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Oscillation processes in synuclein-KO mouse skin microcirculation: a pilot study

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ABSTRACT

The article is devoted to the analysis of skin perfusion oscillation processes with wild-type and synuclein-deficient mice with three knockout (KO) types: αγ-KO, β-KO, and αβγ-KO. The role of synucleins in tissues that exclude the central nervous system remains sparsely studied. Laser Doppler flowmetry was used to investigate the potential involvement of synucleins in murine microcirculation functioning This method is widely used to analyze microcirculatory disturbances in a variety of pathologies and has proven to be sensitive to the manifestation of abnormalities of normal function. The study revealed that deficiency of β-synuclein affects oscillation processes more than αγ-synuclein deficiency.

Keywords: laser doppler flowmetry, microcirculation, synucleins.

1. INTRODUCTION

Synucleins have several functions in the dopaminergic system and are expressed mostly in the brain and spinal cord. Additionally, the expression of synucleins has also been found in red blood cells, endothelium, muscle tissue, etc. While the role of synucleins in the central nervous system has been relatively studied[1,2], especially in the context of synucleinopathies[3], the role in other tissues remains undiscovered.

Regarding the transmission of nerve impulses, the role of synucleins in synaptic function, including vesicular trafficking and neurotransmission, and in neuron viability is discussed[4,5]. From a cardiovascular viewpoint, the role of synucleins in aortic contractile in mice was revealed comparing α-synuclein deficient mice and mice with overexpression of α-synuclein[6]. In another publication, the role of αγ-synucleins on pain sensitivity and myelination of peripheral neurons localized in the skin were investigated[7]. It was shown that αγ-synuclein deficiency does not lead to myelination disorders, there are no behavioral distortions and no disturbances of pain reception. There is also no increased expression of β-synuclein, which, hypothetically, could duplicate and replace the functions of the missing proteins. Partially, this result is confirmed in the publication[8] that the functioning of skin sensory fibers is not impaired during synucleinopathies.

It is crucial to appreciate the role of synucleins in peripheral nerve fibers. From the peripheral nervous system viewpoint, nociceptive C-fibers and sensory-peptidergic fibers are involved in the formation of oscillatory processes in microvessels[9,10]. To summarize the published works, there is a possible indirect effect of synucleins on microcirculation, but, so far, there have been no studies to the best of our knowledge on how they are represented in perfusion rates and microcirculatory oscillation processes. Therefore, this study proposed identifying the knockout type that most affects the microcirculation based on the laser Doppler flowmetry (LDF). LDF is a well-known method widely used for non-invasive blood perfusion monitoring in various cases such as type 2 diabetes mellitus[11], rheumatic diseases[12], etc. A unique feature of this method is the analysis of blood flow oscillations associated with the following types of rhythms: endothelial, neurogenic, myogenic, respiratory and cardiac[13]. Previously, LDF was implemented to diagnose noradrenergic cooling responses of tissue blood microcirculation during Parkinson's disease[14]. Thus, the involvement of this method in the analysis of potential alterations in the microcirculation due to synuclein deficiency is relevant.

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2. MATERIALS AND METHODS

We involved 11 mice in our study: 3 with wild-type (WT), 3 with knockout by αγ-synucleins (αγ-KO), 3 with triple knockout (αβγ-KO) and 2 with knockout by β-synuclein (β-KO). Transgenic animals were provided and supported by Bioresource Collection of IPAC RAS and Centre for Collective Use IPAC RAS facilities and equipment was used to maintain animals in the framework of the State Assignment of IPAC RAS (No. 0090-2019-0005). The experiment protocol was approved by the institutional Ethics Committee of the Institute of Cell Biophysics RAS. All experiments were carried out according to international regulations listed in the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (ETS 123) and ICB RAS Manual for Working with Laboratory Animals №57 (30.12.2011), ethical protocol №2019/5. For data analysis, we grouped our sample by two principles with overlapping occurrences: by the presence of β-synuclein and by the presence of α- and γ-synucleins. Measurements were performed with preliminary sedation with combined anesthesia by Zoletil® ("Virbac Sante Animale", France) injection and N_2O:O_2 (77%:23%) mixture inhalation to maintain the effect. The temperature in the experimental room was stabilized at 25 °C. For all experiments, ECG, body temperature and breathing rate were monitored with custom-developed hardware and software. Each mouse had at least 10 days before the experiment for adaptation to laboratory conditions, water, and food. Mice received drink and food *accesso libero*.

Skin perfusion of the hind limbs of mice were recorded using a developed in-house LDF device (with a central wavelength of laser emission 1064 nm, power 4 mW) with custom signal processing[15] (Fig. 1).

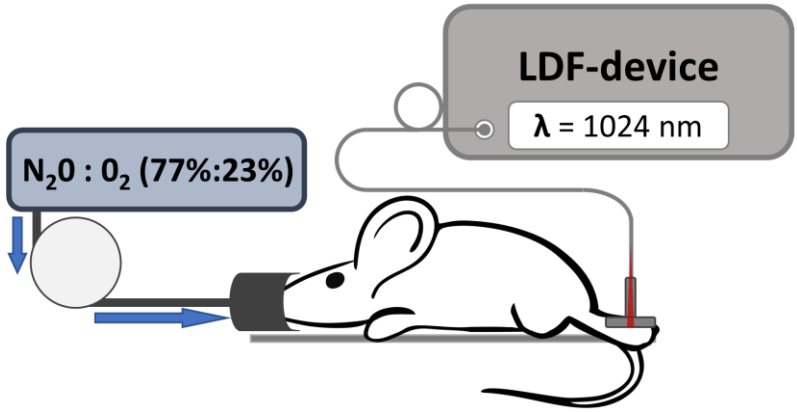


Figure 1. Experimental scheme of perfusion measurement

Blood perfusion was calculated according to the following expression:

$$PU = \int_{f_1}^{f_2} f \cdot P(f) df \ , \tag{1}$$

where, *PU* – blood perfusion measured in perfusion unit (p.u.); *f* – Doppler shift frequency; *P(ω)* – Power spectrum calculation; f_1, f_2 – frequency range of integration procedure (from 0 to 12800 Hz, respectively, in this study). Spectral analysis was performed based on wavelet transform with complex-valued Morlet wavelet and averaging of real part in the time domain. To avoid the influence of motion artifacts for proper signal analysis, subsamples with no artefacts lasting about 10 minutes for each occurrence were selected from the 15-minute experimental recordings. Perfusion values were recorded with a sample rate of 0.05 c. As described in the previous studies[16] for experiments on mice, the average amplitudes of blood flow oscillation were calculated for the following frequency ranges: 0.01-0.02 Hz for endothelial oscillations, 0.02-0.08 Hz for neurogenic, and 0.08-0.2 Hz for myogenic oscillations. These parameters were used for creating classifiers by described grouping approaches.

3. RESULTS AND DISCUSSION

A classifier based on linear discriminant analysis (LDA) was used to determine the influence of the β -synuclein or αγ-synuclein factor on active oscillation processes of microcirculation. As an assumption, it was considered that the

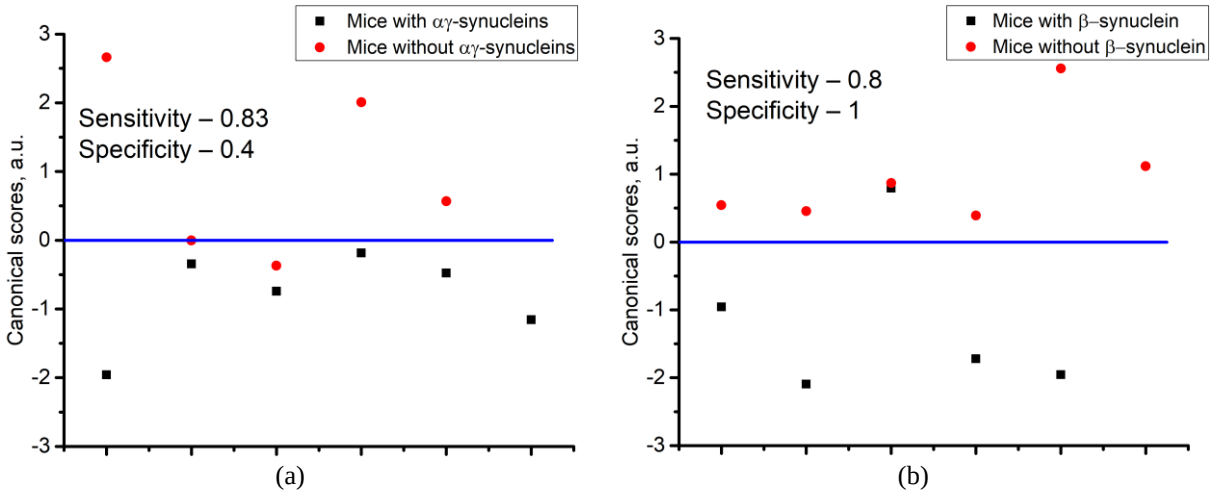
synucleins do not interact or replace each other's functions, and act on blood perfusion oscillations independently. Sensitivity and specificity were chosen as a metric to describe the presence of synucleins deficiency in microcirculation. Statistical analysis of the main parameters by a group is shown in Table 1. No statistical differences by the Mann-Whitney (p<0.05) test were found between chosen parameters.

Table 1. Analysis of average perfusion parameters and oscillation amplitudes by grouping according to the presence of β-synuclein and αγ-synucleins.

Grouping by expression of αγ-synucleins		
	Without αγ-synucleins	With αγ-synucleins
Average perfusion, p.u.	743.2 (552.4 ÷ 1237.6)	536.8 (482 ÷ 748.5)
Average endothelial amplitude a.u.	95.9 (48.5 ÷ 180.3)	143.1 (124.4 ÷ 445.3)
Average neurogenic amplitude, a.u.	171.7 (107.1 ÷ 422)	436.1 (339.6 ÷ 594.6)
Average myogenic amplitude, a.u.	155.7 (87.2 ÷ 278.1)	489 (230.9 ÷ 553.1)
Grouping by expression of β-synuclein		
	Without β-synuclein	With β-synuclein
Average perfusion, p.u.	867.5 (607.4 ÷ 1237.6)	536.8 (425.9 ÷ 688.8)
Average endothelial amplitude, a.u.	330.2 (84.8 ÷ 462.2)	111.9 (77 ÷ 131.8)
Average neurogenic amplitude, a.u.	436.1 (192.3 ÷ 781.2)	219 (145.1 ÷ 433.4)
Average myogenic amplitude, a.u.	241.9 (123.7 ÷ 582.6)	187.9 (138.1 ÷ 510.4)

Data are presented as medians as well as 25% and 75% in brackets.

Afterwards, we analyzed the ability of the linear classifier with cross-validation by the leave-one-out method to separate groups according to the described parameters of blood flow oscillations. Canonical scores for classifiers are shown in Figure 2 with cross-validated sensitivity and specificity.



(a) (b)

Figure 2. Canonical scores for LDA-classifiers for two grouping methods and cross-validated by leave-one-out method sensitivity/specificity values. (KO-groups are positive)

As a result, we obtained different sensitivity and specificity in the analysis of synuclein deficiency factors. If the effect of αγ-synuclein deficiency in the skin tissue is not clearly observed regarding the state of peripheral neurons according to previously published studies, then the microcirculation in general may also be unaffected. To the best of our knowledge, there is no evidence revealing a role for β-synucleins in microvascular oscillations. However, despite the incomplete statistics, the results of grouping by the presence of β-synucleins show better accuracy characteristics of a linear classifier. On this basis, we can assume that β-synuclein knockout affects microcirculatory function more prominently than αγ-synuclein knockout.

4. CONCLUSION

This study demonstrates that the β-synuclein expression factor may have a more significant effect on microcirculation than the deficiency of αγ-synucleins if the accuracy characteristics of a linear classifier were used as the metric. The

results obtained require reproduction on large samples. However, despite this, amid the lack of studies on the role of synucleins (and β-synuclein, in particular) this work is an initialized step in analyzing the role of synucleins in microcirculatory function. Furthermore, the involvement of functional tests of the microcirculation state (drug, heat, electric stimulation, and others) is suggested for a more detailed analysis of the synuclein effect on the microcirculation responses caused by specific systems, such as the endothelium or nerve fibers.

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