Review TRENDS in Neurosciences Vol.28 No.3 March 2005

Orchestrating time: arrangements of the brain circadian clock

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Daily oscillations in physiology and behavior are regulated by a brain clock located in the suprachiasmatic nucleus (SCN). Individual cells within this nucleus contain an autonomous molecular clock. Recent discoveries that make use of new molecular and genetic data and tools highlight the conclusion that the SCN is a heterogeneous network of functionally and phenotypically differentiated cells. Neurons within SCN subregions serve distinctly separate functions in regulating the overall activity of the circadian clock: some cells within the SCN rhythmically express 'clock' genes, whereas others exhibit induced expression of these genes after the organism has been exposed to a light pulse. The coordinated interaction of these functionally distinct cells is integral to the coherent functioning of the brain clock.

Introduction

One of the earliest controversies in neuroscience concerned localization of function in the brain. Pierre Flourens and Karl Lashley, advocates of the aggregate field theory that all brain regions participate in all mental functions, tested localization of function by making lesions throughout the brains of rats in an attempt to eliminate specific behaviors. These crude experiments led to the conclusion that observed deficits were a consequence of the size, rather than the location, of the lesion. Modern neuroscience now rejects the aggregate field theory, based on convincing evidence for localization of function. However, although the suprachiasmatic nucleus (SCN) is possibly one of the best examples of localized neural function, the argument of localized versus distributed function has been recapitulated as our understanding of brain clock assembly has evolved.

Keeping time: how to synchronize 20 000 clocks

Biological processes exhibit daily rhythms that enable organisms to exploit temporal niches in their environment and coordinate physiological processes to optimize metabolic efficiency. In mammals, these rhythms exhibit periods of about a day and are endogenously generated by the 20 000 neurons [1] that constitute the master circadian pacemaker located in the SCN. This conclusion rests on decades of research from numerous laboratories [2].

Circadian rhythmicity is abolished by SCN lesions [3,4] and restored by SCN transplants [5]. The restored behavioral rhythm expresses properties of the donor, not the host [6,7]. These studies led to a dispute reminiscent of the classic aggregate-field versus localization-of-function debate because the lesion results suggest that, as long as 15–25% of the SCN remains intact, rhythmicity persists [8–11]. When the lesion data were integrated with the observation that individual neurons within the SCN are rhythmic and exhibit a range of circadian periods [12–17], it appeared that an organism needed only a small number of clock cells to exhibit overt rhythmicity. In determining whether a lesion would eliminate rhythmicity, location of the lesion and identity of the cells spared or destroyed appeared to be less important than the amount of tissue removed. This led to the question of how a single unified output emerged from this assembly of independent oscillators. In one model, individual cells within the SCN are weakly coupled such that the oscillators become synchronized and oscillate with the mean period of the population [13].

The past decade has witnessed rapid breakthroughs in our understanding of how the circadian clock functions at the cellular and molecular levels (Figure 1). Individual SCN neurons express self-sustained circadian oscillations [12] driven by autoregulatory transcription–translation feedback loops [1,18]. The discovery of core molecular components of the cellular circadian clock enables examination of SCN function at high spatial and temporal resolution, revolutionizing our understanding of how the different cell populations within the SCN are arranged to form separate functional units. These studies challenge in two ways the view that the SCN is functionally homogeneous. First, some SCN cells are not endogenously rhythmic with respect to clock gene expression; only some cells receive direct retinal input and immediately express clock genes following photic stimulation. Second, individual clock cells within the SCN exhibit various phases and free-running periods [15,17,19]. These phenomena are difficult to explain by coupling alone. An alternative 'network' model suggests that it is precisely this heterogeneity of the SCN that is integral to keeping the

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Available online 21 January 2005

 0166-2236/$ - see front matter doi:10.1016/j.tins.2005.01.003

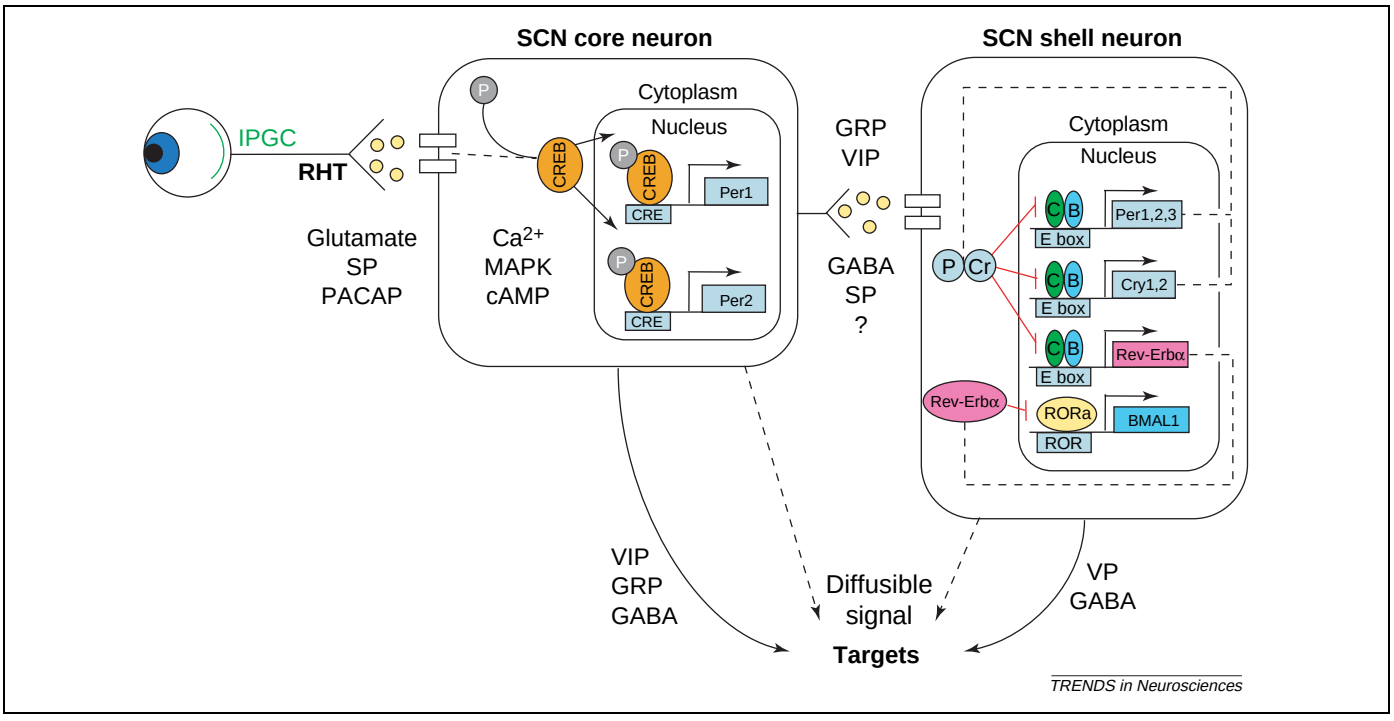

Figure 1. Molecular regulation of the intracellular circadian clock. Light is transduced into a neural signal by intrinsically photoreceptive ganglion cells (IPGCs) in the retina and conveyed to the SCN core along the retinohypothalamic tract (RHT), resulting in the release of the neurotransmitter glutamate and the neuromodulators substance P (SP) and pituitary adenylyl cyclase activating peptide (PACAP) onto retino-recipient cells in the SCN core. Glutamate activates NMDA receptors, causing an influx of Ca^{2+}, which activates kinases such as mitogen-activated protein kinase (MAPK), resulting in phosphorylation of cAMP-response-element-binding protein (CREB). Activated CREB binds to the Ca^{2+}/cAMP response element (CRE) in the promoter region of both *Per1* and *Per2*, activating their transcription. Neurons in the SCN core communicate with the rhythmic SCN shell and SCN targets using a variety of neurotransmitters, including vasoactive intestinal polypeptide (VIP), gastrin-releasing peptide (GRP) and SP. Additionally, almost all SCN cells are GABAergic [24]. Cells in the rhythmic SCN shell contain molecular clocks driven by an autoregulatory transcription–translation loop [1,69]. CLOCK (C) and BMAL (B) dimerize and bind to E-boxes in the promoter region of *Period* (*Per*) genes, *Cryptochrome* (*Cry*) genes and *Rev-Erbα*, activating their transcription. As PER (P) and CRY (Cr) proteins accumulate in the cytoplasm, they dimerize and translocate to the nucleus where they inhibit the activity of CLOCK–BMAL, thus inhibiting their own transcription. At the same time, transcription of BMAL is regulated by an RAR-related orphan receptor (ROR) element that is activated by RORa and inhibited by Rev-Erbα [70]. SCN shell neurons communicate with SCN targets using VP and GABA as neurotransmitters. Additionally, the SCN communicates with some target sites using a diffusible signal [68].

population of oscillators ticking in a coherent fashion [20]. This model hypothesizes that the phases of individual oscillators are synchronized by a daily signal from a subset of SCN cells. By pulling the phases closer together, the overall output of the system remains rhythmic. Central to this model are recent discoveries demonstrating that the morphological and peptidergic heterogeneity of the SCN reflects its functional heterogeneity.

Working in concert: every cell has a role

Classically, the SCN has been subdivided into a dorsomedial shell and a ventrolateral core, based initially on retinal innervation patterns and later on the observation that these regions are defined by phenotypically distinct cell types [21] (Figure 2). This general arrangement has been noted in hamsters, mice, rats and humans [22–25]. Although the hamster SCN will form the framework for the following discussion, some important species-specific differences are noted. Phenotypically, the SCN shell is delineated by vasopressin (VP)-containing cells [22–25], whereas the SCN core contains a variety of cell phenotypes. The most ventral populations is a group of vasoactive intestinal polypeptide (VIP)-containing neurons [23–25]. In hamsters, the caudal, ventrolateral SCN contains a cluster of calbindin (CalB)-expressing cells [26]. Dorsal to the CalB-containing cells is a group of neurons in which the rhythm of phosphorylation of the extracellular signal-regulated kinase 1/2 (p-ERK) is driven by the retina [27]. These cells are called 'cap' cells owing to their appearance, in hamsters, of sitting above CalB-containing cells [28]. Gastrin-releasing peptide (GRP)-containing cells overlap with cap cells (Han S. Lee, PhD thesis, University of Cincinnati, 2003) and CalB-containing cells in hamsters [29], and with VIP-containing cells in rats [30,31]. The spatial relationship of these phenotypically distinct regions is presented schematically in Figure 2, and Figure 3 highlights the phenotypic heterogeneity.

The rhythm section of the orchestra: the vasopressin region

Some cells within the SCN exhibit self-sustained rhythmicity [12] driven by the autoregulatory transcription–translation feedback loop that regulates expression of the *Period* (*Per*) and *Cryptochrome* genes [1]. Rather than being uniformly distributed in the SCN, these intrinsically rhythmic cells are largely confined to the SCN shell, occupying roughly the same area as VP-containing cells (Figure 2) [32–34]. This region of the SCN receives little retinal innervation [21,24] and displays delayed clock gene expression following phase-shifting light exposure [32].

Conducting the show: a role for the SCN core

Cells in the SCN core receive direct retinal innervation [35,36] and express c-fos, *Per1* and *Per2* in response to phase-shifting light pulses [26,32,33,37–39]. Among the

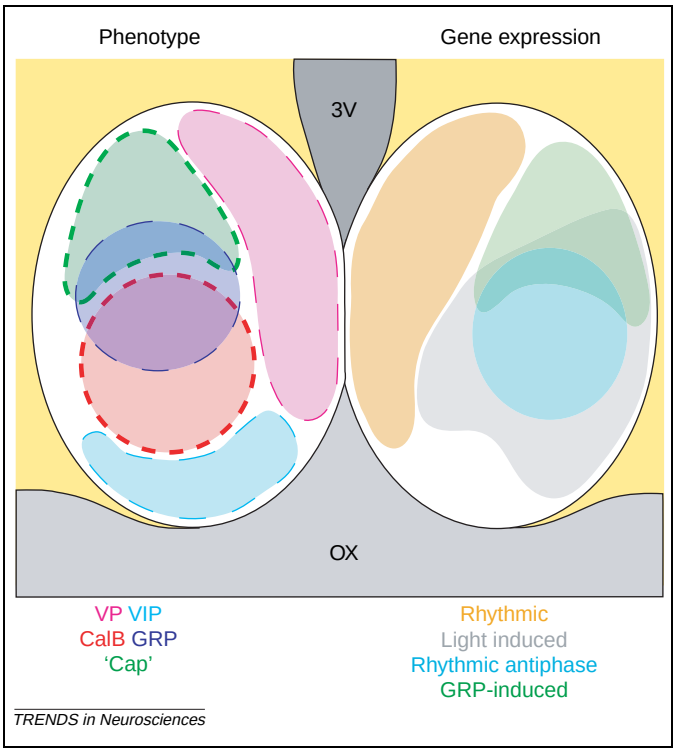

Figure 2. Spatial organization of the different cell populations in hamster SCN at the mid-caudal level. The left SCN depicts the different phenotypic subregions. The SCN shell is delineated by vasopressin (VP)-expressing cells (pink). The SCN core is composed of four cell groups: gastrin-releasing peptide (GRP)-expressing cells (dark blue), calbindin (CalB)-expressing cells (red), vasoactive intestinal polypeptide (VIP)-expressing cells (light blue), and 'cap' cells (green). VIP-containing cells are located along the ventral margin of the SCN; CalB-containing cells lie just dorsal to VIP. The phenotype of the cap cells, so called as they resemble a cap sitting just dorsal to the CalB subregion, has not yet been identified. The cap cells are GRP-responsive and contain phosphorylated extracellular signal-regulated kinase 1/2 (p-ERK) during the night. In hamsters, GRP-containing cells overlap with both the CalB-positive and the cap cell populations. In rats, GRP-expressing cells overlap with those expressing VIP. The right SCN depicts the different regions as defined by *Period* gene expression. Cells that rhythmically express *Period* genes are located in the pale orange region; the gray region within the SCN represents cells that show light-induced *Period* gene expression, and cells in the green region express the *Period* genes following GRP administration. In mice and rats, a small group of cells (blue) expresses *Period* genes rhythmically in antiphase to the expression pattern observed in the SCN shell. Abbreviations: 3V, third ventricle; OX, optic chiasm.

different cell populations in the core, CalB-expressing cells lack both rhythmic electrical activity [40] and rhythmic expression of clock genes [32,34]. Although mice do not have a cluster of CalB-expressing cells in their SCN core, they nevertheless have SCN cells with the same features as those of the CalB region of hamsters. In mice, GRP-expressing cells in the SCN core are light-inducible [41]. It has been observed that cells in the core of the mouse SCN either lack rhythmic expression of *Per1* and *Per2* [41] or express these genes in antiphase to the expression in the SCN shell [42]. Cells in the core oscillate in their responsiveness to photic input: although light exposure always increases firing rates in SCN neurons [43], light induces clock gene expression in the SCN only during the night [1,44]. This gene expression pattern is mirrored by changes in the subcellular localization of CalB, with the cellular nucleus devoid of CalB during the night [44]. Decreasing CalB levels using antisense oligonucleotides eliminates behavioral and molecular responses to nocturnal light pulses [44]. This suggests that CalB-expressing cells function as gates that relay photic signals when open, and that block these signals when closed.

Despite a lack of intrinsic rhythmicity in clock gene expression, the CalB-expressing subregion is essential for SCN function as the master circadian pacemaker. Animals lose all locomotor, hormonal and physiological rhythms following partial SCN ablations that spare up to 50% of cells in the rhythmic SCN shell but eliminate the CalB region [45,46]. Larger lesions that spare the CalB region do not result in loss of rhythmicity. A recent model suggests that this region provides a daily signal that maintains synchronization among the clock cells in the SCN shell [20]. It is predicted that without this signal, oscillators become desynchronized, yielding an arrhythmic system. This phenomenon has recently been demonstrated *in vitro* using coronal brain slices from transgenic animals in which luciferase reports *Per1* expression [17]. Although expression in each individual cell peaks at a particular time of the day that is stable for that cell over several cycles, peak expression for the population as a whole occurs during the middle of the day. When the dorsal third of the nucleus is surgically separated from the ventral portion that contains the core, coordinated rhythmic output persists in only the ventral portion [17]. Individual cells in the dorsal portion remain rhythmic but coherent phase relationships among cells are lost, resulting in a tissue without net rhythmic output.

The gene expression duet: signal spreading from core to shell

For the circadian clock to be phase-shifted or entrained by light, photic information must be relayed from the light-induced cells to the rhythmic cells, which must respond appropriately to the resetting signal (with delays and advances to early-night and late-night light exposure, respectively). Detailed examination of the temporal and spatial patterns of gene expression reveal that *Per1* and *Per2* are regulated separately in a phase-specific and region-specific manner [38,47]. Following phase-shifting light pulses, *Per* expression is first induced in the SCN core [30,32–34,39]. In the mouse, early-night delaying light pulses induce *mPer1* and *mPer2* expression initially in the SCN core, followed later by *mPer2* expression in the SCN shell. Late-night advancing light pulses induce expression of *mPer1* but not *mPer2*. This *mPer1* expression occurs first in the core and later in the shell. Mid-night light pulses that do not alter circadian phase induce *mPer1* expression only in the SCN core. Based on these findings, it appears that the spread of *mPer2* expression to the shell underlies phase delays, whereas a similar spread of *mPer1* underlies phase advances [38,47]. Phase delays to light are impaired in *mPer2* mutant mice [48,49] and following *Per2* antisense treatment [50]. The role of *mPer1* in light-induced phase shifts is less clear [48,49,51], although *Per1* antisense treatment also attenuates phase delays [50].

The soloists: neurotransmitters underlying intra-SCN communication

Given that various SCN regions communicate in a temporally ordered fashion, it becomes important to

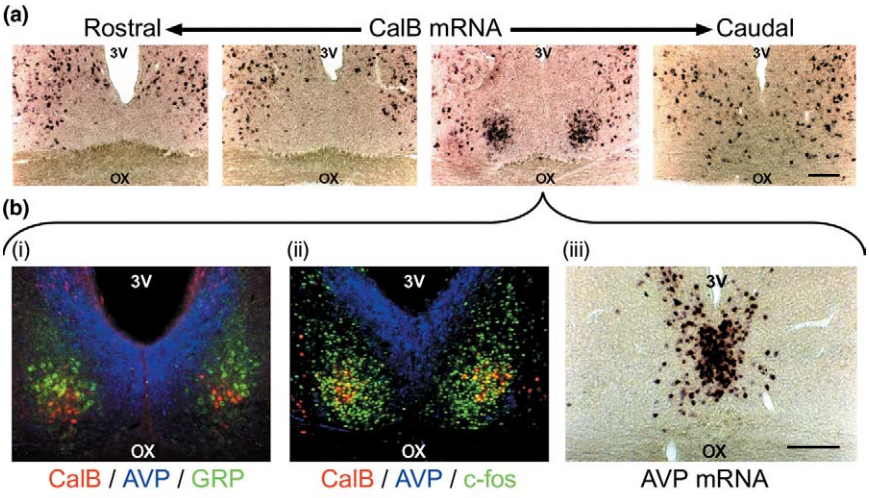

Figure 3. The SCN is heterogeneous in structure and function. **(a)** Calbindin (CalB) expression in the hamster SCN from rostral (left) to caudal (right). **(b)** Heterogeneity of the SCN at the mid-caudal level, as depicted by (i) peptidergic phenotype [expression of CalB, vasopressin (AVP) and gastrin-releasing peptide (GRP)], (ii) functional response (light-pulse-induced c-fos expression, with CalB and AVP as anatomical markers) and (iii) clock-controlled gene expression (AVP mRNA). Abbreviations: 3V, third ventricle; OX, optic chiasm. Panels (a) and (b,iii) are reprinted, with permission, from Ref. [34] © Blackwell Publishing; panels (b,i) and (b,ii) are from unpublished work of M.C. Antle and R. Silver. Scale bar, 100 μm.

understand intercellular communication signals. A phase-resetting signal would be expected to adjust the phase of the circadian clock and to synchronize the activity of clock cells. Impairment of this signal would be expected to result in dampened or arrhythmic output. The neurotransmitters and neuromodulators implicated in intra-SCN communication of photic signals, including substance P (SP), GRP and VIP, will now be discussed in turn.

SP-containing cells are found within the retino-recipient SCN core. In hamsters, almost all SP-positive cells coexpress CalB [29]. When applied to the rat SCN *in vitro*, SP phase-shifts the circadian rhythm in electrical firing rate, with delays during the early night and advances during the late night [52]. SP antagonists attenuate light-induced c-fos expression in the shell but not in the core SCN [53]. These findings are consistent with SP being an output signal of the retino-recipient cells. However, when microinjected into the SCN of hamsters, SP produces small phase delays during only the early night [54]. Together these findings suggest that, although SP might be an intra-nuclear signal from the SCN core, it probably works in concert with other transmitters.

GRP-containing cells are found within the SCN core. In rats they are colocalized with a lateral population of VIP-expressing cells [30], whereas in hamsters GRP-containing cells are located more dorsally and frequently contain CalB [45]. In rats, GRP-containing VIP-positive cells receive retinal input [36] and express c-fos following light exposure [37]. Cells in the GRP-positive region of the rat and mouse also express *Per1* and c-fos following light exposure [30,31,41]. When microinjected to the SCN, alone or in a peptide cocktail, GRP produces phase shifts in locomotor activity rhythms that mimic those produced by light exposure [55,56]. Similarly, when applied to the SCN *in vitro*, GRP produces photic-like phase shifts [57]. Microinjections of GRP lead to transient phosphorylation of ERK1/2 and induce expression of c-fos, *Per1* and *Per2* in the cap cells above the CalB cluster in the SCN [28,58]. Attenuated responses to GRP administration are observed in GRP-receptor-deficient mice and in hamsters in which ERK1/2 phosphorylation has been pharmacologically blocked [28,58]. Although GRP-receptor-deficient mice exhibit attenuated responses to bright light pulses, they have normal responses to dim light pulses and their locomotor behavior is rhythmic in constant conditions [58]. These data suggest that GRP is an output signal of the SCN core that works in concert with other signaling molecules to modulate responses to different light intensities.

VIP-containing cells are found within the SCN core [21], clustered in the ventral SCN adjacent to the optic chiasm. In hamsters, VIP expression is occasionally colocalized with that of CalB. In rats, VIP-containing cells can be divided into a medial GRP-free group and a lateral group that contains GRP [30]. Only the lateral group expresses *Per1* following a light pulse [30]. Few VIP-containing cells rhythmically express *Per1* or *Per2* [31,59]. When VIP is microinjected into the SCN, alone or in a peptide cocktail, it produces photic-like phase shifts [55,56]. When applied to the SCN *in vitro*, VIP produces photic-like phase shifts [60] and induces *Per1* and *Per2* expression [61]. VIP-deficient mice, or knockout mice lacking the VIP receptor $VPAC_2$, have severely disrupted activity rhythms in constant conditions [62,63]. Recent findings suggest that, although these animals and SCN slices prepared from them are frequently arrhythmic, individual SCN cells might continue to be rhythmic in electrical activity [64,65]. These data are consistent with VIP acting as a signal that maintains the phase relationship among clock cells.

The slow arpeggio of rhythmic expression

Cells in the SCN shell have a specific spatial arrangement. Daily rhythmic expression of *Per1*, *Per2* and VP does not occur in all cells simultaneously, but rather spreads and recedes through the SCN similar to water over a tidal

basin [34]. In hamsters this is detected in the rostral half of the SCN, where the shell occupies a much larger area of the coronal SCN than it does at the level of the CalB-expressing subregion. Rhythmic expression of *Per1*, *Per2* and VP starts in a small group of cells located adjacent to the third ventricle in the extreme dorsomedial SCN and spreads ventrolaterally over 4–8 h. It then recedes until expression is again limited to the dorsomedial region [34]. The same pattern of expression has been observed *in vitro* using transgenic mice in which luciferase reports *Per1* expression [17]. The circadian phase of individual cells can be tracked over multiple circadian cycles using this preparation. Expression initially occurs in the dorsomedial periventricular SCN and spreads slowly to the ventral SCN over several hours. Blocking protein synthesis or electrical activity reveals functional aspects of SCN organization. Because the intracellular clock is driven by an autoregulatory transcription–translation feedback loop, individual clocks can all be reset to a common phase by a protein synthesis inhibitor. When the inhibitor is removed, cells begin to oscillate in phase with one another, but re-establish their original phase relationships after several cycles (Figure 4). Furthermore, maintaining this phase relationship requires electrical signaling, either between individual cells or between the core and the shell. When action potentials are blocked with tetrodotoxin, circadian patterns of luminescence in individual cells persist but the system as a whole becomes desynchronized [17]. These data reveal that, although individual cells within the SCN can sustain oscillations, the specific phases of such oscillations are regulated by the network properties of the SCN.

Assembling a symphony from its movements: conclusions and integrations

The circadian system has become a model for understanding neural regulation of complex behaviors. The SCN epitomizes localization of function, but understanding how it functions requires understanding of its different components and their connections. An analogous conundrum occurs in trying to understand functions of cortical interneurons. When classified according to spontaneous electrical activity and morphology, 14 types of cortical interneurons can be identified. However, when the functions of these interneurons are examined, these 14 types collapse into three functional classes [66]. Similarly, although cells within the SCN can be described according to their morphology, peptidergic phenotype, pattern of gene expression, and spontaneous and induced electrical activity, understanding their function will expand our understanding of their role in the circadian network. Several distinct functions can be assigned to cells in the SCN network, including input cells that gate photic information and rhythmic cells that are endogenous oscillators. A recently identified population of light-inducible cap cells that expresses a circadian rhythm driven by the retina forms a third compartment that is liable to have a novel function. It is likely that other aspects of the network remain to be identified.

The next concerto: questions for the future

The SCN is not simply a collection of 20 000 clock cells; rather, it is a heterogeneous structure composed of multiple functional compartments. A new era of work examining gene expression patterns has highlighted the spatial heterogeneity that reflects underlying functional heterogeneity. As each of these separate cell types is investigated further, a picture of how they work together to produce a unified output will emerge. Key to understanding the arrangements of the SCN will be determining the nature of communication among its neurons. Given the spatial pattern of rhythmic expression observed in the SCN shell [17,34], it will be important to determine whether all oscillator cells are equivalent, or whether signals from distinct input regions of the SCN reach only a subset of the oscillator cells. Beyond understanding how the individual components of the SCN are arranged to produce a coherent daily output, it will be essential to understand how this information is communicated to peripheral

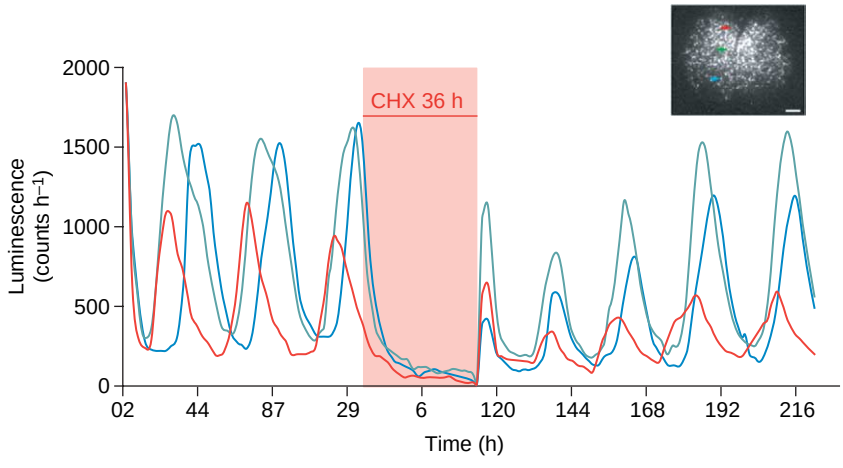

Figure 4. Resetting and reorganizing the synchrony of cell rhythm using cycloheximide (CHX). Recording of bioluminescence of representative individual cells (inset; scale bar, 100 μm). Each cell has a characteristic bioluminescence signal with a specific phase relationship with respect to the other cells. Bioluminescence and circadian rhythmicity is lost during application of CHX. Following removal of CHX, bioluminescence rhythms reappear. The peaks are initially in phase but gradually resume their pre-treatment phase relationships. Modified, with permission, from Ref. [17] © (2003) AAAS (http://www.sciencemag.org).

oscillators and other targets in the body. SCN efferents from both the core and the shell reach each known monosynaptic SCN target site [67], and a diffusible signal from the SCN is sufficient to maintain locomotor rhythms but not endocrine rhythms [68]. Understanding the roles of these distinct signals will be fundamental to understanding how endogenous signals from the SCN are integrated with exogenous environmental cues to produce coherent daily oscillations in functioning of the brain and body.

Acknowledgements

This work was supported by NIH grant NS37919 (R.S.) and by a fellowship from the Canadian Institutes of Health Research (M.C.A.).

References

1 Reppert, S.M. and Weaver, D.R. (2001) Molecular analysis of mammalian circadian rhythms. *Annu. Rev. Physiol.* 63, 647–676
2 Weaver, D.R. (1998) The suprachiasmatic nucleus: a 25-year retrospective. *J. Biol. Rhythms* 13, 100–112
3 Stephan, F.K. and Zucker, I. (1972) Circadian rhythms in drinking behavior and locomotor activity of rats are eliminated by hypothalamic lesions. *Proc. Natl. Acad. Sci. U. S. A.* 69, 1583–1586
4 Moore, R.Y. and Eichler, V.B. (1972) Loss of a circadian adrenal corticosterone rhythm following suprachiasmatic lesions in the rat. *Brain Res.* 42, 201–206
5 Lehman, M.N. *et al*. (1987) Circadian rhythmicity restored by neural transplant. Immunocytochemical characterization of the graft and its integration with the host brain. *J. Neurosci.* 7, 1626–1638
6 Ralph, M.R. *et al*. (1990) Transplanted suprachiasmatic nucleus determines circadian period. *Science* 247, 975–978
7 Sujino, M. *et al*. (2003) Suprachiasmatic nucleus grafts restore circadian behavioral rhythms of genetically arrhythmic mice. *Curr. Biol.* 13, 664–668
8 Davis, F.C. and Gorski, R.A. (1988) Development of hamster circadian rhythms: role of the maternal suprachiasmatic nucleus. *J. Comp. Physiol. [A]* 162, 601–610
9 Rusak, B. (1977) The role of the suprachiasmatic nuclei in the generation of circadian rhythms in the golden hamster, *Mesocricetus auratus*. *J. Comp. Physiol.* 118, 145–164
10 Van den Pol, A.N. and Powley, T. (1979) A fine-grained anatomical analysis of the role of the rat suprachiasmatic nucleus in circadian rhythms of feeding and drinking. *Brain Res.* 160, 307–326
11 Harrington, M.E. *et al*. (1993) Effects of damage to SCN neurons and efferent pathways on circadian activity rhythms of hamsters. *Brain Res. Bull.* 30, 655–669
12 Welsh, D.K. *et al*. (1995) Individual neurons dissociated from rat suprachiasmatic nucleus express independently phased circadian firing rhythms. *Neuron* 14, 697–706
13 Liu, C. *et al*. (1997) Cellular construction of a circadian clock: period determination in the suprachiasmatic nuclei. *Cell* 91, 855–860
14 Herzog, E.D. *et al*. (1997) Circadian rhythms in mouse suprachiasmatic nucleus explants on multimicroelectrode plates. *Brain Res.* 757, 285–290
15 Nakamura, W. *et al*. (2001) Regional pacemakers composed of multiple oscillator neurons in the rat suprachiasmatic nucleus. *Eur. J. Neurosci.* 14, 666–674
16 Nakamura, W. *et al*. (2002) Clock mutation lengthens the circadian period without damping rhythms in individual SCN neurons. *Nat. Neurosci.* 5, 399–400
17 Yamaguchi, S. *et al*. (2003) Synchronization of cellular clocks in the suprachiasmatic nucleus. *Science* 302, 1408–1412
18 Okamura, H. (2004) Clock genes in cell clocks: roles, actions, and mysteries. *J. Biol. Rhythms* 19, 388–399
19 Quintero, J.E. *et al*. (2003) The biological clock nucleus: a multiphasic oscillator network regulated by light. *J. Neurosci.* 23, 8070–8076
20 Antle, M.C. *et al*. (2003) Gates and oscillators: a network model of the brain clock. *J. Biol. Rhythms* 18, 339–350
21 Moore, R.Y. (1996) Entrainment pathways and the functional organization of the circadian system. *Prog. Brain Res.* 111, 103–119
22 Mai, J.K. *et al*. (1991) Evidence for subdivisions in the human suprachiasmatic nucleus. *J. Comp. Neurol.* 305, 508–525
23 Card, J.P. and Moore, R.Y. (1984) The suprachiasmatic nucleus of the golden hamster: immunohistochemical analysis of cell and fiber distribution. *Neuroscience* 13, 415–431
24 Moore, R.Y. *et al*. (2002) Suprachiasmatic nucleus organization. *Cell Tissue Res.* 309, 89–98
25 Abrahamson, E.E. and Moore, R.Y. (2001) Suprachiasmatic nucleus in the mouse: retinal innervation, intrinsic organization and efferent projections. *Brain Res.* 916, 172–191
26 Silver, R. *et al*. (1996) Calbindin-D28K cells in the hamster SCN express light-induced Fos. *NeuroReport* 7, 1224–1228
27 Lee, H.S. *et al*. (2003) The eye is necessary for a circadian rhythm in the suprachiasmatic nucleus. *Nat. Neurosci.* 6, 111–112
28 Antle, M.C. *et al*., (in press) Signaling within the brain's master clock: localized activation of MAP kinase by gastrin-releasing peptide. *J. Neurosci.*
29 LeSauter, J. *et al*. (2002) Calbindin-D(28K) cells selectively contact intra-SCN neurons. *Neuroscience* 111, 575–585
30 Kawamoto, K. *et al*. (2003) Two types of VIP neuronal components in rat suprachiasmatic nucleus. *J. Neurosci. Res.* 74, 852–857
31 Dardente, H. *et al*. (2002) *Per* and neuropeptide expression in the rat suprachiasmatic nuclei: compartmentalization and differential cellular induction by light. *Brain Res.* 958, 261–271
32 Hamada, T. *et al*. (2001) Expression of *Period* genes: rhythmic and nonrhythmic compartments of the suprachiasmatic nucleus pacemaker. *J. Neurosci.* 21, 7742–7750
33 Yan, L. and Okamura, H. (2002) Gradients in the circadian expression of *Per1* and *Per2* genes in the rat suprachiasmatic nucleus. *Eur. J. Neurosci.* 15, 1153–1162
34 Hamada, T. *et al*. (2004) Temporal and spatial expression patterns of canonical clock genes and clock-controlled genes in the suprachiasmatic nucleus. *Eur. J. Neurosci.* 19, 1741–1748
35 Bryant, D.N. *et al*. (2000) Retinal innervation of calbindin-D28K cells in the hamster suprachiasmatic nucleus: ultrastructural characterization. *J. Biol. Rhythms* 15, 103–111
36 Tanaka, M. *et al*. (1997) Direct retinal projections to GRP neurons in the suprachiasmatic nucleus of the rat. *NeuroReport* 8, 2187–2191
37 Romijn, H.J. *et al*. (1996) Differences in colocalization between Fos and PHI, GRP, VIP and VP in neurons of the rat suprachiasmatic nucleus after a light stimulus during the phase delay versus the phase advance period of the night. *J. Comp. Neurol.* 372, 1–8
38 Yan, L. and Silver, R. (2002) Differential induction and localization of *mPer1* and *mPer2* during advancing and delaying phase shifts. *Eur. J. Neurosci.* 16, 1531–1540
39 Yan, L. *et al*. (1999) *Per1* and *Per2* gene expression in the rat suprachiasmatic nucleus: circadian profile and the compartment-specific response to light. *Neuroscience* 94, 141–150
40 Jobst, E.E. and Allen, C.N. (2002) Calbindin neurons in the hamster suprachiasmatic nucleus do not exhibit a circadian variation in spontaneous firing rate. *Eur. J. Neurosci.* 16, 2469–2474
41 Karatsoreos, I.N. *et al*. (2004) Phenotype matters: identification of light-responsive cells in the mouse suprachiasmatic nucleus. *J. Neurosci.* 24, 68–75
42 King, V.M. *et al*. (2003) A *hVIPR* transgene as a novel tool for the analysis of circadian function in the mouse suprachiasmatic nucleus. *Eur. J. Neurosci.* 17, 822–832
43 Meijer, J.H. *et al*. (1992) The relation between light-induced discharge in the suprachiasmatic nucleus and phase shifts of hamster circadian rhythms. *Brain Res.* 598, 257–263
44 Hamada, T. *et al*. (2003) Calbindin influences response to photic input in suprachiasmatic nucleus. *J. Neurosci.* 23, 8820–8826
45 LeSauter, J. and Silver, R. (1999) Localization of a suprachiasmatic nucleus subregion regulating locomotor rhythmicity. *J. Neurosci.* 19, 5574–5585
46 Kriegsfeld, L.J. *et al*. (2004) Targeted microlesions reveal novel organization of the hamster suprachiasmatic nucleus. *J. Neurosci.* 24, 2449–2457
47 Yan, L. and Silver, R. (2004) Resetting the brain clock: time course and localization of mPER1 and mPER2 protein expression in suprachiasmatic nuclei during phase shifts. *Eur. J. Neurosci.* 19, 1105–1109
48 Spoelstra, K. *et al*. (2004) Phase responses to light pulses in mice lacking functional *per* or *cry* genes. *J. Biol. Rhythms* 19, 518–529

49 Albrecht, U. *et al*. (2001) *mPer1* and *mPer2* are essential for normal resetting of the circadian clock. *J. Biol. Rhythms* 16, 100–104

50 Wakamatsu, H. *et al*. (2001) Additive effect of *mPer1* and *mPer2* antisense oligonucleotides on light-induced phase shift. *NeuroReport* 12, 127–131

51 Cermakian, N. *et al*. (2001) Altered behavioral rhythms and clock gene expression in mice with a targeted mutation in the *Period1* gene. *EMBO J.* 20, 3967–3974

52 Kim, D.Y. *et al*. (2001) Substance P plays a critical role in photic resetting of the circadian pacemaker in the rat hypothalamus. *J. Neurosci.* 21, 4026–4031

53 Abe, H. *et al*. (1996) Substance P receptor regulates the photic induction of Fos-like protein in the suprachiasmatic nucleus of Syrian hamsters. *Brain Res.* 708, 135–142

54 Piggins, H.D. and Rusak, B. (1997) Effects of microinjections of substance P into the suprachiasmatic nucleus region on hamster wheel-running rhythms. *Brain Res. Bull.* 42, 451–455

55 Albers, H.E. *et al*. (1991) Interaction of colocalized neuropeptides: functional significance in the circadian timing system. *J. Neurosci.* 11, 846–851

56 Piggins, H.D. *et al*. (1995) Neuropeptides phase shift the mammalian circadian pacemaker. *J. Neurosci.* 15, 5612–5622

57 McArthur, A.J. *et al*. (2000) Gastrin-releasing peptide phase-shifts suprachiasmatic nuclei neuronal rhythms *in vitro*. *J. Neurosci.* 20, 5496–5502

58 Aida, R. *et al*. (2002) Gastrin-releasing peptide mediates photic entrainable signals to dorsal subsets of suprachiasmatic nucleus via induction of *Period* gene in mice. *Mol. Pharmacol.* 61, 26–34

59 Dardente, H. *et al*. (2002) Phenotype of *Per1*- and *Per2*-expressing neurons in the suprachiasmatic nucleus of a diurnal rodent (*Arvicanthis ansorgei*): comparison with a nocturnal species, the rat. *Cell Tissue Res.* 310, 85–92

60 Meyer-Spasche, A. and Piggins, H.D. (2004) Vasoactive intestinal polypeptide phase-advances the rat suprachiasmatic nuclei circadian pacemaker *in vitro* via protein kinase A and mitogen-activated protein kinase. *Neurosci. Lett.* 358, 91–94

61 Nielsen, H.S. *et al*. (2002) Vasoactive intestinal polypeptide induces *per1* and *per2* gene expression in the rat suprachiasmatic nucleus late at night. *Eur. J. Neurosci.* 15, 570–574

62 Harmar, A.J. *et al*. (2002) The $VPAC_2$ receptor is essential for circadian function in the mouse suprachiasmatic nuclei. *Cell* 109, 497–508

63 Colwell, C.S. *et al*. (2003) Disrupted circadian rhythms in VIP- and PHI-deficient mice. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 285, R939–R949

64 Aton, S.J. *et al*. (2004) Circadian rhythmicity in a subset of SCN neurons and synchrony between SCN neurons depends on VIP/VPAC2 signaling. In *Society for Research on Biological Rhythms Abstracts* (Vol. 9), p. 169 (http://www.conferences.uiuc.edu/SRBR/SRBR_04_final1.pdf)

65 Piggins, H.D. *et al*. (2004) Roles of vasoactive intestinal polypeptide in circadian rhythm processes. In *Society for Research on Biological Rhythms Abstracts* (Vol. 9), p. 64 (http://www.conferences.uiuc.edu/SRBR/SRBR_04_final1.pdf)

66 Gupta, A. *et al*. (2000) Organizing principles for a diversity of GABAergic interneurons and synapses in the neocortex. *Science* 287, 273–278

67 Kriegsfeld, L.J. *et al*. (2004) Organization of suprachiasmatic nucleus projections in Syrian hamsters (*Mesocricetus auratus*): an anterograde and retrograde analysis. *J. Comp. Neurol.* 468, 361–379

68 Silver, R. *et al*. (1996) A diffusible coupling signal from the transplanted suprachiasmatic nucleus controlling circadian locomotor rhythms. *Nature* 382, 810–813

69 Okamura, H. (2004) Clock genes in cell clocks: roles, actions, and mysteries. *J. Biol. Rhythms* 19, 388–399

70 Sato, T.K. *et al*. (2004) A functional genomics strategy reveals Rora as a component of the mammalian circadian clock. *Neuron* 43, 527–537