Vision Research 68 (2012) 19–27

Contents lists available at SciVerse ScienceDirect

Vision Research

journal homepage: www.elsevier.com/locate/visres

Structure and spectral sensitivity of photoreceptors of two anchovy species: *Engraulis japonicus* and *Engraulis encrasicolus*

Sergei L. Kondrashev [a,*,1], Valentina P. Gnyubkina [a,1], Lidiya V. Zueva [b,1]

[a] A.V. Zhirmunsky Institute of Marine Biology, Far East Branch, Russian Academy of Sciences, 690059 Vladivostok, Russia
[b] I.M. Sechenov Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences, 194223 St. Petersburg, Russia

ARTICLE INFO

Article history:
Received 28 May 2012
Received in revised form 10 July 2012
Available online 17 July 2012

Keywords:
Retina
Triple cone
Visual pigment
Colour vision
Polarisation detection
Microspectrophotometry

ABSTRACT

The morphology, fine structure and spectral sensitivity of retinal photoreceptors of two anchovy species were investigated using light and electron microscopy and microspectrophotometry. Distinct regional specialisation of cones was observed. Long and short (bilobed) cones were observed in the horizontal retinal belt, including the nasal and temporal retinal zones. Only triple cones with two long lateral components, one small central component were observed in the dorsal and ventro-nasal retinal regions. The long cones presented various lamellar organisation patterns: (1) in parallel along the cell axis in the central retina, (2) oriented transversely at the base of the outer segment, and (3) tilted longitudinally while extending to the tip of the cone in the retinal periphery. In the short cones, the lamellae were always oriented along the cell axis, and their planes were perpendicular to the lamellae in the long cones, providing a structural basis for the detection of polarisation of incident light. The lamellae in all the outer segments of the triple cones are arranged perpendicular to the long cell axis. In both species, the long and short cones from the ventro-temporal retina were slender and more densely packed, and the outer segments of the long cones lay far more sclerad compared with the outer segments of the bifid cones. Microspectrophotometry revealed that in both species the lateral components of the triple cones displayed a maximum absorbance wavelength (λ_{max}) of approximately 502 nm, while the short central components were more shortwave sensitive (λ_{max} = 475 nm). The λ_{max} of all long and short cones in the ventro-temporal zone was 492 nm, compared to 502 nm in other retinal regions. Anchovies are unique among vertebrates in that they contain clear structural basis for both colour and polarisation vision in the same retina.

1. Introduction

The structure of the retina is common to all vertebrate species: rods and cones of several types are arranged in a continuous layer with a distinct morphology and complement of visual pigments in the numerous photoreceptive membranes (lamellae) of the outer segments. Changes in illumination result in the excitation of the photoreceptors and secondary neurons that process primary visual information. It has been suggested that changes in retinal arrangement and morphology are closely related to peculiarities of the specific visual world and tasks of most animals (Lythgoe, 1979; Munz & McFarland, 1977).

The morphologies of the retinas of many representatives of the Engraulidae family are significantly different from the morphologies observed in all fish and other vertebrate species. The short and long cones of anchovies are interlocked in specific units known as "polycones" (Fineran & Nicol, 1978), which are arranged in rows that alternate with rows of rods (Heß et al., 2006; Zueva, 1981). The outer segments of the short cones are bilobed (and sometimes termed "bifid"); in contrast with the transverse pattern observed in other vertebrates, the layers of the lamellae of each cone lobe lie along the cell axis. The long cones are also unusual compared to the cones of other fish; their lamellae are also oriented along the cell's long axis in at least part of the outer segment volume. Moreover, the lamellae of the short and long cones are positioned at right angles to each other, thus providing a morphological basis for polarisation vision (Fineran & Nicol, 1978; Novales Flamarique & Hawryshyn, 1998; Novales Flamarique & Hárosi, 2002; Zueva, 1981).

In addition to short and long cones, some anchovy species have specialised triple cones, described for the first time in European anchovy, *Engraulis encrasicolus* (Zueva & Govardovskii, 1991). The triple cones comprise three photoreceptor components that are bundled together in a group, and appear as a succession of rows in limited zones of the dorsal and ventral retina, where they exhibit a clear regional specialisation. The lamellae of the outer

* Corresponding author. Address: A.V. Zhirmunsky Institute of Marine Biology, Far East Branch, Russian Academy of Sciences, Palchevsky Str. 17, 690059 Vladivostok, Russia. Fax: +7 423 2310900.
E-mail address: navodon@rambler.ru (S.L. Kondrashev).
[1] These authors contributed equally.

0042-6989/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.visres.2012.07.005

segments of the triple cones in species of the genus *Engraulis* are oriented "normally", i.e. transversely to the cell's long axis (Heß, 2009; Novales Flamarique, 2011; Zueva & Govardovskii, 1991). However, not all species of anchovy have triple cones. Despite a thorough investigation of the spatial distribution of cones across the entire retina, the presence of triple cones was not detected in the Bay anchovy, *Anchoa mitchilli* (Fineran & Nicol, 1978; Novales Flamarique & Hárosi, 2002). A study of the retina of the Japanese anchovy, *Engraulis japonicus*, also did not show the presence of triple cones (Awaiwanont et al., 2001). However, other closely related species, such as *E. encrasicolus* and *Engraulis mordax*, have triple cones.

Notably, detailed investigation of the morphology and ultrastructure of the anchovy retina (Fineran & Nicol, 1978; Heß, 2009; Heß et al., 2006; Novales Flamarique, 2011; Zueva, 1981; Zueva & Govardovskii, 1991), the spectral sensitivity of anchovy photoreceptors and the properties of anchovy visual pigments have only been studied by microspectrophotometry (MSP) in the European anchovy (Zueva & Govardovskii, 1991) and the Bay anchovy (Novales Flamarique & Hárosi, 2002). MSP has shown that the outer segments of rods, the short and long cones of the European anchovy have the same λ_{max} and their absorbance spectrum matches rhodopsin template. In contrast, members of the triple cones contain different visual pigments (Zueva & Govardovskii, 1991), potentially providing the basis for colour discrimination in the dorsal and ventral retinal areas. However, these conclusions were based upon the estimation of the half-bandwidth of spectral absorbance data, which provides insufficient information about the nature of visual pigments in these species.

A comparative light microscopy study of museum anchovy specimens revealed a high level of diversity within the retinal structure of 11 anchovy species (Heß et al., 2006). Moreover, detailed studies of the retinal ultrastructure in a limited number of species have yielded contradictory data on the orientation of the lamellae in the long cones (Awaiwanont et al., 2001; Fineran & Nicol, 1978; Novales Flamarique & Hárosi, 2002; Zueva, 1981). The species of the family Engraulidae dwell in various ecological niches, such as seas, estuaries and freshwater basins in temperate, subtropical and tropical zones. Thus, considering the unique organisation of the retina in certain species of anchovy and the inconsistencies found in key parameters, more comprehensive research is necessary. The goal of this study was to investigate the morphology, ultrastructure and spectral sensitivity of retinal photoreceptors in two species of the genus *Engraulis*.

2. Materials and methods

2.1. Fish

Adult Japanese anchovies, *E. japonicus*, were obtained in summer months between July 2008 and August 2011 in the Vostok Bay (Sea of Japan) near Marine Biological Station "Vostok" of the A.V. Zhirmunsky Institute of Marine Biology FEB RAS. The fish were caught at night using a fixed net, placed immediately in a closed thermos containing melted seawater ice and delivered to the laboratory within a half an hour for use in morphological and histological analyses and microspectrophotometry. The fish were immobilised in seawater containing a high concentration of the anaesthetic MS222 (Sigma) and subsequently decapitated. The eyes were enucleated and dissected in small Petri dishes placed on ice under a stereomicroscope.

The fish were treated in accordance with the EU Directive of 2010/63/EU, and the Scientific Council of the Institute of Marine Biology, Far Eastern Branch of the Russian Academy of Sciences (IMB FEB RAS) approved the experimental procedures. The details of the retina of the European anchovy (Black Sea subspecies) *E. encrasicolus ponticus* were described using a collection of semi-thin sections and TEM micrographs, which were obtained in earlier studies (Zueva, 1981; Zueva & Govardovskii, 1991).

2.2. Histology

Extracted eye cups were fixed for several days in a 2% paraformaldehyde/2% glutaraldehyde solution at 4 °C, post-fixed in a 1% osmium tetroxide solution and embedded in an Epon–Araldite mixture. Semi-thin radial and tangential sections (1 μm) of pieces of eye cup, which were precisely oriented relative to the embryonic fissure, were obtained using an ultramicrotome (LK 2B), stained with a 1% toluidine blue solution, and examined under a Olympus BH2 light microscope. Ultrathin sections contrasted with lead citrate were examined under JEM 100B (JEOL) and Zeiss Libra 120 transmission electron microscopes.

For microspectrophotometry and *in situ* studies of the photoreceptors using a light microscope, each eye cup was placed onto a Petri dish containing a chilled physiological saline solution (0.9% NaCl solution in 0.06 M phosphate buffer, pH 7.2) for retinal extraction. A small slice of the retina, which was free of pigmented epithelium, was torn up into tiny fragments using sharp needles on the glass slide in a few drops of physiological saline solution. Afterwards, a drop of methylcellulose (m.v. 4000) solution was added to 1–2 drops of the saline solution containing the isolated photoreceptors to increase the viscosity of the medium and prevent spontaneous movement during microscopic observation. The mixed solution containing suspended photoreceptors was placed between two cover glasses, sealed with Vaseline, and examined under a POLYVAR light microscope (Reichert-Jung, Austria) equipped with Normarski optics or used for microspectrophotometry. Digital photos were obtained using a Canon S50 camera with a Leica DC150 camera adapter.

2.3. Microspectrophotometry

Retinal fragments from 16 fish specimens of *E. japonicus* were used. The eyes were dissected on ice in infrared light (4-LED array) under a stereomicroscope equipped with a high-resolution analogue video camera (WATEC Co., Korea). The image from the video camera was controlled using a black-and-white monitor covered with dark red acrylic glass.

The absorption spectra of the outer segments of photoreceptors, which contain the visual pigments, were measured using a microspectrophotometer equipped with a specially designed "jumping" table (Govardovskii & Zueva, 1988). This is single-beam device; the measured object is placed on the table vibrating at small amplitude and 30 Hz frequency. The measuring beam penetrates alternatively the object and near-cell free area, and electronics makes all next processing. The same device was used during MSP studies of fishes of the Lake Baikal (Bowmaker et al., 1994). It includes a MBR light microscope, a MDR grating monochromator (both instruments were obtained from LOMC, St.-Petersburg, Russia) and a registering attachment with a photomultiplier FEU-79 and an amplifier. A dry quartz-mirror condenser UF 40×0.5 and a 40×0.95 dry objective (both from LOMC) were used. The measuring beam dimensions varied from 2×10 μm to 1×2 μm, depending on the size of the outer photoreceptor segment measured. In some experiments the measuring beam was linearly polarised in the plane of the membranes in the discs of the outer segments using a glass polarisation light filter to enhance the recording.

We measured the absorbance spectra of 190 components of the isolated cones (34 short and 80 long components, and 40 lateral and 36 central components of the triple cones), and 12 groups of rods; the small rod diameters prevented us from obtaining