Opponent Color Responses in Retinal Ganglion Cells

Abstract. The receptive fields of certain ganglion cells in the goldfish retina have been mapped. These fields are "off" center, "on-off" intermediate, and "on" periphery types. The excitatory process controlling the "on" response is stimulated maximally by green light; the "off" response process, inhibitory in nature, is stimulated maximally by red light. The two processes can be light adapted independently.

We have recorded the elecrophysiological responses to monochromatic illumination of one type of ganglion cell in the retina of the goldfish. These cells give "on-off" type responses similar to those found in other vertebrate retinas when illuminated (*1*). Figure 1 is a plot of the intensities necessary to evoke a threshold response at "on" and at "off" for various wavelengths of the stimulating light. For the particular cell shown, "on" thresholds could be obtained from about 425 to about 600 mμ but not at longer wavelengths. "Off" thresholds were obtained from about 530 to about 750 mμ. These thresholds seem to define two separate but overlapping spectral luminosity functions, one associated with the "on" response, the other with the "off" discharge. In the region of overlap of the two functions an increase in the stimulus intensity above that of the "off" threshold diminishes or abolishes the "on" response. Reillumination at any wavelength above the "off" threshold tends to inhibit any "off" discharge following a previous stimulation.

The "on" and "off" processes could be light adapted separately. Adaptation to a steady background of red light raised the threshold of the "off" response, but the threshold of the "on" response was slightly lowered. A blue adapting light made the "on" process less sensitive and the "off" process more sensitive.

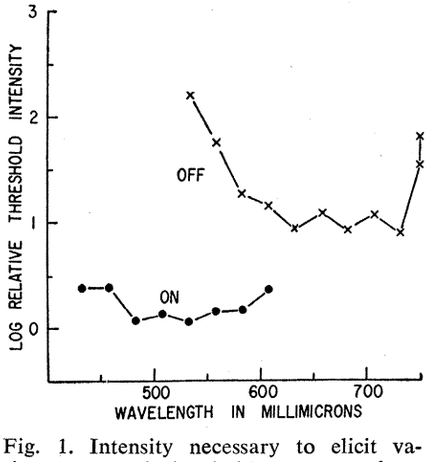

Fig. 1. Intensity necessary to elicit various types of threshold responses from a single ganglion cell at different wavelengths. Each point is an average of four determinations at that wavelength. The duration of the stimulus was 1.0 sec; 0 log units = 5.5×10^{-2} μwatt/cm^2 for all wavelengths. Stimulus diameter, 5 mm.

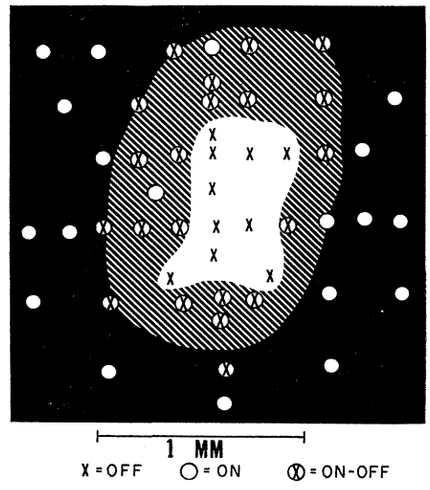

Fig. 2. Receptive field of a single ganglion cell. Central clear area indicates region where only "off" responses were found. Hatched area indicates region where "on-off" type responses were found. "On" responses were found only in peripheral area indicated by solid black. Test stimulus was 153 μ in diameter, wavelength 600 mμ, intensity 18 μwatt/cm^2.

Most of the ganglion cells studied had their "on" maxima at about 525 mμ and their "off" maxima at about 620 mμ, which suggests that this kind of cell may be connected to both porphyropsin and cyanopsin systems (*2*).

Figure 2 is a plot of the response patterns observed when the receptive field was explored with a small spot of 550 mμ wavelength. The "off" center, "on" periphery character is evident.

The two antagonistic processes may indicate the presence of a Hering type opponent color mechanism in the goldfish retina which is encoded not only in terms of wavelength but also spatially in terms of the receptive fields of the ganglion cells (*3*).

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References and Notes

1. See H. G. Wagner, and M. L. Wolbarsht, "Studies on the functional organization of the vertebrate retina," *Am. J. Ophthalmol.* **46**, No. 3, Pt. 2, 46–59 (1958) for examples and bibliography.
2. H. J. A. Dartnall, *The Visual Pigments* (Wiley, New York, 1957).
3. The opinions or assertions contained herein are ours and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large. This research was supported in part by National Science Foundation grants G-3321 and G-7086.

8 December 1959

Chlorophyll-Sensitized Photoreduction in the Thionine-Ferrous System

Abstract. The reduction of thionine in aqueous solution to leucothionine by ferrous ions in light can be sensitized by chlorophyll in the colloidal state, as obtained by diluting alcoholic solution with water.

The ability of chlorophyll in the molecularly dispersed state to catalyze various oxidation-reduction reactions is well known (*1–3*). Recently some evidence was found of a similar ability of chlorophyll in colloidal solutions, prepared by dilution with water of solutions of chlorophyll in alcohol, pyridine, or acetone (*4*).

We have studied the photochemical behavior of the thionine-ferrous iron system in aqueous solution (see *2*, *5*) and have found that, in the presence of colloidally dispersed chlorophyll, reversible photoreduction of thionine can be produced in red light absorbed only by chlorophyll ($\lambda > 650$ mμ).

The ferrous sulfate (analytical reagent grade) was used without further purification; thionine (biological stain of the National Aniline and Chemical Co., Inc.) was purified by repeated recrystallization. Chlorophyll (*a* or *b*) was prepared from fresh spinach by the method of Zscheile and Comar (*6*). The purity of the chlorophyll and of the thionine was checked by chromatography and spectroscopy.

A fresh aqueous solution of thionine ($\sim 5 \times 10^{-5} M$), containing ferrous iron ($\sim 10^{-3} M$), was placed in the main, square optical cell (1 cm^3), while a fresh ethanolic solution of chlorophyll was placed in a side tube, connected to the main cell by a closed capillary. To remove oxygen, both solutions were pumped out on a high-vacuum line; the residual oxygen pressure was $< 10^{-5}$ mm-Hg.

The thionine-Fe^{2+} cell, immersed in a thermostat, was first illuminated in the absence of chlorophyll, by a 1000-watt incandescent lamp, through a glass filter cutting off infrared radiation, and a red interference filter ($\lambda_{max} = 6455$ A). A battery-operated ribbon filament lamp (6 volts, 18 amp), focused first on the sample and then on the entrance slit of a Farrand monochromator, provided the scanning beam. Its intensity was reduced by a variable

aperture until it produced no measurable photolysis. The transmittance of the solution at 600 mμ (thionine band) and 680 mμ (chlorophyll band) was recorded by means of a photomultiplier, using a Tektronix 502 oscilloscope. As expected, in the absence of chlorophyll, the optical density at 600 mμ was not affected by illumination with red light.

The capillary in the side tube was then broken and alcoholic chlorophyll solution was transferred into the aqueous solution in the main cell, giving a colloidal chlorophyll solution with a concentration of $\sim 10^{-5} M$. The color of the mixture was bluish green. Its absorption spectrum, determined with a Beckman DU spectrophotometer, showed a rather flat peak at 670–690 mμ characteristic of chlorophyll in the colloidal state, and the normal sharp absorption peak of thionine at 600 mμ (Fig. 1). The mixture did not undergo decoloration after standing for 2 to 3 weeks in the dark under anaerobic conditions, which indicates that colloidal chlorophyll did not react with thionine or ferrous iron in the dark. After the mixing, the cell was immersed in a thermostat, kept for 5 minutes for temperature equilibration, and then illuminated with red light. No measurable change of optical density at 680 mμ, due to chlorophyll, could be observed in light; but the optical density at 600 mμ, due to thionine, decreased as plotted in Fig. 2.

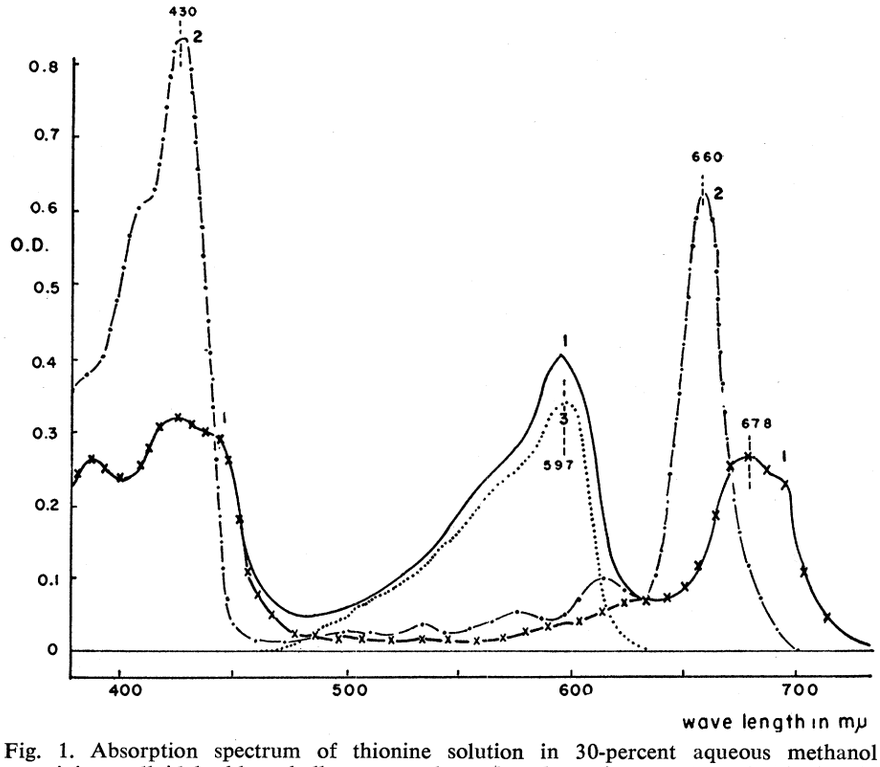

Fig. 1. Absorption spectrum of thionine solution in 30-percent aqueous methanol containing colloidal chlorophyll, compared to the absorption spectra of thionine in alcohol and of chlorophyll solution in ether. [Absorption curve of thionine in 30-percent aqueous alcohol is very similar to that in pure alcohol (see *8*).] **1,** Suspension containing chlorophyll *a* and thionine; 2, chlorophyll *a* in ether 10^{-5} mole/liter; 3 thionine in alcohol 5×10^{-6} mole/liter. Crosses indicate the absorption curve of colloidal chlorophyll solution without thionine.

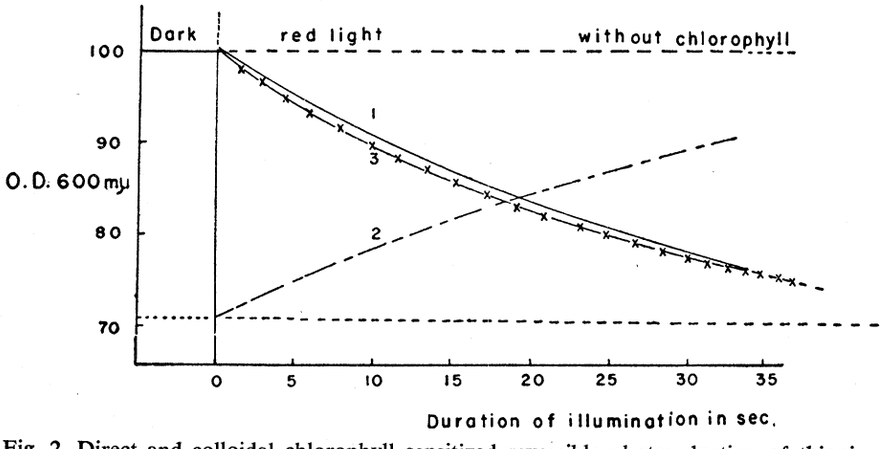

Fig. 2. Direct and colloidal chlorophyll sensitized reversible photoreduction of thionine by ferrous iron. Concentration of chlorophyll, 10^{-5} mole/liter; thionine, 5×10^{-6} mole/liter; ferrous iron, 10^{-3} mole/liter; at 20°C. **1,** Photoreduction of thionine by red light sensitized by chlorophyll; 2, back reaction; 3, photoreduction of thionine by white light (without chlorophyll).

Similarly to the direct photochemical reduction of thionine by ferrous ions, the photosensitized oxidation-reduction of thionine was completely reversible; after several cycles, the absorption spectrum of the suspension was the same as before the illumination.

Sensitization could involve either reversible photoreduction of chorophyll by ferrous ions, as in Krasnovsky's interpretation of chorophyll-sensitized reduction of safranine by ascorbate, or it may be based on energy transfer from chlorophyll to thionine. Such a transfer is impossible in the singlet state, because of the relative position of the excited levels; but if thionine, similarly to the related compound acridine (*7*), has a particularly low-lying triplet state, energy transfer may conceivably occur via the triplet state of chlorophyll.

The capacity of colloidal chlorophyll to photosensitize endergonic oxidation-reduction could be of some interest in relation to its role in photosynthesis.

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