

Short communication

Circadian rhythm in light response in suprachiasmatic nucleus neurons of freely moving rats

J.H. Meijer^{a,*}, K. Watanabe^b, L. D  t  ri^c, J. Schaap^a

^a Department of Physiology, Wassenaarseweg 62, Rijksuniversiteit, Leiden, The Netherlands

^b Department of Physiology, Dokkyo University School of Medicine, Mibu, Japan

^c Department of Comp. Physiology, Eotvos Lorand University, Muzeum krt. 4A, Budapest, Hungary

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Abstract

Long-term recordings of single SCN units were performed in freely moving rats simultaneously with multiunit recordings and evidence is presented for a daily change in light-responsiveness. SCN light response is high during the night and low during the day. We conclude that this difference is caused by a change in sensitivity, with higher sensitivities at night. Moreover, we demonstrate that the circadian rhythm in SCN light response is the result of the integrated behaviour of similarly behaving single SCN units.

Keywords: Suprachiasmatic nuclei; Circadian system; Photic responsiveness; Entrainment; Neurophysiology; Visual system

The suprachiasmatic nuclei of the hypothalamus have been identified as a pacemaker for many circadian rhythms in mammals [19,21,22]. The most important entraining agent of the circadian pacemaker is the external light-dark cycle [15]. The effects of light on the circadian pacemaker are dependent on the intensity and duration of light-exposure and on the phase of the circadian cycle at which light is applied [16,23]. During subjective day, light has no phase shifting effect on the circadian pacemaker whereas during subjective night light causes either a phase delay (early night) or phase advance (late night).

From the retinal photoreceptors, light information is conveyed to the ventral and lateral portions of the SCN via at least two retinofugal pathways, the retinohypothalamic tract and the geniculohypothalamic tract [8,15,20]. Glutamate is the most likely transmitter candidate to mediate light-induced phase shifts [3,4]. Single unit recordings in the retinorecipient areas of the SCN in several anaesthetized mammals of different species have revealed the presence of a subpopulation of light-responsive cells. Most of these respond with an increase and some with a decrease in discharge to an increment in light-intensity.

These cells have large receptive fields, respond in a sustained way to light and have monotonic intensity-response properties with both a threshold and saturation level [6,16–18].

Surprisingly, the responsiveness of SCN neurons to light has never been investigated in intact, unanesthetized animals, neither as a function of light-intensity, nor as a function of circadian phase. We have recorded extracellular multiunit (MUA) activity in 80 freely moving rats with implanted tripolar electrodes (stainless steel, diameter 0.125 mm, Plastics One, Roanoke, Virginia). Two electrodes were aimed at the SCN with a lateral interelectrode distance of 0.4 mm. Recordings were performed through one electrode at a time. The third electrode was cut shorter, its insulation partly removed and placed in the white matter to ground the animals. Until the start of the experiment, the rats were housed in a lighting regimen of 12 h of light (100–150 lux) alternating with 12 h of darkness. At the onset of the experiment the animals were connected to the recording system through a flexible cable that was attached to a counter balanced swivel system to minimize any effect on the animal's freedom of movement. Conventional amplification and recording techniques were applied and resulted in a signal to noise ratio which varied from 3:1 to 8:1 or more. The timing and duration of the light pulses were controlled by a computer. Light intensity was set at 0.15 lux to discriminate maximally between day and night:

* Corresponding author. Department of Physiology, University of Leiden, P.O. Box 9604, 2300 RC Leiden, The Netherlands. Fax: (31) (71) 5276782.

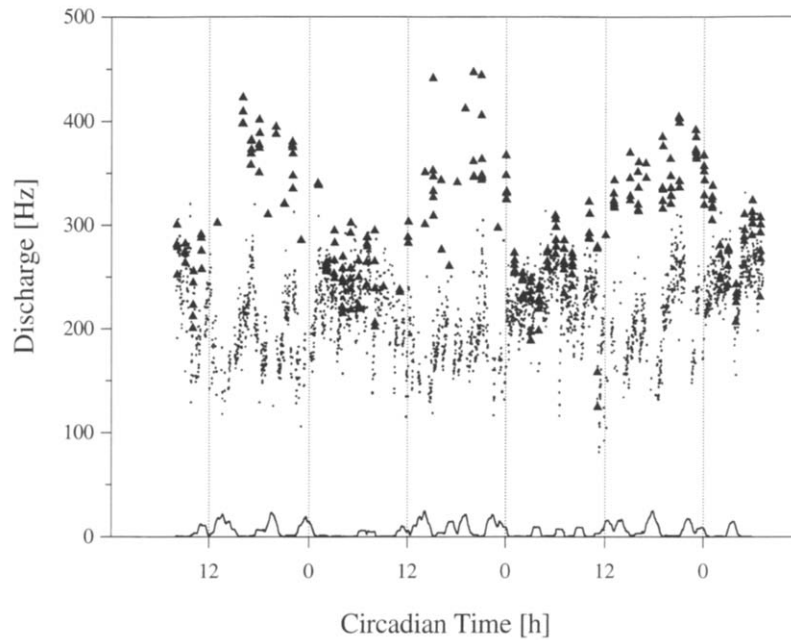


Fig. 1. Light-response of SCN cells as a function of circadian time in a representative animal. Accumulated discharge is indicated per minute during hourly light presentations of 6 min (▲) and in darkness (●). The animal's movements are depicted at the bottom of the figure.

at daytime 0.15 lux is approximately the light threshold whereas at night it evokes a half maximal response. To determine whether the light response of the SCN changed as a function of circadian time, the recording chamber was illuminated for 6 min once every hour. This protocol was followed for at least 2 days. The animal was in complete darkness between the light pulse presentations. Recording

sites were verified at the end of the experiment in 40 μm Cresyl violet stained sections of the brain. To distinguish between the two electrodes, we passed a current (40 μA , 25 s) through one of the electrodes and perfused the animal with a potassium ferrocyanide containing solution to obtain a blue spot at the site of the recording. In nine animals, one of the electrodes appeared to be located in the

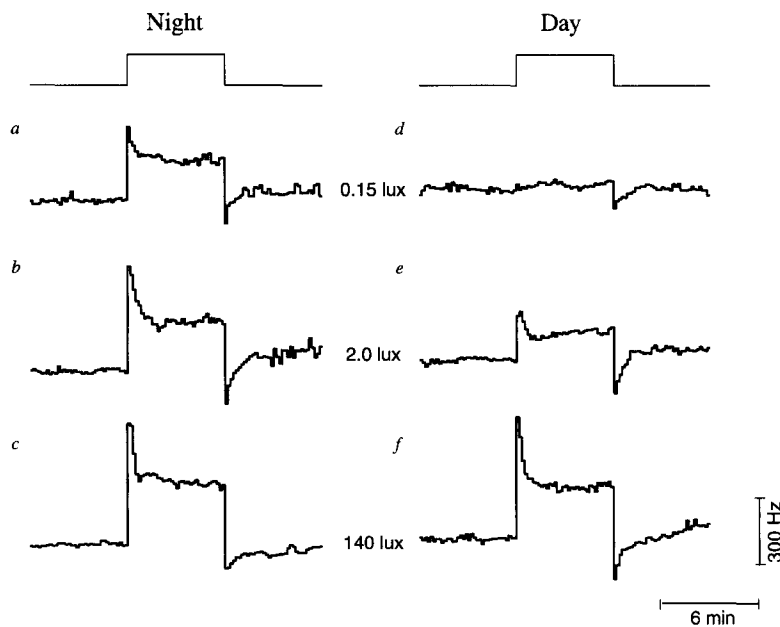


Fig. 2. Light-activated response of the SCN to a 6-min light pulse in a representative animal. Timing of the photic stimulus is indicated above the records. Light intensity was regulated by neutral density filters. Intensities from top to bottom are 0.15, 2 and 140 lux. Depicted on the left are responses during subjective night while on the right responses during subjective day.

SCN. In four more cases with indistinct histology, the circadian discharge pattern and light responses were typical for the SCN and we conclude from the whole these were SCN recordings.

Analysis: neuronal activity was recorded online and processed further off-line. Action potentials were converted to electronic pulses by a window discriminator. These pulses were fed into a computer system that counted the number of pulses per 10 s. A second window discriminator was set to a higher level to detect 'movement' artefacts caused primarily by eating or drinking activity and vigorous head movements. We excluded from analysis every 10-s bin in which we observed also counts on the artefact window discriminator. Lomb periodogram analysis (for non-equidistant data, i.e. data sets with missing values) was applied to investigate the presence of rhythmicity [12]. The level of significance was estimated by a Monte Carlo simulation [13]. During the experiment, the drinking activity was recorded to monitor the animals' circadian rhythm and to determine its circadian phase.

In 13 successful SCN recordings of at least 48 h, MUA was low during subjective night and high during subjective day. This circadian variation in dark discharge level was significant ($P < 0.001$) and is characteristic for the SCN [1,2,7,9,10]. In 12 cases, light induced a sustained increase in discharge rate while in one case a sustained decrease of neuronal firing was observed. Both light-activated and light-suppressed responses retained their respective sign throughout the circadian cycle. However, in the course of the animals' subjective day, light responses became gradually smaller, and in some cases, they became indistinguishable from the background discharge level (Fig. 1). Toward the end of the animals' day, light responses started to increase again and reached a maximum during mid-subjective night. The circadian variation in light response was significant in all animals ($P < 0.001$).

These results may indicate that the SCN is not capable of showing a substantial response to light during the subjective day. Alternatively, there may be a shift in the intensity-response curve as a function of circadian time in such a way that during daytime, higher light-intensities are required to stimulate light-responsive SCN units. To distinguish between these possibilities we measured the light-response of seven animals to four different higher light-intensities (0.5, 2, 17.5, and 140 lux) at all phases of the circadian cycle. An increase in light-intensity resulted in an increment in response at each phase of the circadian cycle (Fig. 2). However, at night (Fig. 2a–c), lower light-intensities were required than at daytime (Fig. 2d–f) to obtain a similar level of SCN discharge. This phenomenon was observed in all the seven animals.

The difference in response between day and night could reflect a similar difference in responsiveness at the level of single SCN units. It could also indicate a difference in proportion of light-activated and light-suppressed cells that contribute to the response at different phases of the cir-

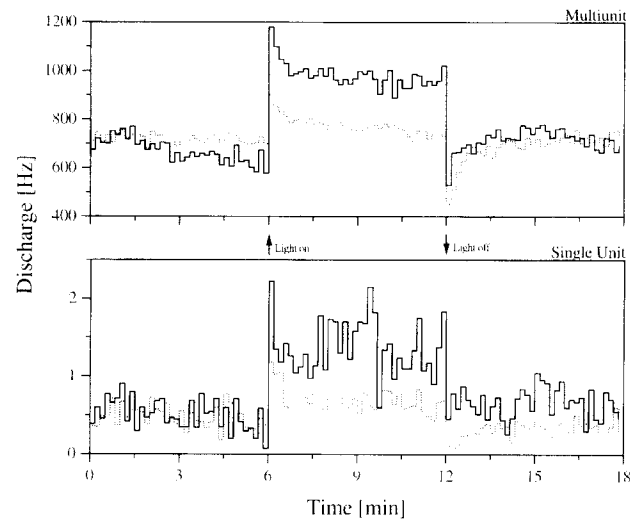


Fig. 3. Multi- and single unit light-response in the SCN of a representative rat to a 6-min light pulse. The multi- and single unit light responses were recorded simultaneously during mid-subjective day (solid line) and during mid-subjective night (dashed line). Light-intensity is 0.15 lux. The light response during day and night are the mean of six and seven trials, respectively.

dian cycle. In seven rats, we were able to isolate a single unit from the multiunit activity and to record from the unit for more than 24 h. These single units appeared to respond similarly, that is, responses to low light-intensities were larger at nighttime than at daytime (Fig. 3). Our results therefore demonstrate a circadian variation in light sensitivity as a result of an integrated response of similarly behaving single units.

Our current finding of a circadian variation in SCN light response is consistent with the rhythm in light response that has been observed in the invertebrate species, *Bulla gouldiana* which functions as an animal model in the study of circadian pacemaker mechanism [14]. In this species, the circadian pacemaker cells are located among the basal retinal neurons. Basal retinal neurons exhibit circadian rhythms in membrane potential with more depolarized potentials during daytime than during night. The circadian rhythm in mammalian SCN discharge rate (high during day and low at night) most likely reflects a similar rhythm in membrane potential [5]. It is noteworthy that light response is high at those phases of the circadian cycle where membrane potential is at its most hyperpolarized phase. As a result of the increased responsiveness at night, the system is optimized to detect light at those phases of the circadian cycle where phase shifts should occur in order to entrain to the light-dark cycle.

A second finding emerging from this study is that light responses exist at each phase of the circadian cycle, both at the level of multi- and single units. This is consistent with qualitative multiunit recordings in rats with a neuronally-isolated SCN where light-activated responses appeared to exist at four different time zones of the circadian cycle [11]. However, this study did not allow for an estimation

of the magnitude of the response at different phases. While light responses in the SCN exist at each phase of the circadian cycle, phase shifts are only possible during subjective night and not during subjective day. In other words, the circadian change in light response of visual SCN cells is very different from the all or none principle by which the circadian pacemaker responds to light. Recent work indicates the hamster retina contains a self-sustained oscillator. The circadian rhythm in light response measured in the SCN might be a product of a circadian rhythm of light response of retinal neurons [24].

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