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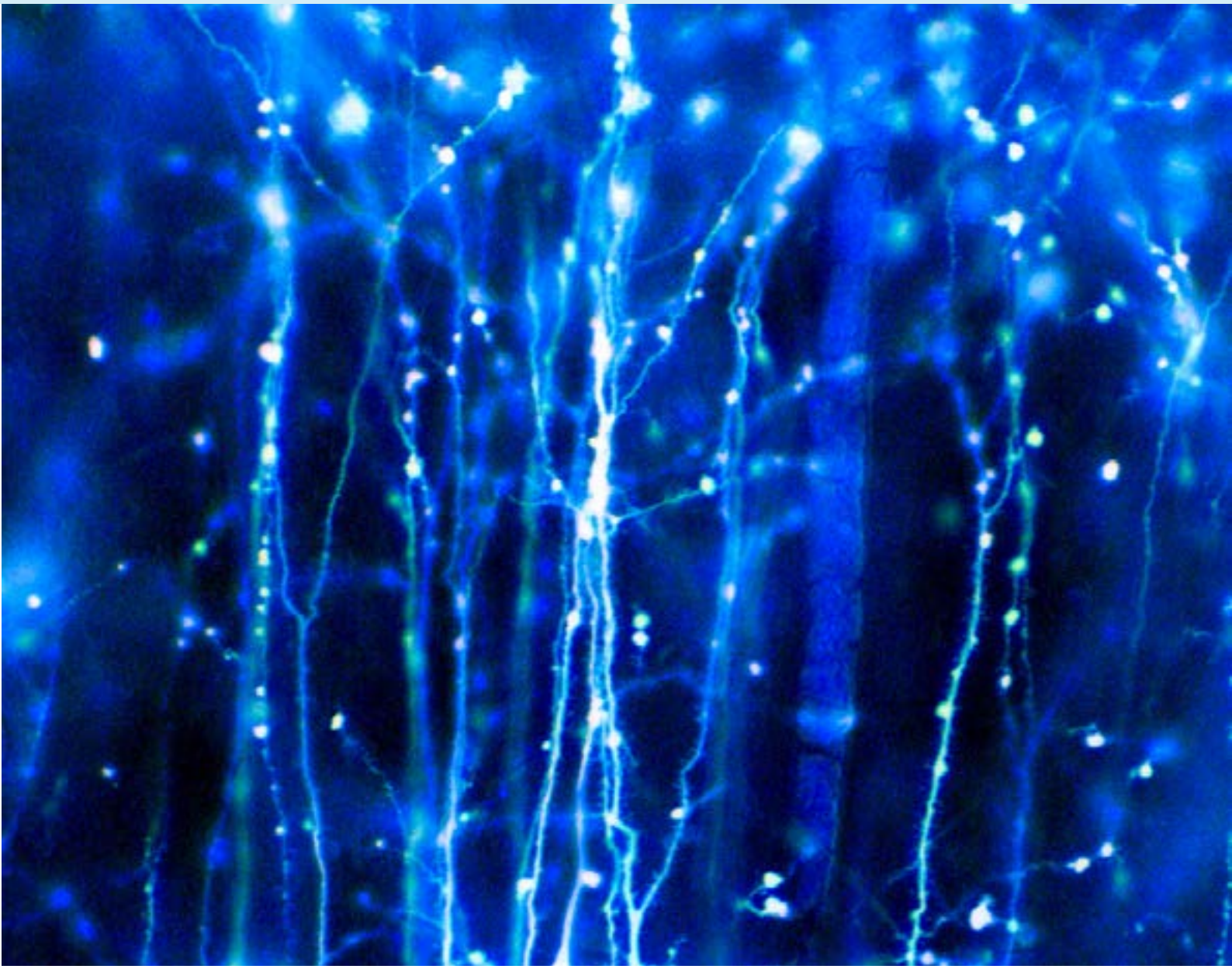

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THE MECHANICAL SIDE OF RESPIRATORY SINUS ARRHYTHMIA

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ABSTRACT

The relationship between the Respiratory Sinus Arrhythmia (RSA) and the Autonomic Nervous System is unquestionable, consequently many authors consider the changes in RSA as an index of autonomic status. This Mini-Review is not intended to contradict this point of view, here we would like to highlight the importance of mechanical processes independent of autonomic reflexes. The present paper reviews previous evidence that strongly support the importance of mechanical influences on RSA and our own experiments with isolated organs or entire animals, in which autonomic reflexes were blocked.

As a conclusion, we can say that RSA's mechanical determinants are also unquestionable; it remains to elucidate its relationship, if any, with autonomic drive.

Keywords: Respiratory sinus arrhythmia. Cardiac autonomic reflexes. Sinus node distension.

RESUMEN

La relación entre la Arritmia Sinusal Respiratoria (ASR) y el Sistema Nervioso Autónomo (SNA) es incuestionable, por lo que muchos autores consideran los cambios en ASR como un índice del funcionamiento del SNA. Esta Mini-Revisión no pretende contradecir este punto de vista, lo que se desea destacar es la importancia de procesos mecánicos independientes del SNA.

En el presente trabajo se revisa bibliografía que apoya fuertemente la importancia de las influencias mecánicas en la ASR y nuestros propios experimentos con órganos aislados o animales enteros, en los que se bloquearon los reflejos autónomos. Como conclusión, podemos decir que los determinantes mecánicos de la ASR también son incuestionables; queda por dilucidar su relación, (si es que la hubiere), con los mecanismos del SNA.

Palabras claves: Arritmia sinusal respiratoria. Reflejos cardíacos autonómicos. Distensión del nódulo sinusal.

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Introduction

The interaction between bodily systems allows the integration of different organs and their connection networks. Ionic channels, genes, molecules, and a number of other structures, work in a concerted manner generating the functional repertoire of a given organ. Meanwhile, these interactions frequently generate "emergent properties" which differ from the activity of each organ separately. Therefore, together with the indispensable analysis of the individual mechanisms, the study of the whole system and its interactions deserves a detailed examination.

Changes in heart rhythm synchronized with respiration (beat intervals are shortened during inspiration and prolonged during expiration) are known as Respiratory Sinus Arrhythmia (RSA). This physiological change of rhythm had been strongly related to Autonomic Nervous System (ANS) activity, particularly with reflexes that discharge trough parasympathetic branch. Nevertheless, there are other aspects of the relationship between respiration and heart rate that could be independent from ANS, specifically those related with mechanical events.

Such is the case of the interaction of the heart and the respiratory system, and more specifically, the interaction between respiratory cycle and heart rhythm*, which will be covered in this Mini Review. A short analysis of the literature is presented. Besides, this paper includes original experiments to prove the point.

Background

The relationship between heart rate and respiration has been recognized since ancient times [1]. Almost a century after the pioneering observation made by Stephen Hales on 1733 [2] who described the changes in blood pressure induced by respiration, Carl Ludwig [3] obtained the first recordings of this relationship. In this work, besides the changes in blood pressure changes Ludwig measured the intervals between beats. Therefore, he was able to show an acceleration of heart frequency during inspiration and a slowing of it during expiration.

A milestone in the field is the work of Francis Bainbridge performed in the early decades of twentieth century. He first studied the relationship between atrial filling and heart rate [4] and later extend his observations to the relationship between respiration and heat rate [5]. His works support the basis of the relationship between ANS, respiration and heart rate regulation, in fact, the changes in heart rate promoted by atrial distention are known as Bainbridge´s reflex.

Beyond the role atrial distention at the beginning of an autonomic reflex, Bainbridge gave us an early picture of the complex mechanisms involved in the heart rate regulation, including lungs movements, circulatory pressure changes, and blood pH, among others. However, all the described mechanisms have a common efferent pathway related to the ANS function, hence producing neural reflexes.

The predominance of the autonomic reflex explanation blurred an important fact, i.e. during respiratory cycle the heart is submitted to mechanical forces derived from its position into the thoracic cage.

In 1956 Blinks [6] stated that a stretch-induced increase in beating rate can be observed in isolated cardiac mammalian heart. Ten years later, Brooks and coworkers [7] observed that the denervation of the heart reduced but did not abolish the response to stretch or release from stretch.

The same group [8] demonstrated that the stretch of an isolated cat sinoatrial node induces an increase of sinus firing and reduced cellular resting potential, whereas the release of stretch gave the opposite effect.
In the last decades of twentieth century, the RSA acquired a new relevance due to an increased interest in the analysis of Heart Rate Variability (HRV). The widespread use of computers in research allowed a more accurate measurement of beat intervals, and hence the measurement of HRV became a fundamental tool for research and diagnostic analysis in many fields (Cardiology, Psychology, Nephrology among others) [9].
The arguments to sustain the dependence of RSA with ANS refer to a number of reflexes that are involved in such dependence [10]. Moreover, there are evidence of neural connections between respiratory and vagal neurons at brain stem [11,12,13].
The changes in the duration of heartbeat intervals that constitutes the HRV occur with different cycles of frequency, among which the most studied is the one having the shortest intervals or High Frequency (HF) band in the spectral analysis. This cycle is unquestionably related with the respiratory cycle, and hence can be ascribed to the RSA [10,9,14].
Today the leading interest for HRV and HF band (or RSA) is its value for the evaluation of the ANS [15]. Nevertheless, this point remains to be elucidated because many other authors raised severe concerns about such a role [16,17].
The work of Bernardi who described the persistence of RSA in recently transplanted (i.e. denervated) hearts [18], represents one of the strongest observations that reinforce the idea of certain independence between respiratory induced changes in heart rate and ANS. Other authors made similar observations ruling out the possibility of reinnervation [17]. Even in transplanted patients after the autonomic blockade, the persistence of RSA was described [19].

In an elegant experiment Horner at al. show the response of in situ pig hearts after a localized stretch of the right atrium [20]. They performed their experiments before and after the vagal section and sympathetic blockade. The main conclusion was that stretch reduces the high frequency ("respiratory") components of the HRV with or without ANS activity.
In the vagotomized rabbit, Perlini et al. [21] signaled the persistence of RSA. They analyzed various mechanisms concluding that this response is related to the mechanical stretch of sinus node produced by the increased venous return during inspiration.
Therefore, it is conceivable to sustain that one of the mechanisms implicated in the persistence of RSA without autonomic drive, is the interaction of respiratory cycle and mechanic atrial distention.
In the present work, we would like to add arguments obtained in our own experimentation to reinforce the importance of the mechanical aspects of the RSA.

Original experiments

Data obtained from isolated Guinea pig hearts.

We used a modified classic Langendorff model [22], hearts isolated from anesthetized Guinea pig (Cavia porcellus), were placed in a system that allows the retrograde perfusion of the aorta, closing the aortic valve, hence directing the perfusion to the coronary arteries with a modified Tyrode solution, (Procedures were approved by "Comisión Honoraria de Experimentación Animal" of the Universidad de la República, Montevideo, Uruguay).
The spontaneously beating hearts were immersed in a double-walled glass jacket, sealed at its ends by rubber plugs. The outer chamber of the glass jacket was filled with warm water pumped from a thermostatized bath to maintain a temperature of 36 ± 1 ºC in the inner

chamber. This inner chamber was filled with the same solution used in the perfusion of the coronary arteries and received the drainage of the coronary veins; the volume added to chamber from the venous drainage was evacuated outside the system.

The upper plug allowed the passage of three tubes: 1) a cannula attached to the aorta, 2) an electromanometer to measure the pressure inside the container and 3) the coronary vein solution drainage. It also allowed the insertion of a thermistor for temperature control of the bath, plus two wires for the recording of the heart´s electrical signals, connected to silver electrodes inserted into the left ventricular wall. Through the lower plug, a catheter and a syringe allowed manual application of suction, to reduce the chamber's pressure.

In this way we obtained a modified Langendorff's preparation with the heart contained into a rigid chamber filled with liquid, simulating the heart and thorax interaction. The pressure around the heart could be cyclically lowered and raised, mimicking the alternation of pressure during the respiratory cycle.

Electrical signals were recorded using a Grass P55 amplifier, pressure was recorded by means of Statham electromanometer connected to a Grass PT11 amplifier. Both signals were acquired into a computer using an A/D converter (Measurement Computing USB-1208FS) and a suitable homemade software.

The hearts (n=5) were allowed to stabilize for 30 minutes in the recording system. Electric activity and pressure were recorded in three different successive conditions: 1) control condition previous to manipulation, 2) rhythmic reductions of pressure were applied to imitate the respiratory changes to which the heart is subjected within the chest, 3) recovery period, following the cessation of manipulation.

Later, in an off-line analysis, the acquired signals were displayed with a suitable software (CED Spike2), and intervals between beats were measured. Figure 1A shows the simultaneous recordings of electrical signal and chamber pressure. As can be seen (Figure 1B), the near rhythmic reduction of pressure, induces changes on heart rhythm, similar to that observed during the SRA. The reduction of pressure in the chamber (namely the "inspiration" phase) induces the shortening of beat intervals, while the release of the maneuver with the consequent increase of pressure ("expiration") led to the prolongation of the intervals.

The square root of the mean of the squares of successive interval differences (rMSSD) was calculated to quantify the changes in beat interval duration. Such index was chosen because it gives a good picture of changes in shorts rows of temporal series [23]. Values were compared using a one-way ANOVA followed by Holm-Sidak's multiple comparisons test (GraphPad Prism software).

A p value < 0.05 was considered significant.

Results

rMSSD significantly increased from 2.64 ± 2.08 to 24.62 ± 17.24 msec. After cessation of the pressure changes (recovery period) rMSSD returned to an average of 11.37 ± 11.53 msec. This value was lower but not significantly different from that observed during the maneuver Our experiment deserves some critical analysis. First, the comparison of changes in pressure with the real changes seen during the respiratory cycle, were not precisely matched with the physiological values. Besides, the heart maintains the autonomic endings and ganglia so the effect of neurotransmitters cannot be ruled out. Finally, the coronary flow could also be affected by the pressure drop, however it is unlikely that such flow changes could induce the observed modifications in the intervals.

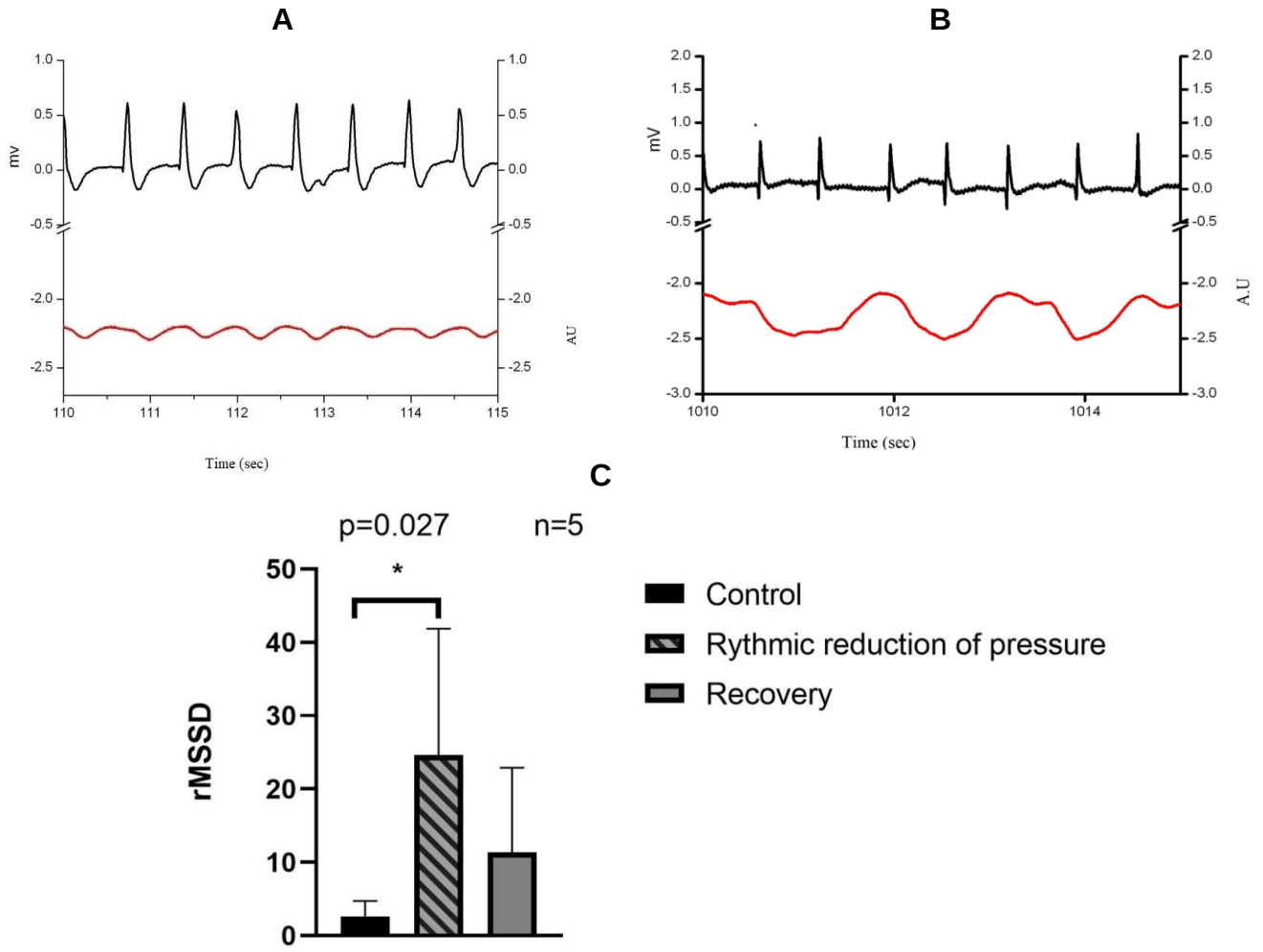

Figure 1. Guinea pig isolated heart (modified Langendorff technique). **1A**. Heart beating spontaneously in control conditions, upper row: recordings of electrical signal , lower row: pressure of the chamber Beat intervals are similar and fluctuations of pressure are coincident with each beat .
1B. Manually appliqued reductions of pressure. Duration of beats intervals are reduced when pressure is reduced and increased when pressure increases.
1C Mean values of rMSSD in each group. The sign * indicates statistically significant differences of values.

Data obtained from anesthetized sheep.

Castrated male sheep (Corriedale Romey Marsh) were prepared for cardiovascular recordings under anesthesia (Procedures were done in accordance with the Guide for Care and Use of Laboratory Animals, published by the US National Institutes of Health (NIH publication No 85-23, revised 1996) and approved by the Laboratory Animal Care and Use Committee of the Favaloro University). A solid state microtransducer (Konigsberg, modelo P 15) was introduced in the right atrium and connected to a Gould amplifier (6600 Series Transducer). A saline-filled catheter was placed in the descending aorta and connected to an electromanometer (Statham P23). To measure the dimensions of the right atrium (RAD), a pair of ultrasonic sensors (5 MHz, 4 mm in diameter with hemispherical lens) was sutured near the sinus node and connected with a sonomicrometer (Triton Technology). Three

subcutaneous electrodes were placed on the chest and connected to an amplifier (BioTach, Gould) to obtain electrocardiographic signals.

Pneumatic cuffs were placed in the descending abdominal aorta and in the posterior cave vein, in its abdominal portion near the diaphragm. Such cuffs allowed the compression of the vessels that induced increments in the pressure of aorta upside the cuff (aortic cuff), or reductions in right ventricle filling (cave vein cuff). The cuffs were manually regulated from outside the body of the animal

Respiratory movements were recorded by means of a chest belt equipped with a latex tube with two piezoelectric crystals inside, thus movements during inspiration increase the distance between the piezoelectric crystals which return to its previous position during expiration. The movements were recorded with the sonomicrometer equipment mentioned before.

Surgery was followed by a recovery period of three weeks. Recovered animals could be recorded in wakefulness without inducing any suffering. Later, sheep were anesthetized and maintained on supine position over a table while mechanically ventilated. Signals were acquired by means of an A/D converter (Data Translation 2801-A) coupled to a personal computer.

Beat intervals duration were obtained from ECG recordings, and then plotted against time as "temporal series" (i.e. intervals vs time); records of respiration, atrial pressure and atrial dimension, were resampled on beat to beat basis following the procedure of Bernardi et. al. [18]. This procedure allows the treatment of each recording as a part of the time series which is suitable for spectral analysis.

For the focus of this work the frequency of cycles its preferred over timing itself, hence, we performed a spectral analysis of the resampled signals. This spectral analysis was used to overcome some mismatching in the timing of recordings derived from the different inertial characteristics of recordings. Beat to beat interval sequence was interpolated with a cubic spline, uniformly resampled (Fs = 4 Hz), and detrended. The power spectral density (PSD) of this signal was estimated by Welch's method, dividing the data in nonoverlapping intervals (512 samples each), windowed with a Hanning window [25].

Results

Figure 2A shows that the dimension of the right auricle (RAD) increases with inspiration and decreases with expiration. Similar behavior is observed with right atrial pressure, not shown. The spectral analysis confirms the coincidence of PSD at the same frequency (Figure 2B). These observations support the concept that besides the ANS reflex, the right atrium dimensions change in synchrony with respiratory movements. In order to prove the influence of non-autonomic mechanisms in SRA, the ANS was blocked using a combination of Propranolol (1 mg/Kg) and Atropine (0.2 mg/Kg), both intravenously. The effectiveness of the blockade was challenged by the elicitation of the baroreflex, after the blockade changes in pressure of the aorta or posterior cave vein did not elicited noticeable modifications on beat to beat intervals.

In spite of the autonomic blockade, the cyclic relationship with breathing was maintained.

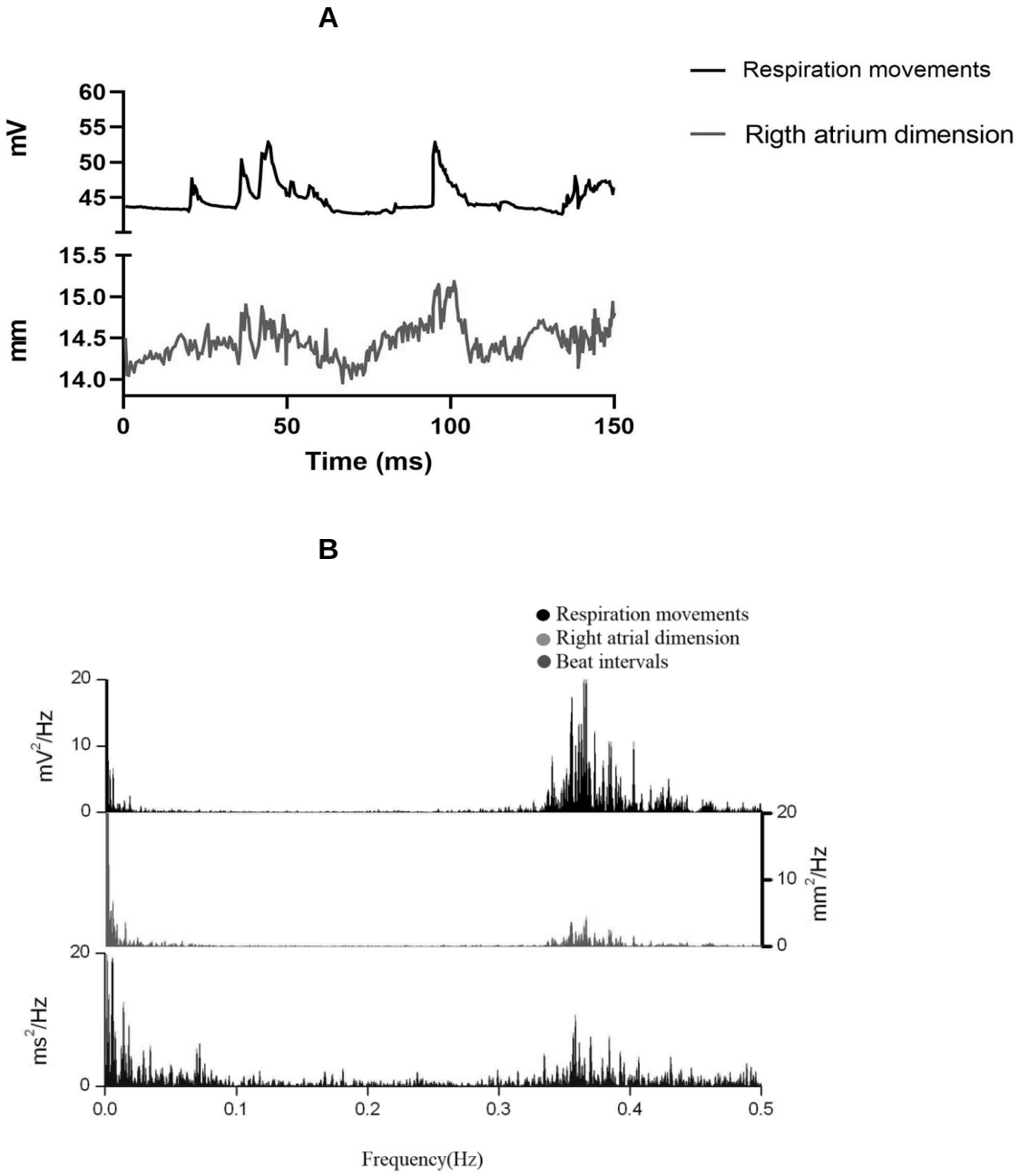

Figure 2. Recordings in a conscious sheep.
A. Respiratory movements (in mV, upper row) and right atrial dimension (lower row). It can be seen that during spontaneous changes in respiration the dimension of the right auricle increases with inspiration and decreases with expiration.
B. Spectral analysis of respiration movements, right atrial dimension and fluctuation of beat intervals. There is a clear correspondence between the fundamental frequency of the three variables.

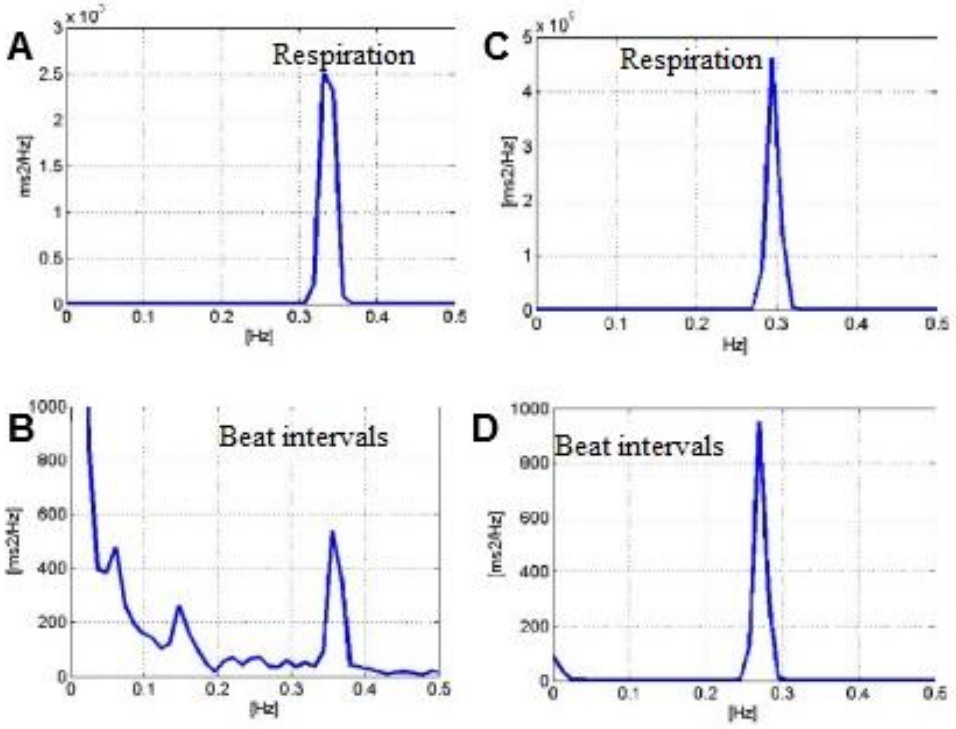

Figure 3. Spectral analysis of respiration and beat intervals fluctuation in an anesthetized sheep.
A-B Sheep without ANS blockade.
A- Power spectrum density of respiration shows a fundamental frequency induced by mechanical ventilation.
B- The beats intervals follows the same frequency.
C-D Sheep with ANS blockade. The behavior of spectral density of both variables is virtually the same

Figure 3 (A and B) shows the spectral analysis of respiration and beat to beat intervals in an anesthetized sheep. The mechanical ventilation allows the regularity of the respiratory movements that in turn induce the changes of beat intervals with the same frequency. This is the expression of the RSA in a mechanical ventilated animal. However, at the Figure 3 (C and D), the same behavior is obtained in an animal with pharmacological blockade of the ANS.

In Figure 4 it can be seen that in a sheep with the blockade, the occlusion of the posterior cave vein lead to a reduction of RAD with the consecutive increment in the duration of beat to beat intervals (bradycardia). When occlusion was released, the blood flow increased RAD which is accompanied by a reduction of the duration of intervals (tachycardia). Considering the blockade of ANS we can say that the observed changes are coherent with a response related to a mechanical distention of right atrium.

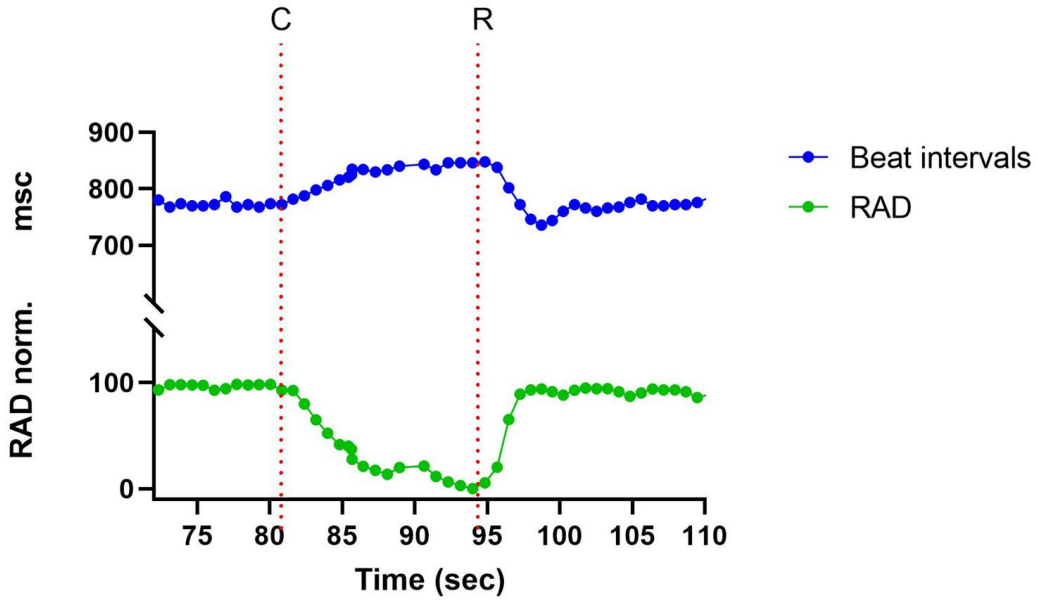

Figure 4. Beat intervals (upper row) and right atrial dimensions (lower row) of an anesthetized sheep with a blockade of ANS.
The line signaled as C indicates the beginning of the compression of the posterior cave vein.
The line signaled as R indicates the end of such compression (see text for discussion).

Conclusions

The relationship between Respiratory Sinus Arrhythmia (SRA) and ANS is well established and indexes of Heart Rate variability (HRV) that reflect SRA (rMSSD, HF band of the spectral analysis, SD1 axis at Poincare's plot etc.) could be good estimators for ANS function. Nevertheless, the role of non-neural mechanisms, particularly those related with the distension of right atrium cannot be ruled out.

Accordingly, with the cited bibliography, our results show that changes in right atrial dimensions in animals with blocked SNA, induce fluctuations in pacemaker rate suggesting that autonomic changes are not the only cause of RSA. However, the exact relationship between such mechanical responses and the ANS reflexes remains to be elucidated.

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REFERENCES

[1] **Billman GE.** Heart rate variability -A historical perspective. *Front Physiol.* 2011;2:1–13.

[2] **Hales S.** Statical Essays: Containing Haemastaticks; Or, An Account of some Hydraulick and Hydrostatical Experiments made on the Blood and Blood-Vessels of Animals. *Meet Counc R Soc* 1733;2:426.

[3] **Ludwig C.** Beitrage zur Kennt- niss des Einflusses der Respriations bewegungen auf den Blutlauf im Aortensysteme. *Arch Anat Physiol* 13, 242–3027. 1847;

[4] **Bainbridge F.** The influence of venous filling upon the rate of the heart. *J Physiol.* 1915;50(2):65–84.

[5] **Bainbridge F.** The relation between respiration and the pulse-rate. *J Physiol.* 1920;54(3):192-202.

[6] **Blinks JR.** Positive chronotropic effect of increasing right atrial pressure in the isolated mammalian heart. *Am J Physiol.* 1956;186(2):299–303.

[7] **Brooks CM, Lu HH, Lange G, Mangi R, Shaw RB, Geoly K.** Effects of localized stretch of the sinoatrial node region of the dog heart. *Am J Physiol* 1966; 211(5):1197–202.

[8] **Lange G, Lu HH, Chang A, Brooks CM.** Effect of stretch on the isolated cat sinoatrial node. *Am J Physiol.* 1966;211(5):1192–6.

[9] Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Heart Rate Variability : Standards of Measurement, Physiological Interpretation, and Clinical Use. *Circulation.* 1996;93:1043–65.

[10] **Pagani M, Lombardi F, Guzzetti S, Rimoldi O, Furlan R, Pizzinelli P, Sandrome G, Malfatto G, Dell'Orto S, Piccaluga E.** Power spectral analysis of heart rate and arterial pressure variabilities as a marker of sympatho-vagal interaction in man and conscious dog. *Circ Res.* 1986;59(2):178–93.

[11] **Kantzides A, Owens NC, De Matteo R, Badoer E.** Right atrial stretch activates neurons in autonomic brain regions that project to the rostral ventrolateral medulla in the rat. *Neuroscience.* 2005;133(3):775–86.

[12] **Spyer KM.** Central nervous mechanisms contributing to cardiovascular control. *J Physiol Annu Rev Prize Lect.* 1994;474:1–19.

[13] **Gilbey M, Jordan D, Ritcher D, Spyer K.** Synaptic mechanisms involved in inspiratory modulation of vagal cardio-inhibitory neurones in the cat. *J Physiol.* 1984;(356):65–78.

[14] **Akselrod S, Gordon D, Ubel F, Shannon D, Barger C, Cohen RJ.** Power spectrum analysis of heart rate control. *Science* 1981;213(4504):220–2.

[15] **Xhyheri B, Manfrini O, Mazzolini M, Pizzi C, Bugiardini R.** Heart Rate Variability Today. *Prog Cardiovasc Dis.* 2012;55(3):321–31.

[16] **Parati G, Mancia G, Di Rienzo M, Castiglioni P, Taylor A, Studinger P.** Cardiovascular variability is/is not an index of autonomic control of circulation. *J Appl Physiol* 2006; 101:690-691

[17] **Kohl P, Hunter P, Noble D.** Stretch-induced changes in heart rate and rhythm: Clinical observations, experiments and mathematical models. *Prog Biophys Mol Biol.* 1999;

[18] **Bernardi L, Keller F, Sanders M, Reddy PS, Griffith B, Meno F, Pinsky MR**. Respiratory sinus arrhythmia in the denervated human heart. *J Appl Physiol* 1989;67(4):1447–55.

[19] **Slovut DP, Wenstrom JC, Moeckel RB, Wilson RF, Osborn JW, Abrams JH.** Respiratory sinus dysrhythmia persists in transplanted human hearts following autonomic blockade. *Clin Exp Pharmacol Physiol.* 1998; 23(5):322-30.

[20] **Horner SM, Murphy CF, Coen B, Dick DJ, Harrison FG, Vespalcova Z, et al.** Contribution to heart rate variability by mechanoelectric feedback. Stretch of the sinoatrial node reduces heart rate variability. *Circulation* 1996;94(7):1762–7

[21] **Perlini S, Solda PL, Piepoli M, Sala-Gallini G, Calciati A, Finardi G, Bernardi L.** Determinants of respiratory sinus arrhythmia in the vagotomized rabbit. *Am J Physiol - Heart Circ Physiol* 1995;269(3 38-3):H909–15.

[22] **Skrzypiec-Spring M, Grotthus B, Szelag A, Schulz R.** Isolated heart perfusion according to Langendorff-Still viable in the new millennium. *J Pharmacol Toxicol Methods.* 2007;55(2):113–26.

[23] **J. von Neumann, R. H. Kent**. HRB and BIH. The mean square succesive difference. *Ann Math Stat.* 1941;12(2):153–62.

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