

Requirements for robust entrainment of weakly forced biological oscillators

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Abstract. Circadian clocks are biological oscillators that synchronize accurately with the day/night cycle by coupling to periodic variations in light and temperature, which may display significant amplitude fluctuations. The requirement for a robust entrainment of circadian clocks raises the theoretical question of which properties of the forced oscillator ensure an efficient entrainment irrespective of the fluctuations in forcing amplitude. To answer this question, we study the dynamics of an oscillatory genetic circuit comprising a simple negative feedback loop and subjected to periodic parameter modulation of variable amplitude. This study reveals that a robust entrainment of the oscillator requires that its period is different from the forcing period and that its phase response curve has a specific shape defined by its first and second derivative near the phase-locking point.

Résumé. L'horloge circadienne est un oscillateur biologique qui se synchronise avec précision au cycle jour-nuit en se couplant aux variations périodiques de la lumière et de la température, lesquelles peuvent présenter d'importantes fluctuations d'amplitude. La nécessité pour l'horloge circadienne de se synchroniser de manière robuste soulève la question suivante : quelles sont les propriétés que doivent posséder un oscillateur forcé pour être entraîné indépendamment des variations d'amplitude du forçage. Afin de répondre à cette question, nous étudions la dynamique d'un circuit génétique oscillant contenant une simple boucle négative et soumis à une modulation périodique d'amplitude variable de ses paramètres. Cette étude révèle qu'un entrainement robuste nécessite que la période de l'oscillateur auto-entretenu soit différente de la période de forçage et que la courbe de réponse de phase associée à la modulation ait une forme spécifique caractérisée par ses dérivés première et seconde près de la phase d'accrochage.

1 Introduction

Endogeneous rhythms are widespread in biological systems, with periods ranging from milliseconds (neuronal pulses) to years (hibernation cycles). Some of these rhythms can be entrained by periodic forcing which corresponds to the simplest case of synchronization [1]. This is the case of circadian clocks, which synchronize with the periodic variation of light and temperature associated with Earth's rotation. However clouds, water or trees can modulate daylight intensities sensed by organisms over several of orders of magnitude. These observations suggest that circadian clocks have evolved mechanisms that ensure a robust entrainment in face to significant daylight fluctuations. This raises a fundamental question : How to design a forced oscillator able to synchronize irrespective of the forcing amplitude ? This question can be addressed to unveil key design principles of circadian clocks as well as to suggest treatment strategies to promote accuracy of body rhythms such as hormonal cycle, cardiac activity or insulin secretion.

To be specific, we consider a schematic model of circadian oscillator based on a negative transcriptional feedback loop. The forcing is represented as a periodic modulation of the model parameters. To analyse the synchronization of the oscillator, we use a standard approach involving a Poincaré map-based description of the dynamics and the powerful concept of phase response curve. We show that the extent of the phase locking regime of frequency ratio 1 : 1 depends on which parameters are modulated : in some cases, the stability of the synchronized state depends little on modulation strength. This result is interpreted in the framework of the phase approximation, in which the perturbed orbit remains in the neighbourhood of the

free limit cycle. It reveals that robust entrainment requires specific properties of both the oscillator and the forcing.

2 Results

Locking properties of a circadian oscillator

Circadian oscillators are implemented at the level of the biochemical machinery of cell comprising genes and proteins interacting through feedback loops. In particular, the clock mechanism of most organisms relies on a core negative feedback loop, through which the clock gene encodes proteins that activate (resp., inactivate) its own transcriptional repressor (resp., activator) [2,3] (example in Figure 1A). The presence of delays or non-linearities along the loop favors the emergence of oscillations in this system [4]. Such basic clock mechanisms can be captured by low-dimensional dynamical model such as those originally proposed by Leloup and Goldbeter for the *Neurospora* clock [5] : a gene is transcribed into mRNA (M) that translates into a protein located in the cytoplasm (P_C), further translocated in the nucleus (P_N) where it inactivates the gene :

$$\begin{cases} \frac{dM}{dt} = s_M \frac{K_I^n}{K_I^n + P_N^n} - d_M \frac{M}{K_M + M} \\ \frac{dP_C}{dt} = s_P M - d_P \frac{P_C}{K_P + P_C} - k_1 P_C + k_2 P_N \\ \frac{dP_N}{dt} = k_1 P_C - k_2 P_N \end{cases} \quad (1)$$

The model includes a Michaelis-Menten type kinetics for the transcription and degradation dynamics, which is required to display oscillations characterized by a free running period T_{FRP} . We consider the following model parameters that give rise to 24-hours oscillations in the dark : $n = 4$, $s_M = 2.185$, $K_I = 1$, $d_M = 0.505$, $K_M = 0.5$, $s_P = 0.5$, $d_P = 1.4$, $k_P = 0.13$, $k_1 = 0.5$, $k_2 = 0.6$. The period of free-running oscillations can be adjusted by changing any parameter value. In addition the effect of light on the circadian oscillator is mediated by a modulating a parameter p , with amplitude ϵ , period T (24 hours) and duration $T/2$:

$$\{ p(t) = p_0 + \epsilon(\Theta(t) - \Theta(t - T/2)) = p(t + T) \quad (2)$$

where Θ is a step function.

The light-dependent perturbation deviates the circadian oscillator from its free limit cycle trajectory during day (example in Figure 1b). If the amplitude deviation is not too large, the stability of the limit cycle ensures that these deviations will decay in the absence of perturbation (following night) while phase changes remain. In such a case, we can define the following Poincare section mapping :

$$\phi_{n+1} = \phi_n - \gamma + V(\phi_n, \epsilon) = F(\phi_n, \epsilon) \quad (3)$$

where ϕ_n is the phase at the time when the light-dependent perturbation is applied during the day n , $\gamma = (T_{FRP} - T)/T_{FRP}$ is the phase change induced by the period mismatch and $V(\phi, \epsilon)$ is the phase change associated with applying the perturbation of amplitude ϵ at ϕ , which corresponds to a phase response curve (PRC). The mapping F has a stable fixed point if there exists ϕ^* that satisfies :

$$\begin{cases} \phi^* = G(\phi^*, \epsilon) \\ -2 < \partial V(\phi^*, \epsilon)/\partial \phi < 0 \end{cases} \quad (4)$$

which corresponds to a phase-locking regime with the existence of stable period-1 limit cycle of the dynamical system described by Eqs. 1. Specifically, the condition $\partial G(\phi^*, \epsilon)/\partial \phi \approx 0$ (equivalent to $\partial V(\phi^*, \epsilon)/\partial \phi \approx -1$) defines a superstable entrainment state. In addition, the entrainment is said to be robust if the stability of entrainment depends little on coupling strength :

$$\frac{\partial}{\partial \epsilon} \frac{\partial V(\phi^*, \epsilon)}{\partial \phi} \approx 0 \quad (5)$$

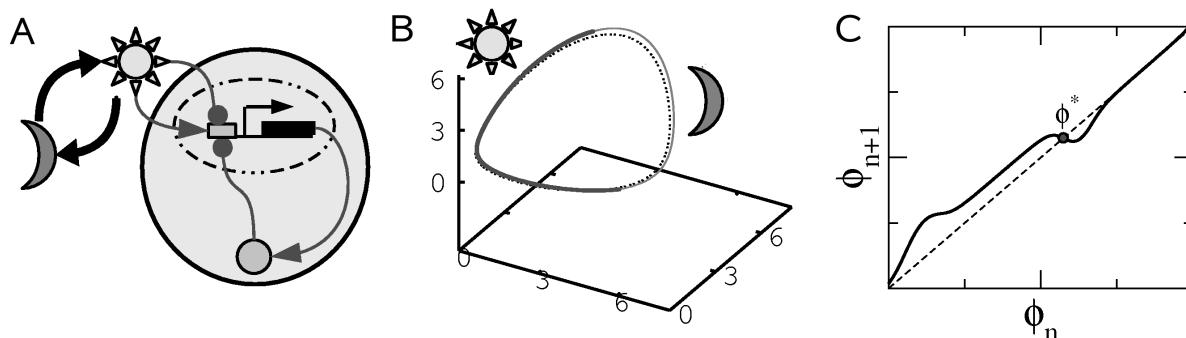


Fig. 1. Representation of the entrainment of a circadian clock. A, Intracellular regulatory circuit based on an autoregulatory gene whose transcription is modulated by daylight. B, Trajectory in the phase space in absence (dashed line) and presence of forcing (thick and thin solid line during day and night respectively). C, Phase return mapping (or phase transition curve) that determines the existence of a stable limit cycle locked with a period ϕ^* to the periodic daylight modulation.

Examples of robust and fragile entrainment

For any parameter set modulated by light, there always exists a range of modulation strengths and of free running periods for which an entrainment state associated with a stable period-1 cycle exists. However, given a certain value of T_{FRP} , quasiperiodicity may occur for too small modulation strength (when $V(\phi, \epsilon) = \gamma$ has no solution) and high-order phase locking may occur for too large modulation strength ($\partial V(\phi_\epsilon^*, \epsilon)/\partial \phi < -2$). In the previous section, we defined a robust entrainment as a phase-locking state whose stability is insensitive to changes in modulation strength. In the following, we show that the robustness of the entrainment state depends actually on which parameters are modulated by light. We consider that the parameter s_M is modulated by light signal (Eq.2). Indeed positive and negative modulations of this parameter provide typical examples of robust and non-robust entrainment.

The phase diagram of Figure 2 shows that both positive and negative modulations of s_M give rise to a stable period-1 cycle in a certain range of modulation strength and period mismatch. However this phase-locked state remains strongly stable for large negative values of modulation strength ϵ if the period mismatch is different from 0 (i.e. $\gamma \neq 0$). The saturation of the function $\partial V(\phi_\epsilon^*, \epsilon)/\partial \phi$ when ϵ increases indicates a robust entrainment according Eq. 5. In contrast, this function does not saturate in the case of positive modulation, which leads to destabilization of the period-1 limit cycle and period-doubling bifurcation, and indicates a non-robust entrainment.

Weak coupling approximation and optimal shape of PRC

If the light-dependent perturbation of the oscillator is small enough, we can assume that the dynamical trajectory remains in a close neighbourhood of the free limit cycle so that only the phase dynamics is perturbed. In the framework of the so-called phase approximation, the effects of perturbations add linearly so that the phase response curve $V_\epsilon(\phi)$ is proportional to modulation strength ϵ :

$$V(\phi, \epsilon) = \frac{\epsilon}{\epsilon_0} V(\phi, \epsilon_0) = \epsilon W(\phi) \quad (6)$$

The phase approximation remains valid for relatively large modulation strengths giving rise to significant phase shift, though the range of validity depends on the phase at which perturbation is applied (Figure 3A). The proportional relationship between the PRC and ϵ is observed for values of ϵ at which $\partial V(\phi_\epsilon^*, \epsilon)/\partial \phi$ saturates (compare Figure 2B and 3A), which suggests that the robustness of entrainment observed in Figure 2 can be interpreted in the framework of the phase approximation. Let consider an oscillator locked with a phase $\phi_{\epsilon_0}^*$ to a periodic modulation characterized by a amplitude ϵ_0 and a period

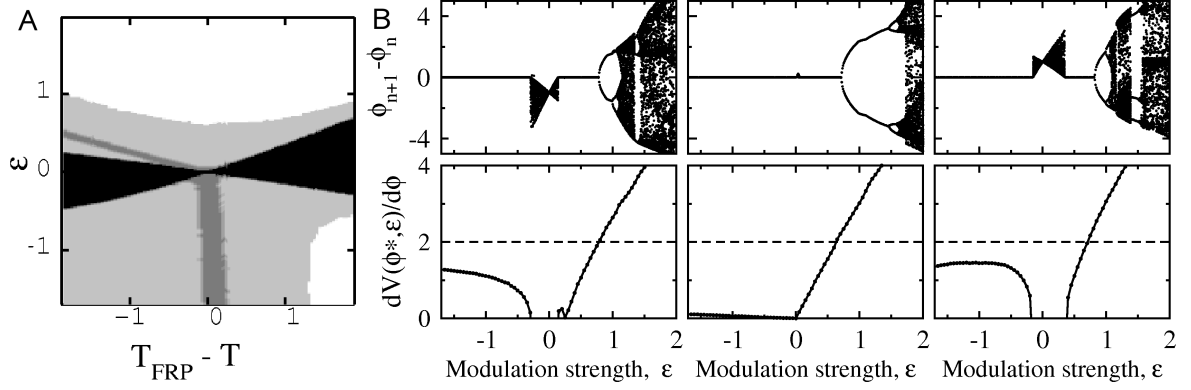


Fig. 2. A, Phase diagram. QP (black) : Quasi-periodicity. P1 (grey) : Phase-locking regime with period-1 cycle. Dark grey indicates a slow local relaxation to the phase-locked state ($|\partial V(\phi^*, \epsilon)/\partial \phi| < 0.2$) Pn : High-order phase locking (period-doubling) and chaos. B, The phase changes between two successive days in the asymptotic regime (top panels) and $\partial V(\phi^*, \epsilon)/\partial \phi$ (bottom panels) as function of ϵ for three values of T_{FRP} (23 h (a), 24 h (b), 25 h (c) respectively)

mismatch γ . From Eqs. 4 and 6, we can compute up to the first order approximation the change of the locked phase induced by a small change in the modulation strength ($\epsilon = \epsilon_0 + \delta\epsilon$) :

$$\phi_\epsilon^* = \phi_{\epsilon_0}^* + \delta\epsilon \frac{\gamma}{\epsilon_0^2 W'(\phi_0)} + 0(\delta\epsilon^2) \quad (7)$$

Combining Eqs. 6 and 7 allows to expand $\partial V(\phi_\epsilon^*, \epsilon)/\partial \phi$ as follows :

$$\partial V(\phi_\epsilon^*, \epsilon)/\partial \phi = \epsilon_0 W' + \delta\epsilon (W'(\phi_{\epsilon_0}^*) - \frac{\gamma W''(\phi_{\epsilon_0}^*)}{\epsilon_0 W'(\phi_{\epsilon_0}^*)}) + 0(\delta\epsilon^2) \quad (8)$$

Therefore a robust entrainment defined by the Eq. 5 requires that the local curvature of the PRC near the locking phase ϕ_ϵ satisfies :

$$\frac{\partial}{\partial \epsilon} \frac{\partial V(\phi_\epsilon^*, \epsilon)}{\partial \phi} = W'(\phi_\epsilon^*) - \frac{W(\phi_\epsilon^*) W''(\phi_\epsilon^*)}{W'(\phi_\epsilon^*)} \approx 0 \quad (9)$$

Defining ϕ_0 the phase satisfying $W(\phi_0) = 0$ and $W'(\phi_0) < 0$, the condition above (Eq. 9) requires that :

1. $W(\phi_\epsilon^*) \neq 0$ (i.e $\gamma \neq 0$) indicating that the modulation period must be different from the free running period.
2. $W(\phi_\epsilon^*) W''(\phi_\epsilon^*) > 0$ indicating that the second derivative of W must be positive (vs negative) for $\phi_\epsilon^* < \phi_0$ (vs $\phi_\epsilon^* > \phi_0$) associated with $\gamma > 0$ (vs $\gamma < 0$).
3. $|W'(\phi_0)|$ is sufficiently small to compensate the vanishing term $W(\phi_\epsilon^*)$ when the modulation strength increases (assuming that the phase approximation still holds).

In addition to these requirements, $|W'(\phi_\epsilon^*)|$ must be sufficiently large so that there exists ϵ not too large satisfying the condition for a strongly stable entrainment $\epsilon W'(\phi_\epsilon^*) \approx -1$.

Figure 3B shows that these requirements on the shape of the function W are satisfied in the case of a negative modulation of s_M . We first define the function $R(\phi) = W'(\phi) - W(\phi)W''(\phi)/W'(\phi)$. Indeed, second derivatives of W are positive for $\phi < \phi_0$ and negative for $\phi > \phi_0$ such that the function $|R(\phi)|$ is close to zero and bound by $|W'(\phi)|$ for a large range of phase in the neighbourhood of ϕ_0 , indicating a robust phase-locked state for both cases of FRP larger and smaller than 24 hours. In contrast, in the case of positive modulation, the sign of second derivative of W (positive for $\phi < \phi_0$ and negative for $\phi > \phi_0$) results in a function $|R(\phi)|$ unbounded by $|W'(\phi)|$, ruling out the existence of a robust phase-locked state.

In the phase approximation, the PRC can be written as a convolution between the phase response function $\mathbf{Z}(\phi)$ and a small light-dependent modulation of the parameter vector $\delta\mathbf{p}(\phi)$:

$$V(\phi, \epsilon) = \epsilon W(\phi) = \epsilon \int_0^{T/2} \delta\mathbf{p}(\phi') \cdot \mathbf{Z}_{\mathbf{p}}(\phi' + \phi) d\phi' \quad (10)$$

$$\mathbf{Z}_{\mathbf{p}}(\phi) = J_{\mathbf{p}}^*(\phi) \cdot \nabla_{\mathbf{X}_{\gamma}} \phi \quad (11)$$

where $J_{\mathbf{p}}^*$ is the transpose of the matrix of partial derivatives of \mathbf{F} with respect to the vector \mathbf{p} evaluated at the phase ϕ of the limit cycle, and \mathbf{X}_{γ} is the stable closed orbit in free running condition. The phase response function is an intrinsic property of the oscillator that does not depend on the properties of the light-dependent modulation of the parameters. A PRC that achieves a robust entrainment requires that the phase response function displays some specific properties. The conditions $|W'(\phi_0)|$ small and $W''(\phi)/W(\phi) > 0$ require that the phase response function $Z(\phi) \approx 0$ for the phase interval during which the perturbation is applied. Such unresponsive interval has been termed dead zone and may be related with saturation or adaptation mechanisms. These mechanisms ensure that the effect of the light-dependent parameter changes on a variable is negligible or is not diffused to other variables in some domain of the phase space.

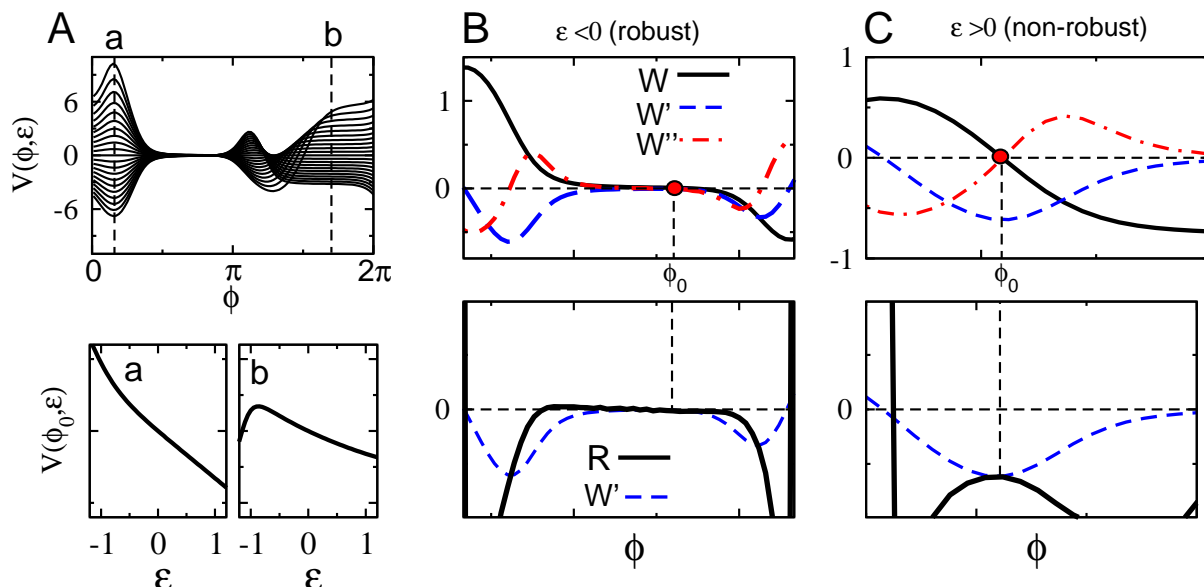


Fig. 3. Relationship between entrainment robustness and shape of the PRC. A, The PRC (top) and the phase response at two specific phase (bottom) for modulation strength ranging from -1.1 to 1.1 . B,C, Local shape of an infinitesimal PRC, $W(\phi)$ and the robustness condition $R(\phi)$ near the locking phase, associated with $\epsilon < 0$ (robust entrainment) and $\epsilon > 0$ (non-robust entrainment).

3 Conclusion

An intriguing characteristic of circadian rhythms is that the free-running period under constant dark conditions is often quite different from 24h in many organisms such as *Neurospora* (≈ 21.5 h), cyanobacteria (≈ 25 h), *Drosophila* (≈ 23.5 h) or mammalian (≈ 24.5 h). In addition, their phase response curves (PRCs) associated with short pulse of light often indicate an insensitivity within certain phase intervals called dead zone. Our study suggests that these two commonly observed properties of circadian clocks can contribute, when combined, to their robustness to daylight amplitude fluctuations. It is possible indeed

that natural selection would evolve an intracellular signaling and regulatory circuit whose kinetics and architecture underlie endogeneous oscillations (i) of a period different from 24 hours and (ii) that are insensitive to specific perturbations in some portion of the limit cycle trajectory.

This interpretation requires to determine the precise relationship between the characteristics of the free-running period and of the PRC in many organisms. It also demands further theoretical investigations to establish whether these properties are compatible with the fact that the clock must also maintain a constant locked phase or adjust it in case of seasonal variation of daylength.

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