

Electrical coupling between bipolar cells in carp retina

(intracellular recording/Lucifer yellow/receptive field/dendritic field)

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ABSTRACT Intracellular recordings were made simultaneously from pairs of neighboring bipolar cells by advancing two independent microelectrodes into retinas of carp (*Cyprinus carpio*). Bipolar cells were identified by their response properties and in several samples were verified by intracellular injection of Lucifer yellow. Current of either polarity injected into one member of the bipolar cell pair elicited a sign-conserving, sustained potential change in the other bipolar cell without any significant delay. This electrical coupling was reciprocal, and it was observed between cell types similar in function and in morphology. Our results strongly suggest that there is a spatial summation of signals at the level of bipolar cells, which makes central receptive field areas much larger than their dendritic fields.

Bipolar cells of the vertebrate retina form a signal pathway from photoreceptors to amacrine or ganglion cells (1, 2) and play a principal role in the early processing of spatial information by the retina (3, 4). A receptive field of a bipolar cell consists of a central area and an antagonistic surround area (3-5). It is generally assumed that the responses of bipolar cells to central illumination are directly transmitted from photoreceptors, whereas those to surround illumination are mediated by horizontal cells. The latter assumption is supported by the findings that (i) the size of the receptive field surround far exceeds the dendritic spread of a bipolar cell (3-5) and (ii) extrinsic currents injected into horizontal cells produce potential changes in bipolar cells (6, 7). The evidence for the former notion, that center responses are directly mediated by photoreceptors, comes from comparisons between the receptive fields determined by physiological methods and the dendritic spreads measured morphologically (3-5). There is, however, a notable discrepancy in such data: the central receptive field is always larger than the dendritic spread (3, 5, 8-10). In the carp retina, for example, a mean diameter of the receptive field centers of on-center bipolar cells is almost 10 times as large as that of their dendritic spreads. Such a large difference between the physiological and morphological field sizes is too great to be accounted for by signal spreads through electrical coupling between photoreceptors (11-13). In this study, we demonstrate directly, by impaling pairs of cells, that bipolar cells in the carp retina are electrically coupled with neighboring bipolar cells. We further propose that the coupling contributes to the enlargement of their receptive field centers.

MATERIALS AND METHODS

Carp (*Cyprinus carpio*) were anesthetized with tricaine methanesulfonate (MS222). The eye was excised and the retina was detached from the pigment epithelium. The isolated retina was mounted flat in a Lucite chamber with the receptor side up and superfused with physiological saline

solution. The solution was continuously aerated with a gas mixture of 98% O₂ and 2% CO₂. The millimolar composition of the medium was as follows: NaCl, 102; KCl, 2.6; CaCl₂, 1.0; MgCl₂, 0.4; NaHCO₃, 20; and glucose, 15.

The retina was illuminated from the receptor side with a white light spot, at an intensity of about 90 lm/m², whose diameter could be changed from 0.3 mm to 2 mm. Usually a light spot of about 1 mm in diameter and 0.5 sec in duration was applied every 5 sec. Annular illumination (0.7 mm in inner diameter and 2.0 mm in outer diameter) was occasionally used to test the center and surround organization of the receptive field. A diffuse background of about 4 lm/m² was given throughout the experiment to maintain the retina under a mesopic condition.

Microelectrodes filled with 2.5 M KCl and having a resistance of 60-120 MΩ were used for intracellular recording and for current injection. Two microelectrodes, with a small separation at the tips, were advanced independently into the retina from the receptor side to record simultaneously from a pair of bipolar cells. After the electrical interaction between the two cells had been studied, the electrodes were withdrawn and the distance between the tips was measured under a light microscope. This distance value was used to determine the separation between the two impaled cells.

We identified the types of penetrated cells by the following criteria: response waveform, recording electrode depth, and center-surround receptive field organization. The adequacy of these criteria was verified by the intracellular dye injection experiment. Glass microelectrodes filled with 5% Lucifer yellow CH were used for this purpose.

Bipolar cells studied are classified into two types according to the polarity of their responses (2, 3). The first type of bipolar cells (on-center cells) is depolarized by illumination of a white light spot. The second type of bipolar cells (off-center cells) is hyperpolarized by the same illumination.

RESULTS

Fourteen pairs of neighboring bipolar cells were recorded successfully. Extrinsic currents of either polarity were injected into each of those cell pairs in order to reveal the potential changes in the neighboring bipolar cells. Electrical interactions were found between 10 pairs (8 pairs of on-center cells and 2 pairs of off-center cells). Typical results of on-center bipolar cell pairs separated by 85 μm are shown in Fig. 1. Each trace represents responses of either member of the pair and consists of responses to illumination of white light spot and to extrinsic current injection into one of these cells. In Fig. 1A, extrinsic currents were injected into an on-center cell whose responses are shown in the lower trace, producing a break in the trace, and potential changes of the other on-center cell were recorded (upper trace). A depolarizing current (about 10 nA) applied to one cell produced a depolarization of about 3 mV in the other cell, whereas a hyperpolarizing current (about 10 nA) produced a hyperpo-

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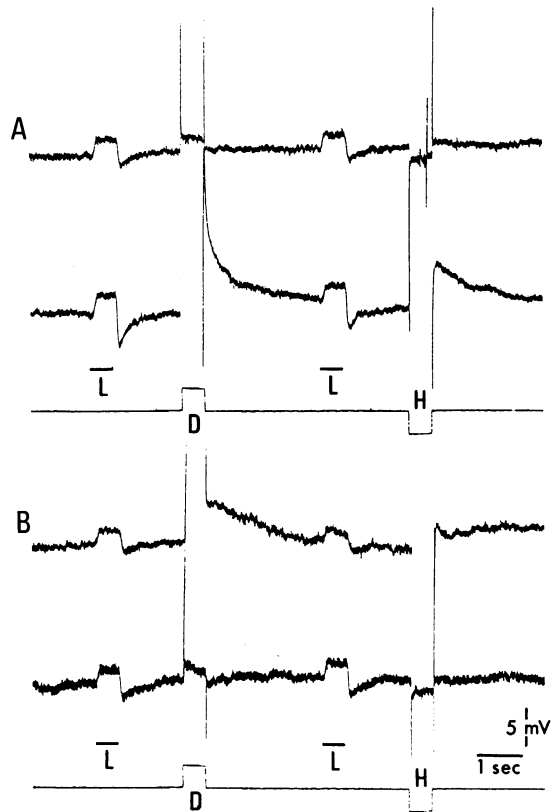


FIG. 1. Simultaneous intracellular recordings from a pair of on-center bipolar cells about $85 \mu\text{m}$ apart (separation measured as described in text). Each trace consists of responses to illumination of a white light spot (L) and to extrinsic current injection into one of these cells, alternately. In A, depolarizing (D) and hyperpolarizing (H) current pulses of about 10 nA were passed into a bipolar cell shown in the lower trace. The depolarization and hyperpolarization of the cell by the applied current, producing a break in the trace, evoked a depolarization and a hyperpolarization of the other cell (upper trace) without any significant delay. In B, the same current pulses were delivered to the cell in the upper trace. The depolarization and hyperpolarization of the cell produced the potential deflections of the same sign in the other cell (lower trace).

larization of about 3 mV . The coupling efficiency was nearly identical for both depolarizing and hyperpolarizing currents. In Fig. 1B, extrinsic currents were injected into the other member of the pair whose responses are shown in the upper trace, and the potential changes of the counterpart were recorded (lower trace). Almost identical results were obtained: the depolarizing or hyperpolarizing current pulses of about 10 nA in one cell produced in the other cell a depolarization or a hyperpolarization of about 3 mV . Similar results were obtained in the remaining seven pairs of on-center bipolar cells separated by $70\text{--}100 \mu\text{m}$. Fig. 2 shows a current-evoked potential recorded at an expanded time scale. From these data, we could not detect the delay that is expected to occur across the chemical synapse.

When the microelectrode was withdrawn from one member of the bipolar cell pair and the current was injected into the extracellular space, only a small potential change (less than 0.5 mV) was recorded from the other bipolar cell. Similar results were obtained when the current-passing electrode was intracellular and the voltage-recording electrode was in the extracellular space. These findings clearly indicate that the current-evoked potential changes are not due to the field potential.

Fig. 3 shows an electrical coupling between off-center bipolar cells. The findings here are similar to those observed in on-center bipolar cell pairs. First, the depolarizing and

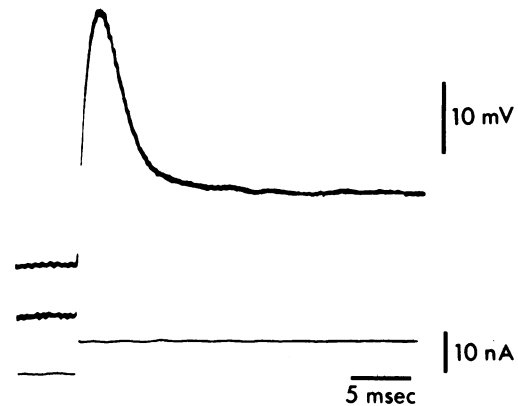


FIG. 2. Current-evoked response recorded at fast time base. Electrodes were inserted into two on-center bipolar cells about $80 \mu\text{m}$ apart. A depolarizing current (about 10 nA) was passed into a bipolar cell shown in the middle trace, producing the break in the trace. The depolarization applied to one cell produced a depolarization of the other cell shown in upper trace. The latency of the response was difficult to measure, since the onset of current-evoked response is masked by the capacitive artifact. However, a smooth transition from the capacitive artifact to coupling potential suggests that there is no delay such as is expected to occur across the chemical synapse (14). The lower trace shows a current step.

then the hyperpolarizing current of about 10 nA injected into a cell (lower trace) produced a depolarization of about 5 mV and a hyperpolarization of about 4 mV in the other cell (upper trace) without any significant delay. When we reversed the conditions with respect to current injection and recording cells, essentially identical results were obtained. The separation between the two cells was about $80 \mu\text{m}$. Another pair of off-center cells separated also by about $80 \mu\text{m}$ also had the coupling, but it was less efficient than that shown in Fig. 3.

When we performed similar experiments on two pairs of on- and off-center bipolar cells separated by about $85 \mu\text{m}$, we could not detect any sign of electrical coupling between them.

It is essential to demonstrate that the coupling described

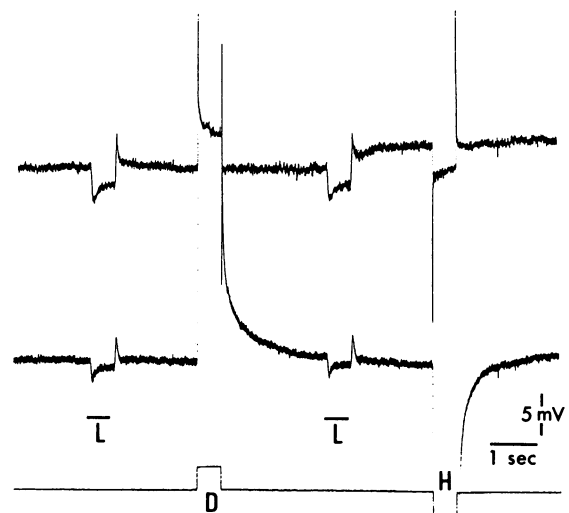


FIG. 3. Simultaneous intracellular recordings from a pair of off-center bipolar cells separated by about $80 \mu\text{m}$. The records are presented according to the convention adopted in Fig. 1. Thus, the response evoked by light stimulation is shown on the left of each current-evoked polarization. When depolarizing and hyperpolarizing current pulses of about 10 nA were passed into the bipolar cell in the lower trace, producing a break in the trace, a depolarization and a hyperpolarization of the other cell (upper trace) were produced without any significant delay.

above is not due to interactions between the two parts within the same bipolar cell, such as the cell body and the axon terminal. To localize the positions of electrode tips, we marked pairs of cells with Lucifer yellow after observing their electrical interactions. Two pairs of on-center bipolar cells were successfully stained. Photomicrographs in Fig. 4 show morphological properties of a pair of cells in tangential sections. Dendrites from the two cells are stained side by side (*a*). The cell on the left was lightly stained due to a relatively low concentration of the dye in the cell. Some of the dendritic processes of the lightly stained cell were missing, because of oblique sectioning. The cell bodies of the two cells were separated by about 70 μm (*b*). Axon terminals of these cells are similar in shape (*c* and *d*). Judging by the big swelling of axon terminals, both cells are likely to be classified as our type I on-center bipolar cells (10). Another Lucifer yellow-labeled pair of cells was also the type I cell. These results suggest that the electrical coupling takes place between neighboring bipolar cells of similar morphological as well as physiological type.

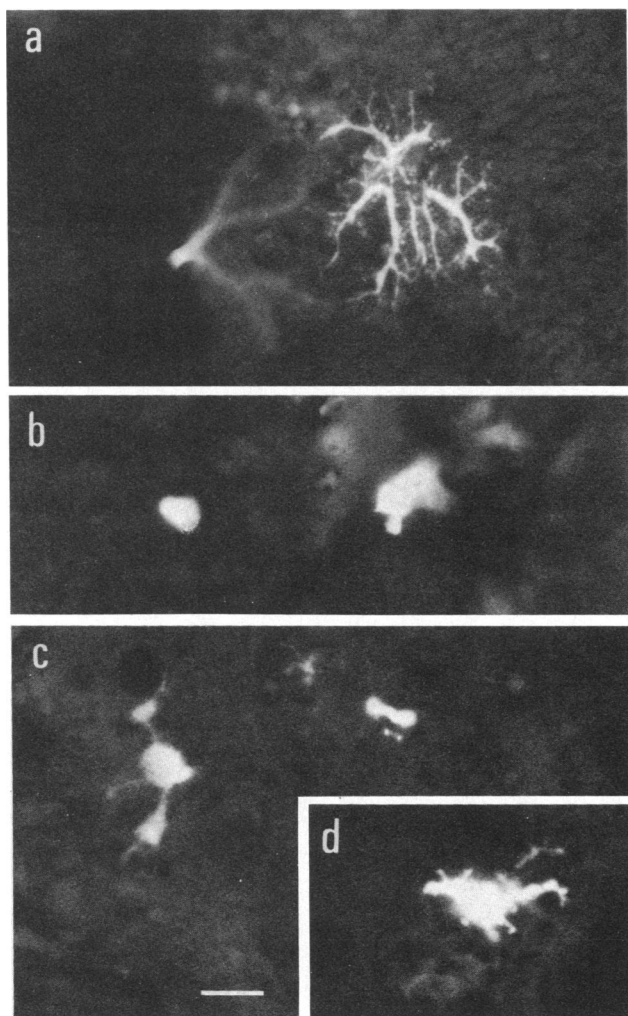


FIG. 4. Photomicrographs showing two on-center bipolar cells identified by intracellular injection of Lucifer yellow after observing their electrical interaction. (*a*) Dendrites, (*b*) cell bodies, (*c* and *d*) axon terminals. Microelectrodes were filled with 5% Lucifer yellow in 100 mM lithium chloride. The cell on the right was marked by passing 3 nA of negative current for 2 min. The lightly stained cell on the left was obtained by passing current of -2.5 nA for 1 min. Two cells were separated by about 70 μm at their cell body. Some of the dendritic processes of the left cell were missing in *a* and two axon terminals are shown in *c* and *d* because tangential sections were obliquely oriented. Scale marker represents 20 μm .

DISCUSSION

The present results demonstrate that in the carp retina, bipolar cells of the same morphological and functional type are electrically coupled to one another and that the coupling is sign conserving. The coupling efficiency, at least over the physiological response range, appears to be almost identical for both depolarizing and hyperpolarizing currents. Since there is no measurable synaptic delay in the observed interaction, the mechanism by which bipolar cells interact may not involve chemically mediated synapses, but direct electrical connections between bipolar cells.

Bipolar cells are both pre- and postsynaptic to amacrine cells (15). It is, therefore, possible that polarizations of one bipolar cell by injecting extrinsic currents could influence another by way of an amacrine cell. This possibility, however, appears not to be the case, because no significant delay was observed in bipolar-bipolar interactions as described above. To investigate bipolar-amacrine interactions, we made simultaneous intracellular recordings from bipolar cells and amacrine cells. When extrinsic currents were injected into a bipolar cell, potential changes were recorded in an amacrine cell with the delay characteristic of chemical synapses (14).

Morphologically, direct interbipolar junctions have been observed in several vertebrate retinas (15–19). These junctions include gap junctions as well as ribbon and conventional chemical synaptic junctions. Gap junctions between neurons are considered to be an anatomical substrate for low-resistance pathways, mediating electrical synaptic transmission. In the fish retina (15, 18), these junctions have been observed at the level of bipolar cell axons, between adjacent axon terminals, or between collaterals that extend outward from the axon terminal. It is, therefore, reasonable to consider that a pathway of the bipolar coupling presented in this study is through their axonal level. However, it does not appear that the two bipolar cells shown in Fig. 4 make direct contacts at their axon terminals or collaterals. Furthermore, although the data are still preliminary, we could occasionally observe similar morphological appearances between some dye-coupled bipolar cells when the Lucifer yellow was injected into a bipolar cell (not illustrated). Therefore, we cannot rule out the possibility that these bipolar cells may communicate with each other through their dendritic processes. As far as we know, however, gap junctions between bipolar cell dendrites in the outer plexiform layer have not been reported. A complete understanding of the interbipolar pathways must await further investigations with electron microscopy of both physiologically and morphologically identified bipolar cells.

There have been several reports that the receptive field centers of bipolar cells are much larger than their dendritic spreads (3, 5, 8–10). For example, in carp bipolar cells identified by both intracellular recording and dye injection (10), we have shown that the mean diameter of the receptive-field centers of type I on-center bipolar cells is 500 μm , while that of their dendritic fields is 56 μm . Such a large disparity between receptive field sizes and dendritic field diameters could be partly due to various experimental errors, such as light scattering, misalignment of electrodes, retinal shrinkages during the histological procedure, and a failure of the dye to infiltrate the smallest dendritic branches. However, it is more likely that the electrical coupling between photoreceptors (11–13), which results in the spatial summation of photoreceptor responses, is responsible for this discrepancy. Indeed, in the carp, the photoreceptors of the same type make contact with both the terminals and the basal processes of neighboring photoreceptors (19). Nevertheless, the observed differences between the dendritic and receptive field diameters are too great to be attributed to signal spreads

through electrical coupling between photoreceptors (20). However, we demonstrated in this study that bipolar-bipolar interactions are both reciprocal and sign conserving. These findings strongly imply that there is a spatial summation of signals at the level of bipolar cells, which would make the disparity between the receptive field and dendritic field sizes even larger.

Direct electrical interactions between bipolar cells observed in the present study, as well as those between photoreceptors in other investigations, would appear to degrade spatial resolutions of the eye. Some undiscovered benefit of these interactions which may offset such a loss of spatial resolution remains to be determined.

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