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Distinct modes of migration position oligodendrocyte precursors for localized cell division in the developing spinal cord

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Abstract

Establishment of the cytoarchitecture of the central nervous system reflects stereotyped cell migration and proliferation of precursor cells during development. In vitro analyses have provided extensive information on the control of proliferation and differentiation of oligodendrocyte precursors, but less is known about the migratory behavior of these cells in vivo. Here we utilize a transgenic mouse line expressing EGFP under the proteolipid protein promoter (PLP-EGFP+) in oligodendrocyte lineage cells to directly visualize their behaviors in developing spinal cord slices. During early development OPCs disperse from their origin at the ventricular zone using saltatory migration. This involves orientation of the cell with a leading edge toward the pial surface, alternating stationary and fast-moving phases and dramatic shape changes. Once cells exit the ventricular zone they exhibit an exploratory mode of migration characterized by persistent translocation without dramatic changes in cell morphology. The control of migration, proliferation and cytokinesis of OPCs appear to be closely linked. In netrin-1 mutant spinal cords that lack dispersal cues, OPC migration rates were not significantly different but the trajectories and number of migrating cells were dramatically reduced. In contrast to DNA replication that occurs at the ventricular zone or throughout the spinal cord neuropil, cell division or cytokinesis of OPCs occurs predominantly at the interface between gray and white matters with the majority of cleavage planes parallel to the midline. These studies suggest that positional cues are critical for regulating OPC behavior during spinal cord development.

Keywords

Oligodendrocyte Precursors; Migration; Proliferation; Spinal Cord; Time lapse microscopy

Introduction

The development of the nervous system is characterized by extensive cell migration that dictates appropriate tissue histogenesis and the development of functional neural units. Regardless of their final destination, immature neurons follow stereotyped patterns of migration. For example, cortical neurons generated in the dorsal ventricular zone of the telencephalon predominantly follow radial pathways towards the pial surface before integrating into the tissue to form the characteristic laminar structures (Hatten 1999). By contrast, inhibitory interneurons that are generated in the ventral forebrain follow tangential pathways to migrate dorsally before integration into cortical layers (Marin and Rubenstein 2001). Premature termination of neuronal cell migration as well as uncontrolled or misdirected

migration results in ectopic cell localization and perturbation of the normal circuitry of the brain. In humans such aberrant migration is thought to contribute to several severe neurological syndromes such as epilepsy and mental retardation (Lammens 2000). The underlying mechanisms mediating neural cell migration are complex. Diverse molecular cues ranging from members of the laminin family (Gudz et al. 2006) to chemokines (Li et al. 2008; Paredes et al. 2006) have been implicated in the regulation of neuronal cell migration and the elucidation of their roles has provided important insights into the mechanisms of neural development (Marin and Rubenstein 2003). For example, studies of neuronal migration using time lapse imaging (Kriegstein and Noctor 2004; Nadarajah et al. 2001) have dissected migrational defects attributable to different genes in the same genetic pathway that result in the same general phenotype (Gupta et al. 2003; Sanada et al. 2004). By contrast, relatively little is known about the distinct pathways or mechanisms of glial cell migration and detailed analyses of oligodendrocyte precursor (OPC) behavior in vivo is missing.

Oligodendrocytes the myelinating cells of the central nervous system (CNS) develop from restricted regions of the germinal zones in a temporally regulated manner and subsequently disperse widely throughout the CNS (Kessaris et al. 2006). For example in the forebrain early in development OPCs arise ventrally followed by consecutive waves of more dorsally derived cells that subsequently intermingle (Kessaris et al. 2006). In the visual system oligodendrocytes of the chick and rat optic nerve are derived from cells at the floor of the third ventricle that subsequently disperse throughout the nerve (Gao and Miller 2006; Ono et al. 1995) while in the spinal cord the majority of oligodendrocytes develop from cells originally located in the ventral ventricular zone (Miller 1996; Pringle et al. 1996), that migrate long distances to populate white matter where they proliferate and mature.

Classical in vitro studies by Pfeiffer and colleagues defined many of the cellular properties of oligodendrocyte precursors. The development of OPCs occurs in a series of stages (Pfeiffer et al. 1993) that reflect different growth factor responsiveness (Gard and Pfeiffer 1990; Gard and Pfeiffer 1993) and antigenic properties (Bansal and Pfeiffer 1992). The differentiation of OPCs into oligodendrocytes is a dynamic property that can be regulated by extrinsic cues (Bansal et al. 1988; Bansal and Pfeiffer 1989) and growth factor influences (Bansal and Pfeiffer 1997). How these processes regulate oligodendrogenesis in vivo is unclear, however limited dilution studies suggest that OPCs colonize regions of the brain substantially in advance of myelination (Barbarese et al. 1983) suggesting that localized cues are important in regulating the behavior of OPCs. In the current study we examine OPC behavior during development by utilizing time-lapse imaging in a transgenic mouse line expressing EGFP (enhanced green fluorescent protein) under the control of the proteolipid protein (PLP) promoter (PLP-EGFP mice) (Mallon et al. 2002). Proteolipid protein is one of the most abundant components of the myelin sheath (Boison and Stoffel 1994), however the short isoform of PLP, DM20, has been shown to be present in early OPCs cytoplasm (Dickinson et al. 1996; Timsit et al. 1992) and this expression allows PLP-EGFP mice to be used as a model system to unambiguously identify OPCs in vivo and follow their cellular behaviors in acute slices of embryonic spinal cord during development.

By real-time confocal imaging, we show that PLP-EGFP+ OPCs in spinal cord slices utilize two major modes of cell migration during development. At E12.5 OPCs arising at the ventricular zone use saltatory migration to move rapidly from their site of origin to the intermediate zone of the expanding neural tube. Saltatory migration is defined as alternating fast moving and stationary periods accompanied by dramatic changes in cell shape. Later in development OPCs utilize both saltatory and exploratory modes of migration throughout gray and white matter. During exploratory migration OPCs synchronize movements of the cell body and the leading edge and do not undergo dramatic changes in cell shape. The trajectory of migration and localization in the developing spinal cord had profound effects on OPC proliferation.

The stereotyped migrational paths taken by neuronal precursors are predominantly orchestrated by multiple guidance molecules. Although our understanding of the guidance of OPC migration is relatively rudimentary several studies suggest they may utilize similar molecular cues such as netrin-1 (Tsai et al. 2006). The rate of migration of PLP-EGFP+ cells was not affected by the lack of netrin-1 however, consistent with previous data (Tsai et al. 2006) we demonstrated a decrease in PLP-EGFP+ cells numbers in netrin-1 mutants concomitant with a decrease in proliferating events.. Remarkably, the vast majority of OPC cytokinesis occurred precisely at the interface between gray and white matter. In the gray matter the plane of division was invariably horizontal to the ventricular zone and perpendicular to the direction of migration.

Materials and Methods

Slice cultures

The PLP-EGFP transgenic mouse line (Mallon et al. 2002) used in this study was maintained in compliance with the animal protocol at Case Western Reserve University with the plug day designated embryonic day 0 (E0). To obtain PLP-EGFP expression in netrin-1 mutant animals the PLP-EGFP transgenic mouse line was crossed with netrin-1 heterozygotes (gift from Dr. Tessier-Lavigne, Genentech, CA) and genotyped for netrin mutant RNA expression (Serafini et al. 1996) and EGFP expression. To prepare living slices, spinal cords embedded in 4% low melting agarose/DMEM were cut into 250 μ m coronal slices or 160 μ m sagittal slices on a vibratome (Leica VT1000S) in ice-cold PBS. Slices were grown in DMEM with 1% FBS, PDGF (platelet-derived growth factor) (10 ng/ml), N2 supplement and 27 mM glucose (Tsai et al. 2006) overnight before imaging. After 12 to 18 hrs, fresh media containing 25 mM HEPES buffer was added and the culture was placed in a heated chamber on LSM 510 confocal system with Axiovert 100M microscope (Zeiss) and a 40x LD objective (NA 0.6) at constant 37°C. Stacks of images at a resolution of 1024 $\times$ 1024 pixels were taken at 10 or 20 min intervals with total recording length for at least 3 hrs. The mean visible thickness of the slice was approximately 40 μ m. All Z axis images were projected into single image and processed into continuous files using Zeiss LSM 510 image browser and Adobe Photoshop. The cell migration tracks were obtained by following cell body movements using Metamorph (Fryer company, Inc, Huntley, IL).

Analyses of purified oligodendrocyte precursor migration in culture

To analyze the migration behaviors of purified OPCs, postnatal day 1 (P1) spinal cord A2B5 + cells were immuno-purified as previously described (Tsai et al. 2002) and plated on laminin-coated (10 μ g/ml) dishes. Fresh medium with 25 mM HEPES was added prior to recording. Images were taken every 5 min by a cooled CCD (Princeton Instruments, Trenton, NJ) and inverted microscope (Axiovert 405M Zeiss) under a 20x LD (NA 0.4) objective. The recordings were repeated 3 times from 3 separate cell preparations.

Data processing

Migrating cells were defined as cells that translocated at least one cell body length during recording. For assessment of cell number, stacks of images taken from the first time point was projected to single image and cells with a clear boundary and the extent of the processes counted. The difference in distribution of cells in ventricular, intermediate, and marginal zones of spinal cord slices and the difference between wild type and netrin-1 mutant animals were tested by chi-square analyses. The migration rates of PLP-EGFP+ cells at different developmental stages and genetic background were tested by one-way ANOVA. PLP-EGFP + cells in wild type spinal cord slices were analyzed from 3 animals at each developmental age and at least 2 slices were recorded. Netrin-1 mutant spinal cord slices from littermates were analyzed from 2 animals at each developmental age and at least 2 slices were recorded.

Results

Early mode of migration of spinal cord OPCs

During development oligodendrocyte precursors can be identified in the ventral ventricular zone of the mouse spinal cord around E12.5 through the expression of PLP-EGFP and many of these cells co-express markers of early OPCs such as Olig2 (Tsai et al. 2006). These cells or their progeny subsequently disperse widely throughout the spinal cord. To observe the early stages of dispersal, the migration of EGFP+ cells was followed using time-lapse microscopy in slice preparations of E12.5 spinal cords. At the ventricular zone PLP-EGFP+ cells were oriented radially. These cells had a leading process projecting toward the pial surface indicating they had developed some level of intrinsic polarity. In the majority of cells the leading process did not project completely to the pial surface but rather was highly dynamic, extending and retracting rapidly. Cell translocation was saltatory and invariably followed the direction of the leading process. In general, the cell body translocated slowly along the length of the process for an extended interval. These periods of uniform movement were interspersed by short intervals of extremely rapid movement as the cell body translocated to a new location. For example, the cell imaged in Figure 1 demonstrated a constant slow rates of migration from 0 min to 80 min followed by sudden collapse and rapid movement of the cell body over a 20 min period (Fig. 1A). Not all PLP-EGFP cells at the ventricular zone demonstrate saltatory migration at the same time. During 3-hour imaging periods from 3 slices, 12% (5/42) of cells underwent saltatory migration and the remainder were relatively sedentary (Fig 1C). Even though not moving at a constant rate, saltatory migrating cells covered significant distances in relatively short time periods. For example, among actively migrating cells the mean rate of migration of saltatory cells was $0.48 \pm 0.8 \mu\text{m}/\text{min}$ while the mean migration rate of all migratory cells was $0.19 \pm 0.02 \mu\text{m}/\text{min}$. Saltatory migration was not restricted to OPCs emigrating from the ventricular zone or to cells in slices. In cell cultures purified A2B5+ OPCs from newborn rat spinal cord cultured on laminin substrate also demonstrated the same saltatory movement of the cell body (Fig. 1B) suggesting this mode of migration is intrinsic to early OPCs and not imposed by the environment.

Exploratory modes of migration

In slices from older animals (E14 to P0) or in regions not closely associated with a germinal zone, OPCs adopted a different mode of migration. In longitudinal slices of dorsal spinal cord OPCs migrating along axonal tracts predominantly demonstrated an exploratory style of translocation. These cells migrated equally in both rostral and caudal directions (Fig. 2). Exploratory migration was characterized by constant and persistent movement of cell bodies and exploratory processes (Fig. 3A–C). The leading processes retained a relatively uniform length but constantly sent out growth cone-like structures to explore their environment (Fig. 3A). This leading process did not appear to be firmly attached to any local substrate or to follow any obvious preformed pathway. Cell translocation in this mode did not result in a total reorganization of cell morphology, rather changes in the identity of the leading process was sufficient to alter the trajectory of the migrating cell. A similar mode of exploratory migrational behavior was seen in purified A2B5+ cells cultured on laminin substrates (Fig. 3B) suggesting that, like saltatory migration, exploratory migration was intrinsic to OPCs. On laminin substrates in vitro OPC migrated at a constant rate and changed their directions repeatedly and kept relatively constant lengths of leading processes during the course of recording (Fig. 3B). Overall, OPCs moving in the exploratory mode did not migrate slower than those using saltatory migration and occasionally cells were seen that switched from one mode to the other.

A less common mode of translocation exhibited by OPCs has been termed the “inchworm” form of migration. This was most often seen in cells engaged in exploratory migration. In one particular cell (Fig. 3D), a bulge formed and moved forward prior to the movement of the cell

body toward the same direction. The same cell demonstrated the migratory behavior twice during the recording (Fig 3D and D'). This migration is reminiscent of nucleokinesis in neuronal migration (Schaar and McConnell 2005) that is largely regulated by cytoskeletal components in the cell and may reflect the fact that OPCs and neurons utilize similar modes of migrational cues.

Consequences of migrational defects

Time-lapse imaging of cell migration can be an informative tool in deciphering migrational defects affected by distinct genes (Gupta et al. 2003; Sanada et al. 2004). We previously demonstrated that the initial OPC dispersal from the ventral ventricular zone is mediated by netrin-1 expression in the ventral midline (Tsai et al. 2003). In netrin-1 mutants OPCs lost the ability to generate directional migration from the ventricular zone, suggesting that netrin-1 is an orientation molecule that establishes cell migration polarity (Tsai et al. 2006). Consistent with this hypothesis, there was an accumulation of PLP-EGFP+ cells in the ventricular and intermediate zones in netrin-1 mutant spinal cords at E14 and E17 suggesting a dispersal defect (Fig. 4A). We previously demonstrated that purified OPCs cultured in the presence of recombinant netrin-1 did not show changes in the rate of migration of chemokinesis (Tsai et al. 2006) and analyses of migrational rates in vivo confirmed those findings. At E14, the migration rate of PLP-EGFP+ cells in wild type slices was $0.24 \pm 0.02 \mu\text{m}$ (n=26) compared with $0.21 \pm 0.02 \mu\text{m}$ (n=17) in netrin-1 mutants. At E17, the migration rate of PLP-EGFP+ cells in wild type slices was $0.21 \pm 0.01 \mu\text{m}$ (n=20) compared with $0.24 \pm 0.02 \mu\text{m}$ (n=30) in netrin-1 mutants indicating no significant differences in OPC migration rates between wild type and mutant slices at E14 and E17. Moreover, no significant differences in OPC migration rate was seen at different developmental stages from E14 to P0 ($p=0.024$). By contrast at P0, the rate of migration of PLP-EGFP+ cells decreased in the mutant spinal cord ($0.14 \pm 0.01 \mu\text{m}$, n=16) compared to wild type slices ($0.19 \pm 0.02 \mu\text{m}$, n=15, $p=0.003$). Together these data support the hypothesis that netrin-1 is a dispersal or orientation factor for spinal cord OPCs but does not affect the rate of migration chemokinesis in vivo.

Localized cytokinesis in the marginal zone

Previous BrdU incorporation studies have shown extensive DNA synthesis of cells at the spinal cord midline (Noll and Miller 1993). By contrast, time-lapse imaging revealed a reproducible pattern of cell division of OPCs in the developing spinal cord. Cytokinesis predominantly occurred at the interface between presumptive gray and white matter. The plane of division depended on the precise location of the dividing cell. Cells undergoing cytokinesis at the gray/white interface had a cleavage plane oriented radially to the ventricular zone (Fig. 5B, black pair of cells) and in most cases one daughter cell continued migration in a radial direction while the other remained stationary (Fig. 5A). By contrast cells undergoing cytokinesis in white matter had a cleavage plane oriented horizontal to the ventricular zone and both cells migrated rapidly after the completion of division (Fig. 5C and D) suggesting that local cues influence both the orientation of the cleavage plane of the cells and the subsequent behavior of daughter cells.

Both the number and the pattern of cell divisions were altered in the absence of netrin-1. Overall fewer cell divisions were observed in netrin-1 mutants and the dividing cells had atypical division planes compared to cells in wild type slices (Fig. 5B–D, compare black to white pair of cells). When total cell numbers were obtained from living spinal cord slices (visible thickness approximately 40 μm), there was a reduction of PLP-EGFP+ cell numbers in the mutant spinal cords and numbers of the dividing cell pairs per slice were also decreased. In order to confirm cell division, stacks of images underwent 3D reconstruction to make sure that the proliferating pair was originally a single cell (data not shown). At E14, there was 1 division event per slice in the mutant spinal cord, and 3.5 division events per slice in the wild type spinal cord during

similar recording intervals. At E17, there were 1.5 division events per slice in the mutant spinal cord, and 6 division events per slice in wild type spinal cord during similar recording interval. By P0, there was one division event per slice in the mutant spinal cord, and 4 division events per slice in wild type spinal cord. These observations indicate that the number of dividing OPCs is compromised in netrin-1 mutant spinal cord and suggests that OPCs that migrate to the gray/white matter interface respond to local signals that stimulate cell division.

Discussion

In this study, we describe the behavior of OPCs in spinal cord slices using time-lapse confocal imaging. We show that PLP-EGFP+ OPCs utilize different modes of migration at different times in development and locations in the spinal cord. Moreover we demonstrate a remarkable restriction in the location of OPC cell division or cytokinesis at the gray-white matter interface suggesting that localized cues trigger cell division and that appropriate migration is critical for subsequent expansion of the OPC population. During their dispersal OPCs migrate in both the coronal plane as well as along the rostral-caudal axis of the spinal cord. Early in development, at E12.5, OPCs predominantly utilize saltatory migration to quickly translocate from the ventricular zone into the marginal zone while later in development the majority of OPCs exhibit exploratory migration. Analyses of the migrational characteristics of OPCs in netrin-1 mutant spinal cords confirms earlier in vitro studies indicating that netrin-1 regulates OPCs orientation or polarity rather than the absolute rate of cell migration. For example, OPCs displayed delayed dispersal from the ventricular zone into the presumptive white matter in netrin-1 mutant spinal cords, although once outside the ventricular zone they had similar migration rates. The initial dispersal from the ventricular zone appears critical to position OPCs for cytokinesis. Cell division and separation of the daughter cells occurred almost exclusively at the gray-white matter interface with a stereotyped orientation of cleavage planes. In the absence of netrin-1, however, the number of dividing cells in this region was substantially reduced.

The migration of neuronal precursor cells in the developing CNS has been extensively investigated and several distinct behaviors and pathways mediated by different molecular mechanisms have been described. In the developing forebrain, cortical neurons generated in the dorsal telencephalic ventricular zone migrate radially to form the classic inside out columnar pattern (Hatten 1999; Rakic 1972) while inhibitory neurons generated in the ventral forebrain migrate tangentially within the subventricular zone (SVZ) or intermediate zone before turning radially to incorporate into specific cortical layers (Angleley et al. 2003; Kriegstein and Noctor 2004; Marin and Rubenstein 2001). During the course of early radial migration uni/bipolar neurons utilize somal translocation and locomotion (Nadarajah et al. 2001) while later radial migration is largely multipolar (Tabata and Nakajima 2003). It seems likely that individual neurons use multiple types of migration, with cells in the lower intermediate zone switching modes of migration as they migrate radially (Nadarajah et al. 2001). More importantly, distinct molecular mechanisms appear to regulate distinct types of migration. For example, the generation of correct cortical lamination involves several proteins, such as p35 (Hunter-Schaedle 1997) and Disabled-1 (Howell et al. 1997). In p35 null animals subsets of radially migrating neurons exhibit branched migration and dissociate prematurely from their normal radial glial substrate (Gupta et al. 2003) while Disabled-1 regulates neuron-glia adhesion properties so that wild-type neurons detach from radial glia at later stages of migration (Sanada et al. 2004).

Like their neuronal counterparts ventricular derived OPCs migrate extensively throughout the developing CNS to populate axonal tracts, although the details of their modes of migration are less clearly understood. Initial OPC migration is radial, but unlike the migration of neurons appears not to rely on preformed glial pathways and exhibits different morphological characteristics. While migrating neurons demonstrate somal translocation that moves the cell

body and shortens attached processes at a constant rate (Nadarajah et al. 2001), oligodendrocyte precursors attach a leading process at the intermediate/marginal zone interface and undergo saltatory movement with process shortening (Fig. 1). Later in development OPCs exhibit exploratory modes while neurons demonstrate saltatory locomotion (Nadarajah et al. 2001). Occasional very slow moving OPCs with multiple processes were observed, however, it was unclear whether these cells migrated towards specific targets. The different modes of migration presumably reflect the combination of intrinsic cell characteristics as well as environmental and substrate cues mediating migration.

The mode of migration utilized by OPCs appears to be largely selected by environmental cues. In developing spinal cord initial migration through presumptive gray matter is largely radial and saltatory and subsequent rostral-caudal or radial migration in presumptive white matter is exploratory. In the forebrain, however, where the histological architecture is reversed with deep white matter and superficial gray matter, the initial migration of presumptive OPCs has been proposed to utilize axon tracts and subsequent migration is radial. In either region it is unclear whether there are specific subpopulations of OPCs that have preferential migratory mechanisms. The expression of some antigenic markers suggests heterogeneity among OPCs (Han et al. 2004) while recent studies suggest that in the forebrain OPCs are generated in spatially and temporally distinct waves (Kessaris et al. 2006). Whether OPCs derived from different regions utilize different migration modes is currently unknown.

The rate of cell migration reflects both cell intrinsic and environmental influences. In general it appears that OPC migration is slower than neuronal precursor migration. The most rapid migration is the chain migration of neuronal precursors at around 122 $\mu\text{m/hr}$ while the rate of radial migration of neuronal precursors is approximately 100 $\mu\text{m/hr}$. By contrast, although glial cell migration in the SVZ of the forebrain occurs at approximately 90 $\mu\text{m/hr}$, in spinal cord OPCs migrate much slower at a rate of 10–12 $\mu\text{m/hr}$. Several factors may contribute to this relatively slow migration. It is conceivable that the environment of the spinal cord may be distinct from that of the cortex. The earlier maturation of spinal cord neurons or the expression of extracellular matrix proteins such as tenascin-C (Garcion et al. 2001) may inhibit migration. The relatively slow migration of spinal cord OPCs may also be intrinsic. In vitro, in the absence of inhibitory cues, these cells migrate at approximately 25 $\mu\text{m/hr}$ (Tsai et al. 2002), substantially slower than that seen in developing forebrain. One important consequence of the rate of OPC migration may be the timing of myelin repair after injury. After demyelination OPCs migrate from surrounding tissue into the lesions (Franklin 2002) and slow rates of migration might substantially compromise neural repair.

The localization of PLP-EGFP+ OPC cell divisions in the developing spinal cord is remarkably restricted (Fig. 5). At E14 in wild type spinal cord, OPCs undergoing cytokinesis are almost exclusively localized to the gray/white interface with the majority of division planes perpendicular to the pial surface. Why cytokinesis is restricted to this location at this period in development is unclear. One possibility is that the gray/white interface is a region rich in OPC mitogens such as PDGF (Calver et al. 1998) and that radially migrating cells are locally stimulated to proliferate in that region. This seems unlikely, however, since there is no evidence of localized expression of PDGF, and analyses of S phase activity by methods such as BrdU incorporation do not reveal a similar distribution (Tsai et al. 2006). Rather, during early development the majority of BrdU incorporation occurs around the midline (Noll and Miller 1993) presumably as a result of local mitogen expression (Pfeiffer et al. 1993). We propose that the relatively precise localization of OPC cytokinesis at the interface between the gray and white matter reflects a local signal that drives cytokinesis that is independent of the mitogenic stimulation that initially provokes the cell to enter the cell cycle. This concept is analogous to the situation in early neuronal development where cycling neuronal precursors undergo cytokinesis selectively at the ventricular surface while DNA synthesis occurs deeper in the

neuropil (Jacobson 1978). The nature of the signals triggering OPC cell division remains to be determined but may be extremely important in facilitating the expansion of the OPCs. In the absence of the dispersal cue netrin-1, OPCs do not migrate to the gray/white interface and fail to undergo cytokinesis resulting in a reduced pool of cells (Tsai et al. 2006). Later in development cytokinesis occurs throughout white matter suggesting the signal is widespread in the white matter and perhaps the initial localization of mitotic cells at the interface reflects the first contact of “proliferation-primed” radially migrating cells with the signal. Given the emerging heterogeneity of OPC populations (Han et al. 2004), however, it may be that not all OPC populations follow the same cytokinesis patterning. Virtually all the cells divided with a cleavage plane vertical to the pial surface resulting in 2 radially oriented daughter cells. Such a plane likely reflects cytokinesis of an originally radially oriented cell such as those undergoing saltatory migration away from the ventricular zone. During neuronal development the plane of cleavage has been suggested to reflect differential daughter cell fates. Symmetric divisions were largely vertical to the ventricular surface and asymmetric divisions were perpendicular (Chenn and McConnell 1995). Similarly, although displaced laterally in presumptive white matter, OPC planes of cleavage are vertical to the ventricular surface and based on the persistent expression of PLP-EGFP it appears that cell division in the white matter generates 2 similar daughter cells.

Understanding the mechanisms mediating the different modes of migration and defining signals that promote cell division and separation of OPCs are likely to provide novel targets for promoting the repair of demyelinating diseases in the CNS that depend on the repopulation of damaged areas with cells capable of remyelination.

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Figure 1.

Saltatory migration mode. (A) At E12.5, OPCs migrate from the ventricular/intermediate zones into the marginal zone using saltatory migration mode with one process toward the pial surface (right edges of each panel). The cell body largely undergoes steady movements from 0 min to 80 min and then suddenly undergoes dramatic morphology change and changes its location at 100 min. (B) The same saltatory mode of migration is utilized purified OPCs cultured on laminin substrates. Note that the OPC with long processes rounds up and suddenly moves from one point to another (55 min to 85 min). (C) Saltatory migration provides fast migration of OPCs. Migration rate changes in 20 min intervals plotted for individual cells demonstrate long periods of inactivity punctuated with short intervals of extremely rapid migration.

Figure 2. Migrational trajectories of PLP-EGFP+ cells in the longitudinal spinal cord slice. PLP-EGFP+ cells in the dorsal spinal cord (A) migrate from the ventral domain dorsally towards the white matter. (B) Once in white matter regions OPCs migrate extensively in the rostral caudal axis.

Figure 3.

Exploratory migration mode. (A) During typical exploratory migration cells migrate at constant speed with relatively constant length of leading processes. Note that in A both cells labeled with asterisks migrate at fairly constant speed. (B) The same migration mode also exists in OPCs cultured on laminin substrates. Note that even though the cell changes migration path and direction during recording it migrates at a constant speed with one leading process. (C) Migration rate changes in every 20 min interval plotted for representative cells in the slice show that OPC using exploratory migration mode holds relatively constant migration rate during the course. (D) A bulge on PLP-EGFP+ cell forms before the cell body movement in slice. In this particular cell, the bulge forms twice (D, D') right before the real cell body movement.

Figure 4.

The distribution of PLP-EGFP+ cells in the wild type and netrin-1 mutant spinal cord. The distribution of PLP-EGFP+ cells in each region in the spinal cord starts similarly between wild type and netrin-1 mutant at E12.5. Progressively, PLP-EGFP+ cells show accumulation in the VZ and IZ of mutant spinal cords at E14 and GM at E17. Although the cell distributions are not significantly different at P0, the PLP-EGFP+ cell number is greatly reduced at P0. The open bars indicate wild type spinal cords and the solid bars indicate netrin-1 mutant spinal cords. VZ, ventricular zone. IZ, intermediate zone. MZ, marginal zone. GM, gray matter. WM, white matter.

Figure 5.

Localized cell division of OPCs in the spinal cord. (A) PLP-EGFP⁺ cell division occurs at the interface of gray and white matters at E14. Even though it is rare for PLP-EGFP⁺ cells to incorporate BrdU in the neuropil the majority of BrdU incorporation occurs at the midline spatially separated from the location of division events. After cell division, the two daughter cells migrate rapidly away from each other. Two dividing pairs are labeled with asterisks and white arrow heads, respectively. (B–D) Schematic representation of localized OPC cell division in the spinal cord slice. (B) At E14, majority of dividing pairs in wild type spinal cords (denoted by black dots) shows cleavage plane parallel to the midline. The number of division events in netrin-1 mutant spinal cord slices (denoted by white dots) is dramatically reduced with aberrant location in the gray matter that has not been observed in wild type slices. (C, D) At E17 and P0, division events in the wild type spinal cord slices are located in the white matter while the number of division events in the mutant spinal cord slices is fewer. The remaining division events in the mutant spinal cord, show aberrant locations in the gray matter. The drawings for B–D are compiled from at least 2 wild type slices at each age and 2 to 3 netrin-1 mutant slices at each age.