Sleep in Alzheimer's disease

The role of the immune and glymphatic system

Essay Master Biomedical Sciences

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Date: 10-11-2023

Abstract

Alzheimer's disease (AD) is a progressive neurodegenerative disorder which affects many areas of the brain, including the hippocampus and cerebral cortex. AD is characterised by amyloid-β plaques, neurofibrillary tangles due to hyperphosphorylated tau, neuronal loss, and brain atrophy. Patients with AD experience many different symptoms, ranging from memory loss, disorientation, and apathy to sleep disturbances. More specifically, between 14-69% of patients with AD experience sleep disturbances and have disrupted sleep. Results from subjective and objective sleep studies show that patients with AD have decreased sleep efficiency, total sleep time, stage 3 of non-rapid eye movement (NREM; slow wave sleep), rapid eye movement (REM) sleep, and fast sleep spindles compared to age-related healthy individuals. In addition, patients with AD experience increased stages 1 and 2 of NREM sleep, more sleep fragmentation, and higher wake after sleep onset. These sleep disturbances are associated with the accumulation of amyloid-β and tau in the central nervous system. In addition, increased levels of amyloid-β and tau are associated with increased wakefulness and decreased REM sleep, NREM sleep, and fast sleep spindles. Thus, there is a positive feedback loop between sleep disturbances and AD pathology. Furthermore, both the immune system and the glymphatic system play an important role in this positive feedback loop. Chronic sleep disturbances lead to an increase in pro-inflammatory cytokines (IL-6, IL-1β, CRP, and TNF-α), microglia, and astrocytes. This neuroinflammation is associated with the accumulation of amyloid-β and tau proteins. Furthermore, chronic sleep disturbances may also cause increased production of amyloid-β, increased release of tau, glial aquaporin-4 (AQP4) depolarization, and astrocyte-induced alteration in perivascular space. The latter two can lead to reduced glymphatic flux and clearance of amyloid-β and tau. The reduction in glymphatic flux is associated with the accumulation of amyloid-β and tau proteins in the central nervous system. Thus, both the immune system and the glymphatic system play an important role in the positive feedback loop between chronic sleep disturbances and AD pathology.

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Introduction

Alzheimer's disease (AD) is currently the seventh leading cause of death worldwide (WHO, 2023). AD is the most common form of dementia, accounting for approximately 60-70% of all dementia cases (WHO, 2023). Worldwide, more than 55 million people have dementia, with every year around 10 million new cases (WHO, 2023). Alzheimer's disease is a progressive neurodegenerative disorder which affects many areas of the brain, including the hippocampus and cerebral cortex (Wang & Holtzman, 2020). Due to the neurodegeneration in the brain, AD causes a progressive decline in cognition and memory (Wang & Holtzman, 2020). The pathology and hallmarks in AD can be observed from a macroscopic and microscopic perspective. At the macroscopic level, there is progressive atrophy of the brain (Holtzman et al., 2011). At the microscopic level, neuronal loss, amyloid-β plaques, and neurofibrillary tangles can be found (Holtzman et al., 2011). Amyloid-β is derived from the gene amyloid-β precursor protein (APP), located on chromosome 21 (Holtzman et al., 2011). Amyloid-β is cleaved from this APP by β-secretase and ɣ-secretase, forming amyloid-β peptides of different lengths (38-43 amino acids) (Holtzman et al., 2011). Normally, amyloid-β is secreted into the brain interstitial fluid (ISF) after being produced by neurons (Kang et al., 2009). However, the soluble monomeric amyloid-β peptides can also self-aggregate and form extracellular insoluble oligomers (β-sheets) and plaques due to the hydrophobic nature of these peptides (Wang & Holtzman, 2020). Tau is synthesized in all neurons and the function of tau is to stabilize microtubules and to bind to tubulin (Holtzman et al., 2011). In AD, tau can become hyperphosphorylated and starts to self-aggregate (β-sheets), forming neurofibrillary tangles (NFT) in the cell bodies of neurons (Holtzman et al., 2011). Tau pathology in the central nervous system has a strong correlation with neurodegeneration and cognitive impairment, unlike amyloid-β (Wang & Holtzman, 2020). There is a familial and sporadic form of Alzheimer's disease. The minority of AD cases is familial and are caused by a genetic mutation in presenilin 1/2 or APP, resulting in an increased production of amyloid-β (Vasciaveo et al., 2023). The majority of AD cases is sporadic and is caused by the accumulation of amyloid- β and tau and a reduced clearance of these proteins from the brain (Vasciaveo et al., 2023).

The symptoms of AD are heterogeneous, with every patient experiencing different types and severity of symptoms. The most common symptoms are memory loss, agitation, apathy, irritability, depression, disorientation, and disturbed sleep (Uddin et al., 2020; Zhou et al., 2019). More specifically, between 14-69% of patients with Alzheimer's disease experience sleep disturbances (Zhou et al., 2019). Sleep changes and amyloid-β aggregation are observed in the preclinical stages of AD before cognitive symptoms are present (Wang & Holtzman, 2020). Further, sleep disturbances and increased wakefulness are predictive risk factors for the development of cognitive decline and neurodegeneration by the accumulation of amyloid-β and tau (Wang & Holtzman, 2020). In contrast, normal sleep is related to the enhancement of cognitive functions, improvement of consolidation of memory, and the clearance of amyloid-β and tau proteins by the glymphatic system (Vasciaveo et al., 2023). The most important effects of normal sleep and chronic sleep disturbances are depicted in Figure 1.
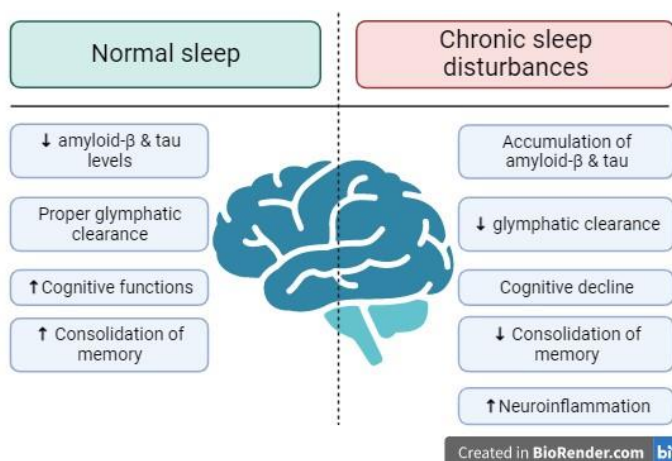

Figure 1: The effects of normal sleep compared to chronic sleep disturbances. Normal sleep is associated with decreased levels of amyloid-6 and tau due to proper clearance of the proteins by the glymphatic system. Further, normal sleep is related to enhancement of cognitive functions and improvement of consolidation of memory. In contrast, chronic sleep disturbances is associated with accumulation of amyloid-β and tau due to decreased clearance of the proteins by the glymphatic system. Further, it is associated with cognitive decline, worse consolidation of memory, and increased activation of neuroinflammation. Sources: (Vasciaveo et al., 2023; Wang & Holtzman, 2020)

The type of sleep is based on the behaviour and physiology of an individual. The behavioural component consists of a specific sleeping position, slow eye movements, reduction in the response to external stimuli, lack of mobility, and impaired cognitive function (Chokroverty, 2010). The physiological component is measured by electroencephalography (EEG) to record brain activity, electromyography (EMG) to record muscle activity, and electro-oculography (EOG) to record eye movements (Chokroverty, 2010). Sleep consists of two stages with different functions: rapid eye movement (REM) sleep and non-rapid eye movement (NREM) sleep. About two-thirds of sleep consists of NREM sleep with four stages and the other one-third of sleep consists of REM sleep (Chokroverty, 2010). REM sleep is characterised by rapid eye movement, fast rhythms and theta waves, and no muscle activity, whereas NREM sleep is characterised by slow eye movements, K-complexes and spindles (stage 2 of NREM sleep), slow wave activity (delta waves; stage 3 of NREM sleep), and reduction in muscle activity (Chokroverty, 2010). REM and NREM sleep follow each other, with each cycle lasting from 90 to 110 minutes in humans (Chokroverty, 2010). Sleep spindles during slow-wave sleep are linked to sleep-dependent cognition and memory consolidation (Cox et al., 2012).

Sleep changes with ageing, resulting in a reduction in slow-wave NREM sleep, an increase in lighter sleep, increased sleep fragmentation, and changes in REM sleep (Wang & Holtzman, 2020). Results from subjective and objective sleep studies show that patients with AD have decreased sleep efficiency, total sleep time, NREM slow wave sleep, and REM sleep compared to age-related healthy individuals. In addition, there is increased latency to REM sleep, stages 1 and 2 of NREM sleep, and wake after sleep onset (Kent et al., 2022; Tadokoro et al., 2020; Zhao et al., 2016; Zhou et al., 2019). Furthermore, the hypothalamus, basal forebrain, midbrain, pons, thalamus, and brainstem are sleep-regulating centres in the brain, which are affected by AD pathology (Holth et al., 2017).

Regular sleep has an important effect on metabolic, endocrine, and immune pathways (Garbarino et al., 2021). Further, AD has a neuroinflammatory component characterised by activated microglia, astrocytes, and pro-inflammatory cytokines surrounding amyloid-β plaques and affected neurons (Green et al., 2020). Sleep disturbances and circadian dysfunction can lead to the activation of microglia and astrocytes, which in turn will lead to the activation of pro-inflammatory cytokines and neuroinflammation (Uddin et al., 2020). The activation of glial cells, pro-inflammatory cytokines, and neuroinflammation are all positively correlated with the accumulation of amyloid-β and tau in the central nervous system (CNS) (Liu et al., 2020; Mander et al., 2022; Vasciaveo et al., 2023). More specifically, the upregulation of pro-inflammatory cytokines leads to increased production and decreased clearance of amyloid-β (Liu et al., 2020). The accumulation of amyloid-β and tau can in turn lead to sleep disturbances, neurodegeneration and neuroinflammation (Mander et al., 2022). There is a positive feedback loop between sleep and inflammation, which will lead to more AD pathology and further disease progression.

The glymphatic system is the waste clearance system of the central nervous system by removing soluble metabolites and proteins, such as amyloid-β and tau, from the CNS (Christensen et al., 2021). The activity of the glymphatic system is the highest during sleep, resulting in more clearance of amyloid-β and tau proteins during sleep and accumulation of these proteins during wakefulness (Kang et al., 2009). The glymphatic system is highly dependent on glial aquaporin-4 (AQP4) water channels (Iliff et al., 2012). Poor sleep quality can cause depolarization of AQP4 and loss of localized AQP4 expression on astrocytic end-feet at the perivascular space (PVS) (Hauglund et al., 2020). Reduced perivascular APQ4 localisation is strongly associated with increased amyloid-β and tau pathology (Zeppenfeld et al., 2017). Thus, sleep disturbances can reduce glymphatic flux by depolarization of AQP4, leading to decreased ability to clear amyloid-β and tau proteins from the CNS, leading to more AD pathology and further disease progression.

The research question addressed in this thesis is: What is the role of the immune system and glymphatic system on sleep in Alzheimer's disease? This thesis aims to summarize the main findings of subjective- and objective sleep studies in patients with Alzheimer's disease, discuss the effect of sleep disturbance on AD pathology, and discuss the role of the immune system and glymphatic system on sleep disturbances in Alzheimer's disease. A better understanding of the role of the immune system and glymphatic system on sleep in Alzheimer's disease can help to find treatment for the observed

sleep disturbances and help to decrease the accumulation of amyloid-β and tau proteins to slow down the progression of the disease.

Main body

Sleep in Alzheimer's disease

Approximately one in four people with Alzheimer's disease experience significant sleep disturbances, including frequent awakenings and various sleep disorders, such as hypersomnia, insomnia, or disruptions in the circadian rhythm (Kang et al., 2017; Moran et al., 2005). To study the possible correlation between sleep and Alzheimer's disease, subjective and objective studies have been performed. Subjective studies use questionnaires to gather information about sleep quality and quantity and sleep disturbances or disorders, whereas objective studies use polysomnography (PSG) or actigraphy to objectively measure sleep. In this section, both subjective and objective studies on sleep in Alzheimer's disease will be discussed. Furthermore, the effect of sleep on amyloid-β and tau levels will also be discussed.

SUBJECTIVE SLEEP STUDIES IN ALZHEIMER'S DISEASE

There are many different questionnaires to assess subjective sleep. Some questionnaires measure sleep quality and quantity, whereas others measure the presence of sleep disorders or daytime sleepiness. See Box 1 for a description of the questionnaire used by the studies discussed in this section. The prevalence of sleep disturbances can be measured by using the Neuropsychiatric Inventory (NPI) questionnaire. A meta-analysis by Zhao et al., (2016) shows that the prevalence of sleep disorders and disturbances in Alzheimer's disease ranges from 14% to 69%, with an overall pooled prevalence of 39%. Other studies report the prevalence of sleep disturbances in Alzheimer's disease patients to be 40% (Yatawara et al., 2018), 35.1% (Fernandez-Martinez et al., 2008), and 48.8% (Zhou et al., 2019). Sleep quality can be measured by the Pittsburgh Sleep Quality Index (PSQI) questionnaire and subjective daytime sleepiness can be measured by the Epworth Sleepiness Scale (ESS) questionnaire. Subjective studies show that patients with AD have a significantly longer sleep duration, longer time in bed, lower sleep quality (higher PSQI score), and lower sleep efficiency (Shin et al., 2014; Zhou et al., 2019). In contrast, other studies have found that patients with AD have higher sleep quality and lower incidences of insomnia (Most et al., 2012; Tadokoro et al., 2020). Another study found no significant difference in sleep quality between patients with AD and healthy-age-related controls (Gorgoni et al., 2016).

Box 1: Description of the most used subjective sleep questionnaires

Questionnaire	Description
Pittsburgh Sleep Quality Index (PSQI) (Buysse et al., 1989)	The PSQI questionnaire consists of nineteen questions and assesses seven components of sleep: sleep quality, sleep and daytime dysfunction, sleep latency, sleep duration, sleep disturbance, and use of sleep medication. Each component is weighted on a 0-3 scale, and the seven components are summed to yield a global PSQI score. The PSQI score can range from 0-21, with a higher score (score above 5) indicating worse sleep quality.
Epworth Sleepiness Scale (ESS) (Johns, 1991)	The ESS questionnaire is used to assess subjective daytime sleepiness. Subjects are asked to rate on a scale of 0-3 how likely they would doze off or fall asleep in eight situations. A score of 10 or higher indicates excessive daytime sleepiness.
Neuropsychiatric Inventory (NPI) (Cummings et al., 1994)	The NPI questionnaire is designed to assess twelve behavioural disturbances commonly observed in individuals with dementia. Frequency and severity of the following symptoms are measured: anxiety, apathy, agitation and/or aggression, aberrant motor activity, disinhibition, dysphoria, delusions, euphoria, eating abnormalities, hallucinations, irritability and/or lability, and sleep disturbances.

OBJECTIVE SLEEP STUDIES ON SLEEP IN ALZHEIMER'S DISEASE

To get a more precise estimate of sleep quality and quantity, objective sleep measurement can be performed. These measurements are done using polysomnography (PSG) or actigraphy. The recordings done during polysomnography are electroencephalography (EEG) to record brain activity, electro-oculography (EOG) to record eye movements, and electromyography (EMG) to record muscle activity (Casagrande et al., 2022). The recordings during actigraphy are done by an actigraph that patients wear on their wrist for a certain period and is based on movement (Martin & Hakim, 2011). Studies show that REM sleep is decreased in patients with AD (Dykierek et al., 1998; Liguori et al., 2014, 2017; Maestri et al., 2015; Montplaisir et al., 1995; Prinz et al., 1982). In addition, REM sleep latency is increased (Liguori et al., 2014, 2017) and REM sleep periods are shorter (Montplaisir et al., 1995). Furthermore, stages 3 and 4 of NREM sleep (slow-wave-sleep) are decreased (De Gennaro et al., 2017; Gorgoni et al., 2016; Liguori et al., 2014; Maestri et al., 2015; Prinz et al., 1982), while stage 1 and 2 of NREM sleep are increased in patients with AD (De Gennaro et al., 2017; Liguori et al., 2014, 2017; Maestri et al., 2015). Moreover, there is a decrease in the density of fast sleep spindles and K-complexes found in patients with AD (De Gennaro et al., 2017; Dykierek et al., 1998; Montplaisir et al., 1995; Rauchs et al., 2008). Further, patients with AD have decreased sleep efficiency (Hoch et al., 1988; Hot et al., 2011; Liguori et al., 2014, 2017), lower total sleep time, more wakefulness after sleep onset, higher sleep latency, and more sleep fragmentation (Hoch et al., 1988; Liguori et al., 2014, 2017; Maestri et al., 2015).

Overall, from both subjective and objective studies, it can be concluded that patients with Alzheimer's disease have many different sleep disturbances and sleep problems. Further, objective studies are more precise and provide more information about the sleep status and sleep problems of patients. However, considering that objective sleep studies need to be performed in the hospital and are expensive, subjective ways to assess sleep should also be used in patients with Alzheimer's disease.

SLEEP, AMYLOID-B AND TAU

Sleep disturbances and increased wakefulness are predictive risk factors for the development of cognitive decline and neurodegeneration by the accumulation of amyloid-β and tau (Holtzman et al., 2011). In a cross-sectional study by Borges et al., (2021), participants underwent a positron emission tomography with Pittsburgh compound, [^{11}C]PiB-PET-CT, to image amyloid disposition and to mark participants amyloid-positive or amyloid-negative. The results of this study show that the amyloid-positive participants (indicative of prodromal Alzheimer's disease) stay for a longer time in bed and have a lower sleep efficiency compared to amyloid-negative participants.

A study by Ju et al., (2013) measured the association between sleep disturbances and the presence of amyloid-β accumulation. Low levels of Aβ42 in the cerebrospinal fluid (CSF) are associated with the presence of amyloid plaques in the brain. The results of this study show that participants with low CSF Aβ42 levels (and amyloid accumulation) have worse sleep quality, lower sleep efficiency, and higher wake time after sleep onset. A study by Chu et al., (2023) measured the association between subjective sleep characteristics and AD-related biomarkers, such as Aβ42 and Aβ40. Low concentrations of plasma Aβ42/Aβ40 ratio or high concentrations of Aβ40/Aβ42 ratio are associated with the presence of amyloid plaques in the brain (Nakamura et al., 2018). The results of this study show that longer sleep duration is associated with higher Aβ burden and lower sleep efficiency is associated with lower Aβ42/ Aβ40 ratio in amyloid-positive participants. In addition, disrupted sleep by sleep deprivation for 36 hours resulted in higher overnight CSF Aβ levels (Lucey et al., 2018) and sleep fragmentation leads to an increase in hippocampal amyloid-β levels (primarily Aβ42) (Duncan et al., 2022).

Furthermore, sleep deprivation causes the increase of non-phosphorylated tau (T181, S202, and T217) and phosphorylated tau (pT181 and pT217) (Barthélemy et al., 2020). Higher sleep latency is associated with higher plasma levels of p-tau-181 (Chu et al., 2023). Importantly, high levels of plasma p-Tau-181 are associated with tau aggregation and amyloid-β pathology (Moscoso et al., 2021). In addition, disruption in the sleep-wake cycle due to sleep deprivation in an AD-mouse model results in altered tau metabolism leading to conformational changes in tau (Di Meco et al., 2014). These

conformational changes represent an early stage of the aggregation of tau and increase the insolubility of tau, inducing neurofibrillary tangles and tau pathology. In an AD-mouse model (5xFAD), sleep fragmentation leads to an increase in amyloid-β accumulation in the lateral septum, retrosplenial cortex, dentate gyrus, and basolateral amygdala and to the presence of tau phosphorylation in the dentate gyrus (Vasciaveo et al., 2023).

On the other hand, the increase in amyloid-β plaques in the hippocampus and striatum leads to disruptions in the sleep-wake cycle (Roh et al., 2012). Specifically, there is an increase in wakefulness and a decrease in REM and NREM sleep. Furthermore, increased levels of amyloid-β and tau are associated with decreased expression of NREM fast sleep spindle located in the frontal cortex in humans (Mander et al., 2022).

The role of the immune system and glymphatic system on sleep in Alzheimer's disease

Based on subjective and objective studies, patients with Alzheimer's disease have many different problems with sleep. Furthermore, disturbed sleep leads to more accumulation of amyloid-β and tau proteins, resulting in more disease progression, memory impairments, and dysregulation of the sleep-wake cycle (Uddin et al., 2020). Both the immune system and the glymphatic system have been linked to both sleep and Alzheimer's disease, providing a possible link between sleep and Alzheimer's disease. In this section, both the role of the immune system and the glymphatic system on sleep in AD will be discussed.

THE ROLE OF THE IMMUNE SYSTEM ON SLEEP IN ALZHEIMER'S DISEASE

Alzheimer's disease has a neuroinflammatory component characterised by activated microglia, astrocytes, and pro-inflammatory cytokines surrounding amyloid-β plaques and affected neurons (Green et al., 2020). Inflammation can have a beneficial role in AD by clearing apoptotic cells and cellular debris (Green et al., 2020). However, amyloid-β and pro-inflammatory cytokines can also activate microglia cells, leading to the release and production of (pro)inflammatory cytokines, chemokines, and cytotoxic factors (Lull & Block, 2010). The overexpression of these pro-inflammatory cytokines leads to disrupted synaptic pruning, disrupted clearance mechanisms, decreased neuronal survival, and disrupted sleep-wake cycles (Green et al., 2020).

A meta-analysis of 72 studies showed that sleep disturbances are associated with higher levels of pro-inflammatory cytokines interleukin-6 (IL-6) and C-reactive protein (CRP) (Irwin et al., 2016). In addition, partial sleep deprivation in humans resulted in higher levels of IL-6 and tumor necrosis factor (TNF-α) monocyte production and a two-fold increase in transcription of TNF-α messenger RNA and a three-fold increase in IL-6 messenger RNA (Irwin, 2006). Furthermore, partial sleep deprivation in humans resulted in greater activation of STAT 1 and STAT 5 and NF-κB (Irwin et al., 2008, 2015). STAT is an abbreviation for signal transducer and activator of transcription and this STAT family promotes an inflammatory microenvironment (Irwin et al., 2015). Nuclear factor (NF) – κB is a transcription factor that plays an important role in the inflammatory signalling cascade (Irwin et al., 2008). In addition, experimental studies performed in rodents show that 24-hour sleep deprivation and chronic sleep restriction lead to increased activation of pro-inflammatory cytokines, including IL-6, IL-1β, and TNF-α, and glial cells (Arjmandi-rad et al., 2022; Liu et al., 2020).

Fragmentation of the sleep-wake rhythm, or sleep fragmentation, leads to increased expression of reactive microglia in the hippocampus in an AD-mouse model (Duncan et al., 2022). In addition, sleep fragmentation in an AD-mouse model (5xFAD) leads to increased activation of microglia in the lateral septum, retrosplenial cortex, motor-sensory cortex, dentate gyrus, thalamus, hypothalamus, and amygdala (Vasciaveo et al., 2023). Sleep fragmentation in the same AD-mouse model leads to an increase in GFAP+ signal in the lateral septum, retrosplenial cortex, dentate gyrus, and basolateral amygdala (Vasciaveo et al., 2023). More GFAP+ signal is an indication of astrogliosis, the activation of reactive astrocytes in the CNS in response to tissue damage, which leads to the formation of glial scar that can inhibit regeneration of axons (Yu et al., 2012). The above-mentioned brain regions in which microglia and astrocytes are activated are all involved in the sleep-wake cycle and regulation

of sleep. Overall, disrupted sleep or sleep fragmentation leads to an increased upregulation of pro-inflammatory cytokines, activation of astrocytes and microglia, and neuroinflammation.

Furthermore, the upregulation of pro-inflammatory cytokines and neuroinflammation observed after sleep disruption is positively correlated with Aβ42 accumulation (Liu et al., 2020). In addition, pro-inflammatory cytokines IL-1β and TNF-α are positively correlated with BACE-1 and RAGE expression, and negatively correlated with LRP-1 expression (Liu et al., 2020). The β-site APP cleaving enzyme 1 (BACE1) is an enzyme that cleaves APP, resulting in the production of amyloid-β (Liu et al., 2020). Lipoprotein receptor-related protein 1 (LRP1) and receptor of advanced glycation end products (RAGE) are important transporters of amyloid-β (Cai et al., 2016). RAGE regulates the influx of amyloid-β from the circulation into the brain, while LRP1 regulates the efflux of amyloid-β from the brain into the circulation via the blood-brain barrier (BBB) (Cai et al., 2016). Increased BACE1 expression leads to increased production of amyloid-β, whereas increased expression of RAGE and decreased expression of LRP1 cause reduced clearance of amyloid-β. Furthermore, the activation of astrocytes and microglial is correlated with the accumulation of amyloid-β in the same brain regions (Vasciaveo et al., 2023). In addition, low-grade CNS inflammation and increased activation of microglia are associated with higher levels of amyloid-β proteins, tau proteins, and neuronal integrity (Mander et al., 2022).

Further, a study by Mander et al., (2022) found that higher levels of amyloid-β proteins and tau proteins are associated with decreased expression of NREM fast sleep spindle located in the frontal cortex in humans. The accumulation of amyloid-β and tau proteins also leads to increased wakefulness and decreased REM and NREM sleep in an AD-mouse model (Roh et al., 2012).

Thus, these results show that there is a positive feedback loop between sleep disturbances, inflammation, and AD pathology. Sleep disturbances may lead to the activation of pro-inflammatory cytokines, microglia, and astrocytes. In turn, this neuroinflammation is associated with increased levels of amyloid-β and tau proteins, which is turn are associated with further sleep disturbances.

The role of the glymphatic system on sleep in Alzheimer's disease

The glymphatic system is the waste clearance system of the central nervous system by removing soluble metabolites and proteins, such as amyloid-β and tau, from the CNS (Christensen et al., 2021). The influx pathway of the glymphatic system is via the para-arterial cerebral spinal fluid (CSF), the clearance pathway via the paravenous interstitial fluid (ISF), and the connection between the two pathways is via the intracellular trans-astrocytic pathway (Iliff et al., 2012). The connection between the pathways (exchange of CSF and ISF) is highly dependent on glial aquaporin-4 (AQP4) water channels (Iliff et al., 2012).

Amyloid-β levels in the ISF in rodents and the CSF in humans fluctuate over a period of 24 hours, with increased levels during the day and decreased levels during the night (Kang et al., 2009). More specifically, amyloid-β levels in the ISF positively correlated with time spent awake and negatively correlated with time spent asleep, with the negative correlation being the strongest with slow-wave sleep (stage 3 of NREM sleep) (Kang et al., 2009). Slow wave delta oscillations are correlated with an increase in glymphatic influx (Hablitz et al., 2019). A study by Kang et al., (2009), measured the effect of sleep deprivation on the ISF amyloid-β levels in mice. The result of this study shows that sleep deprivation increases the ISF levels of amyloid-β above the levels of amyloid-β during the light period. Further, mice spent more time sleeping following sleep deprivation and the sleep induced an immediate reduction in ISF amyloid-β levels. In a study by Lucey et al., (2018), sleep disruption leads to higher overnight levels of CSF amyloid-β levels in humans. More specifically, disruption in slow wave activity is correlated with an increase in amyloid-β levels in the CSF in humans (Ju et al., 2017). Further, tau is also strongly increased in the ISF in mice and the CSF in humans after sleep deprivation (Holth et al., 2019). On the other hand, amyloid-β accumulation in the hippocampus and striatum can reduce the diurnal fluctuation of ISF amyloid-β and worsen the sleep-wake cycle (especially sleep efficiency) in an AD-mouse model (Roh et al., 2012).

The activation of reactive astrocytes by either circadian dysfunction or amyloid-β accumulation leads to depolarization of AQP4 expression, which will decrease the clearance function of the

glymphatic system (Lan et al., 2018). AQP4 knockout mice results in impaired glymphatic function and a reduction of 55% in the clearance of amyloid-β (Iliff et al., 2012). The reduction in amyloid-β clearance could be due to reduced blood-brain-barrier (BBB) transport and/or reduced efflux of CSF to ISF (Rasmussen et al., 2018). In addition, a genetic study performed by Rainey-Smith et al., (2018) found that single nucleotide polymorphisms (SNPs) of the AQP4 gene are related to both increased amyloid-β burden and decreased sleep quality. A study by Vasciaveo et al., (2023) measured the effects of sleep fragmentation in wild-type and 5xFAD mouse models. The 5xFAD mouse model expresses the three mutations of the human APP gene and two mutations of the human presenilin 1 (PSEN1) gene. The results of this study show that sleep fragmentation increases the AQP4 signal in two-months old the AD-mouse model, whereas sleep fragmentation decreases the AQP4 signal in the six-month-old AD-mouse model and the wild-type mice. In addition, poor sleep quality induces AQP4 depolarization, which is the loss of AQP4 expression at the astrocytic end-feet, resulting in impaired glymphatic clearance and amyloid-β accumulation (Liu et al., 2017). Further, patients with AD have a reduction in perivascular AQP4 localisation and this reduction is strongly associated with amyloid-β and tau pathology (Zeppenfeld et al., 2017).

Discussion

SLEEP AND ALZHEIMER'S DISEASE

Subjective studies on sleep in Alzheimer's disease have found that the prevalence of sleep disturbances is around 39% (Zhao et al., 2016). Some subjective studies show that patients with AD have longer sleep duration, longer time in bed, lower sleep efficiency, and lower sleep quality, whereas other studies found higher sleep quality or no difference in sleep quality (Gorgoni et al., 2016; Most et al., 2012; Shin et al., 2014; Tadokoro et al., 2020; Zhou et al., 2019). These inconclusive results can be explained by the fact that subjective sleep questionnaires are dependent on memory processes. Memory is often impaired in patients with Alzheimer's disease, leading to misperception and the tendency to either exaggerate or underestimate the number and severity of symptoms related to sleep. Further, there are also differences in memory impairment of the patients between the studies, leading to overall differences in the outcome. To get a more precise estimate of sleep quality and quantity, objective sleep measurements, such as PSG or actigraphy, can be used.

The objective sleep studies have found that patients with AD have reduced REM sleep (Dykierek et al., 1998; Liguori et al., 2014, 2017; Maestri et al., 2015; Montplaisir et al., 1995; Prinz et al., 1982), reduced stage 3 and 4 of NREM sleep (De Gennaro et al., 2017; Gorgoni et al., 2016; Liguori et al., 2014; Maestri et al., 2015; Prinz et al., 1982), increased stage 1 and 2 of NREM sleep (De Gennaro et al., 2017; Liguori et al., 2014, 2017; Maestri et al., 2015), reduced density of K-complexes and fast sleep spindles (De Gennaro et al., 2017; Dykierek et al., 1998; Montplaisir et al., 1995; Rauchs et al., 2008), lower sleep efficiency, higher sleep latency, and more sleep fragmentation (Hoch et al., 1988; Liguori et al., 2014, 2017; Maestri et al., 2015). These results show that AD patients have many sleep disturbances and circadian rhythm disruptions.

Importantly, sleep disturbances and circadian rhythm disruption, such as longer sleep duration, lower sleep efficiency, worse sleep quality, and sleep deprivation, are associated with increased accumulation of amyloid-β and tau proteins (Barthélemy et al., 2020; Chu et al., 2023; Di Meco et al., 2014; Lucey et al., 2018; Vasciaveo et al., 2023). On the other hand, the accumulation of amyloid-β and tau is associated with increased wakefulness and decreased REM sleep, NREM sleep, and fast sleep spindles (Mander et al., 2022; Roh et al., 2012). The results of these studies indicate a bidirectional relationship between sleep and AD pathology. Further, there is also a positive feedback loop, where both sleep and accumulation of amyloid-β and tau have a negative effect on each other. Thus, sleep disturbances can lead to the progression of neurodegeneration, and in turn, the accumulation of amyloid-β and tau can worsen the sleep-wake cycle (Figure 2).
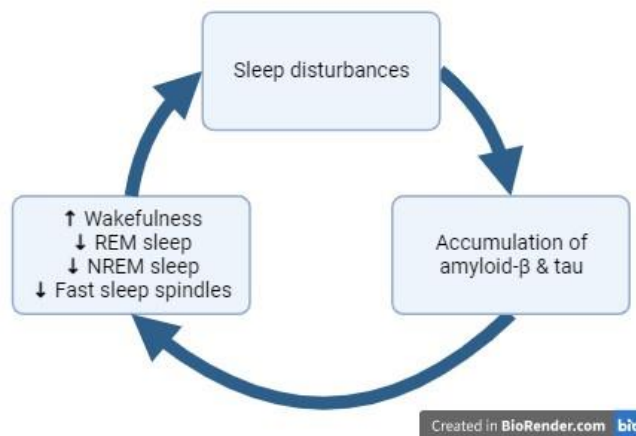
Figure 2: Proposed positive feedback loop between sleep disturbances and the accumulation of amyloid-β and tau. Different sleep disturbances, such as longer sleep duration, lower sleep efficiency, worse sleep quality, and sleep deprivation, are associated with increased accumulation of amyloid-β and tau (Barthélemy et al., 2020; Chu et al., 2023; Di Meco et al., 2014; Lucey et al., 2018; Vasciaveo et al., 2023). The increase in AD pathology is associated with increased wakefulness and decreased REM sleep, NREM sleep, and fast sleep spindles (Mander et al., 2022; Roh et al., 2012).

THE ROLE OF THE IMMUNE SYSTEM ON SLEEP IN ALZHEIMER'S DISEASE

Alzheimer's disease has a neuroinflammatory component which is characterised by activated astrocytes, microglia, and pro-inflammatory cytokines surrounding amyloid-β plaques and affected neurons (Green et al., 2020). Sleep disturbances are associated with higher levels of pro-inflammatory cytokines, such as IL-6, CRP, and TNF-α, in humans (Irwin, 2006; Irwin et al., 2016). Further, chronic sleep restriction may lead to the activation of the pro-inflammatory cytokines IL-6, IL-1β and TNF-α in animal models of AD (Arjmandi-rad et al., 2022; Liu et al., 2017). Patients with AD experience sleep disturbances over a longer period of time, which may induce the increased activation of pro-inflammatory cytokines. In addition, sleep fragmentation leads to the activation of microglia and astrocytes in brain regions associated with the regulation of sleep (Vasciaveo et al., 2023). Sleep fragmentation results in a decrease in NREM sleep and an increase in the amount of sleep/wake shifts (Vasciaveo et al., 2023). Patients with AD also have decreased NREM sleep, more fragmented sleep, and more wakefulness after sleep onset, with the latter two being indicators of the amount of sleep/wake shifts. This shows that sleep problems observed in patients with AD may therefore be directly related to an increase in activation of pro-inflammatory cytokines, microglia, and astrocytes.

On the other hand, an increase in cytokines, such as IL-6 and IL-1β, can cause sleep disturbance and the reduction of both REM and NREM sleep (Garbarino et al., 2021). In addition, the increased activation of pro-inflammatory cytokines, microglia, and astrocytes is associated with higher levels of amyloid-β and tau proteins (Liu et al., 2020; Mander et al., 2022). Further, the pro-inflammatory cytokines IL-1β and TNF-α are positively correlated with BACE-1 and RAGE expression, and negatively correlated with LRP-1 expression (Liu et al., 2020). Increased BACE-1 expression leads to increased production of amyloid-β. The increased expression of RAGE and decreased expression of LRP-1 lead to increased influx of amyloid-β into the brain and decreased efflux of amyloid-β from the brain, respectively. This shows that increased levels of pro-inflammatory cytokines can lead to increased levels of amyloid-β in the central nervous system.

The increased levels of amyloid-β and tau proteins are associated with increased wakefulness, decreased REM and NREM sleep, and specifically decreased NREM fast sleep spindles (Mander et al., 2022; Roh et al., 2012). The reduction in REM sleep, NREM sleep, and fast sleep spindles is also observed in patients with AD. Therefore, AD pathology as a possible consequence of activated neuroinflammation may lead to sleep disturbances. The results of these studies indicate a positive feedback loop between sleep disturbance, activation of the immune system, and AD pathology (Figure 3). Sleep disturbances and sleep fragmentation over a longer period of time may lead to the increased activation of pro-inflammatory cytokines, microglia, and astrocytes. In turn, increased activation of these immune function mediators is associated with the accumulation of amyloid-β and tau proteins. AD pathology may in turn lead to even more sleep-wake cycle problems.
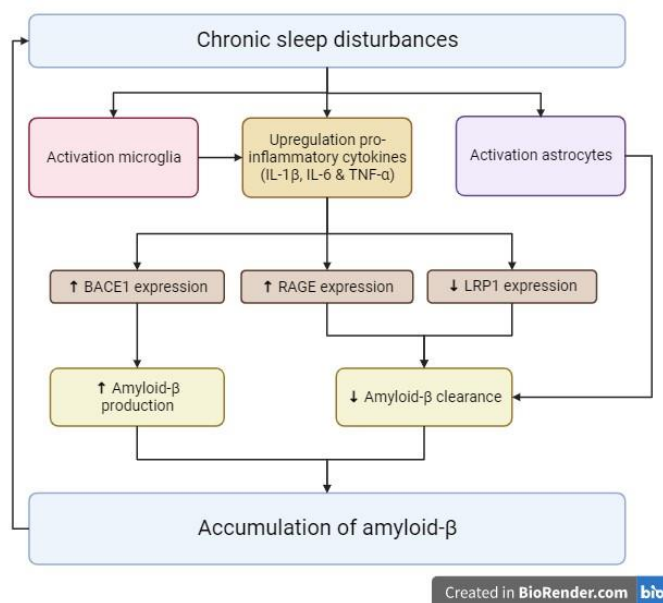
Figure 3: Proposed mechanism on the role of the immune system on sleep in Alzheimer's disease. Chronic sleep disturbances are associated with the activation of microglia and astrocytes, and the upregulation of pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α) (Arjmandi-rad et al., 2022; Irwin, 2006; Irwin et al., 2016; Liu et al., 2017). Additionally, activated microglia cause an upregulation of pro-inflammatory cytokines (Lull & Block, 2010). The pro-inflammatory cytokines IL-1β and TNF-α are positively correlated with BACE1 and RAGE expression, and negatively correlated with LRP1 expression (Liu et al., 2020). The increased expression may lead to increased production of amyloid-β, while increased expression of RAGE and decreased expression of LRP1 may lead to reduced clearance of amyloid-β (Cai et al., 2016; P. Liu et al., 2020). Astrocytes can lead to alterations in the perivascular space size, resulting in decreased glymphatic flux (Vasciaveo et al., 2023; see next paragraph for more detail). The increased production and reduced clearance may lead to the accumulation of amyloid-β in the central nervous system.

THE ROLE OF THE GLYMPHATIC SYSTEM ON SLEEP IN ALZHEIMER'S DISEASE

The glymphatic system is the waste clearance system of the central nervous system (Christensen et al., 2021). The glymphatic flow is the highest during sleep, resulting in increased clearance of amyloid-β and tau proteins during sleep and accumulation of these proteins during wakefulness (Kang et al., 2009). In particular, slow-wave sleep is the strongest associated with increased glymphatic influx (Kang et al., 2009).

Sleep deprivation leads to increased levels of amyloid-β and tau proteins in the ISF/CSF (Holth et al., 2019; Kang et al., 2009; Lucey et al., 2018). More specifically, disruption of slow wave activity is correlated with an increase in amyloid-β (Ju et al., 2017). Patients with AD have a reduction in stage 3 of NREM sleep, characterized by slow brain waves. This observed reduction in slow-wave sleep in AD patients may relate to an increase in amyloid-β levels. Sleep deprivation induces amyloid-β burden in the hippocampus and thalamus, probably resulting from the increase in amyloid-β in the CSF (Shokri-Kojori et al., 2018). On the other hand, amyloid-β accumulation in the hippocampus and striatum is associated with a reduction in diurnal fluctuation, thereby decreasing the amyloid-β clearance from the brain, and disrupting the sleep-wake cycle (Roh et al., 2012).

The glymphatic system is highly dependent on the glial aquaporin-4 (AQP4) water channels (Iliff et al., 2012). AQP4 knockout mice show that without the AQP4 gene, there is an impairment of glymphatic function, with a 55% reduction in clearance of amyloid-β (Iliff et al., 2012). Further, mutations in the AQP4 gene are related to decreased sleep quality and increased amyloid-β burden (Rainey-Smith et al., 2018). This study provides evidence for a link between sleep disturbances, glymphatic impairment, and amyloid-β accumulation. Furthermore, the study by Vasciaveo et al., (2023) shows that sleep fragmentation causes increased AQP4 signal in the two-month-old AD-mouse model, whereas it decreases the AQP4 signal in six-month-old AD-mouse model and wild-type mice.

There is an accumulation of amyloid-β in this AD-mouse model, indicating a reduction in glymphatic flow and glymphatic malfunction. The increase in AQP4 expression is accompanied by an increased activation of astrocytes. The perivascular space (PVS) is lined by end-feet astrocytes and surrounds the cerebral vascular system (Vasciaveo et al., 2023). Increased activation of astrocytes can alter the size of the PVS, thereby reducing the glymphatic flux and clearance (Vasciaveo et al., 2023). One possible explanation is that the increase in AQP4 expression is a compensatory mechanism to accommodate for the loss of glymphatic flux. However, reactive astrogliosis (more active astrocytes) can also induce the loss of polarization of AQP4 at the astrocytic end-feet and increased expression of AQP4 (Mestre et al., 2017). Normally, the expression of AQP4 is highly polarized toward the plasma membrane of the end-feet of the astrocyte (Vasciaveo et al., 2023). The loss of polarization of AQP4 can lead to reduced glymphatic flux (Liu et al., 2017). The decreased expression of AQP4 in the six-month-old AD-mouse model and wild-type mice can be explained by the fact that poor sleep quality and sleep fragmentation induce AQP4 depolarization (Liu et al., 2017). AQP4 depolarization is indicated by the loss of AQP4 expression at the astrocytic end-feet, which can result in impaired glymphatic flux and clearance (Liu et al., 2017). In patients with AD, a reduction of AQP4 expression at the perivascular space is observed and this reduction is strongly associated with amyloid-β and tau accumulation (Zeppenfeld et al., 2017). Sleep fragmentation is accompanied by increased sleep-wake shifts and decreased NREM sleep (Vasciaveo et al., 2023). Patients with AD also have decreased NREM sleep, more fragmented sleep, and more wakefulness after sleep onset, with the latter two being indicators of the amount of sleep/wake shifts. This indicates that sleep fragmentation, which is also observed in AD patients, causes a reduction in glymphatic flux. This may be done either due to increased activation of astrocytes which will alter the PVS size, or reduction of AQP4 expression at the astrocytic end-feet in the perivascular space.

In the study by Lucey et al., (2018), the increase of amyloid-β after sleep deprivation is due to increased production. Further, reduced sleep and increased wakefulness induce increased release of tau and production of amyloid-β (Lucey, 2020). In the study by Ju et al., (2017), slow wave activity induces increased amyloid-β levels by reducing the release of this protein into the interstitial space. In the study by Vasciaveo et al., (2023), amyloid-β accumulation is due to the reduction in glymphatic flux, resulting in the reduction of amyloid-β clearance. Further, the reduced polarization of AQP4 at the astrocytic end-feet is associated with reduced glymphatic flux and the latter is associated with reduced clearance of tau (Harrison et al., 2020). This indicates that chronic sleep disturbances may lead to the accumulation of amyloid-β and tau proteins by increased production and release of these proteins and the reduced clearance of these proteins due to reduced glymphatic flux. In turn, the accumulation of amyloid-β and tau is associated with increased wakefulness and decreased REM sleep, NREM sleep, and fast sleep spindles (Mander et al., 2022; Roh et al., 2012). Thus, the glymphatic system plays an important role in the positive feedback loop between sleep disturbances and increased AD pathology (Figure 4).
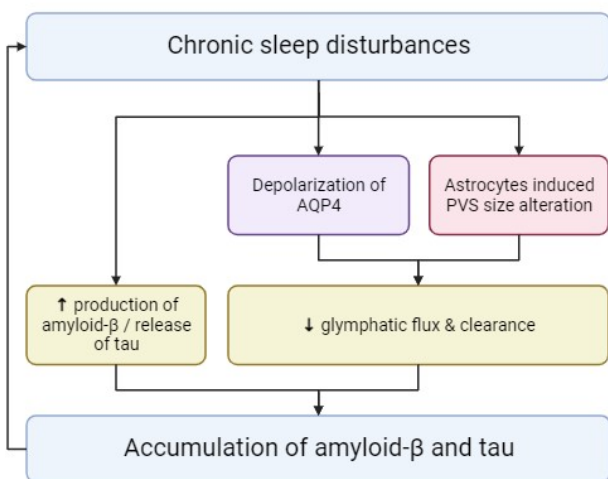

Figure 4: Proposed mechanism on the role of the glymphatic system on sleep in Alzheimer's disease. Chronic sleep disturbances is associated with the increased production of amyloid-β and increased release of tau (Lucey, 2020). Further, chronic sleep disturbances may lead to depolarization of AQP4 at the astrocytic end-feet and may lead to astrocyte induced PVS size alteration (Liu et al., 2017; Vasciaveo et al., 2023). AQP4 depolarization and PVS size alteration may lead to reduced glymphatic flux and reduced clearance of amyloid-β and tau (Liu et al., 2017). Overall, the increased production and release of amyloid-β and tau, and the reduced glymphatic flux and clearance leads to the accumulation of amyloid-β and tau in the central nervous system. AQP4 = glial aquaporin-4; PVS = perivascular space

Some of the studies that investigate the role of the immune system and the glymphatic system on sleep use animal models. These animal studies can be useful to understand the mechanisms of how sleep may affect AD pathology. However, it remains unclear whether the same mechanisms also apply to the human situation. A point for future research is to further investigate the role of the immune system and the glymphatic system on sleep in patients with Alzheimer's disease. By finding the precise mechanisms of how sleep can alter AD pathology via the immune system and glymphatic system, better treatment plans can be made to slow down AD pathology.
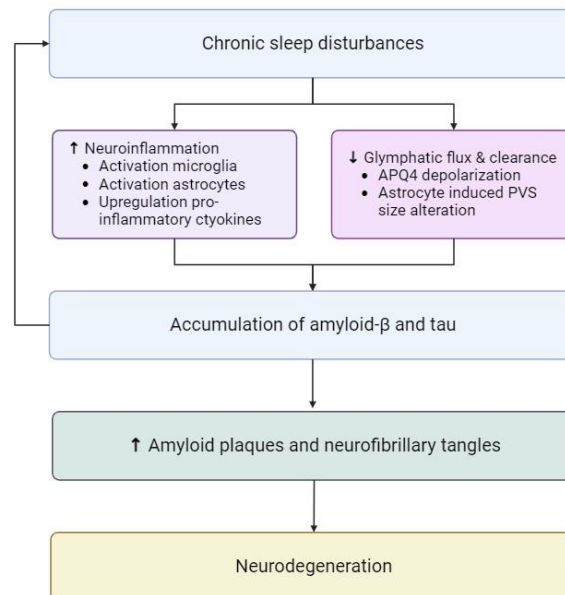

Figure 5: Schematic representation of the potential role of the immune system and glymphatic system on sleep in Alzheimer's disease. *Chronic sleep disturbances may lead to increased neuroinflammation, with activation of microglia, activation of astrocytes, and upregulation of pro-inflammatory cytokines (Arjmandi-rad et al., 2022; Irwin, 2006; Irwin et al., 2016; Liu et al., 2017). Further, chronic sleep disturbances may lead to reduced glymphatic flux and clearance, with AQP4 depolarization and astrocyte induced PVS size alteration (Lucey, 2020; Liu et al., 2017; Vasciaveo et al., 2023). The increased neuroinflammation and reduced glymphatic flux and clearance may lead to the accumulation of amyloid-β an tau in the central nervous system. The accumulation of these proteins is associated with the increase in amyloid plaques and neurofibrillary tangles. The increase of AD pathology may lead to neurodegeneration. In addition, the accumulation of amyloid-β and tau is associated with increased wakefulness and decreased REM sleep, NREM sleep, and fast sleep spindles. Both the immune system and glymphatic system play a role in the positive feedback loop between chronic sleep disturbances and higher AD pathology. AQP4 = glial aquaporin-4; PVS = perivascular space*

Conclusion

The research question addressed in this thesis is: What is the role of the immune system and glymphatic system on sleep in Alzheimer's disease? As a conclusion, patients with Alzheimer's disease experience many different sleep disturbances. The most important sleep disturbances are the reduction in REM sleep, reduction in NREM sleep, reduction of fast sleep spindles, more fragmented sleep and more wakefulness after sleep onset (more sleep-wake shifts). These sleep disturbances and sleep fragmentation may cause the accumulation of amyloid-β and tau proteins. Higher levels of AD pathology are associated with increased wakefulness and decreased REM sleep, NREM sleep, and fast sleep spindles. This indicates a positive feedback loop between sleep disturbances and increased AD pathology. Furthermore, sleep disturbances and sleep fragmentation lead to increased activation of pro-inflammatory cytokines (IL-6, IL-1β, CRP, and TNF-α), microglia, and astrocytes. In turn, this neuroinflammation is associated with higher amyloid-β and tau burden. Specifically, pro-inflammatory cytokines may lead to increased production of amyloid-β via increased expression of BACE-1. Further, it may lead to increased influx and decreased efflux via increased RAGE and decreased LRP1 expression,

respectively. Furthermore, sleep deprivation and sleep fragmentation may also lead to higher levels of amyloid-β and tau proteins in the ISF/CSF due to the increased production or release of these proteins and/or due to reduced clearance due to decreased glymphatic flux. The decrease in glymphatic flux could result from increased activation of astrocytes which will alter the PVS size or the reduction of AQP4 expression at the astrocytic end-feet in the PVS. In turn, the increased accumulation of amyloid-β and tau are associated with a reduction of fast sleep spindles, REM sleep, and NREM sleep and increased wakefulness. Thus, both the immune system and the glymphatic system play an important role in the positive feedback loop between sleep disturbances/fragmentation and increased AD pathology. A schematic representation of the potential role of the immune system and glymphatic system on sleep in Alzheimer's disease is shown in Figure 5.

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