Action Spectrum of Photomechanical Cone Contraction in the Catfish Retina

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The action spectrum for the photomechanical contraction of catfish single cones was found to fit the absorbance spectrum of the visual pigment they contain (determined by microspectrophotometry). This indicates that cone contraction is brought about by a direct effect of light on the cones themselves and not, as is the case for the epithelial pigment, by an indirect effect via the rods. Invest Ophthalmol Vis Sci 25:534–538, 1984

The rods, cones, and epithelial pigment in the retinas of many lower vertebrates undergo positional changes in direct response to changes in the ambient illumination. In the light, the cones are contracted near the external limiting membrane (ELM), while the rods are elongated and are positioned at the back of the eye buried in the dispersed epithelial pigment. On dark adaptation, these positions are reversed, and the cones elongate to take the place of the rods at the back of the eye along with the aggregated epithelial pigment, whereas the rods contract to lie near the ELM. Such migrations serve to position the receptors optimally for the functions they have to perform and to protect the rod visual pigment in the light-adapted retina.[1]

In both the trout[2] and frog,[3] the spectral response curve—or action spectrum—of light adaptive epithelial pigment migration resembles the absorbance spectrum of that animal's scotopic visual pigment. This indicates that isomerization of the rod visual pigment triggers epithelial pigment dispersion. The triggers for rod and cone photomechanical changes, however, are unknown.

For cone contraction, there are two ways in which the light could bring about its effect. The simplest hypothesis is that it occurs in response to a direct effect of light on the cones themselves. The alternative is that like the dispersion of the epithelial pigment, the contraction of cones is triggered by the isomerization of the rod visual pigment. This latter possibility, although at first sight somewhat unusual, could make functional sense, since at dawn the cones are positioned so that they appear to be shielded by a layer of rods, and much of the low level illumination will be absorbed by these rods. If the former alternative is correct, the action spectrum of cone contraction should resemble the absorbance spectrum of the cone visual pigment. However, should cone contraction be rod-triggered, the action spectrum will be fitted best by the rod visual pigment.

The present study determines the contraction action spectrum of cones in the retina of the glass catfish, *Kryptopterus bicirrhis*. This species is particularly suitable for such an experiment since it only has one spectral cone type, a single red cone absorbing maximally in the long wavelength region of the spectrum. The resulting action spectrum is compared with the absorbance spectra of both the photopic and scotopic visual pigments, which are determined by microspectrophotometry.

Materials and Methods

Forty-two catfish (4–5 cm in length) were bought from a local dealer and maintained on a 12 hr light/dark cycle (light phase 05.00–17.00, 1 lux at the water surface) for 36 days at 20 ± 2°C.

Optical System

The basic apparatus is shown in Figure 1. The adapting aquarium was a cylindrical tank (18 cm in diameter, 10 cm in length), flat at both ends and made of transparent Perspex. It was painted white on the inside and black on the outside of all sides except one of the flat ends. This tank was secured in the optical system as shown in Figure 1 so that the circular light beam completely filled the transparent flat vertical front face. A diffuser was placed immediately in front of the tank, ensuring that the fish were bathed in uniform

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monochromatic light from all sides. The light source was a 100 W Halogen lamp. Wavelength and intensity were controlled by the insertion of Schott interference and neutral density filters into the light beam. The box containing the adapting tank was painted mat black on the inside and was completely light tight except for the hole through which the stimulus entered. The complete apparatus was in a darkened room and the light source was shielded additionally by a black box.

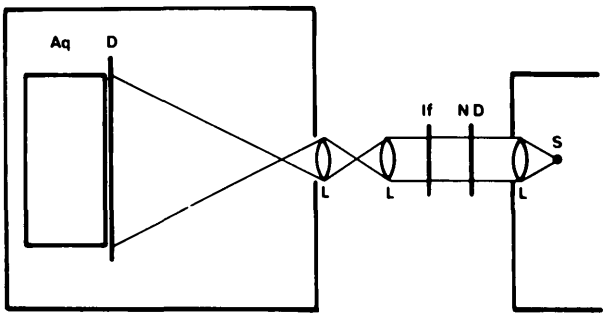

Fig. 1. The optical system used to expose whole fish to uniform 360° monochromatic light. Aq = aquarium; D = diffuser; L = lens; If = interference filter; ND = neutral density filter; S = light source. The aquarium and diffuser as well as the light source were enclosed in black boxes.

Experimental Procedure

All experiments were performed during the middle of the dark phase of the light/dark cycle. This ensured maximal dark adaptation, which often cannot be obtained during the day due to endogenous influences on photomechanical changes.[4] For each experimental exposure, two completely dark-adapted fish were placed in the adapting aquarium and subjected to a particular combination of wavelength and intensity for 45 min. The order of presentation of the test wavelengths (443, 496, 544, 620 nm) and intensities was random.

It is usual in such experiments to use a dim red torch when handling the animals in "darkness." However, as in this case, the contraction of red-sensitive receptors was being investigated, and a dim blue torch was used instead. Control experiments showed that this illumination did not cause any degree of light adaptation by itself.

Pilot experiments had shown that it was not always possible to distinguish catfish, rods, and cones reliably. Therefore, 3 days before each experiment, the eyes of the fish were injected with 2 μl of physiologic saline containing 2 μCi of fucose L- 6-^{3}H (specific activity: 84 Ci/mmol, NEN). This is taken up by the outer segments of red cones but not by the rods.[5] Following autoradiographic processing, it was easy to distinguish the labelled cones from the unlabelled rods. Control experiments leaving one eye uninjected showed no difference in cone positions between injected and uninjected eyes.

Histologic Procedure

Following the experimental adaptation, fish were killed in dim blue light by decapitation—the eyes were enucleated, hemisected, and immersed either in Bouin's or in a phosphate-buffered, 2.5% glutaraldehyde/1% paraformaldehyde fixative for approximately 12 hr. Subsequently, the tissue was washed, postfixed in 2% OsO_4 (2 hr) and dehydrated in a series of alcohols before being embedded in Epon resin. Sections 0.5/1.0 μm thick were then cut on an ultramicrotome and prepared for autoradiography by dipping them in Kodak NTB 2 emulsion and exposing them for 22 days. Slides were developed subsequently in D19 for 3.5 min at 15°C. Following photographic fixation, the sections were stained with Richardson's stain.[6] At least 50 cones were examined from the central area of each retina. The positions of the cones were expressed as a cone index, which is taken as the distance from the ELM to the ellipsoid/outer segment junction divided by the distance from the ELM to Bruch's membrane. Typical light- and dark-adapted cone indices for the glass catfish are 0.15 and 0.45, respectively. All values for a retina were averaged to give an index for that retina. As two fish were used for each wavelength/intensity combination the values shown in Figure 2 are usually the average cone index of four eyes.

Determination of Sensitivity

In the above manner, a curve relating the degree of cone contraction to the intensity of the stimulating light was obtained for each of the four wavelengths tested (Fig. 2). These curves are typically sigmoid in shape, with cones in a fully light-adapted position at high intensities of stimulation and completely dark-adapted at the lowest intensities. However, for the determination of sensitivity, only those levels of stimulation that give an intermediate degree of cone contraction are of interest. Therefore, for each wavelength a regression line was fitted to all cone indices on the descending part of the curve—that is at those intensities of stimulation that gave the last fully light-adapted average cone index, the first fully dark-adapted average cone index, and any intermediate intensities. The inclusion of cone indices obtained at higher and lower intensities of stimulation in the regression line would result in a line that is not a true representation of cone elongation with decreasing light intensity. From these lines, the intensity of light that gave 50% cone contraction was determined for each wavelength. This corresponds to a cone index of 0.3. Since the regression lines are virtually parallel, a different cone index as

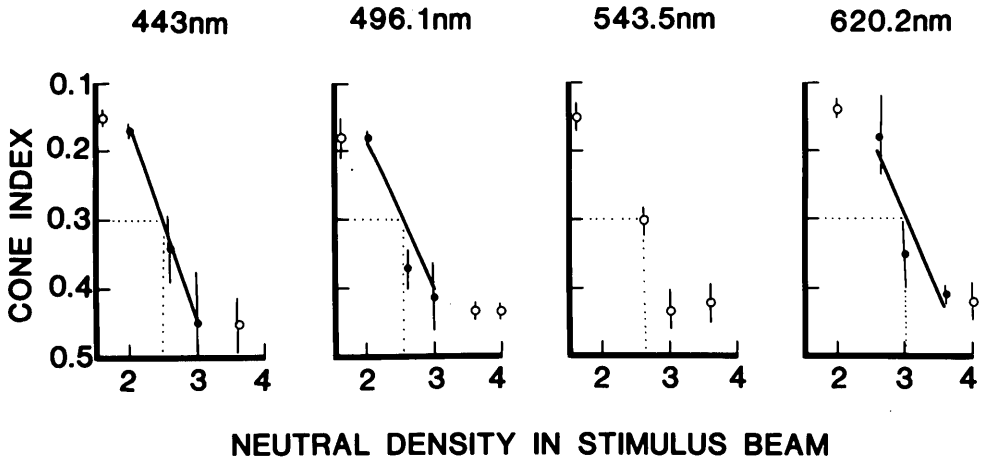

Fig. 2. The degree of cone contraction, as indicated by the cone index, of red cones in the retina of the glass catfish, *Kryptopterus bicirrhis*, following various intensities of monochromatic stimulation at four wavelengths. Low cone indices represent light-adapted cones and high values of neutral density filter in the stimulus beam represent a low light intensity. The solid line is a regression line fitted to the cone indices shown by the solid points (443 nm, $y = -0.39 + 0.28x$; $R^2 = 0.98$; 496 nm, $y = -0.275 + 0.233x$; $R^2 = 0.921$; 620 nm, $y = -0.385 + 0.228x$; $R^2 = 0.869$). Points indicated by the empty circles were not used in the analysis. Vertical bars represent the standard errors of the mean cone indices from four retinas. The dotted line shows the criterion value used to determine the action spectrum. For interference filters transmitting maximally at 443, 496.1, 543.5 and 620.2 nm, 50% of incident light is transmitted at ± 16, 5.8, 5.0, and 6.1 nm, respectively.

the criterion value would result only in a small change in the form of the overall action spectrum.

Unfortunately, the histologic sections obtained for the fish exposed to the second highest intensity of 544 nm stimulation were not good enough to allow an accurate determination of the cone index. Thus, the end of the light-adapted plateau is too uncertain to allow a regression line to be fitted at this wavelength. However, the intermediate intensity of stimulation resulted in a cone index that coincides with that chosen as the 50% criterion value (0.3). This intensity was, therefore, taken directly as representing the criterion value.

The degree of illumination ($\mu W/cm^2$) at the cornea at all wavelength/intensity combinations was measured by a U.D.T. 40× optometer placed in the aquarium. This was also taken as the relative amount of light reaching the retina as the optical media were assumed to be chromatically neutral for the wavelengths of interest here. Fifty percent values that in the first instance were in terms of neutral density (Fig. 2) were, thus, converted to actual intensity values ($\mu W/cm^2$) and expressed as relative quantal sensitivities by dividing their reciprocals by the wavelength of the stimulating light.

Visual Pigments

The absorbance spectra of 16 cone and 17 rod outer segments were determined using microspectrophotometry (MSP). The experimental procedure and analysis of data have been described previously.[7] These spectra are best fitted to a standard porphyropsin absorbance spectrum[8] expressed on a scale of wavelength raised to the power of 0.25.[9,10]

Results

The mean absorbance spectra of the 16 cones and 17 rods are best fitted by standard porphyropsin spectra with maximum absorbances at 607 nm and 540 nm, respectively (Fig. 3).

The highest sensitivity of photomechanical cone contraction was in response to long wavelength stimulation (620 nm). In Figure 4 the contraction sensitivity at 620 nm is taken as 100% and sensitivities at other wavelengths have been plotted relative to this. The resulting action spectrum is much better fitted by the absorbance spectrum of the cone visual pigment than by the rod visual pigment. Specifically, the contraction sensitivity in response to long wavelength stimulation is far too high to be explained by the rod visual pigment.

Discussion

The aim of this study was to determine whether the photomechanical contraction of teleost cones is mediated by the isomerization of the visual pigment within the cones themselves or by the rods. The latter is a possibility since in the dark-adapted retina, the cones are positioned so that they are shielded by a layer of rods and much of the light that could potentially stimulate cone contraction will be absorbed by these rods. If cone contraction is mediated by a direct effect of light on the cones, the action spectrum of cone contraction should resemble the absorbance spectrum of that cone's visual pigment. However, if it is rod-triggered, the contraction sensitivities should fit the absorbance spectrum of the scotopic visual pigment. An action spectrum for the contraction of glass catfish,

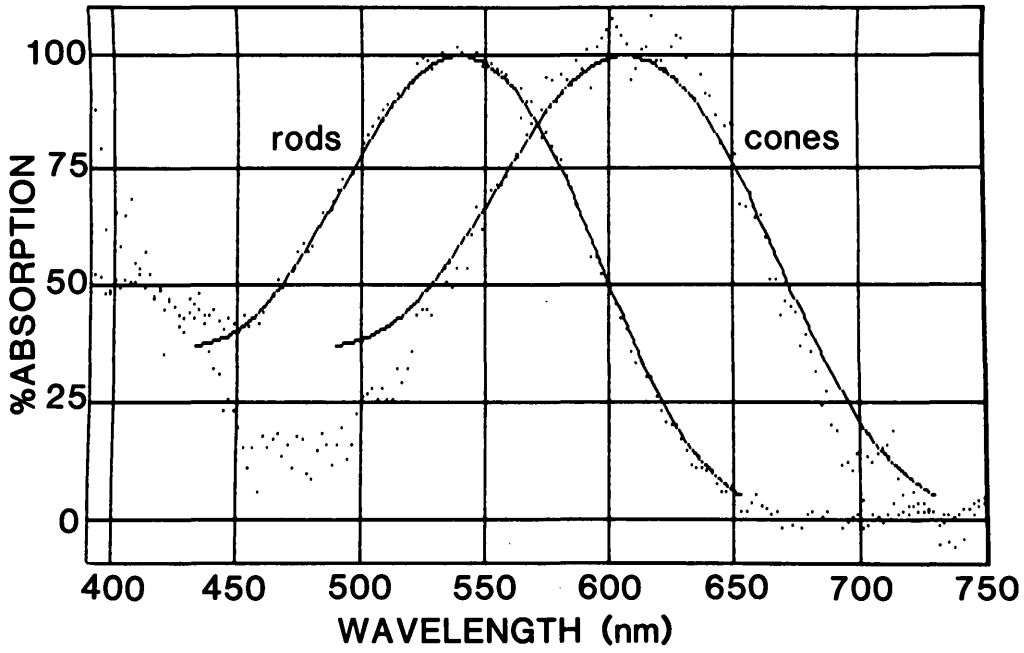

Fig. 3. Mean absorbance spectra of 17 rods and 16 cones from three individual fish. The solid lines are two standard porphyropsin absorbance spectra[8] expressed on a scale of wavelength raised to the power 0.25.[9,10]

red single cones therefore was determined and compared with the absorbance spectrum of its rod and cone visual pigments. Figure 4 shows that the action spectrum of catfish cone contraction is much better fitted by the absorbance spectrum of the cone visual pigment than by that of the rod visual pigment. This is a very strong indication that cone contraction is initiated by a direct effect of light on the cones themselves.

The question must be asked, however, can we be absolutely sure that the species examined here has only one photopic visual pigment? A completely honest answer would be no, since a technique such as MSP may be selective and one could never be sure that a low proportion of some other cone type does not exist without determining the absorbance spectrum of every single cone in the retina. Several things, however, make it fairly likely that the glass catfish does, indeed, contain only one type of cone. Firstly, throughout the whole retina the only cone type seen was uniformly small and single. Secondly, the visual pigments of all 16 cones measured contained the same visual pigment absorbing maximally at 607 nm. This agrees almost exactly with the absorbance spectra of the only 10 cones measured by Levine & MacNichol[11] in *Kryptopterus sp.* It is interesting to note that other families of catfish do not necessarily conform to this arrangement of visual pigments. Within the Ictaluridae, for instance, *Ictalurus punctatus* has a 543 nm rod pigment and a 620 nm pigment in all cones,[12] while *I. nebulosus* has an additional cone visual pigment at 530 nm.[11] *I. melas*, on the other hand, has a single spectrally identical visual pigment in both rods and all cones absorbing maximally at 543 nm.[12] In the case of the latter, it would be impossible, of course, to determine whether cone contraction was rod or cone triggered.

Since cone contraction is triggered by the isomer-

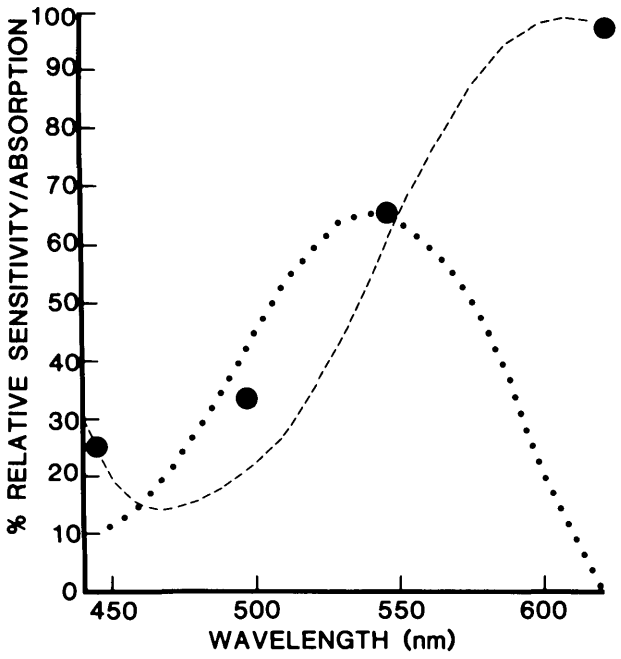

Fig. 4. The relative quantal sensitivities of cone contraction in response to monochromatic stimulation of various wavelengths. The dashed line represents the absorbance spectrum of the cone visual pigment (λ_{max} 607 nm) and the dotted line that of the rod visual pigment (λ_{max} 504 nm).

ization of the visual pigment within the cones, it follows that enough light must reach the cones even in the dark-adapted state when their myoids are elongated and most of the available light is absorbed by the rods. Miller and Synder[13] showed that the light-adapted frog cone myoid, which had a diameter of approximately 2 μm, acted as an efficient light guide. On dark adaptation, when the cone myoid decreased in diameter to about 0.5 μm, this light guiding efficiency was drastically reduced. However, due to the greater size of teleost cone, myoids they are likely to function as light guides even when dark-adapted. This is supported by the psychophysical data of Powers and Easter,[14] who showed a contribution of red cones to the spectral sensitivity of dark-adapted goldfish.

Prior to the present study, several authors had examined the effect of colored lights on photomechanical changes.[15-19] However, in no case were specific cone types containing known visual pigments distinguished. Thus, for instance, the paired outer cones in the guppy treated by Weidemann[18] as a single cone type are made up, in fact, of twin cones, both members of which contain the same visual pigment, and double cones, whose members contain different visual pigments.[11]

If cone contraction is brought about by a direct action of light on the cones, as suggested above, the different cone populations within a single retina containing several spectral cone types might be expected to react differentially to monochromatic stimuli. Thus, for instance, blue light may cause only blue cones to contract. However, such studies are fraught with difficulties: firstly, different spectral cone types cannot always be reliably distinguished by morphologic criteria. Secondly, different cone types migrate to varying degrees. Thus, goldfish double cones move further towards the back of the eye on dark adaptation than single blue cones. This makes experiments involving the selective contraction of specific cone types, such as that of Glickstein,[17] difficult to interpret. Finally, it is possible that there is a light adaptive signal, such as an increase in calcium levels,[20] that is effective for all cone types. Thus, all cones could light adapt even in response to monochromatic stimulation of only one type of cone. All these problems were avoided in the present study by using a photopically monochromatic fish. It would, however, be interesting to repeat such experiments on a species whose retina contains several different cone types.

Key words: teleost, catfish, retina, rod, cone, visual pigment, photomechanical, action spectrum, absorbance spectrum

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