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Origins of Spinal Cord Oligodendrocytes: Possible Developmental and Evolutionary Relationships with Motor Neurons

Key Words
Neuroepithelial cells
Oligodendrocytes
Motor neurons
Spinal cord
Floor plate
Notochord
Sonic hedgehog
Neural patterning
PDGF receptor
Myelin evolution

Abstract
Spinal cord oligodendrocytes develop from migratory glial progenitor cells that are generated by a small subset of neuroepithelial cells in the ventral part of the neural tube. Specification of these neuroepithelial oligodendrocyte precursors, in common with other ventral cells such as motor neurons, depends on morphogenetic signals from the notochord and/or floor plate. The ventrally derived signals can be mimicked in vitro by purified Sonic hedgehog (Shh) protein. Oligodendrocytes and motor neurons are induced over the same range of concentrations of Shh, consistent with the idea that Shh might specify a common precursor of motor neurons and oligodendrocytes. A lineage relationship between motor neurons and oligodendrocytes has previously been suggested by clonal analysis in the embryonic chick spinal cord. We propose a lineage diagram that connects oligodendrocytes and motor neurons and that takes into account the fact that motor neurons and oligodendrocyte precursors are generated at different times during development. Oligodendrocytes might originally have evolved to ensheath motor axons and facilitate a rapid escape response. If so, oligodendrocyte ontogeny and phylogeny might share a common basis.

Introduction to the Oligodendrocyte Lineage

Oligodendrocytes Develop from Migratory Glial Progenitor Cells (O-2A Progenitors)

Oligodendrocytes, the myelinating cells of the central nervous system, are generated during late neurogenesis from proliferative, migratory progenitor cells known as O-2A progenitors. These glial progenitors have been so-named because they can differentiate into either oligodendrocytes or type-2 astrocytes in vitro, depending on the concentration of fetal calf serum (FCS) in the culture medium; type-2 astrocytes develop in the presence of 10% FCS or other inducing factors such as ciliary neurotrophic factor, whereas oligodendrocytes develop in $\leq 0.5\%$ FCS [see 1, 2, for reviews]. It has proved difficult to identify type-2 astrocytes in vivo, so this differentiation pathway might be suppressed during normal development [3]. It is possible that type-2 astrocytes might be generated only in restricted areas of the developing CNS, or in response to injury or disease in the mature CNS.

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O-2A progenitors are highly mobile cells in vitro, and migrate widely through the CNS during embryonic and early postnatal development before differentiating into oligodendrocytes. For example, they migrate into the developing rat optic nerve from the optic chiasm starting just before birth, reaching the eye end of the nerve around postnatal day 5 (P5) [4]. A newborn rat optic nerve is around 3 mm long, implying that O-2A progenitors can migrate at least this distance in a week. There is also evidence for long-range migration of O-2A progenitors in the developing spinal cord and brain. Leber and Sanes [5, 6] marked individual neuroepithelial cells in the embryonic chick spinal cord by infection with a retroviral vector expressing β-galactosidase, and subsequently found clonally related oligodendrocytes or their progenitors dispersed more than 300 μm along the rostro-caudal axis. This is probably an underestimate of their migratory capacity because of the difficulty of assigning more widely dispersed cells to a particular clone of origin. In quail-chick grafting experiments carried out in our own laboratory [Pringle et al., in preparation], graft derived (quail) oligodendrocytes could be found up to 2 mm away from a transplanted spinal cord segment in both the rostral and caudal directions. Levison and Goldman [7, 8] stereotactically injected retrovirus vectors into the subventricular germinal zones at the lateral tips of the lateral ventricles of newborn rats and visualized the progeny of infected cells 3 weeks later. They found oligodendrocytes in the neocortex, subcortical white matter and striatum 1–2 mm away from the presumptive site of virus infection. Thus, while some of the cell dispersion in the experiments quoted above probably results from passive displacement due to growth in size of the CNS, there is clearly also active, long-range migration of oligodendrocytes or (more likely) their progenitors. The nature of the cues that direct migration of these cells is not known; however, it seems likely that radial glial processes and axons might act as substrates for migration at different stages of their journey through the developing CNS.

Where Do Oligodendrocyte Progenitors Come from?

What, and where is the source of the migratory O-2A progenitor cells described above? This has been and still is the subject of debate. We and others have presented evidence that, in the spinal cord at least, most or all oligodendrocyte precursors stem from a specialized subset of neuroepithelial cells in the ventral half of the embryonic neural tube [9–13]. Specification of these neuroepithelial oligodendrocyte precursors, like other ventral cell types in the spinal cord, depends on inducing signals from the notochord/floor plate complex at the ventral midline [14–16]. Recently, Cameron-Curry and Le Douarin [17] presented an alternative view, that oligodendrocyte precursors develop from all parts of the ventricular epithelium, both dorsal and ventral. The arguments for and against these different models are presented below.

Evidence for a Localized Ventral Source of Oligodendrocyte Precursors

Warf et al. [11] bisected embryonic rat spinal cords into dorsal and ventral halves, dissociated the cells and cultured them separately as monolayers. Oligodendrocytes developed in cultures established from both ventral and dorsal cells from embryonic day 16 (E16) rats (counting the date of the vaginal plug as day zero, E0). However, only the ventral, not the dorsal cells from E14 spinal cords gave rise to significant numbers of oligodendrocytes in vitro. Warf et al. [11] interpreted this to mean that oligodendrocyte precursors are generated locally in the ventral half of the spinal cord around E14 and migrate into the dorsal half by E16. An alternative explanation might have been that oligodendrocyte precursors are generated in a ventral-to-dorsal wave under the influence of an inducing factor that diffuses dorsally from the ventral half of the cord. This was considered unlikely because dorsal E14 cells did not give rise to oligodendrocytes even when they were cultured in the same microwell (on a separate coverslip) as ventrally derived cells. Similar experiments have been conducted with avian spinal cord tissue, with analogous results [16].

Further evidence for the ventral origin of oligodendrocyte precursors came from a quite different type of experiment carried out in our laboratory. We were attempting to trace the development of the oligodendrocyte lineage by in situ hybridization with probes that are expressed preferentially in the CNS by oligodendrocyte progenitors. Two probes – against the platelet-derived growth factor alpha-receptor (PDGFRα) and the 'myelin' protein 2′,3′-cyclic nucleotide 3′-phosphodiesterase (CNP) – gave strikingly concurrent results. These probes both revealed a tiny focus of gene expression in transverse sections of rat spinal cord, at the luminal surface in the ventral half of the E14 cord [9, 10]. These foci represent end-on views of narrow, bilateral columns of cells that extend all along the E14 rat spinal cord into the midbrain. After E14, the ($PDGFR\alpha^+$, CNP^+) cells appeared to proliferate and migrate throughout the developing gray and white matter of

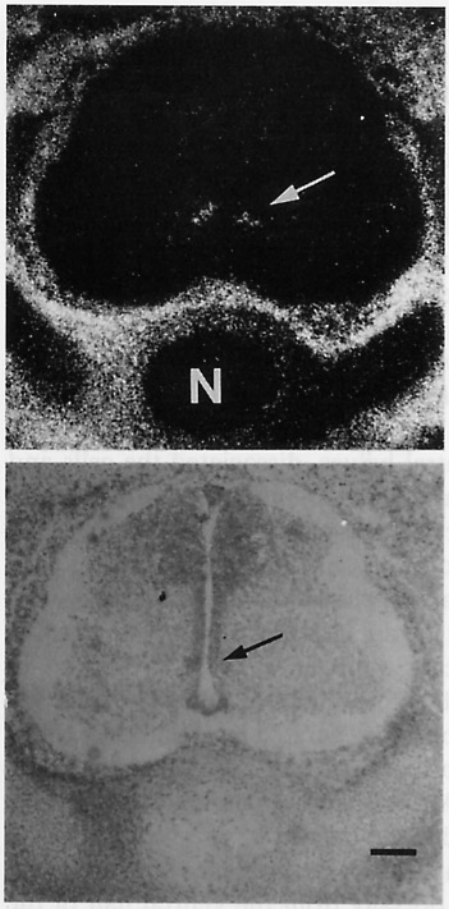

Fig. 1. The origin of oligodendrocyte progenitors in the ventral ventricular epithelium of the embryonic neural tube. Sections of E6.5 chicken spinal cord (wing bud level) were hybridized to a ^{35}S-RNA probe against the 3′ untranslated portion of chicken PDGFRα mRNA, followed by autoradiography and dark field (above) and bright field micrography. PDGFRα is expressed widely outside of the CNS, and in small bilateral foci of oligodendrocyte precursors in the ventral neural tube, approximately 20% of the way from the floor plate towards the roof plate (15% in rodents) (arrows). N = notochord. Scale bar: 200 μm.

the cord, reaching the dorsal half of the cord by E16 and becoming fairly evenly distributed by E18 [9] (fig. 2). Therefore, the spatio-temporal behaviour of these cells closely matched the behaviour of oligodendrocyte progenitors inferred from the culture experiments of Warf et al. [11]. We now have direct evidence that PDGFRα$^+$ cells in the embryonic rat spinal cord are oligodendrocyte progenitors. We immunoselected PDGFRα$^+$ cells from E17 spinal cords with an antibody against the extracellular domain of PDGFRα and studied them in vitro. When maintained in medium containing PDGF-AA and bFGF (to inhibit differentiation), more than 97% of the cells had the morphology and antigenic phenotype of O-2A progenitor cells. As expected for O-2A progenitors, they all differentiated into either oligodendrocytes (in the absence of growth factors and in low concentrations of FCS) or type-2 astrocytes (in the presence of 10% FCS) [44].

Cytological studies with five different oligodendrocyte lineage markers support the view that oligodendrocytes descend from a small group of neuroepithelial cells at the luminal surface in the ventral half of the embryonic spinal cord [9, 10, 12, 13, 18] (fig. 1). Is there anything intrinsically unusual about this microdomain of the neuroepithelium, or is it just one of several (or many) analogous microdomains that express distinct sets of genes and give rise to distinct subsets of differentiated neurons and glia? Very little is known about the origins of most neural cell lineages in the spinal cord, except that ventral cells (such as motor neurons) seem to be derived from ventral neuroepithelium and dorsal cells from dorsal neuroepithelium [19]. It seems possible that the entire ventricular surface of the spinal cord might turn out to be a mosaic of neural precursor cells with diverse specificities [10, 19].

Specification of Oligodendrocyte Precursors by Signals from the Ventral Midline

The ventral location of the neuroepithelial precursors of oligodendrocytes described above raised the question: does formation of these cells, like other ventral cells such as floor plate cells or motor neurons, depend on signals from the notochord/floor plate complex at the ventral midline? Data from several laboratories show that it does.

The Danforth's short tail *(Sd)* mouse carries a semidominant mutation that, in the heterozygous state, causes discontinuous loss of the notochord from caudal regions of the spinal cord with concomitant loss of the floor plate and motor neuron pools [20]. The heterozygous animals are viable and reproduce normally whereas homozygotes die perinatally. We examined spinal cords of heterozygous *Sd* embryos (E12.5–13) and found that foci of PDGFRα$^+$ oligodendrocyte precursors did not appear at the ventricular surface wherever the notochord/floor plate was lacking [14]. In the same animals, in regions of spinal cord that had an associated floor plate, foci of PDGFRα$^+$ cells appeared at the luminal surface just as in wild-type mice.

Transplanting a second notochord next to the neural tube of a developing chicken embryo is sufficient to induce the differentiation of a second floor plate and extra pools of motor neurons [21, 22]. We and others have

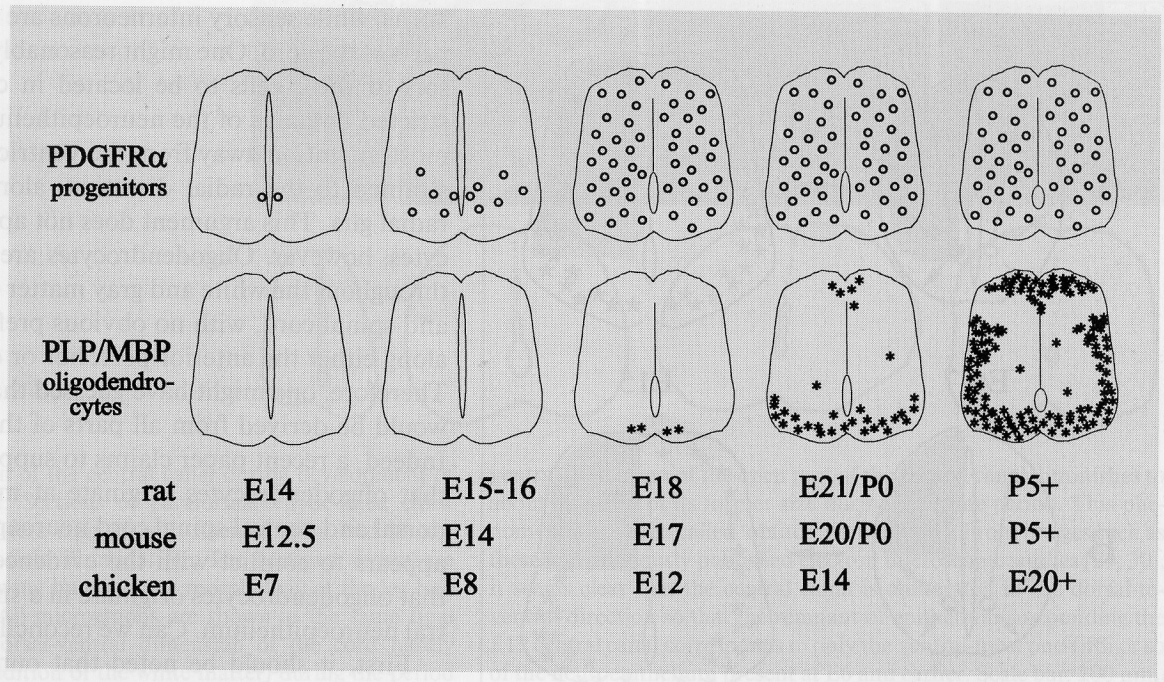

Fig. 2. Distribution of oligodendrocytes and their progenitors at various times during development of the spinal cord. The diagram represents the distributions of PDGFRα+ precursors (top row, open circles) and PLP/MBP+ oligodendrocytes (bottom row, stars) at the indicated developmental stages in mouse, rat and chicken. Referring to the rat: precursors appear at the luminal surface on E14 and proliferate and migrate first through the ventral spinal cord and then into the dorsal cord, reaching a steady state number and distribution around E18, which persists for a few weeks postnatally before numbers decline in the adult (not shown). Oligodendrocytes first appear in the ventral white matter flanking the floor plate around E18, then in the dorsal white matter. Oligodendrocytes increase in number and spread through the lateral white matter during the first postnatal days and weeks. The spread of oligodendrocytes probably represents a wave of differentiation and survival, not proliferation and migration. Note that in mouse, but not rat or chicken, a small number of postmitotic PLP/MBP+ oligodendrocytes differentiate very early (around E14.5) near the ventricular surface very close to the site of first appearance of their PDGFRα+ precursors [18, and unpublished data]. For simplicity, these early oligodendrocytes are not indicated in the figure.

found that such notochord grafts also induce the formation of ectopic groups of oligodendrocyte precursors in vivo [14, 15 and P. Cochard, pers. commun.]. The ability of notochord to induce oligodendrocyte lineage cells can also be demonstrated in vitro. If pieces of dorsal, ventral or intermediate chicken or quail neural plate are cultured on their own in collagen gels, only the ventral explants give rise to floor plate cells, motor neurons oligodendrocytes. However, intermediate or dorsal neural plate explants can be induced to generate all of these cell types if they are co-cultured in contact with fragments of notochord [14–16].

What are the molecules that mediate this activity of the notochord/floor plate? Sonic hedgehog (Shh), a vertebrate homologue of the product of the *Drosophila* patterning gene hedgehog *(hh)*, is expressed in the notochord and floor plate during early neurogenesis [reviewed in 23, 24] and can act in a concentration-dependent manner to induce floor plate cells (higher concentrations of Shh) or motor neurons (lower concentrations of Shh) to form in explant cultures of intermediate avian neural plate [25–27]. We found that Shh could also induce the development of oligodendrocytes in this assay. Oligodendrocytes were induced over the same range of Shh concentrations as motor neurons, suggesting that motor neurons and oligodendrocytes (and perhaps other types of neurons or glia) might descend from the same set of neural precursors, whose specification or survival is influenced by Shh [14]. I return to this idea later.

Is There a Dorsal Source of Oligodendrocytes?

Particular classes of neurons are situated at defined positions along the dorsal-ventral axis of the spinal cord. For example, motor neurons are clustered in the ventral

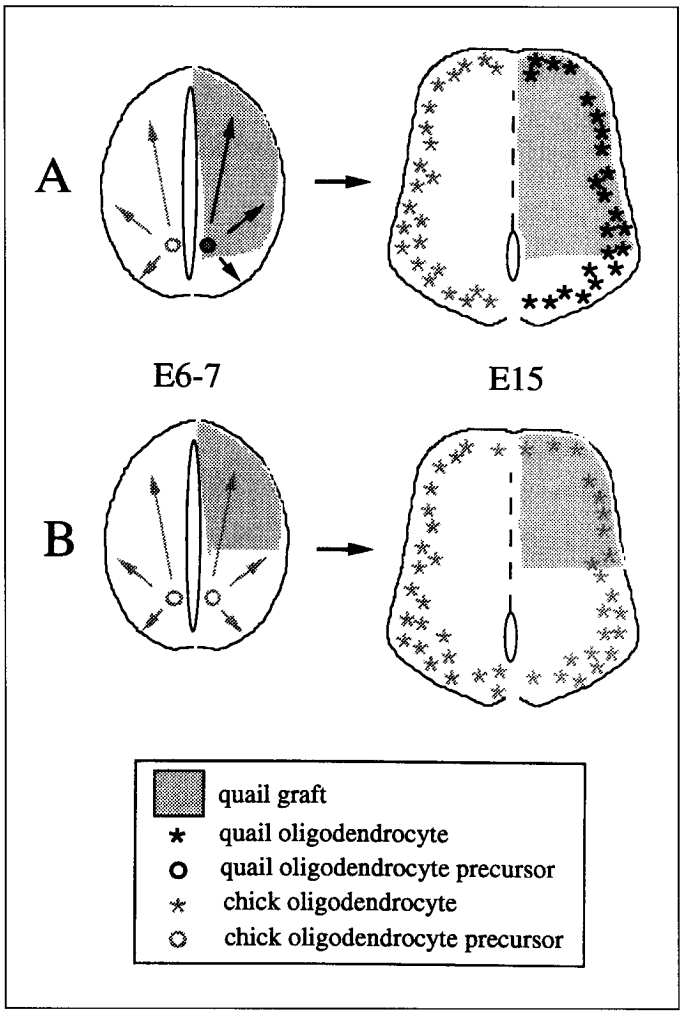

Fig. 3. The developmental fates of neural tube grafts of different dimensions. **A** A quail graft that occupies most of one side of the E6–7 neural tube, including the ventral site of origin of PDGFRα+ oligodendrocyte precursors, goes on to generate oligodendrocytes all through the white matter on that side of the E12 cord. This graft also contributes to the epithelium lining of part of the central canal at E12. This type of graft was classified as 'dorsal' by Cameron-Curry and Le Douarin [17]. **B** A less extensive graft occupying only the dorsal half of the E6–7 neural tube generates neurons and white matter glia in the dorsal regions of the E12 spinal cord, but does not generate oligodendrocytes and does not contribute to the epithelial lining of the E12 central canal [Pringle et al., in preparation]. In our view, these latter grafts demonstrate that oligodendrocytes are not generated uniformly from all parts of the neuroepithelium but arise specifically from ventral neuroepithelium, in agreement with other types of data.

horns, while sensory interneurons are found in the dorsal parts of the cord. One might reasonably expect the precursors of these cells to be located in correspondingly restricted domains of the neuroepithelium, if only because most migration away from the ventricular surface is constrained to the radial direction, along the processes of radial glia. This argument does not apply to oligodendrocytes, however. Oligodendrocytes are distributed widely throughout the white and gray matter of the mature brain and spinal cord, with no obvious preference for position along either the anterior-posterior or dorsal-ventral axes. Therefore, one might have guessed that oligodendrocytes would be derived from all parts of the neuroepithelium. Indeed, a recent paper claims to support this latter view, that oligodendrocytes originate at multiple sites in the dorsal and ventral spinal cord neuroepithelium [17]. This appears to conflict with the evidence, presented above, that oligodendrocytes originate in a small part of the ventral neuroepithelium. Can we reconcile these data?

First, it should be noted that our histochemical evidence for a restricted ventral source of oligodendrocyte lineage cells does not preclude a priori the existence of other oligodendrocyte lineages that might originate elsewhere along the dorsoventral axis. Additional sources of oligodendrocytes simply might not be recognized by our current battery of probes and antibodies, or we might not have noticed them if they did not start to express the known set of markers until after they had moved away from the luminal surface and intermingled with ventrally derived progenitors. There is a suggestion, for example, that cerebellar oligodendrocytes might develop from progenitor cells that do not express the usual set of molecular markers [28]. One would still have to explain why oligodendrocyte lineage cells can develop in cultures of ventral but not dorsal E14 rat spinal cord [11], or E4 chicken spinal cord [16]. However, these data also could be misleading if, for example, the putative dorsally derived oligodendrocyte progenitors had different or more stringent requirements for survival in culture than their ventrally derived counterparts. It is therefore important to examine carefully the evidence for dorsally derived oligodendrocytes.

Cameron-Curry and Le Douarin [17] investigated the origin of oligodendrocytes in the avian spinal cord by grafting dorsal quail neural tube into the equivalent position of an embryonic chicken host, creating a chimeric animal in which the dorsal half of the neural tube was composed entirely of quail cells and the ventral half was composed of chicken cells. The rationale of the experiment is simple: if oligodendrocytes originate from dorsal neuroepithelium, then some or all of the oligodendrocytes

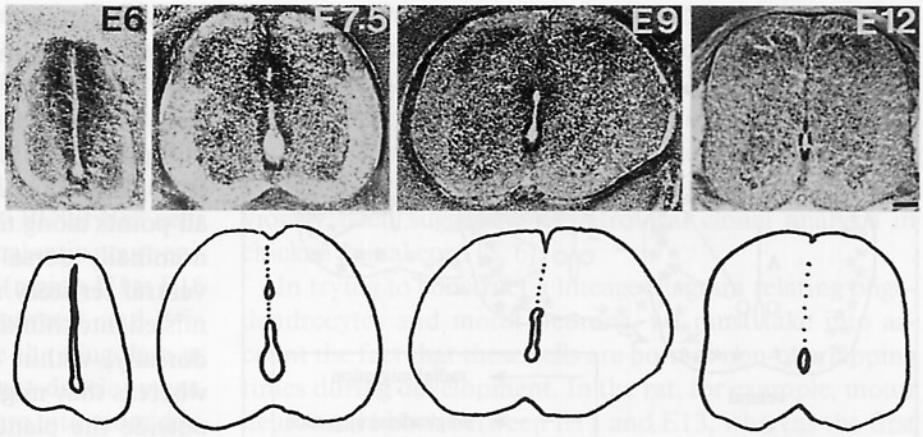

Fig. 4. Obliteration of the central canal during development of the chick spinal cord. Sections of paraformaldehyde-fixed chick embryo spinal cords were stained with haematoxylin and photographed under bright-field illumination. The sections shown are from the level of the wing bud, but the morphology at the leg bud level is very similar. All micrographs are shown at the same final magnification. The dorsal-ventral dimension of the cord hardly changes (except for addition of the white matter) during the period shown, from E6 (stage 29) to E12 (stage 38). However, the dorsal-ventral dimension of the open part of the central canal diminishes to about one-fifth of its former size during the same period. This phenomenon has been called 'obliteration' [29–31]. From images such as these and previously published electron microscopic studies [29, 30], it seems clear that the central canal is obliterated in the dorsal-to-ventral direction so that the remnant of epithelium surrounding the E12 central canal corresponds to only the ventral-most one-fifth or so of the neuroepithelium present at E6 and earlier. Scale bar: 200 μm.

that develop in the chimeric cord should be quail oligodendrocytes, which can be easily distinguished from chicken oligodendrocytes by histochemistry. Cameron-Curry and Le Douarin [17] also performed the reciprocal experiment, in which dorsal chicken neural tube was transplanted into a quail host, thereby allowing oligodendrocytes that originated in the ventral neural tube to be identified unambiguously as quail oligodendrocytes. In both classes of chimera, they found that quail oligodendrocytes developed throughout the spinal cord white matter, suggesting that both dorsal and ventral parts of the neuroepithelium can give rise to migratory oligodendrocyte progenitors. However, interpretation of the experiments is not as straightforward as one might think and, as I argue below, there is reason to be cautious about this conclusion.

The first thing to note is that the neuroepithelial precursor cells are still naive at the time the neural tube grafts are performed (approximately stage 13, E2). If at any point the grafted tissue approaches close enough to the floor plate, it will be induced to adopt a ventral fate. The important thing is not where the grafted tissue originated in the donor embryo but the position it occupies in the chimeric embryo at the time that cell fates are specified, some time between E2 and E6. Moreover, it is not possible to continuously observe the grafted tissue from the time of transplant to the time of analysis which, in Cameron-Curry and Le Douarin's [17] experiments, was nearly 2 weeks later at E12–15. Thus, one is forced to try to infer retrospectively how much of the neural tube were occupied by grafted tissue at early ages when cell fates were being determined, from the position and extent of the graft-derived cells much later at E12–15. Cameron-Curry and Le Douarin [17] made this judgement based on how much of the ventricular neuroepithelium remaining at E12–15 was comprised of graft-derived cells. Grafts that gave rise to ependymal cells lining only the dorsal part of the E12–15 central canal were regarded as 'dorsal' grafts (fig. 3A), while grafts that contributed to the epithelial cells lining only the ventral half of the canal were regarded as 'ventral'. This approach rests on the implicit assumption that the dorsal and ventral limits of the E12–15 central canal correspond to the dorsal and ventral limits of the lumen of the neural tube at E6 and earlier. Unfortunately, this is not true.

During development of the spinal cord the lumen first increases in size as the neural tube grows, then remains constant for a while before decreasing in size once again as the opposing faces of the neuroepithelium on either side of the canal approach one another and 'zip together', dorsal-to-ventral, in a process known as 'obliteration' [29–31]. As illustrated in figure 4, obliteration of the central

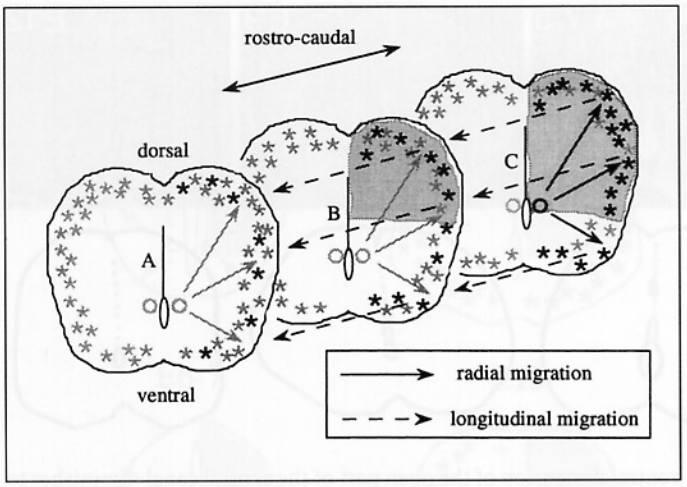

Fig. 5. Oligodendrocyte progenitors from one part of the spinal cord can generate oligodendrocytes in distant parts of the cord by long-range longitudinal migration followed by differentiation. Therefore, if a 'dorsal' graft descends at any point along its length into ventral territory (**C**), graft-derived oligodendrocytes will appear in other parts of the chimeric cord including regions where the graft remains strictly dorsal (**B**). This can create the erroneous impression that oligodendrocytes are generated locally from dorsal neuroepithelium. The same mechanism leads to graft-derived oligodendrocytes even outside the region of the graft (**A**). Symbols are as defined in figure 3.

canal occurs between E8 and E12 in the chicken. As a result, the dorsal-to-ventral dimension of the open central canal at E12 is approximately one-fifth of what it was before E8. What happens to the 'lost' neuroepithelium? The mechanism by which the dorsal neuroepithelium regresses is in general unknown, although there is a report that in the house shrew neuroepithelial cell death plays a role in this process [32]. Whatever the mechanism, the fact remains that four-fifths of the dorsal neuroepithelium disappear between the time that Cameron-Curry and Le Douarin performed their grafts and the time that they analyzed the chimeras. The small remnant of neuroepithelium remaining at E12–15 corresponds to only the ventral-most one-fifth of the neuroepithelium that existed previously. Consequently, the so-called 'dorsal' grafts that generated oligodendrocytes [fig. 3 and 4 in 17] in all probability occupied most of one side of the neural tube, save for the ventral-most one-tenth or so, at earlier times when cell fate specification was taking place. Those grafts would almost certainly have included the ventral foci of ($PDGFR\alpha^+$, $O4^+$) oligodendrocyte precursors described by ourselves and others. This point is illustrated schematically in our figure 3A.

Interpretation of the chick-quail chimeras is further complicated by the propensity of oligodendrocyte progenitors to migrate, not only in the transverse plane but also large distances along the longitudinal axis of the developing spinal cord. Because of this, one must ensure that the transplanted tissue remains entirely dorsal (or ventral) at all points along the length of the graft. For example, if a nominally 'dorsal' graft should at some point descend into ventral territory as illustrated in figure 5, one could be misled into thinking that oligodendrocytes were generated dorsally, within the plane of section being examined, whereas they might in fact have been generated ventrally outside the plane of section. In our experience, dorsal quail grafts frequently end up with an irregular or undulating ventral border, thus contributing to a variable fraction of the spinal cord neuroepithelium at different points along the length of the same graft. Cameron-Curry and Le Douarin [17] also acknowledge this sort of variability within their dorsal grafts; their different 'types' of graft (A1, A2, A3 for example) were the accidental products of such variation, not the deliberate outcome of microsurgery.

In collaboration with Sarah Guthrie (UMDS, Guy's Hospital Medical School, London) we have repeated some of the chick/quail chimera experiments described by Cameron-Curry and Le Douarin [17], taking care to analyze only genuinely dorsal quail grafts that at no point along their length encroach into the ventral half of the chimeric spinal cord. We find that these grafts, illustrated schematically in figure 3B, do not contribute to the ependymal lining of the E15 central canal, as predicted from the discussion above. They give rise to many neurons and white matter glia (presumably astrocytes) in the dorsal parts of the cord, but not to ventral neurons such as motor neurons nor to any type of ventral white matter glia, and not to oligodendrocytes in any part of the spinal cord [Pringle et al., in preparation]. These data provide strong evidence that oligodendrocytes are normally derived from only the ventral half of the spinal cord, in contrast to the conclusions of Cameron-Curry and Le Douarin [17] and in keeping with the bulk of other evidence discussed above.

The Origins of Brain Oligodendrocytes

The brain is fundamentally an extension of the same neural tube that gives rise to the spinal cord; is it possible that similar rules apply to the generation of oligodendrocytes in the brain as in the spinal cord? The columns of $PDGFR\alpha^+$ neuroepithelial oligodendrocyte precursors extend along the E14 rat spinal cord into the hindbrain and

midbrain, ceasing abruptly at the mesencephalic/diencephalic boundary [9]. By analogy with the spinal cord, it seems likely that these precursors provide at least some, if not all of the oligodendrocytes in these brain regions, although this has not been investigated. In the rat forebrain, there is a region in the E15 ventral diencephalon that appears to be a source of migratory PDGFRα$^+$ cells [9]. This is located in the anterior hypothalamic neuroepithelium beneath the foramen of Monro (region H1a; E16 coronal figures 7 and 8 and sagittal figures 1 and 2 in [33]). Note that these authors designate the plug date as P1, not P0 as we do.). During subsequent development, these cells appear to radiate out from there into more dorsal regions of the forebrain, including the medial cerebral cortex, between E16 and birth [9]. It is possible, therefore, that some (or many) cortical oligodendrocytes might develop from progenitor cells that migrate all the way from the hypothalamus, round the lateral tips of the lateral ventricles into the cortex. However, this has not been established.

On the other hand, there is good evidence that several types of glial cells including oligodendrocytes are generated in the subventricular zones surrounding the lateral ventricles of the forebrain during late embryonic and postnatal development. The best evidence for this comes from stereotactic injection of retroviral lineage markers into the subventricular zones beneath the lateral ventricles of newborn rats, following which clones of oligodendrocytes and other glia were subsequently found in the cortex and striatum [7, 8]. It is not known whether oligodendrocyte precursors in the subventricular zones of the forebrain are specified by signals from an organizing centre in the forebrain that is analogous to the notochord/floor plate in the more posterior brain and spinal cord; organizing centres are presumed to be present and to be responsible for establishing restricted domains of gene expression in the forebrain, but the location and nature of the putative organizers is presently unknown.

Relationships between Oligodendrocytes and Motor Neurons

Developmental Relationships

There are some reasons to think that oligodendrocyte precursors and motor neurons might share a particularly close, or obligatory developmental relationship. They are both generated from the neuroepithelium near the floor plate of the spinal cord. They both depend on signals from the notochord/floor plate complex [14–16]. They can both be induced to develop in naive neural tube explant cultures by the same range of concentrations of Shh [14], suggesting that the primary effect of Shh might be to induce the formation of a common precursor of oligodendrocytes and motor neurons. Moreover, a lineage relationship between oligodendrocytes and motor neurons has previously been suggested by retroviral clonal analysis in chicken spinal cord [5, 6].

In trying to construct a lineage diagram relating oligodendrocytes and motor neurons, we must take into account the fact that these cells are born at non-overlapping times during development. In the rat, for example, motor neurons are born between E11 and E13, whereas the first detectable PDGFRα$^+$ oligodendrocyte precursors do not appear until around E14. This suggests that the fates of neuroepithelial precursors might change with time; one particular group of precursors in the spinal cord might first generate only motor neuron progenitors, and subsequently switch to producing only oligodendrocyte progenitors. Other groups of neuroepithelial cells positioned at different points along the dorsal-ventral axis might behave in an analogous way, generating distinct subsets of spinal cord neurons and glia in a sequential manner. Such a scheme was suggested by us previously [34], and is reproduced in figure 6.

What drives fate switching of neuroepithelial cells? It could be either cell-autonomous or determined by the changing environment in the CNS, or a combination of both. For example, once sufficient motor neurons have accumulated in the ventral horns of the spinal cord, factors secreted from those cells might feed back to the luminal surface to stop further motor neuron production and start the production of oligodendrocyte progenitors. It would make teleological sense for neurons to control the production of the glial cells that will eventually come to ensheath them. Factors from motor neurons have already been implicated in the generation of other classes of neurons in the spinal cord [35].

Possible Functional and Evolutionary Relationships between Oligodendrocytes and Motor Neurons: Oligodendrocytes as 'Motor Glia'

Discussion of the developmental origins of oligodendrocytes naturally leads to questions about their evolutionary beginnings. What was the selective advantage offered by the arrival of oligodendrocytes? The obvious answer to this question is that a myelinated axon transmits action potentials at a greatly increased velocity compared to an unmyelinated axon of similar bore. To achieve a given rate of propagation, unmyelinated axons

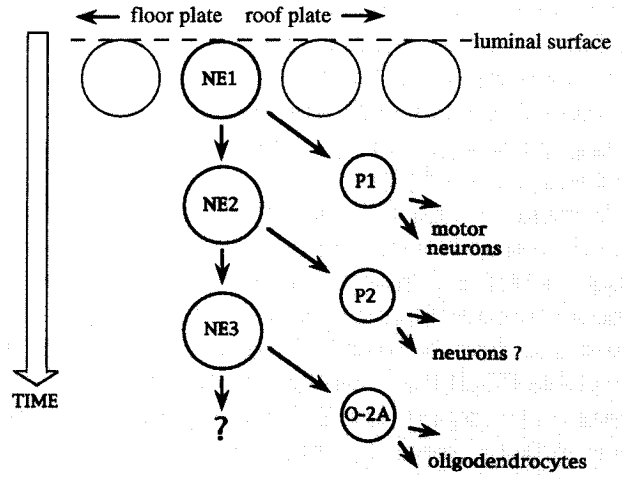

Fig. 6. Hypothetical lineage diagram relating motor neurons (and possibly other types of neurons) and oligodendrocytes. A neuroepithelial precursor cell (NE1) at a defined location at the luminal surface divides asymmetrically to generate a replacement neuroepithelial cell (NE2) and a dedicated motor neuron precursor (P1), which might itself be postmitotic or able to divide a limited number of times before migrating radially and giving rise to motor neurons. The remaining neuroepithelial cell undergoes one or more further rounds of asymmetric division, finally producing one or a pair of dedicated oligodendrocyte progenitors (O-2A). The O-2A progenitor then proliferates and its progeny migrate away in all directions to populate the spinal cord with oligodendrocytes (refer to fig. 2). Thus, motor neurons and oligodendrocytes could share a common ancestry but be generated at different times during development. Other neuroepithelial cells at different positions along the dorsal-ventral axis might similarly generate distinct subsets of spinal cord neurons and/or glia in a sequential manner.

(like the squid giant axon) need to be huge compared to myelinated axons [discussed in 36]. Myelin therefore provides for great space savings and an increase in the speed at which information can be transmitted and processed. This must favour the development of complex nervous systems, and it is certainly true that the mammalian brain would have to be many times larger in volume if it were not for myelin. Indeed, oligodendrocytes offer an even greater space saving over Schwann cells because each Schwann cell myelinates only one internode while a single oligodendrocyte can myelinate many. Thus, it seems obvious that a major advantage of myelin in general, and oligodendrocytes in particular, has been the saving of space (and energy) needed to evolve a complex, portable brain. However, as argued below, myelinating cells might have evolved in the first instance to serve a more primitive function, and might only later have been recruited to help build a better brain.

Myelin-like wrappings of axons by glial cells are present in at least three separate animal groups – annelids, crustacea and vertebrates [36, 37]. In the first two groups, only certain classes of axons are 'myelinated'. In the earthworm, for example, the giant longitudinal fibres that trigger reflex muscular contraction of the animal are myelinated [38, 39]. In prawns, axons involved in the darting escape reaction are myelinated [40] and in crabs, axons involved in triggering rapid retraction of the eye stalks [41]. These creatures cannot be said to possess complex brains but nevertheless do have myelinating cells, which in each case serve to facilitate reflex startle responses. Therefore, even among the vertebrates, myelinating cells might have evolved initially as specialized 'motor glia' whose function was to facilitate a rapid escape response. If so, it might make sense of the notion that oligodendrocytes develop as a late branch of the motor neuron lineage.

Not all vertebrates have myelin. The hagfish and lampreys lack multiple glial wraps in either the CNS or PNS [42]. These animals belong to the group of Agnatha, or jawless fishes, so it has been suggested that myelinating cells first evolved in the PNS in parallel with the jaw (itself a neural crest derivative), and were in some way useful for the evolution of predatory behaviour [43]. It seems equally plausible that the jaw evolved before myelin, and that myelin arose in the CNS to help prey to escape from predators. Once the coordinated program of gene expression required to elaborate myelin had evolved in one cell type, any cell in the body could in theory activate this program given the appropriate cues. Therefore, once myelin-forming cells had evolved in the PNS, they might have followed quickly in the CNS, or vice versa. In any case, it seems likely that the arrival of jawed predators, with or without myelin, would have provided a strong incentive for their preferred vertebrate prey to evolve an effective escape mechanism. Do the Agnatha, lacking myelin, also lack conventional (i.e. motor-driven) escape responses? Adult lampreys seem to be able to swim strongly and rapidly, but they possess large-diameter longitudinal axons that coordinate swimming movements and might help to compensate for their lack of myelin. In contrast, lamprey larvae display a slow 'writhing' form of motion when handled, unlike the rapid 'wriggling' movement characteristic of other fish and eels. This could conceivably relate directly to their lack of myelin. Hagfish are regarded as even

more primitive vertebrates than lampreys. Adult hagfish move sluggishly like lamprey larvae. Their startle response is not to attempt to flee but to secrete prodigious quantities of slime from their body surface, making them extremely difficult to grasp. Both lampreys and hagfish are parasitic on other fish and spend part of their lives attached to or inside their hosts. For this reason, perhaps, they might have experienced less evolutionary pressure than other vertebrates to 'get moving'. Given their lack of oligodendrocytes, it will be interesting to discover whether lampreys or hagfish have PDGFRα$^+$ glial cells in their ventral neural tubes.

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