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INTERACTIONS LEADING TO HORIZONTAL CELL RESPONSES IN THE TURTLE RETINA

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SUMMARY

1. Small responses to large fields of dim monochromatic lights were recorded intracellularly from luminosity horizontal cells (L-cells), chromaticity horizontal cells (C-cells) and cones in the retinae of turtles, *Pseudemys scripta elegans*.

2. Responses of cones to brief flashes applied over steady backgrounds were studied in order to interpret the corresponding responses of horizontal cells. Steady red or green backgrounds make the responses of red-sensitive cones smaller, faster and often diphasic. Green backgrounds have similar effects on the responses of green-sensitive cones to green flashes, but red backgrounds do not change them appreciably. Responses of double cones have properties intermediate between those of red and green cones.

3. L-cells of both type I and type II are hyperpolarized by all visible wave-lengths, and their spectral sensitivity in the linear range resembles that of red cones. Their responses are not invariant with respect to colour, and their sensitivity to green relative to red stimuli increases during red backgrounds. These properties suggest that L-cells are activated mainly by red cones but also receive impingement from the red members of double cones.

4. Spectral properties of red/green C-cells resemble those of green cones as modified by the recurrent action of L-cells. They can be explained assuming that red/green C-cells receive their principal impingement from green cones and subsidiary interactions from green/blue C-cells and the green members of double cones.

5. The spectral sensitivity of the hyperpolarizing responses of green/blue C-cells is ascribed to impingement from blue cones. Their depolarizing responses have complex properties which suggest that they are brought

about by the activity of both L-cells (probably through the blue cones) and red/green C-cells.

6. It is concluded that the main properties of the responses of the horizontal cells can be explained by a simple circuit in which each horizontal cell is connected to a corresponding type of cone and the L-cells have a recurrent impingement on all cones. The scheme is modified by additional interactions which operate on the responses of each horizontal cell type.

INTRODUCTION

The photoreceptors in the vertebrate retina are connected to two types of second-order neurones, the horizontal cells and the bipolar cells. The morphology of these connexions in the turtle has recently been described by Lasansky (1971).

Horizontal cells are usually classified into two main groups: luminosity cells (L-cells) which respond to light of any wave-length with hyperpolarization and chromaticity cells (C-cells) which are hyperpolarized by some wave-lengths and depolarized by others. The L-cells collect activity from numerous photoreceptors and send signals back to them (Baylor, Fuortes & O'Bryan, 1971) and possibly forward to bipolar cells (Werblin & Dowling, 1969). The recurrent loop from horizontal cells modifies the activity of the receptors with the result that receptor responses to flashes of light become different for different colours or patterns of the stimulus (Fuortes, Schwartz & Simon, 1973; O'Bryan, 1973). C-cells have been studied most extensively in fish (MacNichol & Svaetichin, 1958; Svaetichin & MacNichol, 1958; Naka & Rushton, 1966; see also Gouras, 1972 and Daw, 1973, for reviews) and have been classified in accordance with the colours of the stimuli which produce responses of opposite polarity. Thus, cells which are depolarized by red and hyperpolarized by green are called red/green or R/G-cells; others, best depolarized by yellow-green and hyperpolarized by blue, are called Y/B or G/B; and a third group which is depolarized by green and hyperpolarized by both blue and red lights is called G/RB.

It has been suggested that L-cells measure the average illumination of wide areas of the retina while C-cells are involved in the detection and discrimination of colour (see Abramov, 1972 and Gouras, 1972, for reviews). The mechanisms and pathways through which these presumed functions are subserved, however, are not yet understood.

In the turtle two types of L-cells and two of C-cells have been found. L-cells of type I and type II are distinguished by different histological features and receptive fields (Simon, 1973). Of the fifty-one C-cells

encountered in the present study, 65% were R/G and 35% were G/B; C-cells of G/RB type have not been found.

It is clear that C-cell responses, being strongly dependent upon the colour of a stimulus, must be controlled by activities originating in different types of receptors. Colour-dependence is less obvious in L-cells, but its existence is demonstrated by the observation that the shape of their responses changes with wave-length (Fuortes *et al.* 1973).

The purpose of the present study is to describe the properties of the horizontal cells, to identify the interactions operating on them and to determine (as far as possible) what circuits control their activities. It will be concluded that the main features of horizontal cell responses, including the depolarizing responses of C-cells, can be explained by assuming that each horizontal cell type receives impingement from only one type of cone. Additional interactions further modify the responses of each cell type. The proposed interpretations suggest that the distinction between L- and C-cells is not as fundamental as is generally thought since the responses of either cell type are functions of both colour and intensity. Rather, the evidence supports the simple notion that each horizontal cell type reflects primarily the spectral properties of a corresponding cone type.

METHODS

The methods used for stimulating and intracellular recording from retinal cells of the turtle, *Pseudemys scripta elegans*, are the same as described previously by Baylor & Fuortes (1970). Eyecup preparations were kept at room temperature of approximately 21° C in all experiments. Cones and L-cells were identified and classified according to their receptive field and spectral properties following criteria described in previous papers (Baylor *et al.* 1971; Simon, 1973). Cells with large summation areas which were hyperpolarized or depolarized by different wave-lengths were considered to be C-cells. Such cells have been identified in the turtle as horizontal cells by means of intracellular marking experiments (Miller, Hashimoto, Saito & Tomita, 1973).

Stimuli were 10 msec flashes which covered circles 1·25 mm in radius centred about the impaled cell. They were applied either from darkness or over a steady circular background, also 1·25 mm in radius. Monochromatic lights were obtained with narrow-band interference filters (half-widths about 15 nm) attenuated by neutral density filters. The quantity of light delivered by one flash is reported as the number of photons falling over 1 μm^2. In the illustrations numbers near the records measure this quantity in units of 10^3 photons. Dim flashes were used to evoke small responses, and ten to forty such responses were summed on a Mnemotron computer to improve the signal-to-noise ratio. Results were considered only when control runs demonstrated satisfactory stability of the cell throughout the experiment.

In most experiments flashes of different intensities were applied for each colour of the stimulus. In these cases the number of summed responses was inversely proportional to light intensity so that a constant number of photons was delivered in each run. Invariance of the summed responses (as in Figs. 1, 3, etc.) indicates that the average response to one flash is proportional to the strength of the stimulus.

This is an incomplete test of linearity but was considered sufficient for present purposes.

In the figures which show the results of experiments of the type just mentioned the ordinates measure sensitivity, $S(t)$, defined as the voltage, $V(t)$, of the summed response divided by the total number of photons delivered over 1 μm^2 (μV/photon. μm^2). When the illustrations show responses to a single light intensity, the ordinate measures instead the voltage of the response to one flash (mV). In general, even these small responses of horizontal cells were not invariant with respect to wave-length: C-cell responses reversed polarity and L-cell responses often showed consistent differences in time course for different colours. For this reason spectral sensitivity curves could not be constructed using a constant response criterion. Therefore, these curves were constructed by measuring the quantity $S = V_{max}/Q$ where V_{max} is the voltage at the peak of the summed response and Q is the total quantity of light delivered over 1 μm^2. Since only ten filters, separated by approximately 33 nm, were used, there is some uncertainty about the exact values of peak sensitivity (λ_{max}). Restricting the study to small responses evoked by dim flashes avoids many complications which affect the responses to bright lights and, thus, facilitates analysis of the interactions.

RESULTS

L-cell responses

The responses of L-cells to flashes of a given colour are usually linear with respect to stimulus intensity whenever their peak height is less than a few mV (from 2 to 5 mV in different units). They are smooth hyperpolarizing waves similar to the linear responses of red cones but appreciably faster than those of green cones. In a sample of sixty-five cells, times to peak (with standard error of the mean) were as follows: L-cells 106 ± 3 msec; red cones 110 ± 3 msec; green cones 131 ± 2 msec. In most L-cells, both of type I and type II, such small responses were not strictly invariant with respect to wave-length. In some cases response shape changed very slightly with colour (Fig. 1), but in other cells this difference was prominent (Fig. 4). Spectral sensitivity curves (Fig. 2) show a peak between 618 and 650 nm and generally resemble the spectral curves of red cones (see Baylor & Hodgkin, 1973). No appreciable differences were noticed in the linear responses of L-cells of types I or II.

These properties indicate that red cones are the principal cells controlling the responses of L-cells. If they were the only cells involved, however, it would be difficult to explain the strong colour-dependence of L-cell responses which is observed frequently following dim flashes and consistently when brighter flashes are employed (inset of Fig. 1; see also Fig. 6 in Fuortes *et al.* 1973). For this reason one must consider the possibility that cells other than red cones influence L-cell responses even though their contribution is too small to be recognized in the linear measurements of spectral sensitivity.

Useful information on this matter can be obtained making use of

selective adaptation of the retina. It can be shown that, when cones are exposed to a steady background of effectively absorbed light, their responses to a flash become smaller, faster and often diphasic. Deep red backgrounds produce these changes in red but not in green cones. Responses recorded from a red cone are illustrated in Fig. 3*A*. Flashes of

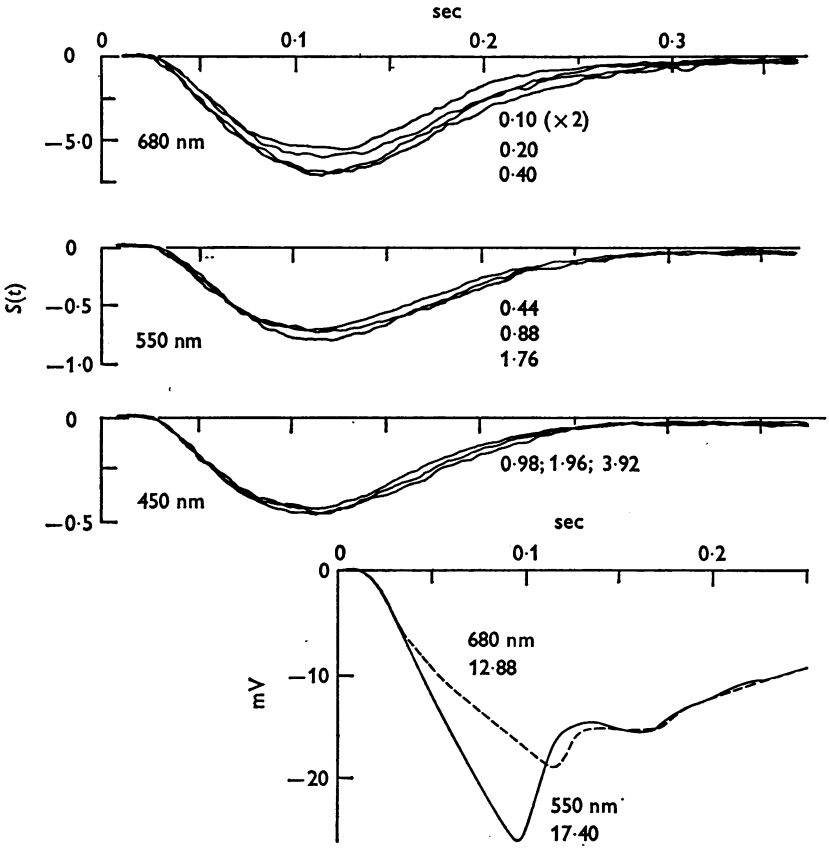

Fig. 1. Responses of an L-cell to monochromatic flashes. The tracings in each set are the sums of 10, 20 or 40 responses to different intensities, the total number of photons delivered remaining the same for all tracings in that set (see Methods). In this and subsequent figures the numbers near each tracing of averaged responses, when multiplied by 10^3, measure the number of photons delivered by one flash over 1 μm^2. The notation (× 2) indicates that a repeat run is included. Ordinates give sensitivity, $S(t)$, as defined in Methods (μV/photon.μm^2). Time zero is the midpoint of a 10 msec flash. Changes in response size as in the top tracings reflect variability during the long time required to complete the measurements rather than systematic deviations from linearity.

Inset: large responses of an L-cell to brighter flashes are clearly different for stimuli of 680 nm (dashes) or 550 nm (continuous).

550 nm applied from darkness produced the usual hyperpolarizing responses; with either a green or a red background sensitivity was decreased and the responses became fast and diphasic. In the green cone of Fig. 3*B* similar changes were evoked when the background was green, but when it was red the responses remained very similar to those elicited from darkness. Thus, if both red and green cones impinge on an L-cell, the relative contribution of the green cones should be greatly increased in the presence of a red background.

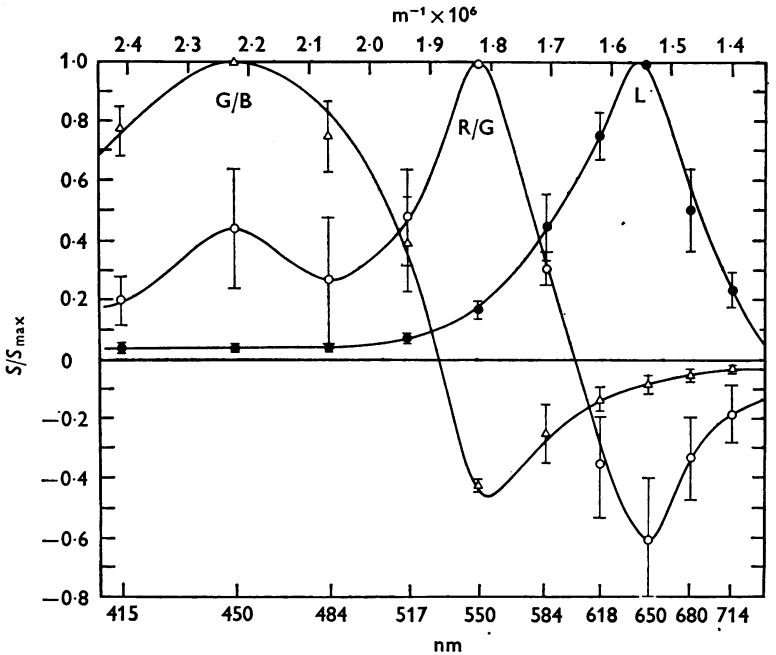

Fig. 2. Spectral sensitivity curves of horizontal cell responses in the linear range. Each point is the mean relative sensitivity measured in twenty-three L-cells, sixteen R/G-cells and four G/B-cells (error bars: standard deviations). Hyperpolarizing responses are plotted up and depolarizing responses are down.

In the experiment of Fig. 4*B*, red and green flashes of relative intensity 1:8·7 were applied from darkness or over a red background. From darkness, the responses of the L-cell had different shape for the two colours and were larger for the red stimulus. In the presence of a red background, the responses became faster for either colour of the flash but sensitivity was decreased more for red. The sensitivity of red cones to red and green flashes was decreased in the same proportion by this background (Fig. 4*A*). Such observations lead to the conclusion that L-cell responses are controlled mostly by the activity of red cones but are also subject to interactions

originating in receptors of other colours as had already been inferred from previous results on non-linear responses (Fuortes *et al.* 1973). It is difficult, however, to identify the cells responsible for this interaction. Green cones (Fig. 3) and R/G-cells (Fig. 6) produce slow responses to green flashes

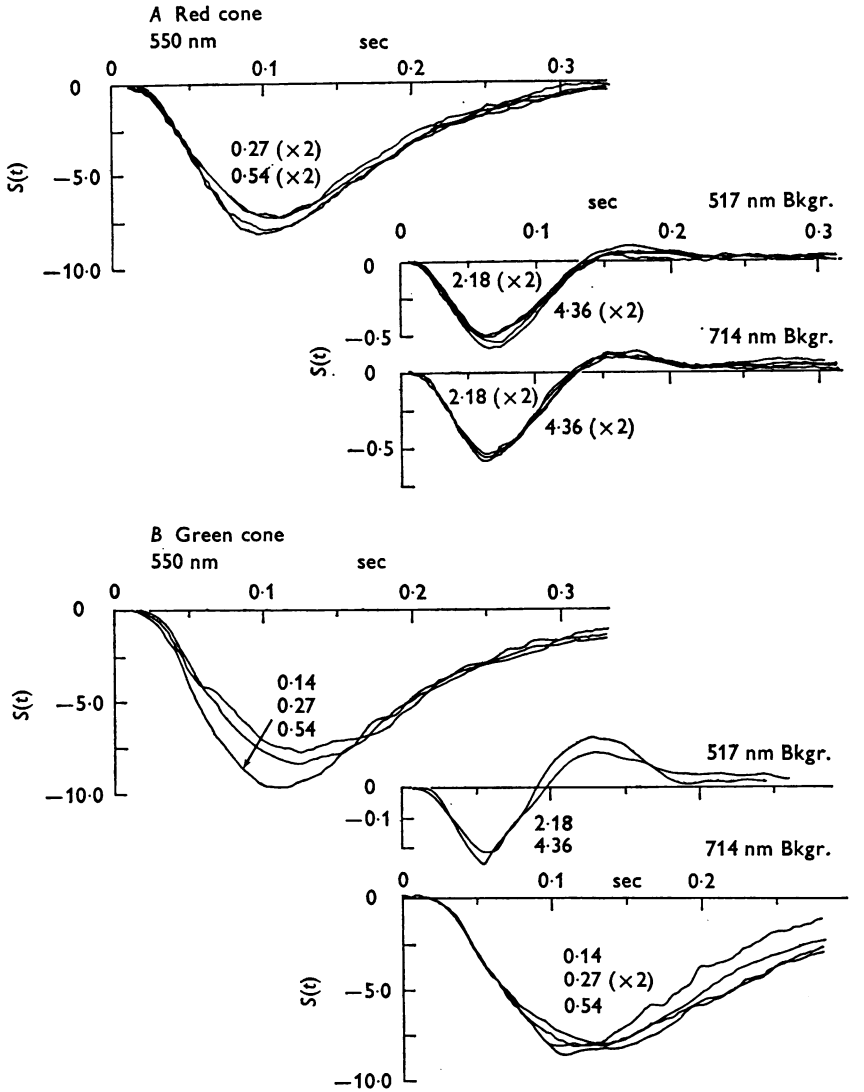

Fig. 3. Effects of background illumination on cone responses. Responses of red (*A*) and green (*B*) cones to 550 nm flashes were applied from darkness and over backgrounds of 517 or 714 nm. In this and subsequent Figures the 517 nm background delivered $1{\cdot}07 \times 10^5$ photons/sec.μm^2 and the 714 nm background, $1{\cdot}19 \times 10^5$ photons/sec.μm^2.

applied over a red background. If they impinge on L-cells, the responses of L-cells would be expected to include some slow component; instead, they are rapid and apparently unrelated to the activity of green cones or R/G-cells. It will be seen later that double cones are a possible source of colour interactions on L-cells.

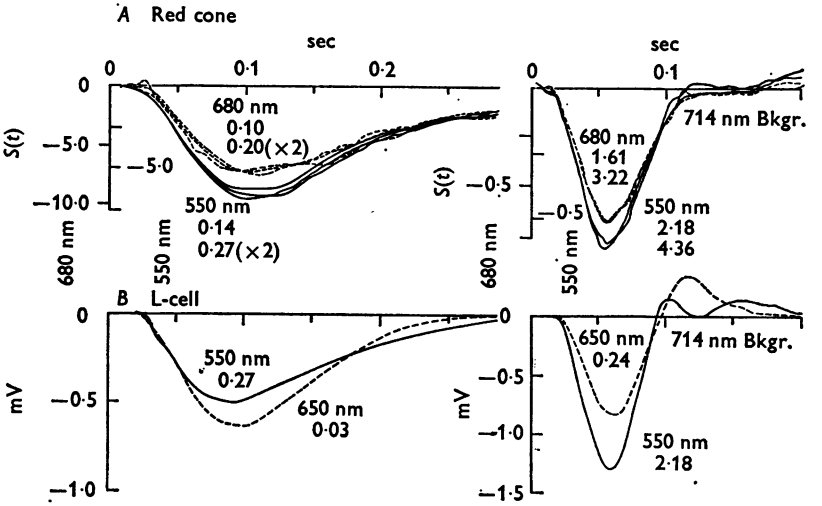

Fig. 4. Comparison of red cone and L-cell responses. *A*, the time courses of linear responses of a red cone are very nearly equal following red (dashed lines) or green (continuous lines) stimuli applied from darkness. Response shape and sensitivities changed when flashes were applied over a red background but the ratio: sensitivity to red/sensitivity to green remained essentially the same. Note separate sensitivity scales in the ordinates for red or green stimuli. *B*, in the L-cell illustrated responses to red or green flashes had a clearly different shape when stimuli were applied from darkness. When flashes were applied over a red background, sensitivity decreased more for the red than for the green stimulus. As only one intensity was used for each colour, ordinate scales give the voltage of single responses.

Responses of R/G-cells

In R/G-cells linear responses to green or blue flashes are smooth hyperpolarizing waves; red flashes evoke instead depolarizing deflexions (Fig. 5). Times to peak, measured in twenty-five cells, were 152 ± 4 msec, 149 ± 4 msec and 117 ± 4 msec for blue, green and red flashes respectively. Linear spectral sensitivity of the R/G-cells is illustrated in Fig. 2. Maximal sensitivity of the hyperpolarizing response is around 550 nm as in the green cones; however, there may be an additional peak at 450 nm corresponding to the peak of sensitivity of blue cones (see Baylor & Hodgkin,

1973). Maximal sensitivity of the depolarizing response is between 618 and 650 nm, as in L-cells or red cones.

When the retina is exposed to a steady green light (517 nm), the hyperpolarizing responses of R/G-cells to green flashes become faster and diphasic as the responses of green cones (Fig. 6*A*). Red backgrounds instead accelerate slightly the decay of the R/G-cell responses but have negligible effect on the green cones. Depolarizing responses of R/G-cells to red flashes are accelerated by either green or red backgrounds; they

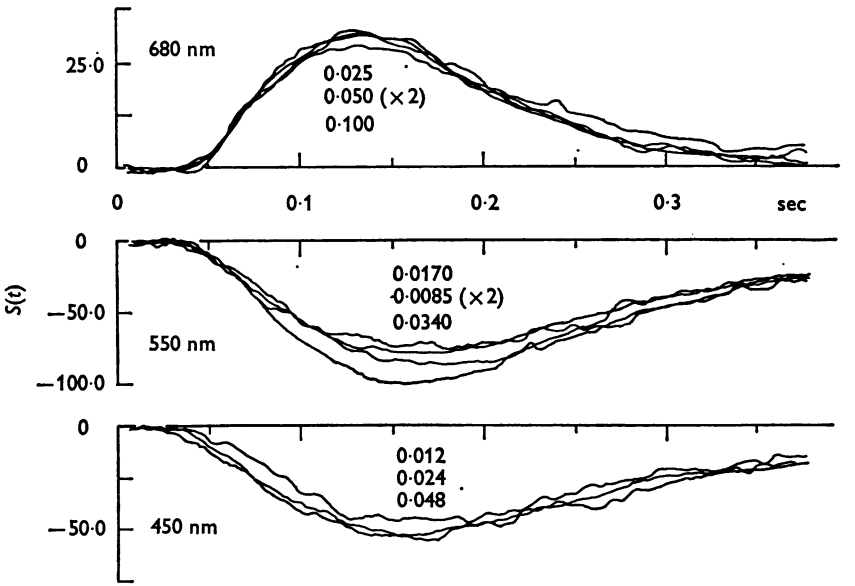

Fig. 5. Responses of an R/G-cell to monochromatic flashes. Experiment was as in Fig. 1. Note slower time course of hyperpolarizing than of depolarizing responses.

resemble then inverted L-cell responses (Fig. 6*B*). Since green cones are depolarized by red lights due to impingement from L-cells (Fuortes *et al.* 1973), it seems possible that the depolarizing responses of R/G-cells arise from the depolarization of the green cones. However, additional impingement from other cells may be required to explain certain discrepancies between the responses of green cones and those of R/G-cells such as the additional blue peak in the spectral sensitivity of R/G-cells (see below).

Responses of G/B-cells

Blue flashes evoke in G/B-cells hyperpolarizing waves analogous to those elicited by green light in R/G-cells (Fig. 7). In a sample of eleven cells, time to peak was 151 ± 5 msec. Responses to longer wave-lengths, instead,

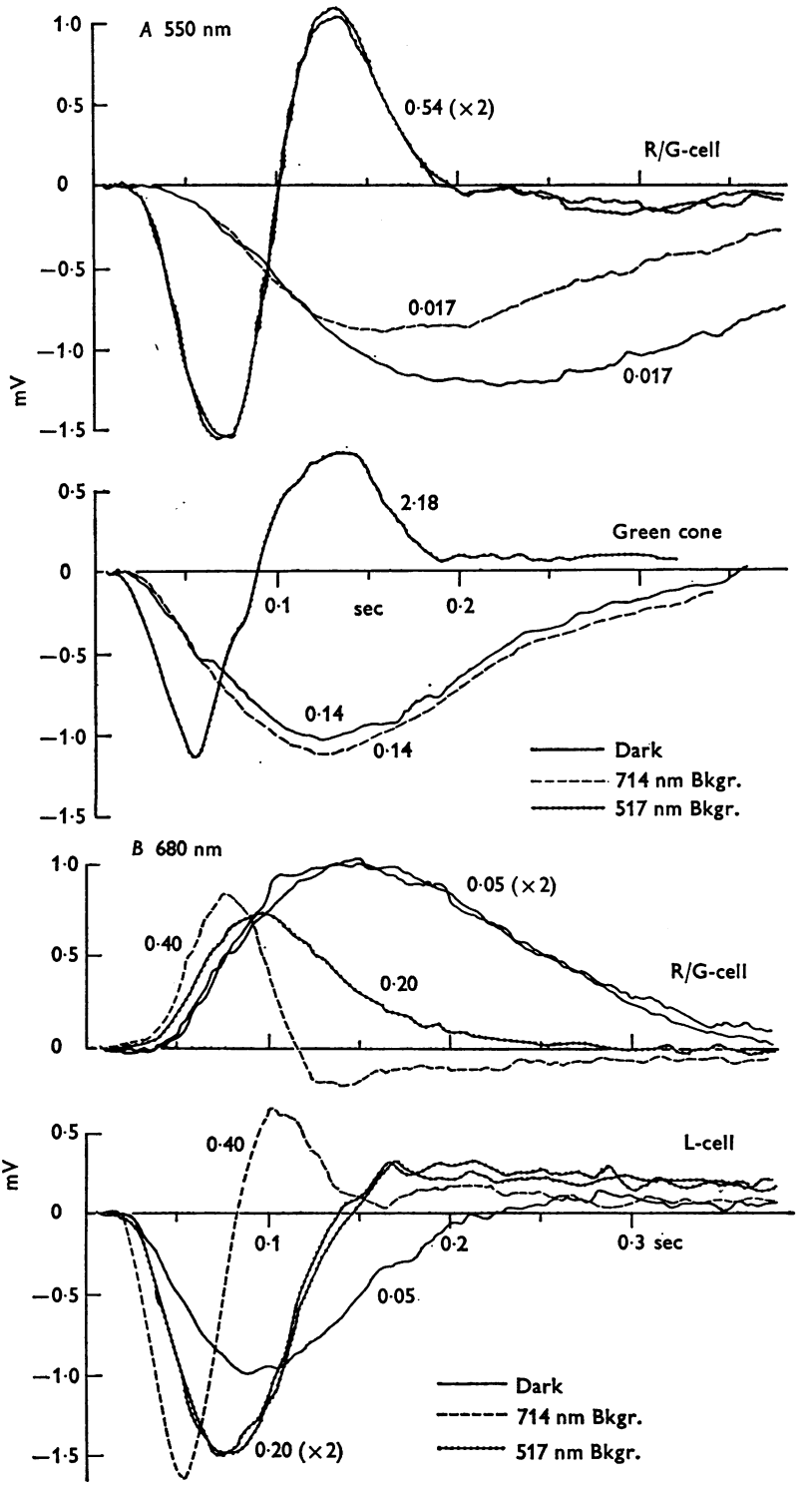

Fig. 6. For legend see facing page.

are conspicuously different: green flashes evoke slow depolarizing responses (time to peak 143 ± 6 msec), and red flashes produce small, brief and irregular depolarizing waves. Their time course changes markedly as intensity is increased, and the early peak may grow more than proportionally to the strength of the stimulus (inset, Fig. 7). These complexities suggest that small responses of G/B-cells to red flashes are already subject to interactions. Peak sensitivity of the hyperpolarizing response is about 450 nm as in blue cones while maximal sensitivity of the depolarizing response is around 550 nm (Fig. 2). With longer wave-lengths sensitivity drops rapidly becoming quite low for the red stimuli which are most effective on red cones and L-cells.

The hyperpolarizing responses to blue flashes are reduced and accelerated by a 517 nm background but are not appreciably altered by a red background (Fig. 8). The depolarizing responses to green flashes are made smaller and faster by green backgrounds. Over a red background their falling phase is accelerated, but they remain much slower than the corresponding responses of L-cells. Instead, these changes are similar to those occurring in R/G-cells under the same experimental conditions (Fig. 6*A*). Finally, the responses to red flashes become faster and smaller on both green and red backgrounds, not unlike the responses of L-cells.

The hyperpolarizing responses of G/B-cells to blue light can reasonably be ascribed to direct impingement from blue cones, but the origin of the depolarizing responses is uncertain. Since their spectral sensitivity has a peak for green light, it might be thought that they are evoked by direct depolarizing impingement from green cones (Naka & Rushton, 1966) or R/G-cells. This interpretation is unlikely because these cells are hyperpolarized by green but are depolarized by red flashes; whereas the responses of G/B-cells are depolarizing for both colours. It seems more probable that depolarization of G/B-cells is brought about by the activity of L-cells but does not follow their spectral sensitivity because of additional interactions.

Receptor control of horizontal cell responses

These results suggest that the main features of horizontal cell responses can be explained by a simple circuit in which each type of cone impinges

Fig. 6. Comparison of responses of R/G-cells, green cones and L-cells. *A*, responses were evoked by green flashes applied from darkness and from red or green backgrounds. The changes produced by the green background are generally similar in the R/G-cell and in the green cone. *B*, depolarizing responses of the same R/G-cell to red flashes were accelerated by both green and red backgrounds. These changes are similar to those occurring in the L-cell under the same experimental conditions.

upon a corresponding type of horizontal cell while L-cells exert a depolarizing action on all cone types. Red light evokes hyperpolarization of red cones and L-cells but is not appreciably absorbed by green or blue cones. L-cell activity will then evoke pure depolarization of green and blue cones, and this action will be reflected in R/G- and G/B-cells respectively. Green light is absorbed well by red and green but only poorly by blue

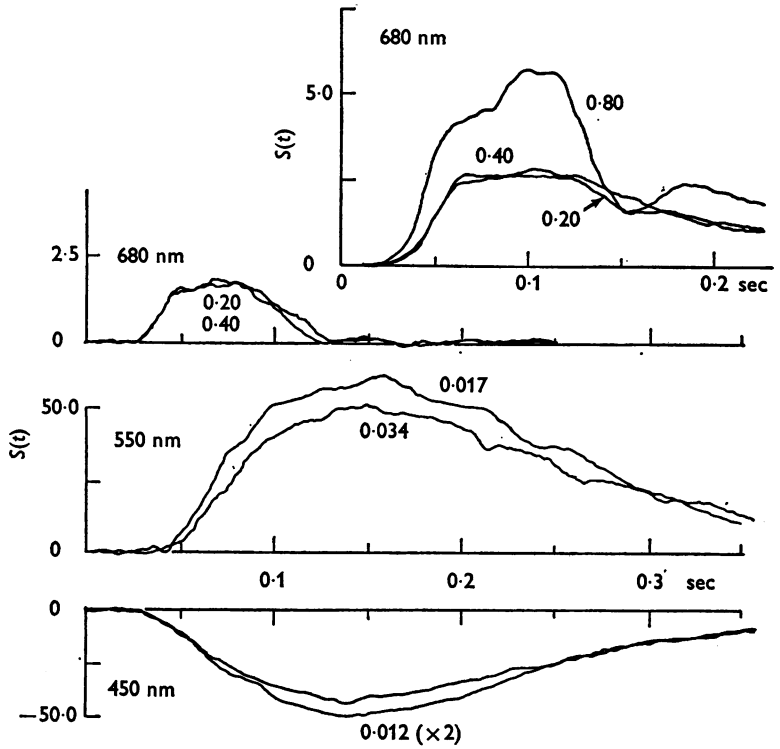

Fig. 7. Responses of a G/B-cell to monochromatic flashes. The experiment was the same as in Figs. 1 and 5. Responses to blue light are similar to those of R/G-cells, and responses to green light resemble those produced in G/B-cells by red light but are somewhat slower. Depolarizing responses to red light are small, short-lasting and irregular. Their peak height may grow more than linearly with light intensity as in the cell illustrated in the inset.

cones; therefore green flashes evoke hyperpolarization of green cones and R/G-cells but depolarize blue cones and G/B-cells. Thus the basic scheme represented by solid lines in Fig. 9 gives rise both to the hyperpolarizing and to the depolarizing responses of C-cells. There are, however, important discrepancies between the predictions of this diagram and the experimental results suggesting the occurrence of additional interactions. These discrepancies will now be considered in some detail.

Modifying interactions

In the basic diagram of Fig. 9 (continuous lines), L-cells receive input only from red cones and red cones only from L-cells. With this arrangement the responses of both cells should be invariant with respect to wavelength since they are controlled by the absorption characteristics of a single pigment (the possible influence of oil droplets is neglected for the present argument). But the results of Fig. 4 (as well as previous findings

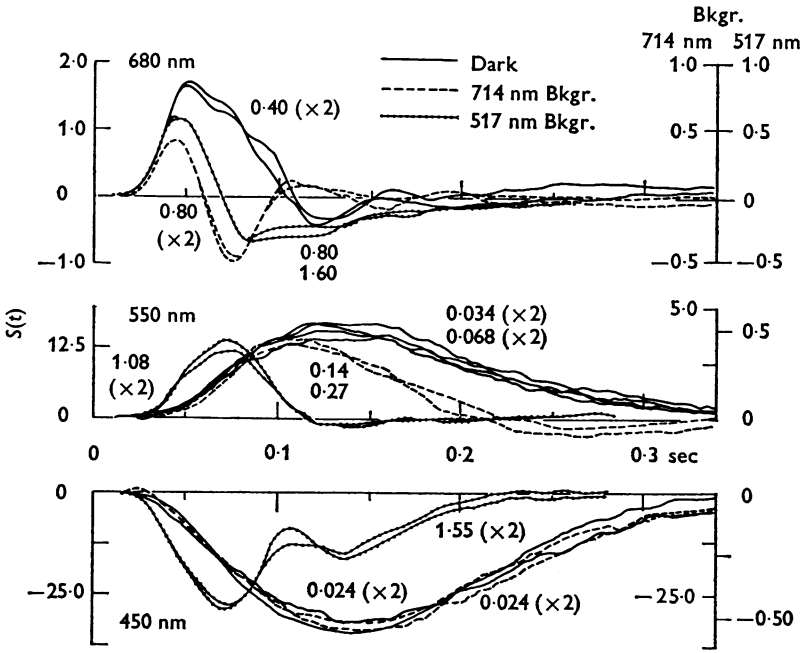

Fig. 8. Effects of backgrounds on G/B-cell responses. Responses were evoked by monochromatic flashes applied from darkness (continuous tracings), over a 714 nm background (dashes) or over a 517 nm background (dots). The red background had no effect on the responses to blue flashes, a moderate effect on the responses to green and a strong effect on the responses to red, but the green background had a major effect on all. Ordinates are labelled for responses from darkness on the left and over the backgrounds on the right.

by Fuortes *et al.* 1973) indicate that L-cell responses are colour-dependent and that this property is not due to a similar colour-dependence of red cone responses. Therefore L-cells must be influenced by some other cell in addition to the red cones. It has already been mentioned that this accessory influence is not likely to originate in green cones or R/G-cells because the time course of their responses to green flashes applied over a

red background differs substantially from the course of the corresponding responses of L-cells (Figs. 4*B* and 6*A*).

There are, however, other cells which appear to have the required properties. The shape of their responses and their receptive fields resemble those of cones. They can be distinguished from red or green cones, however, because the time course of their responses is different for flashes of different colours. Work in progress (A. Richter & E. J. Simon, in preparation) suggests that they are double cones, which are known to consist of two

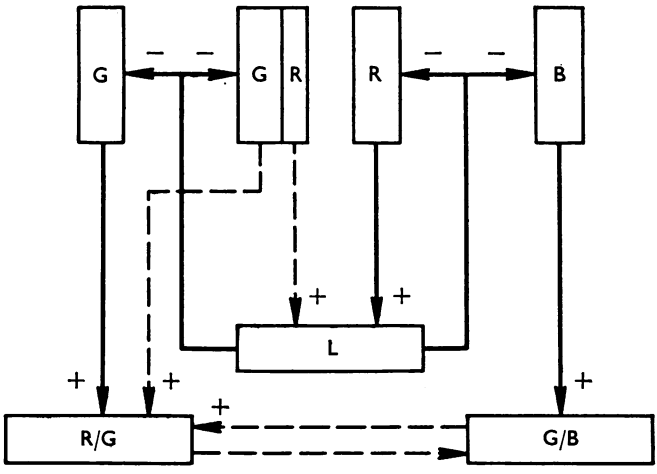

Fig. 9. Diagram of the relations between cones and horizontal cells. The basic connexions responsible for the main properties of the cells are indicated by solid lines; dashed lines represent additional modifying interactions. The symbols + and − denote transmission without or with inversion of polarity. G, R and B are green, red and blue cones respectively; horizontal cells are identified by the usual notation; and the two adjoining R and G receptors represent the double cones. Additional outputs of C-cells could go to receptors, L-cells or bipolar cells; other uncertainties of this scheme are mentioned in Discussion.

tightly associated receptors, each containing one pigment type (Müller, 1857; Liebman, 1972). Fig. 10 is a comparison of responses evoked by green and red flashes in double cones and in L-cells. In both cell types the time course of the responses to the two colours is slightly different when the flashes are applied from darkness. When delivered over a red background, however, the difference of the double cone responses is increased: red flashes evoke rapid, diphasic responses similar to those of red cones (Fig. 4*A*) while green flashes elicit purely hyperpolarizing responses which are considerably faster than the responses produced in the same conditions by green cones (Fig. 3*B*). If these cells were to impinge upon L-cells

together with the red cones, they might well produce the observed colour-dependence of L-cell responses including the changes produced by red backgrounds – a relative increase in the sensitivity to green not associated with a pronounced slow component, which would be expected if the additional impingement originated in the green cones. One may then suggest that L-cells receive excitation not only from red cones but also from double cones (presumably their red members).

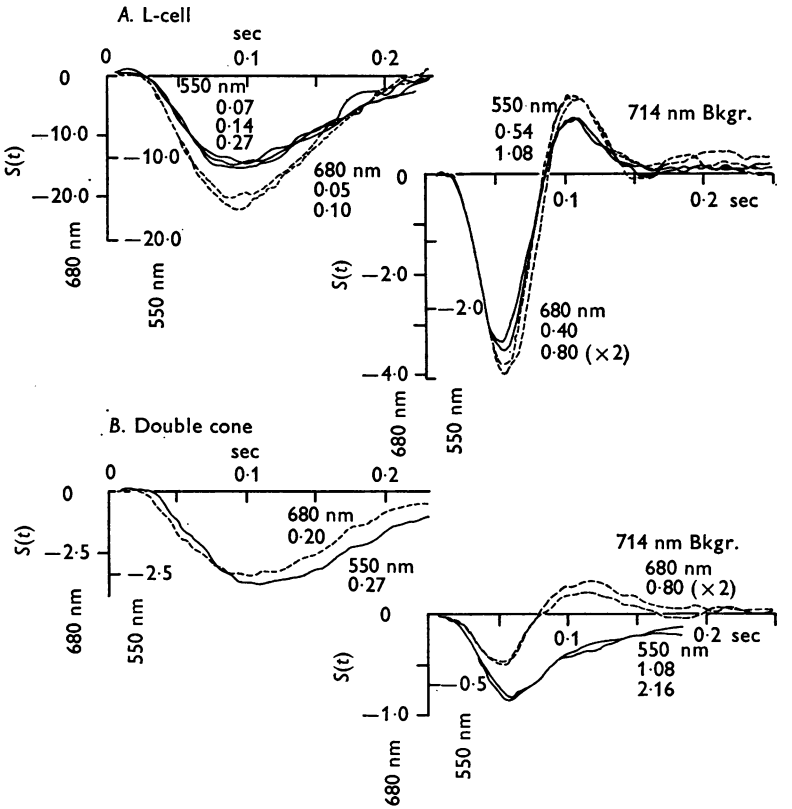

Fig. 10. Comparison of responses of L-cells and double cones. *A*, L-cell responses to green and red flashes applied from darkness and over a 714 nm background. *B*, responses of a double cone to similar stimuli. Responses to green and red flashes become strikingly different when applied over the red background.

The predictions of the basic circuit of Fig. 9 differ from the results also with respect to R/G-cells. It may be argued that the depolarization evoked in green cones by red light is too small to give rise to the large depolarizing responses of R/G-cells, which can exceed 30 mV for bright flashes (not illustrated). Furthermore, all the R/G-cells encountered in the

present work responded with depolarization to dim flashes of 618 nm while the same flashes evoked hyperpolarizing responses in all but one of the green cones studied. These discrepancies might be explained if the microelectrode damaged the synaptic response of a cone more than its direct response to the light it absorbs (see Fuortes *et al.* 1973). In these conditions the L-cell action would evoke only small depolarizations in impaled cones, and net hyperpolarization may result when the recurrent action is superposed on the direct receptor response. Normal cones, responding more vigorously to the synaptic impingement from L-cells, instead would be depolarized by the same stimuli.

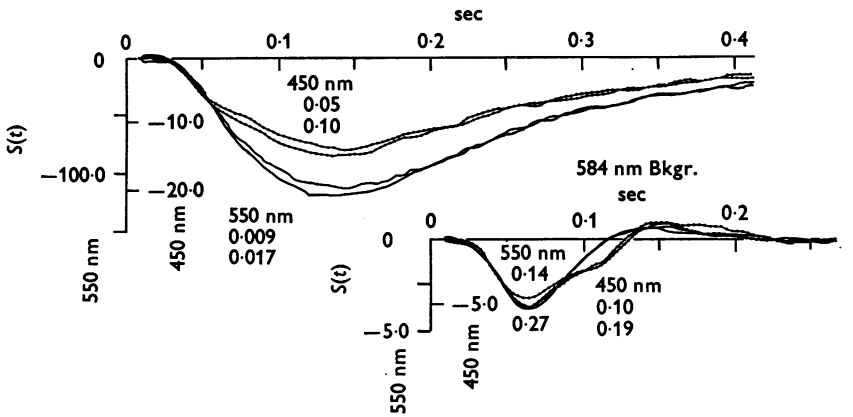

Fig. 11. Responses of an R/G-cell to blue and green flashes. Dotted lines are tracings of responses to 450 nm flashes, continuous lines, to 550 nm flashes. Stimuli were applied from darkness on the left and over a 584 nm background on the right. The 584 nm light delivered $1{\cdot}62 \times 10^5$ photons/sec.μm^2.

It is also possible, however, that R/G-cells receive accessory impingement from blue-sensitive cells, such as the G/B-cells, which are depolarized by 618 nm light. This second alternative is suggested by the high sensitivity of R/G-cells to blue light (Fig. 2) and is supported by results of experiments involving selective adaptation. Light of 517 nm, as used in the experiments of Fig. 6, is unsuitable for this purpose because it is effectively absorbed by both green and blue cones. A background of 584 nm, however, should depress and shorten the responses of the green cones but not of the blue. In the R/G-cell illustrated in Fig. 11 such a background increased the ratio: sensitivity to blue/sensitivity to green by a factor of about 11, making the sensitivity greater for blue than for green. Time courses of the responses to green or blue flashes were similar from darkness. When applied over the background, however, the responses

to blue included a slow hyperpolarizing component reflecting the waveform of blue cones or G/B-cells. These are the results to be expected if blue cones contribute to the hyperpolarizing responses of R/G-cells either directly or through G/B-cells.

If L-cells were the only elements controlling (through the corresponding cone types) the depolarizing responses of R/G- and G/B-cells, then the spectral sensitivity of these depolarizing responses should follow that of the L-cells. The experimental results show that the responses of R/G-cells have the required properties but those of G/B cells do not (Fig. 2). It has already been mentioned that the spectral characteristics of G/B-cells are most easily explained assuming that their depolarizing responses are in fact due to L-cell activity (presumably through blue cones) but are modified by interactions from other cells. A useful clue for identifying this interacting cell is provided by the spectral curves of Fig. 2. The general characteristics of these curves are reproduced by the dashed lines of Fig. 12 (curves 1, 2, and 3). The line with small dots (curve 4) illustrates the spectral sensitivity of blue cones as determined by Baylor & Hodgkin (1973) using small spots of light (100 μm diameter). In the basic scheme of Fig. 9, the hyperpolarizing response of G/B-cells should follow the spectral sensitivity of blue cones and the depolarizing response that of L-cells. In the simplest conditions the entire spectral characteristic of G/B-cells might be expected to be a linear combination of curves 1 and 4. Curve 5 is such a combination (curve 5 = curve $4-[0{\cdot}3 \times$ curve 1]), and it deviates substantially from the plot of experimental measurements. Suppose, however, that R/G-cells also impinge on G/B-cells exerting an action similar to that of the L-cells. This additional impingement will change the spectral sensitivity as shown in curve 6 which is a normalized plot of curve $5-[0{\cdot}35 \times$ curve 2]. The corrected curve follows the experimental data reasonably well supporting the notion that R/G-cells indeed impinge on G/B-cells. Confirming this supposition, it is also found that simple combination of responses of L-cells and R/G-cells to a red stimulus results in a curve which approximates the responses of G/B-cells (inset of Fig. 12).

DISCUSSION

The general conclusions of the present work are summarized in the diagram of Fig. 9. In this scheme each type of cone is connected to a corresponding type of horizontal cell. These connexions are responsible for the hyperpolarizing responses of the three horizontal cell types with sensitivity peaks around 640, 550 and 450 nm, as in red, green and blue cones respectively.

Baylor & Hodgkin (1973) have shown that, in the absence of modifying

interactions, the action spectra of turtle cones are asymmetric, reflecting the properties of the visual pigments and associated oil droplets (Liebman & Granda, 1971; Liebman, 1972). Cone sensitivity, as pigment absorption, decreases sharply for wave-lengths longer than λ_{max} but only moderately (between one and two logarithmic units) for shorter wave-lengths. Thus, red cones respond best to red lights but are also sensitive to green and blue; green cones respond to green and blue but very poorly to red; and blue cones respond well to blue lights only.

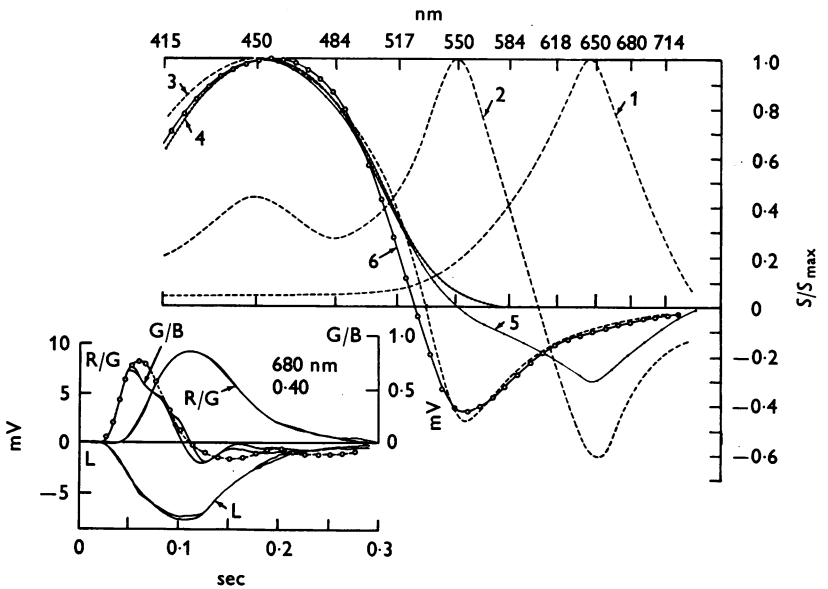

Fig. 12. Simulation of G/B-cell responses. Dashed curves 1, 2 and 3 reproduce the spectral sensitivity curves of Fig. 2 for L-cells, R/G-cells and G/B-cells respectively. Curve 4 (small dots) is the spectral sensitivity of blue cones replotted from the data of Baylor & Hodgkin (1973). Curve 5 (curve $4 - 0{\cdot}3 \times$ curve 1) does not follow the spectral characteristic of the G/B-cells but curve 6 (curve $5 - 0{\cdot}35 \times$ curve 2) does to a good approximation.

Inset: the general characteristics of the response of a G/B-cell to a 680 nm flash are reproduced by simple combination of the responses of an R/G-cell and an L-cell to the same stimulus. The dashed curve with circles was obtained by inverting and adding the responses of the L-cell and the R/G-cell. Ordinates for the R/G-cell and L-cell are on the left and for the G/B-cell are on the right.

If the pathway from each cone type to a corresponding horizontal cell were the only connexion between these cells, then horizontal cells would develop only hyperpolarizing responses, and their spectral sensitivity would be controlled by the absorption of the cone pigments. The situation

is changed, however, by the recurrent loop from L-cells back to cones. Since L-cells are activated by red cones, they respond appreciably to all wave-lengths in the visible spectrum and send depolarizing signals back to all receptors. Red light may elicit hyperpolarization of red cones while evoking negligible direct responses in green and blue cones. The depolarizing impingement from L-cells will then decrease the responses of red cones without reversing their polarity but will evoke pure depolarization of green and blue cones and thereby of R/G- and G/B-cells. Green light evokes hyperpolarization of red cones, L-cells and green cones but not of blue cones. Hence, the blue cones (and through them the G/B-cells) will be depolarized by green light as well as by red. Finally, blue light evokes hyperpolarization of all cones and consequently of all horizontal cell types. Thus, the proposed scheme provides a mechanism by which the responses of R/G- and G/B-cells reverse with wave-length while those of L-cells are always hyperpolarizing. A similar interpretation of C-cell responses was proposed by Gouras (1972).

The depolarization evoked by L-cells, however, is not sufficient to account for the spectral properties of G/B-cells since the sensitivity of their depolarizing responses, with peak in the green rather than in the red, does not follow the spectral characteristic of the L-cells. To explain this discrepancy it is suggested that G/B-cells receive impingement not only from L-cells but also from R/G-cells. Hyperpolarization of R/G-cells by green light evokes depolarization of G/B-cells and increases a similar effect of L-cells. With red lights R/G-cells are depolarized and exert a hyperpolarizing action on G/B-cells antagonizing the effect of the L-cells. The results illustrated in Fig. 12 show that such combined action of L-cells and R/G-cells may be adequate to explain the properties of G/B-cell responses.

It will be noted in addition that the same mechanism might easily give rise to the 'triphasic' responses which have been observed in the G/RB-cells of fish. The responses of L-cells and R/G-cells to red light have opposite polarity and exert opposite action on the blue-sensitive C-cells. When the action of the L-cells predominates, depolarization results; but a stronger action of the R/G-cells could produce hyperpolarization of the blue-sensitive C-cells. With green lights the responses of L-cells and R/G-cells have the same polarity; their combined action will then result in depolarization regardless of their relative effectiveness. The main features of the responses of G/RB-cells (hyperpolarization for red or blue lights and depolarization for green) can thus be accounted for.

Uncertainties

A number of uncertainties remain in the description of the interconnexions between cones and horizontal cells:

(1) The suggestion that double cones impinge upon L-cells is reasonable, but there is no verification that they are indeed the source, or the only source, of the colour-dependence of L-cells. Additional interactions may well be required to explain the strong colour-dependence of L-cell responses to bright flashes illustrated in the inset of Fig. 1.

(2) The high sensitivity of R/G-cells to blue light has been attributed to a connexion reaching them from G/B-cells. This link explains also why R/G-cells are depolarized by light of 618 nm which does not depolarize the green cones. It should be noted, however, that the same results would be obtained if blue cones, rather than G/B-cells, impinged upon R/G-cells. Therefore this proposed connexion should be regarded as tentative.

It is often observed that the responses of double cones to green flashes become faster when the stimuli are applied over red backgrounds (Fig. 10). Thus, the changes brought about by these backgrounds in the corresponding responses of R/G-cells (Fig. 6*A*) can be explained assuming that double cones (perhaps the green members) impinge upon R/G-cells together with single green cones. In turn the shortening of G/B-cell responses under the same conditions (Fig. 8) can be ascribed to impingement from the R/G-cells.

(3) Blue cones have been impaled only on rare occasions, and it has not been possible to show that they are depolarized by L-cells. This action is supposed to occur on the basis of a generalization of the results obtained on red and green cones. Depolarization of G/B-cells might be evoked, however, not by blue cones but directly by L-cells. Conversely R/G-cells could modify the activity of G/B-cells just as well by impinging on the blue cones as by impinging directly on G/B-cells. These matters will remain unresolved until blue cones are investigated satisfactorily.

(4) It has been reported that rods contribute to horizontal cell responses in some animal species (Steinberg, 1969; Kaneko & Yamada, 1972). This action, however, has not been revealed by the experiments performed so far in the turtle, and for this reason rods are not included in the proposed diagram. It is possible, nevertheless, that the experimental conditions were unfavourable for detecting rod activity.

More generally, it must be considered that additional or alternative connexions between cones and horizontal cells might be present. For instance, even though the connexions outlined in the diagram appear to be sufficient to explain the observed responses, parallel pathways linking L-cells or red cones directly to C-cells cannot be excluded. The diagram of

Fig. 9 is probably the simplest but undoubtedly not the only circuit consistent with the results described in this paper.

Terminology

The ideas developed in this work suggest that the terminology usually applied to horizontal cells may be misleading. According to the proposed interpretations there is no sharp distinction between L- and C-cells – all horizontal cells produce colour-dependent responses; therefore, it is reasonable to suppose that all are involved in the detection of colours. The properties of different horizontal cells reflect largely the corresponding properties of different cone types; thus, to a first approximation R/G- and G/B-cells are no more 'red/green' or 'green/blue' than the green and blue cones respectively. Given this situation it may be more appropriate to abandon the subdivision between L-cells and C-cells and classify horizontal cells by the type of cone which is directly presynaptic to them: red, green and blue horizontal cells rather than L-, R/G-, and G/B- or G/RB-cells.

Actions of horizontal cells

In the proposed scheme the L-cells send signals back to all cones. In this way cone activity is modified by an interaction which depends upon both the pattern and colour of a stimulus, and the new signals forwarded by cones to bipolar cells will also be pattern- and colour-dependent. The recurrent connexion from horizontal cells to receptors would be sufficient to give the 'red' horizontal cells a significant role in visual functions. In the diagram of Fig. 9, green and blue horizontal cells send outputs only to one another so the scheme does not explain how their signals are communicated: they might be transmitted to receptors, to 'red' horizontal cells or to bipolar cells. Further work will be required to clarify this point. Nevertheless, it may be concluded that, because of the interactions described in this and prior studies, the responses of each cone and each horizontal cell contain information about both local and general properties of a visual stimulus – intensities, colours and pattern. Even though these different parameters of the stimulus are contained in the responses of each cell type, they are contained differently in each because the connexions of no two types are the same. This organization suggests that information processing in the retina makes use of principles which are significantly different from those usually considered.

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