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Review

Resolving the mysteries of brain clearance and immune surveillance

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SUMMARY

Recent advances are transforming our understanding of brain fluid dynamics, highlighting cerebrospinal fluid (CSF) flow as a key process in brain waste clearance. While debates persist regarding its anatomical

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pathways and regulatory mechanisms, certain core principles have become widely accepted. CSF influx occurs primarily along periarterial spaces, solute clearance follows multiple routes, and glymphatic and meningeal lymphatic systems are functionally connected. Brain clearance is influenced by vascular pulsation, neural activity, and sleep. Immune cells at brain-border niches both monitor solute efflux and modulate flow, linking brain clearance to neuroimmune interactions. A deeper understanding of brain clearance could unlock new therapeutic avenues for autoimmune, neurodegenerative, neoplastic, and psychiatric disorders.

INTRODUCTION

Traditionally, neuroscience has been defined as the study of neurons and neural networks. Over the past two decades, the field has expanded to encompass previously understudied aspects of central nervous system (CNS) physiology, including the role of glial cells as well as the biological mechanisms underlying sleep, fluid transport systems that facilitate waste clearance, and immune surveillance.[1–5] The resultant discoveries have begun to reshape our understanding of fundamental brain physiology and function.

Two major scientific advances in the regulation of brain homeostasis are the discovery of a brain waste clearance system and a revised view of the CNS immune environment. Unlike peripheral organs, the CNS parenchyma lacks lymphatic vessels and instead utilizes perivascular spaces for dynamic fluid transport,[6] a process most active during sleep.[7,8] The identification of this "glymphatic system," a pathway that bypasses the blood-brain barrier (BBB) to export waste from the brain, was initially surprising given the traditional view that CNS barriers limit peripheral solute exchange. Likewise, the long-held assumption of immune privilege,[9] where the brain was thought to be largely isolated from peripheral immunity, has been overturned by studies identifying key sites of neuroimmune interaction within the meningeal membranes.[1,2,10] Bridging these discoveries, the glymphatic system facilitates not only waste clearance but also immune surveillance by delivering brain-derived antigens to antigen-presenting cells located in the meninges bordering the CNS.[10,11] In addition, recent anatomical analyses have revealed previously unrecognized structural features in these regions, including specialized compartments around bridging veins that connect the brain to its meningeal borders.[12] These structures have often been overlooked in standard histological preparations, in which brain removal disrupted critical anatomical relationships.

Given the foundational implications of these discoveries—spanning neuroanatomy, immunity, and sleep biology—it is not surprising that they have provoked scientific debate. This review provides a framework for integrating the diverse data streams now converging on the rapidly evolving field of brain fluid dynamics and immunity. We highlight key unresolved questions and propose experimental strategies by which they may be best addressed. What are the most pressing open challenges, and which methodologies are best suited to resolve them? What definitions and standards are needed to evaluate emerging techniques and reconcile conflicting data? How can insights from rodent models be effectively translated to humans, given current technological limitations? Equally important, we aim to identify those areas of broad consensus so as to accelerate progress and reduce redundancy.

THE MECHANISMS OF BRAIN CLEARANCE

Brain clearance refers to the elimination of waste solutes from the brain parenchyma. Metabolic waste can also be degraded locally through processes such as autophagy and ubiquitination,[13,14] and select solutes, including β-amyloid, can be removed via transport across the BBB.[15] For a comprehensive overview of these mechanisms, we refer readers to.[16] This review rather focuses specifically on brain drainage via glymphatic-lymphatic pathways, which drain the interstitial solutes outside the brain.

In this review, the term "brain clearance" is used to describe three successive steps: (1) the influx of cerebrospinal fluid (CSF) along periarterial spaces into the brain parenchyma, (2) its transport through the brain, and then (3) the exit of fluid containing interstitial waste from the brain.[17,18] A large body of work has now contributed to our understanding of the mechanisms and pathways involved in glymphatic transport since the original hypothesis was proposed in 2012.[6] It is generally accepted that periarterial bulk-flow-driven CSF influx is the primary source of fluid driving the glymphatic system.[6,19] Nonetheless, a potential contribution from vascular fluid traversing the BBB cannot be excluded.[20] This possibility remains unresolved, as robust methodologies for quantifying net vascular water flux across the BBB have yet to be developed. CSF enters the brain parenchyma, facilitated by the dense expression of the water channel aquaporin-4 (AQP4) in astrocytic vascular endfeet, from which it then disperses throughout the interstitial space, collecting metabolic waste as it travels. The waste-containing fluid is subsequently cleared from the brain along several pathways, including perivenous compartments leading to the subarachnoid space and dura mater[12,21] (Figure 1). Glymphatic flow is studied by administration of CSF tracers, normally injected into cisterna magna. Tracer transport depends on molecular weight and other physicochemical properties. We refer to several methodological papers that discuss these factors in detail.[22–25] Within the dura, meningeal lymphatic vessels facilitate the drainage of CSF and solutes toward the cervical lymph nodes.[26–30] Additional efflux routes—such as those along cranial and spinal nerves—also contribute,[31,32] although the relative significance of each pathway remains to be determined.

The glymphatic system is interconnected with CSF flow in multiple ways and at multiple sites, including the choroid plexus (CSF production), ventricular and subarachnoid CSF flow, and CSF egress pathways. Prolonged disruption of meningeal lymphatic drainage results in decreased CSF influx and glymphatic function,[17] whereas enhancing meningeal lymphatic drainage in aged mice leads to improved glymphatic function.[17] Thus, the glymphatic and meningeal lymphatic systems are functionally connected, so that a comprehensive understanding

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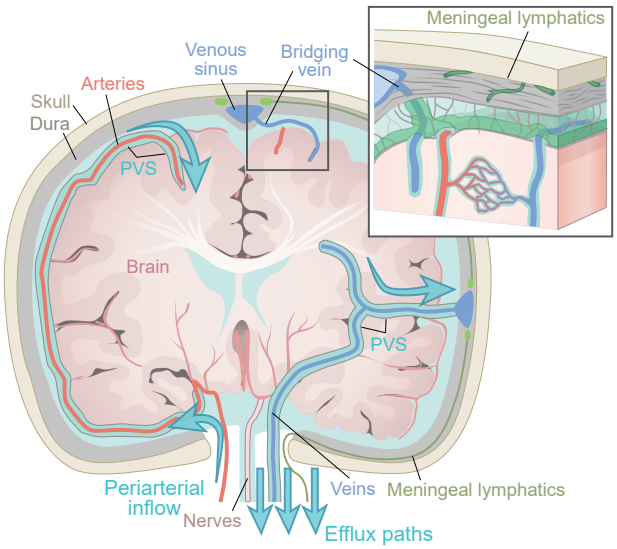

Figure 1. Influx and efflux pathways of the glymphatic system and their connection to meningeal lymphatic vessels

The left hemisphere illustrates cerebrospinal fluid (CSF) influx along periarterial spaces. The right hemisphere shows efflux pathways, including perivenous spaces, bridging veins, cranial nerves, and drainage through meningeal lymphatic vessels.

of brain clearance requires integrating cerebral CSF fluid dynamics, glymphatic, and lymphatic functions.

Drivers of CSF influx

Several mechanisms have been proposed to support brain clearance, many of which are driven by pulsatile changes in vascular diameter.[19] In addition, changes in plasma osmolarity have been suggested to influence brain fluid movement.[33] Pulsatile changes in vascular diameter are generated by cardiac contractions, respiration, and vasomotion—rhythmic oscillations in vessel tone. Vasomotion typically occurs at low frequencies (0.02–0.1 Hz) and has been characterized in both rodent and human studies.[8,34–36] In mice, spontaneous vasomotion can be entrained by neuronal activity through neurovascular coupling and may be driven, at least in part, by infraslow oscillatory activity of the locus coeruleus, which leads to rhythmic norepinephrine (NE) release during non-rapid eye movement (REM) (NREM) sleep. NE acts as a potent vasoconstrictor, producing the large-amplitude fluctuations in vascular diameter that characterize sleep-associated vasomotion.[8] The frequency and amplitude of these pulsations vary by species and physiological state. In mice, vasomotion produces large fluctuations, on the order of ~10%, whereas cardiac pulsations induce arterial diameter changes of only a few percent.[34] Rodent studies have shown that vasomotion predominantly occurs in arteries, not veins,[8,37] but venous vasomotion has been reported in humans. The impact of respiration on arterial diameter changes is less well defined. However, older literature has shown that the negative pressure generated during respiration facilitates venous blood outflow from the cranial cavity, and newer studies indicate that CSF efflux is similarly influenced by respiratory-driven pressure gradients[38] (Figure 2). Individual glial subtypes—including astrocytes, oligodendrocytes, and microglia—are known to regulate cerebral blood flow and may likewise contribute to glymphatic function, although direct evidence remains limited. Neurons are also clearly involved: synchronous neuronal activity induced by optogenetic stimulation has been shown to enhance CSF influx.[39]

CSF turnover, determined by CSF formation and CSF volume, may also affect waste clearance. CSF formation provides the volume input to the glymphatic system and maintains a concentration gradient favoring the exchange of solutes from brain tissue to the CSF.[40] Interestingly, though, the rate of CSF production from the choroid plexus in the lateral ventricles did not predict glymphatic activity in mice.[41]

Neural activity triggers functional hyperemia, which occurs via dynamic changes in arterial diameter and ensuing increases in the total cerebral blood volume. These increases in total blood volume would result, according to the Monroe-Kellie doctrine, in CSF efflux from the cranium.[42] Synchronized neuronal activity can also, via ionic fluxes, drive interstitial fluid motion.[39,43] Similarly, 40 Hz sensory stimulation was found to promote vasomotion and engaged several other parameters related to glymphatic function, including low-frequency vasomotion (0.1 Hz) and AQP4 polarization.[43]

FORCES GOVERNING DIRECTIONAL CSF INFLUX ALONG ARTERIES

The changes in blood flow, as well as the variable hemodynamic vectors across brain regions, are thought to mediate CSF and interstitial fluid flow through local oscillatory volume displacement of the perivascular space. This principle is consistent with the Monro-Kellie doctrine stating that the total volume of brain, blood, and CSF must be constant.[44] Because brain tissue is essentially incompressible, any increase or decrease in blood volume must be compensated by a corresponding displacement of CSF to maintain constant intracranial pressure. As a result, perivascular pumping results in a movement of perivascular and interstitial fluid that enhances waste clearance through mixing.[45] Yet this mixing is not an efficient mechanism of solute clearance[46] and cannot explain the observation that net flow through perivascular spaces exceeds that which may be ascribed to pulsations.[47] Modeling studies have thus focused on the generation of a net flow component from oscillatory changes in vessel diameter, although these studies have remained inconclusive.[48–50] As in other low-pressure systems such as lymphatic vessels and veins, valve-like structures may be necessary to maintain the unidirectional flow observed within perivascular spaces. Astrocytic endfeet are likely candidates to serve as such "valves"—they may become noncontiguous and hence permit fluid entry into the brain parenchyma during vascular dilation, when perivascular pressure exceeds interstitial pressure, yet prevent backflow when tissue pressure becomes higher than perivascular pressure by again tiling their juxtaposed endfeet.[51] Nonetheless, the existence of such an astrocytic valve mechanism remains hypothetical and has yet to be experimentally demonstrated. Definitive validation will require new live imaging approaches, as astrocytic endfeet are smaller than the optical resolution limit of conventional two-photon microscopy.

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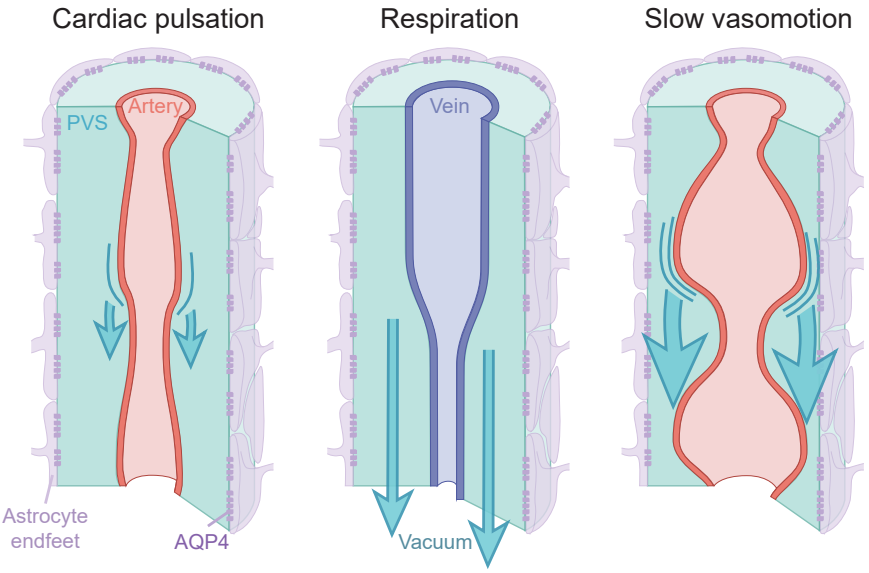

Figure 2. Proposed driving mechanisms of glymphatic flow

Cardiac pulsatility generates high-frequency, low-amplitude arterial wall pulsations that propel cerebrospinal fluid (CSF) through perivascular spaces. Respiratory movements contribute to CSF and venous blood outflow from the cranial cavity by creating negative pressure during inspiration. Slow vasomotion, triggered by infraslow NE oscillations or synchronized neuronal activity, produces large-amplitude changes in arterial diameter that enhance CSF influx, particularly during non-rapid eye movement (NREM) sleep.

INTERSTITIAL TRANSPORT: PASSIVE DIFFUSION OR CONVECTIVE FLOW?

Another debate in the field concerns whether fluid movement within the brain parenchyma is governed solely by diffusion or whether bulk flow also contributes. Evidence supporting both viewpoints has been demonstrated.[52–60] Diffusion will always occur when concentration gradients are present across compartments, and its efficacy increases as the molecular weight of the solute decreases. One modeling study, acknowledging experimental evidence of bulk CSF flow in periarterial spaces,[46] argued that solute clearance should be understood as a dynamic interplay between diffusion and bulk flow. These mechanisms may operate at different spatial scales: solutes might diffuse short distances within interstitial fluid before entering perivascular spaces, whereas bulk flow facilitates their clearance from the brain. Subsequent modeling studies provided additional support for the necessity of bulk interstitial fluid flow, demonstrating that it is required to reconcile experimental observations of tracer distribution, interstitial pressure dynamics, and waste clearance. Also, modeling suggests that surface membranes may be the central bottleneck for clearance.[61,62] The "mixing hypothesis" proposes that bulk flow is restricted to the subarachnoid space, including the perivascular space along large pial arteries, and generating concentration gradients that drive diffusive transport from the tissue toward the pial surface.[45] This model is contradicted by numerous publications that, using time-lapse imaging, have visualized bulk flow in the perivascular spaces.[47,63] Additionally, advective and diffusive transport can interact to produce dispersion, in which spatially heterogeneous velocity fields enhance solute mixing, accelerating homogenization beyond that achievable by diffusion alone.[64,65] The relative roles of diffusion, advection, and dispersion across the parenchymal, perivascular, and subarachnoid compartments—and how these may be influenced by brain state—are thus critically important to understand, as they dictate modeling and thus waste clearance measurements.

INTERSTITIAL FLUID EFFLUX

Solute transport from the brain to the periphery via lymphatic pathways can follow multiple routes, varying across species, brain regions, time of day, body position, circadian timing, and other physiological factors. Bridging (or cortical) veins that drain venous blood and thus connect the brain surface to dural sinuses represent a key route for CSF tracer efflux, termed ACE (arachnoid cuff exit/entrance) points.[12] Additional clearance routes are along spinal nerves[66–68] and along cranial nerves.[26,69,70] ACE points have also been demonstrated in humans,[12] whereas potential additional efflux pathways in humans require further investigation. Lymphatic efflux is also subject to circadian regulation, with higher efflux during the active state and reduced efflux during the inactive state.[71] However, sleep-wake regulation of lymphatic efflux has not been systematically studied, largely due to the invasive nature of current lymphatic imaging methods. In sum, it is important to remember that the exact routes of interstitial fluid efflux have not yet been determined and are likely influenced by multiple factors, including brain state, body position, and other physiological variables. It is also worth noting that CSF tracers or waste products will efflux according to slower kinetics than interstitial fluid itself. The field is rapidly evolving, with ongoing development and refinement of experimental methodologies. We encourage investigators to critically evaluate the technical aspects of these approaches, particularly the availability of noninvasive or minimally invasive methods, and to recognize the importance of carefully choosing (or avoiding) anesthesia, which can profoundly disrupt physiological brain fluid dynamics.[72–74]

Confounders to consider when studying brain clearance

The process of brain clearance involves multiple anatomical structures, barriers, and physiological drivers, many of which remain to be explored. Our limited understanding is partly due to the fact that brain anatomy has traditionally been studied using histological sections. Fluid-filled compartments are particularly vulnerable to artifacts caused by post-mortem pressure changes, fixation, and brain removal from the skull. *In vivo* tracer studies, while informative, can also introduce artifacts: tracer injection can disrupt homeostasis and trigger inflammatory responses. For instance, one study reported that glymphatic flow was suppressed by more than 50% following cannula

implantation but recovered within 1–3 days.[75] Furthermore, tracer injections typically require the use of pressure, which can distort physiological fluid dynamics.[21]

COMPLEX INTERACTIONS MAY UNDERLIE SEEMINGLY CONTRADICTORY OBSERVATIONS

Animal models with disrupted meningeal lymphatics have provided valuable insights into the role of lymphatic transport in maintaining brain homeostasis,[17,18,76,77] though findings remain somewhat conflicting. For example, photoablation of dorsal meningeal lymphatic vessels using verteporfin has been reported by numerous groups to exacerbate β-amyloid pathology in 5xFAD transgenic mice,[17,76,78,79] a model of rapidly progressing Alzheimer's disease. Supporting this, K14-vascular endothelial growth factor receptor 3 (VEGFR3)-Ig transgenic mice—which lack functional meningeal lymphatic vessels due to constitutive expression of a soluble VEGFR-3 decoy receptor—exhibit reduced clearance of tau from the brain parenchyma,[80] another hallmark of Alzheimer's pathology. In humans, phospho-tau-181 and β-amyloid (Aβ40 and Aβ42) have been found enriched in cervical lymph nodes relative to blood plasma,[81] suggesting that these lymph nodes serve as a downstream site of brain waste clearance via lymphatic drainage. Similarly, different animal models of meningeal lymphatic dysfunction have shown their role in aging[17,30,82,83] and numerous neurological disorders, such as Parkinson's disease,[84,85] neuroinflammatory conditions,[18] brain trauma,[86] stroke,[87] migraine,[88] cognitive impairment associated with craniosynostosis,[89,90] brain tumors,[91,92] and others. Moreover, dysfunction of afferent lymphatic vessels (induced by surgical ligation or using genetic models) has been shown to cause cognitive impairment and synaptic input imbalance in the medial prefrontal cortex.[93]

These findings provide a robust demonstration for a critical role for meningeal lymphatic vessels in clearing pathological proteins from the brain. Work from one lab, however, has demonstrated that genetic manipulation of VEGF-C and D levels in a double-transgenic Alzheimer's disease mouse model expressing mutant human amyloid precursor protein (APP)/presenilin-1 (PS1) and 5xFAD mice—models of slowly and rapidly progressing cerebral amyloidosis, respectively—did not significantly alter overall β-amyloid plaque burden.[94] This lack of effect may reflect incomplete regression of lymphatic vessels or confounding effects of VEGF-C and -D depletion on VEGFR2- or VEGFR3-expressing blood endothelial and oligo-lineage cells.[77]

One alternative explanation is that VEGF-C sequestration may paradoxically enhance CSF-to-blood drainage, providing a compensatory route for β-amyloid clearance. Further investigation into different drainage pathways, including perivenous and alternative lymphatic routes, as well as communication between these different drainage routes, is required to fully understand solute clearance mechanisms in the brain.

BRAIN CLEARANCE AND IMMUNE SURVEILLANCE MUTUALLY INFLUENCE EACH OTHER

The glymphatic system serves as a transport pathway for brain-derived antigens with immunogenic potential to enter border tissues such as the meninges[10,11] from which they flow first into lymphatic vessels draining into cervical lymph nodes and thence into the peripheral lymphatic system.[28,29] This drainage pathway creates a continuous system of immune surveillance, where each anatomical compartment—brain parenchyma, perivascular spaces, meninges, and lymph nodes—represents a distinct immunological niche with varying antigen pools and immune cell populations.[11,95] Together, these shape immune interactions and surveillance, which have implications for brain health and disease pathogenesis.[17,76,77,93,96,97,98]

Within the brain, microglia act as resident phagocytes constitutively sampling the microenvironment and clearing cellular debris and soluble antigens, such as β-amyloid.[99,100] While these cells serve as the primary immune sentinels of the parenchyma, microglia express low levels of major histocompatibility complex class II (MHC class II) under homeostatic conditions.[101] However, they can present antigens and initiate robust immune responses in a context-dependent manner, which may help sustain T cell infiltration and reactivity within the parenchyma.[102–104] By contrast, perivascular spaces form interfaces where interstitial fluids meet and converge into CSF, which flows under active surveillance by a rich repertoire of brain-associated border macrophages (BAMs), which include all perivascular, leptomeningeal, choroid plexus, and dural macrophages.[105,106] Unlike microglia, BAMs exhibit higher baseline MHC class II expression and rapidly phagocytose and present CNS-derived antigens—such as α-synuclein and β-amyloid—to T cells.[105,107–109] This antigen presentation capability is supported by chemokine-producing stromal cells—such as those expressing C-X-C motif chemokine ligand 12 (CXCL12)—which serve to anchor T cells and facilitate immune surveillance in these niches proximate to the brain parenchyma.[110] Additionally, endothelial cells lining the cerebral vasculature can directly present antigens to circulating T cells via MHC class I, offering a distinct pathway of antigen exposure.[111,112] This direct endothelial cell antigen presentation may provide an additional layer of immune surveillance that complements antigen presentation within the perivascular and CSF outflow spaces and meningeal lymphatics, highlighting the regional multilayered defense system of CNS immune surveillance.

Putative immunogenic molecules present in the subarachnoid CSF can reach the dura via ACE points.[10,12,18,113] The dura is vastly populated by different types of immune cells that can interact with brain-derived antigens.[10,17,18,76,83,96,113–116] In particular, dural macrophages are constantly phagocytosing molecules in the CSF and have the capacity to efficiently present antigens to nearby T cells[10,113] (Figure 3). Alongside dura-resident B cells and other professional antigen-presenting cells, such as dendritic cells, macrophages have the capacity to shape adaptive immune responses to brain-derived molecules across the entire lifespan and in disease.[10,11,17,83,96,105,117,118]

THE INFLUENCE OF SLEEP AND WAKEFULNESS ON GLYMPHATIC FLOW

Glymphatic transport operates at different rates depending on behavioral state, with most studies finding fluid flow markedly enhanced during sleep compared with wakefulness, and especially so during transitions between the two.[4,5] In mice, tracers

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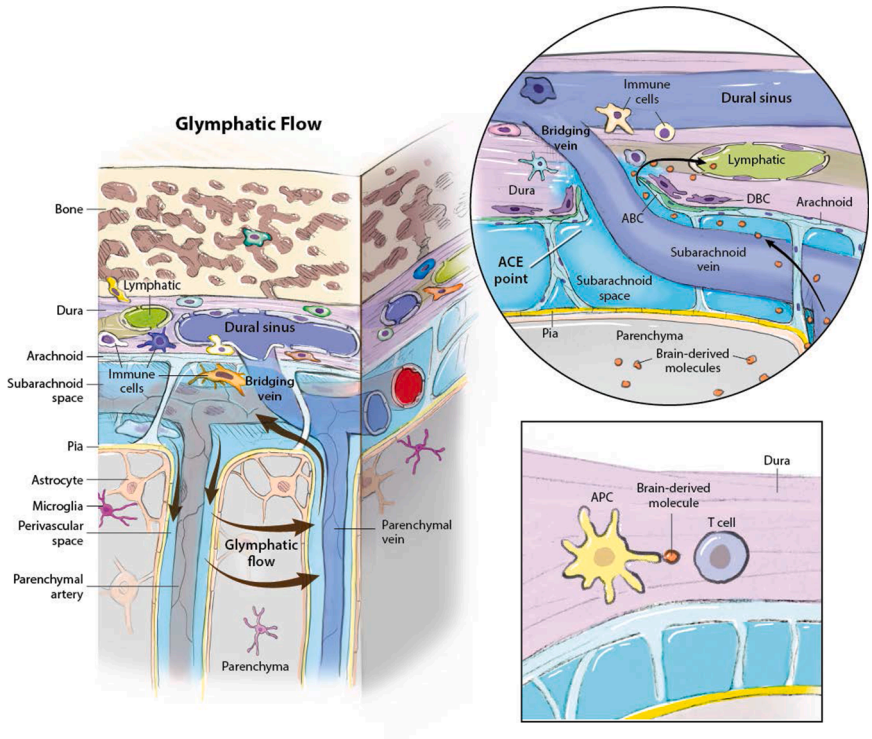

Figure 3. Immune surveillance at the brain's borders

The figure illustrates the anatomical connection between the glymphatic brain clearance and brain drainage via meningeal lymphatic vessels in the dura mater. ACE points (one ACE point is represented on the dorsal part of the brain, but they are spread throughout the entire cranial dura) allow the brain's fluid content to access the dura (upper inset), where prior to drainage through meningeal lymphatics, it is uptaken by dural phagocytes and presented to patrolling immune cells (lower inset), allowing brain immune surveillance.

injected into the cisterna magna entered the cortex more rapidly during sleep than during wakefulness, and cortical clearance of radiolabeled β-amyloid occurred twice as fast in sleeping mice.[7] Similarly, tracer injected into the striatum reached the vascular compartment and cervical lymph nodes more rapidly in sleeping animals, similarly consistent with enhanced clearance during sleep.[71]

Two-photon imaging of awake and sleeping mice revealed that pial arteries exhibit slow, large-amplitude oscillations during NREM sleep, vasodilation during REM sleep, and vasoconstriction upon awakening.[37] These vascular changes were tightly coupled to shifts in perivascular volume: arterial constriction coincided with perivascular expansion, while arterial dilatation heralded the attenuation of perivascular space.[37] Computational models based on these observations predicted that NREM-associated vascular dynamics increase perivascular flow and solute movement.[37]

Sleep-wake-dependent changes in the brain's extracellular space volume may also contribute to the diurnal regulation of glymphatic activity. In mice, the extracellular space expands from approximately 14% during wakefulness to 24% during natural sleep[7] or ketamine-xylazine anesthesia.[119] This expansion reduces resistance to fluid flow, thereby facilitating enhanced glymphatic clearance during sleep. A recent study demonstrated that consolidated sleep is accompanied by a continuous decrease in electrical resistance across the human brain, suggesting a progressive expansion of the interstitial space over the course of 7–8 h of sleep.[120]

Nonetheless, not all findings are in accordance with this picture. One study used fiber photometry in unrestrained, behaving mice to show increased tracer transport from the caudate-putamen to the frontal cortex during wakefulness, relative to sleep or anesthesia induced by either dexmedetomidine, pentobarbital, or ketamine-xylazine.[64] Yet, the use of such within-parenchyma tracer movement as a measure of clearance is problematic. Elevated tracer levels distal to injection sites could reflect increased transport rather than impaired clearance. More broadly, it is crucial to distinguish between solute clearance—defined here as the solute exiting the brain—rather than a simple redistribution within the parenchyma. To advance the field, definitional uniformity and semantic clarity, as well as the standardization of experimental approaches with orthogonal cross-validation using multiple techniques, are all critically needed.

Microarousals during sleep have emerged as particularly potent modulators of fluid flow. Positron emission tomography (PET) imaging in humans showed that tracer clearance correlates with the frequency of NREM microarousals.[121,122] Arousals during light sleep produce large, coordinated surges in CSF flow across multiple brain regions,[123,124] although the precise relationship between these surges and solute clearance remains to be clarified. Intrathecal administration of gadobutrol in humans showed that a single night of sleep deprivation significantly slowed CSF tracer clearance compared with normal sleep,[125,126] reinforcing the importance of sleep for glymphatic function.

One mechanistic hypothesis is that NE levels, low during sleep and elevated during wakefulness, modulate vascular tone and thereby CSF flow. In mice, optogenetic stimulation of the locus coeruleus causes transient NE release and vasoconstriction, demonstrating a link between neural activity and vascular dynamics.[8] In fact, NE oscillations during NREM sleep correlate directly with glymphatic clearance.[8] However, excessive elevations in NE, such as those observed after traumatic brain injury (TBI), suppress glymphatic flow, at least in part by disrupting the regular contractions of cervical lymphatic vessels.[127]

In humans, arousal drops after sleep deprivation are temporally coupled to both pupillary constriction and large CSF flow waves, suggesting that infraslow noradrenergic cycling—and its attendant sympathetic activation—contributes to fluid regulation in states of low arousal and high sleep pressure.[128] Notably, many

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Box 1. Intrathecal contrast-enhanced MRI

Intrathecal administration of GBCAs, which have a molecular size of approximately 600 Da, offers a powerful tool for visualizing solute transport pathways into and out of the human brain.[133] Following lumbar intrathecal injection, the GBCA tracer reaches the cisterna magna within approximately 20 min.[134] after which it propagates along subarachnoid perivascular spaces of large arteries, moving centripetally into the brain parenchyma.[135] Over several hours, the tracer distributes throughout the brain, with regional patterns of enhancement that align with the anatomical distribution of protein aggregates observed in neurodegenerative proteinopathies, including Alzheimer's and Parkinson's disease.[136]

Human glymphatic clearance has been shown to be sleep dependent. Tracer studies reveal slowed interstitial solute clearance following acute sleep deprivation[126] and in individuals with chronic subjective sleep impairment.[137] CSF tracer transport occurs via a combination of diffusion and advective flow mechanisms.[138] Efflux pathways include drainage through the dura mater along the superior sagittal sinus[139] and through skull bone marrow[140] into extracranial lymph nodes.[141] Impaired glymphatic function has been observed in clinical populations, including patients with idiopathic normal pressure hydrocephalus (iNPH),[136] idiopathic intracranial hypertension (IIH),[142] and after subarachnoid hemorrhage (SAH).[143]

Despite its utility, intrathecal contrast-enhanced MRI has several limitations. These include the need for lumbar puncture, the off-label or research use of intrathecal contrast agents, and the relatively low spatial and temporal resolution of conventional MRI. These factors limit the ability to distinguish specific solute transport routes—such as perivascular versus interstitial—and hinder the resolution of dynamic physiological processes (e.g., distinct sleep stages or cerebrovascular oscillations). Nonetheless, multiple safety studies have reported no evidence of neurotoxicity or long-term brain retention of intrathecal gadobutrol at doses ranging from 0.10 to 0.50 mmol.[134,144,145] Importantly, the concentration of gadobutrol in the brain after intrathecal administration is comparable to that achieved with standard intravenous doses.[146]

sleep aids—such as the nonbenzodiazepine γ-aminobutyric acid (GABA)-A agonist zolpidem—do not mimic the natural oscillatory noradrenergic dynamics of sleep and thus fail to promote glymphatic flow.[8]

Together, substantial evidence underscores the critical role of the sleep-wake state in regulating glymphatic transport, though the precise mechanisms and translational implications require further investigation.

ANESTHESIA AND CIRCADIAN REGULATION OF BRAIN CLEARANCE

Anesthetic choice profoundly shapes glymphatic flow. In rodents, ketamine/xylazine, dexmedetomidine, and pentobarbital accelerate CSF influx and solute efflux,[7,72,73,129] whereas volatile agents such as isoflurane and sevoflurane suppress tracer movement in rodents[130] or CSF flow in humans[131] (Box 1). The divergence likely reflects (1) vasodilatory actions of volatile anesthetics that dampen vessel-wall pulsations,[132] (2) distinct electroencephalogram (EEG) signatures—delta power under injectable agents tracks with enhanced tracer entry,[73] and (3) α2-adrenergic agonism of dexmedetomidine and xylazine, which modulates locus-coeruleus NE rhythms thought to drive infra-slow CSF oscillations.[8]

Circadian timing further tunes clearance. In mice, periarterial influx peaks in the light (sleep) phase, coinciding with rhythmic AQP4 redistribution and higher ventricular CSF production.[71] Human MRI confirms greater CSF formation and lower white-matter perivascular diffusivity at night, mirroring sleep-enhanced glymphatic transport.[147,148]

Collectively, brain state (wake, sleep, or anesthesia) and circadian phase converge on vascular pulsatility, neuromodulatory tone, and AQP4 dynamics to set glymphatic throughput. Determining how these variables interact in humans remains an essential next step.

BRAIN CLEARANCE IN HUMANS: TRANSLATING FROM RODENTS

Rodents and humans differ in several key anatomical and physiological features that may influence glymphatic transport. These include differences in brain size, cortical folding, gray-to-white matter ratio, ratio of penetrating cortical arteries for each centrifugally ascending vein, astrocyte morphology and complexity, AQP4 localization and polarization, and systemic parameters such as heart rate, respiratory frequency, and sleep architecture.[149–153] Each of these factors may affect the dynamics of brain fluid flow and must be considered when extrapolating findings from animal models.

From an anatomical standpoint, multiple components of the glymphatic and meningeal lymphatic systems have now been identified in humans.[27,154–157] Perivascular spaces are readily visualized on MRI when enlarged, as in aging and neurological disease,[155] and high-resolution neuroimaging studies have detected perivascular spaces in healthy young adults and adolescents.[154,156] Meningeal lymphatic vessels, first described in rodents, have since been visualized in humans with similar anatomical organization and alignment along dural sinuses.[27,157]

In addition to anatomical parallels, several studies have demonstrated similar physiological properties of brain fluid dynamics in humans as in rodents. Although research in humans is largely limited to noninvasive approaches, the relatively large size and accessibility of the human brain to MRI have enabled novel fluid transport phenomena to be identified in humans and then mechanistically tested in animal models. The most direct clinical extension of rodent work has been the use of intrathecal gadolinium-based contrast agents (GBCAs) in MRI to track glymphatic flow in humans.[146,158] These studies have confirmed key features of glymphatic transport in the human brain, including perivascular tracer

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influx from the subarachnoid space into the parenchyma, bulk flow-dependent solute transport, and CSF efflux along meningeal lymphatics.[84,136,139,159]

Additional imaging modalities—including intravenous GBCA-enhanced MRI, PET/single-photon emission computed tomography (SPECT), and contrast-free MRI techniques—are emerging as valuable tools for studying cranial fluid dynamics.[27,160] For example, GBCA accumulation around bridging veins and in the parasagittal dura suggests perivascular inflammation and impaired clearance in neurological diseases, particularly when coupled with advanced 3D post-processing.[161] However, it remains unclear which of these techniques offer the most direct, reproducible, and clinically relevant assessments of glymphatic function in human populations.

The role of sleep and anesthesia in modulating glymphatic activity has also been investigated in humans, with studies targeting different spatial and temporal scales than those in rodents. Unlike mice, humans sleep in consolidated, ~8-h nocturnal cycles with ~90-min REM/NREM phases. Despite these differences, MRI studies have demonstrated large, rhythmic waves of CSF flow during NREM sleep that are coupled to neural and vascular oscillations.[123,162] Similar sleep-associated modulation of CSF dynamics has been observed in pigeons,[163] suggesting remarkable cross-species conservation.

While few studies have directly measured solute clearance in humans, Eide et al. used intrathecal GBCA injections to show that tracer clearance is sleep dependent.[126] Sleep deprivation modestly reduced clearance, suggesting a conserved—but potentially more complex—relationship between sleep quality and glymphatic function in humans. The implications of human-specific sleep architecture, including sleep fragmentation and altered NREM/REM balance in aging and disease, remain unexplored and warrant further study.

Open questions remain regarding the degree to which other aspects of glymphatic transport are conserved across species. Human brains are larger and gyrencephalic, as opposed to those of rodents, introducing increased complexity in subarachnoid space geometry and potentially longer and more tortuous perivascular pathways. A recent study in ferrets found that cortical folds enhance glymphatic transport efficiency, suggesting that gyrification may facilitate clearance in large brains.[164] In addition, the arteriole-to-venule ratio is significantly higher in humans (~5:1 versus ~1:2 in rodents), which could profoundly influence periarterial flow dynamics and CSF distribution.[149] It has been hypothesized that the increased number of arterioles in humans serves to meet the dual demands of metabolic support and convective CSF transport.

Finally, the precise anatomical pathways of CSF and solute efflux in the human brain remain incompletely defined. While efflux routes have been observed along cranial nerves, dural lymphatics, and bridging veins,[12,84,139,165,166] the relative contribution of these pathways under physiological and pathological conditions is unknown. Future anatomical and physiological studies are needed to map these routes and determine how human-specific features—including cortical folding, vascular density, and sleep physiology—shape the efficiency and adaptability of brain clearance mechanisms.

EMERGING IMAGING STRATEGIES FOR HUMAN FLUID SYSTEMS

There is growing interest in developing contrast-free imaging methods to assess glymphatic transport and meningeal lymphatic drainage by measuring water mobility or brain tissue motion. Such approaches hold promise for evaluating brain clearance in large populations without the need for invasive procedures or advanced contrast agents. The first strategy employed is called magnetic resonance encephalography (MREG)—an ultrafast functional MRI (fMRI) technique that captures whole-brain activity with a temporal resolution of up to 100 ms (10 Hz), significantly faster than conventional fMRI.[167] MREG allows detection of rapid physiological brain pulsations—such as those driven by cardiac cycles, respiration, and vasomotion—in real time. This makes it uniquely suited for studying dynamic movements in the brain, including those involved in the glymphatic system, even though it is not specific to fluid motion.[167] MREG studies have shown marked changes in brain pulsation as a function of the sleep-wake cycle in poor sleepers, in patients with Alzheimer's disease, epilepsy, and more.[168–171] Another strategy involves motion-sensitizing gradients, such as those used in diffusion MRI, which can assess water mobility at multiple spatial scales.[172] Another method leverages phase-contrast or inflow-based MRI to detect CSF movement at sites like the fourth ventricle.[162,173] While these techniques can track fluid dynamics, their ability to reflect glymphatic function, i.e., efflux of neuronal waste materials, remains largely unvalidated. Systematic benchmarking against contrast-enhanced imaging is still in its early stages and rarely extends across diverse physiological or pathological states. In the future, it may be necessary to combine multimodal imaging, including MRI, EEG, and fluid biomarkers, to define glymphatic and meningeal lymphatic function and their impact on disease risk. However, a robust framework for integrating these tools has yet to be established.

A widely used contrast-free method is diffusion tensor image analysis along the perivascular spaces (DTI-ALPSs).[174] This approach calculates the ratio of diffusion components oriented perpendicular and parallel to presumed perivascular spaces in the periventricular white matter. Its popularity is partly due to the accessibility of standard DTI datasets, enabling retrospective analysis. However, DTI-ALPS is not specific to CSF flow and primarily captures water diffusivity in a small white matter region. As such, it is an indirect and potentially imprecise proxy for glymphatic function; its results should be interpreted with caution.[175]

Ultra-high-field MRI enables increased resolution of CSF-specific sequences, allowing more precise measurement of fluid mobility in perivascular spaces surrounding penetrating arteries in white matter.[172,176] Alternatively, tissue displacement associated with CSF dynamics can be measured using techniques such as displacement encoding with stimulated echoes (DENSE-MRI), which captures sub-millimeter tissue deformation.[177]

Water transport across the blood-brain and blood-CSF barriers, which may be mechanistically linked to perivascular glymphatic transport, can also be studied noninvasively using arterial

Box 2. What does the field of brain clearance agree upon?

The review explored opportunities in the emerging field of brain fluid dynamics and immunity. As highlighted, several of the most recent observations require independent validation, yet other core characteristics of brain fluid flow are by now widely accepted within the field. The key consensus points are as follows: (1) CSF inflow occurs primarily along periarterial spaces surrounding large pial arteries, with tracers continuing their influx along penetrating arterioles in both rodent and human brain.[6,7,19,136,158] By contrast, clearance can take multiple paths, including periarteriolar and perivenous compartments, white matter tracts, and cranial and spinal nerves. However, brain fluid will eventually be collected by meningeal and cervical lymphatic vessels and ultimately returned to the venous circulation.[28–30,68–70] (2) The polarized expression of the water channel AQP4 in the astrocytic endfeet supports glymphatic flow.[47] Genetic manipulations and pharmacological approaches have documented that the water channel AQP4 facilitates brain fluid flow.[47,180] In humans, genomic analyses have identified specific single-nucleotide polymorphisms (SNPs) associated with reduced AQP4 membrane localization, sleep disturbances, and an increased risk of Alzheimer's disease.[181–183] The exact mechanism by which AQP4 facilitates CSF influx and interstitial fluid efflux remains unresolved, but several mechanisms have been proposed, including fluid stagnation as a consequence of reduced efflux of interstitial fluid.[184,185]

(3) The glymphatic and lymphatic systems are interconnected, as evidenced by the rapid detection of tracers in cervical lymph nodes within tens of minutes after injection into the brain or CSF.[26,30,69–71] However, the precise efflux routes may vary across species, developmental stages, and experimental conditions. (4) The definition of brain solute clearance was originally introduced by Cserr's group,[186] who proposed that clearance refers specifically to solutes exiting the brain compartment, whichhas since been considered the gold standard in preclinical studies.[7,187] More recent studies have employed real-time imaging strategies using tracers that bind albumin in blood to detect efflux from the brain[75] or, alternatively, have used whole-body SPECT imaging to confirm that radiolabeled tracers exiting the brain accumulate in peripheral tissues.[188] Observing tracer movement within brain tissue as a measure of "clearance" does not directly track the tracer's exit from the brain, and future studies would benefit by including measurements of tracer loss from the brain or accumulation in peripheral tissues. (5) A Prox1-expressing-EGFP-positive membrane exists between the arachnoid barrier layer and the pia mater.[189,190] There is ongoing debate about whether this membrane is always fused with the arachnoid barrier layer or just in certain regions, and if it separates subarachnoid space compartments into two distinct CSF-filled compartments.

spin labeling or other magnetic labeling techniques.[178,179] These studies suggest that water exchange between blood and CSF occurs at multiple brain sites beyond the choroid plexus, challenging the classical model of the "third circulation."

Collectively, these noninvasive MRI-based techniques offer diverse tools to probe components of brain fluid transport in humans. However, none of them captures the entire clearance system, and it is important to emphasize that they typically measure solvent (water) movement or tissue motion, not the clearance efficiency of larger solutes or waste products. As the field advances, rigorous validation, integration of multimodal data, and careful interpretation will be essential to define their relevance to brain health and disease.

CONCLUDING REMARKS

Despite major recent advances in mapping the anatomy and physiology of brain clearance pathways, numerous questions remain about how these systems operate in both health and disease (Box 2). Fundamentally, brain clearance refers to the removal of solutes from the brain parenchyma to peripheral compartments. However, whether this process varies by brain region or follows distinct anatomical routes remains unclear. There is a need to investigate the molecular and cellular crosstalk between vascular barriers (e.g., BBB) and lymphatic/glymphatic interfaces, particularly how dysfunction in one domain may affect the other. Detailed mapping of waste exit routes from different brain regions remains largely uncharted and is one of the more urgent questions to be addressed.

Sleep represents a unique physiological state in humans, involving a transition from an upright, bipedal posture to a recumbent position, accompanied by dramatic changes in neural and astrocytic activity. These changes drive oscillations in cerebral blood flow, which in turn influence the movement of CSF and interstitial fluid. It is likely that sleep-wake differences in brain waste clearance arise from the combined effects of altered cellular and physiological activities, vascular dynamics, and body posture. However, the relative contribution of each factor remains unclear: is the enhancement of clearance during sleep primarily driven by neuronal and glial state changes, vascular pulsatility, positional effects, or an interaction of all three?

Despite the growing recognition of the intimate link between waste clearance and CNS immune surveillance, our understanding of how these processes intersect is still in its early stages. A key issue is to understand the heterogeneity of CNS antigens surveilled by brain-border phagocytes and which antigens are preferentially presented to the adaptive immune system. Understanding this heterogeneity is crucial because the extent to which parenchymal and CSF antigens intermix may vary according to the biophysical properties of the macromolecule, with some remaining sequestered and others being preferentially drained. Additionally, given the distinct immunological niches throughout the brain, how do immune-cell compositions, activation states, and functions vary across distinct brain regions and borders, and to what extent are their activities coordinated?

Despite exciting progress in uncovering the anatomy, physiology, pathways, and regulators of brain fluid dynamics, much remains to be learned. Assessing its relevance to humans and

its impact on disease pathogenesis is crucial, as is exploring whether these unique brain processes offer novel therapeutic opportunities for conditions such as cancer, autoimmunity, neurodegeneration, and psychiatric disorders.

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DECLARATION OF INTERESTS

H.B.: patents: 2012 Nedergaard, Iliff & Benveniste: Methods for evaluating brain-wide paravascular pathway for waste clearance function and methods for treating neurodegenerative disorders based thereon (PCT/US2014/017606). J.I.: serves as the Chair of the Scientific Advisory Board for the company, Applied Cognition. He receives compensation and stock options from this company. J.K.: co-founder of Rho Bio and Pranas Neuro, advisor to MMI, scientific advisory board member to Fibralign, and author and co-author of several patents and provisional applications related to the topic of this review. L.D.L.: inventor on a pending patent application for a method for measuring CSF flow with MRI and a method for modulating CSF flow. L.-H.T.: advisory and consulting positions: Cognito Therapeutics, 4M Therapeutics, TAC Therapeutics, Metro Bio LLC, Cell Signaling Technology, Vandria Therapeutics, and Beren Therapeutics. S.D.M.: patents: modulating lymphatic vessels in neurological disease (University of Virginia Licensing & Ventures Group and PureTech Ventures LLC). S.A.G. and M.N. are co-founders of and consultants to CNS, Inc., which has licensed patents and patent applications from the University of Rochester referable to the glymphatic system and its manipulation.

REFERENCES

1. Rustenhoven, J., and Kipnis, J. (2022). Brain borders at the central stage of neuroimmunology. Nature *612*, 417–429. https://doi.org/10.1038/s41586-022-05474-7.

2. Buckley, M.W., and McGavern, D.B. (2022). Immune dynamics in the CNS and its barriers during homeostasis and disease. Immunol. Rev. *306*, 58–75. https://doi.org/10.1111/imr.13066.

3. Castellani, G., Croese, T., Peralta Ramos, J.M., and Schwartz, M. (2023). Transforming the understanding of brain immunity. Science *380*, eabo7649. https://doi.org/10.1126/science.abo7649.

4. Rasmussen, M.K., Mestre, H., and Nedergaard, M. (2018). The glymphatic pathway in neurological disorders. Lancet Neurol. *17*, 1016–1024. https://doi.org/10.1016/S1474-4422(18)30318-1.

5. Hablitz, L.M., and Nedergaard, M. (2021). The Glymphatic System: A Novel Component of Fundamental Neurobiology. J. Neurosci. *41*, 7698–7711. https://doi.org/10.1523/JNEUROSCI.0619-21.2021.

6. 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. https://doi.org/10.1126/scitranslmed.3003748.

7. 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. https://doi.org/10.1126/science.1241224.

8. Hauglund, N.L., Andersen, M., Tokarska, K., Radovanovic, T., Kjaerby, C., Sørensen, F.L., Bojarowska, Z., Untiet, V., Ballestero, S.B., Kolmos, M.G., et al. (2025). Norepinephrine-mediated slow vasomotion drives glymphatic clearance during sleep. Cell *188*, 606–622.e17. https://doi.org/10.1016/j.cell.2024.11.027.

9. Smyth, L.C.D., and Kipnis, J. (2025). Redefining CNS immune privilege. Nat. Rev. Immunol. *25*, 766–775. https://doi.org/10.1038/s41577-025-01175-0.

10. Rustenhoven, J., Drieu, A., Mamuladze, T., de Lima, K.A., Dykstra, T., Wall, M., Papadopoulos, Z., Kanamori, M., Salvador, A.F., Baker, W., et al. (2021). Functional characterization of the dural sinuses as a neuroimmune interface. Cell *184*, 1000–1016.e27. https://doi.org/10.1016/j.cell.2020.12.040.

11. Kim, M.W., Gao, W., Lichti, C.F., Gu, X., Dykstra, T., Cao, J., Smirnov, I., Boskovic, P., Kleverov, D., Salvador, A.F.M., et al. (2025). Endogenous self-peptides guard immune privilege of the central nervous system. Nature *637*, 176–183. https://doi.org/10.1038/s41586-024-08279-y.

12. Smyth, L.C.D., Xu, D., Okar, S.V., Dykstra, T., Rustenhoven, J., Papadopoulos, Z., Bhasiin, K., Kim, M.W., Drieu, A., Mamuladze, T., et al. (2024). Identification of direct connections between the dura and the brain. Nature *627*, 165–173. https://doi.org/10.1038/s41586-023-06993-7.

13. Palmer, J.E., Wilson, N., Son, S.M., Obrocki, P., Wrobel, L., Rob, M., Takla, M., Korolchuk, V.I., and Rubinsztein, D.C. (2025). Autophagy, aging, and age-related neurodegeneration. Neuron *113*, 29–48. https://doi.org/10.1016/j.neuron.2024.09.015.

14. Choi, I., Wang, M., Yoo, S., Xu, P., Seegobin, S.P., Li, X., Han, X., Wang, Q., Peng, J., Zhang, B., et al. (2023). Autophagy enables microglia to engage amyloid plaques and prevents microglial senescence. Nat. Cell Biol. *25*, 963–974. https://doi.org/10.1038/s41556-023-01158-0.

15. Storck, S.E., Meister, S., Nahrath, J., Meißner, J.N., Schubert, N., Di Spiezio, A., Baches, S., Vandenbroucke, R.E., Bouter, Y., Prikulis, I., et al. (2016). Endothelial LRP1 transports amyloid-β(1-42) across the blood-brain barrier. J. Clin. Investig. *126*, 123–136. https://doi.org/10.1172/jci81108.

16. Rubinsztein, D.C., Codogno, P., and Levine, B. (2012). Autophagy modulation as a potential therapeutic target for diverse diseases. Nat. Rev. Drug Discov. *11*, 709–730. https://doi.org/10.1038/nrd3802.

17. 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. https://doi.org/10.1038/s41586-018-0368-8.

18. 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. https://doi.org/10.1038/s41593-018-0227-9.

19. Iliff, J.J., Wang, M., Zeppenfeld, D.M., Venkataraman, A., Plog, B.A., Liao, Y., Deane, R., and Nedergaard, M. (2013). Cerebral arterial pulsation drives paravascular CSF-interstitial fluid exchange in the murine brain. J. Neurosci. *33*, 18190–18199. https://doi.org/10.1523/JNEUROSCI.1592-13.2013.

20. Wen, Q., Wang, H., Haacke, E.M., Jiang, Q., and Hu, J. (2024). Contribution of Direct Cerebral Vascular Transport in Brain Substance Clearance. Aging Dis. *15*, 584–600. https://doi.org/10.14336/ad.2023.0426.

21. Plog, B.A., Kim, K., Verhaege, D., Kim, M.W., Papadopoulos, Z., Dikranian, K., Dykstra, T., Cao, J., Perrin, R.J., Schwetye, K.E., et al. (2025). A route for cerebrospinal fluid flow through leptomeningeal arterial-venous overlaps enables macromolecule and fluid shunting. Nat. Neurosci. *28*, 1436–1445. https://doi.org/10.1038/s41593-025-01977-4.

22. Xavier, A.L.R., Hauglund, N.L., von Holstein-Rathlou, S., Li, Q., Sanggaard, S., Lou, N., Lundgaard, I., and Nedergaard, M. (2018). Cannula Implantation into the Cisterna Magna of Rodents. J. Vis. Exp. 57378. https://doi.org/10.3791/57378.

23. Ramos, M., Burdon Bechet, N., Battistella, R., Pavan, C., Xavier, A.L.R., Nedergaard, M., and Lundgaard, I. (2019). Cisterna Magna Injection in Rats to Study Glymphatic Function. Methods Mol. Biol. *1938*, 97–104. https://doi.org/10.1007/978-1-4939-9068-9_7.

24. Sigurdsson, B., Hauglund, N.L., Lilius, T.O., Mogensen, F.L.H., Mortensen, K.N., Beschorner, N., Klinger, L., Bærentzen, S.L., Rosenholm, M.P., Shalgunov, V., et al. (2023). A SPECT-based method for dynamic imaging of the glymphatic system in rats. J. Cereb. Blood Flow Metab. *43*, 1153–1165. https://doi.org/10.1177/0271678X231156982.

25. Naganawa, S., and Taoka, T. (2022). The Glymphatic System: A Review of the Challenges in Visualizing its Structure and Function with MR Imaging. Magn. Reson. Med. Sci. *21*, 182–194. https://doi.org/10.2463/mrms.rev.2020-0122.

26. Papadopoulos, Z., Smyth, L.C.D., Smirnov, I., Gibson, D.A., Herz, J., and Kipnis, J. (2025). Differential impact of lymphatic outflow pathways on cerebrospinal fluid homeostasis. J. Exp. Med. *222*, e20241752. https://doi.org/10.1084/jem.20241752.

27. Jacob, L., de Brito Neto, J., Lenck, S., Corcy, C., Benbelkacem, F., Geraldo, L.H., Xu, Y., Thomas, J.M., El Kamouh, M.R., Spajer, M., et al. (2022). Conserved meningeal lymphatic drainage circuits in mice and humans. J. Exp. Med. *219*, e20220035. https://doi.org/10.1084/jem.20220035.

28. Aspelund, A., Antila, S., Proulx, S.T., Karlsen, T.V., Karaman, S., Detmar, M., Wiig, H., and Alitalo, K. (2015). A dural lymphatic vascular system that drains brain interstitial fluid and macromolecules. J. Exp. Med. *212*, 991–999. https://doi.org/10.1084/jem.20142290.

29. 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. (2015). Structural and functional features of central nervous system lymphatic vessels. Nature *523*, 337–341. https://doi.org/10.1038/nature14432.

30. Ahn, J.H., Cho, H., Kim, J.H., Kim, S.H., Ham, J.S., Park, I., Suh, S.H., Hong, S.P., Song, J.H., Hong, Y.K., et al. (2019). Meningeal lymphatic vessels at the skull base drain cerebrospinal fluid. Nature *572*, 62–66. https://doi.org/10.1038/s41586-019-1419-5.

31. Lee, H., Xie, L., Yu, M., Kang, H., Feng, 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. https://doi.org/10.1523/JNEUROSCI.1625-15.2015.

32. Proulx, S.T. (2021). Cerebrospinal fluid outflow: a review of the historical and contemporary evidence for arachnoid villi, perineural routes, and dural lymphatics. Cell. Mol. Life Sci. *78*, 2429–2457. https://doi.org/10.1007/s00018-020-03706-5.

33. Klarica, M., Miše, B., Vladić, A., Radoš, M., and Orešković, D. (2013). ''Compensated hyperosmolarity'' of cerebrospinal fluid and the development of hydrocephalus. Neuroscience *248*, 278–289. https://doi.org/10.1016/j.neuroscience.2013.06.022.

34. van Veluw, S.J., Hou, S.S., Calvo-Rodriguez, M., Arbel-Ornath, M., Snyder, A.C., Frosch, M.P., Greenberg, S.M., and Bacskai, B.J. (2020). Vasomotion as a Driving Force for Paravascular Clearance in the Awake Mouse Brain. Neuron *105*, 549–561.e5. https://doi.org/10.1016/j.neuron.2019.10.033.

35. Mateo, C., Knutsen, P.M., Tsai, P.S., Shih, A.Y., and Kleinfeld, D. (2017). Entrainment of Arteriole Vasomotor Fluctuations by Neural Activity Is a Basis of Blood-Oxygenation-Level-Dependent "Resting-State" Connectivity. Neuron *96*, 936–948.e3. https://doi.org/10.1016/j.neuron.2017.10.012.

36. Rayshubskiy, A., Wojtasiewicz, T.J., Mikell, C.B., Bouchard, M.B., Timerman, D., Youngerman, B.E., McGovern, R.A., Otten, M.L., Canoll, P., McKhann, G.M., et al. (2014). Direct, intraoperative observation of ~0.1 Hz hemodynamic oscillations in awake human cortex: implications for fMRI. Neuroimage *87*, 323–331. https://doi.org/10.1016/j.neuroimage.2013.10.044.

37. Bojarskaite, L., Vallet, A., Bjørnstad, D.M., Gullestad Binder, K.M., Cunen, C., Heuser, K., Kuchta, M., Mardal, K.A., and Enger, R. (2023). Sleep cycle-dependent vascular dynamics in male mice and the predicted effects on perivascular cerebrospinal fluid flow and solute transport. Nat. Commun. *14*, 953. https://doi.org/10.1038/s41467-023-36643-5.

38. Kollmeier, J.M., Gürbüz-Reiss, L., Sahoo, P., Badura, S., Ellebracht, B., Keck, M., Gärtner, J., Ludwig, H.C., Frahm, J., and Dreha-Kulaczewski, S. (2022). Deep breathing couples CSF and venous flow dynamics. Sci. Rep. *12*, 2568. https://doi.org/10.1038/s41598-022-06361-x.

39. Jiang-Xie, L.F., Drieu, A., Bhasiin, K., Quintero, D., Smirnov, I., and Kipnis, J. (2024). Neuronal dynamics direct cerebrospinal fluid perfusion and brain clearance. Nature *627*, 157–164. https://doi.org/10.1038/s41586-024-07108-6.

40. Smets, N.G., Strijkers, G.J., Vinje, V., and Bakker, E.N.T.P. (2024). Cerebrospinal fluid turnover as a driver of brain clearance. NMR Biomed. *37*, e5029. https://doi.org/10.1002/nbm.5029.

41. Liu, G., Mestre, H., Sweeney, A.M., Sun, Q., Weikop, P., Du, T., and Nedergaard, M. (2020). Direct Measurement of Cerebrospinal Fluid Production in Mice. Cell Rep. *33*, 108524. https://doi.org/10.1016/j.celrep.2020.108524.

42. Zimmermann, J., Boudriot, C., Eipert, C., Hoffmann, G., Nuttall, R., Neumaier, V., Bonhoeffer, M., Schneider, S., Schmitzer, L., Kufer, J., et al. (2025). Total cerebral blood volume changes drive macroscopic cerebrospinal fluid flux in humans. PLoS Biol. *23*, e3003138. https://doi.org/10.1371/journal.pbio.3003138.

43. Murdock, M.H., Yang, C.Y., Sun, N., Pao, P.C., Blanco-Duque, C., Kahn, M.C., Kim, T., Lavoie, N.S., Victor, M.B., Islam, M.R., et al. (2024). Multisensory gamma stimulation promotes glymphatic clearance of amyloid. Nature *627*, 149–156. https://doi.org/10.1038/s41586-024-07132-6.

44. Mokri, B. (2001). The Monro-Kellie hypothesis: applications in CSF volume depletion. Neurology *56*, 1746–1748. https://doi.org/10.1212/wnl.56.12.1746.

45. Zhao, L., Tannenbaum, A., Bakker, E.N.T.P., and Benveniste, H. (2022). Physiology of Glymphatic Solute Transport and Waste Clearance from the Brain. Physiology (Bethesda) *37*, 0. https://doi.org/10.1152/physiol.00015.2022.

46. Thomas, J.H. (2019). Fluid dynamics of cerebrospinal fluid flow in perivascular spaces. J. R. Soc. Interface *16*, 20190572. https://doi.org/10.1098/rsif.2019.0572.

47. Mestre, H., Tithof, J., Du, T., Song, W., Peng, W., Sweeney, A.M., Olveda, G., Thomas, J.H., Nedergaard, M., and Kelley, D.H. (2018). Flow of cerebrospinal fluid is driven by arterial pulsations and is reduced in hypertension. Nat. Commun. *9*, 4878. https://doi.org/10.1038/s41467-018-07318-3.

48. Sharp, M.K. (2023). Pulsatile cerebral paraarterial flow by peristalsis, pressure and directional resistance. Fluids Barriers CNS *20*, 41. https://doi.org/10.1186/s12987-023-00445-0.

49. Kedarasetti, R.T., Drew, P.J., and Costanzo, F. (2022). Arterial vasodilation drives convective fluid flow in the brain: a poroelastic model. Fluids Barriers CNS *19*, 34. https://doi.org/10.1186/s12987-022-00326-y.

50. Martinac, A.D., and Bilston, L.E. (2020). Computational modelling of fluid and solute transport in the brain. Biomech. Model. Mechanobiol. *19*, 781–800. https://doi.org/10.1007/s10237-019-01253-y.

51. Gan, Y., Holstein-Rønsbo, S., Nedergaard, M., Boster, K.A.S., Thomas, J.H., and Kelley, D.H. (2023). Perivascular pumping of cerebrospinal fluid in the brain with a valve mechanism. J. R. Soc. Interface *20*, 20230288. https://doi.org/10.1098/rsif.2023.0288.

52. Koundal, S., Elkin, R., Nadeem, S., Xue, Y., Constantinou, S., Sanggaard, S., Liu, X., Monte, B., Xu, F., Van Nostrand, W., et al. (2020). Optimal Mass Transport with Lagrangian Workflow Reveals Advective and Diffusion Driven Solute Transport in the Glymphatic System. Sci. Rep. *10*, 1990. https://doi.org/10.1038/s41598-020-59045-9.

53. Asgari, M., de Zélicourt, D., and Kurtcuoglu, V. (2016). Glymphatic solute transport does not require bulk flow. Sci. Rep. *6*, 38635. https://doi.org/10.1038/srep38635.

54. Cserr, H.F., Depasquale, M., Patlak, C.S., and Pullen, R.G. (1986). Convection of cerebral interstitial fluid and its role in brain volume regulation. Ann. N. Y. Acad. Sci. *481*, 123–134. https://doi.org/10.1111/j.1749-6632.1986.tb27144.x.

55. Smith, A.J., Yao, X., Dix, J.A., Jin, B.J., and Verkman, A.S. (2017). Test of the 'glymphatic' hypothesis demonstrates diffusive and aquaporin-4-independent solute transport in rodent brain parenchyma. eLife *6*, e27679. https://doi.org/10.7554/eLife.27679.

56. Valnes, L.M., Mitusch, S.K., Ringstad, G., Eide, P.K., Funke, S.W., and Mardal, K.A. (2020). Apparent diffusion coefficient estimates based on 24 hours tracer movement support glymphatic transport in human cerebral cortex. Sci. Rep. *10*, 9176. https://doi.org/10.1038/s41598-020-66042-5.

57. Keith Sharp, M., Carare, R.O., and Martin, B.A. (2019). Dispersion in porous media in oscillatory flow between flat plates: applications to intrathecal, periarterial and paraarterial solute transport in the central nervous system. Fluids Barriers CNS *16*, 13. https://doi.org/10.1186/s12987-019-0132-y.

58. Holter, K.E., Kehlet, B., Devor, A., Sejnowski, T.J., Dale, A.M., Omholt, S.W., Ottersen, O.P., Nagelhus, E.A., Mardal, K.A., and Pettersen, K.H. (2017). Interstitial solute transport in 3D reconstructed neuropil occurs by diffusion rather than bulk flow. Proc. Natl. Acad. Sci. USA *114*, 9894–9899. https://doi.org/10.1073/pnas.1706942114.

59. Ray, L.A., Pike, M., Simon, M., Iliff, J.J., and Heys, J.J. (2021). Quantitative analysis of macroscopic solute transport in the murine brain. Fluids Barriers CNS *18*, 55. https://doi.org/10.1186/s12987-021-00290-z.

60. 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. https://doi.org/10.1007/s00401-018-1812-4.

61. Guo, Y., Quirk, K., Kelley, D.H., and Thomas, J.H. (2025). Advection and diffusion in perivascular and extracellular spaces in the brain. J. R. Soc. Interface *22*, 20250010. https://doi.org/10.1098/rsif.2025.0010.

62. Bork, P.A.R., Hauglund, N.L., Mori, Y., Møllgård, K., Hjorth, P.G., and Nedergaard, M. (2024). Modeling of brain efflux: Constraints of brain surfaces. Proc. Natl. Acad. Sci. U.S.A. *121*, e2318444121. https://doi.org/10.1073/pnas.2318444121.

63. Boster, K.A.S., Cai, S., Ladrón-de-Guevara, A., Sun, J., Zheng, X., Du, T., Thomas, J.H., Nedergaard, M., Karniadakis, G.E., and Kelley, D.H. (2023). Artificial intelligence velocimetry reveals in vivo flow rates, pressure gradients, and shear stresses in murine perivascular flows. Proc. Natl. Acad. Sci. USA *120*, e2217744120. https://doi.org/10.1073/pnas.2217744120.

64. Miao, A., Luo, T., Hsieh, B., Edge, C.J., Gridley, M., Wong, R.T.C., Constandinou, T.G., Wisden, W., and Franks, N.P. (2024). Brain clearance is reduced during sleep and anesthesia. Nat. Neurosci. *27*, 1046–1050. https://doi.org/10.1038/s41593-024-01638-y.

65. van Veluw, S.J., Benveniste, H., Bakker, E.N.T.P., Carare, R.O., Greenberg, S.M., Iliff, J.J., Lorthois, S., Van Nostrand, W.E., Petzold, G.C., Shih, A.Y., et al. (2024). Is CAA a perivascular brain clearance disease? A discussion of the evidence to date and outlook for future studies. Cell. Mol. Life Sci. *81*, 239. https://doi.org/10.1007/s00018-024-05277-1.

66. Bechter, K., and Schmitz, B. (2014). Cerebrospinal fluid outflow along lumbar nerves and possible relevance for pain research: case report and review. Croat. Med. J. *55*, 399–404. https://doi.org/10.3325/cmj.2014.55.399.

67. Rzepliński, R., Proulx, S.T., Tarka, S., Stepień, T., and Ciszek, B. (2025). CSF outflow from the human spinal canal: preliminary results from an anatomical specimen-based model. Fluids Barriers CNS *22*, 32. https://doi.org/10.1186/s12987-025-00645-w.

68. Ma, Q., Decker, Y., Müller, A., Ineichen, B.V., and Proulx, S.T. (2019). Clearance of cerebrospinal fluid from the sacral spine through lymphatic vessels. J. Exp. Med. *216*, 2492–2502. https://doi.org/10.1084/jem.20190351.

69. Jin, H., Yoon, J.H., Hong, S.P., Hwang, Y.S., Yang, M.J., Choi, J., Kang, H.J., Baek, S.E., Jin, C., Jung, J., et al. (2025). Increased CSF drainage by non-invasive manipulation of cervical lymphatics. Nature *643*, 755–767. https://doi.org/10.1038/s41586-025-09052-5.

70. Yoon, J.H., Jin, H., Kim, H.J., Hong, S.P., Yang, M.J., Ahn, J.H., Kim, Y.C., Seo, J., Lee, Y., McDonald, D.M., et al. (2024). Nasopharyngeal lymphatic plexus is a hub for cerebrospinal fluid drainage. Nature *625*, 768–777. https://doi.org/10.1038/s41586-023-06899-4.

71. Hablitz, L.M., Plá, V., Giannetto, M., Vinitsky, H.S., Stæger, F.F., Metcalfe, T., Nguyen, R., Benrais, A., and Nedergaard, M. (2020). Circadian control of brain glymphatic and lymphatic fluid flow. Nat. Commun. *11*, 4411. https://doi.org/10.1038/s41467-020-18115-2.

72. Benveniste, H., Lee, H., Ding, F., Sun, Q., Al-Bizri, E., Makaryus, R., Probst, S., Nedergaard, M., Stein, E.A., and Lu, H. (2017). Anesthesia with Dexmedetomidine and Low-dose Isoflurane Increases Solute Transport via the Glymphatic Pathway in Rat Brain When Compared with High-dose Isoflurane. Anesthesiology *127*, 976–988. https://doi.org/10.1097/ALN.0000000000001888.

73. Hablitz, L.M., Vinitsky, H.S., Sun, Q., Stæger, F.F., Sigurdsson, B., Mortensen, K.N., Lilius, T.O., and Nedergaard, M. (2019). Increased glymphatic influx is correlated with high EEG delta power and low heart rate in mice under anesthesia. Sci. Adv. *5*, eaav5447. https://doi.org/10.1126/sciadv.aav5447.

74. Stanton, E.H., Persson, N.D.Å., Gomolka, R.S., Lilius, T., Sigurðsson, B., Lee, H., Xavier, A.L.R., Benveniste, H., Nedergaard, M., and Mori, Y. (2021). Mapping of CSF transport using high spatiotemporal resolution dynamic contrast-enhanced MRI in mice: Effect of anesthesia. Magn. Reson. Med. *85*, 3326–3342. https://doi.org/10.1002/mrm.28645.

75. Plá, V., Bork, P., Harnpramukkul, A., Olveda, G., Ladrón-de-Guevara, A., Giannetto, M.J., Hussain, R., Wang, W., Kelley, D.H., Hablitz, L.M., et al. (2022). A real-time in vivo clearance assay for quantification of glymphatic efflux. Cell Rep. *40*, 111320. https://doi.org/10.1016/j.celrep.2022.111320.

76. Da Mesquita, S., Papadopoulos, Z., Dykstra, T., Brase, L., Farias, F.G., Wall, M., Jiang, H., Kodira, C.D., de Lima, K.A., Herz, J., et al. (2021). Meningeal lymphatics affect microglia responses and anti-Aβ immunotherapy. Nature *593*, 255–260. https://doi.org/10.1038/s41586-021-03489-0.

77. das Neves, S.P., Delivanoglou, N., Ren, Y., Cucuzza, C.S., Makuch, M., Almeida, F., Sanchez, G., Barber, M.J., Rego, S., Schrader, R., et al. (2024). Meningeal lymphatic function promotes oligodendrocyte survival and brain myelination. Immunity *57*, 2328–2343.e8. https://doi.org/10.1016/j.immuni.2024.08.004.

78. Lu, X., Bai, S., Feng, L., Yan, X., Lin, Y., Huang, J., Liao, X., Wang, H., Li, L., Yang, Z., et al. (2025). Cranial bone maneuver ameliorates Alzheimer's disease pathology via enhancing meningeal lymphatic drainage function. Alzheimers Dement. *21*, e14518. https://doi.org/10.1002/alz.14518.

79. Wang, M., Yan, C., Li, X., Yang, T., Wu, S., Liu, Q., Luo, Q., and Zhou, F. (2024). Non-invasive modulation of meningeal lymphatics ameliorates ageing and Alzheimer''s disease-associated pathology and cognition in mice. Nat. Commun. *15*, 1453. https://doi.org/10.1038/s41467-024-45656-7.

80. 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*, 11. https://doi.org/10.1186/s13024-019-0312-x.

81. Al-Diwani, A., Provine, N.M., Murchison, A., Laban, R., Swann, O.J., Koychev, I., Sheerin, F., Da Mesquita, S., Heslegrave, A., Zetterberg, H., et al. (2025). Neurodegenerative fluid biomarkers are enriched in human cervical lymph nodes. Brain *148*, 394–400. https://doi.org/10.1093/brain/awae329.

82. Hitpass Romero, K., Stevenson, T.J., Smyth, L.C.D., Watkin, B., McCullough, S.J.C., Vinnell, L., Smith, A.M., Schweder, P., Correia, J.A., Kipnis, J., et al. (2025). Age-related meningeal extracellular matrix remodeling compromises CNS lymphatic function. J. Neuroinflamm. *22*, 109. https://doi.org/10.1186/s12974-025-03436-0.

83. Rustenhoven, J., Pavlou, G., Storck, S.E., Dykstra, T., Du, S., Wan, Z., Quintero, D., Scallan, J.P., Smirnov, I., Kamm, R.D., et al. (2023). Age-related alterations in meningeal immunity drive impaired CNS lymphatic drainage. J. Exp. Med. *220*, e20221929. https://doi.org/10.1084/jem.20221929.

84. Ding, X.B., Wang, X.X., Xia, D.H., Liu, H., Tian, H.Y., Fu, Y., Chen, Y.K., Qin, C., Wang, J.Q., Xiang, Z., et al. (2021). Impaired meningeal lymphatic drainage in patients with idiopathic Parkinson's disease. Nat. Med. *27*, 411–418. https://doi.org/10.1038/s41591-020-01198-1.

85. Zou, W., Pu, T., Feng, W., Lu, M., Zheng, Y., Du, R., Xiao, M., and Hu, G. (2019). Blocking meningeal lymphatic drainage aggravates Parkinson's disease-like pathology in mice overexpressing mutated α-synuclein. Transl. Neurodegener. *8*, 7. https://doi.org/10.1186/s40035-019-0147-y.

86. Bolte, A.C., Dutta, A.B., Hurt, M.E., Smirnov, I., Kovacs, M.A., McKee, C.A., Ennerfelt, H.E., Shapiro, D., Nguyen, B.H., Frost, E.L., et al. (2020). Meningeal lymphatic dysfunction exacerbates traumatic brain injury pathogenesis. Nat. Commun. *11*, 4524. https://doi.org/10.1038/s41467-020-18113-4.

87. Boisserand, L.S.B., Geraldo, L.H., Bouchart, J., El Kamouh, M.R., Lee, S., Sanganahalli, B.G., Spajer, M., Zhang, S., Lee, S., Parent, M., et al. (2024). VEGF-C prophylaxis favors lymphatic drainage and modulates neuroinflammation in a stroke model. J. Exp. Med. *221*, e20221983. https://doi.org/10.1084/jem.20221983.

88. Nelson-Maney, N.P., Bálint, L., Beeson, A.L., Serafin, D.S., Kistner, B.M., Douglas, E.S., Siddiqui, A.H., Tauro, A.M., and Caron, K.M. (2024). Meningeal lymphatic CGRP signaling governs pain via cerebrospinal fluid efflux and neuroinflammation in migraine models. J. Clin. Investig. *134*, e175616. https://doi.org/10.1172/jci175616.

89. Ma, L., Chang, Q., Pei, F., Liu, M., Zhang, W., Hong, Y.K., Chai, Y., and Chen, J.F. (2023). Skull progenitor cell-driven meningeal lymphatic restoration improves neurocognitive functions in craniosynostosis. Cell Stem Cell *30*, 1472–1485.e7. https://doi.org/10.1016/j.stem.2023.09.012.

90. Matrongolo, M.J., Ang, P.S., Wu, J., Jain, A., Thackray, J.K., Reddy, A., Sung, C.C., Barbet, G., Hong, Y.K., and Tischfield, M.A. (2023). Piezo1 agonist restores meningeal lymphatic vessels, drainage, and brain-CSF perfusion in craniosynostosis and aged mice. J. Clin. Investig. *134*, e171468. https://doi.org/10.1172/jci171468.

91. Song, E., Mao, T., Dong, H., Boisserand, L.S.B., Antila, S., Bosenberg, M., Alitalo, K., Thomas, J.L., and Iwasaki, A. (2020). VEGF-C-driven lymphatic drainage enables immunosurveillance of brain tumours. Nature *577*, 689–694. https://doi.org/10.1038/s41586-019-1912-x.

92. Hu, X., Deng, Q., Ma, L., Li, Q., Chen, Y., Liao, Y., Zhou, F., Zhang, C., Shao, L., Feng, J., et al. (2020). Meningeal lymphatic vessels regulate brain tumor drainage and immunity. Cell Res. *30*, 229–243. https://doi.org/10.1038/s41422-020-0287-8.

93. Kim, K., Abramishvili, D., Du, S., Papadopoulos, Z., Cao, J., Herz, J., Smirnov, I., Thomas, J.L., Colonna, M., and Kipnis, J. (2025). Meningeal lymphatics-microglia axis regulates synaptic physiology. Cell *188*, 2705–2719.e23. https://doi.org/10.1016/j.cell.2025.02.022.

94. Antila, S., Chilov, D., Nurmi, H., Li, Z., Näsi, A., Gotkiewicz, M., Sitnikova, V., Jäntti, H., Acosta, N., Koivisto, H., et al. (2024). Sustained meningeal lymphatic vessel atrophy or expansion does not alter Alzheimer's disease-related amyloid pathology. Nat CardioVasc Res *3*, 474–491. https://doi.org/10.1038/s44161-024-00445-9.

95. Pinho-Correia, L.M., McCullough, S.J.C., Ghanizada, H., Nedergaard, M., Rustenhoven, J., and Da Mesquita, S. (2025). CSF transport at the brain-meningeal border: effects on neurological health and disease. Lancet Neurol. *24*, 535–547. https://doi.org/10.1016/s1474-4422(25)00115-2.

96. Da Mesquita, S., Herz, J., Wall, M., Dykstra, T., de Lima, K.A., Norris, G.T., Dabhi, N., Kennedy, T., Baker, W., and Kipnis, J. (2021). Aging-associated deficit in CCR7 is linked to worsened glymphatic function, cognition, neuroinflammation, and β-amyloid pathology. Sci. Adv. *7*, eabe4601. https://doi.org/10.1126/sciadv.abe4601.

97. Goldman, D.H., Dykstra, T., Smirnov, I., Blackburn, S.M., Da Mesquita, S., Kipnis, J., and Herz, J. (2022). Age-associated suppression of exploratory activity during sickness is linked to meningeal lymphatic dysfunction and microglia activation. Nat Aging *2*, 704–713. https://doi.org/10.1038/s43587-022-00268-y.

98. Kim, M.W., and Kipnis, J. (2025). Glymphatics and meningeal lymphatics unlock the brain-immune code. Immunity *58*, 1040–1051. https://doi.org/10.1016/j.immuni.2025.03.006.

99. Li, Q., and Barres, B.A. (2018). Microglia and macrophages in brain homeostasis and disease. Nat. Rev. Immunol. *18*, 225–242. https://doi.org/10.1038/nri.2017.125.

100. Depp, C., Doman, J.L., Hingerl, M., Xia, J., and Stevens, B. (2025). Microglia transcriptional states and their functional significance: Context drives diversity. Immunity *58*, 1052–1067. https://doi.org/10.1016/j.immuni.2025.04.009.

101. Van Hove, H., Martens, L., Scheyltjens, I., De Vlaminck, K., Pombo Antunes, A.R., De Prijck, S., Vandamme, N., De Schepper, S., Van Isterdael, G., Scott, C.L., et al. (2019). A single-cell atlas of mouse brain macrophages reveals unique transcriptional identities shaped by ontogeny and tissue environment. Nat. Neurosci. *22*, 1021–1035. https://doi.org/10.1038/s41593-019-0393-4.

102. Chen, X., Firulyova, M., Manis, M., Herz, J., Smirnov, I., Aladyeva, E., Wang, C., Bao, X., Finn, M.B., Hu, H., et al. (2023). Microglia-mediated T cell infiltration drives neurodegeneration in tauopathy. Nature *615*, 668–677. https://doi.org/10.1038/s41586-023-05788-0.

103. McGeer, P.L., Itagaki, S., Tago, H., and McGeer, E.G. (1987). Reactive microglia in patients with senile dementia of the Alzheimer type are positive for the histocompatibility glycoprotein HLA-DR. Neurosci. Lett. *79*, 195–200. https://doi.org/10.1016/0304-3940(87)90696-3.

104. Yoshida, T.M., Nguyen, M., Zhang, L., Lu, B.Y., Zhu, B., Murray, K.N., Mineur, Y.S., Zhang, C., Xu, D., Lin, E., et al. (2025). The subfornical organ is a nucleus for gut-derived T cells that regulate behaviour. Nature *643*, 499–508. https://doi.org/10.1038/s41586-025-09050-7.

105. Da Mesquita, S., and Rua, R. (2024). R. Brain border-associated macrophages: common denominators in infection, aging, and Alzheimer's disease? Trends Immunol. *45*, 346–357. https://doi.org/10.1016/j.it.2024.03.007.

106. Iadecola, C., Smith, E.E., Anrather, J., Gu, C., Mishra, A., Misra, S., Perez-Pinzon, M.A., Shih, A.Y., Sorond, F.A., van Veluw, S.J., et al. (2023). The Neurovasculome: Key Roles in Brain Health and Cognitive Impairment: A Scientific Statement From the American Heart Association/American Stroke Association. Stroke *54*, e251–e271. https://doi.org/10.1161/str.0000000000000431.

107. Sankowski, R., Süß, P., Benkendorff, A., Böttcher, C., Fernandez-Zapata, C., Chhatbar, C., Cahueau, J., Monaco, G., Gasull, A.D., Khavaran, A., et al. (2024). Multiomic spatial landscape of innate immune cells at human central nervous system borders. Nat. Med. *30*, 186–198. https://doi.org/10.1038/s41591-023-02673-1.

108. Schonhoff, A.M., Figge, D.A., Williams, G.P., Jurkuvenaite, A., Gallups, N.J., Childers, G.M., Webster, J.M., Standaert, D.G., Goldman, J.E., and Harms, A.S. (2023). Border-associated macrophages mediate the neuroinflammatory response in an alpha-synuclein model of Parkinson disease. Nat. Commun. *14*, 3754. https://doi.org/10.1038/s41467-023-39060-w.

109. Goddery, E.N., Fain, C.E., Lipovsky, C.G., Ayasoufi, K., Yokanovich, L.T., Malo, C.S., Khadka, R.H., Tritz, Z.P., Jin, F., Hansen, M.J., et al. (2021). Microglia and Perivascular Macrophages Act as Antigen Presenting Cells to Promote CD8 T Cell Infiltration of the Brain. Front. Immunol. *12*, 726421. https://doi.org/10.3389/fimmu.2021.726421.

110. McCandless, E.E., Wang, Q., Woerner, B.M., Harper, J.M., and Klein, R.S. (2006). CXCL12 limits inflammation by localizing mononuclear infiltrates to the perivascular space during experimental autoimmune encephalomyelitis. J. Immunol. *177*, 8053–8064. https://doi.org/10.4049/jimmunol.177.11.8053.

111. Aydin, S., Pareja, J., Schallenberg, V.M., Klopstein, A., Gruber, T., Page, N., Bouillet, E., Blanchard, N., Liblau, R., Körbelin, J., et al. (2023). Antigen recognition detains CD8+ T cells at the blood-brain barrier and contributes to its breakdown. Nat. Commun. *14*, 3106. https://doi.org/10.1038/s41467-023-38703-2.

112. Galea, I., Bernardes-Silva, M., Forse, P.A., van Rooijen, N., Liblau, R.S., and Perry, V.H. (2007). An antigen-specific pathway for CD8 T cells across the blood-brain barrier. J. Exp. Med. *204*, 2023–2030. https://doi.org/10.1084/jem.20070064.

113. Drieu, A., Du, S., Storck, S.E., Rustenhoven, J., Papadopoulos, Z., Dykstra, T., Zhong, F., Kim, K., Blackburn, S., Mamuladze, T., et al. (2022). Parenchymal border macrophages regulate the flow dynamics of the cerebrospinal fluid. Nature *611*, 585–593. https://doi.org/10.1038/s41586-022-05397-3.

114. Alves de Lima, K., Rustenhoven, J., Da Mesquita, S., Wall, M., Salvador, A.F., Smirnov, I., Martelossi Cebinelli, G., Mamuladze, T., Baker, W., Papadopoulos, Z., et al. (2020). Meningeal γδ T cells regulate anxiety-like behavior via IL-17a signaling in neurons. Nat. Immunol. *21*, 1421–1429. https://doi.org/10.1038/s41590-020-0776-4.

115. Mrdjen, D., Pavlovic, A., Hartmann, F.J., Schreiner, B., Utz, S.G., Leung, B.P., Lelios, I., Heppner, F.L., Kipnis, J., Merkler, D., et al. (2018). High-Dimensional Single-Cell Mapping of Central Nervous System Immune Cells Reveals Distinct Myeloid Subsets in Health, Aging, and Disease. Immunity *48*, 599. https://doi.org/10.1016/j.immuni.2018.02.014.

116. Fitzpatrick, Z., Ghabdan Zanluqui, N., Rosenblum, J.S., Tuong, Z.K., Lee, C.Y.C., Chandrashekhar, V., Negro-Demontel, M.L., Stewart, A.P., Posner, D.A., Buckley, M., et al. (2024). Venous-plexus-associated lymphoid hubs support meningeal humoral immunity. Nature *628*, 612–619. https://doi.org/10.1038/s41586-024-07202-9.

117. Wang, Y., Chen, D., Xu, D., Huang, C., Xing, R., He, D., and Xu, H. (2021). Early developing B cells undergo negative selection by central nervous system-specific antigens in the meninges. Immunity *54*, 2784–2794.e6. https://doi.org/10.1016/j.immuni.2021.09.016.

118. Rego, S., Sanchez, G., and Da Mesquita, S. (2023). Current views on meningeal lymphatics and immunity in aging and Alzheimer's disease. Mol. Neurodegener. *18*, 55. https://doi.org/10.1186/s13024-023-00645-0.

119. Kress, B.T., Iliff, J.J., Xia, M., Wang, M., Wei, H.S., Zeppenfeld, D., Xie, L., Kang, H., Xu, Q., Liew, J.A., et al. (2014). Impairment of paravascular clearance pathways in the aging brain. Ann. Neurol. *76*, 845–861. https://doi.org/10.1002/ana.24271.

120. Dagum, P., Giovangrandi, L., Levendovszky, S.R., Winebaum, J.J., Singh, T., Cho, Y., Kaplan, R.M., Jaffee, M.S., Lim, M.M., Vandeweerd, C., et al. (2025). A wireless device for continuous measurement of brain parenchymal resistance tracks glymphatic function in humans. Nat. Biomed. Eng. *9*, 1656–1676. https://doi.org/10.1038/s41551-025-01394-9.

121. Pereira, M., Chen, X., Paltarzhytskaya, A., Pacheco, Y., Muller, N., Bovy, L., Lei, X., Chen, W., Ren, H., Song, C., et al. (2025). Sleep neuroimaging: Review and future directions. Published online February 12, 2025. J. Sleep Res. e14462. https://doi.org/10.1111/jsr.14462.

122. Lucey, B.P., McCullough, A., Landsness, E.C., Toedebusch, C.D., McLeland, J.S., Zaza, A.M., Fagan, A.M., McCue, L., Xiong, C., Morris, J.C., et al. (2019). Reduced non-rapid eye movement sleep is associated with tau pathology in early Alzheimer's disease. Sci. Transl. Med. *11*, eaau6550. https://doi.org/10.1126/scitranslmed.aau6550.

123. Picchioni, D., Özbay, P.S., Mandelkow, H., de Zwart, J.A., Wang, Y., van Gelderen, P., and Duyn, J.H. (2022). Autonomic arousals contribute to brain fluid pulsations during sleep. Neuroimage *249*, 118888. https://doi.org/10.1016/j.neuroimage.2022.118888.

124. Lüthi, A., and Nedergaard, M. (2025). Anything but small: Microarousals stand at the crossroad between noradrenaline signaling and key sleep functions. Neuron *113*, 509–523. https://doi.org/10.1016/j.neuron.2024.12.009.

125. Buongiorno, M., Granell, E., Caruana, G., Sansa, G., Vives-Gilabert, Y., Cullell, N., Molina-Seguin, J., Almeria, M., Artero, C., Sánchez-Benavides, G., et al. (2023). Impairments in sleep and brain molecular clearance in people with cognitive deterioration and biological evidence of AD: a report of four cases. BMC Neurol. *23*, 417. https://doi.org/10.1186/s12883-023-03460-8.

126. Eide, P.K., Vinje, V., Pripp, A.H., Mardal, K.A., and Ringstad, G. (2021). Sleep deprivation impairs molecular clearance from the human brain. Brain *144*, 863–874. https://doi.org/10.1093/brain/awaa443.

127. Hussain, R., Tithof, J., Wang, W., Cheetham-West, A., Song, W., Peng, W., Sigurdsson, B., Kim, D., Sun, Q., Peng, S., et al. (2023). Potentiating glymphatic drainage minimizes post-traumatic cerebral oedema. Nature *623*, 992–1000. https://doi.org/10.1038/s41586-023-06737-7.

128. Yang, Z., Williams, S.D., Beldzik, E., Anakwe, S., Schimmelpfennig, E., and Lewis, L.D. (2024). Attentional failures after sleep deprivation represent moments of cerebrospinal fluid flow. Preprint at bioRxiv. https://doi.org/10.1101/2024.11.15.623271.

129. Lilius, T.O., Blomqvist, K., Hauglund, N.L., Liu, G., Stæger, F.F., Bærentzen, S., Du, T., Ahlström, F., Backman, J.T., Kalso, E.A., et al. (2019). Dexmedetomidine enhances glymphatic brain delivery of intrathecally administered drugs. J. Control. Release *304*, 29–38. https://doi.org/10.1016/j.jconrel.2019.05.005.

130. Gakuba, C., Gaberel, T., Goursaud, S., Bourges, J., Di Palma, C., Quenault, A., Martinez de Lizarrondo, S., Vivien, D., and Gauberti, M. (2018). General Anesthesia Inhibits the Activity of the "Glymphatic System". Theranostics *8*, 710–722. https://doi.org/10.7150/thno.19154.

131. Zimmermann, J., Sorg, C., Müller, L., Zistler, F., Neumaier, V., Bonhoeffer, M., Ranft, A., Golkowski, D., Priller, J., Zimmer, C., et al. (2025). Impaired Macroscopic Cerebrospinal Fluid Flow by Sevoflurane in Humans during and after Anesthesia. Anesthesiology *142*, 692–703. https://doi.org/10.1097/aln.0000000000005360.

132. Sullender, C.T., Richards, L.M., He, F., Luan, L., and Dunn, A.K. (2022). Dynamics of isoflurane-induced vasodilation and blood flow of cerebral vasculature revealed by multi-exposure speckle imaging. J. Neurosci. Methods *366*, 109434. https://doi.org/10.1016/j.jneumeth.2021.109434.

133. van Osch, M.J.P., Wåhlin, A., Scheyhing, P., Mossige, I., Hirschler, L., Eklund, A., Mogensen, K., Gomolka, R., Radbruch, A., Qvarlander, S., et al. (2024). Human brain clearance imaging: Pathways taken by magnetic resonance imaging contrast agents after administration in cerebrospinal fluid and blood. NMR Biomed. *37*, e5159. https://doi.org/10.1002/nbm.5159.

134. Edeklev, C.S., Halvorsen, M., Løvland, G., Vatnehol, S.A.S., Gjertsen, Ø., Nedregaard, B., Sletteberg, R., Ringstad, G., and Eide, P.K. (2019). Intrathecal Use of Gadobutrol for Glymphatic MR Imaging: Prospective Safety Study of 100 Patients. AJNR, Am. J. Neuroradiol. *40*, 1257–1264. https://doi.org/10.3174/ajnr.A6136.

135. Eide, P.K., and Ringstad, G. (2024). Functional analysis of the human perivascular subarachnoid space. Nat. Commun. *15*, 2001. https://doi.org/10.1038/s41467-024-46329-1.

136. Ringstad, G., Valnes, L.M., Dale, A.M., Pripp, A.H., Vatnehol, S.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. https://doi.org/10.1172/jci.insight.121537.

137. Eide, P.K., Pripp, A.H., Berge, B., Hrubos-Strøm, H., Ringstad, G., and Valnes, L.M. (2022). Altered glymphatic enhancement of cerebrospinal fluid tracer in individuals with chronic poor sleep quality. J. Cereb. Blood Flow Metab. *42*, 1676–1692. https://doi.org/10.1177/0271678x221090747.

138. Vinje, V., Zapf, B., Ringstad, G., Eide, P.K., Rognes, M.E., and Mardal, K.A. (2023). Human brain solute transport quantified by glymphatic MRI-informed biophysics during sleep and sleep deprivation. Fluids Barriers CNS *20*, 62. https://doi.org/10.1186/s12987-023-00459-8.

139. Ringstad, G., and Eide, P.K. (2020). Cerebrospinal fluid tracer efflux to parasagittal dura in humans. Nat. Commun. *11*, 354. https://doi.org/10.1038/s41467-019-14195-x.

140. Ringstad, G., and Eide, P.K. (2022). Molecular trans-dural efflux to skull bone marrow in humans with CSF disorders. Brain *145*, 1464–1472. https://doi.org/10.1093/brain/awab388.

141. 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*, 7194. https://doi.org/10.1038/s41598-018-25666-4.

142. Eide, P.K., Pripp, A.H., Ringstad, G., and Valnes, L.M. (2021). Impaired glymphatic function in idiopathic intracranial hypertension. Brain Commun. *3*, fcab043. https://doi.org/10.1093/braincomms/fcab043.

143. Eide, P.K., Undseth, R.M., Pripp, A., Lashkarivand, A., Nedregaard, B., Sletteberg, R., Rønning, P.A., Sorteberg, A.G., Ringstad, G., and Valnes, L.M. (2025). Impact of Subarachnoid Hemorrhage on Human Glymphatic Function: A Time-Evolution Magnetic Resonance Imaging Study. Stroke *56*, 678–691. https://doi.org/10.1161/strokeaha.124.047739.

144. Halvorsen, M., Edeklev, C.S., Fraser-Green, J., Løvland, G., Vatnehol, S.A.S., Gjertsen, Ø., Nedregaard, B., Sletteberg, R., Ringstad, G., and Eide, P.K. (2021). Off-label intrathecal use of gadobutrol: safety study

and comparison of administration protocols. Neuroradiology *63*, 51–61. https://doi.org/10.1007/s00234-020-02519-4.

145. Sperre, A., Karsrud, I., Rodum, A.H.S., Lashkarivand, A., Valnes, L.M., Ringstad, G., and Eide, P.K. (2023). Prospective Safety Study of Intrathecal Gadobutrol in Different Doses. AJNR, Am. J. Neuroradiol. *44*, 511–516. https://doi.org/10.3174/ajnr.A7841.

146. Ringstad, G., Valnes, L.M., Vatnehol, S.A.S., Pripp, A.H., and Eide, P.K. (2023). Prospective T1 mapping to assess gadolinium retention in brain after intrathecal gadobutrol. Neuroradiology *65*, 1321–1331. https://doi.org/10.1007/s00234-023-03198-7.

147. Brendstrup-Brix, K., Ulv Larsen, S.M., Lee, H.H., and Knudsen, G.M. (2024). Perivascular space diffusivity and brain microstructural measures are associated with circadian time and sleep quality. J. Sleep Res. *33*, e14226. https://doi.org/10.1111/jsr.14226.

148. Nilsson, C., Ståhlberg, F., Thomsen, C., Henriksen, O., Herning, M., and Owman, C. (1992). Circadian variation in human cerebrospinal fluid production measured by magnetic resonance imaging. Am. J. Physiol. *262*, R20–R24. https://doi.org/10.1152/ajpregu.1992.262.1.R20.

149. Hartmann, D.A., Hyacinth, H.I., Liao, F.F., and Shih, A.Y. (2018). Does pathology of small venules contribute to cerebral microinfarcts and dementia? J. Neurochem. *144*, 517–526. https://doi.org/10.1111/jnc.14228.

150. Eidsvaag, V.A., Enger, R., Hansson, H.A., Eide, P.K., and Nagelhus, E.A. (2017). Human and mouse cortical astrocytes differ in aquaporin-4 polarization toward microvessels. Glia *65*, 964–973. https://doi.org/10.1002/glia.23138.

151. Oberheim, N.A., Takano, T., Han, X., He, W., Lin, J.H.C., Wang, F., Xu, Q., Wyatt, J.D., Pilcher, W., Ojemann, J.G., et al. (2009). Uniquely hominid features of adult human astrocytes. J. Neurosci. *29*, 3276–3287. https://doi.org/10.1523/JNEUROSCI.4707-08.2009.

152. Perlman, R.L. (2016). Mouse models of human disease: An evolutionary perspective. Evol. Med. Public Health *2016*, 170–176. https://doi.org/10.1093/emph/eow014.

153. Mota, B., Dos Santos, S.E., Ventura-Antunes, L., Jardim-Messeder, D., Neves, K., Kazu, R.S., Noctor, S., Lambert, K., Bertelsen, M.F., Manger, P.R., et al. (2019). White matter volume and white/gray matter ratio in mammalian species as a consequence of the universal scaling of cortical folding. Proc. Natl. Acad. Sci. USA *116*, 15253–15261. https://doi.org/10.1073/pnas.1716956116.

154. Piantino, J., Boespflug, E.L., Schwartz, D.L., Luther, M., Morales, A.M., Lin, A., Fossen, R.V., Silbert, L., and Nagel, B.J. (2020). Characterization of MR Imaging-Visible Perivascular Spaces in the White Matter of Healthy Adolescents at 3T. AJNR, Am. J. Neuroradiol. *41*, 2139–2145. https://doi.org/10.3174/ajnr.A6789.

155. Wardlaw, J.M., Benveniste, H., Nedergaard, M., Zlokovic, B.V., Mestre, H., Lee, H., Doubal, F.N., Brown, R., Ramirez, J., MacIntosh, B.J., et al. (2020). Perivascular spaces in the brain: anatomy, physiology and pathology. Nat. Rev. Neurol. *16*, 137–153. https://doi.org/10.1038/s41582-020-0312-z.

156. Lynch, K.M., Sepehrband, F., Toga, A.W., and Choupan, J. (2023). Brain perivascular space imaging across the human lifespan. Neuroimage *271*, 120009. https://doi.org/10.1016/j.neuroimage.2023.120009.

157. 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. https://doi.org/10.7554/eLife.29738.

158. 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. Investig. *123*, 1299–1309. https://doi.org/10.1172/JCI67677.

159. Yamamoto, E.A., Bagley, J.H., Geltzeiler, M., Sanusi, O.R., Dogan, A., Liu, J.J., and Piantino, J. (2024). The perivascular space is a conduit for cerebrospinal fluid flow in humans: A proof-of-principle report. Proc. Natl. Acad. Sci. USA *121*, e2407246121. https://doi.org/10.1073/pnas.2407246121.

160. Absinta, M., Cortese, I.C.M., Vuolo, L., Nair, G., de Alwis, M.P., Ohayon, J., Meani, A., Martinelli, V., Scotti, R., Falini, A., et al. (2017). Leptomeningeal gadolinium enhancement across the spectrum of chronic neuroinflammatory diseases. Neurology *88*, 1439–1444. https://doi.org/10.1212/wnl.0000000000003820.

161. El Kamouh, M.-R., Spajer, M., Singhabahu, R., Sailor, K.A., Bourrienne, M.-C., Mouton, L., Koundal, S., Doukhi, D., Grine, A., Ninneman, J., et al. (2024). Cerebral Venous Blood Flow Regulates Brain Fluid Clearance via Dural Lymphatics. Preprint at bioRxiv. https://doi.org/10.1101/2024.12.12.628127.

162. 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. https://doi.org/10.1126/science.aax5440.

163. Ungurean, G., Behroozi, M., Böger, L., Helluy, X., Libourel, P.A., Güntürkün, O., and Rattenborg, N.C. (2023). Wide-spread brain activation and reduced CSF flow during avian REM sleep. Nat. Commun. *14*, 3259. https://doi.org/10.1038/s41467-023-38669-1.

164. Kameya, N., Sakai, I., Saito, K., Hamabe-Horiike, T., Shinmyo, Y., Nakada, M., Okuda, S., and Kawasaki, H. (2024). Evolutionary changes leading to efficient glymphatic circulation in the mammalian brain. Nat. Commun. *15*, 10048. https://doi.org/10.1038/s41467-024-54372-1.

165. 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*, 2. https://doi.org/10.1186/1743-8454-1-2.

166. Zhou, Y., Ran, W., Luo, Z., Wang, J., Fang, M., Wei, K., Sun, J., and Lou, M. (2022). Impaired peri-olfactory cerebrospinal fluid clearance is associated with ageing, cognitive decline and dyssomnia. EBiomedicine *86*, 104381. https://doi.org/10.1016/j.ebiom.2022.104381.

167. Kiviniemi, V., Wang, X., Korhonen, V., Keinänen, T., Tuovinen, T., Autio, J., LeVan, P., Keilholz, S., Zang, Y.F., Hennig, J., et al. (2016). Ultra-fast magnetic resonance encephalography of physiological brain activity - Glymphatic pulsation mechanisms? J. Cereb. Blood Flow Metab. *36*, 1033–1045. https://doi.org/10.1177/0271678X15622047.

168. Elabasy, A., Suhonen, M., Rajna, Z., Hosni, Y., Kananen, J., Annunen, J., Ansakorpi, H., Korhonen, V., Seppänen, T., and Kiviniemi, V. (2023). Respiratory brain impulse propagation in focal epilepsy. Sci. Rep. *13*, 5222. https://doi.org/10.1038/s41598-023-32271-7.

169. Järvelä, M., Kananen, J., Korhonen, V., Huotari, N., Ansakorpi, H., and Kiviniemi, V. (2022). Increased very low frequency pulsations and decreased cardiorespiratory pulsations suggest altered brain clearance in narcolepsy. Commun. Med. (Lond) *2*, 122. https://doi.org/10.1038/s43856-022-00187-4.

170. Kananen, J., Helakari, H., Korhonen, V., Huotari, N., Järvelä, M., Raitamaa, L., Raatikainen, V., Rajna, Z., Tuovinen, T., Nedergaard, M., et al. (2020). Respiratory-related brain pulsations are increased in epilepsy-a two-centre functional MRI study. Brain Commun. *2*, fcaa076. https://doi.org/10.1093/braincomms/fcaa076.

171. Tuovinen, T., Kananen, J., Rajna, Z., Lieslehto, J., Korhonen, V., Rytty, R., Mattila, H., Huotari, N., Raitamaa, L., Helakari, H., et al. (2020). The variability of functional MRI brain signal increases in Alzheimer's disease at cardiorespiratory frequencies. Sci. Rep. *10*, 21559. https://doi.org/10.1038/s41598-020-77984-1.

172. Rowsthorn, E., Cribb, L., Sinclair, B., Pham, W., Chong, T.T.J., Yiallourou, S., Cavuoto, M., Vivash, L., O'Brien, T.J., Shao, X., et al. (2025). Relationships between measures of neurovascular integrity and fluid transport in aging: a multi-modal neuroimaging study. Fluids Barriers CNS *22*, 59. https://doi.org/10.1186/s12987-025-00664-7.

173. Roefs, E.C.A., Eiling, I., de Bresser, J., van Osch, M.J.P., and Hirschler, L. (2024). BOLD-CSF dynamics assessed using real-time phase contrast CSF flow interleaved with cortical BOLD MRI. Fluids Barriers CNS *21*, 107. https://doi.org/10.1186/s12987-024-00607-8.

174. 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. https://doi.org/10.1007/s11604-017-0617-z.

175. Taoka, T., Ito, R., Nakamichi, R., Nakane, T., Kawai, H., and Naganawa, S. (2024). Diffusion Tensor Image Analysis ALong the Perivascular Space (DTI-ALPS): Revisiting the Meaning and Significance of the Method. Magn. Reson. Med. Sci. *23*, 268–290. https://doi.org/10.2463/mrms.rev.2023-0175.

176. Saib, G., Demir, Z.H., Taylor, P.A., Lalith Talagala, S., and Koretsky, A.P. (2024). Detection of Nanoliter-Scale Cortical Perivascular Spaces Using Heavily T_2-weighted MRI at 7T. Preprint at medRxiv. https://doi.org/10.1101/2024.10.11.24315113.

177. Sloots, J.J., Biessels, G.J., and Zwanenburg, J.J.M. (2020). Cardiac and respiration-induced brain deformations in humans quantified with high-field MRI. Neuroimage *210*, 116581. https://doi.org/10.1016/j.neuroimage.2020.116581.

178. Petitclerc, L., Hirschler, L., Wells, J.A., Thomas, D.L., van Walderveen, M.A.A., van Buchem, M.A., and van Osch, M.J.P. (2021). Ultra-long-TE arterial spin labeling reveals rapid and brain-wide blood-to-CSF water transport in humans. Neuroimage *245*, 118755. https://doi.org/10.1016/j.neuroimage.2021.118755.

179. Taso, M., and Alsop, D.C. (2025). Tissue-to-fluid water-exchange imaging using T_2-selective saturation labeling. Magn. Reson. Med. *94*, 150–165. https://doi.org/10.1002/mrm.30452.

180. Giannetto, M.J., Gomolka, R.S., Gahn-Martinez, D., Newbold, E.J., Bork, P.A.R., Chang, E., Gresser, M., Thompson, T., Mori, Y., and Nedergaard, M. (2024). Glymphatic fluid transport is suppressed by the aquaporin-4 inhibitor AER-271. Glia *72*, 982–998. https://doi.org/10.1002/glia.24515.

181. Burfeind, K.G., Murchison, C.F., Westaway, S.K., Simon, M.J., Erten-Lyons, D., Kaye, J.A., Quinn, J.F., and Iliff, J.J. (2017). The effects of non-coding aquaporin-4 single-nucleotide polymorphisms on cognition and functional progression of Alzheimer's disease. Alzheimers. Dement. (N Y) *3*, 348–359. https://doi.org/10.1016/j.trci.2017.05.001.

182. Liu, L., Zeng, Q., Luo, X., Hong, H., Fang, Y., Xie, L., Zhang, Y., Lin, M., Wang, S., Li, K., et al. (2025). Association Between Single Nucleotide Polymorphisms in the Aquaporin-4 Gene and Longitudinal Changes in White Matter Free Water and Cognitive Function in Non-Demented Older Adults. Hum. Brain Mapp. *46*, e70171. https://doi.org/10.1002/hbm.70171.

183. Sun, X., Tian, Q., Yang, Z., Liu, Y., Li, C., Hou, B., and Xie, A. (2023). Association of AQP4 single nucleotide polymorphisms (rs335929 and rs2075575) with Parkinson's disease: A case-control study. Neurosci. Lett. *797*, 137062. https://doi.org/10.1016/j.neulet.2023.137062.

184. Gomolka, R.S., Hablitz, L.M., Mestre, H., Giannetto, M., Du, T., Hauglund, N.L., Xie, L., Peng, W., Martinez, P.M., Nedergaard, M., et al. (2023). Loss of aquaporin-4 results in glymphatic system dysfunction via brain-wide interstitial fluid stagnation. eLife *12*, e82232. https://doi.org/10.7554/eLife.82232.

185. Pham, C., Komaki, Y., Deàs-Just, A., Le Gac, B., Mouffle, C., Franco, C., Chaperon, A., Vialou, V., Tsurugizawa, T., Cauli, B., et al. (2024). Astrocyte aquaporin mediates a tonic water efflux maintaining brain homeostasis. eLife *13*, RP95873. https://doi.org/10.7554/eLife.95873.

186. 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. *240*, F319–F328. https://doi.org/10.1152/ajprenal.1981.240.4.F319.

187. Groothuis, D.R., Vavra, M.W., Schlageter, K.E., Kang, E.W.Y., Itskovich, A.C., Hertzler, S., Allen, C.V., and Lipton, H.L. (2007). Efflux of drugs and solutes from brain: the interactive roles of diffusional transcapillary transport, bulk flow and capillary transporters. J. Cereb. Blood Flow Metab. *27*, 43–56. https://doi.org/10.1038/sj.jcbfm.9600315.

188. Lilius, T.O., Rosenholm, M., Klinger, L., Mortensen, K.N., Sigurdsson, B., Mogensen, F.L.H., Hauglund, N.L., Nielsen, M.S.N., Rantamäki, T., and Nedergaard, M. (2022). SPECT/CT imaging reveals CNS-wide modulation of glymphatic cerebrospinal fluid flow by systemic hypertonic saline. iScience *25*, 105250. https://doi.org/10.1016/j.isci.2022.105250.

189. Kumar A., Paikra S.K., Kumar R., Narayan R.K., Jha R.K., Yadav S.S., Kumar P., Ganapathy A., Anant M., Bharti S. Structural Validation of the Intermediate Leptomeningeal Layer in the Human Central Nervous System. bioRxiv 2025.07.14.664673. doi:10.1101/2025.07.14.664673

190. Møllgård, K., Beinlich, F.R.M., Kusk, P., Miyakoshi, L.M., Delle, C., Plá, V., Hauglund, N.L., Esmail, T., Rasmussen, M.K., and Gomolka, R.S. (2023). A mesothelium divides the subarachnoid space into functional compartments. Science *379*, 84–88. https://doi.org/10.1126/science.adc8810.