Ref: Ro J Neurol. 2017;XVI(3)
DOI: 10.37897/RJN.2017.3.3

# Individual post-stroke hemodynamic changes and heart rate regulation

**Maxim Mamalyga, Leonid Mamalyga**

[1]Bakulev Scientific Center of Cardiovascular Surgery, Moscow, Russian Federation
[2]Department of Anatomy and Human and Animal Physiology of Institute Biology and Chemistry of Moscow State University, Moscow, Russian Federation

## ABSTRACT

**Objective.** Ischemic stroke increases the risk of cardiovascular diseases. Our study aimed to identify individual hemodynamic changes and the regulation of heart rate after ischemic stroke in animals with different functionalities heart.
**Methods.** The work was carried out on male Wistar rats, weighing 280–320 g. The following methods were used to study: photoinduced local IS, functional tests, telemetry monitoring ECG, echocardiography, magnetic resonance imaging.
**Results.** In the subacute period of IS, animals with low and high heart functional capacity (HFC) show hemodynamic compromises of various severity. During the recovery period, the suppressed activity of the majority of heart rate variability, which took place in the acute and subacute periods, persisted in the animals with low HFC. In animals with high HFC, nature of the autonomic heart regulation did not differ from the controls.
**Conclusion.** Risk of cardiovascular complications and prospect of recovery after stroke are not only associated with character of cerebral lesions, but also with individual heart functional capacities.

**Keywords:** hemodynamics, heart rate variability, ischemic stroke

## INTRODUCTION

Acute dysfunction of cerebral circulation provoking a complex set of neurological disorders is not limited to the brain. Occurring vegetative-visceral dysfunctions impair regulation of cardiovascular system, which greatly exacerbates the neurological condition of patients after ischemic stroke (IS) (1–4). Furthermore, vegetative imbalance regulating functions of the heart induced by IS, is one of the causes of death, even if there are no signs of cerebral pathology after treatment (1,5). Post-stroke neurological disorders along with cardiac pathology provoked by them are characterized as "cerebrocardial syndrome" (6).

Despite the fact that the functional interdependence of the brain and heart is an obvious fact for a long time, but there is no consensus about the threat of heart damage in different periods after IS. Dependence of post-stroke disorders of the heart on its functional capacities has not been studied well enough. This is largely due to the fact that an objective analysis of clinical studies comparing the results is often complicated with a wide variety of brain lesions in different patients, as well as existence of some of these cardiac pathologies before IS (7). Therefore, the study of the fundamental questions of the problem involves not only clinical, but also pre-clinical studies on adequate laboratory animal models where it is possible to control the parameters of ischemic brain damage (severity, localization of the lesion, its size, depth, volume, etc.).

### Study objective

To study the features of hemodynamic and autonomic regulation of heart rate in different periods

*Corresponding author:*
Maxim Mamalyga MD, PhD
*E-mail:* mamalyga83@mail.ru

*Article History:*
Received: 20 August 2017
Accepted: 15 September 2017

after IS, as well as to clarify their relationship with the heart functional capacities.

## MATERIALS AND METHODS

### Object of study

The work was carried out on male Wistar rats, weighing 280–320 g. The study was conducted in the autumn and winter. The animals were housed in a vivarium at five per cage under natural light conditions (the day period lasted for about 8 hours) with free access to food and water. All studies were conducted in strict accordance with the basic bioethical "Rules of work with the use of experimental animals" and ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines

### Functional test

Before study, individual functionality of myocardium was determined in all animals using a conventional stress echocardiography test with dobutamine (8). Dobutamine was infused intravenously to sedated animals using Braun Perfusor Compact infusomat according to the following infusion protocol: 10→20→30→40→50→60→70 μg/kg/min (5 min for each dose). During the study, ECG was recorded online and heart rate variability was assessed. Cardiac output, stroke volume, and EF of the left ventricle were measured by B-, M-, and PW- mode echocardiograms. ST-segment elevation >2 mm in standard lead III was considered as a diagnostic criterion for ischemia.

### Photoinduced ischemic stroke

Focal IS was induced using the method of local photochemical thrombosis of the cerebral cortex vessels (9,10). For this purpose, a hole with a diameter of 2.5 mm was drilled in the skull above the left parietal cortex, leaving a 0.1-mm-thick bone plate through which vessels may be clearly seen in a binocular microscope. Then, a photosensitive agent (Rose Bengal, 20 mg/kg) was administered in the tail vein of an animal. After 2 minutes a light guide with a diameter of 2 mm was inserted into the skull pore, and a laser of a green spectral region (530 nm) was turned on. The density of the beam was 0.64 W/cm$^2$ and the exposure time was 15 min. Rose Bengal is photoactive under green illumination, which leads to platelet aggregation and thrombosis in cortical blood vessels, resulting in focal ischemic brain damage with subsequent necrosis. This model allowed us to control the severity of the IS and the localization of the lesion, as well as its size and depth (10). Animal testing were performed on the next day (the acute period of IS), 5 days (the subacute period of IS), and 35 days (recovery period) after photo-induced thrombosis. The operation was performed under general anesthesia using a Zoletil and Rometar mixture (20 and 10 mg/kg, respectively) in accordance with aseptic and antiseptic rules.

### Telemetry monitoring

All kinds of telemetry monitoring were carried out in freely moving animals in online mode using wireless telemetry system ADInstruments company (Australia) allowing for multi-day recording without and anxiety and stress for animals. ECG registration and recording were performed by transmitter (TR40BB) implanted into rat abdominal cavity. At this, one of the electrodes of the transmitter was fixed to the xiphoid process, the other – to the sternohyoid muscle. ECG monitoring corresponded to standard lead III. Telemetric monitoring of blood pressure in the aorta was performed using the transmitter (TR46SP), which registered systolic (SBP) and diastolic (DBP) blood pressure.

The surgery was performed under general anesthesia with a mixture of zoletil, rometar (20 and 10 mg/kg, respectively) according to aseptic and antiseptic requirements. During the 7 days after the surgery, animals received ketanal and gentamicin.

20 days after surgery IS was simulated, and on the next day (acute period of IS), Day 5 (subacute period of SI) and Day 35 (recovery period), after photothrombosis of vessels, time and spectral analysis of heart rate variability (HRV) was performed (11). Measurement accuracy of R-R interval was 1 ms and sampling frequency was 1024 Hz. Study of HRV included: 1) time analysis of heart rate: heart rate (HR), standard deviation normal to normal (SDNN), root mean square of the successive differences (RMSSD); 2) spectral analysis: total spectrum power (TP), spectral power of high-frequency (HF), spectral power of low frequency (LF) and very low frequency (VLF) of components with a frequency range 0.75-3 Hz, 0.02-0.75 Hz, < 0.02

Hz, respectively; spectrum power in the high frequency range in normalized units (HFnu), spectrum power in the low frequency range in normalized units (LFnu), and sympathovagal index (LF/HF).

### Magnetic resonance imaging

Magnetic resonance imaging (MRI) enabled visualized control of organic brain changes during photo-induced ischemic stroke. Studies were conducted at the Center for Magnetic Tomography and Spectroscopy of Moscow State University named after M.V Lomonosov on tomography scanner "Bruker Biospec 70/30" with the inductance of magnetic field of 7 T and the diameter of induction coil 72 mm. MRI scanning protocol was described previously (12). It included T1 and T2-weighted images in the coronary and axial projections (SP – pulse sequence, SE – spin echo and RARE – rapid acquisition with relaxation enhancement, slice thickness 1.5 mm, resolution 0.1 mm/pixel). The images were processed using the software for work with MRI images: ImageJ, MRIcron, 3D-DOCTOR.

### Echocardiography

Ultrasound examination of the heart was performed using echocardiograph Mindray M5, 10 MHz transducer (Mindray, China). End-diastolic and end-systolic dimensions of the left ventricle were assessed by M-mode by parasternal position with further definition of the shortening fraction. Echocardiography was performed in the same post stroke periods as ECG (Days 1, 5 and 35 days after vascular photothrombosis) (11).

Hemodynamic parameters in different periods after IS were calculated using standard formulas (13). Total peripheral resistance (TPR) – Wetzler Boger formula: 80 • (0.42 • SBP + 0.58 • DBP)/ cardiac output; pulmonary vascular resistance (PVR) – 80 • mPAP/cardiac output; left ventricle stroke work index (LV SWI) – 0.0136 • SI • MBP; cardiac output rate (COR) – cardiac output/ejection period. Mean pulmonary artery pressure (mPAP) was determined using a special table by ratio of accelerating time of flow in the outflow tract of the right ventricle to the ejection time. The average rate of myocardial relaxation (ARMR) is defined as the relation of diastolic endocardial excursion to the time of diastolic relaxation period.

### Statistical analysis

Statistical analyses were performed using "Statistica 6.0" software. The results were expressed as mean values ± standard error of the mean (M ± m). The unpaired Student's t-test was used for statistical analysis. The results were considered statistically significant at $P < 0.05$.

## RESULTS AND DISCUSSION

Numerous clinical studies showed that the efficacy of treatment of patients with ischemic stroke is determined not only by the absence of neurological symptoms, but also by other related somatic disorders provoked by IS. Many stroke treatment programs focus their attention on the identification and prevention of secondary cardiac disease which may be the cause of sudden cardiac death at different moments after neurological recovery (14). So, without cardioprotective therapy, survival of patients with cardiac lesions for 5 years after diagnosis IS was 30-50% (1). In some patients, over 10 years after a stroke, heart failure may be an independent predictor of mortality, or increased risk of the second IS (4,7). High risk of coronary disorders among stroke survivors formed the view that the stroke should be considered as a factor provoking heart disease (1,3,4).

However, some patients after IS did not have serious secondary cardiac complications. Therefore, some authors consider assessment of coronary disorders after stroke as excessive, although they recognize that this issue is not sufficiently studied (15–17). Currently, there is no consensus on the causes of and the level of threat of heart damage at different stages of IS. Recently, the American Heart and Stroke Association pointed out the need to clarify the primary reasons that trigger cardiac regulation at IS (18).

Apparently, the main causes may not only include localization of the lesion in the brain and its value, but the heart functional capacity. To test this hypothesis, in animals with high (I group) and low (II group) heart functional capacities we investigated hemodynamics and autonomic regulation of the heart rate in different periods after IS induced by photochemical effect of rose bengal. As a result, there is a focal ischemic brain damage followed by necrosis (Fig. 1). The average volume of damage is 16.27 ± 1.98 $mm^3$ (from 14.87 to 19.71 $mm^3$).

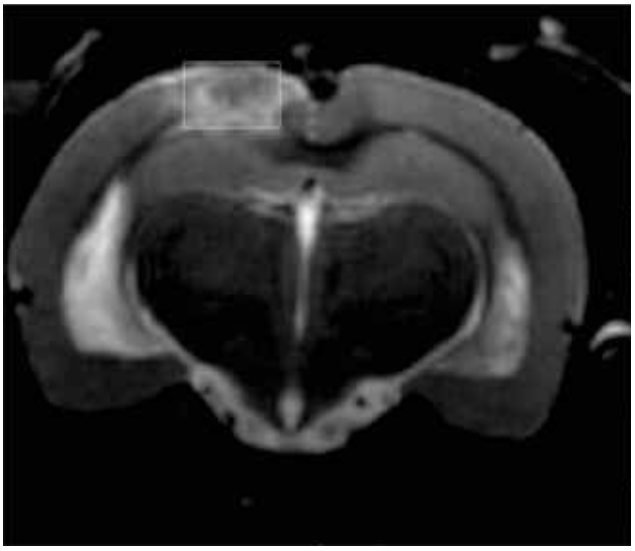

***FIGURE 1.*** *MRI of rat's brain of 35 days after focal ischemic stroke induced by photochemical effect of rose bengal.*

The conventional stress test with dobutamine eneabled to distinguish the two groups of animals: group I had ischemic changes in the myocardium occurred after the administration of dobutamine at a dose of 77 ± 4.95 μkg/kg/min, and in group II – 54 ± 3.08 μg/kg/min (Fig. 2). The diagnostic criterion of myocardial ischemia was ST elevation > 2 mm in the III standard lead.

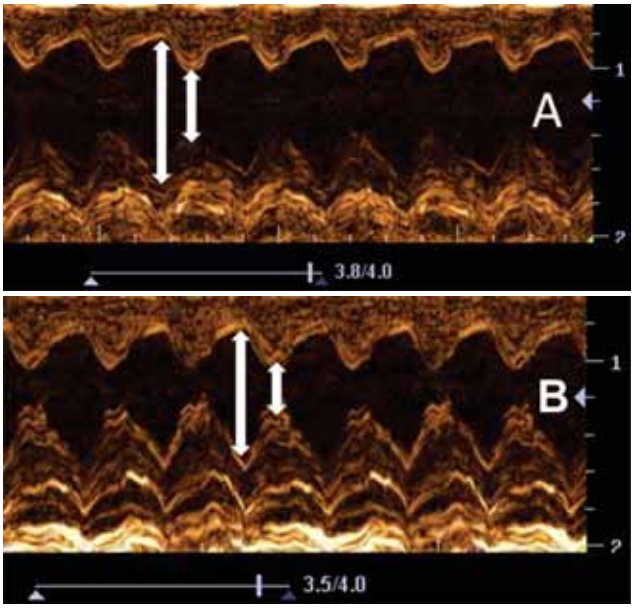


***FIGURE 2.*** *Echocardiography in M-mode on the long axis of the left ventricle during the stress test with dobutamine (50 μg/kg/min) in animals with low (A) and high (B) myocardium functional capacities*

Analysis of the study results on hemodynamics and HRV revealed no differences of related parameters in the control animals and animals from groups I and II. There were no differences between groups in the acute period of IS. Therefore, in tables 16 and 17 corresponding values are presented as mean values of two groups. According to the results of echocardiography (Table 1) the acute phase of IS in animals from groups I and II is accompanied by an increase of cardiac output (CO) and its rate (COR), stroke work index of the left ventricle (LV SWI) and medium pressure in the pulmonary artery (mPAP) suggesting increased functional load experienced by cardiovascular system, regardless of the individual heart functional capacities.

At the subacute stage nature of the hemodynamic changes in the animals from groups I and II was different. Thus, in animals with initially high heart functional capacities, pulmonary vascular resistance (PVR) decreased, but the COR was above the reference level. In animals with low heart functional capacity increase of total peripheral resistance (TPR) and PVR occurred against decrease ($P < 0.01$) of average rate of myocardial relaxation (ARMR). The latter indicates a diastolic heart function disorder. However, in the studied IS periods animals from groups I and II groups did not show significant changes in systolic, diastolic, and mean arterial pressure.

35 days after IS (recovery period) animals from group I did not show changes in hemodynamics, whereas animals from group II still had ARMR reduction (36%), indicating the prolonged disorder of diastolic heart function. Thus, the results indicate that low functional reserves of the myocardium may be a prerequisite for post-stroke disorders of hemodynamics, which persist for a long time after IS.

HRV analysis allowed us to estimate the dynamics of change of the autonomic regulation of heart rate in different periods after IS depending on the individual potential cardiac reserve. Previously, it was suggested that the likelihood of heart damage after stroke, apparently is individual for different patients, although this problem is poorly understood (19).

The findings of HRV study indicate that regulation of heart function mechanisms impairment in acute and subacute period (Table 2). However, no statistically significant differences between HRV parameters in animals from groups I and II were found. Thus, in the acute and subacute periods all

***TABLE 1.*** *The changes of hemodynamic parameters in different periods after IS*

| Parameter | Control | Post-stroke period | | | | |
|---|---|---|---|---|---|---|
| | | acute | subacute | | recovery | |
| | | | group I | group II | group I | group II |
| CO, ml/min | 123±10.71 | 157±11,81 P < 0.05 | 141±12.40 | 113±7.57 | 133±9.84 | 127±8.57 |
| mPAP, mm Hg | 12.0±0.77 | 15.0±1.08 P < 0.05 | 10.0±0.64 | 14.0±0.97 | 11.0±0.71 | 14.0±0.81 |
| PVR, $10^3$ dyne•s• $cm^{-5}$ | 7.81±0.60 | 7.65±0.58 | 5.67±0.41 P < 0.01 | 9.92±0.62 P < 0.01 | 6.61±0.47 | 8,85±0,58 |
| LW SWI, g•m/$m^2$ | 13.2±0.72 | 16.8±1.10 P < 0.05 | 14.4±0.99 | 13.4±0.77 | 15.7±1.18 | 14.1±0.87 |
| COR, ml/ms | 1.80±0.11 | 2.70±0.15 P < 0.001 | 2.17±0.10 P < 0.05 | 1.76±0.14 | 1.90±0.12 | 1.92±0.11 |
| ARMR, sm/s | 3.68±0.24 | 3.43±0.22 | 3.47±0.25 | 2.55±0.13 P < 0.01 | 3.43±0.21 | 2.36±0.18 P < 0.01 |
| TPR, $10^3$din•s• $sm^{-5}$ | 59±4.15 | 55±3.91 | 53±3.47 | 74±4.49 P < 0.05 | 59±3.95 | 63±4.27 |

animals with IS had increase of sympathetic-vagal index, which reflects imbalance of the autonomic regulation of the heart towards the predominance of sympathetic influences. This is supported by a significant decrease in RMSSD (P <0.01), reflecting the decrease of the activity of parasympathetic vegetative regulation. At the same time, total effect (P <0.01) of vegetative regulation (SDNN) is reduced which increases the risk of cardiovascular disorders (20).

Reduction of total power of spectrum (P <0.001) in the acute and subacute periods in animals of both groups indicates decline of cardiac adaptive possibilities. However, in the subacute period, more severe impairments occur. This is evidenced by the simultaneous reduction of the activity of the sympathetic and parasympathetic autonomic regulation (LF and HF-waves), as well as more significant, than in the acute period, decrease (P <0.01) of total power of spectrum. The proportion of the low-frequency component (LF%) is higher than the reference level for the total power of spectrum, while the very-low (VLF%) one was reduced. The latter indicates the preferential violation of neurohumoral link of vegetative regulation. Previous conducted studies suggest that the autonomic dysfunction and

***TABLE 2.*** *Change of heart rate variability in different periods after ischemic stroke*

| HRV | Control | Post-stroke period | | | |
|---|---|---|---|---|---|
| | | acute | subacute | recovery | |
| | | | | group I | group II |
| HR | 258±19.0 | 287±23.2 | 294±23.5 | 246±18.9 | 248±19.1 |
| RMSSD | 5.84±0.53 | 4.01±0.28** | 3.56±0.25 *** | 6.7±0.54 | 4.3±0.29* |
| SDNN | 15.2±1.21 | 10.3±0.76** | 8.8±0.74*** | 17.8±1.39 | 11.4±0.81* |
| TP, $ms^2$ | 70.7±5.01 | 52.4±4.07** | 36.7±3.14 *** | 78.4±6.79 | 59.3±4.08 |
| HF, $s^2$ | 9.40±0.71 | 8.8±0.84 | 5.4±0.43*** | 11.7±0.75 * | 8.76±0.81 |
| LF, $ms^2$ | 34.6±2.83 | 40±2.77 | 27.2±1.95 * | 36.5±2.99 | 42.8±3.46 |
| VLF,$ms^2$ | 26.7±2.39 | 3.61±0.29 *** | 4.1±0.33*** | 30.5±2.95 | 7.74±0.73 *** |
| HF, % | 12.6±0.96 | 16.7±1.35 * | 14.7±1.23 | 14.5±1.12 | 14.7±1.25 |
| LF, % | 47.8±4.06 | 76.3±5.79 *** | 74.1±6.74 ** | 46.5±3.52 | 72±5.33** |
| VLF, % | 36.8±2.78 | 6.87±0.60 *** | 11.2±0.93 ** | 38.9±3.18 | 13.0±1.01 ** |
| HF, nu | 22.1±1.22 | 18.0±1.33* | 16.5±1.28 ** | 23.8±2.07 | 16.9±1.26 * |
| LF, nu | 77.8±6.54 | 81.9±7.78 | 83.4±6.26 | 76.2±6.62 | 83.0±8.38 |
| LF/HF | 3.52±0.30 | 4.54±0.37 * | 5.04±0.41 ** | 3.27±0.25 | 4.89±0.33 * |
| IC | 5.75±0.38 | 4.95±0.41 | 5.79±0.51 | 5.87±0.39 | 5.76±0.45 |

* P < 0.05, ** P < 0.01, *** P < 0.001

decreased HRV are predictive factor of increased cardiac mortality (21–23).

During the recovery period, the animals from groups I and II showed different nature of HRV, reflecting the features of autonomic regulation of the heart rate in animals with low and high heart functional capacities. Thus, 35 days after IS, in animals with high heart functional capacities had increased power of spectrum of high-frequency component ($HFms^2$), which reflects increased activity of parasympathetic regulation. The rest of the HRV parameters returned to control level. In contrast, in animals from group II low myocardium functional capacities predetermined negative impact on the recovery of cardiac autonomic regulation after stroke. These animals retained an unfavorable imbalance of the activity of sympathetic and parasympathetic nervous system toward the sympathetic component of regulation. This is evidenced not only by high values of LF/HF, $LFms^2$, but also decreased RMSSD. This is consistent with clinical studies data, which found that early post-stroke period is associated with worsening of autonomic imbalance towards hyperactivation of sympathoadrenal system and increased diastolic dysfunction. Moreover, in patients with poor prognosis, LF/HF ratio is higher than in those with a good prognosis (24).

Obtained results indicate that in the recovery period animals with low heart functional capacities maintained inhibition of activity of some HRV parameters, which took place in the acute and subacute periods. In animals with high heart functional capacities, heart autonomic regulation 35 days after IS was restored. This gives grounds to conclude that despite the fact that in the early post-stroke subacute period features of impaired autonomic regulation of the heart associated with its functional capacities are not apparent, the latter ones determine the different perspective of their recovery in the animals from groups I and II in 35 days after IS.

Thus, subacute period of IS showed particularities of post-stroke hemodynamics in animals with low and high heart functional capacities, and in the latter changes were more severe. However, the features of HRV occurred only during the recovery period. Given the fact that in both groups of animals IS severity and localization of ischemic lesion were the same, it could be argued that the risk of post-stroke cardiovascular complications and the prospect of their recovery are related not only to the nature of cerebral lesions (foci, their size, etc.) but to individual heart functional capacities. In addition, a comparison of features of hemodynamic changes in animal from groups I and II in the subacute period with the possibility of recovery in the respective groups in 35 days after IS gives reason to believe that in the subacute stage hemodynamic pattern can be a kind of marker that refl ects the perspective of cardiovascular recovery provoked by IS.

This indicates lack of HRV results for objective assessment of the risk of early post-stroke cardiac disorders and prognosis of their recovery, taking into account individual heart functional capacities. The main attention should be paid to the complex research of hemodynamics using tissue Doppler imaging and other techniques that allow more accurate quantifi cation of the state of the cardiovascular system in the early post-stroke period.

**Disclosure**

**No funding or financial support** was obtained for this study.

**Conflict of interest:** None

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