A qualitative model of a motor circadian rhythm

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To Arthur T. Winfree, in memoriam

Abstract

In Nature it is possible to observe diverse rhythms. Because of their adaptive characteristics, the circadian rhythms are of major importance and have been the subject of numerous experimental and theoretical studies. In this article, we give a presentation of the main results we have obtained about the motor circadian rhythm along some years of collaboration between biologists and mathematicians. We present a mathematical model simulating changes in frequency, synchronization and amplitude of the circadian oscillation during two developmental stages of the crayfish, namely, the juvenile and the adult stages. We report also some work in progress on the simulation of the phase response curve and on a simplified model of the rhythm.

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1. General properties of circadian rhythms

Among the established periodical processes driven by one (or more than one) biological clock are the circadian rhythms. The basic rhythmic element of circadian rhythms is an endogenous and innate structure that in constant environmental conditions expresses a period of approximately 24 h, and may be affected by a number of external factors.

Besides the presence of a pacemaker, some empirically derived generalizations have been proposed as descriptions of major features of circadian rhythms: a close dependence on light, in accordance to the well known Aschoff’s rule. Aschoff (1960) proposed that the circadian rhythms of diurnal organisms increase their frequency, amplitude and activity period when the external light increases and that, under this same situation, the opposite (diminution in all of these three parameters) occurs in nocturnal species.

Circadian rhythms are readily phase-shifted by single perturbations in the light or temperature (among others) regimes. The character of the phase shift (phase-lead, phase-lag, and even the absence of change), depends not only on intensity and duration of the perturbing signal, but, especially, on the phase at which the circadian cycle was perturbed. This implies that the pacemaker of the circadian system possesses a differential sensitivity to the perturbing stimuli during the 24-h cycle. Immediately after the perturbing
signal has reached the circadian pacemaker, a “transient phase” appears whose characteristics depend, again, on the circadian time when the perturbing signal reached the pacemaker. After some time, periodic activity is resumed with the same values it had before the perturbing signal. According to Pittendrigh and Minis (1964) and Pittendrigh (1981), the ability of circadian oscillators to delay or to advance when a single stimulus acts on the pacemaker is the basis for the entrainment of circadian rhythms.

The presence of entrainment mechanisms is precisely another essential feature inherent to the circadian oscillator. Entrainment of circadian rhythms is defined as the phenomenon whereby a periodic repetition of some external signal, a light cycle, for example, causes an overt persistent rhythm to become periodic with the same period as the entraining cycle (Aschoff, 1965; Pittendrigh, 1965). A very interesting aspect of the entrainment phenomenon is that there is relatively little energy transfer between the entraining signal and the entrained oscillation.

Entrainment is the basis for the coupling between periodic external signals and innate oscillators, phenomenon that is known as external synchronization, which confers the organisms the ability to show a determined phase relation with individuals of the same species, with organisms of different species and with established external signals.

Two circadian oscillators may mutually entrain each other or one of them may unilaterally entrain the other. This allows various functions either to coincide or to avoid coincidence, providing an organism with an internal temporal order between their oscillating systems. In fact, both external temporal order and internal temporal order are the basis of the clear adaptive features of circadian systems (Pittendrigh, 1965). Because of their adaptive role, circadian rhythms have been recognized as a fundamental aspect of the organization of living systems (Aschoff, 1960; Moore and Sulzman, 1981).

The endogenous nature of the circadian rhythms is attested not only by its period, close but different to 24 h, but by the fact that its motion stops (in suitable systems) as soon as oxygen is withheld and promptly resumes when oxygen is returned (Pittendrigh and Minis, 1964).

From unicellular to vertebrate organisms, structures involved in circadian systems have been categorized into three main groups:

(a) The pacemakers or structures with an innate capacity to generate an oscillation that is communicated to any other element.
(b) The pathways for overt rhythm, i.e. the set of elements with capacity to follow the oscillation imposed by the pacemaker (passive oscillators).
(c) The synchronizers or elements involved in the entrainment or synchronization of pacemaker to external signals.

It is important to consider that potentially, any external stimulus can operate like a synchronizing signal (although light is, by far, the most powerful synchronizer of circadian rhythms). This implies that most of the sensorial receptors and the corresponding afferent fibers, often have been identified as components of synchronizer systems.

1.1. Motor circadian rhythm

We have approached the study of motor circadian rhythm in crayfish by two methods: (a) the analysis of the periodic patterns detected in long term recordings obtained during several developmental stages of crayfish. Some experimental results presented here have been previously reported (Fuentes-Pardo et al., 2001; Viccon and Fuentes-Pardo, 1994). Likewise, the mathematical model presented here is the result of a long development that goes from Lara-Aparicio et al. (1993) to Fuentes-Pardo et al. (2001).

1.2. Ontogeny of the motor circadian rhythm

To obtain information about the organization of oscillators underlying the motor circadian rhythm in crayfish, the motor activity patterns were recorded from very early after hatching until to adult stage.
Different stages in the development were detected from the earliest one, during which the animal shows a clear circadian profile but having very peculiar characteristics (see the following paragraphs) until the adult stage, when the crayfish displays the typical motor patterns of this species. Between these extremes, the activity rhythm progressively changes in amplitude, in prevalent circadian periods, and in light dependence, until it acquires the stability of the adult stage.

The experimental design consisted in the individual recording, during 12 days, of the motor activity of unrestrained crayfish kept at constant temperature ($17\,^\circ C$) and controlled illumination (constant darkness (DD) during 4 days, photoperiodic regimen consisting in 12 h of light alternated with 12 h of darkness (LD 12:12) during the next 4 days and constant light (LL) during the last 4 days). The results were grouped according the age of the animal (from 2 days after hatching until adult age) and were expressed by graphics displaying the oscillation amplitude, the oscillation period, and the amplitude of oscillation, examples of these graphics are shown in Figs. 1 and 3, which show the motor activity recorded from a very young crayfish (Fig. 1) and from an adult crayfish (Fig. 3) during the first days of recording under constant darkness. To visualize the effect of illumination on the main property of the circadian rhythm, namely the circadian period, we plotted the activity time length in each cycle versus the day hour at which it is initiated during the 12 days of the experiment (Figs. 2 and 4).

When very young crayfish (2–20 days old) are put under free-running conditions (i.e. under constant environmental conditions), they show an evident, although noisy, circadian pattern in motor activity with periods close to 24 h. A typical motor recording from a juvenile crayfish kept in DD is shown in Fig. 1. Besides the irregular wave shape of cycles, the activity expresses a circadian rhythm with peaks coinciding with external daytime.

If the juvenile crayfish is kept under a different illumination environment, namely a photoperiodic regimen (12 h of light and 12 h of darkness (12:12 LD) or LL), the period, amplitude and activity period (i.e. time when the activity level is higher than 50% of the oscillation amplitude) change, expressing the dependence on light of the circadian rhythm (Fuentes-Pardo et al., 2001). A summary of these changes is shown in Fig. 2. The horizontal bars represent the activity period length of cycles that in, DD, begin each successive day 1 h before ($\tau = 23\,^\text{h}$). Under the 12:12 LD regimen, the activity period grows considerably, although the circadian period is essentially the same as in DD expressing the failure of circadian rhythm to be synchronized by the photoperiodic regimen. Once the animal is returned to free-running conditions, now under LL, the activity period is shortened and the oscillation...
frequency increases ($\tau = 22.5\, \text{h}$). A similar behavior was observed in 22 young crayfish recorded.

From these results, it is evident that very young crayfish behave, according to Aschoff’s rule, like diurnal animals, i.e. they are more active and have higher frequency of oscillation under constant light than in constant darkness. It is also clear that the circadian rhythm does not adjust its period to the period of the LD regimen, namely, the circadian system is not yet able to be synchronized by external signals.

Fig. 3 shows the main aspects of motor behavior of adult crayfish. The motor activity recorded under constant conditions (DD) shows a circadian pattern with activity peaks during night-time.

The motor activity of an adult crayfish recorded in constant darkness shows a prevalent circadian period of less than 24 h ($\tau = 23\, \text{h}$) (resulting from the analysis of 25 specimens). When the animal is transferred to a photoperiodic regimen (12:12 LD), the circadian period is immediately adjusted to 24 h ($\tau = 24\, \text{h}$). If the animal is recorded again in free-running, now under LL, it shows an increase in the circadian period ($\tau = 25\, \text{h}$) (Fig. 4).

From these results, it is evident that, according the Aschoff’s rule, the adult crayfish has no longer a diurnal behavior but a nocturnal one.

From comparisons of the motor patterns in young and adult crayfish we have concluded that, in crayfish, the motor circadian rhythm is always noisy; and that there are, at least, two groups of oscillators involved in its generation and expression. The first group, appearing very early in ontogeny, would probably be located in the cerebroid ganglion (Fuentes-Pardo et al., 2001), and would be responsible for the generation of circadian motor patterns. A second group, appearing later in ontogeny, would be located in the eyestalk, i.e. in the sinus gland (Fuentes-Pardo et al., 2001), and would be responsible for the synchronization of the motor circadian rhythm to external signals.

1.3. Effect of the pulses of light on the motor circadian rhythm

The effect of single light pulses (22 lx for 15 min), applied at different circadian times, on the motor circadian rhythm of adult crayfish, was measured once...
Fig. 3. Motor recording from an adult crayfish kept under DD. The activity peaks coincide with night-time.

Fig. 4. Changes in the motor circadian rhythm in an adult crayfish under DD, photoperiodic regimen (LD 12:12) and LL. When the LD regimen is applied, the circadian period changes exactly to 24 h indicating a perfect synchronization to this regimen. Once the animal is again in free-running, now under LL conditions, the period changes again to 25 h.
The system is noisy.

The juvenile animals have a diurnal behavior and constructed using experimental data, biological principles and our experience in a previous model of the ontogeny of the ERG. In both these models, each oscillator is based on the simplest dynamical system having a limit cycle, which is a planar system with constant angular speed \( \theta = k \), around a fixed center \((c, 0)\) and where the distance to that center, \(r\), evolves by an autonomous law, \( \dot{r} = er(a^2 - r^2) \), having 0 as an attracting limit cycle. The parameter \(e\) regulates the speed with which solutions approach the limit cycle.

The effect of the coupling of the different oscillators, and the effect of external variables on one of them is assumed to affect directly the essential parameters \(a, c, k\). For example, the effect of light on the above oscillator is modeled by making those parameters dependent on a variable representing the light intensity.

We will always take the coordinate \(x\) as the output of the system. From its construction, this system has an attracting limit cycle: the circle of center \((c, 0)\) and radius \(a\) and frequency \(k\). The parameter \(e\) regulates the speed with which solutions approach the limit cycle.

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The experience has shown that this type of coupling with another system, be it an external variable, a source of noise or another oscillator, models more adequately the corresponding biological coupling. For the purpose of synchronization and noise, the effect of external variables is more effective when they affect the center \((c, 0)\) of the limit cycle, so it is there that we concentrate its action, while in the other parameters sometimes we can just ignore any significant effect.

The first oscillator is assumed to represent a neural oscillator with a higher frequency, sensitive to light and, as we indicated in the biological discussion the motor circadian rhythm, is supposed to be of a noisy character. In accordance with the above principle we assume that its main parameters are affected by a random variable.

The second one is a (hypothetical) hormonal oscillator of a lower frequency that affects the first one, is also sensitive to light, but is not noisy. Its effect on the first oscillator is of an inhibitory character. While the first oscillator is supposed to be active during all the ontogeny process, the second one is assumed to appear only in the adult stages. For the purpose

2. The mathematical model of the motor circadian rhythm

We have constructed a mathematical model of the ontogeny of the motor circadian rhythm, by coupling two oscillators, each having a limit cycle, which was constructed using experimental data, biological principles and our experience in a previous model of the ontogeny of the ERG. In both these models, each oscillator is based on the simplest dynamical system having a limit cycle, which is a planar system with constant angular speed \( \theta = k \), around a fixed center \((c, 0)\) and where the distance to that center, \(r\), evolves by an autonomous law, \( \dot{r} = er(a^2 - r^2) \), having 0 as an attracting limit cycle.

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the rhythm had recovered its steady state formerly perturbed by the light stimulus (see Fig. 8). The phase-shifts, advances or delays, produced by the light stimuli were plotted versus the circadian time, CT, of the crayfish and were the basis to construct a “phase response curve” (Pittendrigh, 1965). The phase response curve summarizes the effects produced by single light pulses on the circadian systems and is the basis for understanding the synchronization process. The phase response curve generated with our data shows a sensitivity peak to light at about 4h CT, which is expressed as a phase advance. During the next hours of the circadian cycle, only small delays could be observed (Fig. 8). The irregular shape of the phase response curves has been interpreted as a remnant of the tidal cycle that is occasionally apparent in some activity rhythms, particularly in aquatic species (Enright, 1970). Viccon and Fuentes-Pardo (1994) consider that the motor circadian rhythm of the crayfish results from the presence of groups of oscillators with different light sensitivity underlying the circadian system. It seems plausible to propose that in the motor circadian rhythm there is a different light sensitivity in the nervous oscillator and the hormonal oscillator.

In summary, the main characteristics of motor circadian rhythm in crayfish that we have considered are:

(a) The circadian character of the motor activity is present from early stages of development.
(b) The juvenile animals have a diurnal behavior and the adult animals are nocturnal. Both groups behave according to Aschoff’s rule.
(c) In the early stage, the motor circadian rhythm is not synchronizable and only becomes so in the adult stage.
(d) The system is noisy.
(e) The light sensitivity changes during the circadian period.
of our analysis it is enough to consider the two extremes stages of the ontogeny so we do not include a simulation of the whole maturation process.

The result of the above considerations, and of some biological criteria to exclude other possibilities is the following system:

\[
\begin{align*}
\dot{x} &= -k_1 \cdot x \cdot (x - c_1) \cdot (x - c_2) \cdot (x - c_3) \cdot \sqrt{r(x - c_4) \cdot (x - c_5) \cdot (x - c_6)} \\
\dot{y} &= k_2 \cdot y \cdot (x - c_1) - a_1 \cdot y \cdot (x - c_2) \cdot (x - c_3) \cdot (x - c_4) \cdot \sqrt{r(x - c_5) \cdot (x - c_6)} \\
\end{align*}
\]

The main parameters \(k_1, k_2, a_1, a_2, c_1, \) and \(c_2\) are the frequencies, radii and centers of the corresponding limit cycles when \(s = 0\), are functions of light intensity and \(c_1\) is affected by a random variable. The parameters \(e_1\) and \(e_2\) are the speeds, given the initial conditions, with which the system reaches the steady state.

The parameter \(s\) can take the value 0 or 1. When the value is 0 the system acts like a single oscillator, corresponding to the juvenile stage. When \(s\) is equal to 1, the system consists of two coupled oscillators in such manner that the system has a nocturnal behavior.

This reflects adequately the main qualitative characteristics of the experimental behavior of adult crayfish: nocturnal character, Aschoff’s rule and synchronization. Consider the effect of a periodic light stimulus on young crayfish. In this case we have to take into account only the first oscillator, and a change of light intensity following a pattern consisting of a first stage of darkness (DD), a stage of periodic switch from darkness to lightness (LD) and a final stage of LL. The result of such a simulation is given in Fig. 5, which has the qualitative properties.

In the adult stage, the hormonal oscillator will be active and affecting the nervous one. Once again, considering the three situations DD, 12:12 LD photoperiod, and LL, the oscillator in the adult stage will look as in Fig. 6 which has the qualitative properties. In both Figs. 5 and 6, we have used the following values for the parameters:

\[
\begin{align*}
&c_1 = 0.009, \quad e_2 = 0.05, \quad a_1 = 1 + 0.5L, \\
&a_2 = 1 - 0.5L, \quad c_1 = 0.4L + r(1.8 + 1.3r), \\
&c_2 = L, \quad k_1 = \frac{3}{24} + 0.003L, \\
&k_2 = \frac{2.5}{23} - 0.0058L.
\end{align*}
\]

![Mathematical simulation of the motor circadian rhythm in juvenile crayfish under DD, LD 12:12, and LL regimens. The lower part illustrates the light intensity; the upper part is the evolution on time of the output variable \(y\) when the hormonal oscillator is not active. The specific values of the parameters appear in the text.](image-url)
Fig. 6. Simulation of the motor circadian rhythm in adult crayfish under DD, LD and LL regimens. As before in Fig. 5, the lower part illustrates the light intensity, the upper part is the evolution on time of the output variable $x_1$, using the whole model with two coupled oscillators (hormonal and neural).

where $L = 0$ in DD, $L$ oscillates between values 1 and 0 in LD and $L = 0.3$ in LL, $r$ is a random variable.

3. Work in progress

Recently, we have been simulating the effect of a single light stimulus on the phase of the motor circadian rhythm. Following the application of this stimulus in the different moments of the circadian day, we obtain an estimate of the advance or lag of the phase after a transition period. This is commonly denoted by $\Delta \Phi$.

The comparison between the experimental and simulated results is shown in Fig. 7. Fig. 7 shows a close correspondence between both curves. Nevertheless, it seems necessary to continue the analysis of this simulation, which will probably include a more precise numerical treatment of the simulation of the phase response curve.

We have also started the analysis of a simplified model of the circadian rhythms. For the construction of this model we make some drastic simplifications of the one described above. First of all we consider only the adult stage and the first oscillator, reducing to a single stimulus on it all the action of the second oscillator and of the direct and indirect light stimuli. Then we simplify the first oscillator by assuming that it always runs along a single cycle (a process that is
made possible by a change of variables that absorbs the variations of the center of the circle), so that after the action of a stimulus an instant return to the circle is assumed (in other words, the parameter $e$ is assumed to have an infinite value).

In the case of a photoperiodic stimulus, the model works as follows:

During the darkness stage, the phase moves at uniform speed from A to B. When the light is turned ‘on’ the point is displaced by the arrow and instantaneously returned to C. In the same way, during the light stage it moves uniformly to D and when the light is turned ‘off’ it jumps to E. This process is repeated periodically.

It is yet to be seen which of the qualitative properties of the adult motor rhythm are reflected in this model. Also, this simplified model is closely related to one due to Guevara and Glass (1982) that has been extensively analyzed. This can be used to obtain theoretical conclusions that give reasonable explanations of some of the characteristics of the complete model and hopefully some clues towards a rigorous proof of them. Further study of this and other simplifications of our model are now in progress.

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