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Precision of a Mammalian Circadian Clock

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Abstract This paper reports study of day-to-day instability in the locomotor activity rhythm of the nocturnal field mouse *Mus booduga*. The free-running period (τ) of this rhythm was estimated in constant darkness in $n=347$ adult male mice. The "onset" and "offset" of locomotor activity rhythm were used as phase markers of the circadian clock. The precision of the onset of locomotor activity was observed to be a non-linear function of τ , with maximal precision at τ close to 24 h. The precision of the offset of locomotor activity was found to in-

crease with increasing τ . These results suggest that the homeostasis of τ is tighter when τ is close to 24 h.

A clock can be useful in measuring the passage of time only if its periodicity remains stable from cycle to cycle [1]. In biological clocks of circadian order, inherent instability sets forth a few challenges for the clock. Such challenges may arise due to extremely labile periodicities which fall beyond the range within which it can synchronize to external cycles (e.g. geophysical periodicity of 24 h). Such lability in the clock periodicity may also affect the most important feature of the biological clock, which is to recognize local time. Even if the lability

in the clock period is so limited that it lies within the range of entrainment, its phase relationship with the local time (ψ , the difference in time between an arbitrary phase of the environmental cycle and a phase of the rhythm) will not be stable. A given phase in the oscillation, appropriately occurring at a given time in the real day, would either phase-lead or phase-lag the target time as the oscillator's frequency varies [2]. In many species of mammals circadian rhythms operate with remarkable precision [1–3]. Although in some mammals the circadian period has been observed to undergo drastic changes during the early stages of development, it typically remains unaltered for the rest of the adult life [4, 5]. Pittendrigh and Daan [1] have reported species differences in period lability. Aschoff and coworkers [6, 7] have also reported that in birds and man the stability of a rhythm depends on its period. However, in these studies the different periods were obtained using light of various illuminations. The general rule which can be formulated from the results of these previous studies is that homeostasis is tighter when the clock period is closer to 24 h. Such a mechanism might re-

Dedicated to the memory of Prof. J. Aschoff

duce the hazards of instability in ψ , which the deviant periodicity entails. Notwithstanding the importance of the day-to-day fluctuation in the clock periodicity, there have been very few efforts to test the relationship between the clock's period and its stability. One of the studies [1] reported observations made on four species and others [6, 7] concern the variability of a modified rhythm. The numbers of animals used in both of the previous studies were low, and the results may also include after-effects of treatments, age-related modulations, etc. We report the relationship between the instability and the clock periodicity in a large number of nocturnal field mice, *Mus booduga*, with free-running period (τ) in constant darkness ranging between 21.9 h. and 24.6 h. Adult male mice ($n=347$) were captured from the fields surrounding the Madurai Kamaraj University campus (9°58' N, 78°10' E). The animals were directly introduced to cages equipped with running wheels. An eccentrically placed magnet in the running wheel facilitated the recording of wheel-running activity by completing/breaking the electrical reed-relay circuit which in turn was picked up by an Esterline Angus Event recorder A620X. Mice feed consisting of grains and water were available ad libitum. Battery-powered torch lights with a combination of filters transmitting red light of wavelength more than 640 nm, which is known to be safe, were used for feeding and other purposes for short intervals. The experimental animals were allowed to free-run under constant dark (DD) conditions in a light-tight, adequately ventilated experimental cubicle ($l \times b \times h = 3.05 \times 2.44 \times 4.27$ m). Temperature ($25 \pm 1^\circ\text{C}$) and humidity ($70 \pm 5\%$) in the cubicle were recorded with a thermohygrograph set-up (Wilh. Lambrecht, Göttingen, Germany) and were found to be nearly constant throughout the experiment. τ was calculated using the times of "onset" and "offset" of the locomotor activity rhythm in DD. The precision of the clock period is defined in this paper as the reciprocal of the standard deviation of τ . Figure 1 illustrates activity records of

six nocturnal field mice kept in DD. The free-running periods (τ) of $n=347$ field mice were estimated while maintaining the animals in DD. The average τ of these mice was found to be 23.36 ± 0.39 h. The precision of the onset and offset of locomotor activity rhythm (presumably reflecting the precision of the circadian clock underlying the locomotor activity rhythm) was plotted as a function of τ . The experimentally obtained data on the precision of onset and offset of the locomotor activity rhythm were then subjected to non-linear regression analysis. The precision of onset of locomotor activity was found to be a non-linear function of τ . The precision of the onset of locomotor activity was observed to increase with increasing τ , reaching a maximum at 23.80 h, and thereafter to decrease with increasing τ ($r^2=0.52$, $P<0.01$; Fig. 2). Although the offset of locomotor activity was less precise than the onset, a non-linear regression on the precision of the offset of locomotor activity shows that the precision increases with τ ($r^2=0.27$, $P<0.01$; Fig. 3). However, in the case of offset of locomotor activity a linear-regression gave an

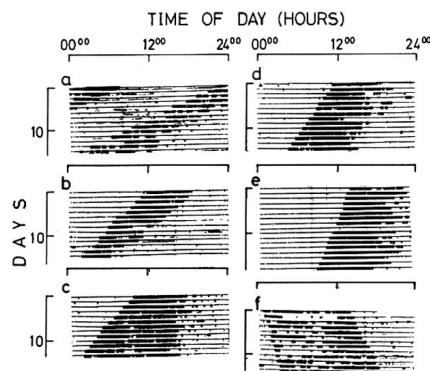


Fig. 1a-f. The activity data of 6 out of 347 animals recorded in constant darkness. The τ in DD were estimated for $n=347$ adult field mice *M. booduga*. The average τ of these mice was 23.36 ± 0.39 h. The τ in DD, taking the onset and offset of locomotor activity as phase marker, respectively, were: a) 22.4 ± 1.4 and 22.38 ± 3.2 h; b) 13.38 ± 0.43 h and 23.14 ± 2.09 h; c) 23.37 ± 0.27 h and 23.46 ± 2.7 h; d) 23.49 ± 0.14 h and 23.60 ± 1.06 h; e) 23.68 ± 0.19 h and 23.87 ± 1.06 h; f) 24.2 ± 0.16 h and 23.36 ± 0.34 h

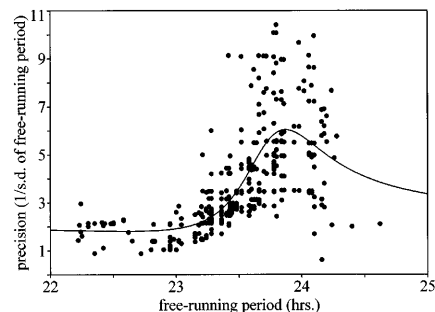


Fig. 2. The relationship between the precision (1/SD) and τ . A non-linear regression curve is drawn to fit the empirical data on precision of τ , calculated using the onset of locomotor activity as phase marker of the circadian clock. The precision of the onset of locomotor activity was first observed to increase with increasing τ , reaching a maxima at 23.80 h and then to decrease with τ ($r^2=0.52$, $P<0.01$)

equally good fit to the data as did the non-linear regression.

The homeostasis of the free-running period (Fig. 2) along with a unique shape of the phase response curve evoked by light [8, 9] could possibly explain the functional importance of the dependency of clock precision on the free-running period. The ψ reflects interaction of the free-running period and the periodicity of the external light/dark cycle through the light-induced phase response curve (with regions of maximum and minimal sensitivity). The instability in τ for values deviating from 24 h is compensated by large phase shifts, whereas stability in τ for values close to 24 h

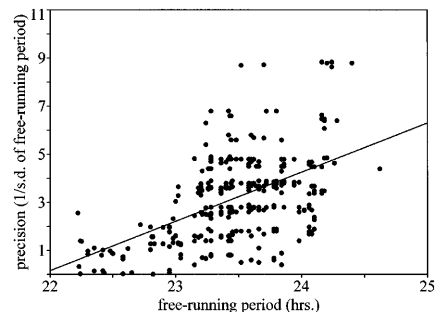


Fig. 3. Illustrates the relationship between the precision (1/SD) and τ . A non-linear regression curve has been drawn for the precision of τ , calculated using offset of locomotor activity as phase marker of the circadian clock. The precision of the offset of locomotor activity was observed to increase with increasing τ ($r^2=0.27$, $P<0.01$)

takes care of the instability in ψ arising due to minimal phase shifts during the subjective day [9]. Thus it can be concluded that the very circadian nature of τ itself has functional significance in the conservation of ψ [1]. It is as though selection pressure for the homeostasis of τ were more stringent when the resonance effect on ψ is involved and relaxed somewhat when τ departs away from 24 h [1, 10]. As a consequence of this, small fluctuations in τ when it is close to 24 h would require a larger adjustment in ψ whereas larger fluctuations in τ when it moves farther from 24 h would require only a slight adjustment to maintain a constant ψ . The results of our experiment suggest that the precision of the onset of locomotor activity is a non-linear function of τ , reaching maximum for τ close to 24 (Fig. 2). The precision of the offset of locomotor activity increased linearly with increasing τ (Fig. 3). This implies that for long τ the offset of activity is more precise than for the onset. Such dependence of precision on τ suggests that in the field mouse indi-

viduals having τ close to 24 h use dusk rather than dawn as their entraining stimulus while those with τ greater than 24 h use dawn rather than dusk.

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Free Energy Reduction by Molecular Interface Crossing: Novel Mechanism for the Transport of Material Across the Interface of Nanoscale Droplets Induced by Competing Intermolecular Forces for Application in Perfluorocarbon Blood Substitutes

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Abstract Perfluorocarbons (PFCs) are inert liquids which can dissolve – and release – approximately 50 times

more oxygen than blood plasma. Oxygen carriers based on PFCs are easy to produce, free of biological components, and more rigorously sterilizable than blood. PFCs injected

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into the body are eliminated by expiration through the lungs. Before reaching the lungs, PFCs accumulate in storage organs such as liver and spleen. In these organs nanoscale PFC droplets reduce their free energy by unifying to microscopic drops, thus indirectly lowering the rate of their expiration.

The model of free energy reduction by molecular interface crossing (FERMIC), a novel emulsion breaking mechanism derived from first principles as presented here, leads to a better understanding of the structure formation processes relevant in perfluorocarbons (PFCs) in vivo.

Especially for reasons of safety to abolish the risk of infecting persons through blood transfusions [1, 2] and because of regional blood shortages, there has been a concerted worldwide effort over the past 10 years to investigate the potential of PFC liquids for