

# Alpha- and Gamma-Synucleins Regulate Energy Metabolism and Xanthine Oxidase Activity in Brain Cells

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**Abstract**—The process of signal transmission and transformation in the central nervous system requires active energy metabolism with high consumption of glucose and oxygen. Reactive oxygen species (ROS) produced as a result of these processes participate in intracellular signaling, but their overproduction leads to oxidative stress. Oxidative stress and  $\alpha$ -synuclein aggregation are recognized as activators of neuronal death in Parkinson's disease. However, much less is known about the physiological role of monomeric synucleins. Using acute brain slices and primary co-cultures of cortical neurons and glial cells derived from transgenic animals with knockout of  $\alpha$ -,  $\beta$ -, and  $\gamma$ -synuclein genes, we investigated the role of these proteins in ROS production and energy metabolism. We found that absence of synucleins leads to the reduced ROS production compared to the wild-type cells. The xanthine oxidase (XO) inhibitor led to the decrease in ROS production in the wild-type cells and the brain slices with  $\beta$ -synuclein knockout, whereas in the slices lacking  $\alpha$ - or  $\gamma$ -synuclein, the XO inhibition was not observed, suggesting possible regulation of this enzyme by these proteins. Knockout of  $\alpha$ - and  $\gamma$ -synucleins resulted in the decrease in mitochondrial membrane potential and reduction in energy capacity (in the form of ATP), which could be one of the mechanisms of XO regulation by synucleins.

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## INTRODUCTION

Brain is characterized by the disproportionately high oxygen and glucose consumption relative to its mass [1]. Their importance is determined by the need to provide energy for the processes occurring in the brain, particularly signal transmission and maintenance of ionic homeostasis [2]. It is also important to consider the role of oxygen as the final electron acceptor in the mitochondrial electron transport chain

(ETC) and as a participant in numerous transformations that produce reactive oxygen species (ROS) – key regulators of intra- and extracellular processes, whose excessive production against the background of limited antioxidant systems causes oxidative stress [3, 4].

One of the possible sources of ROS hyperproduction in the brain cells is xanthine oxidase (XO), which is formed from xanthine dehydrogenase under the action of proteases and converts xanthine through hypoxanthine into uric acid [5]. This enzyme plays a key role in ROS hyperproduction during the second stage of hypoxia, acting after ETC as a source

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of superoxide anion and hydrogen peroxide formation [6, 7]. Activation of XO is directly linked to the changes in bioenergetics, where ATP consumption is coupled with conversion of adenine nucleotides into xanthine and hypoxanthine [8, 9]. It should be noted that studies have shown the role of XO in the development of several neurodegenerative diseases, including Parkinson's disease (PD) [10].

Another component of many brain pathologies is proteinopathies, which involve changes in the functions and/or oligomerization of certain proteins [11]. Among these is  $\alpha$ -synuclein, whose physiological functions and role in PD have been studied in considerable detail [12, 13] compared to other proteins in the synuclein family. Mutations in the  $\alpha$ -synuclein gene are associated with familial forms of PD [14, 15], although this does not exclude the role of the wild-type (WT) protein in the development of the pathology. High level of similarity in amino acid sequences unites  $\alpha$ -synuclein with two other proteins –  $\beta$ - and  $\gamma$ -synucleins, whose significance for normal brain function and its pathologies has been studied to a much lesser extent [16-18].

Models with knockout of the genes encoding these proteins are powerful tools for studying physiological functions of synucleins [17, 19-22]. Using animal models with deficiencies in different types of synucleins has revealed the role of these proteins in regulating redox balance through XO activity: absence of synucleins reduces ROS production, with knockouts of  $\alpha$ - and  $\gamma$ -synucleins leading to the decrease in XO efficiency, reduced mitochondrial membrane potential ( $\Delta\psi_m$ ) and energy capacity of the brain cells. Addition of monomeric exogenous  $\alpha$ -synuclein restored these indicators to the values characteristic of the wild-type cells.

## MATERIALS AND METHODS

**Study object.** In this study, sexually mature mice on the genetic background of the C57BL/6J line with inactivated genes encoding  $\alpha$ -,  $\beta$ -, and  $\gamma$ -synucleins in various combinations were used. Animals were provided by Bioresource Collection of CPT and Centre for Collective Use IPAC RAS (program FFSG-2024-0020). Experimental groups included animals with single knockouts of each of the specified genes, as well as with double knockout of  $\alpha$ - and  $\gamma$ -synuclein genes. The control group consisted of WT C57BL/6J mice without genetic modifications. Genotypes of the animals were confirmed by polymerase chain reaction (PCR) as previously described [23].

**Preparation of primary co-cultures of neurons and glial cells.** Primary co-cultures of neurons and glial cells from the cerebral cortex were obtained

from newborn mice aged P0-P2. The brain was extracted under sterile conditions and placed in a cold phosphate-buffered saline (PanEco, Russia) containing  $\text{Na}_2\text{EDTA}$ . After removal of cerebellum and hippocampus, the cortical tissue was gently minced and incubated in a 0.05% trypsin solution (PanEco) for 15 min at 37°C. Enzymatic action was stopped by triple washing with a Neurobasal medium (PanEco), after which the cells were mechanically dissociated by sequentially passing the suspension through pipettes with decreasing diameters. The cells were then pelleted by centrifugation at 200g for 5 min, and the pellet was resuspended in a fresh Neurobasal medium supplemented with 2% NeuroMax (PanEco), L-alanyl-glutamine (2 mM; Gibco, USA), penicillin (100 units/mL), and streptomycin (100  $\mu\text{g}/\text{mL}$ ) (PanEco). Cells were seeded onto coverslips pre-treated with polyethylenimine (0.05%) at a density of  $\sim 1 \times 10^5$  cells/cm<sup>2</sup>. Cultures were incubated at 37°C in a 5% CO<sub>2</sub> atmosphere. The medium was partially replaced every 3-4 days. Mature cultures on days 10-14 of incubation were used in experiments.

**Assessment of cellular energy capacity using Mag-Fura-2.** To assess changes in intracellular Mg<sup>2+</sup> ion concentration, which correlates with ATP levels, as well as Ca<sup>2+</sup> ions, the membrane-permeable fluorescent indicator Mag-Fura-2 AM (Invitrogen by Thermo Fisher Scientific, USA) was used. Cells were loaded with 5  $\mu\text{M}$  Mag-Fura-2 AM in a Hank's Balanced Salt Solution (HBSS) [composition (mM): 138 NaCl; 1.3 CaCl<sub>2</sub>; 0.4 MgSO<sub>4</sub>; 0.5 MgCl<sub>2</sub>; 5.3 KCl; 0.45 KH<sub>2</sub>PO<sub>4</sub>; 4 NaHCO<sub>3</sub>; 0.3 Na<sub>2</sub>HPO<sub>4</sub>; 10 D-glucose; 20 HEPES (pH = 7.4)] containing 0.04% Pluronic F-127 for 30 min at 37°C. After incubation, the cells were washed twice with HBSS and left for 15 min for complete de-esterification of the dye.

For analysis, a wide-field fluorescence microscope based on the Olympus IX73P1F (Olympus Corporation, Japan) and a fluorescence excitation and detection system Cairn (Cairn Research Ltd., UK) with a 40 $\times$  fluorite immersion objective were used, employing two excitation wavelengths of a xenon arc lamp (340 nm and 380 nm) and detecting emission at 510  $\pm$  30 nm. The fluorescence ratio at 340/380 nm excitation was used to assess changes in Mg<sup>2+</sup> ion concentration in cytosol and to determine the time point of a sharp increase in Ca<sup>2+</sup> ion concentration.

Since Mg<sup>2+</sup> in the cell is predominantly bound to ATP, changes in the Mag-Fura-2 signal after inhibition of mitochondrial ATP synthase with oligomycin A and glycolysis with iodoacetic acid (IAA) were used as an indirect assessment of the rate of macroergic consumption by cells. At the moment of complete ATP depletion, cells lose the ability to maintain the transmembrane gradient of Ca<sup>2+</sup> ions, whose concentration in the cytoplasm sharply increases at this point.

Since the probe used has low affinity for  $\text{Ca}^{2+}$ , only such increase is reflected in the corresponding increase of the analytical signal, and the time between the start of ATP synthesis inhibition and the loss of the  $\text{Ca}^{2+}$  gradient can be considered as a measure of the cells' energy capacity (time to collapse).

**Measurement of  $\Delta\psi_m$  using TMRM.** Magnitude of  $\Delta\psi_m$  was assessed using the potential-dependent fluorescent probe TMRM (Thermo Fisher Scientific), which has the ability to selectively accumulate in mitochondria depending on the magnitude of  $\Delta\psi_m$ . Cells were incubated with 25 nM TMRM dissolved in HBSS for 40 min at room temperature in the dark without subsequent washing. Images of the cells using layer-by-layer scanning mode with a 0.5- $\mu\text{m}$  step were obtained using a Zeiss LSM900 confocal microscope (Carl Zeiss AG, Germany) with an excitation laser with wavelength of 561 nm and detecting the signal in the 565-650 nm range. Assessment of the  $\Delta\psi_m$  level was based on the maximum fluorescence intensity of TMRM in the cell. At least three independent culture replicates ( $n = 3$ ) were used in each experiment, with at least 100 cells analyzed in each replicate.

**Preparation of acute brain slices.** Animals were euthanized by cervical dislocation followed by decapitation. The brain was rapidly removed from the skull and placed in an ice-cold HBSS. Slices 300  $\mu\text{m}$  thick were obtained using a vibratome (Campden 7000smz-2; Campden Instruments Ltd., UK) in the coronal plane in the cerebral cortex region. The slices were then placed in an incubation chamber with HBSS for 30 min at room temperature. Some slices were incubated with oxypurinol (OXY) (20  $\mu\text{M}$ ), an XO inhibitor.

**Assessment of ROS production.** To study the rate of ROS formation, a dihydroethidium (DHE) probe was used, which can penetrate cells and interact with ROS (primarily with superoxide anion) to form a fluorescent product that intercalates with DNA. For the study, slices were incubated in HBSS with addition of 5  $\mu\text{M}$  DHE (Invitrogen by Thermo Fisher Scientific) for 10 min at room temperature. Registration of the fluorescent signal over time was carried out using a Zeiss LSM900 system with an excitation wavelength of 561 nm and emission at 565-620 nm.

The rate of increase in fluorescence intensity served as a marker of the level of intracellular oxidative stress and was used to assess ROS production in brain tissues of different animal genotypes. This parameter was calculated during post-processing using the ZEN Microscopy Software (Carl Zeiss Microscopy GmbH, Germany) and ImageJ. Graphs of the dependence of DHE fluorescence intensity on time were constructed for all regions of interest. For quantitative assessment of ROS production, linear section of the fluorescence intensity growth curve of DHE was analyzed. The rate was calculated as the slope (angu-

lar coefficient) of the straight line of change in the fluorescence intensity of the dye. Values of the ROS production rate were normalized to the average value obtained in the control group without addition of the studied compounds, which was taken as 100%.

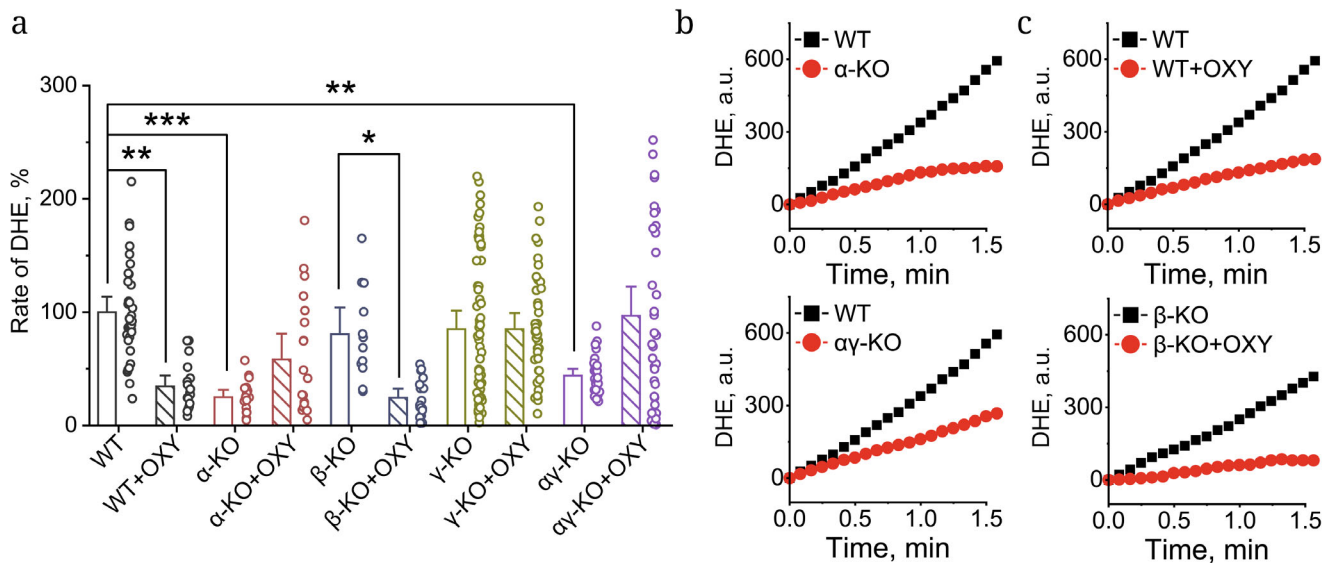
**Statistical analysis.** Analysis of experimental data, graph construction, and calculation of the statistical significance of differences between the groups were performed using OriginPro software. Data were tested for normality using the Shapiro–Wilk test. In some groups, a significant deviation from the normal distribution ( $p < 0.05$ ) was observed, which, together with uneven and relatively small sample sizes, determined the choice of non-parametric analysis methods. The Kruskal–Wallis H-test was used for statistical data processing. When a statistically significant effect ( $p < 0.05$ ) was detected, *post-hoc* analysis was additionally performed using Dunn's test, with Bonferroni correction applied to account for multiple comparisons. All data are presented as a mean  $\pm$  standard error of the mean.

## RESULTS

**Baseline ROS production rate decreases in the absence of endogenous  $\alpha$ -synuclein.** Using DHE as a probe to assess the rate of ROS production in the acute brain slices (Fig. 1), we evaluated the role of different synucleins in maintaining redox balance.

It was found out that the absence of  $\alpha$ -synuclein (brain slices from  $\alpha$ -synuclein knockout mice) leads to the significant decrease in the rate of ROS production to  $25 \pm 3\%$  ( $n = 18$ ;  $p < 0.0001$ ) of the values in the WT mouse slices ( $n = 36$ ; Fig. 1, a and b). It should be noted that the brain slices from the  $\beta$ - and  $\gamma$ -synuclein knockout mice also had a reduced rate of ROS production, but the effects were much less pronounced (80% and 85% of the WT values, respectively) and were not statistically significant. Interestingly, in the cells with simultaneous absence of  $\alpha$ - and  $\gamma$ -synucleins, the rate of DHE oxidation also significantly decreased (to  $43 \pm 3\%$  of the control;  $n = 29$ ,  $p = 0.00137$ ; Fig. 1, a and b), which, considering all our results, suggests a possible regulatory influence of synucleins on the ROS production sites.

One of the enzymes whose activity is accompanied by ROS production is XO, involved in purine metabolism [24]. Considering high level of expression of this enzyme with increased levels of  $\alpha$ -synuclein [25], activation of XO during inhibition of bioenergetic processes [7], and involvement of  $\alpha$ -synuclein in energy metabolism [26, 27], we used an inhibitor of this enzyme (OXY) to determine contribution of XO to ROS production in the brain slices. It can be seen that when the slices were incubated in the presence of the inhibitor (20  $\mu\text{M}$ , 30 min), the level



**Fig. 1.** Rate of reactive oxygen species (ROS) production in the acute brain slices of wild-type (WT) mice and mice with synuclein deficiencies (KO – gene knockout) in various combinations at baseline and with xanthine oxidase (XO) inhibition using oxypurinol (OXY). a) Rate of dihydroetidium (DHE) fluorescence intensity increase in the acute brain slices. Data are normalized to the average value of the baseline rate of DHE fluorescence in WT. b) Representative curves of DHE fluorescence intensity when assessing the baseline rate of ROS production in WT animals and animals with knockouts of  $\alpha$ - and  $\gamma$ -synuclein genes. c) Representative curves of DHE fluorescence intensity when assessing the rate of ROS production under incubation with the inhibitor (OXY) in the WT animals and animals with  $\beta$ -synuclein gene knockout. All data are presented as a mean  $\pm$  standard error of the mean; \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.0001$  – by Kruskal–Wallis test.

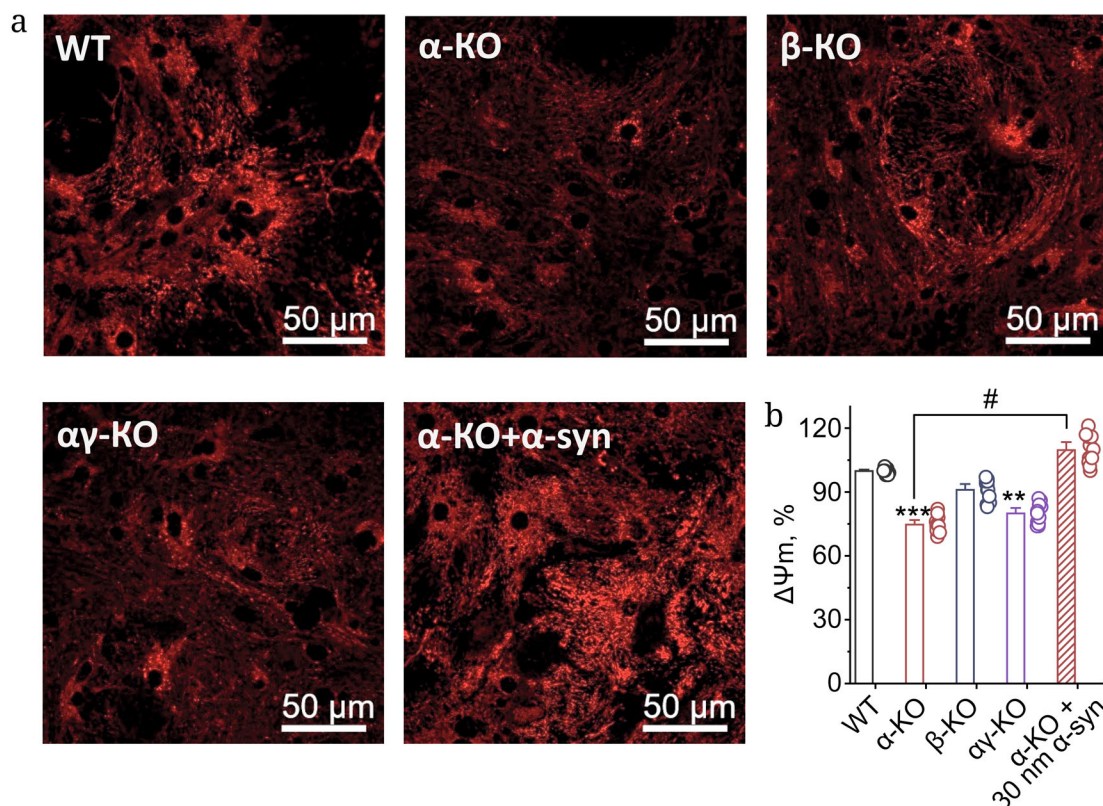
of ROS production in the control group of animals statistically significantly decreased by 65% ( $n = 18$ ;  $p = 0.000105$ ), and in the  $\beta$ -synuclein knockout group by 55% ( $n = 17$ ;  $p = 0.01856$ ; Fig. 1, a and c). This indicates that in these groups of animals, the enzyme retains its physiological activity, since the XO inhibitor reduces ROS production. At the same time, the groups with knockouts of  $\alpha$ - and  $\gamma$ -synucleins, as well as double knockout of both protein genes, did not respond to the addition of OXY, which may indicate possible regulation of XO by  $\alpha$ - and  $\gamma$ -synucleins. One of the factors that could influence XO activity in the cell is change in the energy metabolism and purine balance [24], hence, in order to understand the mechanism of XO regulation by  $\alpha$ - and  $\gamma$ -synucleins, we studied their effect on mitochondrial metabolism.

**Absence of endogenous synucleins decreases mitochondrial membrane potential in the primary neuroglial cultures.** The level of  $\Delta\psi_m$  is a universal indicator of mitochondrial functionality, as most processes in mitochondria are controlled by or depend on this parameter [28]. The results of comparative assessment of  $\Delta\psi_m$  in the cells of primary co-cultures of neurons and glial cells of the cerebral cortex of the WT mice, as well as of the animals with knockout of the synuclein genes, are presented in Fig. 2.

It was found out using the fluorescent indicator TMRM (25 nM) to assess  $\Delta\psi_m$  in the primary brain cortex cells of transgenic mice with different levels

of synucleins that knockout of the genes responsible for  $\alpha$ -synuclein expression leads to the significant decrease in  $\Delta\psi_m$  (to  $74 \pm 0.3\%$  of the WT cell values,  $n = 12$  experiments;  $p < 0.0001$ ; Fig. 2), which is consistent with the previously published data for this genotype [26]. It should be noted that the similar decrease in  $\Delta\psi_m$  was also observed in the cells with double knockout of  $\alpha$ - and  $\gamma$ -synucleins (to  $80 \pm 1.3\%$  of WT cell values,  $n = 12$  experiments;  $p = 0.0025$ ; Fig. 2), but not in the cells with  $\beta$ -synuclein knockout (to  $91 \pm 1.4\%$  of WT cell values,  $n = 12$  experiments;  $p = 0.64512$ ; Fig. 2). To confirm that the effect depends on the absence of  $\alpha$ -synuclein and not on other changes caused by gene modifications, we incubated the cells with 30 nM monomeric  $\alpha$ -synuclein for 1 h, which completely restored the  $\Delta\psi_m$  level in the  $\alpha$ -synuclein knockout cells to the WT values ( $109 \pm 2\%$  of WT cells,  $n = 12$  experiments;  $p = 1$  relative to control;  $p < 0.0001$  relative to  $\alpha$ -KO; Fig. 2). Thus, absence of  $\alpha$ -synuclein and  $\gamma$ -synuclein leads to the decrease in  $\Delta\psi_m$ .

To assess energy capacity of the cells (Fig. 3), the fluorescent probe Mag-Fura-2 was used. ATP in the cells is present predominantly in the form of a magnesium salt, and ATP hydrolysis leads to the increase in  $[Mg^{2+}]$ , which can be recorded through the changes in Mag-Fura-2 fluorescence. Inhibition of glycolysis and oxidative phosphorylation (20  $\mu$ M IAA and 5  $\mu$ M oligomycin, respectively) stops ATP production,



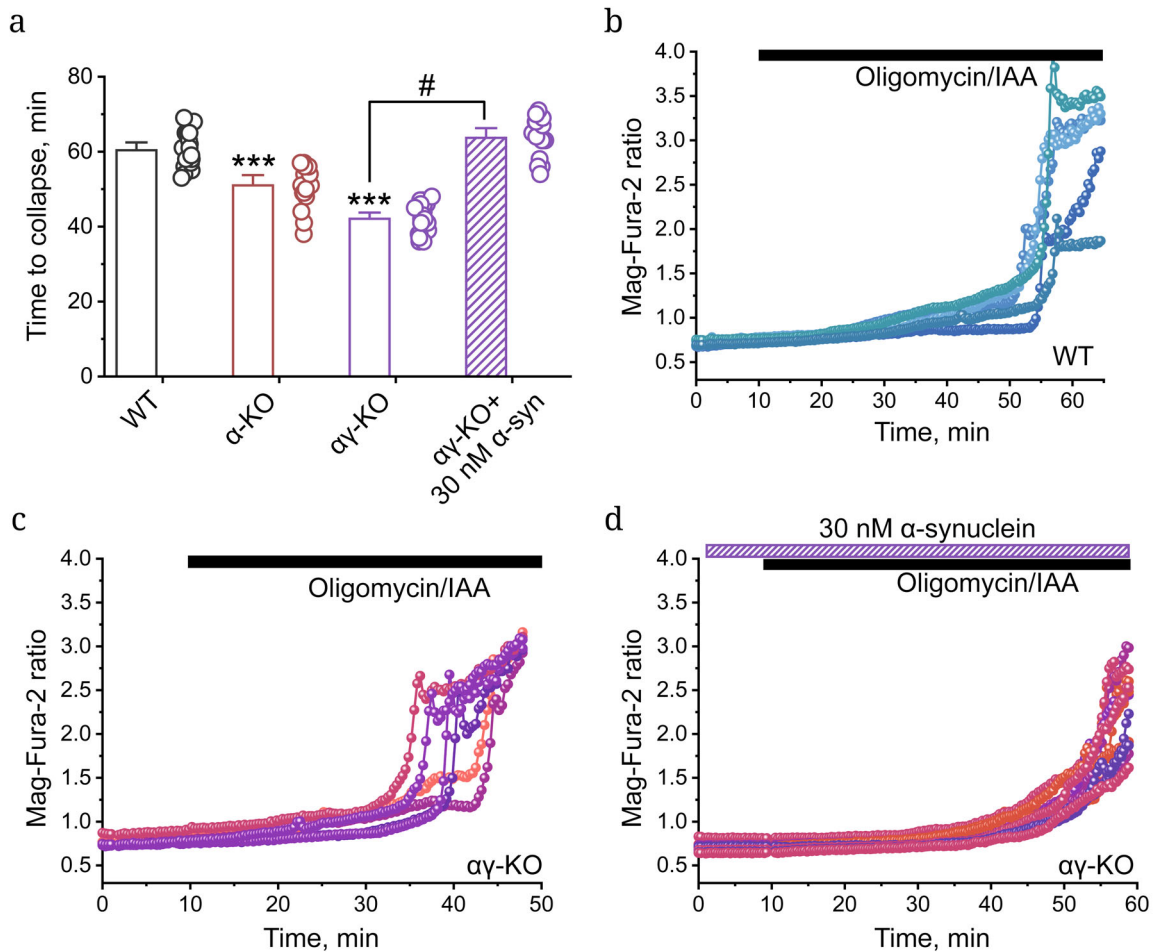
**Fig. 2.** Effect of synuclein absence and exogenous  $\alpha$ -synuclein exposure on  $\Delta\psi_m$ . a) Representative confocal images of neuroglial cultures loaded with TMRM. b)  $\Delta\psi_m$  magnitude normalized to the control group (mean value taken as 100%). Data are presented as a mean  $\pm$  standard error of the mean. \*\*  $p < 0.01$ ; \*\*\*  $p < 0.0001$  – relative to the control group; #  $p < 0.0001$  – by Kruskal–Wallis test. TMRM, tetramethylrhodamine; WT, wild type; KO, gene knockout.

while maintaining its consumption for the cellular needs. Complete ATP consumption leads to the cellular energy collapse with disruption of ionic balance, which can also be recorded as a sharp increase in the Mag-Fura-2 fluorescence, as the probe also has low affinity for  $\text{Ca}^{2+}$ . The time from addition of inhibitors to the cellular collapse (time to collapse) can be taken as a measure of ATP capacity in these cells [7].

Energy collapse in the healthy cells occurred on average after 60 min ( $n = 19$ ; Fig. 3, a and b), while in the animals with  $\alpha$ -synuclein knockout and double  $\alpha,\gamma$ -knockout, this occurred faster – after 51 min ( $n = 17$ ;  $p = 0.01$ ; Fig. 3, a and c) and 42 min ( $n = 22$ ;  $p < 0.0001$ ; Fig. 3a), respectively. This means that in the absence of  $\alpha$ - and  $\gamma$ -synucleins, the ATP reserve in the cells is lower. To test involvement of  $\alpha$ -synuclein in this process, we added 30 nM monomeric  $\alpha$ -synuclein to the culture of cells with simultaneous absence of  $\alpha$ - and  $\gamma$ -synucleins. It was shown that this addition increased energy capacity of the cells with double  $\alpha,\gamma$ -knockout, and energy collapse occurred on average after 63 min ( $n = 16$ ;  $p = 1$  relative to control;  $p < 0.0001$  relative to  $\alpha\gamma$ -KO; Fig. 3, a and d), i.e., at the time that was also characteristic of the control group cells.

## DISCUSSION

The role of aggregated  $\alpha$ -synuclein in ROS generation and oxidative stress has been studied in considerable detail. For example, oligomeric  $\alpha$ -synuclein can produce superoxide anion even in a cell-free environment in the presence of divalent cations [27, 29]. The ability of fibrillar and oligomeric  $\alpha$ -synuclein to activate ROS production by enzymes and mitochondrial ETC, which leads to ferroptosis, has also been demonstrated [30]. However, in all these studies, no change in ROS production in response to monomeric  $\alpha$ -synuclein was observed [29]. In this work, for the first time, we evaluated physiological role of synucleins in regulation of xanthine oxidase and its involvement in maintaining redox balance of neurons and astrocytes. Our results show significant decrease in the ROS production in the cells in the absence of synucleins, and importantly, the most pronounced decrease was observed in the  $\alpha$ -synuclein knockout neurons and astrocytes. The relationship between synucleins and XO was previously shown only in the *Caenorhabditis elegans* model with increased expression of  $\alpha$ -synuclein [25]. In our experiments, absence of the endogenous  $\alpha$ - and  $\gamma$ -synucleins did not lead



**Fig. 3.** Knockout of  $\alpha$ - and  $\gamma$ -synuclein genes affects energy supply level of the brain cells. a) Results of statistical analysis of the time to energy collapse in the brain cells of WT mice, as well as mice with the synuclein gene knockouts. Data are presented as a mean  $\pm$  standard error of the mean; \*\*\*  $p < 0.0001$  – relative to the control group; #  $p < 0.0001$  – by Kruskal–Wallis test. b) Representative graphs of changes in the ratio of Mag-Fura-2 fluorescence intensity with excitation wavelengths of 340 and 380 nm in the WT cells, c) with simultaneous knockout of  $\alpha$ - and  $\gamma$ -synuclein genes without additional exposure, d) with simultaneous knockout of  $\alpha$ - and  $\gamma$ -synuclein genes in the presence of monomeric  $\alpha$ -synuclein. IAA, iodoacetic acid; WT, wild type; KO, gene knockout.

to the decrease in the rate of ROS production in response to the XO inhibitor OXY (Fig. 1). This suggests physiological regulation of XO activity by  $\alpha$ - and  $\gamma$ -synucleins, but not by  $\beta$ -synuclein, and explains that at least part of the decrease in the baseline rate of ROS production in the knockout cells is due to inhibition of ROS production by XO. Allopurinol, and to a lesser extent OXY, has been shown to have antioxidant activity [31, 32]. Nevertheless, in our experiments, the effect of OXY was mainly due to inhibition of XO because ROS production increased in some experiments, which cannot occur due to the action of an antioxidant (Fig. 1).

Regulation of XO by  $\alpha$ - and  $\gamma$ -synucleins could occur either through the direct interaction of the proteins or through the influence of synucleins on mitochondrial metabolism, leading to the changes in purine homeostasis (Figs. 2 and 3). Activation of

XO in various forms of ischemia has been previously demonstrated [6, 7, 33]. The changes in mitochondrial metabolism and energy balance of the neurons and astrocytes in the absence of endogenous  $\alpha$ - and  $\gamma$ -synucleins demonstrated in this study could facilitate rapid changes in the purine levels, which in turn could alter XO activity.

Physiological role of  $\alpha$ -synuclein in mitochondrial metabolism has been shown in our previous study [26], and importantly, it was shown that  $\beta$ -synuclein does not possess the same properties. Although  $\gamma$ -synuclein is less studied, it may also be involved in the regulation of energy and metabolism of nerve cells, as evidenced by our results and the demonstrated effect of this protein on lipid metabolism and dynamics of microtubules and actin cytoskeleton [34–36].

Restoration of energy function in the cells by addition of exogenous monomeric  $\alpha$ -synuclein confirms

the previously shown ability of the exogenous  $\alpha$ -synuclein to restore ATP synthase function and mitochondrial respiration [26]. Importantly, the exogenous  $\alpha$ -synuclein can easily penetrate the cell, which can lead to the prion-like production of  $\alpha$ -synuclein [37]. It has previously been established that the absence of endogenous  $\alpha$ -synuclein disrupts functioning of the mitochondrial ATP synthase, affecting both ATP synthesis processes and the associated mitochondrial respiration [26].

Possible mechanisms for these effects include either direct interaction of  $\alpha$ -synuclein with the XO or indirect influence through the energy metabolism. In the case of  $\alpha$ -synuclein deficiency, normal ATP synthase function is disrupted, reducing ATP production. ATP deficiency initiates compensatory processes of purine catabolism, leading to their degradation to xanthine. Since xanthine is a substrate for XO, this activates its function and shifts metabolic balance toward oxidative reactions [26]. Thus,  $\alpha$ -synuclein functions as a regulator of energy homeostasis, affecting two key links – mitochondrial respiration and purine metabolism. Its presence ensures coordination of the ATP synthase operation with the systems maintaining the cell's energy balance, while its deficiency triggers a cascade of metabolic changes that enhances cellular stress and may contribute to pathological processes.

### Abbreviations

$\Delta\psi_m$	mitochondrial membrane potential
ATP	adenosine triphosphate
DHE	dihydroethidium
HBSS	Hank's balanced salt solution
KO	gene knockout
OXY	oxypurinol
ROS	reactive oxygen species
TMRM	tetramethylrhodamine
XO	Xanthine Oxidase

### Contributions

A. Yu. Abramov – concept and supervision of the work; A. A. Fedulina, A. M. Krayushkina, K. D. Chaprov, and E. S. Seryogina – conducting experiments; A. A. Fedulina, A. Yu. Vinokurov, K. D. Chaprov, and A. Yu. Abramov – discussion of research results and writing the text; A. A. Fedulina, A. Yu. Vinokurov, and A. Yu. Abramov – editing text of the article.

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### Ethics approval and consent to participate

All applicable international, national, and/or institutional guidelines for the care and use of animals were

followed. All procedures were performed in accordance with the principles of good laboratory practice and ethical standards for the humane treatment of animals. The study was approved by the Ethics Committee of Orel State University named after I. S. Turgenev (protocol no. 18, dated 21.02.2020).

### Conflict of interest

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

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