peptide monomerized according to the standard method (Jao, 1997; Džinić, 2018), the 2nd group of cells (control) did not contain any additives, the 3rd group contained 0.1% DMSO (this amount of DMSO was added to the 1st group with Aβ), and the 4th and 5th, in addition to Aβ, also contained D-peptides interacting with various forms of Aβ and its precursors (Bocharov et al., 2021; Van Groen et al., 2008), one these peptides has passed the second phase of clinical trials (Mathew, 2023). After cell cultivation, mitochondria were isolated according to the standard method (Martin, 1998; Daum, 1982). The substrates of complexes I, II and IV and the corresponding inhibitors of the OxPhos complexes were added to isolated mitochondria in separate experiments to focus on studying the change in the activity of each complex induced by Aβ and other substances. Immediately before the beginning of the luminescence measurements, ADP and luciferase with luciferin were added to mitochondria (Lomakina, 2022). For each experimental group, a luminescence graph (RLU) was plotted against time. The maximum values of the first derivatives of the left parts of the obtained bell-shaped curves (ATP production rate) were compared. Peak heights were also compared (they corresponded to the amount of ATP produced by mitochondria). The presence of DMSO led to a slight decrease in the amount and rate of ATP production by human mitochondria compared to the control group. Cultivation of cells with Aβ leads to a decrease in ATP synthesis by mitochondria and a halving of the synthesis rate, while the presence of D-peptides restores these indicators to control values. The work was supported by the Ministry of Science and Higher Education of the Russian Federation (agreement 075-03-2023-106 dated January 13, 2023, subject number FSMG-2021-0002).

S2.139. Changes in metabolic parameters in cells with multiple mtDNA mutations associated with diseases

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Mitochondria play a key role in the development of most intracellular processes. The genetic regulation of its functioning is determined by nuclear DNA (nDNA) and mitochondrial DNA (mtDNA), which contains the genes of 12S and 16S rRNA, tRNA, and individual polypeptides of the electron transport chain (ETC). mtDNA is characterized by a significantly higher level of mutation [1,2]. According to various estimates, the incidence of diseases associated with mtDNA mutations is about one case per 4000-5000 people [3]. Due to the presence of a large number of mtDNA molecules in the cell (it can reach several thousand), the symptoms of pathologies associated with mutations manifest themselves at the level of heteroplasmy of 60-90% [4, 5]. However, it should be noted that these data were obtained as a result of studies containing a limited (usually one or two) number of mtDNA mutations in the cell. While due to the high frequency of mtDNA damage, cases of a significantly higher level of mutational load are not uncommon. So, various interaction effects are possible. The molecular mechanisms of mtDNA mutations have not been sufficiently studied. The nature of changes in ATP synthesis, mitochondrial membrane potential (ΔΨm), the content and activity of ETC proteins, ROS production in various studies differs both qualitatively and quantitatively (even when considering the same mutations), which is probably due to both the values of heteroplasmy and the combination of mutations [6-9]. Thus, the development of research of the relationship between complex combinations of mtDNA mutations and various levels of cellular phenotypic changes is very relevant.

In our work we use lines of cybrids based on THP-1 cells and having 5-8 mtDNA mutations in the MT-RNR1, MT-TL1, MT-TL2, MT-CYTB, MT-ND1, MT-ND2, MT-ND5 and MT-ND6 genes with a heteroplasmy range 1%-68%. At this stage, the studies performed include the analysis of a number of parameters characterizing the bioenergetics of cells (the level and mechanism of formation of ΔΨm; mitochondrial content, the ratio of reduced and oxidized forms, as well as the rate of production of NADH and FADH2; the content and rate of ATP consumption; respiration of cells; formation of ROS; mitophagy level).

The results obtained allow us to make a conclusion about a significant effect of the studied mutations on cellular metabolism, even despite the significantly lower levels of heteroplasmy in comparison with those indicated for the presence of single mutations. In particular, mutations of tRNALeu genes turn out to be significant already at 20% content with the simultaneous presence in the cell of cytochrome b mutations (m.14846G>A) or subunits of the ETC complex I (m.5178C>A, m.14459G>A). All lines are characterized by a significant decrease in the level of ATP in the absence of a positive correlation of this parameter with the time of complete exhaustion of the macroerg when blocking the pathways of its biosynthesis. This indicates various causes of energy deficiency – from decreased ATP formation to hyperactivated actively consuming processes. The combinations of mutations presented in the cybrids are associated with a significant level of dissociation of oxidative phosphorylation, which may be a way to reduce the negative effects of ROS hyperproduction both in the MX matrix and the intermembrane space in the dysfunction of ETC complexes. Disorders of complex I associated with mutations of genes of individual proteins, as well as tRNA genes, are not always compensated by an increase in the expression or activity of succinate dehydrogenase encoded by nDNA, which indicates the limited use of complex II substrates as a tool for protecting cells in the presence of mtDNA mutations. A number of cybrids are characterized by an inverse mode of functioning of the complex V of ETC, which allows maintaining the level of ΔΨm due to consumption of ATP at the same time. Despite the revealed violations, some cybrids are characterized by defective mitophagy, leading to the accumulation of non-functional organelles. In some cases, combinations of mutations can lead to an improvement in the parameters characterizing the state of cells, which is observed, in particular, in the presence of mutations m.13513G>A and m.1555A>G in the MT-ND1 and MT-RNR1 genes, respectively.

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S2.140. Changes in the electrical characteristics of identified neurons in the terrestrial snail as a result of the development of a conditioned situational reflex and reconsolidation of memory for this reflex

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A plethora of experimental data indicate that cellular processes associated with learning are caused by long-term modifications in the efficiency of synaptic transmission and changes in the endogenous properties of the neuron and its membrane [1,2,3]. For a long time, the change in the efficiency of synaptic transmission was recognized as the main learning mechanism until later evidence of non-synaptic mechanisms appeared. Within the framework of such ideas, there is a sufficient number of studies of cellular learning mechanisms [4,5]. Therefore, in many works, studies have been carried out to establish a link between the outcomes of behavioral learning and the excitability of neurons and the electrical characteristics of the membrane [1,2,4,5]. Previously, we have shown membrane correlates (changes in the membrane and threshold potentials of premotor interneurons) for conditioned defensive reflexes of tapping on the shell and aversion to food [2,4,6]. Therefore, the question arose whether such changes are possible during the development of other types of conditioned reflexes. To do this, we studied the possible correlation of the development of a conditioned situational reflex [7], and the reconsolidation of its memory to the dynamics of changes in the electrical characteristics of the premotor interneurons of the defensive behavior LPa3 and RPa3 as well as the serotonin-containing neurons Pd2 & Pd4 of the pedal ganglion, which modulate this reflex in the terrestrial snail,. Therefore, we studied the changes in the membrane and threshold potentials of the premotor interneurons LPa3 and RPa3 of the terrestrial snail after the development of a conditioned defensive reflex to the situation and memory reconsolidation of this reflex.
The experiments were carried out using the mollusc Helix lucorum. In all animals, a situational conditioned reflex was developed according to the contextual paradigm "on the ball" where the animals were rigidly fixed to the shell. Before elaborating the conditioned reflex and after training, the amplitude of the defensive reaction was tested as an indicator of the formed long-term memory. Behavioral responses were tested in two environments (contexts): 1) on a ball (i.e., under standard learning conditions), 2) on a flat surface. In some snails, after the development of a conditioned reflex to the environment, long-term memory of the learning environment was reconsolidated [7]. For the study of reconsolidation, a "reminder" of the learning environment was introduced. The results showed that the membrane potential in neurons LPa3 and RPa3 decreases significantly (about 5 mV) after training. No significant further changes were found in the membrane potential after the reminder (initiation of reconsolidation relative to its post-training level). The threshold potential of these neurons decreased after training and remained unchanged after the reminder. At the same time, after the reminder, the membrane and threshold potentials significantly decrease relative to the initial level (before training). All in all, these neurons can participate in the process of reconsolidation of the situational reflex.
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S2.141. Changes in the process of endocytosis of synaptic vesicles in various types of muscle mice after hindlimb unloading

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Functional unloading of skeletal musculature (immobilization, bed rest, space flight) leads to atrophy, which mechanisms are well studied. Condition of motoneurons, innervating this muscles and launching muscle contraction process, is less studied. Action potential propagation along motoneuron axon provides transmission of excitation from the motor nerve ending to postsynaptic membrane of the muscle fiber by activation of acetylcholine receptors with mediator – acetylcholine. Previously shown, that levels of non-quantum secretion of acetylcholine change, and quantum induced and spontaneous secretion, in rats are changed after antiorthostatic suspension of the hind limbs (support unloading model according to the Morey-Holton method). The intensity of the quantum release of the mediator can be regulated both by exo- and endocytosis. In this work, we analyzed the processes of endocytosis of synaptic vesicles in the nerve endings of muscles of different functional types in mice after 30 days of support unloading (antiorthostatic rear limb suspension (AOS) according to the Morey-Holton method). The experiments were carried out on isolated neuromuscular preparations m. Diaphragma (mixed muscle), m. Soleus (slow muscle), m. EDL (m. extensor digitorum longus fast muscle) of laboratory white mice. The processes of endocytosis of synaptic vesicles were studied using a fluorescent marker FM 1-43 (3μM), which reversibly binds to the presynaptic membrane and during endocytosis of synaptic vesicles is inside the nerve terminal ("loading" of the terminal). An indicator of endocytosis and loading of the fluorescent dye into synaptic vesicles was the appearance of brightly glowing spots inside the nerve ending. The intensity and duration of stimulation of the nerve stump depended on the type of muscle: m. Diaphragma 50 imp/s in 1 minute, m. Soleus – 1 imp/s 5 sec and 10 imp/s 10 sec, m. EDL – 60 sec by hyperpotassic Krebs solution. In the control group of mice with high-frequency stimulation of the motor nerve of the phrenic muscle, the luminescence intensity was 87 r.u. ± 3 r.u. (n=14). In the group of animals after AOS, the terminal luminescence intensity was 75 ±