

Synaptic communication of cellular oscillations in the rat suprachiasmatic neurons

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Abstract

Circadian firing rhythms of cultured rat suprachiasmatic nucleus were measured simultaneously from 4–8 neurons by using a multi-electrode dish and neuronal interactions were examined by a cross-correlation analysis of spontaneous action potentials. Functional connections were detected in the neuron pairs showing synchronized circadian firing rhythms, and when the connections were lost, firing rhythms were desynchronized. After the prolonged treatment with tetrodotoxin, cross-correlation and circadian rhythm synchronization were abolished concomitantly in most neuron pairs. Cellular mechanisms involving Na⁺-channel dependent communication are responsible for the synchronization of the circadian rhythms in individual suprachiasmatic nucleus (SCN) neurons. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

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The suprachiasmatic nucleus (SCN) of the hypothalamus represents the circadian pacemaker in mammals [7] which consists of multiple oscillating neurons [4,5,11,16]. Mutual coupling and integration of cellular and regional oscillators in the SCN are critical for the generation and expression of a single circadian period in various physiological functions. Previously, we demonstrated the circadian firing rhythms of individual SCN neurons of rats in dispersed cell culture on a multi-electrode dish (MED) and reported the period distributed in a wider range from 20.0 to 28.0 h as compared to that in behavioral rhythms [5]. Nevertheless, no statistical difference was detected between the mean periods of single neuronal rhythm and behavioral rhythm. The finding suggests that the circadian period of the SCN in vivo is not determined by a few pacemaker neurons which lead other non-oscillating neurons but by an ensemble of the constituent oscillating neurons. Several mechanisms have been suggested to explain the synchronization of individual SCN neuronal rhythms, such as calcium spikes, paracrine interaction, electrical coupling and ionic interactions [1,8,15]. By a cross-correlation analysis of spontaneous

action potentials, we recently reported that an SCN neuron pair in dispersed cell culture was functionally connected by synapses when their circadian rhythms were synchronized [12]. In the present study, a causal relation between the synaptic communication and synchronization of the circadian rhythm was examined by blocking the Na⁺-channel dependent action potentials. The relation was also examined under synchronized and desynchronized states in some neuron pairs.

The SCN cells of rats from an inbred colony of the Wistar strain were dissociated and cultured by plating 1.0–1.5 × 10⁵ vial cells in the central area of a MED as described elsewhere in detail [5,6,12]. Extracellular action potentials of 4–8 neurons on the same dish were selected for measurement using the criteria of S/N ratio (>5.0) and recorded simultaneously by a multi-channel extracellular recording system (Panasonic) from 1–2 weeks after the start of culture for 2–15 weeks. Spike discharges were recorded every 10 s and the circadian firing rhythm was analyzed using a sequential data array of the mean firing rate in 15 min as described previously [5,12]. The circadian period was calculated in the range 20.0–28.0 h by the chi-square periodogram [13] using the data of at least 5 consecutive days. Neurons which had a single and statistically significant circadian period (*P* < 0.01) were designated as those with

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circadian oscillation. When both of the paired neurons had the firing rhythms with identical circadian periods, the two neurons were designated as synchronized. A cross-correlation analysis was done at different times of the day as described in detail elsewhere [12]. Briefly, spike discharges collected with a multi-channel data recorder (TEAC) were calculated using a micro1401 interface (CED). A spike discharge from one of the paired neurons was used as a reference, and the timing of spikes from the other neuron was examined in 100 μ s bins over the range of ± 1 s until the number of reference spikes exceeded 500. A cross-correlogram was constructed in the neuron pairs with significant circadian firing rhythms. Tetrodotoxin (TTX, Wako) was administered by dissolving in the culture medium at the final concentration of 0.5 μ M. TTX containing culture medium was exchanged every day for 7 days.

Alternations from synchrony to desynchrony and vice versa were observed occasionally in the cultured SCN neurons (Fig. 1a). In such neuron pairs ($n = 9$), cross-correlation was evident during synchronization (Fig. 1b), while it was lost during desynchronization (Fig. 1c). In the particular neuron pair shown in Fig. 1, an action potential of C41 preceded that of C51 by 3.4 ms during synchronization. This relatively long inter-spike-interval (ISI, an interval between the reference time and the peak in the correlogram) suggests a poly-synaptic communication [14]. In addition, the circadian periods in these neurons were changed when the rhythms were synchronized (C41: from 23.2 to 24.0 h, C51: from 24.4 to 24.0 h), suggesting a mutual interaction of the circadian oscillations instead of an unidirectional communication. In another dish, six neurons with synchronized circadian rhythms were split into two groups during long-term recording; three of them with the desynchronized and the remaining three with synchronized circadian rhythms. A cross-correlation was detected between neurons with the synchronized rhythms and lost in the pairs whose circadian rhythms were desynchronized (data not shown).

The result that synchronization of the circadian firing rhythms is always accompanied by the presence of functional communication, and vice versa, strongly suggests the synaptic communication as responsible mechanism for the circadian rhythm synchronization. Therefore, a causal relation between Na^+ -channel dependent neural transmission and rhythm synchrony was examined by prolonged treatment with TTX in seven neuron pairs with synchronized circadian firing rhythms. It has been shown that TTX abolished the overt circadian rhythms in behavior [10] and spontaneous firing of individual SCN neurons [16]. TTX is also shown to be ineffective on the circadian oscillation at the level of the SCN neurons [16]. However, it is an open question whether communication through synapses is necessary for the rhythm synchronization among SCN neurons or not. If the neural communication is critical for rhythm synchrony, a prolonged interruption would lead each neuron to free-run resulting in desynchron-

ization. Therefore, the circadian rhythms reappeared after the washout of TTX were evaluated. TTX completely abolished spontaneous discharges as shown in Figs. 2 and 3. After washing out of TTX, the circadian firing rhythms were desynchronized in 5 neuron pairs out of seven pairs examined and cross-correlation was lost in these pairs (Fig. 2). In the neuron pair shown in Fig. 2a, the spontaneous activity did not appear in one neuron (E39) for 4 days after washout of TTX. The circadian period (24.2 h) of this neuron was not changed by TTX, while that of a paired neuron (E40) was changed from 24.2 to 24.7 h. These findings indicate that the neural communication is necessary for maintaining synchronization of the circadian rhythms in the cultured SCN neurons. Remaining two neuron pairs kept the rhythm synchrony after the TTX treatment. The synaptic

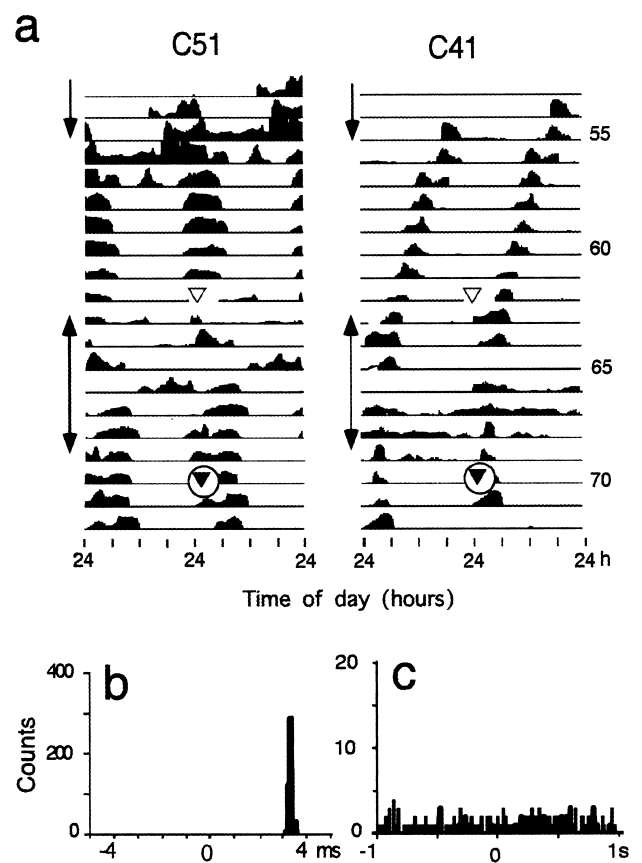


Fig. 1. Alternations from synchrony to desynchrony in the circadian firing rhythms of two neurons. (a) Double-plotted circadian firing rhythms of the paired neurons (C51 and C41). Arrows indicate the time when the two rhythms were in phase. The full scale of firing rate: 6 Hz (C51) and 5 Hz (C41). An interpolar distance between the two electrodes: 335 μ m. Numbers at the right margin of the record are days in culture. Open and closed triangles indicate the time of cross-correlation analysis. The neuron C41 was used as a reference (fired at 0 ms). (b) A cross-correlogram showing an excitatory communication during synchronization of the circadian rhythms. Recording was done at the phase indicated by open triangles in (a). (c) A loss of the cross-correlation during desynchronization. Recording was done at the phase indicated by closed triangles in (a).

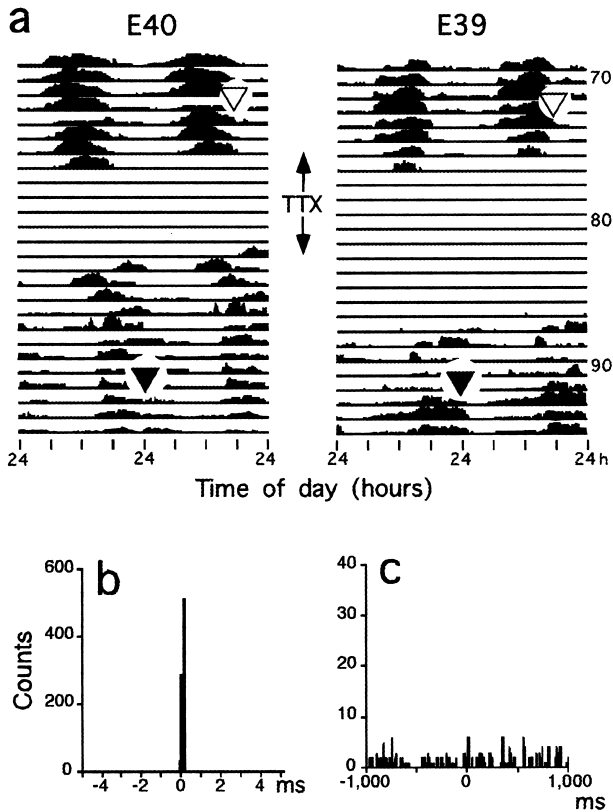


Fig. 2. Desynchronization of firing rhythms after the TTX treatment. (a) Double-plotted circadian firing rhythms of the paired neurons (E40 and E39). Days of the TTX treatment are indicated by arrows. The full scale of firing rate: 7 Hz (E40 and E39, pre-TTX), 3 Hz (E40, post-TTX) and 2 Hz (E39, post-TTX). The two neurons were located on adjacent electrodes. Numbers at the right margin of the record are days in culture. Open and closed triangles indicate the time of cross-correlation analysis. The neuron E40 was used as a reference. (b) A cross correlogram showing an excitatory communication before the TTX treatment. Recording was done at the phase indicated by open triangles in (a). (c) A loss of the cross-correlation after the TTX treatment. Recording was done at the phase indicated by closed triangles in (a).

communication of these neuron pairs was maintained but the ISI was lengthened (one from 1.3 to 1.8 ms, the other from 0.6 to 0.9 ms) (Fig. 3a–c). The circadian firing rhythm resumed at the phase expected from the extrapolation of the rhythm before the treatment. The result suggests the continuation of circadian rhythms during the TTX treatment, as reported by Welsh et al. [16]. The lengthening of ISI indicates that the chronic blockade of neuronal activity by TTX altered the synaptic efficacy, which eventually brought about an inactivation of the functional synapses.

The conclusion does not necessarily contradict the previous studies [3,10] in which the circadian oscillation was demonstrated to persist during TTX treatment. Because, TTX treatment for a short term (<12 h) [3] may not affect the orchestrated circadian rhythm at the SCN level, since the multiple circadian oscillations take many days to be desyn-

chronized completely. Even when TTX is applied for longer time (2 weeks) [10] and the constituent circadian oscillators free-run independently, it is still possible that the reorganized circadian rhythm preserves the phase and period of the oscillation before the treatment. Because the oscillations persist even under TTX treatment, and the properties of circadian oscillation in the multi-oscillator circadian system are predominantly determined by the major oscillations in the constituent oscillations. The latter notion was demonstrated by a mathematical study [9] and proved by the findings that the major periods of individual SCN neurons determine the period of behavioral rhythms [4,5].

The present study demonstrated that synchronization of the circadian oscillation of individual SCN neurons is achieved by the synaptic communication. And TTX

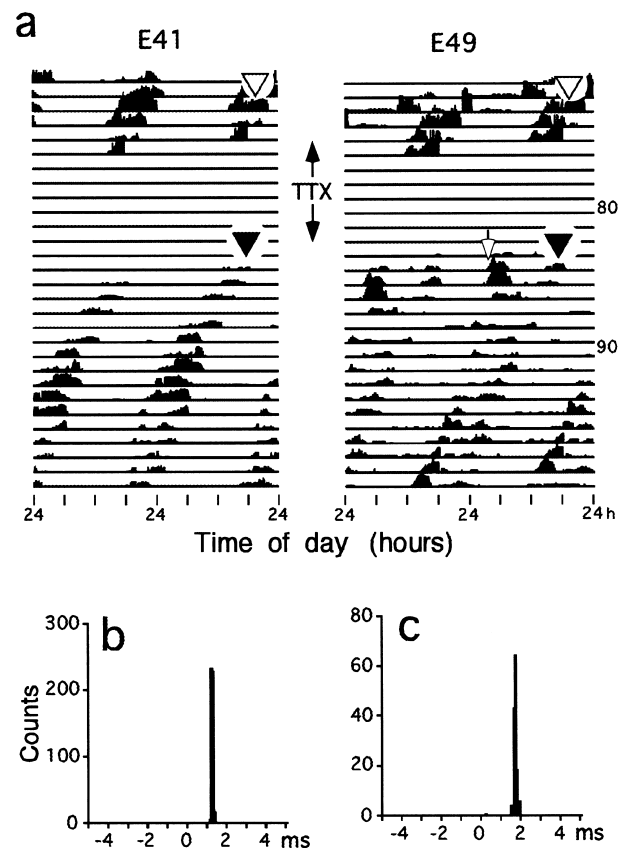


Fig. 3. Persistence of synchronized firing rhythms after the TTX treatment. (a) Double-plotted circadian firing rhythms of the paired neurons (E41 and E49). The circadian period was 22.9 h. An open arrow indicates a transient component with a longer period than the synchronized rhythm appeared in E49 but not in E41. The full scale of firing rate: 4 Hz (E41) and 2 Hz (E49). The two neurons were located on adjacent electrodes. Numbers at the right margin of the record are days in culture. Open and closed triangles indicate the time of cross-correlation analysis. The neuron E41 was used as a reference. (b) A cross-correlogram showing an excitatory communication before the TTX treatment. Recording was done at the phase indicated by open triangles in (a). (c) Lengthening of ISI after the TTX treatment. Recording was done at the phase indicated by closed triangles in (a).

abolishes or attenuates the oscillatory coupling between SCN neurons with synchronized rhythm. Previously we reported the involvement of Ca^{2+} -dependent synaptic transmission in rhythm synchrony by showing a dose-dependent lengthening of an ISI and a loss of cross-correlation with Cd^{2+} , a Ca^{2+} channel blocker [12]. However, neural communications through other mechanisms than chemical synapses are not excluded. Electrical coupling through gap junctions has been suggested in the SCN neurons [2,8]. And halothane, a gap junction blocker, as well as TTX was shown to reduce the dye-coupling between the SCN neurons [2]. The fine neural networks involving gap junctions as well as the excitatory and inhibitory synapses may be advantageous for the multi-oscillator circadian system in the SCN.

It is concluded that Na^+ -channel dependent synaptic communication contributes to the synchronization of circadian rhythms in individual SCN neurons, which is critical process for the circadian rhythm generation in the SCN.

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