CHRONOBIOLOGY INTERNATIONAL, 18(4), 579–600 (2001)

REVIEW

CELLULAR COMMUNICATION AND COUPLING WITHIN THE SUPRACHIASMATIC NUCLEUS

Stephan Michel[1,2] and Christopher S. Colwell[2,*]

[1]Institut für Zoologie, Universität Leipzig, Talstr. 33,
04103 Leipzig, Germany
[2]Mental Retardation Research Center, Department of Psychiatry
and Biobehavioral Sciences, University of California—Los Angeles,
Los Angeles, California

ABSTRACT

In mammals, the part of the nervous system responsible for most circadian behavior can be localized to a pair of structures in the hypothalamus known as the suprachiasmatic nucleus (SCN). Importantly, when SCN neurons are removed from the organism and maintained in a brain slice preparation, they continue to generate 24h rhythms in electrical activity, secretion, and gene expression. Previous studies suggest that the basic mechanism responsible for the generation of these rhythms is intrinsic to individual cells in the SCN. If we assume that individual cells in the SCN are competent circadian oscillators, it is obviously important to understand how these cells communicate and remain synchronized with each other. Cell-to-cell communication is clearly necessary for conveying inputs to and outputs from the SCN and may be involved in ensuring the high precision of the observed rhythm. In addition, there is a growing body of evidence that a number of systems-level phenomena could be dependent on the cellular communication between circadian pacemaker neurons. It is not yet known how this cellular synchronization occurs, but it is likely that more than one of the already proposed mechanisms is utilized. The purpose of this review is to summarize

*Corresponding author. C. S. Colwell, Mental Retardation Research Center, University of California—Los Angeles, 760 Westwood Plaza, Los Angeles, CA 90024-1759. Fax: (310) 206-5060; E-mail: ccolwell@mednet.ucla.edu

briefly the possible mechanisms by which the oscillatory cells in the SCN communicate with each other. (*Chronobiology International, 18(4), 579–600, 2001*)

Key Words: Cellular communication; Cellular oscillations; Circadian rhythms; Coupling; SCN

INTRODUCTION: WHY STUDY COUPLING?

The circadian clock regulates many aspects of an organism's behavior and physiology. In mammals, the part of the nervous system responsible for most circadian behavior can be localized to a pair of structures in the hypothalamus known as the suprachiasmatic nucleus (SCN; for review, see Ref. 1). Importantly, when SCN cells are removed from the organism and maintained in a brain slice preparation, they continue to generate 24h rhythms in electrical activity, secretion, and gene expression (2).

Previous studies suggest that the basic mechanism responsible for the generation of these rhythms is intrinsic to individual cells in the SCN (3) and perhaps to other cell types as well (4). The core molecular machinery driving these cellular oscillations appears to be a negative feedback loop operating at the transcriptional/translational levels (5,6). Given this rapid development in our understanding, it is expected that a great deal of attention will now focus on understanding the intracellular processes responsible for the generation of the circadian rhythm.

Nevertheless, cell-to-cell communication is necessary for conveying inputs to and outputs from the SCN and may be involved in ensuring the high precision of the observed rhythm. Mathematical models already predicted the influence of coupling individual oscillators on the precision of the free-running period τ, adapting to changes of photoperiod and aftereffects of previous zeitgeber cycles (7–10). In addition, there is a growing body of evidence that a number of systems-level phenomena could be dependent on the cellular communication between circadian pacemaker neurons. The ability of the clock to measure changes in day length and the phenomenon of splitting of the activity rhythm in two or more components seems to have a physiological correlate in the coupling of different cell populations within the SCN (11–13). Thus, to our minds, to answer many of the core questions about the mammalian circadian timing system we must develop a better understanding of cellular communication within the SCN.

The SCN are bilaterally paired nuclei located just above the optic chiasm and lateral to the third ventricle (Fig. 1). The number of neurons in the SCN has been estimated to range from 15 to 20,000. SCN neurons have a simple dendritic structure, with most cells exhibiting a bipolar morphology, although cells with monopolar and radial structure were also observed (14).

While it is still difficult to come up with a definitive "circuit diagram" for the SCN, anatomical studies support the subdivision of the rat SCN into at least two subdivisions (15,16). There is a dorsomedial division, or shell, with small

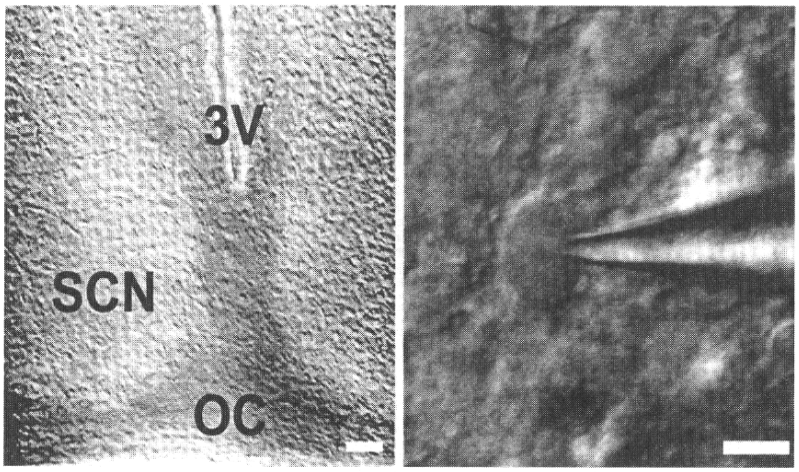

Figure 1. Neurons in SCN brain slices visualized by infrared differential interference contrast (IR-DIC) videomicroscopy. Left: image of SCN under lower power magnification (bar = 100 μM; 3V, third ventricle; OC, optic chiasm). Right: Higher power view of an SCN neuron in same slice during whole-cell patch clamp recording (bar = 10 μm). The IR-DIC microscopy allows a clear view of the soma and, in some cases, processes of SCN cells. We have been using this technology to identify cells in the SCN region for further analysis and to exclude cells from the surrounding hypothalamic regions. Tissue from 14-day-old rat.

cells (10–12 μm in diameter) that typically express vasopressin (VP). These cells are tightly packed, with cell bodies in close contact with their neighbors (somato-somatic appositions). The shell sits atop a ventrolateral division, or core, which contains cells that express vasoactive intestinal peptide (VIP) or gastrin-releasing peptide (GRP). These neurons (12–15 μm in diameter) are found at a lower density, and their cell bodies are in close contact with glia.

Moreover, the inputs and outputs to these two regions also appear to be distinct. Both retinal and thalamic inputs, as well as serotonergic inputs from the raphe, mainly project to cells in the core (17). In contrast, the shell is receiving inputs mainly from the cortex, basal forebrain, and hypothalamus.

Interestingly, a recent study also emphasizes that efferent fibers projecting from the SCN segregate to distinct targets (18). For example, projections from the shell largely target the dorsomedial hypothalamus (DMH) and medial subparaventricular zone (sPVZ), whereas the core projects more to the lateral sPVZ.

Overall, evidence from cytoarchitecture, peptide expression, and projections appear to support the division of the SCN into at least two populations (Fig. 2). However, the question of whether these anatomically defined subdivisions of cells are also physiologically distinct remains to be answered as most electrophysiological studies are not coupled to the analysis of peptide content. One notable exception comes from a recent study by Pennartz and coworkers (19), who described the physiological properties of VP-containing cells using patch-clamp methodology and found them generally to be similar to cells in other regions of the SCN.

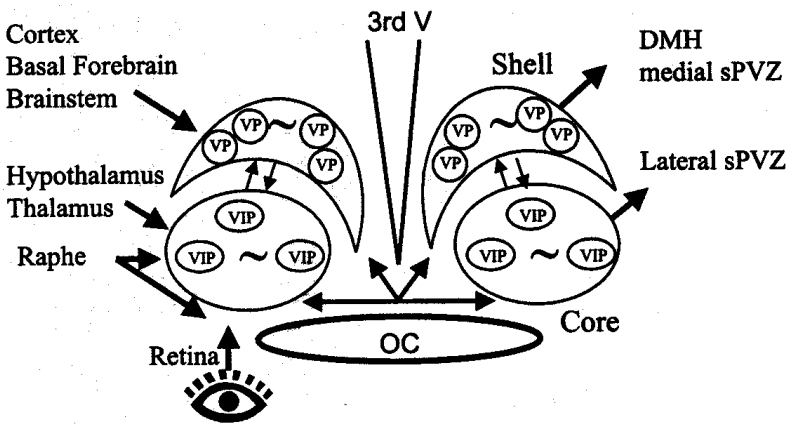

Figure 2. Schematic of anatomical organization of the SCN. This model is based largely on the scheme of Moore and colleagues, who view the SCN as forming two discrete circuits: the core and the shell (see Refs. 17 and 18). Although there are a number of differences between these two cell populations, one of the most apparent is peptide expression. Neurons in the shell largely express vasopressin (VP), while neurons in the core express vasoactive intestinal peptide (VIP). A number of other peptides are also expressed in the SCN. The core receives direct retinal input through the retinohypothalamic tract, indirect photic input through the intergeniculate leaflet of the thalamus (IGL), and serotonergic input from the raphe. The shell receives input from other regions, including the cortex, basal forebrain, and brainstem. The targets of these two regions also appear to be distinct. The shell sends efferents to the dorsomedial hypothalamic nucleus (DMH) and medial subparaventricular zone (sPVZ), while the core largely projects to the lateral sPVZ. Evidence favors reciprocal connections between the core and shell, although the core-to-shell projections appear to predominate. The core and shell of the two SCN nuclei are connected via commissural projections. Evidence suggests that the basic ability to generate circadian oscillations is an intracellular property of SCN neurons in both the core and shell regions.

One of the critical questions about this pacemaker system is whether these cells generate the rhythm through an intracellular or a network process. The answer is not yet known, but several lines of evidence suggest that cell-cell synaptic interactions are not important for the generation of the daily rhythm. First, local injection of tetrodotoxin (TTX) into the SCN region in vivo blocked expression and photic regulation of a circadian rhythm in drinking behavior (20). TTX blocks voltage-sensitive sodium channels and prevents synaptic communication in neural tissue. However, when these treatments ended, the rhythms continued from a phase, which suggested that the SCN oscillator was undisturbed by the experimental manipulation. Similar results were obtained with in vitro SCN preparations in which the expression of circadian rhythms of secretion and spontaneous neural activity were blocked by TTX, but the oscillator itself appeared to be unaffected (3,21,22). In addition, the cells of the SCN generate rhythms in glucose utilization early in the development of the nucleus (23), prior to the majority of synapse formation. Furthermore, circadian rhythm in hibernating ground squirrels are observed at temperatures at which neurons can no longer generate action potentials in the SCN (24). Finally, disassociated SCN cells in

culture retain the ability to generate circadian oscillations (3,25–28). Under these conditions, many of the SCN cells expressed circadian oscillations in spontaneous neural activity that drift out of phase with one another as if each cell contained an independent oscillator. The simplest interpretation of these collective data is that cell-cell interactions are important for the inputs to and outputs from the SCN oscillator, but are not responsible for the generation of the circadian rhythm itself. However, resolution of this issue will have to await the demonstration that single, isolated SCN cells retain the ability to generate circadian oscillations.

The mammalian circadian system did not evolve independently, and comparative evidence is also consistent with the possibility that generation of circadian oscillations is an intracellular, rather than a network, process. Certainly, single-cell organisms like the dinoflagellate *Gonyaulax polyedra* and cyanobacteria exhibit circadian oscillations, so clearly these rhythms can be generated intracellularly (29,30). Further support comes from studies of the marine mollusk *Bulla gouldiana* (31). The eyes of this organism contain an oscillator that drives a circadian rhythm of spontaneous compound action potentials in the optic nerve. A population of electrically coupled cells known as basal retinal neurons (BRNs) is responsible for the generation of this rhythm through a daily cycle in the membrane potential of the BRNs. Isolated BRNs in culture continue to show a circadian rhythm in membrane conductance (32), thus demonstrating that single cells in culture retain the ability to generate a circadian oscillation.

If we assume that individual cells in the SCN are competent circadian oscillators, it is obviously important to understand how these cells communicate and remain synchronized with each other. One striking feature of the circadian rhythms in electrical activity recorded from cultured SCN neurons is the high variability in both the period and phase expressed by the individual cellular oscillators in comparison to rhythms from SCN explants (25) or ultimately behavioral rhythms (27). For this population of SCN oscillators to function adaptively, the clock cells must be synchronized. This immediately raises the question of how this cellular synchronization occurs. The answer to this question is not yet known, although judging from studies on cell signaling pathways in other systems, we would also expect that more than one of the proposed mechanisms is utilized as redundancy in cellular signaling appears to be the rule rather than the exception. The purpose of this review is to summarize briefly the possible pathways by which the oscillatory cells in the SCN communicate with each other (previously reviewed in Refs. 33–35).

INTRINSIC TRANSMITTERS

γ-Aminobutyric Acid as a Putative Coupling Agent

The amino acid γ-aminobutyric acid (GABA) is thought to mediate much of the inhibitory synaptic transmission in the brain. The synaptic release of GABA and subsequent activation of $GABA_A$ receptors opens an ion channel per-

meable to chloride. The resulting hyperpolarization, as well as the underlying increase in conductance, inhibits the cell's electrical activity and makes the neuron less responsive to excitatory input. GABA is the major transmitter used by SCN neurons. Since most, if not all, neurons in the SCN release GABA (36,37), we must start with the assumption that these cells are communicating through inhibitory signaling. Furthermore, since most SCN neurons project to other cells in the SCN, the typical SCN neuron may be best described as an inhibitory interneuron. During the day, SCN neurons are receiving almost continuous inhibitory synaptic input, most of which is mediated by $GABA_A$ receptors (14,38). This presumed increase in GABAergic tone in the SCN during the day may play a role in preventing depolarizing agents, like glutamate, from producing phase shifts during the day. However, in some ways, this brings up a puzzling situation in which the SCN neuron is showing its highest rate of spontaneous activity during the day when it also would be receiving powerful inhibitory synaptic inputs from other cells.

One possible solution to this puzzle was raised by Wagner and coworkers (39) who suggested that GABA can also act as an excitatory transmitter. Utilizing a slice culture preparation, this group reported that, in the SCN, application of GABA can excite cells through a mechanism dependent on $GABA_A$. Interestingly, the effect of GABA on these cells also appears to vary with the time of day as the excitatory response is seen only during the day. The proposed mechanism involves a daily rhythm in the reversal potential of the GABA current (E_{GABA}) such that during the day E_{GABA} is several millivolts positive relative to the resting membrane potential. Accordingly, during the day, when GABA opens the ion channel, it will cause a chloride outflow, leading to depolarization of the membrane potential and generation of action potentials. The opposite happens during the night as E_{GABA} is negative relative to the resting membrane potential. Diurnal changes in intracellular chloride concentration are perhaps the most likely explanation for fluctuations in E_{GABA}. However, other laboratories were unable to reproduce these findings, and three groups published evidence demonstrating that GABA was always inhibitory in day or night (40). We feel that this issue is not yet resolved, and most recent work supports the idea that different populations of SCN neurons may respond to GABA differently. In a population of cultured SCN neurons, 60% of the neurons contained GABA, yet 90% of the synaptic transmission was excitatory, and the GABA antagonist bicuculline decreased the spontaneous firing of some SCN neurons (41). In our laboratory, we have examined the effects of GABA on intracellular calcium levels of SCN neurons in the acute brain slice preparation and found evidence for GABA-induced transient increases in intracellular calcium concentration in at least a subset of the cells (Ref. 42; Fig. 3). These results are consistent with GABA exerting some excitatory effect at least on the level of SCN circuits, if not on individual cells.

In thinking about the possible role of GABA as a coupling signal for synchronizing pacemaker neurons, two recent papers are of special interest (41,43).

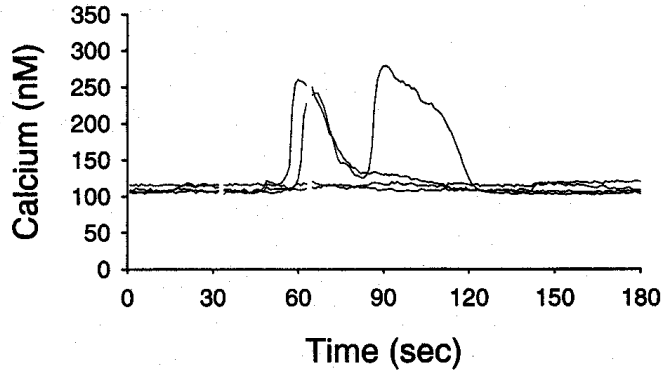

Figure 3. Examples of GABA-evoked transients in intracellular Ca^{2+} concentration $[Ca^{2+}]_i$ measured from SCN cells in a brain slice loaded with the Ca^{2+} indicator dye fura-2. Each line represents data collected from an individual cell. All of these data were collected in the presence of TTX and cadmium, which were included to block voltage-sensitive Na^+ and Ca^{2+} currents. Some SCN cells show an increase in $[Ca^{2+}]_i$ in response to bath application of GABA (100 μM). These GABA-induced Ca^{2+} transients were inhibited by the presence of the $GABA_A$ receptor antagonist bicuculline (data not shown). Data collected during the day from a single SCN slice from a 14-day-old rat.

Both studies maintained cultured SCN cells on multielectrode plates that allow recording circadian rhythms in spontaneous action potentials from several neurons simultaneously. Using this preparation, Liu and Reppert (43) reported that application of GABA inhibited the firing rate and caused phase shifts of individual SCN neurons. They then demonstrated that the daily treatment of these cultured cells with GABA was sufficient to synchronize the cell population. This result suggests that GABA could act as a coupling agent to synchronize SCN oscillators. However, periodic application of any agent hyperpolarizing the membrane potential of the neurons would most likely lead to the same result of synchronized activity. A second study also utilized a similar preparation and found that some pairs of SCN neurons expressed synchronized circadian firing rhythms (41). The vast majority of these pairs (42 of 45) showed a positive cross-correlation, indicating excitatory coupling. Simply put, a positive cross-correlation means that when one of the pairs fired an action potential, it was highly likely that its partner also fired an action potential within a given time window typical for monosynaptic transmission. The underlying mechanism is not yet clear, but the authors suggest a role for GABA based on two experimental observations. First, application of cadmium blocked the positive cross-correlation in 4 of 12 neural pairs. Cadmium is a blocker of voltage-sensitive calcium channels that, through this mechanism, would block most synaptic transmission. Second, the application of the $GABA_A$ receptor antagonist bicuculline blocked the synchronized firing of 2 of 7 pairs. Together, these results are suggestive not only of some role for synaptic transmission and GABA in synchronizing these neurons, but also clearly point out that there must be other mechanisms involved.

Possible Role of Neuropeptides as Coupling Molecules

Many synapses that utilize amino acid transmitters also co-release neuroactive peptides. Nowhere is this more apparent than within the SCN, where it appears that every synaptic connection contains both a classical and a peptide transmitter. Within the SCN, an unusually high number of neuropeptides is expressed. As described above, two of the most prominent are VP and VIP, which help define subdivisions within the nucleus. Some of these peptides, like VP, are secreted into the third ventricle and probably work at a distance. Others, like VIP, are likely to be confined to more discrete regions. In either case, neuropeptides are expected to be released under different conditions and to transmit other types of information than classical transmitters (44). For example, the classical transmitter may be released by low-frequency stimulation, while the peptide requires a burst of action potentials. Once released, the peptides also tend to exert slower postsynaptic effects not necessarily restricted to the synaptic cleft. It is probably fair to say that we are still in the early stages of understanding the physiological roles played by these peptides in the SCN or in the nervous system in general.

The shell (dorsomedial) region of the SCN contains a high density of VP-synthesizing neurons (45–48). These cells release VP with a clear circadian rhythm that has been measured in vivo in the cerebrospinal fluid (e.g., Ref. 49) and in vitro (50,51). A rhythm in the peptide content and mRNA expression has also been characterized (52). Functionally, VP neurons have been suggested to mediate the circadian regulation of neuroendocrine functions like corticosterone release (e.g., Ref. 53) and drinking (54). Within the SCN, VP is likely to play a modulatory role in regulation of electrical activity. Application of VP increased the firing rate of many SCN neurons (around 40%), while application of a V1 receptor antagonist reduced spontaneous activity (55). The cell type of the responding cells was not known, so there could be autoreceptors on the VP-releasing cells that modulate their own firing rate or receptors on VIP-releasing cells.

While VP may function as a modulator, it is unlikely to be required for the generation of circadian rhythms. Studies of two genetically mutated animals have demonstrated that behavioral rhythms can exist in the absence of a rhythm in VP. First, in the VP-deficient Brattleboro rat, normal motor and drinking activity rhythms have been observed (56). Second, in CLOCK mutant mice, VP levels are low and nonrhythmic (57), yet this animal still shows a rhythm for 2 weeks when placed in constant darkness (58). Of course, as in all genetically altered animals, the interpretation of these results has to be tempered by the possibility of compensation occurring by another signaling pathway. Nevertheless, the current data best support a role for VP as a modulator within the SCN and a potential mediator of some of the SCN outputs.

VIP-containing neurons are primarily located in the core (ventrolateral) regions of the SCN. Cells in this region receive input from the retina (both directly and indirectly), as well as from the raphé. The levels of VIP within SCN neurons can be regulated by light, which causes a decrease in protein and RNA (59).

These cells make synaptic connections with other neurons within the SCN and the receptors to VIP, the so-called pituitary adenylate cyclase activating polypeptide (PACAP) type II receptors, are widely distributed within the SCN (60,61). Functionally, the administration of VIP alone or in combination with other peptides may cause phase shifts of the circadian rhythm of wheel-running activity in the hamster SCN (see Ref. 62, but also see Ref. 63). Physiologically, limited information is available about the effects of VIP on SCN neurons. However, VIP is a potent stimulator of adenylate cyclase, and in other CNS regions, VIP has been shown to modulate glutamatergic and GABAergic synaptic transmission, as well as intrinsic voltage-sensitive potassium and calcium currents. Given this background, it would be surprising if VIP did not play an important modulatory role in regulating cell-to-cell communication within the SCN.

In addition to VP and VIP, a number of other peptides and growth factors are expressed in SCN neurons, including GRP (64,65), the peptide histidine isoleucine (45), somatostatin (47), substance P (46), neurotensin (47), nerve growth factor (66), and orphan-FQ/nociceptin (67). In many cases, these peptides appear to be co-localized with amino acid transmitters and presumably function as signaling molecules. One peptide that has recently received some attention is GRP, which is expressed in neurons in the core (ventrolateral) region of the SCN. Application of GRP excited many SCN neurons in vitro and can cause phase shifts of the circadian system in vivo (68). But questions of how this or any other peptide performs in the local SCN circuits and their role in circadian function have not yet been resolved. The role of peptides in the SCN has been reviewed previously (69). The SCN, with its clearly defined circadian function and behavioral outputs, is an excellent location to start exploration of the function of peptide signaling molecules.

GASES: NITRIC OXIDE

Nitric oxide (NO) is a gaseous signaling molecule and, once synthesized, can pass through membranes and alter the biochemistry of surrounding cells. The distance this signal travels is apparently limited by the half-life of NO. Several studies have now shown that the enzyme responsible for the production of this gas, nitric oxide synthase (NOS), is present in the SCN and co-localized with VIP (70–72). Another population of NOS-immunoreactive cells is found among the glial fibrillary acidic protein (GFAP)–immunoreactive astrocytes (73). NOS inhibitors prevent light- and *N*-methyl-D-aspartic acid (NMDA)–induced phase shifts of circadian rhythms in vivo and glutamate- and NMDA-induced phase shifts in vitro (74,75).

While the emphasis of the previous work was on the role of NO in mediating the effect of light on the circadian system, this gas could also be involved in coupling SCN cells. In neurons of the supraoptic nucleus (SON), NO induction caused a fourfold increase in the strength of electrical coupling (76). Any signal

that increases calcium in the NOS-containing cell could trigger the synthesis of NO through a calcium/calmodulin-dependent kinase II mechanism. Once synthesized, NO can diffuse across the cell membrane and regulate the biochemistry of surrounding cells, typically through cGMP-dependent pathways. There has been at least one report that NOS activity expresses a daily rhythm that peaks during the day (77), when resting calcium levels are also at their peak (42). Furthermore, inhibition of NOS disrupts the rhythm of drinking in rats (78). So, while the experimental evidence more clearly indicates a role for NO in mediating the phase-shifting effects of light, it may also have some role in coupling SCN neurons.

LOW-RESISTANCE PATHWAYS: ELECTRICAL COUPLING

Undoubtedly, chemical synaptic mechanisms are probably the predominant way of communication within the SCN, as described above. However, it is important to note that, in a solution with a low concentration of Ca^{2+}, SCN neurons exhibit loosely synchronized bursts of action potentials (79). In addition, a circadian rhythm in glucose utilization is present in the SCN prior to synapse formation in the developing rat (23,80). Reducing Ca^{2+}, as well as the presence of TTX as mentioned above, prevents conventional chemical synaptic transmission; coupled with the developmental results, these studies raise the possibility that another mechanism underlying cell-to-cell communication is in operation within the SCN. We believe that one such mechanism may be the presence of gap junctions linking SCN cell populations.

Gap junction channels form the basis of cell-to-cell electrotonic communication in the nervous system. These channels allow the passage of ions and other small molecules (up to 1 kDa) between coupled cells and function to connect cells both electrically and metabolically. A gap junction channel is formed by two hemichannels, each composed of six connexin (Cx) proteins. Cx are encoded by a multigene family of which Cx26, Cx32, Cx36, and Cx43 are the major isoforms expressed in the developing brain (see, e.g., Refs. 81–85). Recent studies have demonstrated with different techniques that the most likely connexin used in neuronal gap junction in the central nervous system is Cx36 (84,85). Despite the recent progress made by the molecular description of this growing family of gap junction proteins, our understanding of the specific functions of these channels and their regulation is still limited.

Three lines of evidence are consistent with a role for gap junctions in coupling SCN neurons. First, and perhaps most convincingly, SCN neurons exhibit dye coupling in brain slice preparations prepared from both immature (86) and adult (87) rats. Dye coupling is a phenomenon in which a labeled molecule or tracer injected into single cells spreads into surrounding cells. This is a diagnostic feature of cell-to-cell communication mediated by the gap junction. Interestingly, we found that the major subdivisions (ventrolateral/dorsomedial or core/

shell) within the SCN exhibit dye coupling; however, the coupling appears restricted within a subdivision. So, for example, when a neuron from the core was filled with tracer, the label only spread to other cells within the core. This dye coupling can be inhibited by halothane, a known gap junction blocker.

Importantly, our data (86) also suggest that the extent of dye coupling, and presumably gap junction permeability, between SCN cells is activity dependent. The bath applications of two agents (TTX and muscimol) that block electrical activity of SCN neurons through distinct mechanisms both inhibited the extent of dye coupling. These treatments inhibit both electrical activity and synaptic transmission throughout the brain slice. In part to increase the specificity of the manipulation, the voltage clamp was utilized to hold the membrane potential of the originally dye-filled neuron at a more hyperpolarized value than its normal resting potential. As a consequence, these cells exhibited significantly reduced dye coupling. Finally, we took advantage of the naturally occurring circadian rhythm in the frequency of activity in SCN neurons to demonstrate that coupling varies as a function of time of day. The remarkable observation that the amount of coupled cells increased by a factor of 3 during the subjective day provides further support for the proposition that coupling varies as a function of the activity level of these neurons. The mechanisms underlying this regulation are unknown, but must occur quickly on the timescale of minutes. These observations are consistent with a growing body of evidence (e.g., Refs. 88–91) that gap junction channels do not just passively allow the spread of signaling molecules, but instead form an actively regulated communication system. In this context, SCN neurons may provide another example of "state-dependent" variations in gap junction permeability.

Evidence previously reported may be inconsistent with our suggestion that gap junctions serve to couple SCN neurons. First, an electron microscopic (EM) analysis of SCN cells in fixed tissue found gap junctions common between astrocytes, but not neurons (16). However, gap junctions that are small compared to section thickness and infrequent are difficult to identify with EM and may require a different set of techniques. For example, the use of a combination of confocal microscopy and "grid-mapped" freeze fracture EM demonstrated a high incidence of mixed chemical and electrical synapses on neurons within the spinal cord and in different brain areas (84,92). These electrical connections were missed by conventional EM techniques. Second, in a primary cell culture preparation, astrocytes, but not neurons, were found to form gap junctions and to express Cx43 (93). One explanation may be that the preparation of these neurons in cell culture disrupted these direct cell-to-cell connections. While it is possible that SCN cells are coupled via a low-resistance pathway not mediated by gap junctions, it seems more likely that gap junctions are present, but at a low density.

These observations raise the question of the functional significance of the coupling for the circadian timing system; previous studies provide some support for the hypothesis that communication mediated by the gap junction may link

SCN cells. While SCN cells do not exhibit absolutely synchronized action potential generation, the population does show a pronounced daily rhythm, with cells showing spontaneous activity during the day. A careful analysis of firing patterns suggests that the neurons were weakly coupled such that the activity of one cell increases the probability that a neighbor will generate an action potential (79). This weak coupling occurs in the presence of a low Ca^{2+} solution, suggesting a mechanism that does not involve chemical synaptic transmission, but is sufficient to maintain the expression of rhythmic electrical activity. In contrast, in dissociated cultures of rat SCN, it appears that gap junctions couple astrocytes, but not neurons (93). In these conditions, SCN cells continue to express 24h rhythms in electrical activity; however, the cells drift out of phase with each other (3), so that the population can no longer function as a time-keeping system (but also see Ref. 41). This desynchronization occurs in spite of the fact that the cultured cells appear to form functional synaptic connections. In addition, the administration of the gap junction blocker halothane disrupts the circadian rhythms in spontaneous neural activity (94) and in peptide release (95) in rat SCN brain slices, as well as blocks light-induced phase shifts of the circadian locomotor activity rhythm (96).

Comparatively, it is interesting to note that other structures that are known to function physiologically as circadian oscillators are also known to contain cells connected by gap junctions. In vertebrates, this would include the retina and the pineal gland; in nonvertebrates, it would include the eyes of the marine mollusks *Bulla* and *Aplysia*. These observations are at least consistent with the hypothesis that electrical coupling via gap junctions may play a role in the coordination and synchronization of circadian pacemaker cells in the SCN.

Glia

In recent years, there has been a growing appreciation for the complex interactions that can occur between neurons and glia in the nervous system. Glia are certainly influenced by the electrical activity of neurons, and in some cases, glia regulate aspects of cell-to-cell communication between neurons (e.g., Ref. 97).

There are several reasons to think that glia, especially astrocyes, may play a special role in regulating communication between neurons in the SCN. Glial cells stained by GFAP are abundant in the SCN. GFAP, a component of the intermediate filament protein found in the cytoplasm of astrocytes, is a widely used specific marker for astroglial cells. GFAP appears to play a role in astrocyte motility and shape and may be essential in neuron-astrocyte interactions (98). In the mature nervous system, levels of GFAP are generally low unless triggered by events such as neural injury. In contrast, in the SCN, GFAP levels are high and mark a dense astrocytic network (e.g., Ref. 99). Astrocytes in this SCN network have extensive gap junctions and could function as part of a communication pathway linking a rhythmic cell population (100).

Furthermore, astrocytes possess receptors and are regulated by some of the same chemical transmitters that have proven important in regulating SCN neurons, including serotonin and glutamate. This suggestion would be supported by the demonstration of diurnal rhythmicity in some aspects of glial function; indeed, several studies have presented such evidence. The first indication was found by Lavialle and Serviere (101), who described a circadian fluctuation in GFAP distribution in the hamster SCN. Unfortunately, it has been difficult to generalize their observations. For example, an ultrastructural study by Elliot and Nunez (102) did not find time-of-day variation in the amount of glial/neural appositions in the rat SCN. More recently, Moriya et al. (103) reported that they did not find a rhythm in GFAP immunoreactivity in the mouse SCN. Importantly, they also did not see any alteration in the free-running period or in the light-induced phase shift in mice genetically engineered not to express GFAP. Of course, GFAP is just a marker, and the astrocytes may still be performing their normal function for the circadian system.

Glia can modulate communication between neurons in several ways. For example, glia contain transporters for neurotransmitters like GABA and glutamate and are responsible for the uptake of transmitter after release. Regulated changes in transporter function would have the effect of altering the kinetics of a synaptic-evoked response and perhaps the spread of a transmitter outside the bounds of a synaptic connection. In addition, glia can have an impact on neurons through the controlled release of signaling molecules such as NO, as described above. Finally, glia cells may physically alter cell-to-cell contacts. Astrocytes can extend processes that interculate between neurons and alter the physical contacts between cells. This type of physical change can produce relatively fast changes in coupling between neurons. For example, in the SON of the hypothalamus, changes in the reproductive state of the rat are clearly linked to changes in gap junction permeability (89,104). This regulation of gap junction permeability appears to be mediated by rapid changes in glial morphology that "unmasks" gap junction connections and allows synchronized firing among SON neurons. This type of glia/neural interaction could be responsible for rhythmic coupling of neurons in the SCN for there have been several reports of rhythms in astrocyte processes, as described above. Astrocytes can also express cell adhesion molecules, like neural cell adhesion molecule (NCAM), that can regulate cell-cell interactions between neurons.

Polysialylated Form of Neural Cell Adhesion Molecule

In the last few years, there has been an explosion of information suggesting that structural changes in synapse formation can underlie changes in the strength of the synaptic connection between two cells. One of the molecules that has been thought to be involved in these structural changes is a polysialylated form of neural cell adhesion molecule (PSA-NCAM) through its regulation of cell-to-cell

interactions. Interestingly, the SCN is one of the regions of the nervous system that expresses PSA-NCAM at relatively high levels (105). PSA-NCAM-deficient mutant mice show deficits in light entrainment, reduction of the light-induced phase shifts, and the expression of immediate early genes (c-fos), as well as changes in the period of the locomotor activity rhythm in constant darkness (106,107). While there are a number of possible explanations for these results, this last observation is consistent with the hypothesis that PSA-NCAM could be involved in the normal coupling of neurons within the SCN. Mechanistically, PSA-NCAM could regulate the strength of chemical synaptic transmission, as well as mediate structural changes in neurons or glia that could have an impact on electrical transmission via low-resistance pathways, as described above.

Appositions

One of the striking features of SCN morphology is the dense packing of neurons in an unusual way in the central nervous system, although seen in such areas as the granule cell layer of the cerebellum. Anatomical studies have reported somatic apposition in the dorsomedial SCN and axo-dendritic and dendro-dendritic appositions in the ventrolateral SCN (108). These appositions, in which the membranes of two cells are essentially in contact, would certainly make coupling via soluble factors easier and would allow the formation of gap junctions. This physical proximity could also allow communication by more unusual mechanisms, such as changes in electrical fields or changes in potassium concentration in the extracellular space. These types of cellular communication have been discussed previously (33,34). At present, there is no evidence for this kind of supracellular interaction nor the type of laminar organization that would make this sort of effects more likely.

CONCLUSION: CELLULAR COUPLING BETWEEN SUPRACHIASMATIC NEURONS

Coupling is a broad term that describes the mechanisms by which two processes are linked. In terms of the circadian system, there are a number of coupling issues that have occupied the attention of researchers in this field for many years. For example, how is a periodic signal from the environment coupled to the circadian timing system? How is this timing system coupled to its driven outputs, including motor and sensory systems? Finally, given that the circadian system of most organisms appears to be made up of more than one oscillator, how are the multiple circadian oscillators coupled to one another?

With our growing appreciation that the core mechanism involved in generation of circadian rhythms involves a molecular feedback loop that occurs within a single cell, the questions remain, but are now being asked on a smaller, more

detailed scale. For example, we now want to know how signals from the membrane regulate expression of these clock genes and how changes in these gene expression patterns could alter membrane events. Finally, given the anatomical organization of the SCN (cf. Fig. 2), there are a number of questions involving coupling within the SCN that are basically subject to cell-to-cell communication. Studies on coupling between SCN cells should address at least three questions:

1. How are cells within a subdivision coupled?
2. How are cells between different subpopulations coupled?
3. How are cells between the two SCN bilaterally coupled?

Evidence from other systems, as well as work done in the SCN, suggests that the answers to these questions will ultimately implicate multiple, redundant coupling pathways. We believe that understanding these mechanisms will eventually provide us with a physiological explanation for a number of the properties of circadian systems. For example, the long-postulated morning-evening oscillators (8) seem to have their physiological manifestation in different subdivisions of the SCN (11). Another basic feature of circadian clocks, temperature compensation, might be based to some extent on temperature-dependent GABAergic inhibition of pacemaker neurons in the neuronal network (109). And even the magnitude of light-induced phase shifts might be dependent on the degree of coupling (27,106), which again was shown to be modulated actively between day and night (86).

The coupling mechanisms involved in cellular communication within the SCN are not likely to be unique to the circadian system, but this does not diminish their importance. On the contrary, any insights gained as to how cells communicate within the SCN are likely to be broadly applicable to other neural systems. We believe that some features of the circadian system make it an excellent model system to address many core issues in neuroscience research. Many of the behavioral and physiological outputs of the circadian system are precise, quantifiable, and functionally important. This allows the productive use of both neuropharmacologic and genetic approaches. Anatomically discrete and well-defined pathways control these behaviors. Finally, SCN neurons are amenable for detailed cellular and molecular analysis by all of the tools of modern neuroscience. For these reasons, we believe that the circadian system, based in the SCN, is an excellent model system to study cellular communication and then use this knowledge to understand how the nervous system regulates behavior.

ACKNOWLEDGMENT

This work was supported by DFG-Mi 328/2-3 to S. M. and National Institutes of Health grants HL64582, MH59186, HD04612, as well as Whitehall Foundation grant F98P15 to C. S. C.

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