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Asian Journal of Animal and Veterinary Advances



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## The Effect of Intermittent Blue Light on Ultrastructure of the Retinal Photoreceptor Cells in Rat: A TEM Study

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### ABSTRACT

The objective of this experiment was to determine the effect of blue light with different exposure time on ultrastructure of the retinal photoreceptor layer. Fifteen male Wistar rats were divided into three groups: control group and experimental I and II groups. The rats in experimental groups were exposed with intermittent LED blue light for 2/2 h and 3/3 h dark/light cycles with a 60 W LED blue bulb, respectively. Statistical analysis was done using SPSS version 16.00. The thicknesses of photoreceptor layer were analysed with one-way ANOVA. Results showed that in the experimental groups, photoreceptor was damaged apparently. The extent of photoreceptor damages depended on the duration of blue light exposure. Damages in photoreceptor cells were the most evident in the experimental groups I and II. It was concluded that the exposure of photoreceptor cells to LED blue light provokes cell death which may related to photochemical events.

**Key words:** Retina, photoreceptor cell, transmission electron microscope(TEM), LED blue light, rat

### INTRODUCTION

A Part from limitations imposed by the optical properties of the eye, the limits of visual acuity depend on the arrays of neurons in the retinal network. As is characteristic of nocturnal mammals, the rat retina is strongly rod-dominated, with rods making up 97% of all photoreceptors. Cones constitute a much smaller proportion of photoreceptors (Carter-Dawson and LaVail, 1979; Jeon *et al.*, 1998) in the house mouse than in diurnal rodents such as the ground squirrel (Kryger *et al.*, 1998). The structure of the mouse retina has been thoroughly studied anatomically using Nissl staining and electron microscopy (Carter-Dawson and LaVail, 1979; Jeon *et al.*, 1998; Drager and Olsen, 1981; Tsukamoto *et al.*, 2001).

Various histological studies were undertaken on photoreceptor structures of the retina in different animals under light and electron microscope (Braeckevelt, 1983, 1987, 1992, 1993, 1998; Braeckevelt *et al.*, 1996, 1998; Garcia and de Juan, 1999; Haacke *et al.*, 2001).

In an electron microscopic study, Esfandiari *et al.* (2009) observed that the retinal photoreceptor layers of the rabbits exposed to the bright light with different intensities had been damaged, although, there is no ultrastructural evaluation of the effect of intermittent blue light on

photoreceptor layer of the retina. The aim of the present study was to investigate the effect of intermittent blue light with different time exposure on histological changes of photoreceptor layer of the retina in the rat model.

## MATERIALS AND METHODS

All studies were performed in accordance with the Guide to the Care and Use of Laboratory Animals (Olfert *et al.*, 1993).

Fifteen male wistar rats were housed in a 12 h light-dark cycle with an average illuminance of 100 lux in each cage. The rats were fed with commercial pellets and had free access to water. The same room temperature, light history and diet were used before and after the light exposure times.

The light source consisted of 60 W LED blue bulbs. Intensity of the blue light measured with a power meter and was 300-400 lux in each cage. One LED bulb was mounted vertically which stool 170 mm above the floor.

The enclosure for holding the rats was made of two standard wire-bar cages, each of which 450 long, 280 wide and 150 mm high.

The rats were randomly divided into three similar groups 5 of each with the same body weight (Mean $\pm$ SD): control group (CON), experimental group I (EXP-I), experimental group II (EXP-II).

The animals in the CON group were kept in 12 hours light-dark cycles with an average illuminance of 100 lux in the cage. The rats in EXP-I and EXP-II groups were exposed with a 60 W LED blue bulb for 2/2 and 3/3 h dark-light cycle, respectively. After light exposure, the rats were killed with an overdose of pentobarbital. The eyes were removed and marked for orientation. The eyeball fixed in 4% gluteraldehyde and retina separated near the optic nerve. Retina processed for transmission electron microscopy. Thin sections were obtained and examined on a Philips CM-10 (Philips, Eindhoven, Netherlands).

**Statistical analysis:** Statistical analysis was done using SPSS version 16.00. The thicknesses of photoreceptor layer were analysed with one-way ANOVA. The results present as mean $\pm$ SD. The significance level was set at  $p < 0.05$ .

## RESULTS

In the CON group, the rod and cone outer segments were composed of a stack of bimembranous discs surrounded by the cell membranes. The slender mitochondria and round to oval shape, rough endoplasmic reticulum and glycogen particles were presented in the inner segment. The outer limiting membrane appeared normal. The outer nuclear layer consisted of rod and cone nuclei and appeared normal in CON group (Fig. 1).

The histomorphology of photoreceptor layer in the experimental rats were examined after intermittent LED blue light exposure.

As shown in Fig. 2-4 different exposure times of LED blue light had a dramatic effect on photoreceptor layer of retina.

The EXP-I group had minor signs of pathology. The loss of few outer segment and extremely vacuolation in inner segment were obvious (Fig. 2). Condense and pyknotic nuclei of outer nuclear layer were apparent in this group but outer limiting membrane appeared normal (Fig. 2).

The major sign of pathology observed in the EXP-II group. Extensive loss of outer segments was evident. The inner segment was extremely vacuolated and cell swelling and increased extracellular space were obvious (Fig. 3). The inner segment region looked abnormal and disorganized (Fig. 3). The pyknotic and condense nuclei observed in this group (Fig. 4). The outer limiting membrane appeared normal (Fig. 3).

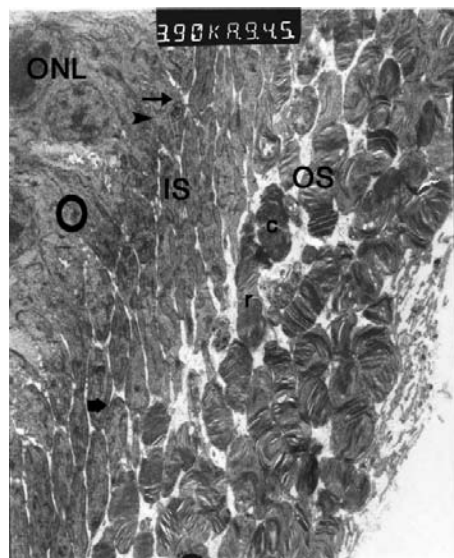


Fig. 1: Electromicrograph of the photoreceptor layer in control group. The outer segment (OS), the outer segment of rod cell (r) and cone cell (c), the inner segment (IS), the mitochondrion (thick arrow) and the outer limiting membrane (circle), (x3900). ONL is the outer nuclear layer

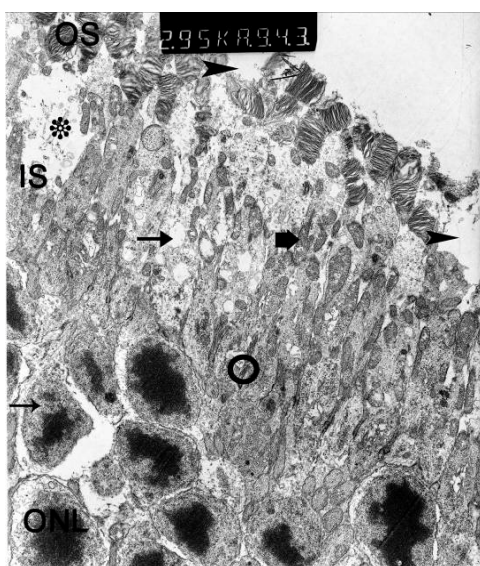


Fig. 2: Electromicrograph of the photoreceptor layer in EXP-I group. The outer segment (OS), the loss of outer segment (arrowheads), the inner segment (IS), the mitochondrion (thick arrow), the large vacuole (asterisk) and small vacuole (arrow), the outer limiting membrane (circle), the outer nuclear layer (ONL) and pyknotic nucleus (thin arrow), (x2950)

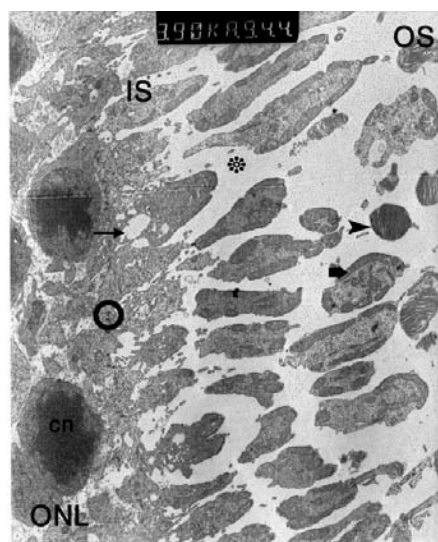


Fig. 3: Electromicrograph of the photoreceptor layer in EXP-II group. The extensive loss of outer segment (OS), the outer segment (arrowhead), the disorganized inner segment (IS), the mitochondrion (thick arrow), the vacuole (thin arrow), the increased extracellular space (asterisk), the outer nuclear layer (ONL) and condense nucleus (cn), (x3900)

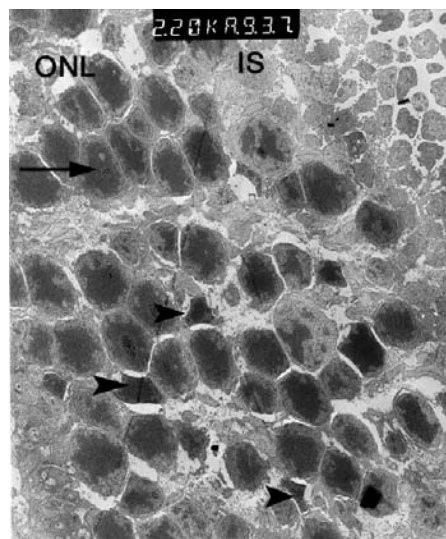


Fig. 4: Electromicrograph of the photoreceptor layer in EXP-II group. The disorganized inner segment (IS), the outer nuclear layer (ONL) and condense nucleus (arrow) and pyknotic nuclei (arrowheads), (x2200)

The mean thickness of the photoreceptor layer was  $85.50 \pm 1.05 \mu\text{m}$  in the CON group, whereas in the EXP-I and EXP-II groups it was  $74.56 \pm 0.85$  and  $61.26 \pm 3.57 \mu\text{m}$ , respectively.

## DISCUSSION

Exposure of the sensory retina to LED blue light with different exposure times resulted in damage to the photoreceptor layer. The quantitative protocol in the present study showed that the reduction in the thickness of photoreceptor layers in experimental groups was due to outer segment loss and karyolysis in outer nuclear layer.

The photoreceptor layer constitutes a functional unit which provides the transducing interface for visual perception (Strauss, 2005). The Retinal Pigment Epithelium (RPE) is also a metabolic complex and active cell layer that is important for local homeostasis and maintenance of the extra-photoreceptor matrix. The role of the RPE in respect to photoreceptor function account for the impact on sight of any abnormality that affects the layer. The RPE transplantation can benefit to the restoration of supporting function for photoreceptor needs. Therefore, optimal functioning of the outer segment of photoreceptor cells dependent on role of the RPE in outer segment turnover (Hansson, 1971).

Vacuoles appeared in inner segment of photoreceptor layer of experimental groups. They are a common response to injury in cells with sublethal damage and are a mechanism by which the cell rids itself of damaged or senescent organelles (Hansson, 1971). In addition, the cell swelling in EXP-II group was obvious. Although, mechanisms of cell swelling are limited, variations in appearance may occur because of differences in cell type and cause of injury. Vacuoles can be caused cell swelling by abnormal storage of carbohydrate or lipid or water. The pyknotic nuclei appeared in EXP-I and EXP-II. This change is common response to the light damage.

These observations confirm numerous previous studies regarding the susceptibility of rats to damage from intense green light (Noell and Albrecht, 1971; Noell, 1979, 1980).

Measurements of photoreceptor layer were significantly lower in EXP-II and EXP-I groups than in the CON group ( $p < 0.05$ ). According to the present results, it can be concluded that exposure to LED blue light can cause retinal photoreceptor damage and increasing of the exposure time extends more retinal damage.

## ACKNOWLEDGMENT

This study was conducted under the sponsorship of the Kazerun branch, Islamic Azad University, Kazerun, Iran.

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