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Abstract

Heart rate variability (HRV) is widely used to characterize cardiovascular autonomic regulation, yet conventional HRV indices are strongly dependent on heart rate (HR). This dependence makes it difficult to determine whether an observed HRV change reflects altered physiological regulation or merely a shift to another operating point along an existing HR–HRV relationship. We investigated this problem using β-adrenergic blockade as a controlled autonomic perturbation and analyzed the resulting changes with a subject-specific heart-rate-dependent Master Curve framework.

Seven laboratory canines underwent treadmill exercise under control conditions and following β-adrenergic blockade with metoprolol. Three animals provided ECG recordings of sufficient quality for quantitative Master Curve reconstruction. For each pair of consecutive RR intervals, the successive difference was assigned to the heart rate corresponding to their pairwise mean. The Master Curve was constructed as the heart-rate-specific root mean square of successive RR differences. Repeated control measurements were used to assess within-subject reproducibility, and β-blockade measurements were compared with the subject-specific control reference at matched heart rates. Heart-rate-normalized successive differences and their local variability were also analyzed over time.

Repeated control Master Curves showed closely overlapping subject-specific HR–HRV relationships, with substantially smaller within-subject deviations than the changes observed after β-blockade. β-adrenergic blockade produced a systematic downward displacement of the Master Curve in all three animals. At matched heart rates, RMSSD was reduced by 54.7–89.0% relative to the corresponding control reference. Normalized variability also decreased markedly after β-blocker administration, particularly during exercise.

These findings demonstrate that β-adrenergic blockade does not merely shift the cardiovascular system along an unchanged HR–HRV relationship but can modify the relationship itself. A mechanistic two-component model suggests that heart rate primarily reflects net chronotropic drive, whereas HRV reflects the magnitude of dynamic regulatory fluctuations around the operating point. The Master Curve therefore

provides a physiological reference framework for distinguishing heart-rate-related scaling effects from genuine changes in regulatory variability.

Keywords

heart rate variability; heart rate; RMSSD; β-adrenergic blockade; metoprolol; Master Curve; modified Poincaré plot; autonomic regulation; normalized variability; canine model

Declarations:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this manuscript.

Introduction

Heart rate variability and the challenge of physiological interpretation

Heart rate variability (HRV) is one of the most widely used non-invasive measures for assessing cardiovascular autonomic regulation. HRV reflects beat-to-beat fluctuations of cardiac rhythm generated by the interaction between intrinsic sinoatrial node properties, sympathetic and parasympathetic modulation, and cardiovascular feedback mechanisms including arterial baroreflex regulation [1–3]. Since the introduction of standardized HRV analysis methods, HRV has become an important tool in cardiovascular research, clinical assessment, exercise physiology, and psychophysiological studies [1].

Among conventional time-domain HRV parameters, the root mean square of successive differences (RMSSD) is commonly interpreted as a marker of short-term, predominantly vagal modulation because it is strongly influenced by respiratory sinus arrhythmia and rapid parasympathetic changes [2,3]. In contrast, the standard deviation of normal-to-normal intervals (SDNN) reflects the overall magnitude of RR interval variability over the analyzed period and integrates regulatory influences acting across multiple time scales, including autonomic modulation, circadian regulation, and slower cardiovascular control mechanisms [1,4].

Despite its widespread use, the physiological interpretation of HRV remains challenging because conventional HRV parameters are strongly dependent on heart rate (HR). Since HR is a nonlinear transformation of the RR-interval,

$$HR \sim \frac{1}{RR}$$

the same absolute fluctuation in heart rate produces different RR interval fluctuations depending on the operating heart rate. Consequently, an observed change in HRV may arise from two fundamentally different mechanisms: (i) a genuine alteration of physiological regulation, or (ii) a displacement along an underlying HR-dependent variability relationship caused by a change in heart rate itself [5–7].

This distinction is particularly important because conventional HRV analysis generally compares measurements between physiological states without reconstructing the underlying HR–HRV relationship. Therefore, an increase or decrease in HRV cannot uniquely determine whether autonomic regulation has

changed or whether the cardiovascular system has simply moved to another operating point along an existing relationship.

β-Adrenergic blockade and the HRV interpretation paradox

Pharmacological autonomic blockade provides an important experimental approach for investigating the relationship between autonomic regulation and HRV. Among these interventions, β-adrenergic blockade represents a particularly informative model because it reduces cardiac sympathetic effects while simultaneously modifying heart rate and cardiovascular control.

By inhibiting cardiac β1-adrenergic signaling, β-blockers reduce sympathetic chronotropic effects, decrease heart rate, and modify cardiovascular reflex regulation [8,9]. However, despite their well-defined pharmacological action, the effects of β-blockade on HRV remain controversial.

Several studies have reported increased HRV following β-blocker therapy. Niemelä et al. demonstrated increased RMSSD and SDNN after β-blockade in patients with coronary artery disease [10]. Similar increases in time-domain and frequency-domain HRV parameters have been reported after myocardial infarction, where β-blocker treatment was associated with increased vagal-related indices and altered autonomic regulation [11,12].

In chronic heart failure populations, β-blockers, particularly carvedilol, have also been associated with increased HRV and improved baroreflex sensitivity. Mortara et al. demonstrated increased SDNN and improved baroreflex gain during carvedilol therapy [13]. Similarly, Bullinga et al. reported increased time-domain and frequency-domain HRV parameters during carvedilol treatment in heart failure patients [14].

More recent studies have confirmed that β-blocker therapy modifies circadian HRV characteristics. Saleem et al. reported altered circadian HRV patterns in β-blocker-treated patients, including increased RMSSD and HF-related measures, suggesting changes in parasympathetic-related variability [15].

However, these observations are not universally consistent. Experimental studies have demonstrated that HRV responses to autonomic interventions depend strongly on the accompanying change in heart rate. Billman emphasized that HRV responses cannot be interpreted independently of simultaneous HR changes, because identical physiological interventions may produce different HRV outcomes depending on the initial operating point [6].

Thus, β-adrenergic blockade creates a fundamental interpretational paradox. Although it reduces sympathetic effects on the heart, conventional HRV measures may increase, decrease, or remain unchanged depending on the associated heart-rate shift. This raises an important question: does β-blockade truly modify physiological variability, or does it simply move the cardiovascular system along an existing HR–HRV relationship?

Heart-rate dependence and current HRV correction approaches

The mathematical dependence between HR and HRV has been recognized for decades. Sacha and Pluta demonstrated that changes in average heart rate alone can alter HRV indices because of the nonlinear relationship between HR and RR intervals [5]. Therefore, a reduction in HR induced by β-blockade may produce an apparent increase in RMSSD and SDNN even without an increase in the underlying physiological variability.

Several approaches have been proposed to correct this limitation. One strategy is direct normalization of HRV parameters by mean RR interval or by empirical power-law relationships:

$$HRV_{corrected} = \frac{HRV}{RR^{p}}$$

where the exponent p is determined empirically [16].

Gąsior et al. demonstrated that different HRV parameters exhibit different dependencies on mean RR interval, showing that RMSSD, SDNN, and frequency-domain indices require different correction approaches [16]. However, such methods assume a global mathematical relationship and may not fully capture individual physiological differences.

Population-based HR–HRV reference models have also been developed. van den Berg et al. analyzed large healthy cohorts and demonstrated nonlinear HR-dependent relationships between HRV parameters and heart rate [17]. These approaches improve correction but remain based primarily on population averages rather than individual physiological trajectories.

More recent approaches have emphasized subject-specific HR-dependent correction models. Yan et al. demonstrated that individual HR-dependent correction may improve interpretation by accounting for personal HR–HRV characteristics [18].

However, most correction methods treat HR dependence primarily as an unwanted mathematical component to be removed. An alternative perspective is that the HR–HRV relationship itself contains physiological information and should therefore be preserved as the reference framework for interpretation.

Pharmacological blockade and the physiological meaning of HRV

Autonomic blockade studies provide important evidence that HRV cannot be interpreted simply as a direct measure of a single autonomic state or sympathovagal balance. Parasympathetic blockade using atropine markedly reduces respiratory sinus arrhythmia and short-term HRV, supporting the dominant contribution of vagal modulation to rapid beat-to-beat variability [19–22]. Classical pharmacological studies demonstrated that abolition or attenuation of vagal cardiac control substantially suppresses HR fluctuations, while later work further showed that HRV reflects the pattern and dynamic modulation of parasympathetic influence rather than parasympathetic tone alone [20–22].

Combined sympathetic and parasympathetic blockade provides an even stronger test of HRV interpretation. Early autonomic blockade studies using propranolol and atropine demonstrated that the heart can be driven toward an intrinsic rate when both major autonomic inputs are pharmacologically suppressed [23]. More recent studies applying full autonomic blockade during rest and exercise showed that suppression of vagal activity is closely related to the disappearance of spontaneous HRV during exercise, while pharmacological blockade modifies both HR and HRV characteristics [24,25]. These findings indicate that variability depends not only on the relative contribution of individual autonomic branches but also on the magnitude, timing, and dynamics of regulatory inputs.

Miyabara et al. quantified the effects of pharmacological blockers on cardiac autonomic regulation and demonstrated that autonomic interventions modify both heart rate and variability parameters, emphasizing that conventional HRV indices alone cannot uniquely identify autonomic states [24]. This

interpretation is consistent with broader reviews of autonomic function, which emphasize that HR, HRV, and recovery indices reflect related but non-equivalent aspects of cardiovascular autonomic regulation [7,26].

Taken together, these observations suggest that HR and HRV represent different aspects of autonomic regulation. Heart rate primarily reflects the net chronotropic effect of opposing autonomic influences, whereas HRV reflects the magnitude and temporal structure of dynamic regulatory fluctuations around this operating state.

Canine models and translational relevance to human cardiac regulation

The present study was performed in canines, which is important for the translational interpretation of the findings. In cardiac electrophysiology, canine preparations are widely used as large-animal models because several electrophysiological properties of canine ventricular myocardium are closer to humans than those of small rodents. Nánási et al. concluded that canine ventricular myocytes represent a reasonably good model for human ventricular cells, with similar action potential configuration and broadly comparable profiles of several major ionic currents, although some interspecies differences remain, particularly in I_{K1} density and I_{to} recovery kinetics [27]. This supports the use of canine models when the aim is to investigate integrated cardiac electrophysiological behavior rather than isolated molecular mechanisms.

The translational relevance of canine models is further supported by recent work from the Szeged cardiovascular electrophysiology group. Polyák, Topal, Varró and colleagues developed a canine model of elite exercise and demonstrated that chronic endurance training induces cardiac electrophysiological remodeling associated with enhanced arrhythmia susceptibility [28]. In that study, 12 sedentary and 12 trained canines were investigated after a 4-month sustained training protocol designed to mimic elite athletic exercise. Training prolonged ventricular repolarization, increased short-term variability of repolarization, enhanced ectopic activity, and increased susceptibility to ventricular fibrillation [28]. These findings provided in vivo, cellular electrophysiological, and molecular evidence that a canine model can reveal mechanisms relevant to malignant ventricular arrhythmias and sudden cardiac death risk in humans.

This is particularly relevant for the present study because our analysis also relies on dynamic cardiac responses across a wide physiological range rather than on isolated resting-state measurements. The treadmill protocol, the wide heart-rate range, and the β-adrenergic perturbation enable reconstruction of the HR–HRV relationship under conditions in which cardiac autonomic regulation is actively challenged. The use of a canine model therefore provides a physiologically meaningful experimental setting for testing whether HRV changes reflect simple movement along an HR-dependent curve or structural modification of the HR–HRV relationship itself.

Thus, although direct extrapolation from canines to humans must be made with caution, canine models offer a strong translational platform for studying cardiac electrophysiological regulation, arrhythmia susceptibility, and heart-rate-dependent variability dynamics. This provides additional justification for using the present dataset to investigate the physiological meaning of HRV through the Master Curve framework.

Toward a heart-rate-dependent Master Curve framework

Recent studies using normalized variability measures have demonstrated that removing heart-rate dependence can reveal autonomic dynamics that are not apparent in conventional HRV indices. Rudics et al. showed that normalized variability exhibits qualitatively different behavior compared with conventional RMSSD and SDNN during stress and relaxation paradigms, emphasizing the importance of separating HR scaling effects from regulatory dynamics [29].

Similarly, nonlinear HRV approaches have suggested that cardiovascular regulation should be interpreted as a dynamic system rather than as a collection of independent HRV parameters [30].

In previous work, we introduced the heart-rate-dependent Master Curve framework based on the relationship between RR variability and heart rate [31]. The Master Curve defines the expected magnitude of variability at each heart rate and provides a physiological reference framework for separating heart-rate-dependent effects from changes in regulatory dynamics.

Unlike empirical correction formulas, the Master Curve does not attempt to remove heart-rate dependence. Instead, it preserves the physiological HR–HRV relationship as the reference coordinate system and allows deviations from this relationship to be interpreted independently of heart rate.

Within this framework, HRV is not considered an absolute physiological quantity, but rather a relative measure defined by the underlying HR–HRV relationship.

Present study

The present study aimed to investigate the effect of β-adrenergic blockade using a heart-rate-dependent Master Curve framework.

Rather than evaluating changes in conventional HRV measures alone, β-adrenergic blockade was used as a controlled physiological perturbation to determine whether autonomic intervention produces:

1. a shift of the operating point along an unchanged HR–HRV relationship, or

2. a structural modification of the HR–HRV relationship itself.

We hypothesized that β-adrenergic blockade produces a systematic displacement of the HR–HRV relationship that cannot be explained solely by the accompanying reduction in heart rate.

Methods

Experimental design and data acquisition

The study was conducted on a cohort of male laboratory canines (N=7) purchased from a licensed experimental animal breeding facility, Ásotthalom, Hungary (approval number of the breeder is XXX/2018, certified by the Department of Animal Health and Food Control of the Ministry of Agriculture and Rural Development, Hungary). All procedures involving canine were carried out in accordance with the European Directive 2010/63/EU and were approved by the Ethical Committee for the Protection of Animals in Resesearch at the University of Szeged, Szeged, Hungary (approval number: I-74-15-2017 and I-74-24-2017) and the Department of Animal Health and Food Control of the Ministry of Agriculture and Rural Development (authority approval: XII/509/2022).

Exercise training in canines

The animals were between 12 and 18 months of age and weighing 9-15 kg, forming a physiologically homogeneous group. The present selective β-adrenergic blockade measurements were performed as part of the previously established canine treadmill-exercise experimental framework developed by the Szeged cardiovascular electrophysiology group [28]. This large-animal model was originally designed to study cardiac electrophysiological remodeling and arrhythmia susceptibility under controlled exercise conditions, and has been described in detail previously [28]. In brief, dogs were familiarized with the treadmill environment and trained to perform controlled running on a programmable canine treadmill system. The animals were gradually conditioned to the training protocol over a 5-week period, in which they were familiarised with research personnel and equipment, and walked on the treadmill apparatus (Dogrunner K9 Racer Treadmill, Dendermonde, Belgium) for 30-40 minutes daily at a maximum speed of 4- 6 km/hr.

Following this acclimatisation period, in the broader experimental protocol, treadmill exercise consisted of both continuous and interval running components. The previously described training paradigm included repeated continuous running sessions with progressively increased speeds and interval sessions alternating between low- and high-speed running. Specifically, the established protocol consisted of two 90-minute continuous running sessions per day at 12–18 km/h and two 50-minute interval running sessions at fixed speeds of 4 and 22 km/h, with treadmill inclination adjusted between 5% and 14% to maintain exercise intensity [28]. Between the daily training sessions, regular resting periods were implemented to ensure adequate hydration and animal welfare. Besides that, the animals received portions of their preferred food as a reward after each training session.

Administration of selective β-adrenergic blocker and Holter ECG monitoring

The present analysis was based on the acute pharmacological testing component of this experimental framework. During the β-blockade experiment, each animal performed treadmill exercise under control conditions and again following β-adrenergic blockade. The acute testing protocol consisted of a progressively increasing load phase followed by an interval phase with alternating low- and high-speed periods. This design was selected to drive heart rate across a wide physiological range, allowing reconstruction of the HR–HRV relationship under both non-blocked and β-blocked conditions.

The two training sessions were separated by a roughly three-hour rest period. Twenty minutes prior to the second training session, selective β-adrenergic blockade was administered prior to the second exercise phase using intravenous metoprolol (Sigma-Aldrich, (±)-Metoprolol (+)-tartrate salt, ≥98% (titration)) at a dose of 1.5 mg/kg body weight. During that time, resting ECG recordings were made to monitor the β-blocker's effects. This intervention was used as a controlled disruption of sympathetic effects on the heart, allowing us to assess whether the HR–HRV relationship was preserved or structurally altered when β-adrenergic effects on the heart were significantly reduced.

Electrocardiographic (ECG) signals were continuously recorded throughout all experimental conditions. During exercise, recordings were obtained using a MOX3 (Maastricht Instruments, The Netherlands) two-lead ECG system with a sampling frequency of 400 Hz. The resulting ECG recordings were used to extract

beat-to-beat RR intervals for conventional HRV analysis, Master Curve reconstruction, and heart-rate-normalized variability analysis.

Although recordings were obtained from seven canines, only three animals provided ECG data of sufficient quality for the final Master Curve analysis. The remaining recordings were excluded because of inadequate signal quality during treadmill running. The main reasons for exclusion were excessive motion artifacts, unstable electrode contact, low signal-to-noise ratio, and noise related to the challenging recording conditions in exercising, fur-covered animals. These recordings did not allow reliable RR interval extraction or stable reconstruction of the HR–HRV relationship and were therefore not included in the quantitative Master Curve comparison.

Master Curve estimation and normalized variability analysis

The subject-specific Master Curve was reconstructed using the previously described modified Poincaré method [31]. For each pair of consecutive RR intervals, the pairwise mean and successive difference were calculated as

$$mRR_i = \frac{RR_i + RR_{i+1}}{2}$$

and

$$SD_i = dRR_i = RR_{i+1} - RR_i$$

Here, SD denotes successive difference, following the terminology of RMSSD, and should not be confused with the statistical standard deviation. Thus, SD_i and dRR_i denote the same beat-to-beat variable.

The corresponding pairwise heart rate was calculated as

$$mHR_i = \frac{60000}{mRR_i}$$

The difference-versus-mean representation assigns each dRR_i value to the midpoint of the corresponding RR transition, providing a symmetric representation of positive and negative beat-to-beat changes. The mHR range was divided into 1-bpm bins, and the root mean square of the successive differences was calculated within each bin:

$$MC(mHR) = RMS(dRR)_{mHR}$$

The resulting discrete relationship was smoothed by spline fitting. The non-blocked subject-specific Master Curve served as the reference for normalization:

$$nSD_i = \frac{dRR_i}{MC(mHR_i)}$$

Thus, nSD is a signed, dimensionless normalized successive difference expressing the actual dRR relative to the fluctuation magnitude expected at the corresponding heart rate. Short-term normalized variability was quantified as the natural logarithm of the local standard deviation of nSD, calculated using a 10-s sliding window

$$\sigma_{nSD} = std_{10s}(nSD)$$

$$LnSD = ln(\sigma_{nSD})$$

Condition-specific Master Curves were compared at matched mHR values, whereas all nSD and LnSD time series were calculated relative to the same non-blocked reference Master Curve.

Condition-specific Master Curves were reconstructed separately for each experimental condition, whereas σ_{nSD} and LnSD time series were calculated relative to the subject-specific non-blocked reference Master Curve. Master Curves were compared at matched heart-rate values, allowing differences in variability to be distinguished from simple displacement along the HR–variability relationship. In addition, the temporal dynamics of heart rate and normalized variability were analyzed in relation to key experimental events, including β-blocker administration and the onset of exercise.

Given the dynamic and heterogeneous nature of the data, the analysis focused primarily on within-subject comparisons and the consistency of observed patterns across conditions. Data segments lacking sufficient Master Curve reference or containing excessive artifacts were excluded from further evaluation. The interpretation therefore emphasizes robust and reproducible physiological trends rather than population-level statistical inference.

The Master Curve was interpreted as a heart-rate-specific reference state of the cardiovascular regulatory system. In this sense, each point of the Master Curve represents the expected level of beat-to-beat variability around a given heart-rate operating point. The system does not remain fixed at this point, but fluctuates around it as a consequence of ongoing regulatory inputs. Therefore, the Master Curve provides a local reference around which short-term variability is expressed.

This interpretation is important because changes in conventional HRV may reflect either movement to another heart-rate operating point or changes in the magnitude of fluctuations around the same operating point. By comparing conditions at matched heart rates, the analysis separates displacement along the HR–HRV relationship from changes in the local variability amplitude itself. A downward shift of the Master Curve therefore indicates that, at the same heart-rate operating point, the system fluctuates with a lower variability magnitude than under the reference condition.

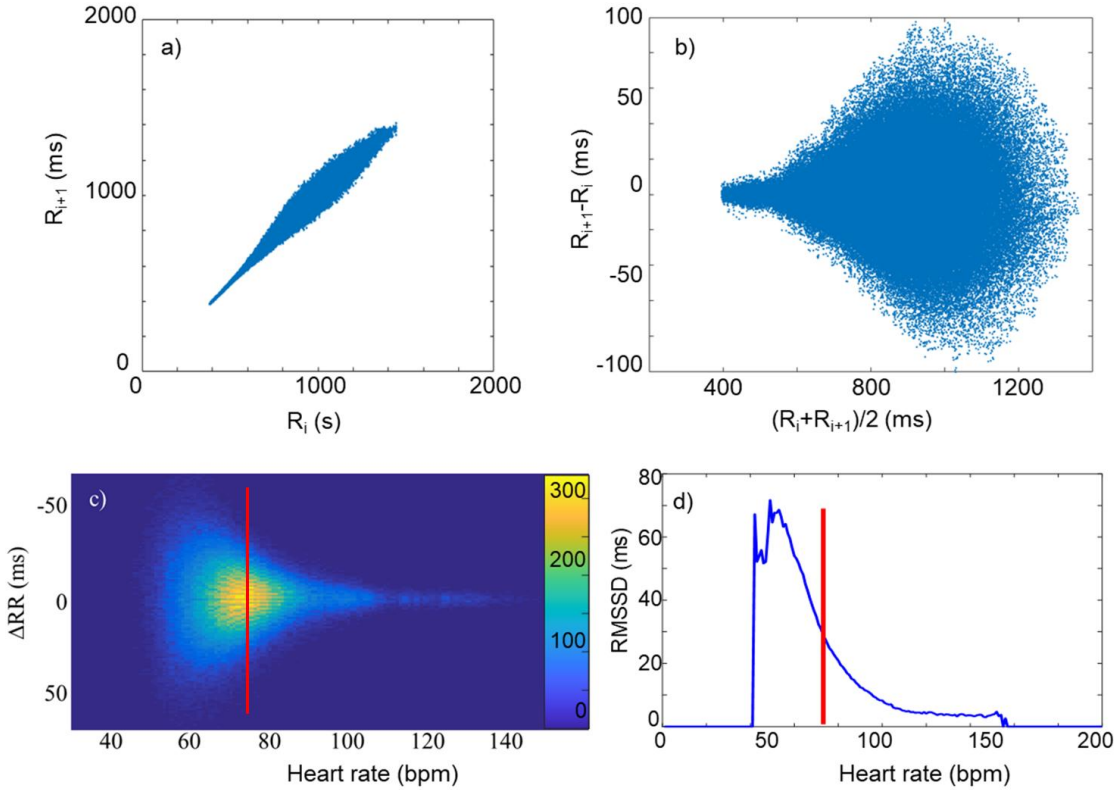


FIGURE 1. Construction of the heart-rate-dependent Master Curve using the modified Poincaré representation. (a) Poincaré plot of a typical RR time series. (b) The same in Bland-Altman-like representation. (c) ΔRR as a function of heart rate (mHR), as calculated from the data in (b). The color code is to show the frequency of the data. (d) The RMSSD versus mHR curve (M-curve), determined from data in (c), as the RMS of the distribution of ΔRR values at each HR. (The red lines stand for illustration of the way of calculation, at an ad hoc mHR value.) Reproduced from Búzás et al. [31].

Statistical analysis of Master Curve displacement

To quantify the effect of β-adrenergic blockade on the Master Curve, comparisons were performed within predefined heart-rate ranges: 90–110, 110–130, 130–150, 150–170, and 170–190 bpm. Within each valid HR bin, the difference between the β-blockade condition and the subject-specific median control Master Curve was calculated as:

$$\Delta ln(RMSSD) = ln\left(RMSSD_{\beta}\right) - median\left(ln(RMSSD_{control})\right)$$

For each predefined HR range, the distribution of $\Delta ln(RMSSD)$ values across valid HR bins was tested against zero using the Wilcoxon signed-rank test:

$$\Delta ln(RMSSD) = 0$$

The test was performed in MATLAB using the signrank(delta) function. Statistical significance was evaluated only in HR ranges containing at least five valid HR bins ($n \geq 5$). Significance levels were indicated as follows: $*p<0.05$, $**p<0.01$, $***p<0.001$, and n.s. for $p \geq 0.05$.

Importantly, the statistical unit was the HR bin, not the individual RR interval or beat-to-beat data point. This approach provides a conservative comparison that is consistent with the Master Curve framework, where variability is evaluated as a function of heart rate rather than as independent beat-level observations.

Results

For better clarity, the characteristic results are shown via individual examples, but across all experiments, qualitatively consistent results were obtained. (See Supporting Information.)

Time-domain behavior before and after β-blockade

Figure 2 shows the temporal evolution of heart rate (HR) and RMSSD throughout the experimental protocol, including the exercise periods (*Running I–IV*), the intervening sleep period, and the subsequent β-adrenergic blockade. The dashed vertical line indicates the time of β-blocker administration.

Before β-blockade, HR and RMSSD exhibited the expected inverse relationship throughout the recording. During the exercise periods (*Running I* and *Running II*), heart rate increased to approximately 200–220 bpm, while RMSSD decreased to only a few milliseconds. During the subsequent sleep period, HR gradually declined to approximately 100–130 bpm, accompanied by a progressive increase in RMSSD, reaching values of approximately 100–200 ms.

Immediately after β-blocker administration, heart rate transiently decreased to approximately 100–110 bpm, while RMSSD remained elevated. During the following exercise period (*Running III*), heart rate increased again but reached substantially lower values than during the corresponding pre-blockade exercise (*Running I*), gradually rising only to approximately 180–200 bpm. At the same time, RMSSD rapidly decreased to very low values and remained markedly suppressed throughout the exercise despite the lower heart rate.

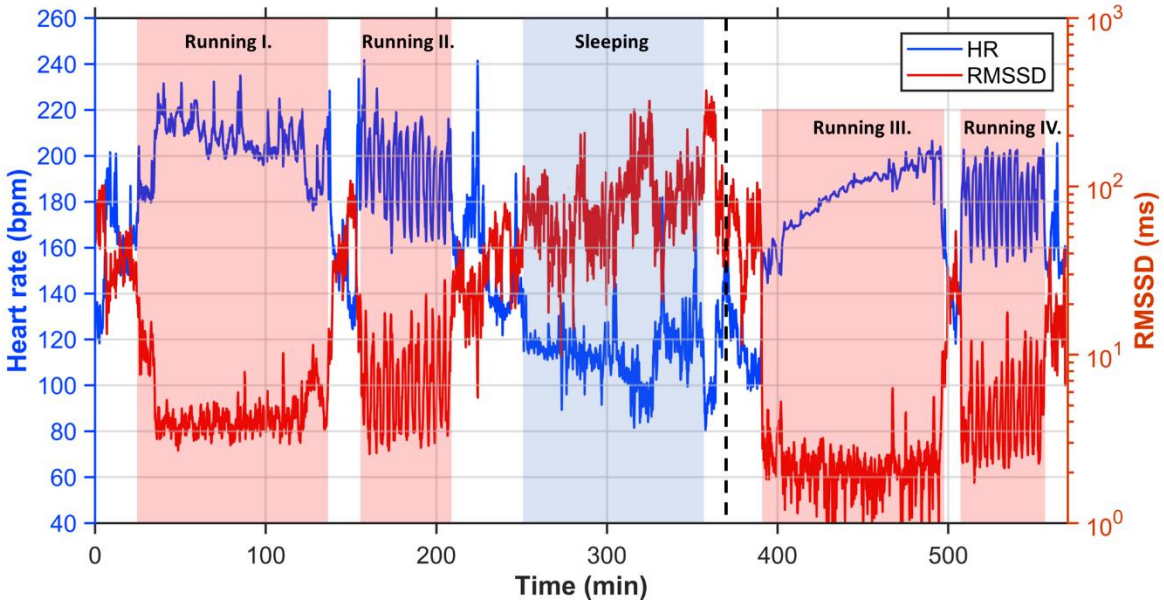


Figure 2. Temporal evolution of heart rate and RMSSD before and after β-adrenergic blockade. Heart rate and RMSSD are shown throughout the experimental protocol. Exercise periods, the intervening resting/sleep period, and β-blocker administration are indicated. The dashed vertical line marks the time of β-blocker administration.

A partial recovery was observed during the final exercise period (*Running IV*), where HR and RMSSD both increased compared with *Running III*. Nevertheless, the maximum heart rate remained below that observed before β-blockade, while RMSSD continued to be substantially lower during exercise than under control conditions.

These observations suggest that the reduction in RMSSD following β-blockade cannot be explained solely by the accompanying changes in heart rate. Instead, they indicate that β-adrenergic blockade modifies the underlying HR–HRV relationship itself, a finding that is examined quantitatively in the subsequent Master Curve analysis.

Master curves during exercise with and without β-blockade

Master Curves were reconstructed separately for repeated control measurements and β-blockade conditions to evaluate whether the HR–HRV relationship was stable under non-blocked conditions and whether it was systematically modified by β-adrenergic blockade (Figure 3).

First, the reproducibility of the Master Curve was assessed using repeated control measurements obtained from the same animals under comparable non-blocked conditions. As shown in the left panels of Figure 3, repeated measurements produced highly similar HR–RMSSD relationships within each subject. Although some scatter was present, the overall shape, slope, and transition region of the Master Curve were preserved across repeated recordings. This demonstrates that the Master Curve represents a reproducible subject-specific HR-dependent variability relationship rather than a transient feature of a single recording.

The control Master Curve showed a characteristic nonlinear dependence on heart rate. On a semi-logarithmic scale, RMSSD decreased approximately linearly with increasing HR in the low-to-mid heart-rate range, corresponding to an approximately exponential decrease in variability. In the higher heart-rate range, the curve deviated from this monotonic decline and, in several animals, showed a partial upward turn at very high HR values. This overall structure resembles the HR–HRV relationship previously observed in humans, although the high-HR increase appears more pronounced in canines [29,31].

A similar nonlinear and reproducible HR–HRV relationship has also been observed in human recordings. In previous human studies, the RMSSD–HR relationship showed the same characteristic nonlinear structure, with an approximately exponential decrease over the low-to-mid heart-rate range and a deviation from monotonic decline at higher heart rates [29,31]. Repeated measurements demonstrated that this relationship is reproducible within individuals, supporting the interpretation that the Master Curve represents a stable, subject-specific HR-dependent reference rather than a recording-specific artifact. The present canine data therefore reproduce the same fundamental HR–HRV organization observed in humans, while extending it to a controlled β-adrenergic perturbation model.

The reproducibility of the control Master Curves is critical for interpreting the β-blockade results. Because repeated non-blocked measurements yielded nearly overlapping Master Curves, the observed post-blockade displacement cannot be attributed simply to measurement variability, fatigue, time-dependent drift, or circadian effects. Instead, the stable control relationship provides a reliable reference against which the effect of β-adrenergic blockade can be evaluated.

Following β-blocker administration, the HR–HRV relationship was systematically displaced downward. In the right panels of Figure 3, β-blockade data are compared with the subject-specific control Master Curve. At matched heart-rate values, RMSSD values after β-blockade were consistently lower than the corresponding control reference. This downward displacement was observed across all animals in the overlapping heart-rate range, although the magnitude of the reduction and the achievable maximum heart rate differed between subjects.

The reproducibility of the control Master Curve was quantified from the same dataset used to generate Figure 3. Pairwise comparisons between repeated control Master Curve estimates within matched HR bins showed small within-subject deviations. The median absolute difference in ln(RMSSD) between repeated control curves was 0.142 in Canine 1, 0.134 in Canine 2, and 0.160 in Canine 3, respectively.

In contrast, the effect of β-adrenergic blockade was substantially larger. Compared with the subject-specific median control Master Curve, β-blockade reduced RMSSD by 73.5% in Canine 1, 89.0% in Canine 2, and 54.7% in Canine 3. The corresponding median shifts Δln(RMSSD) were −1.326 ($p<0.001$), −2.208 ($p<0.001$), and −0.793 ($p<0.001$), respectively.

Thus, the β-blockade-induced displacement of the Master Curve exceeded the normal within-subject variability of repeated control measurements by a large margin. This supports the interpretation that the downward shift was not caused by measurement variability, fatigue, circadian drift, or random day-to-day fluctuation, but reflected a systematic pharmacological effect on the HR–HRV relationship.

HR-range-specific analysis further confirmed the consistency of the effect. For each valid HR bin, the difference between β-blockade and the subject-specific median control Master Curve was calculated. The resulting Δln(RMSSD) values were evaluated within predefined HR ranges using the Wilcoxon signed-rank test, with HR bins serving as the statistical units. Significance was indicated only when at least five valid

HR bins were available within the corresponding range. For example, in Canine 2, RMSSD was reduced by 81.1% in the 110–130 bpm range, 90.7% in the 130–150 bpm range, and 92.1% in the 150–170 bpm range, with all comparisons reaching high statistical significance. These results demonstrate that the β-blockade effect was not confined to a single heart-rate interval, but persisted across the overlapping HR range.

Taken together, Figure 3 demonstrates two central findings. First, the Master Curve is a reproducible subject-specific reference relationship under control conditions. Second, β-adrenergic blockade produces a systematic downward displacement of this relationship. These results show that β-blockade does not merely move the cardiovascular system along an unchanged HR–HRV curve, but modifies the HR–HRV relationship itself.

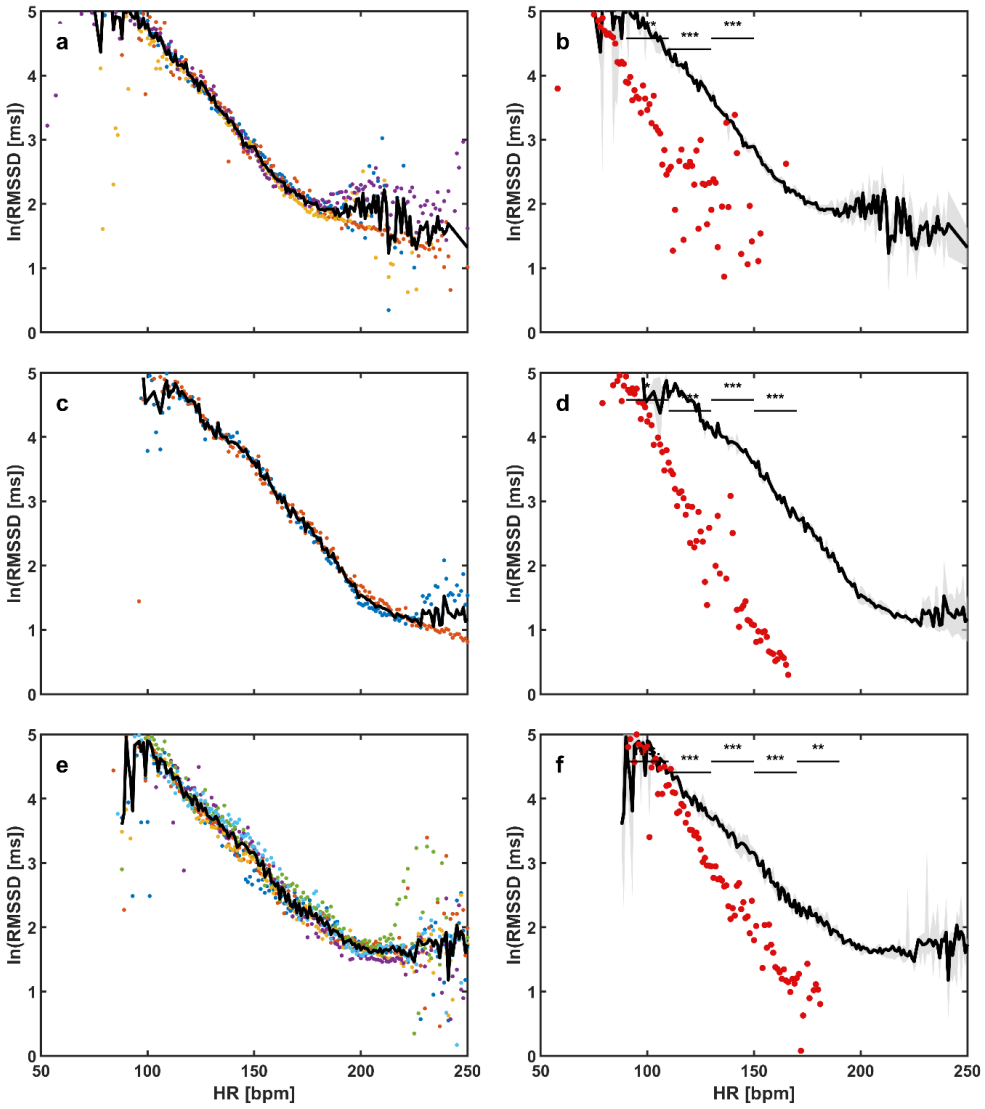


Figure 3. Left panels show repeated control Master Curves obtained from the same animals under non-blocked conditions. The close overlap of repeated measurements demonstrates the reproducibility of the subject-specific HR–RMSSD relationship. Right panels compare the control Master Curve with RMSSD values obtained after β-adrenergic blockade. The black curve represents the control reference Master Curve, while red points indicate post-blockade measurements. β-blockade produces a systematic downward displacement of the HR–HRV relationship, with significantly lower RMSSD values at matched heart rates. This indicates that the reduction in variability cannot be explained by movement along the control curve alone, but reflects a structural modification of the Master Curve itself. Significance markers indicate Wilcoxon signed-rank tests performed on HR-bin-level Δln(RMSSD) values within predefined HR ranges. The statistical unit was the HR bin, not the individual RR interval. Significance was indicated only when at least five valid HR bins were available within the corresponding range. $*p<0.05$, $**p<0.01$, $***p<0.001$, n.s. $p\geq0.05$.

Normalized variability during β-blockade and exercise

Figure 4 shows the temporal evolution of heart rate (HR) together with the normalized variability measures (σ_{nSD} and LnSD) before and after β-adrenergic blockade.

Before β-blockade, heart rate increased during both exercise protocols and gradually declined during the intervening resting/sleep period. Following β-blocker administration, resting heart rate was markedly reduced, while the subsequent exercise-induced increase reached substantially lower values and exhibited smaller fluctuations than under control conditions.

The normalized variability (σ_{nSD}, LnSD) exhibited characteristic transient responses throughout the experimental protocol. Before β-blockade, both constant-load and interval exercise elicited rapid increases in normalized variability, followed by a gradual return toward baseline. During the intervening sleep period, normalized variability remained moderately elevated and increased further upon awakening.

Following β-blocker administration, normalized variability decreased abruptly to approximately 30% of its baseline level. Although a partial recovery occurred during the subsequent resting period, the onset of exercise induced a further marked reduction, with normalized variability falling to only a few percent of the reference level. This strongly suppressed state persisted throughout the exercise period and recovered only gradually thereafter, reaching approximately 50% of the reference value by the end of the experiment.

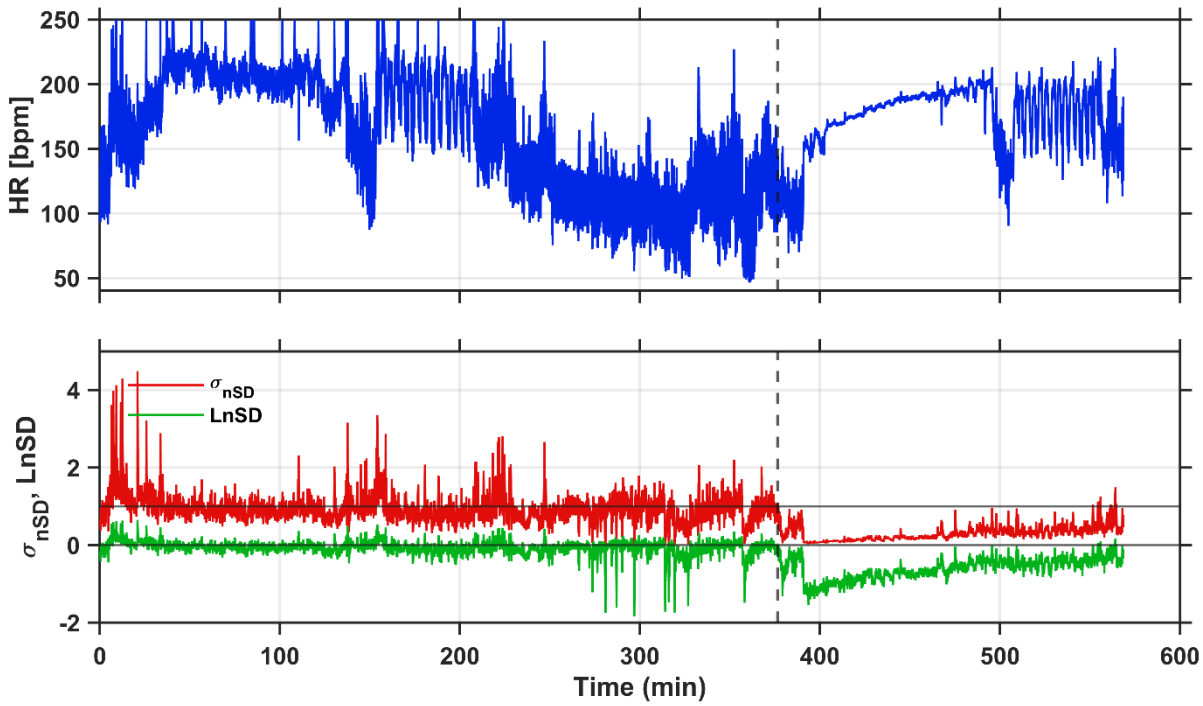


Figure 4. Time series of heart rate and heart-rate-normalized variability before and after β-adrenergic blockade. Heart rate is shown in the upper panel. In the lower panel, the red curve represents the local standard deviation of the normalized successive-difference signal, calculated using a 10s moving window, whereas the green curve represents its natural logarithm (LnSD). The dashed vertical line indicates the time of β-blocker administration. Values of σ_{nSD}=1, corresponding to LnSD=0, represent variability equal to the heart-rate-specific reference level defined by the non-blocked Master Curve.

Discussion

The principal finding of the present study is that β-adrenergic blockade systematically modifies the entire HR–HRV relationship rather than simply reducing heart rate variability. Although conventional time-domain HRV measures remained inversely related to heart rate, the corresponding Master Curve was consistently displaced downward following β-blockade. Consequently, HRV values were lower at comparable heart rates across the physiological range, indicating that the intervention altered the HR–HRV relationship itself rather than merely shifting the operating point along an unchanged curve.

This observation has important implications for HRV interpretation. Conventional HRV analysis is typically based on measurements obtained at a single operating point, implicitly assuming that changes in HRV directly reflect changes in autonomic regulation. However, the present results demonstrate that autonomic interventions may alter not only the operating point, but also the underlying HR–HRV relationship. Consequently, identical changes in HRV may arise either from movement along an unchanged Master Curve or from displacement of the Master Curve itself. Without a heart rate–dependent physiological reference, these fundamentally different mechanisms cannot be distinguished.

This limitation provides a straightforward explanation for the contradictory effects of β-blockade reported in the literature. Studies evaluating HRV at a single heart rate have described both increases and decreases in conventional HRV measures following β-blockade. These apparently conflicting observations are expected if β-blockade simultaneously (i) shifts the operating point because of heart-rate reduction and (ii) displaces the entire Master Curve downward. The observed HRV therefore depends on the relative contribution of these two effects rather than on the pharmacological intervention alone.

Why does β-blockade produce contradictory HRV findings?

A key observation of the present study is illustrated in Figure 5. At first glance, the literature appears contradictory: β-adrenergic blockade has been reported to both increase and decrease conventional time-domain HRV measures such as RMSSD and SDNN. However, we propose that this apparent contradiction is not physiological, but methodological. The fundamental limitation is the absence of a heart rate–dependent physiological reference for interpreting HRV.

As demonstrated by the present results, β-blockade does not simply alter heart rate or reduce HRV at a single operating point. Instead, it modifies the entire HR–HRV relationship. Under control conditions, HRV follows a characteristic heart rate–dependent Master Curve. Following β-blockade, this entire relationship is displaced downward, resulting in lower HRV values across the physiological heart-rate range.

This has an important consequence. β-blockade simultaneously produces two independent effects: it shifts the operating point toward lower heart rates and, at the same time, displaces the entire Master Curve downward. The observed HRV therefore depends on the relative contribution of these two effects rather than on the pharmacological intervention alone.

This concept is illustrated schematically in Figure 5. In case (1), the heart-rate shift dominates, leading to an apparent increase in conventional HRV despite an overall reduction in autonomic modulation. In case (2), the downward displacement of the Master Curve dominates, resulting in a decrease in HRV. Importantly, both outcomes originate from the same physiological mechanism. The apparent inconsistency therefore arises from the way HRV is interpreted rather than from fundamentally different physiological responses.

These observations explain the contradictory findings reported in the literature. Without reconstructing the complete HR–HRV relationship before and after an intervention, HRV measurements obtained at a single operating point remain inherently ambiguous. Single-point comparisons, even under resting conditions, cannot distinguish between movement along an unchanged HR–HRV relationship and structural modification of the relationship itself.

The broader implication extends beyond β-blockade. Conventional HRV analysis implicitly assumes that changes in HRV directly reflect changes in autonomic regulation. However, the present results demonstrate that identical HRV values—or even identical HRV changes—may arise from fundamentally different physiological mechanisms. A given HRV change may simply reflect movement along an unchanged Master Curve caused by heart-rate changes, or it may result from displacement of the Master Curve itself due to altered autonomic regulation. Without a heart rate–dependent physiological reference, these mechanisms cannot be separated.

Reconstruction of the Master Curve provides this missing reference. By describing HRV relative to its expected value at each heart rate, it separates heart-rate–dependent mathematical effects from genuine changes in autonomic regulation. Within this framework, β-blockade consistently produces a downward displacement of the Master Curve, revealing a global reduction in autonomic variability that is largely independent of the instantaneous heart rate.

Importantly, β-blockade should not be viewed as the primary subject of this study, but rather as a controlled physiological perturbation used to reveal the structure of the HR–HRV relationship. The significance of the present findings therefore extends beyond pharmacological blockade itself. Any intervention capable of modifying autonomic regulation—whether pharmacological, physiological, or pathological—should be interpreted within the context of the heart rate–dependent Master Curve rather than from HRV measured at a single operating point.

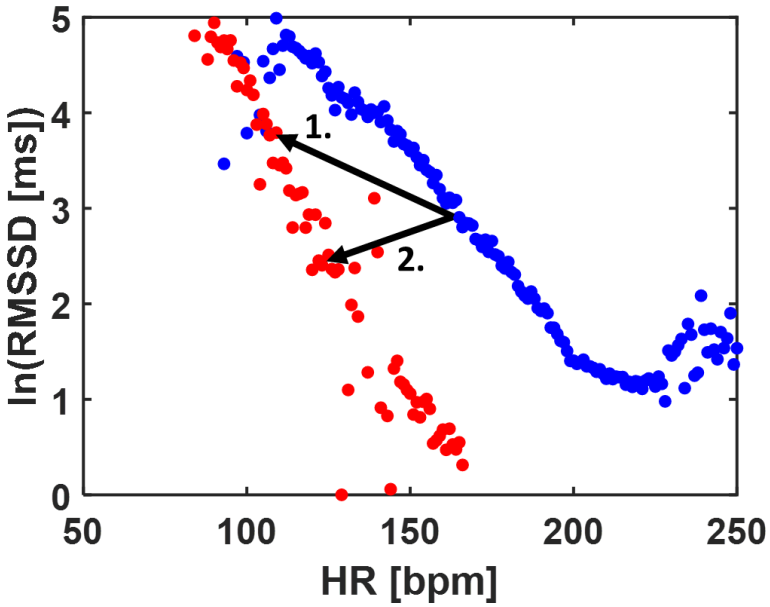


Figure 5. Apparent contradictory HRV responses after β-adrenergic blockade can arise from the same downward displacement of the HR–HRV relationship.

Model

The experimental results demonstrate that β-adrenergic blockade displaces the entire HR–HRV relationship downward rather than merely shifting the operating point along an unchanged curve. To provide a mechanistic interpretation of this observation, we extend our previously introduced stochastic integrate-and-fire framework by assigning physiological meaning to the fluctuation sources underlying the Master Curve [31].

We assume that cardiac rhythm is governed by an intrinsic pacemaker drive together with sympathetic and parasympathetic regulatory inputs. The total effective current driving the cardiac oscillator is written as:

$$I_t = I_i + I_S - I_p$$

where I_i represents the intrinsic (pacemaker) current, I_S the sympathetic contribution, and I_P the parasympathetic contribution. The negative sign reflects the inhibitory effect of the parasympathetic system on the heart rate.

Each component is assumed to fluctuate around a baseline level:

$$I_i = I_{i0}(1 + \gamma\delta_i)$$

$$I_S = I_{S0}(1 + \alpha\delta_S)$$

$$I_P = I_{P0}(1 + \beta\delta_P)$$

where δ_i, δ_P, δ_S are stochastic processes, and α, β, γ represent the relative strength of fluctuations.

Within this framework, heart rate can be approximated as:

$$HR = \frac{60}{\frac{\Delta V_m C_m}{I_t} + APD}$$

while the variability of successive RR intervals can be expressed as:

$$RMS(\Delta RR) \approx \Delta V_m C_m \frac{d}{I_t^2}$$

with

$$d = \sqrt{(\alpha I_S)^2 + (\beta I_P)^2 + (\gamma I_i)^2} \rightarrow HRV$$

Key implication

The above formulation leads to a fundamental conclusion regarding the relationship between heart rate and HRV. HRV is not determined by heart rate alone, but by the magnitude of the underlying autonomic currents. In particular, the variability term depends directly on both sympathetic and parasympathetic contributions, reflecting their combined influence on the system.

In contrast, heart rate is primarily governed by the net chronotropic contribution of these components, which can be approximated as

$$I_S - I_p \rightarrow HR$$

This separation leads to an important and nontrivial result: the same heart rate can be produced by infinitely many combinations of sympathetic and parasympathetic activity, each associated with a different level of HRV. In other words, fixing the heart rate does not uniquely determine the underlying autonomic state.

For instance, a given heart rate may correspond to a condition where both I_S and I_P are high, indicating an elevated overall autonomic activity, or to a condition where both components are low, reflecting a reduced activation state. While these two cases yield identical heart rates, they result in markedly different levels of HRV.

This distinction is essential for the interpretation of experimental results. It implies that heart rate primarily reflects the net chronotropic drive that determines the cardiac operating point, whereas HRV

reflects the magnitude of dynamic regulatory fluctuations around that operating point. This separation provides the missing mechanistic link between the experimentally observed downward shift of the HRV-HR relationship and the physiological effect of β-adrenergic blockade.

Interpretation of the results using the model

β-adrenergic blockade reduces the effective sympathetic contribution acting on the heart. We represent this by introducing a residual sympathetic fraction η:

$$I_S^{\beta} = \eta I_S$$

where $0 \leq \eta < 1$. Complete blockade corresponds to $\eta \approx 0$, while recovery from blockade corresponds to a gradual increase of η toward 1.

Under control conditions, the total effective current is:

$$I_t^C = I_i - I_P + I_S$$

Under β-blockade, it becomes:

$$I_t^{\beta} = I_i - I_P^{\beta} + \eta I_S$$

To compare variability at the same heart rate, the total effective current must be the same:

$$I_t^{\beta} = I_t^C$$

Substitution gives:

$$I_i - I_P^{\beta} + \eta I_S = I_i - I_P + I_S$$

Therefore:

$$I_P^{\beta} = I_P - (1 - \eta) I_S$$

This means that, at a matched heart rate, reduction of the effective sympathetic contribution must be accompanied by a reduction in parasympathetic influence. In physiological terms, if sympathetic chronotropic drive is suppressed, parasympathetic withdrawal is required to preserve the same heart-rate operating point.

The fluctuation magnitude under control conditions is:

$$d_C = \sqrt{(\alpha I_S)^2 + (\beta I_P)^2 + (\gamma I_i)^2}$$

Under β-blockade, the corresponding fluctuation magnitude becomes:

$$d_{\beta} = \sqrt{(\alpha \eta I_S)^2 + [\beta (I_P - (1 - \eta) I_S)]^2 + (\gamma I_i)^2}$$

Because $0 \leq \eta < 1$, both the effective sympathetic term and the compensatory parasympathetic term are reduced at the matched heart rate. Therefore:

$$d_\beta < d_C$$

Since heart rate is matched, the mathematical scaling term I_t^{-2} remains unchanged. Consequently:

$$RMS(\Delta RR)_\beta < RMS(\Delta RR)_C$$

at the same heart rate.

This provides the mathematical basis for the downward displacement of the Master Curve. β-blockade does not merely move the system along the original HR–HRV relationship. Instead, it reduces the magnitude of effective regulatory fluctuations, lowering the expected HRV at comparable heart rates.

Supporting evidence from a human stress–relaxation paradigm

The interpretation provided by the Master Curve framework is not restricted to pharmacological interventions. To examine whether the same principles apply during physiological autonomic transitions, it is useful to compare the present findings with observations obtained from human stress–relaxation paradigms.

Recent studies using heart-rate-normalized variability measures have demonstrated that conventional HRV indices and normalized variability may show qualitatively different responses during autonomic state changes. Rudics et al. showed that conventional HRV parameters, including RMSSD and SDNN, may be strongly influenced by their dependence on heart rate, whereas normalized variability measures reveal dynamics that are not apparent in conventional indices [29].

In these studies, stress induction, associated with increased heart rate and sympathetic activation, resulted in increased normalized variability. Conversely, relaxation, characterized by reduced heart rate and decreased physiological activation, was associated with reduced normalized variability.

At first glance, this behavior appears inconsistent with the traditional interpretation of HRV, where increased variability is commonly associated with lower heart rate and increased parasympathetic activity. However, within the present framework, these findings are expected.

The proposed model distinguishes between the net chronotropic effect determining heart rate and the magnitude of dynamic regulatory fluctuations determining HRV. During stress, increased sympathetic activation is accompanied by dynamic autonomic regulation and compensatory adjustments of opposing influences. As a result, the overall magnitude of regulatory fluctuations increases, producing elevated normalized variability.

In contrast, during relaxation, both sympathetic and parasympathetic influences are reduced in magnitude, despite a relative parasympathetic predominance in the control of heart rate. Because the overall magnitude of autonomic fluctuations decreases, normalized variability is reduced.

Therefore, a lower heart rate does not necessarily imply increased variability. The physiological interpretation depends on whether the system has moved along an unchanged HR–HRV relationship or whether the underlying regulatory dynamics have changed.

Importantly, this physiological example parallels the findings obtained during β-adrenergic blockade. In both cases, conventional HRV measures alone may lead to ambiguous interpretation because changes in heart rate and changes in variability occur simultaneously.

The agreement between pharmacological perturbation and physiological autonomic transitions supports the general applicability of the Master Curve framework. β-blockade therefore serves as a controlled experimental model revealing a broader principle: HRV acquires physiological meaning only when interpreted relative to its heart-rate-dependent reference relationship.

Clinical interpretation: potential dissociation under β-blockade

An important implication of the present framework concerns the interpretation of autonomic state during β-adrenergic blockade.

Within the proposed model, β-blockade represents suppression of the effective sympathetic contribution acting on the heart rather than complete elimination of sympathetic neural activity. Therefore, pharmacological blockade may alter the relationship between autonomic drive and cardiac response.

Under normal physiological conditions, changes in sympathetic and parasympathetic influences are reflected simultaneously in heart rate and HRV. However, when cardiac β-adrenergic effects are attenuated, heart rate may no longer fully represent the underlying sympathetic influence on the organism.

As demonstrated by the model, maintaining a given heart rate under β-blockade requires compensatory reduction of parasympathetic influence. Consequently, the effective autonomic components contributing to cardiac variability are reduced even if central sympathetic activation persists.

This raises the possibility that β-blockade may create a partial dissociation between cardiac measurements and broader autonomic regulation. For example, during exercise or psychological stress, sympathetic drive may remain elevated at the systemic level while its direct chronotropic effect on the heart is pharmacologically limited.

In such conditions, sympathetic activity may continue to influence non-cardiac targets, including vascular, metabolic, and central regulatory systems, despite a reduced heart-rate response.

Therefore, heart rate alone may underestimate the overall level of physiological activation when the relationship between autonomic drive and cardiac response has been modified pharmacologically.

This interpretation remains hypothetical because sympathetic and parasympathetic activity were not measured directly in the present study. Direct validation would require simultaneous assessment of neural autonomic activity, circulating catecholamines, vascular responses, or other independent markers of autonomic state.

Nevertheless, the present framework highlights an important consequence of pharmacological modulation: when the relationship between autonomic input and cardiac output is altered, neither heart rate nor conventional HRV measures alone may provide a complete representation of the underlying physiological state.

Limitations

Several limitations should be considered when interpreting the present findings.

First, the experimental data were obtained from laboratory canines, which may limit direct extrapolation to human physiology. However, the primary observation of the study—the systematic modification of the HR–HRV relationship following β-adrenergic blockade—was consistently observed across the investigated animals. Furthermore, similar qualitative behavior of heart-rate-normalized variability has been reported in human stress–relaxation paradigms, supporting the potential general relevance of the proposed framework [29].

Second, the number of experimental subjects was limited. The present study was designed primarily to investigate the mechanistic relationship between heart rate and variability under controlled conditions, rather than to establish population-level reference values. The consistency of the observed Master Curve displacement across animals supports the robustness of the phenomenon; however, larger datasets will be required to quantify inter-individual variability and determine the general applicability of the framework.

Third, sympathetic and parasympathetic activity were not measured directly. Therefore, the physiological interpretation of the two-component model remains indirect and should be considered a mechanistic hypothesis rather than a direct measurement of autonomic activity. This limitation reflects a broader challenge in HRV research, where the relationship between measured variability and underlying autonomic processes is generally inferred rather than directly observed.

Importantly, the central finding of the present study does not depend on direct measurement of autonomic nerve activity. The experimentally demonstrated result is that β-adrenergic blockade alters the HR–HRV relationship itself, producing a systematic displacement of the Master Curve. The proposed physiological model provides one possible mechanism explaining this observation, but it should not be interpreted as the only possible mechanistic explanation.

Fourth, the present analysis focused primarily on RMSSD and its normalized form. Although RMSSD provides a robust and interpretable measure of short-term RR variability within the proposed framework, a comprehensive validation of the Master Curve approach will require extension to additional HRV parameters, including SDNN, frequency-domain indices, and nonlinear measures.

A further limitation concerns heart-rate coverage. Master Curve reconstruction requires sufficient data across the relevant heart-rate range. Therefore, regions with sparse data, insufficient overlap between conditions, or excessive artifacts may limit direct comparison between control and β-blockade states. In the present study, interpretation was therefore focused on heart-rate ranges where reliable within-subject comparison was possible.

Finally, the degree of β-adrenergic blockade was inferred from the experimental protocol and the observed physiological response rather than independently quantified by pharmacokinetic or receptor-level measurements. Although the heart-rate response strongly indicates substantial cardiac β-adrenergic suppression, future studies including direct pharmacological or biochemical validation could further strengthen the mechanistic interpretation.

Taken together, these limitations do not affect the central empirical observation that β-blockade modifies the HR–HRV relationship. Rather, they define the next steps required to validate the physiological interpretation of this phenomenon across species, conditions, and HRV metrics.

Conclusions

The present study demonstrates that heart rate variability cannot be interpreted independently of heart rate. Using β-adrenergic blockade as a controlled physiological perturbation, we show that autonomic intervention does not merely shift the cardiovascular system along an unchanged HR–HRV relationship, but can modify the relationship itself.

The central finding is that β-blockade produces a systematic downward displacement of the heart-rate-dependent Master Curve, resulting in lower variability at comparable heart rates across the physiological range. This indicates that the observed HRV response after autonomic intervention cannot be interpreted solely from the direction of change in conventional HRV parameters. An apparent increase or decrease in HRV may reflect the combined effects of heart-rate shifts and structural modification of the HR–HRV relationship.

The Master Curve framework separates two fundamentally different mechanisms that are indistinguishable in conventional HRV analysis: movement along an existing HR–HRV relationship caused by changes in operating heart rate, and modification of the relationship itself caused by altered physiological regulation. Without such a heart-rate-dependent reference, HRV changes cannot be uniquely attributed to changes in autonomic modulation.

The proposed two-component model provides a mechanistic interpretation of these observations. Within this framework, heart rate primarily reflects the net chronotropic effect of opposing autonomic influences, whereas HRV reflects the magnitude of dynamic regulatory fluctuations. This separation explains why identical heart rates may correspond to different HRV levels and why similar HRV changes may arise from different physiological mechanisms.

Importantly, β-adrenergic blockade should not be considered the final target of the present investigation, but rather a controlled perturbation that reveals a more general principle. The physiological meaning of HRV emerges only when variability is interpreted relative to its heart-rate-dependent reference relationship.

Overall, the heart-rate-dependent Master Curve provides a physiological reference framework for interpreting HRV across pharmacological interventions, physiological transitions, and potentially clinical conditions. This approach offers a more mechanistically grounded interpretation of HRV than conventional analysis based on isolated variability measures.

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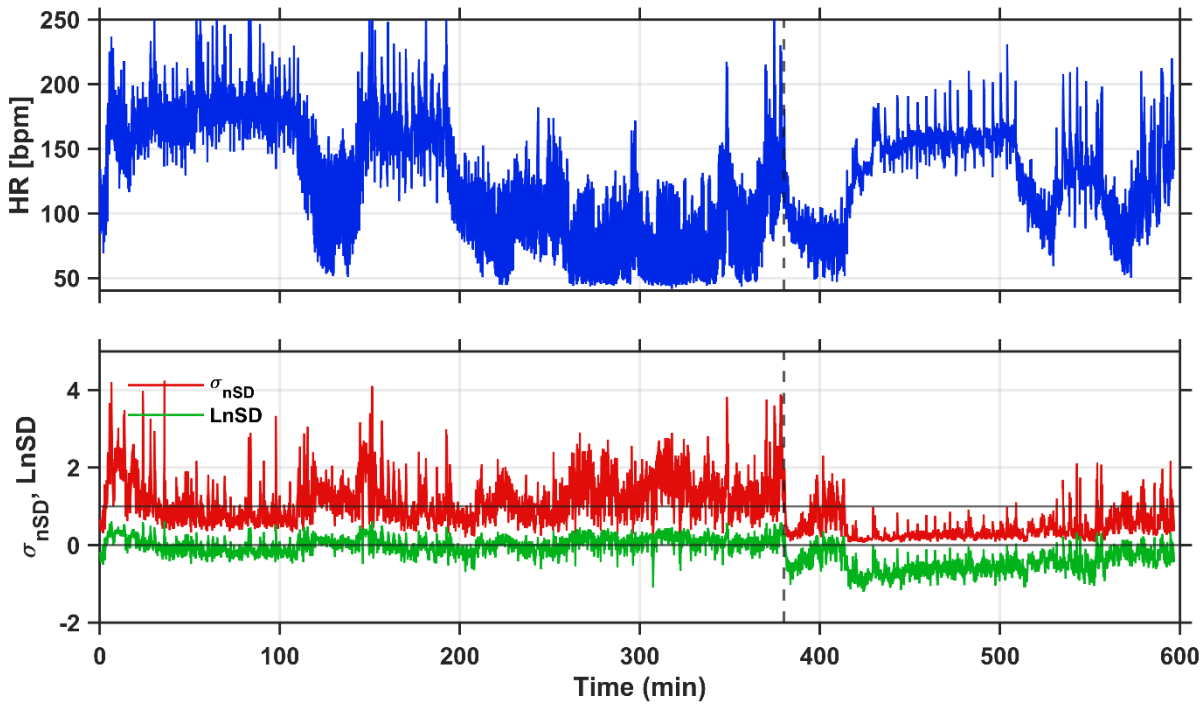
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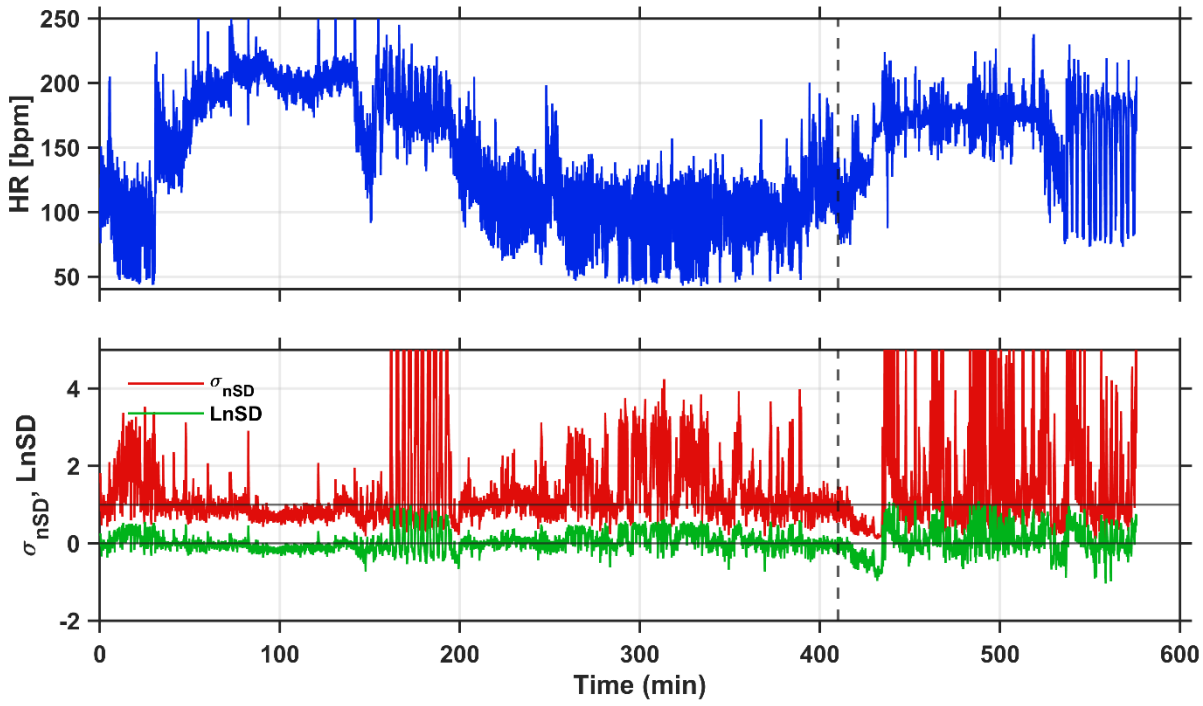
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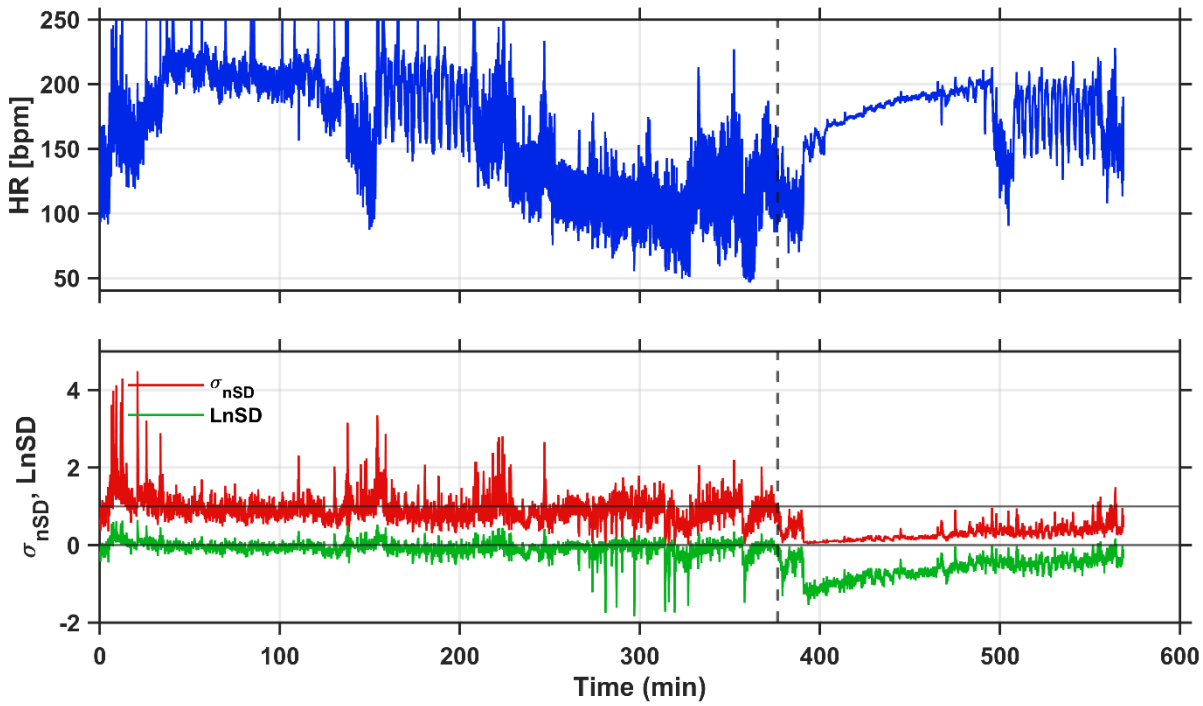
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Supplementary Information







SI Figure 1. Time series of heart rate and heart-rate-normalized variability in the three canines before and after β-adrenergic blockade. Heart rate is shown in blue in the upper panels. In the lower panels, the red curves represent the local standard deviation of the normalized successive-difference signal (σ_{nSD}), calculated using a 10s moving window, while the green curves represent its natural logarithm (LnSD). The dashed vertical lines indicate β-blocker administration. Values of σ_{nSD}=1, corresponding to LnSD=0, represent variability equal to the heart-rate-specific level defined by the non-blocked reference Master Curve.