RESEARCH AND DEVELOPMENT CENTER BIOMEDICAL PHOTONICS

OREL STATE UNIVERSITY named after I.S. Turgenev

SECHENOV UNIVERSITY

OULU UNIVERSITY

SPIE. STUDENT CHAPTER OREL STATE UNIVERSITY

BRAIN METABOLISM CHANGES IN CASES OF IMPAIRED BREATHING OR BLOOD CIRCULATION IN RODENTS EVALUATED BY REAL TIME OPTICAL SPECTROSCOPY METHODS

Gennadii Piavchenko, Evgeniya Seryogina, Ksenia Kandurova, Valery Shupletsov, Igor Kozlov, Dmitry Stavtsev, Olga Stelmashchuk, Evgeny Zherebtsov, Viktor Dremin, Aleksander Alekseyev, Sergey Kuznetsov, Andrey Dunaev, Igor Meglinski

Outline

1. About Us
2. Introduction
3. Materials and Methods
4. Results and Discussion
5. Conclusion and Acknowledgements

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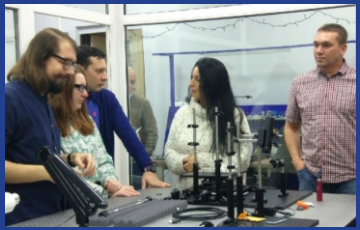
Where Are We From

R&D Center of Biomedical Photonics at Orel State University (Orel, Russia)

Department of Histology, Cytology and Embryology at Sechenov University (Moscow, Russia)

Optoelectronics and Measurement Techniques Laboratory at University of Oulu (Oulu, Finland)

Orel State University



Orel State University (founded in 1919) is a unique and dynamically developing education-science-production complex in the Central part of Russia which integrates qualitative education, perspective science and effective production. More 100 directions of specialist training, more than 20 000 students.

Research interests include the development of biomedical optical noninvasive methods and devices for diagnostics, such as Dynamic Light Scattering methods, Fluorescence, Diffuse Reflectance Spectroscopy and Speckle Contrast Imaging.

Sechenov University

SECHENOV UNIVERSITY
LIFE SCIENCES

Department of Histology, Cytology and Embryology was founded at 1864. It is one of the oldest Histology departments in Russia.

Research interests include the translational research based on structural assessment of cells, tissues and organs functioning.

I.M. Sechenov First Moscow State Medical University (Sechenov University) was founded in 1758 as medical faculty of Imperial Moscow University as the first medical school in Russia. The university is named after Russian physiologist, Ivan Sechenov. Nowadays it is leading medical school in Russia with more than 15000 medical students in it

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Introduction

Source – https://www.mitera.gr/en/department/adult-intensive-care-unit-icu/

Intensive Care Units

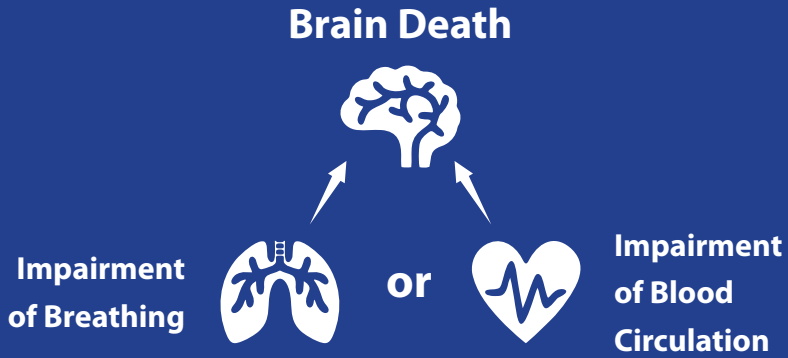
Prediction of Survival

↓

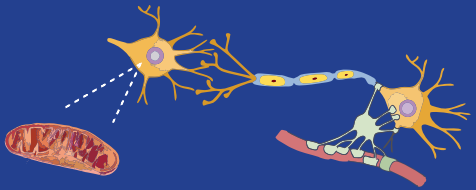
Severity of Disease

** D. Escudero, M. Valentín, J. Escalante, A. Sanmartín, M. Perez-Basterrechea, J. de Gea, … R. Matesanz. Intensive care practices in brain death diagnosis and organ donation. Anaesthesia (2015), 70(10), 1130–1139*

Introduction



Introduction



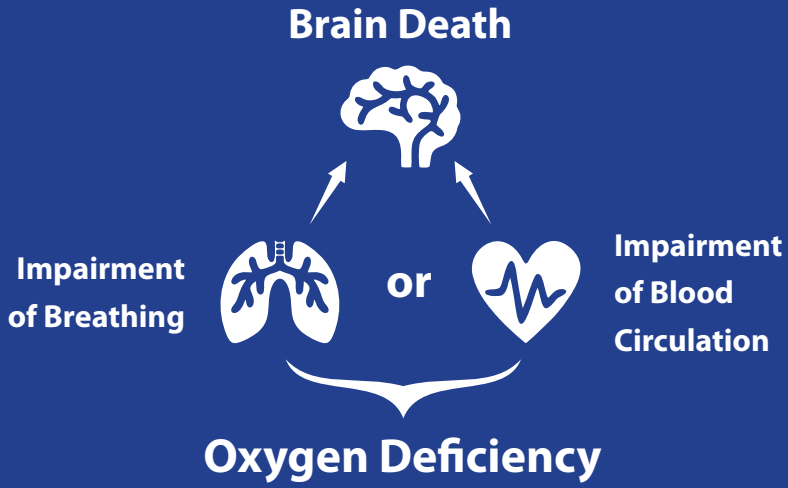
Impairment of Breathing

or

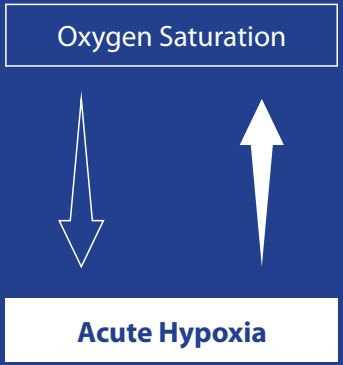
Impairment of Blood Circulation

Oxygen Deficiency

Introduction



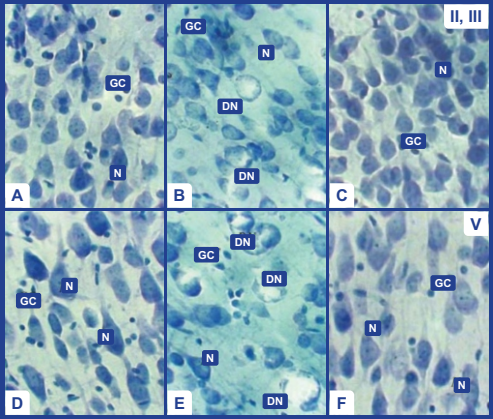
Introduction



** M. Cour, J. Turc, T. Madelaine, L. Argaud. Risk factors for progression toward brain death after out-of-hospital cardiac arrest. Ann. Intensive Care (2019), 9, 45*

Relevance

The development of an acute hypoxic state leads to neurodystrophic processes in the cerebral cortex, in particular, a decrease in the number of neurons



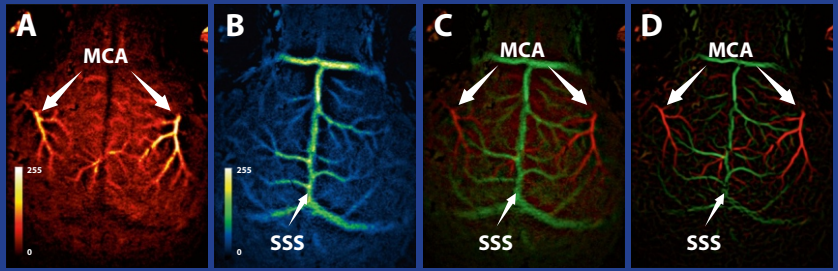
Nervous tissue of layers II and III (a,b,c) and V (d,e,f) of rat brain neocortex at the 20th day of postnatal ontogenesis. (a), (d) Control, (b), (e) after hypoxia at E14, (c),(f) – at E18. N – Neurons, DN – degenerating neurons, GC – glial cells. Nissl's staining. Scale 20 μm.

** Vasiliev D.S., Tumanova N.L., Zhuravina I.A. Structural Changes in the Neocortex Nervous Tissue in Rat Ontogenesis after Hypoxia at Various Terms of Embryogenesis. Journal of Evolutionary Biochemistry and Physiology, (2008), 3 (44), 304—315.*

Relevance

Results of G. Armitage et al.* show that circulatory disorders during ischemia of CC vessels leads to an increase in speckle contrasts after subsequent reperfusion.

Same results were mentioned by V. Kalchenko et al. The authors noted a decrease in blood flow in ischemic areas.**

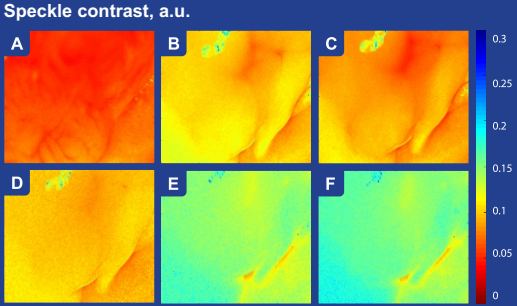


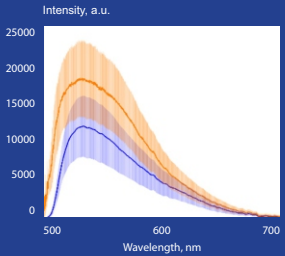
** G. Armitage, K. Todd, A. Shuaib and I. Winship. Laser speckle contrast imaging of collateral bloodflow during acute ischemic stroke. Journal of Cerebral Blood Flow & Metabolism (2010) 30,1432–1436*

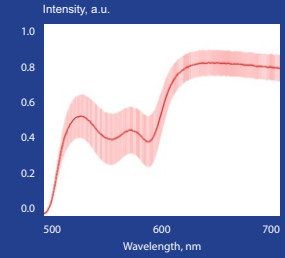
*** V. Kalchenko et al.: A simple approach for non-invasive transcranial optical vascular imaging (nTOVI). J. Biophotonics (2015), 8, No. 11–12, 897–901*

Real-Time Optical Diagnostics

- Fluorescence spectroscopy
- Diffuse reflectance spectroscopy
- Laser speckle-contrast imaging

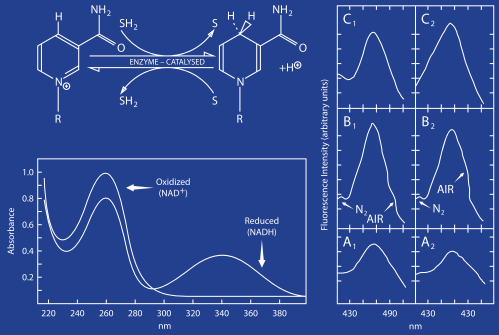






Fluorescence spectroscopy

Fluorescence spectroscopy (FS) changes in the concentration of coenzymes involved in the regulation of brain cell metabolism.



Mitochondria In-Vitro*				Brain In-Vivo**
Respiration Rate	Limiting Substance	NADH %	State #	Metabolic State
0	Oxygen	~100	5	Max. — Anoxia
Slow	ADP	99	4	Hypoxia, Ischemia; Ischenic SD
Fast	Resp. Chain	53	3	Anaesthesia; Awake; HBO, Uncoupler; Siezures
Slow	Substrate	~0	2	Min. — Normoxic SD

NADH

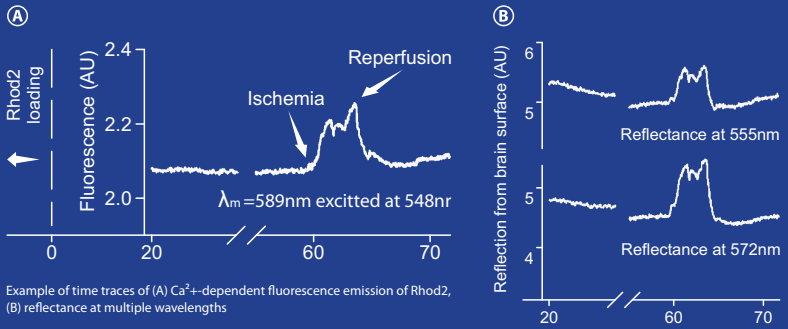
* According to Chance & Williams 1955
** According to Mayevsky 1984

HBO - Hyperbaric Oxygenation
SD - Spreading Depression

** A. Mayevsky and B. Chance. Oxidation-reduction states of NADH in vivo: From animals to clinical use. Mitocondrion (2007), 7, 330-339*

Diffuse reflectance spectroscopy

Diffuse reflectance spectroscopy (DRS) oxidative processes status in the tissue

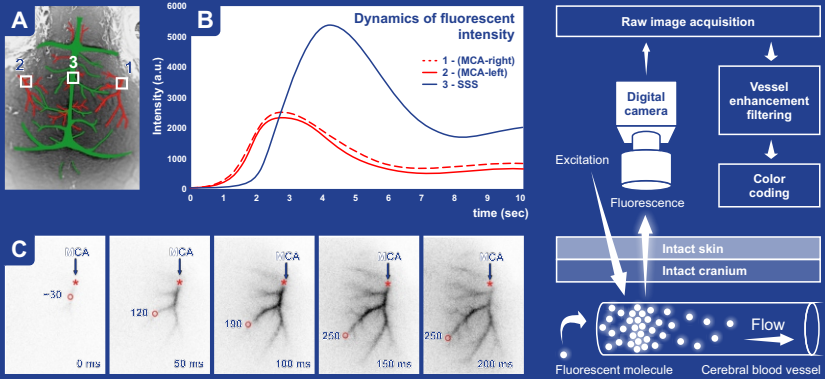


Example of time traces of (A) Ca^2+-dependent fluorescence emission of Rhod2, (B) reflectance at multiple wavelengths

** Du, C., Koretsky, A. P., Izrailtyan, I., & Benveniste, H. Simultaneous detection of blood volume, oxygenation, and intracellular calcium changes during cerebral ischemia and reperfusion in vivo using diffuse reflectance and fluorescence. Journal of Cerebral Blood Flow & Metabolism (2005), 25(8), 1078-1092*

Laser speckle-contrast imaging

Laser speckle-contrast imaging (LSCI) dynamics of changes in blood flow in large and small vessels



*V. Kalchenko, D. Israeli, Yu. Kuznetsov, I. Meglinski, A. Harmelin. Non-invasive transcranial optical vascular imaging (nTOVI). J. Biophotonics (2015), 11-12 (8), 897–901

Aim

to compare metabolic activity and structural changes in the cerebral cortex after acute hypoxia caused by impairment of respiration or blood circulation, so that will show the approach to individualize the post-rehabilitation management of the patients with acute respiratory and circulatory disorders.

Goals

- Modulate acute statement of impairment for respiratory and cardiovascular (CVS) systems.
- Estimate metabolic activity of cerebral cortex tissue (NADH and FAD coenzymes).
- Evaluate oxygen saturation in brain cerebral cortex (O2 to Co2).
- Determine blood flow activity changes during respiratory and CVS disorders.
- Assess the structural post-mortal changes in cortical morphology.

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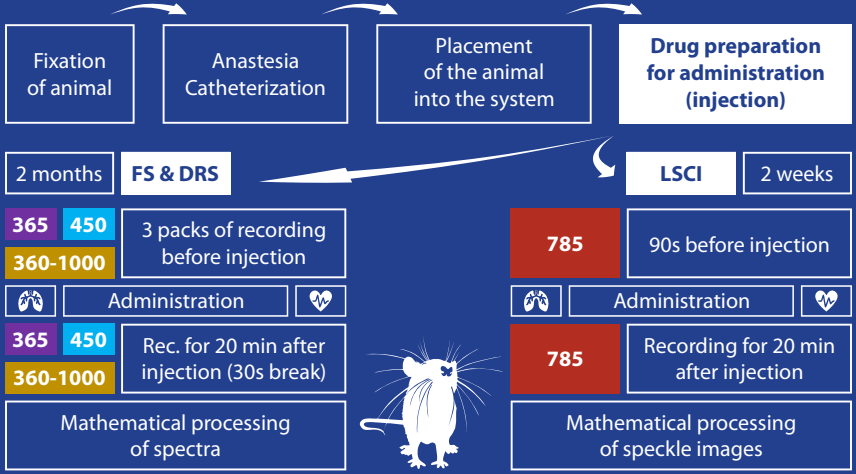
Design of Research



Environment

- **Controlled environmental conditions**

temperature 20–22 °C, humidity of 50–60%, 12-hour lighting cycle 10-times exchange of the room air volume per hour

- **Feeding**

standard balanced granulated nutrition for rodents in accordance with the daily physiological needs. Distilled deionized water ad libitum.

- **Health status evaluation**

veterinarian carried out daily inspection during a two-week quarantine period.

- **Randomization**

random selection using body mass as a leading parameter, mass values did not deviate from the average by more than 10%.

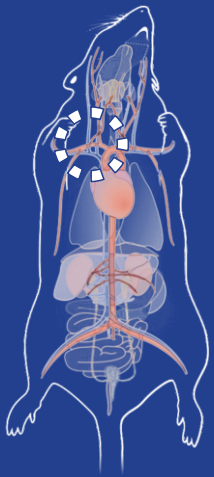
Methods

Anastesia
Zoletil 100 (Vibrac, France), 0.3 ml per 250g animal

Catheterization

jugular veins and arteries
femoral veins and arteries
aorta
carotid artery
etc

Right external jugular vein
PM-60 polymer catheter
(SciCat, Russia, d_{ext}=0.8 mm, d_{int}=0.4 mm)



Catheterization

- Catheter
- Instruments for animals surgery
- Heating table
- LED lamp
- Heparin solution

- Surgical field preparation
- Layered cut
- Mastering ligatures
- Microcision of a vessel
- Catheter insertion
- Ligature dressing
- Catheter insertion
- Withering
- Layer Sewing

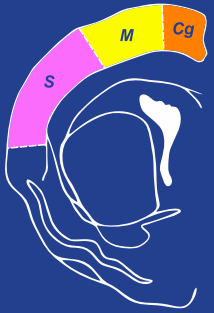
Methods

Operational access to the skull bone dissecting of soft tissues (2 weeks old rats for LSCI method).

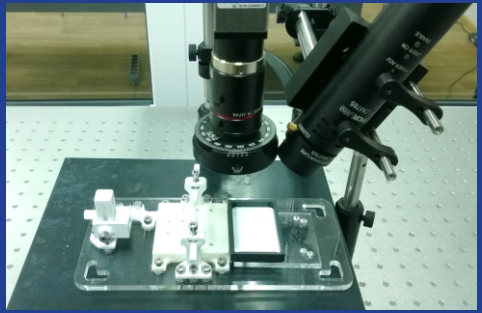
Operational access to the brain cortex drilling a window into the skull (2 months old rats for fluorescence and diffuse reflectance spectroscopy methods).

Methods

Identification of cortical structures
G. Paxinos, C. Watson.
Rat Brain Stereotaxic Atlas. Academic Press (2007).



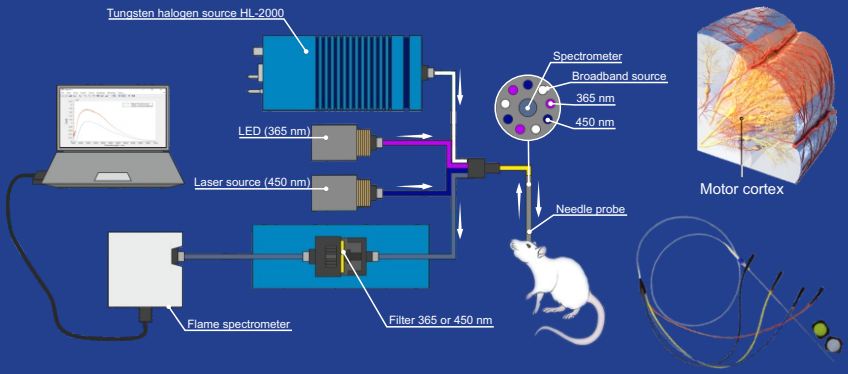
Regions of interest



House-built System with Stereotactic Apparatus

** G. P'yavchenko, L. Shmarkova, V. Nozdrin. Changes in the Number of Neurons in the Rat Motor Cortex and Movement Activity with Age. Neurosci. Behav. Physiol. (2016), 46(3), 270–273*

Fluorescence and diffuse reflectance spectroscopy

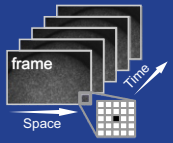


Output power
for 365 nm ex. ≤ 1.5 mW,
for 450 nm ex. ≤ 3.5 mW.
for broadband source ≤ 6.7 mW

Excitation light flux
for 365 nm ex. ≤ 0.16 W·m^{-2}
for 450 nm ex. ≤ 0.37 W·m^{-2}

Fiber numerical aperture
NA = 0.22

Laser speckle contrast imaging



The standard spatial algorithm has been used for speckle contrast images obtaining. Average speckle contrast formula:

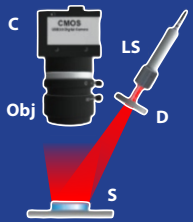
$$K = \frac{\sigma_n}{\langle I \rangle_n}$$

$\langle\ \rangle$ — the symbol of averaging

n — quantity of pixels in stack

$\langle I \rangle_n$ — average intensity in a stack of pixels

σ_n — standard deviation of intensity in a stack of pixels



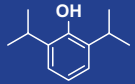
**LS – laser source, D – diffusor,
S – sample, Obj – objective,
C – camera.**

** A. Sdobnov, A. Bykov, A. Popov, E. Zherebtsov, I. Meglinski. Investigation of speckle pattern dynamics by laser speckle contrast imaging. Proc. SPIE (2018), 10685 (9)*

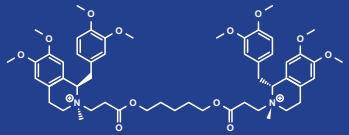
Impairment models

Breathing impairment model

Propofol 0.3 ml



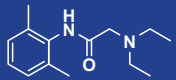
Non-depolarizing muscle relaxant (cisatracurium) in a lethal dose



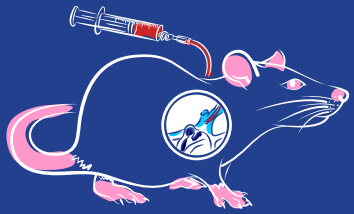
Immediately causes a spasm of the respiratory muscles and respiratory arrest

Blood circulation impairment model

Lidocaine solution (2%) in a dose of 1 ml



Immediately causes a cardiac arrest



Results Processing

- **Speckle contrast parameters**
Matlab® software using a temporal algorithm for averaging over substacks.

- **Fluorescence and diffuse reflectance spectra**
a special algorithm in the Matlab® software environment.

- **Processing of the results**
Origin® Pro.

Methods

Histological analysis
Fixation in buffered formalin.
Brain tissue sections 5 μm thick, hematoxylin and eosin staining.
Identification of signs of cortical hypoxia.

Ethical conclusion
Animal manipulations, as well as experimental conditions, were reviewed and approved by the ethics committee of the Orel State University.

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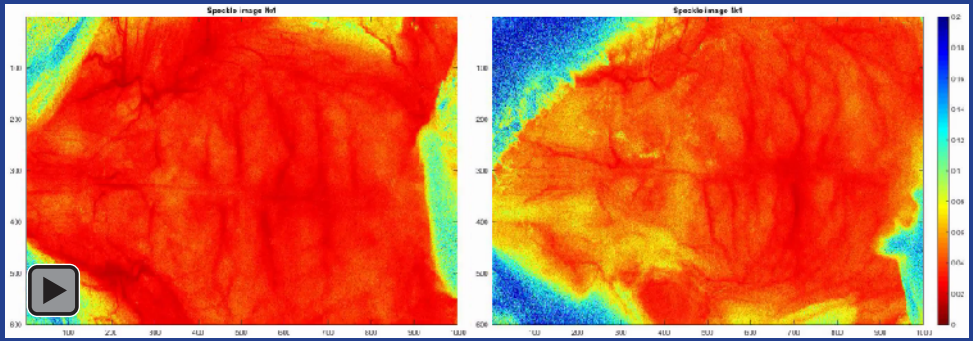
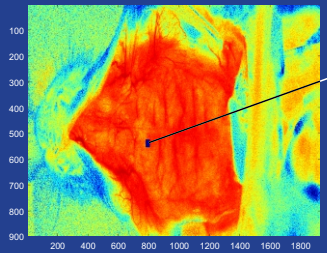
Results – Speckle contrast imaging



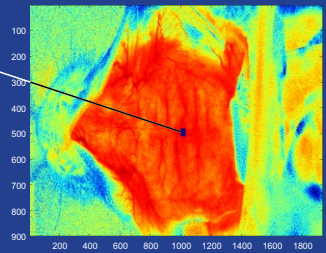
FPS = 40
Exposure time = 20 ms

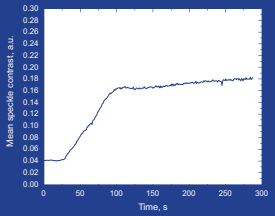
Results – Breath Impairment Case

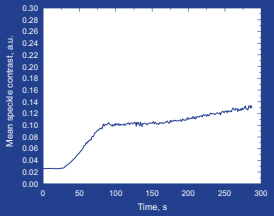


Area of interest

N=40
quantity of pixels in stack



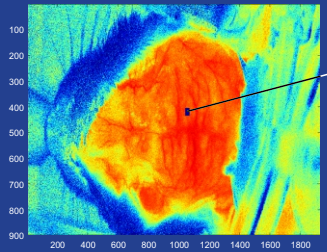




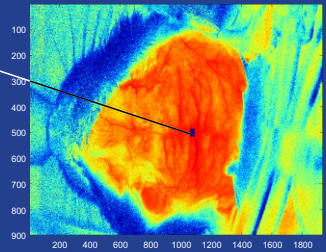
Microcirculation vessel

Venous sinus

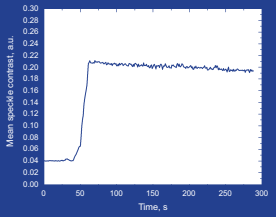
Results – Heart Impairment Case



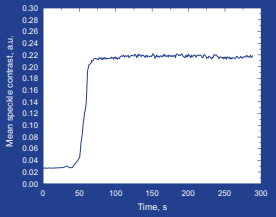
Area of interest



N=40
quantity of pixels in stack

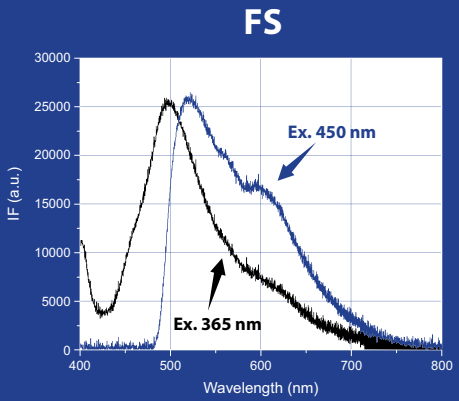


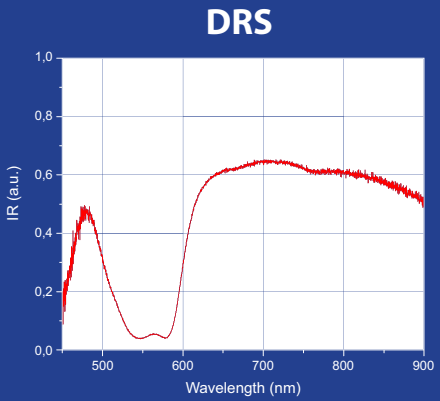
Microcirculation vessel



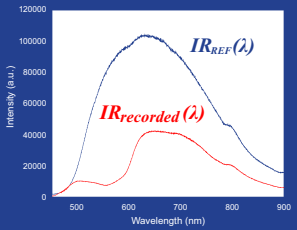
Venous sinus

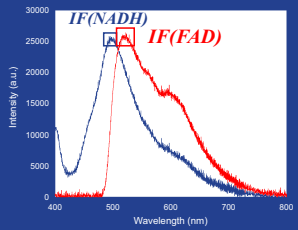
Results – Intact Area Spectra Examples

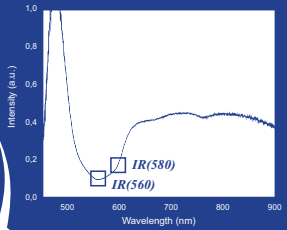




Results – Further Processing







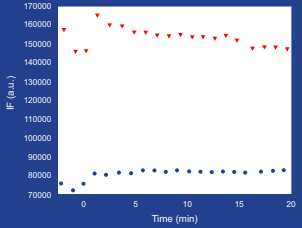
Diffuse reflectance normalization

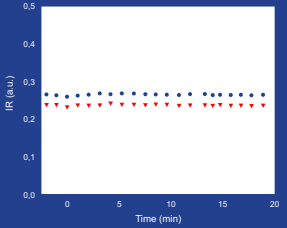
$$IR(\lambda) = \frac{IR_{recorded}(\lambda)}{IR_{REF}(\lambda)}$$

Fluorescence maximum normalization

$$IF_N = \frac{IF_N(NADH)}{IR_N(560)} \quad \text{or}$$

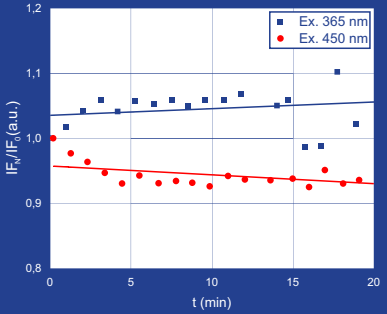
$$IF_N = \frac{IF_N(FAD)}{IR_N(560)}$$



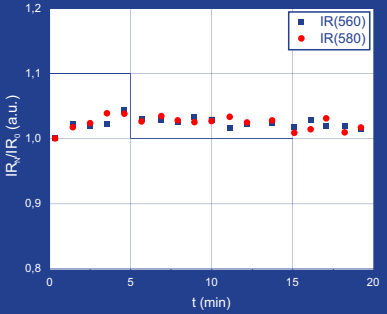


Results – Breath Impairment Case 1

FS



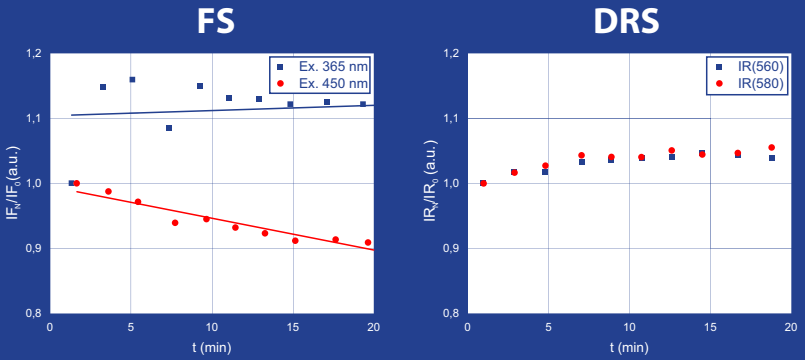
DRS



Linear Fit

Normalization by IF and IR values at appr. 0 s associated with the beginning of the impair

Results – Breath Impairment Case 2

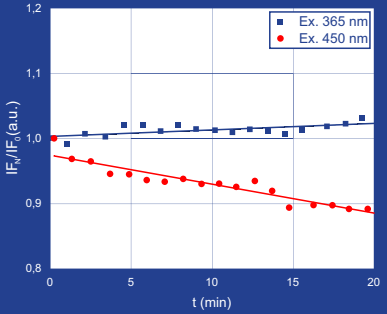


Linear Fit

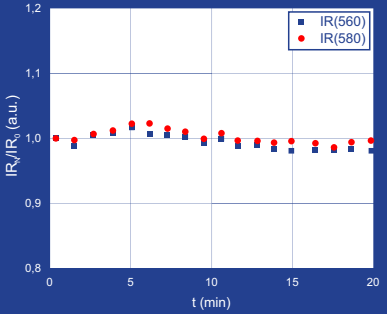
Normalization by IF and IR values at appr. 0 s associated with the beginning of the impair

Results – Heart Impair Case 1

FS



DRS

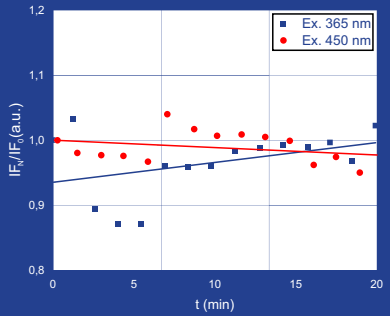


Linear Fit

Normalization by IF and IR values at appr. 0 s associated with the beginning of the impair

Results – Heart Impair Case 2

FS



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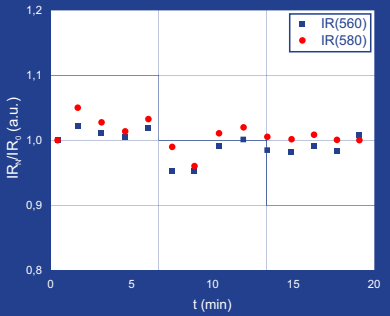
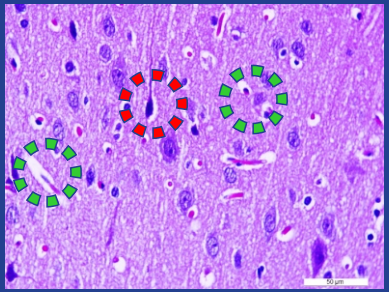


Linear Fit

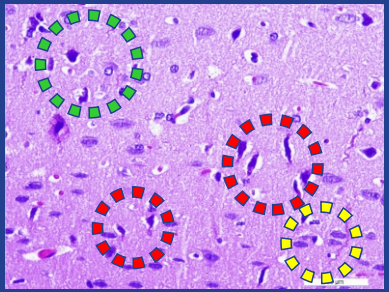
Normalization by IF and IR values at appr. 0 s associated with the beginning of the impair

Results – Morphology changes



Breathing impairment

Motor cortex sections. H&E stain. Signs of numeric hypoxic changes of the neuronal morphology (red) and local perivascular edema (green)



Blood circulation impairment

Motor cortex sections. H&E stain. Huge amount of hyperchromic wrinkled neurons (red), pericellular edema (green), glial nodules & necrobiotic neurons (yellow)

Summary

In acute respiratory failure there is a gradual increase in hypoxic disorders, and a slowdown of hemodynamic processes. All these processes are noted in the first 20 minutes from the start of recording.

In acute circulatory disorders, there is a sharp cessation of the oxygenated blood supply to the brain cortex structures.

Outline

1. About Us
2. Introduction
3. Materials and Methods
4. Results and Discussion
5. Conclusion and Acknowledgements

Conclusion

- **The blood circulation in the vessels of the cerebral cortex stops in case of respiratory arrest after ~ 2 minutes, and when heart arrest occurs after ~ 1 minute.**

- **Changes in the brain tissue oxygen saturation under a heart arrest happen in the first 5 minutes, while an acute breathing impair practically do not change it.**

- **Heart failure is characterized by more acute hypoxic disorders in the cerebral cortex in comparison with processes that occur in the cortex when breathing is impaired.**

- **Acute cardiovascular failure is a more life-threatening process that causes a rapid increase in neuronal metabolic deficit in compare with acute respiratory diseases.**

- **This may be used to personalize the management of patients after acute circulatory disorders and respiratory arrest in the prognosis and rehabilitation*.**

** S. Razvi, I. Bone. Neurological Consultations in the Medical Intensive Care Unit. J Neurol Neurosurg Psychiatry (2003), 74, 16–23*

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Acknowledgements

Press release

Piavchenko awarded John Kiel Scholarship

BELLINGHAM, Washington, USA - May 24, 2019 - Gennadii Piavchenko has been awarded the 2019 John Kiel Scholarship by SPIE, the international society for optics and photonics, for his potential contributions to the field of optics, photonics or related field.

Piavchenko is a PhD student in the Histology, Cytology and Embryology Department at the Orel State University (Russian Federation) where he is supervised by Prof. Vladimir Nozdrin (Orel State Univ.) and Prof. Sergey Kuznetsov (Sechenov Univ.). His research is based on an interdisciplinary approach combining behavioral neuroscience and applied pharmacology with novel trends in biophotonics, supervised by Dr. Andrey Dunaev. He has authored/co-authored 7 peer-review articles and holds one patent for a nervous tissue staining method.

Our team

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O. Stelmashchuk

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I. Kozlov

E. Zherebtsov

V. Dremin

A. Alekseev

RESEARCH AND DEVELOPMENT CENTER BIOMEDICAL PHOTONICS

OREL STATE UNIVERSITY named after I.S. Turgenev

SECHENOV UNIVERSITY

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Thank you!

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