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Effects of Stress on the Brain's Glymphatic System

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Abstract

Psychoneuroendocrine immunology is an integrated field that explores the pathways through which psychological experiences influence immune function and how the immune system, in turn, might alter brain function. The glial-lymphatic system regulates fluid balance and waste removal in the central nervous system, which lacks an independent lymphatic system (glymphatic system). We looked for literature on stress, its consequences, and the glymphatic system in PubMed/MEDLINE, Google Scholar, Scopus, and other online databases. Work, examinations, physical, and mental stress brought on by sickness, trauma, and other everyday occurrences are just a few examples of situations that might be categorized as "stressful." The "fight-flight-freeze response" is the name given to the body's combination of adaptations in reaction to stress. Sleep problems are associated with higher levels of stress. It is plausible to assume that this is the mechanism through which stress affects glymphatic function given that the brain's waste disposal system has been shown to be active during sleep. Studying whether stress reduction can enhance brain waste removal and the prognosis of illnesses marked by metabolite accumulation is promising.

Introduction

The central nervous system (CNS) has evolved a special adaptive mechanism to maintain fluid balance and eliminate interstitial waste, making it the only system in the body without its own anatomically identifiable lymphatic vessels [1].

In 2013, Jessen *et al*. [2] discovered the glymphatic system, a waste removal system consisting of astroglial cells that form perivascular tunnels and promote the removal of soluble proteins, metabolic waste products, and the distribution of glucose, lipids, neuromodulators, amino acids, and growth factors in the brain [2]. Since the discovery of this pathway, much research has been done to further analyze its physiological function and investigate its role in various diseases [3]. This article focuses on the role of stress on the brain. In a biological context, stress is a mental, emotional, or physical factor that can cause physical or mental discomfort. The brain is the major organ involved in the physiological and behavioral responses to stressors and determines whether their effects promote or harm overall health. Changes in the brain, when exposed to stressors, lead to changes in the body's metabolic, cardiovascular, and immune systems and determine the short- and long-term effects of stress [4]. The intimate mechanisms of changes in the glymphatic system under chronic stress are of interest because the brain's waste disposal pathway plays an important role in clearing substances that accumulate and lead to neurodegenerative diseases, such as proteins, including amyloid plaques and tau tangles [5], [6], [7]. The aim of this article is to better understand the link between the glymphatic system and stress.

Methods

We looked through many internet databases, such as Scopus, Google Scholar, and PubMed/MEDLINE. The key search phrases were "glymphatic system" or "glymphatic system" and "stress" and "sleep," and "brain" with filtering enabled, namely publication date from January 1, 1990, to December 31, 2021, and publications published in English.

The glymphatic pathway-the waste cleansing system in the CNS

The cerebrospinal fluid (CSF) and the interstitial fluid in the brain are in constant communication. This connection is made possible by the convective inflow of CSF through the periarterial space [8].

CSF is carried through aquaporin-4 (AQP4) water channels found in the astrocytic end-feet encircling cerebral arteries from the subarachnoid space until it reaches the dense brain parenchyma [8]. The interstitial fluid enters the perivenous areas around the big deep veins as the CSF travels through the parenchyma and exits the brain until it reaches the cervical lymphatics [9].

The glymphatic system regulates the movement of CSF and interstitial fluid within the brain [2]. Originally, in 2012, Iliff *et al*. used two-photon imaging of small fluorescent tracers to detect this exchange and to characterize the dynamics of the glymphatic system [8]. Further studies with fluorescently labeled amyloid beta (Aβ), a pathogenic peptide in Alzheimer's disease, transported through this pathway suggested that the glymphatic pathway is a waste disposal system in the CNS [8]. Later, these properties were confirmed in several articles [10], [11]. Besides Aβ, lactate [12] and tau proteins [13] have also been proposed as metabolites and solutes excreted by this system.

The glymphatic system not simply functions as an interstitial waste disposal system, but is also involved in the transport of nutrients [14], apolipoprotein E, signaling molecules, and tiny lipid molecules [15], [16]. In addition, research has shown that the glymphatic system contributes to the movement of neuromodulators, growth factors, carrier proteins, and other nutrients in the brain [17].

Given the numerous ways in which the glymphatic system affects the deposition and distribution of solutes and metabolites in the CNS, it is reasonable to assume that the proper functioning of this pathway is critical to normal brain function. A particular interest of this review article is the effects of stress on the glymphatic system. Several studies have addressed the effects of traumatic brain injury (TBJ) [18], aging [19], and other diseases on the glimphatic system.

The mechanism behind this link between chronic stress and glymphatic system performance is not entirely known, yet. Wei *et al*. 's study indicated that prolonged stress may impair AQP4-mediated glymphatic transfer through glucocorticoid signaling within the brain [20].

The influence of glucocorticoids on brain physiology

Glucocorticoids, particularly the so-called "stress hormone" cortisol, are secreted as a result of hypothalamic-pituitary-adrenocortical axis activation in response to stressors [21]. When the body reacts to an acute stressor that lasts for a brief amount of time, the release of cortisol is efficient in reestablishing homeostasis, but chronic stress can be damaging to the CNS's ability to operate normally [22]. Numerous publications have examined the effect of glucocorticoids on brain physiology. McEwen *et al*. found that glucocorticoids modify neuronal architecture by causing dendritic retraction or expansion and reduced or heightened synapse density [23].

GCs have been shown to impede glucose transport in glial cells, neurons, and endothelial cells *in vitro* [24], [25] as well as local cerebral glucose utilization in the brain [26].

Prolonged stress has also been reported to lead to hippocampal atrophy, causing the loss of neurons [27], disrupting memory [28], and suppressing appetite [21]. As mentioned above, the effects of chronic stress and stress hormones on the glymphatic system have not been extensively studied, but research by Wei *et al*. suggests that AQP4-mediated glymphatic transport might be influenced by glucocorticoid signaling [20].

Further conclusions about the relationship between stress and impaired glymphatic function can be drawn from evidence of a relationship between stress and alcohol dependence [29]. In addition, there is evidence that chronic intake of 1.5 g/kg ethanol induces reactive gliosis and disrupts glymphatic function [30]. The brain's waste disposal system has been demonstrated to be active during sleep [31]. A rise in stress has been linked to an increase in sleep problems [32], [33], thus it is logical to believe that this is another mechanism by which stress affects the sleep glymphatic system. However, there is still a need for more extensive research and in-depth study of this association.

The functioning of the glymphatic system under the influence of stress

In the modern environment in which we live, one is exposed to multiple situations that disturb the balance of the organism as a whole and its environment.

Many situations in a modern person's daily life might be classified as "stressful," including the workplace, exams, and physical and mental stress brought on by illnesses, traumas, and other medical disorders. The body's reaction to stress is a conglomeration of modifications referred to as the "fight-flight-freeze response" in general [34].

Essentially, this response is a sequence of hormonal and physiological changes made to help the body fight a threat or stay safe [35]. Years of research have also reported the long-term effects of chronic stress on overall health [36]. Chronic stress

has been proven to contribute to increases in blood pressure [37], atherosclerosis [38], obesity-directly or indirectly through less sleep and exercise [39] and brain modifications associated with depression [40], addiction [41], and anxiety [42].

The consequences of prolonged stress on the glymphatic system have not been fully investigated, despite the fact that the effects of stress on the various physiological systems have been the subject of substantial research. The results by Wei *et al*. demonstrate, as has already been indicated, that chronic stress inhibits AQP4-mediated global glymphatic transport inside the CNS, which is characterized by reduced expression and polarization of AQP4, lowered transcription of AQP4, laminin, agrin, and dystroglycan [20]. The sparse literature on the subject suggests a different approach to further investigate the relationship between stress and the functionality of the glymphatic system.

The role of sleep in the glymphatic system (Figure 1)

A very unique feature of the intimate working mechanism of the glymphatic system was identified by Xie *et al*. in 2013 [31]. Their results suggest that sleep is associated with dramatically increased glymphatic clearance, which is suppressed during wakefulness [31]. Using *in vivo* 2-photon imaging of the brain waste disposal system, CSF influx was found to be reduced by 90% compared to awake anesthetized mice [31].

The results were similar when the same experiment was performed with mice in a natural sleeping state [31]. The observations of Xie *et al*. suggest that the sleep state favors convective fluid flow and thus the removal of metabolites in the brain parenchyma. This reveals an important aspect of how the glymphatic system works – it turns on during sleep and eliminates waste products generated in the brain during wakefulness [2]. Considering that the neuromodulator of the arousal state is norepinephrine (NE) [43], Jessen *et al*. suggest that it is also an important regulator of glymphatic activity and may be responsible for the system's suppression during wakefulness [2]. Other studies have also found that glymphatic clearance depends on the activity of noradrenergic receptors, which are considered important regulators of sleep and wakefulness [44]. In another study, using MRI imaging, it was found that the position of the head during sleep also improved the clearance of interstitial solutes [45].

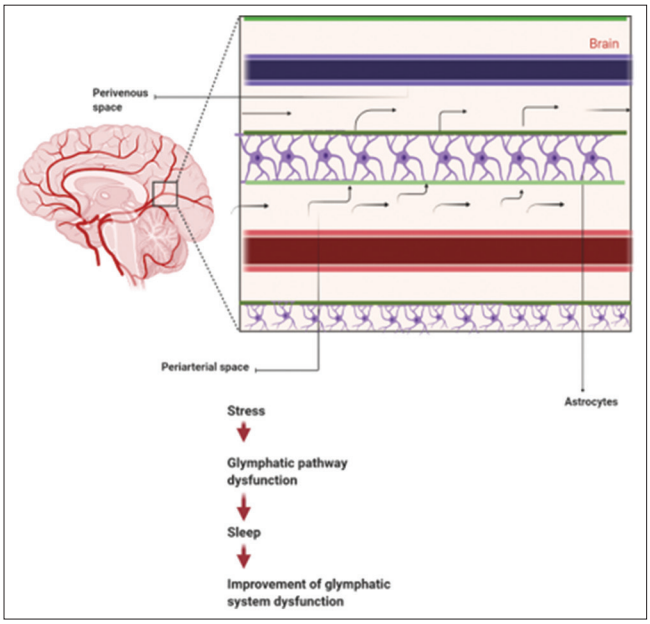

Figure 1: The effects of sleep on the glymphatic system. The figure illustrates the relationship between sleep and improving the brain's glymphatic function

Several studies have examined the close connection between stress and sleep disorders. Stress has been linked to shortened sleep, fragmentation, and a possible reduction in sleep Stages 3 and 4 [46], and disrupted sleep has been shown to increase levels of stress markers such as cortisol, leading to a worsening of the effects of cortisol can stress [46]. With this in mind, in any situation or condition characterized by sleep disturbance, one can expect inadequate glymphatic function due to chronic stress.

The glymphatic system and somatic diseases

Glymphatic drainage of metabolites and solutes can be considered as important in the onset and progression of various diseases. Numerous studies have shown that neurodegenerative disorders such as Alzheimer's disease (AD) and Parkinson's disease (PD) are defined by the buildup of misfolded proteins within or outside of cells, as well as the gradual death of only some types of neurons [47], [48].

There is evidence that the glymphatic system is suppressed in several other diseases-for example, TBI and ischemic and hemorrhagic stroke-and that suppression of glymphatic activity could aid in the pathophysiological mechanisms of these diseases [2].

The underlying etiology of AD is the extracellular accumulation of Aβ [49]. Reduced glymphatic inflow and elimination of Aβ was found in a mouse model of AD [50], and pretreatment of mice with Aβ was shown to result in significant reduction CSF influx, suggesting that AD increases results in impaired glymphatic clearing and deposit of Aβ, further degrading glymphatic function [50].

In addition, Zuroff *et al*. argued that Aβ accumulation in late-onset AD is due to insufficient clearance rather than overproduction [51]. The age-related decrease in glymphatic influx and interstitial clearance of solutes such as Aβ has been attributed

to reduced penetration of arterial pulsations into the aging brain [17], so Aβ clearance has been shown to deteriorate with age [50], so that age-related risk factors would possibly worsen Aβ metabolism [52]. The increase in CSF NE in the elderly [53] would also hypothetically result in decreased glymphatic activity and impaired clearance of Aβ and tau proteins [54], which would accelerate the progression of AD [10]. Musiek *et al*. [55] propose that disruptions in sleep also precede the start of symptoms in AD and may both be fundamental in the pathophysiology and be a consequence of the disease, correlating with the notion that the brain's clearance disposal mechanism is more active while sleeping than during wakefulness [31], [56], [57]. The finding of a significant reduction in glymphatic transport before deposition in the APP/PS1 mouse model of AD [50] suggests that the onset of AD may even be delayed by early restoration of glymphatic flow.

TBI, a recognized risk factor for dementia, including AD, in its early stages, is often characterized by neurofibrillary tangles of aggregated tau protein [13]. Studies by Iliff *et al*. [13] show that glymphatic function is reduced by up to 60% after TBJ and persists mote than a month after damage. When the AQP4 water channel gene is knocked out, the decline in glymphatic function following traumatic brain damage is accelerated and tau protein aggregates are encouraged to form, which causes neurotoxicity in the post-traumatic brain [13]. Damage to the glymphatic system after TBJ may be related to relocalization of AQP4 channels away from astrocytic end-feet [18]. There is work suggesting that changes in glymphatic function after TBJ as well as post-traumatic sleep disorders and consequent damage to neuropeptide clearance may be involved in the pathogenesis of headache after traumatic injury [58]. Chronic dysfunction of the glymphatic system has been suggested as a possible link between repetitive TBI and neurodegeneration [18].

In addition, ischemic stroke and subarachnoid hemorrhage (SAH) have both been researched in relation to lymphatic function. After SAH and during the acute phase of ischemic stroke, there is evidence of severe glymphatic dysfunction impairment, which decreases the clearing of waste products and metabolites [59]. Chronic reduced cerebral blood flow after a stroke has been shown to contribute to the development of dementia after a stroke due to impaired amyloid clearance via the glymphatic system [60]. A crucial factor in the development of PD is the excessive buildup of dangerous substances of synuclein as a result of an imbalance between synthesis and clearance in the brain [61]. The paper by Zou *et al*. [62] showed that glymphatic clearance of synuclein was impaired within the brain of A53T mice, suggesting that glymphatic system dysfunction may be a crucial element in the onset and progression of PD [62]. In addition to this mechanism, there is also evidence of the adverse effects of damaged dopaminergic neurons related to alterations in rapid eye movement sleep, circadian rhythm, and clock gene dysfunction [63].

In Type 2 diabetes mellitus, extracellular solute buildup and subsequent cognitive deterioration result from an imbalance between increases in glymphatic inflow without an equivalent rise in interstitial fluid outflow [64]. Research has been conducted over the years on the effects of stress, from its effects on the cardiovascular system [65] to its effects on metabolism [65], the immune system [66], [67], and the CNS [68]. There is ample evidence linking the glymphatic system to numerous diseases that severely impact the lives of many people around the world. Proper functioning of this waste disposal mechanism in the brain can be important in diagnosing, treating, and preventing these diseases. Therefore, it is important to examine, research, and evaluate the factors that disrupt this system. Although the direct effects of stress on the glymphatic system have not been extensively studied, there is evidence that studying this relationship may be beneficial for the treatment of numerous diseases and conditions affecting the CNS.

Along with the illnesses already mentioned, the glymphatic pathway is connected to autoimmune and infectious conditions. The important innate immune cells that belong in the CNS, microglia, and Type I astrocytes are thought to be part of the glymphatic pathway [69], emphasizing the close relationship between the glymphatic pathway and brain inflammation [69].

SARS-CoV-2 infection is being investigated worldwide to learn more about the effects of infections on the brain's glymphatic system. Microglia and Type I astrocytes infected with murine coronavirus (MHV-A 59), whose pathogenesis is similar to that of SARS-CoV-2, released pro-inflammatory cytokines such as IL-1, IL-2, IL-6 tumor necrosis factor (TNF), and interferons, according to a 2020 study. This study provides important information about the glymphatic pathway and COVID-19 crosstalk [69].

Multiple sclerosis (MS), which is characterized by the invasion of auto-reactive T cells, is one of the most common autoimmune diseases of the CNS [70], [71]. The most common animal model for studying the molecular immune in the CNS is experimental autoimmune encephalomyelitis (EAE). Louveau *et al*. demonstrated that meningeal lymphatic ablation could prevent T cell-APC interaction and activation in dCLNs, resulting in reduced invasion of activated T cells into the brain and amelioration of EAE [70]. They concluded that meningeal lymphatics played an important role in immune surveillance and the regulation of the CNS's inflammatory response [70]. Meningeal lymphatics may thus be a therapeutic target for CNS autoimmune disease. Meningeal lymphatics are crucial in the outcome of an ischemic stroke [72], [73]. Meningeal lymphatics, in addition to the impaired BBB, represent another pathway for peripheral immune cells, such as T and B cells, to invade brain

parenchyma and exacerbate neuroinflammation during ischemic stroke [74], [75]. Mast cells in the meninges may amplify the inflammatory response and worsen stroke patients' prognosis [75]. Besides that, following vascular injury, ingrown meningeal lymphangiogenesis into the injured brain parenchyma can increase interstitial fluid clearance, resolving cerebral edema and promoting vascular renewal [73]. As a result, meningeal lymphangiogenesis appears to be linked to the outcome of ischemic stroke [72].

Through sleep disorders and endocrine abnormalities, the normal physiological process of aging may also cause glymphatic system dysfunction. The change in normal sleep pattern seems to be related to aging [76], and this modification may suppress the clearance role of the glymphatic system by reducing NREM sleep and increasing AQP4 mislocalization [17], [77].

The normal aging process also causes chronic changes in the phenotype of microglia that are similar to the pro-inflammatory response [78], and activated microglia are part of the process that induces the formation of A1-like reactive astrocytes [79], along with cellular senescence characteristics of astrocytes, such as increased expression of cytokines such as TNF, IL-1b, and IL-6 [80], [81], [82], and increased accumulation of proteotoxic aggregates [79]. Lipids may also be secreted autocrinely into PVSs by astrocytes, potentially signaling to other astrocytes and affecting ion fluxes and downstream phosphorylation mechanisms, which, in turn, contribute to modifying the release or uptake of many other signaling pathway agents [83]. As a result, abnormal astrocytes may have a macroscopical effect on neural networks.

Lifestyle and glial-lymphatic system

Diet is the primary cause of obesity, metabolic syndrome, and Type 2 diabetes mellitus, and so these metabolic abnormalities increase the risk of developing AD [84]. Obesity and type 2 diabetes are associated with an increased risk of hypertension as well as other cardiovascular diseases [85], [86]. Hypertension raises the probability of dementia in humans [87], [88], [89]. Although the cause of high blood pressure differs from patient to patient, large amounts sodium intake is widely acknowledged [89].

Both Type 2 diabetes [64] and high blood pressure [90] have been linked to reduced glymphatic function, suggesting one possible explanation for the connection between type 2 diabetes, high blood pressure, and neurodegeneration. One study found that spontaneously hypertensive rats used to have a higher CSF volume but a lower CSF influx [90]. Tracer within the subarachnoid region surrounding the basal brain regions was generally similar in both high blood pressure and normotensive groups, suggesting that disruption of CSF-ISF interactions is most probably due to augmented perivascular flow and clearance [90].

Based on an MRI study of patients with cerebral microvascular disease, decreased cerebrovascular compliance and ability to adapt lead to increased sheer forces generated by the p wave, whereas total flow of blood may actually be reduced as a consequence of the decreased compliance [91]. In patients with small vessel disease following intracerebral hemorrhage, pulsatility (the difference in blood flow between the systolic and diastolic phases) was also found to be increased [92]. CSF pulsatility in the foramen magnum decreased due to an increase in vascular pulsatility [91].

Unexpectedly, many of these patients were also hypertensive, which could explain the lower vascular compliance [91]. Although the precise mechanism is unknown, it appears that variances from normal cerebrovascular function caused by reduced compliance and subsequent high vascular pulsatility impact CSF. Conditions that impair vascular compliance, such as type 2 diabetes or hypertension, are likely to increase disturbances in CSF rhythm, which could lead to dysfunctional CSF-mediated brain clearance and, as an outcome, describe some of the increased dementia risk associated with these conditions.

Essential fatty acids that support vascular health are polyunsaturated fatty acids (PUFAs), especially omega 3 fatty acids (n-3 PUFAs). The key for this function is their antihypertensive, anti-inflammatory properties, regulation of platelet aggregation, and blood lipid metabolism [93]. A type of n-3 PUFA called Docosahexaenoic acid (DHA) has really been linked to a lower risk of Alzheimer's disease and Parkinson's disease, and surprisingly, postmortem studies show DHA deficiency in Alzheimer's patients [94]. Previously, PUFAs have been suggested to improve glymphatic function, with rodents fed a strong PUFA diet demonstrate increased CSF tracer influx and clearance, suggesting PUFAs' contribution to maintaining normal glymphatic function [95], [96].

Moreover, transgenic mouse models with increased amounts of n-3 PUFAs in the brain showed neuronal protection against Aβ-induced neuronal death [95]. In AQP4 knockout mice with the glymphatic system inhibited, this protective effect was not observed [95].

Alcohol intake may influence glymphatic function by interfering with sleep patterns [97]. Excessive alcohol consumption thus has a negative impact on glial-lymphatic function, which is facilitated by both acute and long-term effects on various players in the glymphatic pathway, such as AQP4 polarization and sleep disturbance. Limited consumption of alcohol, to the contrary side, may be beneficial to lymphatic function [97].

Conclusion

This is a topic worth investigating, as the effects of stress on glymphatic function are under-researched. New insights into the mechanisms of development and progression of neurodegenerative diseases would fill the gaps in our understanding of these diseases and open numerous possibilities for their prevention, early detection, and treatment. Chronic stress, which has been identified as a cause of many diseases such as diabetes, obesity, depression, and cardiovascular disease, also undoubtedly plays a role in several CNS diseases and can improve waste clearance in the brain, which in turn would improve disease prognosis, where accumulation of metabolites is an important factor.

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References

1. Trevaskis NL, Kaminskas LM, Porter CJ. From sewer to saviour - targeting the lymphatic system to promote drug exposure and activity. Nat Rev Drug Discov. 2015;14(11):781-803. https://doi.org/10.1038/nrd4608
PMid:26471369
2. Jessen NA, Munk AS, Lundgaard I, Nedergaard M. The glymphatic system: A Beginner's guide. Neurochem Res. 2015;40(12):2583-99. https://doi.org/10.1007/s11064-015-1581-6
PMid:25947369
3. Bacyinski A, Xu M, Wang W, Hu J. The paravascular pathway for brain waste clearance: Current understanding, significance and controversy. Front Neuroanat. 2017;11:101. https://doi.org/10.3389/fnana.2017.00101
PMid:29163074
4. McEwen BS. Protective and damaging effects of stress mediators: Central role of the brain. Dialogues Clin Neurosci. 2006;8(4):367-81. https://doi.org/10.31887/DCNS.2006.8.4/bmcewen
PMid:17290796
5. Weller RO, Subash M, Preston SD, Mazanti I, Carare RO. Perivascular drainage of amyloid-beta peptides from the brain and its failure in cerebral amyloid angiopathy and Alzheimer's disease. Brain Pathol. 2008;18(2):253-66. https://doi.org/10.1111/j.1750-3639.2008.00133.x
PMid:18363936
6. Carare RO, Bernardes-Silva M, Newman TA, Page AM, Nicoll JA, Perry VH, *et al.* Solutes, but not cells, drain from the brain parenchyma along basement membranes of capillaries and arteries: Significance for cerebral amyloid angiopathy and neuroimmunology. Neuropathol Appl Neurobiol. 2008;34(2):131-44. https://doi.org/10.1111/j.1365-2990.2007.00926.x
PMid:18208483
7. Hawkes CA, Härtig W, Kacza J, Schliebs R, Weller RO, Nicoll JA, *et al.* Perivascular drainage of solutes is impaired in the aging mouse brain and in the presence of cerebral amyloid angiopathy. Acta Neuropathol. 2011;121(4):431-43. https://doi.org/10.1007/s00401-011-0801-7
PMid:21259015
8. Iliff JJ, Wang M, Liao Y, Plogg BA, Peng W, Gundersen GA, *et al.* A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid β. Sci Transl Med. 2012;4(147):147ra111. https://doi.org/10.1126/scitranslmed.3003748
PMid:22896675
9. Murtha LA, Yang Q, Parsons MW, Levi CR, Beard DJ, Spratt NJ, *et al.* Cerebrospinal fluid is drained primarily via the spinal canal and olfactory route in young and aged spontaneously hypertensive rats. Fluids Barriers CNS. 2014;11:12. https://doi.org/10.1186/2045-8118-11-12
PMid:24932405
10. Tarasoff-Conway JM, Carare RO, Osorio RS, Glodzik L, Butler T, Fieremans E, *et al.* Clearance systems in the brain-implications for Alzheimer disease. Nat Rev Neurol. 2015;11(8):457-70. https://doi.org/10.1038/nrneurol.2015.119
PMid:26195256
11. Simon MJ, Iliff JJ. Regulation of cerebrospinal fluid (CSF) flow in neurodegenerative, neurovascular and neuroinflammatory disease. Biochim Biophys Acta. 2016;1862(3):442-51. https://doi.org/10.1016/j.bbadis.2015.10.014
PMid:26499397
12. Lundgaard I, Lu ML, Yang E, Peng W, Mestre H, Hitomi E, *et al.* Glymphatic clearance controls state-dependent changes in brain lactate concentration. J Cereb Blood Flow Metab. 2017;37(6):2112-24. https://doi.org/10.1177/0271678X16661202
PMid:27481936

13. Iliff JJ, Chen MJ, Plog BA, Zeppenfeld DM, Soltero M, Yang L, *et al.* Impairment of glymphatic pathway function promotes tau pathology after traumatic brain injury. J Neurosci. 2014;34(49):16180-93. https://doi.org/10.1523/JNEUROSCI.3020-14.2014
PMid:25471560
14. Matsumae M, Atsumi H, Hirayama A, Hayashi N, Sano F, Taiozawa K, *et al.* A new look at cerebrospinal fluid motion. No Shinkei Geka. 2016;44:909-24.
15. Achariyar TM, Li B, Peng W, Verghese PB, Shi Y, McConnell E, *et al.* Glymphatic distribution of CSF-derived apoE into brain is isoform specific and suppressed during sleep deprivation. Mol Neurodegener. 2016;11(1):74.
16. Rangroo Thrane V, Thrane AS, Plog BA, Thiyagarajan M, Iliff JJ, Deane R, *et al.* Paravascular microcirculation facilitates rapid lipid transport and astrocyte signaling in the brain. Sci Rep. 2013;3:2582. https://doi.org/10.1038/srep02582
PMid:24002448
17. Kress BT, Iliff JJ, Xia M, Wang M, Wei HS, Zeppenfeld D, *et al.* Impairment of paravascular clearance pathways in the aging brain. Ann Neurol. 2014;76(6):845-61. https://doi.org/10.1002/ana.24271
PMid:25204284
18. Sullan MJ, Asken BM, Jaffee MS, DeKosky ST, Bauer RM. Glymphatic system disruption as a mediator of brain trauma and chronic traumatic encephalopathy. Neurosci Biobehav Rev. 2018;84:316-24. https://doi.org/10.1016/j.neubiorev.2017.08.016
PMid:28859995
19. Benveniste H, Liu X, Koundal S, Sanggaard S, Lee H, Wardlaw J. The glymphatic system and waste clearance with brain aging: A review. Gerontology. 2019;65(2):106-19. https://doi.org/10.1159/000490349
PMid:29996134
20. Wei F, Song J, Zhang C, Lin J, Xue R, Shan LD, *et al.* Chronic stress impairs the aquaporin-4-mediated glymphatic transport through glucocorticoid signaling. Psychopharmacology (Berl). 2019;236(4):1367-84. https://doi.org/10.1007/s00213-018-5147-6
PMid:30607477
21. Sapolsky RM, Romero LM, Munck AU. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr Rev. 2000;21(1):55-89. https://doi.org/10.1210/edrv.21.1.0389
PMid:10696570
22. Bernstein R. The Mind and Mental Health: How Stress Affects the Brain. California: Touro University Worldwide; 2016.
23. McEwen BS, Bowles NP, Gray JD, Hill MN, Hunter RG, Karatsoreos IN, *et al.* Mechanisms of stress in the brain. Nat Neurosci. 2015;18(10):1353-63. https://doi.org/10.1038/nn.4086
PMid:26404710
24. Horner HC, Packan DR, Sapolsky RM. Glucocorticoids inhibit glucose transport in cultured hippocampal neurons and glia. Neuroendocrinology. 1990;52(1):57-64. https://doi.org/10.1159/000125539
PMid:2118608
25. Virgin CE Jr., Ha TP, Packan DR, Tombaugh GC, Yang SH, Horner HC, *et al.* Glucocorticoids inhibit glucose transport and glutamate uptake in hippocampal astrocytes: Implications for glucocorticoid neurotoxicity. J Neurochem. 1991;57(4):1422-8. https://doi.org/10.1111/j.1471-4159.1991.tb08309.x
PMid:1680166
26. Doyle P, Guillaume-Gentil C, Rohner-Jeanrenaud F, Jeanrenaud B. Effects of corticosterone administration on local cerebral glucose utilization of rats. Brain Res. 1994;645(1-2):225-30. https://doi.org/10.1016/0006-8993(94)91655-1
PMid:8062085
27. Sapolsky RM. Why stress is bad for your brain. Science. 1996;273(5276):749-50. https://doi.org/10.1126/science.273.5276.749
PMid:8701325
28. Newcomer JW, Craft S, Hershey T, Askins K, Bardgett ME. Glucocorticoid-induced impairment in declarative memory performance in adult humans. J Neurosci. 1994;14(4):2047-53. https://doi.org/10.1523/JNEUROSCI.14-04-02047.1994
PMid:8198631
29. Brady KT, Sonne SC. The role of stress in alcohol use, alcoholism treatment, and relapse. Alcohol Res Health. 1999;23(4):263-71.
30. Lundgaard I, Wang W, Eberhardt A, Vinitsky HS, Reeves C, Peng S, *et al.* Beneficial effects of low alcohol exposure, but adverse effects of high alcohol intake on glymphatic function. Sci Rep. 2018;8(1):2246. https://doi.org/10.1038/s41598-018-20424-y
PMid:29396480
31. Xie L, Kang H, Xu Q, Chen MJ, Liao Y, Thiyagarajan M, *et al.* Sleep drives metabolite clearance from the adult brain. Science. 2013;342(6156):373-7. https://doi.org/10.1126/science.1241224
PMid:24136970
32. Kalimo R, Tenkanen L, Härmä M, Poppius E, Heinsalmi P. Job stress and sleep disorders: Findings from the Helsinki Heart Study. Stress Med. 2000;16(2):65-75.
33. Akerstedt T, Kecklund G, Axelsson J. Impaired sleep after bedtime stress and worries. Biol Psychol. 2007;76(3):170-3. https://doi.org/10.1016/j.biopsycho.2007.07.010
PMid:17884278
34. Bernardy NC, Friedman MJ. A Practical Guide to PTSD Treatment: Pharmacological and Psychotherapeutic Approaches. A Practical Guide to PTSD Treatment: Pharmacological and Psychotherapeutic Approaches. United States: American Psychological Association; 2014.
35. Jansen AS, Van Nguyen X, Karpitskiy V, Mettenleiter TC, Loewy AD. Central command neurons of the sympathetic nervous system: Basis of the fight-or-flight response. Science. 1995;270(5236):644-6.
36. McEwen BS. Protective and damaging effects of stress mediators. N Engl J Med. 1998;338(3):171-9. https://doi.org/10.1056/NEJM199801153380307
PMid:9428819
37. Lindquist TL, Beilin LJ, Knuiman MW. Influence of lifestyle, coping, and job stress on blood pressure in men and women. Hypertension. 1997;29(1 Pt 1):1-7. https://doi.org/10.1161/01.hyp.29.1.1
PMid:9039072
38. Kaplan JR, Manuck SB, Clarkson TB, Lusso FM, Taub DM, Miller EW. Social stress and atherosclerosis in normocholesterolemic monkeys. Science. 1983;220(4598):733-5. https://doi.org/10.1126/science.6836311
PMid:6836311
39. Dallman MF. Stress-induced obesity and the emotional nervous system. Trends Endocrinol Metab. 2010;21(3):159-65. https://doi.org/10.1016/j.tem.2009.10.004
PMid:19926299
40. Hammen C. Stress and depression. Annu Rev Clin Psychol. 2005;1:293-319. https://doi.org/10.1146/annurev.clinpsy.1.102803.143938
PMid:17716090
41. Goeders NE. The impact of stress on addiction. Eur

Neuropsychopharmacol. 2003;13(6):435-41. https://doi.org/10.1016/j.euroneuro.2003.08.004
PMid:14636959

42. Shin LM, Liberzon I. The neurocircuitry of fear, stress, and anxiety disorders. Neuropsychopharmacology. 2010;35(1):169-91. https://doi.org/10.1038/npp.2009.83
PMid:19625997

43. Berridge CW, Waterhouse BD. The locus coeruleus-noradrenergic system: Modulation of behavioral state and state-dependent cognitive processes. Brain Res Brain Res Rev. 2003;42(1):33-84. https://doi.org/10.1016/s0165-0173(03)00143-7
PMid:12668290

44. O'Donnell J, Ding F, Nedergaard M. Distinct functional states of astrocytes during sleep and wakefulness: Is norepinephrine the master regulator? Curr Sleep Med Rep. 2015;1(1):1-8. https://doi.org/10.1007/s40675-014-0004-6
PMid:26618103

45. Lee H, Xie L, Yu M, Kang H, Feng T, Deane R, *et al.* The Effect of body posture on brain glymphatic transport. J Neurosci. 2015;35(31):11034-44. https://doi.org/10.1523/JNEUROSCI.1625-15.2015
PMid:26245965

46. Akerstedt T. Psychosocial stress and impaired sleep. Scand J Work Environ Health 2006;32(6):493-501.

47. Fu H, Hardy J, Duff KE. Selective vulnerability in neurodegenerative diseases. Nat Neurosci. 2018;21(10):1350-8. https://doi.org/10.1038/s41593-018-0221-2
PMid:30250262

48. Ross CA, Poirier MA. Protein aggregation and neurodegenerative disease. Nat Med. 2004;10 Suppl: S10-7. http://doi.org/10.1038/nm1066
PMid:15272267

49. Hardy J, Allsop D. Amyloid deposition as the central event in the aetiology of Alzheimer's disease. Trends Pharmacol Sci. 1991;12(10):383-8. https://doi.org/10.1016/0165-6147(91)90609-v
PMid:1763432

50. Peng W, Achariyar TM, Li B, Liao Y, Mestre H, Hitomi E, *et al.* Suppression of glymphatic fluid transport in a mouse model of Alzheimer's disease. Neurobiol Dis. 2016;93:215-25. https://doi.org/10.1016/j.nbd.2016.05.015
PMid:27234656

51. Zuroff L, Daley D, Black KL, Koronyo-Hamaoui M. Clearance of cerebral Aβ in Alzheimer's disease: Reassessing the role of microglia and monocytes. Cell Mol Life Sci. 2017;74(12):2167-201. https://doi.org/10.1007/s00018-017-2463-7
PMid:28197669

52. Qi XM, Ma JF. The role of amyloid beta clearance in cerebral amyloid angiopathy: More potential therapeutic targets. Transl Neurodegener. 2017;6:22. https://doi.org/10.1186/s40035-017-0091-7
PMid:28824801

53. Wang LY, Murphy RR, Hanscom B, Li G, Millard SP, Petrie EC, *et al.* Cerebrospinal fluid norepinephrine and cognition in subjects across the adult age span. Neurobiol Aging. 2013;34(10):2287-92. https://doi.org/10.1016/j.neurobiolaging.2013.04.007
PMid:23639207

54. Szot P. Elevated cerebrospinal fluid norepinephrine in the elderly can link depression and a reduced glymphatic system as risk factors for Alzheimer's Disease. J Aging Sci. 2016;4(2):158.

55. Musiek ES, Xiong DD, Holtzman DM. Sleep, circadian rhythms, and the pathogenesis of Alzheimer's disease. Exp Mol Med. 2015;47(3):e148. https://doi.org/10.1038/emm.2014.121
PMid:25766617

56. Rasmussen MK, Mestre H, Nedergaard M. The glymphatic pathway in neurological disorders. Lancet Neurol. 2018;17(11):1016-24. https://doi.org/10.1016/S1474-4422(18)30318-1
PMid:30353860

57. Mendelsohn AR, Larrick JW. Sleep facilitates clearance of metabolites from the brain: Glymphatic function in aging and neurodegenerative diseases. Rejuvenation Res. 2013;16(6):518-23. https://doi.org/10.1089/rej.2013.1530
PMid:24199995

58. Piantino J, Lim MM, Newgard CD, Iliff J. Linking traumatic brain injury, sleep disruption and post-traumatic headache: A potential role for glymphatic pathway dysfunction. Curr Pain Headache Rep. 2019;23(9):62. https://doi.org/10.1007/s11916-019-0799-4
PMid:31359173

59. Gaberel T, Gakuba C, Goulay R, De Lizarrondo SM, Hanouz JL, Emery E, *et al.* Impaired glymphatic perfusion after strokes revealed by contrast-enhanced MRI: A new target for fibrinolysis? Stroke. 2014;45(10):3092-6. https://doi.org/10.1161/STROKEAHA.114.006617
PMid:25190438

60. Back DB, Kwon KJ, Choi DH, Shin CY, Lee J, Han SH, *et al.* Chronic cerebral hypoperfusion induces post-stroke dementia following acute ischemic stroke in rats. J Neuroinflammation. 2017;14(1):216. https://doi.org/10.1186/s12974-017-0992-5
PMid:29121965

61. Bobela W, Aebischer P, Schneider BL. Alpha-synuclein as a mediator in the interplay between aging and Parkinson's disease. Biomolecules. 2015;5(4):2675-700. https://doi.org/10.3390/biom5042675
PMid:26501339

62. Zou W, Pu T, Feng W, Lu M, Zheng Y, Du R, *et al.* Blocking meningeal lymphatic drainage aggravates Parkinson's disease-like pathology in mice overexpressing mutated α-synuclein. Transl Neurodegener. 2019;8:7. https://doi.org/10.1186/s40035-019-0147-y
PMid:30867902

63. Sundaram S, Hughes RL, Peterson E, Müller-Oehring EM, Brontë-Stewart HM, Poston KL, *et al.* Establishing a framework for neuropathological correlates and glymphatic system functioning in Parkinson's disease. Neurosci Biobehav Rev. 2019;103:305-15. https://doi.org/10.1016/j.neubiorev.2019.05.016
PMid:31132378

64. Jiang Q, Zhang L, Ding G, Davoodi-Bojd E, Li Q, Li L, *et al.* Impairment of the glymphatic system after diabetes. J Cereb Blood Flow Metab. 2017;37(4):1326-37. https://doi.org/10.1177/0271678X16654702
PMid:27306755

65. Hirotsu C, Tufik S, Andersen ML. Interactions between sleep, stress, and metabolism: From physiological to pathological conditions. Sleep Sci. 2015;8(3):143-52. https://doi.org/10.1016/j.slsci.2015.09.002
PMid:26779321

66. Vasileva LV, Saracheva KE, Ivanovska MV, Petrova AP, Marchev AS, Georgiev MI, *et al.* Antidepressant-like effect of salidroside and curcumin on the immunoreactivity of rats subjected to a chronic mild stress model. Food Chem Toxicol. 2018;121:604-11. https://doi.org/10.1016/j.fct.2018.09.065
PMid:30268794

67. Dhabhar FS. Enhancing versus suppressive effects of stress on immune function: Implications for immunoprotection and immunopathology. Neuroimmunomodulation. 2009;16(5):300-17. https://doi.org/10.1159/000216188
PMid:19571591

68. Williams RB, Marchuk DA, Gadde KM, Barefoot JC, Grichnik K, Helms MJ, *et al.* Central nervous system serotonin function and cardiovascular responses to stress. Psychosom Med. 2001;63(2):300-5. https://doi.org/10.1097/00006842-200103000-00016
PMid:11292279

69. Lavi E, Cong L. Type I astrocytes and microglia induce a cytokine response in an encephalitic murine coronavirus infection. Exp Mol Pathol. 2020;115:104474. https://doi.org/10.1016/j.yexmp.2020.104474
PMid:32454103

70. Louveau A, Herz J, Alme MN, Salvador AF, Dong MQ, Viar KE, *et al.* CNS lymphatic drainage and neuroinflammation are regulated by meningeal lymphatic vasculature. Nat Neurosci 2018;21(10):1380-91. https://doi.org/10.1038/s41593-018-0227-9
PMid:30224810

71. Negi N, Das BK. CNS: Not an immunoprivilaged site anymore but a virtual secondary lymphoid organ. Int Rev Immunol. 2018;37(1):57-68. https://doi.org/10.1080/08830185.2017.1357719
PMid:28961037

72. Yanev P, Poinsatte K, Hominick D, Khurana N, Zuurbier KR, Berndt M, *et al.* Impaired meningeal lymphatic vessel development worsens stroke outcome. J Cereb Blood Flow Metab. 2020;40(2):263-75. https://doi.org/10.1177/0271678X18822921
PMid:30621519

73. Chen J, He J, Ni R, Yang Q, Zhang Y, Luo L. Cerebrovascular injuries induce lymphatic invasion into brain parenchyma to guide vascular regeneration in zebrafish. Dev Cell. 2019;49(5):697-710.e5. https://doi.org/10.1016/j.devcel.2019.03.022
PMid:31006646

74. Benakis C, Llovera G, Liesz A. The meningeal and choroidal infiltration routes for leukocytes in stroke. Ther Adv Neurol Disord. 2018;11:1756286418783708. https://doi.org/10.1177/1756286418783708
PMid:29977343

75. Rua R, McGavern DB. Advances in meningeal immunity. Trends Mol Med. 2018;24(6):542-59. https://doi.org/10.1016/j.molmed.2018.04.003
PMid:29731353

76. Mander BA, Winer JR, Walker MP. Sleep and human aging. Neuron. 2017;94(1):19-36. https://doi.org/10.1016/j.neuron.2017.02.004
PMid:28384471

77. Hablitz LM, Vinitsky HS, Sun Q, Stæger FF, Sigurdsson B, Mortensen KN, *et al.* Increased glymphatic influx is correlated with high EEG delta power and low heart rate in mice under anesthesia. Sci Adv. 2019;5(2):eaav5447. https://doi.org/10.1126/sciadv.aav5447
PMid:30820460

78. Tay TL, Savage JC, Hui CW, Bisht K, Tremblay M. Microglia across the lifespan: From origin to function in brain development, plasticity and cognition. J Physiol. 2017;595(6):1929-45. https://doi.org/10.1113/jp272134

79. Clarke LE, Liddelow SA, Chakraborty C, Münch AE, Heiman M, Barres BA. Normal aging induces A1-like astrocyte reactivity. Proc Natl Acad Sci U S A. 2018;115:E1896-905. https://doi.org/10.1073/pnas.1800165115
PMid:29437957

80. Cotrina ML, Nedergaard M. Astrocytes in the aging brain. J Neurosci Res. 2002;67(1):1-10. https://doi.org/10.1002/jnr.10121
PMid:11754075

81. Finch CE. Neurons, glia, and plasticity in normal brain aging. Neurobiol Aging. 2003;24(Suppl 1):S123-7. https://doi.org/10.1016/s0197-4580(03)00051-4
PMid:12829120

82. Campuzano O, Castillo-Ruiz MM, Acarin L, Castellano B, Gonzalez B. Increased levels of proinflammatory cytokines in the aged rat brain attenuate injury-induced cytokine response after excitotoxic damage. J Neurosci Res. 2009;87(11):2484-97. https://doi.org/10.1002/jnr.22074
PMid:19326443

83. Thrane VR, Thrane AS, Plog BA, Thiyagarajan M, Iliff JJ, Deane R, *et al.* Paravascular microcirculation facilitates rapid lipid transport and astrocyte signaling in the brain. Sci Rep. 2013;3:2582. https://doi.org/10.1038/srep02582
PMid:24002448

84. Profenno LA, Porsteinsson AP, Faraone SV. Meta-analysis of Alzheimer's disease risk with obesity, diabetes, and related disorders. Biol Psychiatry. 2010;67(6):505-12. https://doi.org/10.1016/j.biopsych.2009.02.013
PMid:19358976

85. Gaede P, Vedel P, Larsen N, Jensen GV, Parving HH, Pedersen O, *et al.* Multifactorial intervention and cardiovascular disease in patients with Type 2 diabetes. N Engl J Med. 2003;348(5):383-93. https://doi.org/10.1056/NEJMoa021778

86. Seravalle G, Grassi G. Obesity and hypertension. Pharmacol Res. 2017;122:1-7. https://doi.org/10.1016/j.phrs.2017.05.013
PMid:28532816

87. Wysocki M, Luo X, Schmeidler J, Dahlman K, Lesser GT, Grossman H, *et al.* Hypertension is associated with cognitive decline in elderly people at high risk for dementia. Am J Geriatr Psychiatry. 2012;20(2):179-87. https://doi.org/10.1097/JGP.0b013e31820ee833
PMid:21814158

88. Kummer BR, Diaz I, Wu X, Aaroe AE, Chen ML, Iadecola C, *et al.* Associations between cerebrovascular risk factors and Parkinson disease. Ann Neurol. 2019;86(4):572-81. https://doi.org/10.1002/ana.25564
PMid:31464350

89. de Heus RA, Rikkert MG, Tully PJ, Lawlor BA, Claassen JA, NILVAD Study Group. Blood pressure variability and progression of clinical Alzheimer disease. Hypertension. 2019;74(5):1172-80. https://doi.org/10.1161/HYPERTENSIONAHA.119.13664
PMid:31542965

90. Mortensen KN, Sanggaard S, Mestre H, Lee H, Kostrikov S, Xavier AL, *et al.* Impaired glymphatic transport in spontaneously hypertensive rats. J Neurosci. 2019;39(32):6365-77. https://doi.org/10.1523/JNEUROSCI.1974-18.2019
PMid:31209176

91. Blair GW, Thrippleton MJ, Shi Y, Hamilton I, Stringer M, Chappell F, *et al.* Intracranial hemodynamic relationships in patients with cerebral small vessel disease. Neurology. 2020;94(21):e2258-69. https://doi.org/10.1212/WNL.0000000000009483
PMid:32366534

92. Geurts LJ, Zwanenburg JJ, Klijn CJ, Luijten PR, Biessels GJ. Higher pulsatility in cerebral perforating arteries in patients with small vessel disease related stroke, a 7T MRI Study. Stroke. 2019;50:62-8.

93. Lorente-Cebrián S, Costa AG, Navas-Carretero S, Zabala M, Martínez JA, Moreno-Aliaga MJ. Role of omega-3 fatty acids in obesity, metabolic syndrome, and cardiovascular diseases: A review of the evidence. J Physiol Biochem. 2013;69(3):633-51. https://doi.org/10.1007/s13105-013-0265-4
PMid:23794360

94. Bazinet RP, Layé S. Polyunsaturated fatty acids and their metabolites in brain function and disease. Nat Rev Neurosci. 2014;15(12):771-85. https://doi.org/10.1038/nrn3820

PMid:25387473

95. Ren H, Luo C, Feng Y, Yao X, Shi Z, Liang F, *et al.* Omega-3 polyunsaturated fatty acids promote amyloid-b clearance from the brain through mediating the function of the glymphatic system. FASEB J. 2017;31(1):282-93. https://doi.org/10.1096/fj.201600896
PMid:27789520

96. Zhang E, Wan X, Yang L, Wang D, Chen Z, Chen Y, *et al.* Omega-3 polyunsaturated fatty acids alleviate traumatic brain injury by regulating the glymphatic pathway in mice. Front Neurol. 2020;11:707. https://doi.org/10.3389/fneur.2020.00707
PMid:32765412

97. Chan JK, Trinder J, Colrain IM, Nicholas CL. The acute effects of alcohol on sleep electroencephalogram power spectra in late adolescence. Alcohol Clin Exp Res. 2015;39(2):291-9. https://doi.org/10.1111/acer.12621
PMid:25597245