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Drowning stars: Reassessing the role of astrocytes in brain edema

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Abstract

Edema formation frequently complicates brain infarction, tumors and trauma. Despite the significant mortality of this condition, current treatment options are often ineffective or incompletely understood. Recent studies have revealed the existence of a brain-wide paravascular pathway for cerebrospinal (CSF) and interstitial fluid (ISF) exchange. The current review critically examines the contribution of this ‘glymphatic’ system to the main types of brain edema. We propose that in cytotoxic edema, energy depletion enhances glymphatic CSF influx, whilst suppressing ISF efflux. We also argue that paravascular inflammation or ‘paravascularitis’ plays a critical role in vasogenic edema. Finally, recent advances in diagnostic imaging of glymphatic function may hold the key to defining the edema profile of individual patients and thus enable more targeted therapy.

Keywords

cerebral edema; astrocyte; aquaporin-4; glymphatic; paravascular

Unclear waters in brain research?

Brain edema is a potentially fatal accumulation of fluid within the brain tissue, which can be caused by a range of medical conditions, including stroke, traumatic brain injury (TBI), brain tumors or metastases, meningitis, brain abscesses, water intoxication, altitude sickness, malignant hypertension, hypoglycemia and metabolic encephalopathies [1]. In the current review we will primarily discuss the acute causes of brain edema and refer to other texts for coverage on more chronic conditions such as peri-tumor edema [2, 3].

Edema is a pathological phenomenon that may aggravate injury by either causing cellular dysfunction if fluid accumulates intracellularly, or by increasing the distance through which oxygen, nutrients and wastes have to diffuse when it is extracellular. Fluid build-up is more

dangerous in the brain than in peripheral tissues for several macro- and micro-scopic reasons. Macroscopically, the brain is encased within a rigid skull causing any parenchymal swelling to increase intracranial pressure (ICP) and potentially compress other fluid compartments, such as the vasculature. This space limitation can set in motion a vicious cycle where elevated ICP compresses both capillary perfusion and venous drainage, which if unchecked causes further edema, cerebral ischemia, brain herniation and a lethal compression of brainstem cardiorespiratory centers. Brain edema can therefore be thought of as an intracranial compartment syndrome, and this global understanding forms the basis for core therapies such as trephination or surgical decompression, which have been practiced since ancient times [1].

Although several key molecular players that contribute to fluid accumulation have been identified in the last decade, our ‘microscopic’ understanding of brain edema is still incomplete. Key players likely include the water channel, aquaporin-4 (AQP4), the $\text{Na}^+\text{-K}^+\text{-Cl}^-$ cotransporter 1 (NKCC1), sulfonylurea receptor 1 (SUR1)-regulated non-selective cation channels ($\text{NC}_{\text{Ca-ATP}}$), matrix-metalloproteinase 9 (MMP-9), thrombin, substance P, complement receptors, chemokine receptors (e.g. CCR2) and vascular endothelial growth factor (VEGF) (see Glossary) [2, 4, 5, 6]. However, inhibiting or deleting some of these putative molecular targets can be both beneficial and detrimental, depending on when the treatment is initiated and the cause of the edema. We propose that these therapeutic heterogeneities can be at least partly explained by a previously unrecognized contribution from a brain-wide system for cerebrospinal fluid (CSF) and interstitial fluid (ISF) exchange, called the glymphatic pathway.

Composition of major water compartments in brain

To understand the molecular mechanisms that underlie brain edema, we first need to examine physiological water and ion homeostasis in the central nervous system (CNS) [3, 7, 8]. Water and solutes in the brain are distributed into four distinct fluid compartments separated by specialized cellular barriers; the intracellular fluid (ICF), ISF, CSF and vascular compartments (Fig. 1). CSF composition is primarily determined by the choroid plexus, and its production can be experimentally suppressed by inhibition of NKCC1 or carbonic anhydrase [9]. CSF contains a relatively high concentration of sodium to compensate for its low protein content [9]. ICF composition is energy-dependent and set up by the $\text{Na}^+\text{-K}^+\text{-ATPase}$ and several co-transporters relying on the transmembrane Na^+ gradient that this pump generates, such as $(\text{Na}^+)\text{-K}^+\text{-Cl}^-$ glutamate, glucose, $\text{Na}^+\text{-H}^+$ and $\text{Na}^+\text{-Ca}^{2+}$ -transporters [10]. The ICF composition in brain differs in several important respects between different cell types, broadly discussed here as neurons and glia (Table 1) [11, 12]. Neurons, for example, maintain a lower intracellular Cl^- concentration than glia through expression of KCC2, which is important for the hyperpolarizing effect of the inhibitory neurotransmitter, γ -aminobutyric acid (GABA) [13]. Glia have up to four-times greater water permeability due to enrichment with water channel aquaporin-4 (AQP4) [14]. ISF composition is dependent both on solutes exported from brain cells and exchange with CSF, and is very similar in composition to the latter. Conversely, the vascular compartment is largely independent from all the other water compartments in the CNS due to the blood-

brain barrier (BBB), which has a very low permeability to major osmolytes like Na^+K^+ and Cl^- and is impermeable to proteins [9, 15].

Brain, CSF and blood are separated by two concentric barriers

The different elements of the neurovascular unit are closely inter-dependent, and interrupting endothelial tight junctions, pericyte coverage or astrocyte function alone can compromise the entire BBB [16, 17, 18, 19, 20]. The barrier function in most living vertebrates is thought to lie in the vascular endothelium, which expresses abundant tight junctions that prevent solute entry into brain [16, 17, 21]. Unlike in other organs, cerebral endothelial cells are entirely devoid of water channel aquaporin-1 (AQP1) and other aquaporins [3]. However, the cerebral endothelium has extensive transporter expression, along with a potential for selective vesicular transcytosis (pinocytosis) in pathological settings, which can flux large amounts of water along with ions, glucose and amino acids [9, 22, 23]. Most vertebrates also possess a second successive ‘glial barrier’ outside the blood vessel wall [24], which has arisen multiple times during evolution, yet its exact function has long been unclear [25]. In rodents, a recent electron micrographic 3D reconstruction found this second barrier consists primarily of astrocyte end-foot processes, covering 99.7% of the vasculature, with the remaining area being made up of small 20 nm inter-cellular clefts [26]. Pericytes and microglial processes are also scattered in between the vascular wall and astrocytic endfeet [26]. The dimensions of the intercellular clefts would imply that the filter size of the glial barrier is large enough for nearly all mammalian proteins (median size ~35 kDa and diameter 2–3 nm; including serum albumin ~70 kDa and 4–6 nm) [27]. Additionally, the ‘glial barrier’ has a much higher water permeability than the BBB, as almost 40% of its surface area is studded with AQP4 water channels, expressed as large rafts of tetramers called orthogonal arrays of particles (OAPs) [8].

The glymphatic system facilitates rapid interstitial fluid turnover

As an organ, the brain combines a densely cellular tissue with a disproportionately high metabolic rate, indicating a need for rapid fluid turnover within a tight space (Fig. 1) [28]. However, the brain is entirely devoid of a lymphatic drainage system, which normally facilitates ISF turnover in other tissues of the body [29]. Until recently the rapid ISF turnover observed experimentally in brain ($0.15\text{--}0.29\text{ uL min}^{-1}\text{ g}^{-1}$) was thought to occur mainly via passive intercellular diffusion [30]. However, the extracellular compartment in the brain is so narrow and tortuous that it would take albumin-sized molecules 10 hours to diffuse 1 mm [31]. Recent evidence may help answer this puzzle. Data from our group suggests that two distinct barriers (endothelial and glial) at the blood-brain interface may have evolved to delineate a separate paravascular ‘highway’ for fluid exchange [32, 33, 34, 35, 36]. Since the brain is also densely vascularized, with an inter-capillary distance of 17–58 μm in grey matter [37], a slow paravascular circulation provides unique access to all areas. Our results indicate that the paravascular compartment between glial end-foot processes and vascular cells is continuous all the way down along penetrating arterioles to capillaries and continues along veins; permitting ISF influx along almost all penetrating arteries (called the *Virchow-Robin space*), and efflux along a select population of large-caliber ‘deep veins’ [32].

Due to the lymphatic-like function of this system and its dependence on *convective* water movement facilitated by glial cells it was termed the ‘glymphatic’ system [32]. Deleting the glial water channel AQP4 decreases glymphatic solute movement more than 60% [32]. The glymphatic system thus provides a novel explanation to the paradoxical localization of AQP4, which is only expressed on astroglial end-feet abutting the vessel wall, whilst the endothelial barrier is entirely devoid of aquaporins (see Fig. 1) [8]. Several factors have been identified that could drive glymphatic bulk-flow, such as arterial pulse-pressure, which when either pharmacologically or surgically altered can increase paravascular tracer movement by 20–30% [34]. Glymphatic ISF turnover might also be indirectly regulated by other parameters such as neuronal activity, sleep, and anesthesia, which all alter the dimensions of the ISF compartment [31, 35]. Glial control of extracellular matrix tension and thus hydrostatic ISF pressure via cytoskeletal remodeling during physiology and in reactive gliosis could also influence glymphatic function [38]. Finally, the glymphatic system may not only be important for fluid transport, but recent evidence also suggests this system facilitates the movement of lipids, signaling molecules and immune cells [33, 39, 40]. The glymphatic system may therefore represent not only a missing link in our understanding of physiological water and ion homeostasis, but also brain edema [32, 41, 42].

Current concepts regarding brain edema formation

To explain the heterogeneous features of brain edema in different pathologies and the different response to anti-edema therapies most authors distinguish between at least two different types: *cytotoxic* and *vasogenic* (Box 1) [43]. All cells require energy to maintain volume homeostasis, and energy depletion can therefore cause brain cell swelling, termed *cytotoxic* edema [44]. If energy supply is not restored this process will inevitably lead to cell lysis with spillage of all intracellular content, also termed necrotic cell death [43]. However, because there is no net entry of fluid into the brain from the vasculature there should in theory be no overall tissue swelling with ‘pure’ *cytotoxic edema*, merely a fluid redistribution. As we will discuss next, this is not the case, and most authors use the term *ionic edema* for the net entry of water and ions into the brain that accompanies *cytotoxic edema* [43]. We would also argue that the term *cytotoxic* should not be used for intracellular fluid accumulation unrelated to energy depletion (e.g. osmotic), as the mechanisms differ significantly [45]. *Vasogenic* brain edema is traditionally thought to represent the net extravasation of protein-rich fluid into brain following a breakdown of the BBB [46]. This process is believed to involve a widening of the inter-cellular clefts between endothelia and a loss of tight junctions [47, 48]. *Vasogenic* edema will therefore per definition require vascular perfusion, and should be more important when blood flow is increased or normal (e.g. brain tumors and metastases), than for instance at the core of a brain infarct [49]. However, insults that generate mainly *cytotoxic* and *ionic* edema early on (< 24 hours), such as TBI and stroke, are also known to develop a second peak of *vasogenic* edema after 2–4 days; termed the *biphasic* edema response [43, 50, 51].

Reassessing the role of interstitial fluid dynamics in brain edema

The current literature leaves several important questions unanswered about the formation and resorption of brain edema (Box 2). To explore these issues we will next discuss the

potential contribution of the glymphatic system to the different stages of brain edema, using focal ischemia as an example. Cytotoxic cell swelling begins only minutes after an acute infarct [52], whilst overall brain swelling typically occurs more slowly, with intracranial pressure reaching a peak within the first 24 hours for rats and 48–72 hours for humans [3, 43, 46]. How does this acute energy depletion and *cytotoxic* fluid redistribution into cells cause net fluid influx into a tissue that has a severely compromised blood perfusion and microcirculation [53]? The answer is likely that most fluid accumulation occurs outside the infarct core, in a much larger perfused region called the *penumbra*, where cytotoxic edema plays a lesser role [4, 54, 55, 56] (Fig. 2). Previous studies exposing entire brain slices or cell cultures to oxygen and glucose deprivation (OGD) therefore provide a poor model of brain edema. To understand focal brain edema we need to examine the interplay between cytotoxic fluid redistribution in an infarcted core and net fluid entry into a better-perfused *penumbra* [57].

Glymphatic fluid influx could drive ionic brain edema formation

If overall brain swelling or *ionic edema* requires net fluid entry into the tissue surrounding an acute infarct, where does this fluid come from? Arguably, it can only come from blood or CSF [43]. Current theories were developed prior to the discovery of the glymphatic system and suggest that *ionic edema* originates from the vasculature [3]. An interesting recent theory for instance suggests that Na^+ and water influx across the BBB could occur via NKCC1 and $\text{NC}_{\text{Ca-ATP}}$ expressed on endothelial cells in the ATP-depleted core of an infarct [4]. However, we would argue that fluid influx from across the BBB is less likely for several reasons. First, the core of the injury or infarct is poorly perfused, and would therefore exhibit mainly cytotoxic fluid redistribution [58]. Second, the BBB, or more precisely endothelial tight junctions, are relatively intact in the perfused penumbra of an infarct or injury [9, 15]. An intact BBB would imply a low osmotic, hydrostatic and ion permeability (including Na^+Cl^- and K^+) [9]. However, altered endothelial expression of sodium transporter following ischemia could increase ionic and osmotic permeability [4, 59, 60]. Third, transcriptome data suggest that vascular endothelial cells express low levels of NKCC1 and $\text{NC}_{\text{Ca-ATP}}$ during basal conditions in the mouse brain [61].

An alternative explanation might be that edema fluid is entering the brain parenchyma as CSF via the low-resistance para-arterial space. Arguably, this can only happen as a result of increased CSF influx into the parenchyma, decreased ISF efflux or a combination of the two. Glymphatic removal of excess ISF is likely decreased following injury or infarction [62, 63], but the exact mechanisms will be discussed in subsequent sections, as these mechanisms are also important for edema resorption. With regards to CSF influx into the parenchyma, continuous CSF secretion by the choroid plexus is likely the primary source of this fluid [9]. To facilitate this secretion, choroid plexus cells have NKCC1 expression levels that are several orders of magnitude higher than endothelial cells [61], and this expression is further increased after injury [64]. Previous studies showing that NKCC1 inhibition decreases brain edema could therefore, at least in theory, relate to lower choroid plexus production CSF, reducing glymphatic influx [65]. During basal conditions, para-arterial movement of CSF is also thought to be driven by the pulsatility of the vascular wall [34], which is generally increased after a stroke, and can become further increase if intracranial

pressure rises as a result of the *Cushing reflex* [66]. Finally, pericyte constriction and microvascular collapse could force an expansion of the paravascular space, decreasing resistance to fluid influx by this route [67].

Ultimately, edema build-up from either a vascular or paravascular source requires net solute entry into the brain. As is evident from Table 1, the Na^+ concentration in CSF is greater than blood, to compensate for CSF having almost no proteins to exert osmotic pressure. ATP depletion with consequent Na^+ - K^+ -ATPase and Na^+ -cotransporter shut down and cytotoxic cell swelling in the infarct core would cause an extensive redistribution of Na^+ and Cl^- from the ISF to the ICF [49, 68]. $\text{NC}_{\text{Ca-ATP}}$ channels activated by ATP depletion and expressed on parenchymal cells might facilitate this process [4]. Gap junction connections between astrocytes might also rapidly distribute this Na^+ -influx across the glial syncytium [19, 69]. Na^+ and Cl^- redistribution would in turn make ISF slightly hypo-osmolar relative to CSF [49]. As opposed to the BBB, the perivascular glial endfoot barrier provides almost no osmotic resistance to diffusion by virtue of its AQP4 expression, and this osmotic ISF-CSF gradient would in theory pull CSF into the ISF compartment [32]. Influx of Na^+ into the brain parenchyma is usually accompanied by net K^+ efflux that could offset the osmotic Na^+ load [43]. However, in brain edema K^+ efflux is likely to be much smaller than the Na^+ influx for several reasons [4, 15, 58], including negatively charged intracellular proteins retaining ions in the ICF (see Table 1).

Astrocytes: swollen glue or drowning stars?

Having examined the origins of edema fluid; we next want to discuss where it might accumulate. Because of their proximity to the vascular compartment and high-water permeability, astrocytes have long been thought to accumulate most of the edema fluid intracellularly [5]. At first glance, many histological and cell culture studies seem to support this model [5, 70]. However, more recent slice and *in vivo* imaging studies have revealed more contradictory results, with some studies finding that astrocytes swell readily [71, 72, 73, 74], whilst other reports finding that astrocytes regulate volume tightly [75, 76, 77, 78]. What could explain this discrepancy? Technical limitations such as low image resolution or fluorochrome dilution upon swelling could cause imaging results to falsely underestimate or overlook cell volume changes [79]. Conversely, experience from our lab also suggests that it is possible to overestimate volume changes, as ‘unhealthy’ astrocyte subject to phototoxicity, with inadequate energy supply, or in damaged tissue swell much more readily [76, 78].

It is interesting to speculate whether the extensive astrocytic expression of salt and water transporters has been misinterpreted as a pathway for intracellular fluid accumulation, and might instead represent a pathway for paravascular fluid elimination [32]. If ‘energized’ astrocytes are capable of actively regulating their volume across the broad range of osmotic and ionic challenges some studies suggest ($>20\%$ osmolarity drop and $>50 \text{ mM } [\text{K}^+]_o$ increase) [76, 77, 78], edema fluid might at least initially accumulate in the interstitial space of the penumbra; ‘drowning’ the astrocytes by increasing the distance for nutrient and waste diffusion. Consistent with this idea, increasing the resistance to glymphatic circulation by deleting glial AQP4 not only increases ISF volume during basal conditions [80], but also

worsens experimental interstitial edema and causes a higher rate of spontaneous hydrocephalus in knock-out animals [3]. Conversely, ischemia-related cytokines released from the core of an infarct can alter astrocytic solute transporter expression in the penumbra, and potentially compromise astrocyte volume regulation [59]. Unfortunately, no studies have directly examined penumbral ISF volume *in vivo* during brain edema to help us resolve this important point. A more detailed discussion of astrocyte volume regulation is beyond the remit of the current review we refer the reader to other overview articles on this topic [3, 8, 81].

Cerebral paravascuilitis is the hallmark of vasogenic edema

Although the early stages of brain edema formation after an infarct are characterized by salt and water influx (0 to 3 h), the later stages (>3 h to 14 days) also involve BBB opening and significant extravasation of plasma proteins [43, 46, 82, 83]. Traditionally, extensive BBB opening has been thought to cause edema by increased hydraulic conductivity causing blood-pressure-dependent protein extravasation, with osmotic ‘fluid-drag’ [84]. However, experimental measurements have revealed that the direct osmotic effects of albumin extravasation are not temporally correlated with edema formation and that the estimated ‘fluid-drag’ can only account for a fraction of the total water influx (<10%) [58]. Extravasated proteins are also rapidly taken up and subjected to lysosomal degradation by microglia and astrocytes, rather than being deposited in the interstitial space [85]. Thus the osmotic effects of protein extravasation are unlikely to explain *vasogenic edema*. Instead, radioisotope studies indicate that similar to *ionic edema*, *vasogenic edema* also correlates best with $^{22}\text{Na}^+$ influx into the brain. Since the core of an infarct or injury has decreased $^{22}\text{Na}^+$ permeability during the later stages (>3 h), most of this salt and water entry must also occur in the penumbra [58, 86, 87].

What alternative mechanisms might mediate vasogenic edema? We would argue that the delayed onset of vasogenic edema most closely mirrors the paravascular immune response or *paravascuilitis* that can be initiated by necrotic debris, plasma proteins, cytokines and/or tumor cells. Inflammation is a potent trigger and potentiator of brain edema through several mechanisms including hyperemia, BBB opening, leukocyte influx, osmolyte production, immune complex accumulation, reactive oxygen species generation and cytokine-related cell swelling [6, 85]. Several recent lines of evidence support this immune response being primarily paravascular, rather than across the BBB. Firstly, CSF represents a major reservoir for complement factors and immune cells in the CNS, containing up to 3000 leukocytes per ml during normal conditions [88, 89]. Second, perivascular astrocytes and microglia can produce most major complement factors and cytokines, secreting them via the paravascular space directly into CSF [89]. Third, cytokine secretion and leukocyte infiltration is primarily concentrated in the paravascular region of brain parenchyma and in the choroid plexi [90, 91, 92, 93]. Finally, paravascular immune complex accumulation has long been recognized in a variety of brain disorders, including stroke, TBI and even neurodegenerative disorders like Alzheimer disease [40]. Conversely, blood-borne leukocytes would have limited interaction with both CSF and ISF in the perfused regions surrounding an injury or infarct due to the (at least initially) intact BBB discussed above [47]. The only exception to this rule

is intracerebral hemorrhage, where BBB integrity is completely lost causing a spillage of all intravascular cells and mediators [43].

We would therefore suggest that the paravascular space acts as a crucial immune compartment during vasogenic edema formation where astrocytes, microglia, pericytes, endothelial cells and leukocytes can interact directly [33, 94]. Peri-arterial influx and peri-venous efflux of CSF from the parenchyma could drive antigen and leukocyte recirculation to T-helper lymphocytes and dendritic cells in the choroid plexi, which may act similarly to regional lymph nodes [95]. Following an acute brain insult, perivascular astrocytes and resident microglial immune cells are known to rapidly produce pro-inflammatory cytokines such as VEGF-A, IL-1 β , TNF- α and IFN- γ [51, 96, 97]. Genetic variants related to these inflammatory mediators are strongly associated with worse outcomes following TBI or stroke [98, 99, 100].

Although inflammation and edema might be detrimental in the short term due to space constraints, this process may also be necessary for removal of cellular debris and may paradoxically help seal the BBB faster by forming a gliotic scar prior to re-establishment of tight-junctions [101]. It is perhaps therefore unsurprising that both unselective (e.g. dexamethasone) and selective (e.g. blocking IFN- γ , chemokine receptor 2, MMP9, VEGF, IL-6, TNF- α and TGF- β) anti-inflammatory therapies can both improve and worsen brain edema depending on when the treatment is instituted and what the underlying cause is (e.g. infarction, trauma, tumors) [92, 95, 102, 103, 104, 105, 106]. In conclusion, vasogenic edema seems to represent a delayed and long-lasting increase in glymphatic fluid influx of salt and water that may have evolved to facilitate paravascular leukocyte and cytokine delivery.

Brain edema is absorbed into paravascular CSF rather than blood

To restore normal function brain edema needs to be cleared along with excess ions and proteins that have leaked across the BBB in a process that can take many weeks. Traditionally this is thought to occur by clearance via the vascular compartment [42]. However, hydrostatic and osmotic forces would instead favor fluid egress from the vasculature into the brain so long as the BBB is open, and BBB closure often precedes edema resorption [9, 49]. This discrepancy has led some authors to suggest drainage via subarachnoid CSF, but it was unclear how fluid from deep in the parenchyma reaches the brain surface [42]. The recently discovered glymphatic system provides an elegant explanation to this problem [32]. Our data suggest that excess parenchymal fluid and solutes can be effectively cleared from the interstitial space via the paravascular space along a subset of large veins [32]. This glymphatic model may also better explain the long-term consequences of surviving brain edema, such as chronic post-traumatic encephalopathy, syndrome of the trephine and vascular dementia. Structural changes to the glymphatic system, such as perivascular reactive gliosis and AQP4 mislocalization, can persist for almost a month after injury or infarction [63, 82]. Both TBI and stroke can therefore cause a long-lasting impairment of glymphatic clearance of waste products such as β -amyloid [62, 63], and may be responsible for chronically enlarged Virchow-Robin space often seen in these patient groups [107].

Concluding remarks

In summary, most brain disorders generate a complex spatial and temporal pattern of brain edema, involving several distinct mechanisms, which respond very differently to therapies (Box 1). Patients who for instance suffer an ischemic stroke or TBI likely experience some immediate *cytotoxic* edema at the core of the injury, whilst the surround tissue may first develop *ionic* and later *vasogenic* edema (see Fig. 2) [43, 46]. Carefully characterizing the type of brain edema and choosing the time-point at which to intervene might therefore be crucial. Decompressive craniectomy can for example be helpful for the early *cytotoxic* and *ionic* edema phase, whilst it can be detrimental if a *vasogenic* component is present, or during the resorption phase [43]. Similarly, recently suggested therapies targeting water movement via glial AQP4 have been shown to be beneficial in conditions associated with primarily *cytotoxic* and *ionic* edema by limiting water influx (e.g. large ischemic strokes, water intoxication) [5, 14]. Conversely, AQP4 inhibition or deletion adversely affects outcome in some (e.g. tumors, abscesses), but not all (e.g. meningitis), diseases associated with *vasogenic* edema, or when given during the recovery phase of edema [3].

Current tools have limited sensitivity and specificity to accurately determine what ‘type’ of edema a patient has [43]. Brain edema is frequently examined in patients using diffusion-tensor weighted (DWI) magnetic resonance imaging (MRI) looking at the apparent diffusion coefficient (ADC) of isotropic water movement. However, a decreased ADC occurs both in *cytotoxic* edema (ISF shrinkage) and in isolated ISF expansion, making this tool less useful in distinguishing types of brain edema and guiding therapy [108, 109]. It is therefore interesting to speculate whether a recently characterized MRI tracer imaging technique examining glymphatic function might better guide the choice and timing of treatment [41, 110]. In conclusion, further exploration of the critically important paravascular region may hold the key to developing new edema therapies, correctly staging different types of edema and reducing long-term morbidity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Marmarou A. The pathophysiology of brain edema and elevated intracranial pressure. *Cleve Clin J Med*. 2004; 71(Suppl 1):S6–S8. [PubMed: 14964471]
2. Gerstner ER, et al. VEGF inhibitors in the treatment of cerebral edema in patients with brain cancer. *Nat Rev Clin Oncol*. 2009; 6:229–236. [PubMed: 19333229]
3. Papadopoulos MC, Verkman AS. Aquaporin water channels in the nervous system. *Nat Rev Neurosci*. 2013; 14:265–277. [PubMed: 23481483]
4. Simard JM, et al. Newly expressed SUR1-regulated NC(Ca-ATP) channel mediates cerebral edema after ischemic stroke. *Nat Med*. 2006; 12:433–440. [PubMed: 16550187]

5. Manley GT, et al. Aquaporin-4 deletion in mice reduces brain edema after acute water intoxication and ischemic stroke. *Nat. Med.* 2000; 6:159–163. [PubMed: 10655103]
6. Ralay Ranaivo H, et al. Albumin induces upregulation of matrix metalloproteinase-9 in astrocytes via MAPK and reactive oxygen species-dependent pathways. *J Neuroinflammation.* 2012; 9:68. [PubMed: 22507553]
7. Simard M, Nedergaard M. The neurobiology of glia in the context of water and ion homeostasis. *Neuroscience.* 2004; 129:877–896. [PubMed: 15561405]
8. Nagelhus EA, Ottersen OP. Physiological roles of aquaporin-4 in brain. *Physiol Rev.* 2013; 93:1543–1562. [PubMed: 24137016]
9. MacAulay N, Zeuthen T. Water transport between CNS compartments: contributions of aquaporins and cotransporters. *Neuroscience.* 2010; 168:941–956. [PubMed: 19761815]
10. Kirischuk S, et al. Sodium dynamics: another key to astroglial excitability? *Trends Neurosci.* 2012; 35:497–506. [PubMed: 22633141]
11. Rose CR, Ransom BR. Intracellular sodium homeostasis in rat hippocampal astrocytes. *J Physiol.* 1996; 491(Pt 2):291–305. [PubMed: 8866855]
12. Rose CR, Ransom BR. Regulation of intracellular sodium in cultured rat hippocampal neurones. *J Physiol.* 1997; 499(Pt 3):573–587. [PubMed: 9130155]
13. Blaesse P, et al. Cation-chloride cotransporters and neuronal function. *Neuron.* 2009; 61:820–838. [PubMed: 19323993]
14. Haj-Yasein NN, et al. Glial-conditional deletion of aquaporin-4 (Aqp4) reduces blood-brain water uptake and confers barrier function on perivascular astrocyte endfeet. *Proc. Natl. Acad. Sci. U. S. A.* 2011; 108:17815–17820. [PubMed: 21990350]
15. Betz AL, et al. Blood-brain barrier permeability and brain concentration of sodium, potassium, and chloride during focal ischemia. *J Cereb Blood Flow Metab.* 1994; 14:29–37. [PubMed: 8263055]
16. Armulik A, et al. Pericytes regulate the blood-brain barrier. *Nature.* 2010; 468:557–561. [PubMed: 20944627]
17. Daneman R, et al. Pericytes are required for blood-brain barrier integrity during embryogenesis. *Nature.* 2010; 468:562–566. [PubMed: 20944625]
18. Pekny M, et al. Impaired induction of blood-brain barrier properties in aortic endothelial cells by astrocytes from GFAP-deficient mice. *Glia.* 1998; 22:390–400. [PubMed: 9517571]
19. Ezan P, et al. Deletion of astroglial connexins weakens the blood-brain barrier. *J Cereb Blood Flow Metab.* 2012; 32:1457–1467. [PubMed: 22472609]
20. Lien CF, et al. Absence of glial alpha-dystrobrevin causes abnormalities of the blood-brain barrier and progressive brain edema. *J Biol Chem.* 2012; 287:41374–41385. [PubMed: 23043099]
21. Luissint AC, et al. Tight junctions at the blood brain barrier: physiological architecture and disease-associated dysregulation. *Fluids Barriers CNS.* 2012; 9:23. [PubMed: 23140302]
22. Zeuthen T, Macaulay N. Cotransport of water by Na(+)-K(+)-2Cl(−) cotransporters expressed in *Xenopus* oocytes: NKCC1 versus NKCC2. *J Physiol.* 2012; 590:1139–1154. [PubMed: 22250214]
23. Hamann S, et al. Cotransport of water by the Na⁺-K⁺-2Cl(−) cotransporter NKCC1 in mammalian epithelial cells. *J Physiol.* 2010; 588:4089–4101. [PubMed: 20819947]
24. Mayer F, et al. Evolutionary conservation of vertebrate blood-brain barrier chemoprotective mechanisms in *Drosophila*. *J Neurosci.* 2009; 29:3538–3550. [PubMed: 19295159]
25. Bundgaard M, Abbott NJ. All vertebrates started out with a glial blood-brain barrier 4–500 million years ago. *Glia.* 2008; 56:699–708. [PubMed: 18338790]
26. Mathiisen TM, et al. The perivascular astroglial sheath provides a complete covering of the brain microvessels: an electron microscopic 3D reconstruction. *Glia.* 2010; 58:1094–1103. [PubMed: 20468051]
27. Erickson HP. Size and shape of protein molecules at the nanometer level determined by sedimentation, gel filtration, and electron microscopy. *Biol Proced Online.* 2009; 11:32–51. [PubMed: 19495910]
28. Nedergaard M. Neuroscience. Garbage truck of the brain. *Science.* 2013; 340:1529–1530. [PubMed: 23812703]
29. Begley DJ. Brain superhighways. *Sci Transl Med.* 2012; 4:147fs129.

30. Abbott NJ. Evidence for bulk flow of brain interstitial fluid: significance for physiology and pathology. *Neurochem Int.* 2004; 45:545–552. [PubMed: 15186921]
31. Sykova E, Nicholson C. Diffusion in brain extracellular space. *Physiol Rev.* 2008; 88:1277–1340. [PubMed: 18923183]
32. Iliff JJ, et al. A Paravascular Pathway Facilitates CSF Flow Through the Brain Parenchyma and the Clearance of Interstitial Solutes, Including Amyloid beta. *Sci Transl Med.* 2012; 4:147ra111.
33. Rangroo Thrane V, et al. Paravascular microcirculation facilitates rapid lipid transport and astrocyte signaling in the brain. *Sci Rep.* 2013; 3:2582. [PubMed: 24002448]
34. Iliff JJ, et al. Cerebral Arterial Pulsation Drives Paravascular CSF-Interstitial Fluid Exchange in the Murine Brain. *J Neurosci.* 2013; 33:18190–18199. [PubMed: 24227727]
35. Xie L, et al. Sleep drives metabolite clearance from the adult brain. *Science.* 2013; 342:373–377. [PubMed: 24136970]
36. Yang L, et al. Evaluating glymphatic pathway function utilizing clinically relevant intrathecal infusion of CSF tracer. *J Transl Med.* 2013; 11:107. [PubMed: 23635358]
37. Karbowski J. Scaling of brain metabolism and blood flow in relation to capillary and neural scaling. *PLoS One.* 2011; 6:e26709. [PubMed: 22053202]
38. Langevin HM, et al. Cellular control of connective tissue matrix tension. *J Cell Biochem.* 2013; 114:1714–1719. [PubMed: 23444198]
39. Chan WY, et al. The origin and cell lineage of microglia: new concepts. *Brain Res Rev.* 2007; 53:344–354. [PubMed: 17188751]
40. Carare RO, et al. Immune complex formation impairs the elimination of solutes from the brain: implications for immunotherapy in Alzheimer's disease. *Acta Neuropathol Commun.* 2013; 1:48. [PubMed: 24252464]
41. Iliff JJ, et al. Brain-wide pathway for waste clearance captured by contrast-enhanced MRI. *J Clin Invest.* 2013; 123:1299–1309. [PubMed: 23434588]
42. Ohata K, Marmarou A. Clearance of brain edema and macromolecules through the cortical extracellular space. *J Neurosurg.* 1992; 77:387–396. [PubMed: 1380546]
43. Simard JM, et al. Brain oedema in focal ischaemia: molecular pathophysiology and theoretical implications. *Lancet Neurol.* 2007; 6:258–268. [PubMed: 17303532]
44. Thrane AS, et al. Critical role of aquaporin-4 (AQP4) in astrocytic Ca²⁺ signaling events elicited by cerebral edema. *Proc. Natl. Acad. Sci. U. S. A.* 2011; 108:846–851. [PubMed: 21187412]
45. Liang D, et al. Cytotoxic edema: mechanisms of pathological cell swelling. *Neurosurg Focus.* 2007; 22:E2. [PubMed: 17613233]
46. Marmarou A. A review of progress in understanding the pathophysiology and treatment of brain edema. *Neurosurg Focus.* 2007; 22:E1.
47. Krueger M, et al. Blood-brain barrier breakdown after embolic stroke in rats occurs without ultrastructural evidence for disrupting tight junctions. *PLoS One.* 2013; 8:e56419. [PubMed: 23468865]
48. Witt KA, et al. Effects of hypoxia-reoxygenation on rat blood-brain barrier permeability and tight junctional protein expression. *Am J Physiol Heart Circ Physiol.* 2003; 285:H2820–H2831. [PubMed: 12907427]
49. Ishimaru S, et al. Relationship between blood flow and blood-brain barrier permeability of sodium and albumin in focal ischaemia of rats: a triple tracer autoradiographic study. *Acta Neurochir (Wien).* 1993; 120:72–80. [PubMed: 8434521]
50. Rosenberg GA, Yang Y. Vasogenic edema due to tight junction disruption by matrix metalloproteinases in cerebral ischemia. *Neurosurg Focus.* 2007; 22:E4. [PubMed: 17613235]
51. Witt KA, et al. Reoxygenation stress on blood-brain barrier paracellular permeability and edema in the rat. *Microvasc Res.* 2008; 75:91–96. [PubMed: 17651765]
52. van der Toorn A, et al. Dynamic changes in water ADC, energy metabolism, extracellular space volume, and tortuosity in neonatal rat brain during global ischemia. *Magn Reson Med.* 1996; 36:52–60. [PubMed: 8795020]

53. Dawson DA, et al. Temporal impairment of microcirculatory perfusion following focal cerebral ischemia in the spontaneously hypertensive rat. *Brain Res.* 1997; 749:200–208. [PubMed: 9138719]
54. Kohno K, et al. Relationship between diffusion-weighted MR images, cerebral blood flow, and energy state in experimental brain infarction. *Magn Reson Imaging.* 1995; 13:73–80. [PubMed: 7898282]
55. Hossmann KA. Pathophysiology and therapy of experimental stroke. *Cell Mol Neurobiol.* 2006; 26:1057–1083. [PubMed: 16710759]
56. Chen F, Ni YC. Magnetic resonance diffusion-perfusion mismatch in acute ischemic stroke: An update. *World J Radiol.* 2012; 4:63–74. [PubMed: 22468186]
57. Heiss WD. The concept of the penumbra: can it be translated to stroke management? *Int J Stroke.* 2010; 5:290–295. [PubMed: 20636712]
58. Menzies SA, et al. Contributions of ions and albumin to the formation and resolution of ischemic brain edema. *J Neurosurg.* 1993; 78:257–266. [PubMed: 8421208]
59. Jung YW, et al. Altered expression of sodium transporters in ischemic penumbra after focal cerebral ischemia in rats. *Neurosci Res.* 2007; 59:152–159. [PubMed: 17662498]
60. Mokgokong R, et al. Ion transporters in brain endothelial cells that contribute to formation of brain interstitial fluid. *Pflugers Arch.* 2014; 466:887–901. [PubMed: 24022703]
61. Daneman R, et al. The mouse blood-brain barrier transcriptome: a new resource for understanding the development and function of brain endothelial cells. *PLoS One.* 2010; 5:e13741. [PubMed: 21060791]
62. Ren Z, et al. 'Hit & Run' model of closed-skull traumatic brain injury (TBI) reveals complex patterns of post-traumatic AQP4 dysregulation. *J. Cereb. Blood Flow Metab.* 2013; 33:834–845. [PubMed: 23443171]
63. Wang M, et al. Cognitive deficits and delayed neuronal loss in a mouse model of multiple microinfarcts. *J Neurosci.* 2012; 32:17948–17960. [PubMed: 23238711]
64. Lu KT, et al. Inhibition of the Na⁺-K⁺-2Cl⁻ cotransporter in choroid plexus attenuates traumatic brain injury-induced brain edema and neuronal damage. *Eur J Pharmacol.* 2006; 548:99–105. [PubMed: 16962576]
65. Kahle KT, et al. Molecular mechanisms of ischemic cerebral edema: role of electroneutral ion transport. *Physiology (Bethesda).* 2009; 24:257–265. [PubMed: 19675357]
66. He J, et al. Effects of immediate blood pressure reduction on death and major disability in patients with acute ischemic stroke: the CATIS randomized clinical trial. *Jama.* 2014; 311:479–489. [PubMed: 24240777]
67. Hall CN, et al. Capillary pericytes regulate cerebral blood flow in health and disease. *Nature.* 2014; 508:55–60. [PubMed: 24670647]
68. Mies G, et al. Ischemic thresholds of cerebral protein synthesis and energy state following middle cerebral artery occlusion in rat. *J Cereb Blood Flow Metab.* 1991; 11:753–761. [PubMed: 1874807]
69. Rose CR, Ransom BR. Gap junctions equalize intracellular Na⁺ concentration in astrocytes. *Glia.* 1997; 20:299–307. [PubMed: 9262234]
70. Kimelberg HK. Anisotonic media and glutamate-induced ion transport and volume responses in primary astrocyte cultures. *J Physiol (Paris).* 1987; 82:294–303. [PubMed: 3503932]
71. Risher WC, et al. Real-time passive volume responses of astrocytes to acute osmotic and ischemic stress in cortical slices and in vivo revealed by two-photon microscopy. *Glia.* 2009; 57:207–221. [PubMed: 18720409]
72. Nase G, et al. Water entry into astrocytes during brain edema formation. *Glia.* 2008; 56:895–902. [PubMed: 18351631]
73. Risher WC, et al. Persistent astroglial swelling accompanies rapid reversible dendritic injury during stroke-induced spreading depolarizations. *Glia.* 2012; 60:1709–1720. [PubMed: 22821441]
74. Chvatal A, et al. Three-dimensional confocal morphometry - a new approach for studying dynamic changes in cell morphology in brain slices. *J Anat.* 2007; 210:671–683. [PubMed: 17488344]

75. Thrane VR, et al. Ammonia triggers neuronal disinhibition and seizures by impairing astrocyte potassium buffering. *Nat Med*. 2013
76. Takano T, et al. Cortical spreading depression causes and coincides with tissue hypoxia. *Nat. Neurosci*. 2007; 10:754–762. [PubMed: 17468748]
77. Zhou N, et al. Transient swelling, acidification, and mitochondrial depolarization occurs in neurons but not astrocytes during spreading depression. *Cereb Cortex*. 2010; 20:2614–2624. [PubMed: 20176688]
78. Thrane AS, et al. Critical role of aquaporin-4 (AQP4) in astrocytic Ca²⁺ signaling events elicited by cerebral edema. *Proc Natl Acad Sci U S A*. 2011; 108:846–851. [PubMed: 21187412]
79. Zelenina M, Brismar H. Osmotic water permeability measurements using confocal laser scanning microscopy. *Eur Biophys J*. 2000; 29:165–171. [PubMed: 10968208]
80. Haj-Yasein NN, et al. Glial-conditional deletion of aquaporin-4 (Aqp4) reduces blood-brain water uptake and confers barrier function on perivascular astrocyte endfeet. *Proc Natl Acad Sci U S A*. 2011; 108:17815–17820. [PubMed: 21990350]
81. Stokum JA, et al. Mechanisms of Astrocyte-Mediated Cerebral Edema. *Neurochem Res*. 2014
82. Ren Z, et al. 'Hit & Run' model of closed-skull traumatic brain injury (TBI) reveals complex patterns of post-traumatic AQP4 dysregulation. *J Cereb Blood Flow Metab*. 2013; 33:834–845. [PubMed: 23443171]
83. Belayev L, et al. Quantitative evaluation of blood-brain barrier permeability following middle cerebral artery occlusion in rats. *Brain Res*. 1996; 739:88–96. [PubMed: 8955928]
84. Sandoval KE, Witt KA. Blood-brain barrier tight junction permeability and ischemic stroke. *Neurobiol Dis*. 2008; 32:200–219. [PubMed: 18790057]
85. Ivens S, et al. TGF-beta receptor-mediated albumin uptake into astrocytes is involved in neocortical epileptogenesis. *Brain*. 2007; 130:535–547. [PubMed: 17121744]
86. Schielke GP, et al. Blood to brain sodium transport and interstitial fluid potassium concentration during early focal ischemia in the rat. *J Cereb Blood Flow Metab*. 1991; 11:466–471. [PubMed: 1849910]
87. Betz AL, et al. Blood-brain barrier sodium transport limits development of brain edema during partial ischemia in gerbils. *Stroke*. 1989; 20:1253–1259. [PubMed: 2772985]
88. Kivisakk P, et al. Human cerebrospinal fluid central memory CD4⁺ T cells: evidence for trafficking through choroid plexus and meninges via P-selectin. *Proc Natl Acad Sci U S A*. 2003; 100:8389–8394. [PubMed: 12829791]
89. Arumugam TV, et al. Neuroprotection in stroke by complement inhibition and immunoglobulin therapy. *Neuroscience*. 2009; 158:1074–1089. [PubMed: 18691639]
90. Hatterer E, et al. Cerebrospinal fluid dendritic cells infiltrate the brain parenchyma and target the cervical lymph nodes under neuroinflammatory conditions. *PLoS One*. 2008; 3:e3321. [PubMed: 18830405]
91. Chodobski A, et al. Early neutrophilic expression of vascular endothelial growth factor after traumatic brain injury. *Neuroscience*. 2003; 122:853–867. [PubMed: 14643756]
92. Dimitrijevic OB, et al. Absence of the chemokine receptor CCR2 protects against cerebral ischemia/reperfusion injury in mice. *Stroke*. 2007; 38:1345–1353. [PubMed: 17332467]
93. Scholz M, et al. Neutrophils and the blood-brain barrier dysfunction after trauma. *Med Res Rev*. 2007; 27:401–416. [PubMed: 16758487]
94. Williams K, et al. Central nervous system perivascular cells are immunoregulatory cells that connect the CNS with the peripheral immune system. *Glia*. 2001; 36:156–164. [PubMed: 11596124]
95. Kunis G, et al. IFN-gamma-dependent activation of the brain's choroid plexus for CNS immune surveillance and repair. *Brain*. 2013; 136:3427–3440. [PubMed: 24088808]
96. Argaw AT, et al. Astrocyte-derived VEGF-A drives blood-brain barrier disruption in CNS inflammatory disease. *J Clin Invest*. 2012; 122:2454–2468. [PubMed: 22653056]
97. Holmin S, Mathiesen T. Intracerebral administration of interleukin-1beta and induction of inflammation, apoptosis, and vasogenic edema. *J Neurosurg*. 2000; 92:108–120. [PubMed: 10616089]

98. Jordan BD. Genetic influences on outcome following traumatic brain injury. *Neurochem Res.* 2007; 32:905–915. [PubMed: 17342413]
99. Sharma V, et al. Association of ALOX5AP1 SG13S114T/A variant with ischemic stroke, stroke subtypes and aspirin resistance. *J Neurol Sci.* 2013; 331:108–113. [PubMed: 23746795]
100. Koerner IP, et al. Polymorphisms in the human soluble epoxide hydrolase gene EPHX2 linked to neuronal survival after ischemic injury. *J Neurosci.* 2007; 27:4642–4649. [PubMed: 17460077]
101. Willis CL, et al. Partial recovery of the damaged rat blood-brain barrier is mediated by adherens junction complexes, extracellular matrix remodeling and macrophage infiltration following focal astrocyte loss. *Neuroscience.* 2013; 250:773–785. [PubMed: 23845748]
102. Sandercock PA, Soane T. Corticosteroids for acute ischaemic stroke. *Cochrane Database Syst Rev.* 2011;Cd000064. [PubMed: 21901674]
103. Schmidt OI, et al. Closed head injury--an inflammatory disease? *Brain Res Brain Res Rev.* 2005; 48:388–399. [PubMed: 15850678]
104. Kriz J. Inflammation in ischemic brain injury: timing is important. *Crit Rev Neurobiol.* 2006; 18:145–157. [PubMed: 17725517]
105. Lenzlinger PM, et al. The duality of the inflammatory response to traumatic brain injury. *Mol Neurobiol.* 2001; 24:169–181. [PubMed: 11831551]
106. Amantea D, et al. Post-ischemic brain damage: pathophysiology and role of inflammatory mediators. *Febs j.* 2009; 276:13–26. [PubMed: 19087196]
107. Doulal FN, et al. Enlarged perivascular spaces on MRI are a feature of cerebral small vessel disease. *Stroke.* 2010; 41:450–454. [PubMed: 20056930]
108. Kroenke CD, et al. Magnetic resonance measurement of tetramethylammonium diffusion in rat brain: Comparison of magnetic resonance and ionophoresis in vivo diffusion measurements. *Magn Reson Med.* 2003; 50:717–726. [PubMed: 14523957]
109. Badaut J, et al. Brain water mobility decreases after astrocytic aquaporin-4 inhibition using RNA interference. *J Cereb Blood Flow Metab.* 2011; 31:819–831. [PubMed: 20877385]
110. Strittmatter WJ. Bathing the brain. *J Clin Invest.* 2013; 123:1013–1015. [PubMed: 23434595]
111. Hansen AJ. Effect of anoxia on ion distribution in the brain. *Physiol Rev.* 1985; 65:101–148. [PubMed: 3880896]
112. Azevedo FA, et al. Equal numbers of neuronal and nonneuronal cells make the human brain an isometrically scaled-up primate brain. *J Comp Neurol.* 2009; 513:532–541. [PubMed: 19226510]
113. Altura BT, Altura BM. Measurement of ionized magnesium in whole blood, plasma and serum with a new ion-selective electrode in healthy and diseased human subjects. *Magnes Trace Elem.* 1991; 10:90–98. [PubMed: 1844565]
114. Chary SR, Jain RK. Direct measurement of interstitial convection and diffusion of albumin in normal and neoplastic tissues by fluorescence photobleaching. *Proc Natl Acad Sci U S A.* 1989; 86:5385–5389. [PubMed: 2748592]
115. Aukland K, Reed RK. Interstitial-lymphatic mechanisms in the control of extracellular fluid volume. *Physiol Rev.* 1993; 73:1–78. [PubMed: 8419962]

Glossary

Aquaporin-4 (AQP4)	plasma membrane water channel, belonging to the aquaporin family, and primarily expressed by astrocytes in brain on paravascular processes or end-feet.
Blood-brain barrier (BBB)	a selective permeability barrier consisting of vascular endothelial cells and endothelial tight junctions that separates the central nervous system from the vascular compartment.
Convection	collective movement of water and solutes either as a result of diffusion and/or advection. The latter refers to bulk motion of the

	fluid driven by any combination of gradients (e.g. pressure, electrical, thermal, gravitational). In rare instances where the flow of a system = 0, then total convection $\approx$ diffusion. However, in most biologically relevant situations where flow is high, convection $\approx$ advection.
Cushing reflex	severely elevated intracranial pressure (ICP) can cause patients to develop bradycardia, irregular breathing and increased blood-pressure.
Cytotoxic brain edema	swelling of brain cells due to a failure of cellular energy metabolism (i.e. intact blood-brain barrier). Examples: ischemic stroke, traumatic brain injury.
Diffusion	Redistribution of solutes from an area of high to an area of low concentration as a result of Brownian or random molecule movement. Diffusion flux increases as the concentration gradient becomes larger (Fick's first law) and decreases as a function of molecular weight (Graham's law).
Diffusion vs. advection	diffusion is a slow passive process that does not involve bulk movement of fluid, and is highly dependent on concentration gradient and weight of diffusing molecules. Advection relates to rapid directional movement of a fluid, which is largely independent of molecular weight and concentration gradients. Advection is thought to be the main mechanism governing interstitial fluid turnover in peripheral tissues and likely also brain [31, 115].
Glymphatic system	paravascular fluid exchange pathway that enables brain interstitial and cerebrospinal fluid turnover and is facilitated by glial cells.
Hemorrhagic brain edema	brain swelling caused by a complete breakdown of the BBB with leakage of all vascular contents including red blood cells, usually in the context of a hemorrhagic stroke or traumatic brain injury.
Interstitial brain edema	an anatomical term used to describe brain swelling caused by fluid accumulation in the interstitial space, which can occur during both ionic and vasogenic edema.
Ionic brain edema	a functional term used to describe brain swelling caused by net influx of salts (primarily NaCl) and water from the vasculature and/or CSF. Examples: ischemic stroke, traumatic brain injury.
Na⁺-K⁺-Cl⁻ cotransporter 1 (NKCC1)	transmembrane cation-chloride transporter widely expressed in secretory organs, the choroid plexus, and at a lower level in both neurons and astrocytes.
Osmotic brain edema	a subtype of ionic edema where low blood osmolarity forces net water influx to the brain. Example: water intoxication, syndrome of inappropriate antidiuretic hormone secretion (SIADH).

Penumbra	a region of perfused and potentially salvageable tissue surrounding the core of a brain infarct. The penumbra is often defined experimentally by staining for hypoxic tissue with 2,3,5-triphenyltetrazolium chloride (TTC) or clinically by examining the perfusion-diffusion mismatch (PDM) on MRI.
Starling's equation	$J_v = K_f([P_{\text{capillary}} - P_{\text{interstitium}}] - [\pi_{\text{capillary}} - \pi_{\text{interstitium}}])$ i.e. net fluid movement (J_v) = filtration coefficient (K_f) $\times$ ([hydrostatic pressure gradient] – reflection coefficient (σ) $\times$ [oncotic pressure gradient]). A recent reconsideration of this equation by Simard et al. has added separate filtration coefficients for hydrostatic and osmotic forces, to better reflect the unique properties of the BBB.
Sulfonylurea receptor 1 (SUR1)-regulated non-selective cation channels (NC_{Ca}-ATP)	transmembrane cation channels that becomes activated following energy depletion and is linked to cytotoxic, ionic and vasogenic brain edema.
Vasogenic brain edema	a functional term used to describe brain swelling caused by increase BBB permeability, causing leakage of protein, water and salts. Example: ischemic stroke, traumatic brain injury, brain tumor or metastasis, subarachnoid hemorrhage, meningitis.
Virchow-Robin space (VRS)	a term used for macroscopically visible extension of the subarachnoid space that ensheathes arteries as they penetrate the brain parenchyma. The VRS represents the proximal extension of the peri-arterial space implicated in the glymphatic system.

Box 1. Outstanding questions

- **How is glymphatic function affected by common brain insults such as injury, infarction, hemorrhage, infection and tumors?** Several parameters have been identified to date that can increase or decrease glymphatic function, including size of the interstitial space, AQP4 expression / localization, and arterial pulse-pressure. Resistance to CSF-ISF exchange was for instance shown to decrease rapidly during sleep due to an expansion of ISF compartment [35]. Similarly, ISF expansion in the area surrounding an injury or infarct would increase glymphatic flux, whilst cytotoxic ISF shrinkage at the core would compromise glymphatic function. Could astrocytic regulatory volume decrease also represent protective to regulate interstitial size and therefore glymphatic flow [44]?
- **What is the relative contribution of fluid diffusion and convection at the blood-brain and CSF-brain interfaces?** Because of low hydraulic and osmotic permeability at the BBB, we would argue that most fluid movement occurs at the paravascular CSF-ISF interface. When convection of interstitial fluid has been examined in other organs, the contribution of this mechanism relative to diffusion correlates closely with molecular size [114]. Thus larger molecules, such as the waste product immunoglobulins, complement factors or extravasated albumin, would be most sensitive to the function of glymphatic convection.
- **What is the role of the interstitial and paravascular extracellular matrix during physiology and disease?** Similar to the interstitium in other organs, both the ISF and paravascular compartments are likely to be more of a gel- than liquid-phase [38, 115]. The properties of extracellular matrix proteins are therefore important to understanding the real pore size and charge selectivity of the glymphatic system. The extracellular matrix might be known to protect against excessive ISF expansion during edema in other tissues by causing interstitial hydrostatic pressure to build up [115]. Could attachments of the extracellular matrix to glial end-feet open inter-endfoot clefts when ISF pressure increase? How does extensive protein leakage, basal lamina remodeling and reactive gliosis at the gliovascular interface alter the extracellular matrix? Might widening of intercellular clefts between adjacent glial end-foot processes contribute to edema or alter glymphatic function?
- **Is it possible to modulate the function of the glymphatic system by interfering with water or solute transporters?** Would inhibiting this system represent a double-edged therapeutic sword similar to AQP4 deletion, being beneficial in early cytotoxic edema, but detrimental in vasogenic edema and delay edema resorption [8]? What roles do other solute transporters such as those for Na^+ - K^+ - Cl^- -glucose, glutamate, amino acids and the Na^+ - K^+ -ATPase play?

Figure 1.

The glymphatic system regulates cerebrospinal and interstitial fluid exchange in the brain.

(A) Illustration of the main fluid compartments in the brain. (B) Diagram of fluid influx via penetrating arteries and efflux along a subset of large-caliber veins. (C) Diagram of proposed molecular mechanisms governing paravascular CSF-ISF exchange. Abbreviations: Paravascular space (PVS), solute carrier (SLC), zonula occludens (ZO), connexin (CNX) and Na^+ - K^+ -ATPase (NKA).

Figure 2.

Focal brain edema is caused by the interplay of cytotoxic changes in the core of an infarct or injury, and ionic mechanisms in the surrounding tissue or penumbra. **(A)** Illustration of focal brain edema following an ischemic stroke. **(B)** Diagram showing how net influx of Na^+ into dead or dying cells in the core can set up an ionic gradient for water influx into the penumbra, which is incompletely counterbalanced by K^+ efflux. Abbreviations: Plasma membrane (P.m.), end-foot membrane (E.f.m.).

Figure 3.

Phases of brain edema formation and resorption after an acute ischemic stroke. **(A)** Graph showing the likely changes in key parameters such as blood flow and water content after an acute infarct. **(B)** Diagram of suggested mechanisms that might be involved during the different phases of brain edema.

Table 1

Approximate composition of major water-containing compartments in cerebral cortex [10, 31, 111, 112, 113].

	ECF			ICF	
	Blood	CSF	ISF	Glia	Neurons
Volume fraction (%)	10%	10%	12%	41%	27%
Smallest diameter (µm)	8	5	0.04–0.06	0.5 (incl. gap junctions = 0.0015)	
Water (%)	93%	99%	99%	77%	
Sodium (mM)	138	155	143	17–20	4–10
Chloride (mM)	102	125	130	30–35	2–10
Potassium (mM)	4.5	3–4	2–3	100–140	
Bicarbonate (mM)	23	28	10–20	10–15	
Amino acids (mM)	9	1	1	138	
Protein (mg/mL)	70.0	0.4	0.35	200	
Glucose (mM)	5	3–4	3	3	
Calcium (mM)	1.5	1.5–2.0	1.5–2.0	0.0001	
Magnesium (mM)	0.5	0.9–1.3	0.9–1.3	9–11	
pH	7.40	7.38	7.22	7.00	