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Rhythmic firing patterns in suprachiasmatic nucleus (SCN): the rôle of circuit interactions

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Abstract

The suprachiasmatic nucleus (SCN) is believed to contain the main generator of circadian rhythmicity in mammals. In order to obtain further functional details of this, electrophysiological extracellular measurements in vitro were made. By means of an interspike interval distribution analysis, it is shown that there is a novel kind of neuronal firing pattern: the harmonic pattern. From these observations, we have developed a theoretical model based on possible filtering processes occurring during synaptic transmission. The model suffices to infer that regular ultradian oscillators could be an emergent property of circuit interactions of cells in the suprachiasmatic nucleus.

Keywords: Rhythms; Suprachiasmatic nucleus; Electrophysiology; Model

1. Introduction

During the last 30 years the suprachiasmatic nucleus (SCN) of the anterior hypothalamus has become one of the most studied regions of the central nervous system, due to strong evidence showing that the circadian timing system is intrinsic to this region (for a review see [1,2]). Over the last decade in vitro studies with the brain slice technique in the SCN has resulted in rapid progress in determining the endogenous clock properties of the cluster of neurons and glia that comprise the SCN [3].

Based on frequency histograms 3 types of firing patterns have been described in SCN neurons: regular, irregular and bursting, all arising in the same sample simultaneously [4,5]. Although the average firing rate recorded from SCN neurons seems to maintain a circadian rhythm [6–9], little is known about why the firing patterns differ greatly between cells at a given time.

Previous studies, using interspike interval distribution analysis (IIDA), have shown that some SCN neurons show a regular firing pattern that is well fitted with a single narrow Gaussian function

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and, therefore, can be considered as nearly ideal oscillators [5,10]. It has also been shown that the IIDA from those neurons exhibiting an irregular firing pattern can be fitted by a log-norm function [5].

In this paper the presence of a new interspike interval distribution pattern in the rat SCN in vitro is reported. A careful analysis of the different measured patterns leads to a new classification, which enables one to envisage a model that explains the functional relationship between the different neuron behaviours revealed by the experiment. This theoretical model consists of a filtering of neural responses that leads to the generation of regular patterns from totally random firings. Hence, the experimental data are explained in a coherent way.

2. Experimental procedure

In vitro electrophysiological recordings of single neurons in the SCN of 30 adult male Wistar rats (150–200 g) were performed. The animals were housed on a 12:12-h light-dark cycle for 2 weeks with food and water ad libitum. At different circadian times through the day, each animal was beheaded under the lighting condition corresponding to that time, the brain was quickly removed

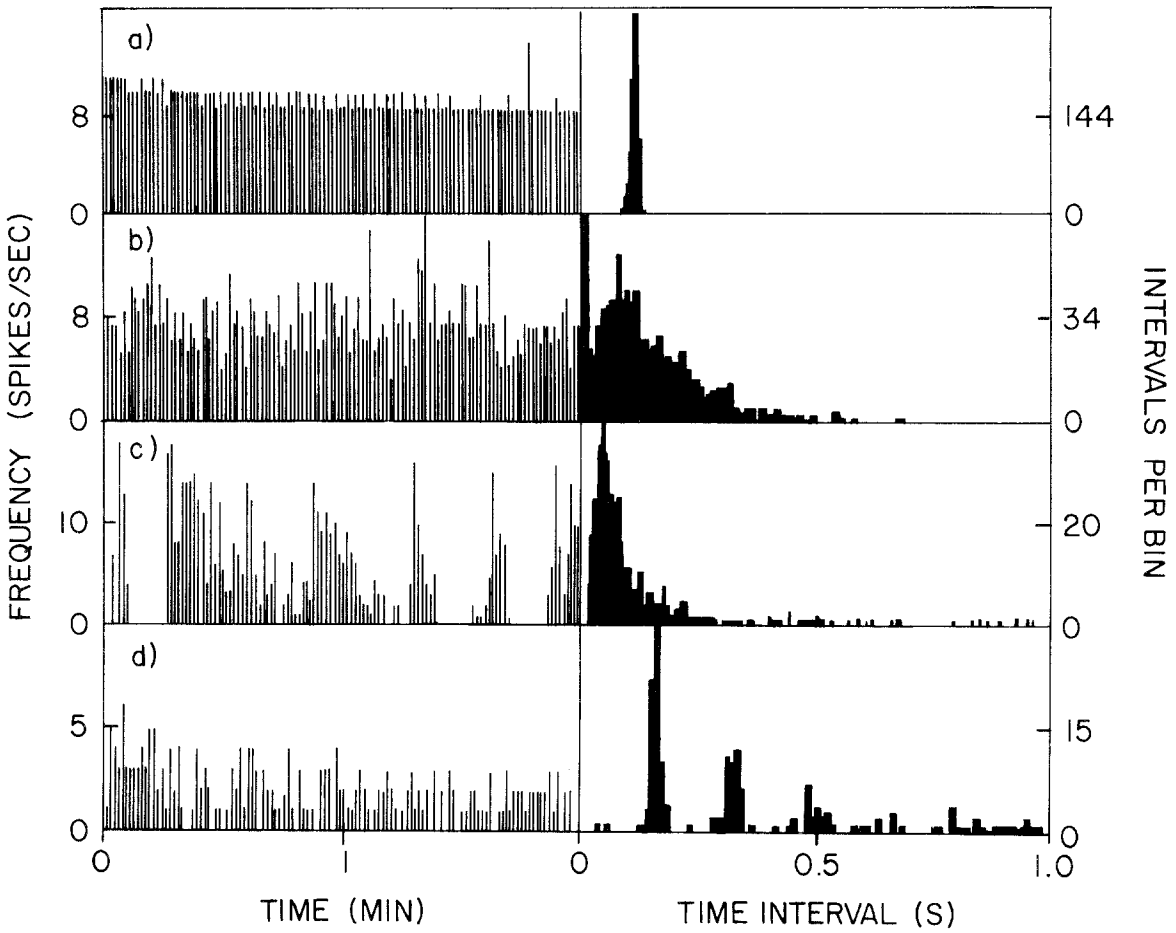

Fig. 1. Examples of recorded frequency of spikes in 4 different neuronal firing patterns in the SCN (left hand side), and the corresponding interspike interval distribution (right hand side). (a) Regular pattern, (b) irregular pattern, (c) bursting pattern, and (d) harmonic pattern.

and dipped in ice-cooled Krebs solution (pH 7.3–7.4, 300 mOsmol) [9]. The pH was finely tuned by adjusting the NaHCO concentration at the desired temperature, and the osmolarity was kept constant by modifying the NaCl proportion. Using a tissue chopper, slices of 400 μm were coronally sectioned from the hypothalamic region, containing the SCN. Once in the recording chamber, the slices were preincubated for 30 min in Krebs solution at 37°C, before recording the extracellular activity of single neurons. Recordings were made with glass micropipettes filled with 2M NaCl (4 to 10 MΩ). During the experiments the slices were perfused continuously at a rate of 3 ml/min with Krebs solution kept at 37°C and bubbled with 95% O_2 and 5% CO_2.

Firing patterns from both SCN were recorded for a total of 472 single cells. Of these, there were 10 to 20 cells per slice. Each cell was monitored with an oscilloscope for about 5 min to attain stability, and then recorded for at least 2 min on a magnetic tape. The data were stored and analyzed by means of a computerized system, which is able to discriminate random signals and to digitize the spikes, making frequency and interval distribution histograms. In order to have a wide and unbiased statistical sample, the recording electrode was advanced in steps of 50 μm and kept in such a position for at least 5 min before moving it to the next position. This procedure allows recording from a number of neurons which were initially silent when the electrode was first placed. Particular care was taken to discriminate the individual recordings by either the frequency and the shape of the responses, or by the location of the cells in the SCN.

3. Experimental results

All the cells recorded could be assigned to one of 4 typical types, according to their firing pattern and IIDA. Fig. 1 illustrates an example from each of the 4 groups. Fig. 1a represents a cell that fires regularly, that is, there is only one interspike interval of ~100 ms with a very small deviation. In Fig. 1b there is a totally irregular pattern, showing a broad distribution of interspike intervals, and in Fig. 1c there is an example of a cell that is quiet for periods greater than 1 s and then exhibits bursting, for small intervals of time. It is difficult to distinguish this type of cell from the previous one from the corresponding IIDA. All these patterns have been reported previously [4,5,10]. The neuron in Fig. 1d shows an irregular firing pattern and a broad distribution of interspike intervals, but the response is grouped around multiples of a basic interval, and the weight of each group of harmonics follows roughly the envelop of the random distribution of Fig. 1b. This latter pattern was reported in a recent abstract [11].

Table 1
Firing rates (mean ± S.D.) from the different neuronal patterns classified according to the bin rate and the IIDA

	Neuron type	Firing frequency (Hz)	Fraction of total (%)
Bin rate	Regular*	5.94 ± 1.89	23.7
	Irregular	3.60 ± 2.56	69.9
	Bursting	2.59 ± 1.89	6.4
IIDA	Tuned**	6.32 ± 2.12	18.0
	Random**	3.38 ± 2.45	65.9
	Harmonic**	4.46 ± 2.64	16.1

Total number of cells = 472.
Overall mean frequency rate = 4.09 ± 2.61.
*Scheffe test $P < 0.05$ with respect to the remaining patterns, $F = 46.41$.
**Scheffe test $P < 0.05$ among the 3 patterns, $F = 35.43$.

For reasons that will become apparent later, based on the IIDA one should consider only 3 qualitatively different patterns: the random broad asymmetrical patterns, as in Figs. 1b and 1c, the harmonic patterns, as in Fig. 1d, and the tuned patterns, as in Fig. 1a. The descriptive statistics on the firing rates from each neuronal type and from the total population are presented in Table 1. For comparison purposes the neurons were classified with either criteria (firing pattern or IIDA). The proportion of each neuronal type varied according to the criteria used for classification. When the IIDA was used, a decrease in the proportion of regular firing neurons (tuned) and an increase in the harmonic ones were observed.

It is worth mentioning that the harmonic patterns differ among themselves not only in the interval of the fundamental harmonic, but also in the

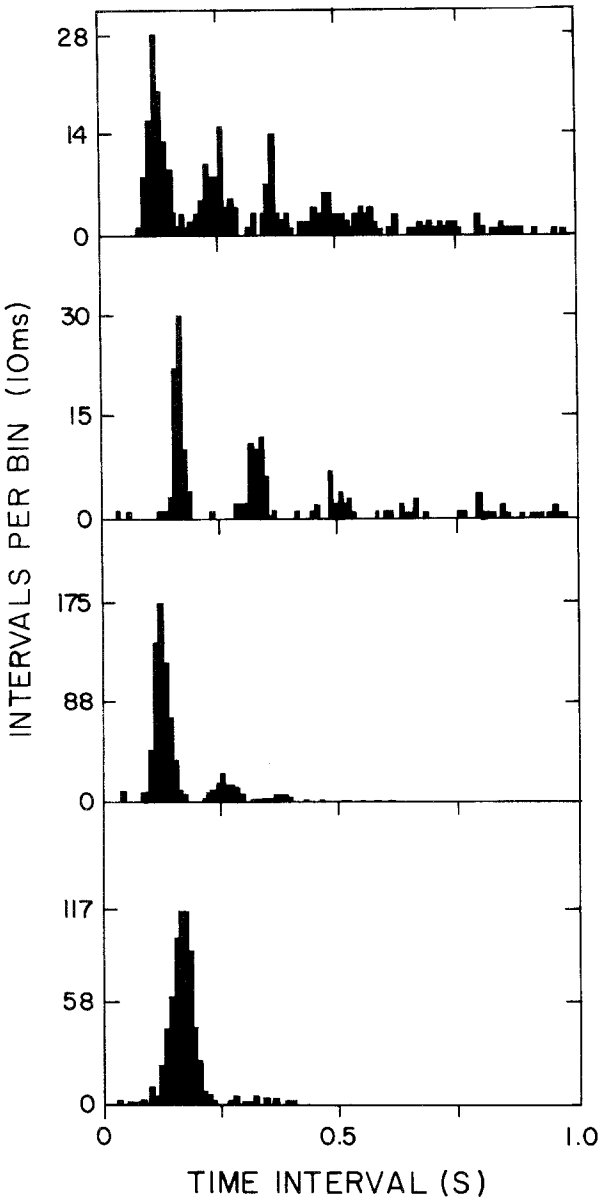

Fig. 2. Four examples of harmonic patterns, as the one shown in Fig. 1d, with decreasing intensity of the long time interval peaks. These sorts of patterns suggest a filtering mechanism of the signals.

relative weight of the subsequent harmonic peaks, in such a way that one could think of a systematic approach to perfect tuning. The patterns of 4 cells are shown in Fig. 2 to illustrate this idea.

4. Theoretical model

The following model suggests a possible mechanism by which one could obtain ideal oscillators (tuned rhythmic cells) from a random distribution of signals. There are 3 basic assumptions:

(1) There are a large number of neurons in the SCN which fire spontaneously, completely at random. These cells act as a source of stimuli to excite other cells. In other words these cells are able to generate their own action potentials, without the need of an external input, rather like the automatism found in myocardial cells. However, these cells are irregular, whereas myocardial cells have a strictly regular firing pattern.

(2) There is another type of neuron which primarily responds to external signals coming from cells interconnected in a network. We shall call these cells 'filters'. The stimuli received by one of these cells could come directly from a source cell or another filter cell, since in the network there would be paths in series and in parallel. We show later that it is not necessary to assume a particular network for the model to work.

(3) The interneuronal transmission of signals is carried out by means of a medium characterized by a transmittance and an absorbance. These characteristics could be changed by the action of an external agent, (e.g. chemical, topological, etc.). Those terms have a precise meaning in optics, when talking about conduction of signals through a medium [12]. In the present context the transmittance and the absorbance are related to the fact that only signals with particular frequencies are absorbed, other frequencies are reflected.

With these assumptions one could imagine a functional relationship between the various elements defined: if one has a source of firings with a broad interspike distribution, one could select a given interval between subsequent firings by means of an appropriate network of neurons that respond with different delay times, depending on the characteristics of the intercellular transmitters which con-

nect them, in a form analogous to a monochromator, or optical filter. At the end one should be able to filter all intervals, except the chosen one.

The first assumption can be readily tested. It is well known [13] that if there is a large number of random events, and that if the probability (p) characterizing a given event is small, then the probability $W_n(\lambda)$ that the event occurs n times in N trials is given by the Poisson distribution

$$W_n(\lambda) = \frac{\lambda^n}{n!} e^{-\lambda} \quad (1)$$

where $\lambda = Np$ is the mean number of events. In the present case, one could safely state that if there are neurons that fire totally at random, then the probability that a given neuron fires 2 consecutive times in exactly an interval of time Δt is very small, and consequently the probability distribution $W_2(\lambda)$ is given by Eq. 1. In this case $\lambda = \Delta t/t_0$, where t_0 is a characteristic time. At this level one should not associate this characteristic time to a biological process and it should be taken as a parameter given by the experiment.

Fig. 3a shows a plot obtained by summing up all the experimental patterns of types 1b and 1c in Fig. 1, that is, all the patterns of the random type. The vertical scale has been normalized by the area under the curve and the horizontal axis is the time interval λ in units of $t_0 = 75$ ms. This characteristic time was given by the maximum of the curve, which should be at $2t_0$. In the same figure the theoretical curve $W_2(\lambda)$ is plotted as a dashed line in order to compare with the experimental data. It is seen that the fit is reasonably good, except for a peak near zero in the experiments and a somewhat large tail at long intervals.

It is difficult to account for events separated by less than 10 ms in the present model. Therefore, one might assume that the discrepancy near zero has to do with processes occurring in the experiment and not considered in the model, such as the possibility of nearly simultaneous external excitations and spontaneous firing of some silent cells, and we shall not comment further on this. Regarding the long tails seen in the experiments, one can say that a slight modification of the ideal conditions of randomness, or an insufficient collection

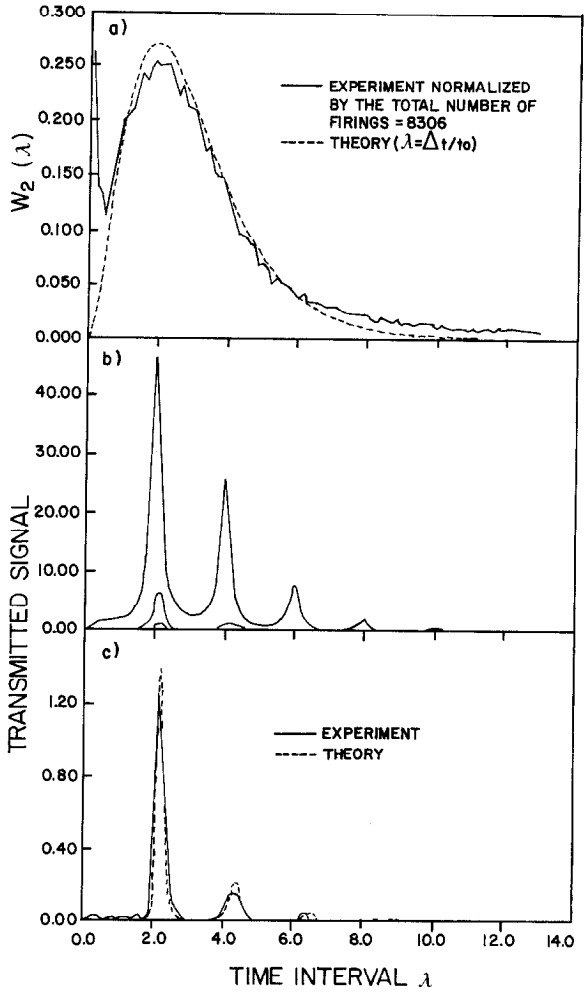

Fig. 3. (a) Comparison of the theoretical interval distribution $W_2(\lambda)$ (dashed line) and the summation of all the measured irregular and bursting patterns, as the ones shown in Figs. 1b and 1c (continuous line). The experimental data were taken from 306 source cells and were normalized to give a unit area under the curve, the time interval scale is measured in units of $t_0 = 75$ ms. (b) Calculated interval distribution patterns after 1, 2 and 3 filtering processes (Eq. 5), showing that the intensity decreases as filters are added. The decreasing intensity after subsequent filtering steps has no experimental significance. (c) Comparison between the results of the theory, after 3 filtering processes and a typical experimental pattern of the kind shown in Fig. 2. The data were multiplied by an arbitrary constant to fit the intensities.

of data, can modify the shape of the ideal curve exactly in this region. In other works [5] this kind of spectrum, obtained with a single neuron, has been adjusted with a log-norm distribution. It is clear

that with a log-norm distribution one can fit an arbitrary asymmetrical curve very well, however, it is preferable to derive the form of the distribution from very simple assumptions, as was done here.

The second step in the model proposes that the functional reason for the existence of a source of random firings is to select specific time intervals, so that there will be some rhythmic signals to be used as components of a circadian oscillator [14,15]. The selection of rhythms is naturally accomplished by taking into account the assumptions 2 and 3 in the following way: it seems reasonable to assume all neurons form a network, and therefore one expects interference between signals arriving at a given neuron, depending on the paths allowed by the network. Obviously, these paths will depend not only on the array, but also on the characteristics of the interneuronal medium, which certainly could be changed at any time by other regulating factors. This would explain the existence of harmonic and tuned firing patterns. The model is illustrated in Fig. 4.

In order to be specific, let us consider a one-dimensional array of 2 media $N - M - N$ (see idealization in Fig. 4), each one characterized by a transmittance T and an absorbance A, which are connected through the relation $T + R = 1 - A$, where R is the reflectivity of medium M, due to the walls separating the 2 media. Then, the transmitted signal from one side of the sandwich to the other is given by the Airy formula [12]

$$\frac{W}{W_0} = \frac{T_{max}}{1 + F\sin^2(\delta/s)} \tag{2}$$

where the maximum transmittance is

$$T_{max} = \frac{T^2}{(1 - R)^2} = \left[1 - \frac{A}{(1 - R)}\right] \tag{3}$$

and the contrast factor is $F = 4R/(1 - R)$. In all problems dealing with interference the important quantity is the phase δ. In the present case this phase should be related to time delays between different paths. Let us write the phase as

$$\delta = 2\pi\frac{\lambda - g}{s + \Delta s} \tag{4}$$

where the variables λ, g and s are times in units of t_0. The parameter s gives the period of the fluctuation, and therefore it should be of the order of the characteristic time. This quantity could be changed by an amount Δs by modifying the chemistry of the medium, or its 'refractive index'. The quantity

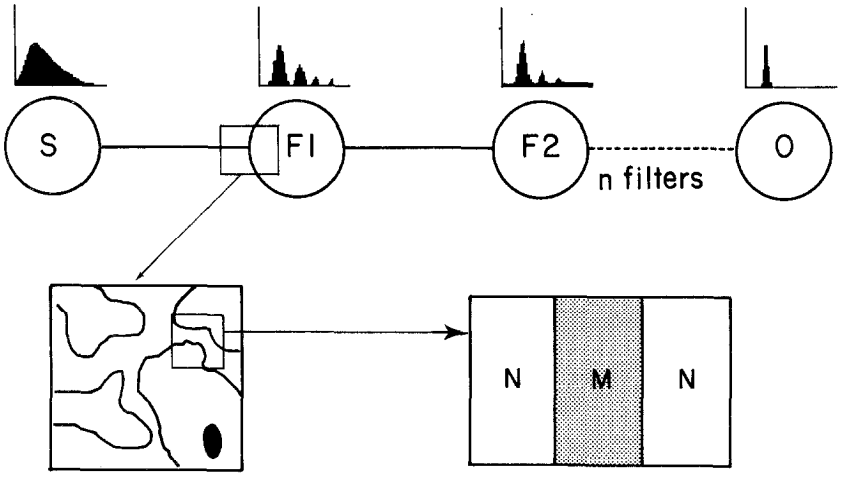

Fig. 4. Sketch showing the idealized filtering process in the model. The source neurons (S) feed the filter ones (Fi), which are interconnected and after n steps a perfectly tuned cell (O) is obtained. The representative spike distribution for each cell is drawn on top, and the inset represents an idealized picture of the synaptic process, which itself is modelled as an optical filter in the form of a sandwich of a medium M between 2 other media N.

g takes into account any phase shifts, or time delays, due to the walls.

Let us now consider a series of sandwiches $N - M_1 - N - M_2 - \ldots - N - M_n - N$, which corresponds to a 1-D transport problem through n finite barriers [16]. The problem of various transport channels in parallel is equally tractable, but for the sake of simplicity, consider only the array in series. Thus, one obtains a recursive formula

$$T_n = \frac{T_{n-1}^2}{(1 - R_{n-1})^2} [1 + F_{n-1} \sin^2(\delta_{n-1}/2)^{-1} \quad (5)$$

The final output ($W = W_0 T_n$) can be very complicated if the parameters of Eq. 2 vary at each step, but the effect of filtering is already present if one considers constant parameters, i.e. identical media. In Fig. 3b we plot the subsequent output after the first, second and third filters. The parameters used were $T = 0.2$, $A = 0.2$, $s = 2.0$, $\Delta s = 0.2$, and $g = 0.01$, and the input signal was given by equation $W_2(\lambda)$, multiplied by an arbitrary factor of 700 to give a reasonable scale. These parameters fit some real data extremely well. This is shown in Fig. 3c, where an experimental pattern is compared with the output of the third filter of Fig. 3b. Different shapes of patterns of the sort shown in Fig. 2 can be adjusted with different number of filters, and the ones in Fig. 1a can be obtained with 5 filters only. Similar filtering, or selection of frequencies, can be obtained with paths in parallel, but the final output depends on the particular configuration of the various elements, and a calculation of this case would not be very informative. The important fact is that the tuning of the firings does not depend on the specific details of the network.

5. Discussion

There are different firing patterns in the cells of the SCN, although at present, there is not a clear relationship between the type of firing pattern and any morphological or functional characteristic of the neurons. The clear finding of a harmonic type of firing pattern immediately suggests that a frequency-filtering mechanism, that leads finally to perfectly tuned cells, is taking place in the SCN. The proposed phenomenological model is able to account for the appearance of all patterns measured in the SCN single cells, and suggests a functional relationship between the different types of behavior.

The main consequence of the model is that one is able to obtain a precise clock from not very precisely defined components. It is worth mentioning that the selection of a function from an originally random process is an efficient and versatile mechanism frequently found in nature, that allows for the function to be performed even if a substantial number of the components are damaged or not working.

It is important to emphasize that the model does not address the question of circadian rhythmicity generation. However, we believe that it provides new ideas which may be applicable to the study of the mechanisms of circadian regulation, and that it also serves as a complementary support to statistical models and simulations [12,13,17]. Previous models have suggested that one could get a circadian cycle of extreme temporal precision, either by an interacting array of oscillators with fixed frequency [13,17,18], or without the need of precise components [12,19], simply as the usual oscillations caused by non-linear behavior found in systems out of equilibrium. None of these studies, however, has addressed the relationship of the model to the actual physiology of any known biological oscillator.

By combining the study of the clock mechanism (not addressed in this work) with the study of the mechanisms leading to the generation of different firing patterns found in the SCN at any given time (which could be explained by the model presented here) it may be possible to gain further insights on the mechanisms of regulation of circadian rhythmicity. At this point, however, the connection between cellular firing and the generation of circadian oscillations, or the appearance of overt rhythmicity, is not straightforward, and beyond the scope of this paper.

It seems that the coherent functional picture given by the model is in agreement with other experimental facts. Firstly, it has been found that in a medium without calcium ions the regular firing patterns disappear, while the irregular ones

persist [20,21]. It is well known [22] that calcium ions are essential for the synaptic transmission in the SCN, therefore, these experiments support the idea that tuned patterns depend on synaptic processes, while the irregular patterns do not, since they do not depend on external stimuli from other cells. There have been reports of the existence of chains of neurons and abundant local circuits within the SCN, which are consistent with the assumption that the neural networks needed for filtering really exist [23,24]. Furthermore, immunohystochemical studies reveal an unusually large number of neuroactive substances and intrinsic connections within the SCN [25]. The unusual abundance of activated glia in the SCN tissue [23,24] could be related to the important function of regulating the ionic composition of the extracellular medium [26,27] and thus could be capable of changing the filter parameters. This is important if one needs a flexible and adaptable clock.

The present model could be extended by investigating the real meaning of the time parameters in it. One could suggest an experiment in which one changes the chemical composition of the extracellular medium, in particular, the concentration of ions that modify the synaptic processes, and compare the experimental observations with the predicted modifications of the harmonic patterns. It is encouraging that the value of g (which gives the frequency of the filtered signal) is $0.01t_0$, that is 0.75 ms. This is within the limits of the measured times a signal takes to cross the synaptic junction (0.5–1.0 ms).

The model implies a modulatory mechanism of synaptic transmission at the level of the filter neurons, which could be either internally or externally regulated. The first case would imply that the filter neuron could have a clock mechanism which would affect the process at the level of receptor dynamic (number, affinity or coupling to ionic channels or second messenger system). In the second case, the filter network would be regulated by inputs from other neurons which could affect the transmission process either by presynaptic (regulation of transmitter release) or post-synaptic (spatial or temporal summation) processes. In both cases the outcome would be interference in the transmission of neuronal impulses from the source to the output neurons.

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