Ionic Mechanisms of Two Types of On-Center Bipolar Cells in the Carp Retina

II. The Responses to Annular Illumination

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ABSTRACT On-center bipolar cells in the dark-adapted carp retina were divided into four types (A, B, C, and D) on the basis of response wave forms, spectral response properties, and electrical membrane properties. Type A and B cells responded to a spot of light with a transient depolarization followed by a plateau, whereas the response of type C and D cells were approximately rectangular in shape. The center and surround responses of type A cells had maximum spectral response of ~525 nm in the lower mesopic range; the polarity of both responses was reversed at positive membrane potentials as the membrane was depolarized by extrinsic current. The center and surround responses of type D cells had a maximum spectral response of ~625 nm in the mesopic or photopic range; the polarity of both responses was reversed at membrane potentials that were more negative than those at the dark level. The results suggest that the center and surround responses mediated by rods are generated by changes in sodium conductance, but in opposite ways; whereas those mediated by red cones are generated by changes in potassium and/or chloride conductances. In type B and C cells, which probably receive inputs from both rods and/or green cones as well as red cones, the center responses were composed of the two ionic mechanisms described above. The surround responses of many type B and C cells were dominated by only one ionic mechanism with a negative reversal potential, but in some type B cells the surround responses were resulted from two ionic mechanisms similar to those of the center responses.

INTRODUCTION

On-center bipolar cells in the *Cyprinid* fish retina respond with depolarization to central illumination, but with hyperpolarization to annular illumination (Kaneko, 1970). In a previous paper (Saito et al., 1979), we attempted to analyze the input from photoreceptors to the receptive field center of on-center bipolar cells in the dark-adapted carp retina, and to determine the ionic mechanisms underlying the generation of their center responses. The

earlier results suggested that rod and red cone synaptic inputs into the bipolar cells may be different in their ionic mechanisms: the rod-related response is generated by an increase in sodium conductance and the red cone-related response is generated by a decrease in potassium and/or chloride conductances.

Electrical membrane properties of on-center bipolar responses to annular illumination have not been studied systematically. Furthermore, membrane resistance measurements have yielded inconsistent results in which membrane resistance of the surround responses increases (carp: Toyoda [1973]; necturus: Nelson [1973]), decreases (Toyoda, 1973), or remains unchanged (Toyoda, 1973; Nelson, 1973). Reversal potentials of the surround response have not been obtained, although some estimated values have been proposed (Toyoda, 1973; Werblin, 1977).

In our recent work on the carp retina (Saito and Kondo, 1978; Toyoda and Tonosaki, 1978 a and 1978 b), we proposed that more than one ionic component was involved in the surround response of certain types of on-center bipolar cells.

This paper provides a more detailed account of the different ionic mechanisms that underlie the various on-center bipolar responses to annular illumination. We also examine the ionic mechanisms of center responses and summarize the results obtained with the center response together with results found with the surround response. Finally, we compare and interpret our present and previous data in terms of the possible mechanisms of the center and surround organization of the bipolar cell receptive field.

METHODS

Experiments were performed on the isolated retina of the carp (*Cyprinus carpio*). The isolated retina was placed receptor-side up in a moist chamber. An Ag-AgCl wire as an indifferent electrode was fixed in the chamber below the retina. Double-barreled microelectrodes (60–150 MΩ resistance) filled with a 2.5-M KCl solution were used for intracellular recording and for the injection of current. The electrode placed at the center of the light spot (400 μm in diameter) was advanced vertically into the retina from the receptor side, while an ~300-ms light flash was presented to the vitreous side every 3 s. An intensity of light of −4.0 log units, which roughly corresponds to 0.3 lm/m^2, was usually used during penetration because it activates both rod and cone systems without a significant change in the state of dark adaptation. The surround response was usually recorded by a concentric annulus with an internal diameter of 0.6 mm and an external diameter of 2.0 mm. The photostimulator used in this study contained three independent channels of light stimulation, a test channel and two background channels, which were provided by separate quartz-iodine lamps. The test channel was used to project the light beam of spots and annuli whose diameter, intensity, and spectral composition could be changed. One background channel was used for selectively adapting the retina with a diffuse white light of 500 or 650 nm. The other background channel was combined with the test channel by a prism and was used for adapting the receptive field center of the bipolar cells with a steady spot of white light to minimize the effect of scattered light from the annulus to the center. Our previous work (Saito et al., 1979) describes our stimulating and recording procedures in more detail.

RESULTS

The responses of on-center bipolar cells to central illumination have been divided into four types on the basis of the response wave forms, spectral response properties, and electrical membrane properties (Saito et al., 1979). The effect of polarizing currents on each of the four types of cells is summarized in Fig. 1. The control responses obtained at a light intensity of −4.0 log units
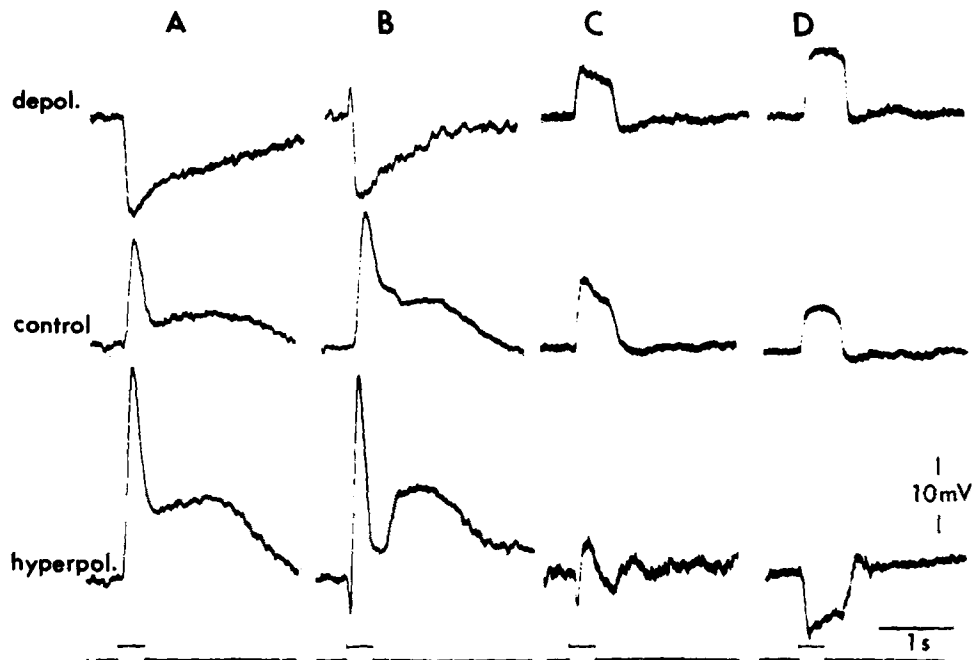

FIGURE 1. Summary of the electrical membrane properties of on-center bipolar cells to central illumination. On-center bipolar cells in the dark-adapted carp retina have been divided into four types on the basis of their response wave forms, spectral response properties, and electrical membrane properties (Saito et al., 1979). The *middle row* shows the four types of responses in the absence of polarizing current. The *upper* and *lower rows* show the effects of depolarizing and hyperpolarizing currents on the response of each type. A type A cell response reversed the polarity at a more positive potential than the zero membrane potential. A type D cell response reversed the polarity at more negative potential than the dark level. Type B and C cells were composed of two voltage components, one showing a positive reversal potential and the other showing a negative reversal potential.

are shown in the *middle row*. The cell shown in record *A* responded to the light with a transient depolarization, followed by a plateau. The wave form of the response is similar to that of the rod response in the retina of cold-blooded animals (Lasansky and Marchiafava, 1974; Norman and Werblin, 1974; Fain, 1976) and is also similar to that of rod-mediated bipolar cells of the dogfish

retina (Ashmore and Falk, 1980). This cell showed a maximum spectral response at ~525 nm under scotopic and mesopic conditions. Thus, it appears that the cell receives a major input from rods, although a minor contribution from green cones cannot be excluded (Stell et al., 1977; Ishida et al., 1980). As the membrane was electrically hyperpolarized, the response increased in amplitude (*bottom trace*), but reversed its polarity as it was depolarized (*top trace*). The reversal potential was about +30 mV in this case. The cell shown in record *D* had a maximum spectral response at ~625 nm, suggesting a major input from red cones. The response wave form was approximately rectangular and similar to that of cones (Tomita, 1965; Burkhardt, 1977). Depolarizing the membrane increased the amplitude of the response (*top trace*), whereas hyperpolarizing the membrane eventually reversed the response polarity (*bottom trace*). The reversal potential was about −54 mV in this case. The wave form of the response of the cell shown in record *B* resembles that of type A cells. It had a maximum spectral response at ~575 nm. The response of this cell became biphasic as the membrane was polarized by extrinsic current; the initial transient hyperpolarization was followed by an enhanced depolarization during hyperpolarization of the membrane (*bottom trace*), but the initial transient depolarization was followed by hyperpolarization during membrane depolarization (*top trace*). The result suggests that the response consists of two ionic components with different time-courses and reversal potentials. The cell shown in record *C* resembles the type D cells in response wave form. It had a maximum spectral response of ~575 nm. The response of this cell decreased in amplitude during either membrane hyperpolarization (*bottom trace*) or depolarization (*top trace*). Recently, we found that the response amplitude of some type C cells was slightly increased during membrane hyperpolarization, although the increment of the responses was much smaller than that of type A and B cell responses (Saito and Kujiraoka, 1982 [in press]). Such complex electrical membrane properties of type C cells might be the result of appropriate interactions between two ionic components with different reversal potentials.

Fig. 2 shows a separation of the two ionic components underlying a type B cell response by using different wavelengths of light. Monochromatic lights of equal quantal flux were successively applied to the retina from 475 to 675 nm in 50-nm steps. Records *a* and *b* show a spectral response pattern in the absence and presence of hyperpolarizing current. Each spectral response was affected differently by the polarizing current. A comparison between the responses at 475 and 675 nm reveals that the amplitude of 475 nm increased with membrane hyperpolarization, whereas the polarity of the response to 675 nm reversed. The response to other wavelengths of light was composed of the two voltage components in which the hyperpolarizing component became prominent at longer wavelengths of light. Records *c* and *d* show the effects of red and green background lights on the spectral response pattern under membrane hyperpolarization. In record *c*, the hyperpolarizing component was completely suppressed in the presence of red background illumination (650 nm). In record *d*, the depolarizing component was suppressed in the presence of green (500 nm) background light.
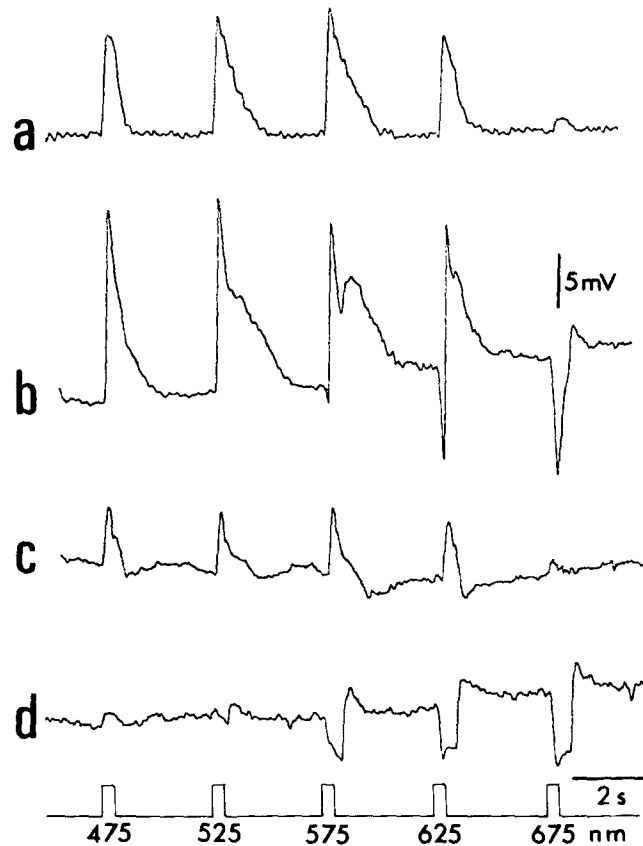
FIGURE 2. Effect of the membrane hyperpolarization on spectral responses of a type B cell in the presence and absence of the chromatic adaptation. (a) Spectral response pattern as the control. (b) Spectral response pattern during the membrane hyperpolarization. Each spectral response was affected differently by the membrane polarization. The response to 475 nm increased in its amplitude, whereas the response to 675 nm reversed its polarity during the membrane hyperpolarization. The responses to other wavelengths were composed of the two voltage components of which the hyperpolarizing component is prominent at longer wavelengths. (c) Spectral response pattern during the membrane hyperpolarization in the presence of 650 nm background light. The hyperpolarizing component was suppressed by the red background light. (d) Spectral response pattern during the membrane hyperpolarization in the presence of 500 nm background light. The depolarizing component was suppressed by the green background light.

The ratio between the hyperpolarizing and depolarizing components during membrane polarization varied from cell to cell within and between preparations. If type A and D cells do indeed receive inputs mainly from rods and red cones, the variety of electrical membrane properties of type B cells may occur through a particular combinations of rod and red cone inputs that differ in their ionic mechanisms. A separation of the two ionic components underlying

the type C cell response has not yet been successful because the cell is not sufficiently stable for a long-term intracellular recording.

Procion yellow or Lucifer yellow dye was injected iontophoretically into 21 type A, 16 type B, 15 type C, and 12 type D cells. Morphological properties of all type A and B cells were like Cajal's large (rod) bipolar cells, which have been characterized by a large cell body and a large, single swelling of their axon terminal (Cajal, 1892). On the other hand, type C and D cells were quite different in morphology from the large bipolar cells. They were characterized by a small cell body and a wide ramification of their axon terminal. The cells with these morphological properties are represented in Cajal's drawings (see plate I, Fig. 1 e of Cajal [1892]) as the small (cone) bipolar cells. In spite of different electrical membrane properties, there were no consistent morphological differences between type A and B cells, or between type C and D cells. The results of an intracellular staining investigation of type C and D cells will appear elsewhere (Saito and Kujiraoka, 1982 [in press]).

In our previous paper (Saito et al., 1979), type A and B cells were referred to as rod-dominant bipolar cells and type C and D cells as cone-dominant bipolar cells.

Because the depolarizing responses to central illumination are contributed by two ionic mechanisms mediated by different synaptic inputs, it is to be expected that the same ionic mechanisms are responsible for generating the hyperpolarizing response to annular illumination. To test this assumption, we measured the reversal potentials of the center and surround responses from the four types of cells described above and compared them.

Fig. 3 shows the effect of polarizing currents on both center and surround responses of a type A cell. The center and surround responses were obtained by alternately applying a spot (0.4 mm in diameter) and annulus (inside diameter, 0.6 mm; outside diameter, 2 mm) at a light intensity of −4.0 log units. The cell had a resting potential of about −20 mV (control) in the dark. The maximum spectral response of the annular surround was ~525 nm. The center response increased in amplitude during membrane hyperpolarization, and polarity was reversed during membrane depolarization. At the membrane potential of +18 mV, the center response became biphasic; there was an initial transient depolarization and a subsequent hyperpolarization. The initial transient depolarization does not correspond with that of the type B cell (see Figs. 1 B and 6), because it is not inverted by membrane hyperpolarization. This suggests that the transient depolarization might reflect an inhomogeneous polarization of the subsynaptic membrane due to an unequal distribution of extrinsic current within the dendritic field.

The control surround response showed a depolarizing hump on its descending phase. When the membrane was hyperpolarized from −20 to −42 mV, the hyperpolarizing response and the depolarizing hump were augmented. Further membrane hyperpolarization, however, resulted in a decrease of the hyperpolarizing response and an increase of the depolarizing hump (not shown). When the membrane was depolarized to +18 mV, the surround response decreased in amplitude. But a further increase in the membrane

depolarization to +27 mV resulted in an unexpected increase rather than the reversal of the surround response. Similar results were obtained in five other type A cells. The depolarizing hump generally became less apparent in dim light, but became prominent in bright light. It is therefore likely that the
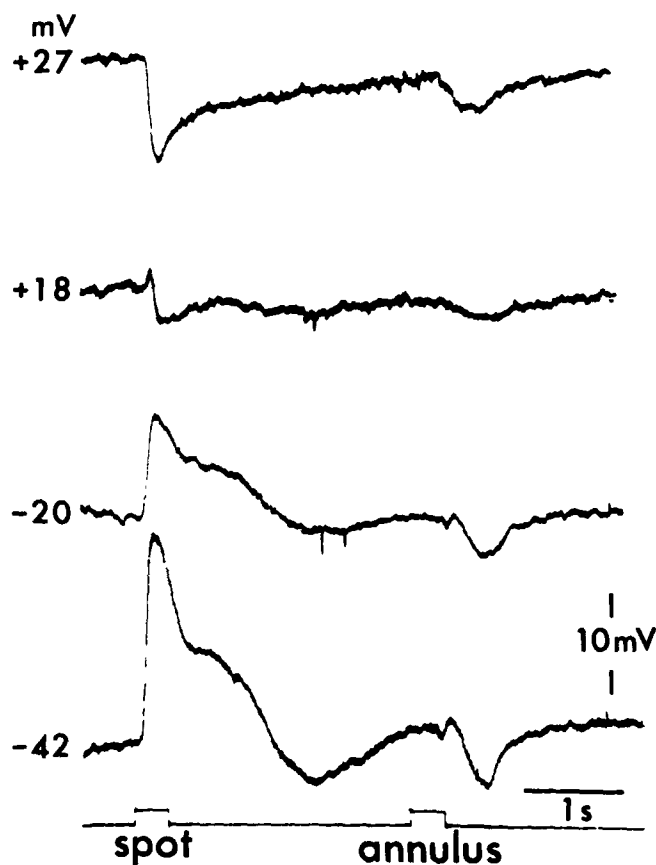

FIGURE 3. Effect of the membrane polarization on the center and surround response of a type A cell. Center and surround responses were obtained by, respectively, light spot of ~0.4 mm in diameter, and an annulus with an inside diameter of 0.6 mm and an outside diameter of 2 mm. The resting potential was about −20 mV in the dark. The center response reversed its polarity at around +12 mV, whereas the surround response did not show any reversal potential. See the text for possible reasons for the failure to obtain reversal potential of the surround response.

hump is an artifact caused by the center response to light scattered from the annulus to the center of the receptive field.

Taking into account the light scattering and the inhomogeneous polarization of the membrane, we suspected that the difficulty in demonstrating the

reversal potential of the surround response could be the result, at least in part, of contamination by the center response. The electrical properties of the surround response were therefore studied in the presence of steady, adapting illumination of the receptive field center to minimize the effect of light scattering. Fig. 4 shows the effect of hyperpolarizing current on the surround response in the presence and absence of the adapting light on the receptive field center. The surround response in the absence of the adapting light was characterized by a depolarizing hump on its recovery phase. The response decreased in amplitude as the membrane was hyperpolarized. Application of the adapting light (indicated by a step of the *bottom trace* in the figure) caused a depolarization of the membrane and a suppression of the hump. The hyperpolarizing surround response in this condition increased in amplitude
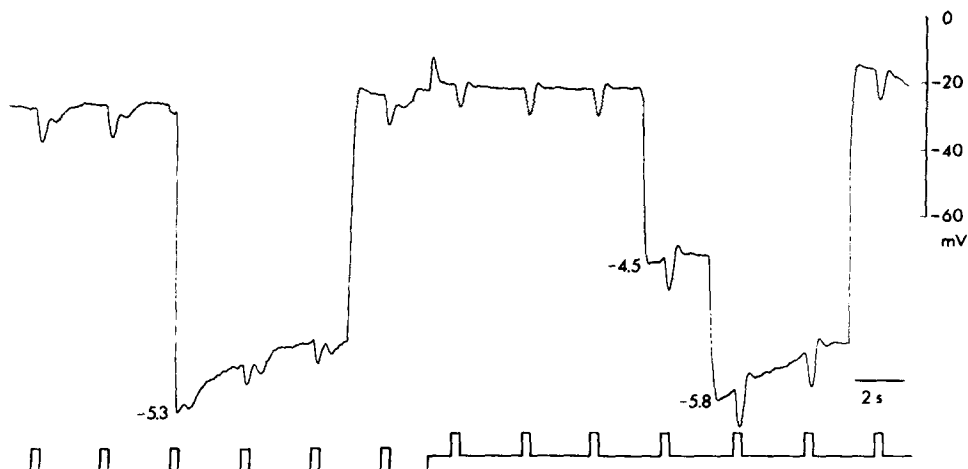

FIGURE 4. Effect of the hyperpolarizing current on the surround response of a type A cell in the presence and absence of a steady adapting light of the receptive field center. The response in the absence of the adapting light was characterized by a depolarizing hump on its recovery phase. This hump became less prominent in the presence of the adapting light (indicated by a step of the stimulus trace). Numbers at the beginning of each polarization indicate the strength of current in nano amperes.

during membrane hyperpolarization. Reversal potential measurement of the type A cell during light adaption of the center is shown in Fig. 5 A. The response was recorded at a light intensity of −4.0 log units. The amplitude of the response increased as the membrane was hyperpolarized from −25 (control) to −75 mV, whereas the polarity of the response was reversed somewhere between +10 and +26 mV as the membrane was depolarized to +46 mV. In seven cells studied under the same light condition, five cells had the reversal potential at a positive potential level. In two cells, however, the response significantly decreased in amplitude during membrane depolarization, but was not reversed. The mean value of reversal potentials measured for five cells was +43 ± 15 mV (SD).

Fig. 6 shows the effect of polarizing current on both center and surround responses of a type B cell. This cell had a resting potential of −30 mV (control) in the dark. The maximum spectral response of the surround was ~575 nm. Characteristics of the center response under polarization of the membrane are similar to those shown in Fig. 1 *B*. The surround response increased in amplitude as the membrane was depolarized and its polarity was reversed as it was hyperpolarized. Similar results were obtained in 12 other type B cells. It is still possible that the inverted surround response during membrane hyperpolarization is due to the contamination by the rod-mediated center response, which increases in amplitude with membrane hyperpolarization. We could minimize contamination by using annuli of red light, because rods
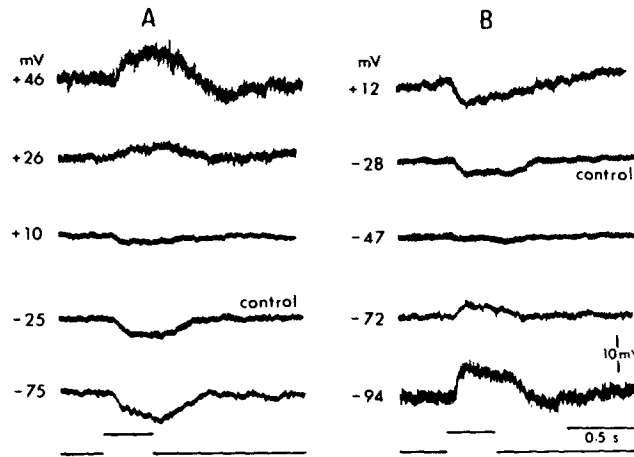

FIGURE 5. Reversal potential measurements for the surround response in the presence of a steady adapting light of the receptive field center. (*A*) The response of a type A cell to light intensity of −4.0 log units. The membrane potential was about −25 mV (control) in the presence of the adapting light of the center. The response reversed its polarity at around +16 mV. (*B*) The response of a type B cell to a light intensity of −3.0 log. The membrane potential was about −28 mV (control) in the presence of the adapting light. The response reversed its polarity at around −47 mV.

are less sensitive to the red region of the spectrum. Fig. 7 shows a typical example of the reversal potential measurement of the type B cell, which is stimulated by 675-nm light flashes. In such a stimulus condition, both center and surround responses reversed their polarity during hyperpolarization of the membrane (record *b*). It is therefore likely that the center and surround responses mediated by red cones have reversal potentials more negative than their resting potentials in the dark. Further support for this claim comes from Fig. 5 *B*, which shows the reversal potential measurement for the surround response of a type B cell in the presence of the adapting light of the center. The response was obtained by annuli of white light at an intensity of −3.0 log units. Depolarizing the membrane from −28 (control) to +12 mV caused an
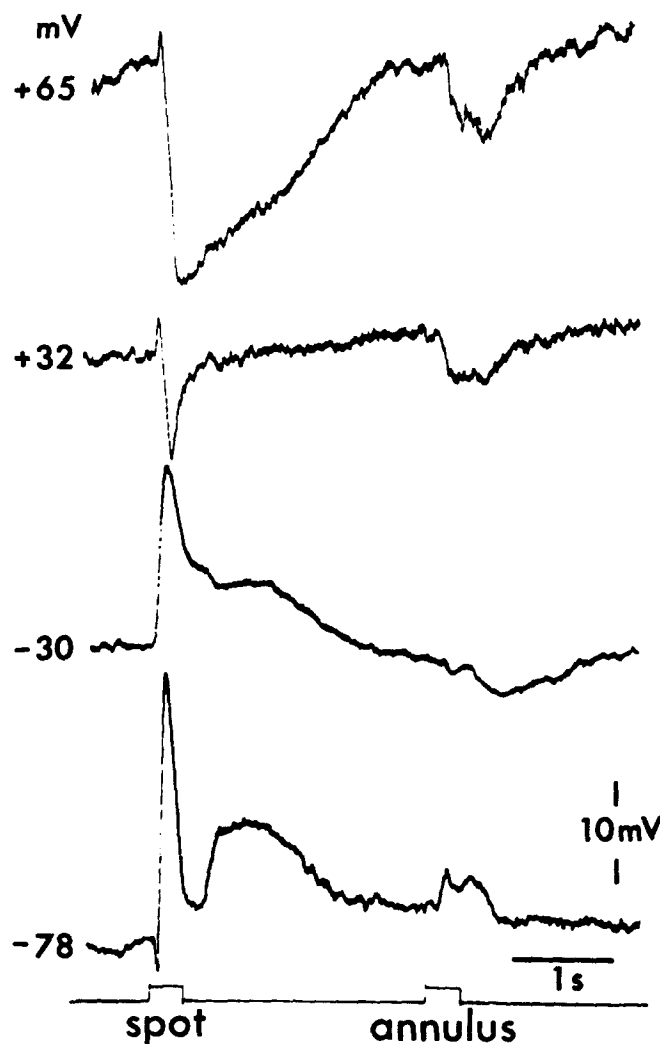
FIGURE 6.   Effect of the membrane polarization on the center and surround responses of a type B cell. The resting potential was about −30 mV in the dark. The maximum spectral response of the surround was ~575 nm. The center response showed complex wave form during the membrane polarization, suggesting that it was composed of two voltage components different in their time-course and in their reversal potential. The surround response reversed its polarity at about −50 mV. Stimulus conditions were same as those in Fig. 3.

increase in amplitude of the response, whereas hyperpolarizing the membrane to −94 mV caused a reversal of the response at about −47 mV.

We measured the reversal potential of 15 type B cells in which the receptive field center was light-adapted. The surround response from 10 of these cells was dominated by a voltage component with a negative reversal potential. A

mean reversal value was −59 ± 11 mV (SD). The surround response from the other five cells was composed of two voltage components with negative and positive reversal potentials. An example of this group is shown in Fig. 8. The response was obtained at a light intensity of −4.0 log units under the adapting light of the center and showed a peak amplitude of ∼575 nm. Hyperpolarizing the membrane by a weak current of −1.8 nA resulted in a small initial transient depolarization, followed by an enhanced hyperpolarization. A stronger hyperpolarizing current of −4.3 nA enhanced the amplitude of both depolarizing and hyperpolarizing components. The effect of diffuse illumination with 500-nm light (indicated by a step of the *bottom trace* in the figure) caused a suppression of only the hyperpolarizing component, so that the
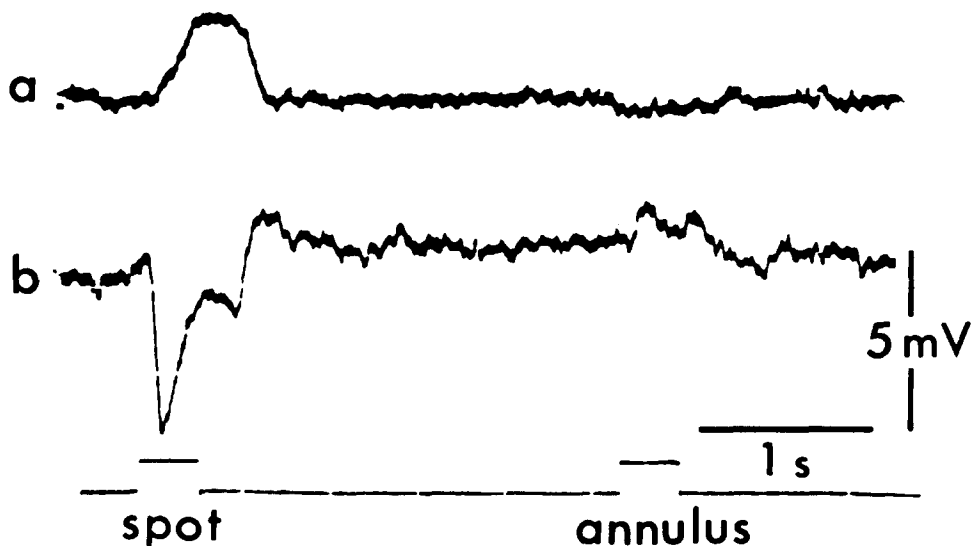

FIGURE 7. Effect of the membrane hyperpolarization on the center and surround responses of a type B cell to a 675-nm light flash. The response was recorded from the same cell studied in Fig. 6. (a) The response as a control. (b) The response during the membrane hyperpolarization. Both center and surround responses reversed their polarity during the membrane hyperpolarization.

depolarizing component dominated the response. The results suggest that the surround response described above might be mediated by the activities of two different types of photoreceptors, such as red cones for the voltage component with a negative reversal potential, and rods or green cones for the component with a positive reversal.

Fig. 9 shows the effect of polarizing current on both center and surround responses of a type C cell. The cell had a resting potential of about −24 mV in the dark. The center and surround responses had a spectral response maximum of ∼625 nm. The characteristics of the center response during polarization of the membrane are similar to those in Fig. 1 C. As the membrane was depolarized, the surround response increased in amplitude, whereas its

polarity was reversed as it was hyperpolarized. Similar results were obtained with two other cells.

Fig. 10 shows a record from a type D cell. This cell had a resting potential of −40 mV in the dark. Both center and surround responses increased in amplitude with membrane depolarization, whereas their polarity was reversed with membrane hyperpolarization. Fig. 11 shows the reversal potential measurement for the surround response of a type D cell during light adaption of the center. As the membrane was displaced from −12 (control) to −82 mV, the response decreased in amplitude and eventually reversed its polarity somewhere between −48 and −70 mV. The maximum spectral response of
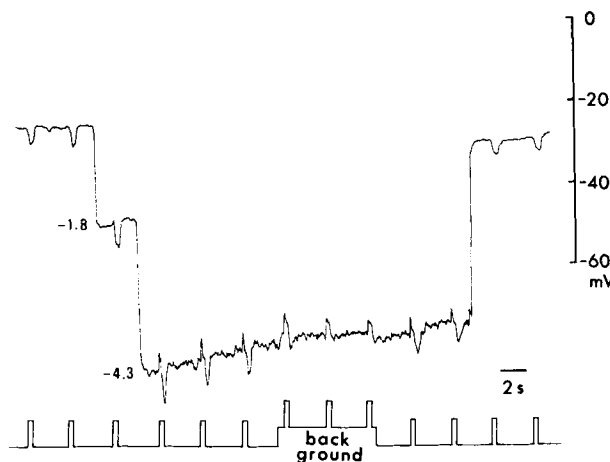

FIGURE 8.   Effect of the hyperpolarizing current on the surround response of a type B cell in the presence of a steady adapting light of the receptive field center. With a strong hyperpolarization the response became biphasic; there was an initial transient depolarization followed by a hyperpolarization. The hyperpolarizing component was suppressed by an application of a diffuse background light of 500 nm (indicated by a step of the *bottom trace*), suggesting that it was mediated by rods or green cones. It is therefore suggested that the depolarizing component is mediated by cones. Numbers at the beginning of each polarization indicate the strength of current in nano amperes.

the surround response of the type D cell was ~575–625 nm. No spectral response measurements of the type D cell were made in combination with the electrical properties because the cells were not sufficiently stable for long-term recording.

DISCUSSION

Response to Central Illumination

The present and previous results (Saito and Kondo, 1978; Saito et al., 1978 and 1979) suggested that there are at least two ionic mechanisms responsible for generating the depolarizing response of on-center bipolar cells to central
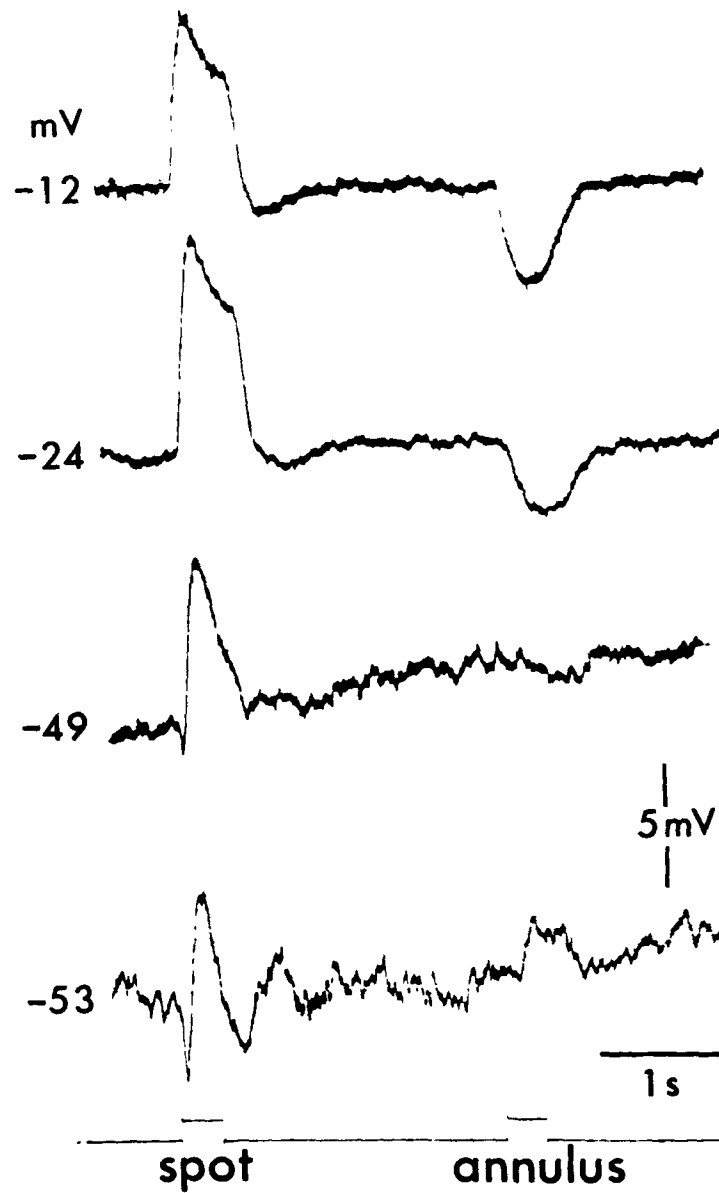
FIGURE 9. Effect of the membrane polarization on the center and surround responses of a type C cell. The resting potential in the dark was −24 mV. The maximum spectral response of the surround was ∼575 nm. The center response was composed of two voltage components with different reversal potential. The surround response reversed its polarity at about −49 mV. Stimulus conditions were same as those in Fig. 3.

illumination in the dark-adapted carp retina. The response of type A cell, which was relatively sensitive to green light in the lower mesopic condition, reversed its polarity at membrane potentials >0 mV, whereas the response of type D cell, which was relatively sensitive to red light in both mesopic and photopic conditions, reversed polarity at membrane potentials more negative than the resting potential in the dark. A large number of bipolar cell responses in the mesopic condition consisted of these two ionic components, although the ratio between them varied considerably from cell to cell. Morphological properties of type A and B cells were like Cajal's large bipolar cells and those of type C and D cells were like Cajal's small bipolar cells (Saito and Kujiraoka,
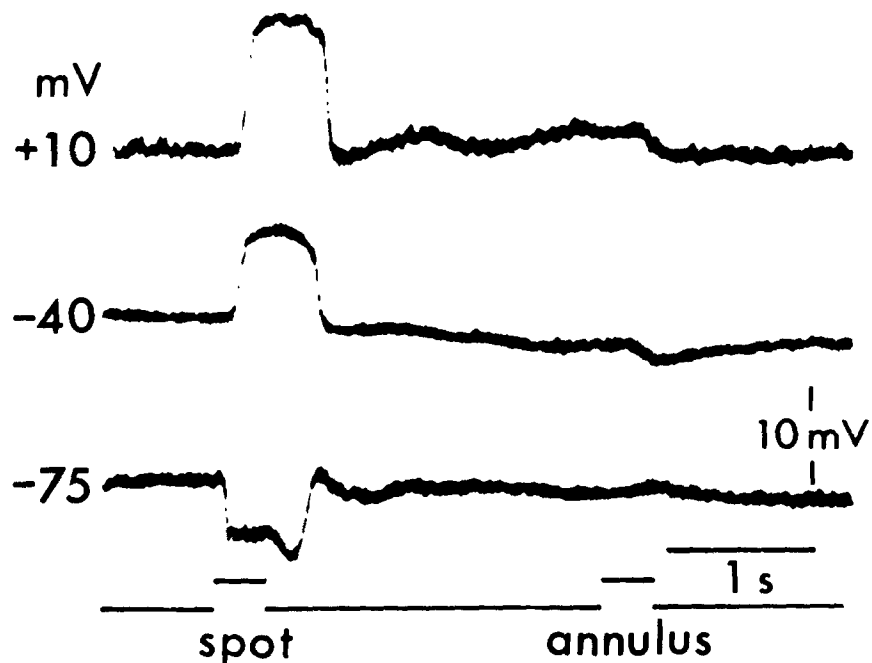

FIGURE 10. Effect of the membrane polarization on the center and surround responses of a type D cell. The resting potential in the dark was −40 mV. Both center and surround responses reversed their polarity at more negative potential than the dark level. Stimulus conditions were same as those in Fig. 3.

1982 [in press]). Measurements of spectral properties (Kaneko and Tachibana, 1978; Saito et al., 1978 and 1979) of Cajal's large bipolar cells under different states of adaptation showed that these bipolar cells had high sensitivities to the green region of spectrum in the scotopic condition and to the red region of spectrum in the photopic condition. This Purkinje shift is consistent with the result of anatomical studies that have demonstrated the convergence of both rod and red cone inputs onto the bipolar cells in the Cyprinid fish retina (Stell, 1967; Stell et al., 1977; Scholes, 1975). Because Cajal's large bipolar cells connect with both rods and cones, Stell et al. (1977) have referred to them as mixed bipolar cells. The fact that the two ionic components could be

separated from each other by applying either green or red background light (Fig. 2) strongly suggests that the synaptic inputs from rod and red cone are different in their ionic mechanisms.

Recent morphological observations (Stell et al., 1977; Ishida et al., 1980) suggest that some mixed bipolar cells of goldfish retina receive inputs from green cones as well. An attempt to isolate green cone inputs from rods was not
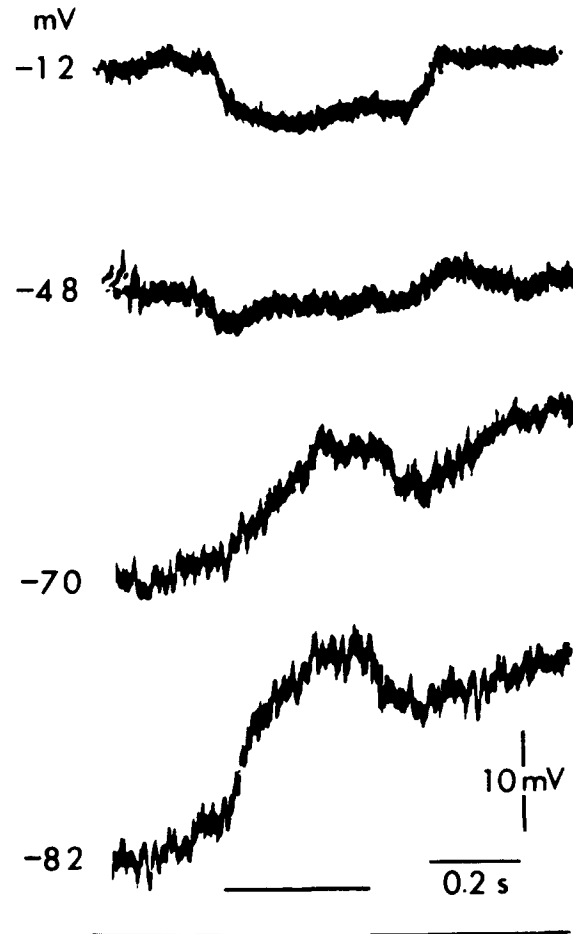

FIGURE 11. Effect of the membrane polarization on the surround response of a type D cell in the presence of a steady adapting light of the receptive field center. The cell had a membrane potential of about −12 mV (control). The response reversed its polarity somewhere between −48 and −70 mV.

made in this study, but it is possible from the result of the chromatic adaptation shown in Fig. 2 that rod and green cone inputs onto the bipolar cell may be mediated by similar ionic mechanisms. The variety of electrical properties of type C cells may be due to an appropriate contribution of the combined inputs from green cones and red cones, although some type C cells

might receive input from rods. The ionic mechanisms for the type C cell responses need further study because of their spectral properties.

If we assume that ionic distributions across the bipolar cell membrane are the same as those of nerve and muscle cell membranes, then the sodium ion is likely to be associated with the positive reversal potential and potassium, chloride, or both ions may be associated with the negative reversal potential. Accordingly, rod-mediated center responses may be generated by an increase in sodium conductance and red cone-mediated response might be generated by a decrease in the conductance of potassium or chloride—or both.

Recently, however, Kaneko and Tauchi (1980) used voltage-clamp technique to study electrical membrane properties of Cajal's large bipolar cells in the carp retina, which are known to have connections with both rods and cones. They reported that bipolar cells in the photopic condition, as well as in the scotopic condition, showed a positive reversal potential. The major difference between their experiments and ours was in the degree of light and dark adaptation of the retina. Most of our experiments were performed on dark-adapted, isolated retinas that had been excised from the animal maintained in darkness. Their experiments mainly used light-adapted, isolated retinas that had been excised from the animal maintained in daylight. It is difficult to explain this discrepancy at present, unless we assume that rods receive an excitation from neighboring cones during light adaptation either by direct connection or some other synaptic pathway (Schwartz, 1975; Copenhagen and Owen, 1976; Nelson, 1977), and/or that rods recover their sensitivity in the course of light adaptation (Dowling and Ripps, 1971; Fain, 1976). The answer to this question must await further investigation of the electrical and spectral properties of the response recorded continuously from a single bipolar cell in a live carp while it undergoes light adaptation.

Response to Annular Illumination

The results from the present and previous studies (Saito and Kondo, 1978) suggest that there are at least two ionic mechanisms responsible for generating the hyperpolarizing surround response, as well as those of the depolarizing center response.

The center and surround responses of type A cells have maximum spectral responses in the lower mesopic range of ~525 nm, suggesting that both responses are mediated by rods. Tachibana (1978) studied the spectral sensitivities for the center and surround responses of the large bipolar cells in the carp retina, and showed that both responses in the scotopic condition have a maximum sensitivity at ~520 nm. The mean reversal potential of type A cell surround response (+43 ± 15 mV, value from eight cells) is different from that of the center response of type A and B cells (+29 ± 13 mV; Saito et al., 1979) in the lower mesopic condition. Taking a large variation in the reversal value of individual cells, this difference may not be essential, but may result from various technical difficulties in the experiment: the effect of light scattering, inhomogeneous distribution of extrinsic current at the center and peripheral parts of the dendritic field, imperfect centering of the light, coupling

resistance of the electrode, rectification of the membrane, and the physiological state of the retina. The positive reversal potential of the surround response of the type A cell suggests that it is mediated by changes in the sodium conductance as with the center response, but in opposite directions.

Electrical properties of the surround response of type C and D cells have not been reported previously. They were relatively sensitive to the red region of the spectrum and both of their surround responses had reversal potentials more negative than the membrane potential in the dark. The similarity between the reversal potentials for the center and surround responses of type D cells (Figs. 10 and 11) suggests that they are generated by changes in the conductance of potassium and/or chloride, but in opposite senses. The different reversal potential for the center and surround responses of type C cells (Fig. 9) could mean that the center response is mediated by inputs from rods and/or green cones as well as red cones, but that the surround is mediated by red cone input alone. However, the number of observations were limited because of the difficulty of recording intracellularly from type C and D cells.

In seven type B cells, the surround response of five cells had maximum spectral response in the mesopic condition at ~575 nm and the remaining two cells showed the same response amplitude to illuminations of 525 and 575 nm. These facts suggest that the spectral response properties of the surround as well as the center are mediated by input from rods and red cones, although the ratio of these inputs may vary in different cells. Kaneko and Tachibana (1978) also reported that the spectral sensitivity of the surround response in some large bipolar cells is similar to that of their center response.

Electrical membrane properties of the surround response of type B cells were rather complicated. In 5 out of a total of 15 cells studied, their surround was composed of two ionic components, one having a reversal at a positive potential and the other at a negative potential. The fact that light adaptation of the cell suppressed the component only with a positive reversal (Fig. 8) suggests that the component with a positive reversal is mediated by rods and the component with a negative reversal is mediated by cones. In the other 10 type B cells, the surround was dominated by an ionic component with a negative reversal potential. The mean reversal potential was −59 ± 11 mV (SD). This value was close to that of the center response in photopic conditions (Saito and Kondo, 1978). It is therefore clear that the surround response of these bipolar cells was mainly mediated by input from red cones. However, minor input from other receptor types can not be excluded, because the surround of these cells had a maximum spectral response between 525 and 625 nm in the mesopic condition. It is necessary to study the electrical membrane properties of these cells in combination with their spectral response properties with more precision.

Center and Surround Organization

It is generally assumed that the response of bipolar cells to annular illumination is mediated by the activity of horizontal cells (Werblin and Dowling, 1969; Kaneko, 1973). Annular illumination, however, cannot isolate the

surround mechanism perfectly, either because light scatters from the annulus to the center of the receptive field or because the center and surround of some cells overlap. On the other hand, extrinsic current through horizontal cells activates the surround mechanism selectively (Maksimova, 1970; Naka; 1971; Marchiafava, 1978; Toyoda and Tonosaki, 1978 a and b). Toyoda and Tonosaki (1978 a and b) studied the effect of injecting currents into two different types of horizontal cells on Cajal's large bipolar cells. According to their results, hyperpolarization of the external L-type horizontal cell mediated by red cones evoked in the bipolar cell a hyperpolarizing response. The polarity of the response was reversed as the bipolar membrane was electrically hyperpolarized. Hyperpolarization of the intermediate horizontal cell mediated by rods also evoked in the bipolar cell a hyperpolarizing response. The amplitude of this response, however, decreased as the bipolar membrane was depolarized, suggesting that the response has a positive reversal potential.

On the basis of the agreement between our results from annular illumination and those of Toyoda and Tonosaki (1978 a and b), it is possible to construct the following tentative model of the generation of the center and surround mechanisms of on-center bipolar cells. A direct synaptic input from the rods brings about on the bipolar cell a depolarizing response mediated by an increase in sodium conductance. This conductance is modulated by the activity of intermediate horizontal cells, which acts through a decrease in sodium conductance. A direct synaptic input from red cones brings about on the bipolar cell a depolarizing response mediated by a decrease in conductance of potassium or of chloride, or both of them. These conductances are modulated by the activity of external L-type horizontal cells, which acts through an increase in potassium and/or chloride conductance. The center and surround responses mediated by both rod and red cone activities seem to result from the two ionic mechanisms described above.

Horizontal cells could exert their antagonistic influence bipolar cells in at least two different pathways: a feedback from horizontal cells to photoreceptors and a "feedforward" from horizontal cells to bipolar cells. There is now convincing evidence for feedback from horizontal to cone cells in the turtle retina from physiological studies in which cone cells are depolarized as a result of either injecting hyperpolarizing current into horizontal cells or by annular illumination (Baylor et al., 1971; Fuortes et al., 1973; Byzov, 1979; Piccolino and Gerschenfeld, 1980). There is some evidence indicating that the feedback may also exist in the fish retina. Burkhardt (1977) showed in the perch retina that the cone decreases in amplitude at the later phase of the response as the stimulus diameter is increased in a certain range. Murakami et al. (1978) reported that transretinal current flowing from the receptor side to the vitreous on the carp retina (which elicits a transient depolarization of the horizontal cell [Byzov and Trifonov, 1968]) evokes a transient hyperpolarization of the cones after a delay. Although there are many pieces of evidence suggesting feedback between horizontal cells and cones, feedback between horizontal cells and rods has not yet been demonstrated.

It is difficult at present to establish whether the surround response is

mediated by the feedback pathway. If the feedback pathway is responsible for the surround, both the center and surround responses should be mediated by the same ionic mechanisms, because they are presumably driven by direct input from the same photoreceptors. Indeed, in the present study, both the center and surround responses mediated by rods reversed their polarity at potentials that were more positive than the membrane potential of 0 mV, suggesting that they are generated by changes in the ionic conductance of sodium channel, and the polarity of the responses mediated by red cones were reversed at potentials more negative than the dark membrane potentials, suggesting that they are generated by changes in the conductance of potassium and/or chloride channels. These results, however, do not exclude the possibility that horizontal cells "feedforward" onto bipolar cells and modulate the same ionic channels as those mediating the center response.

Whatever mechanisms underlie the center-surround organization of the receptive field, it seems reasonable to conclude that rod-mediated center and surround responses are generated by changes in the sodium conductance, but in opposite ways, and that red cone-mediated center and surround responses are generated by changes in the potassium and/or chloride conductances.

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