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## CHARACTERISTICS OF STRUCTURAL CHANGES OF THE RETINA IN EARLY PERIOD OF TRAUMATIC OPTIC NERVE DAMAGE IN THE EXPERIMENT

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*The aim of this study was to characterize the structural changes of the retina in the early period of traumatic damage to the optic nerve.*

*Materials and methods.* The research used a model of crushing the optic nerve. The study was conducted on 30 mature rabbits with a body weight of 2.0 kg to 2.5 kg. Morphological examinations included light and electron microscopy. The retina of the right eye (control) and left eye (lesion side) was evaluated on the 14th day of the experiment.

*The animals were kept and removed from the experiment (through an overdose of narcotic drugs – ketamine) in accordance with the "Bioethical Requirements of the Helsinki Declaration on the Ethical Regulation of Medical Research". Protocol No. 125/22 dated March 24, 2022.*

*The results.* The results of the morphological study showed structural changes occurring in the retina on the 14th day after traumatic damage to the optic nerve, namely the presence of microcirculation disorders and the development of edematous and destructive processes. Neurons exhibit degenerative changes such as karyopyknosis, vacuolization, and colliquative necrosis. Lipofuscin inclusions were recorded in the layer of nerve fibers. On the other hand, presumably restorative and compensatory processes are observed, such as the appearance of young mitochondria and endoplasmic reticulum hypertrophy and hyperplasia.

*The thickness of the layer of ganglion cells of the retina increased by 52 %, and the nerve fibers layer – by 35 % on the affected side compared to the control side. What is found may be the result of the damage itself, but at the same time it can be seen as a protective mechanism and contribute to future recovery.*

*Conclusions.* It was found that changes in cytoarchitectonics of the retina in the early period of traumatic damage to the optic nerve are manifested by a combination of degenerative and restorative processes

*Keywords:* traumatic optic neuropathy, neuroinflammation, retinal ganglion cells, neuroprotection and neuroregeneration

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### 1. Introduction

Structural damage resulting from optic nerve injury is a serious problem that affects vision and quality of life. Traumatic optic neuropathy (TON) is the most common form of optic nerve injury, but to date, the mechanisms underlying its development and consequences are not yet fully understood [1].

The study of structural damage in the retina after optic nerve injury is of great importance. This allows us to gain important insights into the neurodegenerative and regenerative processes that occur because of injury to the retina and optic nerve. Elucidation of the mechanisms of these processes will help to develop more effective methods of treatment and rehabilitation of patients with TON.

The above facts from the literature confirm the importance of studying structural damage in the retina in case of optic nerve injury. Thus, studies [2] have shown that traumatic damage to the optic nerve causes crushing of the retina and leads to the loss of retinal ganglion

cells. This causes disruption of signal transmission from the retina to the brain and leads to a decrease in visual functions. Published studies [3, 4] have shown that structural damage in the retina, such as neuronal loss and changes in microarchitecture, contribute to neurodegeneration and progression of vision loss.

These facts indicate that the study of structural damage in the retina after optic nerve injury is important for understanding the neurodegenerative and regenerative processes that occur in this area. This knowledge may contribute to the development of new therapeutic approaches and treatment strategies aimed at restoring vision and improving the quality of life of patients with traumatic optic neuropathy.

Overall, the study of structural damage in the retina in optic nerve injury is key to understanding pathological processes and developing new treatments. This opens up prospects for improving the diagnosis, prognosis, and treatment of diseases associated with optic nerve

injury, and may have a significant impact on clinical practice in the future.

**The aim of the study** is to characterize the structural changes in the retina in the early period of traumatic optic nerve injury.

**2. Materials and methods**

The optic nerve crush model was used [5]. The study was conducted on 30 sexually mature chinchilla rabbits weighing from 2.0 kg to 2.5 kg, based on the vivarium of the Ivano-Frankivsk National Medical University 2022–2024.

Morphological examinations included light and electron microscopy. The retina of the right eye (control) and the left eye (lesioned side) were assessed on the 14th day of the experiment.

The maintenance of animals and their removal from the experiment (by overdose of narcotic drugs – ketamine) was carried out in accordance with the “Bio-

ethical Requirements of the Declaration of Helsinki on the Ethical Regulation of Medical Research”. Protocol of the Ethics Commission of the Ivano-Frankivsk National Medical University No. 125/22 dated 03/24/2022. Statistical processing of the obtained results was carried out using the parametric criterion of the Student's t-test (for comparison and determination of the significance of the difference) and applied descriptive statistics programs.

**3. Results**

The conducted study at the light-optic level showed an increase in the total thickness of the retina of the affected side by 30.11 μm (12 %), compared with the control side,  $p < 0.05$ . At the same time, the thickness of the GCS layer on the affected side was  $30.61 \pm 6.64$  μm, on the control side –  $20.02 \pm 3.61$  μm, i.e. increased by 10.59 μm, 52.9 %. The nerve fiber layer was  $22.08 \pm 4.35$  μm and  $16.36 \pm 4.72$  μm, respectively, i.e. increased by 5.72 μm, 35 % ( $p < 0.05$ ), Table 1.

Table 1

Comparison of the thickness of the components of the inner layer of the retina on the affected side and the opposite side on the 14th day of experimental optic nerve crush,  $M \pm SD$ , μm

Retinal layers	Affected side	Control side	Difference
RGC	$30.61 \pm 6.64$	$20.02 \pm 3.61$	$\uparrow * 10.59$ . 52.9 %
Nerve fibers	$22.08 \pm 4.35$	$16.36 \pm 4.72$	$\uparrow * 5.72$ . 35 %

Note: \* – significant difference ( $p < 0.05$ )

At the photoptic level, the number of retinal neurons was insignificant on day 14 (Fig. 1, a). Swelling of the inner nuclear layer (INL), outer nuclear layer (ONL) and RGC layer was observed. The

movement of nuclei from the INL to the outer mesh layer (OML) was detected. Microcystic degeneration of ganglion neurons and thickening and clarification of nerve fibers were noted.

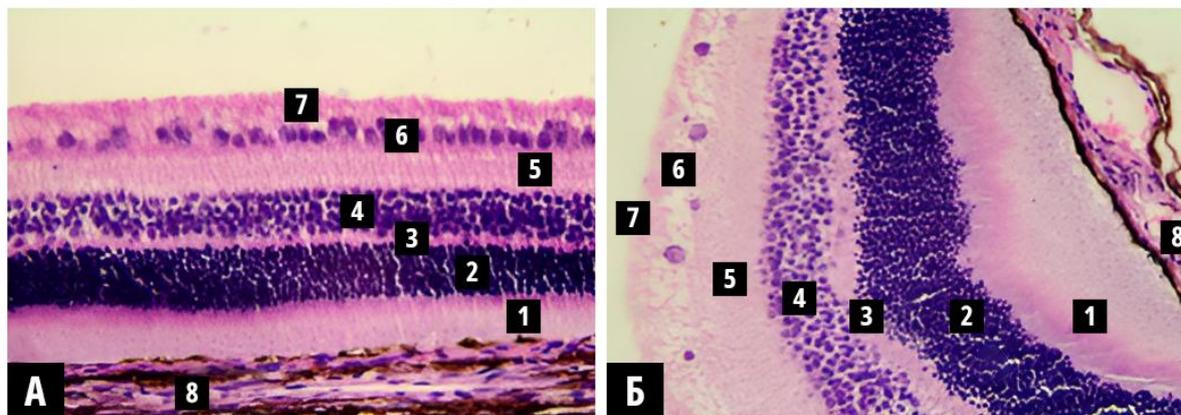


Fig. 1. Histostructure of the retina of the control side (a) and on the lesion side on the 14th day (b) after optic nerve crush: 1 – photoreceptor layer, 2 – ONL, 3 – OML, 4 – INL, 5 – inner retinal layer, 6 – RGC layer, 7 – NFL. Staining with hematoxylin and eosin. Microphotographs. Collection: (a, b, c) 400

According to the morphometric analysis, an increase in the area of the ganglion cell on the affected side was found to be  $71.68 \pm 8.87$  μm<sup>2</sup> compared to the control side of  $58.81 \pm 9.01$  μm<sup>2</sup>, by 12.87 μm<sup>2</sup>, 21.9 %. However, the nuclear-cytoplasmic index (NCI) was  $1.14 \pm 0.36$  and  $1.71 \pm 0.68$ , respectively, i.e. the index decreased by 0.57, 33.3 %,  $p < 0.05$  (Table 2).

At the ultrastructural level, some neurons in the INL show signs of karyopyknosis (nuclear damage), nuclear envelope invagination, cytoplasmic thinning, and vacuole formation.

Some neurons are in a state of colliquative necrosis. Neurons with a normal structure were also found (Fig. 2, a).

Table 2

Comparison of morphometric data of retinal ganglion cells on the affected side and the opposite side on the 14th day of experimental optic nerve crush, M±SD

Indicators	Side of the lesion	Control side	Difference
Ganglionic cell area, $\mu\text{m}^2$	71.68±8.87	58.81±9.01	↑*12.87. 21.9 %
Nuclear area, $\mu\text{m}^2$	37.06±6.20	36.20±6.63	–
NCI	1.14±0.36	1.71±0.68	↓*0.57. 33.3 %

Note: \* – significant difference ( $p < 0.05$ )

In the RGC layer, most neurons have moderate electron density and contain small vacuoles in the cytoplasm. The nuclei of neurons are triangular or irregular in shape due to significant nuclear envelope invagination. There is an expansion of the cisternae of the granular

endoplasmic reticulum, and some of them are damaged, reduced, and contain individual ribosomes. The structure of mitochondria was normal or, in some cases, partial disorganization of cristae and damage to the inner membrane were found (Fig. 2, b).

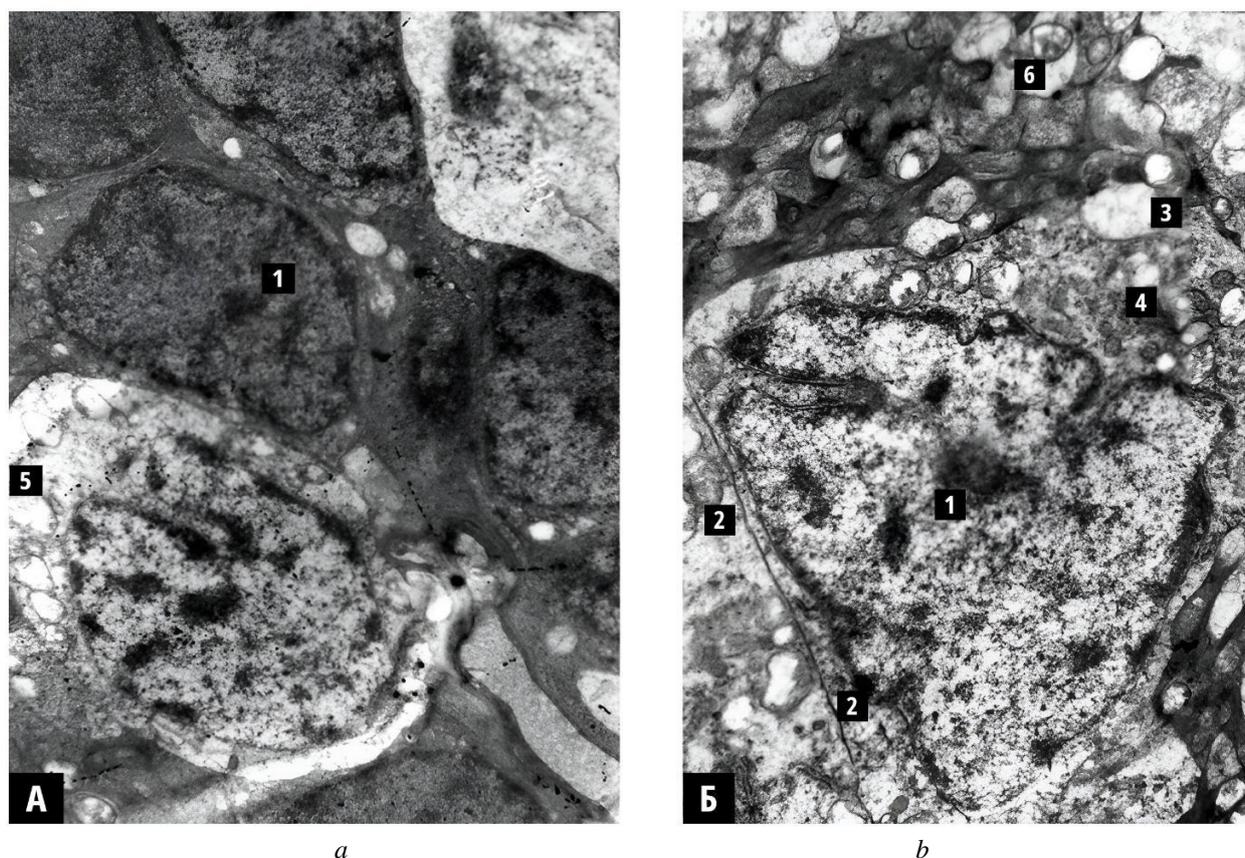


Fig. 2. Changes in bipolar (a) and ganglion neurons on the side of the lesion (b) in rabbits on the 14th day after optic nerve crush: 1 – nuclear envelope, 2 – mitochondria, 3 – vacuoles, 4 – *expanded* cisternae of the granular endoplasmic reticulum, 5 – colliquation necrosis of the neuron, 6 – nerve fiber layer. Electron micrographs. Collection: a – 4800; b – 6400

NFL were altered in four variants. In the first variant, a decrease in the number of neurofilaments, the absence of microtubules, and disorganization and damage to mitochondrial cristae were observed.

In the second variant, young mitochondria were detected in the neuroplasm of axons, and hypertrophy and hyperplasia of the granular endoplasmic reticulum (GER) were also observed.

The third variant was characterized by the presence of lipofuscin inclusions and karyopycnosis in nerve fibers. A significant part of such neurons was also in a state of colliquation necrosis.

Among the changed neurons, individual neurons with a normal structure were also found.

The found damage was observed against the background of retinal microcirculation disorders. The capillaries of the vascular network showed edema of endothelial cells, which led to narrowing of the vascular lumen. In addition, perivascular edema was observed, the arterioles had a slit-like lumen, and the venules were dilated. The vessels were overfilled with erythrocyte sludge (Fig. 3). Microthrombi were recorded in the arterioles and capillaries of the retina, which were formed as a result of adhesion of erythrocytes and platelets to the inner surface

of endothelial cells. The latter had an electron-bright cytoplasm and partially damaged membrane organelles. Kary-

opyknosis, disorganization, and destruction of mitochondrial cristae were observed in myocytes and sericites.



Fig. 3. The lumen of the retinal arteriole of rabbits on the 14th day after traumatic injury to the orbital part of the right optic nerve: 1 – erythrocyte, 2 – platelet, 3 – myocyte, 4 – luminal surface of endothelial cells (5). Electron micrograph. Collection: 8000

#### 4. Discussion

According to the results of morphological studies, structural changes occurring in the retina on the 14th day after traumatic optic nerve injury were revealed.

It was found that the thickness of the retinal ganglion cell layer increased by 52 %, and the nerve fiber layer by 35 % on the affected side compared to the control side. The finding may be a result of the injury itself, but at the same time it can be considered as a protective mechanism and contribute to future recovery.

According to the literature, several studies indicate a possible increase in the thickness of the retinal ganglion cells (RGC) layer in traumatic injuries. Thus, some authors, back in 1996 [6] observed the growth of RGC axons and considered this a sign of regeneration. A similar phenomenon was observed under the influence of therapeutic manipulations by another group of scientists in 2000 [7].

However, it is important to note that not all studies confirm these changes in RGC thickness or have an unambiguous interpretation of these results.

Characterizing the morphometric parameters of the retina on the 14th day after optic nerve crush, an increase in the area of ganglion cells on the side of the lesion by 21.9 % was found, which may be the result of compensatory mechanisms aimed at restoring and compensating for damaged cells. A decrease in NCI by 33.3 % probably reflects changes associated with remodelling of the cell nucleus and cytoplasmic components due to traumatic injury.

At the ultrastructural level, according to the study in the INL and the RGC layer, it can be assumed that neurodegenerative and neuroregenerative processes develop in the retina during optic nerve injury.

In particular, neurodegenerative changes were characterized by neuronal damage, which showed signs of karyopyknosis, nuclear envelope invagination, cytoplasmic thinning, and vacuole formation.

Neuroregenerative processes are also present. Preserved neurons with normal structure and shape are detected.

These changes at the ultrastructural level indicate a complex retinal response to traumatic optic nerve injury. Neurodegenerative processes are manifested in damage and necrosis of some neurons, while neuroregenerative processes are manifested in the preservation and restoration of other neurons.

A decrease in the number of neurofilaments, the absence of microtubules, and disorganization and damage to mitochondrial cristae in part of the nerve fibers were detected in most animals on the 14th day after optic nerve crush in the experiment. Since neurofilaments are important components of the cytoskeleton of neurons, which play an important role in maintaining their morphology and functioning and are also an important element of axonal transport [8]. That is why the dysregulation of neurofilament proteins is widely studied to understand the processes of neurodegenerative and regenerative processes [9].

Disorganization and damage to mitochondrial cristae, which was also recorded in NFL in experimental animals during the study, presumably underlies metabolic damage to the optic nerve. According to modern studies, most optic neuropathies are considered mitochondrial degenerations [10]. It is mitochondrial dysfunction that underlies the loss of RGCs and nerve fibers in the prelaminar part of the optic nerve, which underlies many acute diseases [11].

According to the results of the study, in rabbits on the 14th day after experimental optic nerve damage, lipofuscin inclusions and karyopyknosis in nerve fibers were recorded in NFL. According to the literature, lipofuscin inclusions are areas of lipofuscin accumulation, which is a consequence of the accumulation of oxidation products and other metabolic waste. Lipofuscin accumulation can be considered as one of the biomarkers of toxicity [12] and is therefore studied in the pathogenesis of retinal pigment degeneration [13].

In more severe cases, the results of the study revealed nerve fibers with signs of karyopyknosis and colliculation necrosis. Similar phenomena are observed by a number of authors [14] in sudden disorders of retinal blood circulation (multiple embolization, which also occurred in our experiment). Clinical examination reveals cotton-like exudates, and ultrastructurally it is confirmed by the formation of cytooid bodies.

On the other hand, young mitochondria were detected in the neuroplasm of some axons, hypertrophy and hyperplasia of GER were observed. Since the detection of young mitochondria in the neuroplasm may indicate active processes of mitochondrial biogenesis and repair. Young mitochondria may be the result of the restoration and replacement of damaged mitochondria. This may be an indicator of a positive regenerative response of the organism to injury. According to the observations of the authors [15, 16], experimental enhancement of mitochondrial transport accelerates axonal regrowth in several models of optic nerve injury [17].

Thus, the study of structural changes occurring in the retina on the 14th day after traumatic injury shows the presence of microcirculatory disorders and the development of edematous-destructive processes. Neurons demonstrate degenerative changes, such as karyopyknosis, vacuolization and colliquation necrosis. On the other hand, presumably restorative-compensatory processes are observed, such as the appearance of young mitochondria and hypertrophy and hyperplasia of the GER.

**Study limitations.** The main limitation is the high degree of variability in the modeling of traumatic optic nerve damage and the complexity of statistical data analysis. The differences relate to the initial manifestations and course of traumatic optic neuropathy. All this complicates the classification of the disease and the definition of basic diagnostic criteria.

**Prospects of the research.** A more detailed study of the pathogenetic mechanisms of structural changes

and their correction will contribute to further broader research into this pathology and the development of its effective neuroprotective treatment.

## 5. Conclusions

As a result of the analysis, it was found that changes in the cytoarchitectonics of the retina in the early period of traumatic optic nerve damage are manifested by a combination of degenerative and regenerative processes. An increase in the total thickness of the retina on the affected side by 30.11  $\mu\text{m}$  (12 %), compared with the control side, was found.

At the same time, the thickness of the RGC layer on the affected side was 30.61 $\pm$ 6.64  $\mu\text{m}$ , on the control side – 20.02 $\pm$ 3.61  $\mu\text{m}$ , i. e. increased by 10.59  $\mu\text{m}$ , 52.9 %. The nerve fiber layer was 22.08 $\pm$ 4.35  $\mu\text{m}$  and 16.36 $\pm$ 4.72  $\mu\text{m}$ , respectively, i. e. increased by 5.72  $\mu\text{m}$ , 35 %.

Manifestations of degenerative changes include karyopyknosis, vacuolization, and colliquation necrosis. On the other hand, presumably restorative and compensatory processes include the appearance of young mitochondria and hypertrophy and hyperplasia of GER. The detected changes at the ultrastructural level indicate a complex reaction of the retina to traumatic damage to the optic nerve.

These processes in traumatic damage to the optic nerve in the early stages necessitate a more detailed further study of the features of this pathology at later stages of experimental research.

## Conflict of interests

The authors declare that they have no conflict of interest regarding this study, including financial, personal, authorship or other, that could influence the study and its results presented in this article.

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## Data availability

Data will be provided upon reasonable request

## Use of artificial intelligence tools

The authors confirm that they did not use artificial intelligence technologies when creating the presented work.

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