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Involvement of small-field horizontal cells in feedback effects on green cones of turtle retina

(color coding/receptive field/local circuit)

JACQUES NEYTON*, MARCO PICCOLINO†, AND HERSCH M. GERSCHENFELD*

*Laboratoire de Neurobiologie, Ecole Normale Supérieure, 46, rue d'Ulm, 75230 Paris Cedex 05, France; and †Istituto di Neurofisiologia del Consiglio Nazionale delle Ricerche, Via San Zeno 56100, Pisa, Italy

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ABSTRACT **Light stimuli depolarize green cones of turtle retina through a circuit involving a feedback connection from luminosity horizontal cells (L-HC) to green cones. In turtle retina two types of L-HC have been distinguished: large-field L-HC and small-field L-HC. The spatial properties of the feedback depolarizations of green cones were compared with those of both large- and small-field L-HC. Green cones were found to be more effectively depolarized by relatively small spots of red light than by large red annuli. Moreover, red light stimulation of the periphery of the receptive field could reduce the depolarizing influence of central red stimuli. These spatial properties greatly differ from those of the large-field L-HC, whereas they strongly resemble those of the small-field L-HC. These results suggest that the small-field L-HC mediate the feedback action on green cones.**

Vertebrate photoreceptors have been classically considered as a mosaic of functionally independent cells, each responding only to light impinging on its outer segment. Experimental evidence gathered in the last decade has revealed the existence of complex lateral interactions at this early stage of visual processing. In cones, the increase in diameter of a circular light stimulus up to 120 μm was shown to increase the amplitude of the hyperpolarizing response, whereas further increase of the illuminated area resulted in a reduction of the response (1). The enhancing effect of the stimulation of the near surrounding of the cone was shown to result from electrical coupling to the neighboring cones of the same spectral sensitivity (1, 2), whereas the antagonistic effect of peripheral illumination was shown to be due to the activation of a polysynaptic circuit involving a negative feedback effect from the luminosity horizontal cells (L-HC) on the cones (1). Thus it was demonstrated that the hyperpolarization of the L-HC by either light stimulation or inward current injection (1, 3) could evoke a depolarization of the cones by a synaptic mechanism that at least partially involves an increase in the Ca^{2+} conductance of the cone membrane (4, 5).

In the turtle retina, the feedback action of the L-HC has been shown to affect both red and green cones. In the green cones (6, 7), red lights can induce pure feedback depolarizations, because they are poorly absorbed by the green cone pigment while, at the same time, they can evoke large hyperpolarizations in the L-HC that are mainly driven by the red cones. Two main types of L-HC have been described by Simon (8), according to the extension of the summation area of their receptive field: a large-field type (L1-HC) and a small-field type (L2-HC). It has been shown that only in the L2-HC can peripheral illumination induce an antagonistic effect (9, 10), which is best revealed by using dim light stimulation (10). Leeper (11) compared the morphology of Golgi stain-impregnated horizontal cells of the turtle retina with those stained with intracellular dye injection by Simon (8) and found that the L1-HC and L2-HC actually corresponded, respectively, to the axonal terminal branching and to the somatodendritic region of the same cell, both parts being connected by a slender axon. Moreover, Leeper (12) also showed that only the somatodendritic region establishes contacts with green-sensitive cones and therefore suggested that only the L2-HC are responsible for the feedback effects on green cones.

In the present paper, we have tried to identify the L-HC intervening in the feedback effects evoked by red light in green cones. With this purpose we have compared the receptive field properties of the feedback depolarizing responses evoked by red lights in green cones with those of both L1-HC and L2-HC. We have found that the feedback depolarizations in green cones were evoked more effectively by relatively small central spots than by large red annuli. We have also found that an increase in the illuminated area, to include the periphery of the receptive field, could result in a reduction of the feedback depolarizations in green cones, such an effect being best observed when using dim light stimuli. The spatial properties of the feedback responses of green cones thus correspond to the spatial properties of the light responses of the L2-HC. Our observations are therefore consistent with Leeper's hypothesis that the L2-HC are responsible for the feedback in green cones.

MATERIAL AND METHODS

The experiments were performed on isolated perfused eyecup preparations of the red-eared turtle, *Pseudemys scripta elegans*. The preparations were continuously superfused with a bicarbonate saline (13) of the following composition in mM: NaCl, 110; $NaHCO_3$, 22; KCl, 2.6; $CaCl_2$; $MgCl_2$, 2; glucose, 10; bubbled with a mixture of 95% O_2 and 5% CO_2. In some experiments $SrCl_2$ (3–10 mM) or $BaCl_2$ (1–5 mM) was added to the superfusion medium without compensating for the molarity change. Intracellular recordings were obtained with high-resistance micropipettes (250–600 MΩ) filled with 4 M potassium acetate. The stimuli were circles of light varying in diameter between 190 and 3700 μm or annuli of light of fixed external diameter (3700 μm) whose inner diameter varied between 1710

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Abbreviations: L-HC, luminosity horizontal cells; L1-HC, large-field horizontal cells; L2-HC, small-field horizontal cells.

and 580 μm. These stimuli were provided by a conventional double-beam optical stimulator. Approximately monochromatic lights were obtained by using interference filters (typical bandpass less than 15 nm).

RESULTS

Experiments Performed in Retinas Superfused with Isotonic Saline. Fig. 1 compares the responses of a green cone, a L2-HC, and a L1-HC to the same prolonged stimulation of the retina with deep red light (700 nm) covering different areas of their receptive field. In the green cone a large spot of 3700 μm (Fig. 1, trace a) and a small spot of 1060 μm (Fig. 1, trace b) both evoked sustained depolarizing responses, whereas the illumination with a red light annulus (Fig. 1, trace c) had almost no depolarizing effect. The amplitude of these depolarizing responses of the green cone appeared to be related to the amplitude of the responses elicited in the L2-HC by the same stimuli, because the largest hyperpolarizing responses in this cell were observed after stimulation with both large (Fig. 1, trace d) and small (Fig. 1, trace e) red spots, while the responses evoked by the annulus were much smaller (Fig. 1, trace f). In contrast, no correlation appeared when the depolarizing responses of the green cone were compared to the hyperpolarizations of the L1-HC. The small spot that elicited a large depolarization in the green cone induced a small response in the L1-HC (Fig. 1, trace h), whereas the red annulus, which was practically ineffective on the green cone, evoked a large response in the L1-HC (Fig. 1, trace i).

To allow a better recording of the feedback depolarizations of the green cones, we stimulated them with red light in the presence of a dim background of green light (550 nm) and these responses were then compared with those obtained from the L2-HC and the L1-HC, using similar combinations of stimuli (Fig. 2).

In the presence of such a green background, the feedback depolarizations evoked by small red spots in the green cones (Fig. 2, trace a) were always much larger than the depolarizations elicited by the red annuli which, as can be observed in Fig. 2, trace b, were generally difficult to resolve from the plateau of the hyperpolarization evoked by the green light. Again in these conditions, the amplitude of the feedback depolarizations appeared correlated to the amplitude of the hyperpolarizing responses evoked by the same red stimuli in the L2-HC (Fig. 2, traces c and d), whereas no correlation was observed with the amplitude of the L1-HC hyperpolarizations (Fig. 2, traces e and f).

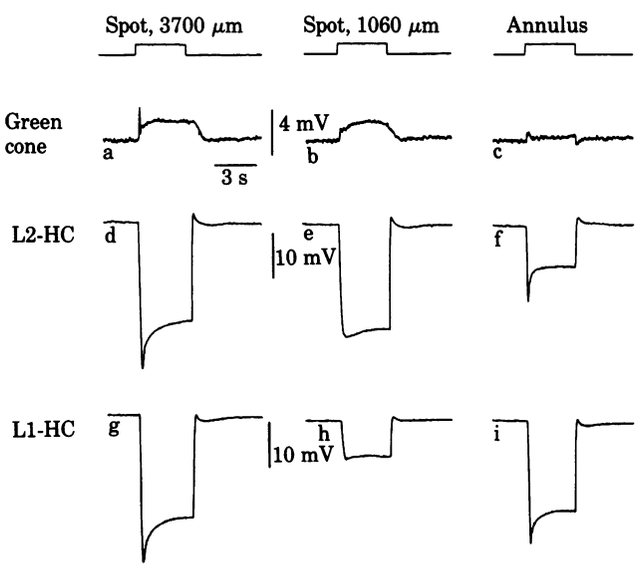

FIG. 1. Intracellular recordings of the light responses of, respectively, a green cone (traces a–c), a L2-HC (traces d–f), and a L1-HC (traces g–i) from different retinas obtained with monochromatic red light stimuli (700 nm) covering different areas of their receptive fields. The diameters of the light spots used to elicit the responses in traces a, d, and g and in traces b, e, and h are indicated above the stimulus trace. The annulus used in traces c, f, and e had an inner diameter of 580 μm. The photon flux was 1.2×10^5 quanta $\mu m^{-2} s^{-1}$.

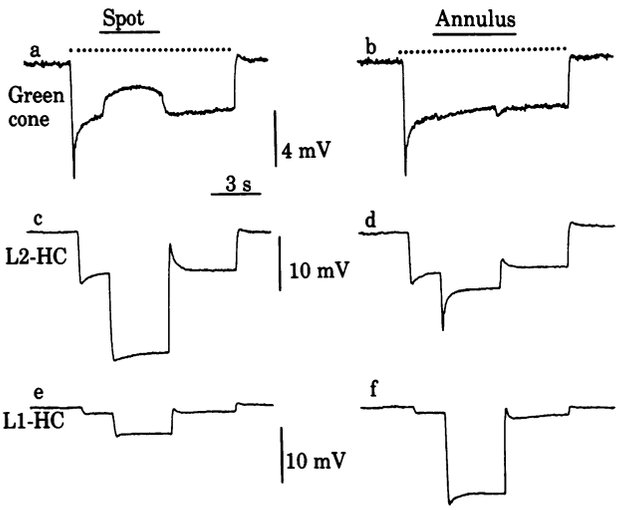

FIG. 2. Intracellular recordings of the light responses of a green cone (traces a and b), a L2-HC (traces c and d), and a L1-HC (traces e and f) obtained with monochromatic red light stimuli (700 nm; 1.2×10^5 quanta $\mu m^2 s^{-1}$) applied in the presence of a background illumination of green light (550 nm; 10^4 quanta $\mu m^{-2} s^{-1}$). The duration of the red light stimuli (870-μm-diameter spot in traces a, c, and e and 870-μm-inner-diameter annulus in traces b, d, and f) is monitored by the full line trace above the cone recordings. The dotted line trace monitors the duration of the green light stimulus (650-μm spot for all recordings).

Analogous observations to those described above were made when comparing the responses of 12 green cones recorded in retinas bathed in normal saline with the responses of the L1-HC and L2-HC.

Experiments on Sr^{2+}- or Ba^{2+}-Treated Retinas. As already stated, the hyperpolarization of the L-HC leads, through a feedback connection, to an increase in the Ca^{2+} conductance of the cones (4, 5). In the presence of Ba^{2+} or Sr^{2+} in the extracellular medium this Ca conductance increase can become regenerative and result in a transient or sustained discharge of spikes (3–5, 7). Because in many untreated green cones the depolarizing feedback responses elicited by red stimuli were of small amplitude and difficult to analyze, Sr^{2+} or Ba^{2+} was applied to facilitate the observation of feedback effects and to study their receptive field properties.

Fig. 3, traces a–c, illustrates the responses to red stimuli of a green cone bathed in a medium containing 10 mM Sr^{2+}. Both a small spot of 870-μm diameter (Fig. 3, trace a) and a large 3700-μm spot (Fig. 3, trace b) of the same bright light evoked in such conditions the repetitive discharge of 25- to 30-mV spikes at 1.5–2 Hz, whereas an annulus (870-μm inner diameter, 3700-μm outer diameter) of the same intensity failed to evoke any spike discharge (Fig. 3, trace c). This is consistent with the observations made in untreated retinas, and here again the properties of the receptive field of the feedback responses paralleled those of the L2-HC, but not those of the L1-HC. Similar results were obtained in 15 green cones bathed in Sr^{2+}- or Ba^{2+}-containing media.

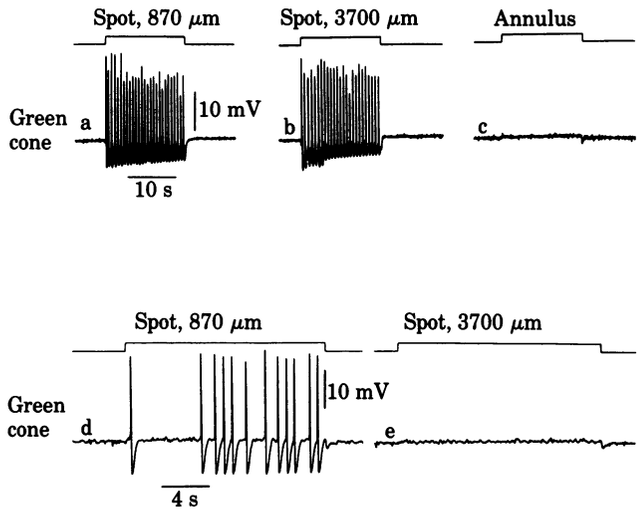

FIG. 3. Intracellular responses obtained with monochromatic red light stimuli (700 nm) in a retina perfused with a 10 mM Sr^{2+}-containing medium. Traces a–c, responses elicited in a green cone by a bright red light (1.2×10^5 quanta $\mu m^{-2} s^{-1}$). Traces d and e, responses obtained with a dim red light (3.5×10^3 quanta $\mu m^{-2} s^{-1}$) from the same green cone. The diameters of the light spots are indicated near the stimulus trace. The inner diameter of the annulus was 870 μm.

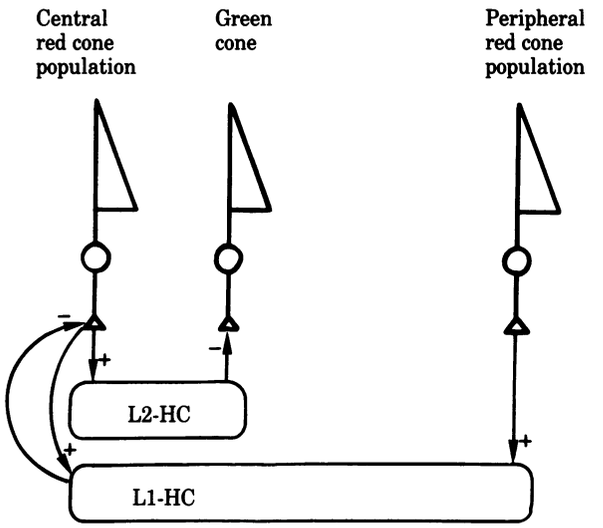

FIG. 4. Diagram of the functional connections between the cones and both types of L-HC. The arrows indicate the direction of transmission and the symbols + and − indicate, respectively, sign-preserving and sign-inverting connections. The central and peripheral red cone populations represented each by a single cone respectively correspond to the red cone pools located inside and outside of the circular central region of the green cone depolarization's receptive field (*ca.* 1000-μm diameter). In order to simplify the schematic representation other connections of both types of L-HC with photoreceptors are ignored.

Another interesting feature of Sr^{2+}-treated cones is that it is possible to detect the feedback effects even when using very dim lights that would generally fail to evoke a feedback response in untreated cones. In the L2-HC an increase of the diameter of the light spot beyond *ca.* 1000 μm results in a decrease of the hyperpolarizing response. This effect is best revealed when the light stimuli are dim. If the feedback depolarizations of the green cones depend on the level of hyperpolarization of the L2-HC, the feedback response should be better evoked by optimal size small spots than by illumination of a large retinal area to include the periphery of the receptive field. In Fig. 3, traces d and e the same cone of traces a and c was stimulated with a 870-μm spot of dim red light (trace d) and with a 3700-μm spot of the same light (trace e). The smaller spot elicited a repetitive discharge of spikes at a lower frequency than in trace a, whereas a large dim spot stimulation became ineffective (trace e).

DISCUSSION

The present results support the view that the L2-HC are the horizontal cells responsible for the feedback depolarizations in green cones. In both untreated and Sr^{2+}- (or Ba^{2+}-) treated retinas these depolarizations were more easily obtained by using relatively small spots than with large annuli (3700 μm, outside diameter). Moreover, in Sr^{2+}-treated retinas it was also possible to show that dim red light illumination of the peripheral area of the receptive field could reduce the feedback effects evoked by illuminating the central area (see Fig. 3, traces d and e), thus showing an unexpected complexity of the spatial properties of the receptive field of the green cones. These receptive field properties of the feedback responses of the green cones correspond to those observed in the L2-HC (9, 10). In contrast, the receptive field properties of the L1-HC are different from those of the feedback depolarizations of the green cones; thus it is unlikely that such cells could play a direct role in the generation of the green cone depolarizations. These results are in complete agreement with the hypothesis of Leeper (12), who excluded on morphological grounds the existence of a direct connection between the L1-HC and the green cones.

However, the absence of a direct connection between the L1-HC and the green cones does not imply that the green cones cannot be affected by the hyperpolarization of the L1-HC. It has been shown that the hyperpolarization of the L1-HC with inward current injection (9) or peripheral light (9, 10) results in a depolarization of the L2-HC, probably through the feedback influence of the L1-HC on red cones. This mechanism would be responsible for the peripheral antagonism of the L2-HC receptive field (9, 10). Thus the reduction of the feedback depolarizations in green cones observed when increasing the area of a dim red illumination (Fig. 3, traces d and e) would be ultimately a consequence of the L1-HC hyperpolarization.

The schematic drawing of Fig. 4 summarizes the interactions between cones and L-HC as discussed above. It shows that both L1-HC and L2-HC receive their main input from red cones, and whereas the L1-HC is responsible for the feedback effects on red cones, the L2-HC intervenes in the feedback depolarization of the green cones.

In Fig. 4, L1-HC and L2-HC are represented as separate elements. As previously mentioned, they actually correspond to different regions of the same cell. In a more general context, the present results also illustrate the complexity attained by local nervous circuits that do not involve spiking elements. In the present case, the soma and the terminal branching of the same neuron, connected between them by a nonconducting axon, not only have rather different connectivity but also function as two different physiological entities.

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