10th International IFAC Symposium on
Computer Applications in Biotechnology
Preprints Vol.2, June 4-6, 2007, Cancún, Mexico

CONTROL OF COUPLED CIRCADIAN OSCILLATORS

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Abstract: Circadian rhythms are endogenous rhythms in physiology or behavior with a cycle length near 24 hours. Circadian rhythms are relevant for many key physiological functions. The periodic light-dark cycle is the dominant environmental synchronizer used to entrain a population of circadian oscillators. In this work we introduce a control approach for both suppression and synchronization of coupled circadian oscillators. The control scheme is based on a modeling error compensation approach. Numerical simulations shows the effectivity of the feedback control law for suppression and synchronization of an array of coupled circadian oscillators via a light-sensitive parameter.

Keywords: Circadian Rhythms, Biological Clocks, Physiological Functions, Robust Control, Synchronization.

1. INTRODUCTION

The biological functions of most living organisms are organized along an approximate 24-h time cycle or circadian rhythm (Goldbeter, 1996). Circadian rhythms, are endogenous because they can occur in constant environmental conditions, e.g. constant darkness. The endogenicity of the circadian rhythms has been demonstrated in microorganisms, in plants and in all kinds of animal species including man (Antle *et al.*, 2003; Dodd *et al.*, 2005; Fu and Lee, 2003). These endogenous rhythms govern daily events like sleep, activity, hormonal secretion, cellular proliferation and metabolism (Buzsa *et al.*, 2004; Goldbeter, 1996; Dodd *et al.*, 2005; Fu and Lee, 2003).

Circadian rhythms are centrally regulated by the suprachiasmatic nucleus (SCN) of the hypothalamus (Goldbeter, 1996). Most neurons in the SCN become active during the day and are believed to comprise the biological clock. Dispersed SCN cells exhibit sustained circadian oscillations with periods ranging from 20 to 28 hours, but on the

tissue level, SCN neurons display a significant degree of synchrony (Fu and Lee, 2003; Kunz and Achermann, 2003). The synchronization property is believed that has physiological relevance. For instance, these synchronized rhythms may influence the pharmacology and the tolerability of anticancer drugs and/or their antitumor efficacy (Fu and Lee, 2003; Levi, 2006). Conversely, a lack of synchronization, or an alteration of circadian clock function can lead to a unpredictable behavior, and may require specific therapeutic measures to restore normal circadian function (Levi, 2006). Over time, the development of a circadian rhythm might impart larger benefits to the organism. In cyanobacteria, for example, matching of the free-running period to the light-dark cycle time provides a selective advantage, which is presumably the basis for its evolution (Ouyang *et al.*, 1998). In Arabidopsis, matching between the circadian period and the light-dark cycle results in plants that fix carbon at a higher rate and grow and survive better than those that lack such a match (Dodd *et al.*, 2005).

Different approaches have been used to couple and synchronize a population of circadian oscillators. Winfree (2002) has suggested that such critical perturbations applied at the appropriate phase of a limit cycle should stop the clock, at least transiently, if the perturbation brings the oscillator back into the vicinity of the steady state. Ueda *et al* (2002) studied a model for circadian rhythms in Drosophila. As a single cell oscillator, they used a more detailed model incorporating 10 variables. They then apply a local coupling through each possible variable, and show that for some of them, synchronization occurs. Interestingly, they assessed the effect of fluctuations in parameter values and show that the coupled system is relatively robust to noise. Another theoretical model of coupled circadian oscillators through local coupling has been proposed by Kunz and Achermann (2003). Using the van der Pol model, they described possible spatial effects, including wave propagation and pattern formation. Gonze *et al* (2005) proved that a mean field approach can be an effective way to couple a population of circadian oscillators, where the global coupling drives oscillators, which would be damped under a constant forcing.

From control and systems theory viewpoints few papers have been addressed the control and synchronization problem of circadian oscillations. Angeli and Sontag (2004) have been establishes global asymptotic stability results using small gain theorems for a single model of circadian oscillations. Kimura and Nishigaki (2005) have been established an analogy of circadian rhythm with the PLL framework. Doyle and co-workers (2004, 2005) have been introduced a robustness analysis and a model predictive control approach for circadian oscillations. Yasuda and Ito (2004) and Takeuchi *et al.* (2006) have been also addressed the generation and suppression of circadian oscillations with control theory tools. To the best of our knowledge, the control and synchronization of coupled circadian oscillations has not been yet addressed from a control theory viewpoint.

In this work, we shown that a feedback control action applied to a light-sensitive parameter is efficient to synchronize an array of coupled circadian oscillators. The coupling is incorporated through a diffusive coupling of frq mRNA concentration. Depending on a pattern of light, which affects the rate of transcription of frq mRNA, the forcing of circadian oscillations may result in suppression and synchronization of the array of coupled oscillators. The control approach is based on modelling error compensation (MEC) techniques. The key feature of MEC control is that modelling error estimation and compensation leads to linear controllers. Moreover, model uncertainty can be explicitly addressed and the nonlinear process model is directly incorporated in the control design, allowing for coupling and nonlinearity of the coupled circadian oscillators to be taken into account. In this way, a simple practical control design with good robustness and performance capabilities is obtained. This work is organized as follows: In Section 2, for the sake of completes of our work, we present the basic circadian oscillator and the corresponding array of coupled oscillators. In Section 3 we introduce our control approach for synchronization of coupled circadian oscillators. Numerical simulations in Sections 4 shows the control performance for suppression and synchronization of the coupled oscillators. Finally, some concluding remarks are given in Section 5.

2. COUPLED CIRCADIAN OSCILLATORS

Various physiological ODE models of individual circadian clocks have been published in the last ten years (Goldbeter, 1996; Antle *et al.*, 2003; Gonze and Goldbeter, 2000; Leloup *et al.*, 1999). They rely on transcriptional regulation, a mechanism possibly yielding limit cycles. We will consider a simple three-variable model proposed for circadian rhythms in Neurospora (Gonze and Goldbeter, 2000). In Neurospora the mechanism of circadian rhythmicity relies on the negative regulation exerted by the protein FRQ on the transcription of its gene frq into the messenger RNA (mRNA), the translation of which leads to the synthesis of FRQ. Thus, the circadian oscillations of the protein FRQ and its mRNA in Neurospora is governed by the system of three kinetic

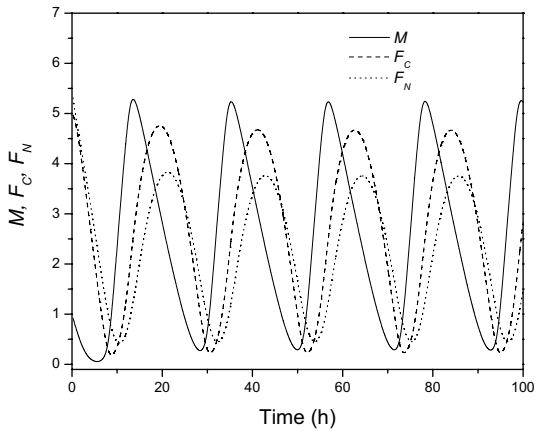

Fig. 1. Dynamical behavior of circadian oscillator at $i = 1$ and parameter values of Table 1.

equations (Gonze and Goldbeter, 2000; Leloup *et al.*, 1999):

$$\frac{dM}{dt} = v_s \frac{K_I^n}{K_I^n + F_N^n} - v_m \frac{M}{K_m + M} \quad (1)$$

$$\frac{dF_C}{dt} = k_s M - v_d \frac{F_C}{K_d + F_C} - k_1 F_C + k_2 F_N \quad (2)$$

$$\frac{dF_N}{dt} = k_1 F_C - k_2 F_N \quad (3)$$

where M, F_C, and F_N denote, respectively, the concentrations (defined with respect to the total cell volume) of the frq mRNA and the cytosolic and nuclear forms of FRQ. Parameter v_s denotes the rate of frq transcription; this parameter increases in the light phase. The other parameters appearing in these equations are the constant K_I related to the threshold beyond which nuclear FRQ represses frq transcription, the Hill coefficient n characterizing the degree of cooperativity of the repression process, the maximum rate v_m of frq mRNA degradation and the Michaelis constant K_m related to the latter process, the apparent first-order rate constant k_s measuring the rate of FRQ synthesis which is assumed to be proportional to the amount of frq mRNA present in the cytosol, the maximum rate v_d of FRQ degradation and the Michaelis constant K_d related to this process, and the apparent first-order rate constants k_1 and k_1 characterizing the transport of FRQ into and out of the nucleus (Gonze and Goldbeter, 2000; Leloup *et al.*, 1999).

In this article, we consider an array of N coupled circadian oscillators resulting from the diffusive coupling of a population of circadian oscillators. In particular, we suppose that the coupling between oscillators is achieved through the formation of a diffusive coupling of frq mRNA concentration. Then, the N oscillators coupled are given by:

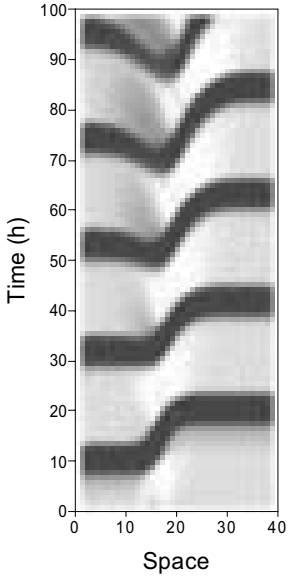

Fig. 2. Surface map of the frq mRNA concentration M.

Table 1. Parameter values for the base cicardian osciilator of Fig. 1 (Taken from Gonze and Goldbeter, 2000)

v_s	1.6	K_I	1.0
v_d	1.4	K_d	0.13
v_m	0.505	K_m	0.5
k_s	0.5	k_2	0.6
n	4.0	k_1	0.5

$$\frac{dM_i}{dt} = \mathcal{C}(M) + v_s \frac{K_I^n}{K_I^n + (F_N^n)_i} - v_m \frac{M_i}{K_m + M_i} \quad (4)$$

$$\mathcal{C}(M) = \sigma(M_{i-1} - 2M_i + M_{i+1}),\ i = 1, ..., N \quad (5)$$

where $\mathcal{C}(M)$ is a diffusive coupling function. The kinetic parameters can differ from one oscillator to the other and thus holds variability in individual circadian oscillators.

Figure 1 and 2 shows the numerical solution of the 40 coupled circadian oscillators (4-5) using parameter values given in Table 1 and different initial conditions. Figure 1 shows M, F_C, and F_N at $i = 10$ which have periods of 21.5 hours approximately. The surface map of behavior of M is shown in Figure 2. Color range form clear to dark at minimum and maximum values of M respectively.

3. SUPPRESSION AND SYNCHRONIZATION OF COUPLED CIRCADIAN OSCILLATORS

The control objective are both suppression and synchronization of the frq mRNA concentration M, by manipulation of the rate of frq transcription v_s, parameter that varies with light and this modulation actually entrains the suprachiasmatic pacemaker (Gonze and Goldbeter, 2000). Thus,

the only necessary communication with the external environment is through a light-sensitive mechanism, which is plausible to be manipulable physiologically and can provide synchrony on the tissue level. In fact, some theoretical and experimental studies have been demonstrated that light induction of clock genes might be a general factor through which the body clock is brought into synchronization with the external environment (Goldbeter, 2006; Gonze *et al.*, 2005). The effect of light on the circadian oscillators would be selected on the basis of the benefit of making the levels of certain gene products lower or higher in daylight than at night, and could be achieved by a light-sensitive protease such as the Cryptochrome of Drosophila before the evolution of the circadian oscillator (Busza *et al.*, 2004).

Thus, let $y^i_{ref}(t)$ be a desired dynamic behavior for the frq mRNA concentration, M. If $y^i_{ref}(t) = (y^1_{ref}(t), ..., y^n_{ref}(t))$ for all $t \geq 0$, where $y^i_{ref}(t)$ is a given single signal, the control problem will correspond to a synchronization problem with respect to synchronization signal $y_{ref}(t)$. The control problem description is completed by the following assumptions:

A1 The frq mRNA concentration $y = M$ and its time-derivative $\dot{y}$ are available for control design purposes.
A2 Kinetics parameters are uncertain.
A3 Coupling function $\mathcal{C}(M)$ is not available for control design.

The following comments are in order:

- A1 is a reasonable assumption, since current experimental test measure proteins composition. For instance, the measurement of frq mRNA concentration can be performed from either a RT-PCR approach (PE Biosystems, Foster City, CA) or microarray analysis. However, even in the absence of such measurements, from the control theory viewpoint, a state estimator can be designed to estimate the frq mRNA concentration from other measurements. On the other hand, from the measurement of frq mRNA concentration, the time-derivative $\dot{y}$ of the controlled variable can be approximated with a linear filter.
- A2 and A3 means that the control input does not rely on a good mathematical model neither on a good knowledge of environmental conditions in order to propose a convenient control.

By Assumptions A2 and A3, Eq. (4) can be written as

$$\frac{dy^i}{dt} = -\left(\widetilde{v}_m \frac{y^i}{\widetilde{K}_m + y^i}\right) + \left(\frac{\widetilde{K}^n_I}{\widetilde{K}^n_I + F^n_N}\right) u_i + \eta_i \tag{6}$$

$$\eta_i = \mathcal{C}_i(y) - \left(v_m \frac{y^i}{K_m + y^i} - \widetilde{v}_m \frac{y^i}{\widetilde{K}_m + y^i}\right) + \left(\frac{K^n_I}{K^n_I + F^n_N} - \frac{\widetilde{K}^n_I}{\widetilde{K}^n_I + F^n_N}\right) u_i \tag{7}$$

where $\left[\widetilde{v}_m, \widetilde{K}_I, \widetilde{K}_m\right]$ are estimated values of parameters $[v_m, K_I, K_m]$, so that η_i are the modeling error functions.

Define the synchronization as $e_i(t) = y^i(t) - y^i_{ref}(t)$ and consider the following feedback control function

$$u_i(t) = -\left(\frac{\widetilde{K}^n_I}{\widetilde{K}^n_I + F^n_N}\right)^{-1} \left[\begin{array}{c} \eta_i(t) - \left(\widetilde{v}_m \dfrac{y^i}{\widetilde{K}_m + y^i}\right) \\ +\varpi_{ci} e_i(t) + \dot{y}^i_{ref} \end{array}\right] \tag{8}$$

where ϖ_{ci} are control design parameters, so that the controlled system is given by

$$\dot{e}_i(t) = -\varpi_{ci} e_i(t) \tag{9}$$

Since $\varpi_{ci} > 0$, such subsystems are asymptotically stable about the zero tracking error; *i.e.*, $e(t) \to 0$ asymptotically. However, by virtue of Assumption A2 and A3, $\eta_i(t)$ are not available for feedback control design. In this way, in order to implement the control function (8) we introduce the following observers to get estimate functions $\widetilde{\eta}_i(t)$ of the uncertain functions $\eta_i(t)$ (Alvarez-Ramirez, 1999; Alvarez-Ramirez *et al.*, 2001):

$$\frac{d\widetilde{\eta}_i}{dt} = \varpi_{ei}(\eta_i - \widetilde{\eta}_i) \tag{10}$$

where ϖ_{ei} are observer design parameters. From (10) and (6) we have

$$\frac{d\widetilde{\eta}_i}{dt} = \varpi_{ei} \left[\begin{array}{c} \dfrac{dy_i}{dt} + \left(\widetilde{v}_m \dfrac{y_i}{\widetilde{K}_m + y_i}\right) - \left(\dfrac{\widetilde{K}^n_I}{\widetilde{K}^n_I + F^n_N}\right) \\ u_i - \widetilde{\eta}_i \end{array}\right]$$

introducing $w_i = \varpi^{-1}_{ei} \widetilde{\eta}_i - y_i$ we have

$$\frac{dw_i}{dt} = \left(\widetilde{v}_m \frac{y_i}{\widetilde{K}_m + y_i}\right) - \left(\frac{\widetilde{K}^n_I}{\widetilde{K}^n_I + F^n_N}\right) u_i - \widetilde{\eta}_i \tag{11}$$

$$\widetilde{\eta}_i = \varpi_{ei}(w_i + y_i)$$

Therefore, a practical feedback control is composed by the modeling error estimator (11) and the following feedback control function:

$$u_i(t) = -\left(\frac{\widetilde{K}^n_I}{\widetilde{K}^n_I + F^n_N}\right)^{-1} \left[\begin{array}{c} \widetilde{\eta}_i(t) - \left(\widetilde{v}_m \dfrac{y^i}{\widetilde{K}_m + y^i}\right) \\ +\varpi_{ci} e_i(t) + \dot{y}^i_{ref} \end{array}\right] \tag{12}$$

Notice that the resulting feedback control (11-12) depends only on measured signals $\{y^i, \overset{\bullet}{y}{}^i_{ref}\}$ and estimated parameters signals, and do not relies on a good mathematical model of system (4-5).

4. NUMERICAL SIMULATIONS

We have carried out some numerical simulations to illustrate both suppression and synchronization capabilities of our control approach. In both cases the control action is activated at $t = 100.0$. We also consider lower and upper limits for the minimum and maximum amplitude of the control inputs as $0.0 \leq u_i(t) \leq 5.0$. Our simulation results indicate good regulation and tracking performance of the closed-loop system.

4.1 Suppression of Coupled Circadian Oscillators

Let the desired controlled behavior be a constant reference value, $y^i_{ref} = 2.5$, *i.e.*, suppression of circadian oscillations. The simulation results are shown in Figure 3. It can be seen from Figure 3-a that we can successfully suppress the oscillatory behavior of the coupled circadian oscillators. Figure 3-b shows that pulse type perturbation of $u_i(t)$ applied at the appropriate phase with the appropriate duration and magnitude can achieve the suppression of the array of coupled oscillator. Such result has been observed experimentally (Honma and Honma, 1999; Jewett *et al.*, 1991; Klante and Steinlechner, 1995). For instance, in circadian rhythms in Drosophila the permanent suppression of circadian rhythmicity was achieved by a single light pulse (Klante and Steinlechner, 1995).

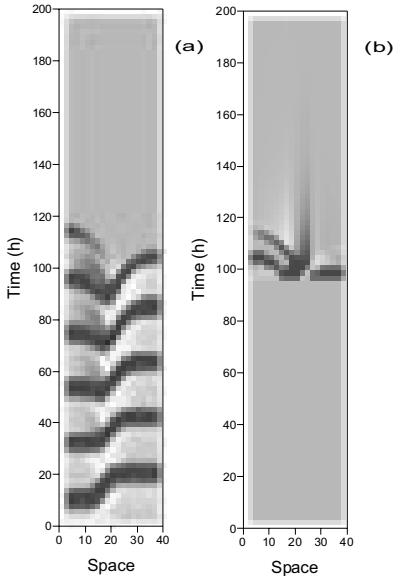

Fig. 3. (a) Surface map of the controlled evolution of $M(x,t)$ to a constant reference. (b) Corresponding surface map of the control input $u(x,t)$.

4.2 Synchronization of Coupled Circadian Oscillators

In this case, let y^i_{ref} be a trajectory generated by a circadian oscillator with parameters given in Table 1 and $v_s = 2.0$. Figure 4 shown the simulation results. Figure 4-a shows that after 10 h, the array of coupled oscillators synchronizes about the desired periodical dynamic behavior. Figure 4-b shows that by using a pattern of a light sensitive parameter, we can force the circadian periodicity. Thus, a light-sensitive parameter periodically forced by light and dark cycles allows to synchronize coupled circadian oscillators.

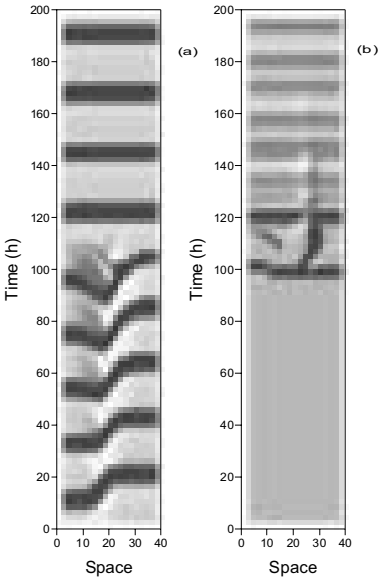

Fig. 4. (a) Surface map of the synchronized evolution of $M(x,t)$ to a given circadian oscillator. (b) Corresponding surface map of the control input $u(x,t)$.

5. CONCLUSIONS

Most organisms, including humans, exhibit daily physiological and behavioral rhythms. In many cases, these rhythms are driven from a circadian oscillator located in the suprachiasmatic nucleus. Although much has been learned in the past 2 decades about the effects of light exposure on the circadian timing system, very little is known about the strength or duration of light needed to achieve suppression and synchrony of coupled circadian rhythms. In this paper, under the assumption of uncertain kinetic parameters and unknown coupling functionalities, a feedback control scheme was designed to achieve both suppression and synchronization of an array of coupled circadian oscillators. Our control approach can be used to study the effect of a pattern of a light-

sensitive parameter to achieve both suppression and synchronization of coupled circadian oscillators. The feedback control law proposed in this work could be implemented experimentally via light-dark changes of variable period and amplitude applied at cellular level.

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