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EFFECT OF INTRACELLULAR POLARIZATION OF HORIZONTAL CELLS ON GANGLION CELL ACTIVITY IN THE FISH RETINA

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Intracellular polarization of horizontal cells of the L-type by 5-10 mv is found to evoke spike responses of the retinal ganglion cells. Four classes of ganglion cells are described, differing in their response to light and, correspondingly, to polarization of the horizontal cells. The observed influence of horizontal cells on activity of the ganglion cells suggests that horizontal cells of the L-type are interneurons organizing the periphery of the receptive field of the ganglion cells, with opposite properties to the center.

Until recently the sources of S-potentials of the fish retina (horizontal cells) have been investigated in isolation, without regard to their connections with other retinal neurons. This approach is partially due to the fact that only the afferent systems of the horizontal cells are known, i.e., their synapses with receptors (1—3). Electrophysiological methods have shown that sources of S-potentials of L- and RG-types receive impulses from two, while triphasic sources receive them from three types of receptor differing in their spectral sensitivity (4). Efferent systems of the horizontal cells are unknown. Many hypotheses have been put forward to explain the role of these cells in analysis of visual stimuli (5—7), but none has been tested by direct experiment. The first deliberate investigation of the physiological role of horizontal cells in direct experiments was undertaken by Byzov on turtles (8). He demonstrated the effect of intracellular polarization of horizontal cells on the local electroretinogram, i.e., on the integral response of cells of the inner nuclear layer (bipolars) to light. The response of bipolars to short hyperpolarizing electrical pulses passed through the horizontal cells was also recorded.

The ganglion cells provide a more interesting and convenient site for investigation of the functions of horizontal cells (although in this case the effect of horizontal cells is also mediated through the bipolars). Ganglion cells generate a multiparametric stimulus, the character of which depends on the color, intensity and spatial distribution of the incoming stimulus (9). Properties of stimuli generated by ganglion cells have been well studied. By investigation of the effect of horizontal cells on various parameters of stimuli generated by ganglion cells, the role of horizontal cells in the formation of the final efferent signal from the retina has been elucidated. Fish provide a particularly convenient object for such investigations because there is no special difficulty in recording single responses of horizontal and ganglion cells simultaneously.

Attempts to discover links between the work of horizontal and ganglion cells were made by Witkovsky (10) and Naka and Kishida (11). In both these investigations simultaneous recordings were made of the S-potential and ganglion cell response to photic stimulation. Witkovsky compared physiological characteristics of ganglion and horizontal cells such as threshold, color discrimination, incremental thresholds, amplitude characteristics, and so on. No similarity was found between the characteristics investigated, and Witkovsky concludes that the horizontal cells are not in the direct line of transmission of stimuli from receptors to ganglion cells. The Japanese workers (11) found a similarity between the relationships of amplitude of the S-potential and duration of the latent period of the ganglion cell response, on the one hand, and stimulus intensity (in the region of high intensities) on the other. On this basis they suggest that latency duration is directly determined by S-potential amplitude. However, if the experiments are performed in this manner, no unambiguous conclusions can be drawn regarding the role of horizontal cells in the ganglion cell response. Correlations found in experiments in which light is the only stimulus may simply be due to the fact that both horizontal and ganglion cells are receiving stimuli from a common source—the receptors.

In this investigation the presence and character of interconnections between horizontal and ganglion cells was investigated by direct electrophysiological experiment: the horizontal cell was polarized directly through an intracellular microelectrode and ganglion cell activity was recorded simultaneously.

METHOD

An isolated pike *(Esox lucius)* retina was placed in a moist chamber cooled with ice. Before the experiment the retina was usually kept for 30 min in an atmosphere of oxygen. The retina was illuminated diffusely on the receptor side by two stimuli of widely different spectra: one of 700 mμ and longer, the other shorter than 470 mμ. The spike response of the ganglion cells was recorded extracellularly by platinized metallic electrodes (12), and the S-potential was recorded by the usual liquid microelectrodes filled with 3M KC1, with an impedance of 5—10 MΩ. Both electrodes were introduced into the retina from the receptor side by means of two independent micromanipulators under visual control. The horizontal distance between the recording electrodes was 300-500 μ.

The horizontal cell was polarized through a separate liquid microelectrode (6—10 MΩ) glued to the recording electrode so that their tips were at the same level vertically and the horizontal distance between them was 10—20 μ. Despite the fact that two relatively thick microelectrodes were inserted into the horizontal cell, they caused little damage to it, as shown by the duration of stable recording of responses (for tens of minutes) and by the amplitude of the recorded potentials (20—50 mv).

Some experiments were carried out with one intracellular microelectrode which was used both for polarization of the horizontal cell and for recording its response. The maximum current passed through the cell was 2.5×10^{-6} amp. Since measurements showed that the input impedance of the horizontal cell was 20—30 KΩ, its maximum possible polarization was of the order of 50—75 mv.

The electrical circuit used in the experiments is illustrated in Fig. 1.

RESULTS

Attention in this investigation was concentrated mainly on determining whether a response to polarization of the horizontal cell arises in the ganglion cell and, if so, on the study of its properties.

Experiments were carried out on 90 pairs of cells (horizontal—ganglion). All responses of horizontal cells in the investigated pairs were S-potentials of the L-type. The maximum response to photic stimulation was 20—50 mv.

Intracellular polarization of the horizontal cell in 56 of 90 cases evoked a spike response of the ganglion cell. As a rule, to produce this response, polarization of the horizontal cell of 5 mv was sufficient. The current passing through the cell in this case was about 2×10^{-7} amp. After withdrawal of the polarizing electrode from the horizontal cell, passage of a current 5—10 times larger did not evoke a ganglion-cell response. This control indicates that the ganglion cell responds to polarization of the horizontal cell itself and not to polarization of the retina as a whole.

Usually if polarization of a horizontal cell by 5—10 mv evoked no response from the ganglion cell, neither could it be evoked by polarization of maximum strength (2.5×10^{-6} amp). Also in this case no response could be produced when the stimulating electrode was brought closer to the recording electrode and introduced into another horizontal cell lying closer. The absence of ganglion cell response to polarization of the horizontal cell was evidently due to properties of the ganglion cell and not of the horizontal. Activity of 34 such ganglion cells was recorded. As a rule, they possessed no spontaneous activity, they had higher photic thresholds, and they responded to any diffuse illumination with short discharges (1—2 spikes).

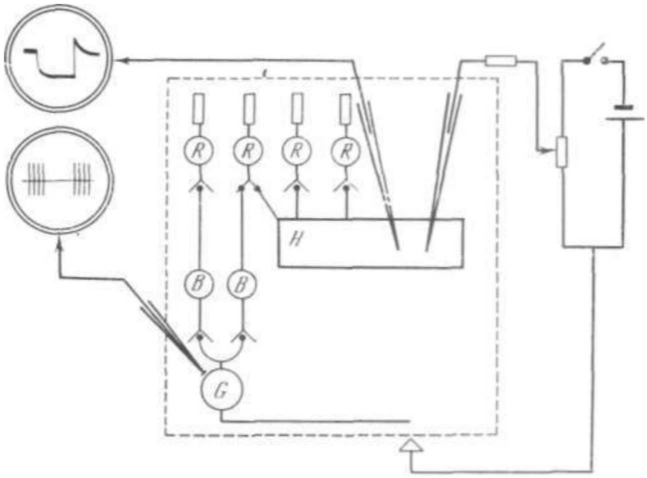

Fig. 1. Circuit for intracellular polarization of horizontal cell and simultaneous recording of responses of ganglion and horizontal cells. R—receptors; H—horizontal cell; B—bipolars; G—ganglion cell.

As mentioned above, 56 ganglion cells responded to polarization of horizontal cells. Practically all possible types of responses were observed: a transient on-response to polarization, spike activity throughout the period of polarization, an off-response to polarization, and slowing or, conversely, quickening of spontaneous activity, and so on. Usually the responses of the ganglion cells to depolarization and hyperpolarization of the horizontal cells were opposite in character, i.e., the on-response to hyperpolarization was of the same type as the off-response to depolarization, and vice versa. The latent period of spike generation by the ganglion cells as a rule depended on the magnitude of polarization of the horizontal cells: the higher the polarization level the shorter the latent period. In cases when a ganglion cell responded throughout the period of polarization, the frequency of spikes in its response increased with an increase in the level of polarization of the horizontal cell (Fig. 2).

Ganglion cells responding to polarization can be subdivided into several classes, depending on the type of response. It was usually found that cells belonging to the same class responded similarly to polarization of horizontal cells.

1. *Cells responding to red light mainly by off-discharges.* Cells of this type give an off-response to red light of low and high intensity; an on-off response to stimuli of intermediate intensities. As a rule these cells respond to blue light by a prolonged on-discharge.

Ganglion cells of this class" give an on-discharge to hyperpolarization and an off-discharge in response to depolarization. This discharge is very similar in configuration to the response to diffuse illumination. In particular, the cell whose response is illustrated in Fig. 3 gave an off-response to red light consisting of 1, 2, or 3 volleys depending on the intensity of the light and the conditions of adaptation. By varying the

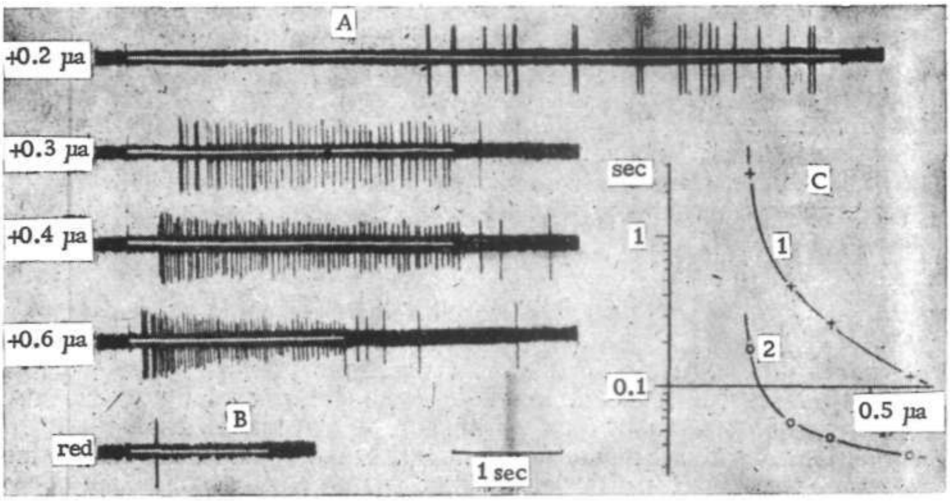

Fig. 2. Response of ganglion cell to polarization of horizontal cell. A) Responses of ganglion cell to depolarization of horizontal by current of increasing strength (magnitude of polarizing currents in μa). White lines inside tracings mark stimulation; B) response of cell to red light (two spikes). Cell did not respond to blue light nor to hyperpolarization of horizontal cell; C) latent period of response (1) and mean duration of intervals between spikes for established discharge (2) as functions of strength of depolarizing current.

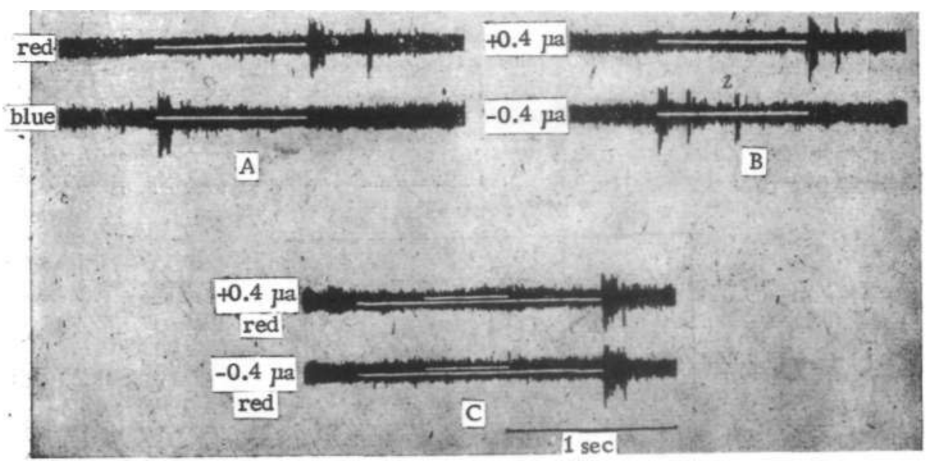

Fig. 3. Cell response of predominantly off-type to red light. A) Cell response to red and blue light; B) cell response to depolarization (1) and hyperpolarization (2) of horizontal cell; C) absence of response to depolarization, against red background (marker of stimulation within curves; upper beam—electrical stimulation, lower beam—red light).

current strength, exactly the same off-responses to depolarization of the horizontal cell could be obtained.

2. *Ganglion cells with spontaneous activity in darkness.* Switching on a red light inhibits, while switching on a blue light quickens the spontaneous activity of these cells.

To red light these cells give an off-response consisting of a transient increase in the frequency of spontaneous activity. Depolarization of the horizontal cell slows while hyperpolarization quickens the spontaneous activity of these ganglion cells. At the time of switching off depolarization of the horizontal cell, spontaneous activity of the ganglion cell quickens for a short time compared with the level in darkness (Fig. 4).

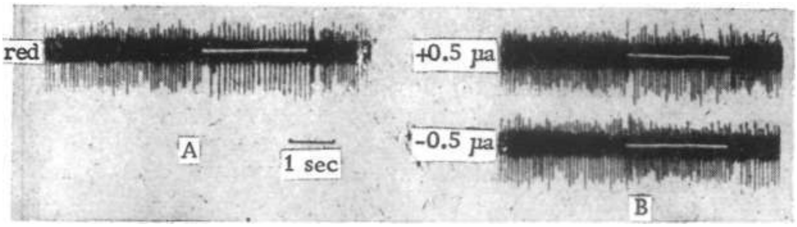

Fig. 4. Ganglion cell with spontaneous activity in darkness. A) Inhibition of spontaneous activity by red light; B) cell response to depolarization (inhibition of spontaneous activity) and hyperpolarization (quickening of spontaneous activity) of horizontal cell.

3. *Cells responding to red light with on-discharge.* These cells are directly opposite to ganglion cells of the first class in the character of their responses. They respond to red light of all intensities by a prolonged on-discharge. To blue light these cells usually respond by an off-discharge. Depolarization of the horizontal cell evokes an off-response in this class of ganglion cells. They also respond to hyperpolarization of the horizontal cells by an off-discharge.

4. *Cells of on-off type.* These cells respond to both red and blue light by on- and off-discharges (Fig. 5A). As Fig. 5B shows, these ganglion cells respond to depolarization and hyperpolarization of the horizontal cells by on- and off-discharges.

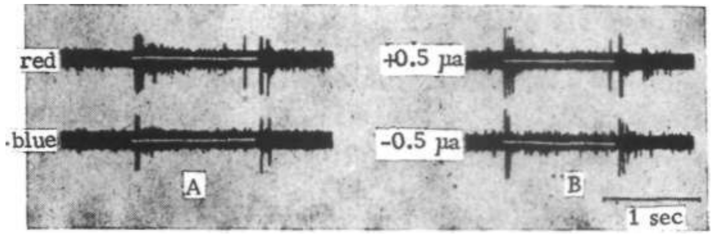

Fig. 5. Ganglion cell of on-off type. A) Cell responses to red and blue light; B) cell responses to depolarization and hyperpolarization of horizontal cell.

When responses of ganglion cells of these different classes to light and to polarization of the horizontal cells are compared, it is clear that depolarization of horizontal cells gives rise to responses in ganglion cells analogous to their responses to red light. The character of the ganglion-cell response to polarization of the horizontal cell is determined purely by properties of the ganglion cell (i.e., it is comparable to the character of its response to light) and is independent of the properties of the horizontal cells, at least horizontal cells of the L-type.

About 20% of ganglion cells responding to polarization of horizontal cells did not fit into this classification. These include both cells belonging to one of these classes in the character of their response to light but responding differently to electrical stimulation, and also cells which could not be placed in any class whatever. For example, in two cases ganglion cells did not respond at all to diffuse illumination but, nevertheless, responded regularly to polarization of horizontal cells. In particular, the cell whose responses are shown in Fig. 2 belongs to this group of "unclassified" ganglion cells.

Hence, in experiments using two independent stimuli (electrical and photic) the ganglion cell was shown to respond to polarization of the horizontal cell by spike generation. Another interesting problem is that of how one stimulus influences the response of the ganglion cell to another stimulus. The effect of one type of stimulation on the other was best seen in experiments in which one stimulus was chosen close to threshold while the other, by contrast, was strong. Preliminary experiments showed that under the influence of a strong red background the response of a ganglion of off-type to intracellular polarization of the horizontal cell is suppressed (Fig. 3C). Changes in the response of ganglion cells of this same class to light can also be observed under the influence of polarization of the horizontal cell. It is possible to select a red stimulus for cells of this class of such an intensity that they change from the off-state to the on-off-state. Depolarization of the horizontal cell in this case abolishes the on-response.

DISCUSSION

The fact should be noted that very slight polarization of the horizontal cell (5—10 mv) evokes a response in the ganglion cell. Potentials of this order (tens of millivolts) arise in horizontal cells during photic stimulation of the retina. Hence, under natural conditions (during photic stimulation of the retina), the effects of horizontal cells on ganglion-cell responses described above must also take place.

Dendritic branches of ganglion cells in different animals do not exceed 1.5 mm in length (13, 14), i.e., they are much smaller than the receptive fields of the horizontal cells, which in fish may extend up to 10 mm (15). Polarization of horizontal cells can thus be produced by illumination of receptors with which the ganglion cell is not directly connected. In this case (just as in experiments with intracellular microelectrodes) the only stimulus for the ganglion cell will be natural hyperpolarization of adjacent horizontal cells. Such polarization, as the present investigation shows, evokes a response in the ganglion cells. This is how the opposite character of the on-off type of center and periphery of receptive fields of ganglion cells can be organized.

This problem will be examined more closely. Ganglion cells were classified purely on the basis of their response to diffuse light. The precise structure of the receptive fields has not been ascertained experimentally. However, according to the work of Wagner et al. (16), cells responding to diffuse red light with an off-discharge have receptive fields with a red off-center and on-periphery; cells responding to this stimulus with an on-discharge have a red on-center and off-periphery. The dimensions of the dendritic branches of ganglion cells are comparable with the dimensions of the center of their receptive fields (17). Consequently, the periphery of the receptive field must be organized from interneurons transmitting excitation horizontally. It can be concluded from the results of this investigation that in fish these interneurons are horizontal cells.

As an example the organization of the receptive field of a ganglion cell with red off-center and on-periphery can be considered. According to the classification used in this paper, this cell belongs to the first class. During illumination of the peripheral parts of the receptive field of the ganglion cell by red light, hyperpolarization arises in the horizontal cells and, as the present experiments show, this evokes an on-discharge in the ganglion cell.*

Horizontal cells of L-type thus are responsible for the property of oppositeness of center and periphery of the receptive field of ganglion cells. However, the results of this investigation are insufficient to explain the color-opposite properties of ganglion cells. Horizontal cells of RG-type evidently play a role in the organization of these properties. Experiments with polarization of horizontal cells of the RG-type can probably shed light on the organization of color properties of receptive fields of the ganglion cells.

*The character of response of the ganglion cell to illumination of the center of its receptive field (in this case, an off-response) is determined by the specific nature of its direct connections with receptors through bipolars.

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