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Imaging Photoplethysmography in Green Light for Assessment of Cerebral Hemodynamics through an Intact Skull

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Abstract—This work demonstrates for the first time a possibility of assessing changes in the parameters of cortical blood flow in mice through the intact cranial bone during pharmacological tests using the method of imaging photoplethysmography with illumination in the green region of the spectrum. This makes it possible to evaluate the effect of tests on hemodynamics without prior surgical intervention on the skull bones. It has been shown that administration of the nitroglycerin can lead to both an increase in cortical blood flow and a decrease in it, which indicates the duality of the effect of this drug on meningeal vasomotor activity.

Keywords: cortical blood flow, imaging photoplethysmography, perfusion index, intact skull, nitroglycerin

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INTRODUCTION

An objective assessment of the functional state of cerebral hemodynamics in the therapy of acute and chronic pathologies of the brain is a difficult and complex medical task, which solution is of paramount importance for adequate preoperative diagnosis of patients with severe cerebrovascular accidents, determining the expected volume and traumaticity of neurosurgical interventions. To assess the parameters of cerebral blood flow in clinical medicine, a wide range of modern diagnostic methods are used, such as magnetic resonance imaging, positron emission tomography, dynamic computed tomography, single-photon emission computed tomography, transcranial Doppler ultrasound, laser Doppler flowmetry, etc.

In most cases, the assessment of cerebral blood flow is carried out with an intact (not trepanned) skull, a rare exception is the use of laser Doppler flowmetry [1, 2] or magnetic resonance imaging [3, 4] during neurosurgical operations or after traumatic brain injury, although such use is very limited and uninformative. At the same time, during the operation it is very important for the surgeon to have accurate and objective information about the functional state of cerebral hemodynamics, the consistency of the mechanisms of autoregulation of cerebral blood flow in various physiological states. This need requires the creation and testing of adequate models of cerebrovascular disorders, the search and study of new potentially active drugs, and the assessment of their action and toxicity in experimental animals. This approach will create the basis for the effective relief of acute conditions and treatment of vascular pathologies of the brain, the development of new diagnostic methods and surgical treatment.

Imaging photoplethysmography (iPPG) has recently been demonstrated in rats to be a reliable, easy-to-use, noncontact method for assessing the functional state of the cortical vasculature in pharmacological and physiological tests. The method is based on illumination of the skull with incoherent green light of in-variable intensity, subsequent intensity modulation of a reflected light due to its interaction with red blood cells, and registration of a sequence of images of the study area. It allows noninvasive assessment of the amplitude-temporal parameters of the microcirculation, depending on the perfusion pressure and the vascular tone [5–7]. In these studies, intravital video recording of the intracranial vessels of animals was

carried out through cranial windows (for both the right and left cerebral hemispheres) in two implementations: with partial grinding of the parietal bone of the skull to optical transparency (closed cranial window) and with trepanation of the cranial bone (open cranial window). It was found that the nature of cerebral hemodynamics in the case of a closed cranial window can differ significantly from hemodynamics during trepanation [5]. Note that cranial windows preparation in both the first and second cases requires certain time, is a nontrivial and technically quite complicated surgical procedure.

In this work, mice were chosen as the object of study. As our preliminary studies have shown, the morphology of the skull bones of this animal allows us to assess cerebral blood flow directly through the parietal bone, without its preliminary thinning or trepanation. The study was carried out using the iPPG method in green light, which is known to acquire the highest modulation at heart rate after interaction with biological tissue containing blood vessels, and allows one to obtain the highest signal-to-noise ratio despite the fact that its penetration depth is not high [8].

This work demonstrates for the first time the possibility of assessing the parameters of cerebral hemodynamics in mice through the intact cranial bone during pharmacological tests using the imaging photoplethysmography method with illumination in the green range of the spectrum, which makes it possible to evaluate the effect of the tests on cortical blood flow under more natural conditions.

ANESTHESIA AND SURGICAL PREPARATION

The pilot study was conducted on a six-month-old male linear laboratory mouse weighing 30 g, prepared in the vivarium of the Center for Hygiene and Epidemiology in the Primorsky krai. The mouse was anesthetized by intraperitoneal injection of a xylazine solution (Bioveta, Czech Republic) at a dose of 5.5 mg/kg and a solution of droperidol (Dalkhimfarm, Russia) at a dose of 4 mg/kg. The adequacy of the anesthesia was checked by the absence of a withdrawal reflex after paw pinching. After reaching the surgical level of anesthesia, the surgical field was prepared. A special depilatory composition (calcium thioglycolate, sodium hydroxide, emulsifier, glycerin, skin softening components) was applied to the parietal part of the animal's head to remove hair and preventing from it getting into the surgical incision, the operation area was disinfected. The animal's head was fixed in a stereotactic device, a skin incision was made on the scalp and the cranial bone was exposed, the soft tissue of the scalp was removed. The surface of the exposed cranial bone was moistened with saline to prevent drying. Steel needle electrodes were inserted into the muscle tissue of a mouse paws to record an electrocardiogram (ECG) during the experiment. An intraperitoneal cannula was installed to administer the study drugs. Throughout all experiments, the animal's temperature was monitored and maintained between 37.5 and 38°C using a heated table with a feedback mechanism. After the preparation procedure, the animal rested for at least 20 min to minimize the reaction effect of the manipulations.

EXPERIMENTAL PROTOCOL

A total of three consecutive experiments (on one animal) were performed to study cerebral blood flow during a pharmacological challenge test with a vasodilator (a drug that dilates blood vessels). The duration of each experiment was 150 s, during which video frames of intracranial structures were recorded continuously and synchronously with the ECG. The baseline of photoplethysmography (PPG) signal at the beginning of the study the was recorded during the first 10 s of each experimental session. At the 10th second, a vasodilator (nitroglycerin (NTG), solution for infusion, Ozon LLC, Russia) was gradually injected intravenously with a cannula at a dose of 10 mg/kg. The time interval between injections was at least 15 min.

EXPERIMENTAL SETUP

Layout of the experimental setup is presented in Fig. 1a, and the chronology of the study is shown in Fig. 1b. Video frames with images of the animal's brain were recorded at a distance of 15 cm from the area under study using a monochrome video camera UI-3060CP-M-GL (Imaging Development Systems GmbH, Germany) with a VIS-NIR 67716 lens (Edmund Optics, United States). Recording was carried out at a frequency of 100 frames per second and a resolution of 760 × 470 pixels. The camera was placed in the center of a lighting module consisting of four diode strip rings (250 LEDs emitting at a wavelength of 530 ± 25 nm) and rigidly mounted on a tripod (Manfrotto, Italy) for precise positioning. To reduce the influence of glare on the measured video signal, the LED emission was linearly polarized, and the reflected light passed through a polarizing film with an orthogonal orientation of the transmission axis. At the same time, a digital electrocardiograph (model Kardiotekhnika-EKG-8, Inkart LLC, St. Peters-

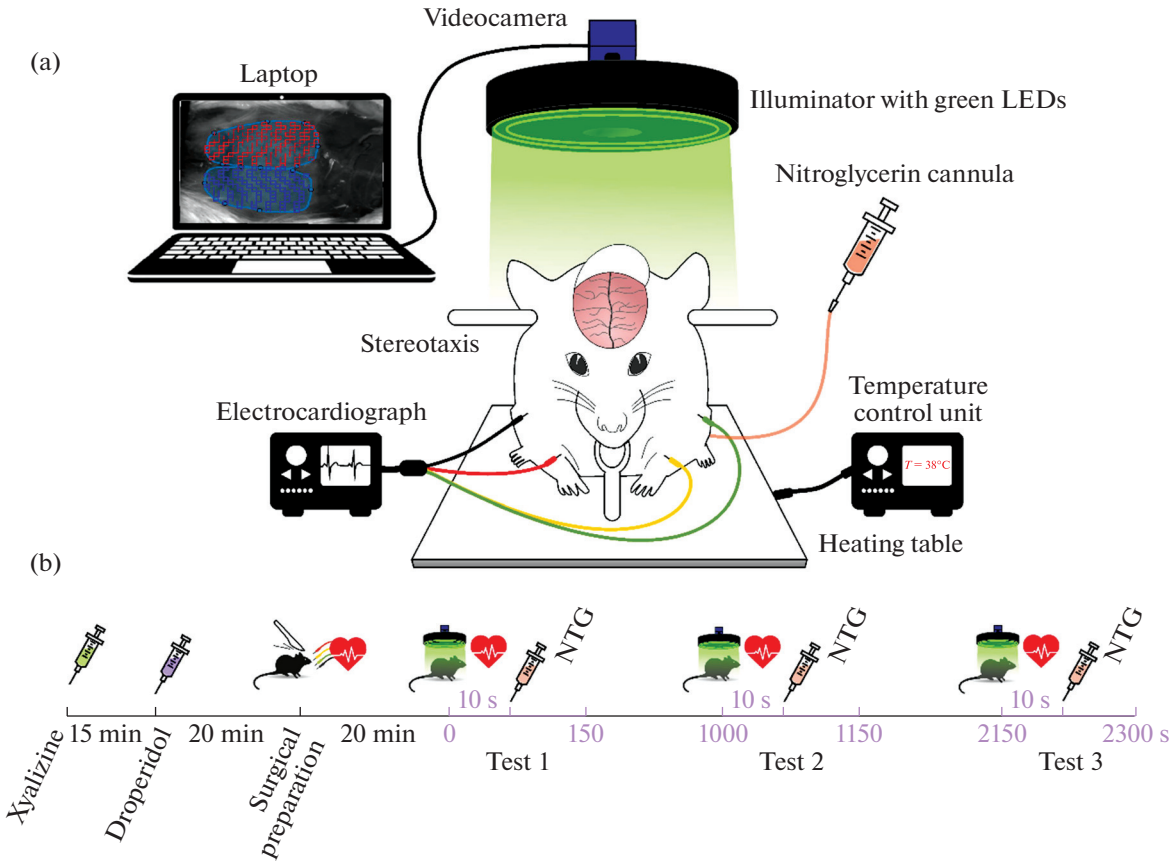

Fig. 1. Layout of the experimental setup and measurement protocol. (a) Scheme of the iPPG setup for monitoring the response of blood flow in the intracranial vessels of the mouse brain to the injection of NTG. The mouse was placed on a table with controlled heating, the animal's head was fixed in a stereotaxic device, and steel needle electrodes were inserted into the paw muscles. The NTG is inserted through an intraperitoneal cannula. Video frames at green illumination of the mouse head were recorded synchronously with the ECG and downloaded in the computer memory. (b) Chronology of the experimental study.

burg, Russia) recorded the mouse electrocardiogram in two leads with a sampling frequency of 1 kHz. The recorded video frames and ECG were synchronized in time with an accuracy of 1 ms and recorded on the hard drive of a personal computer.

DATA PROCESSING

Recorded video frames and ECG data were analyzed in post-processing mode using special software implemented on the MATLAB platform (version R2020a, The MathWorks, Inc., United States). The analysis was carried out over the entire surface of the exposed cranial bone, excluding areas with uncompensated highlights. At each time point, the shape of the PPG signal was assessed in each pixel of the recorded video images using an algorithm described in detail in [5, 7]. Briefly, processing began with digital image stabilization using a sectoral optical flow algorithm [9], then the PPG waveform was calculated for each image point as the frame-by-frame evolution of the pixel value and normalized by calculating the ratio of the alternating component at the heart rate (AC) and the slowly varying (DC) component of the received signal. The duration of each cardiac cycle was determined from the R-peaks of a synchronously recorded ECG. After this, the shape of the averaged PPG signal was calculated by averaging the signals of 15 consecutive heartbeats. Finally, the perfusion index (PI) was calculated as the difference between the maximum and minimum values of the average PPG waveform. The PI parameter was first calculated at each pixel and then averaged over a selected region of interest, for example, the right and left hemispheres. Heart rate (HR) was assessed using ECG data at the same time points.

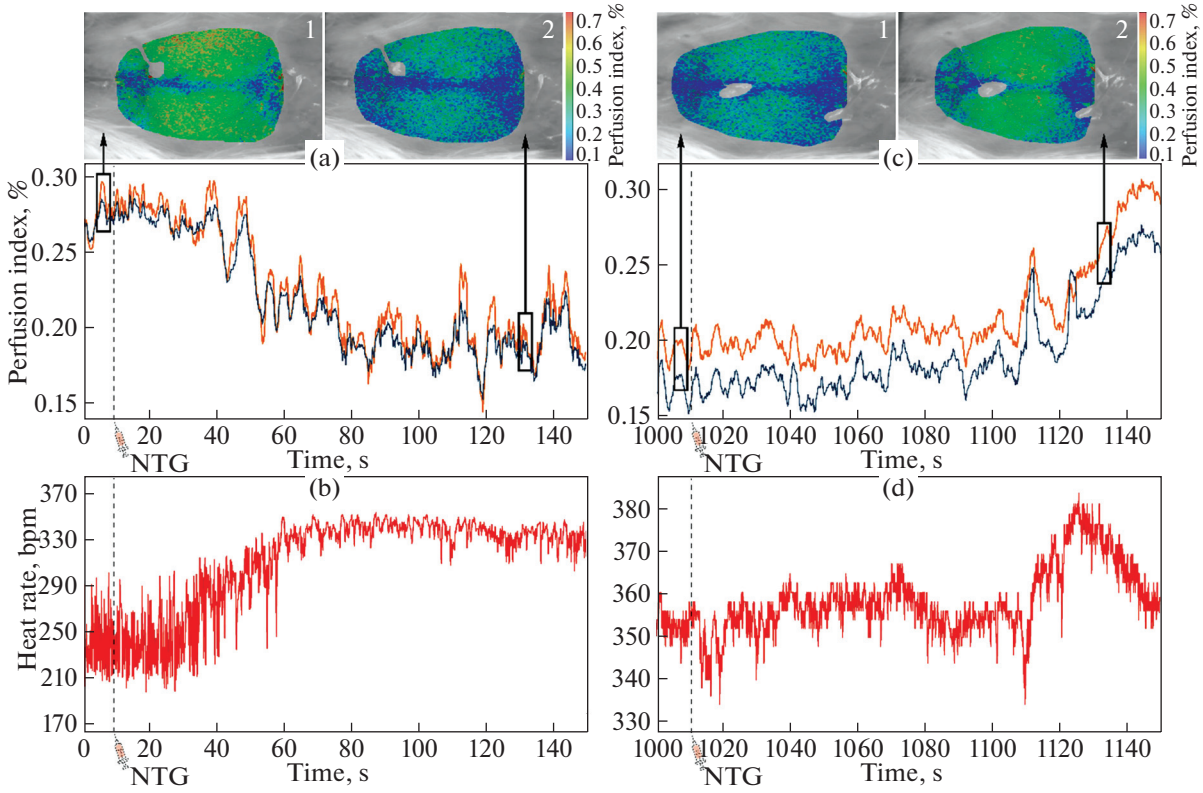

Fig. 2. Experimental graphs obtained by performing two sequential pharmacological tests with intraperitoneal injection of NTG. (a) Time dependence of PI (in orange for the right hemisphere of the brain, blue – for the left) and heart rate – (b), (1) and (2) – maps of the spatial distribution of PI before and after the first injection of NTG, respectively. (c) Time dependence of PI (in orange for the right hemisphere of the brain, blue – for the left) and heart rate – (d), (1) and (2) – maps of the spatial distribution of PI before and after the second NTG injection.

RESULTS AND CONCLUSIONS

For all three consecutive tests with NTG, experimental data were obtained that directly reflect the physiological processes of hemodynamics and the effect of the administered drug on the vascular blood flow of the mouse brain. Figure 2 shows the temporal change in PI and HR (a, c) and (b, d) and maps of the spatial distribution of PI at the beginning (1) and at the end (2) of two consecutive pharmacological tests with the NTG administration.

The drug was administered at 10 s from the start of video recording of each test. From Fig. 2a it can be seen that approximately 30 s after the injection of the vasodilator, there is a significant decrease in PI from a value of ~0.27% at the basal level to ~0.19% in the third minute of the experiment. Maps of the spatial distribution of PI (1) and (2) in pseudo-colors (orange – maximum and blue – minimum value) are presented for 5–9 and 130–134 s of the experiment, respectively. As can be seen, the change in blood flow is significant throughout the entire study area, which is apparently caused by a sharp drop in blood pressure in the vessels of the brain after the administration of NTG. The decrease in blood pressure is compensated by an increase in heart rate (Fig. 2b). Thus, at the basal level, the average heart rate was 239 bpm, and two minutes after the NTG injection, it increased to 325 bpm, which is an indirect sign of the effect of the vasodilator on the vascular system.

The second injection of NTG, as can be seen from the graph in Fig. 2c, led to an increase in PI from basal ~0.18 to ~0.28% by the end of the test. On the distribution maps (1) and (2) presented for 1005–1009 and 1130–1134 s, respectively, a tendency towards an increase in PI is clearly visible. The average heart rate remains high throughout the test – 356 bpm and changes slightly towards the end of the experiment (Fig. 2d). The subsequent, third injection of the vasodilator did not cause a change in the perfusion index, its level was ~0.23%, the average heart rate was 346 bpm.

The results presented in this work are in good agreement with the experimental data obtained in a recently published study of the effect of a vasodilator on cerebral blood flow in rats [9], where it was shown that the injection of NTG can lead to both an increase in the perfusion index and its decrease, which indicate the duality of the effect of this drug on meningeal vasomotor activity.

Thus, this work demonstrates for the first time a possibility of assessing changes in cortical blood flow through the intact cranial bone during pharmacological (potentially also physiological) tests, which makes it possible to evaluate the effect of the tests on cortical blood flow under more natural conditions.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The animal was kept under standard conditions with a natural light regime (12 h light/dark cycle) and free access to water and food. Before the experiment, the animal was kept on a fasting diet for at least 12 h, on the day of the study, drinking was limited. The experiments were carried out in accordance with the ethical principles of the International Association for the Study of Pain and Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes. The study protocol was approved by the decision of the local ethics committee (G.B. Elyakov Pacific Institute of Bioorganic Chemistry, Far Eastern Branch, Russian Academy of Sciences, Vladivostok, Protocol no. 02/23 dated February 27, 2023). Every effort was made to minimize the suffering of the animal.

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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