

Circadian and Homeostatic Factors in Arousal

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In the course of evolution, mechanisms have evolved to anticipate the timing of regularly occurring events. These mechanisms are encompassed in a circadian timing system that include a master clock localized to the suprachiasmatic nucleus of the hypothalamus and “slave” oscillators distributed throughout the body. This system serves multiple functions so as to ensure that various physiological processes occur at optimal and nonoverlapping times, to synchronize our activities to local environmental time, and to permit changes required to respond to new environmental circumstances. We suggest that a generalized concept of arousal (which includes alterations in responsiveness to homeostatic pressures, sensory stimuli and emotional reactivity, and to changes in motor activity) serves as a rubric in which to explore the multiple ways in which the circadian system modulates behavior.

Key words: suprachiasmatic nucleus; hypothalamus; feeding; reproduction; sleep/wake

Introduction: Circadian Rhythms of Arousal

Correct timing is needed for telling good jokes, cooking food, treating illness, making purchases and sales in the stock market, and other activities. *Anticipation* of the timed events is the key to performing optimally when the moment for a response arrives. If we were able to deliberately anticipate timing of important events, how our world would change!

Importantly, in the course of evolution, mechanisms have evolved to anticipate the timing of regularly occurring events. Restated, we have a built-in anticipatory timing mechanism in the *circadian timing system*. It affects our activities, even though we are not generally aware of its actions. This system 1) anticipates regularly recurring upcoming events, 2) ensures that various physiological processes occur at optimal times, 3) enables time-sharing of bodily resources (as neither a cell, nor a tissue, nor an organism can respond continuously or at completely arbitrary times), 4) synchronizes our activities to local environmental time, 5) is sufficiently flexible to permit responses to important environmental events that are not anticipated, and 6) learns to time

new regularly recurring events. A generalized concept of arousal is a useful rubric in which to explore the multiple aspects of circadian rhythms.

Circadian rhythms are biological rhythms that oscillate with a period of approximately 24 h (derived from the Latin words *circa* meaning about, and *diem* meaning day). These rhythms occur throughout phylogeny, from cyanobacteria to unicellular organisms and humans.¹ Arousal encompasses the physiological and psychological components of being awake. It involves the activation of the systems in the brain, the autonomic nervous system, and the endocrine system and leads to increased heart rate and blood pressure, sensory alertness, mobility, and readiness to respond. The thesis of this chapter is that arousal is modulated and, in turn influences, the phase setting of a hierarchically organized circadian system that oscillates with the period of a day. The effects of the circadian system can be explored experimentally in the context of Pfaff's² operational definition of arousal, stated as follows:

An animal or human with a greater degree of generalized CNS arousal

- (i.) shows greater responsiveness to sensory stimuli in all sensory modalities;
- (ii.) emits more motor activity; and
- (iii.) is more reactive emotionally.

Circadian rhythms occur in virtually all physiological and behavioral responses that have been measured.^{3,4} Some rhythms are very salient, such as cycles of sleep and arousal. Other rhythms are less obvious, although they also occur daily. Thus, circadian

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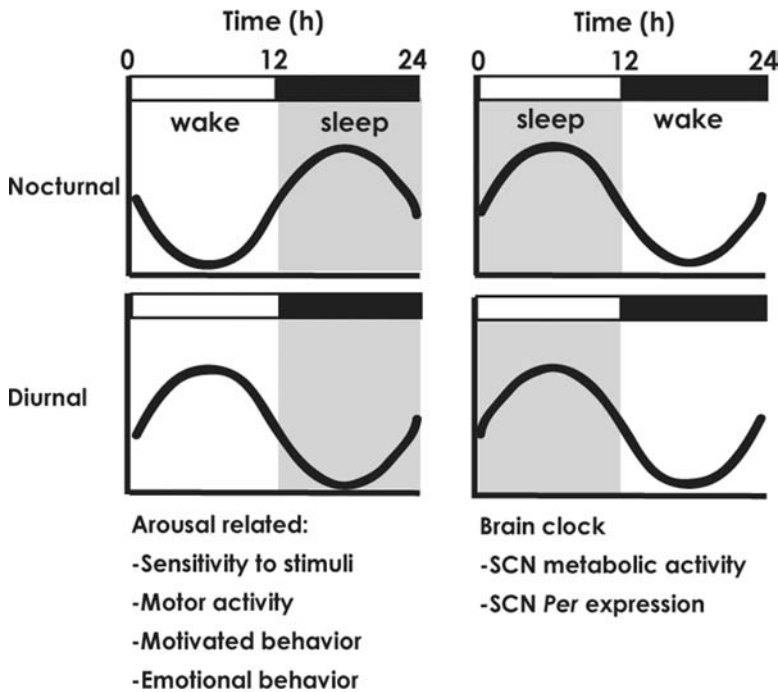


FIGURE 1. Circadian rhythms in diurnal and nocturnal species. In day- versus night-active animals, the timing of arousal-related rhythms, including sensitivity or responsiveness to sensory stimuli, motor responses, and motivated and emotional behavior are opposite in phase. On the other hand, the daily peak and trough of metabolic activity and of clock gene expression in the suprachiasmatic nucleus (SCN) is similar in diurnal and nocturnal animals. For example, glucose utilization and levels of *Per1* and *Per2* expression are high during the day and low at night in both diurnal and nocturnal animals.¹³

rhythms have been reported in sensitivity to external stimuli, motor activity, and motivated and emotional behaviors (FIG. 1). For example, the mouse olfactory bulb has a circadian pacemaker that enhances olfactory responsiveness at night that drives rhythms in the piriform cortex and interacts with the suprachiasmatic nucleus (SCN) to coordinate other daily behaviors.⁵ Circadian rhythms in motor activity are the very measure that led to the discovery of the SCN as a master clock⁶ and continue to serve as the most commonly used index of clock output. Time-of-day modulations affect performance on a wide range of cognitive tasks measuring attentional capacities, executive functioning, and memory.^{7,8} More specifically, there is a daily peak at approximately 4–8 h after wake-up time (and a daily nadir at the latter half of the usual sleep time) in objective and subjective measures of alertness, performance, and attention—indexed by psychomotor, vigilance, and memory tasks, and in attention—indexed by ability to concentrate, subjective alertness, and cognitive performance. A circadian component in learning has been described even in *Aplysia*,⁹ with the circadian system modulating memory formation in phase with the animal's activity period, suggesting a general phenomenon in many types of long-term learning.

Finally, diurnal variations in emotionality have long been reported, and disturbances of the circadian system may be associated with bipolar and other mood disorders.^{10,11} In summary, the circadian timing system modulates each of the aspects of arousal as defined by Pfaff.² In recent years, we have learned a great deal about the mediating mechanisms, as described below.

The Master Clock and the Circadian Timing System

In mammals, timing on the scale of days, seasons, and even years is accomplished by a hierarchically organized system of circadian oscillators that exist in most mammalian cells. Briefly, individual oscillator cells of the body are synchronized to the outside world and to each other by a “master clock” located in the SCN of the hypothalamus. The SCN has dual functions. It receives temporal information with regard to local time in the environment and also with regard to the temporal events from the internal milieu of the body. In turn, it signals circadian time to the rest of the body. In the absence of any external time cues in the environment, circadian rhythms continue to oscillate,

driven by the SCN. The period of the master clock in the SCN is slightly longer or shorter than 24 h, in a manner characteristic of the species. In the presence of timed environmental cues, such as those provided by daily cycles of light and darkness, circadian responses are synchronized to local time and rhythmic responses cycle with a period of precisely 24 h. The understanding of how individual oscillators throughout the body constitute the mammalian circadian timing system has advanced enormously in the past decade through convergent findings in many laboratories analyzing oscillator function in both “bottom-up” whole animal studies and “top-down” cellular/molecular experiments.

The existence of a brain clock was first demonstrated by lesion studies of the hypothalamus.^{6,12} Ablation of the SCN results in the loss of circadian rhythms in sleep/wake, in hormone secretion, as well as in most aspects other of physiology and behavior. That rhythmicity is a property of the SCN itself was demonstrated by the persistence of electrical rhythmicity in brain slices of the SCN *in vitro* and in the SCN isolated within a hypothalamic island *in vivo*.^{14–16} That the SCN is necessary and sufficient to sustain circadian rhythmicity was proven by the demonstration that SCN transplants

placed into the third ventricle sustained rhythmicity in the lesioned host with the period of the donor animal.^{17,18} These converging results confirmed the role of the SCN as the master clock in the body.

The bilaterally symmetrical SCN lies above the optic chiasm on each side of the third ventricle and contains several thousand neurons. Individual SCN cells are autonomous oscillators,¹⁹ and research on the molecular basis of cellular clockworks has had an exciting research trajectory. Circadian clock genes were discovered in 1971 in the fruit fly *Drosophila*.²⁰ Initially, three alleles of the *Period (Per)* gene were identified which had either long (e.g., about 29-h) or short (e.g., about 19-h) periods or no apparent rhythms of eclosion and locomotor activity. In the mid 1980s, the *Per* gene was identified in flies,²¹ and it took another 13 years until orthologs of the *Per* gene were identified in mammals.^{22–24} The identification of the “circadian locomotor output cycles kaput” (*Clock*) gene,²⁵ along with the findings of *Per* genes in mice led to the rapid discovery of the core transcriptional–translational feedback loops underlying the molecular clock mechanism in mammals. Subsequent studies of the nature of the molecular clock advanced rapidly (FIG. 2).²⁶ The

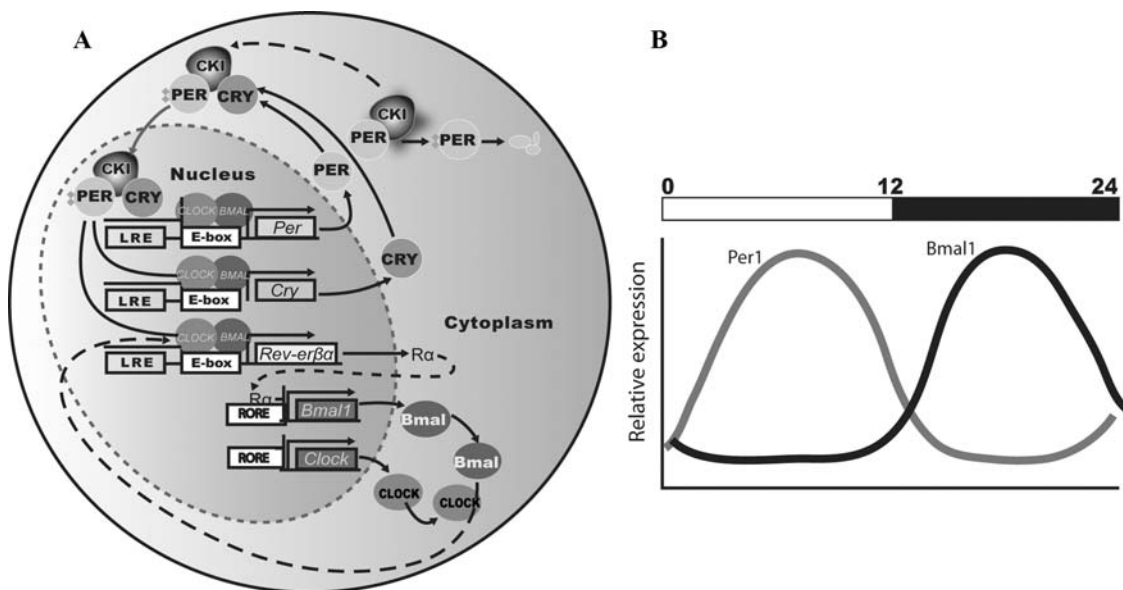


FIGURE 2. Model of the mammalian cellular circadian oscillator. **(A)** The cartoon depicts a simplified version of key elements of the mammalian core circadian oscillator with transcription/translation feedback loops that generate a circadian rhythm. Abbreviations: Bmal, brain and muscle Arnt-like protein-1; CKI, casein kinase I ϵ ; Clock, “circadian locomotor output cycles kaput” mRNA; CLOCK, “circadian locomotor output cycles kaput” protein; CRY, Cryptochrome; E-box, noncanonical 5'-CACGGT-3' E-box enhancer in the clock gene promoters; PER, Period protein; RORE, retinoic acid-related orphan receptor response element; R α , REV-ERB α ; proteins shown in CAPS; mRNA shown in *italics*. See Ref. 29 for further description of clock components. **(B)** The time of day expressed by a cell or a tissue can be assayed by measuring gene or protein expression. The figure depicts SCN expression of *Per1* and *Bmal1* mRNA in the mammalian SCN.

circadian signal is based on feedback loops in which the CLOCK/BMAL1 heterodimers activate the transcription of three *Period* genes (*Per1*, *Per2*, *Per3*) and two *Cryptochrome* (*Cry1*, *Cry2*) genes. In mammals, two cryptochrome genes, *CRY1* and *CRY2*, are highly expressed and rhythmic in the SCN, and a double knockout of *CRY1* and *CRY2* results in loss of rhythmicity when these mice are transferred to constant darkness. PER-CRY dimers act as negative regulators of CLOCK/BMAL1, reducing transcriptional activity of this heterodimer. The orphan receptor genes, such as *rev-erb a* and *rev-erb β*, *ror a* and *ror β* are activated by CLOCK/BMAL1 heterodimers and produce protein products that differentially regulate *Bmal1* transcription. The casein kinase epsilon gene, whose protein product phosphorylates PER proteins, leads to the degradation of PERs in the cytoplasm, slowing accumulation of PER in the nucleus, and thus slowing the pace of the repression of CLOCK:BMAL production.²⁷ Continuing studies add other loops to the circadian molecular machinery to explain some of the dynamics of the rhythmic cycling of clock genes and proteins.^{28,29} Rhythmic expression of clock-controlled genes, such as vasopressin, expressed by cells of the SCN shell, are regulated by the same interlocked feedback loops, and rhythmic vasopressin secretion is disrupted in clock-mutant animals.^{30–33}

The SCN as a whole is thought to produce a coherent circadian signal, and this is puzzling as it was noted even in the first studies of the nucleus that it is heterogeneous. In early experiments, two fundamentally distinct (ventral [or core] and dorsal [or shell]) SCN regions were noted within the nucleus based on cell size and morphology, afferent and efferent connections, and neuropeptide phenotype³⁴ (see Yan *et al.*³⁵ for a review). The SCN also contains a highly characteristic peptidergic arrangement of its neurons (FIG. 3).^{28,35,36} The discovery of the molecular elements of cellular clocks of the SCN enabled the determination of specialized functions of the heterogeneous cells within the nucleus that are associated with core and shell subdivisions and with peptidergically distinct phenotypes. Detailed analysis of clock gene expression within the SCN indicates highly specialized patterns of gene expression within distinct SCN regions that give functional significance to regional anatomical, morphological, and peptidergic differences characteristic of the SCN.^{28,35} Thus, in the shell SCN, most of these clock genes display rhythmic expression with a 24-h period. *Per1* reaches a peak around mid-day and a trough around midnight, and *Per2* has a similar pattern with a delay of several hours in peak and trough times. The ventral SCN lacks or has low amplitude rhythms

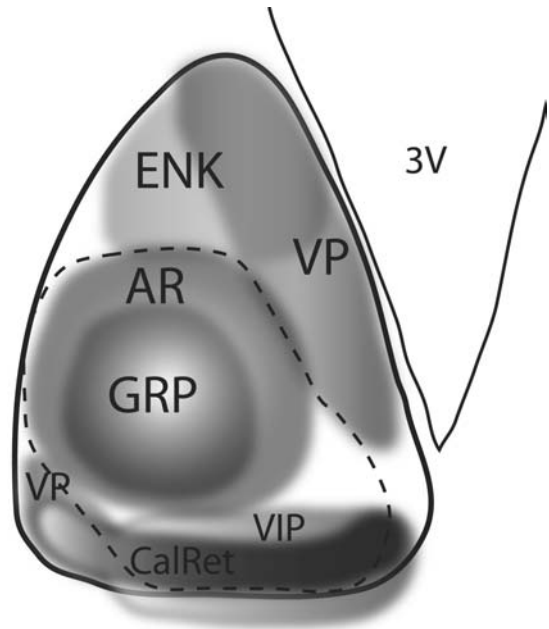


FIGURE 3. SCN neurons are heterogeneous. The cartoon depicts the approximate localization of various peptidergic cells in a coronal section of the caudal mouse SCN at the greatest extent of the core region of the nucleus. The shell contains vasopressin (VP) cells in the dorsomedial and ventrolateral regions and enkephalin (ENK) cells in the dorsal region. The SCN core contains gastrin-releasing peptide (GRP), androgen receptor (AR), vasoactive intestinal polypeptide (VIP), calretinin (CalRet)-containing cells. Sparse distribution of neurotensin cells also occurs in the core SCN.

in *Per1* and *Per2*. The necessary function of the core SCN has been demonstrated in lesion studies demonstrating that animals become arrhythmic following lesions that ablate the core while sparing other compartments of the nucleus.^{37,38} While lesion studies are notoriously difficult to interpret when loss of function is the end point, the conclusion that the core is important if the SCN is to sustain rhythmicity is confirmed in studies of rhythmicity using bioluminescent reporters of clock genes of the SCN in a brain slice preparation. In SCN slices which contain both core and shell, there are strong PER2::LUC rhythms that are structurally stable in all subregions of the nucleus over several circadian cycles and across all slices. In contrast, in SCN slices that lack the core, bioluminescence is of low amplitude and lacks structural stability from cycle to cycle. These studies indicate that despite its lack of (or weak) circadian oscillations, the core region is important for maintaining an organized pattern of synchronized complex spatiotemporal dynamics in

SCN tissue.³⁵ Taken together, these studies indicate that the core region is necessary for the coherent expression of rhythmicity in the SCN. Thus, even though individual cells are clocks, SCN master clock function requires SCN circuits. The key role of the core SCN in sustaining structurally stable rhythms in the animal highlights the importance of inputs to the SCN, as the most important of these reach the core SCN region.

Input to the SCN

The foregoing studies of the relationship between core and shell SCN regions draw attention to the importance of inputs to the core subregion of the nucleus. Photic input from the retina is the most experimentally studied, although hormonal and arousal-related signals are also substantial. The core receives direct input from the retina through the retinohypothalamic tract (RHT). RHT fibers synapse on vasoactive intestinal polypeptide (VIP) and gastrin-releasing peptide (GRP) cells (rat^{39,40}) or on calbindin and GRP cells (hamster⁴¹). Although some retinal fibers may be seen throughout the SCN, the greatest density is in the core region. Functionally, this results in light-induced *Per1* and *Per2* expression first in the core SCN, and then the signal slowly spreads to the shell in a pattern that depends on the precise timing of photic exposure.⁴²

While light is the most salient of resetting stimuli and is among the most reliable of environmental cues available, other stimuli also reach the SCN. Thus, cells of the core contain androgen receptors in male mice, signaling the hormonal state of the animal.^{43,44} In the absence of gonadal hormones, the behavior of the male is reorganized. Gonadectomy alters and androgen treatment restores the period, precision, and overall structure of nocturnal locomotor activity in castrated male mice, showing that these aspects of circadian behavior are modulated by androgens.^{43,45} These results point to a hypothalamic neuroendocrine feedback loop in which the SCN regulates circadian rhythms in gonadal hormone secretion, and androgens act on their receptors within the SCN to alter circadian function. One of the effects of androgens is on overall activity levels. Such arousal-related input to the SCN itself feeds back to alter the activity of the clock, as discussed below.

Output of the SCN

The output signals of the brain clock are the basis of its ability to modulate *generalized CNS arousal* on a circadian basis and to perform the three aspects (sensory, motor, affective) laid out by Pfaff.² The way in which such a tiny hypothalamic nucleus signals time of day to the rest of the body has been of intense interest. SCN efferents are largely restricted to nearby hypothalamic regions, including the subparaventricular

zone, the preoptic area, and the dorsomedial hypothalamus.^{46–48} In addition, humoral signals from the SCN sustain circadian timing of arousal-related responses but not endocrine rhythms. Thus, in animals bearing a “hypothalamic island” created by knife cuts around the nucleus, rhythmic locomotor activity is sustained.¹⁵ Also, circadian rhythms with the donor period are reinstated in SCN-lesioned animals when SCN tissue is transplanted to the third ventricle within a porous polymer capsule that prevents neural outgrowths and permits diffusible signals.⁴⁹ Cell autonomous oscillators are not unique to the SCN; immortalized rat fibroblasts express circadian rhythms.⁵⁰ Studies of transgenic *Per1::luciferase* rats show that circadian clocks exist in most tissues of the body.⁵¹ Considerable interest lies in the fundamental nature of molecular components in central versus peripheral oscillators. Functionally, they are different in that SCN neurons are synchronous in their activity compared to peripheral neurons, although they bear similar genetic elements.⁵² The peripheral clocks can be much more readily reset by glucocorticoids, changes in body temperature, and metabolic cues.⁵³ The stability and precision of SCN neurons (and possibly the fundamental difference between central and peripheral clocks) is the product of the intercellular circuitry and coupling.²⁸ In summary, signaling between SCN and peripheral clocks involves a combination of hormonal cues and cyclic changes in body temperature and food metabolites.⁵²

Homeostatic and Circadian Timing Mechanisms in Sleep and Feeding

Sleep

While the circadian system provides daily modulation, processes such as sleeping and eating, are under homeostatic regulation. The longer one is awake, the sleepier one becomes. The duration of the interval from the last meal is approximately proportional to feelings of hunger. How are these processes monitored? Models describing the interaction of homeostatic and circadian timing systems have been useful in unraveling the interactions between these distinct processes. A major advance in understanding the relationship between circadian and homeostatic mechanisms occurred with the development of the two-process model of sleep regulation.⁵⁴ This model incorporates a homeostatic element, termed *Process S*, and a circadian element, termed *Process C*. The two-process model has proven valuable in mechanistic studies of sleep/wake cycles. The circadian component is most commonly measured by locomotor activity. The homeostatically regulated *Process S* has been measured in delta (1- to 4-Hz)

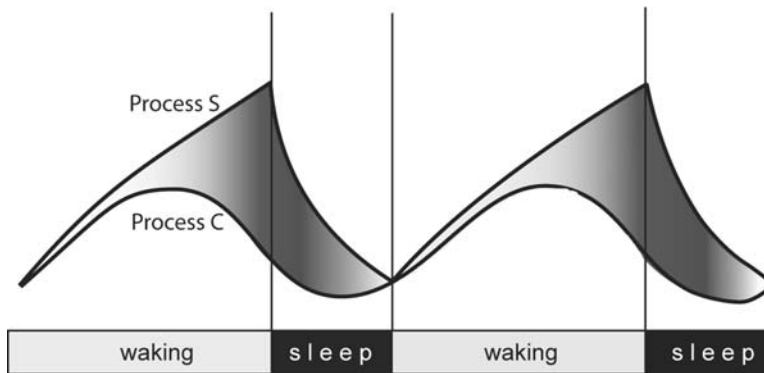


FIGURE 4. The two-process model for regulation of the sleep–wake cycle. Process S represents the need for sleep, which increases during wake and decreases during sleep. Process C represents the circadian system, which oscillates with a period of about 24 h. In this view, the timing of sleep and wakefulness occurs at the peak and trough of these additive processes.

oscillations in the electroencephalogram (EEG) during nonrapid eye movement (NREM) sleep (termed *delta power*). Delta power is high at sleep onset and decreases during sleep. Sleep deprivation increases and naps decrease delta power. At the molecular level, a surprising recent finding regarding Process S revealed in a study using transcriptome profiling in inbred mouse strains is that only a few genes in the brain change reliably following a period of sleep loss, and these genes are part of a specific pathway involved in neuronal protection and recovery after wake-induced glutamate overstimulation.⁵⁵ A schematic of the two interacting processes is shown in FIGURE 4. In this model, sleep occurs when Process S reaches an upper threshold, and wake occurs when Process S is below a lower threshold.

The Two-process Model

In this view, the circadian component controls the thresholds; sleep is initiated at one circadian phase and wake is initiated at another. Thus, the frequency of the sleep/wake cycle depends on the distance between the two thresholds or phases. In Borbely's (1982)⁵⁴ version of the two-process model, homeostatic and circadian processes interact in a mutually synergistic fashion. A variation of the model casts homeostatic and circadian processes as interacting in a mutually inhibitory fashion.⁵⁶ Here, a circadian alerting process opposes wake-dependent sleep-promoting factors to maintain wakefulness across the daytime. A similar opponent process model has been proposed to describe the maintenance of nocturnal sleep in humans.⁵⁷ As sleep pressure dissipates across the night, sleep is protected by the circadian timing system, which achieves maximal levels of sleep propensity during the second half of the habitual sleep period.

Regardless which version of homeostatic/circadian interactions turns out to be mechanistically accurate, the supreme importance of timing is evident in the arousal processes underlying sleep/wake cycles. Mammalian sleep appears during a predictable time in a 24-h cycle. In humans, as daylight decreases and night approaches, increasing feelings of drowsiness eventually lead to the desire to sleep. The timing of sleep and wakefulness, however, is species-specific and follows a general rule. Organisms remain active during hours when the opportunity to acquire food exceeds the risk of predation and sleep during times when the need for vigilance is minimized. The SCN organizes these responses in time, in both diurnal and nocturnal mammals.⁵⁸

Several lines of evidence converge to suggest that the SCN emits a wake-promoting signal, and that this in turn feeds back to alter SCN activity. In animals bearing mutations of the clock genes *Bmal1* and *Cry1/Cry2*, sleep increases and wakefulness decreases.^{59–61} Lesions of the SCN that do not impinge on the preoptic area of the hypothalamus result in an increase in sleep time and a decrease in wakefulness in mice, rats, and monkeys.⁶² The anatomical projections of SCN neurons to arousal areas of the hypothalamus support the possibility of influencing the wake/arousal system.^{46,62,63} There is also feedback to the SCN from the arousal system. Single cell recordings from the freely moving rat indicate that SCN firing rates are altered by vigilance states (monitored by EEG⁶⁴). SCN neuronal activity is elevated during rapid eye movement (REM) sleep and sleep deprivation, and lowered during NREM sleep. Thus, the circadian pattern in electrical activity is modified by afferent information consequent to arousal. Neural pathways mediating these responses

have been described. The circadian sleep-wake cycle controlled by the SCN can be modulated by the dorsomedial hypothalamic nucleus via projections to the locus coeruleus.⁶⁵ In a “flip-flop” switch model,⁶⁶ interactions between the ventrolateral preoptic nucleus and the tuberomammillary nucleus receiving input from orexin cells in the lateral hypothalamus control sleep and wake. This pathway is modulated by the SCN and by emotional and cognitive inputs.

In summary, sleep timing is governed by the interactions between SCN activity and its output to sleep-wake structures, the regulation of circadian clock genes, and feedback to the SCN consequent to this regulation. Further details are available in reviews of SCN outputs to the sleep-wake regulating structures⁶⁷ and circadian clock gene influences on timing and amount of sleep-wake.^{68–70}

Feeding

It seems trivial to point out that energy balance is homeostatically regulated, that organisms seek food to restore depleted energy stores, and that food-seeking behavior usually does not occur during the inactive part of the animal's day. Less obvious is the fact that feeding behavior is modulated by the circadian system.⁷¹ SCN-ablated animals eat, although in a temporally disorganized manner.⁷² The interaction of homeostatic and circadian pressures in seeking food drew substantial interest in studies of biological rhythms

when it was shown that animals bearing complete SCN lesions continue to show food anticipatory behavior with a circadian period.⁷³ That finding set off a hunt that continues to this day for the locus (loci) of a food entrainable extra-SCN circadian clock. The question of whether a food entrainable clock lies in a specific locus or is comprised of a network of cells and circuits is a debate with some heat.^{74,75}

Wherever the food entrainable clock(s) is located, important work has been done on the role of food-related stimuli in setting oscillator phase of bodily tissues. If food is provided during the rodent's inactive phase during the day, this will produce a phase shift in peripheral, but not in SCN, oscillators, reflected in measures of *Per* and other clock genes. The overall conclusion is that in the normal situation (with food available at the animal's active period at night), the SCN signals time to eat, and food-derived signals produced by eating in turn set oscillator phase in peripheral tissues, including liver, heart, and lungs. In the experimental condition in which food is available only during the inactive period, the food-derived signal still sets the phase of peripheral oscillators, while the SCN continues to synchronize to the time indicated by the environmental light/dark cycle. For these reasons, the SCN can be viewed as a light-entrainable oscillator (LEO) and the peripheral oscillators as food entrainable oscillators (FEO; FIG. 5).

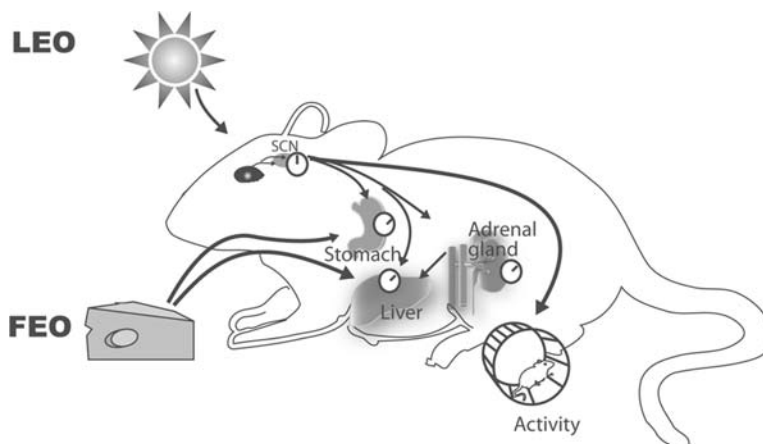


FIGURE 5. Relationship of food entrained oscillator (FEO) to the light entrained oscillator (LEO). Photic information is communicated directly to the SCN via the retinohypothalamic tract. Output signals from the SCN set the phase of extra-SCN oscillators in the brain and, indirectly, in the rest of the body. The SCN determines meal time, and the metabolic signals associated with eating set the phase of bodily oscillators. Under normal circumstances, the signals that derive from the light:dark cycle and those from metabolic cues consequent to eating are in synchrony. If on the other hand, food is available at a time of day when the animal is normally asleep, then food-related cues entrain FEOs, which then enable the animal to predict the timing of meals (measured food anticipatory locomotor activity or elevated body temperature).

Relationship of FEO to LEO

Most of the work on homeostatic factors associated with restricted feeding has been done on nocturnal rodents, although it is obvious that the role of arousing stimuli associated with circadian and homeostatic pressures must differ in phase between diurnal and nocturnal animals. Homeostatic pressures related to eating are highest during the active portion of the day, and this occurs during the dark for nocturnal animals and during the day for diurnal animals. The SCN, however, cycles similarly in nocturnal and diurnal species (FIG. 1). Thus, metabolic and electrical activity, peak and trough phases of clock gene expression, and timing of melatonin secretion is similar in both.^{76,77} This can be understood by the fact that the phase of SCN rhythms are entrained to the light/dark cycle and set by light.⁷⁸ Interesting observations on the importance of arousal emerges from a consideration of the similarities and differences between nocturnal and diurnal animals.⁷⁹

Timing of sensitivity to light is similarly restricted to the nighttime in diurnal and nocturnal species. In contrast, arousal-dependent non-photoc factors, such as activity, or serotonin (levels are activity dependent) induce photic resetting at opposite phases, that is at night in diurnal and during the day in nocturnal animals. In the present context, the importance of this observation is that arousal dependent nonphotoc cues can reset the clock providing a mechanism whereby cues from the body's internal milieu can interact with the circadian system.

Homeostatic pressures, such as those produced by reduced energy reserves, however, are not SCN-regulated nor do such homeostatic cues affect the phase of SCN rhythms. To explore the mechanisms underlying arousal in response to homeostatic food-related pressures, we designed a study in which arousal-related cues produced by the circadian system and the homeostatic effects consequent to depleted energy stores were pitted against each other.⁸⁰ We measured the neuronal activation in 16 different brain regions that have been implicated in food anticipatory activity (FAA) or in eating, using c-fos immunocytochemistry to determine which neurons were the first to be activated in mice exhibiting arousal in anticipation of feeding restricted to their inactive phase. The results indicate that the number of c-FOS-positive neurons was significantly increased in only one of the brain regions studied—the ventromedial nucleus of the hypothalamus (VMH). The activation of VMH neurons thus coincides with the earliest signs of behavioral arousal associated with a change in meal time, leading to the conclusion that VMH activation is involved in the increased arousal

in anticipation of food. In the future, studies directed at exploring the influence of such restrictions of food availability at controlled circadian times can be used to examine the changes in gene expression produced by such homeostatic pressures as has been done for sleep.⁵⁵ It will be interesting to understand the differences in gene expression resulting from such pressures in liver and in brain and consequent to sleep versus food deprivation.

Convergence of Homeostatic and Circadian Regulation

The idea that eating and arousal are related is known—at some level—to all who eat well. The legendary French gastronome Brillat-Savarin noted in his book on the Physiology of Taste (1848) that

Celui qui a besoin de manger ne peut pas dormir; les angoisses de son estomac le tiennent dans un réveil douloureux, et si la faiblesse et l'épuisement le forcent à s'assoupir, ce sommeil est léger, inquiet et interrompu. Celui qui, au contraire, a passé dans son repas les bornes de la discrétion, tombe immédiatement dans le sommeil absolu

We translate this as follows: “A person who is hungry can not sleep; the anguish of the stomach keeps him painfully awake, and if weakness and exhaustion force drowsiness, this sleep is light, worrisome, and interrupted. On the other hand, one who has exceeded the boundaries of discretion in eating, falls immediately asleep.”

It is always gratifying when intuition and experience map onto physiological mechanisms, and here we have a “hit”. The convergence of circadian and sleep regulatory mechanisms on hypocretin represents one of the major breakthroughs of the past decade and provides a link between arousal/sleep and eating. Hypocretin deficiency causes narcolepsy, which is associated with excessive daytime sleepiness, difficulty in maintaining wakefulness, and cataplexy or emotional excitation-induced muscle atonia.^{81,82} The identification of the hypocretin system has uncovered a mechanistic relationship between the circadian and homeostatic systems on the one hand and on the relationship between arousal and eating on the other. Hypocretin injected in the cerebral ventricles stimulates appetite.⁸³ At about the same time, it was reported that orexin (subsequently shown to be the same peptide as hypocretin) has excitatory activity in hypothalamic neurons.⁸⁴ Then, after decades of studies, a mutation in the hypocretin receptor 2 gene was found to be the basis of narcolepsy.^{81,85} These results uncovered a physiologic link between food-motivated behavior and the regulation of arousal/sleep. Hypocretin-1 neuron projections stimulate arousal while antagoniz-

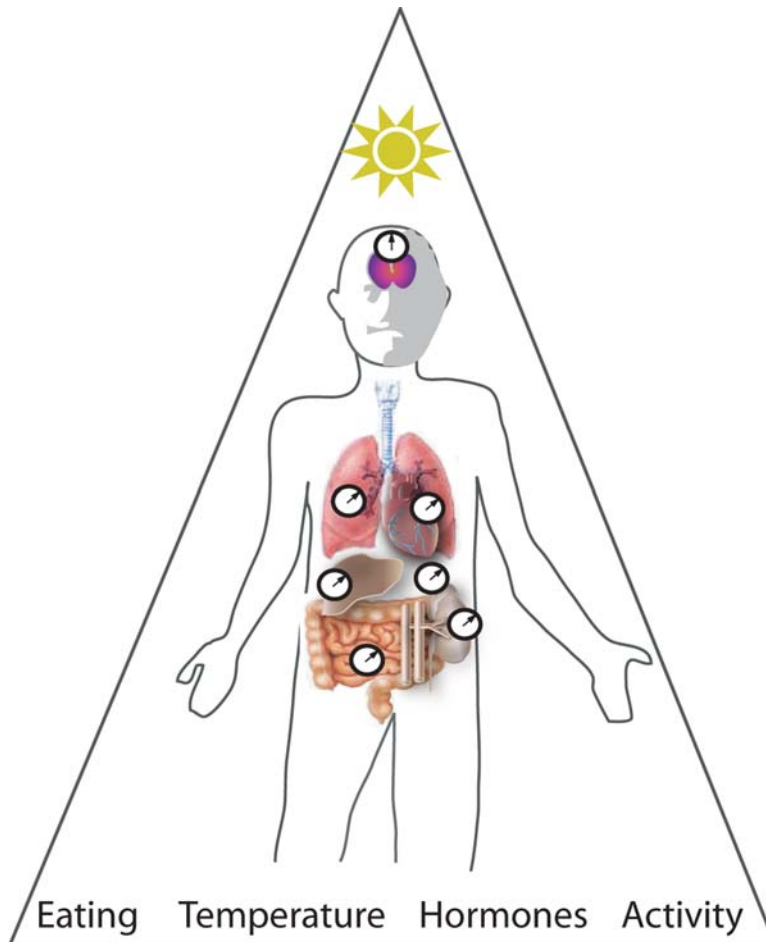


FIGURE 6. The hierarchical organization of the circadian system. The circadian system is a hierarchy of similar cellular clock elements. The master clock of the SCN achieves the “master” role by virtue of the nucleus’s internal circuitry and its ability to send timing signals to the rest of the brain. The SCN itself is entrained to solar time and also receives information from arousal-related activity of the body. Thus, the SCN integrates external and internal information and coordinates the rhythmic activities of peripheral oscillators to produce coherent timing in behavioral, autonomic, and endocrine functions.

ing sleep and muscle atonia.⁸⁶ Direct stimulation of hypocretin cells promote a transition from sleep to wakefulness.⁸⁷ Both circadian and homeostatic factors regulate the rate of hypocretin release with peak cerebro-spinal fluid (CSF) hypocretin-1 occurring at the end of the active period.^{88,89} In SCN-lesioned animals, locomotor activity, body temperature, and CSF hypocretin-1 levels are arrhythmic. Impressively, significant correlations were observed between hypocretin-1 and physical activity both across and within animals. In summary, hypocretin-1 release is under SCN control and is also affected by arousal-related locomotor activity.

Conclusions: Integration of Internal Milieu and External World

The circadian system is made up of a hierarchy of oscillating elements with feedback among levels of organization (FIG. 6). In discussing this system, we have come full circle in discussing the relationship between arousal and the circadian system. We started with the observation that the circadian timing system influences each of the aspects of arousal delineated in Pfaff’s general model.² The fact that the SCN cycles in the same phase in diurnal and nocturnal animals has long been viewed as a problem in discovering the site of signal

inversion of the SCN output signal. If, however, one considers the homeostatic and circadian factors influencing behavior as acting in a coordinate manner, then the nature of the problem can be conceptualized as follows: Arousal cues are derived from the interaction of a battery of factors related to emotions, learning, homeostatic (feeding, temperature, sensory stimuli), and circadian controls. These may act additively, synergistically, or in a mutually inhibitory fashion, as in the two-process models discussed above. Either way, arousal occurs when cues from locomotor activity and from eating and sleep converge appropriately. This system operates with feedback. Arousal itself acts back to modify the photic cues that set the phase of the circadian timing system. The system is flexible, as demonstrated in species that switch between diurnal and nocturnal patterns of activity in response to changes in photoperiod,⁹⁰ changes in hormonal milieu,⁹¹ or even access to activity wheels.⁹² This is a beautifully engineered system where homeostatic and circadian influences at multiple levels are integrated to permit optimal integration of mediators in the internal milieu and external world.

Conflicts of Interest

The authors declare no conflicts of interest.

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