

Short communication

Multunit activity recordings in the suprachiasmatic nuclei: in vivo versus in vitro models

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Abstract

The suprachiasmatic nuclei (SCN) of the hypothalamus continue to oscillate when they are isolated in a brain slice preparation. We recorded multunit activity in the SCN of the rat both in vivo and in vitro to determine the circadian discharge pattern. The variability of the discharge pattern is larger and the amplitude of the rhythm is smaller in vivo than in vitro. Moreover we found evidence for a direct effect of the animal's behavioural activity on electrical activity of the SCN in vivo. These findings may provide an electrophysiological basis for the known effects of behavioural stimuli on the circadian pacemaker. This study underscores the importance of recordings in intact preparations in addition to in vitro work when generalisations to physiological conditions are to be made.

Keywords: Suprachiasmatic nuclei; Circadian system; Multunit recording; In vivo; In vitro; Neurophysiology; Photic; Nonphotic

The suprachiasmatic nuclei (SCN) contain a major pacemaker for the generation of circadian rhythms in mammals [11,17,19]. The pacemaker is entrained to the environmental light-dark cycle via the retina. From here, several retinofugal pathways project to the SCN [6,15,16]. The effects of light on the pacemaker depend on the phase of the circadian cycle at which it is presented. At the beginning of subjective night light causes phase delays and at the end of subjective night it causes phase advances. Light presentation during subjective day does not induce phase shifts.

When the SCN are isolated in a brain slice preparation, they continue to oscillate. Moreover, it is possible to phase shift the rhythm in electrical activity in the slice by pharmacological and electrical stimuli [11]. This means that important attributes of the pacemaker, its rhythm generating mechanism and its phase shifting capacity have been preserved in vitro. Because many variables can now be excluded or controlled an ideal experimental situation is obtained for studies on circadian pacemaker mechanism.

Notwithstanding the merits of an in vitro system, it should be evaluated whether the behaviour of the isolated circadian pacemaker differs from the in vivo condition. To this purpose, we have performed electrophysiological recordings of neuronal discharge of multiple units in the SCN of freely moving rats. We have characterized the neuronal discharge patterns in the SCN and we have compared these with the behaviour of the isolated SCN in brain slices using similar recording techniques and identical off-line analysis.

In vivo recording techniques were as described in Meijer et al. [14]. In short, male Wistar rats were implanted with tripolar electrodes (stainless steel, diameter 0.125 mm, Plastics One, Roanoke, Virginia). Two electrodes were aimed at the SCN with a distance of 0.4 mm between the electrodes. The third electrode was placed in the cortex for reference. The animals were connected to the recording system via flexible cables and a counter balanced swivel system. Measurements were performed through one electrode at a time. During the recording session the animals were in complete darkness in a sound attenuating room.

Neuronal activity was recorded on-line and processed further off-line. On-line, a window discriminator converted the action potentials to electronic pulses. A second window discriminator was set at a higher level to detect artifacts caused by the animal's movements. Action potentials and

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movement artifacts were counted per 10 s bin. Those 10 s bins in which movement artifacts had occurred were excluded from analysis of the discharge pattern. The animal's drinking rhythm was recorded throughout the recording to estimate the animal's circadian phase. At the end of the experiments the animals were sacrificed to verify the recording sites. To obtain a blue spot at one of the electrode tips a current was passed through the electrode and the animal was perfused with a potassium ferrocyanide containing solution.

For the *in vitro* experiments, Wistar rats were entrained to a light-dark cycle (L:D = 12:12). Coronal 400 μm slices were prepared at the beginning of the subjective day and were transferred to an interface chamber. Artificial cerebrospinal fluid (pH 7.4) was perfused at a rate of 1.5 ml/min and contained (in mM): NaCl 122, KCl 3, CaCl_2 1.5, MgSO_4 1.3, NaH_2PO_4 1.25, NaHCO_3 25, glucose 10. Humidified O_2 (95%) and CO_2 (5%) was blown over the slices. Multiple unit recordings were performed at 35°C with 90% platinum/10% iridium electrodes ($R = 0.5 \text{ M}\Omega$). The signal was passed through a high impedance amplifier (bandpass = 0.3 to 3 kHz) and displayed on an oscilloscope. The action potentials were converted to electronic pulses with a window discriminator and were counted by a computer every 10 s.

To determine the variability of the *in vivo* and *in vitro* signal, we used consecutive 10 s bins to calculate the autocorrelation in the signal which provides a measure of variability independent of the waveform. Lomb periodogram analysis for unevenly sampled data was applied to describe the circadian and ultradian components in the discharge pattern [9]; the level of significance was estimated by a Monte Carlo simulation [7].

In seven freely moving rats, multiunit recordings of at least one circadian cycle ($n = 2$) or of more than two cycles ($n = 5$) were performed in the SCN (Fig. 1A). Histological verification of the recording sites confirmed that the electrode tips were inside the SCN in five cases. In the two remaining cases with unclear histology, identical discharge rhythms were observed with maximal electrical activity during subjective day. In previous investigations it has been shown that outside the SCN the peak in electrical activity occurs during subjective night [8,14]. Therefore, we conclude these two recordings must have been from inside the SCN as well. Lomb periodogram analysis revealed a significant circadian discharge pattern in all seven cases ($P < 0.001$).

In seven slices multiunit activity was recorded with a duration between 20 and 27 h (Fig. 1B). In one slice, two recordings were performed simultaneously. These electrodes were placed contralaterally. The Lomb periodogram revealed a significant circadian peak in all eight *in vitro* recordings ($P < 0.001$).

The circadian rhythm of neuronal discharge was low during the night and high during the day both *in vivo* and *in vitro*. However, the appearance of the *in vivo* and *in*

vitro circadian discharge pattern is different. *In vivo* the signal is a sinusoid whereas *in vitro* the activity during the subjective day consists of a sharper peak with near-zero activity at night. Second, a broad band of activity is visible around the circadian component *in vivo*. *In vitro* this band is much smaller.

In accordance with the smaller band in the *in vitro* traces, the autocorrelation *in vitro* appeared significantly higher than *in vivo* (Fig. 2), in other words the variance is lower ($P = 0.002$, Student's *t*-test). To test whether the variance itself displays a circadian variation the datasets have been divided into subjective day and night. The beginning of subjective night is defined as circadian time 12 which corresponds with the onset of the animal's activity. *In vivo*, circadian time was assessed on the basis of the animal's drinking rhythm, *in vitro* on the basis of the previous activity rhythm. The autocorrelation of the resulting datasets revealed a significantly lower variability at night *in vivo* ($P = 0.03$, paired *t*-test) and no difference between day and night *in vitro* ($P = 0.15$, paired *t*-test).

The described differences in variance between *in vivo* and *in vitro* recordings (Fig. 2) may arise from different ultradian components. This has been investigated using the Lomb periodogram to quantify the power of separate periods in the signal (Fig. 3A). This periodogram shows clearly that the power of the circadian component is about two times higher *in vitro* compared to *in vivo*. In contrast, the power of ultradian components is higher *in vivo* (Fig. 3B). A significant ultradian period of 4 h exists *in vivo* and a significant period of 3.5 h *in vitro*. More ultradian periods that approach the level of significance are visible *in vivo*. These periods are about 170, 130 and 100 min. For 2 h or shorter hardly any rhythmicity exists *in vitro*. We conclude that *in vivo* there is more complexity in the signal than *in vitro*.

In vivo, depressions of multiunit activity appeared in the traces such that neuronal activity decreased to nearly 50% of the baseline level in some instances (Fig. 4). Each depression appeared to arise from a particular period in which the animal had been active. However, the depression in multiunit discharge did not occur every time the animal was active and did not depend on the height of the movement score. At a faster timescale we found that multiunit discharge starts to return to normal values after the activity has stopped (Fig. 4b). The return to the initial discharge level proceeds in a nonlinear way and takes about half an hour.

In comparing the discharge patterns *in vivo* and *in vitro* it was demonstrated that *in vitro* the variance was much smaller than *in vivo*. The variance of the signal is a reflection of the neuronal discharge patterns of SCN neurons. However, other factors that may contribute are the local environment around the electrodes and, *in vivo*, possible artifacts that are introduced by the animal's movements. To assure that these movements have not introduced artifacts in our recordings, all those 10 s epochs in

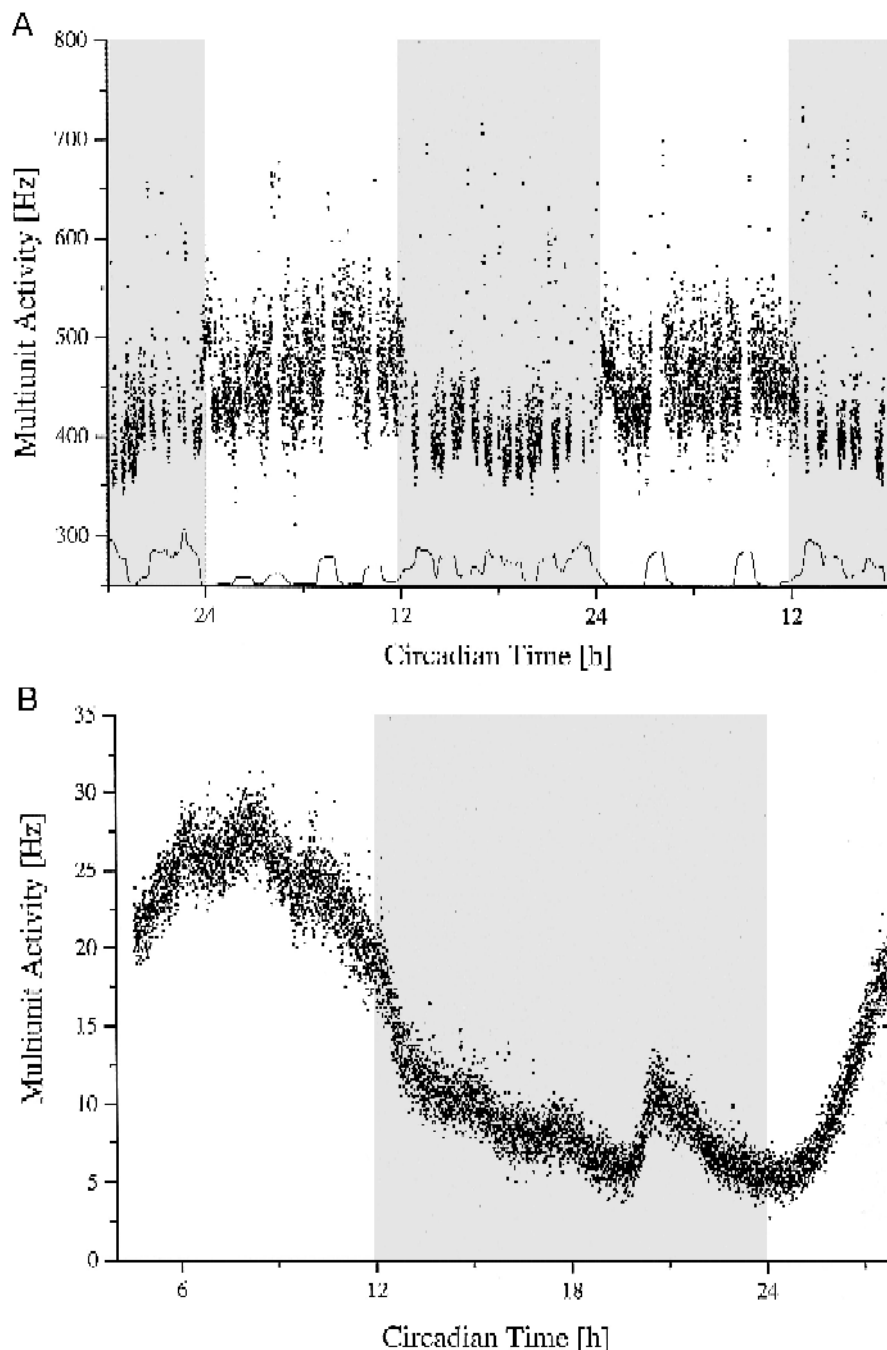


Fig. 1. Discharge pattern of multiunit activity in suprachiasmatic nucleus. Sample rate: every 10 s. Representative examples are shown of recordings (A) in vivo and (B) in vitro. In vivo the animal's movements (filtered by moving average of 5 min) are depicted at the bottom trace at an arbitrary scale. Circadian time has been determined by drinking activity in vivo and by the previous light/dark schedule in vitro. Subjective night has been shaded.

which the animal was active were excluded from analysis.

The local environment of the electrodes is a function of the extracellular resistance and of the recovery time of the tissue after electrode placement. It is expected that on the whole the recording condition in vivo is more favourable than in vitro because extracellular resistance is usually higher and recovery time after electrode placement is longer. Indeed we found a better signal to noise ratio in vivo which supports this expectation. The more favourable

recording condition in vivo will reduce the variability. As an increased variability was found in vivo, this finding cannot be ascribed to the recording condition per se. We conclude that the difference in variance between in vivo and in vitro recordings is caused by a true difference between neuronal discharge patterns of SCN neurons in the in vivo and in vitro preparation.

We attribute this difference to a strong reduction of neuronal interactions in the SCN in hypothalamic slices

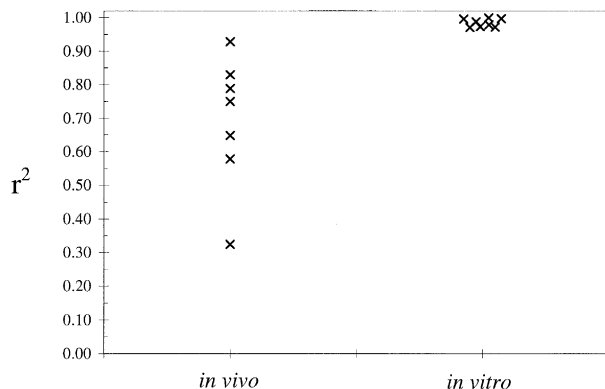


Fig. 2. Autocorrelation of multiunit discharge patterns, in vivo and in vitro. The degree of first order autocorrelation is represented by the r -squared of linear regression. The r -squared of the in vivo traces is significantly lower ($P = 0.0002$; unpaired t -test) compared with the in vitro traces.

where many afferent pathways have been cut off. The absence of afferent pathways will result in an absence of otherwise activated inputs. Another point is that slices are usually prepared with a thickness of 400 μm while the SCN is 500 μm in diameter. This means that at best, a slice contains 80% of the anterior-posterior extent of the SCN so that also connections within the SCN will be disrupted. Both the absence of afferent pathways and the disrupted connections within the SCN will reduce local interactions and will result in a decline in variation.

Our finding of increased variance at daytime in the SCN in vivo could be in accordance with this explanation. At daytime, firing rate of SCN neurons is higher than at night. The rhythm in firing rate most likely reflects a rhythm in membrane potential with more depolarized potentials during the day. It is expected that a decrease in membrane potential is associated with an increase in synaptic activity inside the SCN which will result in an increase in variance.

The variability of the recordings has been characterized not only by autocorrelation but also by Lomb periodogram analysis. The periodograms show broad peaks in the circadian range. This broadness arises from the sampling period, which, in the case of in vitro experiments never exceeded one and a half cycle. This recording time results in relatively few orthogonal periods around 24 h. From the periodograms we conclude that a significant circadian variation in multiunit activity exists both in vivo and in vitro. This is consistent with data from previous multi- and single unit recordings [1,4,8].

A second finding emerging from the periodogram analysis is that the power of the circadian rhythm in vitro is higher than in vivo. In other words the contribution of the circadian components to the signal is in vitro larger than in vivo. This was also evident from the raw data (Fig. 1) where the in vitro rhythms appeared to be the more pronounced.

Finally, the in vivo rhythm shows more complexity in the ultradian range. For these ultradian components, more orthogonal frequencies exist and several independent peaks arise. It is clear that the number of ultradian peaks in vivo is higher. We conclude that the higher variance that we measured in vivo results in part from a multitude of frequencies in the ultradian range.

We have been able to identify one of the sources of variation with a relative low frequency in the in vivo recordings. An increase in the animal's movements appeared to be followed by a strong decline in multiunit discharge. It implies that those brain areas that are associated with the animal's behaviour directly affect the activity of SCN neurons. The duration of these behavioural effects on multiunit discharge is rather long and extends beyond the period of the animal's activity by about half an hour. In this period multiunit activity returns to its baseline level with a curvature that resembles an exponential function.

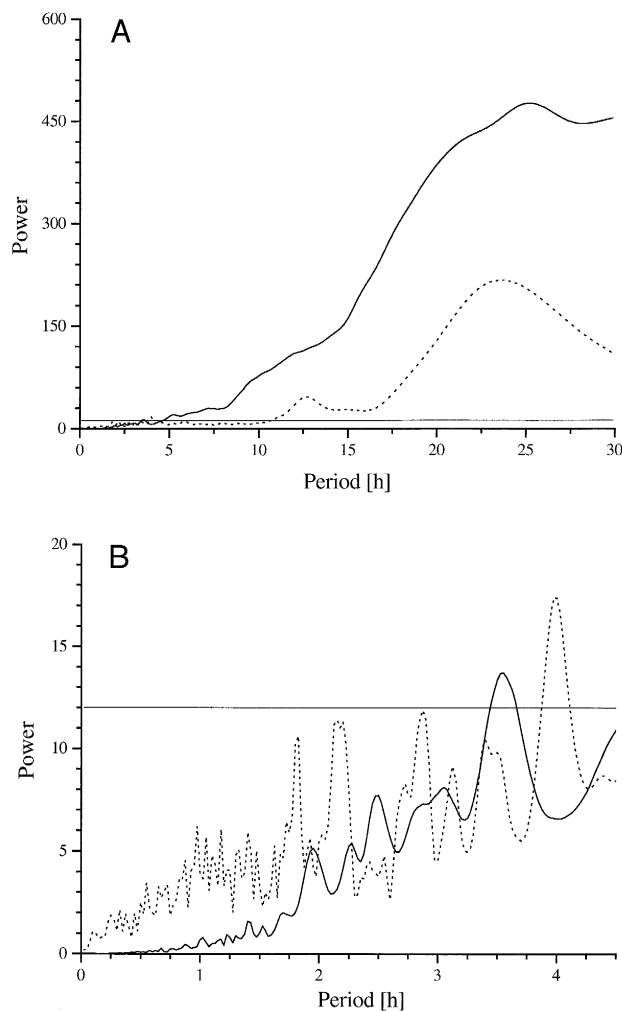


Fig. 3. Lomb periodogram analysis of multiunit discharge patterns. Periodograms of circadian (A) and ultradian (B) periods are means of separately calculated periodograms in vivo (dotted lines; $n = 7$) and in vitro (solid lines; $n = 8$). Horizontal line indicates the 5% level of significance.

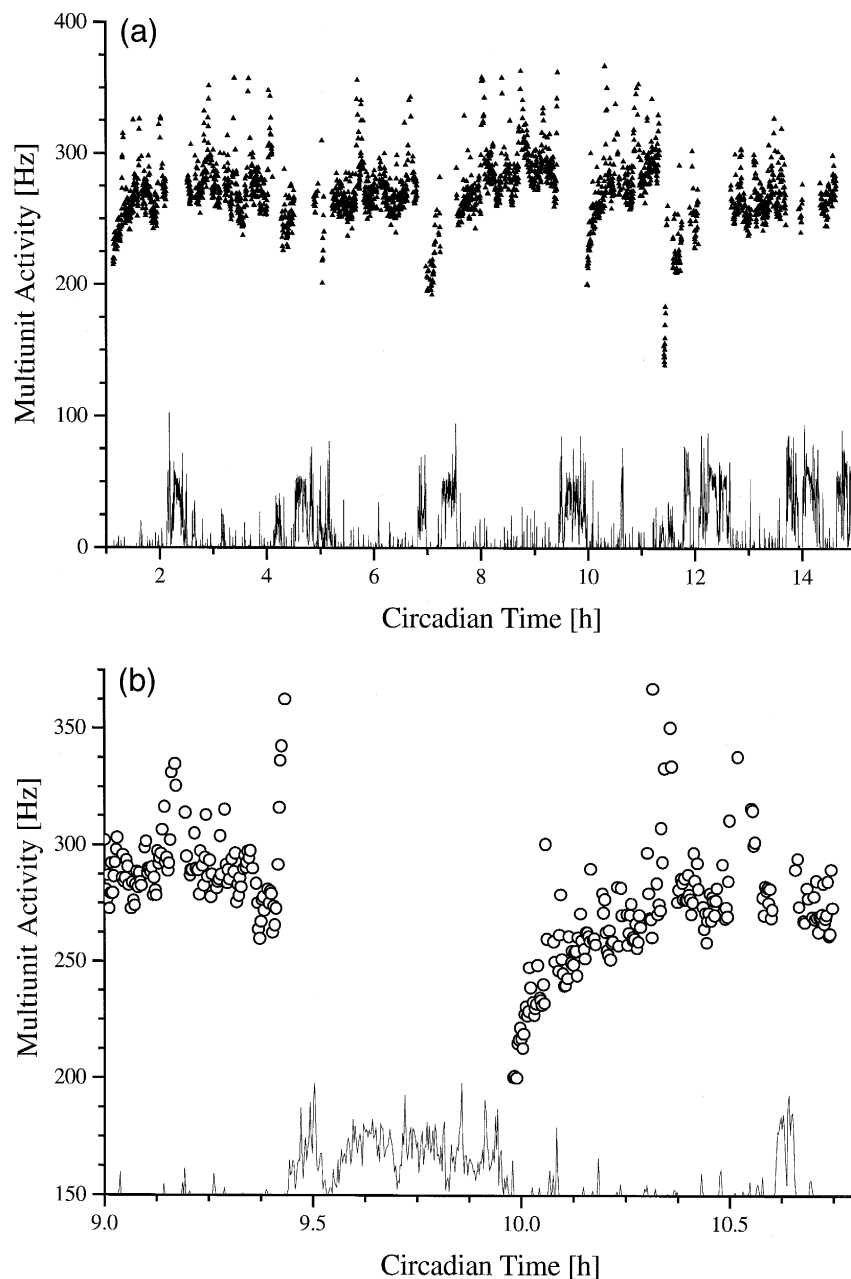


Fig. 4. Example of discharge pattern (a) with obvious depressions of multiunit activity. These depressions are related to the animal's behaviour as they occur predominantly after a period of increased activity. The depressions do not occur after each period of increased activity. One of the depressions in multiunit activity at CT 10 has been magnified in b. Plotting conventions are similar to those of Fig. 1, except for movement artifacts, which have been plotted without filtering.

The duration suggests that at some level a neuromodulator may be involved in this process.

The activity-induced decline in multiunit activity could be observed both during subjective day and subjective night. More data are required to describe this phenomenon as a function of circadian time in a quantitative way. It was noteworthy that not every movement episode was followed by a decline in MUA activity. Possibly, a closer inspection of the animal's behaviour during the recordings could lead to a better predictability of the phenomenon.

Previous electrophysiological studies have characterized the effects of light on the SCN neurons [5,10,12,13]. The observed effects of the animal's activity on the circadian system appear opposite to the effects of light. The main effect of light on multiunit activity in the SCN is excitatory whereas activity results in a decrease in activity. Both activity and light exert their effect throughout the circadian cycle.

These electrophysiological results are interesting with respect to a number of behavioural studies in which it was

shown that the effects of light and activity on the circadian system are opposite. Specific forms of induced activity can phase-advance the circadian wheel-running rhythm in hamsters at the end of subjective day [18,21,22]. In contrast, light results in phase advances at the end of subjective night. Ralph and Mrosovsky [20] have demonstrated that the phase-advancing effects of light can be counteracted by simultaneous activity of the animal. At a cellular level these findings may be explainable by the opposing effects of activity and light on neuronal discharge.

Besides the above mentioned studies several other studies have shown that behavioural parameters can affect the circadian system [2,3,23,24]. All these studies contribute to the notion that a complex system of input pathways determines the integrated response of the circadian pacemaker. The complexity in vivo as compared to the in vitro signal strengthens the notion that in the SCN of intact animals many incoming pathways modify the ultradian and circadian discharge patterns at a neuronal level. Pharmacological manipulation of these pathways in both in vivo and in vitro systems will open new perspectives into the analysis of the pacemaker mechanism.

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References

- [1] Bouskila, Y. and Dudek, F.E., Neuronal synchronisation without calcium-dependent synaptic transmission in the hypothalamus, *Proc. Natl. Acad. Sci.*, 90 (1993) 3207–3210.
- [2] Edgar, D.M. and Dement, W.C., Regularly scheduled voluntary exercise synchronizes the mouse circadian clock, *Am. J. Physiol.*, 261 (1991) 928–933.
- [3] Edgar, D.M., Martin, C.E., Dement, W.C., Activity feedback to the mammalian circadian pacemaker, Influence on observed measures of rhythm period length, *J. Biol. Rhythms*, 6 (1991) 185–199.
- [4] Green, D.J. and Gillette, R., Circadian rhythm of firing rate recorded from single cells in the rat suprachiasmatic brain slice, *Brain Res.*, 245 (1982) 198–200.
- [5] Groos, G.A. and Mason, R., The visual properties of rat and cat suprachiasmatic neurones, *J. Comp. Physiol.*, 135 (1980) 349–356.
- [6] Harrington, M.E., Nance, D.M. and Rusak, B., Double-labeling of neuropeptide Y-immunoreactive neurons which project from the geniculate to the suprachiasmatic nucleus, *Brain Res.*, 410 (1987) 275–282.
- [7] Horne, J.H. and Baliunas, S.L., A prescription for period analysis of unevenly sampled time series, *Astrophys. J.*, 302 (1986) 757–763.
- [8] Inouye, S.-I.T. and Kawamura, H., Persistence of circadian rhythmicity in a mammalian hypothalamic ‘island’ containing the suprachiasmatic nucleus, *Proc. Natl. Acad. Sci. USA*, 76 (1979) 5962–5966.
- [9] Lomb, M.R., Least squares frequency analysis of unequally spaced data, *Astrophys. Space Sci.*, 39 (1976) 447–462.
- [10] Meijer, J.H., Groos, G.A. and Rusak, B., Luminance coding in a circadian pacemaker: the suprachiasmatic nucleus of the rat and the hamster, *Brain Res.*, 382 (1986) 109–118.
- [11] Meijer, J.H. and Rietveld, W.J., Neurophysiology of the suprachiasmatic circadian pacemaker in rodents, *Physiol. Rev.*, 69 (1989) 671–707.
- [12] Meijer, J.H., Rusak, B., Harrington, M.E., Photically responsive neurons in the hypothalamus of a diurnal ground squirrel, *Brain Res.*, 501 (1989) 315–323.
- [13] Meijer, J.H., Rusak, B., Gänshirt, G., The relation between light-induced discharge in the suprachiasmatic nucleus and phase shifts of hamster circadian rhythms, *Brain Res.*, 598 (1992) 257–263.
- [14] Meijer, J.H., Watanabe, K., Détari, L. and Schaap, J., Circadian rhythm in light response in suprachiasmatic nucleus neurons of freely moving rats, *Brain Res.*, 741 1,2 (1996) 352–355.
- [15] Mikkelsen, J.D. and N. Vrang, A direct pretectosuprachiasmatic projection in the rat, *Neuroscience*, 62 (1994) 497–505.
- [16] Moore, R.Y., Retinohypothalamic projection in mammals: a comparative study, *Brain Res.*, 49 (1973) 403–409.
- [17] Moore, R.Y. and Eichler, V.B., Loss of circadian adrenal corticosterone rhythm following suprachiasmatic nucleus lesions in the rat, *Brain Res.*, 42 (1972) 201–206.
- [18] Mrosovsky, N. and Biello, M., Nonphotic phase shifting in the old and the cold, *Chronobiol. Int.*, 11 (1994) 232–252.
- [19] Ralph, M.R., Foster, R.G., Davis, F.C. and Menaker, M., Transplanted suprachiasmatic nucleus determines circadian period, *Science*, 247 (1990) 975–978.
- [20] Ralph, M.R. and Mrosovsky, N., Behavioral Inhibition of Circadian responses to Light, *J. of Biol. Rhythms*, 7 (1992) 353–359.
- [21] Reeb, S.G., Lavery, R.J. and Mrosovsky, N., Running activity mediates the phase-advancing effects of dark pulses on hamster circadian rhythms, *J. Comp. Physiol.*, 165 (1989) 811–818.
- [22] Reeb, S.G. and Mrosovsky, N., Effects of induced wheel running on the circadian activity rhythms of Syrian hamsters: entrainment and phase response curve, *J. Biol. Rhythms*, 4 (1989) 39–48.
- [23] Van Reeth, O., Hinch, D., Tecco, J.M. and Turek, F.W., The effects of short periods of immobilization on the hamster circadian clock, *Brain Res.*, 545 (1991) 208–214.
- [24] Yamada, N.K., Shimoda, K., Takahasi, S. and Takahasi, K., Free-access to a running wheel shortens the period of free-running rhythm in blinded rats, *Physiol. Behav.*, 42 (1988) 87–91.