In: **Basics of Sleep Guide**, M. Opp, Ed., Sleep Research Society, 2005. pp. 131-138.

Chapter 14

Fundamentals of the Circadian System

Martha U. Gillette and Sabra M. Abbott
University of Illinois at Urbana-Champaign

Key Concepts

- Circadian rhythms are the near 24 hour oscillations in behaviors that are found in all organisms.

- In mammals, circadian rhythms are regulated by the suprachiasmatic nucleus (SCN) – a set of paired nuclei in the hypothalamus, directly above the optic chiasm, the position for which they are named.

- The SCN receives glutamatergic input from the eyes, serotonergic input from the midbrain, and cholinergic input from the basal forebrain and brain stem.

- Circadian outputs include projections to arousal centers in the hypothalamus as well as humoral outputs, such as transforming growth factor-α (TGF-α and prokineticin 2 (PK2).

- A 24-hour cycle is maintained in the SCN by a transcription-translation feedback loop consisting of positive elements (*Clock* and *Bmal1*), negative elements (*Period*, *Cryptochrome,* and *Timeless*) and regulatory elements (*Casein kinase I epsilon*, CKIε).

- Animal models, including the genetically tractable fruit fly and various rodent species, have contributed important insights in understanding circadian rhythms.

- According to Borbély's two-process model of sleep regulation, the circadian and homeostatic systems interact to provide consolidated bouts of wakefulness and rest.

Circadian Fundamentals

Organisms exhibit daily variations in a variety of behaviors, which collectively are referred to as the organism's circadian rhythms. In humans, these rhythms extend through multiple levels: 1) general states, such as the daily alternation in sleep and wakefulness, 2) behaviors, such as eating and walking, 3) physiology, such as body temperature, heart rate, or muscle strength, 4) endocrine profiles, such as secretion of cortisol or growth hormone, and 5) tissue/cellular oscillations, such as transcription/translation of liver enzymes. These oscillations in fundamental body processes underlie the predisposition of acute disease states at certain times-of-day, such as the occurrence of heart attacks peaking near dawn.

The cyclic organization of behavior can be observed in the patterning of wheel-running activity of rodent models (Fig. 1). Figure 1A depicts the activity of a nocturnal animal under conditions where the lights are on for 12 hours and off for 12 hours. This phenomenon is called entrainment, in which animals express activity with a fixed phase relationship to environmental conditions.

In the absence of exogenous timing cues, the organism expresses an endogenous rhythm that varies slightly from 24 hours, making it circadian, or 'about a day.' Unperturbed, this circadian rhythm persists, as can be seen in Figure 1B. Due to the slight deviation of the period of the rhythm from 24 hours under these constant environmental conditions, the onset of activity drifts from its original position by a small but constant amount each day. In an aperiodic environment, the animal's activity eventually would become completely out of phase with the environment and then pass back into phase, proceeding at a regular interval with each circadian cycle. This demonstrates that the persistence of rhythmicity is not simply an after-effect of the previous environmental conditions, but rather that an endogenous rhythm is present.

This persistent endogenous rhythm is the expression of an internal circadian clock that keeps time without external timing signals and, in turn, times circadian rhythms in brain and body functions so that oscillations occur with a predictable phase relationship to each other. For example, the circadian clock differentially influences the levels of hormones, so that plasma corticosterone shows a peak shortly before waking, whereas plasma melatonin normally peaks shortly after sleep onset and appears to play a role in regulation of the sleep-wake cycle. Circadian rhythms can also be observed in plasma glucose, with levels rising shortly before the onset of activity. This rise is independent of daily food intake, although feeding and drinking behaviors are themselves circadian rhythms.

A characteristic of circadian clocks is their ability to be reset by environmental signals. The most prominent of these signals is light. When an animal in constant conditions is presented with a brief (~15 min) pulse of light, the response of the animal depends upon the time of light exposure (Figure 1C-E). During the subjective daytime, light has little effect. However, light exposure during subjective early evening delays the onset of activity on the following cycle, and subsequent rhythms continue from this new phase. On the other hand, light exposure during the subjective late night will advance the onset of activity on the following day. A plot called a *phase response curve* (PRC) can be constructed demonstrating the observed circadian response following treatments at various phases of the day/night cycle, as seen in Figure 1F. The PRC to light is similar for both night-active and day-active organisms, such as humans.

The Suprachiasmatic Nucleus

In mammals, circadian rhythms are regulated by a paired brain nucleus located at the base of the hypothalamus, directly above the optic chiasm, hence the name – the suprachiasmatic nucleus (SCN). Multiple experiments have demonstrated the role of the SCN as a central pacemaker for circadian rhythms. Lesioning studies found that selectively damaging the SCN disrupts rhythmicity in corticosterone levels, drinking activity, and wheel-running behavior. This provided the initial evidence that the central pacemaker for the mammalian circadian clock lay within the SCN.

In later work, it was found that transplanting fetal SCN tissue into the third ventricle of animals in which the SCN had been lesioned could restore rhythmicity. Furthermore, if fetal SCN tissue from a wild-type hamster was implanted into a hamster with a genetic alteration that shortened the free-running period, the new free-running period resembled that of the SCN donor rather than the host animal. This evidence suggested that not only was the SCN necessary for generating rhythms, but also that this rhythmicity was an intrinsic property of the SCN cells, which could drive the rhythms of the entire animal.

Based on peptide localization, it is common to divide the SCN into a ventrolateral or 'core' region, and a dorsomedial or 'shell' region. The core neurons are smaller (~30 μm^2) and contain vasoactive intestinal peptide (VIP), while the shell neurons are larger (~45 μm^2) and contain arginine vasopressin (AVP). These divisions appear to be conserved across species, being observed from rodents to humans. Recent studies looking at gene expression in the core and shell suggest that endogenous rhythms are controlled by neurons in the shell, while resetting of circadian rhythms is regulated by neurons in the core.

Neuroanatomical Connections

Inputs

In conjunction with its ability to regulate circadian timing, the SCN is also positioned to receive information about the behavioral and environmental state of the

animal in order to ensure proper setting of the circadian clock. This information is conveyed to the SCN by projections from a variety of different brain regions.

One of the most extensively studied inputs to the SCN comes from subpopulation of retinal ganglion cells whose central projections form the retinohypothalamic tract (RHT). Lesions of the SCN disrupt the development of these neurons and disruption of the RHT results in an inability to respond to resetting light signals. Recent work has found that many of the retinal ganglion cells that comprise the RHT contain a photopigment, melanopsin. These melanopsin-containing cells are photosensitive at the same wavelengths that are most effective for circadian resetting. Additionally, the terminals of the melanopsin-positive retinal ganglion cells collocalize glutamate and pituitary adenylate cyclase-activating polypeptide (PACAP), the putative neurotransmitters of the RHT. Some visually blind humans, who lack awareness of shape, movement or color, do have functional RHTs, enabling them to entrain to day-night cycles.

The RHT also sends projections to the thalamic intergeniculate leaflet (IGL), which in turn sends projections to the SCN through the geniculohypothalamic tract (GHT). The GHT contains neuropeptide Y (NPY) and GABA. NPY is believed to be involved in activity-induced phase shifts during the daytime in nocturnal animals, but also appears to be able to modulate light-induced change in the phase of circadian rhythms. However, while the GHT pathway can transmit photic signals, disruption of this pathway does not prevent entrainment.

While the pineal does not send neuronal projections to the SCN, it exerts humoral influence on the SCN through its production of melatonin. In lower vertebrates, such as fish and some birds, the pineal is actually the primary regulator of circadian rhythms, rather than the SCN. However, in mammals this timekeeping mechanism is consolidated within the SCN, as demonstrated by the fact that removal of the pineal does not significantly disrupt circadian rhythms. While melatonin production by the pineal is not necessary for maintenance of endogenous mammalian circadian rhythms, melatonin is produced at night in the absence of light, providing a means by which the animal can measure night-length. Photoperiod is an important measure for animals, such as hamsters and sheep that are seasonal reproducers.

The SCN also receives serotonergic input, primarily from the median nucleus of the raphe (MnR) in the midbrain. Activation of the median raphe results in an increase in serotonin (5-HT) release at the SCN. 5-HT also shows a strong circadian release pattern in the SCN, with levels of 5-HT and 5-hydroxyindole acetic acid (5-HIAA), the major metabolite of 5-HT, peaking in the middle of the night.

Cholinergic projections to the SCN originate both in the brainstem and basal forebrain in brain nuclei with identified roles in sleep and arousal. Within the brainstem, the parabigeminal nucleus (PBg) is considered a satellite region of the superior colliculus, which appears to play a role in generating target location information as part of saccadic eye-movements, while the laterodorsal tegemental (LDTg) and pedunculopontine tegmental (PPTg) nuclei both are important for regulating the sleep-wake cycle. In the basal forebrain, the substantia innominata (SI) within the nucleus basalis magnocellularis (NBM) contributes to arousal and focused attention. This would suggest that the cholinergic input to the SCN is providing a signal regarding the sleep and arousal states of the animal, and may provide a link between the sleep-wake cycle and circadian rhythms.

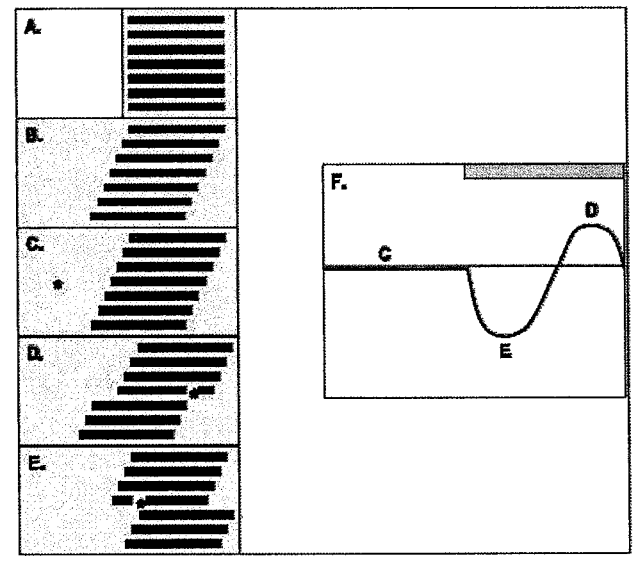

Figure 1. Organization of circadian rhythms of locomotor activity and its regulation by light. Panels A-E are schematic actograms from a nocturnal rodent. Black bars in all figures indicate time when the animal is active measured as running-wheel activity. Gray areas indicate time when the animal is in darkness. Black bars indicate running-wheel activity. A. depicts an animal that has entrained to a light-dark cycle. Activity occurs with a regular 24-h cycle, and primarily during darkness. B. represents the same animal placed under constant conditions, showing a free-running period (*tau*) of less than 24 h. Activity begins slightly earlier each day. C. represents the effect on phase of activity onset in an animal presented with a light pulse (*) during the subjective daytime. No change is observed in the onset of activity on the following day. D. shows the response to a light pulse (*) during the subjective early evening. Activity onset is delayed on the following cycles. E. shows the response to a light pulse (*) during the subjective late evening. The onset of activity occurs earlier than predicted on the following cycles. F. is a *phase response curve* (PRC) for the response to light presented at various circadian phases for animals in constant darkness. The gray bar represents subjective night, and deflection above or below the line represents advances or delays, respectively. Times of treatment for the representative actograms (left panel) are represented by the corresponding letters on the graph. Neuroendocrine Correlates of Sleep/Wakefulness, Cardinali, Daniel P.; Pandi-Perumal, S. R. (Eds.), 2005, 413 p. 110 illus., Hardcover, ISBN: 0-387-23641-4, In press.

Outputs

The SCN exerts its influence on the rest of the body primarily by sending projections to the rest of the hypothalamus, including the subparaventricular zone (sPVHz), ventral tuberal area (VTU), medial preoptic area (MPOA), and the dorsal medial hypothalamus (DMH). The DMH projections are particularly interesting, as many of these neurons appear to be projecting to neurons containing hypocretin/orexin, a peptide well-known for its role in arousal. Also, evidence exists for a multi-synaptic pathway between the SCN and locus coeruleus (LC), an important arousal center in the brain, with the DMH as a relay. The SCN also contains a minor set of efferents to the ventrolateral preoptic nucleus (VLPO), a region which, if lesioned, produces prolonged reduction in sleep duration and amplitude. In addition, the SCN contains projections to the paraventricular nucleus (PVN) and the intergeniculate leaflet (IGL) of the thalamus. The targets of these efferents consist of either endocrine neurons, autonomic neurons, or intermediate neurons that potentially serve to integrate a number of hypothalamic signals. Overall, the SCN appears to be uniquely situated within a network that allows it to interact closely with the regions controlling sleep and arousal states.

One of the major outputs of the SCN appears to be a signal inhibitory for activity. Two recently discovered candidate factors for communicating such a signal include transforming growth factor-α (TGF-α) and prokineticin 2 (PK2). Under normal conditions, TGF-α is expressed rhythmically in the SCN, with a peak during the animal's inactive period and a trough during the active period. When infused continuously into the ventricles, TGF-α inhibits locomotor activity. Conversely, mice lacking the epidermal growth factor (EGF) receptor, making them unable to respond to TGF-α, show an excessive amount of daytime activity. PK2 also is expressed rhythmically in the SCN, again showing peak expression during the animal's inactive period, and it inhibits locomotor activity when infused continuously. This suggests a role for output signals of the SCN in promoting an inactive state that would be permissive for sleep.

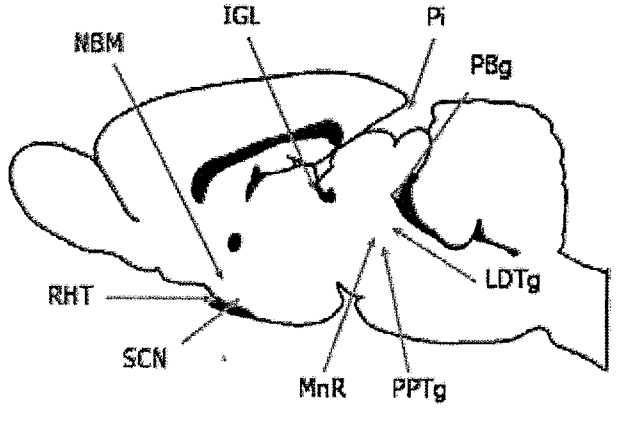

Figure 2. Drawing of a sagittal section through a rat brain demonstrating the location of neuroanatomical projections to the suprachiasmatic nucleus. IGL intergeniculate leaflet, LDTg laterodorsal tegmental nucleus, MnR median raphe, NBM nucleus basalis magnocellularis, PBg parabigeminal nucleus, Pi pineal gland, PPTg pedunculopontine tegemental nucleus, RHT retinohypothalamic tract, SCN suprachiasmatic nucleus

Molecular Biology of Circadian Rhythms

Transcription-Translation Feedback Loop

Much research effort has focused on determining how a biological system keeps 24-hour time. With the discovery that single, dispersed cells can exhibit circadian rhythms, focus turned to understanding cellular processes that generate a near 24-hour timebase. A molecular clockwork appears to generate a ~24-hour rhythm through a feedback cycle involving a set of core clock genes, their mRNAs and proteins. Together they form the molecular clockwork. This cycle consists of a set of interconnected positive and negative feedback loops, and their regulatory elements. Positive elements, which include *Clock* and *Bmal1,* are transcribed into mRNA, which is then translated into proteins that heterodimerize and are translocated into the nucleus. In the nucleus, they activate continued transcription of their own genes, as well as activating transcription of negative elements. The negative elements, which include *Period*, *Cryptochrome,* and *Timeless*, are then transcribed and translated. Proteins of the negative elements also associate in complexes and are translocated to the nucleus, where they feed back to inhibit transcription of the positive elements. These feedback loops are further affected by regulatory enzymes, including casein kinase 1 epsilon (CKIε). The cycle of these feedback loops takes approximately 24-hours to complete, providing a means by which cells within the SCN can maintain a circadian rhythm.

Core clock elements have been found to play a critical role in human sleep disorders. For example, an inherited form of advanced sleep phase syndrome (ASPS) has been associated with a mutation in the *Per2* gene that interferes with a normal phosphorylation site of CKIε. This shortens the cycle through the transcription-translation feedback loop, resulting in a shortened free-running circadian clock in individuals with this form of ASPS. The mechanisms responsible for delayed sleep phase syndrome (DSPS) are less well-known at this point, but also have been linked in some families to gene mutations that interfere with a phosphorylation event by CKIε. Finally, morningness/eveningness phenotypes have been found associated with genetic variations in *Clock.*

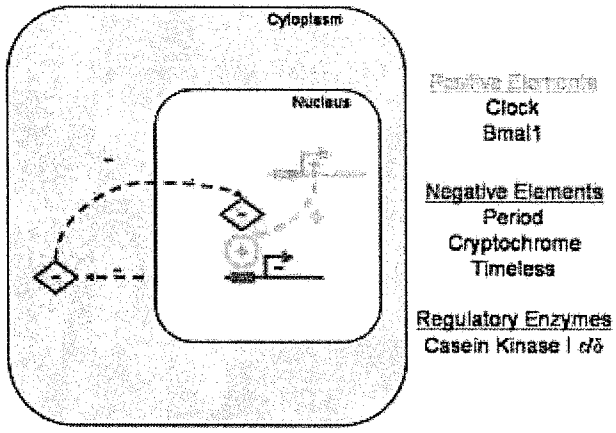

Figure 3. Schematic of the circadian transcription-translation feedback loop within a cell. Positive elements (+) activate the transcription of negative elements (-), which in turn feedback to inhibit their own transcription. Regulatory elements interact with negative and positive elements to produce a near 24-hour cycle. Although one of each element is presented for simplicity, several elements exist as multiple genes in mammals.

Animal Models

Drosophila

The fruit fly, *Drosophila,* is a powerful model for analyzing the genes that underlie behaviors. The first clock genes were discovered by mutagenizing flies and screening them for altered rhythms. The genes discovered in this way in flies have proven to be present in humans, as well, and when altered cause rhythm disturbances with clinical consequences (see above). Recently, *Drosophila* have been found to be useful for sleep studies, as well. *Drosophila* have been shown to exhibit key characteristics of sleep, including prolonged periods of quiescence, during which there is a decreased responsiveness to external stimuli, and homeostatic regulation of these periods.

With the discovery that *Drosophila* do indeed sleep, interactions between the circadian and sleep-wake cycles are beginning to be probed. Recent studies examining the effect of sleep deprivation on wild type flies and those exhibiting clock-gene mutations demonstrate that homeostatic recovery of lost sleep is often disrupted in the absence of a fully functional circadian clock. After undergoing sleep deprivation, wild type flies recover 30-40% of their lost sleep, while flies lacking either *Period*, *Clock* or *Timeless* show a slightly exaggerated sleep rebound following sleep deprivation, recovering close to 100% of their lost sleep. Flies lacking *Cycle* (homologous to *Bmal1*), show even more of an exaggerated response, recovering three times as much sleep as they lost. In addition, the flies that lack *Cycle* begin to die after 10 h of sleep deprivation, a phenomenon observed rarely if at all in wild-type flies. This result seems to be linked to a failure to activate heat-shock genes, a type of stress response, following sleep loss. This suggests a possible genetic link between the circadian and homeostatic drives for sleep. *Drosophila* can be anticipated to continue to be a useful model system for studying these interactions.

Mammals

Nocturnal. While *Drosophila* studies provide useful genetic information, studies of brain organization, physiology and integrative function rely on mammalian models for translation to humans. As a consequence, a large number of circadian studies are done in common laboratory mammals, including mice, rats and hamsters. These animals generally exhibit robust circadian rhythms that can be assessed through wheel-running behavior, telemetric activity measures, neuronal firing rates, and gene expression patterns. With the advent of transgenic technology, mice have become a valuable genetic model for circadian research, as well. It is now possible to observe an animal's circadian phenotype after genetic deletion of key components of the molecular clockwork, as well as to observe protein localization, interaction and expression patterns in transgenic animals containing fluorescently-labeled clock gene products (mRNA or protein).

As with flies, the ability to manipulate the genome allows examination of the role of a specific circadian clock element in the sleep-wake cycle and sleep homeostasis. Studies with transgenic and mutant animals have demonstrated that a disruption in *Clock*, but not the *Period* genes, disrupts sleep homeostasis. Interestingly, mice lacking *Bmal*, the mammalian homolog to *Cycle* in *Drosophila*, do not show the lethal response to sleep deprivation observed in *Drosophila*. While a mainstay for circadian research, nocturnally-active rodents present some difficulties when trying to draw conclusions about circadian rhythms and sleep in diurnal humans.

Diurnal. A diurnal model for circadian rhythms has recently been discovered in the Nile grass rat, *Arvicanthis*. While occasional nocturnal behavior can be elicited by exposure to running wheels, as long as these animals are not provided with running wheels, they maintain a diurnal pattern. *Arvicanthis* exhibits comparable responses to light as are seen with nocturnal rodents, but show differences in responses to GABA, a neurotransmitter often associated with arousal-induced phase shifts. These similarities are understandable, as daylight remains constant regardless of nocturnal or diurnal preference, but the timing of activity and arousal changes. Studies are beginning to be performed examining the molecular clockwork in the SCN of *Arvicanthus*, and so far, the basic core elements of the transcription-translation feedback loop appear to be conserved in this species.

Although SCN of the nocturnal and diurnal mammals are very similar, the major difference between nocturnal and diurnal animals may lie in the brain regions to which the SCN projects, rather than within the SCN itself. This suggests that diurnal models may prove very useful in

studies where the goal is drawing inferences about integrated human circadian rhythms and sleep.

Interactions with the Sleep-Wake Cycle

The two-process model of sleep and circadian interaction

Borbély effectively explained the interaction between the circadian and sleep systems as a two-process model. According to this model, the sleep-wake cycle is regulated by two interacting processes: process S and process C. Process S is related to the homeostatic build-up of sleep debt, resulting in a greater influence of process S the longer one is awake. Process S uses electroencephalographic (EEG) slow-wave activity as a marker. Process C provides a circadian-dependent arousal signal, which, if properly aligned, counteracts the rising homeostatic sleep pressure from process S during the daytime. The converse is true at night, when a declining circadian alerting signal interacts with a decrease in homeostatic sleep pressure. The interaction between these two processes accounts for both the consolidation of sleep and wake under normal conditions, as well as the fact that there is a rhythmic variation in sleep propensity throughout the duration of a period of sleep deprivation. Proper alignment of the sleep and circadian systems contributes importantly to health and well-being.

Sleep and circadian interactions

The interaction between these two processes can be further demonstrated by looking at what happens to one process in the absence of the other. Bilateral SCN lesions have been found to eliminate the circadian rhythm of the sleep-wake cycle. While total time spent in sleep remains the same, the occurrence of sleep becomes randomly distributed between the light and dark phases, rather than being consolidated. However, the ultradian rhythm of slow wave sleep (SWS) cycles remains. Later studies found a slight increase in total SWS and slight decrease in total rapid eye movement (REM) sleep in SCN-lesioned rats.

In order to determine whether the circadian system (process C) influences the homeostatic response (process S), the response of SCN-lesioned animals to periods of sleep deprivation has been examined. While these animals show a homeostatic recovery response to sleep deprivation, they differ in the speed of recovery under different environmental conditions. Animals with an intact SCN placed in an environment free of external circadian cues show the fastest recovery from a period of sleep-deprivation. Animals with SCN lesions or on a regular light-dark cycle require more time than controls to recover the lost sleep. These data suggest that an intact circadian system, without the influence of outside time cues such as light, provides the most efficient means of recovering lost sleep.

Homeostatic sleep drive (Process S) also can influence the circadian system (Process C). If hamsters are sleep-deprived during the latter part of their inactive period, either through exposure to a novel wheel, or by gentle handling, they exhibit a significant advance in onset of wheel-running activity that remains stable as long as they remain in constant conditions. When this sleep-deprivation paradigm is used in mice prior to a light pulse in early night, the phase delay observed is decreased.

Physiological evidence for communication between brain regions regulating sleep-wakefulness and the circadian system can be observed in the correlation between sleep-wake state and neuronal firing rate in the SCN. Early work found that SCN cells undergo changes in firing rate that relate to the arousal state of the animal. More recent studies conducted a detailed analysis of SCN neuronal activity during different sleep stages, and found that the SCN firing rate exhibited a diurnal pattern, but superimposed on it was a change in firing rate dependent on sleep state of the animal. During wake or REM sleep, SCN firing rates were much higher than during NREM sleep. As discussed earlier in this chapter, there are circuits in place for most brain regions that regulate sleep and wakefulness to feed back to the SCN. These studies provide evidence that this feedback actually can influence SCN activity; the functional consequences of this remain to be determined.

Summary

Circadian rhythms describe the patterned occurrence of body processes, such as behavior and physiology, over ~24 hours. In mammals, these rhythms are regulated by the SCN, located in the hypothalamus. The SCN receives input from a variety of brain regions, incorporating information about the animal's lighting conditions, sleep-wake state, and overall environment. The circadian period within the SCN is generated by cell-based transcription-translation feedback loops consisting of positive elements (*Clock* and *Bmal1*), negative elements (*Period*, *Cryptochrome*, and *Timeless*) and regulatory elements (*CKIε*). Many experimental models can be used to examine the effect of disrupting these elements on circadian and sleep properties, including *Drosophila*, nocturnal rodents, and *Arvicanthus*. Through use of these models, it has been established that the circadian cycle plays an essential role in regulating the sleep-wake cycle, and that communication between these two processes is essential for well-being.

Selected Bibliography

Original Articles

Bina, KG, Rusak, B and Semba, K. Localization of cholinergic neurons in the forebrain and brainstem that project to the suprachiasmatic nucleus of the hypothalamus in rat. J Comp Neurol 1993;335:295-307.

Cheng, MY, Bullock, CM, Li, C, Lee, AG, Bermak, JC, Belluzzi, J, Weaver, DR, Leslie, FM and Zhou, Q-Y. Prokineticin 2 transmits the behavioural circadian rhythm of the suprachiasmatic nucleus. Nature 2002;417:405-410.

Daan, S and Pittendrigh, CS. A functional analysis of circadian pacemakers in nocturnal rodents. J Comp Physiol 1976;106:253-266.

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

Dijk, D-J and Czesler, CA. 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 1995;15:3526-3538.

Dudley, TE, Dinardo, LA and Glass, JD. In vivo assessment of the midbrain raphe nuclear regulation of serotonin release in the hamster suprachiasmatic nucleus. J Neurophysiol 1999;81:1469-1477.

Katona, C and Smale, L. Wheel-running rhythms in *Arvicanthis niloticus*. Physiol Behav 1997;61:365-372.

Kramer, A, Yang, F-C, Snodgrass, P, Li, X, Scammell, TE, Davis, FC and Weitz, CJ. Regulation of daily locomotor activity and sleep by hypothalamic EGF receptor signaling. Science 2001;294:2511-2515.

Mistlberger, RE, Bergmann, BM, Waldenar, W and Rechtschaffen, A. Recovery sleep following sleep deprivation in intact and suprachiasmatic nuclei lesioned rats. Sleep 1983;6:217-233.

Moore, RY and Eichler, VB. Loss of a circadian adrenal corticosterone rhythm following suprachiasmatic lesions in the rat. Brain Res 1972;42:201-206.

Ralph, MR, Foster, RG, Davis, FC and Menaker, M. Transplanted suprachiasmatic nucleus determines circadian period. Science 1990;247:975-978.

Shaw, PJ, Tononi, G, Greenspan, RJ and Robinson, DF. Stress response genes protect against lethal effects of sleep deprivation in *Drosophila*. Nature 2002;417:287-291.

Review Articles and Book Chapters

Boivin, DB. Influence of sleep-wake and circadian rhythm disturbances in psychiatric disorders. J Psychiatry Neurosci 2000;25:446-458.

Gillette, MU and Mitchell, JW. Signaling in the suprachiasmatic nucleus: selectively responsive and integrative. Cell Tissue Res 2002;309:99-107.

Lee, HS, Billings, HJ and Lehman, MN. The suprachiasmatic nucleus: a clock of multiple components. J Biol Rhythms 2003;18:435-449.

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

Van Gelder, RN. Recent insights into mammalian circadian rhythms. Sleep 2004;27:166-471.

Acknowledgements

Supported by PHS grants NS35859 (MUG) and NS47802 (SMA).