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The relation between light-induced discharge in the suprachiasmatic nucleus and phase shifts of hamster circadian rhythms

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The role of neurophysiological activation of suprachiasmatic nucleus (SCN) cells in phase shifting the circadian pacemaker of the hamster was investigated in a combined behavioural and electrophysiological study. An electrophysiological study examined the relation between the pattern of light presentation and the induced discharge rate in the SCN. Behavioural experiments examined the relation between the pattern of light presentation and the magnitude of phase shift induced. The combination of these results provides an indirect assay of the relations between induced neural discharge in the SCN and phase shifts of the circadian activity rhythm. The data indicate that the magnitude of phase shifts is monotonically, but not linearly, related to photically induced changes in discharge rate.

INTRODUCTION

At the base of the anterior hypothalamus of mammals reside the suprachiasmatic nuclei (SCN), which function as a circadian pacemaker[17,27]. The photoreceptors responsible for entrainment of the SCN to environmental lighting cycles are found in the retina (see refs. 20,34 and Rusak and Boulos, unpublished results). From the retina, the retinohypothalamic tract (RHT) projects directly to the SCN[5,18,21]. An indirect visual projection, the geniculohypothalamic tract (GHT), conveys photic information to the SCN via the intergeniculate leaflet and ventral lateral geniculate nucleus (see refs. 6,12,22,32,35).

Photic sensitivity of the circadian pacemaker has been assessed in both behavioural and electrophysiological studies. Behavioural studies have used the amplitude of light-induced phase shifts as the dependent measure in assessing pacemaker sensitivity to light. The phase of a rodent's circadian rhythm, freerunning in constant darkness, can be shifted by presenting a single, appropriately timed light pulse. The magnitude of the resultant phase shift depends on the phase of the circadian cycle at which the light pulse is presented and on the duration, intensity and wavelength characteristics of the light[8,19,23,33].

Neurophysiological studies of the SCN using both single-unit and multi-unit recordings have shown that a subpopulation of SCN cells is responsive to retinal illumination[10,13,28]. Their responsiveness to changes in both duration and intensity of illumination has been well documented for the SCN of the rat and the hamster[10,11,16]. Light-responsive SCN cells either increase or decrease their mean discharge rate for the full duration of the light presentation. The change in discharge rate is a monotonic function of the light intensity presented[16]. The cumulative difference in cell firing between a period of illumination and a comparable period of darkness, therefore, is a function of both the intensity and the duration of the light stimulus, parameters which also influence the magnitude of a phase shift.

The question arises how light-induced changes in the firing rates of SCN cells relate to phase shifts of the circadian pacemaker. Lacking a method to directly compare the effects of single light stimuli on firing

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rates and amplitude of induced phase shift, we have used a series of behavioural and electrophysiological experiments which can be related to each other to provide an indirect assay of these relations: (1) the effect of a train of brief light pulses on the discharge patterns of light-responsive SCN cells was investigated and compared with the effects of a single, longer light pulse; (2) the effect of a train of light pulses on the magnitude of a phase shift was studied and compared with its effect on SCN cell firing rates; and (3) the phase-shifting effects of various intensities of a single, continuous light pulse were studied and compared to the intensity–response curve of visual SCN cells (see Meijer et al.[16]).

MATERIALS AND METHODS

Neurophysiology

Twenty-five male Golden hamsters weighing 97–125 g were purchased from Charles River Lakeview, Lakeview, NJ, USA. They were housed in a light/dark (LD) cycle of 14 h of light and 10 h of darkness. Animals were anesthetized by intraperitoneal injections of a 20% urethane solution (initial dose 2 $g \cdot kg^{-1}$) and received additional urethane injections throughout the experiment, as needed. Robinul (0.3 cl, s.c.) was injected to reduce breathing difficulties during the recording session. Visual stimulation was provided as previously described[16], except that stimulation was binocular instead of monocular. The pupils were dilated by application of 1% atropine sulphate to the cornea. Light from a Tungsten incandescent lamp was led through two glass fiber optics to milk glass screens, which had been placed in front of the eyes to provide diffuse illumination of the entire retina. The timing of photic stimulation was controlled by an Apple computer.

Extracellular single unit recordings were made with Tungsten electrodes (tip: 1–3 μm, 1.0–1.7 MΩ). The mean stereotaxic coordinates were 0.7 mm anterior, 0.8 mm lateral (at an angle of 5° to the vertical axis) and 6.7–7.6 mm below dura. The upper incisor bar was 2 mm below the interaural line. The recording electrode was advanced using an electronic microdrive. Signal amplification and recording techniques were similar to those of Meijer et al.[16]. Action potentials were converted to electronic pulses with a window discriminator and stored by an Apple computer.

Neurons in and near the SCN were tested for visual responses to light pulses presented repeatedly for 60–100 s. Recordings were performed at all phases of the subjective night. Cells were classified as visually responsive according to criteria used in previous experiments[16,17]. The eyes were then illuminated with a train of 1-min light pulses alternating with 1 min of total darkness to a total of 15 min of light per sweep. On other sweeps, 15 min of continuous illumination was presented. Spike train statistics and peri-stimulus time histograms were computed on-line and were further processed off-line. At the end of a recording, a small electrolytic current was passed through the microelectrode to mark the recording site. Brain slices 40 μm thick were later obtained and stained with Cresyl violet to verify the locations of recorded cells.

Behavioural studies

Thirty-six male golden hamsters aged 8 weeks were obtained from TNO, Zeist, The Netherlands. The animals were individually housed in cages equipped for recording wheel-running activity. Food and water were available ad libitum. The room temperature was 22°C.

Eighteen animals were entrained to an LD cycle of 14:10 for at least 2 weeks. After release to constant darkness for a period of 7 days, a 15-min white light pulse was presented approximately 6 h after activity onset (circadian time (CT) 18). Light pulses were presented to all animals simultaneously without otherwise disturbing them. Animals were kept in constant darkness for another 12 days following the pulse and were then re-entrained to an LD cycle for 2 weeks. This procedure was repeated seven times with a light pulse of different intensity each time (range 0–260 lx, fluorescent light). Light intensities were measured at the bottom of each cage at the end of every experiment.

The other group of 18 animals was exposed to a train of light pulses at around CT 18. Light pulses were presented similarly to those described above. The train consisted of one, two, four or eight pulses of 1 min, separated by 1 min of darkness and it was presented after 7 days in constant darkness. The light intensity in these experiments was equal to the half-saturation value which was determined in the first behavioural experiment. Animals were allowed to freerun in constant darkness for another 12 days before they were re-entrained to an LD cycle.

Phase shifts were analyzed by eye-fitting a straight line through seven activity onsets before the light pulse and through ten onsets after the pulse. The first two activity onsets immediately after the pulse were not included in the analysis. The fitted lines were extrapolated to the day of the pulse to measure the phase shift. Activity onset was defined as CT 12[23].

RESULTS

Light-evoked discharge in the suprachiasmatic nucleus

In 12 animals, 177 hypothalamic cells were recorded, of which 148 were located just outside the SCN (maximum distance 800 μm) and 29 were within the SCN. Of these cells, eight were consistently activated by light and one was light suppressed. The visually responsive cells were all located within the area of visually responsive neurons described previously in the hamster hypothalamus[16]. Responsive cells were mostly located along the dorsolateral or ventral border of the SCN. One was found 400 μm dorsal to the SCN and three were anterior to the SCN (200 μm, $n = 2$, one of which was light-suppressed and 480 μm, $n = 1$). Inside the SCN, about 17% ($n = 5$) of the recorded cells were light responsive, while outside the SCN only 3% ($n = 4$) responded consistently to light.

Continuous 15-min light pulses induced responses typical of those previously recorded from SCN cells (Fig. 1A,B). In a minority of cases the cell attained its steady-state discharge rate immediately and maintained it throughout the light presentation. In all other cases a phasic component preceded the sustained response to light (see Meijer et al.[16]).

The discharge in the dark was about 40% of that during the light but differed very much among different cells. In those cells showing a phasic component, the mean discharge rate in the first minute of light presentation is, by definition, greater than that in the last minute. The beginning of the light pulse, therefore, contributes more to the overall cumulative change in discharge activity caused by a light pulse than do the later portions of the stimulus. Thus the change in total

neural activity of these units attributable to light exposure does not increase linearly with the duration of a continuous light pulse.

Responses to trains of light pulses were obtained from eight cells (Fig. 1C,D). Trains of 3, 7, 8, 10 or 15 ($n = 4$) 1-min light pulses were used to stimulate these light-responsive cells. In all cases, 1 min light pulses were long enough to clearly activate or suppress the cells. On the other hand, 1 min of darkness between the light presentations did not appear long enough for the cells to recover to their baseline firing rates. The mean discharge of seven light-activated cells during the minutes of darkness was 68% (S.D. = 13.8) of that during the minutes of light exposure. The firing rate during light exposure of the one light-suppressed cell (60% of firing rate in the dark) also fell within this range.

The responsiveness to light did not change with the number of light pulses, neither for cells that responded only in a sustained way to continuous light nor for cells that also had a phasic component (Fig. 2). No exceptions were found to this response pattern throughout the subjective night. Thus, the cumulative change in firing of visual cells always increased linearly with the number of light pulses presented.

Intensity–response curve for phase shifting

A total of 125 light pulses with different intensities were presented to 18 animals. In 35 cases, the activity

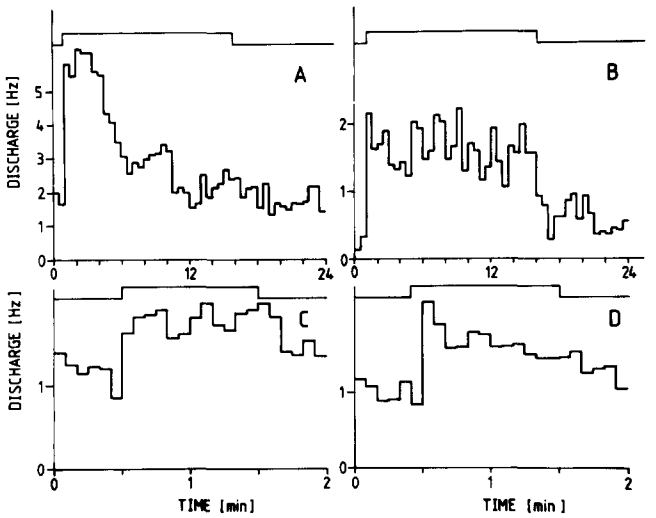

Fig. 1. Responses of two suprachiasmatic neurons to retinal illumination. A and C, respectively, show responses of one cell to a single 15-min exposure to continuous illumination and the averaged response of that cell to 15 1-min light pulses presented with intervening 1-min periods of darkness. B and D show responses of another cell to the same treatments. Note that while the cell in A shows only a transient response to the continuous light stimulus, its responses to the brief pulses are sustained throughout the presentations. The cell in B shows sustained activation to continuous illumination and similarly sustains its response to the brief light pulses (D). The timing of the light pulses is indicated above the records.

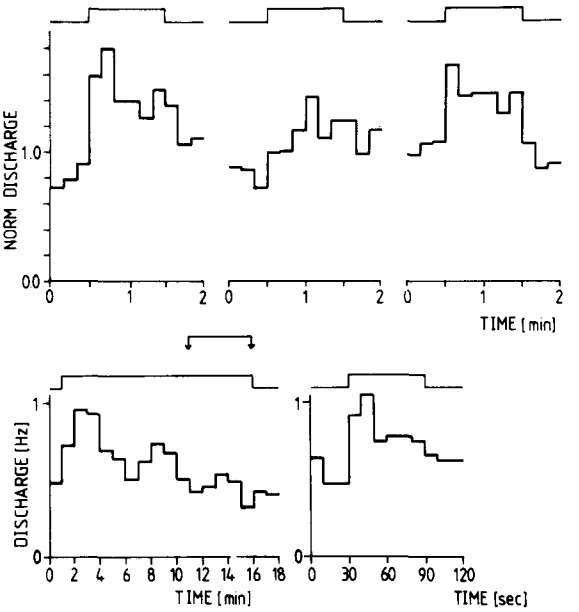

Fig. 2. Upper panel: mean response of a suprachiasmatic nucleus neuron to the first two, intermediate two and last two light pulses of a series of 15 1-min light pulses. Lower panel: responses of a single suprachiasmatic nucleus neuron to illumination presented over a 15-min interval. On the left, the illumination is present continuously as indicated above the record by the upward deflection of the line. Although activation is clear for the first few minutes, the firing rate drops to the baseline level during the last portion of the illumination interval. On the right, a train of eight 1-min light pulses alternating with 1-min dark periods was presented over a 15-min interval. The figure shows the average response to the last three light pulses (corresponding to the time indicated by the arrows in the upper panel). There is still a robust response to light pulses late in the train at a time when the response to a continuous illumination pulse has been lost.

onsets could not be determined unambiguously and these animals were excluded from the analysis. Light pulses ($n = 90$) that were presented at approximately CT 18 generally produced advances of the circadian activity rhythm (Fig. 3). However, in some cases no phase shifts were observed even at the highest light intensities. None of the animals that failed to shift in

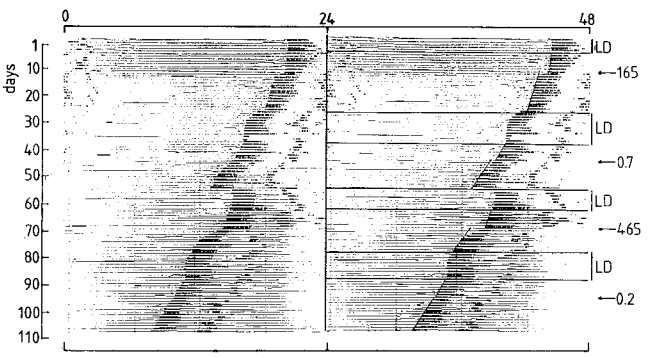

Fig. 3. Double plotted actogram of the running wheel activity of a hamster. The animal was repeatedly entrained to an LD and then released into constant darkness. During each interval of constant darkness, the animal was exposed to a light pulse at CT 18. The four pulses were at intensities of 165, 0.7, 465 and 0.2 lx, in that order.

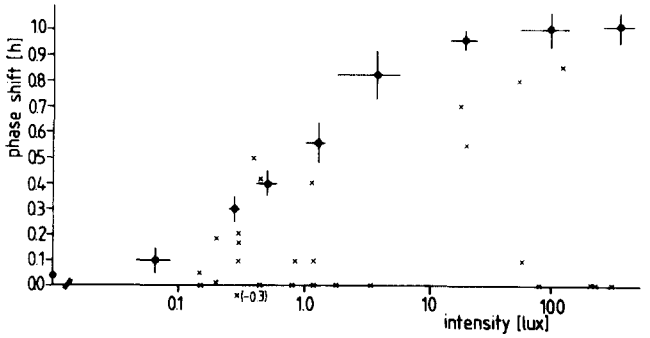

Fig. 4. The mean phase shifts (±S.E.M.) obtained at CT 18 are plotted as a function of the intensity of the light pulses. The marks indicate the individual phase shifts of all those animals that did not display any running wheel activity during the light presentation.

response to the highest intensities showed wheel-running activity during the light pulse. It is possible that these animals were curled up and asleep during the light pulse and may therefore have been exposed to much dimmer light. Approaching this issue from another perspective, we examined the records of 27 animals which were inactive during a light pulse. In 25 of these cases, the observed phase shifts were smaller than the mean phase shifts generated by those pulses in the group as a whole (Fig. 4). It seems likely that inactivity during a light pulse is associated with exposure to a dimmer light than the nominal value presented. We therefore excluded from further analysis all animals which failed to show activity during light exposures.

The magnitude of the advances caused by light at CT 18 depended on the intensity of the pulses. Light pulses of less than 0.1 lx did not produce significant phase shifts. More intense light caused progressively larger shifts until saturation at about 100 lx, which caused approximately 1-h phase shifts (Fig. 4). Further increases in light intensity did not cause larger phase shifts. No substantial differences were observed in the threshold and saturation values of different animals.

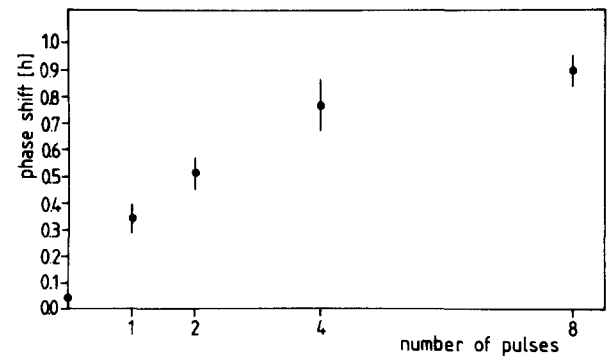

Fig. 5. The mean phase shift (±S.E.M.) of the running wheel activity rhythm that was induced by one, two, four or eight light pulses. The light was presented at CT 18.

TABLE I

The mean response to one, two, four and eight light pulses for those animals for which all values were available

The mean response is proportional to $n^{-0.659}$ for one, two and four pulses, but much less for eight pulses (n = number of pulses).

n	1	2	4	8
mean (h)	0.320	0.497	0.798	0.881
mean (h)$\cdot n^{-0.659}$	0.320	0.315	0.320	0.224

The intensity–response curve for phase shifting was obtained by pooling all phase shifts produced at each light intensity. The curve is characterized by a threshold value of about 0.1 lx and an effective working range of 2 log units. The curve can be fitted by a Michaelis equation $y = x^a/(x^a + b^a)$ with $a = 0.8$ and $b = 0.96$ lx.

Duration–response curve for phase shifting

Series of light pulses with an average intensity of 2.7 lx (range 1.63–3.33) were presented to 18 animals at about CT 18. A single 1-min light pulse at this intensity produced a mean phase shift of 0.34 h (S.D. = 0.23, $n = 15$); two pulses caused a mean shift of 0.49 h (S.D. = 0.26, $n = 14$); four pulses caused a shift of 0.77 h (S.D. = 0.34, $n = 11$); and eight pulses caused a shift of 0.88 h (S.D. = 0.27, $n = 15$). The increment in phase-shift amplitude added by each additional light pulse declined systematically with the number of pulses presented (Fig. 5). For those animals for which all four determinations were available, the geometric mean response was proportional to $n^{-0.659}$ (S.E.M. of this power = 0.034) for 1, 2 and 4 min; the value for 8 min was even lower (Table I). The data obtained from animals exposed to only part of the set of stimuli were generally similar with the exception of one or two cases. Thus, phase-shift amplitude did not increase linearly with the number of pulses presented. Instead, later light pulses in a series contributed less to the net phase shift than early light pulses.

DISCUSSION

SCN cells respond to retinal illumination in a number of ways, including showing changes in firing rates (see Introduction), increases in metabolic activity[25,29] and changes in the expression of c-*fos* and a number of other immediate-early genes[2,9,14,24,26]. It remains to be determined what the relations are between the various responses of the SCN to light and the entraining effects of light on circadian rhythms. As an initial approach we have analysed the relation between the

amount of light-evoked SCN discharge and the magnitude of phase shift induced.

It has previously been shown that SCN discharge follows light intensity according to a sigmoid shaped curve[16]. However, the effects of the duration and temporal pattern of light presentation on the amount of discharge was not described quantitatively in these studies. We have now investigated this issue in a series of electrophysiological experiments. More specifically, the effect of a train of light pulses on SCN discharge was compared with the effect of a single light pulse. Lengthy light pulses induced sustained changes in firing rates in most SCN cells; however, a phasic component preceded the sustained response to light in some cells (see also Meijer et al.[16]). A few cells did not show sustained changes in firing rates during lengthy light presentations. The combination of brief, phasic components in some cells and the gradual loss of firing rate increases in others *ensures* that the average response of the population of SCN cells to light declines gradually during sustained illumination.

Therefore, although longer light pulses may be associated with more SCN activation, the duration of a continuous light pulse is not a linear predictor of the total change in firing rate of the SCN cell population. In contrast, trains of brief light pulses were able to change SCN cell discharge rates without any decline in effectiveness over many minutes of repeated light/dark pulses. For both sustained and more transiently responsive cells, the initial response to light was repeated with each additional pulse. Hence, later pulses contributed equally with earlier ones to the total response to the train and the number of light pulses presented is, therefore, a strong predictor of the total change in SCN firing induced by the train of pulses.

We have previously shown that when light intensity is manipulated at a fixed stimulus duration, neurophysiological responses saturate at an intermediate intensity so that the highest intensities do not evoke any more activity than more moderate intensities[16]. If the total change in cell firing attributable to a light pulse predicts the amplitude of phase shift produced one would make the following predictions based on the neurophysiological results: (1) increasing the intensity of a light pulse over threshold should produce progressively less increment in phase-shift response; (2) the intensity–response curves for neural activity and for phase-shifting should be similar; and (3) increasing the number of pulses in an alternating light/dark series should produce linear increases in the amount of induced phase shift.

Consistent with the first prediction, increasing the intensity of light pulses caused progressively less increment in the amplitude of the phase shift. The intensity–response curve for phase shifting was characterised by a sigmoid shaped curve. Similar results have been obtained by Takahashi et al.[33]. Since this group made use of monochromatic light and expressed light intensity in photon flux, it is difficult to compare their intensity–response curve with the one obtained in this study.

The present study used white light and expressed light intensity in lx to make the results quantitatively comparable with results of electrophysiological SCN recordings in the same species[16]. With respect to the second prediction, we previously reported that the intensity–response curve of light-responsive SCN cells follows a sigmoid function with a working range of about two log units, which can be described by a Michaelis equation $y = x^a/(x^a + b^a)$ with $a = 0.95$ and $b = 70$ lx. The curvature of this function closely resembles the one for phase shifting found in this study ($a = 0.95$ and $a = 0.8$ for the electrophysiological and behavioural studies, respectively) (Fig. 6). However, the value of the half-saturating light intensity for neural responses of visual SCN cells is much higher than that for behavioural phase shifting (70 and 1 lx, respectively).

The difference between the white light sources in the two studies (fluorescent light versus black body radiation) cannot account for so large a difference between the half-saturating light intensities. Other factors which may account for this difference include: (1) the animals used in the electrophysiological studies were anesthetized with urethane during recordings; and (2) during the electrophysiological experiments the animals were exposed to multiple light pulses in order to identify photically sensitive cells, while in the be-

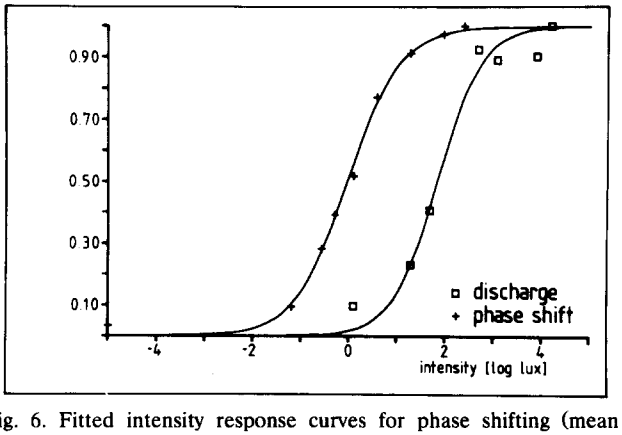

Fig. 6. Fitted intensity response curves for phase shifting (mean values are indicated by squares) and for an electrophysiological response to light (mean values are indicated by plus signs). The curves were fitted with a Michaelis equation $y = x^a/(x^a + b^a)$ with $a = 0.8$ and 0.95, respectively, and with $b = 1$ lx and 70 lx, respectively.

havioural study, a single light pulse was presented after the animals had been exposed to constant darkness for 7 days. We might on this basis expect differences in sensitivity (and therefore in threshold values) in these conditions. We therefore conclude that although the results may be suggestive of a linear relation between change in SCN discharge and magnitude of a phase shift, the results are not conclusive.

With respect to the third prediction, an increase in the number of light pulses did increase the size of the phase shift produced. However, the relation between the number of pulses and the magnitude of the phase shift was not linear. Phase-shift amplitude was proportional to $n^{-0.659}$ for n equal to one, two and four light pulses. For eight light pulses the phase shift was even lower than $n^{-0.659}$, indicating a strong decline in the phase-shifting effectiveness of additional light pulses. This observation is consistent with previous qualitative findings[8]. In that study, 1–5 light pulses were presented near CT 12, with each light pulse lasting 15 min. The results indicated that the phase shift obtained from a series of pulses is larger than the effect of the first light pulse, but smaller than an additive model would predict.

The decline in phase-shifting effectiveness of additional light pulses may be caused, in part, by the fact that the first light pulse causes an instantaneous phase shift of the oscillator to a new phase at which light is less effective. However, this phenomenon does not account for the very strong decline in effectiveness of additional light pulses that we observed. The first light pulse was presented at CT 18. Four light pulses might have caused an instantaneous advance of the pacemaker of approximately 0.8 h (Fig. 5) so that later pulses in the series would have reached the pacemaker at CT 18.8. Since the shape of the PRC is almost flat between CT 18 and CT 20 this phenomenon alone cannot account for the fact that light pulses 5–8 together added only 0.1 h to the phase shift that would have been induced by the first four pulses.

Our behavioural study showed that the magnitude of the phase shift increases at most logarithmically with the number of pulses while the physiological results indicated that the total response of SCN neurons to a series of pulses always increases linearly with the number of pulses. Therefore, these experiments demonstrate that a linear increase in SCN firing does not produce a linear increase in the the magnitude of a phase shift (Fig. 7).

Many visual SCN cells have properties that would permit them to mediate known features of light effects on overt rhythms. They sustain responses to lengthy stimuli, so that longer light pulses would produce more

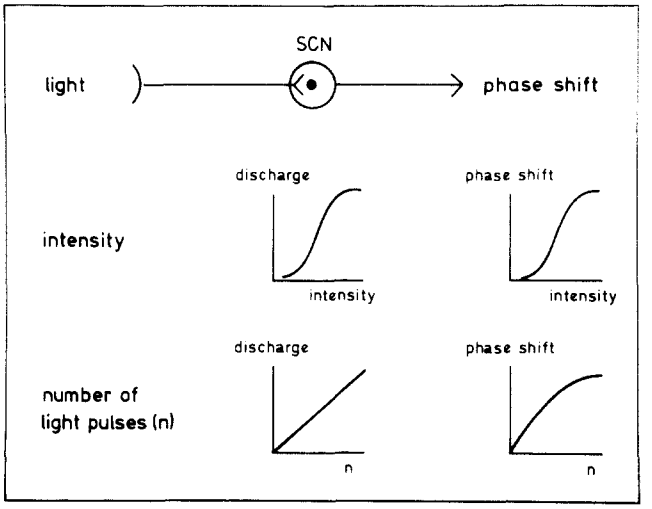

Fig. 7. Summary of protocols and results. Increment in light intensity induces an increment in SCN discharge and in phase shift. The results suggest that discharge is linearly related to the magnitude of a phase shift. An increment in the number of light pulses induces a linear increase in discharge. The increase in phase shift, however, is much less than a linear relation would predict. The discrepancy between the latter two curves indicates that the size of the phase shifts is not linearly related to the degree of increase in discharge rate.

activation, leading to larger phase shifts. SCN cells also code for luminance so that brighter pulses (within their dynamic range) would induce more activation and larger shifts. Finally, some of the differences observed between SCN cells in diurnal and nocturnal species[17] correlate with behavioural differences between these species.

The neurophysiological results do not, however, suggest a mechanism that can account for the characteristics of the phase response curve (PRC). The shape of the PRC suggests that visual cells might respond qualitatively differently to light at different circadian phases, but this was not observed. Instead, both light-activated and light-suppressed cells were encountered at all circadian phases[17]. Another response of SCN cells to light exposure, the induction of Fos-like immunoreactivity, does change with circadian phase[26]. These results indicate the some aspects of SCN responsiveness to light vary with circadian time, but that these are not reflected in changes in neurophysiological responsiveness.

We have now investigated whether the responsivenes of visual SCN cells can account for the magnitude of a phase shift that can be obtained at one circadian phase. We estimated the quantitative relations between discharge in the SCN and the magnitude of phase shift induced. The results reported here indicate that changes in SCN cell firing rates induced by light are related to the phase- shifting effects of light, but under some circumstances there is a clear dissocia-

tion between the effects of light on these two parameters. These findings therefore indicate that firing rate changes are not consistently linked to shifts in pacemaker phase. This dissociation may result from the fact that firing rate changes reflect photic input to the pacemaker system, but that other limitations in the pacemaker itself alter the effectiveness of such input in shifting phase, thereby introducing non-linearities in the relations between these variables.

REFERENCES

1 Abe, B., Rusak, B. and Robertson, H.A., NMDA and non-NMDA receptor antagonists inhibit photic induction of Fos protein in the hamster suprachiasmatic nucleus, *Brain Res. Bull.*, submitted.

2 Aronin, N., Sagar, S.M., Sharp, F.R. and Swartz, W.J., Light regulates expression of a Fos-related protein in rat suprachiasmatic nuclei, *Proc. Natl. Acad. Sci. USA*, 87 (1990) 5959–5962.

3 Cahill, G.M. and Menaker, M., Effects of excitatory amino acid receptor antagonists and agonists on suprachiasmatic nucleus responses to retinohypothalamic tract volleys, *Brain Res.*, 479 (1987) 76–82.

4 Cahill, G.M. and Menaker, M., Kynurenic acid blocks suprachiasmatic nucleus responses to optic nerve stimulation, *Brain Res.*, 410 (1990) 125–129.

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. Physiol.*, 206 (1982) 390–396.

6 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.

7 Colwell, L.S. and Ralph, M.R., Do NMDA receptors mediate the effects of light on circadian behaviour? *Brain Res.*, 523, (1990) 117–120.

8 Decoursey, P.J., Function of a light response rhythm in hamsters, *J. Comp. Physiol.*, 63 (1964) 189–196.

9 Earnest, D.J., Iadarola, M., Yeh, H.H. and Olschowka, J.A., Photic regulation of c-*fos* expression in neural components governing the entrainment of circadian rhythms, *Exp. Neurol.*, 109, (1990) 353–361.

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

11 Groos, G.A. and Meijer, J.H., Effects of illumination on suprachiasmatic nucleus electrical discharge. In R.J. Wurtman, M.J. Baum, O.T. Potts (Eds.), *The Medical and Biological Effects of Light, Vol. 453*, New York Academy of Sciences of the USA, New York, 1985, pp. 134–146

12 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.

13 Inouye, S.T., Light responsiveness of the suprachiasmatic nucleus with the island with the retinohypothalamic tract spared, *Brain Res.*, 294 (1984) 263–268.

14 Kornhauser, J.M., Nelson, D.E., Mayo, K.E. and Takahashi, J.S., Photic and circadian regulation of c-*fos* gene expression in the hamster suprachiasmatic nucleus, *Neuron*, 5 (1990) 127–134.

15 Mason, R. and Rusak, B., NMDA-evoked responses in the Syrian hamster suprachiasmatic nucleus in vitro, *J. Physiol.* 435 (1991) 39P.

16 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.

17 Meijer, J.H. and Rietveld, W.J., Neurophysiology of the suprachiasmatic circadian pacemaker in rodents, *Physiol. Rev.*, 69 (1989) 671–707.

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

19 Nelson, D.E. and Takahashi, J.S., Sensitivity and integration in a visual pathway for circadian entrainment in the hamster (*Mesocricetus auratus*), *J. Physiol.*, 439 (1991) 115–145.

20 Nelson, R.J. and Zucker, I., Absence of extraocular photoreception in diurnal and nocturnal rodents exposed to direct sunlight, *Comp. Biochem. Physiol.*, 69A (1981) 145–148.

21 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.

22 Pickard, G.E., Bifurcating axons of retinal ganglion cells terminate in the hypothalamic suprachiasmatic nucleus and the intergeniculate leaflet of the thalamus, *Neurosci. Lett.*, 55, (1985) 211–217.

23 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.

24 Rea, M.A., Light increases Fos-related protein immunoreactivity in the rat suprachiasmatic nuclei, *Brain Res.*, 23 (1989) 577–581.

25 Rosenwasser, A.M., Trubowitsch, G. and Adler, N.T., Circadian rhythm in metabolic activity of suprachiasmatic, supraoptic and raphe nuclei, *Neurosci. Lett.*, 58 (1985) 183–187.

26 Rusak, B., Robertson, H.A., Wisden, W. and Hunt, S.P., Light pulses that shift rhythms induce gene expression in the suprachiasmatic nucleus, *Science*, 248 (1990) 1237–1240.

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

28 Sawaki, Y., Retinohypothalamic projection electrophysiological evidence for the existence in female rats, *Brain Res.*, 120 (1977) 336–341.

29 Schwartz, W., Davidsen, L. and Smith, C., In vivo metabolic activity of a putative circadian oscillator, the rat suprachiasmatic nucleus, *J. Comp. Physiol.*, 189 (1980) 157–167.

30 Schwartz, W.J., Further evaluation of the tetrodotoxin-resistant circadian pacemaker in the suprachiasmatic nuclei, *J. Biol. Rhythms*, 6 (1991) 149–158.

31 Schwartz, W.J., Gross, R.A. and Morton, M.T., The suprachiasmatic nuclei contain a tetrodotoxin-resistant circadian pacemaker, *Proc. Natl. Acad. Sci. USA*, 84 (1987) 1694–1698.

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

33 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*, 308, (1984) 186–188.

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

35 Zhang, D.X. and Rusak, B., Photic sensitivity of geniculate neurons that project to the suprachiasmatic nuclei or the contralateral geniculate, *Brain Res.*, 504 (1989) 161–164.