The Response Properties of Single Ganglion Cells in the Goldfish Retina

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INTRODUCTION

A cross-section of any vertebrate retina shows a complex pattern in which many types of cells are interconnected. The functions of very few of these cell types are known in any detail. The rods and cones almost certainly absorb the light quanta and are responsible for the initiation of the train of events which culminates in the nerve impulse patterns of the ganglion cells whose axons form the optic nerve. This pattern of nerve impulses is modified by the intensity, shape, and duration of the illumination falling on the retina. In a few types of vertebrate eyes it is also certain that the color of the stimulating light must modify the response patterns of different ganglion cells in a differential fashion. This is a requirement in any system of color vision, and it is fairly certain from behavioral experiments that these animals are able to distinguish colors. Many species of fish apparently can be trained to recognize colors. For this reason the fish retina is an appropriate place to find out how color information is encoded for transmission along the optic nerve.

The response patterns of single ganglion cells of the vertebrate retina have been the subject of extensive study since Hartline's successful isolation of the ganglion cells of the frog retina. These response patterns were classified by Hartline (1938) into "on," "off," and "on-off" types according to the temporal relationship of the discharge to the illumination. These descriptive terms have been generally accepted by subsequent investigators and are in wide usage today. The response patterns of ganglion cells of a wide variety of other vertebrates have been examined by subsequent investigators. This work has indicated that the discharge patterns are basically similar. The "on-off" type is most frequently seen and is looked on as playing the dominant role in the functional organization. The analysis of the response relationship

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The research was supported in part by National Science Foundation Grants G-3321 and G-7086.

to light indicates that very complex influences on the ganglion cell are present. In the visual pathway the ganglion cell is a third order neuron preceded by both the receptor cell and the bipolar cell. Little is known of the response patterns of either bipolar or receptor cells. The graded photopic responses (GPR) of the retinae of some types of fish which have been recorded by Svaetichin and MacNichol (1958) seem to arise from a structure between the photoreceptors and the ganglion cells. Changes in the wave length of the stimulating light vary both the magnitude and sign of the GPR. That is, both depolarizing and hyperpolarizing responses may be found at the same locus as the stimulus changes color. The exact origin of these responses remains to be established. They may arise in the large horizontal cells which are found in the fish retina, or they may be the result of synaptic activity. Somewhat similar responses have been seen in other vertebrate retinae such as the cat (Motokawa *et al.*, 1957, Brown and Wiesel, 1958, Grüsser, 1957). However, only in the teleost retina have they been studied in any detail. If the GPR relates to events that play a dominant role in the excitation of the ganglion cells, then the behavior of these ganglion cells in the fish retina also needs to be examined for possible correlation. The graded responses may be signs of processes that convey information on color as well as brightness. This report contains the results of our studies on the response patterns of the goldfish retinal ganglion cells together with some response patterns of the GPR.

It appears from this work that some wave lengths cause excitation and others inhibition of the same ganglion cells. This suggests that an opposed color mechanism such as that postulated by Hering (1875) is operative at the retinal level.

METHOD

Common goldfish (*Carassius auratus*) from 6 to 8 inches long were used for these experiments. In a manner very similar to that described by MacNichol and Svaetichin (1958), the eye was quickly removed from the head after decapitation, opened, and the retina lifted out after separation from the optic disc. The retina was spread out, receptor side up, upon a transparent glass plate within a small moist chamber. Some loss of rod outer segments almost certainly occurred although as much care and gentleness in this procedure as possible was maintained. No deliberate effort was made to remove these segments. Usually the receptor side was fairly free of melanin pigment; however, patches and scattered granules were often present. Continuous moist oxygen was supplied to the chamber as it appeared to be important for good electrical stability and survival of the retina. The whole operation took less than 3 minutes to accomplish.

Light was projected up through the glass plate and onto the retina from

an optical stimulator in the manner shown diagrammatically in Fig. 1. The optical system was the second of the two systems described by Wagner and Wolbarsht (1958) to which a grating monochromator had been added. The source light was a GE projection lamp featuring a concentrated coiled filament. This was driven at a nominal 17 amperes D.C. and gave a color temperature of approximately 2850°K. The lamp output was monitored by a photocell and maintained constant by adjustment of the filament current.

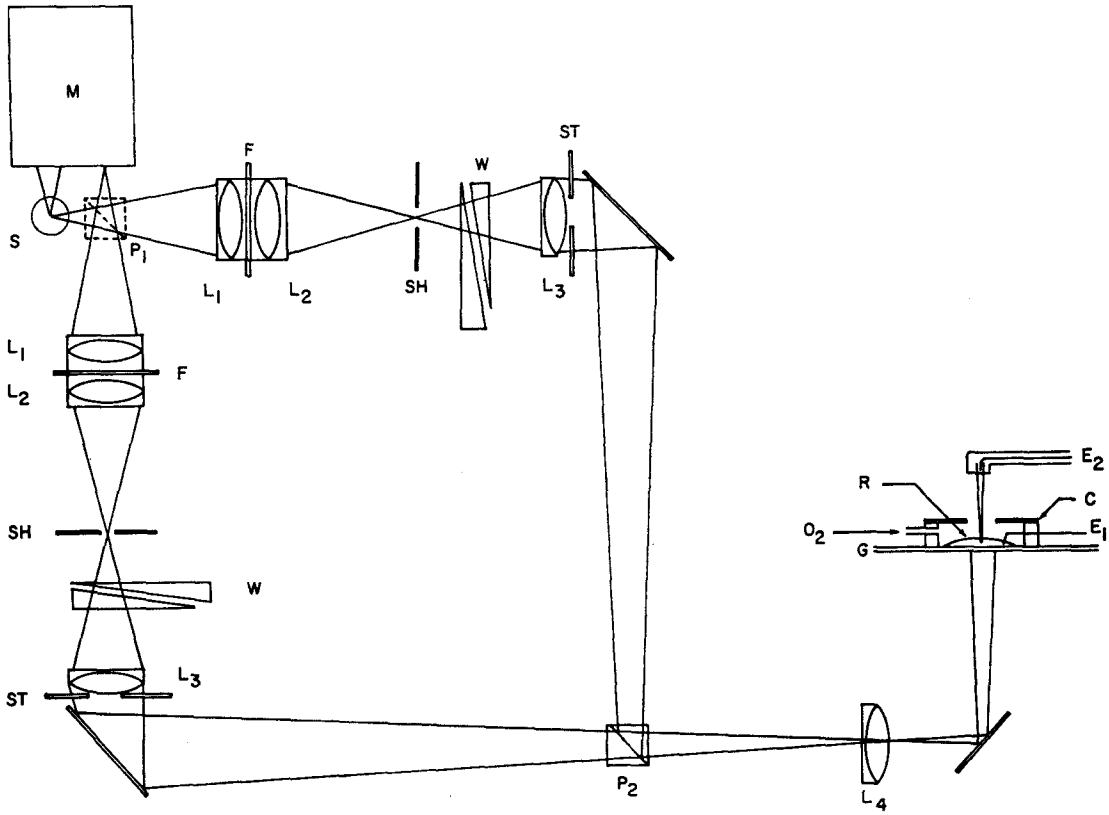

FIGURE 1. Schematic diagram of the optical stimulator and preparation set up. *C*, moist chamber; E_1, indifferent electrode; E_2, recording electrode; *F*, filter; *G*, glass plate; L_1, L_2, collimating lenses; L_3, field lens; L_4, projection lens; *M*, monochromator; O_2, moist oxygen; P_1, separating cube; P_2, combining cube; *R*, retina; *S*, source; *SH*, shutter; *ST*, field lens aperature stop; *W*, neutral density wedge and compensator. For further details see text.

The optical system contained two independent pathways which were essentially equivalent optically. Each pathway has a collimated region (between L_1 and L_2 in the figure) for placement of interference filters, a shutter (*S*) which consists of an electromagnetically driven vane (copy of a design by

H. K. Hartline, personal communication), a neutral density wedge (W) for control of intensity, a field lens (L_3), and an aperature stop (ST) which can be varied in size and position. The two beams are combined in a partially reflecting cube and projected onto the retina by a lens (L_4) in such a manner that an image of the aperture stop (ST) is formed at the plane of the receptor layer of the retina.

The separating cube (P_1) was not used in these experiments. The beam for one of the optical pathways is passed through a grating monochromator of a modified Fastie (1952) design. The grating drive gave a constant rate change

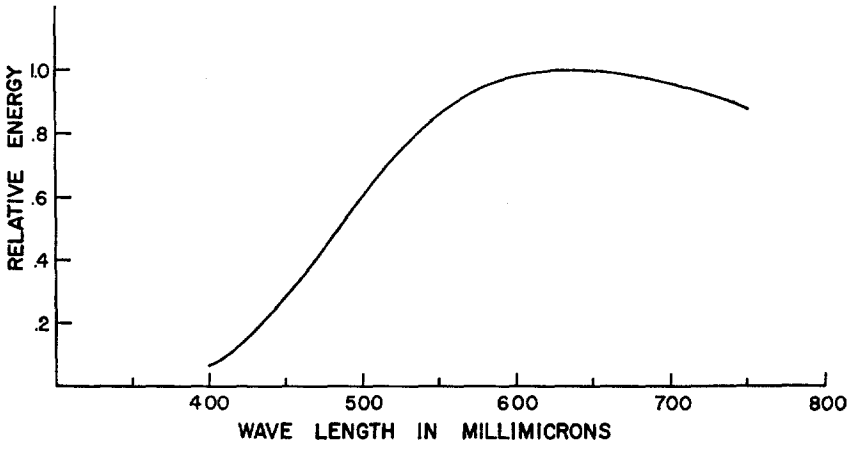

FIGURE 2. Relative energy flux at various wave lengths through the monochromator channel of the optical stimulator as measured with a thermopile in the plane of the retina.

of wave length with time and was directly coupled to a potentiometer in such a way as to furnish a voltage proportional to the wave length. This voltage was used in the experiments on the graded photopic response as the source for the horizontal deflection of the oscilloscope beam (see Fig. 3). Both rate of change and direction are indicated in the figure legend. The spectral radiance of this channel was a function of the wave length. Two principal factors were responsible for this: the change in grating efficiency with wave length and the incandescent tungsten filament source. A plot of the relative flux reaching the retina is given in Fig. 2. Figs. 3 and 4 must be interpreted with these factors in mind; however, the plots in Figs. 7 through 10 were corrected to an equal energy basis.

The spectral characteristics of the second channel were controlled by means of interference filters and cut-off filters. For the red adapting light a Wratten 89B filter was used; for the blue adapting light a B. and L. 470 mμ interference filter with a Wratten W75 filter were used.

The recording electrode for the graded photopic potentials was a 3 M KCl

filled micropipette having a measured resistance in 3 M KCl of at least 20 megohms.

Signals were led through the above salt bridge to an $Ag/AgCl_2$ electrode connected to a negative capacitance type amplifier (MacNichol and Wagner, 1954). A similar metal electrode in contact with the retina served as the source of the reference potential. The output was further amplified by a transistorized D.C. amplifier (MacNichol and Bickart, 1958) and displayed on a double beam oscilloscope.

Inasmuch as micropipettes record ganglion cell spike responses well only occasionally, a number of types of metallic electrodes were tried for this purpose because of their historical success in such situations. Platinum-iridium alloy electrodes, the pointed tip coated with platinum black and the shaft insulated with insl-x enamel, were found to be the most satifactory from the standpoint of electrical stability and ease of isolation of single units. Electrodes could be made rather easily by electropolishing 8–10 mil diameter 70 per cent platinum 30 per cent iridium wire in a solution of 30 per cent NaOH saturated with NaCN. The electrolyzing current was furnished by an A.C. voltage source connected between the wire and a carbon rod. The taper of the point was controlled by the length of wire inserted into the bath. Preliminary shaping of the tip was accomplished at high voltages (6.3 v. A.C.) accompanied by vigorous agitation of the bath. Final polishing with smaller voltages (0.8 v. A.C.) yielded smooth, gradually tapered electrodes having tips of less than 1 micron. The electrode was then coated by pushing the tip through a drop of insl-x enamel supported on a wire hook. The point of the electrode was pushed upward through the drop so that it was coated with a thin layer of enamel and then removed through the open end of the hook. This coat was allowed to dry at room temperature for 24 hours. A second and a third coat were then applied in the same way as the first. Platinizing the tip in a 2 per cent $PtCl_2$ solution just before using was essential. Best results were obtained by passing current from a 15 v. D.C. source in series with a 1 megohm resistor between the electrode and a platinum wire immersed in the solution.

Signals from an electrode inserted into the retina were fed through a blocking capacitor to a conventional cathode follower circuit coupled to the transistor amplifier with rise and holdup time constants both set for .001 second. The indifferent electrode was a platinum-iridium wire placed in contact with the retina at some convenient point.

RESULTS

I. *The Graded Photopic Response*

Using micropipette electrodes, potential changes in response to illumination of the type described by Svaetichin and MacNichol (1958) were obtained in the goldfish retina. Typical spectral response wave forms may be seen in Fig. 3.

These are direct photographs of the oscilloscope screen. The horizontal sweep of the oscilloscope beam was coupled to the monochromator in such a way as to indicate the wave length of the emitted light (see Method). The illumination was on continuously but the wave length was changed at a constant rate. The vertical deflection is proportional to the potential change but inasmuch as the stimulus strength was a function of wave length (Fig. 2) the wave forms are not corrected for an equal energy spectrum. They are, however, qualitatively satisfactory for illustrating the polarity, maxima, the crossover or neutral

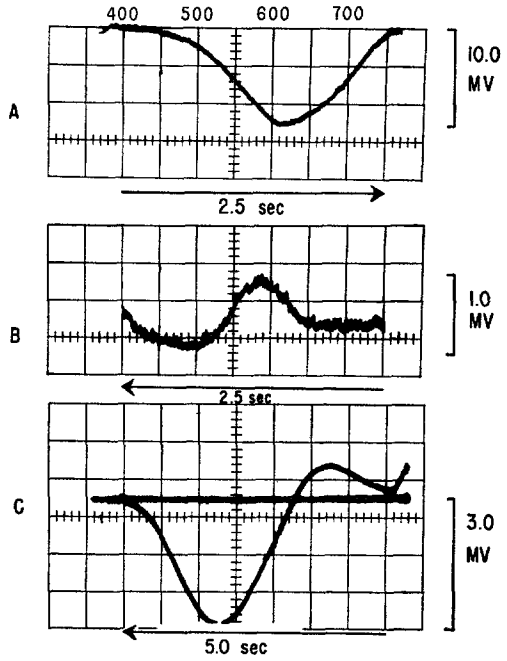

FIGURE 3. Graded photopic response (GPR). In all records positive at the recording electrode is up. The horizontal trace in record C indicates level in absence of stimulus. Sweep direction and duration for each record is indicated by arrow at base. Intensity of stimulus at 600 mμ = 55 μ watts/cm.^{2} See Fig. 2 for energies at other wave lengths.

points. Fig. 3A can be taken as illustrating one type of response which showed a simple hyperpolarization at all wave lengths of the stimulating light. Its maximum sensitivity was in the vicinity of 615 mμ. This response seems equivalent to the "L" type reported by Svaetichin and MacNichol (1958). Figs. 3B and 3C are typical of the wave forms that show a diphasic polarity. Similar responses reported by Svaetichin and MacNichol (1958) were called "chromatic responses."

The record in Fig. 3B has approximately equal positive and negative amplitudes with maxima at about 480 and 580 mμ while the record in Fig.

3C has a much stronger negative phase with its maxima at about 520 and 675 mμ. Wave forms of the type depicted in Fig. 3C are found more frequently than the response recorded in Fig. 3B. Tomita (1957) reported an almost identical wave form to Fig. 3C in the cyprinid fish.

The characterization of these responses to light has been the subject of a number of publications by Svaetichin (see Svaetichin and MacNichol, 1958, for references), Tomita (1957), Mitarai and Yagasaki (1956), and Motokawa *et al.* (1957). Our evidence indicates that the potentials in the goldfish retina

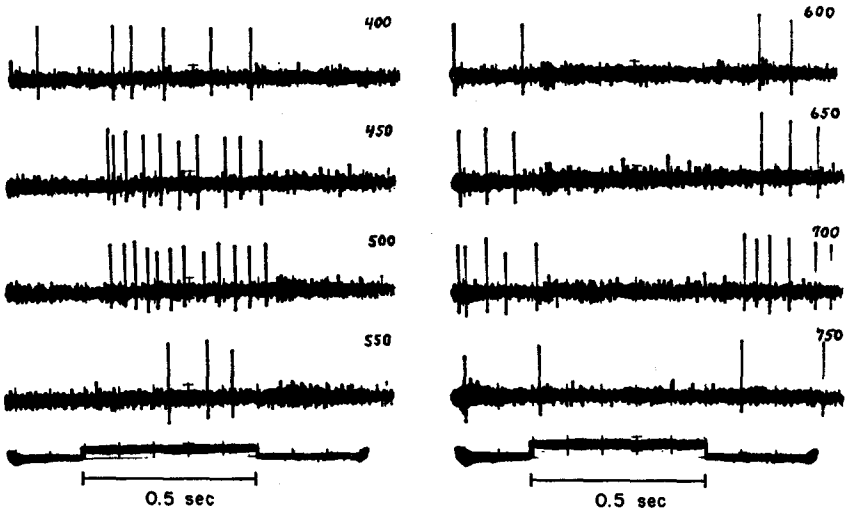

FIGURE 4. Variation of response from a single ganglion cell with change in wave length of stimulus. Wave length of stimulus in mμ at upper right hand of each record. The duration of the stimulus is indicated by the step in the signal trace at the base of each series. Spikes occurring before the onset of the stimulus are "off" responses from a preceding stimulus. Intensity of stimulus at 600 mμ = 55 μ watts/cm.^{2} See Fig. 2 for intensities at other wave lengths.

are governed by the same parameters. Unfortunately, in the goldfish we have found these potentials small in amplitude, difficult to isolate, and subject to rather rapid deterioration so that we have not been able to extend our investigations of these potentials very far.

II. *Ganglion Cell Responses*

1. WAVE LENGTH DEPENDENCY OF THE RESPONSE PATTERN

The response pattern of spike potentials evoked in the ganglion cells of the goldfish retina in response to illumination resembles in many respects the "on," "off," and "on-off" responses observed in other vertebrate retinae. Of these the "on-off" units were observed most frequently. Occasionally units

were found which discharged steadily in the dark but were inhibited during illumination. Other units were observed which gave a steady discharge as long as the retina was illuminated. The studies to be described were concerned with the "on-off" type exclusively.

Illumination of the retina with white light evoked a vigorous burst of impulses in "on-off" units which subsided rather quickly, often ceasing altogether. Extinction of the light was followed by another vigorous burst

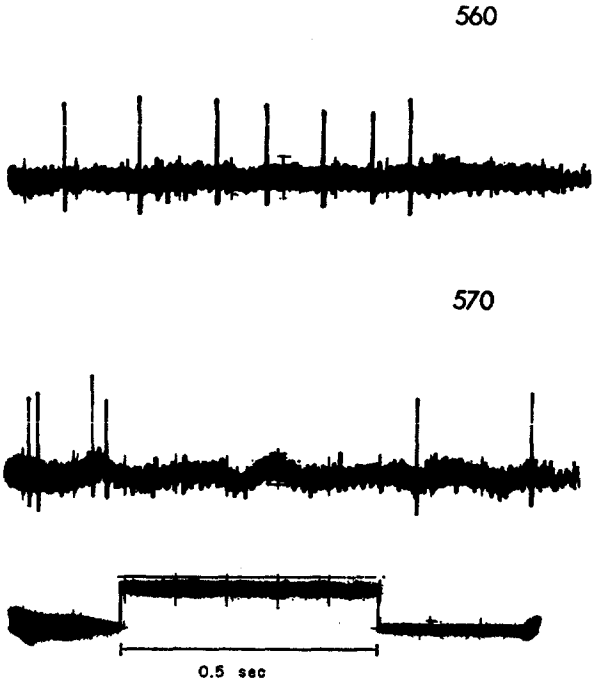

FIGURE 5. Variation of ganglion cell response with a small change in wave length. Wave length of stimulus in mμ is in upper right of each record. The duration of the stimulus is indicated by the step in the signal record at the base of the series. Spikes occurring before the onset of the stimulus are "off" responses from preceding stimuli. Intensity of stimulus 55 μ watts/cm.²

which also tended not to be sustained. The use of spectral illumination, however, revealed a dramatic wave length dependency of the "on-off" pattern. A change in the wave length of the stimulating light converted an essentially pure "on" response to one band of spectral colors into an essentially pure "off" response to illumination in another band of wave lengths. The series of records in Fig. 4 were taken from a ganglion cell preparation which demonstrated this behavior rather well. Light of wave lengths 400 through 550 mμ evoked an "on" response only. To illumination of wave lengths longer than this, the response was essentially a pure "off" effect. The spike activity

preceding illumination in the right hand series of records was actually part of the "off" discharge from a stimulus one second earlier. By this technique it was shown rather clearly that there existed suppression of activity during long wave length illumination. The short wave lengths gave excitation; the long wave lengths had a strong inhibitory influence during illumination followed by an "off" discharge or postinhibitory rebound. The transition

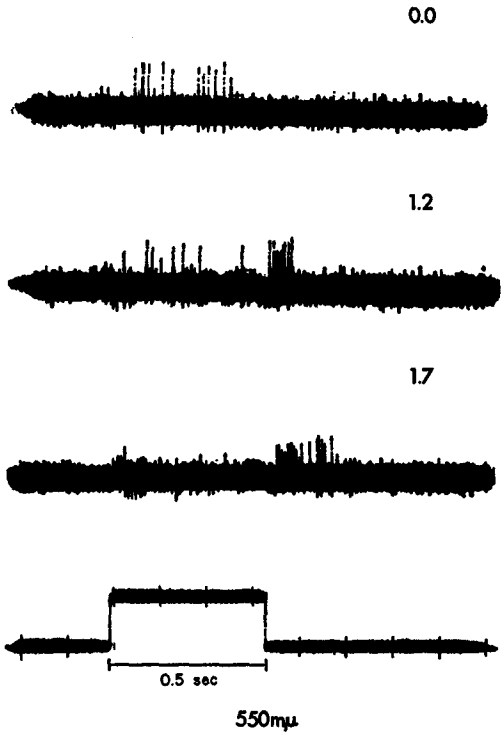

FIGURE 6. Variation of ganglion cell response with change in intensity of stimulus. Duration of stimulus is indicated by step in signal trace at base of the series. Log intensity of stimulus is in upper right of each record. 0.0 log units = 1.7 μ watts/cm.^{2}

from excitation to inhibition was reasonably sharp and could be complete with a change of as little as 10 mμ (see Fig. 5).

2. EFFECT OF INTENSITY

The series of records shown in Fig. 6 is presented to show that at certain wave lengths a change in the intensity also converted a response pattern from one exhibiting excitation ("on" type) to one showing both excitation and inhibition ("on-off") to one with only inhibition evident ("off" discharge only). This was possible only in a limited band of wave lengths and it often required a wide range of intensities to effect this change.

3. EVIDENCE FOR THE EXISTENCE OF TWO OPPOSED COLOR MECHANISMS

The relationship of these two parameters, wave length and intensity, to response pattern became more evident when the spectral luminosity function was determined for the type of ganglion cell shown in Fig. 4. This function was obtained graphically by plotting for each wave length the intensity necessary to evoke a threshold response. The threshold response was taken as the discharge of N or more impulses in response to one-half of a series of test flashes. In most preparations N was taken as one, however occasionally the presence of spontaneous activity required N to be as large as 5 before

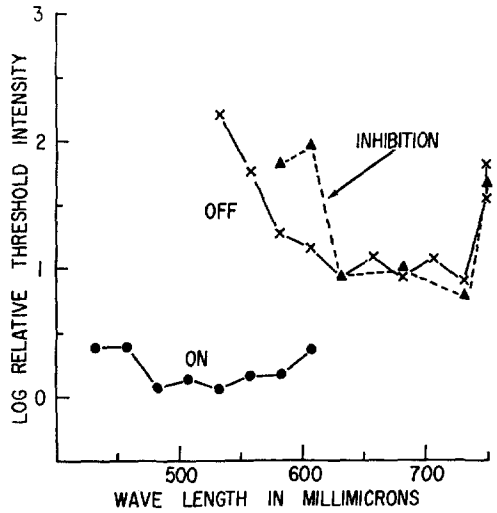

FIGURE 7. Intensity necessary to elicit various types of threshold responses from a single ganglion cell at different wave lengths. Each point is an average of four determinations at one wave length. The duration of the stimulus was 1.0 second, 0 log units = 5.5×10^{-2} μ watts/cm.^{2} for all wave lengths.

consistent and reproducible curves were obtained. Frequency of seeing curves for various N tended to be quite steep so that changes could be made in the threshold criterion without significant differences in the shape of the curves. Threshold criteria were established before a run and remained fixed for the duration of the experimental procedure. Thresholds for both the "on" response and the "off" response were obtained. Fig. 7 illustrates the functions so obtained for a typical ganglion cell. The filled circles are the thresholds for the "on" responses and the crosses the thresholds for the "off" responses. These points when joined by lines appear to define two spectral luminosity functions which are distinct from each other, both in magnitude and in wave length-response maxima. The function defined by "on" thresholds extends from

about 400, through a maximum at about 525, and ending abruptly at about 610 mμ. The second function defined by "off" response thresholds extends from about 530 to 750 with its maximum sensitivity at 620 mμ. The curves overlap from about 530 to 610 mμ. It was within this region that a change in intensity was effective in converting "on" patterns into "on-off" or "off" patterns. There are no points for "on" thresholds at wave lengths longer than 615 mμ because none were found within the range of intensities at our disposal. This abrupt cessation of "on" responses in favor of "off" responses only was quite characteristic and is reflected in the sharp shift in response pattern with change in wave length referred to earlier (see Fig. 5). The suppression

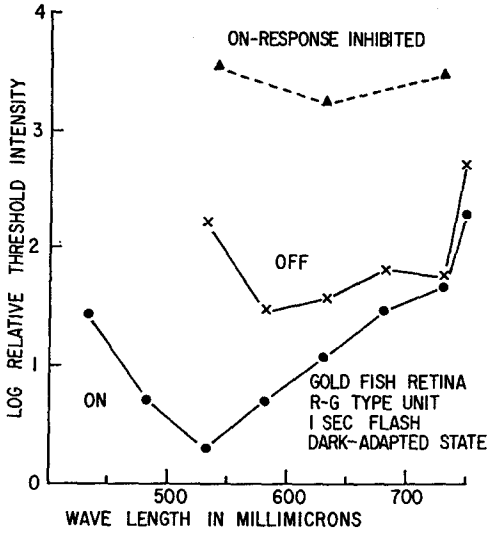

FIGURE 8. Intensity necessary to elicit various types of threshold responses from a single ganglion cell at different wave lengths after dark adaptation. The duration of the stimulus was 1 second, 0 log units = 1×10^{-4} μ watts/cm.^{2} for all wave lengths.

of activity during illumination appeared to coincide closely with the presence of "off" activity at all wave lengths. The triangles in Fig. 7 represent the intensities which caused complete suppression of all activity during illumination. In the region of the overlap of the "on" and "off" functions, this criterion implied that the "on" response present at low intensities was abolished at high intensities, as was illustrated in Fig. 6. At longer wave lengths the inhibition was detected by the interruption of the "off" discharge by re-illumination, and by reduction in the low frequency spontaneous activity that was present. This has been mentioned in connection with Fig. 4 which shows the suppression of activity during illumination at long wave lengths.

In an occasional preparation (Fig. 8), "on" thresholds could be found at all wave lengths from 400 through 750 mμ. With stimuli of somewhat higher

intensity the "off" function also could be identified. At still higher intensities, complete suppression of "on" activity during illumination occurred. This function paralleled that of the "off" process.

4. EVIDENCE FOR THE INDEPENDENCE OF THE TWO MECHANISMS

Although the wave lengths for the maximum sensitivities of the two processes were fairly constant in different ganglion cells, the ratio of sensitivities at these two wave lengths varied greatly. On rare occasions one process was observed to change its sensitivity spontaneously relative to the other processes.

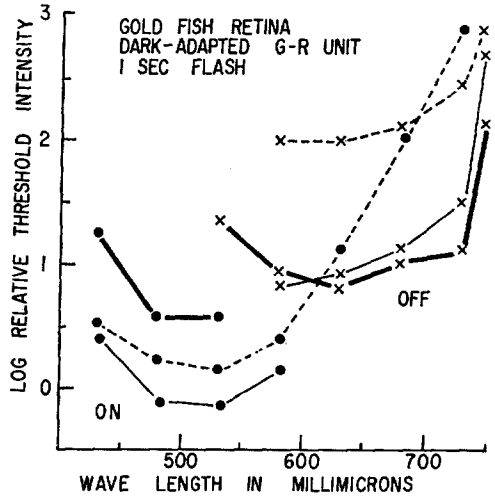

FIGURE 9. Intensity necessary to elicit various types of threshold responses from a single ganglion cell at different wave lengths before, during, and after exposure to a red adapting light. Heavy solid line indicates thresholds before adaptation, dashed lines thresholds during red adapting light, thin solid line indicates thresholds approximately 10 minutes after extinction of the adapting light. Duration of text stimulus 1 second. For intensity of test stimulus 0 log units = 2.3×10^{-2} μ watts/cm.^{2} for all wave lengths. Energy of red adapting light = 5.2×10^2 μ watts/cm.^{2} Duration of adaptation = 27 minutes. See under Methods for further details of red adapting light.

Evidence was also found in the response to light adaptation. Inasmuch as the two processes were displaced with respect to wave length sensitivity, it was possible to influence selectively the state of adaptation of the processes by adapting either to a light of long wave length or to one of short wave length. Fig. 9 illustrates the result observed immediately following several minutes' adaptation to strong red light. Heavy solid lines define the functions of the two processes in the dark adapted or control situation. Broken lines join the points which were obtained in the adapted state. Adaptation caused the inhibitory system to be markedly depressed, while the excitatory system increased its sensitivity. The two systems moved in opposite directions but

their respective maxima were unchanged. Not only was it possible to find excitatory thresholds at long wave lengths previously unobtainable but the inhibitory influence appeared to be sufficiently weakened so that excitatory thresholds could be detected in the presence of fairly strong "off" responses (see 730 mμ, Fig. 9). Removal of the adapting light was followed by a slow but essentially complete recovery of the inhibitory process (see thin continuous line in Fig. 9). Although here the excitatory process continued to increase its sensitivity this was not a constant finding and could be attributed either to instability in this preparation or to failure to allow sufficient time for the preparation to dark-adapt completely before the control period.

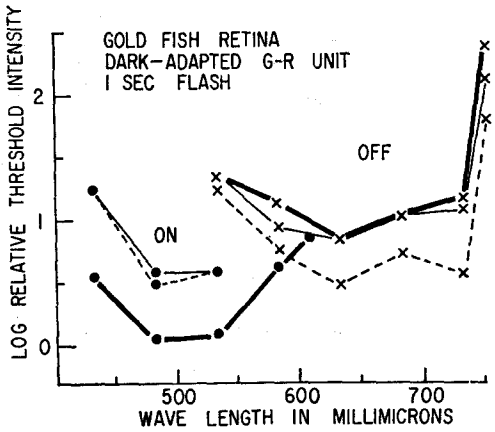

FIGURE 10. Intensity necessary to elicit various types of threshold responses from a single ganglion cell at different wave lengths before, during, and after exposure to a blue adapting light. Heavy solid line indicates thresholds before adaptation, dashed lines thresholds during blue adapting light, thin solid line indicates thresholds approximately 5 minutes after extinction of adapting light. Duration of test stimulus 1 second. For intensity of test stimulus 0 log units = 2.3×10^{-2} μ watts/cm.^{2} for all wave lengths. Energy of blue adapting light = 8.0 μ watts/cm.^{2} Duration of adaptation 15 minutes. See under Methods for further details of blue adapting light.

The effect of short wave length (blue) adapting light is illustrated in Fig. 10. As in the previous figure, the heavy continuous lines represent the dark-adapted spectral luminosity functions of the control. Broken lines represent the functions following several minutes of light adaptation. Again it is evident that the systems moved in opposite directions, the inhibitory system becoming more sensitive and the excitatory system less so. "On" thresholds could not be determined at wave lengths longer than 530 mμ, although in the control they were obtainable out to 605 mμ. The postadaptation control showed a prompt return of the inhibitory influence to the initial conditions, however, the excitatory system remained at about the same level of sensitivity. Similar

experiences along this line suggested that the excitatory influence was appreciably more delicate than the inhibitory system.

5. THE RECEPTIVE FIELD

The experiments described in sections 1 and 2 were made with relatively large field (5 mm. diameter) illumination of the retina. If the stimulus is restricted to very small diameter fields, the response pattern may also be modified. The receptive field of a particular ganglion cell may include an appreciable area of the retina. The spatial limits of this area have usually been estimated by determining the loci of similar responses to a small spot stimulus.

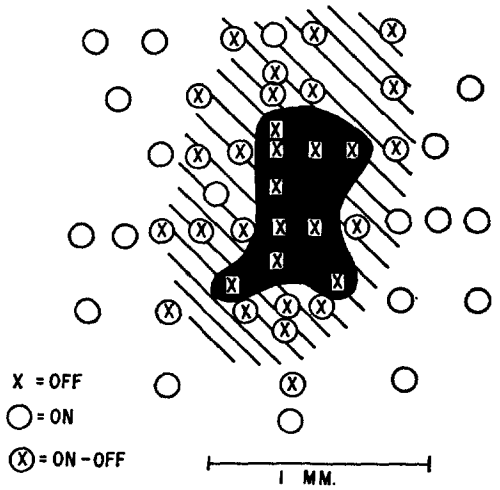

FIGURE 11. Receptive field of a single ganglion cell. Solid black area indicates region where only "off" responses were found. Hatched area indicates region where "on-off" type responses were found. "On" responses were found only on periphery of "on-off" area. Test stimulus was 153 μ in diameter, wave length 600 mμ, intensity 18.0 μ watt/cm.^{2}

Fig. 11 is a spatial plot of the response patterns evoked by small spot illumination at a number of points about the receptive field of one of the chromatically sensitive "on-off" units. These stimuli were all at the same intensity and wave length. Spatial separation of the normal pattern into its separate components is evident. The central area of approximately 0.5 mm. diameter gave essentially a pure "off" response while the periphery gave a pure "on" response. Intermediate regions showed both. The illustration does not indicate the relative strengths of each type of response. However, the prominence of each was graded so that the mixed "on-off" pattern had a very strong "off" component close to the central area, its response fading as more peripheral regions were tested. The opposite was true of the "on" response which was very weak close to the central area, but became increasingly stronger as the more periph-

eral regions were tested. In this illustration, the pure "off" response area has been set off in black. Its irregular shape should not be taken as defining an anatomical delimitation, rather it should be thought of as a functional perimeter as of the moment these stimuli were presented. This receptive field can be called an "off" center "on" periphery unit comparable to the receptive fields obtained in the cat by Kuffler (1953). The spatial division of the "on-off" pattern implies that this receptive field was also chromatically separated. To test this the spectral luminosity function of a small spot placed well within the central area was determined. It matched the inhibitory wave length function found in the full field illumination experiments. Also a plot of the luminosity function for the "on" response of a small area located on the periphery agreed with the earlier plots for the excitatory process using large field stimulation. The central area appears to comprise a fairly limited number of receptors while the peripheral field is extensive, almost as if all peripheral receptors of one class exert an excitatory influence on the ganglion cell. Quantitative measurements of the stimulus-response relationships in central, peripheral, and transitional zones would be necessary for a better understanding of these processes.

DISCUSSION

Color discrimination is ordinarily attributed to cone type photoreceptors, although it has often been proposed that the rods may participate also. Unfortunately, histological differences which would account for chromatic specificity among the cones or among the rods have not been observed so far. In fresh water fish, porphyropsin, a photolabile pigment, has been extracted and interpreted as the effective rod pigment. Its maximum peak of absorption is at about 520 mμ. The extraction of naturally occurring cone photopigments from fish retina has not been reported; however, Wald (1953) synthesized cyanopsin and suggested that it was identical with a naturally occurring cone photopigment. Its maximum absorption occurs at 620 mμ. Recently Hanaoka and Fujimoto (1957) reported spectrophotometric measurements of single cones of the carp (*Cyprinus carpio*) and identified six photopigments with peaks at 425, 495, 530, 570, 630, and 675 mμ. In some cases two were found in a single cone.

We have attempted to relate the published curves on these pigments with the spectral luminosity functions obtained electrophysiologically in fish retinas. Some agreement was found in the presence of the "L" response maximum at about 620 mμ, and the chromatic response maxima at 500, 525, 580, and 675. However, the maxima of our graded photopic response curves need not necessarily match the maxima of the photopigments of Wald or Hanoaka and Fujimoto for a number of reasons. The graded photopic response appears

to arise from some structure or cells between the receptors and the ganglion cells. The chromatic response may be more likely related to bipolar function than to receptor function because of its somewhat deeper location than the "L" response and thus the maxima may be shifted.

Since the significance and site of origin of the GPR are not yet understood, its relationship to the ganglion discharge cannot be determined. Attempts to match published curves for the absorption spectra of porphyropsin and cyanopsin to the "on" and "off" processes of the ganglion cells of the fish retina met with some success also. Unfortunately, our data lack the precision necessary to assert this agreement with confidence. Granit (1941) analyzed the photopic and scotopic spectrum response of carp (*Cyprinus*) using the "a" wave of the electroretinogram. His curves showed two maxima, one at 540 mμ and the other at 600 mμ. In the tench (*Tinca*), another fresh water fish, he was able to record "on" and "off" responses with microelectrodes. The scotopic spectral luminosity response curve of these elements was in close agreement with that of the porphyropsin absorption spectrum. The photopic curve showed a maximum at about 600 mμ.

Not all of the "on-off" response patterns exhibited chromatic selectivity. Units were found with "on" and "off" spectral luminosity response functions parallel to each other which maintained constant separation following chromatic adaptation. Other response patterns were observed to be the reverse of the ones reported in detail in this report, that is, they showed "off" responses following short wave-length stimulation and "on" responses to long wave length light. These observations would imply the presence of considerable specialization in the ganglion cell's relationship to the bipolar and receptor pathways.

Granit (1948) observed changes in the "off/on" ratio to different wave lengths and intensities in the cat. He also recognized the antagonism that existed between the "on" and the "off" response mechanisms (Granit 1951). His conclusion that two types of influence converged upon the same ganglion cell from presumably separate pathways is further supported by the work presented here. Studies by other investigators, notably De Valois *et al.* (1958) who reported that in the lateral geniculate body of the monkey, "on-off" responses could be selectively evoked by blue and yellow light, suggest that the "on-off" wave length discriminating mechanism is a general phenomenon in the vertebrate retina.

Further understanding of the color mechanism may be gained by considering the relationship of the "on-off" responses to the receptive field. The separation of the receptive field into "on" regions and "off" regions was first demonstrated clearly by Kuffler (1953) in the cat. However, chromatic differences in the spatial organization of the same retina were not found (Barlow, Fitzhugh, and Kuffler, 1957). The receptive fields of certain ganglion cells of

the goldfish retina show very definite separation into regions differentially sensitive to color. The central area appears to be a circumscribed group of receptors (in Fig. 11 these were red sensitive) which acted to inhibit the excitation of the ganglion cell. The surrounding area is much more extensive and less easily defined but appears to be homogeneous with respect to the color sensitivity of the receptors and their action on the ganglion cell. This action is opposed to that of the central area and the color sensitivity markedly different. The extent to which this spatial organization further categorizes the function of ganglion cells is not known at this time. Accurate maps of the receptive field sensitivity and extent are difficult to obtain.

It must be realized that all of the present studies were performed in partially dark-adapted retinas and that information regarding changes in spatial organization with adaptation remains to be studied. Although our understanding of the mechanism of color vision in the goldfish retina is far from complete we can certainly say that information about the temporal and spatial characteristics of a chromatic stimulus is transmitted along the optic nerve using an opposed color coding system.

REFERENCES

BARLOW, H. B., FITZHUGH, R., and KUFFLER, S. W., 1957, Change of organization in the receptive fields of the cat's retina during dark adaptation, *J. Physiol.*, **137**, 338.

BROWN, K., and WIESEL, T. N., 1958, Intraretinal recording in the unopened cat eye, *Am. J. Ophth.*, **46**, No. 3, pt. 2, 91.

DE VALOIS, R. L., SMITH, C. J., KITAI, S. T., and KAROLY, A. J., 1958, Response of single cells in monkey lateral geniculate nucleus to monochromatic light, *Science*, **127**, 238.

FASTIE, W. J., 1952, A small plane grating monochromator, *J. Opt. Soc. America*, **42**, 641.

GRANIT, R., 1941, A relation between rod and cone substances based on scotopic and photopic spectra of *Cyprinus*, *Tinica*, *Anguilla*, and *Testudo*, *Acta Physiol. Scand.*, **2**, 334.

GRANIT, R., 1948, The off/on ratio of the isolated on-off-elements in the mammalian eye, *Brit. J. Ophth.*, **32**, 550.

GRANIT, R., 1951, The antagonism between the on-and off-systems in the cat's retina, *ann. Pyschol.*, **50**, 129.

GRÜSSER, O. J., 1957, Rezeptor potentiale einzelner retinaler zapfen der katze, *Naturwissenschaften*, **44**, 522.

HANAOKA, T., and FUJIMOTO, K., 1957, Absorption spectrum of a single cone in carp retina, *Japan J. Physiol.*, **7**, 276.

HARTLINE, H. K., 1938, The responses of single optic nerve fibers of the vertebrate eye to illumination of the retina, *Am. J. Physiol.*, **121**, 400.

HERING, E., 1875, Grundzuge einer theorie des Farbensinnes. *Sitzungsber. k. Akad. Wissensch., Math.-naturwissensch. Cl., Wein.* **70**, Abt. 3, 169.

KUFFLER, S. W., 1953, Discharge patterns and functional organization of mammalian retina, *J. Neurophysiol.*, **16**, 37.

MACNICHOL, E. F., JR., and BICKART, T., 1958, The use of transistors in physiological amplifiers, *IRE Tr. Med. Electronics*, Professional Group on Medical Electronics, **10**, 15.

MACNICHOL, E. F., JR., and SVAETICHIN, G., 1958, Electrical responses from the isolated retinas of fishes, *Am. J. Ophth.*, **46**, No. 3, pt. 2, 26.

MACNICHOL, E. F., JR., and WAGNER, H. G., 1954, A high impedance input circuit suitable for electrophysiological recording from micropipette electrodes, Research Report. Project NM 000 09.03.01, Vol. **12**, 97, Bethesda, Naval Medical Research Institute.

MITARAI, G., and YAGASAKI, Y., 1956, Resting and action potentials of single cone, *Ann. Rep. Research Inst. Environmental Med., Nagoya Univ. Japan*, 54.

MOTOKAWA, K., OIKAWA, T., and TASAKI, K., 1957, Receptor potential of vertebrate retina, *J. Neurophysiol.*, **20**, 186.

SVAETICHIN, G., and MACNICHOL, E. F., JR., 1958, Retinal mechanisms for chromatic and achromatic vision, *Ann. New York Acad. Sc.*, **74**, 385.

TOMITA, T., 1957, Intraretinal action potential of cyprinid fish, *Japan. J. Physiol.*, **7**, 80.

WAGNER, H. G., and WOLBARSHT, M. L., 1958, Studies on the functional organization of the vertebrate retina, *Am. J. Ophth.*, **46**, No. 3, pt. 2, 46.

WALD, G., BROWN, P., and SMITH, P., 1953, Cyanopsin, a new pigment of cone vision, *Science* **118**, 505.