Research Article

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Right vagotomy alters heart rate variability temporarily and increases total choline levels in rats

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Abstract

Objectives: The variability in the time intervals between heartbeats, known as heart rate variability (HRV), serves as a reflection of the intricate interplay between the sympathetic and parasympathetic neural systems. While the potential asymmetric effects of the left and right branches of the vagus nerve remain uncertain, this study aims to investigate the impact of unilateral, bilateral, and atropine interventions on HRV parameters and choline levels within cardiac tissue.
Methods: 40 male adult Wistar albino rats were randomly assigned to the five groups (each n=8): sham-operated, atropine, right vagotomy, left vagotomy, and bilateral vagotomy. Heart rate variability (HRV) analyses were conducted, and the levels of total choline/acetylcholine in heart tissues were quantified. Statistical analyses were performed to assess the results.
Results: The bilateral vagotomy and atropine groups exhibited higher heart rates and high frequency power (HF), along with reduced low frequency power (LF). Total power (TP) remained relatively unchanged. In the bilateral vagotomy group, $DFA\alpha_1$ was significantly elevated while $DFA\alpha_2$ was reduced significantly. SD1 and SampEn were significantly lower in both the bilateral vagotomy and atropine groups. Notably, the right vagotomy group displayed significant changes primarily in the 15th minute, particularly in time-domain parameters, HF, TP, and SD1, with a significant increase observed in total choline levels.
Conclusions: Our results revealed that asymmetrical vagal innervation induces distinct effects on heart rate variability parameters and total choline/acetylcholine levels in heart tissues. Our findings suggest that compensatory hemodynamic recovery, possibly driven by contralateral vagal overactivity, may contribute to these observed results.

Keywords: rat; autonomic nervous system; nervous vagus; heart rate variability; choline

Introduction

Exploring variability within biological systems is a common practice in medicine. Examples include daily fluctuations in chemical messengers, body temperature cycles, and respiratory sinus arrhythmia [1, 2]. The intricate fluctuations in heart rate and rhythm in a healthy individual enable the circulatory system to respond quickly to both physical and psychological stressors that disrupt homeostasis [3]. Heart rate variability (HRV) serves as a method for evaluating autonomic function. It represents the variation in duration between consecutive heartbeats (interbeat-intervals, IBI), which can be assessed through continuous electrocardiographic (ECG) recordings [4]. HRV reflects the function of the autonomic nervous system (ANS) and the extent to which the sympathetic and parasympathetic nervous systems influence heart rate (HR) [5].

Parameters derived from heart rate variability (HRV) analyses encompass time-domain, frequency-domain, and nonlinear analysis [5]. Time-domain HRV analysis involves statistical assessment of the time intervals between consecutive heartbeats [6]. Commonly used parameters in this domain include the root mean square of successive differences between normal heartbeats (RMSSD), which is indicative of vagally mediated changes [5].

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Frequency-domain analysis, on the other hand, evaluates the power of heart rate changes within specific frequency bands [7]. The low frequency (LF) band reflects changes originating from both the sympathetic and parasympathetic nervous systems, while the high frequency (HF) band primarily indicates parasympathetic activity. Consequently, the LF/HF ratio is often used as an indicator of sympathovagal balance [5].

Traditionally, HRV has been examined utilizing time and frequency domain methodologies, which assess the overall amplitude of heart rate fluctuations or fluctuations within specific frequency bands [8]. In order to detect nuanced yet significant alterations and abnormalities within heart rate time series data, novel approaches grounded in nonlinear system theory have been continually evolving. Nonlinear analysis methods are quite different than the traditional methos since they are designed to detect qualitative rather than quantitative properties of heart rate series [9]. Therefore, it is crucial to include these methods in order to better understand the physiological mechanisms that influence HRV parameters.

The pathophysiology of various disorders involving the Autonomic Nervous System (ANS) and their effects on cardiac functions can be better comprehended through Heart Rate Variability (HRV) analysis in experimental animal models. However, as of now, the potential asymmetric effects of the left and right branches of the vagus nerve have not been definitively established. The right branch of the vagus nerve primarily influences the sinoatrial (SA) node, responsible for heart rhythm generation, while the left branch affects the atrioventricular (AV) node [10]. It is probable that the contributions of the left and right branches of the vagus nerve to heart rate variability parameters, especially those associated with the parasympathetic system, will differ.

The levels of certain neurotransmitters directly reflect neuronal function. It is established that vagus nerve stimulation uniformly increases acetylcholine (ACh) release throughout the cardiac muscle [11]. However, the specific contributions of the left and right branches of the vagus nerve to ACh release remain unclear. Therefore, this study aims to investigate the effects of unilateral and bilateral vagotomy, as well as atropine injections, on Heart Rate Variability (HRV) parameters and choline levels in heart tissues.

Materials and methods

Animals and anesthesia

40 male and adult (12–16 weeks old) Wistar albino rats (288.6 ± 34.23 g, n=40) were used as laboratory animals after obtaining the permission from Local Animal Experiments Ethics Committee (No: 77.637.435). Male rats were selected in order to eliminate the potential confounding effects of the menstrual cycle [4]. The animals were kept in the animal care center for at least five days prior to the experiments under 12 h day and night cycle, at 20–22 °C.

Urethane (Sigma-Aldrich, Catalog No. PHA568487) was administered intraperitoneally (i.p.) at a dose of 1.5 g/kg to induce anesthesia before the surgical procedure. This dose was chosen to ensure anesthesia without interfering with cardiac autonomic control [12]. The pedal pain reflex and respiration frequency were continuously monitored to assess the depth of anesthesia following the initial injection of urethane. If necessary, additional doses constituting 25 % of the initial anesthesia dose were administered to maintain the desired level of anesthesia. To prevent hypothermia during anesthesia, the animals were placed on a heating plate, and their body temperature was monitored using a rectal thermometer.

Experimental setting

The 40 rats were divided into five groups (n=8 each) to investigate the asymmetric effects of various interventions on Heart Rate Variability (HRV) and choline levels in the heart: (i) sham, (ii) right vagotomy, (iii) left vagotomy, (iv) bilateral vagotomy, and (v) atropine administration.

Following anesthesia, the rats were positioned supine on a heated thermal plate for electrocardiogram (ECG) recordings. Needle electrodes for ECG were inserted subcutaneously into the extremities. Surface ECG recordings were obtained using the DII derivation [13]. For “R” wave detection, PowerLab (ver 7.0, ADInstruments, Australia) software was used.

Vagotomy and sham procedures

A 2.0 cm incision was made in the middle of the neck on both sides to access the vagus nerve at the cervical level. The sternohyoid and sternomastoid muscles were gently separated using small forceps to expose the vagus nerve. Careful dissection was performed to isolate the vagus nerve from surrounding tissue. Subsequently, either a right, left, or bilateral vagotomy was randomly performed.

In the sham group, the right and left vagal nerves were exposed and stabilized, but no incision was made. Vagal innervation was chemically blocked by administering 5 mg/kg methylatropine bromide (Sigma-Aldrich, Catalog No. M1300000) via injection 1 min after initiating ECG recordings [14].

Heart rate variability analyses

After detecting R waves, the tachogram of RR intervals was created by using Pan-Tompkin's algorithm [15]. These RR tachograms were converted to time series using Berger interpolation. For all HRV analyses the same HRV analysis software (Kubios ver 2.2.1., University of Eastern Finland) was used.

Time domain analysis

RMSSD

The root mean square of successive deviations (RMSSD) between regular heartbeats was obtained by calculating the time difference in milliseconds between each successive heartbeats. After squaring each of the values and summing the result, the square root of the sum was calculated [5].

Stress index

The stress index (SI) was first proposed by Roman M. Baevsky. It reflects the distribution characteristic of the RR histogram (Figure 1). SI, which is suggested to reflect sympathetic activation, is calculated using the following equation (Equations (1) and (2)) [16].

$$\mathrm{SI} = \lfloor \frac{\mathrm{AMo}\ (\%)}{2 \times \mathrm{Mo} \times \Delta\mathrm{RR}} \rfloor \quad (1)$$

$$\mathrm{AMo}\ (\%) = \frac{n}{N} \times 100\% \quad (2)$$

Frequency domain analyses

Five minutes of R-R tachogram data were utilized to represent each recording period. These data were then resampled at 10 Hz to generate time series data. Our frequency-domain analysis employed frequency bands chosen in accordance with Cerutti et al. (1991). These frequency bands were as follows [17]: VLF: 0.02–0.2 Hz; LF: 0.2–0.5 Hz; HF: 0.5–2.5 Hz. Additionally, the LF/HF ratios were also computed. The relative powers of frequency bands were given, as well as the percentage of power spectrum densities (%PSD).

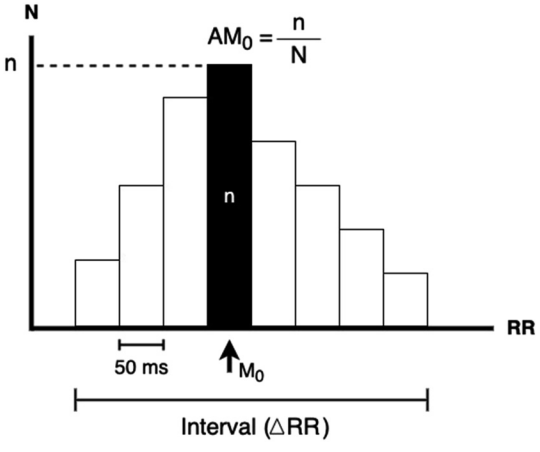

Figure 1: RR histogram used in stress index (SI) calculation.

Nonlinear analyses

Detrended fluctuation analysis (DFA)

The DFA algorithm, developed by Peng et al. (1995), was used to analyze RR time series [18] and the scaling exponents DFA α_1 and DFA α_2 were obtained accordingly. Short-term (DFA α_1) and long-term (DFA α_2) scaling exponents were defined in both groups for different box sizes 4<n<11 and 12<n<64 respectively, which are used for rats [19].

Poincaré plot analysis

An ellipse was drawn around the plotted points to study the Poincaré plot. The Poincaré plot is a statistical visual analysis technique from the perspective of analysis. The coordinates (x, y) of each pair of RR intervals (previous and next) are used to locate them in the rectangular coordinate system, where x represents the RR_n interval value and y represents the RR_{n+1} interval value [20]. The following three parameters are measured using these coordinates:

(i) SD1 is the standard deviation of each point's distance from the y=x axis, which defines the ellipse's width and considered to reflect the sympathetic changes,
(ii) SD2 is the standard deviation of each point from the y=x + average R–R interval, which determines the ellipse's length and considered to reflect the parasympathetic changes,
(iii) SD2/SD1 ratio which is considered as the analogue of LF/HF ratio [5].

Entropy analysis

The goal of sample entropy (SampEn) was to create a less biased and more reliable measure of signal regularity and complexity [21]. It is possible to calculate the SampEn values from a relatively short time series of less than 200 data points. When applied to HRV data, a high SampEn value indicates low predictability in consecutive RR intervals, and a low SampEn value implies that the signal is regular and predictable [21].

Choline/acetylcholine measurements

For choline/acetylcholine analysis, 10 mg of freshly harvested heart tissue was first washed thoroughly in cold PBS. Tissues

were then resuspended in a volume of 500–700 µL of assay Buffer VI/Choline Assay Buffer provided with the kit (Sigma-Aldrich, MAK056) which involves acetylcholinesterase that converts acetylcholine to choline. In the presence of acetylcholinesterase, the kit we used detects total choline (free choline plus acetylcholine). For effective tissue breakdown, the samples were homogenized using an ultrasonic homogenizer, maintained on ice to prevent protein degradation. After homogenization, the samples were centrifuged for a precise duration of 2–5 min at a temperature of 4 °C, employing a cold microcentrifuge set at 11,200 rcf. This step ensures the removal of insoluble material, allowing for the collection of a clear supernatant, which was then meticulously transferred to a clean, sterile tube. Diluted biological samples alongside calibrated kit standards were methodically pipetted into a 96-well plate, each well receiving 50 µl. This was followed by the addition of 50 µl of a meticulously prepared reaction mixture, comprising choline kit solution, an enzyme blend, and a choline-specific probe, into each well. These were then incubated in an uninterrupted dark environment at ambient room temperature for a span of 30 min, ensuring conditions conducive to reaction completion without external light interference. After the incubation period, absorbance readings were accurately measured at 570 nm wavelength. Utilizing these absorbance values, a standard curve was generated for all the kits involved, enabling the quantitative determination of total choline (free choline plus acetylcholine) concentrations in the heart tissue samples [22].

Statistical analysis

To determine if the data were normally distributed, the Shapiro-Wilk test was initially used. Since our data were normally distributed, we used a parametric statistical approach to further examine them. To ascertain whether there was a statistically significant difference between the groups, the independent sample t-test was used, and one-way ANOVA was used for multiple comparisons. The statistical significance level was established at $p<0.05$. The statistical analyses were carried out using IBM SPSS Statistics Version 21.0 (SPSS Inc., Chicago, IL, USA). The format for all data was “Mean SD (Standard Deviation)”.

Results

Time domain analysis

The changes in mean HR, RMSDD and SI between the time periods of before the operation, 15th minute, 30th minute and 45th minute after the operation, respectively were not statistically significant in Sham group ($p>0.05$). In Atropine group mean HR, RMSDD and SI were significantly higher in 15th minute, 30th minute and 45th minute after the injection compared to pre-injection (mean HR and RMSSD: $p<0.05$, SI: $p<0.001$). The changes in mean HR, RMSDD and SI between the time periods of 15th minute, 30th minute and 45th minute after the operation were not statistically significant in left vagotomy group ($p>0.05$). In right vagotomy group mean HR, RMSDD and SI were significantly higher in 15th minute ($p<0.05$), however did not changed significantly in 30th minute and 45th minute after the operation compared to pre-operation ($p>0.05$). In bilateral vagotomy group mean HR, RMSDD and SI were significantly higher in 15th minute, 30th minute and 45th minute after the operation compared to pre-operation ($p<0.05$) (Figure 2).

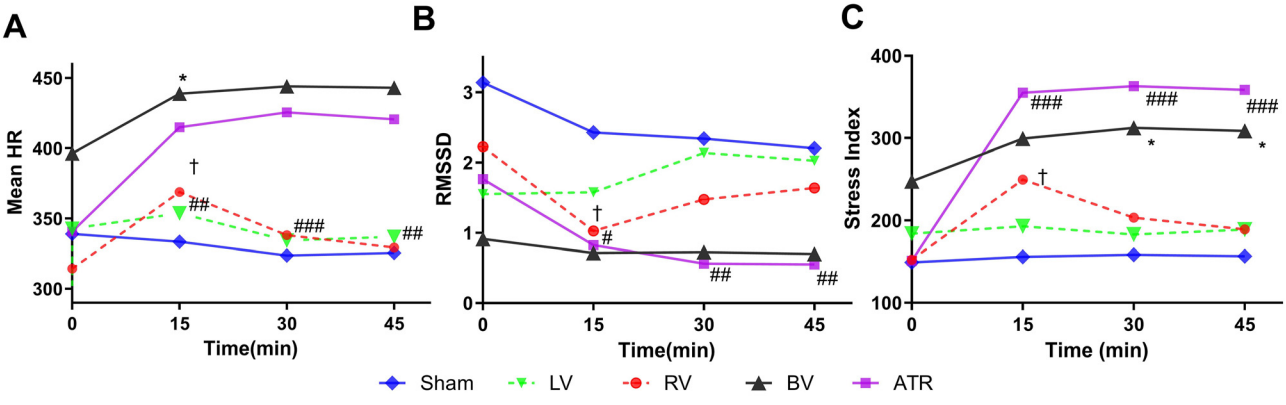

Figure 2: Time domain parameters of experimental groups. Shown are mean heart rate (A), RMSSD (B) and stress index (C). One-way analysis of variance (ANOVA) with post hoc Turkey-Kramer multiple comparison were used for statistical analysis. Data were shown as mean and S.E.M (n=8 per group). (*); $p<0.05$ vs. baseline of bilateral vagotomy (BV) group, (†); $p<0.05$ vs. baseline of right vagotomy (RV) group, (#); $p<0.05$; (##); $p<0.01$ and (###) vs. baseline of atropine treated (ATR) group.

Frequency domain analysis

The changes in mean relative powers of LF and HF, and TP between the time periods of 15th minute, 30th minute and 45th minute after the operation were not statistically significant compared to pre-operation in Sham group ($p>0.05$) (Figure 3). In Atropine group mean relative powers of LF and HF, and TP were significantly higher in 15th minute, 30th minute and 45th minute after the injection compared to pre-injection (mean LF and HF: $p<0.01$, HF: $p<0.001$) (Figure 3). In left vagotomy group, the relative power of LF was decreased significantly in 15th minute ($p<0.05$), 30th minute ($p<0.05$) and 45th minute ($p<0.01$) compared to pre-operation (Figure 3A). Mean relative power of HF was significantly increased in 15th minute, 30th minute and 45th minutes compared to pre-operation ($p<0.01$, Figure 3B), whereas changes in TP were not statistically significant ($p>0.05$, Figure 3C). In right vagotomy group mean relative power of LF was not changed significantly (Figure 3A), mean relative power of HF was significantly higher ($p<0.05$, Figure 3B) and TP was significantly lower in 15th minute ($p<0.05$), however did not change significantly in 30th and 45th minutes compared to pre-operation ($p>0.05$, Figure 3C). In bilateral vagotomy group mean relative power of LF was significantly lower and HF was significantly higher through the recorded period ($p<0.001$, Figure 3A and B), however TP did not change significantly ($p<0.05$, Figure 3C).

Nonlinear analysis

Sample entropy (SampEn)

The changes in mean SampEn between the time periods of pre-operation, 15th minute, 30th minute and 45th minutes after the operation were not statistically significant in Sham group ($p>0.05$). In Atropine group mean SampEn were significantly lower in 15th minute, 30th minute and 45th minutes compared to pre-injection ($p<0.01$). The changes in mean SampEn between the time periods of 15th minute, 30th minute and 45th minutes after the operation were not statistically significant compared to pre-operation in left and right vagotomy groups ($p>0.05$). In bilateral vagotomy group mean SampEn were significantly lower 15th minute, 30th minute and 45th minutes after the operation compared to before the operation ($p<0.001$) (Figure 4A).

Detrended fluctuation analysis (DFA)

The changes in mean differences of $DFA\alpha_1$ and $DFA\alpha_2$ before the operation, 15th minute, 30th minute and 45th minutes after the operation were not statistically significant in sham, atropine, left and right vagotomy groups ($p>0.05$). In bilateral vagotomy group mean $DFA\alpha_1$ was significantly higher while $DFA\alpha_2$ was significantly lower in 15th minute, 30th minute and 45th minutes after the operation compared to pre-operation ($p<0.001$) (Figure 4B and C).

Poincare plot analysis

The changes in mean SD1 between the time periods of pre-operation and after the operation were not statistically significant in Sham group ($p>0.05$). In Atropine group mean SD1 were significantly lower in 15, 30 and 45 min after the injection compared to pre-injection ($p<0.01$). The changes in mean SD1 between pre-operation and post-operation in left vagotomy groups were not statistically significant ($p>0.05$). In right vagotomy group mean SD1 was significantly lower in 15 min after the operation ($p>0.05$), however did not change significantly in 30 and 45 min after the operation compared

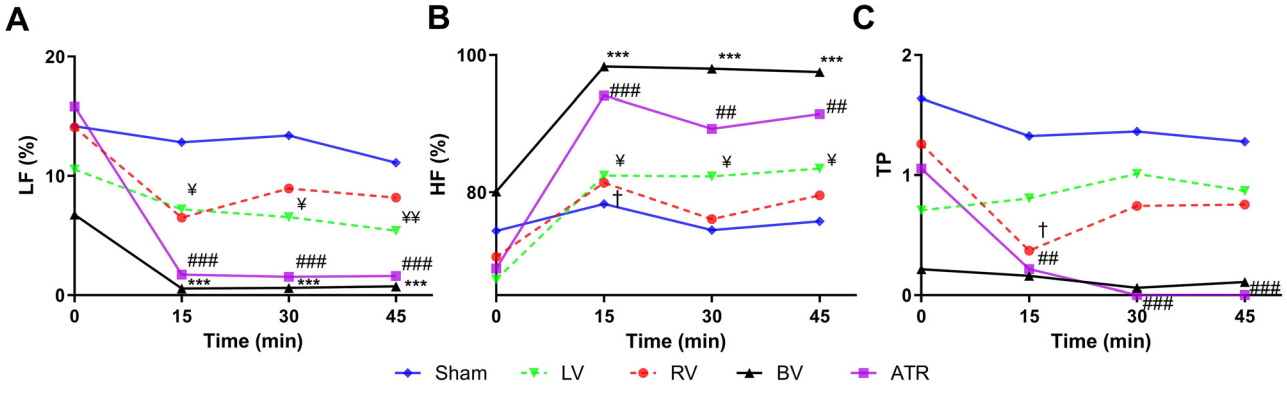

Figure 3: Frequency domain parameters of experimental groups. Shown are LF % (A), HF % (B) and TP ms2 (C). One-way analysis of variance (ANOVA) with post hoc Turkey-Kramer multiple comparison were used for statistical analysis. Data were shown as mean and S.E.M (n=8 per group). (¥); $p<0.05$ and (¥¥); $p<0.01$ vs. baseline of left vagotomy (LV) group, (***); $p<0.001$ vs. baseline of bilateral vagotomy (BV) group, (†); $p<0.05$ vs. baseline of right vagotomy (RV) group, (##); $p<0.01$ and (###); $p<0.001$ vs. baseline of atropine treated (ATR) group.

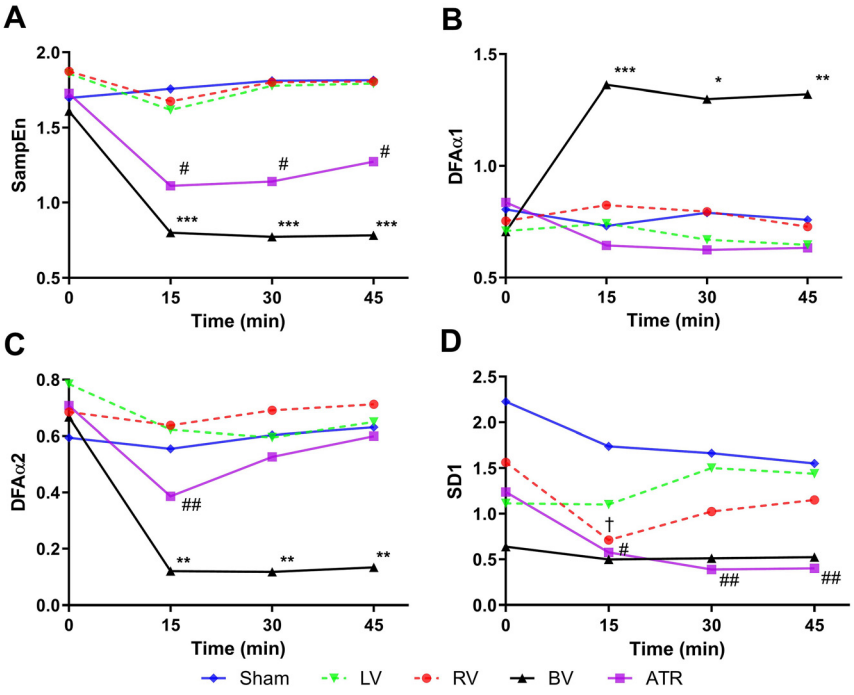

Figure 4: Non-linear parameters of experimental groups. Shown are SampEN (A), $DFA\alpha_1$ (B) $DFA\alpha_2$ (C) and SD1 (D). One-way analysis of variance (ANOVA) with post hoc Turkey-Kramer multiple comparison were used for statistical analysis. Data were shown as mean and S.E.M (n=8 per group). (*); p<0.05; (**); p<0.01; (***); p<0.001 vs. baseline of bilateral vagotomy (BV) group. (†); p<0.05 vs. baseline of right vagotomy (RV) group. (#); p<0.05; (##); p<0.01 vs. baseline of atropine treated (ATR) group.

to pre-operation (p>0.05). In bilateral vagotomy group changes in mean SD1 were not statistically significant in 15, 30 and 45 min after the operation compared to pre-operation (p>0.05) (Figure 4D).

Total choline results

Total choline/acetylcholine levels in groups were given in Figure 5. Total choline/acetylcholine levels were 10.09 ± 6.964 nM in sham group, 8.720 ± 1.923 nM in Atropine group, 12.00 ± 3.588 nM in bilateral vagotomy group, 10.24 ± 4.951 nM in left vagotomy group and 22.05 ± 4.494 nM in right vagotomy group. Total choline/acetylcholine was significantly higher in right vagotomy group compared to other groups (p<0.001).

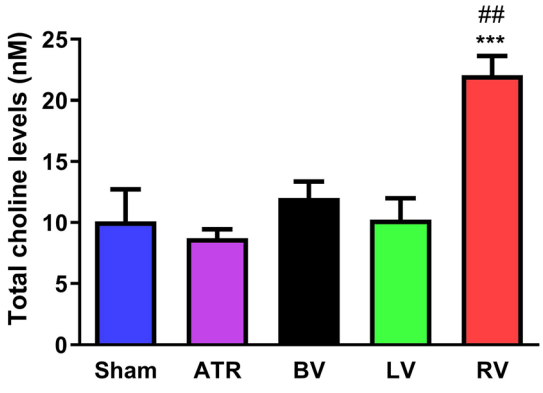

Figure 5: Total choline/acetylcholine levels of experimental groups. Shown are total choline/acetylcholine levels of experimental groups. One-way analysis of variance (ANOVA) with post hoc Turkey-Kramer multiple comparison were used for statistical analysis. Data were shown as mean and S.E.M (n=8 per group). (***); p<0.001 vs. sham, ATR, LV groups, (##); p<0.01 vs. BV group.

Discussion

This study's primary findings can be summarized as follows: (i) left and right acute vagotomy effected HRV parameters asymmetrically, (ii) in right vagotomy, most of the HRV parameters were significantly affected in first 15 min, however trended through "normal" values in time, (iii) acute right vagotomy increased total choline/acetylcholine levels in heart tissues.

HRV is a valuable tool to evaluate the ANS activity, however many characteristics of HRV analysis still remains unclear. In present study, our aim was to evaluate possible asymmetric effects of nervous vagus on HRV parameters of time and frequency domain analyses and nonlinear analysis. Here we observed that, while left vagotomy did not significantly affect HRV parameters, effect of right vagotomy on HRV parameters disappeared after 15 min. Similar to our findings, Mamontov et al. (2022) reported that right vagotomy caused a significant decrease in the spectral characteristics of HRV but left vagotomy did not affect spectral HRV. However they did not continuously analyzed HRV in a time dependent manner [23]. The finding that effect of right

vagotomy on HRV parameters disappeared after 15 min may be caused by a compensatory mechanism that interceded and compensated the withdrawal of the right branch of the vagus nerve. The results of the present study showed that left and right vagal withdrawal effect HRV parameters asymmetrically, moreover as the results of vagal withdrawal, right vagotomy may have triggered a compensatory mechanism.

Bilateral vagotomy and atropine injection, which denote physical and chemical denervation of the vagus nerve, led to a significant decrease in the relative power of LF (LF%) but an increase in the relative power of HF (HF%) during the 45 min experimental period. While it is commonly anticipated that vagotomy would result in decreased HF% and increased LF% due to the removal of parasympathetic tone, it is important to note that LF power reflects the activity of both sympathetic and parasympathetic branches of the autonomic nervous system. Therefore, the observed decrease in LF% post-vagotomy may not solely indicate changes in sympathetic activity but could also be influenced by alterations in parasympathetic tone [7, 24]. Previous studies have demonstrated that total vagal blockage eliminates HF oscillations and reduces power in the LF range [25]. Although HF may reflect vagal function, it may not entirely represent vagus nerve activity [26]. Additionally, another factor contributing to changes in LF% and HF% may be our use of relative power measures instead of natural logarithmic measures. Consequently, the decrease in LF% power may have caused an increase in HF%. Thus, when evaluating LF% and HF%, total power (TP) should also be considered [27]. In the present study both atropine and bilateral vagotomy caused significant reduction in TP as well. Therefore, it can be inferred that power bands in both LF and HF spectrums may have been reduced due to vagotomy and atropine injection, but not proportionally.

Bilaterally vagotomized rats displayed a consistent pattern of persistent loss of complexity and variability. We found eight measures that were significantly changed in vagotomized rats, the majority of which were consistent with the dissenting metrics previously stated. When detrended fluctuation analysis (DFA) considered, $DFA\alpha_1$ increased while $DFA\alpha_2$ increased significantly. Both of these findings could be attributable to possible loss of fractal complexity due to vagotomy. DFA analysis evaluates the fractal components of the heart time series and they provide information regarding fractal complexity [18]. $DFA\alpha_1$ evaluates the heart time series in smaller box sizes ($4<n<11$) than $DFA\alpha_1$ ($12<n<64$). The short-term correlations obtained by smaller box sizes reflect the faster changes by their nature such as baroreceptor reflex, while long-term correlations obtained by using bigger box sizes reflect the regulatory mechanisms that limit fluctuation of the beat cycle [28]. And on the other hand, DFA has been designed compute scaling exponent α, which in the limit of perfect monofractals of infinite length. Signals described by α close to 1 ($\alpha \in [0.75, 1.25]$), are called 1/f noise, which is expected from a healthy heart [29], while α=0.5 describes white noise, and α=1.5 describes Brownian motion [30]. α lower than 0.75 or higher than 1.25 means loss of physiological complexity [9, 31]. Our results showed that only total vagotomy had strong enough effect to change fractal complexity significantly while unilateral vagotomy and atropine did not affect fractal complexity.

In accordance with previous studies, heart rate (HR) was increased with bilateral and right vagotomy, and atropine injection [32–34]. As well known, this positive chronotropic effect is caused by a sudden breakdown in the balance between sympathetic and vagal nerves, resulting in a predominant function of sympathetic nerves in the heart. In line with these changes in the mean HR after vagal withdrawal, HRV analysis also suggest sympatovagal balance to shift towards sympathetic nervous system. that only total vagotomy had strong enough effect to change fractal complexity significantly while unilateral vagotomy and atropine did not affect fractal complexity. This result may be due to compensatory mechanisms since the afferent vagal fiber are still intact in unilateral vagotomy and atropine groups.

In our study, RMSSD, HF, SD1, and $DFA\alpha_1$, parasympathetic nervous system HRV indices [5], and stress index was changed due to vagal withdrawal. These indices, by their nature, reflect relatively fast changes in heart rate time series. Parasympathetic nerves act faster (<1 s) than sympathetic nerves (>5 s). Therefore, these indices reflect parasympathetic activity rather than sympathetic activity [5]. However, in present study relative power of HF was increased following vagotomy, which was expected to be abolished [25]. HF power is a measure of vagal modulation of the heart rate, although it is not a direct measure of vagal tone. If variations in HF power corresponded to changes in vagal tone, average HR should alter accordingly. However, while breathing at varied rates within the 9–24 bpm (in human subjects) range changes HF power, it has little effect on mean HR [5]. In our study, breathing frequency was also recorded via temperature sensor placed in the nose of the animals and adjusted HF band width accordingly to eliminate the effects of breathing frequency on relative power of HF.

It is believed that the release of acetylcholine (Ach) is regulated by pre-synaptic muscarinic receptors on cardiac vagal nerve terminals. The inhibition of ACh release between post-ganglionic vagal neurons innervated by the right and left vagal nerves, respectively, could be caused by a significant overlap for both right and left vagal projections [35, 36]. We hypothesized that different branches of acute

vagotomy may have a specific impact on the levels of acetylcholine released from vagus nerve endings. Previous studies have shown that right vagotomy leads to a decrease in acetylcholine levels in the heart tissue of rats [36]. However, our data (see Figure 5) showed that acute right vagotomy increased total choline/acetylcholine levels in heart tissues compared to sham operated rats. It has been demonstrated that a subacute right cervical vagotomy causes an increase in activity on the opposite side of the vagus nerve, leading to a decrease in hemodynamic parameters [34]. Increased total choline/acetylcholine levels due to increased activity of the left vagal nerve and the activation of compensatory recovery.

The paradigm for relating the HRV frequency components (LF and HF) to the autonomic nerve system divisions (sympathetic and parasympathetic) is particularly too simplistic, and it is the primary source of the general limitation of HRV analyses. HRV is hypothesized to begin in the brain and go through the autonomic nervous system to the heart. HRV analysis can intercept the information for only circulatory control generated by the brain not whole autonomic nervous system [37].

Since we have only evaluated the effects of vagal withdrawal but not vagal stimulation, our study provides no evidence on total autonomic control of the heart. Our findings provide evidence regarding the possible effects of unilateral and bilateral vagotomy on HRV parameters along and acetylcholine levels in heart tissues. Further studies might be conducted to evaluate comprehensive effects of vagal stimulation and the involvement of acetylcholine-mediated compensatory recovery.

Conclusions

For the first time, our study demonstrates that vagal withdrawal affects Heart Rate Variability (HRV) parameters asymmetrically. The observed difference in the impact of unilateral left and right vagotomy supports an asymmetrical effect of vagal innervation on HRV parameters. Specifically, following right vagotomy, compensatory hemodynamic recovery via contralateral vagus overactivity may lead to increased contralateral vagal activity and elevated total choline/acetylcholine levels. These findings further underscore the asymmetrical nature of vagal innervation.

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Research ethics: The study was conducted after the permission from Local Animal Experiments Ethics Committee (No: 77.637.435) in accordance with the Declaration of Helsinki (as revised in 2013).

Informed consent: Not applicable.

Author contributions: HK and HFO planned and carried out the experiments. HK, EB and HFO contributed to sample preparation. All authors contributed to the interpretation of the results. HK took the lead in writing the manuscript. All authors provided critical feedback and helped shape the experiments, analysis and manuscript. MO critically reviewed the manuscript. All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests: The authors state no conflict of interest.

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Data availability: The raw data can be obtained on request from the corresponding author.

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