Trends in Neurosciences

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Review

Spatiotemporal calcium dynamics orchestrate oligodendrocyte development and myelination

Jiaxing Li[1,2,5], Frederic Fiore[3,5], Kelly R. Monk[1,*], and Amit Agarwal[3,4,*]

Oligodendrocyte lineage cells (OLCs), comprising oligodendrocyte precursor cells (OPCs) and oligodendrocytes, are pivotal in sculpting central nervous system (CNS) architecture and function. OPCs mature into oligodendrocytes, which enwrap axons with myelin sheaths that are critical for enhancing neural transmission. Notably, OLCs actively respond to neuronal activity, modulating neural circuit functions. Understanding neuron–OLC interactions is key to unraveling how OLCs contribute to CNS health and pathology. This review highlights insights from zebrafish and mouse models, revealing how synaptic and extrasynaptic pathways converge to shape spatiotemporal calcium (Ca^{2+}) dynamics within OLCs. We explore how Ca^{2+} signal integration across spatial and temporal scales acts as a master regulator of OLC fate determination and myelin plasticity.

Highlights

Oligodendrocyte lineage cells (OLCs) are essential for the formation and maintenance of functional neuronal networks.

Synaptic and extrasynaptic pathways provide inputs to OLCs, which are integrated as Ca^{2+} signals.

Ca^{2+} signals in OLCs exhibit diverse characteristics in amplitude, duration, frequency, location, and size, varying within individual cells and across the lineage based on input nature and strength.

The integration of Ca^{2+} signaling dynamics, referred to as the 'Ca^{2+} code', governs key fate decisions in OLCs, including proliferation, differentiation of oligodendrocyte precursor cells, and myelin sheath formation by mature oligodendrocytes.

OLCs shape circuitry in the central nervous system

OLCs emerge during early developmental stages and populate the entire CNS, persisting throughout an animal's lifespan [1]. These cells exhibit a remarkable diversity of functions, ranging from myelin formation to immune cell modulation [2–4]. The oligodendrocyte lineage encompasses a spectrum of cell states, with at least a dozen distinct cell types identified [5]. Among these, three broad categories have been the focus of extensive research: OPCs, premyelinating oligodendrocytes (pmOLs), and mature oligodendrocytes. OPCs stand out as the sole proliferative and migratory cells within the lineage, capable of forming robust synapses with neurons [1]. Under favorable conditions, OPCs progress through various differentiation states, including pmOLs and oligodendrocytes. Mature oligodendrocytes are responsible for forming and maintaining myelin sheaths on axons over extended periods [6,7]. This myelination process is crucial for modulating axonal conduction velocity, thereby directly influencing signal transduction in neuronal circuits. A particularly intriguing aspect of OLC biology is the presence of unmyelinated axonal segments in several cortical regions of the adult brain [8]. These unmyelinated regions serve as potential sites for neural circuit sculpting and remodeling through a process termed 'adaptive myelination' [9,10]. While the significance of OLCs in shaping neuronal function is evident, the mechanisms governing OLC cell fate progression remain partly understood.

The evolution of OLCs parallels the emergence of myelin in jawed vertebrates approximately 380 million years ago [11,12]. Recent advances in single-cell transcriptomics and high-resolution imaging have propelled our understanding of OLCs across multiple animal models, including zebrafish, mice, rats, and marmosets [1,13,14]. Among these, zebrafish larvae and mice have garnered particular attention due to their amenability to genetic manipulation and the availability of numerous transgenic reporter lines compatible with various *in vivo* optical imaging techniques. Zebrafish larvae, given their transparency, offer an ideal platform for continuous confocal and light-sheet microscopy studies of OLC fate dynamics during early development [15–17]. In contrast, mice allow for the investigation of OLCs through glass windows during adulthood and

[1]Vollum Institute, Oregon Health & Science University, Portland, OR, USA
[2]Department of Neuroscience, Medical University of South Carolina, Charleston, SC, USA
[3]The Chica and Heinz Schaller Research Group, Institute for Anatomy and Cell Biology, Heidelberg University, Heidelberg, Germany
[4]Interdisciplinary Center for Neurosciences, Heidelberg University, Heidelberg, Germany
[5]These authors contributed equally

*Correspondence:
monk@ohsu.edu (K.R. Monk) and
amit.agarwal@uni-heidelberg.de
(A. Agarwal).

in various disease models using multiphoton microscopy [18–20]. These model systems provide unique insights into OLC fate and function across different developmental stages [21], CNS complexities (ranging from about 100 000 neurons in fish larvae to 70 000 000 in mice [22,23]), and pathological states. The use of diverse animal models with distinct experimental advantages is crucial for identifying and analyzing both conserved and species-specific cellular features and functions of OLCs, thereby advancing the current understanding of these enigmatic cells in the nervous system.

OLCs exhibit a complex array of molecular mechanisms that enable them to interact with and respond to neuronal activity. These cells, OPCs in particular, possess a diverse repertoire of ion channels, transporters, and neurotransmitter receptors that allow them to detect and respond to changes in their environment [24–26]. Notably, OPCs form functional synapses with neurons that are structurally analogous to neuron–neuron synapses, with OPCs exclusively occupying the postsynaptic position [27–30]. Unlike in the case of postsynaptic neurons, neurotransmitter release at neuron–OPC synapses does not trigger action potentials in OPCs. Instead, it induces intracellular Ca^{2+} elevations through the activation of Ca^{2+}-permeable ionotropic and metabotropic receptors [29,31–33]. These Ca^{2+} signals are hypothesized to translate neuronal activity patterns into developmental and maturational cues for OPCs [34–37]. Additionally, OPCs and oligodendrocytes express various extrasynaptic ion channels and G-protein-coupled receptors that may influence their fate through intracellular Ca^{2+} signaling [38–44]. Intercellular communication in OLCs extends beyond neuron–OLC interactions. Gap junctions, formed by heterotypic connexin proteins (Cx47–Cx43 or Cx32–Cx30), exist between oligodendrocytes and astrocytes, potentially facilitating the coordination of Ca^{2+} activity between these glial cell types [45–49]. While *in vitro* models have elucidated fundamental Ca^{2+}-dependent regulatory pathways, the spatiotemporal orchestration of Ca^{2+} signals between OLCs and neurons in intact neural circuits remains poorly characterized. In this review, we discuss emerging *in vivo* evidence from zebrafish and mouse models that reveals novel synaptic and extrasynaptic Ca^{2+} signaling modalities governing neuron–OLC interactions during development and adulthood.

OLCs exhibit both local and global Ca^{2+} activity

In *ex vivo* CNS preparations, Ca^{2+} activity in OLCs was reported mostly in either the soma or the entire cell following stimulation of axon bundles or broad pharmacological interventions [50–53]. Recent studies using ultrasensitive genetically encoded Ca^{2+} sensors and high-resolution *in vivo* optical microscopy in zebrafish and mouse preparations illustrate that OLCs actually exhibit Ca^{2+} signals with distinct spatial and temporal characteristics. These studies showed that the majority of Ca^{2+} transients were restricted to small and well-defined areas across the processes of OPCs [54–57] or to individual myelin segments in oligodendrocytes [58,59] (Figure 1). While global Ca^{2+} transients that spread across the entire cell including the soma have been observed in OPCs and oligodendrocytes, such events are rather rare *in vivo* (in both zebrafish and mice) and possibly occur after sustained or global changes in neuronal activity during altered brain states or under pathological conditions [55–58] (Figure 1). Local and global changes in Ca^{2+} activity likely depend on the type of neurotransmitters (synaptic release) and neuromodulators (volumetric release) detected by the cell and the expression levels and distribution (somatic versus processes) of ion channels and receptors at distinct cellular states of OLCs.

OLC Ca^{2+} microdomains

In general, microdomain Ca^{2+} transients are defined as Ca^{2+} signals spatially restricted to small, localized regions of the cellular territory, typically within thin cellular processes, termed 'Ca^{2+} microdomains' (CaMs) [60]. These CaMs arise from Ca^{2+} flux through clusters of channels forming important signaling hubs or 'specializations'. Nevertheless, Ca^{2+} signals in neighboring

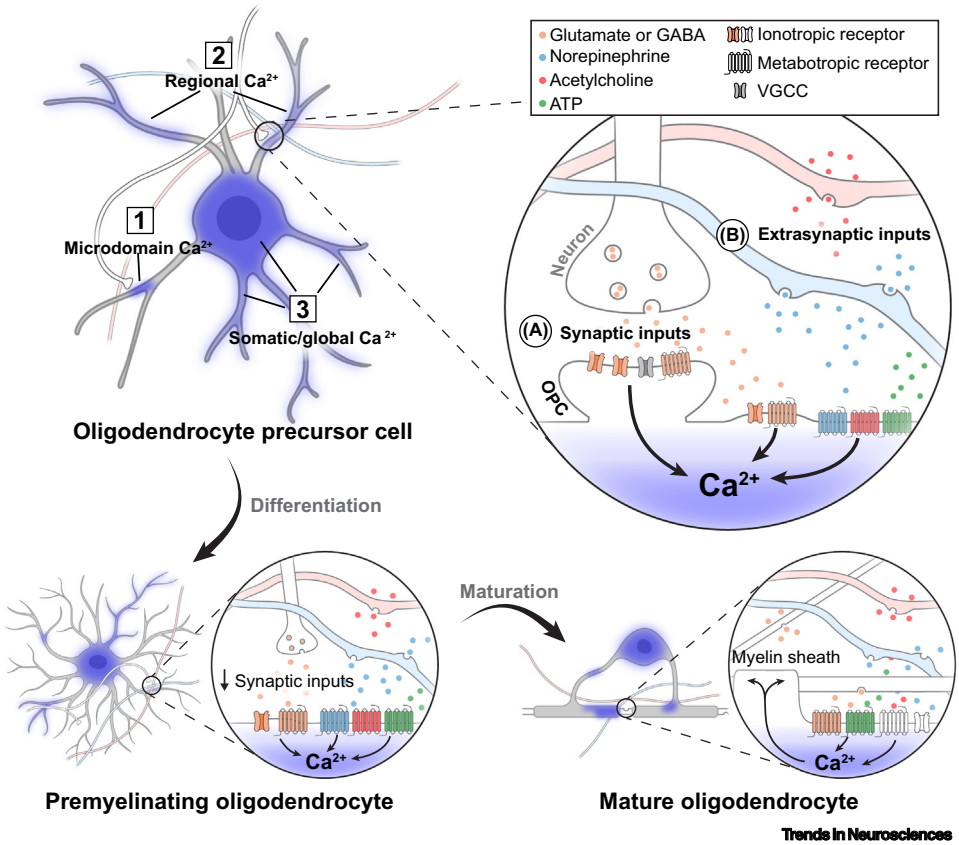

Figure 1. Calcium (Ca^{2+}) signaling shifts modes during fate changes in oligodendrocyte lineage cells (OLCs). Within OLCs, Ca^{2+} events appear at three different levels: (1) microdomain, (2) regional, and (3) somatic/global levels. The majority of Ca^{2+} transients observed *in vivo* take place in microdomains, which are small regions located either at cell processes in oligodendrocyte precursor cells (OPCs) and premyelinating oligodendrocytes (pmOLs) or at myelin sheaths in mature oligodendrocytes (OLs). Occasionally, these microdomain Ca^{2+} transients propagate to fuse into a regional event that can occupy entire branches. In rare occasions, possibly due to intense stimulation, Ca^{2+} events that cover the soma or the entire cell are observed [54–59]. There are two main pathways that induce Ca^{2+} events in OLCs: (A) synaptic and (B) extrasynaptic pathways. These pathways encompass a variety of neurotransmitters (glutamate and GABA) or neuromodulators (norepinephrine, acetylcholine, and ATP), their receptors (ionotropic and metabotropic receptors), and ionic channels (such as voltage-gated Ca^{2+} channels; VGCCs), which collectively shape Ca^{2+} signaling in OLCs [29,39,50,51,55,56,72,74,76,79,87]. Within OLCs, OPCs differentiate into pmOLs and then mature into OLs. During this progression, synaptic pathways attenuate, possibly transitioning to extrasynaptic pathways [5,25,32,38]. The figure was created with Biorender.com.

CaMs can combine to form larger spreading events [55,56,61]. Ca^{2+} originating from both intracellular stores [endoplasmic reticulum (ER) and mitochondria] and the opening of Ca^{2+}-permeable ion channels and receptors on the plasma membrane contribute to microdomain Ca^{2+} transients [61]. Ca^{2+}-binding proteins, Ca^{2+} extrusion pumps, and possibly mitochondrial Ca^{2+} buffering shape the spatial and temporal dynamics of signals in CaMs. Owing to the variable sources and buffering mechanisms, Ca^{2+} events in CaMs emerge in a variety of waveforms with distinct frequencies, amplitudes, and durations. CaM-mediated local Ca^{2+} signaling is well studied in neurons and is known to regulate functions ranging from vesicular exocytosis to synaptic plasticity [62]. Among glial cells, CaMs were initially described in astrocytes [63] and more recently observed in OPCs, pmOLs, oligodendrocytes [54–59], and microglial cells [64]. Recent *in vivo* Ca^{2+} imaging studies suggest that baseline Ca^{2+} events in CaMs occur at varying frequencies across

different glial cell types: more frequently in astrocytes (approximately 0.6 events per minute per CaM in the mouse neocortex [65,66]) than in OPCs (0.5 events per minute per CaM in mouse neocortex [56]) or oligodendrocytes (rarely observed in the mouse neocortex [55,56]). CaMs are often located in close proximity to neuronal processes (axons and dendrites), implying a potential neuronal contribution to the Ca^{2+} activity [57,58,64,67,68]. However, the downstream mechanisms regulating the function of neuronal activity-induced CaMs likely differ between glial cell types. For example, CaMs have been implicated in regulating gliotransmission in astrocytes and process remodeling in microglia [64,69]. With an increasing number of studies now describing CaMs in OLCs [55,56], elucidating the structural basis and stability of these CaMs would contribute to the understanding of their roles in OLC fate decisions [70] and cellular functions, such as myelination and synapse pruning [9].

Neuron–OPC synapses contribute to Ca^{2+} activity in OPCs

Neuron–OPC synapses allow for direct communication between neurons and OPCs. Glutamatergic neuron–OPC synapses are prevalent from development to adulthood [27,71] and have been linked to Ca^{2+} signaling in OPCs [29,50,72] (Figure 1). Through experimentation with brain slice and cell culture electrophysiology, Ca^{2+} activation models were proposed in which AMPA and NMDA glutamate receptors engage the opening of voltage-gated Ca^{2+} channels or in which metabotropic glutamate receptors mobilize intracellular Ca^{2+} stores [50,72–74] (Figure 1). GABAergic neuron–OPC synapses have also been characterized across the CNS [28,38,75]. Although counterintuitive, the neuronal GABA release at these synapses triggers Ca^{2+} elevation by activation of $GABA_A$, and $GABA_B$ receptors thought indirect and direct mechanisms, respectively [76–78]. Adding to these *in vitro* results, recent studies provide *in vivo* evidence supporting the role of synapses in generating Ca^{2+} activity. In mice, an intraperitoneal injection of a mix of ionotropic glutamate (AMPA and NMDA) and $GABA_A$ receptor antagonists led to a reduction of about 40% of the basal Ca^{2+} activity in OPCs in the somatosensory cortex [56]. In zebrafish, blocking synaptic release with tetanus toxin (TeNT), either by neuron-specific expression of TeNT or injection of TeNT into the ventricle of spinal cord, resulted in a 50% reduction of Ca^{2+} transients in spinal cord OPCs [57]. Interestingly, synapses have been observed on OPC processes, but not their soma [72,74], which may explain why Ca^{2+} transients in OPC processes are significantly more frequent than their somatic counterparts [55–57,74]. Overall, the activity of neuron–OPC synapses, regardless of their type, likely contributes to inducing Ca^{2+} signaling in OPCs (Figure 1).

Extrasynaptic pathways modulate OLC Ca^{2+} activity

OPC Ca^{2+} levels can also be induced outside of synapses in response to neuronal activity (Figure 1). For instance, despite the lack of synapse formation in cocultures of OPCs with dorsal root ganglion neurons, OPCs show action potential-induced Ca^{2+} transients in close proximity to axons, albeit with a delayed response onset indicative of nonsynaptic transmission [39]. In addition, specialized structures associated with axon varicosities and vesicles of various sizes were observed at neuron–OPC contact sites [29]. Consistent with this, a significant proportion of postsynaptic scaffold proteins in zebrafish OPCs are located away from presynaptic structures [57], indicating the existence of extrasynaptic communication pathways between neurons and OPCs. Moreover, neuromodulators like norepinephrine and acetylcholine, which engage in volumetric transmission, can also induce Ca^{2+} in mice and cultured human OPCs [55,56,79,80] (Figure 1). In mice, cortical OPCs express all three subtypes of G_q-coupled α1-adrenergic receptors, which were found to mediate arousal-induced CaM activity in OPCs across various brain regions [55,56]. One possible mechanism underlying this Ca^{2+} elevation is the activation of inositol trisphosphate receptors (IP3Rs) on the ER. Two studies in which IP3R type 2 (IP3R2) was selectively disrupted in mouse oligodendrocytes observed altered somatic Ca^{2+} activity *in vitro* and

impaired myelination *in vivo* [81,82]. Taken together, these findings suggest that extrasynaptic pathways, such as the adrenergic receptor–IP3R axis, might regulate OLC development through Ca^{2+} signaling.

Synaptic inputs onto OPCs gradually disappear upon their differentiation [32]; however, oligodendrocytes continue to respond to neuronal activity [26], and CaM activity persists in pmOLs and oligodendrocytes, albeit at a progressively reduced frequency in both zebrafish and mice [55,56,58]. This suggests a transition from synaptic to extrasynaptic communication pathways between neurons and OLCs (Figure 1). Such a transition is also reflected in the changing gene expression profile of cells across the lineage, which downregulate ionotropic, 'synaptic' receptors as they differentiate and mature in zebrafish and mice [5,25,32,54,83]. Consistent with this, a shift from synaptic to extrasynaptic GABA signaling was also observed as OPCs matured [38,84]. In zebrafish and mouse oligodendrocytes, large-amplitude Ca^{2+} elevations are seen in myelin segments [52,58,59], although the sources of such myelinic Ca^{2+} signals are not well understood. Electrical stimulation of *ex vivo* mouse optic nerve explants revealed that elevated extracellular potassium due to neuronal activity contribute to the somatic Ca^{2+} transients in oligodendrocytes, while activation of glutamate and purinergic receptors play a modest role [85]. Conversely, chemogenetic activation of neuronal activity with DREADDs *in vivo* indicates that CaMs in mouse cortical oligodendrocytes are mainly mediated by AMPA receptors and purinergic receptors [86]. Such differences might reflect distinct Ca^{2+} activation pathways used by oligodendrocytes depending on the stimulus and environment. It remains unclear whether these mechanisms mediate Ca^{2+} activity in oligodendrocytes in the absence of external stimulation and how they relate to myelin plasticity and maintenance.

OLC Ca^{2+} activity exhibits a wide range of dynamics

Ca^{2+} signals in OLCs exhibit variable and distinct kinetics. Ca^{2+} events in OLCs differ especially between the soma and microdomains. Whereas somatic Ca^{2+} responses can be generally characterized as slow (e.g., zebrafish spinal cord OPC ~37 s in duration; mouse cortical OPCs ~30 s), CaM activity is comparatively faster (e.g., zebrafish spinal cord OPC ~15 s in duration; mouse cortical OPCs ~1.4 s) [56,58]. Even among CaMs, the kinetics of Ca^{2+} signals in OPC branches are highly diverse (e.g., in mice, OPC CaM duration 330 ms to 12 s [56]) and likely depend on the parameters of the inputs (neurotransmitters and receptors) as well as the intrinsic heterogeneity of OPC properties. For instance, a single depolarizing pulse and a train of consecutive stimulations elicit vastly different Ca^{2+} response patterns in OPCs from mouse brain slices [72]. These distinct Ca^{2+} signals in OPCs might originate from the various neurotransmitters and receptors that are available at a given time and space. For instance, neuromodulators like norepinephrine generate slightly 'delayed' responses relative to the stimulation compared with glutamate [56,87], highlighting the contribution of neurotransmitters to the temporal course of Ca^{2+} events (Figure 2A). Even for a specific neurotransmitter, receptor type can also affect Ca^{2+} kinetics. For instance, compared with AMPA- or NMDA-mediated Ca^{2+} responses, mGluR-mediated Ca^{2+} responses tend to be slower *in vitro* [72,73]. In addition, the presence of other ion channels can also influence the overall kinetics of OPC Ca^{2+} transients *in vitro* [31,50,74] (Figure 2A), illustrating how various synaptic and nonsynaptic components contribute to the wide range of spatial and temporal characteristics of OLC Ca^{2+} responses. These characteristics, including duration and amplitude, are likely tied to the activation of different downstream signaling molecules at either local or global scales. As a result, these Ca^{2+} signals may encode the diverse cellular behaviors and functions of OLCs.

Increasing evidence suggests that OPCs are heterogeneous in gene expression and cellular behavior [88,89]. In mice, for instance, while white and gray matter OPCs do not physiologically

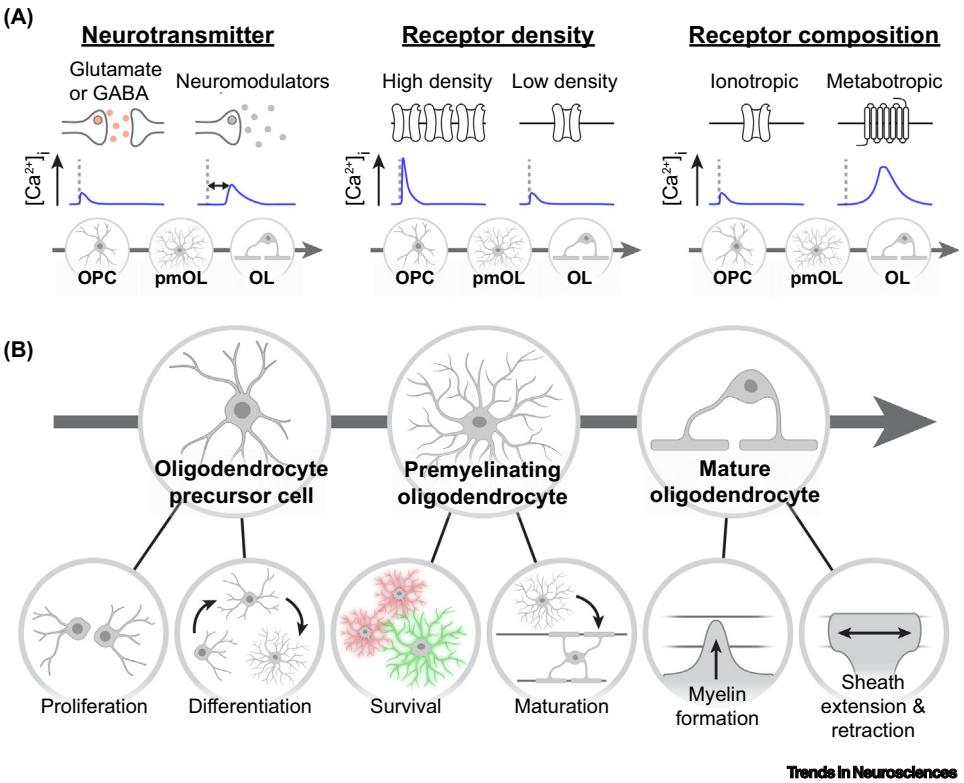

Figure 2. Calcium (Ca^{2+}) signaling exhibits distinct kinetics and roles throughout the oligodendrocyte lineage. (A) Various factors influence the temporal variations of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in oligodendrocyte lineage cells (OLCs). The neurotransmitters inducing the Ca^{2+} transients can affect the timing of the response relative to the trigger. Neuromodulators (which are associated with extrasynaptic pathways) can lead to a delayed Ca^{2+} response in comparison to glutamate or GABA (which are often associated with synaptic pathways) [55,56,87]. Receptor density likely positively impacts the amplitude of Ca^{2+} responses, while receptor composition (e.g., ionotropic versus metabotropic) can shape the kinetics of Ca^{2+} transients in specific cellular compartments [31,50,72–74]. These properties (input modality, receptor density, and receptor composition) vary significantly as oligodendrocyte precursor cells (OPCs) differentiate into an oligodendrocyte (OL), driving the drastic changes in Ca^{2+} transients within the lineage. (B) Ca^{2+} plays multiple roles in OLC behavior and function throughout the lineage. In OPCs, Ca^{2+} regulates proliferation rate [54,56,96–98] and influences their propensity to differentiate into premyelinating oligodendrocytes (pmOLs) [56,81,82]. In pmOLs, Ca^{2+} is possibly involved in cell fate decisions for survival or maturation. In mature oligodendrocytes, Ca^{2+} transients impact myelination, including sheath formation and the extension or retraction of existing sheaths [39,58,59,87,102,103]. The figure was created with Biorender.com.

differ at birth, region-specific properties arise as early as the first postnatal week [90], and *in vivo* imaging suggests that Ca^{2+} varies significantly among individual OPCs [55–57]. Similarly, in zebrafish, two distinct populations of OPCs have been identified based on their location in the spinal cord and their handling of Ca^{2+} [54]. OPCs in soma-rich regions exhibit larger-amplitude Ca^{2+} events and more somatic Ca^{2+} activity than OPCs residing in axon-rich regions. Interestingly, in response to increased neuronal activity, OPCs in neuron-rich regions show enhanced proliferation, while OPCs in axon-rich regions do not [57]. Whether such 'fate-deciding' differences between OPCs also exist in the mouse CNS, however, remains unclear. It is critical to address in the future whether these distinct Ca^{2+} activities are due to the intrinsic properties of OPCs (e.g., types and levels of Ca^{2+} channels) or extrinsic environmental factors (e.g., interactions with different neuronal types). Such investigations will help better understand how Ca^{2+} signaling influences OLC identity, behavior, and function within circuits.

Neurons influence OLC Ca^{2+} activity based on their developmental stage and location

Neurons have a profound impact on OLC Ca^{2+} activity. In an effort to untangle and understand the relationship between the activities of neurons and OLCs, many studies have manipulated neuronal firing through chemical, electrical, or genetic perturbations. In zebrafish larvae, treatment with 4-AP, an agent that depolarizes neurons and enhances neuronal activity, led to an increase in the frequency of Ca^{2+} transients in the processes of OPCs *in vivo* [54]. Similarly, changes in the brain state can activate neurons in the locus coeruleus, elevating Ca^{2+} activity in OPCs *in vivo* [55,56]. In differentiated oligodendrocytes, train stimulation of the isolated mouse optic nerves led to increased somatic Ca^{2+} activity [85], while chemogenetic stimulation using G_q DREADDs led to elevated Ca^{2+} activity in the mouse cortex *in vivo* [86]. Overall, increasing neuronal activity in different parts of the CNS in various animal models consistently elevates Ca^{2+} activity in OLCs.

In contrast, lowering neuronal activity appears to have rather nuanced and context-specific effects on OLC Ca^{2+} signals. For example, in the zebrafish spinal cord, suppression of neuronal activity by tetrodotoxin (TTX; which inhibits neuronal activity by blocking voltage-gated sodium channels) led to a reduced basal frequency of Ca^{2+} activity in early-stage OPCs but not in late-stage OPC *in vivo* [57,58]. Similarly, in developing mouse spinal cord explants, TTX reduced the amplitude and duration of Ca^{2+} events in myelinating oligodendrocytes in the early myelination stage (DIV14), but not in the late myelination stage (DIV21) [91]. These observations likely reflect changes in the sensitivity of OPCs and oligodendrocytes to neuronal activity at different developmental stages. Interestingly, it was also observed in mice that TTX reduced the amplitude and frequency of CaM activity in motor cortex oligodendrocytes *in vivo* [86] but failed to do so in somatosensory cortex oligodendrocytes [52] and OPCs from brain slices [55,56], hinting at possible regional oligodendrocyte heterogeneity in neuronal sensitivity. Alternatively, this discrepancy might reflect differences between *in vivo* (where all connections are intact) and *ex vivo* (where many of those connections have been severed) experimental preparations. Another factor that needs to be taken into account is the basal neuronal activity across the CNS [92]. Indeed, differential levels of neuronal activity could contribute to the observed distinct Ca^{2+} responses in OLCs. Finally, emerging evidence suggests that some Ca^{2+} activity in OPCs is endogenous and independent of any neuronal activity. In cell cultures, spontaneous Ca^{2+} transients occur in OPCs [56] and immature oligodendrocytes [93,94]. These Ca^{2+} events are proposed to be generated by activation of ryanodine receptors [93] or store-operated Ca^{2+} entry [94], possibly requiring coordination between channels on the plasma membrane and the ER. Taken together, these results imply that neuronal effects on Ca^{2+} activity in OLCs, especially regarding baseline activity, depends on the developmental stage and the CNS regions being investigated. Therefore, the results and mechanisms identified across distinct experimental paradigms and model systems should be carefully inferred. Future studies that develop methods to manipulate OLC sensitivity to neuronal activity may help unravel these complex neuron–OLC interactions.

Ca^{2+} impacts OLC proliferation, differentiation, and myelination

Ca^{2+} signals have been closely linked to the development and maturation of OLCs (Figure 2B). A number of studies in recent years have aimed to alter Ca^{2+} signals in OLCs either directly by optogenetic or chemogenetic means or indirectly by modulating neuronal activity. Of note, these studies have produced varied and sometimes contradictory outcomes, emphasizing the context-dependent nature and complexity of Ca^{2+} signaling in OLCs.

Multiple studies suggest a role of Ca^{2+} signals in promoting OPC proliferation. For instance, elevated Ca^{2+} in OPC processes, induced by 4-AP, enhanced OPC proliferation in neuronal soma-rich regions of zebrafish spinal cord, but not in axon-rich areas [54]. Reducing Ca^{2+} in OPCs using

a CalEx pump eliminated 4-AP-induced OPC proliferation *in vivo*, suggesting Ca^{2+} promotes OPC division. Consistent with this, in mice, an OPC-specific deletion of G_q-coupled α1A adrenergic receptors blocked locomotion-induced Ca^{2+} activity in cortical OPCs and reduced their proliferation *in vivo* [55]. Similarly, a high-frequency optogenetic activation of store-operated Ca^{2+} entry in cultured human OPCs enhanced proliferation, suggesting activity-induced Ca^{2+} promotes OPC proliferation [80].

Conversely, other studies indicate a more intricate and nuanced relationship between Ca^{2+} and OPC proliferation. This relationship appears to be influenced by factors such as the duration and intensity of Ca^{2+} signaling, as well as the developmental stage of the organism. During early developmental stages, Ca^{2+} signaling seems to promote OPC proliferation. For instance, the disruption of Cav1.2 (a voltage-gated Ca^{2+} channel) in cultured OPCs isolated from 1-day-old mice resulted in reduced cell proliferation [95]. Similarly, in young mice (2–3 weeks old), increasing Ca^{2+} permeability of AMPA receptors by overexpressing a Ca^{2+}-permeable version of GluA in OPCs *in vivo* led to increased OPC proliferation [96]. In contrast, Ca^{2+} signaling appears to inhibit OPC proliferation in adult mice. For example, reducing Ca^{2+} permeability of AMPA receptors by overexpressing a Ca^{2+}-impermeable version of GluA in OPCs led to increased proliferation *in vivo* [97]. A conditional knockout of Cav1.2 in OPCs resulted in increased OPC proliferation *in vivo* as well [98]. A recent study in adult mice reported that a prolonged Ca^{2+} increases through a direct chemogenetic activation of cortical OPCs using G_q DREADDs suppresses their proliferation [56]. These findings collectively suggest that the impact of activity-induced Ca^{2+} on OPC proliferation is highly context dependent. The relationship between Ca^{2+} signaling and OPC proliferation appears to shift from promoting proliferation during early development to inhibiting it in adulthood. Furthermore, the duration and intensity of Ca^{2+} signaling may play crucial roles in determining its effects on OPC proliferation.

Similarly, studies on the role of Ca^{2+} signaling in OPC differentiation have yielded diverging results, suggesting a complex relationship between the two. In mice, several studies indicate that Ca^{2+} signaling promotes OPC differentiation. For instance, direct chemogenetic activation of Ca^{2+} activity in OPCs or indirect activation through norepinephrine-releasing neurons drives OPC differentiation into oligodendrocytes [56]. In addition, disruption of IP_3R2 in OLCs leads to reduced Ca^{2+} activity and delayed OPC differentiation [81,82]. Conversely, there are also studies suggesting that Ca^{2+} signaling inhibits OPC differentiation. For example, in mice, overexpression of Ca^{2+}-permeable GluA in OPCs reduced their differentiation [96]. Similarly, in cultured human OPCs, optogenetic activation of Ca^{2+} signaling decreased OPC differentiation [80]. To further complicate the matter, OPC-specific disruption of either Cav1.2 channels [98] or $GABA_B$ receptors [99] in mice impaired their Ca^{2+} signaling but had no significant effect on their differentiation.

The varying results discussed above clearly indicate that the relationship between Ca^{2+} signaling and OPC differentiation is not binary but rather influenced by numerous factors. First, the density of receptors (ionotropic or metabotropic) and voltage-gated Ca^{2+} channels on the soma and processes change over time, as cells progress through the lineage or as a broader consequence of the transition between development and adulthood, which can result in distinct levels of Ca^{2+} signaling [24]. Second, the mechanism by which Ca^{2+} is induced in OPCs may determine its impact on differentiation. For instance, ER-mediated prolonged Ca^{2+} activity may play a more significant role than a subtle (and short-duration) ionotropic receptor or voltage-gated Ca^{2+} channel-mediated Ca^{2+} signals in OPC differentiation. Third, OPC differentiation involves a transition between multiple stages (OPC–pmOLs–immature and mature oligodendrocytes) [100,101], and the interplay between Ca^{2+} and the various signaling molecules governing each step remains incompletely understood. Last, and possibly most important, OPC differentiation and proliferation

are interconnected processes, that is, OPC differentiation appears to stimulate proliferation in neighboring OPCs [18]. Consequently, observed effects of Ca^{2+} on one event may be secondary to its influence on the other. Owing to such concurrent changes in proliferation and differentiation, many studies have noted simultaneous changes in both proliferation and differentiation following Ca^{2+} manipulations [56,80,96,98]. For example, direct chemogenetic activation of Ca^{2+} signals in OPCs using G_q DREADDs promoted their differentiation into oligodendrocyte [56]. However, OPCs not expressing G_q DREADDs, whose Ca^{2+} activity was not directly manipulated, transitioned into a higher-proliferation state to replace differentiating OPCs. These findings highlight the importance of investigating proliferation and differentiation events concurrently to accurately assess the responsiveness of OLCs to Ca^{2+} signaling and its role in driving fate transitions. The existence of such complex relationships between Ca^{2+} signaling and OPC differentiation compels further research to elucidate the precise mechanisms and factors involved in this process (see Outstanding questions).

Even after OPCs differentiate into oligodendrocytes, Ca^{2+} activity in oligodendrocytes continues to modulate their further maturation and promote myelination (Figure 2B). Vesicular glutamate release, for instance, has been shown to induce local translation of myelin proteins such as myelin basic protein in cultured mouse myelinating oligodendrocytes [39,87]. In these cultured mouse oligodendrocytes, Ca^{2+} signaling facilitates the expansion of the myelin sheath [94]. Similarly, studies in acute mouse brain slices reveal that Ca^{2+} transients stabilize myelin internodes and mediate their compaction during early remodeling of internodes [52]. *In vivo* imaging studies in zebrafish larvae demonstrate that high-frequency Ca^{2+} transients in nascent myelin sheaths precede elongation, whereas high-amplitude, long-duration Ca^{2+} transients drive sheath shortening [58,59]. Supporting this, axonal vesicular release at heminodes in zebrafish promotes myelin sheath growth, which in turn enhances vesicle release, establishing a feed-forward loop [102]. Furthermore, a recent *in vivo* mouse study using the genetically encoded Ca^{2+} pump CalEx to reduce intracellular Ca^{2+} levels demonstrated that impaired Ca^{2+} signaling results in abnormal growth of myelin sheaths, including shorter internodes and the formation of sheath outfoldings [103]. Collectively, these findings emphasize the critical role of spatiotemporal pattern of intracellular Ca^{2+} signaling in oligodendrocytes for proper myelin formation and maintenance (Figure 2B).

Despite these advances, a major limitation persists: most studies target global rather than local Ca^{2+} activity. As previously discussed, Ca^{2+} activity in OLCs predominantly occurs in subcellular regions such as CaMs, while somatic and global Ca^{2+} activity is rare. Manipulating global Ca^{2+} levels may disrupt multiple signaling pathways and does not necessarily reflect physiological conditions. Therefore, while recent findings have illuminated the roles of Ca^{2+} signaling *in vivo*, a careful interpretation of such results is crucial. Future research should focus on developing tools to manipulate localized CaM activity with greater spatiotemporal precision to unravel the precise role of Ca^{2+} signaling in controlling OLC fate and myelination.

Concluding remarks

The intricate interplay between global and local Ca^{2+} signals in OLC development highlights the complexity of signaling mechanisms regulated with high temporal and spatial precision. These Ca^{2+} signals, generated through synaptic or extrasynaptic pathways, serve as an essential communication tool between neurons and OLCs. Future research directions should focus on elucidating the mechanisms of CaM formation, their consequences, and their interaction with neuronal activity in potentially heterogeneous OLC populations (see Outstanding questions).

To address these knowledge gaps, *in vivo* imaging techniques in zebrafish and mice emerge as vital tools, given that *in vitro* preparations often lead to altered Ca^{2+} activity and aberrant glial

Outstanding questions

What is the structural basis underlying OLC microdomain activity? Are Ca^{2+} events physically confined to these microdomains? Which factors determine the boundaries of these microdomains?

How do OLCs decode distinct Ca^{2+} patterns at both local and global levels? What molecular mechanisms act downstream of Ca^{2+} activity to influence OPC fates and behaviors?

Are mechanical cues integrated into OLC Ca^{2+} signaling through mechanosensitive channels? What role, if any, do these channels play in OLC function and myelination?

Do connexin and pannexin channels mediate intercellular Ca^{2+} activity in OLCs? What is the role of connexin- and pannexin-based channels in establishing basal intracellular Ca^{2+} levels in OLCs?

How does pathogenesis of demyelinating and neurodegenerative diseases, such as multiple sclerosis, affect OLC microdomain Ca^{2+} activity? Do OLC microdomains reorganize during demyelination and remyelination?

responses. The integration of intravital imaging with behavioral monitoring promises to enhance our understanding of OLC Ca^{2+} roles at both cellular and circuit levels. However, a significant challenge lies in the current lack of tools for manipulating intracellular Ca^{2+} with sufficient spatial and temporal precision, which, when developed and combined with *in vivo* imaging, could yield groundbreaking insights. Furthermore, future investigations should expand beyond individual cell Ca^{2+} transients to characterize Ca^{2+} dynamics at the population level and across brain regions, as well as elucidate interactions among OLCs. Given the considerable variability in Ca^{2+} transients among OLCs, research addressing OLC heterogeneity and enabling subtype labeling will be crucial for more accurate Ca^{2+} analysis and comparisons.

While this review primarily focused on neuron–OLC interactions, it is important to acknowledge the potential modulatory roles of other glial cells, such as astrocytes, microglia, and pericytes, on OLC fate *in vivo*. The development of tools for colabeling OLC subtypes with other glial cell types, particularly astrocytes and microglia, will facilitate a more comprehensive understanding of neuron–glia and glia–glia interactions involved in OLC function. These advancements will collectively contribute to a more nuanced and holistic understanding of OLC biology and its implications in neural function and development.

Acknowledgments

This work was supported by grants from the Chica and Heinz Schaller foundation (F.F. and A.A.).

Declaration of interests

The authors report no competing interests

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