Morphology of retinal photoreceptor layer in continuous light-exposed and dark-adapted male cats

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(Received 24 Feb 2007; revised version 4 Jul 2007; accepted 19 Jul 2007)

Summary

The morphology of retinal photoreceptor layer was studied in continuous light-exposed and dark-adapted domestic male cats (*Felis catus*). The eyes of 12 healthy adult cats (4 in continuous light-exposed group, 4 in continuous dark-adapted group, and 4 in control group) were routinely fixed and studied by electron microscope. Results showed that the general structure of photoreceptor layer in this animal was the same as other species; rods were elongated and slender cells. Cone photoreceptors were shorter and stouter than rod photoreceptors. Cats exposed to continuous light for 24 hrs showed increased numbers of melanosome in retinal epithelial layer. The outer segments of rods and cones were long. Vacuoles increased the extracellular space and some condensed nuclei were observed in the inner segments. Continuous dark-adapted group showed a few melanosomes. In this group, the extracellular space, large swelling and condensed nuclei were more than those in other groups.

Key words: Photoreceptor, Continuous light, Continuous dark, Cat

Introduction

Retina plays an important role in visual system. Retina has several layers. One of these layers which is really crucial for visualization, is the photoreceptor layer which contains cone and rod cells. They are sensory retinal receptors (Banks, 1993).

Light rays falling on the eye pass through its refractive media (cornea, lens, anterior and posterior chambers, vitreous body) before reaching the visual receptor cells (the rods and cones) in the retina. The refractive media help focus the image on the retina (Walls, 1942; Duck-Elder, 1958).

Various histologic studies were undertaken on photoreceptor structures of retina in different domestic animals under light and electron microscope (Walls, 1942; Polyak, 1957; Duck-Elder, 1958; Braeckvelt, 1987, 1990, 1992, 1993 and 1998; Braeckvelt *et al*., 1996; Garcia and Dejuan, 1999; Haacke *et al*., 2001).

The traditional separation of retinal photoreceptors into either rods (Stabchen) or cones (Zapfen) was originally proposed by Schultze (1866). In this classical division rods have cylindrical inner and outer segments of more or less the same diameter while typical cones have a shorter conical outer segment and an inner segment of greater diameter. This classification was used exclusively in numerous light microscope studies (Walls, 1942; Polyak, 1957; Duck-Elder, 1958).

In rods, the outer segment discs have all the same diameter while in cones the apical discs are smaller than those of the basal region giving the outer segment a conical shape (Braeckvelt, 1983). 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) and Grenadier anchovy *Coilia nasus* (Haacke *et al*., 2001). Retinal light damage in rats exposed to intermittent light was

compared with continuous light exposure by Daniel *et al*. (1989). They concluded that intermittent light exposure exacerbates type 1 light damage in rats. Mary and Leslie (1987) studied the degree of light damage to the retina with time of day, with bright light exposure in albino rats. They stated that cyclic-light reared rats incurred less retinal damage than dark reared animals. However, no ultrastructural evaluation of the photo-receptor layer under the effect of continuous light exposure and dark adaptation is reported in cats. The present study was undertaken to describe the fine structure of the rods and cones in retina of continuous light-exposed and dark-adapted male domestic cats.

Materials and Methods

Animals: Twelve adult male cats were obtained from animal house of Shiraz University of Medical Sciences. Animals were randomly divided into three groups of control, light-exposed and dark-adapted. The animals were in the normal environmental conditions (12:12 light:dark cycle) for four weeks. The room temperature was kept at approximately 28°C. Exposure to light was accomplished by placing the animal's cages, with their tops open, under white 60-W fluorescent bulbs. Bright light intensity was measured with a power meter which was 500–600 lux. The bulbs were hanged up on the wooden boxes (the distance from the light source was 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 the National Institutes of Health Guide for the care and use of laboratory animals (NIH publication No. 86-23, revised 1985).

Experimental design

1- Four adult cats were exposed to continuous light for 24 hrs from 6:00 am to 6:00 am the next day (light-exposed group).
2- Four adult cats were maintained in the dark room for 24 hrs from 6:00 am to 6:00 am the next day (dark-adapted group).
3- Four adult cats were kept in normal environmental condition for 24 hrs from 6:00 am to 6:00 am the next day (control group—12:12 light:dark cycle).

The histological effects of continuous light exposure and dark adapted were evaluated with transmission electron microscope.

The animals were sacrificed by overdose of xylazine-ketamine injection. The eye balls were quickly removed. The cornea, lens and vitreous body were removed and opened at the equator, then fixed for four hrs in 4% glutaraldehyde buffer (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 an additional one hr. Then, the tissue was washed in sodium cacodylate and cut into pieces less than one mm^2. The tissue was then post-fixed for 1.5 hrs in 1% osmium tetroxide, washed briefly in distilled water, dehydrated through graded ethanol and then cleared in propylene oxide and embedded in agar resin.

Semithin sections of 0.5-µm thickness were obtained using ultramicrotome (Reichert-Jung, Ultracut Austria equipped with glass knives), stained with toluidine blue and were first examined by light microscope. Ultrathin sections of 60-nm thickness were also obtained and collected on copper grids. These sections were stained in aqueous uranyl acetate and lead citrate and were examined with Philips CM-10 transmission electron microscope.

Results

In the control group the retinal pigmented epithelium (RPE) consisted of a single layer of low cuboidal cells. Internally, the retinal pigmented epithelial cells displayed large, vesicular, roughly spherical nuclei with a fairly dispersed chromatin pattern (Fig. 1). Melanosomes were absent. Rods were elongated and slender cells. The rod outer segment was composed of a stack of bimembranous discs. The rod inner segment had few mitochondria, rough endoplasmic reticulum, stack of Golgi apparatus and glycogen particles (Fig. 2). The outer limiting membrane (OLM) appeared normal. The rod's nuclei were round to oval in shape and located at all

levels of the outer nuclear layer (ONL). Cone photoreceptors were shorter and stouter than rods. The cone's nuclei formed a single discontinuous row immediately below the OLM. Cones had the same structural components as rods.

The cats exposed to 24 hrs of continuous light showed a number of melanosomes in the RPE. In the light-exposed group, the outer segment were extremely elongated (Fig. 3). A few bimembranous discs in the outer segment were disorganized. Increased extracellular space and a few vacuolization in the inner segment were evident. Some

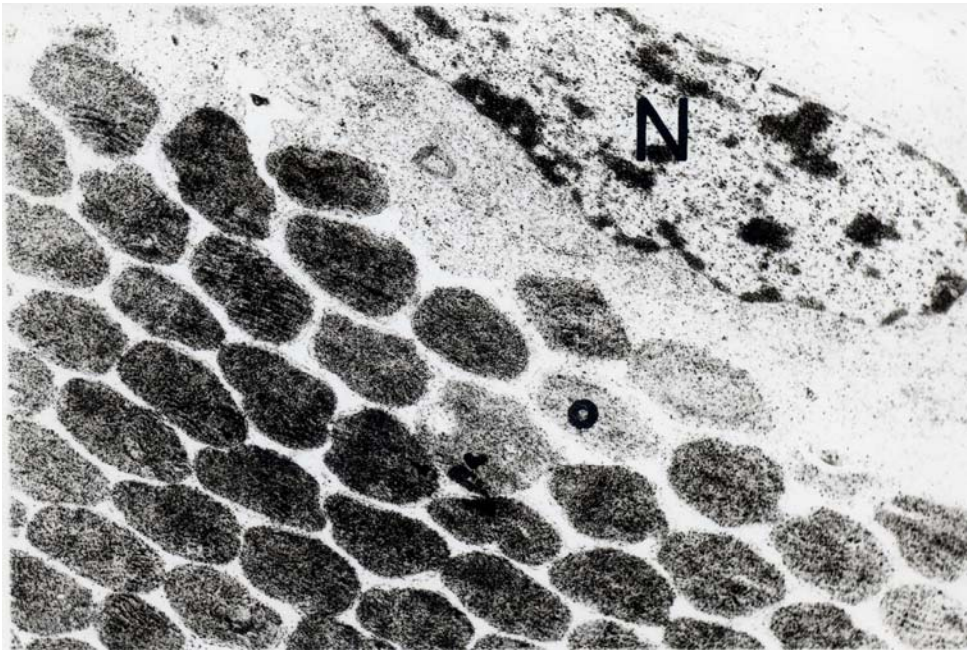

Fig. 1: Micrograph of the retinal pigmented epithelium (RPE) in the control group. N: nucleus; outer segment (thick circle). The RPE appears normal and melanosome was absent. Outer segment (thick circle) (×7000)

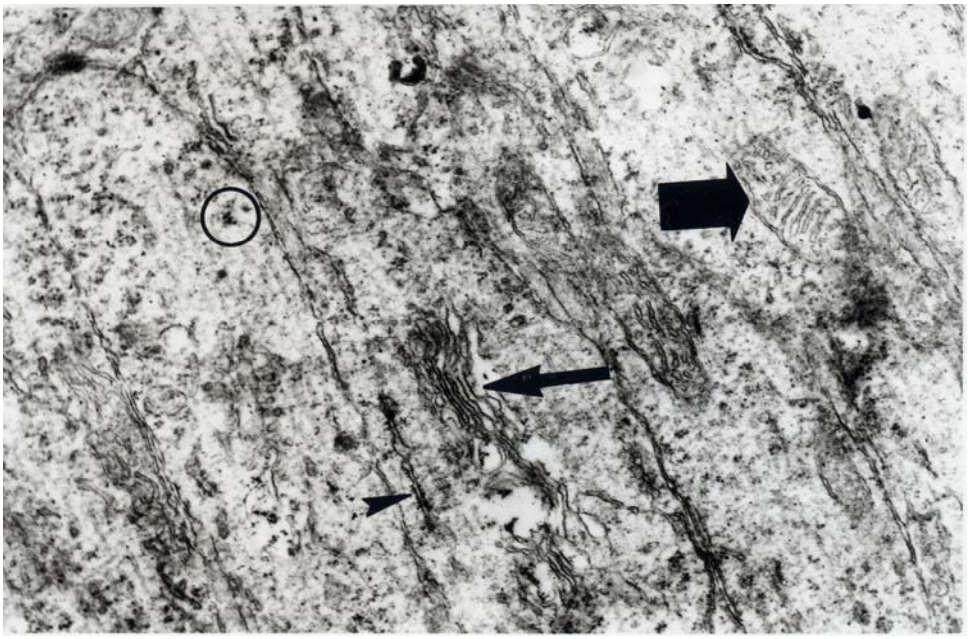

Fig. 2: Micrograph of the inner segment of the rod and cone cells in the control group. Golgi apparatus (arrow), rough endoplasmic reticulum (arrowhead), mitochondrion (thick arrow), glycogen (black circle) (×15500)

condensed nuclei in the ONL and disruption of the OLM were apparent.

The cats adapted to 24 hrs of continuous dark showed a few melanosomes in RPE. Bimembranous discs in the outer segment were more disorganized than in the light-exposed group (Fig. 4). There were extensive swelling, vacuolization and increased extracellular space in the inner segment. The OLM did not exist in several sites and was disrupted in other sites. Extensive condensation of photoreceptor cells nuclei in the ONL was evident.

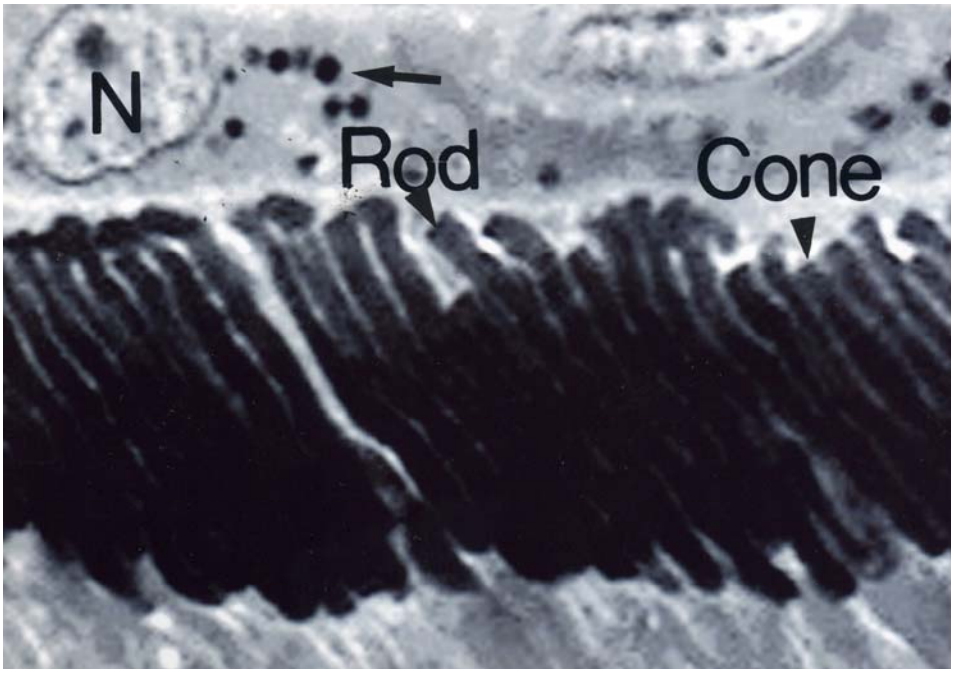

Fig. 3: Micrograph of the retina in the light-exposed group. N: nucleus in RPE; rods and cones are extremely elongated (arrowheads), few melanosomes are present (arrow) (×2000)

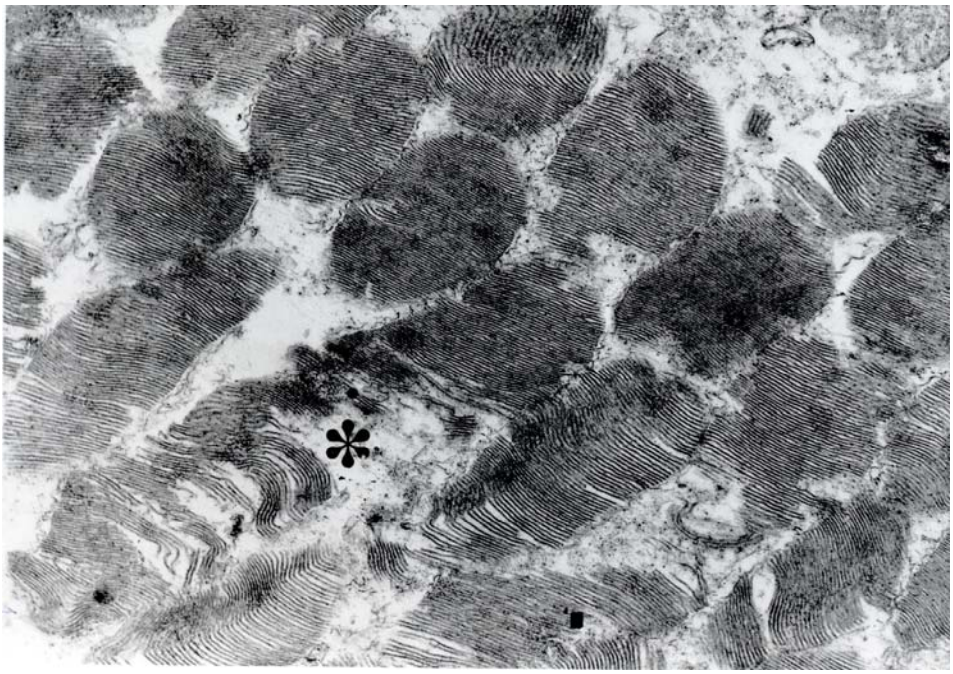

Fig. 4: Micrograph of the outer segment of photoreceptor cells in the dark-adapted group. Disorganized bimembranous discs are also present in the outer segment of rod cell (asterisk) (×14000)

Discussion

Irreversible photoreceptor cell damage from visible light is a complex process which differs in various species (Lai *et al*., 1978). The mechanism of light damage is complicated because age, diet and genetic factors can all influence the susceptibility of retina to the intense light (Lai *et al*., 1978). Our findings demonstrated that the RPE in the control group of male cats consists of a single layer of low cuboidal cells. The large vesicular nuclei were found to have a fairly dispersed chromatin pattern. The RPE of the cat retina, for example, fits the traditional definition very well like other animals (Braeckvelt, 1985, 1986 and 1988). Braeckvelt (1985) believed that the morphology of the RPE and associated structures vary somewhat depending upon retinal location.

In our study, a number of melanosomes were seen in light-exposed and dark-adapted groups. However, further morphometric aspects of RPE should be further examined. Daniel *et al*. (1989) and Mary and Leslie (1987) indicated that in the intermittent light-exposed rats the melanosomes were increased. They stated that cyclic-light-reared rats incurred less retinal pigment epithelial cell damage than dark-reared animals.

Our findings in the control group also correlated with the findings of Braeckvelt (1983, 1992, 1993 and 1998). Braeckvelt *et al*. (1996), Garcia and Dejuan (1999) and Haacke *et al*. (2001) showed that in various species, the outer segment in rods and cones is composed of a stack of bimembranous discs, the inner segment has various organelles, the OLM is formed by a series of zonulae adherents and the ONL consists of rods' and cones' nuclei.

In this study, disorganization of double discs membrane in the light-exposed and dark-adapted groups was observed. It may be due to an effect of intense light and dark condition on the outer segment of the rods and cones. Mary and Leslie (1987) and Daniel *et al*. (1989) concluded that increase in light exposure during the period of rod outer segment phagocytosis enhances photo-receptor damage.

Braeckvelt (1990 and 1998) stated that in the light-exposed group, the outer segments are extremely elongated while in the dark-adapted group they are relatively shorter. The same phenomenon was noticed in the light-exposed group in our study; however, there were no difference in the height of the outer segment between the dark-adapted group and the control group. This may be due to the difference in the exposure time.

There is some disruption in OLM in the light-exposed group. Mary and Leslie (1987) and Daniel *et al*. (1989) concluded that the way or schedule by which intense light is administered would be an important determinant of the extent of retinal damage in rats. In our study, in the dark-adapted group, OLM did not exist in some sites and was disrupted in other sites. Nonetheless, Daniel *et al*. (1989) and Mary and Leslie (1987) stated that disruption occurred only in the dark-adapted group; perhaps, it was due to the difference in the duration of the exposure.

In this study, condense heterochromatin was seen which was increased throughout the nuclei in the dark-adapted and light-exposed groups.

Daniel *et al*. (1989), Mary and Leslie (1987), Braeckvelt *et al*. (1998) and Braeckvelt (1990) stated that visual cell loss occurs largely during the dark periods between or after light exposure; they concluded that both the levels of rhodopsin and visual cell DNA were affected by light and indicated that nuclei in ONL became piknotic and condensed by the affection of intermittent and continuous light- and dark-adaptation.

Acknowledgement

Financial support by the School of Veterinary Medicine of Shiraz University is greatly appreciated.

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