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Laser speckle contrast imaging of cerebral autoregulation in rats at a macro- and microcirculation level

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Abstract. **Using the method of laser speckle imaging for the simultaneous study of macro- and microcirculation in cerebral vessels of healthy rats, we show that the mechanisms underlying cerebral autoregulation depend on the initial condition of the organism and the sex of individual animals. The pharmacological dose-dependent stimulation of the peripheral arterial pressure increase is not accompanied by the cerebral circulation responses of analogous intensity, but manifests itself as 'compensating' reactions, namely, the redistribution of the blood flow at the level of macro- (in females) and microcirculation (in females and males). The obtained results extend our understanding of the capabilities of laser speckle imaging technique in neurophysiological studies of reserve abilities of cerebral circulation autoregulation under the conditions of hypertensive status formation.**

Keywords: *laser speckle contrast imaging, cerebral circulation, macro- and microcirculation.*

1. Introduction

With the development of principally new image recording devices at the end of the XXth century, such as a charge-coupled device (CCD) and a complementary metal–oxide–semiconductor (CMOS), the methods of specklometry began to be intensely introduced into different fields of research. The method of laser speckle imaging of flows became one of the most popular methods, especially in biology. The principle of this technique is based on the finiteness of the frame acquisition time (the exposure time of a CMOS or CCD element) and the presence of fluctuations in the speckle field scattered by the object. The characteristic fluctuation time must be shorter than the camera exposure time. If these requirements are satisfied, the regions of the object where a certain motion occurs, i.e., the motion of red blood cells in a blood vessel, are visualised with locally 'blurred' speckle images. Numerical analysis of the degree of this 'blurring' and particularly its dynamics allows the estimation of the motion parameters of scattering particles, e.g., red blood cells [1].

The laser speckle imaging or, more precisely, laser speckle contrast imaging, became widespread because of the simple hardware, comprising a high-coherence light source and a camera for recording the speckle pattern. The main advantages of the method consist in the possibility to obtain a velocity map of relatively large regions of the object (5×5 mm and larger) without scanning, obtaining the results in real time with high recording rate and the simplicity of combining with other methods for multimodal study of biological objects [2–7].

The advantages of the laser speckle contrast imaging were used in the elaboration of a new toolkit for neurophysiological studies of cerebral blood flow [2, 3, 8–10]. The study of cerebral autoregulation and its independence of the variations of peripheral arterial pressure (PAP) is one of the most urgent problems of neurophysiology, since the disorder of these processes underlies the vascular catastrophes, e.g., the stroke [11]. The maintenance of autoregulation of cerebral blood flow is determined by the processes of redistribution between the macro- and microcirculation, the mechanisms of which remain poorly studied and require focused attention of the researchers. The application of the laser speckle contrast imaging method to the investigation of these processes seems promising, but special innovations are required to extend the capabilities of the method, including those allowing simultaneous study of macro- and microcirculation in the brain vessels.

The above considerations determined the aim of the present research, which consists in the study of reserve capabilities of autoregulation mechanisms of cerebral haemodynamics using the method of laser speckle contrast imaging of macro- and microcirculation under the pharmacological provocation of cerebral circulation response to the PAP variation in male and female rats.

2. Materials and methods of study

To measure the cerebral blood flow index we used the method of laser speckle contrast imaging (LSCI) (Fig. 1). The hardware part of the setup incorporated a source of high-coherence linear polarised (300:1) laser radiation, a Thorlabs HNL210L He–Ne laser (632.8 nm, 21 mW). By means of a single-mode optical fibre (Thorlabs PMC630-50B-APC), the brain cortex of the studied animal was exposed laser light. A CMOS camera (Basler acA2500-14 gm) recorded the subjective speckle pattern of the object, formed by means of a Computar M1614-MP2 objective. For comfortable correspondence to

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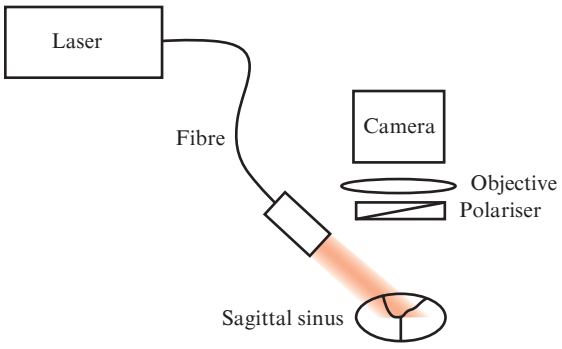

Figure 1. Schematic diagram of the FSCI setup.

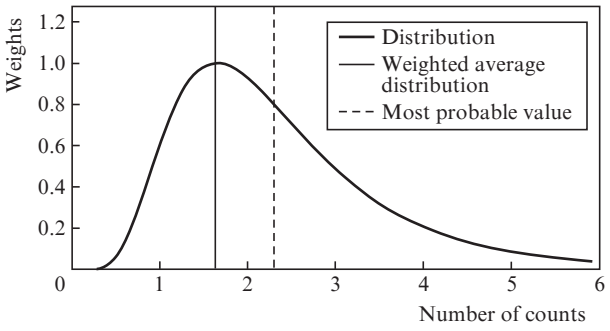

Figure 2. Illustration of the error arising due to averaging of the speckle contrast values over the image area having an asymmetric histogram. This distribution of the speckle contrast can appear when the recorded image has artefacts, e.g., nonuniformity of illumination, as well as reflect the 'abnormal' distribution of speckle contrast values over the vessel. When such images are analysed, the asymmetric distribution arises in their histograms. Simple averaging over such areas will yield an erroneous result, different from the most probable statistical value, thus introducing a systematic error into the measurements.

the Nyquist criterion (more than two pixels per one speckle), the aperture number of the objective was set to be *f*/6. The polarisation filter in front of the objective served to eliminate the direct (uninformative) reflections and the glare from the object surface.

The digital processing of the recorded speckle patterns was implemented using the classical formula for calculating the spatial speckle contrast [1]

$$K = \frac{\sigma}{\langle I \rangle}, \qquad (1)$$

where σ and $\langle I \rangle$ are the standard deviation and the mean value of the speckle pattern intensity in the sliding window. The sliding window is a mask, shifted pixel by pixel along the rows and columns of the image. Physically this algorithm divides the initial image into a set of subimages for calculating the local speckle contrast. In our case, the window size was chosen to be 5×5 pixels, which is a compromise between the preservation of the acceptable spatial resolution and the reliability of the obtained data. The averaging over 50 sequential frames processes in the above way was performed to obtain one image.

To acquire the information about the blood flow index based on the parameters of the recorded speckle contrast pattern we used the algorithm of histogram analysis of the image (or its fragments) [12, 13]. Its main advantage is the possibility of automated differentiation of great vessels and optically unresolvable ones. This technique also allows the exclusion of the error that arises in the case of simple averaging of the speckle contrast over the image fragment having an asymmetric histogram. In this case, the resulting values appear to be shifted from the true one towards the asymmetric 'tail' of the distribution (Fig. 2).

A necessary condition for correct differentiation of vessels is the sufficient number of images of vessels having different size in the studied area. In this case, the histogram will represent a clear multimodal distribution, applicable for further analysis (Fig. 3).

The blood flow index was determined as a quantity, inverse to the speckle contrast. In the control group, including both males and females, it was taken to be 1. The values of the blood flow index in other groups were calculated with respect to the control group.

It is important to comment, what physical meaning we imply using the notion of 'blood flow index'. As mentioned above, the procedure of interpreting the results of the laser speckle contrast imaging is a nontrivial problem.

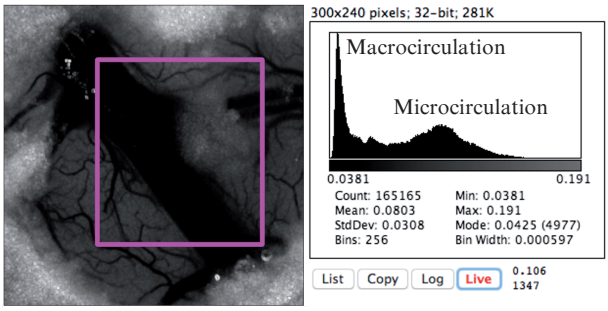

Figure 3. Example of a bimodal histogram in the region of interest (reflecting macro- and microcomponents of blood circulation).

Paper [14], which has already become classical, demonstrated the fundamental difference between the LSCI and Doppler imaging signals in relation to perfusion. This difference is due to the dependence of the speckle-imaging signal both on the velocity of scatterers and on their concentration. Yet a more detailed study of the issue was carried out in Ref. [15], involving such notions as scattering and absorption coefficients. The value of the speckle contrast depends on these two parameters. Thus, the change in the optical properties of the object can be misinterpreted as the change of blood flow parameters. The latest studies have shown that the LSCI technique measures neither the flux, nor the absolute velocity of the blood flow. It yields a certain quantity proportional to the product of the blood flow velocity and the vessel diameter [16]. Therefore, for relatively stable scattering properties of the object, the quantity inverse to the speckle contrast can be used as a qualitative estimate of the blood motion velocity in the vessel.

The possibility of estimating the blood flow dynamics in microvasculature was demonstrated in a few papers [3, 17]. Speaking about microcirculation, we mean the integral assessment of a considerable image fragment, containing a large number of optically unresolvable vessels, rather than the optical resolution of capillaries. In the present paper, we use the algorithm for qualitative assessment of velocity distribution and differentiation of vessels in the image area [12]. The main

criterion of differentiation was the velocity of red blood cells, since it is strongly dependent on the vessel diameter. In the ideal case, the histogram of the contrast distribution is bimodal, with two peaks corresponding to macro- and microcirculation (Fig. 3). When the vessels of intermediate diameter appear in the studied area, the presence of additional peaks is possible; however, they are easily removable by simple redetermination of the image fragment of interest.

The studies were carried out in 10 male and 10 female adult rats. All experimental procedures were performed in correspondence with the Guiding Principles for Research Involving Animals and Human Beings of the Helsinki Declaration [18]. The PAP measurement in awake rats was implemented using a computer-based system for direct monitoring of blood pressure in small animals (PowerLab/400 ML 401, ID Instruments, 2002, Australia) with the Chart 4 software and blood pressure sensors (MLT0699, PowerLab, ID Instruments). A day before the experiments a polyethylene catheter was implanted into the femoral artery of each animal (Fig 4) under the nembutal anaesthesia (0.40 mg kg^{-1}, ip). The standard operation procedure of implanting polyethylene catheters in blood vessels is described in Ref. [19]. In the experiments we used polyethylene catheters (ClayAdams, Parsippany, USA), labelled as BB31695-PE/1 (the external diameter 0.61 mm, the internal diameter 0.28 mm) and BB31695-PE/3 (the external diameter 0.96 mm, the internal diameter 0.58 mm).

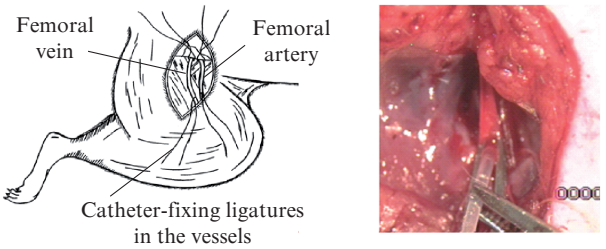

Figure 4. Implantation of polyethylene catheters in arterial and venous vessels: (a) scheme of catheter-fixing ligature application and (b) photograph of surgical implantation of a catheter in the femoral artery.

For post-operation recovery the animals were placed into individual cages (0.3×0.25×0.2 m). The experiments were carried out in 24 h after the operation. Before each experiment, the rats were adapted to the experimental environment during 30 min. In this period, the heparin solution (Biochemi, Vienna/Austria 25000ED/5 mL) was injected via the catheter to each experimental animal to prevent the formation of thrombi at the catheter walls. The preparation was injected as 1000 ED/0.2 mL of heparin (0.2 mL of heparin and 0.8 mL of saline).

With the aim of pharmacological tests, we also implanted a polyethylene catheter into the femoral vein of the animals under the general nembutal anaesthesia (Fig. 4). The experiment was carried out in a day after the operation. For pharmacological provocation of a hypertensive response we used mesaton (Sigma) in the doses of 0.125, 0.25, 0.5 and 1 μg kg^{-1}. The choice of this drug was justified by the necessity to exclude the direct effect on the cerebral vessels, since the mesaton does not penetrate through the blood brain barrier [20, 21].

The drug to awake rats was administered through the venous catheter. The volume of the injected drug was 100 μL per 100 g of the animal weight.

The statistical processing of the experimental data was implemented using the Statistics 5.0 software package. The differences were considered statistically significant for the level of confidence $p < 0.05$.

3. Results of the study

Consider the influence of mesaton on PAP. The intravenous injection of the drug was accompanied by the dose-dependent PAP increase in animals of both sexes (Fig. 5). However, in males the hypertension reactions to mesaton were more expressed. The most striking results were obtained with the dose 1 μg kg^{-1}, for which the difference in PAP increase amplitudes amounted to 24% ($p < 0.05$).

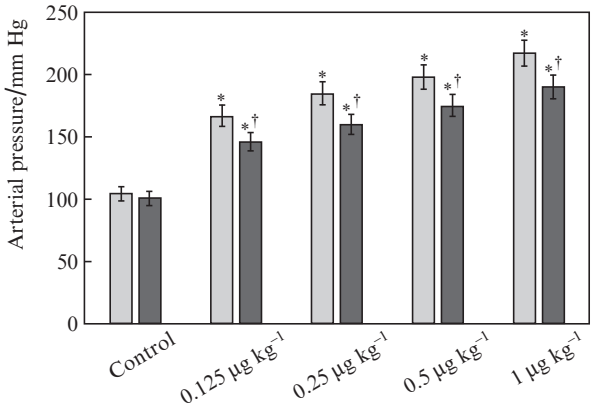

Figure 5. Variations in the peripheral arterial pressure in (■) female and (□) male rats under the pharmacological provocation of hypertensive reactions by injecting mesaton in different doses. The notation of statistical significance: * for $p < 0.05$, the values significantly differ from the control ones; † the values significantly differ between males and females.

The peripheral hypertension reactions, induced by mesaton, were accompanied by the changes of cerebral haemodynamics, different in male and female animals. Thus, in females the increase of PAP from 148±8 to 163±10 mm Hg, which is 46%–61% ($p < 0.05$) of the norm (101±5 mm Hg), was accompanied by an increase in cerebral blood flow by 15%–16% ($p < 0.05$) both in veins and in the brain microvasculature (Fig. 6). In contrast to females, in males under the same con-

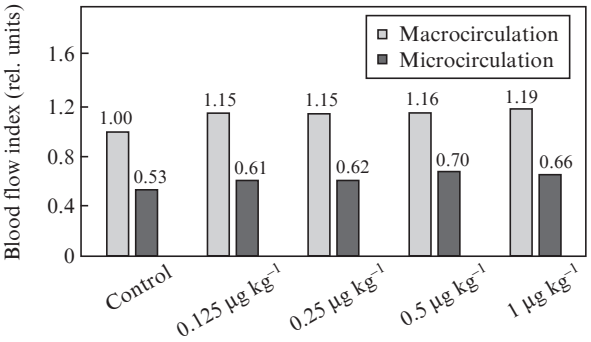

Figure 6. Dynamics of the blood flow index in female rats.

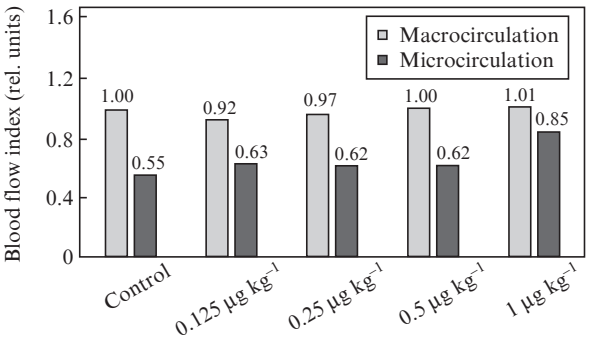

Figure 7. Dynamics of the blood flow index in male rats.

ditions the PAP was higher [168±10 and 177±12 mm Hg, the increase by 60% and 68% ($p < 0.05$) with respect to the norm of 105±7 mm Hg], but the blood supply to the perfusion area confidently increased only in the microvasculature by 13%–14% ($p < 0.05$) (Fig. 7).

The PAP increase to 175±10 mm Hg in females and to 198±12 mm Hg in males was limiting for the former, but not for the latter. Thus, at the mesaton dose 1 μg kg^{-1} in females the maximal changes in cerebral haemodynamics were observed, which were more expressed at the microcirculation level (32%, $p < 0.05$) than in veins (19%, $p < 0.05$). On the contrary, in males the changes of cerebral haemodynamics remained within the normal limits, in spite of the greater PAP increase.

It is interesting to note that the PAP increase 'beyond the limits' (to 182±11 mm Hg in females and to 215±12 mm Hg in males) was not accompanied by the maximal changes of cerebral blood flow in the former, but caused an excessive increase in the blood inflow to the microvasculature in the latter (54%, $p < 0.05$).

Figure 8 presents an example of speckle contrast imaging of a cerebral blood flow change against the background of pharmacological hypertension depending on the sex of the studied animals.

Thus, the hypertensive reactions of the healthy organism at the level of peripheral blood circulation are reflected in cerebral macro- (in females) and microcirculation (in males and females) as an increase in the venous blood flow (in females) and microvasculature perfusion (in males and females).

4. Discussion

In the present research using the laser speckle contrast imaging system we studied the response of the cerebral macro- and microcirculation to the pharmacological stimulation of peripheral hypertension reaction in animals with their sex taken into account. For this aim, we used mesaton that causes the PAP increase at the expense of activation of vascular α1-adrenoreceptors, not penetrating into the brain, i.e., not passing through the blood brain barrier [20, 21]. This fact became a key one in choosing the drug for studying the responses of cerebral haemodynamics, related only to the PAP changes, rather than to the direct effect of vasoactive substances on the cerebral blood flow.

The injection mesaton was accompanied by the dose-dependent PAP increase, the level of which was higher in males than in females. These data agree with the already widely known fact that the hypertensive reactions in a male organism are more intense than in a female one (both in humans and in animals) [22]. In our earlier studies, we have shown that the female type of vascular reactions to the hypertension, i.e., to the increase in the vascular tonus, is more favourable than the male one [23, 24]. This is largely due to the more flexible restoration mechanisms, due to which in the female organism the initial level of PAP is restored faster.

A similar conclusion follows from the analysis of the cerebral blood flow under peripheral hypertension. We revealed that in spite of higher reactivity of the venous component of cerebral haemodynamics and more sensitive changes of the microcirculation in females, in contrast to males, the cerebral blood flow became stabilised after reaching the critical perfusion increase at the microcirculation level (to 32%, $p < 0.05$), when PAP amounted to 175 mm Hg. As a result of these processes, the injection of the maximal dose of mesaton (1 μg kg^{-1}) that caused the greatest increase in PAP, was accompanied by more expressed changes in microcirculation (to 54%, $p < 0.05$) only in males, while in females the changes of macro- and microcirculation corresponded to those at medium doses of the drug.

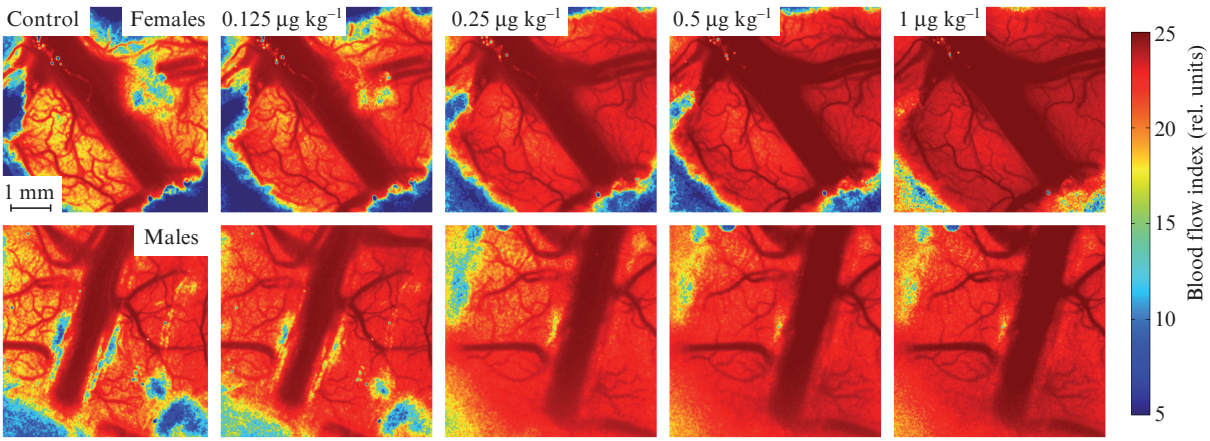

Figure 8. Illustration of the dynamics of increasing blood flow index in female and male rats (the numerical data are presented in Figs 6 and 7).

Thus, the critical changes in the autoregulation of cerebral blood circulation under the dose-dependent increase of PAP occur in females easier (the mesaton dose 0.5 μg kg^{-1}, PAP 175 mm Hg) than in males (1 μg kg^{-1}, 215 mm Hg). However, in females the compensatory mechanisms come into play that suppress the intensity of brain vascular response, whereas in males the vascular reactivity becomes excessive, exceeding that in females by four times ($p < 0.05$).

It is important to note that both in females and in males the cerebral veins less actively react to the PAP increase, than the microvasculature, which is apparently due to the specific features of the cerebral vessels and the arteriovenous shunts, opening when the PAP becomes as high as 140 mm Hg [25]. These processes provide the maintenance of permanent supply of brain tissue with oxygen against the background of significant occlusion of peripheral vessels that tends to hamper blood supply to the brain.

However, it is worth noting that the results were obtained in healthy animals. In our previous studies, using the model of stress-induced development of cerebral infarction, we have shown that the pre-stroke state is characterised by the increased venous blood flow without visible changes in microcirculation, which reacts at the changes only in the post-stroke period [13]. The obtained results show that in females the venous component of cerebral haemodynamics is more sensitive to the peripheral PAP changes, which is an evidence of more efficient redistribution of the cerebral blood flow aimed at the reduction of brain microvasculature load.

Even at minor doses of mesaton in healthy animals, we observed the responses of cerebral haemodynamics at the macro- (females) and microcirculation level (females, males), i.e., when the PAP changes within the limits of physiological values, the cerebral vessels demonstrate response expressed as the increase of the blood flow in them from 13% to 16% ($p < 0.05$). These data are confirmed by the analogous results of other authors that also observed the increase of the cerebral blood flow by 7% when the PAP increased within 10 mm Hg [26]. It follows that the notion of autoregulation of cerebral blood circulation is dynamic, depending on the initial state of the organism and the sex.

5. Conclusions

The practical application of the integral algorithm of the speckle contrast image analysis proved its efficiency in the study of mechanisms of cerebral autoregulation. The multiscale assessment of the blood flow distribution can serve as a means of evaluation of systemic influence of pharmacological agents on the vessels of different size. For a number of problems arising in the study of cerebral blood flow, the rapidness and high quality of results make the LSCI technique comparable with the Doppler flowmetry and Doppler optical coherence tomography (DOCT), and from the point of view of simplicity, the LSCI is even superior.

The main drawback of the speckle contrast method as compared to DOCT is the relatively low dynamic range of velocity measurement, when using one (fixed) exposure, and the rather complicated process of interpreting the results. In spite of the presence of such limitations, the positive features of the present technique, related to its spatial resolution exceeding that of DOCT, allowed us to reveal important regularities in the mechanisms maintaining the 'autonomy' of cerebral blood circulation and reserve capabilities of these processes. We have found that the PAP changes in the beyond-the-limit (from 140 to 215 mm Hg) physiological range do not strongly affect the cerebral blood flow, but manifest themselves in the compensatory mechanisms of cerebral autoregulation. In females, the maximal changes in the cerebral blood flow are faster, but less intense than in males. The achievement of the critical shifts in the cerebral haemodynamics against the background of hypertensive reactions in females is accompanied by the compensatory inertia of the cerebral blood vessels with respect to a further increase in PAP, whereas in males under these conditions one can observe the excessive reactivity of the brain microcirculatory system. In females, the cerebral haemodynamics is more flexible and adaptable to the peripheral hypertensive reactions, which is possibly one of the mechanisms of their greater resistance to the stroke against the background of chronically high PAP.

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