

Review

The Glymphatic System: From Basic Research Advances to Non-Invasive Brain Stimulation Applications-A Narrative Review

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Abstract

The glymphatic system (GS) is a pathway for waste clearance in the brain and a promising therapeutic target for neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD). GS function is governed by multiple interacting factors, such as aquaporin-4 (AQP4) polarization, cerebral arterial pulsatility, and the integrity of meningeal lymphatic vessels (MLVs). Despite growing recognition of the importance of the GS, effective strategies to actively and non-invasively enhance glymphatic function have long been lacking. In recent years, non-invasive neuromodulatory approaches—most notably focused ultrasound (FUS), repetitive transcranial magnetic stimulation (rTMS), and transcranial photobiomodulation (tPBM)—have emerged as novel strategies to modulate GS activity. Growing evidence suggests that these interventions can enhance glymphatic transport by influencing key regulatory mechanisms, including cerebrovascular dynamics, AQP4 expression and localization, and meningeal lymphatic drainage. In this review, we summarize current experimental advances in non-invasive, external alterations of the GS, discuss underlying mechanisms and translational challenges, and highlight the therapeutic potential of GS modulation in neurological diseases characterized by impaired waste clearance. However, further research about the function and dysfunction of the GS in humans is needed to evaluate the findings from animal studies and thereby fill the translational gap in the human pathophysiology of the GS.

Keywords: non-invasive brain stimulation techniques; glymphatic system; meningeal lymphatic vessels

1. Introduction

The glymphatic system (GS) is a critical pathway for the clearance of metabolic waste from the brain and the maintenance of central nervous system homeostasis [1,2]. Accumulating evidence demonstrates that GS dysfunction leads to the impaired removal of neurotoxic proteins, including amyloid- β (A β), tau, and α -synuclein (α -syn), thereby contributing to the pathogenesis of neurodegenerative disorders such as Alzheimer's disease (AD) and Parkinson's disease (PD) [3]. Consequently, modulation of GS function has emerged as a promising strategy for the prevention and treatment of neurological diseases associated with impaired waste clearance. Despite increasing recognition of the importance of the GS, effective approaches to actively regulate glymphatic function remain limited. In recent years, external interventions—particularly non-invasive brain stimulation (NIBS) techniques—have attracted growing interest as potential tools to enhance GS activity predominantly in animal models [4,5,6,7,8,9,10,11]. Moreover, some human data with regard to the GS and its clearing mechanisms including potential augmentations have been discussed by Bosche B et al. [12]. Here, we critically review the structural and functional organization of the GS and its associated pathways, with a particular fo-

cus on meningeal lymphatic vessels (MLVs), key regulatory factors governing glymphatic transport, and emerging evidence supporting the modulation of GS function by NIBS. By integrating current findings across experimental models, this review aims to clarify underlying mechanisms, highlight methodological considerations, and identify future directions for translational research in this rapidly evolving field.

2. Introduction of the Glymphatic System and Meningeal Lymphatic Vessels

2.1 Anatomy and Physiology of Meningeal Lymphatic Vessels

In 1787, Paolo Mascagni first mentioned the existence of MLVs [13], but at that time, his research was largely overlooked by the scientific community. Nearly two centuries later, Földi et al. [14] described the existence of lymphatic connections between the central nervous system (CNS) and the periphery, and found that tracers in the CNS could drain to peripheral tissues via these lymphatic connections, indicating that this connection participates in the molecular drainage of the CNS. In 1996, Li J et al. [15] used scanning electron microscopy to discover and name the meningeal lymphatic pores, considering them part of



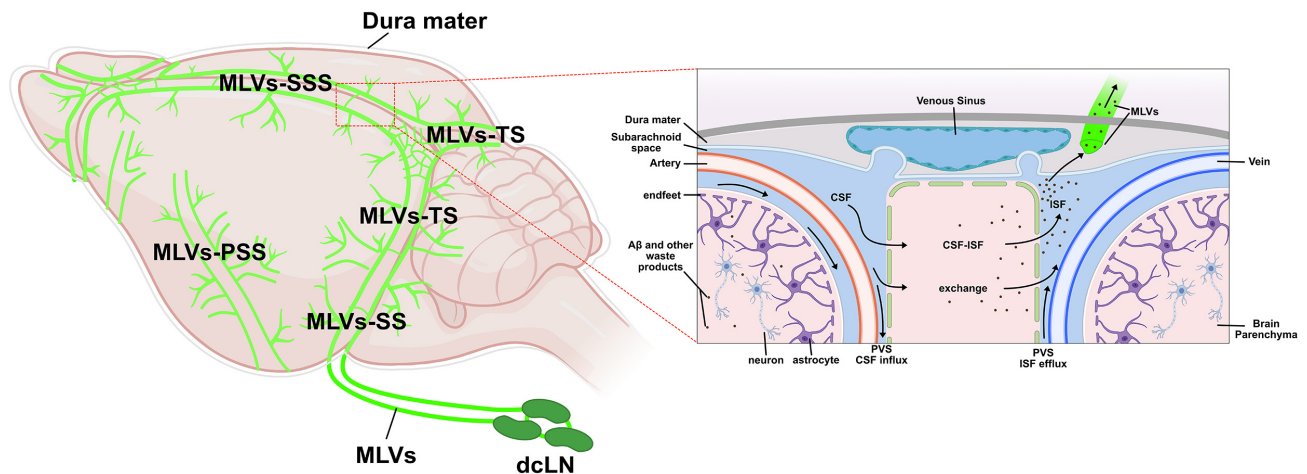


Fig. 1. Schematic diagram of the MLVs in the brain. MLVs located within the dura mater absorb ISF from the adjacent subarachnoid space, and then drain it through the scLVs and dcLVs to the scLNs and dcLNs, thereby establishing a connection between the brain and the peripheral lymphatic system. MLVs, meningeal lymphatic vessels; CSF, cerebrospinal fluid; ISF, interstitial fluid; scLVs, cervical superficial lymphatic vessels; dcLVs, cervical deep lymphatic vessels; scLNs, superficial cervical lymphatic nodes; dcLNs, deep cervical lymphatic nodes; SSS, superior sagittal sinus; TS, transverse sinus; SS, sigmoid sinus; PSS, petrosquamosal sinus; A β , amyloid- β ; PVS, perivascular space.

the anterior lymphatic capillary system of the brain; however, due to technical limitations, they could not determine whether these were actual lymphatic vessels. It was not until 2015 that Louveau A et al. [16] and Aspelund A et al. [17] applied more advanced confocal microscopy imaging techniques to confirm the existence of MLVs in mice, also demonstrating that MLVs serve as the major drainage pathway for cerebrospinal fluid (CSF). Additionally, in 2017, Absinta M et al. [18] used high-resolution magnetic resonance imaging (MRI) to demonstrate the presence of MLVs in both humans and non-human primates.

Structurally, MLVs accompany the dural venous sinuses and converge around the venous sinuses and the cranial base, with the MLVs of the transverse sinus being larger and more complex than that of the superior sagittal sinus [16,19,20]. Although MLVs exhibit anatomical and molecular features characteristic of initial lymphatic vessels, similar to peripheral lymphatics, it also displays certain unique characteristics, such as originating from the optic nerves, traveling near the nasal sinuses to reach above the olfactory bulbs, having small diameters, often being discontinuous, and covering less tissue [17,21]. The specificity of MLVs may be attributed to the high CSF pressure in the CNS, which could influence the branching and dilation of MLVs. Furthermore, the development and maintenance of MLVs depend on the vascular endothelial growth factor C (VEGF-C)/vascular endothelial growth factor receptor 3 (VEGFR3) signaling pathway [22]. Functionally, MLVs serve as a conduit connecting the brain to the peripheral lymphatic system [23], absorbing Interstitial fluid (ISF) from the adjacent subarachnoid space and draining it to the superficial cervical lymph nodes (scLNs) and deep cervical lymph nodes

(dcLNs) through the cranial base openings after exchanging with CSF in the GS [17,24] (Fig. 1). The drainage function of MLVs can facilitate the clearance of toxic proteins such as A β and α -syn from the brain parenchyma, preventing neurodegenerative diseases such as Alzheimer's and Parkinson's diseases [25,26].

2.2 Structure and Basic Function of the Glymphatic System

The proposal of MLVs addressed the issue of how metabolic waste is expelled from the brain, but the question of how metabolic waste is collected and transported within the brain's parenchyma remained unexplained until the discovery of GS. In 2012, Iliff JJ et al. [2] injected small fluorescent tracers into the cisterna magna of mice and utilized two-photon microscopy to observe the flow pathways of these tracers. This groundbreaking study led to the first identification and naming of GS, which is now considered a crucial fluid clearance mechanism in the brain [27,28].

GS is a CSF-ISF exchange system involving aquaporin-4 (AQP4) in astrocytes, relying on arterial and venous perivascular space (PVS) [29]. The circulation pathway of this system is a highly organized fluid transport system, primarily involving the inflow of CSF and the outflow of ISF (Fig. 2). The inflow of CSF refers to its entry into the brain parenchyma from the subarachnoid space via the arterial PVS, where it flows along the PVS into the brain tissue through AQP4 on the end-feet of astrocytes, regulated by CSF pressure and arterial pulsation as the vascular tree branches. The outflow of ISF refers to the exchange of substances between the CSF that has entered the brain parenchyma and ISF, resulting in a cohesive flow that diffuses throughout various brain regions. This flow

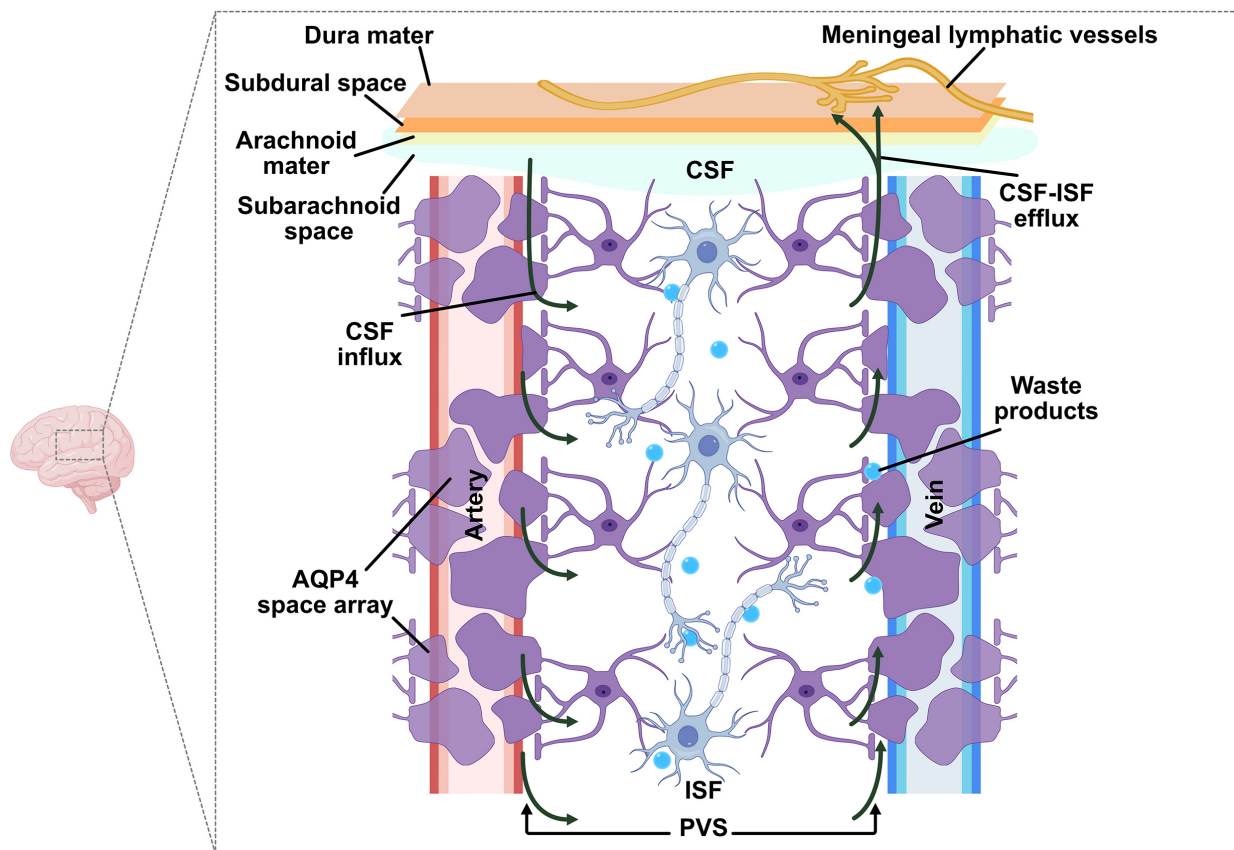


Fig. 2. Schematic Diagram of GS. The circulation of GS mainly includes the inflow of CSF and the outflow of ISF. The inflow of CSF refers to its entry into the brain parenchyma from the subarachnoid space via the arterial PVS, where it flows along the PVS into the brain tissue through AQP4 on the end-feet of astrocytes, regulated by CSF pressure and arterial pulsation as the vascular tree branches. The outflow of ISF refers to the exchange of substances between the CSF that has entered the brain parenchyma and ISF, resulting in a cohesive flow that diffuses throughout various brain regions. This flow carries metabolic waste from the brain and enters the PVS surrounding venous blood vessels through AQP4 on the end-feet of astrocytes, ultimately draining into the MLVs. AQP4, aquaporin-4; PVS, perivascular space; MLVs, meningeal lymphatic vessels; GS, glymphatic system.

carries metabolic waste from the brain and enters the PVS surrounding venous blood vessels through AQP4 on the end-feet of astrocytes, ultimately draining into the MLVs. The primary function of the GS is to clear metabolic waste from the brain and maintain the homeostasis of the brain's internal environment [24,30].

3. Key Determinants of Glymphatic System Function

3.1 Aquaporin-4 (AQP4)

AQP4 is a water channel protein that is highly polarized in the end-feet of perivascular astrocytes [31,32], and its polarity is dependent on the anchoring mediated by the β -sarcoglycan in the dystrophin-associated complex (DAC) and the regulation of endosomal signaling. This mechanism may partly achieve the dynamic transport of AQP4 between vesicles and the plasma membrane, allowing for rapid modulation of channel abundance at the membrane surface without altering total protein expression lev-

els [33,34]. The expression and polarity of AQP4 are regulated by various pathophysiological factors. For example, aging, CNS injuries, traumatic brain injury, and sleep deprivation can downregulate expression and disrupt polarity simultaneously, while hypoxia-ischemia, osmotic imbalance, and hypothermia mainly impair the distribution of polarity [34,35,36,37]. Hypoxic-ischemic injury has been shown to drive the translocation of astrocytic AQP4 to the plasma membrane via calmodulin signaling, thereby altering brain water homeostasis and causing cellular swelling [36]. In the acute phase of CNS injury, astrocytic AQP4 displaces from the end-foot membrane to the cell membrane, leading to cytotoxic edema and impairing glymphatic function; in the chronic phase, AQP4 re-anchors from the cell membrane back to the end-foot membrane, thereby restoring glymphatic clearance [37]. Functionally, the glymphatic mediated influx of cerebrospinal fluid and solute clearance are closely associated with perivascular AQP4 [34]. AQP4 gene knockout can reduce cerebrospinal fluid influx by 70%

and solute clearance by 55% [2]. However, it is important to note that AQP4 depolarization can coexist with inflammation, blood-brain barrier dysfunction, or extracellular matrix remodeling [38,39,40], and current evidence suggests that it is a key associated factor in glymphatic dysfunction [39,41]; thus, its independent causal role still needs to be validated. Furthermore, AQP4 does not directly transport solutes; the mechanism by which its knock-out leads to decreased solute clearance is indirect. The absence of AQP4 hinders the efflux of water produced by astrocytic metabolism, causing end-foot edema that narrows the intercellular spaces and impedes solute diffusion [42]. This mechanism suggests that evaluating glymphatic function requires distinguishing between the convection of CSF within the perivascular space (PVS) and transcellular water exchange through AQP4. Classic fluorescent tracers reflect perivascular convection, while $H_2^{17}O$ MRI can simultaneously visualize transcellular water exchange and perivascular convection [29,43].

The absence of AQP4 can lead to impaired glymphatic drainage function, resulting in the accumulation of A β , tau protein, and α -syn in the brain, thereby causing neurological diseases [44,45,46,47]. Harrison IF et al. [46] and colleagues observed AQP4 impairment along with the accumulation of A β and tau proteins in an AD mouse model. Their findings suggest that AQP4 inhibition impairs the clearance of A β and tau proteins, thereby accelerating the pathological progression of AD [46]. Furthermore, studies have confirmed that the AQP4-mediated GS participates in the removal of α -syn, but this function is suppressed in PD. Zhang Y et al. [44] found that the absence of AQP4 promotes the accumulation of α -syn in mice overexpressing A53T- α -syn, exacerbating PD symptoms. Similar studies have also found that increased expression of AQP4 can enhance hematoma clearance following intracerebral hemorrhage (ICH), aiding in the recovery of brain tissue and improving ICH prognosis [48]. Liu X et al. [47] and Xia M et al. [49] observed decreased expression of AQP4 in the brains of mice subjected to chronic unpredictable mild stress (CUMS), which was accompanied by reduced clearance of A β . The administration of polyunsaturated fatty acids (PUFAs) and the antidepressant fluoxetine was found to alleviate the damage to the GS caused by CUMS [47,49]. Interestingly, early animal studies suggest that depression may contribute to the onset of AD and PD [49,50], potentially through the damage of AQP4 in the GS. Furthermore, one reason for the impaired GS drainage function associated with aging is the decreased expression of AQP4 [51,52]. The polarization of AQP4 can enhance the flow of GS. Recent studies have shown that the enhancement of glymphatic activity during exercise and sleep is partially dependent on the increased polarized expression of AQP4 in astrocytes [53,54]. However, the overexpression of AQP4 in the plasma membrane of astrocytes can also lead to cell swelling, resulting in cerebral edema, which in turn disrupts

the polarized localization of AQP4 at the end-feet of astrocytes and impairs glymphatic function [36,39]. The use of TGN-020 and trifluoperazine to inhibit this process has been shown to alleviate associated symptoms in models of spinal cord injury in rats and stroke in mice [36,39,55]. In summary, AQP4 is an important protein regulating glymphatic function, and its expression may have potential benefits for the treatment of related neurological diseases.

3.2 Arterial Pulsation

There is an interconnection between the CSF in the subarachnoid space and the PVS in the brain [56]. The process of CSF being transported from the subarachnoid space to the brain interstitium via the arterial PVS is pulsatile, primarily driven by the cardiac cycle, with the pulsation speed of cerebral arteries matching the flow rate of the CSF [57]. Specifically, the dilation of cerebral arteries enhanced the influx volume of CSF around the vascular spaces by promoting lateral flow of CSF, while the constriction of cerebral arteries directly increased the flow velocity of CSF [58]. Consequently, researchers consider cerebral arterial pulsation is a pivotal factor in modulating glymphatic drainage.

Mestre H et al. [57] investigated the impact of impaired cerebral arterial pulsatility on glymphatic clearance in a hypertension model characterized by reduced cerebrovascular compliance, finding that the impairment of cerebral arterial pulsatility reduced CSF flow within the PVS and throughout the entire GS, while also decreasing the clearance of A β . Li M et al. [59] also demonstrated that impaired cerebral artery pulsatility inhibits glymphatic clearance by assessing the pulsation of cortical vessels in male wild-type (WT) and microvascular amyloid model (Tg-SwDI) mice with bilateral common carotid artery stenosis (BCAS). Similarly, the impaired glymphatic function in the elderly is also related to the diminished arterial pulsation [52]. On the contrary, the enhancement of cerebral arterial pulsation will enhance the drainage function of the GS. Holstein-Rønso S et al. [58] induced functional hyperemia during anesthesia by stimulating the whiskers, and observed a 1.6-fold increase in the inflow rate of CSF surrounding the arteries in the stimulated hemisphere, enhancing glymphatic function. Their subsequent experiments indicated that the direct increase in CSF inflow was driven by the dilation and contraction of arterial diameter induced by functional hyperemia activating neurons [58]. Exercise serves as a potential intervention through this mechanism, as it not only regulates cerebral pulsatility by improving arterial compliance, thereby directly driving glymphatic drainage [60]; simultaneously, deep breathing during exercise can alter intrathoracic and intra-abdominal pressure, modulating the flow of CSF within the venous PVS and indirectly enhancing glymphatic function [61,62]. Additionally, although the role of sleep in brain clearance remains controversial [53,63], some studies suggest that en-

hanced glymphatic function during non-rapid eye movement (NREM) sleep is partly due to the increased periodic motion of cerebral blood vessels. Specifically, norepinephrine (NE) released during NREM sleep enhances the periodic flow of CSF by driving the periodic motion of cerebral blood vessels, which facilitates the expansion of PVS and the exchange of CSF with ISF, aiding in glymphatic clearance [64]. The aforementioned research results clearly demonstrate the close relationship between arterial pulsation and glymphatic function.

3.3 Others

Whether in a conscious state or under anesthesia, intracranial pressure (ICP) may be a factor affecting GS function. Plog BA et al. [65] have indicated that a reduction in intracranial pressure may facilitate the entry of tracers into the perivascular space PVS. Research by Semyachkina-Glushkovskaya O et al. [66] suggests that regional cerebral oxygen saturation (rScO₂) is also a factor influencing the clearance function of the GS, with increases leading to enhanced production of mitochondrial adenosine triphosphate (ATP), thereby promoting glymphatic drainage and clearance function through stimulating glymphatic contractions. Dysfunction of MLVs and cervical superficial and deep lymphatic vessels can also impair GS function. Research by Da Mesquita S et al. [19] indicates that damage to MLVs can inhibit the inflow of CSF without increasing intracranial pressure, thereby leading to GS dysfunction. The cervical superficial and deep lymphatic vessels are pathways that drains CSF from the GS to the cervical lymph nodes, and their dysfunction can impair the GS by reducing the drainage of CSF from the GS to the periphery [67]. Notably, with aging, the integrity of mLVs and cervical superficial and deep lymphatic vessels and the CSF drainage function undergo degeneration [20,67], suggesting that age-related decline in GS function is also associated with the damage of MLVs and cervical superficial and deep lymphatic vessels. The blood-brain barrier (BBB) exerts a dual effect on GS; excessive openness of the BBB can allow peripheral substances to enter the brain parenchyma, triggering excitotoxicity and inflammatory responses, thereby impairing the polarization of astrocytic AQP4 and causing GS dysfunction [68,69,70,71,72]. Conversely, its proper permeability can facilitate the glymphatic transport of metabolic waste such as A β from GS to dCLNs [73]. Furthermore, in a lipopolysaccharide-induced systemic inflammation mouse model, it was found that inflammation can inhibit the flow of CSF and the exchange between CSF and ISF [74]. Abnormally activated astrocytes and microglia have been shown to exacerbate neuroinflammation by producing large amounts of pro-inflammatory factors such as IL-1, IL-6, and IFN- γ [75,76]; based on this, we hypothesize that the abnormal activation of astrocytes and microglia may impair GS functions through neuroinflammatory responses.

4. Modulating Glymphatic System Function via Non-invasive Brain Stimulation Techniques

NIBS technology, due to its broader applicability and potential for early intervention, represents a cutting-edge approach to treating neurological disorders related to GS dysfunction, focusing on the modulation of GS through various mechanisms (Fig. 3).

4.1 Focused Ultrasound (FUS)

FUS can focus ultrasound energy with millimeter precision on specified focal areas in the brain [77,78], and can produce significant biological effects by adjusting relevant parameters, thereby affecting the CNS [79]. Choi S et al. [80] utilized *in vivo* real-time two-photon and wide-field imaging techniques to demonstrate that low-intensity focused ultrasound (LIFU) applied to the skull base can promote the flow of CSF in PVS. Another study combining real-time MRI and *ex vivo* histological methods has also confirmed that applying FUS to the entire brain significantly increases CSF influx into the brain's PVS [81]. Furthermore, the safety of FUS regulation of glymphatic functions has also been validated in the Sprague-Dawley (SD) rat model and the mouse model of amyotrophic lateral sclerosis [82,83].

The regulatory effect of FUS on GS shows therapeutic potential in models of neurological diseases and aging. Azadian MM et al. [84] found that in the ICH mouse model, the application of LIFU can clear red blood cells from the CNS through GS; in aged mice, LIFU can also reduce neurofilament light chain levels by enhancing glymphatic drainage function. Lee Y et al. [4] and colleagues demonstrated that FUS- microbubbles (MB) primarily enhance the clearance of A β by increasing the excretion of A β from the CSF to the dCLNs via MLVs, thereby delaying the progression of early dementia.

The enhancement of GS function by FUS is related to various factors. Given the ability of AQP4 to form complexes with TRPV4 channels, Wu CH et al. [85] explored the effects of low-intensity FUS on the regulation of TRPV4 channels in astrocytes and its impact on AQP4. They found that low-intensity FUS can activate TRPV4 channels, increasing intracellular Ca²⁺ levels, which in turn activates calmodulin (CaM) and promotes AQP4 polarization [85]. Ye D et al. [86] found that FUS-induced MB expansion and contraction increased the pulsation of arterioles. Moreover, after receiving FUS+MB treatment, the transport efficiency of fluorescently labeled albumin tracers in the PVS surrounding arterioles was significantly enhanced, indicating that the combined application of FUS and MB promotes glymphatic drainage by increasing the pulsation of arterioles [86]. Azadian MM et al. [87] demonstrated in a mouse model of hemorrhagic stroke that FUS can promote the clearance of intracerebral hematoma-like glymphatic fluid, while also observing the inhibition of abnormal

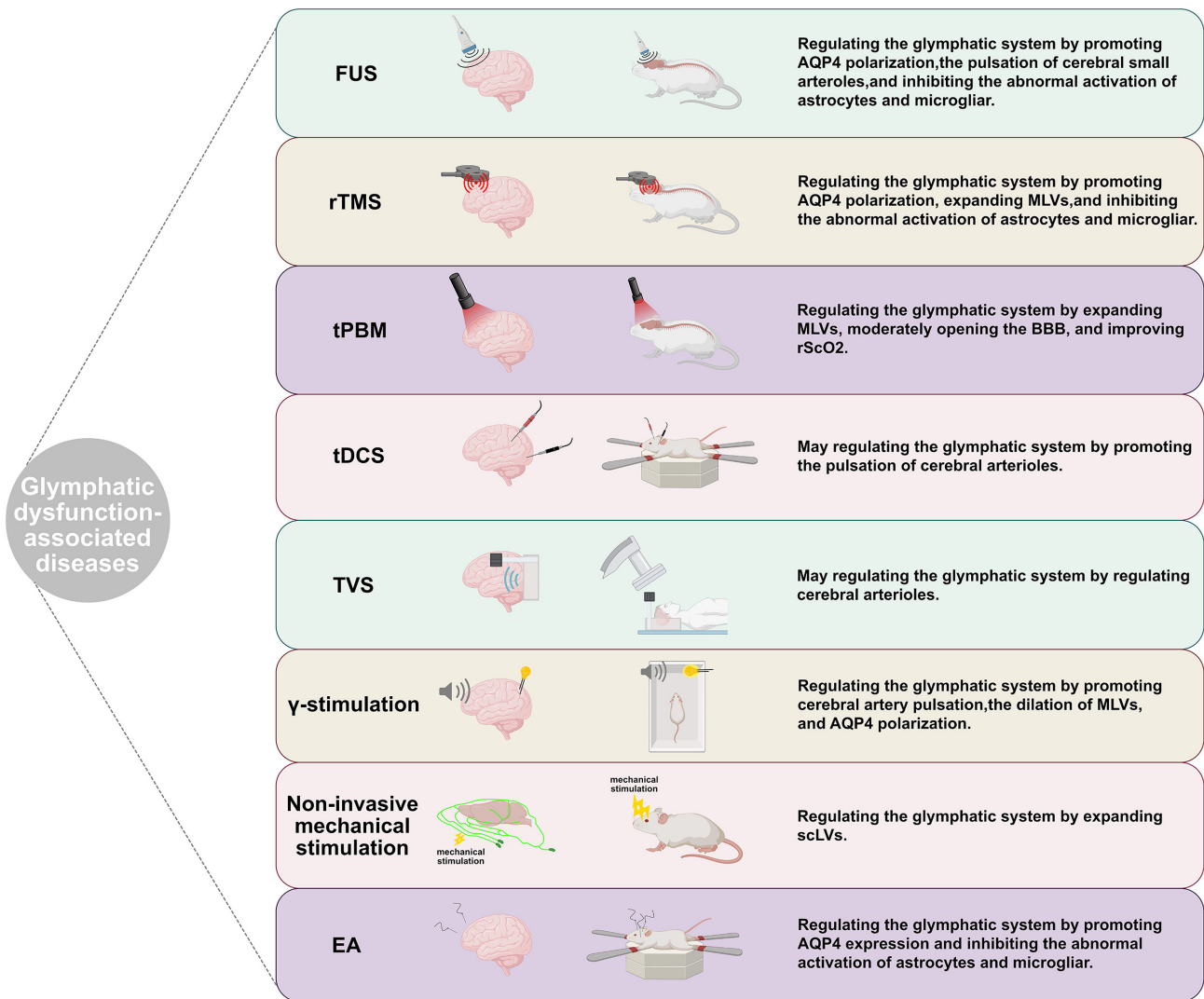


Fig. 3. NIBS technology regulates brain lymphatic function. NIBS, including FUS, rTMS, tPBM, tDCS, TVS, γ -stimulation, non-invasive mechanical stimulation and EA, has been shown to have significant effects in treating diseases associated with brain lymphatic dysfunction by modulating brain lymphatic function. The mechanisms depicted in the figure represent potential pathways through which NIBS modulates the function of the glymphatic system, a point confirmed by current research. Among these, TVS has been conducted on humans, while other experiments primarily involve rodent models. FUS, focused ultrasound; rTMS, repetitive transcranial magnetic stimulation; tPBM, transcranial photobiomodulation; tDCS, transcranial direct current stimulation; TVS, transcranial vibration stimulation; EA, electroacupuncture; BBB, blood-brain barrier; rScO₂, regional cerebral oxygen saturation; NIBS, non-invasive brain stimulation.

activation of microglia and astrocytes, suggesting the correlation between FUS regulation of GS function and its inhibition of abnormal activation of microglia and astrocytes. In conclusion, FUS exerts effects on treating neurological diseases associated with GS dysfunction by regulating GS function, and this effect is related to the increased polarization of AQP4, the increase in cerebral arteriolar pulsatility, and inhibition of Abnormal Activation of Microglia and Astrocytes.

4.2 Repetitive Transcranial Magnetic Stimulation (rTMS)

rTMS has become a NIBS technique that induces excitability changes in the cortex by generating a mag-

netic field that penetrates the scalp, thereby modulating the brain's electrical activity [88,89]. As an important non-invasive option with sustained efficacy [90,91,92], an increasing body of research has demonstrated the potential role of rTMS in regulating the GS and alleviating GS-related neurological disorders. Animal studies have shown that rTMS significantly enhances the removal of CSF tracers from brain tissue after ICH [93] and significantly improves the drainage efficiency of both parenchymal and meningeal lymphatic pathways in a 5xFAD mouse model, reducing A β deposition [5].

The mechanism by which rTMS regulates GS function has also garnered attention. For instance, continuous

theta burst stimulation (cTBS), as a patterned rather than variable form of rTMS [94], recent research reports have indicated that cTBS can significantly improve glymphatic drainage pathways by promoting AQP4 polarization and inhibiting the excessive activation of microglia and astrocytes, thereby significantly ameliorating the pathological damage and clinical cognitive dysfunction associated with diseases related to glymphatic dysfunction, such as AD and mild cerebral infarction, and this effect is associated with the activation of GABAAR [95,96,97]. Furthermore, Li MN et al. [98] found that cTBS can expand MLVs by upregulating meningeal Vascular Endothelial Growth Factor C (VEGF-C), thereby improving the brain's glymphatic clearance function. Anyway, the aforementioned study suggests that rTMS may regulate the brain's glymphatic drainage system by promoting AQP4 polarization, expanding MLVs, and inhibiting the excessive activation of microglia and astrocytes, thereby contributing to the treatment of related neurological disorders.

4.3 Transcranial Photobiomodulation (tPBM)

tPBM is a non-invasive neuromodulation technique that improves brain energy metabolism by affecting the concentrations of oxygenated hemoglobin ($\Delta[\text{HbO}]$) and oxidized cytochrome c oxidase ($\Delta[\text{oxi-CCO}]$), thereby influencing brain function [99,100]. Recent studies have proposed a new perspective that tPBM may be a promising method for treating brain diseases associated with abnormal CSF outflow [101]. Zinchenko E et al. [6] used 32 J/cm^2 tPBM treatment on AD model mice, which does not cause an increase in skull temperature or changes in brain morphology. They found that although the density of large A β plaques remained unchanged, the density of small plaques and overall A β deposition decreased, and the neurological behavior of the mice improved. Further studies revealed that this process is associated with enhanced glymphatic clearance function, which facilitates the efflux of A β to the dcLNs [6]. Furthermore, Li DY et al. [102] used a mouse model of intraventricular hemorrhage (IVH) to demonstrate that tPBM can promote the clearance of red blood cells from the ventricles by enhancing glymphatic activity, thereby improving associated symptoms.

Given that MLVs are a factor influencing GS function, and their disruption can promote the deposition of A β in the meninges and accelerate the progression of AD [103], the study explored the potential benefits of PBM on lymphatic pumping and contractility, and the findings suggest that this is a primary physiological mechanism for fluid transport and tissue waste clearance [104]. Semyachkina-Glushkovskaya O et al. [104] conducted analyses using optical coherence tomography (OCT) and confocal imaging and found that low-dose tPBM expands MLVs by vasodilation, thereby increasing lymphatic endothelial permeability to promote glymphatic drainage function. Additionally, tPBM has also been shown to enhance, rather than dimin-

ish, the cellular connectivity of meningeal lymphatic endothelial cells (mLECs) while improving the clearance capacity of MLVs and enhancing glymphatic drainage function [105]. These findings suggest that tPBM may expand MLVs by vasodilation without compromising the cellular connectivity of mLECs, thereby promoting the drainage and clearance functions of MLVs, which may aid in the treatment of neurological diseases associated with GS dysfunction.

tPBM-mediated BBB opening may be a potential mechanism for activating A β clearance in the brains of AD mice. Zinchenko E et al. [73] investigated this effect and found that tPBM promoted A β glymphatic clearance via the dcLNs. Further analyses indicated that the increased BBB permeability and improved A β clearance mediated by PBM likely result from a reduction in endothelial integrity and decreased expression of TJ proteins [73]. In studies involving AD mice, Semyachkina-Glushkovskaya O et al. [66] explored whether tPBM promotes A β glymphatic clearance by increasing brain energy metabolism. The results indicated that the improvement in brain rScO₂ induced by tPBM may be related to A β clearance in the brain; the increase in rScO₂ led to enhanced mitochondrial ATP production, thereby promoting the drainage and clearance functions of the GS [66]. Overall, these findings provide potential evidence that tPBM improves glymphatic clearance by regulating MLVs, BBB, and rScO₂, thereby treating brain diseases associated with abnormal CSF outflow.

4.4 Transcranial Direct Current Stimulation

Transcranial direct current stimulation (tDCS) is a non-invasive neuromodulation technique that can regulate the excitability of cortical neurons by applying weak electrical currents [106,107]. Wang Y and Monai H [7] first demonstrated the promoting effect of tDCS on CSF-ISF exchange using CSF tracing techniques, and subsequent studies combined electroencephalography (EEG) and inositol trisphosphate receptor type 2 knockout (IP3R2-KO) models to reveal that the mechanism may be related to the enhancement of slow-wave sleep δ waves induced by tDCS, and this process is mediated by IP3R2. Recent studies have also shown that non-rapid eye movement (NREM) sleep with strong δ wave activity can enhance the rhythmic motion of cerebral blood vessels by promoting the release of NE, thereby driving glymph flow [64,108]. Based on this, we speculate that tDCS may promote cerebral arterial pulsatility through the enhancement of slow-wave sleep mediated by IP3R2, thereby improving glymphatic function. It is important to note that this study has not ruled out the influence of anesthesia or sleep state when investigating the effects of tDCS on GS. Whether tDCS still has similar effects in a waking state and its specific mechanisms remain to be further investigated.

4.5 Transcranial Vibration Stimulation (TVS)

Transcranial vibration stimulation (TVS) is a novel non-invasive neuromodulation technique that regulates brain function through electromagnetic vibrations, first applied to human brain research by Kong L et al. [8] and colleagues. The team utilized functional magnetic resonance imaging (fMRI) and discovered that 40 Hz TVS significantly enhances the amplitude of low-frequency fluctuations (ALFF) in various brain regions during wakefulness, as well as the fractional ALFF (fALFF) in the frontal and parietal lobes, indicating its efficacy in stimulating spontaneous brain activity. Meanwhile, cross-correlation analysis of global blood-oxygen-level-dependent (gBOLD) signals and CSF signals revealed that 40 Hz TVS significantly enhances the synchrony between overall brain activity and CSF flow, suggesting that it may promote glymphatic clearance by enhancing neural activity [8]. It is noteworthy that neural activity can dilate cerebral arteries through neurovascular coupling mechanisms [58]. Although studies in mice indicate that neurovascular coupling during wakefulness may not necessarily enhance glymphatic function due to restricted glymphatic drainage, the more complex structure of the human brain and its lower glymphatic clearance efficiency suggest that the drainage limitations affecting GS maintenance of metabolic balance may be weaker than in mice [58,109], suggesting that neurovascular coupling during wakefulness still contributes to enhancing GS function. Therefore, the regulation of GS function by 40 Hz TVS may primarily be achieved by enhancing neural activity, which in turn drives cerebral artery dilation through neurovascular coupling pathways.

4.6 Multisensory Gamma Stimulation

Multisensory gamma stimulation, as a non-invasive neuromodulation technique that enhances cortical neural oscillations through synchronized visual and auditory stimuli, was first demonstrated by Murdock MH et al. [9] in an AD mouse model that 40 Hz stimulation can modulate GS function, reduce amyloid burden, and improve brain function, and this effect was shown to be associated with enhanced brain arterial pulsatility, dilation of MLVs, and increased polarization of AQP4 through techniques such as two-photon imaging, high-resolution volumetric 3D reconstruction, and single-cell RNA sequencing. Immunohistochemical analysis revealed that the effect on brain arterial pulsatility partly depends on vasoactive intestinal peptide (VIP) interneurons. Subsequent preliminary clinical trials also suggested that this intervention strategy can promote the clearance of A β in humans [9]. In conclusion, 40 Hz multisensory gamma stimulation regulates glymphatic function through mechanisms involving VIP interneuron-dependent arterial pulsatility enhancement, MLV dilation, and AQP4 polarization, providing a potential intervention strategy to delay the progression of diseases associated with GS dysfunction.

4.7 Non-Invasive Mechanical Stimulation

Non-invasive mechanical stimulation, as a relatively new non-invasive neuromodulation technique, can influence the flow of CSF by applying force via a mechanical stimulator on intact skin [11]. Jin H et al. [11] explored the potential of this technique to regulate CSF drainage. First, they validated the drainage of CSF to the scLNs using fluorescent tracers in both Prox1-GFP mice and the fiber bundle monkey model, noting that this process was diminished in aged mice due to impaired nitric oxide (NO) signaling. Subsequently, they demonstrated that non-invasive mechanical stimulation of the face and neck could increase the drainage of CSF to scLNs in aged mice by expanding the superficial cervical lymphatic vessels (scLVs), improving the potential feasibility of addressing age-related CSF drainage impairments; notably, this effect is partially dependent on NO signaling [11].

4.8 Electroacupuncture

Furthermore, electroacupuncture (EA) therapy is a form of transcutaneous electrical nerve stimulation (TENS) that combines acupuncture and electrical stimulation [110]. Liang PZ et al. [10] found in mouse models of early to mid-stage AD that EA can reduce A β deposition and alleviate cognitive impairment by improving GS function. Wang LM et al. [111] demonstrated that the mechanism by which EA improves their glymphatic function is related to its inhibition of the activation of astrocytes and microglia, reduction of inflammation, and increase in AQP4 polarization in hippocampal tissue.

5. Comparison of NIBS Methods for Glymphatic System Modulation

Although the effects of various non-invasive techniques on glymphatic function have been described above, direct comparisons between these technologies remain limited, and evidence regarding their relative efficacy, safety, and applicability is scarce. To provide a structured overview of reported effects across NIBS modalities, we summarized changes in GS-related outcomes and expressed them as relative clearance rate estimates where sufficient before–after or treatment–control data were available (Table 1, Ref. [4,6,7,8,9,10,11,66,73,77,78,83,84,93,96,98,103,104,105,106,110,112,113,114,115,116,117,118,119,120,121,122,123,124,125,126,127,128,129,130,131,132,133,134,135]). These estimates were derived by calculating the percentage change in tracer clearance, ISF/CSF transport, or GS-associated surrogate markers reported in individual studies. Importantly, because the underlying evaluation metrics varied substantially among techniques, species, and disease models, these values do not represent standardized or directly comparable measures of glymphatic efficacy and are presented for descriptive and conceptual comparison only.

Table 1. Comparison of various NIBS techniques.

Subject	Technology	Diseases and statuses	Site of application	Application parameters	Evaluation metrics	Advantage	Limitation	Reported glymphatic clearance measure (study-specific)
Mice	FUS	AD, aging	ICH, Brain base, etc.	Cranial 250 kHz, 0.45 MPa, 50 ms pulse width, 20% duty cycle, lasting for 10 minutes [84]; 715 kHz center frequency, 0.15 W sound power, 60 seconds each time, treated once a week, for a duration of 4 or 6 weeks [4], etc.	ELISA, immunohistochemistry of A β and glial cells, Thioflavin S amyloid plaque staining, etc.	Non-invasive, high precision, reversible, accurately acting on deep brain regions, with good compatibility [77,78,83,112,113].	The complex structure of the skull, characterized by multilayering, fluid-filled spaces, and porous non-uniformity, leads to significant phase distortion and energy attenuation as FUS passes through the skull, resulting in shape distortion and positional displacement in the ultrasound focus area. Permanent sensory deficits and gait abnormalities may occur after tFUS ablation treatment [114,115].	36% [4]
Mice	rTMS	ICH, AD, minor CI	Brain	Internal frequency of 50 Hz, inter-cluster frequency of 5 Hz, total pulses 600, each lasting 40 s, once every other day, for a duration of 14 days, or just once [96,98]; 25% maximum output intensity, approximately 0.91 T; stimulation frequency, 10 Hz; stimulation duration, 1.5 s; time interval, 10 s; total number of stimulations, 1560 times, lasting from 3 days to 90 days [93], etc.	Clearance rate of RITC-dextran and FITC-dextran, immunofluorescence, and MLVs diameter measurement, etc.	Non-invasive, re- and producibility, high precision, wide coverage [116,117,118,119,120,121,122,123].	The stimulation depth is relatively shallow, making it difficult to affect deep brain regions. It should be used cautiously in children and pregnant women, and is not suitable for individuals with metal implants in the cranial cavity. Adverse events may include headaches, fainting, hearing damage, localized skin or scalp discomfort, fatigue, and even seizures [116,117,118,119,120,121,122,123].	14% [98]
Mice	tPBM	AD, IVH	Frontal cortex, sagittal sinus, transverse sinus, ventricular region and triangular sinus regions, etc.	Cortical flux: 4–10 J/cm ² , wavelength: approximately 1268 nm, administered once every day (each session: 17 minutes of irradiation, 5 minutes of pause during 61 minutes), lasting for 9 days, or just once [66,73,104]; Cranial flux: 50 J/cm ² , wavelength: 689 nm, duration: 83 seconds [105], etc.	OCT, confocal imaging, immunohistochemistry, rCBF and rScO ₂ , etc.	Non-invasive, with no obvious adverse reactions, applicable to many diseases [124,125].	Delivering sufficient doses of light to the deeper structures of the human brain remains a challenge, with individual heterogeneity present [103,126].	10% [6]
Mice	tDCS	/	Cortex, hippocampus, and dorsal striatum, etc.	0.1 mA current for 10 minutes [7].	EEG, AQP4/CD31 immunostaining, and <i>in vivo</i> dCLNs imaging, etc.	Non-invasive, applicable to multiple diseases [106,127].	Possible adverse reactions include itching, headache, dizziness, tingling sensations, burning sensations, decreased coordination, and emotional instability; those with pacemakers, cranial cavities, or fissures are prohibited from use [127,128,129].	16.8%–25.6% [7]

Table 1. Continued.

Subject	Technology	Diseases and statuses	Site of application	Application parameters	Evaluation metrics	Advantage	Limitation	Reported glymphatic clearance measure (study-specific)
Human	TVS	/	Brain	5 minutes of 40 Hz mechanical vibration [8].	ALFF, fALFF and gBOLD-CSF coupling	Non-invasive [8].	Lack of further research.	5.05%–11.75% [8]
Mice and human	Multisensory AD gamma stimulation		Frontal cortex	1 hour of 40 Hz stimulation [9].	ISF efflux, immunohistochemistry, two-photon imaging, snRNA-seq, and qPCR, etc.	Non-invasive, easy to operate, and highly accessible [9].	Lack of further research.	30% [9]
Mice	Non-invasive mechanical stimulation	Aging	(1) from the periorbital area to the mandible; (2) from the nasal sidewall to the mandible; (3) along the rostral to caudal path of scLV-1 and scLV-2 to the submandibular lymph node.	low magnitude (0.01–0.02 kgf); the treatment includes 1/10/20 sessions of mechanical stimulation, with each session consisting of 20 repetitions lasting 1 minute, including 10 downward sweeping cycles, each lasting 2 seconds, followed by a second cycle of 10 repetitions, and finally a 20-second rest period [11].	Imaging of fluorescent tracers flowing from CSF to scLV and Immunohistochemistry et al.	Non-invasive [11].	Lack of further research.	23% [11]
Mice	EA	AD	GV20, GV26, GV29, BL23	10 minutes of 2 Hz, 1 mA, EA treatment, once a day for the first five days of each week, lasting for 8 weeks [10]; 30 minutes of 2 Hz/15 Hz, 1–2 mA EA treatment, once a day, 6 days a week, lasting for 4 weeks [110].	ELISA, Western blot, contrast-enhanced MRI, and immunohistochemistry, etc.	Non-invasive, applicable to multiple diseases [130,131,132,133].	Corrosion of the needle may cause slight cytotoxicity and cell necrosis during the electrification process, and the small current used may lead to minor tissue damage [134,135].	11% [10]

Abbreviation: ALFF, Amplitude of low-frequency fluctuations; fALFF, Fractional amplitude of low-frequency fluctuations; gBOLD, global blood oxygen level-dependent; ELISA, Enzyme-Linked Immunosorbent Assay; OCT, Optical Coherence Tomography; rCBF, Relative Cerebral Blood Flow; EEG, electroencephalogram; MRI, magnetic resonance imaging; mLEC, meningeal lymphatic endothelial cells; scLVs, superficial cervical lymphatics; GV20, Baihui Point; GV26, Shuigou Point; GV29, Yintang Point; BL23, Shenshu Point; AD, Alzheimer's disease; ICH, intracerebral hemorrhage; RITC, rhodamine B isothiocyanate; FITC, fluorescein isothiocyanate; IVH, intraventricular hemorrhage; qPCR, quantitative polymerase chain reaction.

Explanatory Note: The estimated enhancement rates of glymphatic system-related function were derived from relative changes in study-specific GS-associated outcome measures reported before and after neuromodulation. These measures included tracer clearance, interstitial or cerebrospinal fluid transport, meningeal lymphatic vessel dynamics, A β burden, or validated imaging and biochemical surrogate markers. Owing to heterogeneity in experimental models and evaluation metrics across studies, these estimates should be interpreted as approximate and modality-specific indicators rather than standardized or directly comparable clearance rates.

5.1 Efficacy Trends Across Techniques

NIBS techniques demonstrate considerable heterogeneity in their efficacy for enhancing glymphatic clearance, penetration depth, safety, and translational potential. FUS shows the highest estimated enhancement (~36%) [4], likely owing to its capacity to directly and precisely target deep brain regions; however, skull-induced acoustic distortion and potential neural injury constrain its routine and long-term application [114,115]. In comparison, 40 Hz multisensory gamma stimulation (~30%) and non-invasive mechanical stimulation (~23%) suggest that rhythmic neural entrainment and peripheral lymphatic pumping may facilitate glymphatic transport [9,11]. Traditional modalities, including tDCS, rTMS, and tPBM, yield moderate effects (~10–25%) presumably through indirect modulation of cortical excitability, cerebrovascular dynamics, or meningeal lymphatics rather than direct activation of deep pathways 5,6 [98,104]. TVS and EA appear to induce smaller changes (~5–12%) and remain relatively underexplored [8,10]. Importantly, due to substantial methodological heterogeneity across studies—including differences in tracer types, imaging modalities, and timing of assessment—these numerical estimates should be interpreted cautiously as indicative trends rather than precise measures of efficacy.

It should be noted that, although some NIBS techniques show measurable enhancement of glymphatic clearance, overall efficacy remains moderate rather than high. This underscores the need for caution in interpreting results and highlights the potential value of exploring long-term or repeated applications, particularly in preventive contexts. Furthermore, multi-modal strategies combining complementary NIBS approaches—such as deep-targeted methods (FUS) with surface- or rhythm-based interventions (tPBM, gamma stimulation)—may synergistically improve glymphatic modulation. Future studies should investigate whether such combined or sequential protocols can further increase clearance efficiency, optimize treatment parameters, and maintain safety, particularly in preventive or early intervention contexts.

5.2 Practical Considerations: Feasibility, Safety, and Cost

In addition to their biological effects, operational feasibility, safety, and economic considerations are crucial for the translational potential of NIBS interventions targeting GS. High-precision modalities such as FUS and rTMS require specialized equipment, controlled laboratory or clinical environments, and trained personnel, which limits their applicability for long-term, preventive, or home-based interventions. In contrast, low-intensity or non-contact techniques—including tPBM, tDCS, multisensory γ -stimulation, TVS, and non-invasive mechanical stimulation—are relatively easy to administer, exhibit minimal contraindications, and are suitable for repeated or community- and home-based use.

Safety profiles differ according to energy type and mode of delivery. Low-energy, sensory-oriented, or non-contact interventions generally demonstrate good long-term tolerability, whereas high-intensity electromagnetic or acoustic modalities necessitate careful screening and monitoring to mitigate adverse effects. Economic feasibility is also a key consideration: portable or wearable NIBS devices facilitate outpatient and community deployment, offering promising strategies for early prevention of GS-related disorders in home or community settings, where subclinical glymphatic dysfunction may precede neurological disease. High-cost, facility-bound systems remain largely restricted to hospital or research contexts.

6. Conclusions and Future Directions

GS clears metabolic waste in the brain through ISF-CSF exchange, and its function is regulated by factors such as AQP4 and cerebral arterial pulsation. These NIBS techniques may improve GS function by influencing these regulatory factors, providing potential therapeutic strategies for related brain diseases. Although there are cranial differences between humans and rodents, some studies show that the GS mechanism in both species has certain cross-species consistency (both are regulated by 40 Hz multisensory gamma stimulation and the cholinergic system) [9,136], and most NIBS have demonstrated clinical potential in numerous neurological diseases [137,138,139,140,141,142], suggesting the possibility of regulating human GS.

Current research still has the following limitations: unclear mechanisms of action and key signaling pathways; non-standardized stimulation parameters and lack of a parameter-effect relationship framework; insufficient consideration of individual differences (such as age, gender, disease subtype); experimental models are mostly focused on the acute phase of diseases or healthy states, lacking validation in later stages and chronic conditions; insufficient dynamic monitoring of key indicators such as CSF flow; the long-term safety and durability of effects have not been systematically assessed; clinical evidence is limited and requires validation with large samples. Furthermore, although TGN-020 has been classified as an AQP4 inhibitor based on African clawed frog oocyte assays [143], the false positive rate in oocyte assays is high [144]. Based on this, there is controversy regarding whether TGN-020 directly inhibits AQP4, and further research is needed to determine if the alleviation of brain edema in mice by TGN-020 is through direct inhibition of AQP4 expression.

Current assessment methods for glymphatic function also have limitations. Although classic tracer analysis provides spatial resolution, the reliance on *ex vivo* techniques using fixed tissue samples carries the risk of introducing artifacts [145]. In contrast, *in vivo* imaging techniques can more accurately capture dynamic processes, but each has its limitations: the field of view in two-photon imaging is restricted [145]; conventional MRI tracers only re-

flect perivascular convection; the ALPS index cannot be directly equated with glymphatic dysfunction [146]; and BOLD-CSF coupling only captures unidirectional flow of CSF and is sensitive to the BO magnetic field [147]. The novel imaging probe $H_2^{17}O$ detects water proton signals via MRI, providing the possibility for simultaneous assessment of transcellular water exchange and perivascular convection [43]. Furthermore, the establishment of *in vitro* models such as blood-brain barrier organ chips offers new methods to further investigate the effects of blood flow shear stress and pericyte coverage on glymphatic function [148]. Future research should integrate multimodal neuroimaging techniques and physiological monitoring to further elucidate the specific mechanisms by which NIBS enhances GS functionality and to dynamically observe treatment effects, while maintaining a critical perspective on the differences in results among various methods. Future research should utilize animal models that are closer to humans (such as macaques) for validation and conduct systematic clinical trials. Focus on evaluating the safety, efficacy, and long-term effects of NIBS across different ages and disease stages to promote the standardization of stimulation protocols. It is noteworthy that various other neurological disorders with glymphatic dysfunction, such as depression [47,49], migraines [149], and schizophrenia [150], also benefit from NIBS treatment, and it is necessary to clarify whether improvements in GS function can translate into clinical benefits for these diseases in the future.

Overall, the regulation of GS function by NIBS is a rapidly developing field that still has knowledge gaps. Continuous research to optimize existing strategies and develop new methods is expected to enhance the prevention and treatment effects for GS-related diseases.

Abbreviations

GS, glymphatic system; A β , amyloid- β ; α -syn, alpha-synuclein; AD, Alzheimer's disease; PD, Parkinson's disease; NIBS, non-invasive brain stimulation; MLVs, meningeal lymphatic vessels; CNS, central nervous system; CSF, cerebrospinal fluid; MRI, magnetic resonance imaging; VEGF-C, vascular endothelial growth factor C; VEGFR3, vascular endothelial growth factor receptor 3; ISF, interstitial fluid; scLNs, superficial cervical lymph nodes; dcLNs, deep cervical lymph nodes; AQP4, aquaporin 4; PVS, perivascular space; ICH, intracerebral hemorrhage; CUMS, chronic unpredictable mild stress; PUFAs, polyunsaturated fatty acids; WT, wild-type; Tg-SwDI, microvascular amyloid model; BCAS, bilateral common carotid artery stenosis; NREM, non-rapid eye movement; NE, norepinephrine; ICP, intracranial pressure; rScO₂, regional cerebral oxygen saturation; ATP, adenosine triphosphate; BBB, blood-brain barrier; FUS, focused ultrasound; LIFU, low-intensity focused ultrasound; SD, Sprague-Dawley; MB, microbubbles; CaM, calmodulin; rTMS, repetitive transcranial magnetic stimulation; cTBS, con-

tinuous theta burst stimulation; tPBM, transcranial photobiomodulation; $\Delta[HbO]$, concentrations of oxyhemoglobin; $\Delta[oxi-CCO]$, cytochrome c oxidase; IVH, intraventricular hemorrhage; OCT, optical coherence tomography; mLECs, meningeal lymphatic endothelial cells; tDCS, transcranial direct current stimulation; EEG, electroencephalogram; IP3R2-KO, inositol trisphosphate receptor type 2 knockout; TVS, transcranial vibration stimulation; fMRI, functional magnetic resonance imaging; ALFF, amplitude of low-frequency fluctuations; fALFF, fractional amplitude of low-frequency fluctuations; gBOLD, global blood oxygen level-dependent; VIP, vasoactive intestinal peptide; NO, nitric oxide; scLVs, superficial cervical lymphatics; EA, electroacupuncture; TENS, transcutaneous electrical nerve stimulation; DTI-ALPS, diffusion tensor image analysis along the perivascular space.

Author Contributions

ZJ wrote and revised the manuscript. ZJ, WY, and YZ retrieved and organized the literature. SY, HW, and TL participated in the classification of the literature and contributed to the interpretation of the synthesized findings. SY and TL provided funding support. TL guided the writing of the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflicts of Interest

The authors declare no conflicts of interest.

Declaration of AI and AI-Assisted Technologies in the Writing Process

During the preparation of this work the authors used ChatGpt-3.5 in order to check spelling and grammar. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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