

Possible “Dawn” and “Dusk” Roles of Light Pulses Shifting the Phase of a Circadian Rhythm*

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Summary. A new automatic photoelectric method used in recording the eclosion rate of flies is described. The phase responses of the circadian rhythm of eclosion in *Drosophila pseudoobscura* to light pulses, of 1000 lx intensity and durations varying between 30 min and 12 h, were studied. The rhythm responds selectively either to the “on” or to the “off” transition of light pulses offered during the subjective night. The light pulses shift phase with the “off” transition during the first half of the night (dusk effect) and shift phase with the “on” transition during the second half of the night (dawn effect). The present findings are briefly discussed in the context of the work of other authors in this field.

Introduction

The circadian rhythms in the physiological processes of organisms of diverse levels of organization and complexity display several similarities. Thus the majority of these rhythms are entrained in the laboratory, as indeed in nature, by light/darkness cycles. They persist (free run) even under laboratory conditions of constant light or continuous darkness with a period departing slightly from 24 h. This, for example, is the case with the eclosion rhythm of *Drosophila pseudoobscura* which is the subject of the present report. This circadian rhythm is sensitive to exposure to light while free running in darkness and responds with earlier (advanced) or later (delayed) occurrence of the eclosion (measured with respect to that of a control). These *shifts* in the timings are usually called *phase shifts*. The subjective night part of the cycle is the more sensitive region to light exposure and the phase shift responses here are pronounced. The rhythm responds with delayed phase shifts during the first half of the night and with advanced phase shifts during the second half of the night with a major portion of the subjective day region being fairly unresponsive to light. Information of this nature is contained in *phase response curves*

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now available for several species of plants and animals (Aschoff, 1965; Bünning, 1967; Pittendrigh, 1966). A phase response curve gives "the amount and sign of a phase shift as a function of the phase in which the stimulus is applied" (cf. Aschoff, Klotter and Wever, 1965).

It has been claimed in connection with the incidence of diapause in the pink bollworm *Pectinophora gossypiella* (Adkisson, 1965) and the eclosion rhythm in *Drosophila pseudoobscura* (Chandrashekar, 1967b) that brief light pulses offered during the subjective night convey "dawn" and "dusk" information depending on the time of night they illumine. For the *Drosophila* case the two transitions of a light pulse, the "on" and the "off" were specifically postulated to mediate information of this nature with the "off" (dusk) transition shifting phase during the first half of the night and the "on" (dawn) transition shifting phase during the second half of the night. The experiments reported here were designed to test critically the above assumptions regarding the effects of strong light pulses (1000 lx; for the meaning of "strong" see Winfree, 1970). The evidence on hand points to "on" or "off" of the light pulses variously determining phase shifts as a consequence of the part of the night with which they coincide.

Materials and Methods

Cultures of *Drosophila pseudoobscura* (PU 301 from C. S. Pittendrigh's laboratory) were raised in continuous light of 900 to 1100 lx at $20^{\circ}\text{C} \pm 0.5^{\circ}$ in plastic troughs 22 cm in diameter and 10 cm deep. Two circular windows 3 cm in diameter were cut out of the walls of the trough for ventilation and covered over with fine nylon mesh. Square glass plates 25×25 cm were used as lids for the troughs. The usual cornmeal-agar-yeast-molasses medium was used in rearing the cultures. The larvae creep out of the frequently moistened medium in 17 to 18 days after oviposition and pupate on the inner walls of the troughs and the glass plates. The pupae were harvested about 20 days after oviposition by a floatation method and air dried. For further details see Maier (1972). In all experiments the rhythms were initiated in otherwise arrhythmic populations raised in light by a simple transfer to darkness (red "safe light" of 660 nm). Actual eclosion started, however, 3 to 4 days after transfer by which time it is presumed that the transients would have subsided (Pittendrigh *et al.*, 1958; Winfree, 1970). Each experimental population consisted of 300 unpigmented pupae which were developmentally asynchronous. Eclosion could be followed over 4 to 5 days after which there were too few flies left to eclose. All light pulses contained fluorescent white light of about 1000 lx which was also approximately the intensity in the culture room.

The eclosion was recorded every hour 4 to 5 days in all experiments with the aid of an automatic set-up (Fig. 1) devised by W. Engelmann (together with Arthur T. Winfree). Metal holding-plates 5×10 cm bearing 100 holes held 100 pupae—one pupa in each hole. Fine nylon mesh material glued to one side of the metal plate prevented the pupae falling through. The metal plates were placed over photoelements 5×5 cm and were themselves covered over with sooted glass plates used for slides, to preclude light from reaching the photoelements. Freshly emerging flies, in trying to escape, scraped off the soot above them, allowing red

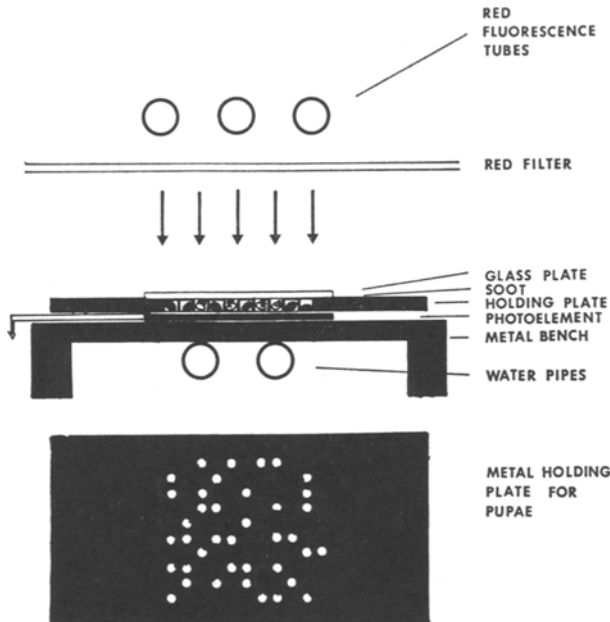


Fig. 1. The device used for recording the rate of eclosion of *Drosophila pseudoobscura* flies. For details see text. The whole set-up is placed in an air-conditioned room and the metal bench supporting the metal plates is additionally kept at a constant temperature by circulating water from a water bath through pipes. Below: top view of the metal holding-plate with the sooted glass plate above it showing the holes in the soot scraped by flies which had emerged

light from above to fall on the photoelements underneath. The flies themselves died in the process out of desiccation. Light illuminated the photoelements in proportion to the number of flies emerging. The voltage of 3 photoelements, united in series and forming a group (bearing 3 metal holding-plates with 300 pupae), was measured every hour with a digital voltmeter of a data logger (Microscan, Dynamco). The voltmeter could scan 100 channels every hour. Data were stored on punch tape and the differences between successive values plotted with the aid of a program (Schelkle, Zentrum für Datenverarbeitung, University of Tübingen).

Results

Two Categories of Experiments Were Performed

Category 1. Experiments in which the subjective night, 12 CT to 01 CT (CT = circadian time of Pittendrigh and Minis, 1964), was systematically scanned by light pulses of varying duration. The “on” and the “off” of the pulses were variously arranged during the experiments

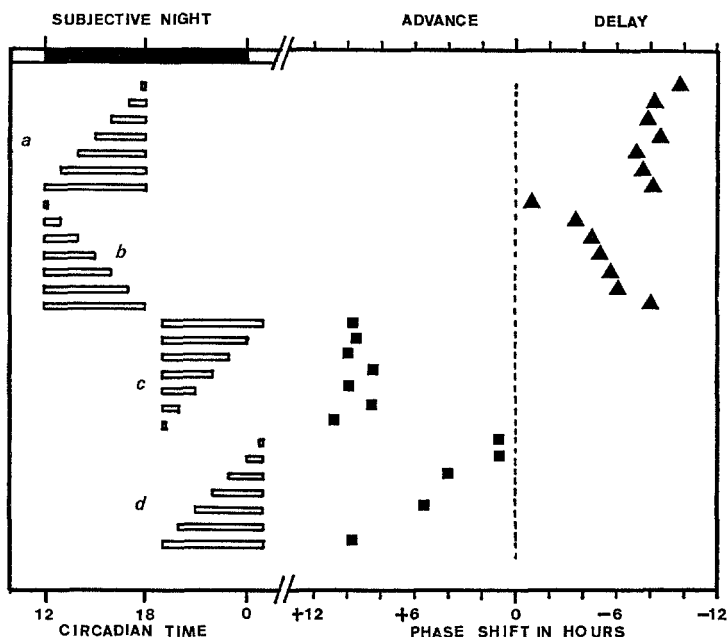


Fig. 2. Shifting the phase of the *Drosophila pseudoobscura* eclosion rhythm with light pulses of 1000 lx and varying duration given in the first and second halves of the subjective night. The light pulses are represented by the unfilled bars and are arranged in 4 batches on the 'circadian time scale' of Pittendrigh and Minis (1964). In batch 'a' the different populations experienced the "on" transition of pulses at different hours but experienced the "off" transition at the same phase (18 CT). In batch 'b' the populations experienced the "on" transition at the same circadian hour (12 CT) but the "off" transition occurred for each population at a different hour. The pulses of batches 'a' and 'b' scan the first half of the subjective night. Batches 'c' and 'd' scan the hours of the second half of the subjective night. In batch 'c' the "on" transitions of all the pulses were in alignment (at 19 CT) with the "off" transition occurring at a different hour for each population. In batch 'd' on the other hand the "on" transitions were systematically staggered and the "off" transitions of pulses aligned. The filled triangles represent averaged median values of eclosion peaks of experimental populations 4-5 days after light treatment, which responded with delay phase shift. The filled squares represent averaged median values of peaks 4-5 days after light treatment showing advancing phase shifts. Apparently the "off" transitions of light pulses determine direction and degree of phase shifts during the first half of the night and the "on" transitions determine phase shifts during the second half of the night

to form batches with one or the other of the two transitions in alignment. The longest pulses did not exceed 6 h since this is the longest duration that would not extend into both parts of the night. Fig. 2 illustrates the

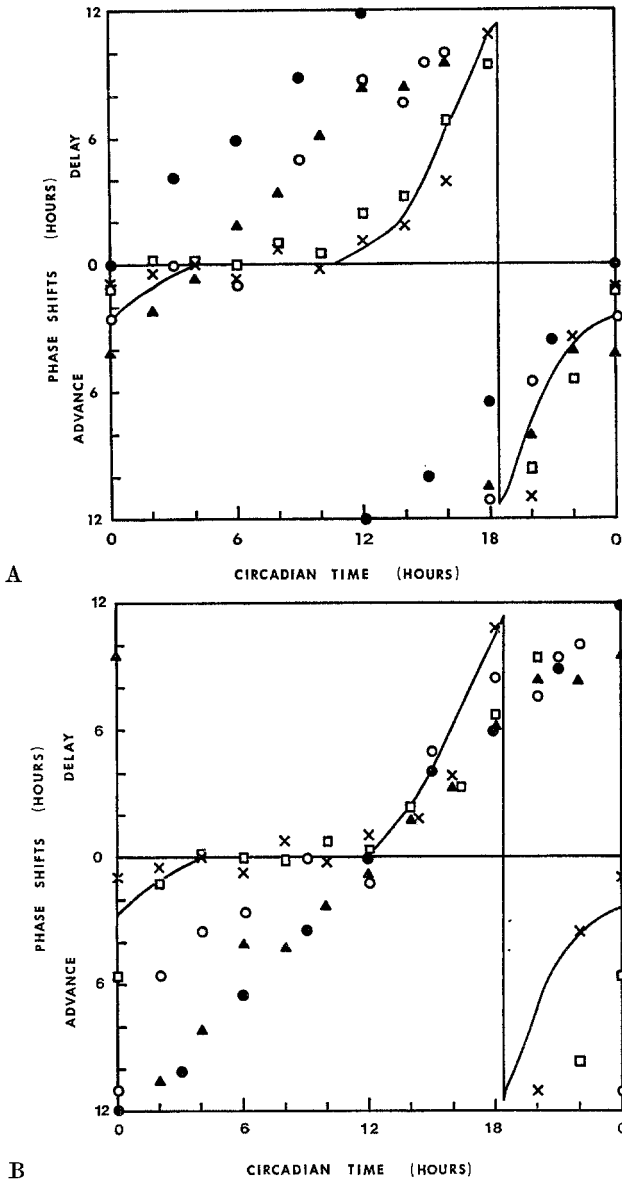


Fig. 3. Phase response curves for the *Drosophila pseudoobscura* eclosion rhythm for light pulses of 1000 lx and durations of 15 min, 30 min, 2 h, 6 h, 8 h, and 12 h. The smooth curve is the standard phase response curve for 15 min light pulses of ca. 1000 lx obtained by Pittendrigh and Minis (1964). ● 12 h; ▲ 8 h; ○ 6 h; □ 2 h; × 30 min light pulses. The phase shifts are plotted against the circadian hour at the "on" transition of the pulses in A and against the circadian hour of "off" transitions of the pulses in B. In A the phase shifts in the region of 19 CT to 01 CT roughly coincide and in B phase shifts in the region of 12 CT to 18 CT coincide regardless of pulse length (with the exception of 12 h pulses)

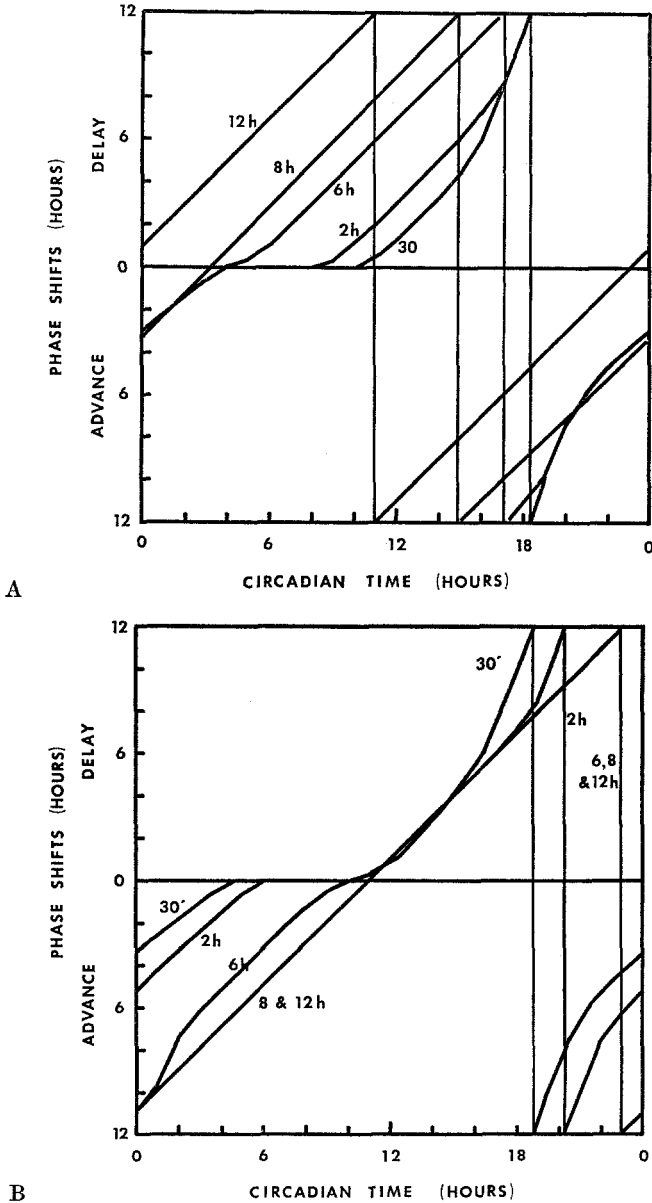


Fig. 4. Theoretically constructed phase response curves for 30 min, 2 h, 6 h, 8 h, and 12 h light pulses derived as explained in the text from the standard 15 min phase response curve. In A responses are plotted against the circadian hour at "on" transitions and in B against "off" transitions of light pulses. The theoretical curves in A and B of this figure are directly comparable to curves in Fig. 3A and B which are based on experimentally obtained data

design of the experiments and the temporal arrangement of the light pulses and presents details of the results obtained. The delay and advance phase shifts are roughly of the same magnitude regardless of the lengths of the pulses when "off" transitions are aligned in the first half of the night and "on" transitions are aligned in the second half of the night (Fig. 2a and c). This tendency is also obvious in other batches in which the "on" and "off" have been systematically staggered to scan the various hours of the two halves of the night (Fig. 2b and d).

Category 2. Experiments to obtain phase response curves for light pulses of about 1000 lx intensity and varying in duration from 15 min up to 12 h. The results of these experiments are presented in Fig. 3 in two arrangements. In Fig. 3A the phase shifts are plotted against the circadian hour of "on" of the pulses. In Fig. 3B the *same* phase shifts are plotted against the circadian hour of "off" of the pulses. It is evident in Fig. 3A that phase shifts in the region of 19 CT to 01 CT roughly coincide in magnitude independent of the pulse duration. Much in the same manner phase shifts in the region of 12 CT to 18 CT coincide as seen in Fig. 3B independent of the pulse-duration. The responses to 12 h light pulses are exceptions to this generalization.

The results may be briefly summarized as follows: When the "on" transition of pulses is used as reference time the phase shifts in the second half of the night (the advance region of the phase response curve) are of the same magnitude for light pulses varying from 15 min to 8 h duration. When the "off" transition is used as reference time in the first half of the night the phase shifts (the delay region of the phase response curve) are of the same magnitude for these pulses.

Pittendrigh and Minis (1964) have worked out the standard phase response curve for *Drosophila pseudoobscura* for 15 min light pulses of 100 ft.c., basing on which the results of several types of light pulse experiments could be predicted (Chandrashekar, 1967a, b; Pittendrigh, 1966; Minis, 1965). The results presented in this paper could be fitted in a general way into the Pittendrigh model as well. With the 15 min standard response curve as a starting point, the responses for longer pulses can be derived. The basic assumption is that, for example, the phase shifts due to a 30 min light pulse can be derived by using the 15 min phase response twice. The phase shift due to one 15 min pulse is calculated first, the CT at the end of the pulse determined. Then the calculation is repeated for the second 15 min pulse following immediately after the first one. The phase shift thus obtained is the phase shift caused by a 30 min pulse. (This method is inherent in the calculations made by Pittendrigh and Minis, 1964. For further details see Johnsson and Karlsson, report of Dept. Electrical Measurements, Lund Institute of Technology, in the press.) Phase response curves for pulses longer than 30 min

are derived analogously. Such theoretically derived curves for pulse lengths *actually used* in this study are presented in Fig. 4A and B. The curves are, therefore, directly comparable to the curves in Fig. 3A and B which illustrate experimentally obtained data. The similarities between the theoretically derived curves and those illustrating experimental data are striking.

Discussion

We have attempted to demonstrate that light pulses of varying duration up to 6 h may evoke responses of comparable magnitude during the subjective night of *Drosophila pseudoobscura* eclosion rhythm. Thus the responses could be plotted in such a manner that either the "on" or the "off" transition of a light pulse appears to determine the amount of phase shift depending on where during the subjective night it was administered. In doing so the transitions apparently mimic environmental "dusk" and "dawn" when pulses coincide with the first half and second half of the subjective night respectively (Chandrashekaran, 1967b).

As pointed out by Aschoff (1965) most workers in circadian rhythm research use the onset of the light pulse as the reference time in plotting response curves. Aschoff suggested that the midpoint of the phase shifting signals be considered as reference points to the exclusion of both the "on" and the "off" transitions. The procedure indeed smoothes out a few angularities in response curve data (Aschoff, 1965). All the same what is implicit in the mid-point approach is the fact that the circadian system registers both "on" and "off" events without doing which it could not *respond* to the midpoint of a signal. The question as to which transition in a strong light pulse, or if it is some other characteristic in it, that is the effective component in phase shifting cannot yet be tested experimentally for very short light pulses (for example of a few seconds duration). In long pulses these transitions may occur in widely different and even antagonistic phases. The responses to 12 h light pulses, however, fit our generalization only to a very limited extent. In this case the peaks appear predictably $n \times 24 + 15$ h after the "off" transitions of the pulses regardless of the *phase* at which this occurs. Pittendrigh (1966) has demonstrated that light pulses longer than 12 h always determined the new course of the rhythm by setting the oscillation in motion with the lights going "off".

We add another qualification to our interpretations of "on" and "off" determining new phase. The interpretations are valid only for the effect of 'strong pulses' (evoking phase response curves of type 0) and indeed if based on the data of Winfree (1970, a and b) our predictions would not work for weak pulses (evoking a phase response curve of type 1, in the terminology of Winfree).

The "on" response can be referred to a special feature of the *Drosophila* type of phase response curves. In the phase response curve of Pittendrigh and Minis (1964) for 15 min pulses for *Drosophila* there is an extended region rather unresponsive to light breaks (region of subjective day). In long light pulses a major portion would fall in this region thus allowing the "on" fraction to assume a dominant role. The responses of the circadian rhythm of the moth *Pectinophora gossypiella*, another organism discussed in literature from our standpoint of dawn and dusk phenomena (Adkinsson, 1965), significantly has also an unresponsive zone in the phase response curve (Minis, 1965).

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