Dynamics of Structural Changes in the Retina during Long-Term Exposure to Bright Light

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Exposure to high-intensity light led to pronounced destructive changes in the retina and focal loss of layers formed by neurosensory cells in rats. Photoinjury led to progressive decrease in the numerical density of nuclei in the outer nuclear layer (by 30% after 2 days, by 75% after 1 week, and more than by 90% after 2 weeks of exposure). After 30 days the photosensory layer completely disappeared, while the outer nuclear layer was presented by solitary nuclei of neurosensory cells.

Key Words: *retina; light*

Photoinjury leads to degeneration of various retinal cells, the degree of this degeneration depends on the intensity and duration of exposure and source of light [1,4]. Today opinions on "priority targets" of retinal photoinjury are different. According to some reports, the initial changes involve the pigmented epithelium [5,10]. Other scientists observed primary dose-dependent changes in the neurosensory cells [2, 11]. There are few studies dealing with morphological changes in all or in the majority of structural elements of the retina after light exposure [9]. The dynamics of morphological changes in the retina during long-term light exposure is little studied. The data of quantitative analysis of populations of neurosensory cells and other retinal neurons and information about disorders in their relationships caused by photoexposure of varying duration are scanty.

We studied the dynamics of injuries to various structural components of the retina during long-term exposure to high-intensity light.

MATERIALS AND METHODS

Experiments were carried out on 35 random-bred adult albino rats of both sexes. The animals were exposed to even illumination for 1, 2, 7, 14, and 30 days with LB-40 fluorescent lamps with the maximum radiation in the violet and green spectra. The exposure was carried out using special devices made from rectangular reflectors with built-in lamps. The illumination intensity was 3500 lux. Control group consisted of 10 intact rats kept under conditions of artificial light/dark regimen (12/12 h) at daytime illumination intensity of 25 lux.

For ultrastructural analysis, central fragments of the posterior wall of the eye were fixed in 2.5% glutaraldehyde on cacodilate buffer (pH 7.4), postfixed in 2% osmium tetroxide, and embedded in epon. Semithin sections were stained with toluidine blue, ultrathin sections were contrasted with uranyl acetate and lead citrate, examined and photographed under a JEM-100 CXII electron microscope. For light microscopy the posterior wall of the eye was fixed in Carnoy fluid. The ratio of neurosensory cells and associative neurons was counted on 5-7-μ transverse sections stained with hematoxylin and eosin. The specific area of retinal layers was determined using Avtandilov ocular grid, the ratio of dots per layer to the total number of dots in the entire retina was estimated. Neurosensory cells with nuclear destruction (pyknosis, rhexis, lysis) were counted per 1000 photoreceptors on semithin sections from each retina, numerical density (per mm^2) and the number of rows of nuclei in the outer nuclear layer were counted. The significance of differences in the mean values was evaluated using Mann—Whitney test.

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RESULTS

A 24-h exposure to intense illumination increased basal plication and hypertrophy of apical processes in the pigmented epithelial cells, vesicular degeneration and rupture of membranes of the outer segments in the photosensory layer. Ultrastructural changes in bipolar and amacrine neurons of the internal nuclear layer consisted in swelling of mitochondria and dilatation of the endoplasmic reticulum cisterns. In radial gliocytes elongated microvilli and increased electron density of desmosome-like contacts of scleral processes were seen. Vitreal processes were vacuolated. The number of polysomes in the ganglionar layer neurons decreased and microvesicles appeared in the cytoplasm.

Changes in the retina after 48-h illumination were heterogeneous. The number of open choroidal capillaries sometimes decreased. The majority of external segments of the neurosensory cells were fragmented. The density of the nuclei distribution in the outer nuclear layer was decreased because of glial processes proliferation. Some neurosensory cell nuclei underwent karyopyknosis, karyorhexis, or karyolysis. Glial layers surrounding destructive photoreceptors were characterized by reduced electron density and moderate hypertrophy (Fig. 1). Synapses of the internal retinal layer were characterized by decreased number and increased osmiophilia of vesicles. Stasis and slugging of formed elements were seen in other areas (choroidal capillaries). Pigmented epitheliocytes were stuffed with phagosomes with fragments of external segments of photoreceptors at different stages of lysis. Internal segments were characterized by mitochondrial edema, dilatation of the endoplasmic reticulum cisterns.

After 7-day intensive illumination specific area and the number of nuclear rows of the outer nuclear layer decreased by more than 50 and 70%, respectively, in comparison with the control (Table 1). The ratio of the neurosensory cells and neurons in the outer nuclear layer decreased to 1:1 *vs.* 6:1 in the control. Somewhere the photosensory layer was absent (50.11± 7.32% retinal section), the perikaryons of the preserved neurosensory cells were surrounded by glial lamellar membranes. The percentage of gliocytes in the internal nuclear layer sharply increased, these cells

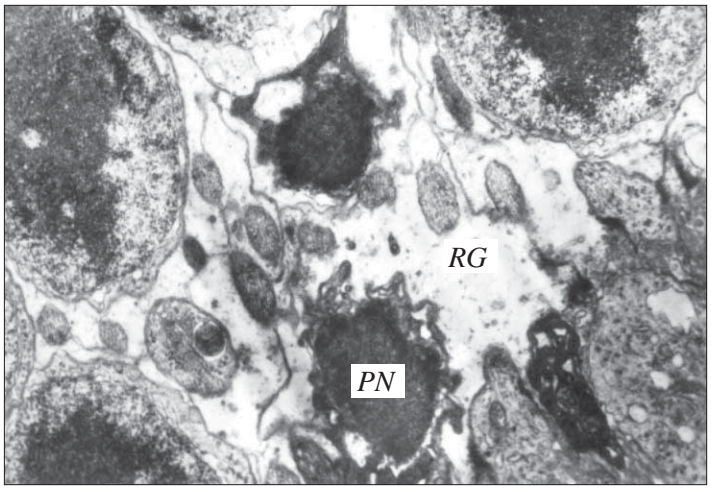

Fig. 1. Focal hypertrophy and decreased electron density of radial glia surrounding destructively modified nuclei of neurosensory cells after 48-h illumination. *RG*: radial glia; *PN*: pyknotic nucleus of a neurosensory cell; ×7200.

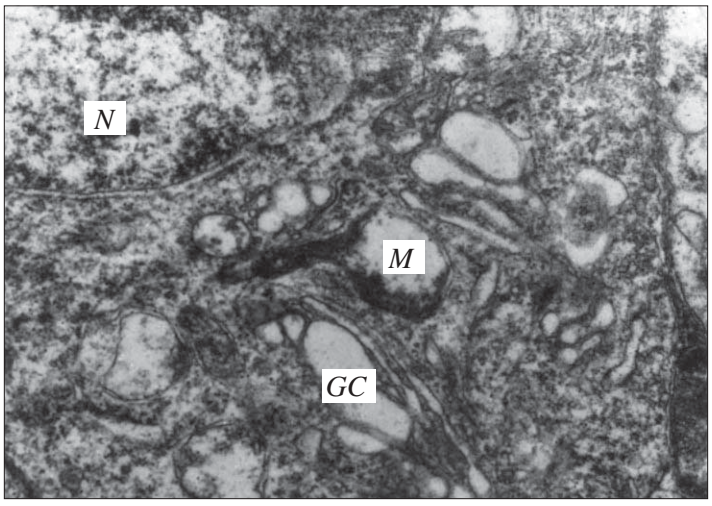

Fig. 2. Cytoplasm vacuolation, dilated cisterns of endoplasmic reticulum, Golgi complex, and destruction of mitochondria in an amacrine neuron after 7-day exposure to intensive light. *N*: nucleus of an amacrine neuron; *M*: mitochondria; *GC*: Golgi complex; ×8000.

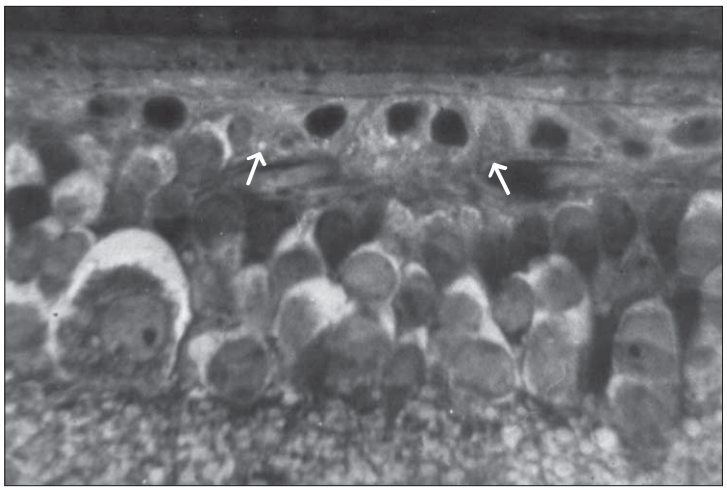

Fig. 3. Disappearance of photosensory and pronounced thinning (shown with arrows) of outer nuclear layer after 14-day intense illumination. Semithin section. Toluidine blue staining, ×900.

were characterized by increased electron density of the cytoplasm and nuclear pyknosis. Many bipolar and amacrine neurons were roughly vacuolated and the majority of their organelles were destroyed (Fig. 2).

In other regions the photosensory layer was filled with destructively modified fragments of the external and internal segments, vacuoles with homogeneous contents. Glial elements exhibited pronounced phagocytic activity towards pyknotic neurosensory cells, (surrounded and penetrated into them). The number of synapses in the inner retinal layer decreased. The remaining contacts were characterized by lowered electron density of presynaptic compartments, disaggregation and destruction of synaptic vesicles, and decreased number of organelles.

After 2-week exposure vacuolation of the cytoplasm, hyperchromia and pyknosis were seen in endotheliocytes of the remaining choroidal blood capillaries. The outer nuclear layer of the majority of sites (90.32±7.63% of retinal section length) was presented by solitary nuclei of neurosensory cells contacting with pigmented epithelium (Fig. 3). The specific area of the outer nuclear layer was 7.000±0.032% (*vs.* 25.00±0.57% in the control), the outer retinal layer disappeared, and the ratio of neurosensory cells to neurons in the internal nuclear layer decreased to 1:3.

After 1-month exposure pigmentoepitheliocytes were hypertrophic and contained numerous residual corpuscles. The basal complex was thickened and stratified. Solitary nuclei of neurosensory cells were seen in intermediate layers of osmiophilic radial glia containing myelin-like and multivesicular corpuscles. The ratio of neurons of the outer and internal nuclear layers decreased to 1:6. Active neovasculogenesis processes were observed between pigmented epitheliocytes and perikaryons of neurosensory cell. New blood capillaries contained large endothelial cells with thick basal membrane. Sites of the retina without pigmented epithelium and layers of neurosensory cells were detected. The internal nuclear layer came into contact with the basal complex.

The state of the nuclear structures is one of the main criteria indicating whether changes in neurosensory cells are reversible (Table 1). The content of degenerative nuclei (pyknosis, rhexis, lysis) in experimental group exposed to 24-h illumination virtually did not differ from that in the control. After 48-h illumination the number of destructively changed nuclei

TABLE 1. Dynamics of Changes in Outer Retinal Nuclear Layer after Intensive Illumination of Different Duration (*M*±*m*)

Duration of exposure	Outer nuclear layer		
	destroyed nuclei, %	numerical density of nuclei per mm^2	number of rows of nuclei
Control	0.20±0.02	4256±206	16.00±2.12
1 day	1.12±0.40*	4237±54	15.40±0.42
2 days	7.56±0.43*+	3016±13*+	15.20±0.33
1 week	7.45±0.53*	1164±147*+	4.07±0.47*+
2 weeks	7.82±0.51*	720±76*	3.40±0.51*
1 month	7.27±0.58*	84±20*+	0.90±0.18*+

Note. $p<0.05$ *compared to the control, +compared to previous term.

of photoreceptors reached 7.7±1.4% and remained at this level until day 30 of illumination, but the dynamics of changes in numerical density and number of layers of nuclei in the outer nuclear layer indicated a progressive reduction of these parameters, which decreased to a minimum on day 30 of exposure.

Hence, intensive illumination of different duration (1, 2, 7, 14, 30 days) leads to changes in the retina of different severity. The most sensitive components are the neurosensory cells. This is explained by the fact that illumination induces excessive accumulation of retinal in the photoreceptors, which induces free radical oxidation of proteins and lipids of the photoreceptor membrane, leading to dysfunction of the retinal antioxidant system [3,8]. Long-term photodamage leads to irreversible decrease of the neurosensory cell population, which is seen from progressive decrease in the number of rows, thickness, numerical density of nuclei of the outer nuclear layer and the ratio of the outer to internal nuclear layers. Alterative changes in the choriocapillaries and pigmented epithelium, leading to disorders in circulation and metabolism, play an important role in the mechanisms of retinal photodegeneration [6,7]. Associative neurons are more resistant to prolonged light exposure than neurosensory cells, which is seen from the dynamics of their quantitative ratio during the exposure.

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