Short Communications

Tracts of putative ultraviolet receptors in the retina of the two-year-old brown trout *(Salmo trutta)* and the Atlantic salmon *(Salmo salar)*

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Summary. We previously showed that the brown trout possesses UV-sensitive cones in its retina that are lost in 2-year-old fish. However, present investigations reveal that in the narrow growth zone along the periphery and the ventral embryonic fissure, the formation of these cones continues in trout and salmon.
Key words. UV-receptors; retina; tetrachromacy; retinal mosaic; development; *Salmo trutta; Salmo salar;* brown trout; Atlantic salmon.

Color vision depends on the presence of more than one cone photopigment. Several teleost fish were shown to have tri- or dichromatic vision, i.e. they possess cone pigments with absorbance peaks at three or two different spectral locations[1]. Tetrachromacy, including as a fourth receptor a miniature cone type absorbing in the near-UV, was found in two cyprinids[2,3].

Recently, we showed that the brown trout also is tetrachromatic, with UV-sensitive single cones forming part of a regular cone mosaic pattern[4,5]. Since these cones occupy the corner positions in the square mosaic units, they are called 'corner cones'. They are slightly shorter than the blue-absorbing single cones found in the center of the mosaic unit (fig. 6). While present over the whole retina from the fry (swimming-up) stage to the one-year-old, the 'corner cones' disappear during the second year[4,5]. Similarly, it was noticed in the Atlantic salmon, *Salmo salar,* that at the age of 125–130 days its eye contained square mosaics with a full complement of cones, whereas in the adult (no age given) corner cones (Zwischenzapfen) were lacking[6].

Unlike mammals, the eyes of teleosts grow throughout life, and new photoreceptors are continuously added in the peripheral zone. Analyses of visual pigments by microspectrophotometry (MSP) and of whole-mounts by lightmicroscopy usually are performed on hemisected eyes, whereby the annular growth zone is being discarded. Therefore, we investigated if, in salmonids, 'corner cones' are still produced in this narrow peripheral zone of cell division when they are no longer present in the rest of the retina. Since the embryonic (choroid) fissure, which in teleosts never closes, represents an extension of the peripheral growth zone, it was included in our study (figs 1, 2).

Material and methods. Due to the curvature of the periphery and the embryonic fissure of the eye, scanning electronmicroscopy was the technique of choice, complemented by lightmicroscopical analyses of whole-mounts of the excised peripheral zone, the fissure and the remaining retina (RM). The eyes of 20 two-year-old brown trout and 5 salmon parr were enucleated and the sclera, choroid and pigment epithelium peeled off while immersed in the fixative (phosphate buffered 1% formaldehyde/3% glutaraldehyde). 1) Whole-mounts were viewed with a Wild M20 Research microscope with drawing tube attachment. 2) Retinae were critical point dried, gold coated[7,8] and viewed with a Jeol 35c Scanning Electronmicroscope.

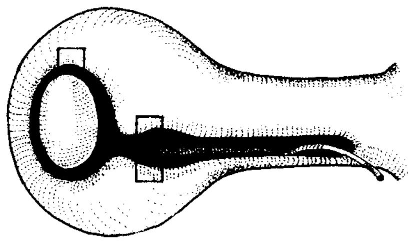
Results and discussion. Analysis of the RM of the two-year-old brown trout showed a square mosaic pattern throughout, devoid of 'corner cones', and confirms, therefore, our previous findings[4,5]. Lightmicroscopy of the retina of the salmon parr gave the same result. The absence of 'corner cones' in the RM yields a double-to-single cone ratio of 4:1 in both salmonids.

The growth zones clearly showed the youngest area with cone inner segments only, followed by cones with connecting cilia and, finally, cones with outer segments (fig. 3). Rods were not seen by surface viewing since they develop at a much lower (vitreal) level than the cones[9]. When fully developed, however, the rods move into a scleral position so that their outer segments now obscure the cone outer segments (fig. 3). Both in the two-year-old trout and the salmon parr, the cones are usually arranged in a row mosaic in the most peripheral region of the circumferential growth zone and the embryonic fissure (fig. 4). (Row mosaics have the subsurface membranes of their double cones arranged in a straight line, while they form a zig-zagging line in the square mosaics.) Our analyses showed rows of double cones separated by rows of single cones corresponding to the central and 'corner cones' of the square mosaic (figs 4, 5). Still within the growth zone, the row mosaic changed into a square mosaic with the shorter single cones taking up a corner position (figs 6, 7).

The cone density in the peripheral growth zone and in the embryonic fissure is greater than in the RM. Due to the presence of the 'corner cones', the ratio of double-to-single cones has changed to 1:1.

Neuronal death during early development seems to be a normal occurrence in vertebrates[10]. Glücksmann, working on tadpoles, was the first to observe a wave of degeneration in the outer nuclear layer at the onset of photoreceptor development before synaptogenesis occurred[11]. In contrast, the loss of photoreceptors observed by us occurs in fully developed retinae of salmonids during their second year. The loss, presumably by death, follows a spatio-temporal gradient (ventro-nasal to ventro-temporal to dorsal region) and affects a specific type of cone only, which occupies a precise location within a regular cone mosaic[4,5]. On the other hand, the UV-cones observed in cyprinids (of unspecified age) are sparse and have not been assigned a location within the cone mosaic, which is of a very irregular square type[2,3]. Scholes[12]

Figure 1. Diagram of eye showing ventral embryonic fissure which in teleosts does not fuse (modified after Portmann). □, denote areas analyzed. Figure 2. Dissected right eye of salmonid, with dorsal iris removed. Photograph taken through lens to highlight the course of embryonic fissure. (×6). Figure 3. Scanning electron micrograph of fissure with growth zones. CIS, cone inner segments only; CC, cone inner segments with connecting cilia; COS, cone outer segments formed; ROS, rod outer segments in a scleral position. They are not visible in the younger zones in surface view since they develop in a more vitreal position to that of the cones. □ Area shown in figure 5. Figure 4. Diagram of row mosaic at the level of cone inner segments. Rows of double cones alternate with rows of two types of single cones. Figure 5. Row mosaic in fissure as viewed with the scanning electronmicroscope. Alternating single cones, corresponding to the central and somewhat shorter 'corner cones' of the square mosaic, are distinct.



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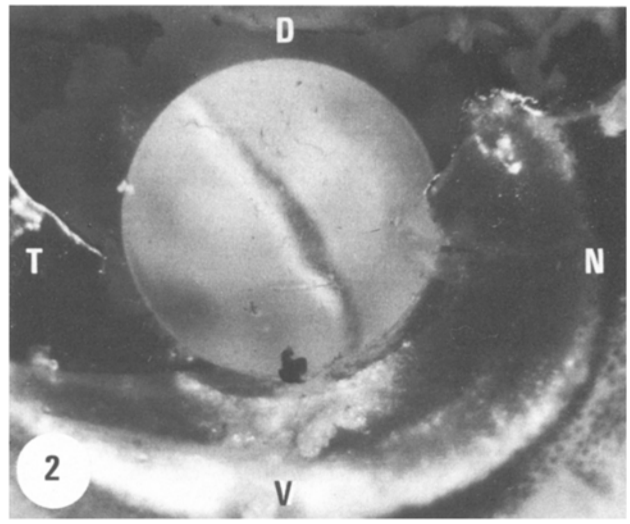

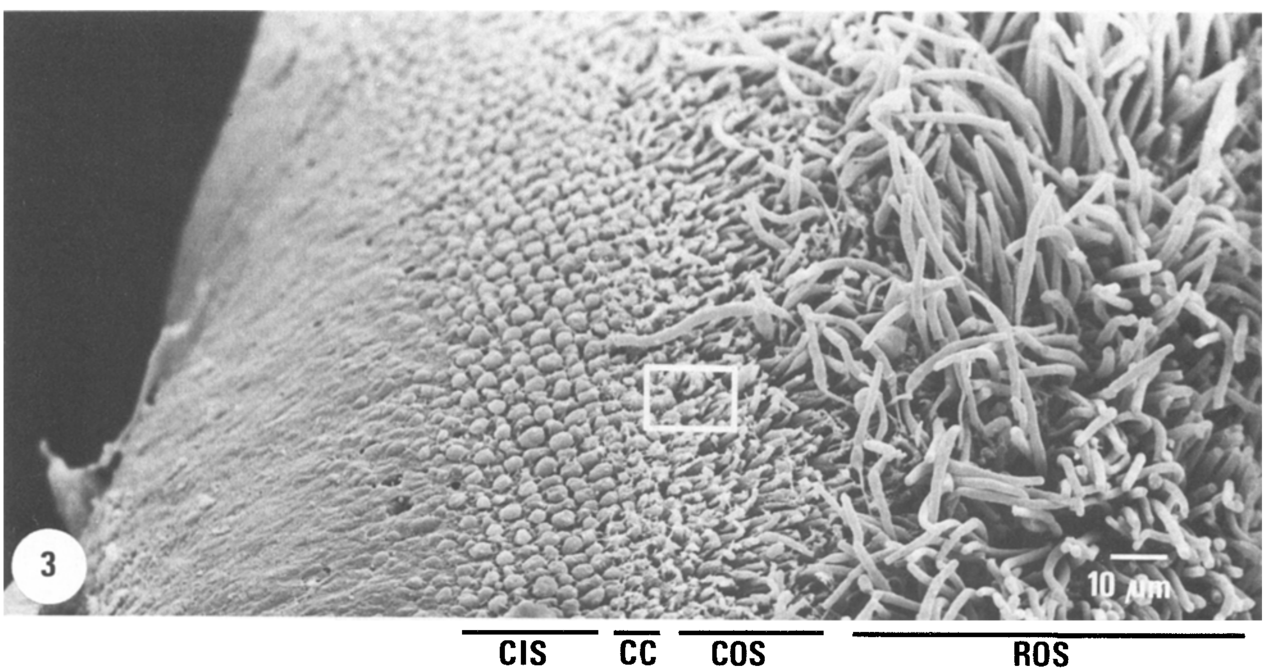

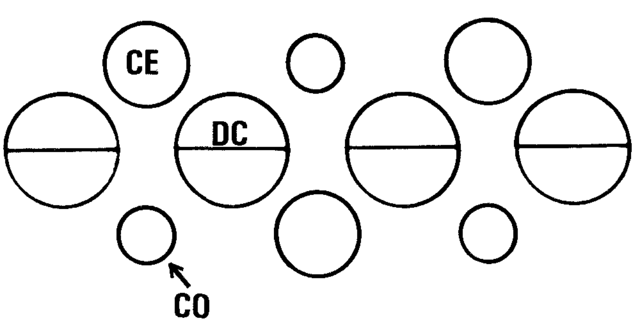

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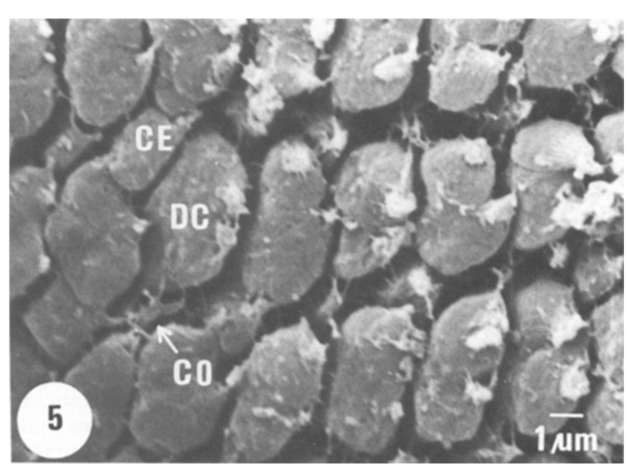

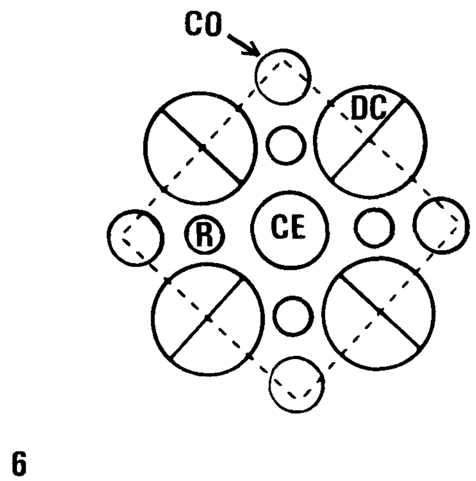

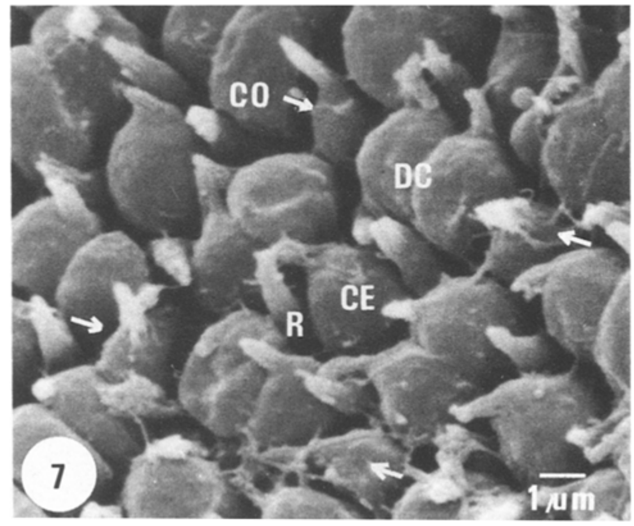

Figure 6. Diagram of square mosaic with shorter single cones in a corner position. Rods are regularly distributed within the cone mosaic unit at this stage of development. Figure 7. Scanning electronmicrograph of square mosaic in fissure. Cone outer segments are well developed.

Abbreviations: CO, corner cones; CE, central cones; CIS, cone inner segments; COS, cone outer segments; D, dorsal; DC, double cones; N, nasal; R, rods; ROS, rod outer segments; T, temporal; V, ventral.

observed in a cyprinid (the rudd) that 'oblique cones', which correspond to our 'corner cones', are absent in older specimens. Our present results show that in older salmonids the formation of 'corner cones' continues but is restricted to a very narrow zone in the periphery and along the embryonic fissure. After formation, the cones are gradually displaced centrally and the 'corner cones' disappear while new ones are being differentiated peripherally.

The fate of disappearing 'corner cones' remains to be established. Adjacent cells, and phagocytes entering from the choroidal blood supply, are likely candidates for debris uptake. Recently, Müller cells have been reported to phagocytose pyknotic cells in the rat[10].

The disappearance of one third of the cones between the ages of 1–2 years of the salmonids poses the further question as to how the loss of synaptic contact with, presumably, horizontal and bipolar cells affects the retinal circuitry, and, finally the retinal mapping in the optic tectum.

While it is still a matter of debate as to what advantages UV-vision conveys to a teleost, the functional significance of the gradual loss of the UV-cones with age over most of the retina is equally enigmatic. Apart from playing a role in selective food uptake[4,5], it may be the stimulation of the UV-sensitive cones which causes the fish to move to deeper waters where the penetration of UV lights is diminished. It has been shown that solar UV-radiation can cause epidermal necrosis in fish kept in surface waters. Such a skin trauma deprives the fish of its protective outer layer and subsequently exposes it to a variety of infection agents[13]. The 'corner cones' in the circumferential strip of the eye cup of older fish are unlikely to play a role in vision. However, the presence of densely packed 'corner cones' along a definite ventral tract, the embryonic fissure, – if they are UV-sensitive – may be instrumental in sun compass orientation. There is growing evidence that fish have an intraocular e-vector analyzer[14]. Therefore, the 'corner cones', may be instrumental in the detection of polarized light. Behavioral experiments, which test the UV-sensitivity and response to plane polarized light of salmonids, may shed further light on what specific cues salmonids use for orientation during their long migration.

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