perhaps the biochemical alteration of the host tissue by one parasite makes it unsuitable for another species, thereby limiting the species involved in double infections.

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Circadian Rhythm in Mammalian Body Temperature Entrained by Cyclic Pressure Changes

Abstract. *A 24-hour cycle of pressure (1.0 to 1.09 atmospheres) can act as a zeitgeber to entrain the endogenous circadian rhythm of body temperature in pocket mice* (Perognathus longimembris) *under constant conditions of environmental temperature and light.*

Many biological rhythms involve events which recur at intervals of approximately a day and have been termed circadian (*1*). Although it has been suggested that external environmental cues are responsible for the persistence of these rhythms (*2*), the bulk of the available evidence supports the usually accepted interpretation that these rhythms are the manifestation of innate oscillations maintained by yet undescribed physiological mechanisms (*3*). Under normal circumstances, circadian rhythms are synchronized and have their phase set by environmental cycles of light and, in some cases, by cycles of temperature (*4*).

Under constant conditions of light and temperature, environmental cycles of sound can also act as weak entraining agents for the circadian rhythms of birds (*5*). Other environmental factors, such as feeding regimes, social interactions, barometric pressure, and magnetic fields have been proposed but not rigorously tested as potential synchronizers (*6*).

We consider the following criteria essential for the demonstration that an environmental cycle can entrain a circadian rhythm: (i) the free-running period of the experimental individuals must be demonstrated to differ from that of the imposed cycle; (ii) the rhythm examined must assume the same period as that of the stimulus cycle; and (iii) it must be shown that the phase of the rhythm, in a subsequent free run, differs from a simple extrapolation of the preentrainment free run (*5*). On the basis of these criteria, we have now demonstrated that large changes in ambient pressure can induce entrainment of a circadian rhythm, when light and temperature are held constant.

The metabolic rate and body temperature of the little pocket mouse have been shown to oscillate with a circadian period (*7*). A manifestation of the daily temperature cycle in isolated animals is a daily torpor during which body temperature drops to near ambient. The duration of the torpor is variable between individuals and ranges from hours to days, depending upon the experiment conditions. The time of arousal from torpor is an easily distinguishable event which is clearly under the control of the circadian system, because the time of arousal is predictable as a function of the circadian period even for animals which may remain in continuous torpor for more than 1 day. Infrequently, some animals will enter torpor and arouse more than once each day. This usually occurs during the transient period in disturbed animals that have not yet stabilized to a new environment. Even less frequently an individual animal may skip a torpor period. For experiments of several weeks' duration, neither of these latter two occurrences detract from the use of the interval between successive times of arousal as a measure of the circadian period in pocket mice.

Table 1. Summary of circadian periods (τ) of time of arousal from torpor measured in *P. longimembris* maintained continuously in conditions of constant dark and constant temperature (20°C) for 41 days. Four of eight mice were exposed to 22 days of atmospheric pressure change, administered at 12 hours at ambient pressure and 12 hours at ambient pressure plus 67 mm-Hg.

Animal No.	Circadian period (hours : min) Day 1–7	Day 8–29*	Day 30–41
	Cyclic pressure change days 8–29		
A	22 : 54	Entrained	23 : 32
B	23 : 22	Entrained	23 : 12
C	23 : 6	Disturbed	23 : 21
D	23 : 10	Disturbed	22 : 52
	No pressure change		
E	23 : 46	22 : 54	23 : 4
F	23 : 26	22 : 41	22 : 41
G	No torpor	23 : 6	21 : 28
H	22 : 38	23 : 14	25 : 8

* See Fig. 1.

Our data are based upon continuous monitoring of body temperature of eight pocket mice for an interval of 6 weeks. Temperature monitoring telemeters were implanted within the abdominal cavity several months prior to the experiment. The animals used included both males and females and all had previously shown a clear tendency for daily torpor when housed under normal laboratory conditions.

Four animals were housed individually in separate sealed chambers provided with incurrent and excurrent airflow systems. A timer and a series of solenoid valves were located outside the chambers and were used to route the excurrent air through a water manometer to pressurize the system. Four control animals were housed in similar chambers except for the excurrent flow control system. Constant conditions of 20°C and complete darkness were provided by an incubator which was sealed for the duration of the experiment. The animals were provided in advance with an adequate supply of mixed seeds. No drinking water is necessary for this species.

The pressure cycle consisted of 12 hours at ambient pressure and 12 hours at ambient plus an additional 67 mm-Hg (1.3 pounds per square inch); the transition to increased pressure took about 30 seconds, and the decrease took about 5 seconds. The pressure cycle began 8 days after the start of the experiment and continued for 22 days, followed by 12 days without the imposed cycle. Both the experimental and the control animals were equally exposed to any residual periodic variables (for example, laboratory noise and normal atmospheric pressure).

With the time of the midpoint of arousal from torpor as a marker in the circadian cycle of body temperature, the results show that two of the four animals receiving cyclic pressure change did entrain to the stimulus (Fig. 1, A and B), as judged by the entrainment criteria described above. The third experimental animal showed a rhythm that was probably entrained for about the first 12 days of the pressure cycle

(Fig. 1C); the fourth animal was clearly not entrained by the cycle, although disturbance of its rhythmicity may have been due to the pressure regime (Fig. 1D). Three control animals manifested free-running periods of less than 24 hours with no perturbations of their rhythmicity during the entire interval that the experimental animals were exposed to the pressure cycle. One control animal spontaneously drifted from a period of less than 23 hours to about 25 hours during the total observation interval. Table 1 contains a summary of the periods of body temperature rhythms of both groups of animals.

The phase relationship of the body-temperature cycle to the stimulus cycle differed markedly between the two animals most clearly entrained. One animal was apparently usually aroused from torpor by the pressure-increase transition, although it anticipated the increase on 8 of the 22 days of treatment; the other animal began arousal some 2 to 4 hours after the transition (Fig. 1B). The rapid increases in pressure which were provided apparently caused some discomfort in the ears of the mice. After these experiments, we administered pressure changes to torpid animals and usually observed a brief pawing at the ears after the pressure was increased, but there was no response to pressure decreases. Time of arousal of the animal shown in Fig. 1B, however, suggests that such discomfort did not act as an immediate stimulus to trigger daily arousal. Although we cannot, at present, rule out the possibility that the ear discomfort was an essential aspect of the entrainment regime, other possibilities include the differences in pressure per se and the concommitant differences in $_pO_2$ and in relative humidity.

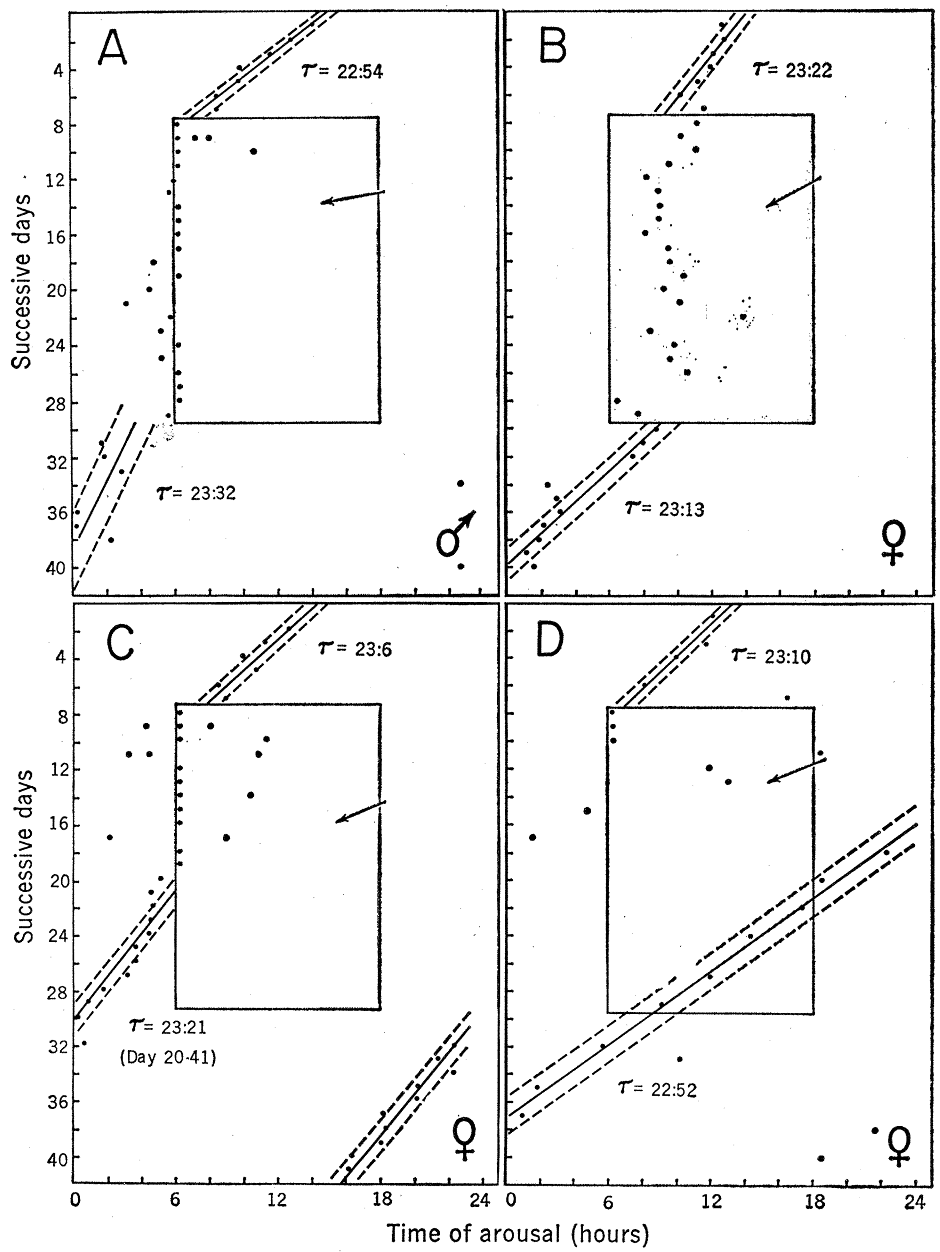

Fig. 1. Arousal from daily torpor in four isolated pocket mice (*Perognathus longimembris*) exposed to a 12-hour increased pressure regime. With the exception of animal C which did not enter torpor on day 1, missing data points indicate that the animal sustained a continuous torpor at a body temperature near 20°C (animal D). Multiple torpors and arousals occurred within single days for animals A and C. Estimates of period length (τ, hours : min) derived from regression lines (solid) and ± 1 S.E. (broken) are indicated for each animal. Arrow indicates the area designating the duration and sequence of the hyperbaric regime.

Regardless of the sensory mechanism involved in producing entrainment, the fact remains that the large-amplitude pressure cycle that we administered was able to produce unequivocal entrainment in only two of the four animals tested. This suggests that the normal daily cycle of barometric pressure is probably seldom, if ever, an adequate synchronizing agent for circadian rhythms.

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