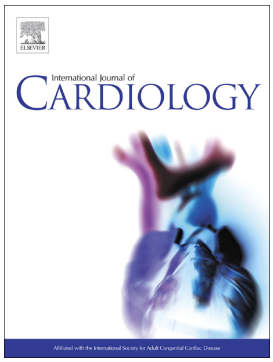

Left cardiac vagotomy rapidly reduces contralateral cardiac vagal electrical activity in anesthetized Göttingen minipigs

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Left Cardiac Vagotomy Rapidly Reduces Contralateral Cardiac Vagal Electrical Activity in Anesthetized Göttingen Minipigs

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All the authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation

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ABSTRACT

Background: The impact of acute unilateral injury on spontaneous electrical activity in both vagus nerves at the heart level is poorly understood. We investigated the immediate neuroelectrical response after right or left cardiac vagal nerve transection (VNTx) by recording spiking activity of each heart vagus nerve (VN).

Methods: Fourteen male Göttingen minipigs underwent sternotomy. Multi-electrode cuffs were implanted below the cut level to record vagal electroneurographic signals during electrocardiographic and hemodynamic monitoring, before and immediately after cardiac VNTx (left: L-cut, n=6; right: R-cut, n=8).

Results: Left cardiac VNTx significantly reduced multi-unit electrical activity (MUA) firing rate in the vagal stump (-30.7% vs pre-cut) and intact right VN (-21.8% vs pre-cut) at the heart level, without affecting heart rate, heart rate variability, or hemodynamics. In contrast, right cardiac VNTx did not acutely alter MUA in either VN but slightly increased ($p<0.022$) the root mean square of successive RR interval differences (rMSSD), an index of parasympathetic outflow, without affecting hemodynamics.

Conclusions: Our study reveals an early left-lateralized pattern in vagal spiking activity following unilateral cardiac vagotomy. These findings enhance understanding of the neuroelectrical response to vagal injury and provide insights into preserving vagal outflow after unilateral cardiac vagotomy. Importantly, monitoring spiking activity of the cardiac right VN may predict onset of left vagal pathway injury, which is detrimental to cardiac patients and can occur as a complication of catheter ablation for atrial fibrillation.

Keywords

Unilateral vagotomy; multiunit electrical activity; cardiac vagus nerve; vagal recording

1. Introduction

The vagus nerve (VN), a long mixed multifascicular autonomic cranial nerve, is composed of predominantly parasympathetic fibers (80% afferent, 20% efferent) with a smaller sympathetic component [1]. This crucial nerve innervates various visceral organs and plays a vital role in the regulation of autonomic functions [3]. Of particular interest is its influence on the heart, an asymmetrical organ where the right heart exhibits a higher density of vagal fibers compared to the left heart [4]. However, the early regulatory mechanisms governing the spontaneous electrical activity between the two vagus nerves at the cardiac level remain unclear. A comprehensive understanding of early neuroelectric adaptations is essential for advancing cardiac bioelectronic medicine applications of neural decoding and stimulation of the VN [5], which is a recognized non-pharmacological therapy to improve heart failure symptoms [6].

While bidirectional transmission of electrical signals along both vagi, originating from the central nervous system and targeting the intrinsic cardiac nervous system (ICNS), primarily elicits chronotropic, inotropic, and dromotropic effects at the heart level [5], the relationship between tonic vagal activity and commonly used heart rate variability (HRV) metrics is inconsistent [7]. Consequently, there is a pressing need to directly measure the physiological electrical activity of the VN at the cardiac level. Although previous studies have primarily focused on recordings of electrical stimulation-evoked responses from each VN [8,9], such measurements fail to capture the nuances of physiological neural signals. While physiological recordings of cervical VN spiking activity have been conducted in small animals and humans [10,11,12], the direct recording of spontaneous vagal electrical activity at the cardiac level remains elusive. Furthermore, the influence of anatomical site on the spontaneous electrical activity of the thoracic VN at the cardiac level is uncertain.

Previous study has demonstrated that subacute right cervical vagal transection (VNTx) primarily affects HRV, while left cervical vagotomy predominantly influences left ventricular (LV) contractility, suggesting the activation of side-dependent compensatory cardiac responses after one week [13]. Conversely, rats subjected to chronic right cervical VNTx exhibited normal cardiorespiratory function but displayed changes in glial morphology in the ipsilateral nucleus tractus solitarii, highlighting the long-term role of afferent fibers on the right side [14]. However, it remains unknown whether contralateral cardiac vagotomy rapidly affects the unilateral spontaneous spiking activity of the intact VN at the heart level. Understanding this phenomenon is critical for identifying early predictors of unilateral vagal injury, which can ultimately lead to chronic left or right cardiac compensatory responses. Considering the spatial anatomical arrangement of fascicles along the VN pathway [1], it is conceivable that the spiking activity of the two vagal trunks at the cardiac level is differentially regulated based on the side of the VNTx, suggesting a left-right electrical influence. Indeed, previous research has indicated that peripheral nerve injury induces comparable changes in the contralateral uninjured nerve over the long term [15]. Therefore, it is plausible that a similar influence occurs in the VN, resulting in rapid changes in spontaneous electrical activity. Of note, prior research has suggested left-lateralization of cortical parasympathetic control of the heart involving afferent and efferent fibers [16]. Based on these last findings, it is likely that the spontaneous electrical activity of the left VN primarily arises from parasympathetic fibers and predominates over that of the right VN in the heart. Furthermore, following an acute left vagus injury, this left-lateralized control would be immediately evident. Nonetheless, the lateralization of vagal spiking activity at the heart level remains unexplored. Although the left and right thoracic VN branches encircle the heart, with ipsilateral collaterals synapsing with contralateral ones [4], the functional dominance of either VN at the heart level

remains uncertain. Our study aims to provide valuable insights into the comprehensive understanding of rapid control processes in cardiac vagus nerves following unilateral acute injury. To achieve this, multi-unit recordings of naturally occurring VN spikes have been conducted using a non-invasive multi-electrode cuff in anesthetized animals, ensuring precise measurements of physiological electrical activity while effectively attenuating background noise [17].

We employed a clinically relevant large animal model, healthy adult male Göttingen minipigs, which is widely recognized for its high homology to humans in heart [18-20] and VN [1,21] research.

2. Methods

Animal Preparation

Fourteen healthy adult male Göttingen minipigs (34.2 ± 1.5 kg body weight) purchased from Ellegaard Göttingen Minipigs A/S (Dalmose, Denmark) were sedated with a cocktail of 1 mg/kg tiletamine hydrochloride and zolazepam hydrochloride (i.m., Zoletil-50+50mg/ml 1fl+solv, Virbac Italia, Milan, Italy) and 1 mg/kg azaperone (i.m., Stresnil, Elanco GmbH, UK). Echocardiography (MyLab30, Esaote, Italy) and continuous electrocardiogram (ECG) monitoring were performed to assess cardiac health and monitor heart rate (HR) and rhythm. An ear vein was cannulated for drug and fluid infusion. General anesthesia was induced with propofol (2-4 mg/kg, i.v., Fresenius Kabi Italia S.r.l.), and the minipigs were mechanically ventilated with 60% oxygen and 40% air via an endotracheal tube, while anesthesia was maintained with 1.0-2.0 Vol% sevoflurane (Sevoflurane Piramal, Piramal Critical Care B.V., Voorschoten, The Netherlands). Mechanical ventilation was adjusted depending on arterial blood gas values to keep the carbon dioxide end-expiratory partial pressure between 35 and 45 mmHg. Minipigs were instrumented in the same way as previously described [22]. Systolic, diastolic, and mean arterial pressures (SAP, DAP, MAP) were measured using a fluid-filled catheter placed via the left femoral artery and linked to a P23ID

strain gage transducer. When MAP was less than 50-60 mmHg, intravenous injection of crystalloid fluids (Ringer's lactate solution) or colloids (succinyl gelatin) was used to maintain intraoperative volemia. While animals were exposed to the warming mattress, their body temperature was monitored using a rectal thermistor. All parameters were continually recorded with an ADInstruments Powerlab 8/35 (ADInstruments, Dunedin, New Zealand) and monitored with the GOULD 5900 Signal Conditioner Cage (Data Sciences International, New Brighton, United States). The double product (DP) was calculated to quantify myocardial oxygen utilization indirectly [22]. The Animal Care Committee of the Italian Ministry of Health approved this study, which was conducted in accordance with Legislative Decree No. 26/2014 (National Competent Authorities for the Implementation of Directive 2010/63/EU of the European Parliament and of the Council on the Protection of Animals Used for Scientific Purposes) and the guidelines for the Care and Use of Laboratory Animals (NIH publication No. 85–23) on the protection of animals used for scientific purposes and with ARRIVE guidelines.

Surgical preparation

Both VNs were surgically isolated at the level of the heart. After incising the layers of skin and subcutaneous tissue, the porcine thorax was opened with a median sternotomy. Pericardiotomy was used to expose the heart, the root of the superior caval vein and the aorta. Then, in the midline of the anterior neck, we performed a vertical incision to expose VN in carotid sheath. From the neck to the root of the superior vena cava and aorta, each VN was meticulously isolated from the surrounding tissue. During continuous electrocardiographic and hemodynamic monitoring, a left or right VNTx was performed at the level of the heart and distal to the detected and preserved recurrent laryngeal nerve (Fig.1A). Cuff electrodes (MicroProbes for Life Science, Gaithersburg, MD, USA) were placed around both VN segments closest to the heart, and each VN was transected 1-2 mm above the cuff.

Epineural recordings of vagal electrical activity before and immediately after unilateral vagotomy

Two multi-electrode cuffs were implanted on both isolated VN at heart level to evaluate the electrical activity of the cardiac VN on both sides (Fig. 1B, C). The electrical activity of each VN was recorded at a sampling frequency of 24.14 kHz using a neural recording device (PZ5 NeuroDigitizer Amplifier, Trucker-Davis Technology, 11930 Research Circle Alachua, FL, USA). For 5 minutes before and immediately after left-sided (L-cut, n=6 animals) or right-sided (R-cut, n=8 animals) cardiac vagotomy, vagal electroneurographic signals (ENG) were collected (Fig.1B). Specifically, we recorded multi-unit spiking activity (MUA) along each VN (Fig.1D, E). MUA recordings are more accurate than other spikes or local field potentials [23] and can be recorded at distance from their origin [24]. MUA have been used to detect spontaneous activity in the intact human right and left vagus nerves [25]. Signal denoising techniques combined with a spike detection algorithm were used to retrieve compound action potentials (CAP) from epineural recordings. To eliminate artifacts caused by ECG signals, we used a fourth-order Butterworth filter-to-filter raw ENG data in the high-frequency range from 1 to 10 kHz. As previously described [25], high frequency signals were denoised using multivariate wavelet denoising. We employed a semi-automatic toolbox called Wave_clus [25] for spike detection, and an expert did visual inspection with maximum a posteriori rejection of artifacts. In brief, we estimated the MUA firing rates on binned spike trains in a rectangular 1-second frame using a Gaussian kernel approach and convolved the resulting spike counts with a Gaussian function with a standard deviation of 3 seconds. The average MUA spike frequencies were then determined across a 5-minute recording in both conditions: before (baseline) and after cardiac vagotomy (both left and right).

Beat-To-Beat Analysis Process

ECG and arterial blood pressure signals

Signals were acquired at a sample frequency of 24.414 Hz to investigate the relationship between spontaneous electrical activity of both VNs, electrocardiography (ECG) and arterial blood pressure (ABP) measures. Fourth-order Butterworth filters were used to zero-phase filter the signals. The following cutoff frequencies were established: A band-pass filter of 0.5÷150 Hz was applied to the ECG signal according to current standards [26], while a low-pass filter of 70 Hz was applied to the blood pressure. The signals were then downsampled by a factor of 15, yielding a final sampling frequency of 1627.6 Hz. QRS were detected using the Pan-Tompkins algorithm [27], which was implemented in the ECGkit MatLab toolbox (Version 1.4.0.0) Arrhythmic beats were excluded from the examination of HRV. After being identified automatically, QRS were thoroughly evaluated and classified by an expert to exclude those caused by arrhythmia. After that, a tachogram was obtained as previously described [28].

Feature extraction of the ECG

The mean HR was calculated as the average of the inverse interbeat N-N interval (IBI). The mean IBI, Q-T interval, NN50 (number of consecutive IBI deviating by more than 50 ms), and pNN50 (NN50 number divided by total number of IBI) were all analysed. The standard deviation of all IBIs in the data set (SDNN) was calculated as an index of total HRV, which includes both sympathetic and parasympathetic contributions. As a specific index for parasympathetic activity, the square root of the differences between adjacent NN intervals (rMSSD) was utilized. In the frequency domain, the LF /HF ratio of the power of the tachogram's low-frequency (LF) and high-frequency (HF) bands was calculated [27]. To account for unequal sampling and missing (i.e., arrhythmic) beats, species-specific criteria for LF (0.010.07) and HF (0.071) were applied, and the Lomb-Scargle method was used to create the periodogram. At rest, the LF/HF ratio should represent the balance of sympathetic and parasympathetic activity and rise when the former exceeds the latter [29]. The rMSSD should give a more robust time-domain measure of parasympathetic activity that keeps its

validity when intervals as small as 10 s are considered [30], without the necessity for frequency domain analysis [30]. To visualize the fluctuations in the period between successive heartbeats, we employed Lorenz diagrams [31]. The duration of the intervals PR, QRS, and QT were manually measured by an expert on representative beats according to current standards [30]. The Fridericia formula was used to adjust QT as follows:

$QTc = \frac{QT(ms)}{\sqrt[3]{\frac{NN(s)}{1\,s}}}$ [30].

All of the hallmarks of HRV outlined above have previously been exploited in a pig model of cardiovascular disease [28,31,32,33] .

Feature extraction of ABP

SAP was calculated by averaging the ABP signal's local maxima (peaks with at least 0.25 of maximum amplitude) and DAP was calculated by averaging the local minima between successive SAP peaks. To calculate MAP, the pressure signal was averaged between two successive DAP minima. Because it corresponds well with invasive gold standard LV measures, femoral dP/dt max (highest rate of arterial pressure rise during systole) was utilized as a index for cardiac contractility [34].

Statistical analyses

GraphPad Prism version 9.4.1 was used to analyze the data. Depending on the data distribution, comparisons were done using the paired Student's t-test or the two-tailed Wilcoxon sign-rank test. We used a one-sided sign-rank test to compare MUA firing rates in the baseline condition and after vagotomy for both sides of the VN to assess the hypothesis of a reduction in MUA firing rates on the intact and severed sides of the VN. As a result, data are reported as mean standard

deviation of the mean or median (interquartile range). Probability levels of $p<0.05$ were regarded statistically significant.

3. Results

Acute effects of left or right cardiac vagotomy on ipsilateral and contralateral vagus nerve multiunit activity (MUA)

The MUA firing rate (% change) of cardiac left (L) VN recorded below the transection was significantly lowered compared to baseline (pre-cut) values, as illustrated in **Fig. 2A, B**. Surprisingly, the MUA firing rate of the intact cardiac right (R) VN was likewise markedly lowered at the same level after cardiac LVN transection. In contrast, spontaneous electrical activity at the cardiac level of the intact LVN was not reduced after acute right cardiac vagotomy **(Fig 2C, D)**. Of note, the MUA value of the RVN recorded below the transection was not reduced below transection.

Acute effects of left or right cardiac vagotomy on heart rate variability and hemodynamic parameters

The LV ejection fraction, a measure of global cardiac contractility was 70±2% in our anesthetized animals. As shown in **Fig.3** and **Table 1**, left acute cardiac vagotomy (L-cut) resulted in no acute hemodynamic or HRV alterations as compared to baseline.

In contrast, acute right cardiac vagotomy (R-cut) significantly increased rMSSD (**Fig. 4** and **Table 2**) without affecting HR, hemodynamic and other HRV measures.

4. Discussion

In our study, for the first time, we investigated the rapid effects of unilateral cardiac vagotomy on the spontaneous electrical activity occurring simultaneously in the ipsilateral stump and the contralateral intact cardiac vagus nerve (VN) in healthy anesthetized adult male minipigs. During continuous electrocardiographic and hemodynamic monitoring, we directly measured physiological spiking activity in both vagus nerves using a multicontact cuff electrode. This allowed us to assess the engagement of vagal fibers directly from both nerves before and immediately after the transection.

Our findings revealed distinct responses depending on the side of transection at the same anatomic level. Left cardiac vagotomy resulted in a decrease in spontaneous electrical signals in the left vagal stump and a simultaneous reduction in action potentials in the intact right VN of the same animal, without significant effects on HRV and hemodynamic values. These results align with a previous study conducted in rats, both under anesthesia and in non-anesthetized conditions [7]. However, when we performed transection of the right VN of the heart in anesthetized and mechanically ventilated minipigs, we observed no immediate impact on spontaneously occurring spikes along the intact contralateral cardiac VN at the same anatomic level. Additionally, hemodynamics and other HRV parameters remained stable. Notably, we did observe a significant increase in rMSSD (root mean square of successive differences) values without any change in HR compared to baseline. rMSSD is a reliable HRV feature associated with parasympathetic activity [30] and less affected by respiration [35]. This increase in rMSSD immediately following right-sided vagotomy suggests a compensatory response due to the preserved integrity of the left VN, which predominantly contributes to parasympathetic outflow [16]. Importantly, rMSSD is more susceptible than HR to homeostatic regulation, leading to larger amplitude alterations following

acute stressors [36]. This finding may support the absence of hemodynamic alterations immediately after each unilateral vagotomy in healthy anesthetized minipigs.

Taken together, our results suggest that the synaptic input from the left VN influences the output of the right VN along fibers projected through the heart, but not vice versa.

Since the structural properties of the each VN vary along its path from the brain to the heart [1], it is conceivable that distinct distribution of fiber types across and within fascicles at cardiac level may contribute left-right influence after transection. To date, there is no consensus on the acute functional implications of unilateral vagotomy due to the lack of simultaneous direct measures of spontaneous electrical activity of both vagus nerves at the heart level in the same animal. Our findings provide valuable insights into the suggested interdependence of spontaneous electrical activity in the two cardiac VN branches. Moreover, the anatomical similarities between the pig vagus nerve and the human vagus nerve [37] make our recordings relevant in the context of implementing cardiac bioelectronic medicine applications.

It is known that the LVN has a higher amount of unmyelinated fibers at the cervical level than the RVN [1]. As a result, the left vagal unmyelinated efferents, whose cardioinhibitory action is delayed compared to vagal B fibers during stimulation [38], may play an important role in maintaining cardiac vagal outflow balance at rest.

Although the thoracic RVN is mainly involved in activating cardiac chronotropic and dromotropic effects [8], transection near the right atrium does not immediately halt electrical activity along the right vagal stump below the cut. This suggests that the spiking activity observed immediately after nerve transection along the right vagus stump may originate from intact left vagal efferents, which do not affect HR significantly as demonstrated by others [39]. Additionally, the stability of spontaneous electrical activity throughout the recording duration, along with the absence of

differences in PR, QT, and QRS durations (indirect measures of vagal tone affected by respiratory activity) [25], can be attributed to the use of volume-controlled mechanical ventilation [40].

We acknowledge some limitations in our study. Firstly, our experiments were conducted under sevoflurane anesthesia, which can affect the autonomic nervous system [41]. ~~However, w~~ We used a low sevoflurane dosage that is unlikely to differentially influence multi-unit activity (MUA) recordings based on the side of unilateral cardiac vagotomy. Additionally, the within-group variability observed in most HRV parameters may explain the absence of significant acute changes during vagal transection. Nevertheless, HR, MAP, cardiac contractility and myocardial oxygen consumption parameters remained stable throughout the recordings without administration of additional cardiovascular drugs. Although the baseline sevoflurane concentrations were equal to those during vagal transection in both groups, we must acknowledge the potential baseline variability between the groups due to the differing sensitivities of individual animals to volatile anesthetics. It is known that 1-2% sevoflurane increases heart rate and reduces SDNN values [42]. In our study, some minipigs exposed to the same concentrations of sevoflurane showed lower heart rate and higher SDNN values than others at baseline. This disparity could be attributed to varying genetically determined respiratory chain activities, resulting in distinct sensitivities to sevoflurane [43-44]. Secondly, we were unable to histologically detect left vagal efferent fibers that regulate the right VN output. It is conceivable that they contribute to network, which has a function in passing down vagal impulses further, and also in integrating parasympathetic and sympathetic information. This complex issue remains a matter of study. However, previous study revealed an early "left-lateralization" pattern in the ventricular inotropic response to the stimulation of the human left thoracic VN near its cardiac branch in the presence of an intact right VN [45]. A neuroimaging study by neural scanning of the heart during simultaneous vagal electrical recording at the time of vagus nerve transection from each side and subsequent stimulation of the

vagal stump below the cut (efferent route) would be helpful to unveil the anatomical trajectory of synaptic input from the left VN influencing the output of the right VN at the cardiac level. Lastly, while multichannel cuff electrodes provide reliable epineural assessment of multi-unit neural activity from various fiber types in each vagus nerve, they have limited selectivity and only provide a global representation of neural signaling [46].

Our results aim to significantly enhance our understanding of the intricate interplay between the autonomic nervous system and the heart. By delving into this complex relationship, they even hold the promise of yielding valuable insights and potential breakthroughs in the field of early predictors for longitudinal vagal stimulation treatment failure in arrhythmia. Ultimately, this knowledge can be leveraged to select patients for autonomic modulation therapies and to optimize the treatment parameters, improving the efficacy and outcomes of vagal activity [47].

5. Conclusion and clinical perspectives

Our study demonstrates that left vagotomy at the cardiac level immediately reduces spontaneous electrical activity in the contralateral VN at the same anatomic level, while the reverse is not observed. These findings reveal an early left-lateralized pattern in vagal spiking activity following unilateral cardiac vagotomy. The rapid changes in physiological electrical activity along the intact right VN at the cardiac level can serve as early predictors of left vagal disease or injury, which may have adverse consequences in patients with heart failure [48], injury in specific regions of the dominant hemisphere [16, 49], following cardiac and lung transplantation [50] and catheter ablation for atrial fibrillation [51]. To avoid esophageal injury, real-time monitoring the spontaneous spiking activity of the right VN during energy delivery to the left atrial posterior wall can prove beneficial. This approach becomes crucial because the vagus nerve plexus, primarily comprising branches from the left vagus nerve, is situated on the anterior surface of the esophagus [52]. Importantly, this plexus is the first to be impacted by radiofrequency, making it

particularly susceptible to thermal damage and consequent effect on contralateral VN electrical activity. Moreover, the simultaneous recording of the cardiac right VN spiking activity could be used as a real-time monitoring to track the effectiveness of catheter ablation of left atrial ganglionated plexi in treating refractory vasovagal syncope [53].

6. Acknowledgment

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7. Data Availability Statement

The data sets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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9. Tables

	BASELINE (n=6)	L-CUT (n=6)	p-value
HR (bpm)	96.67±8.2	97.54±9.4	0.225
SDNN (ms)	2.61± 0.47	7.51± 1.41	0.066
rMSSD (ms)	1.93±0.48	2.11±0.50	0.387
LF/HF	0.49±0.49	1.04±0.51	0.563
PR (ms)	142±11.32	141±14.8	0.892
QRS (ms)	101.67±14.85	108.5±14.64	0.525
QT (ms)	470.67±27.62	479.17±27.69	0.115
QTc (ms)	541.99±16.45	549.27±16.69	0.112
SAP (mmHg)	95.2±9.3	97.8±9.04	0.28
maxSAP (mmHg)	105.5±8.06	110.5±7.96	0.43
DAP (mmHg)	65.7±5.2	69.33±5.9	0.06
PP (mmHg)	29.5±6.5	28.5±6.6	0.72
MAP (mmHg)	74.2±6.3	76±6.8	0.31
DP (mmHg*bpm)	10158.8 ± 1020.5	10967.7 ± 1187.5	0.48
Art dP/dt max (mmHg/s)	0.33±0.09	0.37±0.09	0.198

Table 1. Cardiovascular parameters at baseline and just after left cardiac vagotomy (L-cut). Art dP/dt max, maximum rate of the arterial pressure increase during systole; DAP, diastolic arterial pressure; DP, double product; HR, heart rate; MAP, mean arterial pressure; PP, pulse pressure; QTc, corrected; QT interval; rMSSD, root mean square of successive differences between normal heartbeats; SAP, systolic arterial pressure; maxSAP, maximum systolic arterial pressure; SDNN, Standard Deviation Normal to Normal. Mean values ± S.E.M. n=6.

	BASELINE (n=8)	R-CUT (n=8)	p-value
HR (bpm)	89.54±7.4	92.01±6	0.742
SDNN (ms)	4.1±0.50	4.15±0.52	0.383
rMSSD (ms)	2.42±0.52	2.65±0.53	0.022*
LF/HF	0.27±0.43	0.5±0.74	0.547
PR (ms)	121.5±11.04	123±10.24	0.297
QRS (ms)	81±5.90	79.5±6.41	0.458
QT (ms)	496.25±33.79	494.12±15.13	0.864
QTc (ms)	558.02±27.33	554.02±25.92	0.767
SAP (mmHg)	105.5±4.6	107.875±4.6	0.36
maxSAP (mmHg)	117.2±3.16	120.625±4.62	0.49
DAP (mmHg)	64±4.8	67±4.2	0.36
PP (mmHg)	41.5±7.1	40.9±6.6	0.69
MAP (mmHg)	76.75±5.4	82.25±3.1	0.11
DP (mmHg*bpm)	11550.4 ± 1068.01	11945.7 ± 953.4	0.52
Art dP/dt max (mmHg/s)	0.71±0.15	0.7±0.15	0.735

Table 2. Cardiovascular parameters at baseline and just after right cardiac vagotomy (R-cut). Art dP/dt max, maximum rate of the arterial pressure increase during systole; DAP, diastolic arterial pressure; DP, double product; HR, heart rate; MAP, mean arterial pressure; PP, pulse pressure; QTc, corrected; QT interval; rMSSD, root mean square of successive differences between normal heartbeats; SAP, systolic arterial pressure; maxSAP, maximum systolic arterial pressure; SDNN, Standard Deviation Normal to Normal. Mean values ± S.E.M. n=8. *statistically significant difference.

10. Figure legends

Figure 1. Schematic representation of experimental set up. Multi-electrode cuffs were surgically implanted on left and right vagus nerve (VN) of adult minipigs (a). Unilateral vagotomy was performed at heart level (b) immediately above the multi-electrode cuff (c). The spontaneous multiunit electrical activity of intact contralateral VN and ipsilateral vagus stump was simultaneously recorded during electrocardiographic and hemodynamic monitoring (d-e).

Figure 2. Ipsilateral and contralateral vagal MUA changes compared to baseline (pre-cut) (% change) after left or right cardiac vagotomy. *Left acute cardiac vagotomy (n=6):* MUA change compared to baseline of the left vagus nerve (LVN) at cardiac level **(a)**; MUA change compared to baseline of the right vagus nerve (RVN) at cardiac level **(b)**. *Right acute cardiac vagotomy (n=8):* MUA change compared to baseline of the LVN at cardiac level **(c)**;MUA change compared to baseline of the RVN at cardiac level **(d)** . Bsln, baseline; L-cut, left acute cardiac vagotomy; LVN, left vagus nerve; MUA, multi unit activity; R-cut, right acute cardiac vagotomy; RVN, right vagus nerve. *p<0.05

Figure 3. **Heart rate variability parameters at baseline (Bsln) and after left acute vagotomy (L-cut).** Art dP/dt max, maximum rate of the arterial pressure increase during systole; HR, heart rate; LF/HF, Low Frequency to High Frequency ratio; QTc, corrected QT interval; rMSSD, root mean square of successive differences between normal heartbeats; SAP, systolic arterial pressure; SDNN, Standard Deviation Normal to Normal;. n=6.

Figure 4. Heart rate variability parameters at baseline (Bsln) and after right cardiac vagotomy (R-cut). Art dP/dt max, maximum rate of the arterial pressure increase during systole; HR, heart rate; LF/HF, Low Frequency to High Frequency ratio; QTc, corrected QT interval; rMSSD, root mean square of successive differences between normal heartbeats; SAP, systolic arterial pressure; SDNN, Standard Deviation Normal to Normal. n=8; *p<0.05

Highlights:

- Left cardiac vagotomy immediately reduces spiking activity in the ipsilateral vagal stump and intact right vagus nerve (VN) at the heart level, while right cardiac vagotomy does not.
- Hemodynamic parameters remain unchanged immediately after both left and right cardiac VN transection. However, heart rate variability analysis reveals a rapid increase in parasympathetic outflow after right cardiac vagotomy.
- Physiological spiking activity recorded from the cardiac right VN serves as a predictive indicator for the rapid onset of left vagal pathway injury. Left pathway injury is harmful to cardiac patients and can occur as a complication of catheter ablation for atrial fibrillation.

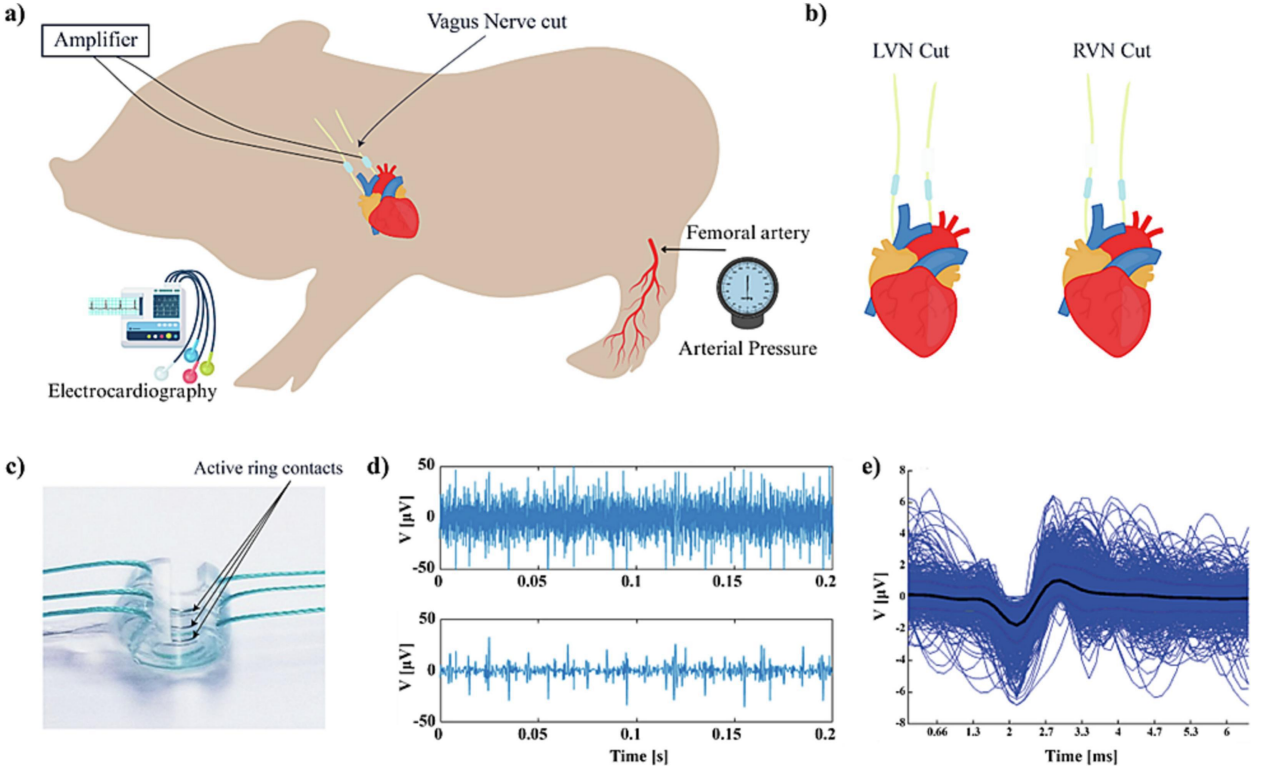
a)
Amplifier
Vagus Nerve cut
Femoral artery
Arterial Pressure
Electrocardiography
b)
LVN Cut
RVN Cut
c)
Active ring contacts
d)
V [μV]
Time [s]
e)
V [μV]
Time [ms]

Figure 1

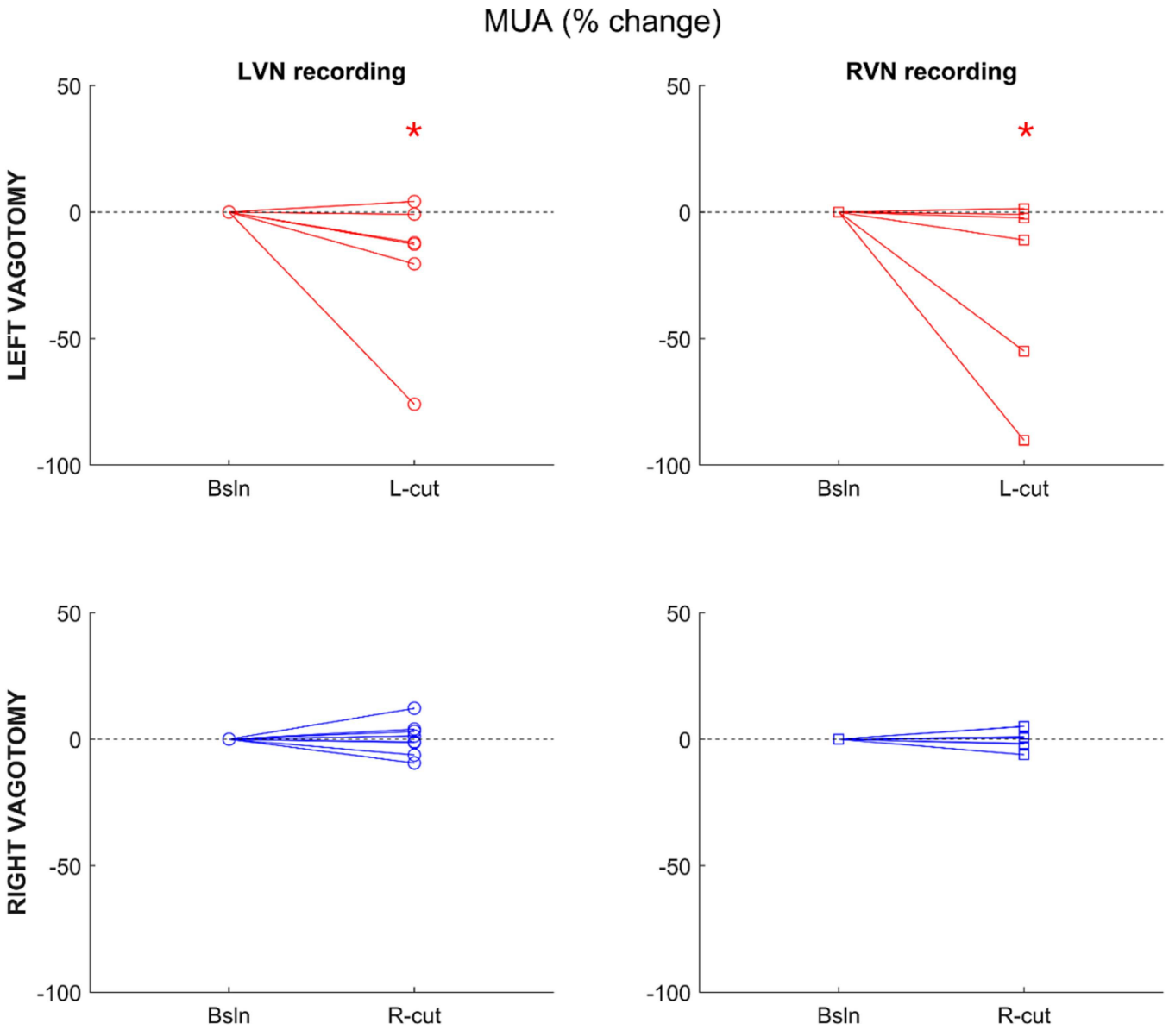
MUA (% change)
LVN recording
RVN recording
LEFT VAGOTOMY
RIGHT VAGOTOMY
50
0
-50
-100
*
Bsln
L-cut
R-cut

Figure 2

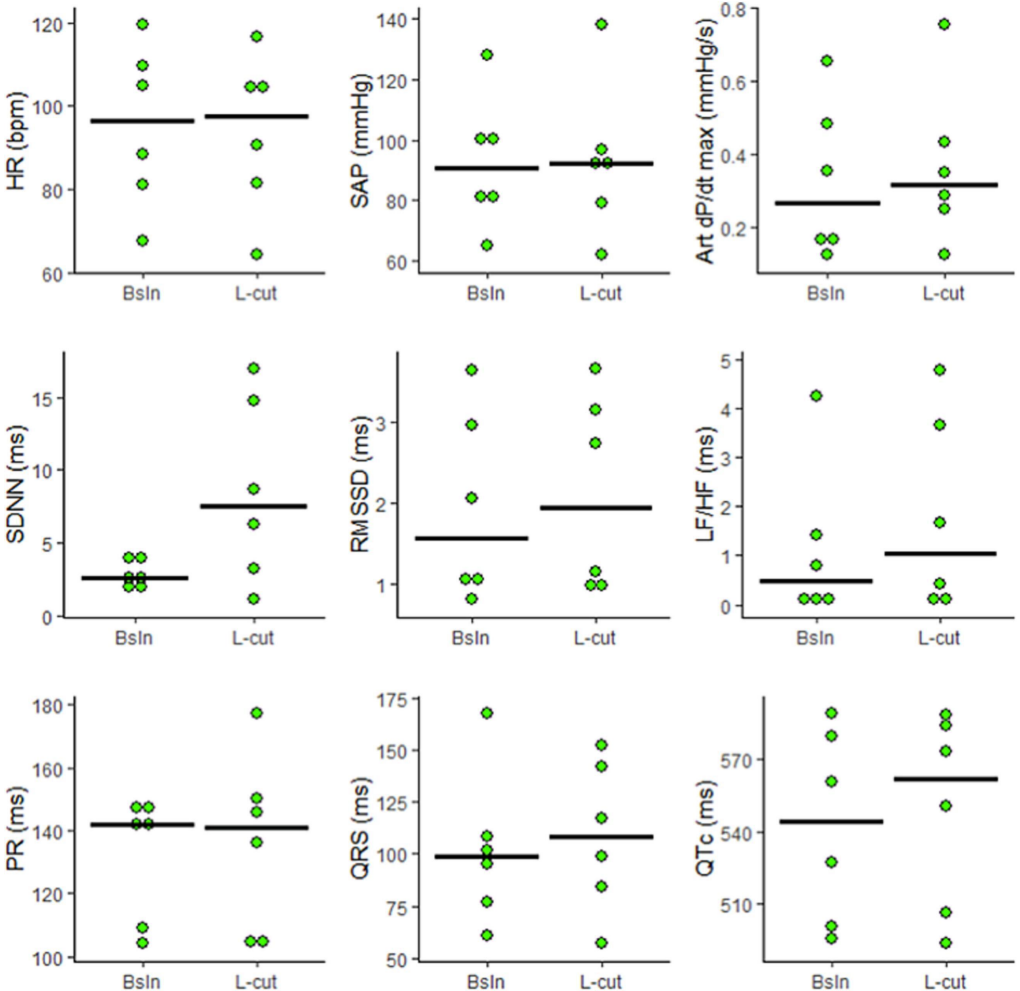
HR (bpm)
120
100
80
60
BsIn
L-cut
SAP (mmHg)
140
120
100
80
60
BsIn
L-cut
Art dP/dt max (mmHg/s)
0.8
0.6
0.4
0.2
BsIn
L-cut
SDNN (ms)
15
10
5
0
BsIn
L-cut
RMSSD (ms)
3
2
1
BsIn
L-cut
LF/HF (ms)
5
4
3
2
1
0
BsIn
L-cut
PR (ms)
180
160
140
120
100
BsIn
L-cut
QRS (ms)
175
150
125
100
75
50
BsIn
L-cut
QTc (ms)
570
540
510
BsIn
L-cut

Figure 3

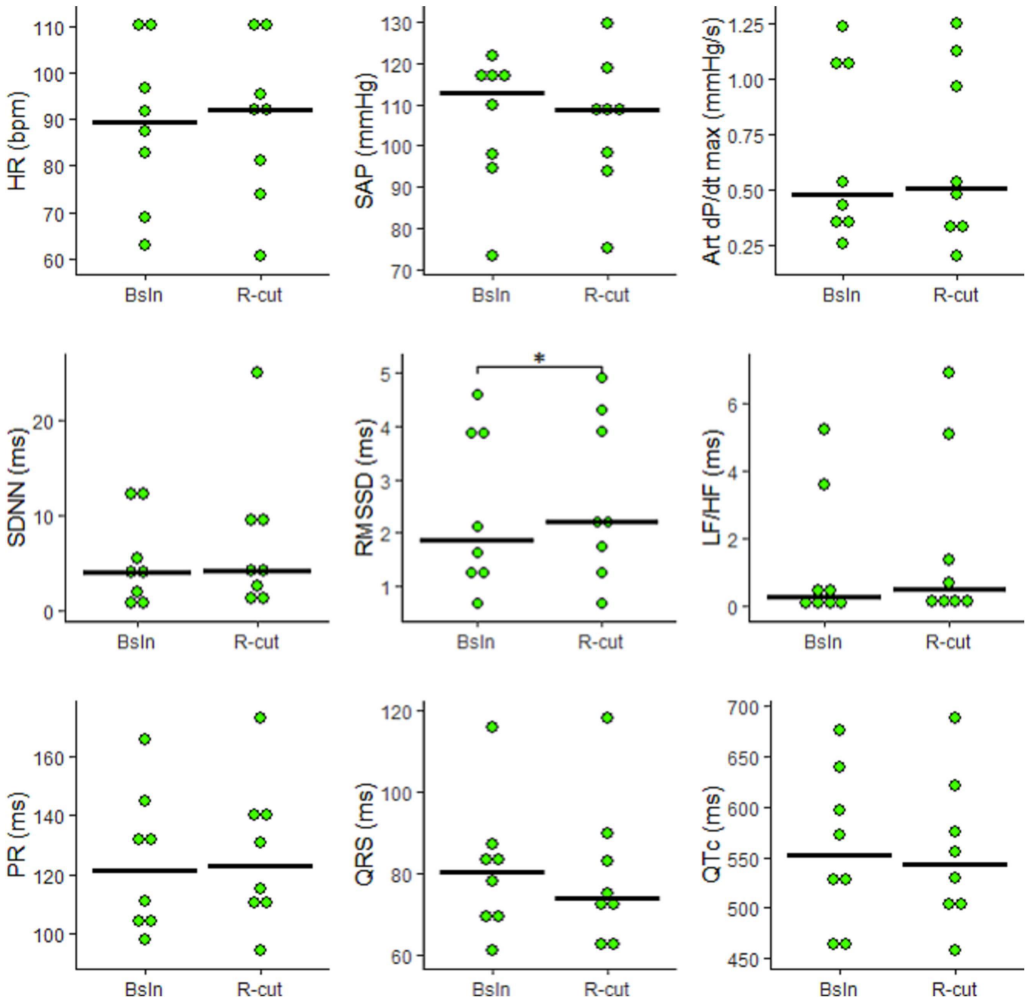
HR (bpm)
SAP (mmHg)
Art dP/dt max (mmHg/s)
SDNN (ms)
RMSSD (ms)
LF/HF (ms)
PR (ms)
QRS (ms)
QTc (ms)
BsIn
R-cut
*

Figure 4