Gap Junctions Between Accessory Medulla Neurons Appear to Synchronize Circadian Clock Cells of the Cockroach Leucophaea maderae

Nils-Lasse Schneider and Monika Stengl

Biology, Animal Physiology Philipps-University of Marburg, Marburg, Germany

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Schneider, Nils-Lasse and Monika Stengl. Gap junctions between accessory medulla neurons appear to synchronize circadian clock cells of the cockroach Leucophaea maderae. J Neurophysiol 95: 1996–2002, 2006. First published November 16, 2005; doi:10.1152/jn.00835.2005. The temporal organization of physiological and behavioral states is controlled by circadian clocks in apparently all eukaryotic organisms. In the cockroach Leucophaea maderae lesion and transplantation studies located the circadian pacemaker in the accessory medulla (AMe). The AMe is densely innervated by γ-aminobutyric acid (GABA)–immunoreactive and peptidergic neurons, among them the pigment-dispersing factor immunoreactive circadian pacemaker candidates. The large majority of cells of the cockroach AMe spike regularly and synchronously in the gamma frequency range of 25–70 Hz as a result of synaptic and nonsynaptic coupling. Although GABAergic coupling forms assemblies of phase-locked cells, in the absence of synaptic release the cells remain synchronized but fire now at a stable phase difference. To determine whether these coupling mechanisms of AMe neurons, which are independent of synaptic release, are based on electrical synapses between the circadian pacemaker cells the gap-junction blockers halothane, octanol, and carbeneoxolone were used in the presence and absence of synaptic transmission. Here, we show that different populations of AMe neurons appear to be coupled by gap junctions to maintain synchrony at a stable phase difference. This synchronization by gap junctions is a prerequisite to phase-locked assembly formation by synaptic interactions and to synchronous gamma-type action potential oscillations within the circadian clock.

INTRODUCTION

In apparently all eukaryotic and at least some procaryotic organisms the temporal organization of physiological and behavioral states is controlled by circadian clocks. In the cockroach Leucophaea maderae lesion and transplantation studies (Reischig and Stengl 2003a; Stengl and Homberg 1994) located the circadian pacemaker in the accessory medulla (AMe), a small neuropil situated in the anterior, ventromedial edge of the medulla of the optic lobe (Homberg et al. 1991; Petri and Stengl 1995; Reischig and Stengl 1996). Similar to the mammalian suprachiasmatic nucleus (SCN) (Moore and Speth 1993; Van den Pol and Tsujimoto 1985), the AMe is densely innervated by γ-aminobutyric acid (GABA)– and peptide-immunoreactive (−ir) neurons (Petri et al. 1995, 2002). Their arborizations form different AMe compartments: the noduli, the internodular, and the shell region (Petri et al. 1995; Reischig and Stengl 1996, 2003b). Both the noduli and the internodular neuropil are densely innervated by the GABA-ir distal tract and by about 25 GABA-ir neurons located in the vicinity of the AMe (Petri et al. 2002; Reischig and Stengl 2003b). Although GABA-ir neurons of the distal tract appear to form the light entrainment pathway from the compound eye (Petri et al. 1995), the GABA-ir neurons in the vicinity of the AMe appear to serve as local interneurons.

Extracellular recordings of the electrical activity of excised accessory medullae (AMae) revealed that circadian pacemaker candidates of the cockroach Leucophaea maderae spike regularly with frequencies between 25 and 70 Hz, reminiscent of the gamma-frequency oscillations of mammalian brain neurons. GABAergic synaptic interactions phase-lock these oscillating neurons and form different assemblies of cells that share the same period (interspike interval) and the same phase (timing of spikes), whereas cells between assemblies differ in phase (Schneider and Stengl 2005). After disruption of synaptic transmission, AMe neurons remained synchronized but obtained a stable phase difference. Therefore additionally nonsynaptic interactions appear to be sufficient for phase coupling of AMe neurons (Schneider and Stengl 2005).

To examine whether gap junctions might be involved in the synchronization of AMe neurons, as in the SCN and other mammalian brain centers (Colwell 2005; Long et al. 2005; Yang and Michelson 2001) we examined the effects of gap-junction blocker on electrical activity in extracellular multunit recordings of excised AMae. The data suggest that synaptic synchronization of AMe neurons occurs through gap-junction-mediated coupling, allowing gamma-type oscillations of the circadian pacemaker network.

METHODS

All experiments were performed on AMae of adult male cockroaches. Breeding colonies of the cockroach (Leucophaea maderae) were kept at the University of Marburg at 30°C and 30% humidity, in light/dark cycles of 12/12 h, with lights on from 7 A.M. to 7 P.M. Animals were provided with unlimited dried dog food, potatoes, and water.

The experimental animals were decapitated and the head capsule was opened to excise the AMe. Details of the preparation were previously described (Schneider and Stengl 2005). The AMe can be recognized at the ventromedial edge of the medulla beneath the bifurcation of a characteristic trachea, as shown in Petri and Stengl (1997, 1999). It was excised with a glass pipette (diameter 150 μm; Flaming/Brown Micropipette Puller, model P-97; Sutter Instruments, Novato, CA). Thus the excised AMe has a diameter and a depth of about 150 μm containing the neuropil of the AMe with about 1,000 associated cells (Reischig and Stengl 2003a,b). As immunocytochemical experiments showed all tissue explants are expected to contain the complete AMe with all associated cells and some associated medulla.

Address for reprint requests and other correspondence: M. Stengl, Biology, Animal Physiology, Philipps-University of Marburg, Karl von Frisch Str., 35032 Marburg, Germany (E-mail: stengl@staff.uni-marburg.de).

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cells. Explants are thus highly enriched for AMe neurons. All experiments were performed at constant light during Zeitgeberzeit ZT 4–9.

A total of 43 AMae (137 experiments) were used for application of vertebrate gap-junction antagonists halothane, carbeneoxalone, and octanol (see following text), or of the chloride channel blocker picrotoxin (PTX). In additional control recordings EtOH (1%) or DMSO (1%) was applied and the ionic conditions were exchanged between normal saline and extracellular solutions without Ca\(^{2+}\) or with high Mg\(^{2+}\) (see following text). All chemicals used were purchased from Sigma-Aldrich (Taufkirchen, Germany).

For the extracellular recordings, the AMe was transferred to a petri dish (diameter 4 cm). The osmolarity of the extracellular saline (156 mM NaCl, 4 mM KCl, 6 mM CaCl\(_2\), 10 mM Hepes, 5 mM glucose, 0.01 g/l phenolred, pH 7.1) was adjusted with mannitol to 380 mOsm. In the Ca\(^{2+}\)-free extracellular solution CaCl\(_2\) was replaced by 1 mM EGTA. In the high-Mg\(^{2+}\) solution CaCl\(_2\) was replaced by 10 mM MgCl\(_2\). Halothane and carbeneoxalone (CBX) were dissolved and diluted in saline or extracellular solution without Ca\(^{2+}\). Octanol and PTX were dissolved in EtOH (final concentration of 0.1–1%) and diluted in the experimental extracellular solutions. The tissue was continuously superfused with 10 ml saline/h at room temperature. Drugs were applied to the tissue by bath application to a chamber with a volume of 5 ml and a flow rate of 30–40 ml/h at the following concentrations: 0.05–0.1% halothane, 0.1–1 mM CBX, 1–2 mM octanol, 0.5 mM PTX.

The extracellular recording technique of excised AMe and data analysis were previously described (Schneider and Stengl 2005). Glass electrodes (0.3–1.5 M\(\Omega\)) connected to an extracellular amplifier (NPI, Tamm, Germany) recorded the extracellular electrical activity of the excised AMe. Multunit action potentials (= events) were recorded as upward and/or downward deflections of the baseline. Recordings sampled different numbers of AMe neurons. Rarely, apparently single-unit recordings were obtained. It cannot be expected that we sampled all AMe neurons at the same time. The output of the amplifier was high-pass filtered (3 Hz) to eliminate electrode offset and low-pass filtered (1.5 kHz) to avoid high-frequency noise and aliasing. The signal was digitized (DIGIDATA 1322A; Axon Instruments, Burlingame, CA) with a sampling rate of 5 kHz, and stored on hard disc for further analysis. Event detection above a given threshold was performed off-line with SPIKE II software (Cambridge Electronic Design, Cambridge, UK).

The mean frequency (number of events/s) was calculated and the interevent-interval distributions were generated using 1 ms bin width, and different periods with a minimum of 100 s, at different time points of the experiment, to identify oscillations and changes in the regularity of the electrical activity.

The oscillation of the firing neurons is defined by the regularity of their action potentials. This means that the time spent between two consecutive action potentials (= the interspike interval) is the period of the oscillation and the time point at which the action potential occurs is the phase of the oscillation. Therefore two neurons are phase-locked (with zero phase difference) if they fire action potentials at exactly the same time point, with the same phase and the same period, or integer multiples of the same period (it would not change the interspike interval if the second cell spikes only at every second action potential of the first cell). Thus their spike amplitudes superimpose in the original recording (larger amplitudes) and a single sharp band occurs in the instantaneous frequency plot (1/interevent interval). Instantaneous frequency (1/interevent interval) plots were calculated over the whole time course of the experiments to visualize the regularity of electrical activity and to analyze the synchrony and phase coupling of the recorded cells. In the instantaneous frequency plot the electrical activity of irregularly spiking neurons, or of neurons spiking with different rates or phases that are not integer multiples of each other, results in a broad cloud of instantaneous frequencies. A defined narrow band of instantaneous frequencies means that all recorded cells fire action potentials very regularly at the same phase and at the same interevent interval or integer multiples thereof (harmonic frequencies). Parallel bands in the instantaneous frequency plot indicate that at least two cells fire with the same or integer multiples of the same interevent interval, but with a stable phase difference. If more than one cell fires very regularly with different noninteger multiples of interevent intervals and with different phase, the bands cross each other (Pikovsk\i et al. 2001). Fusion of parallel bands to one band indicates that the recorded cells now fire with the same phase (action potentials occur at the same time) and the same period (= same interevent intervals). Autocorrelograms were generated, using a bin width of 1 ms for an interval of 1 s over 5- to 30-min periods, to identify rhythmic firing patterns (Grov\e et al. 1978). Regular interevent intervals cause multiple peaks in the autocorrelogram (Tepper et al. 1995). Irregularity eliminates multiple peaks.

RESULTS

In extracellular multiunit recordings of the isolated AMe, the cockroach circadian pacemaker center, the gap-junction antagonists halothane, carbeneoxalone, and octanol were used, to test whether they affect the synchronization of AMe neurons. We show that the gap-junction antagonists desynchronize most, but not all, AMe neurons in the presence and absence of synaptic release. We hypothesize that gap junctions between inhibitory interneurons are essential for phase coupling of circadian pacemaker candidates within the AMe network.

Extracellular recordings of multiunit electrical activity (events) of the excised AMe lasted for several hours. Events were recorded as upward and downward deflections of the baseline (Fig. 1A) depending on the impedance ratio between the recorded neurons, the recording electrode, and the indifferent electrode. Event amplitudes ranged from 50 to 180 \(\mu\)V, whereas the average peak-to-peak noise amplitude was about 40 \(\mu\)V. As described earlier electrical activity within the excised AMe is reversibly blocked by the sodium channel blocker tetrodotoxin (Schneider and Stengl 2005). In about 76% (of 37 preparations) the electrical activity of AMe neurons oscillated regularly (period of the oscillation = interspike interval; phase = occurrence of spikes). Synchronous regular spiking is not recognizable in the mean event frequency (Fig. 1B), but is obvious in instantaneous frequency plots (Fig. 2C), at an extended timescale of the original recordings (Fig. 2D), in interevent-interval distributions (Fig. 2, F–H), and autocorrelation analysis of event times (Fig. 2, I and J). These methods of analysis revealed that recorded AMe neurons were synchronized and fired with constant interevent intervals and at stable phase relationships. Spontaneous activity exhibited one to three distinct parallel bands in the instantaneous frequency plot (see METHODS). Multunit activity resulting in one band in the instantaneous frequency plot originates from synchronized cells firing at the same phase and the same or harmonic frequencies, whereas parallel bands originate from different assemblies of cells firing with the same or harmonic frequencies but with a stable phase difference between assemblies (METHODS).

In 71% of the recordings cells remained synchronized after disruption of synaptic interactions, whereas in 29% of the preparations cells require synaptic interactions for synchronization. Thus as suggested previously, mechanisms of phase coupling in addition to inhibitory synaptic interactions are used to form assemblies, (Schneider and Stengl 2005). To test whether gap-junction–mediated coupling is responsible for
retaining a constant phase relationship after disruption of synaptic release, we applied the gap-junction blocker halothane in the presence of Ca\(^{2+}\)-free extracellular solution (n = 10 in seven preparations) (Fig. 1, A–I). Application of 0.05–0.1% halothane caused a decrease in the amplitude of the electrical events in the original recording (Fig. 1A) and a transient increase and then a strong decrease in the mean frequency (Fig. 1B). The diffusion of distinct bands in the instantaneous frequency plot (Fig. 1C) and simultaneous changes in the mean frequency occurred within 1–3 min in 86% of the preparations.

**FIG. 1.** A–I: gap-junction inhibitors desynchronize accessory medulla (AMe) neurons in the absence of functional synaptic connections. Exchange of extracellular saline to Ca\(^{2+}\)-free extracellular solution (dashed bar) disrupts synaptic release, which results in an increase of activity in the original recording (A) and a rise in the mean frequency (B). In addition the 2 parallel instantaneous frequency bands increase to a higher frequency level in synchrony with a newly appearing third band (C). After additional perfusion with 0.1% halothane (black bar) the mean frequency transiently increases until synchronous activity abruptly ceases (B). Distinct bands in the instantaneous frequency plot rise, broaden, and become diffuse until sudden cease of synchronous activity (C). Original recordings (extended timescales at time points d and e in A, downward deflections of the base line were evaluated) and the autocorrelograms (F–I) show that previously coupled neurons lose phase-locking and fire irregularly during disruption of gap junctions. Same time axes for A–C. Thin bars (f, g, h, and i) indicate the time window for autocorrelation analysis (F, G–I).

**FIG. 2.** A–J: gap-junction antagonist halothane reversibly disrupts phase coupling of AMe neurons in the presence of synaptic interactions. During superfusion with normal saline AMe neurons fire synchronously at the same low frequency and the same phase. This is shown in the original recording (A) and the mean frequency (B). It also becomes apparent in the single band in the instantaneous frequency plot (C), the original trace at an extended timescale at time point D, the sharp peak in the interspike-interval distribution (F), and the multiple peaks in the autocorrelogram (I). During application of 0.1% halothane (black bar in A) the mean frequency increases (B) and the previously distinct band in the instantaneous frequency plot broadens and becomes increasingly irregular with superimposed variable bursts. Expanded timescale (E) and autocorrelogram (J) show that AMe neurons lose stable phase coupling.

(n = 8 in six preparations). The delay of the halothane effect was dose dependent and took longer at lower blocker concentrations (Table 1). Details of the original recordings (Fig. 1, D and E) and autocorrelation analysis after application of 0.1%
halothane revealed that synchronization of neuronal activity is lost (compare Fig. 1, F–I). In 86% of the preparations (n = 7 in six preparations; not shown) the cells started to burst, until complete loss of synchronization occurred. In 71% (n = 5 in five preparations) of the preparations activity ceased completely in the presence of halothane (Fig. 1).

To determine whether coupling by chemical synapses also depends on synchronization by gap junctions (Figs. 2 and 3) we used halothane and other gap-junction blockers in normal saline (n = 40 in 22 preparations). Even in the presence of synaptic interactions, application of 0.1% halothane (n = 20 in 15 preparations) disrupts the synchronization of AMe neurons in 67% of the preparations (Fig. 2; n = 15 in 10 preparations). Halothane changed the amplitude of the electrical events in the original recording (Fig. 2A), first causing an increase of the mean frequency (Fig. 2B) and a broadening of the instantaneous frequency band (Fig. 2C). The diffusion of the instantaneous frequency band was accompanied by the appearance of bursting activity in 40% of these recordings (Fig. 2, C–E). The broadening and shift in the interevent-interval distribution (Fig. 2, F–H) and loss of multiple peaks in the autocorrelogram (Fig. 2: compare I and J) further show loss of coordinated electrical activity. Finally, in almost all preparations, activity ceased completely after application of halothane (not shown). After washout of the gap-junction blocker AMe neurons returned to regular firing with zero phase difference in 70% of the preparations (n = 7 in seven preparations) as can be seen in the narrowing of the instantaneous frequency band (Fig. 2C) and the defined peak in the interevent-interval histogram (Fig. 2H).

To test whether other gap-junction inhibitors mimic the effects of halothane we also used the gap-junction antagonists octanol (n = 4, in three preparations) and carbenoxolone (CBX, n = 14 in nine preparations) in normal saline (Fig. 3). These gap-junction blockers also usually resulted in a dose-dependent, strong, and sustained decline of synchronous electrical activity in normal extracellular saline (Fig. 3, A–D; Table 1). Mostly (but not in all applications) irreversible loss of synchronous activity was caused by application of 0.1–1 mM CBX with dose-dependent time delays (Fig. 3, B and C) (n = 11 in eight preparations). The sustained inhibition of electrical activity caused by CBX was removed and the cells became irregularly active by disrupting synaptic transmission by superfusion with Ca^{2+}-free extracellular solution (Fig. 3D) or by application of the chloride channel blocker PTX (not shown). Also halothane- and octanol-dependent loss of activity could be reversed to irregular activity by Ca^{2+}-free solutions (not shown). Because octanol and PTX were dissolved in EtOH (final concentration: 0.1–1%) respective control experiments were performed. Application of saline with 1% EtOH showed no effects on electrical activity of AMe neurons.

Previous studies showed that GABAergic interneurons coordinate the synchronized, regular spiking of AMe neurons (Petri et al. 2002; Schneider and Stengl 2005). To obtain information whether cells that synchronize AMe neurons independent of GABA are also coupled by gap junctions, we used halothane in the presence of PTX. PTX was previously shown to oppose GABA-dependent inhibitions and thus can be used as GABA_A receptor antagonist in the cockroach (Schneider and Stengl 2005). In the presence of 0.5 mM PTX cells that fired at a low frequency (Fig. 4, A, B, D, F, and J) showed a reversible increase of mean electrical activity after application of 0.1% halothane (Fig. 4, A, B, E, G, and K). In addition, in the presence of PTX halothane resulted in a coordinated broadening of the previously narrow band of instantaneous frequency (Fig. 4B), and reversibly changed the firing mode into regular bursting in 75% of the preparations (n = 8, in three of four preparations) (Fig. 4; compare D and E). Other AMe neurons retained synchrony in the presence of PTX and were not affected by application of halothane (Fig. 4, C, H, I, L, and M) (n = 3, in two preparations).

**DISCUSSION**

Multounit recordings from excised AMae, the circadian pacemaker centers of the cockroach Leucophaea maderae, were used to investigate the effects of gap-junction blockers on synchronization of neuronal spike discharges of circadian pacemaker cells. Evidence is provided that different populations of AMe neurons are coupled by gap junctions maintain-

### TABLE 1. Effects of gap junction antagonists show dose-dependent time delays

<table>
<thead>
<tr>
<th>Blocker</th>
<th>Concentration</th>
<th>Delay, min</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Halothane</td>
<td>0.1%</td>
<td>2.1 ± 1.3</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>0.05%</td>
<td>4.0 ± 0.5</td>
<td>5</td>
</tr>
<tr>
<td>Carbenoxolone</td>
<td>1 mM</td>
<td>2.9 ± 0.2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>0.1 mM</td>
<td>11.9 ± 1.9</td>
<td>6</td>
</tr>
</tbody>
</table>

FIG. 3. A–D: gap-junction antagonists octanol and carbenoxolone (CBX) inhibit AMe neurons in the presence of synaptic interactions. A: application of 2 mM octanol (black bar) reversibly inhibits AMe neurons in normal extracellular saline. B and C: application of CBX causes mostly irreversible inhibitions with dose-dependent time delays. D: neuronal activity inhibited by 0.1 mM CBX resumes in saline without Ca^{2+}, which disrupts synaptic interactions (D).
oscillations with precision on the millisecond scale, firing at frequencies of 25–80 Hz, reminiscent of gamma-frequency oscillations in networks of the mammalian brain.

Electrical synapses appear to be present in the circadian pacemaker center

Our experiments suggest that AMe neurons use gap-junction–mediated coupling as additional mechanism of synchronization. Because different vertebrate gap-junction blockers caused the same effects, dose dependently and reversibly, it is very likely that these gap-junction blockers specifically block cockroach gap junctions. Additional evidence for the presence of gap junctions between cockroach AMe neurons comes from intracellular recordings in the AMe of L. maderae (Loesel and Homberg 2001). Because multiple stainings were obtained in the vast majority of these intracellular recordings it is likely that the injected dye was spreading through gap junctions. Further evidence for the specificity of the vertebrate gap-junction blockers in the invertebrates comes from a comparison of their effects between invertebrate and vertebrate preparations. In the SCN several experimental approaches demonstrated the presence of electrical synapses. Two-cell patch-clamp recordings showed that mammalian SCN neurons are electrically coupled by gap junctions and promote synchronization of electrical activity on the millisecond scale, similar to cockroach AMe neurons (Long et al. 2005). Furthermore, dye coupling experiments, immunocytochemical studies, and mutant analysis showed that gap-junction molecules are present in the SCN and are necessary for synchronization of circadian pacemaker candidates (Colwell 2000; Jiang et al. 1997; Long et al. 2005; Schaap et al. 2003; Shinohara et al. 2000).

Which cells are connected by gap junctions in circadian pacemaker centers?

Neurons of the cockroach circadian pacemaker center exhibit regular spike discharges with precision on the millisecond scale. They form assemblies of phase-locked, synchronously firing AMe cells by GABA release at synapses and by unknown non-(chemically)synaptic mechanisms (Schneider and Stengl 2005). Because in 29% of the preparations AMe neurons desynchronized after disruption of synaptic transmission at least some assemblies appear to be formed solely by chemical synapses. The majority of the AMe cells, however, rely on additional mechanisms of synchronization. Gap-junction blockers caused persistent inhibitions in normal saline, in the presence of functional chemical synapses. These inhibitions could be released by interruption of synaptic transmission and by block of GABA<sub>A</sub> receptors in the presence of gap-junction blockers. Therefore the inhibitions most likely arose from blocker-dependent desynchronization of GABAergic interneurons. Thus apparently at least subpopulations of GABAergic interneurons are synchronized by gap junctions and the electrical synapses are a prerequisite to GABA-dependent assembly formation; however, also in the presence of PTX, when all GABA<sub>A</sub>-receptor–dependent synchronization was already lost, remaining synchronization mechanisms were interrupted and inhibitory interactions were released by gap-junction blockers. Therefore next to GABAergic interneurons there are additional inhibitory interneurons that appear to be coupled by gap

![Image of a figure showing neuronal activity](https://jn.physiology.org/doi/pdf/10.1152/jn.00964.2005)

**FIG. 4.** A–M: in the presence of chloride channel blocker picrotoxin (PTX) the gap-junction antagonist halothane increases and desynchronizes the electrical activity of a subgroup of recorded AMe neurons, whereas other neurons are unaffected. Upward deflections from the baseline are evaluated in C, H, I, L, and M. During the constant presence of 0.5 mM PTX synchronized assemblies of cells could be recorded (A), which formed the sharp bands in the instantaneous frequency plots (B, C). Same time axis is used in A-C. When 0.1% halothane was added (black bar in A) the assembly in B increased its instantaneous frequency and started to burst, whereas the synchronized assemblies in C remained phase locked to a stable phase difference. Original trace at an extended timescale at time points d and e in A further illustrates that bursting develops after addition of halothane. Thin bars in B and C indicate the time windows for autocorrelation analysis and interevent distribution (F–M).
junctions. Very rarely GABA<sub>B</sub>-dependent inhibitions were observed in the AMe, but more often peptide-dependent inhibitions were obtained. Thus it is likely that subpopulations of inhibitory peptidergic neurons of the AMe (such as neurons containing pigment-dispersing factor) might be coupled by electrical synapses (Schneider and Stengl 2005).

The mammalian circadian pacemaker cells also remained weakly coupled after removal of extracellular Ca<sup>2+</sup>, but lost coupling in the presence of gap-junction blockers (Bouskila and Dudek 1993; Colwell 2000; Jiang et al. 1997; Long et al. 2005; Shinohara et al. 2000). As in the cockroach, gap junctions in the SCN couple circadian pacemaker candidates to synchronized phase-locked assemblies. The assemblies of different regions of the SCN are then coupled by synaptic interactions such as by GABAergic and peptidergic neurons (Albus et al. 2005; Colwell 2005; Itri and Colwell 2003). Therefore it is assumed in the circadian pacemaker centers of mammals and, shown here in the cockroach alike, that electrical synapses are a prerequisite for synchronous, regular action potential oscillations with precision on the millisecond scale. However, the functional significance of gap-junction–dependent electrical synchrony in the ultradian range is largely unknown in the circadian pacemaker centers.

Functions of gap-junction–dependent synchronized action potential oscillations in circadian pacemaker centers

More about the function of electrical synapses in neuronal networks is known in other regions of the vertebrate brain. In the locus ceruleus electrotonic coupling synchronizes spontaneous firing of neurons dependent on their firing rates (Alvarez et al. 2002). Computational modeling demonstrated that coupling of nonoscillating cells by gap junctions can generate synchronized membrane potential oscillations (Loewenstein et al. 2001). Thus electrical synapses are widely used in the mammalian brain to generate synchronized neuronal activity, also in the range of 30–80 Hz (Jedlicka and Backus 2005). This so-called gamma rhythm was proposed to be essential for induction of spike-timing–dependent synaptic plasticity, for memory processes, and for “binding” of sensory object features into coherent conscious percepts (Engel and Singer 2001; Jedlicka and Backus 2005).

It is largely unresolved why it is important for circadian pacemaker neurons to express synchronized action potential oscillations with high precision on the millisecond scale in the range of 25–80 Hz, reminiscent of the mammalian gamma rhythms. For the cockroach circadian clock Schneider and Stengl (2005) suggested that neuropeptide-dependent phase control of these regular oscillating action potential rhythms are used for gating different circadian outputs such as circadian locomotor activity rhythms by resonance. It is suggested that the main task of circadian clocks is to synchronize, to determine, and to maintain the phase relationships of the different physiological processes in the body within the 24-h cycle of light and dark. Thus the clock is active throughout the day to determine at what sequence and with what phase relationship all the different physiological processes in the body take place, in synchrony with external rhythms. Because information processing in the brain depends on mechanisms of temporal encoding with precision on the millisecond scale, the circadian clock as part of the brain is also based on these principles.

Neuropeptide-dependent gating might also be used in the mammalian circadian clock because the neuropeptide vasoactive intestinal polypeptide (VIP) mediates rhythmicity and synchrony in the SCN (Atont et al. 2005) and VIP-receptor knockouts show impaired locomotor activity rhythms (Harmar et al. 2002). Furthermore, in connexin 36 knockout mice without gap junctions circadian activity rhythms dampened and delayed in constant darkness (Long et al. 2005). Not only on the network level, but also for single circadian pacemaker cells, electrical activity on the millisecond scale and circadian rhythms appear to be intimately interconnected because electrical silencing stops clock gene rhythms (Nitabach et al. 2002). Further experiments will test whether gap-junction–mediated synchronous oscillations of AMe neurons constitute a necessary prerequisite to circadian clock gene rhythms and to circadian locomotor activity outputs in the cockroach.

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R E F E R E N C E S


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