Modeling the time-resolved modulations of cardiac activity in rats: A study on pharmacological autonomic stimulation.

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

Sorbonne Université, Paris Brain Institute (ICM), CNRS UMR7225, INRIA Paris, INSERM U1127, Hôpital de la Pitié Salpêtrière AP-HP, 75013, Paris, France.

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

Abstract

Assessing cardiac dynamics over time is essential for understanding cardiovascular health and its parallel patterns of activity with the brain. We present a methodology to estimate the time-resolved sympathetic and parasympathetic modulations of cardiac dynamics, specifically tailored for the rat heart. To evaluate the performance of our method, we study a dataset comprising spontaneously hypertensive rats and Wistar-Kyoto rats. 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. We further compared our method with standard measures of low-frequency (LF) and high-frequency (HF) components, which are commonly used, although debated, for sympathetic and parasympathetic activity estimation. 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 brain-heart connection, particularly in cases involving neurodegeneration, brain injuries, and cardiovascular conditions.

Key points

- We developed a method for time-resolved estimation of sympathetic and parasympathetic modulations in rat cardiac dynamics, validated against standard LF and HF measures.
- We used a cohort of spontaneously hypertensive rats and Wistar-Kyoto rats, with dobutamine administration to induce autonomic responses.
- Our method accurately depicted time-resolved sympathetic reactivity similar to autonomic changes during physical exercise.
- Our findings suggest potential for future clinical and translational studies on the brain-heart connection, particularly in cardiovascular conditions.

Keywords

Autonomic nervous system, heart rate variability, dobutamine, Poincare plot

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 (Ruffolo, 1987; Spyer, 1990; Mahoney *et al.*, 2016), being these disruptions typically manifested in the heart rate and rhythmicity (or heart rate variability; HRV) (Macpherson *et al.*, 2009). Standard HRV analysis in rats typically relies on 2-3 minute time-averaged measures, such as mean heart rate or standard deviation of R-R intervals (Kuwahara *et al.*, 1994; Rowan *et al.*, 2007). However, this approach overlooks the rich oscillatory nature of cardiac dynamics, which could provide further insights about the sympathetic and parasympathetic modulations of cardiac dynamics.

Recent advancements in humans have shown that time-resolved analysis of cardiac rhythmicity is a valuable tool for understanding concurrent changes in brain activity (Candia-Rivera *et al.*, 2022; Sargent *et al.*, 2024), highlighting the advantages of the time-resolved analysis over time-averaged measures. Animal models have provided important insights into brain-heart interactions (Hsueh *et al.*, 2023; Jammal Salameh *et al.*, 2024), but the exploration of cardiac changes has largely been limited to simple increases or decreases in heart rate, overlooking the contextual shifts in cardiac rhythmicity.

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 (Candia-Rivera *et al.*, 2025). Our method addressed a recurrent debate about the specificity of different cardiac rhythms with respect to sympathetic or parasympathetic mechanisms. The frequency domain analysis of HRV, typically relying on the estimation of low and high frequency ranges (LF, HF), has been challenged because of the inaccurate estimation of sympathetic activity within the LF range (Goldstein *et al.*, 2011). To address this issue, the proposed method performs an individual-specific separation of the slow and fast HRV oscillations in combination with concurrent changes in the baseline heart rate, enabling a more precise estimation of sympathetic and parasympathetic modulations of cardiac dynamics.

This study aimed to demonstrate the effectiveness of our individual-specific approach in rats. We hypothesized that adapting the cardiac sympathetic and parasympathetic indices could accurately track short-term changes in cardiac rhythmicity during autonomic elicitation, outperforming standard spectral measures such as LF and HF. We validate the efficacy of our method using a dataset of rats exposed to dobutamine (Hazari *et al.*, 2012, 2017), where

dobutamine emulates a non-resting state condition similar to the physiological responses due to exercise due to sympathetic activations and parasympathetic deactivations (Sharma *et al.*, 2015). We applied our method to spontaneously hypertensive rats (SHR), known for their rapid sympathetic reactivity (Shanks *et al.*, 2013), 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 (Hazari *et al.*, 2012, 2017), 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 measure interbeat interval (IBI) or R-to-R peak segment durations (RR series).

The rats were catheterized intravenously in the left jugular vein for the administration of dobutamine. Dobutamine (Sigma-Aldrich, St. Louis, MO, USA) was infused 0.2 ml/min for 2 min in awake rats, after a 5-min baseline period (Hazari *et al.*, 2012, 2017). Administered doses were 20, 40, 80, 160 and 320 µg/kg.

Half of the cohort consisted of rats exposed to diesel exhaust, while the other half was exposed to purified air. However, our primary focus was not on comparing these two conditions. Instead, we concentrated on the instantaneous changes triggered by dobutamine administration. The effects of diesel exhaust on cardiovascular physiology remain inconclusive in the literature. Research suggests that significant effects are generally observed with long-term exposure (Bradley *et al.*, 2013) or in individuals with pre-existing conditions (Carll *et al.*, 2013). Diesel exhaust exposure has been linked to an increased risk of cardiac arrhythmias (Hazari *et al.*, 2017; Rossi *et al.*, 2021). However, these findings have not been consistently replicated in other models (Peretz *et al.*, 2008; Mills *et al.*, 2011).

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.

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). For further detail on the dataset, please refer to the original studies (Hazari *et al.*, 2012, 2017).

Estimation of cardiac sympathetic and parasympathetic indices

The estimation of cardiac sympathetic and parasympathetic indices was based on a previously developed model for humans (Candia-Rivera *et al.*, 2025). The model is based on the Poincaré plot, a method that depicts the fluctuations on the duration of consecutive IBI or RR series (Woo *et al.*, 1992), as shown in Figure 1A. Notably, this method eliminates the need to resample the IBI series, unlike traditional HRV approaches, such as spectral estimations.

Three features are extracted from the Poincaré plot: the cardiac cycle duration measured as the distance to the origin (CCD), and the minor (SD1) and major (SD2) ratios of the ellipse that quantify the short- and long-term fluctuations of HRV, respectively (Sassi *et al.*, 2015).

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

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

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

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

where $\lambda_{\Omega_t}(i)$ denotes the ith the eigenvalue of the covariance matrix of $IBI_{\mathrm{i},\ldots,\mathrm{n}-1}$ and $IBI_{\mathrm{i}+1,\ldots,\mathrm{n}}$, with Ω_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” (Candia-Rivera *et al.*, 2025). 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 (Schäfer & Strimmer, 2005). 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 (Rowan *et al.*, 2007).

The distance to the origin CCD_0 and ellipse ratios SD_{01} and SD_{02} for the whole experimental duration are computed to re-center the time-resolved estimations of CCD, SD1 and SD2. The Cardiac Parasympathetic Index (CPI) and the Cardiac Sympathetic Index (CSI), are computed as follows:

$$D(t) = \overline{\mathrm{CCD}}(t) + CCD_0 \tag{4}$$

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

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

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.

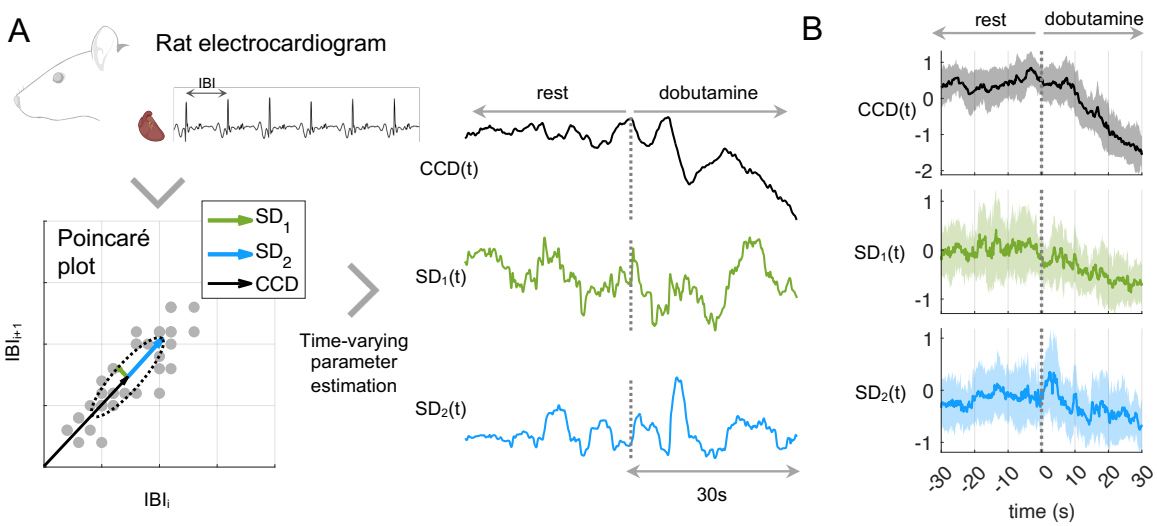

Figure 1. Pipeline for the estimation of the fluctuating parameters of the Poincaré plot. (A) The Poincaré plot illustrates the consecutive changes in interbeat intervals (IBI). The parameters

computation includes the distance from the ellipse center to the origin (CCD), and the minor (SD1) and major ratios (SD2). (B) Group median and median absolute deviation in bold curve and shaded areas of CCD, 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 (Orini *et al.*, 2012). First, the IBI series were evenly re-sampled using spline interpolation and demeaned. The re-sampling frequency was set to 20 Hz, which is commonly set at 10 Hz or higher in rodents (Aubert *et al.*, 1999). The pseudo-Wigner–Ville algorithm consists of a two-dimensional Fourier transform with an ambiguity function kernel to perform two-dimensional filtering (Orini *et al.*, 2012). 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 (Konopelski & Ufnal, 2016). 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 (Rowan *et al.*, 2007). 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.

Post-hoc exploratory analyses were conducted to assess the contribution of each variable—rat type (SHR or WKY), exposure (purified air or diesel exhaust), and dobutamine dose (20, 40, 80, 160 and 320 μg/kg)—as well as their potential interaction effects on the differences in CSI and CPI values. These differences were calculated as the change in values 30 seconds after dobutamine administration compared to 30 seconds before. Model performance was evaluated using the Akaike Information Criterion (AIC), assessing both the individual contributions of the variables and their interactions in fitting the model. The model with the lowest AIC value was considered the best in each case.

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.

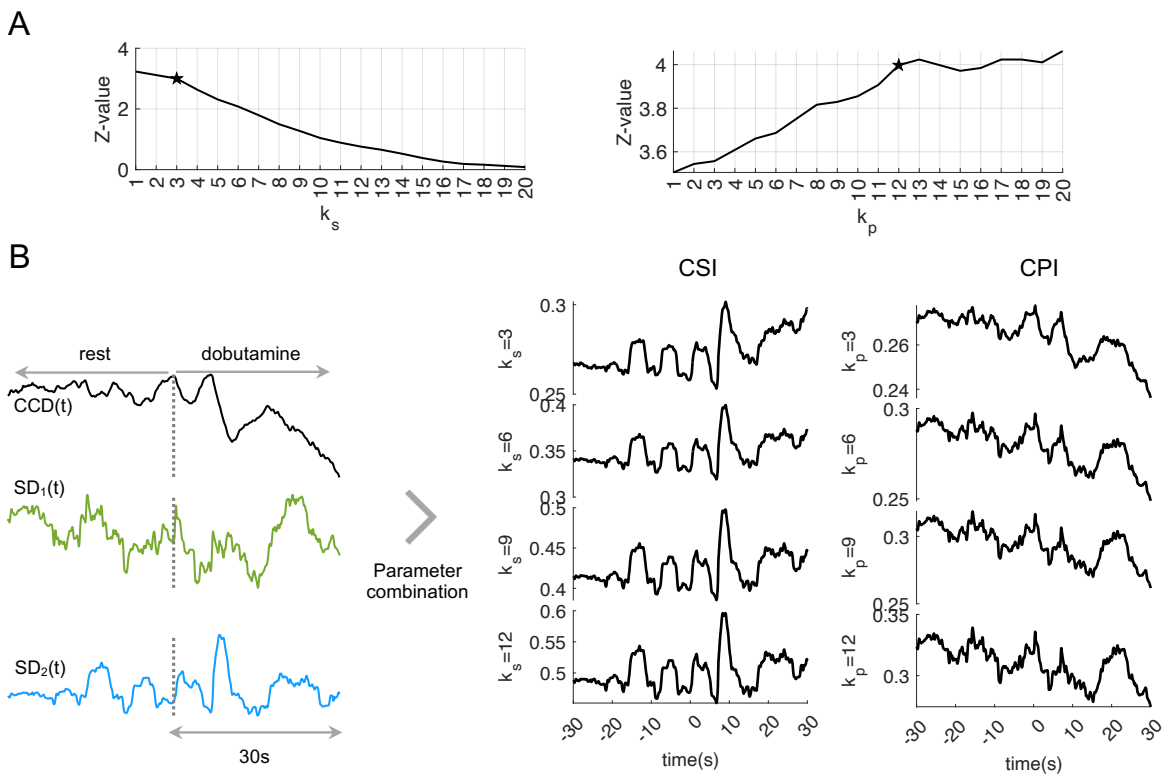

Figure 2. Estimation of the parameters k_p and k_s for the estimation of CSI and CPI in the SHR strain. (A) The Z-value from the Wilcoxon test comparing CSI and CPI for the conditions rest vs dobutamine, as a function of k_p and k_s values. The star indicates the inflection point from which k_s =3 and 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 Figure 3. 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 also 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 WKY strain displays no significant change in HF after dobutamine infusion, with CPI emerging as an effective 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.

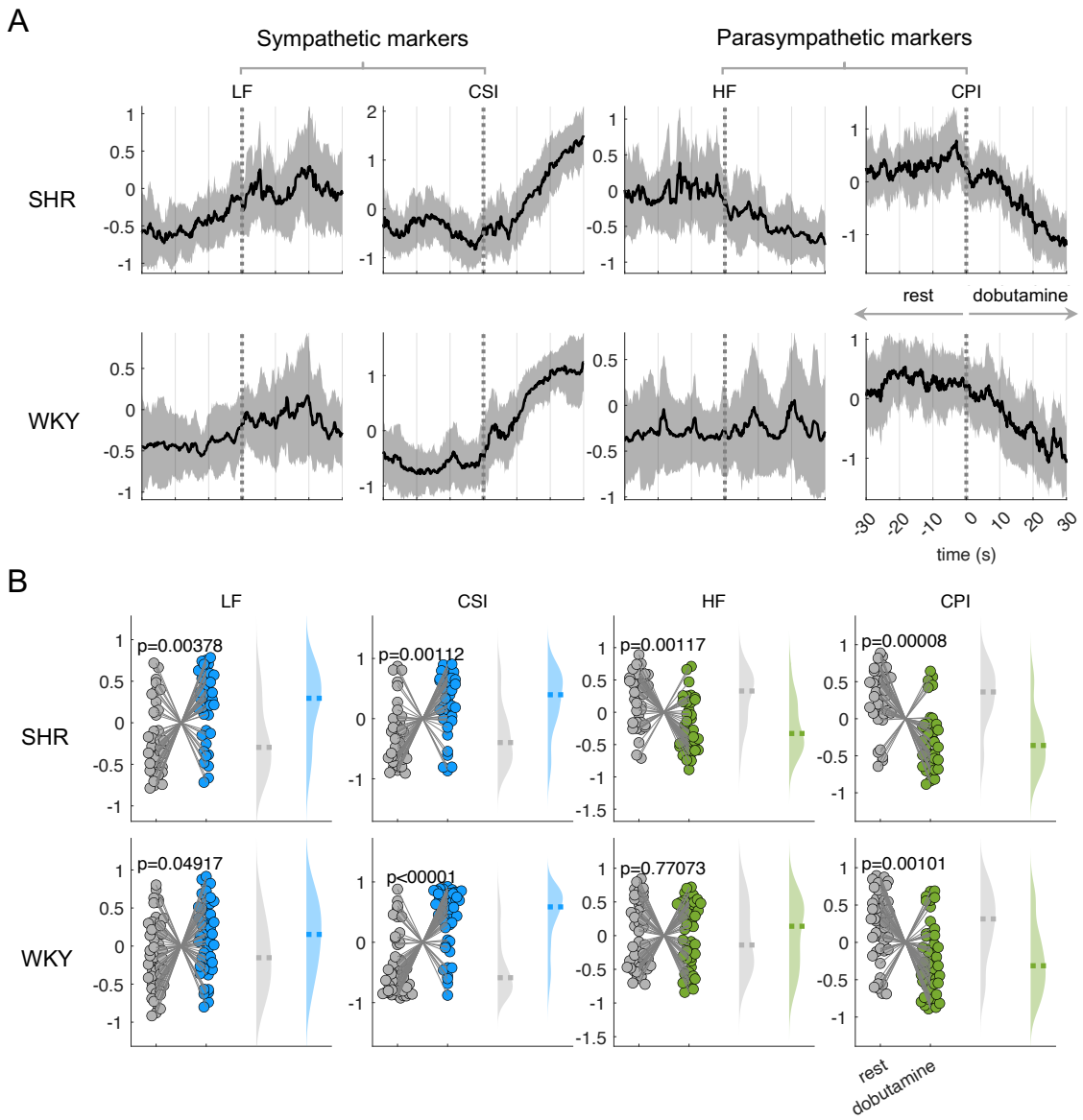

Figure 3. Cardiac autonomic indices CSI and CPI, and their spectral counterparts LF and HF under the rest to dobutamine transition. (A) Time course of the computed indices between -30 to 30 s with respect to the condition change onset. The plot indicates the group median and the shaded areas the median 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.

As part of the exploratory analysis, various linear mixed models were fitted to examine CSI and CPI values as functions of rat type (SHR or WKY), exposure (purified air or diesel exhaust), and dobutamine dose (20, 40, 80, 160, and 320 µg/kg).

For CSI, in two-factor models (N = 1, 2, 3), the lowest AIC value was observed when the dose variable was excluded, indicating that CSI is primarily influenced by diesel exhaust exposure and rat type. In three-factor models (N = 4 to 8), the best fit was achieved when dose was treated as an independent variable alongside the interaction between diesel exhaust exposure and rat type. This suggests that dose does not significantly interact with the other variables regarding its effects on CSI.

For CPI, in two-factor models, diesel exhaust exposure and dose appeared to have a stronger influence on CPI values. In three-factor models, CPI was best explained by considering the interaction among all three factors. These findings, although limited by the number of data points, suggest that CSI and CPI are influenced differently by the analyzed factors. Further studies with larger datasets are needed to confirm these observations.

Table 1. Linear Mixed Model fitting Cardiac Sympathetic Index (CSI)

N model	Formula	AIC
1	**CSI ~ 1 + Exposure + RatType + (1|ID)**	**-406.7596**
2	CSI ~ 1 + Exposure + Dose + (1|ID)	-368.4319
3	CSI ~ 1 + RatType + Dose + (1|ID)	-366.8458
4	CSI ~ 1 + Exposure + RatType + Dose + (1|ID)	-376.7366
5	**CSI ~ 1 + Dose + Exposure*RatType + (1|ID)**	**-498.3792**
6	CSI ~ 1 + RatType + Exposure*Dose + (1|ID)	-346.9463
7	CSI ~ 1 + Exposure + RatType*Dose + (1|ID)	-341.2821
8	CSI ~ 1 + Exposure * RatType * Dose + (1|ID)	-403.9011

ID={1, …, 22}, Exposure={Air, Diesel}, RatType={SHR, WKY}, Dose={20, 40, 80, 160, 320 μg/kg}. AIC: Akaike Information Criterion (lowest value in bold indicates best fitting)

Table 2. Linear Mixed Model fitting Cardiac Parasympathetic Index (CPI)

N model	Formula	AIC
1	CPI ~ 1 + Exposure + RatType + (1|ID)	85.0815
2	**CPI ~ 1 + Exposure + Dose + (1|ID)**	**84.1944**
3	CPI ~ 1 + RatType + Dose + (1|ID)	85.9075
4	CPI ~ 1 + Exposure + RatType + Dose + (1|ID)	78.1941
5	CPI ~ 1 + Dose + Exposure*RatType + (1|ID)	62.9700
6	CPI ~ 1 + RatType + Exposure*Dose + (1|ID)	59.6205
7	CPI ~ 1 + Exposure + RatType*Dose + (1|ID)	63.8296
8	**CPI ~ 1 + Exposure * RatType * Dose + (1|ID)**	**-44.6750**

ID={1, …, 22}, Exposure={Air, Diesel}, RatType={SHR, WKY}, Dose={20, 40, 80, 160, 320} μg/kg. AIC: Akaike Information Criterion (lowest value in bold indicates best fitting)

Discussion

Heart rhythmicity analysis has the potential to be a valuable noninvasive technique for assessing autonomic nervous activity in rats (Kuwahara *et al.*, 1994). Standard time-averaged HRV measures often fail to capture ongoing physiological dynamics because the averaging process, typically spanning a few minutes, masks fluctuations that occur at the level of seconds. For instance, invasive recordings on the left cervical vagus nerve in rats have revealed that tonic vagal activity does not correlate with commonly used HRV metrics (Marmerstein *et al.*, 2021). This indicates the need for further development of methods to understand the modulation of sympathetic and parasympathetic activity on cardiac dynamics.

We developed and validated a method using a rat heart dataset, where pharmacological autonomic elicitation was induced with dobutamine. This drug, a positive inotropic agent, is known to mimic sympathetic activation and parasympathetic deactivation, as observed in conditions like physical exercise (Mahoney *et al.*, 2016). In our proposed method, previously validated in humans (Candia-Rivera & Chavez, 2024*a*, 2024*b*; Candia-Rivera *et al.*, 2025), 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 notable differences on the effects in the WKY with respect to the SHR strain, although sympathetic hyperreactivity is expected in the SHR strain, as compared to WKY (Luft *et al.*, 1986). However, the sympathetic reactivity has been reported only in changes in the baseline heart rate, and not necessarily in the slow fluctuations in HRV (Ricksten *et al.*, 1984). Importantly, our approach surpasses the spectral counterparts, LF and HF, in the description of sympathetic and parasympathetic activities, respectively.

Our method offers various advantages, as compared to state-of-the-art methods. First, it is based on the Poincaré plot, which is a computationally efficient tool to depict the beat-to-beat alterations in IBIs (Woo *et al.*, 1992; Brennan *et al.*, 2001). Second, it considers parallel autonomic modulations in both baseline heart rate and HRV. It is important to study baseline heart rate in conjunction with HRV to fully comprehend sympathetic dynamics (Sacha *et al.*, 2013), 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 (Gonçalves *et al.*, 2010). Third, our proposal focuses on developing a time-resolved estimation

method, which could reduce the need for several minutes of averaging to assess a single experimental condition, but also enabling a comprehensive exploration of the dynamic shifts in autonomic regulations occurring in parallel with other organs, such as the brain (Samuels, 2007; Bashan *et al.*, 2012; Silvani *et al.*, 2016), This underscores the importance of modeling brain-heart interactions to better understand multisystem dysfunctions (Chen *et al.*, 2021).

Further exploration is needed to elucidate the dynamics of rat cardiac activity that extend beyond baseline heart rate or variations in HRV. Specially in the context of diesel exhaust exposure, which can potentially trigger cardiac arrythmias that can be capture as nonlinear patterns of activity (Hazari *et al.*, 2017; Rossi *et al.*, 2021). Linear mixed models suggest that cardiac sympathetic activity is influenced by the interaction between diesel exhaust exposure and the SHR rat type. While the available data may be limited, we cannot exclude the possibility that diesel exhaust exposure induces short-term changes in cardiac sympathetic or parasympathetic indices, potentially driven by cardiac conduction instability and inflammatory processes (Hazari *et al.*, 2017; Rossi *et al.*, 2021). 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 (Silva *et al.*, 2017), while also indicating that increased sympathetic modulation is likely the main cause of lower entropy (Silva *et al.*, 2016).

Although our study has some limitations, such as a small sample size or the inclusion of all-male strains, the results demonstrate the effectiveness of our estimation of autonomic system parameters. Another limitation of this study is the inability to determine whether specific dobutamine doses or their cumulative effects after repeated administrations influence CSI or CPI values. Linear mixed models revealed that CSI was best explained by the interaction between exposure and rat type, while CPI was best explained by the interaction among all three factors studied. These findings suggest that a dose effect, although minimal, cannot be ruled out. Such an effect would likely manifest as rapid sympathetic and parasympathetic modulation of the heart, occurring immediately after a perturbing element, within a complex interplay of regulatory loops spanning multiple timescales (Pathak *et al.*, 2006).

Our proposal has not been tested under other standard conditions of autonomic elicitation, such as monitoring heart rate during cold exposure (Kuwahara *et al.*, 2009), 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 (Coleman, 1980; Kuwahara *et*

al., 1994; Mangin *et al.*, 1998; Aubert *et al.*, 1999; Ohnuki *et al.*, 2001; Ramaekers *et al.*, 2002*a*; Beckers *et al.*, 2006; Bernat *et al.*, 2023).

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 HRV, a key marker of sympathetic activity. In future studies, 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. 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. To optimize organ perfusion, sympathomimetic agents like dobutamine, a β1-adrenergic agonist, have been developed to enhance heart contractility and output (Dubin *et al.*, 2017). However, a comprehensive understanding of this intricacy requires further rigorous investigations in well-controlled in vivo settings.

Future research may include the description of our developed markers under different stressors (Ramaekers *et al.*, 2002*b*; Inagaki *et al.*, 2004; Raffai *et al.*, 2006; Park *et al.*, 2017), and cardiovascular conditions (Henze *et al.*, 2008; Scridon *et al.*, 2012; Han *et al.*, 2014). Further research could deepen our understanding of the fluctuations of cardiac rhythmicity in the context of circadian rhythms, which are typically limited to describing long-term changes in the baseline heart rate alone (Van Den Buuse, 1994).

Our development may also enable the investigation of disruptions that may impact the connection between the brain and heart, and their subsequent effects on neural functions. For instance, in the study of severe brain damage resulting from cardiovascular conditions like stroke (Kodama *et al.*, 2018) or anoxia (Gonçalves *et al.*, 2008), as well as in cases of damage to the spinal cord (Inskip *et al.*, 2012). 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 European Commission, Horizon MSCA Program (grant n° 101151118).

Author contributions

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

References

Aubert AE, Ramaekers D, Beckers F, Breem R, Denef C, Van de Werf F & Ector H (1999). The analysis of heart rate variability in unrestrained rats. Validation of method and results. *Computer Methods and Programs in Biomedicine* **60,** 197–213.

Bashan A, Bartsch RP, Kantelhardt JW, Havlin S & Ivanov PC (2012). Network physiology reveals relations between network topology and physiological function. *Nature Communications* **3,** 702.

Beckers F, Verheyden B, Ramaekers D, Swynghedauw B & Aubert AE (2006). Effects of Autonomic Blockade on Non-Linear Cardiovascular Variability Indices in Rats. *Clinical and Experimental Pharmacology and Physiology* **33,** 431–439.

Bernat B, Erdelyi R, Fazekas L, Garami G, Szekeres RM, Takacs B, Bombicz M, Varga B, Sarkany F, Raduly AP, Romanescu DD, Papp Z, Toth A, Szilvassy Z, Juhasz B & Priksz D (2023). Drug Candidate BGP-15 Prevents Isoproterenol-Induced Arrhythmias and Alters Heart Rate Variability (HRV) in Telemetry-Implanted Rats. *Pharmaceuticals* **16,** 359.

Bradley JM, Cryar KA, El Hajj MC, El Hajj EC & Gardner JD (2013). Exposure to diesel exhaust particulates induces cardiac dysfunction and remodeling. *Journal of Applied Physiology* **115,** 1099–1106.

Brennan M, Palaniswami M & Kamen P (2001). Do existing measures of Poincare plot geometry reflect nonlinear features of heart rate variability? *IEEE Transactions on Biomedical Engineering* **48,** 1342–1347.

Candia-Rivera D, Catrambone V, Thayer JF, Gentili C & Valenza G (2022). Cardiac sympathetic-vagal activity initiates a functional brain–body response to emotional arousal. *Proceedings of the National Academy of Sciences* **119,** e2119599119.

Candia-Rivera D & Chavez M (2024*a*). Freezing of gait in Parkinson's disease increases sympathetic and parasympathetic indices. In *2024 13th Conference of the European Study Group on Cardiovascular Oscillations (ESGCO)*, pp. 1–2.

Candia-Rivera D & Chavez M (2024*b*). Tracking the physiological responses in sleep apnea using robust cardiac sympathetic activity estimation. In *2024 13th Conference of the European Study Group on Cardiovascular Oscillations (ESGCO)*, pp. 1–2.

Candia-Rivera D, de Vico Fallani F & Chavez M (2025). Robust and time-resolved estimation of cardiac sympathetic and parasympathetic indices. *Royal Society Open Science*; DOI: 10.1098/rsos.240750.

Carll AP, Lust RM, Hazari MS, Perez CM, Krantz QT, King CJ, Winsett DW, Cascio WE,

Costa DL & Farraj AK (2013). Diesel Exhaust Inhalation Increases Cardiac Output, Bradyarrhythmias, and Parasympathetic Tone in Aged Heart Failure–Prone Rats. *Toxicological Sciences* **131,** 583–595.

Chen WG, Schloesser D, Arensdorf AM, Simmons JM, Cui C, Valentino R, Gnadt JW, Nielsen L, Hillaire-Clarke CSt, Spruance V, Horowitz TS, Vallejo YF & Langevin HM (2021). The Emerging Science of Interoception: Sensing, Integrating, Interpreting, and Regulating Signals within the Self. *Trends in Neurosciences* **44,** 3–16.

Coleman TG (1980). Arterial baroreflex control of heart rate in the conscious rat. *American Journal of Physiology-Heart and Circulatory Physiology* **238,** H515–H520.

Dubin A, Lattanzio B & Gatti L (2017). The spectrum of cardiovascular effects of dobutamine - from healthy subjects to septic shock patients. *Revista Brasileira de Terapia Intensiva* **29,** 490–498.

Goldstein DS, Bentho O, Park M-Y & Sharabi Y (2011). 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* **96,** 1255–1261.

Gonçalves H, Henriques-Coelho T, Bernardes J, Rocha AP, Brandão-Nogueira A & Leite-Moreira A (2010). Analysis of heart rate variability in a rat model of induced pulmonary hypertension. *Medical Engineering & Physics* **32,** 746–752.

Gonçalves H, Henriques-Coelho T, Bernardes J, Rocha AP, Nogueira A & Leite-Moreira A (2008). Linear and nonlinear heart-rate analysis in a rat model of acute anoxia. *Physiol Meas* **29,** 1133.

Han SY, Gray E, Hughes G, Brown CH & Schwenke DO (2014). Increased sympathetic drive during the onset of hypertension in conscious Cyp1a1-Ren2 rats. *Pflugers Arch - Eur J Physiol* **466,** 459–466.

Hazari M, Callaway J, Winsett D, Lamb C, Haykal-Coates N, Krantz Q, King C, Costa D & Farraj A (2012). Dobutamine "Stress" Test and Latent Cardiac Susceptibility to Inhaled Diesel Exhaust in Normal and Hypertensive Rats. *Environmental health perspectives* **120,** 1088–1093.

Hazari MS, Lancaster JL, Starobin JM, Farraj AK & Cascio WE (2017). Diesel Exhaust Worsens Cardiac Conduction Instability in Dobutamine-Challenged Wistar–Kyoto and Spontaneously Hypertensive Rats. *Cardiovasc Toxicol* **17,** 120–129.

Henze M, Hart D, Samarel A, Barakat J, Eckert L & Scrogin K (2008). 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* **295,** H29–H38.

Hsueh B et al. (2023). Cardiogenic control of affective behavioural state. *Nature* **615,** 292–299.

Inagaki H, Kuwahara M & Tsubone H (2004). Effects of Psychological Stress on Autonomic Control of Heart in Rats. *Experimental Animals* **53,** 373–378.

Inskip JA, Ramer LM, Ramer MS, Krassioukov AV & Claydon VE (2012). Spectral Analyses of Cardiovascular Control in Rodents with Spinal Cord Injury. *Journal of Neurotrauma* **29,** 1638–1649.

Jammal Salameh L, Bitzenhofer SH, Hanganu-Opatz IL, Dutschmann M & Egger V (2024). Blood pressure pulsations modulate central neuronal activity via mechanosensitive ion channels. *Science* **383,** eadk8511.

Kodama T, Kamata K, Fujiwara K, Kano M, Yamakawa T, Yuki I & Murayama Y (2018). Ischemic Stroke Detection by Analyzing Heart Rate Variability in Rat Middle Cerebral Artery Occlusion Model. *IEEE Transactions on Neural Systems and Rehabilitation Engineering* **26,** 1152–1160.

Konopelski P & Ufnal M (2016). Electrocardiography in rats: a comparison to human. *Physiol Res* **65,** 717–725.

Kuwahara M, Kawaguchi T, Ito K & Tsubone H (2009). Effects of Taurine on Cardiovascular and Autonomic Nervous Functions in Cold Exposed rats. In *Taurine 7*, pp. 533–540. Springer, New York, NY.

Kuwahara M, Yayou K, Ishii K, Hashimoto S, Tsubone H & Sugano S (1994). Power spectral analysis of heart rate variability as a new method for assessing autonomic activity in the rat. *Journal of Electrocardiology* **27,** 333–337.

Luft FC, Demmert G, Rohmeiss P & Unger T (1986). Baroreceptor reflex effect on sympathetic nerve activity in stroke-prone spontaneously hypertensive rats. *Journal of the Autonomic Nervous System* **17,** 199–209.

Macpherson AK, Neti S, Chutakositkanon C, Averbach M & Macpherson PA (2009). The variation of dobutamine induced heart stress with heart rate. *WIT Transactions on Biomedicine and Health* **13,** 17–26.

Mahoney L, Shah G, Crook D, Rojas-Anaya H & Rabe H (2016). A Literature Review of the Pharmacokinetics and Pharmacodynamics of Dobutamine in Neonates. *Pediatric Cardiology* **37,** 14–23.

Mangin L, Swynghedauw B, Benis A, Thibault N, Lerebours G & Carré F (1998). Relationships Between Heart Rate and Heart Rate Variability: Study in Conscious Rats. *Journal of Cardiovascular Pharmacology* **32,** 601.

Marmerstein JT, McCallum GA & Durand DM (2021). Direct measurement of vagal tone in rats does not show correlation to HRV. *Sci Rep* **11,** 1210.

Mills NL, Finlayson AE, Gonzalez MC, Törnqvist H, Barath S, Vink E, Goudie C, Langrish JP, Söderberg S, Boon NA, Fox KAA, Donaldson K, Sandström T, Blomberg A & Newby DE (2011). Diesel exhaust inhalation does not affect heart rhythm or heart rate variability. *Heart* **97,** 544–550.

Ohnuki K, Moritani T, Ishihara K & Fushiki T (2001). Capsaicin Increases Modulation of Sympathetic Nerve Activity in Rats: Measurement Using Power Spectral Analysis of Heart Rate Fluctuations. *Bioscience, Biotechnology, and Biochemistry* **65,** 638–643.

Orini M, Bailón R, Mainardi LT, Laguna P & Flandrin P (2012). Characterization of dynamic interactions between cardiovascular signals by time-frequency coherence. *IEEE Trans Biomed Eng* **59,** 663–673.

Park SE, Park D, Song K-I, Seong J-K, Chung S & Youn I (2017). Differential heart rate variability and physiological responses associated with accumulated short- and long-term stress in rodents. *Physiology & Behavior* **171,** 21–31.

Pathak A, Velez-Roa S, Xhaët O, Najem B & Van De Borne P (2006). Dose-dependent effect of dobutamine on chemoreflex activity in healthy volunteers. *British Journal of Clinical Pharmacology* **62,** 272–279.

Peretz A, Kaufman JD, Trenga CA, Allen J, Carlsten C, Aulet MR, Adar SD & Sullivan JH (2008). Effects of diesel exhaust inhalation on heart rate variability in human volunteers. *Environmental Research* **107,** 178–184.

Raffai G, Kocsis L, Mészáros M, Monos E & Dézsi L (2006). Inverse-Orthostasis May Induce Elevation of Blood Pressure due to Sympathetic Activation. *Journal of Cardiovascular Pharmacology* **47,** 287.

Ramaekers D, Beckers F, Demeulemeester H & Aubert AE (2002*a*). Cardiovascular Autonomic Function in Conscious Rats: A Novel Approach to Facilitate Stationary Conditions. *Annals of Noninvasive Electrocardiology* **7,** 307–318.

Ramaekers D, Beckers F, Demeulemeester H, Bert C, Denef C & Aubert A (2002*b*). Effects Of Melanocortins On Cardiovascular Regulation In Rats. *Clinical and Experimental Pharmacology and Physiology* **29,** 549–558.

Ricksten SE, Lundin S & Thoren P (1984). Spontaneous variations in arterial blood pressure, heart rate and sympathetic nerve activity in conscious normotensive and spontaneously

hypertensive rats. *Acta Physiol Scand* **120,** 595–600.
Rossi S, Buccarello A, Caffarra Malvezzi C, Pinelli S, Alinovi R, Guerrero Gerboles A, Rozzi G, Leonardi F, Bollati V, De Palma G, Lagonegro P, Rossi F, Lottici PP, Poli D, Statello R, Macchi E & Miragoli M (2021). Exposure to nanoparticles derived from diesel particulate filter equipped engine increases vulnerability to arrhythmia in rat hearts. *Environmental Pollution* **284,** 117163.
Rowan WH, Campen MJ, Wichers LB & Watkinson WP (2007). Heart rate variability in rodents: uses and caveats in toxicological studies. *Cardiovasc Toxicol* **7,** 28–51.
Ruffolo RR (1987). Review: The pharmacology of dobutamine. *American Journal of the Medical Sciences* **294,** 244–248.
Sacha J, Barabach S, Statkiewicz-Barabach G, Sacha K, Müller A, Piskorski J, Barthel P & Schmidt G (2013). How to strengthen or weaken the HRV dependence on heart rate — Description of the method and its perspectives. *International Journal of Cardiology* **168,** 1660–1663.
Samuels MA (2007). The Brain–Heart Connection. *Circulation* **116,** 77–84.
Sargent KS, Martinez EL, Reed AC, Guha A, Bartholomew ME, Diehl CK, Chang CS, Salama S, Popov T, Thayer JF, Miller GA & Yee CM (2024). Oscillatory Coupling Between Neural and Cardiac Rhythms. *Psychol Sci* **35,** 517–528.
Sassi R, Cerutti S, Lombardi F, Malik M, Huikuri HV, Peng C-K, Schmidt G, Yamamoto Y, Reviewers D, Gorenek B, Lip GYH, Grassi G, Kudaiberdieva G, Fisher JP, Zabel M & Macfadyen R (2015). 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* **17,** 1341–1353.
Schäfer J & Strimmer K (2005). A shrinkage approach to large-scale covariance matrix estimation and implications for functional genomics. *Stat Appl Genet Mol Biol* **4,** Article32.
Scridon A, Gallet C, Arisha MM, Oréa V, Chapuis B, Li N, Tabib A, Christé G, Barrès C, Julien C & Chevalier P (2012). Unprovoked atrial tachyarrhythmias in aging spontaneously hypertensive rats: the role of the autonomic nervous system. *American Journal of Physiology-Heart and Circulatory Physiology* **303,** H386–H392.
Shanks J, Manou-Stathopoulou S, Lu C-J, Li D, Paterson DJ & Herring N (2013). Cardiac sympathetic dysfunction in the prehypertensive spontaneously hypertensive rat. *American Journal of Physiology-Heart and Circulatory Physiology* **305,** H980–H986.
Sharma R, O'Driscoll JM, Saha A, Sritharan M, Sutton R & Rosen SD (2015). Differing autonomic responses to dobutamine stress in the presence and absence of myocardial ischaemia. *J Physiol* **593,** 2171–2184.
Silva LEV, Lataro RM, Castania JA, da Silva CAA, Valencia JF, Murta LO, Salgado HC, Fazan R & Porta A (2016). 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* **311,** R150–R156.
Silva LEV, Lataro RM, Castania JA, Silva CAA, Salgado HC, Fazan R & Porta A (2017). Nonlinearities of heart rate variability in animal models of impaired cardiac control: contribution of different time scales. *Journal of Applied Physiology* **123,** 344–351.
Silvani A, Calandra-Buonaura G, Dampney RAL & Cortelli P (2016). Brain-heart interactions: physiology and clinical implications. *Philos Trans A Math Phys Eng Sci*; DOI: 10.1098/rsta.2015.0181.
Spyer KM (1990). The Central Nervous Organization of Reflex Circulatory Control. In *Central Regulation of Autonomic Functions*, ed. Loewy AD & spyer KM, p. 0. Oxford University

Press.
Van Den Buuse M (1994). Circadian rhythms of blood pressure, heart rate, and locomotor activity in spontaneously hypertensive rats as measured with radio-telemetry. *Physiology & Behavior* **55,** 783–787.
Woo MA, Stevenson WG, Moser DK, Trelease RB & Harper RM (1992). Patterns of beat-to-beat heart rate variability in advanced heart failure. *Am Heart J* **123,** 704–710.