Measuring the time-dependent effects of dobutamine on the modulations of cardiac activity in rats

Short title: Measuring the time-dependent modulations of cardiac activity in rats

Diego Candia-Rivera*, Sofia Carrion-Falgarona, Fabrizio de Vico Fallani, Mario Chavez

Sorbonne Université, Paris Brain Institute (ICM), CNRS UMR722, INRIA Paris, INSERM U1127, AP-HP Hôpital Pitié Salpêtrière, 75013, Paris, France.

** Correspondence: diego.candia.r@ug.uchile.cl*

Abstract

The measurement of the autonomic modulations of cardiac dynamics plays a crucial role in the understanding of cardiovascular health and disease. We aimed to estimate the time-resolved sympathetic and parasympathetic modulations of cardiac dynamics in a rat models. In this study, we present the adaptation of the method for rat cardiac analysis and compare it with standard measures of low-frequency (LF) and high-frequency (HF) components, which are commonly used for sympathetic and parasympathetic activity estimation, respectively.

To evaluate the performance of our method, we study a dataset comprising spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY). These rats were administered dobutamine to elicit autonomic dynamics. The results obtained from our method demonstrated accurate time-resolved depiction of sympathetic reactivity induced by dobutamine administration. These responses closely resembled the expected autonomic alterations observed during physical exercise conditions, albeit emulated pharmacologically. The comparisons with LF and HF measures further confirmed the effectiveness of our method in better capturing autonomic changes in rat cardiac dynamics. Our findings highlight the potential of our adapted method for time-resolved analysis in future clinical and translational studies involving rodents' models. The validation of our approach in animal models opens new avenues for investigating the relationship between ongoing changes in cardiac activity and parallel changes in brain dynamics. Such investigations are crucial for advancing our understanding of the intricate brain-heart connection, particularly in cases involving neurodegeneration, brain injuries, and cardiovascular conditions.

Introduction

Cardiac activity is regulated in the short term through two synergistic pathways: the sympathetic system, which acts as a positive ionotropic, and the parasympathetic system, with negative ionotropic effect [1–3]. In severe conditions such as cardiogenic shock or heart failure, a significant alteration in arterial tension can critically compromise hemodynamics, potentially leading to organ failure. Research spanning human and rodent models suggest that disruptions in cardiac tropism may manifest through alterations in heart rate variability [4]. To optimize organ perfusion, sympathomimetic agents like dobutamine, a β1-adrenergic agonist, have been developed to enhance heart contractility and output [5]. However, a comprehensive understanding of this intricacy requires further rigorous investigations in well-controlled in vivo settings.

Standard heart rate variability (HRV) analysis in rats involves time-wrapped measures such as mean heart rate, standard deviation of R-R intervals or power density features, which are purposed to provide insights on the sympathetic and parasympathetic modulations of cardiac dynamics [6]. Recent evidence in rats has shown that activations in vagal activity does not correlate with commonly used HRV metrics [7]. This indicates the need for further development of HRV methods to understand the modulation of sympathetic and parasympathetic activity on cardiac dynamics in rats. Previously, nonlinear methods have been suggested as an alternative for investigating autonomic dynamics in rats, however, these methods have produced conflicting results [8,9].

Recent advancements in human studies, where it was gathered a relationship between parallel fluctuations in brain and cardiac dynamics, highlighted the advantages of the time-resolved analysis of cardiac dynamics. However, there has been limited exploration in this area regarding animal models, particularly in examining the real-time shifts in autonomic dynamics in rats, which have been largely overlooked. We recently proposed a time-resolved measurements of sympathetic and parasympathetic modulations of cardiac dynamics in humans, which was validated in different experimental conditions demonstrating its robustness [10]. Our method surpassed the standard approach that estimates sympathetic and parasympathetic modulations of heartbeat dynamics in the frequency domain, where the low frequency (LF) and high frequency (HF) bands are integrated as an approximation of the sympathetic and parasympathetic modulations, respectively [11].

The frequency domain analysis of HRV in humans has been challenged because of the inaccurate estimation of sympathetic activity within the LF range [12]. To address this issue,

the method we recently introduced a method that effectively separates slow and fast HRV oscillations in combination with changes in baseline heart rate, enabling a more precise estimation of sympathetic and parasympathetic modulations of heartbeat dynamics. In this study, we present an adapted version of our previously developed method for humans, specifically tailored for analyzing HRV in rats. We validate the efficacy of our method using a dataset of rats exposed to dobutamine [13,14], where dobutamine emulates a non-resting state condition similar to the physiological responses due to exercise due to sympathetic activations [15]. We applied our method to spontaneously hypertensive rats (SHR), known for their rapid sympathetic reactivity [16], and then tested on a group of Wistar-Kyoto rats (WKY). The validation of our approach in a rat model opens new avenues for advancing our understanding of cardiovascular dynamics and their relationships with ongoing neural dynamics in different clinical conditions.

Methods

Dataset description

Open access datasets were used in this study [13,14], comprising 22 rats from two different strains were included in this study: Wistar–Kyoto (WKY) and spontaneously hypertensive rats (SHR), Charles River, Wilmington, MA, USA. The rats were 12-week-old, all males, and they weighted between 300–400 g. All rats were aseptically implanted with radiotelemeters (Model TA11CTA-F40, Data Sciences International, St. Paul, MN, USA) for continuous measurement of the electrocardiogram (ECG). ECGAuto software (EMKA technologies USA, Falls Church, VA, USA) was used to visualize individual ECG signals and analyze and measure RR segment durations.

The rats were catheterized intravenously in the left jugular vein for the administration of dobutamine. Dobutamine (20, 40, 80, 160 and 320 μg/kg; Sigma-Aldrich, St. Louis, MO, USA) was infused 0.2 ml/min for 2 min, after a 5-min baseline period [13,14].

The experimental protocols were approved by and in accordance with the guidelines of the Institutional Animal Care and Use Committee of the United States Environmental Protection Agency, Research Triangle Park, North Carolina. The data were gathered from the United States Environmental Protection Agency website (https://doi.org/10.23719/1503098).

Half of the cohort consisted of rats exposed to diesel exhaust, while the other half was exposed to purified air. However, our main goal was not to examine differences between these two conditions. This decision was based on current evidence, which suggests no short-term effects of diesel exhaust on cardiovascular dynamics [17,18]. Most reported effects typically correspond to either long-term exposure [19] or influence individuals with pre-existing conditions [20].

For simplicity, the various doses of dobutamine administration were combined in the statistical analyses. Due to the substantial variability in the duration of both pre- and post-infusion periods, we established a minimum duration of 30 seconds for each. This criterion led to 41 trials for all SHR rats and 52 trials for all WKY rats. For further detail on the dataset, please refer to the original studies [13,14].

Estimation of cardiac sympathetic and parasympathetic indices

The estimation of cardiac sympathetic and parasympathetic indices was based on a previously developed model for humans [10]. The model is based on the Poincaré plot, a method that depicts the fluctuations on the duration of consecutive IBI [21], as shown in Figure

1A. Three features are extracted from the Poincaré plot: the distance to the origin (R) that quantifies baseline heart rate, and the minor (SD1) and major (SD2) ratios of the ellipse that quantify the short- and long-term fluctuations of HRV, respectively [22].

The time-varying fluctuations of the distance to the origin and the ellipse ratios were computed with a sliding-time window, as follows:

$$R(t) = \sqrt{mean(IBI_{i,\ldots,n-1})^2 + mean(IBI_{i+1,\ldots,n})^2} \quad (1)$$

$$SD_1(t) = \sqrt{\lambda_{\Omega_t}(1)} \quad (2)$$

$$SD_2(t) = \sqrt{\lambda_{\Omega_t}(2)} \quad (3)$$

where $\lambda_{\Omega_t}(i)$ denotes the ith the eigenvalue of the covariance matrix of $IBI_{i,\ldots,n-1}$ and $IBI_{i+1,\ldots,n}$, with $\Omega_t: t - T \leq t_i \leq t$, and n is the length of IBI in the time window Ω_t.

The original method included the implementation of four different approaches to compute the covariance matrices: "exact", "robust", "95%" and "approximate" [10]. In this study, the robust approach was used, which computes the covariance matrix using a shrinkage covariance estimator based on the Ledoit-Wolf lemma for analytic calculation of the optimal shrinkage intensity [23]. That sliding time window to compute the indices (T) was fixed in 3 seconds, which takes into account that typical interbeat interval of the rat is around 100 ms and slow fluctuations occur in the range 0.1 – 1 Hz [24].

The distance to the origin R_0 and ellipse ratios SD_{01} and SD_{02} for the whole experimental duration are computed to re-center the time-resolved estimations of R, SD1 and SD2. The Cardiac Parasympathetic Index (CPI) and the Cardiac Sympathetic Index (CSI), are computed as follows:

$$D(t) = \overline{\mathrm{R}}(t) + R_0 \quad (4)$$

$$CPI(t) = \mathrm{k}_p \cdot (\overline{SD_1}(t) + SD_{01}) + \widetilde{D}(t) \quad (5)$$

$$CSI(t) = \mathrm{k}_s \cdot (\overline{SD_2}(t) + SD_{02}) + \mathrm{D}(t) \quad (6)$$

where $\overline{SD_x}$ is the demeaned SD_x and $\widetilde{D}$ is the flipped D with respect the mean.

The coefficients k_p and k_s determine the influence of fast and slow HRV oscillations relative to changes in the baseline heart rate. In this study, these values were experimentally determined using the SHR dataset and subsequently validated in the WKY dataset. The coefficients were established by comparing the experimental conditions (rest vs. dobutamine) across a range of k_p and k_s values (from 1 to 20). Statistical differences were assessed based on paired comparisons using the Wilcoxon test.

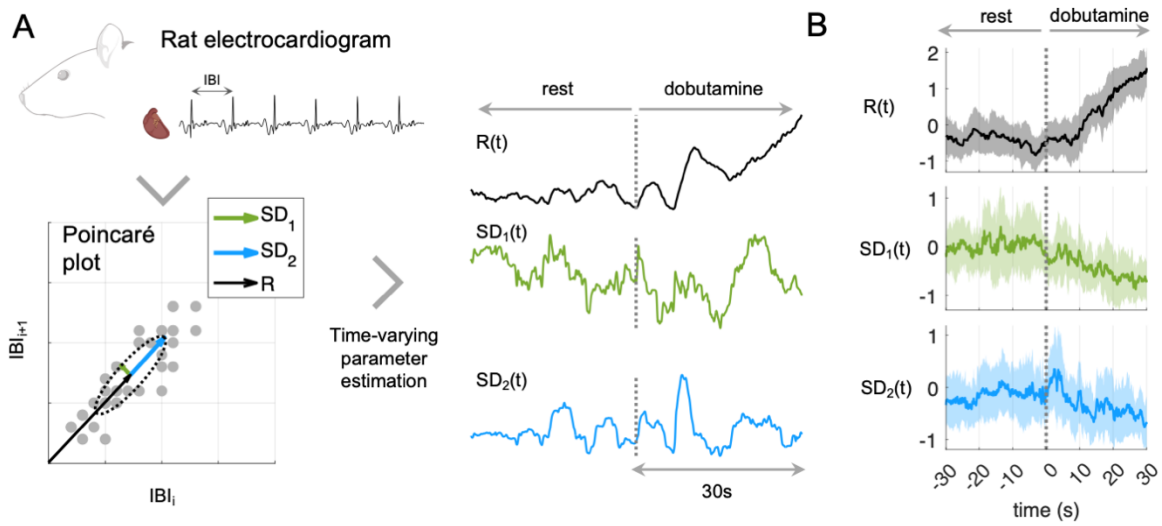

Figure 1. Pipeline for the estimation of the fluctuating parameters of the Poincaré plot. (A) The Poincaré plot illustrates the consecutive changes in interbeat intervals (IBI). The parameters computation includes the distance (R) from the ellipse center to the origin, and the minor (SD1) and major ratios (SD2). (B) Group median and median absolute deviation in bold curve and shaded areas of R, SD1 and SD2.

Estimation of sympathetic and parasympathetic indices using spectral components

The spectral HRV analysis was computed following the adapted Wigner–Ville method, for estimating the LF and HF time series [25]. First, the RR series were evenly re-sampled to 20 Hz using the spline interpolation and demeaned. The pseudo-Wigner–Ville algorithm consists of a two-dimensional Fourier transform with an ambiguity function kernel to perform two-dimensional filtering [25]. It is important to note that in rats, the inter beat interval (IBI) can be as short as 100 ms, compared to the typical range of 600 to 1000 ms in humans [26]. As a result, the LF and HF bands in rat studies have been redefined multiple times, typically as 0.1-1 Hz and 1-3.5 Hz (or alternatively 0.1-1.5 Hz and 1.5-5 Hz), respectively [24]. In this study, the frequency bands were defined as LF: 0.1-1 Hz and HF: 1-3.5 Hz.

Statistical analysis

To statistically evaluate the performance of the two methods (time-frequency and Poincaré plot) in discerning the experimental conditions, we used a two-sided non-parametric Wilcoxon signed-rank test for paired samples. The time-resolved information for all the estimated features was condensed as the average value for each experimental session (30 s duration each condition), and the group-wise descriptive measures are expressed as medians and median absolute deviations. Multiple comparisons were corrected using the Bonferroni rule.

Results

We investigated cardiac dynamics in two rat strains subjected to autonomic elicitation through dobutamine infusion. Our method, based on Poincaré plot descriptors of RR intervals, computed cardiac sympathetic and parasympathetic indices (CSI and CPI). Using the SHR strain, we determined parameters k_p and k_s, which define the weight of HRV components with respect to the changes in baseline heart rate. Parameter definition involved testing k_p and k_s values to effectively distinguish between rest and dobutamine conditions for CSI and CPI estimation, respectively. The Z-value from the Wilcoxon test comparing both conditions served as an indicator for optimal parameters. The inflection point for CSI was identified at k_s =3, and for CPI, it was at k_p=12, as illustrated in Figure 2A. Figure 2B provides an example of a single rat, depicting the estimation of CSI and CPI for various values of k_p and k_s.

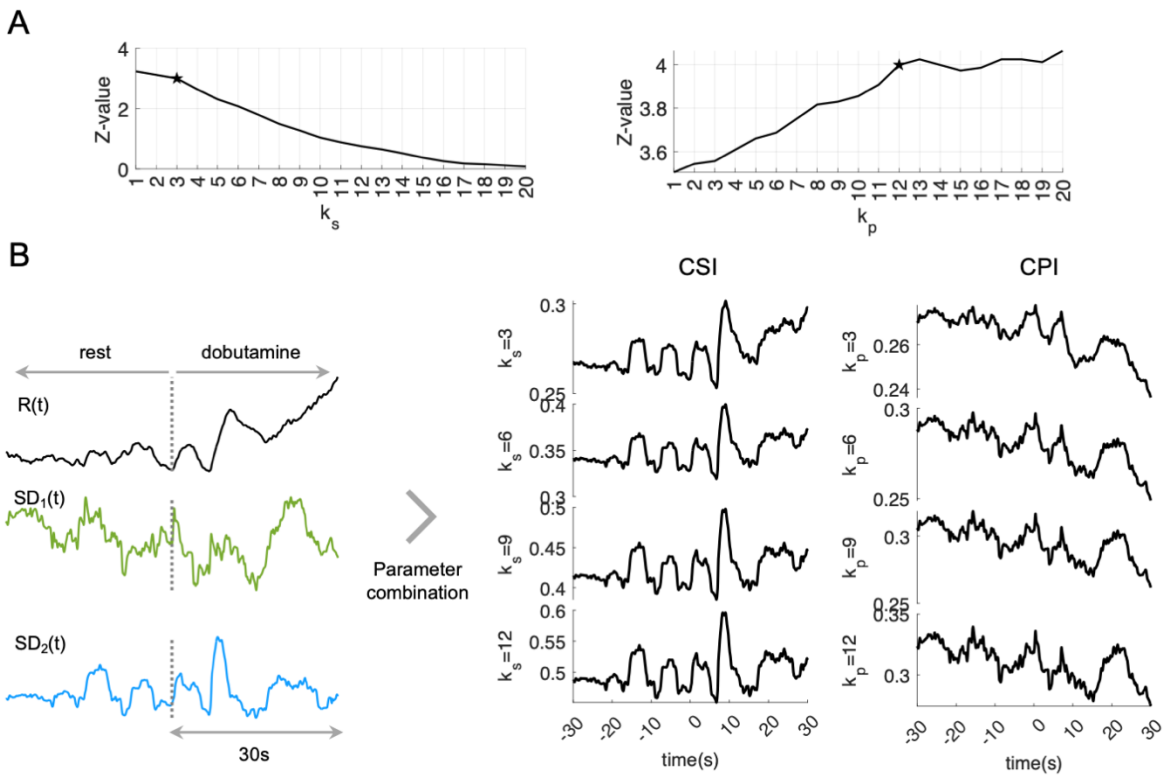

Figure 2. Estimation of the parameters k_p and k_s for the estimation of CSI and CPI in the SHR strain. (A) The Z-value from the Wilcoxon test comparing CSI and CPI for the conditions rest vs dobutamine, as a function of k_p and k_s values. The star indicates the inflection point from which k_s =3 and k_p=12 were used for the rest of the study. (B) Changes in R, SD1 and SD2 for one rat in the transition from rest to dobutamine infusion, and their respective CSI and CPI estimation based on different values of k_p and k_s.

Using the optimal parameters identified in Figure 2, we present the results of the statistical comparison between rest and dobutamine conditions for the two rat strains in Table 1. Furthermore, we include comparisons of their spectral counterparts, LF and HF. Our findings indicate a consistently higher distinguishability of conditions for CSI and CPI compared to LF and HF.

Figure 3 illustrates time-resolved transitions for all autonomic markers, segmented by rat strain. In Figure 3A, the SHR strain exhibits a distinct increase in sympathetic markers following dobutamine infusion, with CSI surpassing LF. Simultaneously, there is a reduction in parasympathetic activity, where CPI proves more pronounced than HF. Similar sympathetic trends are observed in the WKY strain, while the WKT strain displays no significant change in HF after dobutamine infusion, with CPI emerging as a more robust marker in this scenario. The distribution of averaged autonomic markers during rest and dobutamine periods in Figure 3B further underscores the robustness of CSI and CPI in comparison to their spectral estimates counterparts.

Table 1. Wilcoxon test rest vs dobutamine

	Sympathetic markers		Parasympathetic markers	
	LF	CSI	HF	CPI
SHR	**p = 0.00378** **Z =** 2.8962	**p = 0.00112** **Z =** 3.2590	**p = 0.00117** **Z =** -3.2461	**p = 0.00008** **Z =** -3.9458
WKY	p = 0.04917 Z = 1.9671	**p < 0.00001** **Z =** 4.6718	p = 0.77073 Z = 0.2914	**p = 0.00101** **Z =** -3.2876

Bold indicates significance after multiple-comparisons correction using Bonferroni rule, defined at $\alpha = 0.05/4 = 0.0125$

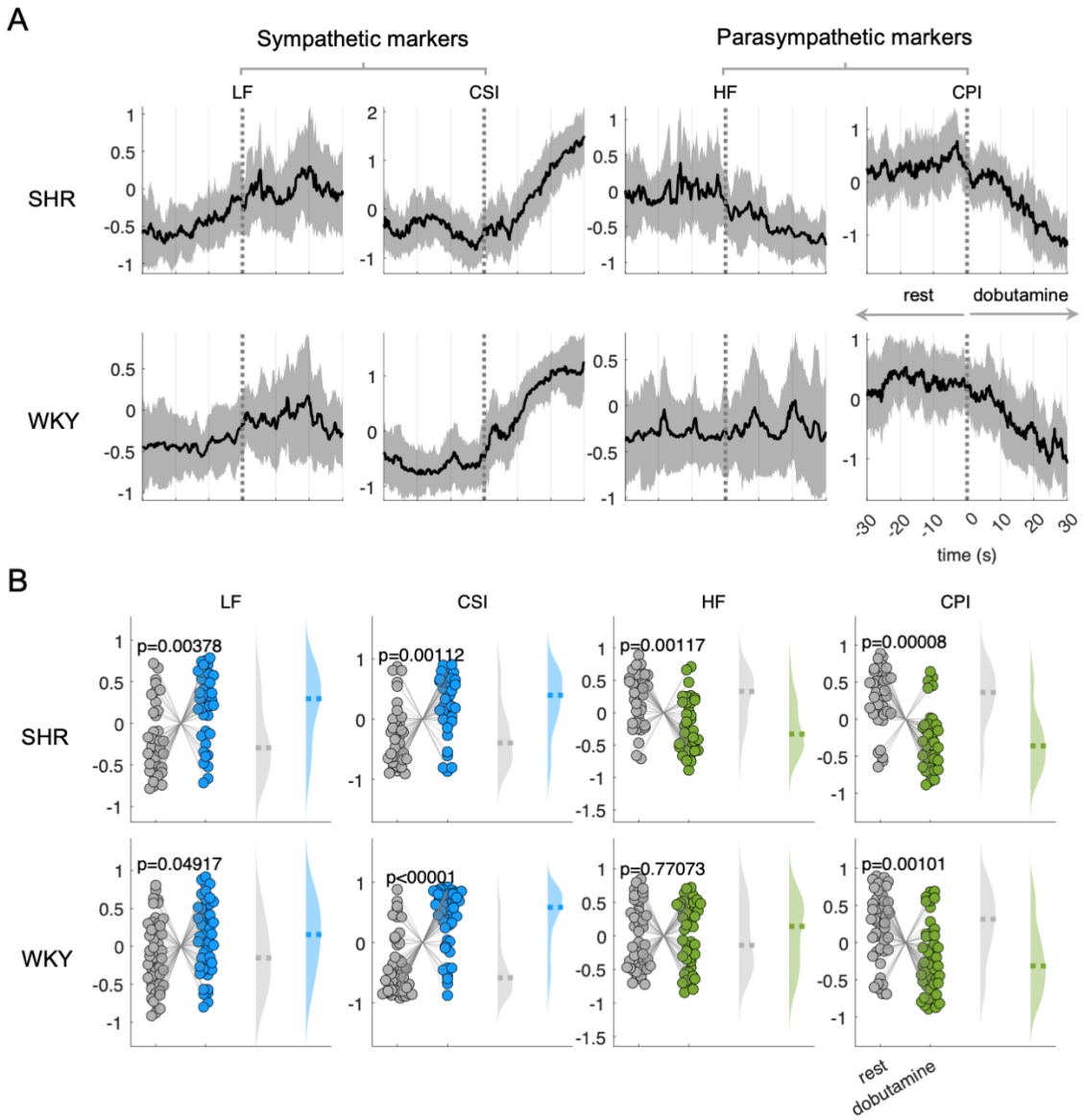

Figure 3. Cardiac autonomic indices CSI and CPI, and their spectral counterparts LF and HF under the rest to dobutamine transition. (A) Time course of the computed indices between -30 to 30 s with respect to the condition change onset. The plot indicates the group median and the shaded areas the median absolute deviation. Time series were z-score normalized per subject before averaging for visualization purposes. (B) Statistical comparison using a signed rank Wilcoxon test, comparing the mean 30 s after the condition change with respect to the 30 s before. Dashed lines indicate the group median. All signal amplitude units are arbitrary units.

Discussion

Heart rate variability (HRV) analysis using power spectral analysis has the potential to be a valuable noninvasive technique for assessing autonomic nervous activity in rats [6]. However, invasive recordings on the left cervical vagus nerve in rats have revealed that tonic vagal activity does not correlate with commonly used HRV metrics. This indicates the need for further development of HRV methods to understand the modulation of sympathetic and parasympathetic activity on cardiac dynamics in rats [7].

Inotropic factors are widely used to compensate cardiac deficiencies. Dobutamine acts as a positive ionotropic, known as sympathomimetic [2]. In our proposed method, previously validated in humans [10,27], we accurately show the time-dependent effects of autonomic elicitation induced by dobutamine in rats, unraveling some of the key components of cardiac regulation by both sympathetic and parasympathetic systems. As expected, our results show that dobutamine triggers an increase in sympathetic activity, shown as a sudden increase in slow fluctuations in HRV, followed by a transient increase in the baseline heart rate. In parallel, a transient decrease in parasympathetic activity occurs. We did not observe different effects in the WKY with respect to the SHR strain, although sympathetic hyperreactivity is expected in the SHR strain, as compared to WKY [28]. However, the sympathetic reactivity has been reported only in changes in the baseline heart rate, and not necessarily in the slow fluctuations in HRV [29]. Importantly, our approach surpasses the spectral counterparts, LF and HF, in the description of sympathetic and parasympathetic activities, respectively. Findings suggest that time-resolved estimation of autonomic modulations of cardiac activity holds great potential for the study of the dynamic changes of the brain-heart crosstalk.

In this study, we present an adapted and validated method for the rat that has been successfully used in humans under various standard conditions, allowing for the measurements of the modulation of cardiac sympathetic and parasympathetic activities. Our method offers the advantage of considering autonomic modulations in both baseline heart rate and HRV. It is important to study baseline heart rate in conjunction with heart rate variability to fully comprehend sympathetic dynamics, as sympathetic modulations of heartbeat dynamics may be reflected solely in changes in the baseline heart rate, rather than changes in HRV (e.g., LF or LF/HF ratio), and vice versa [30].

Further exploration is needed to elucidate the dynamics of rat cardiac activity that extend beyond baseline heart rate or variations in HRV. Nonlinear methods have been suggested as an alternative for investigating autonomic dynamics. However, these methods have produced

conflicting results, suggesting that increased sympathetic modulation may contribute to nonlinear dynamics [9], while also indicating that increased sympathetic modulation is likely the main cause of lower entropy [8].

Although our study has some limitations, such as a small sample size or the inclusion of all-male strains, the results demonstrate the robustness of our estimation of autonomic system parameters. Cumulative effects of increasing doses of dobutamine injection were not explored in this study, and their potential long-lasting impact was not investigated. However, the sympathetic and parasympathetic modulation of heart rate occurs rapidly following a perturbator element, amidst a complex interplay of regulatory loops spanning multiple time scales [31]. Our study deliberately focused on short-term modulation effects to minimize any potential enduring impact on our experimental approach. Our proposal has not been tested under other standard conditions of autonomic elicitation, such as monitoring heart rate during cold exposure [32], which constitutes the main limitation of our study. However, our proposal constitutes a first attempt for a developing field of research on time-resolved brain-heart analysis, applied to animal models. It is worth mentioning that the study under pharmacological interventions to modulate autonomic nervous system activity remain a standard approach [6,33–39]. For accurate time-resolved estimation, it is crucial to set a sliding time window that captures cardiac rhythm oscillations. We established a 3-second window to balance high time resolution with the ability to capture slow fluctuations in heart rate variability, a key marker of sympathetic activity. Determining this window size should rely on the known physiological priors, considering expected response latency and the involved frequencies.

Our method opens new research opportunities for clinical and translational studies to explore the potential of using heartbeat dynamics as biomarkers for assessing conditions characterized by disrupted autonomic dynamics. Future research may include the description of our developed markers under different stressors [40–43], and cardiovascular conditions [44–46]. Our development may also enable the investigation of disruptions that may impact the connection between the brain and the heart [47], and their subsequent effects on neural functions. For instance, in the study of severe brain damage resulting from cardiovascular conditions like stroke [48] or anoxia [49], as well as in cases of damage to the spinal cord [50]. These conditions present compelling areas of interest for further exploration in which the time-resolved estimation of autonomic dynamics can be a hallmark in the comprehension of nervous-system-wise complex dynamics.

Conclusions

Our study presents a robust approach for time-resolved estimation of sympathetic and parasympathetic modulations in rat cardiac dynamics, demonstrating its efficacy in capturing autonomic responses triggered by pharmacological interventions. The application of our method in animal models paves the way for future research aimed at unraveling the complex interplay between cardiac and brain activity in various pathological conditions.

Acknowledgements

This research was supported by the French Agence Nationale de la Recherche (ANR-20-CE37-0012-03).

Author contributions

Conceptualization: DCR; Figures: DCR; Data Analysis: DCR; Writing – first version of the manuscript: DCR; Writing – review & editing: DCR, SCF, FDVF, MC.

References

1. Ruffolo RR. Review: The pharmacology of dobutamine. American Journal of the Medical Sciences. 1987;294: 244–248. doi:10.1097/00000441-198710000-00005
2. Mahoney L, Shah G, Crook D, Rojas-Anaya H, Rabe H. A Literature Review of the Pharmacokinetics and Pharmacodynamics of Dobutamine in Neonates. Pediatric Cardiology. 2016;37: 14–23. doi:10.1007/s00246-015-1263-9
3. Spyer KM. The Central Nervous Organization of Reflex Circulatory Control. In: Loewy AD, spyer KM, editors. Central Regulation of Autonomic Functions. Oxford University Press; 1990. p. 0. doi:10.1093/oso/9780195051063.003.0010
4. Macpherson AK, Neti S, Chutakositkanon C, Averbach M, Macpherson PA. The variation of dobutamine induced heart stress with heart rate. WIT Transactions on Biomedicine and Health. 2009;13: 17–26. doi:10.2495/BIO090021
5. Dubin A, Lattanzio B, Gatti L. The spectrum of cardiovascular effects of dobutamine - from healthy subjects to septic shock patients. Revista Brasileira de Terapia Intensiva. 2017;29: 490–498. doi:10.5935/0103-507X.20170068
6. Kuwahara M, Yayou K, Ishii K, Hashimoto S, Tsubone H, Sugano S. Power spectral analysis of heart rate variability as a new method for assessing autonomic activity in the rat. Journal of Electrocardiology. 1994;27: 333–337. doi:10.1016/S0022-0736(05)80272-9
7. Marmerstein JT, McCallum GA, Durand DM. Direct measurement of vagal tone in rats does not show correlation to HRV. Sci Rep. 2021;11: 1210. doi:10.1038/s41598-020-79808-8
8. Silva LEV, Lataro RM, Castania JA, da Silva CAA, Valencia JF, Murta LO, et al. Multiscale entropy analysis of heart rate variability in heart failure, hypertensive, and

sinoaortic-denervated rats: classical and refined approaches. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 2016;311: R150–R156. doi:10.1152/ajpregu.00076.2016
9. Silva LEV, Lataro RM, Castania JA, Silva CAA, Salgado HC, Fazan R, et al. Nonlinearities of heart rate variability in animal models of impaired cardiac control: contribution of different time scales. Journal of Applied Physiology. 2017;123: 344–351. doi:10.1152/japplphysiol.00059.2017
10. Candia-Rivera D, de Vico Fallani F, Chavez M. Robust and time-resolved estimation of cardiac sympathetic and parasympathetic indices. bioRxiv. 2023; 2023.11.18.567211. doi:10.1101/2023.11.18.567211
11. Task Force of the European Society of Cardiology the North American Society of Pacing. Heart Rate Variability: Standards of Measurement, Physiological Interpretation, and Clinical Use. Circulation. 1996;93: 1043–1065. doi:10.1161/01.CIR.93.5.1043
12. Goldstein DS, Bentho O, Park M-Y, Sharabi Y. Low-frequency power of heart rate variability is not a measure of cardiac sympathetic tone but may be a measure of modulation of cardiac autonomic outflows by baroreflexes. Exp Physiol. 2011;96: 1255–1261. doi:10.1113/expphysiol.2010.056259
13. Hazari M, Callaway J, Winsett D, Lamb C, Haykal-Coates N, Krantz Q, et al. Dobutamine "Stress" Test and Latent Cardiac Susceptibility to Inhaled Diesel Exhaust in Normal and Hypertensive Rats. Environmental health perspectives. 2012;120: 1088–93. doi:10.1289/ehp.1104684
14. Hazari MS, Lancaster JL, Starobin JM, Farraj AK, Cascio WE. Diesel Exhaust Worsens Cardiac Conduction Instability in Dobutamine-Challenged Wistar–Kyoto and Spontaneously Hypertensive Rats. Cardiovasc Toxicol. 2017;17: 120–129. doi:10.1007/s12012-016-9363-1
15. Sharma R, O'Driscoll JM, Saha A, Sritharan M, Sutton R, Rosen SD. Differing autonomic responses to dobutamine stress in the presence and absence of myocardial ischaemia. J Physiol. 2015;593: 2171–2184. doi:10.1113/JP270063
16. Shanks J, Manou-Stathopoulou S, Lu C-J, Li D, Paterson DJ, Herring N. Cardiac sympathetic dysfunction in the prehypertensive spontaneously hypertensive rat. American Journal of Physiology-Heart and Circulatory Physiology. 2013;305: H980–H986. doi:10.1152/ajpheart.00255.2013
17. Peretz A, Kaufman JD, Trenga CA, Allen J, Carlsten C, Aulet MR, et al. Effects of diesel exhaust inhalation on heart rate variability in human volunteers. Environmental Research. 2008;107: 178–184. doi:10.1016/j.envres.2008.01.012
18. Mills NL, Finlayson AE, Gonzalez MC, Törnqvist H, Barath S, Vink E, et al. Diesel exhaust inhalation does not affect heart rhythm or heart rate variability. Heart. 2011;97: 544–550. doi:10.1136/hrt.2010.199042
19. Bradley JM, Cryar KA, El Hajj MC, El Hajj EC, Gardner JD. Exposure to diesel exhaust particulates induces cardiac dysfunction and remodeling. Journal of Applied Physiology. 2013;115: 1099–1106. doi:10.1152/japplphysiol.00343.2013
20. Carll AP, Lust RM, Hazari MS, Perez CM, Krantz QT, King CJ, et al. Diesel Exhaust Inhalation Increases Cardiac Output, Bradyarrhythmias, and Parasympathetic Tone in Aged Heart Failure–Prone Rats. Toxicological Sciences. 2013;131: 583–595. doi:10.1093/toxsci/kfs295
21. Brennan M, Palaniswami M, Kamen P. Do existing measures of Poincare plot geometry reflect nonlinear features of heart rate variability? IEEE Transactions on Biomedical Engineering. 2001;48: 1342–1347. doi:10.1109/10.959330
22. Sassi R, Cerutti S, Lombardi F, Malik M, Huikuri HV, Peng C-K, et al. Advances in heart rate variability signal analysis: joint position statement by the e-Cardiology ESC Working Group and the European Heart Rhythm Association co-endorsed by the Asia Pacific Heart

Rhythm Society. Europace. 2015;17: 1341–1353. doi:10.1093/europace/euv015
23. Schäfer J, Strimmer K. A shrinkage approach to large-scale covariance matrix estimation and implications for functional genomics. Stat Appl Genet Mol Biol. 2005;4: Article32. doi:10.2202/1544-6115.1175
24. Rowan WH, Campen MJ, Wichers LB, Watkinson WP. Heart rate variability in rodents: uses and caveats in toxicological studies. Cardiovasc Toxicol. 2007;7: 28–51. doi:10.1007/s12012-007-0004-6
25. Orini M, Bailón R, Mainardi LT, Laguna P, Flandrin P. Characterization of dynamic interactions between cardiovascular signals by time-frequency coherence. IEEE Trans Biomed Eng. 2012;59: 663–673. doi:10.1109/TBME.2011.2171959
26. Konopelski P, Ufnal M. Electrocardiography in rats: a comparison to human. Physiol Res. 2016;65: 717–725. doi:10.33549/physiolres.933270
27. Candia-Rivera D, Boehme R, Salamone PC. Autonomic modulations to cardiac dynamics in response to affective touch: Differences between social touch and self-touch. bioRxiv; 2024. p. 2024.02.20.581146. doi:10.1101/2024.02.20.581146
28. Luft FC, Demmert G, Rohmeiss P, Unger T. Baroreceptor reflex effect on sympathetic nerve activity in stroke-prone spontaneously hypertensive rats. Journal of the Autonomic Nervous System. 1986;17: 199–209. doi:10.1016/0165-1838(86)90057-3
29. Ricksten SE, Lundin S, Thoren P. Spontaneous variations in arterial blood pressure, heart rate and sympathetic nerve activity in conscious normotensive and spontaneously hypertensive rats. Acta Physiol Scand. 1984;120: 595–600. doi:10.1111/j.1748-1716.1984.tb07425.x
30. Gonçalves H, Henriques-Coelho T, Bernardes J, Rocha AP, Brandão-Nogueira A, Leite-Moreira A. Analysis of heart rate variability in a rat model of induced pulmonary hypertension. Medical Engineering & Physics. 2010;32: 746–752. doi:10.1016/j.medengphy.2010.04.018
31. Pathak A, Velez-Roa S, Xhaët O, Najem B, Van De Borne P. Dose-dependent effect of dobutamine on chemoreflex activity in healthy volunteers. British Journal of Clinical Pharmacology. 2006;62: 272–279. doi:10.1111/j.1365-2125.2006.02657.x
32. Kuwahara M, Kawaguchi T, Ito K, Tsubone H. Effects of Taurine on Cardiovascular and Autonomic Nervous Functions in Cold Exposed rats. In: Azuma J, Schaffer SW, Ito T, editors. Taurine 7. New York, NY: Springer; 2009. pp. 533–540. doi:10.1007/978-0-387-75681-3_55
33. Aubert AE, Ramaekers D, Beckers F, Breem R, Denef C, Van de Werf F, et al. The analysis of heart rate variability in unrestrained rats. Validation of method and results. Computer Methods and Programs in Biomedicine. 1999;60: 197–213. doi:10.1016/S0169-2607(99)00017-6
34. Beckers F, Verheyden B, Ramaekers D, Swynghedauw B, Aubert AE. Effects of Autonomic Blockade on Non-Linear Cardiovascular Variability Indices in Rats. Clinical and Experimental Pharmacology and Physiology. 2006;33: 431–439. doi:10.1111/j.1440-1681.2006.04384.x
35. Bernat B, Erdelyi R, Fazekas L, Garami G, Szekeres RM, Takacs B, et al. Drug Candidate BGP-15 Prevents Isoproterenol-Induced Arrhythmias and Alters Heart Rate Variability (HRV) in Telemetry-Implanted Rats. Pharmaceuticals. 2023;16: 359. doi:10.3390/ph16030359
36. Coleman TG. Arterial baroreflex control of heart rate in the conscious rat. American Journal of Physiology-Heart and Circulatory Physiology. 1980;238: H515–H520. doi:10.1152/ajpheart.1980.238.4.H515
37. Mangin L, Swynghedauw B, Benis A, Thibault N, Lerebours G, Carré F. Relationships Between Heart Rate and Heart Rate Variability: Study in Conscious Rats. Journal of Cardiovascular Pharmacology. 1998;32: 601.

38. OHNUKI K, MORITANI T, ISHIHARA K, FUSHIKI T. Capsaicin Increases Modulation of Sympathetic Nerve Activity in Rats: Measurement Using Power Spectral Analysis of Heart Rate Fluctuations. Bioscience, Biotechnology, and Biochemistry. 2001;65: 638–643. doi:10.1271/bbb.65.638
39. Ramaekers D, Beckers F, Demeulemeester H, Aubert AE. Cardiovascular Autonomic Function in Conscious Rats: A Novel Approach to Facilitate Stationary Conditions. Annals of Noninvasive Electrocardiology. 2002;7: 307–318. doi:10.1111/j.1542-474X.2002.tb00179.x
40. Raffai G, Kocsis L, Mészáros M, Monos E, Dézsi L. Inverse-Orthostasis May Induce Elevation of Blood Pressure due to Sympathetic Activation. Journal of Cardiovascular Pharmacology. 2006;47: 287. doi:10.1097/01.fjc.0000203973.51608.58
41. Inagaki H, Kuwahara M, Tsubone H. Effects of Psychological Stress on Autonomic Control of Heart in Rats. Experimental Animals. 2004;53: 373–378. doi:10.1538/expanim.53.373
42. Park SE, Park D, Song K-I, Seong J-K, Chung S, Youn I. Differential heart rate variability and physiological responses associated with accumulated short- and long-term stress in rodents. Physiology & Behavior. 2017;171: 21–31. doi:10.1016/j.physbeh.2016.12.036
43. Ramaekers D, Beckers F, Demeulemeester H, Bert C, Denef C, Aubert A. Effects Of Melanocortins On Cardiovascular Regulation In Rats. Clinical and Experimental Pharmacology and Physiology. 2002;29: 549–558. doi:10.1046/j.1440-1681.2002.03690.x
44. Henze M, Hart D, Samarel A, Barakat J, Eckert L, Scrogin K. Persistent alterations in heart rate variability, baroreflex sensitivity, and anxiety-like behaviors during development of heart failure in the rat. American Journal of Physiology-Heart and Circulatory Physiology. 2008;295: H29–H38. doi:10.1152/ajpheart.01373.2007
45. Han SY, Gray E, Hughes G, Brown CH, Schwenke DO. Increased sympathetic drive during the onset of hypertension in conscious Cyp1a1-Ren2 rats. Pflugers Arch - Eur J Physiol. 2014;466: 459–466. doi:10.1007/s00424-013-1338-5
46. Scridon A, Gallet C, Arisha MM, Oréa V, Chapuis B, Li N, et al. Unprovoked atrial tachyarrhythmias in aging spontaneously hypertensive rats: the role of the autonomic nervous system. American Journal of Physiology-Heart and Circulatory Physiology. 2012;303: H386–H392. doi:10.1152/ajpheart.00004.2012
47. Samuels MA. The Brain–Heart Connection. Circulation. 2007;116: 77–84. doi:10.1161/CIRCULATIONAHA.106.678995
48. Kodama T, Kamata K, Fujiwara K, Kano M, Yamakawa T, Yuki I, et al. Ischemic Stroke Detection by Analyzing Heart Rate Variability in Rat Middle Cerebral Artery Occlusion Model. IEEE Transactions on Neural Systems and Rehabilitation Engineering. 2018;26: 1152–1160. doi:10.1109/TNSRE.2018.2834554
49. Gonçalves H, Henriques-Coelho T, Bernardes J, Rocha AP, Nogueira A, Leite-Moreira A. Linear and nonlinear heart-rate analysis in a rat model of acute anoxia. Physiol Meas. 2008;29: 1133. doi:10.1088/0967-3334/29/9/010
50. Inskip JA, Ramer LM, Ramer MS, Krassioukov AV, Claydon VE. Spectral Analyses of Cardiovascular Control in Rodents with Spinal Cord Injury. Journal of Neurotrauma. 2012;29: 1638–1649. doi:10.1089/neu.2011.2145