J Comp Physiol A (1985) 156:189–198

Journal of Comparative Physiology A Sensory, Neural, and Behavioral Physiology

Spectral sensitivity of the photoreceptors responsible for phase shifting the circadian rhythm of activity in the bat, *Hipposideros speoris* *

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Accepted November 5, 1984

Summary. 1. The spectral sensitivity of the photoreceptors responsible for phase shifting the circadian rhythm of flight activity in the bat, *Hipposideros speoris* was investigated. For this purpose we studied the phase shifts evoked with 15 min and 2.77 h pulses of monochromatic light at various phases of the rhythm freerunning in DD.

2. A PRC for the circadian rhythm of flight activity in *H. speoris* was constructed with white light pulses (1,000 lx for 15 min) against DD background (Fig. 1). In the first set of experiments 15 min monochromatic light pulses of varying intensities were administered to two phases of the rhythm: the phase of the rhythm at which maximal phase advances occur CT 4, and the phase of the rhythm at which maximal phase delays occur CT 18. The intensities of the 15 min monochromatic light pulses required to produce 50% of the phase shifts evoked with white light pulses (1,000 lx for 15 min) at these two phases were determined. The spectral sensitivity curve for advance phase shifts has a maximum at the wavelength 520 nm and the spectral sensitivity curve for delay phase shifts has a maximum at the wavelength 430 nm (Fig. 5).

3. In the second set of experiments 2.77 h monochromatic light pulses of equal energy of 100 $\mu W/cm^2$ were used. We studied the wavelength dependent phase shifts at four phases of the rhythm: CT 2, CT 4, CT 12 and CT 18. The pulses of 430 and 520 nm evoked unequivocal delay and advance phase shifts, respectively, at all four phases (Fig. 7). These results suggest that at this photopic level of pulse energy, there might be a clear antagonism between the two photoreceptor classes, one having a maximum at the wavelength 430 nm and the other having a maximum at the wavelength 520 nm.

4. We suggest that there may exist two different classes of photoreceptors in the retinas of *H. speoris*. The S photoreceptors (short wavelength sensitive) having a maximum at the wavelength 430 nm and the M photoreceptors (middle wavelength sensitive) having a maximum at the wavelength 520 nm that mediate delay and advance phase shifts, respectively.

Introduction

Spectral sensitivity studies for psychophysical and physiological parameters and for processes of light-induced entrainment and phase shifts of circadian rhythms have been reported for mammals. The spectral sensitivity for phase shifting the circadian rhythm of body temperature in the pocket mouse, *Perognathus penicillatus* (Gordon and Brown 1971) and in rats (McGuire et al. 1973) have been investigated. The spectral sensitivity and colour vision in the golden-mantled ground squirrel, *Spermophilus lateralis* were also studied (Jacobs 1978). The spectral characteristics of the retinal photoreceptors of four species of microchiropteran bats have been determined by ERG studies (Hope and Bhatnagar 1979). Nuboer et al. (1983) have investigated in great detail the effect of colour changes in light regimen as synchronizers of circadian activity in the wild rabbits. Recently Takahashi et al. (1984) studied the spectral sensitivity of the photoreceptors responsible for phase shifting the circadian rhythm of locomotor activity in the hamster, *Mesocricetus auratus*. Their studies, however, were restricted to the phase of the rhythm at which maximum phase advances occurred. No

Abbreviations: *DD* constant darkness; *PRC* phase response curve; τ period of circadian rhythm; *CT* circadian time

* This paper is dedicated to Professor Jürgen Aschoff

published report seems to describe in any detail the spectral sensitivity studies on light-induced phase shifts at various phases of the rhythm for any mammalian circadian system.

In adult mammals the retinal photoreceptors are the only known route for perception of entraining light cycles and blinding results in freerunning circadian rhythms that cannot be entrained or phase shifted by any light cycle (Richter 1967; Nelson and Zucker 1981). So in adult mammals it is quite possible to determine the spectral sensitivity of the retinal photoreceptors that mediate the phase shifts of the circadian rhythm. This could be achieved by studying the phase shifting action of monochromatic light pulses varying in intensity, duration and spectral quality.

In the present studies, we have investigated the spectral sensitivity of the photoreceptors responsible for phase shifting the circadian rhythm of flight activity in the cave-dwelling bat, *Hipposideros speoris*, at different phases of the rhythm. The chronobiological and ecological data are available for these bats (Joshi and Chandrashekaran 1982, 1983, 1984a, b; Marimuthu 1984). In nature these bats exist in a strange paradoxical light-darkness regimen of experiencing absolute darkness during daytime inside natural caves where they roost, and some amount of light (starlight, moonlight) during night time when they forage in the open. They are exposed to skeletal pulses of dim twilight of 4 to 40 lx in intensity once during dawn and again during dusk of the same day that constitute the major phase-resetting stimuli responsible for entrainment by the natural light cycle (Marimuthu 1984). Our recent studies show that dawn twilight is effective in advancing the onset of activity whereas dusk twilight of the same duration is effective in delaying the onset of activity (Joshi and Chandrashekaran, in preparation). We suggest that changes both in the spectral composition and in light intensity of the natural dawn and dusk twilights may be responsible for such differential responses. We have therefore investigated the phase shifting action of light pulses of varying wavelength, intensity and duration. We have designed two sets of experiments to test the wavelength dependence of the phase shifts. Initially we constructed a phase response curve (PRC) for cool fluorescent white light pulses. In the first set of experiments we employed monochromatic light pulses of 15 min duration and tested the spectral sensitivity at two phases of the rhythm: the phase of the rhythm at which maximum phase advances occur (circadian time 4 h: CT 4), and the phase of the rhythm at which maximum phase delays occur (CT 18). In the second set of experiments we employed monochromatic light pulses of longer duration of 2.77 h and of equal energy of 100 μW/cm^2 and tested the spectral sensitivity at four phases of the rhythm: CT 2 (the switch-over point from delays to advances of the fluorescent light PRC), CT 4, CT 12 (the onset of activity) and CT 18.

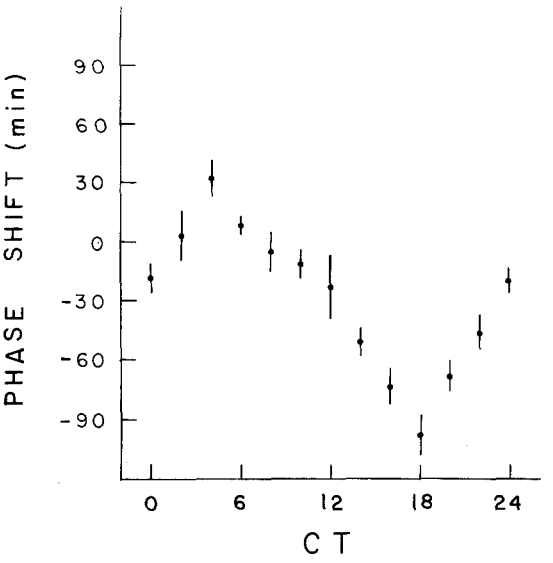

Fig. 1. A PRC for the circadian rhythm of flight activity in the bat, *Hipposideros speoris* evoked with fluorescent white light pulses (1,000 lx for 15 min) against DD background. Phases are calculated in circadian time (*CT*). CT 12 represents onset of activity, hence onset of subjective night. The delay phase shifts were observed during late subjective day (CT 8 and CT 10) and during subjective night (from CT 12 to CT 24). The advance phase shifts were observed during early subjective day (from CT 2 to CT 6). Solid circles: means; vertical bars: S.D. for $n=5$ or 6. Data presented are derived from 71 phase responses for 27 bats

Materials and methods

Animals and housing. Animals studied were adult male bats (*Hipposideros speoris*) freshly captured from a nearby cave on their return flight during dawn hours and brought immediately to the laboratory and housed individually in flight activity swing-cages (30 × 30 × 30 cm) made of light aluminium frames and mosquito mesh netting. The cages were suspended by means of sturdy springs and separated by fibre-wood partitions. The bats resorted to short bouts of flapping flight during activity time which jiggled the cages. Such movements would depress a microswitch and activate the writing pen of an Esterline Angus Recorder located outside the experimental room. The activity-rest pattern of as many as 20 bats in separate swing-cages was assayed concurrently. The temperature was 28°±1 °C in the experimental dark room and relative humidity 85±5%. The bats were hand-fed with minced cockroaches for the first 3–5 days, then they learnt to accept the cockroaches when offered with forceps. They were always fed at random hours and

water was available ad lib. During maintenance and feeding, very dim indirect red light (ca. 2.1×10^{12} photons/cm^{2}s) of $\lambda > 640$ nm was used to which the bats seemed to be insensitive, since the freeruns during unperturbed state were smooth. The bats were in DD from the first day of the experiment except for the light perturbation. Interindividual differences in τ (period of circadian rhythm) led them to become out of phase with each other in about 10 days. Actograms were obtained by pasting 24 h strips chronologically one below the other and 'double plotted'. The onset of activity has been chosen as reference point for computation of τ during freerun and for phase shifts. τ was computed by fitting a least square regression line to 8–10 successive activity onsets immediately preceding the perturbation and extrapolated forward to give the predicted time of the activity onset. The steady-state phase shift was obtained in minutes or hours which denoted the difference in time between the projected activity onset on day 5 after the pulse and the actual fifth post-pulse activity onset on day 5 after the pulse (given by the post-pulse regression line) after all transients had subsided.

White light PRC. Fluorescent cool daylight (Philips tube light, 6500°K, 20 W) was used in constructing the white light PRC against DD background. The phases are expressed in hours of circadian time (CT) after multiplying real time values by $24/\tau$. CT 12 denotes the onset of the subjective night and hence the onset of the activity. The fluorescent light as well as the monochromatic light perturbations were administered by removing individual bats at the desired phases in a small cage ($7 \times 7 \times 7$ cm) made of aluminium frames and white mesh netting. Then this cage was transferred to a light tight small chamber of the same experimental room where all light pulses were administered. For constructing the white light PRC, the bat was exposed to fluorescent white light of 1,000 lx for 15 min. Onset of the pulse was the reference point for the phase at which the white light and the monochromatic light pulses were administered. After the delivery of the pulses the bat was returned to its own activity cage. Appropriate control experiments established that only the light pulse per se and none of the associated disturbances such as handling of the bats etc. produced phase shifts of rhythms. In all phase shift experiments the bats were further allowed to freerun after the perturbation for a period of at least 7–10 days. Untreated bats were allowed to freerun at least for 30–50 days.

Monochromatic light perturbations. The monochromatic light perturbations were achieved essentially by the same procedure that was employed for the fluorescent light perturbations as described above. The individual bat was exposed to light of a particular wavelength and intensity obtained from the monochromator. We used Zeutschel Systematic Monochromator System 'M3' (Dr. Schoser) with narrow band Schott (Mainz) interference filters (7–15 nm at 50% transmission). The wavelengths employed were 430, 480, 520, 580 and 654 nm.

Light from a Halogen Bellaphot lamp (Osram, 64634, 15 V 150 W; made in Germany) was collimated by condenser and projection lenses. Light was filtered with narrow band interference filters described above. Direct monochromatic light was projected onto the bat kept in the beam of monochromatic light ca. 30 cm from the illuminated interference filter. The diameter of the monochromatic light beam was 4 cm, so the bat's head and thorax were fully exposed to monochromatic light. During exposure to light perturbation each bat was awake with eyes open, facing the light source and hanging in the upside-down posture in a small cage ($7 \times 7 \times 7$ cm). The cage dimensions did not permit the bat to change its posture.

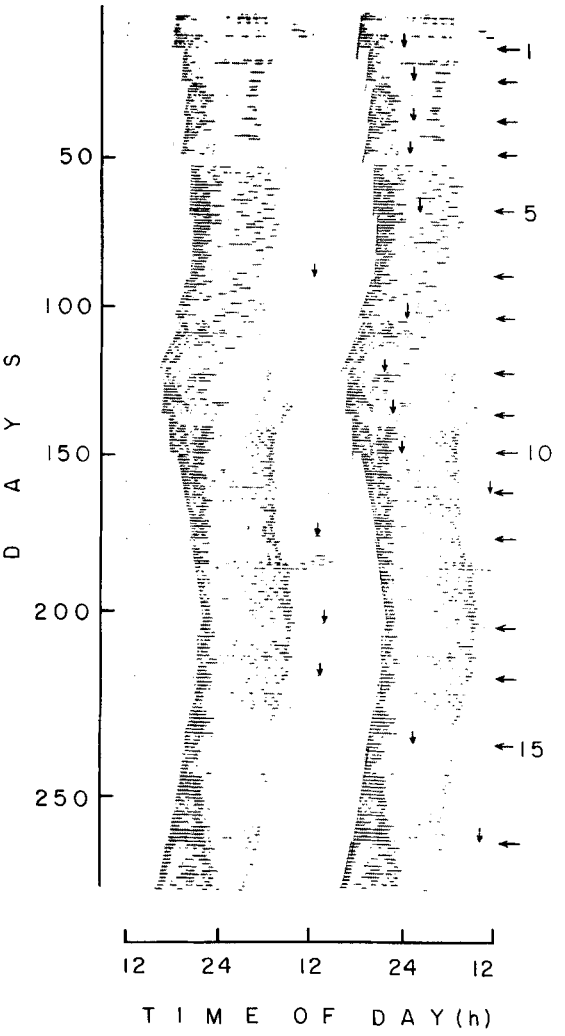

Fig. 2. Flight activity record of a bat in DD exposed to 15 min pulses of white light (1,000 lx for 15 min at CT 18, pulse 1) and monochromatic light (pulses 2 to 16). The pulse treatment is shown in the right half of this actogram and unedited data in the left half. The onset of the pulse was taken as the reference phase. Vertical arrows: phase of perturbation; horizontal arrows: days on which the pulses were administered (extreme right). Pulses presented at CT 18 at wavelengths 430 nm (pulse 2: 5.4×10^{10} photons/cm^{2}s; pulse 4: 2.2×10^{14} photons/cm^{2}s; pulse 10: 1.7×10^{14} photons/cm^{2}s); 480 nm (pulse 7: 2.9×10^{11} photons/cm^{2}s; pulse 8: 2.4×10^{14} photons/cm^{2}s; pulse 9: 1.3×10^{14} photons/cm^{2}s); 520 nm (pulse 3: 2.4×10^{13} photons/cm^{2}s; pulse 5: 6.8×10^{13} photons/cm^{2}s; pulse 15: 1.7×10^{13} photons/cm^{2}s) evoked delay phase shifts. Pulses presented at CT 4 at the wavelengths 430 nm (pulse 14: 2.2×10^{14} photons/cm^{2}s); 480 nm (pulse 16: 1.9×10^{11} photons/cm^{2}s); 520 nm (pulse 12: 2.6×10^{10} photons/cm^{2}s; pulse 6: 2.4×10^{14} photons/cm^{2}s; pulse 13: 2.6×10^{14} photons/cm^{2}s) evoked advance phase shifts. Wavelength 654 nm induced neither advance nor delay phase shift at CT 18 or CT 4 phase (pulse 11) even at 3.3×10^{14} photons/cm^{2}s. The actogram also shows long lasting changes in τ that followed some phase shifts. Data lost for days 48–51 due to failure of equipment

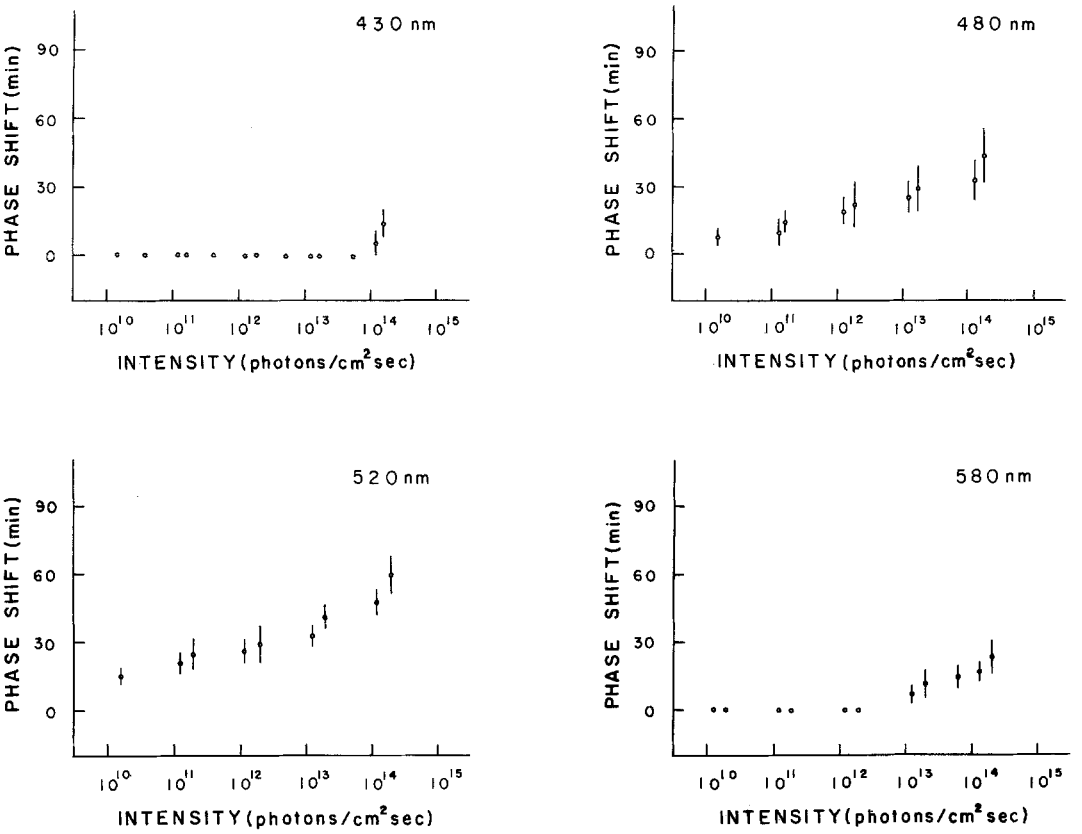

Fig. 3. Intensity-response curves obtained with 15 min monochromatic light pulses for four different wavelengths: 430, 480, 520 and 580 nm. Monochromatic light pulses were administered at phase CT 4. Open circles: means; vertical bars: SD for $n=4$ to 5. Data presented derived from 189 phase responses for 20 bats

The intensity of the monochromatic light was directly measured in $\mu W/cm^2$ with a UDT 40X Optometer (Santa Monica, CA, USA) using a radiometric filter. In order to compare different wavelengths, light intensity was normalized to photons/cm^2s. The maximum energy of the monochromatic light that could be obtained from this monochromator was limited to 100 $\mu W/cm^2$ for all wavelengths.

We carried out two sets of experiments. In the first set of experiments 15 min monochromatic light pulses were administered to two phases of the rhythm: the phase of the rhythm (CT 4) at which maximum phase advances of +30 min (SD$\pm$ 10, $n=6$) occurred and the phase of the rhythm (CT 18) at which maximum phase delays of -97 min (SD$\pm$11, $n=5$) occurred. The intensities of the monochromatic light pulses were systematically altered until the steady-state phase shifts on day five after the pulse attained 50% of the phase shifts evoked with the 15 min fluorescent white light control pulses. The effectiveness of the 15 min monochromatic light pulses were expressed as reciprocal of the light intensity values.

In the second set of experiments, we used monochromatic light pulses of longer duration of 2.77 h and of equal energy of 100 $\mu W/cm^2$. However, the equivalent quantum intensity calculated in photons/cm^2s at each wavelength was as follows: 430 nm: 2.2×10^{14}; 480 nm: 2.4×10^{14}; 520 nm: 2.6×10^{14}; 580 nm: 2.9×10^{14}; 654 nm: 3.3×10^{14}. We investigated the wavelength dependent phase shifts at four phases of the rhythm: CT 2, CT 4, CT 12 and CT 18.

Results

Phase shifting action of 15 min monochromatic light pulses

Figure 1 shows the white light pulse PRC (1,000 lx for 15 min against DD background) for the circadian rhythm of the flight activity in *H. speoris*. This PRC has the general time course and wave form of the PRCs of nocturnal animals (Pittendrigh 1981). We used large wavelength intervals to determine the spectral sensitivity of the photoreceptors in the present studies. Figure 2 illustrates the flight activity record of a bat in DD for 275 days. This bat was exposed to white light control pulses and monochromatic light pulses at CT 4 and CT 18. The actogram also shows the long lasting changes in τ that followed some phase shifts. The magnitudes of phase shifts in response to 15 min monochromatic light pulses of increasing intensity for four different wavelengths were determined at CT 4 and CT 18 phases (Figs. 3 and 4

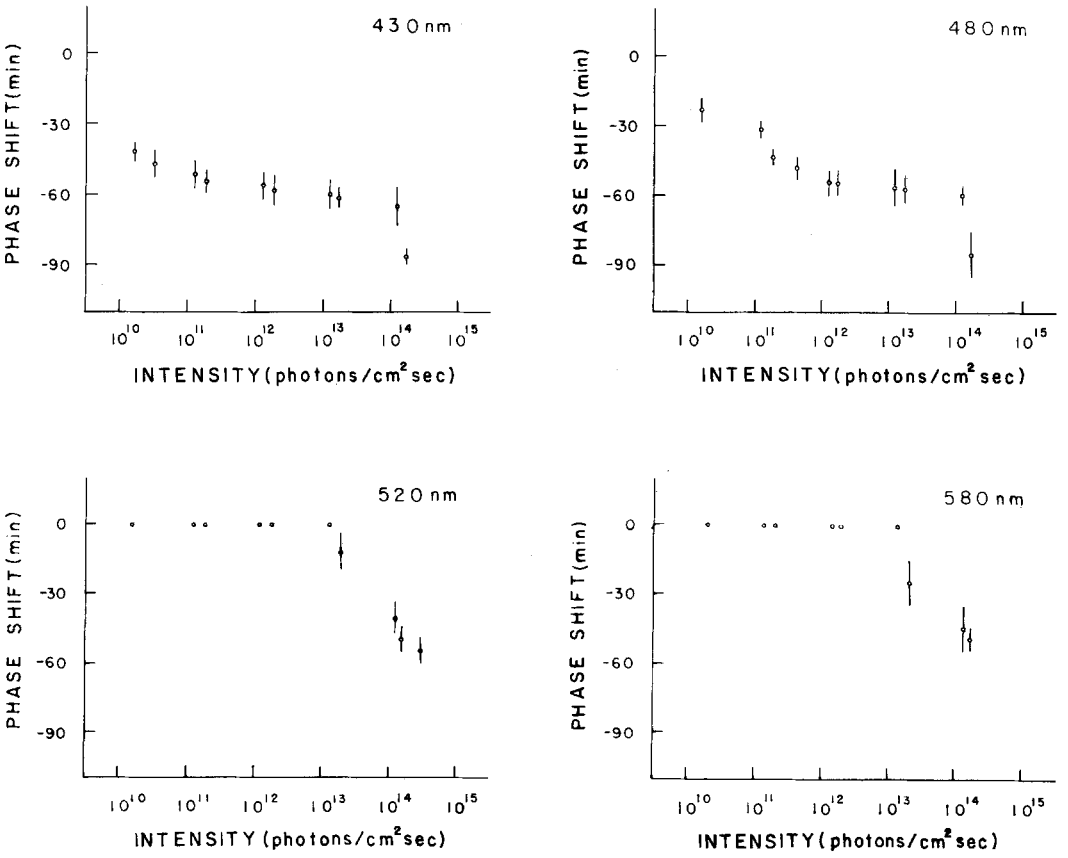

Fig. 4. Intensity-response curves obtained with 15 min monochromatic light pulses for four different wavelengths: 430, 480, 520 and 580 nm. Light pulses were administered at phase CT 18. Open circles: means; vertical bars: SD for $n=4$ to 5. Data presented derived from 192 phase responses for 23 bats

respectively). At any of these two phases difference in the wavelength did not affect the sign of phase shift for 15 min pulse perturbation used here. The magnitude of resultant phase shifts increased with increasing light intensity beyond threshold. The spectral sensitivity curves (Fig. 5) are not based on wavelength interval of $\leqq 10$ nm, therefore the maxima of both curves are indicated rather only as an approximation. The spectral sensitivity curve for the advance phase shifts (solid line) has a maximum at the wavelength 520 nm and the responses to 480 and 430 nm progressively diminished in magnitude. Furthermore the light intensity required to produce 50% of the maximal phase shift at 430 nm had to be ca. 10000 times the light intensity at 520 nm. The wavelength 654 nm induced neither advance nor delay phase shift, even at 3.3×10^{14} photons/cm^{2}s intensity (Fig. 2, pulse 11). The spectral sensitivity curve for the delay phase shifts (dashed line), has a maximum at the wavelength 430 nm. The spectral sensitivity curves for advance and delay phase shifts are not similar. The spectral sensitivity curve for delay phase shifts has shifted towards the shorter wavelengths.

Phase shifting action of 2.77 h monochromatic light pulses

When monochromatic light pulses of higher and equal energy of 100 μW/cm^2 for longer duration of 2.77 h were used, the results are entirely different from those of the 15 min monochromatic light pulses. The sign of the phase shifts evoked with 2.77 h pulses were dependent on the wavelength and independent of the phase of the rhythm.

Figure 6 illustrates the flight activity record of a bat in DD for 214 days. This bat was exposed to monochromatic light pulses of equal energy of 100 μW/cm^2 for 2.77 h. The actogram also shows the transients and long lasting changes in τ that followed some phase shifts. Figure 7 illustrates the

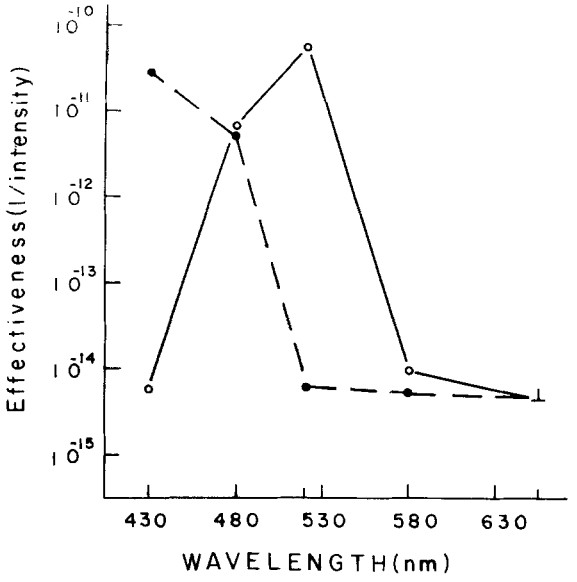

Fig. 5. Spectral sensitivity curves obtained with 15 min monochromatic light pulses that evoked approximately 50% of the maximal advance and delay phase shifts evoked by white light pulses at CT 4 and CT 18 phases, respectively. The spectral sensitivity curve for advance phase shifts (solid line) has a maximum at 520 nm. The spectral sensitivity curve for the delay phase shifts (dashed line) has a maximum at 430 nm. Abscissa: wavelengths of the pulse light; ordinate: measure of effectiveness of 15 min monochromatic light pulses expressed as the reciprocal of light intensity. Symbols used: open circles-value of monochromatic light intensity to obtain 50% of advance phase shifts at CT 4; filled circles-value of monochromatic light intensity to obtain 50% of maximal delay phase shifts at CT 18; ⊥-light intensity that induces neither advance nor delay phase shifts. For each point at least four measurements were taken. Data presented are derived from 156 phase responses for 36 bats

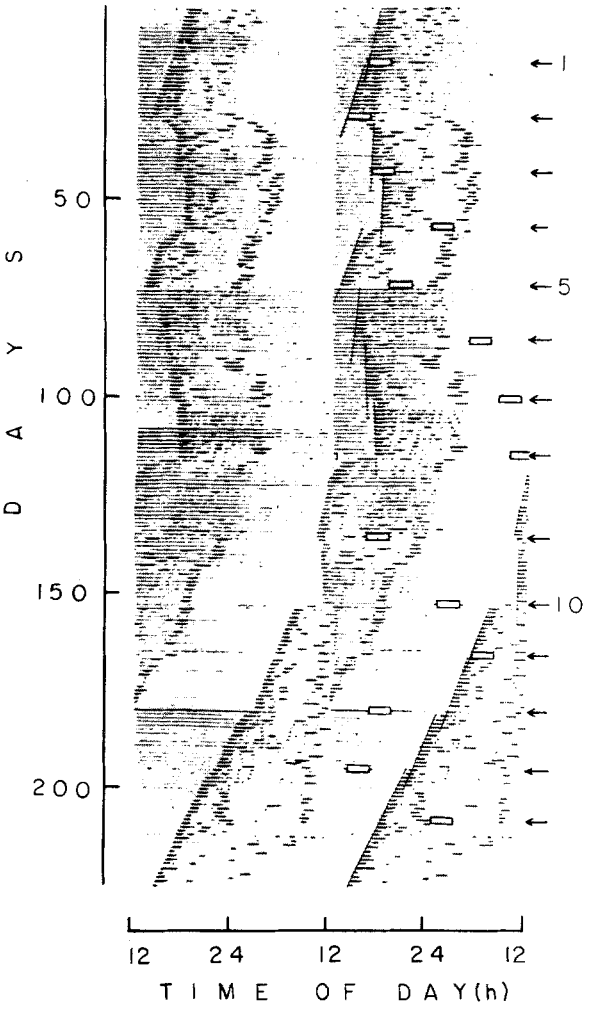

Fig. 6. Flight activity record of a bat in DD that was exposed to monochromatic light pulses of equal energy of 100 μW/cm² for 2.77 h at four critical phases of the rhythm: CT 2- switch-over point from delays to advances for fluorescent light PRC, CT 4, CT 12- the onset of activity and CT 18. Onset of the pulse is taken as reference phase. Time of pulse indicated by hollow rectangles on the right half of the plot and unedited data on the left half. Days on which pulses were administered are shown by horizontal arrows (extreme right). Sign of the phase shifts was dependent on the wavelength, not on the phase of the rhythm. Thus pulses of 430 nm evoked delay phase shifts at CT 2 (pulse 6), at CT 4 (pulse 7), at CT 12 (pulse 2) and at CT 18 (pulse 5). Pulses of 480 nm also evoked delay phase shifts of relatively lesser magnitude at CT 12 (pulse 3) and at CT 18 (pulse 9). Pulses of 520 nm evoked advance phase shifts at CT 2 (pulse 10), at CT 4 (pulse 8), at CT 12 (pulse 1) and at CT 18 (pulse 4). Pulses of 580 nm also evoked advance phase shifts of relatively lesser magnitude at CT 2 (pulse 12), at CT 4 (pulse 13) and at CT 12 (pulse 11). Pulses of 654 nm induced neither advance nor delay phase shifts at CT 18 (pulse 14)

wavelength dependent phase shifts at four critical phases of the rhythm. At CT 2 (upper left panel), the most effective wavelength is 520 nm which evoked advance phase shifts of about 4.3 h; 430 and 580 nm are less effective in evoking delay and advance phase shifts respectively. At CT 4 (upper right panel), the most effective wavelength, however, is again 520 nm which evoked maximum advance phase shift; 580 nm also evoked advance phase shift though of less magnitude, but 480 and 430 nm evoked progressively diminishing delay phase shifts of feeble magnitude. At CT 12 (lower left panel) the most effective wavelength is 430 nm that evoked maximum delay phase shifts, 480 nm also evoked delay phase shifts of lesser magnitude, whereas 520 nm evoked advance phase shifts of a lesser magnitude. At CT 18 (lower right panel) both 430 and 520 nm are equally effective in evoking phase shifts of comparable magnitude but having opposite signs, thus 430 nm evoked delay phase shifts whereas 520 nm evoked advance phase shifts. The wavelength 654 nm evoked neither advance nor delay phase shift at any of the four phases.

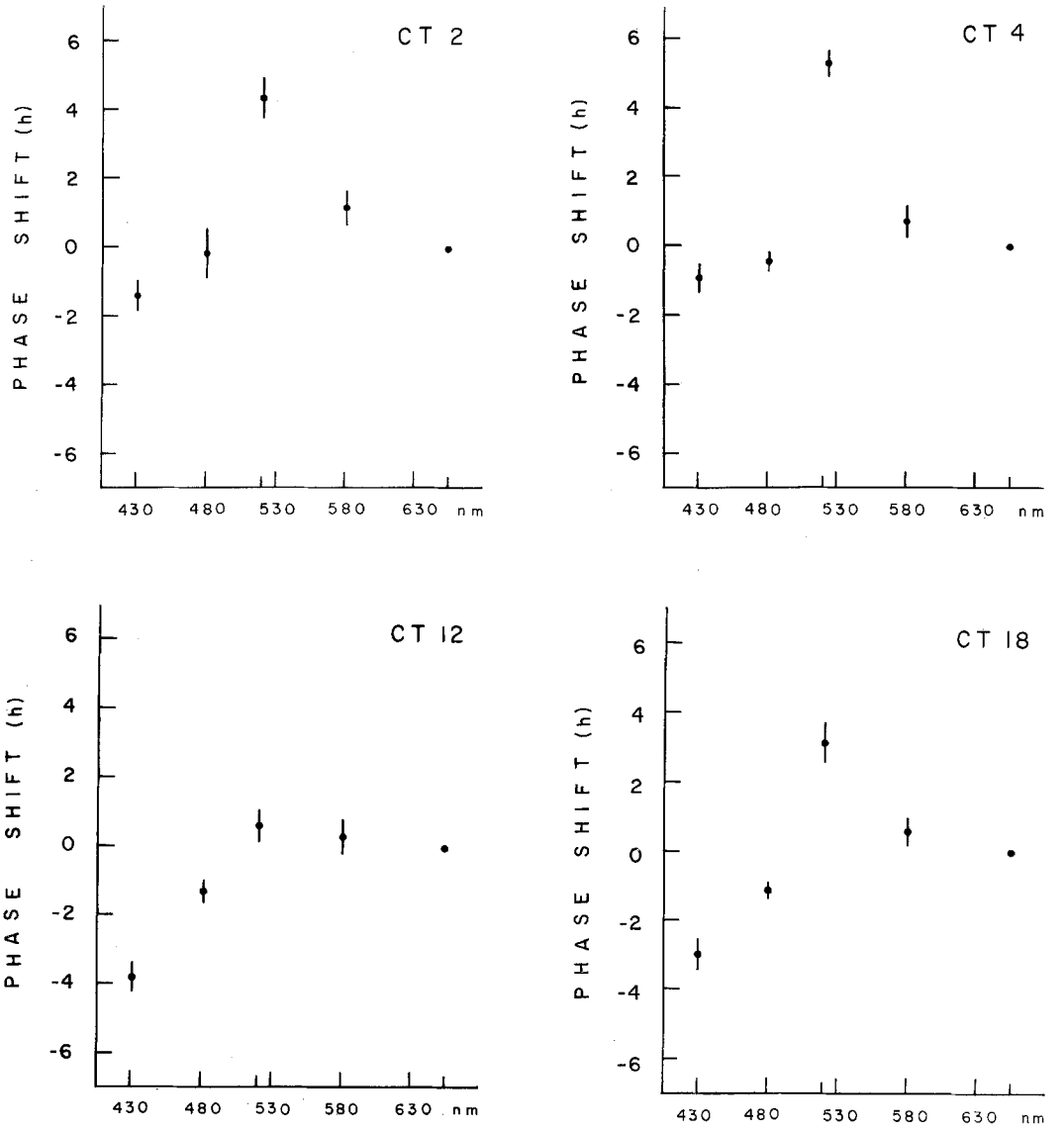

Fig. 7. Wavelength dependent phase shifts of the circadian rhythm of flight activity in *H. speoris* at four phases of the rhythm caused by monochromatic light pulses of equal energy of 100 μW/cm² for 2.77 h. At CT 2 (upper left panel) the most effective wavelength is 520 nm which evoked maximal advance phase shift. At CT 4 (upper right panel) the most effective wavelength is 520 nm which evoked maximum advance phase shift. At CT 12 (lower left panel), the most effective wavelength is 430 nm that evoked maximum delay phase shift. At CT 18 (lower right panel) both 430 and 520 nm are equally effective which evoked phase shifts of comparable magnitude but differing in sign. Wavelength 654 nm induced neither advance nor delay phase shifts at all four phases. Solid circles: means; vertical bars: SD for $n=5$. Data presented are derived from 106 phase responses for 26 bats

Transients and changes in τ caused by 15 min and 2.77 h monochromatic light pulses

15 min monochromatic light pulses produced small phase shifts virtually without any transients; the changes in τ (lengthening or shortening) had no systematic relationship to the phase of perturbation or the wavelength. However, 2.77 h pulses produced large phase shifts with advancing or delaying transients depending on the wavelengths. One to four advancing transients always ensued when 520 nm (Fig. 6, pulses 1, 4, 8, 10) and 580 nm (Fig. 6, pulses 11, 12, 13) wavelengths were employed. Two to four delaying transients always ensued when 430 nm (Fig. 6, pulses 2, 5, 6, 7) and 480 nm (Fig. 6, pulses 3, 9) wavelengths were employed. It might further be emphasized that the shortening of τ was associated with advance phase

shifts caused by 520 nm (Fig. 6, pulses 1, 4, 8, 10) and 580 nm (Fig. 6, pulses 11, 12, 13) wavelengths whereas the lengthening of τ was associated with delay phase shifts caused by 430 nm (Fig. 6, pulses 2, 5, 6, 7) and 480 nm (Fig. 6, pulses 3, 9) wavelengths.

Discussion

The present studies shed some light on the nature of the spectral sensitivity of the retinal photoreceptors in *H. speoris*. The spectral sensitivity curves derived by the brief 15 min pulses of monochromatic light for the maximal advance and delay phase shifts are dissimilar. Thus the spectral sensitivity curve for the advance phase shifts has a maximum at the wavelength 520 nm and that of the delay phase shifts has a maximum at the wavelength 430 nm. Moreover, monochromatic light pulses of 430 and 520 nm at the photopic level (100 $\mu W/cm^2$ for 2.77 h) evoked phase shifts having opposite signs at the same phase of the rhythm. Thus 430 nm unequivocally evoked delay phase shifts whereas 520 nm evoked advance phase shifts at CT 2, CT 4, CT 12 and CT 18 (Fig. 7). It therefore appears that two photoreceptor classes might be present in the retinas of *H. speoris*: S photoreceptors (short wavelength sensitive) having a maximum at the wavelength 430 nm and M photoreceptors (middle wavelength sensitive) having a maximum at the wavelength 520 nm.

There is anatomical evidence for the second class of photoreceptors in microchiropteran retinas (Suthers 1970; Chase 1972). Electrophysiological studies by Hope and Bhatnagar (1979a) revealed that the relative amplitude of the retinal response to spectral stimulation in 4 species of microchiropteran bats could be accounted for by assuming two photopigments: rhodopsin (500 nm) and a second pigment absorbing at about 560–580 nm. It must be emphasized that the present results of the spectral sensitivity studies for *H. speoris* do not give a clear demonstration of the presence of either of the above photopigments or photoreceptors in the retinas of these bats. The relationship between the maxima of spectral sensitivity curves at a given wavelength and the absorption of the photopigment at that wavelength has not yet been investigated for microchiropteran retinas. However, rhodopsin seems to be the likely pigment responsible for advance phase shifts having a maximum at 520 nm. Recently Takahashi et al. (1984) investigated the spectral sensitivity of the photoreceptors responsible for phase shifting the locomotor rhythm in the hamster, *Mesocricetus auratus*. Their studies revealed that the spectral sensitivity curve for the advance phase shifts had a maximum near 500 nm similar to the absorption spectrum for rhodopsin. It would also be quite likely that the two photoreceptors, S and M might be two different classes of rods in the rod dominated retina of an exclusively nocturnal mammal like *H. speoris*. In the retina of the frog, *Rana pipiens*, two different classes of rods were described; the 'green' rods having a maximum at 433 nm, and the 'red' rods having a maximum at 502 nm (Liebman and Entine 1968).

The onset and end of activity in nocturnal animals have been postulated to be controlled by two mutually coupled hypothetical oscillators: E, evening oscillator and M, morning oscillator respectively (Pittendrigh and Daan 1976). Two different photoreceptor classes M and S appear to mediate advance and delay phase shifts respectively in *H. speoris* with differential responses to monochromatic light. Moreover, when we analyse the phase shifting effect of monochromatic light pulses at the photopic level (ca. 2.5×10^{14} photons/cm^2s for 2.77 h), the functional dominance of the S and M photoreceptors during subjective morning and evening, respectively, becomes evident. Thus at the end of activity, i.e., the subjective morning (CT 2 and CT 4), the M photoreceptors are functionally dominant in mediating advance phase shifts whereas the S photoreceptors become relatively less effective in mediating delay phase shifts (Fig. 7, upper left and right panels respectively). On the other hand, at the onset of activity, i.e. the subjective evening (CT 12), the S photoreceptors are functionally dominant in mediating maximal delay phase shifts whereas the M photoreceptors become relatively less effective in mediating advance phase shifts (Fig. 7, lower left panel).

It is tempting to assume that such a dual oscillatory system as mentioned above might underlie the responses we report here. This could be explained by assuming that the M photoreceptors have greater control over the M oscillator, and the S photoreceptors have greater control over the E oscillator.

We have also observed that the phase shifts differed in sign when evoked with pulses in the photopic range of white light of differing spectral distribution but of equal intensity and duration (1,000 lx for 15 min). Thus white incandescent cool light (spectral intensity distribution curve extending from 380 to 800 nm with a single peak at 580 nm) evoked mostly advance responses (except for the phases from CT 6 to CT 12), possibly giving

adequate stimulus to the M photoreceptors (responsible for advance phase shifts) and relatively inadequate stimulus to the S photoreceptors (responsible for delay phase shifts) (Joshi, unpublished experiments). White fluorescent light (spectral intensity distribution curve extending from 380 to 730 nm with two distinct peaks at 460 and 580 nm respectively) evoked mostly delay responses (except for the phases from CT 2 to CT 6, Fig. 1) possibly giving relatively higher stimulus to the S photoreceptors than to M photoreceptors.

It has been reported that the onset of activity depended on the colour and intensity changes in the light regimen. Krüll (1976) suggested that the daily colour variation in the arctic sky during polar summer was able to entrain the activity of the diurnal birds. Nuboer et al. (1983) also reported that the onset of circadian activity in the wild rabbits depended on the colour and intensity changes in light regimen. Blue light intensity increments were more effective in advancing the onset of activity than the blue decrements; and yellow decrements were more effective in delaying the onset of activity than yellow increments.

Hope and Bhatnagar (1979b) reported that the retinas of four species of microchiropteran bats were able to function at progressively higher light levels and showed progressively greater ERG responsiveness at longer wavelengths. They revealed that these results correlated well with bats' natural behaviours (roosting, emergence, foraging) and with the ambient light intensities to which they were exposed in nature. It is tempting to compare the results of ERG responses to longer wavelengths with the results of phase shifting behaviour of the tropical bats that we studied. Such greater responsiveness in phase shifting – both advancing and delaying – to longer wavelengths of 600, 650, 700 and 750 nm at constant energy (100 $\mu W/cm^2$ for 1,000 s) were observed in another microchiropteran bat, *Taphozous nudiventris kachhensis* (Sripathi 1982). In contrast the responses of *H. speoris* to longer wavelengths are entirely different. The longer wavelengths from 610 to 750 nm are apparently perceived by the circadian system as darkness, since in this range of wavelengths phase shifts could not be induced in *H. speoris* by intense monochromatic light. Such differential responses to longer wavelengths may be explainable in terms of their strikingly different preferences for ambient light levels while roosting in nature. Members of *T. n. kachhensis* roost in crevices in rocks where they are exposed to daylight of higher intensity and even to direct sunlight (Sripathi 1982). On the other hand, *H. speoris* roost predominantly in absolute darkness in natural caves during daytime (Marimuthu 1984). The bats used in our experiments were captured from one such natural cave where absolute darkness prevailed.

It would be interesting to determine the spectral sensitivity of the photoreceptors mediating the entrainment of flight activity rhythm of this bat and compare it with the spectral sensitivity of the photoreceptors responsible for phase shifting the flight activity rhythm. Such a comparison may reveal whether or not the same retinal photoreceptor classes are involved in mediating the entrainment and phase shifts in *H. speoris*.

Acknowledgements. We are grateful to Prof. G. Neuweiler, Dr. S. Daan, Dr. J.F.W. Nuboer, Dr. S. Bose and two referees for critically reading the manuscript, making many useful comments and suggesting improvements. Generous financial support from the UGC and DST (Government of India) under the IRHPA scheme and an SRF to DJ from the CSIR, New Delhi are gratefully acknowledged. This work benefitted from equipment obtained under the Indo-German Project on Animal Behaviour.

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