The Glymphatic System

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Introduction

The glymphatic system is a recently discovered macroscopic waste clearance network that uses a unique complex of perivascular tunnels, formed by astroglial cells, to promote efficient elimination of soluble proteins and metabolites from the CNS. Besides waste elimination, the glymphatic system may also function to help distribute nonwaste compounds, such as glucose, lipids, amino acids, and neurotransmitters related to volume transmission, in the brain. Intriguingly, the glymphatic system functions mainly during sleep and is largely disengaged during wakefulness. The biological need for sleep across all species may therefore reflect that the brain must enter a state of activity that enables the elimination of potentially neurotoxic waste products, including amyloid-beta (Aß). The concept of the glymphatic system is relatively new, and this syllabus chapter reviews its basic structural elements, organization, regulation, and functions. We will discuss recent studies indicating that glymphatic function is suppressed in various diseases and that failure of glymphatic function, in turn, might contribute to pathology in neurodegenerative disorders, traumatic brain injury, and stroke. This chapter also addresses recent findings and discusses them within the broader context of what is known about immune function and waste elimination from the CNS.

The Glymphatic Pathway

Clearance of excess fluid and interstitial solutes is critical for tissue homeostasis. In the peripheral tissues, soluble material, proteins, and fluid from the interstitial space are returned to the general circulation by the lymphatic system (Liao and Padera, 2013). The lymphatic network extends throughout all parts of the peripheral tissues, and the density of lymph vessels correlates with the rate of tissue metabolism. Although the brain and spinal cord are characterized by a disproportionally high metabolic rate (Wang et al., 2012), and synaptic transmission is exquisitely sensitive to changes in the environment, the CNS lacks conventional lymphatic vessels. Recently, lymphatic vessels have been identified in the fibrous membranes, meninges, and dura lining the subarachnoid space. These vessels express all the classical lymphatic endothelial cell markers and carry leukocytes, including T-lymphocytes (Aspelund et al., 2015; Louveau et al., 2015). CSF drains into these lymph vessels, which thereby act as a waste clearance path for the glymphatic system.

CSF and interstitial fluid (ISF) continuously interchange. From the subarachnoid space, CSF is driven into the perivascular space (also known as the Virchow–Robin space) by a combination of arterial pulsatility, respiration, and CSF pressure gradients. Thus, the loose fibrous matrix of the perivascular space can be viewed as a low-resistance highway for CSF influx. In addition to being a pathway for the influx of CSF, the perivascular spaces are important sites for delivering energy substrate and regulating blood flow. In pathological conditions, such as multiple sclerosis and stroke, the innate inflammatory response and edema formation are initiated in the perivascular spaces (Ge et al., 2005; del Zoppo et al., 2016). The transport of CSF into the dense and complex brain parenchyma from the perivascular space occurs in part through aquaporin 4 (*AQP4*) water channels expressed in a highly polarized manner in astrocytic endfeet that ensheathe the brain vasculature (Fig. 1). (Iliff et al., 2012; Iliff and Nedergaard, 2013). CSF interchanges with ISF within the brain parenchyma and facilitates a flow of CSF–ISF fluid, along with metabolites and waste solutes from the brain, to the lymphatic system via CSF drainage sites. These sites include the arachnoid villi, cranial and peripheral nerves, perivascular routes, and the newly discovered meningeal lymphatic vessels (Szentistványi et al., 1984; Johnston et al., 2004; Iliff et al., 2012, 2013b; Louveau et al., 2015; Morris et al., 2016). Whether the exit route from the parenchyma follows along veins (Iliff et al., 2012, 2013b) or along arteries (Morris et al., 2016) is still debated (Bakker et al., 2016).

This highly polarized macroscopic system of convective fluid fluxes with rapid interchange of CSF and ISF was described by Rennels and colleagues more than 30 years ago (Rennels et al., 1985). However, it was entitled the "glymphatic system" only in 2012, when the dynamics of CSF influx and clearance were characterized for the first time *in vivo* using two-photon microscopy in mice (Iliff et al., 2012). The name the "glymphatic system" was based on its similarity to the lymphatic system in the peripheral tissue in function, and on the important role of glial *AQP4* channels in convective fluid transport.

Drivers of Glymphatic Influx and Clearance

Glymphatic transport is driven by multiple mechanisms. Entry of CSF along the perivascular space is an energy-requiring process crucial for facilitating CSF–ISF exchange and clearance function. The initial proposal that arterial pulsation is the driving force of the convective CSF movement through the parenchyma (Iliff et al., 2012) was recently challenged (Jin et al., 2016) and elaborated on. The most recent computational modeling reports suggest that although arterial pulsation cannot generate enough force to

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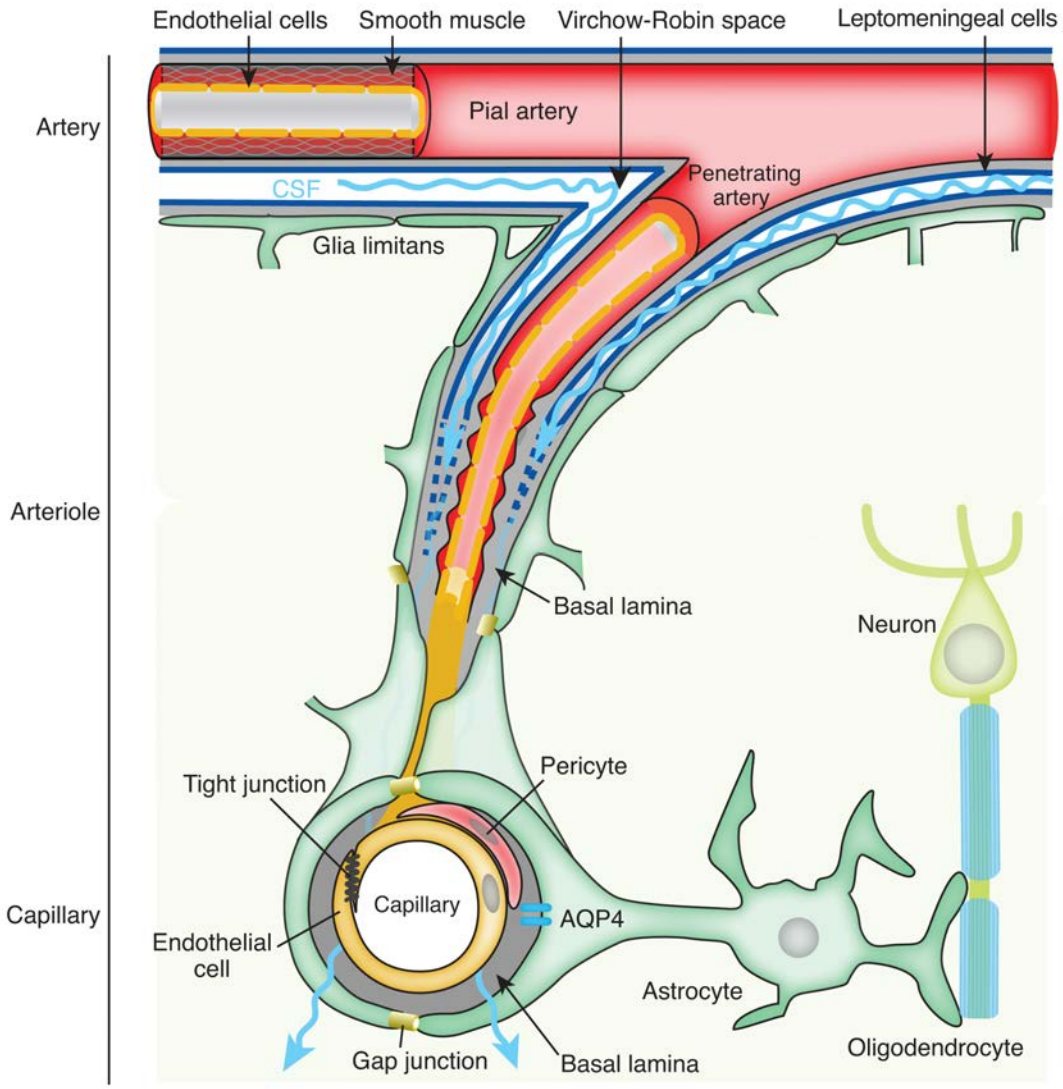

Figure 1. The neurovascular unit. The structure and function of the neurovascular unit allow bidirectional communication between the microvasculature and neurons, with astrocytes playing intermediary roles. Pial arteries in the subarachnoid space bathed in CSF become penetrating arteries upon diving into the brain parenchyma. The perivascular space around penetrating arteries is termed the Virchow–Robin space. As the penetrating arteries branch into arterioles and capillaries, the CSF-containing Virchow–Robin spaces narrow and finally disappear. However, the perivascular space extends to arterioles and capillaries to venules, where it is made up by the basal lamina's ECM that provides a continuity of the fluid space between arterioles and venules. Astrocytic vascular endfeet expressing *AQP4* surround the entire vasculature and form the boundary of the perivascular spaces. Reprinted with permission from Jessen NA et al. (2015), Fig. 3. Copyright 2015, Springer US.

drive convective bulk flow, it can still propagate fast solute transport in the periarterial space through a combined effect of mixing and diffusion (Asgari et al., 2015, 2016). Thus, arterial pulsation, respiration, and the pressure generated through a constant production of CSF by the choroid plexus all drive glymphatic fluid transport, in combination with intracellular astrocytic water flow through *AQP4* channels and a yet unidentified force. The molecular mechanism of CSF flow across astrocytic endfeet is poorly understood. Insight into the role of the connexins (43 and 30) and sodium transporters expressed at the endfeet could provide further knowledge of the ionic and molecular basis of solute movement across the endfeet (Simon et al., 2017).

Similar to the glymphatic flow in the brain parenchyma, a glymphatic system of the eye has recently been proposed. CSF surrounds the optic nerve, and studies have reported some level of exchange between the CSF and ISF of the optic nerve in the anterior part (Denniston and Keane, 2015; Wostyn et al., 2016).

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Further exploration of this pathway during various physiological and pathological conditions might help us to understand the implications of several ocular diseases, including glaucoma (Wostyn et al., 2017) and papilloedema secondary to raised intracranial pressure (Denniston et al., 2017).

The Glymphatic System Is Turned On During Sleep and After Exercise

In rodents, glymphatic activity is dramatically enhanced during sleep and suppressed during wakefulness. Using *in vivo* two-photon imaging, it was shown that the CSF influx in mice in the awake state was reduced by 90% compared with sleeping and anesthetized mice (Xie et al., 2013). The sleep–wake difference in glymphatic influx correlated with the volume fraction of interstitial space that was 13–15% in the awake state and expanded to 22–24% in both sleeping and anesthetized mice. Thus, the increase in interstitial space volume in the sleep state reduces tissue resistance to convective flow, permitting CSF–ISF exchange and clearance of metabolites. In contrast, applying norepinephrine significantly suppressed the glymphatic influx and decreased the interstitial volume fraction. This suggests that the burst release of norepinephrine during arousal increases the cellular volume fraction, resulting in a decrease in the interstitial space (O'Donnell et al., 2012). The concerted effect of norepinephrine thus acts via different mechanisms on both fluid availability and convective fluxes to suppress glymphatic function; therefore, norepinephrine can be considered a key regulator of both the switch between the sleep and wakeful state and solute clearance from the brain.

The suppressing effects of wakefulness on glymphatic clearance of metabolites are further enhanced by sleep deprivation (Plog et al., 2015), which correlates with abolishment of *AQP4* polarization at the astrocytic endfeet parallel to the vasculature (Liu et al., 2017). *AQP4* and its polarization to astrocytic endfeet are important for maintaining fluid and ion homeostasis and crucial to glymphatic influx and clearance in the young mouse brain. The genetic deletion of *Aqp4* was previously shown to impair CSF–ISF exchange by approximately 65% (Iliff et al., 2012), and loss of polarization occurs in parallel with reactive astrogliosis during pathology and aging (Kress et al., 2014; Plog et al., 2015; Gleiser et al., 2016). Interestingly, a recent report showed that aged mice (14–16 months old) that had been running voluntarily for 6 weeks did not have suppressed glymphatic influx or loss of *AQP4* polarization as their age-matched littermates did (He et al., 2017). The molecular mechanisms behind the beneficial effects of exercise on brain cognition are still poorly defined, but this finding reveals a favorable role for exercise in maintaining *AQP4* polarization and glymphatic activity during aging.

Convective CSF Fluxes in Aging and Pathology

Glymphatic activity decreases sharply during aging

Assessment of glymphatic function in old versus young mice showed a dramatic ~80–90% reduction in aged compared with young mice (Figs. 2*A*,*B*) (Kress et al., 2014). The suppression of glymphatic activity included both reduced influx of CSF tracers and reduced clearance of radiolabeled Aß and inulin. As mentioned above, the decreased glymphatic activity during aging could be attributed to increased reactive gliosis. Gliosis is defined by hypertrophy of *GFAP*-positive astrocyte processes (Sabbatini et al., 1999), which appears in parallel with loss of *AQP4* polarization. Other factors perhaps contributing to the reduction of glymphatic activity due to aging are the declines in CSF production by 66% and CSF pressure by 27% (Chen et al., 2009; Fleischman et al., 2012; Iliff et al., 2013b). Aging is also accompanied by stiffening of the arterial wall, leading to a reduction in arterial pulsatility—one of the drivers of glymphatic influx (Iliff et al., 2013b). The observation of age-related decline in glymphatic activity is important because the greatest risk factor identified for neurodegenerative diseases is aging.

Glymphatic flow is reduced before Aß aggregation

The failure of the glymphatic system in aging might thus contribute to the accumulation of misfolded and hyperphosphorylated proteins. In this way, it renders the brain more vulnerable to developing a neurodegenerative pathology or perhaps escalates the progression of cognitive dysfunction. Indeed, all prevalent neurodegenerative diseases are characterized by accumulation of aggregated proteins (Ross and Poirier, 2004). A macroscopic clearance mechanism of brain interstitial solutes may be of particular importance for clearing proteins from the ISF to prevent aggregate formation in neurodegenerative diseases including Alzheimer's disease (AD) (Weller, 1998). Several studies in rodents have reported that Aß is rapidly cleared from the mouse brain along the glymphatic pathway and that this clearance is enhanced during anesthesia and sleep and suppressed by sleep deprivation (Iliff et al., 2012; Xie et al., 2013; He et al., 2017). Also,

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apolipoprotein E is transported via the glymphatic system from the choroid plexus CSF to neurons (Achariyar et al., 2017). Together with the recent finding of a significant reduction in glymphatic transport before deposition of Aß in the *APP/PS1* mouse model of AD (Peng et al., 2016), this suggests that AD onset might be postponed by early restoration of glymphatic flow.

Reduced glymphatic clearance of Aß in animal models (Fig. 2C) (Peng et al., 2016) correlates with abnormally enlarged perivascular space volume, which is also observed in the brains of AD patients compared with aged-matched control subjects (Shinkai et al., 1995; Roher et al., 2003). Recent approaches to investigate the perivascular space volume and CSF flow in humans using magnetic resonance imaging

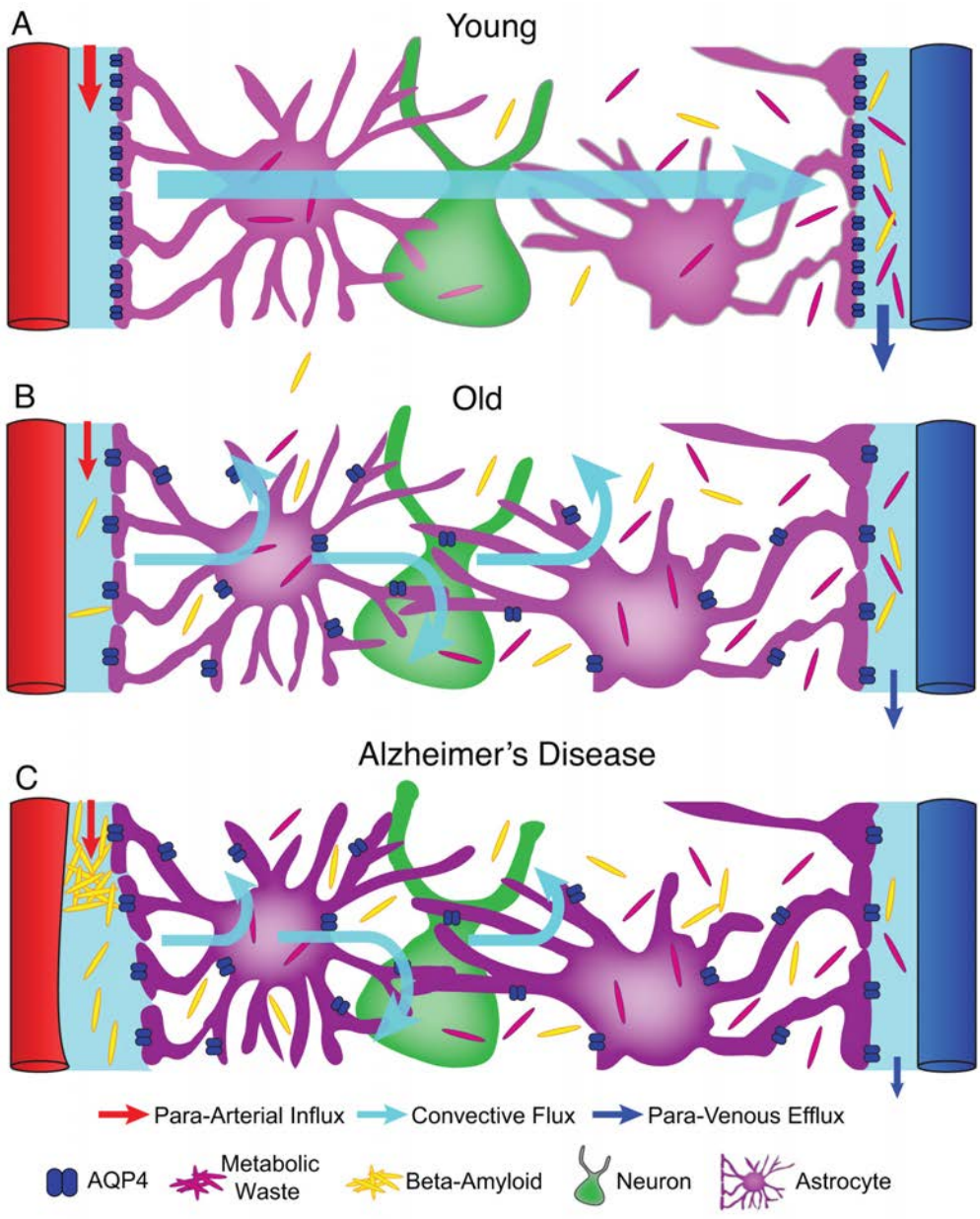

Figure 2. Model of glymphatic function in the young, old, and AD brain. ***A,*** In young and healthy people, CSF enters the brain parenchyma via periarterial pathways, washes out solutes from the interstitial space, and empties along the veins. ***B,*** With aging, glymphatic function is reduced, possibly owing to astrocytes becoming reactive and *AQP4* depolarized from the vascular endfeet to parenchymal processes. ***C,*** In AD, the perivascular space of penetrating arteries is subject to accumulation of Aß peptides. We hypothesize that accumulation of Aß might be caused by impairment of the glymphatic system and that the perivascular pathways are further blocked by protein aggregates such as Aß. In this model, the resulting changes in the perivascular environment lead to abnormal enlargement of perivascular space downstream, which further decreases glymphatic clearance. Reprinted with permission from Jessen NA et al. (2015), Fig. 5. Copyright 2015, Springer US.

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(MRI) and diffusion tensor image analysis likewise reported dilated Virchow–Robin spaces, especially in the white matter (Chen et al., 2011; Ramirez et al., 2016) and decreased diffusivity along the perivascular space (Taoka et al., 2017) in the brain of human AD patients. Decreased diffusivity likely reflects decreased influx of CSF along the arteries and therefore a decrease in glymphatic flux. In relation to the increased glymphatic activity during sleep, work from Holtzman and colleagues documented that the concentration of Aß in CSF follows the sleep–wake cycle in healthy human subjects; however, this fluctuation is disrupted in AD families with increased fibrillar Aß deposits caused by autosomal dominant inheritance of the *PSEN1 E280A* mutation (Roh et al., 2012). It is known that quality of sleep is lower in AD patients (Ju et al., 2013), and a recent study estimated a negative correlation between quality of sleep and the Virchow–Robin space volume in patients evaluated for cerebrovascular disease (Berezuk et al., 2015). Thus, we speculate that reduced quality of sleep in AD patients might be the result of dilated perivascular space that is likely caused by accumulating Aß deposits (Roher et al., 2003) and perivascular macrophages (Hawkes and McLaurin, 2009) clogging the glymphatic pathway.

Decreased glymphatic influx was also recently identified in a mouse model of type 2 diabetes (Jiang et al., 2017). Patients with diabetes have been reported to have accumulated amylin oligomers and plaques in the perivascular spaces of the brain parenchyma (Jackson et al., 2013). In rats overexpressing human amylin in the pancreas, the perivascular amylin accumulation in the brain has been found to induce an inflammatory response with activation of microglia and astrocytes (Srodulski et al., 2014). This finding could suggest that the glymphatic impairment in the diabetic mouse model is caused by the same mechanism as in AD but that amylin instead of Aß accumulates and clogs the perivascular CSF influx pathway.

Glymphatic influx is impeded in conditions of adaptive immune cell infiltration

Dilated perivascular spaces are evident not only in neurodegenerative diseases but also in small-vessel disease (Doubal et al., 2010) and diseases of the CNS associated with a neuroinflammatory response, such as traumatic brain injury (Inglese et al., 2005), multiple sclerosis (Wuerfel et al., 2008), and subarachnoid hemorrhage (Gaberel et al., 2014; Luo et al., 2016; Golanov et al., 2017; Goulay et al., 2017). In these conditions, the Virchow–Robin space turns into an immunological space that expands as the blood–brain barrier is disrupted, and the Virchow–Robin space becomes infiltrated with hematopoietic cells of the adaptive immune system (Esiri and Gay, 1990; Prinz and Priller, 2017). Recent reports have evaluated the glymphatic perivascular flow after traumatic brain injury in mice (Plog et al., 2015) and after subarachnoid hemorrhage in mice (Golanov et al., 2017), nonhuman primates (Goulay et al., 2017), and humans (Gaberel et al., 2014); they found reduced glymphatic influx in all cases.

Glymphatic flow is reduced after cortical spreading depression: a possible new mechanism

The perivascular space can be restricted by factors other than deposition of aggregated proteins and infiltration of immune cells, influencing the glymphatic flow. Following cortical spreading depression (or spreading depolarization), the perivascular space of pial and penetrating arteries and veins was recently found to rapidly and substantially decrease in volume. The restriction lasted several minutes and recovered gradually over 30 min (Schain et al., 2017). During propagation of the depolarizing waves, extracellular K^+ increases and the extracellular space volume is reduced. The increase in extracellular K^+ during spreading depression is slowed in *Aqp4* knock-out animals, probably owing to a larger basal extracellular space volume in these animals, which limits the swelling rate and wave propagation velocity (Yao et al., 2015). Thus, the decrease in perivascular space volume and glymphatic clearance after spreading depression is likely mediated by a reduction in extracellular space volume. Cortical spreading depression is associated with migraine, as well as traumatic brain injury and stroke. An understanding of how the glymphatic system is impaired in these conditions could provide the means for preventing, intervening, or treating the headache that is often a long-term consequence of this condition.

Future Directions

Preventive treatment

Evidence from animals and the few clinical studies performed so far suggest that lessened CSF flow through perivascular Virchow–Robin spaces is associated with reduced function of the glymphatic system. In a mouse model of AD, it was recently found that the glymphatic system was reduced at an early stage before major aggregations of Aß could be detected (Peng et al., 2016). The order of events is important because it indicates that early intervention by improving glymphatic flow could delay onset

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of AD or slow down AD progression. Impaired glymphatic flow leads to reduced clearance of waste metabolites (e.g., Aß); it also leads to impaired distribution of lipids (Rangroo Thrane et al., 2013), glucose (Lundgaard et al., 2015), and probably electrolytes, macromolecules, and other larger compounds important for homeostasis that enter the brain predominantly via the blood–CSF barrier at the choroid plexus. Loss of *AQP4* polarization at astrocytic endfeet is an early event in the aging brain; however, it was recently found not to occur in physically active animals (He et al., 2017), pointing toward one way in which the glymphatic system can be maintained. Further research into the molecular mechanisms behind the beneficial effect of exercise on glymphatic flow maintenance might lead to the development of new, preventive treatment options in the future. Therefore, diagnosis of early disease states is increasingly necessary.

Clinical assessment of glymphatic flow

Clinical assessment of abnormal glymphatic flow could be the key to making early diagnostic tests. Studies using contrast-enhanced MRI provide the experimental groundwork for evaluating glymphatic pathway function in the human brain, and in future, could assess whether failure of CSF fluxes contributes to disease progression. Benveniste's group has made headway toward developing a glymphatic diagnostic test based on MRI scans. By delivering contrast agent into the cisterna magna, the movement of CSF can be followed in real time across the entire brain (Iliff et al., 2013a; Lee et al., 2017). Subsequent studies showed that intrathecal lumbar injections with MRI gadolinium contrast, which are routinely used in clinical myelographic studies, provide a viable alternative route to assess the basic parameters of glymphatic function (Yang et al., 2013; Eide and Ringstad, 2015). However, development of a safe and minimally invasive imaging approach to visualize glymphatic function is necessary for future translational efforts.

Recent work using less invasive positron emission tomography scanning with a tracer for tau pathology, ^{18}F~THK5117, used CSF time activity as a biomarker for CSF clearance. With this approach, researchers were able to see that CSF clearance was reduced in patients with AD and that clearance was inversely associated with Aß deposition. Even less invasive is MRI without the use of contrast agent. By using a magnetic resonance encephalography (MREG) sequence, Kviviniemi and colleagues (2015) were able to detect cardiac, respiratory, and low-frequency pulsations—three mechanisms affecting CSF pulsations. Implementing MREG for diagnostics could offer a less invasive, novel approach for early detection of fluid dynamics and, thus, glymphatic fluxes.

New roles for the glymphatic system

Future studies that focus on the glymphatic system are expected to identify functions of convective CSF fluxes beyond the removal of metabolic waste products. We speculate that the glymphatic system might serve as a pathway for delivery and distribution of drugs, including cancer drugs, within the brain (Hadaczek et al., 2006). Further, we expect that growth factors produced by the choroid plexus, as well as neuromodulators released by several brain stem nuclei positioned close to the ventricular system, are distributed widely across the CNS by the glymphatic system. Thus, in addition to microscopic release of neuromodulators from local nerve terminals followed by local diffusion, volume transmission may involve circulation by macroscopic convective CSF fluxes via the glymphatic system.

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