

# Analysis of Synaptic Inputs to ON-OFF Amacrine Cells of the Carp Retina

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**ABSTRACT** To elucidate the synaptic transmission between bipolar cells and amacrine cells, the effect of polarization of a bipolar cell on an amacrine cell was examined by simultaneous intracellular recordings from both cells in the isolated carp retina. When either an ON or OFF bipolar cell was depolarized by an extrinsic current step, an ON-OFF amacrine cell was transiently depolarized at the onset of the current but no sustained polarization during the current was detected. The current hyperpolarizing the OFF bipolar cell also produced the transient depolarization of the amacrine cell at the termination of the current. These responses had a latency of ~10 ms. The amplitude of the current-evoked responses changed gradually with current intensity within the range used in these experiments. They were affected by polarization of the amacrine cell membrane; the amplitude of the current-evoked responses as well as the light-evoked responses was increased when the amacrine cell membrane was hyperpolarized, while the amplitude was decreased when the cell was depolarized. These results confirm directly that ON-OFF amacrine cells receive excitatory inputs from both ON and OFF bipolar cells: the ON transient is due to inputs from ON bipolar cells, and the OFF transient to inputs from OFF bipolar cells. The steady polarization of bipolar cells is converted into transient signals during the synaptic process.

## INTRODUCTION

The amacrine cells in the teleost retina are third-order neurons in the sense that they receive inputs from bipolar cells. They can be classified into sustained and transient types according to their response to light. The sustained type responds to light either with sustained depolarization (ON type) or with sustained hyperpolarization (OFF type), while the transient type responds to light with transient depolarization at both the onset and the termination of light (ON-OFF type) (Kaneko, 1973; Naka and Ohtsuka, 1975; Chan and Naka, 1976).

Synaptic mechanisms of amacrine cells have been studied by measuring electrical membrane properties of amacrine cells (Toyoda et al., 1973). These studies have suggested that the synaptic transmission from bipolar to amacrine cells is excitatory. It then follows that ON amacrine cells receive inputs from depolarizing (ON) bipolar

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cells and OFF amacrine cells from hyperpolarizing (OFF) bipolar cells since the response polarity is conserved in excitatory synapses. ON-OFF amacrine cells have been suggested to receive inputs from both types of bipolar cells.

Another method of studying synaptic mechanisms is to examine the effects of ions or chemicals. Slaughter and Miller (1981), for instance, have reported a selective blocking action of 2-amino-4-phosphonobutyric acid on ON bipolar cell responses and on the ON activity of ON-OFF amacrine cells that takes place without affecting OFF bipolar cells or the OFF activity of ON-OFF amacrine cells. These observations provide another piece of evidence for the hypothesis that ON-OFF amacrine cells receive inputs from both ON and OFF bipolar cells. Morphologically, the dendrites of ON-OFF amacrine cells in the carp retina are bistratified (Naka and Ohtsuka, 1975; Murakami and Shimoda, 1977) and terminate in both the distal and the proximal part of the inner plexiform layer where axon terminals of OFF bipolar cells and ON bipolar cells terminate, respectively (Famiglietti et al., 1977). These observations also support the above hypothesis. However, it is difficult to explain the transient nature of ON-OFF responses simply by an algebraic sum of sustained ON and OFF bipolar cell responses. Mechanisms of conversion from sustained to transient responses remain to be solved. It has been reported that in the catfish retina horizontal cell axon terminals make direct synaptic contacts on amacrine cells and that either depolarizing or hyperpolarizing current steps injected into these cell axon terminals elicit, through such synapses, an ON-OFF response in the transient amacrine cells (Sakai and Naka, 1985). In this sense, a possibility that ON-OFF responses are produced by an input from either ON or OFF bipolar cells alone has to be tested.

It is the aim of the present study to clarify the mechanism that induces the generation of transient activity in the ON-OFF amacrine cells. For this purpose, we recorded responses of a bipolar cell and an amacrine cell simultaneously with intracellular microelectrodes, and examined the effect of artificial polarization of the bipolar cell by extrinsic current on the amacrine cell. Characteristics of current-evoked amacrine responses were compared with those of light-evoked responses. Preliminary results on a part of this study have been reported (Kujiraoka et al., 1986).

#### METHODS

Intracellular recordings were performed on isolated retinas of the light-adapted carp, *Cyprinus carpio* (25–30 cm in total body length). The animals were maintained in aerated water at 20–22°C and were adapted to room light. After an animal was anesthetized with *m*-aminobenzoic acid ethylester methanesulfonate (MS 222; Sankyo Inc., Tokyo, Japan), the eye was enucleated and hemisected. The retina was detached from the pigment epithelium and placed on a piece of black filter paper with the photoreceptor side up. The isolated retina was mounted in a lucite chamber and superfused with a physiological saline saturated with 100% oxygen (flow rate at 1–2 ml/min). The solution had the following composition (in millimolars): 102 NaCl; 2.6 KCl; 2.0 CaCl<sub>2</sub>; 0.8 MgCl<sub>2</sub>; 20 NaHCO<sub>3</sub>; 15 dextrose; 5.0 Tris-(hydroxymethyl)aminomethane, adjusted to pH 7.8 with HCl.

The retina was illuminated from its receptor side with a white light spot at an intensity of about 90 lm/m<sup>2</sup>. Usually, for the test flash, a light spot of ~1 mm in diameter and 500 ms in duration was illuminated every 5 s. Annular stimulation (2.5-mm outer diameter, 1.0-mm inner diameter) was occasionally used to test the center and surrounding organization of the

receptive field. A diffuse illumination of about  $4 \text{ lm/m}^2$  was given during the course of the experiment to maintain the retina in a photopic condition.

Microelectrodes were filled with either 3 M potassium chloride or 4 M potassium acetate. Their resistances were 60–120 M $\Omega$  as measured in the above solution. Two electrodes were mounted on separate micromanipulators and aligned under the microscope at a tip distance  $\sim 100 \mu\text{m}$ . The electrodes were advanced independently into the retina from its receptor side until simultaneous intracellular recordings were made from a bipolar cell and an ON-OFF amacrine cell.

When responses from the two cells were simultaneously recorded, the bipolar cell membrane was polarized by a current step through a bridge circuit built in the preamplifier. The intensity of the stimulating current was within a range of  $\pm 20 \text{ nA}$  and was 500 ms in duration. Since the current often generated a large voltage drop across an electrode beyond the level of the bridge balance, no special care was taken to eliminate such a voltage drop. The data were monitored on a CRT (VC-10; Nihon Kohden Inc., Tokyo, Japan), and were stored on a magnetic tape for subsequent analysis.

We identified the type of penetrated cells by several criteria, including the response waveform, the depth of recording, and the receptive field organization. The adequacy of these criteria has been confirmed by preliminary dye injection experiments.

## RESULTS

Simultaneous intracellular recordings were made from pairs of a bipolar cell and an ON-OFF amacrine cell to examine the effects of artificial polarization of the bipolar cell by current on the amacrine cell. The effect was detectable in 9 out of 17 pairs on ON bipolar and ON-OFF amacrine cells, and 5 out of 6 pairs of OFF bipolar and ON-OFF amacrine cells. No detectable effect was observed in 3 pairs that were  $>200 \mu\text{m}$  apart. Although the radius of amacrine cell dendritic fields were  $200 \mu\text{m}$  or more (Murakami and Shimoda, 1977), the location of the soma from which the recordings were made was not always in the center of the receptive fields. Thus in some pairs, the bipolar cell recorded could have been out of the dendritic field of the amacrine cell. In 3 pairs of bipolar and amacrine cells, the effect of passing current through the ON-OFF amacrine cell on the bipolar cell was examined. In all these pairs, the effect of polarization of the amacrine cell was not detectable in the bipolar cell.

### *Interaction between ON Bipolar Cells and ON-OFF Amacrine Cells*

Fig. 1 shows an example of simultaneous recordings from an ON-OFF amacrine cell (trace A) and an ON bipolar cell (trace B). After confirming the responses to light, depolarizing current was injected into the bipolar cell at the timing indicated in the figure. An injection of 12.8 nA of current elicited a transient depolarization of  $\sim 6 \text{ mV}$  in the amacrine cell at the onset of current (indicated by an arrow). This current-evoked depolarization was not sustained in spite of the injection of steady depolarizing current into the bipolar cell. The membrane potential returned rapidly to the resting level. Fig. 2 shows responses of the same amacrine cell at various current intensities. The current necessary to produce a detectable response in the amacrine cell was  $\sim 6 \text{ nA}$  in this case. The response amplitude then became larger as the current intensity was increased. It was almost proportional to the current intensity and had a range of 6–18 nA. These potential changes cannot be an artifact

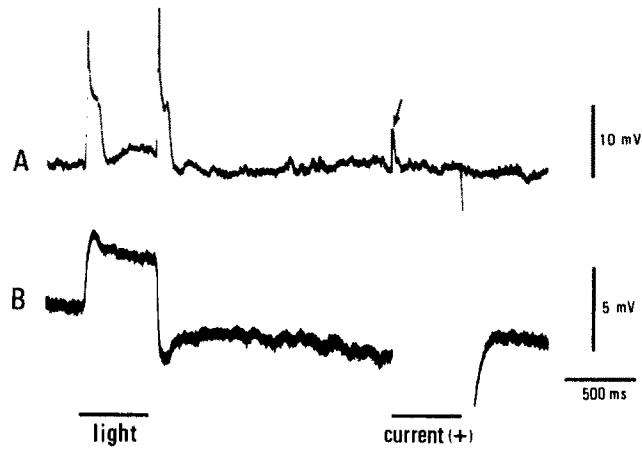


FIGURE 1. Simultaneous intracellular recordings from an ON-OFF amacrine cell (trace A) and an ON bipolar cell (trace B). Each trace shows responses to light and to the depolarizing current injected into the bipolar cell. The timing of light and current stimuli is indicated at the bottom. Depolarization of the bipolar cell elicited a transient depolarization in the amacrine cell at the onset of current pulse (indicated by an arrow). Sustained potential changes during current injection and "OFF responses" at the termination of current were not detectable.

since they disappeared when either one of the electrodes was withdrawn from the cell. The effect of hyperpolarizing current injected into an ON bipolar cell was also tested in the dark. It did not produce any detectable potential changes in the amacrine cell (not illustrated).

Fig. 3 compares the effect of artificial depolarization of an ON bipolar cell on an

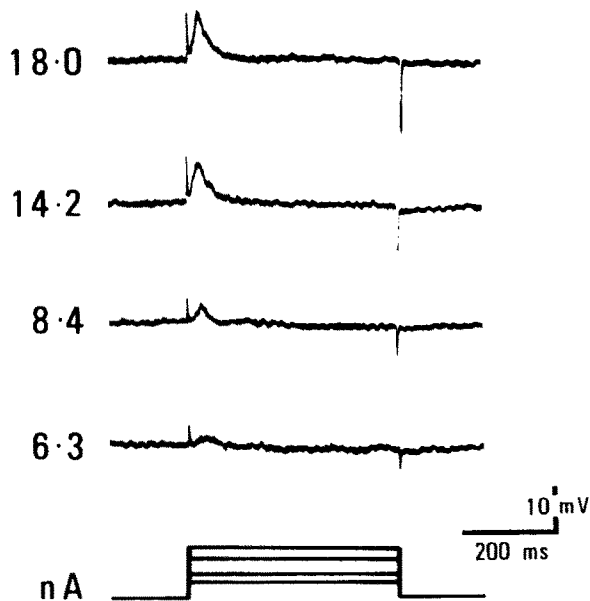


FIGURE 2. Records from the same pair of cells as shown in Fig. 1. Series of the responses were recorded from the amacrine cell while the ON bipolar cell was polarized by currents of various intensities. The current-evoked depolarizing responses were graded and their amplitude was almost proportional to the current within the range shown in the figure. The intensity of the current (in nanoamperes) injected into the bipolar cell are indicated to the left of each response.

ON-OFF amacrine cell with and without background light. In the dark, the transient depolarization, which was similar to that shown in Fig. 1, was elicited when a sustained depolarizing current was applied to the bipolar cell. Switching on the background light produced a transient depolarization followed by a sustained depolarization of  $\sim 5$  mV in this amacrine cell. Under the background light, the current-evoked response was suppressed, leaving the capacitive artifacts at onset and offset of the current. If the release of a transmitter substance is facilitated by membrane depolarization as is generally assumed, the background light will act to release the transmitter from ON bipolar cells. Then further depolarization of the bipolar cell by current would be less effective in increasing the transmitter release than the depolarizing current injected without background light. The present results strongly suggest that the current-evoked responses are mediated by chemical synapses.

The latency of the current-evoked responses was difficult to measure, since the onset of the responses was usually masked by the capacitive artifact. However, in

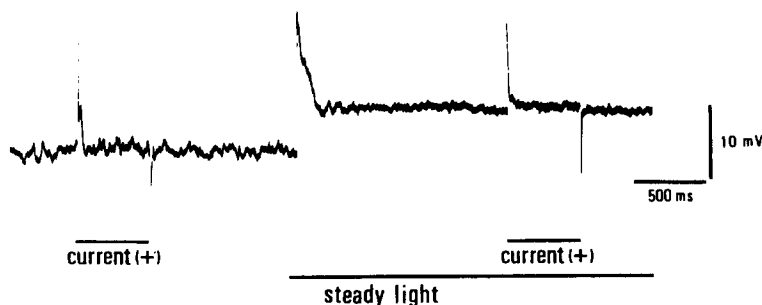


FIGURE 3. The effect of background illumination on an amacrine cell response elicited by polarization of an ON bipolar cell. The responses of the latter are not shown. The intensity of current used in this experiment was +18 nA. The timing of light and current stimuli is indicated at the bottom. The intensity of background light was  $\sim 900$   $\text{lm}/\text{m}^2$ .

two records in which the artifact was relatively small and decayed rapidly, a synaptic delay was estimated to be  $\sim 10$  ms. When the latency of the light responses of the bipolar and the amacrine cell (which was recorded in pairs) was compared, there was a delay of 6–12 ms, which was close to the value estimated by current injection.

Fig. 4 shows the effect of steady hyperpolarization of an amacrine cell membrane on its light-evoked (left) and current-evoked responses (right). When the membrane potential of the amacrine cell was hyperpolarized, both the light- and current-evoked responses were increased in amplitude. The fact that the amplitude of the current-evoked amacrine responses, as well as that of the light-evoked responses, is affected by membrane polarization of the amacrine cell in the same manner, may suggest that both the current- and light-evoked responses are mediated by similar ionic mechanisms.

#### *Interaction between OFF Bipolar Cells and ON-OFF Amacrine Cells*

Fig. 5 shows an example of simultaneous intracellular recordings from an ON-OFF amacrine cell (trace A) and an OFF bipolar cell (trace B). Since OFF bipolar cells

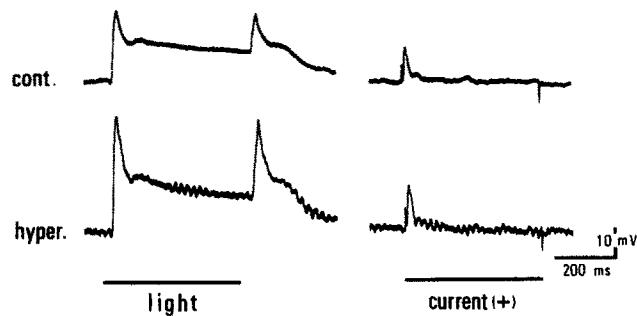


FIGURE 4. The effect of the polarization of an ON-OFF amacrine cell on the responses elicited by light and by a current into an ON bipolar cell. The upper two records are the control (cont.) responses elicited by light (left) and by a current into the bipolar cell (right). The intensity of the current was +19 nA. The responses of the lower trace were recorded under the steady hyperpolarization (hyper.) of the amacrine cell with a current of  $-2.2$  nA. The amacrine cell responses to light and current stimuli were increased in amplitude by hyperpolarization of the membrane.

respond to light spots with hyperpolarization, the effect of artificial hyperpolarization of bipolar cells by current on amacrine cells was examined first. In the figure, after recording responses to light, hyperpolarizing current of about  $-18.6$  nA was injected into the bipolar cell. The current did not elicit a detectable response in the amacrine cell at its onset but evoked a transient depolarization of  $\sim 10$  mV just after its termination. When the bipolar cell was depolarized by current, a transient depolarization was elicited in the amacrine cell at the onset of current, but there was no

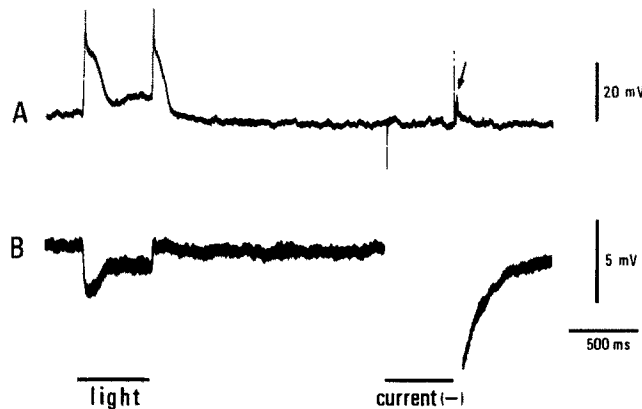


FIGURE 5. Simultaneous intracellular recordings from an ON-OFF amacrine cell (trace A) and an OFF bipolar cell (trace B). Each trace shows responses to light and to hyperpolarizing current injected into the bipolar cell. The timing of light and current stimuli is indicated at the bottom. Hyperpolarization of the bipolar cell elicited a transient depolarization in the amacrine cell at the termination of the current pulse (indicated by an arrow). No steady potential changes during current stimulation nor an "ON response" at the onset of current were detectable.

response when the current was turned off. The amplitude of the response evoked by the depolarizing current, however, was smaller compared with that evoked at the termination of a hyperpolarizing current of the same intensity. This point will be further analyzed in connection with Fig. 7.

Fig. 6 shows the amacrine cell responses evoked by hyperpolarizing currents of different intensities. The amplitudes of the current-evoked responses were graded and, in a range of  $-9$  to  $-19$  nA was almost proportional to the current. In this case, a detectable transient response was produced by a current injection of  $\sim -8$  nA. These potential changes disappeared when either one of the electrodes was withdrawn from the cell.

Present results showing that the amacrine cell response is elicited either at the offset of the hyperpolarizing current or at the onset of the depolarizing current into bipolar cells indicate that the excitatory transmitter is released from bipolar cells when they are depolarized. Fig. 7 shows the amacrine cell responses evoked by depolarizing (left) or hyperpolarizing (right) a current into the bipolar cells, with

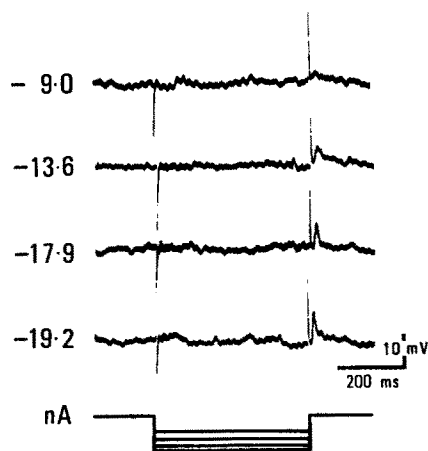
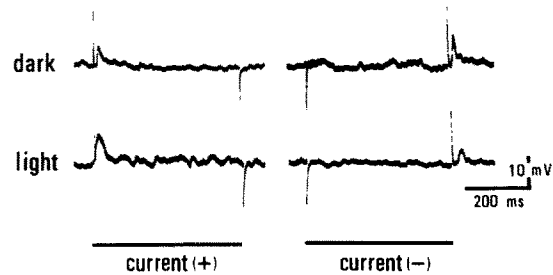


FIGURE 6. Records from another pair of an amacrine cell and an OFF bipolar cell. Series of the responses were recorded from the ON-OFF amacrine cell while the OFF bipolar cell was polarized by currents of various intensities. The responses elicited in the amacrine cell were graded and their amplitude was nearly proportional to the current in the range shown in this figure. The intensities of the currents are indicated to the left of each response in nanoamperes.

and without background light. When a depolarizing current was injected into the bipolar cell it produced a transient depolarization in the amacrine cell at the onset of the current. This response was larger in amplitude in the light than in the dark. When the hyperpolarizing current was injected into the bipolar cell it produced a transient depolarization in the amacrine cell at the offset of current. The amplitude of this response was larger in the dark than in the light. Since OFF bipolar cells are kept depolarized in the dark and hyperpolarized by light, the release of a transmitter substance from OFF bipolar cells will continue in the dark and will be suppressed by light. As was discussed in the previous section, the effect of depolarizing current on increasing the transmitter release would be smaller if the release had already been facilitated by depolarization of the presynaptic terminals. On the other hand, the effect of hyperpolarizing current, which acts to suppress the transmitter release, would be larger if the release had already been facilitated. Therefore the depolarizing response as a rebound from hyperpolarization would be larger in the dark when

FIGURE 7. The effect of background light on the responses of an ON-OFF amacrine cell that were evoked by polarization of an OFF bipolar cell. Records on the right show the responses elicited by hyperpolarizing current into the bipolar cell with (lower trace) and without (upper trace) steady background light.



The response evoked by hyperpolarizing current was suppressed by light. Records on the left show the responses evoked by depolarizing current into the bipolar cell. The response elicited by depolarizing current was augmented in the presence of background light. The intensities of depolarizing and hyperpolarizing current in the bipolar cell were  $+17.5$  and  $-18.0$  nA, respectively. The intensity of background light was  $\sim 90$   $\text{lm/m}^2$ .

OFF bipolar cells are relatively depolarized. The results agree well with those anticipated.

The latency of the current-evoked response was generally difficult to measure because of the large artifact. But in one record the latency of the depolarizing response elicited at the offset of current was estimated as  $\sim 15$  ms, which was slightly longer than that estimated for the transmission from ON bipolar cells to ON-OFF amacrine cells. It is difficult, however, because of the small sampling data, to judge whether or not this difference in latency is significant. Unfortunately, the latency of the response elicited at onset of depolarizing current could not be estimated because of the artifact.

Fig. 8 shows the effect of polarization of an ON-OFF amacrine cell on both the response to light and the current-evoked response. The middle row shows the con-

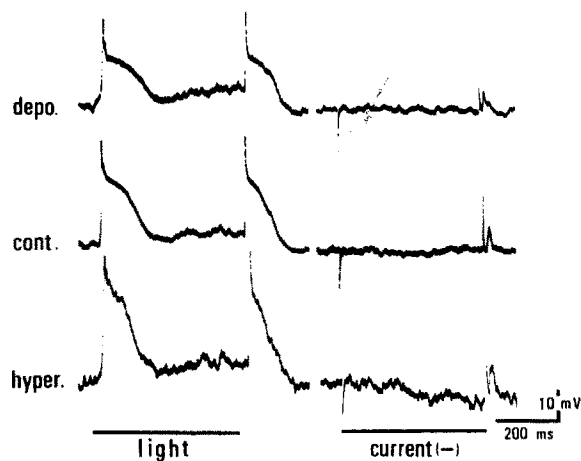


FIGURE 8. Effect of polarization of an ON-OFF amacrine cell on the responses to light (left) and the responses to hyperpolarizing current into an OFF bipolar cell (right). The intensity of the hyperpolarizing current was  $-15.3$  nA. Control (cont.) responses are shown in the middle. The amplitude of the current-evoked response and the response to light was increased by hyperpolarization (hyper.) of the amacrine cell, but was decreased by depolarization (depo.). The polarizing current in the amacrine cell was  $-2.8$  and  $+2.5$  nA, respectively.



rol responses without current injection. When the membrane of the amacrine cell was hyperpolarized, its responses to current as well as to light were increased in amplitude (bottom). When the amacrine cell was depolarized, both responses became smaller (top). These results, as well as those in Fig. 4, appear to indicate that the current-evoked amacrine cell responses are elicited by ionic mechanisms similar to those modulated by light.

## DISCUSSION

### *Characteristics of Current-evoked Amacrine Cell Responses*

We demonstrated, by impaling pairs of bipolar and amacrine cells, that the sustained membrane polarization of the bipolar cell, brought about by extrinsic current injection, causes the transient potential change in the ON-OFF amacrine cell. The current necessary to produce the potential change of several millivolts in the amacrine cell was  $\sim 10$  nA. Unfortunately, the current generated a large artifact across the high resistance of the current-passing electrode. The artifact made it impossible to measure the potential drops that occurred in the bipolar cell through which the current was passing. If the input resistance was 8–40 M $\Omega$ , as measured in other experiments (Toyoda et al., 1977; Saito et al., 1984), then a current of 10 nA produces a voltage change of 80–400 mV in the stimulated cells. One must conclude that this value is out of the physiological potential range. However, a simple product between the injected current and the input resistance may have overestimated the amount the voltage drops in a stimulated cell because of the following two reasons. (a) Bipolar cells are interconnected by low-resistance pathways (Kujiraoka and Saito, 1986; Saito and Kujiraoka, 1988). If a single bipolar cell is injected by the current, a part of the current will flow across the plasma membrane of neighboring bipolar cells via the network of connections. Therefore, the polarization potentials in the bipolar cell will be shunted by the coupling pathways. Recently, a large input resistance value in the giga-ohm range has been reported on the solitary bipolar cells using a single suction electrode with a giga seal (Kaneko and Tachibana, 1985). Such a large input resistance may result from not only an elimination of bipolar-bipolar coupling but also a reduction in nonspecific leakage current produced by the electrode penetration. (b) The bipolar cells are outward rectifying, which may shunt the responses in the depolarizing direction (Toyoda et al., 1977; Saito and Kaneko, 1983). In whole cell patch-clamp analysis of solitary bipolar cells, Kaneko and Tachibana (1985) have found an outward K<sup>+</sup> current, activated by depolarization, that is in the physiological potential range. It is, therefore, likely that the outward rectifying property gives a stabilizing effect to the unphysiological depolarization.

Sustained depolarization of either ON or OFF bipolar cells by a current elicits a transient depolarization of the ON-OFF amacrine cells at the onset of the current. The current hyperpolarizing OFF bipolar cells in the dark also produces the transient depolarization of the amacrine cells at the termination of the current. These current-evoked responses appear to be characteristic of chemical synapses between bipolar and transient amacrine cells because sustained depolarization of ON bipolar

cells by current elicits a sustained depolarization in sustained ON amacrine cells (Kujiraoka et al., 1986).

When the amacrine cell membrane was polarized by extrinsic current, both the current- and light-evoked responses were similarly affected. They increased in amplitude when the amacrine cell membrane was hyperpolarized, while their amplitude decreased when the cell was depolarized. These results directly confirm that ON-OFF amacrine cells receive excitatory inputs from both ON and OFF bipolar cells; the ON transient is due to inputs from ON bipolar cells and the OFF transient is due to OFF bipolar cells. In other words, bipolar cells release an excitatory transmitter when they are depolarized.

#### *Formation of Transient Responses*

Although a convergence of inputs from ON and OFF bipolar cells have been suggested from a number of data, the mechanisms of transient responses have not been well understood. Toyoda et al. (1973) suggested that ON-OFF responses could be produced by the algebraic sum of synaptic inputs from ON and OFF bipolar cells, provided that the initial transient at each depolarizing phase was somehow augmented by synaptic characteristics. Miller (1979) proposed a similar model in which he introduced a threshold level at which the transmitter is released from bipolar cells. Marchiafava and Torre (1978), on the other hand, suggested that the transmitter from bipolar cells would be released transiently.

In these experiments, sustained depolarization of the bipolar cell membrane by extrinsic current produced a transient depolarization in the ON-OFF amacrine cell. Thus there must be some mechanisms in the bipolar-amacrine synapses that convert sustained signals into transient ones. There are at least two possibilities for such conversion mechanisms.

The first possibility is that the transmitter is released transiently from bipolar cells at the beginning of their depolarization. At the squid giant synapse, Katz and Miledi (1971) demonstrated that strong depolarization of the presynaptic terminal beyond the equilibrium potential for calcium ions causes transient release of the transmitter at both the onset and the cessation of current. However, this explanation may not apply to the present cases, because ON-OFF amacrine cells always responded in a transient manner irrespective of the amount of current injected. Also, they only responded to the onset of depolarizing current. Furthermore, our previous study on the sustained ON amacrine cells showed that they respond with a sustained depolarization to a steady depolarization of ON bipolar cells (Kujiraoka et al., 1986). Since there is a sustained release of transmitter from ON bipolar cells to ON amacrine cells, it is unlikely that the same bipolar terminals release a transmitter transiently.

The second possibility is that there is a sustained release of a transmitter from bipolar cell terminals during their depolarization, but that ON-OFF amacrine cells somehow respond to it transiently. Such a conversion mechanism may be present at the subsynaptic membrane. Recently, Toyoda and Fujimoto (1984) studied the effect of repetitive transretinal current pulses on amacrine cell responses. They found that repetitive current pulses, after blocking the receptor-bipolar transmission, elicited a sustained depolarization in both sustained and transient amacrine

cells. They concluded that postsynaptic membrane properties were not responsible for the transient nature of the ON-OFF responses.

Thus, there are no positive data that support either one of the two possibilities so far. There may be more than one type of bipolar cells that have different transmission characteristics. In this connection, it appears important to know whether or not the same bipolar cells send information to both sustained and transient amacrine cells.

#### *DC Components*

Transient ON-OFF amacrine cell responses occasionally show a distinct DC potential shift during illumination (Toyoda et al., 1973; Murakami and Shimoda, 1977; Werblin, 1977). The amplitude and polarity of the DC potential differ not only in individual cells but also in the same cell under various stimulus conditions.

In the present experiments, a current step injected into bipolar cells did not elicit a detectable DC potential shift in ON-OFF amacrine cells. But DC components observed in their light responses must be somehow transmitted to these amacrine cells. One possibility is that there is a small DC potential that is hardly detectable under the present experimental conditions but is summed to produce a detectable DC potential if a greater number of bipolar cells are simultaneously polarized by current. Another possibility is that the DC component comes from other retinal neurons not recorded in this study. The bipolar cells most frequently recorded by intracellular studies are Cajal's large bipolar cells (Saito and Kujiraoka, 1982; Saito et al., 1985). Since there are several varieties of bipolar cell types in the cyprinid fish retina (Cajal, 1972), a certain type of bipolar cells, not studied in the present experiment, may send a sustained input to the ON-OFF amacrine cells. It has been noted that the sustained component of the amacrine cell response is often depolarizing to long wavelengths and hyperpolarizing to short-wavelength light (Djamgoz and Rudok, 1983; Watanabe and Murakami, 1985). Therefore, it is possible that ON-OFF amacrine cells receive sustained inputs from color-coded bipolar cells or from color-coded horizontal cells. Direct synaptic inputs from some horizontal cells to amacrine cells have been suggested in the catfish (Sakai and Naka, 1985). Such direct synaptic contacts, however, have not been reported in the carp retina. Cone-driven bipolar cells of the fish retina belong to Cajal's small bipolar cells and, therefore, there will be less of a chance of recording from them. It is also possible that ON-OFF amacrine cells receive sustained inputs from other amacrine cells, since there are mutual synaptic contacts among amacrine cells (Witkovsky and Dowling, 1969).

The present results have demonstrated directly that ON transients of amacrine cells are due to the inputs from ON bipolar cells and OFF transients are due to the inputs from OFF bipolar cells. They have also indicated that steady polarization of bipolar cells is converted into transient responses during the synaptic process. The precise mechanism of synaptic transmission from bipolar cells to amacrine cells, however, still remains a challenging problem.

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