Accuracy of circadian entrainment under fluctuating light conditions: contributions of phase and period responses.

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ABSTRACT

The accuracy with which a circadian pacemaker can entrain to an environmental 24-h zeitgeber signal depends on (a) characteristics of the entraining signal and (b) response characteristics and intrinsic stability of the pacemaker itself. Position of the sun, weather conditions, shades, and behavioral variations (eye closure, burrowing) all modulate the light signal reaching the pacemaker. A simple model of a circadian pacemaker allows researchers to explore the impact of these factors on pacemaker accuracy. Accuracy is operationally defined as the reciprocal value of the day-to-day standard deviation of the clock times at which a reference phase (0) is reached. For the purpose of this exploration we have used a model pacemaker, characterized solely by its momentary phase and its momentary velocity. The average velocity determines the time needed to complete one pacemaker cycle and, therefore, is inversely proportional to pacemaker period. The model pacemaker responds to light by shifting phase and/or changing its velocity. We further assumed that phase and velocity show small random fluctuations and that the velocity is subject to after-effects. After-effects were incorporated mathematically in a term allowing period exponentially contract to a stable steady state value, with a time constant of 69 days in the absence of light. The simulations demonstrate that a pacemaker reaches highest accuracy when it responds to light by simultaneous phase shifts and changes of its velocity. Phase delays need to coincide with slowing down and advances with speeding up, otherwise no synchronization to the zeitgeber occurs. At maximal accuracy, the changes in velocity are such that the average period of the pacemaker under entrained conditions equals 24 hours. The results suggest that during entrainment, the pacemaker adjusts its period to 24 hours, after which daily phase shifts to compensate for differences between the periods of the zeitgeber and the clock are no longer necessary. On average, phase shifts compensate for maladjustments of phase and velocity changes compensate for maladjustments of period.
INTRODUCTION

The stability of circadian entrainment is of primary functional significance (Pittendrigh 1981b). Indeed, the accuracy with which circadian systems are able to maintain constant phase angle differences ($\psi$) with the rotation of the earth is probably a prime target of natural selection on timing. No matter which circadian function is crucial for the individual’s survival, whether time memory, sun compass orientation, or the measurement of daylength, it will generally benefit from greater accuracy.

Theoretical understanding of the stability of entrainment is based on two approaches. The first is the analysis of simple (phase only) models using empirical phase response curves (PRCs) for brief light pulses interacting with periodic pulses (Pittendrigh and Daan 1976b). The second approach is the study of the dynamics of oscillatory differential equations (Wever 1966; Pavlidis 1967; Pittendrigh 1981c; Kronauer and Czeisler 1993) or other specific pacemaker architectures (Enright, 1980) under the influence of light in complete photoperiods. One important outcome of these studies is that stability is dependent on both the endogenous period of the entrained oscillator ($\tau$), and the shape of the (PRC).

So far, theory has dealt exclusively with on-off light schedules and has hardly considered how noise, in both the endogenous pacemaker and the entraining signal, affects the accuracy with which the phase relationship is maintained. Natural fluctuations in light intensity across the light-dark (LD) cycle are of particular interest in this context, since few organisms can escape these fluctuations. They must have played a role in evolutionary selection on the accuracy of entrainment. These fluctuations arise as a consequence of both varying degrees of cloud cover and behavioral variations. Animals moving in and out of burrows and shades in daytime or closing their eyes (e.g., during sleep) continuously modulate the light intensity seen by their circadian pacemaker (Roenneberg and Foster 1997). The high accuracy of circadian entrainment in nature maintained in the presence of such a variable zeitgeber signal is rather remarkable. Examples can be found in the onset of morning song (the “dawn chorus”) in songbirds (Scheer, 1952; Aschoff and Wever 1962) or in the evening flight in bats (Voûte et al. 1974). Classic PRC theory, although never exploited to explore this situation, would lead one to expect repeated phase shifts at each change in perceived light intensity due to sensitivity of the system to LD transitions. High sensitivity of circadian systems to naturalistic LD cycles (with fluctuating light intensity) would be expected to lead to an increase in the day-to-day variance in phase and, thus, to a decrease in accuracy of entrainment, just as much as it would increase the accuracy in an artificial, rectangular LD cycle. We were inspired to explore this problem theoretically when faced with the fact that European ground squirrels emerge from their burrows hours after dawn and retire long before dusk. Never seeing the twilights, their pacemakers are exposed only to major light transitions that are due to the animal’s own behavior (Hut et al. 1999a). Superimposed on these self selected variations in light exposure are only minor temporal trends in the afternoon light intensities. To exploit these as zeitgeber signals, the animals should somehow be able to integrate light information over several days.

Indeed, circadian systems potentially have developed ways of integrating light information over several days to overcome the stochastic hazards of instantaneous responses. This would require a form of memory and a computational mechanism of filtering day-to-day fluctuations.
One plausible mechanism to propose is one in which the velocity of the pacemaker (inversely proportional to period $\tau$) is affected by light in a phase dependent manner. This suggestion is inspired by the phenomenon of after effects of entrainment (Pittendrigh and Daan 1976a). After an episode of entrainment, $\tau$ may initially be more than 1 hour away from its true long-term endogenous value, and it may require up to 100 days to contract again to this value. It has been suggested before that such effects may contribute to stabilization of the phase angle difference (Pittendrigh and Daan 1976b; Enright 1980; DeCoursey 1989).

A systematic exploration of this proposition with stochastic variations in light intensity has never been made. In this paper we explore how the magnitude of phase ($\phi$) and period ($\tau$) responses, either alone or in combination, to the same random daylight fluctuations affect the accuracy of $\psi$. We use a simple phase-only model, characterizing the circadian pacemaker by its momentary phase, $\phi$, its instantaneous velocity, $360^\circ/\tau$ (in degrees per hour), and a PRC and $\tau$ response curve ($\tau$RC). We generated these response curves from literature data on the pulse PRC in diurnal mammals, mainly from the PRC-atlas (Johnson 1990) with a few species added recently. Diurnal mammals were chosen because they (1) are naturally exposed to the fluctuations in daytime light intensity and (2) provide the best available material for the $\tau$RC. We investigate the effects of amplitude of both the PRC and $\tau$RC as scaled down from the same PRC. This approach is based on evidence (see below) that $\tau$ increases and decreases in response to a light pulse are associated with phase delays and phase advances, respectively. A velocity response curve to light has previously been postulated to account for entrainment to weak sinusoidal light cycles (Swade 1969), for entrainment in general (Enright 1980) and for the dependence of $\tau$ on constant light intensity (Daan and Pittendrigh 1976a; Daan 1977). In those propositions velocity was considered to be a function of instantaneous light intensity. In the present approach we postulate that velocity changes in response to light, and slowly decays to its long-term dark-dark (DD) value as we know from after effects.

Apart from fluctuations in daytime light intensity, we must also take into account spontaneous fluctuations in the internal state of the circadian pacemaker. In the absence of stimulation, it is unlikely that the period of the pacemaker remains exactly constant or that the phase of the pacemaker remains exactly proportional to the passage of time. Inevitably there will be some variation, and this must have its influence on the accuracy of the system (Enright 1980). We investigate the impact of intrinsic variation of $\tau$ and $\phi$ on pacemaker accuracy.

On the basis of simulated entrainment patterns derived from this model we conclude that accuracy is maximized at submaximal amplitudes of the PRC and $\tau$RC, that accuracy can be nearly doubled by the $\tau$ response, and that the $\tau$ response is a highly functional property of circadian pacemakers.

**A SIMULATION MODEL**

In our simulations, the state of the model circadian pacemaker is represented by a point moving along a circle: At each moment, $t$, in time, pacemaker state is characterized by its phase position $\phi_t$, and its velocity $v_t$. $\phi_t$ is expressed in degrees, and $v_t$ in degrees per hour. $\tau_t = 360^\circ/v_t$ is the instantaneous value of the period of the pacemaker in hours. Pacemaker state
is calculated in 4-minute intervals, such that $\phi$ goes on average through $360^\circ$ in 360 time units during stable entrainment to 24 hours. In the absence of light, every 4 minutes phase proceeds by $24/\tau_t$ degrees. Simultaneously, $\tau_t$ is reset to a new value to take into account the slow return of the pacemaker’s velocity to its long-term stable value of $360^\circ/\tau_0$ during prolonged free-run in DD. In the model this is realized by letting the difference between actual $\tau_t$ and $\tau_0$ be reduced by a factor $c$. We use for $c$ the empirical value of $0.991/360$. The consequence of this is that the difference between $\tau$ and its intrinsic value is reduced exponentially to 50% of its initial value after 69 days without any light stimulation. This rate of contraction was chosen to approximate the data for mice reported by Pittendrigh and Daan (1976a, Figure 8).

In the absence of stimulation, a biological pacemaker will not be capable of maintaining its period exactly constant or its phase angle exactly proportional to time. We therefore incorporated small perturbations in the model. This was done by adding a random number to $\tau$ at the end of each 4 minute interval, and another random number to $\phi$. The random numbers are drawn from even distributions with zero mean, the ranges of which will be indicated below.

The responsiveness of the model system to light is defined on the basis of a PRC and a $\tau$RC, the amplitudes of which are varied systematically in a series of simulations. The shapes of these response curves are based on PRC data of 7 diurnal mammal species, as described in the next section. We scaled this PRC (Figure 9.1A) by two factors ($r_\phi$ and $r_\tau$, respectively) to derive the response to light at any time in the cycle. We further assumed that the magnitude of the phase shift is proportional to the duration of the light pulse. This is approximately the case in hamsters for light pulses with a duration of around 5 min (Nelson and Takahashi 1991).

In summary, the model is described by the following two recursive equations:

\[
\begin{align*}
\phi_{i+1} &= \phi_i + \frac{24}{\tau_i} + \varepsilon_\phi + r_\phi \cdot \Delta \phi(\phi_i) \cdot I_t \\
\tau_{i+1} &= \tau_i - c \cdot (\tau_i - \tau_0) + \varepsilon_\tau + r_\tau \cdot \Delta \phi(\phi_i) \cdot I_t
\end{align*}
\]

where

- $\Delta \phi(\phi_i) =$ average diurnal mammal PRC
- $r_\phi =$ scaling factor for sensitivity to light in terms of phase shifts
- $r_\tau =$ scaling factor for sensitivity to light in terms of $\tau$ changes
- $I_t =$ log of applied light intensity at time $t$
- $c =$ rate of contraction of $\tau$ to $\tau_0$
- $\tau_0 =$ long-term steady-state value of $\tau$ in DD
- $\varepsilon_\phi, \varepsilon_\tau =$ random numbers, drawn from rectangular distributions of zero mean and width = $\eta_\phi, \eta_\tau$

This model is subjected to a simulated LD cycle with fluctuating light intensity, $I_t$. We start from the empirical curve describing the change of light intensity of the zenith of a clear sky as a function of time of day (Daan and Aschoff 1975). Simulations in this paper are restricted to a standard day: equinox at the equator. We use a log-transformed light intensity value to account for general light sensitivity characteristics of visual systems (Nelson and Takahashi 1991). By doing so, we effectively attenuate the sensitivity of the model pacemaker to changes.
in light intensity at the higher light intensities. The logarithmic transformation results in the highest sensitivity to changes in light intensity to occur near dawn and dusk, just as might be expected for a circadian pacemaker. Based on sensitivity data of circadian systems (Nelson and Takahashi 1991; Shimomura et al. 1998) we assumed that the sensitivity of the input system of the pacemaker encompasses 7 log units, ranging from the zenith clear sky value to 7 log units below this. Light intensities used to measure the PRC are in the order of 1000 lux which is about 2 log units below maximum clear sky values. The range of log-transformed values is converted to a linear scale, attributing a value of 1 to the light level used for measuring the PRC and a value of 0 to the lowest visible light intensity. Accordingly, the zenith clear sky light intensity value is set to 1.4, and each log unit of light intensity corresponds to 0.2 units on the new scale.

We then introduced simple stochastic variation in the actual light intensity observed by subtracting a random number in the range from 0 to 0.6 from the momentary maximum value. This is done at 28-minute intervals to account for some persistence in the fluctuations. Successive 4-minute values are linearly interpolated. The range from 0 to 0.6 corresponds to 3 log units of light intensity, say 1 log unit to account for eye closure and 2 log units for overcast and shades. Figure 9.2 presents an example of the so defined input signal to the pacemaker. The effects of burrowing behaviour obviously introduces light intensity reductions exceeding 3 log units (Hut et al. 1999a). Elsewhere we will present analyses specifically directed at the influence of burrowing behaviour on pacemaker accuracy.

Figure 9.1 (A) Average phase response curve (PRC) for day active mammals, based on seven species. (B) Average \( \tau \) response curve (\( \tau \)RC) for dayactive mammals, based on five species. This includes data for Microtus arvalis, which exposes itself to light in daytime by above ground feeding (Gerkema et al., 1993). In both panels, species fitted curves (sinewave + two subharmonics) are shown, as well as the average curve for all the species.
The behavior of the model system is investigated for various combinations of the PRC and τRC amplitudes, \( r_\phi \) and \( r_\tau \). The output variable under study is the accuracy of the pacemaker system. For that purpose the standard deviation of the clock times at which the circadian phase equals zero is calculated. The accuracy of the pacemaker system is defined as the reciprocal value of this standard deviation in hours, and has the dimension \( h^{-1} \). In each simulation the standard deviation and, hence, accuracy was computed over 1000 days of the model system, after skipping the initial 100 days to allow for transients to disappear. The average value of the intrinsic period of the model system was computed over the same interval of 1000 days.

**THE MODEL PRC**

For the basic shape of the PRC and τRC in our analysis we used data on the phase response to brief light pulses from seven species of diurnal mammals, five *Sciurid* rodents and two primates (see Table 9.1). For species where PRCs for different pulses were available, we selected the set with pulse duration closest to 1 hour. Circadian timing of the light pulse was determined in

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**Figure 9.2** Example of applied light intensity profiles (continuous lines) and available light on clear days (dashed lines). Subsequent days are plotted beneath each other as well as adjacently. Light intensity is plotted logarithmically. The range is from 0.01 to 100,000 lux. Ticks along the vertical bar each indicate a tenfold change in light intensity.
each case with reference to activity onset (= circadian time (ct) 0). *Octodon degus* data (Lee and Labyak 1997) were not included in this set, since it is debatable whether this is a diurnal species (Kas and Edgar 1998). Human PRC data (Honma and Honma, 1988; Minors et al., 1991; Beersma and Daan 1993) were not included, since in this case a different phase reference (body temperature minimum) was used. Other human phase shift data in the literature are based on the shift on the first day after the pulse, and not on steady-state phase shifts as in the animal literature, or they refer to the effect of multiple pulses given in 24-h intervals rather than single pulses (Czeisler et al. 1989; Jewett et al., 1991, 1994; Boivin et al. 1996; Boivin and Czeisler, 1998). In each case we applied Fourier analysis to describe the primary waveform (period 24 circadian hours) plus its first two subharmonics (periods 12 and 8 h). The seven curves obtained were subsequently averaged (Figure 9.1A). The average curve has zero-crossing points at ct 4.8 and 17.4. The maximal phase delay (-2.50 h) occurs at ct 13.8, the maximal advance (+2.05 h) at ct 20.5. The curve is monotonically declining from ct 20.5 until ct 13.8 and monotonically rising from ct 13.8 until ct 20.5.

Phase delays in response to single light pulses are often associated with an increase in \( \tau \), while phase advances are associated with a decrease in \( \tau \) (Pittendrigh and Daan 1976a). The opposite is true for the response to some nonphotic stimuli (Mrosovsky 1993), and we will comment on this discrepancy in the discussion. To evaluate whether there is sufficient empirical basis to assume proportionality between the \( \phi \) and \( \tau \) responses following a single brief light pulse, we treated \( \tau \)RC data in the same way as PRC data. Obviously, this data base is much more restricted. There are four published \( \tau \) response curves for light pulses, for the sciurids *Tamias striatus*, *Tamiasciurus hudsonicus* (Kramm and Kramm 1980) and *Ammospermophilus leucurus* (Pohl 1982), and for the day- and night-active vole *Microtus arvalis* (Gerkema et al. 1993). In addition, there are unpublished data for the chipmunk *Eutamias sibiricus* (see Table 9.1). The raw data for these \( \tau \)RCs were subjected to the same Fourier analysis with 2 subharmonics, and the results are shown in Figure 9.1B. The average curve in this case has zero-crossings at ct 3.1 and ct 16.5. The maximal period lengthening (-0.373 h) occurs at ct 13.7, and the maximal period shortening (+0.325 h) occurs at ct 19.2. The curve is not monotonic.

### Table 9.1 Phase response curve and \( \tau \) response curve data used in Figure 9.1.

<table>
<thead>
<tr>
<th>Species</th>
<th>Pulse</th>
<th>PRC</th>
<th>( \tau )RC</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ammospermophilus leucurus</em></td>
<td>0.25</td>
<td>100</td>
<td>+</td>
<td>Pohl (1982)</td>
</tr>
<tr>
<td><em>Tamiasciurus hudsonicus</em></td>
<td>1</td>
<td>1200</td>
<td>+</td>
<td>Kramm and Kramm (1980)</td>
</tr>
<tr>
<td><em>Tamias striatus</em></td>
<td>1</td>
<td>1200</td>
<td>+</td>
<td>Kramm and Kramm (1980)</td>
</tr>
<tr>
<td><em>Funambulus palmarum</em></td>
<td>1</td>
<td>1000</td>
<td>-</td>
<td>Navaneethakannan and Chandrashekaran (1986)</td>
</tr>
<tr>
<td><em>Eutamias sibiricus</em></td>
<td>3</td>
<td>400</td>
<td>+</td>
<td>Vermij and Dijk (n.d.)</td>
</tr>
<tr>
<td><em>Saimiri sciureus</em></td>
<td>1</td>
<td>600</td>
<td>-</td>
<td>Hoban and Sulzman, cited in Johnson (1990)</td>
</tr>
<tr>
<td><em>Callithrix jacchus</em></td>
<td>1</td>
<td>1100</td>
<td>-</td>
<td>Wechselberger and Erkert (1994)</td>
</tr>
<tr>
<td><em>Microtus arvalis</em></td>
<td>0.25</td>
<td>30</td>
<td>+</td>
<td>Gerkema et al (1993)</td>
</tr>
</tbody>
</table>
from maximum to minimum due to a minor secondary trough around ct 6. We are not convinced that this secondary dip would remain present in a larger data set. It might well be due to the vagaries of harmonic analysis on a relatively small number of data points. The circadian times of the minimum and maximum of the curve, as well as of the zero crossing in the subjective night, closely match those in the average PRC. Moreover, the two curves are roughly proportional in shape: the maxima have a ratio (PRC/τRC) of +2.05/+0.325 = 6.67, whereas the ratio of the minima is 2.50/-0.373 = 6.70. We therefore feel confident to assume in our model that PRC and τRC have the same shape; that is, that they can both be derived by a linear transformation of one basic curve. For this basic curve we used the average PRC for diurnal mammals as depicted by the solid line in Figure 9.1A.

RESULTS

Figure 9.3 presents the model’s accuracy for a set of simulations in which the contributions of phase shifts and velocity responses are systematically varied. The x-axis presents the applied variation (rφ) in PRC strength, ranging from -1 to 1 in steps of 0.05. The y-axis represents the applied variation (rτ) in τRC strength, ranging from -0.2 to 0.2 in steps of 0.01. The plot presents accuracy for each combination of rφ and rτ in the form of contour plots: the lines connect points of equal accuracy. In the simulations of Figure 9.3 the ranges ηφ and ητ of the random values εφ and ετ of equations (1) and (2) were set to zero; hence, there was no intrinsic variation of pacemaker state. The results demonstrate that pacemaker accuracy varies considerably as a function of rφ and rτ. At rφ = 0 and rτ = 0, the model system free runs at its intrinsic period (τ0) of 23.5h, and thus its phase relationship with the LD cycle is therefore extremely inaccurate. Maximal accuracy of 22 h⁻¹ is observed at rφ = 0.05 and rτ = 0.01. The corresponding standard deviation is almost 3 minutes. Simulations with smaller steps near rφ = 0 and rτ = 0 demonstrate that the actual peak in the profile is even higher about 51 h⁻¹ and occurs at rφ = 0.015 and rτ = 0.0008. This accuracy is much higher than the best obtainable accuracy of a system without responsiveness of τ to light (rτ = 0). In that case, maximal accuracy (12 h⁻¹) is found at rφ = 0.25. From the profile of Figure 9.3, it can be seen that the maximum accuracy is smaller (5 h⁻¹) if the system does not phase shift in response to light (rφ = 0) and only responds to light by changing its τ at a strength of rτ = 0.01. Apparently, accuracy can increase substantially when responses to light, in both φ and τ, occur simultaneously.

The simulations also demonstrate that no stable entrainment occurs whenever positive values of rφ are combined with negative values of rτ, or vice versa. Apparently, phase delays must coincide with a lengthening of τ and phase advances with shortening of τ to obtain stable entrainment. Choosing negative values of rφ in combination with negative values of rτ leads to stable entrainment, but with lower pacemaker accuracy as compared to positive rφ and rτ values. This is because the shape of the PRC is altered by the inversion. Entrainment in this quadrant of the rφ, rτ plane occurs at opposite ψ values.

The absence of any intrinsic pacemaker variation is, of course, unrealistic for biological systems. It is likely that a pacemaker will show small fluctuations of its period and its phase angle in the course of time. We incorporated such noise by arbitrarily adding a random number ετ
between -0.001 h and +0.001 h (\(\eta_\tau = 0.002\)) to \(\tau\) and a random number \(\varepsilon_\varphi\) (\(\eta_\varphi = 0.0003\) h) to \(\varphi\) at 4-minute intervals. The results are presented in Figure 9.4. First, it is obvious that the accuracy of the system is reduced because of the introduction of random fluctuations. Peak accuracy values in this example are reduced from 51 h\(^{-1}\) in Figure 9.3 to about 13 h\(^{-1}\) in Figure 9.4. Second, the peak in accuracy shifts to higher values of \(r_\varphi\) (=0.20) and \(r_\tau\) (=0.05). It is clear that accuracy is again much better under the combined influence of \(\varphi\) and \(\tau\) responses as compared to either response alone. Only phase shifts yield at best an accuracy of 9 h\(^{-1}\) at \(r_\varphi = 0.4\), \(r_\tau = 0\). Only period changes yield at best an accuracy of 3 h\(^{-1}\) at \(r_\varphi = 0\), \(r_\tau = 0.04\). We also tested the effects of incorporating random fluctuations only in \(\tau\) or only in \(\varphi\). The values of \(\eta_\tau\) and \(\eta_\varphi\) of Figure 9.4 were chosen to reduce maximum pacemaker accuracy by the same amount. With \(\eta_\tau = 0.002\) and \(\eta_\varphi = 0\) maximum accuracy was 14 h\(^{-1}\) at \(r_\varphi = 0.15\), \(r_\tau = 0.06\). With \(\eta_\tau = 0\) and \(\eta_\varphi = 0.0003\), maximum accuracy was 14 h\(^{-1}\) at \(r_\varphi = 0.1\), \(r_\tau = 0.01\).

The ratio between the amplitude of the phase shifts and that of the period changes at maximal pacemaker accuracy, \(K = r_\varphi r_\tau\), is of particular interest, since it may be compared with empirical data. In the simulations with fully deterministic \(\tau\), in Figure 9.3, this ratio is 0.015/0.0008 = 19. In Figure 9.4, under stochastic error in both cycle length and phase angle,
$K = 0.2/0.05 = 4$; under stochastic error in phase angle only, $K = 10$; and under stochastic error in $\tau$ only, $K = 3$. As reported above, the ratio between PRC and $\tau$RC of diurnal mammals is about 6.7.

Each of the simulations presented in Figure 9.4 yielded an average value of the model system’s intrinsic $\tau$. This value may differ from 24 hours even under entrained conditions, since additional phase shifts may compensate for the difference between $T$ and $\tau$. For the area where $r_\tau > 0$ and $r_\phi > 0$, the results for the average values of $\tau$ are plotted in Figure 9.5. Along the line $r_\tau = 0$, (i.e., in the absence of any responsiveness of $\tau$ to light) we find that the average $\tau$ approximates $\tau_0$ (=23.5 h). At values of $r_\tau > 0$, the system’s mean $\tau$ value progressively approaches 24.0 hours. At maximal accuracy of the model pacemaker, the value of $\tau$ turns out to be 24.0 hours.

**DISCUSSION**

The simulations show first that if circadian systems were to entrain only by phase shifts ($r_\tau = 0$), their accuracy would not benefit from a high-amplitude PRC. Instead, accuracy is maximized at submaximal PRC. Too high a sensitivity exposes the system too much to the vagaries of the daily light intensity variations. Second, the same thing would happen if entrainment
were to work only by period control ($r_\phi = 0$): submaximal $r_\tau$ maximizes accuracy. Too high a sensitivity increasingly leads to oscillations of the system around its preferred phase and, therefore, to increasing inaccuracy. Additional simulations (data not shown) revealed that the decreases in accuracy at high values of $r_\phi$ and $r_\tau$ are due to stochastic fluctuations in light intensity. No such decreases occur in the absence of stochastic fluctuations. Third, and most important, the simulations demonstrate that the accuracy of a pacemaker model can be increased considerably by letting the model respond to light not only by phase shifts but also by $\tau$ changes. This improved accuracy occurs only if phase advances coincide with shortening of $\tau$ and if phase delays coincide with lengthening of $\tau$. This means that if light is perceived by the system at an unexpectedly early time, this is “interpreted” as being the result of an incorrect phase position of the system, which is adjusted, but which also might be caused by an incorrect velocity of the system, which is also adjusted. The coexistence of phase and period responses of diurnal species to light (Figure 9.1) is therefore likely to have evolved to improve pacemaker accuracy in natural conditions. The net result of the velocity responses is that the average period of the system under entrainment is approximately 24 hours (Figure 9.5).

A series of assumptions form the basis of the model used for the simulations of this article, beginning with the particular type of model chosen. We have taken our model to respond to light by changing its phase and velocity, but not the amplitude of its output waveform. Jewett et al. (1991) published evidence suggesting amplitude responses of the pacemaker signal to light in humans (c.f. Beersma and Daan 1993). Such amplitude changes, if present, occur in particular when light is applied during the subjective night. Our model pacemaker is virtually always synchronized to the LD cycle and does not receive light during the subjective night. So, even if the human pacemaker system responds to light by modifying the amplitude of its output waveform, the results of the simulations will not change drastically since the amplitude changes under our simulation conditions will be very small.

In the simulations of Figures 9.3 and 9.4 we have assumed that the pacemaker under study had an intrinsic period $\tau_0$ of 23.5 h and that $\tau$ slowly contracts to this value in the absence of light. The small intrinsic noise in the pacemaker applied in Figure 9.4 is sufficient to largely mask the impact of $\tau_0$ and the impact of the contraction. Because there is some arbitrariness in the choice of both $\tau_0$ and of the rate of contraction, we repeated the simulations of Figure 9.4, first setting $\tau_0$ to 24 h and then setting the rate of contraction ($c$) to 0 (i.e. $\tau_0$ has no influence whatsoever on the behavior of the system). In both sets of simulations the result is virtually identical to Figure 9.4. This means that if an animal responds to light by adjusting its pacemaker period, the actual value of the intrinsic period of the pacemaker has very little influence on the accuracy of entrainment and on the average $\tau$ during entrainment. Extending $\tau_0$ to 24.5 h yields a contour plot of pacemaker accuracy that is very similar in shape to Figure 9.4. For each combination of $r_\phi$ and $r_\tau$ the accuracy values are, however, slightly higher than in Figure 9.4. This is most likely due to the difference in phase position between the two sets of simulations.

Another set of assumptions concerns the shape of the light signal that we applied. To what extent does it match actual light intensity perception by an animal’s pacemaker? Is a logarithmic transformation of available light intensity a correct characterization of the transfer characteristics of light sensitivity of a pacemaker? The range of 7 log units we used extrapolates the 5 log units measured by Nelson and Takahashi (1991) and Shimomura et al. (1998). Is this
correct? To what extent is the induced phase shift proportional to the duration of the light pulse? To what extent do the frequency characteristics of the fluctuations in light intensity mimic natural fluctuations? Are the postulated sizes of these fluctuations realistic? For most of these questions no precise answers are currently available. We can only argue that the way we simulated the temporal pattern of light is more realistic than applying a square wave 12:12 LD cycle as is frequently done in simulations. Careful measurements of the light actually perceived, which is done in studies by Okudaira et al. (1983) and Savides et al. (1986) in humans and by Hut et al. (1999a) in ground squirrels, are needed to clarify these issues.

The model system studied here under equinoctial (LD ca 12:12) LD cycles shows maximal pacemaker accuracy at $r_\phi = 0.20$ and $r_\tau = 0.05$. This implies that the amplitude of the PRC required to generate maximal accuracy is about 5 times as small as the amplitude of the PRC as determined by artificial light pulses (where $r_\phi = 1$ by definition). There are various ways to deal with this discrepancy. First, one might argue that the eye of the dark-adapted animal receiving its 1-hour artificial light pulse in a PRC experiment is much more sensitive to light than the light-adapted eye of the animal living outside. Because little is known about effects of dark adaptation on pacemaker sensitivity to light in diurnal mammals (see Winfree 1972, for slow dark adaptation in Drosophila, and Shimomura et al. 1998, for dark adaptation effects in $\tau$ mutant hamsters), there is no way for independent optimal estimation of the magnitude of the intrinsic pacemaker fluctuations. The amount of noise in the state of the pacemaker in our

![Figure 9.5 Three-dimensional profile of average $\tau$ values as a function of phase response curve (PRC) amplitude and $\tau$ response curve ($\tau$RC) amplitude for the parameter settings of Figure 9.4. For this purpose, $\tau$ values were averaged over the 1000 days of the simulations.](image)
simulations might be underestimated. Choosing a wider range of random values in equations (1) and (2) will shift the maximum accuracy to higher $r_\phi$ and $r_\tau$ values, which may solve the discrepancy. Statistical analysis of free-running locomotor behaviour in mice (*Mus musculus*) have led Pittendrigh and Daan (1976a) to estimate the standard deviation of pacemaker period to be about 8 minutes, so accuracy would be 7.5 h$^{-1}$. If such accuracy values were to hold for the diurnal species we attempt to simulate, this would suggest again that the values of $\eta$ in our simulations should be increased.

The simulations demonstrate that the coexistence of phase delays and shortenings of $\tau$, as well as the coexistence of phase advances and lengthenings of $\tau$ lead to instability of the system. As demonstrated in Figures 9.3 and 9.4, small negative values of $r_\tau$ are sufficient to preclude entrainment of the model system to the applied LD signal. This finding is important to note in relation to Mrosovsky’s findings (1993) with respect to the impact of motor activity on phase and period of the circadian pacemaker. In those experiments, changes in $\tau$ and changes in phase do show such inverse relationship. The function of the responses of the circadian pacemaker to pulses of motor activity must therefore be distinctly different from improving circadian accuracy.

As the simulations demonstrated, the ratio $K$ of $r_\phi$ and $r_\tau$ at which maximal accuracy occurs is sensitive to the relative amount of random fluctuations in the phase angle and pacemaker velocity. If each of those amounts of noise is chosen to have a similar effect on pacemaker accuracy, the value of $K$ under the combined action of both sources of fluctuation equals 4.0, which is slightly less than the empirical value of about 6.7. Because the relative contributions of both sources of fluctuations are not known, we can only conclude that the empirical value of $K$ lies well within the range from 3 to 10 that results from the simulations.

In a subsequent publication, we will explore the situation specifically for diurnal and nocturnal burrowing animals that sequester themselves in darkness during nighttime and daytime, respectively. They thereby perceive very different light information under natural conditions, which has important consequences for optimal entrainment by phase and period adjustment. Here, it suffices to emphasize that period adjustment is a very important and insufficiently appreciated ingredient of entrainment. It changes our view of the basic mechanism from one in which a systematic discrepancy between $\tau$ and $T$ is corrected by a phase shift each day to one in which the system adjusts its $\tau$ to 24 hours, so that net daily phase shifts for period adjustment are hardly necessary in the natural world.

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