Timing and Consolidation of Human Sleep, Wakefulness, and Performance by a Symphony of Oscillators

Derk-Jan Dijk[1] and Malcolm von Schantz

Surrey Sleep Research Centre and Centre for Chronobiology, School of Biomedical and Molecular Sciences, University of Surrey, Guildford, United Kingdom

Abstract Daily rhythms in sleep and waking performance are generated by the interplay of multiple external and internal oscillators. These include the light-dark and social cycles, a circadian hypothalamic oscillator oscillating virtually independently of behavior, and a homeostatic oscillator driven primarily by sleep-wake behavior. Both internal oscillators contribute to variation in many aspects of sleep and wakefulness (e.g., sleep timing and duration, REM sleep, non-REM sleep, REM density, sleep spindles, slow-wave sleep, electroencephalographic oscillations during wakefulness and sleep, and performance parameters, including attention and memory). The relative contribution of the oscillators varies greatly between these variables. Sleep and performance cannot be predicted by either oscillator independently but critically depend on their phase relationship and amplitude. The homeostatic oscillator feeds back onto the central pacemaker or its outputs. Thus, the amplitude of observed circadian variation in sleep and performance depends on how long we have been asleep or awake. During entrainment to external 24-h cycles, the opposing interplay between circadian and homeostatic changes in sleep propensity consolidates sleep and wakefulness. Some physiological correlates and mediators of both the circadian process (e.g., melatonin and hypocretin rhythms) and the homeostat (e.g., EEG, slow-wave activity, and adenosine release) have been established, offering targets for the development of countermeasures for circadian sleep and performance disorders. Interindividual differences in sleep timing, duration, and morning or evening preference are associated with changes of circadian or sleep homeostatic processes or both. Molecular genetic correlates, including polymorphisms in clock genes, of some of these interindividual differences are emerging.

Key words EEG, slow-wave sleep, sleep spindles, diurnal preference, memory, *Period* genes

1. To whom all correspondence should be addressed: Derk-Jan Dijk, PhD, Surrey Sleep Research Centre, HPRU/MRC, Egerton Road, Guildford GU2 7XP UK; e-mail: d.j.dijk@surrey.ac.uk.

JOURNAL OF BIOLOGICAL RHYTHMS, Vol. 20 No. 4, August 2005 279-290
DOI: 10.1177/0748730405278292

Sleep and waking performance represent complex behaviors generated by elaborate mechanisms involving many brain areas, including brainstem, hypothalamic, thalamic, and neocortical structures (Pace-Schott and Hobson, 2002). Sleep-wake cycles are also associated with changes in many physiological and endocrine systems. The multitude of these associations further illustrates the complexity of sleep-wake states. Sleep and wakefulness as well as performance rhythms are among the few diurnal rhythms that we consciously experience on a daily basis, and this may be why these rhythms, despite their complexity, are examples of circadian rhythmicity that capture the popular imagination. Other factors contributing to the interest in sleep-wake cycles include the dramatic changes in sleep-wake organization over the life span and the impact of sleep disruption and sleep duration on health, quality of life, and life expectancy (Kripke et al., 2002; Groeger et al., 2004). How and why sleep and waking performance oscillate, and how many oscillators contribute to this phenomenology, have been topics of interest to biological rhythm researchers and psychologists ever since the observations by Patrick and Gilbert in 1896 that during a prolonged period of sleep deprivation, sleep propensity waxes and wanes with a period of approximately 24 h (Patrick and Gilbert, 1896).

SLEEP AND WAKE CYCLES ARE HIGHLY CONSOLIDATED COMPLEX STATES

Key characteristics of the sleep-wake cycle of adult humans exposed to environmental and social rhythms include an approximately 8-h sleep and 16-h wake episode that recur at around the same time every day. In healthy adults, the sleep and wake states are highly consolidated. In young adults, approximately 90% of an 8-h nocturnal rest episode is spent asleep as measurable by polysomnography.

During the consolidated sleep episode, sleep structure nevertheless varies dramatically. Non–rapid eye movement sleep (non-REM sleep) and rapid eye movement sleep oscillate with a period of approximately 90 min, an ultradian rhythm that is generated by reciprocal interaction of aminergic and cholinergic brainstem structures. Slow-wave sleep declines during the sleep episode, whereas sleep spindle activity, the duration of REM sleep episodes, and the density of rapid eye movements increase as the nocturnal sleep episodes progresses (Pace-Schott and Hobson, 2002).

During the 16-h wake episode alertness and performance on a variety of tasks remain nearly stable, particularly when studied without knowledge of clock time. The occasionally observed reduction in performance and increase in sleepiness in the afternoon are minor compared to the decrements that occur when wakefulness is extended into the biological night (Dijk et al., 1992; Cajochen et al., 1999). Less than 10% of respondents to surveys report napping for more than an hour (Groeger et al., 2004).

Many physiological and endocrine rhythms such as body temperature, heart rate, cortisol, melatonin, and growth hormone change in close association with the sleep-wake cycle, and these rhythms may contribute to the variations in sleep-wake propensity, be dependent on the sleep-wake cycle, or both.

DAILY RHYTHMS IN HUMAN SLEEP AND PERFORMANCE ARE GENERATED BY THE INTERPLAY OF MULTIPLE EXTERNAL AND INTERNAL OSCILLATORS

The mechanisms underlying the daily rhythms in sleep and waking performance have been investigated in laboratory studies, in which environmental cycles and sleep-wake cycles were altered. Early studies by pioneers in the field, Aschoff and Wever in Germany and Weitzman, Czeisler, and Kronauer in the United States, established that multiple oscillators contribute to human sleep-wake regulation. This insight was contingent on the simultaneous measurement of several physiological and behavioral variables in healthy adults, rather than the measurement of 1 single marker. Assessment of numerous variables allowed the quantification of the phase relationships between them, as well as their free-running periods. This provided early evidence for the possible existence of multiple oscillatory processes (for a review, see Aschoff et al., 1967; Czeisler and Dijk, 2001).

In the absence of externally imposed light-dark and social cycles, sleep-wake cycles remain consolidated but desynchronize from the 24-h day (external desynchrony). This loss of entrainment is accompanied by a dramatic change in the internal phase relationship between the sleep-wake cycle and the body temperature rhythm. The sleep-wake cycle shifts approximately 4 to 6 h later, and most sleep initiations now occur at the body temperature nadir rather than 6 h before the temperature nadir. This change in the inter-

nal phase relationship suggests that separate oscillators drive the sleep-wake cycle and body temperature rhythm. The phenomenon of spontaneous internal desynchrony, during which the sleep-wake cycle oscillates with a period much longer or shorter than the rhythms of core body temperature, urine volume, and other physiological variables, provides stronger evidence for the existence of multiple oscillators. Under these conditions, the timing of waking activities, such as meals, and the subjective estimation of hourly time intervals remain associated with the period of the sleep-wake cycle and do not appear to be governed by the hypothalamic circadian rhythms (Aschoff et al., 1986; Aschoff, 1985). Results from these and other studies led to the concept that human circadian rhythms, including the sleep-wake cycle, are the product of the interaction of multiple oscillators, primarily a deep and strong oscillator and a weak labile oscillator (Moore-Ede and Czeisler, 1984). During entrainment, these two oscillators are synchronized, presumably through light input to one or both oscillators, but this synchronization can be lost in the absence of an externally imposed light-dark cycle.

Although there is no debate about the contribution of multiple oscillatory processes to sleep-wake regulation, the nature of the oscillators has been discussed. There is agreement that a strong self-sustaining circadian oscillator is a major determinant of sleep-wake propensity. In humans and other mammals, this pacemaker is now thought to be located in the SCN, which also drives "hardwired" rhythms such as the melatonin and cortisol rhythm. To describe the nature of the other process, some investigators have used the oscillator metaphor, emphasizing the weak self-sustaining characteristics of this oscillator driving the period of the sleep-wake cycle (Kronauer et al., 1982). Others have emphasized that the process is driven by sleep-wake behavior and use the term *homeostat* or *hourglass oscillator* (Daan et al., 1984). The latter terminology was particularly suited to describe the results of sleep deprivation studies and other studies in which the sleep-wake cycle was manipulated. The results of such studies could be readily explained by the hourglass concept, provided that circadian factors were controlled for. These studies consistently showed that an extension of wakefulness or a restriction of sleep led to an increase in sleep propensity and deep (i.e., slow-wave) sleep, as well as low-frequency electroencephalographic oscillations during non-REM sleep and wakefulness (Dijk et al., 1987; Borbély et al., 2001).

Research over the past two decades has partially replaced the semantic discussion about hourglasses versus oscillators with physiological observations. There is, however, a renewed interest in the overlap in the mechanisms generating these two distinct oscillatory processes because some of the clock genes thought to be responsible for generating the strong circadian rhythms have also been implicated in aspects of the homeostatic oscillator at least in rodents (for a discussion, see Dijk and Franken, 2005).

A SCHEME

The factors affecting sleep-wake regulation in humans and the potential interactions between these factors are summarized in Figure 1. Social factors are a major determinant of sleep-wake behavior. Many of us often wake up in the morning not because of the circadian clock or sleep homeostat but because we have to go to work. The impact of these social factors is even stronger in shift workers, in whom social factors are the main driving force of sleep-wake timing. Note that in this scheme, social factors impinge on the timing of sleep-wake behavior only and do not directly affect the circadian pacemaker or the homeostat. This can lead to a conflict between socially dictated sleep timing and sleep propensity driven by circadian physiology.

Evidence that the light-dark cycle is the strongest synchronizer of circadian rhythmicity and also directly affects many aspects of physiology and behavior in humans is discussed elsewhere in this volume. As far as the circadian aspects of sleep-wake regulation are concerned, light synchronizes the circadian pacemaker, which in humans and other mammals is located in the SCN. Wake- and/or sleep-promoting signals emerging from or driven by the circadian pacemaker are thought to impinge on other brain areas (maybe acting through subservient oscillators), which leads to changes in sleep-wake propensity. This circadian pacemaker oscillates nearly independent of sleep-wake behavior (e.g., a sudden 6-h shift in the sleep-wake cycle does not lead to an immediate and comparable change in the phase of the circadian pacemaker). Many effects of the sleep homeostat on different brain areas are known, but its anatomical localization is not. It has been shown to be functionally and anatomically independent of the SCN because it survives SCN lesions (Trachsel et al., 1992). The sleep homeostat is likely to be a diffuse system, represented

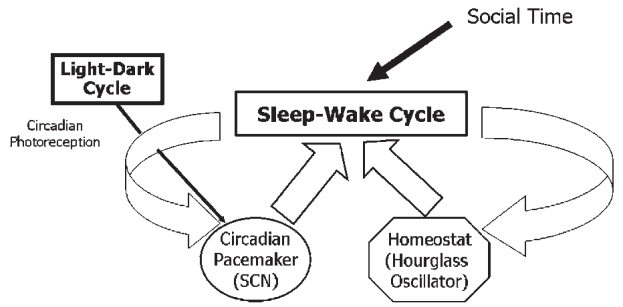

Figure 1. Regulation of sleep-wake cycles by the light-dark cycle, social time, a circadian pacemaker, and the sleep homeostat. Please note that light input reached the SCN via the feedback loop between the sleep-wake cycle and the SCN. Light input to extra SCN areas that may be involved in sleep, wakefulness, and performance is not represented in this scheme. Modified from Dijk and Lockley (2002).

by, for example, neurochemical factors (e.g., adenosine; Porkka-Heiskanen et al., 1997), accumulating during wakefulness or changes in synaptic weight that may occur as a result of neuronal activation during wakefulness and that need to be reversed during sleep (Tononi and Cirelli, 2003).

Whatever their neuroanatomical and neurochemical basis may be, both the circadian and homeostatic signals contribute about equally to sleep-wake propensity (Dijk and Czeisler, 1995). The homeostatic signal/oscillator is driven primarily by sleep-wake behavior (i.e., it keeps track of the history of sleep and wakefulness). Thus, the sleep-wake cycle provides a strong feedback onto the sleep homeostat (see Fig. 1). In this scheme, the sleep-wake cycle also feeds back onto the circadian pacemaker. This is first of all because sleep is associated with altered light input to the light-sensitive pacemaker: we turn off the lights, close the curtains, and close our eyes when we go to sleep. Such feedback from the sleep-wake cycle onto the circadian pacemaker may also occur independently of the gating of light input (see below).

This scheme of the circadian regulation of the human sleep-wake cycle is a hybrid of schemes summarizing models by Kronauer et al. (1982) and Daan et al. (1984) and will be useful for organizing research questions and data in the remainder of this review.

SEPARATION OF THE CONTRIBUTION OF THE TWO INTERNAL OSCILLATORS: PROTOCOLS

The multitude of factors affecting sleep-wake cycles dictates the development of protocols to disentangle their separate contributions. A first step was to separate the contribution of external and internal factors by removing the externally imposed light-dark and social cycles. A second step was to keep the light-dark cycle constant (i.e., study sighted individuals in constant darkness or nonsynchronized blind individuals). Overt endogenous rhythms observed under these paradigms must be generated by internal oscillators. However, because under those conditions, many physiological and behavioral variables oscillate, including sleep-wake behavior, it remains unclear to what extent observed variations are directly driven by one internal oscillator or are in part secondary to rhythmic behavior, the history of sleep and wakefulness, or the composite output of multiple oscillators with a specific phase relationship.

Approaches taken to investigate this latter question include protocols in which the sleep-wake cycle is replaced by constant wakefulness (i.e., a constant routine) or the sleep-wake cycle is scheduled to noncircadian periods.

In the constant-routine protocols, the direct effects of behavioral state on rhythmic variables (masking) are removed. The effects of sleep-wake history are not. Thus, assessments of alertness taken after 4 and 28 h of wakefulness are taken at approximately the same circadian phase, and yet they are very different. This implies that every assessment of performance or sleep propensity during a constant routine represents the combined influence of circadian phase and sleep-wake history. Similar arguments apply for the assessment of sleep or performance parameters in other protocols. Disentangling the separate contributions of sleep-wake history and circadian phase cannot be achieved in constant-routine protocols because only a limited number of combinations of sleep-wake history and circadian phase are obtained. This disentanglement can be achieved in forced desynchrony and ultra-short sleep-wake protocols in which multiple internal phase relationships between sleep-wake cycles and circadian rhythms are realized. Such protocols generate observations that can be quantified by

two independent variables: status of the sleep-wake homeostat and phase of the circadian pacemaker. By varying the duration of the sleep-wake cycle and by conducting the experiments to complete several beat cycles of the circadian and sleep-wake oscillator, a large section of the homeostatic-circadian parameter space is sampled. This allows estimation of the relative contribution of the two oscillators as well as their interaction.

DURING ENTRAINMENT, THE OPPOSING INTERPLAY BETWEEN CIRCADIAN AND HOMEOSTATIC CHANGES IN SLEEP PROPENSITY CONSOLIDATE SLEEP AND WAKEFULNESS AND AFFECT MANY VARIABLES

The relative contribution of the two processes has been estimated for many aspects of sleep and wakefulness in many different "forced desynchrony protocols" (reviewed in Dijk and Franken, 2005). Most if not all variables are influenced by both processes. The direction of sleep-wake dependent effects and the phase and waveforms of the circadian rhythms for some of these variables can be understood by considering the phase relationships between the two oscillators under normal entrained conditions. For example, the propensity to fall asleep depends on time awake and on circadian phase. During a normal waking day, the homeostatic propensity to fall asleep will increase, but this increase is counteracted by the circadian increase in wake propensity (Dijk and Czeisler, 1994). The circadian drive for wakefulness, as indexed by sleep latency and the duration of awakenings within sleep, increases during the biological day to reach a maximum at the end of the biological day, just prior to the nocturnal onset of melatonin secretion (Dijk et al., 1999). This phase had been previously identified as the wake maintenance zone in spontaneous desynchrony (Strogatz et al., 1987) and as forbidden zone for sleep in ultra-short sleep-wake cycles (Lavie, 1986). After leaving this wake maintenance zone, the circadian drive for wakefulness dissipates rapidly. Under entrained conditions, the homeostatic drive for sleep will be high at this circadian phase, and in combination with the dissipation of the circadian drive for wakefulness, this will lead to a dramatic increase in the propensity to fall asleep: a gate to sleep is opened.

The homeostatic propensity for sleep decreases after sleep is initiated. This is countered by an increase in the circadian drive for sleep, which reaches a maximum at the end of the biological night at around the core body temperature nadir (Dijk and Czeisler, 1994).

The propensity to wake up from sleep is also modulated by sleep structure, such that we are much more likely to wake up from REM sleep, particularly REM sleep with a high density of REMs (Barbato et al., 1994), than from non-REM sleep. REM sleep, long known to be modulated by circadian phase, is also affected by homeostatic sleep pressure, such that REM propensity and the density of REMs increase with the time we have been asleep (Khalsa et al., 2002). The combination of these two influences leads to a maximum propensity for REM sleep approximately 2 h after the core body temperature nadir at the time of habitual awakening! In other words, during a nocturnal sleep episode, the sleep-dependent dissipation of sleep propensity is counteracted by a circadian increase in sleep propensity, thereby facilitating sleep consolidation until the very end of the habitual sleep episode. Subsequently, a gate to wakefulness is created by the combined circadian and sleep-dependent promotion of REM sleep and REM density at the appropriate time. The phase relationship between the homeostatic and circadian oscillator during entrainment generates a near-square wave of sleep-wake propensity required for rapid state transitions. When the phase relationship between the two oscillators breaks down, as observed in many blind individuals or in sighted individuals who are jet-lagged or working night shifts, the consolidation of both sleep and waking performance is compromised.

MELATONIN AND OTHER MEDIATORS OF THE CIRCADIAN SLEEP-PROPENSITY RHYTHM

Some physiological and endocrine mediators of the circadian variation in sleep propensity are emerging. The onset of nocturnal melatonin secretion is closely associated with the opening of the nocturnal sleep gate (Lavie, 1997; Dijk and Cajochen, 1997), and even minor changes in the timing of the scheduled sleep-wake cycle relative to the endogenous melatonin rhythm lead to sleep disruption, particularly in the elderly (Dijk et al., 1999). Administration of melatonin during the biological day (i.e., when endogenous melatonin levels are very low) leads to a dramatic increase in the ability to sleep during this phase (Hughes and Badia, 1997; Rajaratnam et al., 2004). These effects of melatonin on early evening sleep are

comparable to the effects of hypnotics administered at this time (Stone et al., 2000). The circadian pacemaker drives the melatonin rhythm, and melatonin receptors are present in the human SCN. This constitutes a feedback mechanism, which contributes to the rapid dissipation of the drive for wakefulness just after the wake maintenance zone (Sack et al., 1997).

The excitatory hypocretin/orexin system is another downstream mediator of the circadian wake-sleep propensity rhythm. Narcoleptic patients have a diminished ability to maintain consolidated wakefulness in particular (Dantz et al., 1994), and cerebrospinal fluid concentrations of orexin/hypocretin are low (Peyron et al., 2000). Postmortem studies indicate severe loss of hypocretin/orexin neurons (Thannickal et al., 2000). Assessments of the diurnal variation of hypocretin/orexin in the cerebrospinal fluid of controls and depressed patients have shown that orexin/hypocretin concentrations reach a maximum at the very end of the waking day/beginning of the biological night (Salomon et al., 2003). Studies in squirrel monkeys, which are diurnal and have a highly consolidated sleep-wake cycle similar to humans, have confirmed the existence of an endogenous circadian rhythm of orexin/hypocretin with a peak at the end of the waking day (Zeitzer et al., 2004), and in rats, SCN lesions abolish this rhythm (Deboer et al., 2004). In both species, motor activity and/or wakefulness stimulates hypocretin/orexin secretion (Deboer et al., 2004; Zeitzer et al., 2004), and this may provide a mechanism by which wakefulness/activity provides a positive feedback onto this wake consolidating system.

SCOPE OF CIRCADIAN AND HOMEOSTATIC REGULATION

Experiments conducted during the past two decades have greatly expanded the range of variables of the sleep-wakefulness continuum shown to be affected by the circadian and sleep-wake oscillators.

Sleep spindles in non-REM sleep are 12- to 14-Hz oscillations observed in the EEG. Their incidence, frequency, amplitude, and duration are modulated by the circadian pacemaker in close association with, and indeed in part mediated by, the melatonin rhythm, such that there are more sleep spindles, with a lower frequency and of longer duration during the biological night and after melatonin administration (Dijk et al., 1997; Wei et al., 1999; Rajaratnam et al., 2004). The circadian modulation of sleep spindles provides the first evidence that the circadian pacemaker modulates major architectural features of the non-REM sleep EEG independently of the sleep homeostat.

Sleep spindles, primarily generated and driven by thalamic nuclei, are thought to play a role in plasticity and learning and memory as well as in sleep maintenance by inhibiting sensory information to reach the cortex. Other frequencies in the EEG in non-REM sleep are also modulated by the circadian pacemaker, although not to the same extent as sleep spindles (Dijk, 1999). The sleep-dependent influence on EEG power varies greatly with frequency. Low frequencies (e.g., slow-wave activity) are very much dependent on time asleep. This is in accordance with early observations obtained during spontaneous desynchrony, which indicated that slow-wave sleep (SWS) is virtually independent of circadian phase and primarily dependent on the sleep process (Dijk et al., 1997). The data are also in accordance with the proposition that activity in these frequencies is a marker of the non-REM sleep recovery process, which is independent of the circadian pacemaker (Borbély, 1982). The EEG during REM sleep, particularly alpha activity, is also modulated by time asleep and circadian phase (Dijk et al., 1997).

In summary, these EEG analyses demonstrate that sleep (both non-REM and REM sleep) is not a stable or univariant state. Rather, this state, as quantified by EEG analysis, is modulated by circadian phase as well as by the duration of sleep. This implies that when sleep occurs during the biological day, such as in nightshift workers, not only is sleep of shorter duration, but sleep structure and the sleep EEG, as well as the endocrine milieu, are very different from nocturnal sleep.

The very different wakefulness EEG varies with circadian phase and time awake, even when assessed under carefully controlled constant behavioral conditions, such as in the Karolinska Drowsiness Test. These variations can be attributed to circadian phase and time awake (Cajochen et al., 2002). Alpha activity (i.e., 8- to 12-Hz oscillations), recorded at both frontal-central derivations and parietal-occipital derivations, exhibits a very pronounced circadian variation, with a nadir late in the biological night and a peak in the middle of the biological day. With increasing duration of wakefulness, alpha activity is reduced. Higher frequencies in the waking EEG display a similar circa-

dian variation, but the exact timing of maxima and minima differs between fronto-central and occipito-parietal derivations. The influence of time awake differs between frequency bands. The data demonstrate that the waking state, as monitored by analysis of brain oscillations, is not a uniform state and is affected by both oscillatory processes. Whether these influences are mediated by circadian modulation of diffuse modulatory systems (such as the noradrenergic, serotonergic system, or histaminergic systems), circadian modulation of cholinergic systems implicated in EEG activation, or direct circadian modulation of the thalamo-cortical system remains to be established. Positron emission tomography (PET) studies comparing regional cerebral blood flow at various times of day are now being applied to identify brain regions that may mediate these circadian and homeostatic changes (Buysse et al., 2004), although it remains challenging to implement such brain-imaging techniques in protocols in which the contribution of the two oscillatory processes can be separated.

PERFORMANCE

Although behavioral and performance correlates of changes in EEG oscillations are not fully established, several studies have demonstrated that such changes correlate with changes in performance and subjective sleepiness (Cajochen et al., 1999).

Direct assessment of the contribution of the homeostat and circadian oscillator to performance and mood variables has been achieved for a variety of performance tests. These include subjective alertness, sleepiness, throughput measures (e.g., digit symbol substitution, addition tasks), sustained vigilance (e.g., the psychomotor vigilance test), visual search tests, probed recall memory test, and procedural memory such as sequence learning (Johnson et al., 1992; Folkard and Åkerstedt, 1987; Dijk et al., 1992; Wyatt et al., 1999; Wyatt et al., 2004; Horowitz et al., 2003; Cajochen et al., 2004; Graw et al., 2004; Monk et al., 1997). For all these variables, separate contributions of time awake or sleep pressure and circadian phase have been established. The circadian modulation of these variables may not come as a surprise: we perform worse at night than during the day, even when we control for the confounding effects of time awake. The circadian peak of performance appears late in the biological day for most variables. Assessment of the wake-dependent modulation in the absence of the circadian confound has revealed that our performance is much more impaired by being awake than could be expected from data obtained during normal entrained conditions. As little as 6 to 10 h of wakefulness dramatically impairs a variety of performance measures (Wyatt et al., 1999).

Which physiological variables mediate these circadian and homeostatic changes in performance? Core body temperature has long been associated with changes in performance, and the circadian rhythm of core body temperature more or less parallels the rhythm of many performance variables. Interestingly, even when circadian modulation of core body temperature is controlled for, variation in core body temperature continues to be associated with variation of a variety of performance measures, including working memory and visual attention, manual dexterity, serial search, and verbal reasoning (Monk and Carrier, 1998; Wright et al., 2002). Such residual variations in performance are also associated with variation in motivation and alertness (Hull et al., 2003).

What is the physiological basis of the homeostatic variation in performance? Adenosine has been implicated in the homeostatic regulation of sleep. Animal studies have demonstrated increases in extracellular adenosine concentration in basal forebrain regions, and adenosine agonists induce (slow-wave) sleep. Many people consume large quantities of caffeine, which acts as an antagonist at adenosine receptors. The popularity of caffeine may be related to its success of intervening with the physiological regulation of sleepiness (Landolt et al., 2004). Does it also intervene with the homeostatic regulation of performance? High-frequency, low-dose administration of caffeine during a forced desynchrony protocol was shown to be remarkably effective in countering the wake-dependent deterioration of some but not all performance measures (Wyatt et al., 2004). Measures related to throughput appear to benefit the most, particularly when performance is required under high sleep pressure during the latter part of the biological night. Such a high-frequency, low-dose caffeine administration regime is also very effective in countering unscheduled sleep episodes, providing further evidence that adenosine is a promising target for the development of countermeasures for unintended sleep episodes that may occur in shift-work situations.

Few attempts have been made to understand the circadian and homeostatic variation in performance

within current psychological or psychophysiological models of performance and attention. One noticeable exception is an analysis of the impact of circadian phase and time awake on visual selective attention. It appears that such effects are not mediated by an impaired detection of relevant stimuli but rather by an impairment of decision making (Horowitz et al., 2003).

THE HOMEOSTATIC OSCILLATOR FEEDS BACK ONTO THE CIRCADIAN PACEMAKER OR ITS OUTPUTS

The amplitude of the observed circadian variation in sleep and performance parameters depends on the status of the sleep homeostat (i.e., on how long we have been asleep or awake). Thus, we cannot simply add up the circadian and homeostatic component. Such a change in the amplitude of the circadian waveform may be interpreted as a feedback of the sleep-wake cycle or status of the homeostat onto the circadian pacemaker or its outputs. The magnitude of these changes in amplitude is quite extraordinary for many variables during sleep and wakefulness: sleep spindles, sleep consolidation, REM sleep duration, REM density, subjective alertness, digit symbol substitution, psychomotor vigilance task, and mood (Dijk et al., 1992; Dijk and Czeisler, 1995; Boivin et al., 1997; Wyatt et al., 2004). The implications of these interactions are far reaching from both a practical and theoretical perspective. Knowledge of circadian phase does not allow prediction of performance without knowledge of the status of the homeostat. Performance at 6 a.m. can be well within the acceptable range, provided that sleep pressure is only minor, but will be at very low levels if sleep pressure is high. These nonadditive interactions may be a further mechanism by which the interaction of the two oscillators can generate very rapid changes in vigilance state.

Other evidence for feedback of the sleep-wake cycle onto the circadian pacemaker stems from studies in volunteers living in a very dim light-dark cycle. Such studies have shown that altering sleep-wake timing can have a small effect on circadian phase, and this effect is larger than what may be expected from the light exposure associated with the sleep-wake cycle (Danilenko et al., 2003; Cajochen et al., 2003).

These data in humans, as well as the inverse association between EEG slow-wave activity and multiple unit activity in the SCN of rats (Deboer et al., 2003) and the effects of sleep deprivation on circadian phase in hamsters (Antle and Mistlberger, 2000), indicate that the sleep-wake cycle as well as the status of the homeostat do indeed feed back onto the circadian oscillator. Thus, although functionally and neuroanatomically independent, the two oscillators interact.

INTERINDIVIDUAL DIFFERENCES IN SLEEP TIMING, DURATION, AND MORNING OR EVENING PREFERENCE AND THEIR MOLECULAR GENETIC CORRELATES

Interindividual differences have been observed in multiple sleep and sleep-related parameters such as sleep timing, sleep duration, sleep structure, or preferred timing for physical or mental activity. Such differences may reflect differences in the intrinsic properties of the circadian pacemaker (e.g., its period or amplitude), entrainment pathways (e.g., light sensitivity), differences in the intrinsic properties of the homeostat (long sleep need), or the phase relationships between the homeostat and circadian pacemaker.

Age and sex explain a large proportion of the interindividual variance. Sleep timing, sleep intensity, and sleep duration change dramatically over the life span and differ between the sexes (Carrier et al., 2001; Van Cauter et al., 2000; Wever, 1984). Diurnal preference determined by the validated Horne-Östberg questionnaire or the Munich Chronotype Scale also varies with age and sex. Morning preference (as represented by a higher score) increases with age and is higher in women than in men at any given age (Duffy et al., 1999; Robilliard et al., 2002; Carrier et al., 1997; Roenneberg et al., 2004). Whereas the sex differences may in part be related to differences in average intrinsic period, the age-related changes are unlikely to be driven only by changes in this circadian parameter, which in fact does appear not to shorten at all over the life span, whether assessed cross-sectionally or longitudinally (Czeisler et al., 1999; Kendall et al., 2001). There is evidence that morningness is associated with a shorter intrinsic period (Duffy et al., 2001). The change in sleep timing as observed in morning and evening types, as well as the change in sleep timing in

the elderly and in delayed sleep phase syndrome, is not always accompanied by an equivalent change in the timing of reliable markers of the circadian pacemakers. Variation in sleep timing and diurnal preference (i.e., variation in external phase relationships) can be accompanied by a change in internal phase relationships (i.e., between the sleep-wake cycle and the circadian pacemaker). The mechanisms underlying these changes remain unclear. Interestingly, comparison of men and women, morning types and evening types, delayed sleep-phase syndrome and controls, and young and older people has indicated differences in characteristics of the sleep EEG that can be interpreted as changes in the sleep homeostat (reviewed in Dijk and Lockley, 2002).

Studies comparing mono- and dizygotic twins have demonstrated a high degree of heritability for several sleep parameters and the EEG and slow-wave sleep in particular (reviewed in Tafti and Franken, 2002). This genetically determined variance that cannot be explained by age and sex will be referred to as true interindividual variation. The extremes of this variation may be so debilitating that they are characterized as sleep disorders, some of which have a circadian component.

A systematic assessment of the separate contribution of circadian and homeostatic factors to interindividual differences has been conducted for sleep duration. The circadian night, as indexed by the duration of melatonin secretion, is shorter in short sleepers (Aeschbach et al., 2003). Whether this difference is causing the short sleep or is a consequence of it and the associated short dark period remains unclear. Analysis of the waking and sleep EEG has suggested that short sleepers may live under a higher homeostatic sleep pressure than long sleepers (Aeschbach et al., 2001). Quantification of the parameters of the increase and decline of sleep pressure, however, has not revealed statistically significant differences between the groups (Aeschbach et al., 1996). Thus, as for the difference in the circadian process, it may be that the differences in average sleep pressure are a consequence of the difference in sleep duration. To which degree these differences have a genetic basis remains to be proven, although studies in inbred mouse strains indicate a substantial genetic component (Franken et al., 2001).

Just as the anatomical locus of the circadian pacemaker is much more well defined than that of the sleep homeostat, we know and understand much more about the genes that create circadian rhythmicity, as well as the mechanisms through which they do so, than about those whose products are involved in the sleep homeostat. Although quantitative trait loci (QTL) analysis in mice indicates that there are additional loci for circadian period length that remain to be characterized (Shimomura et al., 2001), most important clock gene components are probably known. Mutations in and targeted knockouts of these genes result in abnormalities in, or abolition of, free-running circadian periodicity in rodent models (Lowrey and Takahashi, 2004).

Thus, circadian rhythmicity is perhaps the one system in which variability defined by behavioral parameters can be most strongly and directly correlated with variability in a small and defined group of genes. The most striking example of this is the analysis of a familial advanced sleep phase syndrome (ASPS) pedigree. After the initial demonstration that this condition was associated with a severely shortened circadian period length (Jones et al., 1999), the condition was subsequently found to associate with a missense mutation in *PER2*, a key component in the molecular oscillator (Toh et al., 2001) and with a mutation in the casein kinase I delta (Xu et al., 2005). Such high-penetrance clock gene alleles can be confidently predicted to be quite rare. The determinants of the normally distributed phenotypic variability in human chronotype parameters are likely to be a number of more frequent, low-penetrance polymorphisms. The first such polymorphism to be reported as associating with diurnal preference within the normal population was a variable-number tandem repeat polymorphism in the coding region of *PER3* (Archer et al., 2003). The longer allele of this gene was demonstrated to associate with morning preference and the shorter with evening preference (as determined by the Horne-Östberg questionnaire), a finding that has recently been replicated in a South American population (Pereira et al., 2005). Such associations, once firmly established, may provide new ways to investigate the physiological mechanisms underlying interindividual differences in sleep timing and duration.

IMPLICATIONS OF SYMPHONY OF OSCILLATORS FOR RESEARCH APPROACHES AND METHODOLOGIES

The interaction of circadian and homeostatic processes in shaping sleep-wake and performance rhythms implies that the quantification of endo-

phenotypes, which ultimately may help identify genotypes and novel physiology, requires protocols that take these internal interactions into account. Only then will it be possible to attribute phenotypic variation to either variation in the circadian or homeostatic oscillator or both, so that the symphony of internal and external oscillators may eventually be heard.

ACKNOWLEDGMENTS

The authors' current research is supported by the Wellcome Trust and the BBSRC.

REFERENCES

Aeschbach D, Cajochen C, Landolt H-P, and Borbély AA (1996) Homeostatic sleep regulation in habitual short sleepers and long sleepers. Am J Physiol 270:R41-R53.

Aeschbach D, Postolache TT, Sher L, Matthews JR, Jackson MA, and Wehr TA (2001) Evidence from waking electroencephalogram that short sleepers live under higher homeostatic sleep pressure. Neuroscience 102:493-502.

Aeschbach D, Sher L, Postolache TT, Matthews JR, Jackson MA, and Wehr TA (2003) A longer biological night in long sleepers than in short sleepers. J Clin Endocrinol Metab 88:26-30.

Antle MC and Mistlberger RE (2000) Circadian clock resetting by sleep deprivation without exercise in the Syrian hamster. J Neurosci 20:9326-9332.

Archer SN, Robilliard DL, Skene DJ, Smits M, Williams A, Arendt J, and von Schantz M (2003) A length polymorphism in the circadian clock gene *Per3* is linked to delayed sleep phase syndrome and extreme diurnal preference. Sleep 26:413-415.

Aschoff J (1985) On the perception of time during prolonged temporal isolation. Hum Neurobiol 4:41-52.

Aschoff J, Gerecke U, and Wever R (1967) Desynchronization of human circadian rhythms. Jpn J Physiol 17:450-457.

Aschoff J, von Goetz C, Wildgruber C, and Wever RA (1986) Meal timing in humans during isolation without time cues. J Biol Rhythms 1:151-162.

Barbato G, Barker C, Bender C, Giesen HA, and Wehr TA (1994) Extended sleep in humans in 14 hour nights (LD 10:14): Relationship between REM density and spontaneous awakening. Electroencephalogr Clin Neurophysiol 90:291-297.

Boivin DB, Czeisler CA, Dijk D-J, Duffy JF, Folkard S, Minors DS, Totterdell P, and Waterhouse JM (1997) Complex interaction of the sleep-wake cycle and circadian phase modulates mood in healthy subjects. Arch Gen Psychiatry 54:145-152.

Borbély AA (1982) A two process model of sleep regulation. Hum Neurobiol 1:195-204.

Borbély AA, Dijk D-J, Achermann P, and Tobler I (2001) Processes underlying the regulation of the sleep-wake cycle. In *Circadian Clocks*, Takahashi JS, Turek FW, and Moore RY, eds, pp 457-479. New York: Kluwer Academic/Plenum.

Buysse DJ, Nofzinger EA, Germain A, Meltzer CC, Wood A, Ombao H, Kupfer DJ, and Moore RY (2004) Regional brain glucose metabolism during morning and evening wakefulness in humans: Preliminary findings. Sleep 27:1245-1254.

Cajochen C, Jewett ME, and Dijk D-J (2003) Human circadian melatonin rhythm phase delay during a fixed sleep-wake schedule interspersed with nights of sleep deprivation. J Pineal Res 35:149-157.

Cajochen C, Khalsa SBS, Wyatt JK, Czeisler CA, and Dijk D-J (1999) EEG and ocular correlates of circadian melatonin phase and human performance decrements during sleep loss. Am J Physiol 277:R640-R649.

Cajochen C, Knoblauch V, Wirz-Justice A, Krauchi K, Graw P, and Wallach D (2004) Circadian modulation of sequence learning under high and low sleep pressure conditions. Behav Brain Res 151:167-176.

Cajochen C, Wyatt JK, Czeisler CA, and Dijk D-J (2002) Separation of circadian and wake duration-dependent modulation of EEG activation during wakefulness. Neuroscience 114:1047-1060.

Carrier J, Land S, Buysse DJ, Kupfer DJ, and Monk TH (2001) The effects of age and gender on sleep EEG power spectral density in the middle years of life (age 20-60 years old). Psychophysiology 38:232-242.

Carrier J, Monk TH, Buysse DJ, and Kupfer DJ (1997) Sleep and morningness-eveningness in the 'middle' years of life (20-59y). J Sleep Res 6:230-237.

Czeisler CA and Dijk D-J (2001) Human circadian physiology and sleep-wake regulation. In *Circadian Clocks*, Takahashi JS, Turek FW, and Moore RY, eds, pp 531-569. New York: Kluwer Academic/Plenum.

Czeisler CA, Duffy JF, Shanahan TL, Brown EN, Mitchell JF, Rimmer DW, Ronda JM, Silva EJ, Allan JS, Emens JS, Dijk D-J, and Kronauer RE (1999) Stability, precision, and near-24-hour period of the human circadian pacemaker. Science 284:2177-2181.

Daan S, Beersma DGM, and Borbély AA (1984) Timing of human sleep: Recovery process gated by a circadian pacemaker. Am J Physiol 246:R161-R178.

Danilenko KV, Cajochen C, and Wirz-Justice A (2003) Is sleep per se a zeitgeber in humans? J Biol Rhythms 18:170-178.

Dantz B, Edgar DM, and Dement WC (1994) Circadian rhythms in narcolepsy: Studies on a 90 minute day. Electroencephalogr Clin Neurophysiol 90:24-35.

Deboer T, Overeem S, Visser NA, Duindam H, Frölich M, Lammers GJ, and Meijer JH (2004) Convergence of circadian and sleep regulatory mechanisms on hypocretin-1. Neuroscience 129:727-732.

Deboer T, Vansteensel MJ, Detari L, and Meijer JH (2003) Sleep states alter activity of suprachiasmatic nucleus neurons. Nat Neurosci 6:1086-1090.

Dijk D-J (1999) Circadian variation of EEG power spectra in NREM and REM sleep in humans: Dissociation from body temperature. J Sleep Res 8:189-195.

Dijk D-J and Lockley SW (2002) Invited review: Integration of human sleep-wake regulation and circadian rhythmicity. J Appl Physiol 92:852-862.

Dijk D-J, Beersma DGM, and Daan S (1987) EEG power density during nap sleep: Reflection of an hourglass measuring the duration of prior wakefulness. J Biol Rhythms 2:207-219.

Dijk D-J and Cajochen C (1997) Melatonin and the circadian regulation of sleep initiation, consolidation, structure, and the sleep EEG. J Biol Rhythms 12:627-635.

Dijk D-J and Czeisler CA (1994) Paradoxical timing of the circadian rhythm of sleep propensity serves to consolidate sleep and wakefulness in humans. Neurosci Lett 166:63-68.

Dijk D-J and Czeisler CA (1995) Contribution of the circadian pacemaker and the sleep homeostat to sleep propensity, sleep structure, electroencephalographic slow waves and sleep spindle activity in humans. J Neurosci 15:3526-3538.

Dijk D-J, Duffy JF, and Czeisler CA (1992) Circadian and sleep/wake dependent aspects of subjective alertness and cognitive performance. J Sleep Res 1:112-117.

Dijk D-J, Duffy JF, Riel E, Shanahan TL, and Czeisler CA (1999) Ageing and the circadian and homeostatic regulation of human sleep during forced desynchrony of rest, melatonin and temperature rhythms. J Physiol (Lond) 516:611-627.

Dijk D-J and Franken P (2005) Interaction of sleep homeostasis and circadian rhythmicity: Dependent or independent systems. In *Principles and Practice of Sleep Medicine*, Kryger MH, Roth T, and Dement WC, eds, pp 418-434. Philadelphia: Elsevier.

Dijk D-J, Shanahan TL, Duffy JF, Ronda JM, and Czeisler CA (1997) Variation of electroencephalographic activity during non-rapid eye movement and rapid eye movement sleep with phase of circadian melatonin rhythm in humans. J Physiol (Lond) 505:851-858.

Duffy JF, Dijk D-J, Hall EF, and Czeisler CA (1999) Relationship of endogenous circadian melatonin and temperature rhythms to self-reported preference for morning or evening activity in young and older people. J Investig Med 47:141-150.

Duffy JF, Rimmer DW, and Czeisler CA (2001) Association of intrinsic circadian period with morningness-eveningness, usual wake time, and circadian phase. Behav Neurosci 115:895-899.

Folkard S and Åkerstedt T (1987) Towards a model for the prediction of alertness and/or fatigue on different sleep/wake schedules. In *Contemporary Advances in Shiftwork Research*, Oginski A, Pokorski J, and Rutenfranz J, eds, pp 231-240. Krakow: Medical Academy.

Franken P, Chollet D, and Tafti M (2001) The homeostatic regulation of sleep need is under genetic control. J Neurosci 21:2610-2621.

Graw P, Krauchi K, Knoblauch V, Wirz-Justice A, and Cajochen C (2004) Circadian and wake-dependent modulation of fastest and slowest reaction times during the psychomotor vigilance task. Physiol Behav 80:695-701.

Groeger JA, Zijlstra FR, and Dijk D-J (2004) Sleep quantity, sleep difficulties and their perceived consequences in a representative sample of some 2000 British adults. J Sleep Res 13:359-371.

Horowitz TS, Cade BE, Wolfe JM, and Czeisler CA (2003) Searching night and day: A dissociation of effects of circadian phase and time awake on visual selective attention and vigilance. Psychol Sci 14:549-557.

Hughes RJ and Badia P (1997) Sleep-promoting and hypothermic effects of daytime melatonin administration in humans. Sleep 20:124-131.

Hull JT, Wright KP, and Czeisler CA (2003) The influence of subjective alertness and motivation on human performance independent of circadian and homeostatic regulation. J Biol Rhythms 18:329-338.

Johnson MP, Duffy JF, Dijk D-J, Ronda JM, Dyal CM, and Czeisler CA (1992) Short-term memory, alertness and performance: A reappraisal of their relationship to body temperature. J Sleep Res 1:24-29.

Jones CR, Campbell SS, Zone SE, Cooper F, DeSano A, Murphy PJ, Jones B, Czajkowski L, and Ptacek LJ (1999) Familial advanced sleep-phase syndrome: A short-period circadian rhythm variant in humans. Nat Med 5:1062-1065.

Kendall AR, Lewy AJ, and Sack RL (2001) Effects of aging on the intrinsic circadian period of totally blind humans. J Biol Rhythms 16:87-95.

Khalsa SBS, Conroy DA, Duffy JF, Czeisler CA, and Dijk D-J (2002) Sleep- and circadian-dependent modulation of REM density. J Sleep Res 11:53-59.

Kripke DF, Garfinkel L, Wingard DL, Klauber MR, and Marler MR (2002) Mortality associated with sleep duration and insomnia. Arch Gen Psychiatry 59:131-136.

Kronauer RE, Czeisler CA, Pilato SF, Moore-Ede MC, and Weitzman ED (1982) Mathematical model of the human circadian system with two interacting oscillators. Am J Physiol 242:R3-R17.

Landolt HP, Retey JV, Tonz K, Gottselig JM, Khatami R, Buckelmuller I, and Achermann P (2004) Caffeine attenuates waking and sleep electroencephalographic markers of sleep homeostasis in humans. Neuropsychopharmacology 29:1933-1939.

Lavie P (1986) Ultrashort sleep-waking schedule III: "Gates" and "forbidden zones" for sleep. Electroencephalogr Clin Neurophysiol 63:414-425.

Lavie P (1997) Melatonin: Role in gating nocturnal rise in sleep propensity. J Biol Rhythms 12:657-665.

Lowrey PL and Takahashi JS (2004) Mammalian circadian biology: Elucidating genome-wide levels of temporal organization. Annu Rev Genomics Hum Genet 5:407-441.

Monk TH, Buysse DJ, Reynolds CF, Berga SL, Jarrett DB, Begley AE, and Kupfer DJ (1997) Circadian rhythms in human performance and mood under constant conditions. J Sleep Res 6:9-18.

Monk TH and Carrier J (1998) A parallelism between human body temperature and performance independent of the

endogenous circadian pacemaker. J Biol Rhythms 13:113-122.

Moore-Ede MC and Czeisler CA (1984) *Mathematical Models of the Circadian Sleep-Wake Cycle*. New York: Raven.

Pace-Schott EF and Hobson JA (2002) The neurobiology of sleep: genetics, cellular physiology and subcortical networks. Nat Rev Neurosci 3:591-605.

Patrick GTW and Gilbert JA (1896) On the effects of sleep loss: Studies from the Psychological Laboratory of the University of Iowa. Psychol Rev 3:468-483.

Pereira DS, Tufik S, Louzada FM, Benedito-Silva AA, Lopez AR, Lemos NA, Korczak AL, D'Ameida V, and Pedrazzoli M (2005) Association of the length polymorphism in the human Per3 gene with the delayed sleep-phase syndrome: Does latitude have an influence upon it? Sleep 28:29-32.

Peyron C, Faraco J, Rogers W, Ripley B, Overeem S, Charnay Y, Nevsimalova S, Aldrich M, Reynolds D, Albin R, et al. (2000) A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. Nat Med 6:991-997.

Porkka-Heiskanen T, Strecker RE, Thakkar M, Bjørkum AA, Greene RW, and McCarley RW (1997) Adenosine: A mediator of the sleep-inducing effects of prolonged wakefulness. Science 276:1265-1268.

Rajaratnam SM, Middleton B, Stone BM, Arendt J, and Dijk D-J (2004) Melatonin advances the circadian timing of EEG sleep and directly facilitates sleep without altering its duration in extended sleep opportunities in humans. J Physiol 561:339-351.

Robilliard DL, Archer SN, Arendt J, Lockley SW, Hack LM, English J, Leger D, Smits MG, Williams A, Skene DJ, and von Schantz M (2002) The 3111 *Clock* gene polymorphism is not associated with sleep and circadian rhythmicity in phenotypically characterized human subjects. J Sleep Res 11:305-312.

Roenneberg T, Kuehnle T, Pramstaller PP, Ricken J, Havel M, Guth A, and Merrow M (2004) A marker for the end of adolescence. Curr Biol 14:R1038-R1039.

Sack RL, Hughes RJ, Edgar DM, and Lewy AJ (1997) Sleep-promoting effects of melatonin: At what dose, in whom, under what conditions, and by what mechanisms? Sleep 20:908-915.

Salomon RM, Ripley B, Kennedy JS, Johnson B, Schmidt D, Zeitzer JM, Nishino S, and Mignot E (2003) Diurnal variation of cerebrospinal fluid hypocretin-1 (Orexin-A) levels in control and depressed subjects. Biol Psychiatry 54:96-104.

Shimomura K, Low-Zeddies SS, King DP, Steeves TD, Whiteley A, Kushla J, Zemenides PD, Lin A, Vitaterna MH, Churchill GA, and Takahashi JS (2001) Genome-wide epistatic interaction analysis reveals complex genetic determinants of circadian behavior in mice. Genome Res 11:959-980.

Stone BM, Turner C, Mills SL, and Nicholson AN (2000) Hypnotic activity of melatonin. Sleep 23:663-669.

Strogatz SH, Kronauer RE, and Czeisler CA (1987) Circadian pacemaker interferes with sleep onset at specific times each day: Role in insomnia. Am J Physiol 253:R172-R178.

Tafti M and Franken P (2002) Invited review: Genetic dissection of sleep. J Appl Physiol 92:1339-1347.

Thannickal TC, Moore RY, Nienhuis R, Ramanathan L, Gulyani S, Aldrich M, Cornford M, and Siegel JM (2000) Reduced number of hypocretin neurons in human narcolepsy. Neuron 27:469-474.

Toh KL, Jones CR, He Y, Eide EJ, Hinz WA, Virshup DM, Ptacek LJ, and Fu YH (2001) An *hPer2* phosphorylation site mutation in familial advanced sleep phase syndrome. Science 291:1040-1043.

Tononi G and Cirelli C (2003) Sleep and synaptic homeostasis: A hypothesis. Brain Res Bull 62:143-150.

Trachsel L, Edgar DM, Seidel WF, Heller HC, and Dement WC (1992) Sleep homeostasis in suprachiasmatic nuclei-lesioned rats: Effects of sleep deprivation and triazolam administration. Brain Res 589:253-261.

Van Cauter E, Leproult R, and Plat L (2000) Age-related changes in slow wave sleep and REM sleep and relationship with growth hormone and cortisol levels in healthy men. JAMA 284:861-868.

Wei HG, Riel E, Czeisler CA, and Dijk D-J (1999) Attenuated amplitude of circadian and sleep-dependent modulation of electroencephalographic sleep spindle characteristics in elderly human subjects. Neurosci Lett 260:29-32.

Wever RA (1984) Sex differences in human circadian rhythms: Intrinsic periods and sleep fractions. Experientia 40:1226-1234.

Wright KP Jr, Hull JT, and Czeisler CA (2002) Relationship between alertness, performance, and body temperature in humans. Am J Physiol Regul Integr Comp Physiol 283:R1370-R1377.

Wyatt JK, Cajochen C, Ritz-De Cecco A, Czeisler CA, and Dijk D-J (2004) Low dose, repeated caffeine administration for circadian-phase dependent performance degradation during extended wakefulness. Sleep 27:374-381.

Wyatt JK, Ritz-De Cecco A, Czeisler CA, and Dijk D-J (1999) Circadian temperature and melatonin rhythms, sleep, and neurobehavioral function in humans living on a 20-h day. Am J Physiol 277:R1152-R1163.

Xu Y, Padiath QS, Shapiro RE, Jones CR, Wu SC, Saigoh N, Saigoh K, Ptacek LJ, and Fu YH (2005) Functional consequences of a *CKI*δ mutation causing familial advanced sleep phase syndrome. Nature 434:640-644.

Zeitzer JM, Buckmaster CL, Lyons DM, and Mignot E (2004) Locomotor-dependent and -independent components to hypocretin-1 (orexin A) regulation in sleep-wake consolidating monkeys. J Physiol 557:1045-1053.