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DOGFISH GANGLION CELL DISCHARGE RESULTING FROM EXTRINSIC POLARIZATION OF THE HORIZONTAL CELLS

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SUMMARY

1. Ganglion cell discharges were evoked by extrinsic polarization of the horizontal cells in the retina of the smooth dogfish (*Mustelus canis*). Depolarization of the horizontal cell gave rise to a discharge similar to that evoked by a spot of light (centre type response) and hyperpolarization of the horizontal cell, a discharge similar to that by an annulus (surround type response).

2. Procion dye injection established that the current-passing electrode was sometimes located in the external horizontal cell. Other possibilities, such as middle and internal horizontal cells, were neither confirmed nor excluded.

3. Activation of ganglion cells by current was possible under completely dark-adapted conditions and for several log units above this level.

4. Depolarizing current enhanced the ganglion cell response evoked by a light spot in the centre of its receptive field; hyperpolarizing current antagonized the response to the same flash.

5. The results are consistent with the supposition that a potential change in the horizontal cell, irrespective of its polarity, or whether produced by light or current, spreads within a laminar layer (the S-space). The effect of the potential change is to modulate the response of bipolar cells and their input into the ganglion cell.

INTRODUCTION

Maksimova (1970) has shown that in the pike retina, polarization of the horizontal cell by extrinsic current elicited ganglion cell discharges. She further noted that the current-induced discharges were very similar to

those evoked by photic stimuli. In the catfish (Naka & Nye, 1971) it was demonstrated that a depolarization of the horizontal cell by extrinsic current gave rise to a ganglion cell discharge similar to that evoked by a spot of light placed at the receptive field centre, a hyperpolarization by current elicited a discharge very similar to that evoked by annular light.

These results have established beyond any doubt that in the teleost retina horizontal cells are involved in the organization of the ganglion cell receptive field. Maksimova's results in pike retina pertain to light-adapted, colour coded ganglion cells and the ganglion cells studied in the catfish were also driven by the cone system (Naka & Nye, 1970).

In this paper, using techniques similar to those just described we examine the role of the horizontal cell in the smooth dogfish (*Mustelus canis*), known to possess an almost all-rod retina (Stell, Wagner & Wolbarsht, 1970; Stell & Witkovsky, 1972). We will conclude that the part played by the horizontal cell in the organization of the ganglion cell receptive field is practically the same in the rod-dominated dogfish retina under scotopic conditions as in the light-adapted teleost retinas.

METHODS

The eyecup preparation of the dogfish, *Mustelus canis*, was used throughout the experiments. The eyecup was prepared under room light and was then placed in a dark chamber into which cold, moist oxygen was introduced. The preparation was usually dark-adapted 15–30 min before beginning the experiment. However, in some cases, as will be described later, care was taken to dissect the eye under red light and the eye was subsequently completely dark-adapted. Spike discharges and S-potentials were generated for up to 5 hr by the eye cup preparation.

Standard electrical and photographic equipment was used to record the electrical responses. Ganglion cell discharges were picked up by a low resistance platinum plated tungsten electrode. Polarization of the horizontal cell was accomplished by injecting current through a 2 M potassium citrate-filled glass pipette (a series resistance of 1000 MΩ was provided to stabilize the current). The magnitude of the current was measured across the preparation and the ground by a chopper stabilized amplifier (Model 231 amplifier, Analog Devices, Cambridge, Mass.). The photostimulus was obtained from a tungsten lamp and the intensity of the flash controlled by a balanced neutral wedge. The irradiance of the flash at the retinal surface was 3·68 $\mu W.cm^{-2}$ with no attenuation by the wedge.

RESULTS

Response of the ganglion cells evoked by a photic stimulus

Ganglion cells fired either at 'on' or 'off' to small spots (less than 2·0 mm in diameter) located at the point of maximum sensitivity. Annular flashes typically evoked responses of the opposite mode, i.e. centre 'on' and surround 'off', or the reverse. Centre-surround antagonism was

generally demonstrable. We made no systematic study of the receptive field dimensions or properties but our findings are in accord with those of Stell, Detwiler, Wagner & Wolbarsht (1971).

In comparison with the catfish, dogfish ganglion cells were more variable in their responses to light flashes. Threshold latencies varied from less than 100 msec to several hundred msec in different units. Discharge patterns for suprathreshold stimuli varied from tonic to highly phasic from cell to cell.

Ganglion cell responses evoked by extrinsic current

Any dogfish ganglion cell could give rise to spike discharges in response to a polarization of the horizontal cells (see Discussion). The magnitude of the current required to give the discharge was of the order of 5–20 nA when the injecting electrode was located approximately 0·3 mm away from the spike recording site. This is comparable to the value obtained in the catfish retina. The magnitude of current to give a fixed number of discharges was clearly a function of the distance between two electrodes, although no systematic examination of this point was attempted.

We have already mentioned the fact that photic stimuli gave rise to various types of discharge patterns from the ganglion cells. Current injection, on the other hand, produced a clearer picture, in that any given ganglion cell could be classified as one of two distinct types according to its response to extrinsic polarization of the horizontal cells. Adopting the convention used for classification of catfish neurones (Naka & Nye, 1971), we will refer to them as type *A* and *B* ganglion cells.

Records shown in Fig. 1*A* were from a type *A* ganglion cell where a depolarization of the horizontal cell gave rise to an 'on' discharge, a hyperpolarization, an 'off' discharge, and a central spot of light, an 'on' discharge. Although it is not shown in this record, an annulus gave rise to an 'off' discharge. Similar records in Fig. 1*B* show responses from a type *B* cell (an annulus gave 'on' discharges). The discharge characteristics of the type *A* and type *B* cells are summarized in Table 1 which is practically identical to a comparable Table for the catfish ganglion cell. It is seen that, as in the catfish, the two types of ganglion cells showed a complementary discharge to both light and current stimulation. According to the classification adopted, the type A ganglion cell is an 'on' centre unit while the type *B* cell is an 'off' centre unit.

In Fig. 2 are shown results of an experiment in which discharges from three ganglion cells were recorded simultaneously. The spikes with the largest amplitude are marked as no. 1, the medium-size spikes as no. 2 and the small spikes as no. 3. Polarization of a horizontal cell caused all three ganglion cells to discharge. A depolarization (Fig. 2*A*) evoked an 'on'

discharge of no. 3 spikes and an 'off' discharge of nos. 1 and 2 spikes; a hyperpolarization reversed the response patterns of all three cells (Fig. 2*B*). In the dogfish retina, as in the catfish, polarization of a horizontal cell can evoke responses in both type *A* and *B* ganglion cells, thus excluding the possibility that a particular horizontal cell activates only one type of ganglion cell. The difference in the organization of type *A* and type *B* receptive fields may already be established in the bipolar cells (Werblin & Dowling, 1969; Kaneko, 1970; N. Matsumoto & K.-I. Naka, in preparation).

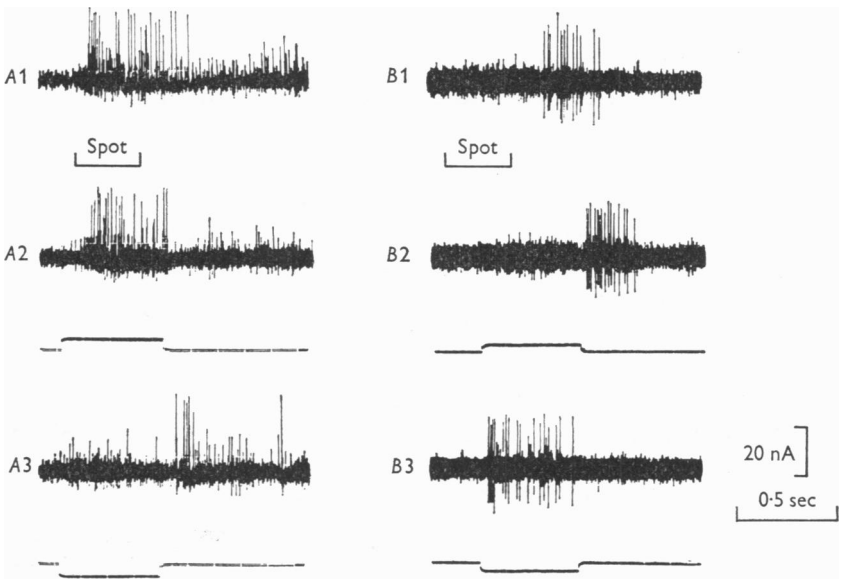

Fig. 1. Comparison of the dogfish ganglion cell discharges evoked by a spot of light (*A* 1 and *B* 1) and discharges evoked by the extrinsic polarization of the horizontal cells (*A* 2 and 3, *B* 2 and 3). The records in column *A* were from a type *A* ganglion cell; the records in the column *B* were from a type *B* ganglion cell. The lower trace is the current record. Upward deflexion of the current trace indicates a depolarization, downward deflexion a hyperpolarization, of the horizontal cells. Approximate timing of the spot flash is indicated. In all records (except those in Fig. 3) the distance between the tips of the current injecting and spike recording electrodes was approximately 0·3 m.

In the dogfish, *Mustelus canis*, Kaneko (1971) has shown that the horizontal cells in a given layer are electrically coupled so that we may think of a given horizontal cell layer as forming a laminar structure (the S-space of Naka & Rushton, 1967), If this is the case polarizing current should be effective in driving a ganglion cell wherever it is injected within the layer, provided its strength is sufficient. In Fig. 3 are shown results from an experiment in which current was injected at six different locations,

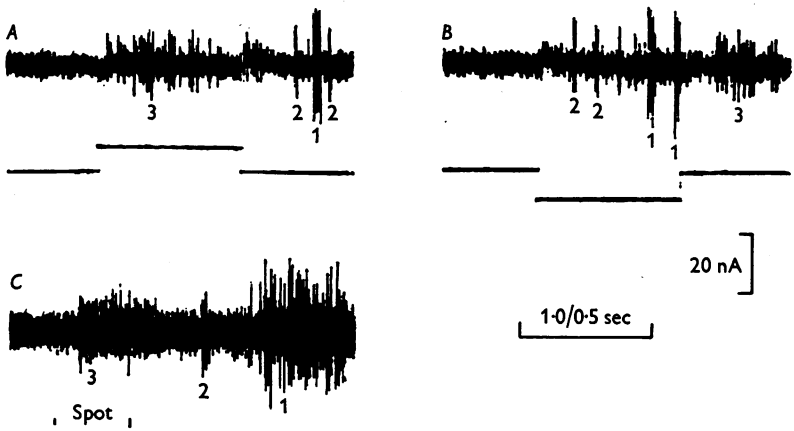

Fig. 2. A multiunit recording of dogfish ganglion cell discharges. In the record three groups of spike discharges are shown. The spikes with the largest amplitude are marked as '1', those of medium amplitude as '2' and the spike discharges of the smallest amplitude as '3'. It is probable that the spikes '3' represent activities of several ganglion cells. Record '*A*' is for a depolarization and '*B*' for a hyperpolarization of the horizontal cells. Record '*C*' is to a spot of light placed at the receptive field centre, recorded at a slower sweep speed. Notice the extremely long latency of the spikes '1'.

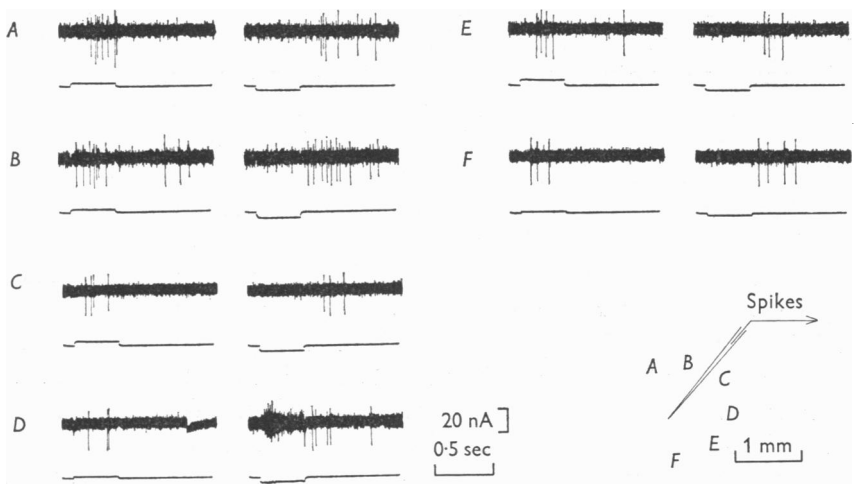

Fig. 3. Records showing the ganglion cell discharges evoked by extrinsic currents injected into the horizontal cells at six different locations. The left column is for a depolarization and right column is for a hyperpolarization. The diagram illustrates the approximate locations of the current injection electrode relative to the spike recording site. The burst of spike-like noise in record '*D*' was due to instability of the current injecting electrode, as seen from corresponding noise in the current record (lower trace).

each approximately 0·8 mm away from the spikes recording site. Currents injected at all locations were effective in inducing the cell discharge and in all cases a depolarizing current induced 'on' discharges while a hyperpolarizing current elicited the opposite response. We repeated this experiment several times and in all cases it was possible to drive the ganglion cell.

Results from these experiments suggest that in the dogfish, as in the tench or in the catfish, the horizontal cells form a laminar structure through which a potential change, whether induced by light or by extrinsic current of either polarity, can spread to exert its influence on all ganglion cells. Thus polarization of any given horizontal cell will influence numerous bipolar cells.

Interaction between light and extrinsic current

In Fig. 4*A* the interaction between the light stimulus and current was examined in a type *A* cell, in which a central spot of light gave rise to an 'on' discharge. A weak depolarizing current, which by itself failed to evoke any response enhanced the 'on' discharge evoked by the light stimulus (Fig. 4*A* 2). A hyperpolarizing current was delivered during the illumination, resulting in a depression of the 'on' discharge to the light flash alone (Fig. 4*A* 3). Records in Fig. 4*B* are from a type *B* cell where a central spot of light gave rise to an 'off' discharge. A depolarizing current delivered during the 'off' discharge depressed the discharge whereas a hyperpolarizing current enhanced it. Table 1 summarizes the results of the interaction experiments. It shows that the results of any interaction experiment in a type *A* unit can be predicted as follows: depolarization by extrinsic current enhances the discharge evoked by a central light flash ('on' or 'off' discharge) whereas hyperpolarization depresses the light induced response. In a type *B* unit these relationships are reversed.

In the catfish retina, both polarities of current passed through the S-space were effective in driving the ganglion cell; one polarity elicited 'on', the other 'off' discharges. In the dogfish, however, it was sometimes found that a current of one polarity could induce a ganglion cell discharge whereas the current of the opposite polarity failed to elicit spikes. In these cases it was always depolarizing current that evoked spike activity.

Nevertheless, the current which by itself failed to evoke spike discharges still depressed the ongoing discharge to a photic stimulus and, under such conditions, the current may elicit an 'off' discharge. Fig. 5 illustrates these two points. Records in Fig. 5*A* and *B* show that a depolarizing current induced spike discharges whereas a hyperpolarizing current failed to do so. Notice that this same hyperpolarizing current evoked responses from another ganglion cell producing spike potentials of a much smaller amplitude. This excludes the possibility that the failure of

the current to evoke a response was due to an inability of the electrode to carry a current of a given polarity. In Fig. 5*C* is shown the 'on' discharge of the cell resulting from illumination by a centrally located spot (indicating that this cell was a type A unit). In Fig. 5*D* and *E*, hyperpolarizing currents were delivered during the 'on' discharge evoked by

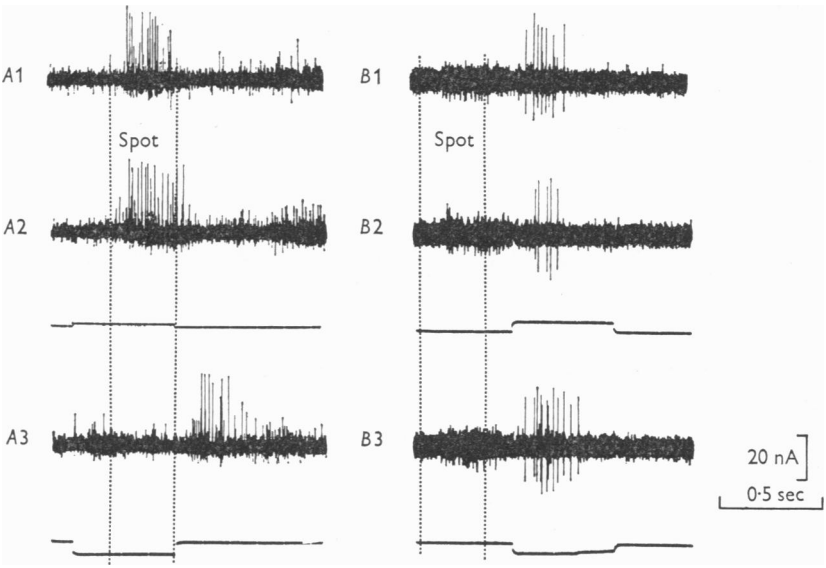

Fig. 4. Interaction between the light input (a spot of light) and artificial polarization of the horizontal cells. Column '*A*' shows that the 'on' discharge to a spot of light (type *A* ganglion cell) was enhanced by a depolarization of the horizontal cells (*A* 2, current alone failed to evoke any discharge) and was depressed by a depolarization (*A* 3). Column *B* shows that similar interaction can be seen for the 'off' discharges in a type *B* ganglion cell. Approximate period of the flash is shown by dotted lines.

TABLE 1. Discharge mode of the dogfish retinal ganglion cells evoked by photic or extrinsic current stimulation

		Spot or depolarization	Annulus or hyperpolarization
Type A	Response	On	Off
	Interaction	Enhancement	Depression
Type B	Response	Off	On
	Interaction	Depression	Enhancement

the photic stimulus. The current, although not effective when given alone as shown in Fig. 5*B*, depressed the ongoing discharge and, at the cessation of the current stimulus, 'off' discharges were evoked. This observation does not contradict the argument that the stimulus responsible for the

'off' discharge gives rise to an inhibitory response and that the 'off' discharge results from a rebound from hyperpolarization (see Discussion). The 'off' discharge evoked by current in Fig. 5*D* and *E*, can be explained by assuming it is a rebound of the depressed light induced excitatory period.

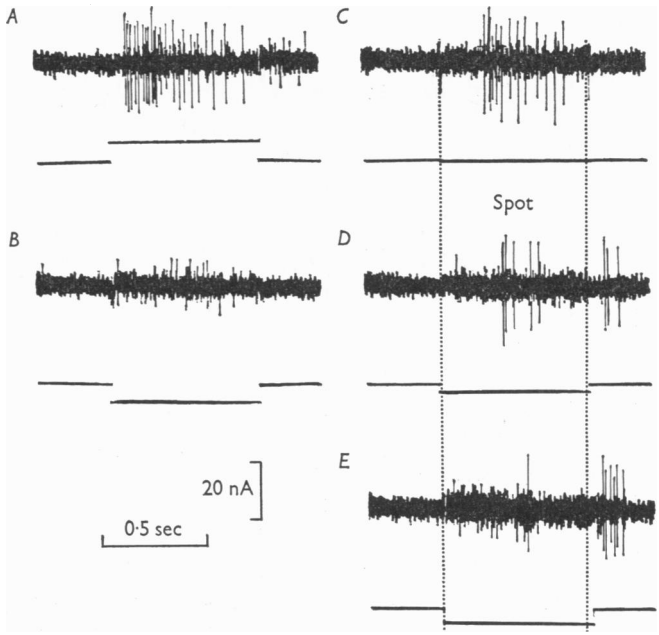

Fig. 5. Records showing an asymmetry of the current induced ganglion cell discharge in the dogfish. A depolarization of the horizontal cell gave rise to 'on' discharges (*A*) similar to that by a spot of light (*C*). However, a hyperpolarizing current failed to give rise to any response (*B*). Note that the spike discharges of much smaller amplitude were elicited by the hyperpolarizing current. In '*C*' and '*D*' currents were superposed on the spot flash which resulted in a decrease in the number of the light induced spike discharges accompanied by an appearance of the 'off' discharges from the current injection.

Effect of adaptational state

Although few in number, cones are found in the smooth dogfish retina (J. Dowling, personal communication). This raises the possibility that the spike discharge observed so far might have been associated with the cone system.

This possibility is considered to be remote on the following grounds. Assuming a colour temperature of the tungsten lamp to be around 2500° K, the radiant distribution of the lamp plus heat filters can be calculated (it makes little difference to the final result if values of 2000 or 3000° K are assumed). The maximum energy produced at the retinal surface was 3·68 μW/cm^2, measured photometrically. For the lamp distribution taken above, this is equivalent to about $1{\cdot}1 \times 10^{13}$ quanta/cm^2.sec.

Ganglion cell thresholds averaged 5·0 log units below this level. Taking the rod diameter as 2 μm, and assuming perfect tapetal reflexion, no absorption by pre-retinal media, a photopigment density of 0·3 and an absorption coefficient for the white light of 0·19, about 1 rod in 7 absorbs a quantum at ganglion cell threshold (the pigment density and difference spectrum of the rod pigment were kindly supplied by H. Ripps). Thus our data were obtained at light levels clearly in the rod operating range. Our stimulus diameter was 1·2 mm, an area encompassing $3·6 \times 10^5$ rods, so at threshold about 5×10^4 quanta were absorbed during a 0·5 sec stimulus.

To test further this possibility we prepared the eyecup, located a ganglion cell by a tungsten electrode and positioned a glass pipette on the retina under dim red light. The preparation was then dark-adapted for 30 min, after which time a horizontal cell was located using a dim test flash. A current delivered through the horizontal cell electrode under these conditions could still evoke ganglion cell discharges. We did not notice any significant difference in the magnitude of the current to induce spike discharges nor in the mode of its action. After further dark adaptation of 30 min, during which the ganglion cell threshold had decreased by 0·8 log units, there was no appreciable difference in the action of extrinsic current. We therefore conclude that the effects of extrinsic polarization of the horizontal cell operate under scotopic conditions when rods are the active photoreceptor elements.

Localization of the current-passing electrode

Anatomical studies reveal that there are three classes of horizontal cells in the smooth dogfish retina: external, intermediate and internal, all of which send processes towards receptor bases (W. K. Stell & P. Witkovsky, in preparation). Kaneko (1971) has shown with Procion marking methods that cells in the external and one of the other two layers respond to light with typical hyperpolarizing S-potentials. Any or all of these cell layers are therefore candidates for the present findings. We applied the Procion dye injection technique to clarify this question. Of nine horizontal cells stained, eight cells were identified as the external horizontal cells and the nature of the ninth cell could not be identified definitely.

DISCUSSION

The retina of dogfish shark, *Mustelus canis*, has been the subject of recent studies on the receptive field organization of ganglion cells (Stell *et al.* 1970, 1971), on the electrical coupling of horizontal cells (Kaneko, 1971) and light- and electronmicroscopic studies (W. K. Stell & P. Witkovsky, in preparation; P. Witkovsky & W. K. Stell, 1971, in preparation). Although a few cones have been identified in the retina, it can be described as a predominantly rod retina morphologically and functionally. The spectral

sensitivity of both the ganglion cell discharge and the horizontal cell response correspond closely to a rhodopsin pigment spectrum (Stell *et al.* 1970; P. Witkovsky, unpublished data). This is in contrast to the two previous studies on teleost fish, where the horizontal cell-ganglion cell interaction has been investigated in the cone dominated system (Maksimova, 1970; Naka & Nye, 1971). However, in both the dogfish and the catfish the prominent feature of the retina is the large horizontal cells which occupy nearly two thirds of the inner nuclear layer.

In the pike retina Maksimova (1970) has reported that of the ninety pairs of horizontal cell-ganglion cell recordings the discharge could be induced in fifty-six pairs, so that some ganglion cells could have received signals through a pathway not involving the horizontal cells. On the other hand, in the catfish retina the findings suggest that all ganglion cells could be activated through polarization of the horizontal cells (Naka & Nye, 1971). In the dogfish where more than 100 pairs have been tested we failed to establish a clear case where polarization of the horizontal cell could not evoke a ganglion cell discharge. Although it is impossible to exclude an exception, we conclude that in the retina of the dogfish all ganglion cells receive signals from the horizontal cells.

Kaneko (1971) has shown elegantly that the horizontal cells of a given layer in the dogfish retina are electrically coupled, indicating that they form a laminar structure referred to as the S-space in the teleost fish (Naka & Rushton, 1967; Naka, 1971). The electrical coupling of horizontal cells is the simplest assumption which can explain the observation made in this paper, namely that currents of both polarity can spread in all directions within the layer formed by horizontal cells. The anatomy is consistent with the coupling data in that gap junctions are seen between horizontal cells of the same horizontal layer (P. Witkovsky & W. K. Stell, in preparation).

The results obtained in this paper, as summarized in Table 1, are remarkably similar to what was seen in the catfish (and to a lesser degree in the pike) suggesting that the role of the horizontal cell and the mechanisms to produce the basic receptive field response (of the ganglion cell and probably of the bipolar cell, too) are identical in the retinas so far studied by the current injection experiment. The fact that dogfish ganglion cell receptive fields are more diverse in their functional characteristics than those observed in the catfish, may be related to the greater morphological diversity of bipolar and ganglion cells observed in the dogfish retina (P. Witkovsky & W. K. Stell, in preparation; W. K. Stell & P. Witkovsky, submitted, and work in preparation). Comparable anatomical studies of catfish retina have not yet been carried out. The functional diversity referred to may be seen in Fig. 2 where the latency of discharge of two, simultaneously

recorded, ganglion cells to a current pulse passed through the horizontal cells, differ greatly. Secondly, in contradistinction to the catfish data, current of one polarity often failed to evoke responses while current of the opposite polarity did induce spike firing.

Nevertheless, the basic rule as indicated in Table 1, which governs ganglion cell discharge mode, is derived in dogfish retina. The results in Table 1 are consistent with a simple interpretation expressed as follows: The stimulus of one mode (a spot of light or depolarization of the horizontal cell in a type *A* unit) gives rise to a depolarization in a bipolar cell whereas the stimulus of opposite mode (annulus or hyperpolarizing current) gives rise to a hyperpolarization. The ganglion cell 'on' discharge or an enhancement of the ongoing discharge results from the bipolar cell depolarization, a hyperpolarization depresses the ongoing discharge. The 'off' discharge results from the rebound from hyperpolarization, a phenomenon seen by Kaneko (1970) in the goldfish bipolar cell. The rebound from a depolarization can give rise to a period of depression, and such a depression (a period of hyperpolarization) has been seen in the goldfish bipolar cell and in the catfish ganglion cell discharges (Figs. 9 and 10 in Naka & Nye, 1971).

The argument presented above tacitly assumes that the ganglion cell activity reflects the polarization of the bipolar cell membrane, a reasonable assumption in a situation where a simple stimulus configuration is employed.

It is supposed that the current injected into the horizontal cell spreads in the laminar S-space and produces in the bipolar cells a potential change similar to that evoked by a photic stimulus. The pathway whereby bipolar cells are activated by horizontal cells is uncertain. It has to be effective for both de- and hyperpolarization of the presynaptic (horizontal cell) membrane, although this does not necessarily indicate an electrical coupling between the two cell types. In both the dogfish rod spherules and in the catfish cone pedicles, a bipolar cell process is flanked by horizontal cell processes. Thus polarity changes in the horizontal cell might affect receptor to bipolar cell transmission in the sequestered extracellular space of the photoreceptor base. Alternatively the bipolar cell might sense the difference between two independent inputs, one from the receptor and the other from the horizontal cell, an hypothesis favoured by Naka (1971).

It is interesting to note that in both the cone-dominated and rod-dominated retinas, two distinct classes of signals are processed in the external plexiform layer. One is the local signal (cf. Naka & Nye, 1970) which can be replaced by a depolarization of the horizontal cell, the other is the integrating signal which relies on the geometrical configuration of the laminar structure formed by the horizontal cells. The potential inside the laminar structure reflects the average intensity received by a particular

area at a particular moment, whereas the local signal is a direct receptor input to the bipolar cells. These two signals interact within the bipolar cell and the results of such interaction are transmitted to the ganglion cells. Probably this is one of the basic functions of the external plexiform layer.

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