Sustained feedback effects of L-horizontal cells on turtle cones

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Prolonged stimulation of the periphery of their receptive field can evoke in turtle cones sustained complex depolarizations or sustained membrane oscillations. In cones in which such effects of prolonged peripheral stimulation are not apparent, the injection of short depolarizing pulses can reveal a sustained increase of electrical excitability in response to prolonged peripheral illumination. The sustained effects of prolonged peripheral illumination have characteristics similar to those of the feedback depolarizations evoked by flash peripheral stimulation: they are labile in untreated retinas, can be blocked by either hyperpolarization, Co²⁺ or agents that depolarize the L-horizontal cells. They are associated with a decrease in the membrane input resistance. In retinas bathed in Sr²⁺- or Ba²⁺-containing media, prolonged peripheral illumination evokes a sustained repetitive discharge of spikes. These experiments demonstrate that the feedback effects of the L-horizontal cells on the cones are not only transient but also sustained and that the sustained effects of peripheral stimulations are associated with an increase in membrane Ca²⁺ conductance. The possible nature of the feedback connection between L-horizontal cells and the cones is discussed.

INTRODUCTION

In the preceding paper (Piccolino & Gerschenfeld 1980) it has been shown that short illumination of the periphery of the receptive field of turtle cones probably leads to an increase in their Ca²⁺ conductance which can become regenerative. This can result in a graded depolarization or in a spike, both generated via a polysynaptic circuit involving negative feedback from the L-horizontal cells, first described by Baylor et al. (1971). The feedback spikes were observed in one-fifth of the cones of untreated retinas in response to peripheral illumination. They were shown to be labile, but they could be stabilized and/or induced in all cones by Sr²⁺- or Ba²⁺-containing media. They were shown to be blocked by hyperpolarization of the cones, by agents blocking Ca²⁺ channels and by drugs depressing L-horizontal cell responses to light stimulation (Piccolino & Gerschenfeld 1978, 1980).

In the present paper the effects on turtle cones of prolonged illumination of large areas of their receptive field periphery are described. It will be shown that

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prolonged peripheral light stimulation induces diverse sustained feedback effects from the L-horizontal cells to cones, namely, complex long depolarizations, prolonged increases in electrical excitability and sustained oscillations. It will also be demonstrated that these sustained responses have the same characteristics as the feedback spike: they are blocked by cone hyperpolarization and \( \text{Ca}^{2+} \)-channel blocking agents, and, in untreated retinas, they show the same lability. Moreover, \( \text{Sr}^{2+} \)- or \( \text{Ba}^{2+} \)-containing media also facilitate these sustained responses and/or convert them to a prolonged discharge of repetitive spikes.

It can therefore be concluded that, like the transient responses, the effects of prolonged peripheral illumination on the cone are most probably due to an increase in \( \text{Ca}^{2+} \) conductance resulting from the negative feedback effect from the L-horizontal cells to the cone. The nature of the feedback mechanism and its physiological significance will be discussed.

Some of these results have been presented, in preliminary form, to the Physiological Society (Gerschenfeld & Piccolino 1978).

**Methods**

The present experiments were performed in the superfused eye cup preparations from the turtle *Pseudemys scripta elegans*. The general procedures of preparation of the eye cup, composition of the solutions, superfusion technique, cell recording and light stimulation were the same as in the preceding paper (Piccolino & Gerschenfeld 1980).

**Results**

*Effects of prolonged peripheral light stimulation on untreated cones*

*Sustained complex depolarizations*

When the periphery of the receptive field of cones of untreated turtle retinas was submitted to prolonged stimulation with bright white light, the cones showed a variety of responses. The first type of response was a sustained depolarization with a rather complex time course, as illustrated in figure 1. The responses of both a cone and an L-horizontal cell to prolonged illumination with the same bright annulus (figure 1a, a') and the same spot (figure 1b, b') were recorded in the same electrode track. Figure 1c shows the response of the cone to a long peripheral stimulus while the cone was maintained polarized with a background of central light. In both records of figure 1a, b, the light stimuli were adjusted to approximately match the amplitudes of the initial peak hyperpolarizations (in the first instance due to light scattered from the annulus to the centre of the receptive field). Therefore, the amount of light that illuminated the centre was about the same in both. However, while the hyperpolarization evoked by the spot had the usual features (figure 1b), the response to the annulus appeared complex: the initial peak of hyperpolarization appeared to be followed by a depolarization that
almost completely suppressed the hyperpolarization plateau (figure 1a). At the end of the light stimulus a depolarizing rebound was observed. A likely explanation of the long duration of the peripheral response is provided by the effects of the same illumination on the L-horizontal cell (figure 1a'). It is evident that the L-horizontal cell response lasts longer than the peripheral stimulus and corresponds

![Diagram](image)

**FIGURE 1.** Sustained feedback effect in an untreated cone. Cone responses to (a) an annulus of unattenuated light, 430 μm inner diameter, (b) a spot of light attenuated by 1 logarithmic unit and (c) a combination of both stimuli. In (c), repetitive pulses of inward current of 10^{-16} A were injected through the recording micropipette. (a'), (b') Responses of an L-horizontal cell, penetrated in the same electrode track, to the same stimuli as in (a) and (b) respectively. In this experiment, as in all experiments described in this paper, the external diameter of the light annulus was 3600 μm.

in duration to the cone response. The mechanism of the sustained depolarizing peripheral response was analysed in figure 1c. Here, the peripheral stimulus was applied against a background of adapting central illumination and the resulting steady depolarization outlasted the duration of the peripheral stimulus. The injection through the cone membrane of short hyperpolarizing pulses revealed that, during the sustained depolarization evoked by the annular stimulation, the cone showed a decrease in membrane resistance (figure 1c).

As with the feedback spikes in untreated retinas (Gerschenfeld & Piccolino 1980),
Figure 2. Effects of inward current on the central and peripheral responses of an untreated cone. Each trace shows the responses to the indicated stimulus before (left) and during (right) the injection of a pulse of hyperpolarizing current. The light intensity of all stimuli was attenuated by 2 logarithmic units. The current used was $2 \times 10^{-10}$ A and its duration is marked by the capacitative artefacts. The numbers that appear on the left hand side of the figure refer to the outer diameters of the spots and to the inner diameters of the annuli in μm.

These sustained depolarizing effects of peripheral illumination were particularly labile and could be reobtained by combining the peripheral illumination with the injection of outward current through the cone membrane.

Like the transient feedback responses, the sustained depolarizations evoked by
prolonged peripheral stimulation were blocked by passing inward current through
the cone membrane. Figure 2 illustrates this for a cone that was stimulated with
concentric light spots, and with annuli of the same intensity but varying area. This
cell, which responded to a small centred light spot with a classical direct response
(figure 2a), developed a sustained complex depolarization when illuminated with
a larger spot or with annuli (figure 2b–e, left). When the same stimuli were repeated

![Figure 2](image)

Figure 2. Membrane oscillations in an untreated cone in response to prolonged peripheral
illumination. (a), (c) Stimulation with an annulus of light attenuated by 2.4 logarithmic
units, inner diameter 150 μm. (b) Stimulation with a spot of light attenuated by 3
logarithmic units, 250 μm in diameter.

while passing inward current (figure 2b–e, right), the complex, sustained depolar­
ization was abolished and normal direct responses were recorded.

All the properties of these complex, sustained depolarizations described above,
namely, their exclusive appearance in response to peripheral light stimuli, the
relation between their time courses and those of the L-horizontal cell responses,
their lability, their association with a decrease in membrane input resistance and
their sensitivity to hyperpolarizing current, strongly suggest that they also
originate from the L-horizontal cell feedback mechanism and that, therefore, the
feedback effects can be not only transient but also sustained in character.

Feedback membrane oscillation

Another pattern of sustained feedback effect in untreated turtle retinas is
illustrated in figure 3. In this experiment, the intensities of both central and
peripheral light stimuli were adjusted to evoke direct peak hyperpolarizing re­
sponses of the same amplitude (figure 3a, b). In this cone, very small voltage
fluctuation could be observed at the dark potential level, which appeared to
decrease during the hyperpolarizing response to a light spot (figure 3b; see Simon et al. 1975; Lamb & Simon 1976; Schwartz 1977). In contrast, the illumination of the periphery of the receptor field evoked the appearance of large and regular, low amplitude, slow oscillations (figure 3a, c). At the same time, the plateau potential was less negative than in the spot responses.

The record shown in figure 3c was obtained from the same cone and is a response to the same annulus observed a short time later; a spike transient appeared just after the peak hyperpolarization and was immediately followed by a 300 ms period in which no oscillation was observed, probably because of the increase in conductance associated with the feedback spikes (O'Bryan 1973; Piccolino & Gerschenfeld 1980), after which the oscillation reappeared and persisted until the end of the light response.

Similar oscillations in response to peripheral illumination were observed in 18 cones of untreated retinas. Depending on the light intensity and thus on the amplitude of the direct hyperpolarization, these fluctuations could appear as oscillations or as repetitive small spikes. Such responses were never observed when cones were illuminated with small spots.

The fluctuations elicited by peripheral illumination could be better observed when moderately bright annuli were used as stimuli. Like the feedback spikes and the sustained depolarizations, they could be suppressed by hyperpolarizing the cones with current or with intense central light stimulation.

Detection of the sustained feedback effects by current pulse injection

The effects of current pulses were investigated in those cones where the sustained feedback effects were scarcely detectable. Byzov & Cervetto (1977) reported that, during centre spot stimulation, depolarizing pulses produced larger electrotonic potentials than hyperpolarizing pulses of the same current intensity, and that such asymmetry was suppressed by Co²⁺. This point was reinvestigated with the use of small spots and large annuli as light stimuli. In figure 4, short pulses of both polarities were passed through the membrane of a cone alternately stimulated with concentric light annuli and with spots. In the dark, the short depolarizing pulses evoked deflections showing an initial transient peak (figure 4a, b). When the same pulses were injected during the response to peripheral illumination, they elicited large spike transients (figure 4a). Such spikes were not observed when the same depolarizing pulses were injected during central illumination, even when the amplitudes of the direct hyperpolarizing responses were matched. In contrast, there was little difference between the hyperpolarizing potentials during the responses to central and peripheral light stimuli (figure 4c, d). These results demonstrate that peripheral illumination produces a sustained increase in the electrical excitability of the cone membrane. Byzov (1979) has recently re-analysed the problem, arriving at results similar to those described above.

The spike responses elicited by depolarizing current pulses during peripheral illumination, like the feedback spikes, involve a Ca²⁺ mechanism.
Application of 4 mM Co\(^{2+}\) almost suppressed the spike transients resulting from the injection of depolarizing pulses during prolonged peripheral illumination without much altering the direct hyperpolarizing responses.

Co\(^{2+}\) also attenuated the small peak transients appearing in the dark (figure 4a, b). Agents, such as nicotine, that depolarize the L-horizontal cells and thus block the feedback effects (Piccolino & Gerschenfeld 1978, 1980) also abolished the differences between the effects of hyperpolarizing and depolarizing pulses during peripheral illumination.

**Effects of prolonged peripheral stimulation of cones bathed in high concentrations of divalent cations**

The results presented in the previous sections indicate that the feedback effects from L-horizontal cells can affect the electrical properties of the cones in a sustained way. It was also shown that, in untreated retinas, these sustained feedback effects are very labile, which makes them difficult to study. As with the transient feedback

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**Figure 4.** Effect of current pulses applied during central and peripheral illumination of an untreated cone. (a), (c) Responses to a 430 \(\mu\)m inner diameter annulus of unattenuated light. (b), (d) Responses to a 150 \(\mu\)m spot of light attenuated by 1 logarithmic unit. Both the outward current pulses (a, b) and the inward current pulses (c, d) were: \(2 \times 10^{-10}\) A. The arrows indicate the initiation and the break of the current application.
effects, the sustained effects can be unmasked, stabilized and amplified by Sr\textsuperscript{2+}- or Ba\textsuperscript{2+}-containing media.

**Experiments in Sr\textsuperscript{2+}-containing media**

Figure 5 shows the responses to prolonged and peripheral illumination of a cone bathed in an extracellular medium containing 10 mM SrCl\textsubscript{2}. The intensity of the two kinds of stimuli was adjusted to match the peak amplitude of the direct responses (figure 5a, b, attenuation 2 logarithmic units; figure 5c, d, attenuation 1 logarithmic unit). In both instances, only the peripheral stimulation induced a repetitive discharge of spikes (figure 5a, c). The frequency of the spikes was higher when the brighter light annulus was used (figure 5c), but in both cases abortive spike-like responses and a background of small voltage fluctuation could be observed (figure 5a, c). The spikes had larger undershoots for the dimmest illumination, probably because the direct response involved less hyperpolarization and thus the driving force for K\textsuperscript{+} ions was larger (figure 5a).

Such repetitive spike discharges in response to prolonged peripheral illumination were consistently observed in cones treated for short periods with 10–12 mM Sr\textsuperscript{2+}.
or for long periods with lower Sr$^{2+}$ concentrations. In certain cones some variability was observed in the amplitude of the feedback spikes during the repetitive discharge. As for feedback spikes evoked by annular flashes, the discharge of repetitive feedback spikes, as well as the light potential fluctuations, was diminished or totally abolished by hyperpolarizing the cone either by passing current (figure 6a, b) or by an intense central light background (not shown).

Figure 6. Effects of membrane hyperpolarization by current on the repetitive spikes evoked by peripheral stimuli in a 10 mM Sr$^{2+}$-treated cone. Responses to a 430 $\mu$m inner diameter annulus of unattenuated light; during the spike discharge, inward current was injected through the recording electrode (intensity indicated above, duration as marked by the dotted lines).

An increase in the dark potential fluctuations (dark noise) was frequently observed in Sr$^{2+}$-bathed cones (figure 7). The fluctuation of the dark potential in the untreated retina was very small (figure 7a) and increased progressively shortly after the application of 6 mM Sr$^{2+}$ (figure 7b), the trace becoming oscillatory, with appearance of spontaneous small spikes after prolonged Sr$^{2+}$ application (figure 7c). Hyperpolarization evoked by central illumination abolished both oscillations and abortive spikes (as in figure 7d), while illumination with a dim
annulus resulted in a dramatic increase in the voltage fluctuations and in a spike discharge, without any preceding direct hyperpolarizing response (figure 7d). A strong light annulus flashed against a background of central illumination evoked a feedback spike (figure 7d).

Experiments in Ba\(^{2+}\)-containing media

It was also possible to reveal the sustained character of the feedback action of the L-horizontal cells on cones by exposing the retina to Ba\(^{2+}\) ions.

The recordings in figure 8 were obtained 4 min after applying 4 mM Ba\(^{2+}\) to a cone that did not previously show overt signs of feedback or voltage fluctuations in the dark. The recording in figure 8a was obtained by stimulating the cone with a light annulus and a spot evoking direct hyperpolarizations of comparable peak amplitude. The peripheral stimulus evoked a slow membrane oscillation but the central spot did not. Moreover, even when the peaks of both direct responses were similar, the plateau level was less hyperpolarized during the annulus stimulation. The recording in figure 8b shows that the oscillations were also evoked when the
peripheral stimulation was superimposed on a prolonged hyperpolarization induced by dim central illumination. Beside accentuating the voltage fluctuations and oscillations, Ba\(^{2+}\) also facilitated the repetitive discharge of spikes during sustained peripheral illumination, in a similar manner to Sr\(^{2+}\).

transient versus sustained feedback effects

Baylor et al. (1971) demonstrated that transient hyperpolarization of the L-horizontal cell, evoked by inward current injection, caused a depolarization of the cone membrane, thus proving the existence of a feedback input from the L-horizontal cell to the cone. In the preceding paper (Piccolino & Gerschenfeld 1980), it was shown the activation of this feedback input evokes a regenerative activation of the cone Ca\(^{2+}\) conductance.

In the present work, prolonged stimulation of the periphery of the cone receptive field was shown to evoke depolarizing and oscillatory effects in both untreated retinas and retinas bathed in high concentrations of divalent cations. Table 1

Discussion

Transient versus sustained feedback effects

Baylor et al. (1971) demonstrated that transient hyperpolarization...
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compares the electrophysiological features of the responses evoked by short or prolonged peripheral illumination. The basic features of both types of responses are rather similar and it is therefore evident that the sustained cone responses to prolonged peripheral stimulation are also due to the activation of the feedback input from the L-horizontal cell to the cones. The sustained, like the transient, responses involve an increase in the cone membrane conductance.

### Table 1. Comparison between the properties of the transient and sustained feedback effects of L-horizontal cells on cones

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<th>Transient</th>
<th>Sustained</th>
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<td>Effects of agents depolarizing the L-horizontal cells</td>
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Nature of the feedback connections from L-horizontal cells to cones

The nature of such connections is unknown at present. Two main mechanisms have been posulated:

(a) **Chemical transmission.** The increase in membrane conductance evoked in the cone by the feedback input activation could be an argument in favour of the existence of a chemical synapse from the L-horizontal cell processes to the cone pedicle. Since light responses of the L-horizontal cells are hyperpolarizing and associated with a decrease in conductance, it is highly likely that the release of transmitter from their processes might be similar to that observed in the photoreceptors, i.e. a continuous release of transmitter in the dark, which becomes reduced or suppressed by light (see Trifonov 1968; Dowling & Ripps 1973; Cervetto & Piccolino 1974; Haneko & Shimazaki 1975; Dacheux & Miller 1976). Therefore, the L-horizontal cell transmitter released in the dark would close Ca²⁺
channels in the cone membrane. Although it has been reported that some chemical transmitters increase the Ca^{2+} conductance (e.g. adrenalin, on vertebrate cardiac muscle fibres; Reuter & Scholz 1977), no transmitter effect involving a decrease in Ca^{2+} conductance has been so far demonstrated. An alternative hypothesis is that L-horizontal cell transmitter released in the dark may increase the K^+ or Cl^- conductance of the cone synaptic ending membrane and thus control the Ca^{2+} conductance, preventing its regenerative activation. The decrease of transmitter release during the L-horizontal cell hyperpolarization by light would therefore decrease such cone K^+ or Cl^- conductance and consequently the Ca^{2+} conductance would increase and eventually become regenerative. In this connection, it may be recalled that Katz & Miledi (1969) have shown that, in TTX-treated presynaptic endings of the squid giant synapse, the blockade of both Cl^- and K^+ conductances allows the appearance of a regenerative increase of the Ca^{2+} conductance.

Some predictions of this model remain to be proven. Even if the membrane potential change expected from a decrease of the cone Cl^- or K^+ conductance, a depolarization, can be observed in the cones when the L-horizontal cell is hyperpolarized (Baylor et al. 1971), this depolarization should reverse when the cone becomes hyperpolarized beyond the Cl^- (or the K^+) equilibrium potential (see Weight & Votava 1970; Gerschenfeld & Paupardin-Tritsch 1974). In the preceding and in the present paper it has repeatedly been shown that when the cone is hyperpolarized a gradual decrease and disappearance of the feedback depolarization is observed, but never a reversal of the feedback effect. However, it is not known at present if the disappearance and lack of reversal are not due to the development of a voltage-dependent conductance.

The ultrastructural findings are not helpful in ascertaining the existence of a chemical transmission. Although vesicles have been observed inside the tips of L-horizontal processes, no vesicles approaching or contacting their membranes facing the cone endings in lower vertebrate retinas have been visualized (see, for example, Dowling & Werblin 1969; Lasansky 1971; Schaeffer & Raviola 1975; Stell 1976).

In summary, the evidence supporting the existence of a chemical feedback synapse appears at present to be rather circumstantial.

(b) Electrical field effect transmission. An alternative mechanism to account for the feedback effects on the cones could be an electrical interaction between the L-horizontal cell processes and the cone endings, as recently proposed by Byzov et al. (1977). If so, the current generated by the membranes of the L-horizontal processes during their hyperpolarization by light would flow through some particular area of the synaptic ending and thus evoke a potential drop across its membrane and a consequent conductance change. No electrical coupling between the cells would be necessary for such interaction, which would be similar to that operating between the Mauthner cells and some small surrounding neurons in the fish medulla, which gives rise to the 'extrinsic hyperpolarizing potential' in the Mauthner cells (Furukawa & Furshpan 1963) or to the 'passive hyperpolar-
izating potential' in the small neurons surrounding them (Korn & Faber 1975). As in these two examples, therefore, the existence of such feedback electric field on the cones would imply a high electrical resistance of the extracellular space separating the L-horizontal cell processes from the cone ending. No information on this point is available at present.

The electrical field effects observed in the fish medulla mentioned above are of short duration. No sustained interactions between neurons through field effects have been reported. However, if the feedback effects result from an electric field interaction it is not difficult to imagine that the depolarization is able to activate the cone Ca\(^{2+}\) conductance.

It is difficult at present to decide experimentally which of the described mechanisms is responsible for the feedback effects. Since these effects include in their origin at least two Ca-dependent steps (transmission from peripheral cones to L-horizontal cells, increase in Ca\(^{2+}\) conductance of the cone membrane), alteration of the Ca\(^{2+}\) extracellular concentration or application of Co\(^{2+}\), both of which have helped in other instances to decide whether the connection between two neurons was chemical or electrical, are of little use in this case because they affect both steps.

Marc et al. (1978) have recently reported that goldfish L-horizontal cells take up GABA and have suggested that these cells release this amino acid. If this is confirmed, the pharmacological demonstration of GABA involvement in the feedback mechanism could be a good confirmation of the chemical character of the feedback connection.

**Physiological significance of the feedback spikes and sustained effects**

Since the discovery of the feedback effects by Baylor et al. (1971), it has been thought that such a mechanism could play a role in the organization of the receptive field of higher order neurons of the retina. Fuortes & Simon (1974; see also Richter & Simon 1975) supported the idea that the feedback effects may intervene in the organization of the pattern-dependent and colour-dependent properties of both bipolar and horizontal cells, namely, the centre-surround antagonism in the receptive field of bipolar cells and the mechanism underlying the depolarizing responses of chromaticity-type horizontal cells.

These properties of second order neurons, which would depend on the feedback mechanism, require that this can affect the cone in a sustained way. The main findings of the present paper are that: (1) the feedback mechanism activated by prolonged peripheral illumination can affect the cone in a sustained way; (2) this sustained influence results in an increase in the cone membrane conductance to Ca\(^{2+}\) ions. The connection of the cones to the second order neurons being chemical, the release of their transmitter depends on Ca\(^{2+}\), and it must, then, be expected that the feedback input, by modifying the cone membrane Ca\(^{2+}\) conductance, will affect its transmitter release and thus modify the responses of the cells postsynaptic to the cone, even when the effects of the feedback on the cone membrane potential appear to be small.
Therefore, the present evidence supports the idea that the feedback circuit plays a role in the organization of the responses of the second order neurons.

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References


