cells

MDPI

Review

Involvement of Astrocytes in the Formation, Maintenance, and Function of the Blood–Brain Barrier

Gabriella Schiera [1], Carlo Maria Di Liegro [1], Giuseppe Schirò [2,3], Gabriele Sorbello [2] and Italia Di Liegro [2,*]

[1] Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (Dipartimento di Scienzee Tecnologie Biologiche, Chimiche e Farmaceutiche) (STEBICEF), University of Palermo, 90128 Palermo, Italy; gabriella.schiera@unipa.it (G.S.); carlomaria.diliegro@unipa.it (C.M.D.L.)
[2] Department of Biomedicine, Neurosciences and Advanced Diagnostics, University of Palermo, 90127 Palermo, Italy; giuseppeschiro1994@gmail.com (G.S.); gabriele96@gmail.com (G.S.)
[3] Neurology and Multiple Sclerosis Center, Unità Operativa Complessa (UOC), Foundation Institute "G. Giglio", 90015 Cefalù, Italy
* Correspondence: italia.diliegro@unipa.it

Abstract: The blood–brain barrier (BBB) is a fundamental structure that protects the composition of the brain by determining which ions, metabolites, and nutrients are allowed to enter the brain from the blood or to leave it towards the circulation. The BBB is structurally composed of a layer of brain capillary endothelial cells (BCECs) bound to each other through tight junctions (TJs). However, its development as well as maintenance and properties are controlled by the other brain cells that contact the BCECs: pericytes, glial cells, and even neurons themselves. Astrocytes seem, in particular, to have a very important role in determining and controlling most properties of the BBB. Here, we will focus on these latter cells, since the comprehension of their roles in brain physiology has been continuously expanding, even including the ability to participate in neurotransmission and in complex functions such as learning and memory. Accordingly, pathological conditions that alter astrocytic functions can alter the BBB's integrity, thus compromising many brain activities. In this review, we will also refer to different kinds of in vitro BBB models used to study the BBB's properties, evidencing its modifications under pathological conditions.

Keywords: blood–brain barrier; brain capillary endothelial cell; astrocytes; in vitro BBB models; extracellular vesicles (EVs)

Citation: Schiera, G.; Di Liegro, C.M.; Schirò, G.; Sorbello, G.; Di Liegro, I. Involvement of Astrocytes in the Formation, Maintenance, and Function of the Blood–Brain Barrier. *Cells* **2024**, *13*, 150. https://doi.org/10.3390/cells13020150

Academic Editors: Daniele Nosi and H. Rheinallt Parri

Received: 8 December 2023
Revised: 8 January 2024
Accepted: 11 January 2024
Published: 12 January 2024

1. Introduction

The blood–brain barrier (BBB) is a structural and biochemical barrier responsible for the selective passage of molecules from the blood to the brain and for maintaining ion homeostasis in the brain microenvironment [1,2]. As we will discuss below, the central role of the BBB in the transport of metabolites and nutrients from the blood to the brain and vice versa also means that the disruption of its function is involved in most neurological pathologies.

Brain capillary endothelial cells (BCECs), astrocytes, pericytes, microglial cells, and neurons participate in the genesis of the BBB and regulate its properties (Figure 1) [3]. The term neurovascular unit (NVU), which was used for the first time in 2002 [4], refers to the set of all the cellular and molecular components that induce and regulate the formation and maintenance of the BBB [5–15]. In other words, even if the basic structural constituents of the BBB are the BCECs, which form tight junctions with each other and lie on a basal lamina, the other surrounding perivascular brain cells, and in particular astrocytes, play a fundamental role in the formation and maintenance of the BBB, both during brain development and in adult life.

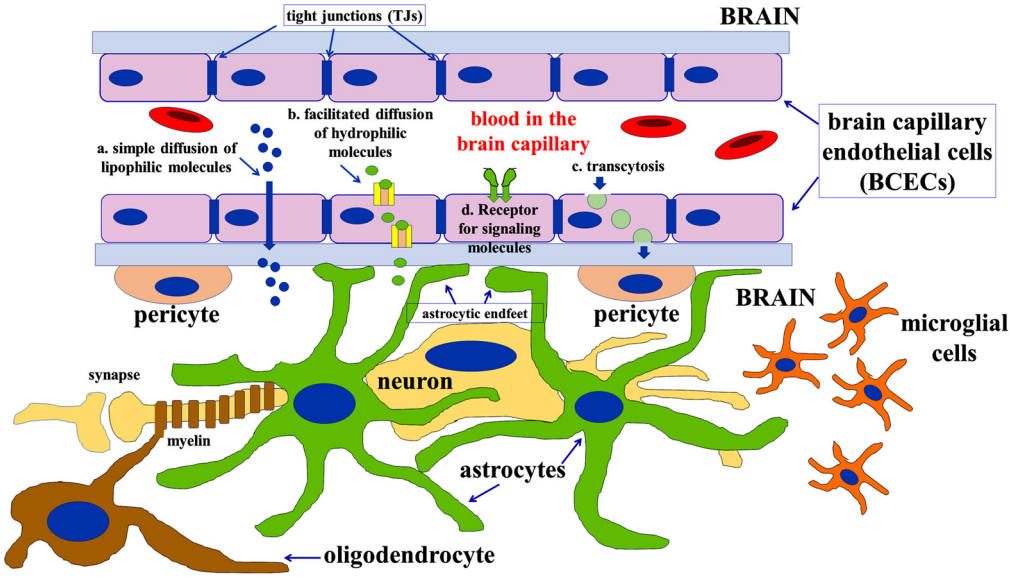

Figure 1. Schematic view of all the components of the neurovascular unit (NVU). The brain capillary is structurally composed of the brain capillary endothelial cells (BCECs) bound together by tight junctions (TJs). The formation of the blood–brain barrier depends, however, on all the cells present around the capillary: neurons (yellow in the picture), oligodendrocytes (brown in the figure), microglial cells (orange in the picture), pericytes (light red in the picture), and, especially, astrocytes (green in the picture). All these cells communicate with each other and with the BCECs by releasing soluble factors that, as discussed below, can be also conveyed by extracellular vesicles. Astrocytes, in particular, directly contact BCECs through the so-called astrocytic endfeet, which also contains aquaporins (see the text for further details).

BCECs are held together by two types of junctions: adherent junctions (AJs) and tight junctions (TJs). The AJs perform the function of maintaining cell-to-cell contacts and are also attached to the cytoskeleton. TJs seal the spaces between cells, determine cell membrane polarity, and limit the permeability of the BBB, giving rise to a high transendothelial electrical resistance (TEER) barrier that blocks ions and small charged molecules. And, indeed, measuring TEER using a cell voltmeter is an extremely frequently used method in order to evaluate the formation of a barrier in BBB in vitro models [16].

TJs form a sort of zipper that closes inter-endothelial spaces [1,2]. They consist of integral membrane proteins, such as occludins and claudins, and membrane-associated proteins, such as zonula occludens (ZO-1) [17]. The role of these proteins is of paramount importance for BBB formation [18–22], and, indeed, alterations in their expression or the presence of mutations that compromise their assembly can be pathogenic [23,24].

The transmembrane protein occludin appears to be a major member of the BBB's TJs, with an important role in the barrier's function [19,25]. Interestingly, during BBB development, its appearance in TJs is late and, thus, its presence indicates the final maturation of these structures; as a consequence, in BBB models obtained in vitro, its peripheral locations in BCECs are an indication of TJ formation and are frequently accompanied by the appearance of BBB properties in these cultured cells [26,27]. Importantly, TJ components can also bind to other cytoplasmic molecules, and these interactions may contribute towards their assembly [28].

BCECs are rich in specific membrane transporters that are involved in the uptake of nutrients, in the removal of waste substances and neurotoxins, and the passage of ions and other molecules. In relation to their different cellular location, there are three main types of transporters and receptors: (a) bidirectional transporters and receptors expressed both apically and basolaterally, for example, glucose transporters (e.g., GLUT-1); (b) unidirectional transporters present in both regions and responsible for the transport of substances either inside or outside the CNS, for example, the insulin or transferrin

receptors; and (c) unidirectional transporters located either in the luminal zone or in the ab-luminal zone, which contribute to the polarity of cerebral endothelial cells; the latter category includes the multi-drug-resistant proteins (MDR1s), which are located in the luminal region and prevent the entry of drugs into the CNS by actively expelling them [29].

Moreover, BCECs express a low amount of leukocyte adhesion molecules in order to limit the passage of lymphocytes and other components of the immune system [30].

All these properties appear at different times during brain development and probably require the synergistic effect of all the brain cells. On the other hand, maintaining the integrity of the BBB is of vital importance since its alteration or loss of function underlies various pathologies affecting the CNS, such as neurodegenerative diseases, brain tumors, and stroke.

In this review, we will consider, in particular, the effects of astrocytes on both the formation and maintenance of the BBB.

2. Developmental Appearance and Maintenance of the Barrier's Function

The exact timing of the appearance of the BBB during development is much debated. One of the most important events that can be associated with the development of cerebral capillaries in rodents is the disappearance of fenestrations and the appearance of TJs in the endothelium between the 11th (E11) and 13th (E13) embryonic days. However, pial vessels show low transendothelial resistance (TEER) up to E20; this suggests that the formation of the BBB is only completed after birth [31].

Actually, the formation of the BBB can be considered a two-step process [32]: (i) cerebral angiogenesis is initiated with the entry of the capillaries into the neuroectoderm and the formation of intraneural vessels. This process is defined as "inductive" since BCECs move towards the neuroectoderm, following a vascular endothelial growth factor (VEGF) concentration gradient. VEGF, produced by neuroepithelial cells, binds to the so-called fetal liver kinase receptor 1 (flk-1), also known as VEGF receptor-2, a receptor present on the surface of BCECs; this event directs the BCECs towards the differentiated phenotype (commitment); (ii) consequently, as a result of the interactions with the surrounding cells (e.g., neurons, pericytes, and glial cells), the BCECs will acquire the final phenotype, characterized by the formation of TJs and by the expression of specific molecules. During the development of the cerebral cortex, angiogenesis is followed by vasculogenesis, where vessels originate from pluripotent endothelial cells [33].

In addition to VEGF, essential factors for the genesis and maintenance of the BBB are the Wingless-related integration site (Wnt)/beta-catenin pathway, the G protein-coupled receptor 124 (GPR124), an orphan member of the G protein-coupled receptor family, and the Sonic Hedgehog (SHH) pathway [32].

2.1. The General Metabolic Role of Brain Astrocytes

In vivo, astrocytes constitute a sort of protective physiological filter that regulates the flow of metabolites to neurons. Circulating glucose (the main metabolic substrate for the brain) directly reaches the neurons in a very low amount, while it is taken up in a greater percentage by the astrocytes that are in contact with the blood vessels. In other words, astrocytes are 'intercalated' between the neurons and glucose, transported into the endothelial cells from the blood by specific transporters (such as GLUT-1). Astrocytes form a large network that embraces many neurons, also at a distance from the BBB; thus, they can transfer glucose to them on a large scale; in addition, in astrocytes, glucose is metabolized by glycolysis into pyruvate and then into lactate, which, once released into the extracellular fluid, can be absorbed by neurons and directly used as an energy source for oxidative metabolism after transforming it back into pyruvate. Interestingly, astrocytes are also the only cells of the nervous system that are capable of storing glucose in the form of glycogen and, therefore, represent an important energy reserve for neurons. Therefore, while the amount of glucose metabolized by astrocytes varies according to the degree of brain activity, the supply of lactate to the neurons is kept almost constant

thanks to its continuous release by astrocytes. This process is called astrocyte–neuron lactate shuttle (ANLS), and it seems to be especially active in association with excitatory neurotransmission [34–44]. It has been recently discovered that the consumption of lactate by neurons is essential for long-term memory consolidation but not, probably, for short-term memory [45–50]. Interestingly, lactate has also been reported to act as a signaling molecule. A G protein-coupled receptor (GPR81), also named hydroxyl-carboxylic acid 1 (HCA1) or hydroxyl-carboxylic acid receptor 1 (HCAR1), has indeed been discovered and has also been proposed to be involved in processes such as learning, memory, and neuroprotection [51–54].

Among other functions related to their ability to control metabolite traffic across the BBB, astrocytes have been recently reported to be able to also regulate iron transport into the brain thanks to their production of hepcidin, a peptide that had been considered a liver-specific regulatory factor. This astrocytic ability is extremely important since iron accumulation beyond the amounts required for metabolic activities is considered one of the causes of oxidative stress and, as a consequence, neurodegeneration, while hepcidin is able to control the amount of entering iron, probably by acting, like in the gut, on the ferroportin 1 (FPN1) transporter, present in BCECs [55–59]. As discussed below, they also release growth factors that are able to influence other brain cells and, in particular, BCECs that will form the BBB.

In addition to these important metabolic functions, astrocytes have a fundamental impact on neurotransmission as they are also able to respond to neurotransmitters as well as release their own transmitters (called gliotransmitters) [49,60–65]. In other words, a bidirectional exchange of information among astrocytes and neurons also exists at the level of nerve impulses. At a primary level, each synapse is indeed enwrapped by an astrocyte, thus forming what has been called a "tripartite synapse" in which an astrocyte contributes to neurotransmission by not only taking back neurotransmitters, such as glutamate, from the synaptic cleft but probably also by producing and releasing modulatory factors. Moreover, given the particular morphology of astrocytes and their ability to form a web, thanks to the gap junctions (GJs) that bind them to each other, astrocytes can embrace many different synapses and neurons, thus forming a network probably responsible for the many aspects of learning and memory [60–65]. More details on this particular aspect of astrocytic function, as well as on the involvement of extracellular vesicles (EVs) in these processes, can be found in a recent review centered on brain cell-to-cell contacts in learning and memory [49].

2.2. Astrocytes and BBB Formation and Maintenance

On the basis of the many different observations that suggested an exchange of solutes and water between the interstitial fluid (ISF) and the cerebrospinal fluid (CSF), years ago, the existence of a brain-specific kind of tissue circulation was proposed and called the "Glymphatic System" (GS) [66–69]. More recently, it is becoming increasingly clear that the GS is essential for maintaining a healthy brain, and, indeed, GS alterations are associated with most neuropathologies [70–72].

Now, fundamental for water trafficking across cell membranes are the water channel-forming proteins known as aquaporins (AQPs), but AQPs do not seem to be present in BCECs. On the other hand, AQP 4 is present in astrocytes, in which it is highly polarized, as it is specifically localized at the so-called astrocytic endfeet that contacts the BBB, at the level of very special structures named orthogonal arrays of particles (OAPs) [69].

Astrocytic endfeet are very important structures that contain, among other organelles, microtubules, mitochondria, and intermediate filaments composed of glial fibrillary acidic protein (GFAP) [73].

Given its importance for the physiological water trafficking across the BBB, it is crucial that AQP4 proteins are represented at the right levels in astrocytes and that, first of all, they are correctly localized; both alterations in AQP4 expression and its delocalization have indeed been related to pathology. For example, AQP4-deficient mice show significantly higher brain water contents when they are infused with artificial CSF into the brain extracel-

lular space [74]. In addition, it has been reported that the localization of AQP4 also depends on the correct organization of the astrocytic endfeet, which, in turn, depends on the assembly of the gap junctions (GJs), formed by connexins 43 and 30 (Cx43 and Cx30, respectively), between the endfeet; microhemorrhages are indeed more frequent in C43-deficient mice [7]. Actually, brain edema has been evidenced in many pathological conditions, from cancer to stroke, and, in many cases, altered production and/or localization of AQP4 has been found, while, at the same time, the involvement of AQP4 in all these alterations suggests this protein and its organization as a therapeutic target [75–79]. Recently, an AQP4 variant with a C-terminal extension (AQP4x) has been also isolated, and it has been shown that it plays a role in BBB integrity [80]. On the other hand, endothelial cells express the autophagy-related 7 gene (Atg7), an E1-like ubiquitin-activating enzyme that is involved in autophagy [81,82]; Atg7 has been recently found to also regulate the interaction between the astrocytic endfeet and the basal membrane (BM), the extracellular structure through which BCECs and other brain cells communicate: indeed, the dissociation of astrocytes from microvessels in the brain of a transgenic mouse with a conditional deletion of Atg7 in BCECs has been uncovered [83]. Interestingly, Atg7 is involved in the regulation of fibronectin expression, and fibronectin seems to be crucial for the adhesion of astrocytes to the GM [83]. Two other genes, the expression of which is instead astrocytic and, in any case, necessary for astrocyte adhesion to the GM, are those encoding laminin and the laminin receptor [84].

In addition to the mentioned studies concerning the GS and AQPs, many further observations have been suggesting, since a long time ago, a central role of astrocytes in regulating the development and maintenance of the BBB's functions. For example, in 2008, it was reported that the BBB properties of the brain endothelium in vivo in adult mice depend on signaling mechanisms that involve bone morphogenic proteins (BMPs), which are specifically activated in astrocytes, and the disruption of which causes a loss of the barrier's function [85]. More recently, it was found that after the tamoxifen-induced apoptotic death of astrocytes in adult mice, BBB damage became evident because of a clear modification of the transport across the BCEC layer [86]. Moreover, in the vessels located close to the apoptotic astrocytes, the expression of TJ proteins (and in particular of ZO-1) was downregulated [86]. It has been recently reported that astrocytes also play a crucial role in BBB maintenance by controlling pH homeostasis through astrocyte-specific proteins, such as the electrogenic sodium-bicarbonate cotransporter 1 (Slc4a4) [87].

Among the astrocytic activities that affect both the developmental formation and maintenance of the BBB, a central role should be attributed to specific growth factors. As we will discuss below, many years ago, by setting an in vitro model of the BBB, we found, for example, that astrocytes (and also neurons) can induce a BBB phenotype in a BCEC cell line when cultured together for a few days [26,27,88,89]. Further studies on this in vitro system allowed us to find out that both cell types produce and release VEGF and fibroblast growth factor 2 (FGF2), which were probably responsible for the effects on the cultured endothelial cells [90,91]. The effects of the astrocyte-derived VEGF as well as of the transforming growth factor beta 1 (TGFβ1) have been further confirmed in recent years [92]. It has been also reported that the astrocyte-derived TGFβ1 can induce, in BCECs, the expression of the TJ protein ZO-1 [93]. Fundamental for BBB maintenance seems also to be the release from astrocytes of Wnt growth factors, which act by stimulating the Wnt/β-catenin pathway, with an important effect on astrocytic endfeet structure too [94]. Another factor recently reported to have a protective effect on the BBB in aged mice after pathological conditions, such as ischemic stroke, is the mesencephalic astrocyte-derived neurotrophic factor (MANF); the authors suggest that the recognition of these MANF functions might offer new ways for approaching ischemic stroke [95].

Finally, both astrocytes and their corresponding glial enteric cells are capable of inducing barrier properties in intestinal epithelial cells; S-nitrosoglutathione (GSNO) has been identified as a molecular mediator of this effect [96].

Interestingly, in spite of what has been said above, some aspects of the BBB become functional in vivo before the appearance of the astrocytes during development [97,98]. In this regard, it is interesting to note that the embryonic neural progenitor cells are able to induce BBB properties in BCECs [99].

2.3. The Role of Extracellular Vesicles (EVs) in the Formation and Maintenance of the BBB

One of the most intriguing discoveries of the last few decades is that all cells, from bacterial cells to the cells of the mammalian nervous system, are able to release extracellular vesicles (EVs) that contain proteins of many kinds and functions, lipids, metabolites, a variety of RNAs, such as microRNA (miRNAs), messenger RNAs (mRNAs), long non-coding RNAs (lncRNAs), even DNA, and sometimes organelles [49,100–103]; for example, ribosomes have been evidenced in EVs released from oligodendrocytes/Schwann cells and targeted to neuronal axons [104–106]. Interestingly, this latter observation has been used to better understand how pre-localized mRNAs, which are a part of RNA–protein complexes, can be translated locally, along the axons and especially at the level of synapses, in response to specific signals [49].

Interestingly, in the brain, all the cell types are able to produce and release EVs (Figure 2) that, once received by other cells, can modify their activities and properties both under physiological and pathological conditions [49,107–110].

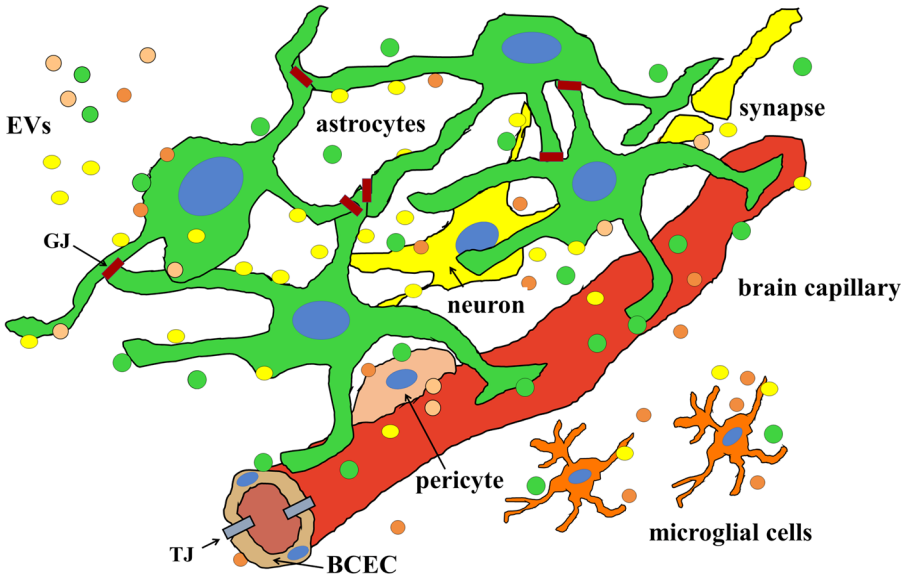

Figure 2. All the components of the neurovascular unit (NVU) are able to release and receive extracellular vesicles (EVs). As discussed in the text, the walls of the brain capillaries are structurally composed of the BCECs bound together by tight junctions (TJs); the formation of a functional blood–brain barrier depends, however, on all the cells present around the capillary. All these cells communicate with each other both through direct contacts and secreted molecules, many of which are delivered by the EVs. For clarity, EVs coming from different cell types have been represented in the same color as the producing cells. Notably, astrocytes form a sort of large web thanks to their ability to form gap junctions (GJs) with each other.

On the other hand, all the brain cells are also able to catch materials delivered through the EVs under both physiological and pathological conditions [110–119]. The release of the EVs is not always identical; different signals can indeed modify this process; among other stimuli, it has been, for example, found that hypoxia can induce modifications in the size and total amount of EVs released by both neurons and glial cells as well as in the quality of their cargoes [120]. Similarly, under both central and peripheral stress conditions, many modifications take place in the brain, many of which are spread among cells via EVs [121].

Notably, EVs produced by brain cells have been found to also cross the BBB and appear in the peripheral blood; similarly, EVs born in other organs can reach the brain by crossing the BBB [110,122,123]. Although it is not completely clear which are the specific functions of this EV exchange among the brain and the peripheral organs, it is probable that it has a role in the regulation that the brain exerts on all the organs of the body, as well as in the communication to the brain of the status of the different organs [124]. EVs from the periphery have also been found to have beneficial effects on the brain under pathological conditions, as happens, for example, for the EVs released from the mesenchymal stem cells of the bone marrow [124–127]. On the other hand, EVs that are released from the adipose tissue seem to be responsible for cognitive impairments in diabetes [128].

Even inside the brain, the precise roles of EVs are not completely understood, even if novel activities are continuously discovered or suggested; one of the most important activities of EVs released from both neurons and glial cells (especially from astrocytes) seems to be to modulate synaptic plasticity and hence complex functions such as learning and memory [49,129–131].

As long as it concerns the formation of the BBB, EVs seem to have a central role; as reported above, years ago, our group found that both neurons and astrocytes produce and release VEGF and FGF2 and that these factors are probably involved in the formation of the BBB in an in vitro system; interestingly, we found these factors in EVs [90,91]. Actually, EVs are released from all the cell types involved in the formation of the NVU, and all these EVs have been suggested to transport molecules important for the formation and maintenance of the BBB, as well as molecules to be eliminated from the brain into the blood [132–135]. Moreover, the mentioned ability of the EVs to cross the BBB offers the possibility to use circulating EVs as diagnostic biomarkers because of the presence of a few cell-specific surface markers. For example, recently, it has been discussed the possibility that some small non-coding RNAs, present in EVs recognizable as astrocyte-derived (ADEVs), appear to be dysregulated under neurodegeneration-prone conditions [136].

Notably, under pathological conditions, EVs may also contribute to altering the BBB and to spread diseases since they can contain toxic proteins, such as amyloid peptides, hyper-phosphorylated Tau proteins, prions, and aggregated α-synuclein [137–144]. In other words, the ability of brain cells to exchange a variety of cell components under physiological conditions is not lost under pathological conditions, but it becomes a sort of weapon that can cause "infection" of neighboring cells.

2.4. In Vitro Models Used to Study the Formation of the BBB

In the attempt to clarify the mechanisms involved in the induction of the BBB, various studies have been conducted on both in vivo and in vitro models. It is clear, anyway, that the barrier properties of BCECs are induced during CNS development by the microenvironment in which these cells are placed. Experimental studies have shown that BCECs, if isolated from their natural context and maintained in culture, lose their "barrier" phenotype, probably because of the lack of the epigenetic signals present in vivo and supplied by neurons, astrocytes, and the extracellular matrix (ECM) [145].

2.4.1. Co-Culture Models for Studying BBB Formation and Maintenance In Vitro

Many studies aimed at understanding the role of the different components of the neurovascular unit were based on in vitro co-culture systems, very often taking advantage of the so-called transwell system (Figure 3). This latter system is based on the use of special culture plates that can also host inserts with a porous membrane at their bottom. Endothelial cells are plated inside the insert, on the porous membrane, enriched with proteins of the basement membrane existing in vivo. Other brain cells can be cultured either at the bottom of the wells (i.e., at a distance from the BCECs) or on the outside of the insert (i.e., very close to the BCECs, even if on the other side of the porous membrane).

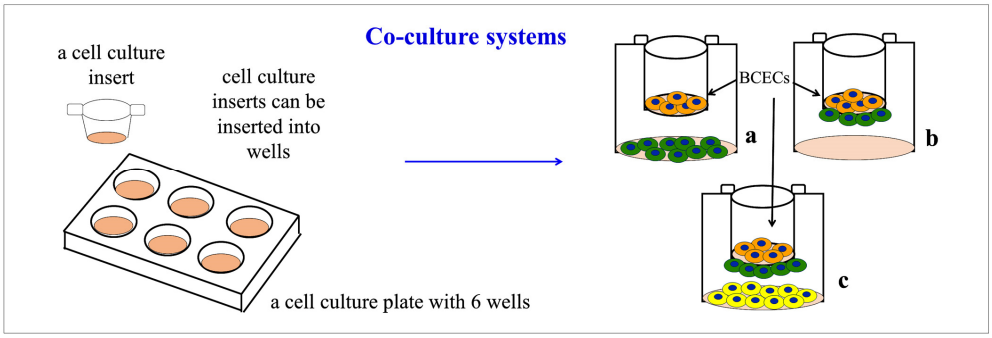

Figure 3. Simple co-culture systems (also called transwell systems) used for studying the role of different brain cell types on BBB formation. This system is based on culture plates with wells that can also host inserts with a porous membrane at their bottom. Endothelial cells (orange in the figure) are plated inside the insert, on the porous membrane, enriched with proteins of the basement membrane existing in vivo. Other brain cells (green in the figure) can be cultured either at the bottom of the wells (**a**) or on the outside of the insert, that is on the other side of the porous membrane (**b**). This system can also be used for studying synergistic effects on the barrier formation of different brain cell types (for example, neurons and astrocytes, or pericytes and astrocytes). These cells can be cultured as a mixed population or separated by culturing one cell type at the bottom of the wells (yellow cells in the figure) and on the outside of the inserts of the other one (green cells in the figure) (**c**).

Like other groups, by using this transwell system, we set up, in the past, an in vitro model of the BBB, in which the BCECs were initially only co-cultured with neurons [88]. We found that neurons are able to induce a cell line derived from the rat cerebral microvessels (RBE4.B cells) to synthesize and localize occludin to the cellular periphery, and that such a localization is modulated both by the composition of the substrate and by soluble signals released by cortical neurons. These effects do not require close physical contacts between cells; in fact, in the co-culture transwell system used in these experiments, the two cell populations laid at least 1 mm apart [88,89].

In vivo studies identified transport systems for the transfer of amino acids through the endothelial cells co-cultured with neurons; neutral amino acids experience the highest rates of transport [146,147]. On the other hand, the catechol groups provide the molecules with hydrophilicity; for this reason, dopamine and its related catecholamines do not cross the barrier. However, L-3,4-dihydroxyphenylalanine (L-DOPA), the precursor of dopamine, enters the brain from the blood more easily than expected from its fat solubility because of its affinity for the neutral amino acid transporters [148]. It has been reported that both L-DOPA and L-tryptophan permeate the barrier with time-dependent saturable kinetics [149–151]. The transfer of L-tryptophan, dopamine, and L-DOPA across the barrier was evaluated by our studies, and it has been seen that the system behaves as a selective interface that excludes dopamine while allowing the passage of L-tryptophan and L-DOPA. Thanks to a mathematical approach, both L-tryptophan and L-DOPA have been shown to move through the BCEC layer through a saturable process, thus indicating that, in the used model, specific carriers are always present.

Permeation studies have confirmed that the described co-culture system possesses the permeability limits characteristic of the BBB [89]. However, cells must be co-cultured with neurons for at least one week in order to observe a peripheral localization of occludin in endothelial cells [88]. Subsequently, we set up a more complex system with three cell types again based on brain capillary endothelial cells (RBE4.B) and neurons, but also including astrocytes (the system was identical to the one shown in Figure 3c, with astrocytes plated on the outside of the inserts and neurons at the bottom of the plate wells) [26]. These preliminary experiments showed that, in the presence of astrocytes, neuron-induced synthesis and peripheral localization of occludin are earlier (5 days of culture) when compared with the time required when BCECs are cultured with neurons only (7 days of culture). This observation suggested the existence of synergistic effects

played by neurons and astrocytes on the barrier's formation. BCECs grown alone or with neurons and/or astrocytes have shown a different ability to prevent the paracellular passage of sucrose from the donor compartment (i.e., the one to which the sucrose was added) to the acceptor compartment (i.e., the lowest one, in the transwell system, that is separated from the donor compartment by the endothelial cell layer); in particular, it was reported that both astrocytes and neurons are able to independently reduce the paracellular passage of sucrose [27]. Thus, the effects of the two cell types are additive: when BCECs are cultured with astrocytes and neurons, their paracellular flux is reduced by one-third. However, the synergistic effect is only visible after 5 days of co-culture. The quality and/or intensity of the effects of astrocytes and neurons on the BCECs depend on how long the cells have been cultured together [27].

The anti-occludin antibodies used in the experiments reported above have a target in an internal region of human occludin (amino acids 132–411); this antibody recognized two different proteins, of which only one (p60) had been expected. The second protein (p48) had never been described before. This protein increases during development even more than p60 and, most interestingly, is enriched in the BCECs cultured with both astrocytes and neurons for 5 days, i.e., under those conditions in which the BCEC layer shows its highest efficiency as a barrier to the passage of sucrose. Although it cannot be excluded that it derives from the degradation of occludin, its abundance, together with the fact that the concentration of p48 changes significantly during development and under different cultural conditions, in relation to the establishment of a functional barrier, suggests that it has a role to play in the formation and maintenance of the BBB [27]. A three-cell-type culture system, including human BCECs, neurons, and astrocytes, and also based on the transwell system, has been more recently described by Barberio et al. [152], who clearly demonstrated the relationship among the presence of astrocytes and neurons and the highest TEER measurements, confirming the existence of a crosstalk between these two cell types and the endothelial cells. Similarly, Ledwig and Reichl reported that three-cell-type models, obtained by using all cell types coming from the same species, also provide a suitable tool for analyzing the permeation properties of new potential brain-targeted drugs [153].

In addition, the interactions between astrocytes and endothelial cells have been studied in a system that also considers the possible effects of a fluid flow (that simulates the blood flow) on BCEC differentiation [154]. In this system, bovine aortic endothelial cells are cultured inside hollow tubes in which the culture medium is allowed to pass, while outside the tubes, there is a chamber that contains the astrocytes (glioma C6 cells). Thanks to this model, it has been shown that this flow plays an essential role in the differentiation of the ECs: it indeed causes an arrest of cell divisions, thus preventing growth on multiple layers; this latter effect is of paramount importance since in vivo the BCECs organize themselves in a single layer. The effect of the flow, added to that of the astrocytes, leads to the reduction in cellular permeability and allows to obtain a system that mimics the real organization of the BBB.

Actually, in vitro co-culture systems allowed for the important demonstration of the effects of different brain cells on the expression of the proteins involved in TJ formation. For example, in a non-human co-culture system in which monkey BCECs were cultured with both rat pericytes and astrocytes, the formation of a barrier was reported based on an increased expression of junctional proteins, such as claudins, ZO-1, and occludin; at the same time, they found an increase in the levels of glucose transporters and ATP-binding cassette-containing (ABC) efflux transporters [155]. The use of an in vitro BBB model also allowed to demonstrate the ability of metformin to cross the BBB, especially under conditions of oxygen–glucose deprivation [156].

Interestingly, co-culture systems prepared from different mammals have shown different levels of TEER; in spite of that, the BBB properties are identical, thus suggesting that different properties, including the species-specific size of BCECs, can be important for determining the ability to control the transendothelial traffic [157].

2.4.2. In Vitro Models Aimed at Studying Modifications of the BBB's Function under Pathological Conditions as well as the Ability of Drugs to cross a Functional BBB

Recently, in vitro BBB models have been also used to study BBB alterations under pathological conditions, such as cancer, neurodegeneration, or brain injuries [158–162].

As discussed above, the interaction between brain tissue and blood circulation actually takes place on the neurovascular units (NVUs), structures that include different cell types, and their associated extracellular matrix (ECM). In order to create NVU-like structures, pluripotent stem cells (iPSCs) can be induced to differentiate in the NVU cell types and then cultured together in an ECM-like environment [163–165]. A similar model, comprising neural and endothelial cells from newborn rats capable of self-assembly in a 3D structure that included a Matrigel ECM, was found to present vascular and BBB-like structures. Strikingly, the obtained NVU could induce vessel formation in the brains of rats suffering from cerebral ischemia [164].

Wevers and colleagues developed a stroke model 'on-a-chip' consisting of human BCECs co-cultured with astrocytes and neurons derived from induced pluripotent stem cells (iPSCs). In this model, endothelial cells produce functional transporters and neurons are able to fire. This pathological model was created by inducing hypoxia and hypoglycemia and by blocking the normal fluid flow. Interestingly, under 'stroke' conditions, the BCECs increase their permeability and reduce their mitochondrial potential [166].

These models have been utilized in the case of ischemic stroke, trying to recreate the cell interactions and the circulatory flow typical of the brain neurovascular units (NVUs) [165].

Recently, a new method for the simultaneous isolation of all the NVU cell types from the murine ischemic brain has been described (EPAM-ia method) [167]. Thanks to this method, a NVU transcriptome database was constructed, and it was possible to demonstrate that an osteopontin gene (Spp1) is upregulated after stroke. Interestingly, the increase of osteopontin was also found in stroke patients. Moreover, it was shown that the injection of anti-osteopontin antibodies in the mice reduced brain edema and protected the BBB, opening the possibility that these antibodies could be used in acute ischemic stroke therapy [167].

Notably, the use of both mono- and co-culture in vitro systems, as well as the use of media conditioned by brain cells such as astrocytes and pericytes, also suggested that, after an injury, both cell types can release inhibitory molecules that downregulate the production of TJ proteins, thus prolonging BBB dysfunction [168].

In general terms, the development of in vitro models of the BBB gives the possibility to study in a controlled environment the relationship among the different components of this very complex system. These in vitro models, and specifically the microfluidic-based ones, are particularly useful for studying pathological conditions because vascular dysfunctions might be directly related to disease progression. This could lead to the possibility of evaluating potentially active drugs and possibly elaborate new therapeutic strategies [169].

In vitro testing platforms, named microphysiological systems (MPSs), have been, in particular, used for the analysis of the ability of drugs to cross the BBB [170,171]. A microfluidic in vitro BBB model (BBB-on-a-chip) has been used to evaluate the possibility to study, in a human in vitro system, the permeability of nanoparticles loaded with therapeutic agents [158,172]. Microfluidic devices have been also used to study the properties of the BBB with intact TJs [173]. In general terms, the use of in vitro transwell systems can be of much help in setting up methods allowing for the transfer of drugs, natural molecules, or even cells across the BBB [11,174]. In one of these systems, the possibility of selecting recombinant adeno-adenoassociated viruses (rAAVs) able to mediate the delivery of recombinant genes to the brain has been successfully studied [175]. In another system, a microfluidic platform has been set up in order to study the ability of lymphocytes to cross the BBB under pathological conditions and how to restore BBB integrity in order to prevent this influx into the brain [176].

In conclusion, we can tell that, in general, the BBB in vitro models could be very useful for not only studying the molecular and cellular mechanisms underlying BBB formation and maintenance but also for studying the capacity of a variety of prodrugs to cross the BBB and acquire a functional structure on the "trans" side of the barrier.

It is, however, clear that these systems might only partially reproduce the properties of the BBB in a series of contexts. Thus, it is of paramount importance to test in vivo systems in order to confirm the data obtained in the experimental in vitro models.

3. Pathological Alterations of the BBB

As discussed above, the BBB is a fundamental structure for ensuring and protecting brain functions. However, BBB integrity can be compromised by many pathological conditions, many of which directly arise in the nervous system, while others can be linked to peripheral disorders. Concerning this latter case, it has been found, for example, that chronic kidney disease can cause cognitive impairments by influencing BBB integrity because of an activation of matrix metalloprotease 2 (MMP2) by the high levels of circulating urea [177]. Similar effects have been observed under hepatic encephalopaty, in which hepatic dysfunction affects the integrity of the neurovascular unit [178]. Moreover, hyperglycemic conditions can affect the production of connexin 43 (Cx43), an important component of the gap junctions that bind astrocytes with each other, thus affecting astrocytic properties and, hence, BBB integrity [179]. In addition, it has been found that the depletion of intestinal microbiota, due to antiobitotic use in adult mice, alters the BBB [180]. Notably, some viruses can invade the brain, also causing BBB disruption [181–186].

On the other hand, in all the neurological pathologies, from cancer [187–190] to traumatic brain injury and stroke [191–195], from neurodegeneration (see below) to epilepsy [196], and even in disorders depending on sleep deprivation [197], alterations of the BBB's integrity have been observed, and in most cases, astrocytes appear to play a central role. As important cellular elements of the neurovascular unit, astrocytes indeed play a prominent role in mediating connections between the endovascular system and neurons and between the immune system and neurons. As a consequence, they also affect brain functioning under pathological conditions, and, in particular, BBB integrity.

Below, we will first consider the astrocytic alterations observed in Alzheimer's disease (AD), the most common neurodegenerative disease, and multiple sclerosis (MS), an example of an inflammatory disease. Then, in the third paragraph, we will review some cases of astrocytic alterations under other pathological conditions.

3.1. Alzheimer's Disease and BBB Alteration: A Focus on Astrocytes

AD is the most common neurodegenerative disease in the world, as well as the first cause of dementia; it is mainly characterized by the deposition of extracellular plaques of β-amyloid (Aβ) and by intracellular neurofibrillary tangles (NFTs) of hyperphosphorylated Tau protein. Among other effects, β-amyloid can damage the BBB by aggregating around the vessels, thus also causing glucose transport dysfunction [198]. Moreover, the aggregates of Tau proteins can also alter BBB integrity [199]. In addition, in recent years, other pathological mechanisms have been shown to have a role in the pathophysiology of diseases, such as microvascular disorders, alteration of cerebral blood flow (CBF), and compromised integrity and permeability of the BBB [200,201]. For example, it has been reported that high levels of heparanase in astrocytic endfeet, by inducing an excessive fragmentation of heparan sulfate, can alter the normal process of drainage across the vessel wall [202]. In addition, β-amyloid seems to also be able to stimulate astrocytes to release soluble factors that affect BBB stabilization [203], while inhibiting, on the other hand, the release of FGF-2 [204]. In general terms, altering the ECM components seems to have a role in mediating the damage to both astrocytes and pericytes and, as a consequence, BBB properties in AD [205].

Thus, it seems that BBB dysfunction plays a role in both the onset and progression of AD.

Notably, different structural alterations of astrocytes have been found in AD, probably depending on the microenvironment. Studies on post-mortem brains of familial AD subjects have shown atrophy of astrocytes contributing to the disruption of the BBB, while, in mouse pathology models, around amyloid plaques, astrocytes appear to take on a hypertrophic appearance, with increased cell bodies and processes [206]. The presence of astrocytes around the plaques is probably due to their normal role in phagocytosing amyloid, although in the AD brains, some membrane proteins that internalize amyloid, such as the low-density lipoprotein receptor-related protein 4 (LRP4), are reduced in the reactive astrocytes [207].

Among the mechanisms causing BBB damage, one of the most accredited is metabolic damage, such as, for example, hypercholesterolemia and hypertension [208]. Apolipoprotein E (APOE) isoform 4, a molecule synthesized in the CNS by astrocytes and microglia, and in the periphery by the liver starting from cholesterol precursors, is indeed a risk factor for the onset of AD. It was recently demonstrated, in a specific knockin mouse model, that the expression of APOE4, and not the expression of other isoforms such as APOE2 and APOE3, reduces the astrocyte-mediated protection of the cerebral vessels. The knockout of astrocyte-derived APOE4 restores BBB integrity [209]. Thus, specific protein expression changes in astrocytes can cause an alteration of their functions, affecting, in particular, their ability in maintaining homeostasis of the extracellular environment.

Moreover, in the brain, astrocytes also play a role in controlling neuroinflammation through their interactions with the cells of the immune system. In particular, it has been shown that due to the increased expression of endothelial adhesion molecules, CD4+ and CD8+, T lymphocytes massively infiltrate the areas of the CNS typically affected in AD, and their presence is increased around to sites of Aβ plaque deposition [210]. However, the role of lymphocytes once they have entered the CNS is not yet completely clear. There are, indeed, both reports of deleterious actions and reports of protective functions. It has been shown that CD4+ cells primed with Aβ are able to interact with astrocytes and modify the expression of synaptic proteins in human neuronal-like SH-SY5Y cells, such as by decreasing the expression of synaptophysin. However, a reduction in inflammatory cytokines and a modification of BBB function with changes in claudin and ICAM-1 expression were also observed in astrocytes co-cultured with CD4+ cells [211].

3.2. Multiple Sclerosis: Astrocytes of the BBB

MS is a chronic inflammatory and degenerative disease of the central nervous system (CNS). Although the exact pathogenetic mechanism of this disease is not yet known, numerous cell types, both resident or not in the CNS, have been shown to be involved at varying degrees in the mechanisms underlying this disease. These cell types can be grouped into three categories: (i) cells of the nervous system, including neurons, oligodendroglia, astroglia, and microglia; (ii) cells of the immune system, in particular CD8+ T lymphocytes, CD4+ T helper-1 and T helper-17 T lymphocytes, and B lymphocytes; and (iii) cells of the vascular system, including the endothelial cells. The crossroad between the immune, vascular, and nervous systems is located at the BBB [212]. Nevertheless, BBB alterations are a hallmark of MS and represent the main pathogenetic mechanism of the relapses. The breakdown of the BBB has a radiological counterpart in lesions evident in magnetic resonance imaging [213]. All the cells that are part of the BBB are involved in the dysregulation of the BBB in MS. Astrocytes, in particular, have shown several pathological involvements in this disease both during the recurrence of relapses and during its clinical progression. In fact, astrocytes interact with cells of the immune system, including self-reactive lymphocytes, and adapt themselves by assuming an inflammatory phenotype [214,215]. The alteration of astrocytes have two main effects, schematically grouped into those resulting from a loss of their homeostatic function and those connected to the acquisition of pathogenic properties. In MS, dysfunctions of the neurovascular unit is also due to the activation of astrocytes that detach from the vessels [216]. In both active and chronic lesions of MS, astrocytes downregulate their production of VEGFA, with a consequent inability to support

endothelial growth and stabilization [215]. Moreover, the VEGFA released by astrocytes under the conditions of neuroinflammation could also be deleterious and mediate the leakage of the BBB and endothelial damage [217].

It has been shown that pathogenic lymphocytes, particularly Th1 and Th17 lymphocytes, migrate into the CNS and interact with astrocytes at the BBB, suppressing their physiological functions in BBB regulation. In particular, activated astrocytes cause the breakdown of the BBB [215] through the production of different mediators, such as astrocytic thymidine phosphorylase and vascular endothelial growth factor A, that have been shown to disrupt the BBB in the presence of CNS inflammatory lesions [218]. Moreover, reactive astrocytes are also present on the edges of chronically active lesions associated with high clinical disability and increased risk of progression in patients with MS [219]. They have also been found in apparently normal white matter [220], suggesting that astrocytes may infiltrate different areas of the CNS before the onset of damage and are somehow early mediators of the pathology.

Interestingly, EVs released from all the cells of the vessel microenvironment have been reported to have an impact on BBB damage [221], perhaps as carriers of microRNAs (miRs) able to inhibit the expression of mRNAs encoding fundamental factors for BBB maintenance. For example, it has been reported that miR-155 is involved in BBB integrity disruption by inhibiting the synthesis of proteins important for the correct formation of junctional complexes at the BBB [222].

3.3. Astrocyte and BBB Alteration under Other Pathological Conditions

As mentioned at the beginning of this paragraph, it has been clearly demonstrated that many pathological conditions, either born in the nervous system or in the periphery, have an effect on BBB integrity. For example, it has been recently found that in methamphetamine (METH)-induced neurotoxicity, neurons not only transfer an excessive amount of aggregated α-synuclein (α-syn) to other neurons but also to astrocytes, where it can induce a decrease in the nuclear receptor-related protein 1 (Nurr1), thus causing an increase in pro-inflammatory factors and BBB damage [223]. Actually, an excess of aggregated α-syn is also normally produced in Parkinson's disease (PD); thus, also in PD, in which BBB integrity loss is also evident, similar effects on astrocytes and, as a consequence, on the BCEC barrier function might be envisaged.

Similarly, in intracerebral hemorrhage, further damage derives from the formation of perihematomal edema (PHE), which, in turn, causes BBB disruption. It has been found that in PHE, AQP4 expression in the astrocytic endfeet is highly decreased, probably as a response to the increase of reactive oxygen species (ROS) due to the hemorrhage [224].

Another pathological condition that involves AQP4 is neuromyelitis optica spectrum disorder (NMOSD), in which patients produce anti-AQP4 antibodies that target astrocytes, thus damaging the BBB, and allowing for further damage to arise due to the chemoattraction of polymorphonuclear leukocytes; notably, however, BBB repair precedes repopulation by astrocytes [225].

Interestingly, it has also been found that when some astrocytes are damaged, the surrounding ones can substitute for them by extending processes that can reach and cover the vascular walls left exposed by lost astrocytes [226].

It is also important to underline that senescence with age of the cells that constitute the NVU, and especially of astrocytes, will contribute to BBB damage and, in turn, to neurotoxicity and inflammation [226–229].

Finally, it is worth noting that it has been reported that ethanol consumption during pregnancy and/or lactation can modify BCEC properties and, in turn, astrocyte gene expression and activities, thus inducing permanent damage to the BBB [230]. Similarly, tobacco smoking and even electronic cigarettes during pregnancy can alter the normal expression of most structural BBB elements, resulting impaired CNS functions, including learning and memory abilities [231].

4. Conclusions and Perspectives

In conclusion, we can certainly describe the BBB as a fundamental structure for the maintenance of the environment that allows for all the functions of the central nervous system, from the control of all the body's physiological activities to more complex abilities such as learning and memory. All the cells that contribute to the microenvironment of the brain capillaries have a function in their structure and function, with many activities, as discussed above, to be attributed to astrocytes. A remarkable observation is that most pathological disorders directly affecting the central nervous system, but also many disorders primarily affecting other organs, can have an impact on the structure and function of the BBB, thus causing, in any case, a worsening of brain activities. Actually, efficient therapies for BBB disruption are not yet available. We suggest that in the near future, an interesting tool might derive from the use of extracellular vesicles (EVs) that might be loaded with a variety of molecules (proteins, metabolites, and drugs, as well as both coding and non-coding RNAs); these EVs might counteract the activities of other molecular species underlying the observed BBB alterations. Importantly, the discussed ability of EVs to cross even an intact BBB in both directions might allow them to treat neurological diseases in very early moments, well before the emergence of pathology-dependent BBB alterations.

Author Contributions: Conceptualization and artwork, I.D.L.; writing and editing, all the authors. All authors have read and agreed to the published version of the manuscript.

Funding: The authors did not receive any external funding.

Acknowledgments: The authors are supported by the Università degli Studi di Palermo (University of Palermo), Palermo, Italy.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Ballabh, P.; Braun, A.; Nedergaard, M. The blood-brain barrier: An overview: Structure, regulation, and clinical implications. *Neurobiol. Dis.* **2004**, *16*, 1–13. [CrossRef]
2. Abbott, N.J.; Rönnbäck, L.; Hansson, E. Astrocyte-endothelial interactions at the blood-brain barrier. *Nat. Rev. Neurosci.* **2006**, *7*, 41–53. [CrossRef] [PubMed]
3. Serlin, Y.; Shelef, I.; Knyazer, B.; Friedman, A. Anatomy and physiology of the blood-brain barrier. *Semin. Cell Dev. Biol.* **2015**, *38*, 2–6. [CrossRef] [PubMed]
4. Harder, D.R.; Zhang, C.; Gebremedhin, D. Astrocytes function in matching blood flow to metabolic activity. *News Physiol. Sci.* **2002**, *17*, 27–31. [CrossRef] [PubMed]
5. Blanchette, M.; Daneman, R. Formation and maintenance of the BBB. *Mech. Dev.* **2015**, *138*, 8–16. [CrossRef] [PubMed]
6. Archie, S.R.; Al Shoyaib, A.; Cucullo, L. Blood-Brain Barrier Dysfunction in CNS Disorders and Putative Therapeutic Targets: An Overview. *Pharmaceutics* **2021**, *13*, 1779. [CrossRef]
7. Cibelli, A.; Stout, R.; Timmermann, A.; de Menezes, L.; Guo, P.; Maass, K.; Seifert, G.; Steinhäuser, C.; Spray, D.C.; Scemes, E. Cx43 carboxyl terminal domain determines AQP4 and Cx30 endfoot organization and blood brain barrier permeability. *Sci. Rep.* **2021**, *11*, 24334. [CrossRef]
8. Kugler, E.C.; Greenwood, J.; MacDonald, R.B. The "Neuro-Glial-Vascular" Unit: The Role of Glia in Neurovascular Unit Formation and Dysfunction. *Front. Cell Dev. Biol.* **2021**, *9*, 732820. [CrossRef]
9. McConnell, H.L.; Mishra, A. Cells of the Blood-Brain Barrier: An Overview of the Neurovascular Unit in Health and Disease. *Methods Mol. Biol.* **2022**, *2492*, 3–24. [CrossRef]
10. Naranjo, O.; Osborne, O.; Torices, S.; Toborek, M. In Vivo Targeting of the Neurovascular Unit: Challenges and Advancements. *Cell Mol. Neurobiol.* **2022**, *42*, 2131–2146. [CrossRef]
11. Zidarič, T.; Gradišnik, L.; Velnar, T. Astrocytes and human artificial blood-brain barrier models. *Bosn. J. Basic Med. Sci.* **2022**, *22*, 651–672. [CrossRef] [PubMed]
12. Chen, L.; Zhen, Y.; Wang, X.; Wang, J.; Zhu, G. Neurovascular glial unit: A target of phytotherapy for cognitive impairments. *Phytomedicine* **2023**, *119*, 155009. [CrossRef]
13. Cresto, N.; Janvier, A.; Marchi, N. From neurons to the neuro-glio-vascular unit: Seizures and brain homeostasis in networks. *Rev. Neurol.* **2023**, *179*, 308–315. [CrossRef]
14. Gnanasekaran, R.; Aickareth, J.; Hawwar, M.; Sanchez, N.; Croft, J.; Zhang, J. CmPn/CmP Signaling Networks in the Maintenance of the Blood Vessel Barrier. *J. Pers. Med.* **2023**, *13*, 751. [CrossRef] [PubMed]

15. Hourfar, H.; Aliakbari, F.; Aqdam, S.R.; Nayeri, Z.; Bardania, H.; Otzen, D.E.; Morshedi, D. The impact of α-synuclein aggregates on blood-brain barrier integrity in the presence of neurovascular unit cells. *Int. J. Biol. Macromol.* **2023**, *229*, 305–320. [CrossRef] [PubMed]
16. Fan, F.; Jiang, H.; Hou, Y.; Zhang, Y.; Zhao, Q.; Zeng, Y.; Meng, X.; Wang, X. Barrier Functional Integrity Recording on bEnd.3 Vascular Endothelial Cells via Transendothelial Electrical Resistance Detection. *J. Vis. Exp.* **2023**, *199*, e65938. [CrossRef] [PubMed]
17. Stamatovic, S.M.; Keep, R.F.; Andjelkovic, A.V. Brain endothelial cell-cell junctions: How to "open" the blood brain barrier. *Curr. Neuropharmacol.* **2008**, *6*, 179–192. [CrossRef]
18. Furuse, M.; Sasaki, H.; Fujimoto, K.; Tsukita, S. A single gene product, claudin-1 or -2, reconstitutes tight junction strands and recruits occludin in fibroblasts. *J. Cell Biol.* **1998**, *143*, 391–401. [CrossRef]
19. Tsukita, S.; Furuse, M. Occludin and claudins in tight-junction strands: Leading or supporting players? *Trends Cell Biol.* **1999**, *9*, 268–273. [CrossRef]
20. Mooradian, A.D.; Haas, M.J.; Chehade, J.M. Age-related changes in rat cerebral occludin and zonula occludens-1 (ZO-1). *Mech. Ageing Dev.* **2003**, *124*, 143–146. [CrossRef]
21. Van Itallie, C.M.; Rogan, S.; Yu, A.; Vidal, L.S.; Holmes, J.; Anderson, J.M. Two splice variants of claudin-10 in the kidney create paracellular pores with different ion selectivities. *Am. J. Physiol. Renal Physiol.* **2006**, *291*, F1288–F1299. [CrossRef] [PubMed]
22. Tsukita, S.; Tanaka, H.; Tamura, A. The Claudins: From Tight Junctions to Biological Systems. *Trends Biochem. Sci.* **2019**, *44*, 141–152. [CrossRef] [PubMed]
23. Chiba, H.; Ichikawa-Tomikawa, N.; Imura, T.; Sugimoto, K. The region-selective regulation of endothelial claudin-5 expression and signaling in brain health and disorders. *J. Cell Physiol.* **2021**, *236*, 7134–7143. [CrossRef] [PubMed]
24. Hashimoto, Y.; Greene, C.; Munnich, A.; Campbell, M. The CLDN5 gene at the blood-brain barrier in health and disease. *Fluids Barriers CNS* **2023**, *20*, 22. [CrossRef] [PubMed]
25. Lapierre, L.A. The molecular structure of the tight junction. *Adv. Drug Deliv. Rev.* **2000**, *41*, 255–264. [CrossRef]
26. Schiera, G.; Bono, E.; Raffa, M.P.; Gallo, A.; Pitarresi, G.L.; Di Liegro, I.; Savettieri, G. Synergistic effects of neurons and astrocytes on the differentiation of brain capillary endothelial cells in culture. *J. Cell Mol. Med.* **2003**, *7*, 165–170. [CrossRef]
27. Schiera, G.; Sala, S.; Gallo, A.; Raffa, M.P.; Pitarresi, G.L.; Savettieri, G.; Di Liegro, I. Permeability properties of a three-cell type in vitro model of blood-brain barrier. *J. Cell Mol. Med.* **2005**, *9*, 373–379. [CrossRef]
28. Bazzoni, G.; Martínez Estrada, O.; Dejana, E. Molecular structure and functional role of vascular tight junctions. *Trends Cardiovasc. Med.* **1999**, *9*, 147–152. [CrossRef]
29. Abbott, N.J.; Patabendige, A.A.; Dolman, D.E.; Yusof, S.R.; Begley, D.J. Structure and function of the blood-brain barrier. *Neurobiol. Dis.* **2010**, *37*, 13–25. [CrossRef]
30. Ransohoff, R.M.; Engelhardt, B. The anatomical and cellular basis of immune surveillance in the central nervous system. *Nat. Rev. Immunol.* **2012**, *12*, 623–635. [CrossRef]
31. Kniesel, U.; Wolburg, H. Tight junctions of the blood-brain barrier. *Cell Mol. Neurobiol.* **2000**, *20*, 57–76. [CrossRef] [PubMed]
32. Obermeier, B.; Daneman, R.; Ransohoff, R.M. Development, maintenance and disruption of the blood-brain barrier. *Nat. Med.* **2013**, *19*, 1584–1596. [CrossRef] [PubMed]
33. Risau, W.; Wolburg, H. Development of the blood-brain barrier. *Trends Neurosci.* **1990**, *13*, 174–178. [CrossRef] [PubMed]
34. Swanson, R.A. Physiologic coupling of glial glycogen metabolism to neuronal activity in brain. *Can. J. Physiol. Pharmacol.* **1992**, *70* (Suppl. S1), S138–S144. [CrossRef] [PubMed]
35. Pellerin, L.; Magistretti, P.J. Glutamate uptake into astrocytes stimulates aerobic glycolysis: A mechanism coupling neuronal activity to glucose utilization. *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 10625–10629. [CrossRef] [PubMed]
36. Schurr, A.; Miller, J.J.; Payne, R.S.; Rigor, B.M. An increase in lactate output by brain tissue serves to meet the energy needs of glutamate-activated neurons. *J. Neurosci.* **1999**, *19*, 34–39. [CrossRef] [PubMed]
37. Smith, D.; Pernet, A.; Hallett, W.A.; Bingham, E.; Marsden, P.K.; Amiel, S.A. Lactate: A preferred fuel for human brain metabolism in vivo. *J. Cereb. Blood Flow Metab.* **2003**, *23*, 658–664. [CrossRef]
38. Pellerin, L.; Magistretti, P.J. Sweet sixteen for ANLS. *J. Cereb. Blood Flow Metab.* **2012**, *32*, 1152–1166. [CrossRef]
39. Dienel, G.A. The metabolic trinity, glucose-glycogen-lactate, links astrocytes and neurons in brain energetics, signaling, memory, and gene expression. *Neurosci. Lett.* **2015**, *637*, 18–25. [CrossRef]
40. Proia, P.; Di Liegro, C.M.; Schiera, G.; Fricano, A.; Di Liegro, I. Lactate as a Metabolite and a Regulator in the Central Nervous System. *Int. J. Mol. Sci.* **2016**, *17*, 1450. [CrossRef]
41. Barros, L.F.; Weber, B. CrossTalk proposal: An important astrocyte-to-neuron lactate shuttle couples neuronal activity to glucose utilisation in the brain. *J. Physiol.* **2018**, *596*, 347–350. [CrossRef] [PubMed]
42. Magistretti, P.J.; Allaman, I. Lactate in the brain: From metabolic end-product to signalling molecule. *Nat. Rev. Neurosci.* **2018**, *19*, 235–249. [CrossRef]
43. Dembitskaya, Y.; Piette, C.; Perez, S.; Berry, H.; Magistretti, P.J.; Venance, L. Lactate supply overtakes glucose when neural computational and cognitive loads scale up. *Proc. Natl. Acad. Sci. USA* **2022**, *119*, e2212004119. [CrossRef] [PubMed]
44. Bhatti, M.S.; Frostig, R.D. Astrocyte-neuron lactate shuttle plays a pivotal role in sensory-based neuroprotection in a rat model of permanent middle cerebral artery occlusion. *Sci. Rep.* **2023**, *13*, 12799. [CrossRef] [PubMed]
45. Suzuki, A.; Stern, S.A.; Bozdagi, O.; Huntley, G.W.; Walker, R.H.; Magistretti, P.J.; Alberini, C.M. Astrocyte-neuron lactate transport is required for long-term memory formation. *Cell* **2011**, *144*, 810–823. [CrossRef]

46. Adamsky, A.; Goshen, I. Astrocytes in Memory Function: Pioneering Findings and Future Directions. *Neuroscience* **2018**, *370*, 14–26. [CrossRef]
47. Adamsky, A.; Kol, A.; Kreisel, T.; Doron, A.; Ozeri-Engelhard, N.; Melcer, T.; Refaeli, R.; Horn, H.; Regev, L.; Groysman, M.; et al. Astrocytic Activation Generates De Novo Neuronal Potentiation and Memory Enhancement. *Cell* **2018**, *174*, 59–71.e14. [CrossRef]
48. Harris, R.A.; Lone, A.; Lim, H.; Martinez, F.; Frame, A.K.; Scholl, T.J.; Cumming, R.C. Aerobic Glycolysis is required for spatial memory acquisition but not for memory retrieval in mice. *eNeuro* **2019**, *6*, ENEURO.0389-18.2019. [CrossRef]
49. Schiera, G.; Di Liegro, C.M.; Di Liegro, I. Cell-to-Cell Communication in Learning and Memory: From Neuro- and Glio-Transmission to Information Exchange Mediated by Extracellular Vesicles. *Int. J. Mol. Sci.* **2019**, *21*, 266. [CrossRef]
50. Akter, M.; Hasan, M.; Ramkrishnan, A.S.; Iqbal, Z.; Zheng, X.; Fu, Z.; Lei, Z.; Karim, A.; Li, Y. Astrocyte and L-lactate in the anterior cingulate cortex modulate schema memory and neuronal mitochondrial biogenesis. *Elife* **2023**, *12*, e85751. [CrossRef]
51. Lauritzen, K.H.; Morland, C.; Puchades, M.; Holm-Hansen, S.; Hagelin, E.M.; Lauritzen, F.; Attramadal, H.; Storm-Mathisen, J.; Gjedde, A.; Bergersen, L.H. Lactate receptor sites link neurotransmission, neurovascular coupling, and brain energy metabolism. Cereb. *Cortex* **2014**, *24*, 2784–2795. [CrossRef] [PubMed]
52. Morland, C.; Lauritzen, K.H.; Puchades, M.; Holm-Hansen, S.; Andersson, K.; Gjedde, A.; Attramadal, H.; Storm-Mathisen, J.; Bergersen, L.H. The lactate receptor, G-protein-coupled receptor 81/hydroxycarboxylic acid receptor 1: Expression and action in brain. *J. Neurosci. Res.* **2015**, *93*, 1045–1055. [CrossRef] [PubMed]
53. de Castro Abrantes, H.; Briquet, M.; Schmuziger, C.; Restivo, L.; Puyal, J.; Rosenberg, N.; Rocher, A.B.; Offermanns, S.; Chatton, J.Y. The lactate receptor HCAR1 modulates neuronal network activity through the activation of Gα and Gβγ subunits. *J. Neurosci.* **2019**, *39*, 4422–4433. [CrossRef]
54. Colucci, A.C.M.; Tassinari, I.D.; Loss, E.D.S.; de Fraga, L.S. History and Function of the Lactate Receptor GPR81/HCAR1 in the Brain: A Putative Therapeutic Target for the Treatment of Cerebral Ischemia. *Neuroscience* **2023**, *526*, 144–163. [CrossRef] [PubMed]
55. Vela, D. Hepcidin, an emerging and important player in brain iron homeostasis. *J. Transl. Med.* **2018**, *16*, 25. [CrossRef] [PubMed]
56. Yanase, K.; Uemura, N.; Chiba, Y.; Murakami, R.; Fujihara, R.; Matsumoto, K.; Shirakami, G.; Araki, N.; Ueno, M. Immunoreactivities for hepcidin, ferroportin, and hephaestin in astrocytes and choroid plexus epithelium of human brains. *Neuropathology* **2020**, *40*, 75–83. [CrossRef]
57. Zhang, X.; Gou, Y.J.; Zhang, Y.; Li, J.; Han, K.; Xu, Y.; Li, H.; You, L.H.; Yu, P.; Chang, Y.Z.; et al. Hepcidin overexpression in astrocytes alters brain iron metabolism and protects against amyloid-β induced brain damage in mice. *Cell Death Discov.* **2020**, *6*, 113. [CrossRef]
58. You, L.; Yu, P.P.; Dong, T.; Guo, W.; Chang, S.; Zheng, B.; Ci, Y.; Wang, F.; Yu, P.; Gao, G.; et al. Astrocyte-derived hepcidin controls iron traffic at the blood-brain-barrier via regulating ferroportin 1 of microvascular endothelial cells. *Cell Death Dis.* **2022**, *13*, 667. [CrossRef]
59. Davaanyam, D.; Lee, H.; Seol, S.I.; Oh, S.A.; Kim, S.W.; Lee, J.K. HMGB1 induces hepcidin upregulation in astrocytes and causes an acute iron surge and subsequent ferroptosis in the postischemic brain. *Exp. Mol. Med.* **2023**, *55*, 2402–2416. [CrossRef]
60. Gordleeva, S.Y.; Tsybina, Y.A.; Krivonosov, M.I.; Ivanchenko, M.V.; Zaikin, A.A.; Kazantsev, V.B.; Gorban, A.N. Modeling Working Memory in a Spiking Neuron Network Accompanied by Astrocytes. *Front. Cell Neurosci.* **2021**, *15*, 631485. [CrossRef]
61. Linsambarth, S.; Carvajal, F.J.; Moraga-Amaro, R.; Mendez, L.; Tamburini, G.; Jimenez, I.; Verdugo, D.A.; Gómez, G.I.; Jury, N.; Martínez, P.; et al. Astroglial gliotransmitters released via Cx43 hemichannels regulate NMDAR-dependent transmission and short-term fear memory in the basolateral amygdala. *FASEB J.* **2022**, *36*, e22134. [CrossRef]
62. Foubert, D.; Cookson, F.; Ruthazer, E.S. Capturing a rising star: The emerging role of astrocytes in neural circuit wiring and plasticity-lessons from the visual system. *Neurophotonics* **2023**, *10*, 044408. [CrossRef] [PubMed]
63. Goenaga, J.; Araque, A.; Kofuji, P.; Herrera Moro Chao, D. Calcium signaling in astrocytes and gliotransmitter release. *Front. Synaptic Neurosci.* **2023**, *15*, 1138577. [CrossRef]
64. Purushotham, S.S.; Buskila, Y. Astrocytic modulation of neuronal signalling. *Front. Netw. Physiol.* **2023**, *3*, 1205544. [CrossRef] [PubMed]
65. Rasia-Filho, A.A.; Calcagnotto, M.E.; von Bohlen Und Halbach, O. Glial Cell Modulation of Dendritic Spine Structure and Synaptic Function. *Adv. Neurobiol.* **2023**, *34*, 255–310. [CrossRef] [PubMed]
66. Rennels, M.L.; Gregory, T.F.; Blaumanis, O.R.; Fujimoto, K.; Grady, P.A. Evidence for a "paravascular" fluid circulation in the mammalian central nervous system, provided by the rapid distribution of tracer protein throughout the brain from the subarachnoid space. *Brain Res.* **1985**, *326*, 47–63. [CrossRef]
67. Iliff, J.J.; Wang, M.; Zeppenfeld, D.M.; Venkataraman, A.; Plog, B.A.; Liao, Y.; Deane, R.; Nedergaard, M. Cerebral arterial pulsation drives paravascular CSF-interstitial fluid exchange in the murine brain. *J. Neurosci.* **2013**, *33*, 18190–18199. [CrossRef]
68. Aspelund, A.; Antila, S.; Proulx, S.T.; Karlsen, T.V.; Karaman, S.; Detmar, M.; Wiig, H.; Alitalo, K. A dural lymphatic vascular system that drains brain interstitial fluid and macromolecules. *J. Exp. Med.* **2015**, *212*, 991–999. [CrossRef]
69. Maugeri, R.; Schiera, G.; Di Liegro, C.M.; Fricano, A.; Iacopino, D.G.; Di Liegro, I. Aquaporins and Brain Tumors. *Int. J. Mol. Sci.* **2016**, *17*, 1029. [CrossRef]
70. de Leon, M.J.; Li, Y.; Okamura, N.; Tsui, W.H.; Saint-Louis, L.A.; Glodzik, L.; Osorio, R.S.; Fortea, J.; Butler, T.; Pirraglia, E.; et al. Cerebrospinal fluid clearance in Alzheimer disease measured with dynamic PET. *J. Nucl. Med.* **2017**, *58*, 1471–1476. [CrossRef]

71. Lv, T.; Zhao, B.; Hu, Q.; Zhang, X. The Glymphatic System: A Novel Therapeutic Target for Stroke Treatment. *Front. Aging Neurosci.* **2021**, *13*, 689098. [CrossRef] [PubMed]
72. Generoso, J.S.; Thorsdottir, S.; Collodel, A.; Dominguini, D.; Santo, R.R.E.; Petronilho, F.; Barichello, T.; Iovino, F. Dysfunctional Glymphatic System with Disrupted Aquaporin 4 Expression Pattern on Astrocytes Causes Bacterial Product Accumulation in the CSF during Pneumococcal Meningitis. *mBio* **2022**, *13*, e0188622. [CrossRef] [PubMed]
73. Díaz-Castro, B.; Robel, S.; Mishra, A. Astrocyte Endfeet in Brain Function and Pathology: Open Questions. *Annu. Rev. Neurosci.* **2023**, *46*, 101–121. [CrossRef] [PubMed]
74. Bloch, O.; Manley, G.T. The Role of aquaporin-4 in cerebral water transport and edema. *Neurosurg. Focus* **2007**, *22*, E3. [CrossRef]
75. Valente, O.; Messina, R.; Ingravallo, G.; Bellitti, E.; Zimatore, D.S.; de Gennaro, L.; Abbrescia, P.; Pati, R.; Palazzo, C.; Nicchia, G.P.; et al. Alteration of the translational readthrough isoform AQP4ex induces redistribution and downregulation of AQP4 in human glioblastoma. *Cell Mol. Life Sci.* **2022**, *79*, 140. [CrossRef] [PubMed]
76. Salman, M.M.; Kitchen, P.; Halsey, A.; Wang, M.X.; Törnroth-Horsefield, S.; Conner, A.C.; Badaut, J.; Iliff, J.J.; Bill, R.M. Emerging roles for dynamic aquaporin-4 subcellular relocalization in CNS water homeostasis. *Brain* **2022**, *145*, 64–75. [CrossRef] [PubMed]
77. Yao, X.Y.; Gao, M.C.; Bai, S.W.; Xie, L.; Song, Y.Y.; Ding, J.; Wu, Y.F.; Xue, C.R.; Hao, Y.; Zhang, Y.; et al. Enlarged perivascular spaces, neuroinflammation and neurological dysfunction in NMOSD patients. *Front. Immunol.* **2022**, *13*, 966781. [CrossRef]
78. Mader, S.; Brimberg, L.; Vo, A.; Strohl, J.J.; Crawford, J.M.; Bonnin, A.; Carrión, J.; Campbell, D.; Huerta, T.S.; La Bella, A.; et al. In utero exposure to maternal anti-aquaporin-4 antibodies alters brain vasculature and neural dynamics in male mouse offspring. *Sci. Transl. Med.* **2022**, *14*, eabe9726. [CrossRef]
79. Liu, S.; Li, H.; Shen, Y.; Zhu, W.; Wang, Y.; Wang, J.; Zhang, N.; Li, C.; Xie, L.; Wu, Q. Moxibustion improves hypothalamus Aqp4 polarization in APP/PS1 mice: Evidence from spatial transcriptomics. *Front. Aging Neurosci.* **2023**, *15*, 1069155. [CrossRef]
80. Mueller, S.M.; White, K.M.; Fass, S.B.; Chen, S.; Shi, Z.; Ge, X.; Engelbach, J.A.; Gaines, S.H.; Bice, A.R.; Vasek, M.J.; et al. Evaluation of gliovascular functions of Aqp4 readthrough isoforms. *bioRxiv* **2023**. [CrossRef]
81. Tanida, I.; Mizushima, N.; Kiyooka, M.; Ohsumi, M.; Ueno, T.; Ohsumi, Y.; Kominami, E. Apg7p/Cvt2p: A novel protein-activating enzyme essential for autophagy. *Mol. Biol. Cell.* **1999**, *10*, 1367–1379. [CrossRef]
82. Komatsu, M.; Waguri, S.; Ueno, T.; Iwata, J.; Murata, S.; Tanida, I.; Ezaki, J.; Mizushima, N.; Ohsumi, Y.; Uchiyama, Y.; et al. Impairment of starvation-induced and constitutive autophagy in Atg7-deficient mice. *J. Cell Biol.* **2005**, *169*, 425–434. [CrossRef] [PubMed]
83. Liu, H.; Wei, J.Y.; Li, Y.; Ban, M.; Sun, Q.; Wang, H.J.; Zhao, D.; Tong, P.G.; Wang, L.; Wang, K.J.; et al. Endothelial depletion of Atg7 triggers astrocyte-microvascular disassociation at blood-brain barrier. *J. Cell Biol.* **2023**, *222*, e202103098. [CrossRef] [PubMed]
84. Chen, Z.; Kelly, J.R.; Morales, J.E.; Sun, R.C.; De, A.; Burkin, D.J.; McCarty, J.H. The alpha7 integrin subunit in astrocytes promotes endothelial blood-brain barrier integrity. *Development* **2023**, *150*, dev201356. [CrossRef]
85. Araya, R.; Kudo, M.; Kawano, M.; Ishii, K.; Hashikawa, T.; Iwasato, T.; Itohara, S.; Terasaki, T.; Oohira, A.; Mishina, Y.; et al. BMP signaling through BMPRIA in astrocytes is essential for proper cerebral angiogenesis and formation of the blood-brain-barrier. *Mol. Cell Neurosci.* **2008**, *38*, 417–430. [CrossRef] [PubMed]
86. Heithoff, B.P.; George, K.K.; Phares, A.N.; Zuidhoek, I.A.; Munoz-Ballester, C.; Robel, S. Astrocytes are necessary for blood-brain barrier maintenance in the adult mouse brain. *Glia* **2021**, *69*, 436–472. [CrossRef] [PubMed]
87. Ye, Q.; Jo, J.; Wang, C.-Y.; Oh, H.; Tiffany J Choy, T.J.; Kim, K.; D'Alessandro, A.; Reshetnyak, Y.K.; Jung, S.Y.; Chen, Z.; et al. Astrocytic Slc4a4 regulates blood-brain barrier integrity in healthy and stroke brains via a NO-CCL2-CCR2 pathway. *bioRxiv* **2023**. [CrossRef]
88. Savettieri, G.; Di Liegro, I.; Catania, C.; Licata, L.; Pitarresi, G.L.; D'Agostino, S.; Schiera, G.; De Caro, V.; Giandalia, G.; Giannola, L.I.; et al. Neurons and ECM regulate occludin localization in brain endothelial cells. *Neuroreport* **2000**, *11*, 1081–1084. [CrossRef]
89. Cestelli, A.; Catania, C.; D'Agostino, S.; Di Liegro, I.; Licata, L.; Schiera, G.; Pitarresi, G.L.; Savettieri, G.; De Caro, V.; Giandalia, G.; et al. Functional feature of a novel model of blood brain barrier: Studies on permeation of test compounds. *J. Control. Release* **2001**, *76*, 139–147. [CrossRef]
90. Schiera, G.; Proia, P.; Alberti, C.; Mineo, M.; Savettieri, G.; Di Liegro, I. Neurons produce FGF2 and VEGF and secrete them at least in part by shedding extracellular vesicles. *J. Cell Mol. Med.* **2007**, *11*, 1384–1394. [CrossRef]
91. Proia, P.; Schiera, G.; Mineo, M.; Ingrassia, A.M.; Santoro, G.; Savettieri, G.; Di Liegro, I. Astrocytes shed extracellular vesicles that contain fibroblast growth factor-2 and vascular endothelial growth factor. *Int. J. Mol. Med.* **2008**, *21*, 63–67. [CrossRef] [PubMed]
92. Tabata, H. Crosstalk between Blood Vessels and Glia during the Central Nervous System Development. *Life* **2022**, *12*, 1761. [CrossRef] [PubMed]
93. Fu, J.; Li, L.; Huo, D.; Zhi, S.; Yang, R.; Yang, B.; Xu, B.; Zhang, T.; Dai, M.; Tan, C.; et al. Astrocyte-Derived TGFβ1 Facilitates Blood-Brain Barrier Function via Non-Canonical Hedgehog Signaling in Brain Microvascular Endothelial Cells. *Brain Sci.* **2021**, *11*, 77. [CrossRef] [PubMed]
94. Guérit, S.; Fidan, E.; Macas, J.; Czupalla, C.J.; Figueiredo, R.; Vijikumar, A.; Yalcin, B.H.; Thom, S.; Winter, P.; Gerhardt, H.; et al. Astrocyte-derived Wnt growth factors are required for endothelial blood-brain barrier maintenance. *Prog. Neurobiol.* **2021**, *199*, 101937. [CrossRef]
95. Han, D.; Li, F.; Zhang, H.; Ji, C.; Shu, Q.; Wang, C.; Ni, H.; Zhu, Y.; Wang, S. Mesencephalic astrocyte-derived neurotrophic factor restores blood-brain barrier integrity of aged mice after ischaemic stroke/reperfusion through anti-inflammation via TLR4/MyD88/NF-κB pathway. *J. Drug Target.* **2022**, *30*, 430–441. [CrossRef] [PubMed]

96. Savidge, T.C.; Newman, P.; Pothoulakis, C.; Ruhl, A.; Neunlist, M.; Bourreille, A.; Hurst, R.; Sofroniew, M.V. Enteric glia regulate intestinal barrier function and inflammation via release of S-nitrosoglutathione. *Gastroenterology* **2007**, *132*, 1344–1358. [CrossRef] [PubMed]
97. Haseloff, R.F.; Blasig, I.E.; Bauer, H.C.; Bauer, H. In search of the astrocytic factor(s) modulating blood-brain barrier functions in brain capillary endothelial cells in vitro. *Cell Mol. Neurobiol.* **2005**, *25*, 25–39. [CrossRef]
98. Saunders, N.R.; Ek, C.J.; Habgood, M.D.; Dziegielewska, K.M. Barriers in the brain: A renaissance? *Trends Neurosci.* **2008**, *31*, 279–286. [CrossRef]
99. Weidenfeller, C.; Svendsen, C.N.; Shusta, E.V. Differentiating embryonic neural progenitor cells induce blood-brain barrier properties. *J. Neurochem.* **2007**, *101*, 555–565. [CrossRef]
100. Schiera, G.; Di Liegro, C.M.; Di Liegro, I. Extracellular Membrane Vesicles as Vehicles for Brain Cell-to-Cell Interaction in Physiological as well as Pathological Conditions. *Biomed. Res. Int.* **2015**, *2015*, 152926. [CrossRef]
101. Mateescu, B.; Kowal, E.J.K.; van Balkom, B.W.M.; Bartel, S.; Bhattacharyya, S.N.; Buzás, E.I.; Buck, A.H.; de Candia, P.; Chow, F.W.N.; Das, S.; et al. Obstacles and opportunities in the functional analysis of extracellular vesicle RNA—An ISEV position paper. *J. Extracell. Vesicles* **2017**, *6*, 1286095. [CrossRef] [PubMed]
102. Meldolesi, J. Exosomes and Ectosomes in Intercellular Communication. *Curr. Biol.* **2018**, *28*, R435–R444. [CrossRef] [PubMed]
103. Lee, H.J. Microbe-Host Communication by Small RNAs in Extracellular Vesicles: Vehicles for Transkingdom RNA Transportation. *Int. J. Mol. Sci.* **2019**, *20*, 1487. [CrossRef]
104. Court, F.A.; Hendriks, W.T.J.; MacGillavry, H.D.; Alvarez, J.; Van Minnen, J. Schwann cell to axon transfer of ribosomes: Toward a novel understanding of the role of glia in the nervous system. *J. Neurosci.* **2008**, *28*, 11024–11029. [CrossRef] [PubMed]
105. Twiss, J.L.; Fainzilber, M. Ribosomes in axons–scrounging from the neighbors? *Trends Cell Biol.* **2009**, *19*, 236–243. [CrossRef]
106. Sotelo, J.R.; Canclini, L.; Kun, A.; Sotelo-Silveira, J.R.; Calliari, A.; Cal, K.; Bresque, M.; Dipaolo, A.; Farias, J.; Mercer, J.A. Glia to axon RNA transfer. *Dev. Neurobiol.* **2014**, *74*, 292–302. [CrossRef]
107. Karnati, H.K.; Garcia, J.H.; Tweedie, D.; Becker, R.E.; Kapogiannis, D.; Greig, N.H. Neuronal enriched extracellular vesicle proteins as biomarkers for traumatic brain injury. *J. Neurotrauma* **2019**, *36*, 975–987. [CrossRef]
108. Ruan, Z.; Pathak, D.; Venkatesan, K.S.; Yoshii-Kitahara, A.; Muraoka, S.; Bhatt, N.; Takamatsu-Yukawa, K.; Hu, J.; Wang, Y.; Hersh, S.; et al. Alzheimer's disease brain-derived extracellular vesicles spread tau pathology in interneurons. *Brain* **2021**, *144*, 288–309. [CrossRef]
109. Gabrielli, M.; Prada, I.; Joshi, P.; Falcicchia, C.; D'Arrigo, G.; Rutigliano, G.; Battocchio, E.; Zenatelli, R.; Tozzi, F.; Radeghieri, A.; et al. Microglial large extracellular vesicles propagate early synaptic dysfunction in Alzheimer's disease. *Brain* **2022**, *145*, 2849–2868. [CrossRef]
110. Zhou, W.; Zhao, L.; Mao, Z.; Wang, Z.; Zhang, Z.; Li, M. Bidirectional Communication Between the Brain and Other Organs: The Role of Extracellular Vesicles. *Cell Mol. Neurobiol.* **2023**, *43*, 2675–2696. [CrossRef]
111. Bakhti, M.; Winter, C.; Simons, M. Inhibition of myelin membrane sheath formation by oligodendrocyte-derived exosome-like vesicles. *J. Biol. Chem.* **2011**, *286*, 787–796. [CrossRef] [PubMed]
112. Wang, S.; Cesca, F.; Loers, G.; Schweizer, M.; Buck, F.; Benfenati, F.; Schachner, M.; Kleene, R. Synapsin I is an oligomannose-carrying glycoprotein, acts as an oligomannose-binding lectin, and promotes neurite outgrowth and neuronal survival when released via glia-derived exosomes. *J. Neurosci.* **2011**, *31*, 7275–7290. [CrossRef] [PubMed]
113. Huang, S.; Ge, X.; Yu, J.; Han, Z.; Yin, Z.; Li, Y.; Chen, F.; Wang, H.; Zhang, J.; Lei, P. Increased miR-124-3p in microglial exosomes following traumatic brain injury inhibits neuronal inflammation and contributes to neurite outgrowth via their transfer into neurons. *FASEB J.* **2018**, *32*, 512–528. [CrossRef] [PubMed]
114. Pei, X.; Li, Y.; Zhu, L.; Zhou, Z. Astrocyte-derived exosomes suppress autophagy and ameliorate neuronal damage in experimental ischemic stroke. *Exp. Cell Res.* **2019**, *382*, 111474. [CrossRef] [PubMed]
115. Lombardi, M.; Parolisi, R.; Scaroni, F.; Bonfanti, E.; Gualerzi, A.; Gabrielli, M.; Kerlero, D.R.N.; Uccelli, A.; Giussani, P.; Viani, P.; et al. Detrimental and protective action of microglial extracellular vesicles on myelin lesions: Astrocyte involvement in remyelination failure. *Acta Neuropathol.* **2019**, *138*, 987–1012. [CrossRef] [PubMed]
116. Datta, C.A.; Dasgheyb, R.M.; DeVine, L.R.; Bi, H.; Cole, R.N.; Haughey, N.J. Stimulus-dependent modifications in astrocyte-derived extracellular vesicle cargo regulate neuronal excitability. *Glia* **2020**, *68*, 128–144. [CrossRef]
117. Nogueras-Ortiz, C.J.; Mahairaki, V.; Delgado-Peraza, F.; Das, D.; Avgerinos, K.; Eren, E.; Hentschel, M.; Goetzl, E.J.; Mattson, M.P.; Kapogiannis, D. Astrocyte- and neuron-derived extracellular vesicles from Alzheimer's disease patients effect complement-mediated neurotoxicity. *Cells* **2020**, *9*, 1618. [CrossRef]
118. Li, Z.; Song, Y.; He, T.; Wen, R.; Li, Y.; Chen, T.; Huang, S.; Wang, Y.; Tang, Y.; Shen, F.; et al. M2 microglial small extracellular vesicles reduce glial scar formation via the miR-124/STAT3 pathway after ischemic stroke in mice. *Theranostics* **2021**, *11*, 1232–1248. [CrossRef]
119. Durur, D.Y.; Tastan, B.; Ugur, T.K.; Olcum, M.; Uzuner, H.; Karakulah, G.; Yener, G.; Genc, S. Alteration of miRNAs in small neuron derived extracellular vesicles of Alzheimer's disease patients and the effect of extracellular vesicles on microglial immune responses. *J. Mol. Neurosci.* **2022**, *72*, 1182–1194. [CrossRef]
120. Jiang, H.; Zhao, H.; Zhang, M.; He, Y.; Li, X.; Xu, Y.; Liu, X. Hypoxia induced changes of exosome cargo and subsequent biological effects. *Front. Immunol.* **2022**, *13*, 824188. [CrossRef]
121. Makrygianni, E.A.; Chrousos, G.P. Extracellular Vesicles and the Stress System. *Neuroendocrinology* **2023**, *113*, 120–167. [CrossRef]

122. Chen, C.C.; Liu, L.; Ma, F.; Wong, C.W.; Guo, X.E.; Chacko, J.V.; Farhoodi, H.P.; Zhang, S.X.; Zimak, J.; Ségaliny, A.; et al. Elucidation of Exosome Migration across the Blood-Brain Barrier Model In Vitro. *Cell Mol. Bioeng.* **2016**, *9*, 509–529. [CrossRef] [PubMed]
123. Morad, G.; Carman, C.V.; Hagedorn, E.J.; Perlin, J.R.; Zon, L.I.; Mustafaoglu, N.; Park, T.E.; Ingber, D.E.; Daisy, C.C.; Moses, M.A. Tumor-Derived Extracellular Vesicles Breach the Intact Blood-Brain Barrier via Transcytosis. *ACS Nano* **2019**, *13*, 13853–13865. [CrossRef] [PubMed]
124. Wang, D.; Guan, S.; Lu, P.; Li, Y.; Xu, H. Extracellular vesicles: Critical bilateral communicators in periphery-brain crosstalk in central nervous system disorders. *Biomed. Pharmacother.* **2023**, *160*, 114354. [CrossRef] [PubMed]
125. Brown, C.; McKee, C.; Halassy, S.; Kojan, S.; Feinstein, D.L.; Chaudhry, G.R. Neural stem cells derived from primitive mesenchymal stem cells reversed disease symptoms and promoted neurogenesis in an experimental autoimmune encephalomyelitis mouse model of multiple sclerosis. *Stem Cell Res. Ther.* **2021**, *12*, 499. [CrossRef] [PubMed]
126. Liu, S.; Fan, M.; Xu, J.X.; Yang, L.J.; Qi, C.C.; Xia, Q.R.; Ge, J.F. Exosomes derived from bone-marrow mesenchymal stem cells alleviate cognitive decline in AD-like mice by improving BDNF-related neuropathology. *J. Neuroinflamm.* **2022**, *19*, 35. [CrossRef] [PubMed]
127. Yari, H.; Mikhailova, M.V.; Mardasi, M.; Jafarzadehgharehziaaddin, M.; Shahrokh, S.; Thangavelu, L.; Ahmadi, H.; Shomali, N.; Yaghoubi, Y.; Zamani, M.; et al. Emerging role of mesenchymal stromal cells (MSCs)-derived exosome in neurodegeneration-associated conditions: A groundbreaking cell-free approach. *Stem Cell Res. Ther.* **2022**, *13*, 423. [CrossRef]
128. Wang, J.; Li, L.; Zhang, Z.; Zhang, X.; Zhu, Y.; Zhang, C.; Bi, Y. Extracellular vesicles mediate the communication of adipose tissue with brain and promote cognitive impairment associated with insulin resistance. *Cell Metab.* **2022**, *34*, 1264–1279.e8. [CrossRef]
129. Morris, G.P.; Clark, I.A.; Zinn, R.; Vissel, B. Microglia: A new frontier for synaptic plasticity, learning and memory, and neurodegenerative disease research. *Neurobiol. Learn. Mem.* **2013**, *105*, 40–53. [CrossRef]
130. Luarte, A.; Henzi, R.; Fernández, A.; Gaete, D.; Cisternas, P.; Pizarro, M.; Batiz, L.F.; Villalobos, I.; Masalleras, M.; Vergara, R.; et al. Astrocyte-Derived Small Extracellular Vesicles Regulate Dendritic Complexity through miR-26a-5p Activity. *Cells* **2020**, *9*, 930. [CrossRef]
131. Cano, A.; Ettcheto, M.; Bernuz, M.; Puerta, R.; Esteban de Antonio, E.; Sánchez-López, E.; Souto, E.B.; Camins, A.; Martí, M.; Pividori, M.I.; et al. Extracellular vesicles, the emerging mirrors of brain physiopathology. *Int. J. Biol. Sci.* **2023**, *19*, 721–743. [CrossRef] [PubMed]
132. Busatto, S.; Morad, G.; Guo, P.; Moses, M.A. The role of extracellular vesicles in the physiological and pathological regulation of the blood-brain barrier. *FASEB Bioadv.* **2021**, *3*, 665–675. [CrossRef] [PubMed]
133. D'Souza, A.; Burch, A.; Dave, K.M.; Sreeram, A.; Reynolds, M.J.; Dobbins, D.X.; Kamte, Y.S.; Zhao, W.; Sabatelle, C.; Joy, G.M.; et al. Microvesicles transfer mitochondria and increase mitochondrial function in brain endothelial cells. *J. Control. Release* **2021**, *338*, 505–526. [CrossRef]
134. Ramos-Zaldívar, H.M.; Polakovicova, I.; Salas-Huenuleo, E.; Corvalán, A.H.; Kogan, M.J.; Yefi, C.P.; Andia, M.E. Extracellular vesicles through the blood-brain barrier: A review. *Fluids Barriers CNS* **2022**, *19*, 60. [CrossRef] [PubMed]
135. Sharma, K.; Zhang, Y.; Paudel, K.R.; Kachelmeier, A.; Hansbro, P.M.; Shi, X. The Emerging Role of Pericyte-Derived Extracellular Vesicles in Vascular and Neurological Health. *Cells* **2022**, *11*, 3108. [CrossRef]
136. López-Cepeda, L.; Castro, J.D.; Aristizábal-Pachón, A.F.; González-Giraldo, Y.; Pinzón, A.; Puentes-Rozo, P.J.; González, J. Modulation of Small RNA Signatures by Astrocytes on Early Neurodegeneration Stages; Implications for Biomarker Discovery. *Life* **2022**, *12*, 1720. [CrossRef] [PubMed]
137. Fevrier, B.; Vilette, D.; Archer, F.; Loew, D.; Faigle, W.; Vidal, M.; Laude, H.; Raposo, G. Cells release prions in association with exosomes. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 9683–9688. [CrossRef]
138. Rajendran, L.; Honsho, M.; Zahn, T.R.; Keller, P.; Geiger, K.D.; Verkade, P.; Simons, K. Alzheimer's disease beta-amyloid peptides are released in association with exosomes. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 11172–11177. [CrossRef]
139. Emmanouilidou, E.; Melachroinou, K.; Roumeliotis, T.; Garbis, S.D.; Ntzouni, M.; Margaritis, L.H.; Stefanis, L.; Vekrellis, K. Cell-produced alpha-synuclein is secreted in a calcium-dependent manner by exosomes and impacts neuronal survival. *J. Neurosci.* **2010**, *30*, 6838–6851. [CrossRef]
140. Shi, M.; Liu, C.; Cook, T.J.; Bullock, K.M.; Zhao, Y.; Ginghina, C.; Li, Y.; Aro, P.; Dator, R.; He, C.; et al. Plasma exosomal α-synuclein is likely CNS-derived and increased in Parkinson's disease. *Acta Neuropathol.* **2014**, *128*, 639–650. [CrossRef]
141. DeLeo, A.M.; Ikezu, T. Extracellular Vesicle Biology in Alzheimer's Disease and Related Tauopathy. *J. Neuroimmune Pharmacol.* **2018**, *13*, 292–308. [CrossRef]
142. Kaur, S.; Verma, H.; Dhiman, M.; Tell, G.; Gigli, G.L.; Janes, F.; Mantha, A.K. Brain Exosomes: Friend or Foe in Alzheimer's Disease? *Mol. Neurobiol.* **2021**, *58*, 6610–6624. [CrossRef] [PubMed]
143. Kushwaha, R.; Li, Y.; Makarava, N.; Pandit, N.P.; Molesworth, K.; Birukov, K.G.; Baskakov, I.V. Reactive astrocytes associated with prion disease impair the blood brain barrier. *Neurobio. Dis.* **2023**, *185*, 106264. [CrossRef] [PubMed]
144. Soukup, J.; Moško, T.; Kereïche, S.; Holada, K. Large extracellular vesicles transfer more prions and infect cell culture better than small extracellular vesicles. *Biochem. Biophys. Res. Commun.* **2023**, *687*, 149208. [CrossRef]
145. Stewart, P.A.; Wiley, M.J. Developing nervous tissue induces formation of blood-brain barrier characteristics in invading endothelial cells: A study using quail–chick transplantation chimeras. *Dev. Biol.* **1981**, *84*, 183–192. [CrossRef] [PubMed]
146. Joó, F. The cerebral microvessels in culture, an update. *J. Neurochem.* **1992**, *58*, 1–17. [CrossRef] [PubMed]

147. Terasaki, T.; Tsuji, A. Drug delivery to the brain utilizing blood–brain barrier transport systems. *J. Control. Release* **1994**, *29*, 163–169. [CrossRef]
148. Wade, L.A.; Katzman, R. Synthetic amino acids and the nature of L-DOPA transport at the blood-brain barrier. *J. Neurochem.* **1975**, *25*, 837–842. [CrossRef]
149. Pardridge, W.M. CNS drug design based on principles of blood-brain barrier transport. *J. Neurochem.* **1998**, *70*, 1781–1792. [CrossRef]
150. Dehouck, M.P.; Méresse, S.; Dehouck, B.; Fruchart, J.C.; Cecchelli, R. In vitro reconstituted blood–brain barrier. *J. Control. Release* **1992**, *21*, 81–92. [CrossRef]
151. Gomes, P.; Soares-da-Silva, P. Interaction between L-DOPA and 3-O-methyl-L-DOPA for transport in immortalised rat capillary cerebral endothelial cells. *Neuropharmacology* **1999**, *38*, 1371–1380. [CrossRef] [PubMed]
152. Barberio, C.; Withers, A.; Mishra, Y.; Couraud, P.O.; Romero, I.A.; Weksler, B.; Owens, R.M. A human-derived neurovascular unit in vitro model to study the effects of cellular cross-talk and soluble factors on barrier integrity. *Front. Cell Neurosci.* **2022**, *16*, 1065193. [CrossRef] [PubMed]
153. Ledwig, V.; Reichl, S. Isolation and Cultivation of Porcine Endothelial Cells, Pericytes and Astrocytes to Develop an In Vitro Blood-Brain Barrier Model for Drug Permeation Testing. *Pharmaceutics* **2023**, *15*, 1688. [CrossRef] [PubMed]
154. Cucullo, L.; McAllister, M.S.; Kight, K.; Krizanac-Bengez, L.; Marroni, M.; Mayberg, M.R.; Stanness, K.A.; Janigro, D. A new dynamic in vitro model for the multidimensional study of astrocyte-endothelial cell interactions at the blood-brain barrier. *Brain Res.* **2002**, *951*, 243–254. [CrossRef] [PubMed]
155. Watanabe, D.; Nakagawa, S.; Morofuji, Y.; Tóth, A.E.; Vastag, M.; Aruga, J.; Niwa, M.; Deli, M.A. Characterization of a Primate Blood-Brain Barrier Co-Culture Model Prepared from Primary Brain Endothelial Cells, Pericytes and Astrocytes. *Pharmaceutics* **2021**, *13*, 1484. [CrossRef]
156. Sharma, S.; Zhang, Y.; Akter, K.A.; Nozohouri, S.; Archie, S.R.; Patel, D.; Villalba, H.; Abbruscato, T. Permeability of Metformin across an In Vitro Blood-Brain Barrier Model during Normoxia and Oxygen-Glucose Deprivation Conditions: Role of Organic Cation Transporters (Octs). *Pharmaceutics* **2023**, *15*, 1357. [CrossRef]
157. Thomsen, M.S.; Humle, N.; Hede, E.; Moos, T.; Burkhart, A.; Thomsen, L.B. The blood-brain barrier studied in vitro across species. *PLoS ONE* **2021**, *16*, e0236770. [CrossRef]
158. Gonzales-Aloy, E.; Ahmed-Cox, A.; Tsoli, M.; Ziegler, D.S.; Kavallaris, M. From cells to organoids: The evolution of blood-brain barrier technology for modelling drug delivery in brain cancer. *Adv. Drug Deliv. Rev.* **2023**, *196*, 114777. [CrossRef]
159. Park, J.S.; Choe, K.; Khan, A.; Jo, M.H.; Park, H.Y.; Kang, M.H.; Park, T.J.; Kim, M.O. Establishing Co-Culture Blood-Brain Barrier Models for Different Neurodegeneration Conditions to Understand Its Effect on BBB Integrity. *Int. J. Mol. Sci.* **2023**, *24*, 5283. [CrossRef]
160. de Rus Jacquet, A.; Alpaugh, M.; Denis, H.L.; Tancredi, J.L.; Boutin, M.; Decaestecker, J.; Beauparlant, C.; Herrmann, L.; Saint-Pierre, M.; Parent, M.; et al. The contribution of inflammatory astrocytes to BBB impairments in a brain-chip model of Parkinson's disease. *Nat. Commun.* **2023**, *14*, 3651. [CrossRef]
161. Stanton, A.E.; Bubnys, A.; Agbas, E.; James, B.; Park, D.S.; Jiang, A.; Pinals, R.L.; Truong, N.; Loon, A.; Staab, C.; et al. Engineered 3D Immuno-Glial-Neurovascular Human Brain Model. *bioRxiv* **2023**. [CrossRef]
162. Bolden, C.T.; Skibber, M.A.; Olson, S.D.; Zamorano Rojas, M.; Milewicz, S.; Gill, B.S.; Cox, C.S., Jr. Validation and characterization of a novel blood-brain barrier platform for investigating traumatic brain injury. *Sci. Rep.* **2023**, *13*, 16150. [CrossRef] [PubMed]
163. Potjewyd, G.; Kellett, K.A.B.; Hooper, N.M. 3D hydrogel models of the neurovascular unit to investigate blood-brain barrier dysfunction. *Neuronal Signal.* **2021**, *5*, NS20210027. [CrossRef] [PubMed]
164. Wang, H.; Yang, H.; Shi, Y.; Xiao, Y.; Yin, Y.; Jiang, B.; Ren, H.; Chen, W.; Xue, Q.; Xu, X. Reconstituting neurovascular unit with primary neural stem cells and brain microvascular endothelial cells in three-dimensional matrix. *Brain. Pathol.* **2021**, *31*, e12940. [CrossRef]
165. Liu, Z.; Tang, Y.; Zhang, Z.; Liu, Q.; Wang, M.; Li, W.; Yang, G.Y. Engineering Neurovascular Unit and Blood-Brain Barrier for Ischemic Stroke Modeling. *Adv. Healthc. Mater.* **2023**, *12*, e2202638. [CrossRef] [PubMed]
166. Wevers, N.R.; Nair, A.L.; Fowke, T.M.; Pontier, M.; Kasi, D.G.; Spijkers, X.; Hallard, C.; Rabussier, G.; van Vught, R.; Vulto, P.; et al. Modeling ischemic stroke in a triculture neurovascular unit on-a-chip. *Fluids Barriers CNS* **2021**, *18*, 59. [CrossRef]
167. Spitzer, D.; Guérit, S.; Puetz, T.; Khel, M.I.; Armbrust, M.; Dunst, M.; Macas, J.; Zinke, J.; Devraj, G.; Jia, X.; et al. Profiling the neurovascular unit unveils detrimental effects of osteopontin on the blood-brain barrier in acute ischemic stroke. *Acta Neuropathol.* **2022**, *144*, 305–337. [CrossRef]
168. Stafford, P.; Mitra, S.; Debot, M.; Lutz, P.; Stem, A.; Hadley, J.; Hom, P.; Schaid, T.R.; Cohen, M.J. Astrocytes and pericytes attenuate severely injured patient plasma mediated expression of tight junction proteins in endothelial cells. *PLoS ONE* **2022**, *17*, e0270817. [CrossRef]
169. Floryanzia, S.D.; Nance, E. Applications and Considerations for Microfluidic Systems to Model the Blood-Brain Barrier. *ACS Appl. Bio. Mater.* **2023**, *6*, 3617–3632. [CrossRef]
170. Galpayage Dona, K.N.U.; Ramirez, S.H.; Andrews, A.M. A Next-Generation 3D Tissue-Engineered Model of the Human Brain Microvasculature to Study the Blood-Brain Barrier. *Bioengineering* **2023**, *10*, 817. [CrossRef]

171. Nakayama-Kitamura, K.; Shigemoto-Mogami, Y.; Toyoda, H.; Mihara, I.; Moriguchi, H.; Naraoka, H.; Furihata, T.; Ishida, S.; Sato, K. Usefulness of a humanized tricellular static transwell blood-brain barrier model as a microphysiological system for drug development applications—A case study based on the benchmark evaluations of blood-brain barrier microphysiological system. *Regen Ther.* **2023**, *22*, 192–202. [CrossRef] [PubMed]
172. Palma-Florez, S.; López-Canosa, A.; Moralez-Zavala, F.; Castaño, O.; Kogan, M.J.; Samitier, J.; Lagunas, A.; Mir, M. BBB-on-a-chip with integrated micro-TEER for permeability evaluation of multi-functionalized gold nanorods against Alzheimer's disease. *J. Nanobiotechnol.* **2023**, *21*, 115. [CrossRef] [PubMed]
173. Kadry, H.; Cucullo, L. Evaluation of Barrier Integrity Using a Two-Layered Microfluidic Device Mimicking the Blood-Brain Barrier. *Methods Mol. Biol.* **2024**, *2711*, 77–88. [CrossRef]
174. Xiao, T.; Pan, M.; Wang, Y.; Huang, Y.; Tsunoda, M.; Zhang, Y.; Wang, R.; Hu, W.; Yang, H.; Li, L.S.; et al. In vitro bloodbrain barrier permeability study of four main active ingredients from Alpiniae oxyphyllae fructus. *J. Pharm. Biomed. Anal.* **2023**, *235*, 115637. [CrossRef] [PubMed]
175. Song, R.; Pekrun, K.; Khan, T.A.; Zhang, F.; Paşca, S.P.; Kay, M.A. Selection of rAAV vectors that cross the human blood-brain barrier and target the central nervous system using a transwell model. *Mol. Ther. Methods Clin. Dev.* **2022**, *27*, 73–88. [CrossRef]
176. Lauranzano, E.; Rasile, M.; Matteoli, M. Integrating Primary Astrocytes in a Microfluidic Model of the Blood-Brain Barrier. *Methods Mol. Biol.* **2022**, *2492*, 225–240. [CrossRef]
177. Matsuki, H.; Mandai, S.; Shiwaku, H.; Koide, T.; Takahashi, N.; Yanagi, T.; Inaba, S.; Ida, S.; Fujiki, T.; Mori, Y.; et al. Chronic kidney disease causes blood-brain barrier breakdown via urea-activated matrix metalloproteinase-2 and insolubility of tau protein. *Aging* **2023**, *15*, 10972–10995. [CrossRef]
178. Claeys, W.; Van Hoecke, L.; Lefere, S.; Geerts, A.; Verhelst, X.; Van Vlierberghe, H.; Degroote, H.; Devisscher, L.; Vandenbroucke, R.E.; Van Steenkiste, C. The neurogliovascular unit in hepatic encephalopathy. *JHEP Rep.* **2021**, *3*, 100352. [CrossRef]
179. Garvin, J.; Semenikhina, M.; Liu, Q.; Rarick, K.; Isaeva, E.; Levchenko, V.; Staruschenko, A.; Palygin, O.; Harder, D.; Cohen, S. Astrocytic responses to high glucose impair barrier formation in cerebral microvessel endothelial cells. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2022**, *322*, R571–R580. [CrossRef]
180. Sun, N.; Hu, H.; Wang, F.; Li, L.; Zhu, W.; Shen, Y.; Xiu, J.; Xu, Q. Antibiotic-induced microbiome depletion in adult mice disrupts blood-brain barrier and facilitates brain infiltration of monocytes after bone-marrow transplantation. *Brain Behav. Immun.* **2021**, *92*, 102–114. [CrossRef]
181. Ju, J.; Su, Y.; Zhou, Y.; Wei, H.; Xu, Q. The SARS-CoV-2 envelope protein disrupts barrier function in an in vitro human blood-brain barrier model. *Front. Cell Neurosci.* **2022**, *16*, 897564. [CrossRef] [PubMed]
182. Hernández-Parra, H.; Reyes-Hernández, O.D.; Figueroa-González, G.; González-Del Carmen, M.; González-Torres, M.; Peña-Corona, S.I.; Florán, B.; Cortés, H.; Leyva-Gómez, G. Alteration of the blood-brain barrier by COVID-19 and its implication in the permeation of drugs into the brain. *Front. Cell Neurosci.* **2023**, *17*, 1125109. [CrossRef] [PubMed]
183. Guo, Y.; Chen, J.; Ji, W.; Xu, L.; Xie, Y.; He, S.; Lai, C.; Hou, K.; Li, Z.; Chen, G.; et al. High-titer AAV disrupts cerebrovascular integrity and induces lymphocyte infiltration in adult mouse brain. *Mol. Ther. Methods Clin. Dev.* **2023**, *31*, 101102. [CrossRef] [PubMed]
184. Kempuraj, D.; Aenlle, K.K.; Cohen, J.; Mathew, A.; Isler, D.; Pangeni, R.P.; Nathanson, L.; Theoharides, T.C.; Klimas, N.G. COVID-19 and Long COVID: Disruption of the Neurovascular Unit, Blood-Brain Barrier, and Tight Junctions. *Neuroscientist* **2023**, *11*, 10738584231194927. [CrossRef] [PubMed]
185. Kaur, G.; Pant, P.; Bhagat, R.; Seth, P. Zika virus E protein modulates functions of human brain microvascular endothelial cells and astrocytes: Implications on blood-brain barrier properties. *Front. Cell Neurosci.* **2023**, *17*, 1173120. [CrossRef] [PubMed]
186. Segura-Collar, B.; Mata-Martínez, P.; Hernández-Laín, A.; Sánchez-Gómez, P.; Gargini, R. Blood-Brain Barrier Disruption: A Common Driver of Central Nervous System Diseases. *Neuroscientist* **2022**, *28*, 222–237. [CrossRef] [PubMed]
187. Burn, L.; Gutowski, N.; Whatmore, J.; Giamas, G.; Pranjol, M.Z.I. The role of astrocytes in brain metastasis at the interface of circulating tumour cells and the blood brain barrier. *Front. Biosci.* **2021**, *26*, 590–601. [CrossRef]
188. Wang, P.; Wu, Y.; Chen, W.; Zhang, M.; Qin, J. Malignant Melanoma-Derived Exosomes Induce Endothelial Damage and Glial Activation on a Human BBB Chip Model. *Biosensors* **2022**, *12*, 89. [CrossRef]
189. Li, X.; Li, L.; Zhou, K.; Zhang, H.; Maalim, A.A.; Chen, X.; He, X.; Ding, X.; Xu, C.; Wang, Y. Glioma Shapes Blood-Brain Barrier Integrity and Remodels the Tumor Microenvironment: Links with Clinical Features and Prognosis. *J. Clin. Med.* **2022**, *11*, 5863. [CrossRef]
190. Zhao, Z.; Zhang, Y.; Li, C.; Li, X.; Chu, Y.; Guo, Q.; Zhang, Y.; Xia, W.; Liu, P.; Chen, H.; et al. Microenvironment-tailored micelles restrain carcinoma-astrocyte crosstalk for brain metastasis. *J. Control. Release* **2022**, *349*, 520–532. [CrossRef]
191. Michinaga, S.; Koyama, Y. Pathophysiological Responses and Roles of Astrocytes in Traumatic Brain Injury. *Int. J. Mol. Sci.* **2021**, *22*, 6418. [CrossRef] [PubMed]
192. Han, G.; Song, L.; Ding, Z.; Wang, Q.; Yan, Y.; Huang, J.; Ma, C. The Important Double-Edged Role of Astrocytes in Neurovascular Unit after Ischemic Stroke. Front. *Aging Neurosci.* **2022**, *14*, 833431. [CrossRef] [PubMed]
193. Qin, X.; Wang, J.; Chen, S.; Liu, G.; Wu, C.; Lv, Q.; He, X.; Bai, X.; Huang, W.; Liao, H. Astrocytic $p75^{NTR}$ expression provoked by ischemic stroke exacerbates the blood-brain barrier disruption. *Glia* **2022**, *70*, 892–912. [CrossRef] [PubMed]
194. Zhang, Y.; Zhao, X.; Zhang, Y.; Zeng, F.; Yan, S.; Chen, Y.; Li, Z.; Zhou, D.; Liu, L. The role of circadian clock in astrocytes: From cellular functions to ischemic stroke therapeutic targets. *Front. Neurosci.* **2022**, *16*, 1013027. [CrossRef] [PubMed]

195. Michinaga, S.; Hishinuma, S.; Koyama, Y. Roles of Astrocytic Endothelin ETB Receptor in Traumatic Brain Injury. *Cells* **2023**, *12*, 719. [CrossRef] [PubMed]
196. Reiss, Y.; Bauer, S.; David, B.; Devraj, K.; Fidan, E.; Hattingen, E.; Liebner, S.; Melzer, N.; Meuth, S.G.; Rosenow, F.; et al. The neurovasculature as a target in temporal lobe epilepsy. *Brain Pathol.* **2023**, *33*, e13147. [CrossRef]
197. Sun, J.; Wu, J.; Hua, F.; Chen, Y.; Zhan, F.; Xu, G. Sleep Deprivation Induces Cognitive Impairment by Increasing Blood-Brain Barrier Permeability via CD44. *Front. Neurol.* **2020**, *11*, 563916. [CrossRef]
198. Kyrtata, N.; Emsley, H.C.A.; Sparasci, O.; Parkes, L.M.; Dickie, B.R. A Systematic Review of Glucose Transport Alterations in Alzheimer's Disease. *Front. Neurosci.* **2021**, *15*, 626636. [CrossRef]
199. Canepa, E.; Fossati, S. Impact of Tau on Neurovascular Pathology in Alzheimer's Disease. *Front. Neurol.* **2021**, *11*, 573324. [CrossRef]
200. Zenaro, E.; Piacentino, G.; Constantin, G. The blood-brain barrier in Alzheimer's disease. *Neurobiol. Dis.* **2017**, *107*, 41–56. [CrossRef]
201. Soto-Rojas, L.O.; Campa-Córdoba, B.B.; Harrington, C.R.; Salas-Casas, A.; Hernandes-Alejandro, M.; Villanueva-Fierro, I.; Bravo-Muñoz, M.; Garcés-Ramírez, L.; De La Cruz-López, F.; Ontiveros-Torres, M.Á.; et al. Insoluble Vascular Amyloid Deposits Trigger Disruption of the Neurovascular Unit in Alzheimer's Disease Brains. *Int. J. Mol. Sci.* **2021**, *22*, 3654. [CrossRef] [PubMed]
202. Zhang, X.; O'Callaghan, P.; Li, H.; Tan, Y.; Zhang, G.; Barash, U.; Wang, X.; Lannfelt, L.; Vlodavsky, I.; Lindahl, U.; et al. Heparanase overexpression impedes perivascular clearance of amyloid-β from murine brain: Relevance to Alzheimer's disease. *Acta Neuropathol. Commun.* **2021**, *9*, 84. [CrossRef] [PubMed]
203. Yue, Q.; Zhou, X.; Zhang, Z.; Hoi, M.P.M. Murine Beta-Amyloid (1-42) Oligomers Disrupt Endothelial Barrier Integrity and VEGFR Signaling via Activating Astrocytes to Release Deleterious Soluble Factors. *Int. J. Mol. Sci.* **2022**, *23*, 1878. [CrossRef]
204. Nakamura, T.; Hashita, T.; Chen, Y.; Gao, Y.; Sun, Y.; Islam, S.; Sato, H.; Shibuya, Y.; Zou, K.; Matsunaga, T.; et al. Aβ42 treatment of the brain side reduced the level of flotillin from endothelial cells on the blood side via FGF-2 signaling in a blood-brain barrier model. *Mol. Brain.* **2023**, *16*, 15. [CrossRef] [PubMed]
205. Anwar, M.M.; Özkan, E.; Gürsoy-Özdemir, Y. The role of extracellular matrix alterations in mediating astrocyte damage and pericyte dysfunction in Alzheimer's disease: A comprehensive review. *Eur. J. Neurosci.* **2022**, *56*, 5453–5475. [CrossRef]
206. Yue, Q.; Hoi, M.P.M. Emerging roles of astrocytes in blood-brain barrier disruption upon amyloid-beta insults in Alzheimer's disease. *Neural Regen Res.* **2023**, *18*, 1890–1902. [CrossRef]
207. Zhang, H.; Chen, W.; Tan, Z.; Zhang, L.; Dong, Z.; Cui, W.; Zhao, K.; Wang, H.; Jing, H.; Cao, R. A role of low-density lipoprotein receptor-related protein 4 (LRP4) in astrocytic Aβ clearance. *J Neurosci.* **2020**, *40*, 5347–5361. [CrossRef]
208. Duong, M.T.; Nasrallah, I.M.; Wolk, D.A.; Chang, C.C.Y.; Chang, T.-Y. Cholesterol, Atherosclerosis, and APOE in Vascular Contributions to Cognitive Impairment and Dementia (VCID): Potential Mechanisms and Therapy. *Front. Aging Neurosci.* **2021**, *13*, 647990. [CrossRef]
209. Jackson, R.J.; Meltzer, J.C.; Nguyen, H.; Commins, C.; Bennett, R.E.; Hudry, E.; Hyman, B.T. APOE4 derived from astrocytes leads to blood-brain barrier impairment. *Brain* **2022**, *145*, 3582–3593. [CrossRef]
210. Zenaro, E.; Pietronigro, E.; Della Bianca, V.; Piacentino, G.; Marongiu, L.; Budui, S.; Turano, E.; Rossi, B.; Angiari, S.; Dusi, S.; et al. Neutrophils promote Alzheimer's disease-like pathology and cognitive decline via LFA-1 integrin. *Nat. Med.* **2015**, *21*, 880–886. [CrossRef]
211. Spampinato, S.F.; Merlo, S.; Fagone, E.; Fruciano, M.; Sano, Y.; Kanda, T.; Sortino, M.A. Reciprocal Interplay Between Astrocytes and CD4+ Cells Affects Blood-Brain Barrier and Neuronal Function in Response to β Amyloid. *Front. Mol. Neurosci.* **2020**, *13*, 120. [CrossRef] [PubMed]
212. Troili, F.; Cipollini, V.; Moci, M.; Morena, E.; Palotai, M.; Rinaldi, V.; Romano, C.; Ristori, G.; Giubilei, F.; Salvetti, M.; et al. Perivascular Unit: This Must Be the Place. The Anatomical Crossroad between the Immune, Vascular and Nervous System. *Front. Neuroanat.* **2020**, *14*, 17. [CrossRef] [PubMed]
213. Montagne, A.; Toga, A.W.; Zlokovic, B.V. Blood-Brain Barrier Permeability and Gadolinium: Benefits and Potential Pitfalls in Research. *JAMA Neurol.* **2016**, *73*, 13–14. [CrossRef]
214. Sanmarco, L.M.; Polonio, C.M.; Wheeler, M.A.; Quintana, F.J. Functional immune cell-astrocyte interactions. *J. Exp. Med.* **2021**, *218*, e20202715. [CrossRef]
215. Kunkl, M.; Amormino, C.; Tedeschi, V.; Fiorillo, M.T.; Tuosto, L. Astrocytes and Inflammatory T Helper Cells: A Dangerous Liaison in Multiple Sclerosis. *Front. Immunol.* **2022**, *13*, 824411. [CrossRef]
216. Cashion, J.M.; Young, K.M.; Sutherland, B.A. How does neurovascular unit dysfunction contribute to multiple sclerosis? *Neurobiol. Dis.* **2023**, *178*, 106028. [CrossRef] [PubMed]
217. Argaw, A.T.; Asp, L.; Zhang, J.; Navrazhina, K.; Pham, T.; Mariani, J.N.; Mahase, S.; Dutta, D.J.; Seto, J.; Kramer, E.G.; et al. Astrocyte-derived VEGF-A drives blood-brain barrier disruption in CNS inflammatory disease. *J. Clin. Investig.* **2012**, *122*, 2454–2468. [CrossRef]
218. Chapouly, C.; Tadesse Argaw, A.; Horng, S.; Castro, K.; Zhang, J.; Asp, L.; Loo, H.; Laitman, B.M.; Mariani, J.N.; Straus Farber, R.; et al. Astrocytic TYMP and VEGFA Drive Blood-Brain Barrier Opening in Inflammatory Central Nervous System Lesions. *Brain* **2015**, *138*, 1548–1567. [CrossRef]
219. Absinta, M.; Maric, D.; Gharagozloo, M.; Garton, T.; Smith, M.D.; Jin, J.; Fitzgerald, K.C.; Song, A.; Liu, P.; Lin, J.P.; et al. A lymphocyte-microglia-astrocyte axis in chronic active multiple sclerosis. *Nature* **2021**, *597*, 709–714. [CrossRef]

220. Moll, N.M.; Rietsch, A.M.; Thomas, S.; Ransohoff, A.J.; Lee, J.C.; Fox, R.; Chang, A.; Ransohoff, R.M.; Fisher, E. Multiple sclerosis normal-appearing white matter: Pathology-imaging correlations. *Ann. Neurol.* **2011**, *70*, 764–773. [CrossRef]
221. Dolcetti, E.; Bruno, A.; Guadalupi, L.; Rizzo, F.R.; Musella, A.; Gentile, A.; De Vito, F.; Caioli, S.; Bullitta, S.; Fresegna, D.; et al. Emerging Role of Extracellular Vesicles in the Pathophysiology of Multiple Sclerosis. *Int. J. Mol. Sci.* **2020**, *21*, 7336. [CrossRef] [PubMed]
222. Maciak, K.; Dziedzic, A.; Miller, E.; Saluk-Bijak, J. miR-155 as an Important Regulator of Multiple Sclerosis Pathogenesis. A Review. *Int. J. Mol. Sci.* **2021**, *22*, 4332. [CrossRef] [PubMed]
223. Huang, J.; Ding, J.; Wang, X.; Gu, C.; He, Y.; Li, Y.; Fan, H.; Xie, Q.; Qi, X.; Wang, Z.; et al. Transfer of neuron-derived α-synuclein to astrocytes induces neuroinflammation and blood-brain barrier damage after methamphetamine exposure: Involving the regulation of nuclear receptor-associated protein 1. *Brain Behav. Immun.* **2022**, *106*, 247–261. [CrossRef]
224. Jeon, H.; Kim, M.; Park, W.; Lim, J.S.; Lee, E.; Cha, H.; Ahn, J.S.; Kim, J.H.; Hong, S.H.; Park, J.E.; et al. Upregulation of AQP4 Improves Blood-Brain Barrier Integrity and Perihematomal Edema Following Intracerebral Hemorrhage. *Neurotherapeutics* **2021**, *18*, 2692–2706. [CrossRef] [PubMed]
225. Winkler, A.; Wrzos, C.; Haberl, M.; Weil, M.T.; Gao, M.; Möbius, W.; Odoardi, F.; Thal, D.R.; Chang, M.; Opdenakker, G.; et al. Blood-brain barrier resealing in neuromyelitis optica occurs independently of astrocyte regeneration. *Clin. Investig.* **2021**, *131*, e141694. [CrossRef]
226. Mills, W.A., 3rd; Woo, A.M.; Jiang, S.; Martin, J.; Surendran, D.; Bergstresser, M.; Kimbrough, I.F.; Eyo, U.B.; Sofroniew, M.V.; Sontheimer, H. Astrocyte plasticity in mice ensures continued endfoot coverage of cerebral blood vessels following injury and declines with age. *Nat. Commun.* **2022**, *13*, 1794. [CrossRef]
227. Preininger, M.K.; Kaufer, D. Blood-Brain Barrier Dysfunction and Astrocyte Senescence as Reciprocal Drivers of Neuropathology in Aging. *Int. J. Mol. Sci.* **2022**, *23*, 6217. [CrossRef]
228. Lin, S.; Zhou, F.Q.; Cheng, J.B.; Sun, X.D.; He, G.Q. Editorial: The role of astrocyte in vascular aging. *Front. Aging Neurosci.* **2022**, *14*, 961288. [CrossRef]
229. Knopp, R.C.; Erickson, M.A.; Rhea, E.M.; Reed, M.J.; Banks, W.A. Cellular senescence and the blood-brain barrier: Implications for aging and age-related diseases. *Exp. Biol. Med.* **2023**, *248*, 399–411. [CrossRef]
230. Siqueira, M.; Araujo, A.P.B.; Gomes, F.C.A.; Stipursky, J. Ethanol Gestational Exposure Impairs Vascular Development and Endothelial Potential to Control BBB-Associated Astrocyte Function in the Developing Cerebral Cortex. *Mol. Neurobiol.* **2021**, *58*, 1755–1768. [CrossRef]
231. Archie, S.R.; Sifat, A.E.; Zhang, Y.; Villalba, H.; Sharma, S.; Nozohouri, S.; Abbruscato, T.J. Maternal e-cigarette use can disrupt postnatal blood-brain barrier (BBB) integrity and deteriorates motor, learning and memory function: Influence of sex and age. *Fluids Barriers CNS* **2023**, *20*, 17. [CrossRef] [PubMed]