Mechanisms of increased infection risk by sleep deprivation

How short sleep makes you sick

Michelle Luxwolda

S2792656

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Neurobiology group

R. Havekes, Ph.D.

Abstract

Sleep and immunity are two complex processes that interact on many levels. Adaptive responses to an infection are increased fatigue and sleepiness. Conversely, sleep deprivation in a healthy situation can have detrimental effects on the risk of infection. The potential mechanisms behind the effect of sleep deprivation on increased infection risk are complex and versatile. To provide an overview of current knowledge in this field, four sub questions are addressed. First, the interplay between sleep change and immune activation is discussed. Being asleep naturally reduces the number of environmental interactions leading to an innate response of the immune system. Therefore, more time spent awake increases the risk for environmental interactions potentially leading to the activation of patterns recognition receptors (PRRs) by pathogen-associated molecular patterns (PAMPs) and danger/damage-associated molecular patterns (DAMPs) that will initiate inflammatory processes. Next discussed are the main effects of sleep deprivation on immune parameters. In general it can be stated that sleep reduces and sleep deficiency increases leukocyte quantities in the blood. Pro-inflammatory and T helper 1 (Th1) cytokine production is favoured over anti-inflammatory and T helper 2 (Th2) cytokine production by sleep. The tightly regulated Th1/Th2 balance can be disturbed by sleep deprivation, mainly favouring a pro-inflammatory response. The third sub question focuses on the effects of decreasing non-rapid-eye-movement (NREM) sleep amount on clearance systems in the brain. Slow-wave-sleep (SWS) is part of NREM-sleep and has a significant anti-inflammatory function. Clearance of neuronal metabolites by the glymphatic system is high during SWS. Sleep deprivation results in disturbances in metabolic clearance which can lead to dangerous protein aggregations. Similar effects occur in the periphery and are discussed in the last sub question. Like in the brain, the lymphatic system follows carefully the sleep/wake cycle. A lack of sleep causes disruption in the diurnal fluctuations of lymph drainage resulting in accumulation of DAMPs, increasing the risk for infectious disease. Disturbed sleep may also gradually impair normal functioning of the HPA axis, increasing its activity. The apparent protective role of sleep is to guard homeostatic balance by counteracting low-grade inflammation. Sufficient homeostasis of the immune system fails when sleep is disturbed, and mild inflammation may escape protection and the condition may worsen. Prolonged sleep loss increases the risk of reaching a chronic inflammatory status, which in turn has a disturbing effect on sleep. The negative interplay between sleep and the immune system then feeds into a vicious circle. Given that sleep has initial control over a healthy situation, makes it a valuable means of supporting immunity.

Table of contents

Introduction

‘Sleep is the best medicine’ is an old wisdom that has been borne out by modern research. Fortunately, we don't have to do much for this, as our bodies naturally seek rest when we don't feel well. An infection causes tiredness and increases the desire to sleep. When we sleep during an infection we spend more time in the stage with non-rapid-eye-movement (NREM) than in healthy situations (Majde & Krueger, 2005). An early study in rabbits showed that more NREM sleep amount and intensity led to more favourable prognosis and less severe clinical signs following infection with different microbes (Toth, Tolley & Krueger, 1993). The sleep response in these rabbits was an indicator of survival chance. Sleep has long been hypothesized to be an important acute phase response of the body to help fight infections (Toth & Krueger, 1988). The hypothesis for the supportive effect of sleep is that it has an energy allocation function to the immune system (Schmidt, 2014). Given this statement, could deprived sleep be expected to be detrimental to the immune system and increase the risk of an infection?

While the role of enhanced sleep in infectious situations is to promote immunological health, sleep loss is not beneficial to the risk of infection outcome. This is supported by experimental studies on animals with manipulated sleep. Genetically reducing sleep duration in *Drosophila* resulted in a decreased bacterial load compared with flies with normal sleep duration. Interestingly, survival rate was not affected in both groups. Flies with a genetically prolonged sleep duration showed an increased resistance to infection, as measured by bacterial load. Also these flies showed the same survival rate as control animals. The decreased bacterial load in the short sleepers was therefore interpreted as a reduced tolerance to infection (Kuo & Williams, 2014). Negative effects of deprived sleep on the outcome of a bacterial infection are also reflected by rodent studies. Disturbed sleep in mice experimentally induced with sepsis resulted in increased mortality compared with a control group with normal sleep (Friese, Bruns & Sinton, 2009). In rats, sleep deprivation resulted in the translocation of commensal bacteria to extra-intestinal sites. These bacteria are usually harmless, but under the circumstances of prolonged sleep deprivation could lead to general sepsis and even death (Bergmann et al., 1996; Everson & Toth, 2000). Hence, sleep is not only important in fighting infections caused by foreign bacteria, but also has an essential role in the homeostasis of the body’s own naturally harmless bacteria. These studies in flies and rodents show how disturbed sleep results in a bacterial disbalance and an increased mortality risk.

Where animal studies show a causal role of sleep in infection outcome, human studies show an association between short sleep duration and infection risk. Experimentally induced infection by nasal drops containing a rhinovirus increased the risk of developing a clinical cold in people reporting a short sleep duration in the preceding weeks of administration of the drops (Cohen et al., 2009). Equal results were found with objectively measured sleep duration by actigraphy (Prather et al., 2015). In otherwise healthy adolescents, objectively measured short sleep duration was associated with more frequent acute illnesses including cold, flu and other common infectious diseases (Orzech et al., 2014). People habitually

sleeping 5 hours or less (self-reported) are associated with more frequent reports of respiratory infection within the past month (Prather et al., 2016) compared with 7-8 hours sleepers. Also, they are associated with an increased risk for developing pneumonia in the next 2 years (Patel et al., 2012). It has to be said that inter-individual differences in sleep needs make up for differences in amount of sleep duration leading to increased risk of infection. A short sleep duration can be sufficient for optimal immune function in people with lower sleep needs. This is shown by people reporting a ≤5 hour night of sleep, but also indicating that they feel this is adequate, who did not have an increased pneumonia risk (Patel et al., 2012). However, from both animal and human studies can be concluded that sleep deprivation in general increases the risk of infection. Since sleep and immunity are both very broad topics, with still relatively little known about sleep, it can be difficult to pinpoint the exact mechanisms behind sleep deprivation leading to an increased infection risk. However, a lot of in-depth research has already been done in both fields which makes it possible to bring the knowledge together.

This essay will look into the theories behind an increased infection risk by short sleep. The research question that will be answered is: What are the potential mechanisms of sleep deprivation leading to an increased risk of infection? Multiple sub questions will be addressed to provide an overview of some of the processes that are responsible for the complicated interplay between short sleep and the immune system. First, the focus will be set on sleep change and immune activation. Second, the effects of sleep deprivation on immune parameters will be discussed. For the third question, I will look into clearance systems of the brain affected by decreased NREM sleep. The last question will relate to the periphery and how the protective role of sleep is inhibited by sleep deficiency. While much is still unknown, the answers to these questions will provide a general overview of the current status in science about the relationship between sleep deprivation and infection risk.

1. What is the interplay between sleep change and immune activation?

The body is constantly exposed to an environment full of potential infectious threats. Many of these threats are blocked by the physical barriers of the mucosal and skin epithelia. These barriers contain antimicrobial peptides and complement factors that form the first line of defence. If a pathogen manages to pass these barriers, specific conserved pathogen-associated molecular patterns (PAMPs) from microbes like bacteria or viruses are recognized by patterns recognition receptors (PRRs). Other than “non-self” PAMPs also endogenous danger/damage-associated molecular patterns (DAMPs), which are released by stressed or injured cells, can be recognized by PRRs. Numerous cells (including neutrophils, macrophages, dendritic cells, endothelial cells, but also epithelial cells and lymphocytes) contain PRRs. These cell-associated PRRs are located on the cell membrane, in the cytoplasm or incorporated into the membrane of endosomes and it is their task to recognize foreign invaders or damaged cells (Bianchi, 2007; Chu & Mazmanian, 2013).

The body wants to contain the spread of pathogens, clean up the damage of defected (endogenous) cells and get healing as quickly as possible. In order to do this it can start an inflammatory response which is characterized by redness, swelling, heat and pain. When

PAMPs and DAMPs are detected by PRRs, complex intracellular signalling pathways are triggered that lead to activation of leukocytes and the production of pro-inflammatory cytokines. Together they orchestrate the early host response to infection. Innate defence mechanisms of phagocytosis by neutrophils and macrophages is the first step in destroying invading or damaged cells. Natural Killer cells can also be triggered to program apoptosis in those cells that have become infected with viruses. Specialized mast cells in the connective tissue send out histamine molecules causing vasodilation which creates redness and heat. Histamines and other inflammatory chemical signalling molecules (including fatty acid prostaglandins, kininogen and other plasma proteins, complement factors and cytokines) also increase the permeability of blood vessels causing nearby capillaries to release protein rich fluids. This causes swelling and increased activation of the lymphatic system. Phagocytes and lymphocytes can now easily escape leaky capillaries to destroy pathogens and clean up dead cell material at local sites. With a more major infection, neutrophils and macrophages release pyrogen chemicals that trigger the hypothalamus and raise the body temperature to cause a systemic fever (Medzhitov, 2008).

Being asleep naturally reduces the number of environmental interactions leading to an innate response of the immune system as described above, simply due to a 7-8 hour stretch of time spent at the same (mostly) habitual spot. Any additional time spent awake can therefore be seen as an enhancement of the number of interactions with a changing environment. Likewise, acute sleep deprivation causes an increase in DAMPS in blood, CSF and the brain (Fig. 1; Benedict et al., 2014; Ooms et al., 2014; Shokri-Kojori et al., 2018). It is the factor time that increases the risk for environmental interactions, potentially leading to the activation of PRRs by PAMPs and DAMPs that will initiate inflammatory processes.

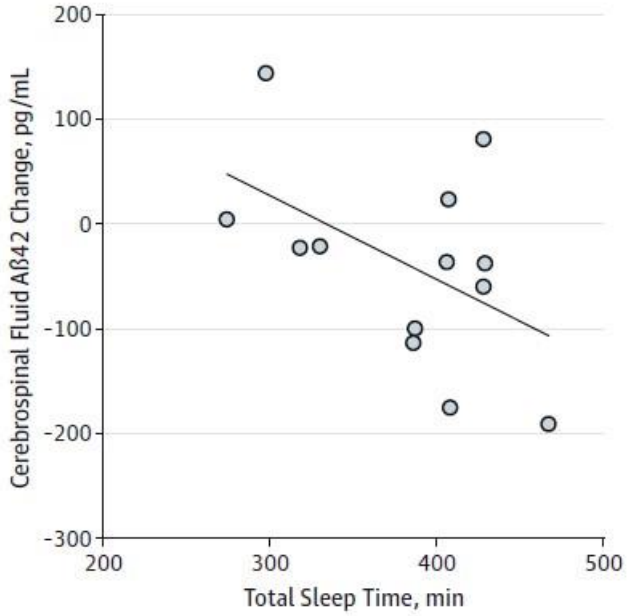

Figure 1. The relationship between Total Sleep Time in minutes and reduction in β-Amyloid 42 (Aβ42) in cerebrospinal fluid. Aβ42 was measured between 5PM and 10AM (r=-0.5, P=0.04). Ooms et al., 2014.

As outlined above, foreign or endogenous (microbial) challenges trigger an innate inflammatory response. Adaptive responses from the central nervous system to an infection related to sleep are increased fatigue and sleepiness, which presumably stimulate recovery. Indirectly, both symptoms promote a less active behavioural state and thereby create the advantage of energy conservation (Verburg-van Kemenade et al., 2017). More directly, the structure of sleep changes. When the immune system is activated after acute infection, sleep is altered by increasing the amount of NREM sleep and often decreasing rapid-eye-movement (REM) sleep (Majde & Krueger, 2005). Although the NREM sleep response driven by infection is independent from the fever response (Krueger & Opp, 2016), increases in core body temperature can promote NREM sleep (Sato et al., 2015). While NREM sleep duration and intensity is increased by infection processes, a concomitant suppression of REM sleep occurs. Thermo-regulatory effector processes like shivering cannot occur during REM sleep. Therefore, suppression of REM sleep may indirectly stimulate immunity by promoting the generation of fever (Imeri & Opp, 2009). A more long term explanation for the function of sleep in immunity challenges is to support the formation of memory in the nervous and the immune system. Although REM sleep might also support adaptive immune functions, a specific role is attributed to stage 3 of NREM sleep when slow- wave sleep (SWS) occurs. A unique pro-inflammatory endocrine milieu present during SWS supports the cross-talk between Antigen Presenting Cells (APCs) and T cells, linking innate and adaptive immune responses. This interaction results in a stronger immunological memory (Westermann et al., 2015). Overall, an infection-driven boost of sleep is assumed to promote host defence (Besedovksy, Lange & Haack, 2019). Which immune parameters are involved in these processes and how they change when sleep changes is described in the next paragraphs.

2. What are the main effects of sleep deprivation on immune parameters?

Leucocytes are the main cells of the immune system that coordinate to provide defence against infectious disease. There are five distinct classes of leukocytes: neutrophils, eosinophils, basophils, monocytes and lymphocytes. Studies on the effect of sleep and sleep loss on leukocytes show discrepancies in their findings. While a number of studies show a reduction of leukocyte counts in sleep situations compared to total or partial sleep deprivation (Boudjeltia et al., 2008; Lasselin et al., 2015; Liu et al., 2009; Ruiz et al., 2012), other researchers do not share these conclusions (Ackermann et al., 2012; Chennaoui et al., 2017; Costa et al., 2010; Irwin et al., 1996; Wilder-Smith et al., 2013). A reason for these differences in results could be found in the timing of cell counting. Leukocyte quantities are variable throughout a daily 24-hour cycle (Born et al., 1997). This means that when cells are counted at just a few time points, results can vary greatly between studies with differentiating time points used for measurements. Many of the before mentioned studies do not account for the circadian rhythms in leukocyte densities. Nevertheless, an important note here is that in none of the studies an increase in leukocyte numbers due to sleep was found. In general it can be stated that sleep reduces and sleep deficiency increases leukocyte quantities in the blood.

Studies on cytokines, important cell signalling molecules, also show very mixed results. Depending on the type of cytokine, sleep has diverse effects. Acute phase cytokines that are mostly investigated in the context of sleep research are Interleukin 6 (IL-6), Tumor Necrosis Factor (TNF) and Interleukin-1 (IL-1). One night of restricted sleep or no sleep at all can show increasing (Vegontzas et al., 2007), decreasing (Faraut et al., 2015) or no effects (Chennaoui et al., 2011) on IL-6 levels compared to normal sleep in humans. Prolonged sleep deprivation shows more consistent findings of increased IL-6 levels compared to normal sleep in both humans (Shearer et al., 2001) and animals (Hu et al., 2003). However, there are also studies of prolonged sleep deprivation in humans (Shearer et al., 2001) and animals (Hirotsu et al., 2012) that report no effects on IL-6 levels. Similar results are found in studies investigating TNF levels. Acute sleep restriction/deprivation showed no changes in plasma TNF levels in healthy humans (Ruiz et al., 2012). One study on prolonged sleep restriction of 6 hours per night for 1 week revealed an increase in plasma TNF levels (Vgontzas et al., 2004), while other studies found no effects (Ruiz et al., 2012; Shearer et al., 2001). Animal studies show similar findings of an increase of (Hu et al., 2003) or no effect (Hirotsu et al., 2012) on plasma levels of TNF after prolonged sleep deprivation or restriction. Interestingly, in humans, total sleep deprivation of 4 nights increased levels of TNF receptor I, an inhibitor and regulator of TNF signalling ((Shearer et al., 2001), while a single night without sleep showed no effects on either TNF receptors I and II (Haack, Pollmächer & Mullington, 2004). Upscaling of the TNF receptors may reflect a homeostatic response to increased levels of TNF. Results are somewhat reversed in studies on the effects of sleep on plasma levels of IL-1. Human studies showed that one night of sleep deprivation resulted in an acute increase in IL-1 (Frey, Fleshner & Wright, 2007; Moldovsky et al., 1989). However, prolonged sleep deprivation did not replicate these findings (Ruiz et al., 2012). Similar mixed findings were reflected in animal studies, where increases of IL-1 following sleep deprivation (Everson, 2005; Hu et al., 2003) and no effects of sleep (Hirotsu et al., 2012; Trammell & Toth, 2015) were shown. Previous examples show that it is hard to pinpoint the exact effects of sleep and sleep loss on cytokine levels, which could possibly be caused by methodological issues.

Methodology used for inducing sleep deprivation and the measurement of cytokine production cause for complexity in assessing the impact of sleep on cytokine levels. Many different protocols can be used for disturbing sleep, ranging from deprivation of a few hours per night up to total wakefulness or only the disruption of REM sleep. Likewise, the number of consecutive nights implementing such protocols can vary among studies while all titling prolonged sleep deprivation. The assessment of cytokine production forms another major issue. Assays used usually include incubation of cells which are stimulated with high doses of non-physiological stimulants. Many important steps in the normal physiology of the effects of sleep on cytokine production are skipped by making use of these in vitro techniques. For example the sources of cytokine production are also affected by sleep, thereby potentially having an impact on the results of cytokine measurements. Cytokines have short half-lives and are often produced locally and momentary. Hence, especially blood measurements make it difficult to detect changes. And maybe not surprisingly, time is also a factor of influence. Not only do many cytokine levels show circadian fluctuations, also the body is geared to maintain a homeostatic state at all times. Any effect of sleep deprivation can be

expected to be counteracted during or close to timing of the event. Therefore, timing of measurements has great impact on the observed effects of sleep on cytokine levels. Altogether, different aspects of the methods used to analyse cytokines in the context of sleep cause for a certain sensitivity which makes it difficult to define exact results.

One general conclusion that can be drawn is that sleep favours pro-inflammatory and T helper 1 (Th1) cytokine production over anti-inflammatory and T helper 2 (Th2) cytokine production (Besedovsky, Lange & Haack, 2019). Type 1 immunity is achieved by Th1 cells releasing mainly Interferon-γ (IFN-γ), IL-2 and TNF-α. Th1 cells respond to intracellular viral and bacterial signals and support cellular type 1 responses like macrophage activation. Type 2 immunity involves IL-4, IL-5, IL-10 and IL-13 which drive humoral type 2 defence via stimulating mast cells, eosinophils and B cells against extracellular pathogens. Both processes are tightly regulated by a homeostatic response. More specifically, Th1 dominance over Th2 is reversed at the second half of sleep in humans (Dimitrov et al., 2004). In figure 2 is shown how early sleep increases and late sleep decreases the ratio of IFN-γ to IL-4 production by CD4+ cells, indicating the Th1/Th2 balance.

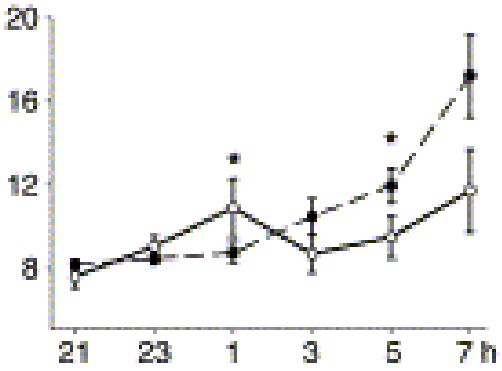

Figure 2. The ratio of IFN-γ to IL-4 producing CD4+ cells during sleep (solid lines) and wakefulness (dashed lines). Values are mean ± SEM, *p<.05. Dimitrov et al., 2004.

The reversal step halfway sleep is quite an important one, because an excessive Th1 response leads to uncontrolled tissue damage by autoimmune responses. Th2 immune responses counteract the Th1 immune responses (Berger, 2000). Deprivation of sleep usually causes people to only go through the first half of sleep and not make it to or only partially go through the second half. Sleep deprivation therefore increases the risk of disturbing the Th1/Th2 balance and mainly favouring a pro-inflammatory response. This is reflected in figure 2 by the effect of wakefulness on the ratio of IFN-γ/Il-4 producing CD4+ cells, showing no decrease during late night compared to the sleep condition. In chronic situations the Th1/Th2 imbalance caused by sleep deprivation could have detrimental health consequences. In conclusion, it can be stated that the overall effect of sleep deprivation on immune parameters is causing an imbalance in tightly regulated ratios of immune cells and their secretion products.

3. What is the effect of decreasing NREM sleep amount on clearance systems in the brain?

Normal sleep alternates between REM and NREM sleep stages with the majority of time spent in NREM sleep. Stage three of NREM sleep is called slow-wave sleep (SWS) or deep sleep (Borbély & Achermann, 1999). SWS is considered important for memory consolidation, but it also has a significant anti-inflammatory function. Several SWS-dependent processes might actively prevent inflammation processes in the brain. One important example is the clearance of amyloid beta during sleep by the glymphatic system. During SWS synaptic activity is minimal and clearance of neuronal metabolites is high (Varga et al., 2016). This makes it an ideal state to regulate levels of amyloid beta, which is released into interstitial fluid (ISF) after cleavage of amyloid precursor protein spanning membranes of neuronal and glial cells. In the ISF, amyloid beta can remain soluble or aggregate into insoluble plaques. The latter can be quite detrimental, because amyloid plaques induce inflammation and ROS production (Clark & Vissel, 2015). The glymphatic system clears the brain parenchyma from amyloid beta during sleep by expanding the cortical interstitial space by more than 60% (Fig. 3; Boespflug & Iliff, 2018; Xie et al., 2013). In this state, proteins and metabolites can be easily flushed away from the central nervous system (CNS).

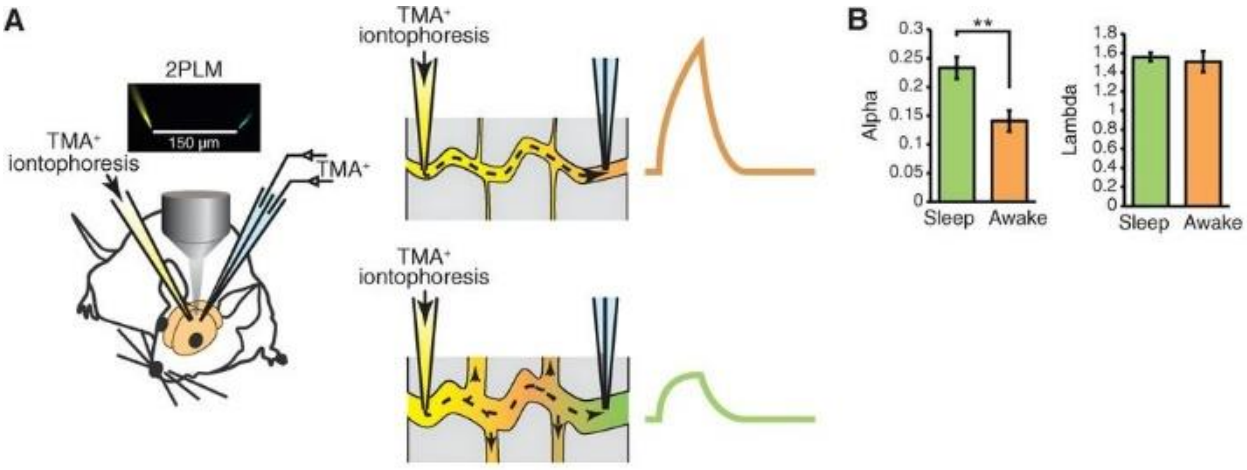

Figure 3. Volume of the cortical interstitial space as measured by real-time TMA^+ iontophoretic quantification technique. A: One iontophoresis microelectrode delivering TMA^+ (left) and one microelectrode at ~ 150µm distance sensitive to TMA^+ (right) are positioned in the extracellular space in the cortex. High TMA^+ levels reflect reduced TMA^+ dilution which is the result of a smaller extracellular space in awake (top) compared to sleeping mice (bottom). **B**: The extracellular space is significantly smaller (alpha) in awake than in sleeping mice, whereas the tortuosity (λ) remained unchanged; n = 4 to 6 mice; **P < 0.01, t test. Xie et al., 2013.

Glymphatic function is primarily active during sleep, which results in a circadian rhythm of fluctuating amyloid beta levels. During waking, amyloid beta levels increase while during sleep amyloid beta levels decline (Fig. 4). This homeostatic system prevents excessive protein aggregation, which is the pathological feature of many neurodegenerative diseases.

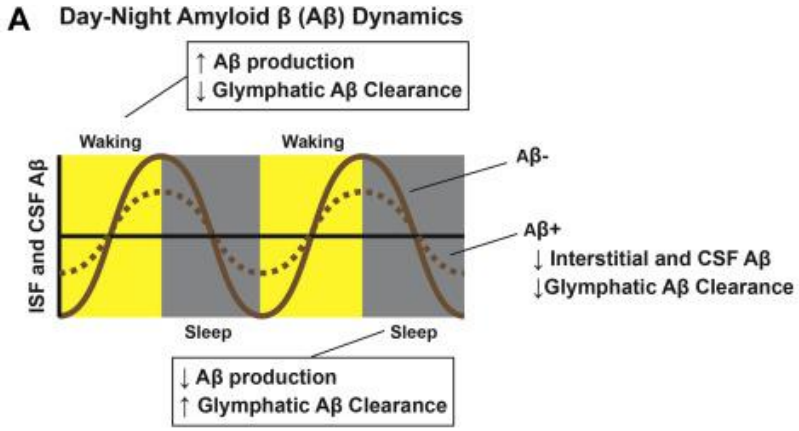

Figure 4. Circadian rhythm of amyloid beta levels (Aβ) in interstitial fluid (ISF) and cerebrospinal fluid (CSF). Boespflug & Iliff, 2018.

Following a night of regular sleep, amyloid beta levels in the brain are lower compared with a night of total sleep deprivation (Fig. 5; Ooms et al., 2014; Shokri-Kojori et al., 2018), or SWS disruption (Ju et al., 2017). The loss of sleep induces higher levels of amyloid beta which increases the chance of the formation of insoluble plaques. Accumulation of insoluble plaques is the main characteristic of Alzheimer's disease. It is logically conceivable that this problem occurs not only with amyloid beta, but also with other soluble proteins and metabolites in the CNS. Sleep deprivation, of especially the SWS containing NREM sleep phase, results in disturbances in metabolic clearance which can lead to protein aggregation in the brain parenchyma. In the long term, this means an increased risk for neuroinflammation and the development of neurodegenerative diseases.

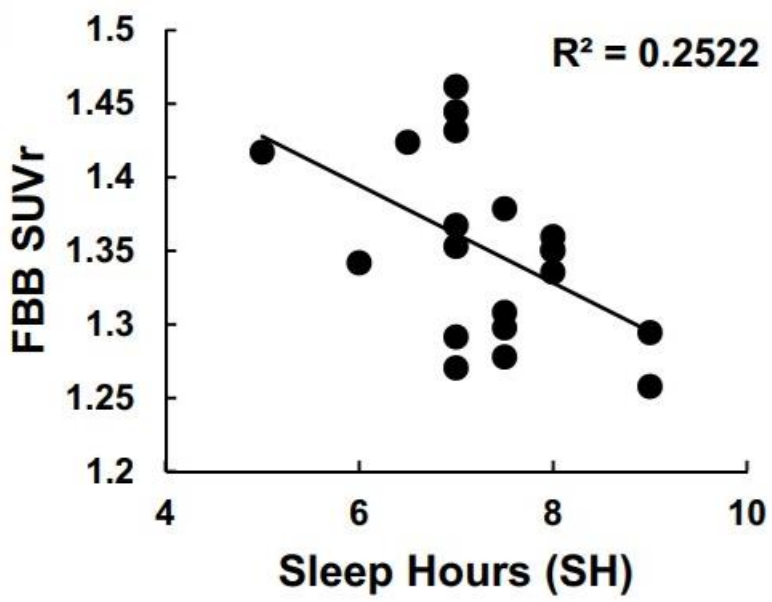

Figure 5. Regression of amyloid beta as indexed by FBB SUVr against sleeping hours (SH). Shokri-Kojori et al., 2018.

4. What protective role of sleep is inhibited by sleep deficiency in the periphery?

It is not only the glymphatic system that is active in the drainage of proteins. In the periphery, proteins from tissues and organs are redirected into lymph nodes by the lymphatic system. From these nodes, proteins from both the glymphatic and lymphatic systems end up into the blood circulation for final clearance by the liver or kidneys (Louveau et al., 2017). Peripheral drainage of proteins by the lymphatic system could be subject to sleep associated changes in the vasculature, as well as the horizontal positioning of the body during sleep. Already in the 70's it was shown that concentrations of proteins and blood cells in legs significantly increase in the early hours after a night sleep (Engeset et al., 1977). Research in sheep shows that during sleep, lymph flow and output are reduced compared to wakefulness (Fig. 6; Dickstein et al., 2000).

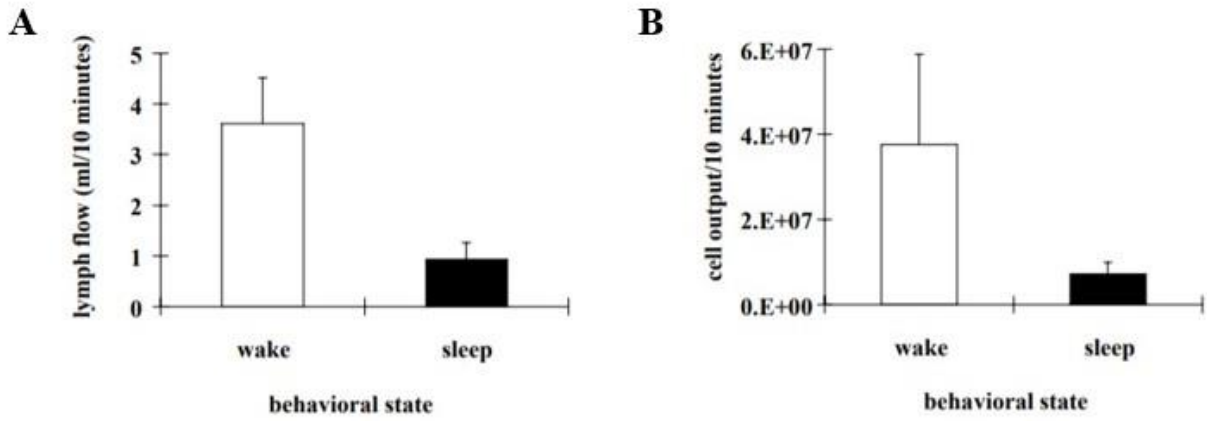

Figure 6. Lymph flow rate and efferent output of sheep during sleep and wakefulness. A: Flow rate of efferent prescapular lymph during wake (n=9) and sleep (n=11). Statistical difference of lymph flow during sleep compared to wake = $p<0.0001$. **B:** Efferent prescapular lymph output during wake (n=9) and sleep (n=11). Statistical difference of lymph output during sleep compared to wake = $p<0.0004$. Error bars indicate SD. Dickstein et al., 2000

Diurnal fluctuations of hormones could have a regulating role in these processes. During the night, growth hormone concentrations peak while cortisol peaks in the morning. These opposing rhythms separate anabolic effects by growth hormone and catabolic effects by cortisol. Growth hormone increases interstitial flow resistance by increasing numbers and volumes of cells and synthesis of interstitial material. Cortisol induces tissue catabolism which reduces the interstitial flow resistance back towards normal (Kurbel, 2005). Such an integrative drainage system presumably follows carefully the sleep/wake cycle, although the specific role of sleep is still unknown. When sleep changes this could have detrimental effects on the well-orchestrated drainage system of the periphery, with an increased waste product load increasing the risk for infectious disease.

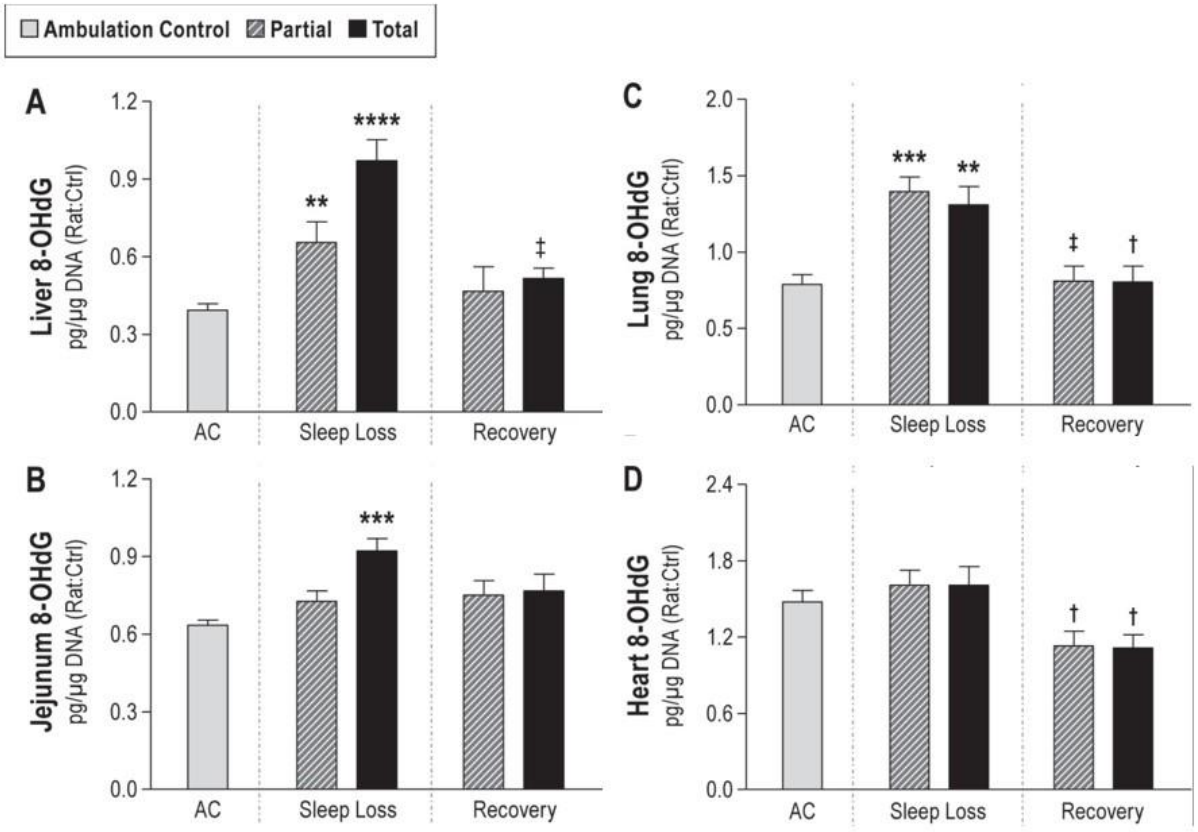

Figure 7. DNA damage indicated by 8-hydroxydeoxyguanosine (8-OHdG) concentrations. Rats were grouped in ambulation controls (ACs; grey bar, n=11) and partially (hatched bar) and totally (solid bar) sleep deprivation during 10 days of sleep loss (n=8–11 per group), and after 2 days of recovery sleep after sleep loss (n=6–7 per group). Concentrations of 8-OHdG in pg/µg DNA were measured in **A:** liver, **B:** jejunum, **C:** lung and **D:** heart tissue. Values are normalized to intra-assay controls (Ctrl) and expressed as means ± standard error. ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$ for the comparison between sleep loss and AC conditions. † $P < 0.05$ and ‡ $P < 0.01$ for the comparison between recovery and sleep loss conditions. Everson et al., 2014.

A study in rats shows that partial and total sleep deprivation for 10 days induced oxidative stress, overall and organ-specific DNA damage and increased cell death (Fig. 7; Everson et al., 2014). These symptoms reflect a condition of increased DAMP levels and DAMP-driven inflammation. Possibly a lack of sleep causes disruption in the diurnal fluctuations of lymph drainage resulting in accumulation of DAMPs. The rats showed the most pronounced cell injury in the intestine, which could eventually lead to a leaky gut. This increases the risk for translocation of gut bacteria and perchance even PAMP-driven systemic inflammation. Sleep loss is associated with impairment of host defence (Krueger, 2016), but whether intestinal dysbiosis and, accordingly, low-grade inflammation form direct causes is still unclear (Benedict et al., 2016; Poroyko et al., 2016; Zhang et al.,2017).

Another integrative system depending on hormones that is affected by sleep loss is the HPA axis. Transient sleep loss causes mild temporary increased activity of the HPA axis while sustained lack of sleep results in elevated evening cortisol levels (Meerlo, Sgoifo & Suchecki, 2008). Chronically elevated glucocorticoid levels have been associated with reductions in

neuronal plasticity and neurogenesis (Sapolsky, 2000), which shows how recurrent increases in glucocorticoid levels by chronic sleep deprivation may add up to a dangerous threshold for certain pathophysiology. Thus, a lack of sleep may gradually impair normal functioning of the HPA axis. Indeed, a study in humans shows that prolonged sleep restriction dysregulated the interplay between inflammatory and stress markers at the cell level (Fig. 8; Simpson et al., 2016).

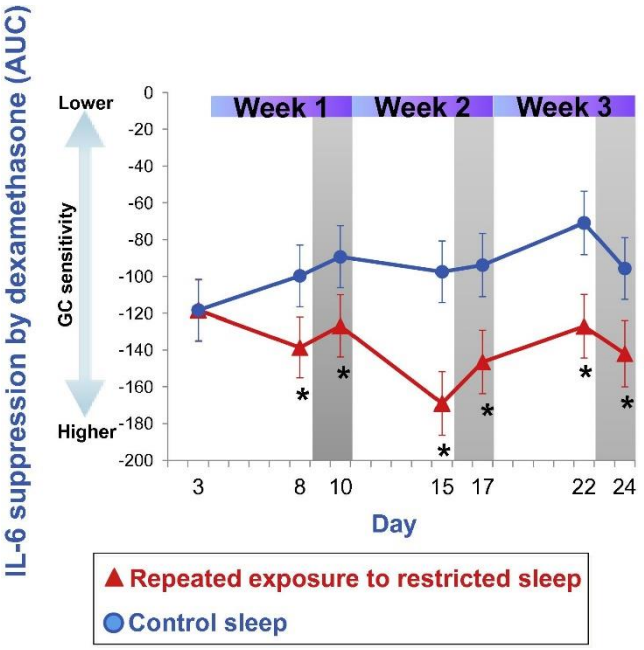

Figure 8. Glucocorticoid (GC) sensitivity of monocytes. GC sensitivity was determined by the ability of dexamethasone to suppress IL-6 expression in monocytes and calculated as AUC of IL-6 suppression. Higher IL-6 suppression by DEX indicates higher GC sensitivity. The experimental design consisted of three weeks of 5 consecutive nights of 4h sleep followed by two nights of 8h recovery sleep. Values are mean ± SEM based on mixed model analysis,*$p < 0.05$ between conditions. Simpson et al., 2014

Sleep seems to serve a protective role, guarding homeostatic balance by timed drainage and retaining normal functioning of complex regulatory processes. When sleep is disturbed, these general physiological processes also get disturbed which could have serious consequences for health.

Discussion

The potential mechanisms of sleep deprivation leading to an increased infection risk are quite versatile. Previous sections show how complicated the interplay between sleep and the immune system is. Both systems interact on different levels with each interaction causing a chain of effects. Changing conditions at one end of the chain logically affect the rest of the chain. In general it can be stated that sleep counteracts low-grade inflammation, thereby maintaining immune homeostasis (Fig. 9). When sleep is disturbed, sufficient homeostasis of the immune system fails. In these circumstances, low-grade inflammation can escape its safeguard and flare up into more critical conditions.

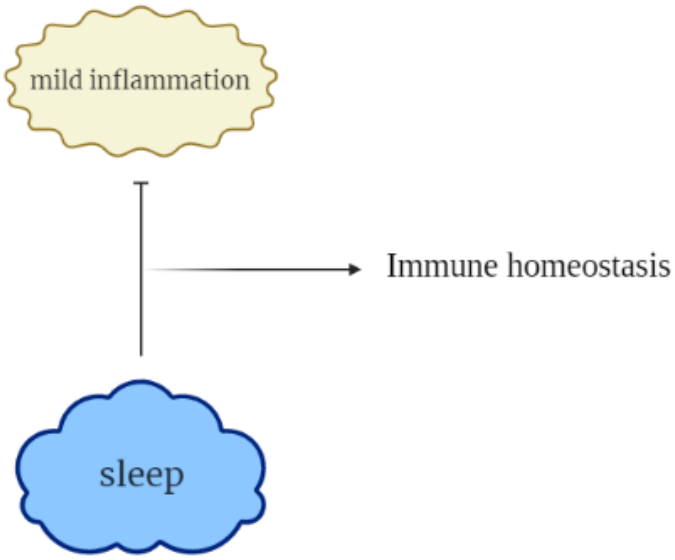

Figure 9. Schematic overview of a healthy interconnective system between sleep and the immune system. Sleep has the protective function of inhibiting mild inflammatory conditions, hereby maintaining immune homeostasis. Created in BioRender.com.

An indirect mechanism by which disturbed sleep can lead to an increased risk of infection can be explained by a changing environment. Environmental interactions potentially leading to an infection are increased by decreasing the amount of sleep. Time spent awake increases the occurrence of PAMPs and DAMPs binding to PRRs which trigger a cascade of intracellular signalling pathways leading to immune activation. This brings us to a more detailed level, where leukocytes are being activated and cytokine production increases. Sleep deprivation causes a disbalance in leukocyte densities, increasing the concentrations which are normally low in sleep conditions. Ratios in cytokine production by immune cells are tightly regulated by sleep and quickly show imbalances when sleep is disrupted.

Disturbed sleep has detrimental effects on equally tightly regulated homeostatic clearance systems in the brain and periphery. Simply stated, sleep serves a protective role in the prevention of accumulation of waste products. Glymphatic and lymphatic systems carefully follow the sleep/wake cycle and flush soluble proteins and metabolites away from the brain

parenchyma and lymph nodes to later be processed by the liver and kidneys. Interference in this process by sleep loss results in dangerous protein aggregations, which is a pathological hallmark for neurodegenerative diseases, but also in general means an increase in DAMP levels. This shows the interconnectivity of different systems affected by sleep loss, resulting in an increased infection risk (Fig. 10).

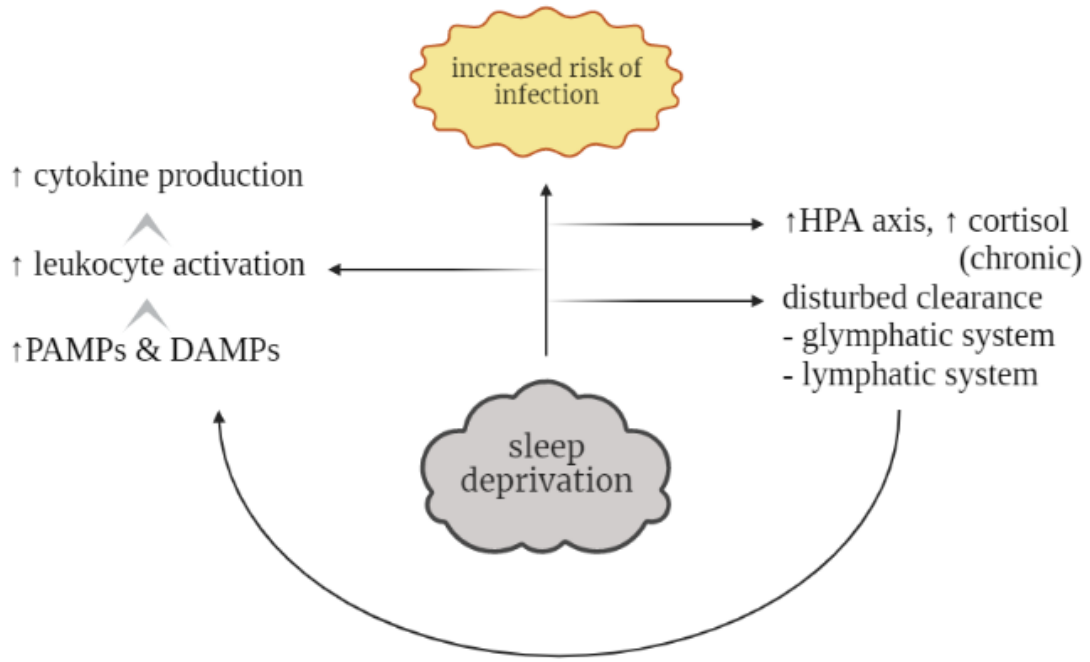

Figure 10. Conceptual model of the mechanisms behind sleep deprivation leading to increased infection risk. More wake time augments environmental interactions which increase the occurrence of PAMPs and DAMPs activating leukocytes. Tightly regulated concentrations of leukocytes and their cytokine secretion products become unbalanced and trigger a cascade of intracellular signalling pathways leading to immune activation. Clearance of waste products by the glymphatic and lymphatic systems gets disturbed, leading to an increase in DAMP levels. In chronic situations, increased activity of the HPA axis may lead to elevated cortisol levels. Created in BioRender.com.

Many different complex systems that may or may not influence each other make it a challenge to deliver a complete and detailed description of all the mechanisms behind increased infection risk by sleep deprivation. Therefore, only a few aspects of the systems that influence the increased risk of infection due to sleep deprivation are described in this essay. For example, changing leukocyte concentrations caused by sleep loss are not mentioned in detail as to how the increased levels negatively influence infection risk. Leukocyte activation per se does not necessarily lead to an infectious state, so there could be a certain threshold that needs to be reached in order to get sick. Or there could be certain types of leukocytes that dominate infection risk over others. These same thoughts could hold for the changes in cytokine levels. Only a few cytokines are discussed, but signalling pathways often include a large variety of signalling molecules as well as crosstalk between other complex signalling systems (Wajand & Siegmund, 2019). This causes for a lot of complexity in assessing the exact effects of cytokine changes caused by sleep loss.

One substance that has not been discussed but may play an essential role in the mechanisms underlying the association between sleep deficiency and low-grade inflammation are prostaglandins (PGs). PGs mediate fever and pain which makes them a key target for nonsteroidal anti-inflammatory drugs (NSAIDs), preventing the synthesis of PG's (Vane, 1971). Other than promoting inflammation, the PG system also plays an essential role in inhibiting inflammation (Serhan, 2017), and it is involved in human sleep physiology. Precursors of PGs have been reported to show nocturnal peaks and daytime troughs, and sleep deprivation reverses this circadian pattern (Jordan et al., 2004). Since research in PGs in the context of sleep in humans is scarce, further investigations of the PG system, possibly including NSAID practice, could elucidate more critical knowledge on the mechanisms behind increased infection risk following sleep deprivation. Looking further into brain waste clearance and sleep raises another interesting thought. Could there be a connection between shorter sleep and a less efficient glymphatic system in the ageing brain? Age-related changes in sleep include shortened nocturnal sleep duration and decreased SWS (Taillard et al., 2021). The glymphatic system is directly dependent on SWS, and also degenerates with age (Benveniste et al., 2019). An exciting concept would be if the process of neurodegeneration could be delayed by improving sleep quality. Exploring such ideas would not only improve knowledge about the mechanisms behind sleep and immunity, but also put sleep itself more in the spotlight as a solution to health problems.

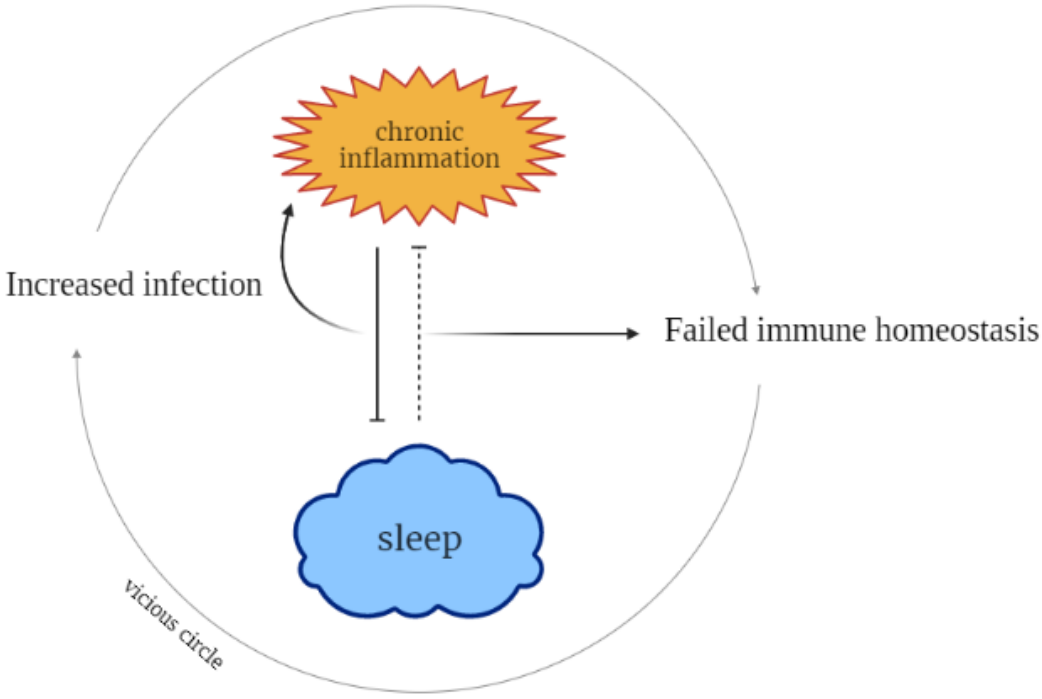

Figure 11. Schematic overview of how a situation of chronic inflammation has a disturbing effect on sleep, thereby failing the protective function of sleep for immune homeostasis. The infection status worsens and the interplay between sleep and the immune system feeds into a vicious circle. Created in BioRender.com

Conditions of increased infection regularly also have negative effects on sleep (Besedovksy, Lange & Haack, 2019), and with disturbed sleep corrupting immune function there is the chance of feeding into a vicious circle. The relationship between disturbed sleep and infection risk can therefore at some point be seen as bidirectional. Starting with a healthy situation, a bit of sleep loss has negative effects on the immune system which do not immediately lead to more disturbed sleep caused by the increased state of infection (Fig. 9). Conditions worsen with prolonged sleep loss increasing the grade of infection. When the inflammatory condition turns chronic this could lead to disturbed sleep, which creates a vicious circle (Fig. 11). What started off as an initial situation of disturbed sleep leading to increased infection is now reversed and both sleep and the immune system counteract each other. At this point we are very far from a healthy situation which is quite hard to return to. Looking at this problem from a philosophical point of view, one could try to answer the question of which came first: the chicken or the egg? The starting point is a healthy interaction between sleep and immunity. What makes this interaction unhealthy is when disturbed sleep drives inflammatory status to a critical point where the inflammation takes over power and starts dominating the field. Therefore, sleep can be seen as the match lighting up the fire. Stretching this metaphor, when you remove the match the fire will keep burning; inflammatory status is at risk of aggravation because of derailed homeostasis. Trying to improve sleep probably not provides a solution to return to a healthy status and we might need medicine (e.g. fire extinguisher) to overcome sickness. Coming from this view on the interplay between sleep and the risk of infection it is sleep that has initial control of a healthy situation. Taking care of good sleep therefore could serve as a tool to conserve immunity status.

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