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Cytological responses involved in neurovascular remodeling after cerebral ischemia in the aged rat brain

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Abstract

Neurovascular unit (NVU) is an elaborated multicellular brain-vessel-blood interface supporting a controlled blood-brain communication through the selective-permeable blood-brain barrier (BBB) and an adequate neurovascular and neurometabolic coupling. Impairment of NVU during aging and neurologic disorders is accompanied by microvascular dysfunction, BBB opening, neurovascular uncoupling, and neuroinflammation, with deleterious effects on brain microenvironment and neuronal signaling. After stroke, neurons are usually lost in the infarct core and astrocytes become reactive and proliferative, dysregulating the balance between neuronal and non-neuronal cells of the NVU in the lesioned area. In this review, we present major cytological responses of the NVU to cerebral ischemia with an emphasis on the aged brain. Early responses of the neurovascular unit to chronic hypoxia include neutrophils infiltration, brain edema, blood vessel disintegration, astrocytes and endothelial cells proliferation as well as conversion of resident microglia to phagocytic microglia. Later responses include the confinement of the peri-infarcted region by a scar tissue composed mainly of reactive astrocytes and endothelial cells, and angiogenesis. However, the newly formed capillary network is disorganized and the blood vessels are leaky making a successful regeneration of the damaged area, unlikely.

Key words:

neurovascular unit, aging, stroke, remodeling

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Introduction

Brain functioning and its responses to various physiologic or pathologic conditions depend on an elaborated cross-talk between its constituting cells, both neuronal and non-neuronal. The neurovascular unit (NVU) is a multicellular assembly of cerebral microvascular endothelium, pericytes, myocytes, perivascular astrocytes, microglia, and neurons, surrounded by extracellular matrix (Fig. 1). Each cell type from this heterogeneous unit essentially contributes to NVU function, and all together are structurally and functionally interconnected to sustain homeostasis of the cerebral microenvironment and brain function (Abbott and Friedman, 2012; Attwell et al., 2010).

NVUs are complex brain-vessel-blood interfaces that form the selective-permeable blood-brain barrier (BBB) and promote the neurovascular and neurometabolic coupling by appropriately adjusting the local blood flow and nutrient delivery to the neuronal activity and metabolism (Abbott, 2002). The fine-tuning of this coupling is facilitated by the small distance of about 8-25 µm between capillaries and neurons, thus a small number of neurons within one NVU (Abbott, 2004).

All these aspects confer to NVUs essential roles for the health and function of the central nervous system (CNS) (Zagrean et al., 2017).

Impairment of NVU cells during aging and diseases like stroke or neurodegenerative diseases, triggers microvascular dysfunction, BBB permeabilization, alteration of neurovascular coupling, and neuroinflammation, with deleterious effects on brain microenvironment, neuronal excitability and connectivity, and cellular metabolism and cell viability. Finding more aspects of cellular interrelations within the NVU and their shaping in aging and neurological diseases could promote the development of more efficient therapeutic approaches to prevent or mitigate the BBB dysfunction and brain homeostasis impairment (Attwell et al., 2015).

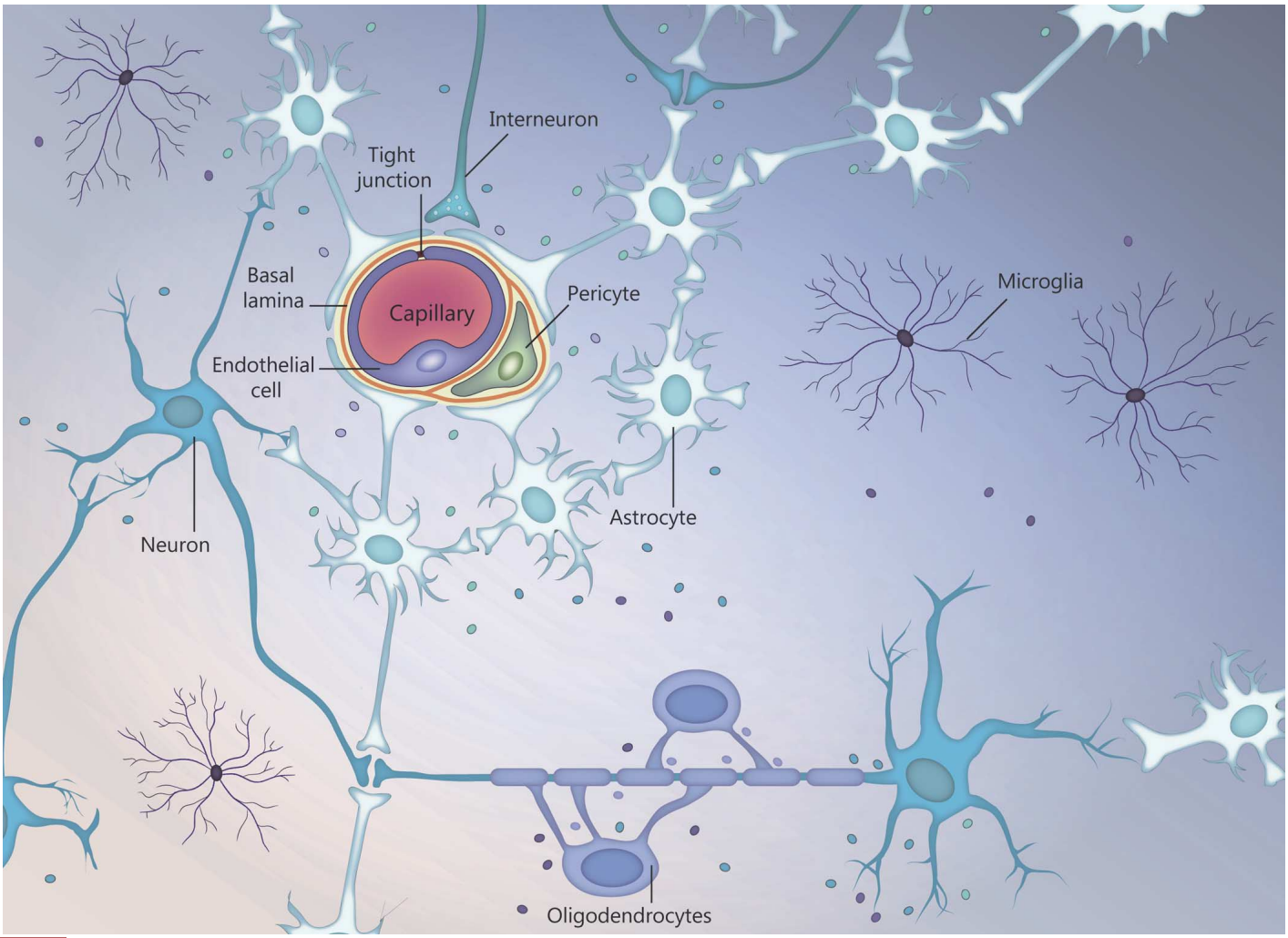

Figure 1
The neurovascular unit (NVU) of the brain. Schematic representation of the NVU, showing the complex interconnections between different cell types – endothelial cells of the capillary with their tight junctions, pericytes, basal lamina, perivascular astrocytes, microglia, neurons – directly connected or communicating through the exosomes released from all cells (shown as small circles in the interstitial space).

Cellular cooperation and coordination in the nervous system – the neurovascular unit

The NVUs are elaborated multicellular functional modules that work on the basis of a dynamic cellular interdependence, cooperation and coordination. Within this assembly consisting of endothelial cells, pericytes, astrocytes, microglia and neurons, the structural and functional particularities of each cell type, but also the way they keep close communication, contribute to the whole NVU function (Fig. 1).

The intercellular communication consists in direct cellular contacts, including gap junction, ligand-receptor interactions and paracrine signaling (Goodenough et al., 1996), but also in direct cell-to-cell transfer of biological information through membrane vesicles, especially exosomes and microvesicles, released extracellularly from all cell types (Valadi et al., 2007) (Fig. 1). The exosomes are the smallest nano-sized (30-100 nm) vesicles that facilitate cell-to-cell communication by freely passing through cell membranes, impacting on target cells function. By unrestricted bidirectional transport through all membrane barriers as BBB, the exosomes facilitate an intricate intercellular cross-talk, both locally, within NVU microdomains, and systemically, between blood and brain (Krämer-Albers and Hill, 2016). The exosomes are currently researched for their therapeutic potential in pathologies missing an efficient treatment, like stroke, but also as biomarkers cargo (Barile and Vassalli, 2017; Zagrean et al., 2018).

The endothelial cells of the cerebral microcirculation are closely interconnected by adherend and tight junctions to form the BBB. The continuous layer of BBB endothelial cells prevents the paracellular transport and uncontrolled fluctuations in the composition of brain interstitium consecutive to changes in the blood content (Abbott, 2013). Instead, the BBB allows a highly-selective transcellular transport by membrane transport systems such as ion transfer, carrier- and receptor-mediated transport, adsorptive-mediated passage, efflux carriage, fluid-phase endocytosis (Zlokovic, 2008). The cerebral endothelium has a dynamic phenotype in response to signaling from perivascular astrocytic end-feet, pericytes, neurons and microglia. In turn, cerebral endothelial cells exert a modulatory effect on neurogenesis and neuronal cells, by secreting vascular endothelial growth factor, nerve growth factor and brain-derived neurotrophic factor (Ward and Lamanna, 2004). The cerebral capillary endothelium is in contact at its abluminal side with pericytes embedded in an extracellular matrix, further coated by astroglial end-feet processes (glia limitans perivascularis) (McArthur et al., 2016).

Within the NVU, perivascular astrocytes connect neurons and cerebral microvessels. In the gray matter, gap junctions interconnected protoplasmic astrocytes form a functional syncytium, rising the contacted synapses up to 160.000 (Cabezas et al., 2014). The astrocytic syncytium also contacts endothelial cells through gap junctions and facilitates transmission of Ca^{2+} waves to signal within NVU. Various transduction pathways are activated consecutive to Ca^{2+} signaling, impacting on cytoskeletal proteins and tight junctions (Abbott et al., 2006). Moreover, Ca^{2+} activates constitutive nitric oxide (NO) synthase that produces NO and vasodilation. In the case of inflammation, the inducible NO synthase is activated, resulting in large amounts of NO that further increase the compromised BBB permeability already altered by the inflammatory process (Kuhnline Sloan et al., 2012). Another facet of perivascular astrocytes role within NVU is the water redistribution within the interstitial fluid and perivascular space, enabled by the presence of aquaporin 4 (AQP_4) in their membrane (Abbott, 2002).

Pericytes are contractile cells surrounding and contacting the capillary endothelial cells by gap junctions (Pardridge, 1999). Through their extensive contacts in microvascular domains, pericytes enable a quick vasodilation effect in response to neuronal activation (Hall et al., 2014), thus participating in the neurovascular coupling. Also, pericytes contribute to basement membrane formation and BBB integrity (Saeed et al., 2014).

List of Symbols and Abbreviations:

neurovascular unit, NVU
blood-brain barrier, BBB
matrix metalloproteases, MMP
central nervous system, CNS
nitric oxide, NO
glial fibrillary acidic protein, GFAP
tumor necrosis factor, TNF
5-bromo-2-deoxyuridine, BrdU
vascular endothelial growth factor, VEGF||

Microglia are constantly scanning the brain environment and through the synaptic pruning, they mediate remodeling of synapses in response to physiological stimuli (Davalos et al., 2005; Nimmerjahn et al., 2005; Salter and Beggs, 2014; Schafer et al., 2012). In addition, byproducts of neuronal activity are cleaned by microglia, avoiding their detrimental accumulation in the CNS.

Active neurons can also directly signal to the endothelial cells within NVU by releasing transmitters like histamine, a process known as neurobarrier coupling. Along with increasing glucose transport into the brain, this neuron-to-endothelial cell coupling transiently modifies the permeability of tight junctions, making the BBB more permissive to larger molecules like antibodies or growth factors (Abbott et al., 2006).

Neurovascular unit in aging and disease

During aging, the NVU is subjected to structural and functional impairments that make it more vulnerable to various pathophysiologic processes, like stroke and neurodegenerative diseases (Cai et al., 2017). The aging-related cerebral changes encompass a lower microvascular density and diminished regional and global cerebral blood perfusion, accompanied by a lower metabolic rate, as confirmed by neuroimaging assessment (Marques et al., 2013).

Aging also entrains an impairment of pericytes and endothelial cells of the NVU, decreased occludin-1 and other tight junction proteins (Elahy et al., 2015), increased BBB permeability, white matter lesions alterations secondary to plasma protein leakage and low activity of the P-glycoprotein efflux transporter (Popescu et al., 2009), and endothelial oxidative stress and inflammation, accompanied by deficient memory and learning (Enciu and Popescu, 2013).

During neurologic disorders, morphologic and functional changes in the BBB and NVU entrain changes in the barrier permeability with significant implications on brain functions and cognitive status. In acute ischemic stroke, there is a BBB disruption that triggers severe consequences. An initial, still reversible phase of the BBB impairment is related to the activation of matrix metalloproteases 2 (MMP-2) and continues with a secondary irreversible disruption related to MMP-3 and MMP-9 activation (Yang and Rosenberg, 2011).

Secondary to stroke, neurons are usually lost in the infarct core. Moreover, astrocytes become reactive and proliferative, dysregulating the balance between neuronal and non-neuronal cells of the NVU in the lesioned area, especially in the aged brain. Therefore, restoring the balance between neurons and non-neuronal cells within the post-stroke perilesional area is crucial for post-stroke recovery. In addition, glial cells become reactive and proliferate, building up gliotic scars which have an initial protective role by confining the damaged area. In the long-term, however, the gliotic scar is deleterious by acting as a barrier to neural regeneration. “Melting” glial scars have been attempted for decades with little success. Alternative strategies include transforming inhibitory gliotic tissue into an environment conducive to neuronal regeneration and axonal growth. The latter idea has gained momentum following the discovery that *in vivo* direct lineage reprogramming in the adult mammalian brain is a feasible strategy for reprogramming non-neuronal cells into neurons. This exciting new technology emerged as a new approach to circumvent cell transplantation (Gresita et al., 2019; Muraoka et al., 2014; Tsunemoto et al., 2015). However, the potential of this new methodology has not been tested to improve the restoration of structure and function in the hostile environment caused by the fulminant inflammatory reaction in the brains of aged animals following stroke.

The NVU disruption in various pathologic conditions may have devastating consequences for the CNS and the organism, considering that the integrity of NVU is essential for the brain normal functioning.

Early responses of the neurovascular unit to cerebral ischemia

Overt injuries like cerebral ischemia causes the damage of the BBB and the disruption of NVU functionality. The increased fragility of aged blood vessels due to decreases in distensible components of the microvessels such as elastin (Hajdu et al., 1990) may lead, upon ischemic stress, to fragmentation of capillaries, promoting the leakage of hematogenous cells into the infarct area (Popa-Wagner et al., 2007; Wang et al., 2020) (Fig. 2A). Hypoxia-induced

vessel dilation leads to the release of pericytes (Fig. 2B), smooth muscle cells and fibroblasts from pre-existing vessels. These changes prime the vasculature for an angiogenic response by rising vascular permeability to proteases, angiogenic myeloid cells or inflammatory cytokines (Buga et al., 2014; Popa-Wagner et al., 2006). The detachment of the endothelial cells from the basal lamina is caused by hypoxia-activated matrix metalloproteases (MMPs) and perivascular microglia. Thus, endothelial cells are phagocytized during the brain blood vessel disintegration, a process that can be visualized by labelling with BrdU and pericytes markers, like prolyl 4-hydroxylase beta (Jolivel et al., 2015; Popa-Wagner et al., 2006; Sabeh et al., 2009) (Fig. 2B).

The detachment of endothelial cells from the basal lamina is paralleled by the proliferation of neuroepithelial cells having a BrdU-positive nucleus, in aged rats. Thus, at three days following the ischemic event, we detected clusters of nestin-immunopositive cells in the ischemic hemisphere of young and aged rats (Fig. 2C). Traditionally, nestin has been reported to be expressed by neuroepithelial cells during development, and by reactive astrocytes after injury (Schwab et al., 2001). The nestin-positive cells overlapped, in part, with glial fibrillary acidic protein (GFAP)-positive cells. Indeed, the vast majority of cells expressed both nestin and GFAP with a BrdU-positive nucleus, suggesting that these cells were still proliferating (Fig. 2D). We assumed that those cells displaying mixed nestin-GFAP phenotype are capillary-derived, differentiating cells that migrate and populate the infarct area, whereas GFAP-positive cells represent simply local astrocytes (Popa-Wagner et al., 2006). Nestin is regarded as a marker for neuroepithelial stem cells of mesenchymal origin (Michalczyk and Ziman, 2005). However, we have shown that after stroke nestin-positive cells arise from the capillary wall, according to the current model of vascular wall structure (Jain, 2003) (Fig. 2D). Nevertheless, the infarct core was not fully developed and delimited by thread-like, nestin-reactive processes until day 14 post-stroke.

Following hypoxic insult, stressed but viable neurons may reversibly expose the 'eat-me' signal phosphatidylserine (PS) on neuronal surface. Migrating microglia detect exposed 'eat-me' signals and engulfment of neurons or parts of neurons exposing such signals follows. This process is known as primary phagocytosis or „phagoptosis" (Fuhrmann et al., 2010; Neher

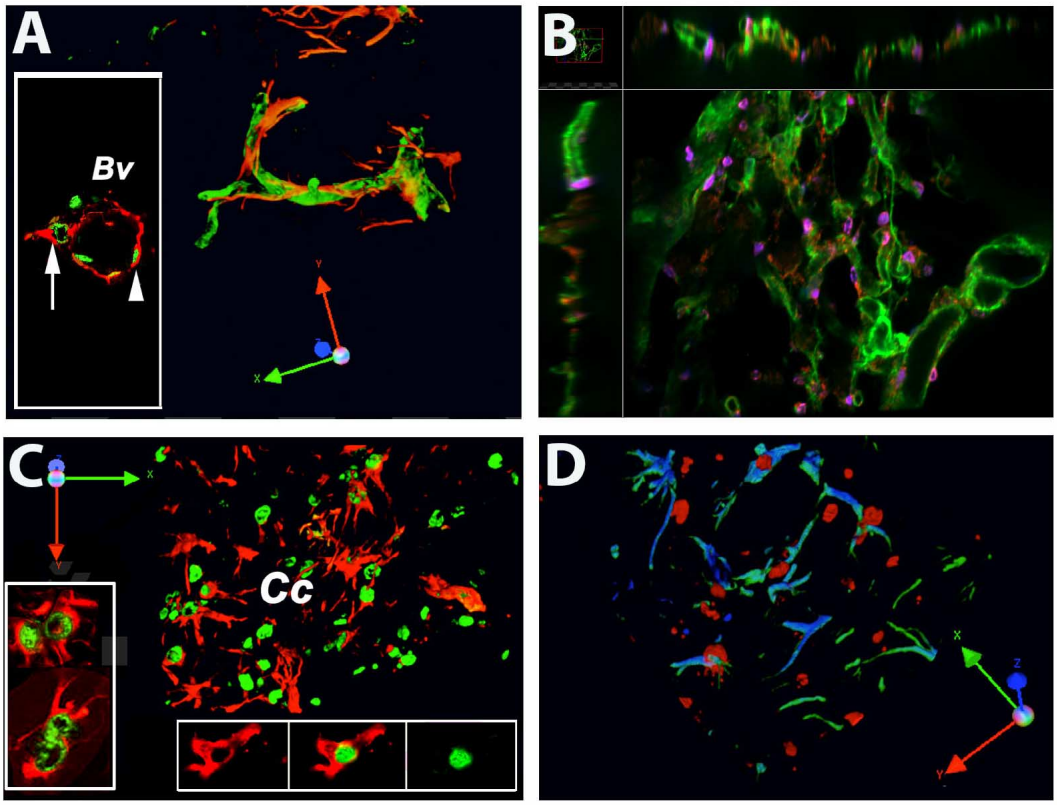

Figure 2
Early responses of the neurovascular unit to cerebral ischemia.
(A) Disintegration of brain vasculature in the hypoxic area.
(B) Detachment of endothelial cells from the vascular wall.
(C) Proliferation of neuroepithelial cells in the corpus callosum (Cc).
(D) Proliferation of nestin- and GFAP-positive cells in the peri-infarcted (PI) area.
Abbreviations:
Bv, blood vessel;
Cc, corpus callosum.

et al., 2011). Toxic neuronal insults, such as dying neurons after stroke, irreversibly expose the 'eat-me' signal recognized by primed microglia, with subsequent phagocytosis of dead neurons, known as secondary phagocytosis (Brown and Neher, 2014) (Fig. 3A). As a consequence, early events following the cortical insult include invasion of blood-born inflammatory cells at the damage site, the buildup of brain edema and activation of astrocytes, resulting in the confinement of the necrotic zone by a barrier formed of activated astrocytes, fibroblasts and a network of blood vessels, constituting the so-called scar tissue (Fig. 3B, D) (Popa-Wagner et al., 2006). Activated microglia and macrophages were shown to release the pro-inflammatory cytokine tumor necrosis factor-alpha (TNF-alpha) within the infarct area, which induces a number of pro-inflammatory changes. These, in turn, increase

Late responses: Neurovascular unit reconstruction

After the build-up of the scar, neurovascular remodeling is noted and involves multiple cellular signals, including trophic factors, guidance molecules, axonal sprouting, and matrix proteases that dissolve the necrotic environment to make a place for the emergence of new blood vessels and proliferation of astroglial cells.

Hypoxia powerfully stimulates angiogenesis. During the activation phase of angiogenesis, endothelial cells (ECs) and fibroblasts migrate into extracellular space, proliferate and release cytokines and angiogenic chemokines like CXCL1, CXCL12/CXCR4 and vascular endothelial growth factor (VEGF), which in turn activate circulating endothelial progenitor cells (EPCs), which eventually integrate into tube structures to further differentiate into mature EC.

Tissue hypoxia activates VEGF-A and VEGF-C

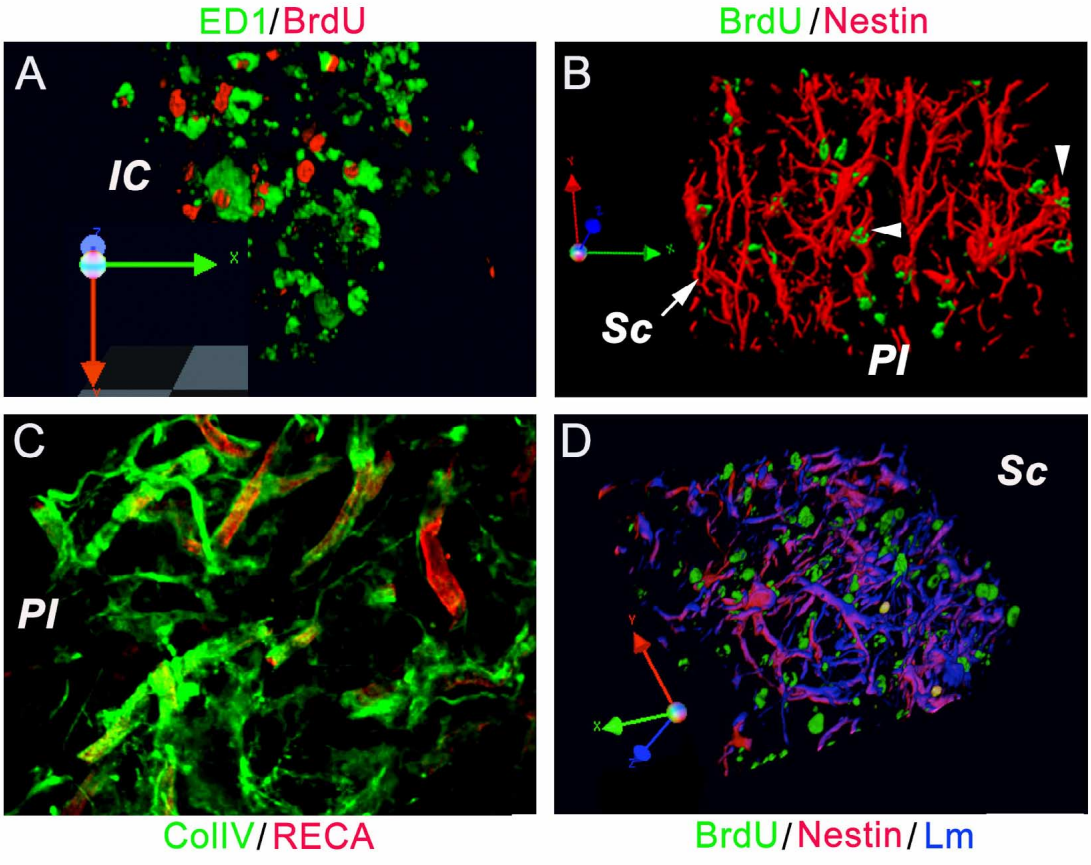

Figure 3
Late responses of the neurovascular unit to cerebral ischemia.
(A) Macrophages in the infarct core (IC) will remove dead neurons and cellular debris.
(B) Buildup of the scar tissue from proliferating endothelial cells (Popa-Wagner, 2006).
(C) Reconstruction of the basement membrane of the blood vessel in the infarcted area.
(D) The scar tissue is both nestin- and GFAP-positive suggesting the dynamic formation of this structure.
Abbreviations:
Pi, peri-infarct;
Sc, Scar;
IC, infarct core;
ED1, CD68 marker for activated microglia,
ColIV, collagen IV;
RECA, rat endothelial cell antigen;
BrdU, 5-bromo-2-deoxyuridine.

leukocyte adhesion, transendothelial migration, vascular leakage, and edema formation (Buga et al., 2014). During the early events, numerous neuronal cells are dying and are removed from the necrotic zone by brain macrophages.

expression and other angiogenic factors such as angiopoietin 2 (Angpt2), angiopoietin-like 2 (Angptl2) and 4 (Angptl4), and the endothelial and smooth muscle cell (SMC) chemoattractant Cxcl1 and its receptor Cxcr2 on EC (Buga et al., 2014). During the resolution phase, migration

and proliferation of ECs is stopped, and the basal lamina is reconstructed based on an increased expression of collagen type IV (Fig. 3C), alpha 2 (Col4a2), fibronectin 1 (Fn1), laminin gamma 1 (Lamc1), nidogen 2 (Nid2) and podoplanin 3 (Plod 3) mRNAs (Buga et al., 2014). Subsequent to proliferation of ECs and EPCs, the new cells start reorganizing into three-dimensionally capillary-like tubular structures. During vascular maturation, ANGPT2-TIE2 induces EC apoptosis and participates, along with VEGF-C and the tumor necrosis factor, member 10 (Tnfsf10) in lymphatic patterning.

The cerebral vascular system in adults is considered to be steady under normal physiological conditions. However, we have recently shown that in uninjured adult rat brain there is a continuous remodeling of the brain vasculature showed by the incorporation of BrdU into the nuclei of endothelial cells lining the lumen of existing blood vessels. Following injury, blood vessels in the remote areas relative to the infarct core and in the contralateral non-lesioned cortex, showed co-labelled BrdU/RECA+ endothelial cells shortly after the BrdU injection, which strongly suggests a bone marrow origin of the endothelial cells. Such cells probably entered the brain from the circulation via leptomeningeal blood vessels. In the peri-infarcted area, double labelling with BrdU/prolyl 4-hydroxylase beta, a marker of proliferating endothelial cells in the close proximity to collagen IV-labelled basement membrane, suggests that in addition to bone-marrow derived endothelial cells, the disintegrating vascular wall itself could also be a source of proliferating endothelial cells (Surugiu et al., 2018). In addition, the patchy distribution of newly incorporated endothelial cells in the mature blood vessels of the adult rat brain is highly suggestive of random incorporation of the new endothelial cells into the mature cerebral blood vessels (Surugiu et al., 2018).

The newly formed neurovascular unit is imbalanced

Unlike angiogenesis which is initiated early after stroke, the post-stroke NVU consists of numerous oligodendroglia, astrocytes and activated microglia embedded in a disorganized network of blood vessels. Neurogenesis is a rare event in the damaged cortical areas located far from the neurogenic zones - the subventricular zone and the hippocampus. Occasionally, axonal sprouting from the surviving neurons is noted. It seems that only the damaged area nearby the subventricular zone, including the striatum, becomes populated with neurons migrating from the adjacent neurogenic zone. In view of the complexity of these systems, the development of neurorestorative therapies is a true challenge, in which age aspects carefully need to be considered (Hermann et al., 2015; Hermann and Chopp, 2012).

Conclusions

An intact NVU is essential for the normal functioning of the CNS. Therefore, NVU disruption may have devastating consequences for the CNS and the organism. Overt injuries like cerebral ischemia cause the damage of the BBB and the disruption of NVU functionality. Early responses of the NVU to chronic hypoxia include neutrophils infiltration, brain edema, blood vessel disintegration, astrocytes, and endothelial cell proliferation, as well as the conversion of the resident microglia to phagocytic microglia. Later responses include the confinement of the peri-infarcted region by a scar tissue composed mainly of reactive astrocytes and endothelial cells, and angiogenesis. The newly formed capillary network is, however, disorganized and the blood vessels are leaky, making a successful regeneration of the damaged area, unlikely.

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Competing interests/conflict of interests

The authors declare no conflict of interest.

References

Abbott AJ (2013) Blood-brain barrier structure and function and the challenges for CNS drug delivery. J Inherit Metab Dis. 36: 437–449. https://doi.org/10.1007/s10545-013-9608-0

Abbott NJ (2004) Evidence for bulk flow of brain interstitial fluid: significance for physiology and pathology. Neurochem Int. 4: 545–552. https://doi.org/10.1016/j.neuint.2003.11.006

Abbott NJ (2002) Astrocyte–endothelial interactions and blood–brain barrier permeability. J Anat .200: 629–638. https://doi.org/10.1046/j.1469-7580.2002.00064.x

Abbott NJ, Friedman A (2012) Overview and introduction: the blood-brain barrier in health and disease. Epilepsia. 53 Suppl 6: 1–6. https://doi.org/10.1111/j.1528-1167.2012.03696.x

Abbott NJ, Rönnbäck L, Hansson E (2006) Astrocyte-endothelial interactions at the blood-brain barrier. Nat Rev Neurosci 7: 41–53. https://doi.org/10.1038/nrn1824

Attwell D, Buchan AM, Charpak S, et al (2010). Glial and neuronal control of brain blood flow. Nature 468: 232–243. https://doi.org/10.1038/nature09613

Attwell D, Mishra A, Hall CN, et al (2015). What is a pericyte?: Journal of Cerebral Blood Flow & Metabolism. https://doi.org/10.1177/0271678X15610340

Barile L, Vassalli G (2017). Exosomes: Therapy delivery tools and biomarkers of diseases. Pharmacol Ther. 174: 63–78. https://doi.org/10.1016/j.pharmthera.2017.02.020

Brown GC, Neher JJ (2014). Microglial phagocytosis of live neurons. Nat Rev Neurosci. 15: 209–216. https://doi.org/10.1038/nrn3710

Buga AM, Margaritescu C, Scholz CJ, et al (2014). Transcriptomics of post-stroke angiogenesis in the aged brain. Front Aging Neurosci. 6: 44. https://doi.org/10.3389/fnagi.2014.00044

Cabezas R, Avila M, Gonzalez J, et al (2014). Astrocytic modulation of blood brain barrier: perspectives on Parkinson's disease. Front Cell Neurosci. 8: 211. https://doi.org/10.3389/fncel.2014.00211

Cai W, Zhang K, Li P, et al (2017) Dysfunction of the neurovascular unit in ischemic stroke and neurodegenerative diseases: An aging effect. Ageing Res Rev. 34: 77–87. https://doi.org/10.1016/j.arr.2016.09.006

Davalos D, Grutzendler J, Yang G, et al (2005) ATP mediates rapid microglial response to local brain injury in vivo. Nature Neuroscience. 8: 752–758. https://doi.org/10.1038/nn1472

Elahy M, Jackaman C, Mamo JC, et al (2015) Blood-brain barrier dysfunction developed during normal aging is associated with inflammation and loss of tight junctions but not with leukocyte recruitment. Immun Ageing. 12: 2. https://doi.org/10.1186/s12979-015-0029-9

Enciu A-M, Popescu BO (2013) Is there a causal link between inflammation and dementia? Biomed Res Int. 2013: 316495. https://doi.org/10.1155/2013/316495

Fuhrmann M, Bittner T, Jung CKE, et al (2010) Microglial Cx3cr1 knockout prevents neuron loss in a mouse model of Alzheimer's disease. Nat Neurosci. 13:411–413. https://doi.org/10.1038/nn.2511

Goodenough DA, Goliger JA, Paul DL (1996) Connexins, connexons, and intercellular communication. Annu Rev Biochem. 65: 475–502. https://doi.org/10.1146/annurev.bi.65.070196.002355

Gresita A, Glavan D, Udristoiu I, et al (2019) Very Low Efficiency of Direct Reprogramming of Astrocytes Into Neurons in the Brains of Young and Aged Mice After Cerebral Ischemia. Front Aging Neurosci. 11: 334. https://doi.org/10.3389/fnagi.2019.00334

Hajdu MA, Heistad DD, Siems JE, et al (1990) Effects of aging on mechanics and composition of cerebral arterioles in rats. Circ Res. 66: 1747–1754. https://doi.org/10.1161/01.res.66.6.1747

Hall CN, Reynell C, Gesslein B, et al (2014) Capillary pericytes regulate cerebral blood flow in health and disease. Nature. 508:55–60. https://doi.org/10.1038/nature13165

Hermann DM, Buga A-M, Popa-Wagner A (2015) Neurovascular remodeling in the aged ischemic brain. J Neural Transm (Vienna). 122 Suppl 1: S25-33. https://doi.org/10.1007/s00702-013-1148-0

Hermann DM, Chopp M (2012) Promoting brain remodelling and plasticity for stroke recovery: therapeutic promise and potential pitfalls of clinical translation. Lancet Neurol. 11: 369–380. https://doi.org/10.1016/S1474-4422(12)70039-X

Jain RK (2003) Molecular regulation of vessel maturation. Nature Medicine. 9: 685–693. https://doi.org/10.1038/nm0603-685

Jolivel V, Bicker F, Binamé F, et al (2015) Perivascular microglia promote blood vessel disintegration in the ischemic penumbra. Acta Neuropathol. 129: 279–295. https://doi.org/10.1007/s00401-014-1372-1

Krämer-Albers E-M, Hill AF (2016) Extracellular vesicles: interneural shuttles of complex messages. Curr Opin Neurobiol. 39: 101–107. https://doi.org/10.1016/j.conb.2016.04.016

Kuhnline Sloan CD, Nandi P, Linz TH, et al (2012) Analytical and biological methods for probing the blood-brain barrier. Annu Rev Anal Chem (Palo Alto Calif) 5: 505–531. https://doi.org/10.1146/annurev-anchem-062011-143002

Marques F, Sousa JC, Sousa N, et al (2013) Blood-brain-barriers in aging and in Alzheimer's disease. Mol Neurodegener. 8: 38. https://doi.org/10.1186/1750-1326-8-38

McArthur S, Loiola RA, Maggioli E, et al (2016) The restorative role of annexin A1 at the blood-brain barrier. Fluids Barriers CNS. 13: 17. https://doi.org/10.1186/s12987-016-0043-0

Michalczyk K, Ziman M (2005) Nestin structure and predicted function in cellular cytoskeletal organisation. Histol Histopathol. 20: 665–671. https://doi.org/10.14670/HH-20.665

Muraoka N, Yamakawa H, Miyamoto K, et al (2014) MiR-133 promotes cardiac reprogramming by directly repressing Snai1 and silencing fibroblast signatures. EMBO J. 33: 1565–1581. https://doi.org/10.15252/embj.201387605

Neher JJ, Neniskyte U, Zhao J-W, et al (2011) Inhibition of microglial phagocytosis is sufficient to prevent inflammatory neuronal death. J Immunol. 186: 4973–4983. https://doi.org/10.4049/jimmunol.1003600

Nimmerjahn A, Kirchhoff F, Helmchen F (2005) Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science. 308:1314–1318. https://doi.org/10.1126/science.1110647

Pardridge WM (1999). Blood-brain barrier biology and methodology. J Neurovirol. 5: 556–569. https://doi.org/10.3109/13550289909021285

Popa-Wagner A, Badan I, Walker L, et al (2007) Accelerated infarct development, cytogenesis and apoptosis following transient cerebral ischemia in aged rats. Acta Neuropathol. 113: 277–293. https://doi.org/10.1007/s00401-006-0164-7

Popa-Wagner A, Dinca I, Yalikun S, et al (2006) Accelerated delimitation of the infarct zone by capillary-derived nestin-positive cells in aged rats. Curr Neurovasc Res. 3: 3–13. https://doi.org/10.2174/156720206775541732

Popescu BO, Toescu EC, Popescu LM, et al (2009) Blood-brain barrier alterations in ageing and dementia. J Neurol Sci. 283: 99–106. https://doi.org/10.1016/j.jns.2009.02.321

Sabeh F, Li X-Y, Saunders TL, Rowe RG, et al (2009) Secreted versus membrane-anchored collagenases: relative roles in fibroblast-dependent collagenolysis and invasion. J Biol Chem 284: 23001–23011. https://doi.org/10.1074/jbc.M109.002808

Saeed AA, Genové G, Li T, et al (2014) Effects of a Disrupted Blood-Brain Barrier on Cholesterol Homeostasis in the Brain. J Biol Chem. 289:23712–23722. https://doi.org/10.1074/jbc.M114.556159

Salter MW, Beggs S (2014). Sublime Microglia: Expanding Roles for the Guardians of the CNS. Cell. 158: 15–24. https://doi.org/10.1016/j.cell.2014.06.008

Schafer DP, Lehrman EK, Kautzman AG, et al (2012) Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner. Neuron. 74: 691–705. https://doi.org/10.1016/j.neuron.2012.03.026

Schwab JM, Beschorner R, Nguyen TD, et al (2001) Differential cellular accumulation of connective tissue growth factor defines a subset of reactive astrocytes, invading fibroblasts, and endothelial cells following central nervous system injury in rats and humans. J Neurotrauma. 18: 377–388. https://doi.org/10.1089/089771501750970930

Surugiu R, Glavan D, Popescu M, et al (2018) Vasculature Remodeling in a Rat Model of Cerebral Ischemia. The Fate of the BrdU-Labeled Cells Prior to Stroke. Front Neurol. 9: 1014. https://doi.org/10.3389/fneur.2018.01014

Tsunemoto RK, Eade KT, Blanchard JW, et al (2015) Forward engineering neuronal diversity using direct reprogramming. EMBO J. 34: 1445–1455. https://doi.org/10.15252/embj.201591402

Valadi H, Ekström K, Bossios A, et al (2007) Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nature Cell Biology. 9: 654–659. https://doi.org/10.1038/ncb1596

Wang C, Börger V, Sardari M, et al (2020) Mesenchymal Stromal Cell-Derived Small Extracellular Vesicles Induce Ischemic Neuroprotection by Modulating Leukocytes and Specifically Neutrophils. Stroke. 51: 1825–1834. https://doi.org/10.1161/STROKEAHA.119.028012

Ward NL, Lamanna JC (2004) The neurovascular unit and its growth factors: coordinated response in the vascular and nervous systems. Neurol Res. 26: 870–883. https://doi.org/10.1179/016164104X3798

Yang Y, Rosenberg GA (2011) Blood-brain barrier breakdown in acute and chronic cerebrovascular disease. Stroke. 42: 3323–3328. https://doi.org/10.1161/STROKEAHA.110.608257

Zagrean A-M, Hermann DM, Opris I, et al (2018) Multicellular Crosstalk Between Exosomes and the Neurovascular Unit After Cerebral Ischemia. Therapeutic Implications. Front Neurosci. 12. https://doi.org/10.3389/fnins.2018.00811

Zagrean A-M, Ianosi B, Sonea C, et al (2017) Blood-Brain Barrier and Cognitive Function. Book chapter in "The Physics of the Mind and Brain Disorders. Integrated Neural Circuits Supporting the Emergence of Mind". Eds I. Opris, M.F. Casanova. Part of the Springer Series in Cognitive and Neural Systems book series (SSCNS, volume 11). Springer International Publishing Switzerland p 713-740, ISBN: 2363-9105.

Zlokovic BV (2008). The blood-brain barrier in health and chronic neurodegenerative disorders. Neuron. 57: 178–201. https://doi.org/10.1016/j.neuron.2008.01.003

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