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Communication and synchronization of circadian rhythms in insectivorous bats

G MARIMUTHU and M K CHANDRASHEKARAN

Department of Animal Behaviour, School of Biological Sciences, Madurai Kamaraj University, Madurai 625 021, India

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Abstract. There is communication and social synchronization of the circadian rhythm in the flight activity of the microchiropteran cave-dwelling bat *Hipposideros speoris*. Thus captive bats surrounded by free-flying conspecifics synchronize their activity to the colony activity. The circadian rhythm of a solitary bat in a solitary cave freeruns. Even the rhythm of an 'alien' bat (*Taphozous nudiventris kachhensis*) held captive in the hipposiderid bat cave freeruns. But the rhythms of a closely-related species, *Hipposideros fulvus* partially entrain to social cues from *Hipposideros speoris*. Social synchronization of circadian rhythms in bats may be species-specific. This synchronization is abolished when continuous light of 10–20 lux is shone inside the natural cave.

Keywords. Circadian rhythms; bats; ultrasonics; communication; synchronization.

1. Introduction

Circadian rhythms, in nature, entrain to the light-dark cycles (LD cycles) generated by sunrise/sunset. If the organisms displaying such entrained 24 hr rhythms are brought into constant conditions of continuous light (LL) or constant darkness (DD) and invariant temperature of laboratories they 'freerun' (Moore-Ede *et al* 1982). Freerunning circadian rhythms have also occasionally been observed to be induced by the LL of the arctic summer and DD of the arctic winter (Swade and Pittendrigh 1967; Mueller 1968; Erkinaro 1969). LD cycles are clearly the most universal and dominant of all zeitgebers (synchronizers). It is now becoming clear, however, that there may be zeitgebers other than LD cycles. Cyclic variations in temperature (Hoffmann 1968), cyclic availability of food (Aschoff 1981), noise-silence cycles (Sulzman *et al* 1977), the state of the tides in the oceans and even the phases of the moon may act as zeitgebers (Neumann 1981). Apart from these abiotic fluctuating factors there are also biotic factors such as bird songs (Gwinner 1966; Menaker and Eskin 1966), mother-pup interaction (Davis 1981; Viswanathan and Chandrashekaran 1984) and such other social stimuli that can synchronize circadian rhythms. In humans social zeitgebers are more effective than physical zeitgebers (Conroy and Mills 1970; Aschoff 1981; Minors and Waterhouse 1981). We have been investigating (Marimuthu *et al* 1978) how the members of a colony of microchiropteran bats inhabiting a true cave under DD and constant temperature and constant humidity conditions still time their activity. Such conditions normally as already explained release circadian rhythms into freeruns.

Abbreviations used: LD, Light-dark cycles; LL, Continuous light; DD, Constant darkness; τ, Period length (time between onset of activity from one day to the next); CF, constant frequency; FM, frequency modulation.

Experiments with trapped bats and flight activity monitoring inside a few such caves indicate that there is clear-cut evidence for social synchronization of the circadian rhythm in the bat *Hipposideros speoris* (Marimuthu *et al* 1978, 1981). Bats held captive some 40 m inside a cave in DD still began their nightly activity to coincide with the onset of the foraging activity of the colony.

We now have information that the circadian rhythm of *Hipposideros speoris* is indeed being synchronized by social communication and that such social communication is inexplicably operant in DD but not in LL.

2. Materials and methods

2.1 *Study site*

The site where the observations and experiments were carried out is a 'true cave' (cave 1), i.e. a cave with just one opening (Twente 1955), situated in a rock complex close to the Madurai Kamaraj University campus (lat. 9°58′ N, long. 78°10′ E). The cave has several labyrinthine ramifications 15–50 m from the cave mouth, which opens on the northern flank of the rock complex. The bats use several of these pockets as their day-time roosting place. We chose a site, ca. 40 m inside the cave, that showed great constancy of temperature (27°C ± 0·5°) and relative humidity of 95% (Lambrecht-Goettingen thermohygrographs) and was absolutely dark (no light measurable over periods of 1,000s even on the energy scala log scale of a United Detector Technology Optometer). A second cave (cave 2) was situated in the same rock complex but with the cave mouth on the southern side. The conditions obtained in cave 2 were very similar to the conditions in cave 1; temperature was constant at 30°C, relative humidity was 85% and darkness complete. Since the depth of cave 2 was only approximately 5 m an artificial mud wall had to be constructed and a black cloth curtain erected to ensure absolute darkness.

2.2 *Recording techniques*

Our behavioural observations and feeding of captive experimental bats were made using a noctovision apparatus with a far-red source of light projection and a viewing scope-screen. The bats did not respond to the switching on of the noctovision by either turning their heads towards the light source or flying away. We also used battery-powered torch lights with a combination of filters transmitting red light of > 610 nm, which seemed not to disturb the animals as much as white light.

Bats were captured on their return flight to the cave in the early hours of the morning before sunrise and placed in light aluminium-framed activity cages wrapped on all sides with synthetic gauze material. The dimension of the cubical cages (30 × 30 × 30 cm) permitted flapping flight. Bouts of flight jiggled the cages, which were suspended from the arms of a metal column held by retort stands. The movements of the cages were transferred directly to mechanically wound thermohygrograph drums with the aid of bamboo strip stylets fitted with felt writing tips. Observations and experiments were made with a minimum of disturbance to the bat population within this rather restrictive cave, in which the ceiling in its roomiest region was 1–1·5 m from the floor. The captive bats were hand-fed at irregular hours of day and night with minced cockroaches.

LL conditions were created by using incandescent bulbs and an automobile lead-acid battery. The incident light at the cage level varied between 1 and 20 lux. The battery was changed after 36–60 hr at random intervals for recharging, by replacing a second freshly charged battery. The caged bats could obviously hear orientation and/or communicative sounds from their unrestrained conspecifics.

3. Results

The hipposiderid bats awaken well before sunset. They then stretch, preen themselves and undertake short flights within the dark recesses. The bats then fly to a chamber proximal to the cave mouth. The bats fly around in this chamber 'sampling light' as other bats have been reported to do (Twente 1955; Voûte *et al* 1974). When it gets sufficiently dark outside after sunset they begin flying out to forage. Figure 1A, setting out the results of a 50-day experiment inside cave 1, indicates that 3 captive bats placed and experimented upon 40 m deep into the cave and surrounded by free-flying conspecifics still 'knew time'. They timed their flight within their confining cages, to the flight pattern of the colony. In further confirmation of the need for conspecific-communication, the results of another 50-day experiment with a solitary bat (in cave 2) with no conspecifics (figure 1B) reveal an impressive 'freerun'. No free-flying conspecifics, no social communication and no synchronization.

Interestingly even the rhythm of an 'alien' bat (*Taphozous nudiventris kachhensis*, an emballonurid species) held captive in the hipposiderid bat cave (cave 1) freeran. Figure 2 shows that even though the social cues emitted by the members of the colony of *H. speoris*, during their outflight and return before sunrise, were available to the captive emballonurid bat *T. n. kachhensis* the locomotor (flight) activity of the latter exhibited a spectacular freerunning rhythm with a τ shorter than 24 hr. But the rhythms of a closely related species *H. fulvus* partially entrain to social cues of *H. speoris*. Figure 3 illustrates how the flight activity rhythm of only one of three members of captive *H. fulvus* showed, synchronization to the colony activity of its sympatric species *H. speoris*.

To find out the effect of constant illumination on the social synchronization of the circadian rhythms we have recorded the flight activity of *H. speoris* inside cave 1 in LL. The circadian rhythms of *H. speoris* freeruns in LL in the laboratory. LL would tend in itself to induce a freerun and the social input would tend to entrain it. Figure 4 shows that under LL conditions of 1 lux two of the three captive bats still entrained to the social cues of their free-flying conspecifics, but the rhythm of the third bat freeran with a τ more than 24 hr. Since we suspected that the LL intensities reaching the bats might have been sub-threshold, the experiment was repeated with an increased light intensity of 10–20 lux. Figure 5 sets forth data for one bat. During the initial period of DD its daily activity rhythm coincided precisely with that of the free-flying conspecifics thus undergoing social entrainment. However, the same bat (and 3 other bats whose activity data are presented in figure 6) freeran in LL of 10–20 lux in spite of the social cues still available to them. The τ is longer than 24 hr in LL. LL seems to have abolished the social synchronization of the circadian rhythm in this bat. Bats in figures 6A, B exhibited in addition to freerunning rhythms, brief activity bouts for 28 days and 13 days, respectively, corresponding to onset of activity of the conspecifics. The bats flew inside activity cages for about 11–100 min just as the conspecifics flew out of the cave to

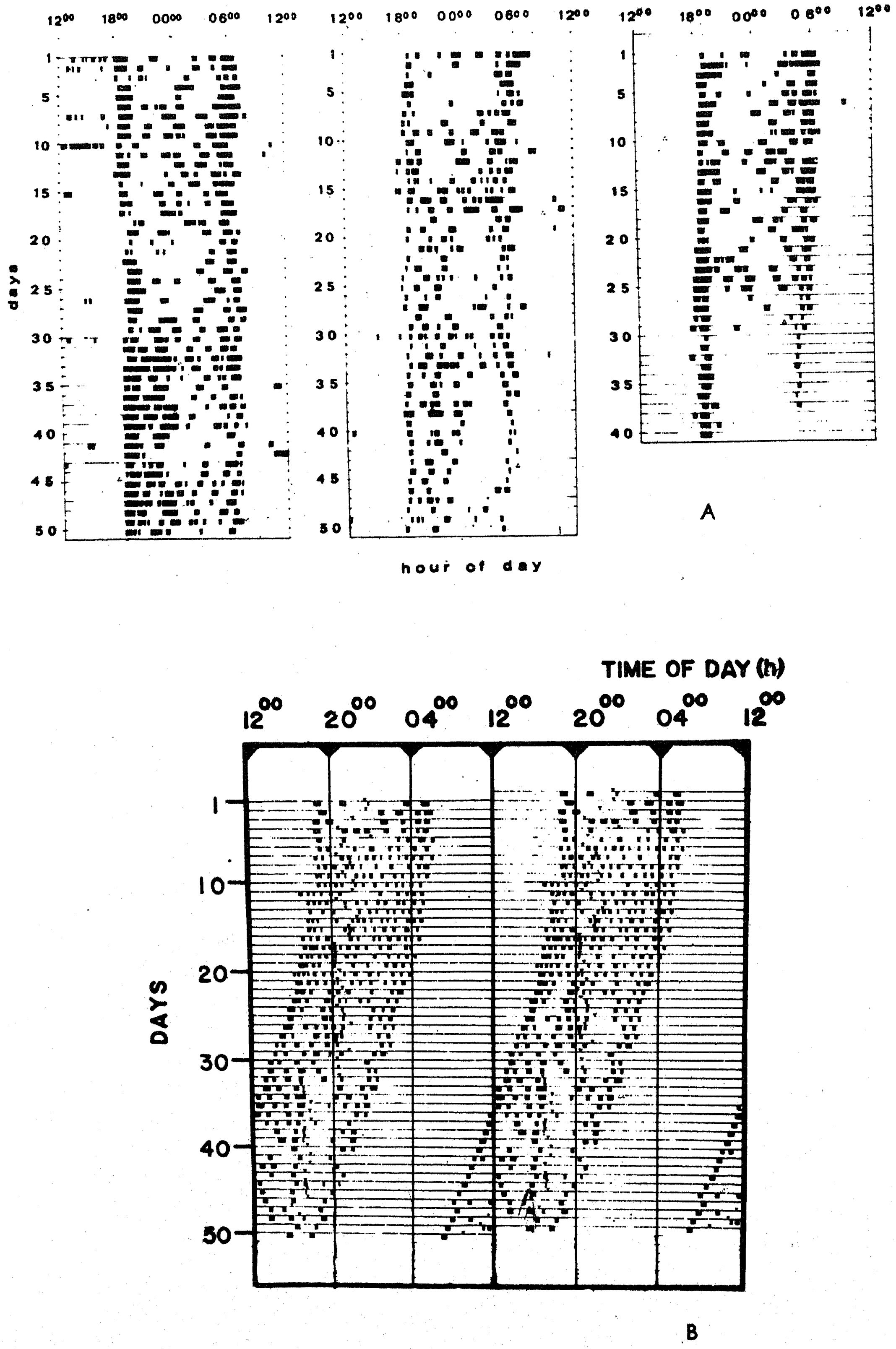
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Figure 1.

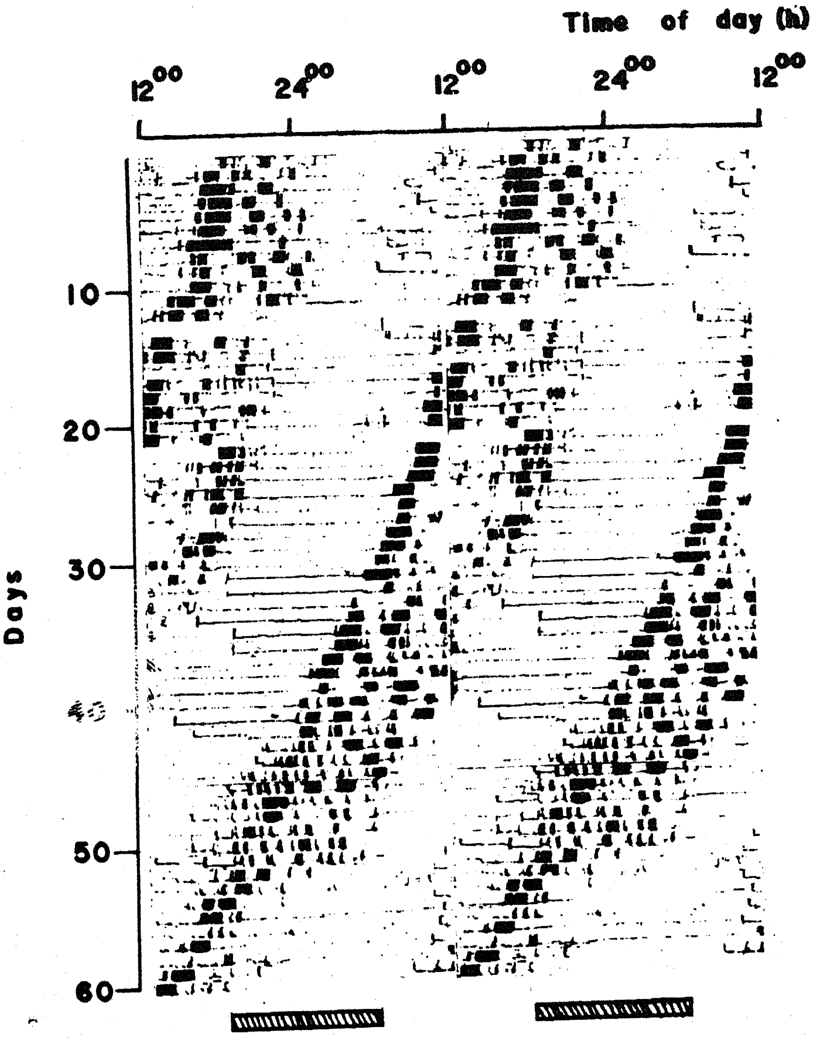

Figure 2. A typical example of the freerunning of the flight activity pattern of an emballonurid bat confined in a hipposiderid cave for 60 days. The original felt pen tracings are double-plotted. The hatched area at the bottom of the figure indicates the time over which the members of the hipposiderid colony would be active, leaving the cave in a mass exodus during early night and returning individually for the rest of the night. Other details are as in figure 1 (after Marimuthu and Chandrashekaran 1983b).

Figure 1. **A.** The flight activity patterns of 3 captive bats of *Hipposideros speoris* for 40 days in one case and 50 days in the other two cases recorded 40 m inside a narrow 'true cave' in Madurai. The bats could fly within the flight activity cages and the movements of the cages were directly recorded. Activity bouts are indicated by vertical patches and the horizontal lines indicate rest. The activity/rest data are schematized from original data and presented one below the other for successive days (after Marimuthu *et al* 1981). **B.** A double plot of activity/rest pattern of a solitary male *H. speoris* recorded in a cave without any conspecifics over a period of 50 days. The activity data for day 1 leading horizontally to data for day 2, data for day 2 to data for day 3 etc to facilitate visual evaluation. Triangles indicate feeding time. The details are schematized from original felt pen tracings. Other details are as in "A" (after Marimuthu *et al* 1981).

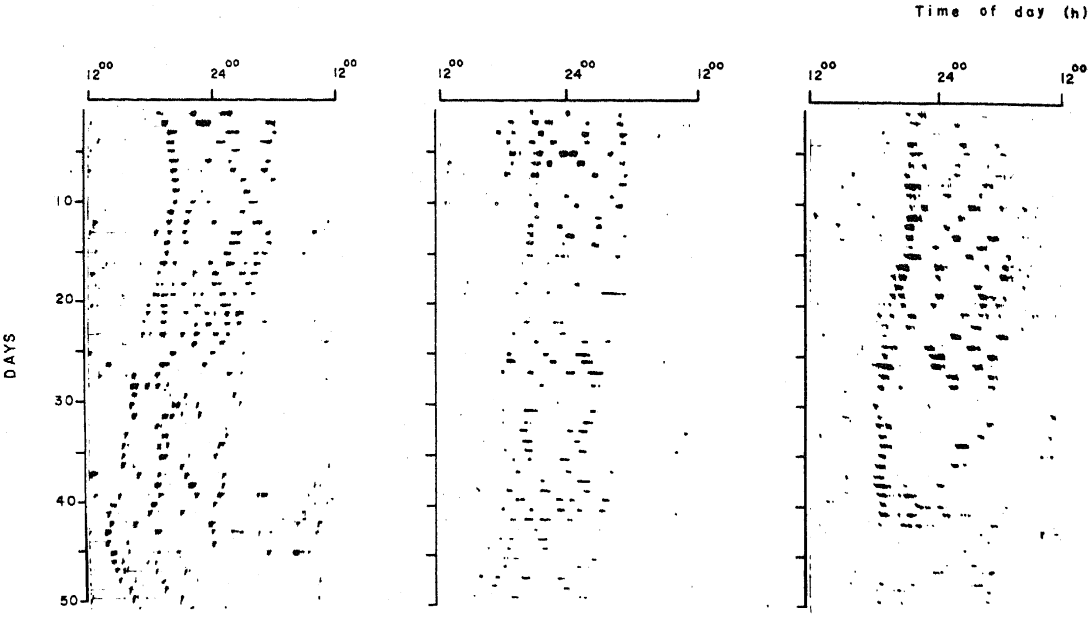

Figure 3. The flight activity patterns of three captive bats of *Hipposideros fulvus* confined in *H. speoris* cave for 50 days. Activity bouts indicate the original felt pen tracings. Other details are as in figure 1.

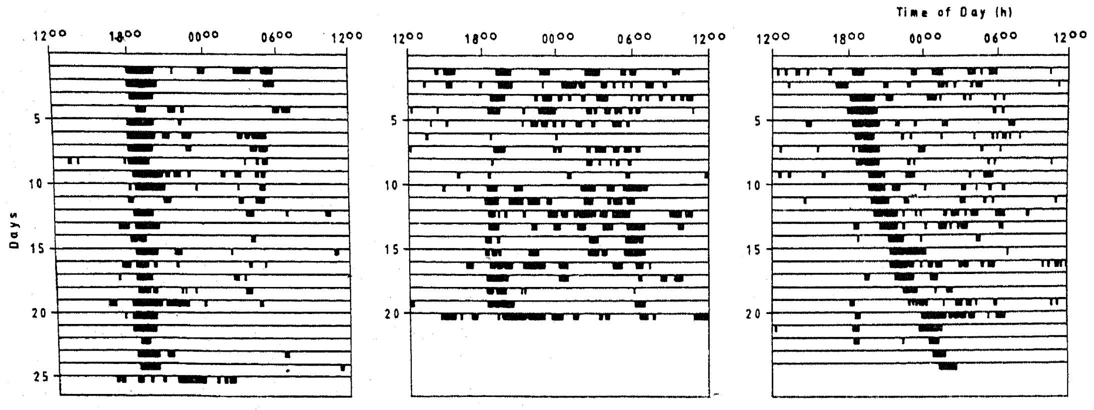

Figure 4. The flight activity patterns of 3 captive bats of *H. speoris* in LL of 1 lux. The activity/rest data are schematized from original felt pen tracings. Other details are as in figure 1.

forage. Such flights of these bats coinciding with the onset of the colony activity account for an exogenous component which expresses itself regardless of the phase of the freerunning rhythms. These exogenous components merge into the activity bouts when the rhythm crosses them during the freerun. The onset of the freerunning oscillatory component for bat in figure 6A crosses the exogenous component around days 37 and 38 without any sign of even a temporary synchronization, i.e. there is no 'relative coordination' (Holst 1939) during the whole run. The freerunning rhythm re-entrained to the social cues as seen in figure 5 when DD was restored. The light was turned off at phase which was 180° off course relative to colony activity. Re-entrainment set in after a few 'transient cycles' with the onset component of the re-entrained rhythm coinciding

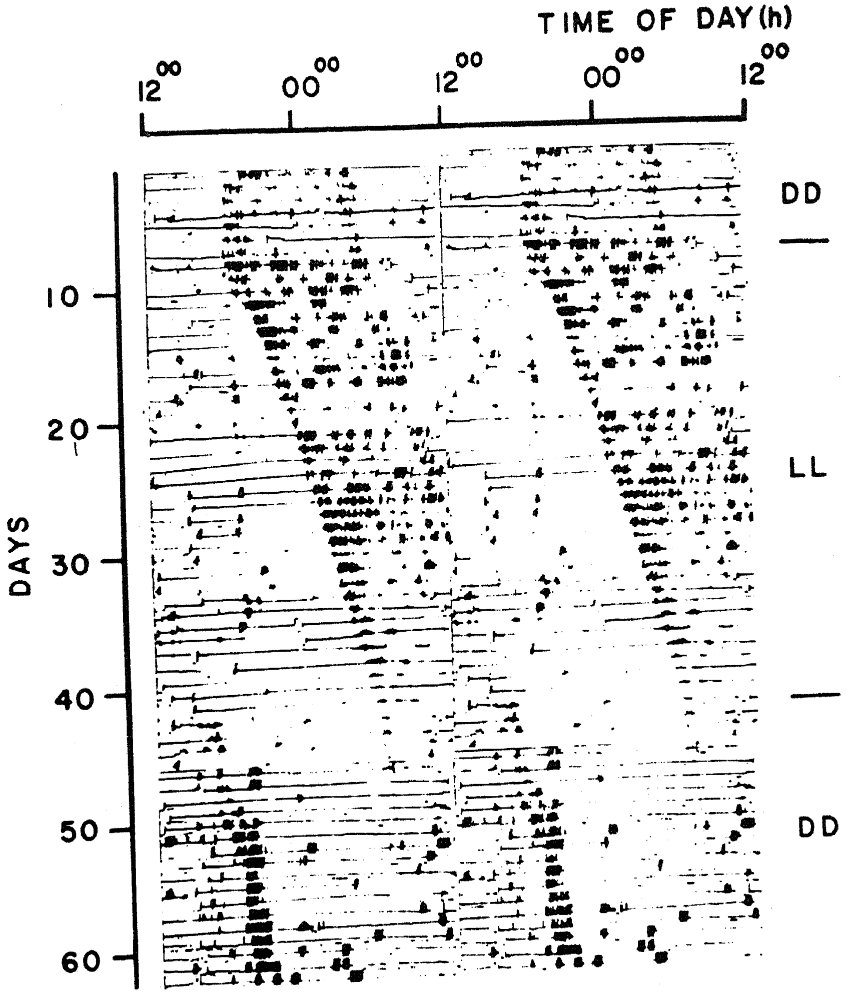

Figure 5. The flight activity patterns of a captive bat for 62 days. Days 1–7, DD; days 8–41, LL of 10–20 lux; days 42–62, DD. The activity/rest data containing the original felt pen tracings are double-plotted and other details are as in figure 1. (after Marimuthu and Chandrashekaran 1983a).

with the onset of colony activity. Another possibility that cannot be ruled out, however, is that the freerunning rhythm persists even after turning LL off and what is seen in figure 5 is some form of 'masking' (Aschoff 1965).

4. Discussion

Literature on communication and synchronization of circadian rhythms is altogether sparse. Even those of the reports that impute social synchronization are only rarely based on rigorous experimentation. One of the earliest reports to impute social synchronization among conspecifics was that of Johnson (1926) for the mice of the genus *Peromyscus*. Subsequent reports described similar effects for blinded mice *Mus musculus* (Halberg *et al* 1954), male chevrotain antelopes (Dubost 1975), wolf-coyote hybrids (Roper and Ryan 1977), beaver colonies of *Castor canadensis* (Potvin and Bovet 1975), deer mice (Crowley and Bovet 1980), macaque monkeys (Rohles and

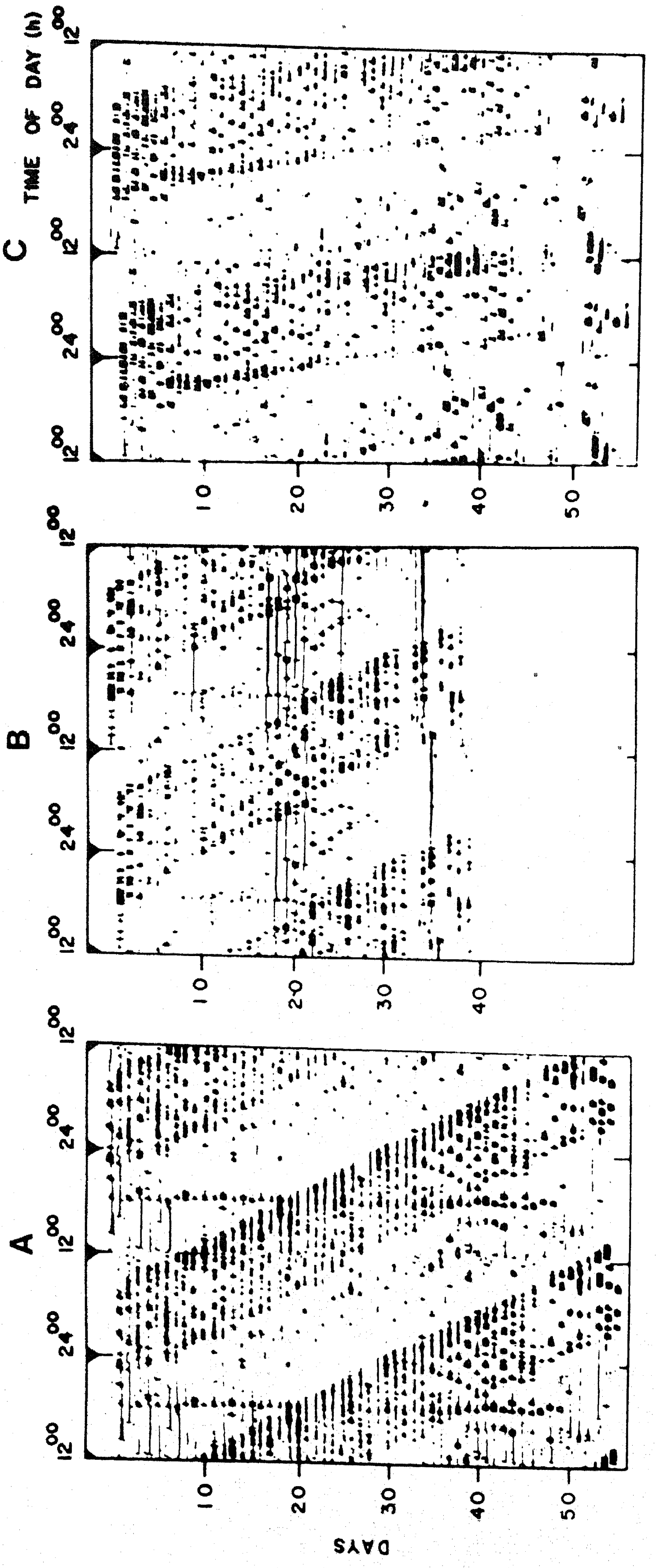

Figure 6. The flight activity patterns of 3 captive bats for 39 days (B) and 55 days (A and C) in LL of 10–20 lux. The activity/rest data containing the original felt pen tracings are double-plotted and other details are as in figure 1 (after Marimuthu and Chandrashekaran 1983a).

Osbaldiston 1969), sexual cyclicity of female mammals (Rusak and Zucker 1979) and so on. Several other reports seeking to causally connect social cues with circadian rhythms are anecdotal (Rusak 1981; Chandrashekaran 1982). The case we present here for synchronization of the circadian rhythm by social communication for the cave-dwelling microchiropteran bat *H. speoris* incorporates the outcome of our researches on this subject since 1977. We have applied the methodologies of the field ethologist and experimental chronobiologist. To this extent we may be justified in believing that our results are relatively free of experimental/laboratory artifacts.

The synchronization of the activity rhythm of all three captive bats illustrated in figure 1A, is absolute and stable. We conclude that the synchronization arises through communication. The communication could be in the form of (i) flight noise, (ii) pheromones and (iii) acoustics. Owing to the close intermingling of these factors in the cave environment, laboratory studies alone can resolve which of these factors plays a dominant role. It is also possible that all these factors have to be simultaneously available. Another feature (figure 1A) re-inforces the case for social synchronization. The captive bats while entraining show an unequivocal bimodality in their activity bouts. The first peak coincides with outflight of the colony and the second peak coincides with the return of the colony.

Figure 2 illustrates that an 'alien' bat (*T. n. kachhensis*) in a hipposiderid cave fails to synchronize. We postulate that the freerunning results from a 'communication gap' between the hipposiderid bat (*H. speoris*) which emits CF ultrasonics of 135 kHz and the emballonurid alien (*T. n. kachhensis*) which emits pulses consisting of a family of harmonics up to 80 kHz. Interestingly audiogram studies reveal that the bat *T. n. kachhensis* cannot indeed 'hear' (white) noise of 135 kHz which is the species specific emission frequency of *H. speoris* (Neuweiler *et al* 1984). Threshold of hearing may not be the only criterion for even the wing-beat noise of hipposiderid bats, well within the hearing range of the emballonurid bat, was apparently unable to synchronize.

A further ethological observation must be described in this context. In the experiment in cave 2 using a solitary bat we would often hear crows and mynas at a nearby waterhole during day and stridulation of crickets during night. These non-specific cues did not synchronize the activity rhythm. This finding contrasts with those of Lohmann and Enright (1967) on birds, and Wever (1979) on humans; bird and human circadian rhythms also entrain to buzzers and other non-specific noise.

It is tempting to assume that communication and synchronization of the circadian rhythm in bats may even be species specific. Thus evolutionarily closely related species, *H. speoris* and *H. fulvus* seem to be able to communicate even if only partially, in the context of synchronization of their rhythms. *H. fulvus* emits CF-FM ultrasonic pulses of 154 kHz with the downward sweeps touching 115 kHz.

Figures 4–6 represent the responses of the bat circadian system when exposed to a conflicting zeitgeber situation. This, however, almost never occurs in nature. Circadian rhythms of the captive bats freerun in LL of 10–20 lux in spite of the social cues available to them. LL thus apparently abolished the social synchronization of the circadian rhythm. The exogenous components exhibited by two individuals (figures 6A, B) represent a stimulus-response situation and may not merit oscillator status. This may be the phenomenon of response that was termed 'positive masking' (Aschoff 1965, 1981; Daan and Aschoff 1975). Figure 4 obviously mirrors a situation of subthreshold LL influence. This explains why two bats synchronized and the third did not.

The artificial light that shone inside the cave scared away a few bats that roosted otherwise in and around the area inside the cave where the recording was carried out. To that extent the 'social cues' might indeed have attenuated in the immediate vicinity. However, within a week or so the bats returned to their original roosting sites, closer to the captive bats. The exogenous component seen so clearly in bats in figures 6A, B is further evidence that the onset of the activity of the bat colony had made itself felt, even though it could not synchronize the rhythm. The freeruns indicate that whatever social cues that prevail are not 'reaching the clock'. In LL the circadian rhythm apparently uncouples from social 'zeitgebers'. The reentrainment of the rhythm by the social cues after the light was turned off (figure 5) further confirmed that it is LL that uncoupled the circadian rhythm from the social cues. Sulzman *et al* (1977) reported that the locomotory activity of squirrel monkeys in noise/silence cycles of 24 hr periods did not synchronize the rhythm in LL of 600 lux, but the food cycles did synchronize it. It would be of great interest in the context of our findings to know whether squirrel monkeys would have entrained to the noise cycles in DD.

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