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Extracellular vesicles as modulators of cell-to-cell communication in the healthy and diseased brain

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Homeostasis relies heavily on effective cell-to-cell communication. In the central nervous system (CNS), probably more so than in other organs, such communication is crucial to support and protect neurons especially during ageing, as well as to control inflammation, remove debris and infectious agents. Emerging evidence indicates that extracellular vesicles (EVs) including endosome-derived exosomes and fragments of the cellular plasma membrane play a key role in intercellular communication by transporting messenger RNA, microRNA (miRNA) and proteins. In neurodegenerative diseases, secreted vesicles not only remove misfolded proteins, but also transfer aggregated proteins and prions and are thus thought to perpetuate diseases by 'infecting' neighbouring cells with these pathogenic proteins. Conversely, in other CNS disorders signals from stressed cells may help control inflammation and inhibit degeneration. EVs may also reflect the status of the CNS and are present in the cerebrospinal fluid indicating that exosomes may act as biomarkers of disease. That extracellular RNA and in particular miRNA, can be transferred by EV also indicates that these vesicles could be used as carriers to specifically target the CNS to deliver immune modulatory drugs, neuroprotective agents and anti-cancer drugs. Here, we discuss the recent evidence indicating the potential role of exosomes in neurological disorders and how knowledge of their biology may enable a Trojan-horse approach to deliver drugs into the CNS and treat neurodegenerative and other disorders of the CNS.

1. Introduction into extracellular vesicles

Extracellular vesicles (EVs) are a heterogeneous group of membrane vesicles of endosomal and plasma membrane that are classified based on biogenesis but also upon the cell type from which they originate (table 1).

Exosomes are perhaps the best-known extracellular membranous vesicles of endocytic origin and typically measure 40–100 nm in diameter as judged by electron microscopy (EM). Exosomes were first reported in 1983 by Johnstone and co-workers [2] while culturing reticulocytes. Reticulocytes are immature erythrocytes containing cytoplasmic ribosomes and some remnants of organelles, such as mitochondria, endoplasmic reticulum, Golgi apparatus and the endosomal system. During their differentiation into mature erythrocytes, these ribosomes and other organelles are lost and several plasma membrane proteins are cleared resulting in a decrease in size and loss of some specific plasma membrane activities [7]. Exosome secretion has been described to be the mechanism involved for shedding this 'unwanted' material [7,8]. Reticulocytes themselves appear to benefit from exosome secretion, but to date the role and the fate of exosomes after secretion *in vivo* remains unclear. Exosomes contain proteins, lipids and microRNAs (miRNAs) that can mediate various signalling functions [9]. Exosomes and other EVs have been found in cell-culture media and many bodily fluids suggesting that most, if not all, cell types can produce exosomes. Exosomes derive from intraluminal vesicles (ILVs) that are formed at the limiting membrane of multivesicular bodies

Table 1. Terminology of extracellular vesicles (EVs) and subclasses.

name	size	description/formation	contents
EVs	40 nm – 1 μm	all vesicles shed by cells apart from oncosomes and apoptotic material	proteins, lipids, nucleic acids (mRNA, small RNA) [1]
exosomes	40 – 100 nm	originate through inward budding from the limiting membrane of MVBs and are released upon fusion with the plasma membrane [2]	cholesterol, ceramide, tetraspanins, GPI proteins, small RNA [3]
ectosomes, microvesicles (MV), shed vesicles/particles	100 – 200 nm	formed directly at the plasma membrane [4]	cholesterol, mRNA tetraspanins, GPI proteins [5]
oncosomes	1 – 10 μm	tumour-derived microvesicles	reflect largely plasma membrane domains of cancer cells [6]

(MVBs) through inward budding. Therefore, the luminal content of ILVs is presumably comparable to that of the cell's cytoplasm containing typical cytoplasmic biomolecules, including RNAs and protein [10]. While the true function of EVs and exosomes are still debated [11], it is clear that these vesicles can alter the physiology of the producing (autocrine) and recipient (paracrine) cells and have a major role in immune responses [12].

Exosomes have been shown to be released *in vitro* by a wide variety of cell types of haematological origin such as B lymphocytes, dendritic cells (DCs), mast cells, T lymphocytes and platelets, the latter being the precursors of the bulk of EVs found in human serum. Exosomes are also released from cells of non-haematological origin, including epithelial cells, tracheobronchial cells, hepatocytes and, the topic of this review, neuronal and glial cells [13–15]. Of direct relevance to central nervous system (CNS) disorders, tumour-derived cell-lines, including tumour cells derived from brain cells [16], produce large amounts of exosomes. In addition to neurons and glia, production of EVs from endothelial cells in the brain could potentially be a route for 'externalizing' brain-specific markers into the blood [17]. The notion that CNS-derived exosomes are released into physiological biofluids such as the cerebrospinal fluid (CSF) [18] and blood suggests that exosomes can be used as diagnostic tools due to their disease-specific content. Despite recent advances, neuronal cells seem to produce a rather limited amount of EVs compared with other cell types [19] and their composition and function are only recently becoming better understood [20].

2. Detection methods

Many optical and non-optical methods are applied for the detection and characterization of exosomes [21]. However, since the discovery of exosomes [2], the detection of EVs and exosomes has been greatly hampered due to a multitude of reasons some of which have led to misconceptions about their composition and function. Only slowly these problems are being recognized in the EV community. Owing to the biological complexity of body fluids, isolation of circulating EVs and exosomes has proved difficult. The first complication is size; exosomes are typically 100 nm and the use of standard differential ultracentrifugation techniques to isolate these EVs risks the possible co-isolation of many other subcellular particles of similar size, including lipoprotein particles, small platelets, cellular debris, protein complexes, fibrinogen and albumin. As a consequence, recovery of exosomes is hard to quantify and current isolation protocols have not been standardized [21]. A major task is to establish robust methods that discriminate between exosomes, microparticles and other types of EVs in biofluids such as urine, blood and CSF that are frequently used for biomarker studies. Differences in properties such as size, morphology, buoyant density and protein composition seem insufficient for a clear distinction [12]. Further characterization of isolated EVs by density requires supplementary techniques such as biochemical approaches, notably Western blotting. Purified exosomes float in sucrose gradients at 1.13–1.19 g ml^{-1} and are most often analysed by EM without sectioning on EM grids. Exosomes collapse during sample dehydration, resulting in a cup-shaped morphology, which is often erroneously used as a typical feature of exosomes [22]. Quickly frozen, vitrified vesicles analysed by EM show that exosomes are naturally rounded in shape [23]; nanoparticle tracking analysis allows determination of the size distribution of exosomes based on the Brownian motion of vesicles in suspension [24]. Finally, flow cytometers can be used but special high-resolution flow cytometry-based methods have only recently been developed for quantitative analysis of individual (immunolabelled)-nanosized vesicles [25].

3. Extracellular vesicles in the healthy central nervous system

While many studies refer to microparticles, exosomes or microvesicles, for the purpose of this review we will generally use the term EVs as defined by the International Society of Extracellular Vesicles [1].

(a) Oligodendrocytes and myelin

During development, oligodendrocyte progenitor cells differentiate into mature oligodendrocytes that enwrap axons forming myelin sheaths necessary for salutatory conduction of impulses. The process of myelination, which relies on two-way signalling from the unmyelinated axons and oligodendrocytes, is critical for maintaining axon integrity and axonal survival. This is clearly exemplified in demyelinating diseases where myelin damage is strongly associated with neuronal and axonal degeneration. That EVs play a role in

Table 2. EV composition in the CNS in health and disease.

cell type	health	disease
neurons	miRNA 124a, adhesion protein L1 Tsg101, Alix, GADPH, ubiquitin, HSP70, AMPA receptors, adapter protein Ndfip1	APP, APP metabolites and cleaving enzymes, prion proteins, aggregated proteins, including Aβ, α-synuclein, phosphorylated tau
oligodendrocytes	myelin proteins, HSP70, HSP90 and HSP71, metabolites, glycolytic enzymes, mRNA and miRNA	cholesterol
astrocytes	HSP70, EAAT, mitochondria, growth factors, angiogenic factors	HSP70, mutant SOD1, PARA4, ceramide, miRNA
microglia	late endosomal markers, MHC-class II, CD13	interleukins, co-stimulatory molecules IL-6, iNOS, COX-2

maintaining axonal integrity is indicated by circumstantial evidence, such as the presence of MVBs containing proteolipid protein (PLP) in the innermost myelin layers in close contact with axons during myelination [26]. Intriguingly, Rab35 was identified as one of the most prevalent Rab proteins in exosomes secreted by oligodendroglia cells [27]. Further studies by these authors revealed that the Rab GTPase-activating proteins TBC1D10B, RN-tre, TBC1D10A, TBC1D10C and TBC1D15 induced a lower release of PLP via EV. However, these regulators are not universally applicable to cells of different origin. *In vitro* application of oligodendroglial EVs improves neuronal viability while inhibiting differentiation of oligodendrocytes and myelin formation [26,28]. Also, production of oligodendrocyte-derived EVs is dramatically reduced by conditioned neuronal medium. This suggests a role for EVs in the bidirectional communication between neurons and oligodendrocytes. Detailed studies of oligodendrocyte-derived EVs reveal the presence of enzymes, chaperones and signalling molecules in addition to myelin components and exosomes markers [15,29] (table 2). While many factors may trigger EV release from oligodendrocytes, one is the neuronal activity-dependent release of the neurotransmitter glutamate that triggers oligodendroglial EV secretion through NMDA and AMPA receptors [26]. That oligodendroglia EVs are preferentially taken up by microglia that do not have antigen-presenting capacity, indicates their role in removing debris in a silent manner avoiding induction of the inflammatory response [30]. As well as secreting EVs, oligodendrocytes take up EVs released by neighbouring glia and endothelial cells [31]. These findings indicate that oligodendroglial EVs participate in a novel mode of bidirectional neuron–glia communication contributing to neuronal integrity, at least in an experimental setting. The exact impact of 'pathogenic' EV release from oligodendrocytes during disease *in vivo* requires more detailed studies.

(b) Neurons and axons

In healthy neurons, EVs may act as a well-controlled mechanism for local and possibly systemic inter-neuronal transfer of information within functional brain networks. In the CNS, movements of MVBs to synapses are tightly linked to synaptic plasticity [32] and exosomes deriving from these compartments may exert alternating functions. Owing to their molecular make-up, EVs are believed to relay complex messages, superior to those of direct cell-to-cell contacts or secreted soluble factors [20].

Several lines of evidence reveal that neurons secrete EVs and that depolarization of neurons *in vitro* dramatically increases this release. Such neuronal-derived exosomes, which contain specific neuronal markers (table 2), are detectable in the CSF [33] indicating neuronal release of small vesicles into the extracellular space. Whether such EVs reach the bloodstream is still unclear, a finding that will be crucial if EVs can act as biomarkers of CNS disorders as maybe inferred from prion-infected cells [34]. In the CNS, neuronal EVs shed at the synapses are internalized by neighbouring cells by endocytosis [10]. Support for uptake of EVs was demonstrated upon injection of oligodendroglia-derived exosomes into the mouse brain, resulting in the functional retrieval of the EV cargo in neurons. As mentioned above, supply of cultured neurons with oligodendroglial EVs improves neuronal viability under conditions of cell stress supporting the contention that EVs may play a key role in neuronal glia interactions during disease.

Moreover, secreted Wnt seems to be transported across synapses by Evi-containing EVs with characteristics of exosomes. The exosome-like vesicles contain the Wnt-binding protein Evenness Interrupted/Wntless/Sprinter (Evi/Wls/Srt) and in the *Drosophila* larval neuromuscular junctions, presynaptic vesicular release of Evi is required for the secretion of the Wnt, Wingless (Wg). Secreted Evi may also act cell-autonomously in the postsynaptic Wnt-receiving cell to target dGRIP, a Wg-receptor-interacting protein, to postsynaptic sites [35]. Although MVBs are suggested to move into the presynaptic structure [36], little is known of how MVBs fuse with the plasma membrane to transmit Wnt signals; possibly RAB11 and syntaxin 1A may have a role [37].

(c) Astrocytes

In the CNS, astrocytes form the blood-brain barrier (BBB), regulate synaptic transmission, as well as being intricately involved in neuronal growth and survival by producing neural growth factors. An important role is in scavenging extracellular glutamate through membrane excitatory amino acid transporters (EAAT). While the functions make use of cell–cell contact and production of, e.g. growth factors, emerging evidence indicates that these functions may also take place via release of EVs. As with other cell types contents of astrocyte-derived EVs vary depending on the environment (table 2) but are known to include factors involved in angiogenesis, MMPs, mitochondria, lipids [38] as well as EAAT. Under stress conditions, astrocytes also release heat shock proteins and synapsin I to maintain homeostasis [39].

(d) Microglia

Early studies demonstrated that the protein content of microglia-derived EVs includes enzymes, chaperones, tetraspanins

and membrane receptors previously reported in B cells and DC-derived exosomes. In addition, microglia-derived MV expressed CD13 and MCT-1 [40]. Intriguingly, microglia-derived EVs stimulate excitatory transmission of neurons *in vitro* and *in vivo* [41]. As discussed above, internalization of EVs by microglia occurs by a macropinocytotic mechanism without inducing a concomitant inflammatory response [30]. Microglia-generated EVs can contain MHC-class II antigens following interferon-γ treatment [40], while DC-derived EVs that contain the invariant CD74 chain may activate NF-κB signalling in microglia [42]. These studies combined clearly show that under certain conditions such EVs could augment inflammatory responses in the CNS. Upon ATP stimulation, microglia release EVs carrying IL-1β and the IL-1β-processing enzyme caspase-1 [43].

4. Extracellular vesicles in central nervous system diseases

(a) Infections

Viral infections alter the host cell composition and such changes are reflected in the composition of EV release from infected cells [44]. Several studies on viruses known to infect the CNS, including HIV-1, HTLV-1 and Epstein-Barr virus, reveal that EVs from infected cells contain viral miRNAs, viral transactivators and cytokines that can control the course of infection. That such EVs are pathogenic has been demonstrated *in vitro*, where treatment of cultured astrocytes with pathogenic HIV Tat protein decreases neuronal viability.

(b) Prion disorders

During prion disorders, the EVs released have a distinct signature containing cellular prion protein, PrP^{C}, and the abnormal infectious form, as well as specific miRNA that in some cases have specific ultrastructural features [45]. These EVs that carry proteinase-K resistant PrPsc are infectious, indicating that EVs may well play a major role in the spread of prion proteins throughout the host [34,46,47].

(c) Neurodegenerative diseases

Apart from mediating normal brain function (for which there is still little evidence), it is clear that EVs have the ability to 'spread' pathology in the brain. This is particularly relevant to prion disorders (see above) and neurodegenerative disorders, where EVs have been reported to sequester and spread pathogenic proteins such as α-synuclein, amyloid precursor protein (APP) and phosphorylated Tau, which are involved in Parkinson's and Alzheimer's diseases, respectively [48,49]. In support of this, a recent study showed that myeloid-derived MVs from Alzheimer's patients are neurotoxic *in vitro*. Such MVs promote the formation of soluble amyloid-β (Aβ) species as well as the uptake of neurotoxic Aβ into MVs [50]. In contrast to being pathogenic, there is also evidence that sphingolipid-modulated EV secretion promotes clearance of Aβ by microglia, an event relevant for Alzheimer's disease. In this case, neuron-derived EVs drive conformational changes in Aβ to form non-toxic amyloid fibrils and promote uptake of Aβ by microglia [51]. These authors also showed that EV secretion was increased following treatment with SMS2 siRNA and enhanced Aβ uptake into microglial cells thereby significantly decreasing extracellular levels of Aβ. Amyloid protein is well known to induce neuronal cell death, and amyloid peptides are known to activate caspase 3 and induced apoptosis in primary cultured astrocytes. That such apoptosis could also be mediated by EVs including exosomes indicates that they might well be crucial in the development of Alzheimer's disease (for an overview, see figures 1 and 2).

Activation of innate immune receptors by host-derived factors exacerbates CNS damage, but the identity of these factors remains elusive. Apart from secretion of misfolded protein aggregates, unconventional release of pathogenic miRNAs, in particular miRNA let-7, may also have a role. miR-let7 is a highly abundant regulator of gene expression in the CNS, which appears to activate Toll-like receptor 7 (TLR7), inducing neurodegeneration. Notably, CSF from individuals with Alzheimer's disease contain increased amounts of miR-let7b, and extracellular introduction of let7b into the CSF of wild-type mice by intrathecal injection causes neurodegeneration while this is not observed in mice lacking TLR7. These studies suggest that miRNAs can function as signalling molecules contributing to the spread of CNS damage, a process possibly mediated by RNA protected in and transmitted by EVs such as exosomes [52,53].

(d) Multiple sclerosis

Recent evidence indicates perturbed interactions between axons and myelin/oligodendrocytes in the early stages of MS [54]. In an intriguing manner, this links with the known emergence of oligodendrocyte stress at the earliest stages of lesion development. Altered communication between axons and myelin/oligodendrocytes, placing the latter under stress, may well therefore be a key part of the early changes in MS [55,56]. It is known that stressed oligodendrocytes communicate in part by secretion of EVs including exosomes containing proteins, lipids and regulatory RNAs. That oligodendrocytes in MS express HSPB5, that such expression is related to activated microglia, and that HSPB5 activates microglia *in vitro* indicates that, hypothetically, such communication between oligodendrocytes and microglia may well occur via EV transfer [57] (figure 2), although this remains to be determined. In human MS and experimental autoimmune encephalomyelitis (EAE), an animal model of MS, the numbers of EVs increase relative to microglia activation. That some of these EVs released are beneficial has been shown in EAE, where in this case exosomes in the serum of young mice have been shown to protect against disease indicating a rejuvenating role [58]. Such a protective role has also been observed in serum from pregnant animals that may explain the temporary resolution of MS during pregnancy [59]. In part, this ability of exosomes to aid oligodendrocyte differentiation is dependent on exosomes delivery of miR-219 that suppresses factors known to inhibit oligodendrocyte progenitors [58]. In addition, EVs released by DC contain miRNA species that reduce oxidative stress and improve remyelination following acute lysolecithin-induced demyelination. Such EVs are preferentially taken up by oligodendrocytes and promote remyelination, although it is unclear whether microglia can also function in this way.

(e) Brain tumours

Small vesicles emanating from cancer cells, either arising as primary brain tumours or as metastases, contain proteins

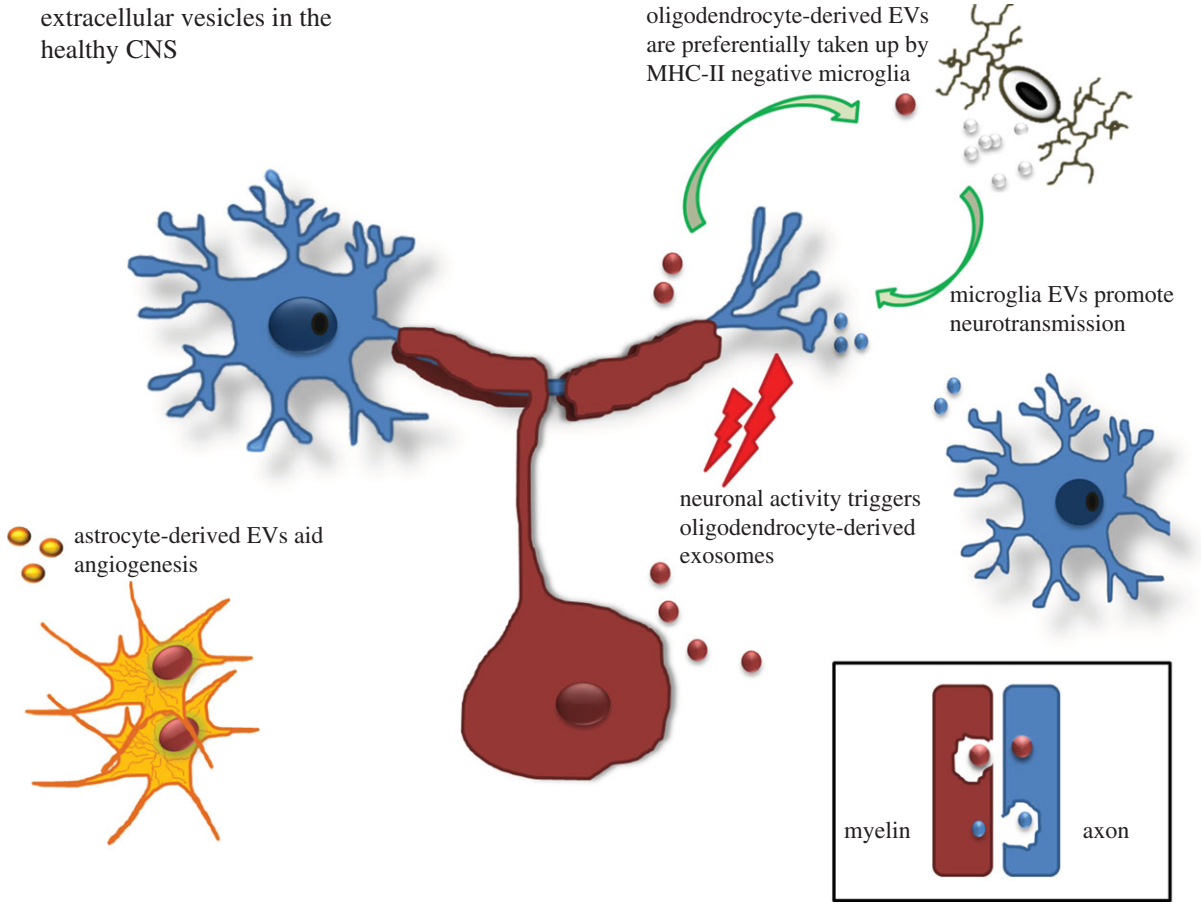

Figure 1. The role of extracellular vesicles in the healthy CNS. EVs carry signatures of the cell in question as well as specific EV-related factors. The impact of such release depends upon the cell type releasing the EVs and the cell type taking up the particles (see text for more details). (Online version in colour.)

associated with malignancy. For this reason, these exosomes are often referred to as oncosomes and act as biomarkers of the disease. For example, oncosomes may be comprised Myc, Ras, HER2 and other cancer-related molecules, including nucleic acids and proteins. Proteomic studies have been performed on metastatic and primary brain tumours and represent an attractive platform to detect and monitor brain tumours as well as study oncogenic pathways and responses to therapies. (For excellent reviews, refer to [60–62].)

(f) Lipid storage diseases

To date, little is known about the role of EVs in lipid storage diseases. In one disorder, namely Niemann-Pick type C1 disease, it has been suggested that EV release of cholesterol may be beneficial [63]. *In vitro* the authors showed that such release may serve as a cellular mechanism to partially bypass the traffic block due to toxic lysosomal cholesterol accumulation. Furthermore, it was also suggested that secretion of cholesterol by EVs helps to maintain cellular cholesterol homeostasis.

5. Exosomes as biomarkers of neurodegenerative diseases

Clinical symptoms present rather late in the pathogenesis of neurological disorders. Although underlying molecular responses are present years before the clinical onset [64], current diagnostic tools are not sensitive enough to detect these early changes.

As discussed above, EVs reflect the state of cells in the brain, both under healthy and damaged conditions, and are present in the brain's extracellular fluid. Therefore, EVs might contain crucial information about these early biochemical changes, and thus represent a new reservoir for biomarker discovery. The CSF has been shown to be a rich source of biomarkers in chronic neurological disorders, since the CSF compartment is in close anatomical contact with the brain interstitial fluid. A brief study using five patients undergoing thoraco-abdominal aortic aneurysm repair, identified exosomes in the CSF using specific antibodies in Western blotting and immune-EM. The latter technique is necessary to examine their structure (cup-shaped) as well as expression of markers [65]. In AD patients, exosomes in the CSF reveal disease-associated proteins. The pathogenesis of AD is related to the hyperphosphorylation of the protein tau. In mild AD, the proportion of phosphotau in the exosomal fraction of the CSF was significantly higher compared with non-AD controls. A preliminary analysis of proteins co-purified with tau in secreted exosomes identified several known to be involved in tau misprocessing [48]. Likewise, in MS patients, higher levels of microglia-derived exosomes can be detected compared to age-matched controls. MS can be characterized by its fulminant activation of microglia resulting in myelin damage. Interestingly, the concentration of these microglia-derived exosomes increased upon brain inflammation and was closely related with disease course, pointing to a clinical value to record disease activity [66]. In brain tumours, CSF-derived exosomes are postulated to give insights into the origin of the malignancy, possible mutations and the response

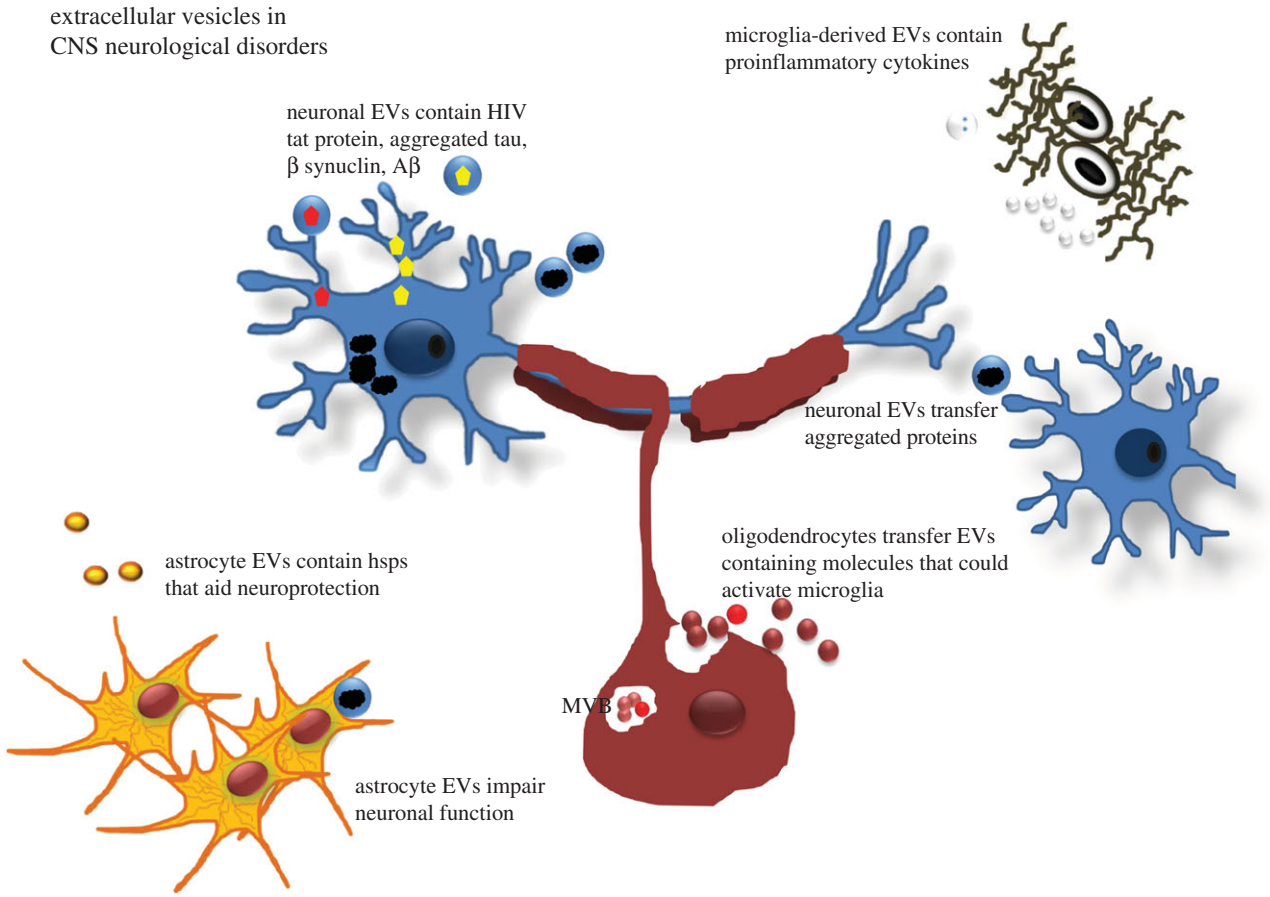

Figure 2. Extracellular vesicles in neurological disorders: proposed actions. In neurodegenerative disorders, neurons, and in some cases astrocytes, produce and release aggregated proteins such as α-synuclein, APP and phosphorylated tau and, in the case of prion disorders, pathogenic PrPSc protein. The EVs released may act as 'seeds' that spread the damage throughout the brain. In demyelinating disease, myelin-stressed oligodendrocytes produce altered myelin proteins and heat shock proteins (hsps) that may (hypothetically) be released in EVs. The 'disease-associated' proteins activate microglia that may augment disease or alternatively affect neurons and axons leading to dysfunction. (Online version in colour.)

to therapy [67,68]. Examples of molecules found tumour-derived exosomes are oncogenetic growth factors, suppressor genes and miRNAs [68]. Changes in the regulation of specific miRNAs has been described in brain tumours, but also in neurodegenerative diseases and neuropsychiatric disorders, including schizophrenia [64,67]. Interestingly, miRNAs are not only detectable in the CSF, but are also present in serum, plasma and urine. Exosomes are able to transport these brain-specific miRNAs across the endothelial cellular layer of the BBB into the circulating blood [17]. Recently, a comprehensive inventory of the EV proteome in human CSF was compiled, revealing enrichment of exosome markers, heat shock proteins, as well as brain-derived proteins, underscoring the biomarker potential of EVs [33]. In this way, brain-specific information can be obtained in a non-invasive manner, underlining exosome-profiling as a perfect candidate for diagnostic tool in the early onset of neurological diseases.

6. Therapies

Since it is likely that EVs contribute to the local propagation of neurodegenerative diseases, targeting EVs or exploiting the nature of EVs as natural carriers of miRNAs and drug delivery devices has been investigated. Neurons communicate through the secretion of EVs contributing to local synaptic plasticity, but EVs may also allow longer range communication within the CNS and influence neuronal networks located at a distance [20]. Targeting EVs directly to sites for inhibiting deleterious effects seems an attractive approach. Adeno-associated viruses (AAVs) encapsulated in EVs that harbour viral capsid proteins can deliver genetic cargo into recipient cells. Gene delivery is dependent on the AAV transgene and not EV-bound mRNA or protein transfer [69]. Indeed, viruses have frequently been used in modified form for drug delivery purposes, however, we suggested previously that naturally produced exosomes equipped with specific viral proteins may serve as optimal delivery devices for functional RNA [70,71]. In an experimental model of stroke in rats, intravenous administration of cell-free MSC-generated exosomes has been shown to greatly improve functional recovery as well as enhancing neurite remodelling, neurogenesis and angiogenesis [72]. EV-mediated delivery offers multiple advantages as these vesicles are biocompatible, can be autologous (i.e. patient-derived) and appear to have the unique ability to cross biological barriers, notably the BBB. In one breakthrough study, exosomes were harnessed with exogenous siRNA by electroporation and engineered to expose a brain-specific peptide (rabies virus glycoprotein-derived peptide). Specific mRNA knockdown was observed throughout the brain but was negligible in the liver and spleen. The therapeutic potential of EV-mediated siRNA delivery was demonstrated by protein (62%) knockdown of BACE1, a therapeutic target in Alzheimer's disease. These findings represented a first *in vivo* example of how to exploit exosome

physiology in a therapeutic setting [73]. Despite the current realization that siRNA loading into exosomes is at best very inefficient [74] and although many technical hurdles need to be overcome, targeting of exosomes to the brain, a major previous biological barrier, seems at least possible.

In another recent study, exosome-mediated transfer of miRNA (in particular, miR-124a) was shown to have a role in neuron to astrocyte signalling. Exosomes isolated from neuron conditioned medium contained small RNAs and were internalized into astrocytes, increasing astrocyte miR-124a and GLT1 protein levels. GLT1 in humans is an important glutamate transporter and its expression is selectively lost in amyotrophic lateral sclerosis (ALS) also known as Lou Gehrig's disease [75]. Intrastriatal injection of specific antisense against miR-124a into adult mice lead to a reduction in GLT1 protein expression and glutamate uptake levels in striatum without reducing GLT1 mRNA levels. miR-124a is selectively reduced in the spinal cord tissue of end-stage SOD1 G93A mice, a mouse model of ALS. Strikingly, exogenous delivery of miR-124a *in vivo* through stereotaxic injection seemed to prevent further pathological loss of GLT1 proteins in SOD1 G93A mice [76].

In conclusion, there is strong evidence that EVs, and in particular endosome-derived exosomes, contribute to homeostasis in the CNS. In CNS-associated diseases EV biogenesis, transfer or composition can alter, causing pathology. A deeper understanding of EV-mediated cell–cell communication and details on their biogenesis and release may lead to improved diagnosis and novel therapeutic options in CNS diseases.

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