NEURON–GLIA INTERACTIONS — OPINION

Myelination and the trophic support of long axons

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Abstract | In addition to their role in providing myelin for rapid impulse propagation, the glia that ensheath long axons are required for the maintenance of normal axon transport and long-term survival. This presumably ancestral function seems to be independent of myelin membrane wrapping. Here, I propose that ensheathing glia provide trophic support to axons that are metabolically isolated, and that myelin itself might cause such isolation. This glial support of axonal integrity may be relevant for a number of neurological and psychiatric diseases.

The interaction of neurons and glia is a feature of virtually all nervous systems. As brains have become larger during vertebrate evolution, the proportion of brain cells that are glia has increased[1]. The oligodendrocytes of the CNS and the Schwann cells of the PNS are best known for making the myelin that ensheaths neuronal axons. The neurodegenerative phenotypes of mouse strains that carry mutations affecting these specialized glia have revealed that the ensheathing cells are essential not only for myelin assembly but also for the functional integrity and long-term survival of axons[2–5]. This requirement is also reflected in the axonal degeneration observed in human neurological diseases in which glial support fails.

The evolution of myelin (BOX 1) reduced the energy required for neuronal communication and boosted the speed of impulse propagation, allowing complex nervous systems to operate quickly and efficiently[6]. However, the myelin sheath itself could be a 'double-edged sword'. The nearly complete insulation of axons by myelin restricts their access to extracellular metabolic substrates. In this Perspective, I combine observations that have emerged from the analysis of mouse mutants carrying glia-specific defects with those of the pathology of human myelin diseases into a larger picture of unrecognized glial functions. These data suggest that oligodendrocytes (which are coupled to astrocytes) and Schwann cells preserve fast axonal transport and long-term axonal integrity in the nervous system. More speculatively, I propose that long axonal tracts depend on these glia to meet the metabolic demands and energy requirements of rapid impulse propagation and axonal transport.

Myelin in health and disease

Myelin is a striking example of cellular specialization. Oligodendrocytes in the CNS and Schwann cells in the PNS generate large amounts of myelin as an extension of their cell membrane that is wrapped around an axonal segment many times. This leaves a tightly compacted and insulating sheath that separates the highly specialized nodes of Ranvier[7,8]. At the nodes of Ranvier, voltage-dependent Na^+ channels are clustered in the axonal membrane, flanked by a paranodal axo-glial junction that provides a strong (although not complete) diffusion barrier[9,10]. In the adjacent juxtaparanodal region, fast K^+ channels are concentrated. The entire nodal region is organized and maintained by a set of adhesion and scaffolding proteins (for a recent review, see REF. 11). During development, axonal activity influences myelination, at least for oligodendrocytes[12]. The physiological function of myelin in promoting saltatory impulse conduction has been known for a long time. However, the subcellular mechanisms of myelin membrane growth[13] are still not understood.

Loss of intact myelin is the cause of several neurological disorders, including multiple sclerosis[14], inherited leukodystrophies of the CNS[15] and various peripheral neuropathies[16]. However, in addition to the primary axonal degeneration that occurs in some forms of multiple sclerosis and neuropathies, secondary axonal degeneration — rather than a slowing of conduction velocity — seems to be the major cause of persistent clinical impairment. Subtle myelin abnormalities may also contribute to more complex disorders, as suggested by the increasing recognition of myelin and white-matter differences in patients with psychiatric diseases, such as schizophrenia[17].

Axon degeneration in myelin disease

In many myelin diseases (TABLE 1), axons themselves are at risk of degeneration. Multiple sclerosis was long considered to be a myelin-specific disease that spares CNS axons; however, the analysis of postmortem brains revealed frequent axonal transections and progressive axon loss in multiple sclerosis lesions[18,19]. In combination with brain atrophy and ventricular enlargement, this secondary axonal dysfunction almost certainly accounts for the transition from the relapsing–remitting form of multiple sclerosis to the chronic progressive course of the disease[14].

Progressive axon loss also contributes to the clinical phenotype of inherited myelin disorders. Leukodystrophies, such as Pelizaeus–Merzbacher disease (PMD), are characterized by dysmyelination of the CNS and poor motor development, beginning in early childhood. In older patients with PMD, the gradual decline of previously acquired motor skills is likely to involve axonal neurodegeneration, as observed in mouse models of the disease[2,20]. A similar decline in motor skills over time is seen in patients with other leukodystrophies, including Pelizaeus–Merzbacher-like disease (PMLD), adrenoleukodystrophy and metachromatic leukodystrophy.

Axonal degeneration is equally important in peripheral neuropathies. In demyelinating Charcot–Marie–Tooth disease type 1 (CMT1),

Box 1 | **The evolution of vertebrate myelin**

Oligodendrocytes and Schwann cells are embryologically and morphologically distinct, reflecting more than 300 million years of parallel glia evolution. Axon-engulfing cells that do not generate myelin membranes appeared much earlier in evolution and are found in most invertebrates[137]. Schwann cells can be considered ancestral glia, with features that may have diverged during CNS evolution into astroglial and oligodendroglial functions. In the vertebrate PNS, non-myelinating Remak cells, which engulf several C-fibre axons without myelinating them, are the simplest axon-associated cells that share features with axon-engulfing glia in invertebrates. Do Remak cells support axon function as do myelinating glia, and does their presence reflect a more fundamental support function that precedes even myelination in evolution? Observations in mutant mice[138] with a sensory neuropathy and progressive loss of C-fibre axons suggest that unmyelinated axons also depend on engulfment by glia.

Support of axon function by glia may be less important for short-lived organisms with short axons, for local interneurons, or when neurons are studied in culture. However, for projection neurons the axons of which are centimetres (or metres) in length, glial support becomes a necessity. This would explain why conditions as diverse as peripheral neuropathies and leukodystrophies exhibit a progressive length-dependent loss of axons as a final common pathway of disease.

many genes causing the disease are exclusively expressed in Schwann cells. These include those encoding peripheral myelin protein 22 (*PMP22*) and myelin protein zero (*MPZ*; also known as *P0*), the main structural proteins of compact myelin, as well as genes that have essential roles in myelinating Schwann cells[21,22]. Developmental hypomyelination and loss of myelin from single internodes (segmental demyelination) explains the reduced nerve conduction velocity (NCV) that precedes the clinical onset of the disease. However, the secondary axonal pathology and degeneration typically begin later, leading to functional denervation, progressive muscle weakness and sensory deficits, which are more clinically important than reductions in NCV[23]. In genetic diseases of myelinating glia, axon loss is often length dependent: it affects first those PNS fibres that innervate the most distal muscle groups, or the longest CNS fibre tracts within the spinal cord[16,24].

How does myelin loss cause axon degeneration? For multiple sclerosis, which is an immune-mediated disease, there could be more than one answer to this question, as the interaction between neurodegenerative and inflammatory mechanisms is complex[25]. Most researchers studying multiple sclerosis use animal models, such as experimental allergic encephalomyelitis, in which demyelination is secondary to severe inflammation. In this model, it seems likely that myelin membranes physically protect CNS axons from activated autoreactive T cells. *In vitro*, $CD8^+$ T cells can also attack unmyelinated axons that express major histocompatibility complex class I proteins on their surface, and this effect may also operate in acutely inflamed multiple sclerosis lesions[26].

In addition to the ability of cytotoxic T cells to lyse axons, inflammation impairs axonal survival owing to the generation of nitric oxide by activated microglia. Nitric oxide readily diffuses into demyelinated axons and perturbs mitochondrial ATP generation[27,28]. At the same time, impulse conduction along demyelinated axons uses considerably more energy per unit length than that along myelinated axons[29] — a result, in part, of channel redistribution and higher than normal Na^+ channel density[30]. Thus, inflammation and demyelination, when combined, might reduce the axonal energy balance below a tolerated threshold level (leading to 'virtual hypoxia'[28]). This threshold is reached when the Na^+/Ca^{2+} exchanger fails and reverses the direction of ionic flow, filling the axon with Ca^{2+}.

Table 1 | **Myelin diseases causing secondary axonal loss**

Human myelin disease	Frequency and causes	Glia and myelin pathology	Secondary axonal involvement	Animal models in research
Multiple sclerosis (MS)	• Relatively common autoimmune disease • Primary cause unknown • Viral origin and genetic risk factors suggested	• CNS-specific disease • Inflammatory lesions in white-matter tracts cause oligodendrocyte death, extensive demyelination and macroscopic plaques	• Axonal swellings and transections in white- and grey-matter lesions • Axon loss associated with permanent disability at later disease stages	• Experimental allergic encephalomyelitis for modelling autoimmunity against myelin epitopes and the inflammatory phase of MS
Inherited leukodystrophies	• Very rare genetic disorders • Several single-gene defects identified, such as in *PLP1* (for PMD), *GJC2* (for PMLD), *ABCD1* (for adrenoleukodystrophy) and *ASA* (for metachromatic leukodystrophy)	• Defects of terminal oligodendrocyte differentiation and myelin formation • Or defects of myelin maintenance with demyelination (also secondary inflammation in adrenoleukodystrophy)	• Perturbation of axonal transport followed by Wallerian degeneration • Purely axonal forms of leukodystrophies: SPG2 (associated with *PLP1* defects) and adrenomyeloneuropathy (associated with *ABCD1* defects)	• Rodents with mutations corresponding to the human disease gene, such as a *Plp1* point mutation or *Plp1* overexpression (for PMD), *Plp1* knockout (for SPG2) and *Abcd1* knockout (for adrenomyeloneuropathy)
Inherited demyelinating neuropathies	• Rare genetic disorders, such as CMT • Many disease genes identified, such as *PMP22* (for CMT1A), *MPZ* (for CMT1B) and *GJB1* (for X-linked CMT)	• Defects of Schwann cell differentiation and peripheral myelination, or myelin maintenance • Segmental demyelination with formation of 'onion bulbs'	• Slowly progressive and length-dependent axon loss causing denervation, sensory deficits and muscle weakness — the clinical hallmarks of CMT	• Rodents with mutations in or overexpression of genes corresponding to human disease genes, such as *Pmp22* (for CMT1A), *Mpz* (for CMT1B) and *Gjb1* (for X-linked CMT)

ABCD1, ABC binding cassette family D member 1; *ASA*, aryl sulphatase A; CMT, Charcot–Marie–Tooth disease; *GJB1*, gap junction protein β1; *GJC2*, gap junction protein γ2 (also known as *GJA12* and *CX47*); *MPZ*, myelin protein zero (also known as *P0*); *PLP1*, proteolipid protein 1; PMD, Pelizaeus–Merzbacher disease; PMLD, Pelizaeus–Merzbacher-like disease; SPG2, spastic paraplegia type 2.

High Ca^{2+} levels (augmented by glutamate excitotoxicity and the release of Ca^{2+} from endogenous stores[28]) trigger Ca^{2+}-dependent proteolytic processes that lead to protein degradation, further impairment of mitochondrial function and eventually Wallerian axon degeneration[31,32] (FIG. 1). Ca^{2+} entry is also harmful to the slender processes of oligodendrocytes, which express Ca^{2+}-permeable NMDA (*N*-methyl-D-aspartate) receptors[33]. Given that axonal degeneration releases glutamate, which can cause Ca^{2+}-mediated injury to oligodendrocytes[33,34], secondary glutamate excitotoxicity might contribute to a vicious cycle of oligodendrocyte dysfunction and axon loss in a broad range of white-matter diseases. Intact myelin sheaths may limit the access of glutamate to axonal glutamate receptors[35,36], and the access of metalloproteinases released from inflammatory cells to the axon[37].

Thus, in inflammatory brain diseases, demyelination contributes to axon loss. However, myelin deficiency may not cause axonal degeneration unless it is combined with inflammation and oligodendroglial injury. Indeed, in multiple sclerosis lesions, it seems impossible to separate demyelination from inflammatory injury to oligodendrocytes. What happens to axons that 'only' face the absence of myelin, and what are the consequences of acute glial injury without demyelination?

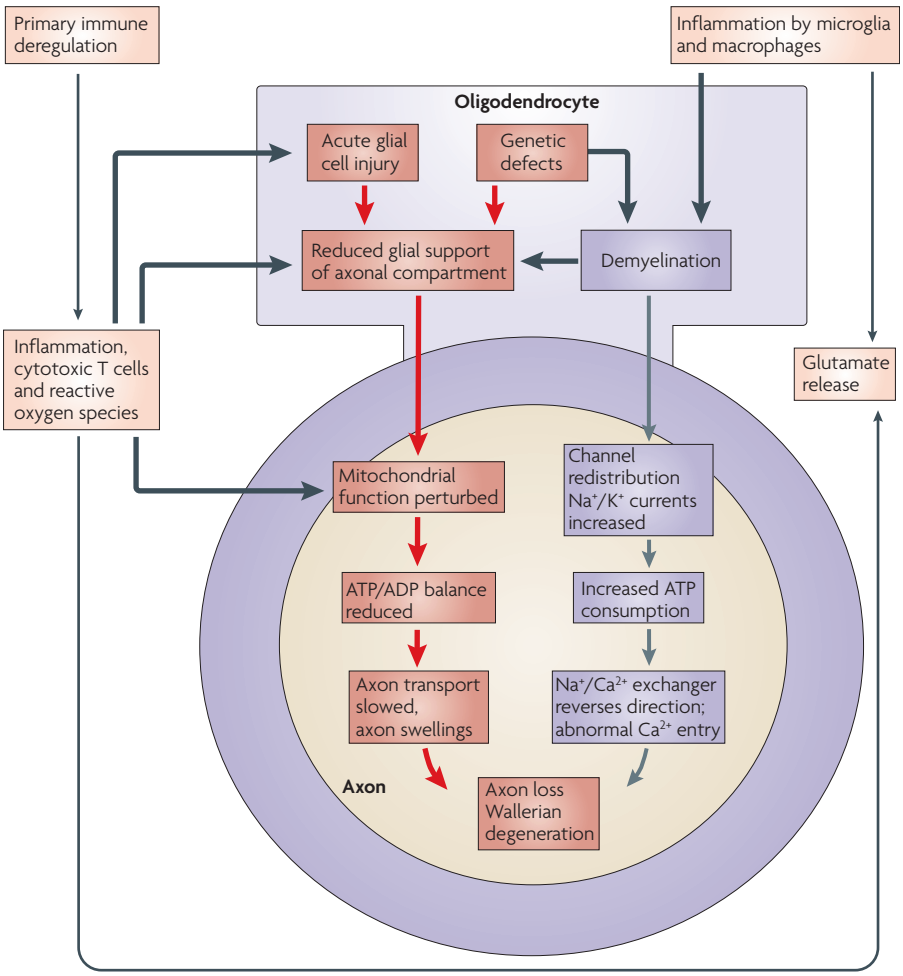


Figure 1 | **Axonal degeneration following oligodendroglial defects and the loss of myelin.** Schematic representation of two pathways hypothesized to explain the secondary axonal degeneration that occurs in diseases that affect myelinating oligodendrocytes in the CNS. Route 1 (shown in red) depicts the perturbation of an axonal support system that is independent of myelin, but may involve glial–axonal metabolic coupling. It is proposed that loss of normal axonal support causes a pathologically reduced energy balance, which leads to a slowed transport rate, axonal swellings and ultimately Wallerian degeneration. Route 2 (shown in blue) depicts the consequences of demyelination, usually in the context of inflammation and mitochondrial damage caused by reactive oxygen species (shown in pink). Increased ion currents and the reorganization of ion channel expression on denuded axon leads to energy depletion and Ca^{2+}-mediated axonal decay. The Ca^{2+}-mediated axonal decay is promoted by excitoxicity and initiated by glutamate release from the injured axon, astrocytes and immune cells. Note that the two pathways are linked. In this comprehensive model, axonal energy depletion marks the final common pathway of a diverse group of CNS diseases that perturb the integrity of myelinating oligodendrocytes.

Axon degeneration independent of myelin loss. Myelin not only speeds impulse conduction but also affects the architecture of the axon[38,39]. Mice that have myelin defects in the absence of inflammation offer a different view of the role of oligodendrocytes and myelin in axonal integrity. For example, oligodendrocytes in *shiverer* mice, which carry a mutation that causes myelin deficiency, make no more than a few non-compacted myelin-like wraps[40,41] (many large-calibre axons remain essentially unmyelinated[41,42]) but show no signs of oligodendrocyte degeneration[42]. Moreover, although axonal mitochondria increase in number[43] (presumably as a response to higher energy expenditure) and the axonal cytoskeleton fails to fully mature[44], there is no axon loss in adult *shiverer* mice[2,42]. This strongly suggests that the near absence of myelin is tolerated provided oligodendrocytes survive and provide a minimal axonal ensheathment.

The situation is similar in the PNS of mouse mutants in which Schwann cells cannot synthesize cholesterol[45,46]. Cholesterol deficiency impairs peripheral myelin growth. Similar to *shiverer* mice, there is dramatically impaired axonal conduction, but no axonal loss[46] (K.-A. N., unpublished observations). Together, these observations suggest that the absence of myelin does not necessarily result in secondary axonal defects.

By contrast, there is evidence that myelinating glia support axonal functions independently of myelin. This was discovered in mouse mutants with specific oligodendrocyte defects that barely impaired myelin synthesis[2–4,47–49]. For example, the proteolipid protein 1 (*Plp1*)-null mouse is a model of human spastic paraplegia, a disease that involves progressive axonal loss in the long spinal tracts. In the absence of PLP1 (referring to the tetraspan membrane proteins PLP and DM20), oligodendrocytes assemble compact myelin that has only ultrastructural abnormalities and provides rapid impulse propagation sufficient for normal motor development[47,50]. However, after a few months, focal blockage of axonal transport leads to numerous axonal swellings[2] and subsequent Wallerian

degeneration of the distal axon. All white-matter tracts are affected in a predominantly axon length-dependent fashion[2,20]. As the absence of PLP1 from oligodendrocytes cannot be compensated for by the transgenic expression of MPZ, PLP1-mediated neuroprotection clearly requires more than the physical stability of myelin[51].

The Wallerian degeneration in PLP1-deficient mice is preceded by a slowing of fast anterograde and retrograde axonal transport[48]. This adds support to the hypothesis that the axonal defects, which are morphologically similar to those in mitochondrial disease models[52,53], are caused by an energy imbalance in myelinated axons. Does the phenotype of *Plp1*-mutant mice reflect a role of oligodendrocytes in supporting mitochondrial energy metabolism in axons[2]?

A similar axon transport defect that precedes axon swellings and degeneration is found in mouse mutants of the myelin-specific glial gene encoding 2′,3′-cyclic nucleotide 3′ phosphodiesterase (*Cnp*; also known as *Cnp1*)[3]. CNP-deficient mice develop without motor problems and exhibit apparently normal myelination. Axonal transport defects and axon swellings occur several weeks earlier than in *Plp1* mutants[49]. Abnormal enlargement of the inner tongue processes of myelin[3] occurs with the first axonal defects[49]. Later, there is a degeneration of paranodal junctions, which normally seal the internodal compartment[54]. The central importance of paranodal junctions for axonal integrity[10] is also supported by studies of mice with mutations in the gene encoding contactin-associated protein 1 (*Cntnap1*; also known as *Caspr1*). In these mice, loss of paranodal junctions causes the degeneration of Purkinje cell axons[55]. This and the phenotypes of mice with other genetic defects in oligodendrocytes[4,56–58] suggest that supporting axonal integrity is a complex function of glia that is easily perturbed.

There is also evidence that Schwann cells support axonal integrity independently of myelin itself. Mice lacking the myelin-specific adhesion protein MAG have normal myelination and no motor symptoms[59,60]; however, their sciatic nerve fibres (and also some central axons) exhibit axonal degeneration and reduced axon calibres[5,61]. That inherited demyelinating neuropathies, such as CMT1, are associated with axon loss is well documented[62]. By contrast, patients with CMT2 have normal NCVs (indicating intact myelination), but reduced amplitudes of compound muscle action potentials, indicating only axon loss. Typically, the genes that cause CMT2 are expressed in neurons; however, intriguing exceptions are specific mutations in *MPZ*[63,64], a gene exclusively expressed by Schwann cells. Most *MPZ* mutations cause a demyelinating neuropathy (CMT1B). It is unclear how *MPZ* mutations cause CMT2; however, to date they seem to always affect the topology of the extracellular domain of MPZ[22].

Theories about myelin-independent axon loss. If widespread axonal degeneration can result from an oligodendrocyte-specific defect but demyelination is not the cause, what are the mechanisms involved? Abnormal axon to glia signalling could affect the degree of post-translational modification of axoskeletal proteins; these include neurofilaments, microtubules and their associated proteins (such as microtubule-associated proteins and tau), which are major determinants of axonal calibre and the Achilles heel of transport processes[65]. Indeed, following normal myelination, axons increase rapidly in calibre: this correlates with glial engulfment but not necessarily with myelination[39]. Do PLP1-deficient glia lack the ability to signal to axons, and could a minor failure of radial axon growth suffice to cause subsequent axonal degeneration? Probably not, because the aberrantly myelinated CNS axons in *shiverer* mutants are significantly smaller in calibre, with narrowly spaced non-phosphorylated neurofilaments and microtubules[44,66], but do not degenerate. This suggests that reduced neurofilament spacing is not the sole cause of axon loss. Unmyelinated axons in *shiverer* mice develop swellings only when oligodendrocytes are further compromised by the absence of PLP1 (REF. 2).

It is theoretically possible that myelin sheaths that are not properly assembled are good insulators but exert a 'toxic' effect on the myelinated axon. There is no experimental support for this idea or for a link between myelin membranes and known toxic proteins (such as Fas ligand (also known as CD95L)). However, there is some indirect evidence for such an effect. PLP1-deficient and CNP-deficient oligodendrocytes have been engrafted into the spinal cord of *shiverer* mice, where they began wrapping unmyelinated but physically intact axons. The axons that were ensheathed by the mutant oligodendrocytes developed swellings several months later[48,49]. Although this observation provides evidence that lack of PLP1 and CNP can cause axon pathology, it does not prove a mechanism for toxicity. Direct toxicity is also difficult to reconcile with the length-dependent axon loss that is seen in mice and humans[20].

A conceptually related idea is that there is an imbalance in normal myelin-to-axon signalling when PLP1 (or CNP) is absent. If oligodendrocytes continuously send both death and survival signals to axons, the lack of PLP1 may selectively reduce survival signalling, resulting in axon death. Although this theory cannot be ruled out, it also lacks experimental support and would not explain axon length-dependent effects. Myelin-derived inhibitors of axon growth such as MAG could also send 'negative' signals for axon survival. However, these proteins are not pro-apoptotic and the genetic ablation of MAG worsens (rather than rescues) the axonal degeneration phenotype of PLP1-deficient mice[67].

A third model, proposed below, holds that oligodendrocytes and Schwann cells provide trophic support to the axon that they ensheath. According to this model, specific myelin proteins are not themselves trophic, but are required by glia to execute their trophic functions. Classical neurotrophic functions involve cell–cell signalling. Although oligodendrocytes release neuronal growth factors and neurotrophic cytokines — such as brain-derived neurotrophic factor, neurotrophin 3, insulin-like growth factor 1 and glial cell line-derived neurotrophic factor *in vitro*[68–70] — their role as axon survival factors *in vivo* remains to be determined.

Trophic support by myelinating glia

Plp1-null mice exhibit axonal pathology that is strikingly similar to mitochondrial diseases[52,53]. This raises the question of whether oligodendroglial trophic support is required for mitochondrial energy metabolism in axons. In this hypothetical model, myelinating glia require specific gene products (including PLP1 and CNP) to support energy generation by axonal mitochondria. This support may be constitutive or may be activated when the energy demands are exceptionally high or when long axons undergo age-related changes at their distal end. Lack of such support may therefore constitute a major risk of axonal degeneration (FIG. 1). The observation that thinner axons, such as those of the optic nerve[2,49], have a higher risk of degeneration than thicker axons supports this hypothesis. Simply normalizing the axonal ATP content (that is, the axon volume) to the number of ATP-consuming $(Na^+ + K^+)$ATPases (that is, axon surface), the

lower volume/surface ratio in thinner axons than thicker axons suggests that the thinner ones have a reduced 'energy reserve'.

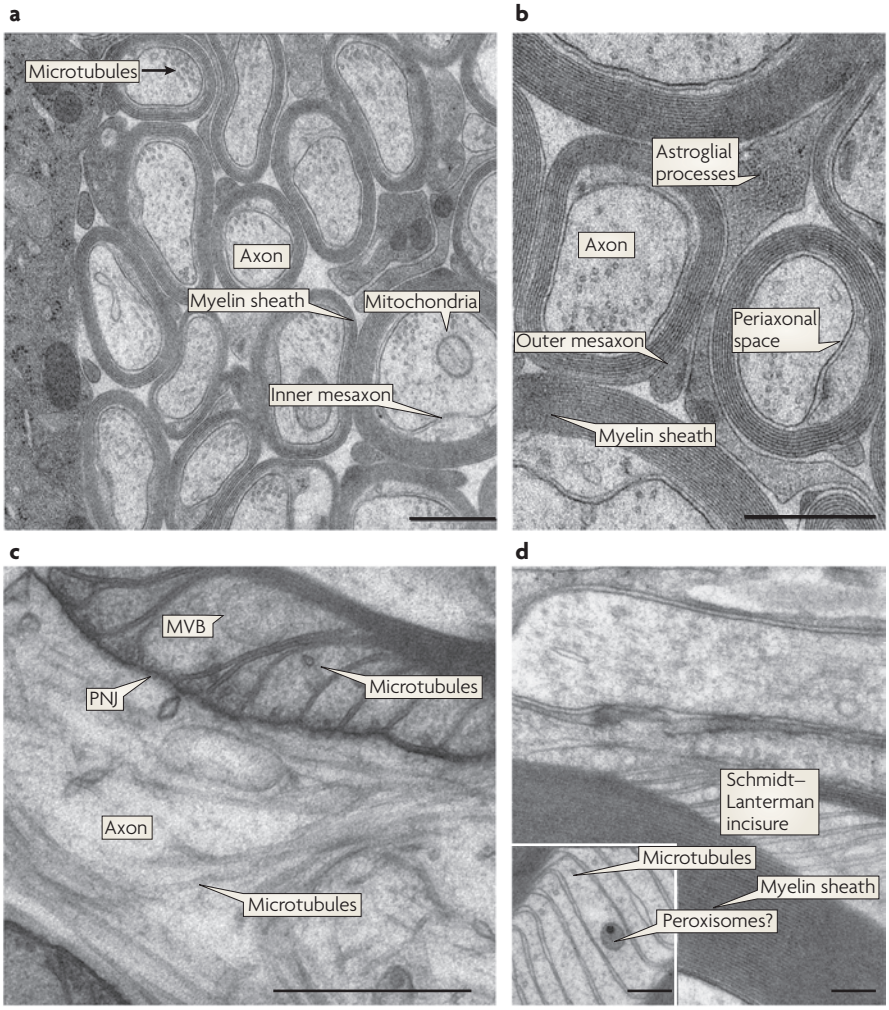
Why would axons require metabolic support? Neuronal cell bodies and distal axonal segments are distinct biochemical compartments, at least with respect to metabolic reactions and the maintenance of a physiological energy balance. Neuronal ($Na^+ + K^+$)ATPases, which use most axonal ATP, are present along the entire internodal membrane[71,72], suggesting that axonal energy demands are not strictly clustered around the nodes. It is theoretically possible that oligodendrocytes and Schwann cells provide energy-rich metabolites[73] to axons. Here, I propose a trophic function of glia-derived glycolysis products (pyruvate, lactate or its derivates) for mitochondria in long fibre tracts. This is highly speculative at present but should be considered for several reasons.

Although glycolysis has not been measured in axons without the associated glia, it presumably occurs throughout the neuronal axoplasm. Glycolytic enzymes synthesized in the soma move anterogradely by slow axonal transport[74–76]. Assuming a rate of movement of 2 mm per day, the distal internodes of an axon 1 m long would receive glycolytic enzymes that had been active *en route* for 500 days. How stable are metabolic enzymes at 37 °C? Estimates from red blood cells (which, unlike axons, are free of lysosomes) revealed a functional half-life for the glucose 6-phosphate dehydrogenase (G6PD) of 48 days[77]. Extrapolated to 500 days, the distal end of the 1 m axon would experience less than 0.1% of the original G6PD activity. In liver cells, glycolytic enzymes have a half-life of only a few days[78]. Although these extrapolations are based on unproven assumptions and must be interpreted cautiously, it is possible that the efficiency of axonal glycolysis is limited in a length-dependent fashion. A length-dependent slowing of fast axonal transport has also been seen in an experimental model of diabetic neuropathy[79] and in animals given a toxic insult that inhibits intermediate metabolism[24]. These observations are compatible with a length-dependent 'loss of energy' in peripheral neuropathies[80] and suggest that distal axons may require additional metabolic support.

Do myelinating glia provide metabolic support? The white matter carries out more glycolytic metabolism than oxidative metabolism[81]. Could ensheathing glia provide glycolysis products locally to axonal mitochondria? A precedent for such a supportive relationship among cells is given by mammalian oocytes, which are associated with cumulus cells that produce pyruvate and lactate[82]. Pyruvate has also been identified as a trophic factor for cultured neurons in glia-conditioned medium[83] and the rescue of neuronal energy metabolism by pyruvate is independent of its antioxidative effect[84]. The role of astrocytes in brain energy metabolism is well known[85]. Cultured astrocytes release lactate[86], and it has been hypothesized that lactate supports the local energy demands of glutamatergic synapses in the cortex[87]. However, this view has been challenged[88], there are conflicting observations[89]

Box 2 | **The ultrastructure of myelin: more than electrical insulation**

The figure shows electron micrographs depicting an overview of myelinated axons (**a** and **b**: optic nerve) and some structural details of the myelin ensheathment (**c** and **d**: sciatic nerve). Axons in cross section, containing microtubules and mitochondria, are separated from the myelin sheath by a thin periaxonal space — a cylindrical liquid-filled volume in which the axon resides. The innermost layer of the myelin sheath — the inner collar — runs parallel to the axon, is often non-compacted and widens at its tip. Thus, non-compacted myelin constitutes a channel-like system within a sheath that runs along the axon, includes the inner and outer collar, and is continuous with the paranodal loops and the glial cytoplasm. In the CNS, non-compacted myelin is coupled to astrocytes. The internodes of peripheral myelin are longer (up to 2 mm) than those in the CNS and are additionally interrupted by Schmidt–Lanterman incisures, which are local stacks of non-compacted myelin spiralling around the axon, similar to paranodal loops. Both loops and incisures also provide a direct route from the glial soma to the lining of the periaxonal space, as adjacent membranes contain gap junctions. The channel system also contains microtubules (inset in **d**) that may serve motor-driven transport functions for organelles such as peroxisomes or multivesicular bodies (MVBs). Scale bar: 500 nm (**a**–**c**) and 200 nm (**d**).



and *in vivo* evidence is still weak[90]. Similarly, in the optic nerve, astrocytes store glycogen and are thought to provide a local energy reservoir for maintaining axon function under hypoxic and low-glucose conditions[91–93].

Oligodendrocytes and Schwann cells contain no glycogen. However, they completely engulf long axonal segments and can thus condition the extracellular milieu in which myelinated axons reside (BOX 2). By comparison, astrocytes have only small points of axonal contact at the nodes of Ranvier[94]. Interestingly, astrocytes and oligodendrocytes are coupled by gap junctions[95–97], which provides a possible route for small metabolites from the blood–brain barrier to myelin and the glial cytoplasm that lines the periaxonal space (inner collar) (FIG. 2). Genetic evidence suggests that this astroglia–oligodendroglia coupling is crucial for myelination: the severe leukodystrophy PMLD is caused by mutations in the human gap junction protein γ2 gene (*GJC2*; also known as *GJA12* and *CX47*)[98,99] — not to be confused with PMD, which is caused by a mutation in the *PLP1* gene. That glia uncoupling impairs axon survival is suggested by the phenotype of some patients with *GJC2* mutations, which is similar to spastic paraplegia[99]. Similarly, deletion of oligodendroglial connexins in mice leads to severe demyelination[100–103].

Glia ensheathment triggers changes in the membrane architecture of the enwrapped axon[104], which could prepare the axon for long-term metabolic interactions with glia. The oligodendroglial turnover of *N*-acetylaspartate (NAA), a metabolite synthesized in neuronal mitochondria, is evidence that axons and oligodendrocytes can exchange small metabolites[105]. In oligodendrocytes, NAA is hydrolysed to aspartate and acetate — a process required for myelin lipid synthesis during development[106]. The flux of NAA between axons and oligodendrocytes is therefore extensive, at least during development. It might seem that exchange of small metabolites would occur in both directions, provided the necessary transporters are in place. However, expression of monocarboxylic acid and dicarboxylic acid transporters along myelinated axons[107] has not been systematically analysed. The observation that loss of paranodal junctions in the PNS results in the accumulation of mitochondria in the nodal axoplasm[108] is compatible with defects of axon–glia metabolic coupling in this region.

If ensheathing glia are metabolically linked to axons, the movement of metabolites between the two compartments is not a trivial problem. The glial cytosol is connected to the periaxonal space by long and narrow channels, such as Cajal bands, Schmidt–Lanterman incisures, the lumina of the paranodal loops and the internodal adaxonal space (BOX 2; FIG. 2). These seem to be crucial for metabolic exchange. For the optic nerve, it has been suggested that this glial channel system allows the murine Theiler's virus to travel from axons to oligodendrocytes[109]. Can simple diffusion account for the efficient transport of metabolites, enzymes or even viral particles? The cytosolic channel system of healthy

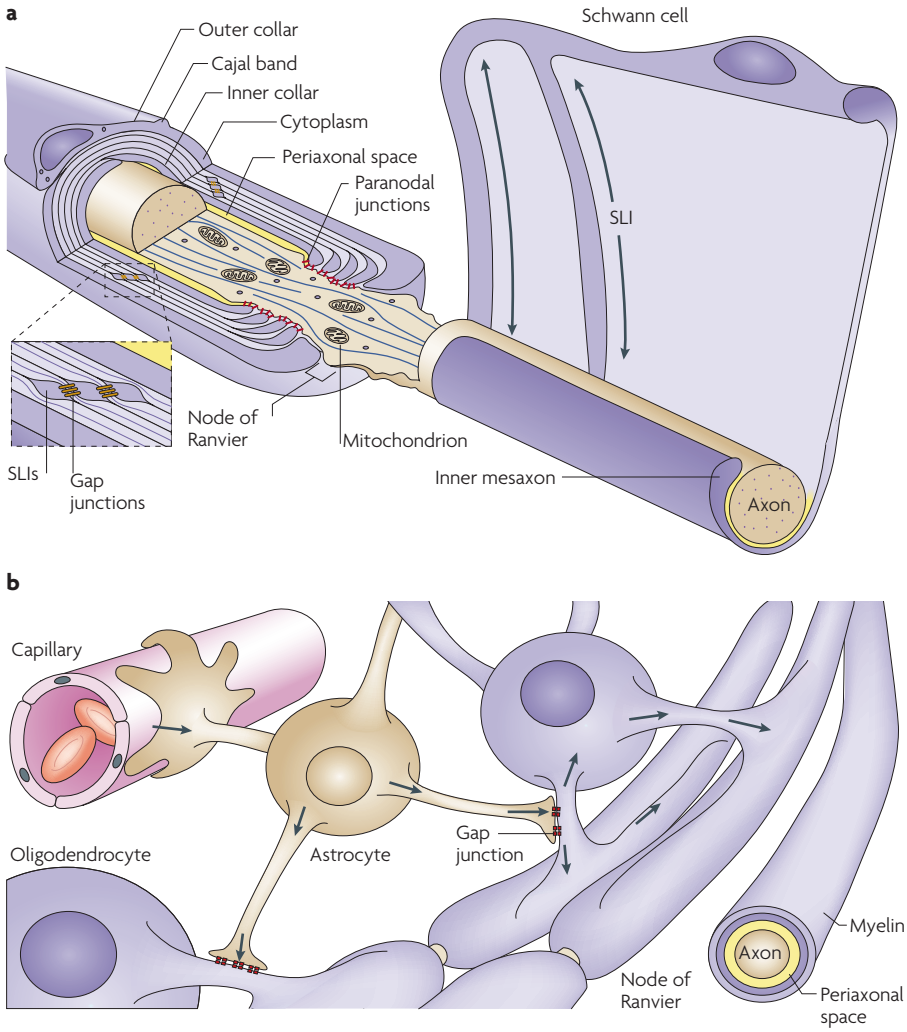


Figure 2 | **Do myelinating glia maintain a system for the trophic support of axons?** **a** | Hypothetical schema depicting a myelinated axon with the unrolled sheath of a Schwann cell shown on the right. Compacted myelin (shown in pale blue) serves as an insulator that inevitably deprives the axoplasm (shown in brown) of free access to nutrients in the extracellular milieu, except for at widely spaced short gaps (nodes of Ranvier). The encapsulated axon is proposed to reside in a milieu conditioned by the glia that can thereby support axonal energy metabolism. Non-compacted myelin defines a channel system (shown in dark blue) that is continuous with the glial cytoplasm and brings cellular metabolites close to the periaxonal space (shown in yellow). The Schmidt–Lanterman incisures (SLIs) — local stacks of non-compacted myelin that spiral around the axon — are linked by gap junctions when stacked. **b** | Oligodendrocytes are also coupled by gap junctions to astrocytes that reach the blood–brain barrier. In addition to simple diffusion, cytosolic 'perfusion' could attenuate concentration differences of metabolites with high axonal turnover. Similar to fast axonal transport, glial intracellular transport may depend on tubular tracks and the fine architecture of this channel system. For the postulated transfer of metabolites across the internodal axon, the adjacent glial and axonal compartments may function as a counter-current system. Metabolic fuelling of axonal mitochondria by glia is compatible with the axonal swellings observed in oligodendrocyte-specific defects[2–4] that resemble the defects in mitochondrial disorders. Part **a** is modified, with permission from REF 139 © (2003) Elsevier.

oligodendrocytes can be dye-filled within minutes[110]. But would intracellular diffusion[111] be fast enough to prevent the formation of steep concentration gradients (which would reduce the efficiency of exchange) if glia deliver metabolites to axons that serve as a sink? In experimental anoxia, the ATP reservoir of myelinated axons fails in less than a minute[89,93], which is about the time it takes to dye-fill the inner and outer mesaxon of live oligodendrocytes by diffusion[112].

It has been postulated that large cells are not bags of water but require an intracellular convection system[113], with molecular motors and tubulin tracts, to overcome the structural constraints of free diffusion. One extreme of such motor-driven intracellular movement is fast axonal transport (2–5 μm per s). In myelinating glia, microtubules and motor proteins may similarly contribute to the movement of proteins and organelles within the extended cytosolic channels — that is, cellular processes and 'non-compacted' myelin (FIG. 2). If we assume that axon–glia metabolic exchange is crucial, minor ultrastructural defects in this oligodendroglial architecture (such as those associated with inflammation[114]) might have a considerable effect on free intracellular diffusion, cytoplasic flow and ultimately the metabolic support of axons.

Interestingly, both PLP1 and CNP have been indirectly associated with the tubulin network of oligodendrocytes, which contributes to the myelin ultrastructure. PLP1 is essential for the post-translational transport of NAD-dependent deacetylase sirtuin 2 (SIRT2) into the myelin compartment[115]. In oligodendrocytes, SIRT2 is most abundant in the adaxonal and abaxonal channel systems of myelin[115–117]. One of the reported SIRT2 target proteins is α-tubulin[118]. CNP, a membrane-anchored protein, binds to tubulin[119,120] and may help anchor tubulin tracks in cytosolic channels of non-compacted myelin. Thus, loss of PLP1 and CNP might affect different aspects of the myelin ultrastructure and tubulin-based transport functions. Indeed, in *Plp1*$^{-/-}$*Cnp*$^{-/-}$ double-knockout mice, axonal survival is lower than in either of the parental single-knockout mice[49].

Independent evidence that glial support of axons has a metabolic component comes from conditional mouse mutants with deficiencies in peroxisomes. These ubiquitous organelles not only detoxify H_2O_2 and synthesize plasmalogens, they also degrade fatty acids, which can support axonal transport[121]. Inactivation of peroxisomal biogenesis factor 5 (PEX5) in oligodendrocytes and Schwann cells renders glial peroxisomes non-functional[4]. Although myelination is normal, the animals exhibit axonal swellings, Wallerian degeneration of axons and premature death. It has been hypothesized that the unusual degree of secondary inflammation in the *Pex5* transgenic mice results from an additional requirement of glia peroxisomes for degradation of inflammatory mediators[122].

Other far-reaching observations of axon–glia coupling have been made, including classical studies on the transfer of radiolabelled proteins from Schwann cell-like glia into the giant axon of the squid[123–125] (with unknown functional implications), or the transfer of horseradish peroxidase from oligodendrocytes to ganglion cell axons in the mammalian optic nerve[126]. More recently, the transfer of entire ribosomes from Schwann cells into the axoplasm was reported, adding to a long-standing debate on axonal mRNA translation[127].

Conclusions

The 'risk' of myelination. The idea that myelinated axons use glial glycolysis products is speculative. However, even if the metabolism of the axons included all glycolysis steps, the requirement for free exchange of metabolites (such as glucose) between the extracellular milieu and the axoplasm might pose a serious problem. The myelin sheath creates a diffusion barrier for ions and small molecules and is interrupted only by the nodes of Ranvier, which are less than a micron in length[128]. Nodes are often several hundred microns apart, and paranodal junctions, although not making an absolute barrier[10,50], help to seal the internodal axon. This can lead to a 'risk' of myelination, because more than 99% of the surface area of the axon is lost with respect to free metabolic exchange. Myelinated axons are largely deprived of free access to extracellular metabolites, residing instead in a milieu that is probably defined by the ensheathing cells. I propose that myelinating glia are able to compensate for the physical insulation of the axon by providing a suitable extracellular milieu. To this end, the channel system is used to physically connect the glia cytosol to the inner collar and paranodal loops that face the periaxonal space (BOX 2). An efficient transfer of metabolites along concentration gradients between axon and glia may depend on intracellular transport within both axons and glia, analogous to a counterflow system. In the CNS, intracellular perfusion within oligodendroglial processes may be particularly important as these cells are coupled to astrocytes, which provide a direct connection to the blood–brain barrier (FIG. 2).

Does oligodendroglial injury — such as that found in inflammatory multiple sclerosis lesions or as a result of glutamate-mediated excitotoxicity — interfere with this trophic function? Perturbations of energy metabolism have consequences within minutes to hours of injury. In a PLP1 transgenic model of PMD, axon loss is associated with local inflammation rather than demyelination *per se*[129]. Is an absence of myelin perhaps better than a myelin sheath generated by an injured oligodendrocyte? The answer to these questions is relevant to all myelin diseases, in which demyelination and axon loss is preceded by inflammation, excitotoxicity and oligodendroglial injury.

Implications for brain ageing and disease. The perception of myelin has gradually changed from that of an inert membrane sheath to that of a metabolically active glial 'organelle' that provides both axonal insulation and neuroprotection. It is intriguing that some normal, age-related changes in the primate brain involve morphological myelin abnormalities[112,130] and slowing of axonal transport rates[131,132]. Given that neurodegeneration can result from primary oligodendrocyte dysfunction that barely alters the myelin phenotype, it is possible that oligodendrocytes also have important bystander effects in neurodegenerative diseases that are not associated with demyelination. The loss of axonal transport contributes to disorders as diverse as Alzheimer's disease[133], polyglutamine diseases[134] and amyotrophic lateral sclerosis[135]. Oligodendrocytes are affected by toxic β-amyloid[136] and may differ in their ability to maintain the viability of axons under the stress of a disease. The hypothetical relevance of oligodendroglial support as a disease-modifying factor should be experimentally addressed with the large number of neuronal and glial mouse mutants that have been generated.

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Competing interests statement

The author declares no competing financial interests.

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