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PERIOD SHIFT INDUCTION BY INTERMITTENT STIMULATION IN A *DROSOPHILA* MODEL OF PER PROTEIN OSCILLATIONS

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ABSTRACT

PER protein circadian oscillations in *Drosophila* have been described by Goldbeter according to a five-dimensional model that includes the possibility of genetic mutation described by changing one parameter, the maximum degradation rate of the PER protein. Assuming that, in a mutant *Drosophila* this parameter is unreachable, we modify another parameter, the translation rate between the mRNA and the nonphosphorylated form of PER protein, by periodic intermittent activation or inhibition. We show how such a modification, simulated in the model by a periodic, on/off, piecewise constant stimulation (which increases or decreases this parameter) allows the entrainment of oscillations exactly at, or close to, a desired period. In a different context, this suggests that some diseases may be corrected using pharmacological agents according to specific periodic delivery schedules. (*Chronobiology International*, 17(1), 1–14, 2000)

Key Words: Control theory—Chronotherapy—Entrainment—Oscillations—Periodic intermittent stimulation

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INTRODUCTION

Biological systems are nonlinear by nature and often show stable periodicities. In particular, circadian rhythms have been observed in many plants or animals and in human beings (Sweeney 1969; Bünning 1973; Winfree 1980; Touitou and Haus 1992; Goldbeter 1995; Vanden Driessche et al. 1996; Boissin and Canguilhem 1998; Hartmann et al. 1998). Some diseases, called *dynamical diseases* by Bélair et al. (1995), are observed when one or more of these rhythms are altered. Changes in the characteristics of these rhythms may be the result of a mutation, modifying biochemical oscillations, and cellular rhythms (Goldbeter 1996), as is the case with the insect *Drosophila*. In previous articles (Claude and Nadjar 1994; Claude 1995) and in another biological context, the problem of shifting a pathological dynamic system toward a physiological one is tackled. Here, we consider the problem of shifting a mutant *Drosophila* PER protein cycle (per^l or per^J) toward a normal ("wild") one using distribution laws common in applied pharmacokinetics.

THE MODEL AND ITS CONTROL

In a recent article (Goldbeter 1995), Goldbeter proposed a model for the circadian oscillations of the PER protein in *Drosophila* (Fig. 1). This five-dimensional biochemical model describes the cycle of production and degradation of the various forms (nonphosphorylated, monophosphorylated, or diphosphorylated) of the PER protein and its mRNA.

This model is described by the following set of equations:

$$\begin{cases} \frac{dM}{dt} = v_s \frac{K_I^n}{K_I^n + P_N^n} - v_m \frac{M}{K_m + M} \\ \frac{dP_0}{dt} = k_s M - V_1 \frac{P_0}{K_1 + P_0} + V_2 \frac{P_1}{K_2 + P_1} \\ \frac{dP_1}{dt} = V_1 \frac{P_0}{K_1 + P_0} - V_2 \frac{P_1}{K_2 + P_1} - V_3 \frac{P_1}{K_3 + P_1} + V_4 \frac{P_2}{K_4 + P_2} \\ \frac{dP_2}{dt} = V_3 \frac{P_1}{K_3 + P_1} - V_4 \frac{P_2}{K_4 + P_2} - k_1 P_2 + k_2 P_N - v_d \frac{P_2}{K_d + P_2} \\ \frac{dP_N}{dt} = k_1 P_2 - k_2 P_N \end{cases} \quad (1)$$

where M is PER mRNA, and P_0 , P_1 , P_2 , and P_N are the nonphosphorylated, monophosphorylated, diphosphorylated, and nuclear forms of the protein, respectively. The parameters of the model were taken from Goldbeter (1995):

$$\begin{aligned} v_s &= 0.76 \mu\text{M h}^{-1}, K_I = 1 \mu\text{M}, n = 4, v_m = 0.65 \mu\text{M h}^{-1}, K_m = 0.5 \mu\text{M}, k_s = 0.78 \text{ h}^{-1}, \\ V_1 &= 3.2 \mu\text{M h}^{-1}, K_1 = 2 \mu\text{M}, V_2 = 1.58 \mu\text{M h}^{-1}, K_2 = 2 \mu\text{M}, V_3 = 5 \mu\text{M h}^{-1}, \\ K_3 &= 2 \mu\text{M}, V_4 = 2.5 \mu\text{M h}^{-1}, K_4 = 2 \mu\text{M}, k_1 = 1.9 \text{ h}^{-1}, k_2 = 1.3 \text{ h}^{-1}, K_d = 0.2 \mu\text{M}, \\ v_d &= 1.6 \mu\text{M h}^{-1} \text{ (wild)}, = 2 \mu\text{M h}^{-1} \text{ (per}^l\text{)}, \text{ or } = 0.5 \mu\text{M h}^{-1} \text{ (per}^J\text{)} \end{aligned}$$

We illustrate the oscillatory behavior of the PER production cycle by a phase representation in the (M, P_t) plane, where $P_t = P_0 + P_1 + P_2 + P_N$; asymptotically, for $t \rightarrow +\infty$,

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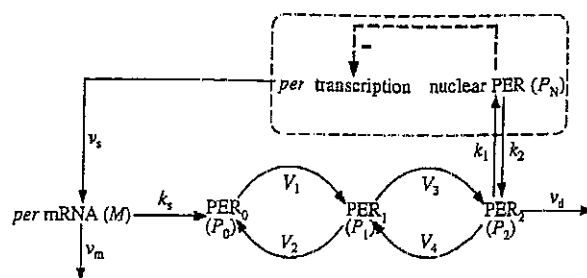


FIGURE 1. The biochemical pathways of PER protein synthesis, phosphorylation, and degradation (After Goldbeter 1995, Fig. 1)

one thus obtains a closed curve representing a stable limit cycle, which is a geometrical characteristic of the system (Goldbeter 1996)

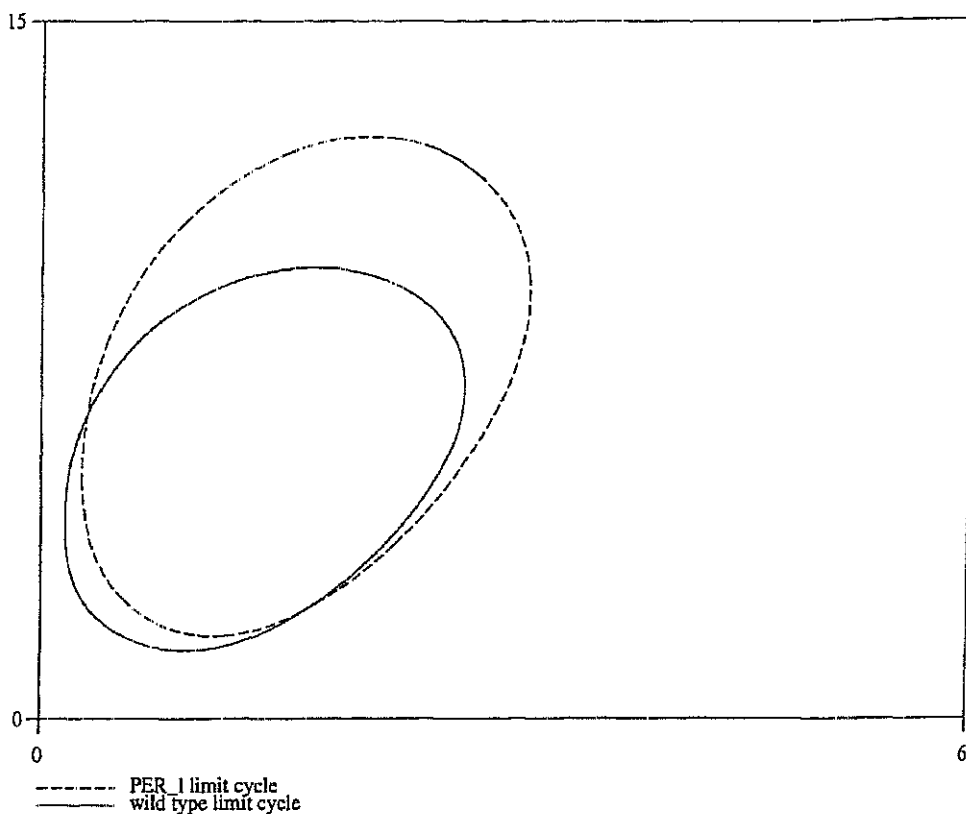
In a subsequent publication (Leloup and Goldbeter 1997), this dynamic system (PER) was coupled to another one (TIM), which allowed modeling of PER protein oscillations in a more complete way. This more elaborate system also shows a circadian rhythm and offers, in the light of experimental observations, other explanations, associated with changes in different parameters, for the mutant phenotypes. However, in the present paper, we only considered the simpler model, given by Eq. 1 above, in which nonmutant (wild) and mutant *Drosophila* strains were characterized tentatively by different values of v_d ($v_d = 1.6 \mu\text{M h}^{-1}$ for the wild type, resulting in an endogenous period of 23.71h, $v_d = 2 \mu\text{M h}^{-1}$ for the long per^L type, with a period of 28.5h, and $v_d = 0.5 \mu\text{M h}^{-1}$ for the ultrashort per^U type, with a period of 16h), the values of the other parameters being the same. These different types exhibit different limit cycles (see Fig. 2)

Here, we consider the problem of shifting a mutant *Drosophila* PER cycle toward the wild-type *Drosophila* PER cycle, setting its period precisely to 24.00h. We use the present model as a paradigm to explain how we can restore altered rhythms by a general method, bearing in mind possible applications to applied pharmacokinetics.

In the present model (unlike the more elaborate one designed by Leloup and Goldbeter 1998), the v_d parameter represents the only "genetic" component, specific for the *Drosophila* type considered here: wild, long (per^L), or ultrashort (per^U). If it were possible to modify the value of v_d in a mutant *Drosophila*, for instance, by gene therapy, to recover the wild-type value of $1.6 \mu\text{M h}^{-1}$, then this problem would be resolved directly. We assumed here that the parameter v_d cannot be recovered by pharmacological or other physical means, and that we had to find an indirect way to change the period of the limit cycle.

A modification of temperature may be considered, but it would also change other parameters of the system (Leloup and Goldbeter 1997), and from the model, we cannot derive information on the temporal variation of all the parameters with respect to temperature.

Since we are dealing with a closed-loop system, we could a priori act on any step of this biochemical pathway. However, from our control law on the system, we also wished to achieve sufficient *robustness* with respect to the inevitable errors that affect the programming of either quantity or time when applying any stimulation scheme



(a)

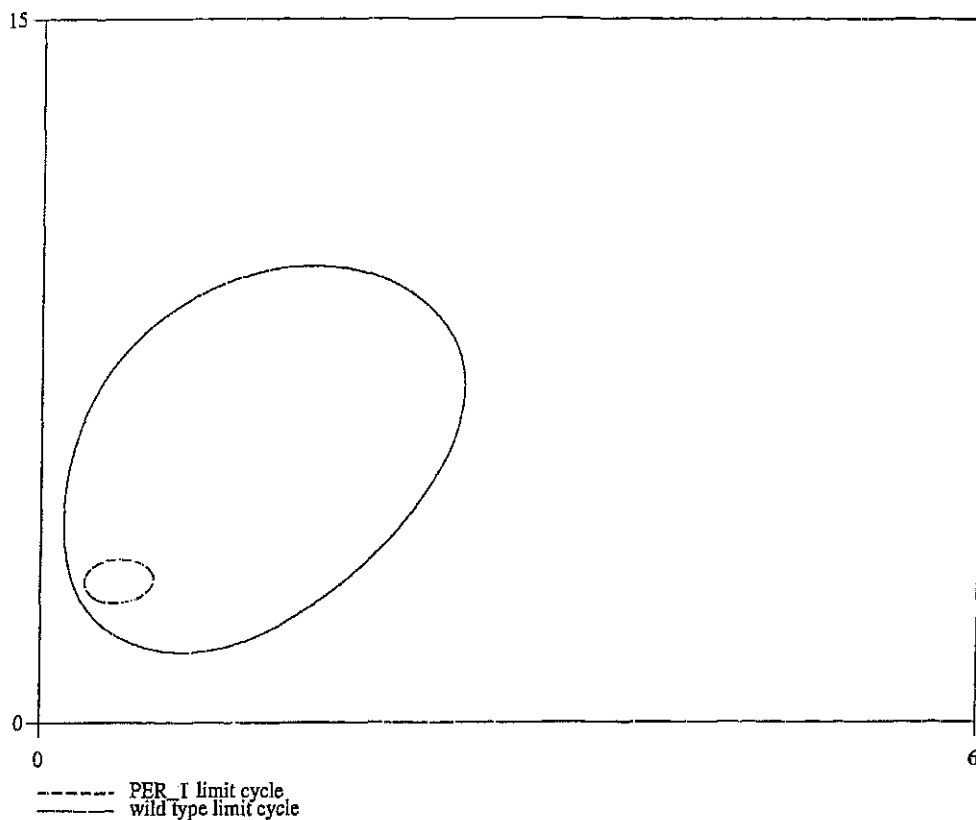
FIGURE 2. (a) The *per*¹ mutant *Drosophila* limit cycle (dashed line) compared with the wild-type limit cycle (solid line) for PER protein oscillations; representation in the (M, P_t) plane without any external control (see text for details) (b) The *per*¹ *Drosophila* limit cycle (dashed line) compared with the wild-type limit cycle (solid line) for PER protein oscillations; representation in the (M, P_t) plane without any external control (see text for details).

X

Considering the biochemical loop described in Fig. 1, we chose to focus on the two main parameters that appear between M and $P_t = P_0 + P_1 + P_2 + P_N$, namely, the constants v_s and k_s (see Eq. 1). These parameters represent plausible pharmacological targets: v_s is a chemical reaction velocity, and k_s is a rate constant (here, a factor multiplying mRNA concentration M). These two parameters could be changed or adapted in the real physical world using a chemical activator or inhibitor.

We controlled both parameters, but do not present results for v_s here. Indeed, v_s , which measures the transcription rate from DNA to mRNA, proved to be more delicate to handle and was thus less robust with respect to possible errors in the amplitude, period, or phase of the stimulation scheme.

Starting from mutant *Drosophila* parameter values, we focused on the activation or inhibition of the translation rate of the PER mRNA into the nonphosphorylated form



(b)

FIGURE 2. Continued.

of the PER protein, which is measured by the parameter k , in the above set of equations.

Based on control laws common in applied pharmacokinetics, we used three different stimulation time schemes, each with activating or inhibiting effect on the control parameter:

1. Constant stimulation
2. 24h periodic bolus (one quasi-instantaneous on-off stimulation of very short duration (3 or 6 minutes) every 24h)
3. Rhythmic intermittent stimulation with a period of 24 00h and effective long durations of 1h, 2h, 3h, 4h, 6h, 8h, or 12h (see Fig. 3), at peak or trough time for P_i in the original mutant model.

Activation or inhibition was obtained, starting from the basis value of 0.78h^{-1} for k , given by Goldbeter in 1995, by adding or subtracting a constant level of amplitude ranging between 0.2h^{-1} and 2.0h^{-1} for activation and between -0.2h^{-1} and -0.7h^{-1} for inhibition.

Thus, our first aim was to obtain, by modifying k_s , an endogenous rhythm close to 24h for a mutant *Drosophila* and, if possible, phases (peak or trough time, counted from zero, modulo 24h) and oscillation values for the different dynamic variables of the controlled mutant model, which should be close to the ones observed in the nonmutant model.

Second, although we used an open-loop control, we wished to obtain a control law that was sufficiently robust with respect to possible errors in amplitude, period, or phase.

Third, inasmuch as a 24h period was in fact obtained for the controlled limit cycle, we also desired to reach it with transients as short as possible, preferably within 1 week.

Finally, we wanted to obtain transients that were close enough to the final (controlled) limit cycle, thus discarding control laws that showed transient behaviors that were too large or erratic.

RESULTS

The aforementioned system of ordinary differential equations was solved numerically using the public domain software SCILAB (scilab@inria.fr), with integration step = 0.01h (36 seconds, occasionally 0.005h = 18 seconds in case of a brief bolus) and total integration time 216h (= 9 days), occasionally 432h or even 648h. For each mutant (*per^L* and *per^T*), two sets of initial values were used; one corresponding to the maximum value of the total PER protein P , for the noncontrolled cycle (peak) (Fig. 4) and the other one to its minimum value (trough).

Most Common Modes of Control: Bolus and Constant Stimulation

None of these modes of control reached the objective of setting the period of PER oscillations to 24h in a robust way.

First, starting from the *per^L* type v_d value of $2.0 \mu\text{M h}^{-1}$, constant stimulation by setting $k_s = 0.78 + 0.5 = 1.28 \text{ h}^{-1}$ leads to a period of 26.5h, closer to 24h, but with oscillation amplitudes much higher than those obtained for the wild-type cycle (with $v_d = 1.6 \mu\text{M h}^{-1}$), whereas inhibition by setting $k_s = 0.28 \text{ h}^{-1}$ leads toward oscillations with a lower amplitude and a limit cycle of period 37.5h (Fig. 4a). These oscillation values are thus incompatible with the wild-type values that are our target.

In the same way, starting from the *per^T* type v_d value of $0.5 \mu\text{M h}^{-1}$, stimulation by setting $k_s = 0.78 + 0.5 = 1.28 \text{ h}^{-1}$ suppresses the oscillations (Fig. 4b), and inhibition by setting $k_s = 0.28 \text{ h}^{-1}$ leads to a period of 22.6h, with a very low amplitude of the oscillations.

In both cases, when applying various stimulation amplitudes according to a 0.1 h^{-1} grid, we could never obtain a 24h periodicity by such a constant stimulation. However, with the *per^T* type, taking $k_s = 0.78 - 0.5375 = 0.2425 \text{ h}^{-1}$, we obtained a controlled limit cycle with exactly a 24h period. We did not find this control law to be robust enough to be retained. First, such a control law would demand too high precision, incompatible with the real conditions in applied pharmacokinetics. Second, even if a slight modification in the control parameter values causes only a small period shift in our case, it may give rise to drastic changes and may even lead to a bifurcation in other nonlinear systems.

We then used a brief stimulation by a periodic bolus of 3-minute or even 6-minute duration and a 24h periodicity. Trying various stimulation amplitudes and phases (stimu-

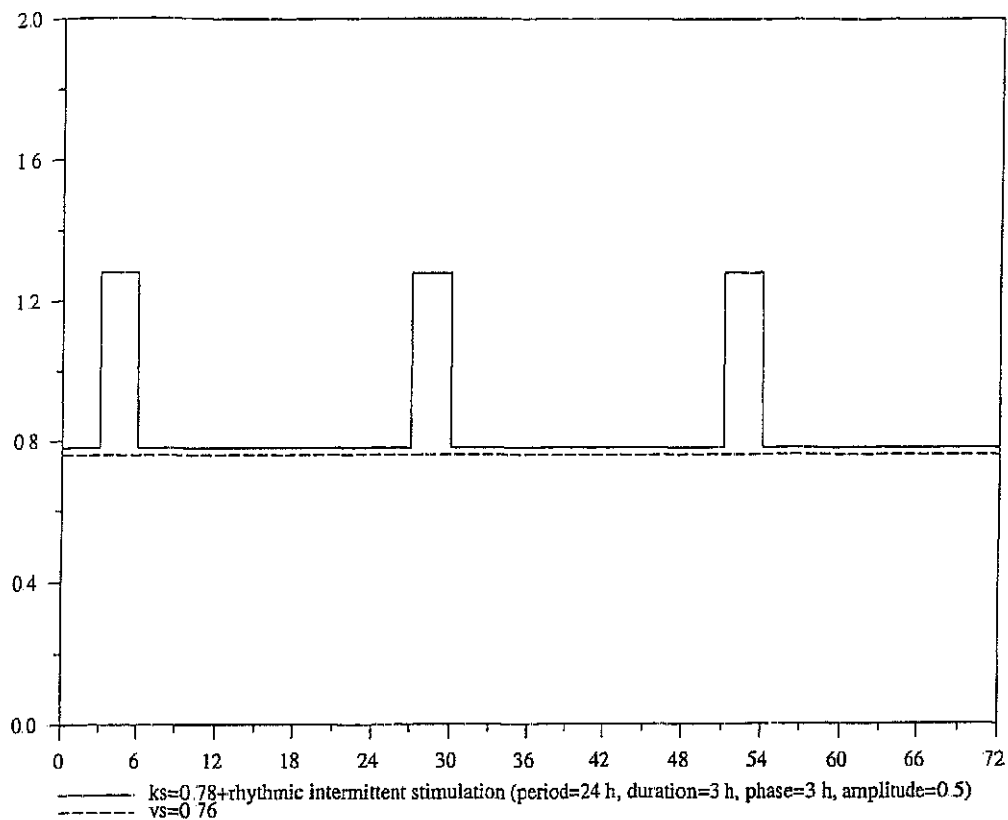
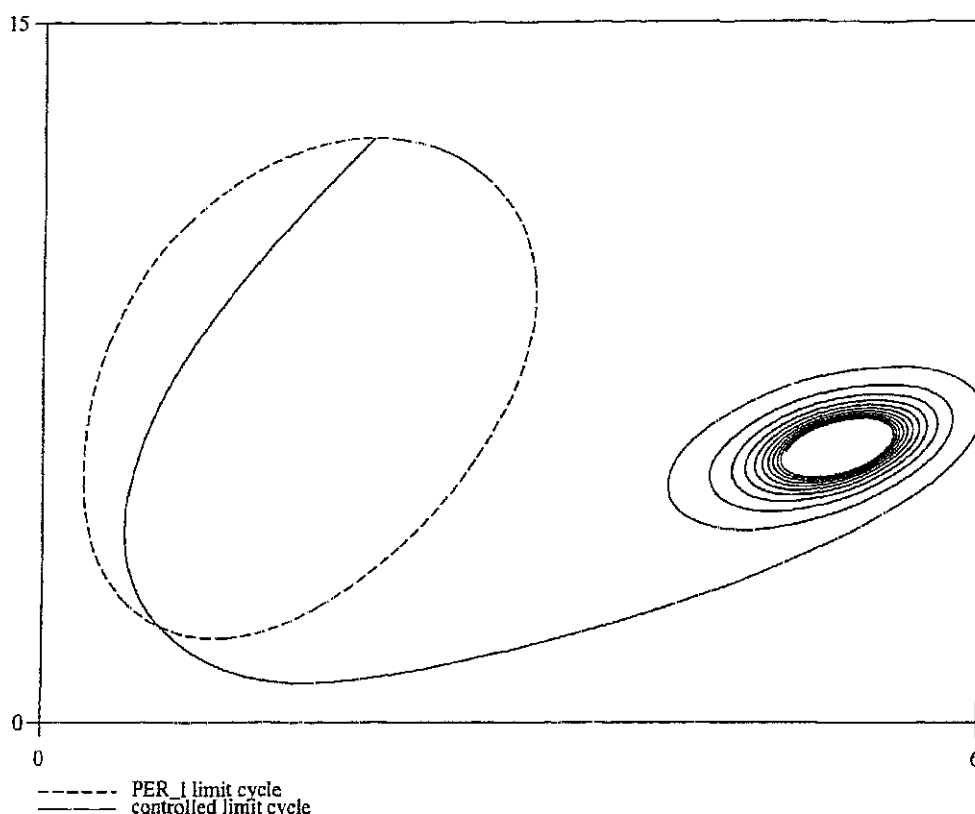


FIGURE 3 Control law applied to the parameter k_s ; square wave characterized on this figure by period = 24h, effective duration = 3h, amplitude (activation) = $0.5h^{-1}$, phase from origin = 3h. Bolus would be represented in the same way, but with a much shorter effective duration (3 or 6 minutes) of the stimulus

lation at peak or trough of the noncontrolled cycle), we could never obtain any significant deviation from the initial limit cycle. This probably illustrates the stable nature of the limit cycle, whether for the per^l or the per^T mutant.

Forcing PER Oscillations by Periodic Intermittent Stimulation

We finally used a long 24h periodic stimulation, according to the rhythmic intermittent schemes mentioned above, in amplitude, phase, and time (long time, between 1h and 12h, unlike 0.05h or 0.1h in the bolus scheme) In both mutants and both modes of stimulation, we obtained good entrainment at the desired 24h rhythm in particular zones of the stimulus amplitude and effective stimulation time. The existence of such zones (and not only well-defined values) ensures natural robustness of the periodic intermittent stimulation with respect to errors in stimulus period, amplitude, duration, and initial phase (peak or trough or another phase).



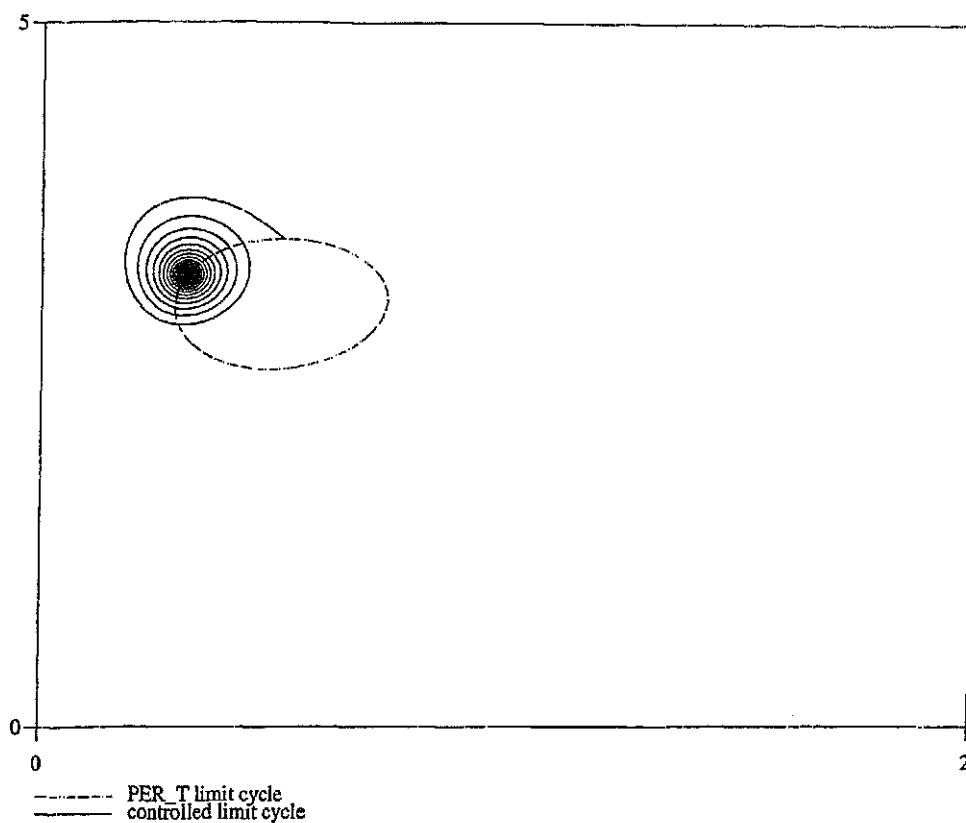
(a)

FIGURE 4. (a) The per^l mutant *Drosophila* limit cycle controlled by constant inhibiting stimulation (solid line), with stimulus amplitude $-0.5h^{-1}$, starting at peak time of the noncontrolled per^l limit cycle (dashed line) (b) The per^r mutant *Drosophila* limit cycle controlled by constant activating stimulation (solid line), with stimulus amplitude $0.5h^{-1}$, starting at peak time of the noncontrolled per^r limit cycle (dashed line)

We present, in Tables 1 and 2, lower and upper bounds for the intervals of values added to k_1 , in which entrainment was obtained and some additional intermediate values within these intervals. Outside these intervals, various types of behavior were observed: no disruption at all, apparent period doubling with a limit cycle appearing as an invaginated eight (Pascal's "limaçon"), or even completely erratic.

For per^l (see Fig. 5a and Table I), the activation proved to be much better than inhibition, with a good 1-1 entrainment, a satisfying limit cycle, and satisfying transient behavior obtained from only 1h of effective activation, provided that stimulus amplitude was chosen high enough ($1.5h^{-1}$ or $2h^{-1}$), up to 12h of effective activation, with lower amplitude levels (from $0.3h^{-1}$ to $0.7h^{-1}$, for 12h, 8h, and 6h), whereas inhibiting entrainment demanded at least 4h of effective inhibition, with an amplitude as low as $-0.3h^{-1}$, down to $-0.4h^{-1}$, for 12h. The different stimulation schemes shown in Table I produced

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(b)

FIGURE 4. Continued

Table 1. 24h Periodic Intermittent Stimulation of *per*¹: Exposure Duration Versus Amplitude of the Activating (Positive Values) or Inhibiting (Negative Values) Stimulus Producing a 1-1 Entrainment, Regardless of the Chosen Initializing Phase, Peak, or Trough of the Original Mutant *P*, Protein Concentration

	Activating						Inhibiting					
1h	—	—	—	—	—	1.5	2	—	—	—	—	—
2h	—	—	—	0.7	1	1.5	—	—	—	—	—	—
3h	—	—	—	0.7	1	—	—	—	—	—	—	—
4h	—	—	0.5	0.7	1	—	—	—	—	—	—	-0.7
6h	0.3	0.4	0.5	0.7	—	—	—	—	—	—	-0.5	-0.7
8h	0.3	0.4	0.5	—	—	—	—	-0.35	-0.4	-0.5	-0.7	—
12h	0.3	0.4	—	—	—	—	-0.3	-0.35	-0.4	—	—	—

Table 2. 24h Periodic Intermittent Stimulation on *per^T*: Exposure Duration Versus Amplitude of the Activating (Positive Values) or Inhibiting (Negative Values) Stimulus Producing a 1-1 Entrainment Regardless of the Chosen Initializing Phase, Peak, or Trough of the Original Mutant *P*, Protein Concentration

	Activating			Inhibiting			
4h	—	—	—	—	—	—	-0.6
6h	—	—	2	—	-0.45	-0.5	—
8h	—	1.5	2	-0.4	-0.45	—	—
12h	1.2	1.5	—	-0.4	-0.45	—	—

a 1-1 entrainment whatever the chosen initial phase was, but usually gave rise to faster convergence if the stimulation started at peak time rather than at trough time, except for the long exposure duration (8h or 12h).

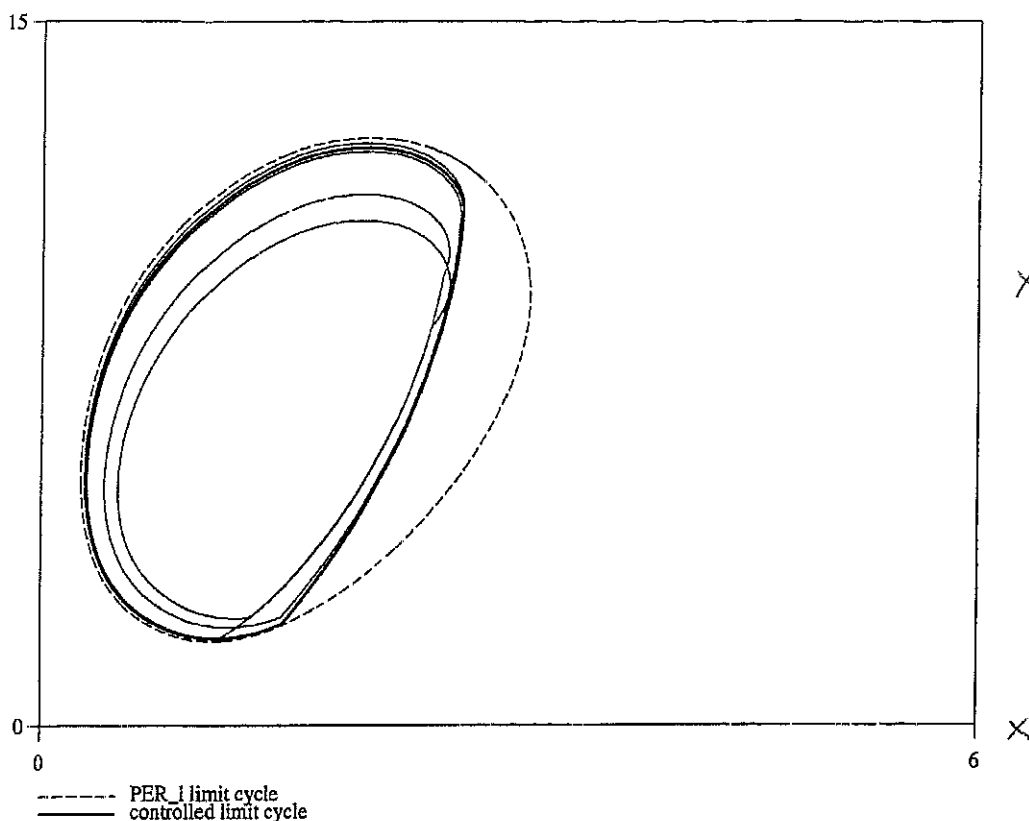
On the other hand, for *per^T* (see Fig. 5b and Table 2), inhibition proved to be easier than activation. Entrainment was obtained with only 4h of exposure for inhibition (versus 6h for activation) and an amplitude within a narrow range (-0.4h^{-1} to -0.5h^{-1} for high exposure durations of 6h, 8h, and 12h), whereas activating entrainment was obtained only for a stimulus amplitude over 1. Stimulation at peak time usually gave rise to faster convergence than at trough time, with shorter transients and oscillation amplitudes for the limit cycle and transients that were closer to those of the wild type.

DISCUSSION

We could not obtain a 24h period by constant or periodic bolus stimulation in a robust way. From a mathematical point of view, a permanent change in stimulus amplitude may produce a deformation of the limit cycle, modification of its period, or even bifurcation of the dynamic system; as for a brief bolus, it usually produces only phase resetting (cf. Winfree 1980).

The inspection of the good entrainment zones suggested the possibility of a "law of areas" that may define a daily dose for an activator or inhibitor of the reaction: A 1-1 entrainment exists only if the total delivered dose during 24h (the amplitude times the effective stimulation time) lies within an "efficacy interval." Outside this interval, we observed the following unadapted types of behavior:

- too long transients
- no entrainment at all, with the cycle returning to the original mutant cycle and consequently to its original period
- a limit cycle with amplitude values or shape that were too far away from the wild type
- 1-2 entrainment with a 48h period for the controlled limit cycle
- even more complicated trajectories in the (M, P_t) phase plane, with a very disorderly appearance



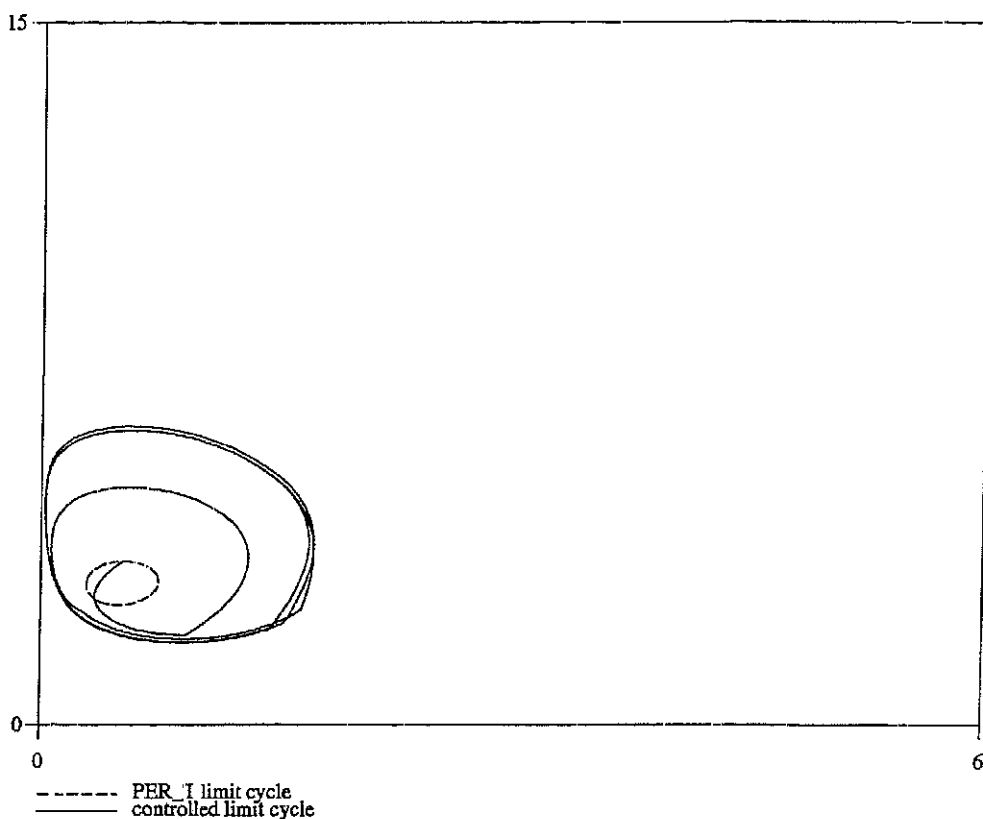
(a)

FIGURE 5 (a) The per^l mutant *Drosophila* limit cycle controlled by 24h periodic intermittent activating stimulation (solid line), with stimulus amplitude $0.4h^{-1}$, exposure duration 6h, starting at trough time of the noncontrolled per^l limit cycle (dashed line). Successful 1-1 entrainment was obtained, with a period of exactly 24h for the controlled per^l limit cycle. (b) The per^r mutant *Drosophila* limit cycle controlled by 24h periodic intermittent inhibiting stimulation (solid line), with stimulus amplitude $-0.4h^{-1}$, exposure duration 8h, starting at peak time of the noncontrolled per^r limit cycle (dashed line). Successful 1-1 entrainment was obtained, with a period of exactly 24h for the controlled per^r limit cycle.

(continued)

Beginning stimulation at peak time or at trough time usually gave the same entrainment results, but not at an equal speed: Very long and/or disorderly transients were observed when the initial phase (peak or trough) was not adapted.

We thus obtained a very good 1-1 entrainment at the desired period of 24h when particular parameters for the control law (stimulus amplitude, effective duration of stimulation, and initial phase) were chosen. We may also fit the phase of the controlled cycle by choosing the pair (amplitude, effective stimulation time). For instance, with the per^l mutant, a good 1-1 entrainment was obtained for a total "delivered daily dose" (of an activator or inhibitor of the reaction) equivalent to a stimulus amplitude for k_r of $+0.8h^{-1}$



(b)

FIGURE 5. Continued.

for 3h, or $+0.6h^{-1}$ for 4h, or $+0.4h^{-1}$ for 6h, or $+0.3h^{-1}$ for 8h, with corresponding P_i peaks at 8.27h, 8.85h, 10.27h, and 12.35h, modulo 24h, from initial time regardless of the choice of the initial phase, peak, or trough time for P_i .

Thus, in this low-dimensional model, which may be taken as a general paradigm of the numerous biological systems in which protein synthesis regulation is involved, we have shown that, using an adapted daily dose, one can shift the period of a "pathological" cycle toward a "physiological" one by varying three factors: total dose, exposure duration, and time of start of exposure (phase). Later, we may improve this tool by frequency coding (Goldbeter 1991, 1993) of the entrainment stimulus, using intermittent burst delivery.

Chronotherapy, until now, has consisted mostly of the rhythmic delivery of medications to improve treatment tolerance and/or efficacy (Lévi 1997; Lévi et al. 1997). Nevertheless, rhythmic alterations of physiological variables have been reported in several diseases, including cancer (Canon and Lévi 1992; Mormont and Lévi 1997) and could also benefit from chronotherapeutic strategies, based on the principle of periodic intermittent drug delivery, developed in this paper.

ACKNOWLEDGMENTS

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