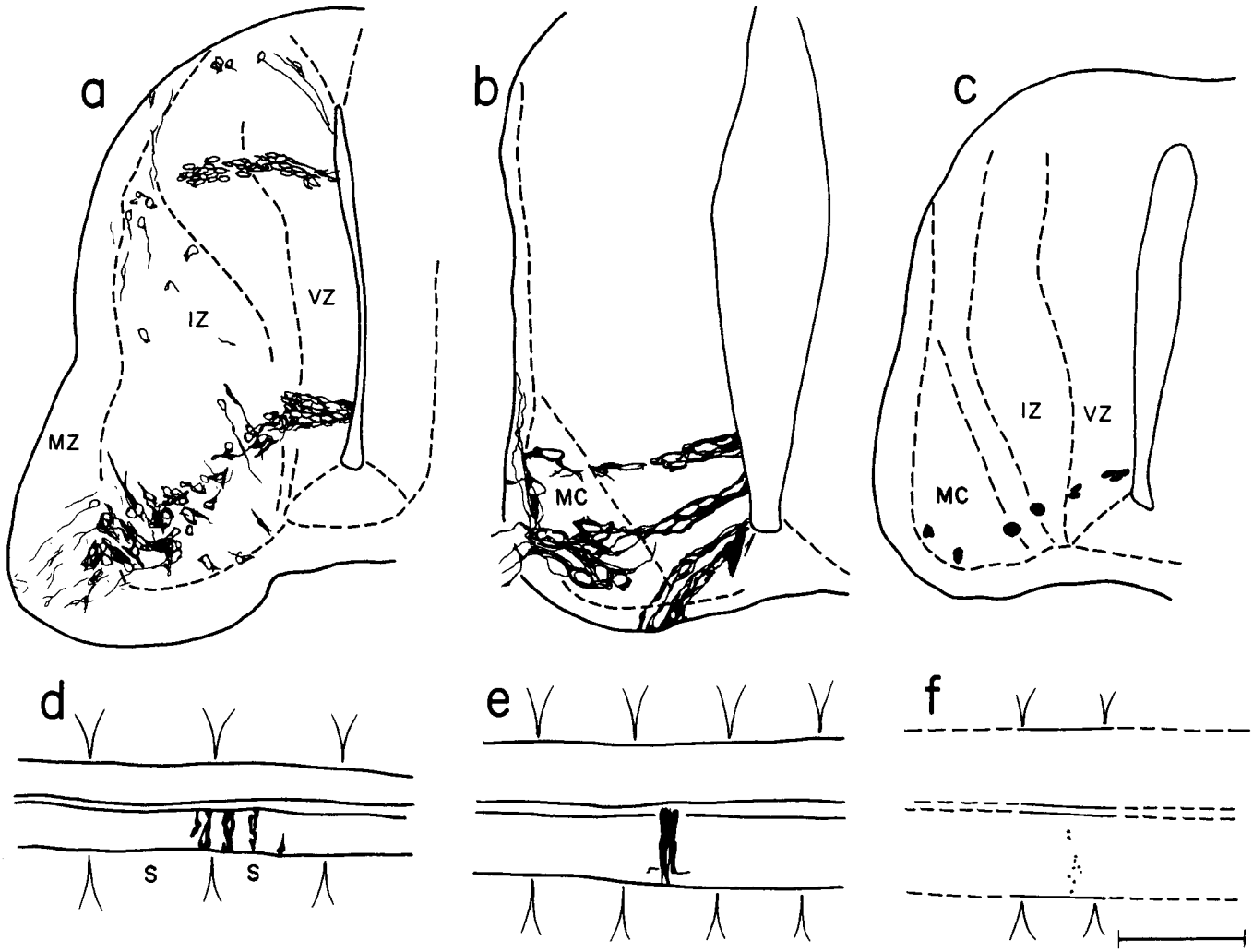

Figure 2. Clones vary in size and spread along the dorsoventral and rostrocaudal axes. *A,* Large clone with three streams of cells stemming from widely separated regions of the VZ. Some cells are migrating ventrally in the IZ. Virus was injected at stage 8, and the animal was killed at stage 29. *B,* A clone with three streams of cells occupying the ventral eighth of the VZ. The clone also contains motoneurons, identified by extension of their axons into the ventral root. Virus was injected at stage 8, and the animal was killed at stage 22. *C,* Small clone with single radial stream of cells from the VZ into the motor column. The most lateral of three motoneurons (identified by criteria detailed in Leber et al., 1990) has moved slightly dorsal of the parental stream. Virus was injected at stage 13, and the animal was killed at stage 25. *D,* Large clone with extensive longitudinal spread, crossing segmental and midsegmental boundaries of somite (*S*). Individually labeled cells cannot be distinguished at this magnification. Virus was injected at stage 8, and the animal was killed at stage 22. *E,* Clone consisting of two streams of cells separated rostrocaudally. Virus was injected at stage 8, and the embryo was killed at stage 28. *F,* Small clone consisting of a single stream of cells. Virus was injected at stage 13, and the embryo was killed at stage 29. Camera lucida drawings from 20 μm sections in *A–C,* and from intact spinal cords in *D–F.* See Figure 4 legend for abbreviations. Scale bar, 100 μm in *A*; 50 μm in *B* and *C*; 350 μm in *D*; 400 μm in *E* and *F*.

most of the dorsoventral extent of the VZ (Fig. 2*A*). Half (27/53) of the clones labeled at these stages consisted of at least two strands originating in separate rostrocaudal positions within the VZ, and 60% (32/53) consisted of at least two strands originating in separate positions along the dorsoventral axis. The percentage of multistranded clones gradually decreased with later stages of injection until almost all clones consisted of single strands (Fig. 3). The size of clones and the dispersal of individual strands along both the rostrocaudal and dorsoventral axes were closely related to the stage of viral injection but not to the stage of analysis or to the interval between injection and analysis (not shown). The increased frequency of multistranded clones at earlier stages of injection suggests that neuroepithelial cells were initially capable of moving tangentially through the VZ, but later lost this ability. Presumably, cells infected with early injections gave rise to daughters that moved away from each other, but then stopped moving and generated progeny that were constrained to move in a radial direction. Cells equivalent to these daughter cells were the ones labeled with injections at later stages.

The gradual restriction of mixing along both the rostrocaudal and dorsoventral axes raised the question of whether they occur simultaneously. In 50% (36/72) of these multistranded clones, the individual subclones were separated along both the dorsoventral and rostrocaudal axes, but in almost as many (42%; 30/72), the individual radial arrays comprising a single clone were separated along the dorsoventral axis but tightly clustered along the rostrocaudal axis (Fig. 1*D*). In contrast, only 8% (6/72) of the multistranded clones consisted of parallel strands of cells separated along the rostrocaudal axis but aligned within a single horizontal plane. This comparison (Fig. 3*C*) suggests that the rostrocaudal restriction may occur earlier than the dorsoventral.

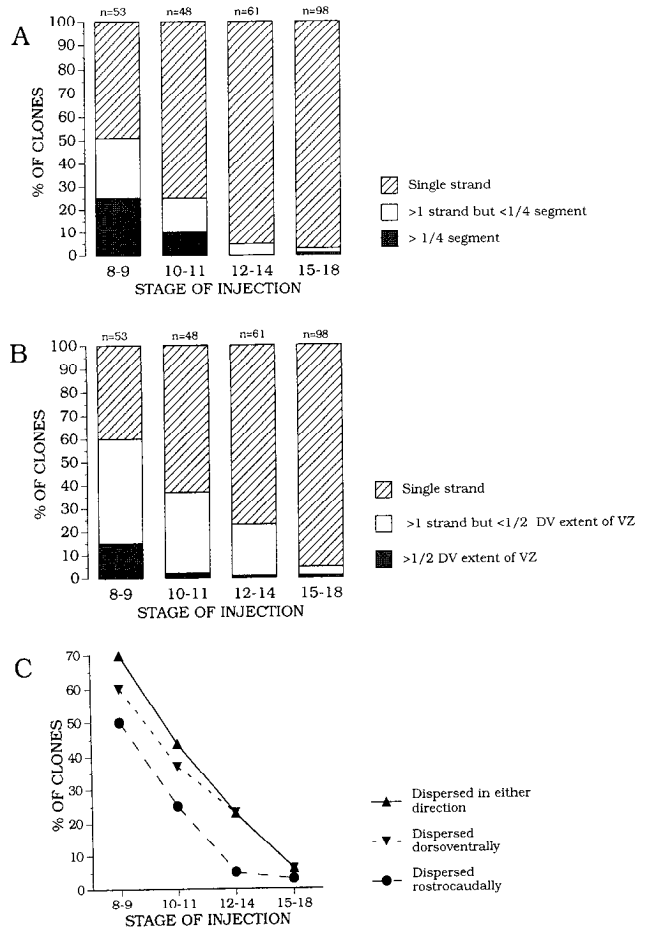

Figure 3. Progressively restricted dispersal of cells in the VZ. *A*, Dispersal along the rostrocaudal axis. Clones were classified as single strands within the VZ (or multiple strands in the same transverse plane), as multiple strands spread over < 1/4 of the length of a spinal segment, or as multiple strands spread over > 1/4 of a segment length. The number of clones analyzed at each stage is indicated. *B*, Dispersal along the dorsoventral axis. Clones were categorized as single strands within the VZ (or multiple strands in the same horizontal plane), or as multiple strands spanning variable proportions of the dorsoventral extent of the VZ. *C*, Overall dispersal compared with dispersal along each axis. Clones were categorized as "dispersed" if they were > 1 strand wide. Because some clones were dispersed along both axes, the sum of the dorsoventrally and rostrocaudally dispersed clones is not equal to the total number of multistranded clones. In *A–C*, clones were analyzed at stages 19–29.

Migration

The restricted tangential movement of cells within the VZ leads to the initial deployment of cells in radial arrays. Later, however, clonal cohorts show a slab-like planar distribution (Leber et al., 1990). To learn how this transformation arose, we analyzed embryos at various times after injection of virus at a fixed time (stages 12–14). In these experiments, we looked only at static images of spinal cord and did not directly visualize migration. We also could not be certain as to whether a particular cell was still in the process of migration. Nevertheless, because almost all clones of cells labeled at these stages originated at single sites within the VZ (Fig. 3), the migratory route of a group of cells could be inferred from the cells' distribution as they left their common site of origin. (Migratory patterns were more difficult to determine after injections at earlier and later stages but were generally consistent with those inferred from injections at stages 12–14.) In general, the orientation of individual cells was consistent with the direction of migration (e.g., radial or tangential) inferred from the distribution of cells in the clone.

Radial migration. At stages 19–21, the earliest examined, the predominant mode of migration was radial. Of the clones that contained cells outside of the VZ, 90% (19/21) were deployed in linear arrays extending from the ventricular surface directly toward the external limiting membrane. The cells in these arrays were usually bipolar in shape and oriented radially (similar to those in Figs. 1*A*; 2*A,B*; 4*B*). Eighty-five percent (16/19) of the clones with cells migrating out of the VZ at these stages were ventral in location, consistent with the early differentiation of the basal plate.

Circumferential migration. Although many clones continued to be strictly radial in arrangement at later stages, nonradial neuronal migration became increasingly prominent. For example, many cells that migrated radially out of the VZ then turned and followed a tangential pathway, parallel to the surface of the spinal cord (Fig. 4*A,B*). By stages 27–29, about half (23/49) of clones contained tangential as well as radial components (Fig. 5). The majority migrated from dorsal to ventral, but some migrated from ventral to dorsal. The ventrally migrating cells originated at all dorsoventral levels of the spinal cord, but were most likely to originate in the dorsal region (Fig. 6). Most of these cells were unipolar in shape, with leading processes and axons extended in the direction of apparent migration (Fig. 4*A,B*).

As they migrated ventrally, the neurons followed the path of a group of circumferentially coursing axons, some of which cross the floor plate to the contralateral side and are among the first axons to form in the spinal cord (Ramón y Cajal, 1960; Holley, 1982; Oppenheim et al., 1988; Yaginuma et al., 1990). These commissural axons arise from a group of very early born interneurons that, like the ventrally migrating cells, are located in the dorso- and intermediolateral regions of the spinal cord and have unipolar, leading processes (Langman and Haden, 1970; Holley, 1982; Yaginuma et al., 1990). Some of the ventrally migrating neurons contributed axons to the commissural path-

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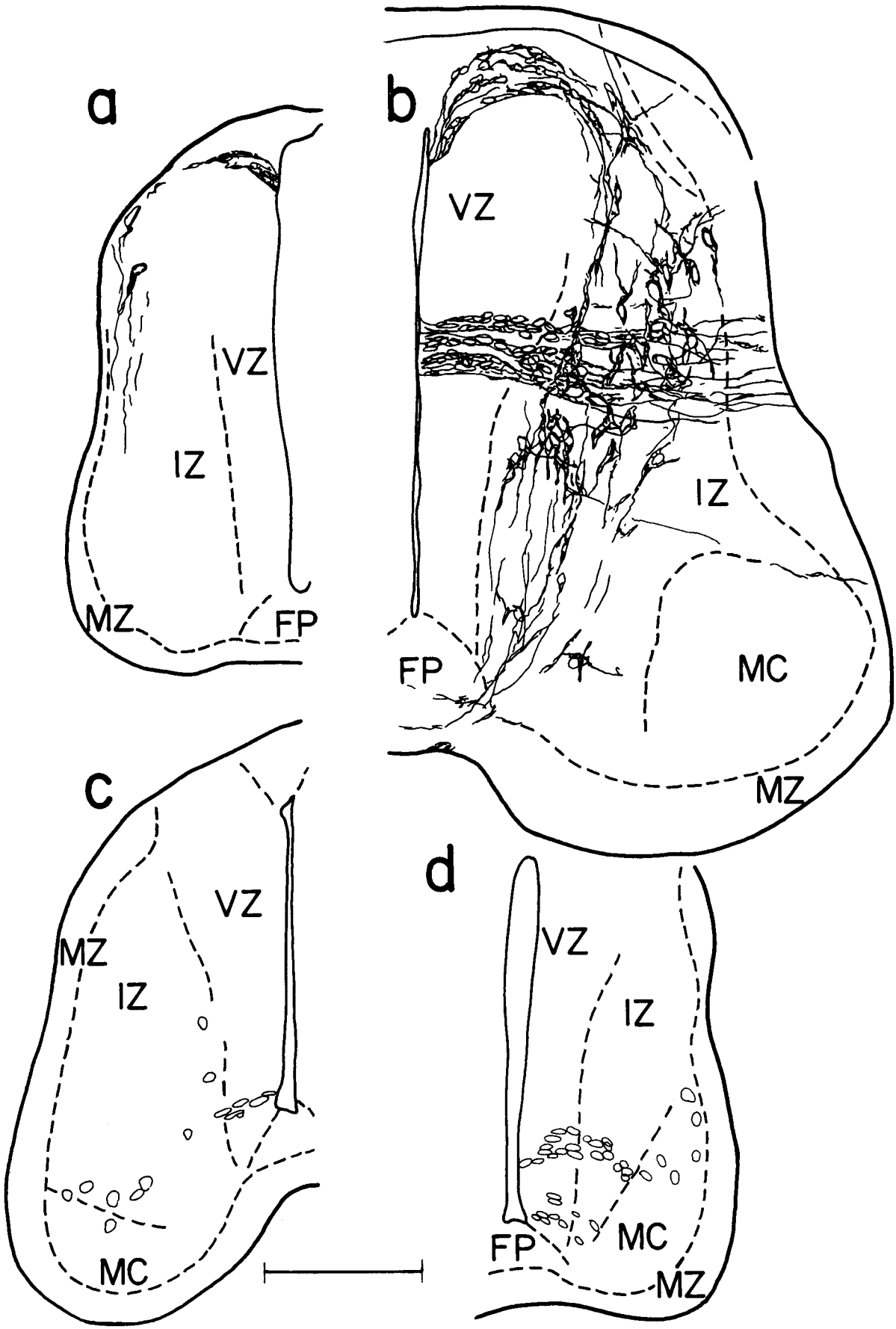
Figure 4. Tangential migrations. *A*, Simple dorsal-to-ventral tangential migration. A pair of unipolar cells originating dorsally have turned and moved ventrally. Their leading processes and axons and those of other more dorsal cells project in a circumferential direction. Virus was injected at stage at stage 12, and the animal was killed at stage 25. *B*, Complex dorsal-to-ventral tangential migration. A broad stream of cells has migrated ventrally from the more dorsal of the two radial arrays that comprise this clone. Many of the ventrally migrating cells give rise to commissural axons. A focus of label (not shown) in the contralateral white matter indicated that the axons crossed the floor plate. Some longitudinally coursing axons could be traced up to four segments rostrally. All of the cell bodies in this clone were within a 40 μm thick transverse slab. Virus was injected at stage 9, and the embryo was killed at stage 29. *C*, Ventral-to-dorsal migration in the IZ. Two cells with labeled nuclei have migrated dorsally just outside the VZ, perhaps on their way to the column of Terni. Other cells are migrating radially. Virus was injected at stage 13, and the embryo was killed at stage 29. *D*, Ventral-to-dorsal migration in the motor column. Three cells with labeled nuclei have migrated dorsally from the distal end of their parent radial stream, but remain within the motor column. Virus was injected at stage 11, and the embryo was killed at stage 25. *FP*, floor plate; *IZ*, intermediate zone; *MC*, motor column; *MZ*, marginal zone. Scale bars, 100 μm.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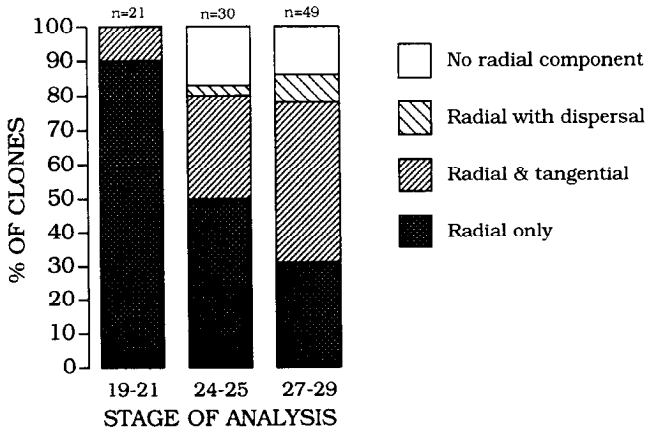

Figure 5. Migratory pattern as a function of the stage of analysis. Clones were labeled with injections at stages 12–14. Some clones consisted exclusively of cells aligned radially. Others had a cluster of radially aligned cells, but also had cells aligned tangentially (parallel to the surface of the spinal cord or to the medial surface of the motor column). Others consisted of a cluster of cells at the end of a narrow, radial stream. Others were either tangential or clustered in arrangement, but lacked a radial component. The number of clones in each group is indicated.

way (Fig. 4*B*), implying that they themselves were commissural interneurons. Not all ventrally migrating neurons were commissural, however. Some sent axons into the white matter lateral to the motor column (Fig. 7). They may have been the ipsilaterally projecting circumferential neurons described by Yaginuma et al. (1990). Nevertheless, they too were unipolar and migrated ventrally without appreciable rostrocaudal movement.

The first cells to migrate ventrally did so in a narrow stream

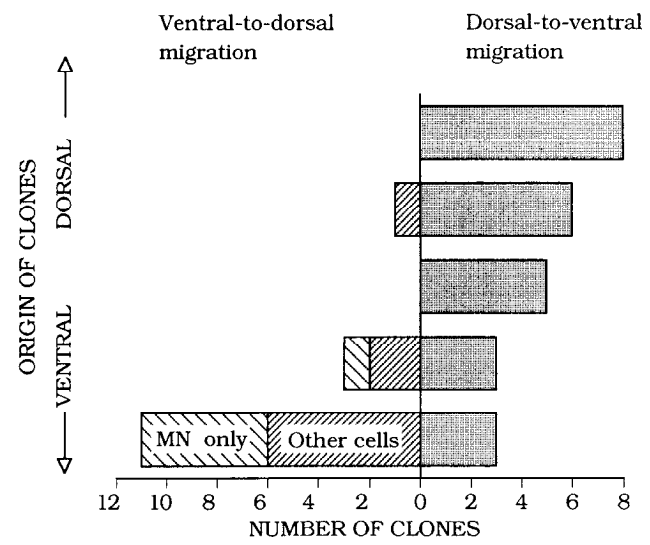

Figure 6. Histogram comparing the direction of tangential migration and the point of origin of clones within the VZ. Clones labeled at stages 12–14 and containing clones with tangentially migrating cells at stages 19–29 were included; n = 38. The VZ was divided into five equal bins along its dorsoventral axis, and the bin of origin and direction of tangential migration were recorded for each clone. Clones with dorsally migrating cells were also classified as to whether these cells consisted exclusively of motoneurons (MN). Three clones with cells migrating both dorsally and ventrally were included on both sides of the vertical line. Dorsally migrating cells originated almost exclusively in the ventral regions of the VZ, whereas ventrally migrating cells tended to come from more dorsal regions but could originate anywhere in the VZ.

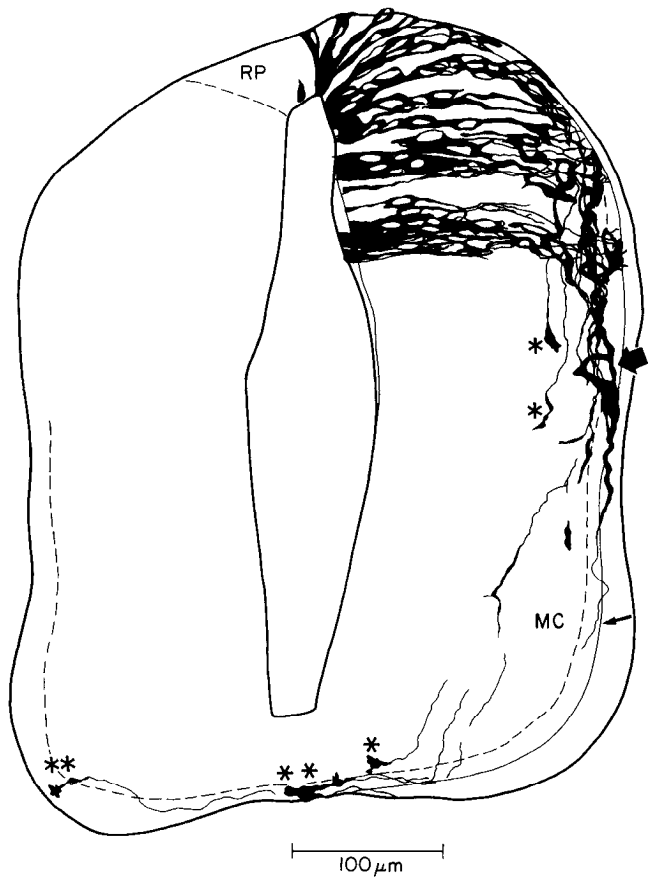

Figure 7. Some noncommissural neurons migrate ventrally. This clone of broad dorsal origin includes cells in the roof plate (*RP*), cells with commissural axons (*) and one large, ventrally migrating cell (*large arrow*) with its leading process (*small arrow*) in the marginal zone, lateral to the motor column (*MC*). Virus was injected at stage 8, and the animal was killed at stage 22. Scale bar, 100 μm.

subjacent to or, rarely, just within the marginal zone (Fig. 4*A*). At later stages, cells often migrated ventrally across the full thickness of the IZ, with some located close to the marginal zone or motoneuron pool and others just outside the VZ (Fig. 4*B*). This mediolateral dispersal of ventrally migrating cells could have resulted from a simple broadening of the fascicle of commissural axons, with ventrally migrating cells now having a greater choice of locations at which to turn orthogonally from their parent radial arrays. Alternatively, the ventral migrants may have continued to turn ventral at the border of the marginal zone, with the oldest cells following the earliest-formed, deepest-lying commissural axons (Holley, 1982) and the younger cells migrating along younger, more superficial axons.

Just as most of the ventrally migrating cells originated from the dorsal VZ, dorsally migrating cells emanated almost exclusively from ventral regions of the spinal cord (Figs. 4*C,D*; 6). Moreover, the dorsal migration differed from the ventral in several ways. First, it was less prevalent, occurring both in fewer clones and in fewer cells per clone. By stages 27–29, for example, the ratio of clones containing ventrally migrating cells to clones with dorsally migrating cells was ~2.5:1. Second, dorsal migrants generally traveled shorter distances than ventral migrants. In fact, about half of the dorsally migrating cells were motoneurons that, although aligned in cords with orientations suggesting a directed migration, nevertheless remained within the

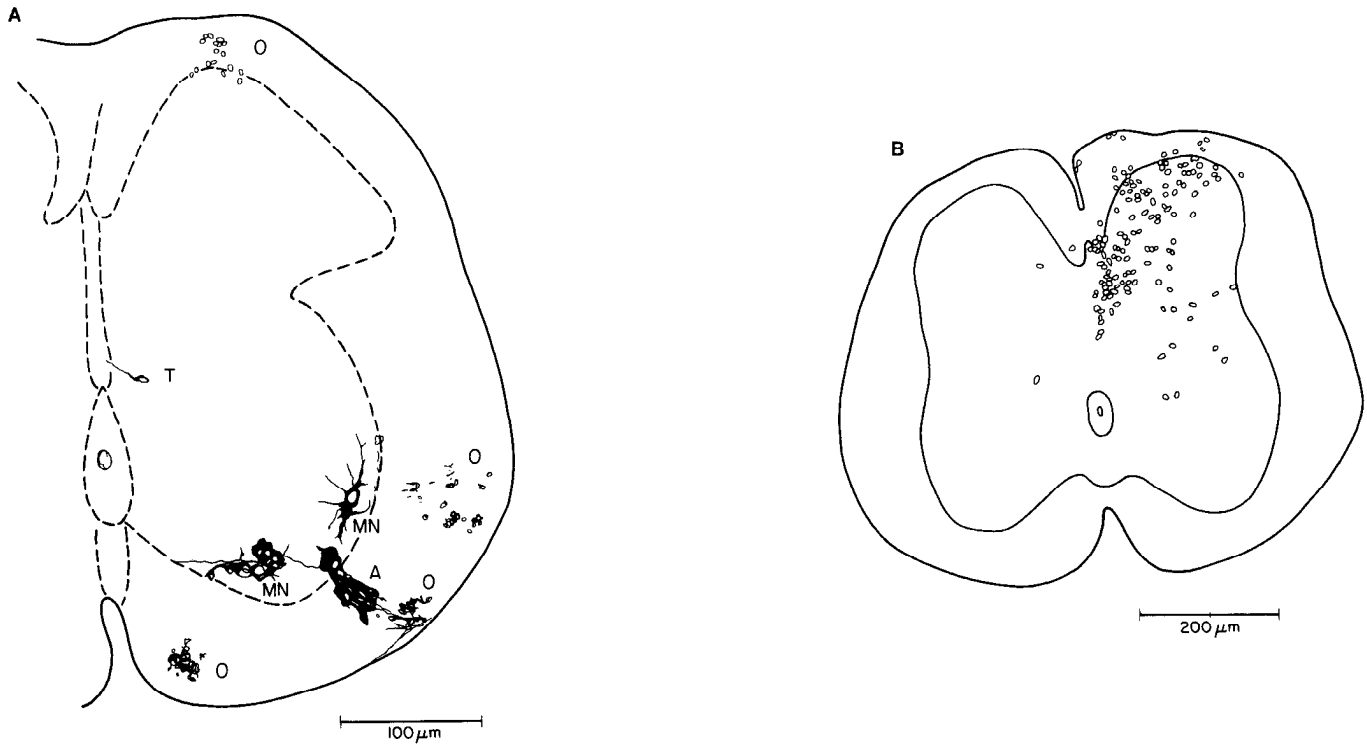

Figure 8. Distribution of clonally related cells in mature spinal cords. *A,* A clone in the thoracic spinal cord containing motoneurons (*MN*), a neuron in the column of Terni (*T*), a cluster of cells likely to be astrocytes (*A*), and four longitudinal arrays of white matter cells that are probably oligodendrocytes (*O*). The cells within the gray matter and the clump of astrocytes were confined to < 100 μm along the longitudinal axis, whereas the oligodendrocytes were spread over 320 μm. Virus was injected at stage 15, and the animal was killed at stage 41. *B,* A large, dorsally based clone labeled with the LZ12 virus. The clone is essentially wedge shaped, with a number of cells having moved into the dorsal white matter. In addition, three cells are found on the contralateral side. The clone is 130 μm thick along the longitudinal axis. Virus was injected at stage 13, and the animal was killed at stage 41. The morphological features by which cell phenotypes were identified were discussed in Leber et al. (1990).

motor columns. Finally, most of the dorsally migrating nonmotoneurons were located deep within the neuropil, just outside the VZ. They separated from their parental radial stream proximally, with clonal relatives continuing radially past the point of divergence. In contrast, the ventrally migrating cells typically turned away from the distal end of their parent radial stream.

Dispersal. As differentiation proceeded, not all clones consisted exclusively of radial and tangential migrants. By stages 24–25, ~15% of clones lacked any sort of radial component (Fig. 5), suggesting that all the cells of the clone had completed their movement away from the VZ and were dispersing in nonradial directions. In another 5% of clones, labeled cells still were arranged in a radial stream as they left the VZ but the more distal cells lacked any obvious tangential or radial arrangement. The cluster of cells at the end of a narrow, radial stream gave the clones a "lollipop" appearance. Such clones were seen only in the more advanced ventral half of the spinal cord, and generally the distal cells were motoneurons (identified as described in Leber et al., 1990). The motoneurons in these clones lacked the alignment and uniform directionality characteristic of the dorsally migrating motoneurons described above.

By stages 31–41, although most clones retained the basic radial or planar arrangement seen at earlier stages, the cells within the planes appeared to be more randomly arranged than they had been at earlier stages (Fig. 8; Leber et al., 1990). In most cases, directed radial and tangential migratory trails were no longer evident. This dispersal, like the dispersal of the distal cells in the "lollipop-shaped" clones described above, probably resulted, in large part, from passive displacement of cells with growth. In general, clonally related cells tended to neighbor each other and to stay within the ventral, intermediate, or dorsal regions of the spinal cord, but the neurons and glia in many clones (particularly those of dorsal origin) were more widely dispersed along the dorsoventral axis (Fig. 8; see also Leber et al., 1990, their Figs. 5, 6).

Longitudinal migration. During the second week of incubation, a third type of directed migration occurred. By stages 31–41, cells that we have tentatively identified as oligodendrocytes or their progenitors on the basis of their scant, distinctly demarcated cytoplasm and short processes that paralleled the axon tracts (Leber et al., 1990) became dispersed in longitudinal arrays within the marginal zone, parallel to the underlying axon tracts, and extended up to several segments rostrally or caudally (Fig. 9). They usually originated from radial or planar arrays of cells in the gray matter, giving the clones a "T"-shape, and suggesting that the oligodendrocytes or their precursors initially migrated radially from the VZ into the marginal zone and then turned and followed axons in either a rostral or caudal direction.

Floor and roof plates

About 20 of the > 1000 clones we analyzed contained cells in the floor plate. Only one of these also contained other cells in the neural tube. Thus, in agreement with Schoenwolf et al. (1989), floor plate and ventral horn cells appear to arise from lineages that had diverged by the time of our earliest injections. Moreover, floor plate clones differed from the other spinal cord clones

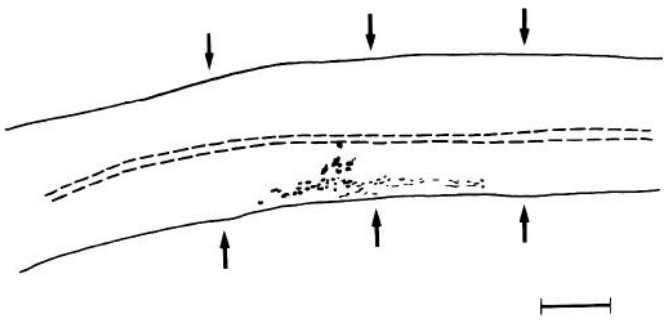

Figure 9. Longitudinal migration of white matter glia. Camera lucida drawing of a dorsal view of a whole mount of a spinal cord showing origin of a clone near the ventricle (*dotted lines*) and widespread longitudinal dispersal in the dorsolateral marginal zone. Segmental boundaries are indicated with *arrows*.

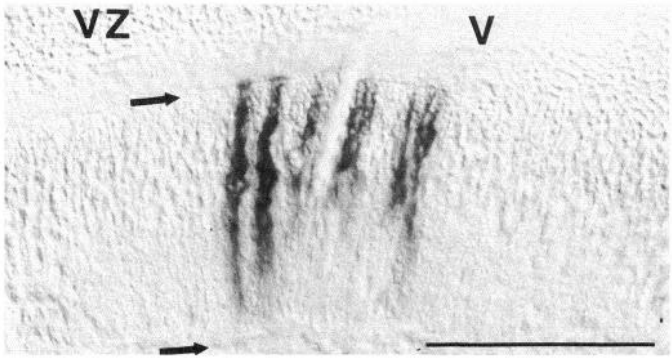

Figure 10. Sagittally oriented floor plate clone. Dorsal is oriented up rostral to the left in this 20 μm thick section. The clone spread ove 180 μm longitudinally, but only 100 μm appears in this section. Thi clone contained no cells outside of the floor plate. *Arrows* indicate th dorsal and ventral edges of the floor plate. The section is somewha oblique and contains both ventricle (*V*) and more dorsal VZ cells (*VZ*) Virus was injected at stage 8, and the embryo was killed at stage 27 Scale bar, 100 μm.

in that labeled cells were aligned rostrocaudally, lying along or near the median sagittal plane (Fig. 10). They were thereby oriented in planes perpendicular to the transverse sheets formed by clones in the rest of the spinal cord. Floor plate clones labeled at early stages (8–9) spanned up to one segment, but became progressively restricted to single strands by stages 16–18.

Clones containing roof plate cells were even less commonly labeled. They, too, were arranged in approximately sagittal planes, but too few were labeled to determine when they became restricted. Occasional clones contained cells both in the roof plate and in the dorsal horn of the spinal cord (e.g., Fig. 7).

Discussion

In this study, we have documented cell movements within the VZ and IZ of the spinal cord. Our main results and the conclusions we draw from them are as follows (Fig. 11). (1) Many clones labeled with injections at stages 8–9 were dispersed widely along both the rostrocaudal and dorsoventral axes. Thus, at early times, cells in the VZ can move in all directions. (2) Clones labeled at stages 15–18 were usually restricted to single sites within the VZ. Thus, the ability of neuroepithelial cells to move within the plane of the VZ decreases to the point where little or no movement occurs. (3) Many labeled clones spread in the transverse but not in the longitudinal plane. Therefore, the decrease in movement within the VZ probably occurs somewhat earlier along the rostrocaudal axis than along the dorsoventral. (4) After movement within the plane of the VZ stops, clonally related cells remain radially arranged within the VZ. This arrangement may result, in part, from the interkinetic movement of cells as they progress through the cell cycle. (5) As they leave the VZ, postmitotic neurons migrate into the IZ in an almost exclusively radial direction. The cords of labeled cells that extend radially from the ventricular surface toward the pia were thus composed of two components: dividing cells in the VZ and postmitotic cells migrating radially toward the external limiting membrane. (6) At the end of their initial radial movement, many cells turn ~90° and migrate ventrally or dorsally, parallel to the surface of the spinal or to the medial edge of the motoneuron pool. Many of these cells differentiate early, are large, and have axons, indicating that they are neurons. (7) A final phase of directed cell movement consists of longitudinal migration of glial cells in the white matter. These cells migrate up to several segments rostrally or caudally, apparently following longitudinal axon tracts. (8) As cells differentiate, they undergo some dispersal, probably as a result of passive displacement secondary to growth of their own processes and of adjacent axons and dendrites. (9) In contrast to most cells of the spinal VZ, clonally related cells within the floor plate epithelium disperse in an exclusively longitudinal direction. With time, however, their ability to move rostrally or caudally also decreases.

Cell movement and spinal cord histogenesis

Results from our earliest injections suggest that cells can initially move tangentially within the VZ. In fact, such tangential movements have been shown more directly by Schoenwolf et al. (1989; Schoenwolf and Alvarez, 1989; Schoenwolf and Sheard, 1989, 1990) and Hilfer et al. (1990), using quail-chick grafts and tracers at stages 3–11. Such tangential cell movement within the VZ may result from both rostrocaudally oriented cell division and intercalation of cells from more medial or lateral positions (Schoenwolf and Alvarez, 1989; Kimmel et al., 1994). Following later injections, however, we find that clones are more restricted, indicating that tangential movement within the VZ diminishes as development proceeds. Consistent with this, Stern et al. (1991) showed that cellular movement is more restricted in the rostral spinal cord VZ than in more caudal, less mature regions. This developmental trend supports the notion of a rapid decrease in rostrocaudal mixing during neurulation, and suggests that the gradual decrease in mixing between stages 8 and 18 that we have documented represents the tail-end of the phenomenon.

Moreover, our results taken together with those cited above suggest that the mixing of VZ cells along the rostrocaudal axis precedes mixing along the dorsoventral axis. Schoenwolf, Hilfer, and collaborators demonstrated that rostrocaudal movements occur at early stages, while movements in the transverse plane are rather limited at the same stages. In contrast, we have shown that at later stages, movements in the transverse plane are not only prominent but continue for a time after the rostrocaudal movements have stopped.

Two forms of radial migration have been reported in spinal cord. Wentworth and Hinds (1978; Wentworth, 1984a,b) and Dorado et al. (1990) suggested that many of the earliest differentiating neurons ("type I" neurons) in the spinal cord (the motor, commissural, and association neurons) migrate by a process of perikaryal translocation. They start as bipolar cells ex-

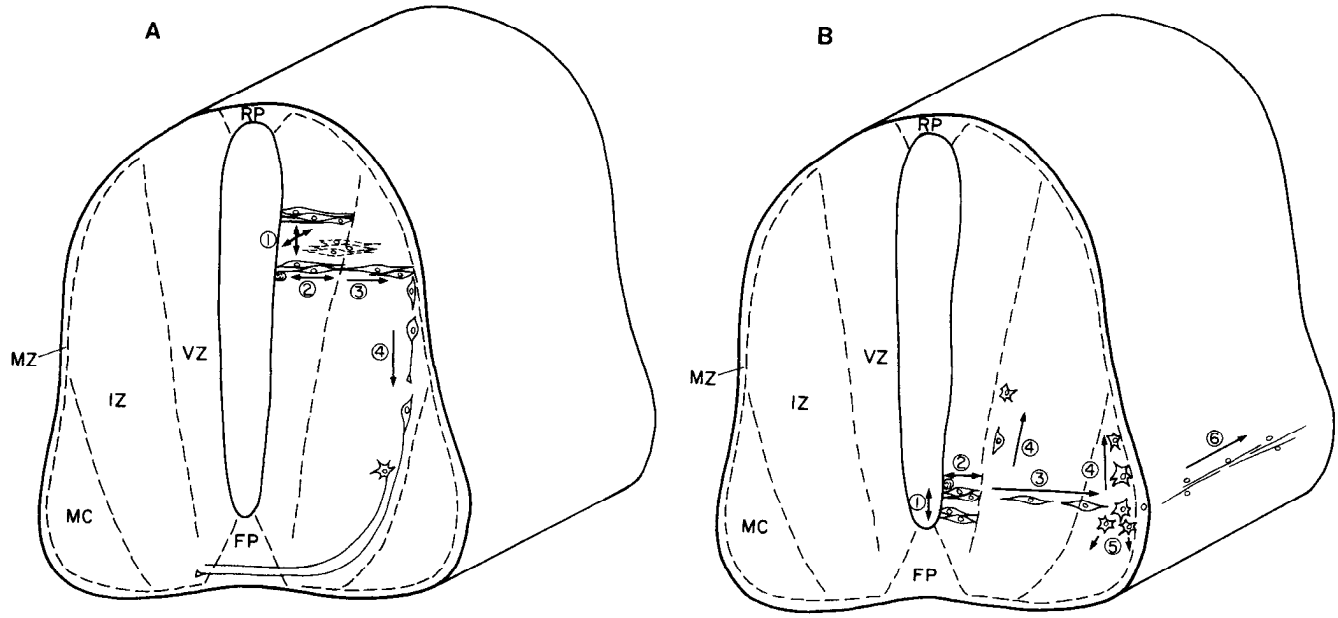

Figure 11. Summary of migratory patterns in dorsal (*A*) and ventral (*B*) chick spinal cord. Major steps ae as follows. (1) Mixing in the VZ. Mixing first occurs in both dorsoventral and rostrocaudal axes (shown only in the dorsal clone for clarity); then dorsoventrally only (shown only in the ventral clone). (2) Radial stacking in the VZ. (3) Radial migration into IZ. (4) Tangential migration. (5) Dispersal of differentiating cells. (6) Longitudinal migration of glia. *FP,* floor plate; *IZ,* intermediate zone; *MC,* motor column; *MZ,* marginal zone; *RP,* roof plate; *VZ,* ventricular zone.

tending processes to both the pial and ventricular surfaces. The ventricular attachment is then lost, the apical end retracts, and the nucleus is displaced laterally toward the marginal zone. Dorado et al. (1990) also showed, however, that other unidentified "type II" ventral horn cells lose both their ventricular and pial attachments and migrate "freely," often with leading, growth cone-like processes. Radial glia exist in the spinal cord (e.g., Choi, 1981; Edwards et al., 1990; Holder et al., 1990; Phelps et al., 1993; Yang et al., 1993), but evidence for their role in guiding neuronal migration remains indirect (Chu-Wang et al., 1981; Liuzzi et al., 1985). Our results are consistent with both forms of migration, and both are likely to occur.

One form of nonradial migration has been recognized for many years. Chick preganglionic neurons originate ventrally, but later migrate dorsally to form the columns of Terni (Levi-Montalcini, 1950; Prasad and Hollyday, 1991). Migration of preganglionic neurons in the rodent spinal cord differs considerably from that in the chick, but is also characterized by a prominent ventral-to-dorsal component (Markham and Vaughn, 1991; Phelps et al., 1993). Previously, we demonstrated that autonomic and somatic motoneurons are often clonal relatives (Leber et al., 1990). It is likely that some of the cells we see migrating from ventral to dorsal just outside the VZ in the present study are such preganglionic neurons en route to the column of Terni.

We found that some somatic motoneurons also migrate dorsally. Motoneurons, whose lineage we described in detail previously (Leber et al., 1990), appear to arise in the ventral part of the VZ, although our data cannot be used to prove this point. After they migrate radially out of the VZ into the motor column, some turn tangentially but take a more superficial route than the autonomic preganglionic neurons and move only short distances from their parental radial stream. This movement may account for an apparent dorsal expansion of the motor column relative to the ventral root (Barron, 1946). The dorsal movement of the somatic motoneurons closely resembles the early phase of dorsal migration of the autonomic preganglionic neurons in rat (Markham and Vaughn, 1991) and may be mechanistically related.

More prominent than the dorsal migration is ventral migration of neurons along the path of the commissural axons. Such a migration was suggested by Langman and Haden (1970) and Ericson et al. (1992), and shown directly by Carpenter and Hollyday (1992) using chick-quail chimeras. We have now shown that at least some of the ventrally migrating cells are commissural neurons. Of note is the existence of several distinguishable groups of commissural neurons in the ventral spinal cord in the chick (Ramón y Cajal, 1960) and rat (Silos-Santiago and Snider, 1992); some of these cells may be positioned by tangential migration, and others by direct radial migration.

Tangential migrations may result either from fibroblast-like locomotion along axonal guides ("neurophilic migration"; e.g., Puelles and Privat, 1977; Rakic, 1985) or from axonal elongation with subsequent translocation of the soma down the axon (e.g., Moody and Heaton, 1983; Bourrat and Sotelo, 1988; Book and Morest, 1990). The dorsal-to-ventral circumferential migration we have described seems most likely to be a manifestation of perikaryal translocation: the early commissural cells have circumferentially projecting axons early in their differentiation, before they even leave the VZ (Ramón y Cajal, 1960; Wentworth, 1984b; Silos-Santiago and Snider, 1992), and use somatic translocation to move their perikarya out of the VZ (Wentworth and Hinds, 1978; Wentworth, 1984a,b). On the other hand, the commissural axons appear to guide the migration of the dorsally moving preganglionic cells in the rat spinal cord (Phelps et al., 1993), so neurophilic migration is possible for the ventrally migrating chick cells as well.

Longitudinal migration of oligodendrocytes or their precur-

sors along axons has previously been reported in spinal cord only during remyelination (Gout et al., 1988). To the best of our knowledge, our reports are the first to describe the process in normal development, although such processes have been reported elsewhere in the central nervous system (Small et al., 1987; Gumpel et al., 1989). In rat spinal cord, oligodendrocyte precursors originate in the ventral part of the VZ and migrate dorsally within the VZ and laterally into the IZ (Warf et al., 1991; Noll and Miller, 1993; Pringle and Richardson, 1993). Oligodendrocytes also originate ventrally in the chick, but the dorsal migration probably takes place exclusively in the IZ (R. H. Miller, personal communication). Whether the dorsal migration is guided by axons, as our results suggest the longitudinal may be, is unknown.

Comparison of migratory patterns in brain and spinal cord

The combination of progressively restricted cell mixing in the VZ, radial migration through the IZ, and tangential migration along axons is not unique to the spinal cord. The brainstem, for example, has many structural and organizational features in common with the spinal cord, and several similarities in their migratory patterns are worth noting. First, as in the spinal cord, cell movements in the chick rhombencephalic VZ decrease with time. Restrictions first affect rostrocaudal movement across rhombomeric boundaries, but eventually increase to limit VZ mixing along both axes. Second, cells migrate out of the VZ into the IZ in a radial fashion. Third, extensive tangential migration has also been demonstrated in the rhombencephalic IZ of the chick. Fourth, as in the spinal cord, movement in the transverse plane predominates over longitudinal movement, at least before E8. Fifth, in both the brainstem and spinal cord, alar-to-basal migration predominates and ventrally migrating cells follow the paths of circumferentially directed axons (Fraser et al., 1990; Tan and Le Douarin, 1991; Hemond and Glover, 1993). Finally, in both spinal cord and brainstem (Heaton et al., 1978; Moody and Heaton, 1983; Covell and Noden, 1989), motoneurons migrate from basal to alar, against the predominant flow. Similar results have been described in the brainstem of rodents (Bourrat and Sotelo, 1988, 1990; Ono and Kawamura, 1989, 1990) and primate (Rakic, 1985).

In contrast to the brainstem, the optic tectum differs vastly from the spinal cord in its structure and organization. Surprisingly, however, the two tissues share a remarkable number of migratory patterns. Both retroviral labeling (Gray et al., 1988) and chick-quail chimeras (Martínez et al., 1992) demonstrate that considerable tangential movement and mixing occurs in the tectal VZ at early stages, but that this mixing becomes progressively restricted. In both tissues, the most prominent pattern of migration is radial, but distinct early and late patterns of nonradial migration also occur. The first-born tectal cells, the multipolar ganglion cells, like the early-born spinal commissural neurons, initially migrate out of the VZ radially but then turn orthogonally and migrate considerable distances tangentially, parallel to the ventricular surface (Senut and Alvarado-Mallart, 1987; Gray and Sanes, 1991; Martínez et al., 1992). In both tissues, the path taken by the tangentially migrating cells matches that followed by their axons, consistent with either migration along homotypic axons or somatic translocation down the leading axonal processes. In the spinal cord, a distinct dorsal migration of preganglionic autonomic neurons occurs; in the tectum, horizontal neurons in more superficial layers undergo an extensive tangential migration (Martínez et al., 1992). Finally, in both tissues, cells believed to be glia migrate away from the pial ends of their radially arrayed clonal relatives (Gray and Sanes, 1991).

A combination of radial and nonradial migration also occurs in the mammalian cerebral cortex. Direct visualization of DiI-labeled cells in the embryonic day (E) 15 mouse telencephalon demonstrates tangential mixing of cells in the VZ (Fishell et al., 1993). Retroviral injections at E14–15, however, label clones of cells that are aligned in single radial arrays in late embryos (Luskin et al., 1988; Walsh and Cepko, 1993), suggesting that tangential cell spread in the VZ becomes quite restricted soon after E15 and that cells leave the VZ in radial arrays. As in other areas of the nervous system, tangential dispersal of cells also occurs at later stages (Walsh and Cepko, 1993; Luskin, 1993), though its timing and extent remain subjects of uncertainty. Although some of this spread may occur in the VZ, a substantial minority of cells in the cortical IZ migrate in nonradial directions (O'Rourke et al., 1992, 1993). Whether these cells follow axons, extracellular matrix substrates, or other cues is still unknown.

The similarities in the development of the spinal cord and the brainstem are not surprising, given their similar structural and functional organization. The remarkable number of developmental features they share with the chick optic tectum and the mammalian cerebral cortex was unexpected, however. A progressive restriction of neuroepithelial cell mixing, an initial radial migration, and a subsequent tangential migration all have roles in the histogenesis of both the laminated and nonlaminated tissues. In the spinal cord, brainstem, and tectum, the tangential migration appears to be follow axon tracts and to involve "type I" cells. Less is known about tangential migration in the cortex. As diverse as these tissues may be, they appear to use a limited repertoire of migratory building blocks, putting these together in region-specific patterns to generate unique neural structures.

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