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REVIEW

Period Responses to Zeitgeber Signals Stabilize Circadian Clocks During Entrainment

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ABSTRACT

Circadian clocks with characteristic period (τ) can be entrained to light/dark (LD) cycles by means of (i) phase shifts which are due to D/L "dawn" and/or L/D "dusk" transitions, (ii) period changes associated with long-term light exposure, or (iii) by combinations of the above possibilities. Based on stability analysis of a model circadian clock it was predicted that nocturnal burrowing mammals would benefit less from period responses than their diurnal counterparts. The model further predicted that maximal stability of circadian clock is reached when the clock slightly changes both its phase and period in response to light stimuli. Analyses of empirical phase response curve (PRC) and period response curve (τRC) of some diurnal and nocturnal mammals revealed that PRCs of both diurnal and nocturnal mammals have similar waveform while τRCs of nocturnal mammals are of smaller amplitude than those of diurnal mammals. The shape of the τRC also changes with age and with increasing strength of light stimuli. During erratic fluctuations in light intensity under different weather conditions, the stability of phase of entrainment of circadian clocks appears to be achieved by an interplay between phase and period responses and the strength of light stimuli.

Key Words: PRC; τRC; Transient; Nonparametric; Parametric; Entrainment.

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INTRODUCTION

Almost all organisms living in periodic environments exhibit a 24h rhythmicity in behavioral and/or physiological processes. Under constant laboratory conditions, the period length of these rhythmicities are often deviant from exactly 24h, hence the name circadian, from 'circa'—about, and 'dies'—a day (Pittendrigh, 1960). Circadian rhythms are most often studied in constant darkness (DD), and sometimes in constant light (LL), where temperature and all other factors that could possibly impart information about the environmental 24h periodicity are kept constant. In constant conditions the circadian clocks free-run expressing their endogenous periodicity referred to as the "free-running period" (τ) (Pittendrigh, 1960). For the same group of individuals, the τ_s measured in DD (τ_{DD}) and in LL (τ_{LL}) typically differ (Aschoff, 1979). I will use the symbol τ to refer to τ_{DD} throughout the text unless explicitly stated otherwise. Organisms living in a 24h environment have not only evolved circadian clocks, they also have evolved a response to periodic environmental factors. The response to cyclic environmental factors (zeitgebers) entrains circadian clocks and thus times the expression of several biological processes, which help organisms to optimize their activities in a fluctuating environment (Sharma and Joshi, 2002). The response of circadian clocks to Zeitgebers, which can be assessed as phase response curves (PRCs) obtained by exposing organisms to brief stimuli of the periodic factor of the environment, is itself periodic, i.e., high at certain times of the day and low at others. The PRCs represent plots of phase shifts as a function of phases at which animals are exposed to brief perturbations (e.g., light pulses) (Johnson, 1995). Rigid adjustment of biological processes to day/night changes is believed to be achieved by circadian timing systems and is primarily due to rapid-phase shifting responses of circadian clocks to periodic light/dark (LD) cycles of the environment (Sharma et al., 2000). Although I shall restrict myself to LD cycles to exemplify entrainment of circadian clocks, one can use other cyclic abiotic and biotic factors as an alternative.

NONPARAMETRIC MODEL OF ENTRAINMENT (PRC MODEL)

According to the nonparametric model, entrainment of circadian timing systems (clocks) to LD cycles is believed to be the result of daily discrete phase shifts, equal to the difference between the periodicity of LD cycles and the τ of circadian clocks (Pittendrigh, 1981). This model of entrainment is based on two key properties of circadian clocks, the PRC and τ, and it further assumes that these two properties remain unchanged during the entrainment process. The stability of phase of entrainment of circadian clocks according to this model depends on τ and the shape of PRC (Beersma et al., 1999; Pittendrigh and Daan, 1976). This model explained several aspects of entrainment to brief light stimuli in *Drosophila* (Pittendrigh, 1981) and in mice (Beersma et al., 1999; Pittendrigh and Daan, 1976), and successfully predicted the maximal and minimal period lengths of LD cycles to which circadian clocks can entrain (limits of entrainment), and the phase relationships between circadian rhythms and LD cycles (Daan, 2000; Sharma and Chidambaram, 2002). The model could even predict precisely regions of bistability when skeleton photoperiods (two brief light pulses per cycle to mimic "dawn" and "dusk") were used for entrainment (Pittendrigh, 1981). The nonparametric model could also explain the functional

relationship between phase relationship (ψ) and τ, the occasional lack of entrainment, and the dependence of "minimum tolerable night" on τ in the nocturnal field mouse *Mus booduga* (Sharma and Chidambaram, 2002; Sharma et al., 1997; 1998a,b,c). The nonparametric model, however, fails to predict the complete compression of activity in LD cycles with longer photoperiods, perhaps caused due to "masking", i.e., direct activating or inhibiting effects of light (Elliott, 1981). In the nocturnal field mouse *M. booduga*, ψ and τ of the locomotor activity rhythm shows a sigmoidal relationship (Sharma and Chidambaram, 2002), similar to the one predicted by the nonparametric model of entrainment for brief light pulses (Pittendrigh and Daan, 1976). However, when complete LD cycles were used for entrainment instead of brief light pulses, the relationship between ψ and τ becomes linear and did not match the predicted relationship (Sharma and Chidambaram, 2002; Sharma et al., 1998b). Furthermore, the nonparametric model requires that animals be exposed to at least one of the LD transitions during twilights (i.e., during "dawn" or "dusk"). This appears improbable since results of a recent experiment suggest that organisms that do not experience any twilight can still entrain to LD cycles (Hut et al., 1999). Therefore, the entrainment mechanisms of circadian clocks appear to be more complex than the tenets implicit in the nonparametric model.

τ of circadian clocks is often regarded as a rigid feature of a species, with the τ of individual animals being approximately normally distributed around the species mean, usually with a fairly small variance (Daan and Beersma, 2002). However, there is evidence that τ of circadian clocks vary in response to different environmental conditions, often reflecting residual effects of prior environmental conditions, typically referred to as "after effects" (Christensen, 1978; Page and Block, 1980; Pittendrigh, 1960; Sheeba et al., 2002; Sokolove, 1975). Mice assayed in DD after being exposed to LD cycles of either 20h or a 28h period length continue to exhibit rhythmic locomotor activity with τ close to the periodicity of the LD cycles for about 100 days (Pittendrigh and Daan, 1976). The after effects of LD cycles may have some functional significance as they might help organisms perform various behavioral and physiological functions at appropriate time of the day even when the environmental LD cycle is perturbed, for example due to clouds, and thus contribute toward the stabilization of the phase relationship between environmental cycles and circadian clocks (Beersma et al., 1999; Enright, 1980; Pittendrigh and Daan, 1976). After effects have also been observed in DD, in animals previously exposed to LL or to LD cycles of varying photoperiods, but the results were equivocal in experiments with different rodent species (Pittendrigh, 1960) and insects (Christensen, 1978; Sheeba et al., 2002; Sokolove, 1975). The entrainment to LD cycles, therefore, cannot be explained by the nonparametric model alone, because τ of circadian clocks can change during entrainment, residual effects of which can be measured subsequently in constant conditions.

PARAMETRIC MODEL OF ENTRAINMENT (τRC MODEL)

Aschoff (1963) suggested that circadian clocks entrain to periodic LD cycles by changing τ, light lengthens or shortens τ, while it simultaneously changes the average level around which circadian timing systems oscillate. According to this model of entrainment (parametric), circadian clocks with short τ should show a more precise onset of activity,

and clocks with long τ should show a more precise offset of circadian activity. Maximal precision for both onset and offset of activity was predicted at intermediate τ values (Aschoff et al., 1971; Sharma and Chandrashekaran, 1999). According to the parametric model, entrainment of circadian clocks to LD cycles occurs due to phase-dependent changes in τ (Aschoff, 1963). Therefore, just as the phase response is a prerequisite for entrainment in the nonparametric model, period response, i.e., changes in period as a function of phase at which perturbations are presented, is essential for entrainment in the parametric model.

PERIOD RESPONSE CURVES (τRCs)

Phase shifts evoked by brief light stimuli, used to construct PRCs, are often associated with long-lasting changes in τ (Daan and Pittendrigh, 1976; Pittendrigh and Daan, 1976; Sharma and Daan, 2002). Light stimuli appear to slow down circadian clocks when causing phase delays and accelerate clocks while advancing their phase. Such changes in τ of circadian clocks influence the time course and form of PRCs (Sharma and Daan, 2002), which is also known to change as a function of τ (Elliott, 1981; Pittendrigh and Daan, 1976; Sharma, 1996). In summary, all basic properties of circadian clocks undergo changes during phase shifting and entrainment, which suggests that entrainment of circadian clocks to LD cycles occurs by combined changes of phase and period (Aschoff, 1963; Beersma et al., 1999; Daan, 2000; Sharma and Daan, 2002).

The changes in τ of circadian clocks associated with phase shifts due to light stimuli administered in constant darkness pose a methodological problem in the estimation of phase shifts. In the classical paradigm phase shifts evoked in circadian rhythms due to perturbations—such as exposure to a brief light pulse—are measured by comparing two regressions (or eye-fitted lines), drawn through two series of phase reference points, one preceding and the other following perturbations. The phase shifts are then estimated as the displacement of these lines relative to each other. As long as τ remains constant, the two regression lines run parallel to each other, and the phase shift measured on any day remains the same. If τ changes due to the perturbation, the two regression lines diverge, and it becomes important therefore to precisely define the day on which the phase shifts were estimated. It has been a common practice among rhythm researchers to take the time of the first expected phase reference point after the perturbation to calculate phase shifts. However, it is well known that τ rarely stabilizes at a new value immediately after perturbation, as most often transient cycles follow, which typically appears as cycles with gradually changing period and which do not mimic the state of circadian clocks (Sharma et al., 2000). What should then be the appropriate measurement of phase shift in this situation? This issue has been discussed at great length elsewhere (Sharma and Daan, 2002), and therefore I will only discuss it briefly here.

The number of transient cycles and extent of phase and period changes can be determined by performing multiple regression analyses on the postpulse events. Evidence of significant contributions from quadratic components would suggest presence of transient cycles. Transient cycles can be avoided by omitting the first few cycles from the analysis, and by establishing that contributions from quadratic components become insignificant. The intercept and slope of the regression lines would then provide an

estimate of phase shifts and period changes evoked by the perturbations (Sharma and Daan, 2002).

Pittendrigh and Daan (1976) reported in four species of nocturnal mammals small effects of brief light stimuli on τ after perturbation. In the Syrian hamster, τ following advancing light pulses was shorter than the pre-pulse τ (Elliott, 1981). Moreover, complete τRC have so far been published for only five diurnal mammals (Beersma et al., 1999; Hut, 2001; Kramm and Kramm, 1980; Pohl, 1982; Fig. 1), three nocturnal animals (Sharma and Daan, 2002; Weinert and Kompauerova, 1998; Fig. 2) and for the common vole *Microtus arvalis* (Gerkema et al., 1993), a species that is neither nocturnal nor diurnal but is characterized by ultradian feeding activity throughout 24 hours. Light pulse τRC of most animals had similar time course and waveform as the light pulse PRC (Table 1; Figs. 1 and 2). The τRCs of diurnal animals show a symmetric waveform with maximal lengthening and shortening of similar magnitude occurring during the early and the late subjective nights respectively. Although, most evidence in support of the nonparametric model of entrainment have come from studies in *Drosophila* or in nocturnal rodents, complete τRCs have been reported only in three nocturnal rodents, of which two were wild caught animals (*Mus booduga* and *Mus platythrix*) and one laboratory outbred mice (*Mus musculus*) (Sharma and Daan, 2002; Weinert and Kompauerova, 1998). The light pulse PRCs and τRCs of these three nocturnal rodents had similar time course and waveform (Table 1; Fig. 2). The phase shifts and period changes in the two species of nocturnal field mice *M. booduga* and *M. platythrix* showed significant positive correlation (Sharma and Daan, 2002). Similar correlation between phase and period responses has also been reported for the laboratory mouse *M. musculus* (Weinert and Kompauerova, 1998). The correlations between phase and period responses

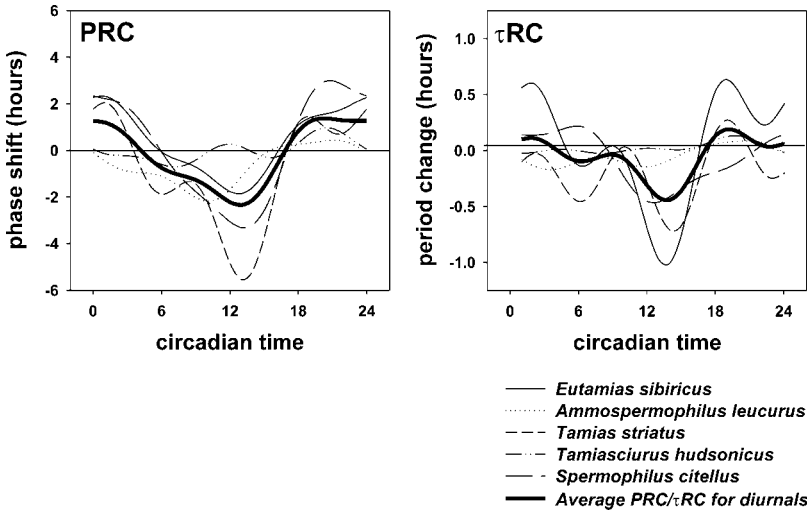

Figure 1. Phase response curves (PRC) and period response curves (τRC) of five species of diurnal mammals. The average PRC and τRC are shown as thick curves. The curves were obtained by fitting raw data to curves represented as sine waves with two subharmonics.

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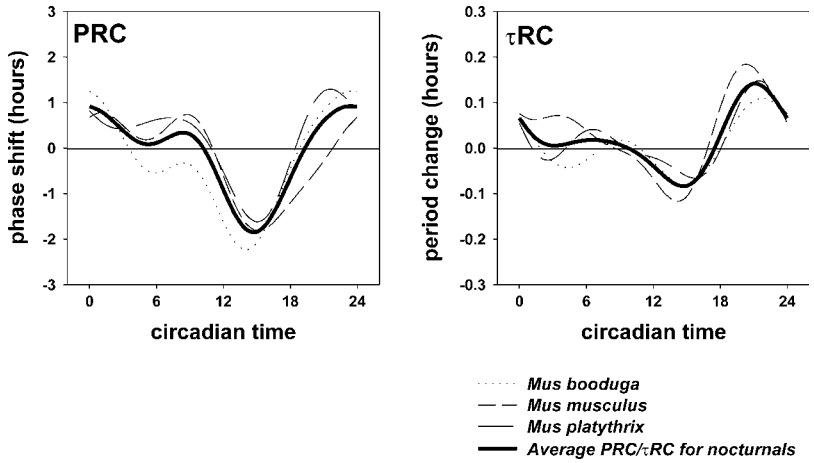

Figure 2. Phase response curves (PRC) and period response curves (τRC) of three species of nocturnal mammals. The average PRC and τRC are shown as thick curves. The curves were obtained by fitting raw data to sine waves with two subharmonics.

are often weak but nevertheless nontrivial, as we are dealing with very small changes in τ, which can accumulate over several days causing large phase shifts (Sharma and Daan, 2002). Furthermore, the changes in τ described here are distinct from transient cycles, because we have demonstrated in a previous study that the changes in τ following a single light stimulus are stable and are maintained for more than 15 days (Sharma and Daan, 2002). The presence of τRC in a number of nocturnal mammals confirms earlier suggestions (Pittendrigh and Daan, 1976) that the circadian clocks of nocturnal mammals may also be characterized by period responses. In few other studies, the period changes due to light pulses were small, irregular, and did not depend upon the circadian phase (Rappold and Erkert, 1994; Wechselberger and Erkert, 1994).

A number of features of circadian clocks undergo changes during ontogenetic development and aging (Aschoff, 1994; Brock, 1991; Sharma, 2001; Weinert, 2000). Age-dependent changes in τ of circadian rhythms have been reported in a number of studies (Aschoff, 1994; Brock, 1991; Sharma, 2001; Sharma and Chandrashekaran, 1998; Weinert, 2000). Some studies report shortening of τ with age, some report lengthening and in some cases no significant difference was recorded with advancing age. A more or less general trend that emerges from all these studies is that the changes in τ are significant between juvenile and adult animals, that τ is stable during adult stage, while the rhythms become unstable during old age (Sharma and Chandrashekaran, 1998). Besides τ, the phase relationship between LD cycles and circadian rhythms changes considerably due to aging (Sharma, 2001; Weinert, 2000). With increasing age the circadian activity rhythm phase advanced, i.e., the activity onset occurred significantly earlier in older animals compared to younger ones. The light pulse PRCs in older animals show decreased phase advances and delays compared to younger animals (Aschoff, 1994; Provencio et al., 1994; Weinert and Kompauerova, 1998). On the other hand, in some studies, either no change in the shape of the PRC was observed with age (Pohl, 1984) or in older animals phase

Table 1. Details of phase response curves (PRCs) and period response curves (τRC) for eleven PRCs and τRCs from four diurnal and three nocturnal mammals. The table provides data on maximal phase delay (max-d), maximal phase advance (max-a), phases of maximal phase delay, maximal phase advances, and phases of upward and downward crossing of the PRCs. It also provides information about τRCs such as maximal period lengthening (max-l), maximal period shortening (max-s), phases of maximal period lengthening and shortening and phases of upward and downward crossing. The ratio of maximal phase delay of the PRCs to maximal period lengthening in the τRCs (K_d) and the ratio of maximal phase advance in the PRCs to maximal period shortening in the τRCs (K_a) are also provided.

	Phase response curve (PRC)				Period response curve (τRC)						
	Maximum phase		Phases of		Maximum period		Phases of		Beersma et al.'s K values		
Species	delay max-d	advance max-a	max-d/ max-a	up/down crossing	lenth. max-l	shor. max-s	max-l/ max-s	up/down crossing	K_d	K_a	Data source
E. sibiricus	−1.85	+2.32	ct13/c11	ct16/ct6	−1.01	+1.64	ct14/ct19	ct17/ct5	1.4	1.8	Beersma et al. (1999)
A. leucurus	−2.18	+0.44	ct10/ct21	ct16/ct24	−0.18	+0.08	ct4/ct21	ct6/ct0	5.5	12.1	Pohl (1982)
T. striatus	−5.55	+2.05	ct13/ct1	ct17/ct4	−0.18	+0.27	ct14/ct19	ct17/ct21	7.6	7.8	Kramm and Kramm (1980)
T. hudsonicus	−0.70	+0.95	ct7/ct20	ct17/ct5	−0.03	+0.13	ct8/ct20	ct10/ct23	7.3	23.3	Kramm and Kramm (1980)
S. citellus	−3.32	+2.98	ct13/ct21	ct17/ct6	−0.46	+0.14	ct13/ct24	ct8/ct21	7.2	22.6	Hut (2001)
M. platythrix 1000 lx IL	−1.62	+1.25	ct15/ct22	ct19/ct11	−0.07	+0.14	ct16/ct22	ct18/ct10	23.1	8.9	Sharma and Daan (2002)

(*continued*)

Table 1. Continued.

Species	Phase response curve (PRC)				Period response curve (τRC)				Beersma et al.'s K values		Data source
	Maximum phase		Phases of		Maximum period		Phases of				
	delay max-d	advance max-a	max-d/ max-a	up/down crossing	lenth. max-l	shor. max-s	max-l/ max-s	up/down crossing	K_d	K_a	
M. booduga 1000 lx DL	−2.25	+1.26	ct14/ct23	ct19/ct4	−0.08	+0.11	ct15/ct20	ct18/ct11	28	11.6	Sharma and Daan (2002)
M. booduga 1000 lx FL	−2.05	+0.82	ct15/ct22	ct18/ct4	−0.16	+0.17	ct14/ct22	ct18/ct10	13	4.8	Sharma (2003)
M. booduga 1000 lx FL	−1.46	+0.62	ct15/ct21	ct18/ct10	−0.06	+0.07	ct14/ct21	ct17/ct2	24.3	8.9	Sharma (2003)
M. musculus juvenile	−2.33	+1.08	ct16/ct23	ct20/ct3	−0.16	+0.29	ct10/ct22	ct16/ct2	14.56	3.72	Weinert and Kompauerova (1998)
M. musculus adult	−1.81	+0.68	ct15/ct24	ct22/ct11	−0.12	+0.18	ct14/ct20	ct17/ct9	15.08	3.78	Weinert and Kompauerova (1998)
M. musculus presenile	−2.25	+0.15	ct16/ct22	ct21/ct10	−0.05	+0.18	ct15/ct20	ct17/ct12	42.8	1.17	(Weinert and Kompauerova (1998)

advances of larger magnitude compared to those of younger animals were observed (Rosenberg et al., 1991). In the laboratory mouse *M. musculus*, light pulse PRC and τRC were estimated in juvenile, adult, and presenile animals (Weinert and Kompauerova, 1998). Although the changes in both PRC and τRC were small, light stimuli evoked mostly period shortening with increasing age, and the τRC became increasingly flat in presenile animals (Table 1; Fig. 3a,b). The delay portion of the PRC remained unchanged while the advance portion decreased in animals of increasing ages.

Intensity of light stimuli also influences the shape of the τRC in the nocturnal field mouse *M. booduga* (Sharma, 2003). Light stimuli of higher intensity (1000 lux) evoked greater period lengthening at CT14 and greater period shortening during the late subjective night (CT20) compared to lower light intensity (100 lux) (Table 1; Fig. 3c–d). Furthermore, the phase shifts obtained with light stimuli of 1000 lux were positively correlated with the changes in period, but with lower light stimuli (100 lux), phase and period responses were not correlated. The lack of correlation for 100 lux light stimuli suggests that parametric effects might be characteristic of stronger zeitgebers.

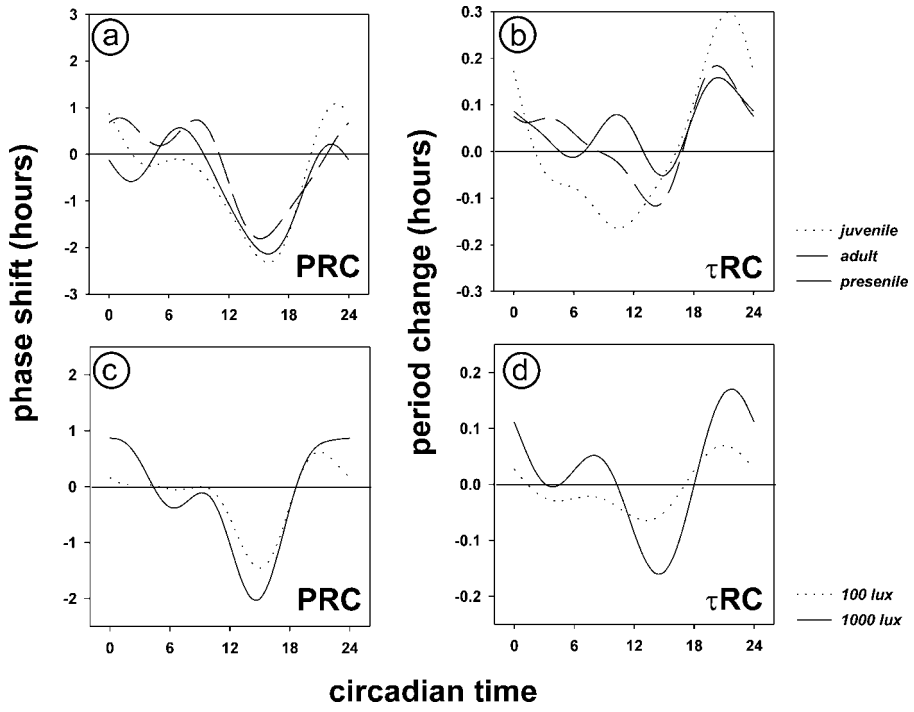

Figure 3. (a) Phase response curves (PRC) and (b) period response curves (τRC) of the laboratory outbred mouse *M. musculus*. The PRC and τRC were constructed using animals of three different age groups (juvenile, adults, and presenile). (c) PRC and (d) τRC of the nocturnal field mouse *M. booduga*, constructed using fluorescent light stimuli of 100 lux and 1000 lux intensity for 15 min duration. The curves were obtained by fitting raw data to sine waves with two subharmonics.

A COMBINED PRC AND τRC MODEL

In a recent paper Beersma et al. (1999) have discussed the stability of circadian clocks in situations where contributions of both phase and period responses occur with stochastic noise. They used a model circadian clock characterized by its instantaneous phase and instantaneous velocity which was assumed to respond to light stimuli with both phase shifts and period changes. The performance of the model was evaluated using their stability in entrained conditions and defined as the ratio of the amplitudes of the PRC and τRC.

In situations where the phase shifts are assumed to occur without changes in period, the stability of the model circadian clock increased with increasing amplitude of the PRC (Beersma et al., 1999). On the other hand, based on the assumption that entrainment occurs due to changes in period without change in phase, the stability of the clock increased with increase in amplitude of the τRC (Beersma et al., 1999). However, the stability was maximal when the amplitude of the PRC was about six times higher than the amplitude of τRC, which closely corresponds to the empirically observed ratios in diurnal mammals (Table 1). The results of simulations demonstrated that stability of the clock is high only when phase advances occur with shortening of τ and phase delays with lengthening of τ (Beersma et al., 1999; Daan, 2000). Furthermore, the stability was maximal in the absence of random fluctuations in the phase and period of the oscillator and when the oscillator responded to light stimuli with both phase and period changes of small magnitude. In the presence of random fluctuations in phase and period of the oscillator, the stability of the oscillator under entrained condition decreased drastically. Another very interesting finding of the simulations is the fact that the stability of the model oscillator was affected more strongly when stochastic noise was introduced in the period of the oscillator rather than in the phase.

Based on the results of simulations it was predicted that nocturnal burrowing animals would benefit less from period responses than their diurnal counterpart (Beersma et al., 1999). Comparison of the stability of circadian clocks in diurnal burrowers, nonburrowers, and nocturnal burrowers, revealed that the stability was maximal in diurnal burrowers at relatively high values of amplitude of the PRC as well as the τRC (Daan, 2000). Optimal stability for the nocturnal burrowers was obtained at much smaller amplitude of τRC. It appears that circadian clocks enhance their stability in ever fluctuating environments by a number of mechanisms, which include phase and period responses to light stimuli (Daan, 2000).

The empirically observed ratio ("K"; Beersma et al., 1999) of maximal phase advance in the PRC and the maximal shortening of τ in the τRC (henceforth will be referred as K_a), which was taken as a measure of stability of circadian oscillators, ranged between 1.8 and 23.3 with an average of 11.25 for diurnal animals (Table 1). The K values for the maximal phase delay in the PRC to maximal lengthening of τ in the τRC (henceforth will be referred as K_d) ranged between 1.4 and 7.6, respectively, with an average of 5.45 in diurnal animals (Table 1). In the nocturnal animals, the K_a values ranged between 1 and 11.6 with a mean of 4.6, and the K_d values had an average of 24.12 and ranged between 13 and 45. The K_d values of nocturnal mammals were significantly higher compared to diurnal mammals. As the PRCs of diurnal and nocturnal animals did not differ much in amplitude the differences in K_d values seems to be mostly due to the differences in the amplitude of

the τRCs (Beersma et al., 1999). Therefore, it appears that nocturnal mammals depend less on period changes compared to phase shifts for entrainment than the diurnal mammals, which supports the predictions made by Beersma et al. (1999) (Figs. 1 and 2).

The stability coefficient *K* depends upon the strength of the light stimuli (Sharma, 2003). In *M. booduga*, the K_d values with 100 lux and 1000 lux light stimuli were 24.3 and 13, whereas the K_a values were 8.9 and 4.8, respectively (Table 1; Figs. 1 and 2). The *K* value also depends upon the age of the animals (Weinert and Kompauerova, 1998). The K_d values were highest in the presenile animals (42.8), followed by adult and juvenile animals (15.08 and 14.56 respectively). The K_a values, however, did not differ between the animals of different age groups (1 to 4.1) (Table 1). A careful analysis of the PRC and τRC of the animals of different age groups suggests that the changes in the K_d values were mostly due to the changes in the amplitude of the τRC and not that of the PRC (Table 1; Figs. 1 and 2).

POSSIBLE MOLECULAR MECHANISMS BEHIND τRCs

Although several genes in the molecular mechanism of the mammalian circadian clock are homologous to those of the *Drosophila* circadian clock, there are several differences in the structure and function of the genes and proteins involved [for review see Cermakian and Sassone-Corsi (2000), Harmer et al. (2001), Rensing and Ruoff (2002), and Reppert and Weaver (2002)]. The mammalian clock genes include three *period* homologues (*mPer* 1, 2, 3) a *clock* homologue (*mClk*) and a *cycle* homologue (*Bmal1* or *Mop3*) of *Drosophila*. The *mPer* mRNA and protein oscillate, with high levels of protein during the end of the subjective day and beginning of subjective night (~CT10–13). In rodents, the *Bmal1* mRNA and protein levels oscillate with high levels close to subjective night (CT18), while *mClk* mRNA is known not to oscillate.

The mPER proteins inhibit mCLK/BMAL1-mediated gene activation to some extent. It is however believed that the two mammalian cryptochromes, mCRY1 and mCRY2, inhibit mCLK/BMAL1 severely, probably in a complex with mPER. *Bmal1* mRNA cycles in antiphase to the mRNA of m*Per* genes. It appears that a positive feedback also is operational between mPER2 (along with CRY1 and CRY2) and the activation of *Bmal1*, while BMAL1/CLOCK represses *Bmal1* through the protein Rev-Erbα [for review see Reppert and Weaver (2002)].

In mammals, light stimuli from the NMDA-receptors reach the clock mechanism via a number of intracellular signaling pathways [for review see Rensing and Ruoff (2002)] and eventually activate genes such as those for the transcription factor AP-1 (*c-fos*, *c-jun*, and *jun-B*) and the genes *mPer1* and *mPer2*. In rats, in addition to the induction of *mPer1* transcription, light also triggers the degradation of the protein BMAL1 (Tamaru et al., 2000). Light signals are transmitted through the influx of calcium, which in turn activates nitric oxide synthase (Ding et al., 1994). This is followed by phosphorylation of cAMP-response element binding protein (CREB) by protein kinase A, and the activation of Ca^{2+}–calmodulin-dependent kinase, and MAP kinase. It is believed that phospho CREB directly induces transcription of the *mPer1* promoter, which contains multiple cAMP-response-elements (CREs). Slow induction of *mPer1* can be facilitated by several factors

like protein kinase C, glucocorticoid hormones, and Ca^{2+}. Phosphorylation of mPER1 by a casein kinase (CKIε; Eide and Virshup, 2001) is involved in the nuclear transport and its stability. The proteins mPER1 and mPER2, CLOCK and BMAL1 exhibit robust circadian variation in phosphorylation (Lee et al., 2001). Overexpression of CKIε leads to decreased mPER1 protein half-life and alters the nucleocytoplasmic localization of proteins. CKIε binds to PER proteins and possibly also to higher-order complexes with CRY1, CRY2, and BMAL1, which are also phosphorylated by this enzyme (Eide et al., 2002).

Light exposure during the early subjective night causes phase delay via glutamate acting at *N*-methyl-D-aspartate receptors (Colwell and Menaker, 1992), which in turn releases intracellular calcium via ryanodine receptors (Ding et al., 1998). The calcium is believed to activate calcium/calmodulin-dependent kinases, MAP kinase, and other kinases phosphorylate CREB and finally bring about induction of gene expression via calcium/cAMP response elements (Ca/CREs) (Gillette and Tischkau, 1999; Obrientan et al., 1998). Light exposure during the late subjective night on the other hand causes phase advances in vivo (or glutamate activation in vitro). Glutamate appears to activate nitric oxide synthesis, activate soluble guanylyl cyclase, increase cGMP, activate cGMP-dependent protein kinase, and phosphorylate CREB (Ding et al., 1997; 1998). The nocturnal response of the circadian clocks of mammals to light may be explained partly by the fact that CREB phosphorylation is a circadian clock–controlled phenomenon and is gated only during night, and hence the ability of light to induce CREB-P is also limited to the subjective night (Ding et al., 1997; Ginty et al., 1993). Among the three proteins (mPER1, mTIM, mCRY) studied for their responses to light exposure during the subjective night, only the levels of mPER1 were found to be increased slightly due to light (Field et al., 2000).

Most of the data on the molecular mechanisms suggest that stabilization of mCRY1 by mPER1, which perhaps extends the duration of the negative feedback loop of the circadian clock, causes phase delays in circadian rhythms (Field et al., 2000). Exposure to light during the late subjective night yields a small increase in the mPER1 levels, which in turn results in small phase advance in the circadian rhythms. The small increase in the mPER1 levels, might explain the occurrence of type 1 PRCs in mammals.

Period lengthening of circadian clocks might occur due to residual changes in the nature of the feedback loops. Long-lasting effects of light stimuli on either the mPER/CRY level or on kinase activities may lead to changes in the period of circadian clocks. Light may also alter the rate of the PER/CRY nuclear entry by a long-term change of kinase activity. Alternatively different directions of phase and period changes during early and late subjective night may result from differing affinities of these proteins for other clock components and/or different levels, rates of protein expression and/or induction that alters the overall kinetics of the feedback loops. The transients on the other hand could be a result of instantaneous phase resetting of the light sensitive oscillators of the suprachiasmatic nuclei (SCN) (Best et al., 1999) and a subsequent gradual shift of the light insensitive oscillators in the SCN.

The effect of light on phase shifts and period changes should also be investigated in the light of phosphorylation of mPER1 and mPER2, CLOCK and BMAL1 due to the action of CKIε and/or other kinases. Further, studies aimed to assess the levels of the clock mRNA and proteins in organisms with large amplitude of PRCs and τRCs might provide some insight into the molecular basis of phase resetting and period changes of the circadian

clocks. Given the importance of mPER1 induction in the phase resetting mechanism, one should also investigate phase and period resetting in *mPer1* mutant animals.

It appears that circadian clocks of both diurnal and nocturnal animals entrain to LD cycles using both phase and period responses, phase responses correct maladjustments in phase, and period responses correct maladjustments in period of circadian clocks. However, for a functional explanation of τRC, one may need to compare the τRC between diurnal and nocturnal animals. Unfortunately, such a comparison is limited by the small amount of data on τRC. It is surprising that PRCs have been published in a number of organisms across all taxa while data on τRC are almost nonexistent. I hope this article will stimulate studies on τRCs in more organisms. Unless τRC data are compiled in a manner similar to the PRCs, and experiments to study the effects of age, strength of light stimuli, and τ on τRCs are conducted, our understanding of entrainment of circadian clocks by LD cycles will remain incomplete.

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REFERENCES

Aschoff, J. (1963). Comparative physiology: diurnal rhythms. *Annu. Rev. Physiol.* 25:581–600.

Aschoff, J. (1979). Circadian rhythms: influences of internal and external factors on the period measurement in constant conditions. *Z. Tierpsychol.* 49:225–249.

Aschoff, J. (1994). On the aging of circadian systems. In: Hiroshige, T., Honma, K. I., eds. *Evolution of Circadian Clock*. Sapporo: Hokkaido University Press, pp. 23–44.

Aschoff, J., Gerecke, E., Kureck, A., Pohl, H., Rieger, P., Von Saint Paul, U., Wever, R. (1971). Interdependent parameters of circadian activity rhythm in birds and man. In: Menaker, M., ed. *Biochronometry*. Washington, DC: Natl. Acad. Sci., pp. 3–29.

Beersma, D. G. M., Daan, S., Hut, R. A. (1999). Accuracy of natural entrainment under fluctuating light conditions: contributions of phase and period responses. *J. Biol. Rhythms* 14:320–329.

Best, J. D., Maywood, E. S., Smith, K. L., Hastings, M. H. (1999). Rapid resetting of the mammalian circadian clock. *J. Neurosci.* 19:828–835.

Brock, M. A. (1991). Chronobiology and aging. *J. Am. Geriatr. Soc.* 39:74–91.

Cermakian, N., Sassone-Corsi, P. (2000). Multilevel regulation of the circadian clock. *Mol. Cell Biol.* 1:59–67.

Christensen, N. D. (1978). The Circadian Clock of *Hemediena thoracica* as a System of Coupled Oscillators. M.Sc. Thesis, University of Auckland, New Zealand, 239.

Colwell, C. S., Menaker, M. (1992). NMDA as well as non-NMDA receptor antagonists can prevent the phase-shifting effects of light on the circadian system of the golden hamster. *J. Biol. Rhythms* 7:125–136.

Daan, S. (2000). Colin Pittendrigh, Jürgen Aschoff, and the natural entrainment of circadian systems. *J. Biol. Rhythms* 15:195–207.

Daan, S., Beersma, D. G. M. (2002). Circadian frequency and its variability. In: Kumar, V., ed. *Biological Clocks*. New Delhi/Berlin: Narosa Publishers/Springer-Verlag, pp. 24–37.

Daan, S., Pittendrigh, C. S. (1976). A functional analysis of circadian pacemakers in nocturnal rodents III, heavy water and constant light: homeostasis of frequency? *J. Comp. Physiol.* 106:267–290.

Ding, J. M., Dong, C., Weber, E. T., Faiman, L. E., Rea, M. A., Gillette, M. U. (1994). Resetting the biological clock: mediation of nocturnal circadian shifts by glutamate and NO. *Science* 266:1713–1717.

Ding, J. M., Faiman, L. E., Hurst, W. J., Kuriaskina, L. R., Gillette, M. U. (1997). Resetting the biological clock: mediation of nocturnal CREB phosphorylation via light, glutamate, and nitric oxide. *J. Neurosci.* 17:667–675.

Ding, J. M., Buchanan, G. F., Tischkau, S. A., Chen, D., Kuriashkina, L., Faiman, L. E., Alster, J. M., McPherson, P. S., Campbell, K. P., Gillette, M. U. (1998). A neural ryanodine receptor mediates light-induced phase delays of the circadian clock. *Nature* 394:381–384.

Eide, E. J., Virshup, D. M. (2001). Casein kinase I: another Cog in the circadian clockworks. *Chronobiol. Int.* 18:389–398.

Eide, E. J., Vielhaber, E. L., Hinz, W. A., Virshup, D. M. (2002). The circadian regulatory proteins BMAL1 and cryptochromes are substrates of casein kinaseIε (CKIε). *J. Biol. Chem.* 277:17248–17254.

Elliott, J. (1981). Circadian rhythms, entrainment and photoperiodism in the Syrian hamster. In: Follett, B. K., Follett, D. E., eds. *Biological Clocks in Seasonal Reproductive Cycles*. Bristol: Wright, pp. 203–217.

Enright, J. T. (1980). *The Timing of Sleep and Wakefulness*. Berlin: Springer-Verlag.

Field, M. D., Maywood, E. S., O'Brien, J. A., Weaver, D. R., Reppert, S. M., Hasting, M. H. (2000). Analysis of clock proteins in mouse SCN demonstrates phylogenetic divergence of the circadian clockwork and resetting mechanisms. *Neuron* 25:437–447.

Gerkema, M., Daan, S., Wilbrink, M., Hop, M., Van der Leest, F. (1993). Phase control of ultradian and circadian rhythms in the common vole (*Microtus arvalis*): the roles of light and the circadian system. *J. Biol. Rhythms* 8:151–171.

Gillette, M. U., Tischkau, S. A. (1999). Suprachiasmatic nucleus: the brain's clock. *Recent Prog. Horm. Res.* 54:33–59.

Ginty, D. D., Kornhauser, J. M., Thompson, M. A., Bading, H., Mayo, K. E., Takahashi, J. S., Greenberg, M. E. (1993). Regulation of CREB phosphorylation in the suprachiasmatic nucleus by light and a circadian clock. *Science* 260:238–241.

Harmer, S. L., Panda, S., Kay, S. A. (2001). Molecular bases of circadian rhythms. *Annu. Rev. Cell Dev. Biol.* 17:215–253.

Hut, R. A. (2001). Natural Entrainment of Circadian Systems; a Study in the Diurnal Ground Squirrel *Spermophilus citellus*. Ph.D. thesis, University of Groningen, The Netherlands.

Hut, R. A., Van Oort, B. E. H., Daan, S. (1999). Natural entrainment without dawn and dusk: the case of the European ground squirrel. *J. Biol. Rhythms* 14:290–299.

Johnson, C. H. (1995). Phase response curves: what they can tell us about circadian clocks? In: Hiroshige, T., Honma, K., eds. *Fourth Sapporo Symposium on Biological Rhythms*. Sapporo: Hokkaido University Press, pp. 2–48.

Kramm, K. R., Kramm, D. A. (1980). Photoperiodic control of circadian activity rhythms in diurnal rodents. *Int. J. Biometeorol.* 24:65–76.

Lee, C., Etchegaray, J. P., Cagampang, F. R., Loudon, A. S. I., Reppert, S. M. (2001). Posttranslational mechanisms regulate the mammalian circadian clock. *Cell* 107:855–867.

Obrientan, K., Impey, S., Storm, D. R. (1998). Light and circadian rhythmicity regulate MAP kinase activation in the suprachiasmatic nuclei. *Nat. Neurosci.* 8:693–700.

Page, T. L., Block, G. D. (1980). Circadian rhythmicity in cockroaches: effects of early post-embryonic development and ageing. *Physiol. Entomol.* 5:271–281.

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

Pittendrigh, C. S. (1981). Circadian systems: general perspective. In: Aschoff, J., ed. *Handbook of Behavioural Neurobiology*. New York: Plenum Press, pp. 95–124.

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

Pohl, H. (1982). Characteristics and variability in entrainment of circadian rhythms to light in diurnal rodents. In: Aschoff, J., Daan, S., Groos, G., eds. *Vertebrate Circadian Systems*. Berlin-Heidelberg: Springer, pp. 239–246.

Pohl, H. (1984). Differences in response of the circadian system to light in the Syrian hamster. *Physiol. Zool.* 57:509–520.

Provencio, I., Wong, S. Y., Lederman, A. B., Argamaso, S. M. (1994). Visual and circadian responses to light in aged retinally degenerate mice. *Vision Res.* 34:1799–1806.

Rappold, I., Erkert, H. G. (1994). Re-entrainment, phase-response and range of entrainment of circadian rhythms in owl monkeys (*Aotus lemurinus g.*) of different age. *Biol. Rhythm Res.* 25:133–152.

Rensing, L., Ruoff, P. (2002). Temperature effects on entrainment, phase shifting and amplitude of circadian clocks and their molecular bases. *Chronobiol. Int.* 19:807–864.

Reppert, S. M., Weaver, D. R. (2002). Coordination of circadian timing in mammals. *Nature* 418:935–941.

Rosenberg, R. S., Zee, P. C., Turek, F. W. (1991). Phase response curves to light in young and old hamsters. *Am. J. Physiol.* 261:R491–R495.

Sharma, V. K. (1996). Light-induced phase response curves of the circadian activity rhythm in individual field mice *Mus booduga*. *Chronobiol. Int.* 13:401–409.

Sharma, V. K. (2001). Do biological clocks age like their owners? *Proc. Indian Natl Sci. Acad.* B67:373–388.

Sharma, V. K. (2003). Effect of light intensity on the phase and period responses in the nocturnal field mouse *Mus booduga*. *Chronobiol. Int.* 20:223–231.

Sharma, V. K., Chandrashekaran, M. K. (1998). Age-dependent modulation of circadian parameters in the field mouse *Mus booduga*. *J. Exp. Zool.* 280:321–326.

Sharma, V. K., Chandrashekaran, M. K. (1999). Precision of a mammalian circadian clock. *Naturwissenschaften* 86:333–335.

Sharma, V. K., Chidambaram, R. (2002). Intensity-dependent phase-adjustments in the locomotor activity rhythm of the nocturnal field mouse *Mus booduga*. *J. Exp. Zool.* 292:444–459.

Sharma, V. K., Daan, S. (2002). Circadian phase and period responses to light stimuli in two nocturnal rodents. *Chronobiol. Int.* 19:659–670.

Sharma, V. K., Joshi, A. (2002). Clocks, genes and evolution: the evolution of circadian organisation. In: Kumar, V., ed. *Biological Clocks*. New Delhi/Berlin: Narosa Publishers/Springer-Verlag, pp. 5–23.

Sharma, V. K., Singaravel, M., Chandrashekaran, M. K., Subbaraj, R. (1997). Relationship between free-running period and minimum tolerable light pulse interval in skeleton photoperiod in field mice *Mus booduga*. *Chronobiol. Int.* 14:237–245.

Sharma, V. K., Singaravel, M., Chandrashekaran, M. K. (1998a). Period-dependent oscillatory free-run in the locomotor activity rhythm of the field mouse *Mus booduga* under skeleton photoperiodic regimes. *Biol. Rhythm Res.* 29:197–205.

Sharma, V. K., Chandrashekaran, M. K., Singaravel, M. (1998b). Relationship between period and phase angle differences in the tropical field mouse *Mus booduga* under gradual and abrupt light–dark transitions. *Naturwissenschaften* 85:183–185.

Sharma, V. K., Geetha, L., Chandrashekaran, M. K. (1998c). Entrainment properties of the circadian locomotion activity rhythm of the field mouse *Mus booduga* under complete and skeleton photoperiodic regimes. *Biol. Rhythm Res.* 29:237–246.

Sharma, V. K., Chidambaram, R., Chandrashekaran, M. K. (2000). Probing the circadian pacemaker of a mouse using two light pulses. *J. Biol. Rhythms* 15:67–73.

Sheeba, V., Chandrashekaran, M. K., Joshi, A., Sharma, V. K. (2002). Developmental plasticity of the locomotor activity rhythm of *Drosophila melanogaster*. *J. Insect Physiol.* 48:25–32.

Sokolove, P. G. (1975). Locomotory and stridulatory circadian rhythms in the cricket *Teleogryllus commodus*. *J. Insect Physiol.* 21:537–558.

Tamaru, T., Isojima, Y., Yamada, T., Okada, M., Nagai, K., Takamatsu, K. (2000). Light and glutamate-induced degradation of the circadian oscillating protein BMAL1 during the mammalian clock resetting. *J. Neurosci.* 20:7525–7530.

Wechselberger, E., Erkert, H. G. (1994). Characteristics of the light-induced phase response curve of circadian activity rhythms in common marmosets, *Callithrix j. jacchus* (Primates-Cebidae). *Chronobiol. Int.* 11:275–284.

Weinert, D. (2000). Age-dependent changes of the circadian system. *Chronobiol. Int.* 17:261–283.

Weinert, D., Kompauerova, V. (1998). Light-induced phase and period responses of circadian activity rhythms in laboratory mice of different age. *Zoology* 101:45–52.

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