NEURAL-IMMUNE INTERACTIONS IN THE REGULATION OF SLEEP

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1. ABSTRACT

Interactions between sleep and the immune system have been recognized for millennia. The lethargy and increased desire to sleep that accompany mild infections such as colds or "the flu" are common experiences. These experiences have fostered the belief that sleep promotes recovery from infectious challenge. Another common belief is that the lack of sleep increases susceptibility to infectious disease. However, despite these age-old and widespread beliefs, surprisingly little empirical evidence supports the hypotheses that increased sleep aids recovery from, and lack of sleep increases susceptibility to, infections. Although research conducted over the last 30 years has clearly demonstrated that sleep is altered during the course of infection, few experiments have directly tested the functional impact of sleep on responses to immune challenge. We will review relevant literature documenting that sleep patterns do indeed change during states of infectious disease, discuss potential mediators of these alterations in behavior, and finally address the issue of whether sleep or sleep loss impacts the ability of the host to mount an appropriate immune response.

2. IMPACT OF IMMUNE ACTIVATION ON SLEEP

The changes in sleep that occur in response to infection depend on the specific microorganism and the route of infection. The quantitative and temporal changes in sleep that develop throughout the course of an infectious challenge have been characterized for several bacterial strains, viruses, and parasites.

2.1. Bacterial infections and sleep

Rabbits have been used to demonstrate alterations in sleep in response to bacterial inoculation (reviewed in (1,2)). In general, bacterially-infected rabbits show an initial increase in the amount of time spent in slow-wave sleep (SWS), which is then followed by a period of SWS suppression. Rapid-eye-movement sleep (REMS) is normally reduced for the duration of the infection. The changes in the amount of SWS are usually paralleled by changes in delta-wave amplitudes (DWA) during SWS, which is generally considered to reflect sleep intensity. Therefore, both the amount and the depth of SWS change in a biphasic manner during infection. The enhancement of SWS in bacterially-infected rabbits typically develops concurrent with fever and other pathophysiologic signs of infectious disease. However, these changes in SWS are not a byproduct of the febrile response as the fever generally persists beyond the period of enhanced sleep, thus, sleep and fever can be dissociated. Viable bacteria are not necessary to induce alterations in sleep. Killed bacteria and isolated bacterial components also elicit enhanced SWS sleep, although higher doses may be required (reviewed in (1,2)). In addition, treatment of animals with bacteriocidal antibiotics attenuates, but does not eliminate the development of microbially-induced sleep alterations. Thus, bacterial replication is not essential for sleep to be altered.

The specific patterns of sleep alterations that accompany bacterial infections may be related to the structural differences between bacterial species. For example, lipid A, which is a component of endotoxin from Gram-negative bacteria, increases SWS in rabbits within an hour after intravenous injection, whereas muramyl dipeptide, a synthetic analog of the monomeric muramyl peptide component of bacterial cell wall peptidoglycan, increases sleep after a longer latency but for a longer duration (3,4). Similarly, Gram-negative bacteria induce enhanced sleep more rapidly than do Gram-positive

bacteria, although the duration of the sleep enhancement induced by Gram-negative bacteria is generally shorter. Variations in sleep patterns may also reflect differences in the disease process. For example, rabbits made septicemic by intravenous inoculation with Pasteurella multocida exhibit a different pattern of sleep responses than do animals in which the same bacteria species is administered intranasally, which causes pneumonia (5). The qualitative and temporal variation in sleep patterns after microbial challenge may explain the apparently inconsistent changes in sleep reported in some studies of humans with spontaneous infections (6,7). Administration of endotoxin also affects sleep in humans (8) and in animals (9,10).

2.2. Viral infections and sleep

The two viral pathogens that have been studied most extensively with respect to sleep are immunodeficiency viruses (HIV in humans and FIV in cats) and influenza virus. Polysomnographic studies of asymptomatic HIV-infected men reveal several alterations in normal sleep patterns. Most prominent is a significant increase in the percentage of SWS during the second half of the night, frequent nighttime awakenings and abnormal REMS architecture are also common (11,12). These altered sleep patterns are not related to psychological, social, or medical etiologies, and they precede the onset of secondary infections or overt neurologic involvement (11,12). As the HIV infection progresses to AIDS, patients tend to develop severe reductions in SWS, marked fragmentation of sleep, and an extreme disruption of normal sleep architecture. These severe changes could be related to HIV-induced encephalitis or to the development of opportunistic infections or aberrant immune responses. Like HIV-infected humans, cats infected with FIV, a feline retrovirus, develop changes in the normal temporal pattern of SWS, an increased frequency of arousal from sleep, and a decrease in the amount of REMS (13). These viral-induced alterations in sleep may be mediated by envelope glycoproteins (14). For example, the envelope glycoproteins gp41, gp120, and gp160 all increase NREM sleep of rats (15,16) in a manner that is similar in some respects to the alterations in sleep of HIV seropositive individuals that are not symptomatic for AIDS (17).

Like infection with immunodeficiency viruses, infection with influenza virus also alters normal sleep patterns in humans and animals. Human volunteers experimentally infected with rhinovirus or influenza virus sleep less during the incubation period, but sleep longer during the symptomatic period, sleep quality and the number of awakenings are not affected (18). Influenza-infected mice develop marked sleep enhancement that is most apparent during the dark portion of the circadian cycle (19,20), when mice are normally most active. In rabbits, intravenous administration of influenza virus induces a brief but consistent period of sleep enhancement (21). Mice and rabbits differ in that influenza virus can replicate completely in mice but undergoes only partial replication in rabbits. Mice inoculated intraperitoneally with the avian paramyxovirus Newcastle disease virus (NDV) develop transient sleep enhancement similar to that of influenza-inoculated rabbits (22), but like influenza virus in rabbits,

NDV cannot replicate in mice. This inability to induce active infection may contribute to the relative brevity of the sleep and temperature effects induced by such challenges. Furthermore, killed influenza virus does not promote sleep in either species (21,23). These observations suggest that in viral infections, unlike bacterial infections, microbial replication may be necessary to influence sleep. Double-stranded RNA (dsRNA), which is produced in infected host cells during viral replication, induces cytokines that are likely to mediate sleep alterations during viral infections. Intracerebroventricular administration of dsRNA extracted from the lungs of influenza-infected mice induces sleep in rabbits, and synthetic dsRNA (polyinosinic:polycytidilic acid, or poly I:C) induces increased sleep in both rabbits and mice (21,22).

2.3. Other organisms

Subspecies of the protozoan Trypanosoma brucei cause the chronic clinical condition known as "sleeping sickness" in humans. In rabbits, subcutaneous inoculation with Trypanosoma brucei brucei increases sleep after a latency of several days, coincident with the onset of fever and other clinical signs of illness (24). The initial period of enhanced sleep gradually abates, although periods of increased somnolence continue to occur in association with the episodic recrudescence of parasitemia (24). In addition to such episodes of hypersomnolence, loss of the normal circadian organization of sleep also develops during chronic trypanosomiasis in rabbits, rats, and humans (25,26,27,24).

Fungal infections can also alter sleep patterns in animals. Subcutaneous administration of live brewer's yeast to rats significantly increases the time spent in SWS during both light and dark portion of the circadian cycle (28). Intravenous inoculation of rabbits with Candida albicans induces sleep alterations similar to those elicited by Gram-positive bacteria (29).

Prion-related pathologies are also associated with alterations in sleep. Rats inoculated with brain homogenate from scrapie-infected animals demonstrate unusual spiking patterns in the EEG about four months after inoculation during periods of quiet wakefulness, later, SWS and active wakefulness are reduced, and drowsiness is increased (30,31). Cats inoculated intracerebrally with brain homogenate from a human with Creutzfeldt-Jakob disease after about 20 months demonstrate increased SWS time, reduced wakefulness, and abnormal EEG and physiological correlates of REMS (32). In humans, the condition known as fatal familial insomnia is associated with prion-related thalamic neurodegeneration. Mutations in the prion protein, a glycoprotein on neuronal membranes and in astrocytes, may underlie the pathological changes that accompany this condition (33). Mice that genetically lack the prion protein gene demonstrate alterations in both sleep and circadian rhythms (34,35).

3. MEDIATORS OF RESPONSES TO IMMUNE CHALLENGE

As reviewed above, a substantial literature describes specific alterations in sleep amount and

architecture that occur during infections with a variety of pathogens. The mechanisms that mediate the sleep responses to infection have not been conclusively identified at present. However, a compelling hypothesis is related to the fact that microbial infections elicit immune responses that alter the basal expression levels of numerous immunomodulatory substances. Research that began in the 1970s demonstrates that one class of immune effectors, cytokines, are powerful modulators of sleep-wake behavior. As such, cytokines represent one of several candidate systems that are likely to be involved in mediating behavioral responses to infection.

Cytokines are low molecular weight proteins that mediate many aspects of the host defense response, inflammation, and tissue remodeling. Although most cytokines were originally described as products of the peripheral immune system on the basis of their immune-modulatory properties, many also modulate multiple central nervous system (CNS) processes. Although low levels of circulating cytokines are capable of altering CNS function (36), and there are several documented mechanisms by which peripheral cytokines may signal the brain (37), cytokines and their receptors are now known to be produced within the CNS (38,39). Observations that cytokine expression and protein levels are altered in response to microbial infection, that cytokines and their receptors are synthesized in brain, and that they are active in brain suggest cytokines as potential mediators of infection-induced alterations in sleep.

3.1. Interleukin-1 and Tumor Necrosis Factor

A substantial body of literature has accumulated over the last 20 years concerning the role of cytokines in sleep regulation. The vast majority of these reports focus on two families of cytokine gene products, interleukin (IL)-1 and tumor necrosis factor (TNF). These pleiotropic cytokines overlap in their biological activities to a great extent, they induce each other's synthesis, and they often are synergistic (39). The interactions between IL-1 and TNF may also be relevant for sleep regulation (40). For example, TNF-induced SWS responses are attenuated in animals pretreated with a soluble IL-1 receptor, and TNF antagonists inhibit IL-1-induced enhancement of SWS.

The IL-1 gene family includes IL-1-beta, IL-1-alpha, the IL-1 receptor antagonist (IL-1ra), and two receptors (IL-1R1, IL-1R2). Although IL-1-alpha enhances SWS, most studies have evaluated the effects of IL-1-beta, which we will designate simply as IL-1. Abundant evidence indicates that IL-1 is involved in the regulation of sleep, but much of this evidence is indirect. For example, IL-1 enhances SWS in every species thus far examined, irrespective of route of administration. IL-1 receptors are widely distributed throughout the CNS, including important sleep-regulatory regions such as the hypothalamus (41). IL-1-containing neurons are also present in the hypothalamus (42,43). IL-1 mRNA can be detected in many brain regions. In rat brain, IL-1 mRNA expression exhibits a diurnal variation, expression is highest during the sleep phase of the circadian cycle and lower during the active phase (44). Sleep deprivation

increases IL-1 mRNA expression (45), and IL-1 protein in plasma is detected more frequently after sleep deprivation than during spontaneous sleep (46). IL-1-like activity in cerebrospinal fluid of cats exhibits fluctuations that are in phase with the sleep-wake cycle (47), plasma IL-1 levels in humans peak at sleep onset (48), and IL-1 is detected more frequently in plasma samples taken from humans during sleep than during waking (49). Stimulated whole blood cell cultures from samples taken during sleep produce more IL-1 than those from cultures taken during waking (50). Generally speaking, those substances or manipulations that induce IL-1 synthesis or release increase SWS, whereas substances that inhibit the synthesis or actions of IL-1 inhibit SWS (reviewed (51,52)).

Direct evidence also indicates that IL-1 is involved in sleep regulation. Interfering with the binding of IL-1 to its receptor by administering the IL-1ra, antibodies directed against IL-1, or a soluble IL-1 receptor fragment reduces spontaneous SWS in otherwise normal animals (53,54,55). Furthermore, sleep deprivation-induced enhancement of SWS is attenuated or blocked when experimental animals are pretreated with anti-IL-1 antibodies or with an IL-1 receptor fragment (46,56,57). Collectively, these data provide strong evidence that IL-1 is involved in the regulation of sleep under normal conditions.

The two forms of TNF are TNF-alpha and TNF-beta. TNF-alpha (cachectin) is a product primarily of macrophages, whereas TNF-beta (lymphotoxin) is a product of lymphocytes. These TNF proteins share about 30% amino acid sequence homology, and have similar, though not identical, biological activities. TNF is produced by astrocytes, and TNF immunoreactive neurons exist in the CNS. As with IL-1, both forms of TNF enhance SWS, although most studies have evaluated TNF-alpha (hereafter denoted as TNF). TNF receptor mRNA is expressed in normal brain, and the soluble TNF receptor is normally present in cerebrospinal fluid. The two cell surface TNF receptors, designated 55 kDa and 75 kDa TNF receptors, mediate distinct actions. The effects on sleep of TNF appear to be mediated by the 55 kDa receptor (58).

The evidence supporting a role for TNF in sleep regulation is similar to that for IL-1. Plasma TNF concentrations correlate with EEG slow wave activity in humans (59). In rat brain, TNF mRNA (60) and TNF protein concentrations (61) exhibit diurnal variation, with peaks occurring during circadian periods associated with sleep. Similarly, peak concentrations of TNF protein in human plasma occur during sleep (49). TNF increases SWS in rabbits (62), mice (58), and rats (63,64), as well as the amplitudes of EEG slow waves (62). Increased SWS after TNF administration is generally accompanied by reduced REMS, although in mice, low doses that promote SWS do not affect REMS. Direct intervention in the TNF system with antibodies (65), binding proteins (66), or soluble receptors or receptor fragments (66) reduces SWS in otherwise normal animals. Mice that lack the 55 kDa TNF receptor sleep less than background strain controls (58). The enhanced SWS that occurs after sleep deprivation (67) or in response to an increased ambient

temperature (68) is attenuated in experimental animals that are pretreated with TNF antagonists. Collectively, these data support the hypothesis that TNF is involved in the regulation of sleep.

3.2. Interferon

The first demonstrated link between cytokines and sleep was the observation that sleep deprivation enhanced the ability of leukocytes to produce anti-viral interferon (IFN) (69). Relative to IL-1 and TNF, the role of IFNs in the regulation of sleep has received less study, and evidence suggesting a role for IFN in the regulation of normal sleep is limited. However, both type I (anti-viral, or alpha/beta) and type II (immunocyte, or gamma) IFNs are known to modulate sleep. Human recombinant leukocyte IFN (alpha/beta) induces cortical EEG synchronization in the rat (70), increases SWS in rabbits (71), and reduces latency to REMS in monkeys (72). Anecdotally, humans undergoing therapy with leukocyte IFN complain of excessive sleepiness (73,74) although sleep may be disrupted rather than enhanced (75). Variations in dosage or in the underlying disease state may contribute to such differences. Human recombinant IFN gamma induces increased non-REM sleep in rabbits (76). When stimulated *in vitro*, blood cells collected during sleep produce more gamma-IFN than do cells collected during waking (50).

Type I IFNs are well known as antiviral cytokines and may be particularly important as modulators of viral-induced alterations in sleep. Influenza, HIV, FIV and NDV all induce alterations in sleep. IFN-alpha receptors are found in brain, and in response to viral infection, almost all nucleated cells can produce type I IFN. Viral components that induce IFN-alpha/beta enhance sleep and concurrently increase plasma concentrations of IFN alpha/beta (21). Collectively, these observations suggest that type I IFNs may be important mediators of sleep alterations that occur during viral infections.

The degree to which sleep patterns change during viral infection may depend on the ability of the organism to mount a type I IFN response. In mice, IFN-alpha/beta production is regulated in part by the *If1* gene. C57BL/6 mice produce relatively high levels of IFN-alph/beta in response to various challenges (77) and exhibit increased SWS in response to influenza infection (78), whereas BALB/c mice produce lower levels in response to similar challenges (77) and do not show influenza-related sleep enhancement (22). B6.C-*H28* mice, which have the BALB/c allele for low IFN alpha/beta production on the C57BL/6 genetic background, show C57BL/6-like sleep responses after challenge with influenza virus but BALB/c-like responses after challenge with Newcastle disease virus (22). Thus, the critical factor mediating alterations in sleep can vary depending on the challenge organism. However, because the *Ifl* allele also influences the expression of TNF-alpha and IL-6, as well as IFN-alpha/beta (79), the precise mediator of these effects on sleep remain uncertain.

3.3. Other cytokines, chemokines and growth factors

Several other cytokines, chemokines and growth factors have been tested for effects on sleep-wake behavior.

These include the cytokines IL-2, IL-4, IL-6, IL-10, IL-13, IL-15, IL-18, the beta chemokine MIP1-beta, and growth factors such as transforming growth factor beta, fibroblast growth factor, nerve growth factor, and granulocyte-macrophage colony-stimulating factor (80,81,82,83,84,85,86,87,88). In general, each of these cytokines, chemokines and growth factors has been the subject of one or a few studies. Conclusions that these cytokines are involved in sleep regulation are often based on correlative data rather than direct evidence. Nevertheless, these types of observations may suggest future directions of research. Such may be the case for IL-6. Although IL-6 has been implicated as a mediator of sickness behaviors in animal models (e.g., (89)), and mediates many of the responses to IL-1 and/or TNF (90,91,92,93), there is little direct evidence implicating IL-6 as a modulator / regulator of sleep. The initial study of the potential somnogenic properties of IL-6 did not reveal any sleep modulation subsequent to central or peripheral administration of human recombinant IL-6 in rabbits (94). Subsequent studies of healthy human volunteers indicated that subcutaneous administration of IL-6 increased slow wave sleep during the latter half of the night (95). In addition, IL6 concentrations in plasma exhibit rhythms in phase with sleep-wake behavior (96), and IL-6 concentrations in plasma increase during sleep deprivation (97,98).

Other regulatory mechanisms for cytokines may also contribute to the precise alterations of sleep in response to immune challenge. For example, studies in animals have demonstrated that infection-induced alterations in sleep are often bi-phasic, periods of sleep enhancement are followed by periods of sleep suppression. Preventing rabbits from sleeping during the phase of E. coli-induced sleep enhancement does not markedly alter the development or duration of the subsequent phase of suppressed sleep (20). Thus, the sleep suppression appears to be actively driven, rather than representing a simple compensatory response to the previous phase of excessive sleep. The sleep-suppression phase could reflect the influence of cytokine feedback mechanisms on sleep-wake behavior. For example, the cytokines IL-10 and IL-4 inhibit the synthesis of IL-1 and TNF and thereby modulate the pathophysiological effects of these pro-inflammatory cytokines. Central administration of IL-10 or IL-4 reduces spontaneous sleep in rats and rabbits (88,86), and mice that lack a functional IL-10 gene exhibit more sleep than genetically-intact mice (9). Because many types of infections induce the expression of IL-10 and IL-4, these inhibitory cytokines, or other regulatory feedback mechanisms that function to limit immune responses to infection, could mediate infection-related suppression of sleep.

4. EFFECTS OF SLEEP AND SLEEP LOSS ON THE IMMUNE SYSTEM

As reviewed above, a significant literature supports the view that immune challenge alters sleep patterns of animals and probably of humans as well. The potential association between infection-related release of

sleep-modulatory cytokines and alterations in sleep raises an important question. Is altered sleep merely a by-product of infectious disease and the immune response, or does sleep in some way facilitate recovery from microbial infections? Some observations suggest that sleep patterns reflect the progression of the disease process, the prognosis, or the clinical outcome. For example, in rabbits inoculated with *E. coli*, *S. aureus* or *C. albicans*, a prolonged phase of enhanced sleep after microbial challenge is associated with a more favorable prognosis and less severe clinical signs than is a short period of enhanced sleep followed by a prolonged reduction in sleep (99,100). In addition, rabbits that eventually die exhibit less sleep than rabbits that survive the infection (100). These observations suggest a prognostic value for sleep during infectious disease. Analogously, HIV-infected humans who are seropositive but are otherwise healthy demonstrate excess Stage 4 sleep (12), but sleep deteriorates and becomes disrupted as the disease progresses (101). Some epidemiological studies of human populations also support a relationship between insomnia or unusually short nighttime sleep durations and decreased life expectancy (102,103), although others do not (104). Associations between absent or diminished sleep, reduced EEG amplitude, and imminent death also occur in aged mice prior to spontaneous death (105), in mice with fatal experimental rabies infections (106,107), and in rats that die subsequent to chronic sleep deprivation and septicemia (108,109,110). The total amount of sleep and the EEG amplitude during sleep also gradually decline in rabbits with trypanosomal infections (111), which are eventually fatal.

Despite these provocative correlations, the impact of sleep loss on immune competence is difficult to assess experimentally. One important concern is distinguishing between effects attributable to a lack of sleep *per se* and those associated with non-specific stress. Stress-related immune impairments are well documented and impact the response to microbial challenge in humans and animals (112,113,114). Experimental models of sleep deprivation in animals generally attempt to minimize non-specific stress and frequently incorporate physiologic measures that are thought to reflect non-specific stress. Some approaches to inducing sleep loss in animals elicit relatively few of the classical physiological signs of non-specific stress (e.g., (115)), whereas increases in markers traditionally thought to reflect responses to stressors (e.g., elevated catecholamines or glucocorticoids) are a prominent feature of other approaches (e.g., (116,117)). However, increased catecholamines or glucocorticoids are generally not present in humans undergoing sleep deprivation (118,119,120,121,69,122,123). Because humans can voluntarily choose to go without sleep, studies of immunologic effects of sleep loss in humans may generate data that will be difficult to replicate in animal models using forced waking (124). Nonetheless, sleep loss that humans experience as a "normal" facet of life is frequently associated with stressors or environmental factors that necessitate or otherwise contribute to loss of sleep (e.g., examinations, bereavement, shift work, depression). Thus, the concern for moderate amounts of non-specific stress in

animal models may be unduly emphasized in some respects.

Another problem in the evaluation of sleep-related alterations in immune function is validation of the biologic or clinical significance of modest but statistically significant changes in immune indices (125,126). Furthermore, most reports evaluating the impact of sleep loss on the immune system have studied healthy animals or people that are not experiencing a substantive immunological challenge coincident with sleep loss. Finally, methods used to induce sleep deprivation, the selection of specific immune indices, and the manner in which the selected immune indices are measured vary substantially between laboratories. These differences likely contribute to the numerous discrepancies that pervade the human and the animal literature pertaining to sleep, sleep loss, and immune function.

Several human studies have examined various aspects of immune function after varying periods of partial or total sleep deprivation (e.g. (118,127)). However, because the studies typically use small numbers of subjects and vary substantially in terms of the parameters measured and the methodology used, the time of blood collection, the environment of the subjects, and the degree of sleep loss, drawing conclusions is complicated and numerous inconsistencies pervade the literature (124). For example, some laboratories report that sleep deprivation alters particular immune parameters (128,118,120,69), whereas others do not find significant perturbations or health impairments associated with sleep loss (126,129) . Recent studies have often used natural killer (NK) cell numbers or activity as measures, some groups report increases in these measures when evaluating samples from sleep deprived subjects (118), whereas others report decreases (120,130,130). Others detect altered responses to antigens or mitogens (128,118,120,69). An increased infection rate (131) and increased respiratory illness or asthma (132) have been reported in some studies of sleep-deprived subjects, but not in others (118). However, such studies probably do not stringently test the impact of sleep or sleep loss on disease incidence or exacerbation because subjects are typically young and healthy, periods of sleep loss are relatively short, and the subjects are not challenged by overt exposure to disease.

Sleep fragmentation, non-restorative sleep, and inadequate sleep may have few significant adverse immune consequences in young, healthy individuals, but could nonetheless severely impact elderly or patient populations. For example, chronic stress that is associated with sleep loss impairs immune responses to influenza vaccination in elderly humans (113). Sleep disruption can be profound in hospitalized patients and nursing home residents (133,115,134). For example, slow-wave sleep occupies less than 1% of the night during the 5 to 8 days after open-heart surgery (133,135). Sleepiness and sleep quality also broadly influence measures of general health status, particularly impacting perceptions about energy and fatigue. Some correlation studies have found significant relationships between sleep and mortality in humans (136),

although others do not (137,136,104,138,139,102,103). Furthermore, host defense responses such as antibody production or microbial clearance could be altered by sleep loss *per se*, but might also be impacted by stress- or illness-induced activation of the glucocorticoid system (e.g., (10)). Greater knowledge about such interactions could have important health implications for hospitalized patients and nursing home residents, who commonly experience severe disruptions in normal patterns of sleep.

Studies in rabbits and mice indicate that reduced or fragmented sleep is associated with a poor prognosis after microbial challenge, whereas increased sleep is correlated with favorable clinical outcomes (100,23). Rats subjected to chronic sleep deprivation did not show changes in splenocyte responses to mitogens, although circulating lymphocyte numbers were reduced (140). However, studies of sleep-deprived rodents undergoing antigenic challenge indicate that sleep loss may induce functionally significant immune perturbations. For example, secondary antibody responses to antigenic challenge are impaired in sleep-deprived mice and rats (141,142). Sleep loss is also reported to retard both viral clearance and the development of a protective antibody response in influenza-infected mice (141), but others have not confirmed this finding (143,144). Sleep deprivation may also modulate other physiologic responses related to immune or acute phase responses. For example, sleep deprivation exacerbates fever in *E. coli*-inoculated rabbits (20), non-pyrogenic doses of sheep red blood cells elicit fever when administered to sleep-deprived rats (145), and intracerebroventricular administration of saline or immunoglobulins induces fever in sleep-deprived but not in rested rats (46). Sleep deprivation is also reported to exacerbate anticoagulant-induced anemia (146) and to retard tumor growth in rats (147).

The hypothesis that chronic (if not acute) sleep loss impairs immune competence is most strongly supported by the observation that chronic sleep deprivation of rats results in intestinal bacterial proliferation, microbial penetration into lymph nodes, septicemia, and eventually death (108,109,115,110,148,149,147). The penetration of bacteria into normally sterile tissues during prolonged sleep deprivation implies the development of immune insufficiency and abnormal host defense, and suggests that sleep loss could render healthy individuals susceptible to disease, as well as exacerbate existing disease or complicate recovery in patient populations. The data that are accruing from both animal and human studies appear to suggest that short-term sleep loss may be accompanied by some enhancement of non-specific host defense mechanisms, whereas chronic or prolonged sleep loss may result in immune suppression (150,151). Similar arguments have been posed for the relationship between immune function and stress, particularly as reflected by elevations in circulating glucocorticoid levels (152). Sleep deprivation has also recently been reported to cause death in *cyc*[01] mutant *Drosophila melanogaster* in association with reduced expression of heat-shock genes after sleep loss (153).

5. SUMMARY AND CONCLUSIONS

Intriguing data support significant interactions between sleep, sleep loss, immune function, and infectious or inflammatory disease. These findings also indicate important directions for future work, such as identifying the mechanisms by which infectious disease alters sleep, determining whether sleep directly promotes recovery from infectious illness, and considering whether infectious disease should represent a contraindication for performing activities that require a high degree of vigilance. Resolution of these issues is critical not only for advancing our basic understanding of sleep but also for addressing significant problems of human welfare. Sleep and sleepiness are major economic and public health considerations. Circadian periods characterized by sleepiness are associated with greater numbers of accidents, from individual mishaps to catastrophic tragedies (154). In addition, poor sleep or excessive sleepiness causes significant economic loss through reduced productivity and compromises the quality of life of many people who suffer from sleep disorders (155). An improved understanding of the factors that mediate sleep and sleepiness may contribute to the alleviation of these important public health concerns.

6. ACKNOWLEDGMENTS

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