Alzheimer's Disease and Sleep Disturbance: Neuroinflammation, Glymphatic Clearance and the Noradrenergic Mechanism

by

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Abstract

Ageing is an important factor for the development of sleep disorders and neurodegenerative disorders, such as Alzheimer's disease (AD). One characteristic of AD is increased sleep disturbances. In addition, sleep loss or disturbances are associated with different conditions or mechanisms affecting or causing AD. In this review, three different aspects associated with AD and affected by sleep loss are covered. This includes neuroinflammation, the glymphatic clearance pathway and the role of the locus coeruleus (LC) and its neurotransmitter noradrenaline (NA). Neuroinflammation is known as a characteristic of AD, resulting in neuronal cell loss and the accumulation of amyloid-beta (Aβ). The glymphatic clearance mechanism is a system in the brain regulating the clearance of waste products and possible toxic metabolites, including Aβ. Hence, when this system is disturbed it can contribute to AD pathology. The LC-NA mechanism is known for its mediating function in arousal and stress. Next to that, NA has possible neuroprotective and anti-inflammatory effects. When this system is disturbed it can contribute to AD pathology. All these three conditions/mechanisms are affected or mediated by sleep. Sleep loss is associated with increased neuroinflammation, dysfunctioning of the glymphatic clearance and dysfunctioning of the LC-NA system. Therefore, sleep loss contributes directly and indirectly to AD pathology. I suggest a feedback circle of sleep disturbance, neuroinflammation, glymphatic clearance and the LC-NA system to contribute to AD pathogenesis or accelerate AD pathology. In addition, I also think AD further contributes to all these different aspects of this feedback circle. I think restoring sleep is an interesting target to tackle for new or improved therapeutic measures to prevent or slow down AD pathology.

Keywords: Alzheimer's disease, sleep, neuroinflammation, glymphatic clearance, locus coeruleus, noradrenaline

Introduction

Sleep is critical for the proper functioning of many organs, especially the brain. Decline of sleep is a physiological hallmark of ageing. Ageing also increases the prevalence of developing sleep disorders such as insomnia and sleep apnoea (Mander, Winer, Jagust, & Walker, 2016). Next to that, disturbance of sleep has been associated with many neurological and psychiatric diseases, including different forms of dementia (Musiek, Xiong, & Holtzmann, 2015). Sleep impairments are amplified in patients with mild cognitive impairment (MCI) and Alzheimer's Disease (AD) (Mander, Winer, Jagust, & Walker, 2016; Pak, et al., 2020).

AD is the most common form of dementia and ageing is the greatest risk factor for its development (Vanderheyden, Lim, Musiek, & Gerstner, 2018). The earliest measurable hallmark of AD is the accumulation of amyloid-beta (Aβ) molecules, forming amyloid plaques in the brain (Ju, et al., 2013). Aβ accumulation begins 10-15 years before clinical symptoms of AD become visible, this is called preclinical AD (Ju, Lucey, & Holtzmann, 2014; Roh, et al., 2012). The continuous circulation of cerebrospinal fluid (CSF) and interstitial fluid (ISF) in the brain is critical for the clearance of interstitial molecules, such as Aβ. Because the brain lacks conventional lymphatic vessels, this system of interstitial solute clearance is called the glymphatic clearance pathway (Iliff, et al., 2012; Jessen, Finmann-Munk, Lundgaard, & Nedergaard, 2015). Disruption of this pathway leads to failure of clearing Aβ molecules from the brain, leading to amyloid plaque formation. In result, this disruption of the glymphatic pathway contributes to the pathogenesis

of AD (Weller, Subash, Preston, Mazanti, & Carare, 2008). Another AD mechanism is the role of the locus coeruleus (LC) and its neurotransmitter noradrenaline (NA). The LC is a brain region affected by AD resulting in neuronal cell death. LC degeneration results in decreased levels of NA. Disruption of the LC-NA system can affect the Aβ concentration, leading to amyloid accumulation and plaque formation, further contributing to AD pathology (Ross, McGonigle, & Bockstaele, 2015). In patients with severe noradrenergic deficiency due to great neuronal cell loss, their pathology progress is even correlated with very severe AD symptoms and earlier death (Bondareff, Mountjoy, & Roth, 1981).
During wakefulness, Aβ concentration increases and during sleep, Aβ concentration decreases, giving sleep a role in Aβ clearance (Ju, et al., 2013; Roh, et al., 2012). Sleep disturbance is also associated with other AD pathology hallmarks, like tau hyperphosphorylation and aggregation, and neuronal and synaptic dysfunction and degeneration. It is vague whether sleep disturbance is already present in early stages of AD and possibly contributes to the pathology of different AD hallmarks. However, evidence suggests that sleep disturbances may be early indicators of Aβ pathology and may precede the onset of measurable cognitive symptoms in AD (Musiek, Xiong, & Holtzmann, 2015).
Enhancing sleep quality is a promising target for developing potential treatments against early AD pathogenesis, however the relationship between sleep and AD pathology is still unclear (Sprecher, et al., 2017). Sleep and AD seem to have a bidirectional relationship. Sleep deprivation increases the concentration of soluble Aβ in the brain, and once Aβ accumulates, sleep abnormalities will occur more frequently (Ju, Lucey, & Holtzmann, 2014; Mander, Winer, Jagust, & Walker, 2016).
On the basis of this information, in this review I would like to further highlight the research question: what is the effect of sleep disturbance in Alzheimer's disease? To answer this, three different questions regarding aspects or mechanisms associated with AD will be discussed: What is the effect of sleep disturbance on neuroinflammatory factors? What is the effect of sleep disturbance on the glymphatic clearance pathway? And, what is the effect of sleep disturbance on the noradrenergic mechanism?

Sleep, AD and neuroinflammation

In this part the effect of sleep on AD-associated neuroinflammation will be covered. First, different neuroinflammatory factors influencing or provoking AD will be discussed. Then, the influence of sleep and sleep disturbances on neuroinflammation will be discussed.

Neuroinflammation in Alzheimer's Disease

The accumulation of Aβ is assumed to be the primary driver of AD pathogenesis, resulting in the formation of amyloid plaques. Next to that, tau molecules forming neurofibrillary tangles are another hallmark of AD. Aβ and tau are two molecules that can activate inflammatory pathways and promote the production and release of inflammatory factors. Neuroinflammation is an inflammatory reaction of the central nervous system (CNS). Due to influence of the blood-brain barrier (BBB), neuroinflammation in the CNS is different than a normal inflammatory response elsewhere in the body. Normal neuroinflammation is a protective reaction to an injury or infection in the CNS, but inordinate neuroinflammatory reactions can cause neurodegeneration, as seen in AD (Hong, et al., 2018). Amyloid plaque formation in AD is associated with immune-mediated neuroinflammation (Chen, et al., 2012). In the CNS the inflammatory response is mainly driven by astrocyte

and microglia interactions (Hong, et al., 2018). Amyloid plaques are often surrounded with activated microglial cells and reactive astrocytes, and these astrocytes in turn produce pro-inflammatory factors, like cytokines and chemokines (Acosta, Anderson, & Anderson, 2017; Kisler, Nelson, Montagne, & Zlokovic, 2017).

Astrocytes are the most numerous type of glial cells in the CNS and play a role in neurotransmitter uptake, brain blood flow, excitability of neurons and synaptic plasticity. Astrocytes can be distinguished because of their expression of surface markers, such as glial fibrillary acidic protein (GFAP) (Acosta, Anderson, & Anderson, 2017; Hong, et al., 2018). Astrocytes can be activated by certain cytokines and cause them to express complement proteins, contributing to neuroinflammation and synapse attenuation (Vanderheyden, Lim, Musiek, & Gerstner, 2018). Reactive astrocytes can also undergo gliosis, resulting in scar tissue formation and neurodegeneration. This process is mediated via upregulated GFAP levels (Acosta, Anderson, & Anderson, 2017; Fukuyama, Izumoto, & Fushiki, 2001). Astrogliosis can lead to different processes, including different neuroinflammation processes and Aβ production. However, Aβ can also trigger reactive astrocyte gliosis, suggesting both Aβ production and astrogliosis can trigger each other and contribute to the development of AD hallmarks (Acosta, Anderson, & Anderson, 2017).

Microglia are the macrophages of the brain, because they are the main innate immune cells within the CNS. Microglia have as main role to drive the innate immune response via recognition of pathogen-associated molecular patterns (PAMPs) or dangerous-associated molecular patterns (DAMPs). Next to that, microglia regulate phagocytosis for the clearance of Aβ molecules (Hong, et al., 2018). Impaired microglial phagocytosis can contribute to Aβ accumulation, because of reduced microglial clearance (Acosta, Anderson, & Anderson, 2017).

Important inducers and modulators of neuroinflammation are PAMPs, DAMPs, cytokines and chemokines. Aβ is a DAMP and induces inflammatory responses via the activation of toll-like receptors. Both PAMPs and DAMPs can induce neuroinflammation in AD. Cytokines mediate inflammation and are released by glia cells, including astrocytes and microglia. There are different cytokines with elevated levels in the CSF which have an associated contribution to AD pathology. Four important cytokines are IL-1β, IL-12, TNF-α and TGFβ. Chemokines are inflammatory mediators and recruit immune cells to the CNS and activate glial cells. CX3C and CCL2 are two chemokines that contribute to neuroinflammation and are associated with AD, however their precise function in AD pathogenesis is still not fully understood (Hong, et al., 2018).

Next to the above mentioned different neuroinflammatory cell types or modulators, other studies discovered the role of the inflammasome complex on AD (Hong, et al., 2018). Inflammasomes are multiprotein complexes and they are proposed to be activated by Aβ (Salminen, Ojala, Suuronen, Kaarniranta, & Kauppinen, 2008). When activated, they in turn activate the inflammatory cascade causing neuroinflammation, contributing to AD pathology (Hong, et al., 2018).

To summarize, neuroinflammation is caused by different cell types and neuroinflammatory factors, including astrocytes, microglia, cytokines, chemokines and inflammasome mechanisms. Most of these different factors contribute to the accumulation of Aβ into amyloid plaques, contributing to the pathogenesis or acceleration of AD. In addition, the development of AD can also contribute to the activation of neuroinflammatory factors and mechanisms, suggesting a bidirectional relationship of activation between neuroinflammation and AD.

Sleep and AD-associated neuroinflammation

Sleep is a biological process that undergoes change with age. Ageing increases the prevalence of sleep disturbance, because homeostatic and circadian rhythm processes become more fragile (Rissling & Ancoli-Israel, 2009). Disruption of sleep is suggested to be a component of the pathway causing AD pathogenesis, and accelerating AD progression. Sleep may be sensitive during preclinical AD, and sleep disturbance may be a component of potential AD risk and be a contributing factor to AD progression. As mentioned in the introduction, sleep disturbance may have an effect on AD pathology, but AD pathology might also affect sleep quality. Sleep disruption is suggested to promote a build-up of Aβ, resulting in accumulation into plaques (Sprecher, et al., 2017). A bidirectional relationship between sleep disturbances and AD pathology is hypothesized (Vanderheyden, Lim, Musiek, & Gerstner, 2018).

It is important to understand two common sleep stages: rapid eye movement (REM) sleep and non-rapid eye movement (NREM) sleep. NREM sleep in humans can be divided in three stages: N1, N2 and N3. The last stage is known as slow wave sleep (SWS). NREM sleep is associated with general decrease of synaptic activity. Patients with early AD hallmarks show a decline in both REM and NREM sleep duration. Alterations in SWS and synaptic activity have been observed in patients with early AD (Havekes, et al., 2019).

A function of astrocytes is the regulation of metabolic demands of neurons and this mechanism is linked to the sleep-wake cycle. Disruption of normal astrocyte activation via GFAP deletion induces Aβ accumulation. Increased wakefulness and neuronal activity regulate extracellular Aβ levels. Clearance of wakefulness-associated Aβ is suggested to be facilitated during sleeping periods by astrocytes and prevent amyloid plaque formation. The decline in sleep with ageing can result in slower astrocyte-derived clearance of Aβ, and hence its contribution to early hallmarks of AD (Vanderheyden, Lim, Musiek, & Gerstner, 2018). Next to sleep disturbance effects on astrocytes, sleep loss can also elevate microglial activation (Sprecher, et al., 2017). Concluding, sleep disturbance-induced effects in both astrocytes and microglia can contribute to elevated neuroinflammatory factors activating AD pathogenesis and/or contribution to AD progression.

Different cytokines, including TNF-α and IL-1β are associated with non-rapid eye movement (NREM) sleep regulation, and other molecules like prolactin and nitric oxide, are associated with REM sleep regulation. All these molecules are sleep enhancers, because they act on sleep regulatory circuits to promote sleep (Krueger, Churchill, & Rector, 2009). A theory about sleep suggests that, because of the usage of synapses and neuronal circuits during the day, ATP is released and induces release of sleep-regulatory substances (SRS) from glia cells. SRS release can be in the form of cytokines, like TNF-α and IL-1β. This cytokine release is enhanced by neural activity during the day and is responsible for synaptic structuring (Krueger, Majde, & Rector, 2011). IL-1β and TNF-α concentrations are involved in the regulation of sleep and their serum concentrations are found to be elevated in AD patients. Daytime sleepiness in mild and moderate AD is associated with elevated serum levels of TNF-α (Krysta, Krzystanek, Bratek, & Krupka-Matuszczyk, 2015). This elevated secretion of TNF-α might be caused by overexpression via microglia. Since TNF-α is an important mediator for neuroinflammation in AD and pathological sleepiness, it might explain a part of the bidirectional relationship of sleep disturbances and AD pathology (Chen, et al., 2012). Sleep deprivation also increases the expression of NLRP3 inflammasomes and the serum levels of IL-1β in astrocytes. Next to that, sleep deprivation also increases neuronal apoptosis. As mentioned before, sleep loss elevates levels of

SRS like IL-1β and TNF-α, but injections of these substances can also induce symptoms associated with sleep deprivation. This suggests a bidirectional relationship between sleep loss and elevated neuroinflammatory factor expression (Xia, et al., 2018).
In summary, sleep disturbance has different effects on neuroinflammation. It can promote the aggregation of Aβ into amyloid plaques, it can elevate astrocyte activation, it can slow down astrocyte-derived clearance of Aβ, and it can elevate microglial activation of neuroinflammatory factor expression, like TNF-α and IL-1β. Altogether, these events can separately or together result in enhanced AD pathogenesis and further AD progression. In addition, different mechanisms and factors regarding neuroinflammation in AD can also induce different forms of sleep disturbances. Therefore these different findings suggest that both sleep disturbance and AD-associated neuroinflammation have reciprocal effects on one another.

Sleep, AD and the glymphatic clearance pathway

In the next part of this review, the effect of sleep on the glymphatic clearance pathway and its association with AD will be highlighted. First, the normal glymphatic clearance pathway will be discussed. Then, the influence of sleep disturbances on the clearance pathway and how this is associated with AD will be discussed.

Glymphatic clearance

Clearance of excess fluid and possible accumulating or harmful substances is a critical mechanism of homeostasis. Protein production and clearance are tightly regulated and reflect normal physiology, but also disease states (Bateman, et al., 2006). The CNS contains two important fluids involved in clearance: the CSF and the ISF. Recently it has been discovered that these two fluids continuously interchange and this mechanism is titled as the glymphatic clearance pathway, based on its similarity to the lymphatic system and the important role of glial channels (Iliff, et al., 2012; Jessen, Finmann-Munk, Lundgaard, & Nedergaard, 2015). In short, this pathway is characterized by the movement of the CSF and ISF around the cerebral vasculature into and through the brain parenchyma facilitated by the clearance of interstitial metabolites to drainage pathways, such as the lymph system. The brain parenchyma is the functional tissue in the brain, consisting of neurons and glial cells. The glymphatic system utilizes a unique system of perivascular tunnels through this parenchyma, and this system is formed by astroglial cells (Jessen, Finmann-Munk, Lundgaard, & Nedergaard, 2015). These astroglial cells contain important water channels, called aquaporin-4 (AQP4). Animals lacking these AQP4 channels exhibit slower CSF influx through the system and a big reduction in solute clearance (Iliff, et al., 2012; Xie, et al., 2013). Because of the high sensitivity of neurons to their environment, the removal of waste products from neural metabolism needs to be quick and efficiently (Xie, et al., 2013).
Because of studies observing the same effects in sleeping and anaesthetized mice, it is suggested the differences in clearance and interstitial space volume compared to wakefulness are not circadian rhythm mediated, but mediated via the sleep-wake state (Xie, et al., 2013). So, the glymphatic clearance system is controlled by the sleep-wake system and is primarily active during sleep and suppressed during wakefulness (Lundgaard, et al., 2017). CSF influx in the awake state of mice is 90% reduced compared to anaesthetized and sleeping mice. Next to that, the volume of interstitial space is decreased during wakefulness and increased

during sleep (Xie, et al., 2013). During NREM sleep, glial cells can shrink up to 60%, resulting in a facilitated flow of CSF through the increased volume of interstitial space (Mander, Winer, Jagust, & Walker, 2016). This indicates that sleep is beneficial for fluid fluxes due to glial shrinkage and is thereby beneficial for clearance of metabolites. In short, this states that a major function of sleep appears to be the activation of the glymphatic clearance pathway to clear the brain from possible toxic waste products produced during wakefulness.
A major driver of arousal is noradrenaline (NA) and this neurotransmitter might also be responsible for suppression of glymphatic clearance during wakefulness (Jessen, Finmann-Munk, Lundgaard, & Nedergaard, 2015). Brain lactate is considered a great biomarker of the sleep-wake cycle. The highest concentration of lactate is during wakefulness and declines during sleep. Lactate plays an important role in the intake and release of glucose in activated brain tissue (Ball, Cruz, Mrak, & Dienel, 2010) and it is well-known that the energy metabolism of the CNS relies almost exclusively on glucose. Lactate production is mediated via the break-down of astrocytic glucose and glycogen and increases the noradrenergic drive via the locus coeruleus (LC). The glymphatic clearance system flushes excess lactate out of the brain (Lundgaard, et al., 2017). The combination of this information forms a possible sleep-wake mechanism in combination with the glymphatic system. The production of lactate in the brain activates the LC, which in turn elevates NA levels resulting in wakefulness and arousal. Next to that, NA suppresses the glymphatic clearance during wakefulness. When NA concentrations decline during the day, the glymphatic clearance will slowly be activated and sleep is induced. This results in the clearance of lactate and other waste products. Giving both NA and lactate an important role in the glymphatic clearance pathway (Jessen, Finmann-Munk, Lundgaard, & Nedergaard, 2015; Lundgaard, et al., 2017). More effects of NA on this and other mechanisms will be discussed later on.
To summarize, the glymphatic system is a mechanism for the clearance of waste products, e.g. lactate, and other possible toxic metabolites. The activity of this clearance system is high during sleep because of decreased glial cell volume and increased interstitial fluid volume, resulting in more fluid flow. During wakefulness, the clearance system is suppressed due to different factors, among which is the influence of noradrenergic input.

Glymphatic clearance: effect of sleep disturbance and AD

Ageing is associated with a decline in glymphatic function. This decline might be attributable to dysregulation of astroglial water transport, due to an altered function of AQP4s. Other factors associated with ageing are the decline in CSF production, CSF pressure and stiffening of the arterial walls. This age-related decline in clearance contributes to accumulation of protein aggregation, such as Aβ (Jessen, Finmann-Munk, Lundgaard, & Nedergaard, 2015). Ageing is the greatest risk factor for AD, however, the glymphatic clearance mechanism is of great importance for neurodegenerative diseases like AD. So, a decline in the function of the glymphatic pathway next to and due to ageing can further contribute to AD. Because AD is characterized by the accumulation of Aβ and tau, the clearance of these proteins via the glymphatic clearance pathway is an interesting target for treatment therapies (Jessen, Finmann-Munk, Lundgaard, & Nedergaard, 2015). The hyperphosphorylation of tau which develops into neurofibrillary tangles mostly occurs in neurons. The accumulation of Aβ occurs in the brain tissue and in the walls of cerebral vessels. Soluble and insoluble forms of Aβ are both present in brains of AD patients (Weller, Subash, Preston, Mazanti,

& Carare, 2008). It is the level of soluble Aβ rather than insoluble Aβ and plaques that is associated with the severity of dementia in AD. Deposition of insoluble Aβ in cerebral artery walls blocks the elimination of soluble Aβ, resulting in the accumulation of Aβ into insoluble plaques in the perivascular pathway which drains ISF from the CSF (Preston, Steart, Wilkinson, Nicoll, & Weller, 2003). The failure of elimination of both soluble and insoluble Aβ from the brain along perivascular drainage pathways is associated with AD pathology, because it results in plaque formation (Weller, Subash, Preston, Mazanti, & Carare, 2008). Progressive accumulation of Aβ in AD could bulk perivascular fluid flow and negatively influence the distribution and clearance of glucose, lactate, and other metabolites within the brain (Ball, Cruz, Mrak, & Dienel, 2010). Next to that, the ageing brain also displays increasing reactive gliosis in astrocytes, defined by hypertrophy of GFAP astrocyte processes, which is associated with AD development. Altered AQP4 expression in reactive astrocytes can result in gliosis and may contribute to disrupted interstitial flow, resulting in clearance failure of metabolites such as Aβ (Iliff, et al., 2012; Jessen, Finmann-Munk, Lundgaard, & Nedergaard, 2015). In short, ageing and cerebral artery wall stiffness result in the failure of the glymphatic clearance. This results in lower drainage of Aβ, resulting in amyloid plaque formation in the perivascular drainage pathway. These plaques further block the drainage pathway, resulting in failure of drainage of all other metabolites. This can cause a loss in homeostasis and neuron deterioration and can contribute to AD pathogenesis (Weller, Subash, Preston, Mazanti, & Carare, 2008) (Fig. 1).

AD is correlated with sleep disturbances, and sleep disturbances are associated with AD pathogenesis. As mentioned above, the sleep-wake state influences the glymphatic clearance pathway. Naturally, disturbances in sleep due to AD might cause disturbances in the glymphatic clearance pathway, and therefore further contribute to AD. The interstitial concentration of Aβ is higher during wakefulness compared to concentrations during sleep. This suggests that wakefulness is associated with increased Aβ production. The awake brain-state is linked to a reduction in the interstitial space volume, because of increased resistance to convective fluid movement and suppressed CSF influx. (Xie, et al., 2013). The reduced CSF flow during wakefulness may be due to vasoconstriction of the vessels surrounding the brain tissue, and this could contribute to the reduced glymphatic fluid transport. Failure of glymphatic CSF influx into the brain, due to sleep deprivation, can contribute to neurodegenerative diseases such as AD (Achariyar, et al., 2016). Low activity of the glymphatic system, possibly due to sleep loss, could be a major risk factor for developing AD (Jessen, Finmann-Munk, Lundgaard, & Nedergaard, 2015).

To summarize, ageing is associated with a decline in glymphatic clearance due to different factors. Glymphatic clearance is regulated via the sleep-wake state, and disturbances in sleep negatively affect glymphatic clearance. Loss of glymphatic clearance results in Aβ accumulation, contributing to AD. This means ageing affects AD directly, and indirectly via the glymphatic clearance, and both these processes are enhanced by poor sleep.

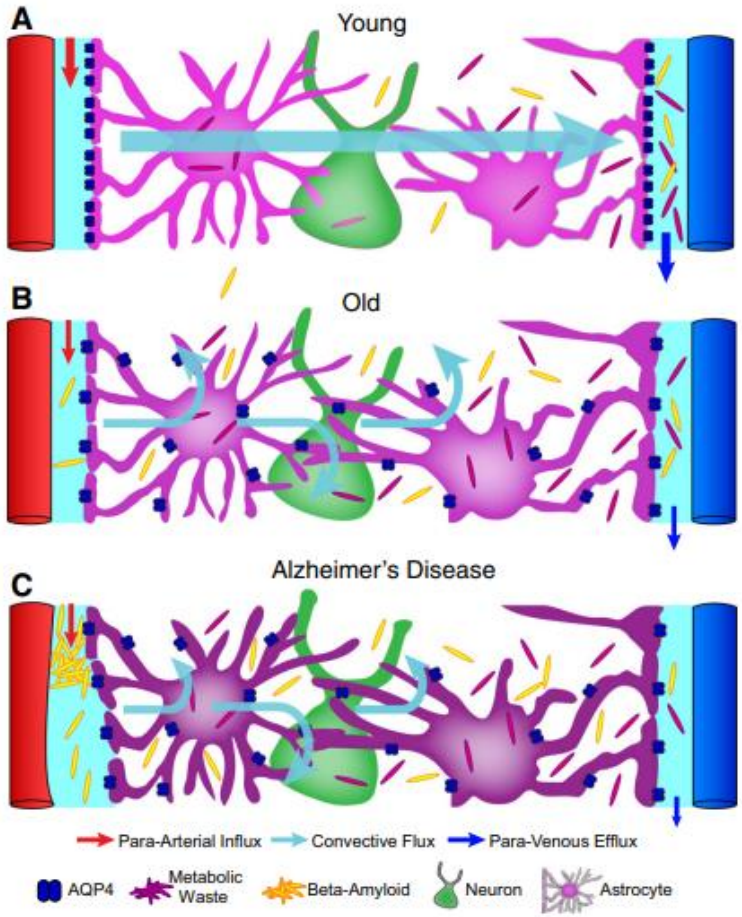

Fig. 1: Model of glymphatic function in young, old and in Alzheimer's disease. **A** In young and healthy people, CSF enters the brain parenchyma via periarterial pathways, washes out solutes from the interstitial space and empties along the veins. **B** With ageing, glymphatic function is reduced, possibly due to astrocytes becoming reactive and AQP4 alterations. **C** In Alzheimer's disease perivascular space of penetrating arteries are subject to accumulation of Aβ. It is hypothesized that accumulation of Aβ might be caused by impairment of the glymphatic system and that the perivascular pathways are further blocked by protein aggregates such as Aβ. In this model, the resulting changes in the perivascular environment lead to abnormal enlargement of perivascular space downstream, which further decreases glymphatic clearance (Jessen, Finmann-Munk, Lundgaard, & Nedergaard, 2015).

Sleep, AD and the noradrenergic system

In this last part of this review, the effect of sleep on the noradrenergic mechanism and its association with AD will be covered. First, the noradrenergic system with the role of the LC and NA will be discussed. Then, the role of the LC-NA system in sleep and the influence of sleep disturbances on this mechanism will be covered, and how this is associated with AD.

The noradrenergic system

The locus coeruleus (LC) is one of the smallest nuclei in the brain, projecting throughout the entire neuronal axis (Aston-Jones & Waterhouse, 2016) (Fig. 2). It is the primary source of noradrenaline (NA) synthesis in the forebrain and the only source of NA in the cortex and hippocampus. These last two regions control cognition, memory and complex behaviours together with the cerebellum. The LC is relatively homogenous in its production, meaning all neurons synthesize NA, and all these neurons have projections to functionally diverse targets. Tonic LC activity is positively associated with arousal, and selective tonic LC activity is even correlated with active waking. Selective inactivation results into low frequency

activity resembling sleep. Phasic LC activity is more closely associated with behavioural output in response to task-related stimuli. This difference in tonic and phasic LC activity is in balance, in that the LC promotes and maintains an optimal level of arousal that is necessary for processing different forms of information. Higher tonic LC activity is associated with stress (Valentino & Van Bockstaele, 2008). NA is important for the retrieval of memories in the hippocampus. Other regions that need NA for memory processes include the amygdala and prefrontal cortex (PFC). These two brain regions are also involved in the integration of the stress response (Ross, McGonigle, & Bockstaele, 2015).
LC neurons express receptors for a wide variety of transmitters and peptides, including NA, orexin and more, which regulate the LC system in conditions like wakefulness, arousal and stress. Once NA is released from the LC, it mostly acts on β-adrenergic receptors present on neurons and glia cells in the CNS.

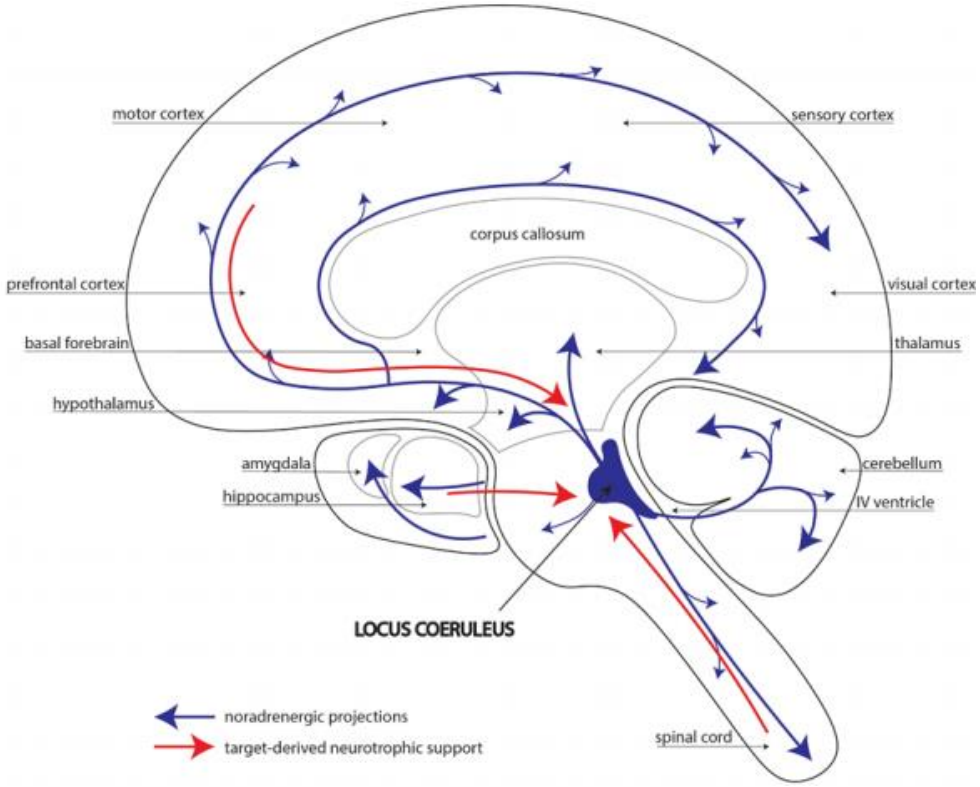

Fig. 2: Locus coeruleus neurons project widely throughout the central neuraxis. The locus coeruleus (LC), is the main source of noradrenaline (NA) in the CNS and sends projections to virtually every region of the CNS (blue arrows). Maintenance of noradrenergic innervation at some regions, most notably the forebrain and spinal cord, depends largely upon target-derived neurotrophic signalling (red arrows) (Feinstein, Kalinin, & Braun, 2016).

Next to the role in arousal and stress, NA has potent anti-inflammatory and neuroprotective effects. Treatment with NA reduces different microglial and astroglial inflammatory responses. Next to that, NA can also suppress pro-inflammatory gene induction by other inflammatory agents, like Aβ. In contradiction to the suppressed inflammatory responses, NA can also activate inflammatory responses in other cell types, like macrophages and smooth muscle cells. This effect may also reflect a protective response, resulting in macrophage-mediated inflammatory responses, controlling the inflammation (Feinstein, Kalinin, & Braun, 2016).
To summarize, the LC-NA system is a mechanism which involves LC projections through the entire neuronal axis. The released neurotransmitter NA induces

different conditions, including arousal and stress. NA also has potent anti-inflammatory and neuroprotective effects.

The LC-NA system: effect of sleep disturbance and AD

As mentioned above, the LC is known to play a physiological role in promoting wakefulness and arousal. It has been found that LC neurons are most active during waking and that their activity is decreased during sleep (Aston-Jones & Waterhouse, 2016; Berridge & Waterhouse, 2003). LC cells have tonic activity during wakefulness and reduced firing rates during sleep. LC activity during wakefulness regulates neuronal transcription to facilitate synaptic potentiation and memory acquisition, while LC inactivity during sleep plays a broad role to enhance protein synthesis in the brain (Cirelli & Tononi, 2011). Next to the adrenergic neurons, there are more neuronal groups involved in the modulation of wakefulness. Among these are orexinergic neurons (Zhu, Fenik, Zhan, Xin, & Veasey, 2015). Orexin, or hypocretine, is a neuropeptide which can innervate nuclei implicated in sleep regulation (Schwartz & Roth, 2008). Under normal physiological conditions, orexin stimulates the LC neurons in producing and releasing NA, which facilitates the transition from sleep to wakefulness (Ross, McGonigle, & Bockstaele, 2015; Schwartz & Roth, 2008). Electrophysiological recordings of orexinergic neurons show that they are relatively silent during sleep compared to wakefulness (Carter, et al., 2012). The LC is one of the regions in the brain most densely populated with orexinergic receptors, therefore orexin plays an important role in the stimulation of LC neurons and wakefulness (Ross, McGonigle, & Bockstaele, 2015). Activation of LC neurons firing by orexin is essential for the regulation of normal sleep and necessary to maintain a waking state (Feinstein, Kalinin, & Braun, 2016).

Neurodegeneration in AD is characterized by atrophy, synaptic and neuronal loss and gliosis of astrocytes. One of the earliest brain regions affected by AD is the LC, in which neuronal cell death and Aβ deposition is observed. LC degeneration plays an important role in AD pathogenesis, because this has impact on inflammation, synaptic functioning, neuronal metabolism and BBB permeability. The terminal regions of LC neurons contain β_2 and α_2 receptors and have direct influence on synaptic transmission. Both these receptors have been associated with the production and secretion of Aβ. Dysregulation of the LC-NA system in AD might contribute to increased Aβ accumulation in LC terminal regions (Ross, McGonigle, & Bockstaele, 2015). As mentioned before, the breakdown of astrocytic glucose and glycogen increases the noradrenergic drive via the LC. Above that, NA might be responsible for the suppression of glymphatic clearance during wakefulness. This suggests that LC-NA dysfunctioning might contribute to altered glymphatic clearance of toxic waste products and Aβ (Lundgaard, et al., 2017). Increased NA levels may play a role in reducing glymphatic clearance, but it is also possible that NA released during 'sleep deprivation stress' can contribute to reduced clearance, due to decreased ECF and ISF flow (Achariyar, et al., 2016). Next to this, the LC-NA system is critical in mediating the inflammatory response. Normally NA can have neuroprotective effects, but dysregulation of the system can result in pro-inflammatory effects, also induced because of additional Aβ accumulation (Ross, McGonigle, & Bockstaele, 2015). Severe noradrenergic deficiency leads to the fall-out of the LC cells, resulting in severe AD pathology (Bondareff, Mountjoy, & Roth, 1981).

LC damage disrupts normal sleep cycles, which can cause sleep abnormalities in AD. Above that, sleep is necessary for metabolite clearance via the glymphatic pathway. Disruption of sleep caused by LC dysfunctioning can cause altered

metabolite clearance, resulting in Aβ accumulation (Xie, et al., 2013). In addition, LC dysfunction might also contribute to sleep-related problems in AD. Hence, a bidirectional relationship between LC dysfunctioning and sleep disturbance, both contributing to AD pathology.
Normally, orexin modulates the transition from sleep to wakefulness by exciting the LC, resulting in NA synthesis and release. It has been found that AD patients have a loss of orexinergic signalling, because of cell loss and lowered orexin levels. This is correlated with sleep disturbance in these patients (Ross, McGonigle, & Bockstaele, 2015). Sleep disruption or deprivation can predict impaired wakefulness, caused by injury to the LC-NA and orexinergic system. Next to that, sleep disruption results in a reduction in LC and orexinergic neurons. Normal ageing is associated with the decline in these neurons, thus sleep disturbance accentuates or even accelerates the ageing process in these neurons. TNF-α levels are upregulated in the LC and orexinergic neurons exposed to sleep disturbance, contributing to neuronal injury. This might explain the lasting sleep-wake impairments and continued injury in these neurons (Zhu, Fenik, Zhan, Xin, & Veasey, 2015).
To summarize, the LC-NA system plays an important role in promoting wakefulness. The LC is one of the earliest regions affected by AD, resulting in neuronal cell loss and Aβ accumulation. Dysregulation of the LC-NA system can cause reduced glymphatic clearance of Aβ, further contributing to AD. LC damage can disrupt sleep, which results to impaired glymphatic clearance. Sleep disturbance can also cause LC-NA dysfunctioning, also leading to impaired clearance. Orexinergic neuron loss is also correlated with sleep disruption, and sleep disruptions can also cause orexinergic neuron decline. All these findings suggest a bidirectional relationship between dysfunctions of the LC-NA system and sleep disturbance, both contributing to AD.

Discussion

In short, the three different conditions and mechanisms affected by and affecting AD and sleep disturbances covered in this review are neuroinflammation, the glymphatic clearance pathway and the LC-NA system. Neuroinflammation is caused by different cell types and neuroinflammatory factors. These different cell types include astrocytes and microglia. Astrocytes play an important role in neurotransmitter uptake, brain blood flow, neuron excitability and synaptic plasticity. Activated astrocytes can contribute to neuroinflammation and synapse attenuation. Reactive astrocytes can undergo gliosis, resulting in neuronal cell loss. This process is mediated via GFAP, which is a surface marker expressed by astrocytes. Gliosis can lead to neuroinflammation and Aβ production. Microglia are the brain macrophages driving the innate immune response. Microglia regulate phagocytosis of different metabolites, including Aβ. Damage in microglia can result in loss of Aβ phagocytosis. Neuroinflammation is mediated via different pro-inflammatory factors, such as cytokines and chemokines. Both cell types in combination with these pro-inflammatory factors contribute to neuroinflammation, resulting in impairments in Aβ clearance and contributing to Aβ accumulation. Therefore neuroinflammation contributes to AD pathology. When AD hallmarks like Aβ start to accumulate they further activate these impairments in astrocytes and microglia, in which they further contribute to AD. The glymphatic clearance mechanism clears waste and toxic products, such as lactate and Aβ from the brain fluids. Normal ageing is associated with decline in glymphatic clearance, due to dysregulation of water transport, decline in CSF production, decline in CSF pressure, and stiffening of the cerebral arterial walls. Extensive loss of glymphatic

clearance results in Aβ accumulation which contributes to AD pathology. During wakefulness, the glymphatic clearance pathway is suppressed, due to increased input from the noradrenergic system. Sleep causes the glymphatic clearance pathway to clear the brain from metabolites which accumulated during the day. Sleep loss can inhibit this glymphatic clearance, resulting in accumulation of these metabolites, which include Aβ. Therefore, normal glymphatic clearance is of great importance for possible prevention or suppressing the development of neurodegenerative diseases like AD. The LC-NA system projects throughout the entire neuraxis and is an important source of NA synthesis in the forebrain, cortex and hippocampus. LC-NA activity is associated with arousal, and when overexpressed it can result in stress. The LC expresses a lot of orexinergic neurons, which stimulate the LC in producing NA, resulting in wakefulness and arousal. NA has potent anti-inflammatory and neuroprotective effects. The LC is one of the earliest regions affected by AD, in which neuronal cell loss and Aβ accumulation is observed.

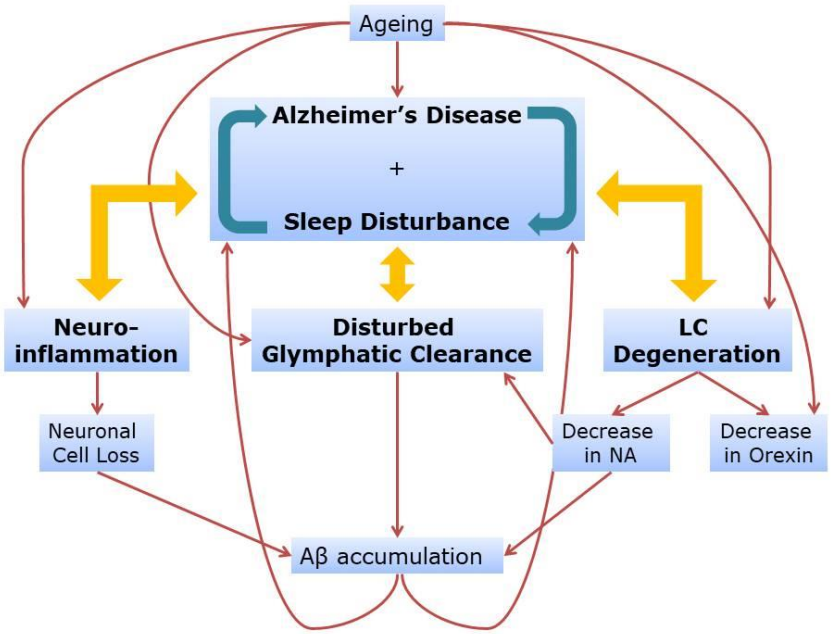

Fig. 3: The feedback circle of Alzheimer's disease (AD), sleep disturbance, neuroinflammation, glymphatic clearance and the LC-NA system. AD and sleep disturbance both affect each other and negatively influence neuroinflammation, glymphatic clearance and the LC-NA system. These three conditions/mechanisms trigger different responses resulting in Aβ accumulation, further contributing to and accelerating AD and sleep disturbance. To a small extent, ageing affects all these different conditions and mechanisms.

To a small extent, normal ageing gradually enhances neuroinflammation and negatively affects glymphatic clearance and the LC-NA system. However, all three conditions/mechanisms can break into the earliest pathogenesis of different neurodegenerative diseases, including AD. In addition, sleep disturbances also negatively affect all these three conditions/mechanisms, which further contribute to AD. Next to this, when AD pathogenesis has started, this also impacts sleep, resulting in sleep disturbances, further contributing to these three conditions/mechanisms and AD. I propose some sort of feedback circle is regulating all of this, suggesting when one of these different mechanisms is affected by AD or sleep loss, it can further contribute to or even accelerate AD pathogenesis and sleep loss (Fig. 3 and 4). I still think ageing is the primary cause of AD, however I think sleep loss can accelerate this normal ageing into AD pathogenesis. When either sleep loss accelerates AD pathogenesis or AD onset is

gradually due to ageing, I think both ways contribute to all three conditions/mechanisms mentioned above, and further negatively affect each other, and again contribute to AD pathogenesis and sleep loss. Next to that, I think sleep loss apart from AD can contribute to neuroinflammation, dysfunctioning of glymphatic clearance, and dysfunctioning of the LC-NA system. Suggesting this would also contribute to early hallmarks of AD and AD pathogenesis.
To conclude, the effect of sleep in Alzheimer's disease is surrounded by a complicated feedback circle, which includes neuroinflammation, glymphatic clearance, LC-NA input and even more factors which are not covered in this review. Sleep loss negatively impacts all these three discussed aspects, contributing to AD. With this insight, maybe new therapeutic measures can be developed to prevent or slow down AD pathogenesis. I think restoring sleep is an interesting target for therapeutic measures, because sleep disturbance is an important characteristic of AD, and sleep loss alone also contributes to a lot of negative effects causing or further accelerating AD.

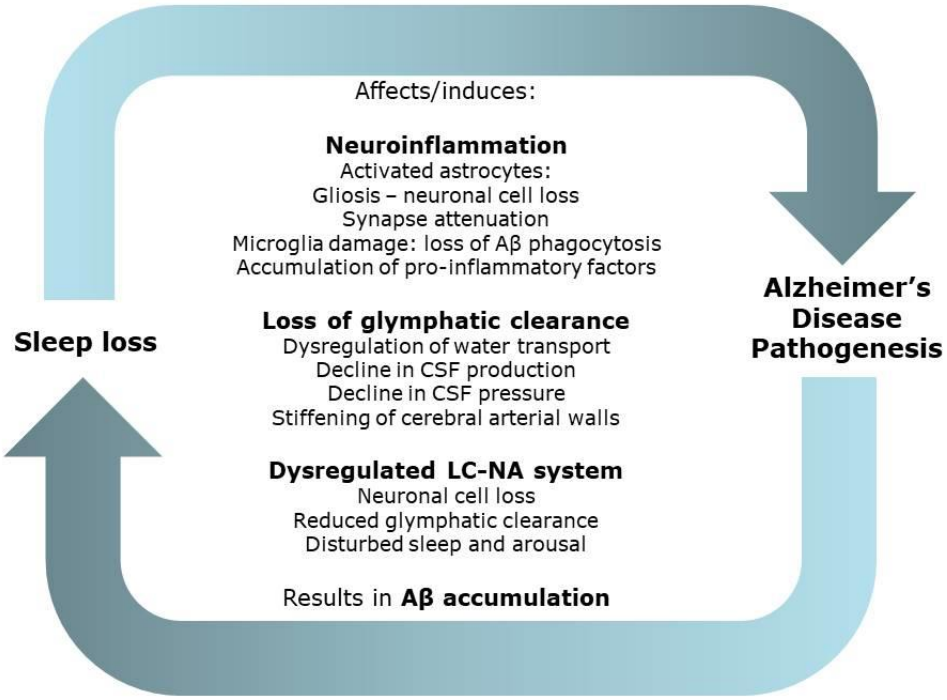

Fig. 4: The relationship between sleep loss and Alzheimer's disease (AD), and what they both can affect or induce. This includes the three different aspects discussed trough this review: neuroinflammation, glymphatic clearance and the LC-NA system. All these different effects can also be induced due to ageing and then also induce or accelerate AD and sleep disturbances.

References

Achariyar, T. M., Li, B., Peng, W., Verghese, P. B., Shi, Y., McConnell, E., . . . Deane, R. (2016). Glymphatic distribution of CSF-derived apoE into brain is isoform specific and suppressed during sleep deprivation. *Molecular Neurodegeneration*, 1-20.

Acosta, C., Anderson, H. D., & Anderson, C. M. (2017). Astrocyte dysfunction in Alzheimer disease. *Journal of Neuroscience Research*, 95: 2430-2447.

Aston-Jones, G., & Waterhouse, B. (2016). Locus coeruleus: From global projection system to adaptive regulation of behavior. *Brain Research*, 75-78.

Ball, K. K., Cruz, N. F., Mrak, R. E., & Dienel, G. A. (2010). Trafficking of glucose, lactate, and amyloid-beta from the inferior colliculus through perivascular routes. *Journal of Cerebral Blood Flow & Metabolism*, 30: 162-176.

Bateman, R. J., Munsell, L. Y., Morris, J. C., Swarm, R., Yarasheski, K. E., & Holtzman, D. M. (2006). Human amyloid-beta synthesis and clearance rates as measured in cerebrospinal fluid in vivo. *Nature Medicine*, 12: 856-861.

Berridge, C. W., & Waterhouse, B. D. (2003). The locus coeruleus-noradrenergic system: modulation of behavioral state and state-dependent cognitive processes. *Brain Research Reviews*, 42: 33-84.

Bondareff, W., Mountjoy, C. Q., & Roth, M. (1981). Selective loss of neurones of origin of adrenergic projection to cerebral cortex (nucleus locus coeruleus) in senile dementia. *The Lancet*, 317: 783-784.

Carter, M. E., Brill, J., Bonnavion, P., Huguenard, J. R., Huerta, R., & de Lecea, L. (2012). Mechanisms for hypocretin-mediated sleep-to-wake transitions. *Proceedings of the National Academy of Sciences*, 39: 2635-2644.

Chen, R., Yin, Y., Zhao, Z., Huang, L., Huang, S., Zhuang, J., . . . Li, P. (2012). Elevation of serum TNF-alpha levels in mild and moderate Alzheimer patients with daytime sleepiness. *Journal of Neuroimmunology*, 244: 97-102.

Cirelli, C., & Tononi, G. (2011). Molecular neurobiology of sleep. *Handbook of Clinical Neurology*, 98: 191-203.

Feinstein, D. L., Kalinin, S., & Braun, D. (2016). Causes, consequences, and cures for neuroinflammation mediated via the locus coeruleus: noradrenergic signaling system. *Journal of Neurochemistry*, 139: 154-178.

Fukuyama, R., Izumoto, T., & Fushiki, S. (2001). The Cerebrospinal Fluid Level of Glial Fibrillary Acidic Protein Is Increased in Cerebrospinal Fluid from Alzheimer's Disease Patients and Correlates with Severity of Dementia. *European Neurology*, 46: 35-38.

Havekes, R., Heckman, P. R., Wams, E. J., Stasiukonyte, N., Meerlo, P., & Eisel, U. L. (2019). Alzheimer's disease pathogenesis: The role of disturbed sleep in attenuated brain plasticity and neurodegenerative disease. *Cellular Signaling*, 64: 1-8.

Hong, Y., Xu, J., Hu, Y., Li, L., Dong, Z., Zhu, T., & Wei, Y. (2018). Neuroinflammation and neuroimmunomodulation in Alzheimer's disease. *Current Pharmacology Reports*, 4: 408-413.

Iliff, J. J., Wang, M., Liao, Y., Plogg, B. A., Peng, W., Gundersen, G. A., . . . Nedergaard, M. (2012). A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid β. *Science Translational Medicine*, 4: 1-11.

Jessen, N. A., Finmann-Munk, A. S., Lundgaard, I., & Nedergaard, M. (2015). The glymphatic system: a beginner's guide. *Neurochemical Research*, 40: 2583-2599.

Ju, Y. E., Lucey, B. P., & Holtzmann, D. M. (2014). Sleep and Alzheimer disease pathology - a bidirectional relationship. *Nature Reviews Neurology*, 10: 115-119.

Ju, Y. S., McLeland, J. S., Toedebusch, C. D., Xiong, C., Fagan, A. M., Duntley, S. P., . . . Holtzman, D. M. (2013). Sleep Quality and Preclinical Alzheimer Disease. *JAMA Neurology*, 70: 587-593.

Kisler, K., Nelson, A. R., Montagne, A., & Zlokovic, B. V. (2017). Cerebral blood flow regulation and neurovascular dysfunction in Alzheimer's disease. *Nature Reviews Neuroscience*, 18: 419-434.

Krueger, J. M., Churchill, L., & Rector, D. M. (2009). Cytokines and other Neuromodulators. In R. Stickgold, & M. Walker, *The Neuroscience of Sleep* (p. 374). Academic Press.

Krueger, J. M., Majde, J. A., & Rector, D. M. (2011). Cytokines in immune function and sleep regulation. *Handbook of Clinical Neurology*, 98: 229-240.

Krysta, K., Krzystanek, M., Bratek, A., & Krupka-Matuszczyk, I. (2015). Sleep and inflammatory markers in different psychiatric disorders. *Journal of Neural Transmission*, 124: 179-186.

Lundgaard, I., Lu, M. L., Yang, E., Peng, W., Mestre, H., Hitomi, E., . . . Nedergaard, M. (2017). Glymphatic clearance controls state-dependent changes in brain lactate concentration. *Journal of Cerebral Blood Flow & Metabolism*, 37: 2112-2124.

Mander, B. A., Winer, J. R., Jagust, W. J., & Walker, M. P. (2016). Sleep: a novel mechanistic pathway, biomarker, and treatment target in the pathology of Alzheimer's disease? *Trends in Neurosciences*, 39: 552-566.

Musiek, E. S., Xiong, D. D., & Holtzmann, D. M. (2015). Sleep, circadian rhythms, and the pathogenesis of Alzheimer Disease. *Experimental & Molecular Medicine*, 47: 1-8.

Pak, V. M., Onen, S., Bliwise, D. L., Kutner, N. G., Russell, K. L., & Onen, F. (2020). Sleep Disturbances in MCI and AD: Neuroinflammation as a Possible Mediating Pathway. *Frontiers in Aging Neuroscience*, 12: 1-11.

Preston, S. D., Steart, P. V., Wilkinson, A., Nicoll, J. A., & Weller, R. O. (2003). Capillary and arterial amyloid angiopathy in Alzheimer's disease: defining the perivascular route for the elimination of amyloid-beta from the human brain. *Neuropathology and Applied Neurobiology*, 29: 106-117.

Rissling, M., & Ancoli-Israel, S. (2009). Sleep in Aging. In R. Stickgold, & M. P. Walker, *The Neuroscience of Sleep* (p. 374). Academic Press.

Roh, J. H., Huang, Y., Bero, A. W., Kasten, T., Steward, F. R., Bateman, R. J., & Holtzman, D. M. (2012). Disruption of the Sleep-Wake Cycle and Diurnal Fluctuation of Amyloid-b in Mice with Alzheimer's Disease Pathology. *Science Translational Medicine*, 4: 1-10.

Ross, J. A., McGonigle, P., & Bockstaele, E. J. (2015). Locus coeruleus, norepinephrine and Aβ peptides in Alzheimer's disease. *Neurobiology of Stress*, 2: 73-84.

Salminen, A., Ojala, J., Suuronen, T., Kaarniranta, K., & Kauppinen, A. (2008). Amyloid-beta oligomers set fire to inflammasomes and induce Alzheimer's pathology. *Journal of Cellular and Molecular Medicine*, 12: 2255-2262.

Schwartz, J. R., & Roth, T. (2008). Neurophysiology of sleep and wakefulness: basic science and clinical implications. *Current Neuropharmacology*, 6: 367-378.

Sprecher, K. E., Koscik, R. L., Carlsson, C. M., Zetterberg, H., Blennow, K., Okonkwo, O., . . . Bendlin, B. B. (2017). Poor sleep is associated with CSF biomarkers of amyloid pathology in cognitively normal adults. *Neurology*, 89: 445-453.

Valentino, R. J., & Van Bockstaele, E. (2008). Convergent regulation of locus coeruleus activity as an adaptive response to stress. *European Journal of Pharmacology*, 194-203.

Vanderheyden, W. M., Lim, M. M., Musiek, E. S., & Gerstner, J. R. (2018). Alzheimer's disease and sleep-wake disturbances: amyloid, astrocytes, and animal models. *The Journal of Neuroscience*, 38: 2901-2910.

Weller, R. O., Subash, M., Preston, S. D., Mazanti, I., & Carare, R. O. (2008). Perivascular drainage of amyloid-beta peptides from the brain and its failure in cerebral amyloid angiopathy and Alzheimer's disease. *Brain Pathology*, 18: 253-266.

Xia, M., Li, X., Yang, L., Ren, J., Sun, G., Qi, S., . . . Li, B. (2018). The ameliorative effect of fluoxetine on neuroinflammation induced by sleep deprivation. *Journal of Neurochemistry*, 146: 63-75.

Xie, L., Kang, H., Xu, Q., Chen, M. J., Liao, Y., Thiagarajan, M., . . . Nedergaard, M. (2013). Sleep drives metabolite clearance from the adult brain. *Science*, 342: 373-377.

Zhu, Y., Fenik, P., Zhan, G., Xin, R., & Veasey, S. C. (2015). Degeneration in Arousal Neurons in Chronic Sleep Disruption Modeling Sleep Apnea. *Frontiers in Neurology*, 6: 1-8.