Galina Yankova*, Olga Bogomyakova and Andrey Tulupov

The glymphatic system and meningeal lymphatics of the brain: new understanding of brain clearance

https://doi.org/10.1515/revneuro-2020-0106
Received September 21, 2020; accepted January 31, 2021;
published online February 23, 2021

Abstract: The glymphatic system and meningeal lymphatics have recently been characterized. Glymphatic system is a glia-dependent system of perivascular channels, and it plays an important role in the removal of interstitial metabolic waste products. The meningeal lymphatics may be a key drainage route for cerebrospinal fluid into the peripheral blood, may contribute to inflammatory reaction and central nervous system (CNS) immune surveillance. Breakdowns and dysfunction of the glymphatic system and meningeal lymphatics play a crucial role in age-related brain changes, the pathogenesis of neurovascular and neurodegenerative diseases, as well as in brain injuries and tumors. This review discusses the relationship recently characterized meningeal lymphatic vessels with the glymphatic system, which provides perfusion of the CNS with cerebrospinal and interstitial fluids. The review also presents the results of human studies concerning both the presence of meningeal lymphatics and the glymphatic system. A new understanding of how aging, medications, sleep and wake cycles, genetic predisposition, and even body posture affect the brain drainage system has not only changed the idea of brain fluid circulation but has also contributed to an understanding of the pathology and mechanisms of neurodegenerative diseases.

Keywords: amyloid; AQP4; glymphatic system; immune cells; meningeal lymphatics.

***Corresponding author: Galina Yankova**, Lavrentyev Institute of Hydrodynamics, Siberian Branch, Russian Academy of Sciences, Novosibirsk 630090, Russia; and Novosibirsk State University, Novosibirsk 630090, Russia,
E-mail: galinayankova2703@gmail.com. https://orcid.org/0000-0002-0782-1819
Olga Bogomyakova, International Tomography Center, Siberian Branch, Russian Academy of Sciences, Novosibirsk 630090, Russia
Andrey Tulupov, International Tomography Center, Siberian Branch, Russian Academy of Sciences, Novosibirsk 630090, Russia; Novosibirsk State University, Novosibirsk 630090, Russia; and Meshalkin National Medical Research Center, Ministry of Health of Russian Federation, Novosibirsk 630055, Russia

Introduction

The lymphatic system is a blindly closed, unidirectional system for transporting interstitial fluid (ISF) from the intercellular space into the venous bed. This system penetrates almost all human tissues and consists of lymph nodes, lymphatic vessels, and lymphatic capillaries. Lymphatic drainage contributes significantly to tissue fluid homeostasis and immune surveillance by helping to remove excess ISF, macromolecules, and immune cells from the intercellular space back into the bloodstream (Schulte-Merker et al. 2011). The lymph movement begins with blindly closed capillaries, and then the flow enters the lymph vessels and lymph nodes ending in the right lymphatic and thoracic ducts (Aspelund et al. 2016).

The lymph movement process through the vessels is provided by the ISF pressure gradient, compression of the lymphatic vessels by the surrounding contracting muscles, arterial pulsation, as well as by the presence of unilateral semilunar valves and a muscular layer in the collecting lymph vessels (Zawieja 2009). The lymphatic system is characterized by a number of different endothelial markers, including vascular endothelial growth factor receptor 3 (VEGFR-3), lymphatic vessel endothelial hyaluronan receptor 1 (LYVE-1), related homeobox 1 (PROX-1), podoplanin (PDPN), cluster of differentiation 31, chemokine ligand 21 (CCL21) - which play an important role in identifying lymphatic vessels (Kong et al. 2017; Norrmén et al. 2011).

The central nervous system (CNS) is previously believed to be devoid of lymphatic vessels. Indeed, despite having a complex circulatory system, the CNS lacks a lymphatic system that could contribute to parenchymal immune traffic, fluid drainage in the brain. Thus, the question arises: how is the CNS effectively cleared of cellular waste products and maintains fluid and tissue homeostasis in the absence of a functional lymphatic or drainage network?

However, recent studies of intracranial clearance showed the presence of meningeal lymphatic vessels, and the glymphatic pathway for the ISF and cerebrospinal fluid (CSF) drainage (Aspelund et al. 2015; Iliff et al. 2012; Jessen et al. 2015; Louveau et al. 2015b). These new results demonstrate the existence of a complex and integrated

system of CSF and ISF drainage pathways and their role in brain cleansing. A better understanding of the role of meningeal lymphatics and the glymphatic system, their interaction, could play a crucial role in understanding and treating many neurodegenerative diseases, age-related brain changes, traumatic brain injuries, circulatory disorders, and tumors (Da Mesquita et al. 2018; Kress et al. 2014; Ma et al. 2017). Body posture and sleep are also known to play a role in the proper functioning of the system (Fultz et al. 2019; Lee et al. 2015; Xie et al. 2013). In this review, we discuss the latest results of these studies.

Methods

A review of the literature was performed to identify current knowledge surrounding the glymphatic system, its visualization by magnetic resonance imaging (MRI) in animals and humans, localization, and function of meningeal lymphatic vessels, their connection with glymphatic system, as well as novel findings surrounding their role in the immunity and pathophysiology of neurodegenerative disease.

The review was performed using the PubMed database. PubMed was searched with the following search terms: ([{meningeal lymphatics} OR {dural lymphatics} AND {disorders}] OR [lymphatic vessels]) OR ([glymphatic system] AND [brain drain]) OR ([glymphatic system] AND [sleep]) OR ([glymphatic system] AND [disorders]) OR ([glymphatic system] OR [meningeal lymphatics] AND [immunity] OR [antigen]). The primary search yielded 315 items. The references of sources found were also reviewed. The search was limited to English manuscripts.

Glymphatic system

Extracellular brain fluids are blood, CSF, and ISF. ISF is formed from fluid and metabolites that are secreted from the tissue and capillaries, partially from the CSF. CSF is thought to secrete in all brain ventricles, then circulates the brain and spinal cord, and absorbed into the bloodstream through arachnoid granulations (Davson and Segal 1996). The CSF circulation issue, its interaction with ISF, and the role in the brain lymphatic drainage system has been the subject of intensive study over the past decades.

The first attempt to study the issue was the glymphatic system theory (Iliff et al. 2012; Jessen et al. 2015). According to this theory, the CSF movement occurs in perivascular spaces delimited between the vessel wall on the inner side and the astrocytic end-feet and the pia mater with the external side. The high concentration of aquaporin channels (AQP4) contained in the terminal sections of the astrocytic end-feet, the influence of pulse wave of arteries, provide a CSF-ISF exchange. Then mixed fluid with the products of neuronal activity leaves the parenchyma along with the perivenous spaces.

Because of the similarity of functions of the given system with peripheral lymphatic system functions, and also the participation of glial cells in its work the term “glymphatic system” has been offered by the group of researchers from Rochester University led by Dr. Maiken Nedergaard in 2012.

This system is important for delivering nutrients throughout the brain, such as glucose, lipids, amino acids, various growth factors, and neuromodulators. Nevertheless, the main role of the glymphatic system is in the elimination of CNS tissue products. The first scientist to describe perivascular spaces in the brain in 1843 was Durand Fardel. Then R. Virchow and Ch.-Ph. Robin described these microscopic spaces as channels in 1851 and 1859, respectively (Kwee and Kwee 2007; Woollam and Millen 1955). Perivascular spaces are believed to connect subarachnoid space (SAS) to the brain parenchyma, thereby providing a pathway for the CSF movement and its exit from the CNS (Hannocks et al. 2018; Iliff et al. 2012). One of the first references of the CNS perivascular pathway appeared in a study on the horseradish peroxidase injection into the lateral ventricles and SAS of the cat’s and dog’s brains. The authors observed a rapid spread of the introduced substance – within 4–10 min. Moreover, the authors suggested that the unidirectional tracer movement is mediated by the pulsation of the penetrating arteries (Rennels et al. 1990, 1985). It was shown that extracellular markers introduced into the rat brain CSF spaces can penetrate into the perivascular spaces of blood vessels near the ventricular wall (Brightman 1965; Brightman and Reese 1969). In study (Ichimura et al. 1991), the authors injected radioiodinated serum albumin into the arterial and venous perivascular space on the brain surface as well as into the rat’s cerebral cortex and SAS and confirmed that perivascular spaces can serve as channels for fluid exchange within the brain. It is worth noting that there is currently no consensus about perivascular spaces: scientists debate both their location and the perivenous space presence (Fayeye et al. 2010; Pollock et al. 1997; Zhang et al. 1990).

It should be noted that in modern literature the terms “perivascular” and “paravascular” are used sometimes interchangeably. This is partly because there is still no clear anatomical understanding of the nature of these spaces and whether they differ from each other.

In 2008, Carare et al. showed that tracer molecules injected into the mouse corpus striatum appear in the basal membranes of brain capillaries and smooth muscle cells of the arterial membrane. Based on these results, the authors put forward an alternative concept for ISF circulation – the

paravascular hypothesis. According to this hypothesis, interstitial waste is drained from the brain between the basement membrane (BM) of smooth muscle cells of the arterial tunica media. The ISF flows in the direction of the cervical lymph nodes (CLNs) in the opposite blood flow direction (Carare et al. 2008). That hypothesis was confirmed by other researchers in later works (Abbott 2013; Arbel-Ornath et al. 2013; Morris et al. 2016).

However, the fluid flow direction in paravascular and perivascular models is controversial. Part of the divergence is due to methodological differences in research: the paravascular flow was caused by experiments with the tracer introduction directly into the brain parenchyma, and the perivascular flow was observed after tracer injection in to CSF (Bakker et al. 2016). Some researchers believe the introduction of the indicator violates the pressure and volume of the brain fluid, which in turn leads to flow direction differences (Hladky and Barrand 2014).

Morris and colleagues proposed a model of bidirectional fluid movement in the brain: CSF inflow via the glymphatic pathway and ISF outflow via the paravascular pathway (Morris et al. 2016). Other studies (Coloma et al. 2016; Sharp et al. 2016) have confirmed this concept, assuming that the orientation of BM structures and the heart pulse wave reflection at the artery branch points may play a role in this reverse transport process.

The aquaporin water channels (AQP4) are another important component of the glymphatic system. Iliff et al. demonstrated significant suppression of both the CSF perivascular influx and the interstitial clearance of amyloid-β using AQP4 KO mice and fluorescent amyloid-β (Iliff et al. 2012). In 2014, researchers showed that the AQP4 gene deletion in mice exacerbates lymphatic dysfunction after a traumatic brain injury and also contributes to the development of neurofibrillary pathology and neurodegeneration in the post-traumatic brain (Iliff et al. 2014). Similar results were obtained by Plog et al. (Plog et al. 2015). A study using MRI showed that the use of the TGN-073 facilitator for AQP4 enhances the ISF transport from glia to the pericapillary space (Huber et al. 2018).

Glymphatic system visualization

MRI allows the entire brain to be examined at once. Iliff et al. were the first to use dynamic contrast-enhanced MRI to visualize the CSF-ISF exchange in the rat brain after intrathecal administration of a paramagnetic contrast agent. The periarterial CSF inflow, the molecular-dependent exchange between CSF-ISF, as well as inflow sites in the pituitary and pineal gland were visualized (Iliff et al. 2013). The study of glymphatic system functions was also performed by intravenous injections of a gadolinium-based contrast agent to rats. It was shown that the gadodiamide distribution in the cerebral cortex and deep cerebellar nuclei depends on both blood and CSF flow (Taoka et al. 2018). This work also assessed the gadolinium residual concentration in the rat brain after repeated gadolinium intravenous administration at different times of the day (morning or evening) and different levels of anesthesia (no, short- and long-term). The results showed the gadolinium concentration in the brain in the morning injection group was significantly lower than in the late injection group. The lowest gadolinium concentration was found in the group administered the substance in the morning under long-term anesthesia. These results can be explained by higher glymphatic system clearance after morning injection and reduced glymphatic system activity after injection in the late afternoon. Gaberel et al. using MRI, it was shown that subarachnoid hemorrhage and the acute phase of ischemic stroke lead to serious disorders of the glymphatic system (Gaberel et al. 2014).

The development of glymphatic system clinical imaging methods contributes not only to the visualization and quantitative determination of the CSF flow but also makes it possible to create fractional anisotropy and diffusion maps to calculate directional diffusion along with perivascular spaces (Dreha-Kulaczewski et al. 2015; Taoka et al. 2017). The MRI of a human brain with intrathecal gadolinium injection is already used to diagnose CSF leak sites in patients with intracranial hypotension, normal-pressure hydrocephalus (NPH) (Akbar et al. 2012; Eide and Ringstad 2015; Ringstad et al. 2017). Ringstad et al. studied CSF dynamics and glymphatic function in patients with NPH and the control group using T1-weighted MRI (Ringstad et al. 2018). In all the subjects, the contrast agent spread anterograde through the large leptomeningeal arteries on the brain surface. Signal amplification in the parenchyma peaked during the night in both groups, although the peak was higher in patients with NTH. These results may indicate that sleep is critical. A reduced gadolinium clearance from SAS and a constant signal enhancement in the brain parenchyma may be signs of a decreased glymphatic system clearance in patients with NTH. In the study (Davoodi-Bojd et al. 2019), the glymphatic system was modeled based on the kinetics of the Gd-DTPA tracer measured by MRI to (1) map the glymphatic system path, (2) derive kinetic parameters of the glymphatic system, and (3) provide quantitative maps of the structure and function of this system. The results of this model exhibit sensitivity to diabetic and control animals and show increased clearance time and binding of tracers in diabetes mellitus compared with control animal brain. In addition, glymphatic system

disorders are associated with neurological diseases: Alzheimer's disease (Reeves et al. 2020; Van De Haar et al. 2016), small vessel disease (Mestre et al. 2017), diabetes (Jiang et al. 2017; Naganawa et al. 2017), traumatic brain injury (Abbott 2013; Jessen et al. 2015; Morris et al. 2016), and stroke (Goulay et al. 2017; Mestre et al. 2020).

As shown in this section, many diseases and pathologies hurt the glymphatic system. The development of non-invasive methods for assessing the glymphatic system functions in humans will contribute to understanding the underlying mechanisms of these disorders, and will subsequently make a major contribution to the development of disease treatment strategies.

Meningeal lymphatic system

Both the brain and spinal cord are known to be protected by the skull and spinal column skeleton respectively, as well as meningeal membranes (dura mater, arachnoid mater, and pia mater).

The supposed meningeal lymphatic system was described in 1787 by Paolo Mascagni (Mascagni 1787). Also, several isolated lymphatic vessels around the cranial nerves and dural blood vessels were previously reported (Furukawa et al. 2008; Killer et al. 1999; Lüdemann et al. 2005). Although in many studies, substances injected into the parenchyma were observed in extracranial (cervical) lymphatic vessels (Abbott et al. 2018; Cserr and Knopf 1992; Cserr et al. 1992) yet the CNS was considered to be devoid of a lymphatic system. However, it was only recently, in 2015, two independent research groups demonstrated that these previously described vessels are lymphatic at the molecular and functional levels (Aspelund et al. 2015; Louveau et al. 2015b). Louveau et al. looked for routes responsible for the recycling of immune cells. They observed high concentrations of immune cells in structures adjacent to the dural sinuses in the meninges of mice. These structures provided a positive response to classical lymphatic markers (PROX-1, LYVE-1, PDPN, VEGFR-3, and CCL21) and their lymphatic nature was further demonstrated by the injection of VEGF-C. The second group of scientists showed the presence of meningeal lymph vessels by examining the CSF drainage scheme to the CLNs. The authors demonstrated that lymphatic vessels absorb CSF from the SAS and ISF through the lymphatic system and then the lymph vessels transport the fluid to the deep cervical lymph nodes (dCLNs). Also, in a model of transgenic mice (K14-VEGFR-3-Ig) with complete aplasia of meningeal lymphatic vessels, a decreased clearance of macromolecules from the brain and the decreased transport of CSF from the SAS to the CLNs were shown.

Meningeal lymphatics consist of thin-walled lymphatic vessels (without valves expressing integrin-α9) with a non-permanent BM compared to peripheral lymphatic vessels. Meningeal lymphatic vessels found in mice are located in proximity to the dural blood vessels and cranial nerves and exit the cranium through an opening along with venous sinuses, arteries, and nerves. Lymphatic vessels crossing the cribiform plate with olfactory nerves are also observed (Aspelund et al. 2015; Louveau et al. 2015b). Since the peripheral lymphatics are responsible for the regulation of ISF homeostasis and immune surveillance, these basic functions were also evaluated in the meningeal lymphatics (Lohrberg and Wilting 2016). The meningeal lymphatic vessels were shown to be able to absorb macromolecules from brain tissue and drain them to the CLNs (Antila et al. 2017; Aspelund et al. 2015; Louveau et al. 2015b, 2017). In the study (Maloveska et al. 2018) was shown the Evans blue movement from the rat SAS into the meningeal lymphatic vessels and then into the peripheral lymphatics for 3 h - 12 days. Based on these data, it can be assumed that meningeal lymphatic vessels are the peripheral lymphatic system extension and the missing link in the transport of macromolecules from brain tissue to extracranial lymphatic vessels (Aspelund et al. 2015; Louveau et al. 2015b).

In 1787, Paolo Mascagni showed the presence of meningeal lymphatic vessels in the human brain (Mascagni 1787). Later, in 1953, Lecco examined the dura mater of 30 people and found lymphatic structures in four of them (Lecco 1953). But all these results were not taken into account, in particular, because of the difficulty in identifying lymphatic vessels in the meninges of the human brain. Convincing evidence for the existence of lymphatic vessels in the brain meninges was presented in studies using animal models. The use of MRI to determine the meningeal lymphatic vessels in humans is shown (Absinta et al. 2017; Jani and Sekula 2018). In this study, the presence of meningeal lymphatics *in vivo* was confirmed in human and non-human primates using MRI enhanced with gadobutrol. The detected lymphatic vessels correspond to mice meningeal lymphatics and were found in the area of the SSS and middle meningeal arteries. The authors suggest the vessels being visualized are ducts since lymphatic capillaries probably won't be detected on an MRI because of the size. In a study of human brain autopsy samples, meningeal lymphatics along the SSS were also found. The morphology of the observed vessels corresponded to the morphology of peripheral lymphatic vessels (Visanji et al. 2018). CSF drainage into the CLNs was investigated by MRI, enhanced

with intrathecal contrast medium. The results of this study also indicated, in contrast with rodents, a more significant role of meningeal lymph vessels in humans in CSF draining into the CLNs than nasal lymph vessels (Eide et al. 2018). The role of the meningeal lymphatic network in the clearance of interstitial amyloid-β was estimated in the study (Goodman et al. 2018). The authors demonstrated that unlike perivascular CSF outflow pathways in Alzheimer's patients, amyloid-β is not deposited in or around meningeal lymph vessels associated with SSS.

Glymphatic-meningeal connection

Based on available data on the glymphatic system and meningeal lymphatics, it can be assumed that there is a relationship between them (Figure 1; see also the videoclip in the online supplementary material). The functional ability of meningeal lymphatic vessels to carry out the CSF outflow was studied by Louveau et al. (2015b). Anesthetized adult mice were simultaneously injected with fluorescein intravenously and with fluorescent tracer dye (QDot655) into the brain ventricles. The lymphatic vessels filled with QDot655, but not with fluorescein, were visible along with the SSS and thus carried out the CSF outflow. Using intracerebroventricular Evans blue dye injections, the authors showed a link between meningeal lymphatics and dCLNs. Half an hour after its injection, the dye was detected in the meningeal lymphatic vessels and the dCLNs and then in the superficial cervical lymph nodes. Ligation of the lymphatic vessels above the CLNs showed the dye absence in the CLNs, and resulted in an increase in meningeal lymph vessels. Notably, the dye injected into the nasal mucosa was not identified in the dCLNs, which means that the meningeal lymph vessels are the primary CSF drainage pathway to the dCLNs. The connection between meningeal lymphatics and CLNs also showed in the works (Antila et al. 2017; Choi et al. 2011; Duarte Torres et al. 2018; Maloveska et al. 2018; Patel et al. 2019). Ma et al. examined the main CSF outflow pathways through the glymphatic system and meningeal lymphatic vessels (Ma et al. 2017). Using near-infrared tracers developed by this team and high-resolution stereomicroscopy, the authors observed the intracerebroventricular mark propagation along the perivascular pathways of the pia mater and cerebral cortex. Tracers were also found to quickly reach lymph nodes by perineural pathways through skull holes. Da Mesquita et al. showed that the glymphatic system functioning is regulated by meningeal lymphatic function, suggesting a direct connection between them through brain fluids without any obvious anatomical connection (Da Mesquita et al. 2018). Using pharmacological, surgical and genetic models, scientists showed that decreased drainage through meningeal lymphatic vessels results in a CSF flow impairment into the brain. One month after the pharmacological ablation of meningeal lymphatic vessels,

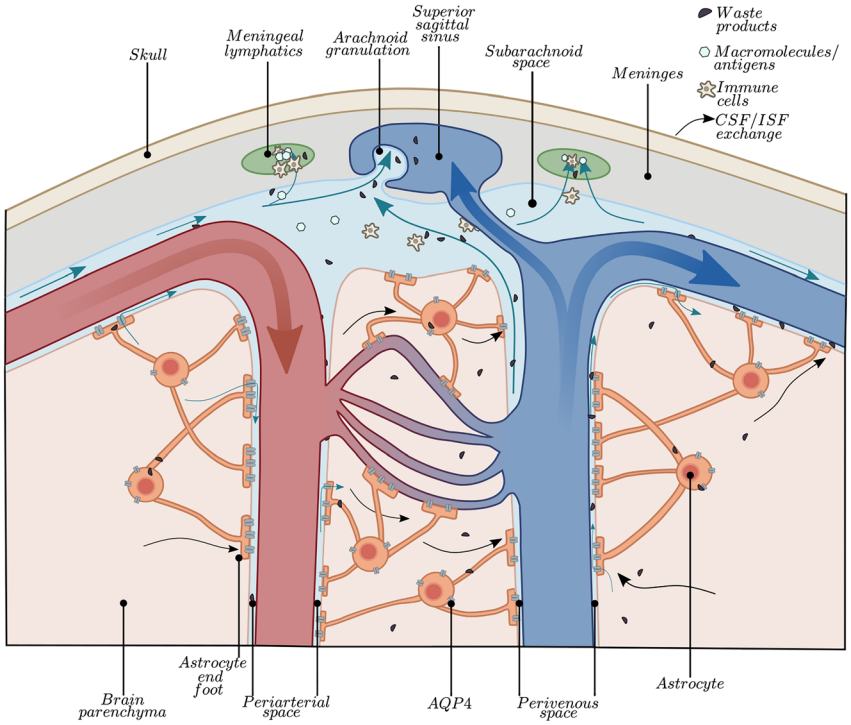

Figure 1: The glymphatic system drains cerebrospinal fluid (CSF) and solutes into the brain via a periarterial pathway, while interstitial fluid (ISF) and solutes leave the brain through the perivenous pathway. CSF can enter the venous bloodstream via arachnoid granulations, can leave brain parenchyma by meningeal lymphatics. CSF macromolecules and immune cells are transported mainly along the dural lymphatic vessels into the lymph nodes and extracranial systemic circulation.

adult mice were found to be learning and memory deficits without any detectable effects on the blood vasculature.

Role of AQP4

The AQP4 pore size is ~0.5 nm (Gutierrez et al. 1995). There are also gaps between the astrocytes end-feet. The fluid and solutes must either seep through the gaps between the astrocyte end-feet or through the AQP4 to get into the extracellular space. Due to the development of the glymphatic theory, much attention is paid to the study of the AQP4 role in this mechanism. The issue of gaps between astrocyte end-feet was investigated in in some studies (Korogod et al. 2015; Mathiisen et al. 2010). According to electron microscopy, it was shown that the astrocytic legs cover almost the entire vascular surface (99.7%), leaving intercellular spaces of less than 20 nm.

Thin slits (20 nm) located between the astrocytic end-feet are a highly resistant barrier to the fluid and solute flow between the perivascular space and the interstitial space. The AQP4 channels were assumed to represent a low-resistance path for convection-mediated fluid penetration and AQP4 strong polarization allows cellular waste and ions to pass through the interstitial and enter the perivenous space (Iliff et al. 2012). In this research (Ringstad et al. 2018), perivascular inflow and outflow were also assumed to have low resistance for CSF-ICF flow since the width of perivascular spaces is believed to be orders of magnitude greater than the width of the interstitial space or spaces between the brain cellular structures. However, glymphatic theory is silent on how solutes present in ISF pass through the perivascular space, as AQP4 is unable to transport most solutes, including macromolecules (Abbott et al. 2018). Mathematical modeling of diffusion and convective transport of fluid into the brain intercellular space was performed (Jin et al. 2016). The study showed that the astrocyte end-feet permeability practically does not affect the speed of convective fluid transport to the intercellular space, since the resistance to the water flow through the astrocyte end-feet is greater than through the gaps between them. Moreover, diffusion (without convection) is the main solute movement mechanism in the brain's extracellular space. The fact that diffusion is a more probable mechanism of fluid and solute movement was also shown in some studies (Asgari et al. 2016; Pizzo et al. 2018; Wolak and Thorne 2013). But, as was noted by Semyachkina-Glushkovskaya et al. (Semyachkina-Glushkovskaya et al. 2018), if diffusion is the dominant process, then all molecules, depending on their size, must have individual diffusion coefficients. However, this was disproved and it was shown the size of molecules does not influence the rate of their elimination (Cserr et al. 1981; Ichimura et al. 1991).

It is worth noting that AQP4 plays various roles in the brain water balance (Badaut et al. 2002), studies in mice with AQP4 gene deletion showed a fluid content increase in the brain (Haj-Yasein et al. 2011), an intracranial pressure increase in induced edema (Papadopoulos et al. 2004). All this shows that AQP4 does play an important role in the water transport between brain regions. And the mechanisms affecting the transport of fluid and solutes are not yet fully explored.

CSF outflow mechanism

Arachnoid granulations were first described by Vesalius in 1553 (Vesalius 1553). In 1705, Pacchioni described arachnoid granulations and reported a connection between the CSF, arachnoid villi, and the SSS (Pacchioni 1705). In 1875, Key and Retzius demonstrated that CSF is absorbed by the arachnoid villi (Key and Retzius 1875). In 1914, Weed confirmed that arachnoid granulations are important sites for CSF absorption (Weed 1914). The results of these studies formed the theory basis that the main CSF absorption place is arachnoid granulations. This theory is accepted today by most researchers. The facts questioning this theory are that venous sinuses in rats appear only 20 days after birth, and arachnoid villi do not exist prenatally in people and sheep and appear only after birth, increasing quantitatively with age (Gomez et al. 1983; Osaka et al. 1980). At the same time, choroidal plexuses produce CSF from the third month of pregnancy (Johnston and Papaiconomou 2002). Therefore, the presence of another pathway for CSF removal is important, especially in the prenatal period.

It is now recognized that there are other CSF outflow pathways, in particular, through the peripheral lymphatic system. The connection between the SAS and the CLNs in dogs and rabbits was shown by Schwalbe in 1869 (Schwalbe 1869). The CSF absorption through the olfactory paths, the cribiform plate to the nasal mucosa, the postpharyngeal lymph nodes and, finally, to the CLNs was demonstrated in some studies (Arnold et al. 1973; Jackson et al. 1979; Szentistvanyi et al. 1984). Johnston and colleagues showed a direct connection between CSF spaces and the lymphatic system of nasal mucosa in primates, including humans (Johnston et al. 2004). But the lymphatic system absence in the nasal mucosa was shown in the research (Lohrberg and Wilting 2016). Gomez et al. confirmed CSF absorption on the periphery of the optic nerve in rabbits (Gomez et al. 1988). The existence of CSF

absorption pathways along the optic nerve was shown in (De La Motte 1978; Erlich et al. 1989; Vonrautenfeld et al. 1994). Da Mesquita et al. found the presence of multiple perineural pathways for CSF outflow (Da Mesquita et al. 2018). These outflow pathways to the peripheral lymphatics appear to be the main exit pathways for both large and small tracer molecules introduced into the CSF. The study (Orešković and Klarica 2010) shows that CSF is produced and absorbed by all surfaces of the CNS that come into contact with it. The exact CSF clearance proportion along each outflow path is not yet known and can adaptively vary in response to other outflow paths (Aspelund et al. 2015; Louveau et al. 2015b). For example, mice born without meningeal lymphatic vessels do not show changes in intracranial pressure or brain fluid content (Aspelund et al. 2015; Louveau et al. 2017). This indicates that CSF outflow increased along another path to maintaining overall balance. From the multiplicity of identified outflow paths, it follows that fluid and solutes can simply leave the cranial cavity along the low-resistance path.

Location of meningeal lymphatic vessels

In 2015, two independent research teams discovered the meningeal lymphatic vessels. They were shown to be located along the sinuses of the brain, cranial, and spinal nerves. However, questions remain about their location in the meninges. Taking into account modern knowledge, three possible locations of meningeal lymphatic vessels were proposed. They can be located in the dura mater, pass between the dura mater and arachnoid sheath or penetrate the SAS (Raper et al. 2016). The location of meningeal lymphatic vessels along the meningeal arteries and dural sinuses suggests their dural location; however, it is not clear how CSF is absorbed from the SAS. Using the mouse model, input extensions of the meningeal lymphatic vessels were detected, which most likely have a connection with the SAS (Louveau et al. 2018). But it is not confirmed whether these vessels completely penetrate the SAS.

Recently it was shown that CSF tracer introduced by intrathecal injection in a human was detected in the dura mater between SSS and SAS, called parasagittal dura, using MRI (Ringstad and Eide 2020). Authors suggested that parasagittal dura may serve as a link between a human brain and dural lymphatic vessels. It was found the fissures in the dura mater between SSS and SAS (Kutomi and Takeda 2020). Authors suggested that these fissures in the dura mater adjacent to SSS are assumed to be intradural channels in the parasagittal dura to function as CSF drainage pathways in a pig.

Diseases and lymphatic drainage

Aging is known to hurt the function of lymphatic vessels in peripheral organs (Chevalier et al. 1996). Similarly, aging is considered an important factor in the regulation of the brain lymphatic system, since many neurological conditions are associated with this process (Da Mesquita et al. 2018; Ma et al. 2017). It is known that the level of molecular components of the BM of cerebral arteries and capillaries changes with age (Hawkes et al. 2011). The authors found a decrease in drainage of low-molecular dextran, laminin, fibronectin, and perlecan along with the BM of the capillaries and arteries of the hippocampus in old mice compared to adults and young mice. In 1998, laminin was found to be an inhibitor of amyloid-β aggregation and also destabilizes pre-formed amyloid-β fibrils (Bronfman et al. 1998). Thus, age-related changes in the brain vasculature can result in the solutes clearance decrease, in particular, amyloid-β, which leads to its accumulation in the BM of cerebral vessels (Carare et al. 2008; Lardenoije et al. 2015). To show the impairment of the glymphatic system efficiency during aging, the authors examined the transport of intraparenchymally administered amyloid-β and found that its clearance was impaired by 40% in old mice compared to young mice (Kress et al. 2014). The relationship between age-related changes in AQP4 expression and changes in the drainage functions of the glymphatic system is expressed in a sharp CSF-ISF exchange decreasing. Da Mesquita et al. studied the effect of aging and Alzheimer's disease on meningeal lymphatic vessels in three mouse models (Da Mesquita et al. 2018). The use of various strategies for VEGF-C delivering in old mice helped to enhance both lymph drainage to dCLNs and perivascular CSF flow; furthermore, older mice treated with VEGF-C showed improved results in learning and memory tests. And ablation of meningeal lymphatic vessels in young-adult transgenic mice leads to more severe brain amyloid-β pathology, with significant deposits in the meninges. Notably that a similar pattern is observed in the dura mater in patients with Alzheimer's disease. But in another study conducted on human autopsy samples, no amyloid-β deposits were found along the SSS (Goodman et al. 2018). Ligation of dCLNs in 5-month-old transgenic mice demonstrated a more severe brain amyloid-β accumulation, neuroinflammation, synaptic protein loss, impaired AQP4 polarization, and deficits in cognitive and exploratory

behavior (Wang et al. 2019). Decreased clearance of extracellular tau protein from the brain parenchyma in mice with removed and ligated meningeal lymphatic vessels was also observed in studies (Cao et al. 2018; P0atel et al. 2019).

While Alzheimer's disease is the most common cause of dementia, a large percentage of neurodegenerative diseases are associated with small vessel disease, including hypertension. Arteriolosclerosis and vascular wall remodeling alter the pulsation of the wall vessels and can increase backflow and thereby reduce the net flow of CSF into the perivascular spaces and thus disrupt the glymphatic system transport. Confirmation of such hypotheses was shown in studies on hypertensive rats (Mortensen et al. 2019). One-tissue compartment analysis revealed impeded Gd-DOTA glymphatic transport, as well as an increase in the volume of the ventricular system and ventricular reflux of a Gd-containing contrast, which indicates abnormal CSF flow dynamics secondary to congenital hydrocephalus. Similar changes were shown in a mouse model with multiple microinfarcts caused by the intraarterial injection of cholesterol crystals (Wang et al. 2017). CSF global flow violation aggravated with the age of mice along the glymphatic pathway was shown. Such changes at the level of small vessels and the glymphatic system can have a significant effect on brain plasticity, as well as on the development and course of neurodegenerative and vascular diseases.

CNS immune privilege

The CNS was long thought to have immune privilege, which was partly due to the lymphatic drainage lack from the CNS. The identification of the meningeal lymphatic vessels and the glymphatic system has led to an active study of both drainage and fluid flow through the cerebral parenchyma and CNS immune privilege. The interaction ways of immune surveillance and response are actively discussed and studied by the world community (Engelhardt et al. 2016; Louveau et al. 2015a, 2018).

It is now generally accepted that immune cells are present in the meninges and provide CNS immune surveillance. It was shown that immune cells (T-cells, B-cells, and dendritic cells) were present in the meningeal lymphatic vessels under normal conditions Louveau et al. 2015b. It is also likely that meningeal lymphatics is an important route for antigen-presenting cells (APCs) and soluble factors from the brain to the dCLNs (Aspelund et al. 2015; Louveau et al. 2015b. This suggests that the meningeal lymphatic vessels are involved in the movement of immune cells from the CNS.

However, the pathways of APC penetration and movement in the brain parenchyma are still unknown. It is assumed that CSF and ISF can carry antigens or APC to peripheral lymphatics. According to the paravascular hypothesis, ISF from the CNS parenchyma drains to lymph nodes along narrow and restricted pathways that include 100–150 nm thick BM in the walls of cerebral capillaries, arterioles, and arteries (Carare et al. 2008, 2014). It was shown that particles in the range of 15–1 μm injected into the cerebral gray matter do not drain along the intramural pathway but are traced along the outer side of the arteries and are separated by the glia limitans from the vessel walls (Carare et al. 2008; Zhang et al. 1995). Therefore, APC migration from the brain parenchyma with CSF along narrow intramural periarterial pathways is extremely unlikely (Carare et al. 2008, 2014).

Recent studies have demonstrated the glymphatic system that removes intercellular solutes from the brain through CSF-ISF exchange along channels formed between the vein walls and the astrocytic end-feet (Iliff et al. 2012; Yang et al. 2013). In some studies, the T-cells and APC outflow from CSF to the dCLNs was demonstrated (Goldmann et al. 2006; Hatterer et al. 2006, 2008; Kaminski et al. 2012), and Mohammad et al. proposed a different pathway for immune cells leaving the brain parenchyma via the rostral migratory stream (Mohammad et al. 2014). However, it is not known whether immune cells can leave the brain parenchyma by the glymphatic system.

Based on the results of a number of experimental studies carried out on a mouse model of autoimmune encephalomyelitis, the following possible ways for the penetration of encephalitogenic T-cells into the CNS were proposed (Agrawal et al. 2006; Bartholomaus et al. 2009; Engelhardt and Ransohoff 2012; Engelhardt et al. 2016; Schlager et al. 2016): T-cell migration through the leptomeningeal vessels associated with the spinal cord and brain (Bartholomaus et al. 2009), direct release of T-cells into the CSF from leptomeningeal microvessels (Schlager et al. 2016), and penetration of T-cells into the SAS using CSF from the choroid plexus of the ventricles (Ransohoff and Engelhardt 2012; Reboldi et al. 2009). It is worth noting that these penetration routes of T-cells into the CNS require the passage of the blood-brain barrier and then the barrier through the glia limitans, which forms a tissue layer surrounding microvessels in the CNS parenchyma and forms the CNS surface adjacent to the pia mater and SAS (Engelhardt et al. 2016).

Despite the data already available, many questions remain to be answered regarding the functioning of the

CNS immune system. It remains to be determined the functions of the meningeal lymphatic vessels at the molecular level, their role in the regulation of the immune response in the meninges and the parenchyma of the CNS, as well as the relationship between meningeal lymphatics and the general immune system. In addition, the anatomical routes by which APCs take from the CSF and CNS parenchyma to the dCLNs remain to be determined.

Conclusion

In the brain, molecular transport and waste disposal are accomplished using a brain lymphatic drainage system, which can be assumed to consist of the glymphatic system, meningeal lymphatics, drainage paths along with the perineural spaces, and the paravascular path along with the BM of the cerebral arteries.

Since this system was characterized relatively recently, there are many unresolved questions regarding its components. For example, experiments on the presence of the paravascular pathway are done ex vivo, which provides almost no information on the ISF flow dynamics. The glymphatic component of this system is a glia-dependent system of perivascular channels, through which CSF penetrates the brain parenchyma, mixes with the ISF, and then leaves the brain via perivenous pathways (Iliff et al. 2012). Over the past few years, a large amount of experimental data has appeared, indicating its role in cleaning and maintaining brain homeostasis. However, it is still not clear what physical mechanisms control the transfer of solutes in the brain parenchyma: what is the relative contribution of convection and diffusion. Another gap in knowledge about this component is the issue of fluid transport into the perivenous space. The discovery of lymphatic vessels in the mouse brain meninges provides new information about CSF transport, thereby contributing to a deeper understanding of brain drainage pathways (Aspelund et al. 2015; Louveau et al. 2015b). Also, this discovery lifts the veil of CNS immune privilege: now immune cells are considered to present in the meninges and provide immune surveillance of the CNS. However, the mystery of the entire brain lymphatic system is still far from being fully solved.

The brain lymphatic drainage system is known to be influenced by aging, sleep and wake cycles, and genetic factors. The clearance effectiveness depends on various diseases and pathologies that reduce the elasticity of blood vessels, negatively affect the brain aquaporin channels and prevent perivascular flow. Therefore, it is important to understand not only the mechanisms of removal of solutes from parenchyma but also to find out the interaction between the glymphatic and lymphatic systems of the brain. As it was shown in the works (Fultz et al. 2019; Xie et al. 2013), sleep promotes the spread of substances in the brain, and therefore it is necessary to take into account the brain activity state when conducting pharmacological studies. The discovery and further research of the brain lymphatic drainage system will undoubtedly contribute to the development of techniques and approaches to neuroimaging.

Author contributions: All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.
Research funding: G.Y. received support from the Russian Foundation for Basic Research (Grant No. 20-31-90097) in a part of glymphatic system and cerebral circulation. A.T. and O. B. received support from the Russian Science Foundation (Grant No. 19-75-20093) in a part of the analysis of hemodynamic changes in neurology and neurosurgery.
Conflict of interest statement: The authors declare no conflicts of interest with respect to the research, authorship, and/or publication of this article.

References

Abbott, N.J. (2013). Blood–brain barrier structure and function and the challenges for CNS drug delivery. J. Inherit. Metab. Dis. 36: 437–449.

Abbott, N.J., Pizzo, M.E., Preston, J.E., Janigro, D., and Thorne, R.G. (2018). The role of brain barriers in fluid movement in the CNS: is there a 'glymphatic'system? Acta Neuropathol. 135: 387–407.

Absinta, M., Ha, S.K., Nair, G., Sati, P., Luciano, N.J., Palisoc, M., Louveau, A., Zaghloul, K.A., Pittaluga, S., Kipnis, J., et al. (2017). Human and nonhuman primate meninges harbor lymphatic vessels that can be visualized noninvasively by MRI. eLife 6: e29738.

Agrawal, S., Anderson, P., Durbeej, M., van Rooijen, N., Ivars, F., Opdenakker, G., and Sorokin, L.M. (2006). Dystroglycan is selectively cleaved at the parenchymal basement membrane at sites of leukocyte extravasation in experimental autoimmune encephalomyelitis. J. Exp. Med. 203: 1007–1019.

Akbar, J.J., Luetmer, P.H., Schwartz, K.M., Hunt, C.H., Diehn, F.E., and Eckel, L.J. (2012). The role of MR myelography with intrathecal gadolinium in localization of spinal CSF leaks in patients with spontaneous intracranial hypotension. Am. J. Neuroradiol. 33: 535–540.

Antila, S., Karaman, S., Nurmi, H., Airavaara, M., Voutilainen, M.H., Mathivet, T., Chilov, D., Li, Z., Koppinen, T., Park, J.-H., et al. (2017). Development and plasticity of meningeal lymphatic vessels. J. Exp. Med. 214: 3645–3667.

Arbel-Ornath, M., Hudry, E., Eikermann-Haerter, K., Hou, S., Gregory, J.L., Zhao, L., Betensky, R.A., Frosch, M.P., Greenberg, S.M., and Bacskai, B.J. (2013). Interstitial fluid drainage is impaired in ischemic stroke and Alzheimer's disease mouse models. Acta Neuropathol. 126: 353–364.

Arnold, W., Ritter, R., and Wagner, W.H. (1973). Quantitative studies on the drainage of the cerebrospinal fluid into the lymphatic system. Acta Otolaryngol. 76: 156–161.

Asgari, M., De Zélicourt, D., and Kurtcuoglu, V. (2016). Glymphatic solute transport does not require bulk flow. Sci. Rep. 6: 38635.

Aspelund, A., Antila, S., Proulx, S.T., Karlsen, T.V., Karaman, S., Detmar, M., Wiig, H., and Alitato, K. (2015). A dural lymphatic vascular system that drains brain interstitial fluid and macromolecules. J. Exp. Med. 212: 991–999.

Aspelund, A., Robciuc, M.R., Karaman, S., Makinen, T., and Alitalo, K. (2016). Lymphatic system in cardiovascular medicine. Circ. Res. 118: 515–530.

Badaut, J., Lasbennes, F., Magistretti, P.J., and Regli, L. (2002). Aquaporins in brain: distribution, physiology, and pathophysiology. J. Cerebr. Blood Flow Metabol. 22: 367–378.

Bakker, E.N., Bacskai, B.J., Arbel-Ornath, M., Aldea, R., Bedussi, B., Morris, A.W.J., Weller, R.O., and Carare, R.O. (2016). Lymphatic clearance of the brain: perivascular, paravascular and significance for neurodegenerative diseases. Cell. Mol. Neurobiol. 36: 181–194.

Bartholomaus, I., Kawakami, N., Odoardi, F., Schlager, C., Miljkovic, D., Ellwart, J.W., Klinkert, W.E., Flugel-Koch, C., Issekutz, T.B., Wekerle, H., et al. (2009). Effector T cell interactions with meningeal vascular structures in nascent autoimmune CNS lesions. Nature 462: 94–98.

Brightman, M.W. (1965). The distribution within the brain of ferritin injected into cerebrospinal fluid compartments. II. Parenchymal distribution. Am. J. Anat. 117: 193–219.

Brightman, M.W. and Reese, T.S. (1969). Junctions between intimately apposed cell membranes in the vertebrate brain. J. Cell Biol. 40: 648–677.

Bronfman, F.C., Alvarez, A., Morgan, C., and Inestrosa, N.C. (1998). Laminin blocks the assembly of wild-type Aβ and the Dutch variant peptide into Alzheimer's fibrils. Amyloid 5: 16–23.

Cao, X., Xu, H., Feng, W., Su, D., and Xiao, M. (2018). Deletion of aquaporin-4 aggravates brain pathology after blocking of the meningeal lymphatic drainage. Brain Res. Bull. 143: 83–96.

Carare, R.O., Bernardes-Silva, M., Newman, T.A., Page, A.M., Nicoll, J.A.R., Perry, V.H., and Weller, R.O. (2008). Solutes, but not cells, drain from the brain parenchyma along basement membranes of capillaries and arteries: significance for cerebral amyloid angiopathy and neuroimmunology. Neuropathol. Appl. Neurobiol. 34: 131–144.

Carare, R.O., Hawkes, C.A., and Weller, R.O. (2014). Afferent and efferent immunological pathways of the brain. Anatomy, function and failure. Brain Behav. Immun. 36: 9–14.

Chevalier, S., Ferland, G., and Tuchweber, B. (1996). Lymphatic absorption of retinol in young, mature, and old rats: influence of dietary restriction. Faseb. J. 10: 1085–1090.

Choi, I., Chung, H.K., Ramu, S., Lee, H.N., Kim, K.E., Lee, S., Yoo, J., Choi, D., Lee, Y.S., Aguilar, B., et al. (2011). Visualization of lymphatic vessels by Prox1-promoter directed GFP reporter in a bacterial artificial chromosome-based transgenic mouse. Blood Adv. 117: 362–365.

Coloma, M., Schaffer, J.D., Carare, R.O., Chiarot, P.R., and Huang, P. (2016). Pulsations with reflected boundary waves: a hydrodynamic reverse transport mechanism for perivascular drainage in the brain. J. Math. Biol. 73: 469–490.

Cserr, H.F. and Knopf, P.M. (1992). Cervical lymphatics, the blood-brain barrier and the immunoreactivity of the brain: a new view. Immunol. Today 13: 507–512.

Cserr, H.F., Cooper, D.N., Suri, P.K., and Patlak, C.S. (1981). Efflux of radiolabeled polyethylene glycols and albumin from rat brain. Am. J. Physiol. Ren. Physiol. 240: F319–F328.

Cserr, H.F., Harling-Berg, C.J., and Knopf, P.M. (1992). Drainage of brain extracellular fluid into blood and deep cervical lymph and its immunological significance. Brain Pathol. 2: 269–276.

Da Mesquita, S., Louveau, A., Vaccari, A., Smirnov, I., Cornelison, R.C., Kingsmore, K.M., Contarino, C., Onengut-Gumuscu, S., Farber, E., Raper, D., et al. (2018). Functional aspects of meningeal lymphatics in ageing and Alzheimer's disease. Nature 560: 185–191.

Davoodi-Bojd, E., Ding, G., Zhang, L., Li, Q., Li, L., Chopp, M., Zhang, Z.-G., and Jiang, Q. (2019). Modeling glymphatic system of the brain using MRI. Neuroimage 188: 616–627.

Davson, H., and Segal, M.B. (1996). *Physiology of the CSF and blood-brain barriers*. CRC Press, Boca Raton, USA.

De La Motte, D.J. (1978). Removal of horseradish peroxidase and fluorescein-labelled dextran from CSF spaces of rabbit optic nerve. A light and electron microscope study. Exp. Eye Res. 27: 585–594.

Dreha-Kulaczewski, S., Joseph, A.A., Merboldt, K.D., Ludwig, H.C., Gärtner, J., and Frahm, J. (2015). Inspiration is the major regulator of human CSF flow. J. Neurosci. 35: 2485–2491.

Duarte Torres, E.N., Abdurashitov, A.S., Namykin, A.A., Shirokov, A., Shushunova, N., Sarantseva, E., and Semyachkina-Glushkovskaya, O. (2018). Lymphatic meningeal role in processes of brain clearing: in vivo visualization. Izv. Saratov Univ. (N. S.), Ser. Chem. Biol. Ecol. 18: 433–438.

Eide, P.K. and Ringstad, G. (2015). MRI with intrathecal MRI gadolinium contrast medium administration: a possible method to assess glymphatic function in human brain. Acta Radiol. Open 4, 2058460115609635.

Eide, P.K., Vatnehol, S.A.S., Emblem, K.E., and Ringstad, G. (2018). Magnetic resonance imaging provides evidence of glymphatic drainage from human brain to cervical lymph nodes. Sci. Rep. 8: 1–10.

Engelhardt, B. and Ransohoff, R.M. (2012). Capture, crawl, cross: the T cell code to breach the blood-brain barriers. Trends Immunol. 33: 579–589.

Engelhardt, B., Carare, R.O., Bechmann, I., Flügel, A., Laman, J.D., and Weller, R.O. (2016). Vascular, glial, and lymphatic immune gateways of the central nervous system. Acta Neuropathol. 132: 317–338.

Erlich, S.S., McComb, J.G., Hyman, S., and Weiss, M.H. (1989). Ultrastructure of the orbital pathway for cerebrospinal fluid drainage in rabbits. J. Neurosurg. 70: 926–931.

Fayeye, O., Pettorini, B.L., Foster, K., and Rodrigues, D. (2010). Mesencephalic enlarged Virchow–Robin spaces in a 6-year-old boy: a case-based update. Childs Nerv. Syst. 26: 1155–1160.

Fultz, N.E., Bonmassar, G., Setsompop, K., Stickgold, R.A., Rosen, B.R., Polimeni, J.R., and Lewis, L.D. (2019). Coupled electrophysiological, hemodynamic, and cerebrospinal fluid oscillations in human sleep. Science 366: 628–631.

Furukawa, M., Shimoda, H., Kajiwara, T., Kato, S., and Yanagisawa, S. (2008). Topographic study on nerve-associated lymphatic vessels in the murine craniofacial region by immunohistochemistry and electron microscopy. Biomed. Res. 29: 289–296.

Gaberel, T., Gakuba, C., Goulay, R., De Lizarrondo, S.M., Hanouz, J.-L., Emery, E., Touze, E., Vivien, D., and Gauberti, M. (2014). Impaired glymphatic perfusion after strokes revealed by contrast-enhanced MRI: a new target for fibrinolysis? Stroke 45: 3092–3096.

Goldmann, J., Kwidzinski, E., Brandt, C., Mahlo, J., Richter, D., and Bechmann, I. (2006). T cells traffic from brain to cervical lymph nodes via the cribroid plate and the nasal mucosa. J. Leukoc. Biol. 80: 797–801.

Gomez, D.G., Ehrmann, J.E., Potts, D.G., Pavese, A.M., and Gilanian, A. (1983). The arachnoid granulations of the newborn human: an ultrastructural study. Int. J. Dev. Neurosci. 1: 139–147.

Gomez, D.G., Manzo, R.P., Fenstermacher, J.D., and Potts, D.G. (1988). Cerebrospinal fluid absorption in the rabbit. Graefes Arch. Clin. Exp. Ophthalmol. 226: 1–7.

Goodman, J.R., Adham, Z.O., Woltjer, R.L., Lund, A.W., and Iliff, J.J. (2018). Characterization of dural sinus-associated lymphatic vasculature in human Alzheimer's dementia subjects. Brain Behav. Immun. 73: 34–40.

Goulay, R., Flament, J., Gauberti, M., Naveau, M., Pasquet, N., and Gakuba, C. (2017). Subarachnoid hemorrhage severely impairs brain parenchymal cerebrospinal fluid circulation in nonhuman primate. Stroke 48: 2301–2305.

Gutierrez, A.M., Gonzalez, E., Echevarria, M., Hernandez, C.S., and Whittembury, G. (1995). The proximal straight tubule (PST) basolateral cell membrane water channel: selectivity characteristics. J. Membr. Biol. 143: 189–197.

Haj-Yasein, N.N., Vindedal, G.F., Eilert-Olsen, M., Gundersen, G.A., Skare, Ø., Laake, P., Klungland, A., Thorén, A.E., Burkhardt, J.M., Ottersen, O.P., et al. (2011). 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. 108: 17815–17820.

Hannocks, M.J., Pizzo, M.E., Huppert, J., Deshpande, T., Abbott, N.J., Thorne, R.G., and Sorokin, L. (2018). Molecular characterization of perivascular drainage pathways in the murine brain. J. Cerebr. Blood Flow Metabol. 38: 669–686.

Hatterer, E., Davoust, N., Didier-Bazes, M., Vuaillat, C., Malcus, C., Belin, M.F., and Nataf, S. (2006). How to drain without lymphatics? Dendritic cells migrate from the cerebrospinal fluid to the B-cell follicles of cervical lymph nodes. Blood 107: 806–812.

Hatterer, E., Touret, M., Belin, M.F., Honnorat, J., and Nataf, S. (2008). Cerebrospinal fluid dendritic cells infiltrate the brain parenchyma and target the cervical lymph nodes under neuroinflammatory conditions. PLoS One 3: e3321.

Hawkes, C.A., Härtig, W., Kacza, J., Schliebs, R., Weller, R.O., Nicoll, J.A., and Carare, R.O. (2011). Perivascular drainage of solutes is impaired in the ageing mouse brain and in the presence of cerebral amyloid angiopathy. Acta Neuropathol. 121: 431–443.

Hladky, S.B. and Barrand, M.A. (2014). Mechanisms of fluid movement into, through and out of the brain: evaluation of the evidence. Fluids Barriers CNS 11: 1–32.

Huber, V.J., Igarashi, H., Ueki, S., Kwee, I.L., and Nakada, T. (2018). Aquaporin-4 facilitator TGN-073 promotes interstitial fluid circulation within the blood–brain barrier: [^{17}O] H_2O JJVCPE MRI study. Neuroreport 29: 697–703.

Ichimura, T., Fraser, P.A., and Cserr, H.F. (1991). Distribution of extracellular tracers in perivascular spaces of the rat brain. Brain Res. 545: 103–113.

Iliff, J.J., Chen, M.J., Plog, B.A., Zeppenfeld, D.M., Soltero, M., Yang, L., Singh, I., Deane, R., and Nedergaard, M. (2014). Impairment of glymphatic pathway function promotes tau pathology after traumatic brain injury. J. Neurosci. 34: 16180–16193.

Iliff, J.J., Lee, H., Yu, M., Feng, T., Logan, J., Nedergaard, M., and Benveniste, H. (2013). Brain-wide pathway for waste clearance captured by contrast-enhanced MRI. J. Clin. Invest. 123: 1299–1309.

Iliff, J.J., Wang, M., Liao, Y., Plogg, B.A., Peng, W., Gundersen, G.A., Benveniste, H., Vates, G.E., Deane, R., Goldman, S.A., 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: 147ra111.

Jackson, R.T., Tigges, J., and Arnold, W. (1979). Subarachnoid space of the CNS, nasal mucosa, and lymphatic system. Arch. Otolaryngol. 105: 180–184.

Jani, R.H. and Sekula, R.F. (2018). Magnetic resonance imaging of human dural meningeal lymphatics. Neurosurgery 83: E10–E12.

Jessen, N.A., Munk, A.S.F., Lundgaard, I., and Nedergaard, M. (2015). The glymphatic system: a beginner's guide. Neurochem. Res. 40: 2583–2599.

Jiang, Q., Zhang, L., Ding, G., Davoodi-Bojd, E., Li, Q., Li, L., Sadry, N., Nedergaard, M., Chopp, M., and Zhang, Z. (2017). Impairment of the glymphatic system after diabetes. J. Cerebr. Blood Flow Metabol. 37: 1326–1337.

Jin, B.J., Smith, A.J., and Verkman, A.S. (2016). Spatial model of convective solute transport in brain extracellular space does not support a "glymphatic" mechanism. J. Gen. Physiol. 148: 489–501.

Johnston, M. and Papaiconomou, C. (2002). Cerebrospinal fluid transport: a lymphatic perspective. Physiology 17: 227–230.

Johnston, M., Zakharov, A., Papaiconomou, C., Salmasi, G., and Armstrong, D. (2004). Evidence of connections between cerebrospinal fluid and nasal lymphatic vessels in humans, non-human primates and other mammalian species. Cerebrospinal Fluid Res. 1.

Kaminski, M., Bechmann, I., Pohland, M., Kiwit, J., Nitsch, R., and Glumm, J. (2012). Migration of monocytes after intracerebral injection at entorhinal cortex lesion site. J. Leukoc. Biol. 92: 31–39.

Key, A. and Retzius, G. (1875). *Studien in der Anatomie des Nervensystems und des Bindegewebes*. Stockholm: Samson and Wallin.

Killer, H.E., Laeng, H.R., and Groscurth, P. (1999). Lymphatic capillaries in the meninges of the human optic nerve. J. Neuro Ophthalmol. 19: 222–228.

Kong, L.L., Yang, N.Z., Shi, L.H., Zhao, G.H., Zhou, W., Ding, Q., Wang, M.H., and Zhang, Y.S. (2017). The optimum marker for the detection of lymphatic vessels. Mol. Clin. Oncol. 7: 515–520.

Korogod, N., Petersen, C.C., and Knott, G.W. (2015). Ultrastructural analysis of adult mouse neocortex comparing aldehyde perfusion with cryo fixation. eLife 4: e05793.

Kress, B.T., Iliff, J.J., Xia, M., Wang, M., Wei, H., Zeppenfeld, D., Xie, L., Kang, H., Xu, Q., Liew, J., et al. (2014). Impairment of paravascular clearance pathways in the aging brain. Ann. Neurol. 76: 845–861.

Kutomi, O. and Takeda, S. (2020). Identification of lymphatic endothelium in cranial arachnoid granulation-like dural gap. Microscopy 69: 391–400.

Kwee, R.M. and Kwee, T.C. (2007). Virchow-Robin spaces at MR imaging. Radiographics 27: 1071–1086.

Lardenoije, R., Iatrou, A., Kenis, G., Kompotis, K., Steinbusch, H.W.M., Mastroeni, D., Coleman, P., Lemere, C.A., Hof, P.R., van den Hove, D.L.A., et al. (2015). The epigenetics of aging and neurodegeneration. Prog. Neurobiol. 131: 21–64.

Lecco, V. (1953). Di una probabile modificazione delle fissure linfatiche della della parte dei seni venosi della dura madre. Arch. Ital. Otol. Rinol. Laringol. 64: 287–296.

Lee, H., Xie, L., Yu, M., KangFeng, H.T., Deane, R., Logan, J., Nedergaard, M., and Benveniste, H. (2015). The effect of body posture on brain glymphatic transport. J. Neurosci. 35: 11034–11044.

Lohrberg, M. and Wilting, J. (2016). The lymphatic vascular system of the mouse head. Cell Tissue Res. 366: 667–677.

Louveau, A., Harris, T.H., and Kipnis, J. (2015a). Revisiting the mechanisms of CNS immune privilege. Trends Immunol. 36: 569–577.

Louveau, A., Herz, J., Alme, M.N., Salvador, A.F., Dong, M.Q., Viar, K.E., Herod, S.G., Knopp, J., Setliff, J.C., Lupi, A.L., et al. (2018). CNS lymphatic drainage and neuroinflammation are regulated by meningeal lymphatic vasculature. Nat. Neurosci. 21: 1380–1391.

Louveau, A., Plog, B.A., Antila, S., Alitalo, K., Nedergaard, M., and Kipnis, J. (2017). Understanding the functions and relationships of the glymphatic system and meningeal lymphatics. J. Clin. Invest. 127: 3210–3219.

Louveau, A., Smirnov, I., Keyes, T.J., Eccles, J.D., Rouhani, S.J., Peske, J.D., Derecki, N.C., Castle, D., Mandell, J.W., Lee, K.S., et al. (2015b). Structural and functional features of central nervous system lymphatic vessels. Nature 523: 337–341.

Lüdemann, W., von Rautenfeld, D.B., and SamiiBrinker, M.T. (2005). Ultrastructure of the cerebrospinal fluid outflow along the optic nerve into the lymphatic system. Childs Nerv. Syst. 21: 96–103.

Ma, Q., Ineichen, B.V., Detmar, M., and Proulx, S.T. (2017). Outflow of cerebrospinal fluid is predominantly through lymphatic vessels and is reduced in aged mice. Nat. Commun. 8: 1–13.

Maloveska, M., Danko, J., Petrovova, E., Kresakova, L., Vdoviakova, K., Michalicova, A., Kovac, A., Cubinkova, V., and Cizkova, D. (2018). Dynamics of Evans blue clearance from cerebrospinal fluid into meningeal lymphatic vessels and deep cervical lymph nodes. Neurol. Res. 40: 372–380.

Mascagni, P. (1787). *De lymphaticis profundis capitis et colli. Vasorum lymphaticorum corporis humani historia et ichnographia. Pars Prima section Siena: Pazzini Carli VII, Art. VI*. Pazzini Carli, Siena.

Mathiisen, T.M., Lehre, K.P., Danbolt, N.C., and Ottersen, O.P. (2010). The perivascular astroglial sheath provides a complete covering of the brain microvessels: an electron microscopic 3D reconstruction. Glia 58: 1094–1103.

Mestre, H., Du, T., Sweeney, A., Liu, G., Samson, A., and Peng, W. (2020). Cerebrospinal fluid influx drives acute ischemic tissue swelling. Science 367, https://doi.org/10.1126/science.aax7171.

Mestre, H., Kostrikov, S., Mehta, R.I., and Nedergaard, M. (2017). Perivascular spaces, glymphatic dysfunction, and small vessel disease. Clin. Sci. 131: 2257–2274.

Mohammad, M.G., Tsai, V.W.W., Ruitenberg, M.J., Hassanpour, M., Li, H., Hart, P.H., Breit, S.N., Sawchenko, P.E., and Brown, D.A. (2014). Immune cell trafficking from the brain maintains CNS immune tolerance. J. Clin. Invest. 124: 1228–1241.

Morris, A.W., Sharp, M.M., Albargothy, N.J., Fernandes, R., Hawkes, C.A., Verma, A., Weller, R.O., and Carare, R.O. (2016). Vascular basement membranes as pathways for the passage of fluid into and out of the brain. Acta Neuropathol. 131: 725–736.

Mortensen, K.N., Sanggaard, S., Mestre, H., Lee, H., Kostrikov, S., Xavier, A.L.R., Gjedde, A., Benveniste, H., and Nedergaard, M. (2019). Impaired glymphatic transport in spontaneously hypertensive rats. J. Neurosci. 39: 6365–6377.

Naganawa, S., Nakane, T., Kawai, H., and Taoka, T. (2017). Gd-based contrast enhancement of the perivascular spaces in the basal ganglia. Magn. Reson. Med. Sci. 16: 61–65.

Norrmén, C., Tammela, T., Petrova, T.V., and Alitalo, K. (2011). Biological basis of therapeutic lymphangiogenesis. Circulation 123: 1335–1351.

Orešković, D. and Klarica, M. (2010). The formation of cerebrospinal fluid: nearly a hundred years of interpretations and misinterpretations. Brain Res. Brain Res. Rev. 64: 241–262.

Osaka, K., Handa, H., Matsumoto, S., and Yasuda, M. (1980). Development of the cerebrospinal fluid pathway in the normal and abnormal human embryos. Pediatr. Neurosurg. 6: 26–38.

Pacchioni, A. (1705). *Dissertatio epistolaris ad Lucam Schorekium deglandulis conglobatis dome meninges humanac*. Rome: Buagni.

Papadopoulos, M.C., Manley, G.T., Krishna, S., and Verkman, A.S. (2004). Aquaporin-4 facilitates reabsorption of excess fluid in vasogenic brain edema. Faseb. J. 18: 1291–1293.

Patel, T.K., Habimana-Griffin, L., Gao, X., Xu, B., Achilefu, S., Alitalo, K., McKee, C.A., Sheehan, P.W., Musiek, E.S., Xiong, C., et al. (2019). Dural lymphatics regulate clearance of extracellular tau from the CNS. Mol. Neurodegener. 14: 1–9.

Pizzo, M.E., Wolak, D.J., Kumar, N.N., Brunette, E., Brunnquell, C.L., Hannocks, M.-J., Abbott, N.J., Meyerand, M.E., Sorokin, L., Stanimirovic, D.B., et al. (2018). Intrathecal antibody distribution in the rat brain: surface diffusion, perivascular transport and osmotic enhancement of delivery. J. Physiol. 596: 445–475.

Plog, B.A., Dashnaw, M.L., Hitomi, E., Peng, W., Liao, Y., Lou, N., Deane, R., and Nedergaard, M. (2015). Biomarkers of traumatic injury are transported from brain to blood via the glymphatic system. J. Neurosci. 35: 518–526.

Pollock, H., Hutchings, M., Weller, R.O., and Zhang, E.T. (1997). Perivascular spaces in the basal ganglia of the human brain: their relationship to lacunes. J. Anat. 191: 337–346.

Ransohoff, R.M. and Engelhardt, B. (2012). The anatomical and cellular basis of immune surveillance in the central nervous system. Nat. Rev. Immunol. 12: 623–635.

Raper, D., Louveau, A., and Kipnis, J. (2016). How do meningeal lymphatic vessels drain the CNS? Trends Neurosci. 39: 581–586.

Reboldi, A., Coisne, C., Baumjohann, D., Benvenuto, F., Bottinelli, D., Lira, S., Uccelli, A., Lanzavecchia, A., Engelhardt, B., and Sallusto, F. (2009). C–C chemokine receptor 6-regulated entry of TH-17 cells into the CNS through the choroid plexus is required for the initiation of EAE. Nat. Immunol. 10: 514–523.

Reeves, B.C., Karimy, J.K., Kundishora, A.J., Mestre, H., Cerci, H.M., Matouk, C., Alper, S.L., Lundgaard, I., Nedergaard, M., and Kahle, K.T. (2020). Glymphatic system impairment in Alzheimer's disease and idiopathic normal pressure hydrocephalus. Trends Mol. Med. 26: 285–295.

Rennels, M.L., Blaumanis, O.R., and Grady, P.A. (1990). Rapid solute transport throughout the brain via paravascular fluid pathways. Adv. Neurol. 52: 431–439.

Rennels, M.L., Gregory, T.F., Blaumanis, O.R., Fujimoto, K., and Grady, P.A. (1985). Evidence for a 'paravascular'fluid circulation in the mammalian central nervous system, provided by the rapid distribution of tracer protein throughout the brain from the subarachnoid space. Brain Res. 326: 47–63.

Ringstad, G. and Eide, P. (2020). Cerebrospinal fluid tracer efflux to parasagittal dura in humans. Nat. Commun. 11: 1–9.

Ringstad, G., Valnes, L.M., DalePripp, A.M.A.H., Vatnehol, S.-A.S., Emblem, K.E., Mardal, K.-A., and Eide, P.K. (2018). Brain-wide glymphatic enhancement and clearance in humans assessed with MRI. JCI Insight 3: e121537.

Ringstad, G., Vatnehol, S.A.S., and Eide, P.K. (2017). Glymphatic MRI in idiopathic normal pressure hydrocephalus. Brain 140: 2691–2705.

Schlager, C., Korner, H., Krueger, M., Vidoli, S., Haberl, M., Mielke, D., Brylla, E., Issekutz, T., Cabanas, C., Nelson, P.J., et al. (2016). Effector T-cell trafficking between the leptomeninges and the cerebrospinal fluid. Nature 530: 349–353.

Schulte-Merker, S., Sabine, A., and Petrova, T.V. (2011). Lymphatic vascular morphogenesis in development, physiology, and disease. J. Cell Biol. 193: 607–618.

Schwalbe, G. (1869). Der Arachnoidalraum, ein Lymphraum und sein Zusammenhang mit dem Perichorioidalraum. Zentralbl. Med. Wiss. 7: 465–467.

Semyachkina-Glushkovskaya, O., Postnov, D., and Kurths, J. (2018). Blood–brain barrier, lymphatic clearance, and recovery: Ariadne's thread in labyrinths of hypotheses. Int. J. Mol. Sci. 19: 3818.

Sharp, M.K., Diem, A.K., Weller, R.O., and Carare, R.O. (2016). Peristalsis with oscillating flow resistance: a mechanism for periarterial clearance of amyloid beta from the brain. Ann. Biomed. Eng. 44: 1553–1565.

Szentistvanyi, I., Patlak, C.S., Ellis, R.A., and Cserr, H.F. (1984). Drainage of interstitial fluid from different regions of rat brain. Am. J. Physiol. Ren. Physiol. 246: F835–F844.

Taoka, T., Jost, G., Frenzel, T., Naganawa, S., and Pietsch, H. (2018). Impact of the glymphatic system on the kinetic and distribution of gadodiamide in the rat brain: observations by dynamic MRI and effect of circadian rhythm on tissue gadolinium concentrations. Invest. Radiol. 53: 529–534.

Taoka, T., Masutani, Y., Kawai, H., Nakane, T., Matsuoka, K., Yasuno, F., Kishimoto, T., and Naganawa, S. (2017). Evaluation of glymphatic system activity with the diffusion MR technique: diffusion tensor image analysis along the perivascular space (DTI-ALPS) in Alzheimer's disease cases. Jpn. J. Radiol. 35: 172–178.

Van De Haar, H.J., Burgmans, S., Jansen, J.F., van Osch, M.J.P., van Buchem, M.A., Muller, M., Hofman, P.A.M., Verhey, F.R.J., and Backes, W.H. (2016). Blood-brain barrier leakage in patients with early Alzheimer disease. Radiology 281: 527–535.

Vesalius, A. (1553). *De humani corporis fabrics lihri septem*. Basel: Oporinus.

Visanji, N.P., Lang, A.E., and Munoz, D.G. (2018). Lymphatic vasculature in human dural superior sagittal sinus: implications for neurodegenerative proteinopathies. Neurosci. Lett. 665: 18–21.

Vonrautenfeld, D.B., Kaiser, H.E., Foeldi, M., Maher, N., and Trienekens, A. (1994). The leptomeningeal sheath of the optic-nerve as an area of lymphatic resorption of cerebrospinal-fluid. Lymphology 27: 685–687.

Wang, L., Zhang, Y., Zhao, Y., Marshall, C., Wu, T., and Xiao, M. (2019). Deep cervical lymph node ligation aggravates AD-like pathology of APP/PS1 mice. Brain Pathol. 29: 176–192.

Wang, M., Ding, F., Deng, S.Y., Guo, X., Wang, W., Iliff, J.J., and Nedergaard, M. (2017). Focal solute trapping and global glymphatic pathway impairment in a murine model of multiple microinfarcts. J. Neurosci. 37: 2870–2877.

Weed, L.H. (1914). Studies on Cerebro-Spinal Fluid. No. III: the pathways of escape from the subarachnoid spaces with particular reference to the arachnoid villi. Am. J. Pathol. 31: 51–91.

Wolak, D.J. and Thorne, R.G. (2013). Diffusion of macromolecules in the brain: implications for drug delivery. Mol. Pharm. 10: 1492–1504.

Woollam, D.H.M. and Millen, J.W. (1955). The perivascular spaces of the mammalian central nervous system and their relation to the perineuronal and subarachnoid spaces. J. Anat. 89: 193.

Xie, L., Kang, H., Xu, Q., Chen, M.J., Liao, Y., Thiyagarajan, M., O'Donnell, J., Christensen, D.J., Nicholson, C., Iliff, J.J., et al. (2013). Sleep drives metabolite clearance from the adult brain. Science 342: 373–377.

Yang, L., Kress, B.T., Weber, H.J., Thiyagarajan, M., Wang, B., Deane, R., Benveniste, H., Iliff, J.J., and Nedergaard, M. (2013). Evaluating glymphatic pathway function utilizing clinically relevant intrathecal infusion of CSF tracer. J. Transl. Med. 11: 1–9.

Zawieja, D.C. (2009). Contractile physiology of lymphatics. Lymphatic Res. Biol. 7: 87–96.

Zhang, E.T., Inman, C.B., and Weller, R.O. (1990). Interrelationships of the pia mater and the perivascular (Virchow-Robin) spaces in the human cerebrum. J. Anat. 170: 111–123.

Zhang, E.T., Richards, H.K., Kida, S., and Weller, R.O. (1995). Directional and compartmentalised drainage of interstitial fluid and cerebrospinal fluid from the rat brain. Acta Neuropathol. 83: 233–239.

Supplementary Material: The online version of this article offers supplementary material (https://doi.org/10.1515/revneuro-2020-0106).