Retinomotor Movement of All Spectral Cone Types of Red Sea Bream *Pagrus major* in Response to Monochromatic Stimuli and UV Sensitivity*¹

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This paper examines the hypothesis that spectral cone types exhibit different retinomotor movements in response to monochromatic radiation and can thus be identified histologically in the retina. Red sea bream *Pagrus major*, 79–105 mm in total length, were dark adapted in a glass aquarium, then exposed overhead to various intensities of white light and monochromatic stimuli with peaks at 337, 368, 466, 551 and 609 nm. The retinæ from fish from each stimulus were examined histologically and scored for thickness of the cone layer and the pigment layer relative to the total retinal thickness. The red sea bream tested showed retinomotor response to 368 nm but not to 337 nm, and therefore were considered sensitive to ultraviolet stimulus. As additional single cones were absent in the cone mosaic, UV perception by central single cones or twin cones was suggested. White light and three other monochromatic stimuli elicited the retinomotor movement of all single and twin cones. These results and similar data from the literature show that the light-adaptive retinomotor movement of different spectral cone types is triggered by light perception in rods and thus do not vary by wavelength of visual stimulus between 368 and 609 nm.

Key words: red sea bream, retina, retinomotor movement, UV sensitivity, cone type, monochromatic stimuli

In fish eyes, retinal photoreceptors and melanin pigment granules exhibit characteristic retinomotor movements in response both to ambient illumination and signals from an endogenous circadian clock. Both the light-induced and circadian-driven movements of cone myoids occur in the absence of efferent input from higher centers.¹⁾ Circadian cone movements are regulated locally within the eye and mediated by endogenous dopamine.¹⁾ Color discrimination is mediated by three photoreceptor substances contained in the outer segments of the cones, each maximally sensitive to a different region of the spectrum: red, green and blue.^{2,3)} Some teleost fishes are known to have single cones sensitive to ultraviolet (UV) with $\lambda_{\max}$ 350 to 370 nm.⁴⁾ If monochromatic stimulation brings about retinomotor movement of one cone type only and not the other, it may be possible to identify respective cones by histological examination of the retina. Many authors have investigated the mechanical responses of fish retinæ to monochromatic stimuli of equal energies and determined the spectrum visible to the fishes concerned, but they have not considered the different cone types.⁵⁾ The objective of this study is to examine the retinomotor movements in response to monochromatic stimuli and the UV sensitivity in the red sea bream *Pagrus major*. This fish is known to be color sensitive.⁶⁾

Materials and Methods

Red sea bream (79–105 mm in total length TL) were ob-

tained from a local fish farmer and kept in a stock tank. Two juveniles were placed in a barrel-like glass trough measuring 12 × 23.5 cm, in water 16 cm deep. The fish were dark-adapted for at least 1 h, and exposed to an overhead monochromatic radiation and white light for 30 min at water temperature 25.0–26.3°C. The sides and bottom of the trough were covered with wrinkled aluminum foil to diffuse the radiation in the trough. Since cones respond to circadian signals, the experiment was done at night.

The light source was a stabilized DC 65V 150W xenon lamp bulb in a lamp house with a condenser lens (Irie, HXA-500), the intensity of which was varied by neutral-density filters (Toshiba, ND). Monochromatic radiation was obtained by interposing a narrow-band monochromatic interference filter between the condenser lens and the neutral density filters. Nippon Shinku Kogaku monochromatic interference filters with peaks at 337 and 368 nm (UV), and Toshiba interference filters with peaks at 466, 551 and 609 nm (visible light) were used. The UV intensity was measured with an UV meter (Minolta, UM-1) and visible light intensity with a photon meter (Meiwa, LICORLI-1000, sensitivity range at 400 to 800 nm). The intensity of stimuli varied from 2.2×10^{-4} to $2.3 \mu\text{M m}^{-2} \text{s}^{-1}$ for the visible light and from 1.3×10^{-6} to $1.7 \times 10^{-2} \text{mW cm}^{-2}$ for the UV.

For histological preparation of the retinæ, fish were killed by cutting the head prior to removal of both eyes. The enucleated eyes were then placed in Bouin's fixative for one day and transferred into 70% alcohol. Tissue from

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the naso-ventral retina, the area to which the overhead lights were presented, was excised and dehydrated, cleaned in xylene, and embedded in paraffin. Cross and tangential sections were cut at $6\text{ }\mu\text{m}$ thick, subsequent to staining with haematoxylin and eosin.

The thickness of the pigment and cone layers provides a good measure of rates of light- and dark-adaptation and was measured with an ocular micrometer. The pigment layer was measured from the edge of the choroid to the tips of pigment protuberances. Since the cones undergo photomechanical changes in the portion of the cell proximal to the external limiting membrane, the thickness of the cone layer was measured from the membrane to the tips of the myoids. Measuring the rods was difficult due to their smallness and the masking effects of the pigment epithelial cells in the light adapted retinae. As the thickness of the layers varied with the size of the retina, each measurement was expressed as a percentage of the total retinal thickness, or retinal index.⁷⁾

In addition to the above-mentioned histological experiments, earlier preparations of retinae of juvenile red sea bream in a previous study⁸⁾ were reexamined for possible changes in cone types and arrangement with growth after metamorphosis of this fish.

Results

Cone Types and Arrangement

In tangential sections of the retinae of fish smaller than 31 mm TL, single and twin cones were found to form a mosaic in a square arrangement. A central single cone was surrounded by four twin cones, with an additional single cone at a corner of the square mosaic, thus the twin cone:single cone ratio was 1:1. In fish 35 mm TL, the additional single cones were absent in the square mosaic and the twin cone:single cone ratio was 2:1. In fish (79–105 mm TL) used in the retinomotor movements, the square cone mosaic was composed also of twin cones and central single cones, with no additional single cones (Fig. 1).

Retinomotor Movements in Response to Visible Light

All eyes exposed to 466, 551, 609 nm and white light at intensities 2.2×10^{-4} to $4.0 \times 10^{-3} \text{ }\mu\text{m}^2 \text{ s}^{-1}$ were dark-adapted. The cones lay close to the contracted pigment, which was in the form of a dense compact strip adjacent to the choroid. Contrary to the expectation, the monochromatic radiation induced the movement of all single and twin cones and pigment in the entire retina; all cones were completely contracted adjacent to the external limiting membrane, and pigments had migrated fully into the processes of the pigment epithelial cells almost enveloping the outer segments of the cones. The light-adaptation took place at intensities $0.02\text{--}0.042 \text{ }\mu\text{m}^2 \text{ s}^{-1}$ at 466 nm, 551 nm and white light and at $0.11 \text{ }\mu\text{m}^2 \text{ s}^{-1}$ at 609 nm (Fig. 2). The cones exposed to 609 nm radiation migrated almost fully to the external limiting membrane at an intensity of $0.0022 \text{ }\mu\text{m}^2 \text{ s}^{-1}$ whereas the cones and pigments exposed to the other wavelengths did not do so.

Retinomotor Movements in Response to UV Stimuli

The retinomotor response of the all cones and pigment was also found in the eyes exposed to 368 nm UV at an ir-

Fig. 1. Tangential section showing the square cone arrangement in the retina of a 79 mm TL red sea bream.

T, twin cone; S, central single cone. Scale bar, $20\text{ }\mu\text{m}$.

Fig. 2. Retinal index (% of total retinal thickness) of the epithelial pigment and cone layer in the eyes of red sea bream exposed to monochromatic light and white light at various intensities.

radiance 0.046 mW cm^{-2} (Figs. 3A and B). Retinomotor movement was absent at 337 nm UV at irradiance 1.3×10^{-6} to $1.7 \times 10^{-2} \text{ mW cm}^{-2}$ (Figs. 3C and 4).

Discussion

When the retina of fish exposed to a particular wavelength is light-adapted then that wavelength is visible to the fish; when the retina remains dark-adapted the fish cannot see in that light.⁷⁾ The observed light-adaptation in response to 368 nm radiation indicates that this wavelength is visible to the red sea bream.

The lack of retinomotor response to 337 nm radiation even at an irradiance 0.016 mW cm^{-2} , indicates poor or no sensitivity to this wavelength. UV sensitivity of fish is known to be mediated by short single cones or additional single cones.^{9–13)} While the additional single cones are present in the square cone mosaic of early juvenile red sea bream,⁸⁾ they disappear around 35 mm TL. The red sea bream used in the retinomotor movement tests had no additional single cones in the retina. However, red sea bream larger than 35 mm TL are still UV-sensitive. Contrary, an independent cone photoreceptor mechanism sensitive to UV is present during the early juvenile stages but is lost during normal development in brown trout *Salmo trutta*,⁹⁾ Atlantic salmon *S. salar*,¹⁰⁾ rainbow trout *Oncorhynchus mykiss*,¹¹⁾ rudd *Scardinius erythrophthalmus*,¹²⁾ and yellow perch *Perca flavescens*.¹³⁾ The UV sensitivity of the retina

Fig. 3. Cross sections showing the retinal adaptation in UV radiation. A, Retina in the process to light-adaptation, 368 nm light at $4.6 \times 10^{-3} \text{ mW cm}^{-2}$; B, Light-adapted retina, 368 nm light at $4.6 \times 10^{-2} \text{ mW cm}^{-2}$; C, Dark-adapted retina, 337 nm light at $1.3 \times 10^{-2} \text{ mW cm}^{-2}$. PE, Pigment epithelial cell layer; CO, cone layer. Scale bar, 50 μm .

Fig. 4. Retinal index (% of total retinal thickness) of the epithelial pigment and cone layer in the eyes of red sea bream exposed to UV radiations at various intensities.

without additional single cones in the red sea bream might indicate the mediation by central single cones or twin cones.

The cones made light-adaptive movements at a lower intensity light of 609 nm than any other visible light used. This result means a higher sensitivity of the cones to 609 nm wavelength, inconsistent with recordings of L-type S-potential from the retina of the red sea bream¹⁴ and the general pattern in retinomotor movements in other fishes.⁵ The L-type S-potential indicates a peak visual sensitivity at around 522 nm and low sensitivity to wavelengths longer than 612 nm. In other fishes, the onset of migration of cones and pigments takes place at the same light intensity. The inconsistency of the present result with other findings merits further careful investigation.

In red sea bream, all cones showed light-adaptive retinomotor movement in response to monochromatic radiation at all wavelengths. Ali⁵ speculated that the inner synaptic relationships might bring about a reaction of all cone types even when a stimulus of only one wavelength was given for an extended period. Kirsch *et al.*¹⁵ electrophysiologically examined the retina of the cichlid *Aequidens pulcher* and concluded that light-adaptive cone contraction was triggered by light absorption in rods. In general, fishes have

duplex retinæ in the adult stage but pure-cone retinæ in the larvae, the rods developing at metamorphosis.^{8,16,17} Retinomotor movements only commence after metamorphosis when the rods develop in herring *Clupea harengus*, pilchard *Sardina pilchardus*, haddock *Melanogrammus aeglefinus*, and lemon sole *Microstomus kitt*.¹⁶

In the red sea bream, therefore, the retinomotor response also seems to be triggered by rods. Thus all spectral cone types exhibit light-adaptive retinomotor movements in response to monochromatic radiation regardless of wavelength. This is understandable from an adaptive point of view. The offshore clean water acts as a monochromatic blue filter and the optic environment of fish offers a blue stimulus.¹⁸ If only the blue cones react in the environment, color sensitivities would be lost even if fishes concerned are sensitive to color.

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