Chapter
36

Physiology of the Mammalian Circadian System

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Chapter Highlights

- The *suprachiasmatic nucleus (SCN)* of the anterior hypothalamus is the master circadian pacemaker for the expression of circadian rhythms. The SCN coordinates these approximately 24-hour rhythms throughout the brain and body and adjusts them to environmental changes. The SCN is highly peptidergic, exhibiting well-defined patterns of regional expression of neuropeptides.
- The *circadian timekeeping system* orchestrates and integrates body rhythms. This system has three components: (1) *the central circadian clock in the SCN* that generates a near–24-hour time base, which it adjusts when it receives signals reporting desynchronization, (2) *input pathways* that transmit information about environmental and body state, and (3) *output pathways* that disseminate information about the timing status of the SCN and mediate the daily expression of rhythms of behavior and physiology.
- Circadian rhythms emerge from *transcriptional/translational feedback loops* comprising a limited number of circadian clock genes and their protein products. Although rhythmic clock gene expression was first identified in the SCN, subsequent work found similar molecular clocks in cells and tissues throughout the body. Nevertheless, coherence of rhythms within a tissue decays in the absence of the SCN, whereas SCN tissue is tightly coupled, even in tissue explants.
- The circadian pacemaker is *entrained by environmental light-dark cycles* and other salient periodic events. Light entrainment depends upon intrinsically photosensitive retinal ganglion cells that express the blue-light photopigment, melanopsin, and that give rise to the retino-hypothalamic tract, terminating in the SCN and certain other nonvisual brain areas. The SCN restricts its own sensitivity to inputs to discrete temporal windows throughout the circadian day-night cycle.
- The mammalian circadian master pacemaker in the SCN synchronizes and coordinates downstream circadian clocks distributed throughout the brain and body. Internal desynchronization or failure of internal entrainment to the environment appears to be both a cause and an effect of disease-related pathophysiology. Thus, the balance between health and disease is strongly dependent on the proper coordination within and between central and peripheral circadian oscillating systems.

AN INTERNAL CLOCK FOR OPTIMAL FUNCTION

Physiologic modulation at the neural level relies on the ability of neurons to respond to salient stimuli. Among the most relevant stimuli for life on Earth are the alternating environmental states of day and night. Day-night changes in metabolism, physiology, and behavior are orchestrated by an endogenous time-keeping system that oscillates with a circadian (*circa*, about, and *dies*, a day) period. Behavioral outputs change significantly so that some behaviors occur in the day, others occur at night, and some are expressed at dawn and dusk.

Internal daily oscillations are a result of adaptation to this major environmental variable: the ever-changing cycle of day and night. Early organisms that optimized cycles of behavior to these changes held a competitive advantage—they could predict environmental state changes. Behaviors that anticipated rather than only reacted to rhythmic environmental changes offer significant benefits. Adaptation to these needs occurred through the emergence of a circadian system capable of optimizing behavioral, physiologic, and metabolic processes with respect to this light-dark cycle. The circadian system organizes body systems so that they occur in 24-hour rhythms. Uninterrupted, this circadian rhythm persists in the absence of exogenous timing cues, such as light, food availability, or social cues.

Outputs of these circadian rhythms can be used as a marker of circadian phases. Patterning of the sleep-wake cycle as well as core body temperature are often used as markers

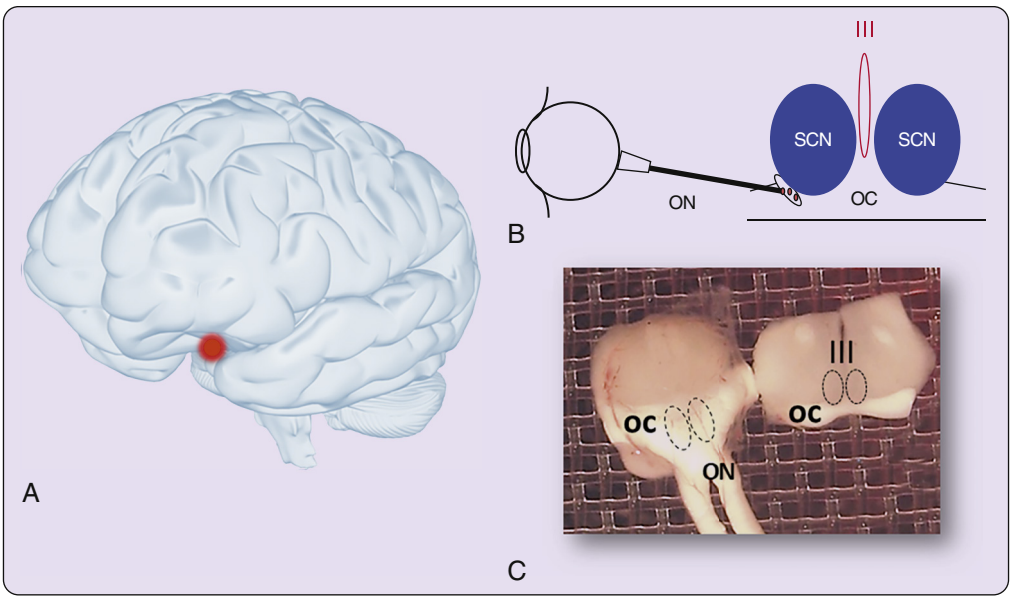

Figure 36.1 Anatomy of the suprachiasmatic nucleus (SCN), site of the master circadian clock. **(A)** The human SCN is located in the mediobasal hypothalamus, as indicated by the *red dot*. **(B)** The SCN receives light information from intrinsically photosensitive retinal ganglion cells via the retinohypothalamic tract of the optic nerve (*ON*). Light provides the primary timing cue to the SCN. The paired SCNs are nestled above the optic chiasm (*OC*) on either side of the third ventricle (III). **(C)** Two orientations of rat hypothalamic brain slices; the SCN is demarcated by *dashed ovals*. The horizontal brain slice (*left*) preserves the ONs, whereas in the coronal slice (*right*) of the SCN is identified by its medial position embedded in the OC directly lateral to the third ventricle (*III*) and the ventral displacement of the area of the ON bordering the paired nuclei.

of circadian phase. In addition, numerous endogenous hormones oscillate with a predictable phase relationship to day and night (reviewed by Van Cauter[1]). Hormonal rhythms can be complex, as the circadian pacemaker, homeostatic state of the organism (activity level, sleep, and feeding), and the pulsatile nature of secretion can affect their oscillations. Nevertheless, clear diurnal patterns of secretion have been reported. Plasma melatonin,[2] growth hormone,[3] prolactin,[4] thyrotropin-releasing hormone,[5] luteinizing hormone,[6] and leptin[7-9] all are elevated during the night. Conversely, adrenocorticotropic hormone and cortisol peak during the day.[10,11] These oscillations in hormone secretion are clock regulated; they continue in a constant environment. Overall, circadian rhythmicity appears to be present at virtually every level of function studied.

This chapter focuses on the neurobiology of circadian timekeeping, describing what is known about the master pacemaker for circadian rhythmicity; the generation of circadian time-keeping via expression of transcription-translation feedback loops; roles of neural activity; how various biologic systems can provide input to the endogenous biologic timing; and how the pacemaker can, in turn, influence the physiology and behavior of the individual. We discuss how the circadian system can adapt to a changing environment by resetting the circadian clock in response to a variety of inputs, including changes in light, activity, and the sleep-wake cycle.

SUPRACHIASMATIC NUCLEI AS PACEMAKER

In mammals, circadian rhythms are regulated by the suprachiasmatic nucleus (SCN), a paired set of brain nuclei located at the base of the hypothalamus, directly above the optic chiasm (Figure 36.1). Each nucleus contains approximately 10,000 cells.[12] The SCN acts as the central pacemaker, coordinating circadian rhythms throughout the brain and body. Lesioning the SCN disrupts rhythmicity in corticosterone levels, drinking, and wheel-running behavior.[13,14] This provided the initial evidence that the central pacemaker for the mammalian clock lay within the SCN. Transplanting fetal SCN tissue in situ or into the third ventricle of animals in which the SCN had been lesioned demonstrated that restoring the SCN could restore rhythmicity.[15,16] Furthermore, when fetal SCN tissue from a wild-type hamster was implanted into the third ventricle of 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. Hence, not only is the SCN necessary for generating rhythms, but also the period of this rhythmicity is an intrinsic property of the SCN cells—the presence of SCN is sufficient to drive the animal's peripheral rhythms.[17,18]

The circadian timekeeping system can be categorized into three major components: (1) a central clock mechanism that generates circadian rhythms, (2) input pathways that synchronize the clock, and (3) output pathways that regulate the daily expressions of behavior and physiology. This simple three-part model has provided a solid foundation for our understanding of circadian timekeeping.

GENERATION OF CIRCADIAN RHYTHM

Molecular Basis for Rhythm Generation

The nature of the biologic substrates of circadian timekeeping that could generate a time base as lengthy as 24 hours was for many years a mystery. Many creative and talented scientists pursued numerous approaches in an array of organisms before timekeeping was proven to be embedded in the

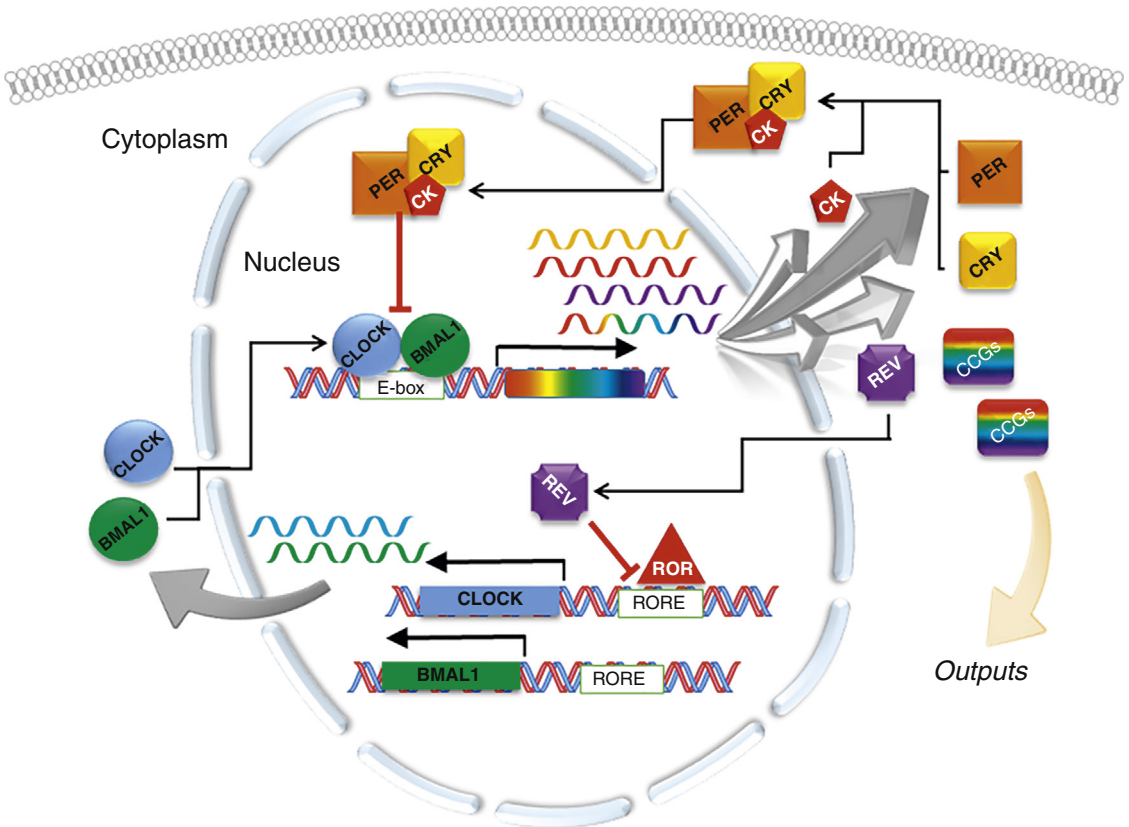

Figure 36.2 Transcriptional/translational feedback loops (TTLs) of the mammalian circadian clock. BMAL1 and CLOCK, the key positive elements of the core circadian clock, heterodimerize and activate transcription of the *Per* and *Cry* genes by binding to the E-box elements in their promoter regions. Their translational products, PER and CRY proteins, heterodimerize in the cytoplasm and then translocate to the nucleus where they interact with the BMAL1/CLOCK complex to inhibit their own transcription. A secondary autoregulatory feedback loop involves retinoic acid receptor-related orphan receptor element (RORE)-mediated transcription, which retinoic acid receptor-related orphan receptor (ROR) activates and REV-ERB represses. Additionally, this TTL-based clock mechanism also controls rhythmic expression of numerous clock-controlled genes (CCGs), which perform biochemical or physiologic roles that vary with circadian timing.

genome. Timekeeping was found to be an emergent property of a negative transcription-translation feedback loop of highly conserved "clock genes." Key elements of proof are based on elegant experiments in mutagenized fruit flies, *Drosophila*. Altering a single gene, *Period,* changes the period of rhythms of locomotory activity permanently. The 2017 Nobel Prize in Physiology or Medicine was awarded to three chronobiologists, Jeffrey Hall, Michael Rosbash, and Michael Young, for their discovery of molecular mechanisms controlling circadian rhythms. This work along with the discovery that single, dispersed mammalian cells can exhibit circadian rhythms,[19] established the important molecular processes within single cells that generate a near 24-hour rhythm.

The approximately 24-hour rhythm emerges from a feedback cycle involving a set of core clock genes, their messenger RNAs (mRNAs), and proteins.[20-22] This cycle involves of a set of interconnected positive and negative feedback loops, and their regulatory elements (Figure 36.2). The core molecular feedback loop in mammals includes positive elements, *Clock* and *Bmal1,* which are transcribed into mRNA, translated into proteins that heterodimerize in the cytoplasm, and then translocate into the nucleus. The proteins CLOCK and BMAL1 are members of a family of transcription factors containing the basic helix-loop-helix (bHLH)-PAS motif by which they bind E-box enhancer sequences of gene promoters.[23-25] This enables them to activate transcription of their own genes, as well as activating transcription of negative regulatory elements. The negative elements, which include the *Period (Per) homologs (Per1, Per2, Per3)*, and the *Cryptochrome (Cry) homologs (Cry1, Cry2)* and *Rev-erb*α, are then transcribed and translated. Proteins of the negative elements also associate in complexes and translocate to the nucleus, where they feed back to inhibit transcription of the positive elements.[20-22]

In addition to the core molecular feedback loop, there is an additional interlocking loop involving *Bmal1*. *Bmal1* is regulated by a retinoic acid receptor–related orphan receptor (ROR) enhancer site located upstream of the *Bmal1* gene; ROR binding activates gene expression, while REV-ERBα binding inhibits transcription.[26] REV-ERBα is regulated by an E-box enhancer located upstream of its transcription start site, resulting in an expression pattern that subsequently puts expression of *Bmal1* completely out of phase with the negative elements of the core molecular feedback loop.[27] These feedback loops are further affected by regulatory enzymes, including casein kinase 1 epsilon (CKIε) and glycogen synthase kinase (GSK),[28-30] and small intracellular regulatory molecules, such as calcium and cyclic adenosine monophosphate (cAMP) with established roles in signal transduction.[31,32] The cycle of these feedback loops takes approximately 24 hours to complete.

Beyond this sequence of interactions, the molecular clockwork modulates and is modulated by cellular redox state. The BMAL1/CLOCK heterodimer regulates the expression

of nicotinamide phosphoribosyltransferase, a rate-limiting enzyme in the nicotinamide adenosine dinucleotide (NAD+) salvage pathway. This relationship is the driving force for rhythmic levels of NAD+, which in turn activate NAD+-dependent histone deacetylases, sirtuin 1 (SIRT1), and SIRT3.[33,34] SIRT1, an important element of metabolic control, displays circadian oscillatory activity and alters PER2 stability and CLOCK function.[33,35] SIRT1 is localized in the mitochondrial matrix, where it mediates the deacetylation of metabolic enzymes.[32,36,37] Additional bHLH transcription factors, *Dec1 (Bhlhe40/Stra13/Sharp2)* and *Dec2 (Bhlhe41/Sharp1)*, can repress their own transcription by directly binding to the BMAL1 protein as well as occupying E-Box enhancer sequences and, thus, inhibiting transcription of other clock-controlled genes.[38,39]

A number of mutations in the genes of the circadian molecular feedback loop result in alterations in circadian phenotypes. A dominant-negative form of the *clock* gene causes lengthening and gradual loss of rhythms under continuous dark (DD) conditions.[40] However, loss of *clock* in null mutants results in shortening of circadian rhythms,[41] likely because of the substitution of the NPAS2 protein for CLOCK in its complex with BMAL1.[42] There is an immediate loss of rhythmicity with null mutation of BMAL.[43] Disruption of *Per1* or *Per2* genes results in a shortened period. *Cry1*$^{-/-}$ mice have a shorter period length, and *Cry2*$^{-/-}$ mice have a significantly longer period length, but both maintain rhythmic behavior.[44,45] These observations indicate that the molecular clockwork is extremely sensitive to alterations in its elements.

Electrical and Redox Rhythms

The SCN expresses additional intrinsic rhythmic properties, including a circadian rhythm in spontaneous electrical activity. When the SCN is isolated by knife-cut from inputs from the rest of the brain[46] or in a brain slice in vitro,[47] circadian rhythms of neuronal firing continue. This property is essential to the function of the circadian timing system,[48-50] peaking in midday in both diurnal and nocturnal mammals. The neurons of the SCN exhibit limited electrical activity during the night.[51,52] Underlying this change in action potential generation is a day/night rhythm in resting membrane potential. The resting membrane potential of SCN neurons is most depolarized and thus more likely to reach threshold for an action potential, during midday and most hyperpolarized in the early night.[52] Differences in membrane potential and ionic conductances between day and night persist even in the absence of synaptic activity.[53] To maintain oscillations in spontaneous activity in the absence of synaptic input, the intrinsic currents must change intrinsically to alter the membrane potential over the day-night cycle.

A potential regulator of intrinsic currents is cellular redox state. Redox state, the potential of molecular substrates to receive or donate electrons, is the manifestation of cellular metabolic state. Near 24-hour oscillations of redox state in the SCN have been identified. They revealed that cellular metabolic state could modulate neuronal excitability via modification of redox-sensitive K+ channels.[52,54] Interestingly, imposed changes in redox state cause immediate changes in excitability.[55] Thus, unlike the transcriptional-translational relationship of changes in the molecular clock to SCN physiology, redox modulation is tightly coupled to neuronal excitability.[54]

Reciprocal interaction between redox state, the molecular clock, and neuronal excitability has been proposed.[52,55]

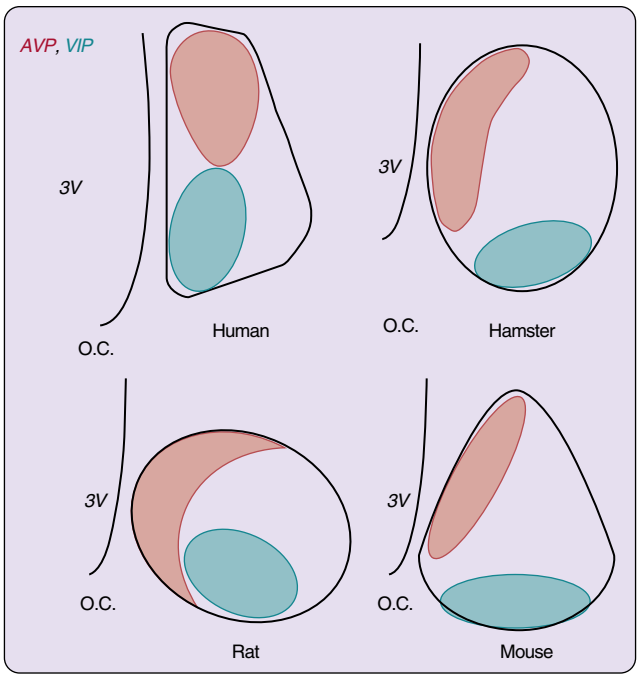

Figure 36.3 Cellular organization of the suprachiasmatic nucleus (SCN). Patterns of neuropeptide immunoreactivity reveal that the SCN has distinct regions of neuropeptide content. Localizations of vasoactive intestinal polypeptide (VIP) and arginine vasopressin (AVP) are salient and highly conserved features across species. The ventrolateral core (*blue*) and a dorsomedial shell (*orange*), both densely packed with somata of small neurons (8–12 μm in diameter), are marked by VIP and AVP, respectively. Light signals from the eyes are conveyed to the ventral core by the retinohypothalamic tract (RHT). The majority of output projections from the SCN originate from the dorsomedial shell. (Modified from Abrahamson EE, Moore RY. Suprachiasmatic nucleus in the mouse: retinal innervation, intrinsic organization and efferent projections. *Brain Res.* 2001;916:172–91; Antle MC, Silver R. Orchestrating time: arrangements of the brain circadian clock. *Trends Neurosci.* 2005;28:145–51; Moore RY, Speh JC, Leak RK. Suprachiasmatic nucleus organization. *Cell Tissue Res.* 2002;309:89–98; Hofman MA. The human circadian clock and aging. *Chronobiol Int.* 2000;17:45–259.)

Reduced forms of the redox cofactors NAD(H) and NADP(H) enhance the DNA-binding activity of the core clock proteins BMAL1 and CLOCK, whereas their oxidized forms inhibit it.[56] Even minute changes in cellular redox state can affect binding activity of these circadian transcriptional activators.

The probable linkage between gene expression, redox state, and electrical activity is further supported by the association of clock-gene expression with intracellular signaling pathways regulating neuronal membrane potential and firing rate. Reciprocally, at the cell membrane, these ionic currents may be necessary for self-sustainment of the molecular clock and could influence the molecular clock via similar intracellular signals.[57]

FUNCTIONAL ARCHITECTURE

More than 300 neuropeptides have been identified in the SCN by mass spectrometry.[58-61] The SCN can be subdivided into two regions based on distribution of neuropeptides: the ventrolateral core region and a dorsomedial shell region (Figure 36.3). The core region receives external input and acts as an integrator, communicating phase-resetting information to the rest of the SCN. The neurons of the core exhibit low amplitude rhythms that are susceptible to clock-resetting cues.[62,63]

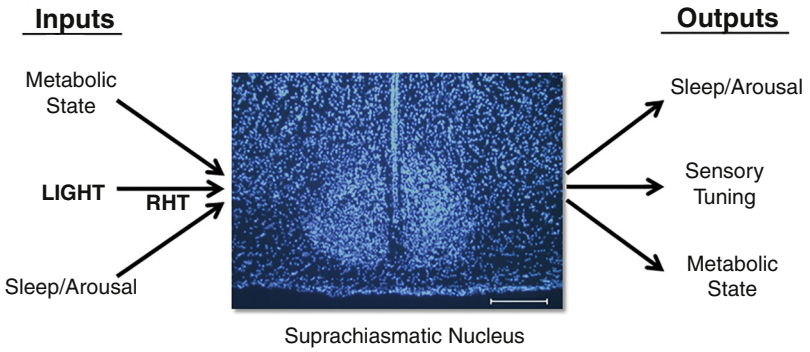

Figure 36.4 Organization of the circadian timing system. Circadian rhythms are orchestrated by a single site in the mammalian brain, the suprachiasmatic nucleus (SCN). The SCN is an endogenous oscillator, spontaneously generating near–24-hour rhythms of neuronal firing rate, output signals, and sensitivity to incoming signals. A coronal section of the medial part of the rat SCN is seen here as two brightly staining clusters of Nissl-positive cells at the base of the third ventricle. The SCN produces output signals that coordinate circadian rhythms of physiology and behavior, including metabolic state, sensory tuning, and sleep and arousal. Phasing of the SCN clock can be adjusted by a range of inputs, including those that communicate metabolic state, environmental light (via the retinohypothalamic tract [RHT]), and sleep/arousal states. Windows of sensitivity to phase-resetting signals are gated by the SCN clock so that signals communicating loss of desynchronization with day-night or output targets adaptively reset SCN clock phasing. Bar = 300 μm.

The core is often demarcated by expression of the neuropeptide vasoactive intestinal peptide (VIP). It also contains neurons that express gamma-aminobutyric acid (GABA) and calretinin. Neurons of a central region, often integrated into the core region, express gastrin-releasing peptide (GRP) colocalized with GABA, and the little SAAS neuropeptide.[12,64-67] The shell region generates robust circadian oscillations in neural activity, neuropeptide release, and *cfos* and *Per* gene expression.[68-71] It comprises larger neurons that express arginine vasopressin (AVP), met-enkephalin, angiotensin II, prokineticin 2 (PK2), and GABA.[12,65,66] There are topographic connections between all regions of the nucleus, as well as bilateral communication between the two nuclei of the animal.[72]

Although the human SCN is larger and less compact than in rodents, its peptidergic organization is similar (Figure 36.3). The dorsal and medial regions contain neurophysin/vasopressin neurons. The central region contains calbindin, synaptophysin, and VIP neurons, whereas the ventral and rostral regions contain synaptophysin, calbindin, and substance P.[73]

Inputs

The SCN is positioned in the mediobasal hypothalamus directly above the optic chiasm, where axons of the optic nerve cross over, which enables stereoscopic vision. The paired nuclei lie adjacent to the walls of the third ventricle (Figures 36.1 and 36.4). Thus the SCN is central to hypothalamic nuclei that control homeostatic physiology and behavior. In addition, the SCN receives inputs and sends outputs to many more distant brain regions; for a more detailed presentation of the anatomy of this system, see Chapter 35. The SCN performs a central integrating role, receiving input via projections that communicate temporal state beyond the SCN (Figure 36.4). This role complements SCN's timekeeping function and enables appropriate alignment of the circadian clock to brain, body, behavioral, and environmental states.

Retinohypothalamic Tract

The primary cue in establishing entrainment to the world is environmental light. Light signals are communicated from the eye to the SCN via a direct projection from the retina, the retinohypothalamic tract (RHT). The RHT is both necessary and sufficient for photic entrainment,[74,75] as disruption of the RHT results in an inability to respond to resetting light signals[76,77] and RHT stimulation can mimic light-resetting cues.[67,78] A subpopulation of retinal ganglion cells is intrinsically photosensitive (ipRGCs), a property conferred by their expression of the blue-light photopigment, melanopsin.[79] These melanopsin-containing retinal ganglion cells are photosensitive at wavelengths that are most effective for circadian resetting.[80] Terminals of melanopsin-positive retinal ganglion cells co-localize glutamate (GLU) and pituitary adenylate cyclase-activating polypeptide (PACAP),[81] the neurotransmitters of the RHT.[82,83]

Melanopsin-containing retinal ganglion cells are distinct from those giving rise to the primary visual pathway.[84] Animals lacking visual photoreceptors (rods and cones), both hereditarily retinal-degenerate strains of mice[85] and genetically modified mice that lack rods and cones,[86] still exhibit normal circadian responses to light. There is redundancy in the circadian photoreception system in the retina. Circadian entrainment is maintained in mice lacking the gene for melanopsin.[87,88] Only when both classical and melanopsin-based photoreception are eliminated is entrainment abolished.[79,89,90]

Intergeniculate Leaflet of the Thalamus

The RHT sends projections to the thalamic intergeniculate leaflet (IGL), which sends projections to the SCN through the geniculohypothalamic tract (GHT). Neurons of the GHT express neuropeptide Y (NPY) and GABA. Retinal signals are conveyed to the IGL, in part by bifurcating axons of the ipRGCs of the RHT.[91] The IGL/GHT provides an indirect, auxiliary pathway by which photic information reaches the SCN. Disruption of the GHT does not prevent entrainment,[92] yet it can result in subtle modifications in the response to light-shifting effects on circadian phase and period.[93,94] The IGL has been suggested to be involved in a more refined photic entrainment as in seasonally altered daylengths or dim nighttime illumination.[95,96]

The IGL also plays a role in in the nonphotic regulation of the circadian system by arousal-related stimuli. IGL

lesions abolish phase-shifting effects of novelty-induced wheel-running[97,98] and benzodiazepine administration in hamsters.[76,99,100] Lesion of the IGL results in shortening of the intrinsic circadian period (*tau*)[101] and interferes with the entrainment effect of scheduled daily treadmill activity in mice.[102] IGL neurons are sensitive to metabolic signals and the GHT may mediate the effects of such signals on the SCN pacemaker.[103,104] This is noteworthy because NPY contributes to integrating metabolic and appetite-related signals within other hypothalamic circuits.[4] Orexins/hypocretins modulate the activity of neurons in the IGL.[105] 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 phase shifts.[106-108]

Raphe

The SCN receives direct serotonergic input from the median raphe, and indirectly via a raphe-to-IGL pathway.[32,109-112] Serotonergic projections to the SCN and IGL have been implicated in (1) modulation of photic effects on the circadian pacemaker during the subjective night[113] and (2) mediation of nonphotic effects on the pacemaker during subjective day.[114] Serotonin depletion results in photic phase shifting that is potentiated, while effects of nonphotic phase-shifting stimuli are impaired.[115,116] Conversely, elevating serotonin, either by electrical stimulation of the serotonergic raphe or injecting serotonergic agonists onto the SCN, inhibits photic phase shifting during subjective night and evokes nonphotic phase shifting during subjective day.[111,117-119]

The SCN may influence the regulation of sleep-wake states by communicating its circadian signal through indirect pathways to the raphe nuclei.[120] Projections from the dorsal and median raphe may convey feedback information to the SCN regarding the vigilance state of the animal. Such reciprocal interactions between the circadian and sleep-wake regulatory systems may contribute to the stable yet adaptive rhythmicity of daily sleep-wake cycles.

Evidence suggests that differential effects of serotonin on the SCN are mediated through different 5-hydroxytryptamine (5-HT) receptors with distinct localizations. Facilitatory effects are mediated by 5-HT1A/7 receptors within the SCN,[117,119] while serotonergic inhibition of light-induced phase-shifts acts through 5-HT1B receptors located presynaptically on RHT terminals.[121,122] Whereas light itself has little phase-shifting effect during mid-subjective day, light at night can block the phase-shifting effects of a serotonin agonist.[123] This suggests that nonphotic and photic (glutamatergic) serotonergic inputs to the SCN are mutually inhibitory.

Brainstem and Basal Forebrain

Cholinergic projections to the SCN originate both in the brainstem and basal forebrain in brain nuclei with identified roles in sleep and arousal[124] and were demonstrated to also be present in diurnal animals.[125] Within the brainstem, these cholinergic projections arise from three nuclei. The parabigeminal nucleus 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.[126] The laterodorsal tegmental (LDTg) and pedunculopontine tegmental (PPTg) nuclei both are important for regulating the sleep-wake cycle.[127] In the basal forebrain, the substantia innominata within the nucleus basalis magnocellularis (NBM) contributes to arousal and focused attention.[128] Unlike the RHT, IGL, and raphe projections described earlier, which generally form overlapping terminal fields in the SCN core, these afferents preferentially project onto the SCN shell.[129,130] The LDTg, PPTg, and NBM are interconnected, and all play roles in regulating the sleep and arousal states of the animal. Stimulation of the LDTg or PPTg releases acetylcholine (ACh) onto the SCN and adjusts behavioral rhythms in a time-of-day dependent manner.[131] These observations suggest that the cholinergic inputs to the SCN may provide a signal regarding the sleep and arousal states of the animal, providing a link between the sleep-wake cycle and circadian rhythms.

Notably, the SCN receives input from approximately 35 areas identified by retrograde tracing and summarized in Morin.[132] Additional sleep-wake input to the SCN may come from the tuberomammillary nucleus.[133] Histamine is a regulator of the sleep-wake cycle, primarily providing a signal of wakefulness. Noradrenergic projections from the locus coeruleus (LC) may provide afferent inputs to the circadian system. The extent of the potential input from these additional monosynaptic and multisynaptic projections onto the SCN allows for an enormous capacity for modulation by a wide range of stimuli.[132]

Outputs

Although circadian physiology influences almost every aspect of physiology and behavior, target sites of SCN efferent projections are relatively small and local, primarily at the level of the hypothalamus. These targets are well-established relays to the autonomic and neuroendocrine systems as well as to structures regulating affective, sensory, and motor processes.[134-136] Neurons from the ventral regions of the SCN project to the lateral region of the hypothalamic subparaventricular zone (sPVHz), the perisuprachiasmatic area, and the ventral tuberal area. The dorsal region of the SCN projects to multiple hypothalamic sites: the medial preoptic area (MPOA), medial sPVHz, dorsal parvocellular paraventricular nucleus (dPVN), and the dorsal medial hypothalamus (DMH).[137] Targets of efferents to the dPVN include endocrine, autonomic, and intermediate neurons, thereby allowing integration of a number of hypothalamic signals.[135] SCN efferents emerge from both the core and shell subnuclei and release both neurotransmitters and peptides, including GABA, GLU, and AVP.

In addition to neuronal efferents, the SCN regulates certain rhythmic processes through diffusible paracrine signals. The presence of a diffusible SCN output signal was first suggested by the finding that complete surgical isolation of the SCN within a "hypothalamic island" abolished SCN-dependent neuroendocrine responses but allowed for persisting locomotor activity rhythms in the same animals.[138] Although this surprising finding conflicted with prior studies suggesting that the ability of SCN transplants to restore rhythmicity in SCN-lesioned hosts depended on anatomic integration with the host brain,[139-141] evidence that transplantation of SCN tissue encapsulated within semipermeable capsules could restore locomotor rhythms provided strong confirmation of the paracrine hypothesis.[18] Several diffusible candidate molecules now have been implicated as circadian output signals, including PK2, tumor necrosis factor-α, and AVP.[138]

Many SCN projection sites are regulators of sleep and arousal. The DMH projections are especially interesting, as many of these neurons appear to project to neurons containing hypocretin/orexin, a peptide well known for its role in arousal.[142,143] In addition, evidence exists for a multisynaptic pathway between the SCN and LC, an important arousal center in the brain, mediated by orexin,[144] with the DMH as a relay.[145] A minor set of SCN efferents project to the ventrolateral preoptic nucleus, a region that, if lesioned, produces prolonged reduction in sleep duration and amplitude.[146] The SCN projects to the paraventricular thalamic (PVT) nucleus and IGL of the thalamus. Both nuclei project back to the SCN. The PVT loop is proposed to provide assessment of sleep/arousal states and SCN modulation, whereas the IGL loop is thought to provide the SCN with information from higher, integrative visual centers.[93,147,148] The PVN acts as a relay between the SCN and the amygdala, which may provide a link between the circadian system and affective states.[149] Overall, the SCN is uniquely situated within a network that enables close interaction with brain regions controlling sleep and arousal states.

CIRCADIAN RESETTING

Timekeeping is a cellular process.[150,151] The expression of independently phased circadian firing rhythms from individual neurons dissociated from neonatal rat SCN cultured on an electrode array provides compelling evidence for the cellular nature of the clock.[152] It follows, then, that the properties gating sensitivity of the circadian clock within the SCN to resetting stimuli and phase resetting must be cellular. The range of responses of the clock must be restricted so that activation of select signaling pathways can occur only at the appropriate time in the circadian cycle.[32,153-155] How does the SCN clock temporally regulate its own responsiveness of specific signaling pathways?

In an attempt to define and understand the underlying control mechanisms subserving clock-gated windows of sensitivity, the SCN under constant conditions was exposed either in vivo or in vitro to treatments that activate elements of specific signaling pathways. Treatments were administered at various discrete points in the circadian cycle, and effects on the time-of-peak in subsequent intrinsic rhythms, such as neuronal activity or clock gene activity, were assessed. If the time-of-peak appears earlier during cycle(s) after treatment compared with controls, the phase of the rhythm is advanced. If the time-of-peak appears later than in controls, then the phase is delayed by the treatment. Assessing the changing relationship between the circadian time of treatment and its effect on phasing of an oscillation can generate a phase-response curve (Figure 36.5). This relationship graphically presents the temporal pattern of SCN sensitivity to activation of specific signaling pathways and, in fact, defines the window of sensitivity to phase resetting via this pathway. Timing of the peak activity after experimental reagents are administered at specific circadian times is compared with the time-of-peak activity in media-treated controls. The permanence of the phase shift is examined by evaluating the time of the peak in the oscillation of activity over the days after a treatment.

Temporal domains identified as sensitive to phase resetting via specific first and second messenger pathways coincide with discrete portions of the circadian cycle. Based on these temporal windows of sensitivities, the circadian cycle can be divided into several temporal states, or domains, of the clock: day, night, dusk, and dawn.[153,154] These studies not only contribute to defining the properties of the clock's temporal domains but also emphasize the complexity of control that the clock exerts over signal integration and phase-resetting within the SCN. These properties have been incorporated into clock-gated regulatory pathways. Each is discussed in the context of the clock domain that is regulated.

Subjective day and night are distinct with respect to their sensitivities and response characteristics under constant environmental conditions. Each correlates with specific neurotransmitter systems that impinge upon this hypothalamic site as evidenced by a large body of neuroanatomic studies.[156] This permits speculation regarding the function of pathways that gain access to and regulate the biologic clock at different points in the circadian cycle. We now consider, in turn, the major identified domains of clock sensitivity (Figure 36.6).

CIRCADIAN CLOCK REGULATORS

Daytime

A number of neurotransmitters and neuropeptides are important in resetting circadian rhythms during the daytime, including 5-HT, PACAP, NPY, and GABA. The majority of these experiments were performed in nocturnal rodents, so daytime is defined as the time during which the lights are on, and/or the model rodent is inactive. As a result, the functional context of this regulation appears tied to arousal-induced resetting, often referred to as nonphotic resetting.[157,158] Nonphotic signals cover a wide variety of phenomena, including sleep deprivation, dark pulses during the light period, and activity associated with exposure to a novel wheel or cage. The unifying factor in nonphotic signals is that they involve arousal during a time when the animal would normally be inactive.

Serotonin, or 5-HT, is believed to play a role in nonphotic, activity-induced phase shifts during the daytime. Increasing 5-HT in the SCN during the *subjective day* of an animal free running in a constant environment induces an advance in peak electrical firing rate in vitro or onset of wheel running in vivo.[111,159] 5-HT levels in the SCN are increased in vivo by electrical stimulation of the dorsal or median raphe.[111,160] Forced wheel running or sleep deprivation during the day also increases 5-HT in the SCN,[161,162] which suggests a role for 5-HT in nonphotic phase shifting. However, depleting 5-HT from raphe projections does not prevent this nonphotic daytime shift,[163] and serotonergic antagonists are not able to attenuate this phase shift,[164] providing mixed evidence for the role of 5-HT. This suggests modulation by additional messengers, possibly neuropeptides.

A second daytime modulator of the SCN clock is PACAP. PACAP is not intrinsic to the SCN but instead is released from synapses of the RHT, where it colocalizes with GLU.[165] Levels of PACAP oscillate throughout the day in SCN samples, which include synaptic terminals of the RHT, but not in other brain regions.[166] If PACAP alone is applied to the SCN brain slice in micromolar quantities, it elicits an advance in peak neuronal firing during the day but has little effect during the night.[81] In vivo findings, however, conflict with this, as attempts to block or remove PACAP's contribution to clock-resetting at night have generated phenotypes that differ in their responses to light or GLU.[167-170] These data suggest that

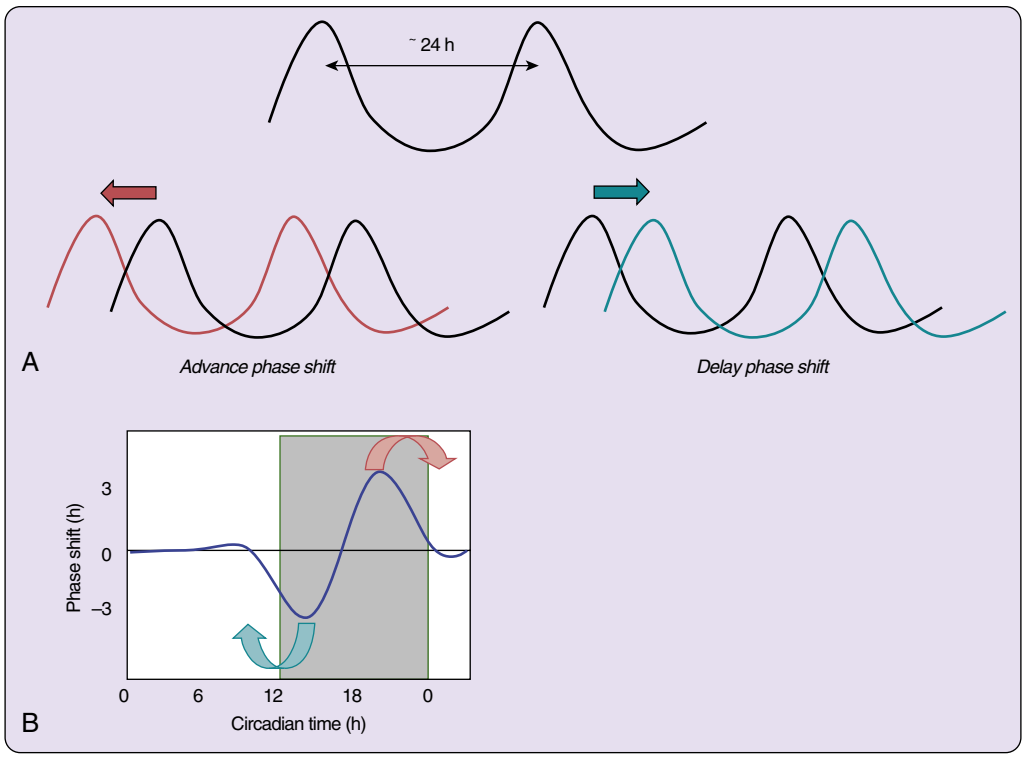

Figure 36.5 Schematic of phase resetting of circadian rhythms. **(A)** Circadian rhythms can be studied as a reportable, endogenous rhythm of approximately 24 hours. The period of the rhythm is measured at an identifiable, reproducible point, which in this example is the time of peak in the neuronal activity rhythm (*top*). A phase advance (*in orange*) occurs when phasing of the rhythm, measured here as the peak activity, appears earlier than in controls (*lower left*). Conversely, a phase delay (*in green*) results from a stimulus that causes the rhythm, again measured at the peak, to occur later than in controls (*lower right*). **(B)** The phase-response curve graphically relates the response to a stimulus with the time it was encountered (under constant conditions where the clock is free-running). This example plots the response in the suprachiasmatic nucleus neuronal firing rhythm to glutamate, the excitatory neurotransmitter of the optic nerve, or in wheel-running of rats to a pulse of light.[82] Notice that there is a temporal change in the sensitivity to light or glutamate. During subjective daytime under these constant conditions, the stimuli have no effect of phasing of the rhythm. Indeed, the circadian system is synchronized to the environment when it senses light during daytime. Within the nighttime domain, in grey, these stimuli can activate downstream signaling targets during restricted periods of sensitivity. This gating of sensitivity/responsiveness results in a phase delay in early subjective night (negative deflection) and a phase advance in late subjective night (positive deflection), respectively.

further study of PACAP's effects on the clock in the context of GLU signaling during the day is warranted.

A third daytime regulator of the clock, NPY, appears to play a dual role in the SCN, resetting the circadian clock both during the daytime and at night. NPY is released from the GHT, the projection from the IGL to the SCN. When NPY is applied during the daytime either to an SCN brain slice in vitro[106] or directly to the SCN in vivo,[171,172] it induces a phase advance. Additional in vivo studies stimulated the IGL, presumably inducing the release of NPY at the SCN. These stimulations also produced advances in wheel-running behavior during the daytime.[173] Interestingly, exposing an animal to light[174] and applying GLU to the brain slice[175] were both capable of blocking the response to daytime application of NPY. The addition of the $GABA_A$ antagonist bicuculline can inhibit the action of NPY,[176] suggesting that the effects of NPY are linked to GABAergic signaling.

A common feature of daytime signaling pathways is their ability to act via cAMP. In the hypothalamic brain slice, cAMP or cAMP analogues applied during the daytime induce phase-advances in the circadian clock, whereas at night they have little effect.[51,177] In addition, endogenous cAMP is high during late day and late night,[178] suggesting a role for cAMP in the transition periods between day and night. It can be hypothesized that by increasing cAMP, these daytime resetting signals are moving the animal to a state that resembles late day, thus resetting the clock to that time.

Dawn and Dusk

The primary resetting signal associated with dawn and dusk is melatonin. This "hormone of darkness" 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 hamster and sheep, that are seasonally reproductive. Melatonin is produced by the pineal gland, and in lower vertebrates, such as fish, lizards, and some birds, the pineal, rather than the SCN, is the primary regulator of circadian rhythms. However, in mammals this timekeeping mechanism has moved to the SCN, as demonstrated by the fact that removal of the pineal does not significantly disrupt circadian rhythms of rats.[179]

Although the pineal is not necessary for maintenance of mammalian circadian rhythms, it is possible to entrain free-running rats with daily injections of melatonin. Entrainment appears to work best if the melatonin injections are timed to occur shortly before the onset of the animal's active period. This entrainment appears to be working through the SCN, as

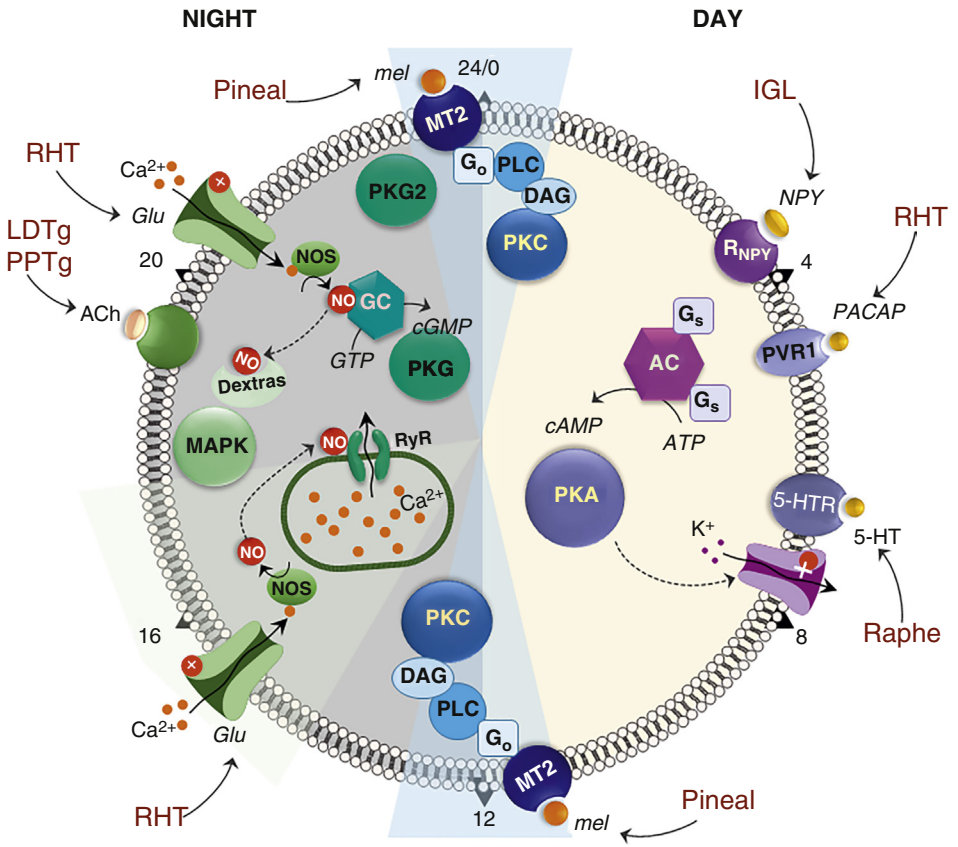

Figure 36.6 Temporally restricted sensitivity of the suprachiasmatic nucleus (SCN) to activation of discrete pathways with distinct effects on clock phasing. Schematic representation of the 24-hour circadian cycle comprising four major time domains (daytime, dawn/dusk, early night, and late night) with respect to temporal sensitivities of the SCN to various signaling pathways. The circadian clock controls the opening and closing of the windows of sensitivity to activation of these pathways so they are temporally relevant and thus convey a signal that is imbued with temporal information. AC, Adenylate cyclase; ACh, acetylcholine; cGMP, cyclic guanosine monophosphte; DAG, diacylglyceride; G_o, G-protein o; G_s, G-protein stimulating AC; GC, guanylate cyclase; GTP, guanosine 5′-triphosphate; 5-HT, 5-hydroxytryptamine/serotonin; IGL, intergeniculate leaflet of the thalamus; LDTg/PPTg, laterodorsal tegmentum and pediculopontine tegmentum of the brainstem; MAPK, mitogen-activated protein kinase; mel, melatonin; MT2, melatonin receptor type 2; NO, nitric oxide; NOS, nitric oxide synthase; NPY, neuropeptide Y; PKA, cAMP-dependent protein kinase; PKC, protein kinase C; PKG, cGMP-dependent protein kinase; PLC, phospholipase C; PVR1, PACAP/VIP receptor type 1; RHT, retinohypothalamic tract; RyR, ryanodine receptor.

lesioning the SCN, but not the pineal, abolishes the ability of a rat to entrain to melatonin injections.[180]

Evidence that melatonin can entrain circadian rhythms led to a number of studies looking at the direct effect of melatonin on the SCN. Melatonin application immediately before dusk in rat or hamster tissue in vitro decreases SCN metabolic or neuronal activity, measured by 2-deoxy-[1-^{14}C]glucose (2-DG) uptake or neuronal firing rate.[181-183] Additionally, melatonin applied to SCN brain slices at either dawn or dusk advances the peak in neuronal firing. Melatonin is ineffective when applied at other times of day.[184,185] This resetting pattern is reproduced by direct activation of protein kinase C (PKC) and can be blocked by inhibitors of PKC, suggesting that PKC is a downstream component of this resetting pathway.[185] In addition, phase-resetting effects of melatonin are inhibited by antagonists specific for the MT-2 type melatonin receptor.[186] In humans, circadian sensitivity to melatonin also occurs at dawn and dusk, but the effect is to advance the circadian system at dusk and to delay it at dawn, in antiphase to the effects of light at night.

Nighttime

In the nighttime domain, there are two known key neurotransmitters, GLU and acetylcholine (ACh), as well as a number of modulatory substances associated with these signals. As was discussed previously, considerable evidence supports GLU as the neurochemical signal transmitting photic stimuli from the retina to the SCN. Cholinergic innervation of the SCN comes from brainstem regions involved in sleep regulation and from the basal forebrain.[131,187,188]

The GLU signaling pathway is similar to many of the pathways that already have been discussed in that it resets the circadian clock at a discrete time of day and in a specific direction. The GLU signaling pathway can either advance or delay the clock, depending on what time of day the signal is presented.[82,189] The GLU resetting pathway has been demonstrated both in vitro and in vivo to be mediated through an

N-methyl-D-aspartate (NMDA) receptor–mediated rise in intracellular Ca^{2+}, followed by nitric oxide synthase activation and production of nitric oxide (NO).[82,190-193] Downstream, the early and late night pathways diverge. During the early night, GLU induces delays in the circadian clock through ryanodine receptor (RyR)-mediated Ca^{2+}-induced Ca^{2+}-release.[194] GLU exposure during the late night, however, advances the circadian clock through a cyclic guanosine monophosphate/protein kinase G (cGMP/PKG) signaling cascade followed by cAMP response element-binding (CREB) protein-activated transcription.[194-196]

Although GLU alone is capable of resetting circadian rhythms, there are many substances that modulate this resetting. These can be divided into two categories: those that decrease the amplitude of the phase-resetting effect of GLU during both the early and late night, which include NPY and GABA,[107,159] and those that have differing effects on GLU-induced phase shifts, depending on what time of night they are applied.

This second category of time-dependent modulators includes 5-HT and PACAP. If animals are depleted of 5-HT, they show increased phase delays in response to light.[197,198] Co-application of a PACAP antagonist, however, either in vitro or in vivo, decreases the phase delay in early night, and when applied during late night, increases the amplitude of the phase advance in both rat and hamster.[199,200] When PACAP is administered in conjunction with GLU in early night, it increases the amplitude of delay, but in late night it decreases the phase advance. This is similar to the effects seen after application of cAMP analogues to the hypothalamic brain slice, suggesting that the effects of PACAP may be mediated via a cAMP pathway.[200,201]

The role of ACh in resetting circadian rhythms has been unclear, with much of the confusion arising from the fact that its effects vary depending on the site of application. The first evidence that ACh may play a role in resetting the circadian clock was discovered when Zatz and Brownstein examined whether pharmacologic manipulation could affect circadian rhythms.[202] They found that injections of the ACh agonist carbachol into the lateral ventricle of Sprague-Dawley rats at CT 15 caused phase delays that were similar to, but not as large as, the phase delays produced by light.[202] Carbachol injections into the lateral ventricle were also later repeated in mice[203] and hamsters,[204] where it was found that administration of carbachol during early night caused phase delays, whereas late night administration caused phase advances.

This pattern of sensitivity and responses is similar to that previously demonstrated in response to light or GLU. Support for the involvement of ACh in the light response came from studies looking at ACh levels in the rat SCN using a radioimmunoassay.[205] Using this technique, no significant oscillation in ACh levels was found under constant conditions, but light pulses administered at CT 14 were found to increase ACh levels in the SCN. However, only one time-point was examined, so it is not known whether this increase was simply a response to exposure to light or if there was actually a circadian pattern to the light-stimulated release. The implication of these studies was that ACh could be the primary neurotransmitter providing the signal of light to the clock.

However, significant evidence began to emerge, indicating that ACh was unlikely to be the primary signal of light. First, whereas it had previously been determined that the RHT transmitted the signal of light from the eye to the SCN, it was found that choline acetyltransferase was not present in this projection.[206] This made it anatomically unlikely that ACh was the primary neurotransmitter involved in this signal.

Additional evidence against ACh being the signal of light came from experiments that found intracerebroventricular (*icv*) injections of hemicholinium, which significantly depletes ACh stores in the brain, did not block the ability of the animal to phase shift in response to light.[207] There was also evidence that injecting NMDA receptor antagonists could block carbachol-induced phase shifts, suggesting that although ACh may play a role in the light response, it must be upstream of a glutamatergic signal.[208] Finally, Liu and Gillette,[209] using extracellular recording in vitro, found that microdrop applications of carbachol directly to the SCN caused only phase advances, regardless of whether the carbachol was applied early or late in the night.

In an attempt to explain these contradicting data, it was hypothesized that the dual response pattern of the SCN to cholinergic stimulation was a result of the location of stimulation. Note that in the initial in vivo studies, carbachol was injected into the lateral or third ventricle, where the drug could have diffuse effects, while in the in vitro studies carbachol was applied in microdrops directly to the SCN. If the in vivo experiments were performed by injecting carbachol directly into the SCN rather than into the ventricle, a phase response pattern similar to that observed in the in vitro experiments using microdrop applications resulted.[187] This suggests that ACh has at least two different effects on the circadian clock, depending upon the site of application. There is an indirect response, working through ventricular pathways, that is likely an upstream of a glutamatergic signal, and a direct response in the SCN that is mediated by the M_1AChR.[210] Based on the anatomic studies tracing cholinergic projections to the SCN that originate in the LDTg and PPTg, the current hypothesis is that this cholinergic signal may be involved in linking sleep and wakefulness with circadian cycles.[124]

COUPLING OF CENTRAL AND PERIPHERAL CLOCKS

Because circadian clocks are fundamental components of cells, it follows that there are myriad individual oscillating clocks in the body. In an intact organism, these clocks are aligned so that each individual tissue maintains a stable phase relationship to the SCN so that clock genes are expressed at the same time each day. When the SCN is removed or the phase is shifted, cells of various tissues maintain their individual circadian rhythms, but they quickly fall out of phase with each other.[211-213] This indicates a hierarchic relationship in which the SCN is the master regulator that synchronizes and aligns the rest of the body's clocks. Many studies of the coupling of extra-SCN clocks to the central pacemaker have been undertaken; several examples are highlighted in the following discussion.

Early SCN-isolation studies established the SCN's role as the master clock necessary for orchestrating the rest of the body clocks. These studies also hint at the various means by which the SCN exerts control over peripheral structures. When the SCN is surgically isolated from the rest of the hypothalamus in rats, oscillations in serum corticosterone levels continue, while locomotor rhythms are lost.[214] Additionally, surgical cuts between the SCN and PVN abolish

reproductive rhythms in hamsters, but rhythmic locomotor activity is maintained in hamsters[215,216] and rats.[217] These findings provided early evidence for both synaptic coupling of SCN to output tissues, as well as the possibility that humoral signals entrain peripheral tissues. This idea was furthered by transplantation studies in which an encapsulated fetal SCN is transplanted into an animal with SCN lesions.[18] Fenestrations in the encapsulating polymer were too small to permit neurite passage, and, indeed, no neural connectivity to the recipient brain could be found. The transplant restored locomotor, feeding, drinking, body temperature, and sleep-wake, but not endocrine, rhythms to the lesioned animal. Clearly, some non-SCN rhythms require physical connections and some do not.

Many brain regions are coupled to the SCN by synaptic connections. Anatomic studies have shown SCN projections that extend to several hypothalamic nuclei, including the organum vasculosum of lamina terminalis (OVLT), MPOA, and PVN, forming direct synapses with gonadotropin-releasing hormone and corticotropin-releasing hormone neurons in these regions.[218-220] Additionally, the neuronal networks connecting the SCN to the IGL and PVT of the thalamus mediate with bi-directional communication between the SCN and these sleep/arousal modulatory regions.[93,147,148]

The SCN is one of many regulators of sympathetic and parasympathetic autonomic signals to peripheral organs. Anatomic studies using retrograde tracers injected into peripheral organs, such as liver, adrenal gland, pancreas, and adipose tissue, reveal a multisynaptic pathway connecting these tissues to autonomic centers in the spinal cord, brainstem, PVN, DMH, SCN, and other hypothalamic regions.[221-224] Tracing of either sympathetic or parasympathetic tracts identifies SCN neurons in overlapping areas of the nucleus, however these neurons seem to be involved in signaling to one or the other of these pathways.[222,223] Light from the external environment can affect these two pathways through SCN-mediated control. For example, exposure of rats to light at night results in increased sympathetic activity and suppressed parasympathetic activity. When the SCN is abolished, this effect is lost.[225] Also, heart rate decreases after light exposure at night in a nocturnal rodent, whereas SCN-lesioned animals do not exhibit this response.[226] Thus the SCN plays a role in modulating autonomic signals to the periphery, but it works in concert with many other regions of the brain, including those regulating body temperature, metabolism, reproductive state, and other physiologic functions.

A growing body of evidence supports a role for humoral signaling in coupling of rhythms between the SCN and other regions. In brain-slice cultures containing PVN tissue, an electrical rhythm emerges in the PVN only after co-culture with an SCN brain slice. The lack of neuronal connections between the two slices in vitro strongly supports a diffusible factor as mediator of the electrical oscillation of the PVN.[227] Additionally, parabiosis experiments connecting the circulatory system of an intact mouse to that of an SCN-lesioned mouse indicate that diffusible signals from the intact animal can entrain peripheral organs (liver and kidney) in the lesioned recipient.[228] Co-culturing SCN tissue with peripheral cells or tissue induces rhythms in these cells that follow the SCN under culture conditions that prevent synaptic connections.[229-231]

These studies indicate that diffusible signals can modulate rhythms between the brain and body. Neuropeptides are abundant in the SCN[59] and are good candidates for humoral signals. As described previously, major neuropeptides found in the SCN include VIP, GRP, little SAAS, and AVP, among others. These peptides are released from the SCN in a circadian fashion,[58,65,71] and each has been implicated in a physiologic role in some aspect of circadian biology.[65,67,71,232-238] Identification of the diffusible signals that couple other tissues to the SCN is currently the subject of intense study, with high therapeutic potential.

Another role for diffusible factors from the SCN may be to provide a signal inhibitory to behavioral activity. Two candidate factors for communicating such signals include transforming growth factor-α (TGF-α) and prokineticin 2 (PK2). Under normal conditions, TGF-α peptide 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 cerebral ventricles, TGF-α fully inhibits locomotor activity. Conversely, mice lacking the cognate receptor, epidermal growth factor (EGF) receptor, are unable to respond to TGF-α and show an excessive amount of daytime activity.[239] PK2 also is expressed rhythmically in the SCN, again showing peak expression during the animal's inactive period, and can inhibit locomotor activity when infused continuously.[240] This suggests a role for output signals from the SCN in promoting an inactive state that would be permissive for sleep.

Some tissues appear to require both synaptic and humoral signals to synchronize to the SCN. When autonomic innervation to the liver is severed, plasma insulin and corticosterone levels remain rhythmic, but plasma glucose levels do not.[241] However, liver tissue from an SCN-lesioned mouse with surgical parabiosis with an intact animal recovers and continues to maintain rhythmicity from that point onward.[228] This suggests that control of liver timing requires both neural and diffusible signals that coordinate separate physiologic functions. Dissecting the intricacies of circadian regulation among peripheral tissues requires careful study.

Coupling of the SCN to peripheral targets, regardless of the manner of this connection, has important implications for health. This interaction allows for synchronization of internal systems to environmental light signals, both on a day-by-day basis and to adjust the animal to seasonal changes. Modern human activities, such as shift work and transcontinental flight, result in significant desynchronization of the central internal clock and various body tissues. This circadian disarray can have significant negative consequences for human health, including increased risks of various cancers, reproductive health, stroke, metabolic syndrome, cardiovascular disease,[242-244] and overall mortality in older individuals.[245]

HEALTH AND DISEASE

Disturbance of the circadian timing system is linked to adverse health effects associated with a loss of synchronization between central and peripheral oscillations. Estimates suggest 10% to 20% of the entire genome displays rhythmic expression in any given tissue or organ,[246,247] and this provides a link to the circadian system to health and disease.[248] A number of core clock elements play critical roles in human sleep disorders. For example, inherited forms of advanced sleep-wake phase disorder are associated with a mutation in the *Per2* gene that alters a normal phosphorylation site of CKIδ/ε[249] or with a mutation in CKIδ.[250] Delayed sleep-wake phase disorder,

which is prevalent in almost 10% of the population,[251,252] has been found to be associated with specific polymorphisms of PER3,[32,253,254] CRY1,[255] and a missense variant of PER2.[256] PER3 expression patterns in human leukocytes correlate with sleep-wake timing, particularly in those individuals with a preference for morningness.[257] Further, morningness or eveningness preferences have been associated with polymorphisms of the human *Clock* gene.[32,258,259]

A number of clock gene mutations in rodents have demonstrated adverse physiologic effects. $Clock^{\Delta 19}$ mice are moderately more susceptible to cancer and display a marked metabolic phenotype involving obesity, dyslipidemia, hepatic steatosis, and hyperglycemia.[260,261] Interestingly, the $Clock^{-/-}$ mice (mice deficient in the *Clock* gene because of targeted gene knockout) do not exhibit the same phenotype as the mice with a partial deletion, $Clock^{\Delta 19}$. $Clock^{-/-}$ mice have a reduced life span, age-related cataract development, and increased risk for dermatitis.[41,42,262] Differences between $Clock^{\Delta 19}$ and $Clock^{-/-}$ mouse models likely arise from the fact that the $Clock^{\Delta 19}$ mice have a dominant negative mutation in CLOCK, whereas $Clock^{-/-}$ are deficient of CLOCK altogether and may experience compensation from upregulation of NPAS2.

Humans often voluntarily override signals from their circadian clock and disconnect their sleep-wake and feeding cycles from their external environment. Under such circumstances, irregular phase relationships are expressed between rhythmic process (such as sleep-wake behaviors and feeding-fasting states) and the circadian clock. Although the internal desynchronizes that occur with jet lag and shift work may be the most dramatic, they are not the only examples of real-world circadian disruption. Widespread occurrence of social jet lag resulting from social overstimulation, work schedules, and the use of artificial lighting may occur in people living under relatively stable entrained conditions. Indeed, many phase-shift their sleep and feeding times they when return to the workdays after free-running according to their clock-time during weekends and holidays.[263] In sum, our around-the-clock society and our interaction with it tend to oppose human evolutionary selection toward diurnality, often with negative health consequences.

CONCLUSION

Circadian rhythms, the near–24-hour oscillations in brain and body functions such as core body temperature, hormone release, and the sleep-wake cycle are embedded in the physiology of cells and tissues. The master pacemaker regulating these rhythms, the SCN, is optimally situated in the hypothalamus to receive input about environmental light, sleep-wake state, and activity status. The SCN alters its phasing in response to changes in environmental conditions and internal states. This change in SCN state, in turn, alters the timing of output signals that regulate the timing of rest/activity and behavioral cycles.

The core mechanisms of timekeeping are encoded in transcription/translation feedback loops of evolutionarily conserved clock genes. The molecular clockwork comprises both positive and negative elements, coupled with other intracellular elements associated with signaling events. Proteins encoded by clock genes are targets of molecular tools to further study clocks in diverse tissues and to treat desynchronized conditions.[264] They are revealing how the SCN synchronizes these various body clocks to environmental cycles and imposed work schedules, and what changes with disease. Circadian-rhythm sleep phenotypes as well as sleep disorders correlate with abnormalities in the genes regulating circadian rhythms. Why internal desynchrony of peripheral tissues with the SCN has negative consequences for human health and longevity remains unknown. New research is necessary for discovering therapeutic mechanisms for timing disorders of the SCN and peripheral clocks as well as treatments that resynchronize the molecular clockworks in health and disease.

CLINICAL PEARL

Circadian rhythms emerge from molecular clockworks that bear the tremendous genetic diversity of the human family and are present throughout the human body. This genetic diversity, as well as contemporary lifestyles, can lead to circadian dysregulation that initiates or exacerbates chronic and acute disease. Dysregulation of the circadian system is linked to increased risk of metabolic, cardiovascular, immunologic, and neurologic disorders, and, notably, disruption of sleep-wake cycles. Light occurring at inappropriate times during the dark phase of the circadian cycle as well as alteration in neurotransmitters and small-molecule profiles can alter sleep-wake patterns, leading to decrements in overall health. Coordination of circadian rhythms, both internally and with respect to the environment or disease state, is necessary for robust health and longevity.

SUMMARY

The SCN of the hypothalamus is the master pacemaker for the mammalian circadian system. The many coupled cellular oscillators within the SCN generate rhythmicity through dynamic, cell-based, transcriptional/translational feedback loops. These molecular feedback loops are formed of positive and negative transcriptional elements that regulate the clock genes that encode them. The activity of the core molecular loop interacting with cellular redox state generates circadian rhythms in SCN electrical activity and neurotransmitter and neuropeptide release, resulting in the transmission of circadian timing signals to downstream oscillators throughout the brain and body. The circadian pacemaker is entrained by environmental light-dark cycles as well as other rhythmic stimuli by restricting its own sensitivity to various entraining signals to discrete temporal windows throughout the circadian cycle. The circadian system regulates the timing of sleep and wakefulness, and disorders can arise within this system. Good health, well-being, and longevity depend on robust internal synchronization both within and between tissues throughout the brain and the body.

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REVIEW QUESTIONS

1. Lesioning of the suprachiasmatic nucleus (SCN) results in which of the following?
   - **A.** Loss of coherent free-running circadian rhythms
   - **B.** Loss of the ability of light-dark cycles to synchronize circadian rhythms
   - **C.** Loss of behavioral activity rhythms but not of physiologic rhythms
   - **D.** Loss of physiologic rhythms but not of behavioral rhythms

2. Approximately how much of the genome is expressed rhythmically under the control of the molecular clockwork oscillator in any given tissue or organ?
   - **A.** 1% to 2%
   - **B.** 10% to 20%
   - **C.** 40% to 60%
   - **D.** 75% to 90%

3. Which of the following statements best describes the distribution of autonomous cellular circadian clocks?
   - **A.** Circadian clock cells are found only in the SCN.
   - **B.** Circadian clock cells are found only in the SCN and in a small number of other regions within the central nervous system.
   - **C.** Circadian clock cells are found in the SCN, in other regions within the central nervous system, and in peripheral tissues and organs.

4. Which of the following best describe(s) elements responsible for circadian light entrainment in mammals?
   - **A.** Melanopsin-containing retinal ganglion cells that respond to blue-light wavelengths
   - **B.** Glutamatergic/PACAPergic projections via the retinohypothalamic tract to the SCN
   - **C.** Insensitivity of the circadian clock during daytime to light signals from the eye
   - **D.** All of the above

5. Which of the following best describes the function of the SCN?
   - **A.** Imposition of circadian rhythmicity throughout the brain and body
   - **B.** Sustainment of circadian rhythmicity throughout the brain and body
   - **C.** Synchronization of circadian rhythmicity throughout the brain and body

ANSWERS

1. A
2. B
3. C
4. D
5. C