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FEED-BACK MODULATION OF CONE SYNAPSES BY L-HORIZONTAL CELLS OF TURTLE RETINA

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SUMMARY

Light stimulation of the periphery of the receptive field of turtle cones can evoke both transient and sustained increases of the cone Ca^{2+} conductance, which may become regenerative. Such increase in the cone Ca^{2+} conductance evoked by peripheral illumination results from the activation of a poly-synaptic pathway involving a feed-back connexion from the L-horizontal cells (L-HC) to the cones. Thus the hyperpolarization of a L-HC by inward current injection can evoke a Ca^{2+} conductance increase in neighbouring cones. The cone Ca^{2+} channels thus activated are likely located at its synaptic endings and probably intervene in the cone transmitter release. Therefore the feed-back connexion between L-HC and cones by modifying the Ca^{2+} conductance of cones could actually modulate the transmitter release from cone synapses. Such feed-back modulation of cone synapses plays a role in the organization of the colour-coded responses of the chromaticity type-horizontal cells and probably of other second order neurones, post-synaptic to the cones. The mechanisms operating the feed-back connexion from L-HC to cones are discussed.

Introductory remarks

Cones are the main class of photoreceptors in the retina of the red-eared turtle (*Pseudemys scripta elegans*). According to their spectral properties and morphology they are classified as either red, green, blue and double cones (Baylor & Hodgkin, 1973; Richter & Simon, 1974). Green cones are much more sensitive to green lights than to deep red lights, while red cones are moderately more sensitive to deep red lights than to green ones. When turtle cones are impaled by a microelectrode they show, in the dark, membrane potentials of 25–35 mV (dark potential). Photoisomerization of their pigment by light impinging on them induces a graded hyperpolarization which may reach amplitudes up to 15–25 mV according to the light intensity (Fig. 1*a*).

Turtle cones are connected through a complex synaptic ending (Lasansky, 1971; Schaeffer & Raviola, 1975, 1978) to the two main classes of second-order neurones: bipolar cells and horizontal cells. These connexions are located in the outer plexiform layer. Bipolar cells constitute the second relay in the visual pathway proper. By their responses to small light spot stimulation of the direct input they receive from cones, two types of bipolar cells have been recognized: hyperpolarizing ('off-centre') bipolars, which are hyperpolarized by stimulation with small centred light spots, and depolarizing ('on-centre') bipolars, which are depolarized by the same stimuli

(Werblin & Dowling, 1969; for the turtle retina see Schwartz, 1974; Richter & Simon, 1973).

Horizontal cells play an interneuronal role in the outer plexiform layer of turtle retina (see below). They respond to light stimuli with graded potentials and are characterized by the great extension of their receptive field. According to their spectral properties two main classes of horizontal cells have been recognized (Svaetichin & MacNichol, 1958): the 'luminosity' horizontal cells (L-HC), maximally sensitive to red lights, which respond by graded hyperpolarizing responses to light stimuli of any wavelength, and the 'chromaticity' horizontal cells, which respond to light by either a depolarization or a hyperpolarization according to the wavelength of the stimulus.

Synaptic transmission from cones to second order cells

In contrast with the majority of synapses known in the central and peripheral nervous system of vertebrates and invertebrates, the synapses connecting cones to second-order cells are of the *tonic* type. In the dark they release transmitter continuously, whilst light (the physiological stimulus) hyperpolarizes the cone, thus depressing or suppressing the transmitter release (Trifonov, 1968). The sustained transmitter release in the dark is Ca^{2+}-dependent: when the ionic content of the extracellular medium is altered by removing Ca^{2+} or adding Co^{2+} or other divalent cations which block Ca^{2+} conductance, the transmitter release from the cone synapses is blocked. Therefore Ca^{2+} channel-blocking agents mimic the effects of light stimulation on the second-order neurones. This has been thoroughly demonstrated in turtle retina (Cervetto & Piccolino, 1974) as well as in other vertebrate retinas for both cones and rod synapses (Dowling & Ripps, 1973; Kaneko & Shimazaki, 1975; Dacheux & Miller, 1976).

When transmitter release from cone synapses is suppressed by light hyperpolarization different effects are observed in the second order neurones: the L-horizontal cells are hyperpolarized as a result of a decrease in their membrane conductance (Trifonov, Byzov & Chailahian, 1974) probably to Na^{+} ions (Waloga & Pak, 1978), the hyperpolarizing bipolar cells are also hyperpolarized and their membrane conductance is also decreased, the depolarizing bipolar cells are depolarized and their membrane conductance increased (Nelson, 1973; Toyoda, 1973; Werblin, 1977; Saito, Hondo & Toyoda, 1979). The effect of the cone transmitter on the C-horizontal cells will be discussed in the last section of this paper. The synaptic transmitter (or transmitters?) of the cones has (have ?) not yet been identified. However, the cone transmitter(s) should be expected to promote effects opposite to those observed when the transmitter release is suppressed. Thus, the synaptic transmitter(s) released from the cones could be expected to (*a*) depolarize the horizontal cells by increasing their membrane conductance, (*b*) depolarize the hyperpolarizing bipolar cells by increasing their membrane conductance and (*c*) hyperpolarize the depolarizing bipolar cells by *decreasing* their membrane conductance.

Antagonistic organization of the turtle cone receptive field

The amplitude and time course of the hyperpolarization of the cones by light depend on the light intensity, the duration of the stimulus and on the area of illumination. The outer segment of the turtle cone has a width of less than 10 μm. However,

the cone response to a centred spot of a given intensity gradually increases in amplitude as the diameter of the spot increases up to 120 μm (Baylor, Fuortes & O'Bryan, 1971). This signifies that a rather large population of neighbouring cones contributes to the response to each single cone through electrotonic coupling (Baylor *et al.* 1971) across gap-junctions (Raviola & Gilula, 1975; see Raviola, 1976). This electrical coupling is very specific, the red cones are exclusively coupled to other red cones, the green cones to other green ones, etc. (Baylor & Hodgkin, 1973; Detwiler & Hodgkin, 1979).

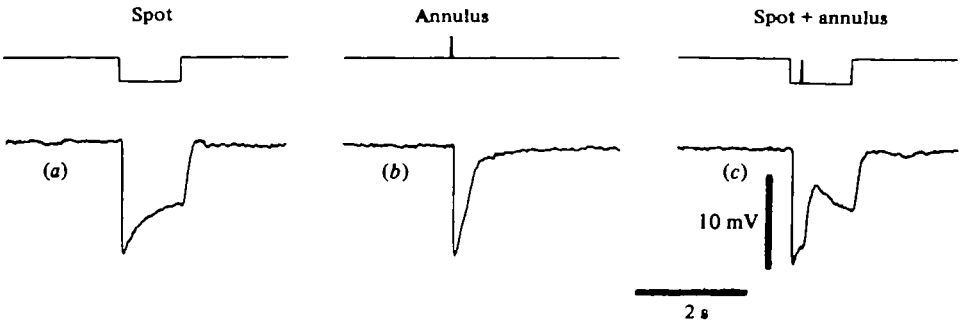

Fig. 1. Depolarizing response of a turtle red cone to peripheral stimulus. (*a*) Hyperpolarizing response of the cone to a centred spot of white light (250 μm diameter, attenuation $-2{\cdot}3$ log units). (*b*) Fast hyperpolarization evoked by a white light annulus flash (inner diameter 430 μm, attenuation $-0{\cdot}3$ log units), due to scattering of light from the annulus to the centre of the receptive field. (*c*) Feed-back depolarizing potential evoked by the annulus flash when properly combined with the spot stimulation. In these experiments, as well in those shown in the other figures, the external diameter of the light annuli used was 3600 μm. In all the experiments the flux density of the white unattenuated light on the retina, measured inside the limits of the visual range, was about $2{\cdot}5\text{–}5 \times 10^{-5}$ μW.μm^{-2}.

Light stimuli covering areas of diameters larger than 120 μm can exert an antagonistic effect on the cone response, which may result in both a reduction of the amplitude and a change in the time course of their response or in the appearance of a depolarizing potential. An example of this antagonistic effect is shown in the cone of Fig. 1, which responded by a classical hyperpolarizing response to a centred spot of light (Fig. 1*a*). The flashing of a large annulus of intense white light, concentric to the spot, also evoked a fast hyperpolarization due to a direct response of the cone to light scattered from the annulus to the centre of its receptive field (Fig. 1*b*). When the same annulus was flashed during the stimulation of the cone by a centred spot (Fig. 1*c*) it evoked a depolarizing potential.

Such depolarizing responses to large light stimuli were discovered by Baylor *et al.* (1971) who showed that they were due to the activation of a polysynaptic circuit: a large population of peripheral cones feed their signals to L-horizontal cells which are electronically connected between them (Kaneko, 1971; see Raviola, 1976) and which feed-back their signals to the central cones, depolarizing them. This circuit, therefore, involves a feed-back connexion between the L-HC processes and the cones, opposite in direction to the synapse between the cones and the second-order cells.

In elegant experiments using two independent microelectrodes, Baylor *et al.* (1971) simultaneously impaled both a cone and a neighbouring L-HC. In these experiments, ward current injection which hyperpolarized the L-HC evoked a depolarization in

the cone. These authors interpreted this depolarization as a synaptic potential resulting from the activation of the feed-back connexion between L-HC and cones. O'Bryan (1973) found that following the depolarizing potential evoked in cones by peripheral stimuli the membrane resistance was decreased. These observations further supported the view that depolarizing potentials could be classical synaptic potentials involving an increase in membrane conductance.

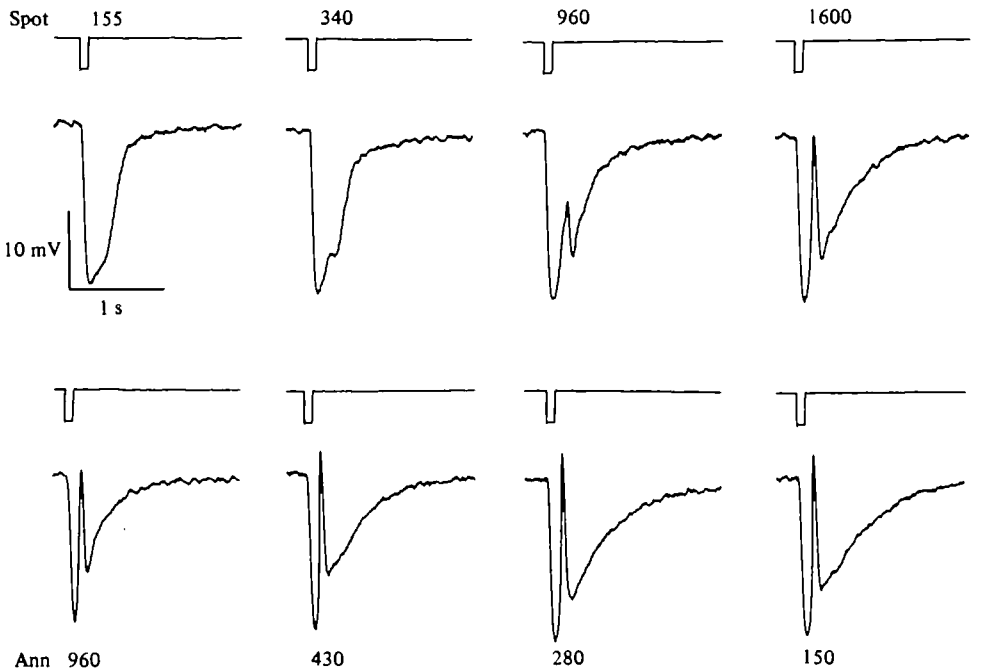

Fig. 2. Stimulation of the periphery of the receptive field evokes spike responses in a red turtle cone of an untreated retina. The figures above the upper records correspond to the diameters of the unattenuated light spot stimuli whereas the figures below the lower records are the inner diameters of the unattenuated light annuli (in μm).

Spikes evoked by peripheral illumination

Some useful indications for the understanding of the nature of the peripherally evoked depolarizing potentials in cones resulted from the study of particular responses to peripheral illumination that showed the characteristics of action potentials. Fuortes, Schwartz & Simon (1973) first observed that stimulation of large retinal areas with red light evoked in green cones a spike-like response which these authors attributed to the activation of the feed-back connexion from L-HC to green cones. Such spike responses were also observed by O'Bryan (1973) when stimulating the periphery of the receptive field of a red cone with annuli of bright white light. We have recently analysed the properties, ionic basis and the possible synaptic mechanisms involved in the generation of these spikes which were observed in a 20% of the cones studied in our experiments (Piccolino & Gerschenfeld, 1978, 1980; Gerschenfeld & Piccolino, 1980; Piccolino, Neyton & Gerschenfeld, 1980). These cone action potentials evoked by peripheral stimulation probably have no physiological significance for retinal

function. But as with other exaggerated manifestations of activity evoked in various cells by stimuli at the limits of the physiological range, their study gave new information on certain synaptic phenomena taking place at the cone endings. Thus, these spikes were shown to result from a regenerative increase of cone membrane conductance to Ca^{2+} ions. We will summarize in the next sections the experimental evidence supporting this view and will discuss the functional implications of our findings.

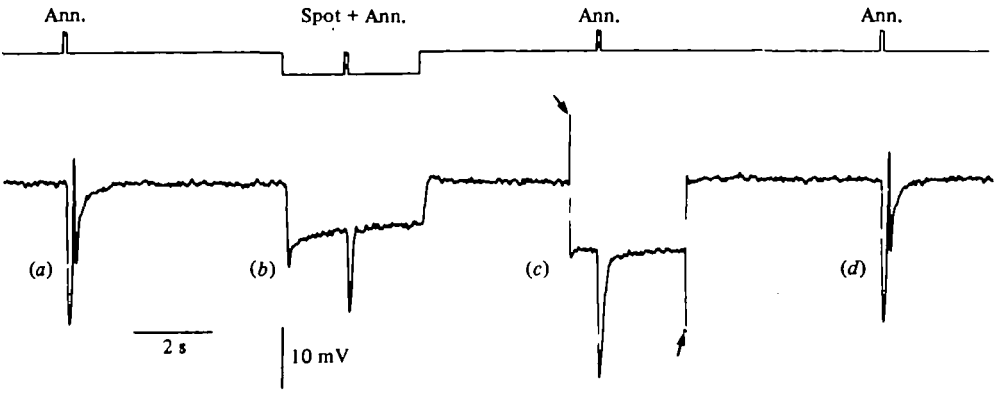

Fig. 3. Hyperpolarization block of the spike evoked by peripheral illumination of a turtle cone. (*a*) Stimulation with a light annulus (430 m inner diameter, unattenuated) evokes a spike. (*b*) Background illumination with a centred spot (250 μm diameter, attenuation $-2{\cdot}2$ log units) evokes a hyperpolarization which blocks the spike. (*c*) The spike is also blocked by hyperpolarizing the cone by injecting inward current (between the arrows, 10^{-10} A), through the recording microelectrode. (*d*) Control recording showing the persistence of the spike.

Properties of cone spikes evoked by peripheral stimulation

As already mentioned, Fig. 2 shows that stimulation with bright light of the periphery of the receptive field of cones evokes spike responses. In Fig. 2(*c*, *b*) the stimulation with centred spots of 340 and 960 μm already elicits, following the direct hyperpolarizing response, depolarizing potentials that increased in amplitude with the increase in the spot diameter. Spots of diameters beyond 1 mm evoked spike-like responses (Fig. 2*d*). When annuli of bright light were used (in all cases their external diameter was 3·6 mm), they also evoked a direct hyperpolarizing response due to the light scattered to the centre of the receptive field, which in all cases was followed by a spike response (Fig. 2*e*, *h*).

Such spike-like responses were shown to have the properties of actual regenerative responses: they showed a critical threshold level and they were blocked by membrane hyperpolarization. Fig. 3 shows that cone membrane hyperpolarization by either inward current injection or by central illumination blocked the spikes. In the cones in which only a small depolarizing potential was recorded in response to peripheral stimuli they could be converted into spikes by depolarizing the membrane by outward current injection and also blocked by hyperpolarizing current.

Ionic mechanism of the spikes evoked in cones by peripheral light stimulation

In the majority of excitable cells two main ion species have been shown to carry the currents responsible for the action potentials: Na^+ and Ca^{2+}. It was therefore natural to investigate if one of these cations was involved in the generation of the cone

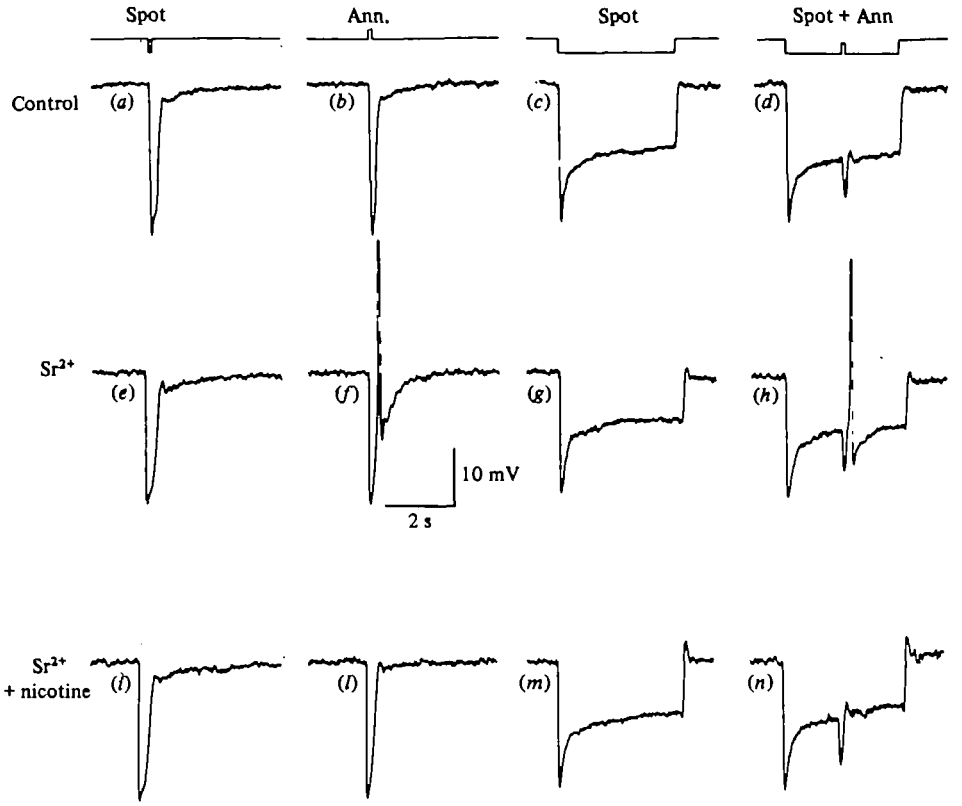

Fig. 4. (*a–d*) Responses of a cone bathed in normal saline to different light stimuli as indicated in the upper traces. (*e–h*) responses of the same cone to the same sequence of stimuli recorded during bath application of 10 mM-$SrCl_2$. Note the discharge of spikes following the annuli stimulation. (*i–n*) The spikes evoked by peripheral stimulation are blocked by nicotine (5 mM) added to the Sr^{2+}-containing medium. Stimulation parameters: (*a, e, i*) centred spot, 250 μm diameter, attenuation $-0{\cdot}5$ log units; (*b, f, l*) concentric annulus, 430 μm inner diameter, unattenuated; (*c, g, m*) centred spot, 250 μm diameter, attenuation $-1{\cdot}5$ log units; (*d, h, n*) combination of a spot such as in (*c*) with an annulus like in (*b*). (Piccolino & Gerschenfeld, 1978.)

spikes responses to peripheral illumination. Tetrodotoxin (TTX), a poison known to block Na^+ channels involved in spike generation, did not affect cone spikes even after prolonged extracellular applications at concentrations up to 10 μM.

Manipulating the Ca^{2+} content of the extracellular medium of the retina to study these spikes was more difficult than in other preparations. On one hand, it was not possible to decrease the extracellular Ca^{2+} concentration because this could alter the synaptic function in the circuit responsible for the spikes. On the other hand, the increase in external Ca^{2+} concentration had effects on the membrane of the outer segment of the cones which became hyperpolarized and showed a decrease or complet

block in the responses to light (Bertrand, Fuortes & Pochobradsky, 1974). Increasing the extracellular Ca^{2+} concentration was however shown to facilitate the spike evoked by peripheral illumination, before the hyperpolarization blocked them. More interesting, in the presence of a high Ca^{2+} environment (15–20 mM), cones which did not previously respond with either a spike or a depolarizing potential to peripheral stimuli responded by an action potential to light annuli until the hyperpolarizing effect of high Ca^{2+} media blocked them.

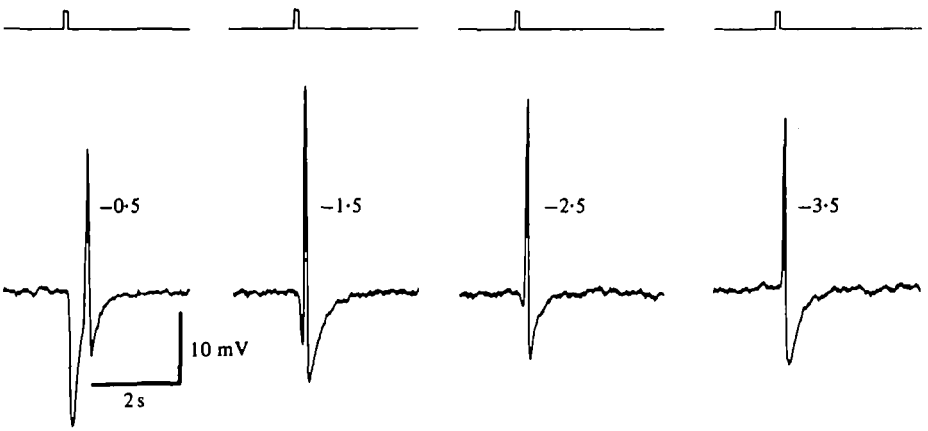

Fig. 5. Spike responses to peripheral stimulation in a Sr^{2+}-treated cone stimulated with annuli (inner diameter 430 μm) of decreasing light intensity. The figures besides the responses indicate the light attenuation in log units.

Fortunately, Sr^{2+}, a divalent cation known to permeate Ca^{2+} channels and to replace Ca^{2+} in synaptic transmission (Fatt & Ginsborg, 1958; Miledi, 1966; Katz & Miledi, 1969*a*; Dodge, Miledi & Rahamimoff, 1969; Meiri & Rahamimoff, 1971) affects neither the cone membrane potential nor the synaptic transmission between cones and second order cells (Piccolino, 1976). In the presence of 4–10 mM-Sr^{2+} in the extracellular environment, spikes evoked in the cones by peripheral illumination became increased in amplitude. Moreover, *every* cone in the turtle retina gave a spike response to an annulus flash in the presence of Sr^{2+} ions in the extracellular medium. Fig. 4 shows an example of a cone which did not show any depolarizing response in a normal medium, when flashing an annulus either alone (Fig. 4*b*) or during a period of central illumination (Fig. 4*d*). After bathing the retina in 10 mM-Sr^{2+} for some minutes, the annulus stimulation by itself (Fig. 4*f*) or in combination with a centred spot (Fig. 4*h*) evoked a spike. Stimulation with a small centred spot (100–300 μm) was not able to evoke a spike in Sr^{2+}-treated retinas (Fig. 4*e*, *g*). In Sr^{2+}-containing media, a dim light annulus could produce a spike without any preceding hyperpolarization, so that the spike arose at the dark potential (Fig. 5*d*). In such cases the latency of the spike was found to be *ca.* 100 ms.

Ba^{2+} ions, as in other cells (see review in Reuter, 1973), also facilitated the feed-back spikes and, as Sr^{2+}, induced in *all* the cones of the retina the appearance of spikes in response to peripheral illumination, regardless whether or not they previously showed depolarizing response to such stimulation. The spikes obtained in a normal medium

or in the presence of Sr^{2+} or Ba^{2+} were all blocked by agents known to block Ca^{2+} channels such as Co^{2+}, Mg^{2+} and D-600.

These results show that light stimulation of the periphery of the receptive field evoked in turtle cones spike responses due to a regenerative increase in Ca^{2+} conductance. Moreover, the experiments with Ba^{2+} and Sr^{2+} show that every cone of the turtle retina is able to respond to peripheral illumination with a spike response.

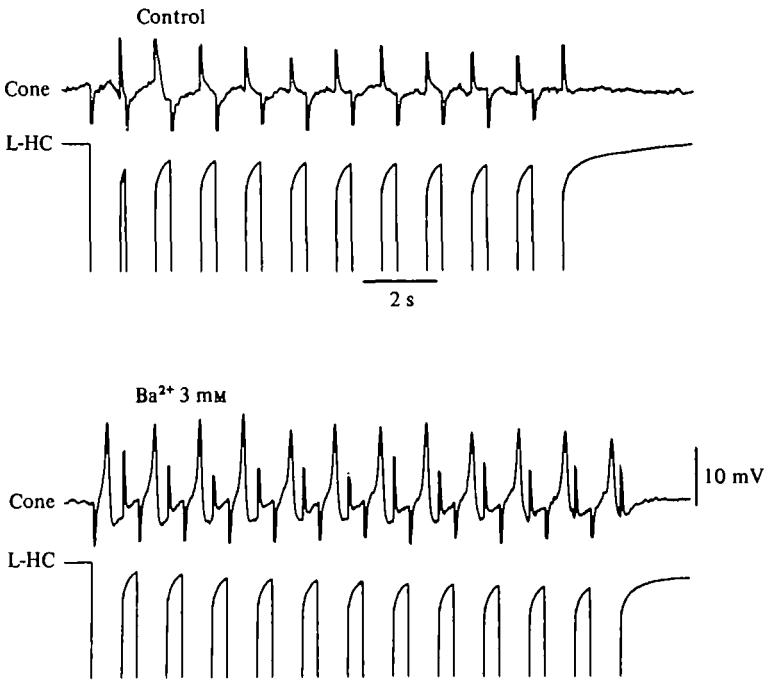

Fig. 6. Simultaneous recoding with two independent single micropipettes from both a cone and a neighbouring L-horizontal cell. The two upper records were obtained when the retina preparation was superfused with normal saline. Using a bridge circuit, pulses of inward current (20 nA) were injected through the recording micropipette impaling the L-HC. No effect is observed in the cone, except for the capacitative artifacts. The two lower traces are recordings from the same two cells obtained during the superfusion of the preparation with a saline containing 3 mM-$BaCl_2$. At this time, each inward current pulse injected in the L-HC evokes a spike in the cone.

Synaptic mechanisms involved in the generation of the spikes evoked by peripheral stimulation

The experiments of Fuortes *et al.* (1973) and of O'Bryan (1973) already mentioned, strongly suggested that the spikes evoked in cones by peripheral stimulation involved the activation of the same feed-back connexion between L-HC and cones described by Baylor *et al.* (1971).

Our experiments confirmed such hypotheses. First, we have tested on Sr^{2+}-treated cones a series of pharmacological agents (nicotine, glutamate, etc.) that are known to depolarize the L-HC and to block their responses to light. Such agents, as expected, blocked the spike responses to peripheral stimulation (Fig. 4*i–n*). However, such experiments could be criticized because of some of the eventual effects of pharmaco–

logical agents on the cone membrane itself. A new series of experiments have brought more direct evidence on the participation of the feed-back connexion of L-HC to cones in the generation of the cone spikes. In these experiments, as in Fig. 6, we followed a protocol similar to that of Baylor *et al.* (1971) commented on above. We impaled both a cone and a neighbouring L-HC with independent microelectrodes and injected inward current pulses to directly hyperpolarize the L-HC.

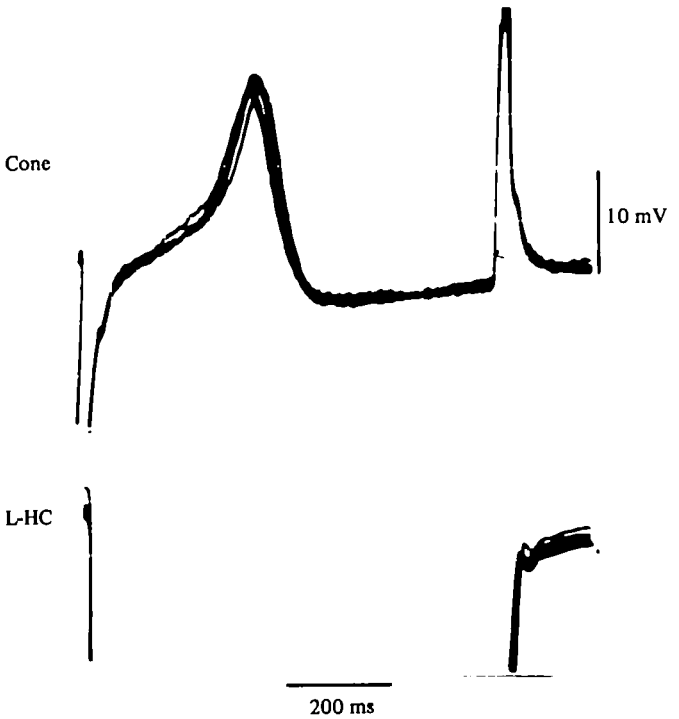

Fig. 7. Superimposed recordings of the responses of the L-HC and the cone as in Fig. 6. Each pulse of inward current injected in the L-HC evoked a constant latency spike in the cone.

In four experiments in which no apparent effects of the L-HC hyperpolarization were observed in the cones when bathed in a normal medium (Fig. 6, top), after addition of either 4 mM-Sr^{2+} or 3 mM-Ba^{2+} the inward current-induced hyperpolarization of the L-HC evoked in the cone the discharge of a spike (Fig. 6, bottom). The superimposed recordings from both cells in Fig. 7 show that all the cone spikes obtained in these conditions showed a constant latency of less of 100 ms and undoubtedly resulted from the L-HC hyperpolarization, since removing the pipette from the L-HC and passing the same current through it had no effect on the cone membrane potential.

It can therefore be concluded that the regenerative increase in Ca^{2+} conductance evoked by peripheral stimulation in turtle cones are the consequence of the hyperpolarization of the L-HC which affects the cone through a feed-back connexion.

Prolonged effects of peripheral illumination on the cone membrane conductance

From experiments as in Figs. 6 and 7 it follows that under appropriate ionic conditions there is a direct relationship between the L-HC hyperpolarization and the cone Ca^{2+} spike. What is the important signal across the feed-back connexion? Is it the variation of the L-HC potential? Or is it the attainment of a certain level of hyperpolarization of the L-HC? In other words, are the effects of L-HC hyperpolarization on the cone membrane conductance transient or sustained?

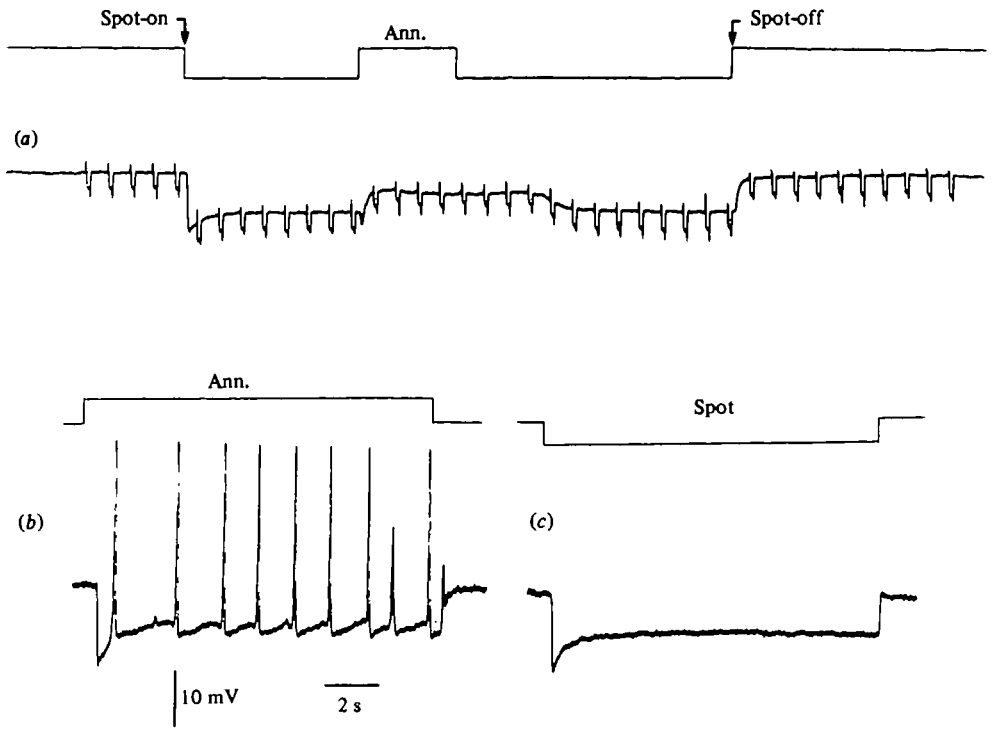

Fig. 8. Sustained effects of peripheral stimulation on the membrane conductance of a turtle cone. (*a*) Prolonged stimulation with a central light spot (250 μm diameter, attenuation -1 log unit) is combined with peripheral illumination by a concentric annulus (430 μm inner diameter, unattenuated light). A sustained depolarization is observed. Square inward current pulses (10^{-10} A) were passed through the cone membrane to measure the membrane resistance, which becomes markedly decreased during the depolarization.

(*b*) Recordings from another cone bathed in 10 mM-$SrCl_2$; prolonged stimulation with a light annulus (150 μm inner diameter, attenuation -1 log units) evokes a repetitive discharge of spikes, whereas in (*c*) the stimulation with a centred spot (250 μm, attenuation -1.3 log units), which evokes a peak hyperpolarization similar to the one elicited by the annulus, does not induce any spike.

Experiments as in Fig. 8 give a precise answer to such questions. Fig. 8(*a*) is the recording from a cone bathed in a normal extracellular medium. During a prolonged illumination of the cone with a small centred spot, an annulus of bright light was also presented for a few seconds. The peripheral stimulation evoked a steady depolarization of the cone. Before, during and after the whole illumination period short square pulses of inward current were passed through the cone membrane to measure its resistance

It is evident that the depolarization evoked by the annulus stimulation was associated with a sustained increase in membrane conductance. In other cases (see Gerschenfeld & Piccolino, 1980) different patterns of responses to sustained activation of the feed-back connexion were observed. In all these cases, as well as in cones that did not show manifest effects of peripheral stimulation, addition of Sr^{2+} ions to the extracellular medium induced the appearance of a repetitive discharge of spikes. An example of such an experiment is shown in Fig. 8(*b*) for a cone which did not show a feed-back response, in a normal medium. The discharge of spikes could be observed as long as the peripheral stimulus was applied. Such repetitive spike discharge never appeared in response to prolonged stimulation with a centred spot of light.

These and other effects of prolonged peripheral stimulation of the cones (see Gerschenfeld & Piccolino, 1980) demonstrate that stimuli able to keep the L-HC hyperpolarized for prolonged periods of time induce sustained increases in the cone membrane conductance to Ca^{2+} ions.

Mechanism of the feed-back connexion between L-horizontal cells and cones

From the evidence presented above it appears that a feed-back connexion between L-HC and cones, opposite in direction to that from cones to L-HC, is able to modify in a sustained way the Ca^{2+} conductance of the cone membrane. Where are located the Ca^{2+} channels affected by this connexion? How does the connexion operate?

The contacts between L-HC processes and cones take place at the synaptic regions of the cone pedicle. In invertebrate photoreceptors (see Ross & Stuart, 1978) it has been shown that Ca^{2+} channels located in the synaptic areas can generate action potentials. It is not therefore too far-fetched to assume that the Ca^{2+} channels involved in the cone depolarizations evoked through the feed-back connexion of the L-HC are situated in the synaptic endings of the cone and possibly are the same Ca^{2+} channels intervening in the transmitter release from the cone synapse. If so, the feed-back connexion from the L-CH would actually modulate the transmitter release from cone endings.

Two main hypotheses have been considered for the mechanism by which the feed-back connexion operates. The first proposes an electrical mechanism (Byzov & Golubtzov, 1977; Byzov, 1979). According to such model the current generated at the membranes of the hyperpolarized L-HC processes would partly flow across the membrane of the cone synaptic ending and generate a potential drop across, depolarizing it. No particular membrane junctions would be necessary for such interaction to take place, the particular geometry of the L-HC processes inside the invaginated cone synapses being highly favourable to such electric field interactions.

The second model postulates the existence of a chemical feed-back synapse between L-HC processes and cones (Gerschenfeld & Piccolino, 1980). If such would be the case it is likely that the L-HC, as the cones, releases its transmitter continuously in the dark. Light stimuli evoking an hyperpolarization of the L-HC would decrease or suppress this transmitter release. In such case it can be postulated that the effects of the chemical transmitter released from the L-HC would be opposite from the described feed-back effects, (i.e. it would directly or indirectly turn-off Ca^{2+} channels in the cone endings).

There are some examples of known transmitters that act by turning-off Ca^{2+}

channels. Thus it has been found that invertebrate heart acetylcholine and muscarinic agonists cause a decrease in the duration of the action potential. In the frog atrial muscle fibres it has been found that such effect is associated with a decrease in Ca^{2+} conductance (Giles & Noble, 1976; Garnier *et al.* 1978). Moreover, in dorsal root ganglion cells in culture, Dunap & Fishbach (1979) have also recently observed that GABA, serotonin decreased the Ca^{2+} component of the action potential.

However, also other possible chemical synaptic mechanisms could result in a decrease of cone Ca^{2+} conductance. For instance, the L-HC transmitter when released in the dark could induce an increase in a K^+ conductance (as inhibitory transmitters do in the CNS) or a voltage-dependent K-conductance, thus keeping the voltage-dependent Ca conductance of the cone ending under control, thus impeding regenerative increase. The decrease in the L-HC transmitter release evoked by peripheral light stimulation could turn-off these K^+ channels, the Ca^{2+} conductance thus increasing and becoming regenerative. In relation with such an idea it may be remembered that Katz & Miledi (1969) observed Ca^{2+} regenerative responses in the TTX-treated squid giant synapses endings after blocking K^+ conductance.

It is difficult to conclude, at present, which mechanism operate the feed-back connexion between L-HC and cones. Electron-microscope studies have not been very helpful in this direction. Neither in the turtle retina (Lasansky, 1971; Schaeffer & Raviola, 1975) nor in those of other lower vertebrates (see for example Dowling & Werblin, 1969; Stell, 1976) synaptic specialization or synaptic vesicles have been observed in relation with the membrane of the L-HC processes facing the cone endings. Moreover, in contrast with classical synapses of the 'phasic' type in which, either manipulation of the extracellular Ca^{2+} content or Ca^{2+} blocking agents can help to clarify the chemical nature of a connexion, these procedures cannot be used in the study of the feed-back connexion because more than one step involving Ca^{2+} ions intervene in the feed-back circuit.

L-HC have been recently shown to be able to take up GABA through a high affinity mechanism (Marc *et al.* 1978) and experiments by Lam, Lasater & Naka (1978) suggest that bicuculine, a GABA antagonist, blocks some effects of the L-HC feed-back on the cones. Picrotoxin, another GABA antagonist, has been shown to block some of the possible repercusions of the L-HC feed-back mechanism on second order neurones (Djamgoz & Ruddock, 1979).

Further pharmacological experiments on the depolarizations evoked on the cones by the feed-back connexion from the L-HC may help to give support to the chemical hypothesis.

Physiological consequences of the feed-back modulation of the cone transmitter release

If through its feed-back connexion with the cone the L-HC modulates the transmitter release from the cone, and, if such modulation operates in all the cones, it can be expected that the responses of all the second-order neurones in the retina should be affected by this feed-back mechanism. We have recently explored such hypothesis on one of the second-order neurones – the red/green horizontal cell (R/G-HC), a chromaticity type horizontal cell (see above) that shows colour-coded responses.

As Fig. 9 recalls, whereas the L-horizontal cell responds to light of all wavelengths by an hyperpolarization (Fig. 9, bottom records), the responses of the R/G-H

pend on the wavelength of the stimulus. These cells are hyperpolarized by red stimuli (Fig. 9, top records, 700 and 650 nm) and hyperpolarized by stimuli of shorter wavelength.

Fuortes & Simon (1974) postulated that the different polarity of the responses of the R/G-HC could be accounted for by the following mechanism: (*a*) R/G-HC receive a direct input from green cones but not from red cones (this has been confirmed by

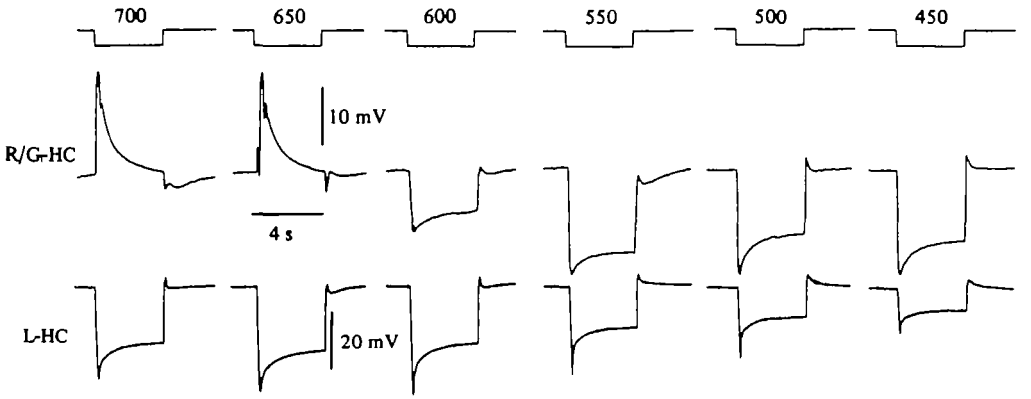

Fig. 9. Spectral sensitivity of horizontal cells of turtle retina. Top recordings, responses of a red/green chromaticity type horizontal cell (R/G-HC) to large spots (3600 μm diameter) of light of different wavelength. The wavelengths are indicated over the timing traces in nm. Bottom recordings, responses of a L-HC to same series of monochromatic stimuli.

morphological studies, see Leeper, 1978); (*b*) thence, green light which hyperpolarizes green cones also evokes the hyperpolarization of the R/G-HC, (*c*) green cones are unaffected by red light impinging on them, (*d*) red illumination of a large area of the retina hyperpolarizes the red cones that feed their signal on the L-HC, which, in turn, feed-back their signal on the green cones depolarizing them, (*e*) as a consequence of the feed-back depolarization of green cones the R/G-HC are depolarized in response to red light.

However, this hypothesis does not appear too convincing in its original formulation since no correlation is generally observed between the amplitude of the depolarizing responses to red light in the R/G-HC (up to 25–30 mV) and the amplitude of the responses of the green cones to such stimuli (which are usually of small amplitude and, in some cones, even undetectable). Moreover, in most green cones, prolonged stimulation with red lights induces only a depolarizing transient at the onset of the stimulus while the R/G-HC responses to the same stimulus generally show a sustained component.

However, this apparent lack of correlation between the effects of red light on green cones and R/G-HC can be discarded when the results of our own experiments are taken into account. Thus we can assume: (1) that red light stimuli evoke an increase of transmitter release from the green cone due to an increase of Ca^{2+} conductance in their endings due to the activation of the feed-back connexion from L-HC on green cones; (2) this transmitter release would evoke a depolarization in the R/G-HC even when only small changes in the green cones potential would be detected, and (3) when

using prolonged red light stimulation, the increase in Ca^{2+} conductance and the consequent increase in transmitter release from green cones would be sustained.

To test such assumptions, experiments were performed in retinas bathed in either Sr^{2+}- or Ba^{2+}-containing media. We knew that if in such conditions a prolonged red light stimulation depolarized the green cones through the feed-back connexion from L-HC, we should observe a repetitive discharge of spikes in green cones. That was in

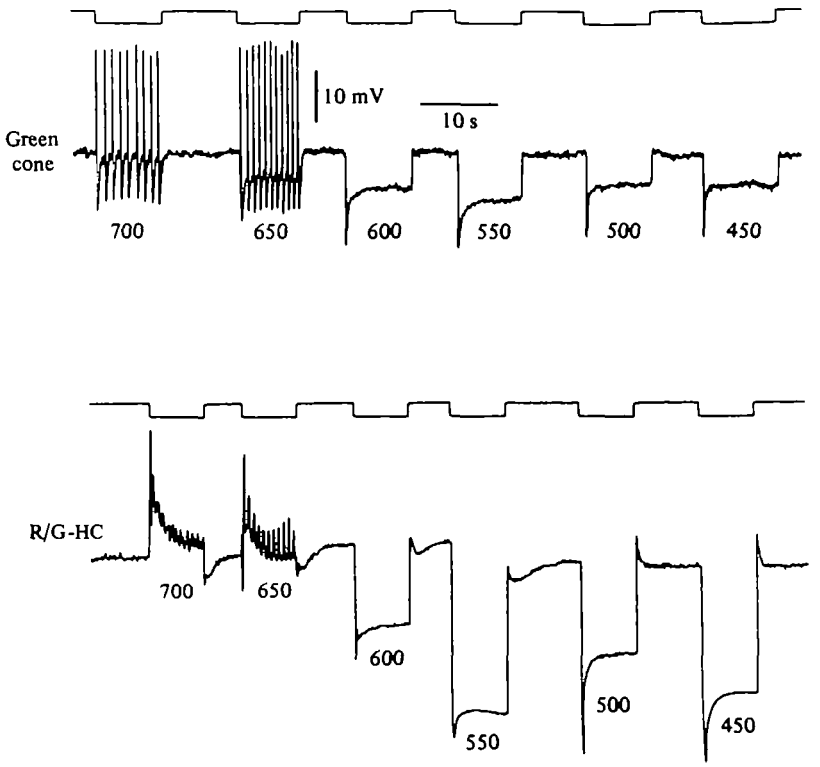

Fig. 10. Recordings from both a green cone and a R/G-HC obtained from different retinas bathed in a medium containing 6 mM-$SrCl_2$. The wavelength of the light stimuli in nm are indicated below each response. The quantum flux for all the light stimuli was $1{\cdot}7 \times 10^5$ photons $\mu m^{-2}.s^{-1}$.

fact what we observed as illustrated in Fig. 10, top records, where red light stimuli (700 and 650 nm) actually evoked a repetitive discharge of spikes in a green cone of a Sr^{2+}-treated retina. What was the repercussion of such phasic increases of conductance across Ca^{2+} channels in the green cones on the R/G-HC of the same retina? It could be expected that such repetitive Ca^{2+} spikes would evoke repeated phasic releases of transmitter from the green cone. Indeed, when a R/G-HC was recorded from the same retina (Fig. 10, bottom) red light stimuli that evoked Ca^{2+} spikes in the green cones also evoked depolarizing potentials in the R/G-HC. The depolarizing potentials in the R/G-HC were found to show the properties of depolarizing synaptic potentials (Piccolino *et al.* 1980): they increase in amplitude when the R/G-HC is hyperpolarized, they become blocked when the spikes in the green cones are blocked by hyperpolarization evoked by intense green light stimulation.

It can be concluded that these results give strong support to the Fuortes & Simon

974) hypothesis in our modified formulation. They clearly show that stimuli which depolarize the green cones through the feed-back connexion from L-HC to those cones, actually modulate the Ca^{2+} conductance, evoking an additional release of transmitter which depolarizes the R/G-HC. These results, therefore, support the idea that the feed-back modulation of the cone Ca^{2+} conductance by the feed-back connexion from the L-HC does actually play a physiological role in the generation of responses of the second-order neurones, postsynaptic to the turtle cones.

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