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# Studies on Phase-Shifts in Endogenous Rhythms

## II. The Dual Effect of Light on the Entrainment of the Eclosion Rhythm in *Drosophila pseudoobscura* *

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*Summary.* Cultures of *Drosophila pseudoobscura* pupae raised in 12:12 hours L/D cycles were subjected to brief light pulses and light steps during early and late subjective night phases.

1. A light pulse and a light step during early subjective night evoke *dissimilar* responses, the pulse effecting a delay phase-shift and the step an advance phase-shift. But a pulse and a step in the latter part of the subjective night evoke a *similar* response from the system and advance the phase.
2. The results are explained by assuming a differential light sensitivity of the underlying system during the subjective night phase itself, with a phase-point of maximum light sensitivity.
3. It is postulated that the light "off" fraction of a pulse acts as a new "dusk" in the early subjective night and that the "on" fraction acts as a new "dawn" in the late subjective night.
4. The results of an experiment where a light pulse and a light step combined to form the light treatment bear out the assumptions made above and indicate that the photoinducible phase itself is not phase-locked to environmental time.

## Introduction

The ability of plants and animals to utilise the environmental parameters for information to control the course and timing of growth, development and reproduction is now well known. Among such of the environmental factors as are consulted, photoperiod is the most reliable. Literature regarding the role of the photoperiod in programming the course of biological processes is extensive (vide reviews in Bünning, 1963; Withrow, 1959; Danilevskii, 1965; and Lees, 1955, 1966).

Bünning suggested as early as 1936 that endodiurnal oscillations in the organisms are causally involved in and mediate photoperiodic phenomena. A vast array of subsequent experimental evidence has supported this view, although the details implicit in the evidence were not always clearly spelled out. Bünning has himself unambiguously set forth frequently the implications inherent in his hypothesis namely that the light-cycles phase the endodiurnal system on the one hand

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and reveal the photoperiodic responsiveness of the organism, on the other. Thus, Bünning states in a publication of 1946: "Der Phasenwechsel erfolgt zwar endogen, aber äußere Reize wirken doch stark regulierend. So kann durch kurzdauernde äußere Anstöße bestimmt werden, zu welchem Zeitpunkt die Phasen der inneren Rhythmik auftreten. Wirksame Reize sind dabei z.B. die Temperatur und namentlich das Licht. Außerdem wird durch den tagesperiodischen Wechsel von äußeren Faktoren, namentlich wieder durch den tagesperiodischen Licht-Dunkel-Wechsel, erreicht, daß die endogene Rhythmik genau die 24-Stunden-Periode einhält, von der sie bei konstanten Außenbedingungen etwas abweichen kann." Pittendrigh (1964, 1966) has further stressed this dual role of light in phasing the oscillation and effecting photoperiodic induction. Among the published results of recent experiments those of Went (1959), Withrow (1959), Bünsow (1959) and Hamner (1960) also elucidate this dual effect of light. It is then obvious that the 'photophil' and 'scotophil' half-cycles of Bünning representing the differential light sensitivity of the circadian oscillation would themselves undergo concurrent shifts when phase-shifts are effected in the underlying oscillation.

Owing to the extensive investigations of Pittendrigh and colleagues (vide references in Pittendrigh, 1966) the time course as well as the light sensitivity of the basic oscillation underlying the eclosion rhythm in *Drosophila* are well known. A simultaneous analysis of the circadian rhythm in this organism and its responses to light pulses and light steps appeared to be a feature of considerable interest. The experimental approach was to expose phase-points in the subjective night phase of pupal cultures in the entrained steady state to light steps. The phase-point of maximum sensitivity and response to light could thus be ascertained. It was further investigated if a solitary light signal in the form of a light pulse could shift this point of maximum response in the steady state oscillation. The results obtained are in agreement with the concept of endodiurnal oscillations participating in time measurement and in programming the temporal sequence of development and growth.

## Experiments and Results

Cultures of *Drosophila pseudoobscura* were raised at a temperature of 20° C from the egg stage onwards upto the pupal stage, in light/darkness (L/D) cycles of 12:12 hours. On the 20th day when the pupal populations were ready to eclose the clock-controlled light switches were turned off shortly after the onset of the last period of darkness (DD). This last onset of DD marked the beginning of the experiment i.e., '0' hr. The intensity of the light of the entraining regime as well as that of the pulses and steps was 3000 lux. The hours at which the light pulses and light steps were administered are expressed in numbers of hours after the onset of darkness and in the corresponding hours on the Subjective Circadian Time scale of Pittendrigh and Minis (1964).

In Fig. 1 are presented the data obtained from an experiment in which a population was exposed to a light pulse of 15′ duration, 27.5

hours after onset of DD i.e., 15.5 hr SCT (curve A). Another population (Fig. 1, curve B) was exposed at the *same* hour as that for the pulse in the above case, to a light *step*. The medians of the eclosion peaks in DD of the entrained control populations are given in the form of dashed vertical lines to facilitate estimation of phase-shifts effected in the oscillation by the light pulse in one case and the light step in the other.

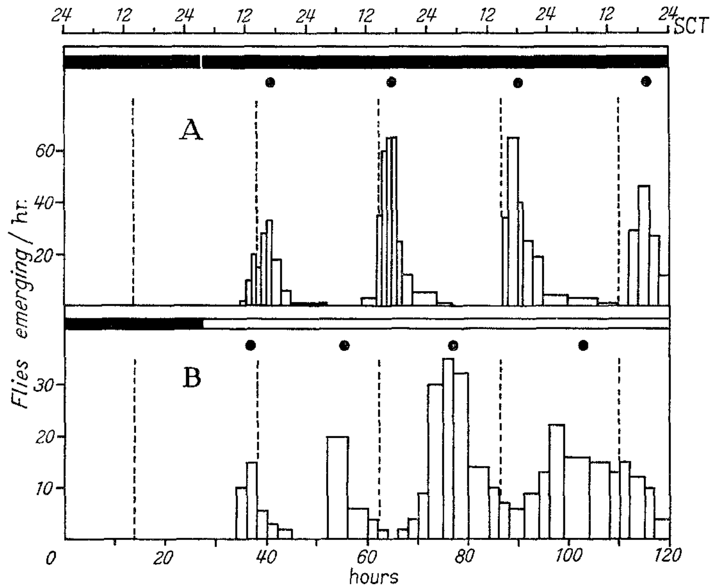


Fig. 1. The effect on *Drosophila* eclosion rhythm of a light pulse of 15′ duration and 3000 lux intensity (curve A) and a light step (curve B) offered in the early subjective night phase 27.5 hours after onset of DD or at 15.5 hr SCT. Solid circles: indicate the calculated medians of the eclosion peaks of the experimental population. Dashed vertical lines: approximately 24 hours apart designate the calculated medians of the eclosion peaks of the control population held in DD following prior entrainment to 12:12 hour L/D cycles. Entrainment of pupal populations and the experiments were performed at 20° C. Abscissa: Below: Hours after pupal populations were released into DD after previous entrainment by L/D cycles. Above: Time for the duration of the experiment given in Subjective Circadian Time-scale (after PITTENDRIGH and MINIS, 1964). Ordinate: Number of flies emerging out of pupal cases in an hour. The light regime during the experiment is indicated by the bars above each curve. Dark shaded portions depict DD. Duration of light interruption not shown to scale for the sake of clarity

It is evident from Fig. 1A that a light pulse falling 27.5 hours after onset of DD generates a delay phase-shift of 5 hours in the steady state, with intervening transient cycles. But a light step on the other hand, i.e., the light "on" without the light "off" component, given during the same phase as the pulse above, causes a distinct advance of 7 hours in the steady state with only one intervening transient (see Fig. 1, curve B). This apparently contradictory action of a light pulse and a light step at the same phase-point may be explained on the following assumption, schematically presented in Fig. 2. Here the original state oscillation in the entrained steady state is depicted in the form of the standard

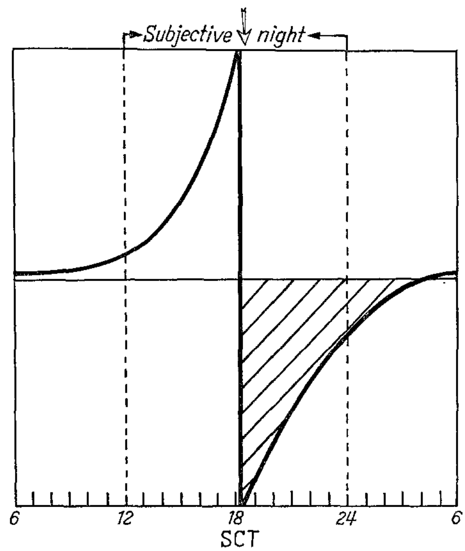


Fig. 2. Schematic diagram of the basic light sensitive oscillation in the entrained steady state in *Drosophila*. The time course and wave form of the oscillation have been depicted in the form of the standard response curve for *Drosophila* (after PITTENDRIGH and MINIS, 1964). The segment of the oscillation bound by the vertical dashed lines — 12.0 hr SCT to 24.0 hr SCT — denotes the subjective night phase. The phase point of maximal sensitivity and response to light is indicated by the arrow close to 19.0 hr SCT. This phase point itself is defined as a function of the preceding L/D regime or light treatment. Hatched area of the oscillation represents the late subjective night phase in the steady state

response curve of PITTENDRIGH and MINIS (1964). The period between 12.0 hr SCT and 24.0 hr SCT would then characterise the subjective night phase for the population. The results of the light step experiment are explainable if it could be presumed that only the latter half of the subjective night phase is sensitive to exposure to light. In effect it is an approximation to BÜNNING's 'scotophil' phase, with the difference that it does not extend over the span of a half cycle. In the step experiment, thus, the response would have first set in when light scanned the point of phase jump — subjective midnight — resulting in maximum response. Any brief exposure to light in the form of a pulse during the early subjective night phase would be taken for a "dusk" information by the population where only the "off" fraction of the light would be effective. This explains the delay phase-shift effected by the pulse falling in the early subjective night. According to the present assumption, the light sensitive latter half of the subjective night would react alike to a light pulse as to a light step.

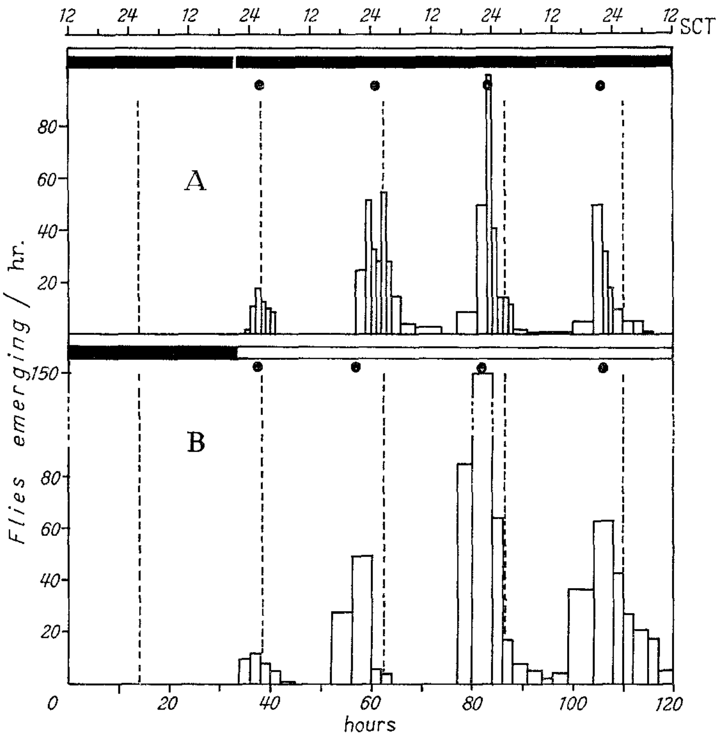


Fig. 3. The effect on *Drosophila* eclosion rhythm of a light pulse of 15′ duration and 3000 intensity (curve A) and of a light step (curve B) offered 33.5 hours after onset of DD or at 21.5 hr SCT. Light treatment in this experiment was administered in the late subjective night phase. Other details as in Fig. 1

The data of another experiment where the light pulse and the light step were given to two populations again at the *same* hour, but this time in the advancing phase of the oscillation which coincides with the latter half of the subjective night, are presented in Fig. 3 curves A and B. It may be seen that a light pulse and a light step evoke a similar response here as postulated above and advance the peaks by about the same number of hours.

In Fig. 4 are set forth the data obtained in an experiment where a brief light "off" information was coupled to a light step, which in effect is a combination of a light pulse and a light step. The addition of the light "off" information which lasted only 5 minutes, to the light step purported to verify the postulated effectiveness of the light "off" fraction of a pulse in the early subjective night phase. Hence the light treatment was offered 27.5 hours after onset of DD. The light treatment itself, as explained above, was a combination of the conditions illustrated

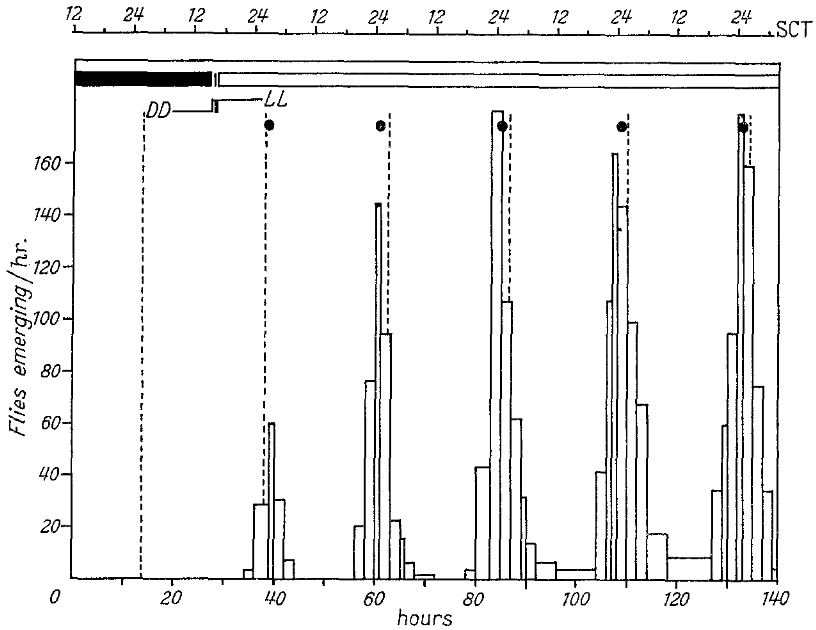


Fig. 4. The effect on *Drosophila* eclosion of a complex light treatment comprising of a pulse and a light step administered in the early subjective night phase at 15.5 hr SCT. Below the bar describing the illumination programme are details of the light treatment consisting of a light pulse of 15′ the "off" fraction of which lasted 5′ after which the light (3000 lux) was turned on. Other details as in Fig. 1

in Fig. 1, A and B. In the present case, as may be seen from Fig. 4, the subsequent peaks appear earlier than in the control. If the population had indeed reacted to the light pulse i.e., to the "off" information in it as presumed, the underlying oscillation would have been instantaneously phase delayed by 5 hours. Owing to this phase-shift of the basic oscillation to the right the light step would cross the altered phase of maximal sensitivity to light about 5 hours later than in the light step experiment described in Fig. 1, curve B. The pronounced advance generated here would counteract and overcome the delay effected by the pulse fraction in the light treatment and would result in a subdued advance phase-shift, as is indeed the case (Fig. 4).

## Discussion

The results of the experiments presented here clearly exemplify the dual effect of light signals and support BÜNNING's original view that endodiurnal oscillations participate in time measurement.

In the entrained steady state oscillation in *Drosophila* the midpoint of the subjective night phase, which is also the point of phase jump, responds most pronouncedly to exposure to light. But any prior interruption falling in the early subjective night phase, alters the time course of the basic oscillation and consequently the phase in the oscillation of maximal light sensitivity and response. Thus the phase of maximum response to light is itself not a feature of the system that is rigidly phase-locked to the environmental time. Thus PITTENDRIGH's (1964) plea that the 'scotophil' phase, or the location of the s-max after his explicit version of the BÜNNING model, be defined in terms of SCT time stresses the modifiability of the underlying oscillation by light and reaffirms BÜNNING's own views.

The results of experiments presented in Fig. 3B and Fig. 4 indicate that the pupae take any light interruption in the early subjective night phase for a new "dusk" and any in the later subjective night phase for a new "dawn". ADKISSON (1964) came to similar conclusions with his diapause studies on *Pectinophora gossypiella*. The events in the light step experiments are not strictly comparable to those in light pulse experiments owing to possible action of continuous light in the former case. Earlier experiments in this laboratory (unpublished) indicate that when a rhythm is induced in a population of *Drosophila* pupae reared in DD by a sudden transfer to LL, the periods of at least the first 3 cycles appear progressively shortened. But the similar effects evoked by a light pulse as by a light step in the latter half of the subjective night phase (Fig. 3) indicates that the basic assumptions made in this paper are valid.

The central point of interest, however, is the fact that a brief exposure of the underlying oscillation to light effectively shifts the light sensitive phase of it to a new phase position. The location of the "photoinducible phase" itself is the function of the entraining illumination regime or preceding light treatment. Thus, light phases the mode of the basic circadian oscillation and entrains the rhythmic system expressing itself in developmental processes.

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