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Sick and tired: how molecular regulators of human sleep schedules and duration impact immune function

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Why do we need to sleep? What regulates when we sleep? And what dictates the number of hours we require? These are often viewed as three separate biological questions. Here, we propose they share molecular etiologies, whereby regulators of sleep schedules and sleep duration also govern the physiological purposes of sleep. To support our hypothesis, we review Mendelian human genetic variants sufficient to advance sleep-wake onset (*PER2*) and shorten sleep length (*DEC2*), and evaluate their emerging roles in immune responses that may rely on a sound night of slumber.

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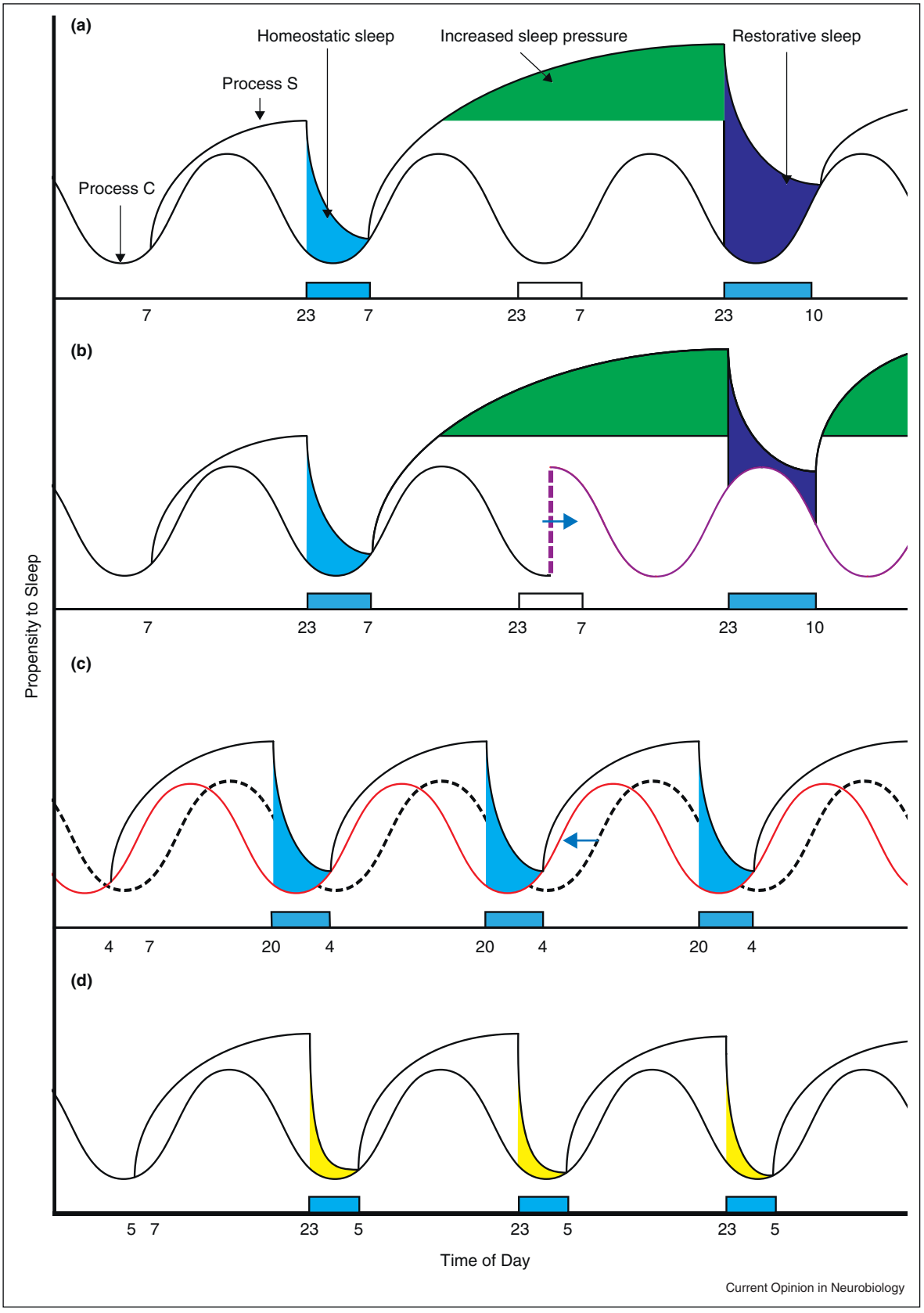
Introduction

Sleep is an innate behavior that is evidently circadian for modern humans because it is usually a daily, consolidated event with predictable timing. Quality of sleep is of utmost importance, but it remains difficult to define because its purpose is highly debated in light of many intriguing possibilities [1,2]. Besides restorative biological processes, optimal sleep in conventional society also takes into account its timing (i.e. sleep schedules relative to the time of day, also known as Process C due to its association with circadian rhythmicity) and its duration (i.e. the number of hours that yield satiety, also known as sleep homeostasis or Process S) (Figure 1a) [3]. Sleep disorders such as insomnia or sleep deprivation distort the relationship between Processes C and S and affect both (Figure 1b). Other variations in sleep patterns include those that specifically affect Process C such as advanced sleep phase (where affected individuals feel sleepy in the late afternoon and wake up before sunrise, though the total amount of sleep remains conventional) (Figure 1c), and those that perturb only Process S such as natural short sleep (Figure 1d) [4]. Therefore, understanding the biological underpinnings of Processes C and S may lead to targeted treatment for sleep disorders.

It is a common belief that there are separate molecular pathways for Processes C and S, and there may be coordinated mechanisms between them that together ensure 'optimal' sleep quality. Therefore, the molecular basis of the two-process model is sometimes simplified as a Venn diagram with two partially intersecting circles (Figure 2a). Process C is better understood compared to Process S because it is associated with the 'molecular core clock,' which defines a series of mechanisms that allow a cell to maintain circadian rhythmicity. The most defined aspects of the molecular core clock are a series of transcription-translation negative feedback loops that take approximately 24 hours to complete [5,6]. But despite a remarkable correlation between cellular and behavioral circadian periods (the time it takes to complete one cycle) [7], it is not clear how the molecular core clock regulates timing of sleep onset and offset. The molecular basis of Process S is even more nebulous because it is challenging to define and assay sleep homeostasis *in vitro*. Therefore, identifying molecular components sufficient to alter sleep timing and duration is of high research interest.

Through the identification of Mendelian human sleep traits, genetic mutations that result in advanced sleep phase (*PER2*) and shortened sleep duration (*DEC2*) were found [8–10]. With regard to the two-process model, *PER2* appear to participate in Process C and *DEC2* in Process S. Interestingly, emerging evidence suggests an intimate relationship between Processes C and S, and clinical outcomes related to immune responses. Is it possible that instead of directly sharing molecular mechanisms, Processes C and S may instead coordinate through participating in physiological reasons for sleep such as immune function? Here we posit that *PER2* and *DEC2* may function as regulators of sleep timing and duration respectively, yet both simultaneously impact the immune system via separate mechanisms (Figure 2b). Together, this hypothesis explains the observed correlation between sleep and immune responses, and also supports an alternative view of the two-process model. Finally, we discuss the challenges of untangling the molecular basis of sleep regulation (how much sleep

Figure 1


(a)
Homeostatic sleep
Increased sleep pressure
Restorative sleep
Process S
Process C
7
23
7
23
7
23
10
(b)
7
23
7
23
7
23
10
(c)
4
7
20
4
20
4
20
4
(d)
5
7
23
5
23
5
23
5
Propensity to Sleep
Time of Day
Current Opinion in Neurobiology

and when), its physiological function (why we sleep), and the consequences (or feedback) of impaired sleep on molecular mechanisms underlying Processes C and S.

Human genetics of sleep timing and duration

The first Mendelian human circadian rhythm trait characterized was Familial Advanced Sleep Phase (FASP), a highly penetrant autosomal dominant trait [8,11]. Affected individuals awaken and go to bed exceptionally early to maintain a normal quantity and quality of sleep, and attempts at modifying circadian tendencies (such as the use of phototherapy) are usually unsuccessful [12,13]. This advancement in sleep phase is accompanied by a shortening of free-running activity period, which measures the endogenous behavioral sleep-wake cycle in the absence of environmental cues such as light, food timing, and social interaction [11,14].

Using human genetic methods, it was determined that PER2-S662G is associated with FASP in this pedigree. To demonstrate that *PER2*-S662G is sufficient to advance sleep timing, BAC transgenic mice carrying *PER2*-S662G and *PER2*-S662D were generated, with the latter mimicking phospho-serine at the same site because S662 was hypothesized to be a phosphorylation site. Remarkably, *PER2*-S662G mice recapitulate advanced activity onset and shorter free-running period observed in FASP individuals, whereas *PER2*-S662D mice demonstrate longer free-running periods [9]. Further supporting the importance of PER2 phosphorylation in sleep timing, an additional genetic variant identified for FASP is located in *CSNK1D*, which reduces enzymatic activity and therefore hypophosphorylates PER2 *in vitro* [15,16]. In addition, constitutive expression of PER2 reversibly disrupts circadian rhythms of activity [17].

Together, these findings suggest that the circadian oscillation of PER2 may be sufficient for regulating sleep timing [6]. However, FASP individuals do not exhibit overt changes in Process S according to EEG measures of sleep architecture [11]. In addition, Per2 knockout (KO) mice are reported to exhibit no significant differences in sleep homeostasis [18], suggesting that *PER2* may be responsible for only Process C. But since *PER2* and *CSNK1D* mutations appear to be sufficient for advancing sleep phase and transmit in a Mendelian manner, it was hypothesized that a rare genetic variant that changes sleep duration may also exist. Indeed, Familial Natural Short Sleepers (FNSS) were found to sleep 6–6.5 hours per night (~2 hours less than controls), and they do not report a sense of sleep deprivation. These FNSS individuals carry a mutation in *DEC2/BHLHE41*, which encodes a transcription repressor that belongs to the Hairy/Enhancer of Split subfamily [19]. The mutation replaces a proline at position 384 with arginine, and BAC transgenic mice carrying *DEC2*-P384R exhibit altered sleep homeostasis. Specifically, *DEC2*-P384R mice undergo shorter duration of rapid eye movement (REM) (~2%) and non-REM (NREM) (~6%) sleep, and recover more readily from sleep deprivation. Together, these findings suggest that *DEC2*-P384R is sufficient for reducing sleep length [10].

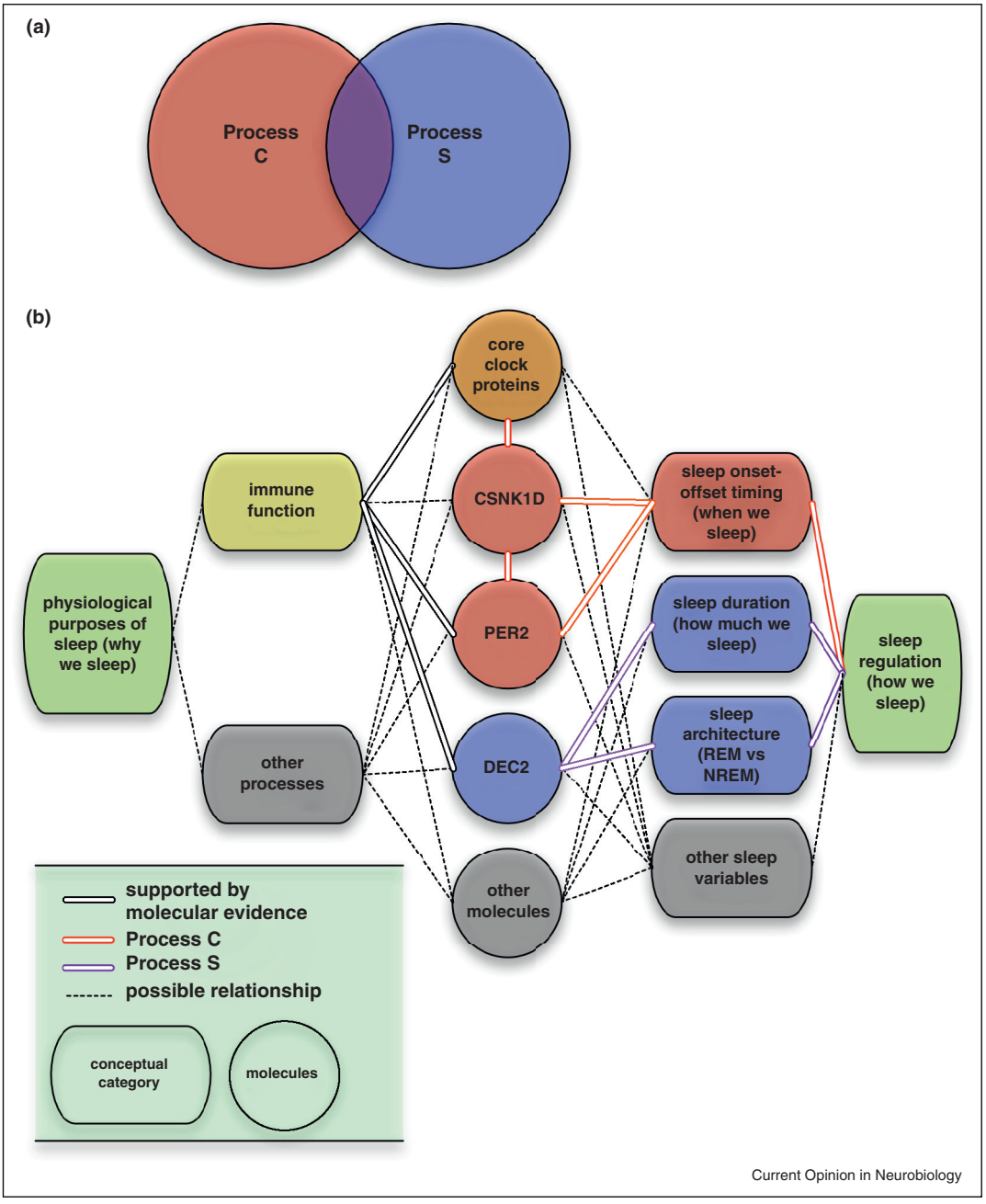
How does the *DEC2*-P384R mutation reduce sleep quantity? Early evidence suggests that DEC2 participates in the molecular core clock, but it is unclear whether circadian molecular mechanisms are responsible for its effects on sleep quantity [20–22]. P384R is located in a novel proline-rich domain with no known circadian function. In addition, unlike *PER2*-S662G, neither *DEC2*-P384R nor *Dec2* KO mice demonstrate a change in free-running period of activity [10,23]. These findings suggest that at least on a behavioral level, sleep quantity and timing are separate processes. In addition, the evidence presented so far seems to suggest that PER2 regulates sleep timing through the molecular core clock, whereas DEC2 alters sleep duration through other pathways that require further investigation. Therefore, Processes C and S may not directly share molecular mechanisms responsible for co-regulating sleep timing and duration. However, recent studies demonstrate a clinical correlation between sleep and immune responses. Perhaps PER2 and DEC2 both exert distinct effects on immune responses, resulting in co-regulation of a potentially important function of sleep? To address this possibility, we explore immune-related roles of PER2 and DEC2 in the next section.

Genes that make you tick can make you sick

While there are numerous studies aimed at understanding circadian effects on metabolism, cardiovascular function, and other physiological processes, similar research for immunological responses is just beginning to emerge [5,24]. Clinical observations suggest that the time of day influences susceptibility to disorders of human immunologic activity, which implies that circadian molecular mechanisms may regulate immune function. For example, the risk of mortality in human patients suffering

(Figure 1 Legend) The Integration of Process C and Process S. Figure not drawn to scale to emphasize theoretical changes. **(a)** The hypothetical two-process model of sleep as described by [3] integrates the daily (circadian) oscillation of sleep propensity (Process C) with the homeostatic sleep propensity accumulated in the awake state and relieved by restorative sleep (Process S). This model of integration assumes that Process C is unaltered in the setting of sleep deprivation. **(b)** A model of sleep integration whereby sleep deprivation also alters Process C (purple line) causing increased dys-synchrony between the two-processes and reconciliation of sleep need. **(c)** A model of integration where Process C is advanced (red line) over normal (dashed line) and Process S is unperturbed. **(d)** A model of integration where homeostatic sleep component of Process S is shortened and Process C is undisturbed. Blue bars, sleep. White bars, sleep deprivation.

Figure 2



Molecular basis for the two-process model of sleep regulation. **(a)** The two-process model has been and remains instrumental for understanding the dynamics of sleep regulation, and recent research is focused on finding molecular correlates for these processes, with an implicit assumption that there are shared molecular mechanisms as well as separate pathways. **(b)** Our proposed model for understanding the purposes and regulation of sleep based on the two-process model [3]. Recent evidence suggests that PER2 and CSNK1D may be sufficient to alter sleep onset-offset timing, and DEC2 may be sufficient to reduce sleep duration and modify sleep architecture by reducing NREM sleep more than REM sleep. Emerging findings point to additional roles for PER2 and DEC2 in various aspects of immune function, which may contribute to the physiological purposes of sleep.

from sepsis is increased between the hours of 2 am and 6 am [25]. Some rheumatic arthritis and asthma patients experience daily cyclical variations in the severity of symptoms [26–29]. Similarly, changes in sleep quantity can interfere with appropriate immunologic responses. For instance, adults with short sleep duration had lower secondary antibody responses to Hepatitis B vaccine, resulting in decreased predicted clinical protection [30]. In turn, this observation is associated with increased T lymphocyte activation and reduced natural killer (NK)

cell activity [31]. Together, these findings suggest that sleep timing or duration and some aspects of immune function may be regulated by common molecular mechanisms.

PER2 and DEC2 appear to be sufficient for altering sleep timing and duration respectively, and these proteins are recently implicated in immune processes, potentially one of the many purposes for sleep. Indeed, *Per2* KO mice demonstrate resistance to endotoxemic shock compared to wild-type mice after intraperitoneal injection with lipopolysaccharide (LPS) [32], and this finding is attributed to reduced oscillations and absolute quantities of IFN-γ and IL-1β cytokines. Furthermore, peritoneal macrophages of *Per2* KO mice downregulate the expression of Toll-like receptor 9 (TLR9), a pattern recognition receptor that participates in both innate and adaptive immunity. When challenged with TLR9 agonist, peritoneal macrophages from *Per2* KO animals had reduced IL6 and TNFα production *in vitro*. These effects likely involve the molecular core clock, because *in vivo* challenges of wild-type mice performed at the peak of TLR9 oscillation revealed increased morbidity and mortality [33••].

Supporting the involvement of the molecular core clock in immune responses, other core clock components are also implicated to regulate inflammatory potential. When injected with LPS, both systemic *Rev-Erbα* KO and macrophage restricted *Bmal1* KO mice had elevated cytokine production by abolishing the robust time-sensitive generation of IL-6 compared to controls [34••]. Macrophages from *Cry1/2* double KO mice have increased nuclear factor kappa B (NF-κB) activity, causing elevated baseline cytokine expression *in vitro* and generating greater inflammation when challenged with LPS *in vivo* [35••]. In addition, in the absence of Clock, T cells fail to proliferate in a circadian manner [36••]. As many immune relevant transcription factors, such as members of the signal transducer and activator of transcription family (STATs) and NF-κB, also fall in the domain of circadian regulation, it is likely that the molecular core clock drives downstream activities of immune responses, with PER2 responsible for a subset that remains to be fully elucidated [37].

Similar to PER2, DEC2 also exerts influences on immune function. Specifically, DEC2 is involved in the maturation of T helper type 2 (T_H2) cell lineages associated with humoral (antibody mediated) immunity. T_H2 cells highly express DEC2 compared to other T-cell lineages [38••,39••]. *Dec2* KO mice demonstrate defective T_H2 responses after repeated stimulation with OVA peptide, decreased alveolar infiltrate and reduced T_H2 cytokine production after exposure to an *in vivo* model of allergic asthma [39••]. DEC2 overexpression in undifferentiated T cells drives a T_H2 cell polarization while *in vivo* allergic asthma challenges yield increased T_H2 cytokin production and increased lung interstitial infiltrate compared to WT animals [38••]. To our knowledge however, there are no current studies addressing the effect of Dec2 on T_H2 cell populations or the larger immune system in the context of circadian timing or, more relevantly, distortion of sleep length. As DEC2 has been shown to affect pathways as diverse as cellular proliferation, differentiation, apoptosis and responses to hypoxia, DEC2 may exert its effects on the immune system and sleep duration outside of the molecular core clock. Further research is necessary for defining pathways downstream of DEC2, and it will be interesting to see whether *DEC2*-P384R confers beneficial or detrimental immune outcomes in addition to its role in reducing sleep duration.

Our hypothesis assumes that one of the main purposes of sleep is immune-related, and most findings that investigate the relationship between sleep and immune responses do so through disrupting sleep. Transcriptome based analysis of human subjects after sleep deprivation (disruption of Processes C and S) reveal significant changes in multiple immune related pathways [40,41••]. Supporting these findings, sleep deprivation in animal models decreases circulating lymphocyte populations, reduces the cellularity of the spleen and bone marrow [42,43], and acutely elevated inflammatory markers [44]. Simulated jet-lag models (which attempt to mimic disruptions of Process C) altered coordinated rhythmic expression of cytolytic factors and cytokines in NK cells, leading to deficient cytolytic function [45•] and markedly decreased survival after LPS injection [46•]. Interestingly, when compared to control, a group of genes that maintain circadian oscillation after sleep deprivation were explicitly immune related, hinting at the critical nature of circadian immune regulation since they are relatively preserved [41••]. Together, these findings support a correlation between sleep and immune function, and we conclude with a further discussion of this association in relation to our hypothesis.

Conclusions

Although it is difficult to study both adaptive and innate immune responses simultaneously, it is plausible that Process C has a broader effect on immunity encompassing both adaptive and innate functions, whereas Process S may more specifically affect adaptive immunity and T cell polarization. Sleep deprivation with its effect on both Processes C and S would then encompass the broadest range of immune alteration. However, even though these data allude to a hierarchical model for sleep and immunity (i.e. *disruption of Processes C and S results in impaired immune function*), a forced alteration in sleep timing/duration may not be the same as natural/habitual short sleep that satisfies homeostatic requirements. For instance, sleep deprivation may disrupt both the normal physiological purposes of sleep *and* affect molecular cues for initiating and enforcing sleep.

As an alternative viewpoint, here we propose that Processes C, Process S and certain immune responses (that may contribute to the physiological necessity of sleep) share molecular components such as PER2 and DEC2. As FASP and FNSS models exhibit stable and inherent alterations of sleep timing and duration, the characterization of their immune responses may address this hypothesis. Furthermore, identification of novel genes for FASP and FNSS may provide additional molecular correlates for sleep timing and duration beyond PER2 and DEC2. Ultimately, physiological reasons for sleep and regulation of sleep itself may require a delicate balance of shared molecular events. Therefore, tuning these pathways using both KO/haploinsufficient animals (loss-of-function) and transgenic mice carrying human mutations (gain-of-function) may reveal the answers to age-old questions of how, when, and why we sleep.

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• The first study linking chronic jet-lag to increased susceptibility to LPS endotoxemia in mice. Chronic jet lag alters levels of inflammatory cytokines after LPS injections, with a subsequent reduction in survival. Chronic jet-lag also changes expression of clock genes and circadian period in different tissues.