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14 December 1983; accepted 29 February 1984

Direct Excitatory Interactions Between Cones of Different Spectral Types in the Turtle Retina

Abstract. *Cone linear sensitivities to red and green stimuli were measured intracellularly in the dark- and light-adapted turtle retina. Test flashes of small diameter were used to minimize horizontal cell feedback. Light adaptation was achieved with either green or red background illumination. The ratio of cone sensitivities to red and green light depended on the color of the background light and differed from the ratio measured in the dark. Electron microscope studies of Golgi-stained turtle cones revealed direct synaptic connections between red and green cones mediated by cone telodendria. These data indicate that the red cone photoresponse is not univariant as has been previously supposed and suggest that mixing of signals from different spectral classes of cones can occur via direct excitatory connections between cones.*

The processing of color information by the vertebrate visual system has yet to be fully understood. A fundamental tenet in this processing is the principle of univariance; the isomerization of photopigment is solely a function of the number of quanta absorbed by the pigment and is independent of the wavelength of the incident illumination (*1*). It has generally been believed, and the evidence to date has supported the notion, that cone photoreceptors manifest univariant photoresponses when stimulated with stimuli of small diameter (*2–5*). This feature of the cone photoresponse was attributed to the univariance of the photochemistry in each class of cones and to the finding that cellular interactions between neighboring cones appeared to be limited to cones containing the same visual pigment (*2, 3, 6, 7*). If double cones are excluded (*8*), nonunivariant photoresponses have been observed in single cones only when they were stimulated by test flashes of large diameter, a stimulus condition that would produce negative feedback from horizontal cells onto cones (*3, 7, 9*). We describe in this report electrophysiological and anatomical evidence which suggests that the photoresponses of the "red" cones in the turtle retina are not univariant even when evoked by stimuli of small diameter.

Recordings were made in the cones of the red-eared turtle, *Pseudemys scripta elegans*, according to standard intracellular techniques (*10*). Cone identification was based upon previously published criteria of receptive field size (*11*) and cone spectral type was established by measuring sensitivities to red and green test flashes (*12*). The results described below show the effects of colored backgrounds on the kinetics and sensitivities of red cone "linear responses" (*13*) to red and green test flashes. Because our results run counter to previous published results, our experimental procedure (*14*) was designed to ensure reliable observations. To minimize the effects of horizontal cell feedback on cone sensitivity and yet fully stimulate the cone's excitatory receptive field, all test flashes were either 180 or 320 μm in diameter (*15*). Test flashes of these diameters, and of the intensities used in this study, evoked horizontal cell responses less than 1 mV in amplitude. The color and intensities of the test and background illumination were controlled with narrow-band interference filters and neutral density filters, each of which was calibrated at the wavelengths of the interference filters. The test flash duration was 50 msec. The colored background light illuminated a circular region approximately 3 mm in diameter.

Univariance of the cone photoresponse implies that (i) the intensities of two different colored test flashes can be adjusted such that they elicit linear range photoresponses that are indistinguishable, and (ii) the ratio of sensitivities measured with these two different colored stimuli is independent of the state of adaptation (*1, 3, 4*). Figure 1A shows dark-adapted photoresponses when the intensity of the green flash was adjusted to obtain a response as similar as possible to that evoked by the dim red flash. Although the peak amplitudes of the two responses can be perfectly matched, the kinetics of the responses differ. The differences in kinetics, although small, were observed in all ten red cones studied. These differences are inconsistent with the principle of univariant photoresponses.

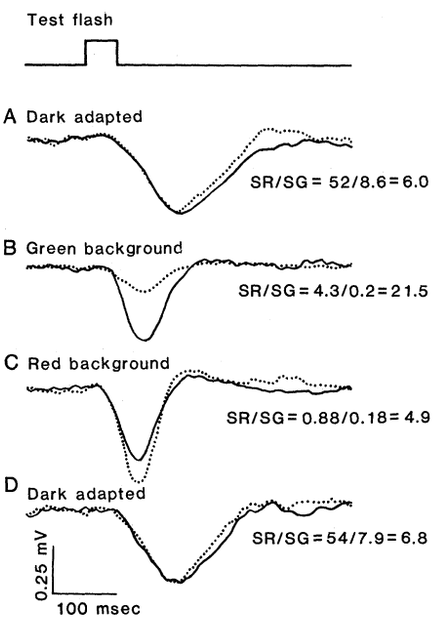

Fig. 1. Intracellular linear range responses measured in a red cone to red (694 nm, solid lines) and green (546 nm, dotted lines) test flashes. The numbers to the right of each set of responses are the red and green flash sensitivites (in microvolts of peak response per quanta per square micrometer on the retina) and the ratio of these cone sensitivities. The uppermost trace describes the timing and duration of the test flashes. From 30 to 40 responses were averaged in each of the displayed responses. (A) Responses recorded in the dark-adapted cone. The intensity of the green flash was adjusted to provide a response equal in amplitude to that evoked by the red flash. (B) Responses recorded in the same cone that had been light adapted with a green (500 nm) background light of an intensity of 1.4×10^{-6} quanta sec^{-1} μm^{-2}. The intensities of the red and green flashes have been increased by a factor of 12.1 over the values used in the dark-adapted retina. (C) Responses recorded in the same cone that had been light-adapted with a red (700 nm) background light of an intensity of 8.11×10^{4} quanta sec^{-1} μm^{-2}. The test flash intensities have been increased by a factor of 59 over the values used in the dark-adapted retina. (D) Control responses recorded in the dark-adapted retina after the periods of light adaptation.

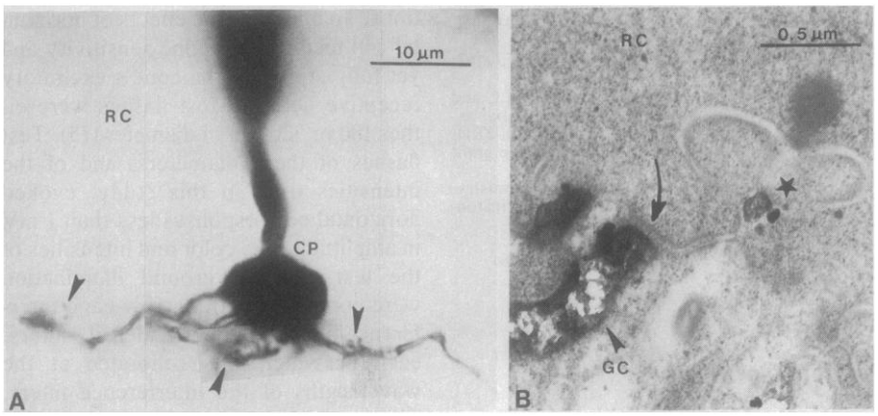

Fig. 2. (A) Light micrograph of a vertical view of a Golgi-stained red single cone (*RC*) in the turtle retina. The cell body (out of focus above) has a constricted short axon that expands into a large cone pedical (*CP*). Numerous radiating telodendria arise from the cone pedicle base and run 10 to 15 μm, ending in clusters of terminals (arrowheads) that project to surrounding cone pedicles. The figure is a montage of several photographs of the same cell at different depths of focus to show several telodendria. (B) Electron micrograph showing contacts made by a Golgi-stained telodendron (arrowhead) from a green member of a double cone (*GC*) with a red single cone pedicle (*RC*). The telodendritic contact forms a basal junction with the red cone base (arrow). Another telodendritic ending is partially cut in section and enters the ribbon synaptic complex to form a central element (star).

Figure 1B shows linear responses from the same red cone under conditions of light adaptation with a green (500 nm) background. Both the red and green test flash intensities were increased by an identical factor of 12.1 over the values used in the dark-adapted retina. The response to the red flash was much larger than that evoked by the green flash, in contrast to the predictions from univariance. The ratio of sensitivities indicates that the cone was 3.5 times as sensitive to the red relative to the green test flashes than it was when dark adapted.

When a red background (700 n*M*) was used, both the red and green test flash intensities were increased by a factor of 59 over their dark-adapted values (Fig. 1C). As for the green background, the linear response amplitudes differed; now, however, the response to the red flash was smaller than that to the green flash. The ratio of sensitivities indicated that the sensitivity to red with respect to green was 0.80, the value obtained under dark-adapted conditions. Figure 1D shows control responses measured after the periods of light adaptation. The nonunivariant behavior shown in Fig. 1 was observed in all ten red cones studied even though the absolute sensitivities of these cones varied from cell to cell (*2*), as did the ratio of red to green sensitivity in each cone (*2*). For these ten cones, the mean dark-adapted red and green flash sensitivities were 36 (standard deviation, 26) and 4.8 (3.2), respectively; the dark-adapted, green-adapted, and red-adapted sensitivity ratios of red flash to green flash were 7.9 (4.7), 19.3 (9), and 5.7 (4.4), respectively.

The results suggest that mixing of signals between cones of different spectral class can occur at the photoreceptor level. Whether the interactions leading to this color mixing are excitatory or inhibitory can be deduced from the differential effects of colored backgrounds on the red cone sensitivity to red and green test flashes. Green background illumination preferentially desensitizes the green cones and reduces the green cone contribution to the red cone response. Since this background increases the ratio of red to green flash sensitivities over the value measured in the dark-adapted state, we conclude that the interaction must be excitatory. This conclusion is consistent with the effect of red background illumination, which produces greater desensitization of red than green cones. In this condition, the relative contribution of the excitatory input of green cones onto red cones is augmented. Involvement of horizontal cells in this color mixing can be excluded since (i) horizontal cells exert only inhibitory influences onto cones (*3, 4, 7, 9*) and (ii) all sensitivity measurements were done with small (180 to 320 μm in diameter) test flashes to minimize the contributions of horizontal cells to cone photoresponses.

Our anatomical studies in the turtle retina have revealed a possible pathway for the mixing of cone signals (*16*). Light microscopic observations have shown that all Golgi-stained (*17*) red and green single cones in the turtle retina have prominent telodendria radiating from the bases of their pedicles for distances of 10 to 15 μm (Fig. 2A). These telodendria appear to converge on neighboring cone pedicles, where they end as small clusters of terminals (arrowheads in Fig. 2A). Electron microscopy shows that stained telodendria end either as central elements of ribbon synapses at distal junctions (*18*) (starred processes in Fig. 2B) or as basal junctions with the cone pedicle surface (arrow in Fig. 2B).

To determine the color specificity of these telodendritic connections, ultrathin serial sections were cut through the vertical extent of Golgi-stained cones. Identification of the spectral types of both origin and target cells was based on the shape and density of their oil droplets (*19*). Red and green single cones are connected indiscriminately by telodendritic contacts to red and green single cones (Fig. 2B) and to red and green members of the double cone.

Telodendritic contacts have been described between unidentified cones in the turtle retina (*18*) and between red and green cones in the Rudd retina (*20*). These processes ended as the central elements of ribbon synapses. This type of contact appears to be more involved with the horizontal cell dendrite at the triad synapse in the target cone than with the target cone itself. The basal junction type of telodendritic contact described in this report is less numerous than the central element type and has a strikingly morphological similarity to the cone to bipolar synapse (*18*) in the vertebrate retina. We thus suggest that this basal junction may be the synaptic site at which color signals of one photoreceptor type are transmitted directly to an adjacent photoreceptor of different spectral type.

Signals could also be conducted between different spectral types of cones via gap junctions, which have been demonstrated in the retinas of a variety of animals (*18, 21*). These gap junctions are not color-specific in the turtle visual streak, and they join all spectral types of cones and rods (*22*). However, our physiological measurements were made in the peripheral retina, where the spacing between neighboring cones is large. Our preliminary anatomical observations (*22*) indicate that this spacing is so large that peripheral cones probably do not communicate with each other via this pathway. Thus, gap junctions do not seem to play a role in the color mixing between cones.

Our conclusion of excitatory interac-

tions between cones of different spectral types contradicts previous reports (*2–4, 6*). These studies were mainly based on matching linear responses evoked by colored stimuli in the dark-adapted state (*2–4*). Only in one case (figure 4 in *4*), was the effect of a red background on linear responses to red and green stimuli examined. We studied ten red cones in detail using colored backgrounds of different intensities and observed that the effect of the background varied from cell to cell and depended on background intensity. These variations may explain the discrepancy between our and previous reports. The apparent lack of coupling between red and green cones described by others (*6*) suggests that the interaction may be too weak to be measured with simultaneous recordings from two single cones; it can be measured when the summated contributions of many cones impinge upon the test cone, however.

The direct excitatory input between cone photoreceptors of differing spectral types described here adds an additional degree of complexity to information processing at the first synaptic level of the turtle retina. This new pathway must be considered in the retinal mechanisms subserving color vision.

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12. Because excitatory interaction have been demonstrated between red and green members of double cones (*8*), we ensured that impaled "red" cones were not the red members of double cones by measuring their red and green flash sensitivities. The ratio of these sensitivities is consistent with the values measured by others (*2*) for red single cones. Occasionally red members of double cones were impaled; their ratio of red to green sensitivity was approximately 1/8 that of single red cones.
13. Dim test flashes delivered to either the dark- or light-adapted retina evoke photoresponses that are linearly related to the quantal content of the test flash; doubling the quanta in a test flash will double the entire response [(*2, 4*); R. A. Normann and P. J. Anderton, *Vision Res.* **23**, 1731 (1983)]. Accordingly, we tested for response linearity at each background by subtracting eight responses to a dim test flash intensity from four summed responses of twice this intensity. If this operation resulted in no net response (indistinguishable from the baseline noise), all 12 responses were in the cones' linear range.
14. Under each state of adaptation, the set of green test flashes was interleaved between two sets of red test flashes, and the data were rejected if the amplitudes of the two sets of averaged red flash responses differed by more than 10 percent. Further, dark-adapted red and green sensitivities were measured before and after the periods of light adaptation to verify that the cell's sensitivity had not deteriorated over the course of the experiment. This procedure was successfully followed in experiments conducted on ten red cones.
15. Linear sensitivity measurements can be meaningfully applied to a cone only if its excitatory receptive field is fully illuminated by the test spot. For most cones studied, 180 μm (diameter) test spots were large enough to fully illuminate the receptive field of the cone. In some cones, however, enlarging the spot to 320 μm produced a slight increase in the linear response amplitude. In these cones, 320 μm test spots were used.
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23. Supported by National Eye Institute grants EY 03748 (to R.N.) and EY 03323 (to H.K.).

15 September 1983; accepted 6 April 1984

Long-Term Potentiation of Hippocampal Synaptic Transmission Affects Rate of Behavioral Learning

Abstract. *Electrical stimulation techniques were used to produce a long-lasting potentiation of synaptic transmission in the hippocampus of naïve rabbits. Animals were then classically conditioned. Long-term potentiation of the hippocampus before training increased the rate at which animals subsequently learned the conditioning task. This result has significance for potential cellular mechanisms of associative learning.*

Identifying cellular mechanisms that are the basis for neural plasticity and its mediation of behavioral learning is a major goal of research in the neurosciences (*1*). Experimental models range from (i) awake, learning animals in which changes in cellular function can be compared directly with changes in behavior to (ii) semi-intact or isolated preparations of the nervous system in which changes in cellular function represent neural analogs of the learning process. Classical conditioning of the rabbit nictitating membrane (NM) response has recently become widely used to study mammalian learning (*2, 3*). In a series of studies, my colleagues and I have found that substantial changes in the activity of hippocampal pyramidal neurons occur during classical conditioning of the NM response and do not occur under other, nonlearning conditions (*4, 5*). This neural plasticity is long-lasting (at least days), robust, and functionally related to the animal's learned behavior (*6*). Foremost among the isolated central nervous system (CNS) preparations is the mammalian hippocampal explant or slice (*7*), which has been used to study long-term potentiation (LTP) (*8*). Such LTP is characterized by a long-lasting (hours to days) increase in synaptic efficacy produced by high-frequency stimulation of afferents to either the hippocampal granule or pyramidal cell populations. The latter effect is also robust and long-lasting and is currently being studied as a model of neural plasticity in the mammalian brain (*9*).

Much debate has centered on whether LTP or an LTP-like phenomenon is the basis for cellular plasticity observed in the intact, behaving animal (*10*). Resolution of this issue would have important implications because several mechanisms already identified as underlying LTP (*11–15*) may be generalizable to associative learning in the intact animal. In previous studies we have noted similarities between the characteristics of hippocampal pyramidal cell plasticity during NM conditioning and the cellular plasticity characteristic of LTP. For example, LTP and the changes in pyramidal cell activity that occur during NM conditioning develop with similar time