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Delivery on call: exosomes as "care packages" from glial cells for stressed neurons

Introduction

In the nervous system, glial cells actively participate in numerous processes. The view of glia as originally proposed by Rudolf Virchow as a kind of glue that sticks together the brain has long been outdated. In fact, the list of glial capabilities is expanding continuously. Microglia, astrocytes and oligodendrocytes are of fundamental importance for the development as well as routine functions of the mature brain. They regulate axonal outgrowth, supply neurons with nutrients, modulate signaling events, provide electrical insulation (myelin formation) and participate in immune responses. Coordination of these processes depends in particular on the communication between glia and neurons. According to the classical view, intercellular communication occurs either through direct cell contact or paracrine action of soluble mediators. Research in the last decade revealed a novel mode of cell communication that involves the exchange of secreted extracellular vesicles between cells. These vesicles contain a collection of biomolecules, which can be transported as a complete package to target cells. After delivery, the molecules can operate in the recipient cell [13]. Recently, a role for these vesicles as shuttles for biomolecules between cells of the nervous system has been suggested [5].

In general, vesicles released by cells are termed extracellular vesicles (EVs) and differ in origin, composition and size. These include shedding microvesicles pinching off directly from the plasma membrane, secreted exosomes originating from the endosomal system, and apoptotic bodies [11]. While the latter are released during apoptosis, microvesicles and exosomes are secreted by healthy cells (**Fig. 1**). A mixture of EVs is detected in nearly all body fluids, i.e. in plasma, urine, or in cerebrospinal fluid (CSF). Due to their cell type-specific content, they exhibit great potential as biomarkers in noninvasive diagnostics. A revolutionary breakthrough in EV research was the discovery of ribonucleic acids in the vesicular cargo and, importantly, the proof that RNA molecules are functional and impact target cells upon internalization. The transfer of mRNA leads to newly synthesized proteins (possibly not represented in the recipient cell proteome before), whereas miRNAs inhibit the production of certain endogenous proteins. Thus, horizontal transfer of genetic information by EVs can affect the target cell phenotype.

In principle, all types of neural cells generate EVs, which are detectable in both the CSF and the supernatant of cultured glial cells and neurons. However, to which extent these vesicles take part in cell–cell communication in the CNS is only rudimentarily understood to date. Recent advances shed light on the role of exosomes in the reciprocal interaction of oligodendrocytes and neurons. Briefly, electrically active neurons stimulate exosome secretion by oligodendrocytes, which in turn are internalized by neurons enabling them to sustain stress conditions. In the context of CNS complexity, the vesicle-dependent cell communication opens up interesting and versatile perspectives.

Origin of microvesicles and exosomes

In a homeostatic state, cells mainly secrete microvesicles and exosomes in constitutive and regulated fashion (**Fig. 1**). Microvesicles pinch off from the plasma membrane and exhibit a heterogeneous size distribution (up to 1000 nm in diameter). Exosomes are smaller (50–100 nm) and stem from the endosomal system. They relate to the intraluminal vesicles (ILVs) of multivesicular endosomes (MVEs) and are secreted into the extracellular milieu upon fusion of MVEs with the plasma membrane. Thus, the biogenesis of exosomes requires the formation of MVEs, which are generated by inward budding of the limiting endosomal membrane. Protein sorting to exosomes takes place at the endosomal membrane involving the ESCRT (endosomal sorting complex required for transport) complex. In addition, the enzyme sphingomyelinase catalyzes the local generation of ceramide, which facilitates the budding of ILVs in an ESCRT-independent fashion. Moreover, a class of transmembrane proteins, so-called tetraspanins, may also participate in the process of ILV formation. The docking of MVEs to the plasma membrane is a prerequisite for subsequent fusion and secretion of exosomes and is controlled by small GTPases of the Rab family (Rab11, Rab27 and Rab35). Exosomes contain characteristic lipids, various RNA species (mainly mRNA and miRNA), endosomal proteins, tetraspanins, integrins and heat shock proteins. Furthermore, exosomes carry cell type-specific components reflecting the identity as well as the state of the host cell but lack ER and mitochondri-

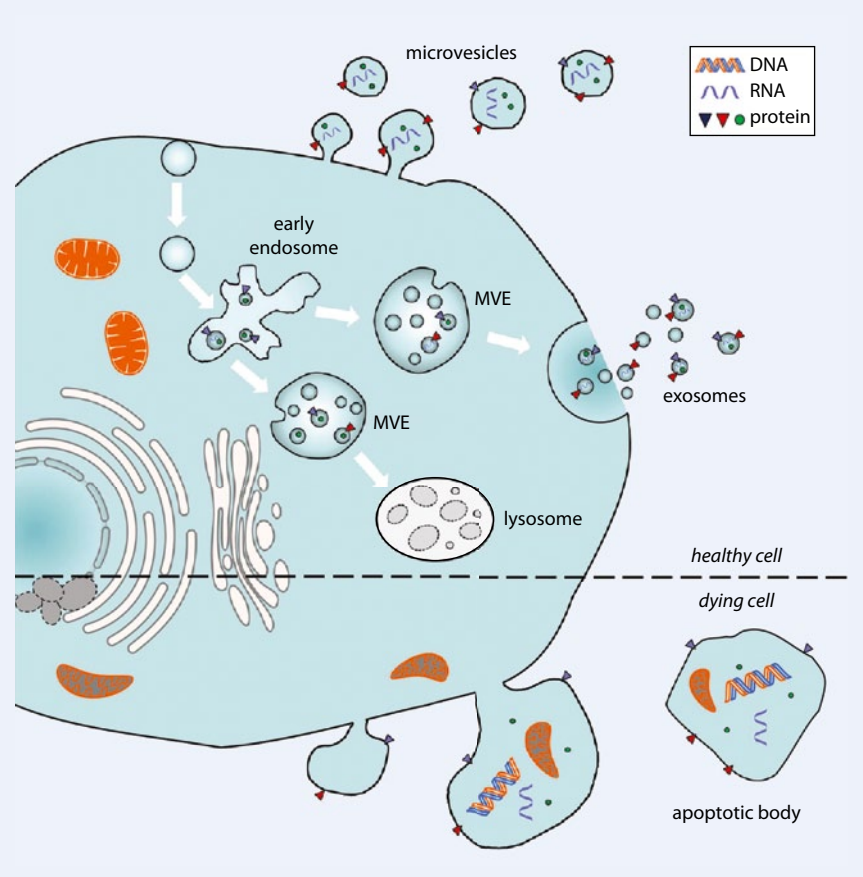

Fig. 1 ▲ Mechanism of exosome and microvesicle secretion. Microvesicles shed directly from the plasma membrane, while exosomes derive from the endosomal system and are released upon fusion of MVEs with the plasma membrane. Other MVEs fuse with lysosomes for degradation. Both exosomes and microvesicles carry proteins and RNA. Dying cells release apoptotic bodies containing DNA, RNA, and fragments of mitochondria

al proteins. In comparison, less is known about the composition of microvesicles. However, factors playing a role in exosome biogenesis seem to be also involved in the formation of microvesicles. Thus, the discrimination between these vesicles in biofluids is challenging and according to present standards should include the determination of combined markers, density and size.

General biological functions of exosomes

Since their discovery about 30 years ago, numerous functions have been attributed to EVs. One of the first described functions was the elimination of nonrequired membrane proteins via exosome secretion during cell maturation of reticulocytes. By this means, cells can release obsolete cellular components in order to avoid an overload of the intracellular recycling system. It has long been known that microvesicles derived from thrombocytes play a major role in coagulation. Research on the functions of EVs in cell communication originated in the fields of immunology and tumor biology [1]. In the immune system, antigen-presenting cells and B cells secrete exosomes carrying MHC class II molecules, which are able to stimulate T cells suggesting a role in the adaptive immune response. Tumor cells release a proportionally large bulk of microvesicles and exosomes, which appear to be heterogeneous in composition and function. On the one hand, they can trigger tumor suppressive immune responses by the interaction with antigen-presenting cells. On the other hand, tumor-derived extracellular vesicles are also able to enhance tumor growth and metastasis by influencing the tumor microenvironment. Since tumor-derived exosomes circulate in the bloodstream, they may provide prognostically relevant information on tumor progression and therapeutic success in noninvasive diagnostics. This is also the case for microvesicles and exosomes released by aggressive brain tumors. In fact, in some cases glioblastoma-derived vesicles carrying tumor markers are capable of passing the blood–brain barrier and are detectable in the circulation.

Furthermore, exosomes have been implicated in fundamental developmental processes, for example in spreading morphogens [6]. Exosomes secreted by drosophila and human cells carry the morphogen Wnt and induce Wnt signaling in target cells. In general, communication by extracellular vesicles appears to be an evolutionarily conserved route used by bacteria, fungi, plants, invertebrates and vertebrates. Unicellular parasites such as leishmania, trypanosoma and plasmodia take advantage of exosomes to communicate with host cells and to transfer virulence factors.

Relevance of exosomes and microvesicles in the nervous system

In the nervous system, evidence exists that vesicles are transferred among neurons as well as between glial cells and neurons ([5] and references therein). Transfer of neuronal exosomes is mainly discussed in the context of synaptic plasticity. Substantial observations were made in the fruit fly. At the Drosophila larval neuromuscular junction presynaptic exosomes are employed to transmit the signaling protein Wnt and its receptor protein Evi/WIs as well as synaptotagmin 4 to post-synaptic muscles. Synaptotagmin 4 transfer enables retrograde signaling, which in turn controls synaptic growth. Furthermore, an involvement of exosomes in synaptic processes has also been suggested for the mammalian nervous system. Cultured cortical neurons release exosomes bearing AMPA receptor subunits in response to glutamatergic activity possibly resulting in a reduction of functional postsynaptic AMPA receptors and excitability of postsynaptic neurons. Thus, activity-dependent release of exosomes from neuronal

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Abstract
Communication between cells is a basic requirement for proper nervous system function. Glial cells execute various functions, operating in close coordination with neurons. Recent research revealed that cell communication is mediated by the exchange of extracellular vesicles, which are also secreted by glial cells and neurons. Extracellular vesicles comprise exosomes and microvesicles, which deliver proteins and ribonucleic acids to target cells. As a result of transfer, the vesicle cargo components can modulate the phenotype of recipient cells. Here, we discuss the characteristics and functions of extracellular vesicles in general and in particular in the central nervous system, where myelinating oligodendrocytes release exosomes in response to neurotransmitter signals, which are internalized by neurons and exhibit neuroprotective functions.

Keywords
Oligodendrocytes · Extracellular vesicles · Exosomes · Cell–cell communication · Axon–glia interaction

postsynaptic somatodendritic areas might regulate signal transmission intensity and represent a mechanism of synaptic plasticity [2]. Hence, exosomes may be implicated in transsynaptic communication in vertebrates and invertebrates.

In addition, microglia and all types of macroglia secrete exosomes and/or microvesicles. Microglia are the resident macrophages in the brain, which maintain tissue homeostasis. After activation, they participate in immune defense and tissue repair. Astrocytes are part of the blood–brain barrier, regulate the extracellular ion balance and partake in repair and scarring processes after brain injury in concert with microglia. Upon activation of $P2X_7$ receptors, microglia and astrocytes shed microvesicles in response to purinergic signals [10]. Microglia-derived microvesicles amplify neurotransmission and thus impact synaptic activity of neurons. Intriguingly, microglial microvesicles carry the pro-inflammatory cytokine interleukin-1β and may induce and propagate inflammatory reactions in the CNS. In the healthy brain, various types of vesicles derived from all glial cells are detectable in the CSF. Upon inflammation, the amount of microglial MVs increases dramatically, for example in the case of multiple sclerosis.

Astrocytes generate a highly heterogeneous population of EVs. However, their function is poorly understood yet. In addition to different protective or trophic factors, astrocyte-derived EVs include enzymes of the energy metabolism and even mitochondrial components. Hence, they are associated with protective functions or metabolic support for neurons.

Oligodendroglial exosomes and axon–glia communication

Oligodendrocytes generate the myelin sheath enabling saltatory impulse conduction. A continuous and reciprocal signal exchange between glia and neurons facilitates the enwrapping of axons with a multilayered membrane and is essential for the formation of a functional axon–myelin unit. Maintenance of axonal integrity necessitates the support of oligodendrocytes and interruption of this external supply causes axonal degeneration. Glial support may depend on the delivery of energy-rich substrates to the axon via specific transport channels. After myelination, oligodendrocytes obtain their energy mainly from glycolysis and may provide glycolytic substrates such as lactate to energy-demanding axons [8]. In addition, axonal maintenance possibly requires the transfer of oligodendroglial exosomes to axons [4, 7].

Oligodendrocytes secrete exosomes upon fusion of MVEs with the plasma membrane (**Fig. 2**, [5]). In addition to the classical exosome-associated proteins and RNAs, oligodendrocyte-derived exosomes comprise myelin proteins such as PLP and CNP as well as several enzymes. These exosomes can be internalized by resting microglia via macropinocytosis without provoking any immunological or inflammatory reaction. Furthermore, oligodendroglial exosomes may have the ability to inhibit myelin synthesis in an autocrine manner. Nevertheless, nothing was known about their action on neurons. Intriguingly, electron microscopy analysis of myelinated nerve fibers reveals exosome-containing MVEs predominantly located in the innermost noncompacted layer of the myelin sheath in close proximity to the axon (**Fig. 2a**). This finding has raised the question whether exosomes are secreted into the periaxonal space and contribute to axon–glia interaction. Indeed, neurons control exosome secretion from oligodendrocytes by neurotransmitter-mediated signals. Electrical activity of excitatory neurons induces the release of the neurotransmitter glutamate, which activates oligodendroglial ionotropic glutamate receptors provoking Ca^{2+} entry and stimulation of exosome release. Receptors of the NMDA subtype seem to play an essential role in this process. Subsequently, neurons internalize the secreted exosomes at somatodendritic and axonal regions (**Fig. 3**), most likely by clathrin- and dynamin-dependent endocytosis. The uptake of oligodendroglial exosomes by neurons seems to be selective since astrocytes and oligodendrocytes internalize these exosomes only to a minor extent.

A crucial question is whether the cargo of oligodendroglial exosomes is functionally retrieved by the internalizing target neuron. In fact, cargo retrieval has been demonstrated by an experimental reporter strategy. The ectopically expressed enzyme Cre-recombinase is sorted to oligodendroglial exosomes and transported to target neurons in a co-culture assay. After transfer, Cre activates a Cre-dependent reporter gene in the recipient neuron (**Fig. 3b**). Importantly, reporter gene activation also occurs in neurons upon injection of Cre-containing exosomes into the mouse brain (**Fig. 3c**). Hence, these experiments provide a proof-of-principle that transfer of oligodendroglial exosomes occurs not only in vitro but also in the brain. This finding is strengthened by the observation that sporadic recombination of neurons in various brain regions is detectable in mice expressing Cre under the control of an oligodendrocyte-specific promoter.

What is the functional impact of exosomes on neuronal target cells? Experiments with cultured neurons showed that application of oligodendroglial exosomes

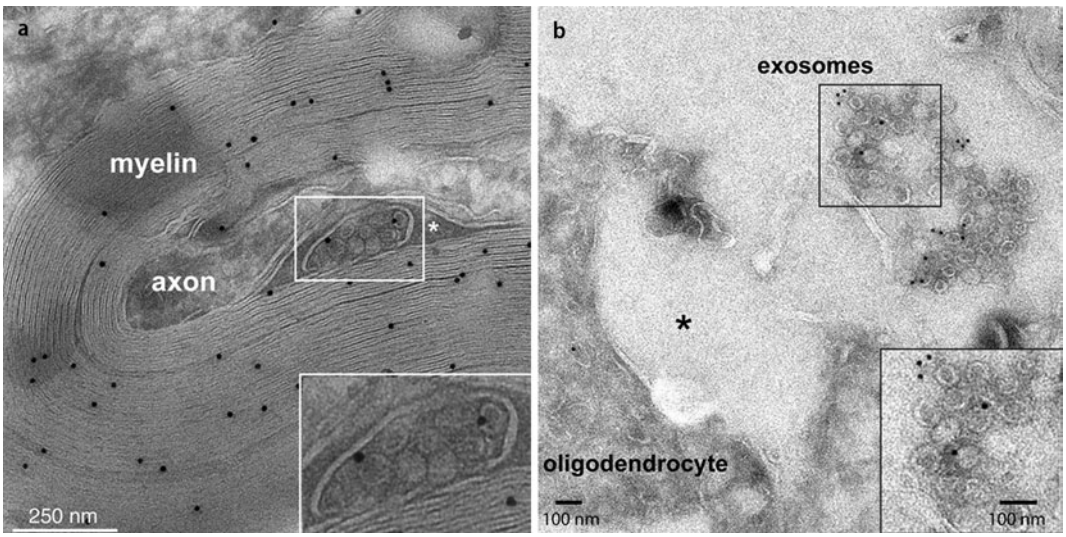


Fig. 2 ▲ Immunoelectron microscopy of exosomes and their cellular precursor stage, the MVEs. **a** Cross-section of a myelinated nerve fiber showing the profile of a MVE (enlargements depicted in insets), which is located in close proximity to the axon suggesting the release of exosomes directly to the axon. MVEs are frequently located in areas opposite to the axon. **b** Exosomes secreted into the extracellular space by cultured oligodendrocytes (*asterisk* marks the potential fusion site of the former MVE). Oligodendroglial exosomes exhibit a highly homogeneous appearance and are smaller than 100 nm in diameter. (Adapted from [3, 4])

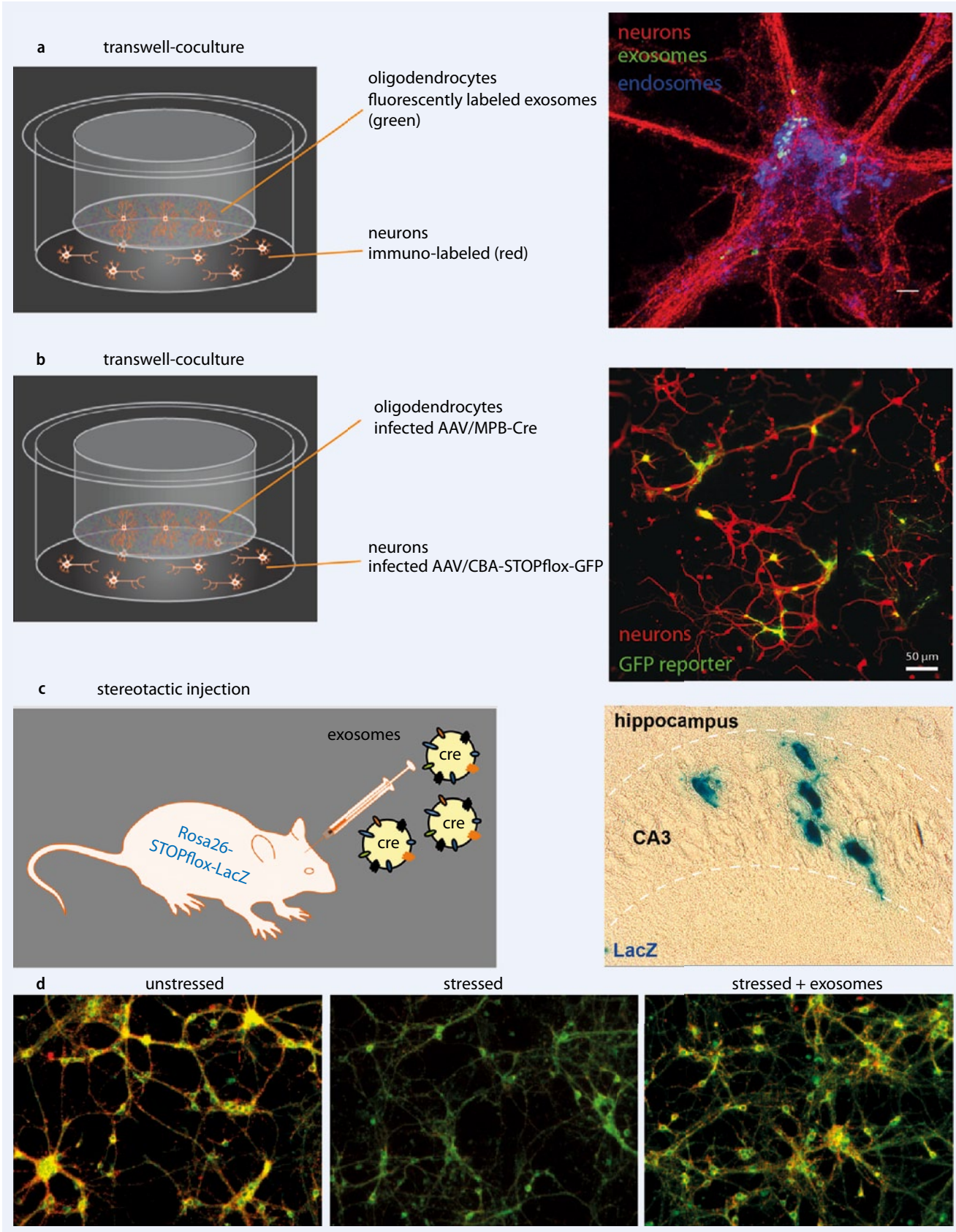
improves neuronal metabolic activity particularly in case of nutrient deficiency or oxidative stress (◘ **Fig. 3d**). In the peripheral nervous system (PNS), evidence suggests that myelinating Schwann cells ship supportive substances to axons via exosomes facilitating regeneration in the case of an injury. Oligodendroglial exosomes thus seem to act as "care" packages with neuroprotective properties, following the principle "delivery on call" (◘ **Fig. 4**). Since electrically active neurons are especially prone to energy deficits and oxidative stress, it appears reasonable to link exosome secretion to neurotransmitter signaling. Hence, active neurons signal to oligodendrocytes that they require external supply with biomolecules. Subsequently, "care" packages in the form of exosomes deliver metabolites, protective proteins, such as heat shock proteins and glycolytic enzymes, mRNA and miRNA, which presumably support the axonal metabolism and thus contribute to axonal maintenance and integrity [4].

Knockout mice lacking the genes encoding the myelin proteins CNP and PLP exhibit phenotypic characteristics demonstrating that trophic support of neurons is provided by oligodendrocytes. These mice develop axonal swellings and subsequent secondary axonal degeneration [9]. Since both proteins are packed into exosomes, one could speculate that their function in glial support is related to exosome transfer. For instance, the absence of PLP and CNP might influence exosome secretion and hence the supply of axons with trophic factors. The analysis of oligodendroglial exosome secretion in PLP and CNP knockout mice will help to clarify this issue. Furthermore, the functional components with supportive effects on neurons need to be determined. Exosomes comprise a range of potentially protective substances such as stress-alleviating proteins and RNA species. To date, it is open whether RNA transfer via exosomes indeed results in local translation in the axon.

Clinical relevance

Utilizing the strategy of a Trojan horse, vesicle-dependent transfer of proteins might foster the spreading of CNS pathologies. Pathogenic proteins, for example prions, amyloid beta, superoxide dismutase (SOD1), and tau, are released in association with exosomes. These proteins exhibit the ability to promote the formation of aggregates, which cannot be degraded by cells. By means of exosome transfer, pathogenic proteins may be able to spread like infectious agents throughout the nervous tissue [12]. How-

Fig. 3 ▶ Uptake and recovery of glial exosomes by neurons. **a**, **b** Exosome transfer from oligodendrocytes to neurons can be demonstrated in a transwell culture system enabling exchange through the culture medium without direct contact between both cell types (cartoon on the *left*). Oligodendrocytes grow on a filter membrane with pores of defined size allowing exosomes to pass. Neurons are placed in the subjacent culture chamber. Specific labeling of oligodendroglial exosomes with a fluorescent dye (**a**) or a functional enzyme (**b**) confirms the transfer to neurons (illustration of immunolabeled neurons on the *right*). **b** Infection of oligodendrocytes with a viral vector carrying Cre recombinase results in exosome-mediated transfer of Cre to neurons and Cre-mediated recombination of a GFP reporter gene in the target neurons. Expression of GFP in recipient neurons illustrates glia-to-neuron transfer and, moreover, the functional retrieval of the exosome cargo. **c** Stereotactic injection of Cre-bearing oligodendroglial exosomes into the brain of transgenic mice carrying the Cre reporter gene results in reporter gene expression in neurons (i.e. in the hippocampus, illustration on the *right*). **d** Staining of neurons with a fluorescent dye detecting energy-producing mitochondria. Neurons stressed by nutrient deprivation possess a low mitochondrial membrane potential (illustration in the *middle*, *green*). The presence of exosomes rescues the mitochondrial membrane potential (illustration on the *right*, *red* or *yellow*, respectively) and protects neurons. (Modified after [4])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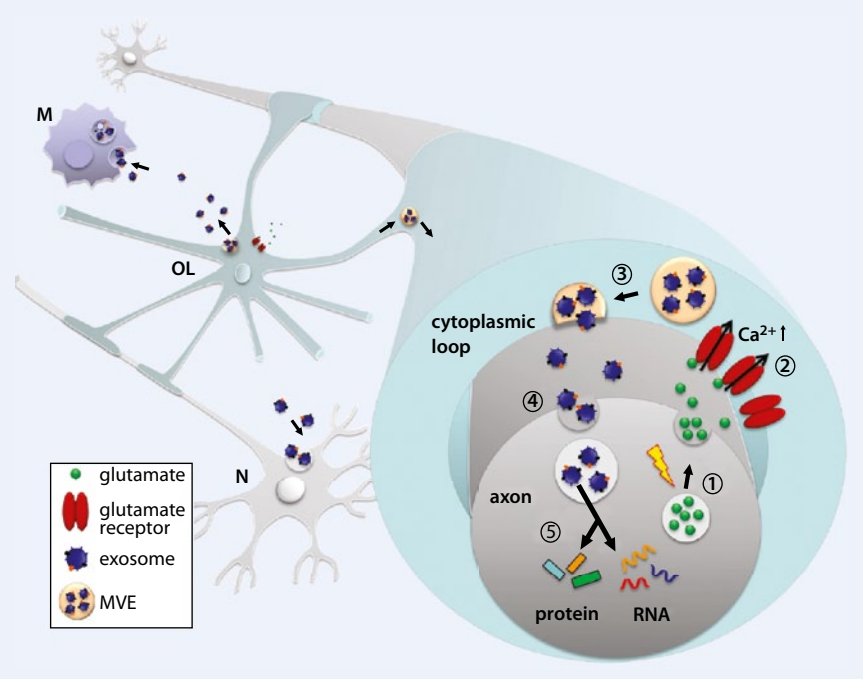

Fig. 4 ▲ Regulated release of exosomes by oligodendrocytes and their role in axon–glia interaction. Electrically active axons release the neurotransmitter glutamate (*1*) provoking calcium entry through oligodendroglial glutamate receptors (*2*). Elevated intracellular calcium concentrations trigger exosome secretion by oligodendrocytes (*OL*, *3*). Neurons (*N*) internalize these exosomes along the axon or at cell bodies (*4*) and utilize the protein and RNA cargo (*5*). Oligodendroglial exosomes are also taken up by microglia (*M*). (Modified from [4])

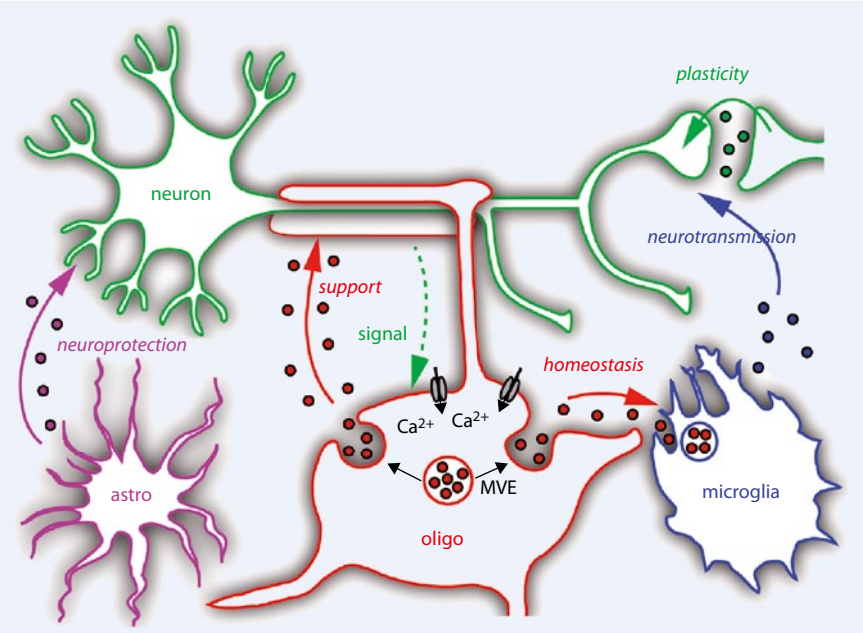

Fig. 5 ▲ Extracellular vesicles and cell communication in the CNS. Cells of the nervous system secrete different types of extracellular vesicles. Neurons release exosomes with a potential role in synaptic plasticity. Microglia modulate neurotransmission via microvesicles. Astrocytes secrete exosomes carrying neuroprotective cargo. Oligodendrocytes release exosomes in response to neurotransmitter signaling, which are internalized by neurons and provide metabolic support. Microglia degrade oligodendroglial exosomes. (Modified after [3])

ever, the mechanisms of exosome-dependent pathogen spreading (such as target cell uptake and processing) are only poorly understood. On the other hand, the tumor-promoting effects of microvesicles/exosomes derived from glioma cells are well defined. These EVs carry the oncogenic form EGFRvIII of the EGF receptor, various RNA species as well as angiogenic factors, which facilitate transformation of target cells and improve the growth conditions of the glioma tumor.

Inflammatory processes in the CNS, as occurring during multiple sclerosis, lead to increased amounts of vesicles in the CSF mostly derived from microglial cells. The relative presence of these vesicles appears to correlate with clinical symptoms and they possibly exhibit pro-inflammatory effects [10]. By all means, vesicles (and their specific content) present in the CSF are promising potential markers for the diagnosis of CNS diseases. However, collecting liquor samples by lumbar puncture is rather an invasive procedure. Whether the detection of CNS-derived vesicular cargo in blood plasma is indeed suitable for diagnostic purposes needs to be confirmed. Furthermore, exosomes may serve as vehicles for targeted drug delivery to the CNS. After systemic injection, biotechnically engineered exosomes from dendritic cells are capable to pass the blood–brain barrier and achieve RNA-mediated gene silencing in the CNS. Tropism can be assigned by targeting defined receptor molecules to the exosomal surface. Intriguingly, exosomes loaded with an anti-inflammatory drug and applied intranasally to mice reach the brain via a yet not characterized pathway, where they are subsequently taken up by microglia and reduce neuroinflammation. Although a substantial amount of fundamental research needs to be carried out on the biology of exosomes and microvesicles, extracellular vesicles bear prospects to be implemented as "cure" packages for the therapy of CNS diseases in the future.

Conclusion

In current research, microvesicles and exosomes emerge as prominent actors in the CNS (◘ Fig. 5). These vesicles influence neurotransmission, support neurons, spread pathogens, and promote inflammation. Keeping with the slogan "delivery on call", oligodendrocytes release exosomes in response to neuronal signals, which transfer protective biomolecules to neurons and possibly contribute to axonal integrity. As most of the fundamental findings presently are based on cell culture experiments, their significance needs to be validated in future in vivo, for example in genetic mouse models. The research field of vesicle-based cell communication is at the beginning and opens up exciting new perspectives in neuroscience.

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Compliance with ethical guidelines

Conflict of interest. E.-M. Krämer-Albers and C. Frühbeis state that there are no conflicts of interest.

All national guidelines on the care and use of laboratory animals have been followed and the necessary approval was obtained from the relevant authorities.

References

1. Bobrie A, Colombo M et al (2011) Exosome secretion: molecular mechanisms and roles in immune responses. Traffic 12(12):1659–1668
2. Chivet M, Javalet C et al (2013) Exosomes as a novel way of interneuronal communication. Biochem Soc Trans 41(1):241–244
3. Frühbeis C, Fröhlich D et al (2012) Emerging roles of exosomes in neuron-glia communication. Front Physiol 3:119
4. Frühbeis C, Fröhlich D et al (2013) Neurotransmitter-triggered transfer of exosomes mediates oligodendrocyte-neuron communication. PLoS Biol 11(7):e1001604
5. Frühbeis C, Fröhlich D et al (2013) Extracellular vesicles as mediators of neuron-glia communication. Front Cell Neurosci 7(182)
6. Gross JC, Boutros M (2013) Secretion and extracellular space travel of Wnt proteins. Curr Opin Genet Dev 23(4):385–390
7. Lewis S (2013) Glia: transporting cargo from A to B. Nat Rev Neurosci 14(9):589
8. Morrison BM, Lee Y, Rothstein JD (2013) Oligodendroglia: metabolic supporters of axons. Trends Cell Biol 23:644–651
9. Nave KA (2010) Myelination and support of axonal integrity by glia. Nature 468(7321):244–252
10. Prada I, Furlan R et al (2013) Classical and unconventional pathways of vesicular release in microglia. Glia 61(7):1003–1017
11. Raposo G, Stoorvogel W (2013) Extracellular vesicles: exosomes, microvesicles, and friends. J Cell Biol 200(4):373–383
12. Schneider A, Simons M (2012) Exosomes: vesicular carriers for intercellular communication in neurodegenerative disorders. Cell Tissue Res 352(1):33–47
13. Thery C (2011) Exosomes: secreted vesicles and intercellular communications. F1000 Biol Rep 3:15