Indian Journal of Biochemistry & Biophysics
Vol. 57, August 2021, pp. 381-384

Effect of constant light on the morphology of the retina in domestic chick (*Gallus g. domesticus*): A scanning electron microscope study

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Received 12 February 2021; *revised 06 March 2021*

Light induces variable levels of damage to the retina and often, death of the photoreceptor cells. It is reported that chick retina, having abundant melanin in the retinal pigment epithelium, undergoes no or minimal damage from exposure to light. However, the morphology of cone and rod photoreceptor cells after continuous exposure of light has not been studied in detail. The present study investigated the detailed changes of photoreceptor cells, by scanning electron microscopy (SEM). Domestic chicks (*Gallus g. domesticus*) from post-hatch day (PH) 1 to 7 were initially reared at 700 lux at 12 h light (L):12 h dark (D). From PH 7 to 18, they were exposed to 12L:12D (control) and 24L:0D (constant light) cycles at 700 lux. At PH18, chicks were sacrificed, their eyes enucleated and preserved in Karnovsky's fixative. The retina was then separated from the adherent choroid, mounted on photoreceptor side up and processed for SEM. There was significant damage to rod and cone outer segments from both dorsal and ventral part of the retina and a decrease in the abundance of cells of the inner nuclear layer in constant light, compared to that found in 12L:12D cycles. It is likely that constant light affects the retinal cells in the vertical pathway, which could alter the phototransduction process.

Keywords: Chick retina, Cones, Ganglion cell layer (GCL), Outer plexiform layer, Rods, Vertebrate retina

Vertebrate retina is a highly differentiated, complex layer of neuronal tissue that develops as an outgrowth of the developing brain. It is the innermost layer of the eye which responds to light. The structure of the mammalian retina has been studied extensively[1-3]. Light induces damage to the retina and in extreme situations, the death of photoreceptor cells. Normal light exposure (about 12 h) in the 24 h light-dark cycle is essential for maintaining a normal circadian rhythmicity, whereas prolonged exposure or exposure to different intensities can significantly modulate many undesirable physiological processes. Prolonged or continual exposure to light could be an important factor for light induced retinal degeneration (LIRD).

In experimental vision research, rodents have been used routinely in LIRD experiments[4]. In these experiments, the animals are subjected to either acute intense light exposure for a while or to chronic exposure to light of moderate intensities for some long period (long-term exposure; 7 days to 3 months). However, since rodents are nocturnal and having rod dominated retina, their vision does not equate the cone-dependent human vision. Since chicks are used as a model in vision research, they appear suitable in LIRD experiment as they have cone–dominated retina (86% cones) and with different kinds of colour sensitive cones (violet, blue, green and red), similar to human retina containing blue, green and red cones. Earlier reported showed that chick retina, though pigmented, undergoes damage after continuous light exposure[5]. Chick photoreceptor cells have been studied by various researchers[6,7]. However, their gross morphology after continuous exposure of light has not been studied, and so the present study was designed to see these changes in the retina after continuous light insult.

Materials and Methods

Rearing of eggs

Fertile eggs (50-60 g weight) of white leghorn chicken (*Gallus g. domesticus*) were incubated at 36 ± 1°C temperature in an incubator at a relative humidity of 65-70%. Hatching took place after 21 days of incubation. From Post-hatch (PH) day 1, chicks were exposed to artificial light using fluorescent white light (40-Watt, intensity: 700 lux), positioned one foot high above the animal cages. The experiments were conducted in accordance with ARVO statement for the

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use of animals in Ophthalmic and Vision Research and approved by Institutional Animal Ethics Committee (approval number: IAEC 911/2015).

Experimental approach

From PH 1 to 7, chicks (n=12) were initially acclimated to artificial white light of 700 lux under 12 h light and 12 h dark (12L:12D) cycles. On PH 8, chicks were divided into an experimental and a control group (n=6 each). Control group was exposed to 12L:12D, while the experimental group was exposed to constant light photoperiod (24L:0D), until PH 18.

Histological assessment of the retina by light microscopy

To understand histological changes in the retina after light exposure in normal photoperiod (12L:12D) and constant light photoperiod (CL, 24L:0D) at PH 18. For this, eyes were fixed in 4% par formaldehyde for 3 h at 4°C. After fixation, samples were washed with 0.1 M phosphate buffer. Optic cups were then transferred into 15% sucrose for 4-6 h and then into 30% sucrose overnight. They were embedded in OCT medium and 14 µm thick sections were cut in a cryocut. Sections were mounted on gelatin-coated glass slides and stained in hematoxylin and eosin (H & E) stain. Retinal histological changes were noted under an optical microscope (Leica DM 6000B) and images acquired with software.

Scanning electron microscopy

At PH18, chicks were sacrificed, their eyes enucleated and fixed in 2.5% glutaraldehyde and 2% paraformaldehyde. Subsequently, the retinas were separated out from the rest of the eyeball, washed with phosphate buffer, flat-mounted and dehydrated with ethanol. The tissues were critical point-dried, mounted onto stubs with photoreceptor side up, and sputter-coated with gold. Specimens were observed under an EVO18 scanning electron microscope (Carl Zeiss) at an operated voltage of 15 kV.

Quantification of damaged outer segments of cones in normal and constant light photoperiod

Light exposure causes shortening of the photoreceptor outer segments. The length of damaged cone outer segments was measured in both normal and experimental chick retinas on digitized images across 145 µm length of the retina by ImageJ software (NIH, USA). Cone outer segment length less than 4 µm was considered damaged due to light exposure, and their number was reckoned and percentage of damaged cones calculated in relation to the normal cones having a length range of 8-12 µm. Rod outer segments length could not be ascertained in SEM micrographs and hence was not considered.

Statistical analysis

The data are represented as mean ± S.E.M. Comparisons were made between control (12L:12D) and experimental group (24L:0D). Unpaired *t* test was performed to compare the data. Analyses were carried out using GraphPad Prism and $P< 0.05$ was considered statistically significant.

Results and Discussion

SEM of photoreceptor cells in normal and constant light condition

Figure 1 depicts normal retinal architecture of chick (Fig. 1A) and changes after exposure to constant light photoperiod (24L:0D, Fig.1B). Shrunken outer nuclear layer (ONL) and inner nuclear layer (INL) was seen after light exposure (Fig. 1B) to the central retina (close to optic disc). A low magnification scanning electron micrograph of the retina and the associated underlying pecten is shown in (Fig. 2). In normal light condition

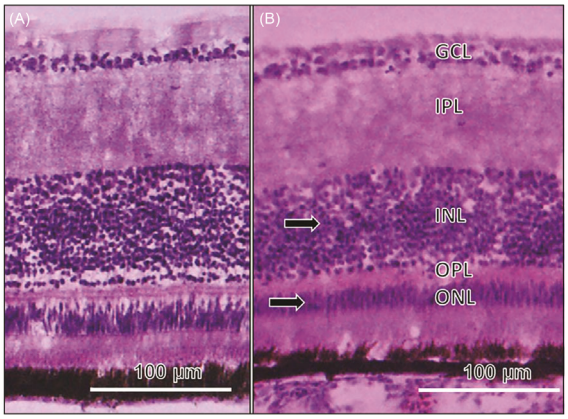

Fig. 1 — Light micrographs showing various layers of chick retina in normal (12L:12D, 1A) and constant light photoperiod (24L:0D, 1B). Shrunken outer nuclear layer (ONL) and inner nuclear layer (INL) is indicated (arrows) after light exposure in Fig. 1B. GCL, ganglion cell layer; IPL, inner plexiform layer; OPL, outer plexiform layer. From central retina close to optic disc. Stained with H & E

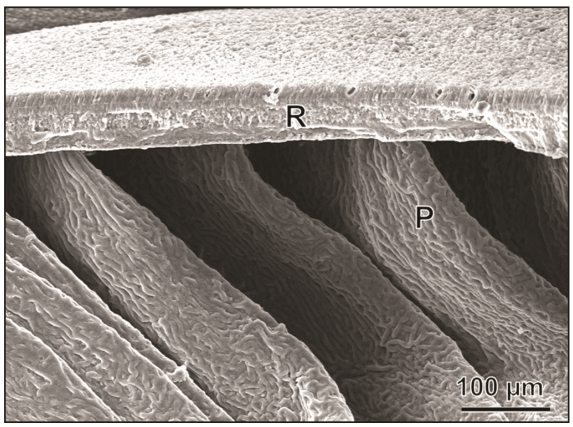

Fig. 2 — Scanning electron micrograph showing chick retina (R) with a portion of the associated pecten (P, comb like structure in bird eye)

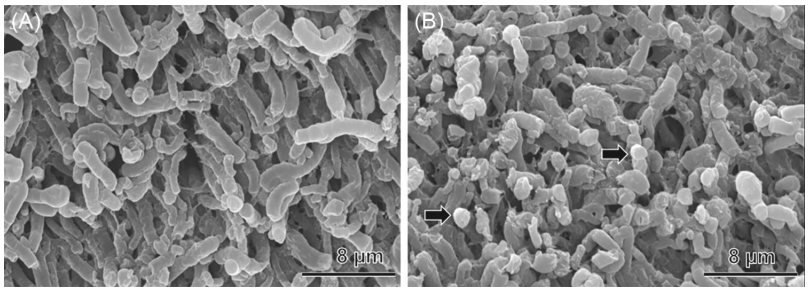

Fig. 3 — Scanning electron micrograph of rods in normal (12L:12D, A) and constant light photoperiod (24L:0D, B). In the latter situation, there is a damage to rod outer segments (appear as convolutions, arrows, B). From the dorsal part of retina, above the optic disc

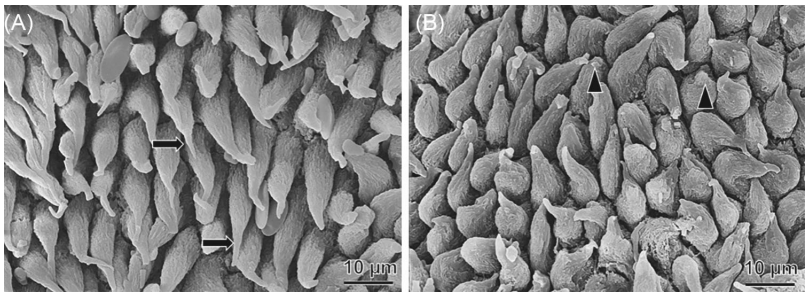

Fig. 4 — Scanning electron micrograph of cones in normal (A) and constant light photoperiod (B). The outer segments of cones are normal in 12L:12D photoperiod (A, arrows), but are damaged in constant light condition (B, arrowheads). From central (A, close to pecten) and adjacent part (2 mm away from pecten) of the retina

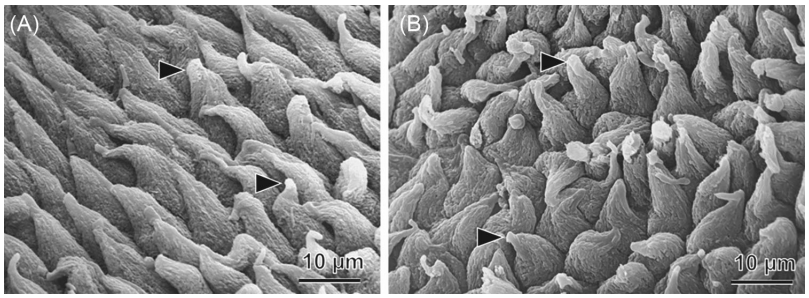

Fig. 5 — Scanning electron micrograph of cones in constant light photoperiod. The outer segments of cones are shorter in constant light condition (arrowheads). From dorsal part of the retina above the pecten

(12L:12D, Fig. 3A), rod photoreceptor outer segments appeared normal (long), whereas in constant light photoperiod (24L:0D), there was convolutions in their outer segments (Fig. 3B). Normal shape and size of the cone outer segments (length range: 8-12 µm) were observed in 12L:12D cycles (Fig 4A), but in constant light photoperiod (24L:0D, Figs. 4B and 5A & B), their outer segments were shorter in length (<4 µm). Quantifications revealed about 50-60% of the cone outer segments to be damaged in experimental retinas (Fig. 6). In normal photoperiod (12L:12D, Fig. 7A), cells in inner nuclear layer were uniformly distributed and more in number, whereas, in constant light photoperiod (24L:0D, Fig. 7B), there were empty spaces in the inner nuclear layer, reflecting cell loss in this layer.

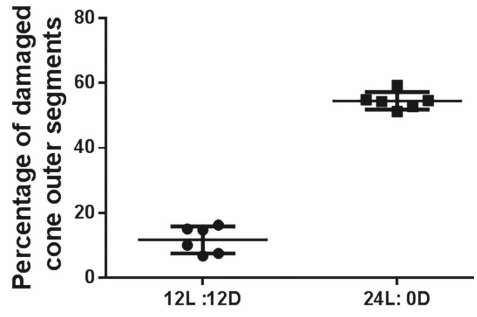

Fig. 6 — Histogram showing percentage damage of cone outer segments (in terms of reduction in length <4 µm) of chick retina in normal (12L:12D) and constant light photoperiod (24L:0D, 3B) across 145 µm length of the retina. In constant light condition, there is a significant damage and reduction to cone outer segment length (50-60%; $P< 0.05$)

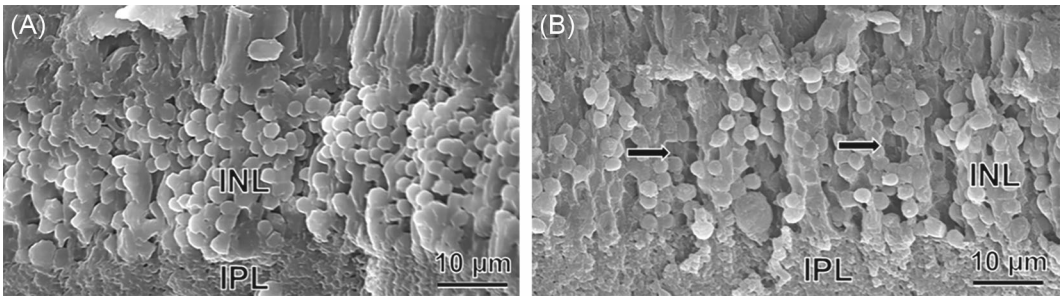

Fig. 7 — Scanning electron micrograph of inner nuclear layer of the central part of retina in normal (12L:12D, A) and constant light photoperiod (24L:0D, B). In constant light condition (B), there are empty spaces (arrows), reflecting cell loss due to light toxicity. IPL, inner plexiform layer

These observations indicate that due to extended light phase in the circadian light dark cycle the adverse effects of light on the retina are inevitable. Thus, a prolonged light phase can have adverse effects not only in animals, but also in the human life span. Earlier studies on a variety of species (zebrafish, rat, cat and monkey indicated that continuous light exposure leads to retinal photoreceptor cell death[8-10]. This study shows that photoreceptor outer segments are damaged in continual light exposure over the dorsal to ventral part of the retina. Chicks were used because they are highly diurnal, possess cones of different spectral types and their vision exclusively depend on cones. While rods show normal morphology in 12L:12D cycle, in constant light condition, their normal structure was lost and damage was seen in the form of convolutions of their outer segments. Also, constant light significantly altered the length of the cone outer segments. Changes were evident in both dorsal to ventral part of the retina.

Diurnal vision is equipped with not only an abundance of cones, but also with neurons in the inner retina for transmitting the signals. In chicks, the inner nuclear layer is normally packed with cells, which are arranged into 8-10 rows of cells. In 24L:0D cycles, there were empty spaces in this retinal layer, implying that constant light damaged the inner nuclear layer cells, perhaps by altering their membrane properties, which can lead to their death.

Conclusion

In domestic chicks, compared with the observations made in normal 12 h light:12 h dark circadian cycles, exposure of the retina to constant fluorescent light (24 h light:0 h dark) at 700 lux leads to damage and a reduction in the length of cone outer segments, as occurred when exposed from post-hatch day 7 to 18. These changes were accompanied by a decrease in the abundance of inner nuclear layer cells in constant light.

Acknowledgement

The Electron Microscopy work was carried out at the Sophisticated Analytical Instrumentation Facility (SAIF-New Delhi), AIIMS, New Delhi. The work was partially supported by funds from the Department.

Conflict of interest

All authors declare no conflict of interest.

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