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Basis of Robustness and Resilience in the Suprachiasmatic Nucleus: Individual Neurons Form Nodes in Circuits that Cycle Daily

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Abstract

How the cellular elements of the SCN are synchronized to each other is not well understood. We explore circadian oscillations manifest at the level of the cell, the tissue, and the whole animal to better understand intra-SCN synchrony and master clock function of the nucleus. At each level of analysis, responses to variations in operating environment (robustness), and following damage to components of the system (resilience), provide insight into the mechanisms whereby the SCN orchestrates circadian timing. Tissue level rhythmicity reveals circuits associated with an orderly spatiotemporal daily pattern of activity that is not predictable from their cellular elements. Specifically, in stable state, some SCN regions express low amplitude or undetectable rhythms in clock gene expression while others produce high amplitude oscillations. Within the SCN, clock gene expression follows a spatially ordered, repeated pattern of activation and inactivation. This pattern of activation is plastic and subserves responses to changes in external and internal conditions. Just as daily rhythms at the cellular level depend on sequential expression and interaction of clock genes, so too do rhythms at the SCN tissue level depend on sequential activation of local nodes. We hypothesize that individual neurons are organized into nodes that are themselves sequentially activated across the volume of the SCN in a cycle that repeats on a daily basis. We further propose that robustness is expressed in the ability of the SCN to sustain rhythmicity over a wide range of internal and external conditions, and that this reflects plasticity of the underlying nodes and circuits. Resilience is expressed in the ability of SCN cells to oscillate and to sustain activity-related rhythms at the behavioral level. Importantly, other aspects of pacemaker function remain to be examined.

Keywords

circadian; coupling; connectome; emergent properties; network plasticity; node

“We have discussed elsewhere the difficulties confronting current attempts to discover the concrete nature of the temperature-compensated, self-sustaining oscillation that underlies and controls the persistent daily rhythms universal in living cells. We have argued that it is therefore profitable to develop in as explicit and restrictive a form as possible the oscillator model for such rhythms. The

quantitative development of our formal model might provide a lead to the concrete nature of the oscillating system”

(Pittendrigh et al., 1958, p. 965).

In their landmark works primarily based on *Drosophila* eclosion, Pittendrigh and colleagues demonstrated that free-running and resetting of circadian rhythms obeyed properties of an oscillator (Pittendrigh et al., 1958; Pittendrigh, 1960). To guide the search for such a biological oscillator, Pittendrigh developed an input-output schematic consistent with behavior that restricted the degrees of freedom available to candidate mechanisms (Fig. 1; Pittendrigh and Bruce, 1957; opening quotation). In this era, the locus and cellular elements of the circadian clock were unknown. The challenge became to fit plausible mechanisms into the black box as phenomenological observations multiplied. The idea of a light-sensitive clock focused the search for a mammalian brain clock to areas that receive retinal input and led to the SCN of the hypothalamus. Many convergent lines of evidence, including SCN lesions, transplants, and demonstrations of rhythmicity in vivo and in vitro, have definitively established the existence of a master brain clock and its residence in the SCN. The history and sequence of these discoveries from initial identification of a retino-recipient region in the hypothalamus in 1972 is beautifully told in Weaver (1998). Our goal in this review is to explore the integration of the “one or more endogenous self-sustaining oscillators” conceptualized in Pittendrigh’s model (Fig. 1), and whose characterization at multiple levels of analysis continues today.

Top-down qualitative models keep us rooted in the phenomena to be explained and offer the advantage of limiting the degrees of freedom required. At the other extreme, bottom-up models offer the benefit of potential quantification by building upon known or predicted biophysical and dynamic mechanisms and may invoke many more degrees of freedom. The circadian system is ideally suited for each of these strategies, as rhythmicity can be assessed by several methods, at multiple levels of organization. By examining the meeting points of top-down and bottom-up strategies, we seek to avoid the problems of the 6 blind men, in the poem by John Godfrey Saxe,¹ who misidentify an elephant based on what body part they happen to touch. Although we, like they, might “dispute loud and long,” various experimental strategies provide insights in which each is partly in the right and all are partly in the wrong.

Risking the success and failure of being partly right and partly wrong, we put forth two suggestions. First, we hypothesize that in stable conditions, clusters of individual SCN oscillators form discrete and identifiable nodes² that are phase locked and linked into circuits that repeat a daily sequence of activity. This sequence of activated nodes functions as a circadian pacemaker that sets the phase of rhythms in the rest of the brain and body. The specific pattern of node activation within the SCN circuit is plastic, and is modulated by internal cues from the body and stimuli from the environment.

Our second proposal is that robustness and resilience are manifest at multiple levels of the circadian system, and that at each level it is important to discriminate between these two properties. Robustness is the ability of a system to sustain function in the face of variations or perturbations in the operating environment. Resilience is the ability to maintain an acceptable level of function in the face of various insults that challenge full, normal operation. Robustness is exemplified by temperature compensation of circadian period at

¹*The Blind Men and the Elephant*, by John Godfrey Saxe. Six blind men each touch an elephant and misidentify it based on what each felt: tusk as spear, tail as rope, etc. “And so these men from Indostan/Disputed loud and long, /.../ Though each was partly in the right,/And all were in the wrong!” (Saxe, 43–48).

²A node is a spatially discriminable cluster of synchronized cellular oscillators. This terminology is used to describe heart pacemaker tissues (Kamino, 1991).

both the organismal and cellular level (Izumo et al., 2003; Pittendrigh and Bruce, 1957). Robustness is also observed in entrainment to different photoperiods (Gorman et al., 1997). Resilience is revealed in rhythms of animals and cells bearing mutations or knockout of clock genes (summarized in Baggs et al., 2009; Liu et al., 2007). It is also revealed in sustained circadian rhythmicity in electrical oscillations or in wheel running after knife cuts that isolate the SCN from the brain (Hakim et al., 1991; Inouye and Kawamura, 1979), and following changes in global cellular processes such as transcription rate (Dibner et al., 2009).

The SCN is ideally suited to the analysis of robustness and resilience because of the availability of several indices of circadian function (electrical, genetic, metabolic, and behavioral) in cells, slices, and whole animals. This enables the experimental study of the features that emerge at each level of analysis of pacemaker function. In turn, understanding SCN robustness and resilience can reveal how circadian rhythms are generated and regulated, and can be addressed profitably using both bottom-up and top-down strategies in empirical and modeling studies.

Most studies of SCN circadian function have focused on oscillation as an end point. Behavioral studies indicate that very few SCN cells and efferents are necessary for the expression of circadian rhythmicity in wheel running (Harrington et al., 1993). In SCN-lesioned animals, whole tissue grafts restore rhythmicity, although the coherence and precision is less than that of the intact animal (Lehman et al., 1987). Grafts of dissociated cell suspensions are also sufficient to sustain rhythmicity, but the success rate of such grafts, and the period and precision of the rescued behavior is less than that achieved by whole tissue grafts (Silver et al., 1990). It is noteworthy that SCN grafts can restore locomotor rhythmicity with a very high success rate, and this has been done by many labs using tissues of various ages and in multiple species (reviewed in Ralph and Lehman, 1991). In contrast, neural tissue grafts are far less successful in other systems such as transplants of dopamine-secreting cells (Bjorklund et al., 2003) and of gonado-tropin-releasing hormone neurosecretory neurons (Rogers et al., 1998). Such findings speak to the resilience of the circadian pacemaker, and suggest the possibility that there may be a “low barrier” for achieving some aspects of rhythmicity.

The presence or absence of rhythmicity, however, is but one index of pacemaker activity. The SCN performs many functions in addition to oscillation; it entrains, phase shifts, displays aftereffects, and is sensitive to light intensity and duration among other internal and external stimuli (Pittendrigh, 1960). SCN output also coordinates the phase of oscillators throughout the body (reviewed in Kalsbeek et al., 2006b). As noted above, very little SCN organization is needed to sustain rhythmicity, perhaps not surprising given that self-sustained temperature compensated circadian oscillation can be reconstituted from three proteins and ATP (Nakajima et al., 2005). The consequences of altered SCN function on properties other than circadian oscillation of the tissue and of the animal have been relatively little studied, but are germane to understanding emergent properties of the networks supporting circadian timing systems (Vansteensel et al., 2008).

SCN INPUTS, OUTPUTS, AND HETEROGENEITY

Top-down and bottom-up analyses both require understanding of components of the circadian system. In all mammalian species examined, the SCN is characterized by two regions that differ in cell size, ultrastructural features, and projections (Card and Moore, 1991; Cassone et al., 1988; Morin and Allen, 2006; van den Pol, 1991). There has been substantial difficulty in developing a consensus nomenclature to characterize subregions of the SCN (pointed out by Morin and Allen, 2006). Here, we have adopted the core-shell

terminology first proposed by a team of circadian researchers including Miller, Morin, Schwartz, and Moore (Miller et al., 1996). Ideally, the terms are best used, in empirical studies, accompanied by the criteria used to delineate these regions. While core-shell terminology is an oversimplification, we suggest that these terms are conceptually useful and convenient for discourse, even as we acknowledge that they do not describe boundaries as clearly defined as the walls of Jerusalem.

Inputs

At a gross level, the core and shell are distinguished by their inputs, outputs, and peptidergic content. Major inputs to the SCN—projections from the eye, the intergeniculate leaflet (IGL) of the thalamus, and the midbrain raphe nucleus—converge primarily although not exclusively on the core (Morin and Allen, 2006). The circadian system is also modulated by endocrine cues, acting on discrete SCN regions. For example, in mice, androgen receptors are highly expressed in the SCN core, and gonadectomy markedly reduces circadian precision and alters period (Karatsoreos et al., 2007). Estrogens also affect circadian rhythms but unlike androgen receptors, estrogen receptors are sparsely expressed through the shell (Vida et al., 2008).

Outputs

Tracing outputs of the SCN further underscores the heterogeneous organization of the nucleus. The SCN can communicate time to the body through both neural and diffusible modes. Evidence for diffusible signals comes from the fact that SCN grafts encapsulated in semipermeable membranes are sufficient to reestablish rhythms of activity-related responses in SCN-lesioned host hamsters. Such grafts (encapsulated or not) provide evidence of the limits of SCN resilience as they do not reestablish endocrine rhythms of any kind (Lehman et al., 1987; Meyer-Bernstein et al., 1999; Silver et al., 1996). Nor do grafts reestablish responses to entraining photic signals, even though there is electron microscopic evidence that retinohypothalamic tract fibers make synapses within the graft (Lehman et al., 1987). Noteworthy is the fact that there are both neural and diffusible outputs and that their distinct functions in the intact animal are not well understood. The nature of the diffusible signals has been pursued with a variety of methods, and thus far, transforming growth factor- α and prokineticin-2 have been identified as candidates (Cheng et al., 2002; Kramer et al., 2001). Whether these signals support endocrine rhythms as well as activity has not been tested.

In contrast to diffusible signals, neural efferents of the SCN have been extensively mapped in rat, hamster, and mouse (Abrahamson and Moore, 2001; Kriegsfeld et al., 2004a; Leak et al., 1999). These projections are topographically organized, as revealed by retrograde and anterograde tracing studies. The specific output mechanisms, transmitter identity, timing of transmitter or neuromodulator release, and the response of target tissues to these signals are being actively explored (e.g., Kalsbeek et al., 2006a).

Peptides

Within core and shell regions, another well-established feature of the SCN is its peptidergic heterogeneity. Detailed descriptions of regional organization of SCN peptidergic phenotypes are available for many species (Moore and Silver, 1998; Morin and Allen, 2006). Generally, arginine vasopressin (AVP) cells lie in a shell region, while vasoactive intestinal polypeptide (VIP) and gastrin-releasing peptide (GRP) cells are restricted to the core. Given the current interest in mouse, we note that Abramson and Moore (2001) provide a detailed analysis the SCN. They describe the shell as neurons synthesizing GABA, calbindin (CalB), AVP, angiotensin II (AII), and met-enkephalin (mENK), and the core as neurons synthesizing GABA, CalB, VIP, calretinin (CALR), GRP, and neurotensin (NT). While there is debate as to the precise localization of cells bearing specific peptidergic phenotypes to core versus

shell locations, there is consensus that the SCN contains a diversity of peptidergic cell types whose function are poorly understood.

Neurons expressing VIP and GRP receive afferent inputs and are important in coordinating rhythms of SCN oscillators in the shell. GRP mediates the effects of the light on the SCN, and in propagating these signals to the shell (Antle et al., 2005; Gamble et al., 2007). VIP cells are similarly retinorecipient, and VIP treatment resets circadian phase (Piggins and Cutler, 2003). Eliminating VIP signaling, by knocking out the peptide or the VPAC₂ receptor, attenuates behavioral rhythms by disrupting phase synchrony of individual oscillators (Aton et al., 2005; Brown et al., 2005).

GABA is also implicated in coordination of SCN rhythmicity. In a rat slice preparation where the SCN has been cut in half, GABA inhibits electrical activity in the ventral SCN but excites neurons in the dorsal SCN during the late day and early night (Albus et al., 2005). Regional differences in the sign of the GABA effect may reflect differences in the Cl⁻ equilibrium. Notably, a K⁺-Cl⁻ cotransporter, KCC2, is abundantly expressed in VIP and GRP cells but absent from AVP cells, consistent with greater GABAergic excitation in AVP cells (Belenky et al., 2008). In support of the importance of Cl⁻ extrusion, blocking the Na⁺-K⁺-2Cl⁻ cotransporter NKCC1 abolishes GABAergic excitation (Choi et al., 2008).

SCN Connectome—The differences among SCN regions in peptidergic phenotypes awaken interest in intra-SCN circuitry because this enables understanding of properties that emerge from the interaction among SCN cells under various conditions. Surprisingly little is known of the SCN connectome, with some evidence from tracing of individual cells, immunoelectron microscopy, and immunofluorescent confocal microscopy.

Core-shell interconnections

With timed tract tracing using the pseudorabies virus injected into two distinct SCN target regions, Leak et al. (1999) report that the core and shell each project to homologous contralateral areas, and that the core projects densely to the shell with little evidence of reciprocal innervation. Core to shell projections arise from several phenotypic classes. In rat, immunoelectron microscopy shows that VIP-labeled axons synapse on AVP neurons (Daikoku et al., 1992). Somatostatin neurons lie along the boundary between AVP- and VIP-delineated regions, make synapses with both, and have been suggested to act as relays (Daikoku et al., 1992). In hamster, the dendrites of CalB cells course dorsally within the SCN, and are positioned to integrate signals from the shell (Jobst et al., 2004). In mouse, the same pattern of dorsally extending dendrites is observed in biocytin-filled GRP cells of the core (Drouyer et al., in press).

Neurons of the shell, both AVP and non-AVP, have varicose dendrites that extend into the core and may release transmitters or peptides (rat, Pennartz et al., 1998). Double-label immunochemistry for various SCN peptides has been done in studies of rat and hamster, although with this method, synapses cannot be confirmed, and dendritic and axonal projections cannot be readily distinguished (hamster, LeSauter et al., 2002; rat, Romijn et al., 1997; van den Pol and Gorcs, 1986).

Within core and shell

Connections within the core are characterized by reciprocal contacts among neuron types. In hamster, CalB cells contact both GRP and VIP cells of the core (LeSauter et al., 2002). In rats, there are many appositions between fibers and cell bodies immunoreactive for VIP and GRP. VIP and somatostatin cells exhibit aut synaptic contacts (Daikoku et al., 1992; Romijn et al., 1997). In mouse, many GRP dendrites are confined to the core (Drouyer et al.,

in press). Neurons of core are also dye coupled, suggesting gap junctions (Jobst et al., 2004; Drouyer et al., in press). Less is known about intrashell wiring. AVP varicose dendrites extend through the shell, and AVP-AVP autosynaptic contacts are observed (Daikoku et al., 1992; Pennartz et al., 1998). In this region, too, gap junctions are revealed by electron microscopy and electrical recordings (Long et al., 2005; Rash et al., 2007).

Given the anatomical and functional heterogeneity of the SCN, and the evidence for emergent tissue-level properties, mapping connectivity within the SCN tissue and among peptidergically and spatially identified elements is necessary for understanding the SCN connectome. The increasing availability of transgenic mice with cell-specific fluorescent or bioluminescent reporters will enable delineation of these circuits.

INTERCELLULAR COUPLING AMONG AUTONOMOUS OSCILLATORS

At the cellular level, rhythms depend on sequential activation and expression of clock genes (Schibler, 2007). Two transcriptional activators, CLOCK and BMAL1, lie at the core of the cellular oscillator. These heterodimerize and bind to E-box elements in the promoters of target genes, including two families of transcriptional repressors, the PERIOD (PER) and CRYPTOCHROME (CRY) proteins, among others. When PER and CRY accumulate in the cytoplasm, they enter the nucleus and inhibit their own expression by repressing CLOCK/BMAL1-mediated transcription. Many other proteins regulate the transcription, binding, and nuclear entry of these proteins (Baggs et al., 2009).

For experimentalists, a question of substantial interest is how signals from autonomous oscillators are integrated to produce a coherent output that sets the phase of behavioral and physiological rhythms (Aton and Herzog, 2005). Individual, dissociated SCN neurons show great variability in period length (Herzog et al., 2004; Honma et al., 2004; Honma et al., 1998; Liu et al., 1997). Rhythmicity, measured by period variance and onset precision, is most tightly controlled in the whole animal, least tightly controlled in single cells, and intermediate in tissue slices. Intercellular weak coupling can synchronize oscillators that vary in period, phase, and amplitude, and is thus an attractive solution to explain SCN coherence (Fig. 2A, Liu et al., 1997). Candidate coupling mechanisms include synaptic communication via GABA, neuropeptide signaling via VIP and GRP, and by gap junctions (reviewed in Aton and Herzog, 2005).

For modelers, synchronization of individual oscillators has long held fascination and many approaches to the SCN are afforded (Indic et al., 2007). A basic model of weak coupling³ of limit-cycle oscillators is computationally convenient, for amplitude variation can be discounted, and phase variation alone considered (Winfree, 1967). Such models are inspired by the ubiquitous synchronization observed in biology, and are successfully applied to the oscillators of the SCN (Liu et al., 1997; Winfree, 1967). More recent models have incorporated information about clock biochemistry (Gonze et al., 2005), heterogeneity in SCN function (Antle et al., 2007), and heterogeneity in coupling strength within the network (Vasalou et al., 2009).

Coupling can account for not only synchronization of neurons to the same phase, but also for stably distributed phase locking of neurons (Fig. 2B). In slice culture, Yamaguchi et al. (2003) show that the phase relationships among individual SCN oscillators are ordered and predictable. The original phase relationship of individual cells is reestablished over a few cycles after rhythms are transiently stopped by cycloheximide. Thus, intercellular coupling

³Winfree (1967) defines coupling as weak when the maximum change in phase of an oscillator due to its interactions with other oscillators is less than 10% of its period.

can explain phase locking of oscillators with distributed phases through the circadian cycle, or synchronization of oscillators cycling in phase (compare Fig. 2A and B).

FUNCTIONAL HETEROGENEITY AND THE SCN CONNECTOME

We have described the anatomical bases of SCN heterogeneity and now turn to spatial-temporal organization aspects of its specialized subregions, as revealed by mapping gene expression patterns over time and in response to light. In rats, hamsters, and mice, the core and shell differ substantially in their expression of endogenous rhythms and response to light. Retinal projections primarily innervate the core with sparser innervation dorsally, although the clarity of demarcation depends on species (Hattar et al., 2006; Johnson et al., 1988; Muscat et al., 2003). Following a light pulse, molecular signatures of photic input are observed initially in regions with the greatest density of retinal terminals. Immediate early genes are expressed in the core but not shell SCN after a subjective night light pulse in rats; in contrast, endogenous FOS expression rhythms may be restricted to the SCN shell (Schwartz et al., 2000; Sumova et al., 1998). Similarly, *Per1* and *Per2* show high amplitude endogenous rhythms in the shell SCN and light inducibility in the core SCN (Yan et al., 1999). In hamsters, *Per1*, *Per2*, and *Per3* cycle with high amplitude in the AVP (shell) region. None of these are detectably rhythmic in the CalB-delineated region of the core, with *Per1* and *Per2* expressed at low levels and *Per3* at high. In response to light pulses, however, *Per1* and *Per2*, but not *Per3*, are induced in the core CalB region during the night (Fig. 3A; Hamada et al., 2001). In addition to lacking detectable clock gene rhythms, hamster CalB cells lack daily rhythms of electrical activity (Jobst and Allen, 2002), although rhythmic changes in cellular localization of CalB protein and a temporally gated response to light pulses are observed (Hamada et al., 2001; LeSauter et al., in press). In mice, the region delineated by GRP also shows a gated photic response and undetectable or low amplitude endogenous rhythmicity (Karatsoreos et al., 2004); GRP and CalB are coexpressed in mouse SCN in the post-natal period (Drouyer et al., in press). Thus, a phenomenology of low amplitude or absent endogenous rhythms in the core with gated photic response is observed in three species.

The interplay of retinorecipient core cells and shell oscillators parallels phase-shifting behavior. A detailed time course analysis of *Per* message expression after light pulses reveals a marked core to shell pattern of activation. *Per* is always expressed in the core at night after a light pulse, but behavioral phase shifts only occur at times when *Per* expression spreads from core to shell (Fig. 3A; Yan and Silver, 2002). The results indicate a correlation between *Per* expression in the shell and behavioral phase shifts, and suggest that photic cues can be gated at the level of core to shell signaling. Furthermore, lesions that include the core subregion abolish circadian rhythmicity at the organism level (Kriegsfeld et al., 2004b; LeSauter and Silver, 1999). A “gates and oscillators” model conceptualizes the interactions of nonrhythmic light-sensitive gate cells and endogenously rhythmic cells, representing the core and shell, respectively (Antle et al., 2003, 2007). The key idea in this model is that two functionally different populations interact to produce the network output. A strength of the model is that the same mechanisms are invoked to account for free-running, entrainment to light pulses, and defined limits of entrainment.

By several measures there is a core region in the SCN that is not detectably rhythmic. By other measures, however, the core is rhythmic (Albus et al., 2005; Davidson et al., 2009). There are likely several reasons for discrepancies. Fewer cells exhibit electrical rhythms in the core compared with the shell SCN (Nakamura et al., 2001). Tissue sections used in acute and organotypic preparations may be 300 to 500 μm , and tissue labeled as ventral may or may not include both core and shell regions; depending on species, anteroposterior location of a section, and flattening of tissue over time, the location of the core is inconsistent among

preparations. These are each common problems in visualizing a three-dimensional volume in a two-dimensional snapshot.

CIRCUITS OF THE SCN

Connecting the Cell into Nodes

As noted in the opening quotation by Pittendrigh, it is “profitable to develop in as explicit and restrictive a form as possible the oscillator model for such rhythms.” On the basis of the experimental evidence discussed below, we introduce a working model in which SCN pacemaker function derives from a sequence of nodes that are connected into circuits, and are activated across space and in time in the nucleus. This sequence of activation of nodes normally takes ~24 h to complete. Restated, just as rhythms at the cellular level depend on sequential expression and interaction of clock genes, rhythms at the tissue level depend on sequential activation of local oscillating nodes. Though the sequence of activation is altered, interfering with one node does not eliminate rhythmicity in the remaining nodes. This is consistent with evidence that in many cases, partial SCN lesions do not abolish locomotor rhythms (Harrington et al., 1993). The ordered spatiotemporal activation of oscillators in the brain’s master clock is the basis of its function as a continuously consulted clock (Cain et al., 2004), enables it to serve as a daily clock and as a seasonal calendar (Hazlerigg and Wagner, 2006), and supports entrainment, phase shifting, and sensitivity to light intensity and duration among other circadian properties.

Sequences of Activity in the SCN

Circadian rhythms of gene expression are characterized by an ordered progression of activation across the tissue (Fig. 3B), although many different descriptions of spatiotemporal changes in phase and amplitude have been reported. These range from lateral to medial, dorsal to ventral, and ventral to dorsal progressions (Hamada et al., 2004; Hughes et al., 2008; Naito et al., 2008; Quintero et al., 2003; Yamaguchi et al., 2003; Yan et al., 2007; Yan and Okamura, 2002). Electrical recordings also indicate multiply phased clusters of rhythmic cells (Albus et al., 2005; Brown and Piggins, 2009; Schaap et al., 2003). The extent to which phase differences at the molecular level correspond to phase differences in electrical activity remains unanswered (Schaap et al., 2001). While the patterns described differ among studies, there is agreement that changes in time and space occur within the nucleus. Discrepancies in the specific spatiotemporal accounts may arise from differences in resolution of measures used, criteria used to define subregions, and tissue preparations. The development of methods for visualizing three-dimensional spaces, such as are available for imaging of human brains, will help to resolve these issues.

Weak coupling is not a sufficient explanation for the slow and orderly sequence of changes seen in the volume of the SCN. We estimate the spread of activation through the SCN at ~30 $\mu\text{m}/\text{h}$ given a 12- to 15-h spread of gene expression (Yamaguchi et al., 2003) over a dorsoventral distance of 350 μm (Abrahamson and Moore, 2001) in a two-dimensional brain slice. The problem then is to explain how the daily cycle of activation-inactivation of nodes takes 24 h. One possibility is that there are regional differences in the duration of clock gene expression that may or may not be based on differences within individual cells. Within the SCN, in addition to waves of activity across the tissue, tidal patterns of activity have been observed. Waves and tides differ significantly from each other, and reflect qualitatively different underlying mechanisms. In a wave, a first on–first off (FOFO) pattern is seen with activity initiated and terminated in one area of the SCN followed by activation in an adjacent area (e.g., Yamaguchi et al., 2003; Yan and Okamura, 2002). In a tide, gene expression spreads out and then recedes back to a point of origin and the area activated first is last to turn off (first on–last off, FOLO; e.g., Hamada et al., 2004; Yan et al., 2007). The FOFO

pattern implies equal duration of gene expression in SCN regions, while the FOLO pattern implies prolonged clock gene expression in the initiation regions, and an orderly decrease in activity duration with distance from the initiation point.

Plastic Reordering of SCN Circuits

Intra-SCN phase relationships are plastic and responsive to environmental cues (Fig. 3C). The effects of photoperiod on the SCN provide a case in point. The amplitude and duration of SCN electrical activity and gene expression both depend on photoperiod (Lincoln et al., 2003). In hamsters and mice in long days, the phase of clock gene expression is reordered along the rostrocaudal axis, with the caudal SCN phase leading the rostral SCN by up to 12 h, while in short days, all regions peak together (Hazlerigg et al., 2005; Inagaki et al., 2007; Naito et al., 2008; Yan and Silver, 2008). It is unclear whether or not photoperiodic changes in the SCN are also accompanied by alterations in waveform of individual oscillators. Photoperiod encoding may be strictly a consequence of the structural heterogeneity of the nucleus (comprehensively reviewed by VanderLeest et al., 2007). On the other hand, long days may lengthen the duration of single unit activity in the dorsal SCN (Brown and Piggins, 2009). This latter work again emphasizes the importance of resolution; when the dorsal and ventral data were pooled, the photoperiodic effects on single unit activity were masked. The phase dispersion may determine melatonin secretion duration and possibly allows for multiple discrete outputs that are flexibly entrained (Fig. 3C). Other evidence of plasticity abounds, and includes novel patterns of SCN organization in response to jet-lag-like conditions and forced desynchrony (Albus et al., 2005; Davidson et al., 2009; de la Iglesia et al., 2004; Nagano et al., 2003).

Opportunities and Challenges in Exploring Neural Circuits

While it was initially thought that the SCN produces a single coherent daily oscillation, it is obvious from the numerous lines of evidence that there is a distinct spatiotemporal pattern of clock gene and clock-controlled gene and protein expression within the nucleus. As an example, in real-time analyses of bioluminescence in SCN slices, gene expression follows a stable sequence. Regionally discrete nodes are activated in a stable order, and the duration of gene expression varies regionally. Subregions of the SCN also differ in amplitude of gene expression, and in the dynamics of on and off phases (Yan et al., 2007). We hypothesize that this daily sequence is the basis of a robust and resilient pacemaker that is capable of oscillation and, more importantly, is able to produce an information rich circadian output.

In summary, 4 elements of the circuit level organization of the SCN await mechanistic exploration, including 1) characterization of oscillator cells within nodes, 2) connections among nodes that support the spatiotemporal changes, 3) regional or cellular differences in clock gene expression, and finally, 4) the basis of a daily sequence of signals within the SCN tissue. We briefly consider the challenges and opportunities in each. 1) In understanding the organization of circuits within SCN nodes, it will be important to identify the phenotype of cells as well as their interconnections. Of particular interest is whether spatiotemporal differences in gene expression among nodes is a property of the individual cells or an emergent property of their place in the circuit. The availability of transgenic mice with reporters for specific cell types allows precise targeting of cells of interest for electrical, molecular, and anatomical studies, and the deployment of membrane-bound reporters can reveal projection patterns of identified neurons. 2) Understanding the coupling mechanisms between nodes should shed light on how steady-state phase relationships are established and maintained and how they are altered by environment. Identifying the internode connections may also reveal whether nodes are the unit of plasticity in response to environmental changes. 3) Rhythms in different SCN subregions may differ in phase, duration of activity, and amplitude, but whether these are due to tissue level or cellular properties, or both, is

unclear, and the extent to which the phenotype of the individual cell versus its connections within the network determines functional differences remains unexplored. 4) Slow spreading of activation within the SCN is not likely mediated by electrochemical signaling. The basis of the daily time course of propagation of signals within the SCN remains open to investigation. Several mechanisms and models have been explored to understand signals in development, and these may apply in the adult nervous system as well. Possibilities include concentration gradients in development, for example, the segmentation clock (Pourquie, 2003), Turing reaction-diffusion models that can produce spatially periodic phenomena (Nakamasu et al., 2009), or cAMP coupling of cellular differentiation that lead to waves within cellular slime mold aggregations (Gerisch, 1987). At this point, there is increasing evidence of sequential, structurally stable, phase-locked circuit activation within the SCN that calls for further experiments, new heuristics, and a renewed focus on systems level analysis.

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Figure 1. Pittendrigh and Bruce's clock-oscillator model depends on one or more endogenous self-sustaining oscillators (ESSO) that either entrain to the environment (A) or free-run in the absence of 24-h cues (B). *Note:* Light and temperature may affect phase of the overt free-running rhythm. This model explicitly frames a research program based on the idea of rhythms derived from an oscillator. Modified from Figure 1 Pittendrigh and Bruce (1957).

Figure 2.

(A) Electrical recordings of SCN neurons in dispersed and organotypic slice culture reveal cell autonomous rhythmicity. Single unit activity of two cells in anti-phase are shown (black and gray traces; reprinted from *Neuron*, v. 14, Welsh et al., Individual neurons dissociated from rat suprachiasmatic nucleus express independently phased circadian firing rhythms, p. 702, ©1995, with permission from Elsevier). A model of intercellular coupling that synchronizes the heterogeneous population of oscillators is shown on the right. When oscillators interact, moving from left to right, coupling synchronizes phase (arrow direction) and period (not shown; adapted from *Cell*, v. 91, Liu et al., Cellular construction of a circadian clock: period determination in the suprachiasmatic nuclei, p. 859, ©1997, with permission from Elsevier). (B) Yamaguchi et al. tracked three individual neurons (circle, triangle, and square on left half of SCN) and showed that after cycloheximide treatment, their original phase relationships were restored (Yamaguchi et al., 2003. Synchronization of cellular clocks in the suprachiasmatic nucleus. *Science* 302:1408–1412. Reprinted with permission from the American Association for the Advancement of Science). On the right, the intercellular coupling model is modified to indicate stable phase locking of oscillators.

Figure 3.

Building functional circuits in the SCN. (A) The functional differentiation of core and shell underlies a gate-oscillator model of circadian function (Antle et al., 2003, 2007). In constant darkness, both *Per1* and *Per2* are rhythmic in the shell (line) but not in the core (black bars). Nevertheless, the core exhibits a gated response to a light pulse (white bars; from Hamada et al., 2001, with permission). Behavioral phase shifts to light are associated with the spread of gene expression from core to shell. *Per1* is induced in the core at both CT19 (no phase shift) and CT22 (advance), but only observed in the shell during the advance (Yan and Silver, 2002). In the model, cells in the core gate photic input and organize oscillators in the shell via a daily resetting pulse. Oscillator output reaches the rest of the brain and feeds back to the core gate cells. (B) Activation of cells proceeds in a slow ordered progression across the SCN (AVP mRNA from Hamada et al., 2004, Temporal and spatial expression patterns of canonical clock genes and clock-controlled genes in the suprachiasmatic nucleus. *Eur J Neurosci*, ©Wiley-Blackwell; PER2:LUC bioluminescence from Yan et al., 2007, Cold Spring Harbor Symposia on Quantitative Biology, v. 72, p. 536, ©Cold Spring Harbor Laboratory Press). These data imply a sequence of locally organized nodes that produce a phase-ordered pattern of activity. (C) The spatiotemporal patterns of rhythmicity are plastic and can be reorganized by photoperiod. Acrophases are synchronous in short days but differ by as much as 12 h in long days along the rostrocaudal axis (from Yan and Silver, 2008, Day-length encoding through tonic photic effects in the retinorecipient SCN region. *Eur J Neurosci*, ©Wiley-Blackwell). Circuit level properties of the SCN are a product of the interaction of tissue and its environmental inputs.