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Müller glial cells of the primate foveola: An electron microscopical study

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ABSTRACT

Previous studies on the ultrastructure of the primate foveola suggested the presence of an inverted cone-like structure which is formed by 25–35 specialized Müller cells overlying the area of high photoreceptor density. We investigated the ultrastructure of the Müller cells in the foveola of a human and macaque retina. Sections through the posterior poles of an eye of a 40 years-old human donor and an eye of an adult cynomolgus monkey (*Macaca fascicularis*) were investigated with transmission electron microscopy. The foveola consisted of an inner layer (thickness, 5.5–12 μm) which mainly contained somata (including nuclei) and inner processes of Müller cells; this layer overlaid the central Henle fibers and outer nuclear layer. The inner layer contained numerous watery cysts and thin lamelliform and tubular Müller cell processes which spread along the inner limiting membrane (ILM). The cytoplasm of the outer Müller cell processes became increasingly dispersed and electron-lucent in the course towards the outer limiting membrane. The ILM of the foveola was formed by a very thin basal lamina (thickness, < 40 nm) while the basal lamina of the parafovea was thick (0.9–1 μm). The data show that there are various conspicuous features of foveolar Müller cells. The numerous thin Müller cell processes below the ILM may smooth the inner surface of the foveola (to minimize image distortion resulting from varying light refraction angles at an uneven retinal surface), create additional barriers to the vitreous cavity (compensating the thinness of the ILM), and provide mechanical stability to the tissue. The decreasing density of the outer process cytoplasm may support the optical function of the foveola.

1. Introduction

The vertebrate retina has an inverted structure with respect to the light path that allows an efficient trophic and structural support of photoreceptors by the retinal pigment epithelium. Consequently, light must pass the entire depth of the neuroretina to reach the receptors. As in other tissues, retinal cells, their processes, and organelles are phase objects which scatter light (Zernike, 1955). In particular, synapses in the plexiform layers and nerve fibers at the inner retinal surface have dimensions close to 500 nm, i.e., within the wavelength range of visible light (380–770 nm), which make them light-scattering structures (Tuchin, 2000). The inherent light reflection allows visualization of retinal layers with optical coherence tomography (OCT). Light scattering reduces the visual acuity and decreases the signal-to-noise ratio of the visual signal (Agte et al., 2011). In addition, retinal blood vessels impede the light path to the receptors (Snodderly et al., 1992). As site of high acuity vision, the retina of haplorrhine primates contains a fovea which is a specialization of the horizontal area centralis. In the center of the fovea (the foveola), the inner retinal layers are shifted peripherally and blood vessels are absent (Müller, 1856; Duke-Elder, 1958); this reduces light scattering at neuronal structures and vessels, and allows a more direct illumination of the receptors. The extreme packing and elongation of the cone photoreceptors in the foveola in association with the midget system of neurons in the foveal slope and parafovea are the main basis of the high-acuity vision provided by the fovea (Polyak, 1941; Hendrickson, 2005).

Müller cells of the primate fovea have a characteristic 'z-shape' which results from the centrifugal shift of the inner Müller cell processes and the centripetal displacement of the photoreceptors and outer Müller cell processes (Reichenbach and Bringmann, 2010). Both

Abbreviations: HFL, Henle fiber layer; ILM, inner limiting membrane; OCT, optical coherence tomography; OLM, outer limiting membrane; ONL, outer nuclear layer; OPL, outer plexiform layer; PBS, phosphate-buffered saline

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movements cause the elongation of the Henle fibers (Ahnelt, 1998; Sjöstrand et al., 1999). Henle fibers are formed by photoreceptor axons that run obliquely or horizontally between the photoreceptor cell somata in the outer nuclear layer (ONL) and the synaptic terminals in the outer plexiform layer (OPL); the axons are surrounded and bound together by Müller cell processes.

It has been described that (in addition to the 'typical' Müller cells of the fovea) there are 25–35 unique Müller cells in the macaque foveola (Reichenbach and Bringmann, 2010). These Müller cells are suggested to form the 'Müller cell cone', i.e., the inverted cone-like structure that overlies the area of high photoreceptor density in the primate foveola (Yamada, 1969; Gass, 1999). The outer processes of the foveolar Müller cells do not leave the foveola and fail to join the course of the photoreceptor axons into the Henle fiber layer (HFL); instead, they run more or less straight towards the innermost layer of the foveola where the somata are located (Reichenbach and Bringmann, 2010). These foveolar Müller cells have no contact to synapses or neuronal elements other than cones. The reduced expression of key enzymes of glio-neuronal transmitter recycling, glutamine synthetase and glutamate uptake transporter GLAST (Bringmann et al., 2013), in the foveola (Nishikawa and Tamai, 2001; Nishikawa, 2006–07) supports this finding. Müller cells of the foveola may have various functional roles. Because they contain macular pigment at high density, they reduce the effects of chromatic aberration and protect the central photoreceptors from light damage by absorbing short-wave-length light (Reading and Weale, 1974; Snodderly et al., 1984). In addition, Müller cells were suggested to provide the structural stability of the fovea (Gass, 1999). The latter function is recognizable in central cystoid macular edema where the detached inner Müller cell layer of the foveola often holds the foveal slopes together; disruption of this layer results in macular hole formation (Gass, 1999).

Müller cells of the primate foveola, which form the 'Müller cell cone', are little characterized. In particular, data on the ultrastructure of these cells are still limited. The aim of the present study was to characterize in more detail the ultrastructural features of the foveolar Müller cells. We found that the somata of foveolar Müller cells extend numerous thin inner processes which spread horizontally below the basal lamina of the inner limiting membrane (ILM); these processes may improve the optical properties of the retinal tissue by smoothing the inner surface of the foveola, may create additional barriers to the vitreous cavity, and may provide mechanical stability to the foveola. We also observed that, in contrast to the cytoplasm of the Müller cell somata and inner processes, the cytoplasm of the outer processes, lying in the ONL of the foveola, displays a dispersed and watery appearance.

2. Materials and methods

2.1. Foveal tissues

The study followed the tenets of the Declaration of Helsinki for research involving human subjects. The use of human material was approved by the Ethics Committee of the University of Leipzig (#745, 07/25/2011). Two eyes were used in the study: one eye of a 40 years-old human male without apparent intraocular disease (enucleation after chemical burn of the eyeball) and one eye of an adult cynomolgus monkey (*Macaca fascicularis*; Covance Preclinical Services, Münster, Germany). A written informed consent from the human donor for the use of retinal tissue in basic science was obtained. The macaque was euthanized in the course of independent experiments, and the eyes were enucleated.

2.2. Tissue preparation

Immediately after enucleation, the anterior parts of the eyes and the vitreous were removed. The posterior poles of the eyes (10 × 10 mm) with the foveas were excised at room temperature using a binocular and a scalpel. The tissues were fixed by immersion for 2 h at 4 °C in a mixture of 4% paraformaldehyde and 0.3% glutaraldehyde in Tyrode's solution which contained (in g/L) 8 $NaCl_2$, 1 glucose, 1 $NaHCO_3$, 1.79 NaH_2PO_4, and 0.2 KCl. After several washing steps with 0.1 M phosphate-buffered saline (PBS; pH 7.4), the tissue blocks were postfixed in 1% OsO_4 (Sigma-Aldrich, Taufkirchen, Germany) in 0.1 M PBS for 1 h at 4 °C. After washing in PBS, the tissues were dehydrated through 30 and 50% acetone at room temperature (15 and 30 min, respectively). Thereafter, the tissue blocks were contrasted with 1% uranyl acetate in 70% acetone for 45 min at room temperature. Then, the tissues were further dehydrated using 90 (30 min) and 100% acetone (30 min twice) at room temperature and embedded in Durcupan (Sigma-Aldrich), before preparation of semi- (thickness, 3 μm) and ultrathin sections (thickness, 60–65 nm).

2.3. Light and transmission electron microscopy

Semithin sections were stained with toluidine blue; images were recorded with a water-immersion objective (50×; numerical aperture, 1.0; Zeiss), a Jenaval light microscope (Zeiss, Oberkochen, Germany), and a CCD camera (Kappa Optronics, Gleichen, Germany). Ultrathin sections were mounted on Pioloform (Wacker Chemie, Munich, Germany)-coated nickel grids and contrasted at room temperature with uranyl acetate for 10 min and Reynold's lead citrate for 5 min. Images were recorded with a Zeiss 912 OMEGA transmission electron microscope equipped with a slow-scan CCD camera (Proscan, Lagerlechfeld, Germany), and the Atlas software (Zeiss).

2.4. Image analysis

The raw images were cut with Adobe Photoshop 6.0 and remained unprocessed for creating the figures. The center of the foveola was defined as the site of the smallest thickness of the tissue. The thickness of the foveola was measured between the ILM and outer limiting membrane (OLM). The rim of the foveola was defined as the site between the area in which only single scattered somata of inner nuclear layer (INL) neurons were found and the area in which somata of INL neurons were regularly present (Fig. 1). It should be noted that absolute

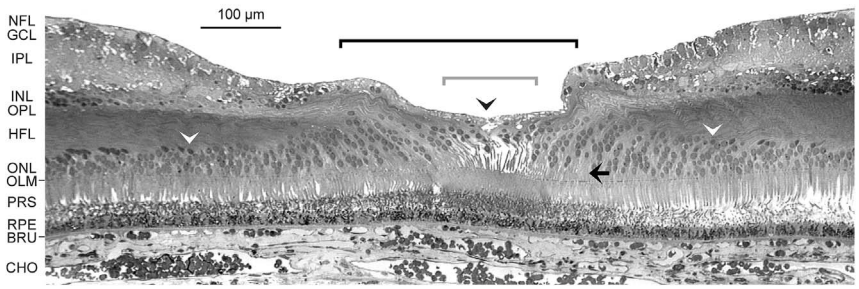

Fig. 1. Structure of the human fovea. The image shows a toluidine blue-stained semithin cross-section through the fovea. The foveola (*black bracket*) is composed of a thin inner layer, which contains the somata and inner processes of specialized Müller cells and that lies in front of the outer nuclear layer (ONL) and the fovea externa which contains the elongated central cone photoreceptors (*gray bracket*). The ONL of the foveola is composed of obliquely arranged rows of cone cell somata (including nuclei) which are separated from the outer limiting membrane (OLM) by a layer which contains the outer fibers of cone cells (OFC) and Müller cell processes (*arrow*). *White arrowheads* indicate the centralmost rod nuclei. The *black arrowhead* indicates the deepest point of the foveal pit. BRU, Bruch's membrane; CHO, choroidea; GCL, ganglion cell layer; HFL, Henle fiber layer; INL, inner nuclear layer; IPL, inner plexiform layer; NFL, nerve fiber layer; OPL, outer plexiform layer; PRS, photoreceptor segments; RPE, retinal pigment epithelium.