REVIEW

Glymphatic failure as a final common pathway to dementia

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Sleep is evolutionarily conserved across all species, and impaired sleep is a common trait of the diseased brain. Sleep quality decreases as we age, and disruption of the regular sleep architecture is a frequent antecedent to the onset of dementia in neurodegenerative diseases. The glymphatic system, which clears the brain of protein waste products, is mostly active during sleep. Yet the glymphatic system degrades with age, suggesting a causal relationship between sleep disturbance and symptomatic progression in the neurodegenerative dementias. The ties that bind sleep, aging, glymphatic clearance, and protein aggregation have shed new light on the pathogenesis of a broad range of neurodegenerative diseases, for which glymphatic failure may constitute a therapeutically targetable final common pathway.

Little can replace the rejuvenating feeling of a good night's sleep. Our mood and affect, as well as our ability to attend, focus, and problem-solve, are all directly linked to how well we sleep. The benefits of sleep are cumulative; they are not restricted to the morning hours or even to a given day. Good sleepers live longer, weigh less, have a reduced incidence of psychiatric disorders, and remain cognitively intact longer (*1*–*4*).

Why do we sleep?

The idea that our brains rest during sleep to preserve energy was both posited and rejected in the 1950s, when electroencephalographic (EEG) recordings of brain activity made it clear that rapid eye movement (REM) sleep, which comprises ~20% of normal sleep, is linked to cortex-wide neuronal activation (*5*, *6*). Indeed, energy consumption declines by only 15% in the remaining non-REM (NREM) periods of sleep. Borbély proposed 40 years ago that the sleep-wake cycle is determined by the interaction of two processes: a circadian oscillator, which cycles with the solar day, and a homeostatic drive for sleep (*7*). A key element in that model is that a sleep deficit (i.e., sleep deprivation) causes a quantifiable "pressure to go to sleep." Subsequent NREM sleep is both longer and deeper than normal, and the antecedent sleep loss can be identified post hoc by an increase in EEG slow-wave activity during recovery sleep (*8*). Slow-wave activity is characterized by a wave of synchronous local neural firing that typically begins in the frontal cortex and propagates posteriorly, occurring roughly every second during NREM sleep (*9*). One of the predictions of the Borbély model is that daytime sleep is lighter, because it is not aligned with the circadian clock, and hence fails to fulfill the homeostatic function of sleep. This prediction has been supported by numerous studies of night-shift workers, who as a group are predisposed to stress, obesity, cognitive deficits, and an elevated risk of neurodegenerative diseases (*10*–*13*). One of the most prominent current models of sleep posits that the purpose of sleep is to restore synaptic homeostasis (*14*). The synaptic homeostasis hypothesis of sleep is based on the observations that wakefulness is associated with the sustained potentiation of excitatory transmission, as well as with the structural expansion of postsynaptic dendritic spines (*15*, *16*). The larger size of spines during wakefulness increases their postsynaptic currents and thereby strengthens excitatory transmission. This model is supported by the observation that sleep deprivation is linked to an increased risk of seizures in predisposed individuals (*17*). It is only during subsequent recovery sleep that excitatory transmission tone and spine volume fall, each returning to its sleep-associated baseline (*18*).

Recent studies in mice have offered molecular insights into the synaptic homeostasis hypothesis by mapping the impact of the sleep-wake cycle on synaptic gene expression (*19*, *20*). These studies showed that genes involved in synaptic signaling were predominantly transcribed before the mice woke up, whereas transcripts of genes involved in metabolism rose a few hours before their expected bedtime. Thus, the circadian clock dictates the transcription of genes in anticipation of the tasks appropriate for the time of day. Similarly, translation of mRNAs into proteins largely followed transcription, so that proteins involved in synaptic signaling were produced during wakefulness, whereas those with a role in metabolism were translated during sleep. Surprisingly, when the mice were kept awake longer than normal, the translation of proteins involved in synaptic signaling continued during sleep deprivation, concurrently with suppressed production of proteins associated with metabolism (*19*, *20*). Thus, the behavioral state, rather than the circadian clock, controls synaptic protein production. Under continued wakefulness, proteins involved in synaptic signaling are continuously produced, whereas proteins needed for restorative metabolic processes are not translated. Thus, extended wakefulness is associated with a dysregulation of translation that enables the sustained potentiation of excitatory transmission; this supports a critical homeostatic role of sleep that cannot occur in the awake state. It is intriguing to speculate that the depth of recovery sleep, detected as slow-wave activity, controls the translation of proteins needed to restore metabolic homeostasis.

The glymphatic and lymphatic systems

A fundamental tenet of brain homeostasis is that protein clearance must approximate protein synthesis. Is removal of protein waste also controlled by the sleep-wake cycle? Until 2012 it was believed that the brain, singular among organs, was recycling all of its own protein waste (*21*). Only a small number of proteins were known to be transported across the blood-brain barrier, and these did not include most of the primary proteins made or shed by brain cells (*22*). In the absence of lymphatic vessels or any overt pathways for fluid export, it was unclear how protein waste might exit the mature brain parenchyma. The default conclusion was that the classical cellular protein degradation pathways—autophagy and ubiquitination—must be responsible for all central nervous system (CNS) protein recycling (*23*).

This supposition, that the brain must recycle its own waste, was questioned after the discovery of the glymphatic system (*24*). The glymphatic system is a highly organized cerebrospinal fluid (CSF) transport system that shares several key functions, including the export of excess interstitial fluid and proteins, with the lymphatic vessels of peripheral tissues (Fig. 1A). Indeed, both the brain's CSF and peripheral lymph are drained together into the venous system, from which protein waste is removed and recycled by the liver (*25*). Yet brain tissue itself lacks histologically distinct lymphatic vessels. Rather, fluid clearance from the brain proceeds via the glymphatic pathway, a structurally distinct system of fluid transport that uses the perivascular spaces created by the vascular endfeet of astrocytes (*26*). The endfeet surround arteries, capillaries, and veins, serving as a second wall that covers the entire cerebral vascular bed. The perivascular spaces are open, fluid-filled tunnels that offer little resistance to flow. This is in sharp contrast to the disorientingly crowded and compact architecture of adult brain tissue, the neuropil, through which interstitial fluid flow is necessarily slow and restricted—akin to a marsh, flowing to the glymphatic system's creeks and then rivers (*27*). The glymphatic system's perivascular tunnels are directly connected to the

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subarachnoid spaces surrounding the brain, from which CSF is rapidly driven into deep regions of the brain by the cardiac rhythm–linked pulsations of the arterial wall (*28*). The vascular endfeet of astrocytes, a primary subtype of glial cells, surround the perivascular spaces and can be regarded as open gates for fluid influx into the neuropil. The astrocytic endfeet are connected by gap junctions, and almost 50% of their plasma membrane facing the vessel wall is occupied by square arrays composed of the water channel protein aquaporin-4 (AQP4) (*29*). Deletion of AQP4 channels in mice reduces both the influx of CSF tracers and the efflux of solutes from the neuropil (*24*, *30*, *31*). Given this pathway's functional similarities to the peripheral lymphatic system, we termed this astrocyte-regulated mechanism of brain fluid transport the "glymphatic (glial-lymphatic) system."

Notably, fluid transport through the glymphatic system is directionally polarized, with influx along penetrating arteries, fluid entry into the neuropil supported by AQP4, and efflux along the perivenous spaces, as well as along the cranial and spinal nerves (*24*, *32*–*34*). In addition to its vectorial nature, glymphatic clearance is temporally regulated, and cyclically so, whereby fluid transport is enabled by sleep and suppressed during wakefulness. Brain fluid transport initiates and proceeds during NREM sleep, and CSF tracer influx correlates with the prevalence of EEG slow-wave activity (*35*, *36*). Fluid flow through the glymphatic system is thus inextricably linked with sleep, to the extent that flow appears to stop with the onset of wakefulness. In this regard, slow-wave activity predominates in the early hours of sleep and is a direct measure of sleep pressure, increasing with antecedent sleep deprivation (*8*). As such, waste removal is likely most efficient in the early hours of sleep and especially during recovery sleep after prolonged wakefulness (*37*). Yet it is easy to imagine why the awake state might be incompatible with active parenchymal fluid flow. Wakefulness relies on the precision of synaptic transmission in both time and space. Active flow might be expected to increase glutamate spillover during synaptic activity, resulting in bystander activation of local synapses and hence a loss of both the temporal and spatial fidelity of synaptic transmission. A recent analysis showed that glymphatic flow is also regulated by circadian rhythmicity, such that fluid transport peaks during the sleep phase of diurnal activity and falls during the active phase, independent of the light cycle. This rhythm is supported by the temporally regulated localization of AQP4 via the dystrophin-associated complex, providing a dynamic link to the molecular circadian clock (*38*).

A functionally integrated unit

Upon discovery and characterization of the glymphatic system, it quickly became apparent that glymphatic efflux pathways needed to be more comprehensively defined. Then came the reports that classical lymphatic vessels draining brain interstitial CSF might also be identified in the dura, the fibrous external layer of the meningeal membranes (*39*, *40*). The meningeal lymphatic vessels are separated from CSF by the arachnoid membrane, an internal meningeal layer whose cells constitute a tight fluid barrier by virtue of their dense expression of tight junctions, identified by their expression of claudin-11 (*41*). Yet the glymphatic and meningeal lymphatic systems are clearly connected: CSF tracers can exit the CNS via the meningeal lymphatic vessels, particularly by way of the lymph vessels of the ventral aspect of the brain draining to the cervical lymph nodes (*39*, *40*, *42*). CSF exit from the CNS by way of the meningeal lymph vessels, as well as via both cranial and spinal nerve roots, is rapid; contrast agents can be detected

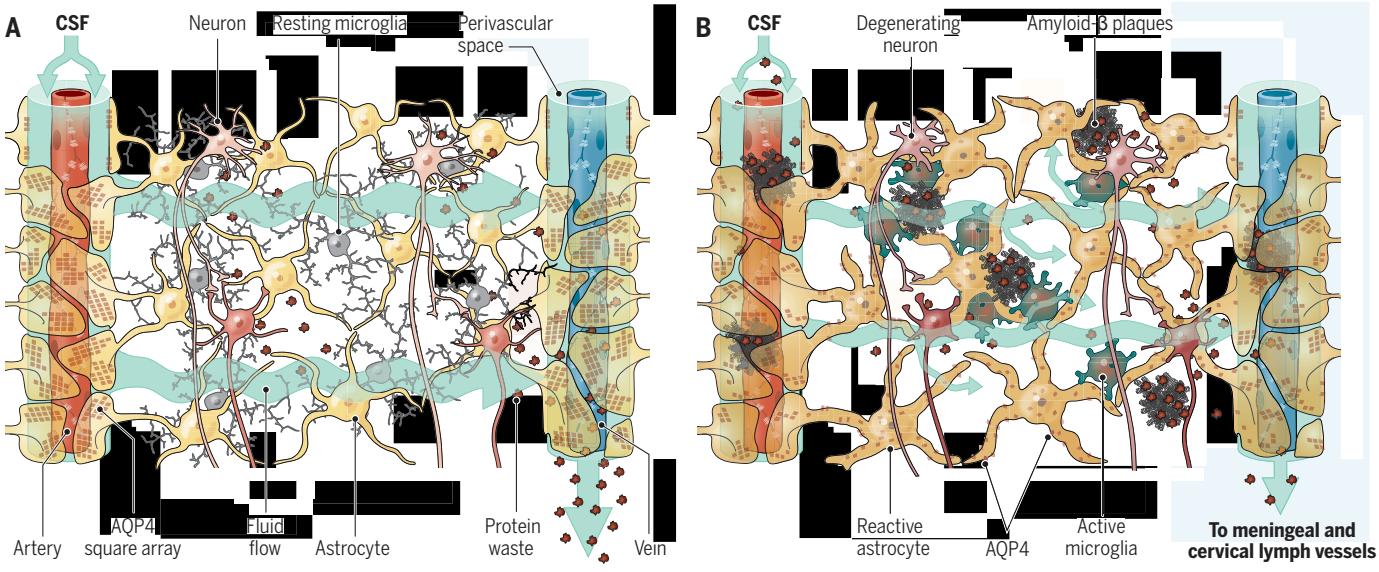

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Fig. 1. The brain glymphatic system is a highly organized fluid transport system. (**A**) Vascular endfeet of astrocytes create the perivascular spaces through which CSF enters the brain and pervades its interstitium. CSF enters these perivascular spaces from the subarachnoid space and is propelled by arterial pulsatility deep into the brain, from where CSF enters the neuropil, facilitated by the dense astrocytic expression of the water channel AQP4, which is arrayed in nanoclusters within the endfeet. CSF mixes with fluid in the extracellular space and leaves the brain via the perivenous spaces, as well as along cranial and spinal nerves. Interstitial solutes, including protein waste, are then carried through the glymphatic system and exported from the CNS via meningeal and cervical lymphatic vessels. (**B**) Amyloid-β plaque formation is associated with an inflammatory response, including reactive micro- and astrogliosis with dispersal of AQP4 nanoclusters. Age-related decline in CSF production, decrease in perivascular AQP4 polarization, gliosis, and plaque formation all impede directional glymphatic flow and thereby impair waste clearance. Notably, vascular amyloidosis might be initiated by several mechanisms. Amyloid-β might be taken up from the CSF by vascular smooth muscle cells expressing the low-density lipoprotein receptor-related protein 1 (LRP1) (*111*). Alternatively, amyloid deposition might be initiated by the backflow of extracellular fluid containing amyloid-β into the periarterial space from the neuropil, rather than proceeding to the perivenous spaces, because of an increase in hydrostatic pressure on the venous side or an inflammation-associated loss of AQP4 localization to astrocytic endfeet.

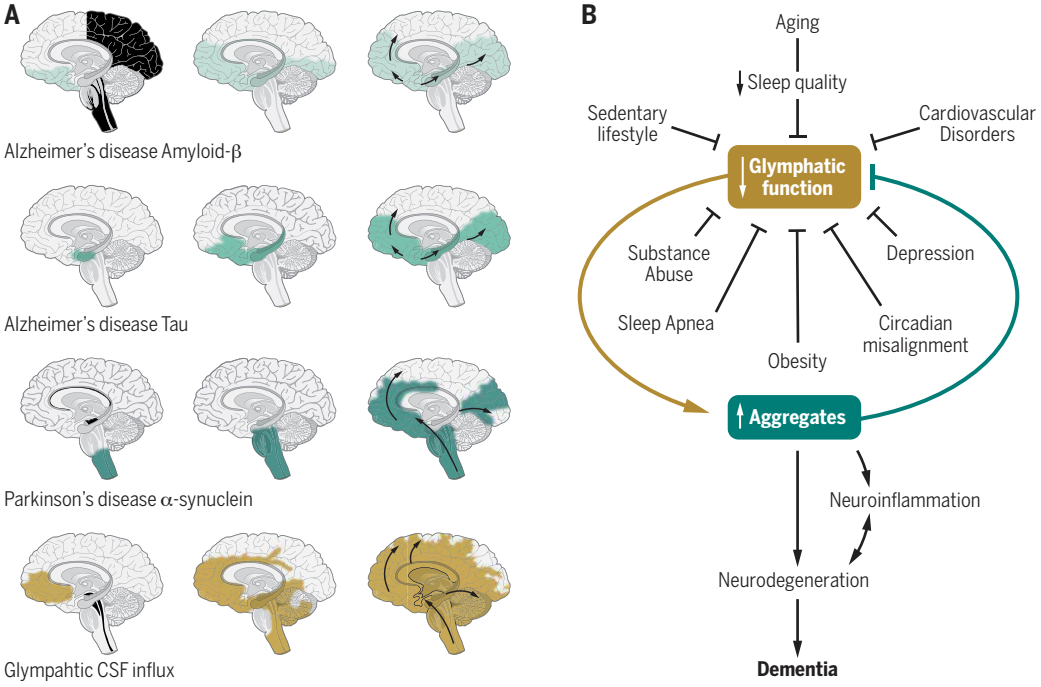

Fig. 2. Prion-like spread of protein aggregates and proposed role of glymphatic transport. (**A**) Seeding and prion-like spread of protein aggregates (amyloid-β and tau) in Alzheimer's disease and of α-synuclein in Parkinson's disease, relative to the distribution of glymphatic influx of a CSF tracer after intrathecal delivery (*67*). Prion-like spread of protein aggregates includes an extracellular component and, hence, the possibility that the seeds are transported by the glymphatic system. (**B**) In this model, the glymphatic system resides at the intersection of a broad scope of disorders, which share an association with diminished brain fluid clearance. Normal aging is also linked to a sharp decline in sleep quality and decreased glymphatic flow. In turn, the stagnation of glymphatic flow, and hence that of extracellular proteins, contributes to protein aggregation, with misfolding and seeding, leading to local inflammation, neuronal loss, and ultimately dementia.

in the deep cervical lymph nodes within minutes after CSF delivery (*42*–*45*). Nonetheless, proteins and tracers can circulate back into the brain along the periarterial spaces, which suggests that our understanding of flow vectors in the CNS is incomplete. More work is needed to comprehensively account for all of the paths by which extracellular fluid and its solutes are cleared from the adult brain (*46*).

Regardless of its precise efflux pathways, CSF ultimately drains into the cervical lymphatic vasculature, by which it returns to the venous system. In a mouse model of Alzheimer's disease (AD), amyloid-β was present in high concentrations in the cervical and axillary lymph nodes, at levels analogous to those in the brain, and yet was either undetectable or barely so in the spleen and other peripheral tissues (*47*). A large proportion of brain waste proteins and metabolites might then be expected to pass through and be cleared by the cervical lymphatics. Lymphatic vessels undergo atrophy in aging (*48*, *49*); thus, lymphatic drainage of CSF may pose a checkpoint—and with aging, a bottleneck—for brain protein clearance. In this regard, overexpression of vascular endothelial growth factor C induced sprouting of the meningeal lymphatic vessels and slowed cognitive decline in a mouse model of AD (*50*). Conversely, both ultraviolet photoablation of meningeal lymphatic vessels and mechanical ligation of cervical lymphatics aggravated amyloid plaque formation in the same mouse models of AD (*50*, *51*). Therefore, the glymphatic and lymphatic systems are intimately connected, both structurally and functionally, such that interference with fluid transport at any segment or node risks upstream fluid stasis and, hence, the aggregation of proteins otherwise destined for clearance.

Why do proteins aggregate?

Aging is also associated with a steep fall in glymphatic flow in the brains of both rodents and humans. CSF inflow of larger tracers is reduced by up to 85% in aged wild-type mice, whereas contrast agent clearance in human brain tissue was inversely correlated to age in all individuals studied (*50*, *52*–*54*). The decrease in glymphatic flow in old mice is partly mediated by mislocalization of AQP4 away from the vascular wall (*52*) and by possible atrophy of meningeal lymphatic vessels (*42*). In addition to age-related decreases in brain fluid transport, glymphatic CSF influx and CSF clearance are each reduced in early stages of amyloid-β deposition in the APP/PS1 model of AD compared with in littermate controls, and CSF clearance continues to decline as the amyloid burden increases (Fig. 1B). Infusion of amyloid-β into CSF acutely reduced glymphatic activity in wild-type mice, suggesting a direct toxic effect (*50*, *55*).

The suppressive effects of both age and amyloid-β overexpression on glymphatic flow can be extended to other experimental rodent models of neurodegeneration: Both traumatic brain injury and Parkinson's disease are similarly linked to a sustained reduction of glymphatic fluid transport (*56*–*58*). Notably, most of these age-related primary neurodegenerative diseases involve disorders of protein processing and aggregation. The hallmark features of these proteinopathies are the fibrillary aggregates of misfolded or hyperphosphorylated proteins (*59*). The protein aggregates can range in size from oligomers to large fibrillary structures. These aggregation-prone proteins include amyloid-β in AD; phosphorylated tau in frontotemporal dementia (FTD), chronic traumatic encephalopathy, and AD; α-synuclein in Parkinson's disease, Lewy body disease, and the multisystem atrophies; mutant huntingtin in Huntington's disease; and TAR DNA-binding protein 43 (TDP-43) in amyotrophic lateral sclerosis and FTD (*60*). Although the specific protein species differ in the different neurodegenerative disorders, in most cases their protein aggregates are formed in part by the interactions of intermolecular β-sheet–rich strands. Once a seed is formed, the aggregates attract monomers of the same protein, as well as other proteins, which may be preferentially bound and entrapped (*60*).

To understand why aging predisposes organisms to these proteinopathies, we need to consider those conditions that favor nucleation, the growth of protein aggregates, and their subsequent seeding to neighboring cells. Protein self-assembly and aggregation depend on a number of factors, among which are structure, concentration, ionic strength, and local pH, as well as their interactions with nucleating interfaces, such as phospholipid membranes (*61*, *62*). Ex vivo aggregation can be induced by simply mixing hydrophobic nanoparticles into an aqueous solution that contains proteins (*63*). A lack of fluid flow (stagnation) or its opposite (shear stress) can also promote aggregation (*64*, *65*), which can occur at a distance from the protein source—for example, along the cerebral vasculature (Fig. 1) (*66*). Depending on the protein, each of these factors, alone

or in combination, can lead to self-aggregation with the formation of stable β-sheet–rich strands. Reduced glymphatic clearance might then be predicted to increase the risk of protein aggregation, given the combination of locally stagnant fluid flow and elevated extracellular concentration of the protein of interest.

Spread of protein aggregates

The recent discovery that specific misfolded and aggregated proteins can propagate and spread in a prion-like fashion has sparked considerable interest (*67*). It has been generally posited that seeding occurs across regions that are synaptically connected (*68*). However, the evidence for synaptic spread is largely based on post hoc analysis of anatomic networks; it remains unclear how synaptic relationships by themselves can mediate seeding. The arguments for synaptic spread are somewhat weakened by the fact that aggregate spread happens in both antero- and retrograde directions across regions that are anatomical neighbors (*68*). An alternative hypothesis is that aggregates simply spread via the extracellular spaces and that the age-dependent reduction in glymphatic flow, with its attendant fluid stagnation, raises the local protein concentration to a level that favors aggregation. In support of this hypothesis, the suppression of glymphatic flow by deletion of AQP4 water channels sharply increased both amyloid-β plaque formation and cognitive deficits in a mouse model of AD (*69*). Similarly, in humans, efflux of CSF containing amyloid-β and phosphorylated tau is reduced in patients with AD compared with age-matched controls. The suppression of CSF clearance in AD is so substantial that it can possibly serve as a biomarker (*70*).

What do we know about the spread of protein aggregates on a macroscopic scale? In AD, amyloid-β deposition typically first occurs in the basal portions of the frontal, temporal, and occipital lobes. Later, the plaques spread to include the hippocampus and posterior parietal cortex, initially sparing both the motor and sensory cortices. These latter regions are first recruited in the final stages of the disease, along with subcortical gray matter regions. Yet the cognitive decline of AD patients correlates more closely with the later-occurring tauopathy and microglial activation than with the earlier amyloid-β plaque formation (*71*, *72*). In the initial stages of AD, phosphorylated tau deposits in the entorhinal cortex, followed by the hippocampus and dorsal thalamus, whereas the neocortex becomes involved later. In Parkinson's disease and Lewy body disease, α-synuclein aggregates initially spread through the brainstem and olfactory bulb, followed by limbic structures, and only then to the neocortex (Fig. 2A). In each of these cases, the aggregates initially deposit at the ventral base of the forebrain and midbrain and then extend rostrally and dorsally to the cortex.

How does this pattern of spread compare to glymphatic CSF inflow (Fig. 2A) (*67*, *73*)? Neuroimaging studies have shown that intrathecally delivered contrast agents are first propelled into the brain along the large cerebral arteries, entering the mediobasal frontal lobe and cingulate cortex along the anterior cerebral artery, the insula via the middle cerebral artery, and the limbic structures (including the hippocampus and entorhinal cortex) via the posterior circulation. The contrast agent remains trapped in the same regions for prolonged periods of time, especially if an underlying pathology is present (*74*, *75*). The accumulation of low–molecular weight CSF contrast agents (<1 kDa) supports the idea that much larger proteins also get trapped in the tortuous extracellular spaces of deep brain regions.

Although the conditions by which pathogenic proteins may become entrapped and aggregate in glymphatic channels remain unclear, the geographic spread of aggregates in AD and Parkinson's disease clearly mirrors the pattern of glymphatic inflow in the human brain, as mapped by magnetic resonance imaging. Indeed, the geographic pattern of macroscopic aggregate formation closely resembles that of entrapped CSF contrast agents during restriction of glymphatic flow in those brains (Fig. 2B). On that basis, we propose that trapping of aggregation-prone proteins in the extracellular space, rather than synaptic connectivity, is responsible for the patterns of protein spread in at least some proteinopathies. As such, the regional variations in the path of seeding across the different types of neurodegenerative diseases may reflect region- and patient-specific variability in the rates of neuronal production of amyloid-β, tau, and α-synuclein.

Notably, although proteins associated with neurodegenerative diseases may normally be either intracellular or extracellular in nature, all are present in the extracellular space. Efforts to sample CSF and extracellular fluid have shown that amyloid-β, tau, and α-synuclein are present outside the cytosol. These proteins all lack N-terminal signal sequences, so unconventional mechanisms must be responsible for their release (*76*). In each of these cases, it is unclear whether oligomers or the larger protein aggregates constitute the principal neurotoxic species (*60*). Although no consensus has been reached, several studies have highlighted the critical role of oligomers as directly toxic and as a nidus for macromolecular aggregation. Immune therapies have attempted to clear the extracellular space and CSF of amyloid-β in AD patients. The failure of such clinical trials may reflect the relatively late initiation of treatment or that the antibody load was not sufficient to clear enough amyloid-β to yield clinical benefit. Alternatively, it is possible that the underlying model of direct, aggregation-associated neurotoxicity is fundamentally incorrect, in AD as well as more broadly (*77*).

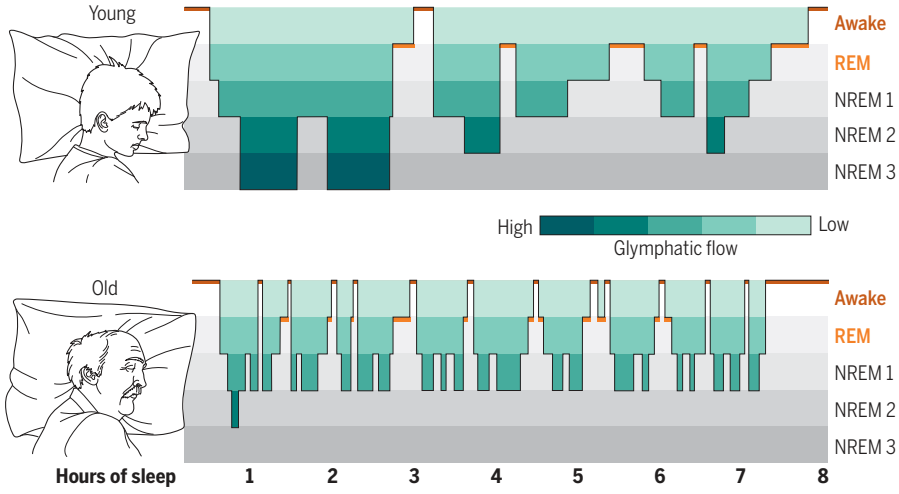

Fig. 3. Sleep architecture in young and old individuals. Hypnograms are constructed from EEG recordings and display the cyclic transitions between sleep stages. The two schematic hypnograms illustrate the sleep architecture of young and old individuals who transition spontaneously between the awake state, REM sleep, and NREM (stages 1 to 3) sleep. Stage 1 NREM sleep is light sleep, whereas stage 3 NREM sleep is the deepest sleep stage and is characterized by slow-wave EEG activity. For young people, deep (stage 3) NREM sleep dominates in the early phases of sleep, whereas REM sleep is more frequent in the later phases. Sleep spindles are most frequent in stage 2 NREM sleep. By contrast, for people older than 60 years of age, sleep is often interrupted by short awake episodes, and older individuals do not typically enter stage 3 NREM sleep. Total sleep time decreases by 10 min for each decade of life (*79*). Green shading indicates the proposed efficacy of glymphatic clearance on the basis of data collected in rodents (*35*, *36*). The lack of stage 3 NREM sleep, the frequent interruptions of stage 1 and 2 NREM sleep, and the shorter total sleep time all serve to decrease glymphatic activity in aging. Critically, a number of disorders and conditions can suppress glymphatic function during NREM sleep, further exacerbating the effects of glymphatic dysfunction in neurodegenerative disease.

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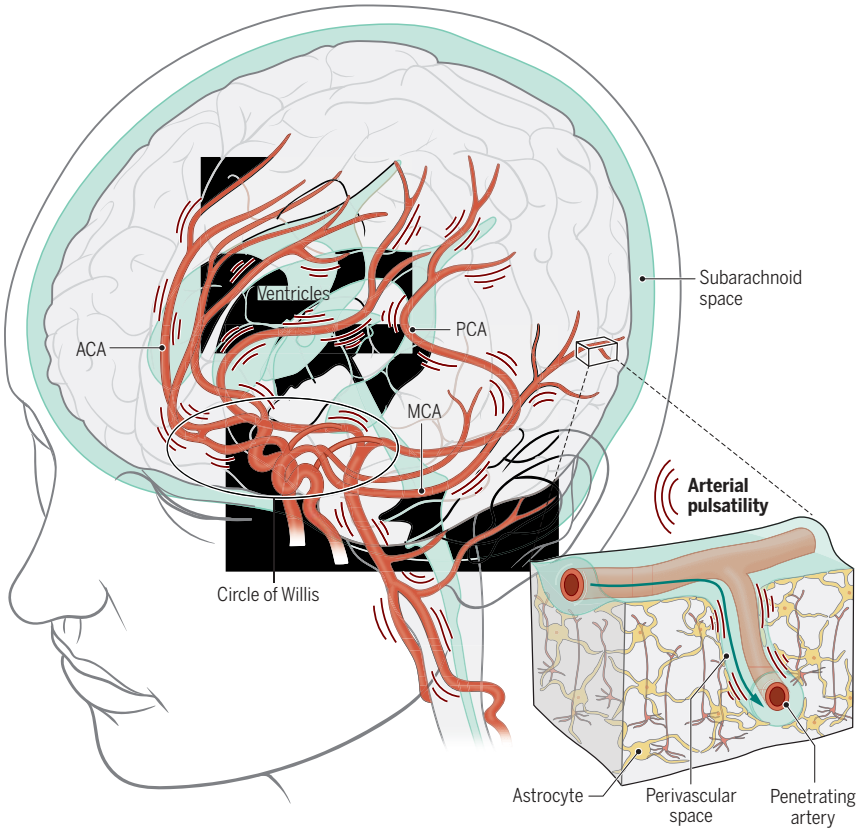

Fig. 4. Arterial pulsatility propels fluid flow in the brain. The brain receives 20 to 25% of a person's cardiac output but constitutes only ~2% of total body weight. The large-caliber arteries of the circle of Willis are positioned in the CSF-containing basal cisterns below the ventral surface of the brain. Arterial pulsatility provides the motive force for CSF transit into the perivascular spaces surrounding the major arteries, whereas respiration and slow vasomotion contribute to sustaining its flow (*112*). The anterior (ACA), middle (MCA), and posterior (PCA) arteries transport CSF to the penetrating arteries (inset), from which CSF is then driven into the neuropil via the still-contiguous perivascular spaces. Cardiovascular diseases associated with reduced cardiac output, such as left heart failure and atrial arrhythmias, reduce arterial wall pulsatility, resulting in diminished CSF flow. In addition, thickening of the arterial wall in SVD, hypertension, and diabetes reduces arterial wall compliance and, hence, pulsatility. Each of these fundamentally cardiovascular disorders serves to attenuate glymphatic flow, providing a potential causal link between these vascular etiologies and AD (*113*).

Sleep, aging, neurodegeneration, and the glymphatic system

The most substantial risk factor for developing protein aggregation, as for developing dementia, is age (*78*). With the glymphatic system in mind, it is notable that sleep quality decreases as a function of normal aging. Insomnia is more frequent with increasing age, and total sleep duration becomes shorter and more interrupted. Perhaps more critically, older individuals rarely enter deep NREM (stage 3) sleep. Most NREM sleep in people older than 60 years of age is light, consisting of the more superficial stages 1 and 2 (*79*) (Fig. 3). Thus, the aged brain spends less time in NREM sleep, potentially causing a catastrophic decline in clearance of brain waste, as the efficacy of glymphatic fluid transport correlates directly with the prevalence of slow-wave activity (*36*). The age-related impairment in sleep quality may thus be causally related to the increased incidence and accelerated course of neurodegenerative disease in older people, whose disrupted sleep architecture may sharply diminish the clearance of brain fluid and its attendant export of protein waste, thus leading to the stagnant interstitial flow that favors aggregate formation.

In addition to the deterioration of sleep architecture in aging, the neurodegenerative diseases—including AD, Parkinson's disease, Huntington's disease, the multisystem atrophies, and the FTDs—are all associated with sleep disturbances (*80*). The best characterized among these are the sleep pathologies associated with Parkinson's disease, in which REM sleep disturbances often precede the onset of motor symptoms by several years or even decades (*80*, *81*). Future work should define whether sleep disturbances that preceded the clinical diagnosis contribute to aggregate seeding and whether sleep disturbances during disease progression accelerate aggregate spread. It would seem axiomatic that a stronger focus on age-related impairment of sleep quality should benefit the aging population.

AQP4 polymorphisms

The polarized expression of AQP4 in the vascular endfeet of astrocytes facilitates glymphatic fluid transport and amyloid-β export in rodents (*24*, *30*) (Fig. 1). In humans, genetic variation in *AQP4* affects both sleep and amyloid-β burden (*82*). A recent study established a link between AQP4, sleep, and the effects of prolonged wakefulness on cognitive function. The study demonstrated that a common single-nucleotide polymorphism (SNP) of *AQP4* was linked to changes in slow-wave activity during NREM sleep that were mirrored by changes in daytime sleepiness as well as in altered reaction times during extended wakefulness (*83*). Yet AQP4 SNPs have also been associated with the rate of cognitive decline in longitudinally followed cohorts of AD patients (*84*). Patients with two specific *AQP4* SNPs exhibited slower cognitive decline after AD diagnosis, whereas cognitive decline progressed more rapidly in individuals with two other *AQP4* SNPs (*85*). Structurally, the integrity of perivascular AQP4 localization was found to degrade with AD, whereas it was preserved in patients older than 85 years of age who remained cognitively intact (*84*). Similarly, the expression of a cluster of transcripts encoding proteins associated with astrocytic endfeet predicted lower amounts of cortical phosphorylated tau in humans (*86*). Indeed, a recent study reported that deletion of *Aqp4* accelerated amyloid plaque formation in a mouse model of AD (*69*). Thus, although AQP4 is expressed only in astrocytes, and not in amyloid-producing neurons, considerable evidence indicates that AQP4 modulates sleep architecture, tolerance to sleep deprivation, amyloid-β accumulation, and the progression of AD. Targeting the brain's waste removal system may thus be an attractive approach for alleviating the waste burden of the proteinopathies because aggregation-prone proteins are removed by bulk flow, without the requirement for specific transporters.

Links to cardiovascular disease

Neurodegenerative diseases are not the only cause of dementia. It has been known for decades that poor cardiovascular health negatively affects cognitive abilities (*87*, *88*), whereas cardiovascular fitness positively correlates with cognition in young adults (*89*) and preserves cognitive performance in aging individuals (*90*). Why is a healthy heart so important for higher brain function? It has been shown that glymphatic function is suppressed in hypertensive rats (*91*, *92*). It is also well established that sleep quality is compromised in cardiovascular diseases (*93*), perhaps providing a link to impaired glymphatic clearance and subsequent protein aggregation and dementia (*94*).

We also propose that a healthy cardiovascular system, besides its role in delivering energy metabolites to the brain, plays a hitherto

unappreciated role in the clearance of neurotoxic waste from the brain. In particular, we have found that the brain's fluid transport system is designed to take advantage of cardiac pulsatility to drive CSF transport in the neuropil (*28*). The ejection pressure of blood from the left ventricle is partly absorbed by the elastic arterial wall of the aorta. As the ejected blood transits the arteries, it enlarges the arterial diameter as its pulse wave propagates downstream (*28*). About 20 to 25% of the total ejected blood volume enters the CNS via the paired internal carotid and posterior cerebral arteries. Pulsatility in these large-caliber arteries constantly transmits pressure waves along the axis of the major vessels, as well as through the soft brain tissue (Fig. 4). The motion of the brain is locally supplemented by the pulsatility of the penetrating arteries, as they enter the brain from the CSF-filled subarachnoid space, thereby driving CSF into the neuropil along the periarterial spaces (*24*). It should not be surprising that heart diseases associated with reduced cardiac output, including congestive heart failure and atrial dysrhythmias (*95*), are also associated with diminished glymphatic flow, because the pulsatility of the cerebral arteries and hence the driving forces within the glymphatic system are reduced. Indeed, the cognitive decline frequently noted in patients with a low cardiac ejection fraction, often attributed to low cerebral perfusion, may also reflect poor glymphatic flow and incomplete waste clearance, as well as a consequent predisposition to aggregate formation and still-slower glymphatic flow (*95*).

Small vessel disease (SVD) is a vascular disorder that targets the small cerebral vessels, in which penetrating arterioles undergo progressive thickening of their walls (*96*). Deterioration of the vascular bed may occur alone or in combination with other pathologies (*97*), leading to progressive demyelination and loss of white matter (*98*). SVD is common in patients with hypertension, many of whom are concurrently diabetic or smokers, and it progresses silently for years before dementia is clinically evident (*99*). Hypertension induces hypertrophy of vascular smooth muscle cells, with a stiffening of the arterial wall that dampens arterial wall pulsatility and compliance, thus reducing convective perivascular flow (*94*, *100*). The stiffening of the perivascular glycocalyx of diabetic patients has a similar effect (*101*), and the two disorders are in frequent combination as the incidence of obesity, a predisposing factor and comorbidity to both, increases worldwide. SVD is linked to glymphatic dysfunction in experimental models (*91*) and may potentiate the progression of neurodegenerative dementias in the same patients at risk for SVD-associated vascular dementia. It is not surprising, then, that the clinical distinctions between AD and the vascular dementias are often blurred by their frequent co-association (*102*).

Outlook

Fundamentally, the studies discussed here highlight the benefits of a good night's sleep. Sleep is an evolutionarily conserved mechanism that serves multiple purposes, with benefits to the homeostatic support of the cardiovascular system, immune system, and memory (*103*–*105*). Yet the most fundamental incentive for the brain to sleep lies in its own self-preservation: Only the sleeping brain is capable of efficiently clearing the waste products generated during active wakefulness. Amyloid-β, tau, and α-synuclein are all present in the brain extracellular fluid and CSF at higher concentrations during wakefulness than during sleep, and sleep deprivation further increases these levels (*106*–*108*). Indeed, positron emission tomography imaging has shown that a single night of sleep deprivation resulted in a significant increase in amyloid-β burden in the hippocampus and thalamus (*109*). Humans need sleep to clear proteins from the brain extracellular space, or these proteins will aggregate, impede fluid flow, and potentiate further fibril polymerization. Together with local inflammation, this process may be expected to progressively suppress glymphatic flow in the most affected regions.

Overall, these observations suggest a causal linkage between the sleep-wake cycle and its regulation of fluid flow via the glymphatic system, and thereby the modulation of the balance between protein clearance and aggregation. As such, the observations suggest a basis for the increased incidence of protein aggregation–related disorders that occur with aging, the appearance of which tracks age-related declines in both vascular health and glymphatic patency. The neurodegenerative dementias may thus be viewed as the products of a final common pathway that integrates the dysfunction of any and all of these closely interdependent upstream mechanisms (Fig. 3). These various processes are linked in their regulation by the brain's glymphatic system, the directed regulation of which may, in turn, present new therapeutic opportunities for the disease-modifying treatment of patients with these disorders (*75*). In particular, the development of small-molecule agonists of glymphatic efflux might present opportunities to slow disease progression in the aggregation disorders, just as the optimization of cardiovascular health might be expected to delay disease onset. These systems are intimately connected such that modulation of glymphatic flow, and hence protein clearance from the brain, will ultimately require a deeper understanding of the dependence of both glymphatic and lymphatic flow on intracardiac pressures.

Recent advances in neuroimaging have provided multiple approaches to map the human glymphatic system and to assess its functional competence in the context of disease, as well as the effects thereof on sleep-dependent glymphatic cycling (*72*, *73*, *108*, *110*). The diagnostic neuroimaging of glymphatic function via such "glymphograms" may provide both a means to predict the risk of developing proteinopathies and an approach by which to evaluate the efficacy of glymphatic flow–directed treatments as they are developed. Until then, the most assured means of preserving effective glymphatic clearance is to get a good night's sleep.

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