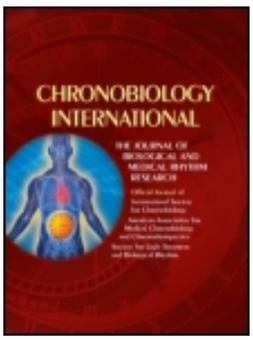

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REVIEW

Electrophysiology of the Circadian Pacemaker in Mammals

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ABSTRACT

The neurons of the mammalian suprachiasmatic nuclei (SCN) control circadian rhythms in molecular, physiological, endocrine, and behavioral functions. In the SCN, circadian rhythms are generated at the level of individual neurons. The last decade has provided a wealth of information on the genetic basis for circadian rhythm generation. In comparison, a modest but growing number of studies have investigated how the molecular rhythm is translated into neuronal function. Neuronal attributes have been measured at the cellular and tissue level with a variety of electrophysiological techniques. We have summarized electrophysiological research on neurons that constitute the SCN in an attempt to provide a comprehensive view on the current state of the art.

MOLECULAR BASIS FOR CIRCADIAN RHYTHMICITY

The circadian rhythm of individual suprachiasmatic neurons is driven by intracellular processes. By manipulation of genes, much information has been gained on the intracellular machinery. Clock genes have been discovered in a wide variety of organisms, ranging from prokaryotic bacteria, plants, and fruit flies to mammals, including man

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(Lakin-Thomas, 2000). Especially among animals, there is striking similarity between these clock genes. A common feature in the functioning of these genes is the transcriptional-translational feedback loop that comprises both clock genes and their protein product. The genes are transcribed to mRNA, and the mRNA is translated into cytoplasmic nuclear proteins. The protein products are transported back into the nucleus, where they affect the transcription of genes.

In mammals, several clock genes have been identified. The main clock genes are the mammalian *Period* (*Per*) family, the *Cryptochrome* (*Cry*) family, *Clock* and *Bmal* (for review see Reppert and Weaver, 2002). Recently, *Dec*1 and *Dec*2 have also been identified (Honma et al., 2002). The *Per* and *Cry* genes are controlled by E-Box elements. Dimers of the BMAL1 and CLOCK proteins bind to the E-Box, thereby activating the transcription of the genes. The cytoplasmic PER and CRY are phosphorylated and transported to the nucleus. Di- and/or trimers of PER and CRY bind to the BMAL1/CLOCK dimers in the nucleus. They prevent transcription of their own genes. As a result, less PER/CRY products are generated, and after degradation PER1, PER2, and CRY no longer inhibit the BMAL1/CLOCK dimer. This allows transcription of the clock genes to reinitiate the cycle.

These processes have not been entirely elucidated, and its very likely the proposed model has to be adjusted in the near future. For example, two interacting feedback loops exist, one with a positive and one with a negative loop (Shearman et al., 2000). The positive loop is connected with the negative limb through the orphan nuclear receptor REV-ERB alpha (Preitner et al., 2002). Moreover it has become clear that the function of the three mammalian *Per* genes is not redundant (Bae et al., 2001). Unknown is whether all processes that generate a rhythm in the circadian range are accounted for, especially in view of the amount of time needed to complete individual steps. Additional processes may be involved to account for the full circadian cycle. Another unresolved issue is why clock genes lead to overt pacemaker activity in the suprachiasmatic nucleus, and not in other tissues where circadian expression profiles have been observed. Circadian rhythms of *Per* in peripheral tissues appear to be dampened in contrast to *Per* oscillations in the SCN (Yamazaki et al., 2000). Advancing insights in these cellular processes are under constant review (Daan et al., 2001; Hastings, 2001; King and Takahashi, 2000; Lowrey et al., 2000; Reppert and Weaver, 2001; 2002).

SECOND MESSENGER SYSTEMS

The intracellularly generated rhythm must be communicated to the membrane system of the neuron to become effective. The attributes of the electrical discharge rhythm depend on the presence of clockgenes (Albus et al., 2002; Herzog et al., 1998; Liu et al., 1997; Nakamura et al., 2002). This communication could be established by a rather large number of candidate cellular processes, for example direct translational/transcriptional regulation of membrane proteins by clockgenes or by activation of second-messenger cascades. One of the possible intracellular messengers is calcium. Colwell (2000a) has shown that the level of intracellular calcium in suprachiasmatic neurons varies with the circadian cycle. Neurons of the SCN have a high calcium content during the day and a low content during the night. This circadian rhythm highlights calcium as a second

messenger that could relay the rhythm of the clock genes to systems at the output level of the neuron, such as the membrane and its ion channels (Colwell, 2000a; Michel et al., 1993). Calcium could also be involved in the transmission of photic information to the intracellular pacemaker. Colwell (2001) has demonstrated elevated levels of calcium levels in response to NMDA application, with higher responses during the day. These studies permit us to hypothesize that calcium has dual attributes in mammalian pacemaker neurons. Calcium probably acts as a second messenger in response to photic input, mediating input to the molecular pacemaker, while it transduces the intracellularly generated pacemaker rhythm to mediate the output. A dual role of calcium has been proposed earlier on the basis of findings in *Bulla gouldiana* (Block et al., 1993).

Various second messenger systems have been implicated in the Zeitgeber signal transmission to the circadian pacemaker. Activation of glutamate receptors leads to an increase in intracellular calcium and subsequent nitric oxide production, followed by either a calcium response via the ryanodine receptor or a cGMP transduction cascade. These cascades converge since both lead to phosphorylation of the Ca^{2+}/cAMP response element binding protein CREB (see Ding et al., 1994; 1998; Obrietan and van den Pol, 1999; de Vries et al., 1994). Inositol-trisphosphate is involved in this cascade, but it is not known at which stage (Hamada et al., 1999). A parallel pathway involves adenyl cyclase, cAMP, and protein kinase A. This parallel pathway appears to gate the first pathway (Tischkau et al., 2000) and can be stimulated by at least the neurotransmitters pituitary adenylyl cyclase-activating polypeptide (depending on the concentration used), 5-HT, and NPY (Biello et al., 1997; Chen et al., 1999; Harrington et al., 1999; Tischkau et al., 2000). During subjective day, NPY acts via protein kinase C (Biello et al., 1997). Phase-shifting of SCN neurons is apparently under control of several second-messenger systems, which suggests fine-tuning of phase responses to Zeitgeber signals.

CIRCADIAN RHYTHM OF NEURONAL FIRING RATE

Welsh et al. (1995) have shown that single neurons kept in culture can fire in a circadian pattern for prolonged periods. The neurons in this study were dispersed from the SCN, i.e., the neurons were extracted from homogenized suprachiasmatic tissue. The study of Welsh has demonstrated that the generation of circadian electrical activity rhythms is not a neuronal network property in which feedback information from one neuron to the other is required for the presence of circadian rhythmicity. Instead, this study led to the important insight that individual cells have the capacity to generate circadian rhythms.

Circadian rhythms in SCN electrical activity were measured for the first time by Inouye and Kawamura (1979). Their recordings were performed *in vivo* with implanted multiunit electrodes, revealing electrical activity of populations of neurons. The population discharge rate is high during subjective day and low during subjective night, which is opposite to the animals' behavioral activity rhythm. Neuronal activity outside the SCN expressed reversed rhythms, with high discharge rates during the night and low discharge rates during the day (Inouye and Kawamura, 1982). The rhythm in multiunit activity of the SCN and other hypothalamic areas has been confirmed in the rat and hamster by two other laboratories (Meijer et al., 1996; 1997; 1998; Yamazaki et al., 1998).

One other nucleus, the bed nucleus of the stria terminalis, was found to have a circadian discharge pattern in phase with that of the SCN, i.e., the discharge levels were higher during subjective day (Yamazaki et al., 1998).

The rhythm of SCN multiunit activity has also been measured in so-called hypothalamic islands. The hypothalamus was disconnected from other brain areas by a knife cut that spared only the optic nerve (Inouye and Kawamura, 1982). As a result, the animals lost their circadian activity patterns but the neuronal activity rhythm inside the SCN was not affected and remained entrainable to light pulses. This experiment demonstrates that the SCN contains an oscillator that can generate circadian rhythms independently of neuronal input from nonhypothalamic areas.

To establish that electrical activity rhythms in the SCN are self-sustained and do not depend on humoral or temperature cues, the SCN was isolated from the brain and kept alive for at least one circadian cycle. Three different groups independently published the first studies on self-sustained neuronal activity rhythm in brain slices containing the SCN (Green and Gillette, 1982; Groos and Hendriks, 1982; Shibata et al., 1982). All three groups measured neuronal activity with single unit electrodes, by which single unit activity was sampled for short periods of time. The in vitro studies confirmed that electrical activity rhythms were self-sustained, and all authors reported peaks in electrical activity rhythms during mid-subjective day. The brain slice model provides an excellent opportunity to test the effect of pharmacological agents on the SCN, because (i) the SCN can be studied in the absence of most, if not all, afferent connections such as the RHT and the GHT, (ii) drugs can be delivered directly to the SCN, and (iii) all conditions can be held constant, thereby preventing confounding effects by parameters other than the passage of time.

The in vitro neuronal rhythm can also be measured with multiunit electrodes (Bouskila and Dudek, 1993; Gribkoff et al., 1998; Jagota et al., 2000; Meijer et al., 1997). The long-term multiunit measurements are more difficult to perform than the short-term single unit method described because a high mechanic stability of both slice and recording electrodes is required (Gribkoff et al., 1998; Jagota et al., 2000; Meijer et al., 1997). One advantage of the technique, however, is the possibility to monitor the activity rhythm of a localized subpopulation of SCN neurons for two to three circadian cycles. A second advantage of the multiunit measurements is that they can be performed with stationary electrodes, whereas single unit recordings require continuous electrode replacement.

The in vivo and in vitro recording techniques have demonstrated robust circadian rhythms in neuronal firing of SCN neurons. The population discharge pattern reaches a maximum during mid-subjective day, usually at Zeitgeber or circadian time 6 to 7, while the minimum is observed during mid-subjective night. By simultaneous recordings with two stationary electrodes inside the SCN it has been demonstrated that different subpopulations of the SCN exhibit different circadian discharge patterns that oscillate out of phase. Phase differences were observed in time of maximal firing, and in half maximum times, which suggests that there are true differences in phase among neuronal oscillations (Schaap et al., 2001). The phase differences showed a large range, from almost zero to about 4 hours, depending on the experiment. Whether these phase differences in the SCN are able to carry information on, for instance, photoperiod measurement remains to be determined. In hypothalamic slices that are cut along a horizontal plane, phase differences of about 8 hours have been observed in times of maximal neuronal activity. One peak corresponded with the moment of dawn, and the other with the occurrence of

dusk (Jagota et al., 2000). The data differed from the ones by Schaap et al. (2001) in that the two peaks (the dawn and dusk peak) were recorded from a single electrode, while Schaap showed phase differences between two locations within the SCN.

Circadian rhythms in neuronal activity of the SCN in the hypothalamic slice have also been measured with intracellular methods. Moreover, they have been recorded with other techniques that indirectly measure neuronal activity patterns of populations of neurons such as metabolic measurements (Schwartz et al., 1980; Shibata and Moore, 1993b), and imaging measurements of membrane potential (Senseman and Rea, 1994). Similar rhythms were recorded from single cultured neurons (Herzog et al., 1997; Honma et al., 1998; Welsh et al., 1995). The rhythms of these neurons in culture have been followed over weeks. These studies all confirm that SCN-neurons are stable, self-sustained oscillators.

The suprachiasmatic neuronal rhythm has been compared between in vivo and in vitro multiunit measurements (Meijer et al., 1997). Since the electrophysiological methodology, the examined species, the data analysis procedures, and the experimenters were identical, an unconfounded comparison between in vivo and in vitro was made. While the amplitude of the rhythm appeared to be the larger in vitro, many high-frequency components were observed in vivo. A strong reduction of neuronal connections in the hypothalamic slice probably underlies the decreased complexity of the in vitro signal. Both afferent pathways and interneuronal communication can contribute to the increased variance of the in vivo signal. Slice preparation disrupts neuronal connections with other brain areas, but also connections within the SCN are lost.

ION CHANNELS INVOLVED IN CIRCADIAN PACEMAKER FUNCTION

The electrical activity of SCN neurons is critical in relaying the pacemaker rhythm to the central nervous system and peripheral functions areas (Schwartz et al., 1987). The electrical activity of SCN neurons consists of action potential firing involving different ion channels. Ion channels are classified into two general classes, voltage-gated and leakage channels. The resting state of both types of channels regulates the resting membrane potential together with ion pumps. The voltage-gated channels that generate action potentials have been described in detail. The most common channels in the central nervous system are the fast sodium channels, the A-type and M-type potassium channels, the delayed rectifier potassium channels, and the calcium-sensitive potassium channels. Furthermore, voltage-gated calcium channels may contribute to the action potential. A typical neuron can be expected to contain fast sodium channels and several potassium channels.

The normal, fast sodium channel mediates the rising flank of the action potential in the SCN (Jiang et al., 1997b). Thomson and West (1990) investigated the effects of calcium channel blockade on the action potential of SCN neurons. Their findings suggest that voltage-gated calcium channels contribute partially to the rising flank of the action potential. Calcium-sensitive potassium channels were found to be active during the declining flank of the action potential, and to mediate the fast depolarizing phase of the afterhyperpolarization. These channels appeared to be not involved in the late afterhyperpolarization or the generation of long-lasting interspike intervals, in contrast to pacemaker neurons in other brain areas (Jahnsen and Llinas, 1984; Thomson and West, 1990). Two other potassium channels that are active during action potentials have been

characterized in the SCN, a delayed rectifier and an A-type current (Bouskila and Dudek, 1995).

Currents that are active during the interspike interval are potential regulators of the circadian rhythm in spontaneous firing rate in the SCN together with currents that determine the resting membrane potential. Three currents have been mentioned in this respect. The aforementioned A-current might be involved since it is activated at membrane potentials observed during the interspike interval (Bouskila and Dudek, 1995). The second current is a slowly inactivating sodium current (Pennartz et al., 1997). This current probably mediates the depolarizing ramp preceding action potentials (Akasu et al., 1993; Pennartz et al., 1997; Thomson and West, 1990). The third current is the H-current, the mixed cationic inward rectifier. Older studies refer to it as the Q-current or as the inward rectifier. This current is carried by sodium and potassium, is activated at hyperpolarized membrane potentials, and tends to change the membrane potential from a hyperpolarized to the resting state.

The H-current in the SCN has been associated with the inclination of the membrane potential during the interspike interval (Akasu et al., 1993), but this finding could not be replicated by de Jeu and Pennartz (1997). In the latter study it was shown that H-current is not different between subjective day and night (de Jeu and Pennartz, 1997) and therefore does not contribute to the rhythm in discharge rate. Both studies reported activation potentials of -60 mV or lower. As the membrane potential of SCN neurons is less negative than 60 mV during the subjective day, this explains why the H-current is not activated during daytime discharge rates. The voltage response of the H-current strongly correlates with the input resistance, which was higher during subjective day (Schaap et al., 1999). This high response during the day probably is a consequence of the higher input resistance, in accordance with the indifferent H-current reported by de Jeu and Pennartz (1997). The H-current is modulated by the cAMP-system (Akasu and Shoji, 1994; Akasu et al., 1992). Conflicting reports about a modulation by melatonin were published (Jiang et al., 1995; van den Top et al., 2002).

Two ATP-sensitive currents have been reported (Hall, 1997). Both are K^+-currents, one glybenclamide-sensitive and the other Ca^{2+}-dependent. Tonic modulation of these channels appears to alter the output of SCN neurons while having little effect on the phase of pacemaker. NPY and melatonin also activate unidentified potassium current(s) (Hall et al., 1999; Jiang et al., 1995). Since the current activated by NPY is sensitive to glybenclamide it may be the same current as the ATP-sensitive current.

The current that is induced by melatonin has been characterized in more detail recently (van den Top et al., 2002). This current appears to be an open rectifier. It was insensitive to tolbutamide and therefore is different from the ATP-sensitive current described by Hall (1997). The amplitude of the melatonin-induced current was not different between subjective day and night.

CIRCADIAN RHYTHM OF MEMBRANE PROPERTIES

Neurons of the SCN are among the smallest neurons in the CNS (van den Pol, 1980). Since small neurons are difficult to investigate electrophysiologically, relatively few studies have assessed the electrophysiological properties of these neurons in the brain slice

preparation. Especially intracellular studies are difficult to perform since the chance of successfully impaling a neuron gets increasingly smaller with decreasing neuronal size. The patch-clamp technique is more successful in this respect, and a number of studies with this technique have been published.

It has not been possible to measure the circadian rhythm in spontaneous firing rate with techniques that record intracellular currents until recently (Jiang et al., 1997b; Kim and Dudek, 1992; Pennartz et al., 1998; Wheal and Thomson, 1984). Using perforated (de Jeu et al., 1998) and whole-cell patch-clamp techniques (Schaap et al., 1999) the measurement of intracellular circadian rhythms became possible. We compared whole-cell properties measured directly after break-in of the seal to properties in cells measured after a stabilization period (Schaap et al., 1999). The circadian rhythm appeared to be lost after the stabilization period. This may explain why earlier attempts to measure these rhythms were unsuccessful.

The membrane potential and input resistance were found to be more depolarized and respectively larger during subjective day than during subjective night when measured directly after break-in. This rhythm is in phase with the rhythm in firing rate (de Jeu et al., 1998; Schaap et al., 1999). A high input resistance was measured during the day, indicating closure of ion channels. Since a lower input resistance is associated with membrane hyperpolarization, the closed channels are likely to mediate outward currents. As closure of outward currents leads to a more depolarized membrane, the observed rhythm in input resistance is consistent with the depolarized membrane potential measured during subjective day (de Jeu et al., 1998; Schaap et al., 1999). The state of outward currents may be regulated by intracellular messengers such as calcium (Colwell, 2000a).

Two other electrophysiological properties, the afterhyperpolarization and adaptation rate also express a circadian modulation (Schaap et al., 1999). These characteristics were measured on evoked spike trains (Pennartz et al., 1998; Schaap et al., 1999). The neuronal firing rate is high at the beginning of such trains and decreases toward the end. The rate of this decrement appeared to be higher during subjective day (Schaap et al., 1999). Light pulses typically result in an excitatory response with high firing frequencies. These high firing rates probably are modulated by the same mechanism that causes spike train adaptation. The circadian rhythm in spike train adaptation therefore may contribute to the rhythm in light response of SCN neurons in vivo.

When the current evoking a spike train is stopped, an SCN neuron is hyperpolarized for some time. This afterhyperpolarization is similar to the action-potential after-hyperpolarization, but is longer in duration and larger in magnitude of hyperpolarization. The magnitude of the afterhyperpolarization appeared to be higher during subjective day (Schaap et al., 1999). The spike train afterhyperpolarization and adaptation are likely to be mediated by the same current, possibly a calcium-sensitive potassium current (Sah, 1996; Schaap et al., 1999).

A new attribute of calcium currents in the SCN has been reported recently by Pennartz et al. (2002) in perforated patch recordings. After suppression of fast sodium channels by TTX, which blocks action potentials, the SCN neuronal membrane potential appeared to oscillate with a frequency between 2 and 8 Hz. These oscillations were observed during the day but not during the night. Stationary oscillations furthermore could not be induced during the night by depolarizing the membrane potential by current injection. Amplitude and frequency of the oscillations could be reduced by calcium-channel blockers and were

specifically inhibited by the L-type Ca^{2+}-blocker nimofidipine. The L-type calcium current appeared to have a large amplitude during the day compared to the night. This current was also shown to enable normal sustained discharge patterns in SCN neurons during the day. These data reveal that the electrophysiology of the neuronal membrane in the SCN differs between subjective day and night in at least two types of channels, an identified L-type calcium current and an unidentified outward current. The latter current underlies the day-night difference in tonic membrane potential and was already referred to by de Jeu et al. (1998) and Schaap et al. (1999). The diurnal variation of the L-type current suggests that expression of this current is controlled by the clockgenes.

In summary, the membrane properties of SCN neurons change during the circadian cycle. This membrane rhythm drives the circadian pattern of firing rate. The rhythm in input resistance and tonic membrane potential indicates that hyperpolarizing channels are closed during the subjective day. Closure of outward channels causes a larger contribution of inward channels to the membrane potential. The observed depolarized membrane potential increases the excitability of the neuron. Changes in excitability result in a circadian rhythm in neuronal activity. The rhythm in L-type calcium current, possibly together with other currents, complements this change in excitability by enabling sustained firing patterns during the subjective day.

HETEROGENEITY OF SCN NEURONS

On the basis of afferent and efferent connections, the SCN can be subdivided into two sections, the ventrolateral and dorsomedial SCN. The ventrolateral area is characterized by a dense termination from the RHT, which converges with afferents from the IGL and the raphe. The dorsomedial section contains more efferent fibers, mostly projecting to other hypothalamic areas (Moga and Moore, 1997; Moore, 1996). The dorsomedial and ventrolateral sections are also different in their neuropeptide content. The ventrolateral section contains vasoactive intestinal polypeptide (VIP), and the dorsomedial area contains arginine vasopressin (AVP) (van Esseveldt et al. (2000); Moore, 1996).

Neurons of the SCN are small ($\pm 8\,\mu$m diameter), have small to modest dendritic arbors (van den Pol, 1980), and are packed in relatively compact nuclei. Because there is little variation in the anatomy of SCN neurons compared to the hippocampus or the neocortex, only a few anatomical distinctions can be made. SCN neurons can be divided into four groups: monopolar, bipolar, curly, and radial neurons (Jiang et al., 1997a; van den Pol, 1980).

In electrophysiological measurements, a heterogeneity among SCN neurons can be observed. Heterogeneity was first observed with extracellular single unit measurements. SCN neurons appeared to have several types of firing characteristics (Groos and Hendriks, 1979). The firing patterns could be classified as either regular, irregular, or burst-like (Cahill and Menaker, 1989; Groos and Hendriks, 1979; Shibata et al., 1984). Thus far it has not been possible to couple the firing patterns to other properties such as the presence of circadian rhythmicity. Manipulation of firing rate by current injection in intracellular studies showed that the regularity of firing is strongly dependent on the firing rate itself (Kim and Dudek, 1993; Pennartz et al., 1998). A regular firing neuron can be transformed into an irregular one by injection of hyperpolarizing current, and vice versa by

depolarizing current. But when the firing rate is in the range of 1.5–5.0 Hz, firing patterns are heterogeneous, and a distinction between regular and irregular firing can be made (Pennartz et al., 1998).

Robust electrophysiological classification of SCN neurons requires an intracellular recording technique. Two approaches of electrophysiological classification have been published. Jiang et al. (1997a) combined voltage-clamp measurements with neuroanatomical morphology. A discrimination was made between monopolar, bipolar, radial and curly bipolar neurons using conductance, holding current and H-current as classifying properties.

Pennartz et al. (1998) used properties measured in current-clamp recordings to separate the neurons into clusters. The grouping was confirmed with statistical techniques and revealed three different clusters. Cluster I neurons are characterized by irregular firing patterns, a monophasic afterhyperpolarizing potential and a small rebound depolarization. Cluster II neurons are regular in intermediate discharge ranges (1.5 to 5.0 Hz), and have biphasic afterhyperpolarizing potentials and small rebound depolarizations. Cluster III neurons are either regularly firing or cannot be brought to intermediate discharge rates at all, have biphasic afterhyperpolarizing potentials, and can be distinguished from cluster II neurons by their large rebound potentials. Cluster I neurons were the most abundant in the SCN (Pennartz et al., 1998).

HETEROGENEITY IN THE EXPRESSION OF CIRCADIAN RHYTHMS

The clustering method has been correlated with the presence of circadian rhythm in membrane properties (Schaap et al., 1999). Cluster I neurons appeared to be rhythmic. Cluster II neurons on the other hand did not have a higher spontaneous firing rate or membrane resistance during subjective day. More data are needed to substantiate this notion, but it can be tentatively concluded that cluster II neurons do not participate in the expression of circadian rhythmicity. The number of cluster III cells in this study was too low to make any statements about their contribution to circadian rhythmicity.

We have been able to identify other subtypes of SCN neurons on the basis of immunohistochemical staining for AVP. Neurons that were positive for AVP generally belonged to cluster I (Pennartz et al., 1998). Moreover, the circadian rhythm in spontaneous firing rate and membrane potential was higher in these neurons than in AVP-negative neurons. Many studies indicate that AVP plays a (modulatory) role in transducing the SCN circadian pacemaker rhythm to other areas (van Esseveldt et al., 2000; Kalsbeek et al., 1996; Jansen et al., 2000; Gerkema et al., 1994; Palm et al., 1999). The stronger rhythms in the AVP-positive neurons are consistent with these assumptions.

INTERCELLULAR COMMUNICATION

GABA is the dominant neurotransmitter in the SCN, as has been shown by immunohistochemical studies (Castel and Morris, 2000; Moore and Speh, 1993; van den Pol and Gorcs, 1986). GABA-ergic hyperpolarizing synaptic responses (Kim and Dudek, 1992) and outward currents (Jiang et al., 1997b) have been shown in electrophysiological

studies. Using patch-clamp recordings, Strecker et al. (1997) found that neurons in the SCN are interconnected by GABA-ergic synapses. There exists some controversy about the precise effect of GABA on the membrane potential or firing rate of SCN neurons.

GABA is a common inhibitory neurotransmitter in the central nervous system. In the SCN however, GABA has been shown to evoke excitatory responses during developmental stages (Chen and van den Pol, 1996). Moreover, Wagner et al. (1997) found excitatory effects of GABA during subjective day, and inhibitory effects during subjective night. These result were not consistent with other studies, showing that most SCN neurons respond to GABA with a decrease in firing rate, without circadian variation (Gribkoff et al., 1999). Exclusively inhibitory responses were found in cultured neurons (Liu et al., 2000). In gramacidin perforated-patch recordings de Jeu and Pennartz (2002) measured reversal potentials of GABA during subjective day and night but also described the effects of GABA when applied exogenously and when it was released by focal stimulation of the SCN. They found that the reversal potential of GABA was more hyperpolarized during the day. As a consequence GABA showed an inhibitory action during the subjective day while being either inhibitory or excitatory during the night. It was also demonstrated that exogenous application of GABA during the night resulted in a blockade of action potentials even when GABA evoked excitatory responses and when the membrane potential was depolarized by GABA. This spike arrest can be explained by a massive shunting effect and by inactivation of sodium channels when situated in a depolarizing shunt.

Liu et al. (2000) furthermore assessed GABA-induced phase responses of single-cultured neurons. The neurons appeared to undergo stable phase shifts, and a phase response curve was constructed. GABA induced phase advances roughly from mid-subjective day to mid-subjective night, and phase delays at other phases. A dead zone of more than 4h was observed during mid-subjective night. These phase responses were mimicked by muscimol and not by baclofen, indicating that $GABA_A$, not $GABA_B$, receptors mediate the phase shifts.

Although GABA appears to be the primary neurotransmitter for communication within the SCN, other neurotransmitters may be involved. Both excitatory and GABA-ergic inhibitory neurotransmission have been demonstrated electrophysiologically in putative inter-SCN communication (Jiang et al., 1997a; Wheal and Thomson, 1984). However, excitatory responses may have been induced by stimulation of glutamatergic fibers extrinsic to the SCN, and these could also play a role in synchronization of SCN neurons (Lundkvist et al., 2002). Strecker et al. (1997) did observe GABA-ergic inhibitory neurotransmission but did not observe any excitatory responses. Intra-SCN projection by neurotransmitters other than GABA, predominantly VIP, has been indicated by neuroanatomical and neurohistological evidence but has not been confirmed thus far by electrophysiological techniques (Abrahamson and Moore, 2001; Jacomy et al., 1999; Moore, 1996). Finally, there is indication for a role of nitric oxide for communication within the SCN (Ding et al., 1994).

NON-SYNAPTIC COMMUNICATION

Communication among SCN neurons could be mediated by means other than synaptic communication. Functional evidence is available to support this notion. Schwartz et al.

(1987) have demonstrated that overt circadian rhythms reappear undisturbed after prolonged periods of TTX infusion into the SCN region in vivo. Likewise, shorter TTX infusions did not have significant effects on the time of maximal firing rate in hypothalamic slices (Shibata and Moore, 1993a; see however, Honma et al., 2000). Blockade of neurotransmission by low/high Ca^{2+} in vitro resulted in synchronous bursts of activity in a multiunit population (Bouskila and Dudek, 1993). Together, these studies demonstrate the existence of a nonsynaptic mechanism of synchronization.

Several nonsynaptic forms of communication have been reported to exist in the SCN, and these have been reviewed elsewhere (van den Pol and Dudek, 1993; Shirakawa et al., 2001). Various techniques revealed glia-glia and glia-neuronal interactions (Shinohara et al., 1995; Tamada et al., 1998) and neuronal gap junctions (Colwell, 2000b; Jiang et al., 1997b; Shinohara et al., 2000). Other forms of nonsynaptic forms of communication are possible, including regulation of extracellular fluid composition (van den Pol and Dudek, 1993) and ephaptic interactions. Ephaptic interactions may occur when the membranes of neighbouring neurons are apposed. These appositions have been observed by electron microscopy (Guldner and Wolff, 1996). The discovery of neuro-astrocyte interactions furthermore suggests the intriguing possibility of direct astrocytical signaling in the SCN (Parpura and Haydon, 2000). New evidence demonstrates that an undefined diffusable substance released by cultured SCN neurons is able to drive circadian oscillations (Allen et al., 2001). Cultured fibroblasts are only capable of expressing transient circadian rhythms, i.e., rhythms that dampen after a few cycles (Balsalobre et al., 1998). The continuing rhythm in the presence of an SCN culture therefore has to be driven by the SCN-culture. Since the membrane that was used to separate SCN culture from the fibroblasts prevented physical contact, a diffusable substance was postulated for relaying the SCN rhythm to the fibroblasts. Such a substance could also function to synchronize neurons within the SCN. A recent study pointed out that Prokineticin2 as a excreted protein may have these functions (Cheng et al., 2002).

CONCLUSIONS

In this paper the electrophysiological attributes of SCN neurons have been summarized, starting with intracellular processes and ending with interneuronal communication. Some gaps in our knowledge have been discussed; for example, the way in which SCN neurons communicate with each other, or the link between the feedback loop in gene expression and the electrical rhythm in membrane processes. While much information about the SCN and its electrophysiology has been gained over the last two decades, researchers are challenged to integrate the electrophysiological findings both with the genetic machinery and with the functional organization of the circadian timekeeping mechanism.

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.

Akasu, T., Shoji, S. (1994). cAMP-dependent inward rectifier current in neurons of the rat suprachiasmatic nucleus. *Pflüeg. Arch.* 429:117–125.

Akasu, T., Shoji, S., Ishimatsu, M. (1992). Forskolin enhances inward rectifier current in neurons of rat suprachiasmatic nucleus. *Kurume Med. J.* 39:213–217.

Akasu, T., Shoji, S., Hasuo, H. (1993). Inward rectifier and low-threshold calcium currents contribute to the spontaneous firing mechanism in neurons of the rat suprachiasmatic nucleus. *Pflüeg. Arch.* 425:109–116.

Albus, H., Bonnefont, X., Chaves, I., Yasui, A., Doczy, J., van der Horst, G. T. J., Meijer, J. H. (2002). Cryptochrome-deficient mice lack circadian electrical activity in the suprachiasmatic nuclei. *Curr. Biol.* 12:1130–1133.

Allen, G., Rappe, J., Earnest, D. J., Cassone, V. M. (2001). Oscillating on borrowed time: diffusible signals from immortalized suprachiasmatic nucleus cells regulate circadian rhythmicity in cultured fibroblasts. *J. Neurosci.* 21:7937–7943.

Bae, K., Jin, X., Maywood, E. S., Hastings, M. H., Reppert, S. M., Weaver, D. R. (2001). Differential functions of mPer1, mPer2, and mPer3 in the SCN circadian clock. *Neuron* 30:525–536.

Balsalobre, A., Damiola, F., Schibler, U. (1998). A serum shock induces circadian gene expression in mammalian tissue culture cells. *Cell* 93:929–937.

Biello, S. M., Golombek, D. A., Schak, K. M., Harrington, M. E. (1997). Circadian phase shifts to neuropeptide Y in vitro: cellular communication and signal transduction. *J. Neurosci.* 17:8468–8475.

Block, G. D., Khalsa, S. B., McMahon, D. G., Michel, S., Guesz, M. (1993). Biological clocks in the retina: cellular mechanisms of biological timekeeping. *Int. Rev. Cytol.* 146:83–144.

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

Bouskila, Y., Dudek, F. E. (1995). A rapidly activating type of outward rectifier K7 + current and A-current in rat suprachiasmatic nucleus neurones. *J. Physiol.* 488:339–350.

Cahill, G. M., Menaker, M. (1989). Responses of the suprachiasmatic nucleus to retinohypothalamic tract volleys in a slice preparation of the mouse hypothalamus. *Brain Res.* 479:65–75.

Castel, M., Morris, J. F. (2000). Morphological heterogeneity of the GABAergic network in the suprachiasmatic nucleus, the brain's circadian pacemaker. *J. Anat.* 196(Pt 1):1–13.

Chen, G., van den Pol, A. N. (1996). Multiple NPY receptors coexist in pre- and postsynaptic sites: inhibition of GABA release in isolated self-innervating SCN neurons. *J. Neurosci.* 16:7711–7724.

Chen, D., Buchanan, G. F., Ding, J. M., Hannibal, J., Gillette, M. U. (1999). Pituitary adenylyl cyclase-activating peptide: a pivotal modulator of glutamatergic regulation of the suprachiasmatic circadian clock. *Proc. Natl. Acad. Sci. USA* 96:13468–13473.

Cheng, M., Bullock, C., Li, C., Lee, A., Bermak, J., Belluzzi, J., Weaver, D., Leslie, F., Zhou, Q. (2002). Prokineticin 2 transmits the behavioural circadian rhythm of the suprachiasmatic nucleus. *Nature* 417:405–410.

Colwell, C. S. (2000a). Circadian modulation of calcium levels in cells in the suprachiasmatic nucleus. *Eur. J. Neurosci.* 12:571–576.

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

Colwell, C. S. (2001). NMDA-evoked calcium transients and currents in the suprachiasmatic nucleus: gating by the circadian system. *Eur. J. Neurosci.* 13:1420–1428.

Daan, S., Albrecht, U., van der Horst, G. T., Illnerova, H., Roenneberg, T., Wehr, T. A., Schwartz, W. J. (2001). Assembling a clock for all seasons: are there M and E oscillators in the genes? *J. Biol. Rhythms* 16:105–116.

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.

Ding, J. M., Buchanan, G. F., Tischkau, S. A., Chen, D., Kuriashkina, L., Faiman, L. E., Alster, J. M., McPherson, P. S., Campbell, K. P., Gillette, M. U. (1998). A neuronal ryanodine receptor mediates light-induced phase delays of the circadian clock. *Nature* 394:381–384.

van Esseveldt, K. E., Lehman, M. N., Boer, G. J. (2000). The suprachiasmatic nucleus and the circadian time-keeping system revisited. *Brain Res. Brain Res. Rev.* 33:34–77.

Gerkema, M. P., Van der Zee, E. A., Feitsma, L. E. (1994). Expression of circadian rhythmicity correlates with the number of arginine-vasopressin-immunoreactive cells in the suprachiasmatic nucleus of common voles, Microtus arvalis. *Brain Res.* 639:93–101.

Green, D. J., Gillette, R. (1982). Circadian rhythm of firing rate recorded from single cells in the rat suprachiasmatic brain slice. *Brain Res.* 245:198–200.

Gribkoff, V. K., Pieschl, R. L., Wisialowski, T. A., van den Pol, A. N., Yocca, F. D. (1998). Phase shifting of circadian rhythms and depression of neuronal activity in the rat suprachiasmatic nucleus by neuropeptide Y: mediation by different receptor subtypes. *J. Neurosci.* 18:3014–3022.

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., Hendriks, J. (1979). Regularly firing neurones in the rat suprachiasmatic nucleus. *Experientia* 35:1597–1598.

Groos, G., Hendriks, J. (1982). Circadian rhythms in electrical discharge of rat suprachiasmatic neurones recorded in vitro. *Neurosci. Lett.* 34:283–288.

Guldner, F. H., Wolff, J. R. (1996). Complex synaptic arrangements in the rat suprachiasmatic nucleus: a possible basis for the "Zeitgeber" and non-synaptic synchronization of neuronal activity. *Cell Tissue Res.* 284:203–214.

Hall, J. C. (1997). Circadian pacemakers blowing hot and cold—but they're clocks, not thermometers. *Cell* 90:9–12.

Hall, A. C., Earle-Cruikshanks, G., Harrington, M. E. (1999). Role of membrane conductances and protein synthesis in subjective day phase advances of the hamster circadian clock by neuropeptide Y. *Eur. J. Neurosci.* 11:3424–3432.

Hamada, T., Liou, S. Y., Fukushima, T., Maruyama, T., Watanabe, S., Mikoshiba, K., Ishida, N. (1999). The role of inositol trisphosphate-induced Ca^{2+} release from IP3-receptor in the rat suprachiasmatic nucleus on circadian entrainment mechanism. *Neurosci. Lett.* 263:125–128.

Harrington, M. E., Hoque, S., Hall, A., Golombek, D., Biello, S. (1999). Pituitary adenylate cyclase activating peptide phase shifts circadian rhythms in a manner similar to light. *J. Neurosci.* 19:6637–6642.

Hastings, M. (2001). Modeling the molecular calendar. *J. Biol. Rhythms* 16:117–123, discussion 124.

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

Herzog, E. D., Takahashi, J. S., Block, G. D. (1998). Clock controls circadian periods in isolated suprachiasmatic nucleus neurons. *Nat. Neurosci.* 1:708–713.

Honma, S., Ikeda, M., Abe, H., Tanahashi, Y., Namihira, M., Honma, K., Nomura, M. (1998). Circadian oscillation of BMAL1, a partner of a mammalian clock gene clock, in rat suprachiasmatic nucleus. *Biochem. Biophys. Res. Commun.* 250:83–87.

Honma, S., Shirakawa, T., Nakamura, W., Honma, K. (2000). Synaptic communication of cellular oscillations in the rat suprachiasmatic neurons. *Neurosci. Lett.* 294:113–116.

Honma, S., Kawamoto, T., Takagi, Y., Fujimoto, K., Sato, F., Noshiro, M., Kato, Y., Honma, K-I. (2002). Dec1 and Dec2 are regulators of the mammalian molecular clock. *Nature* 419:841–844.

Inouye, S. T., Kawamura, H. (1979). Persistence of circadian rhythmicity in a mammalian hypothalamic "island" containing the suprachiasmatic nucleus. *Proc. Natl. Acad. Sci. USA* 76:5962–5966.

Inouye, S. T., Kawamura, H. (1982). Characteristics of a circadian pacemaker in the suprachiasmatic nucleus. *J. Comp. Physiol. [A]* 146:153–160.

Jacomy, H., Burlet, A., Bosler, O. (1999). Vasoactive intestinal peptide neurons as synaptic targets for vasopressin neurons in the suprachiasmatic nucleus. Double-label immunocytochemical demonstration in the rat. *Neuroscience* 88:859–870.

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.

Jahnsen, H., Llinas, R. (1984). Ionic basis for the electro-responsiveness and oscillatory properties of guinea-pig thalamic neurones in vitro. *J. Physiol. (Lond)* 349:227–247.

Jansen, K., Van der Zee, E. A., Gerkema, M. P. (2000). Being circadian or not: vasopressin release in cultured SCN mirrors behavior in adult voles. *Neuroreport* 11:3555–3558.

de Jeu, M. T., Pennartz, C. M. (1997). Functional characterization of the H-current in SCN neurons in subjective day and night: a whole-cell patch-clamp study in acutely prepared brain slices. *Brain Res.* 767:72–80.

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.

de Jeu, M., Hermes, M., Pennartz, C. (1998). Circadian modulation of membrane properties in slices of rat suprachiasmatic nucleus. *Neuroreport* 9:3725–3729.

Jiang, Z. G., Nelson, C. S., Allen, C. N. (1995). Melatonin activates an outward current and inhibits Ih in rat suprachiasmatic nucleus neurons. *Brain Res.* 687:125–132.

Jiang, Z. G., Yang, Y., Liu, Z. P., Allen, C. N. (1997a). Membrane properties and synaptic inputs of suprachiasmatic nucleus neurons in rat brain slices. *J. Physiol. (Lond)* 499 (Pt 1):141–159.

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

Kalsbeek, A., van der Vliet, J., Buijs, R. M. (1996). Decrease of endogenous vasopressin release necessary for expression of the circadian rise in plasma corticosterone: a reverse microdialysis study. *J. Neuroendocrinol.* 8:299–307.

Kim, Y. I., Dudek, F. E. (1992). Intracellular electrophysiological study of suprachiasmatic nucleus neurons in rodents: inhibitory synaptic mechanisms. *J. Physiol. (Lond)* 458:247–260.

Kim, Y. I., Dudek, F. E. (1993). Membrane properties of rat suprachiasmatic nucleus neurons receiving optic nerve input. *J. Physiol. (Lond)* 464:229–243.

King, D. P., Takahashi, J. S. (2000). Molecular genetics of circadian rhythms in mammals. *Annu. Rev. Neurosci.* 23:713–742.

Lakin-Thomas, P. L. (2000). Circadian rhythms: new functions for old clock genes. *Trends Genet.* 16:135–142.

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

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

Lowrey, P. L., Shimomura, K., Antoch, M. P., Yamazaki, S., Zemenides, P. D., Ralph, M. R., Menaker, M., Takahashi, J. S. (2000). Positional syntenic cloning and functional characterization of the mammalian circadian mutation tau. *Science* 288:483–492.

Lundkvist, G. B., Kristensson, K., Hill, R. H. (2002). The suprachiasmatic nucleus exhibits diurnal variations in spontaneous excitatory postsynaptic activity. *J. Biol. Rhythms* 17:40–51.

Meijer, J. H., Watanabe, K., Detari, L., Schaap, J. (1996). Circadian rhythm in light response in suprachiasmatic nucleus neurons of freely moving rats. *Brain Res.* 741:352–355.

Meijer, J. H., Schaap, J., Watanabe, K., Albus, H. (1997). Multiunit activity recordings in the suprachiasmatic nuclei: in vivo versus in vitro models. *Brain Res.* 753:322–327.

Meijer, J. H., Watanabe, K., Schaap, J., Albus, H., Detari, L. (1998). Light responsiveness of the suprachiasmatic nucleus: long-term multiunit and single-unit recordings in freely moving rats. *J. Neurosci.* 18:9078–9087.

Michel, S., Geusz, M. E., Zaritsky, J. J., Block, G. D. (1993). Circadian rhythm in membrane conductance expressed in isolated neurons. *Science* 259:239–241.

Moga, M. M., Moore, R. Y. (1997). Organization of neural inputs to the suprachiasmatic nucleus in the rat. *J. Comp. Neurol.* 389:508–534.

Moore, R. Y. (1996). Entrainment pathways and the functional organization of the circadian system. *Prog. Brain Res.* 111:103–119.

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

Nakamura, W., Honma, S., Shirakawa, T., Honma, K.-I. (2002). Clock mutation lengthens the circadian period without damping rhythms in individual SCN neurons. *Nat. Neurosci.* 5:399–400.

Obrietan, K., van den Pol, A. (1999). $GABA_B$ receptor-mediated regulation of glutamate-activated calcium transients in hypothalamic and cortical neuron development. *J. Neurophysiol.* 82:94–102.

Palm, I. F., Van Der Beek, E. M., Wiegant, V. M., Buijs, R. M., Kalsbeek, A. (1999). Vasopressin induces a luteinizing hormone surge in ovariectomized, estradiol-treated rats with lesions of the suprachiasmatic nucleus. *Neuroscience* 93:659–666.

Parpura, V., Haydon, P. G. (2000). From the cover: physiological astrocytic calcium levels stimulate glutamate release to modulate adjacent neurons. *Proc. Natl. Acad. Sci. USA* 97:8629–8634.

Pennartz, C. M., Bierlaagh, M. A., Geurtsen, A. M. (1997). Cellular mechanisms underlying spontaneous firing in rat suprachiasmatic nucleus: involvement of a slowly inactivating component of sodium current. *J. Neurophysiol.* 78:1811–1825.

Pennartz, C. M., De Jeu, M. T., Geurtsen, A. M., Sluiter, A. A., Hermes, M. L. (1998). Electrophysiological and morphological heterogeneity of neurons in slices of rat suprachiasmatic nucleus. *J. Physiol. (Lond)* 506(Pt 3):775–793.

Pennartz, C., de Jeu, M., Bos, N., Schaap, J., Geurtsen, A. (2002). Diurnal modulation of pacemaker potentials and calcium current in the mammalian circadian clock. *Nature* 416:286–290.

van den Pol, A. N. (1980). The hypothalamic suprachiasmatic nucleus of rat: intrinsic anatomy. *J. Comp. Neurol.* 191:661–702.

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

van den Pol, A. N., Gorcs, T. (1986). Synaptic relationships between neurons containing vasopressin, gastrin-releasing peptide, vasoactive intestinal polypeptide, and glutamate decarboxylase immunoreactivity in the suprachiasmatic nucleus: dual ultrastructural immunocytochemistry with gold-substituted silver peroxidase. *J. Comp. Neurol.* 252:507–521.

Preitner, N., Damiola, F., Molina, L. L., Zakany, J., Duboule, D., Albrecht, U., Schibler, U. (2002). The orphan nuclear receptor REV-ERBalpha controls circadian transcription within the positive limb of the mammalian circadian oscillator. *Cell* 110:251–260.

Reppert, S. M., Weaver, D. R. (2001). Molecular analysis of mammalian circadian rhythms. *Annu. Rev. Physiol.* 63:647–676.

Reppert, S. M., Weaver, D. R. (2002). Coordination of circadian timing in mammals. *Nature* 418:935–941.

Sah, P. (1996). Ca^{2+}-activated K^+ currents in neurones: types, physiological roles and modulation. *Trends Neurosci.* 19:150–154.

Schaap, J., Bos, N. P., de Jeu, M. T., Geurtsen, A. M., Meijer, J. H., Pennartz, C. M. (1999). Neurons of the rat suprachiasmatic nucleus show a circadian rhythm in membrane properties that is lost during prolonged whole-cell recording. *Brain Res.* 815:154–166.

Schaap, J., Albus, H., Eilers, P. H., Detari, L., Meijer, J. H. (2001). Phase differences in electrical discharge rhythms between neuronal populations of the left and right suprachiasmatic nuclei. *Neuroscience* 108:359–363.

Schwartz, W. J., Davidsen, L. C., Smith, C. B. (1980). In vivo metabolic activity of a putative circadian oscillator, the rat suprachiasmatic nucleus. *J. Comp. Neurol.* 189:157–167.

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

Senseman, D. M., Rea, M. A. (1994). Fast multisite optical recording of mono- and polysynaptic activity in the hamster suprachiasmatic nucleus evoked by retinohypothalamic tract stimulation. *Neuroimage* 1:247–263.

Shearman, L. P., Sriram, S., Weaver, D. R., Maywood, E. S., Chaves, I., Zheng, B., Kume, K., Lee, C. C., van der Horst, G. T., Hastings, M. H., Reppert, S. M. (2000). Interacting molecular loops in the mammalian circadian clock. *Science* 288:1013–1019.

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

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

Shibata, S., Oomura, Y., Kita, H., Hattori, K. (1982). Circadian rhythmic changes of neuronal activity in the suprachiasmatic nucleus of the rat hypothalamic slice. *Brain Res.* 247:154–158.

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

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

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

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

Strecker, G. J., Wuarin, J. P., Dudek, F. E. (1997). $GABA_A$-mediated local synaptic pathways connect neurons in the rat suprachiasmatic nucleus. *J. Neurophysiol.* 78:2217–2220.

Tamada, Y., Tanaka, M., Munekawa, K., Hayashi, S., Okamura, H., Kubo, T., Hisa, Y., Ibata, Y. (1998). Neuron-glia interaction in the suprachiasmatic nucleus: a double labeling light and electron microscopic immunocytochemical study in the rat. *Brain Res. Bull.* 45:281–287.

Thomson, A. M., West, D. C. (1990). Factors affecting slow regular firing in the suprachiasmatic nucleus in vitro. *J. Biol. Rhythms* 5:59–75.

Tischkau, S. A., Gallman, E. A., Buchanan, G. F., Gillette, M. U. (2000). Differential cAMP gating of glutamatergic signaling regulates long-term state changes in the suprachiasmatic circadian clock. *J. Neurosci.* 20:7830–7837.

van den Top, M., Buijs, R., Ruijter, J., Delagrange, P., Spanswick, D., Hermes, M. L. (2002). Melatonin generates an outward potassium current in rat suprachiasmatic nucleus neurones in vitro independent of their circadian rhythm. *Neuroscience* 107:99–108.

de Vries, M. J., Treep, J. A., de Pauw, E. S., Meijer, J. H. (1994). The effects of electrical stimulation of the optic nerves and anterior optic chiasm on the circadian activity rhythm of the Syrian hamster: involvement of excitatory amino acids. *Brain Res.* 642:206–212.

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.

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.

Wheal, H. V., Thomson, A. M. (1984). The electrical properties of neurones of the rat suprachiasmatic nucleus recorded intracellularly in vitro. *Neuroscience* 13:97–104.

Yamazaki, S., Kerbeshian, M. C., Hocker, C. G., Block, G. D., Menaker, M. (1998). Rhythmic properties of the hamster suprachiasmatic nucleus in vivo. *J. Neurosci.* 18:10709–10723.

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.