Neurobiology of Circadian Rhythm Regulation

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KEYWORDS

• Circadian • Pacemaker • Suprachiasmatic nucleus • Entrainment • Clock genes

KEY POINTS

- The suprachiasmatic nucleus (SCN) of the anterior hypothalamus has been firmly established as the master circadian pacemaker in mammals.
- The SCN circadian pacemaker is synchronized (entrained) by environmental light-dark cycles via photoreceptors and neural pathways distinct from those mediating visual perception.
- The cellular-molecular basis of circadian rhythm generation involves several circadian clock genes expressed not only in the SCN but also throughout the brain and peripheral tissues and organs.
- The SCN serves as a central pacemaker atop a hierarchically organized, anatomically distributed circadian timing system and entrains downstream circadian clocks via neural and neuroendocrine pathways.
- System-wide circadian coordination is necessary for optimal physiologic function and maintenance of physical and mental health.

IDENTIFICATION OF THE SUPRACHIASMATIC NUCLEUS CIRCADIAN PACEMAKER

The initial demonstrations that lesions of the SCN severely disrupt or abolish circadian rhythms in behavioral and endocrine functions were published in the early 1970s.[1,2] Following these initial demonstrations, extensive subsequent research involving lesions, in vivo and in vitro electrophysiology, functional metabolic mapping, fetal tissue transplant, and molecular analyses revealed that the SCN is capable of autonomous, self-sustained circadian rhythmicity at both the single-cell and tissue levels. These now-classic studies are summarized in the published report of a meeting held to evaluate the state of SCN research on the 25th anniversary of its discovery.[3]

SUPRACHIASMATIC NUCLEUS: A NETWORK OF CLOCK CELLS

Studies using a variety of in vitro models, including electrophysiological recording and optical monitoring of SCN cell and tissue cultures, have provided compelling evidence that circadian oscillation is fundamentally a cell-autonomous process, expressed in many, but probably not all, individual SCN neurons.[4–7] Nevertheless, individual SCN clock cells normally interact to produce coherent circadian signals at the tissue (and behavioral) level.[8–10] Despite the capacity of individual SCN neurons for autonomous rhythmicity, recent studies have revealed that neuronal network interactions increase the frequency of rhythmic cells detected in culture, as well as the amplitude of their

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oscillation, and contribute to the overall robustness of SCN pacemaker function.[11,12]

Early studies suggested that SCN clock cells could maintain intercellular synchrony in the absence of sodium-dependent action potentials,[13,14] suggesting that gap junctions, glial coupling, calcium-dependent signaling, or local diffusible signals might be responsible for synchronizing the network of clock cells.[15] In contrast, however, more recent studies have found that blocking action potentials in SCN tissue slices or cell cultures can disrupt intercellular phase synchrony,[10] thus reviving interest in the possible synchronizing role of synaptic transmission. Both γ-aminobutyric acid (GABA) and vasoactive intestinal peptide (VIP) neurotransmission, among other signaling mechanisms, have now been implicated in the maintenance of coupling among SCN clock cells, as well as among subpopulations of SCN clock cells.[16,17]

MOLECULAR BASIS OF THE SUPRACHIASMATIC NUCLEUS CIRCADIAN PACEMAKER

A critical role for protein synthesis in the mammalian circadian pacemaker was established in the late 1980s,[18,19] and elucidation of the fundamental molecular genetic oscillatory mechanism began in earnest about 10 years later. The first mammalian circadian clock gene, *Clock*, was identified in a forward-genetics mutagenesis screen,[20] and this discovery was followed quickly by the identification of several other core molecular clock components, some of which were homologous to previously discovered circadian clock genes in the fruit fly.[21,22] In addition to *Clock*, other recognized mammalian clock genes include the 3 period (*Per*) genes (*Per1, Per2, Per3*), 2 cryptochrome genes (*Cry1* and *Cry2*), *Bmal1* (also known as *Arntl1 and Mop3*), *CK1e* (*Casein kinase 1 epsilon*), *Rev-erba,* and *Fxbl3*, all of which are expressed in SCN neurons. The specific functions of these various genes within the interlocking molecular feedback loops that generate circadian signals at the cellular level have been reviewed extensively elsewhere, and are not discussed here.

Mutations or deletions of any of these genes produce alterations in circadian phenotype at the behavioral level. The most devastating effects on clock function are seen in *Bmal1* knockout mice, which express immediate loss of rhythmicity in the absence of a light-dark cycle.[21,22] In contrast, the original *Clock* mutation, which codes for a dominant-negative CLOCK protein, dramatically lengthens free-running period and often leads to a gradual loss of rhythmicity under long-term free-running conditions.[21,22] Surprisingly, however, unlike the original *Clock* mutation, *Clock*-null (knockout) mice express robust and persisting circadian rhythms, with only a modest shortening of circadian period[23]; it was subsequently found that NPAS2 can substitute for CLOCK as a dimerization partner for BMAL1 within the SCN, thus maintaining circadian pacemaker function.[24] Regarding the Per genes, several distinct mutations have been studied by different laboratories, but in general, *Per1* or *Per2* disruption shortens circadian period and reduces the robustness of free-running rhythms.[25,26] Similarly, *Cry* mutant mice also exhibit alterations in the free-running period, whereas *Cry1/Cry2* double mutants are rendered arrhythmic.[27,28] In contrast to other clock genes, the circadian clock function of *Ck1e* was discovered by genetic analysis of a spontaneous single-gene mutation that dramatically shortens free-running period in the hamster, originally called the *tau* mutation.[29] Cloning of the *tau* gene revealed its identity as *Ck1e*, and subsequent transgenic insertion of this allele into mice recapitulated the hamster short-period phenotype, whereas deletion of *Ck1e* in mice lengthened the circadian period.[30] More recently, mutations of the *Fxbl3* gene have been shown to lengthen the free-running period.[31,32] Like *Ck1e, Fxbl3* influences circadian period by regulating the posttranslational stability of other clock proteins such as PER and CRY.[33] Of course, in order for the molecular clock to drive circadian rhythmicity in physiology and behavior, clock gene expression must be linked to intracellular signaling pathways regulating neuronal membrane potential, and ultimately, firing rate. Remarkably, recent research demonstrates that ionic events at the cell membrane influence the molecular clock via some of the same intracellular signals that convey clock signals to the membrane, and in some cases, these ionic currents may be necessary for self-sustainment of the molecular clock.[34] Such results—at a minimum—serve to blur the distinction between the core clock mechanisms and the so-called hands of the clock.

In addition to their effects on circadian behavior, some circadian clock gene mutations also affect sleep-wake homeostasis, and several forms of affective behavior, suggesting possible molecular links between the circadian, sleep regulatory, and motivational systems of the brain.[35–37]

FUNCTIONAL ARCHITECTURE OF THE SUPRACHIASMATIC NUCLEUS

Although the SCN was initially characterized as being composed of distinct ventrolateral and

dorsomedial subdivisions,[38] this scheme has been recast to include SCN core and shell subnuclei, a concept that may better accommodate species differences in the anatomic distribution of neuropeptides, afferent terminal fields, and gene expression patterns in the SCN.[39] The SCN core is associated with a high concentration of VIP-positive and gastrin-releasing peptide (GRP)–positive neurons, while the shell is associated with the presence of arginine vasopressin–positive neurons. Beyond this basic organization, however, clear species differences have been noted, and it has been argued that the popular distinction between SCN core and shell may be such an extreme oversimplification as to impede understanding of the functional organization of this critical structure.[40,41]

Although the specific functions of chemically defined SCN cell populations are not fully known, VIP and GRP neurons of the SCN core appear to collate afferent signals relevant to pacemaker entrainment, whereas vasopressinergic (or other) neurons of the SCN shell may have the primary responsibility of generation of self-sustaining circadian oscillations.[42] A preeminent role for the SCN core in pacemaker entrainment is supported by findings that major SCN afferent systems converge in the core subnucleus, administration of SCN core peptides such as VIP and GRP can mimic both light-induced phase shifting and *Per* gene expression in the SCN in vivo and in vitro, and light-evoked changes in SCN physiology and gene expression spreads over time from core to shell. Conversely, evidence for a preeminent role of the SCN shell in pacemaking includes findings that the core projects robustly to the shell, but not vice versa, spontaneous circadian rhythmicity in neuronal activity, neuropeptide release, and gene expression is seen more reliably in the shell than in the core, and spontaneous rhythmicity in SCN gene expression seems to flow from the most dorsomedial toward more central-lateral regions over the course of the circadian cycle. On the other hand, the view that SCN core and shell underlie discrete entrainment and pacemaking functions is probably too simplistic, because (1) several arousal-related afferents of limbic and brainstem origin target the SCN shell,[39] (2) in vitro studies have revealed independent free-running rhythmicity in the secretion of core and shell peptides from the same tissue explant,[43] and (3) SCN core and shell can exhibit stable dissociation of rhythmic gene expression in vivo under certain conditions.[44] Furthermore, studies using microlesions indicate that the integrity of the SCN core is essential for the maintenance of high-amplitude behavioral and molecular-level rhythmicity, suggesting that rhythmic signals from the core serve a permissive gatelike role in sustaining oscillatory function in the shell.[42]

LIGHT INPUT TO THE SUPRACHIASMATIC NUCLEUS: THE RETINOHYPOTHALAMIC TRACT

Although stimuli such as temperature, sound, food, and social cues seem to contribute to phase control, the 24-hour environmental light-dark cycle is the primary cue for circadian entrainment in most mammalians (and other vertebrate taxa). A specialized retinal projection system, referred to as the retinohypothalamic tract (RHT), is both necessary and sufficient for photic entrainment of the circadian pacemaker[45,46] (**Fig. 1**). The RHT originates from a distinct subset of retinal ganglion cells, separate from those giving rise to the primary visual pathways,[47] and terminates mainly in the SCN, as well as more sparsely in the anterolateral hypothalamus, subparaventricular zone, and supraoptic region.[48,49] In addition, RHT axon collaterals also project to the thalamic intergeniculate leaflet (IGL), which, as discussed later, is an important component of the circadian system.

Remarkably, retinally degenerate strains of mice, in which nearly all classic photoreceptors (ie, rods and cones) are lost by early adulthood, exhibit normal circadian responses to light.[50] More recently, similar findings have been reported in genetically engineered mice with a total developmental absence of both rods and cones, demonstrating conclusively that circadian light entrainment can be mediated by a novel, nonrod, noncone photoreceptor system.[51] Circadian entrainment in the absence of rods and cones is maintained by a population of intrinsically photosensitive retinal ganglion cells that use the peptide melanopsin as a photopigment.[52,53] Nevertheless, circadian entrainment is maintained in melanopsin knockout mice[54,55] and is only fully abolished when both classical and melanopsin-based photoreception is eliminated demonstrating redundancy in the circadian photoreception system in the retina.[56–58]

RHT terminals release the excitatory amino acid neurotransmitter, glutamate, which acts through both *N*-methyl-D-aspartate (NMDA) and non-NMDA receptors and a variety of intracellular signaling pathways to increase *Per* gene expression. These changes in gene expression, when superimposed on the ongoing circadian transcription-translation cycle, correspond functionally to phase shifts of the circadian oscillator.[59–62] In addition to glutamate, RHT terminals also release 2 identified peptide cotransmitters,

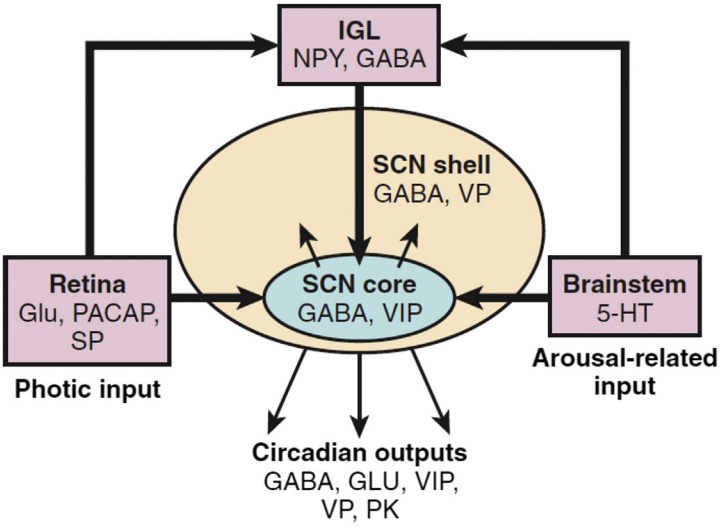

Fig. 1. Overview of functional neuroanatomic pathways in the mammalian circadian system. Major SCN afferent systems originating in the retina and raphe nuclei also target the intergeniculate leaflet (IGL) of the thalamus, which itself projects to the SCN. Retinal projections to the SCN and IGL mediate photic input to the circadian system, whereas raphe projections to the SCN and IGL mediate the effects of certain nonphotic, behavioral state–related signals. Furthermore, raphe-IGL-SCN circuits also modulate and integrate photic and nonphotic signaling to the SCN pacemaker. SCN outputs mainly target the hypothalamus and diencephalon and appear to include both neural efferents and secreted paracrine signals. 5-HT, 5-hydroxytryptamine; GLU, glutamate; NPY, neuropeptide Y; PACAP, pituitary adenyl cyclase–activating peptide; PK, prokineticin; SP, substance P; VP, arginine vasopressin; VIP, vasoactive intestinal polypeptide.

substance P (SP) and pituitary adenyl cyclase–activating peptide (PACAP). SP seems to play an important role in RHT transmission because selective SP antagonists block light-induced phase shifting and immediate-early gene expression in vivo, as well as glutamate receptor–mediated phase shifting in vitro, whereas SP administration can itself induce circadian phase shifts.[63–65] In contrast, PACAP administration has been reported either to antagonize or to mimic the effects of glutamate on circadian phase shifting and *Per* gene expression in vitro, depending on the dose and the circadian phase of administration.[66–68]

OTHER FUNCTIONAL INPUTS TO THE SUPRACHIASMATIC NUCLEUS

An additional major SCN afferent system arises from the IGL, a distinct retinorecipient region of the lateral geniculate complex, intercalated between the dorsal and ventral lateral geniculate nuclei[69–71] (see **Fig. 1**). The projection from the IGL to the SCN is referred to as the geniculohypothalamic tract (GHT), and GHT neurons release both neuropeptide Y and GABA. Retinal signals are conveyed to the IGL in part by axon collaterals of RHT neurons,[72] and GHT and RHT terminal fields are largely coextensive within the SCN core. Thus, the IGL/GHT system provides a secondary, indirect pathway by which light signals can reach the circadian pacemaker. Although the IGL is clearly not necessary for photic entrainment, lesions of the IGL/GHT system result in subtle modifications in the photic control of circadian phase and period.[70,71] Furthermore, the IGL may have a significant role in entrainment under more naturalistic lighting conditions (eg, regimens including twilight transitions, seasonally changing photoperiod, or simulated moonlight), relative to the square-wave light-dark cycles commonly used in the laboratory.[72]

In addition to providing a secondary, indirect source of photic signaling to the circadian clock, the IGL also plays a preeminent role in the regulation of the circadian system by nonphotic, arousal-related stimuli. Thus, IGL lesions abolish the phase-shifting effects of novelty induced wheel running and benzodiazepine administration in hamsters, as well as the period-shortening effect of running-wheel access in rats and the entrainment effect of scheduled daily treadmill activity in mice.[73–78] More recently, evidence has been presented that IGL neurons may mediate the effects of metabolic signals on the SCN pacemaker.[79,80]

A third major afferent system converging mainly on the SCN core originates from the serotonergic midbrain raphe, especially the median raphe nucleus[81–84] (see **Fig. 1**). In addition, ascending serotonergic projections originating in the dorsal raphe nucleus innervate the IGL, providing a second route for serotonergic regulation of the SCN circadian pacemaker.[81,82] As for the IGL itself, extensive evidence has implicated serotonergic projections to the SCN and IGL in modulation of photic effects on the circadian pacemaker, as well as in the mediation of nonphotic effects on the pacemaker. These effects seem to be mediated via 5-hydroxytryptamine $(5\text{-HT})_{1A}$ and 5-HT_7

receptors within the SCN, the IGL, and the raphe nuclei, and by 5-HT_{1B} receptors located presynaptically on RHT terminals.[85–89]

In addition to the RHT, GHT, and 5-HT projections, several other identified pathways provide afferent input to the circadian system, including noradrenergic projections from the locus coeruleus, cholinergic projections from the basal forebrain and pontine tegmentum, and histaminergic projections from the posterior hypothalamus.[90,91] Like serotonergic projections, noradrenergic and cholinergic projections also innervate the IGL, providing an alternate pathway by which these transmitter systems could alter SCN function. However, unlike the RHT, GHT, and 5-HT projections to the SCN, which form generally overlapping terminal fields in the SCN core, noradrenergic, cholinergic, and histaminergic SCN inputs preferentially target the SCN shell. Beyond these systems, a recent review concluded that the SCN receives direct monosynaptic projections from at least 35 distinct brain areas,[41] revealing enormous potential for circadian pacemaker modulation by a wide range of extrinsic and intrinsic stimuli.

SUPRACHIASMATIC NUCLEUS OUTPUT PATHWAYS

Perhaps surprisingly, first-order SCN efferents innervate a relatively small number of target areas concentrated mainly in the diencephalon and basal forebrain.[92,93] These SCN targets then relay circadian timing signals to autonomic and neuroendocrine systems, as well as to central structures regulating affective, sensory, and motor processes, as well as to sleep-regulatory brain regions.[94] SCN efferents emerge from both the core and shell subnuclei and release several neurotransmitters and peptides including GABA, glutamate, and vasopressin (see **Fig. 1**). Remarkably, anatomically distinct populations of SCN neurons seem to innervate specific efferent targets, providing multiple waves of neuronal signals that regulate circadian phase in a target-specific manner. Despite earlier evidence that neuronal efferents were exclusively responsible for conveying SCN output signals,[95] it now appears that the SCN regulates certain rhythmic processes via diffusible paracrine signals. Evidence for a diffusible SCN output signal was first suggested by the finding that surgical isolation of the hamster SCN within a hypothalamic island abolished SCN-dependent neuroendocrine responses but allowed for persisting locomotor activity rhythms in the same animals.[96] The hypothesis for paracrine signaling from the SCN in the regulation of rhythmic processes was strengthened considerably by the finding that transplant of SCN tissue encased within a semipermeable capsule could restore locomotor but not neuroendocrine rhythms to SCN-lesioned arrhythmic hosts.[97] Several diffusible candidate molecules have now been implicated as circadian output signals, including prokineticin-2, tumor necrosis factor-α, and vasopressin.[97]

MULTIOSCILLATOR NATURE OF THE CIRCADIAN SYSTEM

The evidence reviewed above amply justifies the use of the term pacemaker to describe the SCN's role in circadian rhythmicity. Nevertheless, the larger circadian system is now known to be composed of a multiplicity of circadian oscillators distributed widely in the brain and body. Although extensive physiologic studies conducted in the premolecular era revealed several varieties of rhythmic dissociation and disruption that strongly implied the existence of a multioscillatory circadian system,[98] these studies provided little evidence regarding the anatomic localization and distribution of the circadian system.

After the elucidation of the core clock genes, it soon became apparent that the expression of these genes was not restricted to the SCN but was in fact widely distributed in the brain and periphery. Nevertheless, it was generally believed that non-SCN cellular oscillators were highly damped and possessed little or no capacity for self-sustainment in the absence of periodic SCN input. More recently, however, the finding that circadian clock genes express persistent rhythmically in several surgically isolated brain regions[99,100] and in numerous cultured peripheral tissues and cell types[101,102] has provided compelling evidence for a whole-body, anatomically distributed network of cellular- and tissue-level circadian clocks. Thus, the current conception of the circadian system is that the SCN pacemaker entrains rhythmicity in downstream central and peripheral clocks via neural and neuroendocrine signals, as well as less directly via its control of rhythmic feeding, activity, and body temperature,[103] which in turn entrain other behavioral, physiologic, and cellular rhythms (**Fig. 2**). In this way, a broad network of direct and indirect controls ensures that the SCN and other central and peripheral clocks maintain specific, and presumably optimal, phase relationships with the external and internal environment, resulting in the overall temporal coordination of the system.

What is the advantage of a distributed system of independent circadian clocks, as opposed to a passive system that strongly depends on SCN signals for the sustainment of periodicity? Perhaps

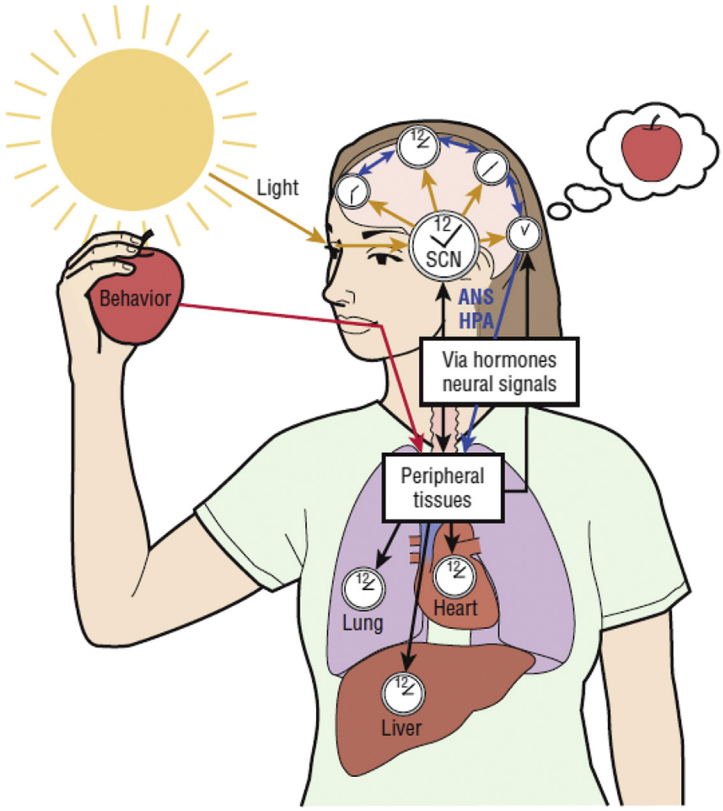

Fig. 2. The multioscillatory circadian timing system includes a large number of autonomously rhythmic circadian clock cells distributed within both central and peripheral tissues. The circadian pacemaker resides in the SCN, is entrained by light-dark cycles and other environmental stimuli, and in turn, serves to entrain and synchronize peripheral clocks. While some peripheral tissues can exhibit persistent autonomous rhythmicity, others may be damped in the absence of periodic SCN signals. Together, SCN and non-SCN central neural oscillators result in rhythmic behavior (such as food intake and motor activity), autonomic nervous system (ANS) function, and hypothalamic-pituitary-adrenal (HPA) axis hormone secretion. These behavioral and physiologic rhythms in turn can give rise to other rhythmic signals (eg, glucose availability, corticosterone levels, and body temperature) that serve to maintain phase synchrony among peripheral oscillators, probably in a tissue-specific manner. In turn, the activity of peripheral oscillators may give rise to rhythmic signals (eg, peripheral hormones, autonomic afferents, and metabolic signals) that contribute to the synchronization of the SCN pacemaker and other central oscillators.

the advantage lies in the ability of such a system to display a degree of plasticity in internal phase relationships under certain environmental conditions, such as when sleep and wakefulness occur at abnormal clock times or when feeding is restricted to atypical temporal windows. Under such conditions, a system of largely self-sustaining clocks can maintain largely undamped rhythmicity at the cellular and tissue levels. Thus, although temporal coordination throughout the circadian system is generally assumed to be physiologically optimal, the overall circadian system may be designed to allow downstream oscillators to display adaptive phase adjustments under certain natural circumstances. Of course, such plasticity may become maladaptive when humans choose to chronically disconnect their feeding and/or sleep-wake cycles from the SCN, as so often occurs in the current 24/7 society.

THE CIRCADIAN TIMING SYSTEM IN HEALTH AND DISEASE

This new picture of the circadian timing system has raised important questions about the potential adverse health effects that may be associated with a loss of normal synchronization between and among central and peripheral oscillations. Thus, circadian disruption at the molecular and systemic levels has been linked to sleep disorders, obesity and diabetes, heart disease, cancer, and psychiatric disorders.[103–106] Given estimates that approximately 10% to 20% of the entire genome is expressed rhythmically in any given tissue or organ,[107,108] many other mechanisms linking circadian synchrony and desynchrony to health and disease will undoubtedly emerge in the next few years and beyond.

Circadian dysregulation certainly occurs quite often in humans, who can override their circadian clock and exert substantial volitional control over their sleep-wake and feeding cycles. Under such circumstances, abnormal phase relationships are expressed between sleep-wake behaviors (and other rhythmic processes tightly linked to sleep or wake states, as well as feeding or fasting states) and the circadian clock (and rhythmic processes tightly linked to those behavioral processes). Although the internal desynchronies that occur with jet lag and shift work may be the most

dramatic, they are not the only examples of real-world circadian disruption. Indeed, social constraints, work schedules, and the use of artificial lighting may result in the widespread occurrence of social jet lag even in people living under relatively stable entrained conditions but who phase shift their sleep and feeding times on weekends and holidays relative to the weekdays.[109] Regardless of work or travel schedules, humans in the modern, round-the-clock society are certainly becoming less strictly diurnal, opposing millions of years of evolutionary selection.

SUMMARY

The SCN contains a master circadian pacemaker that entrains, coordinates, and contributes to the sustainment of a large population of cellular- and tissue-level circadian clocks located throughout the brain and body. Furthermore, the SCN itself contains a large number of normally coupled, but potentially autonomous, cellular oscillators that interact to underlie its pacemaker function. These cellular oscillations are generated by a set of circadian clock genes that interact via negative and positive feedback and ultimately drive circadian expression of a large number of clock-controlled genes, which in turn regulate downstream cellular and physiologic processes. Anatomically, the SCN is characterized by a complex regional organization that is not fully understood. The SCN pacemaker is entrained by several convergent afferent pathways that signal photic and nonphotic stimuli that mediate pacemaker entrainment to periodic environmental events. SCN outputs serve to synchronize non-SCN clocks and to impose rhythmicity on otherwise nonrhythmic processes. While it is becoming increasingly clear that proper coordination of the overall circadian system is critical for maintenance of health and well-being, it may also be the case that adaptive uncoupling among circadian clocks may allow for temporal adaptation to challenging environments.

REFERENCES

1. Moore RY, Eichler VB. Loss of a circadian adrenal corticosterone rhythm following suprachiasmatic lesions in the rat. Brain Res 1972;42(1):201–6.
2. Stephan FK, Zucker I. Circadian rhythms in drinking behavior and locomotor activity of rats are eliminated by hypothalamic lesions. Proc Natl Acad Sci U S A 1972;69(6):1583–6.
3. Weaver DR. The suprachiasmatic nucleus: a 25-year retrospective. J Biol Rhythms 1998;13(2):100–12.
4. Welsh DK, Logothetis DE, Meister M, et al. Individual neurons dissociated from rat suprachiasmatic nucleus express independently phased circadian firing rhythms. Neuron 1995;14:697–706.
5. Herzog ED, Geusz ME, Khalsa SB, et al. Circadian rhythms in mouse suprachiasmatic nucleus explants on multimicroelectrode plates. Brain Res 1997;757:285–90.
6. Colwell CS. Circadian modulation of calcium levels in cells in the suprachiasmatic nucleus. Eur J Neurosci 2000;12:571–6.
7. Kuhlman SJ, Quintero JE, McMahon DG. GFP fluorescence reports Period 1 gene regulation in the mammalian biological clock. Neuroreport 2000;11:1479–82.
8. Low-Zeddies SS, Takahashi JS. Chimera analysis of the Clock mutation in mice shows that complex cellular integration determines circadian behavior. Cell 2001;105:25–42.
9. Shirakawa T, Honma S, Honma K. Multiple oscillators in the suprachiasmatic nucleus. Chronobiol Int 2001;18:371–87.
10. Yamaguchi S, Isejima H, Matsuo T, et al. Synchronization of cellular clocks in the suprachiasmatic nucleus. Science 2003;302:1408–12.
11. Welsh DK, Takahashi JS, Kay SA. Suprachiasmatic nucleus: cell autonomy and network properties. Annu Rev Physiol 2010;72:551–77.
12. Mohawk JA, Takahashi JS. Cell autonomy and synchrony of suprachiasmatic nucleus circadian oscillators. Trends Neurosci 2011;34:349–58.
13. Schwartz W, Gross RA, Morton MT. The suprachiasmatic nuclei contain a tetrodotoxin-resistant circadian pacemaker. Proc Natl Acad Sci U S A 1987;84:1694–8.
14. Shibata S, Moore RY. Tetrodotoxin does not affect circadian rhythms in neuronal activity and metabolism in rodent suprachiasmatic nucleus in vitro. Brain Res 1993;606:259–66.
15. Miche S, Colwell CS. Cellular communication and coupling within the suprachiasmatic nucleus. Chronobiol Int 2001;18:579–600.
16. Aton SJ, Herzog ED. Come together, right … now: synchronization of rhythms in a mammalian circadian clock. Neuron 2005;48:531–4.
17. Herzog ED. Neurons and networks in daily rhythms. Nat Rev Neurosci 2007;8:790–802.
18. Takahashi JS, Turek FW. Anisomycin, an inhibitor of protein synthesis, perturbs the phase of a mammalian circadian pacemaker. Brain Res 1987;405:199–203.
19. Inouye ST, Takahashi JS, Wollnik F, et al. Inhibitor of protein synthesis phase shifts a circadian pacemaker in the mammalian SCN. Am J Physiol 1988;255:R1055–8.
20. Vitaterna MH, King DP, Chang A-M, et al. Mutagenesis and mapping of a mouse gene, Clock,

essential for circadian behavior. Science 1994;264:719–25.
21. Allada R, Emery P, Takahashi JS, et al. Stopping time: the genetics of fly and mouse circadian clocks. Annu Rev Neurosci 2001;24:1091–119.
22. Ko CH, Takahashi JS. Molecular components of the mammalian circadian clock. Hum Mol Genet 2006;15:R271–7.
23. Debruyne JP, Noton E, Lambert CM, et al. A clock shock: mouse CLOCK is not required for circadian oscillator function. Neuron 2006;50:465–77.
24. DeBruyne JP, Weaver DR, Reppert SM. CLOCK and NPAS2 have overlapping roles in the suprachiasmatic circadian clock. Nat Neurosci 2007;10:543–5.
25. Shearman LP, Zylka MJ, Weaver DR, et al. Two period homologs: circadian expression and photic regulation in the suprachiasmatic nuclei. Neuron 1997;19:1261–9.
26. Zheng B, Albrecht U, Kaasik K, et al. Nonredundant roles of the mPer1 and mPer2 genes in the mammalian circadian clock. Cell 2001;105:683–94.
27. Miyamoto Y, Sancar A. Circadian regulation of cryptochrome genes in the mouse. Brain Res Mol Brain Res 1999;71:238–43.
28. Van der Horst GT, Muijtjens M, Kobayashi K, et al. Mammalian Cry1 and Cry2 are essential for maintenance of circadian rhythms. Nature 1999;398:627–30.
29. Lowrey PL, Shimomura K, Antoch MP, et al. Positional syntenic cloning and functional characterization of the mammalian circadian mutation tau. Science 2000;288:483–92.
30. Meng QJ, Logunova L, Maywood ES, et al. Setting clock speed in mammals: the CK1 epsilon tau mutation in mice accelerates circadian pacemakers by selectively destabilizing PERIOD proteins. Neuron 2008;58:78–88.
31. Godinho SI, Maywood ES, Shaw L, et al. The after-hours mutant reveals a role for Fbxl3 in determining mammalian circadian period. Science 2007;316:897–900.
32. Siepka SM, Yoo SH, Park J, et al. Circadian mutant Overtime reveals F-box protein FBXL3 regulation of cryptochrome and period gene expression. Cell 2007;129:1011–23.
33. Maywood ES, Chesham JE, Meng QJ, et al. Tuning the period of the mammalian circadian clock: additive and independent effects of CK1epsilonTau and Fbxl3Afh mutations on mouse circadian behavior and molecular pacemaking. J Neurosci 2010;31:1539–44.
34. Colwell CS. Linking neural activity and molecular oscillations in the SCN. Nat Rev Neurosci 2010;12:553–69.
35. Naylor E, Bergmann BM, Krauski K, et al. The circadian clock mutation alters sleep homeostasis in the mouse. J Neurosci 2000;20:8138–43.
36. Tafti M, Franken P. Functional genomics of sleep and circadian rhythm. Invited review: genetic dissection of sleep. J Appl Physiol 2002;92:1339–47.
37. Rosenwasser AM. Circadian clock genes: non-circadian roles in sleep, addiction, and psychiatric disorders? Neurosci Biobehav Rev 2010;34:1249–55.
38. Moore RY. Entrainment pathways and the functional organization of the circadian system. Prog Brain Res 1996;111:103–19.
39. Moore RY, Silver R. Suprachiasmatic nucleus organization. Chronobiol Int 1998;15:475–87.
40. Morin LP. SCN organization reconsidered. J Biol Rhythms 2007;22:3–13.
41. Morin LP. Neuroanatomy of the extended circadian rhythm system. Exp Neurol 2013;243:4–20.
42. Antle MC, Silver R. Orchestrating time: arrangements of the brain circadian clock. Trends Neurosci 2005;28:145–51.
43. Shinohara K, Honma S, Katsuno Y, et al. Two distinct oscillators in the rat suprachiasmatic nucleus in vitro. Proc Natl Acad Sci U S A 1995;92:7396–400.
44. de la Iglesia HO, Cambras T, Schwartz WJ, et al. Forced desynchronization of dual circadian oscillators within the rat suprachiasmatic nucleus. Curr Biol 2004;14:796–800.
45. Johnson RF, Moore RY, Morin LP. Loss of entrainment and anatomical plasticity after lesions of the hamster retinohypothalamic tract. Brain Res 1998;460:297–313.
46. Golombek DA, Rosenstein RE. Physiology of circadian entrainment. Physiol Rev 2010;90:1063–102.
47. Moore RY, Speh JC, Card JP. The retinohypothalamic tract originates from a distinct subset of retinal ganglion cells. J Comp Neurol 1995;352:351–66.
48. Johnson RF, Morin LP, Moore RY. Retinohypothalamic projects in the hamster and rat demonstrated using cholera toxin. Brain Res 1998;462:301–12.
49. Levine JD, Weiss ML, Rosenwasser AM, et al. Retinohypothalamic tract in the female albino rat: a study using horseradish peroxidase conjugated to cholera toxin. J Comp Neurol 1991;306:344–60.
50. Foster RG, Argamaso S, Coleman S, et al. Photoreceptors regulating circadian behavior: a mouse model. J Biol Rhythms 1993;8(Suppl):S17–23.
51. Freedman MS, Lucas RJ, Soni B, et al. Regulation of mammalian circadian behavior by non-rod, non-cone, ocular photoreceptors. Science 1999;284:502–4.
52. Hattar S, Liao HW, Takao M, et al. Melanopsin-containing retinal ganglion cells: architecture, projections, and intrinsic photosensitivity. Science 2002;295:1065–70.
53. Berson DM, Dunn FA, Takao M. Phototransduction by retinal ganglion cells that set the circadian clock. Science 2002;295:1070–3.

54. Ruby NF, Brennan TJ, Xie X, et al. Role of melanopsin in circadian responses to light. Science 2002; 298:2211–3.
55. Panda S, Sato TK, Castrucci AM, et al. Melanopsin (Opn4) requirement for normal light-induced circadian phase shifting. Science 2002;298:2213–6.
56. Hattar S, Lucas RJ, Mrosovsky N, et al. Melanopsin and rod-cone photoreceptive systems account for all major accessory visual functions in mice. Nature 2003;424:76–81.
57. Drouyer E, Rieux C, Hut RA, et al. Responses of suprachiasmatic nucleus neurons to light and dark adaptation: relative contributions of melanopsin and rod-cone inputs. J Neurosci 2007;27:9623–31.
58. Guler AD, Altimus CM, Ecker JL, et al. Multiple photoreceptors contribute to nonimage-forming visual functions predominantly through melanopsin-containing retinal ganglion cells. Cold Spring Harb Symp Quant Biol 2007;72:509–15.
59. Gillette MU. Regulation of entrainment pathways by the suprachiasmatic circadian clock: sensitivities to second messengers. Prog Brain Res 1996;111:121–32.
60. Kornhauser JM, Ginty DD, Greenberg ME, et al. Light entrainment and activation of signal transduction pathways in the SCN. Prog Brain Res 1996; 111:133–46.
61. Shigeyoshi Y, Taguchi K, Yamamoto S, et al. Light-induced resetting of a mammalian circadian clock is associated with rapid induction of the mPer1 transcript. Cell 1997;91:1043–53.
62. Moriya T, Horikawa K, Akiyama M, et al. Correlative association between N-methyl-D-aspartate receptor-mediated expression of period genes in the suprachiasmatic nucleus and phase shifts in behavior with photic entrainment of clock in hamsters. Mol Pharmacol 2000;58:1554–62.
63. Challet E, Dugovic C, Turek FW, et al. The selective neurokinin 1 receptor antagonist R116301 modulates photic responses of the hamster circadian system. Neuropharmacology 2001;40:408–15.
64. Kim DY, Kang HC, Shin HC, et al. Substance P plays a critical role in photic resetting of the circadian pacemaker in the rat hypothalamus. J Neurosci 2001;21: 4026–31.
65. Piggins HD, Rusak B. Effects of microinjections of substance P into the suprachiasmatic nucleus region on hamster wheel-running rhythms. Brain Res Bull 1997;42:451–5.
66. Chen D, Buchanan GF, Ding JM, et al. Pituitary adenylate cyclase-activating peptide: a pivotal modulator of glutamatergic regulation of the suprachiasmatic circadian clock. Proc Natl Acad Sci U S A 1999;96: 13468–73.
67. Harrington ME, Hoque S, Hall A, et al. Pituitary adenylate cyclase activating peptide phase shifts circadian rhythms in a manner similar to light. J Neurosci 1999;19:6637–42.
68. Hannibal J, Jamen F, Nielsen HS, et al. Dissociation between light-induced phase shift of the circadian rhythm and clock gene expression in mice lacking the pituitary adenylate cyclase activating polypeptide type 1 receptor. J Neurosci 2001;21:4883–90.
69. Moore RY, Card JP. Intergeniculate leaflet: an anatomically and functionally distinct subdivision of the lateral geniculate complex. J Comp Neurol 1994;344:403–30.
70. Morin LP. The circadian visual system. Brain Res Rev 1994;67:102–27.
71. Harrington ME. The ventral lateral geniculate nucleus and the intergeniculate leaflet: interrelated structures in the visual and circadian systems. Neurosci Biobehav Rev 1997;21:705–27.
72. Pickard GE. Bifurcating axons of retinal ganglion cells terminate in the hypothalamic suprachiasmatic nucleus and in the intergeniculate leaflet of the thalamus. Neurosci Lett 1982;55:211–7.
73. Wickland CR, Turek FW. Lesions of the thalamic intergeniculate leaflet block activity-induced phase shifts in the circadian activity rhythm of the golden hamster. Brain Res 1994;660:293–300.
74. Janik D, Mrosovsky N. Intergeniculate leaflet lesions and behaviorally-induced shifts of circadian rhythms. Brain Res 1994;651:174–82.
75. Johnson R, Smale L, Moore RY, et al. Lateral geniculate lesions block circadian phase-shift responses to a benzodiazepine. Proc Natl Acad Sci U S A 1988;85:5301–4.
76. Meyer EL, Harrington ME, Rahmani T. A phase-response curve to the benzodiazepine chlordiazepoxide and the effect of geniculo-hypothalamic tract ablation. Physiol Behav 1993;53:237–43.
77. Kuroda H, Fukushima M, Nakai M, et al. Daily wheel running activity modifies the period of free-running rhythm in rats via intergeniculate leaflet. Physiol Behav 1997;61:633–7.
78. Marchant EG, Watson NV, Mistlberger RE. Both neuropeptide Y and serotonin are necessary for entrainment of circadian rhythms in mice by daily treadmill running schedules. J Neurosci 1997;17: 7974–87.
79. Pekala D, Blasiak T, Raastad M, et al. The influence of orexins on the firing rate and pattern of rat intergeniculate leaflet neurons–electrophysiological and immunohistological studies. Eur J Neurosci 2011;34:1406–18.
80. Saderi N, Cazarez-Marquez F, Buijs FN, et al. The NPY intergeniculate leaflet projections to the suprachiasmatic nucleus transmit metabolic conditions. Neuroscience 2013;246:291–300.
81. Meyer-Bernstein EL, Morin LP. Differential serotonergic innervation of the suprachiasmatic nucleus and the intergeniculate leaflet and its role in circadian rhythm modulation. J Neurosci 1996;16: 2097–111.

82. Morin LP. Serotonin and the regulation of mammalian circadian rhythmicity. Ann Med 1999;31:12–33.
83. Rea MA, Pickard GE. Serotonergic modulation of photic entrainment in the Syrian hamster. Biol Rhythm Res 2000;31:284–314.
84. Mistlberger RE, Antle MC, Glass JD, et al. Behavioral and serotonergic regulation of circadian rhythms. Biol Rhythm Res 2000;31:240–83.
85. Ehlen JC, Grossman GH, Glass JD. In vivo resetting of the hamster circadian clock by 5-HT7 receptors in the suprachiasmatic nucleus. J Neurosci 2001;21:5351–7.
86. Mintz EM, Gillespie CF, Marvel CL, et al. Serotonergic regulation of circadian rhythms in Syrian hamsters. Neuroscience 1997;79:563–9.
87. Prosser RA. Serotonergic actions and interactions on the SCN circadian pacemaker: in vitro investigations. Biol Rhythm Res 2000;31:315–39.
88. Pickard GE, Rea MA. TFMPP, a 5HT1B receptor agonist, inhibits light-induced phase shifts of the circadian activity rhythm and c-Fos expression in the mouse suprachiasmatic nucleus. Neurosci Lett 1997;231:95–8.
89. Smith BN, Sollars PJ, Dudek FE, et al. Serotonergic modulation of retinal input to the mouse suprachiasmatic nucleus mediated by 5-HT1B and 5-HT7 receptors. J Biol Rhythms 2001;16:25–38.
90. Rosenwasser AM. Neurobiology of the mammalian circadian system: oscillators, pacemakers, and pathways. Prog Psychobiol Physiol Psychol 2003; 18:1–38.
91. Rosenwasser AM, Turek FW. Physiology of the mammalian circadian system. In: Kryger MH, Roth T, Dement WC, editors. Principles and practice of sleep medicine. 5th edition. St Louis (MO): Elsevier-Saunders; 2011. p. 390–401.
92. Kalsbeek A, Perreau-Lenz S, Buijs RM. A network of (autonomic) clock outputs. Chronobiol Int 2006; 23:521–35.
93. Kalsbeek A, Palm IF, La Fleur SE, et al. SCN outputs and the hypothalamic balance of life. J Biol Rhythms 2006;21:458–69.
94. Rosenwasser AM. Functional neuroanatomy of sleep and circadian rhythms. Brain Res Rev 2009;61:281–306.
95. LeSauter J, Silver R. Output signals of the SCN. Chronobiol Int 1998;15:535–50.
96. Hakim H, DeBernardo AP, Silver R. Circadian locomotor rhythms, but not photoperiodic responses, survive surgical isolation of the SCN in hamsters. J Biol Rhythms 1991;6:97–113.
97. Silver R, LeSauter J, Tresco PA, et al. A diffusible coupling signal from the transplanted suprachiasmatic nucleus controlling circadian locomotor rhythms. Nature 1996;382:810–3.
98. Rosenwasser AM, Adler NT. Structure and function in circadian timing systems: evidence for multiple coupled circadian oscillators. Neurosci Biobehav Rev 1986;10:431–48.
99. Abe M, Herzog ED, Yamazaki S, et al. Circadian rhythms in isolated brain regions. J Neurosci 2002;22:350–6.
100. Granados-Fuentes D, Tseng A, Herzog ED. A circadian clock in the olfactory bulb controls olfactory responsivity. J Neurosci 2006;26:12219–25.
101. Yoo SH, Yamazaki S, Lowrey PL, et al. PERIOD2::-LUCIFERASE real-time reporting of circadian dynamics reveals persistent circadian oscillations in mouse peripheral tissues. Proc Natl Acad Sci U S A 2004;101:5339–46.
102. Welsh DK, Yoo SH, Liu AC, et al. Bioluminescence imaging of individual fibroblasts reveals persistent, independently phased circadian rhythms of clock gene expression. Curr Biol 2004;14:2289–95.
103. Hastings MH, Reddy AB, Maywood ES. A clockwork web: circadian timing in brain and periphery, in health and disease. Nat Rev Neurosci 2003;4:649–61.
104. Takahashi JS, Hong HK, Ko CH, et al. The genetics of mammalian circadian order and disorder: implications for physiology and disease. Nat Rev Genet 2008;9:764–75.
105. Karatsoreos IN. Effects of circadian disruption on mental and physical health. Curr Neurol Neurosci Rep 2012;12:218–25.
106. Zelinski EL, Deibel SH, McDonald RJ. The trouble with circadian clock dysfunction: multiple deleterious effects on the brain and body. Neurosci Biobehav Rev 2014;40:80–101.
107. Akhtar RA, Reddy AB, Maywood ES, et al. Circadian cycling of the mouse liver transcriptome, as revealed by cDNA microarray, is driven by the suprachiasmatic nucleus. Curr Biol 2002;12:540–50.
108. Panda S, Antoch MP, Miller BH, et al. Coordinated transcription of key pathways in the mouse by the circadian clock. Cell 2002;109:307–20.
109. Wittmann M, Dinich J, Merrow M, et al. Social jetlag: misalignment of social and biological time. Chronobiol Int 2006;23:497–509.