Features, fates, and functions of oligodendrocyte precursor cells

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Oligodendrocyte precursor cells (OPCs) are a central nervous system resident population of glia with a distinct molecular identity and an ever-increasing list of functions. OPCs generate oligodendrocytes throughout development and across the lifespan in most regions of the brain and spinal cord. This process involves a complex coordination of molecular checkpoints and biophysical cues from the environment that initiate the differentiation and integration of new oligodendrocytes that synthesize myelin sheaths on axons. Outside of their progenitor role, OPCs have been proposed to play other functions including the modulation of axonal and synaptic development and the participation in bidirectional signaling with neurons and other glia. Here, we review OPC identity and known functions and discuss recent findings implying other roles for these glial cells in brain physiology and pathology.

In this review, we provide a brief overview of oligodendrocyte precursor cell (OPC) development, fate, and touch on many of their known and proposed functions. Since their discovery, OPCs have been referred to by several names. This list includes small branching cells, type 1 oligodendrocytes, O-2A cells, NG2 cells, NG2 glia, polydendrocytes, synantocytes, and a few others. In this review, we will refer to these cells as OPCs to indicate their primary function. To be specific, these are the central nervous system (CNS) resident population of highly branched and tiled glial cells that express genes encoding the NG2 chondroitin sulfate proteoglycan (*Cspg4*) and the alpha receptor for platelet derived growth factor (*Pdgfra*). OPCs can generate oligodendrocytes and may play other roles in the nervous system. Before covering these many features, we first outline the historical context for the discovery, identification, and evolving definition of OPCs.

A brief history of the discovery of OPCs

OPCs were first described at the turn of the 20th century by pioneering anatomists. Remarkably accurate sketches from dog and human brain tissue of cells resembling OPCs were made by the Scottish pathologist William Ford Robertson in 1899. Robertson called the cells "small branching cells" (Robertson 1899). Subsequently, Del Río-Hortega used silver carbonate stain to correctly discriminate between microglia and oligodendrocytes (Río-Hortega 1921) and later classified oligodendrocytes into four types, referring to Robertson's cells as the "first type" (Río-Hortega 1928). This "first type" likely included some OPCs and a subpopulation of oligodendrocytes. Even with such prescient descriptions, these "small branching cells" remained relatively unrecognized and not studied for almost a century.

In the late 1970s to early 1980s, scientists used the A2B5 monoclonal antibody that recognizes a ganglioside to differentially mark two types of GFAP+ astrocytes in cultures taken from rodent optic nerves (Raff et al. 1983a). It was found that A2B5+ GFAP- cells differentiated into GalC+ oligodendrocytes when maintained in chemically defined serum-free medium, while the same cells differentiated into A2B5+ GFAP+ type 2 astrocytes when cultured in the presence of serum (Raff et al. 1983b). This suggested that A2B5+ cells give rise to both astrocytes and oligodendrocytes and were thus called bipotential O-2A (oligodendrocyte-type 2 astrocyte) progenitor cells. Similar studies found that the NG2 chondroitin sulfate proteoglycan, previously identified to label a population of cultured glia, was also expressed by the A2B5+ O-2A progenitor cells and was downregulated as the cells differentiated into GalC+ oligodendrocytes (Stallcup and Beasley 1987). In the presence of serum, NG2 expression persisted on the type 2 astrocytes. These findings indicated that NG2 is an antigen expressed by O-2A progenitor cells, but a long debate followed as to the *in vivo* correlates of these cultured cells.

At the end of the 1980s, it was discovered that platelet-derived growth factor alpha (PDGF-AA) was the predominant mitogen for O-2A progenitor cells, and that its receptor PDGFRA was responsible for mediating the mitogenic effect of PDGF-AA on O-2A progenitor cells (Pringle et al. 1992). These studies also showed the first appearance of *Pdgfra* mRNA+ cells in germinal regions of brain tissue, which was shortly followed by their migration out of the germinal zone and expansion in the parenchyma. *Pdgfra* expression was downregulated in cells that underwent terminal differentiation into oligodendrocytes but persisted on some cells into adulthood. Thus, *Pdgfra* mRNA expression seemed to mark OPCs.

Subsequent studies investigated the co-expression of *Cspg4* and *Pdgfra* revealing that there was an almost complete overlap between PDGFRA+ and NG2+ cell populations (Nishiyama et al. 1996). The notion that NG2+ cells and *Pdgfra* mRNA+ cells were the same cells and likely to be OPCs became accepted in the late 1990s; however, at that time, available techniques did not allow direct demonstration that OPCs could generate oligodendrocytes, as both NG2 and PDGFRA are lost upon their terminal differentiation into oligodendrocytes. Nonetheless, the establishment of these molecular markers and the characterization of these cells in culture and in tissues established them as the fourth major glial cell population in the CNS.

OPC development and fate

During mammalian brain development PDFRA and NG2 positive OPCs are generated from distinct progenitor domains within the ganglionic eminences, ventricular zones, and spinal cord. The neural progenitors populating these regions are characterized by the production of specific transcription factors and give rise to OPCs that migrate throughout the brain and spinal cord (Kessaris et al. 2006; Cai et al. 2005; Rakic and Zecevic 2003; Huang et al. 2020).

Direct evidence for the oligodendrocyte fate of OPCs in mice has come mainly from cre-lox fate mapping showing that NG2 and/or PDGFRA positive cells are indeed OPCs (Kessaris et al. 2006; Zhu et al. 2008; Dimou et al. 2008; Rivers et al. 2008; Kang et al. 2010; Zhu et al. 2011; Nishiyama et al. 2009). In addition to oligodendrocytes, these studies showed that OPCs also generate protoplasmic astrocytes in the gray matter of the ventral forebrain (Zhu et al. 2008). This suggested that some OPCs in prenatal CNS behaved like the culture identified bipotential O-2A cells, however OPCs never generated fibrous astrocytes in white matter, and the astrocyte fate of OPCs is specifically restricted to early developmental stages (Zhu et al. 2011; Huang et al. 2019). Other studies initially suggested that a small number of neurons in the anterior piriform cortex were generated from *Pdgfra*-expressing cells (Rivers et al. 2008), but a follow-up study did not support the original conclusion (Clarke et al. 2012). Neuronal fates were also reported in *Plp1*-creER mice (Guo et al. 2010), however there is reported non-specific activation of the *Plp1* promoter in cells other than OPCs (Michalski et al. 2011). Neuronal fates were not observed in *Cspg4*-creER mice (Zhu et al. 2011), in a different line of *Pdgfra*-creER mice (Kang et al. 2010), or in *Olig2*-creER mice (Dimou et al. 2008). Thus, the current perspective is that, except for some protoplasmic astrocytes generated prenatally, OPCs

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only generate oligodendrocytes, at least during normal development and throughout life (Young et al. 2013; Tripathi et al. 2017; Hill et al. 2018; Hughes et al. 2018).

OPC residency and morphology

OPCs reside in almost all regions of the CNS. They exhibit a tiled distribution with complex multi-branched arborization (**Fig. 1**). This tiling is established early in development and is found even in regions where no oligodendrocytes or myelin sheaths are generated (Lin et al. 2005; Goebbels et al. 2017). The widespread distribution raises several questions related to how and why this patterning is established and maintained and whether all OPCs are indeed progenitors for oligodendrocytes or if they serve other roles in the brain. For example, it is possible that there are different genetic and/or functional classes of OPCs participating in progenitor and non-progenitor roles (Dimou and Simons 2017; Beiter et al. 2022; Marisca et al. 2020).

The tiling of OPCs is regulated through a balance of local proliferation, oligodendrocyte differentiation, and programed cell death (Raff et al. 1993; Trapp et al. 1997). A mix of growth factor signals are critical for the developmental establishment of the resident OPC populations including PDGF-AA, as established initially in cultured OPCs but confirmed in knockout and overexpressing mouse models (Noble et al. 1988; Richardson et al. 1988; Pringle et al. 1992; Calver et al. 1998), fibroblast growth factor (McKinnon et al. 1990; Baron et al. 2000), and neurotrophins (Cohen et al. 1996; Casaccia-Bonnefil et al. 1996) among others (Barres et al. 1992). Cellular sources for these growth factors include neurons, other glia, and signals released from vascular endothelial cells. For example, migrating OPCs use the vasculature as a scaffold to populate the developing nervous system (Tsai et al. 2016; Lepiemme et al. 2022). Contact mediated signaling is also involved in OPC separation after cell division (Huang et al. 2020), highlighting the balance between diffusible environmental cues and OPC cell surface specific signals. When OPCs do not receive permissive signals for differentiation and integration, they initiate programmed cell death pathways associated with cellular autophagy and apoptosis. When these pathways are disrupted the balance between OPC self-renewal, death, and oligodendrocyte differentiation is skewed (Sun et al. 2018; Meireles et al. 2018). Thus, the coincidence of sufficient growth factor availability, permitting tissue substrates, and enhancing developmental migration results in the establishment of lifelong residency by OPCs throughout the CNS.

In the adult brain, it is less clear which signals maintain OPC tiling, but it is likely that a combination of diffusible and membrane tethered cell-cell contact signals regulate the local populations. Imaging studies of OPC process dynamics in zebrafish spinal cord and mouse cortex have found evidence for contact repulsion when neighboring OPC processes touch (Kirby et al. 2006; Hughes et al. 2013). This proposed ability for OPCs to sense their neighbors results in rapid OPC replacement via division and local migration when single OPCs die or differentiate into myelinating oligodendrocytes (Kirby et al. 2006; Hughes et al. 2013; Hill et al. 2017; Hughes et al. 2018). This process likely also accounts for the rapid replacement of OPCs when widespread OPC specific genetic based cell ablation approaches are used (Xing et al. 2023). Moreover, like the developmental role for PDGF-AA, adult OPC population maintenance is dependent on signaling through PDGFRA (Đặng et al. 2019). This means that sustained environmental cues from neurons, other glial cells, and the neurovascular unit contribute to the tiling behavior of OPCs in the adult.

Once OPCs are resident, they exhibit diverse morphologies dependent on the brain region and physiological context. For example, at baseline, gray and white matter OPCs differ in their process arborization, complexity, and size (Osorio et al. 2023). Similar differences are found between OPCs in neuron soma-rich vs axon-rich regions of the developing zebrafish (Marisca et al. 2020). The different morphologies observed in the zebrafish were associated with different fates and cellular activity suggesting a connection between OPC form and function. Genetic underpinnings for this diversity are not clear but an association with cell division history was found as the emergence of a new morphological phenotype occurred almost exclusively after a cell division event instead of a single cell directly changing morphology without first dividing (Marisca et al. 2020). Without definitive genetic markers, connecting OPC morphological and functional heterogeneity has been challenging and whether similar connections between morphology and function are present in developing and adult mammals is not clear. Future intravital imaging approaches allowing longitudinal investigations of OPC shape and function could reveal how closely these features are linked in other settings.

After acute injury, in neurodegenerative contexts, and even some psychiatric conditions, OPCs display morphological transformations often characterized by hypertrophy and increased branching (Ong and Levine 1999; Vanzulli et al. 2020; Yu et al. 2022; Chapman et al. 2023). These shape changes are generally considered to be a reactive phenotype contributing to the glial scar, however a direct role for OPCs in this injury response is not clear. The best studied OPC morphological injury response is in the context of spinal cord injury and neocortical focal injury via mechanical or laser mediated lesions (Hughes et al. 2013; von Streitberg et al. 2021). These studies indicate that OPC processes polarize toward the lesion within hours followed by cell soma migration and cell division (**Fig. 2**). This response is thought to contribute to the barrier that is established with astrocytes and microglia, both by the presence of OPC processes and via the deposition of extracellular matrix. Inhibition of injury induced OPC proliferation leads to deficits in wound closure suggesting that this OPC-specific response is beneficial for injury containment and tissue regeneration (von Streitberg et al. 2021). Overall, OPCs can quickly alter their morphology in response to cellular and tissue damage, hinting at another link between OPC morphology and function.

Intrinsic and adaptive generation of oligodendrocytes

The primary function of OPCs is the generation of oligodendrocytes, a process that can occur in various contexts depending on the demands of the tissue. Many recent articles have extensively covered this topic highlighting that the signals that induce oligodendrocyte generation are multifaceted (Bechler et al. 2018; Xin and Chan 2020; Monje 2018; Chapman and Hill 2020; Almeida and Lyons 2017). These signals range from biophysical cues such as axon caliber to activity-dependent and/or sensory-dependent release of signals from neurons and other cells in the brain (**Fig. 2A**) (Mayoral et al. 2018; Bechler et al. 2015; Gibson et al. 2014; Mitew et al. 2018; Hill et al. 2014; Liu et al. 2012; Makinodan et al. 2012; Hughes et al. 2018). Whether there are distinct programs that are initiated for developmental, intrinsic, and/or activity modulated generation of oligodendrocytes is an active area of investigation in the field.

The generation of new oligodendrocytes has been shown to be important for specific motor, learning, and memory tasks (McKenzie et al. 2014; Xiao et al. 2016; Pan et al. 2020; Steadman et al. 2020; Wang et al. 2020). These experiments have all relied on a genetic trick to block new oligodendrocyte generation via the inducible OPC-specific deletion of transcription factors such as *Myrf* and *Olig2*, which are required for proper differentiation in adult animals. While powerful, additional methods to explore the precise requirement for new myelin from the generation of new oligodendrocytes and the specific neural circuits that they modify will help further our understanding of the necessity for adaptive myelination in these and other learning paradigms. There is extensive literature demonstrating that OPCs are the major source of remyelinating oligodendrocytes in demyelinating and neurodegenerative contexts. The signals inducing OPC differentiation after demyelination are likely a combination of the intrinsic and adaptive programs used in development and the adult coupled with an injury response and reactive OPC phenotypes. Remyelination by OPCs is discussed below.

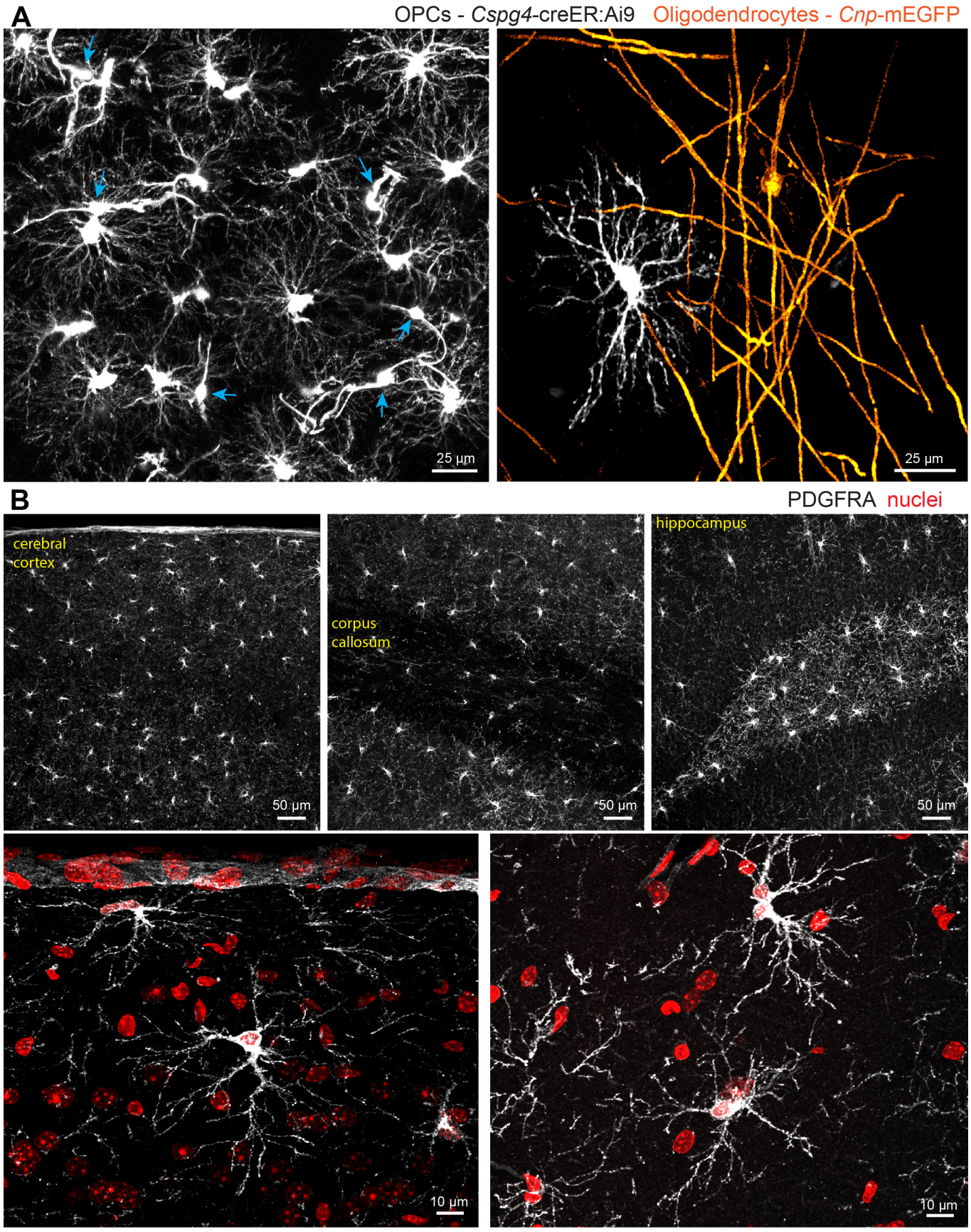

Figure 1 | OPC morphology and residency in the brain. (A) OPCs imaged in the cerebral cortex of a living transgenic mouse with tdTomato fluorescent protein labeling (Ai9 (Madisen et al. 2010)) in all cells with *Cspg4* activity at the time of Cre recombination (Cspg4-creER (Zhu et al. 2011)). Both OPCs and vascular mural cells (blue arrows) are labeled, but OPCs have distinct multibranched and ramified morphologies. This mouse model allows titration of cellular labeling such that single OPCs (shown in gray) can be visualized as in the image on the right. Sparse labeling allows for analysis of OPC morphology in contrast to a myelinating oligodendrocyte (shown in orange) labeled with membrane tethered EGFP (Cnp-mEGFP (Deng et al. 2014)). **(B)** Immunohistochemistry for OPC specific alpha receptor for platelet derived growth factor (PDGFRA) in various brain regions of the mouse forebrain shows widespread tiled distribution and ramified morphology for individual cells across the tissue.

Synaptic input to OPCs

In addition to generating oligodendrocytes that myelinate axons, OPCs themselves have extensive interactions with regions of axons that are unmyelinated. This includes direct neuronal synaptic input allowing OPCs to monitor neuronal activity via neurotransmitter receptor-mediated signaling (Bergles et al. 2000, 2010). All postnatal OPCs are thought to receive this form of synaptic input, which is lost once they differentiate into myelinating oligodendrocytes (Kukley et al. 2010; De Biase et al. 2010). The reason for such specific and specialized signaling is not clear, but the ability to sense patterns of neuronal activity is potentially linked to OPC fate decisions and successful integration during oligodendrocyte generation (**Fig. 2**). While neurotransmitter exposure and pharmacological manipulations can change OPC behavior and fate (Gallo et al. 1996; Pende et al. 1994; Li et al. 2013; Lundgaard et al. 2013; Zonouzi et al. 2015), direct evidence for how physiological neurotransmitter receptor activation is linked to OPC behavior and fate is inconsistent. Thus far, cell type specific *in vivo* manipulations have mainly involved glutamatergic receptor subtypes NMDA and AMPA and GABAergic receptor subtypes.

Deletion of NMDA receptors in OPCs shows no effects on OPC fate (De Biase et al. 2011; Guo et al. 2012), but instead causes altered axonal metabolism followed by delayed myelin degeneration (Saab et al. 2016), suggesting a more prominent role for the NMDA receptor in myelinating oligodendrocytes. Manipulations of AMPA receptors in OPCs have variable outcomes. One study demonstrated that OPC deletion of *Gria2*, *Gria3*, and *Gria4* genes that encode AMPA receptor subunits results in decreased oligodendrocyte survival and integration during differentiation without impacting OPC proliferation (Kougioumtzidou et al. 2017). *Gria2* regulates channel conductance and limits calcium permeability for AMPA receptors, therefore other studies have focused specifically on altering the functionality or expression of *Gria2* to link AMPA mediated changes in intracellular calcium with OPC behavior (Chen et al. 2018; Khawaja et al. 2021). OPC-specific *Gria2* overexpression showed no effects on the generation of oligodendrocytes during development but increased OPC differentiation after injury (Khawaja et al. 2021). This finding is consistent with work showing that non-specific AMPA receptor antagonism increases oligodendrocyte generation after white matter injury (Gautier et al. 2015). Viral mediated overexpression of modified *Gria2* causes changes in OPC proliferation with some minor decreases in oligodendrocyte generation (Chen et al. 2018). Therefore, common outcomes of these manipulations point towards *Gria2* modulating OPC proliferation in adult animals with minimal or inconsistent outcomes on oligodendrocyte differentiation (Kukley 2023). Studies in zebrafish have shown that *gria4a* (an orthlog of *Gria4*) decreases OPC migration and the number of myelin sheaths made by mature oligodendrocytes (Piller et al. 2021), however, it is difficult to make a direct link between this result and the *Gria2* manipulations in the mouse given the differences in the subunits and experimental readouts used. The connection between direct glutamatergic synaptic input signaling through NMDA and/or AMPA receptors and OPC fate outcomes is yet to be resolved. Altogether, how synaptic glutamate release impacts OPC fate requires more detailed investigations using consistent methodologies, molecular manipulations, and animal models.

OPCs also express a variety of genes encoding GABA receptors including ionotropic GABAA and metabotropic GABAB receptors with a variety of subunit compositions (Habermacher et al. 2019). Moreover, OPCs receive direct synaptic input from interneurons in the developing hippocampus and cerebral cortex (Lin and Bergles 2004; Vélez-Fort et al. 2010; Balia et al. 2015). In fact, OPCs and parvalbumin (PV)- positive fast-spiking interneurons arise from similar germinal niches, and this developmental source predicts PV synaptic input to lineage related OPCs (Orduz et al. 2019). Deletion of GABAB receptors in OPCs results in decreased oligodendrocyte differentiation (Fang et al. 2022). However, it is unclear if this is due to the lack of OPCs differentiating or that there are also fewer PV axons to myelinate since, intriguingly, loss of GABABR in OPCs also results in decreased survival of PV-positive neurons. In contrast, conditional OPC GABA γ2 subunit deletion does not cause a change in OPC proliferation or oligodendrocyte generation (Balia et al. 2017). However, γ2 deletion in OPCs does result in a change in myelin patterning and targeting on PV positive axons (Benamer et al. 2020), suggesting a more subtle but important contribution of GABAergic signaling through GABA receptors containing the γ2 subunit in OPCs. Like the story with glutamatergic signaling, additional experiments are needed to more clearly define how synaptic input from GABAergic neurons impacts OPC behavior.

Separate from direct synaptic input from glutamatergic and GABAergic neurons, OPCs also possess many other neurotransmitter receptors including cholinergic, adrenergic, dopaminergic, purinergic, among others (Káradóttir and Attwell 2007; Akay et al. 2021). For example, muscarinic acetylcholine receptors have recently emerged as drivers of OPC fate for remyelination therapy as discussed below (Deshmukh et al. 2013; Mei et al. 2014; Green et al. 2017). Recent experiments have also shown the norepinephrine signaling onto OPCs can regulate local calcium signals and OPC fate in vivo (Fiore et al. 2022; Lu et al. 2022). Given the number of receptor subunits expressed by OPCs, deletion of one or two may not be sufficient to fully block the downstream signaling. A recent zebrafish study, also linking synaptic release to local calcium signals, shows that disruption of major postsynaptic organizers (PSD-95 or Gephyrin) impairs OPC differentiation and oligodendrocyte myelination (Li et al. 2022). As is the case of growth factor signaling in modulating OPC behavior and fate, the same is true for these neurotransmitter signals that have many roles and specific contexts for how and when these neuromodulators impact OPC behavior.

OPCs in axon plasticity, phagocytosis, and immune signaling

OPCs also exhibit roles for modulating axonal growth, plasticity, and regeneration after injury. There is extensive literature suggesting that the OPC response to tissue damage, like spinal cord injury, contributes to the glial scar and an inhibitory environment for regeneration and repair (Levine 2016; Bradbury and Burnside 2019). This idea primarily comes from the increased production of extracellular matrix molecules in damaged tissue, some of which are thought to derive from OPCs (Asher et al. 2002; Garwood et al. 2004). These findings initially suggested that OPCs could be a repelling source for axons, however, as was just discussed, OPCs make extensive synaptic contacts with axons and also have been shown to attract axons in culture and some injury contexts (Yang et al. 2006; Busch et al. 2010; Filous et al. 2014). Thus, OPCs might limit axon regeneration due to their adhesion with growing axons instead of their repulsion via production of NG2 and/or other secreted chondroitin sulfate proteoglycans known to limit axon regeneration (Duncan et al. 2020). Further understanding of the mechanisms impacting synapse formation between OPCs and growing axons and how reactive OPCs vary from homeostatic OPCs in their axon interactions will help resolve this question.

During development, several studies show that OPCs can modulate axon and synapse plasticity. Taking advantage of the zebrafish optic tectum where OPCs are resident without a local myelinating oligodendrocyte population, OPC ablation was found to increase axonal arborization and complexity, resulting in a behavioral change in prey capture (Xiao et al. 2022). Another recent study discovered that OPCs contain a significant amount of axon-derived debris identified via serial electron microscopy, suggesting that these cells are involved in the engulfment / phagocytosis of axons (Buchanan et al. 2022). Similarly, immunohistochemistry in the developing mouse cortex provides some evidence for synaptic debris in OPCs proposing a role for these cells in developmental synaptic pruning (Auguste et al. 2022). Finally, intravital imaging of OPCs adjacent to neuronal cell death events revealed a targeted rearrangement of OPC processes surrounding the dying neuron (Damisah et al. 2020). Altogether, these studies suggest a potential role for OPCs in modulating axonal and synaptic structure and participating in cell debris processing through paracrine or phagocytic mechanisms independent of myelination (**Fig. 2D-E**). Many questions remain for whether these observations are connected and the molecular signaling pathways involved. For example, there was no evidence for OPC

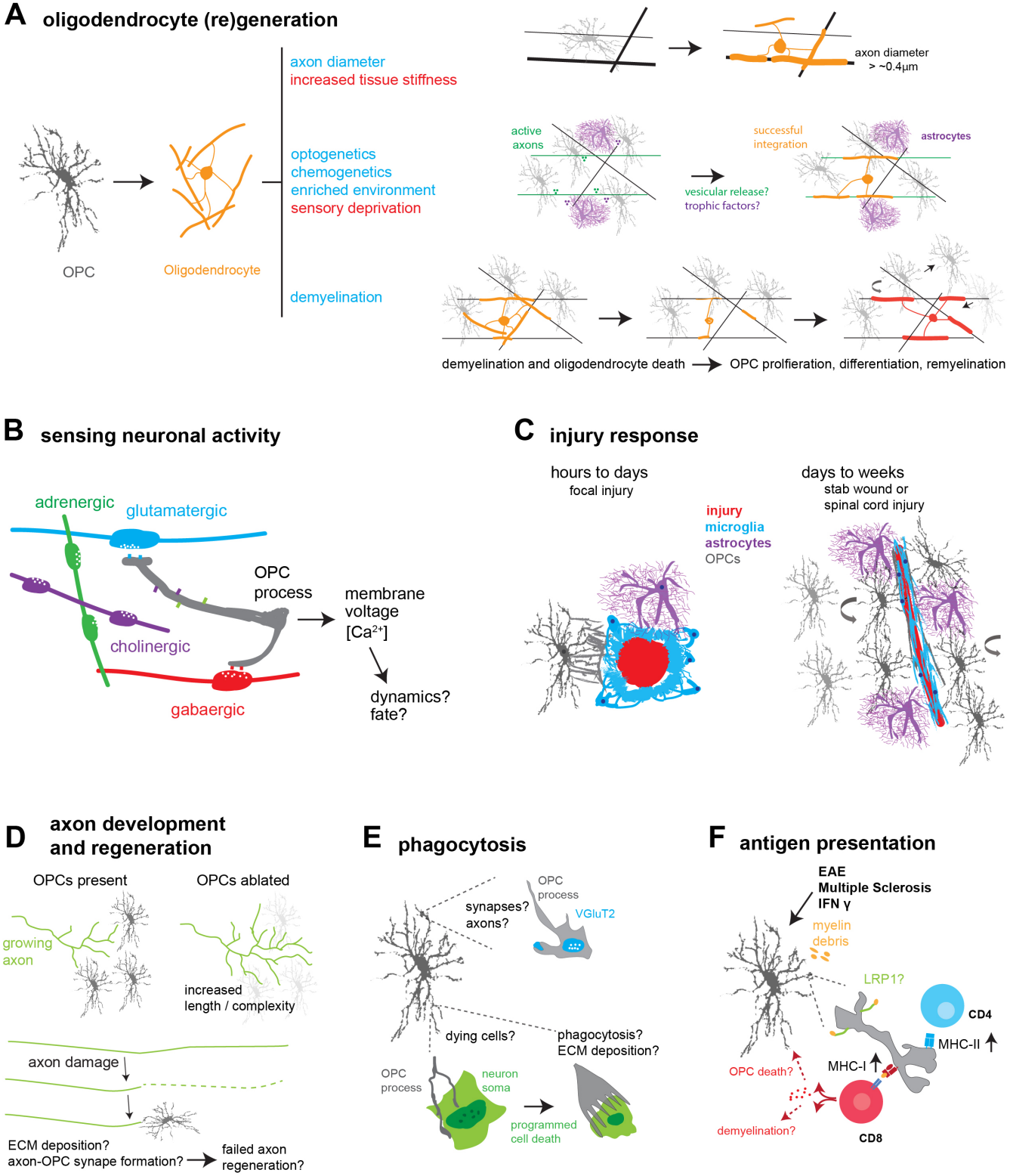

Figure 2 | Established and proposed features and functions of OPCs. (A) The primary function of OPCs is to generate oligodendrocytes across the lifespan. This can be modulated by various environmental factors ranging from biophysical cues to neuron activity-dependent signaling. Factors that enhance oligodendrocyte generation are indicated in cyan and factors that decrease oligodendrocyte generation are shown in red. Similar processes are initiated in response to oligodendrocyte death and demyelination, where OPCs serve as the main source of oligodendrocytes that are generated after myelin loss. **(B)** OPCs have many neurotransmitter receptors and receive direct synaptic input from glutamatergic and GABAergic neurons. These inputs initiate distinct patterns of OPC membrane depolarization and rises in intracellular calcium; however, the primary functional outcome of these inputs is not resolved. **(C)** After injury OPCs respond within hours with process rearrangement and contribution to the glial scar with microglia and astrocytes. Depending on the scale of the damage, OPCs continue to respond via cell migration and proliferation. **(D)** OPCs might play a role in axon growth and regeneration. During development OPC ablation can result in altered axonal arborizations and after injury OPCs might inhibit axonal regeneration either via deposition of extracellular matrix (ECM) or via synaptic connectivity with regenerating axons. **(E)** OPCs exhibit phagocytic-like behavior with some evidence showing synaptic and axonal debris in OPCs and directed OPC process rearrangement when neighboring neurons die. **(F)** OPCs upregulate MHC-encoding transcripts in various diseases and experimental contexts and engage in antigen presentation and activation of CD8 T cells, potentially initiating and/or exacerbating disease.

specific axonal engulfment in the zebrafish study (Xiao et al. 2022) and whether the engulfment of the axonal debris in the electron microscopy study was passive or active could not be determined (Buchanan et al. 2022). There is some evidence that OPCs express genes that encode phagocytic receptors such as *Mertk*, *Ptprj*, and *Lrp1* (Buchanan et al. 2022) however a direct connection between these genes and OPC phagocytosis and/or engulfment of debris is lacking.

Potentially downstream of phagocytosis, other work has discovered that OPCs can participate in immune signaling through specific activation and antigen presentation (Kirby et al. 2019; Falcão et al. 2018; Meijer et al. 2022; Harrington et al. 2023; Fernández-Castañeda et al. 2020). These observations initially came from single-cell RNA sequencing in experimental autoimmune encephalomyelitis and multiple sclerosis (MS) tissues and demonstrated that a subpopulation of oligodendrocyte lineage cells upregulate transcripts associated with antigen presentation including those encoding MHC-I and MHC-II (Falcão et al. 2018; Jäkel et al. 2019). Similar signals were detected in cultured OPCs exposed to Interferon γ, suggesting that this is a cell autonomous response by some OPCs to cytokine exposure (Jäkel et al. 2019; Kirby et al. 2019). The upstream signal leading to the recognition, engulfment, and MHC-I antigen presentation is not well defined in OPCs, but one study demonstrated a role for OPC production of the phagocytic receptor LRP1 in this process (Fernández-Castañeda et al. 2020). A particularly detrimental outcome of this response and increased MHC-I production by OPCs is the activation of CD8 T cells and resulting cytotoxic OPC (and oligodendrocyte) death, potentially depleting the pool available for remyelination and exacerbating autoimmune-mediated demyelination (**Fig. 2F**).

OPCs in neuropathology and aging

In diseases such as MS, myelin-producing oligodendrocytes undergo cell death resulting in demyelination. Following this demyelinating injury, a spontaneous regenerative response can lead to the production of new oligodendrocytes and myelin. Genetic fate-mapping has conclusively shown that these new oligodendrocytes are generated from OPCs both in the surrounding parenchyma as well as from neurogenic zones (Tripathi et al. 2010; Samanta et al. 2015). Remyelination can restore action potential propagation and protect from axonal injury (Smith et al. 1979; Mei et al. 2016). Therefore, several screens for compounds that promote differentiation of OPCs were conducted to identify regenerative approaches to treat demyelinating injuries (Deshmukh et al. 2013; Mei et al. 2014; Najm et al. 2015; Hubler et al. 2018; Early et al. 2018). Some of these candidate drugs had positive results in animal models of demyelination and moved to human clinical trials where they continue to be evaluated for promoting remyelination (Reviewed in (Lubetzki et al. 2020)). Continued validation of electrophysiological and diagnostic imaging biomarkers of remyelination will aid in determining the efficacy of therapies that target restoration of function elicited via OPC differentiation to drive myelin repair (Caverzasi et al. 2023). Patients with MS show varying levels of oligodendrocyte generation (Yeung et al. 2019), suggesting that therapies focused on remyelination via harnessing the regenerative capacity of OPCs may be essential for promoting functional recovery.

While the survival rates of myelinating oligodendrocytes are remarkably high across lifespan (Tripathi et al. 2017), gradual oligodendrocyte death and myelin degeneration are associated with aging (Hill et al. 2018). Furthermore, the capacity of oligodendrocyte and myelin regeneration also declines with age (Psachoulia et al. 2009; Shields et al. 1999; Chapman et al. 2023) mediated in part by age-associated changes in OPCs (Sim et al. 2002; de la Fuente et al. 2020). During development, OPCs start out as a homogeneous population but become functionally heterogeneous across brain regions and aging (Marques et al. 2016; Spitzer et al. 2019). Age-associated transcriptomic and electrophysiological changes of OPCs lead to a decreased potential for differentiation and generation of myelinating oligodendrocytes (Shen et al. 2008; Spitzer et al. 2019). Changes to extrinsic factors such as tissue stiffness in the aged microenvironment may limit the capacity of OPCs to generate new oligodendrocytes (Segel et al. 2019). Recent studies show blood- and CSF-derived factors from young animals promote OPC proliferation and differentiation via youthful monocytes and FGF17 respectively (Ruckh et al. 2012; Iram et al. 2022). In addition, intrinsic factors such as TET1-mediated DNA hydroxymethylation result in an age-dependent decline in myelin repair (Moyon et al. 2021), suggesting multiple factors lead to age-related decline in OPC functions. Recent studies sought to identify therapeutics that increase the capacity for oligodendrogenesis and myelin regeneration in the aged nervous system. The small molecule fasting mimetic, metformin, which modulates the AMPK pathway, restores the differentiation and regeneration capacity of aged OPCs (Neumann et al. 2019). Genetically or pharmacologically enhancing oligodendrogenesis and myelination can reverse age-related memory decline and neurodegeneration (Wang et al. 2020; Chen et al. 2021). Future work continuing to focus on the effects of aging on OPCs and exploration of interventions that facilitate OPC function will help to counteract age-related decline as well as treatment of neurodegenerative diseases.

Concluding remarks

OPCs are well recognized as a separate glial cell population in the brain, and we know a great deal about their role as the precursors for oligodendrocytes. Many molecular signals regulating this process have been discovered and several are now being applied in clinical settings to enhance oligodendrocyte regeneration in neurodegenerative contexts. Even with this extensive and rich literature, many questions remain regarding when, where, and how these molecular signaling cascades result in the initiation, differentiation, and successful integration of a mature myelinating oligodendrocyte in the intact nervous system (Hughes and Stockton 2021). Precise molecular and cellular manipulation coupled with *in vivo* assays will continue to reveal the signals involved in these OPC fate decisions.

Other remaining questions have been highlighted throughout this review. These include heterogeneity within the OPC population along with further investigations into the functions played by OPCs beyond their progenitor role (Dimou and Simons 2017). When considering heterogeneity, it is important to be clear whether the heterogeneity arises from genetic diversity within the OPC population due to developmental source or other intrinsic predetermined genetic programs. There is little evidence for source dependent or genetic heterogeneity within OPCs. The different transcriptome subtypes of oligodendrocytes revealed via RNA sequencing primarily indicates the continuum of differentiation states from OPC to premyelinating oligodendrocytes to fully mature myelinating oligodendrocytes, with more evidence suggesting diversity in the myelinating stage compared to the OPC stage also somewhat complicated by the cell cycle in OPCs (Marques et al. 2016). Even without clear genetic diversity, there is more evidence for OPC functional heterogeneity likely representing plasticity of OPC states in response to cues from the local microenvironment (Kamen et al. 2022). These include differences in OPC properties and functional responses by brain region (Viganò et al. 2013; Hill et al. 2013; Sherafat et al. 2021; Marisca et al. 2020) and across different ages (Shen et al. 2008; Spitzer et al. 2019; Neumann et al. 2019). Contributions from the local microenvironment, whether they be from physiological signaling from neighboring neurons or glia or activation of OPCs in response to pathological conditions, likely drive these heterogenous states. This is not to say that these microenvironmental impacts cannot not have long term consequences for OPC function, even when these cells are placed in a new environment. It just asks the question of whether OPC heterogeneity is genetically predetermined during development or if these different states emerge through environmental influences. Future work is sure to further clarify the duration, functional implications, and reversibility of these OPC functional states.

As we have highlighted throughout, another major remaining question is: what are other functions (beyond their progenitor role) played by OPCs in nervous system physiology and pathology? These range from engaging in bidirectional signaling with

almost every other cell type in the brain and potentially even the peripheral immune system, to responding to damage and disease with distinct behaviors. Overall, recent work has established that OPCs are multifunctional glia that likely contribute significantly towards nervous system development, plasticity, and neurodegeneration on top of their primary role of making myelinating cells.

Acknowledgments
This work was supported by the following funding sources: National Institutes of Health R01 NS122800 and the Esther A. & Joseph Klingenstein Fund and Simons Foundation to R.A.H. National Institutes of Health R01 NS073425 and NS116182 to A.N. National Institutes of Health R01 NS115975 and NS125230 to E.G.H.

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