Structure of the Retina in the Growing Teleost, *Tilapia zillii*

Ghada Alomari, Mohammad- Amin Al-Adhami and Janti Qar*

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Abstract

The light- and dark-adapted retina was studied in four developmental stages of *Tilapia zillii*, a pelagic freshwater, mouth breeder teleost. The retina in the stages studied have a very large falciform process and a curved, open embryonic fissure indicating an asymmetrical retinal growth carried out by the marginal germinal zone. The retina of the species under study is, like that of the majority of teleosts, of the duplex type, i.e. contains both rods and cones. The cones recorded are single, double and triple cones. Vitreally a rod ends into a rod spherule with a single synaptic ribbon and a cone ends into a large cone pedicle with two-five synaptic ribbons.

The retinal pigment epithelium shows a clear reaction to light and dark. In the dark-adapted retina the apical process and melanosomes are retracted, so that the photoreceptor outer segments are exposed, while in the light-adapted retina the apical process with their melanosomes contents extend vitreally to shield the outer segments. Similarly, the photoreceptors exhibit retinomotor movements. Hence, in the light-adapted retina, the rods are extended and the cones show a square mosaic arrangement. In dark-adapted retina, however, the rods are withdrawn (exposed) and the cones show linear arrangement.

The stages studied here witness signs of retinal growth including decreased thickness of the retina and its constituting layers and decreased cellular density. The rod: cone ratio increases with age.

Introduction

The vertebrate retina is an accessible part of the brain. During development, the forebrain evaginates laterally into two optic vesicles. These, then, invaginate to form the optic cups and their connections to the fore brain constrict thus forming the optic stalks (Ennis and Kunz, 1986, Kunz and Callaghan, 1989). A median, ventral
groove, the embryonic fissure (EF), runs from the outer rim of the optic cup into the optic stalk (Schmitt and Dowling, 1994, 1999). This fissure is closed in the majority of vertebrates. Some teleost species, however, retain it throughout life. Its shape may give an indication of the pattern of retinal growth (Douglas et al., 1987, Easter, 1992, Cameron, 1995, 1996). According to Ennis and Kunz (1986) and Kunz and Callaghan (1989), it plays a role in retinal growth due to the fact that the marginal germinal zone (MGZ) extends in to it. In those species, which retain an open EF, a highly vascular fold projection emerges from the fissure into the vitreal cavity. This fold, the falciform process (FP), helps ventilating the eye.

Teleostean retina, like other vertebrate retinas (Douglas et al., 1987), is composed of ten layers. The majority of teleosts have duplex retinas, i.e. have both rods and cones. The latter may be present in several forms: short and long single cones, unequal double cones and twin cones. Triple cones have been rarely recorded (Cameron and Easter, 1995).

The retinas of fish grow throughout life (Ali, 1964, De Miguel Villegas et al. 1997). This is achieved by two mechanisms: 1) A balloon-like stretching of the different retinal layers helps expanding the retina (Johns and Easter, 1977, Shand, 1997). 2) The MGZ at the peripheral rims of retina have an aggregation of pluripotent cells that continually add new neurons and glial cells to the retina (Johns, 1977, Meyer, 1978). Another source of neurogenesis is represented by the addition of new rods by rod precursors found in the ONL (Johns and Fernald, 1981).

In this work we studied the structure of the adult retina in the fresh water teleost, *Tilapia zillii* (Gervais), at the light and electron microscopic levels. We also investigated the structural changes associated with the balloon-like stretching of the retina observed in four different sizes of the fish.

**Materials and Methods**

**Fish samples**

Thirty individuals of the teleost fish, *Tilapia zillii* were collected from Zeitlab dam, north of Jordan. Collections were made between May and July 1999, by using manual sweeping nets. In the laboratory, the fish were categorized into four sizes groups according to their total length. These are 3, 5, 7 and 11 cm. They were then kept in aquaria until the time of fixation. For dark-adaptation the fish-containing aquarium was kept in the darkroom for at least three hours. The eyes were dissected out there and transferred to dark vials containing the fixative.

**Light Microscopy**

The fish was killed by decapitation. The eyes were dissected out, enucleated and bisected under a Wild M3 dissecting microscope. Fixation was done in Bouin's
fluid. The material was then washed in 70% ethanol, dehydrated in upgraded ethanol and cleared in xylene. Paraffin-embedded material was then serially sectioned by a Wild microtome. Stains used were either Ehrlich hematoxylin and eosin or Malory's triple stain. The 7μm-thick sections were examined on Leitz Laborlux 11 microscope and photographed by WILD MPS 51. Cell count for the three cellular layers was made in the fundic retina of all four stages studied. Ten replicas were studied for each.

**Electron Microscopy**

Enucleated eyes were bathed with the primary fixative (2.5% gluteraldehyde in 0.1m sodium cacodylate buffer; pH 6.4-7.4) and minced under a dissecting microscope. Small pieces of the retina were transferred to small vials containing new amounts of the fixative and kept under room temperature for 2 hrs. They were washed with the buffer and postfixed in 1% osmium tetroxide in the same buffer for three hours. Sectioning was made by LKB ultratome. Semithin sections were stained with methylene blue, while ultrathin sections were stained with uranyl acetate and lead citrate and examined under Zeiss EM 10 CR.

**Statistical Analysis**

Data were analyzed using Microsoft Excel 97 and MINITAB program. The relations between the increment of fish total length on one hand and change in retinal thickness or cellular densities on the other were analyzed using student t-test.

**Results**

The antero-laterally directed eyes of *Tilapia zillii* are situated above the midline of the head. The retina is composed of eight layers and two membranes, characterizing all vertebrate eyes. Beginning with scleral side, these are: retinal pigment epithelium (RPE), visual cell or photoreceptor layer (VCL), outer nuclear layer (ONL), outer plexiform layer (OPL), inner nuclear layer (INL), inner plexiform layer (IPL), ganglion cell layer (GCL) and nerve fiber layer (NFL). The scleral and vitreal extensions of Muller cells form the external and internal limiting membrane (ELM and ILM), respectively (Fig.1).

Choriocapillaries closely abut the outer surface of Bruch's membrane (complexus basalis). Similarly, the inner surface of the retina is lined with small vitreal blood vessels (Fig.2), that branch from the hyaloid artery which enters the eye along with the optic nerve through the mid ventral line. The embryonic fissure is persistent with a well-developed falciform process, and is lined with pigment epithelium (Fig.3).

The RPE is a simple cuboidal-columnar epithelium, composed of mononucleated cells with round to oval basally located nuclei. Spherical and rod-
shaped melanosomes are scattered throughout the pigment cell body. The mitochondria are spherical to oval in shape. Smooth endoplasmic reticulum (SER), phagosomes, and myeloid bodies are also scattered in the cell body. The RPE is separated from choriocapillaries by a trilaminar Bruch's membrane (Fig.4). This layer shows retinomotor (photomechanical) movement in response to light and dark conditions. In the light adapted state the rod-shaped melanosomes occupy the vitreal processes of pigment epithelial cells, while the spherical granules remain in a scleral position. In the dark, the pigment epithelial processes retract and the rod-shaped granules are intermingled with the spherical granules.

The retina of Tilapia zillii is a duplex retina. Three types of cones are observed: single cones, double cones and triple cones. Their nuclei are ovoid with their apexes extending to the external limiting membrane. Rod nuclei are smaller and more vitreally placed. Visual cells are arranged in a square mosaic patterns in which the sides of the square unite are formed by double cones, while a single cone occupy the center of the square (Figs. 6, 7). This arrangement is changed into a row pattern in the dark condition (Fig. 8).

A complex network of neuronal interaction made up of pre- and post-synaptic terminals of visual, bipolar and horizontal cells form the OPL, which range between 5-10μm in thickness. The junctions between these neurons are of two types, synaptic ribbons and surface contacts. The bulbous vitreal end of rod myoids, the rod spherule, possesses a single synaptic ribbon (Fig. 9), while its homologous in the cones, the cone pedicle possesses 2 – 5 synaptic ribbons (Fig.10). The INL is composed of one scleral row of horizontal cells, several rows of bipolar cells, Muller cells and two vitreally situated rows of amacrinal cells (Figs. 5, 11). Somata of the ganglion cells (3-5 μm in diameter) are arranged in one row.

Vitreal processes of Muller cells divide the GCL and NFL into fascicles. This feature is particularly clear in the fundic region of the retina (Fig. 11), while at the peripheral region is indistinct (Fig. 1). The Muller cells processes from the ILM on the vitreal surface of the NFL (Fig. 11).

In the four size groups studied here, the increment of the fish total length is associated with a significant reduction in thickness of the whole retina (P<0.024) due to its stretching (Table 1, Fig.12), thus contributing to the overall increase in the size of the eye, including the retina. Another indication for retinal growth is that the absolute thickness of some of the retinal constituting layers shows almost an equivalent decrease. This is particularly obvious in the INL (P < 0.055) (Table 1, Fig. 13).

The ratio of rods to cones in the youngest stage studied here is 2: 1. It gradually increases to reach the ratio of 2.8: 1 in the eldest studied fish (Fig. 14).
Discussion

The eyes of fish and amphibians, unlike those of higher vertebrates, grow continuously. In a number of teleost species, the addition of new neurons and glial cells by the MGZ is asymmetrical. In the nasal side of the retina the addition of new cells goes on at a higher rate than in the temporal retina (Hagedorn and Fernald, 1992; Schmitt and Dowling, 1996). In order to amend this unbalanced growth, more neurons (photoreceptors) are added on the temporal side of the EF thus causing it to curve on that side. During the present work, the retina of the studied stages of T. zillii retains an open, curved EF indicative of asymmetrical growth (Easter, 1992, Cameron, 1995, 1996).

The other mechanism of retinal growth, the balloon-like stretching, was also evident during the present work, indicated by a progressive thinning of the retina and its constituting layers. This mechanism has been recorded almost in all teleost species studied (Ali, 1964; Johns and Easter, 1977, Fernald and Johns, 1980, 1981, Shand, 1997).

The third mechanism of retinal growth noticed during this study is not a widely recorded one. In the retinas of some fish species, new neurons have been noticed to be added by the different cellular layers of the retinas (Marcus, et al. 1999; Schmitt and Dowling, 1999). Evans and Fernald (1993) suggest that the GCL contains rod progenitors that traverses the retina and form vertical bands in the OPL. Similar observation has recorded by De Miguel Villegas et al. (1997). During the present study a few cells have been observed extending across the IPL an observation that favors such an origin for rods.

Like the retinas of the vast majority of fish, the retina of T. zillii is of the duplex type. Four types of photoreceptors are evident. These are: rods, single cones, double cones and triple cones. The ratio of cones: rods is clearly high compared to most other species studied (Meer, et al. 1995, Collin, et al. 1996). These facts represent a good visual acuity and, consequently, a good adaptation for vision oriented predation mode of life (Meer, et al. 1995; Collin, 1997; Shand, 1997). An acute vision is also necessary for the species understudy being a mouth-breeder.

Ziglab dam is an oligotrophic water body with a high transparency. This fact is reflected on fish eye function. The retina shows an efficient retinomotor movement involving both the RPE and photoreceptor as an accommodation to the great fluctuation in the light intensity of such a water body. This observation is in accordance with similar results on the majority of teleost fish studied (Kunz, 1980, Kunz and Ennis, 1983, Braekevelt, et al. 1998a,and b). Some teleosts studied, however, do not show such a diurnal change and retain the square mosaic arrangement through the dark (Kunz et al., 1985)
Acknowledgement

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اتركيب الشبيكة في سمكة المنش

Tilapia Zillii

أحمد العمري و محمد أمين الأعظمي و جانتي قار

التاريخ

درست الشبيكة الكيفية للضوء للفظام في أربع مراحل النمو في سمكة العظمية Tilapia Zillii وقد وجد أن الشبيكة في المراحل المدروسة تمتلك زائدة منجلية وشقاً جنوباً يدل على نمو غير متزن للمشبك تقوم به المنطقة الجرثومية الحافية.

بينت الدراسة أن شبكية السمكة موضوع البحث، مثل تلك التي ل غالبية الأسماك العظمية. من النوع المزدوج الذي يمتلك قضاءاً ومخاريط. وتحت المخاريط مفردة أو ثنائية أو ثلاثية. ينتهي كل قضاء داخلياً بكرية ذات شريط وصلي واحد. بينما ينتهي المخاريط بسوق ذي 2-3 أشرطة وصلية.

يظهر النسيج الطلائي المصفي الشبكية استجابة للضوء والظلام. ففي الشبكية الكيفية للظلام تنسحب الزوائد اللمبية الخدمات الصباغية لتعزز القطع الخارجية للمستقبلات الضوئية. بينما تمتد هذه الزوائد وتنتشر فيها الجسيمات الصباغية في الضوء للفتح القطع الخارجية. ويتضح انها تنسحب القضبان وتظهر المخاريط مпублиعاً في الضوء، بينما تتسرب القضبان وتتظم المخاريط خطياً في الظلام.

شهد مراحل النمو التي درست هنا زيادة نسبة القضبان إلى المخاريط ونقص الكثافة الخلوية وسمك الشبكية وطباتها الثانية.

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References


**Table 1.** The relationship between fish total length on one hand, and retinal thickness, INL cell density, ganglionic cell density and rode : cone ratio, on the other.

<table>
<thead>
<tr>
<th>Fish total length (cm)</th>
<th>Retinal thickness (μm)</th>
<th>INL cells/0.01mm</th>
<th>Ganglionic cells / 0.01mm</th>
<th>Rod:cone ratio</th>
</tr>
</thead>
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<tr>
<td>3</td>
<td>212.98±10.95</td>
<td>178.0 ±20.69</td>
<td>20.6 ±6.70</td>
<td>2:1</td>
</tr>
<tr>
<td>5</td>
<td>189.60±9.03</td>
<td>134.5 ±18.37</td>
<td>18.2 ±3.27</td>
<td>2.2:1</td>
</tr>
<tr>
<td>7</td>
<td>178.00±5.75</td>
<td>97.6 ±10.07</td>
<td>9.7 ±3.27</td>
<td>2.4:1</td>
</tr>
<tr>
<td>11</td>
<td>157.60±16.70</td>
<td>70.9 ±10.46</td>
<td>6.7 ±2.00</td>
<td>2.8:1</td>
</tr>
</tbody>
</table>

* Measurements are expressed as mean ± S. D.

α P<0.024 (student t-test)

β P<0.041 (student t-test)

χ P<0.055 (student t-test)
Fig. 1. Photomicrograph of the light-adapted retina of *T. zillii* showing the different layers. Abbreviations: GCL, ganglion cell layer; INL, inner nuclear layer; OPL, outer plexiform layer; PE, retinal pigment epithelium, arrow, external limiting membrane. (x: 1,000).

Fig. 2. Light-adapted retina showing vitreal blood vessels (small arrow) attached to the vitreal surface of the inner limiting membrane (large arrow). (x: 400).
Fig. 3. The embryonic fissure (arrow) piercing the retina. The vascular tissue that makes the core of the fissure emerges into the vitreal cavity as a falciform process (FP). (x: 250).

Fig. 4. Electron micrograph of outer retina (light-adapted). Choriocapillaries (asterisk) enclose Bruch’s membrane (arrow) sclearally. The vesicular nuclei (N) of the pigment cells are basally situated. Spherical-elongated melanosomes are abundant within the supranuclear cytoplasm and the apical processes of these cells. Note that the apical processes extend between the outer segments (OS). Liposomes (L) are also present. (x: 5,000).
Fig. 5. The different layers of the retina excluding the retinal pigment epithelium. Ellipsoids (large arrow), outer plexiform layer (OPL) and inner nuclear layer (asterisk) are clearly shown. Note the relatively low cellular density of the three cellular layers, particularly the ganglion cell layer (small arrow). (x: 1,250).
Fig. 6. Electron micrograph of the double cone ellipsoids of the light-adapted retina manifesting the square mosaic arrangement. Four double cones (arrows indicating subsurface cisternae separating members of double cones) make up the corners of the square and a single cone occupies its centre. OS, outer segment. (x: 6,250).

Fig. 7. Tangential section of the light-adapted outer retina showing the square arrangement (square) of the double cones. (x: 1,000).
Fig. 8. Row arrangement of double cones in the dark-adapted retina. (x: 1,000).

Fig. 9. Rod spherule with a single synaptic ribbon (arrow) and many spinules. (x: 15,650).
Fig. 10. A diad (arrow) and a synaptic ribbon in a cone pedicle. (x: 25,000).

Fig. 11. Dark-adapted retina of the youngest stage studied. The apical processes of the retinal pigment epithelium are retracted. In the photoreceptor layer, the ellipsoids of single, double (D) and triple (T) cones are shown. The high cellular density of the outer nuclear layer, inner nuclear layer (INL) and ganglion cell layer is clear in comparison to that shown in Fig. 5. An ectopic neuron (small arrow) traverses the inner plexiform layer. Vitreal processes of Muller cells divide the nerve fibre layer (asterisk) into fascicles and emerge vitreally to form the inner limiting membrane (large arrow). (x: 1,000).
Fig. 12. Decreased retinal thickness with changes in the fish total length.

Fig. 13. Changes in the density of INL cells and ganglionic cells (GCs) during fish growth.

Fig. 14. Changes in the rod : cone ratios during fish growth.