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Physical Training Reverses the Impaired Cardiac Autonomic Control and Exercise Tolerance Induced by Right-Side Vagal Denervation

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ABSTRACT Cardiac transplantation results in inevitable denervation. However, it remains poorly understood how important the loss of vagal innervation is for exercise tolerance. It is also unclear whether physical working capacity restoration after denervation is assured during exercise. To assess the effect of denervation on cardiac autonomic control and exercise tolerance, we used an animal model of cardiac denervation by unilateral transection of the vagus nerve. The experimental study was carried out on male Wistar rats ($N = 60$), which were randomly assigned to six groups. One third of animals constituted the control group, the other third underwent right-side vagal denervation, and the rest underwent left-side denervation. After the surgery, half of the animals were trained and the other half were kept sedentary. Electrocardiogram was recorded in all animals, followed by assessment of the heart rate variability. It was shown that the right-side denervation leads to significant decrease in the heart rate variability. The postsurgical maintenance of rats with the right-side denervation in sedentary conditions led to significant decreased exercise tolerance. An increase in the physical working capacity was found in all trained animals but the most significant increase was again observed in rats with the right-side denervation. Moreover, in all trained animals indices of the heart rate variability were significantly higher. These observations allow us to conclude that right-side vagal denervation leads to both a decrease in the cardiac autonomic control and a suppression of physical working capacity. Postsurgical training with submaximal intensity promotes both restoration of the exercise tolerance in all animals and increase in the regulatory neurogenic influences on the sinus node, including rats undergoing vagal denervation.

INDEX TERMS Heart rate variability, heart denervation, neurogenic rhythm regulation, exercise tolerance, physical training.

I. INTRODUCTION

Impairment of the cardiac rhythm regulation disrupts the organism's response to physiological needs, in particular, to physical exercises. This could be a reason for the onset of cardiac insufficiency symptoms in patients with implanted cardiac pacemaker [1]. Notably, clinically less significant signs of hemodynamics problems associated with loss of heart rate control are widespread among patients with the ventricular-based pacing and referenced to as pacemaker syndrome [2]. Loss of the neurogenic control is a serious problem in patients with autonomic neuropathy [3]. However, the lack of this control becomes of the highest importance in patients after heart transplantation [4]. Moreover, while

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sympathetic re-innervation is considered as a possible process in patients in the late period after donor heart transplantation [5], the restoration of the vagus nerve control raises doubts among most researchers even in the late period after transplantation [6]–[8]. Withal, it is well known that in the post-transplant period, heart failure persists in a number of patients, despite the good contractility of the donor heart, thus significantly limiting their rehabilitation [9]. Apparently, vagal innervation of the heart is important in ensuring the physical working capacity, which starts to increase in the early recovery period after physical training [10], and is a prognostic marker in patients with pathology of the circulatory system [11]. Role of the parasympathetic nervous system in maintaining physical working capacitance remains poorly understood. It is assumed that the trophic influences of the vagus nerve on the myocardium are of great importance in the capacitance restoration process, as well as in the whole organism rehabilitation [12]. The absence of basal inhibition of the myocardial tone by the vagus nerve is observed in patients with heart failure, which probably makes an additional contribution to the symptomatology [13]. Additionally, loss of parasympathetic influence leads to an increase in the heart rate that adversely affects both the diastolic function of the myocardium and its metabolism resulting in early incapacity of the transplant [14].

At the same time, it is known that regular physical activity or training of patients with cardiovascular diseases (including chronic cardiac insufficiency) leads to both better exercise tolerance and favorable prognosis [15]. To the date, there are no natural pathophysiological conditions allowing assessing the effect of vagal denervation on the functional state of the myocardium and tolerance to physical exercises. Drug-induced tests with atropine are not specific, and do not meet the conditions of the chronic experiment [16]. To solve this problem, we suggest to use an animal model providing the least traumatic parasympathetic heart denervation in rats so that after such denervation the animals are able to perform physical exercises.

Moreover, there is currently no consensus about the difference in the functional influence of the left and right vagus nerves on the heart. There is observation that right-side vagal transection largely affects the sinus node regulation [17]. However, data on the similarity of the effects due to stimulation of the left and right vagus nerves have also been published[18]. In addition, there is some asymmetry in the indications for the use of the left-side and right-side vagal stimulation. While the right-side is more often recommended during heart failure, the left-side – for the treatment of depression and epilepsy [19]. Besides, the difference in the effects of right-side and left-side vagal denervation on HRV, which reflects the state of the heart autonomic regulation, has not been sufficiently studied.

Our objectives were to assess the effect of the vagal denervation on exercise tolerance and to clarify the influence of the training on autonomic cardiac regulation and the rats' ability to exercise after the denervation.

II. MATERIALS AND METHOD

A. ANIMALS

All experiments were performed in accordance with the ethical guidelines of the International Association for the Study of Pain, the Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes, and reported in compliance with the ARRIVE guidelines 2.0. The study protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the Almazov National Medical Research Centre, prior the experiments (decision No. 21-11PZ#V2 of June 11, 2021). Accordingly, the number of animals in our study was kept to the necessary minimum. Experiments were performed on N = 60 male Wistar rats obtained as a result of breeding in the vivarium of the Almazov Scientific Center, weighing from 250 to 320 g at the beginning of the experiments. Rats were housed by three animals in a light and temperature-controlled environment for at least seven days prior to use, with access to food and water ad libitum.

B. STUDY PROTOCOL

There were eight stages in our experimental study.

1. Initial exercise tolerance test.
2. Division into the groups.
3. HRV assessment.
4. Surgery for the denervation of the heart.
5. Post-surgical estimation of HRV.
6. Physical training of the animals.
7. Final exercise tolerance test.
8. Final assessment of HRV and euthanasia.

1) EXERCISE TOLERANCE TEST

In the first stage of the study, we assessed animals' tolerance to physical exercises by the treadmill test performed on a 5-lane moving road (Maze Engineers, USA). Initially, a training cycle was carried out to adapt the animals to the experimental methodology. The main study was performed one day later. To assess the exercise tolerance, we used a protocol of gradually increasing the treadmill speed every 30 seconds by 5 m/min until a speed of 40 m/min was reached. Two parameters were measured during the test: the duration of the run in minutes, and the traveled distance in meters.

2) DIVISION INTO GROUPS

Sixty rats were divided into six experimental groups depending on the operation type (sham transection in which an appropriate incision was made, but the vagus nerve was not cut, left-side or right-side transection of the vagosympathetic trunk) and postoperative management (physical training or normal feeding). Abbreviated names of the groups are presented in Table 1. Each group consisted of 10 rats.

After the initial tolerance test, all rats were ranged according to the traveled distance in the moving road. Thereafter, the rats were randomly distributed over the groups so that the mean distance was approximately the same in each group

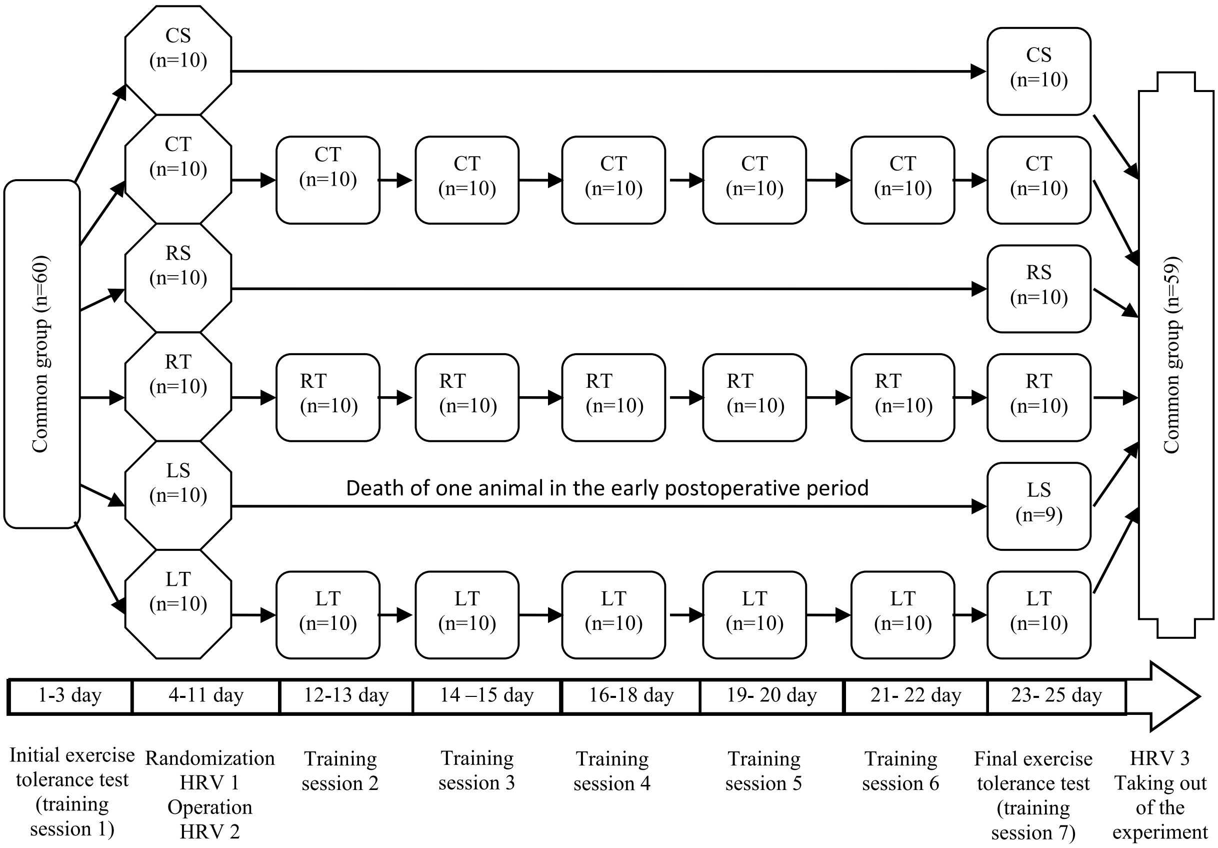

FIGURE 1. Study design. The animals, after being introduced to running on a moving road, were tested for the exercise tolerance and then randomized into six groups. The group names are coded according to the following two-letter designations: C, sham (control) transection; R, right-side denervation; L, left-side denervation; S, sedentary condition; and T, regular physical training. Each group consisted of 10 animals. All animals were assessed HRV before and immediately after denervation. Thereafter, physical exercises were performed only by animals from the groups designated by the letter T in the abbreviated name. Three days after the final assessment of the exercise tolerance, final HRV measurements were carried out, and the animals were withdrawn from the study.

TABLE 1. Groups of Animals Included in the Study.

	Sham transection	Right-side denervation	Left-side denervation
Feeding	CS	RS	LS
Training	CT	RT	LT

TABLE 2. Mean Tolerance Parameters of the Animals in the Groups.

	Distance traveled, m	Duration, s
CS	208 ± 124	471 ± 247
CT	206 ± 117	437 ± 219
RS	200 ± 106	454 ± 210
RT	202 ± 103	436 ± 199
LS	204 ± 112	454 ± 224
LT	204 ± 108	454 ± 224

Data are expressed as mean ± standard deviation.

(see Table 2). According to the statistical dispersion analysis (ANOVA), there was no difference among the groups in terms of distance traveled ($F = 0.006$, $P = 0.99$) or mean duration ($F = 0.031$, $P = 0.99$).

The protocol design is shown schematically in Fig. 1.

Three days after the treadmill test, we anesthetized the animals, measured HRV, and carried out either sham or true resection of a part of the vagus nerve as described in Sect. B4.

3) ASSESSMENT OF HRV

During and after the surgery, electrocardiograms (ECG) were recorded in all rats during narcotic sleep. Initially, a 2.5-min ECG was recorded, and then mechanical stimulation of the vagus nerve was performed for 5 seconds, followed by its transection. After transection, ECG was recorded again for 2.5 min.

Steel needles were inserted into the muscle tissue of the rat limbs to record ECG that was performed by digital electrocardiograph (model KAP-01 "Kardiotekhnika-EKG," Incart Ltd., St. Petersburg, Russia) operating at the sample frequency of 1 kHz. Recorded ECG signals were processed using custom software implemented on the MATLAB platform (Version R2020b, The MathWorks, Inc., MA, USA, 2020). It made it possible to semi-automatically determine the position of R-peaks with the option of visual control and correction of their position on the time scale by the

TABLE 3. Frequency Bands in Humans and Rats.

Frequency bands	Border frequencies, Hz	
	For humans	For rats
very low frequencies, VLF	0.003 – 0.04	0 – 0.2
low frequencies, LF	0.04 – 0.15	0.2 – 0.8
high frequencies, HF	0.15 – 0.4	0.8 – 2.5

operator. The latter was especially useful in the case of a significant change in cardio cycle duration, caused, for example, by mechanical stimulation of the vagus nerve.

To assess both time-domain and frequency-domain parameters of HRV in rats, another software was developed also on the MATLAB platform. All calculations were performed according to the standard methods adopted for the analysis of human HRV. However, due to the fact that the heart rate in rats is about 5 times higher than in humans, some coefficients in the calculations were adjusted to account for this difference [20], [21]. The selected bands and the corresponding standard ranges for spectral HRV parameters in rats are presented in Table 3.

An algorithm based on the fast Fourier transform was used to calculate the frequency parameters. The procedure for calculating spectral indices includes the following steps.

1. Elimination of the outliers in sequences of cardiac intervals. Both manual a removal of single outlier and semi-automatic removal of the outliers that make up a selected percentage of the maximum amplitude are available.
2. Recovery of the heart rate control function using spline interpolation (with a sampling rate of 20 Hz).
3. Calculation of the spectral power density for the selected ECG recordings. For this, three recordings with 100 s duration were selected: before the denervation, immediately after the denervation, and three weeks later.
4. Calculating the total power in each of the VLF, LF and HF frequency bands, as well as the total power (TP), using (1):

$$TP = VLF + LF + HF \tag{1}$$

5. Calculating the normalized value of the high- and low-frequency components, nLF and nHF, and the LF/HF ratio.

4) SURGERY FOR DENERVATION OF THE HEART

a: ANESTHESIA

Rats were anaesthetized by intramuscular injection with a mixture of Zoletil 100 and Xylazine 2% at a dose of 0.08 ml per 100 g of each substance. After the surgical anesthesia, each rat was placed on a thermostatic heating pad to maintain constant body temperature. The pad was pre-cleaned with an antiseptic. The operation on rats was performed in the supine position with spontaneous breathing.

b: SURGERY

After longitudinal skin incision on the anterior surface of the neck, we mobilized the mandibular gland thus exposing own fascia and neck muscles. The fascia was opened up, and the sternocleidomastoid muscle was retracted laterally thus providing access to the neurovascular bundle containing the vagosympathetic trunk and the common carotid artery. The latter were separated from each other, and the vagosympathetic trunk was taken on the holders.

In 40 rats (see Sect. D2 for rats' grouping), the vagosympathetic trunk (either right or left) was crossed with cutting off a part of the trunk 2-3 mm long in order to avoid possible regeneration. The remaining 20 rats formed control groups. They were also subjected to surgery in a similar manner, but for them either the right or the left vagosympathetic trunk was returned in the previous position after being kept in the holders without any additional manipulation. After controlling hemostasis and foreign bodies, the wound was washed with an antiseptic solution and sutured with skin stitches.

c: POST-SURGICAL CONDITIONS

Until the final awakening, the animals were kept in a thermostatic postoperative observation chamber. Thereafter, they were placed in their usual cages. Immediately after the operation and during the next 3 days, the rats were injected with the analgesic drug meloxicam at a dose of 0.1 – 0.2 mg per 100g. In the post-surgical period, the animals were under observation during a week, and then half of the animals were sent to the physical exercises according to the study protocol. Only one rat in the group of right-side denervation died on the second day after the surgery.

5) POST-SURGICAL ESTIMATION OF HRV

Immediately after the surgery we carried out secondary ECG recording of 2.5-min duration to assess changes in HRV.

6) PHYSICAL TRAINING AFTER SURGERY

Half of the animals from the groups of the false, right-side, and left-side denervation were involved in the training process. Physical exercises were carried out on the 8, 10, 12, 15, 17 days after the operation according to the protocol with a gradual increase in the speed of the treadmill by 5 m/min every 30 s until the speed of 40 m/min was reached. The maximum training duration was 360 s, which corresponds to 75% of the average running time of the animals before the surgery. If the animal got tired earlier, the training was stopped with fixing the exact running time. At the end of the second week of the training process, all rats underwent a repeated assessment of exercise tolerance according to a protocol identical to the first study. Therefore, taking into account the initial and final tolerance tests, all groups of trained animals participated in seven regular high-intensity training sessions.

7) FINAL EXERCISE TOLERANCE TEST

At the end of the second week of the exercise process, all rats underwent a repeated assessment of exercise tolerance according to the protocol identical to the initial test.

8) FINAL ASSESSMENT OF HRV

Last ECG recording (of the same duration of about 2.5 min) was carried out two days after assessing the exercise tolerance, also in the state of narcotic sleep of the animal. Right after the last HRV assessment the animals were took out of the experiment and their hearts were removed for further study.

C. STATISTICAL ANALYSIS

The independence of the analyzed data was ensured by the randomization of animals into groups, taking into account the preliminary measured exercise tolerance (see for details Sect II*B*3). The measurement data are presented as mean ± standard deviation. To assess the significance of differences in animals of different groups at different stages, nonparametric criteria for pairwise comparison of dependent samples were used: the sign test and the Wilcoxon test. To compare the differences in indicators in the selected groups, the nonparametric Mann-Whitney and Kolmogorov-Smirnov tests were used. Differences were considered significant at $P < 0.05$. Given the small sample size, we performed a normal distribution analysis, which showed that the normal distribution was not observed in all groups. For this reason, nonparametric statistical methods were used. The HRV datasets were refined according to the three-sigma rule. Because of the outliers' removal, three animals from different groups were not included in the analysis. The statistical analysis of the measured data was carried out by using the software Statistica 10 (StatSoft, Russia).

III. RESULTS

A. EFFECT OF DENERVATION ON HEART RATE REGULATION

Assessment of HRV dynamics before and after the denervation showed that no significant changes in heart rate were observed immediately after the nerve transection. Nevertheless, in the group of animals with the right-side denervation, a significant decrease in HRV was observed in the VLF and HF bands ($P < 0.001$ and $P = 0.049$, respectively). In addition, there was a decrease in TP: 1.94 ± 2.17 and 1.42 ± 2.62 ms^2; $P < 0.001$. The data are presented in Table 4.

It is worth noting that in the groups of sham and left-side denervation, no significant changes in HRV indices were found. None of the groups showed changes in either the normalized parameters HRV bands or LF/HF-ratio. Considering that in each of the three groups (sham, right-side, and left-side denervation) only half of the animals were trained after surgical intervention, in further analysis we compared HRV parameters of trained and untrained animals in respective groups.

TABLE 4. Bands of HRV Spectrum Before and just After Surgery (N = 60).

		Sham	Right-side	Left-side
VLF	Before	0.32 ± 0.39	0.81 ± 0.77	0.30 ± 0.22
	After	0.33 ± 0.71	0.21 ± 0.27*	0.31 ± 0.27
LF	Before	0.21 ± 0.48	0.13 ± 0.17	0.21 ± 0.31
	After	0.15 ± 0.30	0.10 ± 0.11	0.22 ± 0.43
HF	Before	0.53 ± 0.83	0.93 ± 1.66	0.61 ± 0.74
	After	0.44 ± 0.61	0.74 ± 1.19*	0.69 ± 1.05
TP	Before	1.06 ± 1.63	1.87 ± 2.16	1.07 ± 1.09
	After	0.93 ± 1.21	1.05 ± 1.36*	1.20 ± 1.69
nLF	Before	0.20 ± 0.10	0.18 ± 0.11	0.23 ± 0.20
	After	0.19 ± 0.10	0.18 ± 0.14	0.23 ± 0.20
nHF	Before	0.80 ± 0.10	0.82 ± 0.12	0.77 ± 0.20
	After	0.80 ± 0.10	0.82 ± 0.12	0.77 ± 0.20
LF/HF	Before	0.27 ± 0.18	0.32 ± 0.38	0.45 ± 0.64
	After	0.26 ± 0.16	0.30 ± 0.30	0.48 ± 0.81

*Statistically significant difference between ECG recordings before and after the vagus nerve transection.

Data are expressed as mean ± standard deviation.

TABLE 5. Bands of HRV Spectrum Before and 3 Weeks After Surgery for Untrained Animals (N = 29).

		Sham	Right-side	Left-side
VLF	Before	0.51 ± 0.52	1.22 ± 1.11	0.24 ± 0.19
	3 w. after	0.22 ± 0.21*	0.18 ± 0.16*	0.95 ± 0.98*
LF	Before	0.42 ± 0.70	0.25 ± 0.22	0.33 ± 0.40
	3 w. after	0.22 ± 0.53*	0.07 ± 0.08*	0.34 ± 0.57
HF	Before	0.95 ± 1.08	1.81 ± 2.25	0.98 ± 0.93
	3 w. after	0.43 ± 0.64*	0.32 ± 0.25*	0.27 ± 0.16*
TP	Before	1.87 ± 2.25	3.27 ± 2.83	1.56 ± 1.40
	3 w. after	0.87 ± 1.35*	0.58 ± 0.41*	1.57 ± 1.46
nLF	Before	0.22 ± 0.13	0.19 ± 0.13	0.20 ± 0.10
	3 w. after	0.15 ± 0.12	0.17 ± 0.13	0.35 ± 0.25
nHF	Before	0.78 ± 0.13	0.81 ± 0.13	0.80 ± 0.10
	3 w. after	0.85 ± 0.12	0.83 ± 0.13	0.65 ± 0.25*
LF/HF	Before	0.32 ± 0.23	0.26 ± 0.23	0.27 ± 0.17
	3 w. after	0.20 ± 0.23	0.24 ± 0.21	0.87 ± 1.14

*Statistically significant difference between ECG recordings before and after (three weeks later) vagus nerve transection.

Data are expressed as mean ± standard deviation.

It was revealed that among untrained rats in the group of right-side denervation, diminishing in all frequency-band-components of the HRV spectrum became stronger (see Table 5). Note that significant reductions in HRV were also found in untrained controls. Notably that the magnitude of HRV-bands decrease in the group of the right-side denervation was significantly pronounced. Moreover, even in untrained animals from the left-side denervation group, a significant increase in the VLF band and decrease in the HF band were observed. It should also be noted that in the group of left-side denervation there was a significant decrease in nHF from 0.80 ± 0.10 to 0.65 ± 0.25; $P = 0.049$ and a tendency to an increase in the LF/HF-ratio from 0.27 ± 0.14 to 0.87 ± 1.4; $P = 0.068$. We suggest that the changes in the heart autonomic control were probably due to the keeping of animals in sedentary conditions.

Changes in the spectral power of different spectral bands of HRV in untrained rats three weeks after the surgery are shown

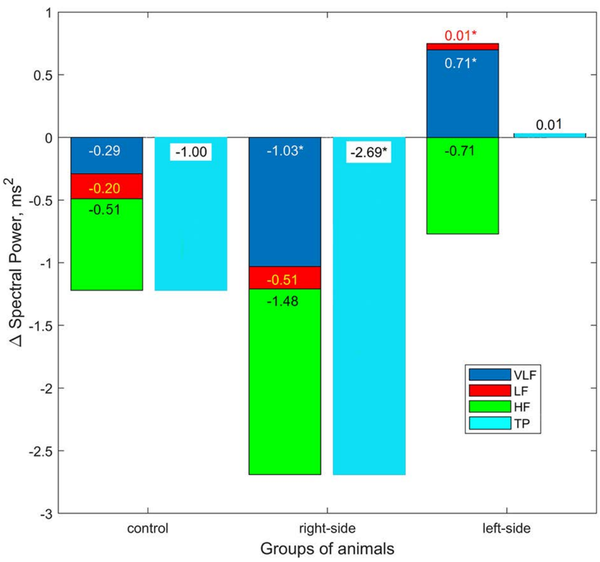

FIGURE 2. Change in HRV spectral power in the groups of untrained animals: sham, right-side denervation, and left-side denervation. Blue bars show mean difference in spectral power three weeks after the surgery for the VLF band, red bars – for the LF band, green bar – for the HF band, and cyan bar – for the total power, TP. Statistically significant difference in respect to that in the sham group is marked by asterisk.

in Fig. 2. In the course of comparative analysis, it was found that in rats with the right-side denervation, the spectrum power significantly decreased in both VLF and TP bands as compared with changes in the sham group. Decrease of the parameters in other spectral bands for these groups of rats was found insignificant. However, in the group of rats with the left-side denervation, a statistically significant increase in the spectral power of VLF and HF bands was observed compared to the sham group. These observations show that under conditions of insufficient physical working capacity of animals, right-side denervation leads to a more pronounced weakening of the cardiac autonomic control.

It is worth noting that the most significant decrease in HRV parameters was observed in rats from the right-side denervation group. In contrast to the sham and right-side denervation groups, there was an increase in HRV indices for the left-side denervation group, with the exception of the HF-band in which statistically significant decrease was observed.

Therefore, it is obvious that the right-side denervation leads to a violation of chronotropic regulation, which manifests itself immediately after the vagus nerve transection and progresses over time. However, keeping animals in sedentary conditions leads to HRV diminishing, which may be a consequence of a not mobile lifestyle.

B. EFFECT OF TRAINING ON EXERCISE TOLERANCE AND CARDIAC AUTONOMIC CONTROL

As stated above, half of the animals in each group underwent a two-week cycle of intense training, at the end of which

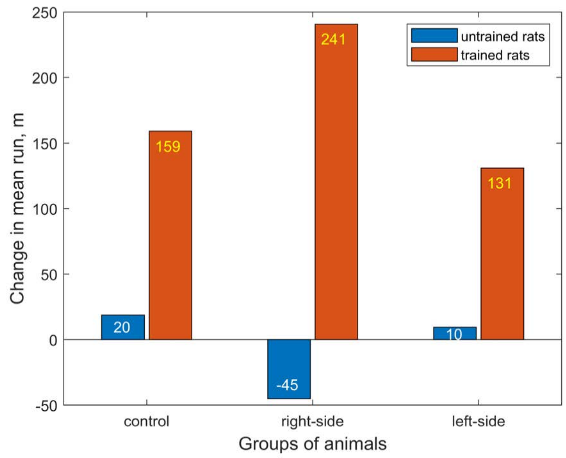

FIGURE 3. Dynamics of exercise tolerance in the sham, right-side and left-side denervation groups.

repeated testing of exercise tolerance was carried out, as well as the cardiac autonomic control was assessed. During each training session, a submaximal load was performed, which was determined from the initial level of tolerance (75 %).

The study revealed that untrained animals of the sham and left-side denervation groups showed a level of exercise tolerance comparable to the initial one, whereas animals with right-side denervation showed a significant decrease in the running distance by 45 ± 89 m; $P = 0.03$ (see Fig. 3). In contrast, trained rats in all groups showed a significant increase in exercise tolerance: 159 ± 233 m in the sham group ($P = 0.007$), 241 ± 228 m in the right-side denervation group ($P < 0.001$), and 131 ± 255 m in the left-side denervation group ($P = 0.03$).

As seen, the right-side denervation leads to a decrease in exercise tolerance, while the animals training is expected to be accompanied by an increase in exercise tolerance, including animals with regulatory dysfunction caused by the right-side denervation.

In the course of a separate study of the effect of training on neurogenic regulation of the heart in groups, it was found that the trained animals had a significant increase in HRV (Table 6). In the sham group, in contrast to the untrained animals, the trained ones showed a significant difference in the HRV dynamics in all spectral bands. In animals with the right-side denervation, significant differences were revealed in the LF and HF bands, as well as in the total power of the spectrum. In the group of trained animals with left-side denervation, the dynamics was similar to the sham group.

At the same time, the ratio of the spectrum components significantly changed only in the group with the left-side denervation, where a statistically significant increase in the parasympathetic component was revealed in the trained animals instead of its decrease, with a corresponding decrease in the LF/HF-ratio.

Therefore, the training process affects not only exercise tolerance, but also HRV in the both sham and sinus node denervation groups.

TABLE 6. Changes in Spectral Parameters of HRV in Groups of Trained and Untrained Animals Three Weeks after Denervation.

		ΔVLF, ms^2	ΔLF, ms^2	ΔHF, ms^2	ΔTP, ms^2
Sham	untrained	-0.29±0.33	-0.20±0.22	-0.51±0.54	-1.00±1.01
	trained	0.32±0.62*	0.16±0.37*	0.18±0.61*	0.66±1.21*
Right-side	untrained	-1.03±1.17	-0.18±0.19	-1.48±2.37	-2.69±3.02
	trained	-0.50±0.80	0.25±0.61*	0.25±1.11*	0.00±1.75*
Left-side	untrained	0.70±3.50	0.05±0.30	-0.77±0.88	-0.02±4.03
	trained	0.33±1.53	0.01±0.11	0.38±0.82*	0.73±1.68

*Statistically significant difference in trained animals compared to untrained ones.

Data are expressed as mean ± standard deviation.

TABLE 7. Changes in Normalized Spectral Parameters of HRV in Groups of Trained and Untrained Animals Three Weeks after Denervation.

		ΔnLF, a.u.	ΔnHF, a.u.	$\Delta(LF/HF)$, a.u.
Sham	untrained	-0.05±0.12	-0.02±0.26	-0.26±0.31
	trained	0.06±0.11	-0.03±0.24	0.37±0.09
Right-side	untrained	-0.01±0.11	-0.01±0.45	-0.03±0.29
	trained	0.09±0.15	0.13±0.25	0.10±0.56
Left-side	untrained	0.03±0.08	-0.24±0.40	0.54±0.96
	trained	0.01±0.9	0.19±0.43*	-0.33±0.82*

*Statistically significant difference in trained animals compared to untrained ones.

Data are expressed as mean ± standard deviation.

IV. DISCUSSION

In this study, an animal model of unilateral vagal denervation demonstrated its effect on the cardiac autonomic control, as evidenced by observed changes in the HRV dynamics after the surgery. It is known that HRV analysis is a fairly reliable method for monitoring neurogenic regulation of the heart, which determines its widespread use for assessing vagosympathetic innervation in the post-transplant period [22], [23].

Our study has shown that only the right-side denervation leads to a statistically significant change in HRV. This indicates the dominant influence on the heart rate of this particular nerve, which innervates the sinus node to a greater extent than the left one [17], [24]. It is noteworthy that immediately after the transection, a decrease in HRV was observed that became more pronounced after three weeks of observation. It also seems natural that the early decrease in HRV affected primarily the high-frequency spectral range (responsible for the respiratory) system, the oscillations in which are modulated mainly by parasympathetic nerves [25]. Whereas the low-frequency range, reflecting neurogenic baroreflex influences on the sinus node, is modulated by means of both parasympathetic and sympathetic nerves [26] that remained intact in our experiment.

Noteworthy that no significant changes in the LF/HF-ratio were observed in most groups. This fact probably indicates the nature of this parameter, on the one hand, and its high intragroup variability, on the other. We attribute this to the fact that the power of both LF and HF components of the HRV spectrum is determined by the function of efferent vagal fibers, which means that transection of the vagus nerve affects both components, leaving their ratio little changed.

At the same time, we have found that after three weeks a decrease in HRV was also observed in the sham group among animals that did not participate in the training process. This was probably due to the keeping of animals in the sedentary conditions, which provoked detraining syndrome. Nevertheless, the severity of the decrease in HRV in the right-side denervation group was significantly greater than in animals of the sham group, which indicated an additional effect of the nerve transection on this indicator.

One of the objectives of this study was to demonstrate the influence of the training process on the dynamics of the cardiac autonomous control after denervation. Considering impossibility to predict the reaction of denervated animals to this process, we chose a rather short study protocol for the following reasons. The first is the possibility of the survival of maximum number of animals, and the second is the possibility of the program being carried out by the majority of animals. The short cycle of the training process was partially compensated by the intensity of the loads. However, despite the limited number of trainings session in the groups of rats being trained, the total runs they performed was seven, which is comparable with the number of sessions used by other research groups [27], [28]. In contrast, only two exercise tests at three-week intervals were performed in the groups of sedentary condition. The large time lag between the loads minimized their effect on the restoration of the cardiac autonomic regulation in these groups, which was confirmed by the significant differences in HRV in trained and sedentary animals.

Two-weeks exercise test revealed that in untrained animals of the sham group and in the group with the left-side vagus nerve transection, the exercise tolerance did not change, while in the group of untrained rats with the right-side transection, a significant decrease in tolerance was observed. This fact underlines the important role of the parasympathetic innervation of the sinus (neither atrioventricular nor ventricle) node in ensuring physical performance.

Moreover, our study revealed that in all groups of animals involved in the training process, the exercise tolerance increased significantly. Interestingly, in the group with the right-side vagus nerve transection, an increase of the tolerance was most pronounced. The reason for this is not entirely clear. However, it can be explained by the influence of sympathetic innervation on the myocardium, which, in the absence of the limiting effects of parasympathetic innervation, led to an increase in contractility due to the trophic effect on the left ventricular myocardium by a mechanism similar to myocardial remodeling in the early stages of hypertension [29].

We have also found that the training process was accompanied by an increase in HRV, which was significantly higher in all spectral bands in the sham (control) group, whereas in the group with right-side transection, an increase was observed only in the LF and HF bands. The most likely reason for the increase in HRV in these groups is an increase in the role of sympathetic influences in the regulation of the heart rate, as well as an increase in parasympathetic influences due to the internal autonomic nervous system, which probably retain their viability after transection the preganglionic fibers that constituent the vagus nerve.

V. STUDY LIMITATIONS

A significant limitation of this work is the impossibility of a priori justified size of the animals groups due to the lack of the published data on the HRV dynamics in rats after unilateral vagal denervation, which could determine the statistical power of the effect. Additional obstacle to the sample size estimation arises from the high physiological variability of the measured parameters in animals. In this regard, the number of animals for the experiment was calculated based on the double cut-off sample size for paired nonparametric tests of comparative analysis, while adhering to the principle of minimizing the number of animals suffering from experimental research. This did not allow us to use of parametric methods of analysis and somewhat reduced the set of statistical tools. However, presented here results on the strength of the HRV response to vagal denervation and physical training can be used for future assessment of the statistical power while planning the experimental study in this direction.

VI. CONCLUSION

In conclusion, the right-side transection of the vagus nerve leads to a persistent violation of the heart rhythm regulation, which entails a process of decreasing exercise tolerance. However, dynamic exercise not only increases physical performance, but also improves the regulation of the heart rate even in animals that have undergone vagal efferent denervation of the sinus node. This makes physical training a promising method in the process of rehabilitation of patients with cardiac autonomic control disorder, including patients after heart transplantation.

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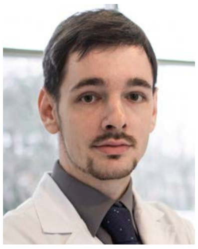

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