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CIRCADIAN AND SEASONAL ADAPTATIONS

Daily and seasonal adaptation of the circadian clock requires plasticity of the SCN neuronal network

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Abstract

Circadian rhythms are an essential property of many living organisms, and arise from an internal pacemaker, or clock. In mammals, this clock resides in the suprachiasmatic nucleus (SCN) of the hypothalamus, and generates an intrinsic circadian rhythm that is transmitted to other parts of the CNS. We will review the evidence that basic adaptive functions of the circadian system rely on functional plasticity in the neuronal network organization, and involve a change in phase relation among oscillatory neurons. We will illustrate this for: (i) photic entrainment of the circadian clock to the light–dark cycle; and (ii) seasonal adaptation of the clock to changes in day length. Molecular studies have shown plasticity in the phase relation between the ventral and dorsal SCN during adjustment to a shifted environmental cycle. Seasonal adaptation relies predominantly on plasticity in the phase relation between the rostral and caudal SCN. Electrical activity is integrated in the SCN, and appears to reflect the sum of the differently phased molecular expression patterns. While both photic entrainment and seasonal adaptation arise from a redistribution of SCN oscillatory activity patterns, different neuronal coupling mechanisms are employed, which are reviewed in the present paper.

Introduction

The rotation of the earth around its axis causes 24-h changes in the environment. Since the beginning of evolution, organisms have been exposed to these changes and have developed internal clocks, which allow them to anticipate environmental alterations. Circadian rhythms are thus an essential property of many living animals, and are observed in both prokaryotic and eukaryotic species. In mammals, an endogenous clock that generates circadian rhythms resides in the suprachiasmatic nucleus (SCN), a relatively small structure of 20 000 neurons in the ventral hypothalamus (Abrahamson & Moore, 2001). Circadian rhythms are generated in individual neurons of the SCN on the basis of molecular feedback loops between clock genes and their protein products (Takahashi *et al.*, 2008). The presence of circadian oscillations in isolated single cells shows the cell autonomous nature of rhythm generation (Welsh *et al.*, 1995). The intrinsic circadian rhythms that are generated by the SCN are transmitted to other parts of the CNS, rendering daily fluctuations in many behavioural, biochemical and physiological events.

For proper functioning of the circadian clock as a chronometer of environmental time, the SCN is responsive to light. Light is a reliable signal to indicate day and night cycles, while changes in day length are a reliable signal to indicate the time of the year. Synchronization of the internal clock to the environmental light–dark cycle can be understood by the phase-shifting effects of light on the clock. Light delays the phase of the clock when perceived during the early night, and advances the phase of the clock during the late night (Daan & Pittendrigh, 1976). The phase-shifting effects of light on the clock are formally described by a phase response curve (PRC; Daan & Pittendrigh, 1976; Pittendrigh, 1981; Nelson & Takahashi, 1991; Fig. 1). The phase-dependent responsiveness of the clock is essential for entrainment to occur, and is a common response property of all living organisms that exhibit circadian rhythms.

Apart from the 24-h changes in the environment, animals are exposed to seasonal changes that arise as a consequence of the rotation of the earth around the sun. Seasonal environmental changes have large impact on animal physiology and induce changes in animals' activity patterns, in their reproductive system and in the fur (Goldman, 2001; Lehman *et al.*, 2002; Ebling & Barrett, 2008; Hazlerigg & Loudon, 2008). For instance in nocturnal rodents, the activity duration during the winter is longer than during the summer, and many animals have offspring at the most optimal time of the year. The after-effects of day length on animal activity patterns are visible when animals are released in constant darkness, showing that the day length information is encoded within the CNS. It has become clear that an important function of the SCN is to measure the annual changes in day length and to pass this information on to downstream targets.

While the molecular basis for rhythm generation is now largely understood, recent lines of evidence indicate that many of the adaptive functions of the clock arise at the network level of organization (for review, see Hastings & Herzog, 2004; Vansteensel *et al.*, 2008; Welsh *et al.*, 2010). The network organization of the SCN also leads to greater stability and robustness of the circadian clock that is absent in

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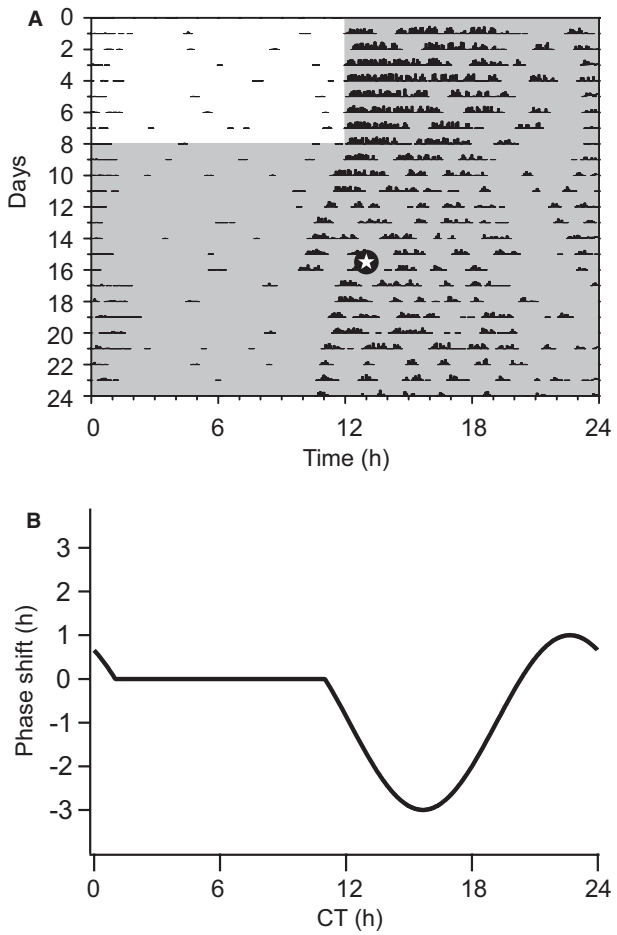

FIG. 1. Phase shifts of running wheel behaviour in the mouse. (A) Example of wheel running actogram for a mouse entrained to a 12 : 12-h light–dark schedule. The actogram shows the wheel running activity of the mouse over the 24-h day. Consecutive days are plotted on successive lines. The light–dark period is depicted by a white (light) and grey (dark) background. On day 8 of the actogram the mouse is placed in constant darkness. A 30-min light pulse was given after seven cycles in constant darkness, 3 h after activity onset (indicated by ✪ in the actogram). Activity onset was defined as circadian time (CT) 12. (B) The PRC summarizes the phase-shifting effect of light pulses, applied at different CTs. The PRC shown here is based on data from Daan & Pittendrigh (1976), and shows large phase delays during the beginning of the night, and smaller phase advances during the end of the night.

peripheral oscillators (Liu *et al.*, 2007). We will review the evidence that photic entrainment to the light–dark cycle and seasonal adaptations of the clock to changes in day length rely on a reconfiguration of neuronal activity patterns within the network of the SCN. We will also review the neuronal mechanisms that are involved in the adjustment of the SCN to daily and seasonal cycles, and will outline that different neurotransmitters and coupling mechanisms are likely to be implicated in the adjustment to daily and seasonal changes.

Adjustment to daily environmental cycles and jet lag

Due to the endogenous nature of circadian rhythmicity, circadian rhythms are preserved in an isolated brain slice, and the use of stationary electrodes allows for recordings of endogenous circadian rhythms in particular areas of the SCN *ex vivo*. With this technique, one can record successfully for up to three circadian cycles. In organotypic cultures of mouse SCN, recordings of luminescence allow for even longer recordings, and have been able to distinguish oscillatory patterns in *period 1::luc* bioluminescence on consecutive circadian cycles in individual cells (Yamaguchi *et al.*, 2003). The electrical and *period 1* activity of the SCN is low during the night (or projected night-time), and high during the day, with a peak in activity at the middle of the day (Schaap *et al.*, 2003; Yamaguchi *et al.*, 2003; Brown & Piggins, 2007; Fig. 2).

The phase-shifting effects of light on the SCN can be mimicked *in vitro* by the application of neurotransmitters that are contained in the retino-hypothalamic tract or by electrical stimulation of the optic nerve (Shibata & Moore, 1993). Bath application of glutamate or its receptor agonists lead to light-like phase shifts in the electrical activity rhythm (Ding *et al.*, 1994; Shibata *et al.*, 1994; Biello *et al.*, 1997). The presence of the peptidergic co-transmitter pituitary adenylate cyclase-activating peptide enhances the postsynaptic effect of glutamate receptor activation (Michel *et al.*, 2006) and increases phase-shifting capability (Colwell *et al.*, 2004). Such studies have revealed that the phase-shifting effects of light on the central clock of the SCN can be mimicked *in vitro*. Thus, the induction of advances vs. delays is not determined by the retinal input, but is a consequence of a postsynaptic phase-dependent responsiveness of the SCN to the stimulus.

Importantly, the brain slice preparation can also be used to study after-effects of environmental conditions on the neurons of the SCN. In other words, the animal can be subjected to a particular environmental condition, and the after-effects of such a condition can be investigated *ex vivo*. A particular manipulation that has been investigated is the influence of a shift of the light–dark cycle (i.e. a jet lag) on the electrical (Vansteensel *et al.*, 2003; Albus *et al.*, 2005) and molecular organization (Yamazaki *et al.*, 2000; Reddy *et al.*, 2002; Nagano *et al.*, 2003; Vansteensel *et al.*, 2003; Davidson *et al.*, 2006b) of the SCN. The influence of abrupt phase changes of the light–dark cycle on the SCN clock of rodents has been the subject of investigation in a number of studies (Reddy *et al.*, 2002; Albus *et al.*, 2005; van Oosterhout *et al.*, 2008; Davidson *et al.*, 2009).

Jet lag within the SCN clock

Adjustments of the clock to the new time are not immediate but require several days. Recordings of electrical impulse activity of the neurons of the SCN following a shift of the environmental light–dark cycle have shown that two subregions of the SCN shift asynchronously (Albus *et al.*, 2005). The lower ('ventral') part of the SCN displays rapid phase adjustment to the new light–dark cycle, while the upper ('dorsal') SCN requires up to 6 days to readjust. In 'intact' slices of the SCN, the two populations result in a bimodal pattern in electrical activity, both in the dorsal and ventral SCN. The bimodal pattern reflects the activity of both a shifted group of neurons and an unshifted group of neurons (Fig. 2). The origin of the two components becomes clear following a surgical cut separating the ventral from the dorsal part. The shifted component is now present exclusively in the ventral SCN, and the unshifted component in the dorsal SCN. As the bimodal activity pattern is recorded at each part of the SCN, the ventral and dorsal areas evidently communicate and transmit their electrical activity patterns to other parts of the SCN.

The asymmetry between the ventral and dorsal SCN is in agreement with studies of molecular expression profiles (Davidson *et al.*, 2009). *In situ* hybridization studies have shown dissociation between rhythms in clock gene expression in the ventral and dorsal SCN following a delaying shift of the light–dark cycle. Oscillations in *period 1*, *period 2* and *cryptochrome 1* in the ventrolateral SCN shift more quickly than in the dorsomedial SCN (Reddy *et al.*, 2002; Nagano *et al.*, 2003; Yan & Silver, 2004; Nakamura *et al.*, 2005; Davidson *et al.*, 2009). The

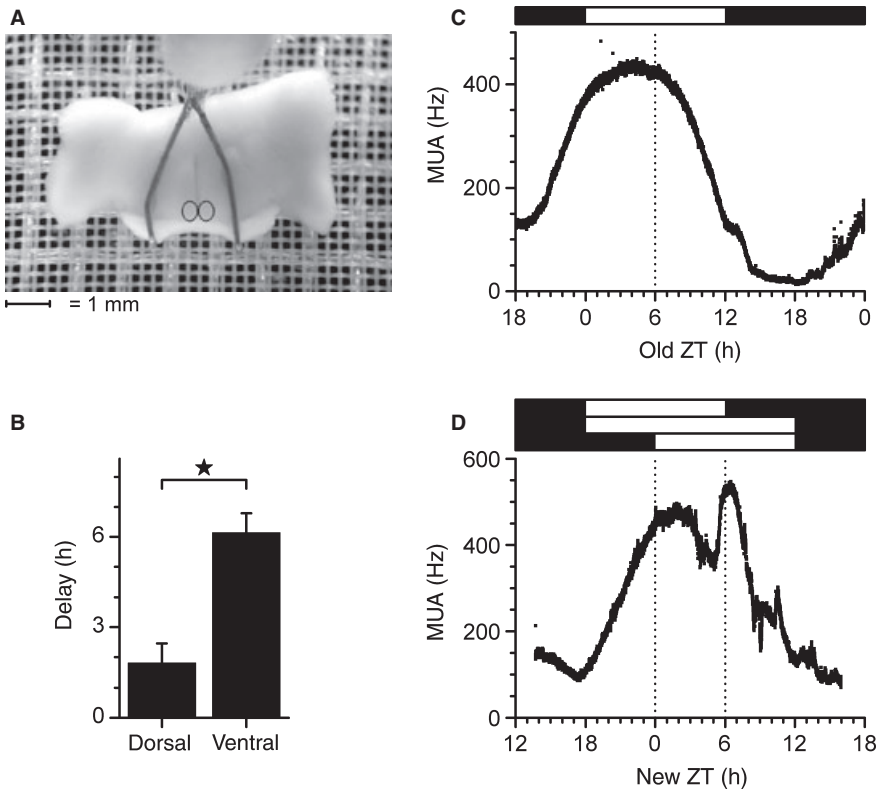

FIG. 2. Extracellular recordings of electrical activity in SCN brain slices. (A) Coronal hypothalamic brain slice of the mouse containing the SCN, indicated by two circles above the optic chiasm. An insulated tungsten fork is used to mechanically stabilize the brain slice in the perfusion chamber to allow for long-term extracellular multiunit recordings. (B) Average delay in the electrical multiunit activity (MUA) peak after a 6-h delay of the light–dark cycle. Recordings were performed on the first cycle after the shift. The graph displays the delay of the two peaks relative to control slices, and shows a delayed ventral component. Of the electrical activity recordings, 67% showed a bimodal electrical activity peak (Albus *et al.*, 2005). (C) Recordings of MUA as a function of Zeitgeber time (ZT) from a brain slice in a control condition. The electrical activity profile is unimodal and has a peak at about midday (Old ZT 6, indicated with vertical dotted line). The previous light–dark cycle is indicated with a black and white bar on top of the graph. (D) Recording of MUA in a brain slice as a function of the new ZT, following a 6-h delay of the light–dark cycle. The shift in the light–dark cycle is indicated by the black and white bars on top of the graph. The heterogeneous phase-shifting response in the SCN can be explained by a fast resetting ventral SCN and a slower resetting dorsal SCN.

dorsal and ventral oscillations in molecular expression can also be forced into desynchrony by exposing rats to short light–dark cycles (de la Iglesia *et al.*, 2004). The regional uncoupling after jet lag is related to a desynchrony of sleep stages (Lee *et al.*, 2009), and to desynchronous behavioural activity patterns (de la Iglesia *et al.*, 2004). Chronic exposure to jet lag increases the mortality rate in aged mice, especially when mice are exposed to advancing shifts (Davidson *et al.*, 2006a). Together, these studies underscore the importance of rhythm coherence for the control of output functions.

Anatomical studies have established that the SCN is a heterogeneous structure and have made an important distinction between the dorsomedial (core) and ventrolateral (shell) part of the SCN, on the basis of neurotransmitter content. The clearest distinction is in rat SCN where the ventral region contains cells that are immunoreactive for vasoactive intestinal peptide (VIP) or gastrin-releasing peptide (GRP). Cells in the shell contain vasopressin or somatostatin (Card & Moore, 1984; Aioun *et al.*, 1998; Abrahamson & Moore, 2001; Moore *et al.*, 2002). Also, afferent and efferent pathways tend to connect with either the dorsal or ventral aspect. We realize that the distinction in ventral and dorsal SCN is an oversimplification, and that transmitter distributions as well as retinal projections show a more complex spatial organization, which is somewhat different among species (Muscat *et al.*, 2003; Morin & Allen, 2006). Nevertheless, we consider this distinction important in a first attempt to understand regional coupling within the SCN.

It has become clear that different subregions of the SCN decode light information differently (Antle & Silver, 2005). Noticeably, the ventral part of the SCN receives most of the retinal input pathways, formed by the retinohypothalamic tract (Ibata *et al.*, 1989; Kiss *et al.*, 2008). The retinorecipient area of the SCN shows light-induced changes in clock gene expression (Yan *et al.*, 1999; Schwartz *et al.*, 2000; Dardente *et al.*, 2002; Kuhlman *et al.*, 2003; Karatsoreos *et al.*, 2004), immediate-early gene expression (Guido *et al.*, 1999) and electrophysiological responses (Groos & Mason, 1980; Shibata *et al.*, 1984a,b; Meijer *et al.*, 1986, 1992; Kuhlman *et al.*, 2003). The neuroanatomical organization of the SCN is therefore merging with the functional organization of the SCN, as revealed under phase-shifting experiments. As re-entrainment after abrupt shifts of the light–dark cycle relies on the phase-shifting effects of light on clock neurons, it may not be surprising that especially the ventral part of the clock shows rapid transitions in phase, while the non-recipient area needs more days to readjust.

Communication mechanisms between the ventral and dorsal SCN clock

The ventral (core) region of the SCN plays a crucial role in re-adjusting the phase of the dorsal (shell) part of the SCN. The underlying mechanisms and neurotransmitters involved in this process are not fully described yet, but we obtained evidence that

γ-aminobutyric acid (GABA) is involved (Albus *et al.*, 2005). Activation of $GABA_A$ receptors in adult neurons will usually cause an influx of Cl^-, resulting in a hyperpolarization of the membrane potential and an inhibitory effect on spike rate (Kaila, 1994). While inhibition is well suited for synchronization within neuronal networks (Van Vreeswijk *et al.*, 1994; Börgers *et al.*, 2010), GABA is not only acting inhibitory in the dorsal SCN but also has an excitatory action (Albus *et al.*, 2005). The mechanisms underlying the excitatory action of GABA in the dorsal SCN have been the subject of investigation in a number of studies. In immature neurons, GABA is causing a Cl^- efflux, resulting in depolarization and excitatory responses (Cherubini *et al.*, 1991). During development, a change in the expression of ion co-transporters regulating the intracellular Cl^- concentration, $[Cl^-]_i$, leads to a lower $[Cl^-]_i$ and a Cl^- influx in response to $GABA_A$ receptor activation (Rivera *et al.*, 1999). The result is the inhibitory GABAergic response typical for adult neurons. The excitatory action of GABA in the SCN has been previously postulated (Wagner *et al.*, 1997; De Jeu & Pennartz, 2002) and debated (Gribkoff *et al.*, 1999), but the functional implication of this excitatory action was not known. Recently, a more detailed analysis of this pathway (Choi *et al.*, 2008) revealed that most excitatory responses were found in dorsal SCN neurons during the night (38%) compared with the day (15%). The switch to excitatory GABAergic responses seems to be achieved by an increase in intracellular Cl^- concentration causing a shift of the Cl^- equilibrium potential (E_{Cl}). The change in $[Cl^-]_i$ is attributed to the activation of the Na^+-K^+-$2Cl^-$ co-transporter (NKCC1), which is expressed in adult SCN neurons (Choi *et al.*, 2008; Irwin & Allen, 2009; Belenky *et al.*, 2010). K^+/Cl^- co-transporters like KCC2 and KCC4 are otherwise keeping $[Cl^-]_i$ low, which results in Cl^- inflow and inhibition as a response to $GABA_A$ activation (Fig. 3).

While clearly the ventral SCN induces shifts in the dorsal SCN, the phase of the ventral SCN is also influenced by the dorsal SCN (Albus *et al.*, 2005). The ventral SCN responds to GABA mainly by inhibition. Following a shift of the light–dark cycle, the large initial shift of the ventral SCN is attenuated, presumably through the inhibitory influence of GABA. We propose that for synchronization by light, only the phase of the ventral SCN neurons is shifted directly by the environmental light–dark cycle by retinal input and that the phase of the dorsal region of the SCN relies on inter-neuronal coupling for entrainment. The ventral SCN is dominant over the dorsal SCN, in that the initial shift of the ventral part is largely adapted by the dorsal part. Understanding the processing of light information between the ventral and dorsal SCN is therefore crucial to understand adaptation to the environmental light–dark cycle.

Although the current lines of investigation have applied jet lag protocols, i.e. large shifts of the environmental light–dark cycle, the underlying mechanisms are also involved in the daily synchronization to the environmental light–dark cycle. The elucidation of the communication mechanisms between dorsal and ventral SCN is essential therefore to understand entrainment of the clock to the external light–dark cycle.

Adjustment to seasonal environmental cycles

Many organisms adapt to seasonal changes in the environment, but the degree of response to different photoperiods varies in different species. Seasonal breeders like sheep and hamsters experience drastic changes in behaviour, reproductive physiology and metabolism (Goldman, 2001; Lehman *et al.*, 2002; Ebling & Barrett, 2008). Other animals, like mice and rats, show alterations of behavioural activity patterns in response to a change in day length (Sumova *et al.*, 2004; vanderLeest

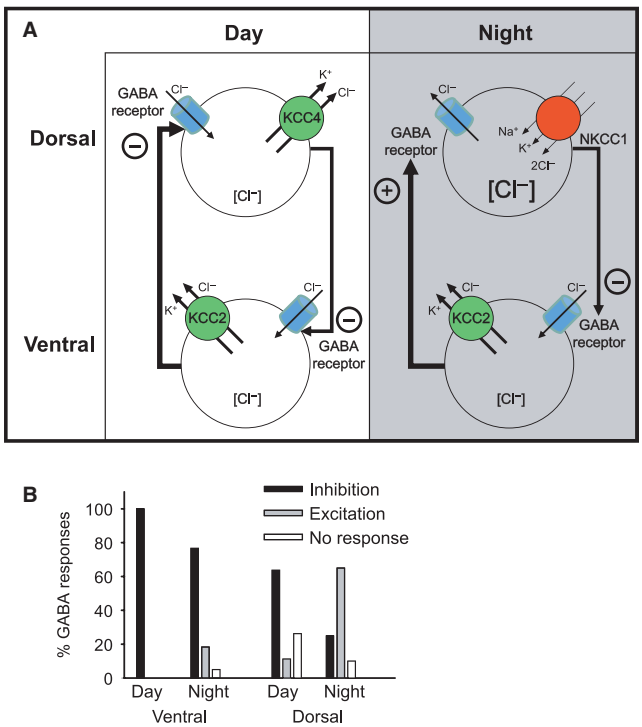

FIG. 3. Putative mechanism of excitatory γ-aminobutyric acid (GABA)ergic transmission in the SCN. (A) The model, based on data by Choi *et al.* (2008), Irwin & Allen (2009), and Belenky *et al.* (2010), suggests that intracellular Cl^- concentration, $[Cl^-]_i$, is regulated by electroneutral cation chloride cotransporters. The balance between Cl^- extruding transporters (KCC2 and KCC4) and Cl^- loading ones (NKCC1) determines $[Cl^-]_i$ and Cl^- equilibrium potential, E_{Cl}. In the ventral SCN neurons, the function of KCC2 is dominating, keeping $[Cl^-]_i$ relatively low, causing Cl^- influx through activated GABA receptors and leading to inhibitory responses. In dorsal SCN neurons, NKCC1 activity at night (grey background) causes higher $[Cl^-]_i$ with a correlated change in E_{Cl}, resulting in outflow of Cl^- through GABA receptors and an excitatory response of the neuron. (B) Bar graph, based on data from Albus *et al.* (2005), showing that the number of excitatory responses in SCN slices differs regionally and over time. The ventral SCN shows mainly inhibitory GABAergic responses. In the dorsal SCN, the inhibitory responses are reduced, and up to 60% of the responses are excitatory during the night.

et al., 2007). The complex system of seasonal regulation has been intensively studied (for review, see Hazlerigg & Loudon, 2008). Here we focus on the role of the SCN in measuring day length to illustrate SCN functional plasticity.

There is accumulating evidence that plasticity in the neuronal network is strongly involved in the day length adjustments of the SCN, and thereby contributes to the regulation of seasonal rhythms. *In vivo* recordings of the SCN have shown that after release in constant darkness, the waveform of the SCN electrical activity is preserved for many cycles (vanderLeest *et al.*, 2007; Houben *et al.*, 2009). Both on long and short days, behavioural activity is triggered or arrested when electrical activity reaches the 50% level, which indicates that the width of the electrical activity peak carries day length information, rather than changes in the threshold level (Houben *et al.*, 2009). The complex interaction between photoperiod-length adjustment and entrainment can be revealed in the SCN *in vitro*, which allows to study the influence of photoperiod on SCN network organization *ex vivo* (Jagota *et al.*, 2000; Mrugala *et al.*, 2000). The electrical activity pattern of the SCN appears strongly dependent on photoperiod, such that the duration of the electrical activity period is extended in long days and compressed in short days (Mrugala *et al.*, 2000). Importantly, these waveform changes are observed at the population level,

which raises the question whether similar waveform changes are observed in individual neuronal activity profiles.

Recordings of single-cell activity patterns in acutely prepared SCN brain slices from rats and mice kept in normal light–dark cycles have shown that single cells are active for surprisingly short periods of time (4–5 h; Schaap *et al.*, 2003; Brown *et al.*, 2006). This is different from the population pattern, which shows a near sinusoidal pattern (vanderLeest *et al.*, 2009b). Some single cells are active at the beginning of the day, some in the midday and some towards the end of the day, and their distribution determines the population waveform (Fig. 4). Electrical recordings of different population sizes have shown that about 50 neurons are required to produce the measured population waveform. With fewer neurons in the recording, the pattern is not fully representative for the actual neuronal distribution within the SCN

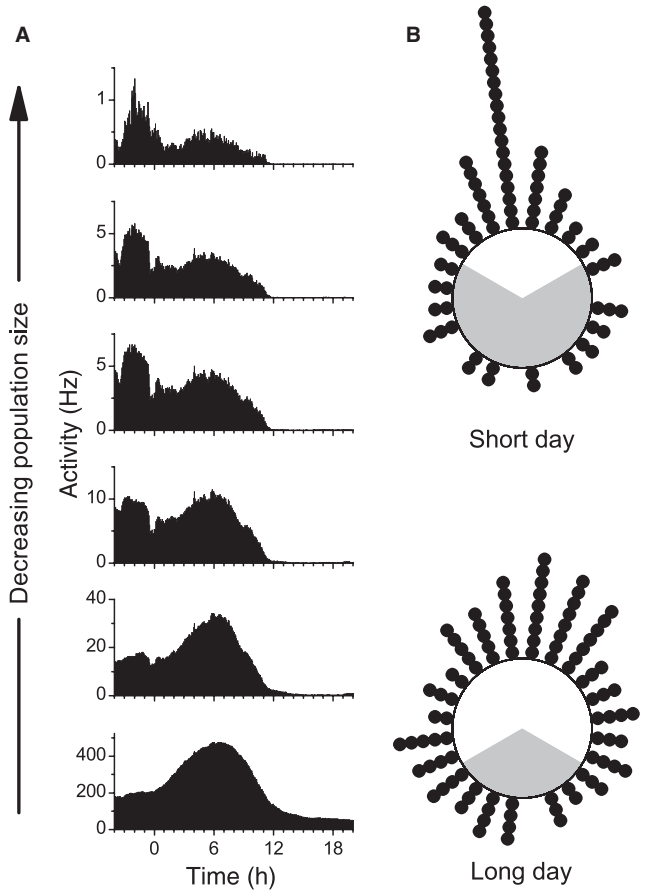

FIG. 4. Recordings of subpopulation electrical activity reveal phase differences between neuronal populations. (A) Recordings of electrical activity with different population sizes in a mouse brain slice, housed under a normal 12 : 12-h light–dark cycle. Population activity was calculated by counting threshold-crossing action potentials in 2-min bins, as a function of the time of day. The top graph shows the electrical activity pattern recorded at near single-unit level, and the lower graphs incorporate increasingly larger populations. When recording a larger population of neurons, the circadian electrical activity pattern has a clear single peak at about midday. At smaller population sizes, it becomes evident that the peak in electrical activity is composed of differently timed neuronal subpopulations. (B) Circular 24-h plots of the number of subpopulation peaks recorded in slices from short and long day length. The subpopulations, as in the top graph of (A), contain the activity of less then five neurons, and peak times are binned and stacked in 1-h intervals. The circles are centred with midday at the top; the grey part represents the dark period. The peak times of the subpopulations are widely distributed in slices from long days and are more centred at midday in slices from short days (B, adapted from vanderLeest *et al.*, 2007).

(Schaap *et al.*, 2003). These results have made it clear that the population pattern is a composite tissue property, and is ultimately determined by the phase relationship among the cells (Schaap *et al.*, 2003; Rohling *et al.*, 2006; vanderLeest *et al.*, 2007; Brown & Piggins, 2009). The logical next question is whether the phase distribution between the SCN neurons has functional significance for seasonal encoding by the SCN. vanderLeest *et al.* (2007) observed that in short days, subpopulations of SCN neurons were relatively synchronized in phase, while in long days the subpopulations are active at different phases of the circadian cycle (Fig. 4). The wide phase distribution under long days results in decompressed electrical activity profiles of the ensemble. The decompressed activity patterns reflect the duration of the long summer day, and serve as an internal representation for the summer. The compressed electrical activity patterns in short days on the other hand reflect the short winter days. No differences are observed between the single-unit activity profiles in long and short days (vanderLeest *et al.*, 2007). Thus, the changes in the waveform of the SCN electrical activity signal result predominantly from alterations in the phase relationship across multiple single-cell oscillators.

Other studies have confirmed these findings and found that a change in phase distribution was the principal mechanism underlying seasonal encoding by the SCN (Brown & Piggins, 2009). Brown & Piggins (2009) additionally observed differences in the electrical activity patterns of individual neurons in the dorsal (but not in the ventral) SCN between long and short days. Computational studies have revealed, however, that these differences in the activity pattern of individual neurons are insufficient to explain the seasonal adjustment of the SCN electrical activity waveform, and that it is especially the plasticity in phase relationship among neurons that accounts for population waveform changes in electrical activity patterns (Rohling *et al.*, 2006; Brown & Piggins, 2009).

Molecular studies also revealed phase synchronization and desynchronization in short and long days, respectively (Hazlerigg *et al.*, 2005; Inagaki *et al.*, 2007; Naito *et al.*, 2008; Sosniyenko *et al.*, 2009). These changes in synchrony underlie the photoperiodic modulation of circadian waveform as expressed at the tissue level, in different clock genes (Nuesslein-Hildesheim *et al.*, 2000; Sumova *et al.*, 2003; Johnston *et al.*, 2005). Importantly, the molecular studies have shown that in long photoperiods, the neurons of the rostral and caudal SCN desynchronize. For instance, *period 1* bioluminescence reporters revealed that *period 1* expression in the rostral SCN shows a bimodal activity pattern in long days, with one component that follows dawn and the other component that follows dusk (Inagaki *et al.*, 2007). In the caudal SCN on the other hand, *period 1* expression is locked to dusk, under all photoperiods (Inagaki *et al.*, 2007). Hazlerigg and coworkers (2005) observed an advance of the peak in *period 2*, *rev-erb* α and *dbp* in the caudal SCN in long days, relative to the rostral SCN. Naito *et al.* (2008) showed multiple peaks in *period 1* bioluminescence in the rostral SCN in long days. In short days, the expression profiles of all genes were in synchrony in all studies. Importantly, Naito *et al.* (2008) showed that the single-cell expression profiles were not different between long and short days, consistent with the electrophysiological studies, and that the changes in waveform are thus a circuit property that is based on phase changes among neurons. A particular subtype of neurons in the retinorecipient area of the hamster SCN could mediate the reorganization of the SCN neuronal network (Yan & Silver, 2008). This group of cells is activated when days get longer and inactivated when days get shorter. Activation of this population may alter the strength of the intercellular connections in the SCN network, and thereby affect the synchrony among neuronal oscillations (Yan & Silver, 2008).

A difference between data from molecular and electrical studies is the observed regional organization. Molecular expression patterns appear region-specific, whereas electrical patterns are transmitted throughout the SCN. Together, the electrical and molecular data support the idea that the ability of the SCN to code for day length is a neuronal network property, rather than a property of single cells, and that waveform changes of the clock output signal are determined by plasticity in the network organization.

Large phase shifts in large-amplitude rhythms induced by short day length; contrast with predictions from limit-cycle oscillator theory

The amplitude of the electrical SCN rhythm is larger when the neurons in the network are more synchronized to each other, as in short day lengths (Schaap *et al.*, 2003; Rohling *et al.*, 2006; vanderLeest *et al.*, 2007). It is a well-established theoretical result that the phase shift of limit-cycle oscillators (like, e.g. the van-der-Pol oscillator) due to external perturbations decreases with an increase in amplitude of the oscillation (Arnol'd & Levi, 1988; Pittendrigh *et al.*, 1991; Winfree, 2000). In other words, it is more difficult to shift high-amplitude rhythms than low-amplitude rhythms by a stimulus of similar strength. Yet, this generic theoretical result seems in contradiction with the experimental observations that large phase shifts are induced in large- rather than small-amplitude rhythms, as observed under short and long days, respectively (vanderLeest *et al.*, 2009a).

We have hypothesized that the apparent contradiction between theory and experiment can be resolved if we focus on the PRC of single cells, and on the phase distribution among the cells (vander-Leest *et al.*, 2009a). We expect that large phase shifts of single cells are reflected in large phase shifts of the SCN as a whole if the single cells are synchronized. In a synchronized population, a stimulus will reach the oscillator cells at the same phase of their cycle, leading to a coherent large shift of the population rhythm. In a desynchronized population on the other hand, the stimulus will reach the cells at different phases of their cycle, leading to diverse phase shifts in single cells and a weaker population response. Using simple simulation studies, where PRC were distributed according to long and short day distributions, the experimental findings could be replicated with great precision. Specifically it could be demonstrated that the phase distribution differences that were experimentally obtained in short and long days lead to changes in the amplitude of the PRC that were experimentally obtained, both in electrical SCN activity as in behavioural activity. The findings were independent of the shape of the single-unit PRC, and were found for both type I and type 0 PRCs (vanderLeest *et al.*, 2009a). While the limit-cycle theory may be valid for individual neurons within the network, the network as an ensemble shows different response characteristics. It appears therefore that the network of the SCN is governed by different rules than individual cells.

Search for neurotransmitters involved in seasonal coding

The neuronal mechanisms responsible for control of seasonal encoded phase distribution between neurons are not identified yet. Several neurotransmitters that are involved in synchronization between SCN neurons may play a role in the capability of the SCN to code for day length. Neurons containing VIP represent the major population of retinorecipient cells, and VIP is therefore a major candidate for distributing light-related phase information within the SCN (Watanabe *et al.*, 2000). Mice deficient of VIP or its receptor vasoactive intestinal peptide receptor 2 ($VPAC_2$) display weak behavioural rhythms with multiple period components and a reduced phase response to light (Harmar *et al.*, 2002; Colwell *et al.*, 2003). Application of VIP can cause light-like phase shifts *in vivo* and *in vitro* (Piggins *et al.*, 1995; Reed *et al.*, 2001), and can restore synchrony in cultured neurons from VIP-deficient animals (Aton *et al.*, 2005). Lastly, lack of VIP or its receptor $VPAC_2$ leads to low-amplitude electrical (Brown *et al.*, 2007) and molecular rhythms in SCN slices (Maywood *et al.*, 2006). These findings suggest that VIP signalling is essential for synchronization within the SCN network to produce high-amplitude circadian rhythms necessary to control behaviour (Vosko *et al.*, 2007).

Another neurotransmitter that may contribute to coupling of individual SCN neurons is GABA. GABA is the most common neurotransmitter in the SCN, and a subset of GABAergic projections within the SCN is functionally excitatory and may play a role in synchronizing ventral and dorsal regions. The role of GABA in the synchronization of individual SCN neurons – as opposed to regional synchronization – is less clear. While it is possible to entrain cultured SCN neurons with repetitive GABA applications (Liu & Reppert, 2000), blocking GABA receptors in SCN slices did not result in a desynchronization of the neurons (Aton *et al.*, 2006).

GRP is able to generate phase shifts in SCN neurons, and is possibly involved in coupling. This cell group is suggested to serve as a relay for communicating photic responses to other SCN cells (Antle *et al.*, 2005; Gamble *et al.*, 2007). Application of GRP, similar to VIP, leads to light-like phase resetting (McArthur *et al.*, 2000) and can restore synchronization in SCN slices of VIP receptor-deficient mice (Maywood *et al.*, 2006). GRP produces an increase in the number of cells that express the phosphorylated form of extracellular signal-regulated kinases (ERK; Antle *et al.*, 2005), and induces *period 1* expression (Gamble *et al.*, 2007). Application of GRP *in vitro* results in an increase in spike frequency (Gamble *et al.*, 2007), and blockade of ERK phosphorylation attenuates GRP-induced shifts (Antle *et al.*, 2005).

It is well documented in rats that some SCN cells communicate via electrical synapses (or gap junctions), although the incidence of this type of coupling is still under debate and varies from estimates of 26% (Long *et al.*, 2005) to less than 5% (Rash *et al.*, 2007). The current opinion is that gap junctions play a role in close-neighbour synchronization and have no influence on coupling of cells between different regions of the SCN (Colwell, 2000).

As VIP may be the major neurotransmitter involved in seasonal adaptation by the SCN, it would be interesting to block VIP signalling in slices from different day lengths. In short days it can be hypothesized that absence of $VPAC_2$ receptor signalling will result in a loss of synchrony between SCN neurons. As different neurons have a slightly different τ (Aton *et al.*, 2005), phase differences will accumulate over time and cells become less synchronized after application of the $VPAC_2$ receptor blocker. This would result in a broader multiunit peak over time.

Concluding remarks: emerging properties in neuronal network

We have summarized different lines of evidence, showing that the SCN neuronal network shows plasticity in order to adapt to changes in the light–dark cycle or to changes in day length. Both daily and seasonal encoding mechanisms are reflected in waveform changes of the SCN rhythm. Plasticity in the SCN neuronal network is apparent from alterations in the phase distribution among SCN neurons or SCN subpopulations, while morphological plasticity or changes at the

receptor level have been less explored. The changes in phase distribution explain the waveform changes that are observed at the population level (Schaap *et al.*, 2003; Hazlerigg *et al.*, 2005; Rohling *et al.*, 2006; Inagaki *et al.*, 2007; vanderLeest *et al.*, 2007; Beersma *et al.*, 2008; Naito *et al.*, 2008; Brown & Piggins, 2009).

While changes in cellular physiology have to underlie the changes in phase distributions, only a few studies have addressed the cellular mechanisms that may determine phase distribution between SCN neurons. Examples are studies on GABAergic postsynaptic effects and the VIP signalling within the SCN. Dynamic and regional regulation of the cellular Cl^- equilibrium potential leads to differential GABAergic responses in dorsal and ventral SCN that may explain the asymmetry in phase-shifting effects observed in these SCN regions (Albus *et al.*, 2005; Belenky *et al.*, 2008; Choi *et al.*, 2008). The $VPAC_2$ receptor activation appears of great importance in cellular synchrony throughout the SCN, and may also play a role in seasonal adaptations of the SCN. It is expected that future studies will focus on cellular mechanisms that determine phase synchrony within the SCN.

We believe that the circadian pacemaker of the SCN offers an attractive model to investigate the functional role of plasticity in neuronal organization. In the SCN, the ensemble behaviour of the interacting oscillator network model can be measured in the network, and the output is defined in terms of phase and period, independent of the level of analysis, allowing for a direct comparison of experimental results. The notion that different properties of the circadian system arise at distinct levels of organization within the animal is of great conceptual interest in view of our goal in understanding the mechanisms underlying human and animal physiology and behaviour.

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Abbreviations

ERK, extracellular signal-regulated kinase; GABA, γ-aminobutyric acid; GRP, gastrin-releasing peptide; PRC, phase response curve; SCN, suprachiasmatic nucleus; VIP, vasoactive intestinal peptide; $VPAC_2$, vasoactive intestinal peptide receptor 2.

References

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

Aioun, J., Chambille, I., Peytevin, J. & Martinet, L. (1998) Neurons containing gastrin-releasing peptide and vasoactive intestinal polypeptide are involved in the reception of the photic signal in the suprachiasmatic nucleus of the Syrian hamster: an immunocytochemical ultrastructural study. *Cell Tissue Res.*, **291**, 239–253.

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

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

Antle, M.C., Kriegsfeld, L.J. & Silver, R. (2005) Signaling within the master clock of the brain: localized activation of mitogen-activated protein kinase by gastrin-releasing peptide. *J. Neurosci.*, **25**, 2447–2454.

Arnol'd, V.I. & Levi, M. (1988) *Geometrical Methods in the Theory of Ordinary Differential Equations*. Springer-Verlag, New York.

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

Aton, S.J., Huettner, J.E., Straume, M. & Herzog, E.D. (2006) GABA and Gi/o differentially control circadian rhythms and synchrony in clock neurons. *Proc. Natl. Acad. Sci. USA*, **103**, 19188–19193.

Beersma, D.G., van Bunnik, B.A., Hut, R.A. & Daan, S. (2008) Emergence of circadian and photoperiodic system level properties from interactions among pacemaker cells. *J. Biol. Rhythms*, **23**, 362–373.

Belenky, M.A., Yarom, Y. & Pickard, G.E. (2008) Heterogeneous expression of gamma-aminobutyric acid and gamma-aminobutyric acid-associated receptors and transporters in the rat suprachiasmatic nucleus. *J. Comp. Neurol.*, **506**, 708–732.

Belenky, M.A., Sollars, P.J., Mount, D.B., Alper, S.L., Yarmon, Y. & Pickard, G.E. (2010) Cell-type specific distribution of chloride transporters in the rat suprachiasmatic nucleus. *Neuroscience*, **165**, 1519–1537.

Biello, S.M., Golombek, D.A. & Harrington, M.E. (1997) Neuropeptide Y and glutamate block each other's phase shifts in the suprachiasmatic nucleus *in vitro*. *Neuroscience*, **77**, 1049–1057.

Börgers, C., Krupa, M. & Gielen, S. (2010) The response of a classical Hodgkin-Huxley neuron to an inhibitory input pulse. *J. Comput. Neurosci.*, **28**, 509–526.

Brown, T.M. & Piggins, H.D. (2007) Electrophysiology of the suprachiasmatic circadian clock. *Prog. Neurobiol.*, **82**, 229–255.

Brown, T.M. & Piggins, H.D. (2009) Spatiotemporal Heterogeneity in the Electrical Activity of Suprachiasmatic Nuclei Neurons and their Response to Photoperiod. *J. Biol. Rhythms*, **24**, 44–54.

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

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

Card, J.P. & Moore, R.Y. (1984) The suprachiasmatic nucleus of the golden hamster: immunohistochemical analysis of cell and fiber distribution. *Neuroscience*, **13**, 415–431.

Cherubini, E., Gaiarsa, J.L. & Ben-Ari, Y. (1991) GABA: an excitatory transmitter in early postnatal life. *Trends Neurosci.*, **14**, 515–519.

Choi, H.J., Lee, C.J., Schroeder, A., Kim, Y.S., Jung, S.H., Kim, J.S., Kim, d.Y., Son, E.J., Han, H.C., Hong, S.K., Colwell, C.S. & Kim, Y.I. (2008) Excitatory actions of GABA in the suprachiasmatic nucleus. *J. Neurosci.*, **28**, 5450–5459.

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

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

Colwell, C.S., Michel, S., Itri, J., Rodriguez, W., Tam, J., Lelievre, V., Hu, Z. & Waschek, J.A. (2004) Selective deficits in the circadian light response in mice lacking PACAP. *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, **287**, R1194–R1201.

Daan, S. & Pittendrigh, C.S. (1976) A functional analysis of circadian pacemakers in nocturnal rodents. II. The variabilliy of phase response curves. *J. Comp. Physiol.*, **106**, 267–290.

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

Davidson, A.J., Sellix, M.T., Daniel, J., Yamazaki, S., Menaker, M. & Block, G.D. (2006a) Chronic jet-lag increases mortality in aged mice. *Curr. Biol.*, **16**, R914–R916.

Davidson, A.J., Yamazaki, S., Arble, D.M., Menaker, M. & Block, G.D. (2006b) Resetting of central and peripheral circadian oscillators in aged rats. *Neurobiol. Aging*, **29**, 471–477.

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

De Jeu, M. & Pennartz, C. (2002) Circadian modulation of GABA function in the rat suprachiasmatic nucleus: excitatory effects during the night phase. *J. Neurophysiol.*, **87**, 834–844.

Ding, J.M., Chen, D., Weber, E.T., Faiman, L.E., Rea, M.A. & Gillette, M.U. (1994) Resetting the biological clock: mediation of nocturnal circadian shifts by glutamate and NO. *Science*, **266**, 1713–1717.

Ebling, F.J. & Barrett, P. (2008) The regulation of seasonal changes in food intake and body weight. *J. Neuroendocrinol.*, **20**, 827–833.

Gamble, K.L., Allen, G.C., Zhou, T. & McMahon, D.G. (2007) 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**, 12078–12087.

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

Gribkoff, V.K., Pieschl, R.L., Wisialowski, T.A., Park, W.K., Strecker, G.J., de Jeu, M.T., Pennartz, C.M. & Dudek, F.E. (1999) A reexamination of the role of GABA in the mammalian suprachiasmatic nucleus. *J. Biol. Rhythms*, **14**, 126–130.

Groos, G.A. & Mason, R. (1980) The visual properties of rat and cat suprachiasmatic neurones. *J. Comp. Physiol. A Neuroethol. Sens. Neural. Behav. Physiol.*, **135**, 349–356.

Guido, M.E., de Guido, L.B., Goguen, D., Robertson, H.A. & Rusak, B. (1999) Daily rhythm of spontaneous immediate-early gene expression in the rat suprachiasmatic nucleus. *J. Biol. Rhythms*, **14**, 275–280.

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

Hastings, M.H. & Herzog, E.D. (2004) Clock genes, oscillators, and cellular networks in the suprachiasmatic nuclei. *J. Biol. Rhythms*, **19**, 400–413.

Hazlerigg, D. & Loudon, A. (2008) New insights into ancient seasonal life timers. *Curr. Biol.*, **18**, R795–R804.

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

Houben, T., Deboer, T., van, O.F. & Meijer, J.H. (2009) Correlation with behavioral activity and rest implies circadian regulation by SCN neuronal activity levels. *J. Biol. Rhythms*, **24**, 477–487.

Ibata, Y., Takahashi, Y., Okamura, H., Kawakami, F., Terubayashi, H., Kubo, T. & Yanaihara, N. (1989) Vasoactive intestinal peptide (VIP)-like immunoreactive neurons located in the rat suprachiasmatic nucleus receive a direct retinal projection. *Neurosci. Lett.*, **97**, 1–5.

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

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

Irwin, R.P. & Allen, C.N. (2009) GABAergic signaling induces divergent neuronal Ca^{2+} responses in the suprachiasmatic nucleus network. *Eur. J. Neurosci.*, **30**, 1462–1475.

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

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

Kaila, K. (1994) Ionic basis of GABAA receptor channel function in the nervous system. *Prog. Neurobiol.*, **42**, 489–537.

Karatsoreos, I.N., Yan, L., LeSauter, J. & Silver, R. (2004) Phenotype matters: identification of light-responsive cells in the mouse suprachiasmatic nucleus. *J. Neurosci.*, **24**, 68–75.

Kiss, J., Csaki, A., Csaba, Z. & Halasz, B. (2008) Synaptic contacts of vesicular glutamate transporter 2 fibres on chemically identified neurons of the hypothalamic suprachiasmatic nucleus of the rat. *Eur. J. Neurosci.*, **28**, 1760–1774.

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

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

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

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

vanderLeest, H.T., Vansteensel, M.J., Duindam, H., Michel, S. & Meijer, J.H. (2009b) Phase of the electrical activity rhythm in the SCN *in vitro* not influenced by preparation time. *Chronobiol. Int.*, **26**, 1075–1089.

Lehman, M.N., Coolen, L.M., Goodman, R.L., Viguie, C., Billings, H.J. & Karsch, F.J. (2002) Seasonal plasticity in the brain: the use of large animal models for neuroanatomical research. *Reprod. Suppl.*, **59**, 149–165.

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

Liu, A.C., Welsh, D.K., Ko, C.H., Tran, H.G., Zhang, E.E., Priest, A.A., Buhr, E.D., Singer, O., Meeker, K., Verma, I.M., Doyle, F.J. III, Takahashi, J.S. & Kay, S.A. (2007) Intercellular Coupling Confers Robustness against Mutations in the SCN Circadian Clock Network. *Cell*, **129**, 605–616.

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

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

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

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

Meijer, J.H., Rusak, B. & Ganshirt, G. (1992) The relation between light-induced discharge in the suprachiasmatic nucleus and phase shifts of hamster circadian rhythms. *Brain Res.*, **598**, 257–263.

Michel, S., Itri, J., Han, J.H., Gniotczynski, K. & Colwell, C.S. (2006) Regulation of glutamatergic signalling by PACAP in the mammalian suprachiasmatic nucleus. *BMC Neurosci.*, **7**, 15.

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

Morin, L.P. & Allen, C.N. (2006) The circadian visual system, 2005. *Brain Res. Rev.*, **51**, 1–60.

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

Muscat, L., Huberman, A.D., Jordan, C.L. & Morin, L.P. (2003) Crossed and uncrossed retinal projections to the hamster circadian system. *J. Comp. Neurol.*, **466**, 513–524.

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

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

Nakamura, W., Yamazaki, S., Takasu, N.N., Mishima, K. & Block, G.D. (2005) Differential response of Period 1 expression within the suprachiasmatic nucleus. *J. Neurosci.*, **25**, 5481–5487.

Nelson, D.E. & Takahashi, J.S. (1991) Sensitivity and integration in a visual pathway for circadian entrainment in the hamster (*Mesocricetus auratus*). *J. Physiol.*, **439**, 115–145.

Nuesslein-Hildesheim, B., O'Brien, J.A., Ebling, F.J., Maywood, E.S. & Hastings, M.H. (2000) The circadian cycle of mPER clock gene products in the suprachiasmatic nucleus of the siberian hamster encodes both daily and seasonal time. *Eur. J. Neurosci.*, **12**, 2856–2864.

van Oosterhout, F., Michel, S., Deboer, T., Houben, T., van de Ven, R.C., Albus, H., Westerhout, J., Vansteensel, M.J., Ferrari, M.D., van den Maagdenberg, A.M. & Meijer, J.H. (2008) Enhanced circadian phase resetting in R192Q Cav2.1 calcium channel migraine mice. *Ann. Neurol.*, **64**, 315–324.

Piggins, H.D., Antle, M.C. & Rusak, B. (1995) Neuropeptides phase shift the mammalian circadian pacemaker. *J. Neurosci.*, **15**, 5612–5622.

Pittendrigh, C.S. (1981) Circadian systems: entrainment. In Aschoff, J. (Ed), *Biological Rhythms*. Plenum Press, New York, pp. 95–124.

Pittendrigh, C.S., Kyner, W.T. & Takamura, T. (1991) The amplitude of circadian oscillations: temperature dependence, latitudinal clines, and the photoperiodic time measurement. *J. Biol. Rhythms*, **6**, 299–313.

Rash, J.E., Olson, C.O., Pouliot, W.A., Davidson, K.G., Yasumura, T., Furman, C.S., Royer, S., Kamasawa, N., Nagy, J.I. & Dudek, F.E. (2007) Connexin36 vs. connexin32, "miniature" neuronal gap junctions, and limited electrotonic coupling in rodent suprachiasmatic nucleus. *Neuroscience*, **149**, 350–371.

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

Reed, H.E., Meyer-Spasche, A., Cutler, D.J., Coen, C.W. & Piggins, H.D. (2001) Vasoactive intestinal polypeptide (VIP) phase-shifts the rat suprachiasmatic nucleus clock *in vitro*. *Eur. J. Neurosci.*, **13**, 839–843.

Rivera, C., Voipio, J., Payne, J.A., Ruusuvuori, E., Lahtinen, H., Lamsa, K., Pirvola, U., Saarma, M. & Kaila, K. (1999) The K^+/Cl^- co-transporter KCC2 renders GABA hyperpolarizing during neuronal maturation. *Nature*, **397**, 251–255.

Rohling, J., Wolters, L. & Meijer, J.H. (2006) Simulation of day-length encoding in the SCN: from single-cell to tissue-level organization. *J. Biol. Rhythms*, **21**, 301–313.

Schaap, J., Albus, H., vanderLeest, H.T., Eilers, P.H., Detari, L. & Meijer, J.H. (2003) Heterogeneity of rhythmic suprachiasmatic nucleus neurons: implications for circadian waveform and photoperiodic encoding. *Proc. Natl. Acad. Sci. USA*, **100**, 15994–15999.

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

Shibata, S. & Moore, R.Y. (1993) Neuropeptide Y and optic chiasm stimulation affect suprachiasmatic nucleus circadian function *in vitro*. *Brain Res.*, **615**, 95–100.

Shibata, S., Oomura, Y., Hattori, K. & Kita, H. (1984a) Responses of suprachiasmatic nucleus neurons to optic nerve stimulation in rat hypothalamic slice preparation. *Brain Res.*, **302**, 83–89.

Shibata, S., Oomura, Y., Kita, H., Liou, S.Y. & Ueki, S. (1984b) Field potentials in the suprachiasmatic nucleus of rat hypothalamic slice produced by optic nerve stimulation. *Brain Res. Bull.*, **12**, 377–379.

Shibata, S., Watanabe, A., Hamada, T., Ono, M. & Watanabe, S. (1994) N-methyl-D-aspartate induces phase shifts in circadian rhythm of neuronal activity of rat SCN *in vitro*. *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, **267**, R360–R364.

Sosniyenko, S., Hut, R.A., Daan, S. & Sumova, A. (2009) Influence of photoperiod duration and light-dark transitions on entrainment of Per1 and Per2 gene and protein expression in subdivisions of the mouse suprachiasmatic nucleus. *Eur. J. Neurosci.*, **30**, 1802–1814.

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

Sumova, A., Bendova, Z., Sladek, M., Kovacikova, Z. & Illnerova, H. (2004) Seasonal molecular timekeeping within the rat circadian clock. *Physiol. Res.*, **53**(Suppl 1), S167–S176.

Takahashi, J.S., Hong, H.K., Ko, C.H. & McDearmon, E.L. (2008) The genetics of mammalian circadian order and disorder: Implications for physiology and disease. *Nat. Rev. Genet.*, **9**, 764–775.

Van Vreeswijk, C., Abbott, L.F. & Ermentrout, G.B. (1994) When inhibition not excitation synchronizes neural firing. *J. Comput. Neurosci.*, **1**, 313–321.

Vansteensel, M.J., Yamazaki, S., Albus, H., Deboer, T., Block, G.D. & Meijer, J.H. (2003) Dissociation between circadian Per1 and neuronal and behavioral rhythms following a shifted environmental cycle. *Curr. Biol.*, **13**, 1538–1542.

Vansteensel, M.J., Michel, S. & Meijer, J.H. (2008) Organization of cell and tissue circadian pacemakers: a comparison among species. *Brain Res. Rev.*, **58**, 18–47.

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

Wagner, S., Castel, M., Gainer, H. & Yarom, Y. (1997) GABA in the mammalian suprachiasmatic nucleus and its role in diurnal rhythmicity. *Nature*, **387**, 598–603.

Watanabe, K., Vanecek, J. & Yamaoka, S. (2000) *In vitro* entrainment of the circadian rhythm of vasopressin-releasing cells in suprachiasmatic nucleus by vasoactive intestinal polypeptide. *Brain Res.*, **877**, 361–366.

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

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

Winfree, A.T. (2000) *The Geometry of Biological Time*. Springer, New York.

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

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

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

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

Yan, L., Takekida, S., Shigeyoshi, Y. & Okamura, H. (1999) Per1 and Per2 gene expression in the rat suprachiasmatic nucleus: circadian profile and the compartment-specific response to light. *Neuroscience*, **94**, 141–150.