Role of Horizontal Cells in Organization of the Catfish Retinal Receptive Field

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THE RECEPTIVE-FIELD organization of the vertebrate retinal ganglion cell has been the subject of numerous studies. However, the cellular mechanism underlying the receptive field has been explored relatively rarely. One exception can be found in a recent study of the mudpuppy retina where responses were recorded from various retinal neurons (17) and it was shown that the polarization of a bipolar cell by a central spot could be antagonized by an annular illumination.

The receptive field in the fish can be as large as 5 mm in diameter (3) and this clearly implies that the signal produced at the periphery of such a field must travel laterally at least 2 mm to reach the receptive field center. The morphology of the vertebrate retina suggests two possible neuronal structures which could account for the lateral transmission of the signal in a receptive field; one is the row of horizontal cells and the other the amacrine cells. Naka and Rushton (13) have shown in the tench that the row of horizontal cells behaves as if it were a laminar conductive medium bounded by two parallel membranes and they have referred to this structure as the S space. Any potential change arising within the S space propagates passively according to a given space constant. It is significant that in a recent paper Kaneko (6) could demonstrate in the dogfish electrical coupling among neighboring horizontal cells.

In a previous paper (12) we analyzed the receptive field of a channel catfish ganglion cell (referred to here as type B) which responded to a spot of light with an "on-off" discharge and to an annulus with a sustained discharge. For receptive-field studies, the channel catfish retina has two distinct advantages; first, the responses do not appear to be color coded, making analysis simple; and second, the ganglion cell gives rise to two distinct discharge patterns closely associated with two different stimulus patterns, namely, a spot and an annulus. In the same paper it was shown that the channel catfish receptive field involved at least two lateral transmission systems: one located at a distal level of the retina and the other at a more proximal level. The distal transmission system possessed distinctive spatial properties suggesting that it relied on the S space for its function.

In this paper we will first present the results of a further study of the catfish receptive field which will lead to a conclusion that there are two types of receptive fields in the retina (types A and B receptive fields) which are complementary in field organization. Our failure to mention the type A receptive field in the previous paper must be attributed to the fact that the type B field can be found more often during the course of an experiment.

In the second part of this paper we describe the results of a series of experiments in which the ganglion cell discharge was artificially induced by polarizing a horizontal cell. It will be shown that the distal transmission system proposed in the previous paper (12) is indeed formed by the rows of horizontal cells and, moreover, that the results of current injection experiments support our earlier suggestion that the row of horizontal cells form a continuous medium referred to as S space (13).

A preliminary description of these results has already been published elsewhere (11).
MATERIALS AND METHODS

The eye-cup preparation of the channel catfish (*Ictalurus punctatus*) retina was prepared in the manner described in previous papers (10, 12). The optical stimulus system and experimental procedure employed were also the same. A general diagram of the electrode arrangement and electrical circuit is shown in Fig. 1.

The S potentials were recorded by one or two glass pipettes filled with 1–2 M potassium citrate. The resistance of the electrodes ranged from 100 to 300 megohms.

A horizontal cell was polarized by passing a current through an intracellular recording electrode. The magnitude of the current passed was monitored at two points in the circuit; one by means of a nanovoltmeter connected between the voltage source and 1 kohm series resistor, and other by measuring the voltage drop across a 1-kilohm resistor placed between the preparation and ground. A Keithley 150 B microvolt-amperometer was used for the former measurement and a Tektronix 3A9 amplifier employed for the latter role. The two measurements agreed to within 2% of their magnitude. Spike discharge was recorded by means of a low-resistance tungsten microelectrode and amplified by a Bioelectric NFI amplifier.

In some cases the tip of the polarizing electrode was located by injecting a Procion dye, type M4RN. The glass pipette was filled with the dye by a method suggested by D.W. Arnett.

PHOTIC STIMULATION AND RESULTS

Receptive-field organization

In this paper the channel catfish receptive field was examined almost exclusively...
by two stimulus patterns: one, a spot of light placed at the center of the receptive field, and the other, an annulus placed concentrically with the spot of light. In the catfish retina these two types of photic stimuli evoke two discharge patterns: the transient discharge and the sustained discharge. As will be shown later, these two discharge patterns are observed to be closely associated with the two stimulus patterns described above and they result from the activation of two mechanisms underlying the catfish receptive field, namely the "local" and the "integrating" mechanisms. Often these two types of discharges were seen in the presence of spontaneous discharge.

Throughout the present experiments the state of adaptation of the retina was not controlled to any precise degree, but it can be assumed that the retina was moderately dark adapted. Under such conditions, the horizontal cell response and the spike discharge have been shown to be dependent on signals originating from a single type of pigment having a maximum absorption at 620 nm (10, 12; also unpublished results).

In a previous paper (12) we gave an extensive description of a type of receptive field in which a spot of light at the center of the field produced a transient discharge while a concentric annulus gave rise to a sustained discharge. It was found that this type of receptive field was the easiest to locate in the channel catfish retina. Since then, another type of receptive field has been found. In this type of field a spot of light placed at the center of the field produces a sustained discharge while an annulus produces a transient discharge. Thus the response pattern is the reverse of the discharge pattern associated with the field reported earlier.

To simplify further description, the receptive field which responds to a spot of light at the center of the field with a sustained discharge will be referred to as a type A field, while the receptive field which responds to a spot of light with a transient discharge will be referred to as a type B field.

It was our impression that the probability of recording from a type B field was roughly 3 times greater than the probability of recording from a type A field. It is probable that both type A and type B cells are ganglion cells, although the origin of the spike discharge was not established. In some small number of cases receptive fields were found which could not be classified into either type A or type B fields. We have not yet made any systematic exploration of these fields.

Records in Fig. 2 illustrate the typical response of types A and B fields to a spot of light and an annulus. Both stimuli were located at the center of the fields and their intensities covered a range of 4.0 log units. The response of a type A field to a spot was a sustained discharge lasting the duration of the stimulus. In the same field an annulus gave rise to transient discharges as the stimulus was switched on and off. As the intensity of the flash increased, the latency of the off-discharge showed a marked increase. However, although the transient discharge was seen most often when the stimulus was switched both on and off, in some fields only an on- or off-discharge appeared. In all other respects these discharge patterns were stable and were seen over a wide range of intensities.

In a type B field the response patterns were the reverse of those seen in a type A field; a spot of light gave rise to a transient discharge similar to that given by an annulus in the type A field, while an annulus gave rise to a sustained discharge similar to that given by a spot in the type A field. Thus a pair of contrasting stimulus patterns were able to evoke very similar discharge patterns in both fields (Table 1).

Additional observations made with threshold stimuli are also of interest. In both types of cell the threshold response to a stimulus, which at moderate intensity levels would give rise to a sustained discharge, was found to be an on-spike. The threshold response to a stimulus which would normally give an on-off response, however, was found to be either an on- or off-response, with the latter appearing more frequently. Repeated stimulation of both fields, by a stimulus giving rise to a transient response, often induced a spontaneous discharge, a phenomenon which can be seen in Fig. 2 (A2 and B1). On the other hand, a repetitive stimulus evoking a sustained discharge often depressed any existing spontaneous discharge. Indeed a clear inhibitory period...
FIG. 2. PST histograms A1 and B1 show the discharge patterns of types A and B ganglion cells in response to a spot placed at the center of the receptive field. Similarly histograms A2 and B2 show responses to an annulus which was placed at the center of the field. In all four records the intensity of the flash spanned 4 log units. The maximum levels of stimulus intensity were $6.3 \times 10^{11}$ photons/mm$^2$ · sec in A1, $1.6 \times 10^{12}$ photons/mm$^2$ · sec in A2, $1.0 \times 10^{13}$ photons/mm$^2$ · sec in B1, and $1.6 \times 10^{11}$ photons/mm$^2$ · sec in B2. The approximate durations of flashes are shown by a bar at the bottom of each display. All records were averages from eight successive runs and spike discharges were counted in bins of 19 msec width. The diameter of the spot was 0.45 mm and the inner and the outer diameters of the annulus were 0.65 mm and 5.0 mm, respectively.

TABLE 1. Discharge patterns of catfish ganglion cells

<table>
<thead>
<tr>
<th>Types of Ganglion Cell</th>
<th>Type A</th>
<th>Type B</th>
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<tbody>
<tr>
<td>Spot</td>
<td>Sustained</td>
<td>Transient</td>
</tr>
<tr>
<td>Annulus</td>
<td>Transient</td>
<td>Sustained</td>
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<tr>
<td>Depolarization</td>
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<tr>
<td>Hyperpolarization</td>
<td>Transient</td>
<td>Sustained</td>
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<tr>
<td>Interaction</td>
<td>Transient inhibits sustained</td>
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FIG. 3. A and B illustrate the suppression of sustained discharges by the stimuli which gave rise to transient discharges. A is from a type A receptive field when a spot of light at the center of the field was flashed at a fixed intensity while intensity of a concentric annulus was increased over a range of 4 log units. The maximum intensity of the annulus was $2.5 \times 10^{12}$ photons/mm$^2 \cdot $sec. The spot illumination (inhibited) fell during the annular illumination (inhibiting). In the type B receptive field shown in B the intensity of an annulus (inhibited) was kept constant while intensity of a spot of light at the center of the field (inhibiting) was increased over a 4 log unit range. The maximum spot intensity was $6.3 \times 10^{11}$ photons/mm$^2 \cdot $sec. The bin width of the PST histogram A was 25 msec and histogram B was 13 msec. Dimensions of stimuli were same as in Fig. 2.

constant intensity fell during the annulus illumination, the intensity of which was increased over 4 log units. This figure shows that as the intensity of the annulus was increased the sustained discharge induced by a spot diminished, eventually being inhibited entirely and replaced by an on-off response. It should be mentioned that Fig. 3A appears almost identical to Fig. 7 of the paper by Naka and Nye (12), which showed that an annulus of light delivered during a spot illumination fails to evoke a sustained discharge in a type B field; i.e., a spot of light inhibits the sustained discharge produced by an annulus. A similar observation could be made even when a spot of light was delivered during the annular illumination and Fig. 3 shows an example of such an experiment in a type B field. In this record the intensity of the spot (inhibiting), which fell during the period of the annular stimulus, was increased over a range of 4 log units, while the intensity of annulus (inhibited), which gave rise to a sustained discharge, was held constant. It can be seen that the sustained discharge was completely inhibited during the period of the spot provided that its illumination was sufficiently intense. Thus the one common feature of the interaction, which occurs in both type A and type B fields, irrespective of the timing of two stimuli, is the inhibitory effect that the transient discharge always exerts on the sustained discharge.

It was shown previously that, in the type B field, a spot of light gives rise to a transient discharge even when the spot of light is placed at some distance away from the center of the receptive field and moreover, that a spot of light falling on the off-center region is also effective in inhibiting the sustained discharge evoked by an annulus (Fig. 8 of ref 12). Similarly, in a type A field a spot of light was found to give rise to a sustained discharge even when it was placed at some distance away from the center of the field. However, it can easily be seen from the description given so far that if the decentered spot were to be enlarged to encircle the receptive field, leaving the center still unilluminated, the resulting discharge should be of an annular type. In the experiments shown in Fig. 4 the interaction between two stimuli was examined: one, a stimulus of fixed intensity in the form of a central spot in Fig. 4A and an annulus in Fig. 4B, and the other, a surround stimulus which occupied variable portions of an annulus of internal and external diameter 1.34 and 2.85 mm, respectively. The surround started as a decentered spot which was expanded in six
fig. 4. Records showing the effect on the sustained discharge in types A and B fields of increasing the area occupied by a surround stimulus. The area occupied by a segment of a surround annulus (inner diameter, 1.34 mm and outer diameter, 2.85 mm) was increased by 0.2 log units for each ascending step along the ordinate axis while its intensity decreased by 0.2 log units. Thus the number of photons falling on the surround was kept unchanged. In A, a spot of light and in B, an annulus, both centered on the receptive field, were flashed at fixed intensities, each giving alone responses shown at the top trace of the corresponding displays. The maximum intensities of the surround segment (bottom trace for each record) were, for A, \(4.0 \times 10^{12}\) photons/mm\(^2\) · sec and for B, \(10^{13}\) photons/mm\(^2\) · sec. Dotted lines indicate parts of responses evoked by the two stimuli. The approximate durations of stimuli are shown at the bottom of each display. Spike discharges were counted in bins of 12.5 msec width in A and 10.0 msec in B.

steps to form, at the sixth step, a complete annulus. Meanwhile the total energy falling on the surround was kept unchanged. This ensured that the change in the response pattern was due to an increase in the area occupied by the surround and was not due to an increase in the stimulus energy. The surround stimulus configuration possessed one other advantage in that all segments were at the same mean distance from the center of the field and any attenuation factor for a given segment could be applied equally to all other segments.

In Fig. 4A the top trace is the response obtained when a type A field was stimulated by a spot at the center of a field, which gave rise to a sustained discharge. In all other traces in the record a segment of the surround annulus preceded the spot and illuminated the field for a period of longer duration. The segment of an annulus first occupied an area comparable to that of the spot located at the center of the field (Fig. 4A, bottom trace). In the lowermost trace, two stimuli (the spot and the annular segment) gave rise to a sustained discharge composed of an exceptionally long on-discharge from the segment followed by the spot response. For each upward step along the ordinate, the intensity of the segment was decreased by 0.2 log units while the area occupied was increased by 0.2 log units, until at the sixth step the segment encircled the field to form an annulus. As the area of the segment increased, the sustained discharge produced by the spot became progressively less prominent until it was almost totally inhibited. Although the results of a similar experiment in a type B field have already been published (12) they are repeated again in Fig. 4B to facilitate comparison with those obtained from a type A field experiment. As in Fig. 4A the area occupied by the surround segment was extended from a decentered spot to an annulus. Initially the segment illumination gave rise to an on-off response which transformed into a sustained dis-
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charge as the segment encircled the field. This can be seen from disappearance of the marked off-discharge. In presence of the illuminated segment, the annulus gave rise to an on-off discharge which gradually transformed into a sustained discharge with an off-response. Finally, when the surround segment formed a complete annulus the off-response disappeared.

In both types of fields, a decentered spot could give rise to a response pattern which was similar to that produced by a spot at the center of the field. However, the same number of photons, distributed in the form of an annulus spread over a larger area, changed the response pattern to that typical of an annulus and, under such conditions, could inhibit the sustained discharge in the type A field and add to the sustained discharge in the type B field.

The results described so far have clearly indicated that the channel catfish retina is equipped with two types of ganglion cells, termed type A and type B, which have complementary response patterns and field organizations.

In the earlier description of the type B field it was stated that the sustained discharge from the surround behaves as if it were generated by a signal which arises from the integration of stimulation received from a wide area of the retina (provided that the central area of the field is not illuminated). It was also pointed out that the characteristics of the integrating mechanism are similar to those of the S space, formed by a row of horizontal cells. The transient discharge properties of the A field described in this paper also possess properties very similar to those of the sustained discharge in the B field, because it also appears to be dependent on input signals integrated over a wide area. The current injection experiment to be described in a following section will justify this assumption and identify the structure as the space formed by the horizontal cells. A spot of light, on the other hand, activates a local mechanism which responds most effectively to changes in the peak local luminance (12).

Artificial polarization of horizontal cell

In the channel catfish it was possible to evoke the discharge of the ganglion cell by artificially polarizing the horizontal cell (11). The possibility that the observed discharge might have been due to a direct excitation of the ganglion cell by extracellular current flow was excluded by the following observations:

1) The magnitude of the current required to evoke a moderate discharge from a ganglion cell was less than 20 nA when the current was injected 0.15–0.2 mm away from the spike-recording site. Furthermore, current as small as 1–5 nA was effective in producing the threshold response from a ganglion cell. This amount of current was comparable to that commonly used to excite a neuron intracellularly.

2) Polarization of the horizontal cell was effective in inducing the ganglion cell discharge only when the electrode was capable of recording a large horizontal cell response (S potential). The same current passed immediately after dislocation of the electrode from the horizontal cell was not effective.

3) As will be shown later, current injected as far as 2 mm away from the spike-recording site was still effective in producing a ganglion cell response. It is difficult to accept the premise that current injected through a glass pipette could directly stimulate a ganglion cell, particularly at such a large distance.

4) Experiments were repeatedly performed in which a Procion dye injected immediately after evoking the ganglion cell discharge could stain a horizontal cell. Identification of the injected neuron clearly established that the stimulation was achieved through the electrode located inside a horizontal cell.

Records in Fig. 5 show the response of types A and B receptive fields to the current injected into the horizontal cells by the electrode placed at about 0.2 mm away from the center of the receptive field. In this figure, A1 and A2 show results of the depolarization and hyperpolarization of the S space in a type A field, and B1 and B2 were obtained by similar polarization of the S space in a type B field. In a type A field depolarization of the S space gave rise to a sustained discharge very similar to that stimulated by a spot of light (cf. Fig. 2A1) while hyperpolarization of the S space gave rise to off-discharges similar to those evoked.
From observations made in Figs. 2 and 5 a general rule can be drawn with respect to the response patterns of the two types of receptive fields when stimulated either by light or by current injection. It is summarized in Table 1 and may be stated as: the depolarization of the S space gives rise to the same response pattern as can normally be produced by a spot of light in the same field, and the hyperpolarization of the S space gives rise to a response pattern similar to that produced by an annulus. As was already discussed (11), this simple relation has an important bearing on the interpretation placed on our observations.

It is also worth mentioning here that in the channel catfish, light always hyperpolarizes the horizontal cells; depolarization of the horizontal cell related to light stimulus has never been observed.

Now we turn our attention to exploring the possible relation between the amplitude of the artificial polarization and the magnitude of the resulting response. We assume that the polarization induced in the experiments was proportional to the magnitude of the current passed. Plots were made only for the sustained discharges because the response magnitude could not be easily defined in the case of the transient discharges. We assume that the polarization induced in the experiments was proportional to the magnitude of the current. Results from two typical experiments (one for an A field and the other for a B field) are shown in Fig. 6. For small currents the response curve was nearly a straight line intersecting the origin, but large currents became progressively less effective in evoking the spike discharge until eventually the response curve reached saturation. At present there is no direct means to evaluate the source of this saturation of the current-induced response. It might have been due to a decrease in the horizontal cell membrane resistance resulting from an excessive intracellular polarization; alternatively, it might have originated by an annulus. In a type B field, depolarization of the S space gave rise to a transient discharge very similar to that generated by a spot of light (cf. Fig. 2B1) while hyperpolarization of the S space gave rise to a sustained discharge which was also similar to that produced by an annulus (cf. Fig. 2B2). However, a minor difference can be seen between the discharges obtained by light and by current, namely the progressive delay of the off-discharge which occurred as the intensity of the flash was increased (Fig. 2A2 and B1). No such delay of the off-response was seen in the case of the transient discharges induced by artificial polarization (Fig. 5A2 and B1). This can be explained by the fact that the decay of the S potential became slower as the intensity of the flash was increased (cf. Fig. 5 of ref 2). In the case of artificial polarization no such increase in delay accompanied the increase in the magnitude of polarizing current.
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FIG. 6. Relationship between the magnitude of the injected current and the number of sustained spike discharges evoked. The ordinate scale gives the number of spikes discharged expressed as a percentage of the maximum discharge and the abscissa shows the magnitude of the injected current. The depolarization (positive current) data were obtained from a type A field and the hyperpolarization (negative current) data were recorded from a type B field. Current scales were adjusted so that both curves could be superposed. Points obtained from the hyperpolarization experiment are shown with their standard deviations. At one or more points in the series of events leading to the firing of the ganglion cell. However, the fact that a similar saturation was seen for both depolarization and hyperpolarization implies that the second suggestion is the more likely.

The foregoing description has shown that in both types A and B fields the responses to a spot and an annulus can interact with one another in such a way that the stimulus giving rise to the transient response will, if strong enough, inhibit the sustained discharge caused by the other. A similar inhibitory interaction could be demonstrated between a light stimulus capable of evoking a transient discharge and a sustained discharge induced by an injected current. An example of such an interaction is shown in Fig. 7. In this experiment, during illumination by a spot of light, current of fixed intensity was delivered through an electrode placed 0.25 mm away from the spike-recording electrode. When the intensity of the spot was sufficiently strong, the sustained discharge, induced by polarization of the S space, was completely inhibited. As in the case of the interaction seen between two photic stimuli (Fig. 3B), a similar inhibition of the sustained discharge could be seen when the temporal sequence of two stimuli was reversed, i.e., a spot of light (inhibiting) delivered during the current induced sustained discharge (inhibited).

The relationship between the intensity of the inhibiting stimulus and the number of (sustained) spike discharges is shown in Fig. 8. These spike discharges were evoked from a type A field either by depolarization of the S space or by stimulation with a spot of light. In the figure the solid line has been redrawn from Fig. 7 of Naka and Nye (12) and represents the inhibition of the sustained discharge produced by a spot of light in a type B field. It can be seen from Fig. 8 that all three cases of interaction (spot-annulus, annulus-spot, and spot-current) have given rise to similar discharge inhibition functions.

Although polarization of the S space could clearly inhibit the current-evoked sustained discharge, it was not so effective in inhibiting the light-evoked sustained discharge. As shown in a previous paper (11) the inhibition was partial even when the polarizing current itself produced a considerable number of off-discharges. This is in marked contrast to the results shown in Fig. 7 where a spot of light completely inhibited the current-evoked sustained discharge. Similarly, as shown in Fig. 10, a current-evoked transient discharge often failed to inhibit a current-evoked sustained discharge.
discharge. In this record, hyperpolarization of the S space produced almost the same number of spike discharges whether it was induced alone or during depolarization from another electrode.

**Multielectrode experiments**

The preceding results have shown that there are two types of receptive fields (types A and B) and that polarization of the horizontal cell (or S space) can evoke a ganglion cell discharge which is similar to that produced by a light stimulus. Histological studies have shown that the fish retina contains at least two classes of horizontal cells; the internal and external horizontal cell. As will be discussed later, we can identify two classes of horizontal cells in the catfish retina, the internal horizontal cells which have a tubular structure and the external horizontal cells which are bricklike in appearance.

These observations indicate two alternative explanations of the possible functional connection between the horizontal cells and the two types of ganglion cells (probably through two types of bipolar cells), namely that: 1) two types of ganglion cells are activated through their own horizontal cell, or 2) both types of ganglion cells could be driven by horizontal cells belonging to both classes. In this section we will present functional evidence to answer this question and in the next section will provide the evidence obtained from dye-injection experiments.

In the experiment shown in Fig. 9 we examine the possibility that current injected into a horizontal cell could drive two types of ganglion cells simultaneously. In this experiment two spike-recording electrodes were placed 350 μ apart on the surface of the retina and a current injecting electrode was placed at the third point of a triangle 350 μ away from both spike-recording electrodes. The electrode arrangement on the retina is shown in Fig. 9C. One spike-recording electrode was recording a train of spike discharge marked spike 1 while the second recording electrode recorded two trains of spike discharges simultaneously, later separated into two individual spike trains by a spike-separation procedure described by Dill et al. (4). PST histograms of these two trains of spikes are marked in Fig. 9 as spike 2 and spike 3. As shown in Fig. 9A hyperpolarization of a horizontal cell (shown by the trace marked i) gave rise to a transient response of the spike 2 while the spike 1 and 3 gave rise to sustained discharges. Depolarization of the same horizontal cell (Fig. 9B, trace i) reversed the discharge patterns of three ganglion cells (Fig. 9, spike 1, 2, 3). From Table 1 we can find that the spike 1 and 3 were from type A ganglion cells and that the spike 2 was from a type B ganglion cell.

In the records shown in Figs. 9 and 10 in which background discharges were present it can be clearly seen that the sustained discharge was followed by an inhibitory period during which the spontaneous discharge was inhibited.

The results shown in Fig. 9 indicate that the polarization of a horizontal cell (through an S space) can drive two types of ganglion cells simultaneously. All repetitions of this experiment gave similar results.
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and judging from the fact that the chances of recording from the two classes of horizontal cell were nearly even (unpublished results), we conclude that both classes of horizontal cell can therefore drive both types of ganglion cell. It may also be presumed that, although the number of neurons recorded in Fig. 9 was limited to three, the number of neurons actually affected by the polarization of a single horizontal cell could be very large.

Both Werblin and Dowling (17) and Kaneko (5) have identified two types of bipolar cells in the mudpuppy and in the goldfish which showed complementary responses to center and surround stimuli. If we assume that the receptor distribution is homogeneous, the simplest scheme required to explain the results shown in Fig. 9 is to postulate the existence of two types of bipolar cells which send their signals to corresponding types of ganglion cells.

Finally, Fig. 10 shows the results of an experiment in which two electrodes, marked i and j, separated by a distance of 250 μ on the surface of the retina, were used to polarize two horizontal cells capable of inducing spike discharges from a ganglion cell. The spike-recording electrode was located at the third point of a triangle 350 μ away from two current-injecting electrodes (the configuration of the three electrodes on the retina is shown in Fig. 10C). Hyperpolarization of either of the two horizontal cells produced a sustained discharge from the ganglion cell (Fig. 10A). The time course of the ganglion cell discharge induced by polarization of the S space was dependent on the waveform of the injected current, and in this record it can be seen that the current i, though smaller in magnitude than j, gave rise to a greater number of spike discharges. In Fig. 10B, a hyperpolarizing current was injected through electrode j during a longer depolarization through electrode i. Depolarization of the S space caused an inhibition of the spontaneous discharge and at the end of the polarization a strong off-discharge was induced. A hyperpolarizing current similar to that passed through electrode j in Fig. 10A was delivered during the depolarization by electrode i. However, although depolarization through electrode i could inhibit the spontaneous discharge, it failed to inhibit the sustained discharged evoked by the current through the j electrode.
Two multielectrode experiments described in Figs. 9 and 10 have shown that a current injected into any horizontal cell can spread 350 μ across the retina to give rise to a ganglion cell discharge at the center of a receptive field; moreover, that both depolarization and hyperpolarization of the S space are effective in inducing a ganglion cell discharge at such a distance. Indeed, in some experiments, the distance involved was as far as 2 mm from the center of the receptive field and both depolarization and hyperpolarization were found to be still effective. The observations can best be interpreted if we assume that the polarization of a horizontal cell could spread passively through the S space according to a characteristic space constant (cf. ref 13). This distance corresponds to a receptive field of approximately 4 mm in diameter and there is ample evidence indicating that receptive fields in the fish retina do cover such a distance (3).

Identification of horizontal cells

The teleost retina possesses at least two classes of horizontal cells, one internal and one external horizontal cell (5, 15). In the channel catfish we found two classes of horizontal cells, the internal horizontal cells having a tubular structure and the external horizontal cells looking somewhat bricklike in structure (unpublished results).

In the course of the present experiments Procion dye was injected after evoking a ganglion cell response through the same electrode. The results of such experiments have shown that both classes of horizontal cell were apparently effective in inducing a ganglion cell discharge. It has been found that a single ganglion cell could be activated by two or three horizontal cells into which dye was injected. The identified cells included both the internal and external horizontal cells.

Together with evidence presented in the preceding section we conclude that both horizontal cells can drive both types of ganglion cells (probably through two types of bipolar cells) to give rise to type A and type B responses. However, this does not experiments will be needed before it will be possible to draw any conclusion.
exclude the possibility that when examined in detail these two types of horizontal cells might show a difference in their functions. Kaneko (5) has reported that the spatial integration was less in the goldfish external horizontal cells when compared with the internal horizontal cells.

**DISCUSSION**

Since the discovery by Kuffler (7) of the concentric organization of the vertebrate retinal receptive field, a substantial number of papers have been published on various types of receptive-field organization. Based on observations of the ganglion cell discharge several authors have proposed possible neuronal mechanisms which could explain the input-output behavior of the retina (1, 14). These proposals have been attractive because the vertebrate retina is organized of distinct layers containing cells of characteristic morphological shapes. However, unless we are able to manipulate the ganglion cell discharge by an artificial stimulation of some interneurons, speculations on the structure of receptive-field mechanisms will always be subject to uncertainty.

There are two questions that must be answered before we can suggest any functionally meaningful receptive-field mechanism: 1) How does a receptive field produce the antagonizing response found in the classical concentric field? 2) The receptive fields extend, in some teleost fish, as far as from 2 to 3 mm from the center of the field; how does the signal find its way over such a distance to the center of the field?

The receptive fields of the catfish retina possess two unique characteristics which show promise of providing answers to these two basic questions. First, when moderately dark-adapted they lack any color-coded response for both the S potential and the ganglion cell discharges behave as if they are driven by signals from a single-cone pigment with a maximal absorption at 625 nm. Second, the ganglion cell produces, over a 2–3 log unit intensity range, two distinct discharge patterns, which are closely associated with the stimulus distribution within the receptive field (10, 12). Thus by virtue of this simpler organization catfish retina provides an opportunity to understand the cellular mechanisms of the vertebrate receptive field.

**Catfish horizontal cell**

As will be discussed later the horizontal cell has been proved to play a major role in the organization of the catfish receptive field. As in other vertebrate retinas, at least two classes of horizontal cells can be seen in the catfish; the internal and external horizontal cell. The internal horizontal cell has a cylindrical structure. Procion dye injected into an internal horizontal cell was observed to diffuse laterally over a distance of 350–500 μ. The external horizontal cells with their bricklike appearance are placed side by side forming a single layer. The perikaryon of the external horizontal cell measured from 30 to 50 μ and very thin axonlike processes are seen to extend laterally from its corners. The number and distance covered by these processes are not known. The spectral responses from both types of horizontal cells are found to be quite similar (unpublished results) suggesting that both classes of horizontal cells are receiving signals from a single-cone population. Morphologically the catfish horizontal cells are almost identical to those found in other teleost fish (5, 15). In the fish, according to Stell (15), the large, flattened, polygonal cells of the external horizontal cell layer form a sheet penetrated by scattered small spaces through which pass radially oriented structures such as bipolar processes, while the cells of the internal layer, with their fine branching processes, form a more open mesh. In the catfish, Procion dye-injection experiments have proved that S potentials can be recorded from both layers of horizontal cells but the responses from the two classes of horizontal cells could not be distinguished from one another. A similar conclusion has already been drawn by Kaneko (5) in the goldfish, although he noted that the external horizontal cells showed less spatial summation than the internal horizontal cells.

Often it was found that current injected more than 2 mm away from the center of the receptive field was still effective in evoking a spike discharge. However, the dimen-
sions of catfish horizontal cells are such that any potential change induced by current injected into a horizontal cell lying at the periphery of a receptive field has to spread across several horizontal cells to reach the field center. In fact, two horizontal cell electrodes placed 300 μ apart could drive simultaneously a ganglion cell (probably through a bipolar cell) lying as far as 300 μ from both injection sites (Fig. 10). These observations can be interpreted if we assume that horizontal cells in a given layer form a space, referred to as the S space in the tenth (13), through which the potential change resulting from the injected current can spread passively.

In the teleost retina tight junctions have been seen among neighboring horizontal cells (18, 19) and the results obtained here indicate that these tight junctions are able to conduct equally both depolarization and hyperpolarization of the presynaptic membrane. It is worth noting here that the spread of a depolarization is a purely artificial phenomenon which is never produced by a light stimulus.

Catfish receptive field

In a previous paper we analyzed in detail the receptive-field organization of the type B field (12). In that analysis we proposed that two response-generating mechanisms underlie the catfish receptive field; one a local and the other an integrating mechanism. The local mechanism which gave rise to a transient discharge in the type B field was assumed to be activated whenever the receptive field is stimulated locally by a spot of light. Thus this mechanism responds to the peak local luminance (PLL) within the field. On the other hand, the integrating mechanism in a type B field gives rise to a sustained discharge. In the paper we concluded that the integrating mechanism must depend on the S space to transmit and to integrate signals arising over a large retinal area. The presence within the receptive field of a spot of light of sufficient strength inhibits the sustained discharge and we interpreted this to mean that the local mechanism can "override" the signal from the integrating mechanism. A dark area at the center of the field was found to be essential for obtaining a sustained discharge to prevent the overriding effect. To account for the fact that decentered spots can also inhibit the sustained discharge we concluded that a second lateral transmission system must exist at a more proximal level of the retina. The purpose of this pathway was that of transmitting signals from local mechanisms activated by decentered spots toward the field's center where the signal interacts with that from the integrating mechanism.

The analysis of the type A field presented in this paper has revealed that its organization precisely complements that of the type B field. The type A field was also found to involve two mechanisms; the local mechanism, which in this case gave rise to a sustained discharge, and the integrating mechanism, which gave rise to a transient discharge. In the type A field an annulus depressed the sustained discharge induced by a spot of light, while in the type B field it was a spot of light which depressed the sustained discharge evoked by an annulus. In both fields, therefore, the stimulus which gives rise to a transient discharge can, if sufficiently strong, always suppress the sustained discharge. The characteristic responses of the catfish receptive field, evoked by light and direct stimulation of the horizontal cells, are summarized in Table I which clearly shows the complementary nature of two fields.

Polarization of horizontal cells

In both types A and B receptive fields, hyperpolarization of the horizontal cells was found to produce a discharge pattern similar to that evoked by an annulus. This result was expected from the analysis of the previous paper (12) because an annulus was found to stimulate only the integrating mechanism which had already been tentatively identified as the S space. We, however, did not expect to find that the depolarization of a horizontal cell would give rise to a ganglion cell discharge similar to that produced by a spot of light. These results showed clearly that the local mechanism could be activated "artificially" through the S space as in the case of the integrating mechanism. Also depolarization of a horizontal cell could propagate passively through the S space in a similar man-
ner to the spread of a hyperpolarization induced by light or by current injection. Indeed, our unpublished results have shown that the magnitude of the threshold current required to produce a ganglion cell discharge was nearly identical for both a depolarizing and hyperpolarizing current. This leads to a very important implication that the local mechanism can be replaced by a depolarization of the S space and, in this sense, the local mechanism could be regarded as being as diffuse as the integrating mechanism. It is apparent that a spot of light must produce locally the same condition as a depolarization of the S space. Procion dye injection has revealed that the polarization of both the internal and external horizontal cell had a similar effect in producing the ganglion cell discharge and, so far, we have failed to notice any conspicuous difference between the effects produced by the polarization of these two horizontal cells.

The morphology of the catfish retina is not known in detail and the structure of the outer plexiform layer is no exception. However, a number of studies have been reported on the outer plexiform layer of the teleost fish and they all agree that bipolar cell dendrites and horizontal cell processes invaginate the cone pedicle (15). Furthermore, only the bipolar cells are thought to pass signals produced in the outer plexiform layer on to the inner plexiform layer where they produce the ganglion cell discharge. In a series of preliminary experiments, intracellular recordings have been made from cells in the inner nuclear layer, probably from bipolar cells. It was found that there were two types of such "bipolar cells" corresponding to the two types of receptive field and an interaction between a spot and an annulus could also be seen in these bipolar cells. These findings suggest that the basic organization of the receptive field is already established at the level of the bipolar cells and that these cells, in turn, activate the ganglion cells forming the two types of receptive field. In both the mudpuppy and the goldfish, two distinct types of bipolar cells have been identified and similar conclusions have already been drawn by Werblin and Dowling (17) and Kaneko (5). To produce the response of a ganglion cell the current injected into a horizontal cell must act either on the receptor terminal where the triad is formed or directly on a bipolar cell. Although the conventional synapses made by the horizontal cells on two other elements, receptors and bipolar cells, are not known in detail, the available evidence in some vertebrates does indicate however that the horizontal cells do make the conventional synaptic contacts on to bipolar processes (18). The results of the current injection experiments suggest that the horizontal cell must relay signals to the receptor or the bipolar cells. They also suggest that the function of these "synapses" is such that both depolarization and hyperpolarization of the presynaptic membrane can produce change in the postsynaptic elements and that the postsynaptic element is capable of producing two distinct responses according to the polarity of the presynaptic change. A simple scheme has been proposed elsewhere to explain these observed effects of horizontal cell polarization (11).

Working on the pike retina Maksimova (9) was the first to succeed in evoking a ganglion cell discharge by polarizing a horizontal cell. Her results agree with ours in that depolarization and hyperpolarization of a horizontal cell could produce a complementary response from a ganglion cell. However, in the pike the magnitude of current necessary to induce the ganglion cell response was approximately 10 times greater than that required in the catfish and the latency of the induced response was also much longer. In the pike the threshold polarization of horizontal cell required to produce the ganglion cell response was 5 mv (9), while our preliminary estimate of threshold polarization in the catfish is about 0.5 mv.

Are two lateral pathways involved?

In a previous paper (12), we have suggested that the channel catfish receptive field incorporated two lateral transmission systems. The results of the current injection experiments have shown that the interaction taking place in the outer plexiform layer could produce the basic receptive-field responses. However, the current injection experiments failed to explain the
fact that a decentered spot could still produce response patterns similar to those evoked by a spot placed at the center of the field. The decentered spot cannot rely on the S space to exert its influence on the receptive-field center because the horizontal cell response produced by a spot of light was of the same polarity as that produced by an annulus and differed only in amplitude. This can be illustrated best by the experiment shown in Fig. 4 in which the pattern of the response to a spot of light placed at the periphery of the field was shown to be a function of the area stimulated. However, during this experiment, as shown in Fig. 11 of the previous paper (12), the potential change inside the S space was likely to be nearly identical for each of the stimulus configurations. If the S space alone yielded the signal leading to the observed change in the response pattern, then we would expect to see the potential inside the S space depolarize in response to a change in the pattern of illumination. However, such a change has never been observed.

Together with the argument presented in the previous paper, we again conclude that one more lateral pathway must be present in the catfish retina and that this lateral transmission system must be located at a level more proximal to the first transmission system, namely the S space.

Conclusion

Since its discovery by Svaetichin (16) the origin and function of the S potential has been a subject of controversy. The earlier suggestion that it is associated with gain control during dark adaptation could not be substantiated. The present study has confirmed Maksimova’s (9) report that the artificial polarization of an S space can activate a ganglion cell and concludes that the horizontal cells are an integral part of the neuronal mechanisms which comprise the catfish receptive field.

SUMMARY

In the catfish retina there are two types of receptive fields, termed type A and type B, which are found to have complementary field organizations. In a type A field a spot of light produces a transient ganglion cell discharge while an annulus gives rise to a sustained discharge. In a type B field the response pattern is reversed. These two discharge patterns produced in the two fields reflect two underlying response mechanisms, namely, a local mechanism which is activated by a spot of light and an integrating mechanism which is activated by an annulus.

The hyperpolarization of the S space by means of a current injected by a horizontal cell electrode was found to produce a response similar to that by an annulus. It was concluded that the S spaces (layers of horizontal cells) serve as an integrating mechanism. The depolarization of an S space was found to produce a response pattern similar to that by a spot of light. Because light cannot depolarize the horizontal cells it was concluded that a spot of light produces a condition similar to that of depolarization of the horizontal cell layer.

It was shown that the depolarization or hyperpolarization of a horizontal cell can spread across the retina to produce a ganglion cell discharge and that the polarization of two separate horizontal cells can interact in producing the receptive field response. The simplest explanation of these observations is that the potential change, whether produced by light or by an injected current, must spread the row of horizontal cells which form a medium known as S space, first described in the tench by Naka and Rushton (13). When a current was injected into either of the two rows of horizontal cells (internal or external cells) they were found to be equally effective in inducing a receptive-field response. So far, we have failed to differentiate the functions of these two classes of horizontal cells.

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