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PHYSICAL ACTIVITY ASSOCIATED WITH AGING RECOVERS MITOPHAGY INITIATION DEFICIENCY ACCORDING TO THE STUDY OF HIPPOCAMPUS, SUBSTANTIA NIGRA AND LOCUS COERULEUS IN ANIMAL MODEL OF SENESCENCE

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Resumo:

Neurodegenerative processes are closely linked to the aging of an individual. The biocellular changes that accompany aging cause protein deposits related to diseases such as Alzheimer's and Parkinson's. In addition, the cognitive decline associated to senescence seems to be retarded by moderated physical activity. Among the neuronal processes apparently affected are mitochondrial traffic, mitophagy and autophagy. The purpose of this study was to analyse Miro-1, Beclin1 and p62 proteins on hippocampus, substantia nigra and locus coeruleus, mostly affected brain areas in Alzheimer's disease and Parkinson's disease cases, on animal model of aging and of aging associated with moderated physical training. Results showed an increased presence of Miro-1 on hippocampi of elderly animals, prevented with physical exercise, and no change on Beclin1 and p62 levels, suggesting the accumulation of nonfunctional mitochondria caused by the deficiency on its arrest and no alterations of autophagy initiation.

Autorização legal: All procedures were authorized by the Ethics Committee on the Use of Animals of IB/USP, protocol nº 271/2016.

Palavras-chave: Miro-1; Lewis rats; autophagy.

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Introdução:

The aging process of the central nervous system is accompanied by cellular and biochemical changes associated with the protein deposit. Such phenomenon is exacerbated in neurodegenerative diseases such as Alzheimer's, Parkinson's and Amyotrophic Lateral Sclerosis, among others. However, it is known that even before the appearance of the protein aggregates, the cells present important deficiencies that culminate in the neuronal death that is common to the senescent encephalon, among them the autophagic deficiency **1 2.**

Sporadic Alzheimer's disease and sporadic Parkinson's disease are closely linked to the aging process. Pathologically, Alzheimer's disease is characterized by loss of neurons in the hippocampus, the region responsible for memory **3**. Classical Parkinson's disease is pathologically characterized by the loss of dopaminergic neurons in the substantia nigra and by the presence of Lewy bodies, intracellular inclusions, in surviving neurons in several areas of the brain, particularly in the substantia nigra **4**. Evidence also indicates loss of neurons of the locus coeruleus in both Alzheimer's disease cases **5** and Parkinson's disease cases **6**.

Initial signaling of macroautophagy involves the Ulk1/Beclin1 complex which generates vesicles with a double lipid layer **7**. Beclin1 has a central role in the maintenance of cellular survival, and its dysfunction may imply neurodegeneration **8**. Autophagy substrates are targeted for degradation by associating with p62, an adaptor protein that connects them to the phagophore. p62 is involved in several cellular processes, and its loss has been linked to accelerated aging and to age-related pathologies **9**.

The accumulation of nonfunctional mitochondria impairs neuronal viability, especially during aging, when there is a natural reduction of autophagic processes. In this same line, it has recently been shown that the blocking of Miro-1 expression, a protein associated with mitochondrial traffic and mitophagy, leads to an increase in the life span of *C. elegans*, which is related to the decrease in the number of mitochondria, but increased organelle efficiency **10**.

Studies of human aging show that physical activity practice protects against cognitive decline, increases attention and performance in cognitive tasks. In a murine model of stroke, aerobic exercise promoted dendritic ramifications and increased motor skills **11**.

Metodologia:

Fifteen male Lewis rats were divided into three groups: five sedentary two-month old rats (Young), five sedentary 11-month old rats (Elderly) and five 11-month rats that underwent moderate exercise training on a treadmill (Trained). Physical training was performed for 3 weeks for adaptation and for 6 more weeks with 50 to 60% of the maximum intensity of training, recalculated every 2 weeks **12**. The hippocampi, substantiae nigrae and loci coerulei were removed and lysed in RIPA. Samples were then quantified by Bradford method and analyzed by Western Blot for labeling of Miro-1, Beclin1 and p62 proteins with β-actin normalization labeling. Statistical analysis was performed by one-way ANOVA and Tukey's multiple comparison test with significance for $p < 0.05$.

Resultados e Discussão:

Many studies point out as a direct consequence of aging the deficiency of the proteasomal system **13** **14**. Experiments with inhibition of proteasome in cultures of neurons show the increase of p62 expression as a consequence **15** - probably as cellular response to promote aggregation and degradation by macroautophagy. The hypothesis for Beclin1 analysis was that it would be diminished with aging; the literature describes the negative regulation of the corresponding gene during this process. The absence of changes in the amount of Beclin1 and p62 in both experimental groups in all analyzed areas (Figure 1) points to the assumption that there is no alteration in macroautophagy, both in its initiation and in the protein aggregation promoted by p62, in this model of aging and aging associated with physical activity.

Analysis of Miro-1 protein, involved in the mitophagy process by being part of the organelle binding complex to the microtubules, revealed a significant increase in its quantity in hippocampal samples from elderly rats and also revealed that this change was reversed with physical training. This difference in the amount of protein was not observed in substantia nigra and locus coeruleus (Figure 1). Such result agrees with published results that associate the decrease of the degradation of Miro-1 to cases of familial and sporadic Parkinson's disease **16** **17**. However, according to these published results such difference should be seen in the analysis of substantia nigra and locus coeruleus samples, areas affected in Parkinson's disease. This leads to the belief that the hippocampus is the area initially affected in non-pathological aging, including proteins of mitochondrial machinery.

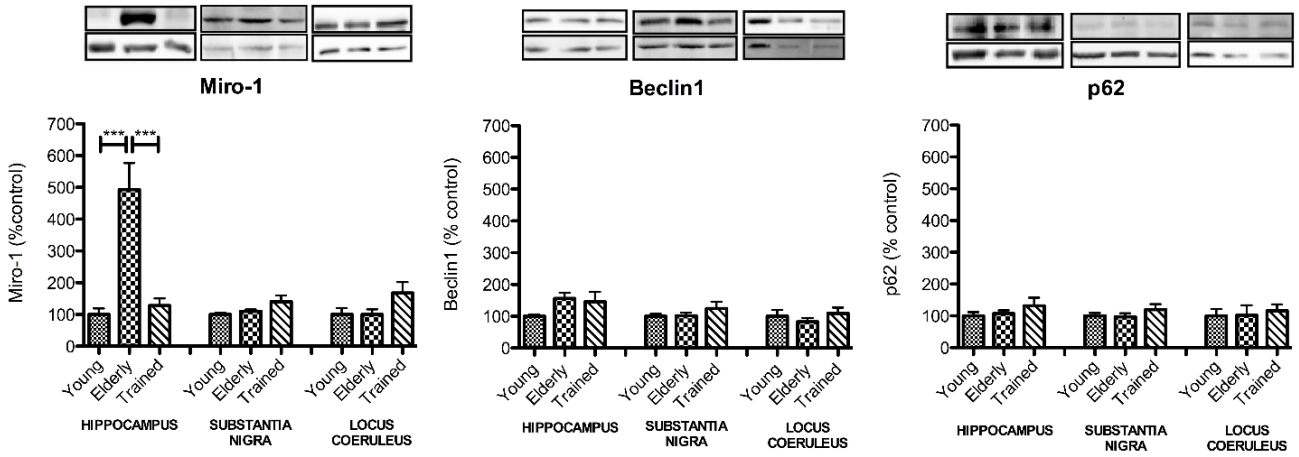

Figure 1. Quantification by percentage of the control - sedentary young animals - of the presence of Miro-1, Beclin1 and p62 by Western Blot in lysate tissue of hippocampus, substantia nigra and locus coeruleus from sedentary elderly and trained elderly animals. One-way ANOVA analysis with Tukey's test showed significance only in the difference between the amount of Miro-1 between Elderly and Young and the amount of Miro-1 between Elderly and Trained (*** $p < 0.001$, $n = 5$).

Conclusões:

The increase of Miro-1 in the hippocampus suggests a higher amount of non-functional mitochondria in elderly rats, which is reversed with physical exercise. The maintenance of Beclin1 and p62 levels suggests that despite the increase of non-functional mitochondria, they are not being further degraded, which may generate their accumulation. Since no significal difference was observed in subtantia nigra and locus coeruleus samples, hippocampal neurons may be more sensitive to accumulation of mitochondria caused by its arrest deficiency on non-pathological aging than the dopaminergic neurons, against the expectation.

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