Deep brain lymphatic vessels: a new player in brain functions, neurodegenerative diseases and psychiatric disorders

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1 Introduction
There are two kinds of vascular systems in the vertebrate body: blood vasculature and lymphatic vasculature. The blood system functions as a closed circulatory system, facilitating the transportation of blood throughout the body and enabling the exchange of salt, water, oxygen, nutrients, hormones, and waste materials between tissues. Conversely, the lymphatic system is a blind-ended, unidirectional system responsible for reabsorbing interstitial fluid that leaks from the vasculature into the extracellular space to complement the blood-cardiac circulatory system [1]. Although the lymphatic system has not been well established compared to the blood-vascular system, it is still widely studied because of its essential role in immune surveillance, lipid resorption and tissue fluid homeostasis regulation [2-7].

In the central nervous system (CNS), identifying a glial-lymphatic (glymphatic) system has significantly advanced our understanding. This system, as demonstrated by Iliff Jeffrey et al. (2012), facilitates exchanges between cerebrospinal fluid and interstitial fluid through specific spaces...
existing between blood vessels, endothelial cells and astrocytes [8]. Later, lymphatic vessels were discovered in the meninges of the mouse brain [9]. Follow-up studies confirmed meningeal lymphatic vessels exist in nonhuman primates and humans [10]. The discovery of meningeal lymphatic vessels and the later follow-up studies redefined the relationship between the immune system and the brain regarding health and disease [11-14]. From neuropathological perspectives, meningeal lymphatic vessels are regulated during normal aging [7, 11], Alzheimer’s disease [11], Parkinson’s disease [15] and following traumatic brain injuries [13].

This review provides a concise overview of the structure and characteristics of lymphatic vessels in three critical peripheral organs. Subsequently, we delve into the distribution and function of the CNS’s glymphatic and meningeal lymphatic systems. Finally, we discuss the recently discovered deep brain lymphatic vessels. Notably, we emphasize the regulation of the brain lymphatic system under the influence of neurodegenerative, stress, and psychiatric disorders.

2 Structure of lymphatic vessels

The lymphatic system comprises initial capillaries that combine to form the collecting lymphatics [16]. The initial lymphatics are blind-ended vessels composed of a single layer of endothelial cells with a discontinuous and often indistinct basal lamina and “button-like” cell junctions [2, 17]. Specialized endothelial cells provide essential conditions for lymphatic formation [18] and unidirectional flow of cells and fluid into the vessel [19]. Collecting lymphatics have intraluminal valves, known as lymphangions, that contract spontaneously to drive flow and several perivascular layers of lymphatic muscle cells that provide vascular tension and rhythmic contraction of blood vessels to allow for active fluid transport against gravity [16, 20–22]. Lymphatic fluid uptake and drainage occur through particular cell junctions between lymphatic endothelial cells that form the lymphatic capillaries. The homeostatic mechanisms regulating junctions opening/closing in peripheral lymphatic vessels have been demonstrated [23]. Cellular and molecular studies show that Rho-Associated Kinase (ROCK) signaling in lymphatic endothelial cells initiates the formation of junction-anchored stress fibers and generates the tension required to pull junctions apart to open for fluid influx. In contrast, activation of vascular endothelial growth factor (VEGF) signaling induces actin stress fiber relaxation, junction zippering and fluid retention by inhibiting ROCK signaling [23].

3 Lymphatic vessels in the heart, liver and kidney

Since lymphatic vessels are fundamentally present in various organs, exploring their structure and function in different organs has been a pivotal research focus [24]. The first cardiac lymphatic sprouts appear in mice at embryonic stages (E12.5). By E18.5, the lymphatic vessels have already entered the heart and continue dilating and projecting to the apex on both the dorsal and ventral sides before developing into a more extensive mature network [25-27]. The cardiac lymphatic system has been linked to heart diseases, especially after myocardial infarction [28]. Knockout of lymphatic vessel endothelial hyaluronan receptor 1 (LYVE1) in mice leads to increased chronic inflammation and long-term decline in cardiac function [29]. On the other hand, promoting lymphatic clearance by overexpression of adrenomedullin improves cardiac edema and protects cardiac functions [30].

The liver is the largest lymph-producing organ, accounting for 25–50% of lymph passing through the thoracic duct [31, 32]. Hepatic
lymphatic vessels can be categorized into three types according to their location: portal, sublobular, and capsular lymphatic vessels [33, 34]. In liver disease, the pressure of lymphatic vessels increases. Patients with cirrhosis have higher peritoneal lymphogenesis (by 30-fold) and thoracic duct lymphatic flow (by 8- to 9-fold) than healthy subjects [35, 36]. Moreover, the number of lymphatic vessels increases during fibrosis, which correlates with vasodilation and disease severity [32, 37, 38].

The renal lymphatic vessels originate from the renal lobular parenchyma and follow the main arteries and veins to the renal hilum and capsule. Renal interlobular lymphatic vessels do not have valves, allowing lymph formed within the kidney cortex to flow in either direction [39]. Interlobular lymphatics are connected to renal hilar lymphatic vessels [40]. Under pathological conditions, lesions in renal tissue often trigger local lymphangiogenesis. On the one hand, the proliferating lymphatic vessels help remove inflammatory cytokines from the injured tissue and slow the progression of renal injury [41, 42]. On the other hand, the newly generated lymphatics might increase systemic inflammation and aggravate renal injury [43, 44]. Furthermore, dysregulation of the renal lymphatic system propagates inflammatory reactions and promotes vascular congestive mechanisms, leading to heart failure [45].

Based on the three examples discussed above, the distribution and structure of lymphatic vessels vary among critical organs. Understanding the distribution, structure, and function of lymphatic vessels in different organs is crucial for understanding their dynamic role in health and disease.

4 CNS glymphatic system

The brain was shown to have a unique drainage system called the glymphatic system [8, 46]. Fluid tracer studies have revealed that cerebrospinal fluid (CSF) flows explicitly into the brain through gaps in specific periarterial areas, almost entirely enveloped by the terminal feet of astrocytes. Astrocyte terminal feet densely express Aquaporin 4 (AQP4) protein, facilitating CSF influx into the brain parenchyma, which intermixes with interstitial fluid [8, 47]. AQP4 is the most abundant water channel in the brain that maintains ion and permeability homeostasis by promoting water diffusion [48, 49]. Consequently, the term “glial-associated lymphatic system or glymphatic system” has been coined for this drainage system [8].

The glymphatic system is crucial in maintaining central nervous system homeostasis by regulating the distribution and transport of nutrients, minerals, water and glucose [50]. The delivery and circulation of apolipoprotein E (apoE), crucial for synaptic plasticity and memory functions, is also inseparable from the critical role of the glymphatic system in maintaining brain health [51]. The ability of glymphatic clearance is enhanced during sleep, leading to decreased brain lactate levels [52]. Furthermore, AQP4 is vital in maintaining normal neural excitation, astrocyte migration, synaptic plasticity, and cognitive and affective functions [53-55]. In pathology, slow clearance or complete occlusion of the glymphatic system was reported during aging [47], inflammatory reactions [56], and stroke [57], as well as in mouse models of Alzheimer’s disease [58], Parkinson’s disease [59] and depression-like symptoms [60]. Furthermore, dysregulation of the circadian expression of AQP4 is associated with depression-like behavior in rodents [61]. AQP4 knockout mice exhibit more accumulation of Aβ plaques [62]. Blocking AQP4 has been used to rescue motor functions after strokes [63] and reverse Parkinson’s disease symptoms in animal models [64]. In
contrast, activation of AQP4 has been shown to alleviate cognitive dysfunctions and provide neuroprotection in a mouse model of Alzheimer’s disease [65].

Thus, the glymphatic system is pivotal for maintaining brain health. Disruptions in its functions might contribute to neurological diseases. Given the critical role of the glymphatic system, further research on mechanisms regulating glymphatic system functions is required, as such mechanisms still need to be discovered. Revealing such regulatory mechanisms could pave the way for developing novel therapeutic interventions to treat neurodegenerative and psychiatric disorders.

5 Meningeal lymphatic system

Meningeal lymphatic vessels have been proposed to exist in humans in 1787 by Mascagni [66]. Eighty years later, Schwalbe provided evidence supporting that hypothesis by using a tracing dye injected in a dog’s subarachnoid space [67]. Csanda and colleagues (1966) further demonstrated the presence of lymphatic vessels in the dura mater of the basal cranial region, establishing their connection with peripheral lymphatic circulation and suggesting a potential role in the drainage of brain waste, nutrients, and other molecules [68]. In 2015, meningeal lymphatic vessels in the mouse brain were comprehensively delineated [9]. The meningeal lymphatic vessels express key markers of lymphatic endothelial cells, including LYVE1, Prospero-related homeobox 1 (PROX1), Podoplanin, and VEGF receptor 3 (VEGFR3). The vessels carry CSF and immune cells down to cervical lymph nodes [9]. In the same year (2015), another study demonstrated that meningeal lymphatic vessels could absorb brain fluids from CSF and interstitial fluid from the subarachnoid space and transport the fluids down to the deep cervical lymph nodes in the base of the skull along the transverse sinus, sigmoid sinus, the retrogonoid vein, the rostral rhinal vein, and the major branches of the middle and anterior meningeal arteries [69]. In the brain, clearance of cellular debris, metabolic waste products and toxic molecules was believed to be mediated by i) transcellular transport mechanisms across the blood-brain and blood-cerebrospinal fluid barriers [70, 71], ii) phagocytosis and digestion by resident microglia and recruited monocytes and macrophages [72, 73] and iii) CSF influx and interstitial fluid efflux by the glymphatic system [8, 47, 58]. Thus, the discovery of meningeal lymphatics redefined the CNS waste clearance pathway.

From pathological perspectives, neurodegenerative diseases such as Alzheimer’s disease and Parkinson’s disease are attributed to protein misfolding, the accumulation of metabolic waste and the buildup of toxic molecules. Therefore, such brain diseases might be closely related to the drainage of meningeal lymphatic vessels and immune regulation [74]. In support of this hypothesis, dorsal and ventral meningeal lymphatic drainage impairment was found in Parkinson’s disease patients [14]. In rodents, ablation of the meningeal lymphatic vessels results in spatial learning deficits and impairments in glycolysis, mitochondrial respiration and oxidative stress processes in the hippocampus [11]. In a mouse model of Alzheimer’s disease, ablation of the meningeal lymphatic vessels increases Aβ depositions in the brain parenchyma. It exacerbates immune and neurovascular responses, affecting the outcome of anti-Aβ immunotherapy [11, 74]. Increases in microgliosis and fibrinogen around the Aβ-plaques reduced Aβ clearance by the anti-Aβ immunotherapy in the mice with ablated lymphatic vessels [74]. Finally, impairment of meningeal lymphatic vessels has been shown to contribute to susceptibility to
chronic psychosocial stress and the development of depression-like symptoms [75]. In conclusion, the meningeal lymphatic system plays a vital role in maintaining brain health, and its dysfunction may contribute to brain disorders, including neurodegenerative and stress/psychiatric disorders.

6 Deep brain lymphatic vessels

As research on the structure, morphology, and distribution of meningeal lymphatic vessels has advanced [76, 77], attention has turned to the potential existence of similar lymphatic structures deep within the brain parenchyma. Potential lymphatic connections between the CNS and the periphery have been described for years [78-80], and the possibility of lymphatic vessels in the brain parenchyma was even suggested earlier [81]. However, one single-day staining protocol study reported the absence of lymphatic vessels in the brain parenchyma or pia mater [69]. In contrast to these findings, LYVE1-positive structures were discovered deep in brain tissue along blood arteries that penetrate deep into the cerebral cortex, suggesting the existence of intraparenchymal lymphatic vessels [82]. However, follow-up studies contested these findings and claimed that the LYVE1 positive cell population exhibits some perivascular macrophage characteristics [83]. Although the presented fluorescent images in this study suggest colocalization/proximity of PROX1, Podoplanin, and CD31 (but not VEGFR3) fluorescent signal with that of LYVE1, the authors still draw their conclusions based on the cells’ molecular composition [83].

Using tissue-clearing techniques, intensive immunostaining protocols, light sheet whole-brain imaging, confocal imaging in thick brain sections and flow cytometry, the existence of lymphatic vessels was demonstrated deep in the brain parenchyma [84]. LYVE1-positive vessel-like structures in the mouse brain were observed deep in brain tissue (examples see Fig. 1 and Video 1, data adopted from [84] with permission.

Fig. 1 Lymphatic vessels in the hippocampus of mice (light sheet microscopy and LYVE1 staining). A representative 3D reconstructed fluorescent image of the deep brain lymphatic vessels network within the hippocampus of mice shows the distribution of deep brain lymphatic vessels according to their diameter (color coded). Data was adapted from reference [84] with permission from the authors. The movies in movie S4 in [84] provide detailed information about lymphatic vessels in hippocampus.
from authors). The LYVE1 positive vessels can also be co-labeled by three other key markers of lymphatic vessels: namely Podoplanin, PROX1 and VEGFR3. Furthermore, flow cytometry identified lymphatic endothelial cells labeled with LYVE1 or Podoplanin (after excluding cells positive for macrophage markers) deep in brain tissue [84]. Like functional lymphatic vessels in the periphery, deep brain lymphatic vessels run alongside and parallel to blood vessels and carry white blood cells but not red blood cell markers [84]. Consequently, the authors concluded these vessels are functional deep brain lymphatic vessels. Structural and distribution analysis revealed that most lymphatic vessels originated from the brain’s outer surface [84]. In support of this discovery, a recent article in Science News highlighted a novel clearing/staining technique called “wildDISCO” and 3D imaging for mapping the mouse body and organs. This technique revealed structures stained with LYVE1, Podoplanin and PROX1 deep in brain tissue. The results show a pronounced PROX1 positive tubular signal extending deep into the cortex from the superior sagittal sinus. Furthermore, LYVE1 and Podoplanin signals cover the lymphatic capillaries on the surface of the mouse brain but also enter the para-thalamic parenchyma [85]. More recently, a third piece of evidence supporting the existence of lymphatic vessels in brain parenchyma was reported in the mouse brain [86].

A careful examination of the immunostaining procedures reveals major methodological differences between studies that reported parenchymal lymphatic vessels [84–86] and earlier studies that failed to detect lymphatic vessels in brain parenchyma [69]. The source, dilution and incubation intervals of LYVE1, VEGFR3, PROX1 and podoplanin antibodies are different. This is a crucial factor in detecting the vessels, as highlighted by the two studies reporting on the lymphatic vessels in brain tissue [84, 86]. Fixing, dehydrating, tissue preparation, and staining procedures are also vastly different. Chang et al. (2023) used slow and exhaustive fixing, dehydrating, tissue clearing, antibody incubating and washing procedures before imaging [84]. The critical roles of perfusion procedures and fluid temperature were also highlighted by the other study that detected lymphatic vessels in the brain parenchyma [86]. The study that failed to detect lymphatic vessels in brain tissue used overnight fixing of whole mount samples followed by washing, staining (on the second day) and imaging (in wild-type mice), or direct imaging in freshly prepared brain tissue without fixation or antibody incubations (in PROX1-GFP transgenic mice) [69]. Such fundamental methodological differences might explain the opposing findings. In conclusion, while additional investigations are necessary, the collective evidence from the studies above strongly supports the existence of deep brain lymphatic vessels. These findings offer new insights into the characteristic features of these vessels and underscore the need for continued research to identify the mechanisms regulating them in both health and disease.

## 7 Regulation of deep brain lymphatic vessels by chronic stress

The CNS plays a crucial role in mediating stress responses [87], including orchestrating the hypothalamic–pituitary–adrenal (HPA) axis in response to stress [88]. The hippocampus, prefrontal cortex and amygdala are among the most investigated brain regions in the field of stress/psychiatric disorders [89–91]. Early research primarily focused on the effects of stress and stress hormones (glucocorticoids: cortisol in humans and corticosterone in rodents) on neuronal
plasticity, including dendritic tree arborization and synapse density/plasticity [92, 93]. Notably, this body of research indicates that stressful life events cause atrophy in the dendritic arborization of CA1 pyramidal neurons and a reduction in axon density of CA3 pyramidal neurons [94]. Similarly, chronic stress diminishes neuronal dendritic branches and length in the prefrontal cortex [87, 95]. In contrast, chronic stress induces opposite effects in the amygdala. Remarkably, chronic stress increases dendritic tree branches, length and spine density in basolateral amygdala [96–99].

On the other hand, studies provide compelling evidence linking chronic stress to a broad spectrum of diseases, including cancer and cardiovascular and immune system-related diseases [100]. Regarding the immune system, chronic stress promotes inflammatory mechanisms and suppresses immune system activity [101]. Research indicates that stress triggers physiological responses, leading to increases in stress hormones, subsequently resulting in elevated levels of inflammatory mediators such as cytokines [102]. Therefore, chronic stress pathology can impact neuronal, cardiovascular, inflammatory and immune functions. Consequently, chronic stress might also impact the lymphatic system in the brain. Indeed, chronic stress has been shown to remodel the complexity of the lymphatic vessel network inside tumors [103] and reduce the flow rate of the lymphatic system circulation in the brain [60]. Disruption of meningeal lymphatic vessels increases susceptibility to chronic psychosocial stress [75]. Regarding deep brain lymphatic vessels, a recent study shows that chronic mild stress or repeated corticosterone injections reduce the areas and length of deep brain lymphatic vessels and lymphatic endothelial cell markers (respectively) in the hippocampus of mice [84]. Besides the intriguing finding of regulating deep brain lymphatic vessels by chronic stress, several important issues must be noted here: 1) Brain region specificity: The effects of stress were found to be specific to certain brain regions. The authors found changes in lymphatic vessels in the hippocampus, thalamus and amygdala but not in the prefrontal cortex, dorsal raphe nucleus or lateral habenula. The distinction lies in the source of lymphatic inputs, with the former three brain regions receiving lymphatic inputs from the basal side. In contrast, the latter areas receive inputs from the dorsal meningeal lymphatic vessels. The findings suggest that ventral meningeal and deep brain lymphatic vessels are more vulnerable to brain pathology. This aligns with recent findings indicating that the ventral meningeal lymphatic system is more vulnerable to changes during aging pathologies than the dorsal part [77]. 2) Differential effects on lymphatic vessel complexity: The authors found that chronic stress reduced lymphatic vessel complexity in the hippocampus while increasing their diameter in the amygdala. This opposing effect of chronic stress mirrors previous reports on spine density and dendritic arborization in both brain regions. 3) Subregional variation within the hippocampus: The authors observed distinct effects of chronic stress within the hippocampus. Chronic stress more substantially affected the dorsal hippocampus’s lymphatic vessels than the ventral hippocampus [84]. Therefore, chronic stress appears to regulate deep brain lymphatic vessels in a region-specific, region-dependent-opposing, and subregion-distinct manner. These findings highlight the complex impact of chronic stress on the regulation of deep brain lymphatic vessels, emphasizing the need for a comprehensive understanding of the regional and subregional dynamic changes that might occur in the brain during psychiatric disorders.

Mechanistically, chronic stress has been shown to regulate various signaling pathways, including
those related to growth factors such as the VEGF signaling pathway [104]. Chronic treatment with the stress hormone corticosterone downregulates VEGF signaling, including its receptors [105]. It is worth noting that VEGF signaling is a major driver of lymphangiogenesis [106]. Mice lacking VEGFC or VEGFR3 are lacking intact and functional meningeal lymphatic vessels [7]. Consistent with the pivotal role of VEGF signaling in mediating stress effects and regulating lymphatic vessels, chronic stress was found to reduce deep brain lymphatic vessels by downregulating VEGFC signaling in the hippocampus [84]. Stress downregulates VEGF signaling by decreasing the expression of VEGF receptors, promoting the production of a soluble fragment of VEGF receptors that neutralizes the growth factor and increasing the amount of neutralized/bound/nonfunctional portion of VEGFC within hippocampal tissue [84]. Although the findings provide intriguing insights into the molecular mechanisms governing the remodeling of brain parenchyma lymphatic vessels during stress-related psychopathologies, additional mechanistic studies are required to further elucidate the specific role of VEGFC signaling in this context.

8 Conclusions and Future Perspectives

Lymphatic vessels exhibit diverse structures and distribution across species and organs. Gaining insights into the distribution, structure and function of the lymphatic vessels in different organs is crucial for comprehending their dynamic roles in health and disease. In the CNS, the glymphatic system is essential for brain health, and dysfunctions in this system might contribute to several neurological diseases/disorders. Similarly, the meningeal lymphatic vessels are necessary for normal brain functions. Notably, the recent discovery of deep brain lymphatic vessels represents a critical advancement, filling a significant gap by connecting the glymphatic system, responsible for the cellular-level microenvironment, with the meningeal lymphatic system, governing the macroenvironment around the entire organ. The functional similarities suggest a close relationship of communication and cooperation among these three systems to maintain a healthy CNS. However, further investigations into the structural features, drainage functions and molecular mechanisms governing the functions of the glymphatic, meningeal, and deep brain lymphatic systems, as well as their communication within the CNS, still need to be discovered. This limits our ability to target the brain lymphatic system as a therapeutic intervention to treat brain disorders. Unraveling these regulatory mechanisms holds the promise of developing innovative therapeutic interventions for treating a broad spectrum of neurological, neurodegenerative, and psychiatric disorders.

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**Author contribution**

B.G., J.C., and N.A. collected literature and wrote the manuscript. All authors commented on all manuscript versions and approved the final version.

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All authors declare no conflict of interest.

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There is no data for this review.

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Not applicable.

**Consent to participate**

Not applicable.

**Supplementary information**

**Supplementary Video 1.** Lymphatic vessels in the hippocampus of mice (light sheet microscopy and LYVE1 staining, movie length: 23 s). Representative 3D reconstruction of the deep brain lymphatic vessels network within the hippocampus of mice showing the distribution of deep brain lymphatic vessels according to their diameter (color coded). Data was adapted from ref [84] with permission from the authors.

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