Myelination-independent functions of oligodendrocyte precursor cells in health and disease

Myelination-Independent Functions of Oligodendrocyte Precursor Cells in Health and Disease

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Abstract

Oligodendrocyte precursor cells (OPCs) are a population of tissue resident glial cells found throughout the central nervous system (CNS), constituting approximately 5% of all CNS cells and persisting from development to adulthood and ageing. The canonical role of OPCs is to give rise to myelinating oligodendrocytes. However, additional functions of OPCs beyond this traditional role as precursors have been suggested for a long time. In this Perspective, we provide an overview of the multiple myelination-independent functions that have been described for OPCs in the context of neuron development, angiogenesis, inflammatory response, axon regeneration, and their recently discovered roles in neural circuit remodelling.

Main text

It is now increasingly appreciated that almost every aspect of central nervous system (CNS) formation and function is critically regulated by glial cells [1]. Glia is used as an umbrella term that encompasses astrocytes, microglia, and oligodendrocytes, each of which have specialised functions in the CNS. Oligodendrocytes myelinate axons to facilitate conduction and provide metabolic support to axons [2]. Myelination by oligodendrocytes is a dynamic process that occurs over extended periods of life [3], and it can change in response to experience and neuronal activity; a process referred to as adaptive myelination [4,5]. New myelin is generally not formed by existing oligodendrocytes, but by differentiation of specialised oligodendrocyte precursor cells (OPCs, Figure 1a) [6]. Interestingly, unlike other undifferentiated stem and progenitor cells, OPCs neither reside in niches nor are they transient cells. Instead, OPCs are evenly distributed throughout the CNS (comprising ~5% of all cells in the adult mouse), thereby tiling the tissue with their process networks (Figure 1b, c) [7–11]. As OPCs reside within the tissue, they constantly integrate neural activity expressing a broad range of neurotransmitter receptors and voltage-gated ion channels [12]. Furthermore, OPCs are uniquely different from other glia in that they can form post-synaptic contacts with neurons, allowing them to integrate neuronal activity with high spatial and temporal precision (Figure 1d) [13,14]. Together, these properties make OPCs a constant cell population that is tightly integrated into neuronal networks, challenging the view that giving rise to myelinating oligodendrocytes is their only function, and raising the question as to whether tissue resident OPCs should be regarded a separate glial cell type (Box 1).

Despite the enduring question of what other roles OPCs have beyond the generation of myelinating oligodendrocytes, definitive answers have remained sparse and somewhat fragmented, especially in the healthy CNS. This is primarily owed to the circumstance that experimental manipulation of OPCs frequently also affects myelin generation, which makes it

difficult to dissect primary OPC effects from secondary effects due to altered myelination. However, a series of recent observations point to specific roles for OPCs in shaping the development and function of neural circuits. In the light of these recent advances, 40 years after the discovery of OPCs by Martin Raff and colleagues [15], it is a prime moment to summarise what is known about the myelination-independent functions of OPCs in the healthy and diseased CNS, and to discuss their established properties in the context of their recently reported roles neural circuit regulation.

Multiple functions of oligodendrocyte precursors in the healthy and damaged CNS

Guidance of migrating neurons. In the developing forebrain, OPCs are generated in successive waves with the first OPCs arising during mid-embryogenesis in the ventral forebrain [16]. Intriguingly, although these ventrally specified OPCs expand and migrate to populate the entire telencephalon by the time of birth, they are subsequently replaced by OPCs that are born later in the dorsal cortex [16]. What is the function of these transient precursors, given their limited contribution to the overall populations of OPCs and myelinating oligodendrocytes in the postnatal brain? Recently, an elegant study by Lepiemme and colleagues has shown that ventrally-born OPCs crucially regulate the migration of interneurons into the cortex [17]. Just like ventrally specified OPCs, cortical interneurons arise from the medial ganglionic eminence, from where they concomitantly migrate dorsally to the cortex. However, these two cell types do so using different migration strategies. OPCs migrate along blood vessels [18], whereas interneurons migrate through the parenchyma following a long-range gradient of the chemokine Cxcl12 that is released from the cortex [19]. However, endothelial cells throughout the CNS also release Cxcl12 and it had remained unclear for a long time how interneurons manage to follow the cortical gradient whilst ignoring the endothelial-derived Cxcl12. Lepiemme et al. showed that ventrally born OPCs once associated with vessels, unidirectionally repel migrating interneurons. This

contact-driven repulsion allows interneurons to solely follow the Cxcl12 gradient coming from the cortex [17]. It is a common biological phenomenon that the same signalling mechanisms are repurposed in different contexts, but it creates the complication that it needs to be ensured that the same signals coming from different sources do not get misinterpreted by any one cell. The finding that ventrally born OPCs act as mediators to ensure migrating interneurons ignore chemokines coming from the vasculature is a beautiful example of how intercellular crosstalk can achieve correct interpretation of such cues. This functionality also demonstrates an important myelination-independent role for transient OPCs to regulate circuit formation by ensuring proper guidance of interneurons.

Angiogenesis. Crosstalk between OPCs and vasculature does not only regulate the migration of newly formed neurons and OPCs to their target territories. In the developing postnatal brain, OPCs themselves regulate local tissue vascularisation by acting as sensors for hypoxia through activity of OPC-encoded hypoxia-inducible factor (HIF) [20,21]. On the one hand, HIF activation stimulates OPCs proliferation and arrests their maturation to myelinating oligodendrocytes. These autocrine effects are mediated through HIF-induced secretion of Wnt7a/7b by OPCs [20]. At the same time, the same Wnt signalling mechanism has paracrine effects and stimulates vessel growth into the hypoxic tissue where OPCs directly contact sprouting vascular endothelial cells [20,21]. Disruption of hypoxia sensing by OPCs in the forebrain leads to insufficient angiogenesis and axon degeneration, thus highlighting the importance of these bi-directional interactions between OPCs and vasculature to ensure healthy CNS development.

Mediator of tissue inflammation. Loss of myelin is a hallmark of demyelinating diseases such as Multiple Sclerosis (MS) and CNS injury. Remyelination of demyelinated axons is one of the few regenerative processes in the CNS that show relatively high efficiency due to

105 the lifelong abundance of OPCs which can, in principle, differentiate to become myelinating
106 oligodendrocytes at any time [22]. Upon demyelination, OPCs respond to inflammatory
107 cytokines and chemokines released by different immune cells, which affect the remyelination
108 process through regulation of OPC proliferation and differentiation [23]. However, several lines
109 of experimental evidence indicate that OPCs do not only respond to inflammation, but that
110 they actively contribute to driving inflammatory processes. OPCs themselves express
111 immune cues in response to demyelination such as CCL-2 and IL-1β [24]. In fact, OPCs are
112 crucial for disease progression in experimental autoimmune encephalomyelintis (EAE). EAE
113 pathogenesis is markedly reduced when NFkb activator 1, a key signal transducer of
114 interleukin 17 required for EAE induction, is selectively depleted from OPCs [25], showing that
115 OPCs themselves actively participate in mediating inflammatory processes. More recently,
116 several reports provided further evidence for active roles of OPCs in inflammatory
117 responses. Active MS lesions show aberrant clusters of perivascular OPCs [26]. These OPC
118 clusters interfere with the integrity of astrocyte endfeet and tight junctions in mouse models,
119 triggering breakdown of the blood-brain barrier and infiltration of inflammatory cells [26].
120 Furthermore, single-cell transcriptomics revealed that demyelination primes
121 oligodendrocytes to express immune genes [27], and induces OPCs to phagocytose and
122 present myelin debris via major histocompatibility complex (MHC) class I and MHC-II [28,29].
123 Together, these works show that tissue resident OPCs not only respond to inflammation, but
124 that they can act as mediators, perpetuators, and even inducers of inflammatory processes
125 in the CNS.
126
127 ***Glial scar formation and inhibition of axon regeneration.*** One of the main reasons why
128 severed CNS axons do not efficiently regenerate in mammals is the formation of a glial scar
129 around the lesion site, in addition to the presence of other inhibitors of axon regeneration like
130 myelin debris [30,31]. Following injury, OPCs, as well as astrocytes and microglia, increase their

proliferation rate and migrate to the site of damage where OPCs show upregulation of a broad set of genes associated with inhibition of axonal growth beyond the scar [32,33]. Chondroitin sulphate proteoglycans (CSPGs) are one major class of axon regeneration inhibitory molecules. Many CSPGs are prominently expressed by OPCs, such as brevican, neurocan, and phosphacan [34]. The commonly used OPC maker NG2 is also such a proteoglycan (encoded by the *cspg4* gene) and has been known for over 20 years as one of the key inhibitors of axonal regeneration within the glial scar [35]. Since then, a series of studies has successfully devised strategies to enhance axon regeneration in spinal cord injury by either perturbating OPC accumulation within the lesion [36], or by targeting NG2 using function-neutralising antibodies to promote regeneration and functional recovery in rat models [37,38]. Besides the expression of inhibitory molecules that prevent axon growth, it has also been suggested that OPCs might entrap dystrophic axons within lesions through the formation of synapse-like contacts [39], which are known to exist between axons and OPCs in the healthy CNS [14]. Even though compelling experimental evidence is lacking, it is tempting to speculate that axon-OPC synapses have stabilizing functions on remodelling axons at the site of transection [39], similar to how neuronal synapses stabilise axon branch dynamics during development [40,41].

The effects on axon growth and sprouting are not necessarily an exclusive property of OPCs in lesions, even though they may be most pronounced in scars characterised by increased density of reactive OPCs. We should bear in mind that NG2-expressing OPCs equally exist throughout the healthy CNS where they express the same growth inhibitory molecules, even if at lower levels. It may therefore be that OPCs in glial scars formed in response to injury of the CNS secrete additional factors to inhibit axonal regrowth. In addition, OPCs may also serve important functions in providing guidance to axons during development and remodelling of neural circuitry in the healthy CNS, as we discuss in the following sections.

Properties that enable oligodendrocyte precursors to participate in the regulation of neuronal circuitry in the healthy CNS

The proper formation of neural circuitry depends on accurate guidance and arborisation of axonal and dendritic arbours, and the establishment of synaptic connections of appropriate number and strength with the right neuronal partners [42]. These processes can be subject to dynamic remodelling as forms of circuit refinement and plasticity. Both formation and refinement of circuit connectivity can be fine-tuned by neuronal activity as one of the driving forces to form, stabilise or enforce some synaptic connections, and to eliminate other synapses [43]. All major types of glia play important roles in regulating different aspects of circuitry during development and plasticity. For instance, astrocytes regulate synaptogenesis and neuronal homeostasis, microglia govern synapse pruning,and oligodendrocytes exert activity-dependent myelination [5,44–46]. Interestingly, OPCs are uniquely integrated into neural circuits. OPCs express receptors for most neurotransmitters and voltage-gated ion channels, and are accurate sensors for extracellular potassium [12]. Furthermore, OPCs are the only glial cell type that forms synaptic contacts with glutamatergic and GABAergic neurons as pre-synaptic partners [47,48]. The function of these axon:OPC synapses is still unclear, but they allow OPCs to detect quantal release of neurotransmitter with high spatio-temporal resolution to potentially discriminate the precise origins of activity over a broad range of firing frequencies that wouldn't be possible to discriminate with ambient concentrations of neurotransmitter surrounding the cell [13]. OPCs are not only listeners to neuronal networks, they also have the capability to communicate to surrounding cells. Indeed, OPCs can use exocytosis as a mechanism to externalise cargo [49–51], and even release exosome vesicles [52]. Myelinating oligodendrocytes exhibit exosome release in response to neurotransmitter signalling [53], which may equally be employed by OPCs to communicate with neurons. Together, these features equip OPCs to monitor neural activity like no other glial cell type,

raising the intriguing question of what OPCs do with the information that they receive from the network that they are integrated in, and how OPCs themselves may contribute to the form and function of networks in development and plasticity [54].

The first direct evidence showing myelination-independent functions of OPCs in the context of neuronal network function came from ex-vivo studies where it was shown that the NG2 proteoglycan is shed from the OPC cell surface in an neural activity-dependent manner, and the inhibition of NG2 shedding impaired long-term potentiation of pyramidal neurons of the somatosensory cortex [55]. This, and several later studies also reported that the manipulation of OPCs and their function results in various behavioural deficits in mice: NG2-deficient mice exhibit altered sensory-motor gating such as reduced pre-pulse inhibition [55], genetic ablation of OPCs from the prefrontal cortex induces depressive-like behaviours [56], depletion of $GABA_B$ receptors from OPCs impairs social cognitive behaviour [51], and the depletion of Kir4.1 potassium channels from OPCs leads to improved spatial memory [57]. Although all these studies show that dysfunctional OPCs ultimately impair circuit function and animal behaviour, they are difficult to interpret because it remains unclear which of these defects result from myelination independent OPC functions, and which ones result from secondary effects of impaired oligodendrogenesis and myelination by dysfunctional OPCs.

To test if OPCs directly regulate circuit form and function *in vivo*, it requires the design of studies where OPCs can be specifically manipulated without indirectly interfering with myelination. One possibility to achieve this specificity is to manipulate OPCs in CNS areas where they do not (or rarely) differentiate to myelinate axons during stages when functional circuits are present. Examples of such regions are the mouse barrel cortex where OPCs accumulate along the septa separating the barrels [58], the molecular layer of the cerebellum [59], as well as in the olfactory bulb where OPCs extend their processes into synaptic

glomeruli [60]. However, the absence of reagents and assays to selectively interfere with OPCs in these regions *in vivo* have thus far limited the investigation of region-specific OPC functions in mammalian models.

Oligodendrocyte precursors directly sculpt circuit structure and function in the visual system. In 2022, we published a study showing that OPCs regulate fine-tuning of neural circuitry in the visual system through regulation of axonal remodelling [61]. They used the optic tectum of young zebrafish as model, as this brain region meets all criteria required to specifically disentangle myelination-independent functions of OPCs. The optic tectum is the primary visual processing centre of zebrafish (equivalent to the lateral geniculate nucleus in mammals) and the site where retinal ganglion cell (RGC) axons form terminal arbours that synapse to dendrites of tectal neurons. We showed that this synaptic region is interspersed with OPCs that interact with surrounding axons and dendrites, but which do not differentiate to myelinating oligodendrocytes, allowing them to study OPC functions without indirectly interfering with myelination. Importantly, during these stages the zebrafish retinotectal system is a functional circuit capable of processing complex sensory-motor transformations [62,63]. We found using genetic global and specific local elimination of OPCs from the optic tectum that RGC arbours showed exuberant sprouting and altered remodelling when OPCs were conditionally eliminated during phases when visual processing is refined. Functionally, these manipulations degraded the acuity of visual processing, meaning that the OPC-mediated effects on RGC arbour remodelling impaired synaptic connectivity in the retino-tectal system [61]. To our knowledge, this was the first study showing unambiguously that OPCs have mature functions in regulating neural circuit function independently of their canonical roles of generating myelinating oligodendrocytes, which opens a plethora of questions. Do axon:OPC synapses and activity integration play a role in regulating axon growth and remodelling, given that these processes can be controlled by neural activity [64]?

Are these constitutive effects of OPCs that can affect all axons regardless of their identity or is there specificity between subpopulations of axons and OPCs? It is known that specificity of circuit connectivity is governed by selective expression of matchmaking molecules between neurons with over 30 classes of retinal ganglion cell axons [65,66], and that OPCs also express many of these matchmaking molecules [67]. Furthermore, what are the cellular morphogenic processes that govern OPC-mediated axon remodelling?

Do OPCs guide or prune axons to regulate circuit connectivity? Overshooting axonal sprouting and faulty synapse formation in the absence of OPCs can occur by two mechanisms which do not need to be mutually exclusive. Lack of inhibition/stabilisation of axon arbours resulting in exuberant sprouting, and/or lack of active removal of surplus connections through phagocytosis. Two recent studies have reported the presence of pre-synaptic axonal material within OPCs, suggesting that OPCs actively prune synapses through phagocytosis [68,69]. Three-dimensional electron microscopy reconstructions in the developing mouse visual cortex found that OPCs frequently surround fine axonal filopodia, and that OPCs themselves contain a high number of phagolysosomes and fragments of axons, indicating that they prune synaptic components [69]. Similarly, a light-microscopic study revealed pre-synapses of thalamocortical axons within OPCs of the visual cortex, and the amount synaptic material within OPCs was dependent on the degree of synaptic remodelling during eye opening [68]. Thus, OPCs have the capacity to ingest axons, which may mean that they actively prune neuronal circuits. In this case, it will be important to elucidate the difference between synapse pruning by OPCs and by microglia, which are thought to be the main phagocytic cells in circuit plasticity [45]. Another important consideration to make here is that each phagocytosed pre-synapse may have been the pre-synaptic partner of an axon:OPC synapse. Such a scenario would also be consistent with the data available to this date, but substantially change the interpretation of the role of the ingested presynaptic

material. In the latter case, the pre-synapses ingested by OPCs would not necessarily affect number of neuronal synapses and thus circuit connectivity, but rather alter how OPCs are connected to neurons through their synapse-like connections, whose roles are still entirely unclear.

Whether or not OPCs prune circuits though phagocytosis of neuronal synapses, our own work suggests that guidance is at least one mechanism that is in place because they observed that axonal filopodia frequently retract upon contact with OPC processes [61], similar to their repulsive properties within glial scars and their role in guiding migrating interneurons [17,35]. Indeed, OPCs express a range of molecules beyond proteoglycans that can guide axons. One such example is semaphorin 5a, which is highly enriched in OPCs and which inhibits growth of retinal ganglion cell axons *in vitro* [70], and synaptogenesis in the hippocampal dentate gyrus *in vivo* [71]. Interestingly, mutations in semaphorin 5a are linked to autism in humans [72], which encompasses a range of complex disorders with defects in neural circuitry. It is tempting to speculate if these defects in synapse formation result from altered regulation of axon remodelling by dysfunctional OPCs, which remains to be investigated in future studies.

Conclusions and outlook

Although suggested for a long time, it is now clear that tissue resident OPCs have a number of functions in the healthy and diseased CNS that are independent of their canonical roles in developmental, adaptive, and regenerative myelination (Figure 2). Should we stop using the term OPC in order to reflect the circumstance that these cells are more than just a precursor? We think that it is neither necessary nor helpful to completely overhaul the terminology. From the literature to date, all OPCs, NG2 cells, or how else they are known (Box 1), are members of the same overall cell type that expresses the same set of core

markers, and they all can in principle give rise to myelinating oligodendrocytes. Intrinsic and extrinsic factors allow segregation of OPCs into different states and even functionally different groups that restrict their roles and propensities to differentiate [11,73,74]. However, despite these potential groupings and the non-canonical roles of this type of glia in the healthy and diseased CNS, there is no evidence that any of these cells isn't also a precursor with the potential to give rise to a myelinating oligodendrocyte.

Irrespective of this opinion on the nomenclature of tissue resident OPCs, the collective findings on how OPCs directly participate in different processes in the healthy and diseased nervous system raise several questions. Are there unifying attributes that collectively describe the non-canonical roles of OPCs independent of their context? We propose that one possibility would be to regard tissue-resident OPCs as sensors of physiological signals, which they integrate to subsequently act as mediators to other cells that locally surround them. Examples are their described roles in sensing hypoxia to stimulate angiogenesis [20], in acting as antigen presenting cells upon demyelination [28,29], and in transducing IL-17 mediated EAE [25]. Along those lines, a very recent study revealed exacerbated microglial reactions following prion infection in the absence of OPCs [75], further corroborating the idea that OPCs act as mediators to surrounding cells.

In addition to their roles as signal integrators and mediators to non-neuronal cells, OPCs also directly talk back to the axons they interact with, whether through regulation of their remodelling in circuit maturation [61], ingestion of pre-synapses [68,69], or direct bi-directional cross talk [51]. It is an imminent open topic to address the role of axon:OPC synapses in this context. Is the ability of OPCs to integrate synaptic and non-synaptic input used to directly shape circuit development and plasticity? One simple possibility is that axon:OPC synapses could act as temporary guideposts or placeholders to stabilise axon terminals in target areas

313 when they are not yet connected to neuronal postsynapses. It is known that synapse
314 formation guides and stabilises growing axons [40,41]. Synaptic contacts to OPCs could
315 facilitate these processes just like they have been proposed to entrap dystrophic axons in
316 lesions [39]. Albeit speculative, OPCs could even employ such contacts to help establish
317 functional boundaries within the CNS. Synaptic specificity and matchmaking between
318 individual neurons are regulated by complex combinations of adhesion molecules that each
319 cell expresses [65]. It will be interesting to investigate if OPCs express these molecules in a
320 similarly variegated manner as neurons do. This would enable OPCs to participate in these
321 matchmaking processes, which would add a new level of complexity into the diversity of
322 oligodendrocytes and their interactions with surrounding axons. Regardless of the precise
323 mechanisms by which OPCs integrate into and shape circuits, the overall role of non-
324 canonical OPC functions in the context of CNS circuit disorders will be an exciting area for
325 future research. For example, it was shown that fifty percent of dysregulated genes in
326 patients who suffered from major depressive disorders were in fact encoded by OPCs [76].
327 How do dysfunctional OPCs contribute to these and other disorders where circuit
328 connectivity and function is disrupted? They may likely lead to faulty myelination especially
329 in areas that show variable degrees of myelination. However, it may also be direct effects of
330 dysfunctional OPCs as they sculpt the nervous system. The continued development of more
331 refined reagents and assays will allow to dissect to these and other questions on the role of
332 neuron:OPC crosstalk in the time to come.

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Competing interests

The authors declare no competing interests.

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Figures Legends:

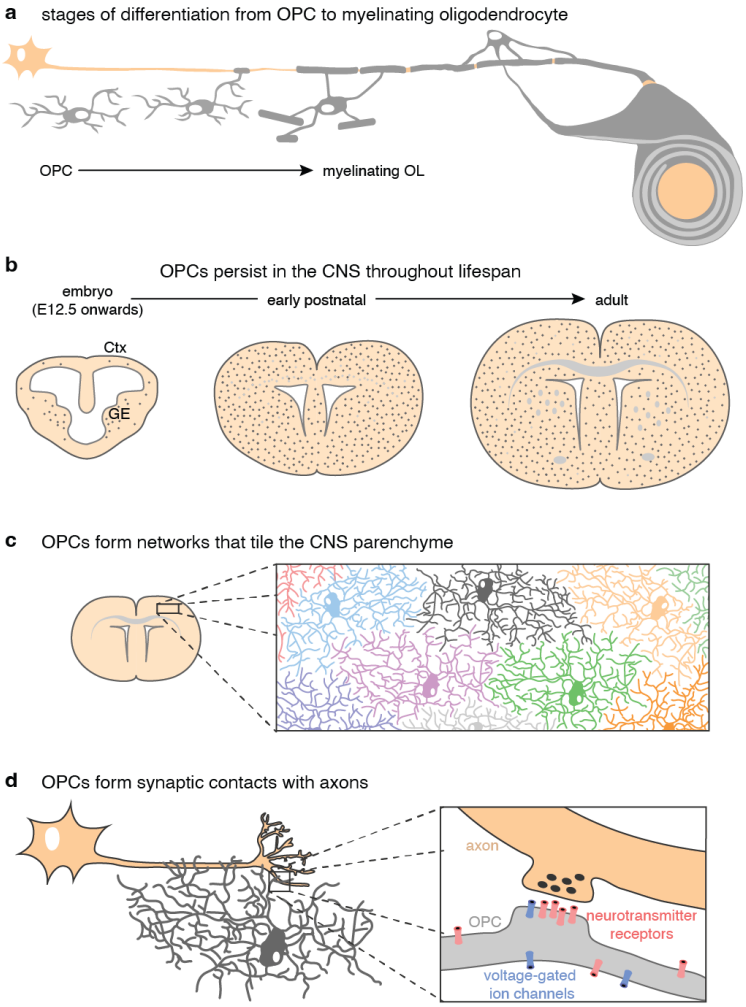
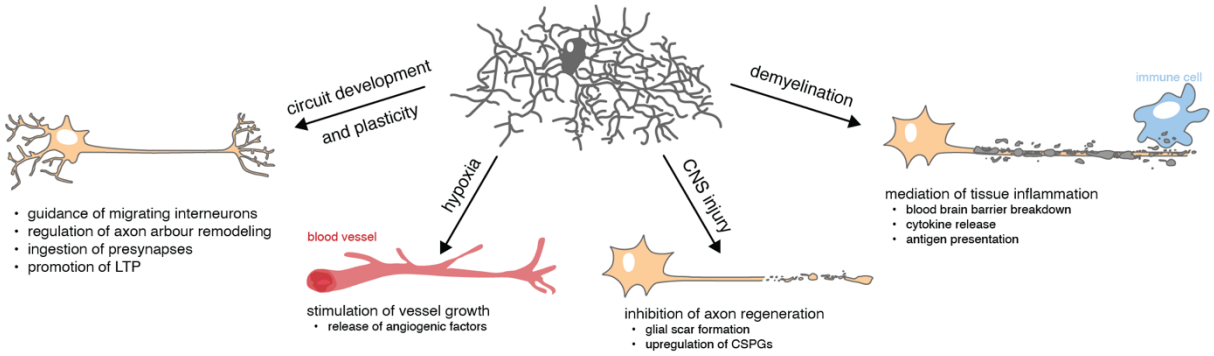


Figure 1: Characteristics of oligodendrocyte precursor cells (OPCs)
a) The canonical role for OPCs is to give rise to myelinating oligodendrocytes in health and disease. OPCs have highly branched processes which they iteratively wrap around axons to form myelin sheaths during their differentiation.
b) OPCs are specified in discrete CNS regions (in the mouse telencephalon starting from the ganglionic eminence (GE) between embryonic day 12.5-15, and from the dorsal cortex (Ctx) from around birth), from where they disperse to evenly distribute throughout the CNS and where they persist into adulthood.
c) With the parenchyma, each OPC forms a highly branched process network, with each cell occupying its own territory. OPCs sense their territory and expand into regions not occupied by an OPC through migration, growth, or proliferation, resulting in a tiling of the CNS.
d) OPCs closely interact with and receive information from axons throughout development and adulthood. They integrate neural activity via neurotransmitter receptors and voltage-gated ion channels and, once settled in the parenchyma, can form synaptic contacts with neuronal pre-synapses.



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576 **Figure 2: Multifunctional OPCs in the healthy and damaged CNS.** Several non-canonical
577 functions have been attributed to OPCs in different contexts as discussed in this
578 Perspective.
579

Box 1: Identity and terminology of oligodendrocyte precursor cells

Tissue-resident oligodendrocyte precursor cells (OPCs) represent about 5% of all CNS cells which tile the tissue with their complex process networks [7]. They can differentiate to myelinating oligodendrocytes throughout life as part of developmental, adaptive, and regenerative myelination, but OPC numbers are maintained in constant homeostasis [4,5,10,22]. Due to the permanent presence of OPCs throughout the CNS it has been speculated for a long time that OPCs are likely more than just a precursor. Hence, over the years OPCs have been given different names to reflect this circumstance, such as synantocytes, polydendrocytes, or simply NG2 glia because all OPCs express the NG2 antigen [77–79]. In fact, at the time of their first discovery in the early 1980s, OPCs were named O-2A progenitors because they can give rise to oligodendrocytes and type 2 astrocytes in culture [15]. Some refer to OPCs as oligodendrocyte progenitor cells because it has been reported that they can give rise to multiple neural lineages [80,81] (even Schwann cells in disease contexts [82]). However, no matter how these cells are referred to, they all are the same cell type that shares a set of key transcription factors and markers, most importantly the two oligodendrocyte lineage determinants Olig2 and Sox10 [83,84]. This gives them the principal ability to give rise to myelinating OLs, even though additional markers may be discovered in the future that distinguish between OPCs that can generate OLs and those that do not, and even though OPCs fulfil a series of additional functions in the healthy and diseased CNS as presented in this article.