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Scanning Electron Microscopy of Photoreceptor Cells in Male Cat under the Effect of Continuous Light Exposure and Dark Adapted

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Abstract: The photoreceptor layer in the retina under the effect of continuous light exposure and dark adapted of male domestic cat (felis catus) was studied by scanning electron microscopy. Two types of receptors were identified: Rods and single cones and no double cones were present. Rods and single cones were present in a ratio of 19:1, respectively in control group, but in experimental groups, the concentration of rods and single cones was reduced. In light exposure group, these cells were extremely elongated while in the dark adapted state their length was a little reduced in comparison with control group. But in control group, there was no remarkable difference between the average of the thickness of rods and cones in comparison with experimental groups.

Key words: Photoreceptor, scanning electron microscopy, light exposure, dark adapted

INTRODUCTION

The fine structure of retinal photoreceptors has been investigated in a variety of vertebrate species because of their position as the first neuron in the visual pathway as well as their heavy involvement in protein production as they continuously regenerate outer segment discs (Braeckvelt, 1982). This report describes the variation in length, thickness and concentration of the rods and cones during continuous light and dark adaptation in the male cat retina.

In rod photoreceptors the outer segment discs are all of the same diameter while in cones the apical discs are smaller than those of the basal region giving the outer segment conical shape (Braeckevelt, 1982). Other scientists studied the fine structure of the photoreceptor layer in different animals such as in the butterfly fish (Braeckvelt, 1990), red-backed salamander (Braeckvelt, 1992), red-tailed hawk (Braeckvelt, 1993), barred owl (Braeckvelt *et al*., 1996) emu (Braeckvelt, 1998), black bass (Garcia and Dejuan, 1999), grenadier anchovy coilia nasus (Haacke *et al*., 2001). Retinal light damage in rats exposed to intermittent light in comparison with continuous light exposure was studied by Daniel *et al*. (1989). They concluded that intermittent light exposure exacerbates type 1 light damage in rats. Mary and Leslie (1987) studied degree of light damage to the retina with time of day of bright light exposure in albino rats. They stated that cyclic-light reared rats incurred less retinal damage than dark reared animals. Braeckvelt *et al*. (1998) studied photoreceptor fine structure in oreochromis niloticus in light and dark adaptation. Braeckvelt (1990) studied photoreceptor fine structure in light and dark adaptation in the butterfly fish and Rohrschneider and Ilsabe (1976) studied photoreceptor cells in the light and dark adaptation of haplochromis burtoni by scanning electron microscopy. In order to understand the retinal response, it is necessary to know the three-dimensional architecture of the photoreceptor layer under conditions of light- and dark-adaptation and the present report describes relevant scanning electron microscopical observations.

MATERIALS AND METHODS

Animals: Twelve adult male cats were obtained from animal house of Shiraz Medical University. Animals were randomly classified in three groups of control, light exposure and dark adapted. The animals were in the normal environmental conditions (12: 12 light-dark cycle) for 4 weeks. Room temperature was kept at approximately 28°C. Exposure to light was accomplished by placing the cages of animal which were open at the top under white 60W florescent lamp. Bright light intensity measured with a power meter, was 500-600 lux. The lamps hanged up on the wooden boxes (the distance from the light source is 110 cm). The wooden boxes were 120 cm wide×170 cm long×130 cm height. Each cage contained two animals. All studies were performed in accordance with National Institues of Health Guide for the care and use of laboratory animals.

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Table 1: Comparison between length, thickness and population of rods and cones in different experimental groups. Mean±SE . (*) Show significant difference

	Control group	Light exposure group	Dark adapted group
Length of rod	25.6±3.55	*39.2±3.55	24.8±2.94
Thickness of rod	1.2±.28	1.8±.28	1±.21
Length of cone	12.8±2.16	*17.4±1.41	11.7±1.63
Thickness of cone	2.1±.24	2.6±.43	2.2±.28
Population of rods and	20	18	19

Experimental design:

- Adult cats (4 in numbers) were exposed to continuous light for 24 h from 6:00 a.m. to 6:00 am next day (Light exposure group).
- Adult cats (4 in numbers) were maintained in the dark room for 24 h from 6:00 a.m. to 6:00 a.m. next day (Dark adapted group).
- Adult cats (4 in numbers) were in the normal environmental condition for 24 h from 6:00 a.m. to 6:00 a.m. next day (Normal group) (12:12 light-dark cycle).

The histological effects of continuous light exposure and dark adapted were evaluated with scanning electron microscope in the department of electron microscopy of Veterinary Medicine of Shiraz University (in summer season).

The animals were sacrificed by overdose of xylazine-ketamin injection. The eye balls were quickly removed. The cornea, lens and vitreous body were removed and opened at the equator then fixed for 4 h in 4% glutaraldehyde buffer to pH 7.3 with sodium cacodylate at 4°C. The posterior half of the eyeball was removed, the retina was separated near the optic nerve and fixed for additional 1 h. Then the RPE layer was removed. Then, the tissue was washed in sodium cacodylate, cut into pieces less than 2 in 2 mm, put in osmium tetroxide solution for 2 h and washed briefly in distilled water. Ascending grades of acetone were used for dehydrating the tissue then the tissue were mounted on specimen stubs and coated with gold under vacuum to insure electrical conductivity of the specimens. All tissues were examined in an oxford 5526 scanning electron microscope. The length and thickness measured of rods and cones by scanning electron microscope (Table 1).

Statistical analysis: The data were finally analyzed by one-way ANOVA and sample pair test, using SPSS soft ware (Version 11.5). Duncan multiple range test was also used to detect significant differences.

RESULTS

The findings represented that rod cells had a cylindrical and long form with equal thickness whereas cone cells were stouter than rod cells and cone-shaped (Fig. 1 on the scale of 5 μm = 3.1 cm).

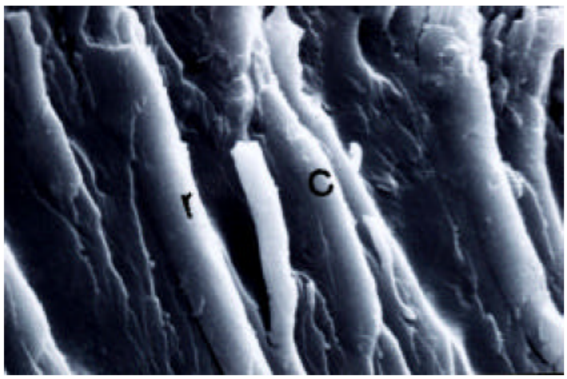

Fig. 1: View of the outer segment of rod (r) and cone (c) in the control group. Magnification×5000 (on the scale of 5 μm = 3.1 cm)

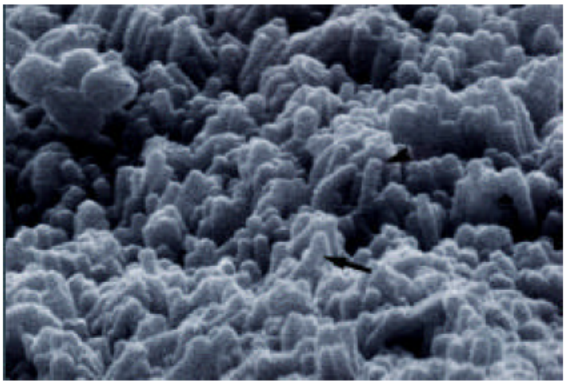

Fig. 2: View of the outer segment of rod (arrowhead) and cone (arrow) in the control group. The retinal pigmented epithelium removed. Magnification ×2500 (on the scale of 5 μm = 0.9 cm)

In control group the average of the length of outer and inner segments of rod cells was 25.6±3.55 μm and the average of thickness was 1.2±.28 μm. The same parameters in cone cells were 12.8±2.16 μm and 2.1±.24 μm. By counting rod and cone cells in 25 μm^2, in average, 19 rod cells were found ratio one cone cell (Fig. 2) (on the scale of 5 μm = 0.9 cm).

Results about continuous light-exposed group show that in comparison with the control group, the length of outer and inner segments of rod and cone cells were dramatically increased, in a shape that the average of the length of outer and inner segments of rod cells was 39.2±3.55 μm and 17.4±1.41 μm in cone cells and the average of their thickness was 1.8±.28 μm in rod cells and 2.6±.43 μm in cone cells (Fig. 3 on the scale of 5 μm = 2.1 cm). By counting rod and cone cells in 25 μm^2, in average, 17 rod cells were found ratio one cone cell.

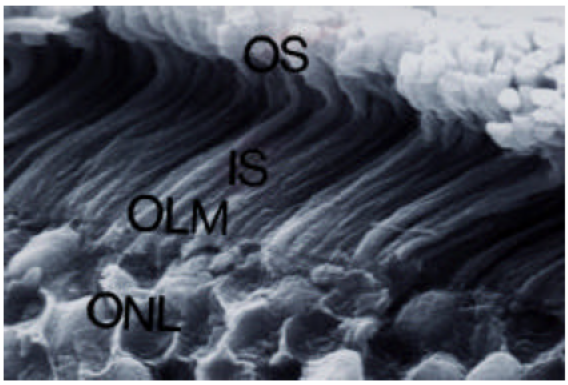

Fig. 3: View of the Outer Segments (OS), Inner Segments (IS), Outer Limiting Membrane (OLM) and Outer Nuclear Layer (ONL) of the photoreceptor layer in the light exposure group. Magnification×3500 (on the scale of 5 μm = 2.1cm)

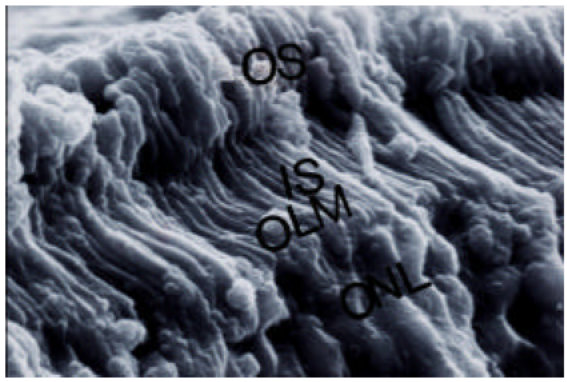

Fig. 4: View of the Outer Segments (OS), inner segments (IS), Outer Limiting Membrane (OLM) and Outer Nuclear Layer (ONL) of the photoreceptor layer in the dark adapted group. Magnification×3500 (on the scale of 5 μm = 2.1cm)

Results in dark-adapted group indicated that there is no remarkable difference between the average of the length and thickness of the outer and inner segments in rod and cone cells in comparison with the control group and even a little reduction was found, in a form that the average of the length of the outer and inner segments of rod cells in this group were 24.8±2.94 μm and the thickness of those was 1±.21 μm and in cone cells were 11.7±1.63 μm and 2.2±.28 μm (Fig. 4 on the scale of 5 μm = 2.1 cm). The same counting in this group was 18 rod cells ratio one cone cell.

DISCUSSION

On the basis of present study, significant difference was noticed in the average of the length of outer segment and inner segment of rods and cones between light exposure group and other groups (control group and dark adapted group), but this difference was not significant between control and dark adapted groups. These results showed that, in light exposure group, the length of photoreceptor cells rose by increasing light intensity and these cells have been activated. But in dark adapted group, these cells were deactivated and even a reduction in length and thickness of these cells was found without a significant difference in comparison with control group. Scientists such as Rohrschnider and Ilsabe (1976), Braeckvelt *et al*. (1998) and Braeckvelt (1990) also state that the length of photoreceptor cells rises by increasing light intensity and were activated that is compatible with this study findings. These scientists also state about dark adapted groups that the length of photoreceptor cells diminishes with significant difference and they are deactivated. The results represented that, although the length of photoreceptor cells was reduced, there was no significant difference in comparison with control group, which can be due to the differences in dark periods and animal species. Also the thickness of rods and cones increased in light exposure group in comparison with control group (without significant difference) and reduced in dark adapted group without significant difference. Also Braeckvelt *et al*. (1998) stated that rods, single cones and double cones in Oreochromis niloticus were present in a ratio of 30:1:2, respectively. But in this study no double cones were present and rods and single cones were present in a ratio of 19:1 in control group, that can be due to the differences in animal species. Our finding showed that the concentration of rods and single cones in light exposure and dark adapted groups was reduced in comparison with control group. This decreased maybe due to the retinal damage caused by ligh and dark period in light exposure and dark adapted groups. Similarly, decrease in concentration of photoreceptor cells and retinal damage in light exposure and dark adapted was report by Mary and Leslie (1987), Daniel *et al*. (1989), Braeckvelt (1990) and Braeckvelt *et al*. (1998).

CONCLUSION

The results of this study, showed that the continuous light can elongated the photoreceptor cells significantly, although the dark adaptation has not significant effect on length and thickness of these cells. However, the continuous light could increase the thickness of these cells, but this result was not significant. It is possible that longer light period need to increase the cell thickness significantly.

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