

## Circadian periods of single suprachiasmatic neurons in rats

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### Abstract

Neuronal activity of a single neuron was monitored continuously for more than 5 days by means of a multi-electrode dish in dispersed cell culture of the rat suprachiasmatic nucleus (SCN). Sixty-seven out of 88 neurons showed a robust circadian rhythm in firing rate. The mean circadian period was 24.2 h, which was almost identical to that of the locomotor activity rhythm in 114 weanling rats blinded on the day of birth. However, the circadian period in individual SCN neurons was scattered from 20.0 to 28.3 h (SD, 1.4 h), while the period of activity rhythm clustered from 24.0 to 24.8 h (SD, 0.2 h). It is concluded that a large number of SCN neurons contain the circadian oscillator, the period of which is more variable than the circadian period of the SCN as a whole. It is suggested that the circadian rhythms in individual SCN neurons are capable of synchronizing to each other and are integrated to constitute a multiple oscillator system(s) within the SCN. © 1998 Elsevier Science Ireland Ltd. All rights reserved

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Circadian rhythms have been demonstrated in the isolated SCN [3,10,15] and in the dispersed SCN cell culture [9,12,17] in rats. Interestingly, the circadian periods of the SCN *in vitro* were somewhat different from those measured *in vivo*. For example, the circadian periods of locomotor activity or corticosterone levels under DD [5–8] are always longer than 24 h, while the peptide rhythms in the SCN tissue or dispersed cell cultures often showed periods shorter than 24 h [9,15]. On the other hand, Welsh et al. demonstrated that the circadian rhythm in firing rate of a single cultured SCN neuron was different from neuron to neuron, suggesting that multiple oscillating neurons are involved in the circadian pacemaker in the SCN [18]. In the present experiment, we recorded circadian rhythms in neuronal activity from single SCN neurons by culturing dispersed SCN cells on a multi-electrode dish and compared the circadian period to that measured in the locomotor activ-

ity rhythm in rats, to see whether there is a parallelism in the circadian period between *in vivo* and *in vitro*.

Rats in an inbred colony of Wistar strain were used. They were bred and raised in our animal quarters where the environmental conditions were controlled (12 h light and 12 h dark, lights on 6000–1800 h, about 100 lux at the rat cage, room temperature  $22 \pm 2^\circ\text{C}$ , humidity  $60 \pm 5\%$ ). They had free access to commercial rat chow (Oriental, Tokyo) and tap water. Methods for the SCN cell dissociation were described in detail elsewhere [9]. Briefly, dissociated SCN cells from 15–20 newborn rat pups were pooled and cultured on 2–4 multi-electrode dishes (Panasonic) at one time. Cell suspension including  $1.5\text{--}1.8 \times 10^5$  vial cells were plated in the central area (5 mm diameter) of each dish. Eight hours after plating the cells, 1 ml of culture medium was overlaid which was supplemented with 2% fetal calf serum and hormones. Constituents of the supplements were the same as those in the previous experiment except the serum [9]. Culture medium was exchanged every second day until the recording was started. Extracellular action potentials of single neurons were recorded by a multi-channel extracellular recording system (Panasonic). A multi-

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electrode dish (22 mm diameter) contains 64 planar embedded microelectrodes on a glass plate which was covered with insulation layer of polyimide. The electrodes were arranged in an  $8 \times 8$  array in an area of  $1 \text{ mm}^2$ . The size of each electrode was  $50 \times 50 \mu\text{m}$  and an interpolar distance between two electrodes was  $150 \mu\text{m}$ . Spontaneous neuronal activity of the cultured SCN neurons was started to monitor from 4 to 10 days after cell culturing and recorded continuously for 2–6 weeks. Action potentials were recorded simultaneously from 4–8 neurons in the same multi-electrode dish. Spike discharge was discriminated using a time-window slicer (Nihon Koden). Numbers of spike were counted every 10 s with signal-processing systems (Contec) and fed into an NEC PC9801 computer. Circadian rhythm was analyzed using the mean spike counts/sec in 15 min. Circadian period was calculated by a chi-square periodogram using data of at least 5 consecutive days.

We analyzed the neuronal activities of 88 neurons from 18 dishes. Sixty-seven neurons showed rhythms with a circadian period, while the remaining 21 neurons did not show the circadian periodicity. Fig. 1 illustrates the circadian rhythms in spontaneous firing rate from two neurons in the same dish. In addition to double-plotted rhythms (Fig. 1B), the patterns of neuronal activity throughout the circadian period were demonstrated (Fig. 1A). Robust circadian rhythms were detected in both neurons, but the phase, as well as the period, were slightly different between the two neurons (the period was 23.4 h in D27–7 and 22.6 h in D27–

58). Fig. 2A indicates the distribution of the circadian period in the dispersed SCN cells. The mean period was 24.2 h and the sample standard deviation was 1.4 h ( $n = 67$ ). The circadian period in individual neurons ranged from 20.0 h to 28.3 h.

The circadian period of locomotor rhythm was also measured in weanling rats ( $n = 114$ ) from our colony. Locomotor activity was monitored by an Animex (Shimadzu, Type III). The principle of locomotion measurement was described elsewhere [6,8]. Male rats were blinded by bilateral ocular enucleation on the day of birth under hypothermic anesthesia. On the 21st postnatal day, they were weaned and singly housed in a polycarbonate cage. Locomotor activity measurement was started immediately after weaning. Some of the results have been published previously (Figs. 1 and 2A,C in [8]). The circadian period was analyzed by a chi-square periodogram using 7-day activity record between the 5th and 6th postnatal weeks. The mean circadian period was 24.4 h and the standard deviation was 0.2 h ( $n = 114$ ; Fig. 2B). The circadian period of individual locomotor rhythm was ranged from 24.0 h to 24.8 h. There was no statistically-significant difference in the mean circadian period between the neuronal activity rhythm in the single SCN neurons and the spontaneous locomotor rhythm (Student's *t*-test). However, the range of period distribution was quite different. The variances in the circadian rhythms of firing rate and of locomotor activity were statistically-significantly different ( $F < 0.025$ ).

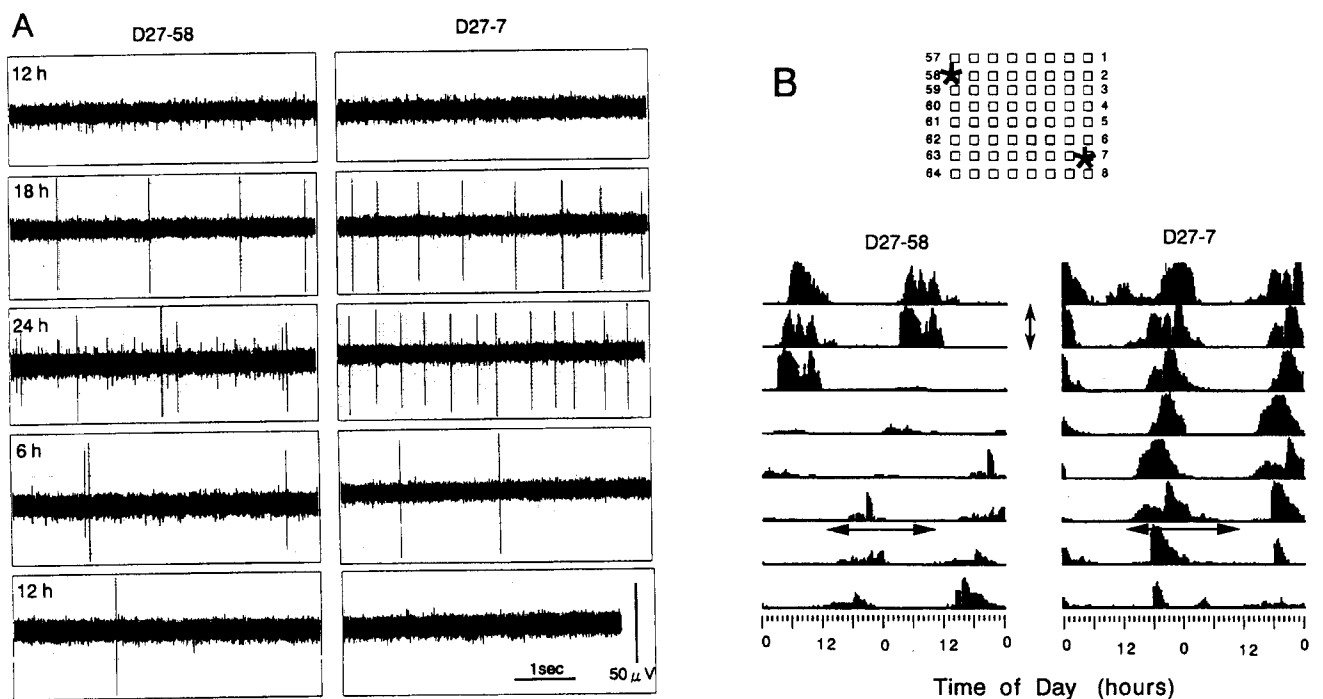


Fig. 1. (A) Circadian change in spontaneous firing of two neurons (D27–7 and D27–58) in a same dish. Local time was indicated in the record of D27–58. Vertical and horizontal bars indicate  $50 \mu\text{V}$  and 1 s, respectively. (B) Double-plotted circadian firing rhythms of the same neurons as in (A) expressed in the mean spike counts/sec in 15 min. A vertical arrow between the two graphs indicates the spike counts (5 spikes/s for D27–7 and 2 spikes/s for D27–58). Horizontal arrows in the graphs indicate the time when neuronal activities of (A) were recorded. The positions of the electrodes (asterisks) from which neuronal activity was monitored are illustrated in the diagram of the electrode array.

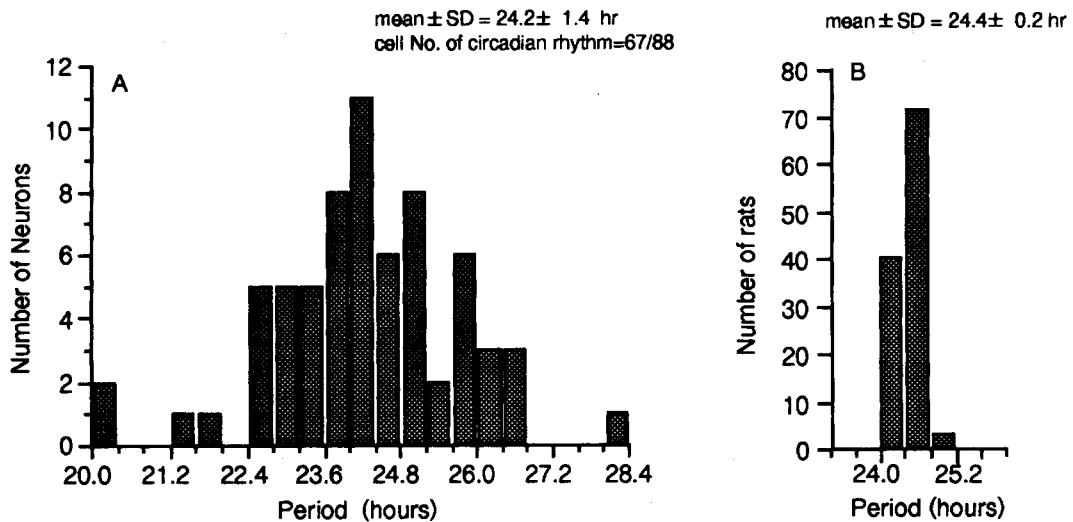


Fig. 2. Distribution of circadian period of neuronal activity rhythm in individual SCN neurons (A), and of the locomotor activity rhythm in weanling rats (B). Ordinate indicates the number of cells (A) and of animals (B). Abscissa indicates the circadian period.

The present results clearly indicate that the circadian rhythm in the neuronal activity of individual SCN neurons is more variable than in locomotor activity. It has been reported that the circadian period varied according to the developmental stage of animals [2,13]. In the present study, the neuronal rhythm in individual neurons was recorded 1–7 weeks after the initiation of cell culture, which corresponds to the postnatal 1.5–7.5 weeks. On the other hand, the mean circadian period of locomotor activity rhythm was 24.4 h in 5–6 week old rats, which was not different from that in 2–9 month old rats ( $24.43 \pm 0.14$  h) [6]. The variability in the circadian period was essentially identical in the two stages of development. Therefore, a large difference in the variability between *in vivo* and *in vitro* does not seem to be due to the stage of development. Recently, a similar wide distribution of the circadian period was reported in the tau mutant hamster, measuring the neuronal activity rhythm of the individual SCN neurons [11].

Enright [4] has previously proposed in his model for the circadian pacemaker that mutual couplings of multiple oscillating neurons might stabilize the circadian periodicity as a whole, and that the larger the number of neurons involved, the more the circadian period becomes stable. The present findings support this model.

The physical mechanism underlying the oscillatory coupling is not known. The SCN neurons communicate to each other by means of synapses or humoral factors. The previous observation suggested the rhythm synchronization without synaptic communication, because a circadian rhythm was detected in the fetal SCN, where there seemed to be no synaptogenesis [14], and neuronal synchronization was observed in the adult SCN slice without calcium-dependent synaptic transmission [1]. The SCN glia might play some role in the oscillatory coupling [16]. We have previously demonstrated that the circadian rhythms in SCN slice culture became internally desynchronized when glial

proliferation was suppressed by antimetotics [15]. On the other hand, the oscillatory coupling in the SCN is possibly mediated by some humoral factor. Recently, we were able to demonstrate that the expression of circadian rhythm in the SCN cell culture depended neither on synaptogenesis nor on cell architecture [9].

It is concluded that a number of the SCN neurons (76%) have their own oscillator. Although the mean circadian period in the individual SCN neuron is essentially the same as that of the circadian locomotor rhythm, the period of neurons varied in a wider range. The results suggest that the circadian rhythm in locomotor activity is a result of the integration of multiple circadian rhythms in the SCN.

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