



# The Role of Vesicle Trafficking and Release in Oligodendrocyte Biology

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## Abstract

Oligodendrocytes are a subtype of glial cells found within the central nervous system (CNS), responsible for the formation and maintenance of specialized myelin membranes which wrap neuronal axons. The development of myelin requires tight coordination for the cell to deliver lipid and protein building blocks to specific myelin segments at the right time. Both internal and external cues control myelination, thus the reception of these signals also requires precise regulation. In late years, a growing body of evidence indicates that oligodendrocytes, like many other cell types, may use extracellular vesicles (EVs) as a medium for transferring information. The field of EV research has expanded rapidly over the past decade, with new contributions that suggest EVs might have direct involvement in communications with neurons and other glial cells to fine tune oligodendroglial function. This functional role of EVs might also be maladaptive, as it has likewise been implicated in the spreading of toxic molecules within the brain during disease. In this review we will discuss the field's current understanding of extracellular vesicle biology within oligodendrocytes, and their contribution to physiologic and pathologic conditions.

**Keywords** Exosomes · Microvesicles · Extracellular vesicles · Sphingolipids · Myelin · Oligodendrocytes · Axon-glia communication · Demyelination · Remyelination

## Introduction

Oligodendrocytes (OLs) are a subtype of glial cells found within the central nervous system (CNS), responsible for the formation and maintenance of specialized myelin membranes which wrap neuronal axons. Myelin sheath enables rapid saltatory conduction down an axon, functioning to allow synchronized signal propagation in a system with maximized spatial and temporal control. Mature myelinating oligodendrocytes may myelinate specific segments in multiple axons, each with unique demands for myelin composition [1]. The development of these vast and elaborate membrane extensions requires precise coordination for the OL to deliver lipid and protein building blocks to the correct location at the right time. OLs incorporate both internal and

external cues to adapt myelination patterns, and thus the reception of these signals also requires tight control.

Extracellular vesicles (EVs) have sparked interest in the neuroscience field because of their potential to facilitate intercellular communication, protect signaling molecules outside of the cell, and the potential to also harbor infectious agents or aberrant disease-causing molecules. Previous reviews have approached the importance of OL trafficking pathways with a particular focus on cell polarization [2] and vesicle transport during myelin formation [3]. In this review, we will provide a brief overview of vesicle-mediated signaling to and from OLs in both health and disease.

## A Brief Overview of Oligodendrocytes and Myelination

### Myelin Composition and Ultrastructure

Myelin is chemically comprised of approximately 70% lipid and 30% protein. CNS and peripheral nervous system (PNS) myelin have compatible lipid composition, however the molar ratios of lipid species enriched in myelin are distinctly unique from that found in other biological membranes. The

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most abundant myelin lipids are cholesterol, galactosylceramides (galactocerebrosides) and glycerophospholipids, with primarily saturated long-chain fatty acids that enable tight packing of lipids in the myelin sheath [4]. Myelin is a multilamellar structure characterized by electron microscopy to contain alternating electron dense and light layers, which are known as the major dense line and intraperiod line, respectively. The major dense line results from the apposition of cytoplasmic surfaces of an expanding myelinating OL process. Myelin basic protein (MBP) is crucial for stabilizing the major dense line, interacting with negatively charged lipids at the cytoplasmic surface of the lipid membrane [5, 6]. In contrast, the intraperiod line represents the apposition of the extracellular leaflets. Myelin proteolipid protein (PLP) and its splice variant DM20 are considered the primary integral proteins that enable tight compaction of normally repulsive extracellular membranes via hydrophilic interactions with other proteolipid molecules and galactocerebrosides [7, 8]. The specifics of how an OL process wraps around an axon to form concentric membrane layers within the CNS is still debated; meanwhile there is a greater consensus for myelin-wrapping by Schwann cells in the PNS. The myelin leading edge spirals and extends down the axon to form an internode, while the lateral edges become paranodal loops that participate in sodium channel clustering characteristic of an axonal Node of Ranvier. Removal of the cytoplasm and myelin membrane layer compaction initiates at the outermost layers and works its way inward toward the axon [9]. Healthy myelin sheaths enable rapid saltatory conduction of neuronal signals by decreasing capacitance and increasing resistance; however, the layers of specialized membrane maintain some level of permeability through cytoplasmic channels to permit vesicular transport and trophic support of both the myelin and axon [10, 11]. For more intensive insight into myelin dynamics, consider the review by Stadelmann et al. or the chapter by Rasband and Macklin [12, 13].

### Oligodendrocyte Origins, Migration and Maturation

Oligodendrocytes undergo many maturation states before reaching their destination and becoming a mature myelinating OL. Oligodendrocyte progenitor cells (OPCs) are proliferative and highly motile precursors derived from glia stem cells in two distinct regions, the ventral ventricular germinal zone (pMN) and the dorsal aspect of the spinal cord, during early embryonic development [14]. OPCs proliferate in response to mitogens such as platelet-derived growth factor (PDGF) and fibroblast growth factor (FGF) and migrate from the germinal foci to form a tiling distribution throughout the CNS [15]. Excess OPCs are removed by apoptosis or become quiescent, maintaining a progenitor pool capable of generating new OLs across the lifespan [16]. As they mature, OLs become less motile and adopt a more complex radial

morphology with ramified processes as they begin to produce a variety of sphingolipids and myelin specific proteins. Mature myelinating OLs are post-mitotic and non-motile with a distinct stellate morphology from fine processes in parallel or connected to myelinated internodes [17].

### Oligodendrocyte Metabolism and Susceptibility

As myelin formation requires high levels of protein production, there is significant metabolic susceptibility for OLs. Endoplasmic reticulum-protein quality control (ERQC) checkpoints ensure that only accurately translated proteins exit the ER, otherwise protein products are transported to the proteasome for degradation. When there is accumulation of aberrant proteins, this triggers the unfolded protein response (UPR) which in turn increases ERQC signaling. Left unresolved, the UPR and ER-stress lead to apoptosis. OLs are susceptible to ERQC failure and such deficits have been identified in a multitude of myelin disorders [18]. Similarly, mitochondria are involved in many pivotal functions of the cell. In addition to energy production, mitochondria play a role in many signaling processes such as calcium homeostasis and the production of molecular signals, exemplified by pro-death proteins. As such, mitochondrial dysfunction has a significant impact on glial cell health and neurological disease development and progression [19]. The review by Reddman et al. provides clarification on the intersection of autophagy, mitophagy, and lysosomal function in normal and aberrant nervous system physiology [20]. The highly organized structure of myelin crafted by OLs underscores their vulnerability to disease and the heavy impact of OL loss on neuronal function [21]. An increasing interest focuses on understanding the mechanisms of myelin-axon cross talk, particularly those involving transfer of information via membranous vesicles [22].

### A Brief Overview of Extracellular Vesicles

#### Standardized Terminology and Research of Extracellular Vesicles

Vesicle secretion is an evolutionarily conserved mechanism that can be utilized by all cell types. While originally thought to function only as waste disposal, the field is now captivated by the capacity of vesicles to deliver signals between cells. Extracellular vesicle (EV) is a generalized term used to describe highly heterogeneous populations of secreted membrane vesicles, which vary in both biogenesis and signaling cargo, which may be comprised of nucleic acids, proteins and lipids. The composition of EVs are influenced by cell type, differentiation stage, age, metabolic status and especially by disease state of the secreting cells [23, 24]. Much

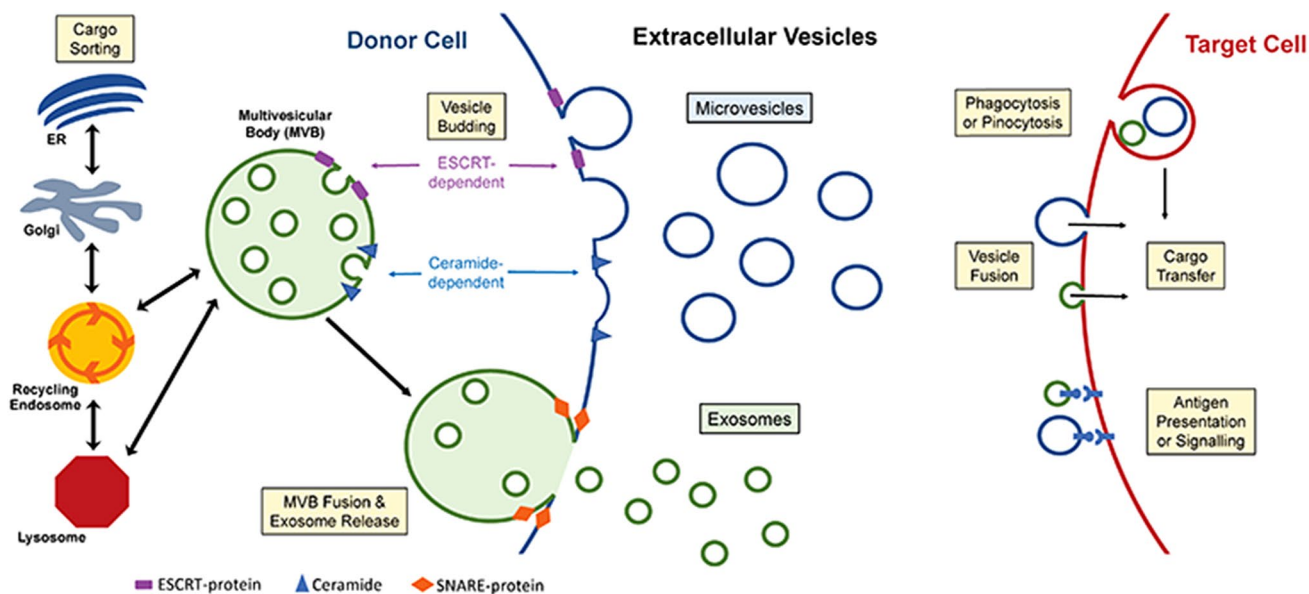
like myelin, current understanding of EV dynamics was established by biochemical analysis and electron microscopy, but there are still many unknowns surrounding the composition, biodynamics and physiological roles of EVs in health and disease. EVs can be subcategorized into 2 broad categories based on their size and biogenesis; exosomes are intraluminal vesicles between 50 and 200 nm released via fusion of a multivesicular body with the plasma membrane (PM) while microvesicles (ectosomes) are between 200 and 1000 nm and are derived from evaginations that lead to PM shedding (Fig. 1) [25–27]. For a deeper review of vesicle biogenesis, release and targeting, see van Niel et al. [28] International societies and databases have been established to consolidate the field behind likened terminology and to standardize sample collection, isolation, storage and analysis of extracellular vesicles (Table 1) [29–34].

### Machinery Involved in Extracellular Vesicle Biogenesis

The process of vesicle formation may follow many different mechanisms which are influenced by the physiology and health status of the cell, however, the progression of events that result in EV release are generalizable. First, cargo are targeted to microdomains at the site of vesicle formation, whether that be an endosomal compartment in the case of exosomes, or the PM for microvesicle-mediated delivery.

These cargo are the first regulators of vesicle formation [35], but generation and targeting of cargo to membrane microdomains is dependent on individual cellular contexts [36]. The Rab family of small guanosine triphosphatases (GTPases), in conjunction with additional effector proteins, are the best studied regulators of vesicle trafficking [37]. Cargo may originate from the Golgi apparatus, endoplasmic reticulum (ER) or through internalization at the PM before they are sorted for vesicle incorporation and secretion (Fig. 1). Cycling through the endosomal sorting compartment is pivotal for proper incorporation of cargo into exosomes or microvesicles, and as such any impairment in endosome cycling, transport, or interconnected degradative organelles will largely impact on vesicle biosynthesis and release.

Exosomes are primarily formed in the endosomal pathway through inward budding of the endosomal membrane to form intraluminal vesicles (ILVs), transforming that endosome into a newly matured multivesicular body (MVB). These MVBs are segregated for either lysosomal degradation or release into the extracellular milieu. Endosomal sorting complex required for transport (ESCRT) proteins play a fundamental role in vesicle biogenesis by clustering ubiquitylated transmembrane cargo (ESCRT-0 and -I) before microdomain budding and fission (ESCRT-III) [38]. While defects in ESCRT proteins impact the capacity of cells to produce exosomes, there are also ESCRT-independent mechanisms of ILV formation (Fig. 1) [39].



**Fig. 1** Extracellular vesicle release and uptake. Within the donor cell, cargo is sorted from various organelles and targeted for extracellular vesicle (EV) incorporation by cargo- and context-dependent mechanisms. EVs are formed by 2 major pathways: inward budding into an endosome to form a multivesicular body (MVB), or by direct outward budding of the plasma membrane (PM). Both methods employ multiple molecular components to alter membrane topology, such as

ESCRT proteins and the lipid ceramide. Vesicles released from the MVB following SNARE protein facilitated fusion with the PM are termed exosomes, while the vesicles derived from PM fission are microvesicles. Heterogenous populations of EVs traverse interstitium and systemic circulation to reach their target, where they may bind to surface receptors, fuse with the cell membrane, or be internalized by the recipient cell to execute their function

**Table 1** Resources for extracellular vesicle research

Resource	Purpose	Website	Source and support	Citation
<b>Databases</b>				
EV-TRACK	Platform to report and assess isolation methodology and characterization of EVs, as well as corresponding datasets	<a href="http://evtrack.org/">http://evtrack.org/</a>	International Consortium of EV experts outlined on their website Last updated: Sept. 2018	[29–31]
EVpedia	Database for profiles of proteins, lipids, RNAs and metabolites identified in various populations of EVs. Search and comparison tools of these datasets are also offered	<a href="http://evpedia.info/">http://evpedia.info/</a>	Pohang University of Science and Technology Last updated: April 2018	[32]
Vesiclepedia	Database of molecular readouts, including lipid, RNA and protein identified in different classes of EVs	<a href="http://microvesicles.org/">http://microvesicles.org/</a>	La Trobe Institute for Molecular Sciences Last updated: May 2019	[33]
ExoCarta	Database for profiles specific to exosomes and their cargo	<a href="http://exocarta.org/">http://exocarta.org/</a>	La Trobe Institute for Molecular Sciences Last updated: May 2019	[34]
<b>Professional societies</b>				
International society for extracellular vesicles	A professional society run by scientists that provides education, event and publication opportunities for those conducting EV research. This society also supports many national societies in the EV field	<a href="https://www.isev.org/">https://www.isev.org/</a>	Founded in 2012, with its headquarters located in the United States. The Executive Board is elected by the General Assembly at the Annual meeting	

Local production of ceramide at the PM is mediated by neutral sphingomyelinase-2 hydrolysis of sphingomyelin and contributes to microdomain formation for vesicle budding [40]. Ceramide is a bioactive lipid that confers a negative (outward) curvature of membranes owing to its biophysical chemistry. Reducing ceramide production either genetically [41] or chemically with the pharmacologic inhibitor GW4869 [40, 42] significantly decreases the capacity of the cell to release extracellular vesicles. Additionally, the formation of tetraspanin enriched membrane (TEM) domains serve as a platform for ESCRT-independent vesicle formation [43, 44]. The tetraspanin family of proteins, including CD63, CD81, CD82, and CD9 are all highly enriched in extracellular vesicles and thus serve as good biomarkers for vesicle populations.

Microvesicles, or ectosomes, are instead formed by evaginations of the PM into the extracellular milieu. There are some commonalities between the machinery employed in exosome and microvesicle biogenesis, but we are only just starting to uncover the unique mechanistic pathways involved in the creation of microvesicles. Significant changes to the abundance and distribution of both protein and lipid species in the plasma membrane are initiated to create an asymmetry that promotes domain formation for microvesicle release. Phospholipids, namely phosphatidylserine, are shifted to the external leaflet of the phospholipid bilayer to enable outward membrane bending and contribute to actin

cytoskeleton restructuring [45, 46]. The enzymes that direct these site changes include aminophospholipid translocases (such as flippase and floppase), scramblases, calpain, and lipid catabolism enzymes [47].

### Machinery Involved in Extracellular Vesicle Fission and Fusion

Microvesicles simply close off and are released from the PM, while exosomes follow a more regulated and complicated pathway to secretion. A fully formed MVB is transported along the cytoskeleton to fuse with the PM to release its ILVs as exosomes into the extracellular space. Fusion events are mediated by SNARE complexes (soluble N-ethylmaleimide-sensitive factor attachment protein receptor), which act by specific pairing of vesicle (R)- and target (Q)-SNAREs (Fig. 1). While these proteins are highly conserved, each cell type has distinct pairings, requiring context-specific investigation to understand the role of SNARE proteins in vesicle trafficking. Feldman et al. provides an extensive outline of SNARE localization and complex formation within Oli-neu cells, primary OLs and myelin isolates [48].

EVs can travel short or long distances through the extracellular matrix (ECM) and into more systemic circulation, as EVs have been found in a variety of biological fluids [49]. Targeting of the EVs is facilitated by specific surface protein interactions on the EV and recipient cell, such as

EV integrins and cellular intercellular adhesion molecules (ICAMs). It has also been demonstrated that the ECM is involved in the process of pairing EVs with their cell targets, acting as a zipper through fibronectin and laminin proteins. Many additional target pairings have been identified, based in a variety of molecular, cellular and systemic specifics [28]. Promoting selective or specific targeting of EVs is an area of intense investigation for the potential use of EVs in therapy.

## EVs as Mediators of Intercellular Communication in the Brain

There are many intrinsic characteristics of EVs which are intriguing in the context of nervous system health and disease. EVs are highly stable in the extracellular space, have the capacity to carry a wide array of molecules, are capable of crossing the blood-brain barrier [50], maintain donor cell markers to indicate their origin, and are often highjacked by invaders to evade immune detection and gain cell entry [51]. In the normal developing CNS, EVs containing morphogens such as Wnt and Hedgehog may contribute to signaling gradients [52, 53], while other EV cargo have been shown to participate in cell-fate determination [54].

However, the cargo of EVs are not always benign or beneficial. Misfolded protein aggregates are now considered as contributors to pathology in most neurodegenerative diseases. Various groups have demonstrated that EVs contain and propagate prion [55],  $\alpha$ -synuclein [56, 57], and amyloid [58] proteins, or similarly, the enzymes that lead to misfolding such as SOD1 [59]. Isolation of EVs has traditionally been limited to biological fluids such as serum or cerebrospinal fluid (CSF) of patients with neurological disorders, which have been found to contain EVs enriched in indicators of pathology [60, 61]. However, more recent studies have identified EVs enriched in indicators of pathology within the interstitium of parenchyma from animal models and human tissues of neurodegenerative diseases [59, 62, 63]. This has opened up the possibility for EVs to serve as valuable biomarkers of disease in diagnosis, prognosis and progression. Removal of pathogenic EVs may also serve to decrease overall disease burden or alter its course, but whether depletion will have this effect in patients remains an open question. On the other hand, EVs have the potential to be manipulated as targeting mechanisms for drug delivery in experimental design and therapeutics of the CNS [64, 65]. EVs may be loaded with therapeutic molecules, such as pharmacologic agents or RNAs, to target delivery to specific cells of interest. The cargo may instead be tumor antigens, so that EVs could serve to activate the immune system against patient cancer cells. Both the negative and positive potential of EVs have significant implications for clinical application.

This becomes especially pertinent to the study of neurological disorders because of the lack of reliable biomarkers and effective treatments for patients suffering from these conditions. A recent review by Basso and Bonetto provides a thorough summary of EV-mediated intercellular communication within the brain with a specific focus on neuron-glia interactions [22] while Lai and Breakefield discuss the therapeutic potential of EVs within the CNS [66].

## Vesicle Compartments of Oligodendrocytes

### Oligodendroglial Endosome Cycling

Our understanding of the mechanisms that mediate the trafficking and recycling of myelin membrane components has advanced in recent years. Myelin is not a uniform structure, but rather a highly complex and dynamic membrane with many different domains comprised of components in a ratio specific to that location. Thus, each OL tightly regulates the temporal and spatial integration of components in myelin sheath formation and maintenance.

Most major myelin proteins are cycled through endosomes as they are trafficked to myelin membranes, but each follows a distinct pathway that employs various vesicular machinery. The myelin protein PLP (found at the intraperiod line) is recycled from myelin via clathrin-independent and ESCRT-independent endocytosis and is enriched in the late endosome/lysosome (LE/Lys) compartment [40, 67, 68]. The proteins myelin-associated glycoprotein (MAG) and myelin-oligodendrocyte glycoprotein (MOG), which localize to periaxonal and outer tongue myelin, respectively, are both internalized by clathrin-dependent endocytosis. However, MAG is sorted to the LE/Lys while MOG traffics to the recycling endosome [69]. While these studies demonstrate the complex specificity of endosomal cycling for different myelin components, the mechanism to target these vesicles and the contextual relevance of specific myelinating conditions remain unanswered. Some preliminary evidence has indicated SNARE proteins as a mechanism utilized by OLs to specifically target myelin cargo. The PLP-containing vesicles in the LE/Lys compartment express the R-SNARE protein VAMP7 and are targeted to myelin membranes through interactions with target Q-SNARE proteins Syntaxin 3/ SNAP23. Conversely, the recycling endosomes which contain PLP are enriched in VAMP3 and are directed to fuse with OL soma membranes via coupling with the Syntaxin 4/ SNAP23 Q-SNARE complex [70]. The coalescence of cargo is the first event to precipitate vesicle formation, and so it is curious that such different vesicle populations with distinct machinery are formed under the same pretext. This begs the question of what other molecular signaling pathways are involved in the fate determinations for these recycled myelin



components and how are those incorporated into the vesicles that deliver the cargo to their intended destination.

The importance of lipid mediators to endosomal cycling have also been identified within OLs. Individual knockouts of the three components that comprise the enzyme complex that regulates phosphoinositides (phosphatidylinositol 3,5-bisphosphate (PI<sub>(3,5)</sub>P<sub>2</sub>)) each lead to hypomyelination due to sequestration of MAG in the LE/Lys compartment [71]. New molecular mechanisms involved in organizing and targeting myelin cargo are still being revealed, each with implications relevant to a specific set of myelin building blocks. With so many mechanisms at play, the field will need to take a systematic approach to fully understand the events involved in segregated trafficking to myelin membranes.

When endosomal cycling becomes dysregulated, there are catastrophic effects on oligodendroglial stability and myelin formation or maintenance. When the ESCRT-I protein TSG101 protein is knocked down within OLs, there was significant swelling of the endosomal compartment and lead to severe white matter vacuolation common to spongiform encephalopathy [72]. The endosomal sorting protein SNX27 is required to sequester the G-protein coupled receptor GPCR17 within recycling endosomes and prevent its degradation in lysosomes. GPCR17 plays a vital role in OL maturation, so when SNX27 is reduced, GPCR17 is degraded and consequentially, increases OL differentiation without corresponding myelin protein expression [73]. Decreased SNX27 gene expression has been reported in patients with Down Syndrome, and SNX27 knock out or knock down mice have similar deficits including cognitive impairment and hypomyelination [74]. Conversely, mutations in the N-myc downstream regulated gene (NDRG1) cause aberrant sequestration of low density lipoprotein receptor (LDLR) in oligodendroglial endosomes, resulting in decreased uptake of LDL in Charcot-Marie-Tooth disease type 4D (CMT4D). [75] NDGR1 silencing was also found to decrease OL differentiation through downregulation of the transcription factor Olig2. These factors likely impact on lipid processing in mature OLs and may explain the abnormal myelination seen in CMT4D. These examples demonstrate that regulation of endosomal compartments is pivotal to proper OL maturation and myelination, and that disruptions to these highly specialized systems which regulate trafficking can lead to devastating disease.

The endosomal compartment coordinates distribution of components within the OL, as well as those that have been internalized from the surrounding environment. OLs synthesize and recycle lipid components from myelin, but it has been shown that external sources of lipid may also contribute to myelin formation by OLs. A study that utilized astrocyte specific knock out of sterol regulatory element-binding protein (SREBP) cleavage-activating protein (SCAP), found hypomyelination of axonal segments with

increased compensatory incorporation of diet-derived sterols into myelin membranes [76]. This suggests that OLs receive lipid products from astrocytes and the environment, posing the question of how these lipids are taken up by OLs. Endocytosis of external cues is especially important to OLs, as it is strongly held that myelination is cued by neuronal activity. Different experimental approaches have been utilized to test whether the neuronal signals are relayed through points of contact with OLs or if there is a secreted factor that recruits oligodendroglial interaction and subsequent myelination [77–79]. While there are a number of repulsive signals, no robust instructive signals have been identified [80]. Neuronal release of glutamate has been shown to cause and influx of Ca<sup>2+</sup> through AMPA and NMDA receptors on OLs, leading to the release of the OL extracellular vesicle pool [81]. These vesicles are endocytosed by neurons, revealing a bidirectional route of communication between the 2 cell types. This endocytic machinery is also commonly used by viruses to infiltrate cells, surpassing the common infection pathway through viral receptors required for entry. One such example is the JC Polyomavirus (JCPyV), which is known to infect both OLs and astrocytes, despite these cells lacking the attachment receptor lactoseries tetrasaccharide c. This virus was recently shown to be packaged inside EVs, which when released from the origin cell, were able to transmit virus to naïve OLs and astrocytes while remaining protected from antisera against viral antigens [82]. This has significant implications for clinical applications of the immunomodulatory drugs used in the autoimmune demyelinating disease Multiple Sclerosis (MS). Some of these therapies increase patient susceptibility to progressive multifocal leukoencephalopathy (PML) caused by JCPyV [83]. Knowing this mechanism of viral infection provides useful information to establish more reliable metrics to predict risk of PML and can inform the development of therapeutics to decrease viral load for patients on immunomodulatory therapies. Introducing a foreign invader, such as JCPyV, into the complex and dynamic endosomal system of OLs poses a large challenge and existential threat to the cell, the myelin membranes it maintains, and the axons that they support.

### Oligodendroglial Exocytosis

The release of EVs has become an important topic for consideration in intercellular communication, broadening the scale of system dynamics that were previously thought to be limited by proximity. EVs have the potential to relay signals between cells and may also serve as a window into the state of cellular health at any given time. In this way, EVs could be used as valuable biomarkers to understand nervous system cell reactivity in health and disease states [84]. OLs have been shown to release high levels of exosome-like vesicles [40, 85]. A screen of small Rab-GTPase proteins

identified Rab35 and its activating protein TBC1D10A–C as important regulators of OL exocytosis. [86] EV release from primary OLs can be induced by calcium treatment and have been found to contain major myelin proteins CNP, PLP, MBP and MOG together with proteins involved in cell-stress relief when analyzed for protein and lipid content [85]. This was taken to mean that these OL-derived EVs may provide trophic support to axons, but no testing of neuronal outcomes following EV treatment were performed in this study. The mechanism by which the EVs were formed was not determined and remains an important question; most often the EV target and functional impact is of primary interest. EVs isolated from rat primary OLs demonstrated an autoregulatory effect that lead to decreased cell surface expansion and myelination through Rho-ROCK-myosin interactions on the receiving OL [87]. Interestingly, these OL-derived EVs counteracted the pro-myelinating effect of neuronal conditioned medium. As mentioned above, neuronal activity is known to influence OL myelination of axons, but this would suggest that communication from other OLs may supersede the neuronal signals to myelinate.

Microglia are a likely candidate to receive EVs due to their function as surveillance cells of the CNS. One study described a Major Histocompatibility Complex (MHC)-II negative subpopulation of microglia that preferentially took up EVs derived from Oli-neu cells via micropinocytosis, without subsequent immune activation [88]. This finding adheres to the common notion that EVs have low immunogenicity, recognized as “self-antigens” when presented to antigen presenting cells (APCs). However, this may change when the same vesicular machinery is being utilized for viral uptake and secretion. A recent study characterized OPCs as antigen presenting cells that are targeted during demyelination [89], and it has been previously demonstrated that EVs from APCs express MHC molecules to stimulate immune activation [90]. These ideas taken together evoke interesting implications for the potential of EVs to propagate antigen presentation in autoimmune demyelinating disorders.

There are many different disease states that affect lipid processing, and as such have a substantial impact on both myelin and vesicle production by OLs [38]. In Krabbe Disease, decreased lysosomal galactosylceramidase activity leads to the toxic accumulation of psychosine (galactosylsphingosine) and global demyelination. Psychosine inserts itself into membranes, disrupting normal fluidity and architecture [91, 92]. These membrane changes were shown to cause myelin membrane vesiculation and an increase in OL vesicle release in both primary OLs and CG4 cells [93]. In the lysosomal storage disorder Niemann-Pick type C1 (NPC), cholesterol accumulates in the LE/Lys compartment resulting in demyelination. In a similar fashion, this leads to increased extracellular vesicle release by Oli-neu cells and patient fibroblasts [94]. Both of these disorders

lead to significant changes in lipid composition in cellular membranes, with consequential increases in EV production. Whether the vesicle secretion is occurring because of changed membrane dynamics or as a purposeful mechanism to unburden the cell remains an open question. By either of these mechanisms, the effect of these lipid-rich EVs on recipient cells is likely to be similarly taxing.

## Myelin Damage and Vesiculation

When myelin membranes become damaged, the physicochemical properties of the charged membranes cause them to fold on themselves, which has been aptly named myelin vesiculation or vacuolation [95]. Damage to myelin in Multiple Sclerosis has been described as having “intramyelinic blebs” which represent lamellar separation and edema. This can affect long stretches of internodal myelin, leaving some portions with an intact lamellar structure [96]. Many neurodegenerative disorders exhibit myelin pathology and loss, with similar histopathologic patterns of myelin decompaction, vacuolation, and fragmentation with vesicles present on both periaxonal and surface myelin [97, 98]. These structural alterations eventually give way to myelin dissolution and debris recruiting phagocytic cells with significant consequences for the exposed axon. Specific proteins found in myelin debris have been shown to decrease OPC differentiation through the Fyn-RhoA-ROCK signaling pathway, thus preventing remyelination from occurring [99]. It is interesting to consider that both OL-derived EVs and myelin debris decrease myelination through a common signaling pathway.

## Future Directions

The field of EV research is growing rapidly, with constant advancement in our understanding of both physiologic and pathologic EV function owing to improved methodologies, data collection standards and analytics. The cellular cross-talk that is potentiated by EVs as well as the propensity for EVs to harbor toxic entities involved in neurodegenerative disease underscore the importance of this research. Many questions still remain regarding the mechanisms that direct cargo to sites of vesicle formation, as well as the targeting of those vesicles from donor to recipient cells. Not only are vesicle populations and their cargo heterogeneous, but their function as effectors to mediate or potentiate disease has been clearly demonstrated in many disorders, including those of the central nervous system. Thorough investigation of EV contents and the pathway they follow from biogenesis on through secretion and target cell uptake is required to elucidate the relevance of EVs and how they may be manipulated to improve outcomes. Furthermore, common and context-specific EV biology will be clarified

as more studies follow recommended standards for EV collection, isolation, characterization and application. The foundation for EV research has been laid using in vivo and in vitro model systems, which have more reliable experimental paradigms to understand EV biogenesis, targeting and impact. EVs are found in most biological fluids and have been isolated from both healthy and diseased patients; given their stability, EVs have a high potential to serve as valuable biomarkers to monitor neurodegenerative disease progression and treatment efficacy. While there have been a number of preliminary studies which isolated EVs from biological fluids of patients with CNS disorders, research on the human implications for EV biology require much more investigative effort. Scientific interest in the study of EVs has expanded rapidly over the last decade, burgeoning improved tools and methodologies to better understand their biologic relevance. Improved standardization and sensitivity will continue to impact on the field's understanding of the role that EVs play in both human patients and models of disease.

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