Published in final edited form as:
Sleep Med Clin. 2009 June 1; 4(2): 99–110. doi:10.1016/j.jsmc.2009.01.005.

BIOLOGICAL TIMEKEEPING

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Keywords

biological rhythms; sleep; circadian; suprachiasmatic nucleus

The daily transition from light to darkness has significantly shaped the evolution of most living species, from unicellular organisms to mammals. Adaptation to this environmental constraint occurred through the emergence of a circadian system capable of adjusting both behavioral and physiological processes to this light-dark cycle. Superimposed upon the daily light-dark cycle is a seasonal influence that modifies the relative durations of day and night over the course of a year. Be they day-active or night-active, all organisms need a means of keeping time in a 24-hour world as well to adapt to the availability of food, and to avoid predators. In addition, they require a means of adjusting to changes in day length or transition times that may occur.

Interestingly, rather than simply reflecting the external day-night cycle, these rhythms in behaviors persist in the absence of exogenous timing cues such as light, food availability, or social cues. Every organism expresses an endogenous rhythm that varies slightly from 24 h, making it *circadian*, or 'about a day.' Uninterrupted, this circadian rhythm persists.

These circadian rhythms can be observed in outputs such as the patterning of the sleep-wake cycle, and in humans, core body temperature is often used as a marker of circadian phase. In addition, numerous endogenous hormones can be used as markers (reviewed by Van Cauter[1]). Although hormonal rhythms exhibit complex waveforms due to combined effects of the circadian pacemaker, organismic state, such as activity level, sleep and feeding, and the pulsatile nature of secretion, clear diurnal patterns of secretion have been reported [2]. Plasma melatonin [3, 4], growth hormone [5], prolactin [6], thyrotropin-releasing hormone [7], luteinizing

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hormone [8] and leptin [9-11] are all elevated during the night, in antiphase to adrenocorticotropic hormone and cortisol [12, 13]. These oscillations in hormone secretion continue in a constant environment, and, therefore, are clock-regulated. Circadian rhythmicity appears to be present at virtually every level of functioning studied. In fact, maintenance of a constant *milieu interior* may be a consequence of a balance among rhythmic, mutually opposed control mechanisms [2].

This review will explain the neurobiology of circadian timekeeping, describing what is known about the master pacemaker for circadian rhythmicity, how various biological systems can provide input to the endogenous biological timing, and how the pacemaker can in turn influence the physiology and behavior of the individual. We will discuss how the circadian system can adapt to a changing environment by resetting the circadian clock in the face of a variety of inputs, including changes in light, activity and the sleep-wake cycle. Finally we will discuss the genetics of circadian time-keeping, highlighting what is currently known about heritable disorders in circadian timing.

I. THE CIRCADIAN CLOCK

In mammals, circadian rhythms are regulated by a paired set of nuclei located at the base of the hypothalamus, directly above the optic chiasm, hence their name – the suprachiasmatic nuclei (SCN) (Fig 1). Multiple experiments have demonstrated the role of the SCN as a central pacemaker for circadian rhythms. Lesioning studies found that damage to the SCN disrupts rhythmicity in corticosterone levels, drinking, and wheel running behavior [14, 15]. This provided the initial evidence that the central pacemaker for the mammalian clock lay within the SCN.

In later work, it was found that transplanting fetal SCN tissue into the third ventricle of animals in which the SCN had been lesioned could restore rhythmicity [16]. Furthermore, if fetal SCN tissue from a wild-type hamster was implanted into a hamster with a genetic alteration that shortened free-running period, the new free-running period resembled that of the SCN donor rather than the host animal. This evidence suggested that not only was the SCN necessary for generating rhythms, but also that this rhythmicity was an intrinsic property of the SCN cells, which could drive the rhythms for the entire animal [17].

In the mouse, each SCN measures approximately 300 μm medial to lateral, 350 μm dorsal to ventral, and spans approximately 600 μm from rostral to caudal end. One SCN contains a total of approximately 10,500 cells [18]. Based on peptide localization, it is common to divide the rodent SCN into a ventrolateral or 'core' region, and a dorsomedial or 'shell' region (Fig. 1). The core neurons are small and contain vasoactive intestinal peptide (VIP), calretinin (CALR), and gastrin-releasing peptide (GRP) colocalized with γ-amino butyric acid (GABA), while the shell neurons are larger and contain arginine vasopressin (AVP), met-enkephalin (mENK), and angiotensin II (AII) [18]. There are topographic connections between the contralateral shells and the contralateral cores, as well as a unidirectional connection between the core and shell within each nucleus [19].

The human SCN is not as compact as the rodent but contains many of the same subdivisions. The dorsal and medial regions contain neurophysin/vasopressin neurons. The core region contains calbindin, synaptophysin and VIP neurons, while the ventral and rostral regions contains synaptophysin, calbindin and substance P [20].

Inputs

In conjunction with its ability to regulate circadian timing, the SCN is also positioned to receive information about the behavioral and environmental state of the animal in order to ensure proper

setting of the circadian clock. This information is conveyed to the SCN by projections from a variety of different brain regions.

One of the most extensively studied inputs to the SCN comes from a subpopulation of retinal ganglion cells whose central projections form the retinohypothalamic tract (RHT). Lesions of the SCN disrupt the development of these neurons [21], and disruption of the RHT results in an inability to respond to resetting light signals [22, 23]. Recent work has found that many of the retinal ganglion cells that comprise the RHT contain a photopigment, melanopsin [24]. These melanopsin-containing cells are photosensitive at the same wavelengths that are most effective for circadian resetting [25]. Additionally, the terminals of the melanopsin-positive retinal ganglion cells collocalize glutamate (GLU) and pituitary adenylate cyclase-activating polypeptide (PACAP) [26], the putative neurotransmitters of the RHT.

The RHT also sends projections to the thalamic intergeniculate leaflet (IGL), which in turn sends projections back to the SCN through the geniculohypothalamic tract (GHT). The GHT contains neuropeptide Y (NPY) and GABA. 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 [27, 28]. However, while the GHT pathway can transmit photic signals, disruption of this pathway does not prevent entrainment [29].

The SCN also receives serotonergic input, primarily from the median raphe, that is primarily involved in activity-induced phase shifts during the daytime. Activation of the median raphe results in an increase in serotonin (5-HT) release at the SCN [30-32]. 5-HT release also shows a strong circadian release pattern in the SCN, with 5-HT release peaking at CT 14, and 5-hydroxyindole acetic acid (5-HIAA), the major metabolite of 5-HT peaking at CT 16 [33]. SCN sensitivity is similar to NPY:5HT causes daytime phase shifts in nocturnal animals and modulates the response to light signals at night [34, 35].

Cholinergic projections to the SCN originate both in the brainstem and basal forebrain in brain nuclei with identified roles in sleep and arousal [36] and were recently demonstrated to also be present in diurnal animals [37]. Within the brainstem, these cholinergic projections arise from three nuclei. The parabigeminal nucleus (PBg) 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 [38], while the laterodorsal tegmental (LDTg) and pedunculopontine tegmental (PPTg) nuclei both are important for regulating the sleep-wake cycle [39]. In the basal forebrain, the substantia innominata (SI) within the nucleus basalis magnocellularis (NBM) in the basal forebrain contributes to arousal and focused attention [40]. The LDTg, PPTg, and NBM are interconnected, and all play roles in regulating the sleep and arousal states of the animal. This would suggest that the cholinergic input to the SCN is providing a signal regarding the sleep and arousal states of the animal, and may provide a link between the sleep-wake cycle and circadian rhythms.

Additional sleep-wake input to the SCN may come from tuberomammillary nucleus (TMN). Studies have shown histaminergic input to the SCN from the TMN[41]. Histamine is a regulator of the sleep-wake cycle, primarily providing a signal of wakefulness.

Outputs

The SCN exerts its influence on the rest of the body primarily by sending projections to the rest of the hypothalamus. Neurons from the core region project to the lateral region of the subparaventricular zone (sPVHz), the peri-suprachiasmatic area (PSCN), and the ventral tuberal area (VTU), all within the hypothalamus. The shell projects to medial preoptic area (MPOA), medial sPVHz, dorsal parvocellular paraventricular nucleus (dPVN) and the dorsal medial hypothalamus (DMH), also all within the hypothalamus [42]. The targets of efferents to

the dPVN consist of either endocrine neurons, autonomic neurons, or intermediate neurons that potentially serve to integrate a number of hypothalamic signals [43]. The DMH projections are particularly interesting, as many of these neurons appear to be projecting to neurons containing hypocretin/orexin, a peptide well known for its role in arousal [44, 45]. In addition, evidence exists for a multi-synaptic pathway between the SCN and locus coeruleus (LC), an important arousal center in the brain, mediated by orexin [46], with the DMH as a relay [47]. The SCN also contains a minor set of efferents to the ventrolateral preoptic nucleus (VLPO), a region which if lesioned produces prolonged reduction in sleep duration and amplitude [48]. In addition, the SCN contains projections to the paraventricular nucleus (PVT) and intergeniculate leaflet (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, integratative visual centers [49-51]. The PVN appears to act as a relay between the SCN and the amygdala, which may provide a link between the circadian system and affective disorders [52]. Overall, the SCN appears to be uniquely situated within a network that allows it to interact closely with the regions controlling sleep and arousal states.

One of the major output roles of the SCN appears to be to provide an inhibitory signal for activity. Two recently discovered 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 ventricles, TGF-α inhibits locomotor activity. Conversely, mice lacking the epidermal growth factor (EGF) receptor, making them unable to respond to TGF-α, show an excessive amount of daytime activity [53]. PK2 is also expressed rhythmically in the SCN, again showing peak expression during the animal's inactive period, and can inhibit locomotor activity when infused continuously [54]. This suggests a role for the output signal of the SCN in promoting an inactive state that would be permissive for sleep.

II. CIRCADIAN RESETTING

Within such a complex neuronal clock structure, there is a consensus that timekeeping is a cellular process. Indeed, 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 this clock [55]. It follows that gating of sensitivity to resetting stimuli and phase resetting must be cellular properties. Moreover, the clock must be able to restrict the range of responses in the cellular repertoire so that activation of select signaling pathways can occur only at the appropriate time in the circadian cycle. We have endeavored to determine how the clock temporally regulates the responsiveness of specific signaling pathways.

In an attempt to define and understand the underlying control mechanisms subserving clock-gated windows of sensitivity, we exposed the SCN-bearing brain slices *in vitro* to treatments that activate elements of specific signaling pathways. Treatments were administered at various discrete points in the circadian cycle, and the time of the peak in the spontaneous rhythm of neuronal activity was assessed over the next one or two circadian cycles *in vitro*. If the time-of-peak appeared earlier during cycle(s) after treatment compared to controls, the phase of the rhythm had been advanced. If the time-of-peak appeared later than in controls, then the phase had been delayed by the treatment. By assessing the changing relationship between the circadian time of treatment and its effect on phase, a phase-response curve (PRC) was generated. 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. The permanence of the phase shift was examined by evaluating the

time of the peak in neuronal activity over one or two days after a treatment. Timing of the peak after experimental reagents had been administered at the maximal point of sensitivity was compared with the time of the peak in media-treated controls.

Temporal spheres identified as sensitive to phase resetting via specific first and second messenger pathways coincide with discrete portions of the circadian cycle. In terms of these temporal restrictions, the circadian cycle can be divided into several discrete temporal states, or domains, of the clock: day, night, dusk and dawn [56]. Our studies not only contribute to defining the properties of the clock's temporal domains, they emphasize the complexity of control that the clock exerts over signal integration and phase resetting within the SCN. These properties have been incorporated into putative clock-gated regulatory pathways. Each will be 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. Furthermore, each correlates with discrete periods of sensitivity to specific neurotransmitter systems that are demonstrated to impinge upon this hypothalamic site as evidenced by a large body of neuroanatomical studies [57]. This permits speculation regarding the nature of pathways that gain access to and regulate the biological clock at different points in the circadian cycle. We will now consider, in turn, the major identified domains of clock sensitivity.

Circadian Clock Regulators

Daytime—A number of signaling molecules appear to be important in resetting circadian rhythms during the daytime, including 5-HT, PACAP, NPY and GABA (Fig. 2). The majority of these experiments have been performed in nocturnal rodents, so daytime is defined as the time in which the lights are on, and/or the animal is inactive. As a result, the functional context of this regulation seems to be tied to arousal-induced resetting, often referred to as non-photic resetting. Non-photic signals cover a wide variety of phenomenon, including sleep deprivation, activity associated with exposure to a novel wheel, or even cage changes. The unifying factor in non-photic signals is that they involve arousal during a time when the animal would normally be inactive.

While 5-HT is believed to play a role in activity-induced or non-photic phase shifts during the day, there is some question about whether this form of phase shifting is entirely due to 5-HT. If serotonergic agonists are applied to the hypothalamic brain slice during the daytime, the peak in electrical firing activity advances, but no change in peak firing rate is seen if the agonists are applied during the night [34],. Similar results are seen *in vivo* if the dorsal or medial raphe is stimulated [32],, a paradigm which has been shown by microdialysis to increase 5-HT release at the level of the SCN [58]. During forced wheel running or sleep deprivation during the daytime, there is also an increase in 5-HT release at the SCN [59, 60]. This suggests a possible link between 5-HT and non-photic phase shifting, but evidence also exists to complicate this assertion. If 85-95% of the serotonin is depleted from the raphe projections to the SCN, animals still are capable of phase shifting in response to daytime forced activity [61]. In addition, these activity-induced phase shifts are not significantly attenuated following injection of serotonergic antagonists [62]. These data suggest that while 5-HT may play a role in non-photic resetting, the full resetting response depends on additional modulatory neurotransmitters, possibly neuropeptides.

PACAP appears to play a dual role in the SCN, producing effects both during the daytime by itself and at night by acting in conjunction with GLU. PACAP is not intrinsic to the SCN; it is released from the RHT, where it is colocalized with GLU [63]. Examining levels of PACAP in tissue samples collected throughout the 24-h cycle revealed that PACAP exhibits a significant oscillation in the SCN, but not in other brain regions, and is lower during the light period than

the dark period [64]. If PACAP is applied to the brain slice at different times of day, micromolar quantities will cause an advance in neuronal firing activity during the daytime, but have relatively little effect during the night [26]. However, the *in vivo* response is more complicated. When PACAP is injected into the SCN of the hamster between CT 4-8, transient phase advances in wheel-running activity are seen during the first day after treatment, paralleling the results seen with the brain slice, but the long-term effects of a PACAP injection appear to produce a delay in wheel-running activity [65]. This suggests that while PACAP has an effect on circadian rhythms during the daytime, further work is needed to determine the precise nature of this signal.

NPY also 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. Studies have examined the effects of either injecting NPY into the SCN region of the intact animal and monitoring wheel running behavior [66, 67] or applying NPY directly to the hypothalamic brain slice and examining the peak in neuronal firing activity [27]. In both cases it was found that when NPY was applied during the daytime, it induced 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 [68]. Interestingly, it has been found that exposing an animal to light [69] or applying GLU to the brain slice [70] were both capable of blocking the response to daytime application of NPY. The addition of the $GABA_A$ antagonist, bicuculline, is also capable of inhibiting the effects of NPY [71], suggesting that the effects of NPY are linked to GABAergic signaling.

One factor that daytime signaling pathways hold in common is that they all appear to be mediated by cyclic adenosine monophosphate (cAMP). In the hypothalamic brain slice, cAMP or cAMP analogs applied during the daytime induce phase advances in the circadian clock, while at night they have little effect [72, 73]. In addition, endogenous cAMP is high during late day, and late night [74], 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.

Dawn and Dusk—The primary resetting signal associated with dawn and dusk is melatonin (Fig. 2). 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 the hamster, that are seasonally reproductive. Melatonin is produced by the pineal gland, and in many vertebrates the pineal is actually the primary regulator of circadian rhythms, rather than the SCN. 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 [75].

While 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 lesioning the SCN, but not the pineal, abolishes the ability of a rat to entrain to melatonin injections [76].

Evidence that melatonin can entrain circadian rhythms led to a number of studies looking at the direct effect of melatonin on the SCN. Using either 2-deoxy-[1-^{14}C]glucose (2-DG) or neuronal activity as a marker of SCN activity, melatonin decreases both 2-DG uptake and neuronal firing activity in the rat or hamster most significantly when applied right before dusk [77-79]. By examining electrophysiological activity *in vitro* in the SCN, melatonin applied at either dawn or dusk advances the peak in neuronal firing, but produces no effects when applied at other times of day [80, 81]. This resetting pattern mimics that seen in response to activation of

protein kinase C (PKC), and was blocked by inhibitors of PKC, suggesting that PKC is a downstream component of this resetting pathway [81]. In addition, this resetting could be inhibited with antagonists specific for the MT-2 type melatonin receptor [82]. In humans, circadian sensitivity to melatonin also occurs at dawn and dusk, but the effect is to advance the circadian system at dusk but to delay it at dawn.

Nighttime—In the nighttime domain there are two known key players, GLU and acetylcholine (ACh), as well as a number of modulatory substances associated with these signals (Fig. 2). As was discussed previously, considerable evidence supports GLU as the neurochemical signal transmitting photic stimuli from the retina to the SCN, but the functional context of the cholinergic resetting signal is still unknown.

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 [83, 84]. 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 calcium, followed by nitric oxide synthase (NOS) induction and resultant production of nitric oxide (NO) [83, 85-88]. Beyond this point, the early and late night pathways diverge. During the early night GLU induces delays in the circadian clock through ryanodine receptor (RyR)-mediated calcium release [89]. 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 protein (CREB)-activated transcription [89-91].

While 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 phase-resetting effect of GLU during both the early and late night, which include NPY and GABA [34, 35], 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 include 5-HT and PACAP. If animals are depleted of 5-HT, they show increased phase delays in response to light [93]. Co-application of a PACAP antagonist however, either *in vitro* or *in vivo*, decreases the phase delay seen in early night, and increases the late night phase advance in both rat and hamster [94, 95]. When PACAP is administered in conjunction with GLU, it increases the early night phase delays, but decreases the late night phase advances. This is similar to the effects seen following application of cAMP analogs to the hypothalamic brain slice, suggesting that the effects of PACAP may be mediated by a cAMP pathway [96].

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 might play a role in resetting the circadian clock came in 1979, when Zatz and Brownstein examined whether pharmacological manipulation of the SCN could affect circadian rhythms, using serotonin N-acetyltransferase (SNAT) activity in the pineal as a marker of circadian phase. SNAT activity has an endogenous rhythm in the pineal that is higher during the night than during the day, and this rhythm was previously found to be reset by light. It was found that injections of carbachol into the lateral ventricle of Sprague-Dawley rats at CT 15 caused phase delays in SNAT activity that were similar to, but not as large as, the phase delays produced by light [97]. Carbachol injections into the lateral ventricle were also later repeated in mice [98] and hamsters [99], where it was found that administration of carbachol during early night caused phase delays, while late night administration caused phase advances.

This pattern of sensitivity and response 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 (RIA) [100]. 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, however, is that ACh might be the primary neurotransmitter providing the signal of light to the clock.

However, significant evidence began to emerge indicating that ACh was not likely to be the primary signal of light. First of all, 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 (ChAT) was not present in this projection [101], making it anatomically unlikely that ACh was the primary neurotransmitter involved in this signal. This evidence might need to be reconsidered, however, as recent studies have found an alternative splice variant of ChAT present in ganglion cells that was not picked up using previous antibodies [102]. Experiments have not yet been published looking at whether this alternative form of ChAT is present in the RHT.

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 [103]. 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 was upstream of a glutamatergic signal [104]. Finally, Liu and Gillette [105], 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 evening.

In an attempt to explain these contradicting data, it was hypothesized by our lab that the dual response pattern of the SCN to cholinergic stimulation was a result of the location of application. Note that in the initial *in vivo* studies, carbachol was injected into the lateral or third ventricle, where the drug could have a diffuse effect, while in the *in vitro* studies carbachol was applied in microdrops directly to the SCN. As was predicted, if the *in vivo* experiments were performed by injecting carbachol directly into the SCN rather than into the ventricle, a similar phase response pattern to that observed in the *in vitro* experiments using microdrop applications resulted [106]. Furthermore, it was found that mice lacking the M_1-type muscarinic receptor (M_1AChR) do not respond to intra-SCN carbachol injections [107], but still exhibit biphasic responses to light and *icv* injections of carbachol [108]. Together this evidence 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 the ventricles, that is likely upstream of a glutamatergic signal, and a direct response that is mediated by the M_1AChR. Based on the anatomical studies looking at cholinergic projections to the SCN that originate in the LDTg and PPTg, as well as the NBM, the current hypothesis is that this cholinergic signal may be involved in linking the sleep-wake and circadian cycles together.

III. GENETICS OF CIRCADIAN RHYTHMS

Much research effort has focused on determining how a biological system keeps 24-hour time. With the discovery that single, dispersed cells can exhibit circadian rhythms, the focus turned towards understanding cellular processes that generate a near 24-hour timebase. A molecular clockwork appears to generate a ~24-hour rhythm through a feedback cycle involving a set of

core clock genes, their mRNAs, and proteins. Together they form the molecular clockwork. This cycle consists of a set of interconnected positive and negative feedback loops, and their regulatory elements. Positive elements, which include *Clock* and *Bmal1*, are transcribed into mRNA, which is then translated into proteins that heterodimerize and are translocated into the nucleus. In the nucleus, they activate continued transcription of their own genes, as well as activating transcription of negative elements. The negative elements, which include *Period*, *Cryptochrome* and *Rev-erbα* are then transcribed and translated. Proteins of the negative elements also associate in complexes and are translocated to the nucleus, where they feed back to inhibit transcription of the positive elements. Additional genes which have been proposed to be involved in the circadian clock include *Rorα* [109], *Timeless (Tim)* [110], *Dec1* and *Dec2* [111] and most recently *SIRT1* [31, 112, 113]. These feedback loops are further affected by regulatory enzymes, including casein kinase 1 epsilon (CKIε) and glycogen synthase kinase (GSK) [114-116], and small intracellular regulatory molecules with established roles in signal transduction [31, 117]. The cycle of these feedback loops takes approximately 24-hours to complete, providing a means by which cells within the SCN can maintain a circadian rhythm.

Core clock elements have been found to play a critical role in human sleep disorders. For example, inherited forms of advanced sleep phase syndrome (ASPS) have been associated with either a mutation in the *Per2* gene that interferes with a normal phosphorylation site of CKIδ/ε [118] or with a mutation in CKIδ [119]. Delayed sleep phase syndrome (DSPS) on the other hand has been found in some cases to be associated with a specific polymorphism of hPER3 [31, 120, 121]. Recently PER3 expression patterns in human leukocytes were found to correlate with sleep-wake timing, particularly in those individuals with a morningness preference [122]. Finally, morningness/eveningness preference has been associated with a polymorphism of the human *CLOCK* gene [31, 123, 124].

IV. CONCLUSION

Circadian rhythms are the near24-hour oscillations brain and body functions, such as core body temperature, hormone release, and the sleep-wake cycle. The master pacemaker regulating these rhythms is located in the suprachiasmatic nucleus (SCN) in the hypothalamus. The SCN is ideally situated to receive input about environmental light, sleep-wake state and activity status. It can be reset in response to these stimuli and, in turn, provide output signals to regulate the timing of activity and behavior. The core mechanisms providing this timekeeping ability are still being elucidated, but appear to be provided by through transcription/translation feedback loops, consisting of both positive and negative elements, coupled with other intracellular elements associated with signaling events. Interestingly, circadian rhythm sleep disorders as well as sleep phenotypes are beginning to be correlated with abnormalities in the genes regulating circadian rhythms.

Acknowledgments

The authors gratefully recognize present and past support from the National Institutes of Health: HL08670, HL67007, NS22155, and NS35859 (MUG) and F30 NS047802 and GM07143 (SMA).

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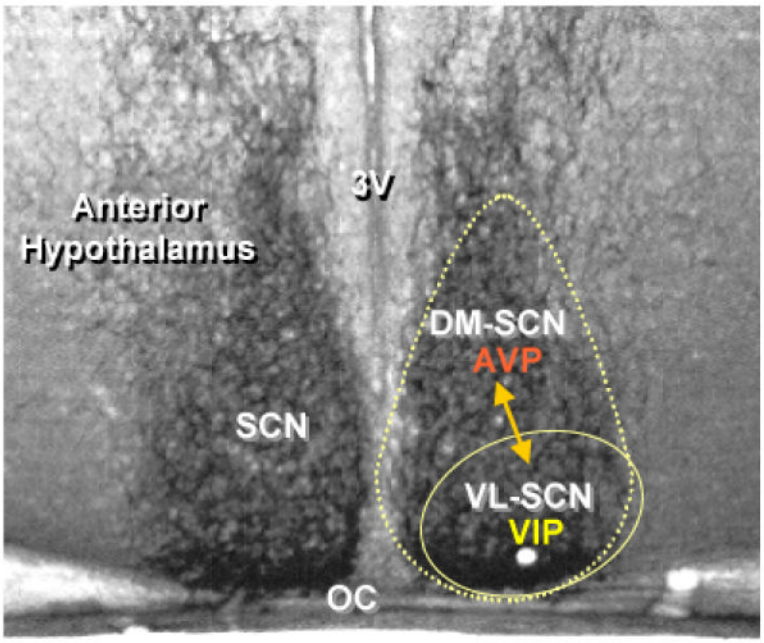

Figure 1.

Anatomy of the mammalian suprachiasmatic nucleus (SCN). This medial; transverse section of the rat anterior hypothalamus shows the bilateral SCN stained darkly with an antibody to an endogenous peptide. The paired SCN are at the base of the brain, flanking the third ventricle (V3) and positioned directly above the optic chaism (OC). The two major subdivisions of the SCN are delineated. The dorsomedial SCN (DM-SCN) is marked by neurons expressing arginine vasopressin (AVP), whereas neurons of the ventrolateral SCN (VL-SCN) express vasoactive intestinal peptide (VIP).

Figure 2.
Circadian changes in temporal windows of SCN sensitivity to phase-resetting signals transmitted from various brain sites. Time-of-day specific signals are presented together with the major sources of SCN innervation by projections bearing these neurotransmitters and neuropeptides. Daytime is marked by sensitivity to serotonin (5-HT), pituitary adenylate cyclase-activating peptide (PACAP), neuropeptide Y (NYP) and GABA. During dusk and dawn, the pineal hormone melatonin can stimulate resetting of the SCN clock. At night, the SCN is sensitivity to phase adjustment by glutamate and PACAP from the eye, as well as by cholinergic inputs from brain regions that regulate sleep and wakefulness.