J. comp. Physiol. 106, 291–331 (1976)

Journal
of Comparative
Physiology · A

# A Functional Analysis of Circadian Pacemakers in Nocturnal Rodents

## IV. Entrainment: Pacemaker as Clock

Colin S. Pittendrigh and Serge Daan *

Department of Biological Sciences, Stanford University, Stanford, California 94305, USA

Received November 7, 1975

**Summary.** 1. In the *first* part of the paper, the model of non-parametric entrainment of circadian pacemakers is tested for the case of nocturnal rodents. The model makes use of the available data on freerunning period ($\tau$) in constant darkness (Pittendrigh and Daan, 1976a) and on phase response curves (PRC) for short light pulses (Daan and Pittendrigh, 1976a). It is tested in experiments using 1 or 2 light pulses per cycle.

2. *Mesocricetus auratus* and *Peromyscus leucopus* entrain to zeitgebers involving 1 pulse (15′ or 60′) per cycle. The phase angle differences between rhythm and light cycle depends on the periods ($\tau$ and $T$) as predicted by the model. Entrainment of *P. leucopus* is unstable due to the after effects on $\tau$ created by the light pulse.

3. The limiting values of zeitgeber period to which the animals entrain are much closer to 24 h than in *Drosophila pseudoobscura*, as the model predicts. However, frequent failures to entrain to $T=23$ and $T=25$ h are only explained if we take considerable interindividual variation in both $\tau$ and PRC into account.

4. With 2 pulses per cycle, the model predicts that entrainment will be more stable when activity is in the longer interval between the pulses than when it is in the shorter interval. This is true in the experimental data, where the phase relationships match predictions for skeleton photoperiods up to ca. 14 h. Increasing asymmetry forces animals into a "phase jump", so that activity shifts from the shorter to the longer interval. These $\psi$-jumps are accurately predicted in the hamster, but they occur at much longer photoperiods than predicted in *P. leucopus.*

5. Thus, the unqualified model, using a rigidly fixed species $\tau$ and PRC, is surely inadequate to explain entrainment. The extent to which variations in $\tau$ and PRC-shape, both "spontaneous" and induced by the entrainment process, can be known or inferred restricts the validity of the predictions. Yet

---

* Present address: Zoologisch Laboratorium, Rijksuniversiteit Groningen, Haren (Gr.), The Netherlands

we conclude, from a good deal of agreement between experiment and prediction (i), from the close correspondence between complete and skeleton photoperiods (ii), and on behavioural grounds (iii), that non-parametric entrainment by short light signals has a major share in the entrainment of nocturnal rodent rhythms in nature.

6. With these restrictions in mind, we analyse in the *second* part of the paper how the empirical regularities concerning $\tau$ and PRC, and reported earlier (Pittendrigh and Daan, 1976; Daan and Pittendrigh, 1976a, b), contribute to the stabilization of the phase angle difference ($\psi$) between the pacemaker and the external world. Use is made of computer simulations of artificial pacemakers with variable $\tau$ and PRC.

7. $\psi$ is most sensitive to instabilities in $\tau$ when $\bar{\tau}$ is close to 24 h. Thus the very *circadian* nature of these pacemakers helps to conserve $\psi$. Selection pressure for homeostasis of $\tau$ has been large in a species *(M. auratus)* where $\bar{\tau} = 24$ h. The effect of $\psi$-instability is further reduced by entrainment with 2 pulses (dawn and dusk), made possible by the PRC's having both an advance and a delay section.

8. To analyze the contributions to $\psi$-conservation with seasonally changing photoperiod, we have assumed that it is of functional significance to conserve the phase of activity with respect to dusk (nocturnal animals) or to dawn (diurnal animals). We distinguish three contributions of nocturnal pacemaker behaviour to this type of $\psi$-conservation: increased amplitude of the PRC (i), asymmetry in the PRC, such that the slope of the delay-part is steeper than the slope of the advance-part (ii), and a short freerunning period in DD (iii).

9. A further contribution must derive from parametric effects of light, which are not traceable by the model, but certainly effective in preventing in complete photoperiods the $\psi$-jump which is seen in skeleton photoperiods. The existence of parametric effects is further demonstrated by the change of $\tau$ with light intensity in LL, described by Aschoff's Rule, which presumably reflects differences in PRC-shape between nocturnal and diurnal animals (Daan and Pittendrigh, 1976b).

10. The paper concludes with an attempt to distinguish the features of circadian clocks that are analytically necessary for entrainment to occur (i), or have functional meaning, either in the measurement of the lapse of time (ii) or in the identification of local time (iii).

## I. Introduction

The model of entrainment that prompted these studies implies that to explain the resultant steady-state one needs only two parameters of the pacemaker: its period ($\tau$) and its phase-response-curve (PRC) for single light pulses of the same duration and intensity as those used to entrain it. We have measured both parameters, and their variability, in the four nocturnal rodents chosen for study (Pittendrigh and Daan, 1976a; Daan and Pittendrigh, 1976a). In this paper we have two goals. First we evaluate the model's utility by asking to what extent a knowledge of $\tau$ and PRC can, in fact, account for the limited information we have on the entrainment of our rodents. Second, we then explore some intrinsic properties of the non-parametric entrainment mechanism in relation to the general clock-like functions of circadian pacemakers.

## II. Entrainment Phenomena

### *a) The Experimental Program*

The experiments we report used the light-tight cabinets and running-wheel cages described previously (Pittendrigh and Daan, 1976a). Every cabinet was equipped with its own clock-controlled light. The animals were subjected to cycles of either 15′ or (in a few cases) 1 h light pulses. There are two major groups of experiments.

In the first group each entraining cycle involved only one light pulse: its period ($T$) was varied to values greater and less than that ($\tau$) of the pacemaker. These experiments had two goals: (i) to determine the dependence of $\psi$, the phase angle difference between pacemaker and light cycle, on both $\tau$ and $T$; and (ii) to measure the limiting values of $T$ to which the pacemaker would entrain.

In the second set of experiments two pulses were given in each cycle (with $T$ constant at 24 h) to create a "skeleton photoperiod" (cf. Pittendrigh, 1966; Ottesen et al., in preparation) whose duration was systematically varied. Again there were two goals: (i) to determine the dependence of $\psi$ on skeleton photoperiod duration; and (ii) to find the maximum photoperiod to which the animal would entrain before being forced into a "$\psi$-jump" (Pittendrigh, in preparation).

The two experimental sets overlapped in some ways. Figures 10–14 illustrate, with raw data, how most were done. An animal was initially allowed to freerun in DD before being entrained by a single light-pulse ($T = 24$ h). When an entrained steady-state was reached, a second light pulse was added, creating a skeleton photoperiod. When the new steady-state entrainment developed, one of the pulses was shifted to lengthen the skeleton photoperiod; successive shifts permitted measurement of the dependence of $\psi$ on the duration of the skeleton photoperiod (PPs) and determination of the maximum PPs before a phase-jump ($\psi$ jump) was enforced. The phase angle difference ($\psi$) was always measured as the distance in time between the onset of the light pulse and the onset of activity. $\psi$ is given a negative sign when activity starts after the light pulse, a positive sign when it anticipates the light.

### *b) The Model's Predictions and Their Testability*

The model of non-parametric entrainment analyzed in this paper, has been described elsewhere for the circadian rhythm of pupal eclosion in fruitflies (Pittendrigh, 1966; in preparation). Without elaborating on the details of the criteria for stable entrainment it yields (Ottesen et al., in preparation), we find it useful to restate the general principle.

According to the model, the circadian rhythm is in equilibrium with a light-dark cycle, consisting of repetitive short light pulses, when each pulse falls at that

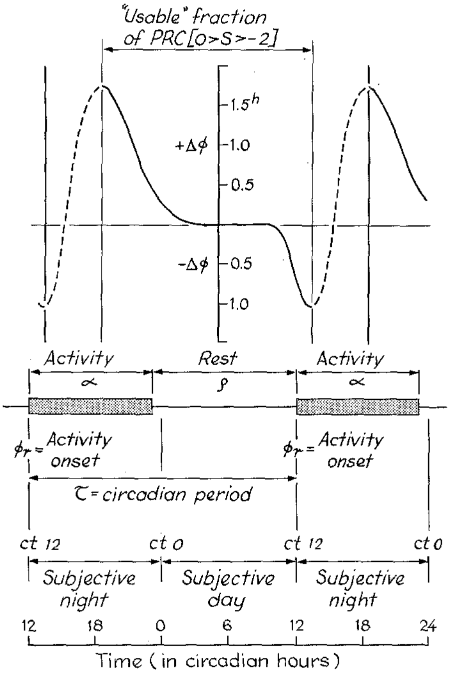

**Fig. 1.** The temporal relationship between the circadian pacemaker (its PRC) and the rhythm of activity ($\alpha$) and rest ($\rho$) which it drives. $S$ is the slope of the phase response curve

phase ($\phi$) of the rhythm, characterized by a response $\Delta\phi(\phi)$ which equals the difference $\tau - T$, where $\tau$ is the period of the circadian oscillator, $T$ is the period of the entraining light cycle, and $\Delta\phi(\phi)$ is the phase shift measured in the phase response curve for the same short light pulses.

In the rodent case, we cannot directly assay the phase ($\phi$) of the circadian pacemaker. The behaviour of a pacemaker relative to an entraining light cycle must be inferred from the rhythm it drives. Figure 1 illustrates the basis for such inference. It uses a generalized PRC (with slope, S) comparable to those of our rodents and relates the time-course of pacemaker responses to the observed cycle of activity ($\alpha$) and rest ($\rho$). The phase where activity onset occurs is arbitrarily chosen as a marker of the pacemaker's circadian time 12 (ct 12); it marks the beginning of the "subjective night", during which light pulses first cause phase-delays and later phase-advances. The "subjective day" is the time from ct 0 to ct 12, and is characterized by minimal response to light.

The general features of entrainment by the interaction of short light pulses ($T = 24$) and a circadian pacemaker with such a response curve, are schematically sketched in Figure 2. When $\tau$ is shorter than $T$ (panel A) a negative, delaying, phase shift is needed every day, and the light pulse will fall in the end of the subjective day; activity will start shortly after the light pulse. When $\tau$ exceeds $T$ (panel B), a daily phase advance is needed, and in equilibrium, the light pulse will fall in the late subjective night, around activity end. When $\tau$ equals $T$ (panel C), the light pulse may fall anywhere in the "dead zone", and a wide range of unstable phase relationships between the light cycle and the rhythm is predicted. With two pulses

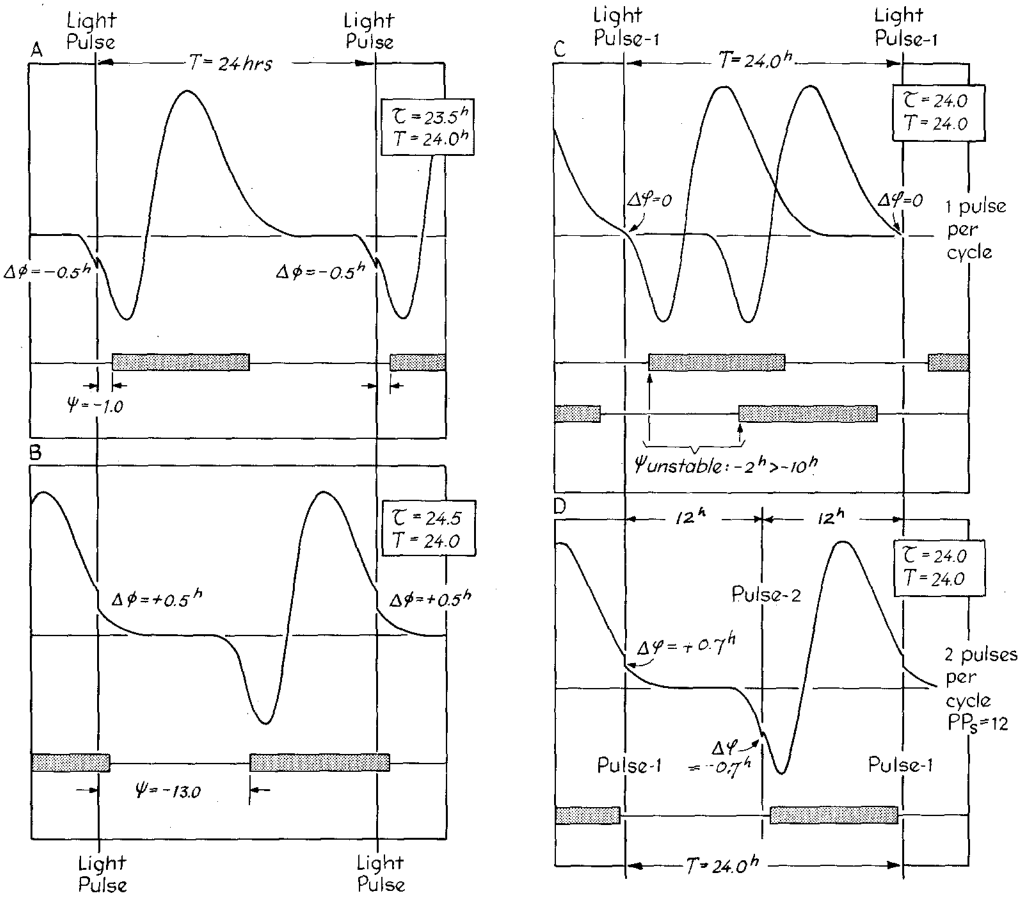

**Fig. 2 A–D.** The model for non-parametric entrainment by brief light pulses. Predicted phase relationships between the rhythm and a T24 light-dark cycle, with one (A, B, C) or two (D) pulses per cycle. (A) An individual with a freerunning period ($\tau$) smaller than 24 h is entrained by a recurrent delaying phase shift ($-\Delta\phi$), with the onset of activity close to the light pulse ($\psi = -1.0$ h). (B) An individual with a freerunning period ($\tau$) larger than 24 h is entrained by a recurrent advancing phase shift ($+\Delta\phi$), with activity end close to the light pulse ($\psi = -13.0$ h). (C) An individual with $\tau = 24.0$ has an unstable phase relationship ($-2 > \psi > -10$) since the light pulse, causing zero phase shift ($\Delta\phi = 0$) can fall anywhere in the "dead zone". (D) This instability of $\psi$ is eliminated by entrainment with two light pulses per cycle, one causing an advance phase shift and one causing a delay phase shift, such that the net $\Delta\phi = 0$

per cycle (panel D, $T = 24$; PPs $= 12$), the pacemaker stabilizes in such a phase relationship with the light cycle that the advance phase shift produced by one pulse is exactly compensated by a delay phase shift due to the other pulse. In practice, it is most efficient to obtain predictions of the equilibrium phase relationships by computer simulation of the entrainment process itself. [Major consequences of variation in $\tau$, $T$ and PRC, however, can also be deduced rapidly using "phase transition curves", which describe the phase to which the oscillation moves as a function of the phase when a light pulse is applied (Ottesen *et al.*, in preparation). The manipulation of phase transition curves allows us immediately to spot the phases hit by repeated light pulses in steady-state entrainment by both skeleton photoperiods and single pulse zeitgebers.].

The variation of both $\tau$ and PRC between individuals of the same species and even within individual animals, raises problems in evaluating the model. We cannot expect to predict all features of the entrained steady-state in the quantitative detail that was possible in *Drosophila* where the individual experiment – measuring $\tau$, PRC, or $\psi$ – involved populations of thousands of insects. A single estimate of any parameter of the *Drosophila* pacemaker is, in fact, an average value derived from a very large population in which the impact of individual differences is minimized. In the rodent work reported here an individual's $\tau$ was usually measured before entraining the animal, but the utility of even that precaution is limited by the extent to which the immediate prehistory and age of the animal was recorded.

In deriving predictions for some entrained steady-state we have used not only the species average period $\bar{\tau}$ but a range of $\tau$ values corresponding with the observed range of variation within species. Thus when we lack precise information on an individual's period we can at least demand that its behavior falls within limits – set by the model – given the assayed range of $\tau$-variation. There is no practical way to further elaborate prediction by allowing for individual differences in PRC shape; thus in all cases predictions were based on the species average curves (Daan and Pittendrigh, 1976a, Fig. 3).

The results obtained allow a comparison of the observation and prediction for only two of our species (*Mesocricetus auratus* and *Peromyscus leucopus*), whose periods ($\tau$) and phase response curves have been measured.

### *c) Phase Angle Difference ($\psi$) as a Function of $\tau$ and $T$*

There is a well known qualitative relationship between the phase angle difference ($\psi$) and the periods of both driving ($T$) and driven ($\tau$) oscillations. In the steady-state entrainment, the driven rhythm phase leads the driver more or phase lags the driver less, the shorter $\tau$ and the longer $T$ is (Aschoff and Wever, 1962; Hoffmann, 1963; Ottesen et al., in preparation). Figure 3 illustrates that in *P. leucopus* $\psi$ does depend on $\tau$ in the expected manner. In animal #1165, in freerun prior to its entrainment, $\tau$ was longer than 24 h (24.30 h); in #1164 $\tau$ was considerably shorter (23.55 h). When each animal was exposed to a 15′ light pulse each day ($T$ 24) they eventually were entrained to this cycle. When steady-state was reached in #1165 the light pulse, as expected, fell in the late subjective night where it effected the necessary $+\Delta\phi$ ($\psi = -12$ h). In the case of #1164 ($\tau$ short) the steady-state had radically different $\psi$(0 h); the light pulse falls in the early subjective night causing the necessary $-\Delta\phi$.

Figure 4 summarizes this relationship. In zeitgebers consisting of either one 15′ pulse or one 1 h pulse per 24 hrs, there is a significant correlation between $\psi$ and $\tau$. The 15′ points are close to prediction based on our phase response curve for *P. leucopus* which was itself derived for 15′ pulses. It should further be noted that the dependence of $\psi$ on $\tau$ is weaker for the stronger (1 h) light pulses. This is again a general property in the entrainment of self-sustained oscillations, analogous to Hoffmann's (1969) demonstration that the dependence of $\psi$ on $\tau$ in lizard

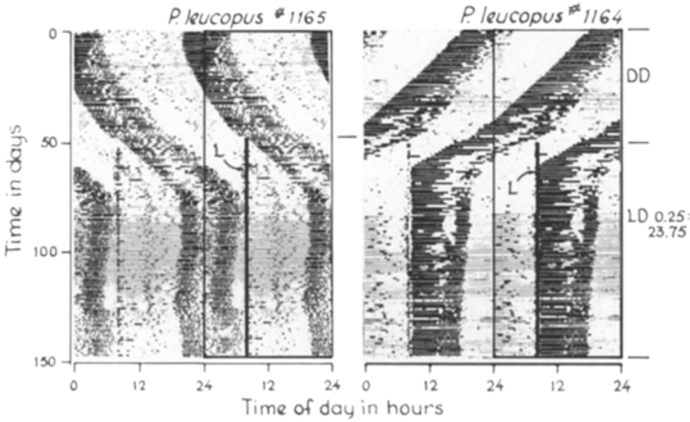

**Fig. 3.** Examples illustrating the dependence of the phase angle difference ($\psi$) on $\tau$. After an initial DD-freerun both white-footed mice were entrained by a daily 15min light pulse. In the animal with $\tau_{DD} > 24$ h (# 1165) the light fell in the entrained steady state at the end of activity, in the animal with $\tau_{DD} < 24$ h, the light fell at the onset of activity

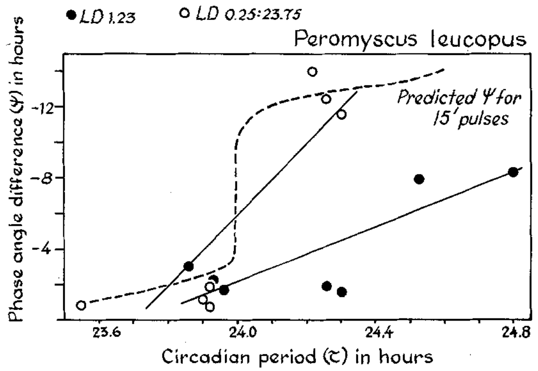

**Fig. 4.** Phase angle difference between the activity rhythm and entraining light pulses as a function of $\tau$, measured in DD prior to entrainment. Lines are linear regressions, with coefficients of correlation $r=0.80$ (1 h pulses) and $r=0.87$ (15′ pulses). The dashed curve shows the predictions for $\psi$ ($\tau$) based on the phase response curve of *P. leucopus* for 15′ light pulses

locomotor activity decreases as the amplitude of the entraining temperature cycle becomes larger.

Records of two deermice (*P. maniculatus*) (Fig. 5) illustrate the comparable dependence of $\psi$ on $T$. Animal #1607 was exposed to a light cycle with a period ($T$) of 23 h (LD 0.25:22.75), below the average $\tau$ for the species; #1507 was exposed to $T=25$ h (LD 0.25:24.75), well above the average $\tau$ for the species. Both animals were entrained by these pulse zeitgebers. With a short zeitgeber period (#1607) the entraining light pulse fell in the late subjective night, i.e., at the end of the activity time; and when $T > \tau$ (# 1507) it fell in the early subjective night, at the beginning of the activity time.

Figure 6 summarizes all our information on the dependence of $\psi$ on $T$ of such single pulse zeitgebers. Each plotted point marks the onset of activity of a single

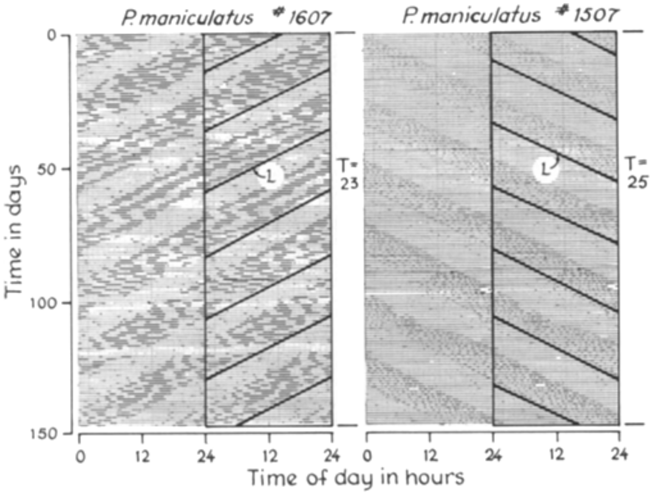

**Fig. 5.** Examples of two deermice, illustrating the dependence of the phase angle difference ($\psi$) on $T$. A 15′ light pulse recurring with a period $T = 23$ h hits the circadian rhythm in the entrained steady state at the end of activity (#1607), a pulse with $T = 25$ h (#1507), entrains the rhythm by falling close to the onset of activity

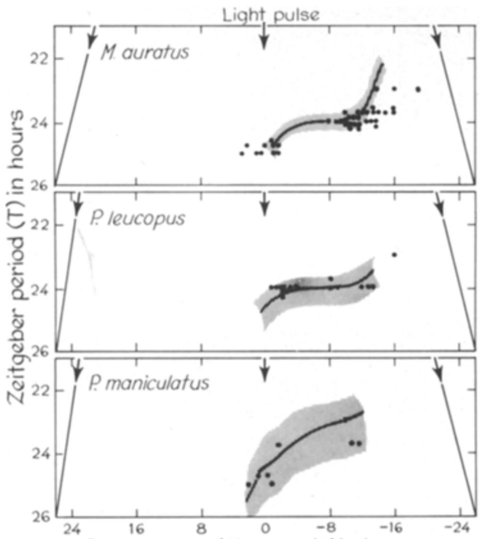

**Fig. 6.** Predicted and observed phase angle differences for entrainment by single short (15′) light pulses applied with various $T$. The distance between the two oblique lines is $2 \cdot T$. Each point represents the observed onset of activity relative to the time of the light pulse for an animal. The drawn line is the phase predicted from the species' phase response curve given the average species $\tau$. The dark area represents the predictions allowing for the known variation in $\tau$ of around the average

animal which apparently achieved steady-state entrainment by the light cycle. While three light pulses are always indicated, the middle pulse is chosen as phase-reference for the zeitgeber. The shaded areas indicate the limits based on computer predictions from the PRC within which the points should fall. The line in the middle is the predicted position for all activity onsets based on two assumptions: 1. that each animal has the species average value of $\bar{\tau}$; and 2. it also has the species average PRC. The limits of the shaded area broaden the "prediction" by allowing for variation of $\tau$ within the 95% limits of all $\tau$-values (Table 2) measured in these studies (Pittendrigh and Daan, 1976a). We have no way of broadening the limits still further by allowing for the known interindividual variation in PRC. Given the uncertainties in PRC-variation and in activity onset as a reliable measure of pacemaker phase during entrainment, we consider the observations an acceptable match to the predictions. It is further obvious that $\psi$ generally increases, becoming less negative or more positive, as $T$ lengthens.

### *d) Relative Coordination*

Table 1 summarizes the behavior of *M. auratus*, *P. leucopus*, and *P. maniculatus* exposed to various light cycles (1 pulse/cycle) greater and less than 24 hrs. The information is limited but it confirms the general prediction that in all 3 species the limits of entrainment are much closer to $\tau$ than is the case in *D. pseudoobscura.* In that insect the PRC yields both $+\Delta\phi$ and $-\Delta\phi$ values of about 6 hrs that still fall in the "usable" part of the curve where the slope lies between 0 and $-2$; and, in fact, *D. pseudoobscura* can be driven, with 15 min pulses, to about $T_{18}$ and $T_{30}$, respectively (Pittendrigh, 1966). In none of the rodents we tested (Table 1) does our best estimate of the species average PRC yield usuable $\Delta\phi$ values of more than 3 h.

It is often difficult to decide whether an animal really was entrained by a light-dark cycle. In some cases, the system appears entrained for a long time, but then breaks away from the light, the activity band crosses the light pulse, and again becomes apparently entrained for a number of cycles (Fig. 8, #1669,

**Table 1.** Ranges of entrainment. The numbers of animals showing stable entrainment, unstable entrainment ("bouncing") and failing entrainment ("relative coordination") are shown for light cycles with 15′ light per cycle, (or, in $T24$, either 15′ or 60′ light per cycle)

| Species | $T23$ | $T23.75$ | $T24$ | $T24.75$ | $T25$ |
|---|---|---|---|---|---|
| *M. auratus* | | | | | |
| Entrained | 3 | 9 | 22 | 5 | 6 |
| Bouncing | – | – | – | – | – |
| Rel. coord. | 8 | – | – | 1 | 7 |
| *P. leucopus* | | | | | |
| Entrained | 1 | 1 | 13 | – | – |
| Bouncing | – | – | 7 | – | – |
| Rel. coord. | – | – | – | 1 | 2 |
| *P. maniculatus* | | | | | |
| Entrained | 1 | 3 | – | 2 | 2 |
| Bouncing | 1 | – | – | – | – |
| Rel. coord. | 1 | – | – | 1 | 1 |

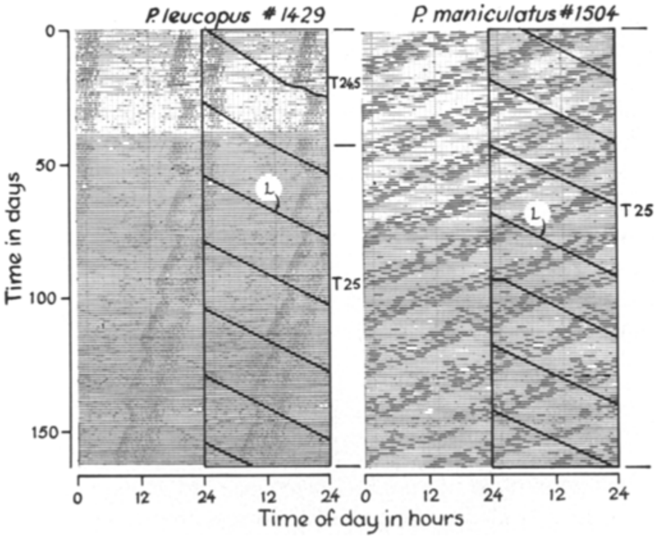

Fig. 7. Examples of relative coordination between a circadian activity rhythm and a long-$T$ light cycle outside the range of entrainment

1671). With the exception of animal #1504 (Fig. 7), all cases of failure to entrain (Figs. 7, 8) are characterized by the now familiar phenomenon of "relative coordination" (von Holst, 1939; Enright, 1965), which was also described by Swade and Pittendrigh (1967) as an "oscillatory freerun". The recurring impact of the zeitgeber on the rhythm is visible as a regular beat phenomenon.

### e) *The Compression of Activity Time*

When entrainment nearly succeeds one sees most clearly a phenomenon probably characteristic of all entrainment to cycles in which $T$ is different from $\tau$. Figure 8 illustrates this "compression of $\alpha$" (Pittendrigh, 1974). When $T$ is 23 h (Fig. 8, top) the light pulse comes to fall in the late subjective night, effecting the necessary phase-advances, while entrainment is nearly achieved. The cut-off of activity shifts forward with the same slope as the light cycle; the onset, however, has a period which, while shorter than that of the animals' freerun, is longer than 23 h. Thus onsets and cut-offs come steadily closer until the activity time ($\alpha$) is compressed, in some cases almost to vanishing point when the whole rhythm clearly "breaks free" from any entrainment at all. The light pulse then begins to fall in the pacemaker's dead zone, and $\alpha$ promptly decompresses, expanding back to a duration close to that characteristic of the freerunning system. However, as the early subjective day and the steadily advancing light pulse converge, $\alpha$ is again compressed when the light pulse repeatedly phase advances the end of $\alpha$ more than it succeeds in advancing the onset. Comparable $\alpha$-compression can be seen when the "beat frequency" is higher in a zeitgeber with period $T = 25$ h (Fig. 8, #1719, 1725).

Figure 9 demonstrates that a compression of $\alpha$ occurs even when entrainment succeeds. In this case *M. musculus* (#1071) was entrained by a steadily length-

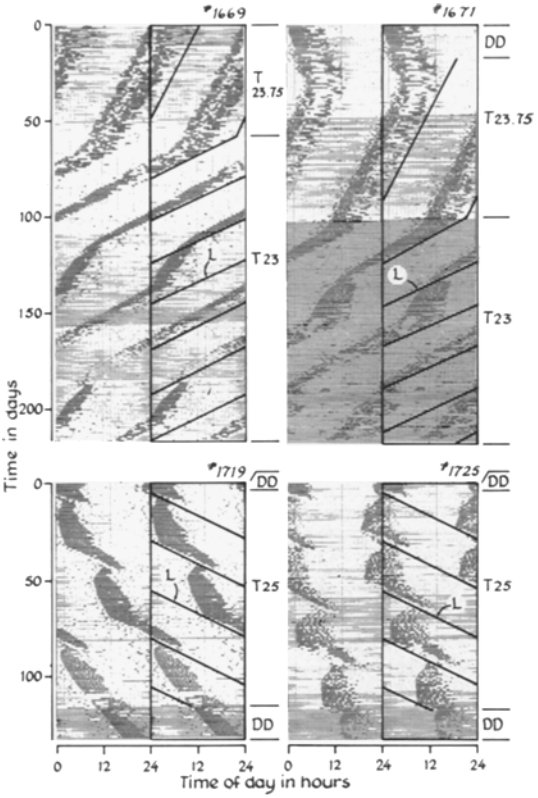

**Fig. 8.** Examples of failing entrainment of golden hamsters by single 15′ light pulses given in periods of *T*23 and *T*25. Animals #1669 and #1671 were initially entrained to *T*23.75. Entrainment to *T*23 produced a gradual decrease in activity time (α), until the light pulse broke through the activity and the animals were temporarily unentrained. This happened twice in both animals. Loss of entrainment was accompanied by a gradual increase (decompression) of α. Animals #1719 and #1725, in *T*25, showed similar compression and decompression of α associated with the "beats" in relative coordination

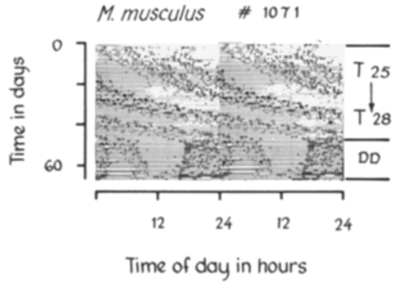

**Fig. 9.** Gradual decompression of α after entrainment of *Mus musculus* # 1071 by an LD 14:14 cycle. After release in DD, α increased from 16 h to 20 h in 14 days by the drifting apart of two clearly distinguishable components

ening light cycle up to $T = 28$ h before being released into DD. Decompression promptly followed release from the daily phase delay of the activity onset. It should be noted that the light cycle in this experiment contained a long photoperiod (14 h of light in $T=28$), which allowed entrainment far beyond the range of zeitgeber-periods with single short light pulses. Finally we note here that some smaller $\alpha$-compression is sometimes also observed when $T$ equals 24 h.

### *f) The "Bouncing" Phenomenon*

A quite different case of unstable entrainment is illustrated by the three white-footed mice *(Peromyscus leucopus)* in Figure 10. All of them initially freeran with a period greater than 24 h. Their first encounter with the light pulse either failed to cause any $\Delta\phi$ because it fell in the "dead zone" (#1172) i.e., the part of the cycle where the pacemaker is virtually insensitive to light, or caused a phase-delay which drove the pacemaker away from the light pulse which then, falling

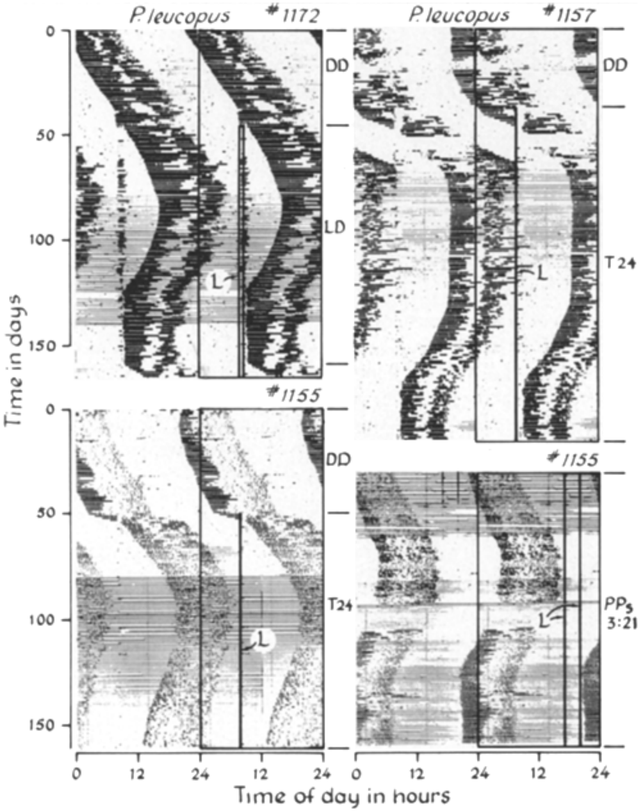

**Fig. 10.** The "bouncing" phenomenon (1). Four activity records of white-footed mice, showing unstable entrainment by very short photoperiods. After an initial freerun in DD, with $\tau$ longer than 24 h, the rhythms are entrained by a single 15′ light pulse, falling at the end of the activity time. As $\tau$ becomes shorter, the activity "bounces off" and drifts forward every day until entrainment is achieved again, now with the light pulse occurring in the early subjective night. *P. leucopus* #1155 showed two such bounces, the second one in a short skeleton photoperiod (PPs 3)

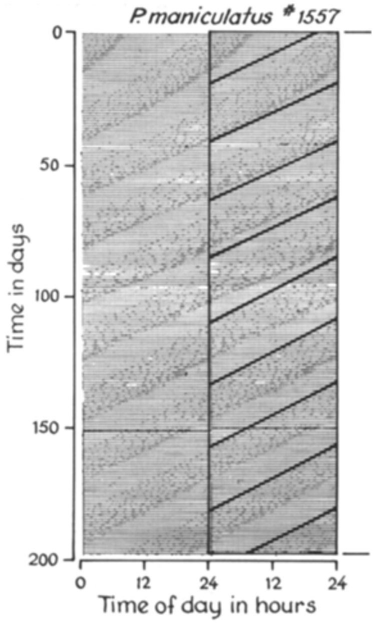

**Fig. 11.** The "bouncing" phenomenon (2). Example of a deermouse, in which $\psi$ changed during prolonged entrainment to an LD 0.25:22.75 cycle. In the first 50 days, $\tau$ was apparently larger than 23 h, and in steady state entrainment activity end locked on to the light pulse. Then $\tau$ became shorter than 23 h and from about day 120 onwards, the onset of activity locked on to the light pulse

in the dead zone, was without effect. Eventually, in all cases, the pacemaker's motion "to the right" brought the end of activity (and hence the phase-advance part of its PRC) into coincidence with the light pulse. The result, in #1157, was entrainment to the light cycle for about ~60 cycles; in #1155 the apparent entrainment was briefer; and in #1172 it was not maintained at all. Thus in all 3 animals the phase-advance effected by the light pulse had an after-effect (Pittendrigh and Daan, 1976a) which made $\tau$ shorter; the pacemaker moved "to the left" and freeran so long as the light pulse fell in the PRC's dead zone. Secondary entrainment then developed when the light pulse fell on the phase-delay section of the PRC, near activity onset. Figure 12 summarizes this interpretation. A fourth case of "bouncing" in animal #1155 in a skeleton photoperiod of two 15′ light pulses, 3 h apart, is shown also in Figure 10 (lower right).

Figure 11 gives an equally clear case of this "bouncing" phenomenon in *P. maniculatus*. The animal was exposed to a cycle ($T = 23$ h) of 15′ pulses every 23 h. At the beginning its own $\tau$ was greater than 23 h and it became entrained when the light pulse was coincident with the phase-advance section of the PRC at the end of the activity. The repeated phase-advances caused by the succession of single pulses evidently imposed an after-effect of $\tau$ which, after nearly 100 cycles of entrainment, became less than 23 h. The animal then freeran with $\tau < 23$ until by day ~120 entrainment was again effected by the light pulse causing phase-delays at the beginning of the subjective night (Fig. 12).

The general lability of $\tau$ that underlies such after-effects of phase-shifts makes it nearly impossible to maintain a true steady-state when entrainment involves only 1 pulse per cycle; and this surely contributes to the variance of observed $\psi$ values recorded in Figure 6.

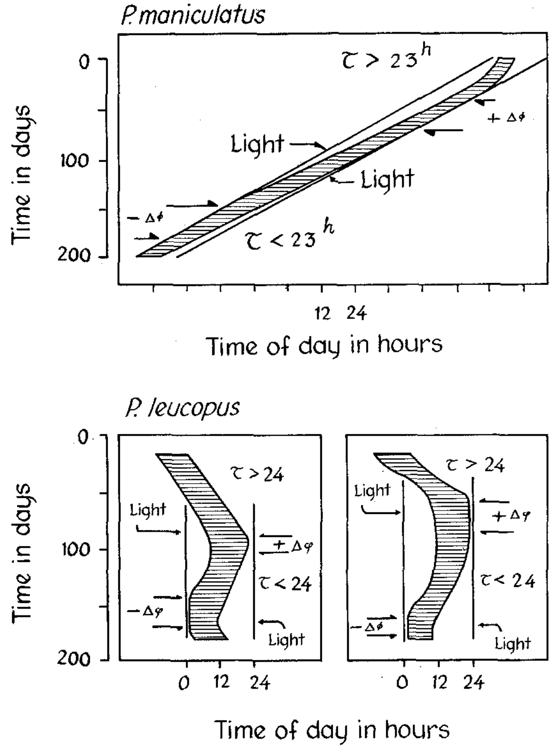

**Fig. 12.** Interpretation of the "bouncing" phenomenon illustrated in Figure 10 and 11 for *P. maniculatus* (#1557) and *P. leucopus* (#1172, left; and #1157, right). See text

### g) Entrainment by Skeleton Photoperiods

We have examined the dependence of $\psi$ on skeleton photoperiods (PPs) holding $T$ constant at 24 h. The animals (19 *M. auratus* and 19 *P. leucopus*) were subjected to entrainment initially by a single pulse per cycle, and then by a succession of cycles involving 2 pulses. In such a protocol, activity typically occurs during only one of the two dark intervals between the light pulses. After an animal had reached apparent steady-state entrainment by a given PPs one of the pulses was shifted to generate a new PPs. Generally, this was done by shifting the pulse falling at the end of the activity time, i.e., at the beginning of the subjective day, forward. Figure 13 gives a partial record for two animals illustrating these protocols. When the experiments were initiated (1963) there was uncertainty that 15′ pulses would be effective phase-shifting signals in these rodents. It was decided, therefore, to use 15′ pulses on some animals and 60′ pulses on others. In Figure 13, #1168 is being driven by 60′ pulses and #1154 by 15′ pulses.

The behaviour of *P. leucopus* (#1154) is qualitatively characteristic of that of all animals in this protocol. The initial freerunning $\tau$ was less than 24 h. The 15′ pulse was initiated at a time when it fell in the dead zone of the pacemaker's PRC; the rhythm continued to move to the left until the light pulse, falling in

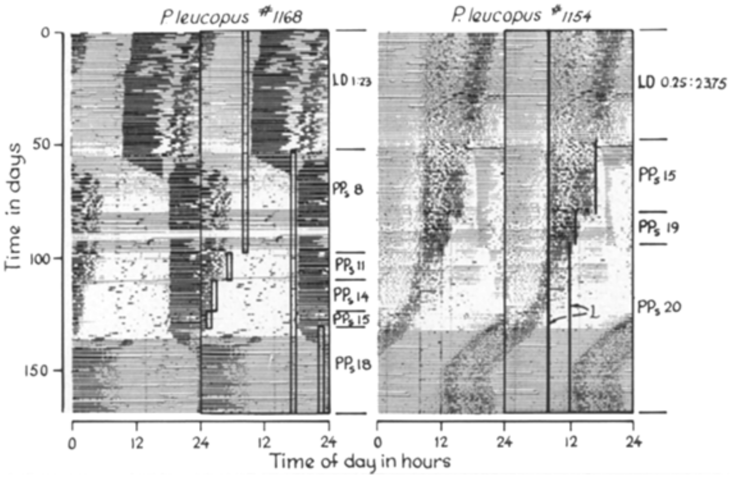

**Fig. 13.** Examples of the experimental protocols used to assay the phase relationship between activity rhythms and "skeleton photoperiods". White vertical bars indicate 1 h pulses, black bars 15′ light pulses (100–200 Lux) in otherwise DD. By decreasing the interval between the two light pulses comprising the activity time $\alpha$ is compressed. Eventually the rhythm performs a "phase jump" to the longer pulse interval and $\alpha$ expands. In #1168 the phase jump was via delay transients, at PPs 18, in #1154 the phase jump was via advance transients, at PPs 20

the early subjective night caused phase-delays. We again see a compression of $\alpha$ as the light pulse held activity onsets to $T=24$ but failed, at first, to stop the advance of activity cut-offs. A second light pulse is then initiated 9 h after the first (PPs 15); it falls in the late subjective night and advances the activity cut-offs—$\alpha$ is further compressed. Thirty-three days later the second pulse is advanced by 4 h, it occurs 5 h after the first and again compresses $\alpha$ still further. The "night" defined by these 2 pulses has been reduced to 4.75 h (PPs 19). When it is reduced to 3.75 h (PPs 20) there is a dramatic change. The activity time apparently cannot be compressed any further, and it "jumps" from the short to the long interval between the two light pulses. After a period of apparent freerun it reaches a new steady-state dependent on a $-\Delta\phi$ caused by the "same" pulse which previously caused $+\Delta\phi$. Such $\psi$-jumps have long been known in the *Drosophila* case (Pittendrigh, 1966).

In #1168, to which 60′ pulses were applied, there is again a compression of $\alpha$ as the pacemaker initially is entrained by a single pulse that effects a phase delay every 24 h. When the second pulse was initiated, it immediately forced the pacemaker into a $\psi$-jump. It assumes a $\psi$ in which the end of the second pulse is taken as sunset, creating $-\Delta\phi$. The original pulse (now marking the end of the night) is then subjected to a series of successive advances which reduce "night" length. Each reduction in night length compresses $\alpha$. It is significant that the second shift of that pulse step already reduces night length to the same value which originally induced a phase-jump. The second time it does not; the only difference is that the second experience was approached gradually. We return to this fact, found in many animals, in later discussion. The final reduction in night length to 6 h (PPs 18) is too much and the pacemaker again phase-jumps assuming

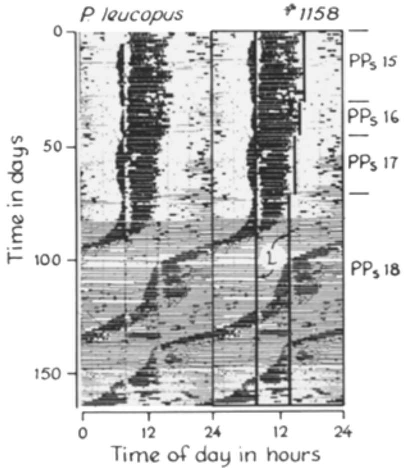

**Fig. 14.** Oscillatory patterns close to the limits of entrainment (PPs 15–17) and "relative coordination" beyond the limit of entrainment (PPs 18) in a white-footed deermouse in skeleton photoperiods

stable entrainment to the cycle with the longer interval between pulses serving as "night".

It is clear from these and all our other results with skeleton photoperiods, that the activity can occur in either of the two intervals between the light pulses and never is spread out over both. In asymmetric skeletons, i.e., when $\mathrm{PPs} \neq 12$, the situation with activity in the long interval is always more stable. Yet, by gradually increasing the asymmetry one can compress $\alpha$ into the short interval up to a certain limit. We have become used to speaking of the "minimum tolerable night" for the shortest interval into which activity can be compressed.

The behaviour of *P. leucopus* (#1158) recorded in Figure 14 is of interest in two respects, reflecting the impact on entrainment of after-effects on $\tau$. During the first 80 days of the experiment it underwent a series of $\alpha$-compressions as the short "nights" to which it was entrained were successively shortened. Its activity onsets always slightly anticipated the light pulse functioning as sunset; they show a clear oscillation, comparable to the behaviour DeCoursey (1972) has recorded for *Glaucomys volans*. It is as though the greater $+\Delta\phi$ effected each time the "morning" pulse is advanced has a transient after-effect shortening $\tau$, thus making $\psi$ even more positive. That after-effect decays more rapidly than it does in a freerun presumably because of the daily $-\Delta\phi$'s which continue to be adequate to maintain a "steady-state". When a final advance of the morning pulse reduced night length to 6 h the pacemaker broke away from entrainment and was never recaptured by the light cycle whose only effect is a pronounced "relative coordination". It is as though the impact of the final short "nights" (long skeleton photoperiods) had such a pronounced after-effect *(shortening $\tau$)* that the period of the pacemaker (characterized in this species by a low-amplitude PRC; Daan and Pittendrigh, 1976a) had been driven beyond the limits of entrainment for this particular Zeitgeber.

Figures 15 and 16 summarize all the information we have on the dependence of $\psi$ on PPs ($T=24$ h) in both *M. auratus* and *P. leucopus*. The lines representing the morning and evening pulses are the midpoints of each pulse (15′ or 1 h) whose

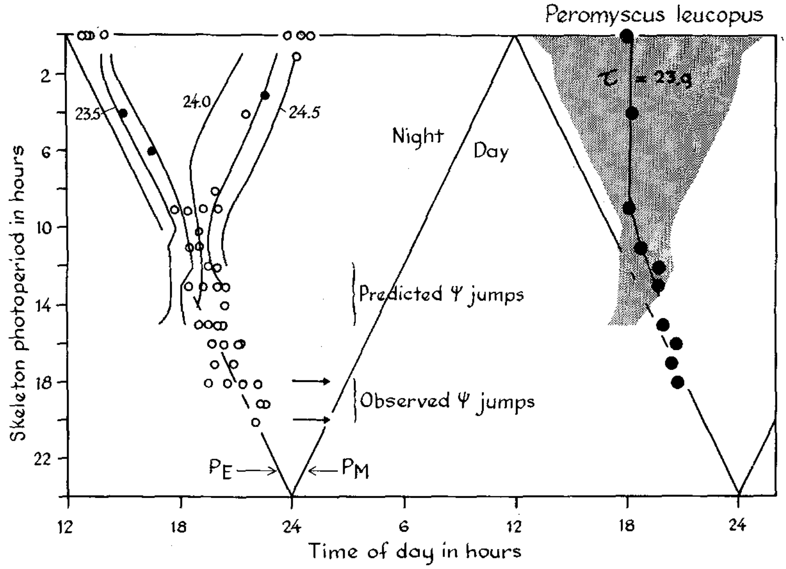

**Fig. 15.** *(Peromyscus leucopus).* The phase-relationship ($\psi$) between activity onsets and skeleton photoperiods. Each photoperiod is created by two 15 min pulses ($P_M$ and $P_E$) every 24 h. *Left:* activity onsets as open circles; solid circles are animals forced into a $\psi$-jump from the complementary long photoperiod indicated by arrows below. The plotted curves are onset times predicted from the model; using five values of $\tau$ (differing by 0.25 h) that cover ~95% of the known interindividual variation in the species. The species average PRC was used in all computations. Each predicted curve extends down to the longest photoperiod before the $\psi$-jump occurs for that value of $\tau$. *Right:* average values for each photoperiod are indicated by the solid circles; the line fitted to them is the model's prediction for $\tau = 23.9$

brief periods are ignored in defining the skeleton photoperiods they create. The left half of Figure 15 shows all the observed phases of activity onset of white-footed mice in various skeleton photoperiods consisting of two 15′ light pulses. The curves are computer-predictions obtained from the phase-response-curve of *P. leucopus*, using five different values of $\tau$ (23.50, 23.75, 24.00, 24.25, 24.50). These lines extend downward to somewhere between PPs 12 and PPs 15 h. According to the simulations, the pacemaker should "jump" to the longer interval between the pulses when the interval where activity occurs is further compressed. The observed $\psi$-values demonstrate that stable phase relationships with much longer skeleton photoperiods are possible: $\psi$-jumps were actually observed in the range between PPs 18 and PPs 20. Equilibrium $\psi$-values in animals after such a jump are shown as black dots. In the right half of Figure 15 the average $\psi$ for every PPs is indicated, together with the range of predicted values for $\tau$'s of 23.5 to 24.5 h. The line connecting the averages coincides best with predictions for $\tau$ = ca 23.9 h, close to the species average of 24.01 h. While, generally, the ranges of predicted and observed $\psi$-values match each other closely up to PPs to 14, the model fails obviously beyond these photoperiods. It is apparently possible by further compression of the activity time to entrain the animals to longer skeleton photoperiods than expected on the basis of a single pacemaker with a fixed phase

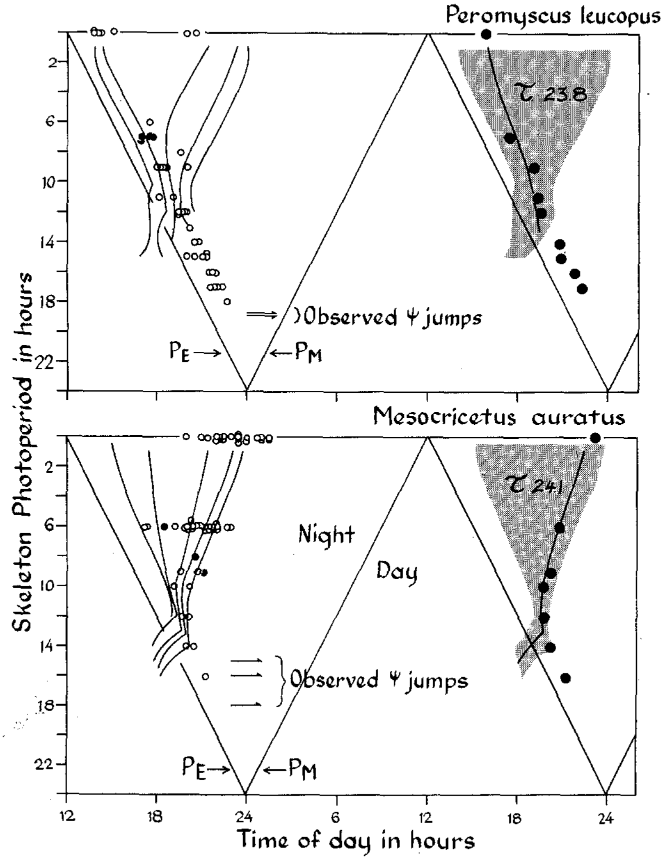

Fig. 16. ψ as a function of skeleton photoperiod duration in *P. leucopus* and *M. auratus*. Skeleton photoperiods defined by 1 h pulses whose midpoints are given by the lines $P_M$ and $P_E$. Other conventions as in Figure 15. The increased "zeitgeber strength" of the 1 h pulse is reflected in the movement of *leucopus* onsets closer to $P_E$ than was the case (Fig. 15) with 15 min pulses. The difference between the species is clear and expected given their average PRCs and $\bar{\tau}$ values

response curve. We return to the meaning of this discrepancy between observation and the model's prediction in the following paper (Pittendrigh and Daan, 1976b).

While we expected less agreement between prediction based on 15′ phase response curves and observation in 1 hr skeleton photoperiods, the trend (Fig. 16) is not much different from Figure 15. Again the observations do not exceed the range of prediction in *P. leucopus* except for skeleton photoperiods beyond 14 h (Fig. 16, upper panel). Hamsters (Fig. 16, lower panel) undergo a phase jump at shorter PPs than *P. leucopus*, and hence show a somewhat better agreement with the model. The model's prediction of a significant interspecies difference (using $\bar{\tau}$ and $\overline{\text{PRC}}$) is well matched by the slopes of the average ψ's for the two species.

### h) Evaluation of the Model

How well can the non-parametric model of entrainment be evaluated in terms of the experiments now summarized? There are several reasons why – using individual rodents – one cannot confront prediction with a body of observation comparable in precision and scope to the facts available for *Drosophila.* Assays of all the relevant parameters ($\tau$, PRC and $\psi$) are based on averages from populations of thousands of pacemakers in the insect case, and potentially major individual variations are thereby obscured. These, however, are sensitively reflected in every rodent experiment. Moreover, individual variations from $\bar{\tau}$ cannot always be established prior to a test and when they are there remains the complication of $\tau$'s lability including its susceptibility to change by the act of entrainment itself. The individual's PRC is never known.

Given the limitations so imposed we judge the results reported to be substantial evidence that the non-parametric model developed for *Drosophila* is a powerful tool in explaining the entrainment of our rodents. Thus for *P. leucopus* and *M. auratus* the *species average* values of $\psi$, as a function of skeleton photoperiod, are in remarkably good agreement (Fig. 16) with the model's predictions (including the interspecific difference) based on species averages for $\bar{\tau}$ and $\overline{\text{PRC}}$. Further, the model itself predicts the greater scatter of $\psi$ values for the shorter photoperiods: the efficacy of the 2 pulse interaction in minimizing the variability of $\psi$ is expected (see Fig. 25) to increase under longer photoperiods where the absolute value $|\Delta\phi|$ of the interacting phase-shifts is greater. The model also accounts well for the observed variation in $\psi$ in animals driven by cycles involving only one pulse (Fig. 4): the variation is directly correlated with the observed differences in the individual's $\bar{\tau}$.

There are two aspects of the data which are less well accounted for by the model. While it certainly predicts that the range of $T$ values to which the rodents will entrain should be smaller than in *Drosophila* (and it is), we cannot readily explain the numerous cases of failure to entrain even to $T = 23$ h and $T = 25$ h (Table 1) unless there is indeed substantial inter-individual variation in PRC amplitude. Nevertheless, that possibility cannot be dismissed and the observation of relative coordination (vs. outright entrainment) at those $T$ values in some animals is not of itself a sufficient basis to reject the model, although it severely limits its usefulness for the prediction of *individual* behaviour.

The second problem it faces is the success of many individuals of *P. leucopus* to entrain to much longer photoperiods than the model predicts (Fig. 15). Here again we cannot reject the possibility that individual variation in PRC is involved but the number of exceptions makes that both unlikely and an unesthetic escape. On the other hand there is good reason to expect this behavior in *P. leucopus* and not – as observed – in *M. auratus.* The model's predictions are based on $\bar{\tau}$ and $\overline{\text{PRC}}$ values of individuals freerunning in DD, but the *P. leucopus* pacemaker (as distinct from that of *M. auratus*) is known to be strongly susceptible to after-effects (Pittendrigh and Daan, 1976a; and see Fig. 9 in Pittendrigh and Daan, 1976b). Thus it seems likely that the experimental protocol we used, in which the duration of the entraining photoperiod was only gradually increased, served to impose on this more labile pacemaker a significant change in $\tau$ and an as-

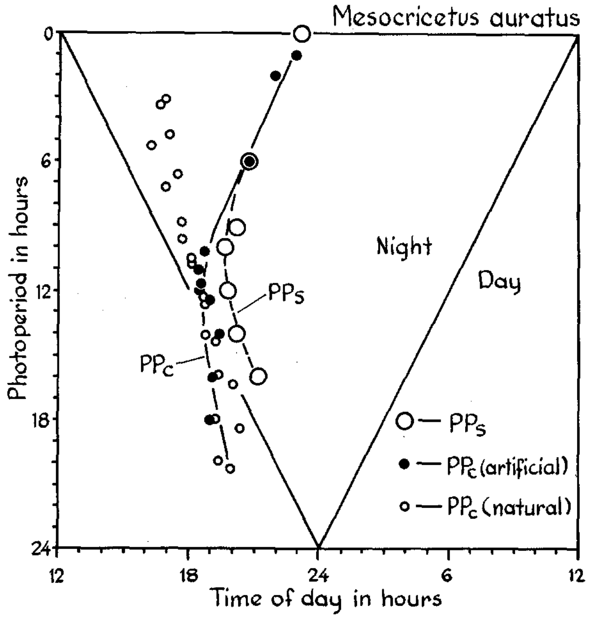

**Fig. 17.** The simulation of complete photoperiods by skeleton photoperiods in *M. auratus*. See text

sociated change in PRC shape. That interpretation is supported by the behavior of animal #1158 (Fig. 14) which, when it did reach a maximum tolerable photoperiod had its $\tau$ so shortened by the long skeleton photoperiods to which it had previously entrained, that when it eventually escaped from control by the long photoperiod, it would not entrain even to the shorter alternative at $T=24$ h. Clearly the model, which assumes *known* values of $\tau$ and PRC shape, cannot be rejected by behaviors in which $\tau$ is known to have changed but to an unknown extent: the model may be inadequate in this respect but not necessarily invalid.

Thus far we have asked only if a knowledge of $\tau$ and PRC yields an explanation of entrainment by the same short pulses used to measure the PRC. We think it does with the qualifications noted. There is, however, a quite distinct sense in which we can ask if the model is relevant to entrainment in the real world: how well do skeleton photoperiods (comprising two short pulses) simulate the action of complete (uninterrupted) photoperiods? Figure 17 makes the appropriate comparison using *M. auratus*, which is the only species for which the necessary data are available. The mean $\psi$'s for skeleton photoperiods are taken from Figure 16. We thank J.F. Elliott for permission to include his measurements of $\psi$ observed when hamsters were exposed in the laboratory to complete photoperiods (Elliott, 1974). The extent to which skeletons simulate complete photoperiods in hamsters is much the same as in *Drosophila:* it is good for all photoperiods up to ~12 h; less good thereafter. And again complete photoperiods protect the animal against the $\psi$-jump which purely non-parametric control imposes on the pacemaker when PPs becomes as long as 15 h. In a 12 h skeleton photoperiod additional light, either as multiple pulses or as a complete photo-

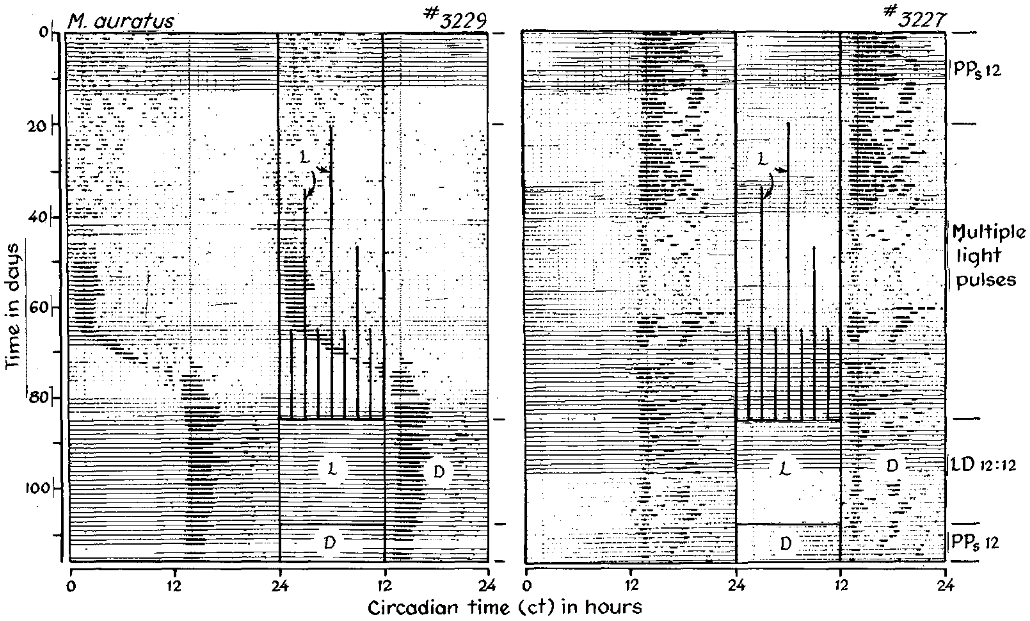

**Fig. 18.** A comparison of rodent behaviour in complete and skeleton photoperiods. The activity records of two hamsters, exposed to the same protocol, are shown: After initial entrainment to a skeleton photoperiod, with 15′ light pulses, spaced 12 h apart, (PPs 12), more light pulses were added in one of the two dark intervals. Then the light was given during this whole interval, and finally, the original PPs 12-schedule was re-established. Animal #3229, which had its activity between 0 and 12, was clearly affected by the extra light pulses, falling in its subjective night, and was finally forced into a phase jump into the other dark interval. Hamster #3227, being active between 12 and 24 h, received the extra light in its subjective day and its rhythm was not noticeable affected. In the lower part of the panels there is no obvious difference in phasing before and after the transition from complete (LD 12:12) to skeleton (PPs 12) photoperiod

period, has no effect whatsoever on the phase of the animal's rhythm when given during the subjective day (Fig. 18, right panel) – although the same light given during the active phase (subjective night) forces the animal into the opposite interval (Fig. 18, left panel). The parametric effect of light during the subjective day, though adequate to protect against the $\psi$-jump, is small.

In Figure 17 we also included $\psi$ values for hamsters exposed to completely natural light cycles throughout the year at the Messaure Biological Station in northern Sweden (Daan and Aschoff, 1975, Fig. 21). These data are less in agreement with skeleton photoperiod measurements than those from artificial photoperiods. In natural daylight, especially at the high latitude (66°42′ N.L.) where these data were obtained, there is of course ambiguity as to which moments of the light-dark cycle should be compared with artificial "lights-on" and artificial "lights-off". Any choice of reference point (we used the beginning and end of civil twilight, i.e., sun positions of 6° below the horizon) is arbitrary and affects both the photoperiod and the $\psi$-axes in the graph.

There is another, important respect in which the data from Messaure must be qualified: the experimental animals had no opportunity to escape to a dark retreat during the daylight hours. Nor had Elliott's animals when exposed to artificial complete photoperiods in the laboratory. Virtually nothing is known about the behavior of our nocturnal rodents in nature as it bears on the question of how much light they see and at what times of day. If, as we suspect, and preliminary data (Pittendrigh and Domingos, in preparation) suggest, they retreat to dark nests soon after sunrise and emerge only much later (just before sunset) the real mechanism in nature indeed rests crucially on the interaction of morning phase-advances, evening phase-delays and their net interaction with $\tau$. Thus a knowledge of $\tau$ and PRC provides a biologically adequate basis for elucidating the essential features of how the pacemaker reaches a steady-state phase-relation to the external world, although we have to accept the restrictions posed by the extent to which variations in $\tau$ and PRC, both spontaneous and induced by the entrainment process itself, can be known.

## III. The Clock-like Properties of the Pacemaker

### *a) The Empirical Regularities*

In the course of these studies we have encountered several empirical regularities about the pacemakers of our nocturnal rodents including:

1. In constant darkness (DD), their period ($\bar{\tau}$) is generally $\leqq 24$ h.

2. There is a trend among the species for PRC shape, expressed as the difference (D-A) between the areas under delay and advance sections of the curve, to change as $\bar{\tau}$ changes; the shorter $\bar{\tau}$ the greater D-A.

3. There is a similar dependence of D-A on $\bar{\tau}$ between individuals within the species.

4. There are indications of a comparable change in phase shift responses as the individual pacemaker's $\tau$ is changed.

5. The stability of $\bar{\tau}$, as expressed by a reduction in its standard deviation, increases the closer $\bar{\tau}$ is to 24 h.

Several of these regularities are new and sufficiently clear to invite interpretation. We have previously noted (Pittendrigh and Daan, 1976a; Daan and Pittendrigh, 1976a) that we believe the rules have functional significance in the context of entrainment phenomena and have now concluded that the non-parametric mechanism accounts for at least the major features of entrainment in our rodents. In subsequent sections we explore some general properties of that mechanism in part by purely analytical considerations and in part by computer simulation of entrainment using model PRCs. Our purpose is to ask whether and how our empirical rules concerning the interdependence of $\tau$, its stability, and PRC shape contribute to one or the other of two theoretically distinguishable clock functions that must be served by circadian pacemakers in their entrained steady-state.

### b) *Two Separable Clock Functions*

#### 1. Measuring the Lapse of Time: The Homeostasis of $\tau$ and Non-parametric Entrainment

The explicit treatment of circadian rhythms as reflecting the presence of daily clocks in animals emerged from the discovery of time-compensated sun-orientation (Kramer, 1950; von Frisch, 1950). Here the principal functional prerequisite which focused attention was the necessity to measure a lapse of time – to compensate for change in the sun's azimuth through the course of the day. It was this function which provoked Pittendrigh (1954) to re-examine the reported temperature dependence of pacemaker frequency and find it was, in fact, temperature-compensated. The more general homeostasis of pacemaker frequency that has since been recognized was also considered principally in this context (Pittendrigh and Caldarola, 1973). In short, an oscillator is useful to measure durations of time only if its angular velocity is reasonably stable from cycle to cycle. It may well be that this particular clock function has made non-parametric entrainment preferable in general: the essence of all parametric mechanisms is a modulation of angular velocity entailing variation in the estimation of the same time intervals.

#### 2. Recognizing Local Time: The Conservation of $\psi$

Circadian pacemakers dictate a preprogrammed pattern of some (not all) metabolic events that is appropriately structured in its temporal sequence to match the daily march of changing conditions in the external world. They create an "internal day" which is repeatedly enacted even in an aperiodic environment. We may well ask (e.g. Enright, 1970) why natural selection has elaborated this strategy of a self-sustaining oscillator whose time-course paces the sequence of metabolic change to cope with the challenge of the daily cycle of environmental conditions. The predictability of that cycle must be largely responsible: while the continuing change of conditions throughout the day is a challenge, its predictability provides an opportunity that natural selection has exploited in developing a temporal program whose utility can be relied upon day after day. Preprogramming itself is, therefore, no riddle; but why has selection gone further to assure its *automatic* re-initiation as soon as the program is finished? Automatic re-initiation is what makes the system an "oscillator" – why do it? A full answer involves more, but surely includes as a major component benefits that derive from the entrainability of all self-sustaining oscillators. A daily program is useless (indeed disadvantageous) unless it can be phased correctly to local time. Thus it is the phase-control, more than the period control, inherent in entrainment which is the principal dividend selection has reaped in converting a daily program into an oscillator by assuring its automatic re-initiation: it is by achieving the entrained steady-state that the pacemaker recognizes local time, because the transformation of $\tau$ to $T$ which characterizes that entrained steady-state occurs only when the pacemaker assumes a unique phase relation ($\psi$) to its zeitgeber.

The adequacy of phase-control has been largely taken for granted but there are, we think, many peculiarities of circadian pacemakers which relate directly to this crucial issue of maintaining a stable phase-relation ($\psi$) between the pacemaker and the external world; those features are what give circadian pacemakers a uniqueness distinguishing them from the plethora of other biological oscillators, and they include the empirical rules about $\bar{\tau}$, PRC and their interdependence we have reported.

In subsequent paragraphs we address two quite distinct "threats" to a stable phase-relation ($\psi$) between pacemaker and external world. The first is the residual instability of $\tau$; this threat exists under any given entraining cycle although, as we show, it is more acute under short photoperiods than longer photoperiods. The second is the systematic, seasonal, change in the wave-form of the entraining light cycle, or more specifically, the seasonal change in photoperiod. These two challenges to conservation of an optimal $\psi$ raise different problems and are treated separately.

### *c) The Conservation of $\psi$: The Problem of $\tau$-Instability*

Figure 19 will serve to define one class of problems involved in the conservation of $\psi$. Other variables affect the phase-relation between a simple harmonic oscillator (with period $\tau$) and a second (with period $T$) that drives it, but the ratio ($\tau/T$) of their freerunning periods plays a major role. The figure illustrates the familiar fact that $\psi$ is positive when $\tau/T<1$ and becomes negative when $\tau/T>1$. The feature most relevant here, however, is that the slope of the dependence of $\psi$ on $\tau$ (holding $T$ constant as it is in nature) is maximal when $\tau$ equals $T$: Thus $\psi$ not

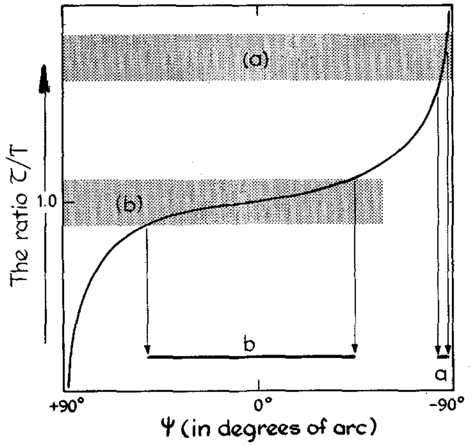

**Fig. 19.** The dependence of the phase-angle-difference, $\psi$, between two oscillators on their periods $\tau$ and $T$. The period of the driver ($T$) is held constant and the period of the slave ($\tau$) is varied. When the ratio $\tau/T$ is less than 1.0 $\psi$ is positive; it becomes negative when $\tau/T>1.0$. The figure considers the effect on $\psi$ of given amount of instability of $\tau$; in case (a) the instability is around a $\bar{\tau}$ value far from $T$ and the consequent $\psi$-variation is small. The same degree of $\tau$-instability when $\bar{\tau}=T$ is considered in case (b), where the consequent $\psi$-instability is much greater

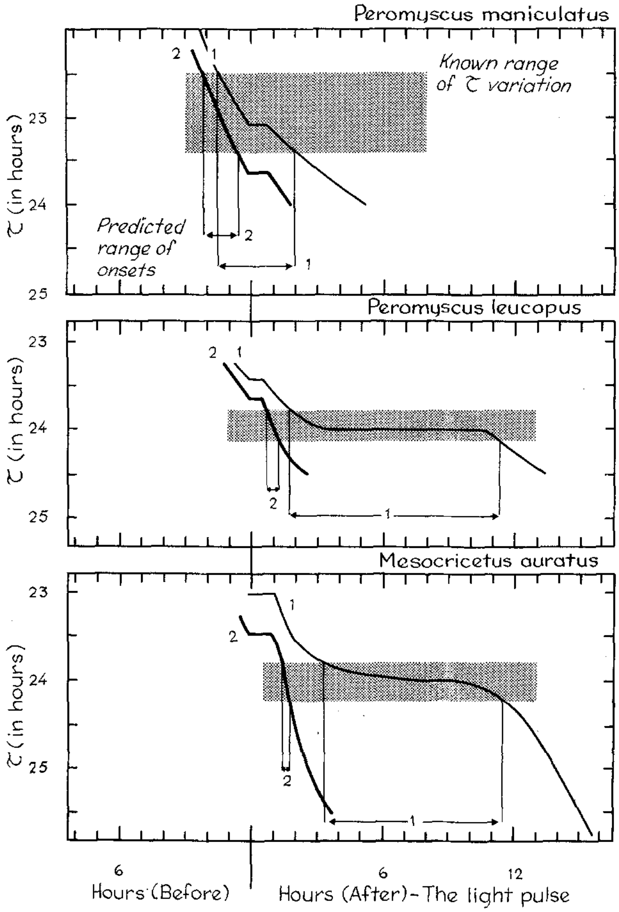

**Fig. 20.** Predicted ranges of variation in the phase of activity onset based on (a) the known range of variation in $\tau$ and (b) the species' average phase response curves. Variation in $\psi$ is much smaller with entrainment by two pulses (2) than with entrainment by one pulse per cycle (1)

only reflects but *amplifies* the consequences of minor fluctuations in $\tau$ when the oscillator is close to resonance with its driver. If the shape of the curve in Figure 19 bears any relation to the dependence of $\psi$ on $\tau$ in the circadian case, where we are of course not dealing with simple harmonic oscillators, then the homeostasis of $\tau$ takes on added meaning. An oscillator with a frequency sufficiently stable to measure the lapse of time (some fraction of the cycle) within tolerable limits can evidently still be inadequate as a useful clock: if its average period ($\bar{\tau}$) matches $T$ precisely, minor fluctuations of $\tau$ around its mean may be acceptable in estimating duration of time but wholly unacceptable because of the fluctuations they generate in the system's estimate ($\psi$) of local time.

Figure 20 shows that this issue is, in fact, relevant in the circadian case. Using measured PRCs of three of our rodents, we have computed the phase angle difference ($\psi$) between pacemaker and light pulse holding $T$ constant at 24 h

(1 pulse/cycle) and varying $\tau$. Again $\psi$ greatly amplifies $\tau$ variation when $\tau/T$ is close to 1.0. The cause, in these circadian cases, is clear: there is a large fraction of each pacemaker's subjective day where the magnitude of the phase shifts effected by light pulses is extremely small. In *P. leucopus* there is indeed a completely "dead zone" of several hours where light has no detectable effect (Daan and Pittendrigh, 1976a, Fig. 2). When the animal is being driven by a single light pulse in each cycle (with $T=\tau$), if *any* fluctuation around $\tau$ is to be compensated by the light pulse $\psi$ must change by the length of the PRC's dead zone. The "bouncing" phenomenon (Fig. 10) is a dramatic illustration of this sensitivity of $\psi$ to small $\tau$-fluctuation.

The computed behavior of the three real circadian pacemakers entrained by single pulses raises the following general question: How, given the mechanism we are evaluating, can $\psi$ be made sufficiently stable to render the pacemaker a useful sensor of local time as distinct from its function in measuring the lapse of time?

The first of several possible contributions to the stabilization of $\psi$ is the homeostasis of $\tau$ itself: that well-known characteristic of a circadian pacemaker is crucial to both of its clock functions. Moreover, one of the empirical regularities we have found (Pittendrigh and Daan, 1976a) in comparing the pacemakers of our four species immediately takes on meaning in this context. The homeostasis of $\tau$ is tighter the closer $\bar{\tau}$ is to 24 h, thus reducing the hazards of $\psi$ instability which that particular $\bar{\tau}$ value entails. It is as though selection pressure for the homeostasis of $\bar{\tau}$ is most stringent when the resonance effect on $\psi$ is involved and relaxed somewhat when $\bar{\tau}/T$ gets away from unity (Fig. 21 and Table 2).

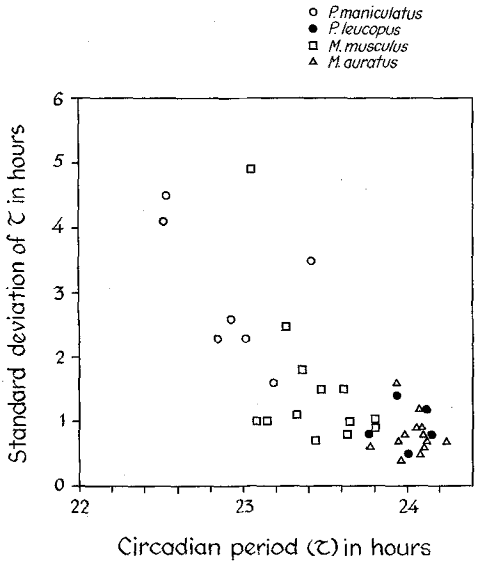

**Fig. 21.** Variability of $\tau$ plotted as a function of average $\tau$ in four species of rodents. Results from the experiments in which phase response curves for brief light pulses were obtained (Daan and Pittendrigh, 1976a)

**Table 2.** Variation in $\tau$ in constant darkness. All values in h

| | $\bar{\tau}$[a] | 95% range[a] | Mean s.d. $\tau$[b] |
|---|---|---|---|
| *M. auratus* | 24.07 | 23.75–24.35 | 0.08 |
| *P. leucopus* | 24.01 | 23.60–24.60 | 0.09 |
| *M. musculus* | 23.50 | 23.70–24.50 | 0.15 |
| *P. maniculatus* | 23.36 | 22.15–24.65 | 0.30 |

[a] From Figure 2 in Pittendrigh and Daan 1976a
[b] From Table 4 in Daan and Pittendrigh 1976b

There is a related alternative strategy: $\psi$ is the more stable (for a given degree of $\bar{\tau}$ instability) the further $\bar{\tau}/T$ is away from unity (Fig. 19). Thus we are tempted to the conclusion that the very *circadian* nature of $\bar{\tau}$ itself has functional significance in the conservation of $\psi$; that the discrepancy between $\bar{\tau}$ and $T$ is not so much tolerable error as a positive element in the system's design reducing instability in the phase of its entrained steady-state. While this is probably true, the full functional meaning of the circadian nature of $\tau$ emerges only later when we consider the impact on $\psi$ of seasonal change in photoperiod.

The third and quite the most important feature of the entrainment mechanism contributing to the stabilization of $\psi$ is that in nature the steady-state is almost surely created by an interaction of two signals in each cycle, not one. Figure 20 includes, for the three pacemakers studied, the computed dependence of $\psi$ on $\tau$ variation when the entraining cycle ($T=24$ h) includes two pulses 12 h apart (PPs *12*). The impact on $\psi$ of $\tau$ instability is greatly reduced. Again the cause is clear: the steady-state now depends primarily on the interaction of positive and negative phase-shifts which are large compared to the variation in $\tau$.

Before leaving this point we should note that with one exception (*Pilobolus*; Bruce et al., 1961) all the known PRCs of circadian pacemakers involve both phase-advances and phase-delays. The fact that in all cases advances are generated in the late subjective night (sunrise) and delays in the early subjective night (sunset) is an analytic necessity: but it is not an analytic necessity, and hence not trivial biologically, that both advances and delays are elicited. Many oscillators – including, as a biological example some neurons – can only be phase-delayed, or advanced, not both. We suggest that the involvement of two-pulse interaction in generating the entrained steady-state of circadian pacemakers is a non-trivial feature of these oscillators contributing significantly to the conservation of $\psi$ and thus enhancing their clock function.

There is one more remarkable feature of the pacemaker that bears on the stabilization of its phase relation to any given light-cycle. That is the finding that even within the individual animal, PRC shape is dependent on $\tau$ (Daan and Pittendrigh, 1976a). In the experiments reported we changed the period of an individual animal exploiting, as a tool, the after-effects of prior entrainment. The $\Delta\phi$ generated as ct 15 (3 circadian hours after activity onset) was assayed when the pacemaker's $\tau$ was shorter and longer than its average $\bar{\tau}$. The phase delay generated was greater when $\tau$ was shorter. While several detailed interpretations of its cause are possible, the phenomenological reality has obvious functional significance: a contribution to $\psi$ conservation in the face of $\tau$ variation is made by a systematic, compensatory, change in the pacemaker's PRC.

### *d) The Conservation of $\psi$; The Problem of Changing Photoperiod*

#### 1. Model Pacemakers; Intrinsic Properties of the Non-parametric Mechanism

The residual instability of frequency which its homeostasis permits is not the only problem – nor for that matter the most challenging – in conserving an appropriate phase between a circadian pacemaker and the external world. Outside of the tropics organisms must cope with seasonal variation in the pattern of the external day. And, though predictable, that seasonal change presents challenges of its own. There are several issues to be addressed in this context. How, for example, can a single preprogram be written to match a wide repertoire of different daily patterns? (See Pittendrigh and Daan, 1976b). We shall, however, restrict present discussion to only one of the questions raised: What is the impact on $\psi$ of seasonal change in the length of photoperiod in each daily cycle? If it threatens to impair some appropriate phase relation ($\psi$), what adaptive adjustments in pacemaker parameters ($\tau$, PRC shape, and general lability) will offset the adverse effect?

We have pursued this question using model circadian pacemakers with simply characterized PRCs (Fig. 22). They are all Type-1 (Winfree, 1970; Ottesen et al., in preparation). The absolute value of the slopes we used, as well as the circadian times at which they change, were set to match what is commonly encountered in the PRCs of real circadian pacemakers. The slopes of the two "usable" parts of each PRC, where the slope is negative and less than $-2$, are designated $S_M$ (the section where positive phase-shifts are generated in the morning) and $S_E$ (negative phase-shifts in the evening). Entrained steady-states for various values of $\tau$, and a full range of 2-pulse (skeleton) photoperiods, were obtained by computer simulation.

It is our purpose, by varying the $\tau$ and PRC shape of these model pacemakers to find general properties intrinsic to the non-parametric mechanism that will shed light on the functional meaning of the empirical rules we listed in Section III, a.

#### 2. Premises and Definitions: $\psi$-Day and $\psi$-Night

In exploring the intrinsic properties of the entrainment mechanism relative to seasonal change we have adopted, as starting premise, the assumption that for diurnal forms the goal is to conserve activity-onset close to sunrise and for nocturnal forms to keep activity-onset near to sunset. We use $\psi_D^*$ and $\psi_N^*$ respectively, to denote the phase-relation, relative to the light-cycle, of day-active and night-active organisms. We recognize that species will surely differ in precisely how close to sun-up or sun-down it is optimal to time activity onset but such differences do not detract from the validity of our analysis. Thus in computing and discussing $\psi_D^*$, the phase of the entire subjective-day is defined by its mid-point (ct 6) and that of the subjective night by its mid-point (ct 18); and the "real phase" (activity onset) of some nocturnal species, e.g., is then obtainable by subtracting some appropriate

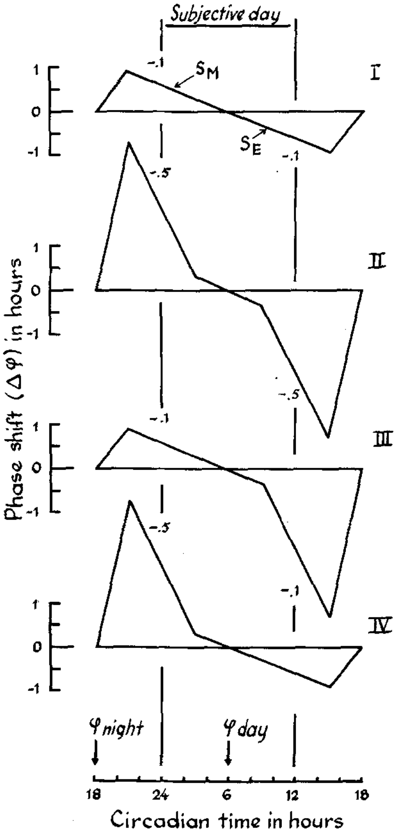

**Fig. 22.** The Phase Response Curves of four model circadian pacemakers whose entrainment behavior is reported in Figure 23. The values of curve slopes in the morning ($S_M$) and evening ($S_E$) are indicated

constant, such as 5 or 6 h depending on the species, from the phase (ct 18) of the midpoint of the subjective night. Clearly we choose sunrise as phase-reference for the light cycle in discussing diurnal forms and sunset in the case of nocturnal forms.

Other notations as follows, facilitate description of the issues involved: (a) $P_M$ and $P_E$ denote morning and evening light pulses; (b) $\phi_m$ and $\phi_e$ denote the phases (circadian times) of the pacemaker at which $P_M$ and $P_E$ fall in steady-state; (c) $\phi_m^*$ and $\phi_e^*$ denote the phases to which the pacemaker is immediately shifted by the morning and evening pulses; (d) $+\Delta\phi_m$ and $-\Delta\phi_e$ are the phase-shifts caused by $P_M$ and $P_E$ falling at $\phi_m$ and $\phi_e$ respectively; (e) $\phi_D$ (=ct 6) and $\phi_N$ (=ct 18) are our phase-reference points for the subjective day and subjective night respectively. In the graphic representations of entrainment they are marked as $\phi_D^*$ and $\phi_N^*$ after they have been displaced in time by the preceding light pulse; (f) finally we use $\psi_N$ to denote the phase-relation between $P_E$ and $\phi_N$, but $\psi_N^*$ for the relation of $P_E$ and $\phi_N^*$. A similar distinction is made between $\psi_D$ and $\psi_D^*$ (Fig. 23).

### 3. Pacemaker Variables Affecting the Conservation of $\psi_D$ and $\psi_N$

Figure 23 summarizes the response of one of our simplest model pacemakers (II in Fig. 22) to changing photoperiods; the shape is fully symmetric and its period ($\tau$)

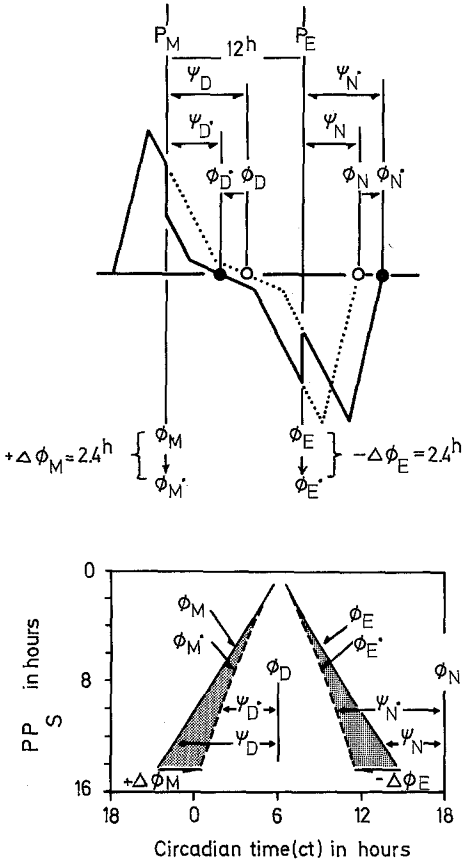

**Fig. 23.** The differential responses of the "Subjective Day" and "Subjective Night" to changing photoperiod. *Upper Panel:* Model pacemaker #II entrained to a 12 h skeleton photoperiod. The morning pulse ($P_M$) falls at $\phi_m$ (=ct 22.8) and phase-shifts ($+\Delta\phi_m=2.4$ h) the pacemaker to $\phi_m^*$ (=ct 1.2). The whole of the subjective day, marked by ct 6 ($=\phi_D$) is consequently pulled towards dawn (phase-advanced) by 2.4 h. On the other hand the evening pulse ($P_M$) falling at $\phi_E$ (=ct 13.2) phase-delays the pacemaker to $\phi_E^*$ (=ct 10.8) and consequently delays the whole subjective night. *Lower Panel:* The solid lines $\phi_m$ and $\phi_e$ mark the circadian times at which $P_M$ and $P_E$ fall on the pacemakers if photoperiod is increased from 1 h to 14 h. $\psi_D$ and $\psi_N$ inevitably change (are not conserved) as photoperiod changes; but the phase advance of $\phi_m$ to $\phi_m^*$ and the delays of $\phi_E$ to $\phi_E^*$ have the effect of partially conserving $\psi_D^*$ and $\psi_N^*$. Thus $\psi_D^*$ is always less than $\psi_D$; $\psi_N^*$ always greater than $\psi_N$

is set equal to $T$(=24 h). The upper panel illustrates the steady-state realized under a 12-hour photoperiod (PPs). The phase-shifts caused by the light pulses ($P_M$ and $P_E$) are illustrated: $\phi_m$ goes to $\phi_m^*$, $\phi_D$ to $\phi_D^*$, etc. A central feature of the mechanism is immediately clear: the morning pulse, causing a $+\Delta\phi$, advances the subjective day, while the evening pulse, causing a $-\Delta\phi$, delays the subjective night. Thus, while an increase in photoperiod duration necessarily changes (fails to conserve) $\psi_D$ and $\psi_N$ (lower panel), the impact of increasing photoperiod on $\psi_D^*$ and $\psi_N^*$ is much less: *some* contribution to the conservation of $\psi_D^*$ and $\psi_N^*$ is an intrinsic

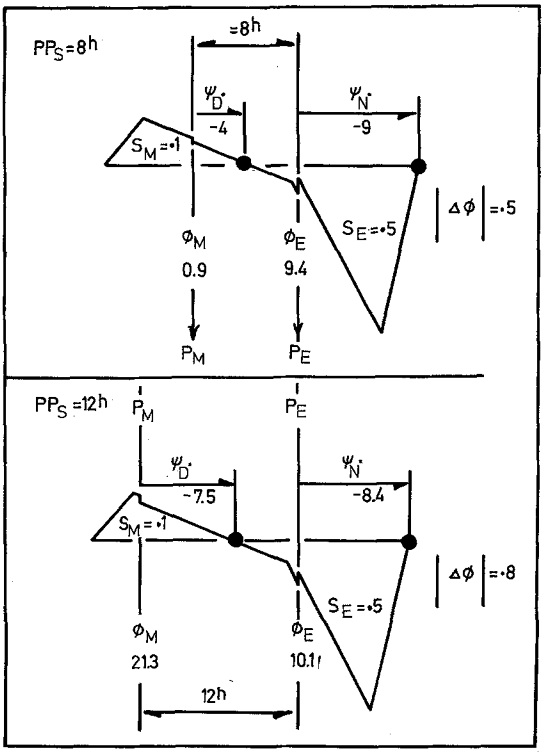

**Fig. 24.** The conservation of $\psi_N^*$ by introducing asymmetry into the PRC. In model pacemaker #III, the slope ($S_M = 0.1$) of the phase advance section of the curve is less than the slope of the phase-delay section ($S_E = 0.5$). When photoperiod is increased from 8 h to 12 h the inescapable increase in $|\Delta\phi|$ is effected by a small displacement of $\phi_E$ (from ct 9.4 to ct 10.1) because $S_E$ is steep; but $\phi_m$ suffers a major displacement (from ct 0.9 to ct 21.3) because $S_M$ is small. The consequence is good conservation of $\psi_N^*$ and very poor conservation of $\psi_D^*$

property of the mechanism whose central feature is an interaction by morning phase advances and evening phase delays.

It is obvious that the steeper the slope (or the higher the amplitude) of the PRC the better are $\psi_N^*$ and $\psi_D^*$ conserved through a wide range of photoperiods. The compensation remains poor, however, no matter how much one increases PRC slopes ($S_M$ and $S_E$) where the morning and evening pulses fall; and, moreover, this unqualified strategy has its own inherent disadvantage: it shortens the maximum photoperiod to which the pacemaker will entrain before being forced into a $\psi$-jump (Fig. 24).

There are, however, two quite different ways in which the behavior of the system can be markedly improved.

The first is by introducing asymmetry into the shape of the PRC, as in #III in Figure 22. With both $\tau$ and $T$ set again at 24 h its response to change in photoperiod is illustrated by Figure 24. Since $\tau = T$ the sum of the phase-shifts caused by the two pulses ($P_M$ and $P_E$) must be zero; and, hence $+\Delta\phi_m$ must equal $-\Delta\phi_e$. The difference in the slopes of phase advance ($S_M$) and phase-delay ($S_E$) sections

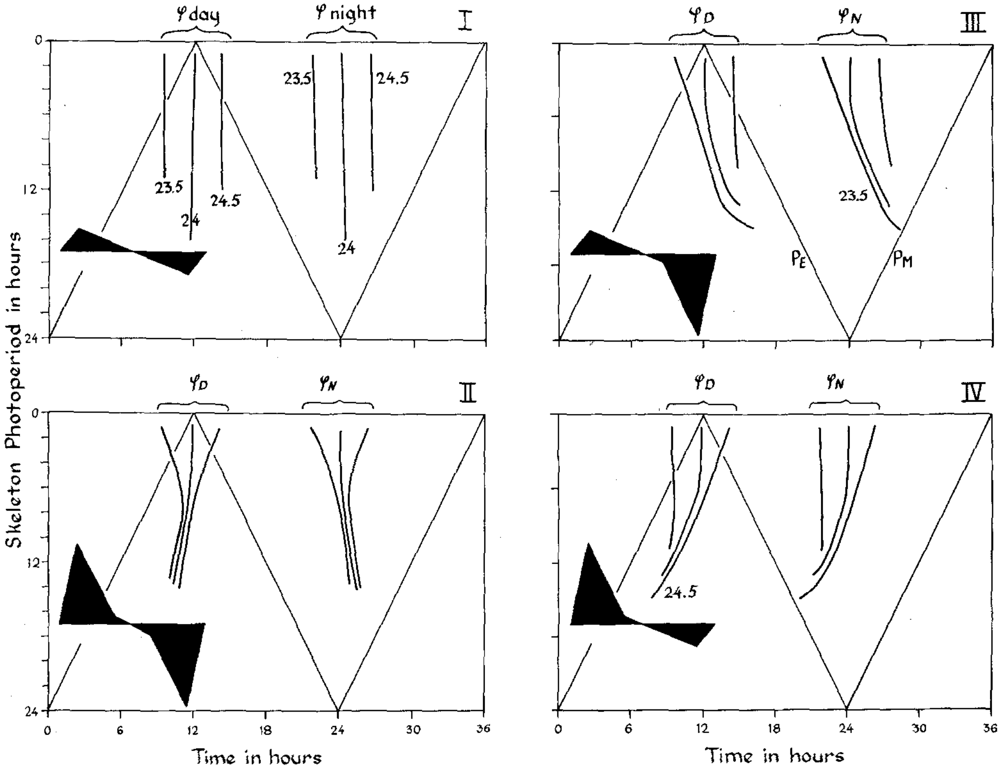

**Fig. 25.** The entrainment behavior of the model pacemakers shown in Figure 22. The curves plot the steady-state phase of ct 6 (marking the pacemaker's subjective day, $\phi_{\mathrm{DAY}}$) and ct 18 (its subjective night, $\phi_{\mathrm{NIGHT}}$). For each pacemaker (whose PRC is shown) steady-states were computed for three values of $\tau$ (23.5; 24.0; 24.5). The oblique lines mark the morning ($P_{\mathrm{M}}$) and evening ($P_{\mathrm{E}}$) pulses that create each skeleton photoperiod; $T = 24$ h

of the PRC has an obvious consequence: when the photoperiod is increased the absolute value $|\Delta\phi|$ of each of the two interacting phase shifts increases; but the difference in slopes ($S_{\mathrm{M}}$ and $S_{\mathrm{E}}$) of the advance and delay sections of the PRC causes an asymmetric displacement on the circadian scale of the times ($\phi_{\mathrm{m}}$ and $\phi_{\mathrm{e}}$) at which the morning and evening pulses must fall to generate equally increased $|\Delta\phi|$ values. The greater slope ($S_{\mathrm{E}}$) of the delay section of the curve yields the necessary increment in $-\Delta\phi_{\mathrm{e}}$ with minimum displacement of $\phi_{\mathrm{e}}$ (the time at which $P_{\mathrm{E}}$ falls): on the other hand to generate the necessary increment in $+\Delta\phi_m$ the time ($\phi_{\mathrm{m}}$) of the morning pulse ($P_{\mathrm{M}}$) has to shift – because $S_{\mathrm{M}}$ is small – much more. The net effect is – compared to PRC #II in Figure 23 – a clearly enhanced stabilization of $\psi^*_{\mathrm{N}}$; but this enhancement is obtained at the expense of $\psi^*_{\mathrm{D}}$, which is now not conserved at all. It is clear that a reciprocally asymmetric PRC (with $S_{\mathrm{M}} > S_{\mathrm{E}}$) will conserve $\psi^*_{\mathrm{D}}$ at the expense of $\psi^*_{\mathrm{N}}$ (Fig. 25).

The conservation of $\psi$ can also be improved by adjusting the pacemaker's period as distinct from its PRC shape (Fig. 25). The principal effect on our model pacemakers of setting $\tau$ different from $T(=24\,\mathrm{h})$ is seen in the range of shorter

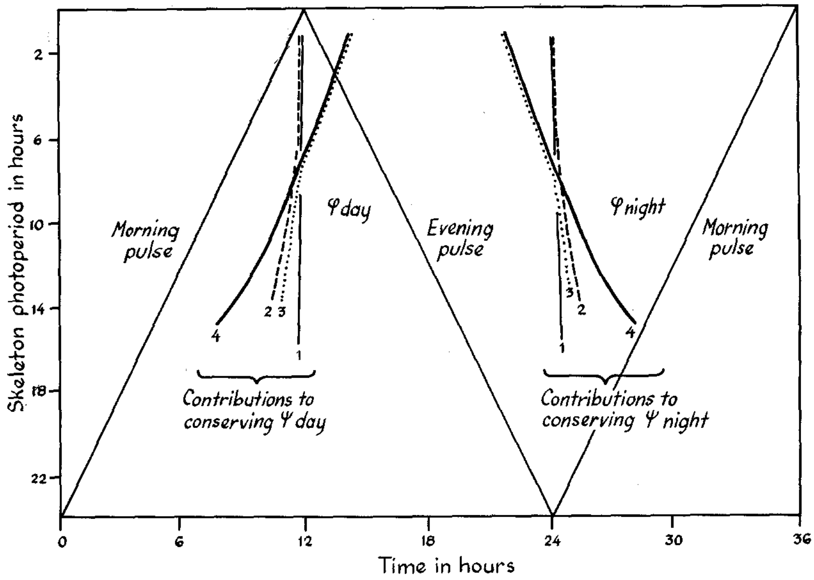

**Fig. 26.** Components in optimal strategies for the seasonal conservation of $\psi$ in diurnal and nocturnal organisms entrained non-parametrically. For the diurnal case the phase of ct 6 (midpoint of the subjective day) is plotted; the phase of ct 18 (its midpoint) marks the subjective night. In each case ($\phi_{\mathrm{DAY}}$, $\phi_{\mathrm{NIGHT}}$) curve 1 is the behavior of model pacemaker I characterized by low amplitude of the symmetrical pacemaker II (Curve 2) reduces the change in both $\psi_{\mathrm{D}}$ and $\psi_{\mathrm{N}}$ when photoperiod increases (cf. Fig. 23). The benefit of increased PRC amplitude is only significant at longer photoperiods. Curve 3 is the consequence of changing $\tau$ while still keeping the PRC (Model II) symmetrical. $\psi_{\mathrm{D}}$ is improved especially, now, in the shorter photoperiods when $\tau > 24$ h; $\psi_{\mathrm{N}}$ is improved when $\tau < 24$ h. Curve 4: $\psi_{\mathrm{D}}$ becomes nearly invariant throughout the range of photoperiods when $S_{\mathrm{M}} > S_{\mathrm{E}}$ and $\tau > 24$ h (PRC IV in Fig. 22); $\psi_{\mathrm{N}}$ is optimally conserved when $S_{\mathrm{M}} < S_{\mathrm{E}}$ and $\tau < 24$ h (PRC III in Fig. 22). That combination of strategies also increases the maximum tolerable photoperiod, after which a $\psi$-jump occurs

($< 6$ h) photoperiods; here the steady-state derives from the interaction of small $\Delta\phi$'s because the two light pulses, falling close together, hit the PRC where its amplitude is small and, in our models, its slopes (both $S_{\mathrm{M}}$ and $S_{\mathrm{E}}$) are equal. The effect of setting $\tau$ less than $T$ amounts, itself, to a daily phase-advance equal to $T-\tau$ and when the entrained steady-state is reached the evening pulse must cause a $-\Delta\phi_{\mathrm{e}}$ that is greater (by an amount $T-\tau$) than the $+\Delta\phi_{\mathrm{m}}$ caused by the morning pulse. This disparity in the $|\Delta\phi|$ required from the two pulses demands that the evening pulse be shifted to the right (increasing $-\Delta\phi_{\mathrm{e}}$) and thus conserving $\psi_{\mathrm{N}}$ and, *a fortiori*, $\psi_{\mathrm{N}}^{*}$.

The effective phase-delay ($-\Delta\phi$) caused by setting $\tau > T$ has a comparable (but reciprocal) consequence in diurnal forms: it demands a relative increase in the $+\Delta\phi_{\mathrm{m}}$ caused by the morning pulse. To effect a larger $+\Delta\phi_{\mathrm{m}}$ the morning pulse ($P_{\mathrm{M}}$) must occur earlier on the circadian time scale and thus enhance the conservation of both $\psi_{\mathrm{D}}$ and, especially, $\psi_{\mathrm{D}}^{*}$.

There is a second dividend in setting $\tau \neq T$. Figures 25 and 26 show that when the "diurnal strategy" is adopted of setting $S_{\mathrm{M}} > S_{\mathrm{E}}$ the maximum photoperiod to which the pacemaker will entrain (before being forced into a $\psi$-jump) is increased when $\tau$ is lengthened. Conversely when $S_M < S_E$ (the most effective strategy to conserve $\psi_N$) the maximum photoperiod is extended by setting $\tau$ less than $T$. Thus the two principal contributions to conserving any appropriate $\psi$ (either $\psi_D$ or $\psi_N$) complement each other in enhancing another aspect of the pacemaker's (non-parametric) entrainment.

Figure 25 summarizes the behavior of all four of the pacemakers whose PRCs are given in Figure 22. For each pacemaker there are two sets of three curves: one set ($\phi_D$) plots the phase of ct 6, the other plots the phase ($\phi_N$) of ct 18. The three curves in each set are derived from computations in which the pacemaker is given $\tau$ values of 23.5, 24.0 and 24.5 h. The termination of each curve marks the maximum photoperiod to which the pacemaker will entrain before being forced into a "$\psi$-jump". $P_E$ and $P_M$ are the lines marking evening and morning light pulses respectively. Combining the shape of PRC #III with $\tau < T$ (23.5) gives the best conservation of $\psi_{NIGHT}$ (heavy curve); the shape of PRC #IV and $\tau > T$(24.5) gives the best conservation of $\psi_{DAY}$.

Figure 26 summarizes the three adjustments of pacemaker parameters ($\tau$ and PRC shape) which make independent, additive, contributions to maximizing the conservation of $\psi_D$ and $\psi_N$ respectively.

### *e) Conservation of $\psi$: Nocturnal Strategies*

#### 1. Adjustment of $\bar{\tau}$ at the Species Level

The responses of model pacemakers to changing photoperiod duration provide the analytic tools necessary for further functional analysis of pacemaker properties in our nocturnal rodents. Along with the value of their average periods ($\bar{\tau}$), their PRC's are reproduced in Figure 27 in a form comparable to those (Fig. 22) of our models.

Both major strategies contributing to the conservation of $\psi_{NIGHT}$ are conspicuous. (1) $\tau$ is never significantly greater than 24 h; it is clearly much less than that in two of the four species; and (2) there is a comparable trend for $S_E$ to exceed $S_M$.

We have summarized all the established measurements of $\bar{\tau}$ for diurnal and nocturnal animals in an earlier paper (Daan and Pittendrigh, 1976b). That current listing re-emphasizes a statistical trend that was an original component of his rule which Aschoff (1960) subsequently regarded as less important than the pacemaker's response to constant light. But the fact is that in the majority of diurnal forms $\tau$ (when measured in DD) exceeds 24 h and in nocturnal forms it is less than 24 h. The behaviour of our non-parametrically entrained models leaves us with little doubt that this empirical regularity reflects widespread adjustment of $\bar{\tau}$ as one adaptive modification of the pacemaker to conserve $\psi$, in the face of seasonal change, either to sunrise ($\bar{\tau} > 24$) or sunset ($\bar{\tau} < 24$).

We noted earlier that in setting $\bar{\tau}$ away from $T$ selection would gain a stabilization of $\psi$ in the face of inherent $\tau$ instability. This is true for analytic reasons and the fact, also reported by Aschoff et al. (1971) for birds and man, that the standard deviation of $\tau$ steadily falls as the mean $\bar{\tau}$ approaches 24 h clearly encourages this interpretation of the circadian nature of pacemaker period – it compensates for

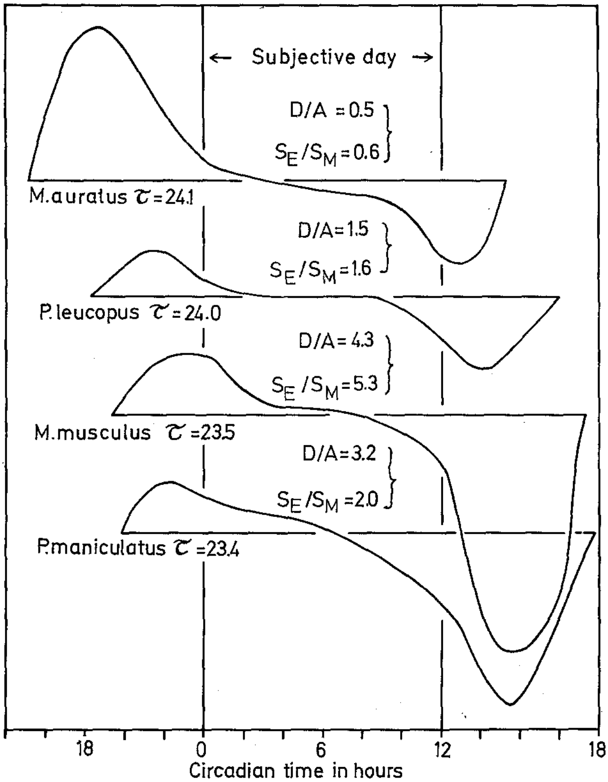

**Fig. 27.** The PRCs of *M. auratus*, *P. leucopus*, *M. musculus* and *P. maniculatus*. Redrawn from the data of Figure 3 in Daan and Pittendrigh (1976a). $D/A$ = area under delay section of curve/area under advance section $S_M$ = average (negative) slope of advance (morning) section of curve. $S_E$ = average (negative) slope of delay (evening) section of curve

(or tolerates) a greater degree of $\tau$-instability. However, there is a wholly distinct selective advantage in setting $\bar{\tau}$ away from 24 h: it enhances the conservation of $\psi$, rendering it more nearly constant throughout the annual cycle of changing photoperiod. The circadian nature of their periods is more than tolerable error in the design of circadian pacemakers; it is a positive contribution to their competence to recognize local time (conserve $\psi$) not only in the face of unpredictable $\tau$-instability (a minor gain) but wholly predictable seasonal change in daylength (the major gain).

## 2. Adjustment of PRC at the Species Level

The second feature characteristic of the pacemakers in our nocturnal rodents was initially described in terms of the areas under the delay (D) and advance (A) sections of the PRC. With the exception of the hamster, D is larger than A in our rodents. We also reported a consistent trend both between and within species for D-A to increase as $\tau$ shortened, and, stimulated by this "rule", we sought and found evidence of a similar change in $\Delta\phi$ responses when $\tau$ of an individual pacemaker was systematically shortened or lengthened (Daan and Pittendrigh, 1976a). This common trend – between species, between individuals and within the individual – may well reflect a truly general aspect of pacemaker structure in our rodents but its function is not necessarily the same in all three cases.

First the behaviour of model pacemakers leads us to expect that D will usually be larger than A in nocturnal species generally. It is, of course, not the two areas (D and A) under the curve that are functionally relevant but the associated slopes, $S_E$ and $S_M$ respectively. The steeper $S_E$ the greater is D likely to be; the steeper $S_M$ the greater is A likely to be. In fact, as Figure 27 shows the trends in (D-A) change are closely paralleled by change in $(S_E - S_M)$. Analysis of our models showed that even for a constant $\bar{\tau}$, increase in $(S_E - S_M)$ made its own major contribution to conserving $\psi_N$ throughout a wide range of photoperiods. We cannot therefore view the interspecific trend (itself imperfect) for $S_E - S_M$ to increase as $\tau$ shortens as *compensatory;* it is, rather, *additive.*

The observed species differences in $\tau$ and $(S_E - S_M)$ will result, if our model is correct, in differences in the precise value of $\psi_N$ and the extent to which it is held constant through the seasons. There is no *a priori* reason why all 4 species should begin activity at precisely the same time relative to sunset nor totally avoid a seasonal modulation of $\psi_N$. Different ecological niches will demand fine adjustment in a basic nocturnal strategy; and that adjustment can be effected in either of the two *complementary* (not *compensatory*) ways our model pacemakers revealed.

## 3. Compensation for Inter- and Intraindividual $\tau$ Variation

Individual animals in a species committed to a common ecological strategy are subject to significant innate variation in $\bar{\tau}$. That variation in itself will entail some desynchronization within the species under any and all photoperiodic regimes although it is minimized (Fig. 25) when photoperiods are long. It is a remarkable fact reported earlier (Daan and Pittendrigh, 1976a) that the shape of the pacemaker's PRC automatically changes in a way that compensates for the systematic $\bar{\tau}$ variation between individuals. The shorter $\bar{\tau}$ the greater (D-A) and, hence, we infer the greater $S_E/S_M$. Here the relationship between $\bar{\tau}$ and (D-A) is not *additive* or complementary as it is in shaping a species' strategy; it is clearly *compensatory* in maintaining the species strategy (attainment of some species $\psi$) in the face of uncontrolled (not designed) $\tau$ variation. Thus, a decrease in $\bar{\tau}$ will, if uncompensated, reduce the negative value of $\psi$; but that reduction is offset when the slope $(S_E)$ of the delay part of the curve is increased and that $(S_M)$ of the advance part reduced.

This is of course the same type of compensation we have already reported to hold for the individual pacemaker (Daan and Pittendrigh, 1976a). It seems that the fundamental structure of the pacemaker entails this interdependence of $\tau$ and PRC shape. We are evidently again seeing something for which natural selection must be responsible: by incorporating a necessary interdependence of $\tau$ and PRC shape into the clock's structure, provision is at once made not only for day-to day instability of its frequency but the inevitable inter-individual variation in its genetic specification with the species. It is also then an oscillator which once committed to either a nocturnal or diurnal strategy – automatically brings *both* devices to bear, additively, in conserving either $\psi_D$ or $\psi_N$ throughout the natural range of photoperiod change.

### *f) After-Effects: The Systematic Lability of $\bar{\tau}$*

The phenomenon we called after-effects (Pittendrigh and Daan, 1976a) reflects a general, systematic lability of pacemaker frequency quite separable from day-to-day instability. Its functional significance is by no means as clear as change in $\bar{\tau}$ or $\overline{\text{PRC}}$ shape and the meaning of strong differences between the species in their susceptibility to after-effects is even less clear.

The behaviour of model pacemakers does however provide one strong suggestion about the role played by the after-effects of photoperiod. It is recalled (Pittendrigh and Daan, loc. cit.) that in both *Peromyscus* and *Mus* the after-effect of long photoperiods is quite different from what we expected on the basis of LL action: *long* photoperiods *shorten* $\bar{\tau}$. Given the extreme nocturnal strategy that combines short $\tau$ and large $S_E - S_M$, our computer simulations with model pacemakers show that the maximum tolerable day-length (before the $\psi$-jump occurs) is lengthened the shorter $\tau$ becomes (Fig. 26). There is at least a suggestion here of a potential utility in one kind of after-effect: the effect of long photoperiods in the extreme "nocturnal" model (Fig. 26) is to enhance (by shortening $\tau$) the pacemaker's competence to entrain non-parametrically to still longer days.

### *g) Entrainment in Nature*

A principal goal of these papers has been to evaluate how well the non-parametric mechanism that accounts for so much of *Drosophila pseudoobscura* behaviour is involved in the entrainment of nocturnal rodents: how generally useful is the model?

Even in *Drosophila* – a diurnal form – the non-parametric mechanism is not the whole story. The simulation of complete (PPc) photoperiods by skeletons (PPs) begins to fail for long photoperiods; it is poor after about 12 h and fails completely at 14 h when the $\psi$-jump phenomenon occurs. Entrainment involving complete photoperiods (PPc) continues to be good even with daylengths at least 18 h long (Ottesen, et al., in preparation). There is, then, a "parametric" action of light in *Drosophila* and its principal functional significance lies in avoiding the $\psi$-jump; the insect remains "diurnal" ($\psi_D$ is conserved) throughout the wide range of photoperiods it encounters in nature.

One way to describe the discrepancy between PPc and PPs entrainment is that the parametric action of continuing light avoids the $\psi$-jump because it eventually (after ~12 h) has driven the pacemaker off the limit-cycle that *characterizes its DD motion* (Pittendrigh, in preparation); it stays off that limit-cycle so long as the illumination continues but re-enters at the phase designated ct 16, four hours after the night has begun. Thus, in nature it is never allowed to receive, *while on its DD limit-cycle*, two non-parametric signals (lights on, lights off) separated by the crucial interval of 14 h.

The discrepancy between PPs and PPc for all photoperiods up to ~12 h is so small that we must conclude the entrainment mechanism is fundamentally non-parametric; that the parametric action is small; and that its principal functional significance is in conserving $\psi_D$ (avoiding the $\psi$-jump) throughout the range of photoperiods it seeks to exploit.

The role of any parametric effects is even less clear in nocturnal forms. Unless night-active species repeatedly interrupt their day-time rest and see the light, there seems no escape from concluding the mechanism *must* be basically non-parametric; their entrainment would rest on the interaction of a light "pulse" seen as they begin their evening activity and another at dawn when they retreat to dark nests. If that were indeed the case the $\psi$-jump phenomenon would be a real hazard. It is inherent in the non-parametric mechanism and can only be avoided for very long photoperiods by an extreme PRC modification whose principal feature will be reduction of the phase-advance part of the curve.

The principal obstacle to progress in elaborating further detail about the basically nonparametric control of nocturnal rodents is the remarkable ignorance that persists about their daytime behaviour in the field. Nothing appears to be known, for either mice or hamsters, about how often they see light – and how much. Preliminary data from a study in progress (Pittendrigh and Domingos, in preparation) indicate that *Peromyscus maniculatus* leaves its dark nest and sees brief light pulses much earlier, and more often in the afternoon than hamsters. If that kind of behavioural difference proves general, as well it might, it is clearly premature to attempt any more detailed evaluation of species differences in PRC shape which is only one component in an overall strategy to conserve a particular $\psi$-night. Daytime "looking" may be a behavioural escape from a $\psi$-jump otherwise inherent in a given PRC shape.

Even the *Peromyscus* behaviour still leaves the mechanism basically nonparametric and the steady-state basically dependent on the interaction of the major $\Delta\phi$'s generated in morning and evening. Figure 18 using hamsters shows that the phase ($\psi$) of steady-state based initially on only two pulses 12 h apart, is not measurably changed by the addition of supplementary pulses throughout the day. They are not however without function: they will be as effective as continuous light in increasing the maximum tolerable "photoperiod" (in avoiding the $\psi$-jump).

We should not however overlook the fact that several species do, in fact, undergo quite discrete switches from night-time to day-time activity at a particular season. Such "seasonal inversion" (Erkinaro, 1961) is known in salmonid fishes (Kalleberg, 1958; Eriksson, 1973) and microtine rodents (Erkinaro, 1961, 1969). It is probably significant that most known cases of inversion come from high northern latitudes where the annual cycle of variation in daylength is maximal.

### *h) Circadian Pacemakers: The Empirical Generalizations*

Table 3 lists eleven empirical regularities that characterize the behaviour of circadian pacemakers. Some, as indicated, rest on a securer, broader base of observation than others. Three of them are analytic necessities by which we mean

**Table 3.** The significance of circadian pacemaker properties. a = analytic necessity; b = has functional significance in the measurement of the lapse of time (stabilization of $\tau$); c = has functional significance in the identification of local time (stabilization of $\psi$ as well as $\tau$); d = extent to which the generality is established

| | a | b | c | d |
|---|---|---|---|---|
| 1. The freerunning period in ($\tau$) is often significantly different from 24 h | − | − | + | ++ |
| 2. In diurnal forms $\tau > T$ is common; in nocturnal forms $\tau < T$ is common | − | − | + | + |
| 3. In diurnal forms $\tau_{LL} > \tau_{DD}$; in nocturnal forms $\tau_{LL} < \tau_{DD}$ | − | − | | ++ |
| 4. $\tau$ is conserved within narrow limits by a homeostatic mechanism | − | + | + | ++ |
| 5. Homeostasis of $\tau$ is greater when $\tau$ approaches 24 h | − | − | + | + |
| 6. Circadian PRCs are characterized by both $+\Delta\phi$ and $-\Delta\phi$ responses | − | − | + | ++ |
| 7. Maximum responses to light occur in the subjective night | + | − | − | ++ |
| 8. Phase-delay responses precede phase-advance responses in the subjective night | + | − | − | ++ |
| 9. The shape of PRCs changes systematically as $\tau$ changes | − | − | + | + |
| 10. $\tau$ is subject to after-effects | − | + | − | ++ |
| 11. When non-parametrically entrained, $\psi$-jumps are inescapable within the natural range of photoperiods | + | − | − | ++ |

they are characteristic of all self-sustaining oscillations (e.g. statement 11), or, given the mechanism of entrainment involved (light/dark cycle) they are inevitable (statements 7 and 8). These analytic necessities are of no special biological interest because they lead to no insights about either mechanism or function. The only exception to that is statement 11, which poses a functional problem.

The majority of those features which are not analytic necessities have clear functional significance. Four merit special notice because they rest on a very broad base of observations.

The first is the circadian nature of pacemaker period. To our knowledge the possible functional significance of $\tau$ so often being different from 24 h has received little or no attention previously. Indeed when the oscillator model for circadian clocks was first explicitly developed, the discrepancy of $\tau$ from 24 h was clearly regarded as tolerable "imperfection"; that all natural selection had demanded was that $\tau$ be close enough to $T(=24\text{ h})$ to be entrainable (Pittendrigh and Bruce, 1957). We have now found, however, that the very *circadian* nature of $\tau$ enhances the utility of the pacemaker as clock in two distinct respects. It reduces the sensitivity of $\psi$ to $\tau$-instability, and it helps conserve both $\psi_{\text{day}}$ (when $\tau > 24\text{h}$) and $\psi_{\text{night}}$ (when $\tau < 24\text{ h}$) throughout the year as photoperiod changes.

The second is that remarkable general homeostatis of their frequency (Pittendrigh and Caldarola, 1973) that was first recognized in the special case of temperature-compensation (Pittendrigh, 1954). There are no known exceptions to the rule of temperature-compensation, which has been looked for and found in the circadian pacemakers of every major group of eukaryotic organisms. The general homeostatic constraint on $\tau$-instability has clear relevance to pacemaker function in the recognition of local time ($\psi$-conservation). The homeostasis of angular velocity it reflects is further functionally important in the measurement of the lapse of time. This stabilization of angular velocity could never be achieved if the entrainment mechanism were basically parametric, since parametric action of the light per definition entails modulations of angular velocity.

The third and fourth are embodied in what Pittendrigh (1960) called "Aschoff's Rule". Its original formulation (Aschoff, 1958) included both statements 2 and 3 in Table 3, and is summarized as follows:

| | *Nocturnal organisms* | *Diurnal organisms* |
|---|---|---|
| (a) | $\tau_{DD} < 24$ h | $\tau_{DD} > 24$ h |
| (b) | $\tau_{DD} < \tau_{LL}$ | $\tau_{DD} > \tau_{LL}$ |
| (c) | $\alpha_{DD} > \alpha_{LL}$ | $\alpha_{DD} < \alpha_{LL}$ |

There have always been exceptions to these statements which Lohmann (1967) and Rensing and Brunken (1967) have reviewed and emphasized; and Aschoff himself dropped (a), above, in his 1960 discussion. Nevertheless, there are clear statistical trends which the three statements accurately report. Like the other empirical regularities we have emphasized in this section we believe that statements (a) and (b) in Aschoff's Rule reflect pacemaker properties that are peculiar to the circadian case and have meaning in their function as biological clocks. Statement (a) in the rule is the same as our statement (2) in Table 3 and its meaning has been noted. The pacemaker behaviors summarized by statements (b) and (c) in Aschoff's Rule have of themselves no functional meaning although Wever (1962) has utilized them in developing a model of parametric entrainment. But, though themselves without functional meaning we think they are consequences of pacemaker features that have major functional significance: we see (b) as a consequence of the different PRC shapes which yield optimum diurnal and nocturnal strategies for the conservation of $\psi$. In the nocturnal case the optimum PRC shape has involved $S_E > S_M$ and as a consequence the area under the delay (D) section of the PRC exceeds that of the advance (A) section. In diurnal forms, where we expect $S_E < S_M$, A is expected to exceed D. We are, of course, here following the original proposals of DeCoursey (1959) and Pittendrigh (1960) that the ratio of the areas under the advance and delay sections of the PRC for pulses predicts, in general, the net parametric effect of continuous illumination. While we think this is still a complex issue, our analysis of the dependence of $\tau$ on light intensity (Daan and Pittendrigh, 1976a) provided substantial encouragement in that the approach yields qualitatively valid predictions. Thus we see in statement (b) of Aschoff's Rule an indication that the second strategy (modification of PRC shape) which enhances the conservation of $\psi_N$ and $\psi_D$ respectively is as widespread as the adjustment of $\bar{\tau}$.

Once again, therefore, strong empirical regularity in biological systems reflects the handiwork of natural selection: the circadian nature of $\tau$ itself, its general homeostasis, and behaviors summarized by Aschoff's Rule are what make circadian pacemakers unequivocally *clocks* and not just another set of oscillations in living things.

## References

Aschoff, J.: Tierische Periodik unter dem Einfluß von Zeitgebern. Z. Tierpsychol. **15**, 1–30 (1958)

Aschoff, J.: Exogenous and endogenous components in circadian rhythms. Cold Spr. Harb. Symp. quant. Biol. **25**, 11–28 (1960)

Aschoff, J., Gerecke, U., Kureck, A., Pohl, H., Rieger, P., von Saint Paul, U., Wever, R.: Interdependent parameters of circadian activity rhythms in birds and man. In: Biochronometry (ed. M. Menaker), Bruce, V., Weight, F., Pittendrigh, C.S.: Resetting the sporulation rhythm in *Pilobolus* with short light flashes of high intensity. Science **131**, 728–730 (1961)

Daan, S., Aschoff, J.: Circadian rhythms of locomotor activity in captive birds and mammals: their variations with season and latitude. Oecologia **18**, 269–316 (1975)

Daan, S., Pittendrigh, C.S.: A functional analysis of circadian pacemakers in nocturnal rodents. II. The variability of phase response curves. J. comp. Physiol. **106**, 253–266 (1967a)

Daan, S., Pittendrigh, C.S.: A functional analysis of circadian pacemakers in nocturnal rodents. III. Heavy water and constant light: homeostasis of frequency? J. comp. Physiol. **106**, 267–290 (1967b)

DeCoursey, P.J.: Daily activity rhythms in the flying squirrel, *Glaucomys volans*. Ph. D. Thesis, University of Wisconsin 162 pp. (1959)

DeCoursey, P.J.: LD ratios and the entrainment of circadian activity in a nocturnal and a diurnal rodent. J. comp. Physiol. **78**, 221–235 (1972)

Elliott, J.A.: Photoperiodic regulation of testis function in the golden hamster: relation to the circadian system. Ph. D. Thesis, University of Texas, Austin 1974

Enright, J.T.: Ecological aspects of endogenous rhythmicity. Ann. Rev. Ecol. Syst. **1**, 221–238 (1970)

Eriksson, L.O.: Spring inversion of the diel rhythm of locomotor activity in young sea-going trout (*Salmo trutta trutta L.*) and Atlantic salmon (*Salmo salar L.*) Aquilo, Ser. Zool. **14**, 68–79 (1973)

Erkinaro, E.: The seasonal change of the activity of *Microtus agrestis*. Acta. oecol. scand. **12**, 157–163 (1961)

Erkinaro, E.: Der Phasenwechsel der lokomotorischen Aktivität bei *Microtus agrestis* (L.), *M. arvalis* (Pall.) und *M. oeconomus* (Pall.). Aquilo, Ser. Zool. **8**, 1–31 (1969)

Frisch, K. von: Die Sonne als Kompaß im Leben der Bienen. Experientia (Basel) **6**, 210–221 (1950)

Hoffmann, K.: Zur Beziehung zwischen Phasenlage und Spontanfrequenz bei der endogenen Tagesperiodik. Z. Naturforsch. **18**b, 154–157 (1963)

Hoffmann, K.: Die relative Wirksamkeit von Zeitgebern. Oecologia (Berl.) **3**, 184–206 (1969)

Holst, E. von: Die relative Koordination als Phenomen und als Methode zentralvervöser Funktionsanalyse. Ergebn. Physiol. **42**, 228–306 (1939)

Kalleberg, H.: Observations in a stream tank of territoriality and competition in juvenile salmon and trout (*Salmo salar* L. and *Salmo trutta* L.) Rep. Inst. Freshwater Res. Drottningholm **39**, 55–98 (1958)

Kramer, G.: Orientierte Zugaktivität gekäfigter Singvögel. Naturwissenschaften **37**, 188 (1950)

Lohmann, M.: Zur Bedeutung der lokomotorischen Aktivität in circadianen Systemen. Z. vergl. Physiol. **55**, 307–332 (1967)

Pittendrigh, C.S.: On temperature independence in the clock system controlling emergence time in *Drosophila*. Proc. nat. Acad. Sci. (Wash.) **40**, 1018–1029 (1954)

Pittendrigh, C.S.: Circadian rhythms and the circadian organization of living systems. Cold Spr. Harb. Symp. quant. Biol. **25**, 159–184 (1960)

Pittendrigh, C.S.: The circadian oscillation in *Drosophila pseudoobscura* pupae: A model for the photoperiodic clock. Z. Pflanzenphysiol. **54**, 275–307 (1966)

Pittendrigh, C.S.: Circadian oscillations in cells and the circadian organization of multicellular systems. In: The neurosciences: Third study program, Vol. 38 (eds. F.O. Schmitt, F.G. Worden), pp. 437–458, Cambridge, Mass.: MIT Press 1974

Pittendrigh, C.S., Bruce, V.C.: An oscillator model for biological clocks. In: Rhythmic and synthetic processes in growth. p. 75–109. Princeton, N.J.: Princeton University Press 1957

Pittendrigh, C.S., Caldarola, P.C.: General homeostasis of the frequency of circadian oscillations. Proc. nat. Acad. Sci. (Wash.) **70**, 2697–2701 (1973)

Pittendrigh, C.S., Daan, S.: A functional analysis of circadian pacemakers in nocturnal rodents. I. The stability and lability of spontaneous frequency. J. comp. Physiol. **106**, 223–252 (1976a)

Pittendrigh, C.S., Daan, S.: A functional analysis of circadian pacemakers in nocturnal rodents. V. Pacemaker structure: A clock for all seasons. J. comp. Physiol. **106**, 333–355 (1976b)

Rensing, L., Brunken, W.: Zur Frage der Allgemeingültigkeit circadianer Gesetzmäßigkeiten. Biol. Zbl. **86**, 545–565 (1967)

Swade, R.H., Pittendrigh, C.S.: Circadian locomotor rhythms of rodents in the Arctic. Amer. Nat. **101**, 431–466 (1967)

Wever, R.: Zum Mechanismus der biologischen 24-Stunden-Periodik. Kybernetik **1**, 139–154 (1962)

Winfree, A.: An integrated view of the resetting of a circadian clock. J. theor. Biol. **27**, 327–374 (1970)