

Relationship Between Period and Phase Angle Differences in *Mus booduga* Under Abrupt Versus Gradual Light-Dark Transitions

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Entrainment of the circadian rhythm of locomotor activity in the field mouse *Mus booduga* was studied under light-dark (LD) cycles in which the LD transitions were either abrupt ($n=18$) or gradual ($n=23$), mimicking “dawn” and “dusk” transitions. The phase-angle-difference (ψ) in both types of LD treatments and the free-running period (τ) in continuous darkness (DD) were computed for each individual. It was observed that the τ and ψ of these animals in LD regime with gradual light to dark or dark to light (LD) transition were significantly correlated ($r=-0.803$; $P<0.001$). On the other hand, τ and ψ of animals in LD regime with abrupt LD transitions did not show any significant correlation ($r=0.199$; $P=0.43$). The results of these experiments clearly indicate that a tight relationship between τ and ψ is exhibited in the regime with gradual LD transitions, whereas there is no such relationship in the regime with abrupt LD transitions. This difference may be due to stronger after-effects on τ of the LD regime with abrupt transitions as compared to the LD regime with gradual transitions.

Circadian pacemakers recognize the local time by assuming a constant phase-angle-relationship (ψ) with its zeitgeber [1]. In steady-state entrainment with the zeitgeber the driving

cycle and the driven rhythm should in principle maintain a rigid ψ [2]; the driven rhythm should lead in phase the driver if the free-running period (τ) of the driven rhythm is shorter than the period of the driver (T). Whether the zeitgeber consisted of one 15 min light pulse or a 1-h light pulse per 24 h, the ψ and τ were significantly correlated. Such relationships become weaker for longer or stronger (>1 h) light pulses [1].

Under natural LD conditions several factors that synchronize the biological rhythm probably remain active simultaneously. Nevertheless, change in light intensity seems to be the most significant among the synchronizers [3]. In nature the patterns of phasing in some organisms may be attributed to variations in both photoperiod and duration of twilight [4]. In many animals of both temperate and tropical regions “dawn” and “dusk” pulses probably act as synchronizers [5]. Both the qualitative and quantitative changes in light that occur during dawn and dusk have been implicated as important time cues for synchronizing animals [6]. However, in several mammals even chopped sinusoidal cycles of light intensity, without spectral shift, effectively entrain circadian rhythms [7]. Moreover, the ability of circadian rhythms to entrain to low amplitude sinusoidal cycles of light is

suggestive of relating two apparently distinct modes of entrainment: entrainment by discrete and entrainment by sinusoidal light pulses [8].

In day-active animals phasing of rhythms is conserved by the “parametric” action of light. Despite this, observations on circadian rhythms and their entrainment have been based mostly on data obtained from studies with rectangular LD cycles that exclude twilight or have only a very crude approximation to situations obtained in nature [9]. Annual records indicate that there are seasonally modulated changes in the circadian rhythms, these being especially pronounced during summer and winter [6]. Such seasonal modulation in τ can be the “after-effects” of entrainment to extremely “short” and “long” photoperiods during winter and summer respectively [1]. The role of the parametric effects of light do not seem to be clear in night-active animals which spend most of the daylight hours in light-excluding shelters, emerging at dusk and regaining shelter at dawn [8]. Studies with bats, flying squirrels, and other nocturnal animals have shown that these animals see very little light, in some cases none at all for some consecutive days [10, 11].

None of the studies conducted so far have focused on the inter-individual variation in the mechanism(s) and consequences of entrainment (measured in terms of τ and ψ). Results of earlier work carried out on the nocturnal field mouse *Mus booduga*, the animal used in the present study, suggest that there exists a considerable amount of inter-individual difference in τ and the shape of the phase-response-curve (PRC) [12]. Furthermore, we have also reported for this species a ψ -jump under extremely long skeleton photoperiods (ca. 18 h) [13, 14]. In this paper we report the difference in the relationship between τ and ψ in *M. booduga* under LD regimes with gradual versus abrupt LD transitions.

Male field mice *M. booduga* were captured from fields near the Madurai Kamaraj University campus ($9^{\circ}58'N$, $78^{\circ}10'E$). These animals ($n=61$) were divided into three groups. Animals of the first group ($n=23$) were individu-

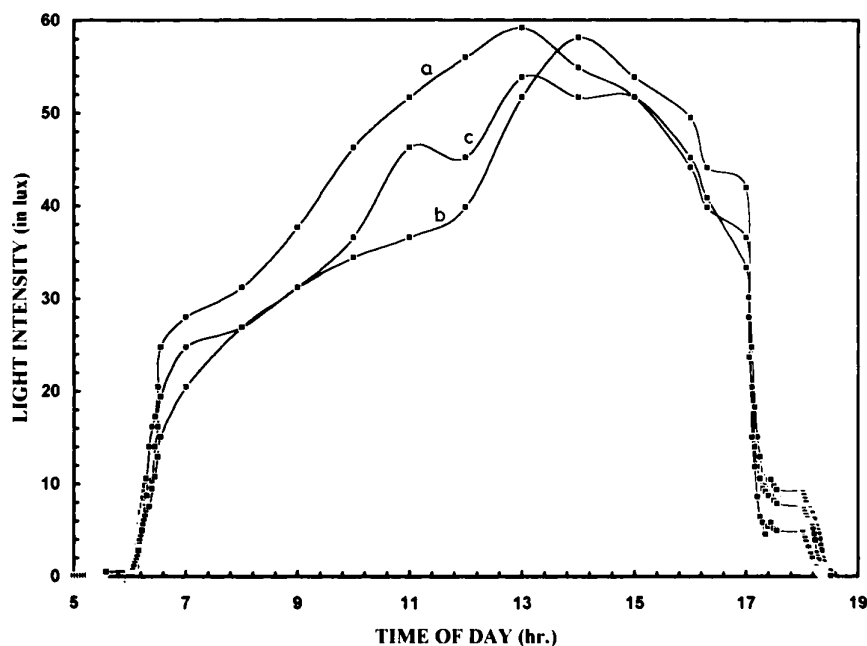


Fig. 1. The intensity profile of the light dark (LD) cycles with gradual light-dark transitions. The three different curves are the intensity records at the beginning of the experiment (a), at the middle (b), and after the experiments (c) were concluded

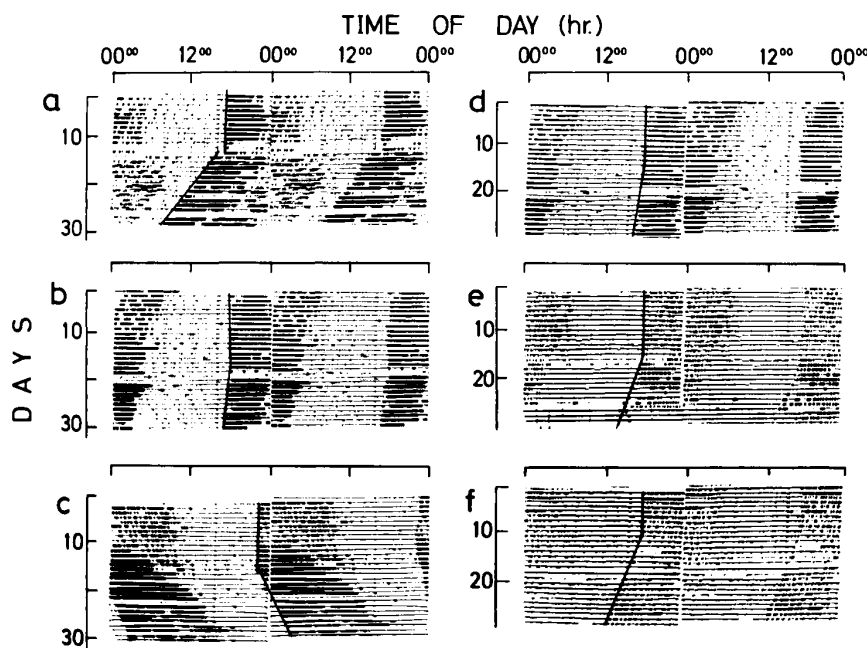


Fig. 2. The activity data of 6 out of 60 animals first entrained to gradual (a, b, c) or abrupt (d, e, f) light dark (LD) cycle and then released into constant darkness (DD)

ally introduced into running wheel in gradual LD cycles in a room with one window. Members of the second group ($n=18$) were introduced into running wheels in abrupt LD of

12:12 h. Members of the third group ($n=20$) were directly introduced into running wheels in constant darkness (DD). In performing these LD experiments (with both gradual and abrupt

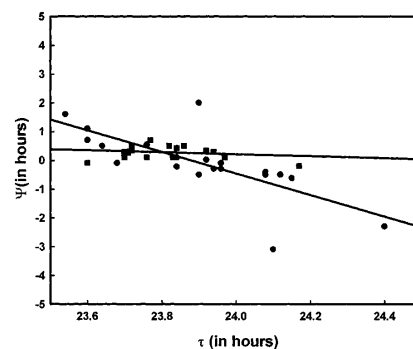


Fig. 3. The relationship between the phase angle difference (ψ) and the free-running period (τ) measured in light dark (LD) cycles with gradual light-dark transitions (●) and abrupt light-dark transitions (■)

regimes) care was taken that maximum light intensities in both cases were comparable (ca. 50 lux in both LD regimes). The locomotor activity of these animals was monitored using an activity running wheel (diameter approx. 20 cm) attached to a transparent plexiglas cage of dimension $0.07 \times 0.11 \times 0.09$ m, with a small opening of 0.02 m diameter. Reed relays attached to the wheels activated the writing stylets of an Esterline Angus A620X Event Recorder when the running mice caused revolutions of the wheel. The activity patterns of as many as 18 mice in separate running wheels, placed on open shelves in the experimental room, could be assayed concurrently. The temperature inside the experimental rooms ($3.05 \times 2.44 \times 4.01$ m), which did not have windows but were gently ventilated, remained more or less constant at $25 \pm 1^\circ\text{C}$ and the relative humidity was $75 \pm 5\%$. Food (millet and grain) and water were available ad libitum. The room was entered at irregular intervals, on an average once in 2 days for purposes of cleaning cages, placing food and water etc. Care was taken that the animals were not disturbed except for inevitable exercises which seldom lasted beyond 5–10 min. Red light of $\lambda > 640$ nm obtained with a combination of red and orange filters fitted to a battery-operated torchlight was used inside the cubicle. Actograms were obtained by pasting 24-h activity/rest strips chronologically one below the other in the standard manner and double plotted. The

onset of activity was used for computation of τ during free-runs. The ψ of the animals in LD is expressed as the difference in time between the activity onset and sunset (18:30 hours) in the zeitgeber time. The phase angle difference is taken as positive when the activity onset anticipated sunset, and negative when it followed sunset.

Light intensity inside the experimental room provided with daylight is shown in Fig. 1. Figure 2 illustrates in actograms ψ attained by 6 out of 61 animals in gradual and abrupt LD cycles, and the subsequent free-running period in DD (τ). The τ and ψ of the animals exposed to gradual LD cycle were negatively correlated ($r=-0.803$, $P<0.001$; Fig. 3). On the other hand, τ and ψ in abrupt LD conditions were not significantly correlated ($r=0.199$, $P=0.43$; Fig. 3). Moreover, the correlations observed under abrupt versus gradual LD regimes were significantly different ($Z=-2.65$, $P=0.004$) [15]. The ψ of the animals in gradual LD cycle ranged from 2 to -3.1 h (-0.12 ± 1.07 h) (mean \pm s.d.). In DD the same animals free-ran with τ ranging between 23.54 and 24.4 h (23.92 ± 0.22 h; Fig. 3). In the abrupt LD schedule the ψ of the animals had a relatively narrow range, falling between 0.7 and -0.2 h (0.28 ± 0.23 h), and these animals in DD free-ran with τ ranging between 23.7 and 24.17 h (23.81 ± 0.13 h; Fig. 3). Our results clearly show that there exists a strong negative correlation between τ and ψ under the gradual LD treatment, whereas under abrupt LD cycles τ and ψ are uncorrelated, indicating that the expected relationship between τ and ψ [1], which is preserved under gradual LD transitions, is ameliorated to the point of being undetectable statistically when the LD transitions are abrupt. This amelioration cannot be merely a consequence of reduced variation in τ under abrupt LD because the symmetry of the relationship between τ and ψ , and therefore a significant correlation between them, could be maintained even under reduced variation in τ . The amelioration of the correlation points to a qualitative alteration of the basic relationship between τ and ψ under the abrupt LD regime.

In a previous study involving sinusoidal LD cycle the lake chub *Couesius plumbeus* displayed significant systematic annual changes in τ , whereas animals entrained to rectangular LD cycles have shown no evidence of seasonal variation in τ [9]. The relative constancy in τ in artificial LD cycle indicates that the cues provided by seasonal changes in daylength (L or D) do not lead to annual variations in τ . These results suggest that changes in ψ arising from entrainment to variable LD cycles in nature are the major determinants of τ , with τ being correlated with ψ , daylength, and twilight duration [8, 9, 16]. In contrast, the responses to the "on" and "off" transitions in natural LD cycles were similar to the responses to sudden changes in light intensity as in rectangular LD cycles, by semifossorial rodents that show no natural seasonal after-effects on τ or no consistent seasonal relations between τ and ψ [17]. The above conflicting observations may be explained in terms of the environments experienced by these animals. Fish and other diurnally active vertebrates are normally exposed to continuously changing light conditions that include distinct dawns and dusks. These animals can show significant seasonal changes in τ , suggesting significant relations between τ and ψ [16, 18, 19].

It is clear from the results reported here that the nocturnal field mouse *M. booduga* apparently differentiated gradual from abrupt LD cycles. The relationship between τ and ψ predicted by the non-parametric model of entrainment [1] was better exhibited only by animals under the gradual LD regime. As a consequence of entrainment to gradual LD cycles the τ of animals subsequently examined in DD was negatively correlated to ψ during previous entrainment (Fig. 3). However, ψ under abrupt LD cycles exhibited no significant correlation with τ measured subsequently in DD (Fig. 3) although both sets of experiments were conducted simultaneously. The fact that some animals caught from the field and directly introduced into running wheel in DD, exhibited ranges of τ similar to the group of animals first entrained to gradual LD cycle and then introduced into DD sug-

gests that the situations in the field and in the laboratory under a gradual LD regime may be similar with regard to their effects upon the covariation in τ and ψ . The reason for uncorrelated τ and ψ in abrupt LD cycles may be the strong coupling resulting between the onset of darkness and the onset of activity [1]. Moreover, rectangular (abrupt) LD cycles that exclude twilight are only a crude approximation to the sinusoidal LD cycles seen in nature. It is of much physiological and functional significance that even in this low latitude, twilight durations at dawn and dusk, lasting barely 12–16 min, should play a role in conserving the relationship between τ and ψ .

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1. Pittendrigh CS, Daan S (1976) A functional analysis of circadian pacemakers in nocturnal rodents. IV. Entrainment: pacemaker as clock. *J Comp Physiol* 106:291
2. Moore-Ede MC, Sulzman FM, Fuller CA (1982) *The clocks that time us: physiology of the circadian timing system*. Harvard University Press, Cambridge
3. Cloudsley-Thompson JL (1973) Entrainment of the "circadian clock" in *Buthotus minax* (Scorpiones: Buthidae). *J Interdiscipl Cycle Res* 4:119
4. Aschoff J (1969) Phasenlage der Tagesperiodik in Abhängigkeit von Jahreszeit und Breitengrad. *Oecologia (Berlin)* 3:125
5. Helfman GS (1979) Twilight activities of yellow perch, *Perca flavescens*. *J Fish Res Board Can* 36:173
6. Kavaliers M (1978) The role of photoperiod and twilight in the control of locomotor rhythms in the lake chub, *Couesius plumbeus* (Agassiz). PhD thesis, University of Alberta, Edmonton, Alta
7. Swade RH, Pittendrigh CS (1967) Circadian locomotor rhythms of rodents in the arctic. *Am Nat* 101:431
8. Hastings JW, Rusak B, Boulos Z (1991) *Neural and integrative animal physiology*. Wiley-Liss, Amsterdam

9. Kavaliers M, Ross DM (1981) Twilight and day length affects the seasonality of entrainment and endogenous circadian rhythms in a fish, *Couesius plumbeus*. *Can J Zool* 59:1326
10. Joshi D, Chandrashekar MK (1985) Spectral sensitivity of the photoreceptors responsible for phase shifting the circadian rhythm of activity of the bat *Hipposideros speoris*. *J Comp Physiol* A156:189
11. De Coursey PJ (1986) Light-sampling and entrainment in flying squirrels. *J Comp Physiol* A159:161
12. Sharma VK (1996) Light-induced phase response curves of the circadian activity rhythm in individual field mice *Mus booduga*. *Chronobiol Int* 13:401
13. Sharma VK, Singaravel M, Chandrashekar MK, Subbaraj R (1997) Relationship between free-running period and minimum tolerable light pulse interval of skeleton photoperiods in field mice *Mus booduga*. *Chronobiol Int* 14:237
14. Sharma VK, Singaravel M, Chandrashekar MK (1997) Period-dependent oscillatory free-run in the locomotor activity rhythm of the field mouse *Mus booduga*. *Biol Rhythm Res* (in press)
15. Rao CR (1952) Advanced statistical methods in biometric research. Wiley & Sons, New York
16. Daan S, Aschoff J (1975) Circadian rhythms of locomotor activity in captive birds and mammals: their variations with season and latitude. *Oecologia* (Berlin) 18:269
17. Kenagy GJ (1978) Seasonality of endogenous circadian rhythms in a diurnal rodent *Ammospermophilus leucurus* and a nocturnal rodent *Dipodomys merriami*. *J Comp Physiol* 128:21
18. Pohl H (1980) Properties of the circadian clock of the redpoll *Carduelis flammea*. I. Stability of period and ranges on entrainment. *Physiol Zool* 52:186
19. Pohl H (1980) Properties of the circadian clock of the redpoll *Carduelis flammea*. II. Overt functions in relation to season. *Physiol Zool* 53:199