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Circadian Rhythms - Genetic Regulation and Clinical Disorders

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INTRODUCTION

Circadian rhythms are endogenously generated rhythms with a period length of about 24-hours. A biological clock in the hypothalamic suprachiasmatic nuclei is responsible for the generation of circadian rhythms. Notable examples of the circadian rhythms include the sleep-wake cycle and rhythms in hormone production. Abnormalities of the circadian system include biological clock lesions that result in arrhythmic behavior and irregular sleep patterns. Abnormalities of the circadian system also occur when there is desynchronization of environmental clock time with the phase of the "internal milieu" resulting in conditions such as "jet lag". Numerous aspects of human physiology are greatly influenced by the time of day, as is the pathogenesis of illness.

This review summarizes our current knowledge of the organization of the circadian system and the generation and regulation of biological clock function. The role the circadian system plays in human physiology along with the detection and treatment of biological clock disorders is also discussed.

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For The Editorial Board,
Robert M. Blizzard, MD
Editor-in-Chief

CIRCADIAN SYSTEM ORGANIZATION

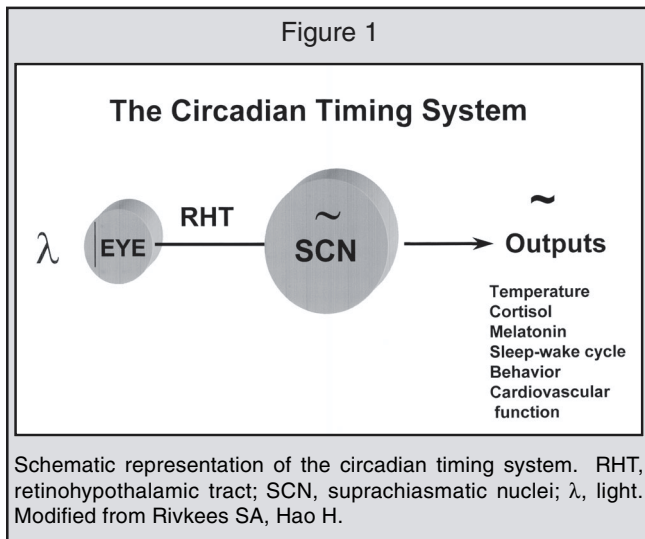
The system responsible for the generation and regulation of circadian rhythms is the circadian timing system. This neural system consists of a biological clock located in the paired suprachiasmatic nuclei (SCN) of the anterior hypothalamus, of an input pathway from the retina, and output pathways from SCN (Figure 1).¹

Because oscillations of the biological clock are not exactly 24-hours, synchronizing (entraining) the circadian pacemaker each day to the 24-hour light-dark cycle is necessary. Otherwise, clock oscillations will drift (free-run) out of phase with that of the environmental cycle. A direct pathway, the retinohypothalamic tract (RHT), from the retina to the SCN mediates photic entrainment of the SCN.¹ Light is the most potent entraining stimulus (Figure 1).

Two types of photic regulation of circadian phase (types 0 and 1) have been described.² In humans, strong (type 0) resetting is observed after very bright light exposure (10,000 lux), and modest (type 1) resetting is observed with ordinary indoor lighting (200 lux). Although cutaneous light has been suggested as influencing circadian function in humans, there is little support for the notion that this or other extraretinal photoreception is important in mammals.³

MOLECULAR BASIS OF CIRCADIAN RHYTHMICITY

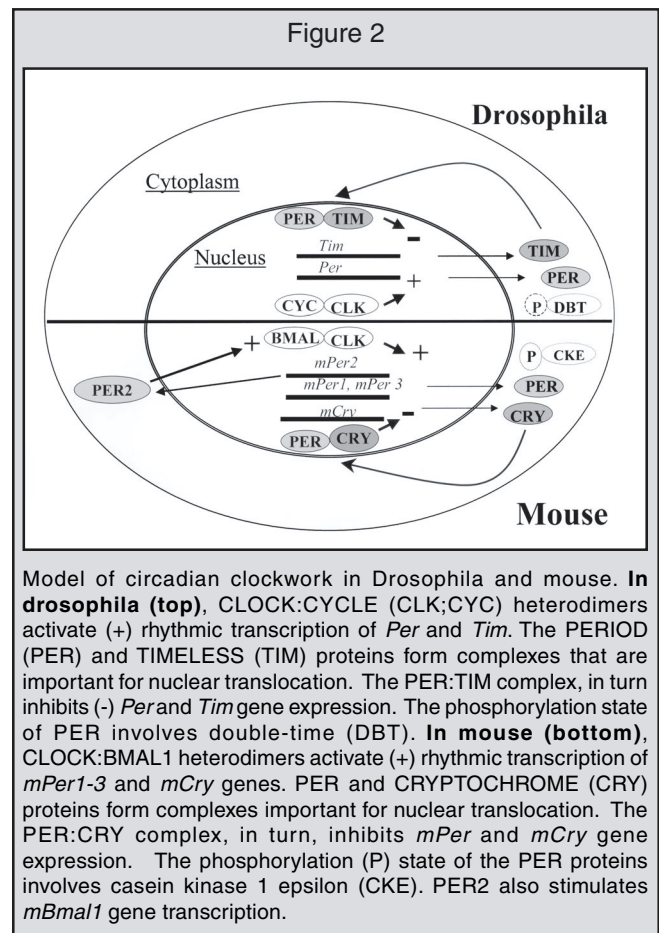
Recent data suggests that the SCN is composed of multiple, single cell circadian oscillators. These oscillate as an ensemble to generate overt rhythms.⁴ Gamma-aminobutyric acid (GABA), an inhibitory neurotransmitter, plays an important role in synchronizing the oscillations of individual clock cells.⁴



Considerable progress has been made over the past several years in defining the molecular mechanisms of clock oscillations.⁵ In yeast, drosophila, and in mammals, it now appears that the molecular clockwork involves interlocking feedback loops that stimulate or inhibit clock gene expression.⁶

The molecular mechanisms leading to circadian rhythm generation were first detailed in drosophila (Figure 2). In these flies, the circadian feedback loop is generated by the transcriptional regulatory proteins PERIOD (PER) and TIMELESS (TIM) encoded by the *per* and *tim* genes. These are activated in the morning, and their two protein products accumulate in the cytoplasm during the day. In the evening, dimerization of PER and TIM occurs and the complex enters the nucleus. After entering the nuclei, the PER-TIM complex inhibits *per* and *tim* gene expression. In addition to feedback inhibition, the proteins CYCLE (CYC) and CLOCK (CLK) dimerize to stimulate *per* and *tim* gene expression in a rhythmic manner. These processes result in a 24-hour cycle of clock protein oscillations.

In the mammalian clock, several clock genes that are homologous to drosophila clock genes have been recently identified and discovered to play similar roles in clock regulation. Homologous mammalian and



Drosophila clock genes are described in Table 1, and their corresponding roles in circadian rhythm generation are illustrated in Figure 2. The rhythmic transcription of *mPer* genes (murine *Pers* 1-3) and *mCry* (Cryptochromes 1 and 2) are driven by the transcriptional activating factors CLOCK and BMAL1, that interact with specific promoter elements. PER and CRY then accumulate in the cytoplasm to form complexes that enter the nucleus. Within the nucleus, CRY will then directly interact with CLOCK and BMAL1 to turn off transcription of the *mPer* and *mCry* genes. As the levels of PER and CRY fall, CLOCK and BMAL1 will dimerize to restart *mPer* and *mCry* transcription restarting the 24-hour cycle.⁵

In addition to PER:CRY feedback inhibition, other processes contribute to the clock mechanisms. For example, PER2 (Figure 2) stimulates BMAL1 expression so that PER and BMAL1 expression are out of phase. Alteration in the phosphorylation status of PER proteins also influences PER stability and cellular localization. In *Drosophila*, the kinase double-time alters PER phosphorylation.⁶ In mammals, casein kinase 1 epsilon⁷ influences PER phosphorylation. Mutations in each of these kinases alter normal rhythmicity.

Evidence suggests that PER proteins also play a role in the photic regulation of clock phase. Following either photic or glutamatergic stimulation of the SCN, a cascade of calcium-mediated events is triggered, leading to activation of the transcriptional regulator CREB.⁴ In turn, CREB binds to cAMP-response-element (CRE) sites within promoter regions to induce the expression of *mPer1* and *mPer2*. Alterations in PER protein expression then play a role in resetting clock phase.

EXPRESSED RHYTHMICITY IN HUMANS AND OTHER MAMMALS

The rhythmic expression of intrinsic clock genes also drives the expression of clock-output genes, which communicate circadian phase to the rest of the organism.⁴ This occurs as E-box elements, which are a binding site for PER, and which are present in promoter regions of other genes.⁴

Mutations in clock genes have been recognized in rodents with abnormal rhythmicity. Very recently, the first mutation of a human clock gene hPER2 has been discovered. This mutation results in the advanced-sleep phase syndrome that is characterized by very early morning awakening.^{8,9} As other individuals with abnormal rhythmicity are identified, it is anticipated that additional clock gene mutations will be found.

Table 1
Homologous Genes in *Drosophila* and Mice that Play a Role in Circadian Clock Regulation

Drosophila	Mouse
period (<i>per</i>)*	mPeriod1 * mPeriod2 * mPeriod3 *
Timeless (<i>tim</i>)* Time-out	None mTimeless**
Cryptochromes (<i>Cry</i>)*	mCry1* mCry2*
clock*	mClock*
cycle*	mBmal1 (MOP3) mBmal2 (MOP9)
double-time*	casein kinases 1 epsilon (TAU)*

*mutation results in arrhythmic behavior
**mutation results in embryonic lethal

Adapted from Reppert and Weaver¹

Outputs of the circadian system have been widely characterized in human clinical studies. Notable examples include the sleep-wake cycle, daily rhythms in body temperature, and day-night rhythms in cortisol production. Day-night differences in gonadotropin, testosterone, growth hormone and thyrotropin secretion are also recognized.¹⁰ Melatonin production by the pineal gland is also regulated by the SCN, with secretion occurring at night in proportion to the duration of darkness. In seasonal breeding species, changes in the duration of nocturnal melatonin production regulates the activity of the reproductive axis.¹¹ Melatonin does not appear to influence the human reproductive axis.¹² In humans, the duration of melatonin secretion is related to the length of days. The role of endogenous melatonin secretion in regulating SCN function is also unclear, as pinealectomized animals exhibit normal circadian rhythmicity and normal phase-shifting responses to light.¹³

Day-night differences are recognized for many homeostatic mechanisms such as body temperature, which has a nadir in the early morning hours. Cardiovascular function exhibits diurnal rhythmicity, as

does platelet function.¹⁴ Rhythms in cognitive ability are recognized, and the productivity of shift workers and health care providers varies with the time of day.

There is also increasing recognition that the circadian cycle influences the pathogenesis of many illnesses. Myocardial infarctions and cerebrovascular events occur most commonly in the morning.¹⁴ Croup and certain forms of asthma are associated with evening- hour exacerbations.¹⁵ In some individuals, seizures are related to the time of day. Sudden infant death syndrome (SIDS) has a strong time related component, occurring most frequently in early morning hours.¹⁶ However, we do not know if the circadian system plays a role in SIDS pathogenesis.

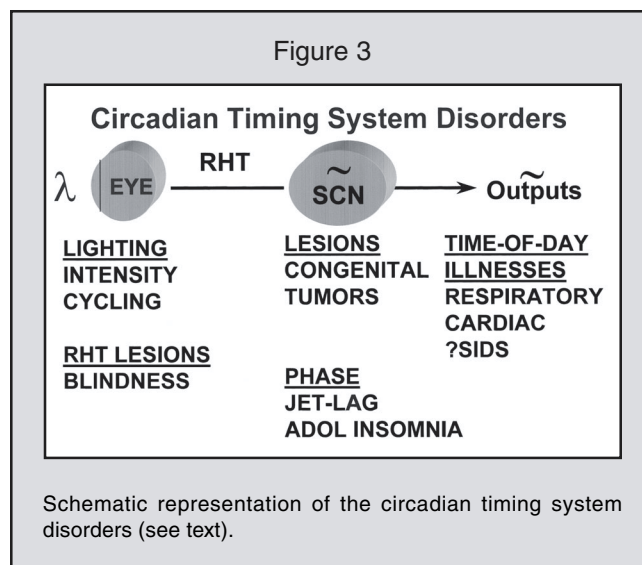
CIRCADIAN SYSTEM ABNORMALITIES

Since the circadian system exerts potent influences on human behavior and physiology, circadian system disorders will have overt clinical manifestations.¹⁷ Circadian system disorders may be related to abnormal clock function or to abnormal entrainment of the clock (Figure 3).

When more than 90% of the SCN is damaged, arrhythmic behavior may result. Thus, congenital or acquired anterior hypothalamic lesions or tumors may result in the loss of expressed day-night rhythms on sleep-wake disorders.¹⁸ Congenital central system abnormalities may also be associated with clock lesions, as we have discovered arrhythmic activity patterns in a child with septo-optic dysplasia.¹⁹

Clock disorders include abnormalities in circadian phase, which relate to the timing of expressed rhythmicity (e.g. the onset and offset of sleep-wake cycles) relative to the 24-hour day. Abnormalities of circadian phase occur when the “hands” of the endogenous clock are out of phase with the environmental light-dark cycle. One notable example of this phenomenon is jet lag, which occurs when circadian clock phase does not match that of light-dark cycle after changing time zones.

Another condition in which abnormal phase relationships occur is in delayed-sleep phase insomnia. In this condition that prominently affects adolescents, clock phase is delayed with resultant late sleep-onset and awakening times. Delayed-sleep phase insomnia should be considered when the individual does not fall asleep until after midnight and awakens late in the morning or in the afternoon. This condition becomes exaggerated when the effected individual is allowed to “sleep in” on weekends. Families with abnormally advanced circadian phase have also been described, some with hPER2



mutations, suggesting a strong genetic component for the setting of circadian phase.^{8,9}

Entrainment disorders may result from inadequate retinal innervation of the SCN. In blind individuals without intact RHT function, the absence of photic information may result in impaired synchronization of endogenous and environmental phases. The circadian phase of such individuals will free-run, resulting in times when the individuals’ sleep-wake cycles do not correspond with the light-dark cycle. Recent evidence shows that timed melatonin administration may help entrain the circadian phase of blind individuals who do not entrain to the 24-hour day. This helps synchronize sleep-wake cycles with the environmental light-dark cycle.²⁰ Surprisingly there are blind individuals who have intact retinal innervation of the SCN. In these individuals, environmental lighting will entrain the circadian clock so that endogenous rhythmicity is in phase with the light-dark cycle.²¹ Unknown non-photoc factors may also entrain circadian phase in blind individuals, as we have observed sleep-wake cycles in perfect synchrony with the light-dark cycle in individuals with anophthalmia.

Another cause of entrainment abnormalities is related to problems in environmental lighting conditions. If individuals are exposed to constant indoor lighting or darkness, or to low-intensity cycled lighting that is not potent enough to shift the clock (<200 lux), expressed rhythmicity will free-run. This situation can occur in constantly illuminated intensive care units where the patient’s circadian phase will drift from that of care providers. This may result in perceptions of abnormal behavior. The interpretation of time-of-day dependent tests e.g., cortisol levels also will be inaccurate in this setting. Thus, to prevent free-running rhythms, cycled lighting of adequate intensity is needed.

DETECTING BIOLOGICAL CLOCK DISORDERS

A history of regular sleep and wake times in an individual is reassuring that the biological clock is functioning normally. The lack of regular sleep or awakening time may reflect abnormal clock function. Surprisingly, despite the socially disruptive effects of arrhythmic behavior, clock-related behavioral problems may not be brought to medical attention. Yet upon inquiry, families will give clear histories of abnormal activity patterns.

To assess clock function, diaries of sleep and waking times are useful. If the time the patient awakens and retires to sleep is consistent from day-to-day, this suggests normal clock function. However, if sleep patterns are irregular, or are out of synchrony with those of other family members, clock lesions may be present.

To provide objective assessments of behavior patterns, periods of rest and wakefulness can be assessed using monitors worn on the wrist that collect activity information for extended periods (actigraphy). Analysis of activity patterns collected over 2-3 week periods (actograms) can then be used to determine if there is normal rhythmicity or altered phase-relationships.

CHRONOTHERAPY

Over the past several years, considerable progress has been made in the treatment of biological rhythm disorders. Light has been recognized to regulate circadian rhythmicity in humans.² Exposure to bright light (10,000 lux) during the night is a strong stimulus that produces rapid shifts in circadian phase in humans.² Not surprisingly, light therapy is now being considered as a potential therapy for jet lag and other circadian phase disorders.

The concept that bright light resets the circadian clock is also important for night-shift workers. By providing an environment with bright light exposure during work at night and darkness during the daytime when the worker rests, it is possible to shift the endogenous circadian cycle to that of the work schedule.²² Light therapy is also used in the treatment of certain forms of depression.²³

Behavioral paradigms can be used to treat circadian-phase disorders. Delayed sleep-phase insomnia can be treated by progressively delaying sleep onset over several days until the patient's sleep-wake cycle is in phase with the desired time of day. Alternatively, imposing regular waking times each morning can help resynchronize circadian phase.

MELATONIN

Melatonin has received much attention as a "chronotherapeutic". Melatonin is an endogenous indolamine that is produced by the pineal gland at night in proportion to the duration of darkness.²⁴ In mammals, melatonin exerts its effects through specific high-affinity receptors that include Mel 1a (mel 1) and Mel 1b (mel 2) receptors.²⁵ These receptors consist of seven transmembrane spanning domains and couple with guanosine nucleotide binding proteins (G proteins).²⁵ In humans, the melatonin receptors have been identified in the SCN.²⁶ In non-human primates, melatonin receptors have been identified in the hippocampus, brainstem, thalamus and cerebral cortex.²⁷

Melatonin has been touted as a therapy for a variety of conditions ranging from aging to cancer. Yet, as reviewed,²⁸ most of these claims have little credible scientific support. Melatonin, however, may have legitimate use in treating sleep disorders. Melatonin has well documented hypnotic properties, and is therefore effective in facilitating sleep onset.²⁹⁻³¹ The hypnotic effects of melatonin are most pronounced when melatonin is given in the evening.³²

It has also been suggested that melatonin can acutely shift circadian phase and may have a role in treating clock disorders such as jet lag.³³ This issue remains controversial. Modest melatonin-induced phase shifts have been detected in some rodent species, but not in others.³⁴

In humans, using the onset of melatonin secretion to mark circadian phase, it has been suggested that melatonin induces small shifts in circadian phase.^{33,35} However, when primates are studied under rigorous conditions that are very difficult to achieve in humans, no phase shifting effects of melatonin are apparent.³² These observations suggest that melatonin action in the treatment of jet lag^{36,37} may be related to hypnotic effects, rather than phase-shifting properties.

Although melatonin may not acutely shift circadian phase,³² melatonin administration at the same time each day may entrain free-running circadian phase. In blind individuals, nocturnal melatonin administration has been shown to entrain activity patterns to the 24-hour day.^{20,37,38}

SUMMARY

Increasing evidence show that the circadian system exerts profound effect on human physiology. In parallel with increases in our understanding of the clinical importance of circadian biology, there has been an explosion in our understanding of the genetic

mechanisms that contribute to the workings of the circadian clock. Elucidation of abnormalities of the circadian system has also led to the discovery of new clinical disorders that can now be identified and treated.

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Letter to the Editor

Ghrelin-induced obesity

The July issue of *Growth, Genetics & Hormones* (Vol. 17, p 34-35) contains a discussion of the ability of this 28 amino acid peptide to induce body fat accumulation in rodents.

But of great importance to students of human obesity is the observation that the lean weight of these obese animals was probably less, certainly not greater, than that of the controls. This finding puts such ghrelin-treated animals clearly at odds with the human state, for the latter usually have an increase in lean weight, most certainly not a decrement.¹ The only clearly documented exceptions to this rule are patients with the Prader-Willi syndrome^{2,3} or Cushing's syndrome. With respect to body composition the human state differs from obesity induced by experimental hypothalamic lesions, from that of the "ob/ob" mouse, and the Zucker rat, all of which are characterized by a subnormal lean weight. Obviously, such animals, and those treated with ghrelin, cannot serve as models for human obesity.

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Gilbert B. Forbes, MD

Editor's Response: Dr. Forbes in his talented analytical way has added significantly to the Abstract, Ghrelin: A Gastrointestinal and Hypothalamic Peptide Affecting Hormone Secretion and Fat Metabolism which dealt with studies in rats and not humans. With his astute commentary he reminds us that we should not necessarily project data obtained in rodents to humans. Neither of the Editors commenting on this article were so astute as to mention this most poignant point.

Thanks very much, Dr. Forbes. The Editorial Board eagerly invites each reader to write and comment on pertinent points, ask questions or query us concerning what is published in *Growth, Genetics & Hormones*.

Robert M. Blizzard, MD
Editor-in-Chief

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