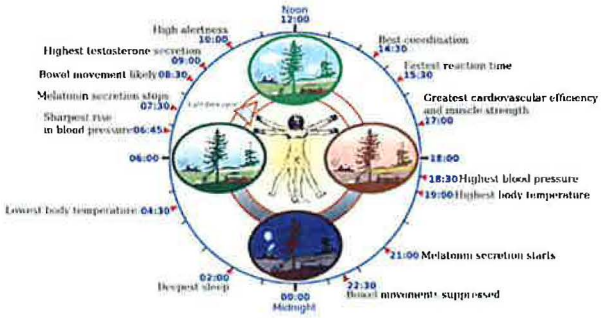
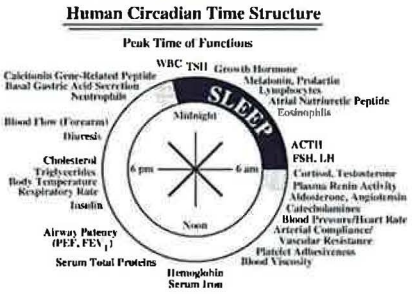
Sleep and Wake: The Circadian Tango of Health





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Internal Medicine Grand Rounds
UT Southwestern Medical Center
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Nilesh B. Dave, MD, MPH has disclosed financial interests or other relationships with commercial concerns related directly or indirectly to this Program. Dr. Dave will not be discussing off-label uses of drugs in his presentation.

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Introduction

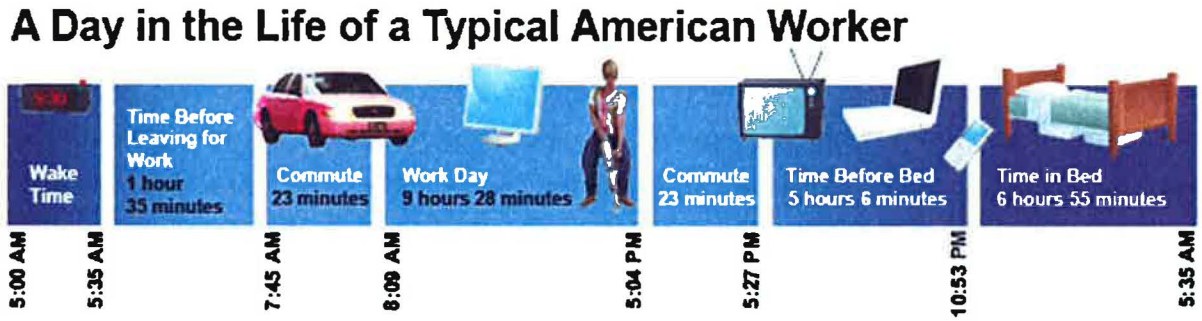
Humans spend approximately one-third of their lifetime sleeping. Given the amount of time occupied by this state, the purpose of sleep is not well understood but it is defined as a reversible state of decreased consciousness. Despite the significant time we spend sleeping, there is inadequate appreciation in clinical medicine and in clinical regarding the pathophysiological changes of a given disease. In fact, no matter what the specialty, there are numerous trials demonstrating no effect of a given intervention. Is it possible that this intervention was timed poorly with respect to the circadian rhythm? Is it possible a drug intervention did not work because it's pharmacokinetics were not timed with the 24 hour peak of its pathophysiologic target?

The practice of medicine is primarily a daytime job. Our approach to therapy is predominantly homeostatic; that is, we focus on managing a patient's problem during their wake ours. We prescribe medications so that they are taken as early as 8:00 AM and as late as 10:00 PM often to maximize therapeutic effect during the day and in other instances, to maximize an adverse effect while asleep.

In this Grand Rounds, I will review the molecular mechanisms that govern our master circadian clock; how this master circadian clock influences peripheral circadian clocks of several organs; highlight several diseases whose pathophysiology and management change with respect to the time of day; and the importance of optimizing disease management and clinical research using a 24 hour clock rather than a 16 hour clock when we are (supposedly) awake and aware of our health.

Clinical Significance

No matter who the patient is--rich or poor, male or female, young or old, having no or many medical problems--he or she can experience a problem with falling asleep, staying asleep, or even struggling to maintain alertness during the day. Like chronic pain, the deleterious effect of poor sleep has no social, economic, cultural, or medical boundaries--it will make anyone miserable. With technological advancement and globalization of commerce and economies, Americans are sleeping far less. The 2008 Sleep in America poll was conducted by the National Sleep Foundation by completing a telephone survey among 1000 Americans and noted a sleep time under 7 hours for working Americans.



The standard 8-hour business day is no longer the norm in America. NSF's 2008 *Sleep in America* poll reports the average American's work day is now 9 hours and 28 minutes. The average time spent in bed is 6 hours and 55 minutes - with 6 hours and 40 minutes spent actually sleeping. NSF recommends getting at least 7 to 9 hours of sleep each night.

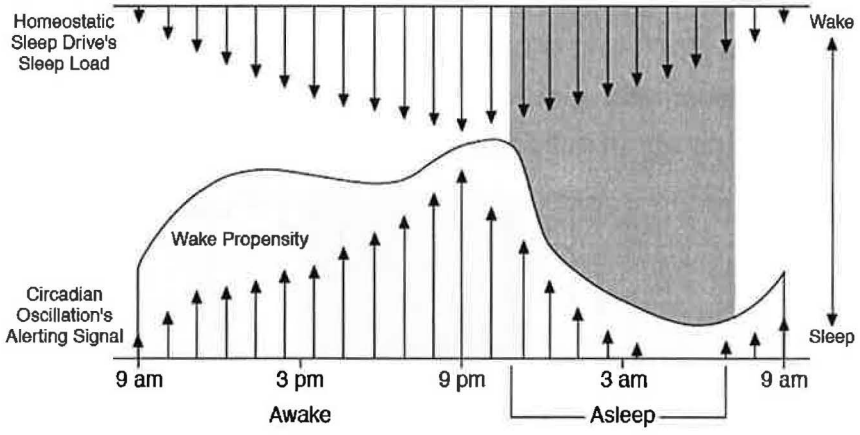
Whether sleep deprivation is from working or due to medical problems, its consequences can alter normal physiology as well as directly affect sleep quality. In fact, various medical conditions have circadian pathophysiology which may respond better to therapy delivered according to the circadian rhythm (chronotherapeutics).

Sleep-Wake Control

Rhythmic physiological processes with a period that is nearly 24 hours are termed circadian rhythms. Sleep-wake control occurs over a 24 hour time period. The two-process of sleep propensity (12) describes the interaction between Process S and Process C. Process S refers to the increasing homeostatic sleep drive/pressure as hours of wakefulness increase and to the change in daytime vigilance due to the synergy with the circadian rhythm, or Process C. In Process S, one factor that may be in-

tricate to this process is adenosine which increases in parallel to Process S. Process C refers to the circadian rhythm (hence the term *circadian* from "circa" meaning "about" and "dies" referring to a "day"). It is thought that this increasing homeostatic sleep pressure is offset by the simultaneous contribution of a diminished circadian sleep drive. Therefore, the drowsiness that occurs with the onset of sleep may be offset by the decreased circadian sleep propensity. The homeostatic sleep drive results in about four hours of sleep and the peaking of the circadian sleep drive adds another 4 hours to help humans achieve eight hours of sleep. From the perspective of sleep hygiene, the importance of waking up at the same time everyday--even on weekends or days off from work--is vitally important in maintaining both processes.

In the diagram below, the peaking of the homeostatic sleep drive and the peaking of wakefulness occur together prior to the onset of sleep. As the homeostatic sleep drive wanes around 2-3:00 AM, the circadian sleep drive peaks resulting in nearly four more hours of sleep. Together, these processes interact to consolidate sleep. Process C sets the daily oscillations in these thresholds and likely helps set the thresholds of the "homeostat" that will lead to the onset of sleep and wakefulness. (41) It should be noted that these two processes are not independent; there is a molecular level of interdependence that is not clearly elucidated. Circadian rhythm disorders, such as delayed-sleep phase syndrome and advanced-sleep phase syndrome, occur when there is a dissociation of the circadian clock from the

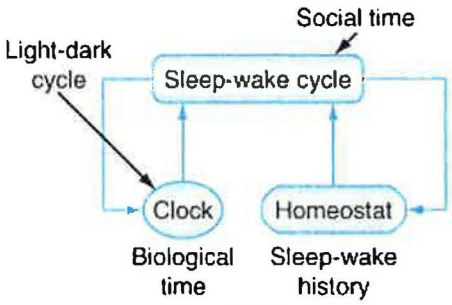


The interaction of Process S and Process C over 24 hours. (65)

chronological clock leading to impaired daytime function and sleepiness outside of the usual sleep time.

Experiments to determine the relative contributions of the homeostatic and circadian sleep processes are challenging. Sleep onset occurs approximately 2 hours

after melatonin secretion; six-seven hours before the nadir of core body temperature; and after being awake for 16 hours. (41) Teasing these factors from each other is challenging. A proposed model of interaction is demonstrated in the figure to the right. The sleep-wake cycle is influenced by homeostatic and circadian factors. External cues such as the light-dark cycle influence the circadian clock whereas social cues help reinforce the sleep-wake cycle. Disturbances in either of these variables will lead to a generalized disturbance in the sleep-wake cycle.

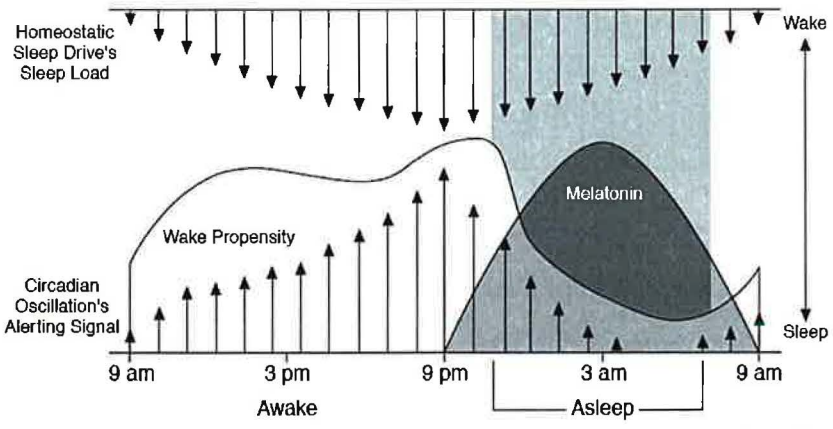


Source: Chapter 34. (41)

Melatonin

Melatonin (N-acetyl-5-methoxytryptamine) is often referred to as the "hormone of darkness." It is the primary product secreted by the pineal gland via autonomic stimulation from the suprachiasmatic nucleus. Melatonin has multiple functions including the regulation of the circadian rhythm and serving as an antioxidant. Exposure to natural light is vital in maintaining melatonin's secretory pattern.

There are four sub-types of the melatonin receptor. Humans have three of them--MT1 and MT2 (located in the CNS) and MT3 which is peripheal--with two subtypes mostly located in the suprachiasmatic nucleus (SCN) of the hypothalamus. (90) Human MT1 receptors inhibit SCN neuronal activity whereas MT2 receptors regulate



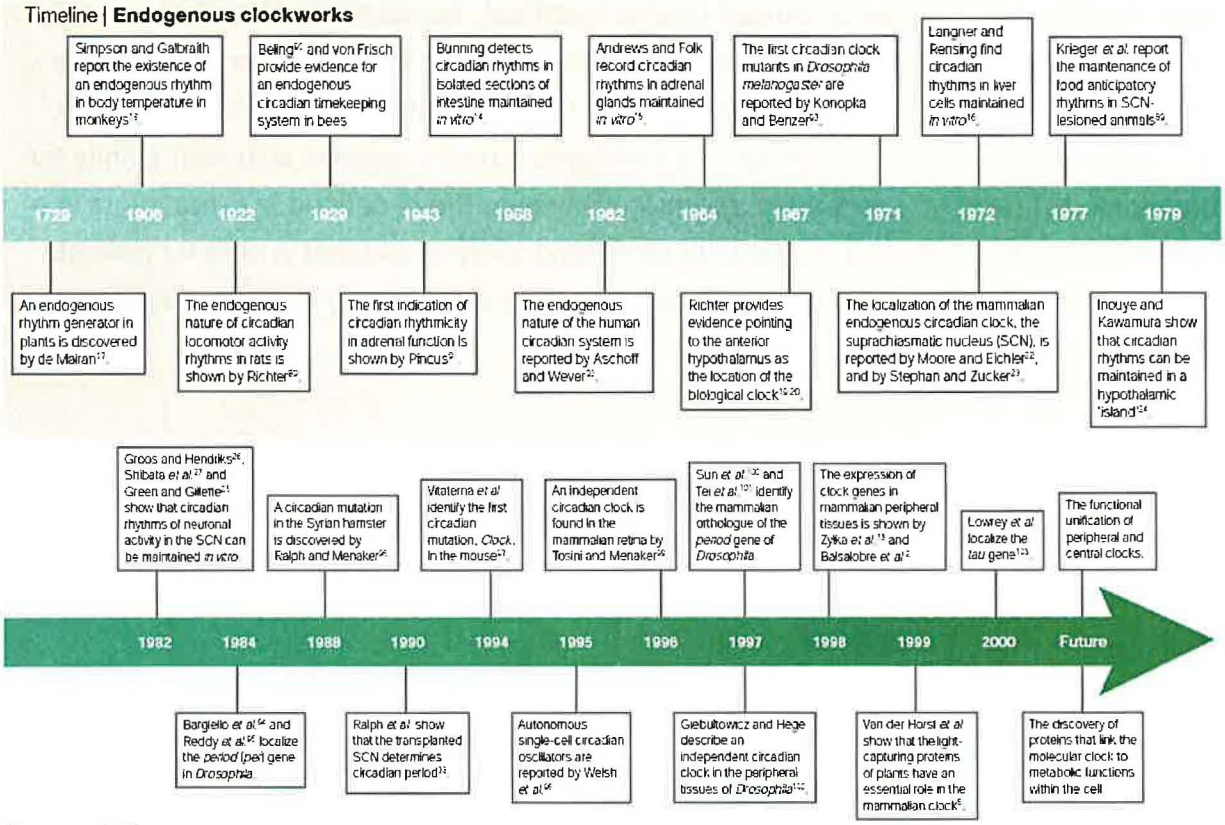
phase shifting. (49). In the diagram below(65), the sudden drop in the circadian alerting

signal occurs with the gradual increase in melatonin which helps to expedite the inhibitory effect on SCN neurons. Ultimately, this interaction facilitates sleep-onset and makes it more predictable.

Interestingly, in patients with comorbid insomnia lasting more than five years, lower peaks of melatonin secretion were observed. (31) The significance of this observation, however, has not been delineated yet. Insomnia can be sub-typed as sleep-onset insomnia and sleep-maintenance insomnia. In a 1996 study, patients with sleep-onset insomnia versus early morning insomnia were more likely to be phase delayed (later bedtime) and phase advanced (earlier bedtime), respectively. (43) The use of timed melatonin to help reverse circadian rhythm changes has not been shown to be effective nor is it a recommended therapy for chronic insomnia due to its lack of FDA regulation. (57) However, ramelteon, a melatonin receptor agonist with high affinity for melatonin MT_1 and MT_2 receptors and selectivity over the ill-defined MT_3 receptors is a non-addictive hypnotic that is helpful in facilitating sleep-onset and is safe for patients with chronic obstructive pulmonary disease. Studies are ongoing regarding the use of ramelteon as a chronobiotic agent.

Master Circadian Clock

The first evidence of a rhythm generator akin to a circadian clock was discovered in plants in 1729. Nearly 200 years later, the discovery of rhythmically controlled temperature changes was reported in 1906 by Simpson. Then, in 1972, Moore and Eichler discovered that the mammalian circadian clock resided within the suprachiasmatic nucleus. Definitive evidence that the suprachiasmatic nuclei are indeed the central clocks occurred when transplantation experiments successfully demonstrated SCN lesioned animals having the unique circadian rhythm of the transplanted SCN. (62)



Source: (16)

Circadian pacemakers have many defining characteristics. Three of these include an endogenous and autonomous rhythmicity that is resilient to periodic changes in the external environment; a period that is very close to 24 hours ; and the ability of the endogenous rhythm to be reset or changed by environmental inputs. (41)

Light as the major *zeitgeiber* of the circadian rhythm

External entrainment of the circadian rhythm is heavily dependent on exposure to daylight, an important zeitgeiber. "Zeitgeiber" is the term reserved for the external environmental clues we use to help us adapt to each day's 24-hour time progression. Zeitgeibers include the rising and setting of the sun, timing of meals, activities of people around us, and various social activities. Individuals who have lost their vision often experience difficulty in adapting to the constraints of the 24-hour daily cycle; this reinforces the importance of visual cues in establishing the internal clock.

Exposure to (blue) light at the wavelength of 446-477 nanometers (nm) and of sufficient brilliance (far more than indoor flourescent lights) that leads to the cessation of melatonin secretion. For visual acuity, the eye's rods and cones process light differently via rhodopsin to ultimately send signals along the optic nerve to the optic chiasm and ultimately to the visual cortex of the occipital lobe. It is worth noting that full-spectrum, bright light is not efficient in stimulating the SCN. This light is often intolerable to the rods and cones leading to headaches and eye strain. (Offices typically have 400 lux light; TV studios 1000 lux; smartphone LCD screen (20-100 lux) and a bright sunny Dallas day, >10,000 lux.) Blue light between 446-477 nm is far more efficient and requires about 1/10th the brilliance of full spectrum light. Full-spectrum light with only 250 lux has been shown to reduce melatonin secretion significantly. (83) Patients with seasonal affect disorder (SAD) have fewer melanopsin-containing retinal ganglion cells and usually respond to properly timed and dosed light therapy. (26) (81) It is not known whether light therapy alone is superior to pharmacologic treatment of SAD.

Light entering the eye takes a different pathway to entrain the circadian rhythm. This

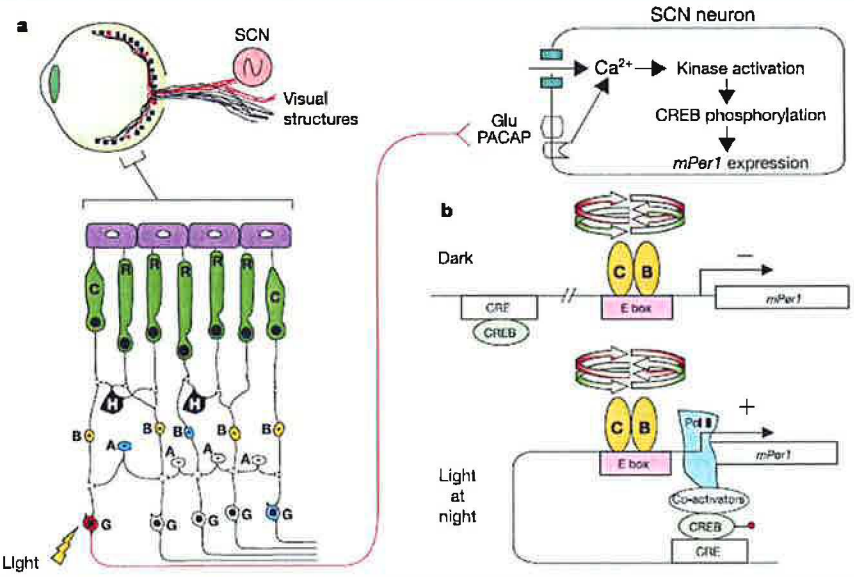


Figure 4 New visual pathway from retina to the SCN. **a**, A small population of widely dispersed, melanopsin-positive ganglion cells (red) form the retinohypothalamic tract that projects to the SCN. These ganglion cells (G) are directly light responsive. They also receive input from rods (R) and cones (C) through bipolar (B) and amacrine cells (A), some of which may contain crytochromes (blue). The precise anatomy of inputs to the melanopsin-positive neurons remains to be established. Glutamate (Glu) and PACAP mediate light effects on the *mPer* genes in SCN neurons. **b**, Light at night activates *mPer1* expression through phosphorylated CREB, independent of CLOCK–BMAL1 E-box control. Red and green arrows indicate interacting negative and positive loops of the clockwork, respectively. **Source:** (64)

pathway is called the retinohypothalamic tract (RHT). The RHT is both necessary and sufficient for entrainment of the circadian pacemaker by light. This tract terminates primarily in the SCN but also has projections to other components of the hypothalamus that are important in the circadian system. Melanopsin is the photopigment contained in the small subset of retinal ganglion cells that form the RHT. Melanopsin is very sensitive to changes in the luminance of natural daylight.

Circadian clock anatomy and molecular function

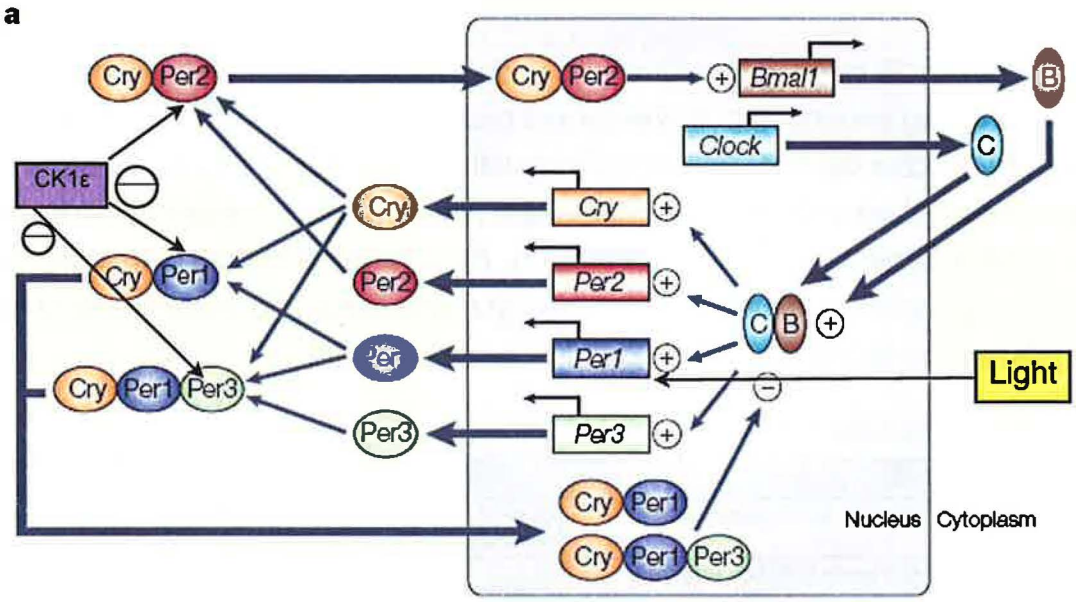
The human circadian clock or rhythm generator resides within the bilateral suprachiasmatic nuclei of the hypothalamus located above the optic chiasm. The SCN have a core and a shell whose nuclei communicate with a handful of neuropeptides. The SCN shell primarily utilizes arginine vasopressin-positive (AV) neurons whereas the SCN core uses vasoactive intestinal peptide (VIP) and gastrin-releasing peptide-positive neurons. Efferent neuronal projections of the SCN primarily populate the core. The shell is responsible for the spontaneous rhythmicity of neuronal activity and the secre-

tion and expression of the *Per* gene and c-Fos. These circadian genes will be discussed in more detail in the next section.

In the last 25 years, our mechanistic understanding of the mammalian circadian rhythm has grown enormously. In the current molecular model of the circadian clock, its maintenance occurs via a transcriptional-translational auto-regulatory positive and negative feedback loop (33) involving the major (known), human circadian clock genes: *Clock*, *BMAL1* (brain and muscle ARNT-like 1), *Per1/Per2/Per3* (3 period genes), *Cry1* and *Cry2* (cryptochrome genes that are blue-light responsive and found in retinal ganglion cells and the SCN), and *CK1ε* (casein kinase 1 epsilon).

Sources: (58), tablet adapted from (41)

Step	Action	Effect
1	CLOCK and BMAL1 form protein heterodimers.	CLOCK is phosphorylated and CLOCK:BMAL1 heterodimers enter the nucleus as a key transcription factor.
2	CLOCK:BMAL1 binds to E-box enhancer elements of *Per*s, *Cry*s, and *Rev-Erbα*. (Light can independently activate *Per1*)	Positive and rhythmic drive of expression of these circadian genes.
3	PER and CRY mRNAs are translated to proteins that form homodimers and heterodimers in the cytoplasm.	CRY:CRY and CRY:PER(1/2/3); dimers enter the nucleus.
4	PER is phosphorylated by CK1ε.	PER1/2/3 proteins are degraded & nuclear accumulation is slowed.
5	PER:CRY and CRY:CRY represses CLOCK:BMAL1-driven transcription of *Per*1/2/3 and *Cry*1 & 2.	Negative feedback of PER and CRY proteins on their own transcription.
6	PER:CRY represses *Rev-Erbα* transcription.	*Rev-Erbα* is regulated by negative feedback of PER:CRY, resulting in a similar oscillation in expression.
7	REV-ERBα enhances *Bmal1* and represses *Cry1* transcription.	REV-ERBα modulation of transcription produces different phases in expression of *Bmal1* and *Cry1*.
8	CRY proteins inhibit H3 histone acetylation by P300.	CRY proteins can modulate transcription via modulations in histone acetylation.
9	The histone acetyl transferase P300 works together with CLOCK:BMAL in promoting *mPer1*, *mPer2*, and *Cry1* transcription.	H3 histone acetylation rhythms in the promoter regions of *mPer1*, *mPer2*, and *Cry1* may regulate timing of the transcription of these genes.

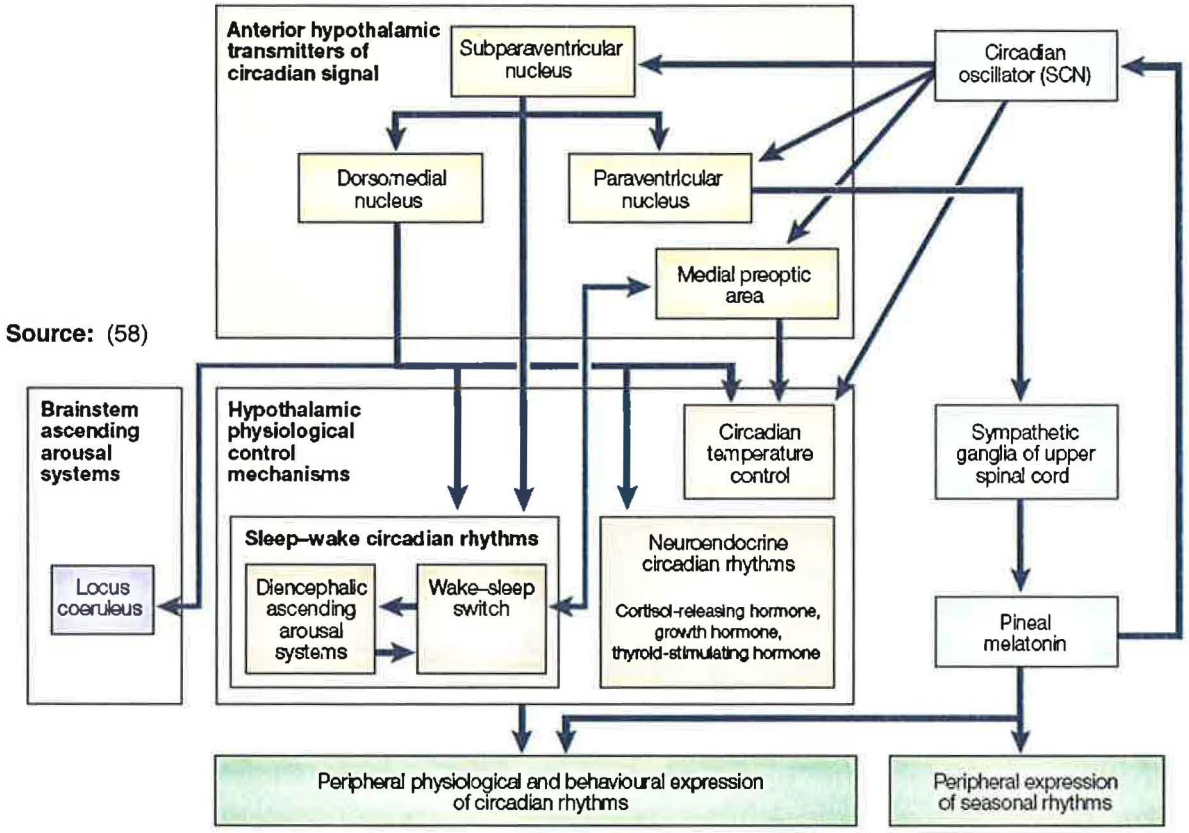


The interaction of these feedback loops results in a tango of molecular signals that occur reliably every 24 hours with individual events occurring consistently at the same point in the 24 hour period.

How does the master clock synchronize peripheral clocks?

Other hypothalamic structures have also been linked in this entrainment process of biological rhythms including the paraventricular nucleus, subparaventricular zone which has a high density of SCN neurons, and the dorsomedial hypothalamus. (52) (3) These structures are part of the anterior hypothalamus which acts as a large relay station to communicate information from the SCN to the reticular activating system, pineal gland, pituitary gland, and to structures responsible for maintaining core body temperature (see diagram below). The SCN does not directly control downstream effects of the circadian rhythm but it clearly has a primary role in being an intermediary between the environment and the body's intrinsic physiology.

From the perspective of evolution, this system clearly had a protective and adaptive benefit that remained preserved but unable to adapt to the influence of current



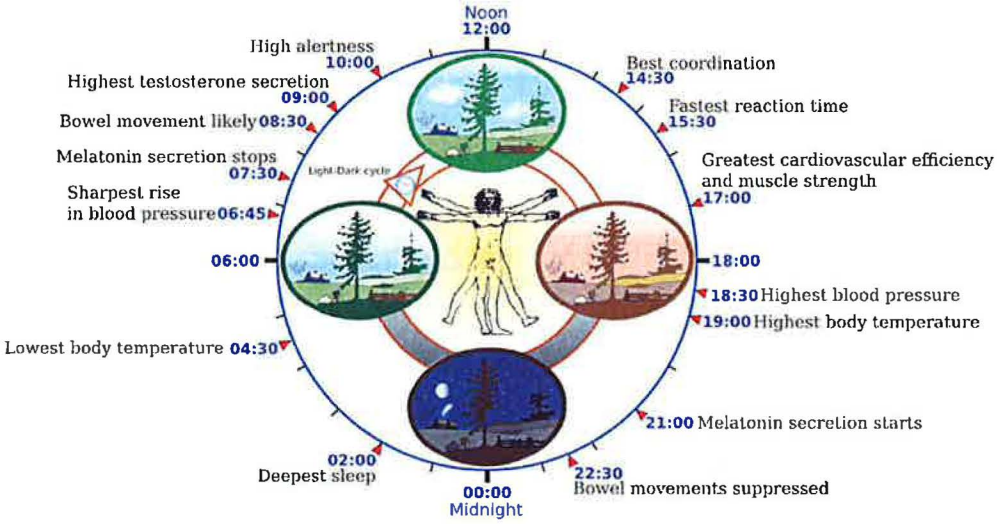
Source: (58)

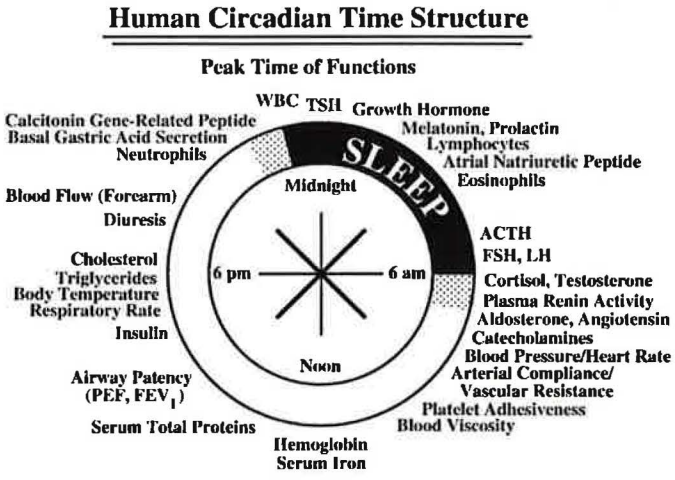
technology. Perhaps we have allowed current technology to overwhelm our adaptive abilities. It is still not known how the SCN synchronizes behaviors like food intake and anticipatory locomotor behavior. But, animal experiments (79) have shown the liver being entrained simply by feeding without SCN influence. What is known is that the SCN has an important role in autonomic control and in controlling hypothalamic neuroendocrine pathways.

A group of investigators (16) has proposed that peripheral clocks--intrinsic periodic rhythms of organs--provide feedback to the SCN via the hypothalamus. The hypothalamus could get feedback from hormones and neuronal signaling.

Circadian rhythms and clinical medicine

Recently, there has been a greater understanding of the circadian influence inherent in the normal functions and physiology of the body. Every organ has not yet been thoroughly investigated to determine exactly what influence the circadian pacemaker has on its own local circadian function. It is beyond the scope of this paper to review the details of every organ's innate circadian rhythm, but it is worthwhile to review several of these. The figures below show the breadth of circadian influence and the table that follows the time structure diagrams gives a cross-section of circadian clock genes found in various organs.





The figure above shows a physiologic summary of basic human functions.(75)

Source: (44)

Expression of clock genes in human peripheral tissues

Tissue	Conditions	Genes	Observations	Ref.
Skin (keratinocytes, fibroblasts, melanocytes)	Cell culture	*CLOCK, PER1*	Expression of mRNA and protein in cell lines	[53]
Oral mucosa; skin	24h sampling Healthy young men $n = 8$	*PER1, BMAL, CRY1, TIM, CLOCK*	Significant ANOVA and cosinor for 3 genes. Early and late wake time peaks in *PER1*, late peaks in *BMAL1*, mid to late peaks in *CRY1*. More variability and lower amplitude in skin than mucosa	[52]
Vascular smooth muscle cells	Cell culture	*PER2, CLOCK, NPAS2*	Rhythmic expression following serum shock or retinoic acid treatment	[119]
PBMCs	2 point sampling (9:00/21:00) Healthy adults: $n = 9$	*CLOCK, PER2*	Change in morning vs. evening expression of *PER2*. Non significant difference for *CLOCK*	[58]
PBMCs	35h CR Healthy young men: $n = 3$	*PER1-3, DEC1*	Rhythmic *PER1-3* expression under constant conditions. Early to mid wake time peaks. Non-significant amplitude for *DEC1*.	[56]
White blood cells (mononuclear and polymorphonuclear)	39h modified CR (limited activity) Healthy young men: $n = 7$	*PER1*	Significant oscillation of expression. Peak in early wake in both populations, secondary peak in polymorphonuclear cells	[57]
Whole blood cells	24h sampling Healthy young men: $n = 12$ N-24: $n = 1$	*PER1-3, CLOCK, BMAL1*	Significant oscillation of expression by ANOVA; Change in expression pre- and post-treatment (light, MLT, B12, methylphenidate)	[60]
Skin; hair root keratinocyte; monocytes	Primary cell culture	*BMAL1*: luciferase reporter	Variability in amplitude based on source tissue. Variability in inter-subject expression of period	[118]
PBMCs	Sampling before and after blue light (5.5-7.2klux). Neonates (12-27d): $n = 61$	*BMAL1, CRY1*	Reduction of *BMAL1*, increase in *CRY1* after light exposure. Inter-subject variability	[61]
PBMCs	~24h sampling Healthy young men: $n = 10$	*PER2, BMAL1, REV-ERBα*	Variability in expression *PER2, BMAL1* oscillate in phase, constant *REV-ERBα* expression. Suggest two "types" based on early vs. late expression of peak	[59]
Colon carcinoma cell lines; colon biopsies	Cell culture; Biopsies from 25 patients	*PER1, PER2, CLOCK*	Detection of all three RNAs in RNA samples from biopsies and cell lines. Detection of PER1 and CLOCK proteins in biopsies by immunofluorescence	[55]
Oral mucosa	24h sampling; Light response (2h, 460 lux at ZT13). Healthy young adults: $n = 9/12$	*PER2*	Variability in expression at all intensities. Significant increase in *PER2* expression with blue light	[54]
Pineal gland tissue	Postmortem pineal tissue: Control (Braak stage 0) $n = 24$; Preclinical AD (Braak stages I-II) $n = 22$; AD (Braak stages V-VI) $n = 22$	*PER1, CRY1, BMAL1, CLOCK*	*PER1, CRY1, BMAL1*, but not *CLOCK*, expressed rhythmically in control subjects. No rhythm detected in preclinical or clinical AD patients	[87]

AD, Alzheimer's disease; B12, vitamin B12; CR, constant routine; MLT, melatonin; N-24, non-24-h sleep–wake disorder; PBMC, peripheral blood mononuclear cell; ZT, Zeitgeber Time (time after lights on).

Neuroendocrine physiology

Figure A below demonstrates the effect of homeostatic and circadian factors on the neuroendocrine system. The data represents 8 healthy men who were part of a 53 hour sleep/wake protocol in which there were 8 hours asleep, 28 hours awake, and 8 hours of daytime sleep. The shaded area is a normal 24 hour period. Growth hormone (GH) and PRL (prolactin) secretion shift with the shifted sleep. Cortisol and TSH remained synchronized to the circadian rhythm.

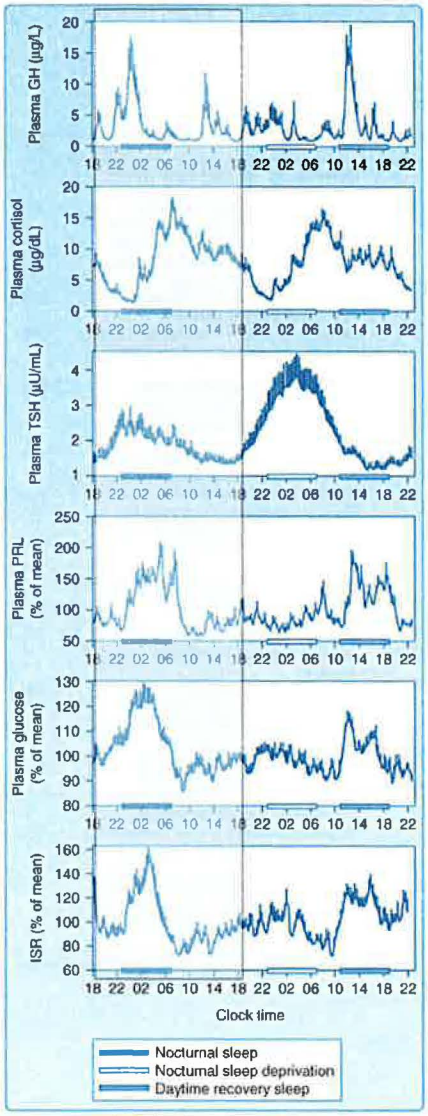


Figure A: Shaded area is a normal 24 hour period. PRL=prolactin. ISR=Insulin secretion rate

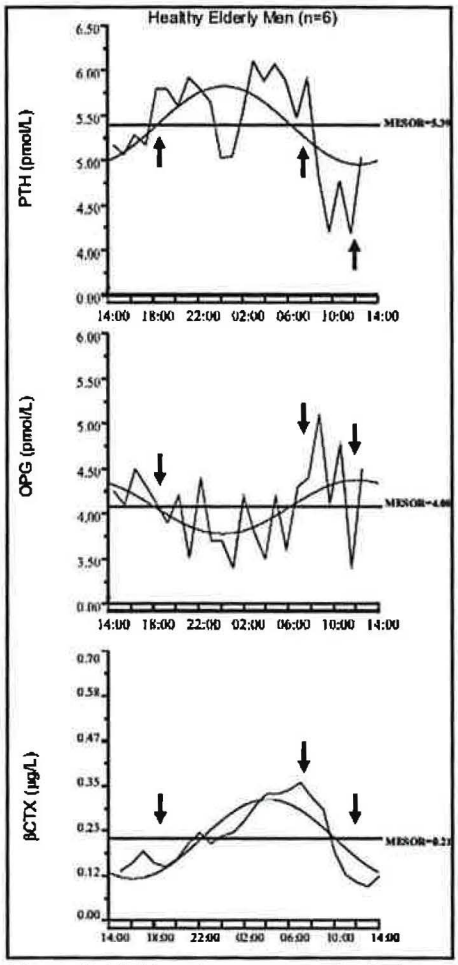
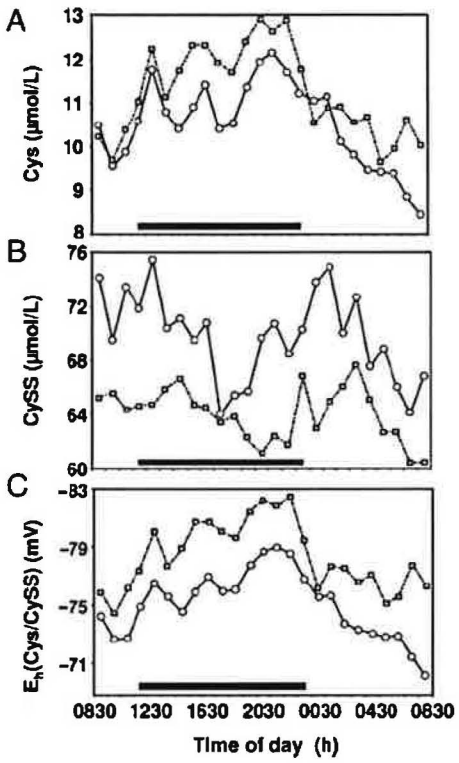


Figure B: PTH and osteoprotegrin.(39)

Figure B above examines the circadian nature of parathyroid hormone (PTH) secretion, osteoprotegrin (OPG, regulator of osteoclasts), and type 1 collagen C telopeptide (βCTX) in healthy elderly men.(39) This study suggested that osteoporosis in women may be due to circadian irregularity.

Blood/Immune System/Stem Cells

Oxidative stress has been implicated in atherosclerosis (48) but these redox changes are also normal in catalytic reactions and protein-protein interactions. Redox states are inherent to methionine and cysteine, amino acids with sulfur groups. A recent study showed diurnal variation among this state in human plasma as noted in the adjacent figure. In figure C, the redox state of plasma cysteine is elevated in men and women.

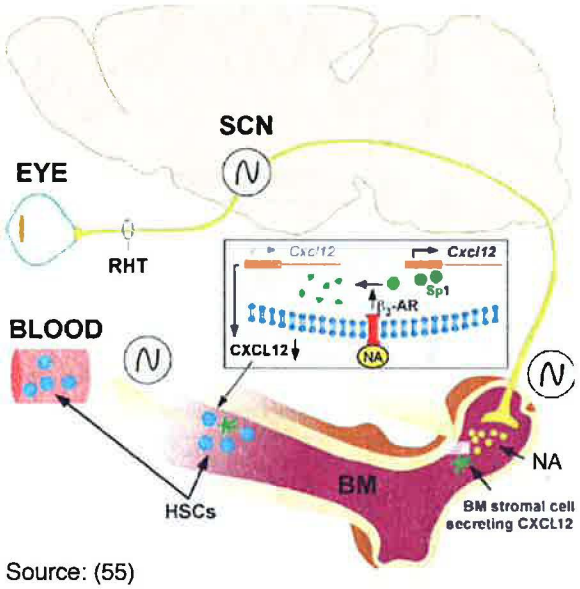


Diurnal variations in plasma cysteine (Cys; *A*), cystine(CySS; *B*), and the Cys/CySS redox state (*C*) in women (○; *n*=17) and men (□; *n*=21).(9)

The effect of the circadian rhythm can be profound. In a recent NEJM study, sudden deaths from cardiovascular disease in sleep apnea patients occurred more commonly from midnight to 6:00 AM than in the general population and compared to patients who died of cardiovascular but did not have sleep apnea (30). It is possible that the higher nocturnal oxidative stress burden may have contributed to the timing of events. Interestingly, in Hawaii, these events are more likely to occur in travelers in the afternoon or evening suggesting that this timing change may be related to circadian rhythm alterations. (20)

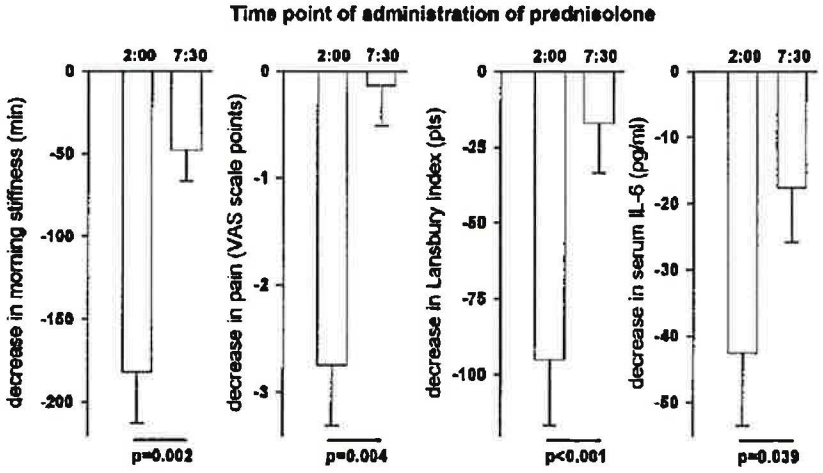
Hemostasis is profoundly affected by the circadian rhythm. Early in the morning, the biorhythms of cellular and vascular signaling plus the presence of coagulation factors coincides with very low levels of pro-fibrinolytic activity. This synergistic situation leads to a higher risk of thrombotic events like acute myocardial infarctions and cerebrovascular accidents. These findings have been inferred from two studies in which a group of patients had constant unfractionated heparin infusion. Therapeutic aPTT levels peaked nocturnally and reached their nadir early in the morning. (42,70,25,35) Also, it is possible that protein C and protein S have circadian rhythms with peak levels occurring early in the morning.(85) Further studies are needed to elucidate these patterns.

With the ongoing debate about stem cell research, there is still much to be learned about optimally using them. A fascinating study was published only a few months ago regarding the mechanisms of physiologic trafficking of hematopoietic stem cells (HSCs). (55) In the study, the investigators used knockout mice including ones with missing *BMAL1* and *Per1* genes. Their experiments demonstrated that HSCs do not have random behavior; their circulation is dictated in a circadian manner after light exposure The SCN causes noradrenergic stimulation of β_3-adrenergic receptors, degradation of transcription factor Sp1, and downregulation of Cxcl12, a chemokine that can induce HSC directed migration (see adjacent figure). These results have implications for optimizing stem cell harvesting.



Source: (55)

The morning symptoms of rheumatoid arthritis can be explained by circadian variations in chemokines and immune complexes. (80) Interferon-gamma, IL-2, TNF-alpha affect the Th1 response (cellular immunity). Interferon gamma and IL-2 have peak levels at midnight and TNF-alpha levels begin to rise between midnight-2:00 AM and peaks



Source: (2)

around 6-8 AM in patients with rheumatoid arthritis. (80,45) One study has shown that

the timing of 5-7.5 mg of prednisolone at 2:00 AM instead of 7:30 AM for 5 consecutive days resulted in improved symptoms and IL-6 levels as noted in the figure below: (2)

This evidence makes a strong argument for the development of chronotherapeutics so that maximal therapeutic benefit is achieved. Fatigue is common in nearly all autoimmune and infectious diseases. TNF-α is probably responsible, and in fact, blockade of TNF-α in rheumatoid arthritis patients resulted in much less fatigue.(93) A recent study investigated how TNF-α may lead to fatigue. This study found that TNF-α impairs the activation of a few circadian clock genes in mice, including *Per1*, *Per2*, and *Per3* and to reduce locomotor activity. (18)

GI Tract

The gastrointestinal tract has a robust circadian presence. Colonic activity is greatest during the day and reaches its nadir during the night. Surges of activity occur upon awakening and after meals. 5-10% of the liver's genes have transcription mediated in a circadian pattern. The most dominant zeitgeiber of the GI tract is feeding. A recent study in mice demonstrated that even blinding the SCN in darkness, the gastrointestinal tract and myenteric plexus clock genes can still be entrained based on altered feeding schedules independent of vagus nerve stimulation or input. (73) (38) These findings suggest the GI tract has its own peripheral circadian rhythm. Interestingly, like the SCN, the myenteric plexus also uses vasoactive intestinal peptide. Clinically, shift worker has been associated with dylipidemia, GI discomfort, reproductive problems, and breast cancer. (10) Breast cancer has been associated with circadian rhythm disruption and decreased circulating melatonin levels (78). often report an increased number of GI symptoms. For example, time zone travel can often lead to GI symptoms, including constipation (so called "vacastipation").

Lungs

Not much is known about the presence of circadian clock genes in bronchial wall epithelial cells; however, it is quite well known that asthmatics are at increased risk for exacerbations between midnight and early morning. It has been postulated that the decreased in cortisol (before its morning surge) may be responsible. There is also evidence that daytime iNOS activity is higher in the vasculature of bronchial tissue among nocturnal asthmatics. (82) Inhaled corticosteroid and beta-agonist therapy has been shown recently to increase transcription of circadian clock genes (*Per1*) (17) but more

studies are needed to determine the significance of this finding. Of course, upper airway obstruction seen in obstructive sleep apnea is a nocturnal phenomenon as is central sleep apnea which is a problem of metabolic ventilatory control.

Mood Disorders

Last, but not least, several mood disorders, such as bipolar disorder, have circadian associations. In depression and bipolar disorder, sleep times can increase or decrease (during manic phases). Light therapy is being explored as a possible therapy for fatigue and associated mood disorders in cancer patients. (51)

Evidence for a new clinical and research paradigm?

Medical school education teaches fundamental concepts of cell metabolism and physiology with a bias towards homeostasis. Ultimately, all organisms will attempt to adapt to its environment in order to maintain a balance of its biological functions but it cannot be ignored that there are circadian rhythms that can become dysfunctional.

Research and clinical medicine often ignore circadian physiology of the human body. Most of us think about the impact of a disease during wake hours; in fact, patients' medications are dosed upon awakening or just before going to bed. Of course, compliance to therapy is problematic. While extended release regimens are convenient, could these medications be counter-productive with respect to the circadian rhythm? Should medications be developed with chronopharmkokinetics in mind? Fortunately, this approach is slowly gaining ground. A recent hypertension study demonstrated nocturnal hypertension that responded to nocturnally dosed anti-hypertensives in chronic kidney disease patients.(56)

Countless research papers report sophisticated methods of tissue extraction from mammalian specimens without any notation of timing. As noted previously, harvesting of GI colonic tissue based on circadian physiology can have huge implications to study findings and conclusions. Studies and trials examining the effect of new therapies often do not explain why an experimental drug's dose timing was chosen or even if circadian physiology could have led to a negative result. Many studies of various medical subspecialties demonstrate negative results but it is interesting to speculate whether any of them were due to poor timing of a therapy based on subjects' circadian rhythms.

Considering the intricate mechanisms that control circadian rhythms; its effect on normal physiology; and the deleterious effect of disease pathophysiology on the circadian rhythm, there is no doubt a need to push research in delineating global and organ-specific circadian physiologies. Moreover, there is a need for pharmaceutical research to shift towards chronotherapeutics and practitioners to truly and specifically manage their patients "24/7."

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