Extracellular Vesicles at CNS barriers: Mode of Action

Eva-Maria Krämer-Albers*

Institute of Developmental Biology and Neurobiology, Biology of Extracellular Vesicles, University of Mainz, 55099 Mainz, Germany

* correspondence to:
Eva-Maria Krämer-Albers
Email: alberse@uni-mainz.de
Phone: +49 6131 39 26257

Summary

The exchange of molecules between the brain and periphery is limited by cellular barriers such as the blood-brain barrier (BBB) and the blood-CSF barrier (BCB). Extracellular vesicles (EVs) secreted by brain cells or circulating in the blood stream interact with these barriers and provide a pathway for brain-periphery communication. This review briefly summarizes the main current concepts of EVs signaling over the BBB/BCB. EVs can either be released by barrier cells upon stimulation, act on barrier cells modulating barrier properties, or cross the barrier transferring cargo between the circulation and the brain. The mechanisms of EV signaling and passage over the BBB are increasingly being explored, with inflammation being a main driver. EVs acting at or through the barriers possess wide-ranging effects on brain-periphery communication in both healthy and pathological states. A deeper understanding of the mechanisms of action is important for translation into biomedical applications for brain diseases.

Key words: extracellular vesicles, exosomes, blood-brain barrier, blood-CSF barrier, cross-organ communication, brain endothelial cells

Short title: Extracellular vesicles interacting with CNS barriers

Introduction

Extracellular Vesicles (EVs) are released from cells by budding off the plasma membrane or secretion from multivesicular bodies (MVBs) and mediate cell-cell communication by interaction with target cells [1]. EVs are involved in both short-distance paracrine signaling and long-distance interorgan communication and, accordingly, are found in all body fluids and the bloodstream [2]. Enclosed by a membrane, EVs are filled with cytoplasmic cargo including proteins, lipids, nucleic acids and other bioactive molecules, which reflect the parental cell physiological state and can have multiple biological effects upon delivery to target cells. The collective term EVs comprises both small EVs (exosomes secreted from MVBs and plasma membrane-derived small EVs) and larger EVs budding from the plasma membrane (microvesicles or microparticles) [3].

In the nervous system, EVs are exchanged between neurons and glial cells contributing to CNS homeostasis and are playing important roles in neuroinflammation and neurodegeneration [4-7]. There is ample evidence to suggest that CNS-derived EVs enter the blood and vice versa, and that EVs play a key role in brain-periphery communication in physiology as well as pathology. Although it is well accepted in the current literature that EVs cross the blood-brain barrier (BBB), it is not clear how, where, and when EVs can overcome this tightly controlled cellular barrier. What mechanisms do EVs use to promote brain-periphery interaction and to pass the BBB and which factors regulate these processes? Extensive research has been conducted on these topics in recent years, despite existing technical challenges in the field of EVs related to the discrimination of distinct EV subtypes, *in vivo* imaging of nanosized EVs, and the availability of genetic models that efficiently and specifically interfere with EV release or transfer. This article summarizes the recent advances and provides an overview of EV signaling at CNS barriers highlighting the main concepts EVs employ in brain-periphery interaction (for more in depth reading, please refer to recent reviews by Ramirez et al., Saint-Pol et al., Pauwels et al., Busattoet al. [8-11].

CNS barriers

CNS barriers separate the bloodstream from the brain parenchyma and the cerebrospinal fluid (CSF). They control the selective entry of substances to protect the brain and regulate brain homeostasis. Three types of brain barriers are distinguished according to their anatomical location and morphology: the BBB, the blood-CSF-barrier (BCB), and the arachnoid barrier [12,13]. The latter will not be further considered here since its involvement in EV signaling and brain-periphery communication has not been reported. The BBB is believed to represent the main barrier in terms of length and protection of the brain from entry of blood-borne substances. It elaborates along brain capillaries and is formed by specialized brain

microvascular endothelial cells (BMEC), whose intercellular spaces are sealed by tight junctions and adherens junctions strongly limiting paracellular transport to small hydrophilic molecules such as water. Pericytes embedded in a basement membrane and astrocytic endfeet cover the basal membrane of BMECs. Together with perivascular neurons, these cells constitute the neurovascular unit (NVU), where all cells directly or indirectly contribute to barrier function and properties [14]. The BCB is formed by the epithelial cells of the choroid plexus, which projects into the ventricles and is responsible for the secretion of CSF. Choroid plexus epithelial cells (CPE) are interconnected by tight junctions and face the CSF at their apical side, while blood vessels lined by a fenestrated endothelium allow for free molecular exchange at the basal side [15]. However, substances that have passed the BCB from the blood to the CSF additionally must overcome the ependymal cells lining the ventricles to enter the brain parenchyma. Altogether, CNS transport across the BBB and BCB largely occurs via highly selective transcellular pathways such as carrier mediated transport, receptor-mediated endocytosis and transcytosis. Barrier permeability is controlled by the barrier cells (BMECs and CPE) and regulated by signals they receive from the blood stream or the microenvironment such as the NVU. In response to such signals or due to mechanical impairment, barrier integrity is often lost during CNS pathology and appears to be compromised in certain peripheral conditions, such as inflammation.

EVs can interact with CNS barriers in three ways: (1) Barrier cells can release EVs that act within the barrier microenvironment, enter the brain parenchyma, or arrive in the blood stream carrying their cargo to more distant sites. (2) Brain-derived or circulating EVs may signal to the CNS barrier to modulate barrier function and properties. (3) EVs can pass the barrier either non-selectively during disease upon its disintegration or through a specific transportation process mediated by the exposure of surface molecules allowing entry through a selective mechanism. Notably, EVs may also enter the brain via the circumventricular organs (CVOs), which are located around the third and fourth ventricles and lack a typical barrier function because of the presence of fenestrated capillaries. CVOs are largely lined by a layer of specialized ependymal cells (tanycytes) limiting the diffusion of substances into the CSF [16]. However, CNS entry of EVs at the CVOs remains speculative and will most likely be the subject of future research.

EVs released by barrier cells

BMECs represent the first line and main physical barrier of the BBB. They release EVs at the apical side into the bloodstream and at the basal side towards the NVU and the brain parenchyma [17]. BMEC-EVs may thus signal to the periphery, mediate communication within the NVU or aid transcytosis of blood-bourne molecules into the brain (Figure 1). Circulating

BMEC-EVs are increased in the plasma of patients with neurological disorders such as multiple sclerosis [18] indicating that these EVs play a role in modulating BBB functions and peripheral signalling during neuroinflammation.

Proteomic profiling of BMEC-EVs collected from human immortalized hCMEC/D3 cells revealed several receptors known to be involved in transcytosis suggesting that the receptor cargo may be delivered into the brain parenchyma via EVs [19]. TNFα-treatment, which is mimicking inflammatory conditions and known to increase BBB-permeability, increases the rate of BMEC-EV release and among the proteomic cargo the abundance of proteins acting downstream of TNFα, such as NF-κB, the pattern recognition receptor PRX3 and the integrin receptors VCAM-1 and ICAM-1, reflecting the activated endothelial state [20]. Moreover, TNFα/INFγ-stimulated BMEC-EVs increasingly contain junctional proteins including claudin-5 (CLN5) [17,21]. $CLN5^+$BMEC-EVs appear to adhere to leukocytes and accumulate at the site of leukocyte attachment to the endothelium *in vivo* [21]. Possibly, VCAM-1 and ICAM-1 located on BMEC-EVs may interact with leukocyte integrins to recruit the $CLN5^+$BMEC-EVs to the leukocyte surface. $CLN5^+$BMEC-EVs were suggested to promote the interaction of leukocytes with endothelial junctional components fostering transendothelial migration of leukocytes. When isolated TNFα/INFγ-stimulated BMEC-EVs are applied to a cultured monolayer of naïve BMECs (hCMEC/D3 model), an increase of leukocyte adhesion and barrier permeability as well as the propagation of the inflammatory endothelial phenotype is observed [22**]. Thus, BMEC-EVs released by the inflamed brain endothelium affect barrier properties and appear to contribute to BBB dysfunction (Figure 1 a).

Furthermore, BMEC-EVs were implicated in regulating peripheral immune processes. Focal inflammatory brain lesions such as traumatic brain injury (TBI) or stroke elicit an acute phase response (APR) directing the peripheral immune response and transmigration of leukocytes into the brain. Injection of IL1-β into the brain parenchyma mimics such lesions and was shown to trigger the release of BMEC-EVs as well as astrocyte-derived EVs into the circulation resulting in activation of the APR in the liver [23,24]. Adoptive transfer of IL1-β-induced BMEC-EVs or astrocyte-EVs into naïve animals was sufficient to drive different aspects of the APR, such as liver cytokine production, neutrophil recruitment, and leukocyte transmigration. Moreover, IL1-β-induced BMEC-EVs were able to activate the typical sickness behaviour associated with injury or infection in mice [24]. Shedding of BMEC-EVs and vascular remodelling is also increased upon *in vitro* mechanical injury of human primary BMECs and in TBI [25]. In summary, BMEC-EVs released from the injured and inflamed brain microvasculature initiate the APR to drive the secondary phase peripheral immune response and promote leukocyte recruitment to inflammatory brain regions [26]. Thus, BMEC-EVs mediate both local effects at the BBB and systemic remote effects.

CPE cells forming the physical barrier of the BCB release EVs at their apical side into the CSF (Figure 1 b) to import nutrients such as folate into the CNS, regulate the maintenance of neural stem cells in the neurogenic niche, and transmit inflammatory signals [27-29*]. Similar to the observation at the BBB, systemic inflammation increases CPE-EV release and elicits a pro-inflammatory phenotype in astrocytes and microglia mediated by the CNP-EV miRNA cargo. In the CSF of Alzheimer disease model mice, CPE-EVs are elevated too and inhibiting EV release prevents Aβ-induced cognitive decline [30**] indicating the relevance of this CPE-EV signalling axis also in neurodegenerative disorders [31]. Interestingly, viruses can hijack CPE-EVs to enter the brain. JC polyoma virus, which causes progressive multifocal leukoencephalopathy (PML), can infect CPE cells and utilize CPE-EVs to invade the CNS and target glial cells that cannot be directly infected by free virions [32].

EVs acting on barrier cells

Barrier cells and barrier function can be modulated by EVs derived from the CNS or the circulation (Figure 2). BBB-associated cells constituting the NVU signal to the brain endothelium and appear to regulate vascular integrity under physiological and pathological conditions [33-36]. Using zebrafish larvae and cultured rodent cells it was shown that neuronal EVs foster expression of the adherens junction protein VE-Cadherin in BMECs via indirect action of miRNA-132 [33]. According to the stimulus, pericytes activated with PDGF-BB or LPS release EVs enriched with pro-regenerative growth factors or pro-inflammatory cytokines, respectively [34]. Although the effects of the distinct pericyte EVs on endothelial cells have not been studied further, these findings indicate that the pericyte phenotype is adopted by EVs and may affect the microenvironment and hence barrier function with implications for both neuroprotection and neuroinflammation. For example, under hypoxic conditions and HIF pathway activation, CNS pericyte EVs promote wound healing of endothelial cells, reflecting the pro-angiogenic activity of these EVs [35]. Together, these findings suggest that NVU-derived EVs and particularly pericyte EVs may be key regulators of brain vascular maintenance and regeneration (Figure 2 a).

EVs originating from the periphery circulating in the bloodstream interact with the brain endothelium and mainly initiate inflammatory processes. Leukocyte-EVs derived from activated platelets, stimulated neutrophils, or immune-challenged monocytes are internalized by cultured BMEC cells and neutrophil- as well as monocyte-EVs promote an inflammatory, activated endothelial phenotype [37-39]. EVs derived from transformed leukemia blast cells in addition to their uptake into the brain endothelium were able to promote blast cell transmigration across BMEC monolayers [40]. Thus, blast cell-EVs apparently contribute to the loss of BBB integrity allowing the blast cells to breach the barrier and invade the CNS. The

ability of tumor EVs to penetrate tissue barriers and to support metastasis is well described and was linked to the EV integrin profile [41]. Whether the support of leukocyte transmigration is a function limited to EVs derived from transformed cells or is also achieved by EVs of benign origin remains unknown.

In contrast to the pro-inflammatory effects of peripheral EVs considered to harm the barrier function, there are also examples of EVs improving barrier properties. These barrier-supporting EVs populations largely originate from stem cells, which exhibit regenerative or protective effects in various injuries and are used in different therapeutic settings. For example, when administered systemically *in vivo* after TBI, EVs isolated from umbilical cord blood-derived endothelial colony forming cells and mesenchymal stem cells increased BBB integrity, reduced brain edema, and stabilized tight junctions [42,43]. Similarly, neural progenitor cell-derived EVs enhance barrier properties in a stroke model *in vitro* and *in vivo* [36].

Less is known about the effects of EVs on the barrier cells of the BCB (Figure 2 b). Lymphoblast entry into the CNS may also occur at the BCB. Indeed, some lymphoblast-derived EVs were shown to promote the transmigration of their parental cells in an *in vitro* BCB model without impairing the barrier properties [44]. However, the ability to transmigrate was specific to the lymphoblast line the EVs were originating from and the overall effects of different lymphoblast-EVs on CPE varied significantly. More research needs to be performed investigating the interaction of EVs with the BCB and their contribution to the exchange between the CSF and the bloodstream.

EVs passing the barrier

It is widely accepted that EVs have the natural ability to cross the CNS barriers (Figure 3). The selectivity and prevalence of EV-transfer (including spatiotemporal aspects) are regulated by factors that are not yet fully understood. Liberation of CNS-EVs into the circulation has been observed primarily under pathological conditions associated with barrier breakdown, such as injury [23] brain tumors [45] or neurodegeneration [46,47]. Similarly, penetration of peripheral EVs into CNS-tissues is promoted by inflammatory processes [48]. EV-Transfer across CNS barriers under healthy conditions appears limited but simply maybe difficult to uncover by experimental means. Transgenic labelling of hematopoietic cell-derived EVs (using Cre-recombinase) demonstrated that *in vivo,* these EVs rarely address neurons under normal conditions but result in high frequency neuronal uptake upon peripheral inflammation or increased neuronal activity mimicking epileptic states [48,49]. Intriguingly, neuronal activity determined the regions and populations of neurons that are targeted by peripheral blood-derived EVs and neuronal uptake was even triggered by the physiological stimulus of a behavioral task [49**]. Possibly, neurovascular coupling within the NVU and the processes

within the NVU promoted by neural activity [50] contribute to the recruitment the EVs over the BBB. Intriguingly, microbiota outer membrane vesicles pass the gut intestinal barrier as well as the BBB and target the brain parenchyma, indicating that EVs may be part of the microbiota gut-brain axis [51*] .

EV-entry into the CNS appears to be selective, involving various receptors on the brain endothelium and the EV-membrane. Paracellular transport has not been described, but a number of *in vitro* and *in vivo* studies suggest transport of EVs by adsorptive transcytosis via different dynamin-, clathrin-, and caveolin-dependent endocytic transcellular routes [52-54]. CD46, transferrin receptor, C-type lectin receptors, and heparan sulfate proteoglycan have been proposed as receptors on the BMEC surface [55-58]. Uptake of macrophage-EVs by BMECs was dependent on ICAM-1 and LFA-1 present in both EVs and BMECs [58]. Comparison of the pharmacokinetics of different EVs derived from macrophages, fibroblasts, T-cells, keratinocytes, and various cancer cell types revealed that EV-types are quite diverse in terms of their entry dynamics and targeted brain regions [53*] . The factors that determine the kinetics of EV-transfer remain largely unexplored. However, it has been suggested that tumor-derived EVs increase their rate of transfer across the BBB by downregulating Rab7 to remove the break that slows transcytosis through the brain endothelium [54].

Overall, the passage of EVs across the BBB and the supportive effect of inflammation have been confirmed in several i*n vivo* and *in vitro* models. While entry into the brain appears universal for EVs of different origins, the mechanism of entry and transfer kinetics are related to specific properties of EV types as well as the surrounding milieu, such as inflammation. Whether EVs can enter or exit the brain via the BCB is unknown, but there is no reason to assume that the BCB cannot be overcome by mechanisms similar to those described for the BBB.

Conclusions

EVs emerge as key players in brain-periphery communication by modulating CNS barrier functions or delivering cargo across the barriers. EVs acting at CNS barriers exhibit versatile signaling functions and mediate tissue crosstalk in the healthy state and more pronounced upon injury and inflammation. The entry into the brain of a combined bundle of bioactive molecules and their targeted delivery to a specific cell population, which can be driven by external cues such as neural activity or local inflammatory processes, is expected to transform our understanding of brain-periphery communication and provide new insights into the mechanisms of neural plasticity and neuroinflammation. The molecular pathways of EV-barrier interactions are beginning to be unraveled and appear diverse, possibly reflecting the molecular heterogeneity of EVs and multiple evolutionary traits. Despite challenges associated

with EV-methodology and experimental modelling of CNS barrier morphological complexity, significant progress has been made by combining *in vitro* cell biology, *in vivo* modelling and transgenic strategies. Technical limitations include EV labelling with dyes that often label non-EV structures [59], classification and purity of EV particles studied, and methods to interfere with EV release that either do not affect all EV subtypes or have off target effects [3]. Awareness of these limitations is critical to the rigorous design and careful interpretation of the studies, as well as to the development of future innovations to cross these boundaries. Further advances in understanding the complex biology of EVs at barriers will on one hand, provide new insights into pathomechanisms of brain diseases, and on the other hand, drive diagnosis and the development of novel drug delivery systems for their treatment.

Acknowledgements

EMKA thanks Dr. Alexandra Brahmer for input and help with literature search. Apologies to all colleagues who's work could not be cited due to space limitations. Research of EMKA is funded by DFG (KR 3668/2-2).

References

1. van Niel G, D'Angelo G, Raposo G: **Shedding light on the cell biology of extracellular vesicles**. *Nat Rev Mol Cell Biol* 2018, **19**:213-228.
2. Yanez-Mo M, Siljander PR, Andreu Z, Zavec AB, Borras FE, Buzas EI, Buzas K, Casal E, Cappello F, Carvalho J, et al.: **Biological properties of extracellular vesicles and their physiological functions**. *J Extracell Vesicles* 2015, **4**:27066.
3. Thery C, Witwer KW, Aikawa E, Alcaraz MJ, Anderson JD, Andriantsitohaina R, Antoniou A, Arab T, Archer F, Atkin-Smith GK, et al.: **Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines**. *J Extracell Vesicles* 2018, **7**:1535750.
4. Schnatz A, Muller C, Brahmer A, Kramer-Albers EM: **Extracellular Vesicles in neural cell interaction and CNS homeostasis**. *FASEB Bioadv* 2021, **3**:577-592.
5. Krämer-Albers EM, Hill AF: **Extracellular vesicles: interneural shuttles of complex messages**. *Curr Opin Neurobiol* 2016, **39**:101-107.
6. Budnik V, Ruiz-Canada C, Wendler F: **Extracellular vesicles round off communication in the nervous system**. *Nat Rev Neurosci* 2016, **17**:160-172.
7. Hill AF: **Extracellular Vesicles and Neurodegenerative Diseases**. *J Neurosci* 2019, **39**:9269-9273.
8. Ramirez SH, Andrews AM, Paul D, Pachter JS: **Extracellular vesicles: mediators and biomarkers of pathology along CNS barriers**. *Fluids Barriers CNS* 2018, **15**:19.
9. Saint-Pol J, Gosselet F, Duban-Deweer S, Pottiez G, Karamanos Y: **Targeting and Crossing the Blood-Brain Barrier with Extracellular Vesicles**. *Cells* 2020, **9**.
10. Pauwels MJ, Vandendriessche C, Vandenbroucke RE: **Special delEVery: Extracellular Vesicles as Promising Delivery Platform to the Brain**. *Biomedicines* 2021, **9**.
11. Busatto S, Morad G, Guo P, Moses MA: **The role of extracellular vesicles in the physiological and pathological regulation of the blood-brain barrier**. *FASEB Bioadv* 2021, **3**:665-675.
12. Abbott NJ, Patabendige AA, Dolman DE, Yusof SR, Begley DJ: **Structure and function of the blood-brain barrier**. *Neurobiol Dis* 2010, **37**:13-25.
13. Kadry H, Noorani B, Cucullo L: **A blood-brain barrier overview on structure, function, impairment, and biomarkers of integrity**. *Fluids Barriers CNS* 2020, **17**:69.
14. Langen UH, Ayloo S, Gu C: **Development and Cell Biology of the Blood-Brain Barrier**. *Annu Rev Cell Dev Biol* 2019, **35**:591-613.
15. Strazielle N, Ghersi-Egea JF: **Choroid plexus in the central nervous system: biology and physiopathology**. *J Neuropathol Exp Neurol* 2000, **59**:561-574.
16. Kaur C, Ling EA: **The circumventricular organs**. *Histol Histopathol* 2017, **32**:879-892.
17. Yun JW, Barzegar M, Boyer CJ, Minagar A, Couraud PO, Alexander JS: **Brain Endothelial Cells Release Apical and Basolateral Microparticles in Response to Inflammatory Cytokine Stimulation: Relevance to Neuroinflammatory Stress?** *Front Immunol* 2019, **10**:1455.
18. Mazzucco M, Mannheim W, Shetty SV, Linden JR: **CNS endothelial derived extracellular vesicles are biomarkers of active disease in multiple sclerosis**. *Fluids Barriers CNS* 2022, **19**:13.
19. Haqqani AS, Delaney CE, Tremblay TL, Sodja C, Sandhu JK, Stanimirovic DB: **Method for isolation and molecular characterization of extracellular microvesicles released from brain endothelial cells**. *Fluids Barriers CNS* 2013, **10**:4.
20. Dozio V, Sanchez JC: **Characterisation of extracellular vesicle-subsets derived from brain endothelial cells and analysis of their protein cargo modulation after TNF exposure**. *J Extracell Vesicles* 2017, **6**:1302705.
21. Paul D, Baena V, Ge S, Jiang X, Jellison ER, Kiprono T, Agalliu D, Pachter JS: **Appearance of claudin-5(+) leukocytes in the central nervous system during neuroinflammation: a novel role for endothelial-derived extracellular vesicles**. *J Neuroinflammation* 2016, **13**:292.

22. Roig-Carles D, Willms E, Fontijn RD, Martinez-Pacheco S, Mager I, de Vries HE, Hirst M, Sharrack B, Male DK, Hawkes CA, et al.: **Endothelial-Derived Extracellular Vesicles Induce Cerebrovascular Dysfunction in Inflammation**. *Pharmaceutics* 2021, **13**.
** In vitro study demonstrating that small EVs collected from inflamed BMECs subjected to a monolayer of naïve BMECs increased leukocyte adhesion and barrier permeability and promoted an inflammatoy phenotype. Leukocyte adhesion was dependent on the expression of the cell adhesion molecule VCAM-1 but not ICAM-1.*

23. Dickens AM, Tovar YRLB, Yoo SW, Trout AL, Bae M, Kanmogne M, Megra B, Williams DW, Witwer KW, Gacias M, et al.: **Astrocyte-shed extracellular vesicles regulate the peripheral leukocyte response to inflammatory brain lesions**. *Sci Signal* 2017, **10**.
24. Couch Y, Akbar N, Roodselaar J, Evans MC, Gardiner C, Sargent I, Romero IA, Bristow A, Buchan AM, Haughey N, et al.: **Circulating endothelial cell-derived extracellular vesicles mediate the acute phase response and sickness behaviour associated with CNS inflammation**. *Sci Rep* 2017, **7**:9574.
25. Andrews AM, Lutton EM, Merkel SF, Razmpour R, Ramirez SH: **Mechanical Injury Induces Brain Endothelial-Derived Microvesicle Release: Implications for Cerebral Vascular Injury during Traumatic Brain Injury**. *Front Cell Neurosci* 2016, **10**:43.
26. Yates AG, Anthony DC, Ruitenberg MJ, Couch Y: **Systemic Immune Response to Traumatic CNS Injuries-Are Extracellular Vesicles the Missing Link?** *Front Immunol* 2019, **10**:2723.
27. Grapp M, Wrede A, Schweizer M, Huwel S, Galla HJ, Snaidero N, Simons M, Buckers J, Low PS, Urlaub H, et al.: **Choroid plexus transcytosis and exosome shuttling deliver folate into brain parenchyma**. *Nat Commun* 2013, **4**:2123.
28. Balusu S, Van Wonterghem E, De Rycke R, Raemdonck K, Stremersch S, Gevaert K, Brkic M, Demeestere D, Vanhooren V, Hendrix A, et al.: **Identification of a novel mechanism of blood-brain communication during peripheral inflammation via choroid plexus-derived extracellular vesicles**. *EMBO Mol Med* 2016, **8**:1162-1183.
29. Lepko T, Pusch M, Muller T, Schulte D, Ehses J, Kiebler M, Hasler J, Huttner HB, Vandenbroucke RE, Vandendriessche C, et al.: **Choroid plexus-derived miR-204 regulates the number of quiescent neural stem cells in the adult brain**. *EMBO J* 2019, **38**:e100481.
** Identifies a mechanism controlling the mainenance of adult neural stem cells involving CPE-derived EVs released into the CSF, which deliver miR-204 to the neurogenic niches of the brain. Inhibition of EV-release from the choroid plexus using GW4869 reduced the amount of miR-2014 in the CSF and reduced the number of neuroblasts generated in a transwell co-culture system of primary choroid plexus and subependymal zone tissue.*

30. Vandendriessche C, Balusu S, Van Cauwenberghe C, Brkic M, Pauwels M, Plehiers N, Bruggeman A, Dujardin P, Van Imschoot G, Van Wonterghem E, et al.: **Importance of extracellular vesicle secretion at the blood-cerebrospinal fluid interface in the pathogenesis of Alzheimer's disease**. *Acta Neuropathol Commun* 2021, **9**:143.
*** Important in vivo work demonstrating that EV-release from the CPE is relevant for neurodegeneration. The study showed that the presence of oligomeric Aβ in the CSF promotes the generation of CPE-EVs with proinflammatory effects on neural cells. Interfering with EV-release using GW4949 protected mice from the development of Aβ-induced cognitive impairment, which was measured using the novel object recognition behavioural test.*

31. Van Hoecke L, Van Cauwenberghe C, Dominko K, Van Imschoot G, Van Wonterghem E, Castelein J, Xie J, Claeys W, Vandendriessche C, Kremer A, et al.: **Involvement of the Choroid Plexus in the Pathogenesis of Niemann-Pick Disease Type C**. *Front Cell Neurosci* 2021, **15**:757482.

32. O'Hara BA, Morris-Love J, Gee GV, Haley SA, Atwood WJ: **JC Virus infected choroid plexus epithelial cells produce extracellular vesicles that infect glial cells independently of the virus attachment receptor**. *PLoS Pathog* 2020, **16**:e1008371.
33. Xu B, Zhang Y, Du XF, Li J, Zi HX, Bu JW, Yan Y, Han H, Du JL: **Neurons secrete miR-132-containing exosomes to regulate brain vascular integrity**. *Cell Res* 2017, **27**:882-897.
34. Gaceb A, Ozen I, Padel T, Barbariga M, Paul G: **Pericytes secrete pro-regenerative molecules in response to platelet-derived growth factor-BB**. *J Cereb Blood Flow Metab* 2018, **38**:45-57.
35. Mayo JN, Bearden SE: **Driving the Hypoxia-Inducible Pathway in Human Pericytes Promotes Vascular Density in an Exosome-Dependent Manner**. *Microcirculation* 2015, **22**:711-723.
36. Zhang L, Graf I, Kuang Y, Zheng X, Haupt M, Majid A, Kilic E, Hermann DM, Psychogios MN, Weber MS, et al.: **Neural Progenitor Cell-Derived Extracellular Vesicles Enhance Blood-Brain Barrier Integrity by NF-kappaB (Nuclear Factor-kappaB)-Dependent Regulation of ABCB1 (ATP-Binding Cassette Transporter B1) in Stroke Mice**. *Arterioscler Thromb Vasc Biol* 2021, **41**:1127-1145.
37. Faille D, Combes V, Mitchell AJ, Fontaine A, Juhan-Vague I, Alessi MC, Chimini G, Fusai T, Grau GE: **Platelet microparticles: a new player in malaria parasite cytoadherence to human brain endothelium**. *FASEB J* 2009, **23**:3449-3458.
38. Dalvi P, Sun B, Tang N, Pulliam L: **Immune activated monocyte exosomes alter microRNAs in brain endothelial cells and initiate an inflammatory response through the TLR4/MyD88 pathway**. *Sci Rep* 2017, **7**:9954.
39. Ajikumar A, Long MB, Heath PR, Wharton SB, Ince PG, Ridger VC, Simpson JE: **Neutrophil-Derived Microvesicle Induced Dysfunction of Brain Microvascular Endothelial Cells In Vitro**. *Int J Mol Sci* 2019, **20**.
40. Kinjyo I, Bragin D, Grattan R, Winter SS, Wilson BS: **Leukemia-derived exosomes and cytokines pave the way for entry into the brain**. *J Leukoc Biol* 2019, **105**:741-753.
41. Hoshino A, Costa-Silva B, Shen TL, Rodrigues G, Hashimoto A, Tesic Mark M, Molina H, Kohsaka S, Di Giannatale A, Ceder S, et al.: **Tumour exosome integrins determine organotropic metastasis**. *Nature* 2015, **527**:329-335.
42. Gao W, Li F, Liu L, Xu X, Zhang B, Wu Y, Yin D, Zhou S, Sun D, Huang Y, et al.: **Endothelial colony-forming cell-derived exosomes restore blood-brain barrier continuity in mice subjected to traumatic brain injury**. *Exp Neurol* 2018, **307**:99-108.
43. Williams AM, Bhatti UF, Brown JF, Biesterveld BE, Kathawate RG, Graham NJ, Chtraklin K, Siddiqui AZ, Dekker SE, Andjelkovic A, et al.: **Early single-dose treatment with exosomes provides neuroprotection and improves blood-brain barrier integrity in swine model of traumatic brain injury and hemorrhagic shock**. *J Trauma Acute Care Surg* 2020, **88**:207-218.
44. Erb U, Hikel J, Meyer S, Ishikawa H, Worst TS, Nitschke K, Nuhn P, Porubsky S, Weiss C, Schroten H, et al.: **The Impact of Small Extracellular Vesicles on Lymphoblast Trafficking across the Blood-Cerebrospinal Fluid Barrier In Vitro**. *Int J Mol Sci* 2020, **21**.
45. Garcia-Romero N, Carrion-Navarro J, Esteban-Rubio S, Lazaro-Ibanez E, Peris-Celda M, Alonso MM, Guzman-De-Villoria J, Fernandez-Carballal C, de Mendivil AO, Garcia-Duque S, et al.: **DNA sequences within glioma-derived extracellular vesicles can cross the intact blood-brain barrier and be detected in peripheral blood of patients**. *Oncotarget* 2017, **8**:1416-1428.
46. Shi M, Liu C, Cook TJ, Bullock KM, Zhao Y, Ginghina C, Li Y, Aro P, Dator R, He C, et al.: **Plasma exosomal alpha-synuclein is likely CNS-derived and increased in Parkinson's disease**. *Acta Neuropathol* 2014, **128**:639-650.
47. Goetzl EJ, Abner EL, Jicha GA, Kapogiannis D, Schwartz JB: **Declining levels of functionally specialized synaptic proteins in plasma neuronal exosomes with progression of Alzheimer's disease**. *FASEB J* 2018, **32**:888-893.

48. Ridder K, Keller S, Dams M, Rupp AK, Schlaudraff J, Turco DD, Starmann J, Macas J, Karpova D, Devraj K, et al.: **Extracellular vesicle-mediated transfer of genetic information between the hematopoietic system and the brain in response to inflammation**. *PLoS Biol* 2014, **12**:e1001874.
49. Kur IM, Prouvot PH, Fu T, Fan W, Muller-Braun F, Das A, Das S, Deller T, Roeper J, Stroh A, et al.: **Neuronal activity triggers uptake of hematopoietic extracellular vesicles in vivo**. *PLoS Biol* 2020, **18**:e3000643.
*** Here, Kur et al build on their previous findings that blood-derived EVs deliver functional cargo across the blood-brain barrier and show that neuronal activity drives the uptake of EVs by neurons also under physiological conditions of blood-brain barrier integrity. The study employs independent experimental strategies including optogenetics and a behavioural paradigm to underscore that neuronal activity alone is sufficient to recruite hematopoietc EVs into the brain and neurons.*

50. Kaplan L, Chow BW, Gu C: **Neuronal regulation of the blood-brain barrier and neurovascular coupling**. *Nat Rev Neurosci* 2020, **21**:416-432.
51. Bittel M, Reichert P, Sarfati I, Dressel A, Leikam S, Uderhardt S, Stolzer I, Phu TA, Ng M, Vu NK, et al.: **Visualizing transfer of microbial biomolecules by outer membrane vesicles in microbe-host-communication in vivo**. *J Extracell Vesicles* 2021, **10**:e12159.
* *Colonizing the intestine with E.coli carrying a genetic tracer (Cre-recombinase) that is shed together with outer membrane vesicles (OMV) results in reporter expression in distant tissues including the brain. First demonstration that OMV cargo can not only pass the gut intestinal barrier but also result in the functional delivery of OMV cargo over the blood-brain barrier.*

52. Matsumoto J, Stewart T, Sheng L, Li N, Bullock K, Song N, Shi M, Banks WA, Zhang J: **Transmission of alpha-synuclein-containing erythrocyte-derived extracellular vesicles across the blood-brain barrier via adsorptive mediated transcytosis: another mechanism for initiation and progression of Parkinson's disease?** *Acta Neuropathol Commun* 2017, **5**:71.
53. Banks WA, Sharma P, Bullock KM, Hansen KM, Ludwig N, Whiteside TL: **Transport of Extracellular Vesicles across the Blood-Brain Barrier: Brain Pharmacokinetics and Effects of Inflammation**. *Int J Mol Sci* 2020, **21**.
**Banks et al perform pharmacokinetics to compare the entry of EVs from different cellular sources into the brain. According to their results, brain entry is a rather universal characteristic of EVs. The rate of BBB-transfer varied strongly and was determined by characteristics specific to the EV-type.*

54. Morad G, Carman CV, Hagedorn EJ, Perlin JR, Zon LI, Mustafaoglu N, Park TE, Ingber DE, Daisy CC, Moses MA: **Tumor-Derived Extracellular Vesicles Breach the Intact Blood-Brain Barrier via Transcytosis**. *ACS Nano* 2019, **13**:13853-13865.
55. Kuroda H, Tachikawa M, Yagi Y, Umetsu M, Nurdin A, Miyauchi E, Watanabe M, Uchida Y, Terasaki T: **Cluster of Differentiation 46 Is the Major Receptor in Human Blood-Brain Barrier Endothelial Cells for Uptake of Exosomes Derived from Brain-Metastatic Melanoma Cells (SK-Mel-28)**. *Mol Pharm* 2019, **16**:292-304.
56. Qu M, Lin Q, Huang L, Fu Y, Wang L, He S, Fu Y, Yang S, Zhang Z, Zhang L, et al.: **Dopamine-loaded blood exosomes targeted to brain for better treatment of Parkinson's disease**. *J Control Release* 2018, **287**:156-166.
57. Joshi BS, Zuhorn IS: **Heparan sulfate proteoglycan-mediated dynamin-dependent transport of neural stem cell exosomes in an in vitro blood-brain barrier model**. *Eur J Neurosci* 2021, **53**:706-719.
58. Yuan D, Zhao Y, Banks WA, Bullock KM, Haney M, Batrakova E, Kabanov AV: **Macrophage exosomes as natural nanocarriers for protein delivery to inflamed brain**. *Biomaterials* 2017, **142**:1-12.

59. Verweij FJ, Balaj L, Boulanger CM, Carter DRF, Compeer EB, D'Angelo G, El Andaloussi S, Goetz JG, Gross JC, Hyenne V, et al.: **The power of imaging to understand extracellular vesicle biology in vivo**. *Nat Methods* 2021, **18**:1013-1026.

Figures and legends

Figure 1

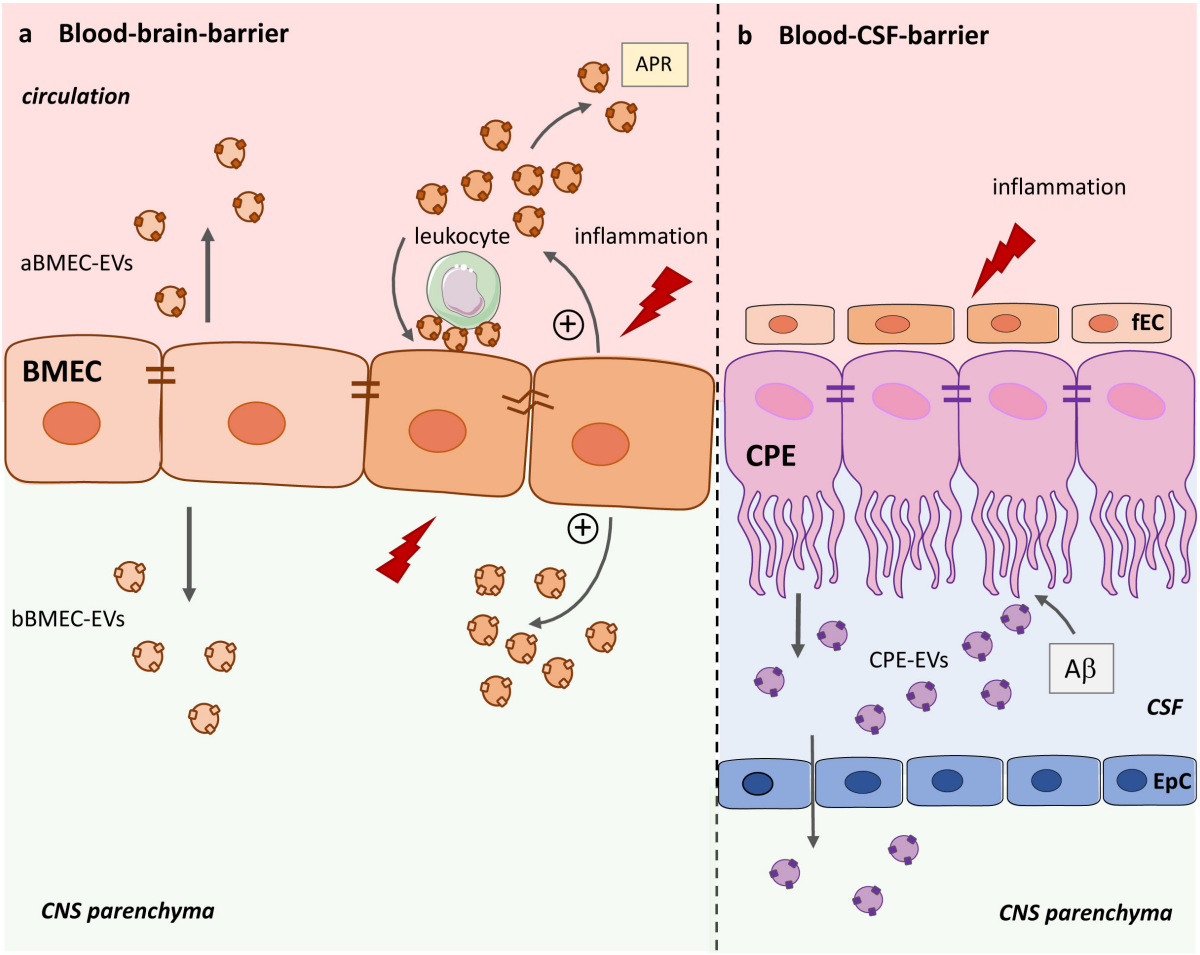

Figure 1: Mode of action of barrier-derived EVs. (a) Endothelial cells **(**BMECs) of the BBB release apical EVs (aBMEC-EVs) into the circulation and basal EVs (bBMEC-EVs) to the neurovascular unit and the brain parenchyma. Inflammation increases EV-release from BMECs, promoting the acute phase response (APR) triggered by CNS inflammation and facilitating leukocyte adhesion at the BBB. Please note that the presentation of the BBB is simplified and lacks pericytes and astrocytes, which contribute to barrier properties at the basal side of the brain endothelium. **(b)** Choroid plexus epithelial cells (CPE) at the BCB release EVs at their apical side into the CSF, which further cross the ependymal cell layer (EpC) and enter the brain parenchyma. Inflammation and the presence of amyloid (Aβ) in the CSF trigger EV secretion by CPE. fEC, fenestrated endothelium.

Figure 2

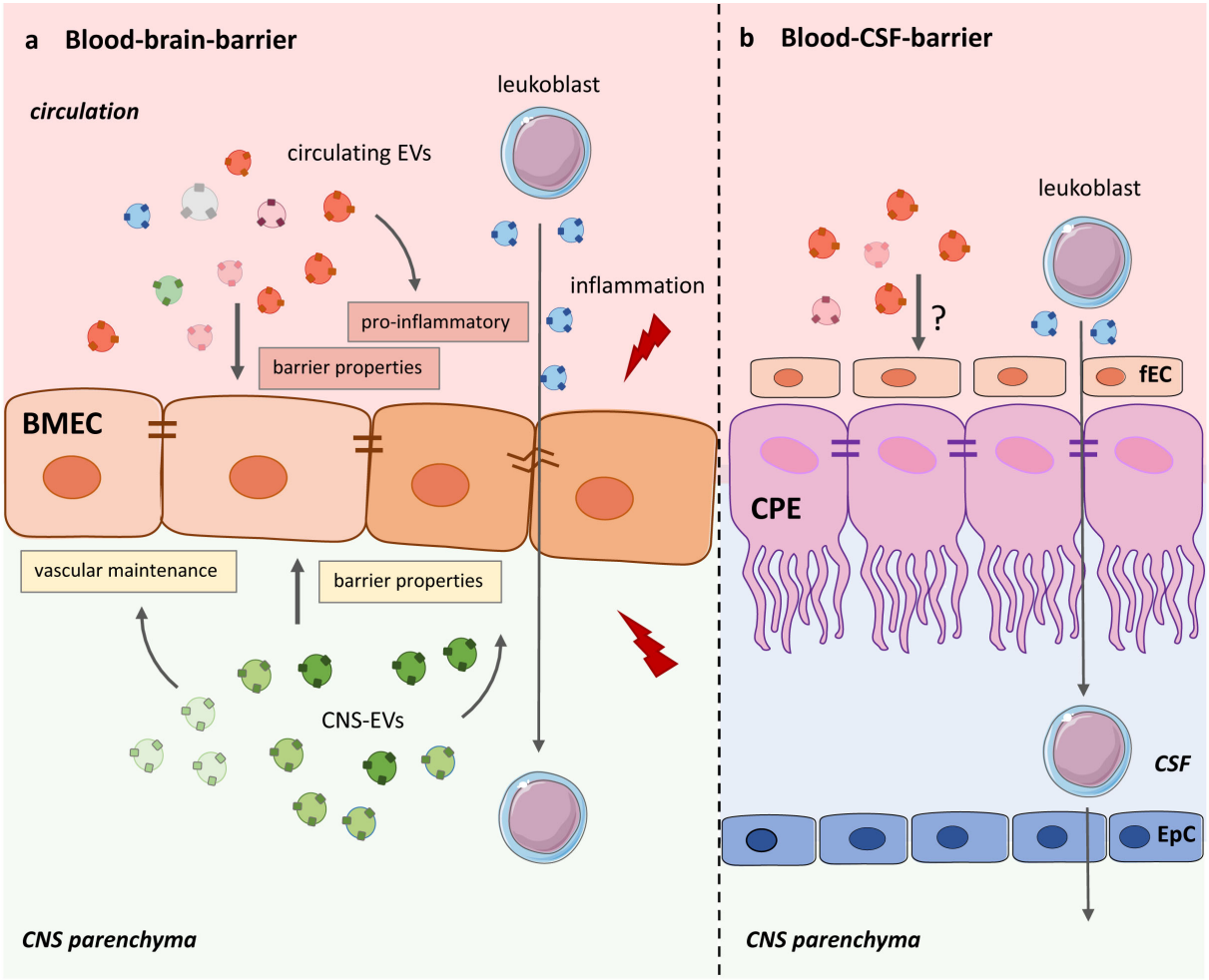

Figure 2: EVs addressing barrier cells. (a) Circulating EVs derived from different types of peripheral cells and brain-derived EVs interact with BMECs and modulate barrier properties and integrity. Tumor-derived EVs can facilitate invasion of tumor cells into the CNS via the BBB and **(b)** BCB.

Figure 3

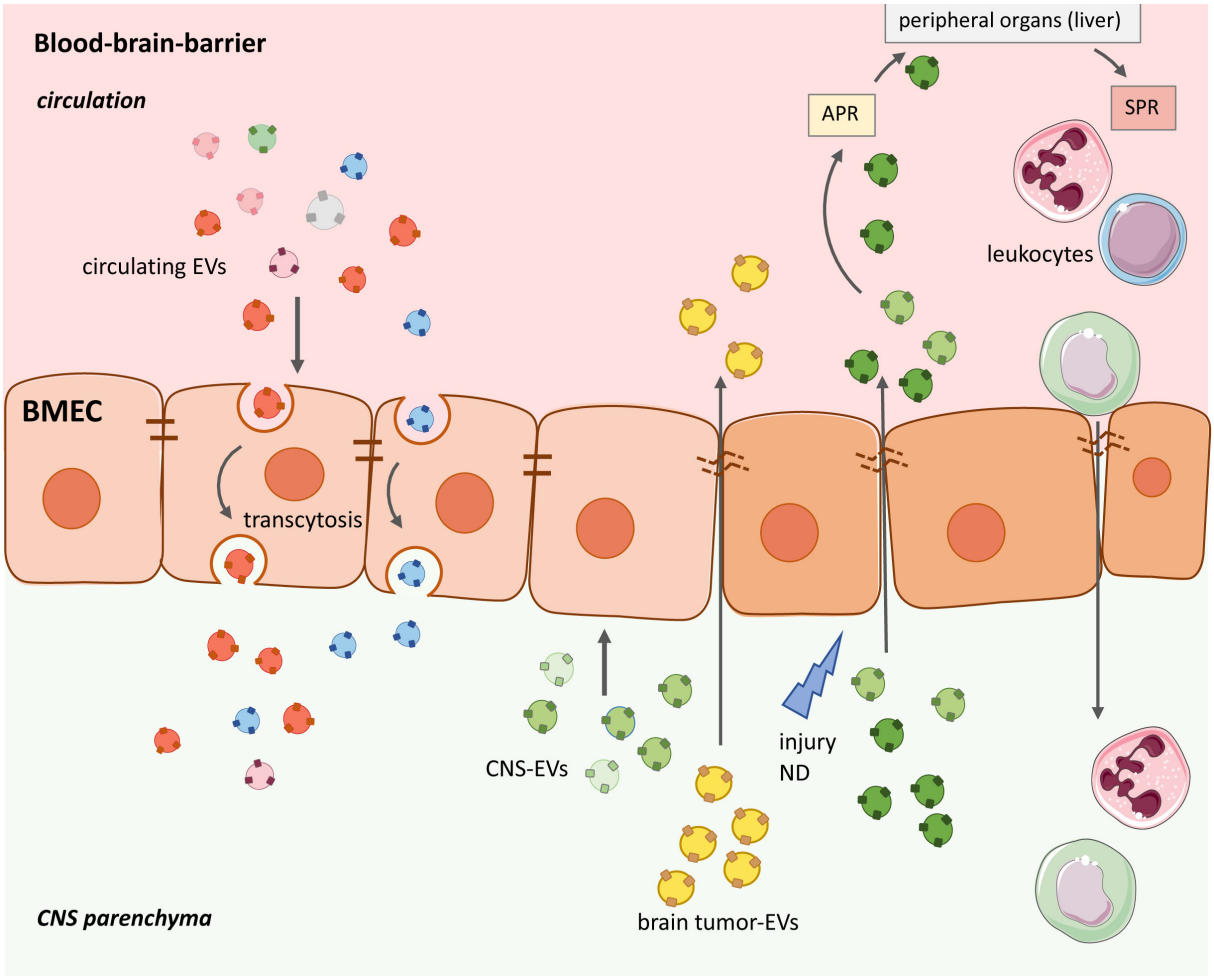

Figure 3: EVs cross the BBB. Several types of circulating EVs (e.g. hematopoetic cell-derived EVs) pass the BBB by adoptive transcytosis and enter the CNS parenchyma. The factors controlling BBB transfer of EVs are not well understood and depend on the EV-type and the physiological context. CNS-EVs enter the circulation when the BBB integrity is impaired, f.e. upon brain tumor malignancy, neurodegeneration (ND) or injury, and may provide a target for liquid biopsy. CNS-EVs can participate in the acute phase response (APR) to injury which promotes a secondary phase immune response (SPR) in the periphery.