

The Colin S. Pittendrigh Lecture

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Colin Pittendrigh, Jürgen Aschoff, and the Natural Entrainment of Circadian Systems

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Tau response curve

It is a great honor for me to present this first Pittendrigh lecture. I do so as the stand-in for Professor Jürgen Aschoff, who of course was invited to speak on the occasion but whose general health at 85 prevented him from coming over from Freiburg. He sends his best wishes to all of you.

The entrainment of circadian systems has been an issue close to the heart of both Colin Pittendrigh and Jürgen Aschoff. It provides a first-rate biological problem indeed. Entrainment is the exquisite functional link between circadian oscillators and the rotation of the earth that shaped their evolution in virtually the entire living world. Entrainment requires special input mechanisms that have provided us with the foremost experimental handle for unraveling the physiology underlying these oscillations.

Colin Pittendrigh, whose pioneering contributions to our field we celebrate today, has been intrigued and challenged by the problem of entrainment throughout his career. His first thoughts about it date back to his war years in Trinidad (Fig.1), where he sat pondering two species of mosquitoes. Each species had its distinct daily activity peak, clearly tuned to daily variations in humidity but equally clearly entrained by something else, in view of the large day-to-day variations in the general level of humidity (Pittendrigh, 1950).

Most of you, I am sure, consider the problem of entrainment basically as solved. Most have sat in courses in which the principle of phase resetting was

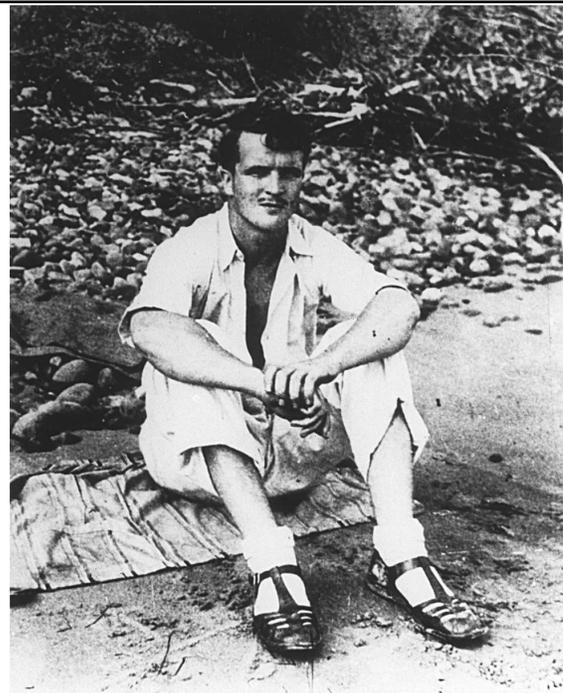


Figure 1. Colin Pittendrigh in Trinidad ca 1940 (photograph in Aschoff's estate).

explained. There is somehow an endogenous oscillation, which runs at a frequency slightly deviating from once per 24 h, and that deviation is corrected each day by an instantaneous, abrupt phase shift in response to light: every evening at dusk and/or every morning at dawn, as dictated by the laws of the phase response

curve (PRC). Most of you have made your own exercises, either graphically or on the computer, and you have experienced that, indeed, entrainment can work this way. But few have been stubborn enough to ask, "Does it work this way in nature?" Colin Pittendrigh, who was the inspired genius behind this whole theory, remained uncertain, and questioning, until his death on March 16, 1996.

In this hour, I want to contrast the approach of Pittendrigh with that of Aschoff. I will briefly restate these approaches to the issue of entrainment. I then address especially the PRC model of Colin Pittendrigh, its success, and its problems. And, finally, I discuss a new proposition that integrates elements from both approaches. If there is anything I can add to the perspective of the two giants of our field—to further perpetuate Isaac Newton's phrase (Merton, 1965)—it is because in my lifetime I had the dwarf's luck of standing on the shoulders of both of them, first as a postdoc, then as a personal friend.

Nonparametric versus Parametric Entrainment

The two approaches are intimately linked to the concepts of nonparametric and parametric entrainment. These concepts refer to the nature of the action of light, whether continuous or tonic in the case of parametric entrainment, or discrete or phasic in the case of nonparametric entrainment (Daan, 1977). Suggestive of phasic effects are the large phase shifts elicited often by very brief light pulses, while the changes in period as a result of different constant light intensities are proof of the existence of tonic effects. Pittendrigh attributed special value to the transitions of light to darkness—and vice versa—that would generate instantaneous nonparametric phase shifts, while Aschoff's starting point was the parametric dependence of circadian period on constant light. They developed their models accordingly, with different emphases:

PITTENDRIGH	ASCHOFF
Nonparametric	Parametric
Discrete	Continuous
Phasic	Tonic
PRC	$\tau = f(\text{light intensity})$
Instantaneous	Gradual
Twilight	Daylight

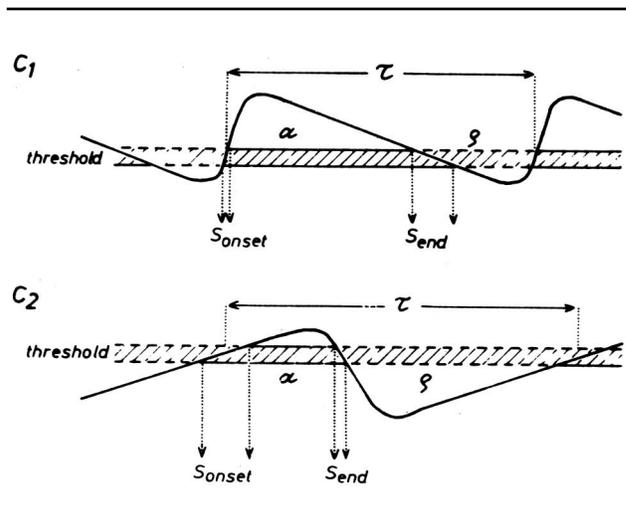


Figure 2. Aschoff's level-threshold approach. An endogenous oscillation triggers activity onset when passing upward through a threshold. The mean level of the oscillation is affected by light intensity along with the period and waveform of the oscillation (from Aschoff et al., 1971).

Pittendrigh started from the PRCs, which had been detected in the late 1950s virtually simultaneously in his own lab (Burchard, 1958; Pittendrigh, 1958) and in those of Woody Hastings (Hastings and Sweeney, 1958) and Ken Rawson (Decoursey, 1960a, 1960b). The entrainment principle developed from this observation, as you all know it, is summarized in the statement $\Delta\phi(\phi) = \tau - T$ (Pittendrigh, 1981a): in stable entrainment, the free-running period τ of the circadian system is corrected each day for the difference between τ and 24 h when the light falls at that particular phase ϕ of the cycle in which a phase shift $\Delta\phi$ equal to this difference is generated.

In contrast, Aschoff suggested that light lengthens or shortens the period of an endogenous oscillation, while at the same time it affects the average level around which this oscillation moves (e.g., Aschoff, 1964). One example (Fig. 2) of his theoretical views is taken from Aschoff's paper in the Friday Harbor meeting of 1971. He suggested that light may affect the period of the circadian oscillation and at the same time modify its shape—or waveform—and the level around which the oscillation moves. The oscillation would interact with an endogenous threshold and induce overt activity as long as the oscillating variable is above the threshold. The approach predicted and explained several interesting features of the precision

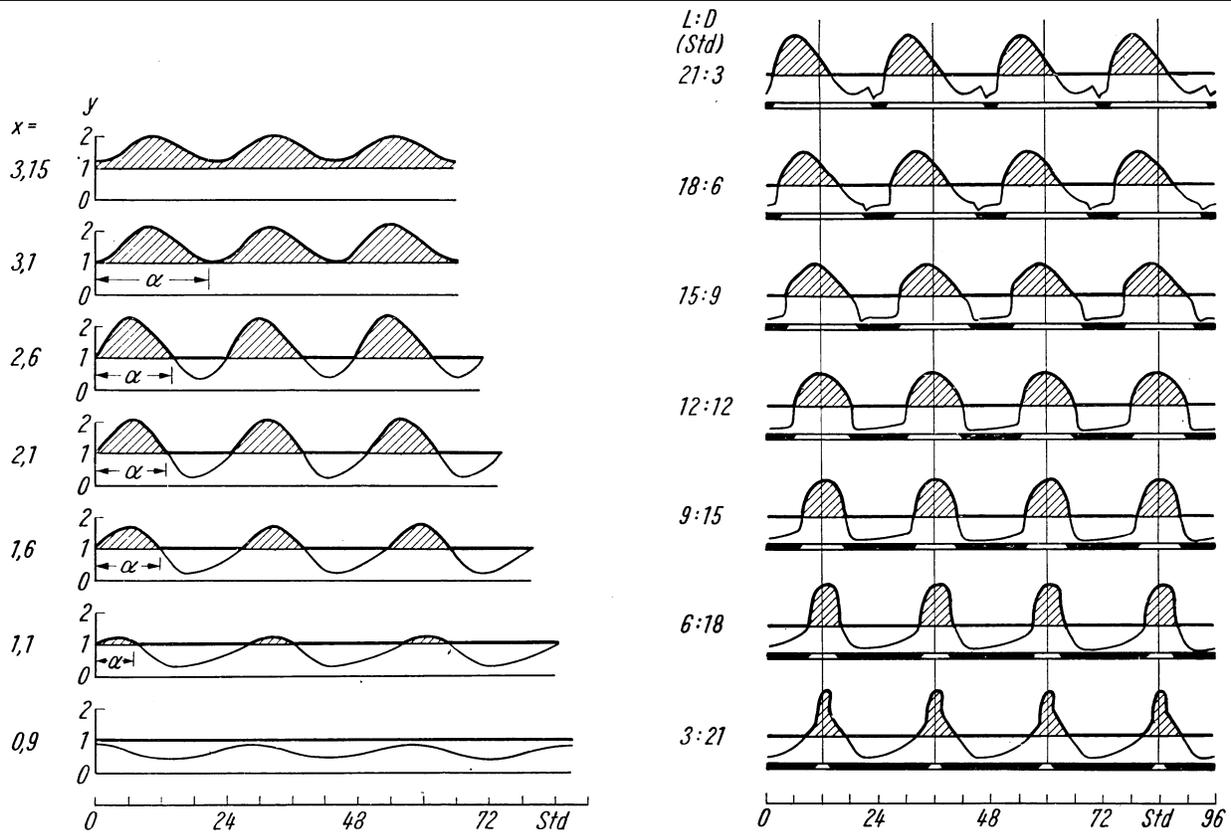


Figure 3. Simulations obtained from Wever's mathematical model. Left: effects of different light intensities on the oscillation, explaining period changes and arrhythmicity under high and low levels of light. Right: entrainment by different photoperiods (from Wever 1966).

of the oscillation, such as higher precision in the onset of activity for short τ s and higher precision in the offset of activity for long τ values. It also predicted maximal precision at intermediate τ values (Aschoff et al., 1971). This simple scheme clearly can generate entrainment as long as the parametric effect of light (on τ) is phase dependent. The entrainment patterns were specifically studied by Aschoff's collaborator Rütger Wever, who designed a mathematical model based on these premises (Wever, 1966). This was essentially a differential equation that generates a self-sustained oscillation in two state variables, S and dS/dt :

$$\frac{d^2S}{dt^2} + 0.5(S^2 + S^{-2} - 3) \cdot \frac{dS}{dt} + S + 0.6 S^2 = L + \frac{dL}{dt} + \frac{d^2L}{dt^2}. \quad (1)$$

Light intensity, represented by L in the equation, affects the period of the oscillation in constant conditions and is also able to generate entrainment in a cycle (Fig. 3). On the left-hand side from bottom to top, the frequency and level of the oscillation increase with increasing L and at the same time cause an increase in

activity time α . In the right-hand panel, we see entrainment by different photoperiods. The virtue of such models is primarily that they connect hitherto unrelated phenomena. For the biologist, they entail the basic problem that the terms in the equation have no concrete physiological interpretation. There is also no way to measure the parameters independently, as we can measure τ , the PRC, and the phase angle difference ψ .

The distinction between the initial approaches of the two gentlemen is surely related to the systems they studied. For Pittendrigh, this was the single instantaneous event of eclosion in *Drosophila*; for Aschoff, it was the continuous modulation of activity in the circadian rhythms of birds, mammals, and humans. I emphasize that each of them had a very open mind toward the other's viewpoint. Aschoff already in 1965 categorized the various ways to measure PRCs (Aschoff, 1965), and even before that he wrote in the *Annual Reviews of Physiology*,

To become entrained, this natural period has to be corrected through advancing or delaying phase-shifts by the Zeitgeber. This may be accomplished by (a) the

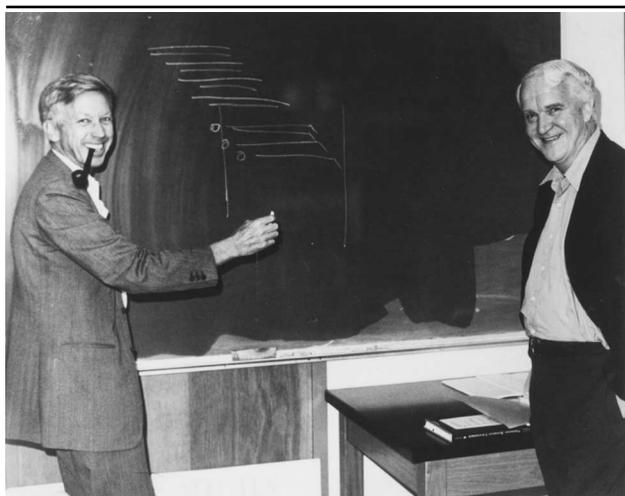


Figure 4. Jürgen Aschoff and Colin Pittendrigh in front of the blackboard in the library of the Max Planck Institut für Verhaltensphysiologie in Erling-Andechs (photograph in Aschoff's estate).

effect of one transition, (b) the difference of the effects of two transitions, (c) by the net (integrated) effects of continuous actions, or (d) by some combination of these possibilities. It seems probable that in the majority of organisms the fourth possibility is realized. (Aschoff, 1963 p. 591)

Pittendrigh later thought of ways to derive predictions on τ as a function of light intensity from the PRC. This led to the proposition of velocity response curves, with continuous light accelerating and decelerating the oscillation in different phases (Daan and Pittendrigh, 1976).

In their private lives, Aschoff and Pittendrigh (Fig. 4) became very close friends, and this friendship included the families. They had met in 1958, when Aschoff made his first trip to the United States. From this visit, the idea of the Cold Spring Harbor Symposium of 1960 was born, as well as a lifelong intense exchange of ideas, letters, and sympathy. Aschoff once noted the ups and downs in Pittendrigh's productivity, and at Pitt's retirement symposium on Mount Hood, Oregon, in 1984, he claimed to have established a multiannual cycle in scientific performance, which then led him to analyze his own production and compare their results (Fig. 5). Aschoff's conclusion from the analysis was, "always in harmony, never in synchrony."

Success and Problems of the PRC model

The nonparametric PRC model has become standard textbook stuff, while few of the present genera-

tion have heard about Aschoff's level-and-threshold theory. Perhaps the greatest success of the PRC entrainment model was the PRC itself. It gave the field its first-rate, clearly defined, experimental tool for probing the physiology of circadian systems.

As far as entrainment is concerned, the PRC model yielded highly accurate predictions for the pattern of entrainment of *Drosophila* eclosion rhythms by brief light pulses. The range of entrainment by different Zeitgeber periods could be predicted, and so could the phase of both the overt rhythm and the underlying oscillator. The match between prediction and observation culminated when Pittendrigh used skeleton photoperiods and was able to precisely predict the so-called zone of bistability and where the oscillator would land depending on initial conditions (Pittendrigh, 1981b).

When I was at Stanford in the early 1970s, we started to look for similar matches in nocturnal mammals. Indeed, there was reasonable agreement between predicted and observed phase in a skeleton photoperiod, for instance, in the white-footed deer mouse (Fig. 6). The lines on the left side indicate predicted onsets of activity, as dependent on skeleton photoperiod duration and on individual τ . The circles are the individual observations. On the right-hand side, the mean predictions and observations are indicated. The circadian rhythm in natural daylight was also reasonably predictable from the behavior in a skeleton photoperiod.

But there were problems too. In a skeleton photoperiod, with two brief light pulses 12 h apart in otherwise darkness, the activity of a nocturnal mammal—its subjective night—will be restricted to one of the two dark intervals. If one compresses this interval, at some point a ψ -jump will occur, as predicted from the PRC. One problem that emerged was that this compression could go much further than predicted: The observed ψ -jumps occurred at skeleton photoperiods of 18 to 20 h, not at 12 to 15 (Fig. 6). We also sometimes observed nearly complete compression of activity time in entrainment by single pulses. Jeff Elliott (1981) has later shown that this compression simultaneously entails a compression of the PRC. That means that the PRC is not a fixed property of the system but is itself modified by entrainment. Also, τ is not a fixed property. We know that there are after effects of prior entrainment: initially, after release from a 24-h regime, τ is very close to 24. Only in prolonged free runs, τ gradually reverts to its stable endogenous value. So the two elements— τ and PRC—on which all

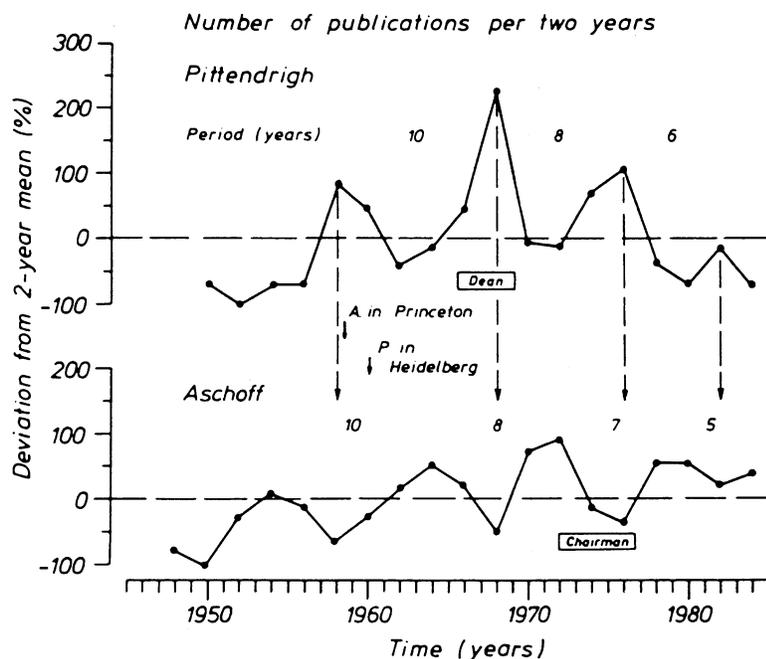


Figure 5. Aschoff's analysis (1984) of oscillations in Pittendrigh's scientific productivity, compared with those in his own publication frequency.

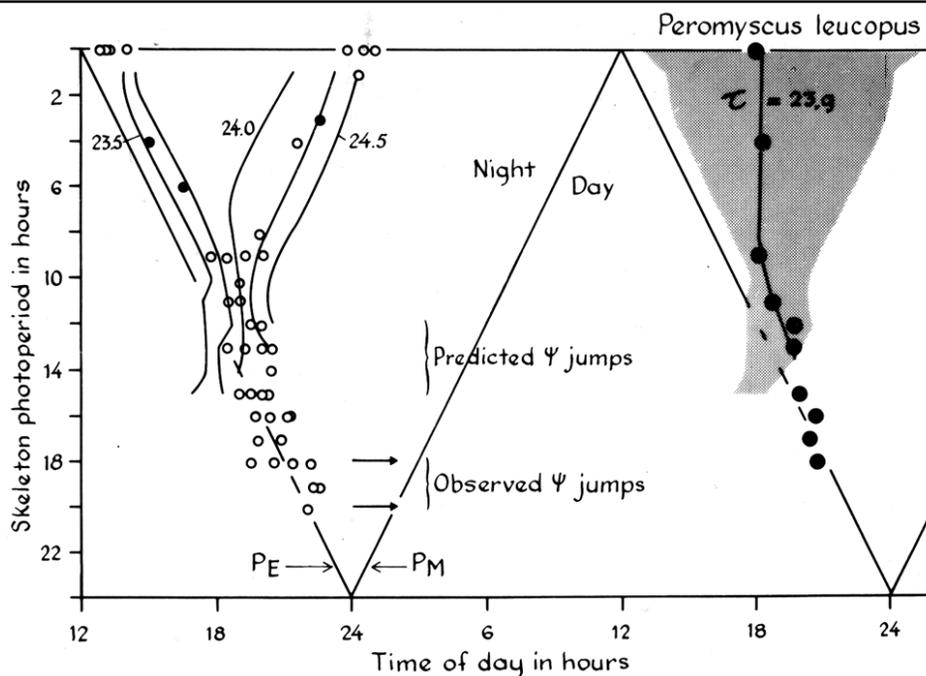


Figure 6. Entrainment by skeleton photoperiods: observed activity onsets in *Peromyscus leucopus*, compared with theoretical predictions from the phase response curve model. Left side: individual mice. Right: group averages (from Pittendrigh and Daan, 1976b).

predictions of entrainment are based on fixed properties of the system, which can be independently assayed. They are themselves continuously affected by the zeitgeber.

Pittendrigh was well aware of the fact that his entrainment model was based on a simplification. He

expressed this on several occasions. In his posthumous papers he writes,

We initially conceived this pacemaker as involving only one state variable (hence "simple clock"). Phase is indeed the only variable in our description (versus explanation) of the steady-state time course, but

phase is, of course, not a state variable, and its use carries no commitment as to how many such variables are involved in the oscillatory dynamics of the pacemaker. No matter how complex (how many state variables a limit-cycle oscillator may involve), its *steady-state motion* remains a succession of phases, which is what we can assay by measuring the phase-shift to a standard strong light pulse. (C. S. Pittendrigh, unpublished)

Pittendrigh clearly did not think of the PRC as an explanation. He also saw the limitations of his approach with respect to entrainment by natural photoperiods. These led him (Pittendrigh, 1981b) to start working with a mathematical pacemaker model not unlike that of Wever. I have put this model (written by Th. Pavlidis) here in the same mathematical form as Wever did, as a single differential equation:

$$\frac{d^2S}{dt^2} + (L - 0.5) \frac{dS}{dt} + (L + 0.3)S - 0.3S^2 + 0.5 = 0. \quad (2)$$

The curious fact is that on close scrutiny, Wever's (1966) model (equation 1) did contain the first and second time derivatives of light intensity and thus contained a phasic, nonparametric element. The Pavlidis equation (2) has only L , light intensity itself, and is thus essentially parametric.

In Pittendrigh's modeling work with this equation, he always had to make the assumption of a second slave oscillator controlling eclosion of *Drosophila*. This oscillator was not independently assessable and its existence thus not easily disproved. Another major problem with the whole PRC approach concerns the value of τ . In many simulations, Pittendrigh demonstrated that the stability of entrainment decreases as τ gets very close to T , the period of the zeitgeber. Evolution should thus have set τ away from 24 h, instead of close to 24. It should be either smaller or larger, and we should expect to find a bimodality of τ values—which empirically has not been substantiated.

There is a last basic problem. While Aschoff's model allowed qualitative predictions on the timing of animal behavior in the natural environment—predictions that were compared extensively by Aschoff with behavioral data (Aschoff and Wever, 1962; Aschoff, 1969; Daan and Aschoff, 1975)—the PRC model has never been tested under natural LD situations. Whatever the accuracy of its predictions, the PRC model always requires that at least one of the light-dark transitions at twilight is seen by the animal:

either dawn or dusk, or both. We have questioned whether this is always true.

There is remarkably little information in the literature on the light patterns actually perceived by animals in nature. Savides et al. (1986) have investigated this issue in humans. They equipped subjects with light sensors and let them go about their own business. What they found is that human beings, even in Southern California, get surprisingly little exposure to daylight, and what they perceive is surprisingly erratic from day to day. Light can hardly be considered a precise zeitgeber, and yet humans are remarkably precise and stable in their circadian organization. Roelof Hut in my lab has set out to measure light perception in the European ground squirrel (*Spermophilus citellus*). He did this by means of a radio collar with a light-sensitive selenium cell in the circuitry. The transmission frequency allowed him to establish when the animal entered its burrow and came above ground. Using this system he could assess above-ground activity of ground squirrels—or *susliks* as they are called—both in nature, in the Hungarian puszta where the animals live naturally, and in a fenced compound near the lab in Holland (Hut et al., 1999).

Figure 7 shows one typical example of such records. The animal emerges usually 3 h after dawn civil twilight and goes underground 2 h before dusk. This holds for all animals, throughout the summer. We know from endoscopic observations of the burrow that they completely block the burrow entrance after their final afternoon retreat (Hut and Scharff, 1998). These animals are hibernators, and in winter they are permanently in constant darkness underground. So *susliks* virtually never in their whole life see the twilight transitions, which are so crucial in PRC entrainment.

So how can their circadian systems remain entrained? Entrainment by other sensory cues than light seems highly implausible. Temperature is a very erratic signal, certainly for an animal moving in and out of a burrow and thereby generating its own temperature cycle. We have briefly thought that they might use the spectral composition. The UV component of light appears much later than sunrise and disappears before dusk. But these diurnal mammals shield off UV with their optic system and unlike hamsters do not entrain to UV cycles (Hut et al., in press). Hence, spectral composition does not appear to be the solution. With visible light, the problems are also enormous. Not only are there sharp transitions each time

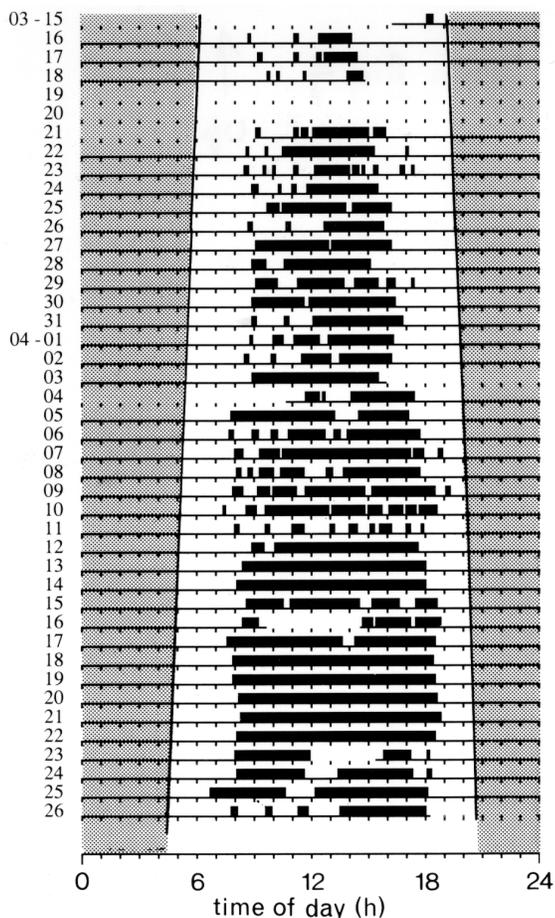


Figure 7. Actogram recording spontaneous above-ground activity of a European ground squirrel in natural conditions. Light area indicates civil daylight (i.e., sun altitude $> -6^\circ$) (Hut et al., 1999).

the animal enters or leaves its burrow, but also the variations in intensity from day to day are considerable. Daily curves of visible radiation show a great deal of variation between days, without any corresponding phase changes in observed activity (Hut et al., 1999). Starting from this situation, we developed an integrated approach to the problem of entrainment by light (Beersma et al., 1999).

A Combined Phase and Frequency Resetting Model

The ground squirrels make two things abundantly clear: (1) they maintain high accuracy of circadian timing without ever seeing the real twilights, and (2) the light they do see varies greatly in intensity from day to day. If that light profile were truly rectangular (upper panel in Fig. 8), it would be theoretically impossible to be entrained by it, because the animal would never

notice any external change in light intensity. It might just as well live in constant light or on a stationary, nonrotating planet facing its sun. The only possibility for it to be entrained by light is to exploit the small changes occurring in daytime while it is still above ground, such as those in the upper-right corner in the lower graph. But these temporal changes are reliable only in the average curves, when integrated over many days. They are not highly reliable on a day-to-day basis. Light intensity varies as much between sunny and cloudy days as it does in the average curve over the time the animal is above ground.

This poses the true dilemma for entrainment. On one hand, these circadian systems should be sensitive toward relatively small changes in light intensity. On the other hand, they can only exploit these changes as averaged over many days rather than respond to each cloud that hides the sun. And they should do so while moving in and out of their burrows and thus generating huge variations in light intensity by their own behavior. Most of the rapid changes in light perceived are self-generated and totally irrelevant as cues with respect to time of day. Evolution must somehow have taken care that animals do not respond to these transitions as zeitgeber signals. But how? The dilemma is that high sensitivity toward small variations in light intensity typically requires rather unstable, weak oscillators with a high-amplitude PRC. On the other hand, the integration of light information over many days would seem to require a strongly self-sustained oscillator, with τ very close to 24 h.

There are two general predictions for a diurnal animal living the life of a ground squirrel. In the first place, the light sensitivity of the system should at least be expressed in that part of the cycle that is, by their behavior, exposed to daylight. This is, in diurnal creatures, the *subjective day*. In nocturnal animals, we are used to a dead zone in the PRC, but that would hardly seem compatible with entrainment for these ground squirrels. In the European ground squirrel, we are currently measuring a PRC, but before that is completed, I can only give a general impression of the PRC in diurnal mammals on the basis of published material. These curves are derived from literature data, compiled in the PRC atlas (Johnson, 1995), with a few species recently added. We computed these by first fitting a harmonic curve plus two subharmonics to the original data in each species and then averaging the values in each circadian hour. There are now usable curves for 7 diurnal and 12 nocturnal mammals. In each case, we selected pulse durations as close to 1 h as available.

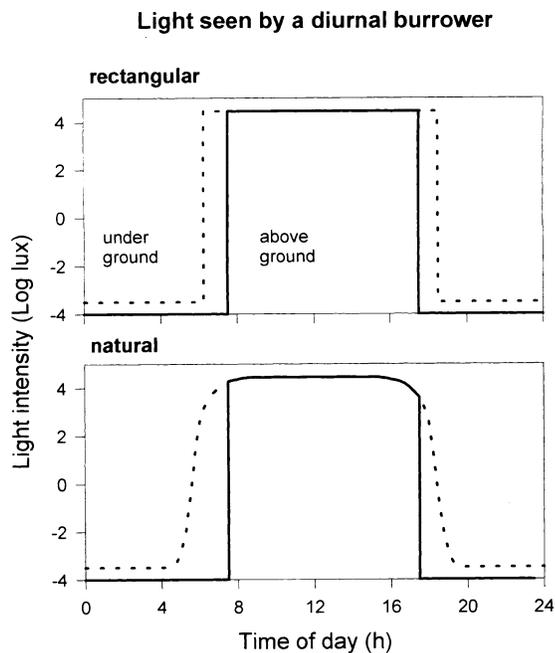


Figure 8. The light seen by a diurnal burrowing mammal: two schemes.

The first impression is that the dead zone might be smaller in the diurnal than in the nocturnal mammals (Fig. 9a). But that may be an illusion. The data suggest that the amplitude of the PRC in diurnal mammals is larger, but we have to realize that pulse intensity and duration were also larger—together by a factor of about 2. Therefore, we have standardized the PRCs to a standard light pulse of 1 h and 1000 Lux, assuming that in this range of pulse strengths, the phase shift is proportional with both duration and intensity. The curves (Fig. 9b) then become remarkably similar. The average diurnal PRC only appears to be shifted to the left by about 1 h relative to the average nocturnal PRC. The phase position of these curves is of course determined by the definition of the phase reference point—activity onset. This was set at ct 0 in diurnal and at ct 12 in nocturnal species, but there is no evidence that activity onset occurs at exactly opposite phases under entrainment. Indeed, the ground squirrel data (Fig. 7) suggest that activity onset may be programmed to occur rather late with respect to lights on. Future analyses may demonstrate systematic differences between the light PRC's of diurnal and nocturnal mammals, but these are so far certainly not impressive. In both cases, the true dead zone is pretty small, certainly smaller than the 10 h a ground squirrel typically spends above ground.

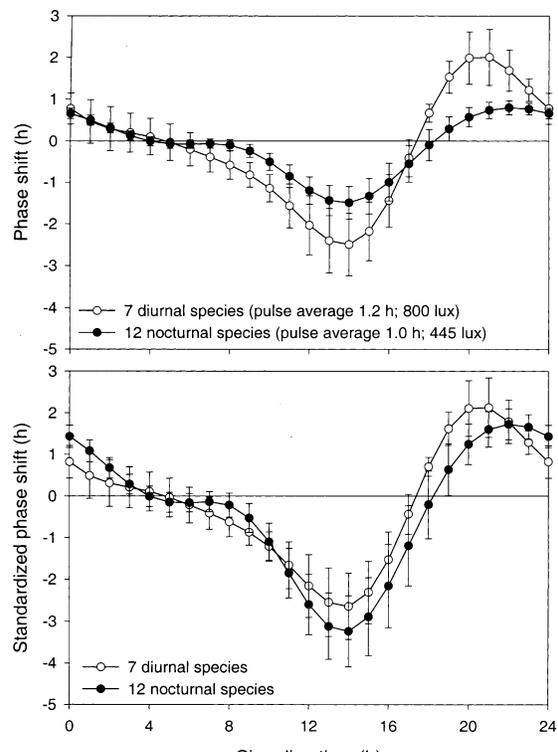


Figure 9. Phase response curves of nocturnal and diurnal mammals. (A) Means \pm 1 SEM of fitted harmonic curves, each with the first two subharmonics. (B) Standardized curves for a 1 h, 1000 Lux pulse.

In the second place, a functional requirement appears to be that the circadian system runs at a period τ very close to 24 h. Our field has of course long appreciated the existence of so-called after effects, which reflect exactly that property. Mice exposed previously to either a 20-h or a 28-h light-dark cycle, and subsequently put in constant darkness, initially have endogenous τ s close to the period of the imposed zeitgeber and only gradually revert to a standard value for mice around 23.5 h. The differences between the two treatment remain measurable until 100 days of free run in DD (Pittendrigh and Daan, 1976a).

In the course of these studies, we also noted that there are after effects of single brief light pulses. There was a slight but over numerous data points significant tendency for advancing light pulses to be followed by a reduction of τ and for delaying pulses to increase τ (Pittendrigh and Daan 1976a, Table 4). Since 1976, this has been studied in more detail by several researchers, notably Kramm and Kramm (1980) and Pohl (1982), and more or less complete τ -response curves (τ -RCs) are now available for five species. We have summa-

rized these in a single graph (see Beersma et al., 1999, Fig. 1). It is interesting that four of these concern diurnal mammals, while the fifth, the common vole, *Microtus arvalis* (Gerkema et al., 1993), is a grass-feeding species that comes above ground both at night and in daytime. In contrast, τ changes are much less pronounced in nocturnal species. They have also much more rarely been reported—in contrast with the PRCs in which the bulk of the material is from nocturnal mammals. The τ -RCs to light are very similar to PRCs. The general shape is the same, and the crossover points from delays to advances and back are similar (Beersma et al., 1999, Fig. 1). They differ in amplitude. The ratio is about 1 to 7 in diurnal mammals. This means that for every hour of instantaneous phase shift in the first cycle, there are 9 min of change in τ , which accumulate to another hour of phase drift after seven cycles! What few data there are in the nocturnal species suggest that the ratio is rather in the order of 1 to 70 (Pittendrigh and Daan, 1976a), and hence it is understandable that the τ changes are less frequently observed.

The question is, do these τ changes contribute to the stability of entrainment? If we take stability of entrainment as a criterion important in the evolution of circadian clock properties, can we understand this difference between diurnal and nocturnal mammals in which the PRCs are so similar? Domien Beersma and I designed a simplistic model to investigate this issue. We wanted to know how the magnitudes of the ϕ -response and of the τ -response contribute to the stability of the system under naturalistic light regimes. This stability was viewed both by Pittendrigh and by Aschoff as the primary adaptive requirement for circadian function.

This model is again a “phase only model.” I know that it is unrealistic to presume that a biological oscillator is so simple that it moves instantaneously back to its limit cycle after a perturbation, as is implicit in a phase-only model. However, all the more “realistic” models based on differential equations have the disadvantage that they employ parameters that have no physiological interpretation, while measurable biological realities such as phase and period remain unspecified. So we simply use an oscillator defined by an intrinsic period τ_0 , say 23.9 h. Every phase—with a resolution of 4 min—is characterized by a response to light intensity as defined by a PRC and a τ -RC, each

scaled down by a factor from the same basic curve in the second panel, and we modify these two factors. They are further linearly scaled with light intensity. Every 4 minutes, the computer takes a random value between the minimum and maximum of a natural logarithmic light-dark cycle. The parameter we investigate is the accuracy of the system. This is the reciprocal of the standard deviation of ψ , the phase angle difference. It reflects how accurately the phase angle difference between the system and the LD cycle is preserved in the face of stochastic variations in light intensity perceived. We do this for three types of mammal: a non-burrower, like a hare or a marmoset, which is continuously exposed to the outside light and darkness; a diurnal burrower, like a ground squirrel—or a human being for that matter, who also tends to withdraw from the natural daylight; and finally a nocturnal burrower. In the last two cases, only part of the response curves will be illuminated.

We varied the scaling factors for the τ -RC and PRC independently and plotted the accuracy over 500 cycles in a three-dimensional plot as a function of these two (Fig. 10). The scaling factors for the τ -RC and PRC on the two horizontal axes are relative measures. If this were a meeting of physicists, I would have defined them in standard units, which might properly be called Aschoffs on the τ -RC axis and Pittendrighs on the PRC axis. But alas, this is noisy biology rather than physics. If both the τ -RC and PRC amplitude are 0, there is of course no entrainment, or accuracy = 0. If there is only a PRC (the τ -RC scaling factor = 0), then the accuracy increases with increasing PRC amplitude, reaching a maximum of about 8 h⁻¹ (or a day-to-day standard deviation of ca 7.5 min). If the PRC is 0, then accuracy increases with increasing τ -RC amplitude, reaching a maximum of about 3 h⁻¹.

The important outcome of the exercise is that there is an intermediate peak in accuracy of the system, in which values of 13 h⁻¹, corresponding with a day-to-day standard deviation of 5 min, are reached. This happens at low amplitudes of PRC and τ -RC in which each one of them alone would be barely sufficient to generate entrainment. By combinations of the two at low amplitude, the maximal precision attainable with each one of them separately can be nearly doubled! If natural selection acts on timing processes such that the least accurate systems are weeded out—through whatever function: sun compass orien-

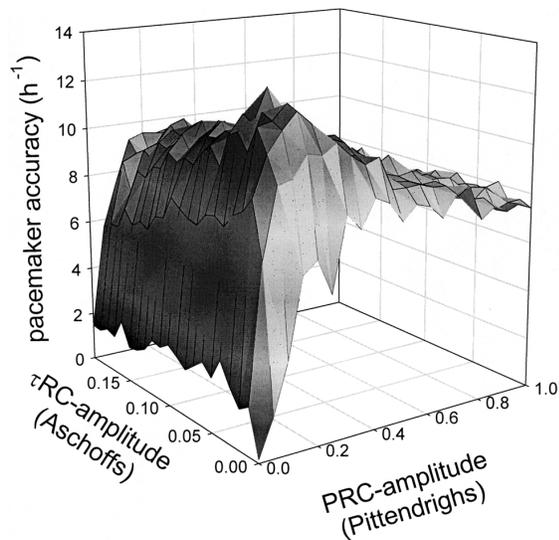


Figure 10. Accuracy of entrainment as a function of phase response curve and τ -response curve amplitude. Based on simulations in Beersma et al. (1999).

tation, time memory, or the precise measurement of day length—we may view this as a fitness landscape, in which the best adapted systems are found at the peak of the landscape.

This is early in our analyses. The details of the peak location and size will undoubtedly turn out to depend on exactly how the noise, the stochastic variation of light intensity, is structured in the natural world. We are currently exploring a series of more naturalistic possibilities, which may move the accuracy peak to different combinations of PRC and τ -RC amplitude. For the time being, however, it is intriguing and gratifying to note that the peak occurs at a relative amplitude of the τ -RC that is about 6 times smaller than that of the PRC. This ratio, of course, corresponds closely to the ratio in measured amplitudes for diurnal mammals (Beersma et al., 1999). I will return to this issue for nocturnal mammals shortly.

This “fitness landscape”—if we may consider accuracy of daily timing as a currency for evolutionary fitness—is for a non-burrower with continuous access to light outside. A diurnal burrower can also remain entrained by letting its τ be modulated even if it sees only a small and erratic part of the daily light intensity curve. Figure 11 gives the simulated pattern of light perceived by an animal that is only above ground from ct 0 until ct 10. It does remain entrained by virtue of adjusting its τ to exactly 24 h, in spite of the fact that it

Diurnal burrower, Simulated

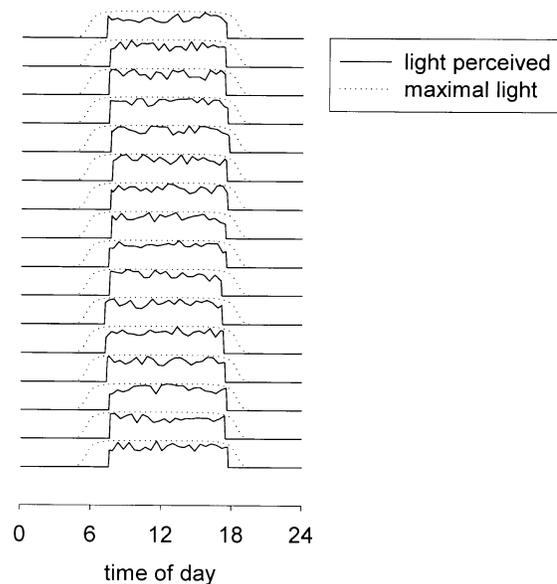


Figure 11. Simulation of stable entrainment of circadian activity by combined phase and period adjustment in a diurnal burrower exposing itself from ct 0 to ct 10 to natural variations in light intensity.

never sees the twilight and that the light signal it receives is enormously variable.

Finally, we can compare the accuracy of entrainment for the three types—non-burrower, diurnal burrower, and nocturnal burrower. So we made two further such 3D landscapes and summarized the locations of the peaks in Figure 12. The solutions turn out to vary. Maximal accuracy is reached in the diurnal burrower at relatively high values of the PRC amplitude as well as the τ -RC amplitude—or at large Pittendrighs and large Aschoffs. The optimal solutions for the nocturnal burrower are found at much smaller τ -RC amplitude. This may provide an adaptive explanation for the fact that the τ after effects of single pulses in nocturnal rodents are probably smaller—and indeed more rarely observed—than in the diurnal species. Also quantitatively the solutions correspond reasonably to our best current estimates of the ratio between phase and velocity responses (lines in Fig. 12). Of more general importance still is that all three solutions are characterized by the fact that during entrainment, the endogenous τ is modulated by the velocity response to exactly 24 h (shaded area in Fig. 12), and thus the system no longer requires a net phase shift each day.

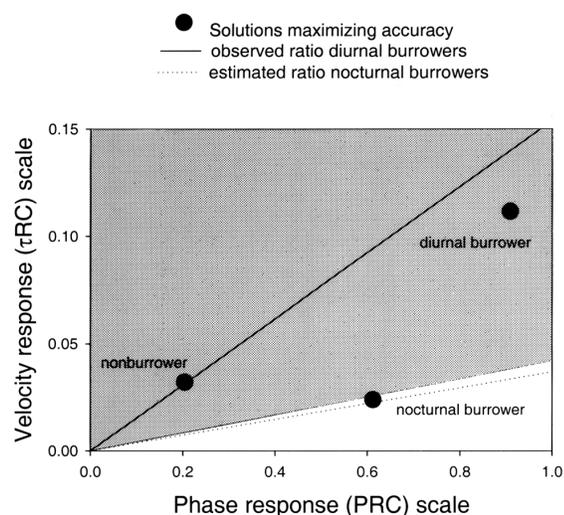


Figure 12. Maximal accuracy solutions for a non-burrowing (continuously exposed) animal and for burrow-dwelling diurnal and nocturnal mammals. The solid line reflects the amplitude ratio of τ -RC/PRC for diurnal mammals; the dotted line reflects the estimated amplitude ratio of τ -RC/PRC for nocturnal mammals. The shaded area indicates all solutions in which mean endogenous τ becomes 24 h exactly during entrainment.

Clearly, the τ -RC both exists and contributes importantly to entrainment, certainly in diurnal mammals and especially in those living underground. The effects of light on both phase and period are purely parametric in this model. Nonparametric or transition effects would completely spoil entrainment each time a ground squirrel comes out of its burrow—or each time a shift of human mine workers emerges above ground. Whether phase shift and τ change are part of the same, parametric mechanism and always bear a proportional relationship to each other is not yet known. This question awaits more complete and precise measurements of τ -RCs.

Be this as it may, I believe that the notion of PRC entrainment by single, discrete phase shifts has suggested to us a mechanism far too coarse for circadian pacemakers. The adjustment of τ by continuous action of light may well turn out to be a fundamental functional property of these pacemakers, allowing animals to finely tune their intrinsic period to that of the earth's rotation without needing to perturb the system each day. Indeed, Terry Page has shown that after effects are an innate property of the pacemaker in the isolated Bulla eye (Page et al., 1997). In addition, we now know

many different feedback loops from peripheral oscillators and overt rhythms, such as the pineal, from locomotor activity and even from carbohydrate ingestion, as Kurt Krauchi has shown at this meeting. All these loops may well contribute to stabilizing the period of the pacemaker at exactly 24 h. I expect that diurnal animals have more necessity for such fine tuning than nocturnals. They are exposed throughout the day to the rather erratic variations in light intensity, and they need to measure the average curve and at the same time suppress their responsiveness not to be knocked about all the time. Nocturnal burrowing mammals can reach higher accuracy because they do not see most of these variations. But also in the nocturnal types, such as the flying squirrel, *Glaucomys volans*, fine-tuning by τ adjustment has been described (DeCoursey, 1989).

In contrast to the prevailing opinion in our field, I am convinced that evolution has not satisfied itself with making sloppy biological clocks, with periods deviating from 24 h, which need resetting once or twice each day. No, evolution has gone the whole hog and has taken care that these clocks under natural conditions run at exactly 24 h without the need for disturbing daily corrections.

I finish by thanking those people who have intellectually contributed a great deal to the substance of this talk: Jürgen Aschoff¹ especially for lending me the slides for the lecture he was originally going to give here today, Roelof Hut for his exquisite ground squirrel work, and Domien Beersma for having been a long-time dedicated sparring partner on these issues and for doing all the modeling. And then prominently Colin Pittendrigh, for his friendship and his clear insights. These insights anticipated everybody else's, as demonstrated in one of his early works of art (Fig. 13) dating back to 1959 when he visited Aschoff in Heidelberg. Everything was in essence already there: both the PRC and the change in τ . The field will eventually look back at these two gentlemen as not only in harmony but also in synchrony.

NOTE

1. A few months after this lecture, Juergen Aschoff suffered a major brain stroke from which he was not to recover. His many contributions to the field have been honored in a special issue of the *Sapporo Symposia on Circadian Clocks* (see Daan, 2000).



Figure 13. Drawing by C. S. Pittendrigh made after his first visit to the Aschoff family in Heidelberg in 1959.

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