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Spectral sensitivity of ON and OFF responses from the optic nerve of goldfish

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Abstract

The vertebrate retina processes visual information in parallel neural pathways known as the ON and OFF pathways. These pathways encode increments and decrements of light independently as excitatory responses. We examined the photopic spectral response of ON and OFF mechanisms in goldfish by measuring the sensitivity of optic nerve responses to the onset and termination of stimuli of various wavelengths. Using various adapting backgrounds, we found that the ON and OFF responses have different spectral sensitivities. The weighting of the cone inputs to the responses was estimated by an algebraic summation model. This model suggests that for the ON response, input from S-cones is stronger and more independent than for the OFF response, and M- and L-cones show stronger antagonism in the ON response than in the OFF response. The OFF response probably receives input from all cone types, but spectral antagonism is weak and its dominant input is from L-cones.

Keywords: Color vision, ON pathways, OFF pathways, Parallel processing, Goldfish

Introduction

The vertebrate visual system detects increments and decrements of light as separate events, and signals the occurrence of these events in two parallel, excitatory pathways known as the "ON" and "OFF" pathways (Hartline, 1938; Hubel & Wiesel, 1960; Werblin & Dowling, 1969; Naka, 1976). Evidence for parallel processing of light increments and decrements was first obtained by Hartline (1938), who classified the responses of retinal ganglion cells he recorded in the frog as ON or OFF, depending on the phase of the light stimulus that excited them. With more sophisticated electrophysiological recording techniques, Werblin and Dowling (1969) found that bipolar cells in the retina of the mudpuppy could also be classified as ON or OFF. The unifying work of Naka (1976) demonstrated that ON and OFF bipolar cells synapse with ON and OFF ganglion cells, respectively, and offered further evidence for the concept of parallel ON and OFF pathways in the vertebrate retina.

The evidence for parallel ON and OFF pathways was strengthened by the combined physiological and anatomical studies of Famiglietti et al. (1977) and Nelson et al. (1978) who discovered that the processes of ON bipolar and ganglion cells were confined to one sublamina of the inner plexiform layer, the processes of OFF bipolar and ganglion cells were segregated into an adjacent sublamina, and the processes of ON–OFF ganglion cells stratified in both sublaminae. Amacrine cells (Famiglietti et al., 1977) and interplexiform cells (Hashimoto et al., 1980) can also be classified physiologically into ON and OFF types, and the processes of these cells conform to the stratification scheme in the inner plexiform layer. The anatomically separate ON and OFF pathways formed by these neurons in the retina remain separate in the lateral geniculate nucleus in the monkey (Schiller, 1984), and in the tree shrew and mink, in striate cortex as well (Kretz et al., 1986; LeVay et al., 1987).

Several studies of single cells in the visual system have suggested that the photoreceptor inputs to the ON and OFF pathways may differ. For example, Witkovsky (1965), in a study of the photopic spectral sensitivity of retinal ganglion cells in carp, found that ON-center cells and ON responses from ON–OFF cells tended to be more sensitive in the short-wavelength region of the spectrum, whereas OFF-center cells and OFF responses from ON–OFF cells were more sensitive to longer wavelengths. Muntz (1962) found that retinal ganglion cell projections to the diencephalon of the frog, which were almost exclusively from ON-center cells, were more sensitive to short wavelengths of light than to long wavelengths.

Similar findings have been reported in mammals. Malpeli and Schiller (1978) reported a relative insensitivity to short-wavelength light in OFF-center retinal ganglion cells and lateral geniculate cells in the macaque. By testing light-adapted animals with full field spots of light, they found that most color opponent cells that were sensitive to a short-wavelength stimulus were ON-center; OFF-center cells were more often red-green color opponent. Similar results were reported by de Monasterio (1979) and by Zrenner and Gouras (1981).

Retinal mass potentials to stimulus onset and termination also exhibit different spectral sensitivities. Wheeler (1979) re-

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corded potentials from the optic nerve of light-adapted goldfish, and found different spectral sensitivities for the responses that followed the onset and termination of the stimulus. Wheeler (1979) found that responses following stimulus increments (ON responses) were relatively more sensitive to short-wavelength light than responses following stimulus decrements (OFF responses), although no attempt was made to determine the specific photoreceptor classes that contribute to the ON and OFF responses of the optic nerve. De Monasterio (1979) found that the *b*-wave of the electroretinogram (ERG), a response to light increments, was more sensitive to short wavelengths of light than was the *d*-wave, which is a response to light decrements. Evers and Gouras (1986) have hypothesized that the majority of S-cones feed into ON-center bipolar cells of the monkey, whereas M-and L-cones feed into both ON- and OFF-center bipolar cells. Mills and Sperling (1990) have also reported different spectral sensitivities for the ERG *b*- and *d*-waves of the monkey, and show that spectral sensitivity of the *b*-wave is fit by a model that incorporates chromatic opponency, whereas the spectral sensitivity of the *d*-wave can be modeled by a non-opponent, additive model.

This paper explores the possibility that retinal processing of increments and decrements of light occurs through different chromatic mechanisms. We measured the spectral sensitivity of the ON and OFF responses from the optic nerve of goldfish under various background conditions, and determined the relative contributions of the various cone types to the ON and OFF responses using an algebraic summation model. The goldfish is a particularly well-suited species for this study. It has at least three separate cone photopigments with widely separated wavelengths of peak spectral absorptance (Marks, 1965; Hárosi, 1976), and it also has trichromatic vision, as demonstrated behaviorally (Yager, 1969; Shefner & Levine, 1976). For the purposes of this study, we have ignored any possible contribution to spectral sensitivity from ultraviolet-sensitive receptors (Hawryshyn & Beauchamp, 1985).

Methods

Subjects and surgery

Common goldfish (*Carassius auratus*, Ozark Fisheries, Stoutland, MO) approximately 10 cm standard body length were maintained on a 12-h-light/12-h-dark cycle (room lights on at 8:00 A.M. and off at 8:00 P.M.) at 20°C for at least 2 weeks prior to use. All recording was performed between 9:00 A.M. and 6:00 P.M. Data from six fish were obtained for each background condition (see below) except for the white background, where seven fish served as subjects. Fish were immersed in 0.04% solution of tricaine methanesulfonate (Sigma Chemical, St. Louis, MO) until deeply anesthetized and then paralyzed with an intramuscular injection of 1.4 mg gallamine triethiodide (Flaxedil, Davis and Geck, Pearl River, NY). The cranium was opened, and the telencephalon was removed to expose the optic chiasm. The right optic nerve was then cut at the chiasm and freed from surrounding connective tissue so that a 2–3 mm segment of the whole nerve could be pulled into a suction electrode (described below). At the end of the experiment fish were sacrificed by pithing.

Stimulation

A two-channel optical system (Fig. 1) was built around a 75-W xenon arc lamp (Photon Technology and Osram). One channel was gated by an electromagnetic shutter (Uniblitz) and formed the stimulus beam while the other channel formed the background beam.

To vary the spectral composition of the stimulus, narrow-band interference filters (Melles-Griot, 8-nm bandwidth at half-

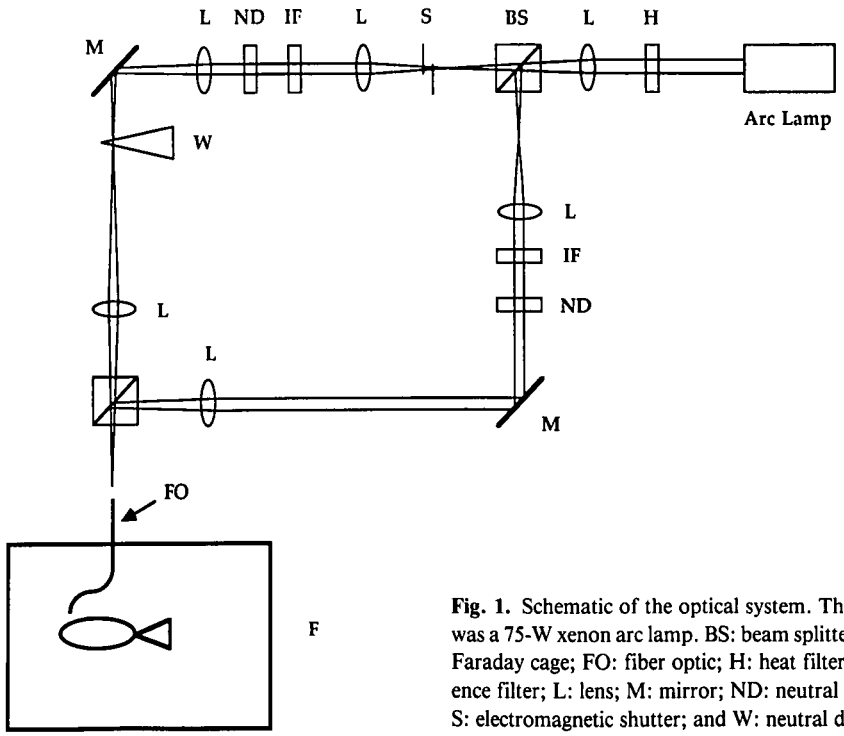

Fig. 1. Schematic of the optical system. The light source was a 75-W xenon arc lamp. BS: beam splitter; F: shielded Faraday cage; FO: fiber optic; H: heat filter; IF: interference filter; L: lens; M: mirror; ND: neutral density filter; S: electromagnetic shutter; and W: neutral density wedge.

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height) were placed in the collimated portion of the stimulus beam. Eleven different wavelengths were used to obtain spectral sensitivity curves; these were (in nm): 400, 430, 449, 490, 519, 551, 581, 620, 641, 670 and 701. Neutral density filters (Oriel) in combination with a 2.0 log unit neutral density wedge (Dyn-Optics) in the stimulus beam controlled the stimulus irradiance. Corneal irradiance was calibrated in absolute radiometric units with a silicon photodiode (United Detector Technologies, Santa Monica, CA, PIN 10DFP). The calibrations were made at the plane of the cornea of the fish and took into account all optics in front of the cornea. The stimulus duration (500 ms) was controlled by the shutter *via* a laboratory computer (Data Translation LSI 11/23). This relatively long duration separated, in time, the ON and OFF responses of the optic nerve.

To vary the background, either an interference filter or a Wratten gelatin filter was placed in the background beam. The spectral composition of the backgrounds was chosen to adapt one or more of three specific cone types found in the retina of the goldfish. The spectral absorptance curves of the pigments found in these cone types is given in Fig. 2A (from Hárosi, 1976). The term "L-cone" will be used to refer to the cone type that absorbs maximally in the long-wavelength region of the visible spectrum (λ_{max} = 625 nm), "M cone" refers to the cone type that absorbs maximally at a mid-spectrum wavelength (λ_{max} = 530 nm), and "S-cone" refers to the cone type that absorbs maximally in the short-wavelength region (λ_{max} = 450 nm). The spectral distribution of the backgrounds used in this study (Fig. 2B) was chosen in accordance with the photopigment curves to differentially adapt these three cone types. The backgrounds were as follows:

1. "White light" provided by the xenon arc-lamp beam with no interference filters, served as a "neutral" adapting background.
2. "Short wavelength" provided by placing a 476-nm interference filter in the background beam to adapt primarily S- and M-cones.
3. "Middle wavelength" provided by placing a 582-nm interference filter in the background beam to adapt primarily M- and L-cones.
4. "Magenta" provided by placing a Wratten #33 filter in the background beam, to adapt at short and long wavelengths.

The integrated spectral energy of all backgrounds was fixed at 1.27 μW/cm^2 at the plane of the cornea using neutral density filters. To estimate the adapting effect of each background on the three cone types, the quantal efficiency of each background was calculated, and these values are shown in Table 1.

Table 1. *Quantal efficiency of each background*

Background	S-Cones	M-Cones	L-Cones
White	26	44	65
476 nm	84	68	31
582 nm	1	70	85
Wratten 33	3	7	72

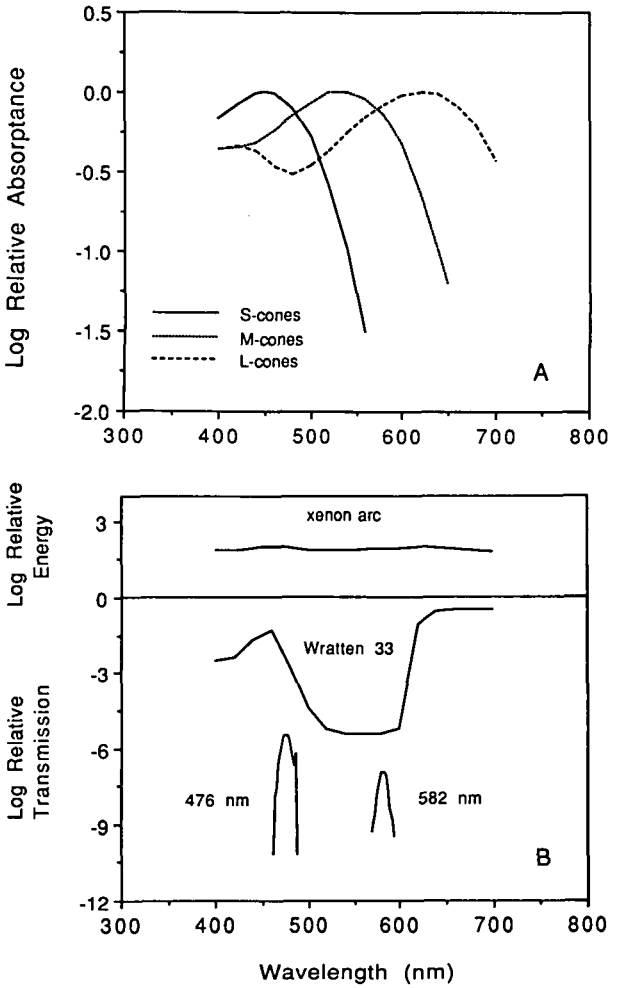

Fig. 2. A: Spectral absorptance curves for the pigments found in goldfish L cones (λ_{max} = 625 nm), M cones (λ_{max} = 530 nm), and S cones (λ_{max} = 450 nm), from Hárosi (1976). B: The spectral distribution of each of the four backgrounds used in this study. Upper curve: white background, produced by the unfiltered xenon arc lamp. Lower curves: chromatic backgrounds produced by various filters. The absolute energy of all backgrounds was 1.27 μW cm^{-2} at the cornea, integrated across the spectrum. Each background has been arbitrarily shifted on the ordinate to emphasize their relative shapes.

Each value represents an estimate of the probability (in percent) of a quantum catch for a given cone type under each background condition. Cones with a high probability of a quantum catch will be adapted more than cones with lower probabilities. The values were found by computing the quantal energy distribution of each background and integrating this with the special sensitivity of the photopigment of each cone type.

Recording

After a fish was surgically prepared, it was positioned between sponges in a Plexiglas aquarium and respired with aerated water that contained 0.005% tricaine methanesulfonate. The eyes rested above the water line. A circular piece of white bond paper, formed to fit the cornea and moistened with fish Ringer's, acted as a diffusing screen and served to keep the cornea moist

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(the optical effects of this diffuser were included in the calibrations). Visual stimuli completely covered this diffusing screen, and because the diffusing screen covered the entire pupil of the fish (mean pupil diameter = 3.57 mm for this study), it is assumed that both background and test stimuli covered the full visual field, which is approximately 185 deg in goldfish (Easter et al., 1977).

Pasteur pipettes were made into suction electrodes by pulling and fire polishing the narrow end of the pipette until an inner diameter of approximately 1 mm was achieved. The end of the pipette was heated and bent slightly to facilitate access to the optic nerve. After the fish was surgically prepared, the free optic nerve was drawn by suction into the pipette until a leakproof seal was established. The pipette was then filled with fish Ringer's and a chlorided silver wire (0.005 in. diameter) was lowered into the Ringer's. This allowed recording of compound action potentials from the whole optic nerve—the optic nerve response (ONR). An additional wire was placed in the skull cavity to serve as a reference electrode.

The ONR was differentially amplified with a bandpass of 0.1–100 Hz (Tektronix 5A22N) and displayed on a storage oscilloscope (Tektronix 5113). Responses were simultaneously recorded by the computer through an A/D converter at a sampling rate of 333 Hz, and stored for off-line analysis.

The optic nerve response

The ONR is a compound action potential representing the summed activity of retinal ganglion cells (Adrian & Matthews, 1927). We used the ONR in this study for several reasons. First, the literature already contains evidence that the photopic spectral sensitivity of the ONR differs depending on whether the stimulus is an increment or a decrement (Wheeler, 1979). Second, the properties of the ON and OFF components of the ONR relate well to the properties of ON and OFF retinal ganglion cells recorded extracellularly. In the cat, for example, irradiance-response curves of ON and OFF responses of the ONR have different slopes, and these correspond to differences in slope found in irradiance-response functions from ON and OFF retinal ganglion cells in that species (Olsen et al., 1986). Third, insofar as the ONR depicts activity from the entire population of retinal ganglion cells, it represents a level of recording intermediate between single units and the integrated behavioral response. The ONR therefore is a reasonable measure to characterize the total retinal output available to the brain, and may be useful in relating physiological responses to behavioral measures of spectral sensitivity. Fourth, the reliability and stability of ONR recordings is quite good, thus allowing sufficient time to present a detailed series of stimuli.

There are, however, potential problems in using the ONR as a dependent measure. Because the ONR is a mass potential, it is difficult to attribute its various components with absolute certainty to specific cell types (i.e. ON-center or OFF-center retinal ganglion cells). Also, because ganglion cell receptive fields have both ON and OFF response areas (Kuffler, 1953; Wolbarsht et al., 1961), and because the light stimulus is full-field, mixing of center and surround responses into one component of the ONR is inevitable. On the other hand, because receptive-field center responses generally dominate the response of a ganglion cell at threshold under full-field stimulation (Kuffler, 1953; Barlow et al., 1957), it is reasonable to assume that, near threshold, ONRs to the onset of a light stimulus are mainly due to excitatory center responses of ON-center retinal ganglion cells. Likewise, responses to the termination of the stimulus probably originate mainly from excitatory center responses of OFF-center ganglion cells.

A related problem is that any given component of the optic nerve response may reflect the sum of the activity from separate ON- and OFF-center cells. For example, ON-center cells respond to light increments with an increase in firing rate, while OFF-center cells respond to the same stimulus with a decrease in firing rate (Falzett et al., 1988); therefore, the ONR to a light increment should be the sum of two types of activity. Although this is an interaction that cannot be avoided, we have noted that the major components of the ONR in light-adapted goldfish retina respond to light stimulation by rising above the baseline noise level, not dropping below it. It is presumed that these responses are reflecting the predominance of units that respond with excitation to the stimulus; i.e. ON-center cells to light increments, OFF-center cells to light decrements, and ON–OFF cells to both. We have also noted that, on occasion, the ONR does drop slightly below baseline at the onset of the light, and then rises considerably above baseline at the termination of the light. This pattern of response mimics that of OFF-center ganglion cells in dark-adapted goldfish retina, where these cells decrease their firing rate to light increments and increase their firing rates to light decrements (Falzett et al., 1988).

Finally, the ON and OFF responses of the ONR are each frequently made up of more than one wave (see Fig. 3). For instance two or three peaks may occur during the ON response or OFF response. Multiple peaks are not usually seen near threshold, but only at suprathreshold intensities. In addition, only three fish showed multiple waves for a given response across all wavelengths. An analysis of the data from these three fish under the white background condition suggests that these components do not differ in spectral sensitivity. That is, all waves observed in the ON response have similar spectral sensitivities, and likewise for the OFF response. In this study, the first component visible above threshold is tracked throughout the response series, and is the component for which spectral sensitivity is reported.

Procedures

The order of presentation of the four backgrounds was randomized for each experiment. Before collecting a series of data for a given background, the fish was allowed to adapt to the background for 15 min. An ascending method of limits was used to present different intensities of the 11 test wavelengths to the fish under each background condition. The order of presentation of the 11 wavelengths was staggered so to minimize adapting any one cone type. At each irradiance-wavelength combination, three recordings of the ONR were averaged by the computer. Software was written so that the waveforms could be viewed later on a personal computer, and the ON and OFF responses could be identified and the peak amplitude of the responses recorded. Irradiance-response functions for a given wavelength were generated by plotting the peak amplitude of the response as a function of the irradiance. Smooth curves were fit to the data by switching the axes (to interpolate for irradiance instead of amplitude), performing a logarithmic transform, and fitting the curve: $y = a + b \log(x)$, using least-squares regression. To derive spectral sensitivity curves, linear interpolation from the regression analysis was used to find the

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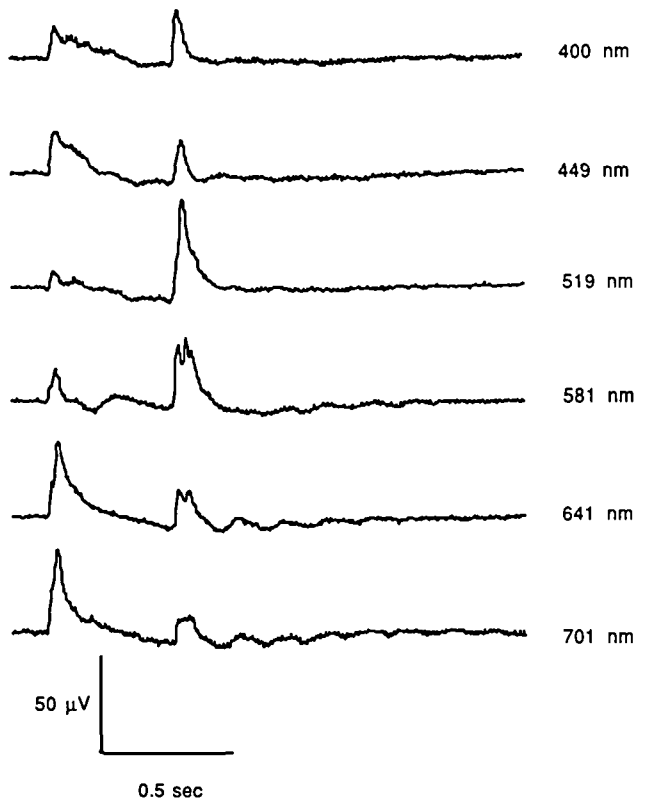

Fig. 3. Representative optic nerve responses from one fish to six of the eleven test wavelengths used in the study. Each response is the average of three trials of a 500-ms stimulus presented on top of a white adapting background. The ON response is the rise in activity that occurs after the onset of the stimulus, and the OFF response is the rise in activity after the termination of the stimulus. The quantal irradiance of the stimulus was held constant within 0.1 log unit to a value of 10.2 log quanta s^{-1} cm^{-2}.

irradiance that would theoretically provide a 10-μV criterion response at that wavelength. Relative spectral sensitivity curves were constructed for both the ON and the OFF responses by normalizing the data relative to 551 nm (except for the white background, which was normalized to 581 nm due to missing data from 1 fish). Absolute sensitivity curves were constructed by plotting the data using the averaged absolute irradiance values for the responses. For the white-light background, the maximum variability in absolute sensitivity between fish was 1.03 log units, collapsed across both the ON and OFF responses.

Modeling and curve fitting

Several methods have been used in the past to examine how the inputs from different photoreceptor classes are combined to produce a particular spectral sensitivity curve. Linear models that allow algebraic summation of the photoreceptor signals are useful in describing opponent interactions between the cone types (Yager, 1967; Sperling & Harwerth, 1971; Thorpe, 1972). Because additivity can be defined algebraically, the linear model can detect subtractive influences between cone types that are antagonistic to each other. The variation of this model used in the present study assumes spectral sensitivity is determined by a sum of the cone inputs to the ganglion cells that create the ON and OFF responses. The model computes a weight for each cone type that can be thought of as a measure of the contribution of the cone type to the spectral sensitivity curve. The equation for the model takes the form:

$$S(\lambda) = P_S W_S + P_M W_M + P_L W_L,$$

where

$S(\lambda)$ = the expected spectral sensitivity at wavelength λ, given the actual spectral sensitivity.

$P_{(S,M,L)}$ = the sensitivity of the corresponding S-cone, M-cone, or L-cone pigment curve at wavelength λ.

$W_{(S,M,L)}$ = the computed weight for the corresponding S-cone, M-cone, or L-cone pigment curve, given the background condition.

To implement the curve-fitting procedure, the photopigment absorptance curves from Hárosi (1976) were transformed from log relative absorptance to percent relative absorptance and normalized to unity. The experimental data were converted similarly. The photopigment curves were altered to correct for light absorption by the ocular media (Bassi et al., 1984), as the experimental data were calculated from corneal irradiance measures. A computer program that employed the Simplex algorithm (Caceci & Cacheris, 1984) was then used to find the best fit of the model to the data. The resulting best-fit curve and the associated pigment weights are used in the discussion of this modeling procedure. The quantitative weights computed by the model will be used in discussing the relative contributions of the cone types to a spectral sensitivity curve. In order to compare how the relative weights of the cone types changed across the different background conditions, the raw weights were normalized to the weight of the L-cones for a given background condition. Therefore, the L-cones will always have a weight of 1.00 and other cone types will be weighted relative to the strength of the L-cone input.

Results

Spectral sensitivity

Figure 3 shows the ONR response to full-field monochromatic stimuli of six different wavelengths presented on a white-light background. All stimuli in this example were equated in corneal irradiance (10.2 log quanta s^{-1} cm^{-2}, $\pm$0.1 log unit range). It is clear that the relative amplitude of the ON and OFF responses depends upon the wavelength of the stimulus. For example, the ON response is larger than the OFF response at 449 nm and at the two longest wavelengths, while the OFF response is larger at 400 nm and the two middle wavelengths.

Figure 4 illustrates the shape of irradiance-response functions for the ON and OFF responses across six of the 11 stimulus wavelengths (symbols). The smooth curves were used to interpolate the criterion response. These curves were obtained from one fish under the white-light background. The curves for the ON response show a slope change with wavelength, whereas the curves for the OFF response show less variability. This suggests that the spectral sensitivity curves derived for the ON response would change with changing criterion, particularly at 519 nm. Specifically, the decrease in sensitivity we observed at mid-spectral wavelengths (see below) would become even

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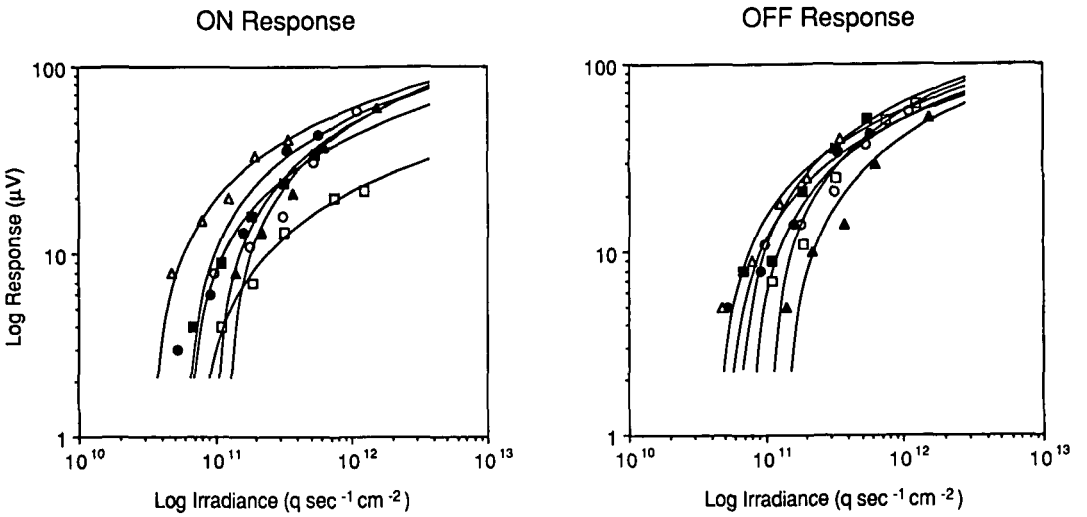

Fig. 4. Representative irradiance-response functions (symbols) for six of the 11 test wavelengths for one fish under the white-light background, plotted on logarithmic axes. The lines show a least-squares fit of the equation $y = a + b \log(x)$, which was used in interpolating the irradiance for a criterion response of 10 μV. Symbols: ○: 400 nm; ●: 449 nm; □: 519 nm; ■: 581 nm; △: 641 nm; and ▲: 701 nm.

greater with a higher criterion (suggesting stronger spectral antagonism). By using a relatively low response criterion (10 μV), we are estimating spectral sensitivity near threshold where the most sensitive elements would respond. The shape of the irradiance-response functions suggest that a higher response criterion would yield a different spectral sensitivity function, but the difference in spectral sensitivity between the ON and OFF responses would remain.

Figure 5 shows the mean (±1 S.E.M.) log relative spectral sensitivity of the ON and OFF response for seven fish measured on the white-light background. The solid line in each graph represents spectral sensitivity based on a linear sum of the responses from S-, M-, and L-cones in the goldfish retina. The values in the lower left of each graph are the weights assigned to each cone type by the model, normalized to the L-cones.

Concentrating on the averaged data in Fig. 5 (open symbols), the ON response is most sensitive in the short- and long-wavelength regions of the spectrum, while the OFF response is more broadly tuned with a single prominent peak in the long-wavelength region. These results are similar to those obtained by Wheeler (1979) under tungsten light adaptation. Under the linear model, S-cones are assigned a relatively strong positive weight for the ON response, and a small, negative weight for the OFF response. The trough in the function of the ON response suggests inhibition from M-cones, which is supported by the negative weighting factor given the M-cones by the model. M-cones are given a small positive weight for their contribution to the OFF response. L-cone input appears to be the main factor determining spectral sensitivity for the OFF response.

Figure 6 shows the effect on spectral sensitivity of imposing the 476-nm background, which was designed to selectively adapt the S-cones and M-cones. The shape of the OFF-response curve is changed little as compared to the white-light background condition. However for the ON response, the 476-nm background had a selective adapting effect on the short and middle wavelength regions of the spectrum, strongest at the

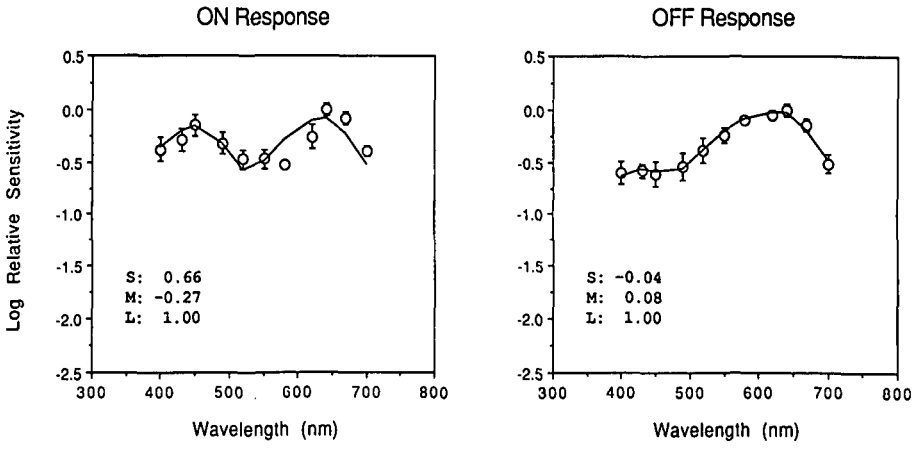

Fig. 5. The mean (± S.E.M.) log relative spectral sensitivity of the ON and OFF responses for seven fish for the white-light background condition (open symbols). The means are normalized to 581 nm for this background, and at 551 nm for other backgrounds. The solid line is the least-squares fit of an algebraic sum of cone inputs. Figs. 6–8 use these same conventions.

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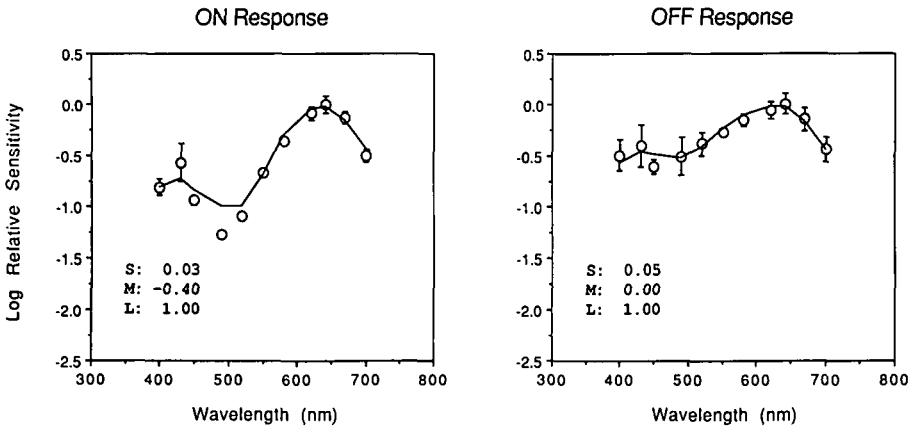

Fig. 6. The spectral sensitivity of the ON and OFF responses for six fish under the 476-nm background condition.

adapting wavelength of the background. For the OFF response, the S-cones and M-cones play little role in the fitted function, whereas the L-cones dominate. For the ON response, S-cones are given little weight, but M-cones are weighted negatively to account for the decrease in spectral sensitivity around the adapting wavelength.

Figure 7 shows the adapting effects of the 582-nm background. Again, the spectral sensitivity of the OFF response remains broadband. In contrast to the OFF response, the spectral sensitivity of the ON response has changed dramatically compared to the white-light background condition. The 582-nm background has suppressed the sensitivity of the L-cones by about 1.5 log units relative to the short-wavelength region of the curve. This background condition dramatically emphasizes the point that the cone inputs to the two responses are different, given the dissimilar adapting effects of this background on the spectral sensitivity of the two responses. The linear modeling underscores the substantial difference in cone inputs to the ON and OFF responses for this background condition. It is evident that the S-cones have a much greater influence in the ON response than the OFF response. M-cones are given a negative weight for the ON response to account for the depression in the function in the range of wavelengths between 580 nm and 620 nm. For the OFF response, weights for S- and M-cones are larger than is the case for the white background, suggesting that the 582-nm background has adapted L-cones, revealing subtle input from other cone types. If L-cones were the only photoreceptor type determining spectral sensitivity for the OFF response, then one would not expect a change in spectral sensitivity for any background condition.

Figure 8 shows the influence of the Wratten 33 background on spectral sensitivity. This background passes both short- and long-wavelength light to adapt the S- cones and L-cones and reveal M-cones. The spectral sensitivity curves for both responses are broadband under this background condition. Both show adaptation in the long-wavelength region of the spectrum, and a peak sensitivity at the middle wavelengths; this would be expected if the M-cones have a significant influence on spectral sensitivity. In this case, it is interesting to note that the OFF response seems to be adapted more at long wavelengths by this background than by the mid-wavelength adapting background (582 nm). Perhaps this is due to the combined adapting effects of the background at the short- and long-wavelength regions of the spectrum, which leaves M-cones relatively unadapted and less affected by suppression from L-cones. Examining the results of the modeling, the weighting factor for the S-cones is larger for the ON response than the OFF response, and the weight for the M-cones is the largest here than for any other background condition. In fact the M-cone weight is positive, and no significant spectral opponency is evident for the ON re-

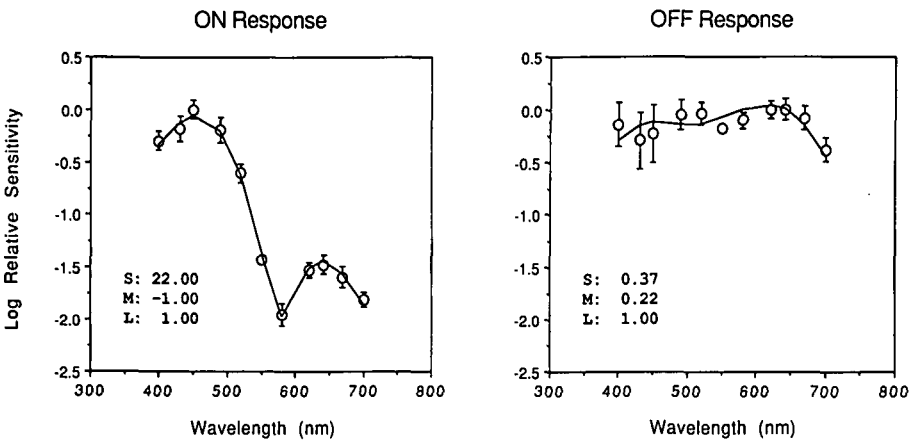

Fig. 7. The spectral sensitivity of the ON and OFF responses for six fish under the 582-nm background condition.

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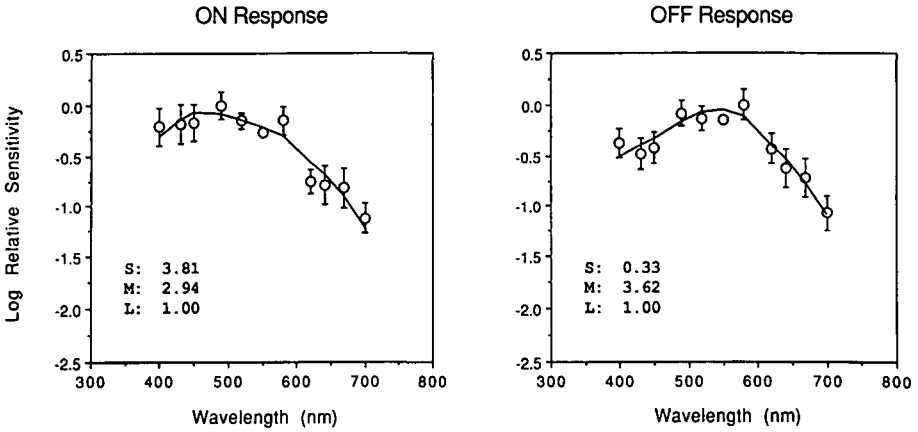

Fig. 8. The spectral sensitivity of the ON and OFF responses for six fish under the Wratten 33 background condition.

sponse. These values are consistent with the shape of the curves and the expected result of the background, which was to reveal contribution of the M-cones to the response. However, the short-wavelength region of the spectrum is relatively unadapted for the ON response because, as Table 1 reveals, this background was less than optimal for adapting S-cones.

In addition to spectral sensitivity, a comparison of absolute sensitivity was made between the ON and OFF responses. Figure 9 shows the spectral sensitivity curves plotted as a function of absolute corneal irradiance for each of the background conditions. In most cases, the spectral sensitivity curves are similar in absolute sensitivity at long wavelengths. This suggests that

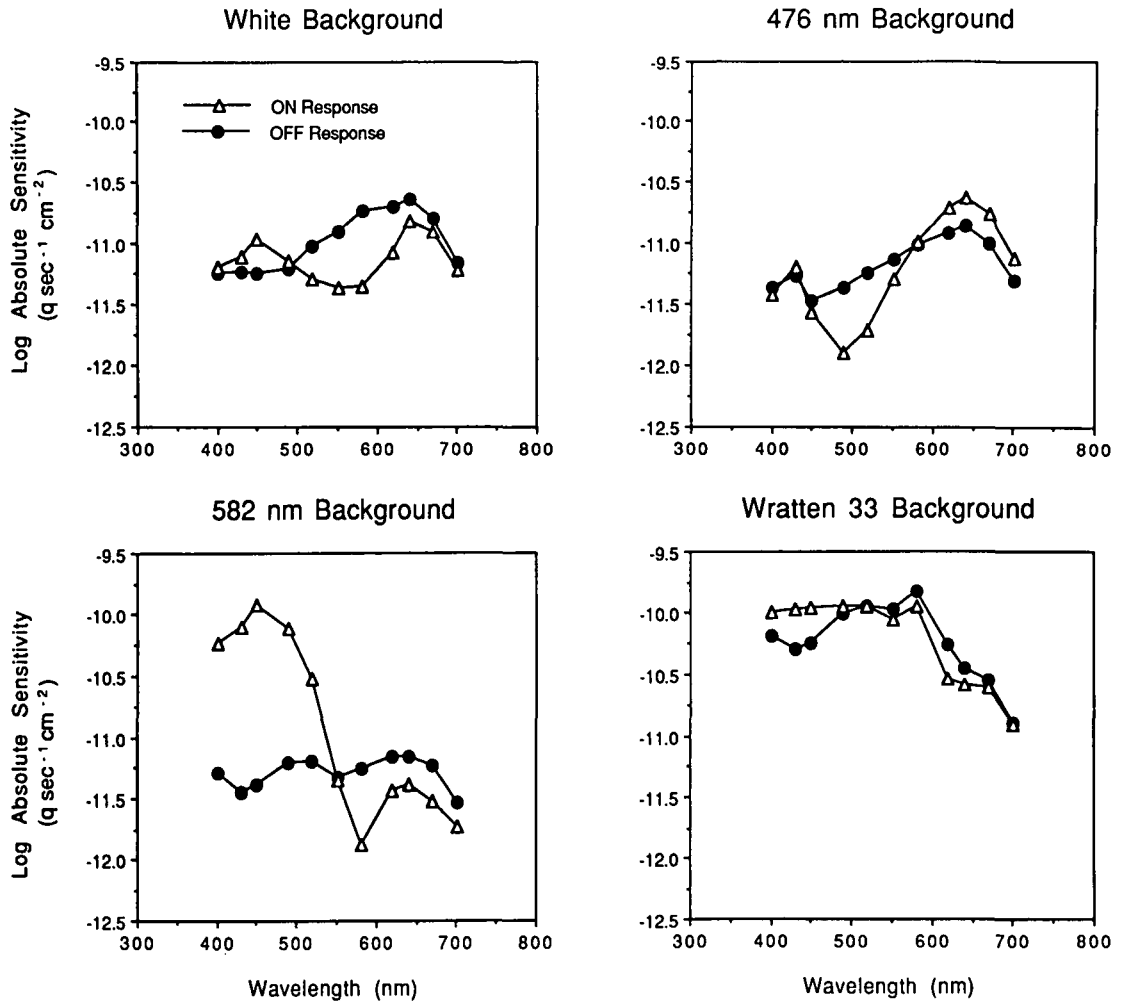

Fig. 9. A comparison of absolute sensitivity, in corneal irradiance, of the ON response (triangles) and the OFF response (solid circles) across the four background conditions. See text for details.

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one photoreceptor type (the L-cones) is determining spectral sensitivity at long wavelengths. Differences in absolute sensitivity for the ON and OFF responses are seen between 520 and 640 nm for the white background condition, where spectral antagonism in the ON response decreases sensitivity relative to the OFF response. A similar effect is seen for the 476-nm background condition at wavelengths between 450 and 550 nm. The 582-nm background shows that S-cone input is very strong in the ON response and is relatively unaffected by adaptation. Because the absolute sensitivity of both responses is similar at long wavelengths, L-cones must contribute to both responses, but S-cones must contribute more strongly to the ON response. The results for the Wratten 33 background show that this condition had similar adapting effects on the two responses, and absolute sensitivity for both responses is similar across all wavelengths.

Discussion

The light-adapted goldfish retina processes increments and decrements of light through different chromatic mechanisms, as determined by the ON and OFF responses of the optic nerve. The difference in spectral sensitivity between the two responses can be attributed to differential weighting of cone contributions to the separate responses.

In general, the spectral sensitivity function of the OFF response is broadband and reflects primary input from L-cones. There is evidence of contributions from M-cones and S-cones, but strong antagonistic interactions between the three cone types are not apparent for the OFF response, at least with the background conditions used in this study. The spectral sensitivity curves for the ON response suggest strong contributions from all cone types, as well as antagonistic interactions between the cone types. The S-cone and L-cone types appear to be particularly independent, as evidenced by the ability to adapt them separately (e.g. see results for the 582-nm background, Fig. 7). The antagonism seen between the M-cones and L-cones (white background, Fig. 5) is consistent with studies of goldfish retinal ganglion cells that show spectral opponency between the two cone types (Beauchamp & Lovasik, 1973; Mackintosh et al., 1987).

Relation of the results to other physiological studies

The results reported here for the white-background condition are consistent with those of Wheeler (1979), who also recorded the relative spectral sensitivity of the ON and OFF responses of the optic nerve in goldfish. Even though he found a relatively lower sensitivity to long-wavelength stimuli than is reported in the present study, this would be predicted because a tungsten source (as he used) has relatively more energy at long wavelengths than a xenon lamp, and such a background would preferentially adapt L-cones and decrease relative sensitivity in the long-wavelength region of the spectrum.

Wagner et al. (1960), Beauchamp and Lovasik (1973), van Dijk and Spekreijse (1984), and Mackintosh et al. (1987) all obtained photopic spectral sensitivity curves from goldfish ganglion cells. All of these authors show cells with input mainly from L-cones, similar to the spectral curves of the OFF response. They also show cells with input from S-cones and L-cones, similar to the spectral curve for the ON response under the 582-nm background condition. However, none of these authors reported consistent differences in spectral sensitivity between ON- or OFF-center cells, although none of the studies specifically searched for this bias. It is unclear whether finding such a difference (or lack thereof) would be a function of sample size and/or experimental procedure for single-unit studies.

Data gathered from recordings of ganglion cells in the monkey retina do show some correlations with the results of the present study. The spectral sensitivity of the ON response is consistent with the findings of studies which suggest that ON-center retinal ganglion cells are particularly sensitive to short-wavelength light. Malpeli and Schiller (1978), de Monasterio (1979), and Zrenner and Gouras (1981) all report a relative insensitivity to short-wavelength light in OFF-center retinal ganglion cells and lateral geniculate cells of the monkey, but ON-center cells exhibited a strong response to short-wavelength stimuli. Evers and Gouras (1986) report evidence for S-cone input to ON-center but not OFF-center bipolar cells, as concluded from macaque ERG data. The spectral sensitivity of the ON response in the present study shows a strong influence from the S-cones, whereas the OFF response does not show overtly the same influence. However, this does not mean that the S-cone contribution to the OFF response is absent. The influence may be weaker in OFF-center cells, or it may be masked by neural interactions with the other cone types making it difficult to isolate through chromatic adaptation. Mills and Sperling (1990) report spectral sensitivity differences between the *b*- and *d*-waves of the monkey ERG that are thought to be due to the presence or absence of spectral opponency between M- and L-cones. The spectral sensitivity of the *b*-wave was modeled by opponent interactions between M- and L-cones, but such opponency was not necessary to model spectral sensitivity of the *d*-wave. However, they did not report significant S-cone input to either waveform. Their findings are consistent with those of the present study in that the ERG response to light increments shows more spectral antagonism than the ERG response to light decrements. Mills and Sperling (1990) suggest that antagonism between M- and L-cones is stronger at the level of ON-center bipolar cells than for OFF-center bipolar cells. Taken with the results of the present study, the question arises of whether these differences are expressed in the population of ON- and OFF-center ganglion cells in the monkey retina.

One human electrophysiological study has suggested contrast sensitivity differences between the ON and OFF pathways. Zemon et al. (1988), recording visual evoked potentials (VEPs), found that VEPs for decremental stimuli generally had a larger amplitude for contrast decrements than for contrast increments. They also found different spatial tuning curves for the two stimuli: VEPs to contrast decrements were larger than those to contrast increments for stimuli that subtended as little as 5 min of arc.

Relation of the results to psychophysics

Because the activity recorded from the ONR represents the responses of the entire retinal ganglion cell population, one can gain insight into its relevance for behavior by comparing the spectral sensitivity of the ON and OFF responses to psychophysical measures of spectral sensitivity obtained from goldfish (Yager, 1967; Yager, 1969; Shefner & Levine, 1976; Beauchamp et al., 1979; Powers, 1978; Neumeyer, 1984; Hawryshyn & Beauchamp, 1985). The several behavioral studies that find broadband monotonic functions under tungsten backgrounds (Yager; 1967, Powers, 1978; Beauchamp & Rowe, 1977) approximate the spectral sensitivity of the OFF response of the ONR under the white-background condition. That is, these

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behavioral curves appear to receive a strong, dominant contribution from the L-cones. These psychophysical studies used several testing paradigms, so no particular paradigm was likely to be responsible for the monotonic functions. Beauchamp et al. (1979), using a yellow adapting background, found evidence of S-cone input at short wavelengths and L-cone input at long wavelengths, similar to the spectral sensitivity curve for the ON response in Fig. 7.

Other studies show more complex spectral sensitivity functions; those with two or more maxima (Hawryshyn & Beauchamp, 1985; Shefner & Levine, 1976) resemble the spectral sensitivity of the ON response recorded here under the white-background condition. Several of these curves showed a decreased sensitivity at mid-spectrum wavelengths, perhaps due to an antagonistic action of the M-cones as proposed in the present study. Neumeyer's (1984) results with a fluorescent white background and several adapting backgrounds produced complex spectral functions, whose shapes suggest complex neural interactions as have been noted in the present study. Why some psychophysical functions resemble the ON response and others the OFF response is unknown. However, Neumeyer and Arnold (1989) also propose that psychophysical spectral sensitivity would be different depending on whether the fish must choose a lighted test field or a darkened test field.

Several human psychophysical studies have addressed the issue of sensitivity to incremental and decremental stimuli. Most of the studies were concerned with the relative sensitivity to increments and decrements, but none of the studies examining photopic processing of increments and decrements have measured the human's spectral sensitivity to these stimuli. Although a few of these studies report no difference in sensitivity to increments or decrements (Herrick, 1956; Rashbass, 1970; Roufs, 1974), several have found that, under certain stimulus conditions, humans are more sensitive to decremental than incremental stimuli (Boynton et al., 1964; Short, 1966; Patel & Jones, 1968; Cohn & Lasley, 1975; Krauskopf, 1980; Bowen et al., 1989). Whether this sensitivity difference is wavelength dependent remains to be determined.

Color processing in the ON and OFF pathways

The results of the present study suggest that the ON- and OFF-center pathways in the goldfish retina may have different spectral sensitivities. At this point, one can only speculate about the functional significance of this differential spectral sensitivity. It is conceivable that the visual system uses the disparity in color information from the two pathways as a means of color coding, just as the distinct spectral information received by individual neurons from the different cone types is believed to be crucial for color discrimination.

The difference in spectral sensitivity between the ON and OFF responses leaves no doubt that the goldfish visual system processes increments and decrements through different chromatic mechanisms. However, ON- and OFF-center cells that are color opponent can generate both ON and OFF responses, depending on the wavelength of stimulation. In particular for the goldfish, double opponent cells exist at the level of the retina, and these cells are capable of producing ON and OFF responses from both the center and surround (Daw, 1967). Cells such as these are classified as ON or OFF depending on the stimulating wavelength (e.g. Mackintosh et al., 1987). Because it is difficult to classify a color opponent cell rigidly as ON or OFF, it is more difficult to specify which cells are generating ON and OFF responses.

To explain the differences in spectral sensitivity reported in this study, one must postulate that either ON- and OFF-center cells receive different photoreceptor inputs, or that the photoreceptor input to one spatial component of the receptive field of all chromatic cells is consistently different.

If the receptive-field center of ON-center ganglion cells contributes exclusively to the ON response, and the receptive-field center of OFF-center ganglion cells to the OFF response, then one might conclude that the cone inputs to the receptive-field centers of these two cell types differ in the goldfish. Although we have attempted to maximize the conditions for measuring activity from receptive-field centers, we have no direct confirmation of the source of the inputs to the ON and OFF responses.

The alternative explanation of our results assumes that the activity reflected in the ON and OFF responses is the result of a mixture of receptive center and surround activity from ON- and OFF-center cells. For example, the ON response would be the result of activity from the receptive-field center of ON-center ganglion cells *plus* activity from the receptive-field surround of OFF-center cells. If this were the case, then the cone inputs to these two spatial components of these two cell types must differ. For example, to explain the results shown for the 582-nm background (Fig. 7), there must be a strong S-cone input to the receptive-field center of ON-center cells *and* to the surround of OFF-center cells (the regions that would be activated by light onset), but a different weighting of S-cone input to the surround of ON-center cells *and* the center of OFF-center cells (the regions that would be activated by light decrement). However, such connectivity is inconsistent with the results shown for the 582-nm background in Fig. 9, which shows that the absolute sensitivity of the OFF response is 1.5 log units lower than the ON response at short wavelengths. Such a reduction in absolute sensitivity could not occur unless the S-cones that contribute to the receptive-field surround of ON-center cells were adapted differently than the S-cones which contribute to the receptive-field center of OFF-center cells. This is unlikely, because all cells must sample from the same population of S-cone photoreceptors, and all S-cone photoreceptors would be adapted in the same manner by this background. Perhaps future studies of the chromatic inputs to single cells of the goldfish retina will provide answers to the origin of this differential spectral sensitivity.

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