Insights into anchovy vision – retinal neuroanatomy of *Engraulis encrasicolus*

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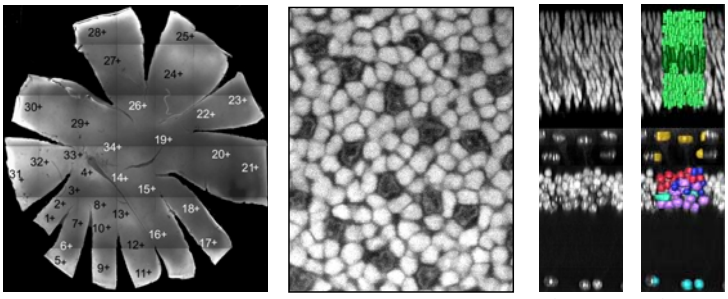
Introduction

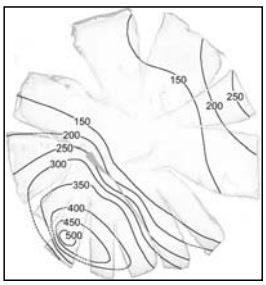
The outer retina of the European anchovy (*Engraulis encrasicolus)* shows an unusual ultrastructure providing specific sensitivity to polarized light. Exceptional features are cone chains (polycones) consisting of two alternating cone types (short and long cones), a radial alignment of lamellae containing visual pigment and a wedge shaped specular tapetum to enhance photon yield. The high regularity of the cone arrangement is expected to be continued down to the level of second and third order neurons, as these structures are synaptically tied to the photoreceptors. To gain more insight into anchovy vision our studies are addressed to the structure of the inner retina applying four main methods.

Material & Methods

European anchovies were obtained from fishermen in Rovinj (Croatia), and eyes were fixed. For the investigation of nuclear patterns, cell topography and correlation maps DAPI stained retinal wholemounts were scanned with a two-photon microscope and nuclei are counted via 3D-reconstruction (1). On the basis of ultrathin section series and TEM the complex structure of the outer plexiform layer is reconstructed in 3D (2). Neuroanatomical stainings with antibodies or lipophilic dyes reveal cell morphology and allow for cell classification (3). The investigation of the inner plexiform layer is carried out by tangential semithin section series and CLSM (4).



A B C C‘



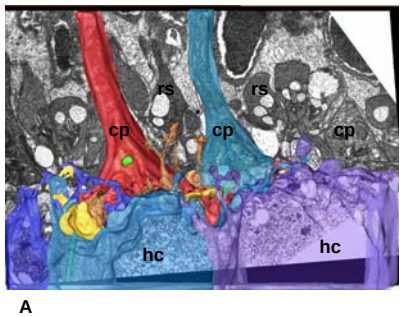
D

1 **A** Retinal wholemount with 34 two-photon-scanning sites.

B Two-photon-scan of DAPI-stained photoreceptor nuclei at site 15; dark cone nuclei arranged in polycones with intermediate bright rod nuclei.

C Brightest point projection of a two-photon-stack (radial view) showing all retinal cell nuclei, **C‘** with 3D-reconstructed cell nuclei for counting them.

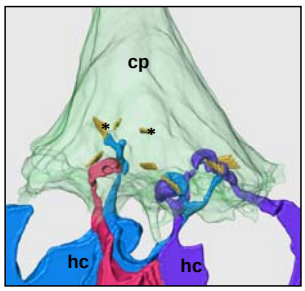
D Isodensity contour map illustrating the distribution of cone photoreceptors.



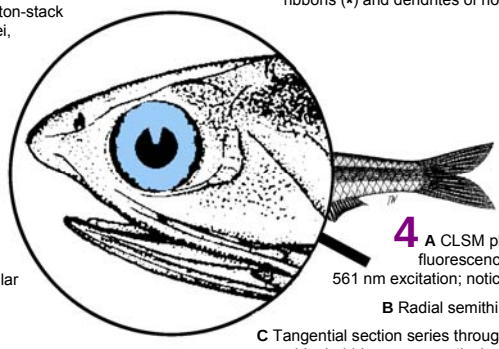
A B

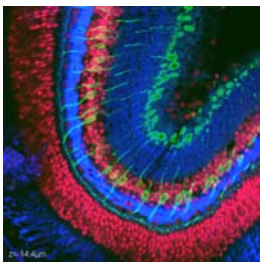
2 **A** 3D-reconstruction of cone pedicles (**cp**) and adjacent horizontal cells (**hc**) from an ultrathin section series; not reconstructed: rod spherules (rs).

B, C Details of a single cone pedicle (**cp**) with synaptic ribbons (*) and dendrites of horizontal cells (**hc**).



C





A

3 **A** Multispectral CLSM photograph of a retinal section near the Ora serrata; ToPro3 (nuclei, red), PKCα-positive bipolar cells (green), Müller cells and IPL (Wheat Germ Agglutinin, blue).

B PKCα-positive bipolar cells and **B‘** 3D-reconstruction of PKCα-positive bipolar cells and bipolar cell nuclei; the **arrow** depicts the same sublayer as in 4A-D.

C Ganglion cells stained with the lipophilic tracer DiI.

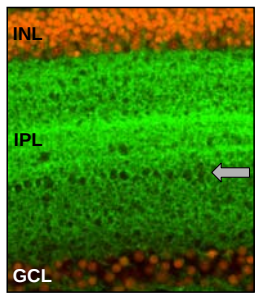
4 **A** CLSM photograph of the auto-fluorescence of the IPL (green) at 561 nm excitation; notice the substratification;

B Radial semithin section of the retina;

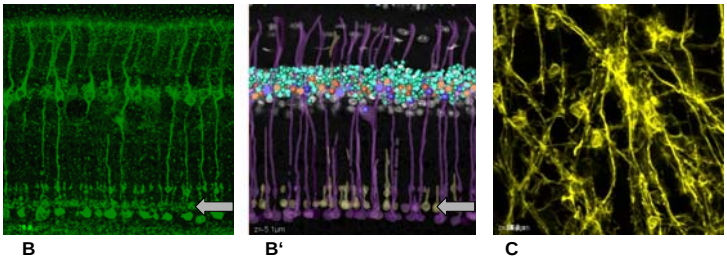
C Tangential section series through the boxed area in B; white bubbles are synaptical endings of bipolar cells.

D Single 3D-reconstructed layer of synaptical endings of bipolar cells depicted by the **arrow** in A-C; notice the regular arrangement!

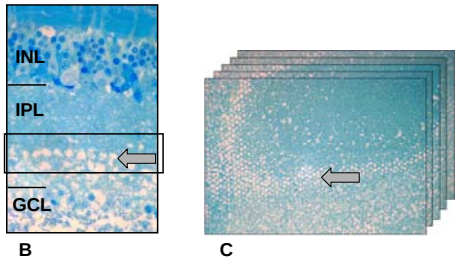
INL: inner nuclear layer, **IPL**: innerplexiform layer, **GCL**: ganglion cell layer; **arrow** depicts the same sublayer, also shown in 3B.



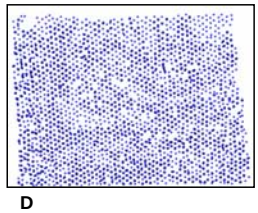
A



B B‘ C



B C



D

Results

Topography and correlations of <u>all</u> retinal cell nuclei are visualized by isocontour maps (1D). The OPL’s meshwork can be described and unravelled by 3D-reconstruction of ultrathin section series (2). In this way, neuronal wiring rules are going to be uncovered. By means of immunohistochemistry (e.g. Anti-PKCα, Anti-Calretinin etc.) the morphology of subpopulations of horizontal, bipolar and amacrine cells are revealed (3). The inner plexiform layer shows a division into several sublaminae consisting of largely regularly arranged synaptical endings that can be correlated with the immuno-histochemical data (4). Liphophilic dyes (DiI, DiO, DiD) provide an insight into ganglion cell’s morphology and network (3C).

Discussion

The ongoing studies represent a multi-method approach to illuminate the inner retina structure of a specialized vertebrate eye. Our data reveal highly regular and complex configurations in both the outer and inner plexiform layer as the synaptic endings mirror the cone arrangement. Different classes of horizontal, bipolar and amacrine cells are defined by cell morphology, antibody-staining specificity, termination depth in the IPL and nuclear characteristics (inner structure, shape, size, position). There is a strong correlation of topographic cell distribution between cone photoreceptors and second/ third order neurons. These findings are essential for future understanding of the mechanisms of polarization vision in vertebrates.