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S-POTENTIALS IN THE DARK-ADAPTED RETINA OF THE CARP

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SUMMARY

1. S-potentials were recorded in the dark-adapted carp retina. After recording, Procion Yellow was injected from the recording electrode to identify histologically the cell recorded.

2. Both L- and C-type S-potentials were found. L-type S-potentials were further classified into two groups. The first group was characterized by a high threshold, fast rise and fall times and a spectral sensitivity peak of about 620 nm. The second group showed a low threshold, slow response time course and a spectral sensitivity peak of about 520 nm.

3. Cells of the first group were identified as external and internal horizontal cells. Cells of the second group were identified as intermediate horizontal cells.

4. In the dark-adapted retina, the spectral sensitivity of the intermediate horizontal cell showed a good agreement with the absorption spectrum of the rod pigment, porphyropsin.

5. Neither a chromatic background nor white background illumination of moderate intensity (1 cd.m^{-2}) shifted the spectral sensitivity of the intermediate horizontal cell.

6. Under a strong background (10 cd.m^{-2}), the intermediate horizontal cell was entirely suppressed. Under the same conditions the external horizontal cell was still capable of responding.

7. From these observations it is concluded that the intermediate horizontal cell receives input from rods only.

INTRODUCTION

The S-potential is a graded, sustained response recorded from the retina of many vertebrates. By electrophoretic dye injection, S-potentials were shown to come from horizontal cells (MacNichol & Svaetichin, 1958;

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Werblin & Dowling, 1969; Kaneko, 1970, 1971; Steinberg & Schmidt, 1970).

In the goldfish two types of horizontal cells were demonstrated to give rise to S-potentials: the external and the internal horizontal cells. From both types of cells, two kinds of S-potentials characterized by different spectral responses were recorded (Kaneko, 1970). The L-type response showed only hyperpolarization to all visible wave-lengths. The C-type response changed response polarity depending on wave-length.

Morphologically, the third type of horizontal cell, the intermediate horizontal cell, was shown to exist in the goldfish (Cajal, 1909; Stell, 1967). Stell showed that intermediate horizontal cells make synaptic contacts with rods, while external horizontal cells make contacts with cones.

In a previous study (Kaneko, 1970) where S-potentials were recorded in the light-adapted retina, Procion Yellow injections were confined to the external and internal horizontal cells. It could well be assumed that the strong light adaptation used there effectively saturated the rods and thus precluded any response from the intermediate horizontal cells. Under those conditions, it is probable that only the cells with cone input were operative.

In the present study, S-potentials were recorded from the dark-adapted carp retina and the cells involved were identified by fluorescent dye injection.

METHODS

Carp, *Cyprinus carpio*, of about 500 g body weight, were obtained from a commercial dealer and stored in an aerated tank before use. The animal was kept in the dark for more than an hour before the experiment.

In order to keep the retina dark-adapted during surgery, the fish was dissected under infrared illumination using image converter tubes. However, in later experiments, it was found that dim illumination (about 0·5 cd.m^{-2}) during dissection did not alter the responses of the retina substantially provided the retina was kept in total darkness for about 5 min before recording. The isolated retina was mounted, receptor side up, in a small dish 10 mm wide and 3 mm deep. The dish was made of Teflon and a bottom piece of thin glass. The stimulating light passed through the transparent bottom and reached the retina from the vitreous side. The dish was mounted in a moist chamber in which the retina was maintained in good condition for more than 2 hr.

Intracellular recordings were made with micro-electrodes filled with 4 M potassium acetate. To identify the recorded cells, Procion Yellow M 4 RAN was injected electrophoretically from the recording electrode by the method developed by Stretton & Kravitz (1968). Micro-electrodes were connected to a cathode follower preamplifier and to an oscilloscope. The records were stored on FM-magnetic tapes for further processing.

Tungsten electrodes (Hubel, 1957) were used to record spike discharges from ganglion cells.

The retina was illuminated with monochromatic light having a half band width of about 5 nm. Light from a tungsten filament lamp (6 V, 18 A) was dispersed into a monochromatic light by a grating monochromator. Wave-lengths of monochromatic

light were changed in 20 nm steps from 400 to 720 nm. The quantum flux of the monochromatic lights was adjusted to the same level by a neutral density wedge driven by a programmed servo-motor. This program was made from intensity measurements using a solar battery whose spectral sensitivity was calibrated against a thermopile. The maximum intensity of the monochromatic light was about $1{\cdot}3 \times 10^{10}$ quanta.sec^{-1}.mm^{-2}. It was attenuated by neutral density filters in 0·25 log unit steps. The difference in spectral transmission in the range of 400–760 nm of these neutral density filters was less than 0·05 log units. The image of an iris diaphragm was focused on the retina. The size and the position of the light spot were changeable. The electrode tip was postioned in the centre of the light spot.

The adapting light obtained from a second small tungsten lamp diffusely illuminated the retina. The intensity was attenuated by neutral density filters and the spectral distribution by coloured glass filters when necessary. (Toshiba VR-62, passing a spectral range longer than 610 nm; VB-48, passing shorter than 540 nm.) The maximum intensity of white background light was 10 cd.m^{-2} (or 1·8 μW.cm^{-2}).

Experiments were performed in a dark room at temperatures of 15–20° C.

RESULTS

S-potentials from the dark-adapted retina

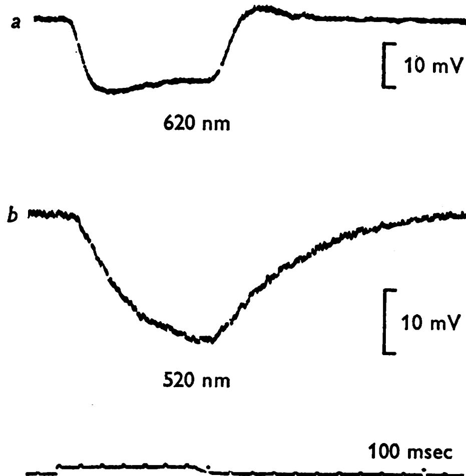
Both L- and C-type S-potentials were recorded from the dark-adapted carp retina. As shown in many previous studies (Wagner, MacNichol & Wolbarsht, 1960; Tomita, 1965; Norton, Spekreijse, Wagner & Wolbarsht, 1968; Kaneko, 1970), the L-type S-potentials recorded from light-adapted carp and goldfish retina showed maximal responses at about 620 nm, which corresponded to the spectral absorption and sensitivity peaks of red-sensitive cones (Marks, 1965; Tomita, Kaneko, Murakami & Pautler, 1967).

In a single penetration of the isolated retina, we usually recorded a series of S-potentials. The L-type S-potentials recorded from the dark-adapted carp retina were classified according to the recording depth. The L-type S-potentials of the first group (Text-fig. 1*a*) were recorded closest to the receptors. They had higher thresholds than the second group (see below). The rise and fall times of these responses were fast with a sustained potential on this time scale. L-type S-potentials of this group also showed a maximum response at about 620 nm to equal quantum monochromatic lights. These S-potentials were thought to be the same type as those commonly recorded from the light-adapted retina.

The L-type S-potentials of the second group (Text-fig. 1*b*) was obtained vitread to the first group. S-potentials of the second group had a lower threshold than the first group. The intensity of a light flash giving rise to a barely detectable response (about 0·1 mV) was 10^5 quanta.sec^{-1}.mm^{-2} which was about 1 log unit above the light intensity for a threshold response in ganglion cells of the dark-adapted goldfish retina (Raynauld, 1969). The responses of this second cell group showed slow rise and fall

times reaching a steady level in more than 600 msec. From the differences in sensitivity and in the time course of the responses, these two groups were readily discernible from each other.

By penetrating the retina more vitread, the L-type S-potentials of the third group were recorded. These responses showed very similar characteristics to the first group.



Text-fig. 1. L-type S-potentials recorded from a dark-adapted carp retina. Note the difference between the two responses in time course and sensitivity. *a*: a response defined as the first group. Diffuse illumination of 620 nm monochromatic light of $1{\cdot}3 \times 10^8$ quanta.sec^{-1}.mm^{-2}. *b*: a response defined as the second group. Diffuse illumination of 520 nm monochromatic light of $2{\cdot}3 \times 10^6$ quanta.sec^{-1}.mm^{-2}.

The recorded cells were identified morphologically by electrophoretic injection of Procion Yellow M4 RAN from the recording electrode. S-potentials of the first group were identified as coming from external horizontal cells. Cells of the second group were identified as intermediate horizontal cells and those of the third group as internal horizontal cells.

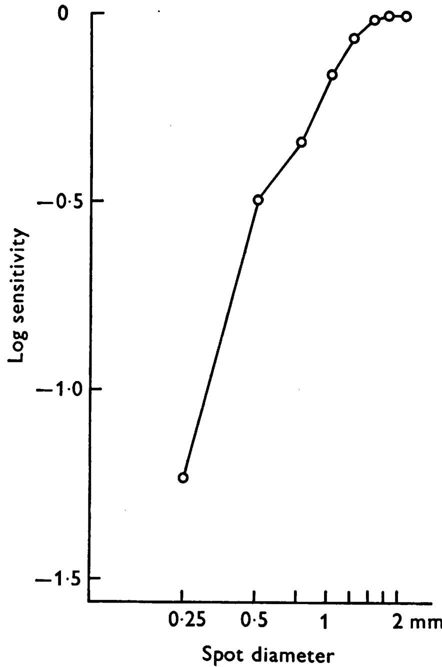
Plate 1*a* is a montage photomicrograph constructed from three serial 10 μm thick sections of an intermediate horizontal cell injected with Procion Yellow. Plate 1*b* is a more complete reconstruction of the same cell by a camera lucida drawing. The cell body is located in a layer between the external and the internal horizontal cells. The intermediate horizontal cell has a flatter cell body with longer dendrites than the external horizontal cells. Dendrites extend sclerad and end at the receptor terminal region. Axons were not observed. In horizontal sections the dendrites were seen spreading in a roughly circular area of about 100 μm in diameter. We found

eleven intermediate horizontal cells after injecting the dye into twenty units. No other types of cells were found.

The C-type S-potentials recorded from the dark-adapted retina came from either external or internal horizontal cell, the same origin as in the light-adapted retina. No substantial difference was found in the spectral responses of C-type units between the dark- and light-adapted states.

Spatial summation in the S-potential from the intermediate horizontal cell

S-potentials from the fish retina are known to have a large spatial summation within the uniform receptive field (Naka & Rushton, 1967; Norton *et al.* 1968). Text-fig. 2 shows a spatial summation curve of an intermediate horizontal cell. The abscissa indicates the spot diameter and the



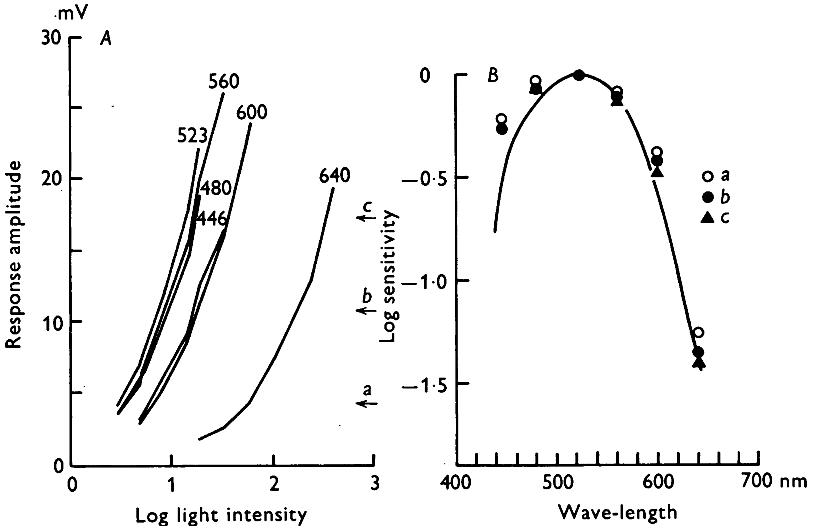
Text-fig. 2. Spatial summation in an intermediate horizontal cell. Abscissa: spot diameter of 520 nm monochromatic light. Ordinate: reciprocal of the light intensity giving rise to a response of criterion voltage (6·8 mV), expressed in a relative value to the maximum.

ordinate the sensitivity of the cell in log units, defined as the reciprocal of the light intensity giving rise to an arbitrary criterion voltage. The curve shows that this cell has a uniform receptive field of about 1·5 mm in diameter. The antagonistic surround seen in bipolar cells (Werblin &

Dowling, 1969; Kaneko, 1970) was not observed here. Similar results were obtained from 9 other intermediate horizontal cells.

Spectral sensitivity of the intermediate horizontal cell in the dark-adapted retina

A common method of analysing the input to a particular cell is to examine the spectral sensitivity and to see if it matches the spectral sensitivity of any known photoreceptor. This method was used in an experiment shown in Text-fig. 3. Here, intensity amplitude curves obtained from an intermediate horizontal cell using six different wave-lengths are shown. The



Text-fig. 3. *A*: relationship between response amplitudes of an intermediate horizontal cell and the intensity of monochromatic light flashes. The wave-length indicated in nm to each curve.

B: spectral sensitivity determined from the curves in *A*. The sensitivity was defined as the reciprocal of the light intensity giving rise to responses of a criterion voltage. Three criteria were set and indicated by arrows in *A* (*a*, 4·5 mV; *b*, 11·4 mV; *c*, 18·2 mV). Points shown with different symbols correspond to three above criteria. The continuous curve indicates the absorption spectrum of porphyropsin calculated from a nomogram proposed by Munz & Schwanzara (1967) for retinene$_2$ photopigments.

curves were almost parallel to each other and could be matched with a single template provided that the template was shifted by an appropriate amount horizontally. The parallelism between the intensity amplitude curves with different wave-lengths is a good indication that only one type of photopigment is involved to generate these responses (Naka & Rushton, 1966).

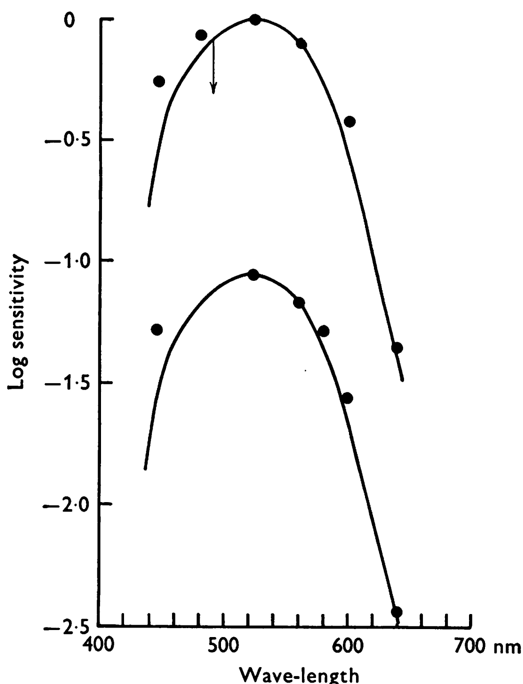
Spectral sensitivity was calculated as the reciprocal of light intensity

giving rise to an arbitrary criterion voltage. Here, three criteria were set and each sensitivity curve was plotted in Text-fig. 3*B* with different symbols. All the three curves appeared very close, indicating that with different light intensities no more than one receptor mechanism was isolated.

The curves agree well with the absorption spectrum of the rod pigment, porphyropsin, determined by spectrophotometry (Munz & Schwanzara, 1967; Bridges, 1967).

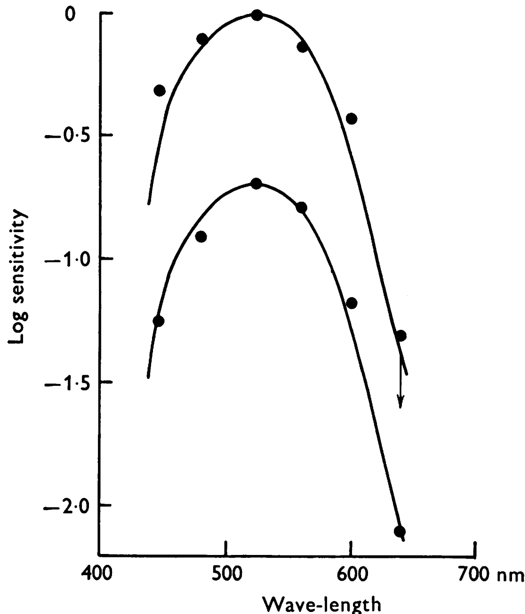
Spectral sensitivity of the intermediate horizontal cell with selective background

The input to the intermediate horizontal cell was further analysed using chromatic background illumination in an attempt to test the possibility of multiple receptor inputs. Experiments shown in Text-figs. 4 and 5 were



Text-fig. 4. Spectral sensitivity curves of the same cell as in Text-fig. 3. The upper plot shows the spectral sensitivity determined in the complete darkness. The lower plot shows the spectral sensitivity under a selective background. The adapting light was obtained from a tungsten bulb through a glass filter (Toshiba VB-48). The spectral composition of the background light was broad, but the approximate wave-length of the peak transmission of the filter was indicated by an arrow. Continuous curves are the absorption spectrum of porphyropsin.

done along this line. In the experiment shown in Text-fig. 4, the spectral sensitivity curve was first obtained in the scotopic state in a similar manner as shown in Text-fig. 3. Again, the sensitivity spectrum (upper plot) agreed well with the absorption spectrum of porphyropsin. Next, a steady blue-green background light (Toshiba filter, VB-48) was given and the cell was steadily hyperpolarized by 26 mV. Superimposed on this steady background, monochromatic light flashes were given and the spectral sensitivity of the cell was examined. The sensitivity of the cell was depressed



Text-fig. 5. Spectral sensitivity curves of another intermediate horizontal cell. Background light was passed through a red glass filter (Toshiba VR-62). Other conditions are the same as in Text-fig. 4.

by about 1 log unit equally in all the wave-lengths examined. Thus the lower plot of Text-fig. 4 also agreed with the absorption spectrum of porphyropsin, provided that the sensitivity curve was shifted vertically by an appropriate amount.

Text-fig. 5 shows a similar experiment with red background light (Toshiba filter VR-62). The spectral sensitivity was first examined without background light (upper plot). Next, a red background was given and the spectral sensitivity was examined. As seen from the lower plot of Text-fig. 5, the sensitivity of the cell was suppressed almost uniformly over the entire spectral region tested. Both spectral sensitivity curves examined

either in the scotopic state or with red background illumination again agreed with the absorption spectrum of porphyropsin.

Good agreement of spectral sensitivity curves (1) without background light, (2) with blue-green background illumination and (3) with red background illumination strongly suggests that in the dark-adapted retina the intermediate horizontal cells receive input from rods containing a single photopigment and not from other types of receptors.

Responses from the intermediate horizontal cell under mesopic and photopic conditions

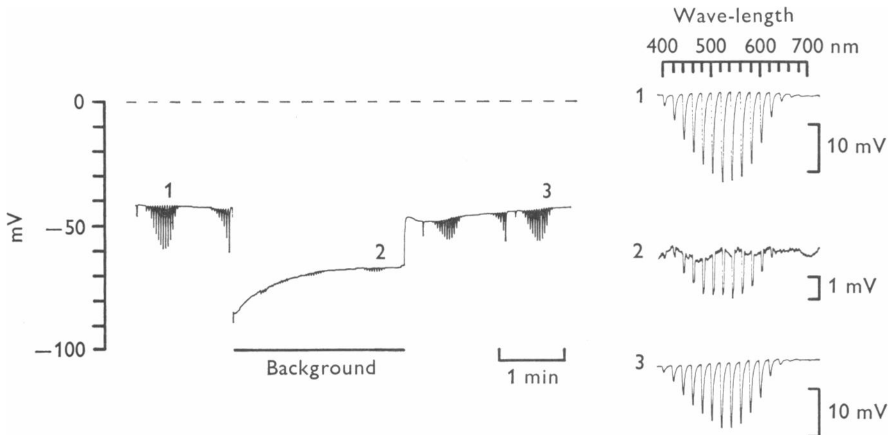
It is now natural to ask if the intermediate horizontal cells receive cone input in the mesopic and photopic states. In the experiment shown in Text-fig. 6, responses from the intermediate horizontal cell were analysed first in the dark-adapted retina and then in the light-adapted state. Here, an intermediate horizontal cell was penetrated and after its spectral responses were recorded, a white background light of 1 cd. m^{-2} was given. As shown in the Figure, the cell was hyperpolarized about 50 mV by the background. Light flashes superimposed on the background illumination did not produce any detectable responses in the initial 1 min. Although the background illumination continued, the membrane potential gradually diminished and the cell began showing hyperpolarizing responses to blue-green light flashes. The membrane potential reached a stable level of −65 mV and here spectral responses were recorded. The cell showed a maximum response to 520 nm monochromatic light. A spectral sensitivity curve obtained from another intermediate horizontal cell under the same experimental conditions agreed well with the absorption spectrum of porphyropsin. The time course of individual responses was much faster than those observed in the dark-adapted retina.

After turning off the background illumination, the membrane potential returned to a level close to that before the background light was given. Spectral responses recorded 2 min after turning off the background was slightly smaller in amplitude than control responses but showed a maximum at 520 nm.

Similar experiments were done on other intermediate horizontal cells using a much stronger background light. As in the previous experiment, an intermediate horizontal cell was penetrated and its spectral responses were recorded. Next, a strong white background light (10 cd. m^{-2}) was given and the cell was hyperpolarized. The background illumination continued and the membrane potential gradually diminished with a similar time course as in the previous experiment, but this time the cell showed no response to light flashes superimposed on the background. This silent period continued as long as the cell was held (7 min). Under this strong

background light, no other S-potentials from intermediate horizontal cells were recorded from this retina. From these observations, it is suggested that the rod activity was entirely suppressed under the strong background illumination.

From the same preparation and under the strong background illumination C-type S-potentials were recorded in several penetrations. This suggests that cones were operative in this retina.



Text-fig. 6. A recording from an intermediate horizontal cell and the effect of white background light of 1 cd.m^{-2}. The horizontal line under the record indicates the period of background illumination. Spectral responses indicated with numbers are reproduced on the right with magnification. Light intensity at 1 and 3, $1{\cdot}3\times10^{7}$ quanta.sec^{-1}.mm^{-2}; at 2, $1{\cdot}3\times10^{8}$ quanta.sec^{-1}.mm^{-2}.

The state of adaptation of the retina under these backgrounds was also examined by recording from ganglion cells using metal electrodes. The goldfish ganglion cells were shown to have rod inputs in the scotopic, and cone inputs in the photopic state (Raynauld, 1969). With the same experimental conditions used for the intermediate horizontal cells, but now performed on an on-centre off-surround ganglion cell, the spectral sensitivity in the receptive field centre was examined. These ganglion cells showed a sensitivity peak at about 520 nm in the dark-adapted state. With the same background illumination as used in Text-fig. 6 (1 cd.m^{-2}), the sensitivity of the cell decreased and its peak shifted to about 620 nm, almost immediately after the background illumination was turned on. This observation indicates that rods were operative in the dark-adapted retina and cones were operative under the background illumination, and confirms the previous report (Raynauld, 1969). Similar results were obtained using the stronger background light (10 cd.m^{-2}).

DISCUSSION

The carp intermediate horizontal cell responded to light flashes with L-type S-potentials. In the dark-adapted retina, the spectral sensitivity of these cells agreed well with the absorption spectrum of porphyropsin (Munz & Schwanzara, 1967; Bridges, 1967): selective background did not reveal any other receptor mechanisms.

Under weak background illumination the intermediate horizontal cell continued to show a spectral sensitivity of the rod pigment. Since the absorption peak of the rod pigment (523 nm) is close to that of green cones (535 nm, Marks, 1965), it is difficult to identify with complete assurance the type of input to the intermediate horizontal cell only from the spectral sensitivity functions. Nevertheless, experiments in the light-adapted retina led us to conclude that the responses of the intermediate horizontal cell seen under low background illumination were contributed by rods. Under strong background illumination, responses from the intermediate horizontal cell were completely suppressed, while the external horizontal cell showing C-type S-potentials was still capable of responding. Cells showing C-type S-potentials are believed to receive input from green cones as well as red cones (Naka & Rushton, 1966). Moreover, ganglion cells in the dark-adapted retina had a simple centre-surround type receptive field and received input from rods (Raynauld, 1969, and this study). These ganglion cells showed input from cones under strong background illumination. These observations suggest that the rods were saturated under the strong background light, and that the intermediate horizontal cells are connected to rods only and not to cones.

One of the characteristics of the responses from the intermediate horizontal cell was its slow rise and decay. However, the time course of these responses was not a fixed feature of the intermediate horizontal cell. With background illumination, rise and decay became faster. Since responses from single carp rods were not reported, direct comparison between responses from intermediate horizontal cells and from rods was not possible. The only intracellular recordings from single rods were made in the gecko (Toyoda, Nosaki & Tomita, 1969) and in the frog (Toyoda, Hashimoto, Anno & Tomita, 1970). They showed that dark-adapted rods of these animals responded with hyperpolarization to light and had a slow time course similar to that observed for the intermediate horizontal cell in the present study. It seems common, therefore, that dark-adapted rods respond with a slow time course even in different animals.

Raynauld (1969) observed in the goldfish a total suppression of retinal activity for 15–20 min in the initial stage of light adaptation. A total suppression was not observed in the present study. A Purkinje shift observed

in ganglion cells in our preparation was prompt, although the intensity of the background light was of the same order. We do not have a good explanation for this difference.

Synaptic contacts between fish horizontal cells and receptors were studied on Golgi-impregnated preparations under the electron microscope (Stell, 1967). He demonstrated in the goldfish that the external horizontal cell makes synaptic contacts with cones, while the intermediate horizontal cell only with rods. The external horizontal cells were not extensively studied in the present experiments, but we have suggestions by Watanabe & Hashimoto (1965) and by Witkovsky (1967) that the external horizontal cells receive input from cones, but not from rods. They observed that L-type S-potentials had spectral sensitivities peaking at about 620 nm and that these cells did not show a Purkinje shift. The present study shows that the intermediate horizontal cells receive input from rods and not from other types of receptors. These studies strongly support Stell's morphological observations (1967) and suggest that at the horizontal cell level the information originating from rods is independent from the information from cones.

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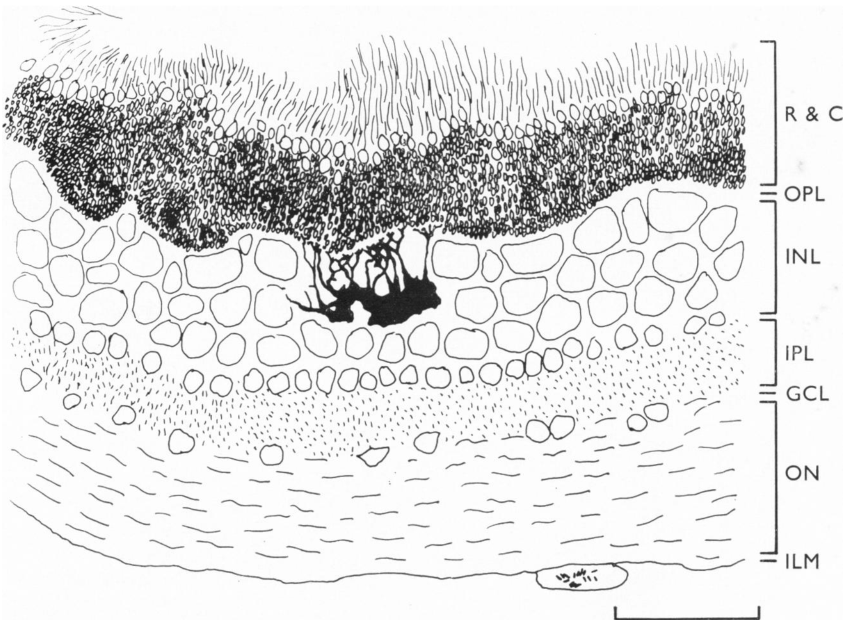

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EXPLANATION OF PLATE

a, A montage photomicrograph of an intermediate horizontal cell injected with Procion Yellow, composed from three serial sections of 10 μm thickness. Calibration 100 μm.

b, A more complete reconstruction of the same cell by a camera lucida. Calibration 100 μm. R and C, layer of rods and cones; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer; ON, optic nerve layer; ILM, inner limiting membrane.