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Calcium Signaling in the Oligodendrocyte Lineage: Regulators and Consequences

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Abstract

Cells of the oligodendrocyte lineage express a wide range of Ca^{2+} channels and receptors that regulate oligodendrocyte progenitor cell (OPC) and oligodendrocyte formation and function. Here we define those key channels and receptors that regulate Ca^{2+} signaling and OPC development and myelination. We then discuss how the regulation of intracellular Ca^{2+} in turn affects OPC and oligodendrocyte biology in the healthy nervous system and under pathological conditions. Activation of Ca^{2+} channels and receptors in OPCs and oligodendrocytes by neurotransmitters converges on regulating intracellular Ca^{2+}, making Ca^{2+} signaling a central candidate mediator of activity-driven myelination. Indeed, recent evidence indicates that localized changes in Ca^{2+} in oligodendrocytes can regulate the formation and remodeling of myelin sheaths and perhaps additional functions of oligodendrocytes and OPCs. Thus, decoding how OPCs and myelinating oligodendrocytes integrate and process Ca^{2+} signals will be important to fully understand central nervous system formation, health, and function.

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INTRODUCTION

Oligodendrocytes are the myelin-producing glial cells of the central nervous system (CNS). Oligodendrocytes differentiate from oligodendrocyte progenitor cells (OPCs), which themselves constitute another major cell type of the CNS that represents 3–10% of the cells of the adult brain and spinal cord (Stadelmann et al. 2019). The role of oligodendrocytes in producing the myelin sheaths that facilitate rapid action potential propagation along axons has long been known, but more recently additional functions have been identified (Stadelmann et al. 2019). Rather than acting as a static insulator, it is now thought that myelin is adaptable and that the number, distribution, length, and thickness of myelin sheaths along axons can be dynamically regulated, including in response to neuronal activity (Almeida & Lyons 2017, Chang et al. 2016). Indeed, activity-driven adaptation to myelin may represent a form of nervous system plasticity because myelin alterations have the capacity to fine-tune conduction and thus refine neural circuit function (Fields 2015, Monje 2018). A role for myelinating oligodendrocytes in providing metabolic support to underlying axons has recently been identified, which may also be tuned to support axons based on their firing properties (Saab & Nave 2017). The dynamic interactions of axons

and myelinating oligodendrocytes are further evidenced by observations that ion homeostasis is regulated by channels localized in the myelin sheath at the axon-myelin interface (Suminaite et al. 2019). Reflecting its status as a ubiquitous signaling molecule and second messenger, regulation of the intracellular concentration of Ca^{2+}, $[Ca^{2+}]_i$, in oligodendrocytes, including locally within myelin sheaths, can directly affect the formation and remodeling of myelin and likely also additional functions that are now being uncovered.

Prior to the formation of myelin sheaths, oligodendrocytes differentiate from OPCs, which colonize the white and gray matter of the CNS through proliferation and migration from germinal zones throughout the developing CNS neuraxis (Bergles & Richardson 2015). Upon completion of migration, OPCs locally interact with one another to take up largely nonoverlapping tiled positions throughout the parenchyma (Hughes et al. 2013, Kirby et al. 2006), where they remain the most proliferative cell of the CNS throughout life, with a lifelong ability to generate oligodendrocytes in both health and disease (Young et al. 2013, Zawadzka et al. 2010). In addition, it is now emerging that OPCs play roles beyond that implied by their name. This is evidenced by their ability to form synapses with axons (Almeida & Lyons 2014), to regulate synaptic function (Almeida & Lyons 2017), and to even serve as antigen-presenting cells in disease (Falcão et al. 2018, Kirby et al. 2019). Although we are only beginning to scratch the surface of their functional repertoire, it is clear, as per myelinating oligodendrocytes, that regulation of $[Ca^{2+}]_i$ affects many aspects of the development of OPCs, including their generation of oligodendrocytes and execution of diverse functions.

Here we review our current understanding of how $[Ca^{2+}]_i$ is regulated in OPCs and oligodendrocytes and how the regulation of $[Ca^{2+}]_i$ in turn affects key stages of OPC and oligodendrocyte formation and function. We divide the review into two principal sections: In the first, we define the key molecular regulators of $[Ca^{2+}]_i$ in OPCs and oligodendrocytes, noting, where data are available, how such factors affect OPCs and oligodendrocytes. In the second part, we discuss how the regulation of $[Ca^{2+}]_i$ in turn affects OPC and oligodendrocyte biology in the healthy nervous system and how dysregulation of $[Ca^{2+}]_i$ can disrupt tissue health and function. We finish by briefly outlining key unanswered questions in the field and approaches that are being taken to address them.

WHAT REGULATES INTRACELLULAR Ca^{2+} CONCENTRATION IN THE OLIGODENDROCYTE LINEAGE?

The divalent cation Ca^{2+} is a ubiquitous signaling molecule/second messenger, whose regulation affects innumerable enzymatic and physiological functions in numerous cell types. In the nervous system, the roles that Ca^{2+} plays in neurons range from broad regulation of gene expression to fine spatial and temporal control of synaptic vesicle release. The channels, transporters, receptors, and exchangers that regulate $[Ca^{2+}]_i$ have been well characterized in neurons, many of which are now known to regulate Ca^{2+} in glia (Allen & Lyons 2018, Rusakov 2015). To date, in the context of the oligodendrocyte lineage, our knowledge primarily pertains to the identification of candidate regulators of Ca^{2+} in OPCs, particularly in response to synaptic input from axons. Synaptic connections between neurons and OPCs have been found in both gray matter and white matter (Bergles et al. 2000, Gallo et al. 2008, Kukley et al. 2007, Mangin & Gallo 2011, Ziskin et al. 2007), with OPCs receiving glutamatergic and GABAergic synaptic input (Bergles & Richardson 2015, Lin & Bergles 2003, Zonouzi et al. 2015). Importantly, in the context of this piece, it has also been shown that glutamate and GABA receptor activation can increase $[Ca^{2+}]_i$ (Karadottir & Attwell 2007, Tanaka et al. 2009). OPCs have even been shown capable of performing linear integration of glutamatergic synaptic inputs manifest as either global or localized Ca^{2+} signaling events in

distal processes (Sun et al. 2016). Transcriptomic data from isolated OPCs demonstrate that these cells express many factors that function in synaptic communication, with a particular enrichment of genes implicated in postsynaptic organization and signaling (Zhang et al. 2014). Interestingly, the expression and function of such synaptic proteins and associated ion channels become generally downregulated in mature myelinating oligodendrocytes (De Biase et al. 2010, Paez et al. 2010, Zhang et al. 2014). Nonetheless, it is now also clear that axon-oligodendrocyte communication continues through myelination (Micu et al. 2018) and that distinct aspects of myelination are regulated by localized Ca^{2+} activity (Miller 2018). We first provide an overview of the key factors in OPCs and oligodendrocytes that regulate Ca^{2+}, with a focus on those influenced by axon-oligodendrocyte lineage communication.

Voltage-Gated Ca^{2+} Channels

The influx of Ca^{2+} through membrane channels is a crucial step in signal transduction pathways involved in regulating growth, maturation, and functional plasticity. Whole-cell recordings from OPCs in conditions appropriate for isolating Ca^{2+} currents revealed the presence of L-type and T-type voltage-gated Ca^{2+} channels (VGCCs) (Fulton et al. 2010, Haberlandt et al. 2011). Further evidence for expression of different isoforms has been obtained through polymerase chain reaction of messenger RNA extracted from single cells in tissue slices and by RNAseq from isolated cortical OPCs, indicating that transcripts for L-type (Cav1.2, 1.3) and T-type (Cav3.1, 3.2) VGCCs are most abundant (Haberlandt et al. 2011, Zhang et al. 2014). Moreover, in vitro and in situ Ca^{2+} imaging experiments have shown that Cav1.2 and Cav1.3 channels are active in both gray matter and white matter OPCs during early postnatal development (Cheli et al. 2015, Paez et al. 2010) and that these channels mediate voltage-gated Ca^{2+} signals in OPCs in response to synaptic activity (Sun et al. 2016).

Voltage-dependent Ca^{2+} entry affects multiple aspects of OPC development. In vitro analyses of isolated OPCs indicate that they undergo spontaneous Ca^{2+} transients while migrating and that these events are inhibited by pharmacological blockade of Cav1.2 function (Paez et al. 2009b). Experimental elevation of Ca^{2+} in OPCs through Cav1.2 channels triggers process retraction, suggesting that these channels are involved in regulating the directed movement of OPCs during early development (Paez et al. 2007, 2009b). The activity of Cav1.2 in OPCs is directly modified by phosphorylation: For example, activation of PKC and tyrosine kinase receptors, such as the PDGF receptor α, enhances Cav1.2 Ca^{2+} influx in OPCs, whereas PKA activity has an inhibitory effect (Paez et al. 2010). These kinase-mediated effects on Cav1.2 activity in turn affect the dynamics of process extension and migration in OPCs (Paez et al. 2010).

A role for Cav1.2 channels in later stages of oligodendrocyte maturation and myelination has also been demonstrated (Cheli et al. 2015, 2016). Cav1.2-deficient OPCs display a simple morphology and low levels of myelin protein expression and are less capable of establishing contacts with axons of cocultured cortical neurons (Cheli et al. 2015). In vivo, significant hypomyelination was found in the brains of conditional knockout mice in which Cav1.2 channels were deleted in OPCs during the first postnatal week (Cheli et al. 2016). The reduced myelination in these animals was accompanied by a decline in the rate of OPC migration and proliferation (Cheli et al. 2016). Furthermore, the conditional deletion of Cav1.2 from adult OPCs does not affect the generation of new oligodendrocytes and/or the synthesis of myelin but significantly disturbs the survival of callosal OPCs and their proliferation rate (Pitman et al. 2020). In contrast, OPCs expressing a gain-of-function mutation in the L-type Ca^{2+} channel Cav1.2 showed greater L-type Ca^{2+} influx and displayed characteristics suggestive of advanced maturation, including a more complex morphology and higher levels of myelin protein expression in culture and

in vivo (Cheli et al. 2018). Consistent with this, expression of Cav1.2 channels bearing this gain-of-function mutation triggered process formation and promoted oligodendrocyte-neuron interaction through the activation of Ca^{2+}/calmodulin-dependent protein kinase II (Cheli et al. 2018).

Numerous studies have indicated that the mechanisms that drive normal oligodendrocyte lineage progression through myelination are often essential for the regeneration of myelin (remyelination) following its loss or damage in disease or injury (Franklin & ffrench-Constant 2017). In line with this, a significant upregulation of Cav1.2 Ca^{2+} channels in OPCs was found in the demyelinated corpus callosum (Paez et al. 2012). Several weeks after cuprizone-induced demyelination, proliferating OPCs in demyelinated areas were found to express high levels of Cav1.2 channels, suggesting that L-type Ca^{2+} entry might play a fundamental role in driving differentiation and/or regulating survival of newly generated oligodendrocytes after demyelination (Paez et al. 2012). Indeed, myelin regeneration was significantly delayed when the Cav1.2 channel was ablated in adult NG2-positive OPCs after cuprizone treatment (Santiago González et al. 2017). This inefficient remyelination was seen in different areas of the mouse brain and was accompanied by a significant reduction in the number of myelinating oligodendrocytes (Santiago González et al. 2017). Altogether, these data indicate that VGCCs, particularly L-type Cav1.2 channels, are essential for OPC and oligodendrocyte maturation during development as well as during remyelination (**Figure 1**).

AMPA and NMDA Receptors

Neuronal activity can regulate OPC proliferation, the formation of oligodendrocytes, myelination, and remyelination. The molecular mechanisms underpinning these effects remain to be defined, but neurotransmitter-mediated axon-OPC/oligodendrocyte communication represents the major class of candidate mediators. It is now clear that OPCs receive abundant glutamatergic and GABAergic input, with most glutamatergic current in proliferating OPCs being mediated through AMPA receptors (AMPARs) and Kainate receptors (Bergles & Richardson 2015, Lin & Bergles 2003, Spitzer et al. 2019). OPCs express both Ca^{2+}-permeable and -impermeable AMPARs, meaning that glutamate can increase OPC $[Ca^{2+}]_i$ directly, or indirectly (**Figure 1**), because AMPAR-mediated Na^+ influx might depolarize the OPC plasma membrane and activate VGCCs. It has been proposed that AMPAR stimulation promotes OPC migration (Gudz 2006) and that AMPAR activation stimulates OPC motility by increasing the cell surface localization of a Ca^{2+}-permeable AMPAR subunit that increases the amplitude and frequency of Ca^{2+} transients in migrating OPCs (Harlow et al. 2015). The role of AMPARs in regulating the transition of OPCs to oligodendrocytes has been extensively studied in vitro and, more recently, in vivo. In one set of experiments, AMPARs with distinct Ca^{2+} permeability properties were overexpressed in oligodendrocytes, and it was found that those with increased Ca^{2+} permeability increased OPC proliferation but reduced differentiation (Chen et al. 2018). However, in triple-knockout mice with effectively no AMPAR-mediated currents in OPCs or oligodendrocytes, it was principally the survival of newly differentiating oligodendrocytes that was affected. In these animals, an increase in the death of newly myelinating oligodendrocytes reduced both oligodendrocyte number and myelination (Kougioumtzidou et al. 2017). Whether the survival of oligodendrocytes downstream of AMPAR signaling is also mediated through changes in $[Ca^{2+}]_i$ remains to be determined. Interestingly, in other contexts, Ca^{2+} is required for the activation of the transcription factor Tfeb (Medina et al. 2015), which was recently found necessary to mediate the programmed cell death of oligodendrocytes during normal CNS development (Sun et al. 2018). In another example of developmental mechanisms being redeployed

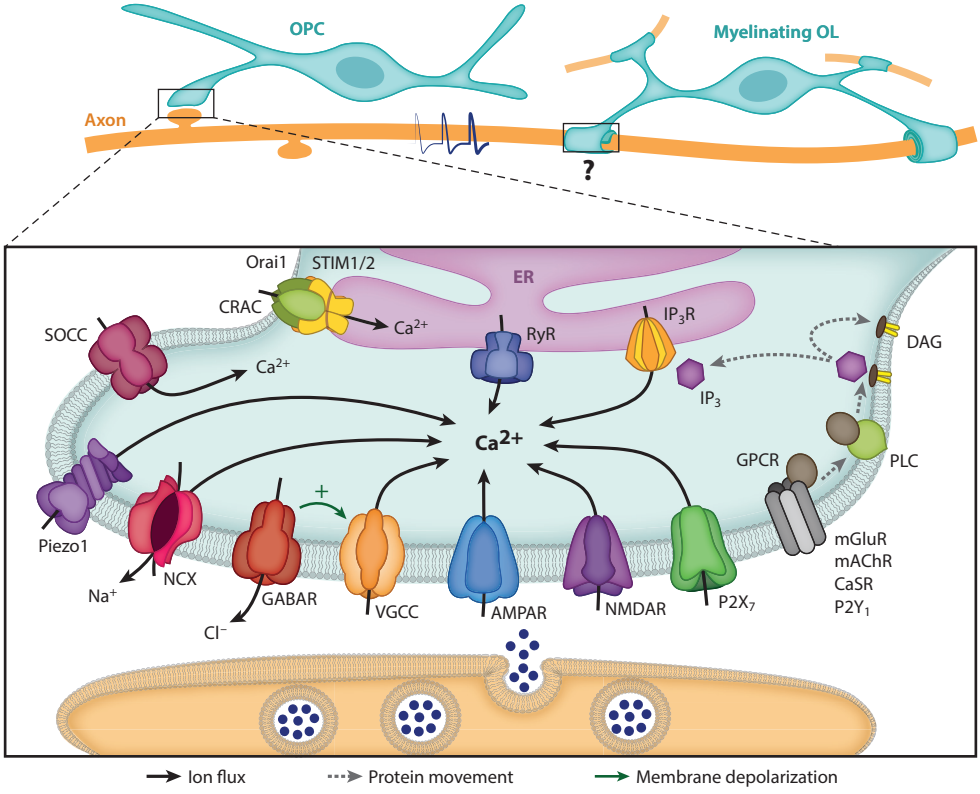

Figure 1

Oligodendrocyte lineage cells, including OPCs (*left cell*) and newly myelinating oligodendrocytes (*right*), can experience an increase in $[Ca^{2+}]_i$, which can be mediated in numerous ways, including the activation of VGCCs; ligand-gated Ca^{2+} channels such as AMPAR, NMDAR, and $P2X_7$; GPCRs such as mGluR, mAChR, CaSR, and $P2Y_1$; and/or Ca^{2+} channels gated by Ca^{2+} release from the ER like SOCCs and CRACs or Ca^{2+} channels located on the ER such as IP_3R and RyR. Additionally, the activation of $GABA_AR$ can potentially depolarize the plasma membrane and activate VGCCs and AMPAR. NCX, which couples the influx of Ca^{2+} to the efflux of Na^+, and the mechanosensitive ion channel Piezo1 are also implicated in Ca^{2+} homeostasis in OPCs/oligodendrocytes. Black arrows indicate the direction of ion flux through channels. Dotted gray arrows indicate protein movement. Note that future studies will be required to define precisely how these, and likely other, factors are dynamically regulated through oligodendrocyte lineage progression or in disease. Abbreviations: AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; CaSR, Ca^{2+}-sensing receptor; CRAC, Ca^{2+} release–activated Ca^{2+} channel; DAG, diacylglycerol; ER, endoplasmic reticulum; $GABA_AR$, γ-aminobutyric acid A receptor; GPCR, G protein–coupled receptor; IP_3, inositol 1,4,5-trisphosphate; IP_3R, inositol 1,4,5-trisphosphate receptor; mAChR, muscarinic acetylcholine receptor; mGluR, metabotropic glutamate receptor; NCX, Na^+/Ca^{2+} exchanger; NMDAR, *N*-methyl-D-aspartate receptor; OPC, oligodendrocyte progenitor cell; $P2X_7$, P2X purinoceptor 7; $P2Y_1$, P2Y purinoreceptor 1; PLC, phospholipase C; RyR, ryanodine receptor; SOCC, store-operated Ca^{2+} channel; VGCC, voltage-gated Ca^{2+} channel.

during remyelination, OPCs recruited following demyelination also received transient glutamatergic inputs from neurons (Etxeberria et al. 2010), and it has been proposed that such input promotes OPC differentiation into myelinating oligodendrocytes via AMPAR activation (Gautier et al. 2015). However, the mechanisms by which this occurs also remain to be determined.

OPCs and oligodendrocytes also express NMDA receptors (NMDARs), with several studies indicating that glutamate can activate NMDA-mediated Ca^{2+} influx in both OPCs and mature oligodendrocytes (Karadottir & Attwell 2007, Karadottir et al. 2005) (**Figure 1**). More recent analyses indicate that NMDAR-mediated current may be restricted to nonproliferative OPCs that are in a quiescent state, or a state primed for differentiation into oligodendrocytes (Spitzer et al. 2019). NMDAR expression continues in oligodendrocytes to mature stages, and NMDAR subunits have even been localized within myelin sheaths at the axon-glial interface, where they could mediate direct axon-myelin communication (Micu et al. 2016, 2018). Nonetheless, the importance of NMDAR in oligodendrocyte lineage progression and myelination has been difficult to unpick. In vitro studies of purified rat OPCs suggested that NMDAR activation facilitates differentiation by enhancing myelin protein expression and morphological maturation (Li et al. 2013), and in cocultures, NMDAR activity can promote the local translation of myelin proteins in response to neuronal activity (Wake et al. 2011). It has also been proposed that neuronal activity can lead to brain-derived neurotrophic factor (BDNF) and neuregulin secretion and that this alters the surface localization of NMDARs on oligodendrocytes, making myelination more sensitive to neuronal activity (Lundgaard et al. 2013). The role of NMDAR in regulating myelination in vivo has been harder to define. Cell type–specific ablation of NMDAR function from the oligodendrocyte lineage does not significantly alter OPC proliferation, oligodendrocyte number, and myelination (De Biase et al. 2011), even following demyelination (Guo et al. 2012). However, NMDAR-deficient oligodendrocytes displayed enhanced Ca^{2+} influx mediated by AMPARs (De Biase et al. 2011), suggesting that NMDARs and AMPARs may exhibit functional redundancy in the oligodendrocyte lineage, e.g., in regulating $[Ca^{2+}]_i$ and downstream cell biological events. More recently, further analyses of mutant mice with disrupted oligodendrocyte lineage NMDARs revealed a transient deficit in myelination and late-onset axonal degeneration, potentially due to impaired metabolic support of axons by myelinating oligodendrocytes (Saab et al. 2016). It was proposed that local vesicular release of glutamate by axons might preferentially bias myelin to more active axons and/or increase metabolic shuttling to axons with a greater metabolic need (Micu et al. 2018). However, how an NMDAR-mediated signal might increase myelination and/or metabolic support of axons in vivo remains to be investigated, as does any involvement of regulation of $[Ca^{2+}]_i$.

Initial investigations of NMDAR function in oligodendrocytes suggested that NMDAR activation occurred in ischemic-like states and resulted in rapid Ca^{2+}-dependent detachment and retraction of oligodendrocyte processes in the white matter of mice (Karadottir et al. 2005, Salter & Fern 2005) (**Figure 2**). Several NMDAR subunits (NR1–3) were detected in mature myelin, and NMDAR antagonism greatly reduced the myelin damage after ischemia (Micu et al. 2006). This view has recently been challenged, because the effects of ischemia on oligodendrocytes can be mediated through proton-gated Ca^{2+}-permeable transient receptor potential (TRP) channels (Hamilton et al. 2016). However, it is possible that different channels might mediate Ca^{2+} influx in distinct models of ischemia or over different time courses (Doyle et al. 2018). The role of NMDAR signaling in remyelination has also been investigated using a focal toxin-induced model of demyelination, wherein fewer axons were remyelinated when NMDARs were blocked with specific inhibitors (Lundgaard et al. 2013). Similarly, the NMDAR antagonist MK801 delays remyelination in the cuprizone model of demyelination by inhibiting OPC differentiation (Li et al. 2013). However, whether these pharmacologically induced effects act directly on oligodendrocytes or indirectly, e.g., through neurons, remains to be clarified. In all of the above cases, how NMDAR activity converges on $[Ca^{2+}]_i$ and how this might in turn mediate any of the responses of OPCs or oligodendrocytes also remain to be investigated.

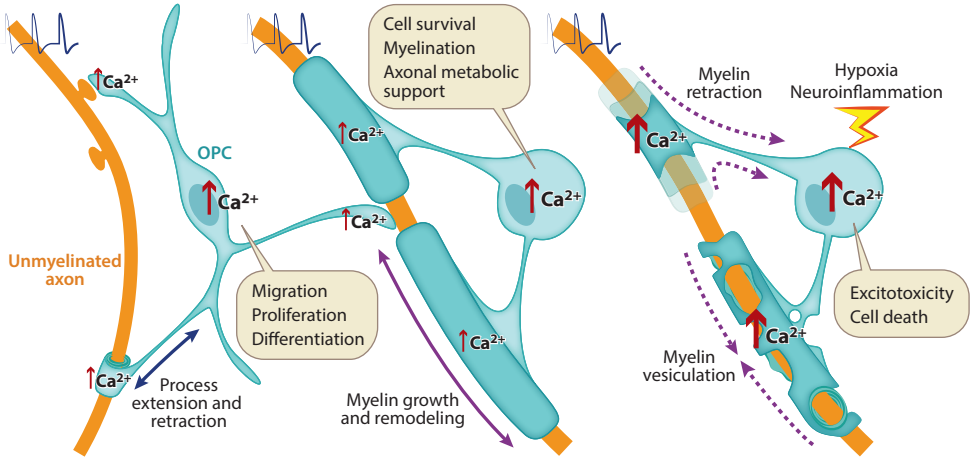

Figure 2

Representation of the multiple roles of Ca^{2+} signaling in oligodendrocyte progenitor cells (OPCs) and oligodendrocytes at different stages of development as well as in pathological situations. Red arrows indicate that Ca^{2+} levels change in different cellular compartments and that increasing Ca^{2+} levels can promote many aspects of oligodendrocyte maturation, but very high levels of Ca^{2+} are often associated with pathological outcomes. However, the complex dynamic changes in Ca^{2+} levels that regulate distinct aspects of oligodendrocyte biology remain to be determined.

Metabotropic Glutamate Receptors

In addition to ionotropic glutamate receptors, OPCs express metabotropic glutamate receptors (mGluRs), which are G protein–coupled receptors (GPCRs). These receptors are highly expressed in OPCs and, albeit to a lesser extent, in mature myelinating oligodendrocytes (Luyt et al. 2003, 2006). Binding of glutamate to these receptors activates phospholipase C and inositol 1,4,5-trisphosphate (IP_3) production and thus drives Ca^{2+} efflux from the endoplasmic reticulum (ER), a major mechanism by which the levels of $[Ca^{2+}]_i$ are regulated (**Figure 1**). Additionally, mGluRs are also known to increase the expression of Ca^{2+}-permeable AMPARs in OPCs (Zonouzi et al. 2011). The function of mGluRs is unclear, but in cultured OPCs, activation of these receptors raises $[Ca^{2+}]_i$ (Luyt et al. 2003), accelerates OPC morphological maturation (Spampinato et al. 2014), and leads to the release of BDNF (Bagayogo & Dreyfus 2009). Like NMDARs, mGluRs can locally regulate myelin basic protein synthesis in response to glutamate (Spampinato et al. 2014, Wake et al. 2011), and in vivo, mGluR antagonists can inhibit the rise in OPC $[Ca^{2+}]_i$ following neuronal stimulation, suggesting a role for these receptors in activity-dependent myelination (Haberlandt et al. 2011). Similar in structure to mGluRs, another GPCR called the Ca^{2+}-sensing receptor (CaSR) is highly abundant in the oligodendrocyte lineage, particularly in newly myelinating oligodendrocytes (Chattopadhyay et al. 2008, Zhang et al. 2014). This receptor can respond to changes in the extracellular concentration of Ca^{2+} and amino acids and can in turn influence $[Ca^{2+}]_i$ through promoting Ca^{2+} release from internal stores (Chattopadhyay et al. 2008) (**Figure 1**). Perhaps surprisingly, the roles of mGluR and CaSR in the oligodendrocyte lineage have not yet been systematically investigated through cell type–specific targeting in vivo, and this remains an important challenge for the future.

GABA Receptors

GABA is the major inhibitory neurotransmitter in the adult CNS. It acts on two distinct types of receptors: ligand-gated ionotropic $GABA_A$ and $GABA_C$ receptors and G protein–coupled $GABA_B$

receptors. OPCs express functional $GABA_A$ receptors (Von Blankenfeld et al. 1991, Williamson et al. 1998) and the sustained expression of these channels is promoted by axonal cues (Arellano et al. 2016). In contrast to its inhibitory effect on mature neurons, GABA depolarizes OPCs, as the chloride reversal potential of OPCs is approximately −40 mV (Lin & Bergles 2003). This depolarization may in turn activate VGCCs and explain the reported GABA-mediated Ca^{2+} entry in OPCs (Haberlandt et al. 2011, Tanaka et al. 2009, Vélez-Fort et al. 2010) (**Figure 1**). $GABA_A$ receptor activation can also influence Ca^{2+} entry through AMPARs (Lin & Bergles 2003), which could be a second way by which $[Ca^{2+}]_i$ changes are induced by $GABA_A$ stimulation in OPCs. In this regard, the activity of GABAergic interneurons in the hippocampus has been shown to increase $[Ca^{2+}]_i$ in OPCs (Haberlandt et al. 2011). Interestingly, the mode of GABA-related axon-OPC signaling changes over time, with synaptic communication giving way to largely extrasynaptic communication (Balia et al. 2017), the relevance of which remains to be fully explored.

New evidence suggests that GABA signaling may play a role in oligodendrocyte lineage progression and thus in myelination and remyelination. In an ex vivo cortical slice preparation, pharmacological impairment of $GABA_A$ increased the proliferation and survival of OPCs and also mature oligodendrocyte number (Hamilton et al. 2017). However, in another experimental paradigm, decrease of $GABA_A$ receptor activity increased OPC proliferation but reduced oligodendrocyte differentiation (Zonouzi et al. 2015). Further highlighting the complexity of GABAergic signaling in OPCs, cell type–specific genetic targeting in OPCs of a $GABA_A$ receptor subunit associated with synaptic activity did not affect OPC proliferation at all but instead regulated cell survival (Balia et al. 2017). OPCs also express functional $GABA_B$ receptors that promote cell proliferation and migration in vitro (Luyt et al. 2007), but how $GABA_B$ affects OPCs or oligodendrocytes in vivo remains to be tested.

It is clear that GABA receptors likely play an important role in OPC and oligodendrocyte biology, but the way in which Ca^{2+} transduces GABA receptor signals is poorly understood. Therefore, deciphering the stage and context-specific roles of GABA and downstream signaling in regulating the oligodendrocyte lineage remains an important challenge in the field (for an in-depth discussion, see Habermacher et al. 2019).

Purinergic Receptors

ATP is released in an activity-driven manner from axons and astrocytes (Hamilton et al. 2010). Using Ca^{2+} imaging techniques, Hamilton et al. (2010) revealed that ATP can induce Ca^{2+} signals in OPCs in isolated mouse optic nerves. Pharmacological experiments demonstrated that these responses were mediated by either $P2X_7$ receptors, which are ligand-gated, nonselective Ca^{2+} channels, or $P2Y_1$ receptors, which are metabotropic receptors linked to G protein activation and IP_3-dependent Ca^{2+} release from internal stores (Hamilton et al. 2010) (**Figure 1**). $P2X_7$ receptor–mediated Ca^{2+} signaling in OPCs is initiated by an increased influx of Na^+ and Ca^{2+} through the activated receptor. But subsequently, Na^+ influx across $P2X_7$ channels can trigger a secondary increase in $[Ca^{2+}]_i$ through VGCCs (**Figure 1**). ATP is also rapidly hydrolyzed to adenosine: Therefore, OPC Ca^{2+} changes may in part be mediated by the activation of A1 and A3 adenosine receptors (Stevens et al. 2002). This pathway can also modulate glutamate signaling by decreasing the postsynaptic expression of Ca^{2+}-permeable AMPARs (Zonouzi et al. 2011). Evidence for the role of purinergic signaling in controlling OPC development derives mostly from in vitro findings. ATP-mediated activation of $P2Y_1$ receptors has been shown to stimulate OPC migration and block cell proliferation in vitro (Agresti et al. 2005). Moreover, ATP released from active neurons stimulates Ca^{2+} signaling in OPCs and inhibits proliferation and increases differentiation of OPCs

(Stevens et al. 2002). Although the in vitro and ex vivo data are compelling, the function of ATP-induced Ca^{2+} signaling in OPCs and oligodendrocytes in vivo requires further investigation.

Muscarinic Acetylcholine Receptors

OPCs express three subtypes of muscarinic acetylcholine receptors (mAChRs), M1, M3, and M4, coupled to Gq proteins that activate phospholipase C (Cohen & Almazan 1994) (**Figure 1**). All are highly active in OPCs and expressed at lower levels in mature myelinating oligodendrocytes (De Angelis et al. 2012, Molina-Holgado et al. 2003, Zhang et al. 2014). As in neurons, activation of mAChR in OPCs promotes Ca^{2+} release from internal stores via IP_3 production (Abiraman et al. 2015, Karschin et al. 1994, Welliver et al. 2018). Activation of these receptors in isolated OPCs enhances cell proliferation and, consistent with this, the expression of PDGF receptor α (De Angelis et al. 2012). In contrast, mAChR antagonists reduce OPC proliferation but stimulate OPC differentiation and myelination (Abiraman et al. 2015, Welliver et al. 2018). M3 receptor knockdown in cultured human OPCs blocks cytoplasmic Ca^{2+} changes following muscarinic agonist treatment and enhances OPC differentiation (Welliver et al. 2018). Importantly, M3-deficient OPCs mature faster and produce more myelin than control cells after transplantation into demyelinated mice and improve remyelination of the adult mouse brain (Welliver et al. 2018). Clemastine, a US Food and Drug Administration–approved antihistamine, is also a mAChR antagonist and was identified in a cell-based phenotypic screen of compounds for their ability to promote oligodendrocyte differentiation (Mei et al. 2014). Clemastine has since been shown effective in promoting remyelination in several mouse models of myelin damage and dysregulation, and it mediates its function through the M1 muscarinic receptor (Cree et al. 2018; Liu et al. 2016; Mei et al. 2014, 2016). Clemastine has even shown promise in clinical trials in promoting optic nerve function in patients with multiple sclerosis (MS) (Green et al. 2017) and in treatment of a patient with white matter injury (Cree et al. 2018). Because clemastine broadly inhibits mAChRs and has other targets in the CNS, it has the potential to lead to cognitive defects (Anagnostaras et al. 2002), and so more specific mAChR antagonists may need to be developed for the long-term treatment of conditions such as MS.

Store-Operated Ca^{2+} Channels and Ca^{2+} Release from the Endoplasmic Reticulum

Store-operated Ca^{2+} channels (SOCCs) are plasma membrane channels regulated by the amount of Ca^{2+} in intracellular stores, most notably the ER. As noted above, a significant portion of Ca^{2+} signaling in OPCs is dependent on IP_3-mediated Ca^{2+} release from the ER, which stimulates the activity of SOCCs to replenish ER Ca^{2+} (**Figure 1**). Two channels thought to mediate store-operated Ca^{2+} influx have been found in OPCs: the canonical transient receptor potential channel subfamily C member 1 (TRPC1) (Paez et al. 2009a, 2011) and the transient receptor potential channel subfamily M member 3 (TRPM3) (Papanikolaou et al. 2017). The expression of TRPC1 in OPCs is developmentally regulated (Paez et al. 2011), and the activity of this Ca^{2+}-permeant channel is essential for OPC proliferation and for the action of PDGF and golli-MBP proteins on OPC development (Paez et al. 2009a, 2011). Specifically, in vitro and in situ proliferation analysis revealed that golli enhanced the PDGF-stimulated proliferation of OPCs through activation of TRPC1. PDGF induced a biphasic increase in OPC $[Ca^{2+}]_i$, and golli specifically increased Ca^{2+} influx during the second SOCC-dependent phase that followed the initial release of Ca^{2+} from intracellular stores (Paez et al. 2009a, 2011). This store-operated Ca^{2+} uptake appeared to be essential for cell division, since specific SOCC antagonists completely blocked the effects of

PDGF and golli on OPC proliferation (Paez et al. 2009a). Furthermore, in vitro Ca^{2+} imaging experiments suggest that golli proteins associate with TRPC1 channels in OPC processes (Paez et al. 2011). These data implicate golli proteins in the regulation of TRPC-mediated Ca^{2+} influx and indicate that extracellular Ca^{2+} entry through TRPC1 is an essential component in the mechanism of OPC proliferation.

Using immunohistochemical techniques and Ca^{2+} imaging of isolated optic nerves and optic nerve cultures, Papanikolaou et al. (2017) demonstrated the presence of functional TRPM3 in oligodendrocytes. They showed that these channels are essential for maintaining normal $[Ca^{2+}]_i$ and ATP-mediated Ca^{2+} signaling in optic nerve oligodendrocytes (Papanikolaou et al. 2017). Additionally, they described the presence of Ca^{2+} release–activated Ca^{2+} channels (CRACs) in optic nerve oligodendrocytes, which are composed of the plasmalemma-spanning channel Orai and the stromal interaction molecule (Stim), which functions as an ER Ca^{2+} sensor (Papanikolaou et al. 2017) (**Figure 1**). Interestingly, golli-MBP has been shown to interact directly with Stim1 and to positively regulate myelin sheet expansion in primary oligodendrocytes via Ca^{2+} signaling (Walsh et al. 2010). In summary, these studies indicate that both TRP and CRAC channels are important mediators of $[Ca^{2+}]_i$ changes in OPCs and oligodendrocytes: These SOCCs participate in almost all Ca^{2+} signaling in which Ca^{2+} release from the ER is induced and, as such, are essential for the transduction of the signaling initiated by metabotropic Ca^{2+} receptors in the OPC/oligodendrocyte plasma membrane. However, the consequences of disrupting store-operated Ca^{2+} entry on OPCs and mature oligodendrocytes in vivo remain to be determined.

The role of Ca^{2+} release from internal stores in regulating oligodendrocyte differentiation has recently been validated. Li et al. (2018) showed that ER Ca^{2+} release via ryanodine receptors (RyRs), particularly isoform 3, is essential for oligodendrocyte differentiation in vitro. In this study, the authors demonstrated that the RyR antagonist ryanodine neutralized the increase in $[Ca^{2+}]_i$ observed in OPCs and reduced the number of mature oligodendrocytes in culture. Furthermore, RyR3 facilitated intracellular Ca^{2+} waves in the soma and in the processes of OPCs after caffeine stimulation, phenomena that disappear in mature oligodendrocytes (Li et al. 2018) (**Figure 1**). In summary, Ca^{2+} release from the ER and Ca^{2+} entry via SOCCs are key regulators of oligodendrocyte differentiation.

Mechanosensitive Ca^{2+} Channels

In addition to molecular signals, oligodendrocyte lineage cells are also responsive to mechanical cues (Klingseisen & Lyons 2017). The influence of biophysical properties on OPC biology was recently illustrated by comparison of the proliferative properties of neonatal and adult OPCs. Upon demyelination, OPCs have the capacity to generate new oligodendrocytes that remyelinate demyelinated areas, but this capacity becomes drastically reduced with age. In comparing OPCs from neonatal and adult stages, Segel et al. (2019) found that their proliferation was greatly regulated by the environmental niche and, quite remarkably, by the age-related stiffness of their substrate: Adult CNS tissue becomes stiffer with age and less hospitable to OPC proliferation. The authors hypothesized that preventing the mechanosensitive ability of OPCs to respond to substrate stiffness might facilitate proliferation in an aged environment (Segel et al. 2019). Remarkably, inhibition of the mechanosensitive ion channel Piezo1 (**Figure 1**) allowed OPCs to exhibit neonatal levels of proliferation on aged substrates. Piezo1 is a Ca^{2+}-conducting channel, and OPCs exhibited Ca^{2+} activity only when placed on aged-like substrates but not when Piezo1 was knocked down (Segel et al. 2019). How Ca^{2+} might in turn regulate proliferation downstream of mechanosensation remains to be observed.

Myelinating oligodendrocytes also respond to biophysical cues, specifically to the shape of targets for myelination. In fact, oligodendrocytes in culture enwrap axon-shaped plastic microfibers with myelin-like sheaths, but they only do so on fibers of a caliber of 400 nm or over (Lee et al. 2012), the size at which axons become myelinated in vivo. Interestingly, oligodendrocytes also make longer myelin sheaths on thicker plastic fibers (Bechler et al. 2015). Whether mechanosensitive channels mediate these effects remains to be determined. How Ca^{2+} might regulate sheath growth and dynamics is discussed below.

Na^+/Ca^{2+} Exchangers

Na^+/Ca^{2+} exchangers (NCXs) are transmembrane proteins involved in the regulation of diverse neuronal and glial cell functions. These transporters couple the influx of Ca^{2+} to the efflux of Na^+ or vice versa (**Figure 1**). Cells of the oligodendrocyte lineage express three different members of the NCX family: NCX1, NCX2, and NCX3. NCX1 and NCX3 were found to be developmentally regulated (Boscia et al. 2012), with NCX1 being highly expressed by OPCs and NCX3 the principal isoform in mature oligodendrocytes. Electrophysiological studies first demonstrated the participation of NCXs in OPC Ca^{2+} signaling. Tong et al. (2009) showed that OPC plasma membrane depolarization induced by $GABA_A$ receptor activation triggers noninactivating Na^+ channels, which in turn induce $[Ca^{2+}]_i$ elevation via NCX1. Furthermore, they suggested that Ca^{2+} signaling mediated by NCX1 is an essential molecular component in the modulation of OPC migration (Tong et al. 2009). The importance of Ca^{2+} signaling mediated by NCX3 in OPC maturation was later investigated and it was found that NCX3 deletion impaired OPC differentiation in vitro, whereas its overexpression led to an upregulation of myelin proteins (Boscia et al. 2012). Furthermore, NCX3-knockout mice exhibited a significant hypomyelination in the spinal cord, accompanied by higher densities of OPCs (Boscia et al. 2012). Together, these findings indicate that Ca^{2+} signaling mediated by NCXs plays a crucial role in oligodendrocyte differentiation and thus myelination. Further studies are, however, necessary to expose whether changes in NCX activity might contribute to dysfunction in demyelinating diseases and whether its modulation might be of therapeutic relevance.

Gap Junctions

In the CNS, gap junctions mediate intercellular communication among oligodendrocytes and between oligodendrocytes and astrocytes, forming glial networks. Gap junctions are formed by members of the connexin (Cx) family of transmembrane proteins, and cells of the oligodendrocyte lineage express Cx29, Cx32, Cx45, and Cx47 (Cisterna et al. 2019, Nualart-Marti et al. 2013). Mutations in the genes encoding Cx32 and Cx47 cause the X-linked form of Charcot-Marie-Tooth disease and Pelizaeus Merzbacher–like disease, respectively, both of which exhibit major disruption to myelination in both humans and corresponding mice models (Cisterna et al. 2019). Disruption to oligodendrocyte Cxs has also been associated with neuroinflammation (Papaneophytou et al. 2019), and it has been found that the expression of Cxs is dysregulated in MS and experimental autoimmune encephalitis (Markoullis et al. 2012a,b, 2014). Gap junctions have numerous functions and have been implicated in the propagation of glial Ca^{2+} waves. Indeed, using laser photostimulation and Ca^{2+} imaging in primary cultures, Parys et al. (2010) demonstrated the presence of bidirectional Ca^{2+} waves from astrocytes to oligodendrocytes that were sensitive to gap junction blockers. These results suggest that disrupted Cx function, and by extension Ca^{2+} waves, may regulate myelination and may contribute to a proinflammatory environment in demyelinating disorders. In myelin sheaths, Cx32 is localized to the paranodal loops, where it may form reflexive gap junctions between different layers of myelin (Balice-Gordon et al. 1998). Such coupling may be

particularly important due to the unique topology of the multilaminar compacted mature myelin, with this type of gap junction representing an intracellular pathway for diffusion of cytoplasmic components and signals, including Ca^{2+}.

We have given an overview of our current understanding of how individual receptors and channels can mediate changes in $[Ca^{2+}]_i$ in oligodendrocytes, but others are likely to be defined through ongoing work. A major challenge for the field, however, will be to understand how these factors interact and collaborate over time to orchestrate the specific localized codes of Ca^{2+} activity that likely drive downstream cellular functions. Indeed, numerous Ca^{2+} sensors and effectors are also likely to transduce Ca^{2+} codes into cellular responses. Such Ca^{2+}-sensitive/responsive proteins are usually involved in many signaling pathways and thus can in turn alter ion channel activity, posttranslational modifications of proteins, changes in the cytoskeleton dynamics, modifications in gene expression, or any combination of the above. As in neurons, the localization, amplitude, and source of Ca^{2+} signals, or Ca^{2+} gradients generated across the cell body or processes, can potentially regulate specific functions in oligodendrocyte lineage cells. However, how the spatial localization of Ca^{2+} signals are regulated and ultimately transduced to specific responses in OPCs and/or myelinating oligodendrocytes and how these Ca^{2+} changes are amplified and sustained over an appropriate timescale to orchestrate OPC development and myelination are largely unknown. Nonetheless, in the second part of this review, we address how changes in $[Ca^{2+}]_i$ can affect cell behavior and neural circuit health and function and outline key outstanding challenges for the field.

HOW DOES REGULATION OF INTRACELLULAR Ca^{2+} AFFECT OLIGODENDROCYTE PROGENITOR CELL AND OLIGODENDROCYTE FORMATION AND FUNCTION?

As noted above, many factors can regulate $[Ca^{2+}]_i$ in OPCs and oligodendrocytes (**Figure 1**). However, it remains relatively unclear how changes in $[Ca^{2+}]_i$ then regulate key aspects of OPC and oligodendrocyte biology, but insights are now beginning to emerge.

Ca^{2+} Signals and Oligodendrocyte Progenitor Cell Morphology

Ca^{2+}-sensitive cell behaviors can be initiated by a wide range of Ca^{2+} sensors such as calmodulin, S100B proteins, Ca^{2+}/calmodulin-dependent protein kinases (CaMKs), and calcineurin. These Ca^{2+} sensors and effectors are particularly effective in controlling the cytoskeleton dynamics via actin and microtubule assembly and disassembly. For instance, S100B is a Ca^{2+}-binding protein that is localized in the cytoplasm and nucleus of some subpopulations of astrocytes. S100B proteins are also present in postnatal and adult populations of NG2-positive OPCs as well as in immature and mature myelinating oligodendrocytes (Hachem et al. 2005). It has been shown that S100B regulates astrocytes shape and migration in a Ca^{2+}-dependent manner via association with actin and microtubular structures (Brozzi et al. 2009, Sorci et al. 2000). Thus, it is tempting to speculate that these Ca^{2+}-sensitive proteins play similar roles in OPCs by transducing Ca^{2+} signals into morphological changes. Likewise, the Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) was found to be involved in regulating OPC morphology through stabilization of the actin cytoskeleton (Waggener et al. 2013). CaMKII is a serine/threonine protein kinase that is controlled by the Ca^{2+}/calmodulin complex. It has been proposed that CaMKII activation initiates a period of intense cytoskeleton reorganization following Ca^{2+} influx (McVicker et al. 2015). CaMKII is able to interact with microtubules in active synapses (Lemieux et al. 2012), and the phosphorylation of profilin (Ryan et al. 2005) and cofilin (Xie et al. 2007) by CaMKII stimulates actin polymerization via independent intracellular pathways. It was recently established that Cav1.2 channels promote

OPC process extension via CaMKII (Cheli et al. 2018) and that CaMKII activity is crucial for the regulation of myelin thickness during developmental myelination (Waggener et al. 2013). Therefore, S100B and CaMKII may play a role in the reorganization of both the actin and microtubule cytoskeleton in OPC as well as in myelin sheaths. The activation of these Ca^{2+}-sensitive proteins could be a central event in which Ca^{2+} influx initiated by diverse Ca^{2+} channels converge to modulate OPC morphology and potentially also myelination.

Activity-Regulated Oligodendrocyte Progenitor Cell Proliferation Is Mediated by Intracellular Ca^{2+}

Ca^{2+} can affect the proliferation of many cell types, but the regulation of cell proliferation by neuronal activity may be specific to OPCs. As noted in the previous section, there is a wealth of evidence that axons provide synaptic and extrasynaptic input to OPCs through numerous factors that converge on regulation of $[Ca^{2+}]_i$ (**Figure 2**). However, whether or how Ca^{2+} actually affects OPC and oligodendrocyte biology is relatively unclear. A recent study using zebrafish employed lineage tracing, RNAseq, and Ca^{2+} imaging to better understand OPC diversity and function in vivo (Hoche et al. 2019). This led to the identification of two broad classes of OPCs, one with highly dynamic processes that had the capacity to directly generate differentiated oligodendrocytes and another with a similarly elaborate but less dynamic network of cellular processes that did not directly generate oligodendrocytes. Interestingly, this second type of OPC had gene expression characteristics of being responsive to neuronal activity and exhibited more frequent and higher-amplitude Ca^{2+} transients in their cellular processes. Furthermore, this class proliferated more upon the induction of neuronal activity, and this activity-driven proliferation was inhibited by expression of the exogenous Ca^{2+}-exporting pump CalEx in OPCs (Hoche et al. 2019). These results indicate that the level of $[Ca^{2+}]_i$ in a specific subset of activity-responsive OPCs mediates activity-regulated OPC proliferation (Hoche et al. 2019). The precise molecular mechanisms mediating axon-OPC communication upstream of Ca^{2+} activity in this context remain to be determined. However, signaling via BDNF through OPC TrkB represents a strong candidate, given that TrkB mediates the effects of optogenetic stimulation of OPC proliferation in mammals (Geraghty et al. 2019) and that TrkB receptor activation can stimulate release of Ca^{2+} from intracellular stores, at least in neurons. It is clear that combining high-resolution imaging of Ca^{2+}, manipulations of neuronal activity and candidate gene function, and monitoring cell fate will accelerate our understanding of how Ca^{2+} regulates OPC biology.

Gene Expression Programs that Drive Oligodendrocyte Differentiation Can Be Regulated by Ca^{2+}

Oligodendrocytes can differentiate in cell culture in the absence of dynamic cues, but extrinsic signals can regulate oligodendrocyte differentiation and the survival of newly differentiated oligodendrocytes in vivo (Klingseisen & Lyons 2017). Whether specific extrinsic signals specifically regulate differentiation per se or simply the survival of newly differentiated oligodendrocytes has been difficult to unpick. However, it now appears that Ca^{2+} signaling may play a direct role in oligodendrocyte differentiation per se. Several key transcription factors, including Sox10, are well known to be essential for oligodendrocyte lineage progression (Bergles & Richardson 2015). A recent study identified the nuclear factor of activated T cells (NFAT) transcription factor as a target of Sox10 that influences the oligodendrocyte differentiation gene expression program in vivo (Weider et al. 2018). The function of NFAT is critically regulated by $[Ca^{2+}]_i$, because the nuclear import, and thus the gene expression–regulating function of NFAT, is controlled by the

Ca^{2+}-dependent phosphatase calcineurin (Hogan et al. 2003). The signals that regulate $[Ca^{2+}]_i$ upstream of calcineurin-dependent stimulation of NFAT remain to be identified.

Localized Codes of Ca^{2+} Activity in Myelin Sheaths Regulate Myelination

OPCs and oligodendrocytes are morphologically complex cells, with OPCs and newly differentiating oligodendrocytes having large networks of cellular processes that interact with multiple cellular targets, including, most notably, neurons and axons. Changes in $[Ca^{2+}]_i$ in OPCs and myelinating oligodendrocytes can take many forms, from being manifest throughout the entire cell (or even across cellular syncitia) to being localized to discrete cellular microdomains (Baraban et al. 2018, Battefeld et al. 2019, Hoche et al. 2019, Krasnow et al. 2018, Micu et al. 2007). Recent live-imaging studies of the Ca^{2+} activity of newly myelinating oligodendrocytes revealed remarkable microdomain specificity in Ca^{2+} activity that prefigured specific cell biological outcomes. Imaging genetically encoded Ca^{2+} indicators in oligodendrocytes during the early stages of myelination in zebrafish, Baraban et al. (2018) showed that the frequency, amplitude, and duration of Ca^{2+} transients in myelinating processes were predictive of whether myelin sheaths remained associated with axons or were retracted (**Figure 2**). During myelin sheath generation, individual oligodendrocytes make numerous short myelin sheaths on many axons but subsequently retract from some axons while maintaining sheaths on others (Czopka et al. 2013). Baraban et al. (2018) found that individual, high-amplitude, long-duration Ca^{2+} transients prefigured sheath retractions and that these were mediated by the Ca^{2+}-dependent protease calpain in oligodendrocytes.

This study and another also found that the frequency of lower-amplitude, shorter-duration myelin Ca^{2+} transients correlated positively with the speed of sheath growth (Baraban et al. 2018, Krasnow et al. 2018). The longitudinal and circumferential growth and wrapping of the myelin sheath around the axon is driven by iterative cycles of actin polymerization and depolymerization (Nawaz et al. 2015). Ca^{2+} is known to regulate actin polymerization, and so its dynamic regulation in myelin is well placed to control sheath growth (**Figure 2**). Indeed, Krasnow et al. (2018) showed that experimental buffering of Ca^{2+} slowed sheath growth. In addition, they found that approximately half of the Ca^{2+} transients that they observed in myelin were driven by neuronal activity, as evidenced by a reduction in transients upon TTX treatment. The factors driving activity-regulated and activity-independent codes of myelin Ca^{2+} activity mediating sheath formation, retraction, and elongation remain to be determined. While these studies in zebrafish characterized Ca^{2+} activity in the myelinating processes of newly forming oligodendrocytes, another study characterized the kinetics of Ca^{2+} activity in myelin sheaths in the mouse cortex. They too observed significant localized Ca^{2+} activity during myelin sheath growth and remodeling, and they observed that this became reduced over time but that it was reactivated upon remyelination (Battefeld et al. 2019). Unlike the observations of Krasnow et al. (2018), Battefeld et al. (2019) did not find evidence that the Ca^{2+} transients they observed were driven by neuronal activity, an apparent discrepancy that remains to be deconstructed. Interestingly, Battefeld et al. showed that the myelin Ca^{2+} transients required the function of the mitochondrial permeability transition pores, suggesting that myelin Ca^{2+} transients may result, at least in part, from local release from mitochondria in myelin (Battefeld et al. 2019). However, the prevalence of mitochondria within myelin sheaths remains to be fully elucidated, and thus direct visualization of Ca^{2+} activity in specific subcellular compartments will require targeting of Ca^{2+} reporters to these domains, as has been demonstrated in astrocytes (Agarwal et al. 2017).

Oligodendrocyte Ca^{2+} and Regulation of Neural Circuit Function

The Ca^{2+}-driven regulation of oligodendrocyte lineage progression and myelination involves cell biological events that are manifest over timescales of minutes, hours, or even days. In neurons,

acute regulation of Ca^{2+} also plays roles in regulating neurophysiology at much finer timescales. For example, Ca^{2+} channels regulate the integration of postsynaptic signals that change gene expression underpinning neuronal plasticity (Dolmetsch et al. 2001, Mermelstein et al. 2000). VGCCs localized in synaptic spines, dendritic shafts, and neuronal cell bodies (Hell et al. 1996) can also affect dendritic spine morphology and postsynaptic stability. Furthermore, Ca^{2+} channels are key components of active signaling complexes in postsynaptic dendrites and dendritic spines, where Ca^{2+} entry can influence many aspects of functional plasticity, e.g., long-term potentiation (Nanou & Catterall 2018). This all begs the question of what, if any, function Ca^{2+} might play in regulating the electrophysiological properties of OPCs and oligodendrocytes and how this might affect neural circuit function.

As noted throughout, OPCs have the ability to respond to neuronal activity, but recent studies have suggested that they might in turn also tune neuronal function by releasing secreted factors (Sakry et al. 2014) and neuromodulators to locally regulate the function of neuronal synapses. For example, it has been proposed that oligodendrocytes can release BDNF, which acts on neuronal presynaptic terminals through TrkB signaling, and that this can fine-tune the release of neurotransmitters from the neuronal presynapse (Jang et al. 2019). OPCs/oligodendrocytes are by all accounts considered a postsynaptic cell from a physiological perspective, but postsynaptic release of BDNF from neurons has been observed and is a Ca^{2+}-driven process. Although we are only beginning to scratch the surface as to the function of potentially bidirectional axon-OPC communication, it is clear that integrating inputs and coordinating functional outputs will likely involve numerous Ca^{2+}-regulated pathways and events. Dissecting how these are spatially and temporally coordinated during nervous system function represents an enormous challenge for the field.

Myelinating oligodendrocytes have physiological functions beyond merely providing insulation to axons. As noted earlier, the regulation of metabolic support of axons may be an activity-regulated process involving Ca^{2+}-permeable NMDARs localized to myelin sheaths (Saab et al. 2016). The kinetics of activity-regulated metabolic support in vivo remain to be determined, but over acute timescales, it is also very likely that neurophysiological function is dynamically regulated at the interface between the axon and myelin sheath. Indeed, we and others have recently postulated that the innermost compartment of the myelin sheath has a rich physiology that we are only beginning to grasp (Micu et al. 2018, Suminaite et al. 2019). For example, much of the K^+ released by axons during action potential firing is extruded into the periaxonal space between the axon and its overlying myelin sheath. Given the very tight adhesion of the myelin sheath to the axon at paranodal junctions, mechanisms must be in place to remove K^+ from this space to allow membrane repolarization. Recent studies have shown that the inwardly rectifying potassium channel Kir4.1 is localized to the innermost compartment of the myelin sheath at the periaxonal space and that deletion of Kir4.1 from the oligodendrocyte lineage disrupts K^+ homeostasis, increases susceptibility to seizures, and results in axonal pathology (Larson et al. 2018, Schirmer et al. 2018). The delivery of K^+ into the periaxonal space may, at least transiently, depolarize the myelin membrane at the periaxonal space, where many ion channels and transporters are proposed to localize. Nonetheless, it remains unclear quite how much active signaling occurs at the axon-glial interface and to what extent this affects acute or long-term neurophysiology.

Dysregulation of Oligodendrocyte Progenitor Cell and Oligodendrocyte Ca^{2+} Can Lead to Pathology

The highly orchestrated regulation of $[Ca^{2+}]_i$ has the consequence of being prone to dysregulation. Unfortunately, due to its ubiquity and importance in many cellular processes, dysregulation of Ca^{2+} can lead to severe pathological outcomes. In depth discussion of how Ca^{2+} can drive

pathologies is beyond the scope of this piece, but particular susceptibilities of OPCs and myelinating cells to Ca^{2+} dysregulation are important to note.

A major consequence of severe preterm birth is neonatal hypoxia, to which OPCs are acutely sensitive. Neonatal hypoxia can greatly dysregulate OPC proliferation and impair the generation of differentiated oligodendrocytes, which is thought to contribute to delayed cognitive development (Salmaso et al. 2014). The effects of neonatal hypoxia are mediated in large part through GABAergic signaling, which, as noted above, can regulate $[Ca^{2+}]_i$ in OPCs (Zonouzi et al. 2015). Indeed, the histone deacetylase sirt1 is a major downstream mediator of the effects of hypoxia on OPC behavior (Jablonska et al. 2016), and its activation can also be regulated by Ca^{2+} activity, suggesting a possible mechanism by which Ca^{2+} drives hypoxia-related pathology.

The expression of Ca^{2+}-permeable channels by myelinating oligodendrocytes has also been proposed to contribute to demyelination and myelin injury. The fact that Ca^{2+}-permeant channels are present in myelin sheaths at the axon-myelin interface may predispose this region to damage, e.g., following excitotoxic insults and ischemia, as noted above (**Figure 2**). Ca^{2+} dysregulation could lead to myelin pathology through numerous mechanisms, from induction of Ca^{2+}-dependent proteases such as calpain, which has been shown to drive myelin sheath retraction during development (Baraban et al. 2018), to induction of cell stress in the ER (Lin & Popko 2009), which is acutely sensitive to dysregulation of Ca^{2+} levels. Indeed, dysregulation of Ca^{2+} can directly influence the structure of the compact myelin sheath itself. Myelin membrane breakdown is a hallmark of many demyelinating diseases, and in a rat model of neuromyelitis optic it has been shown that myelin sheaths break down in an inside out manner, i.e., decompacting at the innermost layer first, with myelin becoming vesiculated. Interestingly, this can be driven by increased Ca^{2+} levels, which disrupt the polymer-like structure formed by myelin basic proteins integral to the compact structure of the myelin sheath (Weil et al. 2016). Therefore, it is possible that elevated Ca^{2+} in myelin could prefigure and trigger myelin vesiculation in disease (**Figure 2**).

OUTSTANDING QUESTIONS AND FUTURE DIRECTIONS

Based in large part on our knowledge of the molecular regulators of Ca^{2+} in other cells, including neurons and astrocytes, we are now beginning to understand the factors that converge on the regulation of Ca^{2+} in the oligodendrocyte lineage. Although further regulators will no doubt be discovered, including most likely additional GPCRs, one key challenge for the field will be to characterize the dynamics of Ca^{2+} regulation at high spatial and temporal resolution in vivo. Genetically encoded Ca^{2+} indicators now provide impressive signal to noise ratios and have properties that, with computational deconvolution, allow kinetics to be inferred at the millisecond scale. Taking advantage of the capacity to localize such reporters in multiple wavelengths and to subcellular compartments will help reveal the nature of Ca^{2+} signaling within and between cells. These reporters, coupled with opto- and chemogenetic strategies to locally and broadly regulate Ca^{2+} levels directly, will in turn allow us to further probe the consequence of manipulating Ca^{2+} signaling. An enormous challenge for the field will be to understand how the many factors that converge on Ca^{2+} interact, collaborate, and compete over time to affect Ca^{2+}. However, the ability to employ CRISPR-based gene manipulation strategies to assess the function of multiple factors in parallel may go some way toward improving our understanding. An arguably greater challenge in many ways, given the plethora of Ca^{2+}-responsive molecular targets, as noted above, will be the deconstruction of how specific codes of Ca^{2+} signaling culminate in specific cellular outcomes. Making real progress on this broad aim, in health and disease, is likely to involve not only molecular genetics and cellular and imaging approaches but also biophysical and modelling studies of the kinetic interactions between Ca^{2+} and its myriad substrates. That this simple cation

can regulate so much fundamental biology is almost incredible and as such provides challenges that will likely keep the community occupied for some time to come.

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