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J Biol Rhythms 2006; 21; 482

DOI: 10.1177/0748730406294627

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Neurobiology of the Sleep-Wake Cycle: Sleep Architecture, Circadian Regulation, and Regulatory Feedback

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Abstract This mini-review article presents the remarkable progress that has been made in the past decade in our understanding of the neural circuitry underlying the regulation of sleep-wake states and circadian control of behaviors. Following a brief introduction to sleep architecture and physiology, the authors describe the neural circuitry and neurotransmitters that regulate sleep and cortical arousal (i.e., wakefulness). They next examine how sleep and wakefulness are regulated by mutual inhibition between sleep- and arousal-promoting circuitry and how this interaction functions analogously to an electronic “flip-flop” switch that ensures behavioral state stability. The authors then discuss the role of circadian and homeostatic processes in the consolidation of sleep, including the physiologic basis of homeostatic sleep drive (i.e., wake-dependent increase in sleep propensity) and the role of the SCN in the circadian regulation of sleep-wake cycles. Finally, they describe the hypothalamic circuitry for the integration of photic and nonphotic environmental time cues and how this integration allows organisms to sculpt patterns of rest-activity and sleep-wake cycles that are optimally adaptive.

Key words suprachiasmatic nucleus, dorsomedial hypothalamic nucleus, REM, NREM, locomotor activity, VLPO

WHAT IS SLEEP?

Although several hypotheses and theories have been advanced as teleological explanations for the function of sleep, a unified theory of sleep function remains elusive. Such limited insight regarding sleep as a biological phenomenon is remarkable when one considers that nearly one third of our lives is spent in this behavioral state. Although we may not fully understand the *why* of sleep, sleep clearly serves an

important function, as indicated by the severe cognitive and physical consequences of sleep deprivation, the evolutionary conservation of sleep in mammals, and the strong “rebound” of sleep following sleep loss (Durmer and Dinges, 2005).

A widely held, albeit simplified, operational definition of sleep is that of a natural state characterized by a reduction in voluntary motor activity, a decreased response to stimulation (i.e., increased arousal threshold), and stereotypic posture. Sleep is readily

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distinguishable from other states of altered “consciousness,” such as coma and anesthesia, in that it is easily reversible and self-regulating. During wakefulness, the cortical EEG typically contains desynchronized high-frequency, low-amplitude waves in the 14- to 30-Hz range (i.e., beta waves), presumably reflecting differences in the timing of processing of cognitive, motor, and perceptual functions. During “quiet rest,” when the eyes are closed, EEG oscillations predominate in the 8- to 12-Hz range and are referred to as alpha waves. At the onset of non-rapid eye movement sleep (NREMS), the waves become larger in amplitude (reflecting increased cortical firing synchrony), and the EEG frequency slows. In humans, NREMS is composed of 4 stages. During stage 1 of sleep, conscious awareness of the external environment gradually disappears, and the EEG slows, with oscillations predominating in the 4- to 7-Hz theta range. Stage 2 sleep is typified by the complete loss of conscious awareness as well as the appearance of “sleep spindles” and “K-complexes” in the EEG. During stages 3 and 4 sleep, commonly termed *deep sleep*, delta waves appear (1-3 Hz; also referred to as slow-wave activity [SWA]) in the EEG. Increasing sleep intensity is thus defined by an increase in total power, amplitude, and incidence of delta waves in the cortical EEG during NREMS. The manifestation of delta waves in the EEG is thought to reflect synchronized oscillations of thalamocortical circuit activity (Steriade, 2003). The neocortex is also capable of generating autonomous delta wave activity—that is, slow oscillations (< 1 Hz, generally 0.5-1.0 Hz).

During REMS, which cycles periodically with NREMS, the cortical EEG transitions to a high-frequency, low-amplitude activity that resembles the desynchronized pattern of stage 1 sleep and wake in humans. In contrast to wake, however, the electrooculogram (EOG) reflects rapid eye movements, and the electromyogram (EMG) evidences profound atonia (of the skeletal muscle tissue, only the extraocular, inner-ear, and respiratory muscles are unaffected). In rodents, hippocampal theta activity (4-8 Hz) is a prominent electrophysiological marker for REMS (and other specific behaviors during wake, e.g., exploration). Theta oscillations are generated by cholinergic and GABAergic inputs from the “theta pacemaker” neurons of the medial septum and vertical limb of the diagonal band, which receive and transform tonic ascending input from the brainstem (Kocsis and Kaminski, 2006). Similar to rodents, theta oscillations have been detected during REMS in the human hippocampus, yet unlike rodents, theta activity is not

continuous (i.e., tonic) during REMS (Cantero et al., 2003). It should be noted that human EEG scalp recordings do not typically reflect hippocampal theta per se as the human hippocampus is located in the medial temporal lobe, which is far from the scalp surface.

Recent studies show that the theta oscillations during REMS are driven by neurons in the precoeruleus area in the pons and that the REMS atonia is caused by neurons in the adjacent sublateralodorsal area (Lu et al., 2006). These “REM-on” zones are inhibited by a nearby “REM-off” region, including the ventrolateral periaqueductal gray matter and lateral pontine tegmentum. The REM-on area also can inhibit the REM-off area, and the mutual inhibition between these regions produces a “flip-flop” switch (see below), which ensures sharp and complete transitions between REM and NREM sleep. This REM switch is in turn modulated by cholinergic neurons (which promote REMS) and noradrenergic and serotonergic neurons (which inhibit REMS) (McCarley, 2004).

The Neuroanatomy of Sleep

Starting with the work of Moruzzi and Magoun (1949), it has been shown that forebrain and cortical arousal/waking behavior is mediated by a set of ascending pathways that originate in the upper brainstem near the pons-midbrain junction (Figure 1A). Classically defined as the *ascending reticular activating system*, it is now clear that several discrete neuronal populations mediate arousal and the cortical desynchrony of wakefulness via projections to the thalamus and basal forebrain (Saper et al., 2005c). Among the most important components of the ascending arousal system are the cholinergic neurons in the pedunculopontine (PPT) and laterodorsal (LDT) tegmental nuclei in the mesopontine tegmentum (Levey et al., 1987). The PPT and LDT send excitatory cholinergic projections to thalamocortical nuclei and the reticular nucleus. At the level of the thalamus, these projections play a critical gating role for thalamocortical transmission by preventing relay neurons from being hyperpolarized and entering into burst mode, thus clearing the way for thalamocortical sensory transmission. Another population of cholinergic neurons is intermixed with noncholinergic (largely GABAergic) neurons in the basal forebrain (including the nucleus basalis and magnocellular preoptic nucleus in the substantia innominata, as well as the medial septal nucleus and nucleus of the diagonal band of Broca) that project to the cortex, and to a lesser extent, the thalamus, and are similarly implicated in waking and

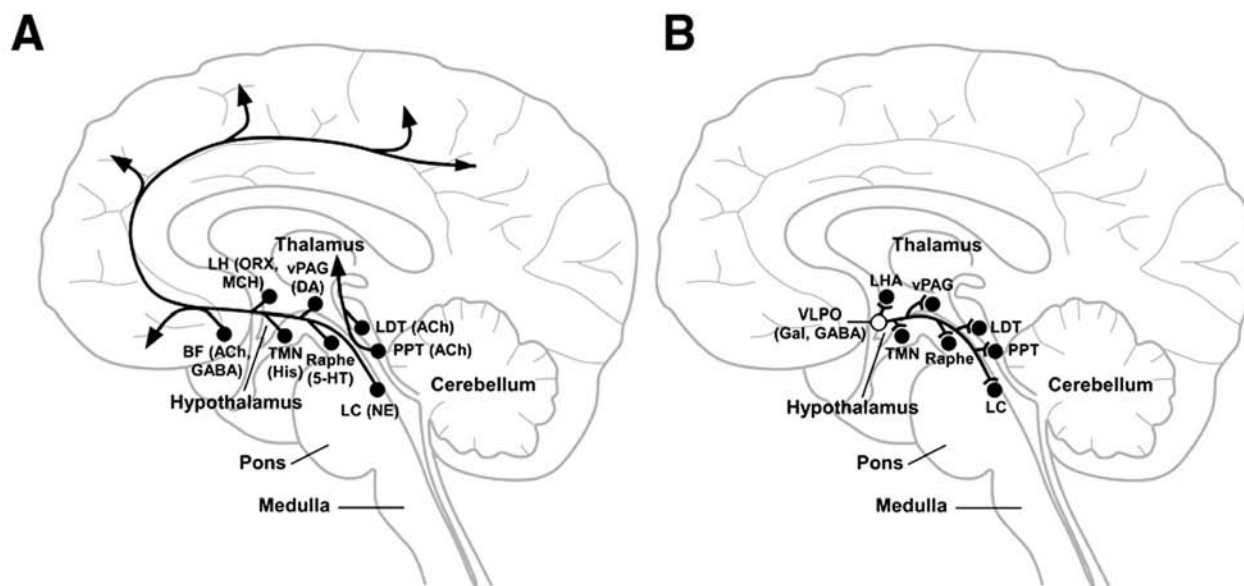


Figure 1. (A) The ascending arousal system consists of noradrenergic neurons of the locus coeruleus (LC), cholinergic neurons in the pedunculopontine and laterodorsal tegmental (PPT/LDT) nuclei, serotonergic neurons in the dorsal raphe nucleus (DR), dopaminergic neurons of the ventral periaqueductal gray matter (vPAG), and histaminergic neurons of the tuberomammillary nucleus (TMN). These systems produce cortical arousal via 2 pathways: a dorsal route through the thalamus and a ventral route through the hypothalamus and basal forebrain. The latter pathway receives contributions from the orexin (ORX) and melanin-concentrating hormone (MCH) neurons of the lateral hypothalamic (LH) area as well as from GABAergic or ACh neurons of the basal forebrain (BF). (B) A schematic of the projections of the ventrolateral preoptic nucleus (VLPO; open circle) to the main components of the ascending arousal system. The VLPO neurons are primarily active during sleep and contain the inhibitory transmitters GABA and galanin.

EEG desynchronization. Lesions of the basal forebrain produce coma (i.e., persistent subdelta < 1-Hz EEG) in experimental animals, thus illustrating the importance of these structures in waking behaviors and consciousness (Buzsaki et al., 1988). In addition to the mesopontine and basal forebrain inputs to the thalamus, a series of wake-promoting monoaminergic cell groups projects to the thalamus, lateral hypothalamus, basal forebrain, and cerebral cortex (Saper, 1984). The monoaminergic systems include the noradrenergic locus coeruleus, the dopaminergic neurons of the ventral periaqueductal gray matter, the serotonergic dorsal and median raphe nuclei, and histaminergic neurons in the tuberomammillary nucleus (for review, see Saper et al., 2005c). In general, neurons in all of these cell groups fire more during wakefulness than during NREMS and show virtually no activity during REMS. More rostrally, the lateral hypothalamus (LH), in addition to receiving input from these ascending monoaminergic systems, projects to the basal forebrain, cerebral cortex, and, in a reciprocal manner, to components of the brainstem arousal systems. The lateral hypothalamus contains at least 2 distinct populations of neurons that contribute to the regulation of wakefulness. LH neurons that

contain orexin (also called hypocretin) are active during wakefulness and increase the firing rates of neurons in the tuberomammillary nucleus, locus coeruleus, and dorsal raphe. Mice lacking orexin demonstrate narcolepsy-like symptoms, including profound behavioral state instability (e.g., frequent state transitions) and cataplexy (Chemelli et al., 1999; Mochizuki et al., 2004). LH neurons that contain melanin-concentrating hormone (MCH) have similar projections to the orexin neurons but are mostly active during REMS, during which time they are thought to inhibit the ascending monoaminergic systems.

As outlined above, projections from cholinergic neurons, monoaminergic cell groups, and LH orexin neurons act in a coordinated manner to produce arousal. But what turns off this arousal system to produce sleep? A series of studies has elucidated a critical role for the ventrolateral preoptic nucleus (VLPO) in inhibiting these arousal circuits during sleep (Sherin et al., 1996) (see Figure 1B). Neurons of the VLPO are sleep active, and loss of VLPO neurons (e.g., cell-specific lesions) produces profound insomnia and sleep fragmentation (Lu et al., 2000). The VLPO contains 2 populations of neurons, the first a cluster of neurons in the "core" of the VLPO that projects most heavily to

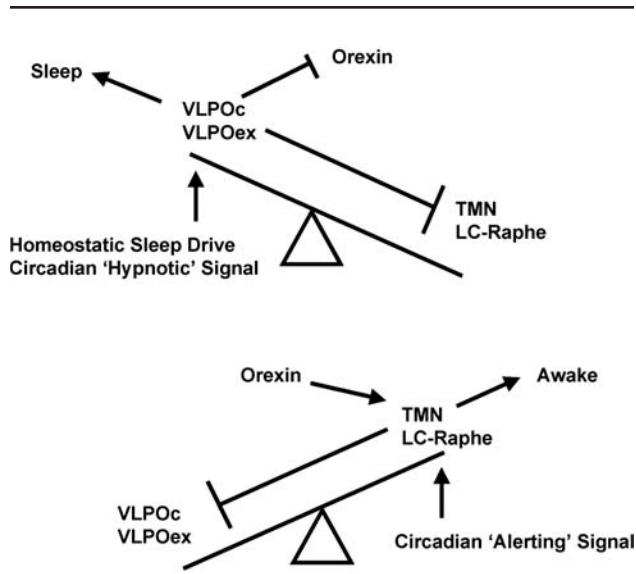


Figure 2. Neurons of the ventrolateral preoptic nucleus (VLPO) are sleep active, and loss of VLPO neurons produces profound insomnia and sleep fragmentation. The VLPO contains 2 populations of neurons, the first a cluster of neurons in the "core" of the VLPO (VLPOc) that projects most heavily to the tuberomammillary nucleus (TMN), whereas the second population is more diffusely located (i.e., extended VLPO; VLPOex) and projects more heavily to the locus coeruleus (LC) and the dorsal and median raphe nuclei. This interaction between the VLPOex and components of the arousal systems is mutually inhibitory, and as such, these pathways function analogously to an electronic flip-flop switch. The lateral hypothalamic (LH) orexin neurons likely play a stabilizing role for the switch. Circadian and homeostatic processes influence both sides of the switch to produce consolidated sleep and wake bouts.

the tuberomammillary nucleus, whereas the second population is more diffusely located and projects more heavily to the locus coeruleus and the dorsal and median raphe nuclei. This interaction between the VLPO and components of the arousal systems has been demonstrated to be mutually inhibitory, and as such, these pathways function analogously to an electronic "flip-flop" switch/circuit (Gallopini et al., 2000; Saper et al., 2001; McGinty and Szymusiak, 2000) (see Figure 2). By virtue of the self-reinforcing nature of these switches (i.e., when each side is firing, it reduces its own inhibitory feedback), the flip-flop switch is inherently stable in either end state but avoids intermediate states. The flip-flop design thus ensures stability of the behavioral state and facilitates rapid switching between behavioral states. Flip-flop switches also possess, at times, the undesirable property of abruptly undergoing unwanted state transitions. The frequency of unwanted state transitions may increase if 1 side of the switch is "weakened," as the weakened side becomes less able to inhibit the other side, thereby

biasing the switch more toward a midpoint where smaller perturbations may trigger a state transition. As an example, it has been suggested that cell loss in the VLPO during aging may weaken the switch, ultimately manifesting in sleep fragmentation and daytime napping, both of which are frequent complaints in the elderly (Saper et al., 2005c). It appears that the LH orexin neurons may play a stabilizing role for the switch, but this influence is likely indirect. Specifically, it appears that LH orexin neurons actively reinforce monoaminergic arousal tone as opposed to having a direct, mutually inhibitory relationship with the VLPO itself. The position of the orexin neurons outside the flip-flop switch permits them to stabilize the behavioral state, which reduces transitions during both sleep and wakefulness, whereas narcoleptic humans or animals who lack orexin have increased transitions in both states (Saper et al., 2001).

HOW IS CONSOLIDATED SLEEP ESTABLISHED?

Thus far, we have described the putative neural pathways by which sleep-wake states are regulated. But how is it that in humans, a consolidated bout of sleep of approximately 8 h is achieved each night? In 1982, Borbely proposed a 2-process model of sleep regulation in which a homeostatic process (i.e., sleep drive) that builds during wakefulness and declines during sleep interacts with a circadian process that is independent of sleep and waking (Borbely and Tobler, 1985). A further elaboration of this model for sleep-wake regulation, the "opponent process" model, was proposed in 1993 (Edgar et al., 1993). Although conceptually similar to Borbely's 2-process model, the opponent process model suggested a role for the circadian pacemaker in the SCN (see below) in actively facilitating the initiation and maintenance of wakefulness and opposing homeostatic sleep tendency during the subjective day. This conceptual model was derived from observations in a group of squirrel monkeys (*Saimiri sciureus*), which demonstrated a profoundly increased daily sleep time, loss of sleep-wake consolidation, and short sleep latencies (at lights-off) following lesions of the SCN. Interestingly, SCN lesions in nocturnal rodents have yielded results contrasting with those from the diurnal squirrel monkey. For example, although SCN lesions in rats eliminate sleep-wake rhythms, there is virtually no effect on total daily sleep duration or component sleep, suggesting that the circadian timing of sleep in rodents may be

independent of homeostatic processes (Mistlberger et al., 1987). One problem in comparing these results is that the primate work inevitably depends on observations in a relatively small number of animals. In addition, all of these experiments were done with electrolytic lesions, and the involvement of the optic chiasm and damage to adjacent areas that are critical for circadian timing, such as the subparaventricular zone (see below), was not assessed. Recently, it has been argued that hypersomnolence in the SCN-lesioned monkeys may have reflected their fragmented sleep and reduced sleep intensity (Mistlberger, 2005), which could have been affected by involving these adjacent regions. As both sleep continuity and sleep intensity are thought to be important for restorative sleep, the lesioned monkeys may have experienced less "restorative" sleep and thus required a longer bout of sleep to meet homeostatic requirements. Thus, in contrast to the opponent-process model, this interpretation supports both a wake- and sleep-promoting role for the SCN in sleep-wake regulation. A resolution of this problem awaits data on selective, cell-specific SCN lesions in each species.

Consistent with the aforementioned models for sleep-wake regulation, in humans, the sleep-wake cycle is regulated by the interaction of homeostatic and circadian processes. Specifically, there is a paradoxical increase in circadian drive for wakefulness during the course of the waking day that opposes the wake-dependent increase in sleep propensity, resulting in a consolidated bout of wakefulness (Dijk and Czeisler, 1995). Similarly, consolidated sleep is thought to occur as the result of an increase in circadian sleep drive during the course of the subjective night that opposes the decline in homeostatic sleep drive during sleep. The concept of a circadian "hypnotic" signal subserving the circadian drive for sleep has also been proposed, but as yet, no endogenous correlate of this putative signal has been uncovered (Dijk et al., 1997).

In addition to the use of SCN-lesion animal models (see above), the interrelationship between circadian and homeostatic processes in sleep-wake regulation has been recently investigated using mutant and recombinant circadian clock gene mouse models (i.e., the regulation of sleep in the absence of an intact circadian clock). The circadian *Clock* mutant mouse, the first circadian mutant tested, demonstrated a significant decrease in total daily sleep (ca. 2 h less in both light-dark and constant-dark conditions) that was largely attributable to a decrease in nocturnal NREMS, without a corresponding decrease in sleep intensity (as assessed by NREM delta power) (Naylor et al., 2000).

These observations in the *Clock* mutant provide support for the concept that the *Clock* mutation influences, in addition to circadian control, homeostatic control of sleep. An analysis of the functional role of circadian clock genes in sleep-wake regulation was also performed using cryptochrome (*Cry1* and *2*) double knockouts (*Cry1^{-/-}/Cry2^{-/-}*). In contrast to the *Clock* mutants, the *Cry1^{-/-}/Cry2^{-/-}* knockout mice demonstrate significant increases in NREMS, consolidation of NREM episodes, and NREMS delta power and an attenuated compensatory response to sleep deprivation (Wisor et al., 2002). It has since been suggested that the divergent NREMS patterns (i.e., decrease vs. increase) in the *Clock*- and *Cry1, 2*-deficient mice may not be conflicting results but rather may reflect the fact that *CLOCK* and *CRY1,2* are positive and negative transcriptional regulators, respectively, of the molecular feedback loops that produce endogenous rhythmicity. Results from the *Clock* mutant and *Cry1,2* knockout mice do, however, contrast sharply with that of the circadian *Period* (*Per1*, *Per2*, and *Per1,2*) knockout mice. Specifically, all *Period* mutant mice demonstrate normal amounts of daily total and component sleep (i.e., NREMS and REMS). Sleep-wake regulation has also recently been evaluated in the *Bmal1* (*-/-*) circadian mutant. *Bmal1* (*-/-*) mutant mice demonstrated increased total sleep time, sleep fragmentation, and sleep intensity (as assessed by NREM delta power) (Laposky et al., 2005). Collectively, with the possible exception of the results of the *Period* knockout mice, the findings from the *Clock*, *Cry1,2*, and *Bmal1* mutant mice support a functionally important role for the circadian clock genes in both the circadian and homeostatic components underlying sleep-wake regulation. In addition to the SCN, clock genes are expressed rhythmically throughout the brain and in the periphery. Therefore, it remains unclear whether clock gene expression in the SCN is required for the circadian and homeostatic regulation of sleep.

HOMEOSTATIC REGULATION OF SLEEP

The nature of "sleep drive" is unknown but has been conceptualized as a homeostatic pressure that builds during the waking period and is dissipated by sleep. This homeostatic process or "sleep homeostat" thus represents the need for sleep—that is, "sleep propensity" and, as indicated above, EEG delta power is considered a marker for this process. The cellular substrate of this homeostatic sleep drive is also unknown, although a putative endogenous somnogen,

adenosine (AD), is thought to play a critical role (Porkka-Heiskanen et al., 1997). AD is a naturally occurring purine nucleoside that is hypothesized to accumulate during wake and, upon reaching sufficient concentrations, inhibits neural activity in wake-promoting circuitry of the basal forebrain and likely activates sleep-promoting VLPO neurons located adjacent to the basal forebrain (Saper et al., 2005a). Consistent with this hypothesis are the observations that intracerebroventricular injections of AD promote sleep, extracellular concentrations of AD increase with prolonged waking and decline with sleep, administration of adenosine agonist near the VLPO increases sleep and induces Fos in VLPO neurons, and nonspecific AD antagonists (e.g., caffeine) potently increase waking and decrease sleep (for review, see Porkka-Heiskanen et al., 2002). Recent data have, however, demonstrated that accumulation of AD in the basal forebrain is not necessary for sleep drive (Blanco-Centurion et al., 2006), suggesting that the cellular basis of the sleep homeostat remains elusive.

Circadian Regulation of Sleep-Wake Cycles

The circadian timing system (CTS) provides temporal organization for most neurobehavioral, physiological, and biochemical variables, including the sleep-wake cycle (Moore-Ede et al., 1982). The fundamental adaptive advantage of this temporal organization is that it allows for predictive, rather than entirely reactive, homeostatic regulation of function. For example, prior to waking, body temperature, sympathetic autonomic tone, and plasma cortisol rise, presumably in anticipation of increased energetic demands.

In mammals, the circadian clock in the SCN in the anterior hypothalamus is critical for establishing the circadian rhythm of sleep-wake. Regulation of sleep-wakefulness by the SCN is evident as the sleep-wake cycle continues on a ca. 24-h basis, even when environmental conditions are constant (i.e., in the absence of environmental time cues), but only if the SCN is intact (Mistlberger, 2005). In humans, a clear circadian variation in sleep propensity and sleep structure has been demonstrated by uncoupling the rest-activity cycle from the output of the circadian pacemaker—that is, a forced desynchrony protocol (Cajochen et al., 2002; Dijk and Czeisler, 1995; Dijk et al., 1997). It has been hypothesized that circadian modulation of the cortical EEG, in particular REMS propensity, may occur through indirect projections from the SCN to the mesopontine tegmental nuclei involved in REMS

generation (McCarley and Massaquoi, 1992). Rather strikingly, despite a demonstrated role for the SCN in governing the timing of sleep, the SCN itself has only minimal monosynaptic outputs to sleep-regulatory centers such as the VLPO and lateral hypothalamus and none at all to brainstem arousal sites. Thus, the circadian regulation of sleep behavior is thought to be mediated by multisynaptic projections from the SCN to sleep-wake centers of the brain (Deurveilher and Semba, 2005; Saper et al., 2005b). The densest projection from the SCN terminates dorsally and caudally in the subparaventricular zone (SPZ) (Watts et al., 1987; Watts and Swanson, 1987). Similar to the effects of SCN ablation, lesions that include the ventral SPZ abolish behavioral circadian rhythms, including rest-activity, sleep-wake cycles, and feeding (as measured in constant darkness; Lu et al., 2001). Lesions of the dorsal SPZ, surprisingly, eliminate the circadian rhythm of body temperature but have little effect on the rhythms of locomotor activity or sleep. The SCN and vSPZ, in turn, project densely to the dorsomedial hypothalamic nucleus (DMH). Lesions of the DMH also abolish circadian rhythms of locomotor activity, sleep-wake, feeding, and corticosteroid secretion in constant darkness (Chou et al., 2003), but they do not eliminate the rhythm of body temperature. These findings suggest that neurons of the SCN regulate circadian rhythms via multiple and divergent pathways. For example, the SCN is thought to regulate the circadian rhythm of melatonin release via a direct projection to the paraventricular hypothalamic nucleus, whereas circadian control of thermoregulation is mediated by a projection from the SCN to the dorsal subparaventricular zone (Saper et al., 2005b). However, the principal neuronal output pathway that determines the timing of circadian behavior is mediated by a primary projection from the SCN to the ventral SPZ, followed by a secondary projection to the DMH. The DMH, which resides at the end of a column of tissue in the hypothalamus that is critical for the circadian regulation of rest-activity and sleep-wake cycles, sends a dense glutamatergic projection to the lateral hypothalamus (overlapping with the field of orexin-containing neurons) and an intense GABAergic projection to the VLPO (Chou et al., 2003). These projections from the DMH neurons to sleep-wake centers of the hypothalamus provide a putative mechanism for the circadian regulation of sleep-wake cycles.

These findings raise the question of why circadian rhythms of behavior and physiology are regulated by a multisynaptic pathway in the hypothalamus, rather than by a direct projection from the SCN to sleep-wake

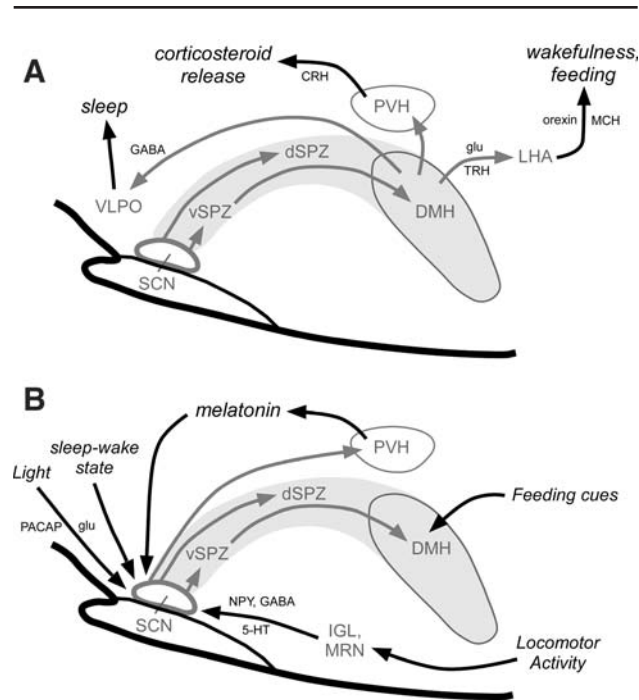


Figure 3. Circadian regulation of sleep-wake cycles. (A) The circadian rhythm of sleep-wake is regulated at multiple levels in the hypothalamus. The circadian clock in the SCN sends an indirect projection to the DMH via the SPZ that is critical for the circadian rhythm of sleep-wake. The DMH, in turn, provides rhythmic output to brain regions critical for the regulation of sleep-wake, hormone synthesis and release, and feeding. (B) This multistage regulation of circadian behavior in the hypothalamus allows for the integration of multiple time cues from the environment to shape daily patterns of sleep-wake. SPZ, subparaventricular zone; 5-HT, 5-hydroxytryptamine (serotonin); CRH, corticotrophin-releasing hormone; GABA, γ -amino butyric acid; glu, glutamate; DMH, dorsomedial hypothalamic nucleus; dSPZ, dorsal subparaventricular zone; IGL, intergeniculate leaflet; LHA, lateral hypothalamic area; MRN, median raphe nucleus; MCH, melanin-concentrating hormone; NPY, neuropeptide Y; PACAP, pituitary adenylate cyclase-activating polypeptide; PVH, paraventricular hypothalamic nucleus; TRH, thyrotropin-releasing hormone; VLPO, ventrolateral preoptic nucleus; vSPZ, ventral subparaventricular zone.

centers of the brain. In both diurnal and nocturnal species, the circadian rhythm of spontaneous neuronal discharge in the SCN peaks during the subjective day, despite oppositely phased behavioral rhythms. Also, the timing of environmental pressures such as food availability and predation does not always track the solar day, even though the SCN normally remains entrained (i.e., phase-locked) to the light-dark cycle. Thus, this series of multiple relays in the hypothalamus (SCN, SPZ, and DMH; see Figure 3) could allow for the integration of light-entrained circadian cues from the SCN with nonphotic environmental time

cues to establish patterns of rest-activity and sleep-wake cycles that are most adaptive to an organism (Saper et al., 2005b).

Consistent with this hypothesis, it was recently shown by cell-specific lesions that the DMH is critical for the expression of food-entrainable circadian rhythms of a variety of basic functions, including body temperature, rest-activity cycles, and sleep-wake cycles (Gooley et al., 2006). Furthermore, in the presence of competing light and food zeitgebers, the rhythm of SCN electrical activity, vasopressin expression, and clock gene expression in the SCN remains entrained to the light-dark cycle (Inouye, 1982; Damiola et al., 2000; Stokkan et al., 2001; Gooley et al., 2006), whereas the rhythm of expression of c-Fos in the DMH synchronizes with a daily meal (Gooley et al., 2006). These data suggest that daily cycles of restricted food availability entrain the daily rhythm of neuronal activity in the DMH, thereby synchronizing behavioral and physiologic rhythms. This is further supported by the finding that restricted daytime feeding selectively induces a robust rhythm of *Per1* and *Per2* gene expression in the DMH (Mieda et al., 2006; Fuller and Saper, unpublished observations). However, it should be noted that in a recent study, lesions in the medial hypothalamus that included the DMH did not block the preprandial increase in locomotor activity, suggesting that some food anticipatory behaviors may be preserved in DMH-lesioned rats (Landry et al., 2006). Nonetheless, the former studies raise the interesting possibility that in animals that are fed ad libitum, the circadian rhythm of feeding that is mediated by the SCN, SPZ, and DMH provides regulatory feedback control of the CTS in the hypothalamus to consolidate the circadian expression of sleep-wake and rest-activity cycles.

FEEDBACK CONTROL OF THE CTS BY REST-ACTIVITY AND SLEEP-WAKE CYCLES

Although the CTS is critical for the establishment of daily patterns of sleep-wake and rest-activity, behavioral activity can, in turn, provide feedback to the CTS to reset the phase of circadian rhythms. Recent work has uncovered several mechanisms by which SCN neuronal activity and the expression of circadian rhythms are regulated by endocrinologic and behavioral outputs of the circadian clock.

The circadian clock in the SCN determines the daily rhythm of melatonin secretion from the pineal gland, which, in mammals, is located in the epithalamus adjacent to the habenular nuclei. The SCN sends

GABAergic projections to cells in the parvocellular hypothalamic paraventricular nucleus, which in turn sends projections to the intermediolateral (IML) cell column of the spinal cord. IML preganglionic cholinergic fibers then project to the superior cervical ganglia cells, which in turn send noradrenergic sympathetic postganglionic axons along the carotid artery back into the skull to reach the pineal gland (Moore, 1996). Circulating melatonin levels are elevated by about 10-fold during the biological night (in both diurnal and nocturnal species) relative to during the biological day. Despite a well-established role for melatonin in mediating photoperiod-dependent changes in seasonal reproduction in some mammals, the role of melatonin in the regulation of natural sleep remains controversial. In humans, exogenously administered melatonin promotes sleep (i.e., earlier sleep onset and longer sleep duration) in humans during the subjective daytime (Mendelson, 1997; Sack et al., 1997; Rajaratnam et al., 2004; Stone et al., 2000), and daily ingestion of melatonin entrains free-running circadian rhythms in blind individuals (Lockley et al., 2000; Sack et al., 2000), suggesting that melatonin provides direct feedback to the CTS. Furthermore, pharmacological suppression of nocturnal melatonin levels produces an increase in total wake time (and a concomitant decrease in NREMS and REMS) (Van Den Heuvel et al., 1997), and the circadian rhythm of plasma melatonin also has a temporal association with the aforementioned circadian rhythms in cortical EEG activity during sleep in humans (Dijk et al., 1997), suggesting a direct influence of melatonin on sleep-wake regulation.

In lower mammals, a clear link between melatonin and sleep has yet to be established. Considering that melatonin levels are highest during the subjective night in both day- and night-active species, it seems unlikely that melatonin plays a role in promoting sleep in nocturnal animals. Furthermore, melatonin-deficient mice do not show any obvious deficits in sleep-wake regulation (see Huber et al., 2000). In rats, daily administration of melatonin entrains the circadian rhythm of locomotor activity but only if the SCN remains intact (Cassone et al., 1986). Thus, melatonin signaling in the SCN is required for melatonin-induced entrainment of behavioral circadian rhythms. Recently, it was shown that melatonin exerts its phase-shifting effects on circadian rhythms via melatonin receptors (MT1 and MT2) expressed in the SCN (Jin et al., 2003; Liu et al., 1997). Hence, the SCN-driven rhythmic release of melatonin could function as an endogenous zeitgeber to reset the circadian phase of

SCN electrical activity, thereby providing a feedback that consolidates the sleep-wake rhythm.

Historically, circadian behavioral rhythmicity in rodents has most frequently been measured with the use of running wheels. However, wheel-running activity itself acutely resets the CTS and alters the circadian period (Yamada et al., 1986; Reeb and Mrosovsky, 1989). In contrast to the effects of light on circadian rhythms, elevated locomotor activity (either enforced or stimulated, i.e., with a novel running wheel) induces phase advances during the subjective day (up to ~4 h) and small phase delays during the late subjective night (< 1 h) (for review, see Mrosovsky, 1996). However, in these studies, it is difficult to distinguish whether wheel running itself is the primary zeitgeber or if the wakefulness and/or stress associated with increased locomotor activity resets the circadian timing system. In Syrian hamsters without running wheels, sleep deprivation by gentle handling induces phase advances during the subjective day, suggesting that enforced wakefulness may be sufficient to reset the circadian clock (Antle and Mistlberger, 2000). Although stressors are thought to have little effect on circadian phase, stressful stimuli have been shown to acutely induce gene expression in the SCN and influence the expression of behavioral and body temperature rhythms for several days (for review, see Meerlo et al., 2002).

In humans, exercise and sleep-wake cycles are relatively weak zeitgebers compared to the light-dark cycle. Exercise during the early subjective night induces small phase delays (< 1 h) of the circadian timing system (Barger et al., 2004; Buxton et al., 2003), and inverting the sleep-wake cycle (and therefore rest-activity and social contact) on a background of dim light does not produce phase shifts significantly different from that expected for the endogenous drift of the circadian pacemaker (~0.2 h/day) (Duffy et al., 1996). In a simulated shiftwork study, sleep episodes that were scheduled before or after a night shift yielded small phase advances (~20 min/day) and phase delays (~60 min/day), respectively, suggesting that scheduling sleep cycles can facilitate adaptation to shiftwork (Santhi et al., 2005). A caveat of these studies is that it was not possible to dissociate the effects of sleep versus darkness on circadian phase resetting. Notably, in rodents, dark pulses administered on a background of constant light reset behavioral circadian rhythms in a phase-dependent manner (Navaneethakannan and Chandrashekar, 1986; Rosenwasser and Dwyer, 2002).

The pathways by which locomotor activity and/or sleep/wake states regulate the CTS are currently

unknown. However, thalamic lesions that include the intergeniculate leaflet (IGL) block circadian phase shifts in response to wheel-running activity (Wickland and Turek, 1994; Marchant et al., 1997; Janik and Mrosovsky, 1994). The IGL, a distinct lamina of neurons interposed between the dorsal and ventral lateral geniculate nuclei of the thalamus, gives rise to a dense neuropeptide-Y (NPY-) and GABA-containing projection that terminates bilaterally in the SCN. The terminal field of the geniculohypothalamic tract (GHT) in the SCN is coextensive with the retinorecipient neurons and is thought to subserve entrainment to locomotor activity (for review, see Harrington, 1997). For example, similar to the effects of wheel running on circadian phase, microinjection of NPY into the SCN induces phase advances during the subjective day (Albers and Ferris, 1984). In addition, NPY and novel running wheel access attenuate light-induced phase advances of behavioral rhythms (Weber and Rea, 1997; Edelstein et al., 2003), and NPY down-regulates expression of the clock genes *mPer1* and *mPer2* in the SCN in hypothalamic slices (Fukuhara et al., 2001). Collectively, these data suggest that NPY-containing neurons in the IGL transmit nonphotic information to the SCN to reset circadian rhythms.

The SCN also receives a major afferent projection from serotonergic neurons in the median raphe nucleus (MRN) of the midbrain (for review, see Morin, 1999). Injection of the neurotoxin 5,7-DHT into the region of the SCN blocks entrainment to daily access to a running wheel or to enforced daily running on a treadmill (Edgar et al., 1997; Marchant et al., 1997). The observation that both the IGL and serotonergic input to the SCN are required for locomotor activity-induced entrainment and phase shifting suggests that NPY and serotonin may act on the circadian clock via a common mechanism.

Recently, it was shown that the firing rate of neurons in the SCN correlates with the sleep state of the animal, indicating that sleep/wake can provide feedback control of SCN electrical activity (Deboer et al., 2003). Superimposed on the circadian rhythm of SCN neuronal activity, SCN neurons fire more rapidly during REM sleep compared to NREM. Furthermore, selectively depriving animals of slow-wave sleep results in an increase in SCN neuronal firing, whereas REM sleep deprivation results in a decrease in the SCN firing rate. These data suggest that sleep states and homeostatic sleep pressure regulate SCN neuronal activity. Additional studies are needed to fully characterize this novel interaction between homeostatic and circadian regulation of sleep at the level of the SCN.

SUMMARY

Remarkable progress has been made in the past decade in our understanding of the neural circuitry underlying the regulation of sleep-wake states and circadian control of behavior. Specific neuronal pathways, transmitters, and receptors have been identified that are now the target of pharmaceutical manipulation for the treatment of sleep disorders. Recent studies indicate that sleep and wakefulness are regulated by mutually inhibitory populations of neurons in the hypothalamus and brainstem, which ensure behavioral state stability and facilitate rapid switching between sleep and wakefulness. Despite significant advances in our understanding of the neural circuitry and molecular mechanisms underlying sleep-wake behavior and the interrelationship between sleep processes and the CTS, significant gaps remain in our knowledge of how circadian and homeostatic processes interact to execute control of the behavioral state. The 2-process model originally proposed by Borbely has served as an important model to conceptualize how the interaction between homeostatic sleep pressure and the circadian rhythm of sleep propensity produces a consolidated bout of approximately 8 h of sleep per night in humans. This model of sleep-wake regulation will be further developed and refined as our understanding of the cellular and molecular bases of sleep-wake and circadian rhythm control continues to improve. Currently, the signaling pathway(s) that mediate the wake-dependent increase in sleep propensity (i.e., homeostatic sleep drive) is unknown, and only recently have SCN efferent pathways been identified that regulate the circadian control of sleep-wake cycles. Regulation of the circadian rhythm of sleep-wake at multiple levels in the hypothalamus may allow for the integration of photic and nonphotic environmental time cues to establish a pattern of sleep-wake behavior that is most adaptive for an organism. Understanding the input pathways by which sleep-wake, rest-activity, endocrinologic, and feeding cycles regulate the CTS and circadian expression of sleep-wake is currently an area of intense investigation.

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