

THE SYNCHRONIZATION OF THE CIRCADIAN CAECOTROPHY RHYTHM WITH DIFFERENT PHOTOPERIODS

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I n t r o d u c t i o n

A circadian rhythm is an endogenous biological oscillation. In the absence of any exogenous entraining agent (synchronizer, Zeitgeber) its period length is closely to but differs significantly from 24 hours (c i r c a d i a n - Halberg 1959). According to Aschoff's rule the length of the endogenous period of nocturnal animals during continuous light conditions (LL) exceeds significantly twenty-four hours. In continuous darkness (DD) it is shorter than twenty-four hours. The opposite holds for diurnal animal species (Aschoff 1960). For animals the main synchronizer is the light-dark (LD) cycle. Other exogenous rhythms (noise, temperature, electromagnetic fields) are of secondary importance.

While during a light-dark schedule the circadian rhythm is 'in phase' with the Zeitgeber, there exists a characteristic time relation between corresponding phases of rhythm and entraining agent, called phase angle difference (p.a.d.). The amount of the p.a.d. depends from 1. the period length of the Zeitgeber, 2. the light intensity of the Zeitgeber, 3. the light-dark-time ratio of the Zeitgeber and 4. the length of the endogenous period of the individual animal.

By means of monitoring the circadian alternation of hard faeces excretion and caecotrophy of the laboratory rabbit typically nocturnal characteristics of this function have been worked out in previous experiments. For example the length of the circadian period increased with increasing light intensity and the time of adaptation was longer after an advance-shift as compared to a delay-shift of the Zeitgeber (Jilge 1976, 1979, 1980a, b). These results accord well with those obtained in different species of nocturnal rodents (e.g. Pittendrigh and Daan 1976a, b; Daan and Pittendrigh 1976; Kenagy and Hoyt 1980), with other parameters obtained in the rabbit (Prud'hon et al. 1978; Reyne et al. 1979) and with the same parameter of the rabbit obtained in other laboratories (Hörnigke and Batsch 1977). No information so far is available concerning the phase relation between a circadian function of the rabbit and different light-dark ratios of a 24 hours Zeitgeber. Therefore the alternation of hard faeces excretion and caecotrophy was registered during eleven different LD schedules in order to find out how a circadian rhythm of the rabbit is synchronized with Zeitgebers of different photoperiod length which are supposed to have different power for synchronization.

M e t h o d s

15 adult male rabbits (himalayan) lived in a sound-isolated animal room in individual solid metal cages on grid floor. Room temperature: 18.0 ± 0.3 C; rel. humidity 60 ± 10 %; air turnover 20 times/hour. Illumination of the room by 10 light bulbs of 40 Watt each; intensity ca. 300 lux. The LD-regimen was adjusted on an electric switch clock.

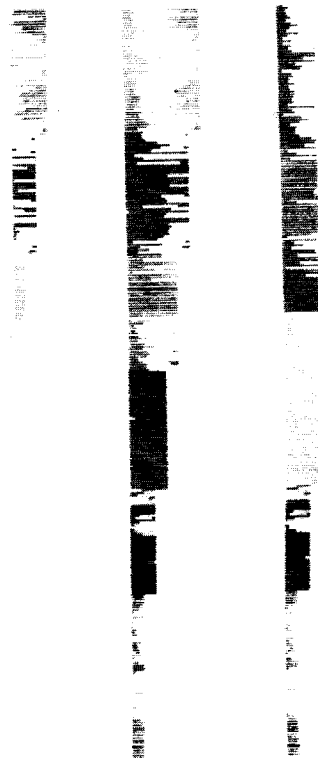
It was controlled by a photocell-contact on the event recorder. The registration of the hard faeces excretion rhythm occurred as described elsewhere (Jilge 1978). Instead of the electromechanical flaps as used originally an electronic photocell-recording system was used which worked at $\lambda=950$ nm. The animals had continuous access to drinking water from bottles and to pelleted rabbit food.

Experimental design: the animals consecutively were exposed to the following light-dark schedules: LD 12:12; LD 14:10; LD 16:8; LD 18:6; LD 20:4; LD 22:2; LD 24:0 (LL); LD 12:12; LD 10:14; LD 8:16; LD 6:18; LD 4:20; LD 2:22; LD 12:12. The changes of the respective LD-schedules were switched by shifting the onset of the photoperiod, the end remaining constant (except for LL and the re-inserted LD 12:12). The time of adaptation to each schedule amounted to at least 45 days (see figures 1, 2, 3). The total duration of the experiment amounted to 1032 days; this paper reports the experiment from day 270 through day 1032. For calculation the end of hard faeces excretion corresponding to the onset of caecotrophy was taken as reference point of the circadian rhythm.

R e s u l t s

During all light dark schedules with a dark-time period longer than two hours all 15 rabbits succeeded to synchronize firmly with the respective LD regimen. After shifting the onset of the light-dark schedule the complete circadian function shifted too significantly and made up a new phase relation to the synchronizer. The extension of the photoperiod was followed by a significant increase of the phase difference between the corresponding phases of synchronizer (light on) an circadian rhythm (end of hard faeces excretion = onset of caecotrophy). Reducing the hours of light the rhythm was shifted almost in parallel to the synchronizer with

Figure 1:
The alternation of hard faeces excretion (horizontal lines) and caecotrophy (blank between horizontal lines) during 6 different LD-ratios (dotted area: hours of darkness; white area hours of light).



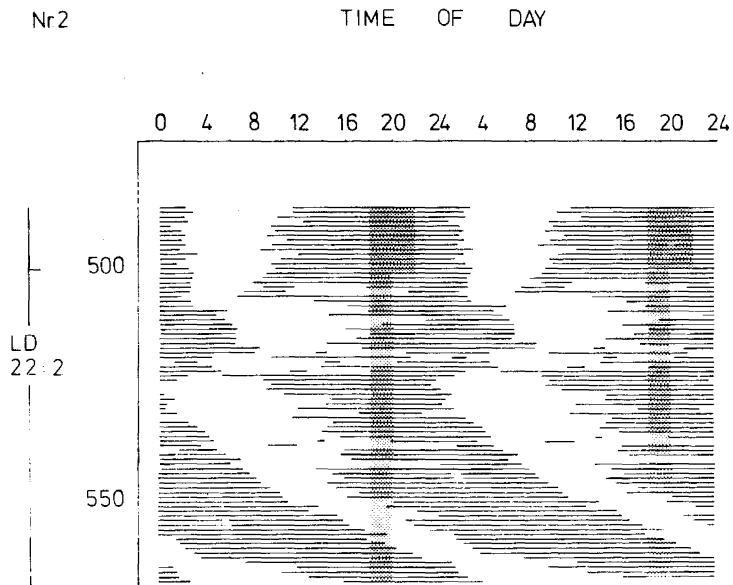


Figure 2: Hard faeces excretion and caecotrophy rhythm during LD 20:4 and 22:2. This animal failed to entrain during LD 22:2

Figure 3:
Original plot of a bi-phasic faeces excretion rhythm running free during continuous light conditions (day 567-637). During decreasing photoperiods both fractions of the bimodal rhythm were shifted almost in parallel to the onset of light. Finally a LD 12:12 was inserted.

The resynchronisation with this schedule occurred by advancing the circadian rhythm.



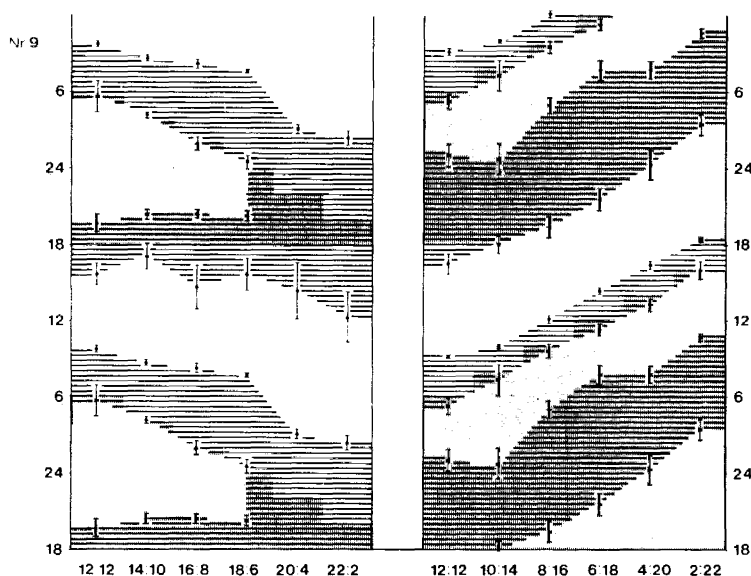


Figure 4:

The faeces excretion pattern of a typical biphasic animal entrained with different LD-ratios.

Horizontal lines: hard faeces excretion; non-lined area: caecotrophy
Shaded area: dark-time; non-shaded area: light-time

Ordinate: time of the day; abscissa: different LD schedules.

The picture is read from the bottom to the top and from the left to the right side. For each of the LD schedules the average-values of onset and end of caecotrophy of either fraction were calculated for $n = 10$ days \pm SD. Note that the increase of photoperiod length (left part of the figure) was responded by reduction and final cessation of the nocturnal caecotrophy-fraction. The main (diurnal) caecotrophy simultaneously became elongated. In the course of the photoperiod reduction (right part of the figure) both fractions of the rhythm were delayed almost in parallel to the delay of light-onset (same animals as figure 3).

a slight but in total significant decrease of the phase difference between synchronizer and rhythm (figures 1, 3, 4, 5).

After complete synchronization with the respective LD-schedule the day-to-day-variability amounted to 0.3-0.5 hours with the exception of LD 22:2 (0.9 ± 0.5 hours). During 70 days on this schedule three animals did not succeed to synchronize with the Zeitgeber but their rhythm showed an 'oscillatory free run' (figure 2). The variability between the animals (interindividual variability) over a wide range of LD ratios was about 1.5 ± 1.9 hours except for the very short photoperiod-schedules LD 6:18, LD 4:20 and LD 2:22 when it was three to four times that during the other schedules (figure 5), the day-to-day-variability nevertheless was 0.3 - 0.5 hours.

In the course of the extension of the photoperiod those animals caecotrophing originally twice per 24 hours changed their caecotrophy pattern and all showed a monophasic pattern in LD 22:2 and in LL.

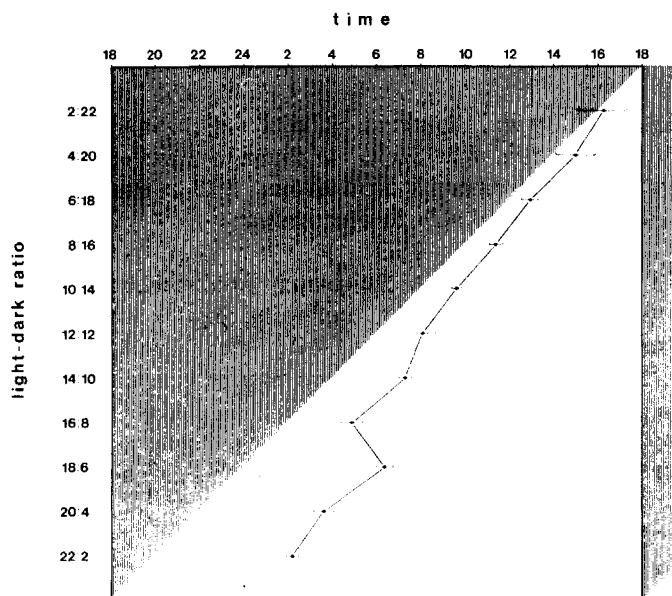


Figure 5:

The onset of caecotrophy at eleven different light-dark ratios.

Shaded area: darkness; white area: light.

Each point marks the average value of 15 animals \pm interindividual variability at the respective LD-regimen after complete synchronization (last 10 days at each schedule).

(In LD 22:2 only 12 animals; 3 animals failed to entrain with this Zeitgeber.)

The second (nocturnal) caecotrophy was abolished by advancing the end of this second fraction of the rhythm. During the reduction of the photoperiod both fractions were phase-shifted in parallel to the onset of the synchronizer.

During the 70 days of continuous light conditions all animals within day 5 to 39 showed a regularly free-running circadian rhythm. The average length of the circadian period amounted to 24.9 ± 0.2 hours. The intraindividual (period-to-period) variability was significantly less (0.01-0.3 hrs/period) than the intraindividual variability of the synchronized rhythm (0.3 - 0.5 hours/day).

These results suggest that for the rabbit as well as for other nocturnal animal species long photoperiods are weaker synchronizers than are short photoperiods. This is indicated by the increased day-to-day-variability and the lack of synchronization of 3 out of 15 animals (during LD 22:2). During short photoperiods animals synchronized with the Zeitgeber at phase relations with high inter-individual variability. The low day-to-day-variability however suggests, that short photoperiods have a higher power of synchronization.

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Summary

Fifteen adult male rabbits were kept in eleven different LD-ratios (LD 2:22, 4:20, 6:18, 8:16, 10:14, 12:12, 14:10, 16:8, 18:6, 20:4, 22:2). By means of monitoring the circadian alternation of hard faeces excretion and caecotrophy it was found that the phase difference between the onset of light and the onset of caecotrophy increased with increasing and decreased with decreasing length of the photoperiod. The very long photoperiod (LD 22:2) did not synchronize at all 3 out of 15 animals. The day-to-day-variability of the other animals was elevated significantly. During the other schedules the intraindividual variability amounted to 0.3-0.5 hours. The variability between the animals was significantly increased during the short photoperiod schedules (LD 6:8, 4:20, 2:22). Thus in the rabbit a short photoperiod evidently seems to be stronger Zeitgeber than a short scotoperiod.

The increasing length of the photoperiod caused a cessation of the second, nocturnal caecotrophy-fraction. During the prolongation of the dark-time both fractions were shifted in parallel to the onset of light.

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