A possible synchronization mechanism of the suprachiasmatic nucleus based on the phase-response curve

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Abstract

The suprachiasmatic nucleus (SCN), the master biological clock in mammals, consists of multiple, single-cell oscillators (i.e. clock cells) with widely dispersed periods. The mechanism underlying synchronization and entrainment of the clock cells is an important issue in nonlinear dynamics as well as Chronobiology. Recently, we have shown that the neurotransmitter *γ-aminobutyric acid* (GABA) mediated cell-to-cell interaction is essential for the clock cell synchronization [1]. Following this observation, we develop a novel model of the SCN — a population of non-identical oscillators responding slowly to GABA in a phase-dependent manner. This model successfully reproduces the two key properties of the SCN: self-organized synchronization and entrainment to external stimuli. Therefore, we propose that the phase-dependent, slow responses of clock cells to GABAergic inputs can be the basis of the SCN synchronization and entrainment.

1. Introduction

The SCN contains thousands of autonomous, circadian oscillator cells having quite different intrinsic periods (20 ~ 28 hrs) [2]. Yet, these cells *in situ* can synchronize themselves to generate a coherent output rhythm and be entrained to external, cyclic environments. Understanding how they achieve these properties is not only an important issue in chronobiology [2] but also a challenging topic of interest in physics [3, 4].

For the SCN to exhibit synchronization and phase-resetting responses, cell-to-cell interactions must exist, and a promising agent mediating the interactions is GABA [5, 6]. However, there has been no explicit demonstration so far that intrinsic GABA has an essential role in the SCN operations. Furthermore, the precise mode of GABA action in clock cell synchronization is unknown. Recently, we have shown that SCN slices bathed in bicuculline-containing medium for 2 days exhibit no circadian rhythms, suggesting that the neurotransmitter *γ-aminobutyric acid* (GABA) mediated cell-to-cell interaction is essential for the clock cell synchronization [1]. We also found, using simultaneous extracellular single-unit recordings of paired SCN neurons and cross-correlogram analysis, that coupling interactions among SCN neurons are significant on a slow timescale (i.e. tens of seconds), not on a fast timescale [1]. Following these observations, in this study, we developed a novel model of the SCN — a population of non-identical oscillators responding slowly to GABA in a phase-dependent manner, and demonstrated that this model successfully reproduces the two key SCN properties: self-organized synchronization and entrainment to external stimuli.

2. Methods

We formulated a hypothesis that the coherent firing activity of the SCN arises from slow, phase-dependent, phase-shifting responses of the individual clock cells to GABAergic stimulus. That is, the circadian phase of the individual oscillator can be either advanced or delayed based on the circadian phase at which a GABA stimulation is given, and the stimulation is provided by the endogenous GABA released from neighboring cells.

Based on this hypothesis, we constructed a simple but novel model for the SCN — a network of 10,000 self-

sustained oscillators (with different intrinsic periods ranging 20 ~ 28 hrs), coupled with the 'GABA phase response curve (PRC)' of Fig. 1. According to this PRC, circadian phase ϕ_i of each oscillator can be advanced (when $\phi_i = 0 \sim \pi/2$ and $3\pi/2 \sim 2\pi$) or delayed (when $\phi_i = \pi/2 \sim 3\pi/2$) upon receiving a GABA stimulus from neighboring cells. The shape of this PRC was modeled after the recent experimental work by Liu and Reppert [7] who examined the phase shifting properties of cultured clock cells to exogenous GABA, applied daily for 1 or 6 hr. In our model, the sum of the firing rates of the neighboring cells (A_i) assumed the role of endogenously released GABA: that is, the more frequently the SCN neurons fired, the greater the amount of GABA released. Then, we further assumed that only when the sum A_i remained larger than a critical value (e.g., A_c=10,000) for a sufficient period of time (e.g., T_c=1 hr), the GABA action through the PRC of Fig. 1 is effective for the ith cell. The total sum A_{tot} of the firing rates of the individual oscillators was monitored as an output signal of the entire network. The particular number (10,000) of oscillators was chosen based on an actual estimate of number of clock cells in rat SCN [2].

The dynamics of our model network is described by the following set of phase equations:

$$\phi_i^{(m+1)} = \phi_i^{(m)} + \omega_i \Delta t + R(\phi_i^{(m)}; A_i^{(m)}) \tag{1}$$

$$A_i^{(m)} = \sum_{j \neq i}^{10,000} \{\sin(\phi_j^{(m)}) + 1\}, \tag{2}$$

Here, $\phi_i^{(m)}$ and ω_i represent the circadian phase at time $m\Delta t$ and the intrinsic frequency of the ith oscillator, respectively. The function R is basically the V-shaped function $\Delta\phi$ of Fig. 1 except that it also depends on the value of A_i, which is the sum of the 'firing rates' $(\sin\phi_j + 1)$ of the individual cells, excluding the ith cell itself. Without the presence of function R, the Eq. (1) describes N linear oscillators that are mutually independent.

At every iteration step of the simulation, A_i was computed and compared with prescribed threshold value A_c. If the value of A_i had stayed larger than a preassigned threshold value A_c for a period longer than a preassigned threshold value T_c, the ith oscillator advances or delays its circadian phase according to function R. Otherwise, ϕ_i increases linearly at its own rates ω_i. Numerical iterations of Eq. (1) were performed with a time step size Δt = 0.1 hr.

3. Results

Figure 2 well illustrates a typical example undergoing self-organized synchronization and subsequent entrainment to external, cyclic stimuli. The simulation initially began with uniformly distributed phases (gray in Fig. 2A) and a normal distribution of intrinsic periods (gray in Fig. 2B). As the oscillators gradually synchronized to each other through the PRC-mediated cell-to-cell interaction, the network produced a coherent rhythm in Atot with a period of 24.8 hrs after a transient period of about 70 hrs (see Fig. 2C). For the final synchronized state, the distribution of the phases (black in Fig. 2A) and periods (black in Fig. 2B) both showed a significant degree of coherence. The behavior of the coupled network stayed essentially the same with different values of Ac and Tc as long as they were within the range Ac = 8,500 - 10,100 and Tc = 0.4 - 3.0 hrs. Thus, the coupling interactions based on the V-shaped PRC can synchronize individual oscillators so as to produce a coherent output rhythm.

The same model also exhibited another important characteristic of the SCN — *entrainment*. The self-organized output rhythm (period=τ) of the network could be entrained to the rhythm (period=τ') of the externally applied stimulus. For the case shown in Fig. 2D, for example, at every hour of a total 6-hour duration the phase of each oscillator ϕ_i was forced to have a shift based on function $R(\phi_i)$ regardless of the value of A_i. This protocol was repeated at every τ'=27.0 hrs. As a result, the period of the initial rhythm (τ=24.8 hrs) gradually altered to match that of the stimulus (τ'=27.0 hrs). The phenomenon of entrainment was consistently observed as long as $23 \leq \tau' \leq 27$ for the same stimulation protocol. The entrainment also occurred even if the external stimulus was delivered before the network had self-organized to produce a coherent rhythm.

We also investigated the minimum coupling range of GABA-mediated intercellular communication necessary for the synchronization of clock cells. The connectivity of our model network was systematically varied from 2 (i.e., nearest neighbor coupling) to 10,000 (i.e., all-to-all coupling). When the number of connected oscillators was less than 20, no clear coherent rhythm emerged at all. But, a stable rhythm always existed when the number of connected oscillators was greater than 30. In other words, a certain level of intercellular connectivity was required for the oscillators to rhyme in a coherent manner. This result may explain the lack of a coherent rhythm in the cultures of SCN cells [8]; in low-density cultures the degree of GABA-mediated coupling can be quite low, although some functional

GABAergic synapses are present [5, 6].

4. Discussion

How can the coupling interaction mediated by a PRC like the one shown in Fig. 1 synchronize the non-identical oscillators? Our heuristic explanation involves two different physical issues: (1*) phase-locking* and (2) *frequency-locking*. The phenomenon of phase-locking is rather easy to understand if one recognizes that the V-shaped PRC has two fixed points (stable O and unstable O'. Point O (O') is stable (unstable) in the sense that all circadian states converge (diverge) to the point as indicated by the arrows, upon receiving a sequence of stimulations. In fact, PRCs having one stable fixed point always bring the phase of each oscillator of the network to a single value, thus resulting in a stable phase-locked rhythm. In the meantime, the differences in the intrinsic periods of the individual oscillators disappear as the system undergoes frequency-locking, a universal phenomenon in nonlinear systems of coupled oscillators [3, 4]. The nonlinearity of our model system originates from the nonlinear shape of the PRC function R and the existence of the threshold values, Ac and Tc.

In summary, we have introduced a new concept that the cell-to-cell coupling interaction of SCN is mediated by a phase response curve of GABA. Two essential properties of the SCN in situ - self-organized synchronization and entrainment - are well captured in the model employing this idea. Thus, we propose that the phase-dependent, slow responses of clock cells to GABAergic inputs are the basis of the SCN synchronization and entrainment.

5. References

[1] Kim, Y.I, Rho, Y.A., Kwak, Y., Kim, D.Y., Jeong, J., Lee, J. & Lee, K.J. Synchronization of the suprachiasmatic nucleus by phase-dependent clock cell responses to GABAergic inputs (submitted)

[2] Reppert, S.M., & Weaver, D.R. (2002) Coordination of circadian timing in mammals. *Nature* 418: 935-941.

[3] Winfree, A.T. (2002) Oscillating systems. On emerging coherence. *Science* 298:2336-2337.

[4] Glass, L. (2001) Synchronization and rhythmic processes in physiology. *Nature* 410:277-284.

[5] 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.

[6] Ralph, M.R. & Menaker, M. (1989) GABA regulation of circadian responses to light. I. Involvement of GABAA-benzodiazepine and GABAB receptors. *J. Neurosci.* 9:2858-2865.

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

[8] 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.

[9] Shirakawa, T., Honma, S., Katsuno, Y., Oguchi, H. & Honma, K. (2000) Synchronization of circadian firing rhythms in cultured rat suprachiasmatic neurons. *Eur J Neurosci*. 12:2833-2838.

Figure 1 Model GABA phase response curve plotting the phase shift $\Delta\phi$ induced by a 'GABA stimulation' as a function of circadian phase ϕ. A positive (negative) value of $\Delta\phi$ means an advance (delay)

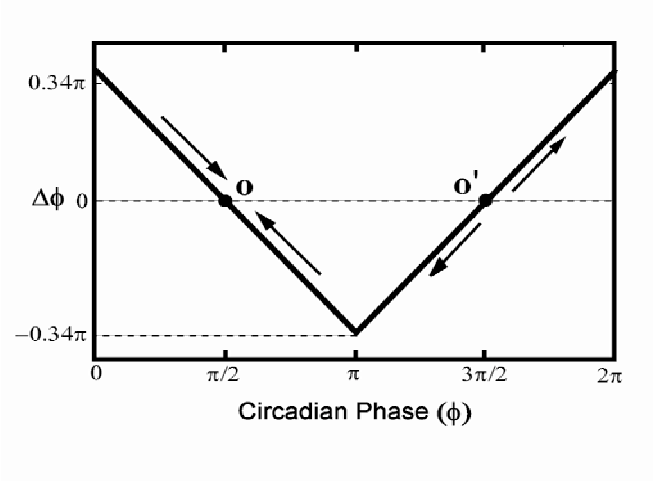

Figure 2 Synchronization and entrainment of the model SCN network. Distributions of phases (**A**) and periods (**B**) of the individual oscillators before and after synchronization. (**C**) shows the emergence of a self-synchronized rhythm in the total sum of the firing rates (A_{tot}). (**D**) shows the entrainment of the self-synchronized network by external, cyclic stimuli (commenced at the position marked by an arrow).