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SENSITIVITY AND INTEGRATION IN A VISUAL PATHWAY FOR CIRCADIAN ENTRAINMENT IN THE HAMSTER (*MESOCRICETUS AURATUS*)

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SUMMARY

1. Light-induced phase shifts of the circadian rhythm of wheel-running activity were used to measure the photic sensitivity of a circadian pacemaker and the visual pathway that conveys light information to it in the golden hamster (*Mesocricetus auratus*). The sensitivity to stimulus irradiance and duration was assessed by measuring the magnitude of phase-shift responses to photic stimuli of different irradiance and duration. The visual sensitivity was also measured at three different phases of the circadian rhythm.

2. The stimulus–response curves measured at different circadian phases suggest that the maximum phase-shift is the only aspect of visual responsivity to change as a function of the circadian day. The half-saturation constants (σ) for the stimulus–response curves are not significantly different over the three circadian phases tested. The photic sensitivity to irradiance ($1/\sigma$) appears to remain constant over the circadian day.

3. The hamster circadian pacemaker and the photoreceptive system that subserves it are more sensitive to the irradiance of longer-duration stimuli than to irradiance of briefer stimuli. The system is maximally sensitive to the irradiance of stimuli of 300 s and longer in duration. A quantitative model is presented to explain the changes that occur in the stimulus–response curves as a function of photic stimulus duration.

4. The threshold for photic stimulation of the hamster circadian pacemaker is also quite high. The threshold irradiance (the minimum irradiance necessary to induce statistically significant responses) is approximately 10^{11} photons cm^{-2} s^{-1} for optimal stimulus durations. This threshold is equivalent to a luminance at the cornea of 0·1 cd m^{-2}.

5. We also measured the sensitivity of this visual pathway to the *total number* of photons in a stimulus. This system is maximally sensitive to photons in stimuli between 30 and 3600 s in duration. The maximum quantum efficiency of photic integration occurs in 300 s stimuli.

6. These results suggest that the visual pathways that convey light information to

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the mammalian circadian pacemaker possess several unique characteristics. These pathways are relatively insensitive to light irradiance and also integrate light inputs over relatively long durations. This visual system, therefore, possesses an optimal sensitivity of 'tuning' to total photons delivered in stimuli of several minutes in duration. Together these characteristics may make this visual system unresponsive to environmental 'noise' that would interfere with the entrainment of circadian rhythms to light–dark cycles.

INTRODUCTION

In vertebrates the process of 'vision' is partitioned into several distinct operations that exist within functionally separate neural pathways and accomplish a variety of visual tasks. Visual pathways project from the retina to the ventral and dorsal thalamus, the hypothalamus, the accessory optic nuclei, the pretectum and the superior colliculus (reviewed in Rodieck, 1979). These visual pathways have been implicated in such diverse operations as object identification and localization, control of pupil diameter and regulation of eye and head movements. In mammals visual projections from the retina to the anterior hypothalamus are thought to carry photic information that synchronizes or entrains circadian rhythms to the daily light–dark cycle (reviewed in Rusak & Zucker, 1979; Moore, 1983; Meijer & Rietveld, 1989). Photic pathways that mediate circadian entrainment, however, are often neglected in a discussion of vision. This study will focus on the physiological characterization of the visual pathways that mediate the photic entrainment of a circadian pacemaker in the golden hamster.

In mammals photic information is transmitted from an ocular photoreceptor to a neural circadian oscillator in the suprachiasmatic nucleus (SCN) where this information is used to entrain endogenous circadian rhythms to the environmental light cycle (reviewed in Rusak & Zucker, 1979; Moore, 1983). This visual system uses two distinct anatomical pathways that connect the retina to the SCN. A neural projection from the retina directly to the SCN has been observed in all mammals (Moore, 1973; Eichler & Moore, 1974). An indirect retinal projection through the intergeniculate leaflet of the lateral geniculate nucleus has also been observed to terminate in the SCN (reviewed in Meijer & Rietveld, 1989). These retinal projections constitute a set of neural pathways that provide the mammalian circadian pacemaker with light to entrain circadian rhythms. Together, retinal photoreceptors, the retino-hypothalamic tract, the intergeniculate pathway and the phase-shifting mechanism of the circadian oscillator constitute the mammalian *photic-entrainment pathway.*

The light sensitivity of the photic-entrainment pathway has been studied electrophysiologically. Retinal illumination induces electrophysiological responses in neurones of the SCN in several mammals (Lincoln, Church & Mason, 1975; Groos & Mason, 1980). The receptive fields of light-responsive SCN units are extremely large, often exceeding 20–40 deg of visual angle, with no detectable centre-surround organization (Groos & Mason, 1980). Many of these cells also respond to continuous light stimulation with sustained increases or decreases in firing rate for periods as long as 30 min or 1 h (Lincoln *et al.* 1975; Meijer, Groos & Rusak, 1986), and are often unresponsive to briefer stimuli of 5 s or less (Groos & Mason, 1978, 1980; Meijer *et al.* 1986). Although anatomical and electrophysiological studies have provided a great

deal of information about this photic-entrainment pathway, *functional* studies of this pathway are necessary for a complete understanding of the physiological properties of this visual system. Functional characterization of this visual pathway is critical for continued study of the electrophysiological and molecular events that mediate the photic entrainment of mammalian circadian rhythms.

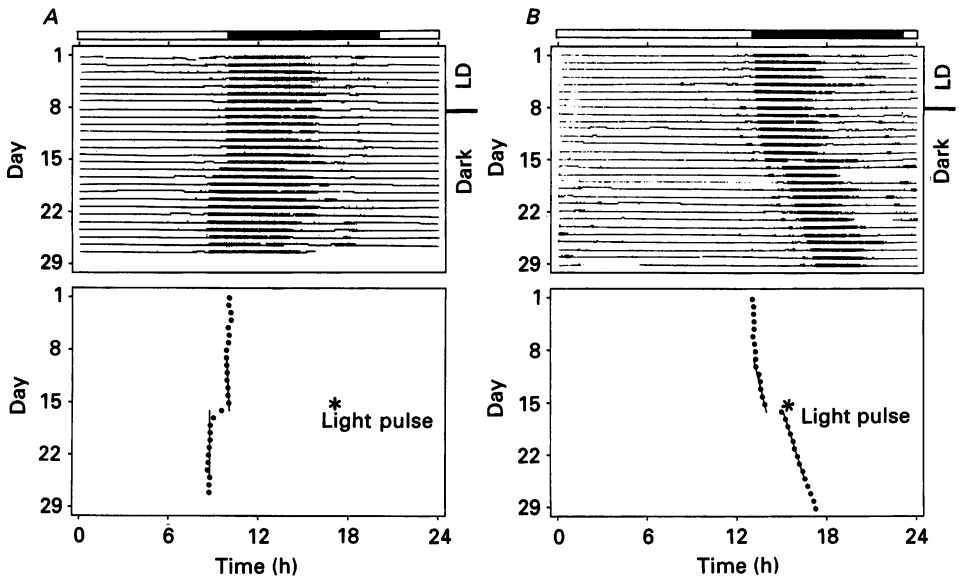

Fig. 1. Records from two golden hamsters showing entrainment, free-run and steady-state phase shifts of the circadian rhythm of wheel-running activity. *A* is an example of a light-induced phase advance and *B* is an example of a phase delay. The upper plots in *A* and B show the actual activity records. Successive days are plotted from top to bottom and the hours of the day from left to right. Pen deflections depict wheel-running activity and intervals without pen deflections are times of inactivity. In the lower plots the activity onsets in each cycle of the rhythm have been indicated by ●. For the first several days the hamsters were entrained to a light–dark cycle (14 h light: 10 h dark: indicated by LD). The light and dark bars at the top of each record show the times of light and dark respectively. Following entrainment the hamsters were maintained in continuous darkness for 1 week. On the seventh day of darkness each hamster received a light pulse at the time indicated by the asterisk and a steady-state phase shift of the activity rhythm was induced. The record on the left is from a hamster that received a 300 s stimulus ($8{\cdot}5 \times 10^{12}$ photons cm^{-2} s^{-1}) at circadian time 19 causing a steady-state phase advance of 106 min. The record on the right is from a hamster that received a 1 h light pulse ($6{\cdot}1 \times 10^{13}$ photons cm^{-2} s^{-1}) at circadian time 14 causing a 66 min phase delay. The steady-state phase used to estimate circadian time 12 before and after the pulse is shown in the lower plots as the regression line (fitted by eye) through the activity onsets immediately before and after the light pulse for each animal. The magnitude of the phase shift in each case is the time between these two phase-reference points before and after stimulation as described in the text.

Measurement of functional light sensitivity

To measure the sensitivity of the visual pathway that conveys light information to the hamster circadian pacemaker, we have used a functional measurement of the light-sensitivity of an output of the system. This assay uses a fundamental feature

of circadian pacemakers; i.e. the induction of steady-state phase shifts of circadian rhythms by light. Entrainment of circadian rhythms to the daily cycle of light and dark occurs by repeated adjustment of the phase and period of the oscillator to maintain a constant phase relationship between the rhythm and the synchronizing

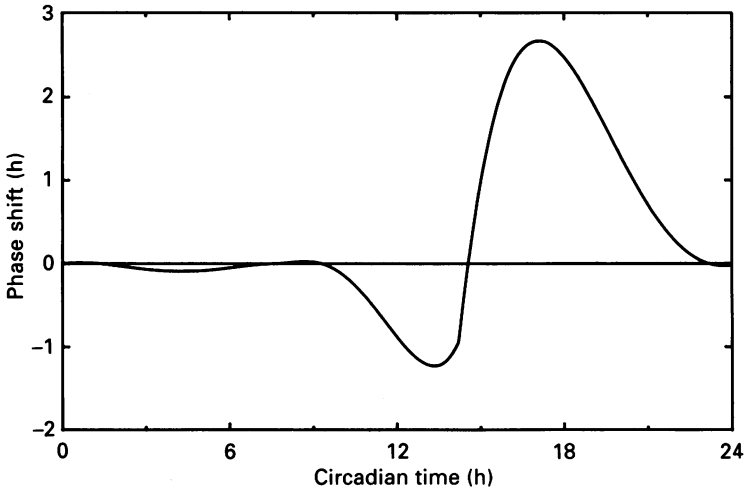

Fig. 2. Diagram of a phase–response curve for 1 h 'saturating' light pulses based upon Takahashi *et al.* (1984). The line represents the magnitudes of steady-state phase shifts as a function of the phase of light stimulation (1 h white light of 350 lx) after 5 days of constant darkness.

light cycle (Pittendrigh, 1981). In the golden hamster these adjustments are temporary light-induced changes in the period (lengthening or shortening) and are evident as phase shifts (phase delays or phase advances) of the overt circadian rhythm of wheel-running activity (Fig. 1). The magnitude and sign (or direction) of light-induced phase shifts depend upon the phase of the circadian oscillator at the time of stimulation (DeCoursey, 1961, 1964; Daan & Pittendrigh, 1976). Plots of these phase shifts as a function of the phase of stimulation are 'phase–response curves' (Fig. 2) and have been used extensively to study the mechanism of entrainment, as well as the formal properties of circadian pacemakers (Daan & Pittendrigh, 1976; Pittendrigh, 1981,).

Because phase shifts of circadian rhythms are induced by light, the photic-entrainment pathway can be characterized using this phase-shift response as an assay. Using this approach, the stimulus–response relationship and the spectral sensitivity have been measured for phase advances of the pacemaker that underlies the circadian rhythm of wheel-running activity in the golden hamster (Takahashi, DeCoursey, Bauman & Menaker, 1984). Using 900 s photic stimuli, the phase-shifting response was shown to have a spectral sensitivity peak near 500 nm and a relatively high threshold for stimulation. A remarkable feature of this system is that light pulses of equivalent numbers of photons, but durations that range from 4 to 45 min, have been found to induce phase shifts of similar magnitudes (J. S. Takahashi, P. J. DeCoursey, L. Bauman and M. Menaker, unpublished observations;

see Takahashi *et al.* 1984). The photic-entrainment pathway, including the circadian pacemaker and the photoreceptive pathway that subserves it, must be capable of integrating continuous light inputs over extended durations of time.

We have performed several experiments to characterize fundamental physiological properties of the circadian pacemaker and the visual system that carries light information to it in the golden hamster. Steady-state phase shifts of the circadian rhythm of wheel-running activity were used as a functional assay of photic sensitivity for this visual pathway subserving photic entrainment. We have quantified the sensitivity of this photic-entrainment pathway to light irradiance, measured the duration of temporal integration of light inputs by this pathway and assessed photic sensitivity of this visual pathway at different phases of the circadian cycle.

METHODS

General procedures. Male golden hamsters (*Mesocricetus auratus*, Lak: LVG [SYR]) were obtained at 3–5 weeks of age and housed in groups in a 24 h light–dark cycle (14 h light and 10 h darkness). Illumination inside the cages during the light phase was provided by fluorescent tubes (General Electric: F40CW) and was adjusted to approximately 250 lx at a level 9 cm above the cage floor. After a minimum of 2 weeks, the hamsters were transferred to individual cages equipped with running wheels and microswitches to monitor wheel-running activity. Activity was recorded for each animal with an Esterline–Angus event recorder that monitored the status of a microswitch attached to each running wheel. Revolutions of the wheel registered as deflections of the recording pen on each hamster's 24 h record. Each record was plotted onto a summary chart with successive days beneath one another (see Fig. 1). These individually housed hamsters were kept in ventilated light-tight enclosures (six cages per enclosure, inside dimensions of the enclosure: $56 \times 44 \times 179$ cm). Within these enclosures the hamsters were subjected to a light–dark cycle identical to that already described.

After a minimum of 7 days the light–dark cycle was discontinued and the hamsters were maintained in constant darkness to allow expression of the endogenous (free-running) circadian rhythm of activity. On the seventh day of darkness a light stimulus was delivered to each hamster at the appropriate phase of the rhythm. By convention, each complete cycle of a circadian rhythm can be divided into 24 h of 'circadian time'. An hour of circadian time is equal to the period of the circadian rhythm measured in an individual animal divided by 24. To calculate circadian time for a nocturnal species such as the hamster, the onset of activity is used as a phase-reference point for circadian time 12. Using this convention, circadian time 19 occurs 7 circadian hours after the estimated onset of activity. Following stimulation, each hamster was returned to constant darkness for 2 weeks, after which the steady-state phase shift was measured. The magnitude of the phase-shift response was estimated by extrapolating the steady-state phase of the oscillation (again using activity onset as the phase-reference point) before and after the stimulation to the cycle following stimulation. The magnitude of the phase shift was equal to the difference between the two steady-state phase-reference estimates on the cycle after the stimulus. For the first several cycles after a phase shift, there are often transient changes in the period length of the rhythm, and the phase reference points during these transient changes may not reflect the steady-state phase of the oscillator (Pittendrigh, 1981). The first four activity onsets after light stimulation were, therefore, excluded from the estimate of the new steady-state phase. At least ten consecutive activity onsets, beginning with the fifth onset following the stimulus, were used to assess the new steady-state phase of the rhythm. The response magnitudes are reported in minutes of circadian time $\pm$ the standard error of the mean (S.E.M.). By convention, shifts in phase that cause the phase of activity onset to occur earlier than expected after stimulation are termed phase advances of the activity rhythm (Fig. 1*A*). Phase shifts that cause the onset to occur later are phase delays (Fig. 1*B*).

Circadian phase of stimulation. In hamsters, as in other species, the magnitude and sign (advance or delay) of light-induced phase shifts of the circadian rhythm are dependent upon the phase of the stimulation (DeCoursey, 1964; Daan & Pittendrigh, 1976). Light pulses induce steady-state phase

shifts of greatest magnitude in the golden hamster when administered between circadian times 18 and 20 (6–8 circadian hours after activity onset; see Fig. 2). In these experiments the most extensive measurements of light sensitivity of the photic-entrainment pathway were performed at circadian time 19. The large phase advances induced at circadian time 19 maximized the sensitivity of our measurement of light responsiveness. For comparison, the photic sensitivity was also measured at circadian time 21, a phase of the rhythm at which smaller phase advances are induced by light, and at circadian time 14, a time at which light induces phase delays. The proper clock time for delivery of each light pulse was determined for each hamster on the day of stimulation. Unless indicated, data from animals in which the actual circadian time of stimulation deviated by more than 30 min from the target circadian time were not included in any analyses. The mean and range of the actual circadian times of the stimulus onsets are reported for each experiment. Animals were used only once, to reduce the response variability caused by prior manipulations, and at the time of photic stimulation the hamsters ranged in age from 6 to 12 weeks.

Photic stimulation. Light from a tungsten–halogen lamp (General Electric: FHS) or a strobe flash (Vivitar: 285) was directed with condensing and projection lenses through a series of infra-red absorbing filters (Schott Glass Technologies: KG 4, KG 1) and an interference filter (Schott: AL; λ_{max} = 503 nm; half-width = 20 nm). Neutral density filters (Schott: NG) and a variable voltage transformer controlled the irradiance of the stimulus and a shutter controlled the stimulus duration. For stimulus durations greater than 90 s a manual single-leaf shutter was used, while for durations from 3 to 90 s an electronic shutter (Uniblitz, Vincent Associates, Rochester, NY, USA) controlled stimulus duration. The strobe flash was used to deliver 3 ms pulses. The effective pulse duration from the strobe flash was measured using a radiometer and oscilloscope to be 2·9 ms (duration of pulse exceeding 25% maximum irradiance).

For stimulation each hamster was transferred to a white plastic chamber that held the animal during stimulus delivery. All transfers were made without visible light, using an infra-red viewer (FJW Optical Systems, Palatine, IL, USA). For stimulus durations less than 3600 s, hamsters were held in a small cylindrical chamber (5·5 cm radius, 10·5 cm height, area = 95 cm^2). Hamsters were held in a similar chamber of larger dimensions that held six animals in six individual compartments for the 3600 s stimuli (each compartment was one-sixth of a cylinder with a 13·2 cm radius and a 10·5 cm height; area available for each hamster = 91 cm^2). The tops of both stimulus chambers were made of flashed-opal glass (3 mm thickness) to act as a translucent light diffuser. When the shutter was opened, light was projected onto the opal glass and the entire stimulus chamber beneath was illuminated.

Stimulus irradiance, illuminance and luminance were measured to facilitate comparisons of sensitivity to other visual responses. These units of measurement are used as described in Wyszecki & Stiles (1982). Irradiance within the stimulus chamber was measured before and after each stimulus using a photodetector placed inside the chamber, centred 5 cm below the diffusing screen. For 3 ms stimuli the irradiance was determined by dividing the total energy delivered in the light pulse by the duration of the pulse. Irradiance was measured directly for pulses greater than 3 ms in duration. Irradiance measurements were made with a radiometer/photometer (model S350, probe no. 248, United Detector Technologies, Hawthorne, CA, USA) equipped with a radiometric filter. Irradiance was measured in $\mu W\ cm^{-2}$ and converted to photons $cm^{-2}\ s^{-1}$ based upon the energy per photon for λ = 503 nm. The average deviation of the irradiance measurements from the reported stimulus mean was $0{\cdot}9 \pm 1{\cdot}2\%$. The maximum deviation of the irradiance from the reported stimulus mean was 8·6%. Illuminance measurements were made with the same instrument using a photometric filter and cosine diffuser and are reported as lux ($lm\ m^{-2}$). Stimulus luminance was measured using a United Detector Technologies photometer (model 40X) with a photometric filter and footlambert lens (model 113) and the threshold luminance is reported as $cd\ m^{-2}$.

Our goal in these studies was to measure the functional sensitivity of the photic-entrainment pathway under conditions that were as 'natural' as possible. For this measurement stimuli were delivered to hamsters freely moving in the stimulus chamber without control of the animals' eyelids or body posture. The strength of the stimulus at the surface of the cornea may differ slightly from the irradiance measurements we have reported, but our observations suggest that the reported light levels are quite similar to the actual levels at the cornea. There are at least two obvious sources of error for this measurement. First, the hamster eyelid absorbs $96 \pm 1\%$ of 503 nm light when completely closed (mean ± S.E.M. for five absorption measurements of 503 nm light, measured

with a small fibre-optic probe positioned under the eyelids of three anaesthetized hamsters). This absorbance could reduce the irradiance at the cornea by as much as 1·4 log units if the hamster's eyes were completely closed for the duration of a stimulus. Second, a hamster's posture could also reduce the actual irradiance at the cornea. Although the walls of the stimulus chamber are very reflective, the hamster is free to cover its eyes during stimulation. The behaviour of the animals in the stimulus chamber was often observed during stimulation and the eyelids remained open for the duration of stimulation (except for normal 'blinks') and no systematic head or eye covering was seen. By leaving a 0·5 cm gap between the upper rim of the stimulus chamber and the opal glass, the normal exploratory behaviour of the hamsters was reinforced and animals tended to poke their noses into the gap, positioning their eyes directly below the opal glass. Even during the very long exposures to high irradiance, hamsters invariably positioned their heads within 1 cm of the opal glass at the top of the stimulus chamber with eyes open and directed upward toward the light source.

Data analysis. The data were statistically fit with a four-parameter logistic equation using the computer programme 'Allfit', developed by DeLean, Munson & Rodbard (1978), to compare the relationships between stimulus strength and the phase-shift response for different stimulus durations and circadian phases of stimulation. Allfit uses a least-squares curve-fitting routine (a form of the Gauss–Newton algorithm) to fit the stimulus–response data with a modified form of the Naka–Rushton equation (Naka & Rushton, 1966). For this function,

$$R = \frac{R_{\min} - R_{\max}}{1 + (I/\sigma)^N} + R_{\max}, \tag{1}$$

where I is the magnitude of the stimulus (the stimulus irradiance or the total number of photons in the stimulus) and R is the steady-state phase shift of the rhythm to that stimulus. $R_{\max}$ is the maximum response to a stimulus (I) of infinite magnitude and $R_{\min}$ is the response when $I = 0$. The stimulus required to induce a response of one-half the maximum response is σ. This term is often referred to as the semi-saturation or half-saturation constant. The parameter for the 'steepness' of the curve, N, corresponds to the slope of the function when plotted on a logit–log plot with I represented as $\ln(I)$. The parameter, N, is a necessary modification of the Naka–Rushton equation when fitting many physiological stimulus–response curves (Boynton & Whitten, 1970). Each parameter of the best-fit function was determined for each set of stimulus–response data with the fewest constraints possible.

Statistical differences were also determined between the individual parameters ($R_{\min}$, N, σ and $R_{\max}$) for the different circadian phases and durations of stimulation using Allfit. An initial fit of the data was made with constraint of as few parameters as possible and subsequent fits were then made while forcing the individual curves to share the parameters in question or to be some constant value (i.e. $R_{\min} = 0$). Significant differences between the resulting fits and the initial fit indicated a fit that was significantly worse and parameters that were statistically different, as described in DeLean *et al.* (1978). Differences between the fits were determined using a significance level of $P < 0{\cdot}05$.

Significant differences between the responses of individual groups were determined using analysis of variance (ANOVA). When significant differences were found the responses of the groups in question were compared using a Tukey–Kramer test or a Student's t test. The statistical package Systat (version 4.0) was used to perform both the ANOVA and the post-hoc tests.

Terminology. The term 'sensitivity' often refers to the reciprocal of the stimulus required to produce a response of a criterion size. Since it is difficult to compare the sensitivities of two responses that have different maxima, we will define physiological sensitivity as the reciprocal of the quantity of light needed to induce a one-half maximum response ($1/\sigma$) for a given set of stimulus conditions. Sensitivity, in this context, is independent of the relative–maximum response of one system compared to another. Instead, sensitivity is dependent upon the operational or functional range of the response. Threshold sensitivity refers to the reciprocal of the stimulus necessary to induce a response of some criterion quantity (or statistical significance). Responsivity is defined as the response of a system to some unit of stimulus.

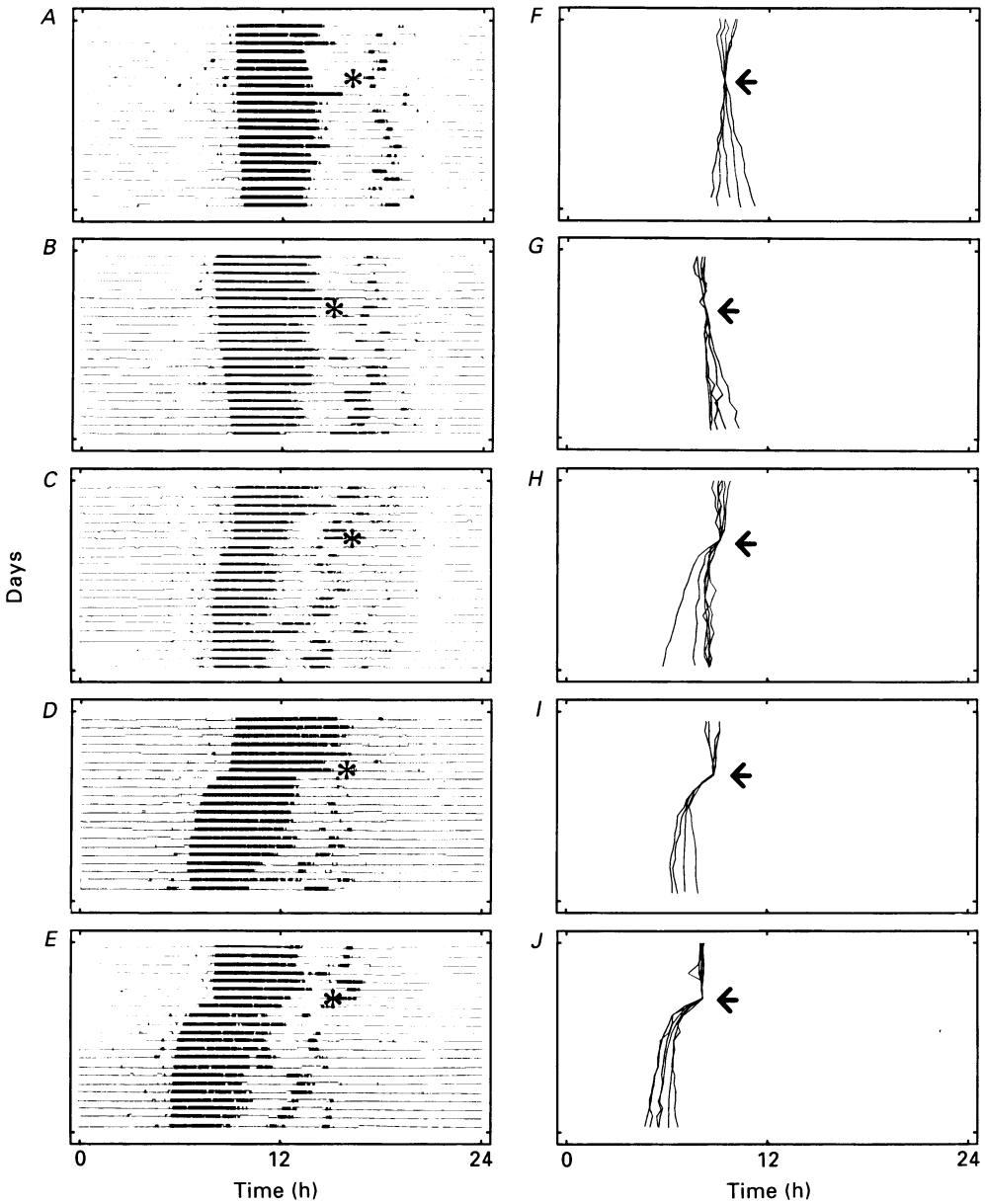

Fig. 3. Steady-state phase advances of the locomotor-activity rhythm induced by 300 s stimuli of different irradiance at circadian time 19. The panels on the left (*A–E*) are the wheel-running records for five hamsters that received light pulses of five different levels of irradiance. The irradiance levels were *A*, no light; *B*, $8{\cdot}0 \times 10^{9}$; *C*, $2{\cdot}6 \times 10^{11}$, *D*, $8{\cdot}7 \times 10^{12}$; and *E*, $3{\cdot}8 \times 10^{14}$ photons $cm^{-2}\ s^{-1}$. Asterisks indicate the time of stimulus onset. The panels on the right (*F–J*) are activity-record tracings from four to six animals that received the stimulus described for the corresponding panels on the left. For these tracings the onsets of activity have been connected to show the responses of individual hamsters to photic stimulation on the day indicated by the arrow. The mean ($\pm$S.E.M.) steady-state phase shifts induced by the light pulses were -4 ± 4, 3 ± 4, 56 ± 8, 100 ± 5 and 114 ± 8 min for *F–J*, respectively.

RESULTS

Photic sensitivity to 300 s stimuli at circadian time 19

As an initial measurement of the sensitivity of the hamster photic-entrainment pathway, the magnitude of the steady-state phase shifts induced by 300 s light pulses was measured at circadian time 19 for different irradiance levels. Stimuli of 300 s in duration were presented to twenty-one groups of animals (three to eight animals per group; total $n = 77$). One of these groups was placed in the stimulus chamber for 300 s, without opening the shutter, to determine the effect of handling alone ($n = 5$). An additional group was left undisturbed in constant darkness and not transferred to the stimulus chamber ($n = 8$). The mean circadian time of stimulation for this experiment was 19 h 01 min (range of circadian times for stimulation was 18 h 40 min–19 h 30 min) (hours and minutes here and throughout the text are circadian hours and minutes; stimulus durations, however, are given in real time not circadian time).

The 300 s light pulses induced statistically significant phase shifts for irradiance levels greater than $7{\cdot}4 \times 10^{10}$ photons cm^{-2} s^{-1} (Tukey–Kramer: d.f. = 88; $P < 0{\cdot}01$). For our stimulus, this threshold is equal to a luminance of 0·093 cd m^{-2}. Examples of steady-state phase shifts induced by 300 s stimuli are shown in Fig. 3. Low-irradiance stimuli did not induce steady-state changes in the phase of the activity rhythm. Higher levels of irradiance ($> 7{\cdot}4 \times 10^{10}$ photons cm^{-2} s^{-1}) induced significant phase advances and the response appeared to saturate at approximately 10^{13} photons cm^{-2} s^{-1}, since no increase in response could be detected above this irradiance. When the magnitudes of the light-induced phase shifts are plotted as a function of the $\log_{10}$-stimulus irradiance (Fig. 4) they can be fitted by a four-parameter curve analogous to stimulus–response curves measured for other visual pathways (Naka & Rushton, 1966). The maximum response for the fit was 114 min and the minimum response was −6 min but not significantly different from 0 (as determined by Allfit). The slope of the function was 0·6 and the irradiance necessary to induce a half-maximum response (57 min) was $3{\cdot}1 \times 10^{11}$ photons cm^{-2} s^{-1}. The steady-state phase of activity rhythms remained unaltered for hamsters left undisturbed in their cages for the duration of the experiment (mean phase shift = 1 ± 3 min, ±S.E.M.). Phase shifts were also quite small in animals transferred to the stimulus chamber for 300 s but not subjected to light (-4 ± 4 min). These shifts were also not significantly different from the phase shifts for hamsters left undisturbed in darkness.

Sensitivity to other stimulus durations at circadian time 19

To determine the photic sensitivity to several other durations of stimulation, the sensitivity of the hamster photic-entrainment pathway was also measured to 3 ms, 3 s, 30 s and 3600 s stimuli. For these different durations of light stimulation the stimulus–response curves reflected differing degrees of sensitivity to light irradiance (Fig. 5).

Sensitivity to stimulus durations greater than 300 s was measured by presenting 3600 s (1 h) light stimuli of different irradiance levels to eight groups of animals (three to seven per group; total $n = 42$). One group was placed in the stimulus

chamber for 1 h without opening the shutter to determine the effect of handling alone for this extended duration. The mean circadian time of stimulation for these animals was 18 h 55 min. The range for the circadian time of stimulation (±30 min) was extended for these groups because of the lengthy stimulus duration, and the actual

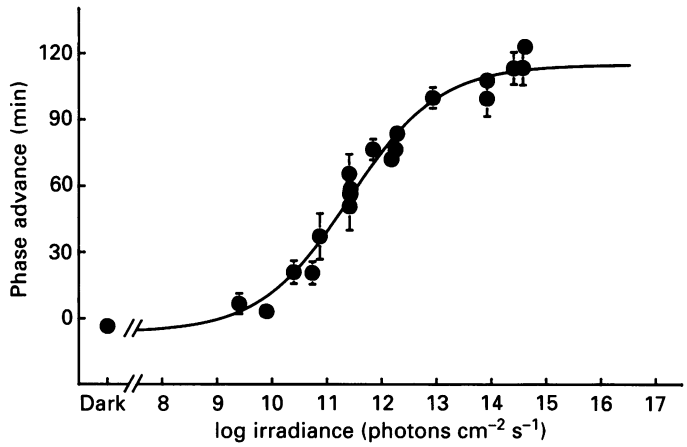

Fig. 4. Phase–advance response as a function of stimulus irradiance for 300 s light pulses at circadian time 19. The points represent the means ± S.E.M. for responses to monochromatic light pulses (503 nm) delivered to twenty-one groups of hamsters. The continuous line is the modified Naka–Rushton function fitted to the data ($R_{min} = -6$ min, not significantly different from 0; $N = 0{\cdot}6$; $\sigma = 3{\cdot}1 \times 10^{11}$ photons cm^{-2} s^{-1}; $R_{max} =$ 114 min).

range was 18 h 16 min–19 h 41 min. For 1 h stimuli, $1{\cdot}6 \times 10^{11}$ photons cm^{-2} s^{-1} (luminance = 0·20 cd m^{-2}) were necessary to induce phase shifts that were significantly different from animals that did not receive light (Tukey–Kramer: d.f. = 35; $P < 0{\cdot}05$). The maximum response to the 1 h stimulus duration was 110 min, but this maximum was not significantly different from the maximum to 300 s stimuli (Fig. 5*A*). The minimum response was 5 min but not significantly different from 0 (Allfit, n.s.). An irradiance of $1{\cdot}4 \times 10^{11}$ photons cm^{-2} s^{-1} was required to induce a one-half maximum response for the 1 h stimuli and the slope of the stimulus–response curve was 0·9. Neither the slope nor the half-maximum differed from those parameters determined for 300 s stimuli (Allfit, n.s.). For hamsters moved to the stimulus chamber and left in darkness for 1 h, phase shifts (8 ± 3 min) were not significantly different from shifts measured for animals left undisturbed in their cages (Tukey–Kramer, n.s.).

As a control for the use of the different stimulus chamber for 1 h light pulses, one group ($n = 5$) was given a light pulse of 300 s ($1{\cdot}5 \times 10^{11}$ photons cm^{-2} s^{-1}) in the stimulus chamber normally used for 1 h pulses. A second group ($n = 5$) received a light pulse of identical irradiance and duration in the chamber normally used for the 300 s pulses. Stimulation for 300 s in this chamber produced phase shifts equal to those in animals stimulated in the chamber normally used for the 300 s pulses (42 ± 8 and 53 ± 4 min respectively; Student's *t* test, n.s.).

The sensitivity of the photic-entrainment pathway to irradiance was lower for light pulses less than 300 s in duration. Phase-shift responses to these brief stimuli did not saturate even for the stimuli of highest irradiance. The modified Naka–Rushton equation was fitted to these data using Allfit as if the maximum

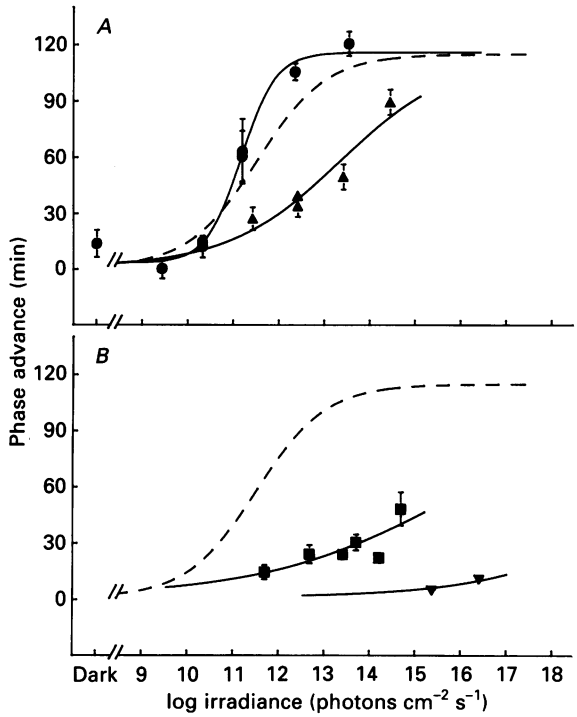

Fig. 5. Phase-advance response as a function of stimulus irradiance for light pulses of 3 ms, 3 s, 30 s, and 3600 s in duration at circadian time 19. *A*, the stimulus–response curves for 3600 and 30 s light pulses are compared to those induced by the 300 s light pulses. The data points shown are the means ± S.E.M. of phase shifts induced by the 3600 s pulses (●) and the 30 s pulses (▲). The continuous lines through each data set are the best fit of the modified Naka–Rushton equation for the data. (For the 3600 s stimuli: R_{min} = 0 min, $N = 0{\cdot}9$, $\sigma = 1{\cdot}4 \times 10^{11}$ photons cm^{-2} s^{-1}, R_{max} = 114 min. For the 30 s simuli: R_{min} = 0 min, $N = 0.3$, $\sigma = 2.0 \times 10^{13}$ photons cm^{-2} s^{-1}, R_{max} = 114 min.) *B*, the means ± S.E.M. are represented for responses to 3 s (■) and 3 ms (▼) pulses. The continuous lines through each of the data sets are the Naka–Rushton function fitted to each data set as described in the text. (For the 3 s stimuli: R_{min} = 0 min, $N = 0{\cdot}2$, $\sigma = 1{\cdot}3 \times 10^{16}$ photons cm^{-2} s^{-1}, R_{max} = 114 min. For the 3 ms stimuli: R_{min} = 0 min, $N = 0{\cdot}2$, $\sigma = 2 \times 10^{19}$ photons cm^{-2} s^{-1}, R_{max} = 114 min.) The curve describing the responses to 300 s stimuli is shown by the dashed line (R_{min} = 0 min, $N = 0{\cdot}6$, $\sigma = 3{\cdot}1 \times 10^{11}$ photons cm^{-2} s^{-1}, R_{max} = 114 min).

responses to each of these stimulus durations were identical to the maximum for the 300 s stimuli. Light pulses of 30 s were presented to five groups of hamsters to measure the photic sensitivity (eight to fourteen per group; total $n = 50$). The mean circadian time of stimulation was 19 h 03 min (range = 18 h 32 min–19 h 29 min). The 30 s pulses of greater than $2{\cdot}6 \times 10^{12}$ photons cm^{-2} s^{-1} (equivalent to 2·9 cd m^{-2})

induced significant phase advances (Fig. 5*A*). The minimum response for the stimulus–response curve fitted to these data was 8 min but this was not significantly different from 0, or from the minimum response to 300 s stimuli (Allfit, n.s.). The irradiance required to induce a one-half maximum response for 30 s light pulses was

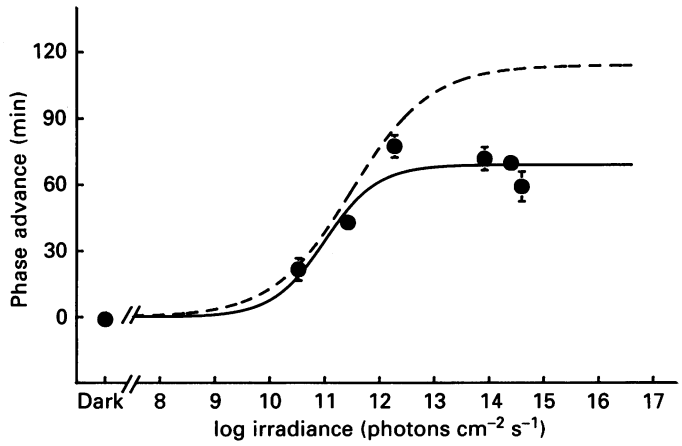

Fig. 6. Stimulus–response curve for 300 s light pulses at circadian time 21. Points represent the means ± S.E.M. of the phase shifts to the 300 s monochromatic light pulses (503 nm) delivered to seven groups of hamsters. The continuous line is the Naka–Rushton function fitted to the data (R_{min} = 0 min; N = 0·9; $\sigma = 1 \times 10^{11}$ photons cm^{-2} s^{-1}; R_{max} = 69 min). The dashed line represents the fit for 300 s light pulses delivered at circadian time 19 (redrawn from Fig. 5).

$2{\cdot}0 \times 10^{13}$ photons cm^{-2} s^{-1} and was significantly greater than the half-saturation constants for the 300 s pulses (Allfit: d.f. = 20, $P < 0{\cdot}0001$). The slope of the stimulus–response curve for the 30 s stimuli was estimated to be 0·3.

Light pulses of 3 s in duration were presented to twelve groups of hamsters (four to six per group; total n = 60). The mean circadian time of stimulation for these groups was 18 h 57 min (range = 18 h 31 min–19 h 11 min). For 3 s pulses the irradiance necessary to induce a half-maximum response was estimated to be $1{\cdot}3 \times 10^{16}$ photons cm^{-2} s^{-1} and the slope of the curve was 0·2 (Fig. 5*B*). The half-saturation constant for the 3 s stimulus–response curve was significantly greater than that measured for 300 s pulses (Allfit: d.f. = 21; $P < 0{\cdot}0001$). For 3 s stimuli $5{\cdot}0 \times 10^{15}$ photons cm^{-2} s^{-1} (equivalent luminance = 4300 cd m^{-2}) induced phase advances that were significantly greater than unstimulated controls (Tukey–Kramer: d.f. = 43; $P < 0{\cdot}05$).

To measure responses to very brief stimuli, light pulses of 3 ms in duration were presented to two groups of hamsters at circadian time 19 (five and twelve per group; total n = 17). The mean circadian time of stimulation for these groups was 19 h 04 min (range = 18 h 50 min–19 h 24 min). Light pulses of 3 ms did not induce significant phase shifts even for irradiance levels of 3×10^{16} photons cm^{-2} s^{-1} (Fig. 5*B*; Tukey–Kramer, n.s.). There were inadequate data for a meaningful estimate of the parameters of the stimulus–response curve for the 3 ms duration. If the stimulus–response curve for 3 ms stimuli were similar to that measured for the

longer-duration pulses, the irradiance of a 3 ms stimulus would have to exceed 10^{19} photons cm^{-2} s^{-1} to induce a 60 min response. This irradiance would be greater than that of direct sunlight, which is approximately 10^{17} photons cm^{-2} s^{-1} (photons between 400 and 700 nm; Wyszecki & Stiles, 1982).

Photic sensitivity to 300 s stimuli at circadian time 21

To determine the sensitivity of the hamster circadian system to light at a phase of the rhythm following circadian time 19, the relationship between stimulus irradiance and the induced phase shift for 300 s pulses was measured at circadian time 21. Light pulses of 300 s durations and different irradiance levels were presented to seven groups of animals (four to six per group; total $n = 36$). One of these groups was transferred to the stimulus chamber without opening the shutter, to determine the effect of handling alone, at circadian time 21 ($n = 5$). The mean circadian time of stimulation for this experiment was 21 h 02 min (range = 20 h 42 min–21 h 15 min). At this phase the stimulus–response curve for 300 s light pulses was quite similar to that obtained at circadian time 19 (Fig. 6). Only the maximum response was reduced at circadian time 21. Light pulses of $2{\cdot}7 \times 10^{11}$ photons cm^{-2} s^{-1} (equivalent luminance = 0·32 cd m^{-2}) delivered at circadian time 21 induced phase shifts that were significantly different from controls that did not receive a light stimulus (Tukey–Kramer: d.f. = 29; $P < 0{\cdot}001$). The phase shifts induced by transferring hamsters to the stimulus chamber at circadian time 21 were small (-1 ± 1 min) and were not different from those in hamsters left undisturbed in their cages (Tukey–Kramer, n.s.). The maximum phase advance for the stimulus–response curve fitted to the responses in hamsters receiving light was 69 min, and the minimum was not different from 0 (Allfit, n.s.). The slope of the function was 0·9 and the irradiance necessary to induce a one-half maximum response was $1{\cdot}0 \times 10^{11}$ photons cm^{-2} s^{-1}. The slope and half-saturation constant for the fit were not significantly different from those parameters measured for the function describing 300 s light pulses delivered at circadian time 19 (Allfit, n.s.). The maximum phase advance for pulses delivered at circadian time 21, however, was significantly smaller than that for pulses at circadian time 19 (Allfit: d.f. = 19; $P = 0{\cdot}01$). The stimulus–response curve generated for the 300 s light pulses delivered at circadian time 21 differed from that at circadian time 19 in only the single parameter of maximum response. The irradiance required to induce a half-maximum response, the threshold irradiance and the slope of the stimulus–response relationship were all similar at circadian time 21. Therefore, even though the maximum *responsivity* of the hamster circadian pacemaker to light is less at circadian time 21 than at circadian time 19 (the maximum phase shift is significantly smaller), the *sensitivities* to light ($1/\sigma$) are equivalent at the two circadian phases.

Photic sensitivity at circadian time 14

Representative experiments were also performed to measure the sensitivity of the photic-entrainment pathway at a phase of the rhythm were phase delays of the oscillator are induced by light. In these experiments the sensitivity to stimulus durations of 1 h, 300 s and 30 s was measured at circadian time 14. The photic sensitivity of the phase-shifting mechanism at circadian time 14 was similar to that

observed at circadian times 19 and 21. These light pulses, however, induced steady-state phase delays of the circadian rhythm of activity. Because of the limited experiments performed at circadian time 14, the responses to stimuli of all three durations were analysed in a single fit using Allfit.

Photic stimuli of 1 h were presented to five groups of hamsters at circadian time 14 (four to seven per group; total $n = 27$). For these animals the mean circadian time of stimulation was 14 h 03 min (range = 13 h 29 min–15 h 02 min). Groups of hamsters were also moved to the stimulus chamber for 300 s and 1 h without opening the shutter, to determine the effects of handling alone for these durations. Stimulus magnitudes of $9{\cdot}1 \times 10^{12}$ photons cm^{-2} s^{-1} (equivalent to a luminance of 10 cd m^{-2}) induced phase shifts that differed significantly from controls (Tukey–Kramer: d.f. = 22; $P < 0{\cdot}02$). The minimum response for the fit of the Naka–Rushton function (Fig. 7*A*) was −7 min (not significantly different from 0; Allfit). The maximum response for the fit was −50 min and the slope was −1·5. An irradiance of $1{\cdot}6 \times 10^{12}$ photons cm^{-2} s^{-1} was necessary to induce a half-maximum response and was not significantly different from that measured for the 1 h light pulses delivered at circadian time 19 (Allfit, n.s.).

The photic sensitivity to 300 s pulses was also measured at circadian time 14 using five groups of hamsters (nine to twelve per group; total $n = 54$). The mean circadian time of stimulation was 14 h 05 min (range = 13 h 32 min–14 h 29 min). The mean response appeared to decrease for the highest irradiance levels of 300 s stimuli at circadian time 14 (Fig. 7*B*). The decreased response, although not statistically significant, complicated the fit of the data with the four-parameter equation and a reasonable fit could not be obtained using Allfit. The data were fitted without using the response to $2{\cdot}5 \times 10^{14}$ photons cm^{-2} s^{-1} and sharing the maximum response with maxima for the 30 s and 1 h stimuli. The maximum response of the resulting fit was −52 min. The minimum response of −9 min was not significantly different from the minima for other stimulus durations at circadian time 14 or different from 0 (Allfit, n.s.). The slope of the curve was −0·6 and the half-saturation constant was 1×10^{12} photons cm^{-2} s^{-1}. The half-saturation constant was not significantly different when the fit was performed on the data that included the response to $2{\cdot}5 \times 10^{14}$ photons cm^{-2} s^{-1} (Allfit, n.s.). The half-saturation constant for 300 s stimuli at circadian time 14 was also not different from those for 300 s stimuli at either circadian times 19 or 21. The half-saturation constant for 300 s stimuli at circadian time 14 was also not statistically different from the half-saturation for 1 h pulses at this phase (Allfit, n.s.).

For the 30 s stimuli, light pulses of different irradiance were presented to four groups of hamsters (three to six per group; total $n = 19$). The mean phase of stimulation for these animals was 13 h 58 min (range = 13 h 25 min–14 h 10 min). Responses to 30 s stimuli at circadian time 14 did not appear to saturate at even the highest irradiance levels (Fig. 7*C*). The maximum response for the fit was determined by constraining it to be shared with the maxima for the 300 s and 1 h stimuli at circadian time 14. The maximum response for the resulting fit was −52 min. The minimum response without constraint was −23 min but was not significantly different from 0, or from the minima for the other durations of stimulation (Allfit, n.s.). The minimum response was −8 min when the minimum was fit by constraining

it to be equal to the minima for the 300 s and 1 h stimuli. The slope of the function was $-0{\cdot}4$ and an irradiance of 6×10^{12} photons cm^{-2} s^{-1} was necessary to induce a half-maximum response.

The stimulus–response curves obtained at circadian time 14 are equivalent to those measured at both circadian times 19 and 21. Extensive comparisons between the

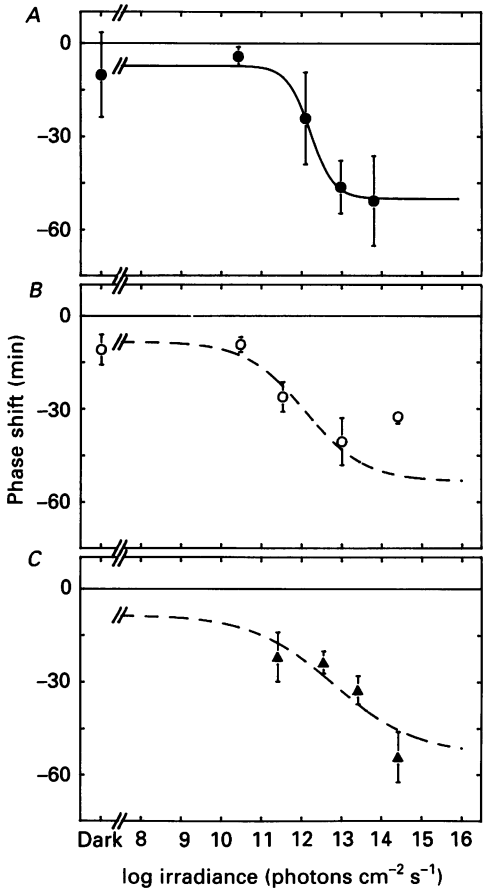

Fig. 7. Stimulus–response curves for light pulse durations of 30, 300 and 3600 s at circadian time 14. *A*, means ± S.E.M. for responses to 3600 s light pulses delivered at circadian time 14 are plotted as a function of the stimulus irradiance. The continuous line is the best-fit Naka–Rushton function for the data ($R_{min} = -7$ min; $N = -1{\cdot}5$; $\sigma = 1{\cdot}6 \times 10^{12}$ photons cm^{-2} s^{-1}; $R_{max} = -50$ min). *B*, means ± S.E.M. for responses to 300 s light pulses delivered at circadian time 14. The dashed line is the Naka–Rushton function fitted to the data set as described in the text ($R_{min} = -9$ min; $N = -0{\cdot}6$; $\sigma = 2{\cdot}5 \times 10^{14}$ photons cm^{-2} s^{-1}; $R_{max} = -52$ min). *C*, means ± S.E.M. for responses to 30 s light pulses delivered at circadian time 14. The dashed line is the Naka–Rushton function fitted to the data set as described in the text ($R_{min} = -8$ min; $N = -0{\cdot}4$; $\sigma = 6 \times 10^{12}$ photons cm^{-2} s^{-1}; $R_{max} = -52$ min).

data obtained at circadian time 14 and from circadian times 19 and 21 were not made because of the limited data. The data suggest, however, that the half-saturation constants for stimulation at circadian time 14 are not significantly different from

those measured at circadian times 19 and 21 for similar stimulus durations. Only the magnitude and direction of the steady-state phase shifts (advance or delay) are different at circadian time 14.

Analysis of temporal integration in the photic-entrainment pathway

These data demonstrate that the photic-entrainment pathway in the golden hamster is not equally sensitive to the irradiance of light pulses of different

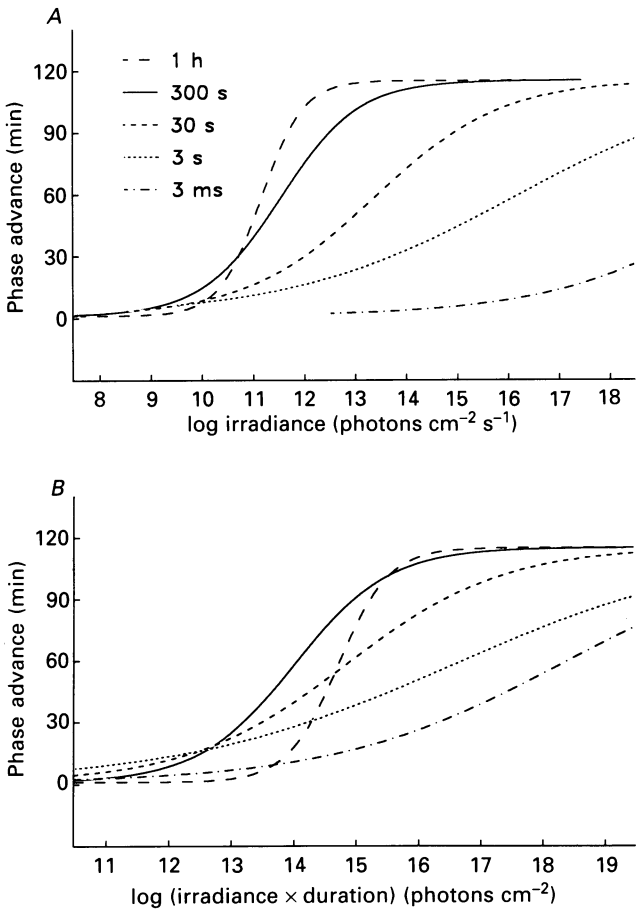

Fig. 8. Stimulus–response curves for stimuli of different durations at circadian time 19 plotted in two different conventions. *A*, the stimulus–response curves for the various durations of stimulation are plotted as a function of the irradiance of the light pulse. *B*, the curves are plotted as the response to the total photons in the stimulus.

durations. Because previous studies have suggested that this visual pathway can integrate photic information over time (Takahashi *et al.* 1984), this pathway may appear less sensitive to irradiance in the shorter durations of stimulation because brief stimuli contain fewer photons than longer stimuli of equal irradiance. A measurement of the photic sensitivity of this pathway, therefore, must consider the sensitivity to the *total* photons in the stimulus (the product of stimulus irradiance

and duration) as well as the sensitivity to irradiance. For comparison, the stimulus–response curves measured at circadian time 19 are plotted as a function of both irradiance and total photons in Fig. 8. Figure 8*A* presents the Naka–Rushton functions previously described for the different durations of stimulation as a function of the irradiance of each stimulus. Figure 8*B* shows the responses plotted as a function of the total photons. If the total number of photons in each pulse were integrated equivalently for each stimulus duration, then the stimulus–response curves describing the responses as a function of total photons should be similar regardless of stimulus duration. The stimulus–response curves for different durations of stimulation, however, remain quite distinct when plotted as a function of total photons (Fig. 8*B*). The curves differ in terms of slope and half-saturation value over this range of stimulus durations.

A striking difference between the stimulus–response curves in Fig. 8*A* and *B* is the change in the curves describing responses to 300 s and 1 h stimuli. When plotted as a function of irradiance, the responses to 1 h pulses are usually larger than those to 300 s stimuli of equal irradiance. It is apparent when the curves are plotted as a function of the total photons that this photic-entrainment pathway is often more responsive to the photons in a 300 s stimulus than to the photons in a 1 h stimulus. Responses to 1 h pulse durations are, for many quantities of light, less than those to 300 s pulses. These results suggest that the ability of the hamster photic-entrainment pathway to integrate inputs over time may be reduced for stimulus durations greater than 300 s.

The relative sensitivities to irradiance and to total photons are plotted for each stimulus duration in Fig. 9. The sensitivity to irradiance ($1/\sigma$) increases with the duration of the stimulus for durations between 3 and 300 s (Fig. 9*A*). A portion of this increased sensitivity to irradiance, however, may be due to temporal integration. If this sensitivity increase was caused by an integration of the total photons in the stimulus then the slope of this increased sensitivity as a function of log duration of the stimulus would be 1·0. The slope of this increase in sensitivity with stimulus durations between 3 and 300 s, however, is 2·3–2·8 (2·8 for the increase in sensitivity between 3 and 30 s). For increasing stimulus durations between 3 and 300 s, there is an increase in the sensitivity of the photic-entrainment pathway that cannot be attributed solely to stimulus integration. Over this range the sensitivity to irradiance increases with duration 20 to 60 times more rapidly than expected if the increase were caused only by the temporal integration of photons. Between 300 s and 1 h the sensitivity increase appears to saturate at a maximum that defines the maximum sensitivity to irradiance for this visual pathway. The hamster circadian system appears unable to integrate additional light information in pulses longer than 300 s and the response of this photic-entrainment pathway is dependent only upon the irradiance of these extended stimulus durations.

A plot of the sensitivity to total photons ($1/\sigma t$) as a function of stimulus duration also illustrates the reduced sensitivity to photons in 30 s and 1 h pulses relative to the sensitivity to 300 s pulses (Fig. 9*B*). The sensitivity to pulses less than 300 s is lower than predicted by temporal integration of photons since for stimuli less than 300 s the slope of the sensitivity curve is 1·3 and complete temporal integration of the stimulus would result in an equal sensitivity to total photons for all durations (slope

= 0). The maximum quantum efficiency for the photic-entrainment pathway is marked by the peak in sensitivity to total photons in 300 s stimuli and suggests that 'linear' temporal integration may occur in stimulus durations surrounding 300 s. The quantum efficiency is also reduced for 1 h stimuli causing an apparent 'tuning' of quantum efficiency in stimuli of approximately 300 s.

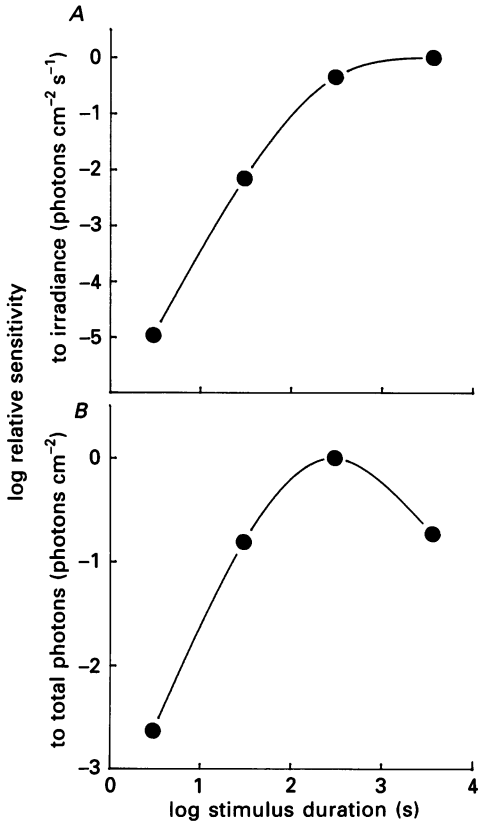

Fig. 9. Sensitivity to irradiance and total photons measured at circadian time 19. *A*, the relative sensitivity to irradiance is presented as a function of the different stimulus durations. *B*, the relative sensitivity to total photons is presented as a function of the different stimulus durations.

Mathematical description of sensitivity for the hamster photic-entrainment pathway

The relationship between phase-shift responses of the hamster circadian system and the irradiance of photic stimulation can be described by a form of the Naka–Rushton equation:

$$r = \left(\frac{i^n}{i^n + s^n}\right) r_{\max}, \tag{2}$$

where r is the response of the pacemaker (the steady-state phase shift) in minutes, i is the irradiance of the photic stimulation in photons cm^{-2} s^{-1}, and $r_{\max}$ is the maximum response measured to a photic stimulus of infinite irradiance at a

particular phase of the rhythm. (This equation is slightly different from eqn (1) and does not include the parameter for the minimum response which is always 0 for this model.) Because the duration of the photic stimulus influences both the half-saturation constant (s) and the slope (n) of the Naka–Rushton curve describing the

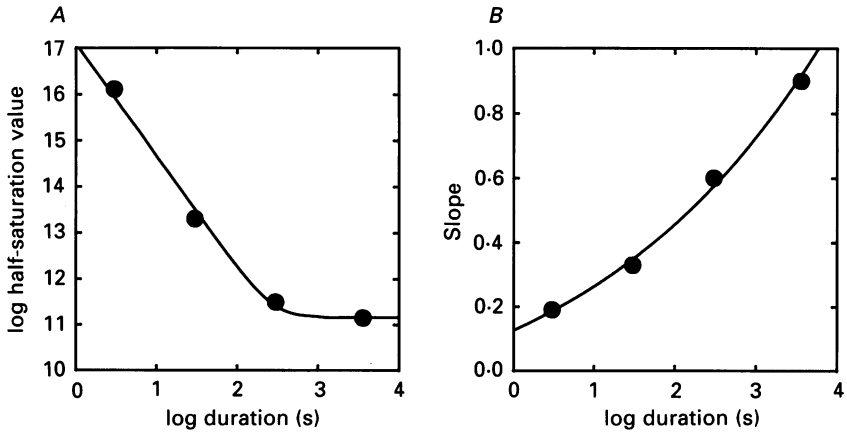

Fig. 10. Functions describing the values of the half-saturation and slope as a function of stimulus duration. A, the half-saturation value (s) as a function of stimulus duration as predicted by eqn (3). For s: $A = 2{\cdot}04 \times 10^{17}$; $B = -2{\cdot}44$; and $C = 1{\cdot}4 \times 10^{11}$. B, the slope (n) of the stimulus–response curves as a function of stimulus duration predicted by eqn (4). For n: $D = 0{\cdot}356$; $E = -0{\cdot}845$; and $F = -0{\cdot}23$. For both plots the data points represent the values of s and n measured experimentally at circadian time 19.

responses, these values for n and s were fitted from the empirical data shown in Figs 4 and 5. The parameters for the half-saturation value (s) and the slope (n) are described by the equations:

$$s = At^B + C, \tag{3}$$

and

$$n = Dt^E + F. \tag{4}$$

For eqn (3) describing the half-saturation value (s) the stimulus duration in seconds is t, and A, B and C are constants. The value of s decreases with increasing stimulus durations between 3 and 300 s (Fig. 10A). The half-saturation value (s) reaches a minimum at approximately $1{\cdot}4 \times 10^{11}$ photons cm^{-2} s^{-1} for stimulus durations greater than 300 s. When the half-saturation value is transformed to *total* photons cm^{-2} ($s\,t$) there is a minimum value of 8×10^{13} photons cm^{-2} for inducing a half-maximum phase shift for stimulus durations from 310 to 370 s. This duration corresponds to the range for maximum quantum efficiency of stimulus integration by the hamster photic-entrainment pathway.

For eqn (4) describing the slope (n) the duration of the stimulus in seconds is t, and D, E and F are constants. There is a gradual increase in n with increased stimulus duration. Over the range of stimulus durations from 3 s to 1 h, n increases from approximately 0·2 to 0·9. This model predicts values for n that are very similar to the values for n measured experimentally (Fig. 10B).

The model presented in eqns (2)–(4), describing the photic responses of the hamster phase-shifting mechanism, assumes that the maximum response (r_{max}) to light for a

given phase of the rhythm is independent of stimulus duration. This assumption appears to be valid for the golden hamster at circadian times 19 and 14 over the range of irradiance levels used in our experiments. However, r_{max} does change as a function of circadian phase (see Fig. 2). At circadian time 19, $r_{max} \approx +110$ min, while $r_{max} \approx$

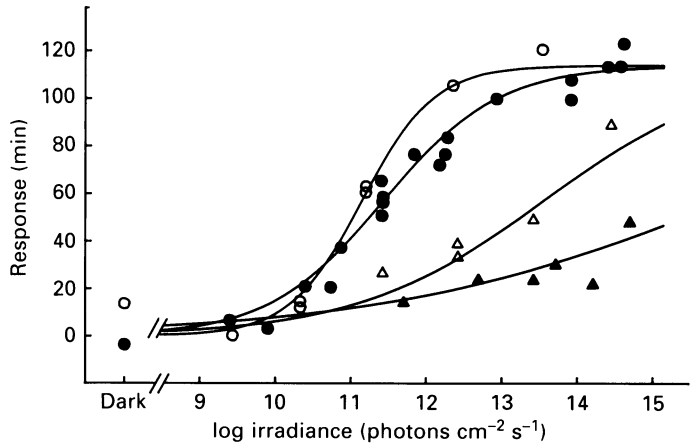

Fig. 11. Fit of the equations describing the value for half-saturation and slope to the phase-shift responses obtained at circadian time 19. The curves represent the Naka–Rushton functions (eqn (2)) for the different stimulus durations using the values for s and n determined by eqns (3) and (4). The data points represent the mean responses to photic stimulation of the indicated irradiance and duration. Responses to 1 h photic stimuli are represented by ○; 300 s stimuli, ●; 30 s stimuli, △; 3 s stimuli, ▲. Lines through the data points are the predicted responses to the photic stimuli based upon eqns (2), (3) and (4). For the curves $r_{max} = 114$ min; $A = 2{\cdot}04 \times 10^{17}$; $B = -2{\cdot}44$; $C = 1{\cdot}4 \times 10^{11}$; $D = 0{\cdot}356$; $E = -0{\cdot}845$; $F = -0{\cdot}23$.

$+70$ min at circadian time 21, and $r_{max} \approx -50$ min at circadian time 14. Figure 11 shows a comparison of the quantitative model with experimental data obtained at circadian time 19. The model predicts responses very similar to the phase-shift responses measured to photic stimuli from 3 s to 1 h in duration and irradiance levels from 10^9 to 10^{15} photons cm^{-2} s^{-1}.

DISCUSSION

The functional physiology of the visual pathway that mediates the entrainment of the hamster circadian pacemaker appears to be quite unique among visual pathways. This photic-entrainment pathway is relatively insensitive to brief light stimulation of 30 s or less in duration and is also unresponsive to irradiance levels below 10^{10} photons cm^{-2} s^{-1}. While other visual pathways are much more sensitive to irradiance and brief durations of stimulation, the hamster photic-entrainment pathway may be optimally sensitive to longer-duration photic signals. The temporal 'tuning' and high threshold measured for this pathway appear to be ideally matched to environmental inputs associated with the daily light–dark cycle that act to entrain the hamster circadian pacemaker (the behaviour of the hamster is discussed below). The unique physiological properties of the photic-entrainment pathway also suggest

that this visual pathway may be fundamentally different from photic pathways that subserve other types of vision.

Temporal integration of photic signals

For the photic-entrainment pathway of the golden hamster, the sensitivity to irradiance is dependent upon the duration of stimulation. Temporal integration of photons is indicated by the relatively constant quantum efficiency measured over the range of durations surrounding 300 s and this integration of light inputs causes the sensitivity to irradiance to be directly dependent upon the duration of these stimuli. Temporal integration of photic stimuli for at least 300 s is very unusual for a visual pathway and may represent the most extended duration of photic integration ever seen in a visual response (temporal integration in other visual pathways is discussed below). In addition, the quantum efficiency for this visual pathway is also smaller for stimuli of 30 s or less in duration. The reduced quantum efficiency for both very brief and very extended stimulus durations establishes a *maximum quantum efficiency* to 300 s stimuli. Although a single mechanism could limit the sensitivity to both brief and extended stimulus durations, this functional tuning may also be the result of separate processes that independently limit the quantum efficiency for short and long stimulus durations.

The photic-entrainment pathway in the golden hamster is relatively insensitive to the photons delivered in stimuli of 30 s or less. A simple explanation for this result is that a component of the neural pathways that carry light information to the circadian pacemaker may be less responsive to these brief durations of photic stimulation. This hypothesis is supported by electrophysiological data from light-responsive cells in the SCN. Light stimulation for less than 5 s does not induce responses in some SCN cells that do respond to longer stimuli (Groos & Mason, 1978, 1980; Meijer *et al.* 1986). This lack of responsiveness to brief stimuli suggests that some component of the pathway between the photoreceptor and the SCN is less sensitive to brief durations of stimulation. On the other hand, we cannot exclude the possibility that the circadian pacemaker itself is also less sensitive to shorter-duration stimuli. It may be necessary for the pacemaker to be stimulated for relatively long durations to induce changes in the phase of the hamster circadian oscillator.

Light saturation of the photic-entrainment pathway may also decrease the quantum efficiency for short durations of stimulation. Because the photic-entrainment pathway appears to integrate photons over time, the irradiance of a brief stimulus must be higher than the irradiance of a longer stimulus to deliver an equivalent number of photons within a brief period of time. Since these irradiance levels are in the range known to cause saturation in cone pathways, these high irradiance levels may saturate the photic-entrainment pathway in the hamster (human-cone saturation occurs at 10^{13}–10^{15} photons cm^{-2} s^{-1}; calculated from Hood, Ilves, Maurer, Wandell & Buckingham, 1978). This saturation could occur at the photoreceptor (e.g. pigment bleaching) or along the neural pathway leading from the photoreceptor to the circadian pacemaker. Changes in the stimulus–response functions with decreasing stimulus duration are consistent with this hypothesis of light saturation. Both the reduced sensitivity to light and the decreased slope of the

stimulus–response curves observed with stimulus durations less than 300 s suggest that light saturation may be occurring at some point in the photic-entrainment pathway. Electrophysiological responses in light-responsive cells in the SCN also suggest that the responsive range of the neural pathway that transmits light information to the SCN extends 2–3 log units above the minimum threshold for irradiance and does not systematically increase due to light adaptation (Groos & Meijer, 1985; Meijer *et al.* 1986). Given the functional and electrophysiological data it is quite likely that the hamster photic-entrainment pathway saturates at irradiance levels greater than 10^{13}–10^{14} photons cm^{-2} s^{-1}.

The sensitivity to total photons is also restricted by some process(es) during 1 h stimulus durations. This relative insensitivity to photons in 1 h stimuli may be due to several processes, including a refractory period following prior stimulation, or even some form of light adaptation. Indeed, a simple explanation of this result is that light adaptation is limiting temporal integration of photons in pulses greater than 300 s. If light adaptation did contribute to the reduced sensitivity to irradiance after 300 s, the time course for this adaptation would be considerably longer than that measured in any other visual system. Light adaptation usually occurs much more quickly with a time course of milliseconds or seconds. Light adaptation is completed within 1 s in the rat as measured by electroretinogram (ERG; Green, 1973). Adelson (1982) has demonstrated that light adaptation in human rods may actually occur in two stages. The first stage is complete within 200 ms and a second slower stage is not complete until 30 s after the onset of light stimulation. Even this slower stage of light adaptation probably occurs too rapidly to reduce the sensitivity to the 1 h stimuli without also reducing the sensitivity to 300 s pulses.

Although light adaptation could theoretically occur at any point along the visual pathway from the photoreceptor to the mechanism of the circadian pacemaker, electrophysiological studies of SCN cells suggest that light adaptation does not occur in the photic pathways connecting the photoreceptor to the SCN. Transient components of visual responses are sometimes observed within the first few seconds after the onset of light in many SCN cells. These light-responsive SCN cells, however, respond primarily with a sustained increase or decrease in firing rate for the duration of light stimulation (Groos & Mason, 1978, 1980; Meijer *et al.* 1986). In addition, no systematic decrease in light sensitivity is observed during stimulation or following prior stimulation. The photic-input pathway to the mammalian circadian pacemaker appears to function as a 'luminance detector' and responds to sustained levels of illumination with little or no apparent change in sensitivity over time. However, specific experiments have not been performed to test for light adaptation in the visual pathways between the retina and the SCN after prolonged stimulation. These electrophysiological studies suggest that the reduced sensitivity to photons after 300 s (and temporal integration) occurs at a point 'down-stream' from the retina and the visual pathways that carry light information to the circadian oscillator. These processes may actually occur within the mechanism of the circadian oscillator.

One down-stream process that may contribute to the reduced quantum efficiency after 300 s is the normal forward 'motion' of the pacemaker during light stimulation. While the maximum response at circadian time 19 is approximately 2 h, the maximum response at subsequent circadian phases is lower (Fig. 2). At circadian

time 21 the maximum phase advance is 69 min (Fig. 6). At later circadian phases the responsiveness decreases further until no phase shifts can be induced by light during the subjective day (Fig. 2). The oscillator may continue in its normal forward motion during the 1 h light pulses to phases past circadian time 19. In addition, any phase-advancing effect of a light pulse that begins at circadian time 19 could supplement this forward motion of the oscillator. The photons delivered over prolonged periods of time may, therefore, be acting upon less responsive circadian phases after circadian time 19. This reduced responsiveness during the latter portions of long-duration pulses would reduce the quantum efficiency for these stimuli.

Temporal integration in other visual pathways

Typically, light pulses of relatively long duration are used to induce phase shifts of circadian oscillations. The range of temporal integration of light information, however, has never been systematically measured for any circadian system. Stimuli of relatively long durations are generally used to induce phase shifts of mammalian circadian rhythms (12 h, Pittendrigh, 1960; 10 min, DeCoursey, 1961; 15 min, Daan & Pittendrigh, 1976; 15 min and 1 h, Takahashi *et al.* 1984), while few studies have used stimuli of more limited durations (Pittendrigh, 1960; Joshi & Chandrashekaran, 1984). None of these studies, however, have quantified the relative sensitivity of a circadian pacemaker to different durations of stimulation.

The most extensive measurements of the effects of stimulus duration on a circadian oscillation have been performed in *Drosophila*. Winfree (1972*a*, *b*) has measured the responsiveness of the circadian rhythm of pupal eclosion in *Drosophila pseudoobscura* to different durations of equal-irradiance stimuli. Measurements were made for both phase advances and phase delays of the rhythm at several circadian phases during the first 72 h following release into darkness from constant light. Once a steady-state rhythm was established, only several seconds of stimulation were needed to induce a maximum response of the circadian rhythm of pupal eclosion. The responsiveness of the phase-shifting mechanism to stimulus duration has also been found to be different for two latitudinal strains of *Drosophila auraria* (Pittendrigh & Takamura, 1989). A northern strain of *D. auraria*, *Hokkaido*, is less sensitive to brief durations of stimulation than a southern strain, *Miyake*. Using light pulses of 50 lx, 2 h of stimulation was required to induce maximum phase shifts in *Hokkaido* while only 15–60 min of stimulation induced equal responses in *Miyake*.

Visual pathways that do not mediate the entrainment of circadian oscillations have a range of temporal integration that is limited to milliseconds or seconds. The limits of temporal integration of photic inputs have been studied in many light-sensitive systems using Bloch's law of temporal summation (Bloch, 1885). Bloch's law asserts that within a 'critical duration' a constant response can be induced as long as the number of photons in the stimuli are equal (the product of the stimulus irradiance and stimulus duration is constant). The upper limit for complete temporal integration in human vision is generally thought to be of the order of 100 ms or less (see Baumgardt, 1972). However, measurements of rhodopsin levels in the human eye have shown that temporal integration for bleaching may be as long as 48 s (Campbell & Rushton, 1955). Probably the longest periods of temporal summation have been found in light-induced responses of the photosensitive pineal gland of non-

mammalian vertebrates. The period of stimulus summation is as long as 128 s for chromatic responses from the frog pineal organ (Dodt & Heerd, 1962). In the pike pineal gland, the same response was found to have a summation period of more than 10 s (Falcon & Meissl, 1981). Since the pineal gland has been implicated as a photoreceptor for entrainment of circadian rhythms in many non-mammalian vertebrates (Menaker & Underwood, 1979), extended durations of temporal integration may be a general property of all photic pathways that subserve circadian pacemakers.

The relationship between photic sensitivity and stimulus duration for the hamster circadian pacemaker is also intriguing from an experimental point of view. The duration of a photic stimulus appears to be extremely important for determining whether or not the circadian pacemaker is responsive to that stimulus. If this differential sensitivity to stimulus duration is due to an effect at the level of the oscillator then all studies that measure the sensitivity of this pacemaker to any stimulus (including pharmacological agents) should recognize that a circadian pacemaker may be differentially sensitive to identical 'doses' of stimulation that persist for different periods of time. Furthermore, if these differential effects of light act on a component of the photic-entrainment pathway, then other agents that effect this pathway could also have differential effectiveness due to the duration of stimulation. For example, pharmacological agents delivered as different doses or using different delivery methods (intraperitoneal injections, intraventricular injections or oral delivery) are likely to be active for quite different durations and measures of the sensitivity of circadian systems to these agents should consider the temporal aspects of their actions. The sensitivity of the hamster circadian pacemaker to any agent may depend upon its duration of action.

Sensitivity to stimulus irradiance

At least 10^{11} photons cm^{-2} s^{-1} are required to induce significant phase shifts of the hamster activity rhythm for even the most effective stimulus durations, making this photic-entrainment pathway quite insensitive to irradiance compared to other visual pathways (Table 1). Golden hamsters can be trained to recognize optical gratings that are much lower in luminance than that required for phase shifting. The visual threshold is less than 5×10^{-4} cd m^{-2} for 'white'-light gratings (Emerson, 1980) and is much lower than the threshold luminance of 10^{-1} cd m^{-2} measured for the photic-entrainment pathway. The threshold for visual responses in the rat is also much lower than that for phase shifts in the hamster. The minimum threshold for the b-wave of the ERG is approximately 2×10^{-4} cd m^{-2} after 12 h of dark adaptation (4×10^{-3} td; Dodt & Echte, 1961). A similar threshold has been measured behaviourally (Silver, 1967) and evidence suggests that the limits of luminance detectability may also be controlled by a circadian rhythm in the rat (Rosenwasser, Raibert, Terman & Terman, 1979). The absolute threshold for visual function in the rat, therefore, is at least 2·5 log units lower than the circadian phase-shifting threshold for the hamster. For comparison, the absolute threshold for human scoptic vision has been estimated to be $8{\cdot}5 \times 10^{-8}$ cd m^{-2} for white light (Denton & Pirenne, 1954) or 10^5 photons cm^{-2} s^{-1} (510 nm, Marriott, 1963; see also Ripps & Weale, 1976 for rod and cone thresholds). The threshold for human rod vision is, therefore, at

least 6 log units lower than the threshold for inducing phase shifts of the hamster circadian pacemaker.

The relative insensitivity of the hamster photic-entrainment pathway is probably due in part to an insensitivity of the visual pathway that conveys light information to this pacemaker. The photic sensitivity of this pathway has been measured

TABLE 1. Visual thresholds for photic systems

			Threshold	
Response	Species	Stimulus	Irradiance (photons cm^{-2} s^{-1})	Luminance (cd m^{-2})
Phase shift	Hamster	500 nm, 300 s	10^{11}	0·1
SCN neurons	Rat	White	—	0·1
Behavioural	Hamster	White, grating	—	$\leqslant$ 0·0005
Rod ERG	Rat	White, 40 ms	—	$\approx$ 0·0002
Cone ERG	Rat	White, 40 ms	—	$\approx$ 2·0
Scotopic vision	Human	510 nm, 1 ms	10^{5}	—
Photopic vision	Human	550 nm, 1·2 ms	10^{9}	—

See text for references.

electrophysiologically in several mammals. The threshold for induction of electrophysiological responses in the hamster SCN is 1–10 lx (Meijer *et al.* 1986; Meijer & Rietveld, 1989) and is somewhat higher than the threshold we have measured for circadian phase shifting in the present study (threshold illuminance for phase shifting = 0·2 lx). This disparity between the thresholds measured for these two responses may be due to the use of anaesthetics, the spectral distribution of the stimulus and/or different durations of stimulation. The photic stimuli generally used in electrophysiological studies are probably shorter in duration than those required to measure the minimum visual threshold for this pathway. The electrophysiological sensitivity of this system to light, therefore, may not have been measured using maximally effective stimuli. Our results suggest that the lowest threshold irradiance for stimulation of the photic-entrainment pathway is measured using stimuli that are at least 300 s in duration, and shorter-duration stimuli may result in a measurement of a minimum threshold for irradiance that is artificially high. The threshold for 30 s stimuli, for example, may be as much as 1–2 log units higher than that measured for 300 s stimuli (Fig. 9*A*) and may explain much of the disparity between the photic thresholds measured in these two different manners. The thresholds for electrophysiological responses in the SCN of the rat and cat are slightly lower than those measured for the hamster (0·1 lx for the rat, Meijer *et al.* 1986; 0·1 cd m^{-2} for the rat and cat, Groos & Mason, 1980) and are very similar to the threshold measured for phase shifts of the hamster circadian pacemaker (minimum threshold luminance for phase shifting = 0·1 cd m^{-2}; threshold illuminance = 0·2 lx).

This pathway's minimum threshold to irradiance may also be influenced by the limited ability to integrate photons over extended stimulus durations. An upper limit of stimulus integration to durations less than 1 h may make this system unresponsive to irradiance levels less than 10^{11} photons cm^{-2} s^{-1}; these levels would

induce phase shifts if the pathway was able to integrate over longer durations of time. In this sense, absolute sensitivity and temporal integration must be intimately coupled within this photic-entrainment pathway.

The photic sensitivity has also been measured for phase shifts of the circadian rhythm of pupal eclosion in *Drosophila pseudoobscura* using single durations of light stimulation. The maximum light sensitivity and spectral sensitivity are both independent of circadian phase and the direction of the phase shift for the photic-entrainment pathway of this species (Frank & Zimmermann, 1969; Klemm & Ninnemann, 1976). The threshold for inducing circadian phase shifts in *D. pseudoobscura* is approximately 10^{11} photons cm^{-2} s^{-1} for 15 min stimuli (454–458 nm, Zimmermann & Goldsmith, 1971; 459 nm, Klemm & Ninnemann, 1976). This threshold is identical to the threshold we have measured for inducing phase shifts in the golden hamster using 503 nm monochromatic light.

Because mammalian rod threshold is much lower than the threshold measured for the hamster photic-entrainment pathway, it is tempting to speculate that a cone-like photoreceptor mediates the phase-shifting response in the hamster. The golden hamster possesses a small complement of cone-like photoreceptors in its predominantly rod retina (discussed in Takahashi *et al.* 1984). Evidence for two types of photoreceptors has also been seen in the increment threshold for increasing background illumination in the rat ERG (Dodt & Echte, 1961; Dowling, 1967; Green, 1973). A Purkinje shift appears at approximately 10^3–10^4 times the absolute threshold for the ERG response measured using 605 nm light (Dodt & Echte, 1961). The threshold for inducing phase shifts is actually within this range of retinal illuminance at which the response from a cone-like photoreceptor becomes predominant over the rod response. Therefore, even though the spectral sensitivity suggests that a photopigment with maximum sensitivity to 500 nm light mediates the effects of light to the circadian pacemaker in the golden hamster (Takahashi *et al.* 1984), this photopigment may be associated with a photoreceptor with a cone-like sensitivity to light.

There is additional evidence from the mouse that cone-like photoreceptors mediate the effects of light to the circadian pacemaker because rod photoreceptors may not be necessary for light-induced phase shifts. A genetic mutation in the mouse (*rd*) results in degeneration of retinal photoreceptors, beginning about 1 week after birth (Sorsby, Koller, Attfield, Davey & Lucas, 1954). At this time the rod photoreceptors begin a rapid degeneration and cones degenerate with a slower time course (Carter-Dawson, LaVail & Sidman, 1978). By 65 days of age virtually all rod photoreceptors are absent from the retina. By 120 days only 20% of the normal complement of cone nuclei can be identified in the peripheral retina and only 2% remain in the central retina. A small number of cone nuclei survive to at least 18 months. Evidence for a few remaining cone outer segments has also been reported in these mice using immunohistochemical probes that bind to cone outer segments (Pickard, Lemmon & Denoso, 1986). These photoreceptor losses result in a deterioration of visual function, as measured by ERG (Noell, 1958), single-cell recordings from the optic tectum (Dräger & Hubel, 1978) and behavioural responses (Bonaventure & Karli, 1961; Nagy & Misanin, 1970). The threshold for inducing all of these visual responses increases dramatically (to sometimes immeasurable levels) in mice with degenerate

retinae. Heterozygous *rd* mice do not express this mutation and retain relatively normal photoreceptors (Carter-Dawson *et al.* 1978). The functional sensitivity of the mouse photic-entrainment pathway has been measured in normal mice and heterozygous and homozygous *rd* mice with degenerate retinae 80 days after birth. Surprisingly, the photic sensitivities ($1/\sigma$) for phase shifting of circadian rhythms in these strains of mice are equivalent (515 nm stimuli of 900 s; R. Foster, D. Hudson and M. Menaker, unpublished observations). Even though virtually all of the rod photoreceptors have degenerated by this age in the *rd* mice and only a few cone remnants may remain, the sensitivity of the circadian system to light is equal to that measured in mice with normal retinae. The photoreceptor that mediates the effects of light to the circadian pacemaker in this species is probably not a rod therefore, and is probably a cone-like photoreceptor or a yet unidentified photoreceptor with cone-like sensitivity characteristics.

Adaptive significance of these physiological properties

It is interesting to speculate about the adaptive significance of the relatively low sensitivity to stimulus irradiance and the insensitivity to brief stimulus durations measured for this photic-entrainment pathway. This phase-shifting mechanism is more sensitive to relatively long stimulus durations and relatively insensitive to other brief photic signals of 30 s or less in duration. This visual pathway, therefore, is insensitive to short-duration stimuli of high irradiance during the subjective night that could interfere with the entrainment of circadian rhythms. An ecological example would be lightning. Although the luminance of lightning can be greater than that of direct sunlight (8×10^{10} cd m^{-2} for lightning *vs.* $1{\cdot}6 \times 10^{9}$ cd m^{-2} for sunlight; Kaufman, 1981), the brief durations of illumination associated with lightning are probably ineffective for inducing responses in the hamster circadian pacemaker.

The relatively high threshold for stimulation also renders this pacemaker unresponsive to light of lower irradiance such as that from starlight or moonlight that may interfere with the photic entrainment of circadian rhythms. The irradiance of unobstructed light from a full moon is approximately 3×10^{10} photons cm^{-2} s^{-1} (total irradiance between 400 and 700 nm; Munz & McFarland, 1977). This irradiance is probably below the threshold for the phase-shifting response of the hamster circadian pacemaker. Similarly, the irradiance of starlight is approximately $9{\cdot}3 \times 10^{8}$ photons cm^{-2} s^{-1} (irradiance between 410 and 700 nm; Munz & McFarland, 1977) and also approximately 2·5 log units below the threshold for inducing responses in the hamster circadian sytem. If the hamster circadian pacemaker and the photoreceptive system that subserves it were sensitive enough to detect starlight or moonlight then the long exposures to these sources of illumination each night could disrupt the stable entrainment of circadian rhythms to the daily solar cycle.

While the circadian system of hamsters is more sensitive to the total number of photons in 300 s light pulses, it is certainly not unresponsive to photic stimulation for longer durations. If the daily exposure to light is not limited in duration, the irradiance alone becomes the critical measure of stimulus strength and this system may be thought of as *more* sensitive to the 1 h light pulses than to the 300 s pulses. For example, a 1 h stimulus will always induce phase shifts similar to or larger than those obtained with a 300 s stimulus of equal irradiance (see Fig. 6*A*). It is interesting

to speculate, however, that there may be a temporal limit to the total duration of light exposure available to the hamster. In a natural environment there may be selection against extended exposure to potential predators during daylight hours and this may limit the time that the nocturnal hamster can be exposed to sunlight for entrainment of circadian rhythms.

Although the photic-entrainment pathway in the hamster is most sensitive to the irradiance in stimuli that persist for at least 300 s in duration, these extended stimuli are not *required* for photic-entrainment of circadian rhythms. Hamsters are routinely housed in laboratory environments in complete photoperiods (such as 14 h light : 10 h dark). The mammalian circadian system can also entrain to short light pulses delivered as a 24 h 'skeleton' photoperiod (twice daily light pulses separated by regular periods of darkness). The hamster circadian rhythm of wheel-running activity can entrain to 1 s light pulses of high illumination delivered in a 14 h skeleton photoperiod (Earnest & Turek, 1983). In addition the reproductive system of hamsters was stimulated by this lighting regimen as if the animals were maintained in a complete light–dark cycle of long days (14 h light). Circadian oscillations in the flying squirrel (*Glaucomys volans*) will also entrain to *single* 1 s light pulses delivered each day (DeCoursey, 1972).

Hamsters in a natural environment probably use several short periods of illumination each day to entrain circadian rhythms to light–dark cycles. In simulated burrows that are subjected to a light–dark cycle, hamsters exit their burrows repeatedly during the light phase and are exposed to brief periods of illumination (approximately five exposures per day, from a few seconds to 3 min per exposure; Pratt & Goldman, 1986). These short exposures are sufficient for the entrainment of the circadian rhythm of wheel-running activity to the light–dark cycle. Similar results have also been obtained with the flying squirrel (DeCoursey, 1986). In this species several light-sampling periods every few days are sufficient for entrainment of circadian rhythms to the light–dark cycle. While the mammalian photic-entrainment pathway is not maximally sensitive to irradiance in stimuli less than 300 s, the circadian pacemaker is able to use the light information in these relatively brief stimuli.

Conclusions

The physiology of the visual pathway that mediates the photic entrainment of hamster circadian rhythms is unique among visual pathways. This visual pathway has a high threshold for stimulation and is unresponsive to light levels below 10^{10} photons cm^{-2} s^{-1}. In addition, this visual pathway is relatively insensitive to stimulus durations of 30 s or less that are quite effective stimuli for image-forming visual pathways in the golden hamster. These unique physiological properties suggest that this visual pathway is well suited to carrying the light information necessary for synchronization of mammalian circadian oscillations to the environmental cycle of light and dark. A useful goal for future studies will be to determine the basis for these unique physiological characteristics within the individual elements that make up this visual pathway that is specialized for the photic-entrainment of mammalian circadian rhythms.

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