



Oxidative Stress and the Neurovascular Unit

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Abstract: The neurovascular unit (NVU) is a relatively recent concept that clearly describes the relationship between brain cells and their blood vessels. The components of the NVU, comprising different types of cells, are so interrelated and associated with each other that they are considered as a single functioning unit. For this reason, even slight disturbances in the NVU could severely affect brain homeostasis and health. In this review, we aim to describe the current state of knowledge concerning the role of oxidative stress on the neurovascular unit and the role of a single cell type in the NVU crosstalk.

Keywords: neurovascular unit; oxidative stress; cell types; molecular mechanisms

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1. Introduction

Although the morphological similarities between the neuronal and the vascular system have already been recognized by the Belgian anatomist Andreas Vesalius in the 15th century, only in the latest two decades the concept of neurovascular unit has emerged [1]. The neurovascular unit (NVU) represents an anatomical and functional whole which includes different cell types, which are intimately and reciprocally linked to each other and acellular elements [2,3].

Cell types include vascular cells (endothelial cells, pericytes, and vascular smooth muscle cells), glial cells (astrocytes, microglia, and oligodendroglia), and neurons. The acellular elements are proteins and enzymes that regulate the composition of the matrix. These elements are structurally and functionally integrated and interdependent and represent a highly efficient system of regulation of cerebral blood flow important to the formation and maintenance of the blood–brain barrier (BBB), which interposed between the systemic circulation and the brain parenchyma [4,5].

This interplay is made by gap junctions between components, and adhesion molecules, such as cadherins and integrins [6–8].

Although both neuronal and endovascular elements are critical and of vital importance, some neuroscientists considered brain cells and cerebral blood vessels distinct entities.

This led to the belief that except when the delivery of blood flow to the brain is impaired, neurons have little to do with the vascular system and vice versa.

Therefore, a rigid distinction has been made between "neurodegenerative diseases" (Alzheimer's disease, Frontotemporal dementia, and Parkinson's disease) and "cerebrovascular diseases" (vascular dementia, ischemic and hemorrhagic stroke). In the last decade, the knowledge about the NVU has been increased regarding structure and function. Currently, the pathophysiology and the clinical relevance of the NVU for cerebrovascular diseases and other neurologic disorders are better known.

In fact, the NVU concept challenged previous assumptions and emphasized the symbiotic relationship between brain cells and cerebral blood vessels, taking on a central role in all aspects of normal brain function and in the pathobiology of a wide variety of brain diseases [9]. Complex communication between neural and vascular structures is required to rapidly and precisely match neuronal metabolism to blood flow within the central nervous system. The neurovascular coupling is a mechanism by which active neurons signal blood vessels to change their diameter and has become increasingly used as a method to evaluate the health and function of the cerebrovascular system and responsiveness of the neurovascular unit.

A number of important studies have identified astrocytes as key intermediaries in neurovascular coupling, following their skill to modulate vascular tone. Astrocytes, via a Ca²⁺-dependent mechanism, engage numerous signaling pathways which lead to the release of vasoactive signals capable of both dilating and constricting arterioles. The kinetics and effectiveness of these signals are, in turn, determined by the metabolic state of the tissue, the level of basal arteriole tone, and/or the magnitude and type of stimulus evoked [10]. Deficits in components of the neurovascular unit have been linked to a wide range of conditions associated with a deteriorating central nervous system structure and function. Neurovascular coupling prepares to become a biomarker in the early detection and monitoring of most neurovascular complications observed in humans [11].

The NVU is prone to protective and damaging events, leading from health to illness, recovery, or death. In this context, systemic, endocrine, neural factors, and oxidative stress can transform all or part of the NVU. Disorders of the NVU can contribute to the initial stroke pathology and the development of a brain injury after a stroke.

Chronic injury to the small penetrating arteries is the leading cause of a lacunar stroke, oxidative stress, vascular dementia, and intracerebral hemorrhages.

Low microvascular density or microvascular dysregulation is likely a major factor that will contribute to eventual brain injury after a large vessel stroke. In the context of an acute stroke due to embolism or thrombosis of the large arteries, the dysfunction of the NVU is also partly responsible for cerebral edema and may lead to the progression of the lesion into the ischemic penumbra [12–14].

Therefore, understanding the mechanisms of neurovascular dysfunction in disease conditions may allow the development of potent and effective therapies for the prevention and treatment of brain diseases.

2. NVU and its Major Components

The NVU is composed of cells from the brain endothelium that interact with pericytes, astrocytes, oligodendroglial cells, microglial cells, neurons, and extracellular matrices in order to form the blood–brain barrier.

2.1. Pericytes

Among the NVU components, mural cells as the pericytes which are in close proximity to endothelial cells, play a vital role in the BBB integrity [15] and regulate the BBB formation and maintenance through secreting inhibitory signals. They control permeability gradients and segregate blood from the brain parenchyma [15–18]. It is yet unknown how pericytes regulate the vesicular pathways across the BBB [19] since the number of vesicles as caveolae and the rate of endothelial transcytosis are lowered. Ben-Zvi and colleagues have identified MFSD2A as a negative regulator of transcytosis in the BBB endothelial cells. Increased expression of this protein turns out to coincide with the onset of endothelial barrier function in mouse embryos and this expression was higher in the BBB endothelial cells compared with peripheral vessels. Under pathological conditions [20– 22], pericytes are highly susceptible to oxidative stress and apoptosis [23–26]. Reactive oxygen species (ROS) are important modulators of cellular functions in pericytes. Although there are many enzymes that can potentially produce ROS, the NADPH oxidase (Nox) family proteins are the predominant source of reactive oxygen species under physiological and pathological conditions. Nox4 is a major superoxide-producing enzyme among the Nox family in human brain pericytes. Nox4 expression level was significantly increased under hypoxic conditions both in vivo and in vitro. Upregulated Nox4 increases the production of MMP-9 probably through NFkB in pericytes and plays a harmful role in the acute phase of ischemic stroke by increasing the size of the infarct area and enhancing the breakdown of the BBB. [27–29].

2.2. Endothelial Cells

The BBB endothelial cells are presented with a highly sophisticated junctional complex consisting of adherens junctions (AJs), gap junctions (GJs), and tight junctions (TJs) and exhibit an extremely high level of continuity, thereby stringently limiting paracellular diffusion of solutes and water between the blood and the brain [30-36]. In fact, an important feature of the BBB endothelial cells is having low rates of nonspecific transcellular vesicular transport (transcytosis), including (macro) pinocytosis, clathrin-dependent and caveolin-dependent endocytosis. Although endothelial cells have a paucity of plasma membranes invaginations, clathrin-coated vesicles present in the capillary endothelium mediate mainly transcytosis in the direction of blood-to-brain [37-40]. In this way, the BBB regulates the entrance of important substances from the bloodstream, such as glucose, and blocks the entry of neurotoxins, such as fibrinogen and peripheral immune cells, maintaining the specialized composition of the brain interstitial fluid [41,42]. In contrast, caveolae are considered negligible in the BBB integrity and seem to be the major transcellular pathway of loss of the BBB function that turns out to increase its permeability in pathological conditions [43,44]. In the last decade, several studies have raised considerable interest for the extracellular vesicles, named exosomes, which can mediate cell-to-cell communication in a variety of biological processes [45,46], as in CNS inflammation, neurogenesis, or neuroprotection [47] and as an efficient transport system of exogenous siRNA across the BBB [48–51].

In the BBB endothelial cells, Ca²⁺ signaling has an important role in enhancing the BBB permeability, and gap junctions (GJs) are also intimately involved in the Ca²⁺ signaling processes [52–54]. The GJs directly connect the cytoplasm of adjacent endothelial cells and facilitate the transfer of ions, metabolites, and second messengers. To date, most of the evidence available points to Ca²⁺ as an important regulator of the paracellular route, contributing to increased vesicular trafficking across the BBB. Indeed, in the BBB endothelium, the junctional protein, as well as the cytoskeletal components, appear to be important targets of the effector protein that are activated downstream of the Ca²⁺ signaling processes [54]. In fact, in the BBB endothelial cells, different connexin (Cx) and pannexin (Panx) proteins form large conductance channels in the endothelial membrane, which can facilitate direct transcellular transport. An endothelial Ca²⁺ increase is a common feature of various pathological conditions that are associated with BBB dysfunction [35,36,54–59]. Their opening is promoted by several types of cellular stress, including mechanical stress during bone remodeling [60], ischemia [61,62], subarachnoid hemorrhage [63,64], and oxidative stress [65].

Novel investigations have recently reported whole gene expression profiling of brain EC, aiming to search for relevant mechanisms regarding the BBB and NVU pathophysiology and for potential treatment options [66].

The endothelial cells of brain vessels (including capillaries) integrate and respond to myriad intravascular, vascular, and extravascular signals. These cells are viewed as a key regulator of blood flow, transport, BBB function, and immune surveillance of normal brain tissue. The endothelial cells have ongoing direct interaction with extravascular (as-

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trocytes, microglia, neurons), vascular (pericytes, smooth muscle), and intravascular (leukocytes, platelets, red blood) cells [12]. It is known that the BBB is a tight barrier for watersoluble molecules that may only enter the CNS via specific transporters. Thanks to the presence of tight junctions among endothelial cells, to the absence of macropinocytosis, and to the loss of fenestrae, the BBB is a highly selective barrier. In pathological conditions, especially in the oxidative stress, the modification of these three elements alter the BBB integrity, preventing the production of an ultrafiltrate through the brain's capillary bed.

In particular, free radicals can directly compromise the BBB integrity via modulation of tight-junction/cytoskeletal integrity [67–69], disrupting both microtubules and actin filaments via matrix metalloproteinase activation [70] and via cellular communication [71]. In neurons, microtubules are of primary importance since they maintain these cells polarization and intracellular trafficking. Particularly, a cytoskeleton-associated protein, MI-CAL (molecule interacting with CasL) binds actin filaments and selectively oxidizes Met 44 and Met 47 in a NADPH-dependent, in order to cause filament severing [72] with consequent cell morphology alteration, to regulate blood vessel sprouting [73]. In addition, collapsin response mediator protein 2 (CRMP2) is sensitive to oxidation in cells exposed to hydrogen peroxide (H₂O₂) [74].

CRMP2 is an important regulator of microtubule stability, and through oxidation and phosphorylation it promotes its dissociation from tubulin and microtubule collapse.

Several studies suggest that oxidative stress and consequential endothelial dysfunction have a critical role in age-related cerebromicrovascular impairment and neurovascular uncoupling [75–79] in different diseases [80–83].

For this reason, along with inflammation, oxidative stress seems to be one of the main inducers of neurodegeneration, causing excitotoxicity, neuronal loss, axonal damage, and promotes cell death. The role of neuroinflammation in many diseases, such as cerebral cavernous malformation (CCM), has been already confirmed [84], but Scimone et al. have suggest novel mechanisms involved in CCM development, through expression profile studies in CCM endothelial cells without functional deficits at the three CCM genes [85]. Studies in vivo and in vitro on mice have also used lipopolysaccharide (LPS) to induce inflammation and the destruction of the BBB. It has been seen that the BBB is relatively resistant to the LPS-induced disruption and appears to be dependent on cyclooxygenase (COX) but not on oxidative stress.

Some brain regions result to be more vulnerable than others; in fact, it appears that astrocytes and pericytes play a minor role in BBB disruption LPS-mediated [35,86,87]. Inflammation also can induce BBB disruption by altering tight junction function, because occludin and claudin-5 appear susceptible to redox changes [88,89]. Both occludin and claudin-5 contain highly conserved redox-sensitive cysteine residues that form disulfide bonds critical to the structure and function of the TJs, thus promoting paracellular opening [90,91] and transcytotic leakage [92,93].

2.3. Astrocytes

Astrocytes, the most abundant type of neuroglia cells are also responsible for regulating transcellular transport across the BBB. Distinct types, including radial astrocytes, fibrous astrocytes, and protoplasmic astrocytes are within the CNS based on structure, distribution, and function, as well as their expression level of the different isoforms and splice variants of the intermediate filament protein, glial fibrillary acidic protein (GFAP) [94,95]. Astrocytes have been implicated in maintaining water and ionic homeostasis, favoring the generation of action potentials in neurons protecting them against oxidative stress associated with their high energy consumption [96,97]. Astrocytes undergo a senescence-like stress response, named "astrosenescence" by Cohen and described as a functional change that has a severe impact on neurodegenerative diseases [98]. Astrocytes play a role in regulating the BBB redox homeostasis, through antioxidants release as glutathione (GSH) [99] and nuclear factor erythroid 2-related (Nrf2)-dependent glutathione [100,101]. Nrf2 is an important example of mitochondrial ROS signaling, which leads to nuclear gene expression changes [102]; in fact, Nrf2 is transferred from the cytoplasm to the nucleus, where it binds the DNA antioxidant response element (ARE) of the genes involved in the antioxidant response [103] and induces secondary defense proteins in oxidative stress condition. This allows astrocytes to be more protected than neurons against moderate levels of oxidative stress [56–58,104–106]. Astrocyte are provided with numerous protrusions that anchor neurons to their blood supply forming a complete layer around cerebral blood vessels known as glial limitans. They regulate immune cell entry, waste clearance, and blood flow [107]. Reactive astrocytes carry mitochondria to their endfeet, driving vascular remodeling, potentially through ROS generation and redox signaling [108].

Astrocyte end-feet also release growth factors involved in the formation of the TJ protein, such as the vascular endothelial growth factor (VEGF), which is likely a key protein in this process through its ability to affect both vascular and neuronal cells [61–64,109–112]. ROS are also able to enhance directly the VEGF transcriptional level in the brain endothelial cells exposed to oxidative stress or thrombin [113]. In fact, once bound to its receptor VEGFR2, VEGF promotes the proangiogenic signaling cascade, leading to the activation of the ERK/MAPK pathway [114,115].

A highly important signaling molecule, by which astrocytes both communicate with each other and with vascular cells, is also ATP and its metabolites, adenosine, and ADP [116,117]. When ATP is released from astrocytes in response to neuronal activation, it contributes to microvascular dilation by triggering the production of endothelial NO [116]. ATP is directly linked to astrocyte metabolism, but in aging, cellular energy metabolism, and ATP production are altered in different types of cells, although little is known about age-related alterations in neurovascular coupling mechanisms and astrocyte dysfunction. In any case, neurovascular uncoupling is reversible in aging by interventions that improve endothelial function and cerebromicrovascular reactivity.

2.4. Oligodendrocytes

Oligodendrocytes are one of the major cell types in the white matter, and within the CNS, they produce a lipid-rich membrane called myelin to enwrap axons for efficient conduction of electrical impulses. This myelinating action is most essential during the nervous system development, but it is also critical in the repair of damaged white matter in the adult brain. It is well known that oligodendrocytes can signal to neurons via myelin-axon interactions.

Recently, it has been demonstrated that oligodendrocytes metabolically support neuronal axons with a particular reference to lactate supply. Furthermore, endothelial–oligodendrocyte interactions may contribute to ongoing angiogenesis and oligodendrogenesis in adult white matter, particularly after brain injury. During the chronic phase after a white matter injury, matrix metalloproteinase (MMP)-9 from oligodendrocytes may promote vascular remodeling [118].

2.5. NVU in White Matter

Although, the NVU concept has been mostly utilized referring to functional cellular units in "gray matter", cell–cell interactions could be also very important for white matter.

In fact, many review articles focus on astrocytes which are interposed between neurons and microvessels. In white matter, however, neuronal axons are covered by myelin. Since oligodendrocytes do not contact microvessels directly, the NVU in white matter should be considered as "neurons–oligodendrocyte–astrocyte–microvessels".

White matter is particularly vulnerable to cerebrovascular injury. This vulnerability has been attributed, in part, to the nature of cerebral vascular anatomy. However, the ac-

tive and contributing role of glial cells in white matter vascular injury has been demonstrated by the inhibition and modulation of these cells in recent experimental models [119].

2.6. Microglia

In the early stages of development, microglia arise from the yolk sac and settle in the brain as the first glial cells, developing in conjunction with neurons in highly plastic cells with mobility. Under physiological or pathological conditions, the microglia continuously monitor the surrounding environment and always responds in the first place to any insult in the SNC.

The intricate relationship existing between the different components of the NVU is expressed very well with regard to microglia, capable of polarizing into distinct pro-inflammatory (M1) or anti-inflammatory (M2) phenotypes. Current data indicates that M1 pro-inflammatory microglia contribute to a BBB dysfunction and a vascular "leak", while M2 anti-inflammatory microglia play a protective role at the BBB [120].

Over the past few decades, microglial cells have been regarded as the main executor of inflammation after acute and chronic central nervous system (CNS) disorders, responding rapidly to exogenous stimuli during acute trauma or infections, or signals released by cells undergoing cell death during conditions such as a stroke, Alzheimer's disease (AD) and Parkinson's disease (PD) [121,122].

2.7. Basement Membrane

The basement membrane (BM) represents the extracellular matrix (ECM) found predominantly underneath endothelial and epithelial cells. The ECM is a structure in constant morpho-functional "remodeling", both in physiological and pathological conditions, based on the functional requests coming from its own interior (through the action of metalloproteases) and from the cells (through the action of adhesion proteins) [123,124]. In the brain, two types of BM are found: an endothelial BM and a parenchymal BM, separated by pericytes. Under physiological conditions, the two BM layers are indistinguishable. The main components of the BM are: collagen IV, laminin, entactin, and heparan sulfate proteoglycan 2, which have a crucial role in vascular integrity. The BM exerts many important functions, including structural support, cell anchoring, and signaling transduction [125]. Metalloproteases (MMPs) are a family of endopeptidases containing zinc and calcium, that show the potential to degrade the protein of the ECM. In particular, the 9 isoform (MMP9) is important in the brain's microvascular environment.

Metalloproteases activated by ROS degrade the BM protein, suggesting that oxidative stress increases the BBB permeability and promotes extravasation of inflammatory factors and ROS in the brain, causing neurodegeneration diseases [126–129].

3. Oxidative Stress and Transporters at the Neurovascular Unit

The NVU transporters are vital for the regulation of normal brain physiology. In pathological conditions, brain edema increases due to the alteration of ion, water, and glutamate transporters, primarily in astrocytes. Drug toxicity is enhanced because of the alteration of efflux transporters (ABC transporters). In addition, the energy metabolism results altered because the glucose transporters are dysregulated.

3.1. ABC Transporters

The brain is lipid-rich compared to other organs, but there is very little known about the potential roles for ATP-binding cassette (ABC) transporters in brain lipid transport. ABC transporters are localized on the blood-facing plasma membrane where they allow unidirectional transport from the cytoplasm to the extracellular space. To date, 48 ABC transporters are known in the human genome. Their principal role is to extrude metabolic waste into the blood and form a selective barrier in order to protect the CNS by limiting entry of xenobiotics, including toxins and a large number of drugs [130,131]. Several genetic diseases are caused by ABC transporter mutations. For example, mutations in ABCA4, whose protein transports retinylidene phospholipid complexes within the rod outer segment of the retina, can cause Stargardt disease and other related eye disorders [132,133]. Others ABC transporters are expressed in the human brain [134,135] with specialized functions in terms of their location at the level of brain region (e.g., ABCA7 in hippocampus), cell type (e.g., ABCA2 in oligodendrocytes), and organelle (e.g., ABCD1 in peroxisomes). ABC transporters have evolved to counteract oxidative stress; indeed, ROS may function as an endothelial signal transduction intermediate promoting cell survival, increasing ABC expression to compensate the increased load of oxidative stress products, or to compensate for the loss of efflux pumps in damaged tissues [136]. Oxidative stress may also lead to increased lipid peroxidation, which is implicated in BBB disintegration, with consequent decrease in ABC expression and activity.

3.2. Glucose Uptake

The brain has almost no storage of glucose, and it must be supplied continuously via the blood circulation [137,138]. Glucose uptake occurs in neurons and astrocytes via different glucose transporters (GLUTs), that are, respectively, GLUT3 and GLUT1 [139,140]. Through GLUT1, glucose supplied by the cerebral circulation can cross the BBB.

Astrocytes play a pivotal role in glucose metabolism. Acute and chronic high-glucose environments activate the glutathione/pentose phosphate pathway (PPP) system in astrocytes, preventing ROS elevation. Chronic high-glucose environments induce endoplasmic reticulum stress through increased hexosamine biosynthetic pathway flux [141].

Astrocytes contain small amounts of glucose in the form of glycogen granules [142].

Unfortunately, glucose derived from astrocytes glycogen cannot cross the neural cell membrane because of its low lipid solubility and, therefore, it cannot be available for neurons.

On the other hand, lactate or pyruvate, the end-products of glycolysis, can exit from astroglia via monocarboxylate transporter 1 (MCT1) and MCT4 and can re-enter into neurons via monocarboxylate transporter 2 (MCT2) and they can be used as an energy source for neuronal tricarboxylic acid (TCA) cycle substrates [143–146].

The metabolic roles of lactate in brain function have long been debated in the literature as an astrocyte–neuron lactate shuttle. Various models attempted to quantify the magnitude of lactate trafficking from astrocytes to neurons, from neurons to astrocytes, and between astrocytes via gap junctional transfer and lactate release from the brain.

Strong evidence points for a glutamate-evoked glycolysis in astrocytes coupled with lactate shuttling in neurons.

The cellular sources of lactate in the brain remain to be established, and neurons have many specific functions fulfilled by glycolysis, so it is risky to assume that lactate is always astrocyte derived. Recent discoveries that revealed novel signaling functions for lactate are significant advances [147,148].

4. Oxidative Stress and Mitochondrial Dysfunction

It is known that proper neuronal activity entails high amounts of energy. In the brain, the amount of energy is very reduced, whereby constant supply of energy substrates are required through blood flow [149]. The NVU maintains energy substrates essential to the fulfilment of the metabolic needs [150], not only through the communication among cell types but also through the mitochondria [151,152]. Mitochondria are fundamental in cell homeostasis due to their involvement in several vital processes, such as cell growth and differentiation, cell cycle control and death [153,154], intermediary metabolism, Ca²⁺ homeostasis and signaling, and apoptosis [155]. Oxidative stress can affect the mitochondrial

respiratory chain function, thereby altering the membrane permeability and calcium homeostasis, along with increasing the heteroplasmic mtDNA and weakening the mitochondrial defense systems [156,157]. Complexes I and III produce radicals, as superoxide anions and hydrogen peroxides, which have various cellular signaling roles and are necessary for cell differentiation, proliferation, survival, and adaptive immunity responses. In the complex IV and during oxidative phosphorylation reactions, the movement of electrons results in the reduction of oxygen to water. Almost all the generated superoxide anions are effectively neutralized by superoxide dismutase (SOD) in order to form hydrogen peroxide, which serves as an important precursor for other free radicals and acts as a secondary messenger with the ability to diffuse across the mitochondrial membrane, through a specialized protein from the aquaporin family. In this way, ROS can modulate the expression of several genes involved in the signal transduction [158–167]. The effects related to ROS signals are different and even opposite, depending on their concentration. At elevated concentrations, they influence the oxidative stress, ultimately leading to cell death, whereas at lower concentrations, they mediate redox signaling events in favor of the progression of disease [168,169] and promoting proliferation, invasiveness, angiogenesis, and metastasis [170–172]. However, despite the presence of mitochondria, the endothelial cells obtain a large proportion of their energy from anaerobic glycolytic metabolism [173]. This suggests that mitochondria serve primarily as essential signaling organelles rather than as manufacturers of ATP in the vascular endothelium [174–176], leading the liberation of vasoactive factors from the endothelium for modulation and maintenance of the BBB integrity and brain homeostasis [177].

5. Possible Therapeutic Target to Protect the NVU

In the last years, preclinical and clinical approaches were designed to protect the NVU. Several molecules with potential therapeutic effects were identified to target ROS, boost mitochondrial function, and decrease free radical production and oxidative damage [178,179], in an attempt to improve neurovascular health.

Noncoding RNAs are implicated in a wide variety of cellular processes, as well as in many disease conditions [180–187] in which the oxidative stress alters ROS homeostasis. Oxidative stress induces the expression of several transcription factors such as NF κ B, AP-1, p53, HIF-1 α , PPAR- γ , β -catenin/Wnt, and Nrf2 that, in turn, activate the expression of hundreds of genes which are associated with growth factors signaling, immune responses, and cell cycle transition. Some lncRNAs that are aberrantly expressed in oxidative stress have functional interactions with miRNAs especially in the neurodegenerative conditions [188]. Therefore, these transcripts could be regarded as biomarkers for the assessment of the levels of oxidative/antioxidative imbalance [189].

In case of intracerebral hemorrhage in combination with intraventricular hemorrhage (IVH), intraventricular recombinant tissue plasminogen activator (rt-PA) represents a good approach to dissolve the blood clot in the ventricular system. However, blood derivatives enter the parenchyma and may still adversely affect functional structures of the brain [42]. In contrast, clinical arterial approaches to lowering blood pressure (and, therefore, cerebral reperfusion pressure) in an acute ischemic stroke have not shown clear benefits [190]. One clinical study demonstrated that lithium exposure in bipolar disorder determines a reduced risk of stroke, potentially being able to protect the endothelium and NVU [58]. Experimental studies suggested that an endothelial Ca2+ overload plays a role in endothelial dysfunction including the BBB opening, a key mechanism in the acute stage of a stroke; therefore, lithium significantly increasing the stability of the BBB [35,36,57]. Through the inhibition of the activity of the phosphoinositol phosphatases, it decreases levels of inositol 1,4,5-trisphosphate, by resulting in possible induction of autophagy [191].

Also the Xestospongin C, a potent inhibitor of the Ins(3)P-sensitive release channel that displays high selectivity over ryanodine receptors, suppress the early Ca²⁺ rise in metabolically inhibited endothelial cells [192].

Low-dose therapeutic lithium concentrations significantly augment the cerebral vessel relaxation, independently of central and autonomic nerve system influences and also stabilized the dynamic thrombin-induced and PAR-1 receptor agonist-induced permeability of human endothelium [35].Instead, lithium accumulation or overdose reduces endothelium-dependent but not endothelium-independent vasorelaxation, suggesting that lithium could have differential effects on the endothelium and the vasculature [193].

Recent clinical trials have demonstrated the efficacy of glibenclamide, a drug that binds the sulfonylurea receptor 1 proteins at potassium channels and may significantly reduce cerebral edema following a stroke [194].

Intravenous glyburide that reduces brain swelling and improves survival in preclinical models of a stroke has shown to be effective also in patients who suffered an ischaemic stroke and a large hemispheric infarction [195].

6. Conclusions

The brain is one of the main organs in the body with the highest metabolic demand and requires a tight regulation of the surrounding environment.

This tight control is exerted by the NVU, comprising different cell types. Even slight perturbations in the NVU might affect, in some cases irreversibly, brain homeostasis and health.

It is also known that in many cases the BBB integrity is deeply affected by oxidative stress. In fact, increased reactive oxygen species (ROS) production contribute to endothelium dysfunction and increased permeability of the BBB [196].

Together with oxidative stress, several pathological factors can cause BBB compromise, mainly increasing BBB permeability.

Direct damage to endothelial cells and the BBB can affect other components of the neurovascular unit, further aggravating BBB damage and dysfunction and eventually leading to neuronal dysfunction, neuroinflammation, and neurodegeneration.

Studying the role of oxidative stress in the pathogenesis of neurological diseases and protecting the BBB in the early stages can be a great help in limiting the progression of the disease.

About the antioxidant's effects of a specific target, to date, the evidence is limited in human endothelial cells [35,87,197], and the use of antioxidant therapy in cerebrovascular disease still needs further details.

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References

- 1. Carmeliet, P.; Tessier-Lavigne, M. Common mechanisms of nerve and blood vessel wiring. Nature 2005, 436, 193–200.
- 2. Wilhelm, I.; Nyul-Toth, A.; Suciu, M.; Hermenean, A.; Krizbai, I.A. Heterogeneity of the blood-brain barrier. *Tissue Barriers* **2016**, *4*, e1143544–e1143548.
- 3. Noumbissi, M.E.; Galasso, B.; Stins, M.F. Brain vascular heterogeneity: Implications for disease pathogenesis and design of in vitro blood-brain barrier models. *Fluids Barriers CNS* **2018**, *15*, 12s.
- 4. Armstead, W.M.; Raghupathi, R. Endothelin and the neurovascular unit in pediatric traumatic brain injury. *Neurol. Res.* 2011, 33, 127–132.
- 5. Abbott, N.J.; Friedman, A. Overview and introduction: The blood-brain barrier in health and disease. *Epilepsia* 2012, 53, 1–6.
- Simard, M.; Arcuino, G.; Takano, T.; Liu, Q.S.; Nedergaard, M. Signaling at the gliovascular interface. J. Neurosci. 2003, 23, 9254– 9262.
- 7. Del Zoppo, G.J. The neurovascular unit, matrix proteases, and innate inflammation. Ann. N. Y. Acad. Sci. 2010, 1207, 46–49.
- 8. Figley, C.R.; Stroman, P.W. The role(s) of astrocytes and astrocyte activity in neurometabolism, neurovascular coupling, and the production of functional neuroimaging signals. *Eur. J. Neurosc.* **2011**, *33*, 577–588.
- 9. Iadecola, C. The neurovascular unit coming of age: A journey through neurovascular coupling in health and disease. *Neuron* **2017**, *96*, 17–42.
- 10. McConnell, H.L.; Zhenzhou, L.; Randall, L.; Mishra, A.; Mishra, W. Astrocyte dysfunction and neurovascular impairment in neurological disorders: Correlation or causation? *Neurochem. Int.* **2019**, *128*, 70–84.
- 11. Squair, J.W.; Lee, A.H.; Sarafis, Z.K.; Chan, F.; Barak, O.F.; Dujic, Z.; Day, T.; Phillips, A.A.Network analysis identifies consensus physiological measures of neurovascular coupling in humans. *J. Cereb. Blood Flow Metab.* **2020**, *40*, 656–666.
- 12. National Institute of Neurological Disorders and Stroke. Report of the Stroke Progress Review Group. 2002. Available online: www.stroke.nih.gov/documents/SPRG_report_042002_508C.pdf (accessed on 29 July 2021).
- 13. Bosche, B.; Dohmen, C.; Graf, R.; Neveling, M.; Staub, F.; Kracht, L.; Sobesky, J.; Lehnhardt, F.G.; Heiss, W.D. Extracellular concentrations of non-transmitter amino acids in peri-infarct tissue of patients predict malignant middle cerebral artery infarction. *Stroke* **2003**, *34*, 2908–2913.
- 14. Liebeskind, D.S.; Jüttler, E.; Shapovalov, Y.; Yegin, A.; Landen, J.; Jauch, E.C. Cerebral Edema Associated With Large Hemispheric Infarction. *Stroke* 2019, *50*, 2619–2625.
- 15. Armulik, A.; Guillem, G.M.M.; Nisancioglu, M.H.; Wallgard, E.; Niaudet, C.; He, L.; Norlin, J.; Lindblom, P.; Strittmatter, K.; Johansson, B.R.; et al. Pericytes regulate the blood-brain barrier. *Nature* **2010**, *468*, 557–561.
- 16. Giannoni, P.; Badaut, J.; Dargazanli, C.; Fayd'Herbe De Maudave, A.; Klement, W.; Costalat, V.; Marchi, N. The Pericyte-glia interface at the blood-brain barrier. *Clin. Sci.* **2018**, *132*, 361–374.
- 17. Daneman, R.; Prat, A. The blood-brain barrier. Cold Spring Harb. Perspect. Biol. 2015, 7, a020412.
- 18. Engelhardt, B.; Sorokin, L. The blood-brain and the blood cerebrospinal fluid barriers: Function and dysfunction. *Semin. Immunopathol.* **2009**, *31*, 497–511.
- 19. Sweeney, M.D.; Ayyadurai, S.; Zlokovic, B.V. Pericytes of the neurovascular unit: Key functions and signaling pathways. *Nat. Neurosci.* **2016**, *19*, 771–783.
- Hill, J.; Rom, S.; Ramirez, S.H.; Persidsky, Y. Emerging roles of pericytes in the regulation of the neurovascular unit in health and disease. J. Neuroimmune Pharmacol. 2014, 9, 591–605.
- 21. Bhattacharya, A.; Kaushik, D.K.; Lozinski, B.M.; Yong, V.V.W. Beyond barrier functions: Roles of pericytes in homeostasis and regulation of neuroinflammation. *J. Neurosci. Res.* **2020**, *98*, 2390–2405.
- 22. Hirunpattarasilp, C.; Attwell, D.; Freitas, F. The role of pericytes in brain disorders: From the periphery to the brain. J. Neurochem. 2019, 150, 648–665.
- Shah, G.N.; Morofuji, Y.; Banks, W.A.; Price, T.O. High glucose-induced mitochondrial respiration and reactive oxygen species in mouse cerebral pericytes is reversed by pharmacological inhibition of mitochondrial carbonic anhydrases: Implications for cerebral microvascular disease in diabetes. *Biochem. Biophys. Res. Commun.* 2013, 440, 354–358.
- Shah, G.N.; Price, T.O.; Banks, W.A.; Morofuji, Y.; Kovac, A.; Ercal, N.; Sorenson, C.M.; Shin, E.S.; Sheibani, N. Pharmacological inhibition of mitochondrial carbonic anhydrases protects mouse cerebral pericytes from high glucose-induced oxidative stress and apoptosis. *J. Pharmacol. Exp. Ther.* 2013, 344, 637–645.
- Ding, X.; Zhang, M.; Gu, R.; Xu, G.; Wu, H. Activated microglia induce the production of reactive oxygen species and promote apoptosis of co-cultured retinal microvascular pericytes. *Graefes Arch. Clin. Exp. Ophthalmol.* 2017, 255, 777–788.
- 26. Rustenhoven, J.; Jansson, D.; Smyth, L.C.; Dragunow, M. Brain Pericytes As Mediators of Neuroinflammation. *Trends Pharmacol. Sci.* 2017, *38*, 291–304.
- Nishimura, A.; Ago, T.; Kuroda, J.; Arimura, K.; Tachibana, M.; Nakamura, K.; Wakisaka, Y.; Sadoshima, J.; Iihara, K.; Kitazono, T. Detrimental role of pericyte Nox4 in the acute phase of brain ischemia. *J. Cereb. Blood Flow Metab.* 2016, 36, 1143–1154.
- Yao, H.; Ago, T.; Kitazono, T.; Nabika, T. NADPH Oxidase-Related Pathophysiology in Experimental Models of Stroke. Int. J. Mol. Sci. 2017, 18, 2123.
- Kuroda, J.; Ago, T.; Nishimura, A.; Nakamura, K.; Matsuo, R.; Wakisaka, Y.; Kamouchi, M.; Kitazono, T. Nox4 is a major source of superoxide production in human brain pericytes. J. Vasc. Res. 2014, 51, 429–438.

- Coisne, C.; Engelhardt, B. Tight junctions in brain barriers during central nervous system inflammation. *Antioxid. Redox Signal.* 2011, 15, 1285–1303.
- Tietz, S.; Engelhardt, B. Brain barriers: Crosstalk between complex tight junctions and adherens junctions. J. Cell Biol. 2015, 209, 493–506.
- 32. Bechmann, I.; Galea, I.; Perry, V.H. What is the blood-brain barrier (not)? Trends Immunol. 2007, 28, 5–11.
- 33. Wolburg, H.; Lippoldt, A. Tight junctions of the blood-brain barrier: Development, composition and regulation. *Vascul. Pharmacol.* **2002**, *38*, 323–337.
- 34. Obermeier, B.; Daneman, R.; Ransohoff, R.M. Development, maintenance and disruption of the blood-brain barrier. *Nat. Med.* **2013**, *19*, 1584–1596.
- 35. Bosche, B.; Molcanyi, M.; Rej, S.; Doeppner, T.R.; Obermann, M.; Müller, D.J.; Das, A.; Hescheler, J.; Macdonald, R.L.; Noll, T.; et al. Low-Dose Lithium Stabilizes Human Endothelial Barrier by Decreasing MLC Phosphorylation and Universally Augments Cholinergic Vasorelaxation Capacity in a Direct Manner. *Front. Physiol.* 2016, 7, 593.
- Haupt, M.; Zechmeister, B.; Bosche, B.; Lieschke, S.; Zheng, X.; Zhang, L.; Venkataramani, V.; Jin, F.; Hein, K.; Weber, M.S.; et al. Lithium enhances post-stroke blood-brain barrier integrity, activates the MAPK/ERK1/2 pathway and alters immune cell migration in mice. *Neuropharmacology* 2020, *181*, 108357.
- 37. Hervé, F.; Ghinea, N.; Scherrmann, J.M. CNS delivery via adsorptive transcytosis. AAPS J. 2008, 10, 455–472.
- Strazielle, N.; Ghersi-Egea, J.F. Physiology of blood-brain interfaces in relation to brain of small compounds and macromolecules. *Mol. Pharm.* 2013, 10, 1473–1491.
- Haupt, M.; Zheng, X.; Kuang, Y.; Lieschke, S.; Janssen, L.; Bosche, B.; Jin, F.; Hein, K.; Kilic, E.; Venkataramani, V.; et al. Lithium modulates miR-1906 levels of mesenchymal stem cell-derived extracellular vesicles contributing to poststroke neuroprotection by toll-like receptor 4 regulation. *Stem Cells Transl. Med.* 2021, *10*, 357–373.
- 40. Kraemer, M.; Lee, S.I.; Ayzenberg, I.; Schwitalla, J.C.; Diehl, R.R.; Berlit, P.; Bosche, B.; Katsarava, Z.; Obermann, M. Headache in Caucasian patients with Moyamoya angiopathy–a systematic cohort study. *Cephalalgia* **2017**, *37*, 496–500.
- Bentz, K.; Molcanyi, M.; Schneider, A.; Riess, P.; Maegele, M.; Bosche, B.; Hampl, J.A.; Hescheler, J.; Patz, S.; Schäfer, U. Extract derived from rat brains in the acute phase following traumatic brain injury impairs survival of undifferentiated stem cells and induces rapid differentiation of surviving cells. *Cell Physiol. Biochem.* **2010**, *26*, 821–830.
- 42. Bosche, B.; Mergenthaler, P.; Doeppner, T.R.; Hescheler, J.; Molcanyi, M. Complex Clearance Mechanisms After Intraventricular Hemorrhage and rt-PA Treatment-a Review on Clinical Trials. *Transl. Stroke Res.* **2020**, *11*, 337–344.
- 43. Klaassen, I.; Van Noorden, C.J.F.; Schlingemann, R.O. Molecular basis of the inner blood-retinal barrier and its breakdown in diabetic macular edema and other pathological conditions. *Prog. Retin Eye Res.* **2013**, *34*, 19–48.
- Reeson, P.; Tennant, K.A.; Gerrow, K.; Wang, J.; Novak, S.W.; Thompson, K.; Lockhart, K.L.; Holmes, A.; Nahirney, P.C.; Brown, C.E. Delayed inhibition of VEGF signaling after stroke attenuates blood-brain barrier breakdown and improves functional recovery in a comorbidity-dependent manner. J. Neurosci. 2015, 35, 5128–5143.
- 45. Urbanelli, L.; Magini, A.; Buratta, S.; Brozzi, A.; Sagini, K.; Polchi, A.; Tancini, B.; Emiliani, C. Signaling pathways in exosomes biogenesis, secretion and fate. *Genes* **2013**, *4*, 152–170.
- 46. Haqqani, A.S.; Delaney, C.E.; Tremblay, T.L.; Sodja, C.; Sandhu, J.K.; Stanimirovic, D.B. Method for isolation and molecular characterization of extracellular microvesicles released from brain endothelial cells. *Fluids Barriers CNS* **2013**, *10*, 4.
- 47. Andaloussi, S.E.; Mäger, I.; Breakefield, X.O.; Wood, M.J.A. Extracellular vesicles: Biology and emerging therapeutic opportunities. *Nat. Rev. Drug Discov.* **2013**, *12*, 347–357.
- Andaloussi, S.E.; Lakhal, S.; Mäger, I.; Wood, M.J.A. Exosomes for targeted siRNA delivery across biological barriers. *Adv. Drug Deliv. Rev.* 2013, 65, 391–397.
- 49. Kumar, L.; Verma, S.; Vaidya, B.; Gupta, V. Exosomes: Natural Carriers for siRNA Delivery. *Curr. Pharm. Des.* 2015, 21, 4556–4565.
- Kalani, A.; Tyagi, A.; Tyagi, N. Exosomes: Mediators of neurodegeneration, neuroprotection and therapeutics. *Mol. Neurobiol.* 2014, 49, 590–600.
- 51. Tsilioni, I.; Panagiotidou, S.; Theoharides, T.C. Exosomes in neurologic and psychiatric disorders. Clin. Ther 2014, 36, 882-888.
- 52. De Bock, M.; Culot, M.; Wang, N.; Bol, M.; Decrock, E.; De Vuyst, E.; da Costa, A.; Dauwe, I.; Vinken, M.; Simon, A.M.; et al. Connexin channels provide a target to manipulate brain endothelial calcium dynamics and blood-brain barrier permeability. *J. Cereb. Blood Flow Metab.* 2011, *31*, 1942–1957.
- De Bock, M.; Culot, M.; Wang, N.; da Costa, A.; Decrock, E.; Bol, M.; Geert Bultynck, G.; Cecchelli, R.; Leybaert, L. Low extracellular Ca2+ conditions induce an increase in brain endothelial permeability that involves intercellular Ca2+ waves. *Brain Res.* 2012, 1487, 78–87.
- 54. De Bock, M.; Wang, N.; Decrock, E.; Bol, M.; Gadicherla, A.K.; Maxime Culot, M.; Cecchelli, R.; Bultynck, G.; Leybaert, L. Endothelial calcium dynamics, connexin channels and blood-brain barrier function. *Prog. Neurobiol.* **2013**, *108*, 1–20.
- 55. De Vuyst, E.; Wang, N.; Decrock, E.; De Bock, M.; Vinken, M.; Van Moorhem, M.; Lai, C.; Culot, M.; Rogiers, V.; Cecchelli, R.; et al. Ca (2+) regulation of connexin 43 hemichannels in C6 glioma and glial cells. *Cell Calcium* **2009**, *46*, 176–187.
- Moccia, F. Calcium Signaling in Endothelial Colony Forming Cells in Health and Disease. Adv. Exp. Med. Biol. 2020, 1131, 1013– 1030.

- Bosche, B.; Schäfer, M.; Graf, R.; Härtel, F.V.; Schäfer, U.; Noll, T. Lithium prevents early cytosolic calcium increase and secondary injurious calcium overload in glycolytically inhibited endothelial cells. *Biochem. Biophys. Res. Commun.* 2013, 434, 268– 272.
- Lan, C.C.; Liu, C.C.; Lin, C.H.; Lan, T.Y.; McInnis, M.G.; Chan, C.H.; Lan, T.H. A reduced risk of stroke with lithium exposure in bipolar disorder: A population-based retrospective cohort study. *Bipolar. Disord.* 2015, *17*, 705–714.
- Marshe, V.S.; Pira, S.; Mantere, O.; Bosche, B.; Looper, K.J.; Herrmann, N.; Müller, D.J.; Rej, S. C-reactive protein and cardiovascular risk in bipolar disorder patients: A systematic review. *Prog. Neuropsychopharmacol Biol. Psychiatry* 2017, 79, 442– 451.
- 60. Siller-Jackson, A.J.; Burra, S.; Gu, S.; Xia, X.; Bonewald, L.F.; Sprague, E.; Jiang, J.X. Adaptation of connexin 43-hemichannel prostaglandin release to mechanical loading. *J. Biol. Chem.* **2008**, *283*, 26374–26382.
- 61. Retamal, M.A.; Schalper, K.A.; Shoji, K.F.; Orellana, J.A.; Bennett, M.V.L.; Sáez, J.C. Possible involvement of different connexin43 domains in plasma membrane permeabilization induced by ischemia-reperfusion. *J. Membr. Biol.* **2007**, *218*, 49–63.
- 62. Dohmen, C.; Bosche, B.; Graf, R.; Reithmeier, T.; Ernestus, R.I.; Brinker, G.; Sobesky, J.; Heiss, W.D. Identification and clinical impact of impaired cerebrovascular autoregulation in patients with malignant middle cerebral artery infarction. *Stroke* **2007**, *38*, 56–61.
- 63. Tso, M.K.; Macdonald, R.L. Subarachnoid hemorrhage: A review of experimental studies on the microcirculation and the neurovascular unit. *Transl. Stroke Res.* **2014**, *5*, 174–189.
- 64. Bosche, B.; Graf, R.; Ernestus, R.I.; Dohmen, C.; Reithmeier, T.; Brinker, G.; Strong, A.J.; Dreier, J.P.; Woitzik, J. Members of the Cooperative Study of Brain Injury Depolarizations (COSBID). Recurrent spreading depolarizations after subarachnoid hemorrhage decreases oxygen availability in human cerebral cortex. *Ann. Neurol.* **2010**, *67*, 607–617.
- 65. Ramachandran, S.; Xie, L.-H.; John, S.A.; Subramaniam, S.; Lal, R. A novel role for connexin hemichannel in oxidative stress and smoking-induced cell injury. *PLoS ONE* **2007**, *2*, e712.
- 66. Tso, M.K.; Turgeon, P.; Bosche, B.; Lee, C.K.; Nie, T.; D'Abbondanza, J.; Ai, J.; Marsden, P.A.; Macdonald, R.L. Gene expression profiling of brain endothelial cells after experimental subarachnoid haemorrhage. *Sci. Rep.* **2021**, *11*, 7818.
- Lochhead, J.J.; McCaffrey, W.; Quigley, C.E.; Finch, J.; DeMarco, K.M.; Nametz, N.; Davis, T.P. Oxidative stress increases bloodbrain barrier permeability and induces alterations in occludin during hypoxia-reoxygenation. *J. Cereb. Blood Flow Metab.* 2010, 30, 1625–1636.
- 68. Wilson, C.; González-Billault, C. Regulation of cytoskeletal dynamics by redox signaling and oxidative stress: Implications for neuronal development and trafficking. *Front. Cell Neurosci.* **2015**, *9*, 381.
- Schreibelt, G.; Kooij, G.; Reijerkerk, A.; van Doorn, R.; Gringhuis, S.I.; van der Pol, S.; Weksler, B.B.; Romero, I.A.; Couraud, P.-O.; Piontek, J.; et al. Reactive oxygen species alter brain endothelial tight junction dynamics via RhoA, PI3 kinase and PKB signaling. *FASEB J.* 2021, *13*, 3666–3676.
- 70. Pun, P.B.L.; Lu, J.; Moochhala, S. Involvement of ROS in BBB dysfunction. Free Radic. Res. 2009, 43, 348–364.
- 71. Quintanilla, R.A.; Orellana, J.A.; von Bernhardi, R. Understanding risk factors for Alzheimer's disease: Interplay of neuroinflammation, connexin-based communication and oxidative stress. *Arch. Med. Res.* **2012**, *43*, 632–644.
- 72. Hung, R.-J.; Terman, J.R. Extracellular inhibitors, repellents, and semaphorin/plexin/MICAL-mediated actin filament disassembly. *Cytoskeleton* **2014**, *68*, 415–433.
- 73. Hung, R.-J.; Pak, C.W.; Terman, J.R. Direct redox regulation of F-actin assembly and disassembly by Mical. *Science* **2011**, *334*, 1710–1713.
- Morinaka, A.; Yamada, M.; Itofusa, R.; Funato, Y.; Yoshimura, Y.; Nakamura, F.; Yoshimura, T.; Kaibuchi, K.; Goshima, Y.; Hoshino, M.; et al. Thioredoxin mediates oxidation-dependent phosphorylation of CRMP2 and growth cone collapse. *Sci. Signal.* 2011, 4, ra26.
- 75. Tucsek, Z.; Toth, P.; Tarantini, S.; Sosnowska, D.; Gautam, T.; Warrington, J.P.; Giles, C.B.; Wren, J.D.; Koller, A.; Ballabh, P.; et al. Aging exacerbates obesity-induced cerebromicrovascular rarefaction, neurovascular uncoupling and cognitive decline in mice. *J. Gerontol. A Biol. Sci. Med. Sci.* **2014**, *69*, 1339–1352.
- 76. Toth, P.; Tarantini, S.; Ashpole, N.M.; Tucsek, Z.; Milne, G.L.; Valcarcel-Ares, N.M.; Menyhart, A.; Farkas, E.; Sonntag, W.E.; Csiszar, A.; et al. IGF-1 deficiency impairs neurovascular coupling in mice: Implications for cerebromicrovascular aging. *Aging Cell.* 2015, 14, 1034–1044.
- 77. Tarantini, S.; Tran, C.H.T.; Gordon, G.R.; Ungvari, Z.; Csiszar, A. Impaired neurovascular coupling in aging and Alzheimer's disease: Contribution of astrocyte dysfunction and endothelial impairment to cognitive decline. *Exp. Gerontol.* 2017, 94, 52–58.
- Park, L.; Hochrainer, K.; Hattori, Y.; Ahn, S.J.; Anfray, A.; Wang, G.; Uekawa, K.; Seo, J.; Palfini, V.; Blanco, I.; et al. Tau induces PSD95-neuronal NOS uncoupling and neurovascular dysfunction independent of neurodegeneration. *Nat. Neurosci.* 2020, 23, 1079–1089.
- Tarantini, S.; Balasubramanian, P.; Yabluchanskiy, A.; Ashpole, N.M.; Logan, S.; Tamas, K.; Ungvari, A.; Nyúl-Tóth, A.; Schwartzman, M.L.K.; Benyo, Z.; et al. IGF1R signaling regulates astrocyte-mediated neurovascular coupling in mice: Implications for brain aging. *Geroscience* 2021, 43, 901–911.
- Fainardi, E.; Castellazzi, M.; Bellini, T.; Manfrinato, M.C.; Baldi, E.; Casetta, I.; Paolino, E.; Granieri, E.; Dallocchio, F. Cerebrospinal fluid and serum levels and intrathecal production of active matrix metalloproteinase-9 (MMP-9) as markers of disease activity in patients with multiple sclerosis. *Mult. Scler.* 2006, *12*, 294–301.

- 81. Brouns, R.; Wauters, A.; De Surgeloose, D.; Marien, P.; De Deyn, P.P. Biochemical markers for blood-brain barrier dysfunction in acute ischemic stroke correlate with evolution and outcome. *Eur. Neurol.* **2011**, *65*, 23–31.
- 82. Montagne, A.; Zhao, Z.; Zlokovic, B.V. Alzheimer's disease: A matter of blood-brain barrier dysfunction? *J. Exp. Med.* 2017, 214, 3151–3169.
- Rao, J.S.; Bhoopathi, P.; Chetty, C.; Gujrati, M.; Lakka, S.S. MMP-9 short interfering RNA induced senescence resulting in inhibition of medulloblastoma growth via p16(INK4a) and mitogen-activated protein kinase pathway. *Cancer Res.* 2007, 67, 4956–4964.
- Peng, W.; Wu, X.; Feng, D.; Zhang, Y.; Chen, X.; Ma, C.; Shen, H.; Li, X.; Li, H.; Zhang, J.J.; et al. Cerebral cavernous malformation 3 relieves subarachnoid hemorrhage induced neuroinflammation in rats through inhibiting NF-kB signaling pathway. *Brain Res. Bull.* 2020, 160, 74–84.
- Scimone, C.; Donato, L.; Alibrandi, S.; Esposito, T.; Concetta Alafaci, C.; D'Angelo, R.; Sidoti, A. Transcriptome analysis provides new molecular signatures in sporadic Cerebral Cavernous Malformation endothelial cells. *Biochim. Biophys. Acta Mol. Basis Dis.* 2020, 1866, 165956.
- Banks, W.A.; Gray, A.M.; Erickson, M.A.; Salameh, T.S.; Damodarasamy, M.; Sheibani, N.; Meabon, J.S.; Wing, E.E.; Morofuji, Y.; Cook, D.G.; et al. Lipopolysaccharide-induced blood-brain barrier disruption: Roles of cyclooxygenase, oxidative stress, neuroinflammation, and elements of the neurovascular unit. J. Neuroinflammation 2015, 12, 223.
- Chan, W.H. Photodynamic treatment induces an apoptotic pathway involving calcium, nitric oxide, p53, p21-activated kinase 2, and c-Jun N-terminal kinase and inactivates survival signal in human umbilical vein endothelial cells. *Int. J. Mol. Sci.* 2011, 12, 1041–1059.
- Ronaldson, P.T.; Demarco, K.M.; Sanchez-Covarrubias, L.; Solinsky, C.M.; Davis, T.P. Transforming growth factor-beta signaling alters substrate permeability and tight junction protein expression at the blood-brain barrier during inflammatory pain. J. Cereb. Blood Flow Met. 2009, 29, 1084–1098.
- Bellmann, C.; Schreivogel, S.; Günther, R.; Dabrowski, S.; Schümann, M.; Wolburg, H.; Blasig, I.E. Highly conserved cysteines are involved in the oligomerization of occludin-redox dependency of the second extracellular loop. *Antioxid. Redox Signal.* 2014, 20, 855–867.
- Knowland, D.; Arac, A.; Sekiguchi, K.J.; Hsu, M.; Lutz, S.E.; Perrino, J.; Gary, K.; Steinberg, G.K.; Barres, B.A.; Nimmerjahn, A.; et al. Stepwise recruitment of transcellular and paracellular pathways underlies blood-brain barrier breakdown in many diseases. *Neuron* 2014, *82*, 603–617.
- 91. Fleegal-DeMotta, M.A.; Dohgu, S.; Banks, W.A. Angiotensin II modulates BBB permeability via activation of the AT1 receptor in brain endothelial cells. *J. Cereb. Blood Flow Metab.* **2009**, *29*, 640–647.
- 92. Mayhan, W.G.; Heistad, D.D. Permeability of blood-brain barrier to various sized molecules. *Am. J. Physiol.* **1985**, 248, H712–H718.
- Ziylan, Y.Z.; Robinson, P.J.; Rapoport, S.I. Blood-brain barrier permeability to sucrose and dextran after osmotic opening. *Am. J. Physiol.* 1984, 247, R634–8.
- 94. Tabata, H. Diverse subtypes of astrocytes and their development during corticogenesis. Front. Neurosci. 2015, 9, 114.
- 95. De Majo, M.; Koontz, M.; Rowitch, D.; Ullian, E.M. An update on human astrocytes and their role in development and disease. *Glia* 2020, *68*, 685–704.
- 96. Jakovcevic, D.; Harder, D.R. Role of astrocytes in matching blood flow to neuronal activity. Curr. Top. Dev. Biol. 2007, 79, 75–97.
- Harder, D.R.; Zhang, C.; Gebremedhin, D. Astrocytes function in matching blood flow to metabolic activity. *News Physiol. Sci.* 2002, *17*, 27–31.
- 98. Cohen, J.; Torres, C. Astrocyte senescence: Evidence and significance. Aging Cell 2019, 18, e12937.
- 99. Agarwal, R.; Shukla, G.S. Potential role of cerebral glutathione in the maintenance of blood-brain barrier integrity in rat. *Neurochem. Res.* **1999**, *24*, 1507–1514.
- 100. Rojo, A.I.; Innamorato, N.G.; Martín-Moreno, A.M.; De Ceballos, M.L.; Yamamoto, M.; Cuadrado, A. Nrf2 regulates microglial dynamics and neuroinflammation in experimental Parkinson's disease. *Glia* **2010**, *58*, 588–598.
- 101. Ronnett, G.V.; Ramamurthy, S.; Kleman, A.M.; Landree, L.E.; Aja, S. AMPK in the brain: Its roles in energy balance and neuroprotection. *J. Neurochem.* 2009, 109, 17–23.
- 102. Whelan, S.P.; Zuckerbraun, B.S. Mitochondrial Signaling: Forwards, Backwards, and in Between. Oxid. Med. Cell Longev. 2013, 2013, 351613.
- 103. Itoh, K.; Wakabayashi, N.; Katoh, Y.; Ishii, T.; Igarashi, K.; Engel, J.D.; Yamamoto, M. Keap1 Represses Nuclear Activation of Antioxidant Responsive Elements by Nrf2 through Binding to the Amino-Terminal Neh2 Domain. *Genes Dev.* 1999, 13, 76–86.
- 104. Vargas, M.R.; Johnson, J.A. The Nrf2-ARE cytoprotective pathway in astrocytes. Expert Rev. Mol. Med. 2009, 3, e17.
- Stewart, V.C.; Stone, R.; Gegg, M.E.; Sharpe, M.A.; Hurst, R.D.; Clark, J.B.; Heales, S.J.R. Preservation of extracellular glutathione by an astrocyte derived factor with properties comparable to extracellular superoxide dismutase. *J. Neurochem.* 2002, *83*, 984– 991.
- Stewart, V.C.; Heales, S.J.R. Nitric oxide-induced mitochondrial dysfunction: Implications for neurodegeneration. *Free Radic. Biol. Med.* 2003, 34, 287–303.
- 107. MacVicar, B.A.; Newman, E.A. Astrocyte regulation of blood flow in the brain. Cold Spring Harb. Perspect. Biol. 2015, 7, a020388.

- Bisland, S.K.; Goebel, E.A.; Hassanali, N.S.; Johnson, C.; Wilson, B.C. Increased expression of mitochondrial benzodiazepine receptors following low-level light treatment facilitates enhanced protoporphyrin IX production in glioma-derived cells in vitro. *Lasers Surg. Med.* 2007, 39, 678–684.
- Lee, S.-W.; Kim, W.J.; Choi, Y.K.; Song, H.S.; Son, M.J.; Gelman, I.H.; Kim, Y.-J.; Kim, K.W. SSeCKS regulates angiogenesis and tight junction formation in blood-brain barrier. *Nat. Med.* 2003, *9*, 900–906.
- 110. Schwarz, Q.; Gu, C.; Fujisawa, H.; Sabelko, K.; Gertsenstein, M.; Nagy, A.; Taniguchi, M.; Kolodkin, A.L.; Ginty, D.D.; Shima, D.T.; et al. Vascular endothelial growth factor controls neuronal migration and cooperates with Sema3A to pattern distinct compartments of the facial nerve. *Genes Dev.* 2004, 18, 2822–2834.
- 111. Miao, H.Q.; Soker, L.S.; Feiner, L.; Alonso, J.L.; Raper, J.A.; Klagsbrun, M. Neuropilin-1 mediates collapsin-1/semaphorin III inhibition of endothelial cell motility: Functional competition of collapsin-1 and vascular endothelial growth factor-165. *J. Cell Biol.* **1999**, *146*, 233–242.
- 112. Hagedorn, M.; Balke, M.; Schmidt, A.; Bloch, W.; Kurz, H.; Javerzat, S.; Rousseau, B.; Wilting, J.; Bikfalvi, A. VEGF coordinates interaction of pericytes and endothelial cells during vasculogenesis and experimental angiogenesis. *Dev. Dyn.* 2004, 230, 23–33.
- 113. Sanchez, A.; Tripathy, D.; Luo, J.; Yin, X.; Martinez, J.; Grammas, P. Neurovascular unit and the effects of dosage in VEGF toxicity: Role for oxidative stress and thrombin. *J. Alzheimer Dis.* **2013**, *34*, 281–291.
- 114. Diebold, L.; Chandel, N.S. Mitochondrial ROS regulation of proliferating cells. Free Radic. Biol. Med. 2016, 100, 86–93.
- 115. Pastukh, V.; Roberts, J.T.; Clark, D.W.; Bardwell, G.C.; Patel, M.; Al-Mehdi, A.-B.; Borchert, G.M.; Gillespie, M.N. An oxidative DNA "damage" and repair mechanism localized in the VEGF promoter is important for hypoxia-induced VEGF mRNA expression. Am. J. Physiol. Cell Mol. Physiol. 2015, 309, L1367–L1375.
- 116. Toth, P.; Tarantini, S.; Davila, A.; Valcarcel-Ares, M.N.; Tucsek, Z.; Varamini, B.; Ballabh, P.; Sonntag, W.E.; Baur, J.A.; Csiszar, A.; et al. Purinergic glio-endothelial coupling during neuronal activity: Role of P2Y1 receptors and eNOS in functional hyperemia in the mouse somatosensory cortex. *Am. J. Physiol. Heart Circ. Physiol.* 2015, 309, H1837–H1845.
- 117. Wells, J.A.; Christie, I.N.; Hosford, R.P.S.; Huckstepp, R.T.R.; Angelova, P.R.; Pirkko, V.; Cork, S.C.; Abramov, A.Y.; Teschemacher, A.G.; Kasparov, S.; et al. A critical role for purinergic signalling in the mechanisms underlying generation of BOLD fMRI responses. J. Neurosci. 2015, 35, 5284–5292.
- 118. Takakuni, M.; Kazuhide, H.; Loc-Duyen, D.P.; Changhong, X.; Eng, H.L.; Ken, A. Biphasic Mechanisms of Neurovascular Unit Injury and Protection in CNS Diseases. *CNS Neurol. Disord. Drug Targets* **2013**, *12*, 302–315.
- 119. Levit, A.; Hachinski, V.; Whitehead, S.N. Neurovascular unit dysregulation, white matter disease, and executive dysfunction: The shared triad of vascular cognitive impairment and Alzheimer disease. *Geroscience* **2020**, *42*, 445–465.
- 120. Ronaldson, P.T.; Davis, T.P. Regulation of blood-brain barrier integrity by microglia in health and disease: A therapeutic opportunity. J. Cereb. Blood Flow Metab. 2020, 40, S6-S24.
- 121. Liu, L.R.; Liu, J.C.; Bao, J.S.; Bai, Q.Q.; Wang, G.Q. Interaction of Microglia and Astrocytes in the Neurovascular Unit. *Front. Immunol.* **2020**, *11*, 1024.
- 122. Hannah, T.; Emmanuel, P. Microglia in the Neurovascular Unit: Blood-Brain Barrier-microglia Interactions After Central Nervous System Disorders. *Neuroscience* **2019**, *405*, 55–67.
- 123. Rosell, A.; Ortega-Aznar, A.; Alvarez-Sabín, J.; Fernández-Cadenas, I.; Ribó, M.; Molina, C.A.; Lo, E.H.; Montaner, J. Increased brain expression of matrix metalloproteinase-9 after ischemic and hemorrhagic human stroke. *Stroke* **2006**, *37*, 1399–1406.
- 124. Pfefferkorn, T.; Rosenberg, G.A. Closure of the blood-brain barrier by matrix metalloproteinase inhibition reduces rtPAmediated mortality in cerebral ischemia with delayed reperfusion. *Stroke* **2003**, *34*, 2025–2030.
- 125. Lingling, X.; Abhijit, N.; Yao, Y. Basement membrane and blood-brain barrier. Stroke Vasc. Neurol. 2018, 4, 78–82.
- 126. Kim, G.W.; Gasche, Y.; Grzeschik, S.; Copin, J.-C.; Maier, C.M.; Chan, P.H. Neurodegeneration in striatum induced by the mitochondrial toxin 3-nitropropionic acid: Role of matrix metalloproteinase-9 in early blood-brain barrier disruption? J. Neurosci. 2003, 23, 8733–8742.
- 127. Ridnour, L.A.; Dhanapal, S.; Hoos, M.; Wilson, J.; Lee, J.; Cheng, R.Y.S.; Brueggemann, E.E.; Hines, H.B.; Wilcock, D.M.; Vitek, M.P.; et al. Nitric oxide-mediated regulation of β-amyloid clearance via alterations of MMP-9/TIMP-1. J. Neuroche. 2012, 123, 736–749.
- Zhang, S.; An, Q.; Wang, T.; Gao, S.; Zhou, G. Autophagy–and MMP-2/9-mediated Reduction and Redistribution of ZO-1 Contribute to Hyperglycemia-increased Blood-Brain Barrier Permeability During Early Reperfusion in Stroke. *Neuroscience* 2018, 377, 126–137.
- Guilfoyle, M.R.; Carpenter, K.L.; Helmy, A.; Pickard, J.D.; Menon, D.K.; Hutchinson, P.J. Matrix Metalloproteinase Expression in Contusional Traumatic Brain Injury: A Paired Microdialysis Study. J. Neurotrauma 2015, 32, 1553–1559.
- Löscher, W.; Potschka, H. Blood-brain barrier active efflux transporters: ATP-binding cassette gene family. *NeuroRx* 2005, 2, 86– 98.
- 131. Soontornmalai, A.; Vlaming, M.L.H.; Fritschy, J.-M. Differential, strain-specific cellular and subcellular distribution of multidrug transporters in murine choroid plexus and blood-brain barrier. *Neuroscience* **2006**, *138*, 159–169.
- 132. Cremers, F.P.M.; Lee, W.; Collin, R.W.J.; Allikmets, R. Clinical spectrum, genetic complexity and therapeutic approaches for retinal disease caused by ABCA4 mutations. *Prog. Retin. Eye Res.* **2020**, *79*, 10086.

- 133. Donato, L.; Scimone, C.; Rinaldi, C.; Pasquale Aragona, P.; Briuglia, S.; Angela D'Ascola, A.; Rosalia D'Angelo, R.; Antonina Sidoti, A. Stargardt Phenotype Associated With Two ELOVL4 Promoter Variants and ELOVL4 Downregulation: New Possible Perspective to Etiopathogenesis? *Investig. Ophthalmol. Vis. Sci.* 2018, 59, 843–857.
- Langmann, T.; Mauerer, R.; Zahn, A.; Moehle, C.; Mario Probst, M.; Stremmel, W.; Schmitz, G. Real-time reverse transcription-PCR expression profiling of the complete human ATP-binding cassette transporter superfamily in various tissues. *Clin. Chem.* 2003, 49, 230–238.
- 135. Ohtsuki, S.; Terasaki, T. Contribution of carrier-mediated transport systems to the blood-brain barrier as a supporting and protecting interface for the brain; importance for CNS drug discovery and development. *Pharm. Res.* **2007**, *24*, 1745–1758.
- Sita, G.; Hrelia, P.; Tarozzi, A.; Morroni, F. P-glycoprotein (ABCB1) and Oxidative Stress: Focus on Alzheimer's Disease. Oxid. Med. Cell Longev. 2017, 2017, 7905486.
- 137. Itoh, Y.; Abe, T.; Takaoka, R.; Tanahashi, N. Fluorometric determination of glucose utilization in neurons in vitro and in vivo. *J. Cereb Blood Flow Metab.* **2004**, *24*, 993–1003.
- Lundgaard, I.; Li, B.; Xie, L.; Kang, H.; Sanggaard, S.; Haswell, J.D.R.; Sun, W.; Goldman, S.; Solomiya Blekot, S.; Michael Nielsen, M.; et al. Direct neuronal glucose uptake heralds activity-dependent increases in cerebral metabolism. *Nat. Commun.* 2015, 6, 6807.
- 139. Vannucci, S.J.; Maher, F.; Simpson, I.A. Glucose transporter proteins in brain: Delivery of glucose to neurons and glia. *Glia* **1997**, 21, 2–21.
- 140. Simpson, I.A.; Carruthers, A.; Vannucci, S.J. Supply and demand in cerebral energy metabolism: The role of nutrient transporters. J. Cereb Blood Flow Metab. 2007, 27, 1766–1791.
- 141. Takahashi, S.; Izawa, Y.; Suzuki, N. Astrogliopathy as a loss of astroglial protective function against glycoxidative stress under hyperglycemia. *Rinsho Shinkeigaku* **2012**, *52*, 41–51.
- Cataldo, A.M.; Broadwell, R.D. Cytochemical identification of cerebral glycogen and glucose-6-phosphatase activity under normal and experimental conditions. II. Choroid plexus and ependymal epithelia, endothelia and pericytes. J. Neurocytol. 1986, 15, 511–524.
- 143. Pierre, K.; Magistretti, P.J.; Pellerin, L. MCT2 is a major neuronal monocarboxylate transporter in the adult mouse brain. *J. Cereb Blood Flow Metab.* **2002**, *22*, 586–595.
- 144. Pellerin, L.; Bergersen, L.H.; Halestrap, A.P.; Pierre, K. Cellular and subcellular distribution of monocarboxylate transporters in cultured brain cells and in the adult brain. *J. Neurosci. Res.* **2005**, *79*, 55–64.
- 145. Pierre, K.; Pellerin, L. Monocarboxylate transporters in the central nervous system: Distribution, regulation and function. *J. Neurochem.* **2005**, *94*, 1–14.
- 146. Takahashi, S. Metabolic compartmentalization between astroglia and neurons in physiological and pathophysiological conditions of the neurovascular unit. *Neuropathology* **2020**, *40*, 121–137.
- 147. Dienel, G.A. Brain Glucose Metabolism: Integration of Energetics with Function. Physiol Rev. 2019, 199, 949–1045.
- 148. Dienel, G.A. Fueling and imaging brain activation. ASN Neuro. 2012, 4, e00093.
- 149. Ohta, S.; Gidö, L.G.; Siesjö, B.K. Influence of ischemia on blood-brain and blood-CSF calcium transport. J. Cereb. Blood Flow Metab. 1992, 12, 525–528.
- 150. Gordon, G.R.J.; Mulligan, S.J.; MacVicar, B.A. Astrocyte control of the cerebrovasculature. *Glia* 2007, 55, 1214–1221.
- Ouyang, Y.-B.; Giffard, R.G.; Alyautdin, R.; Khalin, I.; Nafeeza, M.I.; Haron, M.H.; Kuznetsov, D. Cellular neuroprotective mechanisms in cerebral ischemia: Bcl-2 family proteins and protection of mitochondrial function. *Cell Calcium* 2004, *36*, 303–311
- 152. Alyautdin, R.; Khalin, I.; Nafeeza, M.I.; Haron, M.H.; Kuznetsov, D. Nanoscale drug delivery systems and the blood-brain barrier. *Int. J. Nanomed.* **2014**, *9*, 795–811.
- 153. Osellame, L.D.; Blacker, T.S.; Duchen, M.R. Cellular and molecular mechanisms of mitochondrial function. *Best Pract. Res. Clin. Endocrinol. Metab.* **2012**, *26*, 711–723.
- 154. Carvalho, C.; Correia, S.C.; Perry, G.; Castellani, R.J.; Moreira, P.I. Cerebrovascular and mitochondrial abnormalities in Alzheimer's disease: A brief overview. *J. Neural. Transm.* **2016**, *123*, 107–111.
- 155. Chan, D.C. Mitochondria: Dynamic organelles in disease, aging, and development. Cell 2006, 125, 1241–1252.
- Zhao, R.; Jiang, S.; Zhang, L.; Yu, Z. Mitochondrial electron transport chain, ROS generation and uncoupling. *Int. J. Mol. Med.* 2019, 44, 3–15.
- 157. Donato, L.; Scimone, C.; Alibrandi, S.; Pitruzzella, A.; Scalia, F.; Rosalia D'Angelo, R.; Sidoti, A. Possible A2E Mutagenic Effects on RPE Mitochondrial DNA from Innovative RNA-Seq Bioinformatics Pipeline. *Antioxidants* 2020, 9, 1158.
- 158. Handy, D.E.; Loscalzo, J. Redox Regulation of Mitochondrial Function. Antioxid. Redox Signal. 2012, 16, 1323–1367.
- 159. Janssen-Heininger, Y.M.W.; Mossman, B.T.; Heintz, N.H.; Forman, H.J.; Kalyanaraman, B.; Finkel, T.; Stamler, J.S.; Rhee, S.G.; van der Vliet, A. Redox-Based Regulation of Signal Transduction: Principles, Pitfalls, and Promises. *Free Radic. Biol. Med.* 2008, 45, 1–17.
- Bae, Y.S.; Oh, H.; Rhee, S.G.; Yoo, Y.D. Regulation of Reactive Oxygen Species Generation in Cell Signaling. *Mol. Cells.* 2011, 32, 491–509.
- Rigoulet, M.; Yoboue, E.D.; Devin, A. Mitochondrial ROS Generation and Its Regulation: Mechanisms Involved in H(2)O(2) Signaling. *Antioxid. Redox Signal.* 2011, 14, 459–468.
- 162. Gough, D.R.; Cotter, T.G. Hydrogen Peroxide: A Jekyll and Hyde Signalling Molecule. Cell Death Dis. 2011, 2, e213.

- Wang, Y.; Yang, J.; Yi, J. Redox Sensing by Proteins: Oxidative Modifications on Cysteines and the Consequent Events. *Antioxid. Redox Signal.* 2012, 16, 649–657.
- Murphy, M.P. Mitochondrial Thiols in Antioxidant Protection and Redox Signaling: Distinct Roles for Glutathionylation and Other Thiol Modifications. *Antioxid. Redox Signal.* 2012, 16, 476–495.
- 165. Reczek, C.R.; Chandel, N.S. ROS-Dependent Signal Transduction. Curr. Opin. Cell Biol. 2015, 33, 8–13.
- 166. Schieber, M.; Chandel, N.S. ROS Function in Redox Signaling and Oxidative Stress. Curr. Biol. 2014, 24, R453–R462.
- 167. Sies, H.; Jones, D.P. Reactive Oxygen Species (ROS) as Pleiotropic Physiological Signalling Agents. *Nat. Rev. Mol. Cell Biol.* **2020**, 21, 363–383.
- 168. Sena, L.A.; Chandel, N.S. Physiological Roles of Mitochondrial Reactive Oxygen Species. Mol. Cell. 2012, 48, 158–167.
- 169. Yang, S.; Lian, G. ROS and diseases: Role in metabolism and energy supply. Mol. Cell Biochem. 2020, 467, 1–12
- 170. Wu, W.-S. The Signaling Mechanism of ROS in Tumor Progression. Cancer Metast. Rev. 2006, 25, 695–705.
- 171. Moloney, J.N.; Cotter, T.G. ROS Signalling in the Biology of Cancer. Semin. Cell Dev. Biol. 2018, 80, 50-64.
- 172. Angelova, P.R.; Abramov, A.Y. Role of mitochondrial ROS in the brain: From physiology to neurodegeneration. *FEBS Lett.* **2018**, 592, 692–702.
- 173. Culic, O.; Gruwel, M.L.; Schrader, J. Energy turnover of vascular endothelial cells. Am. J. Physiol. 1997, 273, C205–C213.
- 174. Quintero, M.; Colombo, S.L.; Godfrey, A.; Moncada, S. Mitochondria as signaling organelles in the vascular endothelium. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 5379–5384.
- 175. Tang, X.; Luo, Y.-X.; Chen, H.-Z.; Liu, D.-P. Mitochondria, endothelial cell function, and vascular diseases. *Front. Physiol.* **2014**, 5, 175.
- 176. Busija, D.W.; Rutkai, I.; Dutta, S.; Katakam, P.V. Role of Mitochondria in Cerebral Vascular Function: Energy Production, Cellular Protection, and Regulation of Vascular Tone. *Compr. Physiol.* **2016**, *6*, 1529–1548.
- 177. Busija, D.W.; Katakam, P.V. Mitochondrial mechanisms in cerebral vascular control: Shared signaling pathways with preconditioning. J. Vasc. Res. 2014, 51, 175–189.
- 178. Tong, V.; Teng, X.W.; Chang, T.K.H.; Abbott, F.S. Valproic acid II: Effects on oxidative stress, mitochondrial membrane potential, and cytotoxicity in glutathione-depleted rat hepatocytes. *Toxicol. Sci.* 2005, *86*, 436–443.
- Hamel, E.; Nicolakakis, N.; Aboulkassim, T.; Ongali, B.; Tong, X.-K. Oxidative stress and cerebrovascular dysfunction in mouse models of Alzheimer's disease. *Exp. Physiol.* 2008, 93, 116–120.
- 180. St Laurent, G., 3rd; Faghihi, M.A.; Wahlestedt, C. Non-coding RNA transcripts: Sensors of neuronal stress, modulators of synaptic plasticity, and agents of change in the onset of Alzheimer's disease. *Neurosci. Lett.* **2009**, *466*, 81–88.
- Nunomura, A.; Moreira, P.I.; Castellani, R.J.; Lee, H.-G.; Zhu, X.; Smith, M.A.; Perry, G. Oxidative damage to RNA in aging and neurodegenerative disorders. *Neurotox. Res.* 2012, 22, 231–248.
- Kanagaraj, N.; Beiping, H.; Dheen, S.T.; Tay, K.S.S.W. Downregulation of miR-124 in MPTP-treated mouse model of Parkinson's disease and MPP iodide-treated MN9D cells modulates the expression of the calpain/cdk5 pathway proteins. *Neuroscience* 2014, 272, 167–179.
- 183. Oh, S.E.; Park, H.-J.; He, L.; Skibiel, C.; Junn, E.; Maral Mouradian, M. The Parkinson's disease gene product DJ-1 modulates miR-221 to promote neuronal survival against oxidative stress. *Redox Biol.* 2018, 19, 62–73.
- Pallarès-Albanell, J.; Zomeño-Abellán, M.T.; Escaramís, G.; Pantano, L.; Soriano, A.; Segura, M.F.; Martí, E. A High-Throughput screening identifies microRNA inhibitors that influence neuronal maintenance and/or response to oxidative stress. *Mol. Ther Nucleic Acids* 2019, *17*, 374–387.
- Gamez-Valero, A.; Guisado-Corcoll, A.; Herrero-Lorenzo, M.; Solaguren-Beascoa, M.; Marti, E. Non-Coding RNAs as sensors of oxidative stress in neurodegenerative diseases. *Antioxidants* 2020, 9, 1095.
- 186. Beeraka, N.M.; Doreswamy, S.H.; Sadhu, S.P.; Srinivasan, A.; Pragada, R.R.; Madhunapantula, S.R.V.; Aliev, G. The role of exosomes in stemness and neurodegenerative diseases-Chemoresistant. Cancer Therapeutics and Phytochemicals. *Int. J. Mol. Sci.* 2020, 21, 6818.
- 187. Catanesi, M.; d'Angelo, M.; Tupone, M.G.; Benedetti, E.; Giordano, A.; Castelli, V.; Cimini, A. MicroRNAs dysregulation and mitochondrial dysfunction in neurodegenerative diseases. *Int. J. Mol. Sci.* **2020**, *21*, 5986.
- Donato, L.; Scimone, C.; Alibrandi, S.; Rinaldi, C.; Sidoti, A.; D'Angelo, R. Transcriptome analyses of lncRNAs in a2e-stressed retinal epithelial cells unveil advanced links between metabolic impairments related to oxidative stress and retinitis pigmentosa. *Antioxidants* 2020, 9, 318.
- 189. Ghafouri-Fard, S.; Shoorei, H.; Taheri, M. Non-coding RNAs are involved in the response to oxidative stress. *Biomed. Pharmacother.* **2020**, *127*, 110228.
- 190. Bosche. B.; Macdonald, R.L. Response to Letter Regarding Article, Relevance of Blood-Brain Barrier Disruption After Endovascular Treatment of Ischemic Stroke: Dual-Energy Computed Tomographic Study. Stroke 2015, 46, e200.
- 191. Chiu, C.T.; Chuang, D.M. Neuroprotective action of lithium in disorders of the central nervous system. *J.Cent. South Univ. Med. Sci.* **2011**, *36*, 461.
- 192. Schäfer, M.; Bahde, D.; Bosche, B.; Ladilov, Y.; Schäfer, C.; Piper, H.M.; Noll, T. Modulation of early [Ca2+]i rise in metabolicallyinhibited endothelial cells by xestospongin C. *Am. J. Physiol. Heart Circ. Physiol.* **2001**, *280*, H1002–H1010.

- 193. Bosche, B.; Molcanyi, M.; Noll, T.; Rej, S.; Zatschler, B.; Doeppner, T.R.; Hescheler, J.; Müller, D.J.; Macdonald, R.L.; Härtel, F.V. A differential impact of lithium on endothelium-dependent but not on endothelium-independent vessel relaxation. *Prog. Neuropsychopharmacol Biol. Psychiatry* 2016, 67, 98–106.
- 194. Griepp, D.W.; Lee, J.; Moawad, C.M.; Davati, C.; Runnels, J.; Fiani, B. BIIB093 (intravenous glibenclamide) for the prevention of severe cerebral edema. *Surg. Neurol. Int.* 2021, *12*, 80.
- 195. Sheth, K.N.; Elm, J.J.; Molyneaux, B.J.; Hinson, H.; Beslow, L.A.; Sze, G.K.; Ostwaldt, A.C.; Del Zoppo, G.J.; Simard, J.M.; Jacobson, S.; et al. Safety and efficacy of intravenous glyburide on brain swelling after large hemispheric infarction (GAMES-RP): A randomised, double-blind, placebo-controlled phase 2 trial. *Lancet Neurol.* **2016**, *15*, 1160–1169.
- 196. Enciu, A.M.; Gherghiceanu, M.; Popescu, B.O. Triggers and effectors of oxidative stress at blood-brain barrier level: Relevance for brain ageing and neurodegeneration. *Oxid. Med. Cell Longev.* **2013**, 2013, 297512.
- 197. Tarantini, S.; Yabluchanksiy, A.; Fülöp, G.A.; Hertelendy, P.; Valcarcel-Ares, M.N.; Kiss, T.; Bagwell, J.M.; O'Connor, D.; Farkas, E.; Farzaneh Sorond, F.; et al. Pharmacologically induced impairment of neurovascular coupling responses alters gait coordination in mice. *Geroscience* 2017, 39, 601–614.