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Effect of Infrared Stimulation on Neurons in the Mouse Hippocampus

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ABSTRACT

Introduction: the brain structure clearly distinguishes the regions corresponding to the hippocampus, the fimbria, and the dentate gyrus, which play important roles in consolidating short- and long-term memory and learning and spatial memory processes.

Given the previous research and evidence on cell types with high metabolism, it was important to investigate how hippocampal neurons from Balb/c mice respond to increasing doses of infrared laser. This study used morphometric techniques on transmission electron microscopic images to quantify the volumetric fractions of different cellular components. For this, 20 animals were separated into 4 groups: Group 1 was the control and Groups 2, 3, and 4 received infrared stimulations of 4, 8, and 16 J/cm2, respectively, for 15 consecutive days. After stimulation, the animals were euthanized by excess CO2 and treated with the inclusion technique for observation with transmission electron microscopy. Neurons were micrographed at 4000 X final magnification, and the number and volumetric fractions of cellular constituents such as neuronal size, nucleus, cytoplasm, rough endoplasmic reticulum, mitochondrial size, and number were quantified using ultrastructural morphometric techniques. Based on the analysis of these findings, we can conclude that applying infrared stimulations to hippocampal neurons leads to alterations in all the cellular components assessed. This suggests that these modifications could determine changes in the neuronal metabolism.

Keywords: Neurons; Laser Infrared; Morphometry.

Introduction

The hippocampus corresponds to a structure located in the cerebral hemispheres, specifically in the inner portion of the temporal lobe of the cerebral cortex. From a physiological point of view, the hippocampus and other components make up the limbic system (Olivares *et al*., 2015).

Importantly, the hippocampus is part of the hippocampal formation, an invagination of the parahippocampal gyrus that contains the hippocampus itself, the dentate gyrus, and the subiculum (Kotter *et al*., 1997). Within these regions are cellular strata consisting of molecular, pyramidal, and polymorphic neurons, leading into an upper region characterized by densely packed cells and a lower region with less densely packed neurons (Fitzgerald *et al*., 2022).

It has been shown that the hippocampus is involved in important functions related to emotions, spatial learning, short-term memory, and long-term memory consolidation (Afifi & Bergman, 2006). It has been rightly referred to as "responsible for affective life" (Saavedra *et al*., 2015).

In another context, low-power infrared laser emits stimulations, the irradiations of which at different doses modify the cellular components, ultimately leading to changes in their morphology and functionality.

In consideration of its proven properties, there is evidence of increased mitochondrial ATP synthesis (Xu *et al*., 2008), increased collagen synthesis (Cornejo *et al*., 2013), anti-inflammatory properties (Fikackova *et al*., 2006), regulation of cell proliferation (Vink *et al*., 2003), healing properties (Kreisier *et al*., 2003), and an increase in both protein synthesis (Shefer *et al*.,2003) and deoxyribonucleic acid synthesis (Karu, 2008).

Given this context, this study aimed to identify the structural alterations in the cellular components of mouse hippocampal neurons when exposed to escalating levels of infrared laser stimulation.

Material and Method

Animals

Twenty 3-month-old BALB/c mice, all females, weighing approximately 25 grams, were selected and kept in captivity for 15 days at 22°C under a regime of 12 hours of light and 12 hours of darkness, a diet of concentrated pellets (Alimentos Balanceados Ltda., Chile) and water intake ad libitum.

Experiment design

Four experimental groups were formed, each with 5 animals. Group 1 was the control, and Groups 2, 3, and 4 received infrared doses of 4, 8, and 16 J/cm2, respectively, for the 15 consecutive days.

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Sample processing for electron microscopy

At the end of the irradiation, the mice were euthanized by excess CO2. Then, the braincase was dissected using trepanation to extract biological material, which was then prepared for observation using transmission electron microscopy, fixation of the sample in Karnovski's solution in sodium cacodylate buffer pH 7.4, osmium tetroxide, uranyl acetate, and lead citrate, dehydration with acetone in increasing concentrations, and inclusion in propylene oxide and epoxy resins.

Samples for microscopic observation were obtained with an ultramicrotome, making ultrathin sections of 600 A, which were finally micrographed and studied in an Auriga electron microscope with a final magnification of 4000 X.

Morphometric evaluation of cellular components

We used the dot grid morphometric technique expressed as volumetric fractions to quantify the various neuronal components according to Weibel 1969.

Statistical analysis

The average ± SD obtained in each experimental condition was considered for the statistical analysis. Subsequently, the Kruskal-Wallis statistical test for independent samples was performed using the GraphPad Prism 8.0.2 statistical software. A p value >0.05 was considered statistically significant.

Results

The application of morphometric methods to evaluate the behavior of the different neuronal components in the hippocampus of mice irradiated with increasing infrared laser doses (figure 1 – 4).

It was hypothesized that cell size influenced the thickness of the dentate gyrus, so transmission electron microscopy was used to measure the size and volumetric fractions of the different neuronal components. The following data were obtained from these electron micrographs: In terms of cell size, there was a certain tendency to increase the neuronal size of the irradiated neurons compared to the control group. It could be inferred that the neurons irradiated at a dose of 4 J/cm2 were larger; however, there was no statistically significant difference (figure 5 - 10).

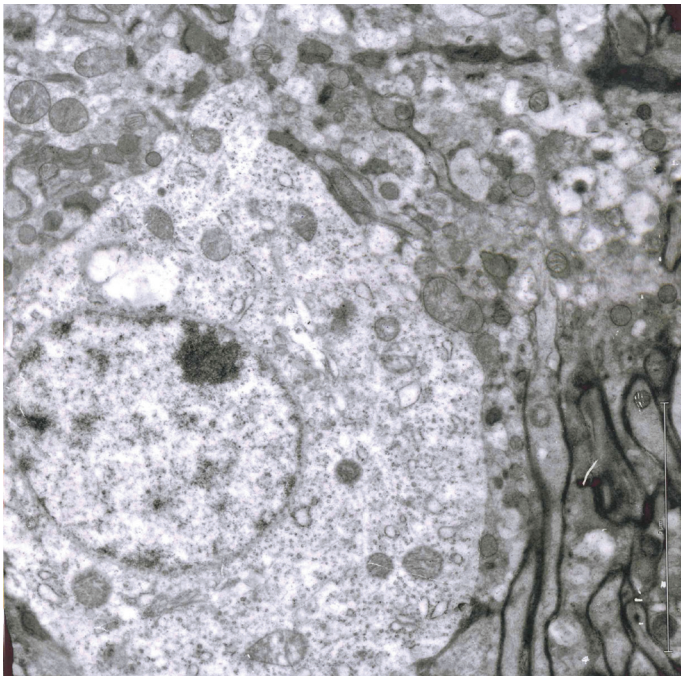

Figure 1. Transmission electron micrograph of the control group, corresponding to the hippocampal neuron of BALB/c mice. 4000x

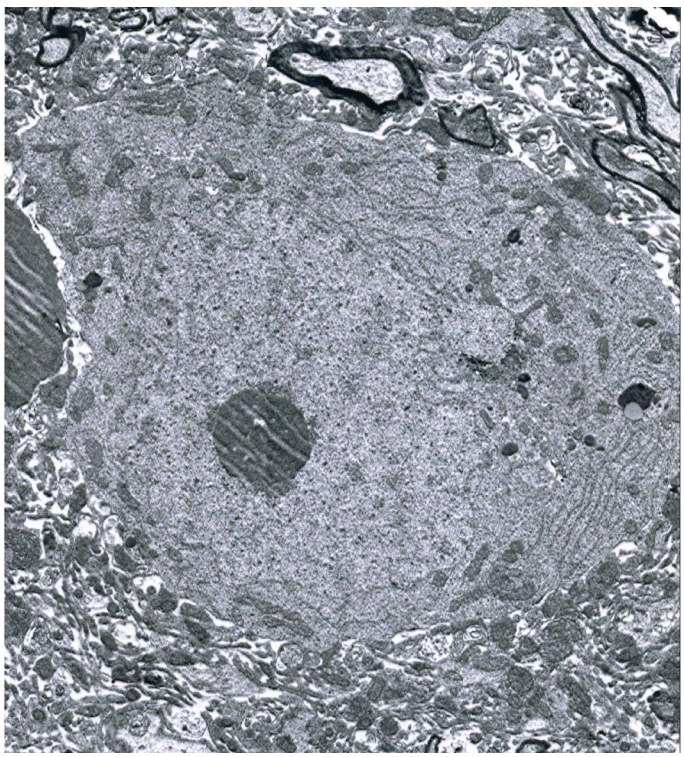

Figure 2. Transmission electron micrograph corresponding to hippocampal neuron from BALB/c mice irradiated with 4 j/cm2. 4000x

Discussion

The analysis of the results clearly shows that the infrared emissions and doses used can alter neuronal components, which could cause variations in their physiology.

Regarding the observed increase in both the quantity and dimensions of mitochondria in mouse hippocampal neurons stimulated with increasing infrared laser emissions, we concur with the findings of Yang *et al.*, 2017. Their study treated rats with infrared emissions for stroke, which resulted in a substantial enhancement in mitochondrial activity, leading to a noteworthy increase in ATP synthesis. The same situation was evidenced in the studies by Ando *et al.*, 2013, where an infrared laser was applied post-injury on spinal cord neurons, achieving increases in ATP and facilitating locomotor function.

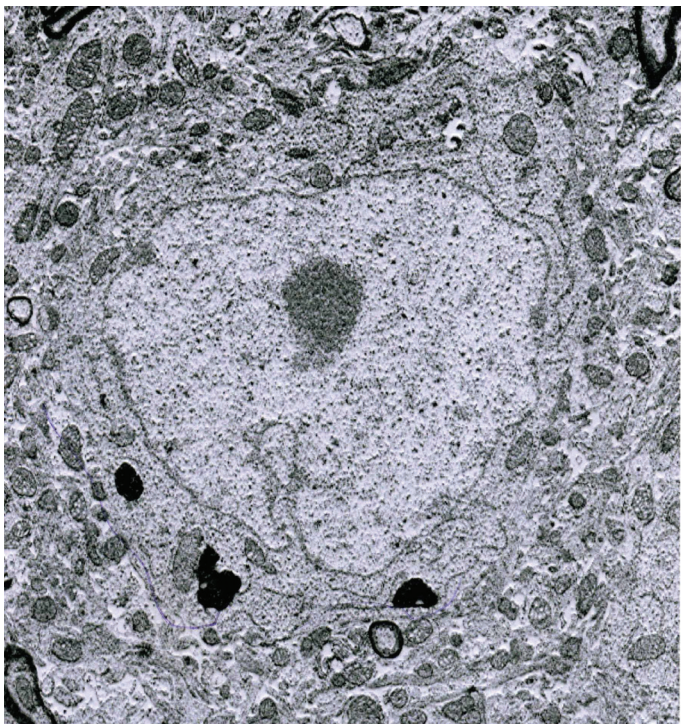

Figure 3. Transmission electron micrograph corresponding to hippocampal neuron from BALB/c mice irradiated with 8 j/cm2. 4000x

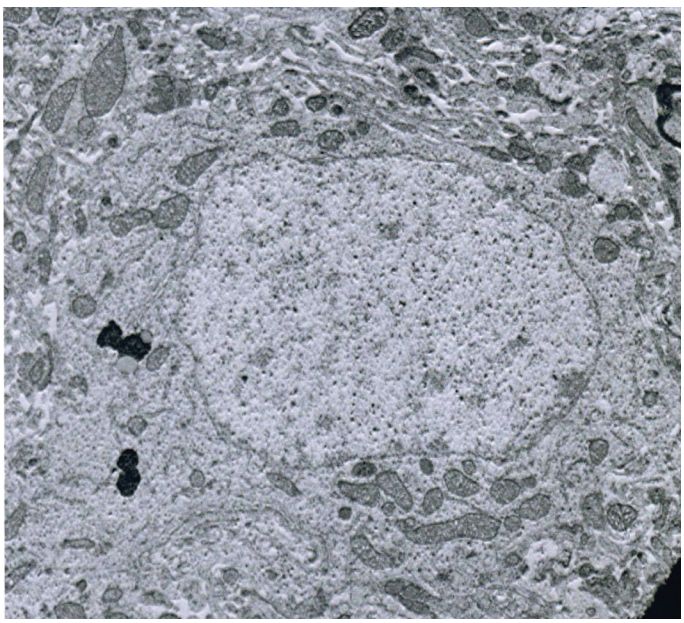

Figure 4. Transmission electron micrograph corresponding to hippocampal neuron from BALB/c mice irradiated with 16 j/cm2. 4000x

Working on hippocampal neurons under the effect of this photomodulatory mechanism, Salehpour *et al.*, 2018, demonstrated in a similar situation that these applications activate the functioning of complex IV of the mitochondrial respiratory chain, increasing ATP synthesis and eventually increasing mitochondrial volume.

Finally, Gao and Xing (2009) determined that photomodulation generates an increase in mitochondrial ATP and activates cytosolic calcium-releasing channels, which determine the activation of transcription factors and gene expression.

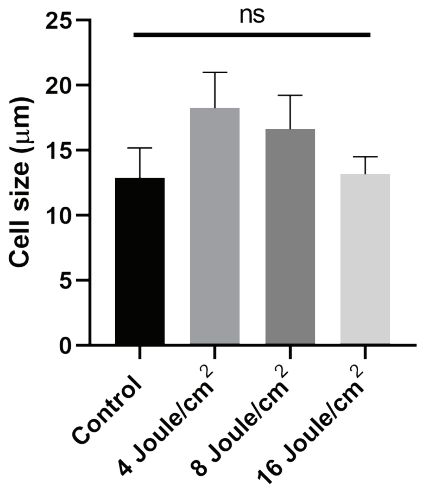

Figure 5. Cell size plot of mouse hippocampal neurons irradiated with increasing doses of infrared laser.
The size of the irradiated neurons increased progressively compared to the control group, and the greatest increase was noted with doses of 4 J/cm2.

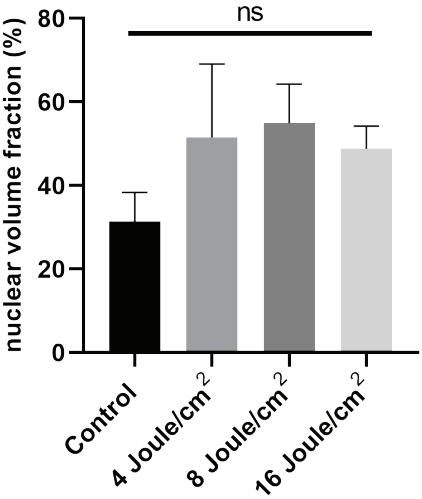

Figure 6. Graph of nuclear volumetric fractions of neurons in the hippocampus of mice irradiated with infrared emissions.
The volumetric fraction of the nucleus in the irradiated groups clearly shows a marked increase compared to the control.

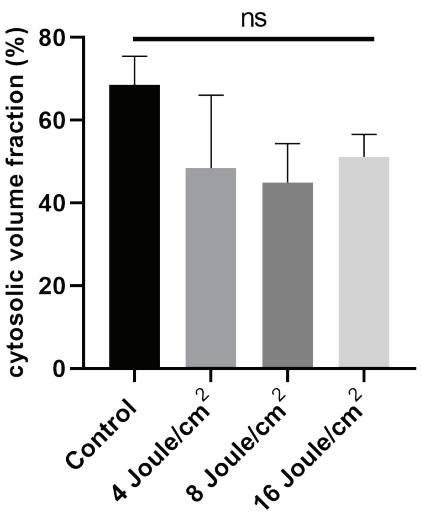

Figure 7. Cytosolic volumetric fractions of control hippocampal neurons and those irradiated with increasing doses of infrared laser.
A clear decrease in the cytosolic volumetric fraction of stimulated neurons is described, which coincides with the increases described for neuronal nuclei.

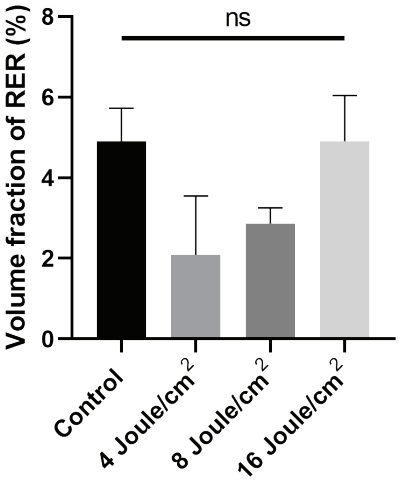

Figure 8. Descriptive graph of the volumetric fractions corresponding to the rough endoplasmic reticulum (RER) in hippocampal neurons of normal mice and those irradiated with increasing doses of infrared laser.
It is evident that the volumetric fraction of RER decreases in the groups irradiated with doses of 4 and 8 J/cm2 compared to the control group; however, as the irradiation dose increases to 16 J/cm2, the volume fraction increases and is equivalent to that shown by the control group.

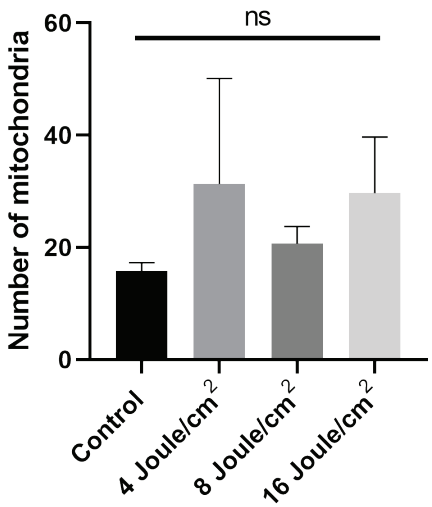

Figure 9. Description of the number of mitochondria quantified in hippocampal neurons from both normal mice and mice irradiated with increasing doses of infrared laser.
The number of mitochondria in the irradiated groups is significantly increased compared to the control; however, the increase is greatest in the group irradiated with 4 J/cm2.

The studies described clearly show the mitochondrial changes that respond to infrared stimulations compared to those of the control group. This situation

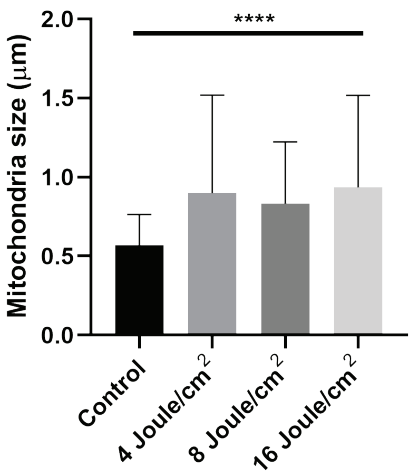

Figure 10. Graph highlighting the mitochondrial size obtained from hippocampal neurons of normal mice and those irradiated with increasing doses of infrared laser.
The mitochondria in the stimulated neurons are drastically larger than those of the control. Furthermore, the neurons stimulated with doses of 4 J/cm2 exhibit even more pronounced differences in this aspect.

is also consistent with the results obtained by Cornejo *et al*. in 2010 and 2013 and Espinoza in 2018, working on liver cells with inductions of 8 and 10 J/cm2, respectively.

The neuronal RER also undergoes modifications, presenting its maximum expression when stimulated with 16 J/cm2, the opposite of those observed by the authors cited above, who noted RER with up to 10 J/cm2, but bearing in mind that hepatocytes and neurons have different functions and therefore require different organelle densities.

Similarly, infrared stimulations do not alter the nuclear volumetric fraction closely related to the cytosolic one, and the nuclear-cytoplasmic ratio is maintained according to the results reported in the previously mentioned studies.

Finally, regarding the discrepancy in size observed in the stimulated neurons, which exhibited a sustained increase with significant evidence at doses of 4 J/cm2, it is important to consider and apply the Arndt-Schulz Rule (1920). This rule aids in interpreting the effects of a weak external factor that leads to substantial cellular modifications, as opposed to intense stimulations that inhibit these modifications and allow the cells to return to their original size at higher doses.

References

1. Olivares J, Juarez E, Garcia F. El hipocampo: Neurogenesis y aprendizaje. Revista Médica Universidad Veracruzana 20-28, 2015
2. Kotter D, Stephan K. The limbic system concept. Rev Neurosci 8, 139-145, 1997.
3. Fitzgerand G, Mtui E, Dockeery P. Neuroanatomia Clínica y Neurociencia. Elsevier España Ed 8.22.2022
4. Afifi A, Bergman R. Neuroanatomia Functional Ed Mcgraw-Hill Interamericana. 2006
5. Saavedra J, Diaz W, Zúñiga L, Navia C, Zamora T. Correlación funcional del sistema límbico y la memoria. Morfolia 7, 2015
6. Xu X, Zhao X, Liu T, Pan H. Low intensity laser irradiation improves the mitochondrial dysfunction of C2C12 induced by electrical stimulation. Photomedicine and laser surgery 26(3):197-202. 2008.
7. Cornejo R, Garrido O, Jaramillo R. Microscopia electrónica de núcleos hepáticos estimulados con dosis crecientes de laser infrarrojo. International Journal of Morphology 33(1): 222-228, 2015.
8. Vink E, Cajnie B, Cornelissen M, Declercq H, Cambier D. Increased fibroblast proliferation induced by light emitting diode and low

power laser irradiation. Lasers in medical science 18:95-99, 2003.
9. Fikackova H, Dostalova T, Vosicka R,Peterova V, Navratil L, Lesak, J. Arthralgia of the temporomandibular joint and low-level laser therapy. Photomedicine and laser surgery 24(4):522-527. 2006.
10. Kreisier M, Christoffers A, Willershausen B, Hoedt B. Effect of low-level GaAlAs laser irradiation on the proliferation rate of human periodontal ligament fibroblasts: an in vitro study. Journal of clinical periodontology 30(4):353-8, 2003.
11. Shefer G, Barash I, Oron U, Havely O. Low energy laser irradiation enhances of novo protein synthesis via its translation-regulatory proteins in skeletal muscle myoblast. Biochimica et Biophysica 1593:131-139, 2003.
12. Karu T. Mitochondrial signaling in mammalian cells activated by red and Near-RR radiation. Photochemistry and Photobiology 84:1091-1099,2008.
13. Weibel E. Stereological principes for morphometry in electron microscopic cytology. Int Rev Cytol,26 235-302, 1969.
14. Yang L, Tucker D, Dong Y, Wu C, Lu Y, Li y. Photobiomodulation therapy promotes neurogenesis by improving post-stroke local microenvironment and stimulating neuroprogenitor cells. Experimental Neurology 299: 86-96, 2018.
15. Ando T, sato S, Kobayashi H, Nahuashiro H, Ashida H, Hamblin M, Obara M. Low-level laser therapy for spinal cord injury in rat: effects of polarization. Journal of Biomedical Optics 18(9):1-6, 2013.
16. Salehpour F, Mahmoudi J, Kamari F, Sadigh Shamblin M. Brain photobiomodulation therapy: A narrative review.Molecular Neurobiology 55:6601-6636, 2018.
17. Gao X, Xing D. Molecular mechanisms of cell proliferation induced by pow power lase irradiation. Journal of Biomedical Science 16(1):4-9, 2009.
18. Cornejo R, Jaramillo R, Garrido O, Vergara L. Efecto del laser infrarrojo sobre células tiroideas. International Journal of Morphology 34(2):498-502, 2016.
19. Espinoza A. Cambios morfológicos en hepatocitos de rata irradiados con laser infrarrojo AsGa. Tesis Magister 2016.

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