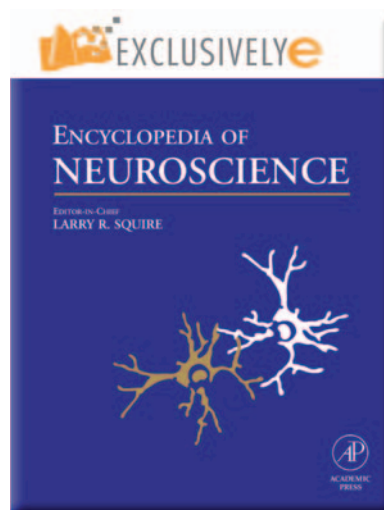


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## Entrainment of Circadian Rhythms by Light

**A M Rosenwasser**, University of Maine, Orono, ME, USA

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### Introduction

In general, the endogenous, inherited period of the circadian pacemaker is close to – but not exactly equal to – 24 h. Indeed, the term *circadian* ('approximately daily') was coined to emphasize this fundamental feature of biological timing. Thus, endogenous circadian pacemakers depend upon signals derived from exogenous daily cycles in order to synchronize, or entrain, to the exact 24 h periodicity of the environment. It is only through this entrainment process that circadian pacemakers acquire their adaptive utility as biological 'clocks,' responsible for coordinating behavior and physiology with local time. While several periodic environmental factors may contribute to or modulate the entrainment of the circadian pacemaker, the most prominent, widespread, and fundamental source of circadian entrainment is the daily alternation of environmental light and darkness. In nature, light intensity and spectral composition change in a complex manner as a function of time of day, and depend not only on solar illumination but also on lunar phase, starlight, latitude, and local climatic conditions. Further, actual exposure to available environmental light depends dramatically on an animal's ecological niche and lifestyle (e.g., time spent in burrows, caves, or deep in the ocean). Nevertheless, simple square-wave ('on-off') light–dark cycles have proved to exert effective circadian entrainment in the laboratory, and much has been learned about the formal, physiological, and molecular bases of light entrainment from studies conducted under such simplified environmental conditions.

### Formal Analysis of Light Entrainment

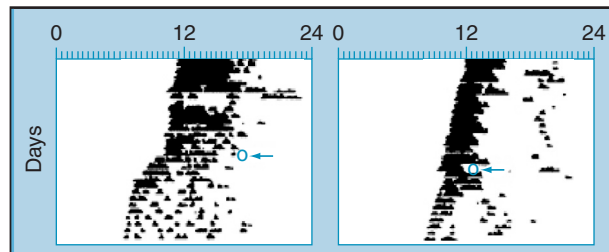
#### Parametric and Nonparametric Entrainment

Brief light pulses can evoke rapid and permanent changes in the phase of the free-running circadian pacemaker. The response of the circadian pacemaker to a light pulse is phase dependent, such that photic stimulation near 'subjective, dusk' or early in the subjective night results in phase delays (i.e., shifts to relatively later timing in subsequent cycles), while photic stimulation late in the subjective night or near dawn results in phase advances (i.e., shifts to relatively earlier timing in subsequent cycles, see [Figure 1](#)).

In contrast, the circadian pacemaker is relatively refractory to photic stimulation during the circadian daytime, indicating that the critical phases of light exposure for entrainment occur near dusk and dawn. The function relating the magnitude and direction of circadian phase shifting to the phase of stimulation for any given stimulus is referred to as the 'phase–response curve' (PRC) for that stimulus (see [Figure 2](#)). Extensive research has shown that the general shape of the light-pulse PRC, as just described, is seen consistently across species, and indeed, across orders, and in both nocturnal and diurnal life-forms. Thus, while the seminal vertebrate PRC experiments were done mainly in nocturnal rodents, more recent analyses have shown that the 'typical' diurnal mammalian PRC very closely resembles that of nocturnal mammals in both amplitude and waveform.

Colin Pittendrigh systematized these observations in the highly influential and extensively validated nonparametric theory of light entrainment, which proposed that phasic responses to brief light exposures occurring at specific critical circadian phases (i.e., near dusk and dawn) are responsible for entrainment of the circadian pacemaker, even under more natural light–dark schedules with 'complete' photoperiods (see [Figure 3](#)). This model may be contrasted conceptually with the parametric entrainment theory, associated with the work of Jurgen Aschoff, and based on extensive observations indicating that the period of a free-running circadian pacemaker changes systematically (either lengthening or shortening, depending on the species) as a function of continuous light intensity. Thus, parametric entrainment theory proposes that the tonic effects of temporally extended light exposure alter the underlying period of the circadian pacemaker, causing it to match the periodicity of the entraining environment.

Although nonparametric entrainment has proved to be a powerful descriptive and predictive model, recent analyses indicate that both phasic, nonparametric effects and tonic, parametric effects may contribute to circadian entrainment, especially under field conditions, or during exposure to more realistic laboratory simulations of the natural light–dark cycle. Thus, some species appear to entrain successfully in the natural environment without ever being exposed to abrupt, dawn- and dusk-related light transitions, while in laboratory experiments, both brief and extended light exposures result in long-term changes in free-running period as measured in continuous darkness (i.e., 'after-effects' of prior entrainment). Further, quantitative simulations incorporating both nonparametric (phase-shifting) and parametric (period-adjusting) effects

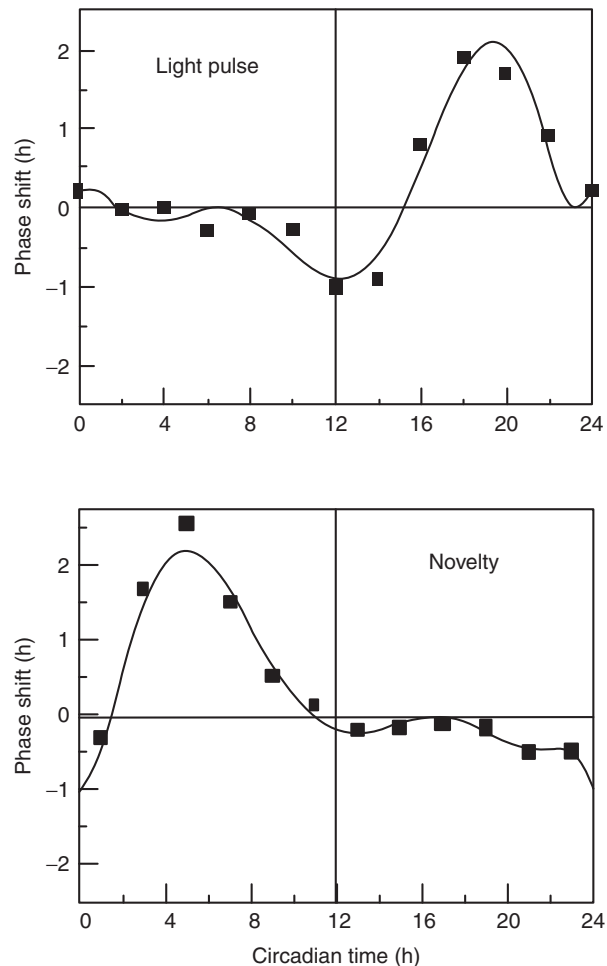


**Figure 1** Examples of phase shifting of free-running circadian activity rhythms by brief light pulses. Left panel: Circadian rhythm of wheel running recorded from a hamster in constant darkness. On the day indicated by the arrow, the animal was exposed to light for 30 min (open circle) beginning at circadian time 20. (By convention, the onset of activity in a nocturnal animal is defined as circadian time 12; thus, circadian time 20 is 8 circadian h after activity onset; where 1 circadian hour = circadian period/24.) On circadian cycles following the light pulse, activity onset occurs earlier than would be predicted by extrapolating from the phase of the preceding free-running rhythm. After several days of 'transient' cycles, as is typical for advancing phase shifts, a stable phase advance of several hours is revealed. Right panel: In this experiment, a similar light pulse was presented at circadian time 14, resulting in a phase delay shift of approximately 1 h, which was complete within one circadian cycle. Phase resetting without transients is typical of light-induced phase delays. Reproduced from Mistlberger RE and Rusak B (2005) Circadian rhythms in mammals: Formal properties and environmental influences. In: Kryger MH, Roth T, and Dement WC (eds.) *Principles and Practice of Sleep Medicine*, 4th edn., pp. 1–30. Philadelphia: Elsevier-Saunders, with permission from Elsevier.

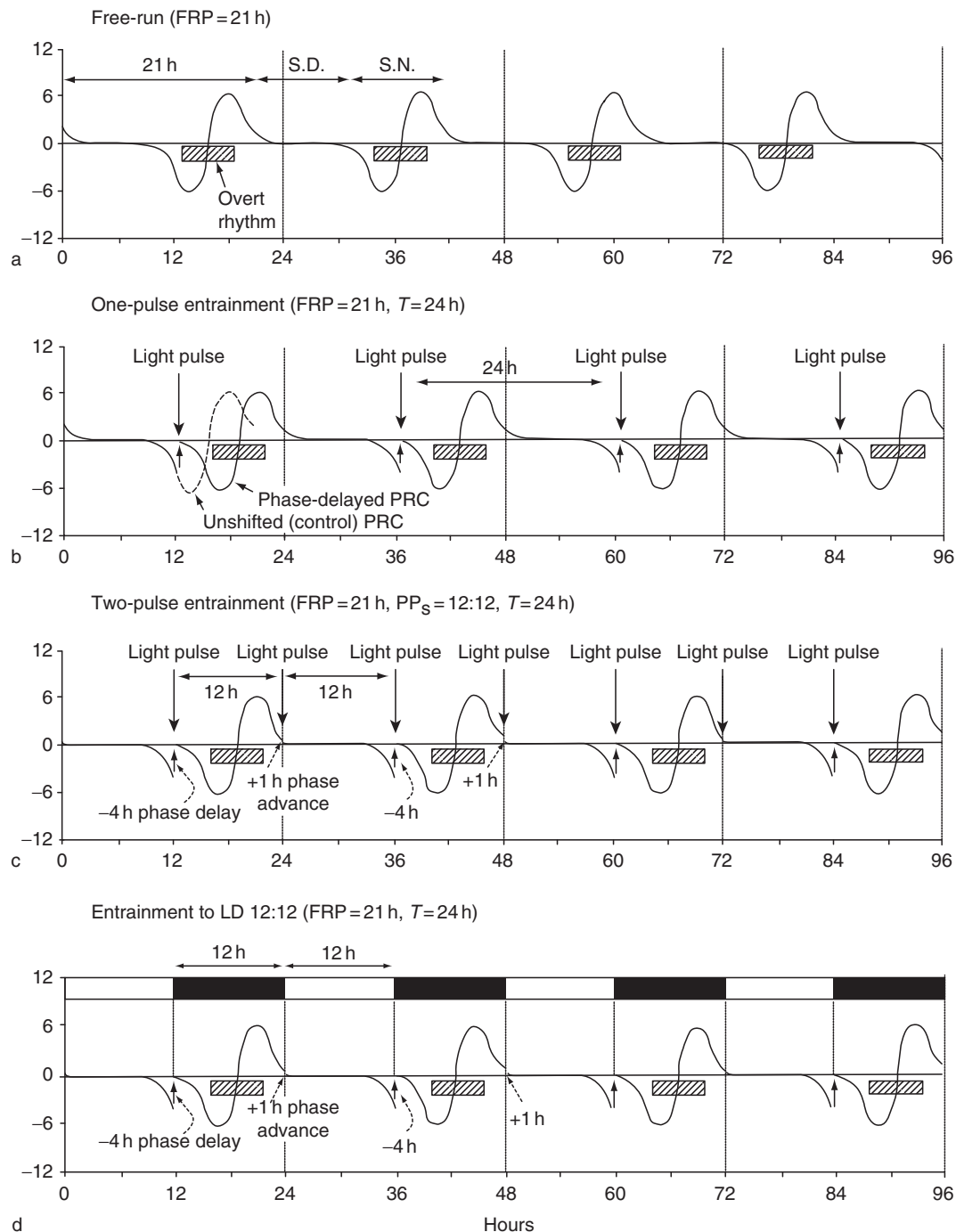
result in more stable entrainment than do models based solely on nonparametric effects.

### Toward Naturalistic Light-Dark Cycles

The importance of parametric effects for natural entrainment is also highlighted by laboratory experiments utilizing simulated gradual twilight transitions, rather than the more typical square-wave light-dark cycles. Acute exposure to brief twilight-like light pulses and brief square-wave light pulses yields identical phase shifts when the two pulse types are equated for photon numbers, indicating that the nonparametric effects of light pulses are independent of pulse 'shape.' In contrast, inclusion of simulated twilight transitions during steady-state entrainment to extended photoperiods alters dramatically the pattern of circadian entrainment relative to standard square-wave light-dark cycles. In addition to altering the timing of daily activity onsets and offsets, exposure to twilights results in more stable and more effective entrainment, and alters the free-running period as measured during subsequent exposure to constant darkness. Together, these studies strongly suggest that parametric effects of twilight exposure on the underlying free-running period increase the entraining efficacy of a light-dark cycle.



**Figure 2** Phase-response curves describing the phase-dependent sensitivity of the hamster circadian pacemaker to photic (top) and nonphotic (bottom) stimulation. (Top) As seen in **Figure 1**, brief (here, 15 min) light pulses result in phase delays when presented near subjective dusk or early in the subjective night, while similar light pulses evoke phase advances when presented late in the subjective night. While the exact timing and relative amplitude of the delay and advance zones of the phase-response curve differ across species, these general features characterize all known photic phase-response curves in both nocturnal and diurnal animals. (Bottom) Phase advances during mid-subjective day and small phase delays during late subjective night are the result of 2 h of exposure of free-running hamsters to a novel environment containing a running wheel. Such nonphotic phase shifting is typically seen only in animals displaying signs of behavioral arousal or activation during the novelty stimulus. Reprinted from Rosenwasser AM (2003) Neurobiology of the mammalian circadian system: Oscillators, pacemakers, and pathways. In: Fluharty SJ and Grill HJ (eds.) *Progress in Psychobiology and Physiological Psychology*, vol. 18, pp. 1–38. San Diego: Elsevier Academic Press, with permission from Elsevier. Original data replotted from (top) Daan S and Pittendrigh CS (1976) A functional analysis of circadian pacemakers in nocturnal rodents. II. The variability of phase response curves. *Journal of Comparative Physiology A* 106: 253–256 and (bottom) Mrosovsky N, Salmon PA, Menaker M, et al. (1992) Non-photic phase shifting in hamster clock mutants. *Journal of Biological Rhythms* 7: 35–41.



**Figure 3** Light entrainment of a free-running circadian pacemaker according to the nonparametric model. In these panels, the phase of the circadian pacemaker is represented by its phase-response curve (PRC) and by the expected timing of locomotor activity. The examples shown in this figure assume a circadian pacemaker of a nocturnal organism that free-runs in constant darkness with a free-running period (FRP) of 21 h. (a) Free-running of PRC and overt rhythm with a period of 21 h. Note that the PRC and activity rhythm recur earlier each day relative to the 24 h objective day. (b) 'One-pulse' entrainment. A brief light pulse is administered to the same organism once every 24 h. During stable entrainment, the light pulse will recur at a circadian phase that results in a daily phase delay of 3 h (in this case, at approximately circadian time 13). (c) Two-pulse entrainment by a 'skeleton' photoperiod (PPs 12:12) in which a brief light pulse is administered once every 12 h. During stable entrainment, the light pulses will occur at circadian phases such that the net steady-state phase (i.e., advance plus delay) will result in a daily 3 h delay (in this case, the net 3 h delay is achieved by a daily 1 h advance combined with a daily 4 h delay). (d) Entrainment to a complete 12 h light-dark photoperiod (LD 12:12). In this case, the dawn transition results in a daily 1 h phase advance, while the dusk transition results in a daily 4 h delay, similar to the effects of the skeleton photoperiod shown in panel (c). According to this model, light occurring between dawn and dusk (i.e., in the daytime) is largely irrelevant for circadian entrainment. From Johnson CH, Elliott JA, and Foster R (2003) Entrainment of circadian programs. *Chronobiology International* 20: 741-774.

Another difference between typical laboratory light–dark cycles and natural light–dark cycles is that the natural nighttime environment includes variable amounts of starlight and moonlight, while the night phase of laboratory light–dark cycles is typically characterized by total darkness. While field studies have frequently concluded that animal activity patterns are altered by such very dim nocturnal illumination, the relevance of these studies for entrainment of the circadian pacemaker is uncertain. Recent laboratory studies have begun to compare circadian entrainment under square-wave light–dark cycles, with or without very dim nocturnal illumination approximating that of starlight or dim moonlight. Even at intensities that fail to evoke circadian phase shifting, dim nocturnal illumination, like twilight transitions, appears to increase the effectiveness of the light–dark cycle, possibly by increasing the pacemaker's sensitivity to daytime light intensities. The possible contribution of parametric modification of the underlying period of the circadian pacemaker to these effects has not been tested directly.

## **Physiology of Light Entrainment**

### **Circadian Photoreception**

In invertebrates and in nonmammalian vertebrates, both ocular and extraocular photoreceptors typically contribute to circadian entrainment. Vertebrate extraocular photoreceptors include both light-sensitive pinealocytes, as well as deep-brain or encephalic photoreceptors. While a number of novel and retinal photopigments have been identified within the vertebrate brain, the functional and anatomical couplings of encephalic photoreceptors to the circadian pacemaker have not been described in any vertebrate model system. In contrast, mammalian circadian photoreception appears to be entirely dependent on retinal mechanisms. Nevertheless, the photoreceptors and the central neural pathways mediating mammalian circadian entrainment comprise a distinct biological system, separate from those mediating visuoperceptual functions.

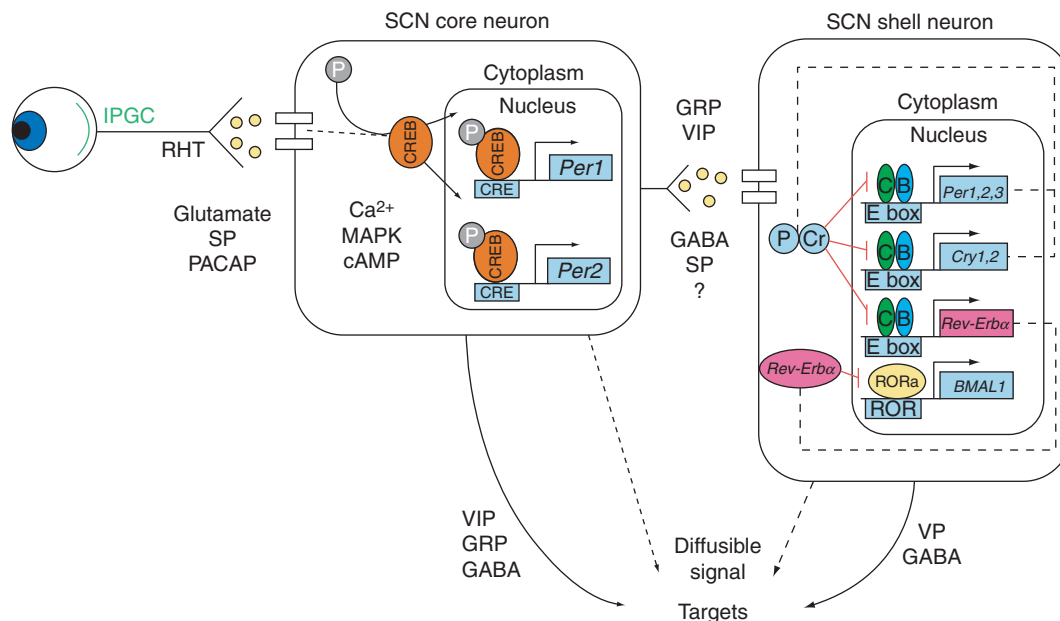
Studies with mice bearing genetic mutations leading to the progressive loss of nearly all classical rod and cone photoreceptors showed that some strains of retinally degenerate mice continue to display normal circadian photosensitivity. Eventually, similar results were obtained in genetically engineered mice completely lacking rods and cones throughout development, demonstrating unequivocally that nonclassical retinal photoreceptors are sufficient to mediate circadian entrainment. Physiological and biochemical experiments identified a novel retinal photopigment,

melanopsin, and showed that this protein was specifically expressed within the small and scattered subpopulation of retinal ganglion cells giving rise to the retinohypothalamic tract (RHT), thus conferring intrinsic photosensitivity on these neurons. The term retinohypothalamic tract has been used traditionally to refer to the near-bilaterally symmetrical retinal projection to the hypothalamic suprachiasmatic nucleus (SCN), site of the primary vertebrate circadian pacemaker. Nevertheless, melanopsin-positive retinal ganglion cells also project to other diencephalic and mesencephalic targets, including other identified components of the circadian timing system (e.g., the intergeniculate leaflet), as well as neural structures mediating oculomotor reflexes and other 'non-visual' (some authors prefer 'non-image-forming') retinal functions.

While melanopsin-positive photosensitive retinal ganglion cells are sufficient to mediate circadian light entrainment, genetic deletion of melanopsin impairs, but does not fully abolish, photic control of the circadian pacemaker. Indeed, light entrainment is abolished only when both melanopsin and classical photoreceptors are deleted, indicating a degree of redundancy in circadian photoreception. Thus, very recent studies show that photosensitive retinal ganglion cells can also respond to light via rod- and cone-mediated neural signals, and that melanopsin expression levels depend on the integrity of the classical photoreceptors. Further, genetic deletion of RPE65, a protein required for the maintenance of rod and cone photosensitivity, results in much more dramatic disruption of circadian entrainment than does elimination of the classical photoreceptors. Together, these findings indicate that classical retinal photoreceptors, while not necessary, do normally contribute significantly to circadian entrainment.

### **Entrainment Pathways, Neurotransmitters, and Intracellular Signaling**

As mentioned earlier, the RHT projects to the SCN, intergeniculate leaflet (IGL), and other 'nonvisual' brain regions. The terminals of this projection release glutamate as the primary neurotransmitter, as well as specific peptide cotransmitters, including pituitary adenylyl cyclase-activating peptide (PACAP) and possibly substance P. Indeed, double-labeling studies of the retina demonstrate that melanopsin and PACAP provide essentially redundant markers for RHT cell bodies. Light-evoked glutamate release from RHT terminals acts through both *N*-methyl-D-aspartate (NMDA) and non-NMDA glutamate receptors to activate a number of second-messenger systems and downstream signaling pathways, leading eventually to the transcriptional changes underlying



**Figure 4** Cellular and molecular bases of light entrainment. Light is transduced into a neural signal by melanopsin-positive intrinsically photoreceptive ganglion cells (IPGCs) in the retina and conveyed to the suprachiasmatic nucleus (SCN) core along the retinohypothalamic tract (RHT), resulting in the release of the neurotransmitter glutamate and the neuromodulators pituitary adenylyl cyclase-activating peptide (PACAP) and substance P (SP) onto retinorecipient cells in the SCN core. Glutamate activates *N*-methyl-D-aspartate (NMDA) receptors, leading to calcium influx and activation of several known calcium-dependent signaling pathways (e.g., mitogen-activated protein kinase; MAPK), resulting in phosphorylation of cyclic AMP (cAMP) response element-binding protein (CREB). Activated CREB binds to the calcium/cAMP response element (CRE) in the promoter regions of both *Per1* and *Per2*, thus activating their transcription. Neurons in the photoreceptive SCN core then communicate with the spontaneously rhythmic SCN shell (and with SCN efferent targets) using a variety of neurotransmitters, including vasoactive intestinal polypeptide (VIP), gastrin-releasing peptide (GRP), SP, and  $\gamma$ -aminobutyric acid (GABA). Cells in the rhythmic SCN shell contain molecular clocks driven by a set of interlocking positive and negative autoregulatory transcription-translation feedback loops (e.g., *Per*, *Cry*, *Rev-Erba*, *BMAL1*). SCN shell neurons communicate with SCN efferent targets using vasopressin (VP) and GABA as neurotransmitters. Additionally, the SCN communicates with some target sites using a diffusible signal. Reprinted from Antle MC and Silver R (2005) Orchestrating time: Arrangements of the brain circadian clock. *Trends in Neurosciences* 28: 145–151, with permission from Elsevier.

phase shifting of the circadian pacemaker (see [Figure 4](#)). Analysis of these mechanisms is complicated, however, by the fact that the pacemaker autoregulates its own entrainment by establishing time-limited windows of sensitivity to environmental entrainment stimuli and to activation of neurotransmitter receptors and second-messenger systems. For example, the circadian pacemaker is sensitive to light-induced phase shifting mainly during the subjective night, as described by the photic PRC. Thus, light entrainment is based on a circadian rhythm in the photic sensitivity of the pacemaker, and like other circadian rhythms, rhythmic light sensitivity is controlled by mechanisms intrinsic to the pacemaker. In addition, while photic stimuli evoke similar changes in gene transcription during early and late subjective night, the signaling pathways coupling glutamate receptor activation to transcriptional activation during these two circadian time windows appear to be at least partially nonoverlapping.

Both *in vivo* and *in vitro* studies have demonstrated that light, glutamate, and/or NMDA evoke

alterations in gene expression and circadian phase shifting only at night. These night-active phase-shifting stimuli appear to access the molecular clock via multiple calcium-dependent and cyclic GMP (cGMP)-dependent signaling pathways, leading to (1) phosphorylation of the transcription factor calcium and cyclic AMP response element-binding protein (CREB), (2) transcription of specific immediate-early genes (IEGs), including *c-fos*, and (3) alterations in the transcription of specific ‘clock’ genes such as the *period* genes (*Per1*, *Per2*), the protein products of which represent state variables encoding the oscillation phase of the circadian pacemaker. While not a primary focus of the present discussion, the circadian pacemaker can also be phase shifted and entrained by nonphotic stimuli linked to behavioral arousal and wakefulness (see [Figure 2](#)), and by agonists at specific subtypes of serotonin,  $\gamma$ -aminobutyric acid (GABA), and neuropeptide Y (NPY) receptors. In contrast to photic entrainment, the circadian pacemaker is sensitive to

these stimuli primarily during the subjective day, and day-active phase shifting stimuli access the molecular clock via cyclic AMP (cAMP)-dependent signaling pathways. Further, night-active and day-active phase-shifting stimuli are mutually antagonistic. Thus, while the circadian pacemaker is refractory to phase shifting by serotonin, GABA, and NPY agonists during the subjective night, these stimuli effectively block nighttime phase shifting by light and glutamate agonists, while in turn, photic stimulation during the subjective day antagonizes nonphotic phase shifting.

In addition to glutamate, RHT neurons also synthesize and co-localize the neuropeptide PACAP. Numerous studies indicate that PACAP plays a significant role in light entrainment of the circadian pacemaker, in part via modulation of glutamatergic signaling. PACAP–glutamate interactions appear to be rather complex, however, and depend on both circadian phase and PACAP levels. Early studies using the *in vitro* SCN slice preparation suggested that PACAP functions mainly as a daytime entrainment signal; despite its retinal origin, PACAP stimulation of the SCN mimicked the effects of nonphotic stimuli, producing cAMP-mediated phase advances during subjective day. While subsequent studies confirmed these observations at relatively high (micromolar) concentrations, PACAP was also shown to mimic the nighttime effects of light and glutamate on circadian phase shifting and clock gene expression at lower (nanomolar) concentrations. Further, at the higher concentrations that evoke daytime phase shifting, nighttime PACAP administration exerts complex modulation of glutamate-induced phase shifting and gene expression. Thus co-application of PACAP is reported to block glutamate-induced phase advances and *period* expression during late subjective night, but to potentiate glutamate-induced phase delays during early subjective night. Similarly, in behavioral experiments, intra-SCN PACAP mimicked – and a PACAP receptor antagonist blocked – the phase shifting effects of light during early subjective night, but PACAP was without effect during late subjective night. The role of PACAP in light entrainment has also been examined using both PACAP-deficient and PACAP receptor (PAC1)-deficient mice, but the effects of genetic deletion of the peptide and the receptor were different. Thus, PACAP-knockout mice showed decreased light-induced phase delays while PAC1-knockout mice showed increased phase delays during early subjective night. Further, while PACAP-knockout mice also showed reduced light-induced phase advances, PAC1-knockout mice actually showed abnormal light-induced phase delays during late subjective night. Finally, the PAC1-knockout mice also displayed surprising dissociation

between light-induced phase shifting and gene expression, in that the potentiated early-night phase delays were accompanied by blunted light-induced *c-fos* and *period* expression.

### **Processing of Entrainment Signals by the SCN**

The initial evidence for the anatomical heterogeneity of the SCN emerged from tract-tracing studies showing that retinal afferents to the rat SCN are generally segregated within the ventral aspect of the nucleus, especially in its more caudal aspects. Subsequently, chemoanatomical studies revealed marked regional heterogeneity in the expression and distribution of specific neuropeptides within the nucleus. Based on the distribution of retinal (and other) SCN afferents, as well as on neuropeptide expression, the rat SCN has been typically characterized as comprising two distinct regions: (1) a ventrolateral zone, defined in part by the presence of retinal terminals along with vasoactive intestinal peptide (VIP)-immunopositive and gastrin-releasing peptide (GRP)-immunopositive cells and (2) a dorsomedial zone, defined mainly by the presence of arginine–vasopressin (AVP)-positive cells. More recent studies, however, have revealed extensive species differences in the intranuclear arrangement of both afferent terminals and neuronal phenotypes, even among nocturnal rodents. In one particularly striking example, Silver and co-workers have identified a small, densely packed and centrally located cluster of calbindin (CB)-positive neurons in the hamster SCN that is not readily apparent in other species. In attempting to better accommodate such species differences, Moore has proposed an alternate view in which the SCN is composed of functionally distinct ‘core’ and ‘shell’ subnuclei, which differ across species in their exact extent and placement. Nevertheless, a two-component core and shell scheme may eventually prove insufficiently complex to account for the full range of species-related variation, or even for the functional compartmentalization of the SCN within a species. Thus, retinal, thalamic, and brain stem SCN afferents, generally described as overlapping in the ventral SCN, may exhibit somewhat different terminal fields within the SCN core, while different SCN peptides clearly display more than two distinct intranuclear distribution patterns. On the other hand, given that the SCN appears to mediate two primary circadian functions (rhythm generation and entrainment), it is appealing to hypothesize the existence of two discrete subnuclei, each associated mainly or exclusively with one of these functions.

Since retinal projections to the SCN are generally coextensive with the core subnucleus, it has frequently

been suggested that this SCN region may play a preeminent role in the photic entrainment of the circadian pacemaker (see [Figure 4](#)). Indeed, retinal afferents form direct synaptic connections with VIP-, GRP-, and CB-positive SCN core neurons. In addition, both *in vivo* and *in vitro* studies show that direct application of SCN core peptides such as VIP and GRP can mimic the cellular and phase shifting effects of light on the circadian pacemaker, but that similar effects are not seen for direct application of SCN shell peptides. Two other major SCN afferent systems converge within the SCN core to form terminal fields that overlap at least partially with the RHT: (1) a projection arising from retinorecipient neurons of the thalamic IGL, and co-localizing NPY and GABA, and (2) a projection consisting of serotonergic (and possibly nonserotonergic) axons of the midbrain raphe. Projections from the IGL to the SCN provide an indirect route for retinal signals to reach the SCN, and while disruption of this pathway fails to abolish photic entrainment of the circadian pacemaker, it does alter the effects of light on circadian period, phase, and coherence. Further, IGL lesions broadly disrupt the ability of arousal-related nonphotic cues to phase shift the circadian pacemaker, indicating that the IGL plays a major role in the integration of photic and nonphotic entrainment signals. Similarly, an important function of serotonergic raphe projections to the SCN involves arousal-dependent modulation of photic input to the circadian pacemaker, via both pre- and postsynaptic interactions with the photic signaling pathway(s).

In accord with the apparent convergence of both photic and photomodulatory signals, acute light exposure increases the expression of the immediate-early gene, *c-fos*, and of the identified clock genes, *Per1* and *Per2*, specifically within the SCN core (see [Figure 4](#)), including in VIP-, GRP-, and CB-containing cells. While some studies have also shown light-induced IEG and *period* expression in the SCN shell, such effects appear to reflect indirect signaling pathways by which photic information is relayed from light-sensitive SCN core neurons to light-insensitive SCN shell neurons. Remarkably, light evoked phase shifts of gene expression in the SCN core appear to occur almost immediately (within hours), while resynchronization of gene expression in the SCN shell tracks closely with the gradual reestablishment of a new steady-state phase at the behavioral level. Both pharmacological evidence and studies with genetically modified mice indicate that VIP and GABA signaling within the SCN are critical for conveying such signals from core to shell, and, further, for maintaining synchrony among individual SCN pacemaker neurons, probably located in both core and shell.

In contrast to the evidence supporting a preeminent role for the SCN core in processing photic entrainment signals, other studies have revealed that the spontaneous expression of cellular and molecular rhythmicity is more widespread and more robust within the SCN shell relative to the core. For example, SCN shell peptides exhibit persisting *in vivo* circadian rhythms at both mRNA and protein levels under constant darkness, while SCN core peptides do not (both core and shell peptides are consistently rhythmic under light–dark cycles). Similarly, the SCN shell displays spontaneous circadian rhythmicity in both *c-fos* and *period* gene expression while the SCN core does not. Surprisingly, however, spontaneous rhythms in normally nonrhythmic SCN core peptides can be ‘unmasked’ by depletion of other SCN neuromodulators, including somatostatin (generally a shell-associated peptide) and serotonin. In the SCN slice preparation, dorsal (presumably shell) SCN neurons are more likely than ventral (presumably core) SCN neurons to exhibit spontaneous circadian rhythms in firing rate, while SCN slices are more likely to display rhythmic AVP release than VIP release. It is important, however, that circadian firing rhythms may in fact be detected throughout the SCN, and that both shell and core peptides may be released rhythmically by SCN slices. Indeed, cultured SCN slices may display simultaneous circadian rhythms in both AVP and VIP release, with different free-running periods, demonstrating that both core and shell subnuclei must contain potentially autonomous oscillatory networks.

Early studies indicated that microsurgical separation of the dorsomedial (shell) and ventrolateral (core) SCN in a hypothalamic slice preparation spared electrophysiological rhythms in the ventrolateral aspect while abolishing rhythmicity in the dorsomedial aspect of the nucleus. Similarly, fetal tissue transplants appear to restore rhythmicity in SCN-lesioned arrhythmic hosts only when the transplants include surviving core-related peptides, such as VIP and/or CB. While these studies may seem to suggest that critical circadian pacemaker cells are located in the SCN core, they are probably better interpreted as confirming the necessary role for SCN core peptides in coordination and synchronization of critical pacemaker cells within the SCN core and shell. Thus, a reasonable hypothesis for photic information processing within the SCN is that retinorecipient light-entrainable cellular oscillators in the SCN core provide neural signals that are necessary for the entrainment and synchronization of light-insensitive cellular oscillators in the SCN shell. According to this view, much of the dynamic complexity of circadian light entrainment revealed

by formal analysis at the behavioral level can be accounted for by interactions among functionally distinct SCN subnuclei.

## Conclusion

Analysis of the mechanisms by which photic signals entrain the circadian pacemaker has provided a rich model system in chronobiology, and more generally, in behavioral neuroscience. Classic and contemporary behavioral analyses have revealed several general quantitative principles, the most powerful of which is the phase-response curve, a complex yet predictable function that characterizes the phase-dependent responsiveness of the circadian pacemaker to light. Recently, an integrative, multidisciplinary approach incorporating physiological, pharmacological, anatomical, and molecular approaches has successfully pried open the 'black box' formalism of PRC analysis and has provided a robust biological understanding of the photic entrainment pathway. These basic scientific studies await further exploitation as applied chronobiologists develop practical interventions that may be effectively deployed in the service of public health and safety.

*See also:* Circadian Oscillations in the Suprachiasmatic Nucleus; Circadian Regulation by the Suprachiasmatic Nucleus; Circadian Gene Expression in the Suprachiasmatic Nucleus; Circadian Organization in Non-Mammalian Vertebrates; Circadian Rhythms: Influence of Light in Humans; Photoreceptor Adaptation; Photoreceptors and Circadian Clocks; Photoreceptors: Physiology.

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