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'Rigid' Internal Timing in the Circadian Rhythm of Flight Activity in a Tropical Bat

R. Subbaraj and M.K. Chandrashekaran

School of Biological Sciences, Madurai University, Madurai–625021, India

Summary. The timing of the emergence activity of a colony of the bat *Taphozous melanopogon* occupying a cave environment in Madurai (lat. 9°58′ N, long. 78°10′ E) and consisting of approximately 150–180 animals of both sexes was studied at 10-day intervals for one year. Even though the time of sunset during the year varied over a range of 41 min, the bats displayed an astonishingly 'rigid' time of emergence that was restricted to a narrow 'gate' of 16 min from 18:25 to 18:41 h. The timing of the termination of the activity was less precise in all seasons. Light/dark cycles are clearly the *Zeitgeber*. Even so, the bats began flying when it was very dark (0.1 lx) during shorter days, and they flew out as the sun was disappearing in the evening horizon (50 or more lx) during long days. Evidently there is no invariant, 'fixed' lower threshold intensity that uniformly elicits the onset of activity throughout the year as reported earlier by other authors (Aschoff, 1969; Erkert, 1974). It is proposed that the bats undergo a systematic seasonal shift in the threshold sensitivity to light in response to the photic *Zeitgeber* of the environment. The result is a remarkable precision in terms of the hours of the civil/calendar day in the emergence of *Taphozous melanopogon*.

1. Introduction

The bodily functions of organisms display rhythmic variations often synchronized by the natural alternation of day and night (Bünning, 1973). Thus, for instance, the circadian organization underlying locomotion in animals results from the interaction of an endogenous rhythm with the external light/darkness cycle, which is the most reliable environmental parameter. The daily activity of bats has been shown to be closely attuned to some form of endogenous rhythm by Griffin and Welsh (1937), Rawson (1960), Menaker (1961), DeCoursey and DeCoursey (1964), and Erkert (1970). In nature the daily emergence behavior of bats from their roost closely corresponds to the time of sunset,

Table 1

Latitude	Difference between longest and shortest days	Ratio between difference and shortest day	Daily increment in day length
Madurai 9°58′N	1.53 h	0.13	0.29 min

which offers itself as an arbitrary phase reference point. A number of external variables such as precipitation, wind, and temperature may directly influence the emergence behavior.

During the seasons, the light/dark cycles undergo systematic changes. Following the seasonal changes in the length of the day, changes occur in the timing of the emergence of bats. In recent years many investigators (Erkinaro, 1972; Daan and Aschoff, 1975; Kenagy, 1976; Laufens, 1969, 1973; Voûte et al., 1974) have made field studies, all in temperate regions, on the timing of various activities in birds, bats, and other small mammals. We observed that in the tropics there are noticeable differences in the activity phases of the endogenous rhythm of bats in natural conditions. It is often thought that day length is practically invariant in the regions close to the equator. The day length, however, varies by about 1 h 32 min (Table 1) over the seasons of the year at Madurai (lat. 9°58′ N, long. 78°10′ E). The aim of this paper is to present some results of field observations and laboratory experiments for evaluating the timing of emergence behavior of a tropical, cave-dwelling bat, *Taphozous melanopogon,* in relation to the almost imperceptibly changing photoperiodic environment in the tropics.

2. Methods

Studies were conducted at Keela Kuyil Kudi caves in the vicinity of the campus of Madurai University occupied by a colony of approximately 150–180 animals of both sexes and mixed ages of the insectivorous species *Taphozous melanopogon.* These bats clung in clusters of 6–8 in the inner recesses of crevices of tall boulders leading to dark, damp caves, some nooks of which were almost inaccessible to humans. Older males and females often chose regions closer to the entrance and were visible for observation during the daytime and were often even exposed to sunlight. Such animals were always solitary. Further features of the clusters such as sex composition, age distribution, heirarchical order, and even numbers could not be studied in detail in the absence of infrared viewers.

Visual observations of onset and termination of activity at the mouth of the cave were made at intervals of 10 days from January 1975 to January 1976. The Keela Kuyil Kudi cave site chosen for the present study faces westward and is perched on the southern slopes of a massive granite rock hill with an east–west axis. The bat counts could be made only by sitting or lying back against the rocky flooring of the caves and looking upward and forward where the bats contrasted against the dimly lit sky and horizon. In any other position it was difficult to count them against the dark backdrop of the rocks. Sunrise and sunset times were obtained from tables published by the Regional Meteorological Centre, Calcutta and were adjusted for longitude and latitude. Phase angle differences $\Psi_{\text{-onset}}$ were calculated from time of sunset to time of onset of emergence activity and $\Psi_{\text{-end}}$ from the time of returning to the roost until the time of sunrise. The temperatures plotted in Figure 1 were measured at the cave mouth and were *always* 8° to

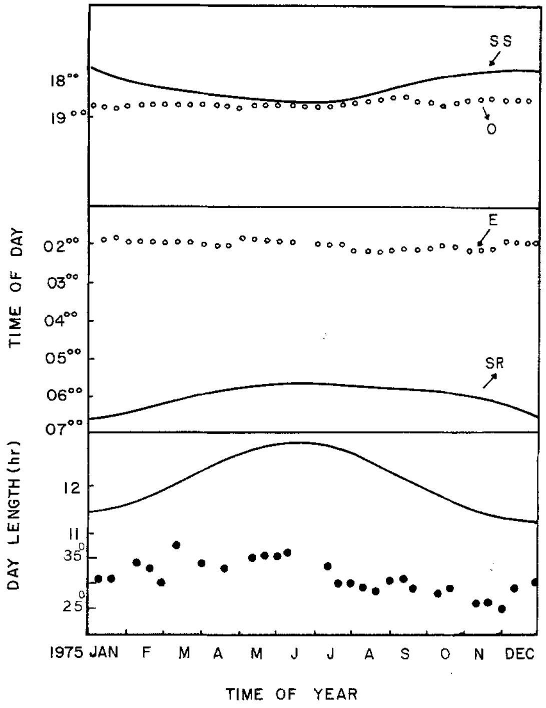

Fig. 1. Field data on time of onset (*O*) and end (*E*) of flight activity of a population of the cave-dwelling species of tropical bat *Taphozous melanopogon*. *SR* and *SS* denote sunrise and sunset. The lower panel depicts variations in day length at Madurai, India (lat. 9°58′N long 78°10′E). *Ordinate:* hour of day for upper panel and number of hours of light in lower panel. *Abscissa:* months. Temperature (●) at the cave mouth at hours of onset of flight activity given in lower panel. Observations made at 10-day intervals from January 1975 to January 1976

9° C higher than deeper into the damp cave. Thermohygrographic recordings have been made for the interior of the caves for a few months of the year and these reveal an amazing constancy within the course of 1 day. Light intensity was measured with a luxmeter (AEG). The lowest level that could be directly and reliably measured was 0.1 lx.

Wooden tilting cages 34 cm × 17 cm × 20 cm poised on knife edges in grooves of appropriate size were used to measure the locomotor (flight) activity of the bats in the laboratory. Slight ambulatory movements tilted the cages, and these tilts were recorded on slow-moving kymograph drums by stylets fitted with ink-writing tips. The dimensions of the cages did not permit actual flight. Conditions of incarceration did not seem to have any effect on the levels of the activity patterns and on the general well-being of the bats. The continuous dim light of 5 lx used in the laboratory experiments came from an incandescent bulb.

3. Results

Voûte et al. (1974) and DeCoursey and DeCoursey (1964) observed that the members of their colonies always crowded together at the entrance to their roost shortly before they set out on their foraging flight. Such 'light-sampling' behavior was not apparent in *Taphozous* even though individuals might have flipped about or moved around on all four limbs within the darker recesses of their cave.

The time of the first flyer from the crevices can be generalized as the index of the time of onset of activity of the colony since in many cases the first flyer is invariably followed by hordes of bats within an average of 2 min (see also Neuweiler, 1969). Interestingly the first flyer was always a solitary

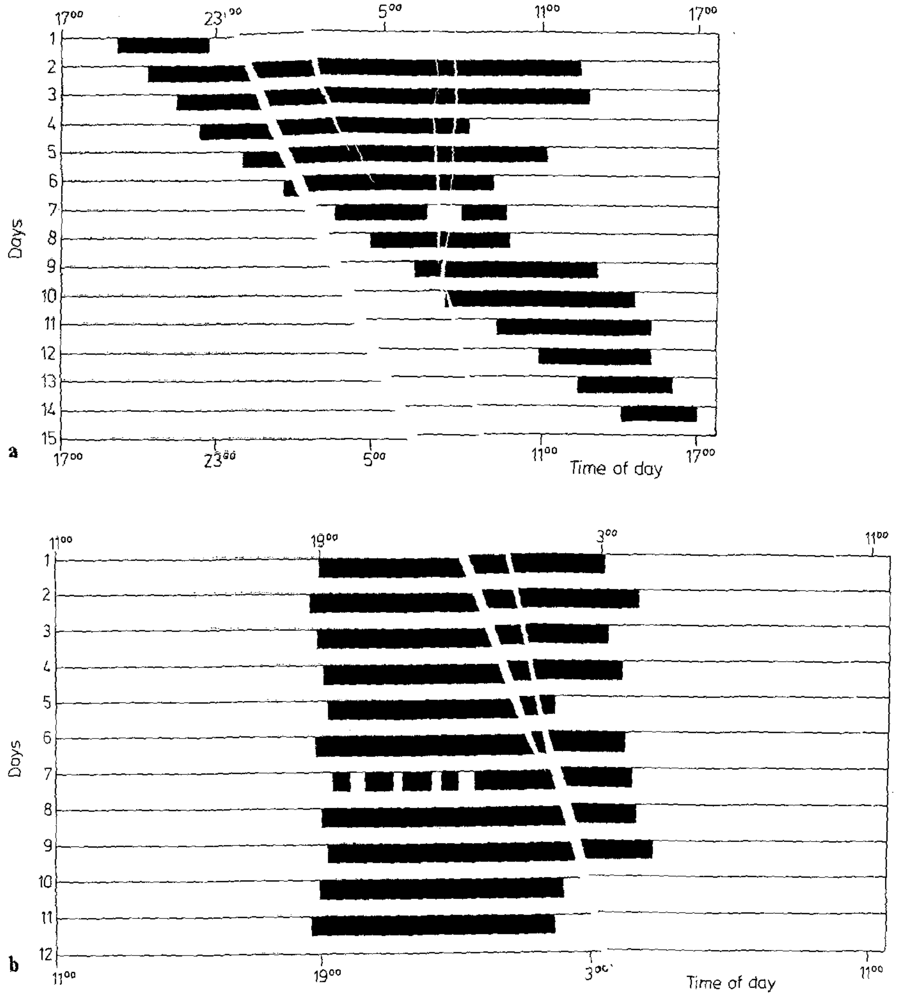

Fig. 2. a Free running circadian rhythmicity in the locomotor activity of a solitary male of *Taphozous* maintained under constant LL of 5 lx and 29 ± 1° C. Thick bands indicate sustained locomotor activity. Thin lines indicate torpor and inactivity. **b** Entrained circadian rhythmicity in locomotor activity of a solitary male *Taphozous* under natural L/D conditions. 29 ± 1° C. *Ordinate:* Number of days. *Abscissa:* Hour of calendar day

bat. It is not known if the first flyer is the same animal every day. The rest of the colony then fly out in bursts of 6–8 animals, each burst apparently representing members of a cluster. Within 15–18 min the entire estimated population of 150–180 bats would have left their roost. Bats, being social mammals, apparently influence one another in synchronizing the phases of their activity and rest. Arousal precedes actual onset of the foraging flight by 1 to 2 h. Audible vocalizations characterize arousal in the innermost reaches of the cave. The onset of the foraging flight of the first flyer under field conditions has been plotted against the months of the year in Figure 1. Each of the 2 sets of 36 dots represents *single* observations on the 'onset' and 'termination' of activity respectively of the activity of the bat colony, made at 10-day intervals for an entire year. Even though the time of sunset varied over a range of 41 min during the year (Table 1), the first flyers showed an astonishingly 'constant' time of emergence. The distribution of time of emergence indicates no parallel seasonal shift with the time of sunset and is restricted within a narrow *gate* of 16 min between 18:25 and 18:41 throughout the year. The end of activity was also precise in all seasons. Yet even $\Psi_{\text{-end}}$ (the relationship between end of activity and sunrise) was not consistent, i.e., even returning-to-the-roost timing did not keep pace with the seasonal progressions in sunrise.

Laboratory experiments revealed, however, that the otherwise rigid 24-h rhythm is 'freed' when recorded in constant dim light (5 lx) with a period of 25.3 h (Fig. 2a). Similarly, the original 24-h constancy of the rhythm is re-established by exposure to light/dark cycles (whether artificial or natural) (Fig. 2b). The '*free run*' establishes the circadian and endogenous origins of the rhythm and its re-establishment in response to light/dark cycles clearly indicates that light is the principal *Zeitgeber* in the laboratory and possibly in nature as well. According to Aschoff et al. (1972) the best way to measure the phase relation in nocturnal animals is to compare the midpoint of activity with the midpoint of dark time. We chose the onset as the reference phase since this component, especially under conditions of confinement in activity cages, was more precise and predictable than the end and hence the midpoint.

Foraging activity was markedly modified by precipitation but the general level of activity (arousal and preforaging flight) occurring in the crevices close to the time of sunset was not inhibited. During spells of rain sustained flight activity was noticed inside the crevices. Cloud cover and wind speed, factors which do not fluctuate much in Madurai, inhibited the activity of the species to a lesser degree.

4. Discussion

The precision of the *entrained* rhythm of emergence from the roost and return after foraging in animals is expressed by the day-to-day variations in their timings, paralleling variations resulting from *Zeitgeber* progression. The time of sunrise and sunset influences the daily onset and end of activity. The legitimate question arises, why should light/darkness be implicated as the *Zeitgeber* for animals that seek darkness during hours of daylight and venture forth only when darkness sets in. The animals live according to a self-imposed schedule, in the dark (or very dim light) both day and night.

In principle a previously synchronized circadian clock can keep the activity/rest phases of *Taphozous* geared to the day/night periodicity of the outside environment. However, our laboratory experiments show that the flight activity is no longer controlled and drifts in constant dim light. In such cases a *Zeitgeber* to 'gate' and regulate activity becomes essential. The two most likely and universal *Zeitgebers* are light and temperature (Bünning, 1973). It is clear from Figure 1 that activity patterns bear *no* resemblance to patterns of temperature. Moreover, temperature is as unreliable a cue in the tropics as it is in temperate regions. In view of these facts and of our positive demonstration that light/dark cycles *entrain* the rhythm, we conclude that light is the *Zeitgeber*. There are possibly cyclic variations in the ambient light intensity strong enough to *entrain* the rhythm, or *alternately*, the animals venture to the mouth of the Cave, where their circadian clock is 'reset' de novo daily. *Entrainment* ensues by means of phase shifts. Any bat attempting flight well before the time when light intensity outside the cave is high experiences a 'delay phase shift' in the onset of activity on the same and subsequent days. Similarly, higher intensities of light experienced for even a few minutes early in the morning cause 'advance phase shifts.' This appears to be precisely the situation, judging from a 15-min (white) light pulse 'phase response curve' we have worked out for *Taphozous melanopogon* (Subbaraj and Chandrashekaran, unpublished). Light shocks during the (subjective) day cause delays. Light shocks during the (subjective) night cause advances.

Neuweiler (1969) reported the activity patterns of a colony of 800–1000 fruit-eating and tree-living bats, *Pteropus giganteus giganteus*, in Madras, India and noted that these free-living bats also abandoned their trees with great precision in timing. On a particular day Neuweiler noted the following temporal phases: the first bats leave at 18:34 h and the whole colony noisily vacates the tree by 18:45 h in a matter of 11 min. At 18:05 h, barely 40 min before the departure of the last bats, the entire colony is still asleep. Kenagy (1976) conducted extensive studies on the periodicity of the daily activity and its seasonal changes in free and captive kangaroo rats of *Dipodomys* spp., which occur between 10° and 51° N latitude. There was great precision and synchrony in the emergence of these rats from their burrows at relatively high light intensities of 200–2000 lx. Kenagy's rats were solitary within their burrows and hence their synchronized emergence could not have been socially contrived. Kenagy suggests that an 'endogenous state of an already synchronized animal may provide a sufficient cue for the onset of activity.' The seasonal changes in the timing of emergence, which parallel the progression of sunset, also implicate light/darkness as a *Zeitgeber*. Aschoff and v. Holst (1960) demonstrated spectacular changes in the timing of the onset and termination of the flight activity in the jackdaw *Coleus monedula* L., which closely paralleled changes in the sunrise and sunset over the period of a year at 49°20′N. Erkert (1974), in his studies on the effect of moonlight on nocturnal mammals, carried out under natural conditions near the equator in Columbia (4°35′N 74°27′W), concludes that activity of African fruit bats living in the open (*Rousettus aepyptiacus*) started 30–60 min after sunset when light intensity varied between starlight to moonlight intensities depending on the phases of the moon. Evidently a *fixed* lower threshold triggers onset of flight. In the cave-dwelling insectivore *Taphozous melanopogon* there is no such fixed lower threshold or seasonal variations in the onset and end of activity with variations in time of sunset and sunrise over the seasons. Consequently, $\Psi_{\text{-onset}}$, $\Psi_{\text{-mid-point}}$, and $\Psi_{\text{-end}}$ undergo marked seasonal variations indicating seasonally varying phase angle features.

Nocturnal phasing of onset of flight ranges from +4 min (before sunset) to −39 min (after sunset) reflecting (Fig. 1) changes in the time of sunset relative to the constancy of the bat activity. Aschoff (1969) proposed that the light-to-dark ratio and the duration of twilight determine the phase(s) of the activity rhythm in *entrainment*. Both parameters vary within very narrow limits in Madurai. Twilight transitions are rapid and last less than 10 min when light intensity drops sharply from 50 lx to 0.1 lx and lower. The important differences in the responses of tropical *Taphozous* are the absence of a 'fixed' lower threshold of activity-releasing light intensity demonstrated in the work of Aschoff (1969) and Erkert (1974) and the 'rigidity' in the timing of daily onset of flight. As a result the bats began flying when it was dark on the shorter days of winter (0.1 lx) and flew even as the sun was visible on the horizon during the longer days of summer (50 lx). Laufens (1969, 1973) found a similar seasonal change in the threshold of light intensity that triggered flight activity in Natterer' bat *Myotis nattereri*. This species, studied in West Germany, varied in size of population from 2 to 30, changed their quarters (man-made shelters with unobtrusive activity recorders) on the average of nearly once a week. Only females were observed by Laufens (1973).

In view of the gradualness of the change in *Zeitgeber* conditions, precision in the activity is not a disadvantage in tropical bats. Other important modifying factors that exercise selective pressure are the presence and activity patterns of birds of prey. The Keela Kuyil Kudi cave region is frequented by eagles, kites, and vultures during hours of daylight.

The precise timing in the flight activity of *Taphozous* reported here apparently arises from a seasonally adjusted threshold to triggering light intensities. Such variations in sensitivity have been reported for *Myotis nattereri* by Laufens (1973) and may well be a more wide-spread phenomenon than suspected. However, such seasonally varying sensitivity to light does not lead to a constancy in timing in animals inhabiting temperate regions, owing to the extreme variations in daylength. On the contrary, in the case of *Taphozous melanopogon* in Madurai, the seasonal compensatory changes in threshold sensitivity inevitably result in an activity pattern reflecting 'rigid' internal timing relative to the hour of the calendar day.

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