Brain Research, 382 (1986) 109–118
Elsevier

BRE 11981

Luminance Coding in a Circadian Pacemaker: the Suprachiasmatic Nucleus of the Rat and the Hamster*

JOHANNA H. MEIJER[1,2], GERARD A. GROOS[2] and BENJAMIN RUSAK[3]

[1]Department of Physiology and Physiological Physics, University of Leiden, Leiden; [2]Department of Zoology, University of Groningen, Haren (The Netherlands) and [3]Department of Psychology, Dalhousie University, Halifax (Canada)

(Accepted February 4th, 1986)

Key words: suprachiasmatic nucleus — photic stimulation — circadian rhythm — neurophysiology — luminance coding

The hypothalamic suprachiasmatic nuclei (SCN) of mammals function as a pacemaker driving circadian rhythms. This pacemaker is entrained to the daily light–dark cycle in the environment via the retina and central retinal projections to the anterior hypothalamus. We carried out a comparative study of the visual properties of rat and hamster SCN neurons. Extracellular single cell activity was recorded in the SCN of urethane-anaesthetized animals. In both species, visual SCN neurons responded to retinal illumination with a sustained increase or a sustained decrease in electrical discharge. The majority (75%) of these cells were activated by light. In both the rat and the hamster SCN, visually responsive cells altered their discharge rate as a monotonic function of luminance. The intensity–response curve could be described by a Michaelis function with a small working range between threshold and saturation (2–3 log units) and a relatively high threshold. Intensity–response curves in both species were occasionally different for increasing as opposed to decreasing luminance. Thus, hysteresis effects of illumination may occur in the SCN. The spontaneous firing rates as well as the responsiveness of visual SCN cells were subject to marked variations between and within cells. The overall photic responsiveness of SCN neurons, however, indicated that they are specialized for luminance coding in the range of light intensities naturally occurring at dawn and dusk. This property makes these cells suitable to mediate photic entrainment of circadian rhythms as well as the measurement of photoperiod.

INTRODUCTION

Lesion studies have indicated that the suprachiasmatic nuclei (SCN) of the hypothalamus are important for the production of many circadian rhythms in mammalian species[35,44]. In rodents, there is additional evidence that the SCN in fact comprise a circadian pacemaker[18,24]. This pacemaker is intrinsically capable of sustaining circadian rhythmicity[14,24] and controls the circadian aspect of many behavioral and physiological processes[28,35,37]. Moreover, the SCN are essential for photoperiodic aspects of seasonal rhythms through the control of pineal melatonin production[4,10,28,33,35]. The SCN pacemaker also participates in timing the ovulation cycle in rodents[35,44]. For their proper timing all these biological rhythms are critically dependent on light. The SCN receive photic information from the environment via the retina[13,21,26,27,29,30,42]. This retinal projection permits the SCN pacemaker to entrain to the daily light–dark (LD) cycle and to measure the daily photoperiod[13,23,24,36].

Two retino-suprachiasmatic pathways exist by which light affects the SCN pacemaker. A retino-hypothalamic tract (RHT) terminates directly in the SCN[21,26,27,29,30] and an indirect projection reaches the SCN via the ventral lateral geniculate nucleus (vLGN)[5,6,13,19,22,30,34,40]. The vLGN receives a retinal input[19,22] while vLGN cells in the terminal field of the retinal fibers project with neuropeptide Y-containing axons to the SCN[5–7,19,30,34]. Although definitive evidence that this retino-geniculo-suprachiasmatic projection carries photic information to the SCN is still

* This manuscript was completed shortly before the death of Gerard Groos on March 25, 1985. His co-authors dedicate this paper to the memory of a close friend and colleague.

Correspondence: J.H. Meijer, Department of Physiology and Physiological Physics, University of Leiden, Wassenaarseweg 62, Leiden, The Netherlands.

lacking, there are strong indications that it is indeed a photic pathway[1,2,13,17,25]. The visual responsiveness of the SCN has been demonstrated by single and multiple-unit recordings[15,16,18,23,24,28,38]. In the rat and cat SCN, two types of visually responsive cells have been found. Ligh-activated cells respond to a stepwise increase in illumination with a sustained increase in their discharge rate whereas light-suppressed cells respond with a sustained decrease in electrical activity[15,16]. Both response types are also observed in the SCN following electrical stimulation of the optic nerve[28,38,39].

Most information concerning the visual properties of SCN cells stems from the rat. The hamster, however, has most often been chosen for investigation of the functional properties of the circadian pacemaker and, in particular, the light-dependence of pacemaker function[3,8–11,31,32,41]. Thus, a phase response curve for single light pulses has been measured most precisely in the hamster[8,32,41] and phase-dependent shifts in free-running activity rhythms to dark pulses have been reported[3,11]. The relation of phase-shifting behavior to entrainment by LD cycles, to tonic stimulation with light and to photo-periodism have also been studied extensively in the hamster[9,10,31].

The circadian system appears to be sensitive to the intensity of light exposure, as well as to its duration. Following presentation of a light pulse, the magnitude of the phase-shifts and the amount of melatonin suppression induced are a function of the magnitude of the light stimulus[4,33,41]. These studies suggest that the visual system of the SCN functions as a luminance detector. We have therefore undertaken an electrophysiological study of light-responsive SCN cells in urethane-anaesthetized rats and hamsters to document the luminance coding properties of this system. This study shows that the visual properties of the rat SCN that specialize it for luminance coding are also present in the SCN of the hamster.

MATERIALS AND METHODS

Animals

One series of experiments was performed on 19 male hamsters (*Mesocricetus auratus*; LGV:lak, purchased from Charles River Lakeview, Lakefield, NJ, U.S.A.) weighing 90–155 g. The hamsters were kept in an artificial photoperiod of 14 h of light (100–140 lux) daily to ensure a functional testicular state. In another experimental series, 37 male Wistar rats (Cpb:WU, TNO, Zeist, The Netherlands) were used with body weights ranging from 280 to 360 g. The rats were housed in a lighting regimen of 12 h of light (100–150 lux) alternating with 12 h of darkness.

Hamster experiments

Before surgery, hamsters, taken from either the light or the dark portion of the light–dark cycle, were anaesthetized by a series of small i.p. injections of a 20% urethane solution (total initial dose: 2 $g \cdot kg^{-1}$). In addition, the animals received a s.c. injection of atropine sulphate (0.04 mg). Small, supplementary doses of urethane were administered as necessary to maintain anaesthesia throughout the 6–13 h recording period. The hamsters were mounted in a stereotaxic instrument with the upper incisor bar 2 mm below the interaural line and cranial surgery was performed. A craniotomy was made to allow vertical lowering of a microelectrode into the suprachiasmatic region of the hypothalamus. The stereotaxic coordinates of the SCN were: 0.7 ± 0.5 mm anterior to bregma, 7.5 ± 0.5 mm ventral to the cortical surface and 0.1 ± 0.1 mm lateral to the midline. Rectal temperature was monitored continuously and maintained at 37 °C. Extracellular single neuron activity was recorded in and near the hamster SCN with platinum-iridium or tungsten electrodes with a 1–3 μm tip and an impedance of 1.0–1.7 MΩ at 1 kHz. The vertical movement of the microelectrode was controlled by a hydraulic or a stepmotor-controlled micromanipulator. At the end of the experiment the last electrode track was marked with electrolytic microlesions (6 μA current for 15 s) spaced at equal distances along the length of the track.

For photic stimulation a tungsten incandescent lamp was used as the light source. A collimated light bundle was passed through a glass fiber light guide. Infrared wavelengths were eliminated using an infrared stepfilter. The pattern and intensity of ocular illumination was regulated using an electronic shutter and neutral density filters. In the majority of experiments, whole-field retinal illumination of the left eye was used, while the right eye was occluded. The pupil of the left eye was maximally dilated with a 1% atropine sulphate solution. In the absence of visual stimulation the hamster was in complete darkness.

The eyes and exposed brain surface were protected by a coating of mineral oil.

Rat experiments

Rats were anaesthetized at various phases of the light–dark cycle with a combination of urethane (0.4 $g \cdot kg^{-1}$, i.p.) and hypnorm (Philips-Duphar, 1 $ml \cdot kg^{-1}$, i.p.). Additional urethane and hypnorm were administered throughout the experiment as required for stable narcosis. Surgery was supported with local anaesthesia. Electrical recording and visual stimulation techniques were as described previously[15,16]. Briefly, a glass micropipette recording electrode (3 M KCl with 2% pontamine sky blue, tip diameter 0.4–0.8 μm) was positioned in the suprachiasmatic region. Parallel tracks were made through the suprachiasmatic area and the optic chiasm using an hydraulic microdrive. The last track of each experiment was marked with pontamine sky blue microionophoresis. Either an electronically controlled glow modulation tube (Sylvania, R1131C) or a tungsten incandescent lamp was used for photic stimulation. In the latter case the light intensity was controlled with calibrated neutral density filters, while the pattern of stimulation was controlled with an electronic shutter. Light was directed to one eye (with the contralateral eye occluded) via a glass fiber light guide. The entire retina could thus be illuminated with spatially unstructured white light. The pupil of the illuminated eye was dilated with 1% atropine sulphate and the cornea was slowly superfused with saline.

Electrical recording and histology

Conventional amplification and processing techniques for extracellular single unit recording were used. Action potentials were converted to electronic pulses with a window discriminator and stored in a PDP 11/10 or an Apple computer, which also controlled the stimulation protocol. Light pulses ranging in length from less than 1 s up to 1.5 h were used, but most cells were tested using pulses varying in length from 10 s to 120 s. Apparent visual responses to briefer stimuli were always confirmed using 60- or 120-s long stimuli. Very short stimuli were used primarily to test for response latencies in cells already identified as visually responsive (Fig. 3). Cells were only identified as visually responsive when they met the criterion of showing a repeatable and consistent response to light on at least 4 consecutive trials. Spike train statistics and post-stimulus time histograms (PSTH) were computed on-line and processed further off-line. After marking the electrode track, the brains of the animals were perfused with formaline, sectioned coronally, and stained with cresyl-violet/luxol fast blue for microscopic reconstruction of the recording sites.

RESULTS

Tonic activation and suppression by light

In 37 rats a total of 543 cells were studied in the retrochiasmatic area and the portion of the anterior hy-

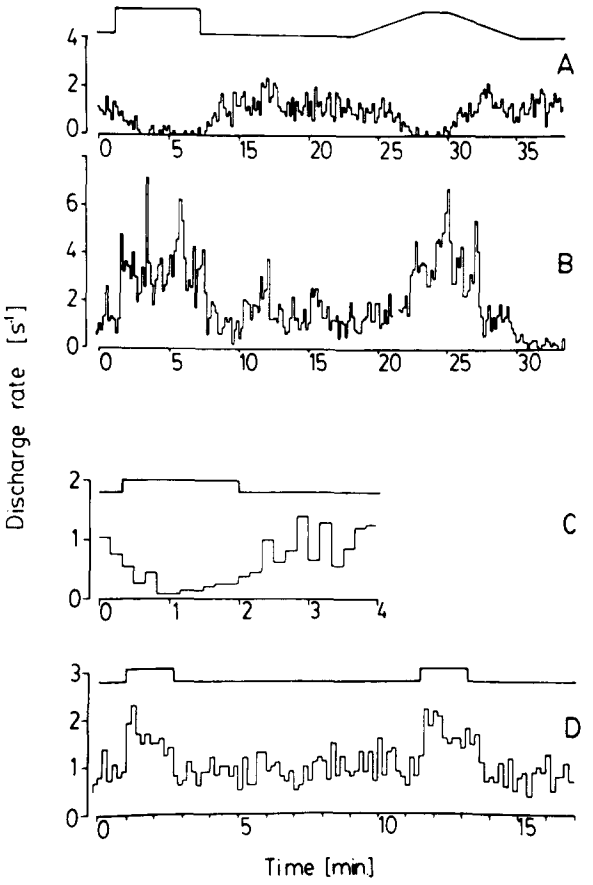

Fig. 1. Tonic activation and tonic suppression of electrical activity in the rat and hamster SCN. Light-suppression (A, C) and light-activation (B, D) are shown for individual neurons recorded in the rat (A, B) and hamster (C, D) SCN. The photic stimulus, indicated above the records, is presented against a dark background. The electrical activity of C and D is the mean activity of two sweeps. Spikes were counted every 10 s.

pothalamus overlying the optic chiasm. Light microscopic examination of the recording sites showed that of these neurons 205 were located in the SCN (including a region approximately 100 μm dorsal and lateral to the boundaries of the nucleus proper), the dorsal part of the chiasm ventral to the SCN, or in the rostral half of the retrochiasmatic area. In the following discussion these cells are referred to as SCN cells.

Of the rat SCN cells, 32% exhibited distinct visual responses. Twenty-seven percent of visual SCN cells were classified as light-suppressed and 73% as light-activated, according to response features described in detail elsewhere[15,16]. Briefly, the activated cells maintained increased mean discharge rates for the entire duration (exceeding 1 h in some rat experiments) of suprathreshold retinal illumination. Suppressed cells responded with a sustained decrease in electrical discharge, sometimes with complete cessation of all activity until the light was turned off. Examples of both types of response are shown in Fig. 1. The majority of these neurons were encountered in the caudal half of the SCN.

Of 219 cells recorded in the ventromedial region of the anterior hypothalamus in 19 hamsters, 50 were located within the SCN. Nineteen cells (38%) were visually responsive. These cells were recorded predominantly in the dorsal, lateral and ventral aspects of the SCN. One visual cell, however, was encountered 150 μm dorsal to the SCN and 4 visual cells were located 40–200 μm rostral to the SCN. Three of these 4 cells were embedded in the optic chiasm (Fig. 2). All visual cells in the hamster responded to light in a tonic on or tonic off fashion and, following the classification used for visual SCN cells in the rat, they were characterized as activated and suppressed cells respectively (Fig. 1). Activated cells in the hamster

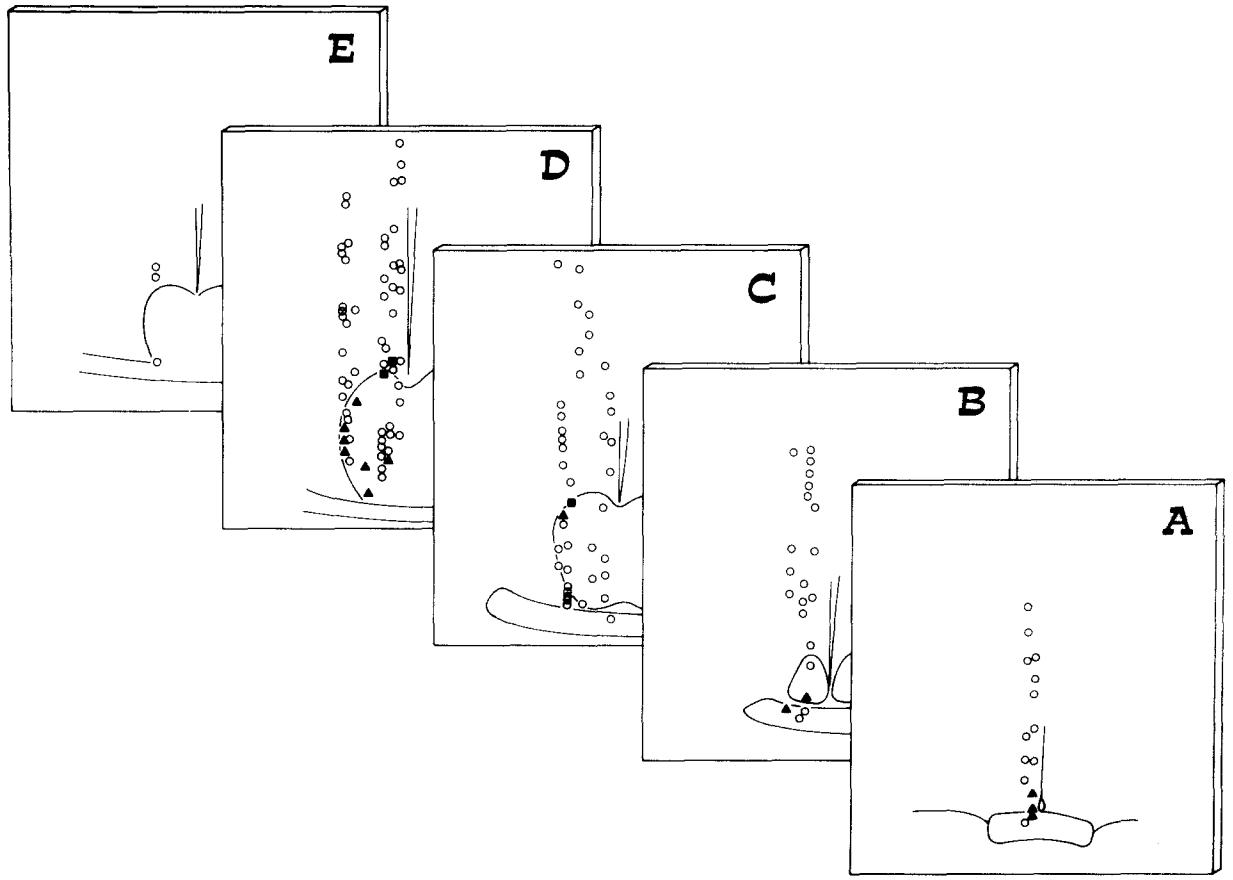

Fig. 2. Localization of light-responsive neurons in the hamster suprachiasmatic region. Visual cells of both the activated (triangles) and the suppressed (squares) types were encountered at various levels along the rostro-caudal axis (A–E). The circles mark the location of visually unresponsive cells. Cells are marked to the left of the third ventricle.

maintained an increased firing rate while the stimulus was on and suppressed cells responded oppositely. The activated cells were more common than the suppressed cells. Only 3 cells (16%) were suppressed while 16 (84%) were activated by light. Thus, the ratios of suppressed to activated visual cells in the suprachiasmatic region were 0.37 and 0.19 for the rat and hamster, respectively. In the hamster the 3 suppressed neurons were at the dorsal SCN border, but it would be premature to conclude that a regional differentiation of response exists. In the rat, both response types could be found in cells located close to one another.

Brief light stimuli (duration < 5 s) did not always evoke a full response in cells that were clearly responsive to longer light exposures. For instance, using a suprathreshold (540 lux) flash of 500 ms duration, one light-activated neuron in the rat SCN required 100 stimulus presentations to allow detection of a visual response in the PSTH. A 2 min light pulse of the same intensity produced a clearly distinguishable response in this cell with only one stimulus presentation. The fact that dark discharge rates of visual cells in either species could be as low as 2.5 min^{-1} contributed to the need for long light pulses to permit recognition of a visual response. Although the light-induced changes in firing rate may be small, the relative increase or decrease in firing rate was always considerable.

Dark discharge, response variability and circadian time

Activated as well as suppressed cells showed a variety of response patterns. Differences between PSTHs were especially apparent at the onset and end of a light pulse. First, the response latency of visual cells in the hamster varied from less than 400 ms to 10 s, response latencies of less than 1 s being most common. In the rat SCN, the visual response was occasionally detectable within 100 ms after light onset but most commonly developed fully between 200 ms and 20 s after the beginning of stimulation. Fig. 3 shows PSTHs to brief (less than 1 s) photic stimuli that illustrate typical response latencies in the rat SCN. Second, a transient component preceded the sustained response in 11 activated cells in hamsters. In this species the duration of this transient initial phase varied from 10 to 80 s (mean: 38.2 ± 18.9 s). A much shorter

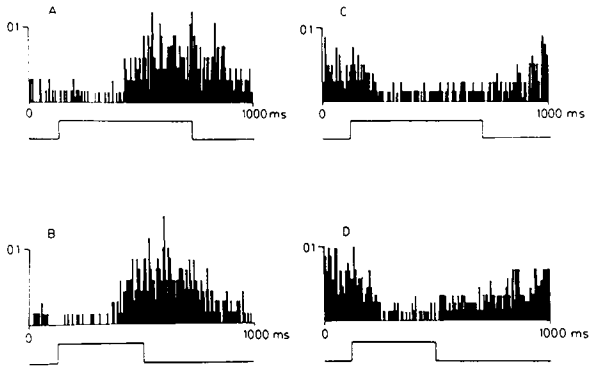

Fig. 3. PSTHs for a light-activated (A, B) and a light-suppressed (C, D) SCN cell in the rat. The cells were stimulated with brief light pulses, indicated below the records, against a dark background.

transient of less than 400 ms preceded the longer transient in all responsive hamster cells located in or on the border of the chiasm (Fig. 4). In the SCN of

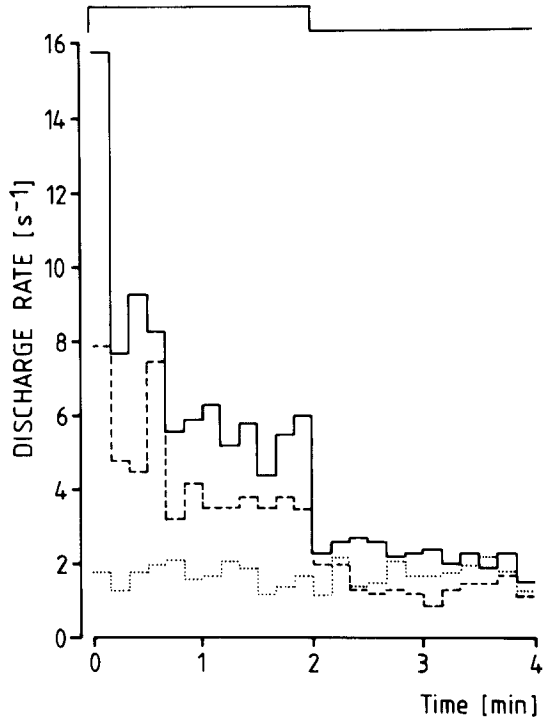

Fig. 4. An example of the transient components preceding the sustained response in an activated cell in the hamster. In the same figure, the response to different light intensities is illustrated. The continuous line represents the response to a light pulse with a duration of 2 min and a light intensity of $14 \cdot 10^3$ lux, the broken line the response to $61 \cdot 10^2$ lux and the dotted line is the discharge rate in continuous darkness. The responses presented are the mean of two sweeps (4 min per sweep) while spikes were counted every 10 s.

the rat, brief transients in the photic response were very rare (cf. ref. 16). The great majority of cells attained their steady state light-adapted discharge gradually. Neither in the rat nor in the hamster were transients encountered in light-suppressed cells. Third, in both species the discharge of visual SCN cells returned to a dark-adapted level at a variable rate within 1 min of the end of stimulation.

Although the general characteristics of the PSTHs varied considerably among different cells, for any particular neuron they were virtually constant. In the hamster, however, 4 cells which were studied for periods of several hours displayed prominent spontaneous fluctuations in their discharge rates. A maximum of 20-fold changes in the spontaneous discharge rate were observed. The absolute amplitude of suppression and activation elicited by a pulse of light varied accordingly (Fig. 5A). An increase in the spontaneous discharge rate always resulted in an increase in the absolute magnitude of activation or suppression.

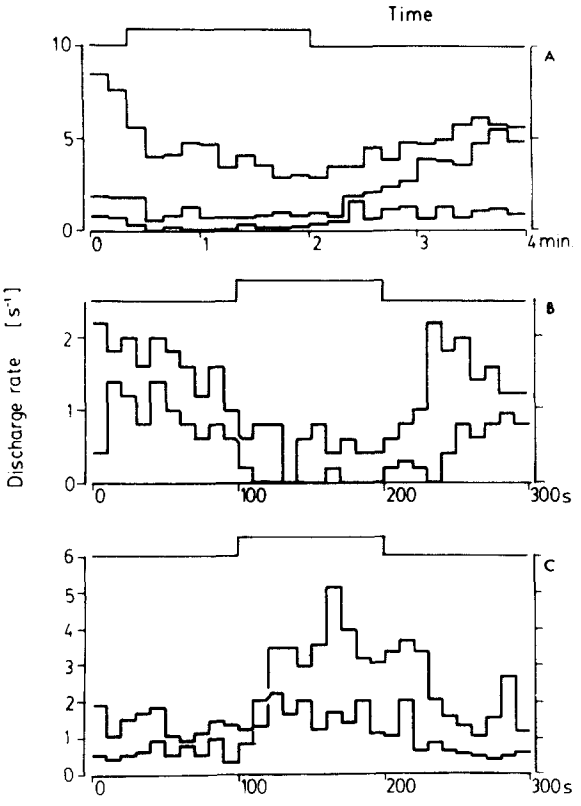

Fig. 5. Variability in absolute photic response magnitude in hamster (A) and rat (B, C) SCN cells. Records A and B show repeated samples from two light-suppressed cells. A light-activated cell is shown in C.

The relative magnitude of the visual response was not constant but varied unpredictably with the overall discharge rate. Similar observations were made for visual cells in the rat SCN (Fig. 5B). Just over half of those light-responsive cells in the rat which were studied for periods exceeding 1 h exhibited slow variations in the dark discharge rate with a concomitant variation in absolute photic responsiveness. Despite these fluctuations, the general response pattern (characterized by latency, duration of the transient component and rate of dark adaptation of the cell) remained stable in each cell. It should be emphasized that the variations in spontaneous electrical discharge and in response magnitude occurred within periods of a few hours. Thus, the variability in visual responsiveness described here was not a result of the circadian pacemaker cycle of the SCN.

The occurrence of suppression or activation was not obviously related to the phase of the LD cycle to which the animals had been exposed before the experiment. Either type of visually responsive cell could be found at any phase of the circadian cycle.

Intensity–response curves and hysteresis

Intensity-response curves were obtained by plotting the mean discharge rate as a function of the intensity of the adaptation luminance. Intensity–response curves were obtained in two different ways. First, single light-pulses of different intensities were presented after the eye had been dark-adapted for at least 2 min. The mean steady-state discharge rate was then recorded as a function of luminance. Second, intensity–response curves were plotted for continuously increasing or decreasing illumination (Fig. 1A) or for a series of ascending or descending steps of light intensity (Fig. 8). Intensity–response functions were obtained in the hamster only for activated cells because the overall discharge rates of the suppressed cells were too low to meaningfully describe the intensity-dependence of the response. Luminance–response curves were measured for both response types in the rat.

In both species the intensity–response curve of activated cells was characterized by a monotonic increase in discharge rate as a function of luminance, irrespective of the method employed to obtain the curves. A threshold existed for low levels of illumination and below this value luminance-dependent activ-

ity could not be distinguished. Above the threshold the cells had a working range within which the discharge increased until saturation was reached at light intensities of approximately 2–3 log units above threshold (Fig. 6). In some of the rat SCN cells saturation was not observed even at the highest intensities (800 lux) that could be presented. The threshold and saturation points in both species were rather constant for a particular visual SCN cell but differed among cells. The mean standardized intensity–response curve for various hamster SCN cells was computed by correcting for the mean dark discharge rate of each cell and subsequently normalizing the luminance-dependent discharge (Fig. 7). This average curve exhibited the same general features as curves for individual cells. The intensity–response curve can be readily described by a Michaelis function (Fig. 7). Similar functions were obtained for the monotonically decreasing curve observed for light-suppressed cells in the rat SCN.

Both gradual and step changes in light-intensity yielded similar, monotonic, intensity–response

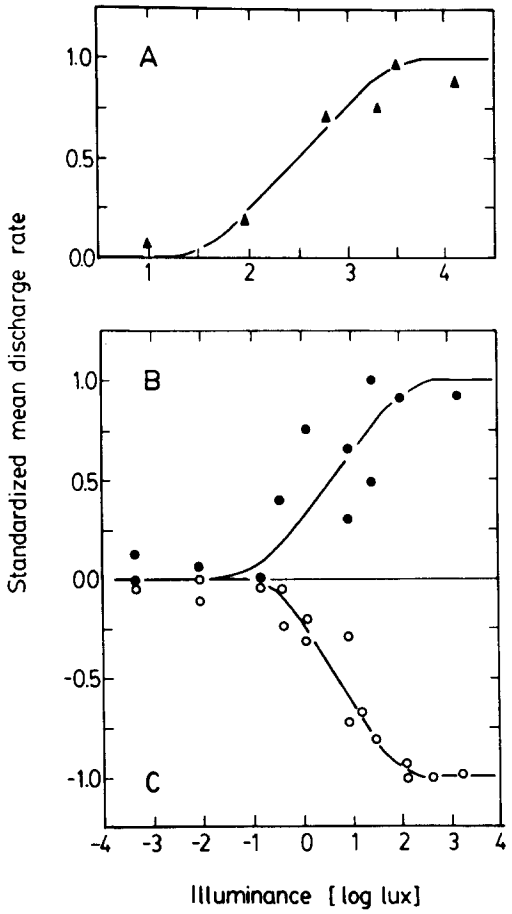

Fig. 6. Examples of luminance-dependent electrical activity of light-activated cells in the hamster (A), and a rat (B) and a light-suppressed cell in the rat (C). For these intensity–response curves the steady-state mean discharge rate during light-pulse stimulation of different intensities (defined as the mean discharge rate 60 s after onset of the light pulse) was corrected for the dark discharge rate (the mean discharge rate prior to the onset and 100 s after ofset of the light pulse) and subsequently normalized with respect to the maximal response. Each curve could be fitted by a Michaelis function with Hill coefficients ranging from 0.6 to 0.9.

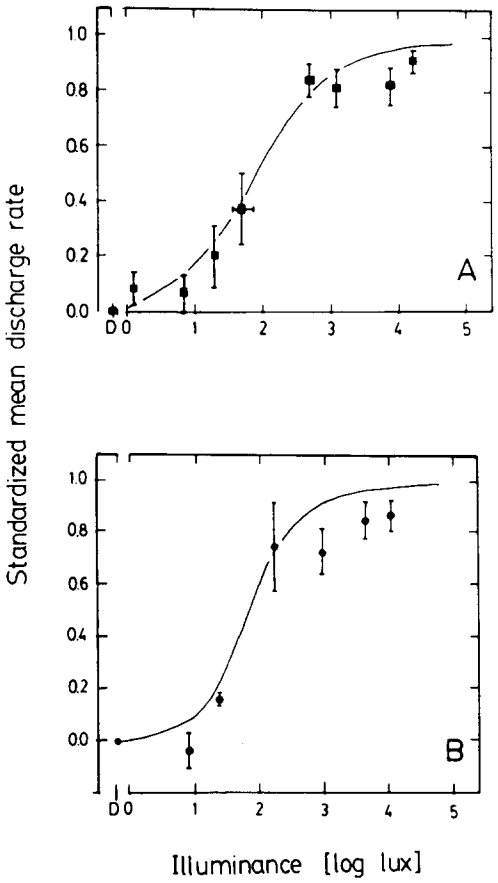

Fig. 7. Average intensity–response curves for several light-activated cells in the hamster. The mean of various measurements at any light intensity is presented with the standard error of the mean. The curve in A is composed for light-activated cells, including those few located just rostrally to the SCN. The curve in B represents only those cells that were located strictly within the boundaries of the SCN. The curves fitted are based on Michaelis functions. As in Fig. 5 the dark discharge rate is subtracted from the steady-state discharge rate at any light intensity. Next, the maximum corrected response is normalized to one.

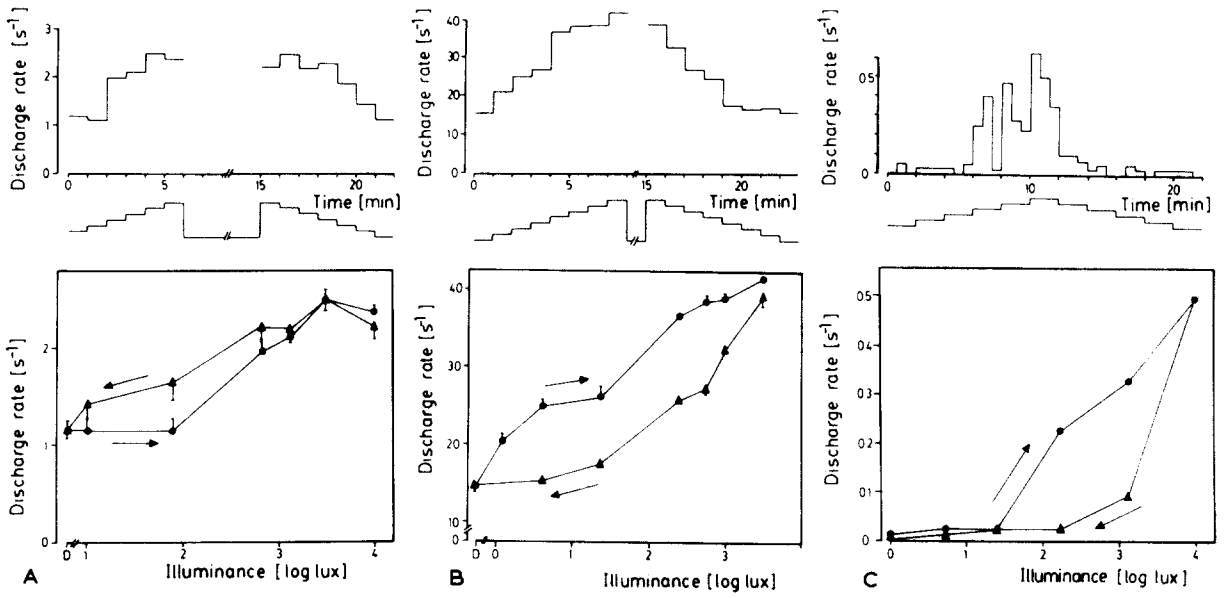

Fig. 8. Hysteresis in intensity–response functions for 2 (B,C) out of light-activated SCN cells in the hamster. The records of electrical activity are shown in the upper row of panels. The staircase stimulus protocol is shown immediately underneath the records. Intensity–response curves corresponding to the records are shown below. D indicates the state of complete dark adaptation. In A, the discharge rate was counted every 1.2 s while the mean discharge rate and the standard error of the mean are computed every minute. In B, the mean and the standard error are computed every 59.4 s over 33 data units. In C, the discharge rate was counted every 1/3 minute in the upper panel and every minute in the lower panel.

curves, exhibiting threshold and saturation behavior. For some cells in both species, however, the intensity–response curve for increasing luminance was slightly different in curvature and position along the intensity axis from that for decreasing luminance (Fig. 8). Thus, the luminance-dependent discharge rate of these cells was affected by the previous adaptation level. This phenomenon shows that hysteresis may occur in the light-intensity coding of visual SCN cells.

DISCUSSION

The circadian pacemaker in the rodent SCN receives a dual optic input[14,36]. It has previously been shown for the rat and the cat that photic information reaching the SCN through these pathways results in activation or suppression of the electrical activity of visually responsive neurons[15,16,23,24,28,38]. The present experiments extend this observation to the SCN of the hamster, an animal which has been the subject of many investigations of the functional organization of photic entrainment and photoperiodic time measurement. In particular, it was shown that in the SCN of the hamster as well as the rat, visual cells function as luminance coders, whose mean discharge rate reflects the level of overall ambient illumination within a restricted range of light intensities. The dependence of their discharge on the history of previous illumination is reflected in the hysteresis often observed in the intensity–response curve. Together, these properties render visual SCN neurons suitable for detecting the transitions in environmental illumination at dawn and dusk and for monitoring the level of maintained external illumination. Behavioral studies have indicated that these features of the photic environment are of primary importance for the control of the circadian pacemaker and photoperiodic clock in the rodent SCN[9,10,31,41].

In both the rat and the hamster SCN, the terminal fields of afferents from the retina and the vLGN overlap to a large extent[5–7,21,27,30,34,40]. Moreover, the RHT is known to terminate in areas rostral and dorsal to the SCN[30] where neuropeptide Y-like immunoreactivity is also seen[5–7,19]. Cells receiving retinal input have been found embedded in the optic chiasm adjacent to the SCN[26]. Thus, the histological reconstruction of the location of rat and hamster vis-

ual cells (Fig. 2) indicates a good agreement with the known termination of the direct and indirect visual afferents of the SCN. In the rat, the predominant presence of visual cells in the caudal portion of the SCN is consistent with the neuroanatomical findings[21,27].

Unfortunately, the common terminal fields of the RHT and the vLGN-SCN projection prohibit any inference from the anatomical location of a recording site as to whether a particular SCN cell is driven through the vLGN pathway, the RHT or through both. Neither can the identity of the relevant pathway be established from characteristic response latencies. The latencies found in this study far exceed those expected on the basis of conduction times and synaptic transmission delays. Previous experiments have demonstrated that suppressed and activated cells are each present in the SCN of optic tract- or vLGN-ablated rats[13,15,17,38]. Moreover, excitatory and inhibitory responses to electrical stimulation of the optic nerve or optic chiasm are observed in in vitro slice preparations of the rat SCN[39]. Thus, activation and suppression can both be mediated by the RHT and, hence, in intact animals the response type is not indicative of which optic input drives a particular SCN cell.

Marked variability was noted in the photic responsiveness of cells in the SCN of hamsters and rats. There was considerable variation in the temporal pattern of response to a light pulse both within and between cells. Short-term fluctuations in the absolute magnitude of the response to various light intensities were similarly present. This variability may conceivably restrict reliable processing of visual information by the circadian pacemaker in the SCN. However, when the standardized intensity–response curves of a number of cells are averaged, it appears that together these cells are capable of a rather precise representation of the level of ambient illumination (Fig. 7). Whether this type of response integration is actually employed by the SCN is unknown. In view of the high degree of interconnectivity of the SCN[43] the notion of visual response integration is at least viable.

Despite differences in response pattern and response magnitude among cells, the same general functional characteristics are encountered. In both species visual SCN cells exhibit a threshold above which the discharge rate increases (activation) or decreases (suppression) monotonically as a function of light intensity, and the responses of most cells saturate at higher levels of illumination. The response threshold in anaesthetized animals (approximately 1 lux in the hamster and 0.1 lux in the rat SCN) is high compared with the threshold for scotopic vision in nocturnal rodents[20]. The working range of these cells, moreover, is narrow compared to the wide range of naturally occurring light intensities ($4 \cdot 10^{-5}$–10^5 lux)[12].

Fluctuations in illumination level below threshold and above the saturation point do not provide information to the SCN pacemaker. Visual SCN cells appear to be specialized to monitor changes in moderate light intensities such as those that occur during the dusk and dawn transitions. Furthermore, they are capable of coding the absolute level of constant illumination only within a limited range of intensities approximately 3 log units above threshold. This interpretation of the present data will depend on the observation in alert animals of intensity–response functions in SCN units that resemble those in urethane-anaesthetized animals. The high threshold and narrow working range are at least consistent with functional studies of the phase-shifting effectiveness of light in alert hamsters[41].

ACKNOWLEDGEMENTS

We are grateful to Mary Harrington and Donna Goguen for their help in the conduct of these studies. This research was supported by grants from Z.W.O. of The Netherlands, NSERC of Canada and the Dalhousie RDFS.

REFERENCES

1 Albers, H.E., Ferris, C.F., Leeman, S.E. and Goldman, B.D., Avian pancreatic polypeptide phase shifts hamster circadian rhythms when microinjected into the suprachiasmatic region, *Science*, 223 (1984) 833–835.

2 Albers, H.E. and Ferris, C.F., Neuropeptide Y: role in light-dark cycle entrainment of hamster circadian rhythms, *Neurosci. Lett.*, 50 (1984) 163–168.

3 Boulos, Z. and Rusak, B., Circadian phase response curves for dark pulses in the hamster, *J. Comp. Physiol.*, 146 (1982) 411–417.

4 Brainard, G.C., Richardson, B.A., King, T.S., Matthews, S.A. and Reiter, R.J., The suppression of pineal melatonin content and N-acetyltransferase activity by different light irradiances in the Syrian hamster: a dose–response relationship, *Endocrinology,* 113 (1983) 293–296.

5 Card, J.P. and Moore, R.Y., Ventral lateral geniculate nucleus efferents to the rat suprachiasmatic nucleus exhibit avian pancreatic polypeptide-like immunoreactivity, *J. Comp. Neurol.,* 206 (1982) 390–396.

6 Card, J.P., Brecha, N. and Moore, R.Y., Immunohistochemical localization of avian pancreatic polypeptide-like immunoreactivity in the rat hypothalamus, *J. Comp. Neurol.,* 217 (1983) 123–136.

7 Card, J.P. and Moore, R.Y., The suprachiasmatic nucleus of the golden hamster: immunohistochemical analysis of cell and fiber distribution, *Neuroscience,* 13 (1984) 415–431.

8 Daan, S. and Pittendrigh, C.S., A functional analysis of circadian pacemakers in nocturnal rodents. II. The variability of phase response curves, *J. Comp. Physiol.,* 106 (1976) 253–266.

9 Daan. S. and Pittendrigh, C.S., A functional analysis of circadian pacemakers in nocturnal rodents. III. Heavy water and constant light: homeostasis of frequency?, *J. Comp. Physiol.,* 106 (1976) 267–290.

10 Elliott, J.A. and Goldman, B.D., Seasonal reproduction. In N.T. Adler (Ed.), *Neuroendocrinology of Reproduction,* Plenum Press, New York, 1981, pp. 377–423.

11 Ellis, G.B., McKlveen, R.E. and Turek, F.W., Dark pulses affect the circadian rhythm of activity in hamsters kept in constant light, *Am. J. Physiol.,* 242 (1982) R44–R50.

12 Hamburger, F.A., *Das Sehen in der Dämmerung,* Springer-Verlag, Wien, 1949.

13 Groos, G.A., The neurophysiology of the mammalian suprachiasmatic nucleus and its visual afferents. In J. Aschoff, S. Daan and G.A. Groos (Eds.), *Vertebrate Circadian Systems,* Springer-Verlag, Heidelberg, 1982, pp. 96–105.

14 Groos, G.A. and Hendriks, I., Circadian rhythms in electrical discharge of rat suprachiasmatic neurons recorded in vitro, *Neurosci. Lett.,* 34 (1982) 283–288.

15 Groos, G.A. and Mason, R., Maintained discharge of rat suprachiasmatic neurons at different adaptation levels, *Neurosci. Lett.,* 8 (1978) 59–64.

16 Groos, G.A. and Mason, R., The visual properties of rat and cat suprachiasmatic neurones, *J. Comp. Physiol.,* 135 (1980) 349–356.

17 Groos, G.A. and Rusak, B., Neurophysiological studies of the ventral lateral geniculate-suprachiasmatic nucleus projection in the rat, *Soc. Neurosci. Abstr.,* 8 (1982) 543.

18 Groos, G.A., Mason, R. and Meijer, J.H., Electrical and pharmacological properties of the suprachiasmatic nuclei, *Fed. Proc.,* 42 (1983) 2790–2795.

19 Harrington, M.E., Nance, D.M. and Rusak, B., Neuropeptide Y immunoreactivity in the hamster geniculo-suprachiasmatic tract, *Brain Res. Bull.,* 15 (1985) 465–472.

20 Hecht, S., Shlaer, S. and Pirenne, M.H., Energy, quanta and vision, *J. Gen. Physiol.,* 25 (1942) 819–840.

21 Hendrickson, A.E., Wagoner, N. and Cowan, W.N., An autoradiographic and electron microscopic study of retino-hypothalamic connections, *Z. Zellforsch.,* 135 (1972) 1–26.

22 Hickey, T.L. and Spear, P.D., Retinogeniculate projections in hooded and albino rats: an autoradiographic study, *Exp. Brain Res.,* 24 (1976) 523–529.

23 Inouye, S.T., Light responsiveness of the suprachiasmatic nucleus within the island with the retino-hypothalamic tract spared, *Brain Research,* 294 (1984) 263–268.

24 Inouye, S.T. and Kawamura, H., Characteristics of a circadian pacemaker in the suprachiasmatic nucleus, *J. Comp. Physiol.,* 146 (1982) 153–160.

25 Meijer, J.H., Rusak, B. and Harrington, M.E., Geniculate stimulation phase shifts hamster circadian rhythms, *Soc. Neurosci. Abstr.,* (1984) 502.

26 Millhouse, O.E., Optic chiasm collaterals afferent to the suprachiasmatic nucleus, *Brain Research,* 137 (1977) 351–355.

27 Moore, R.Y., Retinohypothalamic projection in mammals: a comparative study, *Brain Research,* 49 (1973) 403–409.

28 Nishino, Y. and Koizumi, K., The role of the suprachiasmatic nuclei of the hypothalamus in the production of circadian rhythm, *Brain Research,* 112 (1976) 45–59.

29 Pickard, G.E., Morphological characteristics of retinal ganglion cells projecting to the suprachiasmatic nucleus: a horseradish peroxidase study, *Brain Research,* 183 (1980) 458–465.

30 Pickard, G.E., The afferent connections of the suprachiasmatic nucleus of the golden hamster with emphasis on the retinohypothalamic projection, *J. Comp. Neurol.,* 211 (1982) 65–83.

31 Pittendrigh, C.S. and Daan, S., A functional analysis of circadian pacemakers in nocturnal rodents. IV. Entrainment: pacemaker as clock, *J. Comp. Physiol.,* 106 (1976) 291–331.

32 Pohl, H., Differences in responses of the circadian system to light in the Syrian hamster, *Physiol. Zool.,* 57 (1984) 509–520.

33 Reiter, R.J., Action spectra, dose–response relationships, and temporal aspects of light's effects on the pineal gland. In R.J. Wurtman, M.J. Baum and J.T. Potts, Jr. (Eds.), The Medical and Biological Effects of Light, *Ann. N.Y. Acad. Sci.,* 453 (1985) 215–230.

34 Ribak, C.E. and Peters, A., An autoradiographic study of the projections from the lateral geniculate body of the rat, *Brain Research,* 92 (1975) 341–368.

35 Rusak, B. and Zucker, I., Neural regulation of circadian rhythms, *Physiol. Rev.,* 59 (1979) 449–526.

36 Rusak, B. and Boulos, Z., Pathways for photic entrainment of mammalian circadian rhythms, *Photochem. Photobiol.,* 34 (1981) 267–273.

37 Rusak, B. and Groos, G.A., Suprachiasmatic stimulation phase shifts rodent circadian rhythms, *Science,* 215 (1982) 1407–1409.

38 Sawaki, Y., Suprachiasmatic nucleus neurones: excitation and inhibition mediated by the direct retino hypothalamic projection in female rats, *Exp. Brain Res.,* 37 (1979) 127–138.

39 Shibata, S., Oomura, Y., Hattori, K. and Kita, H., Responses of suprachiasmatic nucleus neurons to optic nerve stimulation in rat hypothalamic slice preparation, *Brain Research,* 302 (1984) 83–89.

40 Swanson, L.W., Cowan, W.M. and Jones, E.G., An autoradiographic study of the efferent connections of the ventral lateral geniculate nucleus in the albino rat and cat, *J. Comp. Physiol.,* 156 (1974) 143–164.

41 Takahashi, J.S., DeCoursey, P.J., Bauman, L. and Menaker, M., Spectral sensitivity of a novel photoreceptive system mediating entrainment of mammalian circadian rhythms, *Nature (London),* 308 (1984) 186–188.

42 Underwood, H. and Groos, G., Vertebrate circadian rhythms: retinal and extraretinal photoreceptors, *Experientia,* 38 (1982) 1013–1021.

43 Van de Pol, A.N., The hypothalamic suprachiasmatic nucleus of rat: intrinsic anatomy, *J. Comp. Neurol.,* 191 (1980) 661–702.

44 Zucker, I., Rusak, B. and King, R.G., Sr., Neural bases for circadian rhythms in rodent behaviour. In A.H. Riesen and R.F. Thompson (Eds.), *Advances in Psychobiology,* Vol. 3, Wiley and Sons, New York, 1976, pp. 35–74.