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Retinal adaptation to dim light vision in spectacled caimans (*Caiman crocodilus fuscus*): Analysis of retinal ultrastructure

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ABSTRACT

It has been shown that mammalian retinal glial (Müller) cells act as living optical fibers that guide the light through the retinal tissue to the photoreceptor cells (Agte et al., 2011; Franze et al., 2007). However, for nonmammalian species it is unclear whether Müller cells also improve the transretinal light transmission. Furthermore, for nonmammalian species there is a lack of ultrastructural data of the retinal cells, which, in general, delivers fundamental information of the retinal function, i.e. the vision of the species. A detailed study of the cellular ultrastructure provides a basic approach of the research. Thus, the aim of the present study was to investigate the retina of the spectacled caimans at electron and light microscopical levels to describe the structural features. For electron microscopy, we used a superfast microwave fixation procedure in order to achieve more precise ultrastructural information than common fixation techniques.

As result, our detailed ultrastructural study of all retinal parts shows structural features which strongly indicate that the caiman retina is adapted to dim light and night vision. Various structural characteristics of Müller cells suppose that the Müller cell may increase the light intensity along the path of light through the neuroretina and, thus, increase the sensitivity of the scotopic vision of spectacled caimans. Müller cells traverse the whole thickness of the neuroretina and thus may guide the light from the inner retinal surface to the photoreceptor cell perikarya and the Müller cell microvilli between the photoreceptor segments. Thick Müller cell trunks/processes traverse the layers which contain light-scattering structures, i.e., nerve fibers and synapses. Large Müller cell somata run through the inner nuclear layer and contain flattened, elongated Müller cell nuclei which are arranged along the light path and, thus, may reduce the loss of the light intensity along the retinal light path. The oblique arrangement of many Müller cell trunks/processes in the inner plexiform layer and the large Müller cell somata in the inner nuclear layer may suggest that light guidance through Müller cells increases the visual sensitivity.

Furthermore, an adaptation of the caiman retina to low light levels is strongly supported by detailed ultrastructural data of other retinal parts, e.g. by (i) the presence of a guanine-based retinal tapetum, (ii) the rod dominance of the retina, (iii) the presence of photoreceptor cell nuclei, which penetrate the outer limiting membrane, (iv) the relatively low densities of photoreceptor and neuronal cells which is compensated by (v) the presence of rods with long and thick outer segments, that may increase the probability of photon absorption. According to a cell number analysis, the central and temporal areas of the dorsal tapetal retina, which supports

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Abbreviations: GCL, ganglion cell layer; INL, inner nuclear layer; IPL, inner plexiform layer; MOSP, myelin oligodendrocyte-specific protein; NFL, nerve fiber layer; OCT, optical coherence tomography; OLIG2, oligodendrocyte transcription factor; OLM, outer limiting membrane; ONL, outer nuclear layer; OPL, outer plexiform layer; PBS, phosphate-buffered saline; RPE, retinal pigment epithelium

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downward prey detection in darker water, are the sites of the highest diurnal contrast/color vision, i.e. cone vision and of the highest retinal light sensitivity, i.e. rod vision.

1. Introduction

Vertebrate species possess an inverted retina which allows an efficient trophic and structural support of photoreceptors by the retinal pigment epithelium (RPE). However, the inversion of the retina has the disadvantage that light traverses the entire neural retina before it arrives at photoreceptors. Cellular structures which have dimensions compatible with the wavelengths of light like cell processes and organelles are phase objects which reflect and scatter light (Zernike, 1955; Land, 1972). The inherent light reflection by the retinal tissue allows the visualization of retinal layers with optical coherence tomography (OCT). In OCT images of human and avian retinas, nerve fiber (NFL) and plexiform layers show the highest reflectivities (Rauscher et al., 2013; Scheibe et al., 2014). This suggests that a considerable proportion of incident light is reflected at neuronal axons and synapses. Further hyperreflective structures are the outer limiting membrane (OLM) and the transition zones between the inner and outer photoreceptor segments which were previously described as "refractive discs" (Detwiler, 1943). Photon scattering reduces the intensity and the signal-to-noise ratio of the light transmitted through the retina (Agte et al., 2011). The resulting decreases in the visual acuity and sensitivity will deteriorate vision particularly under dim light and scotopic conditions under which each absorbed photon is important. It has been shown that, in the mammalian retina, Müller glial cells guide the light with minimal intensity loss through the neuroretina towards the photoreceptor cells; this ensures that the light bypasses the scattering elements of the retina (Franze et al., 2007; Agte et al., 2011; Labin et al., 2014). In the retina of nocturnal mammals, which contains a thick, multilayered outer nuclear layer (ONL), the outer Müller cell processes are very thin. Here, the light is transported by the nuclei of rod photoreceptor cells which are arranged in linear vertical columns (Solovei et al., 2009). These nuclei form a chain of lenses which transmit the light delivered by Müller cells and direct it to the receptor segments (Solovei et al., 2009). However, it is unclear whether Müller cells of nonmammalian species also improve the light transmission through the neuroretina.

Crocodilians are characterized by a semi-aquatic lifestyle. They hunt and feed from the muddy and gloomy bottom of the water, but also make their pickings from the water's edge with bright ambient light conditions. Although crocodilians are arrhythmic (cathemeral) animals, which are active during both day (bright) and night (dark), their eyes are rather structurally adapted for vision under dim light and scotopic conditions. This adaptation is recognizable, for example, in the presence of tapeta lucida and the rod dominance of the retina (Heinemann, 1877; Chievitz, 1889; Abelsdorff, 1898; Garten, 1907; Laurens and Detwiler, 1921; Detwiler, 1943; Duke-Elder, 1958). Tapeta lucida of crocodilians are formed by light-reflecting guanine crystals in the RPE (Chievitz, 1889; Laurens and Detwiler, 1921; Franz, 1934; Braekevelt, 1977; Dieterich and Dieterich, 1978; Ollivier et al., 2004). Generally, the ocular fundus of crocodilians is separated into two regions: a thick streak in the dorsal fundus has a bright appearance because it contains the tapetum lucidum while the ventral fundus is dark and contains a tapetum nigrum (Chievitz, 1889; Abelsdorff, 1898; Garten, 1907; Laurens and Detwiler, 1921; Dieterich and Dieterich, 1978; Ollivier et al., 2004). The presence of a dorsal lucido-tapetal and a ventral nigro-tapetal retina is suggested to represent an adaptation to the hunting behavior of crocodilians (Duke-Elder, 1958). The higher light sensitivity provided by the tapetum lucidum in the dorsal retina allows the animals to hunt and feed from the dim-lighted bottom of the waters (Abelsdorff, 1898; Dieterich and Dieterich, 1978). To prevent dazzle in bright light, crocodilians reduce the amount of light that reaches the retina by the closure of the slit pupils (Laurens and Detwiler, 1921; Walls, 1942; Banks et al., 2015). The retina of caimans contains rods with large diameter inner and outer segments and three types of cones: long single cones sensitive to red light, short single cones sensitive to blue-violet light, and double cones sensitive to red-green and red-red light, respectively (Govardovskii et al., 1988). The spectral sensitivities of cones suggest that caimans have trichromatic vision (Govardovskii et al., 1988).

The tapetum lucidum and the rod dominance of the retina contribute to the high sensitivity of the crocodilian vision in dim ambient light. However, it is unclear whether there are further structural adaptations of the crocodilian retina which improve the light transmission through the neuroretina and thus the visual sensitivity. For example, it is unclear whether Müller cells have a phenotype which allow them to guide the light through the neuroretina. Therefore, we investigated the ultrastructure of the retina of the brown caiman, *Caiman crocodilus fuscus* (COPE, 1868) which is one of four recognized subspecies of the spectacled caiman (Velasco and Ayarzagüena, 2010). We describe various ultrastructural specializations of the caiman retina which may improve the visual sensitivity in dim light environments. In addition to the guanine-based tapetum lucidum, these specializations include the peculiar morphology of photoreceptor cells and the spatial arrangements of neuronal perikarya and Müller cell processes in the inner retina.

2. Materials and methods

2.1. Animals and anesthesia

Experiments were carried out with IACUC approval and in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and the institutional animal care and use guidelines. All efforts were made to minimize the number of animals used and their suffering. A total of three juvenile brown caimans (length, ~70 cm; age, 5–6 years; both sexes), obtained from a local naturally breeding colony (Laguna Tortuguero, Vega Baja, Puerto Rico), were used. After immobilization on ice, the animals were deeply anesthetized with tiletamine/zolazepam (5 mg/kg; i.p.), and the eyeballs were removed.

2.2. Eye fixation

In order to mark the positions of the different retinal areas, two incisions were placed in the dorsal and nasal sclera of the eyeballs. Two eyes were fixed for electron microscopy using two different methods: (i) one eye was fixed overnight at room temperature with 4% paraformaldehyde and 5% glutaraldehyde in calcium- and magnesium-free phosphate-buffered saline (PBS) that consisted of (in mM) 136.9 NaCl, 2.7 KCl, 10.1 Na_2HPO_4, and 1.8 KH_2PO_4 (pH 7.4 adjusted with Trisbase). Thereafter, the eye was washed with PBS and then stored in PBS with sodium azide (0.01%) at 4 °C until further use. (ii) The anterior part including the lens was cut from the other eye. The remaining eyeball was processed by a fixation procedure modified after Schikorski (2014a,b, 2016) with microwave-assisted aldehyde fixation of oxygenated tissue (Kok and Boon, 1992). The fixation solution was prepared with 1.6% paraformaldehyde and 2.5% glutaraldehyde in low-calcium bicarbonate buffer that consisted of (in mM) 136 NaCl, 2.5 KCl, 1.3 Na_2HPO_4, 10 $NaHCO_3$, and 2.6 $MgCl_2$. After bubbling of the solution with carbogen (95% O_2 and 5% CO_2) for 20 min, 0.5 mM $CaCl_2$ was added, and the pH was adjusted to 7.4. The eyeball was transferred into