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A BRIEF BIOMATHEMATICAL HISTORY OF CIRCADIAN RHYTHMS: FROM WIGGLESWORTH TO WINFREE

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Abstract. As we know, Arthur Winfree passed away a relatively short time ago. His memory motived us to investigate his great influence in the biomathematical development of one of the most important branches of Biological Sciences, i.e. the Chronobiology. In this paper we present the "giants", paraphrasing Newton, on whose shoulders Winfree stood up seeing farther about biological rhythms, as well as some of his contemporaries. We can appreciate Winfree's influence in the field observing that when he decided change his interest towards other fields, there appeared a certain fading in the number of people working in circadian rhythms from a mathematical point of view. However, the solid basements built by the founders of this branch of Science have allowed that nowadays not only high quality papers of biomathematical contents continue to appear in important journals, but also papers of pure mathematical problems inspired by circadian rhythms. In fact, the building of the interface of Biology and Mathematics has been reinforced in such a manner that both disciplines have mutually benefited. Finally, we present a section in which it is possible to appreciate a few articles that have appeared after Winfree.

1 Introduction When we learned that our dear professor and friend Arthur Winfree passed away, being aware of the great importance of his contributions to biomathematics, particularly in biological rhythms, we planned to investigate the evolution of the study of circadian rhythms, whose mathematical study began with Wigglesworth, and which received a formidable impulse with Winfree who initially studied biology and then mathematics. In our investigations on rhythms, we form a group of mathematicians and biologists which studied several of Winfrees's papers that surely have influenced the construction of our mathematical models.

2 The mathematical precursors

2.1 Kalmus and Wigglesworth In a Symposium on biological rhythms carried out at Cold Spring Harbor in the United States of America in 1960, several mathematical models were presented in order to simulate properties of the circadian rhythms. Among these papers, one of the most important entitled *Shock excited system as models for biological rhythms* was presented by Kalmus and Wigglesworth (a biologist and a mathematician, respectively). In this paper the authors associated a limit cycle to a circadian rhythm.

The meaning of a phase portrait and a limit cycle was illustrated through the building of a simplified model of two "substances" whose concentrations depend on the time t, $x(t)$, $y(t)$ and the rate of changes of the present total quantities are specified in the following manner:

1. substance y is transformed into substance x at a rate $K(y-x)$, say, depending only on the difference $(y-x)$;

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2. substance y is also renewed (from some "reservoir") at a rate $F(x)$, say, depending only on x;
3. substance x decays at a rate $G(x)$, say, depending only on x.

If the total amounts present are Ax, By, where A and B are constants, we have, by considering the net rates of change,

$$\begin{aligned} A\frac{d(x)}{dt} &= K(y-x) - G(x) \\ B\frac{d(y)}{dt} &= -K(y-x) + F(x). \end{aligned} \tag{1}$$

In order to get a differential equation known in non-linear mechanics, the system can be reduced to a second order linear equation:

$$\frac{d^2(x)}{dt^2} + \left(\frac{(A+B)K}{AB} + \frac{G'(x)}{A}\right)\frac{d(x)}{dt} + \frac{K}{AB}\left(G\left(x\right) - F\left(x\right)\right) = 0,$$

where $G'(x) = \frac{dG(x)}{dx}$.

Furthermore the authors provided conditions for the existence of a stable periodic solution for equation (1). Finally they applied the results to two hydraulic models and built the geometrical phase paths.

2.2 Klotter In these historic *Proceedings* there appeared two articles of K. Klotter. One of them entitled *General properties of oscillating systems* is of a great importance since on the basis of the harmonic oscillator properties and some generalizations, the author determines several concepts, essential to build and understand the mathematical simulation.

Klotter associates autonomous oscillations to "endogenous" oscillations and non-autonomous oscillations to "exogenous" oscillations. Furthermore, he gives a definition of a free system as one that can not oscillate by itself indicating that self-supported oscillations appear only in systems with the capacity of self-oscillation.

In Biology the frequency or period of oscillations is called in diverse forms: Pittendrigh called them *natural oscillations* or free running oscillations. Aschoff called them *spontaneous oscillations.*

According to Klotter, another way to consider endogenous oscillations is to observe if the dissipated energy in a cycle is restored from a source of non-periodic energy or not. If so, the oscillation is autonomous and it is considered as a self-oscillation. For example, in the pendulum of a clock, an energy spring restores the lost energy in each oscillation. This is shown in the Figure 1. According to the quantity of dissipated energy during a cycle, respect to the total energy of the system, self-sustained oscillations are restored oscillations or relaxation oscillations.

Such systems have the property that the amplitude of the oscillation is almost constant and the frequency is almost the same as the natural frequency of the free pendulum.

Respect to relaxation oscillations we can consider as an example the oscillator showed in Figure 2 in which the components are a neon diode, a capacitor, a source of energy and a switch connected as shown and whose graph is like that appearing in the second part of the same figure. As we know, for the study of non-linear oscillations the van der Pol oscillator is fundamental. Its mathematical model is given by the equation:

$$\frac{d^2(y)}{dt^2} - \varepsilon(1-\alpha^2y^2)\frac{d(y)}{dt} + \omega^2 y = 0.$$

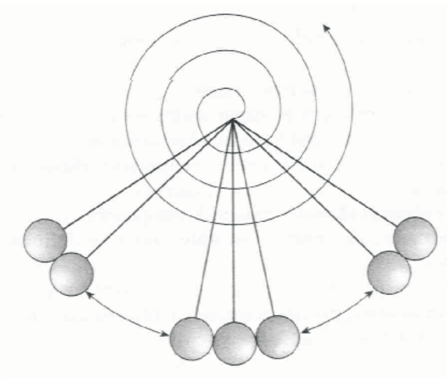

Figure 1: Example of a “recovered” oscillation.

This equation has a solution whose self-sustained oscillation is of two forms:

1. if $\varepsilon \leq 1$ then the oscillation can be recovered, say, as pendulum.
2. if $\varepsilon > 1$ then the oscillation is of relaxation.

Non-autonomous systems are defined in the following way:

If there exists a source of non-constant and periodic energy which depends on time and that acts on a system, then it is called “non-autonomous” and the oscillations are “forced”.

The appearing cases are different and they depend of the forcing frequency acting on a non self-oscillating or on an self-oscillating system.

From the classification that Klotter made inherent to oscillatory systems, one can find mathematical models simulating several aspects of the circadian rhythms.

2.3 Wever One could say that in a form almost parallel to Klotter and Wigglesworth, Wever published papers referring to circadian rhythms, but applied to human beings. From experimental works made by himself at Max Planck Institute of Psychiatry, he built a dynamical model based on a forced oscillator of the van der Pol type as:

$$\frac{d^2(y)}{dt^2} + \varepsilon(y^2 + y - a)\frac{d(y)}{dt} + \frac{dy}{dt} + gy^2 = \frac{d^2(z)}{dt^2} + \frac{d(z)}{dt} + z,$$

where y represents the biological variable under consideration, for example, activity body temperature or the hormonal concentration, and z represents the external control stimulus like the light intensity, the force of social contacts or the intensity of an electric field. This variable can be constant or a function of time t; α, ε and g are parameters which vary depending on the kind of species. Mathematically, ε represents the speed, with initial conditions given, at which the system reaches the stable state.

Figure 3 shows seven graphs in which one can observe how the light influences the circadian rhythms. We will show later a graph where using the mathematical concept of isochrones, Peterson verified Wever’s conclusion.

Wever published 55 papers on this argument ranging a period from 1962 to 1985.

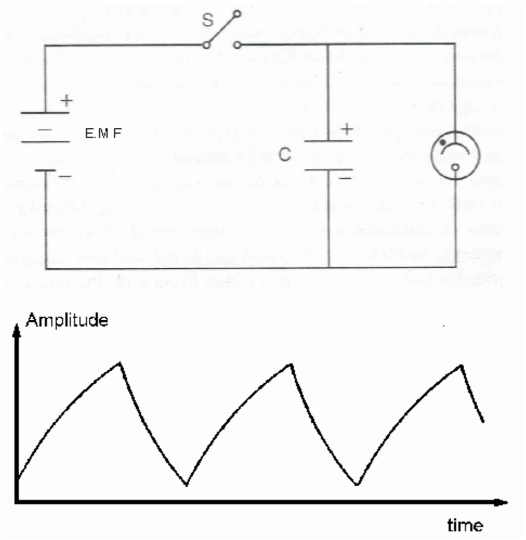

Figure 2: A relaxation oscillator and its output graph.

2.4 Pavlidis Another important mathematician precursor is T. Pavlidis, electrician engineer, who studied diverse aspects of the biological rhythms building furthermore, as far as we know, the first topological model of a circadian rhythm which can be encountered in his treatise titled: *"Biological Oscillators: Their Mathematical Analysis"*. In such a book it is possible to find all the biological and mathematical foundations of the circadian rhythms.

2.5 Winfree There are more investigators that have contributed to the development of the field, until Winfree appears with a profound and vast production. His production makes him a leader of the field as it is shown by his more than 1900 citations mentioned in his fundamental book *"The geometry of biological time"*, published by Springer Verlag in 1979.

Many of the first papers written by Winfree express his interest in the comprehension of some obscure aspects of the circadian rhythms. Thus, he applied mathematical analysis, electronic experiments and digital computer simulation to explain the nature of the periodic processes and the kind of interactions involved in them. He also studied the modes of temporal organization resulting from weak interactions in a population of innately oscillatory devices. Winfree concluded that communities with self-entraining capability may exist within individual metazoan plants and animals as the basis of the temporal organization of their physiological process.

As far we know Winfree was one of the first authors that made possible the mathematical simulation of some characteristics of circadian rhythms. In the article called *Biological Rhythms and the Behavior of Populations of Coupled Oscillators* (1967) Winfree wrote: "The impressive variety of biological rhythms leaves no doubt that autonomously periodic processes contribute to the coordination of life-processes". The question here raised is, "What modes of temporal organization -if any- could result from weak interactions in a population of innately oscillatory devices (e.g. electronic oscillators, secretory cells, spontaneously active neurons, or individual animals)?" For populations of "generalized relaxation oscillators", threshold conditions are discovered for mutual synchronization in any of a variety of modes.

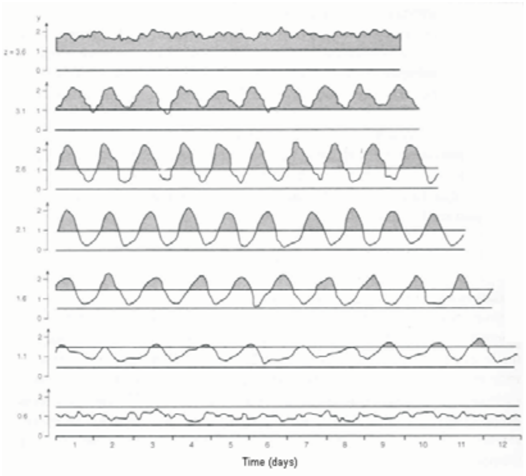

Figure 3: Solutions of the equation with different values of the external force z, overlapping in each one, the very random fluctuation. Both diagrams, upper and bottom, represent solutions out of the oscillation range. Ten successive cycles of each solution are represented. Shadow areas correspond to "active" states (above the fixed threshold in y = 1). [See Wever 1987].

An important work of Winfree was done on the phase resetting of the circadian rhythm of pupal eclosion in populations of the fruit fly *Drosophila pseudoobscura*, in response to a single discrete perturbation (Winfree 1967, Winfree 1970, Winfree 1972 and Winfree 1974) extending and systematizing the observations of Pittendrigh and his coworkers. Using a dim blue light as the resetting stimulus, Winfree plotted the daily eclosion time as a function of the duration of the stimulus at the time at which it is given. The resulting three dimensional graph resembles four turns of a corkscrew linking tilted planes. The corkscrew axis points a unique stimulus time and duration, small variations of which result in radically altered phase resetting. Approximations to this stimulus result in unusual broadening of the daily eclosion peak, even to the extreme of obscuring the circadian rhythm. In other experiments (Winfree 1972), Art found that the eclosion circadian rhythm of populations of *Drosophila* reared at constant temperature, resets its phase by up 12 hours with a brief pulse of light. The new finding is that the rhythm´s susceptibility to resetting depends on the previous time the population was maintained in darkness. This finding is compatible with: a) a linear kinetic model involving a bleachable pigment which very slowly regenerates in darkness and b) any of a diversity of models involving a non-periodic or damped oscillation. Winfree reported that a light stimulus of a critical strength applied at a critical circadian phase could essentially stop the circadian clock in *Drosophila pseudoobscura* by resetting the circadian oscillator close to its singularity (a phaseless position at which the amplitude of circadian oscillation is zero). Since then, similar observations of attenuated circadian amplitude in response to critical stimuli had been limited to unicells, insects and plants. Jewett et al (1991) found that the phase of the human circadian pacemaker can be inverted using an unconventional three-cycle stimulus to investigate whether critically timed exposure to a more moderate stimulus could drive that oscillator towards its singularity. He reported that exposure of humans to fewer cycles of bright light, centered around the time at which the human circadian pacemaker is most sensitive to light-induced phase shifts, can markedly attenuate endogenous circadian amplitude. In some cases there is an apparent loss of rhythmicity, as expected to occur in the region of singularity.

In the following decade, the interest of Winfree in circadian rhythms was oriented toward the study of sleep as well as other aspects related to periodical human behaviors (Winfree 1977, Winfree 1982a, Winfree1982b, Winfree 1982c, Winfree 1987 and Winfree 1991). Example of this is his work with Kawato, Strogatz and other authors about organization of oscillators underlying cycles of sleep-wakefulness (Kawato et al 1982 and Strogatz 1990) and the action of some substances on circadian rhythms (Winfree 1986). Winfree proposed that sleep duration depends mainly on the timing of prior sleep onset relative to a rhythm of 24- to 25-h duration. The author emphasizes four additional points: 1) that the dependence sometimes includes a remarkable discontinuity, 2) that such dependence is characteristic of a rhythmically modulated threshold process; 3) that the rhythm's period gradually changes in some experiments; and 4) that no comparable regularity has been detected in the timing of sleep onset. This last impugns the reliability of models that treat sleep onset and wake onset as complementary but comparable processes.

Some authors as Kawato and Suzuki (1978), Strogatz (1990) and Jewett (1991) apply the proposals of Winfree, generated from his work on *Drosophila*, to study different aspects of the cycle sleep-wakefulness in humans.

Biological oscillators are commonly subjected to a single brief stimulus to perturb the ongoing rhythm. After stimulation, the oscillator can recover although its phase may be advanced or delayed relative to the original cycle. A single transient perturbation can also stop the ongoing rhythm. Arthur Winfree systematically classified these responses to brief perturbations, as determined by the strength of the stimulus, and the phase within the cycle at which the stimulus was given. A natural question arises from Winfree's work. Are certain stimulus shapes better than others at achieving a desired effect? These questions led to Forger and Paydarfar (2004) to the analysis of phase space geometry, calculus of variations, and analysis of responses to noisy perturbations. The authors concluded that the first two methods have limited applicability in biology because they require a detailed mathematical description of the oscillator. The third method is applicable to any oscillator without mathematical prerequisites. They validate this method by finding optimum stimuli that start and stop repetitive firing in a neural pacemaker model, and the optimum light stimulus for resetting the circadian rhythm in a model of the human circadian clock.

Results of Jewett (1991) and his colleagues that bright light can induce strong (Type O) resetting of the human circadian pacemaker, show that the human clock is more responsive to light than has been previously thought. The interpretation of their results is subtle, however, because of an unconventional aspect of their experimental protocol: they measured the phase shift after three cycles of the bright-light stimulus, rather than after the usual single pulse. A natural question is whether the apparent Type O response could reflect the summation of three weaker Type 1 responses to each of the daily light pulses. Strogatz shows mathematically that repeated Type 1 resetting cannot account for the observed Type O response. In this sense the resetting response of humans is similar to that of *Drosophila*, *Kalanchoe*, and *Culex*, and is more complex than that predicted by conventional PRC theory. To describe the way in which light resets the human circadian pacemaker, one needs a theory that includes amplitude resetting, as pioneered by Winfree and developed for humans by Kronauer (Strogatz 1990 and Winfree and Strogatz 1984).

One of the most important article Winfree wrote is "24 hard problems about the mathematics of 24 hours", published by the American Mathematical Society (1979), and can be considered his legacy on circadian rhythms.

We would like to emphasize some facts about circadian rhythms made known by Winfree, which among others helped us to build a mathematical model:

1. Namely, each oscillator reaches its maximum at different moments.
2. In a suitable constant environment, the "clock" of the organism has an autonomous kinetics. That is why the rate of change of each state variable (for example, some biochemical concentrations) is given as a function of the rest of variables. This means that one works with a system $\frac{dx}{dt} = R(x, P)$, where P represents the output of the external parameters from a standard configuration.
3. That system has only a unique attractor limit cycle and returns to it quickly after the most of the transitory perturbation types.
4. A stimulus (as the exposition to light or the cooling during the night) is a change in the parameters P. Many times in laboratory experiments $P(t)$ is equal to zero until a time t_0 from which is constant and, after an interval of time M, it is equal to zero again.

(The following paragraph is of fondamental importance to understand some aspects of the biological evolution)

5 … As far as I know, into the transients one can find the essence of the temporal adaptation and the first aim of the evolutive change. This is due to the fact that circadian rhythms work in an autonomous form only in the laboratory. In Nature they are perpetually governed by the succession of the day and the night, in such a way that its behavior is a little more that a succession constantly renewed of "transients". The dynamics of transients has been universally ignored.

6 … the organism is a lattice of many similar oscillators from which we only observe the collective behavior by means of rough measures of the complete organism …

7 According to point 5, these many individual oscillators maybe not always interact in an adequate manner as for they maintain in a strict synchrony in a common attractor limit cycle. This internal distribution of the phase could be a vital characteristic of the biological rhythms.

Namely, each oscillator reaches its maximum at different moments.

(The above consideration gives a precision to the Aschoff's law which refers to a global circadian behavior although not local, see Aschoff 1960).

We have the feeling that when Winfree, a biologist by training , discovered the "existence" of topology, he foresaw the tremendous potential that this field of mathematics would have in the study of biological phenomena. That is why when dealing with fundamental topics of biological clocks like synchronization, phase resetting and entrainment, Winfree settles out an old source of confusion by defining these concepts with mathematical precision.

One of the most important concepts appearing in the same treatise is the concept of "isochrones". Winfree says that in many biological problems it is required to know the difference in time between two independent oscillators when both are near enough to a common attractor limit cycle "knowing" only an initial state of each oscillator in the attractor basin but not in the attractor cycle.

He affirms that there exists a foliation of the attractor basis in a family of 1-parameter manifolds named isochrones with the following property:

All the states in a determinated isochrone converge in the sense that the rhythms appearing from such initial conditions, eventually, are undistinguished.

Then one says that all these initial conditions have the same “latent phase” ϕ, which is a map of the space of states of the oscillator on the circle of the phases:

$$\phi : R^u \to S^1$$

In the Figure 4, appearing in an article of Peterson (1980), it is shown a simple graph of a system of isochrones in which each straight line corresponds to states in the same isochrone respect to the same light intensity, implying that their phases are the same.

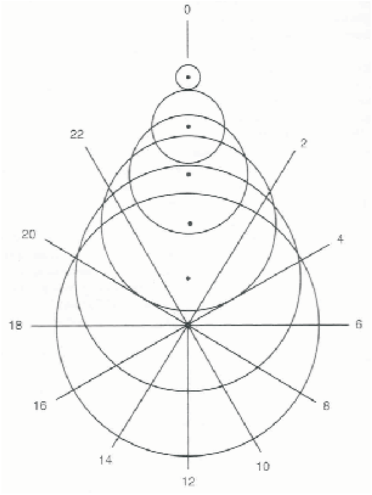

Figure 4: Each partition corresponds to states lying in the same isochrone with respect to a certain light intensity. The greater circle is the limit cycle corresponding to darkness. The ascending succession of limit cycles tends to a point in the higher part, which corresponds to light intensities growing to infinity. Observe that isochrones converge to a critical phaseless point. In this case, one observes that in any time there exists only one limit cycle and a system of isochrones determined by light intensity.

About isochrones, Winfree makes, among others, the following questions:

- Is it possible to say something more about isochrones of the arbitrary kinetics of the limit cycle, at least near to the equilibrium?
- Is it true that every isochrone approximates arbitrarily to every point of the manifold without phase or to its boundary?
- How can one calculate analytically the isochrones?

As an example of Winfree’s enthusiasm in topology we can see the following fragment of the article entitled *Stability of knots in excitable media* (Sutcliffe PM and Winfree AT 2003):

“Through extensive numerical simulations we investigate the evolution of knotted and linked vortices in the FitzHugh-Nagumo model. On medium time scales, of the order of a hundred times the vortex rotation period, knots simultaneously translate and precess with very little change of shape. However, on long time scales, we find that knots evolve in a more complicated manner, with particular arcs expanding and contracting, producing substantial variations in the total length. The topology of a knot is preserved during the evolution, and after several thousand vortex rotation periods the knot appears to approach an asymptotic state. Furthermore, this asymptotic state is dependent upon the initial conditions and suggests that, even within a given topology, a host of metastable configurations exists, rather than a unique stable solution..."

Winfree's work allows us to adopt a more theoretical attitude, underscoring the core of theoretical biology and disregarding irrelevant details. His questions on the problems he worked on can be considered a rich source of investigation themes like the following fragment of an abstract of an article entitled *Starting, stopping and resetting biological oscillators: In search of optimal perturbations* (Forger and Paydarfar 2004):

"... A natural question arises from Winfree's work: Are certain stimulus shapes better than others at achieving a desired effect? ... "

We have seen in the above section the importance of the concept of isochrone introduced by Winfree. In the next abstract of an article entitled *Threshold, excitability and isochrones in the Bonhoeffer-van der Pol system* (Rabinovitch and Rogachevskii 1999), we are able to see the application of such a concept:

"Some new insight is obtained for the structure of the Bonhoeffer-van der Pol system. The problems of excitability and threshold are discussed for all three types of the system classified according to the existing attractors: a focus only, a limit cycle only and a limit cycle together with a focus. These problems can be treated by the T-repellers and the T-attractors of the system which are mutually reciprocal under time inversion. The threshold depends on the structure of the T-repeller (unstable part of integral manifold). This structure is then used to understand the behavior and the properties of the two different types of isochrones: Winfree isochrones (W-isochrones) and regular isochrones. Winfree's description of a W-isochrone is extended to excitable systems. Both W-isochrones and regular isochrones are calculated for the Bonhoeffer-van der Pol system in its limit cycle and excitable regimes. The important role of the T-repeller as an asymptotic limit for both types of isochrones is manifested."

There are lots of examples in which we can appreciate the rilevance of the applications of the ideas in many themes developed by Winfree, as it is possible to observe in the following resume of the article named *Clocks and patterns in myxobacteria: a remembrance of Art Winfree* (Oster 2004):

"At the beginning of their aggregation phase waves of cell density sweep across the surface of myxobacteria colonies. These waves are unlike any other in biology. Waves can be linear, concentric or spiral and when they collide, instead of annihilating one another they appear to pass through each other unchanged. Moreover, the wavelength determines the spacing and pattern of fruiting bodies that will rise up presaging sporulation. The explanation for these waves was suggested by the work of Art Winfree on cellular clocks, and confirmed by a mathematical model that explains all of the observed wave behavior..."

Winfree's work ranged the mathematical modeling of biological phenomena from circadian rhythms and cardiac arrhythmia to the self-organization of slime mold colonies and the study of Belousov-Zhabotinsky reaction. It is worthwhile to mention that we have restricted the actual presentation only to works on biological rhythms mainly to circadian rhythms.

It is very impressive to witness Winfree's great capacity for working, since he published more than a hundred first rate scientific papers and several books. Moreover, he had the ability for reading, assimilating and synthesizing lots of scientific work. For example, in order to improve the first edition of his above mentioned book *The geometry of biological time*, he replaced some sections by others containing more recent information; for this task he had to read about 300 articles, all of them in just a sabbatical semester!

3 After Winfree As we can see, Winfree builds a bridge between the experimental biological facts and pure mathematics. Some papers of Winfree (Winfree 1982a and Winfree 1982b) express precisely the presence of the connection between experiments and theory made by the author Contemporaries and after Winfree as, Strogatz, Rand and Holmes, Kawato, Guckenheimer, Peterson, and many others, have also contributed in an important manner to the development of this branch of the biomathematics.

In the following we review a few models built by different authors but taking only those of qualitative character, each one simulating some aspects of circadian rhythms.

Abstraction is necessary when we try to understand the organization of circadian systems instead to describe the behavior of circadian systems in all its complexity. Beersma (Beersma 2005) for example, forms a global concept as a model of reality. From here, he attempts to transform the conceptual model in a mathematical formulation. On these bases he reviews 4 types of mathematical models based, all of them in well established characteristics or properties of circadian rhythms: models with negative feedback operating in pacemaker cells, coupling between pacemaker cells and cells where the periodic behavior is expressed, oscillator models describing the behavior of the composite circadian pacemaker, and models about how the circadian pacemaker influences behavior.

In 1993 Friesen et al, published a brief review summarizing information about a qualitative scheme proposed by themselves in 1984 (Block 1984). In this qualitative scheme, authors provides a means of summarizing information concerning oscillating systems. One of the most significant aspects of the scheme is that it can be employed to deduce the form of the system of differential equations which show the rate constants that must be deduced through experimentation for the construction of explicit mathematical models.

In 1989, Rusak, published a paper that reviews how the formal analysis of circadian systems, primarily in rodents, set the agenda for physiological studies, and the degree to which this agenda has been fulfilled. It is discussed how physiological analyses of circadian systems can help to redefine issues such as the nature and identity of the pacemaker, the nature of the entrainment process, the roles of photic and nonphotic cues, and the role of feedback in the circadian system.

Ton-That (2002) made the proposal that biological rhythms such as circadian rhythms, biochemical rhythms and neural oscillators are based on the mathematical model of the theory of harmonic oscillators namely solutions of certain second-order differential equations. He affirms that these oscillators can also be viewed as spherical harmonics on the circle in the two-dimensional Euclidean space.

Pedersen and Johnsson (1994) express that, on the basis of physiological experiments, it is possible to propose that circadian rhythms have stable singularities. Therefore, they decide to investigate the stability of the two singularities of the extension of the van der Pol equation given by

$$\frac{d^2y}{dt^2} + 0.5(y^2 + y - 2 - 3)\frac{d^2y}{dt^2} + (1 + 0.6y)y = \frac{d^2z}{dt^2} + \frac{dz}{dt} + z,$$

where y is the oscillating variable, and z is the light intensity assumed to excite the oscillator, which simulates several aspects of the circadian systems. Authors concluded that the model is unsuitable to describe circadian systems which have stable singularities.

In 2001, Fuentes-Pardo et al. attacked the problem of understanding the localization of the main structures involved in the motor circadian rhythm of crayfish. They presented experimental results giving the properties of this rhythm in young and adult crayfish. Then they constructed a mathematical model (based on a previous one for the electroretinogram circadian rhythm in the same species, Lara-Aparicio et al 1993) simulating those properties. In the process of constructing the model the authors clarified and made precise some hypotheses about the biological structures involved in circadian oscillations. They also formulated some hypotheses about the general properties of circadian systems.

Mathematical models have been also proposed as an approach to understanding the molecular basis underlying circadian systems. In 1999, Sheper established a fundamental question in the field of circadian rhythms concerns the biochemical and molecular nature of the oscillator. By studying a mathematical model of molecular clocks based on this general concept, the author sought to determine which features such clocks must have to generate robust and stable oscillations and to allow entrainment by external stimuli such as light. The model produced circadian oscillations as an emergent property even though a time delay in protein synthesis and rate constants of the feedback loop were much shorter than 24 h. Along with the delay in protein production, strong nonlinear interactions in macromolecular synthesis and nuclear feedback appeared to be required for the model to show well-behaved oscillatory behavior. Realistic phase-shifting patterns induced by external stimuli could be achieved by multiple mechanisms namely, up and downward perturbations of protein or mRNA synthesis or degradation rates.

Various kinds of bifurcation phenomena produced in a circadian oscillator model of *Drosophila* were investigated by Tsumoto et al (2005). Circadian oscillations in proteins PER and TIM, result from the negative feedback exerted by a PER-TIM complex on the expression of the *per* and *tim* genes that code for the two proteins. In order to studying circadian oscillations of proteins in *Drosophila*, a mathematical model was proposed. The model cannot only account for regular circadian oscillations in environmental conditions such as constant darkness, but also give rise to more complex oscillatory phenomena including chaos and birhythmicity. Authors identify parameter regions in which the model generates complex oscillations as well as regular circadian oscillations. By incorporating the effect on a light-dark (LD) cycle they detected that the waveform of the periodic variation in the light-induced parameter has a marked influence on the global bifurcation structure or the type of dynamic behavior resulting from the forcing term of the circadian oscillator by the LD cycles.

A mathematical model for the intracellular circadian rhythm generator has been studied by Sheperd et al, (1999) based on a negative feedback of protein products on the transcription phenomena. The delay and nonlinearity in the protein production and the cooperativity in the negative feedback (Hill coefficient) were for this model found to be necessary and sufficient to generate robust circadian oscillations. The similarities between model outcomes and empirical findings establish that circadian rhythmicity at the cellular level can plausibly emerge from interactions among molecular systems which are not in themselves rhythmic.

Mathematical models are useful for providing a framework for integrating data and gaining insights into the static and dynamic behavior of complex biological systems such as networks of interacting genes. Smolen et al (2000) reviewed the dynamic behaviors expected from model gene networks incorporating common biochemical motifs, and conclude that the majority of gene network modeling studies have focused on determining the types of dynamics that can be generated by common biochemical motifs and conclude that qualitative modeling will need to be supplanted by quantitative models or specific systems.

Complex functions as sleep, have been object of many biomathematical studies. In 2003 Acherman proposed a model based on the interaction of two processes, a sleep/wake dependent homeostatic Process S and a circadian Process C. Both processes generate the timing of sleep and waking. The time course of Process S was derived from a physiological variable, EEG slow-wave activity.

In addition to the timing of sleep, changes of daytime vigilance are accounted for by the interaction of the two processes. Strogatz (1978) proposed a model based on the assumption that two coupled non linear oscillators drive the sleep-wake and body temperature rhythms.

The novel aspect of the model is that its equations may be solved analytically.

After the analysis of the spontaneous timing of 359 sleep-wake cycles recorded from 15 internally desynchronized human subjects, Strogatz et al. (1986) proposed that the observed sleep-wake patterns obey a simple rule: The phase of the circadian temperature rhythm at bedtime determines the lengths of both prior wake (alpha) and subsequent sleep (rho). From this rule they derived an average alpha:rho emphasizing the importance of mathematical models in the analysis of the circadian rhythms properties.

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