4-21-2016

In Synch but Not in Step: Circadian Clock Circuits Regulating Plasticity in Daily Rhythms

Jennifer A. Evans
Marquette University, jennifer.evans@marquette.edu

M. R. Gorman
University of San Diego

Accepted version. *Neuroscience*, Vol. 320 (April 2016): 259-280. DOI.

In Synch but Not in Step: Circadian Clock Circuits Regulating Plasticity in Daily Rhythms

J.A. Evans
Department of Biomedical Sciences, Marquette University
Milwaukee, WI
M.R. Gorman
Department of Psychology, University of San Diego
La Jolla, CA

Abstract: The suprachiasmatic nucleus (SCN) is a network of neural oscillators that program daily rhythms in mammalian behavior and physiology. Over the last decade much has been learned about how SCN clock neurons coordinate together in time and space to form a cohesive population. Despite this insight, much remains unknown about how SCN neurons communicate with one another to produce emergent properties of the network. Here we review the current understanding of communication among SCN clock cells and highlight a collection of formal assays where changes in SCN interactions provide for plasticity in the waveform of circadian rhythms in behavior. Future studies that pair analytical behavioral assays with modern

Neuroscience, Vol 320 (April 21, 2016): pg. 259-280. DOI.

neuroscience techniques have the potential to provide deeper insight into SCN circuit mechanisms.

Abbreviations

- GABA, γ-aminobutyric acid;
- AVP, arginine vasopressin;
- DD, constant darkness;
- LD, light:dark;
- LDLD, light:dark:light:dark;
- LL, constant light;
- NRs, nonresponders;
- NWR, novel wheel running;
- PRC, phase response curve;
- SCN, suprachiasmatic nucleus;
- TTX, tetrodotoxin;
- VIP, vasoactive intestinal polypeptide

Key words: circadian; suprachiasmatic nucleus; clock network; coupling; behavior; plasticity

Introduction

Daily rhythms are an important and pervasive feature of life on this planet. In mammals, a wide variety of behavioral and physiological processes fluctuate regularly each day (e.g., locomotor activity, sleep, cognitive performance, hormone secretion, protein synthesis, cell division). These and other essential processes are programed by an endogenous timekeeping system that has evolved to promote survival in a rhythmic environment. The primary evidence for the endogenous basis of daily rhythms is that they persist under constant conditions devoid of environmental time cues. Because the period length adopted under constant conditions is not exactly 24 h, these internally programed rhythms are referred to as circadian (circa dies, about a day). Experimental approaches that track daily changes in behavior and physiology have proven vital for defining formal mechanisms of circadian timekeeping and the process of entrainment to the 24-h environment. Following on this work, the biological generator of daily rhythms was localized to a specific site in the anterior hypothalamus, the suprachiasmatic nucleus (SCN). The discovery that this small region of the brain controls the temporal patterning of behavior and physiology is one of the most striking examples of localized function in the field of neuroscience. Recent

advances have built upon this pioneering work to define the cellular mechanisms of circadian timekeeping, revealing the existence of a molecular oscillator built of "clock genes". We now realize that nearly every cell of the body is a daily clock, and the next major challenge in the field is to define the circuits and signals through which these clocks interact with one another.

In this review, we highlight the evidence that the master circadian clock in mammals contains multiple interacting clock cells organized into a network, as first articulated in 1960 (Pittendrigh, 1960). Importantly, this classic model posits that the master circadian clock contains functionally distinct clocks that coordinate with one another to form a pacemaker. A large body of research supports the fundamental premise of this model, which is striking given that it was first formulated based purely on behavioral data obtained before the discovery of the SCN's role as a pacemaker. Although there is compelling evidence that the SCN does indeed contain multiple cellular clocks, little is known about the circuits, signals, and mechanisms by which SCN cells coordinate with one another. Deeper insight into SCN circuitry is imperative for understanding the intercellular interactions that guide rhythmic neural behavior, which is a common theme emerging throughout neuroscience. Here we will provide a brief summary of clock circuits and then review formal assays where plasticity in circadian behavior is thought to reflect interactions among master clock cells. We hope that re-examining these formal analytical tools through a modern lens will highlight ways that these assays can be used to further define SCN circuits. As with past advances in our field, the continued synthesis of formal mechanistic insight with cutting-edge technical advances is expected to deepen our understanding of principles and mechanisms underlying circadian timekeeping.

Circadian circuits

The circadian timekeeping system: clocks at multiple levels

Multi-clock models can be categorized as hierarchical or complex (Moore-Ede et al., 1976). Hierarchical circadian models postulate that

the body contains multiple circadian clock tissues, with "peripheral" clock tissues regulated by a central pacemaker that maintains internal synchrony within the system (Rosenwasser and Adler, 1986). In contrast, complex circadian models stipulate the existence of multiple, interacting clock cells within the central pacemaker itself (Pittendrigh, 1974 and Pittendrigh and Daan, 1976b). There is a great deal of experimental support for each model, reviewed briefly below, and in both models a critical question is how multiple clock tissues or cells are able to communicate with one another. It is important to note that communication is not required in a multi-clock system. For example, a group of cellular clocks with identical period lengths would remain synchronized without intercellular signaling. Although it is theoretically possible that cellular clocks could be identical clones running at the same speed, this is biologically implausible. Indeed, a large number of studies indicate that the intrinsic period of different tissue and cellular clocks are not identical (Abe et al., 2002, Balsalobre, 2002 and Granados-Fuentes et al., 2004). Even in the face of heterogeneous period lengths, intercellular communication may not be essential given that external input (i.e., zeitgebers, e.g., light) might effectively synchronize a population of non-interacting clock cells. However, under constant conditions devoid of time cues, non-interacting clock cells would be unable to maintain synchrony. Under these circumstances, temporal desynchrony would lead to arrhythmia at the level of the population and multiple, independent periodicities would manifest in the overt rhythms controlled by these clock cells. Since the vast majority of mammalian species do not display multiple periodicities under constant darkness (DD), this implies that the underlying tissue and cellular clocks interact through coupling mechanisms. In the simplest sense, coupling is the ability of one cellular (or tissue) clock to influence the rhythm of another clock through interactions that may be reciprocal or one-directional. In this regard, coupling may influence any rhythmic property (i.e., period, phase, amplitude, precision), although an emphasis is often placed on coupling mechanisms that maintain period synchrony. In addition, intercellular interactions regulate phase synchrony among different clocks. In some cases, coupling may inhibit phase synchrony to prevent the simultaneous expression of opposing biological processes. In this manner, coupling would optimize performance of the system as a whole by preventing "phase locking" (i.e., absolute phase

synchrony). Thus, coupling can have multiple consequences for the expression of rhythmic parameters, which could be mediated by distinct types of signaling mechanisms.

Findings supportive of the hierarchical clock model derive from studies of human and non-human primates, where independent rhythms of behavioral activity and body temperature emerged under constant conditions or after large shifts in the entraining light:dark (LD) cycle (Aschoff, 1965, Moore-Ede et al., 1977, Sulzman et al., 1977, Tapp and Natelson, 1989 and Weibel et al., 1997). Based on the concept that a single oscillator cannot simultaneously express multiple, independent periods, these results were interpreted as reflecting the actions of at least two distinct oscillators disconnected from central control. The existence of multiple clock tissues was also indicated by work demonstrating that food and psychoactive stimulants could restore the expression of daily rhythms in SCN-lesioned animals (Honma et al., 1989, Stephan, 1989 and Tataroglu et al., 2006). Although, it has been very difficult to localize non-SCN food- and drug-sensitive tissue clocks (Davidson, 2006), recent work highlights an important role for dopamine signaling in the dorsal striatum (Gallardo et al., 2014). To date, the most compelling evidence in support of the hierarchical clock model is the discovery that numerous glands and tissues are able to generate endogenous rhythms *in vitro* (Abe et al., 2002, Balsalobre, 2002 and Granados-Fuentes et al., 2004). Endogenous rhythms are also displayed by single somatic cells, which indicates that the basic mechanism for circadian timekeeping operates at the molecular level.

At the cellular level, circadian rhythms are programed by a molecular oscillator consisting of a family of clock genes that regulate their own expression (Mackey, 2007, Zhang and Kay, 2010 and Buhr and Takahashi, 2013). At its core, circadian rhythms are sustained by a ~24-h transcriptional-translational negative feedback loop (Fig. 1A) with positive elements that serve as activators and negative elements that serve as repressors. The positive elements are bHLH-PAS transcription factors CLOCK and BMAL, which form a dimer that initiates transcription of *Period* (*Per1*, *Per2*, *Per3*) and *Cryptochrome* (*Cry1*, *Cry2*) genes (Fig. 1A-1). The corresponding protein products (e.g. PER1-3, CRY1-2) are negative elements that form protein dimers, which feedback to inhibit their own expression by antagonizing the

transcriptional activity of CLOCK-BMAL (Fig. 1A-2). The negative elements are degraded over time, thus relieving repression and allowing transcription to recommence the following day. In addition to this primary loop, there are a number of interconnected accessory loops that act to stabilize and amplify circadian oscillations at the cellular level (Fig. 1, Fig. 2 and Fig. 3). This molecular oscillator functions in nearly every cell of the body to regulate biological processes in a tissue-specific manner (Panda et al., 2002, Storch et al., 2002 and Zhang et al., 2014).

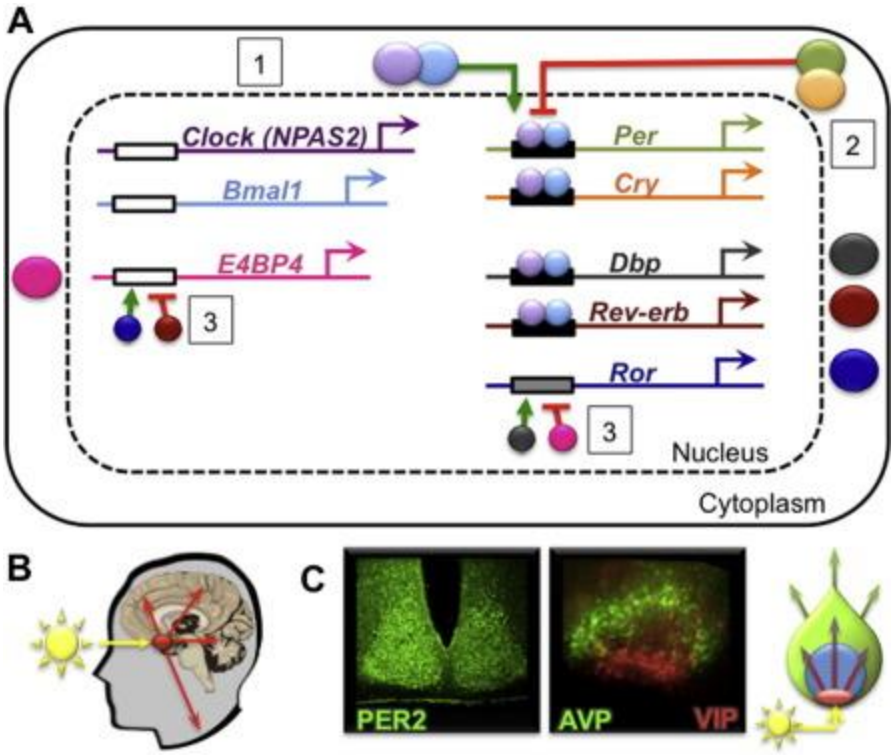

Fig. 1. Circadian clocks at multiple levels. (A) Simplified model of circadian timekeeping at the molecular level depicting clock gene transcriptional-translational feedback loops. 1. The transcription factors CLOCK and BMAL1 bind to E-box elements (black box) within the promoter sequences of a variety of clock genes. Note that NPAS2 is a paralog of CLOCK that functions in some tissues to activate transcription. 2. The protein products of *Period* (*Per*) and *Cryptochrome* (*Cry*) genes form repressors that inhibit their own transcription. Note there are three paralogs of *Per* (*Per1, Per2, Per3*) and two paralogs of *Cry* (*Cry1, Cry2*). 3. Additional feedback loops involve additional clock genes that interact with the elements of the core loop to amplify and stabilize molecular clock function. For example, the protein products of *Rev-erb* and *Ror* genes compete for binding at ROR elements (white box), whereas the protein products of *Dbp* and *E4bp4* compete for binding at D-box elements (gray box). (B) Simplified model of circadian timekeeping at the systems level. The master clock within the SCN (red circle) receives light input that synchronizes it to the 24-h day. The SCN then provides outputs to downstream clocks in the brain and body to

Neuroscience, Vol 320 (April 21, 2016): pg. 259-280. DOI.

coordinate their function. (C) Left: The SCN contains ~10,000 neurons in each lobe that express the clock protein PER2. Middle: SCN neurons can be classified based on neuropeptide expression, with the major groups being those that express Arginine Vasopressin (AVP) and Vasoactive Intestinal Polypeptide (VIP). Right: Simplified model of circadian timekeeping within the SCN network. Briefly, VIP (depicted in red) and other neurons in the SCN core (depicted in blue) receive and process photic inputs, which they then transmit to neurons in the SCN shell (green) that provide outputs to downstream tissues. See text for additional details.

These various tissue clocks are regulated by a master clock within the SCN, a bilateral structure in the anterior hypothalamus located immediately above the optic chiasm (Welsh et al., 2010 and Mohawk et al., 2012). Lesion and tissue-graft experiments have demonstrated that the SCN is both necessary and sufficient for sustained circadian rhythms under most experimental conditions (Weaver, 1998). Consistent with its role as master clock, the SCN as a tissue displays circadian rhythms in many cellular processes, including metabolism, electrical activity, and gene/protein expression (Klein et al., 1991), which it generates intrinsically without the need for input from the environment or other tissues. For example, the whole SCN exhibits circadian rhythms in spontaneous electrical firing and *Period* transcription *in vitro*, with peak expression during the projected daytime hours. Further, these circadian rhythms are reflected in the cellular activity of single SCN neurons, which typically display relatively short ~5-h periods of electrical activity at midday (Schaap et al., 2003 and Brown et al., 2006). In addition to its intrinsic oscillatory capacity, the SCN receives input from the environment (e.g., light) that allows it to synchronize to the 24 h solar day. This photic input is transmitted to the SCN through a direct retinal projection, the retinohypothalamic tract, and several indirect pathways, including the geniculohypothalamic tract from the intergeniculate leaflet of the thalamus (Morin, 1994). The majority of retinal ganglion cells that provide photic input to the SCN expresses the photopigment melanopsin and respond intrinsically to photic stimulation (Berson, 2003, Gooley et al., 2003, Hattar et al., 2003, Morin et al., 2003, Panda et al., 2003 and Brown and Robinson, 2004). Light stimuli at night increase electrical firing and clock gene expression in the SCN, which is ultimately conveyed to downstream targets to adjust their phase. Thus, the SCN serves as the interface between the external and internal milieus, synchronizing the various body clocks to the 24-h world and one another (Fig. 1B).

Neuroscience, Vol 320 (April 21, 2016): pg. 259-280. DOI.

Similar to the evolution of hierarchical models, both formal and physiological analyses support complex clock models that emphasize the existence of multiple, interacting oscillators within the master clock itself (Pittendrigh, 1960 and Pittendrigh and Daan, 1976b). The complex clock model was first proposed based on plasticity in behavioral phenotypes that manifest under various environmental conditions (Pittendrigh, 1960, Pittendrigh, 1974 and Pittendrigh and Daan, 1976b). In contrast to evidence for the hierarchical model, different overt rhythms were modulated in parallel and thus it was concluded that this form of environmentally induced plasticity reflected a global change occurring within the central pacemaker itself. It is now well established that the SCN does indeed contain a network of multiple cellular clocks (Fig. 1C). Individual SCN neurons are self-sufficient cellular clocks that continue to express circadian rhythms even when synaptic communication is disrupted (Bouskila and Dudek, 1993, Shibata and Moore, 1993, Welsh et al., 1995, Herzog et al., 1997, Liu et al., 1997 and Shirakawa et al., 2001). However, when unable to interact, SCN neurons display different period lengths and desynchronize with one another over time. In contrast, when SCN neurons are able to communicate, period synchrony is sustained by intercellular signaling. The ability to maintain period synchrony at the tissue level independent of input appears to be a property that is not shared by other tissue clocks (Yamazaki et al., 2000, Nagoshi et al., 2004 and Welsh et al., 2004). As discussed above, coupling mechanisms within the SCN also regulate phase synchrony, with SCN neurons "preferring" to adopt specific phase relations (Yamaguchi et al., 2003) that are modulated by the environment (Jagota et al., 2000, Ohta et al., 2005 and Inagaki et al., 2007).

For both hierarchical and complex models, there remain fundamental questions concerning the properties and functions of the underlying clock cells or tissues. For the hierarchical model, important questions include: (1) how does the SCN communicate with downstream tissue clocks, (2) does the local clock in downstream tissues play an important role in controlling rhythmic output, and (3) do the cells and tissues of peripheral clocks interact with one another and/or transmit cues back to the SCN? Likewise, there are outstanding questions about the circuitry and function of the SCN complex. In order to understand the master clock network, it will be critical to: (1) address whether SCN neurons differentially contribute to the emergent

properties of the network and (2) define the mechanisms by which SCN communicate with one another to coordinate their rhythmic behavior.

The clock complex: SCN circuitry

The majority of SCN cells are local projection neurons that connect to other neurons in the SCN and surrounding hypothalamus (Abrahamson and Moore, 2001 and Moore et al., 2002). Nearly all SCN neurons produce γ-aminobutyric acid (GABA), yet they can be categorized into distinct subgroups based on neuropeptide expression (Moore and Speh, 1993 and Abrahamson and Moore, 2001). Classic work detailing SCN cytoarchitecture in the rat distinguished two spatially segregated compartments: the dorsomedial shell and the ventrolateral core (Moore and Silver, 1998). Within the SCN shell is a dense population of neurons that express arginine vasopressin (AVP), whereas the SCN core contains several different neuronal subclasses, including those that express vasoactive intestinal polypeptide (VIP). This organization typifies the SCN of most eutherian mammals studied to date, although regional anatomy and chemoarchitecture can vary between species (Cassone et al., 1988 and Morin, 2007). The SCN shell and core are convenient constructs for describing SCN compartmentalization; however, there is a growing appreciation that this organizational scheme belies a deeper complexity that awaits discovery. For instance, the SCN produces dozens of signaling factors, and the list of important SCN peptides continues to expand even to this day (van den Pol and Tsujimoto, 1985, Lee et al., 2013 and Lee et al., 2015). Thus, the SCN remains a complex structure with regions and cell types whose functions are not readily transparent (Moore et al., 2002, Antle et al., 2003, King et al., 2003, Lee et al., 2003 and Antle and Silver, 2005).

In addition to compartmentalization of neuropeptide expression, there are also regional differences in SCN function. For example, SCN neurons display regional differences in phase and inherent period length, which manifest even when considering a single neuropeptide subclass of SCN neuron (Shinohara et al., 1995, Schwartz et al., 2000, Quintero et al., 2003, Yamaguchi et al., 2003, Albus et al., 2005, Noguchi and Watanabe, 2008, Evans et al., 2011 and Myung et al.,

Neuroscience, Vol 320 (April 21, 2016): pg. 259-280. DOI.

2012). Further, light does not indiscriminately excite all SCN neurons; instead, photic stimuli activate only ~25% of SCN neurons and inhibit a smaller population (Meijer et al., 1986, Jiao et al., 1999, Aggelopoulos and Meissl, 2000, Nakamura et al., 2004 and Brown et al., 2011). Tract tracing studies suggest that there is a denser retinal projection to the SCN core than the SCN shell (Abrahamson and Moore, 2001 and Lokshin et al., 2015). These anatomical differences in retinal connectivity map onto functional differences in photic responses, with the SCN core displaying light-induced changes in gene expression that precede those in the SCN shell (Silver et al., 1996, Dardente et al., 2002, Yan and Okamura, 2002, Yan and Silver, 2002, Kuhlman et al., 2003, Nagano et al., 2003 and Yan and Silver, 2004). The current working model of photic signaling is that the SCN core contains first order neurons that receive afferent input, which process and transmit this information to neurons in the SCN shell. On the other hand, the SCN shell is thought to contain strongly rhythmic cells that provide outputs to reset the phase of downstream tissues (Nakamura et al., 2001, Dardente et al., 2002, Zhou and Cheng, 2005, Kalsbeek et al., 2010 and Evans et al., 2015). However, there are aspects of this model that remain unclear. For instance, although some work supports the idea that subclasses of SCN neurons differ in oscillatory capacity (Jobst and Allen, 2002), recent work indicates that cellular rhythmicity is stochastic, relies on network interactions to be sustained stably, and does not segregate with neuropeptide expression (Webb et al., 2009). Further, both AVP and VIP neurons extend processes to target regions in the hypothalamus, thalamus, and forebrain (Abrahamson and Moore, 2001, Buijs and Kalsbeek, 2001 and Kalsbeek and Buijs, 2002), which suggests that both SCN shell and core neurons provide signals to downstream clocks. The functional relevance of signals emanating from SCN neurons within different compartments remain ill defined.

SCN signaling mechanisms

Intercellular communication within the SCN network may involve multiple mechanisms (van den Pol and Dudek, 1993, Michel and Colwell, 2001 and Aton and Herzog, 2005). An important role for synaptic communication in SCN coupling is revealed by work demonstrating that blocking Na^+-dependent action potentials with

tetrodotoxin (TTX) causes SCN neurons to desynchronize *in vitro* (Yamaguchi et al., 2003). But the persistence of SCN timekeeping in the presence of TTX or low Ca^{2+} suggests that the network can use other coupling mechanisms not dependent on synaptic release of signaling molecules (Schwartz et al., 1987, Schwartz, 1991, Bouskila and Dudek, 1993, Dudek et al., 1993 and Shibata and Moore, 1993). In addition, the specific mechanisms employed in coupling may depend on the maturity of the network since the SCN displays circadian rhythms before synaptogenesis (Shibata and Moore, 1987 and Reppert, 1992). Although this plurality of potential coupling mechanisms presents an obstacle to understanding SCN circuitry, several factors have been identified that influence the emergent properties of the network (i.e., VIP, GABA, AVP). To date, significant progress has been made in defining the specific contribution of these coupling factors, although recent work suggests that their precise role in SCN coupling may be influenced by the state of the network itself (Evans et al., 2013, Bedont et al., 2014 and Wang et al., 2014).

VIP

Research conducted by a number of different labs has established a clear role for VIP in SCN coupling (Vosko et al., 2007). Mice deficient in either VIP or its receptor are unable to sustain circadian rhythms in DD, and instead their locomotor rhythms devolve into seemingly random bouts of activity (Harmar et al., 2002 and Colwell et al., 2003). The arrhythmic phenotype that manifests in VIP-deficient mice reflects a loss of neuronal synchrony within the SCN and a decrease in the number of SCN neurons able to maintain high amplitude rhythms (Aton et al., 2005, Maywood et al., 2006, Brown et al., 2007, Hughes et al., 2008 and Ciarleglio et al., 2009). VIP knockout mice also display a range of other phenotypes, including changes in sleep, metabolism, cardiac function, and reproduction (Bechtold et al., 2008, Sheward et al., 2010, Hannibal et al., 2011, Hu et al., 2011, Schroeder et al., 2011, Fahrenkrug et al., 2012 and Loh et al., 2014), which may reflect loss of internal synchrony at the systems level (Loh et al., 2011). Thus, VIP signaling is an important mediator of SCN synchronization whose absence has widespread consequences for behavior and physiology. However, recent work indicates that VIP can also desynchronize the phase of

Neuroscience, Vol 320 (April 21, 2016): pg. 259-280. DOI.

SCN neurons if given at the wrong time or at high doses (An et al., 2013 and Ananthasubramaniam et al., 2014), which highlights the need to define precisely the properties and mechanisms of VIP signaling to better understand how it regulates clock function at the cellular, network, and systems levels.

GABA

Unlike VIP, recent work has revealed that GABA influences circadian coupling by desynchronizing SCN neurons (Evans et al., 2013, Freeman et al., 2013, DeWoskin et al., 2015 and Myung et al., 2015). Normally, this effect of $GABA_A$ signaling is hard to detect because VIP signaling is a potent synchronizing agent. But in the absence of VIP (e.g., when SCN slices are cultured from VIP knockout mice or cultured with VIP antagonists), SCN neurons desynchronize due to $GABA_A$ signaling within the network (Evans et al., 2013 and Freeman et al., 2013). However, the functional role of $GABA_A$ signaling can be influenced by environmentally induced changes in the state of the network (Evans et al., 2013, Farajnia et al., 2014 and Myung et al., 2015). For example, although $GABA_A$ signaling acts to inhibit phase synchronization when SCN neurons are tightly synchronized under standard laboratory lighting conditions, it serves to facilitate network re-synchronization when SCN neurons are desynchronized by light *in vivo* (Evans et al., 2013). Interestingly, the change in the functional role of $GABA_A$ signaling under these lighting conditions is associated with an increase in the number of SCN neurons that display GABA-induced excitatory responses (Farajnia et al., 2014 and Myung et al., 2015). Similarly, $GABA_A$ signaling can synchronize dissociated SCN neurons *in vitro* (Liu and Reppert, 2000) and is involved in the transfer of resetting information from one SCN compartment to another (Albus et al., 2005 and Han et al., 2012). Clearly, further work is required to define the conditions and mechanisms that determine the functional role of $GABA_A$ signaling in SCN coupling.

Other mechanisms

In the absence of VIP signaling, SCN neurons can be synchronized by AVP or GRP (Brown et al., 2005, Maywood et al.,

2006 and Maywood et al., 2011). Although AVP is traditionally viewed as an output signal, AVP neurons provide signals that modulate network function to regulate the rate of recovery from simulated jetlag (Yamaguchi et al., 2013 and Mieda et al., 2015). On the other hand, GRP is mostly known for its role in processing photic signals (McArthur et al., 2000, Dardente et al., 2002, Antle and Silver, 2005 and Gamble et al., 2007), and its potential role in SCN coupling remains unclear. In addition to synaptic communication, electrotonic signaling through low-resistance gap junctions may also influence SCN synchrony by allowing the transmission of charged ions and other small molecules (e.g., cAMP) among cells in close apposition (Bennett et al., 1991, Rash et al., 2000, Rash et al., 2001 and Connors and Long, 2004). Gap junctions can be found in SCN neurons and glial cells, with the diffusion of labeled molecules (i.e., dye coupling) occurring mostly between cells within a SCN region (Welsh and Reppert, 1996, Jiang et al., 1997, Colwell, 2000, Jobst et al., 2004 and Long et al., 2005). When gap junctions are targeted pharmacologically, SCN electrical rhythms become broader, arrhythmic, or bimodal (Prosser et al., 1994, Shinohara et al., 2000a, Shinohara et al., 2000b and Shirakawa et al., 2001). Bimodal rhythms also emerge in the presence of the glial metabolism antagonist, fluorocitrate (Prosser et al., 1994). These changes in the waveform of SCN electrical rhythms are thought to be due to altered communication among SCN neuronal subpopulations (Wang et al., 2014), although it remains unclear how this influences the functional properties of the network. Given the evidence for multiple SCN coupling mechanisms, it will be important to obtain detailed insight into how SCN neurons integrate the various signals produced by the network and the functional consequences of cross-modal interactions revealed by previous work (Colwell, 2000, Shinohara et al., 2000b, Itri et al., 2004 and Wang et al., 2014).

Formal assays for investigating the emergent properties of the complex clock

The complex clock model was first inspired by changes in the waveform of circadian rhythms that manifest under certain environmental lighting conditions, such as seasonal changes in day length and constant light (LL) (Pittendrigh, 1974 and Pittendrigh and Daan, 1976b). Other analytical paradigms discovered since also imply

the existence of multiple, interacting oscillators within the SCN (e.g., simulated jetlag, non-24-h cycles, 24-h light:dark:light:dark (LDLD) cycles). By investigating circadian plasticity at the behavioral level, these formal assays are used to infer the structure of the complex clock and the properties of its constituent oscillators. A renewed focus on defining the neurobiological bases of circadian plasticity at the behavioral level may provide critical insight into SCN circuitry and may be used to test putative SCN coupling factors. However, the formal concepts embodied in the complex clock model should be updated and elaborated in order to provide refutable hypotheses for testing the role of different coupling mechanisms. Here we will use terminology originally adopted under the dual oscillator model for its utility, but this is intended to represent at least two populations of clock cells (Winfree, 1967, Pavlidis, 1973, Enright, 1980a, Strogatz, 2003 and Izhikevich, 2007). In addition to providing insight into the nature and mechanisms of SCN coupling, the formal assays highlighted in this review may provide means to further distinguish SCN neuronal subpopulations. Moreover, further investigation into the bases of flexibility in circadian waveform may lead to novel approaches to mitigate the harm of circadian disruption in humans (Harrison and Gorman, 2012).

Changes in circadian waveform after photoperiodic changes in day length

Photoperiod influences myriad physiological and behavioral rhythms, including both reproductive and non-reproductive processes (Illnerova, 1991, Goldman, 2001 and Gorman et al., 2001a). In fact, there is a suite of diurnally and nocturnally phased events whose duration mirrors the length of the light and night portion of the LD cycle, respectively. The fact that numerous rhythms change in concert is taken as evidence that the central pacemaker itself is sensitive to photoperiod. For instance, the duration of melatonin secretion from the pineal gland is proportional to the length of the night, with a longer duration of release under winter-like short days compared to summer-like long days. Likewise, the duration of locomotor activity (α) compresses under long days and expands under short days (Fig. 2A). Melatonin is an important driver of photoperiodic changes in physiological function in some species, but photoperiodic changes in α

do not require melatonin (Hastings et al., 1987 and Refinetti, 2002). This also is taken as evidence that the SCN itself is altered by changing day lengths. More direct measures of SCN function indicate that it does indeed encode photoperiod. For example, photoperiod modulates the length of the photosensitive phase of the circadian cycle, as determined by light-induced phase shifts and changes in SCN gene expression (Pohl, 1983, Pittendrigh et al., 1984, Pohl, 1984, Binkley and Mosher, 1986, Humlova and Illnerova, 1992, Travnickova et al., 1996, Vuillez et al., 1996, Illnerova and Sumova, 1997, Sumova and Illnerova, 1998 and vanderLeest et al., 2009). Proportional changes in SCN rhythms of diurnally expressed markers are also observed, with long days increasing the duration of SCN electrical firing, endogenous *c-fos* expression, and clock gene/protein production (Sumova and Illnerova, 1998, Jac et al., 2000, Jagota et al., 2000, Messager et al., 2000, Mrugala et al., 2000, Sumova et al., 2003, de la Iglesia et al., 2004b, Johnston et al., 2005, VanderLeest et al., 2007, Naito et al., 2008, Yan and Silver, 2008 and Brown and Piggins, 2009). The process of photoperiodic modulation of overt rhythms and SCN function can also be observed after release from long days to DD, which eliminates the masking influence of light (Fig. 2A). Following transfer to DD, circadian rhythms are said to "free-run" and reflect inherent period length (τ). At the same time, α increases in a systematic fashion after release into DD, with α expansion occurring as activity onset advances and activity offset delays each cycle. Advances in the onset of behavioral activity and melatonin secretion are highly correlated, as are the delays in their offsets, suggesting that a common mechanism underlies increases in both rhythms (Elliott and Tamarkin, 1994).

Neuroscience, Vol 320 (April 21, 2016): pg. 259-280. DOI.

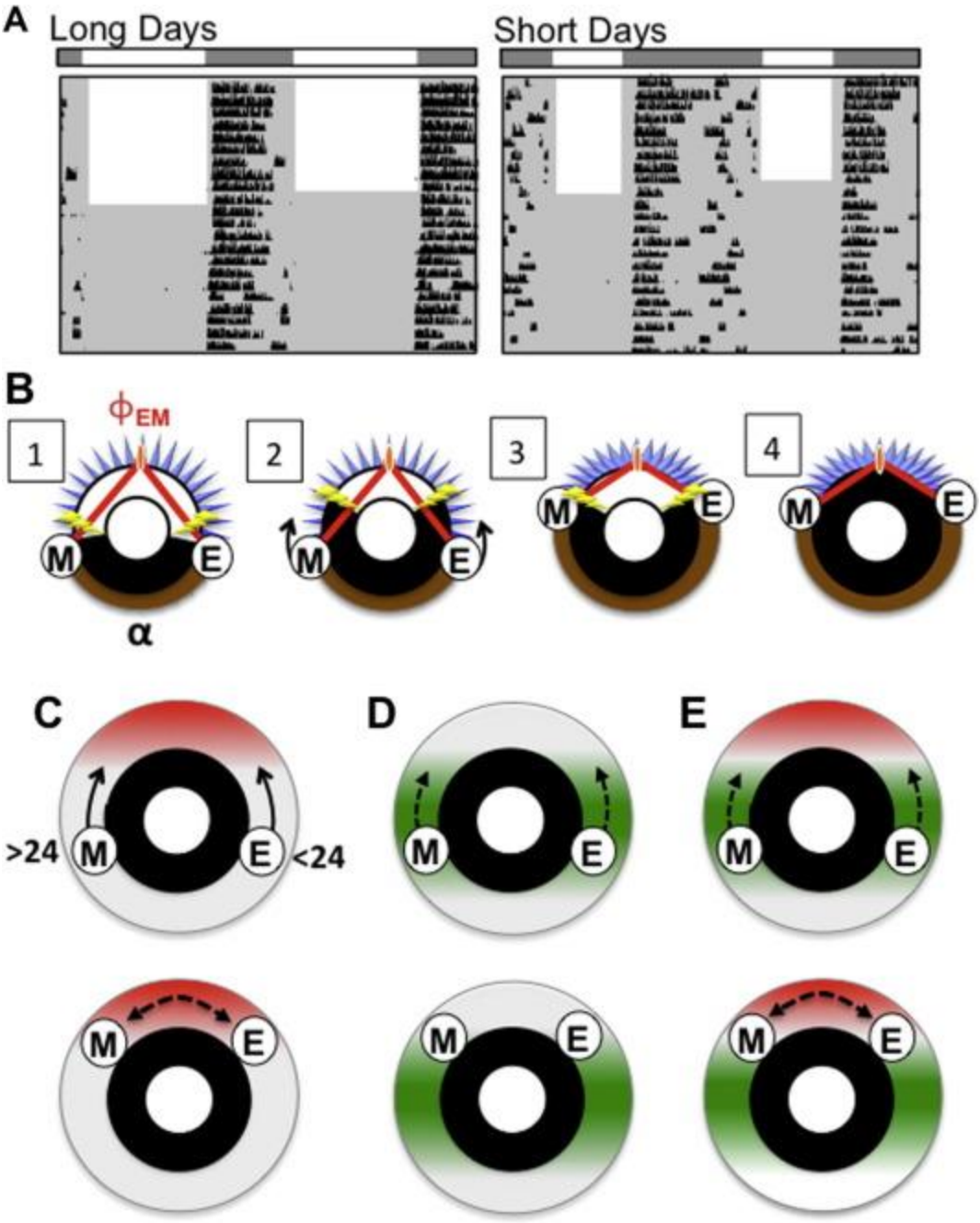

Fig. 2. Photoperiodic modulation of circadian waveform. (A) Representative double-plotted actograms illustrating changes in locomotor activity rhythms of Syrian hamsters held under long day and short day photoperiods before release into constant darkness. White and black bars above each actogram illustrate initial housing conditions, with the internal shading indicating the change in lighting conditions. Data replotted from (Evans et al., 2007). (B) Schematic representation of the complex clock model of Pittendrigh and Daan. 1. Under long day photoperiods, Evening (E) and Morning (M) oscillators define the times of activity onset and offset, respectively. E is phase delayed by light at dusk whereas M is phase advanced by light at dawn (lighting

bolts). The phase angle of E and M (φ_{EM}, internal red angle) determines the duration of nighttime locomotor activity (α, semi-circular brown bar). Importantly, α is inversely related to the duration of electrical and molecular activity of SCN neurons (blue spikes). 2. When transferred to short day photoperiods, E and M oscillators adopt different period lengths, <24 h and >24 h, respectively (solid arrows). Over time, this causes changes in φ_{EM}, increases α, and decreases the phase distribution of SCN neurons. 3. Stability in circadian waveform is established when E and M are re-entrained by light. 4. Maximal expansion under constant darkness resembles that achieved under very short day photoperiods. (C) Conceptual model for a repulsive coupling mechanism. Top: Changes in φ_{EM} are caused by inherent differences in period (E < 24 h, M > 24 h) that cause E and M shift closer to one another over consecutive days (solid arrows). Bottom: Changes in φ_{EM} are ultimately limited by a repulsive coupling interaction between E and M (red shading) that causes each oscillator to shift in the direction opposite of their free-run (dashed arrows). The strength of this repulsive coupling interaction is expected to depend on φ_{EM}, as illustrated by changes in shading. D) Conceptual model for attractive coupling mechanisms. Top: Changes in φ_{EM} is caused by an attractive coupling interaction (green shading) between E and M that causes them to shift close to one another (dashed arrows). The strength of coupling is expected to depend on φ_{EM}, as illustrated by changes in shading. (E) Conceptual model for combined attractive and repulsive coupling mechanisms. In the combined model, both types of coupling interactions influence the steady state of the network. See text for more details.

At the formal level of analysis, photoperiodic changes in circadian waveform are thought to reflect adjustments in the phase relationships between two distinct populations of clocks (Fig. 2B). Based on the differential control of activity onset and offset, these two clock populations have been labeled evening (E) and morning (M) oscillators. Since activity onset advances whereas activity offset delays under DD and short day lengths, it is theorized that E oscillators have τ < 24 h and M oscillators have τ > 24 h. According to this model, the inherent differences in τ alter the phase angle between E and M oscillators (φ_{EM}), which leads to increases in the duration of subjective night (Pittendrigh, 1974, Illnerova, 1991, Elliott and Tamarkin, 1994 and Gorman et al., 1997). It is further predicted that during entrainment, the E oscillator with τ < 24 h is decelerated daily by light at dusk and the M oscillator with τ > 24 h is accelerated daily by light at dawn. When day length changes, these resetting-actions of light would influence φ_{EM} (Fig. 2B), which is expected to alter the waveform of output signals from the SCN and the overt rhythms it programs (e.g., α, melatonin duration). Due to the reduced influence of photic cues under short days and DD, the changes that manifest are thought to due to inherent τ differences between E and M and/or φ_{EM}-dependent coupling.

Typically, the duration of subjective night stabilizes under DD, which would not be expected if its expansion were determined solely by inherent differences in τ. Instead, the rhythms of independent free-running oscillators would periodically diverge and converge to produce a "beating" pattern when monitored over many cycles. In contrast to this prediction, the maximal degree of subjective night expansion is relatively fixed under most conditions (Hastings et al., 1987, Elliott and Tamarkin, 1994 and Gorman et al., 1997). In fact, DD-induced increases in α and melatonin secretion are typically proportional to the length of the scotophase under the previous LD cycle (Illnerova, 1991 and Elliott and Tamarkin, 1994), with little to no further expansion occurring after release from very short days (Fig. 2A). Thus, the systematic yet constrained pattern implies that coupling synchronizes the period of E and M to regulate φ_{EM} and prevent arrhythmia or "beating" from manifesting (Pittendrigh and Daan, 1976b, Illnerova, 1991 and Elliott and Tamarkin, 1994).

Since its conception, this complex clock model has captured the interest of chronobiologists and inspired a search for the location of E and M oscillators. Based on this model, E and M oscillators are predicted to display (1) adjustable phase relationships dependent on photoperiod, (2) differential control over activity onset versus offset, and (3) inherent period differences with $\tau_E < 24$ h $< \tau_M$. Several studies investigating SCN electrical firing rhythms and clock gene/protein expression have identified subgroups of SCN neurons whose phase relationships are modulated by photoperiod, with these neuronal subgroups organized along a rostral-caudal axis and/or dorsal-ventral axis (Jagota et al., 2000, Hazlerigg et al., 2005, Inagaki et al., 2007, Naito et al., 2008, Yan and Silver, 2008, Brown and Piggins, 2009, Evans et al., 2013 and Myung et al., 2015). Furthermore, studies have identified specific subgroups of SCN neurons within the rostral and caudal SCN that differentially control activity onset and offset (Inagaki et al., 2007). Lastly, period differences have been reported for SCN neurons located within discrete regions (Shinohara et al., 1995, Noguchi et al., 2004, Noguchi and Watanabe, 2008 and Myung et al., 2012). Collectively, this work suggests that long photoperiods modulate the phase relationships between SCN neurons, but questions remain about this process and the underlying coupling mechanisms.

Neuroscience, Vol 320 (April 21, 2016): pg. 259-280. DOI.

Important questions concerning the nature of this coupling process need to be addressed by additional studies. For example, the basic premise of τ_E < 24 h < τ_M of the original E-M model does not take into account the fact that α expansion under DD reflects the actions of a coupled system. Thus, DD-induced α expansion could be driven by inherent period differences OR by phase shifts produced by intercellular signaling factors. Although it has been extremely difficult to distinguish these two possibilities, this is a non-trivial issue that hinders insight into how SCN subgroups are interacting during photoperiodic changes in circadian waveform. For example, if α expansion is driven by inherent period differences, then coupling interactions need not engage until a specific φ_{EM} is achieved (Fig. 2C). In this case, coupling reflects an φ_{EM}-dependent interaction that resets E and M oscillators so that they synchronize with a similar period when in a specific relationship. In effect, this would limit further changes in φ_{EM} and prevent phase locking from manifesting (Fig. 2C). Alternatively, α expansion may not reflect inherent period differences but instead could be driven by intercellular interactions that cause E and M to reset one another (Fig. 2D). In this model, phase locking is prevented by φ_{EM}-dependent changes in coupling strength (Fig. 2D). Thus, both models involve a recursive mutual resetting process that is φ_{EM}-dependent (Daan and Berde, 1978 and Oda and Friesen, 2002). Although pitted against one another here, it is worth noting that these two models are not mutually exclusive (Fig. 2E) and may be accounted for by a single coupling process that involves sinusoidal rhythms in resetting (Daan and Berde, 1978). Recent work has validated this general concept by demonstrating that SCN neurons can interact through φ-dependent resetting (Evans et al., 2013), and an important issue for future work is testing whether this type of "coupling response rhythm" varies with SCN subclass and/or environmental conditions.

The conceptual models described above are designed to illustrate possible ways of envisioning the formal process of coupling, which is an important step toward defining its neurobiological basis. Although it remains untested whether the strength or nature of SCN coupling is systematically influenced by neuronal phase relationships, formal analyses may begin to examine this issue by investigating the rate of α expansion following release from different pretreatment conditions. Further, work suggests that distinct SCN factors promote period/phase synchrony (VIP) and desynchrony (GABA), and future

studies should test whether these factors influence photoperiodic plasticity in circadian waveform. It is clear that VIP signaling plays an important role in stabilizing circadian waveform in the absence of environmental time cues (Harmar et al., 2002 and Colwell et al., 2003), but additional work should test whether the DD phenotype of VIP-deficient mice is caused directly by loss of the VIP-related synchronizing cue or indirectly by a GABA-related desynchronizing signal (Freeman et al., 2013). Moreover, VIP knockout mice fail to maintain photoperiodic changes in α and SCN electrical activity upon release into DD (Lucassen et al., 2012), but the strength of rhythms displayed by VIP mice is enhanced by short day entrainment. In addition to short day lengths, rhythms of VIP-deficient mice are improved by LL and use of a running-wheel (Power et al., 2010 and Hughes et al., 2015). Although the neurobiological basis remains unclear, it should be investigated whether these environmental conditions restore rhythmicity in VIP-deficient mice by changing the strength of other SCN coupling factors. When paired with manipulation of specific signaling mechanisms, formal analyses of photoperiodic plasticity in circadian waveform has the potential to shed new light on the process and mechanisms underlying SCN coupling.

Changes in circadian waveform under LL

Support for the complex clock model also derived from the observation that locomotor activity rhythms could dissociate into multiple components under LL conditions (Pittendrigh and Daan, 1976b). In nocturnal rodents, LL lengthens τ, compresses α and decreases locomotor activity levels (Aschoff, 1960). In addition, chronic exposure to LL can induce a temporal reorganization known as "splitting" (Fig. 3), which is characterized by two bouts of rest and locomotor activity per circadian cycle (Morin and Cummings, 1982, Turek et al., 1982, Cheung and McCormack, 1983, Lees et al., 1983, Boulos and Morin, 1985, Meijer et al., 1990, Puchalski and Lynch, 1991b, Pickard et al., 1993 and Lax et al., 1998). While the LL-induced split is emerging, the two activity components may free-run with different circadian periods, one shorter and the other longer than 24 h. However, when the two activity bouts reach antiphase, the split rhythm stabilizes such that two distinct activity components are maintained. After transfer from LL to DD, the two activity bouts rapidly

rejoin, with unsplit activity rhythms re-emerging after a brief interval (Earnest and Turek, 1982). The presence of activity bouts with distinct period lengths during induction and resolution of the LL-induced split was taken as evidence that this reflects the actions of multiple clocks cycling in antiphase. That these clocks were located within the master clock itself was further based on observations that overt rhythms besides locomotor activity are likewise split under LL (Shibuya et al., 1980, Pickard et al., 1984 and Swann and Turek, 1985) and that SCN electrical rhythms were bimodal in LL-split animals (Mason, 1991 and Zlomanczuk et al., 1991). It is worth noting that the incidence of splitting under LL is influenced by many different factors, including LL intensity, species, sex, age and wheel running. In some animals chronic LL will cause the complete loss of circadian rhythmicity (Fig. 3), with ultradian rhythms thought to reflect the independent programs of many desynchronized SCN neurons (Honma and Hiroshige, 1978, Mason, 1991 and Lax et al., 1998). Lastly, some inbred strains of mice display spontaneous splitting under conditions of DD rather than LL, although this phenotype appears to arise due to reorganization of non-SCN clocks (Abe et al., 1999 and Abe et al., 2001).

Neuroscience, Vol 320 (April 21, 2016): pg. 259-280. DOI.

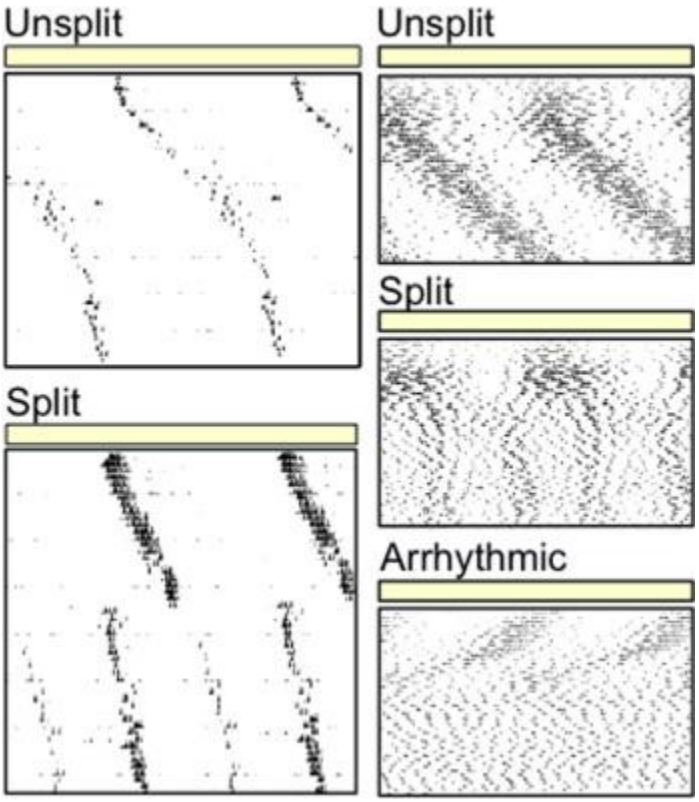

Fig. 3. Modulation of circadian waveform under constant light (LL) conditions. Representative double-plotted actograms illustrating changes in circadian waveform of Syrian hamsters (left) and mice (right) during exposure to LL. White bars above each actogram illustrate lighting conditions. Hamster and mouse data are replotted from (Gorman, 2001) and (Evans et al., 2012b), respectively.

The neurobiological basis of LL-induced split rhythms has been localized to antiphase oscillations of the left and right lobes of the SCN (de la Iglesia et al., 2000, de la Iglesia et al., 2003, Ohta et al., 2005, Yan et al., 2005 and Butler et al., 2012), which are connected by contralateral projections extending across the midline (Moore and Leak, 2001). Consistent with the idea that each split bout is generated by a separate lobe, unilateral SCN lesions can cause the emergence of an unsplit rhythm (Pickard and Turek, 1983), although lesions of non-SCN tissue can produce similar effects (Harrington et al., 1990). However, temporal dissociation of left and right SCN may not be the exclusive means by which splitting arises because animals sustaining unilateral SCN lesions can display LL-induced split rhythms (Davis and Gorski, 1984). Further, Siberian hamsters split by LL do not display antiphase electrical rhythms in the left and right SCN, which suggests rearrangement of SCN neurons within each lobe (Zlomanczuk et al.,

1991). Consistent with this, LL-split hamsters display antiphase rhythms in the shell and core of each SCN lobe as well as antiphase rhythms between lobes (Yan et al., 2005 and Butler et al., 2012); however, it remains unknown if this pattern generalizes to other species.

Although photoperiodic and LL-induced modulations of circadian waveform both provide support for the concept of the SCN being a complex comprised of multiple interacting clocks, some studies suggest that photoperiodic and LL-induced changes in circadian waveform are mediated by distinct neurobiological mechanisms. For instance, E and M oscillators are modeled as differentially controlling activity onset and offset, but the left and right SCN provide redundant programing (Davis and Gorski, 1984 and Davis and Viswanathan, 1996). Also, photoperiod does not alter the phase relationship between left and right SCN (de la Iglesia et al., 2004b) and unilateral SCN lesions do not compromise photoperiodic changes in gonadal function (Hastings et al., 1987). The relationship between photoperiodism and LL-induced splitting remains difficult to test because these two paradigms involve incompatible experimental conditions and bright LL masks many overt rhythms in nocturnal rodents that are highly informative (e.g., suppression of melatonin secretion). Thus, it remains possible that these two forms of plasticity are fundamentally distinct in terms of their underlying coupling mechanisms.

Despite progress in identifying the neuroanatomical substrate(s) for LL-induced rhythms, the process by which the SCN reorganizes under LL remains unclear. Under the original complex clock model, the two split bouts under LL were originally labeled E and M because in some records the split activity bouts appeared to derive from the evening and morning portions of the unsplit activity rhythm (Earnest and Turek, 1982, Morin and Cummings, 1982, Swann and Turek, 1982 and Lees et al., 1983). However, this is not always the case and in some records the origin of the split bouts is unclear (e.g., Fig. 3). Although the neurobiological evidence discussed above indicates that subsets of E and M oscillators likely control each split bout, it was originally hypothesized that LL lengthened τ_E and shortened τ_M to cause reductions in φ_{EM} and α compression (Pittendrigh, 1974 and Pittendrigh and Daan, 1976b). Alternatively, it has been proposed that LL-induced splitting could reflect light-induced changes

in the strength and/or nature of the coupling process (Daan and Berde, 1978, Kawato and Suzuki, 1980 and Oda and Friesen, 2002). It remains unclear precisely what causes the activity rhythm to split into two synchronized bouts that cycle in antiphase, but this may involve changes in coupling that involve either attractive or repulsive interactions (Daan and Berde, 1978 and Oda and Friesen, 2002). Thus, this phenomenon may be modeled using conceptual processes like those postulated to regulate photoperiodic modulation of circadian waveform.

An important issue for future work will be to understand precisely how LL influences coupling between SCN neurons to fragment circadian waveform into split and arrhythmic patterns. Little is known about how SCN signaling changes during LL-induced splitting and arrhythmia, which may reflect the fact that these forms of plasticity develop over a long period time with little experimental control. Previous research has demonstrated that VIP in rats is downregulated by acute light exposure and 3-wk exposure to LL (Albers et al., 1987, Shinohara et al., 1993, Isobe and Nishino, 1998 and Shinohara et al., 1999). However, it is unknown if VIP levels rebound during long-term LL exposure like that necessary to induce splitting and arrhythmia. Further, the depressive effects of light on VIP may not generalize to other rodent species (Dardente et al., 2004). It may be possible to test whether downregulation of VIP under LL provides the impetus for loss of synchronization that occurs during LL-induced arrhythmia, but this mechanism fails to fully account for the observation that split bouts are able to synchronize once aligned into an anti-phase configuration. Another possibility is that the emergence of LL-induced splitting reflects coupling mechanisms that resist light-driven α compression through a repulsive type of interaction. Further work is needed to understand how LL may influence SCN coupling. One way in which this question can be addressed is by systemically evaluating the effects of chronic light on putative SCN coupling factors and testing whether down/up regulation is causally related to LL-induced plasticity in circadian waveform. Given the species differences in the incidence and thresholds for LL-induced splitting, future studies may also benefit from a comparative approach using a variety of nocturnal and diurnal models.

Neuroscience, Vol 320 (April 21, 2016): pg. 259-280. DOI.

Changes in circadian waveform after light-induced resetting

The SCN receives light cues that allow it to synchronize to local time (Meijer and Schwartz, 2003). Photic entrainment is mediated by a circadian rhythm in light sensitivity described in the photic phase response curve (PRC), which illustrates how light applied at different phases of the circadian cycle shifts the phase of overt rhythms (Johnson, 1999). Specifically, light pulses early in subjective night produce phase delays, light pulses late in subjective night produce phase advances, and light pulses during the subjective day produce negligible effects. In nocturnal rodents, light pulses also produce acute changes in circadian waveform that depend on the direction of phase resetting (Fig. 4A). Following late night light pulses, activity/melatonin offset advances readily but activity/melatonin onset requires several cycles to shift completely (Boulos and Rusak, 1982, Honma et al., 1985, Illnerova and Vanecek, 1987 and Meijer and De Vries, 1995). The different resetting kinetics of distinct phase markers causes the emergence of "transient" cycles where subjective night is compressed, which is resolved as activity onset shifts gradually over subsequent days (Fig. 4A). In extreme cases, transient cycles may be characterized by the complete loss of nocturnal events, such as melatonin secretion (Illnerova and Vanecek, 1987). In contrast, α compression is less pronounced during light-induced phase delays because both phase markers reset with similar kinetics following a light pulse applied during early night (Fig. 4A). Direction-dependent transients in circadian waveform also emerge following shifts of the LD cycle that simulate travel across time zones (Fig. 4B), although shifts in activity onset and offset are oftentimes masked by light under these conditions.

Neuroscience, Vol 320 (April 21, 2016): pg. 259-280. DOI.

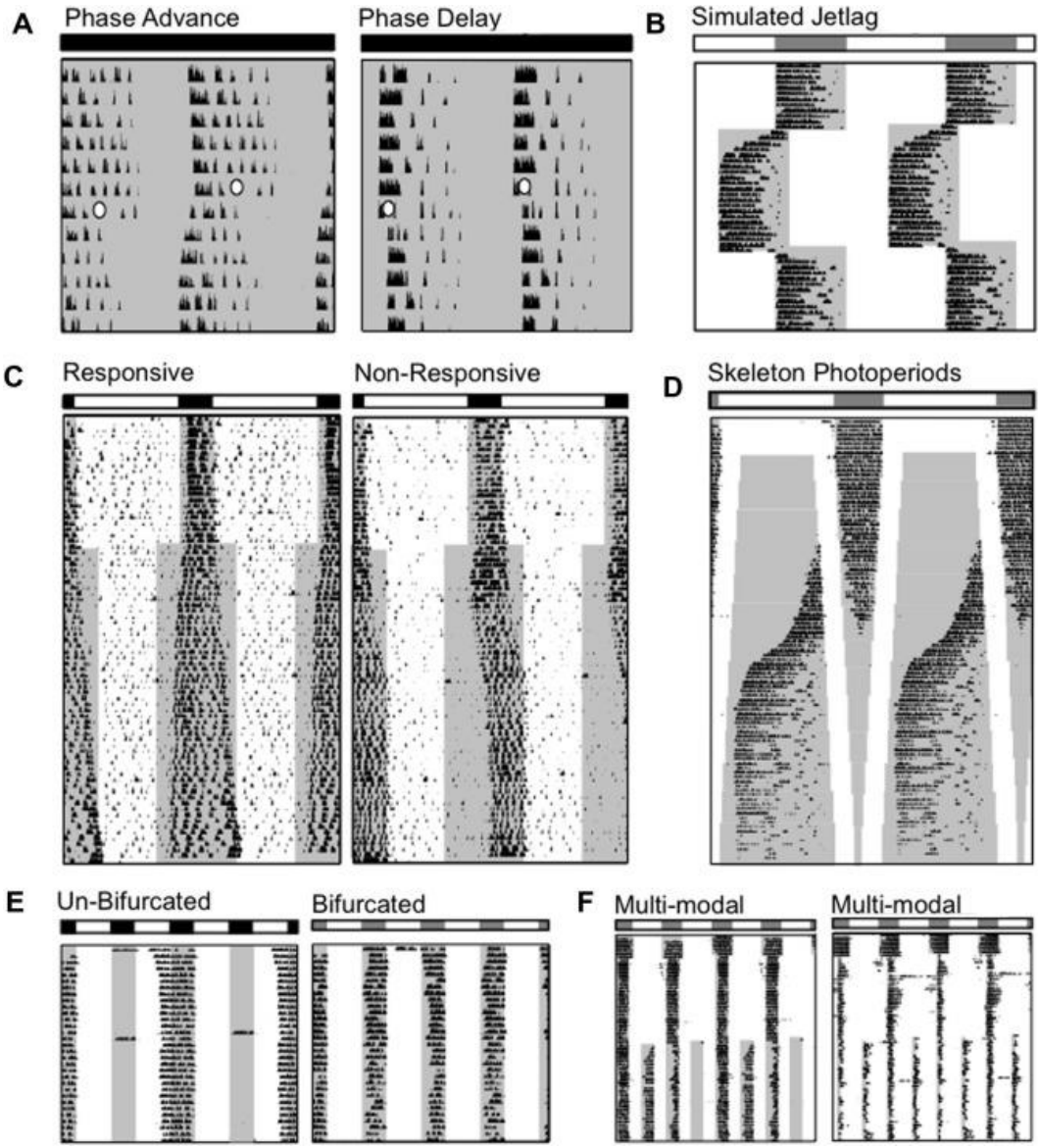

Fig. 4. Plasticity in circadian waveform during light-induced resetting (A), simulated jetlag (B), photoperiodic non-responsiveness (C), skeleton photoperiods (D), LDLD cycles (E), and multi-model lighting conditions (F). Data in panel A are from (Evans et al., 2007), panel B are from (Evans et al., 2009), panel C are from (Gorman and Elliott, 2004), panels D-E are from (Evans et al., 2005), and panel F are from (Evans & Gorman, unpublished observations).

Differential photic resetting also occurs within the SCN itself, with distinct resetting patterns evident between different clock genes and SCN regions. In hamsters, the evening and morning peaks of SCN electrical activity specifically detected in a horizontal slice preparation are differentially shifted by application of glutamate *in vitro* (Jagota et

Neuroscience, Vol 320 (April 21, 2016): pg. 259-280. DOI.

al., 2000). Furthermore, discrepancies in resetting are evident among different SCN clock gene/protein rhythms (Sumova and Illnerova, 1998, Zylka et al., 1998, Reddy et al., 2002, Yan and Silver, 2002 and Yan and Silver, 2004). In addition, SCN regional differences in the rate of re-entrainment have been reported in both the advance and delay direction (Nagano et al., 2003, Nagano et al., 2009, Albus et al., 2005, Nakamura et al., 2005, Davidson et al., 2009, Rohling et al., 2011 and Sellix et al., 2012). Results of bioluminescence imaging with real-time reporters of clock protein indicate that the SCN core shifts faster than the SCN shell after a 6-h advance of the LD cycle (Sellix et al., 2012), but the spatiotemporal kinetics of re-entrainment in the delay direction have yet to be examined with this approach.

At the formal level of analysis, light-induced changes in circadian waveform have been interpreted within the context of the complex clock model. According to this model, direction-dependent resetting kinetics reflect differences in the light sensitivity of E and M and their mutual coupling strength (Boulos and Rusak, 1982, Honma et al., 1985, Illnerova, 1991 and Meijer and De Vries, 1995). In the case of differential light sensitivity, the immediate phase advance of activity offset suggests that M is highly sensitive to light provided during late subjective night, while the sluggish response of activity onset indicates E is less sensitive to light at this phase. That the steady state phase shift of onset often equals that for offset has been interpreted as evidence for coupling between E and M (Illnerova, 1991 and Meijer and De Vries, 1995), but this process remains ill defined. Subsequent decompression of α during transient cycles could be explained by coupling that promotes period synchrony (Fig. 2D) or desynchrony (Fig. 2C) in an φ_{EM}-dependent manner. Conversely, neither process can account for the lack of delaying transients, unless (1) E and M are equally responsive to light presented during early subjective night or (2) there is an asymmetry in the mutual coupling between E and M (Illnerova, 1991).

Given the translational appeal in better understanding this process, there are a number of things that could be addressed in future work. First, a detailed understanding of how putative E and M oscillators respond to photic stimuli could be used to test whether they are characterized by distinct PRCs. Formal analyses may also provide insight by testing whether nonphotic resetting is similarly marked by

transients. The results of such a study may provide insight into whether direction-specific resetting reflects a general feature produced by fundamental differences in the ability of underlying oscillators to reset one another. Conversely, if this phenomenon reflects that E and M have different photic PRCs, then there is little reason to suspect that nonphotic phase shifts will involve transients or that those that might emerge will bear any similarity to light-induced advancing transients. Furthermore, future studies can be designed to compare and contrast the paradigms presented in the proceeding two sections (i.e., changes in circadian waveform after release into DD and after discrete light pulses) in order to resolve whether they reflect identical or disparate processes. Lastly, studies should investigate whether specific SCN coupling factors influence the kinetics of photic re-entrainment. Although transients are not commonly quantified, it is of interest that the rate of re-entrainment is accelerated by either a gain in VIP signaling (Shen et al., 2000 and An et al., 2013) or a loss of AVP signaling (Yamaguchi et al., 2013 and Mieda et al., 2015). A re-examination of light-induced transients in the context of altered SCN signaling may provide insight, as would studies that couple these manipulations with approaches that quantify the associated changes in SCN spatiotemporal organization.

Photoperiodic non-responsiveness

Within many rodent species, some animals fail to adopt the typical short day phenotype of reproductive quiescence, and are commonly referred to as short day nonresponders (NRs) (Nelson, 1987). In Siberian hamsters, insensitivity to short day lengths has a circadian basis (Puchalski and Lynch, 1988, Puchalski and Lynch, 1991a, Puchalski and Lynch, 1991b, Puchalski and Lynch, 1994, Freeman and Goldman, 1997, Gorman et al., 1997, Gorman and Zucker, 1997, Prendergast and Freeman, 1999 and Gorman and Elliott, 2004). NR Siberians express both a short α (Fig. 4C) and a short melatonin signal under short day lengths, with each rhythm phase locked to dawn in the large majority of animals (Puchalski and Lynch, 1986, Margraf et al., 1991, Margraf and Lynch, 1993, Gorman et al., 1997, Gorman and Zucker, 1997, Prendergast and Freeman, 1999 and Gorman and Elliott, 2004). A complementary pattern can be seen in the rhythm of spontaneous electrical activity within the SCN of

NR Siberian hamsters (Margraf et al., 1991). NR Siberian hamsters display short day responses if long melatonin infusions are provided; indicating that peripheral sensitivity is intact but that the appropriate signal is absent in these animals (Margraf and Lynch, 1993). Further, Siberian hamsters from artificially selected NR lines, if raised in DD or a short day photoperiod, can exhibit photoperiodic responsiveness under short day lengths (Stanfield and Horton, 1996, Freeman and Goldman, 1997 and Goldman and Goldman, 2003). Lastly, pre-treatment to very long day lengths can cause unselected animals to display a state of short day nonresponsiveness qualitatively similar to that produced by artificial selection (Freeman and Goldman, 1997, Gorman et al., 1997, Gorman and Zucker, 1997, Prendergast and Freeman, 1999 and Goldman et al., 2000). Collectively, these data suggest that the NR phenotype in Siberian hamsters is caused by a fundamental change in the function of the SCN that limits plasticity in circadian waveform.

The nature of the change in the SCN that causes short day nonresponsiveness remains unclear. It has been proposed that short day nonresponsiveness may reflect a change in τ and/or altered coupling (Puchalski and Lynch, 1991b, Puchalski and Lynch, 1994 and Gorman and Zucker, 1997). As described above, it is theorized that photoperiodic expansion of α reflects inherent differences in the period of E and M, with $\tau_E < 24$ h $< \tau_M$. One possible explanation of photoperiodic non-responsiveness contends that lengthening of τ_E causes it to be > 24 h, which lengthens overall τ and inhibits the ability to expand α under short days. Consistent with this hypothesis, it has been reported that animals from artificially selected NR lines will display gonadal regression under LD cycles >24 h (Puchalski and Lynch, 1994). However, a lengthened τ is not always observed in NR Siberian hamsters, and it has been suggested that a change in the coupling between E and M oscillators may constrain increases in φ_{EM} in these animals. Originally, it was postulated that NR animals have stronger coupling; however, it remains difficult to specify changes in coupling strength given the possibility of both synchronizing and desynchronizing signaling mechanisms. Given the utility of exploring this phenotype further, future studies may benefit from recent advances that could allow for genetic manipulations in this species (Hsu et al., 2014 and Sander and Joung, 2014).

Neuroscience, Vol 320 (April 21, 2016): pg. 259-280. DOI.

Changes in circadian waveform under skeleton photoperiods and the phenomena of "phase jumps"

Under skeleton photoperiods, the full photophase is replaced with two short light pulses simulating light transitions at dusk and dawn (Fig. 4D). These conditions have ecological relevance for nocturnal rodents that primarily receive light exposure as they emerge from and return to darkened burrows. Skeleton photoperiods also serve as useful analytic tools for studying seasonal changes in pacemaker function, since long and short day lengths are simulated with equivalent light exposure. For the most part, entrainment under skeleton photoperiods resembles that elicited by full photoperiods, except when the former simulates very long day lengths (Pittendrigh and Daan, 1976a). A "phase jump" occurs under these conditions (Fig. 4D), where activity onset crosses one of the entraining light pulses and α realigns within the longer of the two available scotophases (Pittendrigh and Daan, 1976a, Rosenwasser et al., 1983, Stephan, 1983, Sharma et al., 1997 and Evans et al., 2005). In contrast, phase jumps rarely occur under comparable full photoperiods, suggesting that the continued light exposure contributes to stable entrainment under very long day lengths (Pittendrigh and Daan, 1976a and Pittendrigh and Daan, 1976b).

Previous models have largely accounted for phase jumps through an asymmetry in the phase delay and advance regions of the photic PRC (Pittendrigh and Daan, 1976a, Stephan, 1983 and Sharma et al., 1997). These early models, however, do not take into account photoperiod-induced changes in the amplitude of the photic PRC, where reduced phase shift magnitude correlates with decreases in α (Pohl, 1983, Pittendrigh et al., 1984, Pohl, 1984 and Shimomura and Menaker, 1994). Therefore, during entrainment to very long day lengths, like those simulated under skeleton photoperiods, light-induced phase shifts are markedly attenuated and less clearly able to generate phase jumps. Mutual coupling mechanisms may account for the emergence of phase jumps under skeleton photoperiods (Pittendrigh and Daan, 1976b and Evans et al., 2005). As α is compressed, a phase jump could emerge as φ_{EM} decreases through either a loss of period synchrony or by more direct repulsive interaction. After the initiation of the phase jump, φ_{EM} would increase,

Neuroscience, Vol 320 (April 21, 2016): pg. 259-280. DOI.

which would allow for synchrony and complete realignment into the alternative scotophase. As the alternative scotophase is typically longer than its counterpart, E and M can then adopt the φ_{EM} that is more conducive to stable entrainment. Very long day lengths incorporating full photophases may inhibit phase jumps by countering the oscillator interactions that instigate the jump. These photic effects may impose long-term changes in circadian function like that described above (e.g., NR phenotype in Siberian hamsters) by increasing τ_E and/or by changing φ_{EM}-dependent mutual coupling mechanisms. Further study of phase jumping may thus serve to characterize changes induced by exposure to simulated long day lengths and examine conceptual models of φ_{EM}-dependent interactions.

Forced desynchrony under non 24-h LD cycles

As discussed in the preceding sections, adaptive phase relationships of oscillators may be the product of coupling processes. But if pacemakers are subjected to non-ecological conditions (e.g., LD cycles markedly longer or shorter that 24 h), coupling may not be sufficient to maintain a coherent rhythm. Indeed, there exists a range of environmental frequencies with which the circadian pacemaker can resonate. The range of non-24-h LD cycles (i.e., T cycles, where T = period of the external cycle) to which a given species of mammals can synchronize can be predicted by τ and the photic PRC typical of that species. Further, a collection of oscillators marked by heterogeneous τ would be predicted to differentially entrain to non-24-h T cycles (Shinbrot and Scarbrough, 1999). For example, oscillators with τ close to T would be expected to entrain, whereas oscillators with τ markedly deviating from T would be expected to free-run. Long-term records of such rhythms would be predicted to have at least two dominant rhythms that beat in and out of phase with one another. Support for these predictions can be found in the activity rhythms of rats held under short T cycles (T21-T22), which is near the lower limit of entrainment for this species (Vilaplana et al., 1997a, Campuzano et al., 1998, Cambras et al., 2000 and Cambras et al., 2004). In these records, a non-entrained activity component with τ > 24 h appears to be superimposed onto another activity rhythm entrained to the lights-off transition of the T cycle. When the two activity bouts cross, they exhibit relative coordination (Schwartz et al., 2009). When T

approaches 24 h, this pattern is altered in that the entrained component becomes more robust and the non-entrained activity band diminishes. Measures of subsequent free-running rhythms and anticipatory activity preceding the lights-off transition indicate that these patterns of activity do not result solely from the masking effects of light. Instead, these "beating" patterns are thought to reflect that the master clock is partially entrained, which has been supported by investigations revealing that the SCN shell and core are desynchronized under these conditions (de la Iglesia et al., 2004a). Importantly, differential gene expression in these SCN regions appears to persist temporarily after release into DD, arguing against a pure masking effect by light and darkness. To date, the forced desynchrony paradigm has been used to provide new insight into the function of outputs specifically produced by SCN shell and core compartments (Lee et al., 2009, Schwartz et al., 2009, Smarr et al., 2012 and Wotus et al., 2013), as well as the propagation of resetting signals within the SCN network (Schwartz et al., 2010). It remains unclear if analogous behavior is observed in other species, but if the rat is unique in its expression of this behavior, then this may provide an interesting comparative approach for studying circuit properties that enable this form of plasticity. In addition, investigating the process of desynchronization and resynchronization using the forced desynchrony model may provide novel insight into SCN coupling mechanisms.

Bifurcated rhythms under 24-h LDLD cycles

Exposure to 24-h LDLD cycles generates bifurcated rhythms, where each activity bout is entrained to one of the daily dark periods (Fig. 4E). A variety of protocols using 24-h LDLD cycles rapidly induces bifurcated rhythms in hamsters and mice (Mrosovsky and Janik, 1993, Gorman, 2001, Gorman and Lee, 2001, Gorman and Elliott, 2003, Gorman and Elliott, 2004, Gorman et al., 2003 and Evans et al., 2005). The first procedure for generating bifurcated rhythms under LDLD cycles utilized scheduled exposure to novel wheel running (NWR) during the subjective daytime (Mrosovsky and Janik, 1993, Gorman and Lee, 2001 and Evans and Gorman, 2002). This protocol is also referred to as behavioral decoupling to acknowledge the role of scheduled wheel running and to distinguish it from LL-induced splitting (Mrosovsky and Janik, 1993). Comparable split rhythms also emerge

without NWR when animals are exposed to a LDLD cycle with two, short scotophases of equal duration (Gorman, 2001). Both activity bouts under LDLD correspond to physiological indices of subjective night (e.g., melatonin secretion and light-induced gene expression) and subjective night markers are absent during the intervening bouts of behavioral quiescence (Gorman et al., 2001b, Edelstein et al., 2003 and Raiewski et al., 2012). Moreover, LDLD-induced bifurcated rhythms rejoin after release into DD through a series of transients, suggesting that each LDLD activity bout is programed by a separate group of oscillators. Of interest, 24-h LD cycles with more than two scotophases induce multi-modal rhythms in rats (Vilaplana et al., 1997b) and hamsters (Fig. 4F). Several factors are postulated to contribute to the emergence of bifurcated rhythms under LDLD (Gorman et al., 2003 and Evans et al., 2005). First, robust novelty-induced wheel running is thought to shift a subset of oscillators into the daytime scotophase through nonphotic resetting (Mrosovsky and Janik, 1993, Gorman and Lee, 2001 and Evans and Gorman, 2002). Second, the short nighttime scotophase under LDLD challenges entrainment and may instigate a phase jump of a subset of oscillators into the daytime scotophase (Pittendrigh and Daan, 1976a, Stephan, 1983 and Evans et al., 2005). As postulated to occur under skeleton photoperiods, a phase jump may be instigated by a change in the interactions between constituent oscillators that cause component oscillators to repel one another. However, under LDLD, the alternative scotophase is not of sufficient length to complete the phase jump, which promotes a stably bifurcated state.

Formal and physiological data indicate that exposure to LDLD produces bifurcated rhythms that are distinct from LL-induced split rhythms. First, LDLD-induced bifurcated activity bouts rejoin upon transfer to LL, which will independently induce splitting within several weeks (Gorman, 2001). Further, there is no indication of a left-right asymmetry in the SCN during LDLD-induced bifurcation, rather light-induced *c-fos* and *Period* gene expression is observed throughout both lobes of the SCN (Gorman et al., 2001b, Edelstein et al., 2003, Watanabe et al., 2007 and Yan et al., 2010). Thus, there is reason to believe that LDLD-induced bifurcation arises from the dissociation of oscillators operating within each lobe of the SCN, although their precise location remains undetermined.

Neuroscience, Vol 320 (April 21, 2016): pg. 259-280. DOI.

Advancing the current working model of the SCN complex

Multiple clock populations within the SCN network

The classic multi-oscillator model first described by Colin Pittendrigh has received wide acclaim since its formal introduction. As described in the preceding sections, there is a large body of neurobiological evidence supporting the theory that the SCN contains multiple independent and heterogeneous oscillators. Analysis of SCN rhythmicity after exposure to changing environmental conditions indicates that the SCN network can be temporally reorganized in a variety of ways, with complex patterns sometimes emerging within the same paradigm. For instance, LL-induced splitting is associated with antiphase oscillations of the left and right SCN lobe, but also antiphase oscillations of shell and core regions within each lobe (Mason, 1991, de la Iglesia et al., 2000, de la Iglesia et al., 2003, Ohta et al., 2005, Yan et al., 2005 and Butler et al., 2012). Further, there is evidence that photoperiodic modulation of circadian waveform corresponds with changes in the phase relationships of SCN neurons within the rostral and caudal poles of the network, but also those within the shell and core compartments (Jagota et al., 2000, Hazlerigg et al., 2005, Inagaki et al., 2007, Naito et al., 2008, Yan and Silver, 2008, Brown and Piggins, 2009, Evans et al., 2013 and Myung et al., 2015). Lastly, forced desynchrony corresponds to dissociated rhythms in the shell and core compartments of the rat, which do not stably reorganize as in other behavioral paradigms (de la Iglesia et al., 2004a). The plurality of ways in which the SCN network can be rearranged suggests that multiple sub-populations exist. Intra- and inter-SCN coupling mechanisms are likely differentially sensitive to factors operating under these distinct behavioral paradigms, which warrants further study. Real-time imaging of molecular rhythmicity has proved to be an important technique for revealing subgroups of SCN neurons; however, it should be noted that ex vivo preparations usually reduce the complexity of the SCN network and therefore may not capture all aspects of its circuitry. Further technological advances that allow for real-time visualization of SCN function while the network is fully intact and integrated into the larger system is expected to provide important insight into network function (Hamel et al., 2015).

Neuroscience, Vol 320 (April 21, 2016): pg. 259-280. DOI.

The formal assays highlighted in this review may be exploited further to distinguish SCN neuronal subpopulations and define the processes by which they interact. If the objective is to localize functionally distinct oscillators within the SCN in order to study their connections, intrinsic differences, and interactions, the best paradigms with which to pursue this goal may be those involving photoperiodism, LDLD-induced bifurcation, and forced desynchrony, since these paradigms appear to disassociate functionally and anatomically distinct oscillators operating within each lobe of the SCN. Convergent analyses using these paradigms may elucidate distinct subgroups of oscillators that regulate overt periodicity and circadian waveform through their interactions. Furthermore, comparative studies are needed to investigate whether the coupling processes modulating circadian waveform are distinct for the different behavioral assays discussed in this review. Previous work using the Siberian hamster demonstrates a clear relationship between plasticity in circadian waveform across three specific behavioral assays (i.e., photoperiodic responsiveness, LDLD-induced bifurcation, and arrhythmia under constant dim lighting conditions), which suggests that these are regulated by a common underlying coupling mechanism (Evans et al., 2012a). In contrast, circadian plasticity in these three assays was not related to changes in circadian waveform that emerged immediately after release from entrained to constant conditions or during light-induced resetting transients. This pattern of results suggests that these latter behavioral assays may reflect the actions of coupling mechanisms that are distinct. Given the known species differences in the behavioral response to the behavioral assays discussed in this review, a comparative approach investigating SCN circuitry in non-murine species may be useful. Future studies may benefit from recently developed methods (Hsu et al., 2014 and Sander and Joung, 2014) that allow for genetic manipulations in non-murine animal models that display interesting behavioral phenotypes indicative of unique coupling of SCN oscillators (e.g., the hamster). Research incorporating both nocturnal and diurnal animal models is likewise of interest.

Influence of light on SCN coupling

Under each of the paradigms presented above, photic stimulation was a critical agent influencing circadian waveform. Under

unconventional photic conditions, bi-stability (LL, LDLD) or desynchronization (LL, non 24-h T cycles) can emerge depending on the intensity and/or duration of illumination (Pickard et al., 1993, Gorman et al., 2003, Gorman et al., 2005, Cambras et al., 2004, Gorman and Elliott, 2004 and Rosenthal et al., 2005). While bright light is necessary for splitting under LL, dim nighttime illumination appears to be a pivotal factor influencing circadian plasticity under short day photoperiods, skeleton photoperiods, simulated jetlag, LDLD, and non 24-h T cycles (Gorman and Elliott, 2004, Evans et al., 2005, Evans et al., 2009 and Gorman et al., 2005). Many questions remain about how light produces these effects. For example, does bright and dim light influence circadian plasticity by directly influencing mutual coupling mechanisms or does it alter the inherent period of SCN neurons? Do different subtypes of SCN neurons exhibit differences in their sensitivity and/or nature of responses to light? How is photic information being processed and propagated through the SCN network (Antle et al., 2003)?

While the present discussion focuses on changes in circadian waveform, the analytical paradigms presented in the preceding sections often produce changes in τ, commonly referred to as circadian aftereffects. For example, exposure to LL both lengthens overt τ and changes circadian waveform. Does light influence α and τ via a common mechanism or are these effects produced through distinct means? Simulations using mathematical models with separate parameters modulating the collective frequency and relative phase of coupled nonlinear oscillators suggest that either parameter can be used to synchronize a population of high frequency nonlinear oscillators (Shinbrot and Scarbrough, 1999). Behavioral and physiological studies addressing circadian responses to light may attempt to disentangle photic effects on τ and circadian waveform in order to further address the role of light in modulating circadian plasticity under the above paradigms.

Alternatives to the complex clock model

Over the years, alternative models to the complex clock have been proposed to account for specific behavioral phenotypes. Prominent among these alternative models are those that posit the

circadian system contains a "gate" that regulates the expression of overt rhythmicity and can be modulated by external conditions to affect circadian waveform. This type of model has been largely invoked to account for photoperiodic changes in circadian waveform, where short day photoperiods may lower the threshold of the gate and thereby produce the expansion of subjective night through seemingly independent periodicities of its onset and offset. Accordingly, short day NR animals could have more rigid thresholds; however, this model is less able to account for the phase angle of entrainment displayed by the majority of NR animals (i.e., locked to lights-on or lights-off). Mathematical models where overt rhythmicity is controlled by the actions of multiple oscillators with variable τ and gated by a threshold have been used to simulate a wide variety of circadian behaviors (de la Iglesia et al., 2004b, Enright, 1980a, Enright, 1980b and Shinbrot and Scarbrough, 1999). Under this premise, a population of oscillators with variable τ could form a highly precise, functional pacemaker through the collective actions of multiple, imprecise short-term oscillators. The emergent "neuronal" rhythm may be used to regulate output from the system through the electrical firing pattern of the SCN neurons themselves (Shinbrot and Scarbrough, 1999), or through the actions of a "discriminator" node that need not be inherently rhythmic (Enright, 1980b). In this latter model, direct interactions between individual oscillators are not necessary, if the discriminator can influence the period and/or phase of all the oscillators within the population (Enright, 1980a). This discriminator model may bear on the organization of the central pacemaker, being similar to one of the functions proposed for the calbindin-immunoreactive sub-nucleus within the hamster SCN (Antle et al., 2003). Thus, it stands to reason that alternative models should be considered as potentially relevant elements of SCN circuits, regardless of the importance and popularity of the complex SCN model.

Conclusions

The behavioral and physiological analyses highlighted in this review indicate that the central pacemaker is composed of a population of self-sufficient clocks that couple together to form a plastic network. The next step is to advance our understanding of the coupling mechanisms that govern pacemaker function and the circuitry

that supports this complex. This review presents models of SCN coupling that can be subjected to empirical tests. In particular, these coupling models posit that SCN neurons assume specific relationships through inherent differences in τ and/or φ_{EM}-dependent interactions. While previous experimental work has focused largely on putative coupling that synchronizes SCN neurons, the forms of circadian plasticity highlighted in this review can be conceptualized as either attractive (Fig. 2D) or repulsive coupling mechanisms (Fig. 2C). Whether "attractive" and "repulsive" coupling processes reflect distinct signaling mechanisms remains to be determined through empirical study, but it is of interest that similar processes have been employed in mathematical models of coupled, nonlinear oscillators (Shinbrot and Scarbrough, 1999). It will be important to gain a better understanding of the source and temporal patterning of coupling signals such as these, which can be tested effectively by synthesizing formal, physiological, and molecular analyses.

Acknowledgments

J.A.E. acknowledges support from NIH grant R01NS091234 and Whitehall Foundation grant 2014-12-65. M.R.G. acknowledges support from NSF grant IBN-0346391, NIH grant NICHD-36460, and ONR grant N00014-13-1-0285.

References

Abe et al., 1999. H. Abe, S. Honma, K. Honma, T. Suzuki, S. Ebihara. Functional diversities of two activity components of circadian rhythm in genetical splitting mice (CS strain). *J Comp Physiol A*, 184 (1999), pp. 243–251

Abe et al., 2001. H. Abe, S. Honma, M. Namihira, S. Masubuchi, K. Honma. Behavioural rhythm splitting in the CS mouse is related to clock gene expression outside the suprachiasmatic nucleus. *Eur J Neurosci*, 14 (2001), pp. 1121–1128

Abe et al., 2002. M. Abe, E.D. Herzog, S. Yamazaki, M. Straume, H. Tei, Y. Sakaki, M. Menaker, G.D. Block. Circadian rhythms in isolated brain regions. J Neurosci, 22 (2002), pp. 350–356

Abrahamson and Moore, 2001. E.E. Abrahamson, R.Y. Moore. Suprachiasmatic nucleus in the mouse: retinal innervation, intrinsic organization and efferent projections. *Brain Res*, 916 (2001), pp. 172–191

Aggelopoulos and Meissl, 2000. N.C. Aggelopoulos, H. Meissl. Responses of neurones of the rat suprachiasmatic nucleus to retinal illumination

under photopic and scotopic conditions. *J Physiol*, 523 (Pt 1) (2000), pp. 211–222

Albers et al., 1987. H.E. Albers, N. Minamitani, E. Stopa, C.F. Ferris. Light selectively alters vasoactive intestinal peptide and peptide histidine isoleucine immunoreactivity within the rat suprachiasmatic nucleus. *Brain Res*, 437 (1987), pp. 189–192

Albus et al., 2005. H. Albus, M.J. Vansteensel, S. Michel, G.D. Block, J.H. Meijer. A GABAergic mechanism is necessary for coupling dissociable ventral and dorsal regional oscillators within the circadian clock. *Curr Biol*, 15 (2005), pp. 886–893

An et al., 2013. S. An, R. Harang, K. Meeker, D. Granados-Fuentes, C.A. Tsai, C. Mazuski, J. Kim, F.J. Doyle 3rd, L.R. Petzold, E.D. Herzog. A neuropeptide speeds circadian entrainment by reducing intercellular synchrony. *Proc Natl Acad Sci*, 110 (2013), pp. E4355–E4361

Ananthasubramaniam et al., 2014. B. Ananthasubramaniam, E.D. Herzog, H. Herzel. Timing of neuropeptide coupling determines synchrony and entrainment in the mammalian circadian clock. *PLoS Comput Biol*, 10 (2014), p. e1003565

Antle and Silver, 2005. M.C. Antle, R. Silver. Orchestrating time: arrangements of the brain circadian clock. *Trends Neurosci*, 28 (2005), pp. 145–151

Antle et al., 2003. M.C. Antle, D.K. Foley, N.C. Foley, R. Silver. Gates and oscillators: a network model of the brain clock. *J Biol Rhythms*, 18 (2003), pp. 339–350

Aschoff, 1960. J. Aschoff. Exogenous and endogenous components in circadian rhythms. *Cold Spring Harb Symp Quant Biol*, 25 (1960), pp. 11–28

Aschoff, 1965. J. Aschoff. Circadian rhythms in man. *Science*, 148 (1965), pp. 1427–1432

Aton and Herzog, 2005. S.J. Aton, E.D. Herzog. Come together, right...now: synchronization of rhythms in a mammalian circadian clock. *Neuron*, 48 (2005), pp. 531–534

Aton et al., 2005. S.J. Aton, C.S. Colwell, A.J. Harmar, J. Waschek, E.D. Herzog. Vasoactive intestinal polypeptide mediates circadian rhythmicity and synchrony in mammalian clock neurons. *Nat Neurosci*, 8 (2005), pp. 476–483

Balsalobre, 2002. A. Balsalobre. Clock genes in mammalian peripheral tissues. *Cell Tissue Res*, 309 (2002), pp. 193–199

Bechtold et al., 2008. D.A. Bechtold, T.M. Brown, S.M. Luckman, H.D. Piggins. Metabolic rhythm abnormalities in mice lacking VIP-VPAC2 signaling. *Am J Physiol*, 294 (2008), pp. R344–R351

Bedont et al., 2014. J.L. Bedont, T.A. LeGates, E.A. Slat, M.S. Byerly, H. Wang, J. Hu, A.C. Rupp, J. Qian, G.W. Wong, E.D. Herzog, S. Hattar,

S. Blackshaw. *Lhx1* controls terminal differentiation and circadian function of the suprachiasmatic nucleus. *Cell Rep*, 7 (2014), pp. 609–622

Bennett et al., 1991. M.V. Bennett, L.C. Barrio, T.A. Bargiello, D.C. Spray, E. Hertzberg, J.C. Saez. Gap junctions: new tools, new answers, new questions. *Neuron*, 6 (1991), pp. 305–320

Berson, 2003. D.M. Berson. Strange vision: ganglion cells as circadian photoreceptors. *Trends Neurosci*, 26 (2003), pp. 314–320

Binkley and Mosher, 1986. S. Binkley, K. Mosher. Photoperiod modifies circadian resetting responses in sparrows. *Am J Physiol*, 251 (1986), pp. R1156–R1162

Boulos and Morin, 1985. Z. Boulos, L.P. Morin. Entrainment of split circadian activity rhythms in hamsters. *J Biol Rhythms*, 1 (1985), pp. 1–15

Boulos and Rusak, 1982. Z. Boulos, B. Rusak. Phase response curves and the dual oscillator model of circadian pacemakers. J. Aschoff (Ed.), *et al.*, *Vertebrate circadian systems*, Springer-Verlag, Berlin Heidelberg (1982), pp. 215–223

Bouskila and Dudek, 1993. Y. Bouskila, F.E. Dudek. Neuronal synchronization without calcium-dependent synaptic transmission in the hypothalamus. *Proc Natl Acad Sci*, 90 (1993), pp. 3207–3210

Brown and Piggins, 2009. T.M. Brown, H.D. Piggins. Spatiotemporal heterogeneity in the electrical activity of suprachiasmatic nuclei neurons and their response to photoperiod. *J Biol Rhythms*, 24 (2009), pp. 44–54

Brown and Robinson, 2004. R.L. Brown, P.R. Robinson. Melanopsin – shedding light on the elusive circadian photopigment. *Chronobiol Int*, 21 (2004), pp. 189–204

Brown et al., 2005. T.M. Brown, A.T. Hughes, H.D. Piggins. Gastrin-releasing peptide promotes suprachiasmatic nuclei cellular rhythmicity in the absence of vasoactive intestinal polypeptide-VPAC2 receptor signaling. *J Neurosci*, 25 (2005), pp. 11155–11164

Brown et al., 2006. T.M. Brown, J.R. Banks, H.D. Piggins. A novel suction electrode recording technique for monitoring circadian rhythms in single and multiunit discharge from brain slices. *J Neurosci Methods*, 156 (2006), pp. 173–181

Brown et al., 2007. T.M. Brown, C.S. Colwell, J.A. Waschek, H.D. Piggins. Disrupted neuronal activity rhythms in the suprachiasmatic nuclei of vasoactive intestinal polypeptide-deficient mice. *J Neurophysiol*, 97 (2007), pp. 2553–2558

Brown et al., 2011. T.M. Brown, J. Wynne, H.D. Piggins, R.J. Lucas. Multiple hypothalamic cell populations encoding distinct visual information. *J Physiol*, 589 (2011), pp. 1173–1194

Buhr and Takahashi, 2013. E.D. Buhr, J.S. Takahashi. Molecular components of the mammalian circadian clock. *Handb Exp Pharmacol*, 217 (2013), pp. 3–27

Buijs and Kalsbeek, 2001. R.M. Buijs, A. Kalsbeek. Hypothalamic integration of central and peripheral clocks. *Nat Rev Neurosci*, 2 (2001), pp. 521–526

Butler et al., 2012. M.P. Butler, M.N. Rainbow, E. Rodriguez, S.M. Lyon, R. Silver. Twelve-hour days in the brain and behavior of split hamsters. *Eur J Neurosci*, 36 (2012), pp. 2556–2566

Cambras et al., 2000. T. Cambras, J. Vilaplana, A. Campuzano, M.M. Canal-Corretger, M. Carulla, A. Diez-Noguera. Entrainment of the rat motor activity rhythm: effects of the light-dark cycle and physical exercise. *Physiol Behav*, 70 (2000), pp. 227–232

Cambras et al., 2004. T. Cambras, J. Chiesa, J. Araujo, A. Diez-Noguera. Effects of photoperiod on rat motor activity rhythm at the lower limit of entrainment. *J Biol Rhythms*, 19 (2004), pp. 216–225

Campuzano et al., 1998. A. Campuzano, J. Vilaplana, T. Cambras, A. Diez-Noguera. Dissociation of the rat motor activity rhythm under T cycles shorter than 24 hours. *Physiol Behav*, 63 (1998), pp. 171–176

Cassone et al., 1988. V.M. Cassone, J.C. Speh, J.P. Card, R.Y. Moore. Comparative anatomy of the mammalian hypothalamic suprachiasmatic nucleus. *J Biol Rhythms*, 3 (1988), pp. 71–91

Cheung and McCormack, 1983. P.W. Cheung, C.E. McCormack. Splitting of the locomotor activity rhythm in rats by exposure to continuous light. *Am J Physiol*, 244 (1983), pp. H573–H576

Ciarleglio et al., 2009. C.M. Ciarleglio, K.L. Gamble, J.C. Axley, B.R. Strauss, J.Y. Cohen, C.S. Colwell, D.G. McMahon. Population encoding by circadian clock neurons organizes circadian behavior. *J Neurosci*, 29 (2009), pp. 1670–1676

Colwell, 2000. C.S. Colwell. Rhythmic coupling among cells in the suprachiasmatic nucleus. *J Neurobiol*, 43 (2000), pp. 379–388

Colwell et al., 2003. C.S. Colwell, S. Michel, J. Itri, W. Rodriguez, J. Tam, V. Lelievre, Z. Hu, X. Liu, J.A. Waschek. Disrupted circadian rhythms in VIP- and PHI-deficient mice. *Am J Physiol*, 285 (2003), pp. R939–R949

Connors and Long, 2004. B.W. Connors, M.A. Long. Electrical synapses in the mammalian brain. *Annu Rev Neurosci*, 27 (2004), pp. 393–418

Daan and Berde, 1978. S. Daan, C. Berde. Two coupled oscillators: simulations of the circadian pacemaker in mammalian activity rhythms. *J Theor Biol*, 70 (1978), pp. 297–313

Dardente et al., 2002. H. Dardente, V.J. Poirel, P. Klosen, P. Pevet, M. Masson-Pevet. *Per* and neuropeptide expression in the rat suprachiasmatic nuclei: compartmentalization and differential cellular induction by light. *Brain Res*, 958 (2002), pp. 261–271

Dardente et al., 2004. H. Dardente, J.S. Menet, E. Challet, B.B. Tournier, P. Pevet, M. Masson-Pevet. Daily and circadian expression of neuropeptides in the suprachiasmatic nuclei of nocturnal and diurnal rodents. *Brain Res Mol Brain Res*, 124 (2004), pp. 143–151

Davidson, 2006. A.J. Davidson. Search for the feeding-entrainable circadian oscillator: a complex proposition. *Am J Physiol*, 290 (2006), pp. R1524–R1526

Davidson et al., 2009. A.J. Davidson, O. Castanon-Cervantes, T.L. Leise, P.C. Molyneux, M.E. Harrington. Visualizing jet lag in the mouse suprachiasmatic nucleus and peripheral circadian timing system. *Eur J Neurosci*, 29 (2009), pp. 171–180

Davis and Gorski, 1984. F.C. Davis, R.A. Gorski. Unilateral lesions of the hamster suprachiasmatic nuclei: evidence for redundant control of circadian rhythms. *J Comp Physiol*, 154 (1984), pp. 221–232

Davis and Viswanathan, 1996. F.C. Davis, N. Viswanathan. The effect of transplanting one or two suprachiasmatic nuclei on the period of the restored rhythm. *J Biol Rhythms*, 11 (1996), pp. 291–301

de la Iglesia et al., 2000. H.O. de la Iglesia, J. Meyer, A. Carpino Jr., W.J. Schwartz. Antiphase oscillation of the left and right suprachiasmatic nuclei. *Science*, 290 (2000), pp. 799–801

de la Iglesia et al., 2003. H.O. de la Iglesia, J. Meyer, W.J. Schwartz. Lateralization of circadian pacemaker output: activation of left- and right-sided luteinizing hormone-releasing hormone neurons involves a neural rather than a humoral pathway. *J Neurosci*, 23 (2003), pp. 7412–7414

de la Iglesia et al., 2004a. H.O. de la Iglesia, T. Cambras, W.J. Schwartz, A. Diez-Noguera. Forced desynchronization of dual circadian oscillators within the rat suprachiasmatic nucleus. *Curr Biol*, 14 (2004), pp. 796–800

de la Iglesia et al., 2004b. H.O. de la Iglesia, J. Meyer, W.J. Schwartz. Using *Per* gene expression to search for photoperiodic oscillators in the hamster suprachiasmatic nucleus. *Brain Res Mol Brain Res*, 127 (2004), pp. 121–127

DeWoskin et al., 2015. D. DeWoskin, J. Myung, M.D. Belle, H.D. Piggins, T. Takumi, D.B. Forger. Distinct roles for GABA across multiple timescales in mammalian circadian timekeeping. *Proc Natl Acad Sci*, 112 (2015), pp. E3911–E3919

Dudek et al., 1993. F.E. Dudek, Y.I. Kim, Y. Bouskila. Electrophysiology of the suprachiasmatic nucleus: synaptic transmission, membrane properties, and neuronal synchronization. *J Biol Rhythms*, 8 (Suppl) (1993), pp. S33–S37

Earnest and Turek, 1982. D.J. Earnest, F.W. Turek. Splitting of the circadian rhythm of activity in hamsters: effects of exposure to constant

darkness and subsequent re-exposure to constant light. *J Comp Physiol A*, 145 (1982), pp. 405–411

Edelstein et al., 2003. K. Edelstein, H.O. de la Iglesia, N. Mrosovsky. *Period* gene expression in the suprachiasmatic nucleus of behaviorally decoupled hamsters. *Brain Res Mol Brain Res*, 114 (2003), pp. 40–45

Elliott and Tamarkin, 1994. J. Elliott, L. Tamarkin. Complex circadian regulation of pineal melatonin and wheel-running in Syrian hamsters. *J Comp Physiol A*, 174 (1994), pp. 469–484

Enright, 1980a. J.T. Enright. Temporal precision in circadian systems: a reliable neuronal clock from unreliable components? *Science*, 209 (1980), pp. 1542–1545

Enright, 1980b. J.T. Enright. *The timing of sleep and wakefulness: on the substructure and dynamics of circadian pacemakers underlying the circadian sleep-wake cycle*. Springer-Verlang Berlin Heidelberg, Berlin, Germany (1980)

Evans and Gorman, 2002. J.A. Evans, M.R. Gorman. Split circadian rhythms of female Syrian hamsters and their offspring. *Physiol Behav*, 76 (2002), pp. 469–478

Evans et al., 2005. J.A. Evans, J.A. Elliott, M.R. Gorman. Circadian entrainment and phase resetting differ markedly under dimly illuminated versus completely dark nights. *Behav Brain Res*, 162 (2005), pp. 116–126

Evans et al., 2007. J.A. Evans, J.A. Elliott, M.R. Gorman. Circadian effects of light no brighter than moonlight. *J Biol Rhythms*, 22 (2007), pp. 356–367

Evans et al., 2009. J.A. Evans, J.A. Elliott, M.R. Gorman. Dim nighttime illumination accelerates adjustment to timezone travel in an animal model. *Curr Biol*, 19 (2009), pp. R156–R157

Evans et al., 2011. J.A. Evans, T.L. Leise, O. Castanon-Cervantes, A.J. Davidson. Intrinsic regulation of spatiotemporal organization within the suprachiasmatic nucleus. *PLoS One*, 6 (2011), p. e15869

Evans et al., 2012a. J.A. Evans, J.A. Elliott, M.R. Gorman. Individual differences in circadian waveform of Siberian hamsters under multiple lighting conditions. *J Biol Rhythms*, 27 (2012), pp. 410–419

Evans et al., 2012b. J.A. Evans, H. Pan, A.C. Liu, D.K. Welsh. *Cry1-/-* circadian rhythmicity depends on SCN intercellular coupling. *J Biol Rhythms*, 27 (2012), pp. 443–452

Evans et al., 2013. J.A. Evans, T.L. Leise, O. Castanon-Cervantes, A.J. Davidson. Dynamic interactions mediated by nonredundant signaling mechanisms couple circadian clock neurons. *Neuron*, 80 (2013), pp. 973–983

Evans et al., 2015. J.A. Evans, T.C. Suen, B.L. Callif, A.S. Mitchell, O. Castanon-Cervantes, K.M. Baker, I. Kloehn, K. Baba, B.J. Teubner,

J.C. Ehlen, K.N. Paul, T.J. Bartness, G. Tosini, T. Leise, A.J. Davidson. Shell neurons of the master circadian clock coordinate the phase of tissue clocks throughout the brain and body. *BMC Biol*, 13 (2015), p. 43

Fahrenkrug et al., 2012. J. Fahrenkrug, B. Georg, J. Hannibal, H.L. Jorgensen. Altered rhythm of adrenal clock genes, StAR and serum corticosterone in VIP receptor 2-deficient mice. *J Mol Neurosci*, 48 (2012), pp. 584–596

Farajnia et al., 2014. S. Farajnia, T.L. van Westering, J.H. Meijer, S. Michel. Seasonal induction of GABAergic excitation in the central mammalian clock. *Proc Natl Acad Sci*, 111 (2014), pp. 9627–9632

Freeman and Goldman, 1997. D.A. Freeman, B.D. Goldman. Evidence that the circadian system mediates photoperiodic nonresponsiveness in Siberian hamsters: the effect of running wheel access on photoperiodic responsiveness. *J Biol Rhythms*, 12 (1997), pp. 100–109

Freeman et al., 2013. G.M. Freeman Jr., R.M. Krock, S.J. Aton, P. Thaben, E.D. Herzog. GABA networks destabilize genetic oscillations in the circadian pacemaker. *Neuron*, 78 (2013), pp. 799–806

Gallardo et al., 2014. C.M. Gallardo, M. Darvas, M. Oviatt, C.H. Chang, M. Michalik, T.F. Huddy, E.E. Meyer, S.A. Shuster, A. Aguayo, E.M. Hill, K. Kiani, J. Ikpeazu, J.S. Martinez, M. Purpura, A.N. Smit, D.F. Patton, R.E. Mistlberger, R.D. Palmiter, A.D. Steele. Dopamine receptor 1 neurons in the dorsal striatum regulate food anticipatory circadian activity rhythms in mice. *eLife*, 3 (2014), p. e03781

Gamble et al., 2007. K.L. Gamble, G.C. Allen, T. Zhou, D.G. McMahon. Gastrin-releasing peptide mediates light-like resetting of the suprachiasmatic nucleus circadian pacemaker through cAMP response element-binding protein and *Per1* activation. *J Neurosci*, 27 (2007), pp. 12078–12087

Goldman, 2001. B.D. Goldman. Mammalian photoperiodic system: formal properties and neuroendocrine mechanisms of photoperiodic time measurement. *J Biol Rhythms*, 16 (2001), pp. 283–301

Goldman and Goldman, 2003. S.L. Goldman, B.D. Goldman. Early photoperiod history and short-day responsiveness in Siberian hamsters. *J Exp Zool A Comp Exp Biol*, 296 (2003), pp. 38–45

Goldman et al., 2000. S.L. Goldman, K. Dhandapani, B.D. Goldman. Genetic and environmental influences on short-day responsiveness in Siberian hamsters (*Phodopus sungorus*). *J Biol Rhythms*, 15 (2000), pp. 417–428

Gooley et al., 2003. J.J. Gooley, J. Lu, D. Fischer, C.B. Saper. A broad role for melanopsin in nonvisual photoreception. *J Neurosci*, 23 (2003), pp. 7093–7106

Gorman, 2001. M.R. Gorman. Exotic photoperiods induce and entrain split circadian activity rhythms in hamsters. *J Comp Physiol A*, 187 (2001), pp. 793–800

Gorman and Elliott, 2003. M.R. Gorman, J.A. Elliott. Entrainment of 2 subjective nights by daily light:dark:light:dark cycles in 3 rodent species. *J Biol Rhythms*, 18 (2003), pp. 502–512

Gorman and Elliott, 2004. M.R. Gorman, J.A. Elliott. Dim nocturnal illumination alters coupling of circadian pacemakers in Siberian hamsters, *Phodopus sungorus. J Comp Physiol A*, 190 (2004), pp. 631–639

Gorman and Lee, 2001. M.R. Gorman, T.M. Lee. Daily novel wheel running reorganizes and splits hamster circadian activity rhythms. *J Biol Rhythms*, 16 (2001), pp. 541–551

Gorman and Zucker, 1997 .M.R. Gorman, I. Zucker. Environmental induction of photononresponsiveness in the Siberian hamster, *Phodopus sungorus. Am J Physiol*, 272 (1997), pp. R887–R895

Gorman et al., 1997. M.R. Gorman, D.A. Freeman, I. Zucker. Photoperiodism in hamsters: abrupt versus gradual changes in day length differentially entrain morning and evening circadian oscillators. *J Biol Rhythms*, 12 (1997), pp. 122–135

Gorman et al., 2001a. M.R. Gorman, B.D. Goldman, I. Zucker. Mammalian photoperiodism. J.S. Takahashi (Ed.), *et al.*, *Handbook of behavioral neurobiology: circadian clocks*, Vol. 12, Kluwer Academic/Plenum Publishers, New York (2001), pp. 481–508

Gorman et al., 2001b. M.R. Gorman, S.M. Yellon, T.M. Lee. Temporal reorganization of the suprachiasmatic nuclei in hamsters with split circadian rhythms. *J Biol Rhythms*, 16 (2001), pp. 552–563

Gorman et al., 2003. M.R. Gorman, J.A. Elliott, J.A. Evans. Plasticity of hamster circadian entrainment patterns depends on light intensity. *Chronobiol Int*, 20 (2003), pp. 233–248

Gorman et al., 2005. M.R. Gorman, M.E. Kendall, J.A. Elliott. Scotopic illumination enhances entrainment of circadian rhythms to lengthening light:dark cycles. *J Biol Rhythms*, 20 (2005), pp. 38–48

Granados-Fuentes et al., 2004. D. Granados-Fuentes, L.M. Prolo, U. Abraham, E.D. Herzog. The suprachiasmatic nucleus entrains, but does not sustain, circadian rhythmicity in the olfactory bulb. *J Neurosci*, 24 (2004), pp. 615–619

Hamel et al., 2015. E.J. Hamel, B.F. Grewe, J.G. Parker, M.J. Schnitzer. Cellular level brain imaging in behaving mammals: an engineering approach. *Neuron*, 86 (2015), pp. 140–159

Han et al., 2012. S. Han, F.H. Yu, M.D. Schwartz, J.D. Linton, M.M. Bosma, J.B. Hurley, W.A. Catterall, H.O. de la Iglesia. Na(V)1.1 channels are critical for intercellular communication in the suprachiasmatic nucleus

and for normal circadian rhythms. *Proc Natl Acad Sci*, 109 (2012), pp. E368–E377

Hannibal et al., 2011. J. Hannibal, H.M. Hsiung, J. Fahrenkrug. Temporal phasing of locomotor activity, heart rate rhythmicity, and core body temperature is disrupted in VIP receptor 2-deficient mice. *Am J Physiol*, 300 (2011), pp. R519–R530

Harmar et al., 2002. A.J. Harmar, H.M. Marston, S. Shen, C. Spratt, K.M. West, W.J. Sheward, C.F. Morrison, J.R. Dorin, H.D. Piggins, J.C. Reubi, J.S. Kelly, E.S. Maywood, M.H. Hastings. The VPAC(2) receptor is essential for circadian function in the mouse suprachiasmatic nuclei. *Cell*, 109 (2002), pp. 497–508

Harrington et al., 1990. M.E. Harrington, G.A. Eskes, P. Dickson, B. Rusak. Lesions dorsal to the suprachiasmatic nuclei abolish split activity rhythms of hamsters. *Brain Res Bull*, 24 (1990), pp. 593–597

Harrison and Gorman, 2012. E.M. Harrison, M.R. Gorman. Changing the waveform of circadian rhythms: considerations for shift-work. *Front Neurol*, 3 (2012), p. 72

Hastings et al., 1987. M.H. Hastings, A.P. Walker, J. Herbert. Effect of asymmetrical reductions of photoperiod on pineal melatonin, locomotor activity and gonadal condition of male Syrian hamsters. *J Endocrinol*, 114 (1987), pp. 221–229

Hattar et al., 2003. S. Hattar, R.J. Lucas, N. Mrosovsky, S. Thompson, R.H. Douglas, M.W. Hankins, J. Lem, M. Biel, F. Hofmann, R.G. Foster, K.W. Yau. Melanopsin and rod-cone photoreceptive systems account for all major accessory visual functions in mice. *Nature*, 424 (2003), pp. 76–81

Hazlerigg et al., 2005. D.G. Hazlerigg, F.J. Ebling, J.D. Johnston. Photoperiod differentially regulates gene expression rhythms in the rostral and caudal SCN. *Curr Biol*, 15 (2005), pp. R449–R450

Herzog et al., 1997. E.D. Herzog, M.E. Geusz, S.B. Khalsa, M. Straume, G.D. Block. Circadian rhythms in mouse suprachiasmatic nucleus explants on multimicroelectrode plates. *Brain Res*, 757 (1997), pp. 285–290

Honma and Hiroshige, 1978. K.I. Honma, T. Hiroshige. Endogenous ultradian rhythms in rats exposed to prolonged continuous light. *Am J Physiol*, 235 (1978), pp. R250–R256

Honma et al., 1985. K. Honma, S. Honma, T. Hiroshige. Response curve, free-running period, and activity time in circadian locomotor rhythm of rats. *Jpn J Physiol*, 35 (1985), pp. 643–658

Honma et al., 1989. S. Honma, K. Honma, T. Hiroshige. Methamphetamine induced locomotor rhythm entrains to restricted daily feeding in SCN lesioned rats. *Physiol Behav*, 45 (1989), pp. 1057–1065

Hsu et al., 2014. P.D. Hsu, E.S. Lander, F. Zhang. Development and applications of CRISPR-Cas9 for genome engineering. *Cell*, 157 (2014), pp. 1262–1278

Hu et al., 2011. W.P. Hu, J.D. Li, C.S. Colwell, Q.Y. Zhou. Decreased REM sleep and altered circadian sleep regulation in mice lacking vasoactive intestinal polypeptide. *Sleep*, 34 (2011), pp. 49–56

Hughes et al., 2008. A.T. Hughes, C. Guilding, L. Lennox, R.E. Samuels, D.G. McMahon, H.D. Piggins. Live imaging of altered *period1* expression in the suprachiasmatic nuclei of *Vipr2-/-* mice. *J Neurochem*, 106 (2008), pp. 1646–1657

Hughes et al., 2015. A.T. Hughes, C.L. Croft, R.E. Samuels, J. Myung, T. Takumi, H.D. Piggins. Constant light enhances synchrony among circadian clock cells and promotes behavioral rhythms in VPAC2-signaling deficient mice. *Sci Rep*, 5 (2015), p. 14044

Humlova and Illnerova, 1992. M. Humlova, H. Illnerova. Resetting of the rat circadian clock after a shift in the light/dark cycle depends on the photoperiod. *Neurosci Res*, 13 (1992), pp. 147–153

Illnerova, 1991. H. Illnerova. The suprachiasmatic nucleus and rhythmic pineal melatonin production. D.C. Kelin (Ed.), *et al.*, *Suprachiasmatic nucleus: the mind's clock*, University Oxford Press, New York (1991), pp. 197–216

Illnerova and Sumova, 1997. H. Illnerova, A. Sumova. Photic entrainment of the mammalian rhythm in melatonin production. *J Biol Rhythms*, 12 (1997), pp. 547–555

Illnerova and Vanecek, 1987. H. Illnerova, J. Vanecek. Dynamics of discrete entrainment of the circadian rhythm in the rat pineal N-acetyltransferase activity during transient cycles. *J Biol Rhythms*, 2 (1987), pp. 95–108

Inagaki et al., 2007. N. Inagaki, S. Honma, D. Ono, Y. Tanahashi, K. Honma. Separate oscillating cell groups in mouse suprachiasmatic nucleus couple photoperiodically to the onset and end of daily activity. *Proc Natl Acad Sci*, 104 (2007), pp. 7664–7669

Isobe and Nishino, 1998. Y. Isobe, H. Nishino. AVP rhythm in the suprachiasmatic nucleus in relation to locomotor activity under constant light. *Peptides*, 19 (1998), pp. 827–832

Itri et al., 2004. J. Itri, S. Michel, J.A. Waschek, C.S. Colwell. Circadian rhythm in inhibitory synaptic transmission in the mouse suprachiasmatic nucleus. *J Neurophysiol*, 92 (2004), pp. 311–319

Izhikevich, 2007. E.M. Izhikevich. Synchronization. E.M. Izhikevich (Ed.), *Dynamical systems in neuroscience: the geometry of excitability and bursting*, MIT Press, Cambridge, MA (2007), pp. 443–505

Jac et al., 2000. M. Jac, A. Sumova, H. Illnerova. C-Fos rhythm in subdivisions of the rat suprachiasmatic nucleus under artificial and natural photoperiods. *Am J Physiol*, 279 (2000), pp. R2270–R2276

Jagota et al., 2000. A. Jagota, H.O. de la Iglesia, W.J. Schwartz. Morning and evening circadian oscillations in the suprachiasmatic nucleus *in vitro. Nat Neurosci*, 3 (2000), pp. 372–376

Jiang et al., 1997. Z.-G. Jiang, Y.-Q. Yang, C.N. Allen. Tracer and electrical coupling of rat suprachiasmatic nucleus neurons. *Neuroscience*, 77 (1997), pp. 1059–1066

Jiao et al., 1999. Y.Y. Jiao, T.M. Lee, B. Rusak. Photic responses of suprachiasmatic area neurons in diurnal degus (*Octodon degus*) and nocturnal rats (*Rattus norvegicus*). *Brain Res*, 817 (1999), pp. 93–103

Jobst and Allen, 2002. E.E. Jobst, C.N. Allen. Calbindin neurons in the hamster suprachiasmatic nucleus do not exhibit a circadian variation in spontaneous firing rate. *Eur J Neurosci*, 16 (2002), pp. 2469–2474

Jobst et al., 2004. E.E. Jobst, D.W. Robinson, C.N. Allen. Potential pathways for intercellular communication within the calbindin subnucleus of the hamster suprachiasmatic nucleus. *Neuroscience*, 123 (2004), pp. 87–99

Johnson, 1999. C.H. Johnson. Forty years of PRCs – what have we learned? *Chronobiol Int*, 16 (1999), pp. 711–743

Johnston et al., 2005. J.D. Johnston, F.J. Ebling, D.G. Hazlerigg. Photoperiod regulates multiple gene expression in the suprachiasmatic nuclei and pars tuberalis of the Siberian hamster (*Phodopus sungorus*). *Eur J Neurosci*, 21 (2005), pp. 2967–2974

Kalsbeek and Buijs, 2002. A. Kalsbeek, R.M. Buijs. Output pathways of the mammalian suprachiasmatic nucleus: coding circadian time by transmitter selection and specific targeting. *Cell Tissue Res*, 309 (2002), pp. 109–118

Kalsbeek et al., 2010. A. Kalsbeek, E. Fliers, M.A. Hofman, D.F. Swaab, R.M. Buijs. Vasopressin and the output of the hypothalamic biological clock. *J Neuroendocrino*l, 22 (2010), pp. 362–372

Kawato and Suzuki, 1980. M. Kawato, R. Suzuki. Two coupled neural oscillators as a model of the circadian pacemaker. *J Theor Biol*, 86 (1980), pp. 547–575

King et al., 2003. V.M. King, S. Chahad-Ehlers, S. Shen, A.J. Harmar, E.S. Maywood, M.H. Hastings. A hVIPR transgene as a novel tool for the analysis of circadian function in the mouse suprachiasmatic nucleus. *Eur J Neurosci*, 17 (2003), pp. 822–832

Klein et al., 1991. D.C. Klein, R.Y. Moore, S.M. Reppert (Eds.), *Suprachiasmatic nucleus: the mind's clock*, University Oxford Press, New York (1991)

Kuhlman et al., 2003. S.J. Kuhlman, R. Silver, J. Le Sauter, A. Bult-Ito, D.G. McMahon. Phase resetting light pulses induce *Per1* and persistent spike activity in a subpopulation of biological clock neurons. *J Neurosci*, 23 (2003), pp. 1441–1450

Lax et al., 1998. P. Lax, S. Zamora, J.A. Madrid. Coupling effect of locomotor activity on the rat's circadian system. *Am J Physiol*, 275 (1998), pp. R580–R587

Lee et al., 2003. H.S. Lee, J.L. Nelms, M. Nguyen, R. Silver, M.N. Lehman. The eye is necessary for a circadian rhythm in the suprachiasmatic nucleus. *Nat Neurosci*, 6 (2003), pp. 111–112

Lee et al., 2009. M.L. Lee, B.E. Swanson, H.O. de la Iglesia. Circadian timing of REM sleep is coupled to an oscillator within the dorsomedial suprachiasmatic nucleus. *Curr Biol*, 19 (2009), pp. 848–852

Lee et al., 2013. J.E. Lee, L. Zamdborg, B.R. Southey, N. Atkins Jr., J.W. Mitchell, M. Li, M.U. Gillette, N.L. Kelleher, J.V. Sweedler. Quantitative peptidomics for discovery of circadian-related peptides from the rat suprachiasmatic nucleus. *Journal of Proteome Research*, 12 (2013), pp. 585–593

Lee et al., 2015. I.T. Lee, A.S. Chang, M. Manandhar, Y. Shan, J. Fan, M. Izumo, Y. Ikeda, T. Motoike, S. Dixon, J.E. Seinfeld, J.S. Takahashi, M. Yanagisawa. Neuromedin s-producing neurons act as essential pacemakers in the suprachiasmatic nucleus to couple clock neurons and dictate circadian rhythms. *Neuron*, 85 (2015), pp. 1086–1102

Lees et al., 1983. J.G. Lees, J.D. Hallonquist, N. Mrosovsky. Differential effects of dark pulses on the two components of split circadian activity rhythms in golden hamsters. *J Comp Physiol*, 153 (1983), pp. 123–132

Liu and Reppert, 2000. C. Liu, S.M. Reppert. GABA synchronizes clock cells within the suprachiasmatic circadian clock. *Neuron*, 25 (2000), pp. 123–128

Liu et al., 1997. C. Liu, D.R. Weaver, S.H. Strogatz, S.M. Reppert. Cellular construction of a circadian clock: Period determination in the suprachiasmatic nuclei. *Cell*, 91 (1997), pp. 855–860

Loh et al., 2011. D.H. Loh, J.M. Dragich, T. Kudo, A.M. Schroeder, T.J. Nakamura, J.A. Waschek, G.D. Block, C.S. Colwell. Effects of vasoactive intestinal peptide genotype on circadian gene expression in the suprachiasmatic nucleus and peripheral organs. J Biol Rhythms, 26 (2011), pp. 200–209

Loh et al., 2014. D.H. Loh, D.A. Kuljis, L. Azuma, Y. Wu, D. Truong, H.B. Wang, C.S. Colwell. Disrupted reproduction, estrous cycle, and circadian rhythms in female mice deficient in vasoactive intestinal peptide. *J Biol Rhythms*, 29 (2014), pp. 355–369

Lokshin et al., 2015. M. Lokshin, J. LeSauter, R. Silver. Selective distribution of retinal input to mouse SCN revealed in analysis of sagittal sections. *J Biol Rhythms*, 30 (2015), pp. 251–257

Long et al., 2005. M.A. Long, M.J. Jutras, B.W. Connors, R.D. Burwell. Electrical synapses coordinate activity in the suprachiasmatic nucleus. *Nat Neurosci*, 8 (2005), pp. 61–66

Lucassen et al., 2012. E.A. Lucassen, H.C. van Diepen, T. Houben, S. Michel, C.S. Colwell, J.H. Meijer. Role of vasoactive intestinal peptide in seasonal encoding by the suprachiasmatic nucleus clock. *Eur J Neurosci*, 35 (2012), pp. 1466–1474

Mackey, 2007. S.R. Mackey. Biological rhythms workshop IA: molecular basis of rhythms generation. *Cold Spring Harb Symp Quant Biol*, 72 (2007), pp. 7–19

Margraf and Lynch, 1993. R.R. Margraf, G.R. Lynch. Melatonin injections affect circadian behavior and SCN neurophysiology in Djungarian hamsters. *Am J Physiol*, 264 (1993), pp. R615–R621

Margraf et al., 1991. R.R. Margraf, P. Zlomanczuk, L.A. Liskin, G.R. Lynch. Circadian differences in neuronal activity of the suprachiasmatic nucleus in brain slices prepared from photo-responsive and photo-non-responsive Djungarian hamsters. *Brain Res*, 544 (1991), pp. 42–48

Mason, 1991. R. Mason. The effects of continuous light exposure on Syrian hamster suprachiasmatic (SCN) neuronal discharge activity *in vitro. Neurosci Lett*, 123 (1991), pp. 160–163

Maywood et al., 2006. E.S. Maywood, A.B. Reddy, G.K. Wong, J.S. O'Neill, J.A. O'Brien, D.G. McMahon, A.J. Harmar, H. Okamura, M.H. Hastings. Synchronization and maintenance of timekeeping in suprachiasmatic circadian clock cells by neuropeptidergic signaling. *Curr Biol*, 16 (2006), pp. 599–605

Maywood et al., 2011. E.S. Maywood, J.E. Chesham, J.A. O'Brien, M.H. Hastings. A diversity of paracrine signals sustains molecular circadian cycling in suprachiasmatic nucleus circuits. *Proc Natl Acad Sci*, 108 (2011), pp. 14306–14311

McArthur et al., 2000. A.J. McArthur, A.N. Coogan, S. Ajpru, D. Sugden, S.M. Biello, H.D. Piggins. Gastrin-releasing peptide phase-shifts suprachiasmatic nuclei neuronal rhythms *in vitro. J Neurosci*, 20 (2000), pp. 5496–5502

Meijer and De Vries, 1995. J.H. Meijer, M.J. De Vries. Light-induced phase shifts in onset and offset of running-wheel activity in the Syrian hamster. *J Biol Rhythms*, 10 (1995), pp. 4–16

Meijer and Schwartz, 2003. J.H. Meijer, W.J. Schwartz. In search of the pathways for light-induced pacemaker resetting in the suprachiasmatic nucleus. *J Biol Rhythms*, 18 (2003), pp. 235–249

Meijer et al., 1986. J.H. Meijer, G.A. Groos, B. Rusak. Luminance coding in a circadian pacemaker: the suprachiasmatic nucleus of the rat and the hamster. *Brain Res*, 382 (1986), pp. 109–118

Meijer et al., 1990. J.H. Meijer, S. Daan, G.J. Overkamp, P.M. Hermann. The two-oscillator circadian system of tree shrews (*Tupaia belangeri*) and its response to light and dark pulses. *J Biol Rhythms*, 5 (1990), pp. 1–16

Messager et al., 2000. S. Messager, D.G. Hazlerigg, J.G. Mercer, P.J. Morgan. Photoperiod differentially regulates the expression of *Per1* and *ICER* in the pars tuberalis and the suprachiasmatic nucleus of the Siberian hamster. *Eur J Neurosci*, 12 (2000), pp. 2865–2870

Michel and Colwell, 2001. S. Michel, C.S. Colwell. Cellular communication and coupling within the suprachiasmatic nucleus. *Chronobiol Int*, 18 (2001), pp. 579–600

Mieda et al., 2015. M. Mieda, D. Ono, E. Hasegawa, H. Okamoto, K. Honma, S. Honma, T. Sakurai. Cellular clocks in AVP neurons of the SCN Are critical for interneuronal coupling regulating circadian behavior rhythm. *Neuron*, 85 (2015), pp. 1103–1116

Mohawk et al., 2012. J.A. Mohawk, C.B. Green, J.S. Takahashi. Central and peripheral circadian clocks in mammals. *Annu Rev Neurosci*, 35 (2012), pp. 445–462

Moore and Leak, 2001. R. Moore, R. Leak. Suprachiasmatic nucleus. J. Takahashi (Ed.), *et al.*, *Handbook of behavioral neurobiology: circadian clocks*, Vol. 12, Kluwer Academic/Plenum Publishers, New York (2001), pp. 141–179

Moore and Silver, 1998. R.Y. Moore, R. Silver. Suprachiasmatic nucleus organization. *Chronobiol Int*, 15 (1998), pp. 475–487

Moore and Speh, 1993. R.Y. Moore, J.C. Speh. GABA is the principal neurotransmitter of the circadian system. *Neurosci Lett*, 150 (1993), pp. 112–116

Moore et al., 2002. R.Y. Moore, J.C. Speh, R.K. Leak. Suprachiasmatic nucleus organization. *Cell Tissue Res*, 309 (2002), pp. 89–98

Moore-Ede et al., 1976. M.C. Moore-Ede, W.S. Schmelzer, D.A. Kass, J.A. Herd. Internal organization of the circadian timing system in multicellular animals. *Fed Proc*, 35 (1976), pp. 2333–2338

Moore-Ede et al., 1977. M.C. Moore-Ede, D.A. Kass, J.A. Herd. Transient circadian internal desynchronization after light-dark phase shift in monkeys. *Am J Physiol*, 232 (1977), pp. R31–R37

Morin, 1994. L.P. Morin. The circadian visual system. *Brain Res Brain Res Rev*, 19 (1994), pp. 102–127

Morin, 2007. L.P. Morin. SCN organization reconsidered. *J Biol Rhythms*, 22 (2007), pp. 3–13

Morin and Cummings, 1982. L.P. Morin, L.A. Cummings. Splitting of wheelrunning rhythms by castrated or steroid treated male and female hamsters. *Physiol Behav*, 29 (1982), pp. 665–675

Morin et al., 2003. L.P. Morin, J.H. Blanchard, I. Provencio. Retinal ganglion cell projections to the hamster suprachiasmatic nucleus, intergeniculate leaflet, and visual midbrain: bifurcation and melanopsin immunoreactivity. *J Comp Neurol*, 465 (2003), pp. 401–416

Mrosovsky and Janik, 1993. N. Mrosovsky, D. Janik. Behavioral decoupling of circadian rhythms. *J Biol Rhythms*, 8 (1993), pp. 57–65

Mrugala et al., 2000. M. Mrugala, P. Zlomanczuk, A. Jagota, W.J. Schwartz. Rhythmic multiunit neural activity in slices of hamster suprachiasmatic nucleus reflect prior photoperiod. *Am J Physiol*, 278 (2000), pp. R987–R994

Myung et al., 2012. J. Myung, S. Hong, F. Hatanaka, Y. Nakajima, E. De Schutter, T. Takumi. Period coding of *Bmal1* oscillators in the suprachiasmatic nucleus. *J Neurosci*, 32 (2012), pp. 8900–8918

Myung et al., 2015. J. Myung, S. Hong, D. DeWoskin, E. De Schutter, D.B. Forger, T. Takumi. GABA-mediated repulsive coupling between circadian clock neurons in the SCN encodes seasonal time. *Proc Natl Acad Sci*, 112 (2015), pp. E3920–E3929

Nagano et al., 2003. M. Nagano, A. Adachi, K. Nakahama, T. Nakamura, M. Tamada, E. Meyer-Bernstein, A. Sehgal, Y. Shigeyoshi. An abrupt shift in the day/night cycle causes desynchrony in the mammalian circadian center. *J Neurosci*, 23 (2003), pp. 6141–6151

Nagano et al., 2009. M. Nagano, A. Adachi, K.H. Masumoto, E. Meyer-Bernstein, Y. Shigeyoshi. *RPer1* and *rPer2* induction during phases of the circadian cycle critical for light resetting of the circadian clock. *Brain Res*, 1289 (2009), pp. 37–48

Nagoshi et al., 2004. E. Nagoshi, C. Saini, C. Bauer, T. Laroche, F. Naef, U. Schibler. Circadian gene expression in individual fibroblasts: cell-autonomous and self-sustained oscillators pass time to daughter cells. *Cell*, 119 (2004), pp. 693–705

Naito et al., 2008. E. Naito, T. Watanabe, H. Tei, T. Yoshimura, S. Ebihara. Reorganization of the suprachiasmatic nucleus coding for day length. *J Biol Rhythms*, 23 (2008), pp. 140–149

Nakamura et al., 2001. W. Nakamura, S. Honma, T. Shirakawa, K. Honma. Regional pacemakers composed of multiple oscillator neurons in the rat suprachiasmatic nucleus. *Eur J Neurosci*, 14 (2001), pp. 666–674

Nakamura et al., 2004. T.J. Nakamura, K. Fujimura, S. Ebihara, K. Shinohara. Light response of the neuronal firing activity in the suprachiasmatic nucleus of mice. *Neurosci Lett*, 371 (2004), pp. 244–248

Nakamura et al., 2005. W. Nakamura, S. Yamazaki, N.N. Takasu, K. Mishima, G.D. Block. Differential response of *Period1* expression within the suprachiasmatic nucleus. *J Neurosci*, 25 (2005), pp. 5481–5487

Nelson, 1987. R.J. Nelson. Photoperiod-nonresponsive morphs: a possible variable in microtine population-density fluctutations. *Am Naturalist*, 130 (1987), pp. 350–369

Noguchi and Watanabe, 2008. T. Noguchi, K. Watanabe. Regional differences in circadian period within the suprachiasmatic nucleus. *Brain Res*, 1239 (2008), pp. 119–126

Noguchi et al., 2004. T. Noguchi, K. Watanabe, A. Ogura, S. Yamaoka. The clock in the dorsal suprachiasmatic nucleus runs faster than that in the ventral. *Eur J Neurosci*, 20 (2004), pp. 3199–3202

Oda and Friesen, 2002. G.A. Oda, W.O. Friesen. A model for "splitting" of running-wheel activity in hamsters. *J Biol Rhythms*, 17 (2002), pp. 76–88

Ohta et al., 2005. H. Ohta, S. Yamazaki, D.G. McMahon. Constant light desynchronizes mammalian clock neurons. *Nat Neurosci*, 8 (2005), pp. 267–269

Panda et al., 2002. S. Panda, M.P. Antoch, B.H. Miller, A.I. Su, A.B. Schook, M. Straume, P.G. Schultz, S.A. Kay, J.S. Takahashi, J.B. Hogenesch. Coordinated transcription of key pathways in the mouse by the circadian clock. *Cell*, 109 (2002), pp. 307–320

Panda et al., 2003. S. Panda, I. Provencio, D.C. Tu, S.S. Pires, M.D. Rollag, A.M. Castrucci, M.T. Pletcher, T.K. Sato, T. Wiltshire, M. Andahazy, S.A. Kay, R.N. Van Gelder, J.B. Hogenesch. Melanopsin is required for non-image-forming photic responses in blind mice. *Science*, 301 (2003), pp. 525–527

Pavlidis, 1973. T. Pavlidis. *Biological oscillators: their mathematical analysis*. Academic Press, New York (1973)

Pickard and Turek, 1983. G.E. Pickard, F.W. Turek. The suprachiasmatic nuclei: two circadian clocks? *Brain Res*, 268 (1983), pp. 201–210

Pickard et al., 1984. G.E. Pickard, R. Kahn, R. Silver. Splitting of the circadian rhythm of body temperature in the golden hamster. *Physiol Behav*, 32 (1984), pp. 763–766

Pickard et al., 1993. G.E. Pickard, F.W. Turek, P.J. Sollars. Light intensity and splitting in the golden hamster. *Physiol Behav*, 54 (1993), pp. 1–5

Pittendrigh, 1960. C.S. Pittendrigh. *Circadian rhythms and the circadian organization of living systems*. Cold Spring Harb Symp Quant Biol, 25 (1960), pp. 159–184

Pittendrigh, 1974. C.S. Pittendrigh. Circadian organization in cells and the circadian organization of multicellular systems. F.O. Schmitt, F.G. Worden (Eds.), *The neurosciences: third study program*, MIT Press, Cambridge (1974), pp. 437–458

Pittendrigh and Daan, 1976a. C.S. Pittendrigh, S. Daan. A functional analysis of circadian pacemakers in nocturnal rodents: IV. Entrainment: Pacemaker as Clock. *J Comp Physiol A*, 106 (1976), pp. 291–331

Pittendrigh and Daan, 1976b. C.S. Pittendrigh, S. Daan. A functional analysis of circadian pacemakers in nocturnal rodents: V. Pacemaker structure: a clock for all seasons. *J Comp Physiol A*, 106 (1976), pp. 333–355

Pittendrigh et al., 1984. C.S. Pittendrigh, J.A. Elliott, T. Takamura. The circadian component in photoperiodic induction. *Ciba Foundation Symposium*, 104 (1984), pp. 26–47

Pohl, 1983. H. Pohl. Strain differences in responses of the circadian system to light in the Syrian hamster. *Experientia*, 39 (1983), pp. 372–374

Pohl, 1984. H. Pohl. Differences in responses of the circadian system to light in the Syrian hamster. *Physiological Zoology*, 57 (1984), pp. 509–520

Power et al., 2010. A. Power, A.T. Hughes, R.E. Samuels, H.D. Piggins. Rhythm-promoting actions of exercise in mice with deficient neuropeptide signaling. *J Biol Rhythms*, 25 (2010), pp. 235–246

Prendergast and Freeman, 1999, B.J. Prendergast, D.A. Freeman. Pineal-independent regulation of photo-nonresponsiveness in the Siberian hamster (*Phodopus sungorus*). *J Biol Rhythms*, 14 (1999), pp. 62–71

Prosser et al., 1994. R.A. Prosser, D.M. Edgar, H.C. Heller, J.D. Miller. A possible glial role in the mammalian circadian clock. *Brain Res*, 643 (1994), pp. 296–301

Puchalski and Lynch, 1986. W. Puchalski, G.R. Lynch. Evidence for differences in the circadian organization of hamsters exposed to short day photoperiod. *J Comp Physiol A*, 159 (1986), pp. 7–11

Puchalski and Lynch, 1988. W. Puchalski, G.R. Lynch. Characterization of circadian function in Djungarian hamsters insensitive to short day photoperiod. *J Comp Physiol A*, 162 (1988), pp. 309–316

Puchalski and Lynch, 1991a. W. Puchalski, G.R. Lynch. Circadian characteristics of Djungarian hamsters: effects of photoperiodic pretreatment and artificial selection. *Am J Physiol*, 261 (1991), pp. R670–R676

Puchalski and Lynch, 1991b. W. Puchalski, G.R. Lynch. Expression of circadian rhythmicity in Djungarian hamsters under constant light: Effects of light intensity and the circadian system's state. *J Comp Physiol A*, 169 (1991), pp. 185–189

Puchalski and Lynch, 1994. W. Puchalski, G.R. Lynch. Photoperiodic time measurement in Djungarian hamsters evaluated from T-cycle studies. *Am J Physiol*, 267 (1994), pp. R191–R201

Quintero et al., 2003. J.E. Quintero, S.J. Kuhlman, D.G. McMahon. The biological clock nucleus: a multiphasic oscillator network regulated by light. *J Neurosci*, 23 (2003), pp. 8070–8076

Raiewski et al., 2012. E.E. Raiewski, J.A. Elliott, J.A. Evans, G.L. Glickman, M.R. Gorman. Twice daily melatonin peaks in Siberian but not Syrian hamsters under 24 h light:dark:light:dark cycles. *Chronobiol Int*, 29 (2012), pp. 1206–1215

Rash et al., 2000. J.E. Rash, W.A. Staines, T. Yasumura, D. Patel, C.S. Furman, G.L. Stelmack, J.I. Nagy. Immunogold evidence that neuronal gap junctions in adult rat brain and spinal cord contain connexin-36 but not connexin-32 or connexin-43. *Proc Natl Acad Sci*, 97 (2000), pp. 7573–7578

Rash et al., 2001. J.E. Rash, T. Yasumura, F.E. Dudek, J.I. Nagy. Cell-specific expression of connexins and evidence of restricted gap junctional coupling between glial cells and between neurons. *J Neurosci*, 21 (2001), pp. 1983–2000

Reddy et al., 2002. A.B. Reddy, M.D. Field, E.S. Maywood, M.H. Hastings. Differential resynchronisation of circadian clock gene expression within the suprachiasmatic nuclei of mice subjected to experimental jet lag. *J Neurosci*, 22 (2002), pp. 7326–7330

Refinetti, 2002. R. Refinetti. Compression and expansion of circadian rhythm in mice under long and short photoperiods. *Integr Physiol Behav Sci*, 37 (2002), pp. 114–127

Reppert, 1992. S. Reppert. Pre-natal development of a hypothalamic biological clock. D. Swabb (Ed.), *et al.*, *Progress in brain research*, Vol. 93, B.V.: Elsevier Science Publishers (1992), pp. 119–132

Rohling et al., 2011. J.H. Rohling, H.T. Vanderleest, S. Michel, M.J. Vansteensel, J.H. Meijer. Phase resetting of the mammalian circadian clock relies on a rapid shift of a small population of pacemaker neurons. *PLoS One*, 6 (2011), p. e25437

Rosenthal et al., 2005. S.L. Rosenthal, M.M. Vakili, J.A. Evans, J.A. Elliott, M.R. Gorman. Influence of photoperiod and running wheel access on the entrainment of split circadian rhythms in hamsters. *BMC Neurosci*, 6 (2005), pp. 41–53

Rosenwasser and Adler, 1986. A.M. Rosenwasser, N.T. Adler. Structure and function in circadian timing systems: Evidence for multiple coupled circadian oscillators. *Neurosci Biobehav Rev*, 10 (1986), pp. 431–448

Rosenwasser et al., 1983. A.M. Rosenwasser, Z. Boulos, M. Terman. Circadian feeding and drinking rhythms in the rat under complete and skeleton photoperiods. *Physiol Behav*, 30 (1983), pp. 353–359

Sander and Joung, 2014. J.D. Sander, J.K. Joung. CRISPR-Cas systems for editing, regulating and targeting genomes. *Nature Biotechnology*, 32 (2014), pp. 347–355

Schaap et al., 2003. J. Schaap, H. Albus, H.T. VanderLeest, P.H. Eilers, L. Detari, J.H. Meijer. Heterogeneity of rhythmic suprachiasmatic nucleus

neurons: Implications for circadian waveform and photoperiodic encoding. *Proc Natl Acad Sci*, 100 (2003), pp. 15994–15999

Schroeder et al., 2011. A. Schroeder, D.H. Loh, M.C. Jordan, K.P. Roos, C.S. Colwell. Circadian regulation of cardiovascular function: a role for vasoactive intestinal peptide. *Am J Physiol*, 300 (2011), pp. H241–H250

Schwartz, 1991. W.J. Schwartz. Further evaluation of the tetrodotoxin-resistant circadian pacemaker in the suprachiasmatic nuclei. *J Biol Rhythms*, 6 (1991), pp. 149–158

Schwartz et al., 1987. W.J. Schwartz, R.A. Gross, M.T. Morton. The suprachiasmatic nuclei contain a tetrodotoxin-resistant circadian pacemaker. *Proc Natl Acad Sci*, 84 (1987), pp. 1694–1698

Schwartz et al., 2000. W.J. Schwartz, A. Carpino Jr., H.O. de la Iglesia, R. Baler, D.C. Klein, Y. Nakabeppu, N. Aronin. Differential regulation of *fos* family genes in the ventrolateral and dorsomedial subdivisions of the rat suprachiasmatic nucleus. *Neuroscience*, 98 (2000), pp. 535–547

Schwartz et al., 2009. M.D. Schwartz, C. Wotus, T. Liu, W.O. Friesen, J. Borjigin, G.A. Oda, H.O. de la Iglesia. Dissociation of circadian and light inhibition of melatonin release through forced desynchronization in the rat. *Proc Natl Acad Sci*, 106 (2009), pp. 17540–17545

Schwartz et al., 2010. M.D. Schwartz, S. Congdon, H.O. de la Iglesia. Phase misalignment between suprachiasmatic neuronal oscillators impairs photic behavioral phase shifts but not photic induction of gene expression. *J Neurosci*, 30 (2010), pp. 13150–13156

Sellix et al., 2012. M.T. Sellix, J.A. Evans, T.L. Leise, O. Castanon-Cervantes, D.D. Hill, P. DeLisser, G.D. Block, M. Menaker, A.J. Davidson. Aging differentially affects the re-entrainment response of central and peripheral circadian oscillators. *J Neurosci*, 32 (2012), pp. 16193–16202

Sharma et al., 1997. V.K. Sharma, M. Singaravel, M.K. Chandrashekaran, R. Subbaraj. Relationship between free-running period and minimum tolerable light pulse interval of skeleton photoperiods in field mice *Mus booduga. Chronobiol Int*, 14 (1997), pp. 237–245

Shen et al., 2000. S. Shen, C. Spratt, W.J. Sheward, I. Kallo, K. West, C.F. Morrison, C.W. Coen, H.M. Marston, A.J. Harmar. Overexpression of the human VPAC2 receptor in the suprachiasmatic nucleus alters the circadian phenotype of mice. *Proc Natl Acad Sci*, 97 (2000), pp. 11575–11580

Sheward et al., 2010. W.J. Sheward, E. Naylor, S. Knowles-Barley, J.D. Armstrong, G.A. Brooker, J.R. Seckl, F.W. Turek, M.C. Holmes, P.C. Zee, A.J. Harmar. Circadian control of mouse heart rate and blood

pressure by the suprachiasmatic nuclei: behavioral effects are more significant than direct outputs. *PLoS One*, 5 (2010), p. e9783

Shibata and Moore, 1987. S. Shibata, R.Y. Moore. Development of neuronal activity in the rat suprachiasmatic nucleus. *Brain Res*, 431 (1987), pp. 311–315

Shibata and Moore, 1993. S. Shibata, R.Y. Moore. Tetrodotoxin does not affect circadian rhythms in neuronal activity and metabolism in rodent suprachiasmatic nucleus *in vitro. Brain Res*, 606 (1993), pp. 259–266

Shibuya et al., 1980. C.A. Shibuya, R.B. Melnyk, N. Mrosovsky. Simultaneous splitting of drinking and locomotor activity rhythms in a golden hamster. *Naturwissenschaften*, 67 (1980), pp. 45–47

Shimomura and Menaker, 1994. K. Shimomura, M. Menaker. Light-induced phase shifts in tau mutant hamsters. *J Biol Rhythms*, 9 (1994), pp. 97–110

Shinbrot and Scarbrough, 1999. T. Shinbrot, K. Scarbrough. Using variability to regulate long term biological rhythms. *J Theor Biol*, 196 (1999), pp. 455–471

Shinohara et al., 1993. K. Shinohara, K. Tominaga, Y. Isobe, S.T. Inouye. Photic regulation of peptides located in the ventrolateral subdivision of the suprachiasmatic nucleus of the rat: daily variations of vasoactive intestinal polypeptide, gastrin-releasing peptide, and neuropeptide Y. *J Neurosci*, 13 (1993), pp. 793–800

Shinohara et al., 1995. K. Shinohara, S. Honma, Y. Katsuno, H. Abe, K. Honma. Two distinct oscillators in the rat suprachiasmatic nucleus *in vitro. Proc Natl Acad Sci*, 92 (1995), pp. 7396–7400

Shinohara et al., 1999. K. Shinohara, K. Tominaga, S.T. Inouye. Phase dependent response of vasoactive intestinal polypeptide to light and darkness in the suprachiasmatic nucleus. *Neurosci Res*, 33 (1999), pp. 105–110

Shinohara et al., 2000a. K. Shinohara, T. Funabashi, D. Mitushima, F. Kimura. Effects of gap junction blocker on vasopressin and vasoactive intestinal polypeptide rhythms in the rat suprachiasmatic nucleus *in vitro. Neurosci Res*, 38 (2000), pp. 43–47

Shinohara et al., 2000b. K. Shinohara, H. Hiruma, T. Funabashi, F. Kimura. GABAergic modulation of gap junction communication in slice cultures of the rat suprachiasmatic nucleus. *Neuroscience*, 96 (2000), pp. 591–596

Shirakawa et al., 2001. T. Shirakawa, S. Honma, K. Honma. Multiple oscillators in the suprachiasmatic nucleus. *Chronobiol Int*, 18 (2001), pp. 371–387

Silver et al., 1996. R. Silver, M.T. Romero, H.R. Besmer, R. Leak, J.M. Nunez, J. LeSauter. Calbindin-D28K cells in the hamster SCN express light-induced Fos. *Neuroreport*, 7 (1996), pp. 1224–1228

Smarr et al., 2012. B.L. Smarr, E. Morris, H.O. de la Iglesia. The dorsomedial suprachiasmatic nucleus times circadian expression of *Kiss1* and the luteinizing hormone surge. *Endocrinology*, 153 (2012), pp. 2839–2850

Stanfield and Horton, 1996. K.M. Stanfield, T.H. Horton. Testicular growth and locomotor activity of Siberian hamsters from short-day-responsive and short-day-nonresponsive lineages. *Biol Reprod*, 54 (1996), pp. 789–799

Stephan, 1983. F.K. Stephan. Circadian rhythms in the rat: constant darkness, entrainment to T cycles and to skeleton photoperiods. *Physiol Behav*, 30 (1983), pp. 451–462

Stephan, 1989. F.K. Stephan. Entrainment of activity to multiple feeding times in rats with suprachiasmatic lesions. *Physiol Behav*, 46 (1989), pp. 489–497

Storch et al., 2002. K.F. Storch, O. Lipan, I. Leykin, N. Viswanathan, F.C. Davis, W.H. Wong, C.J. Weitz. Extensive and divergent circadian gene expression in liver and heart. *Nature*, 417 (2002), pp. 78–83

Strogatz, 2003. S.H. Strogatz. *Sync: the emerging science of spontaneous order*. Hyperion, New York (2003)

Sulzman et al., 1977. F.M. Sulzman, C.A. Fuller, M.C. Moore-Ede. Spontaneous internal desynchronization of circadian rhythms in the squirrel monkey. *Comp Biochem Physiol*, 58A (1977), pp. 63–67

Sumova and Illnerova, 1998. A. Sumova, H. Illnerova. Photic resetting of intrinsic rhythmicity of the rat suprachiasmatic nucleus under various photoperiods. *Am J Physiol*, 274 (1998), pp. R857–R863

Sumova et al., 2003. A. Sumova, M. Jac, M. Sladek, I. Sauman, H. Illnerova. Clock gene daily profiles and their phase relationship in the rat suprachiasmatic nucleus are affected by photoperiod. *J Biol Rhythms*, 18 (2003), pp. 134–144

Swann and Turek, 1982. J. Swann, F.W. Turek. Cycle of lordosis behavior in female hamsters whose circadian activity rhythm has split into two components. *Am J Physiol*, 243 (1982), pp. R112–R118

Swann and Turek, 1985. J.M. Swann, F.W. Turek. Multiple circadian oscillators regulate the timing of behavioral and endocrine rhythms in female golden hamsters. *Science*, 228 (1985), pp. 898–900

Tapp and Natelson, 1989. W.N. Tapp, B.H. Natelson. Circadian rhythms and patterns of performance before and after simulated jet lag. *Am J Physiol*, 257 (1989), pp. R796–R803

Tataroglu et al., 2006. O. Tataroglu, A.J. Davidson, L.J. Benvenuto, M. Menaker. The methamphetamine-sensitive circadian oscillator (MASCO) in mice. *J Biol Rhythms*, 21 (2006), pp. 185–194

Travnickova et al., 1996. Z. Travnickova, A. Sumova, R. Peters, W.J. Schwartz, H. Illnerova. Photoperiod-dependent correlation between

light-induced SCN c-fos expression and resetting of circadian phase. *Am J Physiol*, 271 (1996), pp. R825–R831

Turek et al., 1982. F.W. Turek, D.J. Earnest, J. Swann. Splitting of the circadian rhythm of activity in hamsters. J. Aschoff (Ed.), *et al.*, *Vertebrate circadian systems*, Springer-Verlag, Berlin Heidelberg (1982), pp. 203–214

van den Pol and Dudek, 1993. A.N. van den Pol, F.E. Dudek. Cellular communication in the circadian clock, the suprachiasmatic nucleus. *Neuroscience*, 56 (1993), pp. 793–811

van den Pol and Tsujimoto, 1985. A.N. van den Pol, K.L. Tsujimoto. Neurotransmitters of the hypothalamic suprachiasmatic nucleus: immunocytochemical analysis of 25 neuronal antigens. *Neuroscience*, 15 (1985), pp. 1049–1086

VanderLeest et al., 2007. H.T. VanderLeest, T. Houben, S. Michel, T. Deboer, H. Albus, M.J. Vansteensel, G.D. Block, J.H. Meijer. Seasonal encoding by the circadian pacemaker of the SCN. *Curr Biol*, 17 (2007), pp. 468–473

vanderLeest et al., 2009. H.T. vanderLeest, J.H. Rohling, S. Michel, J.H. Meijer. Phase shifting capacity of the circadian pacemaker determined by the SCN neuronal network organization. *PLoS One*, 4 (2009), p. e4976

Vilaplana et al., 1997a. J. Vilaplana, T. Cambras, A. Campuzano, A. Diez-Noguera. Simultaneous manifestation of free-running and entrained rhythms in the rat motor activity explained by a multioscillatory system. *Chronobiol Int*, 14 (1997), pp. 9–18

Vilaplana et al., 1997b. J. Vilaplana, T. Cambras, A. Diez-Noguera. Dissociation of motor activity circadian rhythm in rats after exposure to LD cycles of 4-h period. *Am J Physiol*, 272 (1997), pp. R95–R102

Vosko et al., 2007. A.M. Vosko, A. Schroeder, D.H. Loh, C.S. Colwell. Vasoactive intestinal peptide and the mammalian circadian system. *Gen Comp Endocrino*l, 152 (2007), pp. 165–175

Vuillez et al., 1996. P. Vuillez, N. Jacob, R. Teclemariam-Mesbah, P. Pevet. In Syrian and European hamsters, the duration of sensitive phase to light of the suprachiasmatic nuclei depends on the photoperiod. *Neurosci Lett*, 208 (1996), pp. 37–40

Wang et al., 2014. M.H. Wang, N. Chen, J.H. Wang. The coupling features of electrical synapses modulate neuronal synchrony in hypothalamic superachiasmatic nucleus. *Brain Res*, 1550 (2014), pp. 9–17

Watanabe et al., 2007. T. Watanabe, E. Naito, N. Nakao, H. Tei, T. Yoshimura, S. Ebihara. Bimodal clock gene expression in mouse suprachiasmatic nucleus and peripheral tissues under a 7-hour light and 5-hour dark schedule. *J Biol Rhythms*, 22 (2007), pp. 58–68

Weaver, 1998. D.R. Weaver. The suprachiasmatic nucleus: a 25-year retrospective. *J Biol Rhythms*, 13 (1998), pp. 100–112

Webb et al., 2009. A.B. Webb, N. Angelo, J.E. Huettner, E.D. Herzog. Intrinsic, nondeterministic circadian rhythm generation in identified mammalian neurons. *Proc Natl Acad Sci*, 106 (2009), pp. 16493–16498

Weibel et al., 1997. L. Weibel, K. Spiegel, C. Gronfier, M. Follenius, G. Brandenberger. Twenty-four-hour melatonin and core body temperature rhythms: their adaptation in night workers. *Am J Physiol*, 272 (1997), pp. R948–R954

Welsh and Reppert, 1996. D.K. Welsh, S.M. Reppert. Gap junctions couple astrocytes but not neurons in dissociated cultures of rat suprachiasmatic nucleus. *Brain Res*, 706 (1996), pp. 30–36

Welsh et al., 1995. D.K. Welsh, D.E. Logothetis, M. Meister, S.M. Reppert. Individual neurons dissociated from rat suprachiasmatic nucleus express independently phased circadian firing rhythms. *Neuron*, 14 (1995), pp. 697–706

Welsh et al., 2004. D.K. Welsh, S.H. Yoo, A.C. Liu, J.S. Takahashi, S.A. Kay. Bioluminescence imaging of individual fibroblasts reveals persistent, independently phased circadian rhythms of clock gene expression. *Curr Biol*, 14 (2004), pp. 2289–2295

Welsh et al., 2010. D.K. Welsh, J.S. Takahashi, S.A. Kay. Suprachiasmatic nucleus: cell autonomy and network properties. *Annu Rev Physiol*, 72 (2010), pp. 551–577

Winfree, 1967. A.T. Winfree. Biological rhythms and the behavior of populations of coupled oscillators. *J Theor Biol*, 16 (1967), pp. 15–42

Wotus et al., 2013. C. Wotus, T.R. Lilley, A.S. Neal, N.L. Suleiman, S.C. Schmuck, B.L. Smarr, B.J. Fischer, H.O. de la Iglesia. Forced desynchrony reveals independent contributions of suprachiasmatic oscillators to the daily plasma corticosterone rhythm in male rats. *PLoS One*, 8 (2013), p. e68793

Yamaguchi et al., 2003. S. Yamaguchi, H. Isejima, T. Matsuo, R. Okura, K. Yagita, M. Kobayashi, H. Okamura. Synchronization of cellular clocks in the suprachiasmatic nucleus. *Science*, 302 (2003), pp. 1408–1412

Yamaguchi et al., 2013. Y. Yamaguchi, T. Suzuki, Y. Mizoro, H. Kori, K. Okada, Y. Chen, J.M. Fustin, F. Yamazaki, N. Mizuguchi, J. Zhang, X. Dong, G. Tsujimoto, Y. Okuno. Doi M, Okamura H Mice genetically deficient in vasopressin V1a and V1b receptors are resistant to jet lag. *Science*, 342 (2013), pp. 85–90

Yamazaki et al., 2000. S. Yamazaki, R. Numano, M. Abe, A. Hida, R. Takahashi, M. Ueda, G.D. Block, Y. Sakaki, M. Menaker, H. Tei. Resetting central and peripheral circadian oscillators in transgenic rats. *Science*, 288 (2000), pp. 682–685

Yan and Okamura, 2002. L. Yan, H. Okamura. Gradients in the circadian expression of *Per1* and *Per2* genes in the rat suprachiasmatic nucleus. *Eur J Neurosci*, 15 (2002), pp. 1153–1162

Yan and Silver, 2002. L. Yan, R. Silver. Differential induction and localization of *mPer1* and *mPer2* during advancing and delaying phase shifts. *Eur J Neurosci*, 16 (2002), pp. 1531–1540

Yan and Silver, 2004. L. Yan, R. Silver. Resetting the brain clock: time course and localization of mPER1 and mPER2 protein expression in suprachiasmatic nuclei during phase shifts. *Eur J Neurosci*, 19 (2004), pp. 1105–1109

Yan and Silver, 2008. L. Yan, R. Silver. Day-length encoding through tonic photic effects in the retinorecipient SCN region. *Eur J Neurosci*, 28 (2008), pp. 2108–2115

Yan et al., 2005. L. Yan, N.C. Foley, J.M. Bobula, L.J. Kriegsfeld, R. Silver. Two antiphase oscillations occur in each suprachiasmatic nucleus of behaviorally split hamsters. *J Neurosci*, 25 (2005), pp. 9017–9026

Yan et al., 2010. L. Yan, R. Silver, M. Gorman. Reorganization of suprachiasmatic nucleus networks under 24-h LDLD conditions. *J Biol Rhythms*, 25 (2010), pp. 19–27

Zhang and Kay, 2010. E.E. Zhang, S.A. Kay. Clocks not winding down: unravelling circadian networks. *Nature reviews Mole Cell Biol*, 11 (2010), pp. 764–776

Zhang et al., 2014. R. Zhang, N.F. Lahens, H.I. Ballance, M.E. Hughes, J.B. Hogenesch. A circadian gene expression atlas in mammals: implications for biology and medicine. *Proc Natl Acad Sci*, 111 (2014), pp. 16219–16224

Zhou and Cheng, 2005. Q.Y. Zhou, M.Y. Cheng. Prokineticin 2 and circadian clock output. *Febs J*, 272 (2005), pp. 5703–5709

Zlomanczuk et al., 1991. P. Zlomanczuk, R.R. Margraf, G.R. Lynch. *In vitro* electrical activity in the suprachiasmatic nucleus following splitting and masking of wheel-running behavior. *Brain Res*, 559 (1991), pp. 94–99

Zylka et al., 1998. M.J. Zylka, L.P. Shearman, D.R. Weaver, S.M. Reppert. Three period homologs in mammals: differential light responses in the suprachiasmatic circadian clock and oscillating transcripts outside of brain. *Neuron*, 20 (1998), pp. 1103–1110

Corresponding author. Address: Marquette University, Department of Biomedical Sciences, 561 N 15th St Schroeder Complex, Room 446, Milwaukee, WI 53233, USA.