



Methodology of monitoring cardiovascular regulation

Metodologija praćenja kardiovaskularne regulacije

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Introduction

Adaptation of an organism to changes in external and internal environments is regulated by the autonomic nervous system (ANS)¹⁻⁴. The ANS is structurally and functionally positioned to interface between the internal and external milieu, coordinating bodily functions to ensure normal homeostasis and adaptive responses to environmental changes⁴. The neural control of cardiovascular (CV) system plays a major role in such adaptations, even if different humoral mechanisms also participate in this control. In fact, dynamic environmental changes contrasting basic functional needs of the organism dramatically challenge the CV adaptive mechanisms. The fact that “cardiovascular diseases are the leading cause of death in the world today and will remain so by the year 2020” (The WHO MONICA Project⁵) strongly supports the need for new insights into CV regulatory mechanisms. This review considers recent studies which focus on the understanding of CV regulation and the methodology for monitoring CV regulation.

Cardiovascular regulation

CV neural regulation occurs through both sympathetic and parasympathetic (vagal) outflow to the heart and vessels. Central autonomic drives act directly from the central nervous system (CNS) on the heart and vessels, while peripheral drives are relayed to the heart and vessels through, among others, the baroreflex function. These drives are relayed to

the heart through sympathetic and parasympathetic outflows and to blood vessels through sympathetic outflow only (Figure 1). Classically, central integration modifies the performance of individual reflexes according to the prevailing behavioural needs (*ie* exercise)⁶. Thus, heart period (HP) is modified together with other controlled hemodynamic variables, such as vascular resistance and, consequently, arterial blood pressure (BP)⁷. Numerous techniques for studying autonomic control of CV system are based on HP and arterial BP analysis (Figure 2). These techniques can be divided in two groups: techniques based on induced fluctuations of arterial BP and techniques based on analysis of spontaneous fluctuations of BP (Figure 2). Here we put emphasis on the techniques of HP and BP spontaneous fluctuations due to their numerous advantages with respect to the techniques based on induced fluctuations of arterial BP. The resulting HP and arterial BP values obtained by this technique reflect the overall interaction between central and peripheral mechanisms of CV regulation, without providing straightforward information on separate central and peripheral contributions being the result of rather complex interplay⁸. As a result of the activity of different mechanisms involved, HP and arterial BP fluctuations are commonly observed in physiological conditions. These fluctuations are present even in the absence of motor behaviour, like in paralysed animals⁹. Such fluctuations in hemodynamic parameters reflect both the presence of a variety of naturally occurring physiological perturbations to CV homeostasis (*i.e.* respiration, postural shifts, thermoregulation) and the dynamic response

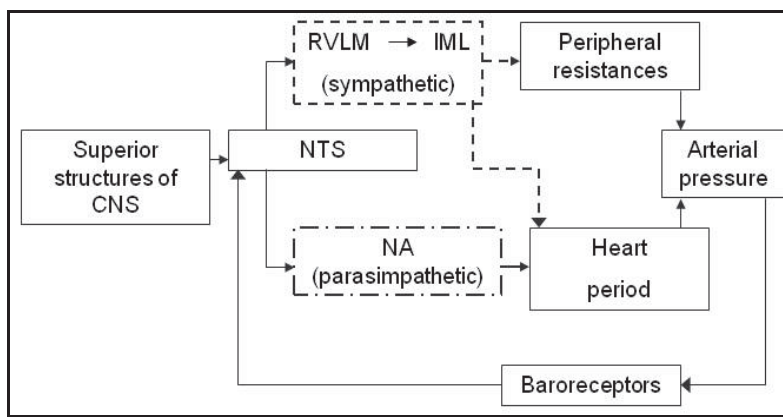


Fig. 1 – Mechanisms of peripheral (baroreflex) and central regulation of cardiovascular system
 NTS – nucleus tractus solitarius; RVLM – rostral ventrolateral medulla; IML – intermediolateral column; NA – nucleus ambiguus.
 Long dash spotted line-parasympathetic nervous system drive, dashed line-sympathetic nervous system drive

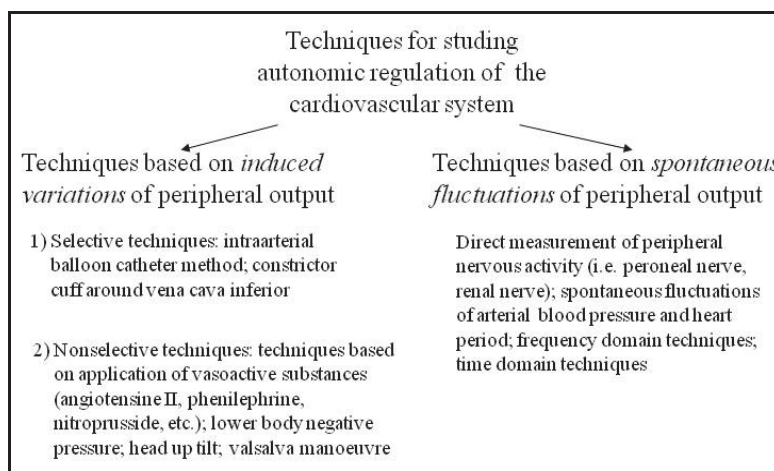


Fig. 2 – Scheme of techniques for studying the autonomic regulation of cardiovascular system

of the CV control systems to these perturbations¹⁰. Increase of arterial BP and HP variability after sinoaortic baroreceptor deafferentation and their decrease after following pharmacological sympathetic and parasympathetic ganglionic block^{9,11} suggest that a significant part of spontaneous, steady-state arterial BP and HP variability is due to the interaction in central and peripheral mechanisms of CV control.

Central control

There is a lack of data on the central pathways subserving “central command” responses (Figure 1). The idea of a “central command” signal originated from the observations that heart rate, ventilation and BP increase almost immediately at the onset of voluntary exercise⁶. “Central command”, in the case of BP and heart rate increase at the beginning of the exercise has a clear functional implication in matching blood flow to increased metabolic rate of an organism¹².

A dramatic demonstration of “central command” as a feed-forward regulation in the absence of muscle activity is shown in the study of Gandevia et al.¹³, in which a paralysed, artificially ventilated human subject attempted to perform isometric contractions. It resulted in marked concomitant increases in arterial BP and heart rate, which were

graded according to the degree of the attempted force. Concomitant hypotensive and bradycardic changes central by origin are found in sleep¹⁴ and opioid anesthesia¹⁵. According to our findings central command has different impact on the organism with respect to the age¹⁶.

A number of cerebral areas appear to be involved in central control of CV function. These areas are mostly located in the frontal cortex and include parts of the cingulate and insular cortex¹⁷, orbitofrontal cortex¹⁸, amygdala¹⁹, dorsomedial hypothalamic nucleus²⁰ and midbrain^{21,22}.

Baroreflex control

Baroreflexes represent classic negative feed-back mechanisms (Figure 1). Changes in baroreceptor input to the brain provoke changes in neural output in two branches of the ANS – sympathetic and parasympathetic branches. The parasympathetic (vagal) system controls about 75% of the fastest baroreflex effector loop – heart rate, up to 100 beats/min. The sympathetic system controls the remaining 25% of this effector, and further controls conductance and contractility of the heart, and total peripheral resistance¹.

The parasympathetic system acts fast and powerfully, and can change heart rate within one beat¹. Due to the different dynamics of neurotransmitter release, different intra-

cellular effector molecular mechanisms and different mechanisms of neurotransmitter removal from neuromuscular synaptic cleft, sympathetic nervous system act slower with respect to parasympathetic system²³.

Blood pressure set point

Baroreceptor reflex performance is modified by various mechanisms of the CNS. Though criticised for erroneous associations it might provoke between biological systems and the servo control system, the term “set point” is in use for description of the “desired level” of BP and baroreflex mechanism²⁴.

The set point (level around which arterial pressure is regulated) varies under different physiological (*ie* exercise)⁶ and pathophysiological (*ie* hypertension)²⁵ conditions. A natural selection appears to favour a control system that regulates arterial pressure around a set point that varies according to an animal's behaviour²⁴.

Baroreflex sensitivity

Baroreflex sensitivity (BRS), or baroreflex gain is defined as a transfer function between a primary (input) change in BP and reflex (output) compensatory change in BP or heart rate²⁴. From the classical studies of baroreflex functioning²⁶ till the recent investigations^{9, 11 27–29} the open loop baroreflex studies were done in anesthetised or pharmacologically treated animals. In conscious animals and humans, it is very difficult to perform an open-loop analysis of baroreflex gain.

Methods for studying mechanisms of cardiovascular regulation-techniques

Techniques for analysis of HP and BP induced fluctuations

These methods apply external stimulus for the evaluation of baroreflex loop and perform so-called “spot” analysis of BRS³⁰.

Many basic laboratory techniques are applicable to experimental evaluation of selective carotide or aortic arch baroreceptors (intravascular occlusion of corresponding artery³¹. Selective unloading of cardiopulmonary baroreceptors is performed by an inflating cuff placed around *vena cava inferior*. All these methods are invasive, demanding anesthesia and applicable only to laboratory animals.

Non-selective tests of baroreceptor function stimulate whole groups of baroreceptors, without any care for their regional and functional differences.

Orthostatic tests like stand test, tilt test and lower body negative pressure (LBNP) test are widely used for clinical and scientific purposes of investigating CV regulation³². The head-up tilt test is the method used for investigation of syncope, presyncope, dizziness and palpitations related to dysautonomia symptoms³³. Lately, the test has been criticized due to great variation in sensitivity and specificity rates in different studies, as well as for its limited accuracy and reproducibility³⁴. In this technique baroreflex stimulus is physiological, but the specificity is limited, due to unloading

of cardiopulmonary baroreceptors and stimulation of vestibular centers³⁵.

LBNP induces, with the depression below iliac crest, fluid shift (blood and interstitial fluids) towards the lower part of the body. LBNP stimulates CV system, in a particular a baroreflex regulation loop by unselective unload of these receptors. The LBNP test can cause syncope and progressive fluid shift can cause CV changes that are not stationary, not providing this important condition for further mathematical analysis of the signal³⁶.

A method for application of vasoactive substances (Oxford method) was founded by Smyth et al.,³⁷ in 1969. It is based on intravascular injection of vasoactive substances, like angiotensin II, phenylephrine or nitroprusside. It is used as the gold standard method for BRS measuring. It is methodologically simple, more specific, but it quantifies only arterial BP-HP baroreflex loop. Vasoactive substances also act directly on the CNS structures, cardiopulmonary receptors, as well as on sinoatrial node³⁸.

Valsalva manoeuvre is based on tachycardic or bradycardic response on the initial decrease or increase of arterial BP appearing during constant expiratory pressure (40 mmHg) lasting for 15–20 s. It is a noninvasive, simple method, but its disadvantages are the involvement of chemoreceptors, cardiopulmonary baroreceptors, muscle receptors and it also requires active collaboration of a patient³⁹.

Techniques for analysis of HP and BP spontaneous fluctuations

A basic methodological advantage of these techniques is continuous measurement of BRS and higher level of sensitivity on baroreflex dysfunction as compared to classical methods^{30, 40, 41}.

Direct measurement of sympathetic nerve activity in peroneal nerve or in renal nerve allows measurement of BRS as the responsiveness of sympathetic nervous activity to the changes of BP⁴². The sympathetic bursts are synchronized with transient reductions of BP and are silenced during increased pressure⁴³.

Spontaneous fluctuations in HP and arterial BP have been explored both in frequency and time domains during the last two decades. Spontaneous sequences of HP and arterial BP beat-to-beat values have been used to study different aspects of CV regulation in physiological and pathophysiological states. An important step in evaluation of CV control came from the recognition that oscillations in HP and BP result both from the operation of feed-back regulatory loops^{41, 44} and from “central commands”^{45–47}. In clinical use, there are different software packages, like Nevrokard[®], compatible with Finapres[®], Portapres[®], Colin[®] and BIOPAC[®] monitors.

Frequency domain techniques

Studies on the frequency domain^{10, 40, 48, 49} have provided a novel insight into the interplay of sympathetic and vagal CV modulations, leading to new tools for studying CV control. The frequency components of these fluctuations can

be assessed by spectral analysis⁵⁰ and reflect major changes in autonomic control of heart and vessels. HP power spectra depict the modulation of autonomic control on sinoatrial node, not its absolute value⁵¹. In many conditions, the modulation amplitude is proportional to its absolute value^{48,52}. In HP power spectra, the low-frequency band (less than 0.15 Hz in humans⁵⁰, 0.45 Hz in rats⁵³, 0.6 Hz in mice⁵⁴), has been associated with the modulation of both sympathetic and parasympathetic outflow, while the high frequency band (greater than 0.15 Hz in humans⁵⁰, 1.04 Hz in rats⁵³, 1.0 Hz in mice⁵⁴) has been associated with the modulation of parasympathetic outflow⁵⁵. The contribution of sympathetic and parasympathetic efferent activity to low frequency and high frequency HP and BP power spectra, respectively, has been confirmed during wakefulness⁵⁶ and sleep⁵⁷ by experiments using selective pharmacological blockade (propranolol, atropine).

Time domain techniques

Analysis of the continuous relationship of beat-by-beat changes in arterial pressure and HP revealed that spontaneous increases or decreases in systolic arterial pressure ("ramps") induce directionally similar reflex changes in HP⁵⁸. On this basis, a novel technique called "spontaneous baroreflex analysis" was developed for dynamic studying of the arterial baroreflex control of the sinus node⁴⁴. This widely accepted method^{45,59-62} is based on a computer scanning of BP and HP time series to identify sequences of spontaneously occurring consecutive beats in which BP and HP change in the same direction, (named "baroreflex sequences") i.e. hypertensive/bradycardic and hypotensive / tachycardic sequences⁴⁴.

Measuring BRS from spontaneous variations in BP and heart rate⁴⁴ has several advantages over methods that artificially induce changes in BP. This method excludes administration of vasoactive compounds or external appliances that could influence the baroreceptor reflex by a direct action on receptor or effector sites⁶³. BRS is measured within physiological BP ranges, allowing computation of the gain at BP close to the operating set point value, with minimal non-specific effects from other efferent nerves⁴⁰. The baroreceptor gain thus obtained is closest to the physiological one. These methods do not arouse subjects or animals, thereby reducing stress-induced effects. In contrast to pharmacological or mechanical methods, they are suitable to assess BRS over prolonged periods of time^{30,41}.

Methods that evaluate BRS from spontaneous changes in BP and HP make use of linear regression analysis of HP vs spontaneously occurring ramps in BP^{44,58,64}, and of spectral analysis⁶⁵ or other statistical relationships between BP and pulse interval changes⁶⁶.

BRS calculated as a slope of HP vs BP linear regression in spontaneously occurring pressure ramps⁴⁴ shows the best correlation to reference pharmacological methods and gives zero value following interruption of the baroreflex arch^{41,44,60}.

Apart from "baroreflex sequences", beat-to-beat analysis of the continuous relationship between spontaneous fluctua-

tions in BP and HP also reveals the occurrence of sequences of consecutive beats in which BP and HP change in the opposite direction (*ie* hypertensive/tachycardic and hypotensive/bradycardic sequences). These sequences have been defined as "non-baroreflex"⁴⁴.

The physiological meaning and thus the possible role of non-baroreflex sequences in evaluation of central command of the CV regulation is still controversial. Oosting et al.⁴¹ include in BRS index calculation all BP sequences, non-baroreflex sequences as well, regardless the direction of HP changes with respect to pressure changes. The idea behind this approach is that the relationship between HP and arterial BP includes both baroreflex and random influences; if baroreflex – mediated effects on HP are present, they should appear as such when averaging over ramps is performed⁴¹. In addition, this technique included $49.8 \pm 4.1\%$ of all the recorded beats in BRS index calculation. Calculation also included a significant number of sequences that corresponded to non-baroreflex ones.

The main limitation of this approach is that the BRS index is mainly a measure of parasympathetic reaction, being calculated on short sequences (9.7 ± 1.6 beats, mean \pm SEM) and with a delay of HP vs arterial BP (3, 4 and 5 beats) that is too short to take account a full sympathetic reaction to an arterial pressure change⁴⁶. HP changes induced by vagal reactions would superimpose upon slow sympathetically induced ones⁴¹.

Furthermore, Legramante et al. demonstrated in anaesthetised rabbits⁶² and humans⁴⁵ that spontaneously occurring non-baroreflex sequences can be considered an expression of autonomic regulatory mechanisms operating with feed-forward features, as it is the case of "central command". They have calculated a baroreflex gain on sequences where heart rate and BP changed in the same direction⁴⁴, while the gain of feed-forward mechanisms was calculated on non-baroreflex sequences. The same authors demonstrated that both branches of the ANS take part in feed-forward mechanisms of short-term CV neural regulation^{45,62}. Recent investigation on conscious freely moving rats⁴⁷ have provided further evidence that non-baroreflex sequences reflect mechanisms feed forward in origin. A complete autonomic pharmacological blockade reduced the number of non-baroreflex sequences, as did sympathetic blockade, selective alpha-receptor blockade did not induce changes, while beta-receptor blockade induced a significant decrease in non-baroreflex sequences occurrence. Moreover, parasympathetic blockade induced increase in non-baroreflex sequences. The results of Pagani et al.⁴⁸ demonstrate that physiological role of non-baroreflex sequences is an expression of feed-forward type of short term CV regulation being in dynamic interaction with feed-back mechanisms of baroreflex origin.

Still, the intrinsic limitation of this method in evaluating feed-forward mechanisms is a small number of beats (in animals $\approx 5\%$ ⁶² and in humans $\approx 7\%$ ⁴⁵) organised in sequences characterised by a non-baroreflex pattern. This finding is in contrast with the fact that feed-forward mechanisms can be engaged for a prominent fraction of time⁶.

Zoccoli et al.⁴⁶ suggest that parallel analysis of both the BRS index of Bertinieri et al.⁴⁴ and the BRS index of Oosting et al.⁴¹, and a novel index in time domain sensitive to slow sympathetic fluctuations would overcome the limitations of the method of Oosting et al.⁴¹ in estimating the relative contribution of feed-back control, and the limitations of the method of Legramante et al.⁴⁷ in estimating feed-forward control over HP and offer a more complete picture of the interrelation between peripheral and central mechanisms in HP control. The index⁴⁶ b_{HPMAP} is calculated as an index of linear regression of arterial BP vs HP 30s sequences. It correlates well with indexes of Bertinieri et al.⁴⁴ and Oosting et al.⁴¹ in quiet wakefulness of the conscious rats, while in active sleep correlates significantly with the sympathovagal index. We have reported that the index b_{HPMAP} can reflect sympathetic changes in the time domain as well⁴⁶. This data suggest that the overall picture of baroreflex-central command interaction can be achieved by comparative analysis of more than one method for calculation of BRS and feed-forward gain proposed in the literature.

Short-comment and conclusion

It is well-known that phasic and tonic increases in central drive to the heart both as impaired baroreflex regulation might increase the incidence and severity of cardiac arrhythmias⁶⁷. An increased central drive is also present in acute stress (classic “defense” or “alerting” response⁶⁸, chronic psychological stress⁶⁹, acute physical stress⁷⁰ as well as during arousal⁷¹, acoustic stimulation⁷²). In all circumstances, the central drive and impaired baroreflex both were positively correlated to the incidence of cardiac arrhythmia in susceptible subjects. On the basis of these results, the techniques of CV monitoring keep an important place in studying pathophysiological mechanisms of arrhythmogenesis.

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R E F E R E N C E S

1. Rowell LB. Reflex control during orthostasis. In: Rowell LB, editor. Human cardiovascular control. New York: Oxford University Press; 1993. p. 37–80.
2. Berne RM, Levy MN. The cardiovascular system: regulation of heart beat. In: Berne RM, Levy MN, editors. Physiology. 3rd ed. St. Louis: Mosby-Year Book; 1993. p. 417–37.
3. Franchini KG, Cowley AW. Autonomic control of cardiac function. In: Robertson D, Low PA, Polinsky RJ, editors. Primer on the autonomic nervous system. San Diego: Academic Press; 1996. p. 42–8.
4. Hamill RW. Peripheral autonomic nervous system. In: Robertson D, Low PA, Polinsky RJ, editors. Primer on the autonomic nervous system. San Diego: Academic Press; 1996. p. 12–25.
5. The World Health Organization. The WHO MONICA Project [updated on 2009 September19]. Available from: <http://www.ktl.fi/monica>. 2002.
6. Rowell LB. What signals govern the cardiovascular responses to exercise? Role of central command. In: Rowell LB, editor. Human cardiovascular control. 1st ed. New York: Oxford University Press; 1993. p. 500.
7. Cavalcanti S, Belardelli E. Modeling of cardiovascular variability using a differential delay equation. IEEE Trans Biomed Eng 1996; 43(10): 982–9.
8. Valentini M, Parati G. Variables influencing heart rate. Prog Cardiovasc Dis 2009; 52(1): 11–9.
9. Dworkin BR, Dworkin S, Tang X. Carotid and aortic baroreflexes of the rat: I. Open-loop steady-state properties and blood pressure variability. Am J Physiol Regul Integr Comp Physiol 2000; 279(5): R1910–21.
10. Akselrod S, Gordon D, Madwed JB, Snidman NC, Shannon DC, Cohen RJ. Hemodynamic regulation: investigation by spectral analysis. Am J Physiol 1985; 249(4 Pt 2): H867–75.
11. Dworkin BR, Tang X, Snyder AJ, Dworkin S. Carotid and aortic baroreflexes of the rat: II. Open-loop frequency response and the blood pressure spectrum. Am J Physiol Regul Integr Comp Physiol 2000; 279(5): R1922–33.
12. Rowell LB. Central circulatory adjustments to dynamic exercise: Regulation of the central circulation. In: Rowell LB, editor. Human cardiovascular control. 1st ed. New York: Oxford University Press; 1993. p. 255–301.
13. Gandevia SC, Killian K, McKenzie DK, Cranford M, Allen GM, Gorman RB, et al. Respiratory sensations, cardiovascular control, kinaesthesia and transcranial stimulation during paralysis in humans. J Physiol 1993; 470: 85–107.
14. Baust W, Bohnert B. The regulation of heart rate during sleep. Exp Brain Res 1969; 7(2): 169–80.
15. Jaffe HJ, Martin WR. Opioid analgesics and antagonists. In: Gilman AG, Goodman LS, Roll TW, Muraol F, editors. The pharmacological basis of therapeutics. 5th ed. New York: Macmillan Publishing Co; 1975. p. 1703.
16. Silvani A, Asti V, Bojic T, Ferrari V, Franzini C, Lenzi P, et al. Sleep-dependent changes in the coupling between heart period and arterial pressure in newborn lambs. Pediatr Res 2005; 57(1): 108–14.
17. Oppenheimer SM, Gelb A, Girvin JP, Hachinski VC. Cardiovascular effects of human insular cortex stimulation. Neurology 1992; 42(9): 1727–32.
18. Dampney RA. Functional organization of central pathways regulating the cardiovascular system. Physiol Rev 1994; 74(2): 323–64.
19. Iwata J, Chida K, LeDoux JE. Cardiovascular responses elicited by stimulation of neurons in the central amygdaloid nucleus in awake but not anesthetized rats resemble conditioned emotional responses. Brain Res 1987; 418(1): 183–8.
20. Dampney RA, Coleman MJ, Fontes MA, Hirooka Y, Horiuchi J, Li YW, et al. Central mechanisms underlying short- and long-term regulation of the cardiovascular system. Clin Exp Pharmacol Physiol 2002; 29(4): 261–8.
21. Schenber LC, Vasquez EC, da Costa MB. Cardiac baroreflex dynamics during the defence reaction in freely moving rats. Brain Res 1993; 621(1): 50–8.
22. Verberne AJ, Owens NC. Cortical modulation of the cardiovascular system. Prog Neurobiol 1998; 54(2): 149–68.
23. Levy MN, Martin PJ. Autonomic Control of cardiac conduction and automaticity. In: Shepherd JT, Vatner SF, editors. Nervous control of the heart. Amsterdam: Harwood Academic Publishers; 1996. p. 201–26.
24. Sagawa K. Baroreflex control of systemic arterial pressure and vascular bed. In: Shepherd JT, Abboud FM, editors. Hand book of physiology. Section 2: The cardiovascular system.

- Bethesda (MD): American Physiological Society; 1983. p. 453–96.
25. Korner P, Bobik A, Oddie C, Friberg P. Sympathoadrenal system is critical for structural changes in genetic hypertension. *Hypertension* 1993; 22(2): 243–52.
 26. Moissejeff E. Zur Kenntnis des Carotissinus reflexes. *Z Gesamte Exp Med* 1926; 53: 693–704.
 27. Porta A, Magagnin V, Bassani T, Tobaldini E, Montano N, van de Borne P. RR-SAP causality in heart transplant recipients. *Conf Proc IEEE Eng Med Biol Soc* 2010; 2010: 3449–52.
 28. Kawada T, Li M, Kamiya A, Shimizu S, Uemura K, Yamamoto H, et al. Open-loop dynamic and static characteristics of the carotid sinus baroreflex in rats with chronic heart failure after myocardial infarction. *J Physiol Sci* 2010; 60(4): 283–98.
 29. Kawada T, Kamiya A, Li M, Shimizu S, Uemura K, Yamamoto H, et al. High levels of circulating angiotensin II shift the open-loop baroreflex control of splanchnic sympathetic nerve activity, heart rate and arterial pressure in anesthetized rats. *J Physiol Sci* 2009; 59(6): 447–55.
 30. Mancina G, Mark AL. Arterial baroreflex in humans. In: *Shepherd JT, Abboud FM*, editors. *Hand book of physiology. Section 2: The cardiovascular system*. Bethesda (MD): American Physiological Society; 1983. p. 755–93.
 31. Horne RS, De Preu ND, Berger PJ, Walker AM. Arousal responses to hypertension in lambs: effect of sinoaortic denervation. *Am J Physiol* 1991; 260(4 Pt 2): H1283–9.
 32. Patel AR, Engstrom JE, Tusing LD, McNeeley KJ, Chelmsky TC. Lower body negative pressure: a test of cardiovascular autonomic function. *Muscle Nerve* 2001; 24(4): 481–7.
 33. Macedo PG, Leite LR, Santos-Neto L, Haubul D. Tilt test-from the necessary to the indispensable. *Arq Bras Cardiol* 2011; 96(3): 246–54. (English, Portuguese, Spanish)
 34. Sheldon R. Tilt testing for syncope: a reappraisal. *Curr Opin Cardiol* 2005; 20(1): 38–41.
 35. Barón-Esquínias G, Martínez-Rubio A. Tilt table test: state of the art. *Indian Pacing Electrophysiol J* 2003; 3(4): 239–52.
 36. Halliwill JR, Lawler LA, Eickhoff TJ, Joyner MJ, Mulvagh SL. Reflex responses to regional venous pooling during lower body negative pressure in humans. *J Appl Physiol* 1998; 84(2): 454–8.
 37. Smyth HS, Sleight P, Pickering GW. Reflex regulation of arterial pressure during sleep in man. A quantitative method of assessing baroreflex sensitivity. *Circ Res* 1969; 24(1): 109–21.
 38. Young CN, Fisher JP, Fadel PJ. The ups and downs of assessing baroreflex function. *J Physiol* 2008; 586(5): 1209–11.
 39. Kardos A, Rudas L, Simon J, Gingl Z, Csanády M. Effect of postural changes on arterial baroreflex sensitivity assessed by the spontaneous sequence method and Valsalva manoeuvre in healthy subjects. *Clin Auton Res* 1997; 7(3): 143–8.
 40. Parati G, Di Rienzo M, Omboni S, Mancina G. Computer analysis of blood pressure and heart rate variability in subjects with normal and abnormal autonomic cardiovascular control. In: *Mathias CJ, Bannister R*, editors. *Autonomic failure: A textbook of clinical disorders of the autonomic nervous system*. 4th ed. New York: Oxford University Press; 1999. p. 211–23.
 41. Oosting J, Struijker-Boudier HA, Janssen BJ. Validation of a continuous baroreceptor reflex sensitivity index calculated from spontaneous fluctuations of blood pressure and pulse interval in rats. *J Hypertens* 1997; 15(4): 391–9.
 42. Hart EC, Joyner MJ, Wallin BG, Karlsson T, Curry TB, Charkoudian N. Baroreflex control of muscle sympathetic nerve activity: a nonpharmacological measure of baroreflex sensitivity. *Am J Physiol Heart Circ Physiol* 2010; 298(3): H816–22.
 43. Delius W, Hagbarth KE, Hongell A, Wallin BG. General characteristics of sympathetic activity in human muscle nerves. *Acta Physiol Scand* 1972; 84(1): 65–81.
 44. Bertinieri G, Di Rienzo M, Cavallazzi A, Ferrari AU, Pedotti A, Mancina G. Evaluation of baroreceptor reflex by blood pressure monitoring in unanesthetized cats. *Am J Physiol* 1988; 254(2 Pt 2): H377–83.
 45. Legramante JM, Raimondi G, Massaro M, Iellamo F. Positive and negative feedback mechanisms in the neural regulation of cardiovascular function in healthy and spinal cord-injured humans. *Circulation* 2001; 103(9): 1250–5.
 46. Zoccoli G, Andreoli E, Bojic T, Cianci T, Franzini C, Predieri S, et al. Central and baroreflex control of heart rate during the wake-sleep cycle in rat. *Sleep* 2001; 24(7): 753–8.
 47. Legramante JM, Sacco S, Raimondi G, Di Lecce VN, Pallante M, Di Nardo P, et al. Investigating feedforward neural regulation of circulation from analysis of spontaneous arterial pressure and heart rate fluctuations in conscious rats. *Am J Physiol Heart Circ Physiol* 2009; 296(1): H202–10.
 48. Pagani M, Lombardi F, Guzzetti S, Rimoldi O, Furlan R, Pizzinelli P, et al. 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.
 49. Malliani A. The Pattern of Sympathovagal Balance Explored in the Frequency Domain. *News Physiol Sci* 1999; 14: 111–7.
 50. Aksefrod S, Gordon D, Ubel FA, Shannon DC, Berger AC, Cohen RJ. Power spectrum analysis of heart rate fluctuation: a quantitative probe of beat-to-beat cardiovascular control. *Science* 1981; 213(4504): 220–2.
 51. Lenzi P, Zoccoli G, Franzini C. Regulation of the cerebral and extracerebral circulation during the wake-sleep cycle. In: *Lugaresi E, Parmeggiani PL*, editors. *Somatic and autonomic regulation in sleep. Physiological and clinical aspects*. Milan: Springer-Verlag; 1997. p. 25–41.
 52. Pomeranz B, Macaulay RJ, Caudill MA, Kutz I, Adam D, Gordon D, et al. Assessment of autonomic function in humans by heart rate spectral analysis. *Am J Physiol* 1985; 248(1 Pt 2): H151–3.
 53. Cerutti C, Gustin MP, Paultre CZ, Lo M, Julien C, Vincent M, et al. Autonomic nervous system and cardiovascular variability in rats: a spectral analysis approach. *Am J Physiol* 1991; 261(4 Pt 2): H1292–9.
 54. Ishii K, Kuwabara M, Tsubone H, Sugano S. Autonomic nervous function in mice and voles (*Microtus arvalis*): investigation by power spectral analysis of heart rate variability. *Lab Anim* 1996; 30(4): 359–64.
 55. Malliani A, Pagani M, Lombardi F, Cerutti S. Cardiovascular neural regulation explored in the frequency domain. *Circulation* 1991; 84(2): 482–92.
 56. Japundžić N, Gricbois ML, Zitoun P, Laude D, Elghozi JL. Spectral analysis of blood pressure and heart rate in conscious rats: effects of autonomic blockers. *J Auton Nerv Syst* 1990; 30(2): 91–100.
 57. Zemaityte D, Varoneckas G, Sokolov E. Heart rhythm control during sleep. *Psychophysiology* 1984; 21(3): 279–89.
 58. Fritsch JM, Eckberg DL, Graves LD, Wallin BG. Arterial pressure ramps provoke linear increases of heart period in humans. *Am J Physiol* 1986; 251(6 Pt 2): R1086–90.
 59. Hughson RL, Quintin L, Annat G, Yamamoto Y, Gharib C. Spontaneous baroreflex by sequence and power spectral methods in humans. *Clin Physiol* 1993; 13(6): 663–76.
 60. Parlow J, Viale JP, Annat G, Hughson R, Quintin L. Spontaneous cardiac baroreflex in humans. Comparison with drug-induced responses. *Hypertension* 1995; 25(5): 1058–68.
 61. Nakazato T, Shikama T, Toma S, Nakajima Y, Masuda Y. Nocturnal variation in human sympathetic baroreflex sensitivity. *J Auton Nerv Syst* 1998; 70(1–2): 32–7.
 62. Legramante JM, Raimondi G, Massaro M, Cassarino S, Peruzzini G, Iellamo F. Investigating feed-forward neural regulation of circulation from analysis of spontaneous arterial pressure

- and heart rate fluctuations. *Circulation* 1999; 99(13): 1760–6.
63. *Coleman TG*. Arterial baroreflex control of heart rate in the conscious rat. *Am J Physiol* 1980; 238(4): H515–20.
64. *Parati G, Di Rienzo M, Bertinieri G, Pomidossi G, Casadei R, Gropelli A*, et al. Evaluation of the baroreceptor-heart rate reflex by 24-hour intra-arterial blood pressure monitoring in humans. *Hypertension* 1988; 12(2): 214–22.
65. *Robbe HW, Mulder LJ, Rüddel H, Langewitz WA, Veldman JB, Mulder G*. Assessment of baroreceptor reflex sensitivity by means of spectral analysis. *Hypertension* 1987; 10(5): 538–43.
66. *Cerutti C, Ducher M, Lantelme P, Gustin MP, Paultre C*. Assessment of spontaneous baroreflex sensitivity in rats a new method using the concept of statistical dependence. *Am J Physiol* 1995; 268(2 Pt 2): R382–8.
67. *DeSilva RA*. Central nervous system risk factors for sudden cardiac death. *Ann N Y Acad Sci* 1982; 382: 143–61.
68. *Sokolov EN*. Perception and the conditioned reflex. Elmsford, NY: Pergamon Press; 1963.
69. *Parker GW, Michael LH, Hartley CJ, Skinner JE, Entman ML*. Central beta-adrenergic mechanisms may modulate ischemic ventricular fibrillation in pigs. *Circ Res* 1990; 66(2): 259–70.
70. *Bjelakovic B, Ilic S, Chouliaras K, Milovanovic B, Vukomanovic V, Bojic T*, et al. Heart rate variability in children with exercise-induced idiopathic ventricular arrhythmias. *Pediatr Cardiol* 2010; 31(2): 188–94.
71. *Verrier RL, Kirby DA*. Sleep and cardiac arrhythmias. *Ann N Y Acad Sci* 1988; 533: 238–51.
72. *Silvani A, Bojic T, Cianci T, Franzini C, Lodi CA, Predieri S*, et al. Effects of acoustic stimulation on cardiovascular regulation during sleep. *Sleep* 2003; 26(2): 201–5.

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