may inhibit snail neurons through two different ionic mechanisms, causing a selective increase of membrane permeability to either Cl^- ions or K^+ ions. These data confirm that 5-HT possesses at least one fundamental property of chemical transmitters—the ability to alter selectively the membrane permeability to specific ions. Further work is still necessary to clarify whether 5-HT is actually involved in the synaptic inputs to the neurons endowed with 5-HT receptors.

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Compound Eyes: Localization of Two Color Receptors in the Same Ommatidium

Abstract. *The compound eye of the cockroach* Periplaneta *has receptors for ultraviolet light (maximum sensitivity at 365 nanometers) and green light (maximum sensitivity at 510 nanometers). Single photoreceptor cells in the compound eye were impaled, identified by spectral response, and marked with dye-filled microelectrodes. Using two different dyes, we showed that both types of receptors can be found in the same ommatidium.*

The retinas of arthropod compound eyes comprise hundreds or thousands of units called ommatidia (*1*). An ommatidium usually contains seven or eight elongate photosensory cells (retinular cells) whose cell bodies surround an attached axial structure known as the rhabdom. The rhabdom is formed by the juxtaposition or interleaving of masses of microvilli of the retinular cells and is the site at which photons are captured in the visual process. Generally each retinular cell sends an axon to the optic ganglia of the brain. The ommatidia are normally separated from one another by dense sleeves of screening pigment, and each possesses a small dioptric structure.

Behavioral tests show clearly that some arthropods have color vision (*2*). Certain other species are presumed to discriminate wavelengths because electrophysiological measurements reveal receptors with differing spectral sensitivities (*3*). Relatively little is known, however, about whether different color receptors are present in the same ommatidium (*4*). We here present evidence, based on individually marked cells, that they can be (*5*).

These experiments were performed on a white-eye mutant of the cockroach *Periplaneta americana* (*6*). This mutant lacks all accessory screening pigments but is otherwise normal. On the basis of spectral responses, there are two kinds of receptor cells in the eye (Fig. 1B), an ultraviolet receptor with maximum sensitivity at 365 nm and a green receptor with maximum sensitivity at about 507 nm (*7*, *8*). The sensitivity of the ultraviolet receptor is down 3 log units at 507 nm, whereas the sensitivity of the green receptor is down about 1.2 log units at 365 nm. Consequently it is a simple matter to decide which kind of cell has been impaled by examining the responses to green and ultraviolet test lights.

Isolated heads were sliced through the cornea so as to expose a row of ommatidia over their full length. The preparation was mounted on soft wax in a short section of quartz tube and covered with artificial saline (*8*). Single cells near the surface of the cut were impaled with micropipette electrodes filled with aqueous solutions of the fluorescent anionic dyes Procion yellow (5 percent) or Procion red (2 percent) (*9*). The pipettes were pulled with several threads of fiber glass in them (to facilitate filling) and were filled from the shank with a hypodermic needle fitted with a 0.2-μm Millipore filter. The procedure was generally to explore with a red-filled pipette until a cell sensitive to ultraviolet light had been impaled and identified. A series of 10- to 20-na pulses up to 100 msec long was then passed through the pipette for several minutes until the cell had visibly stained. By using the white-eye mutant and working close to the surface of the slice, we could usually see which ommatidium had been penetrated. Then a second electrode filled with yellow dye was advanced into the same or an adjacent ommatidium until one or more cells sensitive to green light were located and stained in similar fashion. Heads were fixed overnight and prepared for light microscopy by conventional means (*10*). The tissue was observed and photographed with a fluorescence microscope.

These experiments present technical difficulties. The retinular cells are about 7 μm in diameter, and many of the pipettes that were small enough to enter them without producing appreciable damage would not pass sufficient current to stain the cells. This was particularly true of the pipettes filled with Procion red. Morever, because the cells being studied were close to the surface of the tissue, their relationships to surrounding cells frequently became disrupted during preparation for microscopy. And finally, curvature of the ommatidia limited the number of cross sections that could readily be cut. We have nevertheless obtained slides from four different preparations which convince us that receptors for ultraviolet and for green light can be found in the same ommatidium.

An example is shown in Fig. 1A, which was traced from a projection of the original color transparency (*11*). The individual ommatidia are shown cut in cross section, with the images of the retinular cells arranged around the rhabdoms like the petals of flowers. The heavy lines connecting the centers of adjacent ommatidia have been added to the tracing to delineate ommatidial rows. Cellular debris from

damaged ommatidia is indicated by the broken lines, and the plane through which the eye was opened for recording is indicated approximately by the straight dashed line in the upper right quadrant.

Four units in the section (perhaps five) were stained. Two of these, one red (an ultraviolet receptor) and one yellow (a green receptor), lie together at the top of the section near the middle. Consideration of the pattern of rows of ommatidia indicates that despite the cellular damage near the cut surface, the two cells belong to the same ommatidium. The other stained cells (below and to the right) lie in two other ommatidia.

In the original color transparency there is no ambiguity in distinguishing red-stained cells from yellow ones. The difference is described in Fig. 1C, which shows the transmission spectra of the red and yellow patches of film on the color transparency. In the absence of color reproduction, Fig. 1C therefore provides objective evidence that the cells were stained differently.

This result shows that at least some of the ommatidia of cockroach compound eyes contain both ultraviolet and green receptors. The fine structure of cockroach ommatidia has recently been described (*12*), but our information on stained cells is not sufficiently extensive to assign the units sensitive to ultraviolet and green lights to specific morphological cell types. There is reason to believe, however, that ommatidia in the ventral part of the eye have only green receptors (*7*).

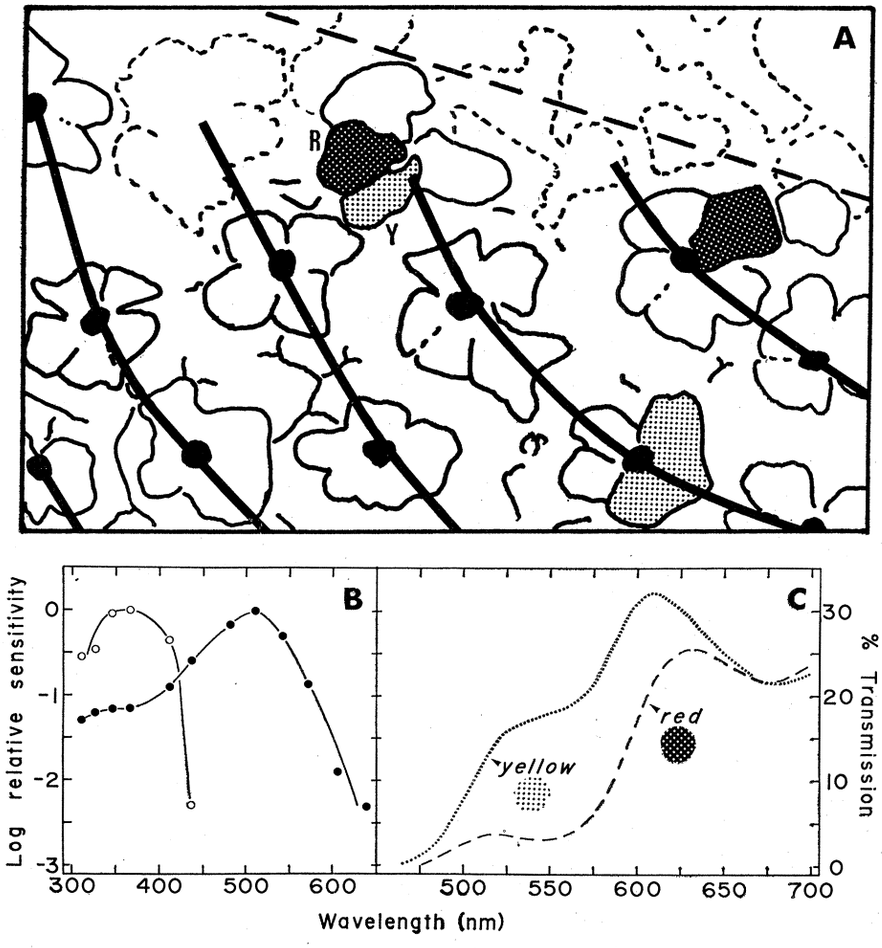

Fig. 1. (A) Tracing of a photomicrograph (original in color) showing several ommatidia cut in cross section. Heavy curved lines have been added to show rows of ommatidia. Several cells have been stained with Procion red or yellow (stippling). Two of these cells lie in the same ommatidium: *R* was an ultraviolet receptor and *Y* a green receptor. The straight dashed line in the upper right marks the exposed surface through which the recording electrodes were brought from above. The electrodes probably entered the cells at a different level from that shown in this cross section, and dye diffused to this region. In the retinula that contains only yellow stain (lower right), the dye was distributed between two cells. This should not be considered evidence for natural coupling between cells, because the recording pipette may have passed through both units. See the text for further details. (B) Spectral sensitivity functions for cells in the cockroach eye which are sensitive to ultraviolet and green light (*8*). (C) Transmission spectra of the photographic images (color transparency) of the adjacent red-stained and yellow-stained cells shown above.

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4. There are two instances in which cells in the same ommatidium have been reported to contain different visual pigments. In the fly *Calliphora*, microspectrophotometric measurements indicate that the two "central cells" in each ommatidium have a 470-nm pigment, whereas the six surrounding cells have a 510-nm pigment [H. Langer and B. Thorell, in *The Functional Organization of the Compound Eye*, C. G. Bernhard, Ed. (Pergamon, New York, 1966), pp. 145–149]. The rhabdoms of Diptera are almost unique in having spatially separated rhabdomeres for each retinular cell instead of a single compact rhabdom. Because of this unusual anatomy, information on other forms seems desirable. More recently, F. G. Gribakin [*Nature* **223**, 639 (1969)] has reported that ommatidia of the honeybee contain four green receptors (530 nm), two blue receptors (430 nm), and two ultraviolet receptors (340 nm). This conclusion is based on differences in rhabdomeric fine structure after adaptation of the living animal to different colored lights.
5. Double staining of retinular and eccentric cells in single ommatidia of *Limulus* has been achieved by M. E. Behrens and V. J. Wulff [*J. Gen. Physiol.* **48**, 1081 (1965); *Vis. Res.* **7**, 191 (1967)] with other experimental questions in mind.
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