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TONIC AND PHASIC EFFECTS OF LIGHT IN THE ENTRAINMENT OF CIRCADIAN RHYTHMS

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There is one specific area where the problems of circadian rhythms intertwine with those of tonic and phasic functions of sensory systems: in the entrainment of these rhythms by the natural cycle of light and darkness.

Numerous factors in most animals' environment vary in a 24-hour pattern. In the course of evolution on an ever rotating planet, circadian rhythms in animal behavior and physiology may have developed ultimately as an adaptation to any or many of these variations. Sleep, for instance, is currently viewed as a behavioral strategy, immobilizing the organism at a time of day when being active would be inefficient in terms of energy gain and expenditure, or even hazardous owing to increased risk of predation.[3] As an evolutionary response to environmental periodicities, innate temporal programs have developed in a wide variety of organisms, down to protozoans and algae. These rhythms include circadian variations in arousal, motivation, and performance. In constant conditions their frequency typically differs from $(24 \text{ hours})^{-1}$ and from any other known environmental periodicity; it varies among individuals and among different genotypes. These facts sufficiently attest to their endogenous nature. No sensory input, whether tonic or phasic, is required to generate these rhythms.

By the same token, circadian rhythms must contain facilities insuring their synchrony with environmental periodicity. Otherwise, they would obviously be useless. The most reliable time cue available in the environment is the daily cycle of light and darkness. Virtually all circadian rhythms studied turn out to be sensitive to the light-dark cycle in the sense that they can be synchronized or *"entrained"* by it.[4] The period of the organism's rhythm is corrected for day after day by smaller or larger shifts induced by the light.

The mechanism of entrainment has received considerable interest. Yet, the problems are far from solved. One of the main obstacles has been our ignorance about both the photoreceptors involved and the sensory pathways to the circadian pacemakers. The very existence of such pacemakers has only recently been demonstrated. It is of tell-tale significance that in every case analyzed they are located close to photosensory organs. In cockroaches, circadian pacemakers controlling the activity rhythm were localized in the optic lobes,[5] in silkmoths a clock in the protocerebrum times pupal eclosion,[6] in the mollusc *Aplysia* there is a pacemaker in the eyes,[7] in sparrows the light-sensitive pineal has a function in driving the bird's daily rhythm of activity and body temperature.[8] In mammals there is increasing evidence that the suprachiasmatic nuclei (SCN) in the hypothalamus are responsible for a diversity of circadian rhythms.[9, 10] A plausible interpretation of the facts available, on the one hand, is that the SCN serve as a master-pacemaker triggering or synchronizing several physiological and behavioral circadian rhythms, some of these probably via the hypothalamic-pituitary regulation centers. They thereby maintain the internal

temporal order. On the other hand, the SCN possibly function to synchronize the organism with the outside world by their direct afferent connection with the retina.[11]

What happens on the afferent pathway is not known. The receptor cells in the retina involved have not been identified; the electrophysiology of their SCN-projections is unknown, and so is the physiology of the pacemaker itself. The analysis of sensory function in entrainment is restricted to a body of behavioral data from experiments in which parameters of the light cycle are manipulated and the behavior of the pacemaker is inferred from one of the rhythms it controls.[e.g. 12-18] Such studies have not been aimed particularly at the analysis of light perception, but they do relate to tonic and phasic effects of light to the extent that the authors were concerned with two theoretically possible modes of entrainment. The circadian system may respond continuously to the level of light intensity with a modulation of its velocity. By ongoing acceleration and deceleration, it may be able to adjust its frequency to that of the environment. In jargon borrowed from physical oscillator theory, this has been baptized "parametric entrainment." On the other hand, one might envisage that the pacemaker is abruptly reset once or twice a day when the environment offers its discrete time cues, viz. at dawn and dusk, just as anybody would adjust their slow watch to a radio time signal each day. This is "non-parametric" entrainment. The continuous observation of light intensity requires some tonic* input from the photoreceptors, whereas the perception of dawn and dusk may be adequately achieved by phasic* responses.

The problem of abrupt (nonparametric, phasic, differential) versus continuous (parametric, tonic, proportional) entrainment of circadian rhythms has long been recognized theoretically,[12] yet no attempts have been made to distinguish experimentally between their contributions to entrainment. Experimentally, it is perfectly feasible to entrain circadian rhythms by repetitive short light stimuli,[17] conveying virtually only phasic information. On the other hand, there is little direct evidence for tonic contributions to entrainment—understandably so, since any entraining agent is a cycle and hence necessarily involves gradients or transitions. Direct evidence for tonic effects on circadian rhythms is thus restricted to unentrained, or free-running, conditions. Continuous illumination consistently affects circadian frequency in most animals. This influ-

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* The terms "tonic" and "phasic" are used here in the strict sense of responses to constant sensory stimulation and to gradients in stimulus intensity, respectively. At the conference, there has been a tendency to describe as tonic effects a variety of long-lasting behavioral responses to sensory input or its interruption, and to distinguish between tonic and phasic responses according to the time constants of their decay. In the present context, such broad usage would have undesirable consequences. It is known that a brief (<1') light stimulus in otherwise constant darkness (DD) may phase shift a circadian oscillation by a couple of hours.[13] Days or weeks later a phase-shifted system will, at any point in time, be in a different phase, and hence behave differently from a control that did not receive the stimulus. While the reset itself is completed in a matter of days, the result can often be observed for as long as an organism's lifetime. The time constant of decay is infinity, regardless of the time course of the initial reset. Describing such effects as tonic would obscure the real issue. The circadian oscillation itself creates a pattern in time, and thereby the perpetuation of the effect of one sudden event. Clearly, from the persistence of a reset something is learned about the circadian oscillation, not about its resetting mechanism.

ence has attracted considerable interest since Aschoff[12] showed that circadian rhythms are slowed down by constant light (LL) in nocturnal animals and accelerated by LL in diurnal animals, and proposed that these tonic effects are of functional significance in the mechanism of entrainment. Comparing six species, Pohl[19] has recently reported a close correspondence between effects of light intensity on τ in unentrained conditions and on phase angle difference between rhythm and zeitgeber in entrained conditions. These data suggest that tonic effects of constant light indeed reflect some aspect of the entrainment mechanism. Dr. C. S. Pittendrigh and I have studied the question of whether these effects are related to phasic entraining stimuli. We obtained evidence for such a relationship in four species of nocturnal rodents. The argument is based on the interspecific and interindividual comparison of two sets of experimental data fully described elsewhere.[1, 2]

The first set of data concerns so-called *phase response curves* for short (15') light stimuli applied in constant darkness (DD) to individual rodents belonging to four species. These curves describe the induced phase shift in the activity rhythm as a function of the phase of the rhythm at the time of the stimulus. The activity rhythm was assayed by monitoring running wheel revolutions. The phase shift was measured by comparing rhythm phases during 10 days before and after the stimulus. Stimuli applied around the onset of activity induce delay phase shifts, those applied around the onset of the daily rest period induce advance phase shifts, as in other nocturnal animals.[14] The magnitude of the responses varies considerably, both among and within species (FIGURE 1). Large delays were observed in species with relatively fast circadian DD-rhythms (*Mus musculus*, average circadian $\tau = 23.43$ hrs; *Peromyscus maniculatus*, $\tau = 22.92$ hrs); small delays in species with a slow free-running rhythm (*Peromyscus leucopus*, $\tau = 23.99$ hrs; *Mesocricetus auratus*, $\tau = 24.04$ hrs). The same trend was found in comparing individuals in the one species (*P. maniculatus*) showing substantial interindividual variation in free-running period.[1]

The second set of data (FIGURE 2) establishes the dependence of the free-running circadian period (τ) in the four species on the intensity (I) of continuous illumination.[2] In species with large delay responses to light pulses (*M. musculus*, *P. maniculatus*), τ is more steeply dependent on I, or, the circadian rhythm is slowed down by constant light more than in *P. leucopus* and *M. auratus*. The same correlation between maximum delay phase shift due to a light pulse and the slow-down effect of constant light was found in the interindividual comparison in deermice (*P. maniculatus*).

Thus, there is a close correlation both in the inter- and intra-species comparisons between the two sets of data. This suggests that the changes in (angular) velocity of the circadian rhythm elicited by (1) brief light pulses in constant darkness and (2) continuous illumination are based on qualitatively similar processes. The response to constant light, when studied on the same basis, must, however, be smaller by at least an order of magnitude. For instance, in *P. maniculatus* the transfer from DD to continuous illumination of 100–200 Lux results in a slight increase in the free-running circadian period by 1.1 hours or 5%.[2] A 15' light pulse of this intensity alone may induce a delay of 2 hours (30°) (FIGURE 1). Using a simple computer algorithm, we showed[2] that the pacemaker had to respond to constant light in any phase with a change in velocity of 0.02 times that of its response to a 15' pulse to produce an overall deceleration of 5%. The proximate hypothesis accounting for this reduced
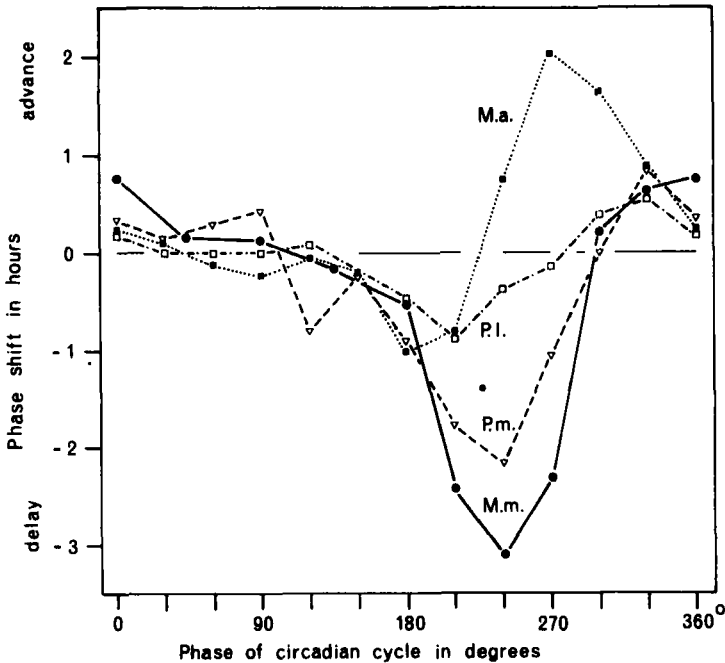
FIGURE 1. Phase response curves for brief (15′) light pulses in DD of circadian activity rhythms in four species of nocturnal rodents. One circadian cycle is divided in 360°, with the onset of activity at 180°. Phase shifts (advance shifts positive; delay shifts negative) were measured in hours. Each symbol is an average of 5–20 measurements. M.a. = *Mesocricetus auratus*, P.l. = *Peromyscus leucopus*, P.m. = *Peromyscus maniculatus*, M.m. = *Mus musculus*. (From Daan & Pittendrigh.[1])

response in continuous light invokes light adaptation of the photoreceptors involved. Actually, by treating the pulse responses as effects on the pacemaker's angular velocity, and by applying simple linear transformations to account for various levels of photoreceptor adaptation, we were able to qualitatively predict from the first set of data the results from the second set.[2]

Until we get concrete information on the physiology of the circadian pacemakers and their afferent connections, these and other[17] behavioral data force us in the opinion that transitions between light and darkness are major contributors to the entrainment of these rodent's circadian rhythms. Presumably, very little influence is exerted by the continuous action of light. This is the more true since the "subjective day" (circadian phases 0–180°)—i.e., that part of the cycle that in stable entrainment coincides with the daily light period—is often characterized by near insensitivity in the phase shifting response to light (FIGURE 1). In animals retreating in dark burrows in daytime, as many nocturnal animals do, there are further behavioral reasons for their reliance on phasic information derived from the twilight transitions.[20]

Thus, the effect of constant illumination on circadian frequency is presently at best considered an artifact, resulting from the entrainment mechanism. By incomplete photoreceptor adaptation to continuous light, residual accelerations
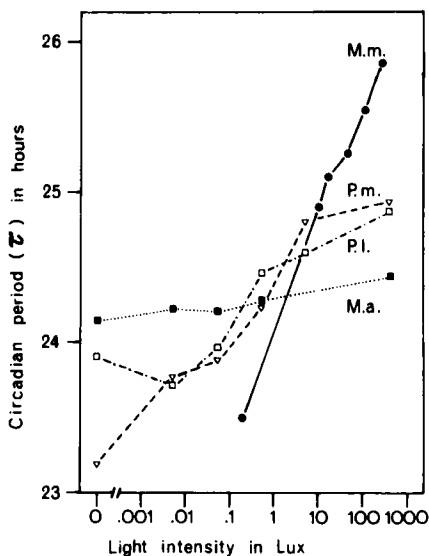
FIGURE 2. The dependence of average free-running circadian period on the intensity of constant illumination (LL) in four species of nocturnal rodents. (Abbreviations as in FIGURE 1). (From Daan & Pittendrigh[2]; Data for *Mus musculus* from Aschoff[22]).

and decelerations in different phases of the circadian cycle may account for a slight but measurable effect on the duration of the complete cycle (τ). This hypothesis is schematically illustrated in FIGURE 3. The hypothesis obviously can be translated into an explicit algorithm. I have not withstood the temptation of simulating various patterns of entrainment on this basis. The Fortran computer program used defines angular velocity in continuous darkness as a constant ($=360/\tau$ degrees per hour) throughout the cycle: 15' of light following darkness changes angular velocity of the rhythm as dictated by an empirical phase response curve; 15' of light following light changes angular velocity as dictated by a linear transformation (say 0.02) of this curve. FIGURE 4 shows that the major features of entrainment by light cycles are easily accommodated by the hypothesis. This does not imply that the assumptions made in the computer program are necessarily correct. It can only be concluded that a system having similar, though quantitatively different, responses to constant illumination and to transitions in light intensity would to a large extent behave as many circadian activity oscillators do. Other features of activity rhythms, most notably "splitting"[13, 18] and "after-effects,"[13, 21] indeed suggest that the circadian pacemaker involved is less rigid that assumed in these simulations and is better described by a system of two coupled oscillators.[13, 18, 22] This refinement does not pertain to the role of photoreceptors in entrainment by light.

The relationship between tonic and phasic effects outlined here clearly remains a theoretical construct. The action of photoreceptors cannot soundly be separated from the rest of the circadian system on the basis of behavioral data alone. Direct electrophysiological analysis is obviously needed. One of the major questions in this area is to what extent modulations of receptor sensitivity are themselves under endogenous circadian control. This is unknown in vertebrates. In several athropods retinal pigment migration is based on an endogenous circadian rhythm. Electroretinograms in crayfish[23, 24] and scor-
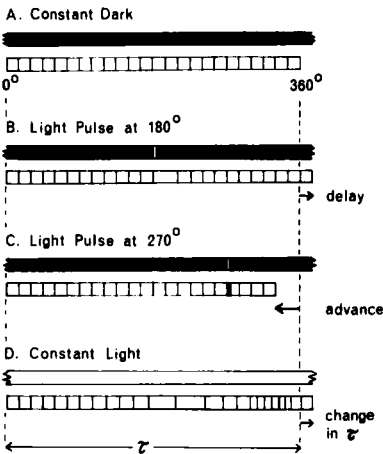
FIGURE 3. A model of the relation between phasic and tonic effects of light on a circadian oscillation.

A. Constant darkness (DD). Angular velocity is defined as a constant $(= \dfrac{360}{\tau}$ degrees/hour) throughout. Vertical lines indicate circadian phases 15° apart.

B. A single 15' light pulse at 180° produces a delay phase shift by sudden reduction of angular velocity.

C. A 15' light pulse at a later phase (270°) produces an advance phase shift by a sudden increase in angular velocity. Changes in angular velocity necessary to produce the empirically measured phase shifts are calculated for every phase point of the rhythm.

D. Constant light (LL). The velocity response curve is reduced by a constant factor X to account for photoreceptor adaptation. The resulting time course of circadian phases is shown using the golden hamster's response curve and X = 0.12. Deceleration occurs at phases with delay phase shifts, acceleration at phases with advance phase shifts, together producing a slight increase in τ.

A. Constant Dark

0°   360°

B. Light Pulse at 180°

delay

C. Light Pulse at 270°

advance

D. Constant Light

change in τ

τ

pions[25] show that the eyes are alternatingly "dark-adapted" and "light-adapted," even in constant darkness. On the basis of this variation alone, circadially changing responses to a stimulus, and hence entrainment, would be expected. It remains a fascinating question how much of circadian synchronization is accomplished by means of the sensory periphery, and how much is a central property of the driving circadian oscillators.

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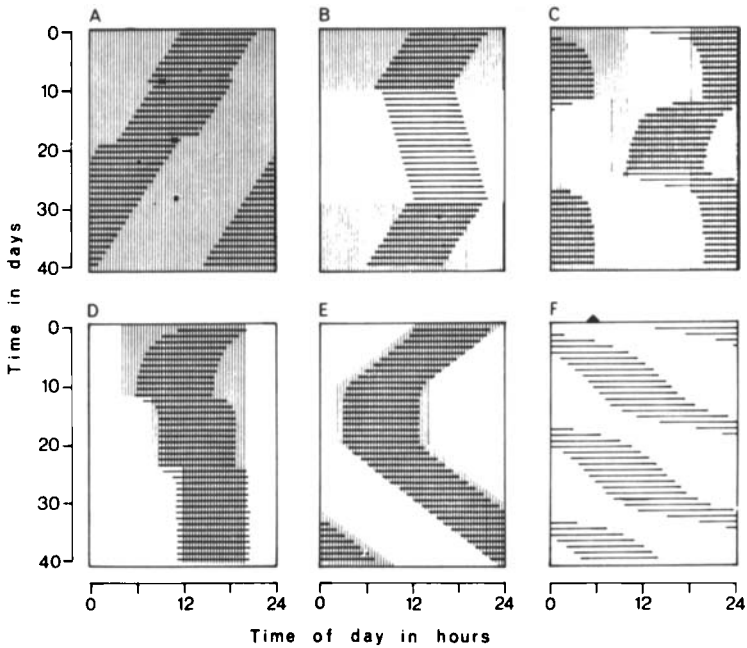2. DAAN, S. & C. S. PITTENDRIGH. 1976. J. Comp. Physiol. **106**: 267–290.

FIGURE 4. A sample of computer simulations of light effects on the circadian rhythm of activity in the golden hamster, using the empirically measured phase response curve (FIGURE 1) and the model of FIGURE 3. Parameters used: $\tau = 23.5$ hrs $X = 0.13$ in L; $X = 0.00$ in D. $X = 0.50$ at each transition from L→D or D→L. Horizontal lines indicate the times during which the oscillation passes through phases 180° to 330° (i.e., the active phase or subjective night). Vertical shading indicates times of "darkness."

A. Constant darkness (DD) with three 15′ light pulses (indicated by dots) falling (1) just after the onset of activity and producing a delay phase shift, (2) close to activity end, resulting in an advance phase shift, and (3) in the rest phase ("subjective day"), producing no effect.

B. A step from DD to LL causes an increase in τ. Upon "switching off the light," i.e., resetting X from 0.13 to 0.00, the rhythm resumes its original τ of 23.5 hours.

C. By a daily period of 8 hours of light the rhythm is entrained with activity occurring during darkness. A 10 hours forward phase shift of the light cycle results in a gradual approach to reentrainment. The same holds for a backward phase shift of the light cycle.

D. Entrainment by zeitgebers with different photoperiods (LD 8:16; LD 12:12; LD 16:8). Activity occurs relatively earlier in long nights than in short nights.

E. Entrainment to different zeitgeber periods (T = 23, 24 and 25 hours), each involving 12 hours of darkness. In T = 23, activity occurs at the end of the "night," in T 24 in the middle of the night, in T 25 at the beginning of the night.

F. Relative coordination in a sine wave light cycle (X varies as a sine wave with period 24 hours between 0.14 and 0.18.) Time of the maximum of the sine wave is indicated by the solid triangle.

All these simulations have their experimental counterpart. See References 1, 2, 12, 26, 27.

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DISCUSSION

DR. EDWARD TAUB (*Institute for Behavioral Research, Silver Spring, Md.*): I think this paper points up very clearly the ambiguities inherent in the term tonic influences on the central nervous system. It's a very interesting borderline case of something that could, if you look at it in one way, be characterized as a tonic influence or, if you look at it in another way, as a phasic influence. I found Dr. Bullock's talk this morning particularly illuminating in specifying some of these ambiguities, and I think the way out is a nonphenomenological approach looking at what is happening within specific centers within the central nervous system.

For example, here we have a 15-minute pulse of light that is producing entrainment of a long-acting or long-continuing locomotor episode. Is it therefore a phasic influence? Or can one conceive of this 15-minute episode of light producing a tonic or activating influence on a particular CNS center, which then releases the locomotor influence?

DR. DAAN: Your question suggests that the resetting of a circadian rhythm may be a slow (tonic) process, since the effect is observed many hours, even days, after application of the resetting stimulus. However, it is quite conceivable that the pacemaker controlling the behavioral rhythm observed itself

is instantaneously reset by the stimulus. If this is true, also its "phase response curve" should be instantaneously reset. This is experimentally measurable. Dr. Pittendrigh at Stanford University has done the experiment, using the *Drosophila* pupal eclosion rhythm, by probing the system with a second light pulse after it had been reset by a first pulse. The outcome is that the reset is virtually instantaneous, even though it may take the system a couple of days to regain steady-state motion. This experiment, if done properly, requires a large investment of time and energy, and remains to be done in rodents. The *Drosophila* experiment indicates that it is incorrect to view any long-term behavioral effect as the result of a tonic sensory process. Purely phasic information may suddenly alter the time course of a series of events, and thus remain tractable long after the response of the afferent pathway has subsided.

DR. T. H. BULLOCK: This stimulates me to suggest a very parallel case that isn't quite circadian, but where we have an interaction between pacemaker and environmental modulation of it. And that's the case of electric fish of the species that has a very regular discharge. Think of a fish that has a pacemaker in its head that's controlling the discharge of its organ about a thousand times per second, or another species about 300 times per second with extreme regularity. The coefficient of variation in these cases is on the order of 0.01%. Now this is a true pacemaker, but we know that it's under modulation of two kinds— what might be called tonic and phasic, if one chooses to face that dilemma of terminology. In both cases the input that comes to the pacemaker is in the form of discrete pulsatile events, nerve impulses, that are arriving. But in the one case, which you call parametric, the modulation is smoothed out over many cycles so that we would think of it as equivalent to a DC input (that is, a very steady influence that can accelerate or decelerate gradually over many cycles.) This is important for the animal because this modulation must occur without adding any jitter, without changing the regularity. Therefore, the modulation must be either this kind, which is very smooth (i.e., essentially DC, and I'm talking about actual neurophysiological mechanisms that we think we know), or of the other kind, which would be also carefully jitter free (i.e., where the pulsatile input comes precisely at a certain phase of the cycle every time, but by shifting that phase smoothly can accelerate or decelerate, thereby again, avoiding the introduction of jitter but accomplishing a very carefully regulated advance or retard in the period). So this would be, I think, a nice example of what you called both the parametric and nonparametric simultaneously occurring, at least in our model of how it's happening in the fish brain. This is prescribed from the types of neurons that we know exist but still have to confirm by looking inside the pacemaker to see that it's really happening that way. We have only so far been able to look inside the pacemaker and see signs of the nonparametric or cycle-by-cycle, carefully phase-locked type of input, which turns out to be a postsynaptic potential of microbial size since the modulations are fractions of a percent.

DR. DAAN: Can you also say what if there is a large difference in the amount of shifting the two processes do? Or are you able to do that in your system?

DR. BULLOCK: Not for sure. We have the hunch that the slower, long-time constant DC kind of effect is more important. But that is not yet confirmed.