Entrainment Properties of the Circadian Locomotor Activity Rhythm of the Field Mouse *Mus booduga* under Complete and Skeleton Photoperiodic Regimes

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ABSTRACT

Predictions for the phase angle differences ($\psi$) between the activity rhythm and the zeitgeber for different skeleton photoperiods based on the phase response curve (PRC) and the free-running period ($\tau$) of the field mouse *Mus booduga* were made. These predictions were based on two assumptions: (i) The PRC for light pulses of 1 h duration and ca 45 lx intensity should resemble the PRC for pulses of 15 min duration and 1000 lx intensity. (ii) One of the two light pulses (LP) constituting the skeleton photoperiod should always impinge upon that zone of the PRC which has a slope of $<-2$. Experiments were performed to compare $\psi$ under skeleton and complete photoperiods and also to test the assumptions made in predicting $\psi$. The results show that the basic oscillation underlying the activity rhythm of the field mouse *Mus booduga* undergoes a “phase-jump” when two brief light pulses (of 1 h duration) were used to mimic a photoperiod of 20 h. The $\psi$ values obtained for skeleton photoperiods closely match the predicted values. Under complete photoperiods, the experimentally obtained values match the predictions only up to 16 h. We conclude therefore that beyond this photoperiod, two discrete light pulses may not be sufficient to simulate the effect of a complete photoperiod.

Abstracting keywords: Circadian rhythms, entrainment, phase-relationship, phase-jump, skeleton photoperiod, transients.

INTRODUCTION

Entrainment of circadian rhythms to cycles of repeated light and darkness (LD) has been demonstrated to result in the natural periodicity of 24 h (Aschoff, 1965; Johnson, 1992). This is achieved by repeated discrete phase shifts of the assayed rhythm by environmental time cues (Pittendrigh & Daan, 1976) resulting in a fairly constant phase angle relationship between the entraining cycle and the entrained rhythm. However, entrainment under highly variable photoperiods entails a fine-tuning of the phase angle relationship between the rhythm and the zeitgeber which implies that the phase angle difference depends on the duration of...
the photoperiods (Daan & Aschoff, 1975). The results of studies conducted in temperate regions on diurnal chaffinches demonstrate that the duration of the activity phase (α) shows a remarkable adjustment to the duration of light (L) (Daan & Aschoff, 1975). This modification of phase angle magnitude and duration of activity may assume significance in the context of entrainment to the highly variable natural photoperiods in temperate regions. Under the relatively constant natural photoperiods of tropical regions, such a modification does not appear mandatory. Therefore, we made some predictions about the phase angle relationships under varying photoperiods in the tropics (although in the tropics not all these photoperiods actually occur) and tested them for the locomotor activity rhythm of the tropical field mouse *Mus booduga*.

Investigating the nature of the phase angle relationship in *Drosophila melanogaster* and four species of nocturnal rodents, Pittendrigh (1966) and Pittendrigh and Daan (1976) have demonstrated that it is possible to simulate the effect of complete photoperiods by two brief light pulses administered at the beginning and at the end of these photoperiods. Such simulation breaks down when extremely long photoperiods are used, resulting in a “phase-jump” of the rhythm (Pittendrigh & Daan, 1976). In making predictions about the phase angle relationship for *Mus booduga*, we postulated that in our experiments when the duration of the photoperiod exceeds 18 h, “phase-jump” should occur. Furthermore, these animals when subjected to shifts of ‘complete’ LD cycles exhibited a marked difference in the re-entrainment, i.e. when the LD cycles were advanced by 11 h and 12 h, re-entrainment ensued by means of delaying transients. Surprisingly, when these LD cycles were advanced by 13 h, re-entrainment ensued by means of advancing transients as opposed to the delaying transients occurring in response to the 11 and 12 h advance shifts of LD cycles (Geetha et al., 1996). This intriguing result can only be explained in terms of a bistability (Pittendrigh, 1966) of the underlying circadian oscillator resulting in a “phase-jump” of the activity rhythm of these animals. Therefore it was of interest to see whether our predictions would be borne out in terms of the phase angle relationships under entrainment effected with complete and skeleton photoperiods. We tried to predict \( \psi_{\text{on}} \) (for the onset of activity) for different photoperiods using the light pulse PRC of this species (Geetha et al., 1996), the species average \( \tau \) and the assumption that one of the two light pulses should fall in that zone of the PRC where the slope is < –2.

**MATERIALS AND METHODS**

Adult male mice *M. booduga* (n=15) captured from the fields surrounding the Madurai Kamaraj University campus (9°58′N lat.78° 10′E long.) were maintained under LD 12:12 h cycles for approximately two weeks and then introduced into
cages fitted with running wheels. An eccentrically placed magnet in the running wheel was used in the recording of wheel-running activity by making or breaking a reed-relay circuit, which in turn activated the writing stylets of an Esterline Angus Event recorder A 620 X. Daily activity/rest data strips were pasted chronologically one below the other and the resulting actograms were double plotted according to conventional methods. Food, consisting of millets and grains, and water were available ad libitum. Battery-powered torch lights with a combination of filters transmitting red light of wavelength > 610 nm (Viswanathan & Chandrashekaran, 1985) were used for short durations for feeding and other purposes.

Complete photoperiods
Animals \((n=6)\) were subjected to schedules of different photoperiods keeping \(T\) constant at 24 h. Different LD duration ratios were achieved by altering the relative durations of L and D by either a sudden and non-recurrent lengthening of L or of D. The various LD ratios administered were: 22:2, 20:4, 18:6, 16:8, 14:10, 12:12, 10:14, 8:16, 6:18, 4:20 and 2:22 h. Only the durations of L or D were altered whereas the intensity of L was kept constant at ca. 45 lx at cage level.

Skeleton photoperiods
Animals \((n=9)\) were entrained to 12:12 h complete photoperiods for about a month. Skeleton photoperiods of 12:12 h were achieved by restricting light in the L phase to 1 h at the onset of the L phase and 1 h at the end of the L phase and it was presumed that the complete photoperiod was thus being simulated. Subsequently, this 12:12 h ratio was varied by systematically shifting one of the brief light pulses by increments of 2 h. The various ratios of LD so obtained were 10:14, 8:16, 6:18, 4:20, 2:22, 20:4, 18:6, 16:8 and 14:10 h. Intensity of the light pulses was maintained at ca. 45 lx at cage level in all cases. \(\psi_{\text{onset}}\) and \(\psi_{\text{end}}\) values were computed as the time interval between lights off and onset of activity \((\psi_{\text{on}})\) and between lights on and end of activity \((\psi_{\text{off}})\).

Prediction of \(\psi_{\text{onset}}\) and “phase-jump”
We used the phase response curve (PRC) of *Mus booduga* (Geetha et al., 1996) to predict the \(\psi_{\text{on}}\) and the photoperiod at which an instability of the circadian system would appear and result in a “phase-jump” of the activity rhythm. The following assumptions were made to predict \(\psi_{\text{on}}\) under skeleton photoperiods of various durations:

(i) The PRC from light pulses of 1 h duration and 45 lx intensity should resemble that from 15 min duration and 1000 lx intensity.

(ii) One of the two light pulses (LP) constituting the skeleton photoperiod should always impinge upon that zone of the PRC which has a slope of \(< -2\).
Taking into consideration the average free-running period ($\tau$) of $23.83 \pm 0.32$ h, the PRC and the above-mentioned assumptions, probable $\psi_{on}$ values were computed for various skeleton photoperiods ranging from 2 to 20 h (Fig. 1).

All data points were plotted with error bars around the mean, representing 95% confidence intervals for visual hypothesis testing.

![Graph](image)

**Fig. 1.** $\psi_{on}$ plotted against duration of darkness. Filled squares denote the predicted $\psi_{on}$ values based on the PRC for all durations of darkness. Dotted lines connect observed $\psi_{on}$ values for skeleton photoperiods and straight lines connect observed $\psi_{on}$ values for complete photoperiods. Error bars represent 95% confidence intervals.
RESULTS

When animals were exposed to complete photoperiods, entrainment occurred with the activity being confined to the dark interval (Figs. 1 and 2). Under skeleton photoperiodic regimes, the activity was confined to the shorter dark intervals when the photoperiods were < 16 h. Beyond this photoperiod, the activity bout shifted to the longer dark intervals (Fig. 3).

Fig. 2. A typical double plotted actogram showing entrainment to complete photoperiods with different durations of L and D in a 24 h cycle. Open bars represent light and black areas denote darkness. The light intensity was ca 45 lx.
The duration of activity exhibited a steady increase when the duration of darkness was increased from 4 to 10 h under both complete and skeleton photoperiodic regimes (Fig. 4). Beyond a photoperiod of 10 h, $\alpha$ did not exhibit much lengthening corresponding to the duration of darkness. Generally $\alpha$ was longer under complete photoperiods than under skeleton photoperiods. There was a fixed phase relationship between lights off and the onset of activity under complete photoperiods irrespective of the duration of darkness. As a result, $\psi_{on}$ (which is close to 0) remained nearly constant throughout (Fig. 1). However, under skeleton photoperiods, the constant phase relationship was maintained only up to 12 h of darkness in that $\psi_{on}$ remained close to 0. Beyond this point, $\psi_{on}$ assumed more negative values (Fig. 1). The phase angle relationship between lights on and the end of activity ($\psi_{off}$) increased with the increase in the duration of darkness under both complete and skeleton photoperiods and became progressively more positive (Fig. 5).

Fig. 3. A typical double plotted actogram showing responses of the rhythm to skeleton photoperiods. Unlike entrainment to complete photoperiods, entrainment to skeleton photoperiods ensues by means of several transients. Note that the animal confines its activity to the longer dark phase when the duration of the photoperiod created by the skeleton pulses was 20 h indicating a “phase jump” in the underlying oscillation. Other details as in Fig. 2.
DISCUSSION

The observed $\psi_{on}$ for skeleton photoperiod, closely matches the predicted one, computed theoretically, for all durations of darkness, thus validating the assumptions made. The only exception was when the skeleton photoperiod contained 6 h of darkness or less which was also the predicted zone for “phase-jump” of the oscillator. Actual “phase-jump” however, occurred with 4 h of darkness in a skeleton photoperiodic regime. This phenomenon of “phase-jump” occurred in *Peromyscus leucopus* when the duration of darkness was reduced to 3.75 h (Pittrendrigh & Daan, 1976). The occurrence of this phenomenon is known to vary between individuals of the same species depending on their endogenous periodicity (Hastings et al., 1991; Sharma et al., 1997). The $\psi_{on}$ under complete photoperiods also matches closely the $\psi_{on}$ for skeleton photoperiods of less than 16 h. Beyond the region where “phase-jump” occurred under skeleton photoperiods, the simulation of complete photoperiods by two skeleton light pulses was no longer possible.

Fig. 4. Duration of activity ($\alpha$) plotted against the duration of darkness under complete and skeleton photoperiodic regimes. Dotted lines connect $\alpha$ values under skeleton photoperiodic regime and the solid lines connect values under complete photoperiods. Mean and 95% confidence intervals are given.
The $\psi_{\text{off}}$ values become more positive with increasing durations of darkness both under complete and skeleton photoperiods. A positive $\psi_{\text{off}}$ value reflects the restriction of activity in spite of the availability of longer durations of darkness. This trend is observed under both complete and skeleton photoperiods. The pattern of modulation of both $\psi_{\text{on}}$ and $\psi_{\text{off}}$ in the present case resembles modulations observed in chaffinches by Aschoff and Wever (1965). It may be noted that for $M. \text{booduga}$ in contrast to the rather rigid $\psi_{\text{on}}$, the $\psi_{\text{off}}$ values appear to be more variable. This probably reflects a stronger coupling between the onset of activity and lights off than that between the end of activity and lights on. It suggests that in nature, the onset of activity may be more directly under the control of circadian

Fig. 5. $\psi_{\text{off}}$ plotted against the durations of darkness under both skeleton and complete photoperiods. Details as in Fig. 4.
time keeping than the end of activity which may be dictated by environmental and exogenous factors (Chandrashekaran et al., 1983). It is interesting that the duration of activity, $\alpha$, does not show much dependence on the duration of darkness under both complete and skeleton photoperiods and that there is a remarkable resemblance in the pattern of $\alpha$ in the entrained state between complete and skeleton photoperiods with light durations below 16 h.

In this context, it is important to mention that in order to make more realistic predictions regarding the phase relationship between LD cycles and activity rhythms, inter-individual variations in $\tau$ and in PRCs ought to be considered (Sharma et al., 1997). The entrainment properties under skeleton as well as complete photoperiods seem to be similar for almost all the durations of light used, resulting in observed values matching the predicted values. This indicates that the mechanism of entrainment under both types of photoperiodic regimes may be similar since the predictions were made using the PRC and the average $\tau$ of the animals. The mismatch between the predicted and observed values under certain durations (> 16 h) of skeleton photoperiods might arise because two discrete light pulses may not be sufficient to simulate the effect of such extremely long complete photoperiods. We have further experimental evidence (Sharma, unpublished) that even in tropical regions (9° 58'N lat. 78° 10'E long.), where photoperiod varies only within a range of 1.53 h, complete natural photoperiods (LD cycles) seem to better conserve phase relationships than artificial LD 12:12 h cycles. This might imply that even in such southerly latitudes dawn and dusk twilight play an effective role in entrainment of circadian systems.

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REFERENCES


