Subthreshold bipolar atrial stimulation affects the discharge rate of the sinus node: an animal study

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***Abstract*—Aim of this study was to verify whether the electrical field generated inside the right atrium by sub-threshold electrical impulses (impulses unable to induce cells activation) may condition the discharge rate of the sinus node cells. An electrophysiological study was performed on seven young farm pigs before and after denervation. After general anesthesia, pigs were stimulated with impulses delivered at constant rate by a bipolar catheter positioned inside the right atrium. The amplitude of the stimulus was set to avoid atrial capture. A 10-minute atrial stimulation was performed at a rate above and below the spontaneous heart rate, both before and after denervation. Three animals showed a conditioning of the sinus rhythm, observed as phase synchronization. The different response, or even the no response, of animals to stimulation could be due to different factors, concerning biological, pharmacological and "geometric" conditions. The important result remains that a perturbation of the sinus rhythm can be induced by a very low electrical field, as the one generated by the activity of artificial pacemakers, and it could help explaining the onset of rhythm disturbances in paced patients.**

***Index Terms*—Sino-atrial node, Synchronization, Phase resetting**

I. Introduction

The interaction of weakly coupled self-sustained periodic oscillators is a well-studied topic in nonlinear sciences; it is of particular interest when giving rise to the phenomena as phase resetting and synchronization [1]. Such phenomena are often encountered even in physiological systems exhibiting an oscillatory behaviour. Above all others, the heart, the biological oscillator *par excellence*, has been object of a number of investigations. At cellular level interesting experiments were carried out on spontaneously beating aggregates of cardiac cells from embryonic chicken heart stimulated with single impulse or impulses of different amplitudes and frequencies [2]. These experiments evidenced the interaction between stimuli and cardiac cells activity in terms of phase resetting (single stimulus) and synchronization (train of impulses). At systemic level, abnormal rhythm such as modulated parasystole is modeled with the paradigm of phase resetting [3]. The weak interaction of an electric stimulus with the sinoatrial node (SAN) has been used to interpret data from patients under cardiac resynchronization therapy [4]. Furthermore, simulation studies performed using a model of the baroreflex loop subjected to external stimulation made possible to detect synchronization under different conditions of the autonomic nervous regulation [5]. Aim of this study was to verify whether the electrical field generated inside the right atrium by sub-threshold electrical impulses (impulses unable to induce cells activation) may condition the discharge rate of the sinus node cells and lead to synchronization phenomena.

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II. Methods

After authorization of our ethics committee, we included this study in a preexistent electrophysiological study protocol on seven young farm pigs. After sedation with tiletamine hydrochloride and zolazepam a catheter for electrophysiological study was inserted in the right atrium and placed as close as possible to the sinoatrial node. The animals were mechanically ventilated at $0.25Hz$. Constant rate stimulation was performed with stimuli ranging between $0.1V$ and $0.5V$, according to the specific atrial threshold of the single animal, and with duration $0.5ms$. The amplitude of the stimulus was set to avoid atrial capture and the bipolar modality of the stimuli administration guaranteed the decay of the electrical field proximally to the catheter. A 10-minute atrial stimulation was performed at higher (*Hfreq*) and lower (*Lfreq*) frequencies compared to the spontaneous heart rate. In order to exclude rhythm variability due to the autonomic activity, the same stimulation protocol was repeated after animal denervation, performed by resection of vagal nerves (*parasympathectomy*) and by the administration of hexamethonium hydrochloride (*sympathectomy*). The effectiveness of the denervation was tested by blood drain and atropine administration. Surface electrocardiogram (ECG) and atrial electrical activity were recorded at 4000 Hz simultaneously with invasive blood pressure and respiration. A semi-automated procedure was implemented in order to detect stimuli, QRS complex occurrence and their relative phases. The effect of the stimuli on the SAN activity was investigated analyzing the relationship between the phase of the stimulus and the length of the RR interval. In particular, the synchronization epochs were detected using the method of synchrogram.

A. The synchrogram

Synchrogram is a widely used graphical tool suitable to detect the presence of synchronized rhythm in periodically stimulated oscillators. This technique uses the so called unwrapped phase, calculated using the equation:

$$\Phi_n = I_{R_{prev}} + \frac{\tau_n - \tau_{R_{prev}}}{\tau_{R_{next}} - \tau_{R_{prev}}} \quad (1)$$

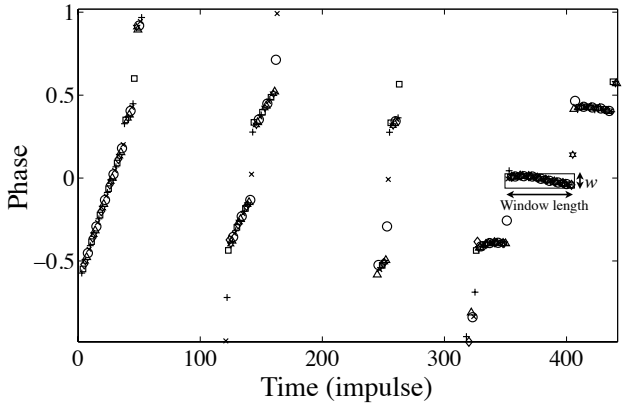

Fig. 1. Quantification of the synchrogram. The subtraction of the average phase over an horizontal sliding window results in a line whose height is the synchronization measure *w*

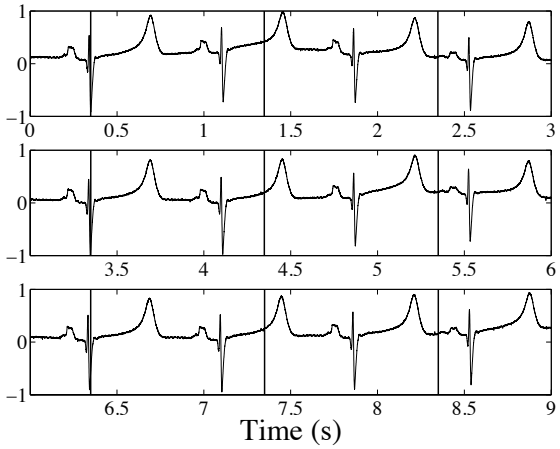

Fig. 2. ECG strip from animal P2 during stimulation at 60 stimuli per minute. The stimulation is synchronized 3:4 with the ECG (i.e. 4 QRS complexes every 3 stimuli). Vertical lines represent the occurrence of the stimuli.

where Φ_n is the phase of the n^{th} stimulus, $I_{R_{prev}}$ is the number of cycles completed by the oscillator (the number of QRS complexes in our case), τ_n is the time of the n^{th} stimulus, $\tau_{R_{prev}}$ is the time of the occurrence of the previous QRS complex, and $\tau_{R_{next}}$ the time of the occurrence of the following QRS complex. This equation leads to an increasing value of the phase. Using the $modulus\ m$, the unwrapped phase can be wrapped around, and in presence of an $n:m$ synchronised rhythm the plot of $\Phi(mod\ m)$ will show an n horizontal line structure.

B. Quantification and testing of the synchrogram

The quantification of the synchronization was performed in accordance with [6]. For each couple of n and m, the phases plotted on the synchrogram are divided in n subgroups alternatively (note that when the synchrogram exhibits an n line structure, every subgroup will coincide with one of the lines), the phase of each group is averaged and the average of each group is subtracted from all the members of the group, eliminating the mean value. In presence of an n line structure this results in a single "noisy" line around zero. The width of the resulting line is a measure of the synchronization between the stimuli and the oscillator. The measure was computed in a sliding window in order to assess the dynamical behaviour of synchronization. Figure 1 shows the result of the subtraction of the average phase from each group. The reliability of the results were then evaluated by replacing the ECG signal under stimulation with the ECG signal recorded at rest in order to exclude synchronization due to casuality.

III. RESULTS

Conditioning of the sinus rhythm was observed in three out of seven animals (indicated as P1, P2, P3). Table I shows the synchronization epochs detected on the three animals. The table shows the stimulation frequency in stimuli per minute, the detected synchronization ratio, the duration of the synchronization epochs and the quantification of the synchronization. P1 shows short epochs of synchronization both before and after denervation. P2 shows long lasting synchronization epochs both before and after denervation and with *Hfreq* and *Lfreq* stimulation frequency. An ECG strip during 3:4 synchronization in P2 is plotted in Figure 2. The vertical lines represent the occurrence of the stimuli. Figure 3 shows a synchrogram from P2; a 5:3 synchronization ratio is observed under *Hfreq* after denervation. Figure 4 shows a synchrogram from P3 data under *Hfreq* after denervation where 9:7 synchronization ratio is observed.

TABLE I
SYNCHRONIZATION EPOCHS OBSERVED IN ANIMALS P1, P2 AND P3.

Animal	Stimuli/minute	Ratio	Duration (stimuli)	Q Synch
P1	55	5:6	13	<.02
	55	5:6	18	<.02
P1 (den)	80	7:5	80	<.02
	80	5:4	25	<.007
P2	60	3:4	270	<.025
	90	8:7	100	<.025
	90	8:7	65	<.025
P2 (den)	60	1:1	>800	<.005
	90	5:3	320	<.03
P3 (den)	75	9:7	140	<.02

IV. CONCLUSION

In this study we analysed the response to subthreshold stimulation of seven anesthetized farm pigs both before and after denervation. The study shows that subthreshold electrical stimulation affects the SAN activity in both intact and denervated animal and that a stimulation with an impulse train can give rise to the phenomenon of synchronization. The different response, or even the no response, of animals to stimulation could be due to different factors, concerning biological (cell membrane permeability), pharmacological (response to anesthetic drugs) and "geometric" (position of the catheter inside the atrium) conditions. The important result remains that a perturbation of the sinus rhythm can be induced

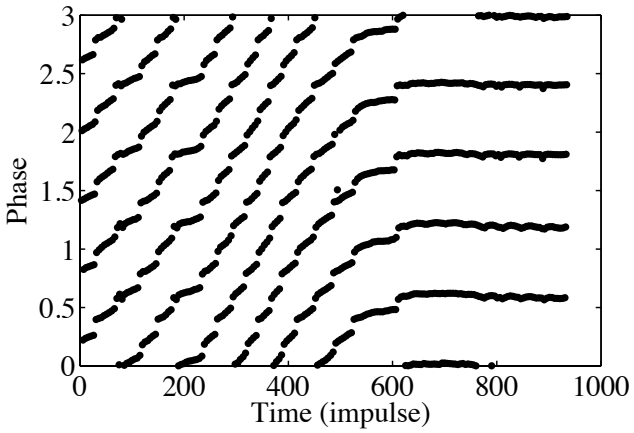

Fig. 3. The synchrogram of P2 under Hfreq after denervation. The 5:3 synchronization is evidently observed starting after about 620 impulses, showing five horizontal lines.

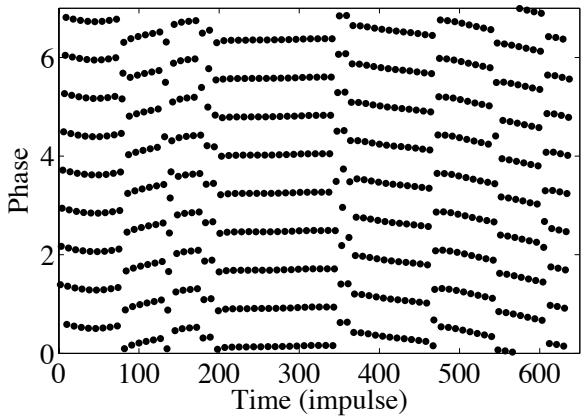

Fig. 4. The synchrogram of P3 under Hfreq after denervation.

by a very low electrical field, as the one generated by the activity of artificial pacemakers. Although in a small subset of experimental animals, these findings may have important clinical implications providing an additional clue to explain the occurrence of rhythm disturbances in paced patients.

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