ANNUAL REVIEWS

Annual Review of Neuroscience

Astrocyte Endfeet in Brain Function and Pathology: Open Questions

Blanca Díaz-Castro,[1,*] Stefanie Robel,[2,*] and Anusha Mishra[3,*]

[1]UK Dementia Research Institute and Centre for Discovery Brain Sciences, University of Edinburgh, Edinburgh, Scotland, UK; email: B.Diaz-Castro@ed.ac.uk

[2]Department of Cell, Developmental and Integrative Biology, University of Alabama at Birmingham, Birmingham, Alabama, USA; email: srobel@uab.edu

[3]Department of Neurology Jungers Center for Neurosciences Research and Knight Cardiovascular Institute, Oregon Health & Science University, Portland, Oregon, USA; email: mishraa@ohsu.edu

Annu. Rev. Neurosci. 2023. 46:101–21

First published as a Review in Advance on February 28, 2023

The *Annual Review of Neuroscience* is online at neuro.annualreviews.org

https://doi.org/10.1146/annurev-neuro-091922-031205

*These authors contributed equally to this article

Keywords

vasculature, astrocyte, endfoot, blood-brain barrier, cerebral blood flow, neurovascular coupling

Abstract

Astrocyte endfeet enwrap the entire vascular tree within the central nervous system, where they perform important functions in regulating the blood-brain barrier (BBB), cerebral blood flow, nutrient uptake, and waste clearance. Accordingly, astrocyte endfeet contain specialized organelles and proteins, including local protein translation machinery and highly organized scaffold proteins, which anchor channels, transporters, receptors, and enzymes critical for astrocyte-vascular interactions. Many neurological diseases are characterized by the loss of polarization of specific endfoot proteins, vascular dysregulation, BBB disruption, altered waste clearance, or, in extreme cases, loss of endfoot coverage. A role for astrocyte endfeet has been demonstrated or postulated in many of these conditions. This review provides an overview of the development, composition, function, and pathological changes of astrocyte endfeet and highlights the gaps in our knowledge that future research should address.

Contents

INTRODUCTION

Astrocytes are glial cells that tile the brain. They enwrap the entire central nervous system (CNS) vascular tree with subcellular structures called endfeet, while their cell body and thousands of fine branches permeate the brain parenchyma and interact with other neural cells to aid brain homeostasis, behavior, and defense responses (Allen & Eroglu 2017, Nagai et al. 2021) (**Figure 1*a–c***). Astrocyte endfeet mediate the communication between brain cells and the vasculature as part of the neurogliovascular unit (Iadecola 2017). Endfeet are also an important component of the blood-brain barrier (BBB), together with brain endothelial cells, vascular smooth muscle cells (VSMCs), pericytes, and the vascular basement membrane (Sweeney et al. 2019) (**Figure 1*c*,*d***).

Studies in the nineteenth century revealed that astrocytes extend large endfoot processes to cover the vasculature, leading Camillo Golgi to hypothesize that they deliver nutrients to neurons and Ernesto Lugaro to propose that they remove the toxic waste products of neuronal metabolism. Santiago Ramón y Cajal further suggested that astrocyte endfoot contraction may dilate blood vessels and that this regulation may underlie attention and sleep-wake cycles (Antonakou & Triarhou 2019, García-Marín et al. 2007, Ramón y Cajal 1895). In the twentieth century, astrocytes were largely ignored due to technical challenges in studying their physiology. Recent development of calcium indicator dyes and genetic manipulation methods has revived interest in astrocytes and confirmed that many astrocyte functions proposed by early neuroscientists hold true. Here, we provide a critical review of current knowledge about the structure, molecular makeup, and function of astrocyte endfeet in the CNS over the life span and in disease. We also highlight fascinating questions about astrocyte biology that remain unanswered, as they may offer fruitful directions for future research.

VASCULATURE AND ASTROCYTE ENDFOOT DEVELOPMENT

Astrocytes are one of the last cell types to be generated in the CNS. After asymmetric division of radial glia, progenitors destined to become astrocytes detach from the ependymal surface and

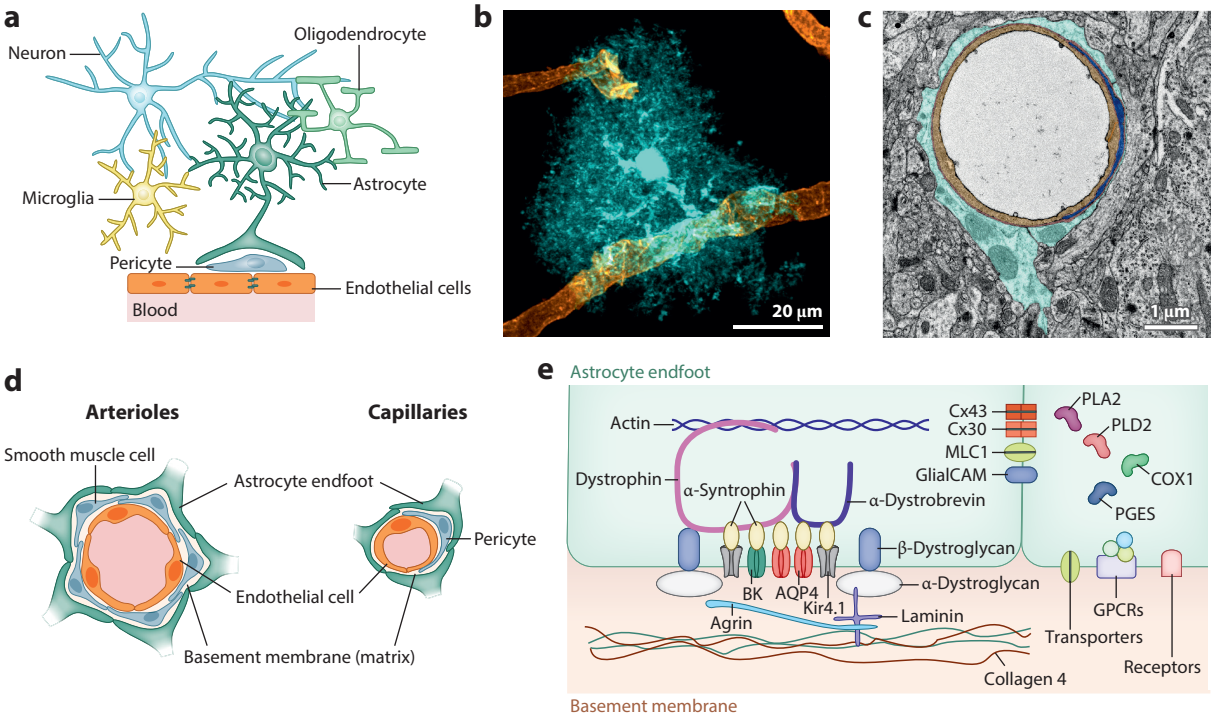

Figure 1

Astrocyte endfoot morphology and molecular composition. (*a*) Schematic representing the known astrocyte interactions with other CNS cell types. (*b*) Confocal image of an astrocyte (*teal*) interacting with brain capillaries (*orange*). Astrocyte endfeet can be seen surrounding the blood vessels. (*c*) Transmitted electron microscopy image of a cross-section of a brain capillary. Brain endothelial cells (*orange*) form the vessel wall. They are partially surrounded by a pericyte (*blue*). Astrocyte endfeet (*teal*) enwrap the vessel. (*d*) Schematic of the BBB components in cross-sections of an arteriole and a capillary. (*e*) Schematic representing a subset of known astrocyte endfoot proteins. Abbreviations: AQP4, aquaporin 4; BK, large-conductance Ca^{2+}-dependent K^+ channel; COX1, cyclooxygenase 1; Cx, Connexin; GPCR, G protein–coupled receptor; Kir4.1, inward-rectifying K^+ channel 4.1; MLC1, megalencephalic leukoencephalopathy with subcortical cysts 1; PGES, prostaglandin E2 synthase; PLA2, phospholipase A2; PLD2, phospholipase D2. Microscopy images in panels *b* and *c* provided by Austėja Čiulkinytė and Isabel Bravo-Ferrer, respectively.

migrate into the cortex. In rodents, astrocyte proliferation occurs mostly within the week before and after birth, followed by differentiation and maturation over the next few weeks, when they establish contact with the vasculature and other brain cells (Clavreul et al. 2019, Ge et al. 2012, Tien et al. 2012) (**Figure 2**). Human astrocyte proliferation follows a similar course, starting prenatally and continuing postnatally until the entire brain is tiled and the vasculature is encapsulated with endfeet (de Majo et al. 2020).

Large cerebral vessels grow into the human and rodent brain in utero (Coelho-Santos & Shih 2020, Marín-Padilla 2012, Streeter 1918). Angiogenesis continues postnatally to generate capillaries and new penetrating vessels, as necessary, to form the mature cerebrovascular network, paralleling cortical expansion (Coelho-Santos & Shih 2020, Marín-Padilla 2012, Tata et al. 2015, Wälchli et al. 2015). In rodents, most of the capillary bed forms between postnatal day (P)8 and P10 (Coelho-Santos et al. 2021, Harik et al. 1993) (**Figure 2**). This period concurs with peak astrocyte proliferation and endfoot formation (Bushong et al. 2004, Clavreul et al. 2019, Farmer et al. 2016, Ge et al. 2012, Lunde et al. 2015), after which astrocytes and the vasculature mature together through weaning (P21) (de Majo et al. 2020, Ge et al. 2012, Lunde et al. 2015,

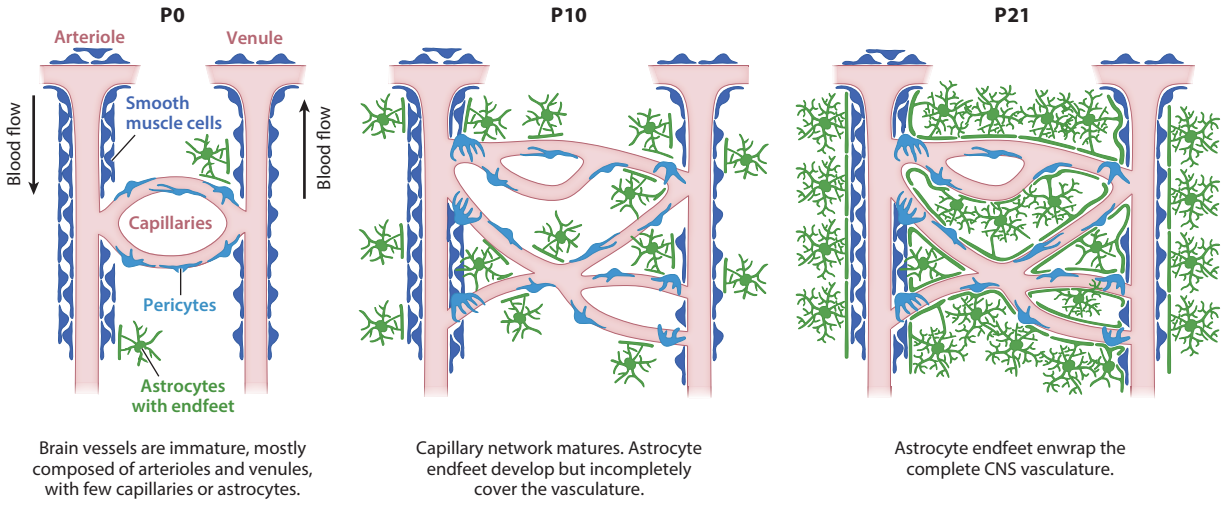

Figure 2

Development of the astrocyte-vascular interface in rodents. A rudimentary network of arterioles and venules, connected by a few capillaries, exists in the rodent brain at birth (P0, *left*). At this time, astrocyte differentiation has just begun; there are only a few astrocytes with immature morphology, and endfeet are largely absent. Peak postnatal angiogenesis occurs between P8 and P10 (*center*), when the mature cerebral capillary network develops, occurring around the same time as peak astrocyte proliferation. Endfeet formation is actively occurring during this time, but the coverage is still incomplete. By P21 (*right*), the vascular structure and astrocyte morphology have reached adult proportions, with endfeet covering essentially the entire vascular tree. The signals that initiate endfoot-vascular interactions during development and govern its maintenance in adulthood are largely unknown. Abbreviations: CNS, central nervous system; P, postnatal day.

Stackhouse & Mishra 2021) (**Figure 2**). Indeed, astrocyte and vascular development are suggested to be inseparable (Marín-Padilla 2012), and mouse studies show that inhibiting astrogenesis disrupts cerebrovascular patterning (Ma et al. 2012, O'Sullivan et al. 2017). As new vessels infiltrate the brain, the extracellular matrix proteins secreted by vascular endothelial cells and astrocyte endfeet form the double-layered basement membrane that encases VSMCs along larger vessels, whereas they merge to form a unified layer encasing pericytes around capillaries (Ma et al. 2012) (**Figure 1*d***).

Structural development of the gliovascular interface is accompanied by endfeet polarization to contain the proteins necessary for astrocyte-vascular interactions (Clavreul et al. 2019, Ge et al. 2012, Lunde et al. 2015) (**Figure 2**). Endfoot expression of aquaporin 4 (AQP4), glucose transporter (GLUT-1), and the inward-rectifying K^+ channel Kir4.1 gradually increases (Daneman et al. 2010, Harik et al. 1993, Seifert et al. 2009). Endfoot maturation also correlates with increased endothelial BBB proteins such as claudin-5 (Gilbert et al. 2019). The signaling pathways that govern the development and maintenance of endfoot-vascular interactions remain largely unknown and warrant a thorough investigation.

ASTROCYTE ENDFOOT MORPHOLOGY AND COMPOSITION

Morphology

Astrocytes have remarkably complex morphology. They possess 5–12 primary branches that arise from the cell body and ramify into thinner branches, leaflets, and endfeet (Chai et al. 2017, Diaz-Castro et al. 2021). Despite morphological differences between brain regions, virtually every

astrocyte in the gray matter contacts one to six blood vessels (Hösli et al. 2022). The cerebrovascular tree—encompassing arterioles, capillaries, and venules—is near-completely wrapped by endfeet that tile the vessel wall, with the endfoot size decreasing as the vessels get smaller (Ramón y Cajal 1913, Wang et al. 2021). Endfeet cover 70–100% of the vascular perimeter, isolating the brain parenchyma from the vasculature (Bertossi et al. 1997, Mathiisen et al. 2010, Mugnaini & Walberg 1965) (**Figure 1*c***). Ablating an astrocyte endfoot leads to rapid replacement within minutes (Kubotera et al. 2019, Mills et al. 2022), and, if endfoot replacement fails, BBB damage ensues (Heithoff et al. 2021). These findings highlight the importance of astrocyte endfeet for brain function.

Composition

Astrocyte endfeet contain mitochondria, rough endoplasmic reticulum, and vesicles (Bertossi et al. 1997, Boulay et al. 2017, Göbel et al. 2020, Mathiisen et al. 2010). Endfeet also enclose microtubules, glycogen granules, and bundles of intermediate filaments composed of glial fibrillary acidic protein (GFAP) (Bertossi et al. 1997, Kacem et al. 1998). Although we lack a complete description of the endfoot proteome, we know it encompasses protein translation machinery, metabolic enzymes, adhesion proteins, and scaffold proteins, which interact with channels, transporters, and receptors that are important for astrocyte-vasculature interactions.

One widely studied endfoot protein is AQP4, an astrocytic water channel enriched in endfeet where it forms multiprotein orthogonal arrays (Landis & Reese 1974, Rash et al. 1998). AQP4 is anchored to the plasma membrane through a scaffolding protein complex called dystrophin-associated protein complex (DAPC) (Culligan & Ohlendieck 2002). DAPC in endfeet comprises dystrophin Dp71, α-dystrobrevin-1, α1-syntrophin, and β- and α-dystroglycan (Belmaati Cherkaoui et al. 2021, Culligan & Ohlendieck 2002, Heuser et al. 2012) (**Figure 1*e***). DAPC anchors the endfoot to laminin and agrin in the basement membrane (Gee et al. 1994), while also forming a scaffold for proteins like AQP4 (Hoddevik et al. 2017, Neely et al. 2001) and the K^+ channel Kir4.1 (Connors et al. 2004) on the endfoot plasma membrane.

Some proteins are enriched at the contact points between astrocyte endfeet (**Figure 1*e***). These include the gap junction proteins Connexin (Cx)43 and Cx30, which may be important in shuttling metabolic substrates throughout the astrocyte network to facilitate delivery to neurons (Landis & Reese 1974, Rouach et al. 2008, Simard et al. 2003). Astrocyte endfoot contact points in mouse and human also contain a multiprotein complex that includes megalencephalic leukoencephalopathy with subcortical cysts 1 (MLC1, a protein of unknown function), GlialCAM (also called HepaCAM, hepatocyte cell adhesion molecule), chloride channel protein 2 (ClC-2, a voltage-gated chloride channel), and zona occludens protein 1 (ZO-1, a tight junction protein) (Gilbert et al. 2019, Hoegg-Beiler et al. 2014, Teijido et al. 2004). Mutations in MLC1 and GlialCAM cause a developmental disease called megalencephalic leukoencephalopathy with subcortical cysts (Leegwater et al. 2001, López-Hernández et al. 2011), in which gliovascular functions are dysregulated (Gilbert et al. 2021), suggesting important roles for these proteins at the endfoot, although the exact nature of their functions remains unclear.

Astrocyte endfeet also express proteins related to blood flow regulation (**Figure 1*e***). They contain phospholipase A2 (PLA2), phospholipase D2 (PLD2), cyclooxygenase 1 (COX1), and prostaglandin E2 synthase (PGES), which synthesize the vasodilatory agent PGE_2 (Gordon et al. 2008, Mishra et al. 2016, Takano et al. 2006), and the cytochrome P450 ω-hydroxylase 4A (CYP4A), which synthesizes vasoconstrictive 20-hydroxyeicosatetraenoic acid (20-HETE) (Gonzalez-Fernandez et al. 2020). Cytochrome P450 2C11 (CYP2C11) epoxygenase produces epoxyeicosatrienoic acids (EETs) and is strongly expressed in endfeet on arterioles to induce

vasodilation (Peng et al. 2002) but is completely absent in endfeet on capillaries (Mishra et al. 2016), suggesting possible vascular segment-specific heterogeneity.

The discovery of endfoot proteins has thus far been serendipitous and limited, but it is now being expanded by omics technologies. Recently, translating ribosome affinity purification was used to identify more than 600 locally translated endfoot genes related to immunity, matrix and cell adhesion, and vascular function (Boulay et al. 2017). Local translation may allow astrocyte endfeet to quickly respond to the vasculature, but whether all endfoot proteins are translated locally remains unknown. Proteomic analysis of biochemically isolated endfeet indicates that many endfoot proteins are part of the mitochondrial electron transport chain (Stokum et al. 2021). However, the difficulty of isolating pure endfeet limits the interpretation of these data. Co-immunoprecipitation assays have also identified transporters, ion channels, adhesion and trafficking molecules, and G protein–coupled receptors that interact with GlialCAM and MLC1 at the endfeet (Alonso-Gardón et al. 2021). What else is present at the endfoot, and what do these proteins do there? New subcompartment-specific proteomics tools may help answer these questions.

ASTROCYTE ENDFOOT FUNCTIONS

Blood-Brain Barrier Development and Maintenance

Astrocytes are thought to help maintain the BBB (**Table 1**). Early studies found that transplanting glial progenitors to peripheral tissues conferred BBB properties to those vessels and prevented tracer leakage (Janzer & Raff 1987). Coculture experiments also showed that astrocytes induce BBB properties in brain endothelial cells (Beck et al. 1986, Tao-Cheng et al. 1987), reflected by increased expression of tight junction proteins and transendothelial electrical resistance. Because astrocyte-conditioned medium was sufficient to induce BBB features in endothelial cell cultures (Canfield et al. 2017, Shivers et al. 1988, Siddharthan et al. 2007), astrocyte-derived secreted factors were proposed to drive BBB formation and maintenance.

However, BBB initiation, maturation, and maintenance may be distinctly regulated processes. During early embryonic development, the BBB is established before astrocytes are differentiated, suggesting that other cells, such as pericytes, likely play a dominant role (Daneman et al. 2010).

Table 1 Astrocyte endfoot functions in health

Area	Astrocyte endfoot functions
Blood-brain barrier	Basement membrane production Development? Maturation? Maintenance
Blood flow regulation	Resting vascular tone Autoregulation Vascular reactivity (CO_2) Neurovascular coupling
Nutrient uptake	Glucose Hormones Lipids Amino acids
Brain waste clearance	Transcellular Glymphatic system Intramural periarterial drainage

Question marks denote hypothesized functions.

However, secreted factors from radial glia (astrocyte-like stem cells) may act on pericytes and/or endothelial cells during BBB formation; this possibility remains unresolved. Furthermore, most of the brain's capillary bed is generated postnatally (Coelho-Santos et al. 2021), when astrocyte endfeet start engaging with the vasculature (Bushong et al. 2004), and may contribute to BBB formation. Conversely, pericytes may also induce endfoot properties in astrocyte processes that reach the vasculature. Pericyte-deficient mice exhibit reduced endfoot expression of α-syntrophin, AQP4, and laminin α2 (Armulik et al. 2010). Astrocytes, pericytes, and endothelial cells may act in concert to induce BBB properties.

Astrocyte ablation in the spinal cord reduces expression of the tight junction protein occludin (Schreiner et al. 2015), indicative of a BBB compromise. However, substantial BBB damage was not observed based on erythrocyte or fibrinogen extravasation into the parenchyma (Schreiner et al. 2015, Tsai et al. 2012). These findings are somewhat inconclusive, as many smaller blood-borne solutes (ions, lipids, small proteins) may still enter the brain following less pronounced BBB disruptions. Live in vivo imaging during and after the ablation of single astrocytes or endfeet has revealed remarkable plasticity, whereby neighboring astrocytes quickly rescue the exposed vessel surface with new endfeet (Kubotera et al. 2019, Mills et al. 2022). No leakage of large tracers was observed in these studies, suggesting no immediate loss of the BBB. In contrast, simultaneously ablating several astrocytes induced BBB damage associated with discontinuous endothelial tight junctions and irregular basement membrane (Heithoff et al. 2021). Interestingly, ablating single pericytes also has a minimal effect on the BBB (Berthiaume et al. 2018). Perhaps astrocytes and pericytes play redundant roles in BBB maintenance, and damage becomes salient only when a large-scale loss of either cell type occurs.

In summary, astrocytes appear necessary for BBB maintenance, but the underlying mechanisms remain unknown. Do astrocytes release molecules that act on endothelial cells directly and, if so, which ones? Or, do astrocytes exert their functions by interacting with other cellular players, such as pericytes? Although BBB damage is often examined as an increase in nonspecific permeability between the blood and the brain, it could also encompass disruptions in specific transport mechanisms, such that nutrient delivery to the brain or toxic waste clearance is hampered. Future studies should consider these possibilities.

Blood Flow Regulation

Astrocytes are ideally positioned to regulate neurovascular coupling (NVC) (**Figure 1*a–d***). They detect neuronal activity with their fine parenchymal processes and release vasoactive molecules onto cerebral vessels via endfeet to change vascular diameter and thus control blood flow (Gordon et al. 2008, Lind et al. 2018, Metea & Newman 2006, Mishra et al. 2016, Paquette et al. 2021, Peng et al. 2002, Zonta et al. 2003) (**Table 1**). When astrocytes sense neuronal activity, they synthesize arachidonic acid (Stella et al. 1994) and metabolize it into various vasoactive molecules. Astrocyte endfeet express all necessary enzymes to synthesize PGE2, which acts on EP4 receptors on VSMCs and pericytes to dilate arterioles and capillaries, respectively (Gordon et al. 2008, Mishra et al. 2016, Zonta et al. 2003). Astrocyte endfeet on arterioles can also release EETs to dilate them (Gebremedhin et al. 1992, Metea & Newman 2006), but owing to a lack of epoxygenase expression in capillary endfeet, EETs do not affect these small vessels (Mishra et al. 2016). Astrocytes also produce 20-HETE, which constricts arterioles (Metea & Newman 2006, Mulligan & MacVicar 2004) and capillaries (Hall et al. 2014), likely via G protein–coupled receptor 75 (GPR75) or GPR39 on VSMCs and pericytes (Garcia et al. 2017, Gonzalez-Fernandez et al. 2020, Methner et al. 2021). Astrocytes may also control NVC via K^+ regulation. During neuronal activity, astrocytes take up extracellular K^+ via Kir4.1 channels, which are abundantly expressed on their fine processes and

endfeet (Djukic et al. 2007). Initially, astrocytes were thought to siphon K^+ from perisynaptic to perivascular spaces during activity, leading to vessel dilation (Paulson & Newman 1987), but this hypothesis was not experimentally supported (Metea et al. 2007). However, an alternate mechanism involving K^+ release via large-conductance Ca^{2+}-dependent K^+ (BK) channels on their endfeet was later shown to dilate vessels in an astrocytic Ca^{2+}–dependent manner (Filosa et al. 2006).

Despite this evidence, the role of astrocytes in NVC remains controversial, as astrocyte Ca^{2+} signals are often observed to be too small or slow (Del Franco et al. 2022, Nizar et al. 2013). However, endfeet Ca^{2+} transients are reliably detected during intense or sustained neuronal activity, when they contribute to NVC (Institoris et al. 2015), and astrocyte recruitment enhances hemodynamic responses in vivo (Gu et al. 2018, Schulz et al. 2012). It is also possible that very small Ca^{2+} signals, below the detection threshold of current technologies, are enough to trigger vasoactive signals, as preventing fast Ca^{2+} signals in astrocytes dampens NVC (Mishra et al. 2016). Activity-dependent Ca^{2+} increases located in astrocyte fine processes may also suffice to influence second messengers that stimulate vasoactive signal release from endfeet.

Astrocytes control brain blood flow in several ways besides NVC. They regulate the resting diameter of vessels via ATP-dependent constriction (Kur & Newman 2014) and prostaglandin-dependent dilation (Rosenegger et al. 2015). In response to increases in blood pressure, astrocytes signal to vessels to increase vascular tone (Kim et al. 2015). Modulating resting tone may allow astrocyte endfeet to determine the polarity and magnitude of NVC (Blanco et al. 2008). Astrocytes also participate in hemo-neural or vasculo-neuronal coupling by detecting changes in blood flow and signaling in the reverse direction to alter neuronal firing (Kim et al. 2016, Moore & Cao 2008). This mechanism may modulate neuronal activity depending on metabolic supply. Furthermore, brainstem and hypothalamic astrocytes modulate the output activity of local neurons to alter peripheral organ function in response to changes in blood CO_2 levels or osmolality, for example, to change breathing rate or water/salt intake (Cleary et al. 2020, Gourine et al. 2010, Wang & Parpura 2018); this could represent a critical mechanism for maintaining whole-body homeostasis.

Brain Waste Clearance

Astrocyte endfeet also play a role in waste clearance. Brain waste is primarily cleared along cerebrovascular conduits, either by transport across the BBB into the vascular lumen or via flow along perivascular spaces and drainage into the lymphatic system (**Table 1**). The glymphatic model hypothesizes that cerebrospinal fluid is pumped into the brain along the penetrating arterioles, where it mixes with interstitial fluid and accumulates waste products, and is cleared out along venules (Jessen et al. 2015). Thus, waste products are transported in the same direction as blood flow and expunged into the cerebrospinal fluid. Mice lacking AQP4 were found to have reduced glymphatic flow, suggesting that endfeet AQP4 facilitates this drainage (Iliff et al. 2012). Alternatively, the intramural periarterial drainage (IPAD) model posits that interstitial fluid containing waste is pumped toward the pial surface along the basement membrane of arterioles in the opposite direction of blood flow (Aldea et al. 2019). Astrocyte endfeet are also proposed to drive IPAD-dependent perivascular waste movement by regulating functional hyperemia and cerebrovascular tone (Diem et al. 2018). Thus, astrocytes could regulate perivascular flow under either the IPAD or the glymphatic model by dynamically altering vessel tone. These mechanisms are important for clearing hazardous waste products, such as aggregates of amyloid-β (Aβ) (Iliff et al. 2012) and potentially tau (Harrison et al. 2020, Iliff et al. 2012). A complete understanding of the mechanisms by which astrocyte endfeet regulate waste clearance remains an important knowledge gap to be addressed.

Table 2 Astrocyte endfoot dysfunctions in pathologies

Condition	Astrocyte endfoot dysfunctions
Aging	Endfoot swelling AQP4 depolarization Delayed restorative plasticity
Traumatic brain injury	Loss of endfoot coverage Endfoot swelling AQP4 depolarization Release of factors damaging the vasculature Lack of BBB repair in mild/diffuse TBI? Dysfunctional neurovascular coupling?
Stroke	Loss of endfoot coverage (with recovery?) AQP4 depolarization Dysfunctional neurovascular coupling Impaired autoregulation
Alzheimer's disease	Loss of endfoot coverage AQP4 depolarization Dysfunctional neurovascular coupling Alteration of waste clearance
Multiple sclerosis	Loss of endfoot coverage Endfoot swelling Alteration of AQP4 expression patterns β-Dystroglycan cleavage Alteration of waste clearance

Question marks denote hypothesized dysfunctions. Abbreviations: AQP4, aquaporin 4; BBB, blood-brain barrier; TBI, traumatic brain injury.

ASTROCYTE ENDFOOT ALTERATIONS WITH AGING, INJURY, AND DISEASE

Aging

Dysfunction of the brain vasculature and astrocytes is associated with aging (Banks et al. 2021, Boisvert et al. 2018, Farrall & Wardlaw 2009, Habib et al. 2020). How aging influences endfoot function, however, remains understudied (**Table 2**). In rodents, astrocyte endfeet grow larger with age, likely due to swelling (Bors et al. 2018). AQP4 expression increases with age in both mouse and human, largely due to increased AQP4 in parenchymal astrocyte processes rather than endfoot changes (Kress et al. 2014, Zeppenfeld et al. 2017). Exogenous tracer clearance is also decreased in aged mice (Kress et al. 2014), prompting the question of whether this is a direct causal effect of AQP4 expression changes.

Although damaged astrocyte endfeet are replaced by neighboring astrocytes in young mice, this process is delayed in aged counterparts (Kubotera et al. 2019, Mills et al. 2022), suggesting a loss of astrocyte plasticity to cope with vascular damage. More research is required to understand how age affects astrocyte endfoot functions in different brain regions and how these changes may prime neurodegenerative disease development.

Traumatic Brain Injury

Traumatic brain injury (TBI) induces damage to the vasculature and/or BBB irrespective of injury type or severity (**Table 2**). In areas with vascular damage, astrocytes respond to blood-borne factors in one of two ways: They become either reactive in response to focal TBI or atypical in response to mild, diffuse TBI.

After focal TBI, signals from the lesion site induce a gradient of different types of reactive astrocytes (Robel 2017) in response to blood-borne factors (Schachtrup et al. 2010) and other cues. Astrocytes closest to the lesion form glial borders (formerly called the glial scar) (Sofroniew 2020), reducing the leakage of immune cells and blood proteins outside the lesion (Anderson et al. 2016, Bush et al. 1999). Border-forming astrocytes change drastically in morphology, with hypertrophic, overlapping processes extending into and around the lesion and loss of domain organization (Oberheim et al. 2008), but little is known about their interactions with the vasculature. Reactive astrocytes farther from the lesion continue to envelop the vasculature, but expression of endfoot proteins is reduced or irregular (Ren et al. 2013). Notably, TBI results in substantial vessel dilation, neurovascular uncoupling, and autoregulatory dysfunction (Szarka et al. 2018, Toth et al. 2016, Villalba et al. 2017). Capillaries compressed by swollen astrocyte endfeet have also been observed in biopsies from TBI patients (Østergaard et al. 2014). However, whether TBI-initiated changes in astrocyte endfeet contribute to these pathologies is unresolved.

Reactive astrocytes release soluble factors that can disrupt BBB integrity, such as matrix metalloproteases (MMPs), or reduce leakage of blood-borne factors (Michinaga & Koyama 2019, Rempe et al. 2016). Many molecules assessed for BBB repair, for example, Sonic hedgehog (Shh), transforming growth factor β (TGFβ), or glial-derived neurotrophic factor (GDNF), also regulate astrocyte reactivity. Leakage of immune cells, plasma proteins, or dyes is often used to determine whether BBB repair has taken place, but these readouts do not distinguish the leakage-reducing function of border-forming astrocytes from the restoration of BBB properties at leaky vessels. In fact, there is a surprising lack of in vivo imaging data supporting the idea that vessels reacquire BBB properties after TBI, even though ruptured vessels are repaired and reperfused (George et al. 2022). Thus, it is unclear whether blood-borne factor leakage is simply contained by the glial border or whether astrocytes initiate BBB repair at leaky vessels by secreting molecules that modulate tight junctions and other barrier properties.

After mild TBI/concussion, BBB leakage can persist for months (George et al. 2022, Johnson et al. 2013, Wang & Li 2016). Why such relatively minor BBB damage is not swiftly repaired remains a mystery, but a surprisingly different atypical astrocyte response induced by mild TBI/concussion may be one mechanism. In contrast to classical astrogliosis, atypical astrocytes rapidly lose many proteins after exposure to blood-borne factors (George et al. 2022), including endfeet-enriched proteins such as AQP4, Kir4.1, Cx43, and Cx30. Unlike focal TBI, where such loss is limited to the endfeet, mild TBI/concussion results in the loss of these proteins in the entire astrocyte (Shandra et al. 2019). Though endfeet typically remain in place at vessels, endothelial tight junctions and the basement membrane are disrupted (George et al. 2022). Early after mild TBI/concussion, BBB disruption is likely a direct consequence of the shear forces on the brain. What is surprising is that neither the tight junctions nor basement membrane are repaired even months after the injury. This lack of repair could be due to the inability of atypical astrocytes to form borders because a clear lesion to cordon off is lacking. The loss of most astrocyte-specific proteins suggests that soluble BBB repair factors may also be missing. Alternatively, atypical astrocytes may release factors like MMPs that keep the BBB open. Further research is needed to determine the exact mechanisms at play.

Stroke

Stroke induces widespread reactive astrogliosis, evidenced by drastic changes in the expression profile of astrocytes that occur within hours and last for weeks (Rusnakova et al. 2013, Zamanian et al. 2012). Border-forming reactive astrocytes that separate the infarct lesion from the surrounding tissue are characterized by AQP4 mislocalization away from the endfoot to the parenchymal

fine processes (Banitalebi et al. 2022), suggesting changes in endfoot functions (**Table 2**). Whether stroke also alters other DAPC-anchored proteins remains unexamined.

Ischemic stroke impairs NVC in patients, even in nonischemic regions extending far beyond the infarct (Krainik et al. 2005, Lin et al. 2011, Salinet et al. 2015). This NVC impairment is partly caused by increased capillary constriction due to 20-HETE (Li et al. 2021). As the enzyme that produces 20-HETE is largely expressed by astrocyte endfeet and its receptor GPR75 is largely expressed by VSMCs and pericytes (Gonzalez-Fernandez et al. 2020), this impairment likely reflects astrocyte dysfunction. NVC is also impaired after hemorrhagic stroke, and increased 20-HETE is observed in patients after both ischemic (Yi et al. 2017) and hemorrhagic (Crago et al. 2011, Donnelly et al. 2015) stroke, where it correlates with worse neurological decline. In animal models of hemorrhagic stroke, a complete inversion of NVC has been observed, whereby neuronal activity results in vasoconstrictions rather than dilations (Balbi et al. 2017, Koide et al. 2012) due to large K^+ efflux from endfeet via BK channels, resulting in VSMC depolarization and vessel constriction (Koide et al. 2012). This increased K^+ efflux is driven by large-amplitude purinergic-mediated Ca^{2+} signals in astrocyte endfeet (Pappas et al. 2015, 2016).

Large astrocytic Ca^{2+} waves also occur during cortical spreading depolarizations (Chuquet et al. 2007), which are common after both ischemic and hemorrhagic stroke. The vasoconstriction and inversion of NVC following cortical spreading depolarization depend on astrocyte Ca^{2+} (Chuquet et al. 2007, Major et al. 2017) and, to some extent, on 20-HETE (Fordsmann et al. 2013). Similar large astrocyte Ca^{2+} signals follow ischemic stroke (Ding et al. 2009, Rakers & Petzold 2017), but whether they increase 20-HETE synthesis or K^+ efflux from astrocyte endfeet to reduce NVC following ischemic stroke remains unknown. However, observations that attenuating astrogliosis mitigates cerebral blood flow defects and improves neurological outcomes after ischemic stroke support this idea (Begum et al. 2018). Astrocytic 20-HETE may be an adaptive response to stimulate angiogenesis after stroke (Liu et al. 2019), although with obvious detrimental consequences to NVC.

Stroke also impairs cerebral autoregulation (Koide et al. 2021, Salinet et al. 2015), as well as perivascular space dynamics and glymphatic clearance (Mestre et al. 2020). The hypoxia and reduced waste clearance resulting from these impairments, in addition to neurovascular uncoupling, may underlie the increased incidence of dementia in stroke patients (Delgado et al. 2022, Levine et al. 2015, Savva et al. 2010). However, the role of astrocytes in these dysfunctions is as yet unknown.

Astrocytes play multifaceted roles in BBB integrity after stroke. Ablating reactive astrocytes after stroke increases BBB permeability (Williamson et al. 2021) and prevents behavioral recovery (Liu et al. 2014), suggesting a supportive role in BBB maintenance. In agreement, loss of endfeet after photothrombotic stroke results in a swift recovery of endfoot coverage by processes of nearby astrocytes (Mills et al. 2022). Furthermore, interventions that protect BBB function are correlated with increased astrocyte endfoot coverage, and astrocyte-secreted neurotrophic factors can restore BBB integrity (Zhou et al. 2022). In contrast, other studies report that abnormal reactive astrocytes underlie BBB dysfunction after stroke (Qin et al. 2022, Zhang et al. 2022), suggesting a potentially detrimental role. These findings indicate that astrocytes can either help maintain or disrupt the BBB after stroke, but when, how, and the precise changes in endfoot proteins that mediate these effects have yet to be determined. Suppressing the detrimental consequences of reactive astrocytes, while preserving or even amplifying their beneficial roles, may be the most effective way to combat cerebrovascular dysfunction following stroke (Escartin et al. 2021).

Alzheimer's Disease

Brain vasculature alterations precede dementia and worsen with disease progression (Sweeney et al. 2019, Wardlaw et al. 2019). Imaging and transcriptomics studies have shown that vascular cells such as brain endothelial cells and pericytes are severely altered in conditions that lead to dementia, such as cerebral small vessel disease or Alzheimer's disease (AD) (Procter et al. 2021). Although recent work has highlighted changes in endfoot protein expression and morphology in AD, we know little about how astrocyte endfoot functions are altered in, and contribute to, neurodegeneration (**Table 2**).

Astrocytes undergo dramatic phenotypical changes in AD (De Strooper & Karran 2016, Perez-Nievas & Serrano-Pozo 2018). Expression of endfoot genes encoding AQP4, MLC1, and dystrobrevin is upregulated in subjects with dementia (Simon et al. 2018). Both human AD brains and mouse models of amyloidopathy show overall AQP4 upregulation in the cortex (Hoshi et al. 2012, Zeppenfeld et al. 2017) and a decrease of endfoot AQP4 polarization with disease progression. This change in AQP4 polarization is largely driven by increased parenchymal AQP4, which is associated with Aβ plaques, rather than a decrease in the endfeet (Kimbrough et al. 2015, Xu et al. 2015, Zeppenfeld et al. 2017). Changes in AQP4 expression are particularly pronounced around vessels exhibiting cerebral amyloid angiopathy (CAA) compared to non-CAA vessels in the same individual (Hoshi et al. 2012, Owasil et al. 2020). *Aqp4* gene knockout exacerbates pathological and behavioral phenotypes in a mouse model of amyloidopathy (Xu et al. 2015), but the role of endfoot versus parenchymal astrocyte AQP4 in this context remains uncertain. Perhaps the disruption of normal AQP4 distribution leads to a failure of Aβ clearance via perivascular/glymphatic waste pathways in AD (Iliff et al. 2012). Intriguingly, the glymphatic waste clearance pathway is more active during sleep (Xie et al. 2013), which is also disrupted in AD (Ju et al. 2013).

Vascular amyloid accumulation has additional consequences for endfoot functions. In CAA, amyloid aggregations form between the endfeet and vascular mural cells, resulting in a swollen endfoot appearance and reduced vessel coverage (Kimbrough et al. 2015). These amyloid aggregations physically distance endfeet from vascular cells, leading to NVC impairment. This impairment likely contributes to neuron starvation and BBB dysfunction, thus worsening pathology in AD (Sweeney et al. 2019). CAA also upregulates angiopoietin-related protein 4 (ANGPTL4), an angiogenic factor normally induced by hypoxia, in endfeet, further contributing to vascular susceptibility (Chakraborty et al. 2018).

Although a causative relationship between endfoot dysfunction and AD initiation and progression is difficult to establish at the moment, the importance of astrocyte endfeet for the interaction of the CNS with the vasculature demands more investigation in this field.

Multiple Sclerosis

Multiple sclerosis (MS) is characterized by white matter lesions that lead to demyelination in the spinal cord and cortex. These lesions are thought to be caused by T lymphocytes crossing the BBB and invading the nervous system (Mapunda et al. 2022). Research in the experimental autoimmune encephalomyelitis (EAE) mouse model of MS has shown that T cells first accumulate between the brain endothelial cells and endfeet. Symptoms develop when T cells pass through the endfoot barrier and enter the parenchyma (Tran et al. 1998). Swollen endfeet and a disrupted endfoot barrier are observed in humans with MS and in EAE mice (Brosnan & Raine 2013, Eilam et al. 2018). Interestingly, endfoot β-dystroglycan cleavage, induced by macrophage-derived MMP-2 and MMP-9, is necessary for endfoot barrier disruption, T cell parenchymal invasion, and disease development in EAE mice. Accordingly, loss of β-dystroglycan has been observed around vessels with T cell parenchymal invasion (Agrawal et al. 2006, Wolburg-Buchholz et al. 2009). In addition,

in both humans and EAE mice, AQP4 is upregulated around cortical and spinal cord lesions compared to healthy tissue (**Table 2**) (Aoki-Yoshino et al. 2005, Fournier et al. 2019, Sinclair et al. 2007). Waste clearance mechanisms are also dysfunctional in MS, and this dysfunction correlates with clinical disability severity, lesion volumes, and brain atrophy (Carotenuto et al. 2022). Further research is needed to inform whether therapies that prevent endfoot disruption could be clinically relevant strategies for treating MS.

CONCLUSIONS AND FUTURE DIRECTIONS

Technical advances in the past few decades have facilitated and intensified the investigation of astrocyte interactions with the vasculature. Yet, many fascinating and imperative questions remain open.

Although astrocyte endfoot development and maturation parallel CNS capillary formation, the role of astrocytes in forming and maintaining the BBB is unclear. In the mature healthy brain, endfeet contribute to NVC, resting vascular tone, and, to some extent, vascular reactivity to blood pressure changes. As structures that form the physical border between CNS tissue and the vasculature, endfeet are also well positioned to regulate brain-body homeostasis. What other signals in the blood, aside from osmolality and CO_2, might endfeet sense? Do endfeet modulate local neural activity in the CNS to regulate additional peripheral physiological processes?

Astrocyte endfoot morphology, function, and molecular makeup are severely altered at early stages of CNS pathologies. Endfoot changes could lead to blood flow dysregulation and BBB dysfunction, which prevents the proper exchange of substances between the blood and CNS. In contrast, some endfoot alterations could protect and prevent further damage in disease. Identifying the damaging and protective roles of endfeet may help design treatments that halt CNS impairment and restore homeostasis.

New technical advances will be essential to answer these open questions. Automated analysis tools for three-dimensional electron microscopy images would provide detailed information about endfoot-vasculature interactions. Molecular sensors designed for astrocytes, for example, voltage-sensitive dyes tuned to hyperpolarized membrane properties, would uncover how ionic changes influence endfoot-vessel communication. Finally, new tools to investigate endfoot protein makeup would facilitate the discovery of proteins relevant to endfoot function and dysfunction.

In summary, astrocyte endfeet play a crucial role in the bidirectional communication between neural tissue and the blood to maintain homeostasis within both the CNS and body. More detailed investigations using novel tools developed to approach the questions we pose here are bound to illuminate the complex role of astrocyte endfeet in brain function and assist future efforts to develop therapeutic interventions for neurological disease.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

To facilitate open access, B.D.-C. has applied a Creative Commons Attribution (CC BY) license to any author-accepted manuscript version arising from this submission. B.D.-C. is supported by the UK Dementia Research Institute. S.R. is supported by the National Institutes of Health (NIH) (R01NS121145, R01NS105807). A.M. is supported by the NIH (R01NS110690, P30AG066518) and the JTMF Foundation.

LITERATURE CITED

Agrawal S, Anderson P, Durbeej M, van Rooijen N, Ivars F, et al. 2006. Dystroglycan is selectively cleaved at the parenchymal basement membrane at sites of leukocyte extravasation in experimental autoimmune encephalomyelitis. *J. Exp. Med.* 203(4):1007–19

Aldea R, Weller RO, Wilcock DM, Carare RO, Richardson G. 2019. Cerebrovascular smooth muscle cells as the drivers of intramural periarterial drainage of the brain. *Front. Aging Neurosci.* 11:1

Allen NJ, Eroglu C. 2017. Cell biology of astrocyte-synapse interactions. *Neuron* 96(3):697–708

Alonso-Gardón M, Elorza-Vidal X, Castellanos A, La Sala G, Armand-Ugon M, et al. 2021. Identification of the GlialCAM interactome: The G protein-coupled receptors GPRC5B and GPR37L1 modulate megalencephalic leukoencephalopathy proteins. *Hum. Mol. Genet.* 30(17):1649–65

Anderson MA, Burda JE, Ren Y, Ao Y, O'Shea TM, et al. 2016. Astrocyte scar formation aids central nervous system axon regeneration. *Nature* 532(7598):195–200

Antonakou EI, Triarhou LC. 2019. "Anatomical mechanism of ideation, association and attention" [1895] and "Certain points in neurological histophysiology" [1896]: Cajal's conjectures, then and now. *J. Chem. Neuroanat.* 104:101702

Aoki-Yoshino K, Uchihara T, Duyckaerts C, Nakamura A, Hauw J-J, Wakayama Y. 2005. Enhanced expression of aquaporin 4 in human brain with inflammatory diseases. *Acta Neuropathol.* 110(3):281–88

Armulik A, Genové G, Mäe M, Nisancioglu MH, Wallgard E, et al. 2010. Pericytes regulate the blood-brain barrier. *Nature* 468(7323):557–61

Balbi M, Koide M, Wellman GC, Plesnila N. 2017. Inversion of neurovascular coupling after subarachnoid hemorrhage in vivo. *J. Cereb. Blood Flow Metab.* 37(11):3625–34

Banitalebi S, Skauli N, Geiseler S, Ottersen OP, Amiry-Moghaddam M. 2022. Disassembly and mislocalization of AQP4 in incipient scar formation after experimental stroke. *Int. J. Mol. Sci.* 23(3):1117

Banks WA, Reed MJ, Logsdon AF, Rhea EM, Erickson MA. 2021. Healthy aging and the blood-brain barrier. *Nat. Aging* 1(3):243–54

Beck DW, Roberts RL, Olson JJ. 1986. Glial cells influence membrane-associated enzyme activity at the blood-brain barrier. *Brain Res.* 381(1):131–37

Begum G, Song S, Wang S, Zhao H, Bhuiyan MIH, et al. 2018. Selective knockout of astrocytic Na^+/H^+ exchanger isoform 1 reduces astrogliosis, BBB damage, infarction, and improves neurological function after ischemic stroke. *Glia* 66(1):126–44

Belmaati Cherkaoui M, Vacca O, Izabelle C, Boulay A-C, Boulogne C, et al. 2021. Dp71 contribution to the molecular scaffold anchoring aquaporine-4 channels in brain macroglial cells. *Glia* 69(4):954–70

Berthiaume A-A, Grant RI, McDowell KP, Underly RG, Hartmann DA, et al. 2018. Dynamic remodeling of pericytes in vivo maintains capillary coverage in the adult mouse brain. *Cell Rep.* 22(1):8–16

Bertossi M, Virgintino D, Maiorano E, Occhiogrosso M, Roncali L. 1997. Ultrastructural and morphometric investigation of human brain capillaries in normal and peritumoral tissues. *Ultrastruct. Pathol.* 21(1):41–49

Blanco VM, Stern JE, Filosa JA. 2008. Tone-dependent vascular responses to astrocyte-derived signals. *Am. J. Physiol. Heart Circ. Physiol.* 294(6):H2855–63

Boisvert MM, Erikson GA, Shokhirev MN, Allen NJ. 2018. The aging astrocyte transcriptome from multiple regions of the mouse brain. *Cell Rep.* 22(1):269–85

Bors L, Tóth K, Tóth EZ, Bajza Á, Csorba A, et al. 2018. Age-dependent changes at the blood-brain barrier. A comparative structural and functional study in young adult and middle aged rats. *Brain Res. Bull.* 139:269–77

Boulay A-C, Saubaméa B, Adam N, Chasseigneaux S, Mazaré N, et al. 2017. Translation in astrocyte distal processes sets molecular heterogeneity at the gliovascular interface. *Cell Discov.* 3:17005

Brosnan CF, Raine CS. 2013. The astrocyte in multiple sclerosis revisited. *Glia* 61(4):453–65

Bush TG, Puvanachandra N, Horner CH, Polito A, Ostenfeld T, et al. 1999. Leukocyte infiltration, neuronal degeneration, and neurite outgrowth after ablation of scar-forming, reactive astrocytes in adult transgenic mice. *Neuron* 23(2):297–308

Bushong EA, Martone ME, Ellisman MH. 2004. Maturation of astrocyte morphology and the establishment of astrocyte domains during postnatal hippocampal development. *Int. J. Dev. Neurosci.* 22(2):73–86

Canfield SG, Stebbins MJ, Morales BS, Asai SW, Vatine GD, et al. 2017. An isogenic blood-brain barrier model comprising brain endothelial cells, astrocytes, and neurons derived from human induced pluripotent stem cells. *J. Neurochem.* 140(6):874–88

Carotenuto A, Cacciaguerra L, Pagani E, Preziosa P, Filippi M, Rocca MA. 2022. Glymphatic system impairment in multiple sclerosis: relation with brain damage and disability. *Brain J. Neurol.* 145(8):2785–95

Chai H, Diaz-Castro B, Shigetomi E, Monte E, Octeau JC, et al. 2017. Neural circuit-specialized astrocytes: transcriptomic, proteomic, morphological, and functional evidence. *Neuron* 95(3):531–49.e9

Chakraborty A, Kamermans A, van Het Hof B, Castricum K, Aanhane E, et al. 2018. Angiopoietin like-4 as a novel vascular mediator in capillary cerebral amyloid angiopathy. *Brain J. Neurol.* 141(12):3377–88

Chuquet J, Hollender L, Nimchinsky EA. 2007. High-resolution in vivo imaging of the neurovascular unit during spreading depression. *J. Neurosci.* 27(15):4036–44

Clavreul S, Abdeladim L, Hernández-Garzón E, Niculescu D, Durand J, et al. 2019. Cortical astrocytes develop in a plastic manner at both clonal and cellular levels. *Nat. Commun.* 10(1):4884

Cleary CM, Moreira TS, Takakura AC, Nelson MT, Longden TA, Mulkey DK. 2020. Vascular control of the CO_2/H^+-dependent drive to breathe. *eLife* 9:e59499

Coelho-Santos V, Berthiaume A-A, Ornelas S, Stuhlmann H, Shih AY. 2021. Imaging the construction of capillary networks in the neonatal mouse brain. *PNAS* 118(26):e2100866118

Coelho-Santos V, Shih AY. 2020. Postnatal development of cerebrovascular structure and the neurogliovascular unit. *Wiley Interdiscip. Rev. Dev. Biol.* 9(2):e363

Connors NC, Adams ME, Froehner SC, Kofuji P. 2004. The potassium channel Kir4.1 associates with the dystrophin-glycoprotein complex via α-syntrophin in glia. *J. Biol. Chem.* 279(27):28387–92

Crago EA, Thampatty BP, Sherwood PR, Kuo C-WJ, Bender C, et al. 2011. Cerebrospinal fluid 20-HETE is associated with delayed cerebral ischemia and poor outcomes after aneurysmal subarachnoid hemorrhage. *Stroke* 42(7):1872–77

Culligan K, Ohlendieck K. 2002. Diversity of the brain dystrophin-glycoprotein complex. *J. Biomed. Biotechnol.* 2(1):31–36

Daneman R, Zhou L, Kebede AA, Barres BA. 2010. Pericytes are required for blood-brain barrier integrity during embryogenesis. *Nature* 468(7323):562–66

de Majo M, Koontz M, Rowitch D, Ullian EM. 2020. An update on human astrocytes and their role in development and disease. *Glia* 68(4):685–704

De Strooper B, Karran E. 2016. The cellular phase of Alzheimer's disease. *Cell* 164(4):603–15

Del Franco AP, Chiang P-P, Newman EA. 2022. Dilation of cortical capillaries is not related to astrocyte calcium signaling. *Glia* 70(3):508–21

Delgado J, Masoli J, Hase Y, Akinyemi R, Ballard C, et al. 2022. Trajectories of cognitive change following stroke: stepwise decline towards dementia in the elderly. *Brain Commun.* 4(3):fcac129

Diaz-Castro B, Bernstein AM, Coppola G, Sofroniew MV, Khakh BS. 2021. Molecular and functional properties of cortical astrocytes during peripherally induced neuroinflammation. *Cell Rep.* 36(6):109508

Diem AK, Carare RO, Weller RO, Bressloff NW. 2018. A control mechanism for intra-mural peri-arterial drainage via astrocytes: how neuronal activity could improve waste clearance from the brain. *PLOS ONE* 13(10):e0205276

Ding S, Wang T, Cui W, Haydon PG. 2009. Photothrombosis ischemia stimulates a sustained astrocytic Ca^{2+} signaling in vivo. *Glia* 57(7):767–76

Djukic B, Casper KB, Philpot BD, Chin L-S, McCarthy KD. 2007. Conditional knock-out of Kir4.1 leads to glial membrane depolarization, inhibition of potassium and glutamate uptake, and enhanced short-term synaptic potentiation. *J. Neurosci.* 27(42):11354–65

Donnelly MK, Crago EA, Conley YP, Balzer JR, Ren D, et al. 2015. 20-HETE is associated with unfavorable outcomes in subarachnoid hemorrhage patients. *J. Cereb. Blood Flow Metab.* 35(9):1515–22

Eilam R, Segal M, Malach R, Sela M, Arnon R, Aharoni R. 2018. Astrocyte disruption of neurovascular communication is linked to cortical damage in an animal model of multiple sclerosis. *Glia* 66(5):1098–117

Escartin C, Galea E, Lakatos A, O'Callaghan JP, Petzold GC, et al. 2021. Reactive astrocyte nomenclature, definitions, and future directions. *Nat. Neurosci.* 24(3):312–25

Farmer WT, Abrahamsson T, Chierzi S, Lui C, Zaelzer C, et al. 2016. Neurons diversify astrocytes in the adult brain through sonic hedgehog signaling. *Science* 351(6275):849–54

Farrall AJ, Wardlaw JM. 2009. Blood-brain barrier: ageing and microvascular disease—systematic review and meta-analysis. *Neurobiol. Aging* 30(3):337–52

Filosa JA, Bonev AD, Straub SV, Meredith AL, Wilkerson MK, et al. 2006. Local potassium signaling couples neuronal activity to vasodilation in the brain. *Nat. Neurosci.* 9(11):1397–403

Fordsmann JC, Ko RWY, Choi HB, Thomsen K, Witgen BM, et al. 2013. Increased 20-HETE synthesis explains reduced cerebral blood flow but not impaired neurovascular coupling after cortical spreading depression in rat cerebral cortex. *J. Neurosci.* 33(6):2562–70

Fournier AP, Gauberti M, Quenault A, Vivien D, Macrez R, Docagne F. 2019. Reduced spinal cord parenchymal cerebrospinal fluid circulation in experimental autoimmune encephalomyelitis. *J. Cereb. Blood Flow Metab.* 39(7):1258–65

Garcia V, Gilani A, Shkolnik B, Pandey V, Zhang FF, et al. 2017. 20-HETE signals through G-protein-coupled receptor GPR75 (Gq) to affect vascular function and trigger hypertension. *Circ. Res.* 120(11):1776–88

García-Marín V, García-López P, Freire M. 2007. Cajal's contributions to glia research. *Trends Neurosci.* 30(9):479–87

Ge W-P, Miyawaki A, Gage FH, Jan YN, Jan LY. 2012. Local generation of glia is a major astrocyte source in postnatal cortex. *Nature* 484(7394):376–80

Gebremedhin D, Ma YH, Falck JR, Roman RJ, VanRollins M, Harder DR. 1992. Mechanism of action of cerebral epoxyeicosatrienoic acids on cerebral arterial smooth muscle. *Am. J. Physiol.* 263(2):H519–25

Gee SH, Montanaro F, Lindenbaum MH, Carbonetto S. 1994. Dystroglycan-α, a dystrophin-associated glycoprotein, is a functional agrin receptor. *Cell* 77(5):675–86

George KK, Heithoff BP, Shandra O, Robel S. 2022. Mild traumatic brain injury/concussion initiates an atypical astrocyte response caused by blood-brain barrier dysfunction. *J. Neurotrauma* 39(1–2):211–26

Gilbert A, Elorza-Vidal X, Rancillac A, Chagnot A, Yetim M, et al. 2021. Megalencephalic leukoencephalopathy with subcortical cysts is a developmental disorder of the gliovascular unit. *eLife* 10:e71379

Gilbert A, Vidal XE, Estevez R, Cohen-Salmon M, Boulay A-C. 2019. Postnatal development of the astrocyte perivascular MLC1/GlialCAM complex defines a temporal window for the gliovascular unit maturation. *Brain Struct. Funct.* 224(3):1267–78

Göbel J, Engelhardt E, Pelzer P, Sakthivelu V, Jahn HM, et al. 2020. Mitochondria-endoplasmic reticulum contacts in reactive astrocytes promote vascular remodeling. *Cell Metab.* 31(4):791–808.e8

Gonzalez-Fernandez E, Staursky D, Lucas K, Nguyen BV, Li M, et al. 2020. 20-HETE enzymes and receptors in the neurovascular unit: implications in cerebrovascular disease. *Front. Neurol.* 11:983

Gordon GRJ, Choi HB, Rungta RL, Ellis-Davies GCR, MacVicar BA. 2008. Brain metabolism dictates the polarity of astrocyte control over arterioles. *Nature* 456(7223):745–49

Gourine AV, Kasymov V, Marina N, Tang F, Figueiredo MF, et al. 2010. Astrocytes control breathing through pH-dependent release of ATP. *Science* 329(5991):571–75

Gu X, Chen W, Volkow ND, Koretsky AP, Du C, Pan Y. 2018. Synchronized astrocytic Ca^{2+} responses in neurovascular coupling during somatosensory stimulation and for the resting state. *Cell Rep.* 23(13):3878–90

Habib N, McCabe C, Medina S, Varshavsky M, Kitsberg D, et al. 2020. Disease-associated astrocytes in Alzheimer's disease and aging. *Nat. Neurosci.* 23(6):701–6

Hall CN, Reynell C, Gesslein B, Hamilton NB, Mishra A, et al. 2014. Capillary pericytes regulate cerebral blood flow in health and disease. *Nature* 508(7494):55–60

Harik SI, Hall AK, Richey P, Andersson L, Lundahl P, Perry G. 1993. Ontogeny of the erythroid/HepG2-type glucose transporter (GLUT-1) in the rat nervous system. *Brain Res. Dev. Brain Res.* 72(1):41–49

Harrison IF, Ismail O, Machhada A, Colgan N, Ohene Y, et al. 2020. Impaired glymphatic function and clearance of tau in an Alzheimer's disease model. *Brain J. Neurol.* 143(8):2576–93

Heithoff BP, George KK, Phares AN, Zuidhoek IA, Munoz-Ballester C, Robel S. 2021. Astrocytes are necessary for blood-brain barrier maintenance in the adult mouse brain. *Glia* 69(2):436–72

Heuser K, Eid T, Lauritzen F, Thoren AE, Vindedal GF, et al. 2012. Loss of perivascular Kir4.1 potassium channels in the sclerotic hippocampus of patients with mesial temporal lobe epilepsy. *J. Neuropathol. Exp. Neurol.* 71(9):814–25

Hoddevik EH, Khan FH, Rahmani S, Ottersen OP, Boldt HB, Amiry-Moghaddam M. 2017. Factors determining the density of AQP4 water channel molecules at the brain-blood interface. *Brain Struct. Funct.* 222(4):1753–66

Hoegg-Beiler MB, Sirisi S, Orozco IJ, Ferrer I, Hohensee S, et al. 2014. Disrupting MLC1 and GlialCAM and ClC-2 interactions in leukodystrophy entails glial chloride channel dysfunction. *Nat. Commun.* 5:3475

Hoshi A, Yamamoto T, Shimizu K, Ugawa Y, Nishizawa M, et al. 2012. Characteristics of aquaporin expression surrounding senile plaques and cerebral amyloid angiopathy in Alzheimer disease. *J. Neuropathol. Exp. Neurol.* 71(8):750–59

Hösli L, Zuend M, Bredell G, Zanker HS, Porto de Oliveira CE, et al. 2022. Direct vascular contact is a hallmark of cerebral astrocytes. *Cell Rep.* 39(1):110599

Iadecola C. 2017. The neurovascular unit coming of age: a journey through neurovascular coupling in health and disease. *Neuron* 96(1):17–42

Iliff JJ, Wang M, Liao Y, Plogg BA, Peng W, et al. 2012. A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid β. *Sci. Transl. Med.* 4(147):147ra111

Institoris Á, Rosenegger DG, Gordon GR. 2015. Arteriole dilation to synaptic activation that is sub-threshold to astrocyte endfoot Ca^{2+} transients. *J. Cereb. Blood Flow Metab.* 35(9):1411–15

Janzer RC, Raff MC. 1987. Astrocytes induce blood-brain barrier properties in endothelial cells. *Nature* 325(6101):253–57

Jessen NA, Munk ASF, Lundgaard I, Nedergaard M. 2015. The glymphatic system: a beginner's guide. *Neurochem. Res.* 40(12):2583–99

Johnson VE, Stewart JE, Begbie FD, Trojanowski JQ, Smith DH, Stewart W. 2013. Inflammation and white matter degeneration persist for years after a single traumatic brain injury. *Brain J. Neurol.* 136(Pt. 1):28–42

Ju Y-ES, McLeland JS, Toedebusch CD, Xiong C, Fagan AM, et al. 2013. Sleep quality and preclinical Alzheimer disease. *JAMA Neurol.* 70(5):587–93

Kacem K, Lacombe P, Seylaz J, Bonvento G. 1998. Structural organization of the perivascular astrocyte endfeet and their relationship with the endothelial glucose transporter: a confocal microscopy study. *Glia* 23(1):1–10

Kim KJ, Iddings JA, Stern JE, Blanco VM, Croom D, et al. 2015. Astrocyte contributions to flow/pressure-evoked parenchymal arteriole vasoconstriction. *J. Neurosci.* 35(21):8245–57

Kim KJ, Ramiro Diaz J, Iddings JA, Filosa JA. 2016. Vasculo-neuronal coupling: retrograde vascular communication to brain neurons. *J. Neurosci.* 36(50):12624–39

Kimbrough IF, Robel S, Roberson ED, Sontheimer H. 2015. Vascular amyloidosis impairs the gliovascular unit in a mouse model of Alzheimer's disease. *Brain J. Neurol.* 138(Pt. 12):3716–33

Koide M, Bonev AD, Nelson MT, Wellman GC. 2012. Inversion of neurovascular coupling by subarachnoid blood depends on large-conductance Ca^{2+}-activated K^+ (BK) channels. *PNAS* 109(21):E1387–95

Koide M, Ferris HR, Nelson MT, Wellman GC. 2021. Impaired cerebral autoregulation after subarachnoid hemorrhage: a quantitative assessment using a mouse model. *Front. Physiol.* 12:688468

Krainik A, Hund-Georgiadis M, Zysset S, von Cramon DY. 2005. Regional impairment of cerebrovascular reactivity and BOLD signal in adults after stroke. *Stroke* 36(6):1146–52

Kress BT, Iliff JJ, Xia M, Wang M, Wei HS, et al. 2014. Impairment of paravascular clearance pathways in the aging brain. *Ann. Neurol.* 76(6):845–61

Kubotera H, Ikeshima-Kataoka H, Hatashita Y, Allegra Mascaro AL, Pavone FS, Inoue T. 2019. Astrocytic endfeet re-cover blood vessels after removal by laser ablation. *Sci. Rep.* 9(1):1263

Kur J, Newman EA. 2014. Purinergic control of vascular tone in the retina. *J. Physiol.* 592(3):491–504

Landis DM, Reese TS. 1974. Arrays of particles in freeze-fractured astrocytic membranes. *J. Cell Biol.* 60(1):316–20

Leegwater PA, Yuan BQ, van der Steen J, Mulders J, Könst AA, et al. 2001. Mutations of *MLC1* (*KIAA0027*), encoding a putative membrane protein, cause megalencephalic leukoencephalopathy with subcortical cysts. *Am. J. Hum. Genet.* 68(4):831–38

Levine DA, Galecki AT, Langa KM, Unverzagt FW, Kabeto MU, et al. 2015. Trajectory of cognitive decline after incident stroke. *JAMA* 314(1):41–51

Li Z, McConnell HL, Stackhouse TL, Pike MM, Zhang W, Mishra A. 2021. Increased 20-HETE signaling suppresses capillary neurovascular coupling after ischemic stroke in regions beyond the infarct. *Front. Cell Neurosci*. 15:762843

Lin WH, Hao Q, Rosengarten B, Leung WH, Wong KS. 2011. Impaired neurovascular coupling in ischaemic stroke patients with large or small vessel disease. *Eur. J. Neurol.* 18(5):731–36

Lind BL, Jessen SB, Lønstrup M, Joséphine C, Bonvento G, Lauritzen M. 2018. Fast Ca^{2+} responses in astrocyte end-feet and neurovascular coupling in mice. *Glia* 66(2):348–58

Liu Y, Li Y, Zhan M, Liu Y, Li Z, et al. 2019. Astrocytic cytochrome P450 4A/20-hydroxyeicosatetraenoic acid contributes to angiogenesis in the experimental ischemic stroke. *Brain Res*. 1708:160–70

Liu Z, Li Y, Cui Y, Roberts C, Lu M, et al. 2014. Beneficial effects of gfap/vimentin reactive astrocytes for axonal remodeling and motor behavioral recovery in mice after stroke. *Glia* 62(12):2022–33

López-Hernández T, Ridder MC, Montolio M, Capdevila-Nortes X, Polder E, et al. 2011. Mutant GlialCAM causes megalencephalic leukoencephalopathy with subcortical cysts, benign familial macrocephaly, and macrocephaly with retardation and autism. *Am. J. Hum. Genet.* 88(4):422–32

Lunde LK, Camassa LMA, Hoddevik EH, Khan FH, Ottersen OP, et al. 2015. Postnatal development of the molecular complex underlying astrocyte polarization. *Brain Struct. Funct.* 220(4):2087–101

Ma S, Kwon HJ, Huang Z. 2012. A functional requirement for astroglia in promoting blood vessel development in the early postnatal brain. *PLOS ONE* 7(10):e48001

Major S, Petzold GC, Reiffurth C, Windmüller O, Foddis M, et al. 2017. A role of the sodium pump in spreading ischemia in rats. *J. Cereb. Blood Flow Metab.* 37(5):1687–705

Mapunda JA, Tibar H, Regragui W, Engelhardt B. 2022. How does the immune system enter the brain? *Front. Immunol.* 13:805657

Marín-Padilla M. 2012. The human brain intracerebral microvascular system: development and structure. *Front. Neuroanat.* 6:38

Mathiisen TM, Lehre KP, Danbolt NC, Ottersen OP. 2010. The perivascular astroglial sheath provides a complete covering of the brain microvessels: an electron microscopic 3D reconstruction. *Glia* 58(9):1094–103

Mestre H, Du T, Sweeney AM, Liu G, Samson AJ, et al. 2020. Cerebrospinal fluid influx drives acute ischemic tissue swelling. *Science* 367(6483):eaax7171

Metea MR, Kofuji P, Newman EA. 2007. Neurovascular coupling is not mediated by potassium siphoning from glial cells. *J. Neurosci.* 27(10):2468–71

Metea MR, Newman EA. 2006. Glial cells dilate and constrict blood vessels: a mechanism of neurovascular coupling. *J. Neurosci.* 26(11):2862–70

Methner C, Cao Z, Mishra A, Kaul S. 2021. Mechanism and potential treatment of the "no reflow" phenomenon after acute myocardial infarction: role of pericytes and GPR39. *Am. J. Physiol. Heart Circ. Physiol*. 321:H1030–41

Michinaga S, Koyama Y. 2019. Dual roles of astrocyte-derived factors in regulation of blood-brain barrier function after brain damage. *Int. J. Mol. Sci.* 20(3):E571

Mills WA, Woo AM, Jiang S, Martin J, Surendran D, et al. 2022. Astrocyte plasticity in mice ensures continued endfoot coverage of cerebral blood vessels following injury and declines with age. *Nat. Commun.* 13(1):1794

Mishra A, Reynolds JP, Chen Y, Gourine AV, Rusakov DA, Attwell D. 2016. Astrocytes mediate neurovascular signaling to capillary pericytes but not to arterioles. *Nat. Neurosci.* 19(12):1619–27

Moore CI, Cao R. 2008. The hemo-neural hypothesis: on the role of blood flow in information processing. *J. Neurophysiol.* 99(5):2035–47

Mugnaini E, Walberg F. 1965. The perivascular elements in the central nervous system. An electron microscopical study. *Acta Neurol. Scand. Suppl.* 13(Pt. 2):629–36

Mulligan SJ, MacVicar BA. 2004. Calcium transients in astrocyte endfeet cause cerebrovascular constrictions. *Nature* 431(7005):195–99

Nagai J, Yu X, Papouin T, Cheong E, Freeman MR, et al. 2021. Behaviorally consequential astrocytic regulation of neural circuits. *Neuron* 109(4):576–96

Neely JD, Amiry-Moghaddam M, Ottersen OP, Froehner SC, Agre P, Adams ME. 2001. Syntrophin-dependent expression and localization of Aquaporin-4 water channel protein. *PNAS* 98(24):14108–13
Nizar K, Uhlirova H, Tian P, Saisan PA, Cheng Q, et al. 2013. In vivo stimulus-induced vasodilation occurs without IP3 receptor activation and may precede astrocytic calcium increase. *J. Neurosci.* 33(19):8411–22
Oberheim NA, Tian G-F, Han X, Peng W, Takano T, et al. 2008. Loss of astrocytic domain organization in the epileptic brain. *J. Neurosci.* 28(13):3264–76
Østergaard L, Engedal TS, Aamand R, Mikkelsen R, Iversen NK, et al. 2014. Capillary transit time heterogeneity and flow-metabolism coupling after traumatic brain injury. *J. Cereb. Blood Flow Metab.* 34(10):1585–98
O'Sullivan ML, Puñal VM, Kerstein PC, Brzezinski JA, Glaser T, et al. 2017. Astrocytes follow ganglion cell axons to establish an angiogenic template during retinal development. *Glia* 65(10):1697–716
Owasil R, O'Neill R, Keable A, Nimmo J, MacGregor Sharp M, et al. 2020. The pattern of AQP4 expression in the ageing human brain and in cerebral amyloid angiopathy. *Int. J. Mol. Sci.* 21(4):E1225
Pappas AC, Koide M, Wellman GC. 2015. Astrocyte Ca^{2+} signaling drives inversion of neurovascular coupling after subarachnoid hemorrhage. *J. Neurosci.* 35(39):13375–84
Pappas AC, Koide M, Wellman GC. 2016. Purinergic signaling triggers endfoot high-amplitude Ca^{2+} signals and causes inversion of neurovascular coupling after subarachnoid hemorrhage. *J. Cereb. Blood Flow Metab.* 36(11):1901–12
Paquette T, Piché M, Leblond H. 2021. Contribution of astrocytes to neurovascular coupling in the spinal cord of the rat. *J. Physiol. Sci.* 71(1):16
Paulson OB, Newman EA. 1987. Does the release of potassium from astrocyte endfeet regulate cerebral blood flow? *Science* 237(4817):896–98
Peng X, Carhuapoma JR, Bhardwaj A, Alkayed NJ, Falck JR, et al. 2002. Suppression of cortical functional hyperemia to vibrissal stimulation in the rat by epoxygenase inhibitors. *Am. J. Physiol. Heart Circ. Physiol.* 283(5):H2029–37
Perez-Nievas BG, Serrano-Pozo A. 2018. Deciphering the astrocyte reaction in Alzheimer's disease. *Front. Aging Neurosci.* 10:114
Procter TV, Williams A, Montagne A. 2021. Interplay between brain pericytes and endothelial cells in dementia. *Am. J. Pathol.* 191(11):1917–31
Qin X, Wang J, Chen S, Liu G, Wu C, et al. 2022. Astrocytic p75NTR expression provoked by ischemic stroke exacerbates the blood-brain barrier disruption. *Glia* 70(5):892–912
Rakers C, Petzold GC. 2017. Astrocytic calcium release mediates peri-infarct depolarizations in a rodent stroke model. *J. Clin. Investig.* 127(2):511–16
Ramón y Cajal S. 1895. Algunas conjeturas sobre el mecanismo anatómico de la ideación, asociación y atención. *Rev. Med. Cir. Pract.* 36:497–508
Ramón y Cajal S. 1913. Sobre un nuevo proceder de impregnación de la neuroglia y sus resultados en los centros nerviosos del hombre y animales. *Trab. Lab. Investig. Biol. Univ. Madrid* 11:219–37
Rash JE, Yasumura T, Hudson CS, Agre P, Nielsen S. 1998. Direct immunogold labeling of aquaporin-4 in square arrays of astrocyte and ependymocyte plasma membranes in rat brain and spinal cord. *PNAS* 95(20):11981–86
Rempe RG, Hartz AMS, Bauer B. 2016. Matrix metalloproteinases in the brain and blood-brain barrier: versatile breakers and makers. *J. Cereb. Blood Flow Metab.* 36(9):1481–507
Ren Z, Iliff JJ, Yang L, Yang J, Chen X, et al. 2013. "Hit & Run" model of closed-skull traumatic brain injury (TBI) reveals complex patterns of post-traumatic AQP4 dysregulation. *J. Cereb. Blood Flow Metab.* 33(6):834–45
Robel S. 2017. Astroglial scarring and seizures: a cell biological perspective on epilepsy. *Neuroscientist* 23(2):152–68
Rosenegger DG, Tran CHT, Wamsteeker Cusulin JI, Gordon GR. 2015. Tonic local brain blood flow control by astrocytes independent of phasic neurovascular coupling. *J. Neurosci.* 35(39):13463–74
Rouach N, Koulakoff A, Abudara V, Willecke K, Giaume C. 2008. Astroglial metabolic networks sustain hippocampal synaptic transmission. *Science* 322(5907):1551–55
Rusnakova V, Honsa P, Dzamba D, Ståhlberg A, Kubista M, Anderova M. 2013. Heterogeneity of astrocytes: from development to injury—single cell gene expression. *PLOS ONE* 8(8):e69734

Salinet ASM, Robinson TG, Panerai RB. 2015. Effects of cerebral ischemia on human neurovascular coupling, CO_2 reactivity, and dynamic cerebral autoregulation. *J. Appl. Physiol.* 118(2):170–77

Savva GM, Stephan BCM, Alzheimer's Soc. Vasc. Dement. Syst. Rev. Group. 2010. Epidemiological studies of the effect of stroke on incident dementia: a systematic review. *Stroke* 41(1):e41–46

Schachtrup C, Ryu JK, Helmrick MJ, Vagena E, Galanakis DK, et al. 2010. Fibrinogen triggers astrocyte scar formation by promoting the availability of active TGF-β after vascular damage. *J. Neurosci.* 30(17):5843–54

Schreiner B, Romanelli E, Liberski P, Ingold-Heppner B, Sobottka-Brillout B, et al. 2015. Astrocyte depletion impairs redox homeostasis and triggers neuronal loss in the adult CNS. *Cell Rep.* 12(9):1377–84

Schulz K, Sydekum E, Krueppel R, Engelbrecht CJ, Schlegel F, et al. 2012. Simultaneous BOLD fMRI and fiber-optic calcium recording in rat neocortex. *Nat. Methods* 9(6):597–602

Seifert G, Hüttmann K, Binder DK, Hartmann C, Wyczynski A, et al. 2009. Analysis of astroglial K^+ channel expression in the developing hippocampus reveals a predominant role of the Kir4.1 subunit. *J. Neurosci.* 29(23):7474–88

Shandra O, Winemiller AR, Heithoff BP, Munoz-Ballester C, George KK, et al. 2019. Repetitive diffuse mild traumatic brain injury causes an atypical astrocyte response and spontaneous recurrent seizures. *J. Neurosci.* 39(10):1944–63

Shivers RR, Arthur FE, Bowman PD. 1988. Induction of gap junctions and brain endothelium-like tight junctions in cultured bovine endothelial cells: local control of cell specialization. *J. Submicrosc. Cytol. Pathol.* 20(1):1–14

Siddharthan V, Kim YV, Liu S, Kim KS. 2007. Human astrocytes/astrocyte-conditioned medium and shear stress enhance the barrier properties of human brain microvascular endothelial cells. *Brain Res.* 1147:39–50

Simard M, Arcuino G, Takano T, Liu QS, Nedergaard M. 2003. Signaling at the gliovascular interface. *J. Neurosci.* 23(27):9254–62

Simon MJ, Wang MX, Murchison CF, Roese NE, Boespflug EL, et al. 2018. Transcriptional network analysis of human astrocytic endfoot genes reveals region-specific associations with dementia status and tau pathology. *Sci. Rep.* 8(1):12389

Sinclair C, Kirk J, Herron B, Fitzgerald U, McQuaid S. 2007. Absence of aquaporin-4 expression in lesions of neuromyelitis optica but increased expression in multiple sclerosis lesions and normal-appearing white matter. *Acta Neuropathol.* 113(2):187–94

Sofroniew MV. 2020. Astrocyte reactivity: subtypes, states, and functions in CNS innate immunity. *Trends Immunol.* 41(9):758–70

Stackhouse TL, Mishra A. 2021. Neurovascular coupling in development and disease: focus on astrocytes. *Front. Cell Dev. Biol.* 9:702832

Stella N, Tencé M, Glowinski J, Prémont J. 1994. Glutamate-evoked release of arachidonic acid from mouse brain astrocytes. *J. Neurosci.* 14(2):568–75

Stokum JA, Shim B, Huang W, Kane M, Smith JA, et al. 2021. A large portion of the astrocyte proteome is dedicated to perivascular endfeet, including critical components of the electron transport chain. *J. Cereb. Blood Flow Metab.* 41(10):2546–60

Streeter GL. 1918. The developmental alterations in the vascular system of the brain of the human embryo. *Contrib. Embryol.* 8:5–38

Sweeney MD, Zhao Z, Montagne A, Nelson AR, Zlokovic BV. 2019. Blood-brain barrier: from physiology to disease and back. *Physiol. Rev.* 99(1):21–78

Szarka N, Pabbidi MR, Amrein K, Czeiter E, Berta G, et al. 2018. Traumatic brain injury impairs myogenic constriction of cerebral arteries: role of mitochondria-derived H_2O_2 and TRPV4-dependent activation of BK_{ca} channels. *J. Neurotrauma* 35(7):930–39

Takano T, Tian G-F, Peng W, Lou N, Libionka W, et al. 2006. Astrocyte-mediated control of cerebral blood flow. *Nat. Neurosci.* 9(2):260–67

Tao-Cheng JH, Nagy Z, Brightman MW. 1987. Tight junctions of brain endothelium in vitro are enhanced by astroglia. *J. Neurosci.* 7(10):3293–99

Tata M, Ruhrberg C, Fantin A. 2015. Vascularisation of the central nervous system. *Mech. Dev.* 138(Pt. 1):26–36

Teijido O, Martínez A, Pusch M, Zorzano A, Soriano E, et al. 2004. Localization and functional analyses of the MLC1 protein involved in megalencephalic leukoencephalopathy with subcortical cysts. *Hum. Mol. Genet.* 13(21):2581–94

Tien A-C, Tsai H-H, Molofsky AV, McMahon M, Foo LC, et al. 2012. Regulated temporal-spatial astrocyte precursor cell proliferation involves BRAF signalling in mammalian spinal cord. *Development* 139(14):2477–87

Toth P, Szarka N, Farkas E, Ezer E, Czeiter E, et al. 2016. Traumatic brain injury-induced autoregulatory dysfunction and spreading depression-related neurovascular uncoupling: pathomechanisms, perspectives, and therapeutic implications. *Am. J. Physiol. Heart Circ. Physiol.* 311(5):H1118–31

Tran EH, Hoekstra K, van Rooijen N, Dijkstra CD, Owens T. 1998. Immune invasion of the central nervous system parenchyma and experimental allergic encephalomyelitis, but not leukocyte extravasation from blood, are prevented in macrophage-depleted mice. *J. Immunol.* 161(7):3767–75

Tsai H-H, Li H, Fuentealba LC, Molofsky AV, Taveira-Marques R, et al. 2012. Regional astrocyte allocation regulates CNS synaptogenesis and repair. *Science* 337(6092):358–62

Villalba N, Sackheim AM, Nunez IA, Hill-Eubanks DC, Nelson MT, et al. 2017. Traumatic brain injury causes endothelial dysfunction in the systemic microcirculation through arginase-1-dependent uncoupling of endothelial nitric oxide synthase. *J. Neurotrauma* 34(1):192–203

Wälchli T, Mateos JM, Weinman O, Babic D, Regli L, et al. 2015. Quantitative assessment of angiogenesis, perfused blood vessels and endothelial tip cells in the postnatal mouse brain. *Nat. Protoc.* 10(1):53–74

Wang M-L, Li W-B. 2016. Cognitive impairment after traumatic brain injury: the role of MRI and possible pathological basis. *J. Neurol. Sci.* 370:244–50

Wang MX, Ray L, Tanaka KF, Iliff JJ, Heys J. 2021. Varying perivascular astroglial endfoot dimensions along the vascular tree maintain perivascular-interstitial flux through the cortical mantle. *Glia* 69(3):715–28

Wang Y-F, Parpura V. 2018. Astroglial modulation of hydromineral balance and cerebral edema. *Front. Mol. Neurosci.* 11:204

Wardlaw JM, Smith C, Dichgans M. 2019. Small vessel disease: mechanisms and clinical implications. *Lancet Neurol.* 18(7):684–96

Williamson MR, Fuertes CJA, Dunn AK, Drew MR, Jones TA. 2021. Reactive astrocytes facilitate vascular repair and remodeling after stroke. *Cell Rep.* 35(4):109048

Wolburg-Buchholz K, Mack AF, Steiner E, Pfeiffer F, Engelhardt B, Wolburg H. 2009. Loss of astrocyte polarity marks blood-brain barrier impairment during experimental autoimmune encephalomyelitis. *Acta Neuropathol.* 118(2):219–33

Xie L, Kang H, Xu Q, Chen MJ, Liao Y, et al. 2013. Sleep drives metabolite clearance from the adult brain. *Science* 342(6156):373–77

Xu Z, Xiao N, Chen Y, Huang H, Marshall C, et al. 2015. Deletion of aquaporin-4 in APP/PS1 mice exacerbates brain Aβ accumulation and memory deficits. *Mol. Neurodegener.* 10:58

Yi X, Lin J, Wang C, Zhou Q. 2017. CYP genetic variants, CYP metabolite levels, and neurologic deterioration in acute ischemic stroke in Chinese population. *J. Stroke Cerebrovasc. Dis.* 26(5):969–78

Zamanian JL, Xu L, Foo LC, Nouri N, Zhou L, et al. 2012. Genomic analysis of reactive astrogliosis. *J. Neurosci.* 32(18):6391–410

Zeppenfeld DM, Simon M, Haswell JD, D'Abreo D, Murchison C, et al. 2017. Association of perivascular localization of aquaporin-4 with cognition and Alzheimer disease in aging brains. *JAMA Neurol.* 74(1):91–99

Zhang Q, Liu C, Shi R, Zhou S, Shan H, et al. 2022. Blocking $C3d^+$/$GFAP^+$ A1 astrocyte conversion with semaglutide attenuates blood-brain barrier disruption in mice after ischemic stroke. *Aging Dis.* 13(3):943–59

Zhou M, Li R, Venkat P, Qian Y, Chopp M, et al. 2022. Post-stroke administration of L-4F promotes neurovascular and white matter remodeling in type-2 diabetic stroke mice. *Front. Neurol.* 13:863934

Zonta M, Angulo MC, Gobbo S, Rosengarten B, Hossmann K-A, et al. 2003. Neuron-to-astrocyte signaling is central to the dynamic control of brain microcirculation. *Nat. Neurosci.* 6(1):43–50