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Ultradian Rhythms Underlying the Dynamics of the Circadian Pacemaker

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1. Introduction

Before discussing rhythms and pacemakers, we would like to begin this chapter by quoting Albert Einstein (Einstein, 1934):

> "Our experience hitherto justifies us in trusting that nature is the realization of the simplest that is mathematically conceivable. I am convinced that purely mathematical construction enables us to find those concepts and those lawlike connections between them that provide the key to the understanding of natural phenomena".

Indeed, it seems that nature can be described and understood in mathematical terms. In the context of physical phenomena nobody can deny the usefulness, applicability and relevance of mathematical models. However, as strange and paradoxical as it might appear, the importance of mathematics in biology is still questioned.

In the case of biological systems it is particularly important to discuss whether this confidence is based on facts. Among life scientists it is a widespread opinion that mathematical models just put in very complicated terms what they already knew.

On the other hand this is in sharp contrast with what Darwin expresses in a letter, regretting not having deepened in some basic mathematical principles (Nowak, 2006).

Mathematical models incorporate experimental information, both, quantitative and qualitative. Models have to mimic the observed behavior, but this is not enough. They have to allow for the understanding of the mechanisms underlying the studied phenomenon and also have to be able to make predictions (again, both qualitative and quantitative).

The construction of a mathematical model is not an unidirectional process. The feedback at the different stages of the process is one of the most important and useful characteristics (see Figure 1). A mathematical model provides the necessary elements to compare and even discard different hypotheses, and, in many cases, to propose new experiments (Fuentes-Pardo et al., 2005).

Just to provide a few examples in which the construction of a mathematical model has been essential in the understanding of a biological process we mention the work by Hodgkin and Huxley in the early fifties, on the existence of selective ion channels on the membrane of a neuron. Their model not only enabled them to test this hypothesis, namely, but also to deduce qualitative properties of the transmission of electrical impulses, leading to the notion of action potential (Hodgkin et al., 1952a; Hodgkin & Huxley, 1952b, 1952c, 1952d, 1952e). In

the past few years, important progress has been made in establishing mathematical models at the basis of the understanding of many biological systems: genetic regulatory networks, ecological dynamics, morphogenetic process and so on. At the same time, it is important to mention the fact that some of these models have been proven efficient tools in the design of new treatments or in implementing new therapies (Murray, 2003a, 2003b).
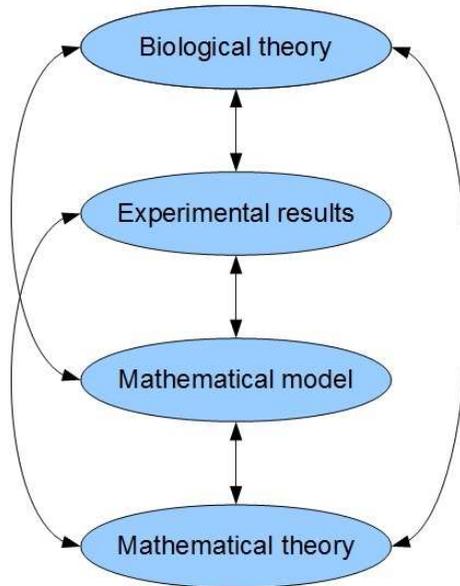

Fig. 1. Relationship among experiments, models, and theories.

As we hope we will make clear in what follows, it is absolutely necessary to incorporate modeling tools in the study of circadian rhythms and their pacemakers. We could use the title of the famous song by George Gershwin "I've got rhythm" to express our conviction that without a solid mathematical framework, we will never have rhythms or at least a good understanding of them.

In the rest of this chapter we are going to explain the main characteristics of a circadian rhythm and the possible organization of the structures in charge of generating the circadian oscillation, i.e. the pacemaker(s). Also we are going to expose the importance of high frequency oscillators (ultradian oscillators) in the generation of the circadian oscillation in the pacemaker and finally we are going to discuss the development of a mathematical model related to the emergence of an oscillation with a period of about 24 hours in a circadian pacemaker and the concomitant phenomenon of synchronization.

The story began a few years ago, when a biologist asked a mathematician to construct a mathematical model for the emergence of the crayfish circadian rhythm. The mathematician asked her what the goal was. The reply can be summarized as follows:

−To understand in depth the observed experimental phenomena in order to explain the mechanisms underlying to the emergence of circadian rhythms in the corresponding pacemaker.

What followed was a close collaboration which involved, among other things, a great deal of patience from both, in order to be able to establish a common language and the possibility of "translating" questions, problems and assertions phrased in a biological way in the mathematical jargon and the other way around. As a result of this collaboration (that still continues) an important point was made:

A focal point to the understanding the generation of the 24 hour oscillation in the circadian pacemaker is a more primeval time-base, one that encompasses both biophysics and biochemistry, both cellular biology and cell reproduction. This is a high frequency clock or ultradian clock (Lloyd et al., 2008).

Considering that the physiological basis for the pacemaker function has to be at the cellular level, it is a fundamental question how individual cells interact among themselves in order to produce a coherent collective behavior that exhibits periodic features. The study of how biological systems organize themselves in time (including the study of biological clocks and biological rhythms), how time and space-time structures and hierarchies arise in living organisms, what we might call space-time morphogenesis, is still far from satisfactory. In what follows we present a concrete attempt to provide a mathematical framework in a specific example.

The chapter is organized as follows. In section 2 we briefly discuss the basic features of circadian rhythms and the circadian pacemaker(s) organization. Section 3 is devoted to the molecular and cellular basis of the circadian pacemaker functioning. In section 4 we provide a short review of the presence of ultradian oscillators that are believed to be responsible for the emergence of oscillatory behavior in the circadian pacemaker. Section 5 deals with the specific case of the crayfish, presenting both experimental results as well as a mathematical model. We present in section 6 a very simple model that might account for the appearance of low frequency oscillations in the (circadian) pacemaker as a result of the coupling of ultradian (high frequency) nonlinear oscillators. We conclude with some general remarks and, more importantly, with open problems and further questions on the subject.

2. What are circadian rhythms?

Many physiological variables change according to some temporal signals like the hour of the day or the seasons during the year. The study of biological rhythms is centered on the understanding of the mechanisms underlying the periodical changes exhibited by the functions of the organisms, as well as the relationship they maintain with external signals.

Among the collection of biological rhythms it can be distinguished those belonging to the *circa* (*lit.* near) class. These rhythms are designed in that way because when periodical signals from the environment are eliminated, the oscillation persists with a period similar, but different from that of the external influence. For example, under constant environmental conditions, the circatidal, circadian, circalunar, and circannual rhythms show a period of approximately 12 hours, 24 hours, 28 days, and 365 days respectively. In natural conditions, however, the external perturbations are present, the period of these rhythms is exactly equal to the period of the external perturbation.

A common feature of *circa* rhythms is that are all the expression of endogenous oscillator networks. In *circa* rhythm there exists a structure, known as the pacemaker, which has the ability to oscillate even under constant environmental conditions. This is true under normal circumstances, when it is part of the organism and even when it has been removed from it.

This structure has also the ability to impose its rhythm to other related units within the organism called effectors.

In this presentation, we discuss circadian rhythms, which are based on a circadian biological clock or circadian pacemaker that has a free-running period of 20-28 hour. This clock is able to synchronize itself to 24-hour environmental cycles, namely the light-dark cycles from the Earth rotation.

Circadian rhythms are relevant to many processes and have received considerable attention. We mention the following two important facts:

1. Their universality. With the exception of bacteria and simpler organisms, all living organisms that have been studied exhibit some kind of rhythm.
2. Their fundamental role in the temporal organization of the physiological and behavioral functions of living organisms.

Although circadian rhythms have been well known since antiquity, the existence of the corresponding biological clocks has only been, relatively speaking, recently accepted (around the sixties). Since then, researchers have gathered evidence in favor of the existence of endogenous biological circadian oscillators or circadian pacemakers.

2.1 Organization of circadian pacemakers

In the early days of chronobiology the notion of circadian organization was quite simple: inside the organism there was a clock (pacemaker) able to be entrained by light via photoreceptors and to impose its rhythm to some structures in the body called effector organs (Zivkovic B., 2006).

This model became difficult to sustain because of new experimental evidence. For example, it was recognized that other signals as temperature, food, or social cues are able to synchronize the pacemaker (Zivkovic B., 2006).

Moreover, experimental results pointed out to the existence of more than one pacemaker inside the organism: in certain environmental conditions circadian rhythms would split into two or more components, each with a different endogenous period; every component observed corresponds to the dynamics of each pacemaker (Zivkovic B., 2006).

In some organisms it appeared that the organization between multiple pacemakers was hierarchical, i.e., there was a master or central pacemaker whose output affects the phase (synchronizes) of the group of peripheral or slaves pacemakers. However in other organisms it appeared that the organization was non-hierarchical, i.e., each pacemaker influences all the others, and as a group, generates an output that drive all the overt rhythms (Zivkovic B., 2006).

The next step was the recognition that the information does not necessarily flow in just one direction: from the environment, via sensory systems, to the pacemakers, to the effector organs. The pacemaker(s) also generate(s) a circadian rhythm in sensory sensitivity. For example the eye may be more sensitivity to light during the night than during the day. Other form of feedback was, for example, the effect of an hormone released from an effector organ over the pacemaker dynamics (Zivkovic B., 2006).

2.2 Synchronization

Other remarkable characteristic of the *circa* rhythms is that, although their oscillations are due to endogenous mechanisms, they have the ability "to follow" the rhythm coming from external signals. An alignment of the period and phase of the *circa* rhythms to the period

and phase of some external rhythms can be observed. This property is known as extrinsic synchronization or synchronization by external stimulus.

In contrast to extrinsic synchronization, in which, as we just mentioned, an external signal plays a fundamental role in inducing a coherent collective behavior, there is another kind of coordinated in time regime. We could call this intrinsic or spontaneous synchronization. In this, there is no distinguished agent.

In order to have this functionality of external synchronization, it has been postulated that the biochronometric system has to possess an internal synchronization system regulating the pacemaker functional units and their interactions with an external synchronization system.

3. How does the circadian pacemaker work?

In several organisms including fungi, plants, worms, crustaceans, insects, mollusca, birds and mammals, circadian pacemakers, centrals and peripherals, have been identified by performing lesion studies, *in vitro* and *in vivo* functional studies, as well as transplant studies. However the way the pacemakers work is far from being understood (Paranjpe & Sharma, 2005).

It is now common to begin from a general assertion the circadian oscillation exhibited by the pacemaker will be describable as a circular list of causes and effects that closes within the bounds of a single cell, even in the most complicated systems as in mammals (Dunlap, 1999).

In an attempt to understand the mechanisms underlying the dynamics of circadian pacemaker, molecular models have been proposed. As in any other self-sustaining oscillatory process, these molecular models are based on negative and positive feedback loops. Clock genes turn on and off because of the proteins they encode. It has been suggested that synchronization of the intracellular clock with the geophysical cycles is due to the degradation of a substance and formation of another, both induced by light (Harmer et al., 2001).

Now, assuming that there is (and that we reasonably understand) "an intracellular synchronizable clock" in each circadian pacemaker cells, it still remains to be elucidated how the molecular time arising from the molecular clock is transferred to the cell in order to promote changes in its excitability and modify the physiology and behavior of the organism. This is a very complicated problem for which, up to the present, there is no adequate model.

Moreover, it was discovered that every cell in the body contain a molecular clock, i.e. every cell is a secondary or slave pacemaker with a molecular machinery very similar to one in the central or master pacemaker. However it is known that if we remove a central pacemaker all overt rhythms finished, while removal a peripheral pacemaker does not; that transplantation of a central pacemaker tissues also transplant the phase and the period of all overt rhythms of the donor to the host, while transplantation of peripheral pacemaker does not do, i.e., the observed period and phase were that of the host; that the central pacemakers cells kept in a dish cycling "indefinitely" while peripheral pacemaker damp into arrythmicity after just a few cycles (Zivkovic, 2006).

Recent results have made evident the important role of electrical signaling at the cellular level. It was shown that in order to maintain a robust circadian oscillation, the molecular feedback loops are necessary but not sufficient. The rhythmic nature of pacemaker cells was traditionally described considering the individual properties of the cells. Nitabatch and cols.

questioned this premise showing that interneuronal communication is necessary to sustain molecular, cellular and systemic rhythms (Nitabach et al., 2002, 2005, 2006).

So, as we can see, the panorama is very complicated.

4. The oscillation in the circadian pacemaker by coupling ultradian oscillations

Research on biological clocks has been centered in the circadian ones. That is why they were frequently employed as a reference point, in particular with respect to the value of its frequency: rhythms that have a frequency less than that of the circadian rhythms were called infradian and those having a bigger frequency were called ultradian rhythms.

As we previously noted, organisms possess a pacemaker that matches physiological functions to the 24 hour cycle of day and night on the Earth. Since 1971 when R. J. Konopka and S. Benzer (Konopka & Benzer, 1971) identified the first clock-gene up to date, there were many significant advances in the comprehension about the mechanisms underlying the generation of circadian rhythms. However the paradigm that mechanisms generating a period of about 24 hours also have a period of 24 hours (that is the fact that every cell in the pacemaker is capable of sustaining a 24 hour oscillation individually in its own activity), every time is less solid because of new experimental evidences that explore the possibility that the circadian pacemaker is an emergent property of circuit interactions. In this section we argue that ultradian oscillators are coupled to yield a composite circadian pacemaker.

As Paetkau and collaborators. pointed out (Paetkau et al., 2006), "the beats" mechanism has been largely ignored because of a number of critical arguments, but most of the criticisms predated gene regulatory model of circadian oscillation.

First of all, 24 hours is a long time in terms of the coherent intracellular dynamics of organisms. We only have to think, for example, of the temporal scale of energy generation, metabolic reactions, transcriptional order, and cell proliferation and development. All these processes make evident the presence and ubiquity of ultradian oscillators in biology: with a period of about 40 minutes, the oxygen consumption and other metabolic processes in *Acanthamoeba castellanii*; similar ultradian clocks were observed in other protists (ciliates and flagellates) and yeast; a 40 minute cycle in general transcriptional activity in yeast; with a period of 69 minutes, respiration in *Dictyostelium*; 3 hour cycles of expression of the mammalian p53 protein; 2 hour periodicity in the expression of the Notch effector Hes1 in cultured cells; a 1.5–3 hour periodicity in the expression of NF-κB signaling molecule in mouse cells in culture, among many others (Lloyd & Murray, 2007; Paetkau et al., 2006).

The complex time structure of organisms requires the synchronized operation of multiple processes in many time domains. To focus exclusively on one time domain and thereby ignoring the full complexity of the system is to risk misconception of underlying mechanisms and to oversimplify the whole phenomenon. Several authors have suggested that at least some circadian pacemakers comprise coupled ultradian ones. Theodosios Pavlidis, in 1969 (Pavlidis, 1969, 1971), proposed the idea of generating slow rhythms from relatively fast biochemical processes by weak coupling of ultradian oscillators. Many other modes of coupling are possible, and the over all period of the whole may be longer or shorter than the free-running period of the longest or shortest component, respectively (Winfree, 1967, 2001).

The presence of 'beats' was noted in several experimental studies and models and it has been suggested as a mechanism for producing circadian oscillations. For example, Patekau

and collaborators (Paetkau et al., 2006) propose a model in which two independent transcriptional-translational oscillators with periods much shorter than 24 hours are coupled to drive a forced oscillator that has a circadian period, using mechanisms and parameters of conventional molecular biology. The *Drosophila* circadian clock can be modeled as a system of coupled ultradian ones based on data showing ultradian peaks in the power spectrum (Dowse & Ringo, 1987). Barrio and cols. (Barrio et al., 1997) developed a theoretical model of ensambles of mutually coupled ultradian oscillators to explain the generation of circadian rhythms in mammals. Díez-Noguera (Díez-Noguera, 1994) propose a functional model of the circadian system based on the degree of intercommunication in a complex system (the circadian pacemaker). The model was conceived to explain previous results concerning the maturation of motor activity in young rats. The maturation of the rhythm shows a predominance of ultradian components just after weaning, which disappear gradually when the circadian rhythm becomes apparent.

5. Experiments and mathematical models from the pacemaker crayfish

Crayfish is a nocturnal freshwater crustacean belonging to the Decapoda Order. With respect to the study of circadian rhythms crayfish has proved to be an excellent model due to its ability to survive in the non-natural conditions of the laboratory.

In this animal it has been detected a great variety of circadian rhythms in both behavior and physiological activity (Aréchiga et al., 1993). An example is the rest-activity (the motor) circadian rhythm and the circadian rhythm of sensitivity to light of retinular cells, (ERG circadian rhythm, (see Figure 2).
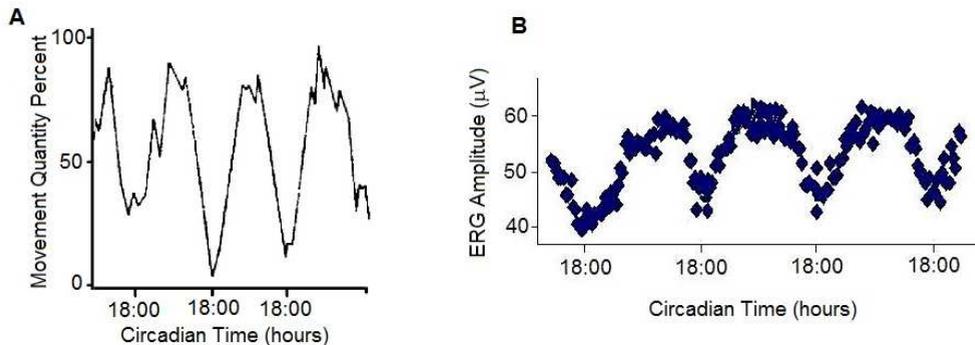

Fig. 2. Crayfish motor circadian rhythm (A) and ERG circadian rhythm (B).

5.1 Pacemaker organization for crustaceans

For crustaceans it has been proposed the existence of a multioscillatory pacemaker system (Aréchiga, 1993; Rodíguez-Sosa, 2008). To define a circadian system of multiple oscillators, identification of its individual components becomes the first task. It must be shown that each of these pacemakers is able to maintain a self-sustained circadian rhythmicity in isolation as well as being in synchrony within the whole circadian system.

Circadian rhythmicity has been demonstrated to be expressed in various isolated structures such as the isolated eyestalk (Sánchez & Fuentes-Pardo, 1977), the neurosecretory X-organ-

sinus gland system and in the retina-lamina ganlglionaris system (Aréchiga & Rodríguez-Sosa, 1998, 2002; Rodríguez-Sosa et al., 1994; Uribe et al., 1998, as cited in Rodríguez-Sosa, 2008). Another possible pacemaker for the crayfish circadian system is the supraesophageal ganglion. Its abblation supresses circadian rhythmicity, however, no experiments *in vitro* have been reported and some rhythms persists after the lesion (Hernández & Fuentes-Pardo, 2001). The way in which these pacemakers interact to generate a synchronous rhythmicity is still unknown (See Figure 3).
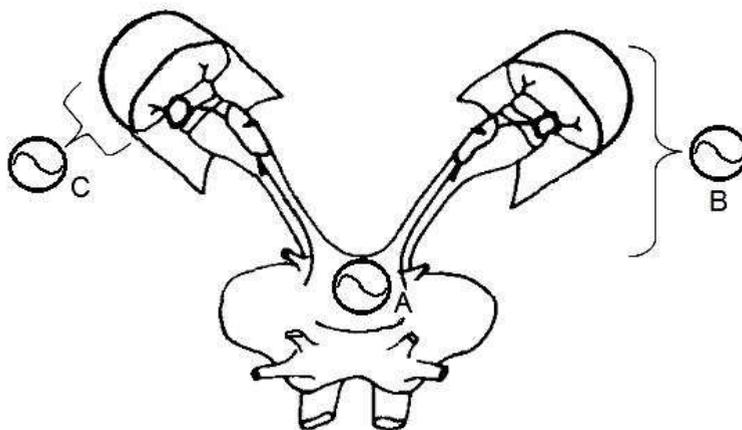
Fig. 3. Schematic representation of the crayfish cerebroid ganglion and eyestalks. A) Cerebroid ganglion, B) Eyestalk and C) Retina are the proposed pacemakers (Modified from Aréchiga et al., 1993).

Moreover, at present, there are no conclusive studies about the origin of the circadian oscillations in one of the pacemakers. An approach to this problem involves the study of the ontogeny of this circadian rhythm.

For the expression of a circadian pattern it is necessary that the anatomical substrate reaches maturity and establishes the necessary structural and physiological relationships between its parts. During development, different structures and functions begin to show some temporal organization that eventually will acquire circadian characteristics. This implies that underlying the sense of time of the organism there are a number of changes in its anatomical and functional organization in the pacemaker. Indeed, the organism exhibits successive changes in period, relative amplitude and activity level of their circadian rhythms during all the ontogeny process. It can be assumed that variation in these parameters involves changes in the structures that participate in the generation of the rhythm, namely, the pacemaker (Fanjul-Moles et al., 1987).

5.2 The presence of ultradian and circadian rhythms in the pacemaker emergent temporal patterns

Ultradian and circadian rhythms have been reported in various studies on crustaceans such as locomotor activity and the photoresponse amplitude in the crayfish retina or cardiac activity (Aguzzi et al.; Fanjul-Moles & Prieto-Sagredo, 2003; Miranda-Anaya et al., 2003b, as

cited in Rodríguez-Sosa, 2008). The mechanisms underlying this interaction have been a matter of discussion in several reports. In what follows we are going to present our experimental results, interpretation and mathematical modeling.

Experimental data show that during crayfish ontogeny, before the complete maturation of its neuroendocrine system, there are only ultradian frequency oscillators in the pacemaker. Because of the influence of some neurosecretions, presumably the pigment dispersing hormone (PDH), high frequency oscillations progressively vanish until they completely disappear and the circadian oscillation appears (Figure 4).
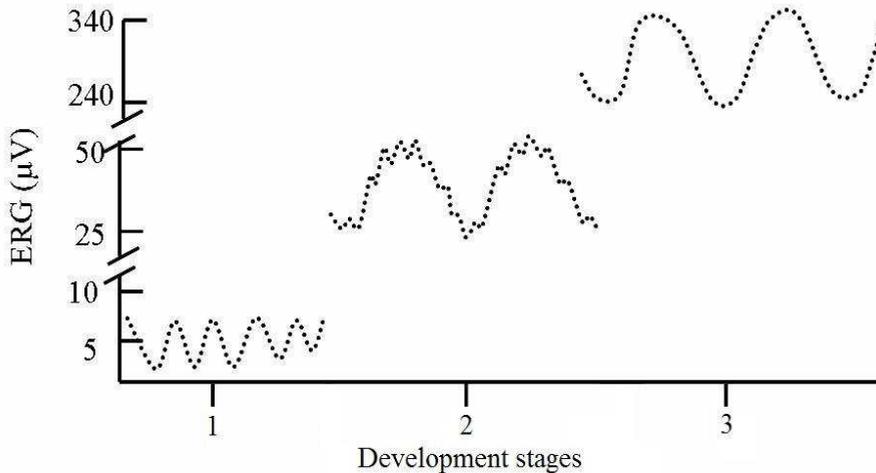

Fig. 4. Ontogeny of the ERG circadian rhythm. In very young crayfish (immediately after hatching), the ERG amplitude is very low (~4 µV) and shows clear ultradian fluctuations with a period ranging from 15 min to 4 h. Four weeks old crayfish express higher ERG amplitude (~50 µV) and, for the first time, the presence of a circadian oscillation. Older animals (around 5 months after hatching) show a progressive increment in ERG amplitude and period length, as well as a progressive disappearance of the high frequency cycles.

The four hour pattern (ultradian oscillations) found in the youngest crayfish is similar to the pattern found in the ERG amplitude recorded from the isolated eyestalk (a presumible pacemaker) of an adult crayfish (Sánchez & Fuentes-Pardo, 1976). In this experimental design, it could be observed that, superimposed on circadian variations in the ERG amplitude, there were high frequency cycles that seem to be correlated with the circadian time, since their amplitude depended on the phase of the circadian rhythm when they appeared.

Another experimental design in which ultradian oscillations appear superimposed to the ERG circadian rhythm was recorded from crayfish deprived of the sinus glands. A plausible interpretation to these results is that the organization of a circadian rhythm is produced as a consequence of the loss of a release from the sinus gland of a neurohormonal substance, presumptively PDH (Moreno-Sáenz et al., 1986).

In addition to the past scenarios, we have observed the presence of ultradian rhythms in experiments in which we used deuterium oxide (D_2O) in long term recordings of the ERG

(Fuentes-Pardo & Moreno-Sáenz, 1988). The authors analyzed the effect of deuterium oxide on the circadian oscillations obtained from both intact animal and isolated eyestalk. Results showed a direct relationship between the D_2O dose and the lengthen of period in both circadian and ultradian cycles. It was proposed that the lengthening of the circadian period is due to the effect of D_2O upon the high frequency oscillations. The lengthening produced by D_2O could result from the diffusion of this substance to all the cells, tissues and organs of the organism, particularly to the cells involved in the generation and expression of the ERG oscillatory activity. These cells would actually be the oscillators that reduce their oscillation frequency produced by the physicochemical changes of D_2O. The results allow us to propose that D_2O affects the oscillatory machinery by its well-established general property of slowing biochemical reaction kinetics due to the "heavy isotope effects".

Finally, the last scenario in which we have observed circadian and ultradian oscillations is when the ERG circadian rhythm recorded from a crayfish kept under constant darkness is perturbed by the presence of a light stimulus, this induces a phase change, an advance or a delay, that can be detected once the rhythm returns to a steady state. The sense and magnitude of change depend on the circadian time of stimulus application. It is worthwhile noticing that immediately after the light application, the ERG circadian rhythm shows a transitory stage characterized by the presence of irregular high frequency (ultradian) oscillations; the characteristics of these, particularly phase as well as the circadian moment when the stimulus was applied, seem to determine whether the rhythm will show advance or delay in the steady state (Fuentes-Pardo et al., 2008).

It is natural to conjecture that the ultradian rhythms observed in each and every one of the above experimental scenarios (ontogeny, sectioned eyestalk, sinus gland deprivation, D_2O and application of a light stimulus) appear as a consequence of the failure (or decreased velocity in the case of D_2O) of the release of PDH. The circadian rhythm in the pacemaker's crayfish is generated by weak coupling of ultradian oscillators between cells. Ultradian oscillation would become apparent under weak coupling or in absence of coupling. The pacemaker period would be a function of the coupling of the ultradian oscillators, increasing as the coupling decreasis.

In the next section we explore this possibility with a simple model.

6. Circadian behavior in the pacemaker emerging from the coupling of ultradian oscillators

As we saw in the previous section, in the ontogenesis of the crayfish circadian rhythm the experiments showed a global ultradian rhythm in very young individuals that evolves into a global circadian rhythm with an intermediate stage in which both rhythms are superposed. How could one construct a pacemaker model based on the coupling of many ultradian oscillators?

The coupling strength of the oscillators in the pacemaker should vary during the development of the rhythm: In the early stages, the coupling should be weak enough to preserve the ultradian character of the resulting output, but strong enough to synchronize all the oscillators (otherwise the result would be a practically constant signal, being the sum of many unsynchronized clocks). At the other end of the process, in the adult stage, the strength of the coupling should be able to produce a global circadian result. And it should also reproduce the intermediate step where both ultradian and circadian rhythms coexist!

In a first stage we have taken a different option, without denying that it would be interesting to search for a simple model whose individual oscillators and modes of coupling evolve naturally in the above manner. Our approach consists in assuming the existence of a well-defined ultradian rhythm in the pacemaker from the beginning of the ontogenesis and of a circadian rhythm that emerges gradually, but whose circadian character is well defined from its first appearance. We concentrate on modeling the relations between the two. This does not exclude the possibility that the circadian oscillator might be the result of the coupling of many ultradian oscillators (c.f. the following section).

The results of this approach can be found in (Lara-Aparicio et al., 1993; Fuentes-Pardo et al., 1995, 2005). The mathematical model has proved useful for the understanding of several characteristics of the circadian rhythm.

At this time we are asking if the pacemaker temporal pattern observed in the crayfish could be the result of the coupling of ultradian oscillators. In order to mimic the described behavior we have to take into account the maturation (hormonal) effect. So in the next, we are going to show how in a simple model, low frequency oscillatory behavior in a pacemaker can be obtained from the coupling of its components, each one being a high frequency oscillator.

In order to fix ideas we will consider that the systems consists of four nonlinear oscillators. In the absence of any coupling, we will simply assume that the oscillators are identical and whose dynamics is determined by the same function f.

In mathematical terms we have a system of four second order nonlinear differential equations in which x_i (i=1,...4) represents the "position" of the i-th oscillator:

$$\ddot{x}_1 = f_1(x_1, \dot{x}_1) + a_{12}x_2 + a_{13}x_3 + a_{14}x_4$$
$$\ddot{x}_2 = f_2(x_2, \dot{x}_2) + a_{21}x_1 + a_{23}x_3 + a_{24}x_4$$
$$\ddot{x}_3 = f_3(x_3, \dot{x}_3) + a_{31}x_1 + a_{32}x_2 + a_{34}x_4$$
$$\ddot{x}_4 = f_4(x_4, \dot{x}_4) + a_{41}x_1 + a_{42}x_2 + a_{43}x_3$$

In other words, even when in principle the f_i could be different, we take them as identical for the sake of simplicity. Moreover, the terms involving the coefficients a_{ij} (linear terms) represent the coupling among the different oscillators in the pacemaker. The coupling is directly related to the PDH presence (see Section 5.2). It is important to point out that in many physical systems, such as masses joined by springs or interacting pendulums the nature of the coupling is relatively well known. On the other hand, in the case of cells, the underlying interactions among cells is due to rather complex communication mechanisms, that in fact, constitute the subject of very active research.

In principle, we don't impose any specific functional form for f although later on we will consider it to be a van der Pol oscillator.

We now adopt the specific functional form for the terms in the equation responsible for the oscillatory dynamics:

$$f_i(x_i, \dot{x}_i) = \mu(1 - x_i^2)\dot{x}_i, \ i = 1,...4.$$

In other words, each separate component is a van der Pol system.

In the following pictures (Figure 5), the behavior of a van der Pol oscillator is depicted. The illustration is taken from Weiisstein, Eric W. "van der Pol Equation." from MathWorld--A Wolfram Web Resource (http://mathworld.wolfram.com/vanderPolEquation.html) and we have left the notation as it appears there, i.e. x and y are taken as independent and dependent variables respectively instead of t and x, so that the van der Pol equation reads

$$y'' - \mu(1 - y^2)y' + y = 0.$$

Moreover, using the language of dynamical systems, each component has the structure of a relaxation oscillation which is very robust (a stable limit cycle). Essentially this means that if the system is perturbed it will recover its oscillations.

After writing the system as a collection of eight equations of first order, rather than four of second order we can plot the positions and velocities of the oscillators

In Figure 6 we show a simulation for this system in which both velocities and positions are represented. Notice that after a transient state, in which individual high frequency oscillations are observed, a regime in which low frequency oscillators appears.

It would be worthwhile, in future to prove if the different perturbations to the pacemaker that we observed experimentally can be modeled by our coupled high frequency oscillators.
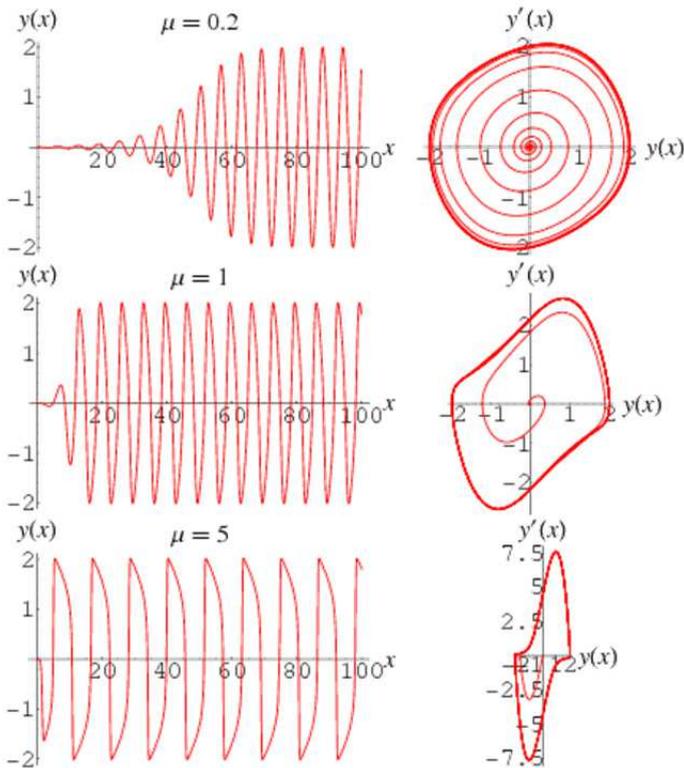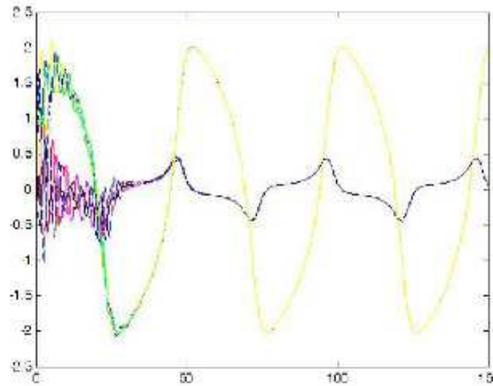

Fig. 5. Van der Pol oscillator.

Fig. 6. Coupled oscillators.

7. Conclusion

The study of the emergent dynamics of circadian pacemaker illustrates the utility and even the necessity to develop mathematical models to understand the biological systems. Remarkable also is the interaction that can be observed among a biological theory, the experimental results, the mathematical model, and the mathematical theory.

In our work, mathematical models have been fundamental to understand the mechanism underlying to some phenomena and suggest us new experiments. When we propose and study the mathematical model to describe the ontogeny of crayfish circadian rhythm, immediately emerged many questions about the origin of circadian rhythms and the possible compatibility with the different proposals and explanations found in the literature.

At this moment our models have a qualitative character, but it is possible to refine them in order to obtain quantitative answers to some questions.

It is important to remember that, in spite of we want to make a simple model, the biological systems exhibits an extraordinary diversity and complexity. That is because is really hard to consider that the modeling process is finished.

8. Acknowledgments

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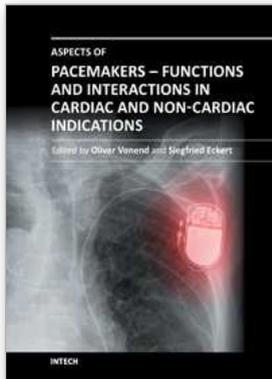
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Outstanding steps forward were made in the last decades in terms of identification of endogenous pacemakers and the exploration of their controllability. New â€œartificalâ€ devices were developed and are now able to do much more than solely pacemaking of the heart. In this book different aspects of pacemaker â€" functions and interactions, in various organ systems were examined. In addition, various areas of application and the potential side effects and complications of the devices were discussed.

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