Intrinsic Cone Adaptation Modulates Feedback Efficiency from Horizontal Cells to Cones

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abstract Processing of visual stimuli by the retina changes strongly during light/dark adaptation. These changes are due to both local photoreceptor-based processes and to changes in the retinal network. The feedback pathway from horizontal cells to cones is known to be one of the pathways that is modulated strongly during adaptation. Although this phenomenon is well described, the mechanism for this change is poorly characterized. The aim of this paper is to describe the mechanism for the increase in efficiency of the feedback synapse from horizontal cells to cones. We show that a train of flashes can increase the feedback response from the horizontal cells, as measured in the cones, up to threefold. This process has a time constant of ~3 s and can be attributed to processes intrinsic to the cones. It does not require dopamine, is not the result of changes in the kinetics of the cone light response and is not due to changes in horizontal cells themselves. During a flash train, cones adapt to the mean light intensity, resulting in a slight (4 mV) depolarization of the cones. The time constant of this depolarization is ~3 s. We will show that at this depolarized membrane potential, a light-induced change of the cone membrane potential induces a larger change in the calcium current than in the unadapted condition. Furthermore, we will show that negative feedback from horizontal cells to cones can modulate the calcium current more efficiently at this depolarized cone membrane potential. The change in horizontal cell response properties during the train of flashes can be fully attributed to these changes in the synaptic efficiency. Since feedback has major consequences for the dynamic, spatial, and spectral processing, the described mechanism might be very important to optimize the retina for ambient light conditions.

key words: retina • synapse • goldfish • calcium current

INTRODUCTION

Most cells in the visual system have receptive fields with antagonistic surrounds. Bipolar cells are the first cells in the visual system with this organization. The antagonistic surround of these cells is generated for a large part by feedback connections in the outer retina. In general, antagonistic surrounds play a role in contrast enhancement and in edge detection. In addition, these feedback connections control the size of the horizontal cell (HC)[1] receptive fields (Verweij et al., 1996b), play a prominent role in the generation of spectral opponent HCs (Stell and Lightfoot, 1975; Kamermans et al., 1991), and form the neural basis of the color constancy (Kamermans et al., 1998).

The strength of the feedback signal from HCs to cones seems to vary strongly with the adaptation state of the retina. For instance, in the light adapted retina,

two types of spectrally opponent HCs can be found, whereas in the fully dark adapted retina, spectrally opponent HCs are completely absent (Weiler and Wagner, 1984). Changes in response properties of ganglion cells can also be found. In a fully light-adapted condition, these cells have antagonistic surrounds. These surrounds are absent in the dark-adapted condition (Raynauld et al., 1979). Given this important role of negative feedback in the signal processing in the retina, variations of the strength of negative feedback will have strong impact on the whole visual system.

The Mechanism of Negative Feedback in the Goldfish Retina

For goldfish, the negative feedback pathway from HC to cones has been described in detail (Verweij et al., 1996b; for review see Kamermans and Spekreijse, 1999). Horizontal cells feed back to cones by modulating the cone Ca-current directly in a γ-aminobutyric acid (GABA)–independent way. Hyperpolarization of the HCs results in a shift of the Ca-current activation function to more negative potentials, yielding an increase in Ca influx in the cone synaptic terminal and thus an increase in glutamate release (Verweij et al., 1996a). The neurotransmitter involved is presently unknown. Although the properties of the negative feed-

back pathway are beginning to be resolved, the way this pathway is modulated during light/dark adaptation is not clear at all.

In this study, we describe a mechanism by which the efficiency of the feedback signal is changed during adaptation. To change the adaptation state of the retina, a train of bright white flashes was used. It will be shown that during this stimulus protocol, the efficiency of the feedback signal increases strongly due to a slight depolarization of the cones. This cone depolarization is due to intrinsic photoreceptor adaptation. This study illustrates that local photoreceptor adaptation yields a strong change in the network properties of the retina.

MATERIALS AND METHODS

Preparation

Goldfish, *Carassius Auratus*, (12–16 cm standard body lengths) were kept at 18°C under a 12-h dark/12-h light regime. Before the experiment, the fish was kept in the dark for 7 ± 1 min. The fish was decapitated, and an eye was nucleated. This eye was hemisected and most of the vitreous was removed with filter paper. The retina was isolated, placed receptor side up in a superfusion chamber and superfused continuously (1.5 ml/min) with oxygenated Ringer's solution (pH 7.8, 18°C). This procedure was done, using infrared ($\lambda = 920$ nm) illumination.

Patch Clamp Measurements and Intracellular Recordings

Optical stimulator. A 450-W Xenon lamp supplied two beams of light. These were projected through Uniblitz VS14 shutters (Vincent Associates), neutral density filters (NG Schott), lenses, and apertures. For the patch clamp measurements, the spots, 20 μm in diameter, were projected through a 40× water immersion objective (N.A. = 0.55) of the microscope and the spots, 3,000 μm in diameter, were projected through the condenser (N.A. = 1.25) of the microscope. The full-field white light stimuli used for intracellular recordings were projected onto the retina through a 2× objective lens (N.A. = 0.08) of the microscope. For all experiments, only white light stimuli were used. The light intensities are expressed in log units relative to 4×10^3 cd/m². Since we used white light stimuli in our experiments, we compared the size of the cone light responses to white light with the responses of M cones to 550-nm light flashes and calculated the amount of effective quanta in our white light to be 1.0×10^5 s⁻¹ μm⁻¹ (550 nm). In goldfish, Malchow and Yazulla (1988) calculated that 50% of the pigment was bleached when the retina was stimulated with 3.16×10^9 photons s⁻¹ μm⁻¹, left on for 4 min. The amount of photons they used exceed our stimulus condition by six log units. This is an indication that in our experimental conditions, bleaching hardly plays a role.

Electrodes and recording equipment. The patch pipettes were pulled from borosilicate glass (GC150TF-10; Clark) and the intracellular microelectrodes were pulled from aluminosilicate glass (o.d. = 1.0 mm, i.d. = 0.5 mm; Clark) with a P-87 micropipette puller (Sutter Instruments Co.).

Patch pipettes had impedances between 5 and 10 MΩ when filled with pipette medium and measured in Ringer's solution. The series resistance during the whole cell recording was <12 MΩ. Electrodes were mounted on an MP-85 Huxley/Wall-type micro manipulator (Sutter Instruments Co.) and connected to a 3900A Integrating Patch Clamp (Dagan Corp.).

Microelectrodes had impedances ranging from 100 to 200 MΩ when filled with 4 M KAc. The intracellular voltages were measured with an electrometer (S7000A; World Precision Instruments) and recorded on paper (Graphtec Linearcorder).

Data acquisition, control of the optical stimulator, and control of the patch clamp were done with a 1401 AD/DA convertor (Cambridge Electronic Design Ltd.) and an MS-DOS–based computer system.

Ringer Solutions and Pipette Medium

The Ringer solution contained (mM): 102.0 NaCl, 2.6 KCl, 1.0 MgCl₂, 1.0 CaCl₂, 28.0 NaHCO₃, 5.0 glucose, and was continuously gassed with ~2.5% CO₂ and 97.5% O₂, yielding a pH of 7.8. Some Ringer solutions contained drugs as indicated in the text, and in the figure legends: flupentixol (50 μM; Research Biochemicals, Inc.), niflumic acid (100 μM; Sigma-Aldrich Chemical Co.), CoCl₂ (2 mM; Merck). In tetraethylammonium (TEA)-Cl Ringer's solution, 15 mM of the NaCl was replaced by 5 mM CsCl and 10 mM TEACl.

The standard patch pipette medium contained (mM): 12.0 KCl, 61.0 d-gluconic-K, 1.0 MgCl₂, 0.1 CaCl₂, 1.0 EGTA, 5.0 HEPES, 5.0 ATP-Na₂, 1.0 GTP-Na₃, 0.2 3':5'-cGMP-Na, 20 Phosphocreatine-Na₂, 50 U/ml creatine phosphokinase. To change the Cl-equilibrium potential, KCl was exchanged for equimolar d-gluconic-K. The pH of the pipette medium was adjusted to 7.25 with KOH. Cesium pipette mediums contained equimolar d-gluconic-Cs and 12.0 CsCl instead of the KCl and d-gluconic-K. All chemicals were obtained from Sigma-Aldrich Chemical Co.

Liquid Junction Potential

The liquid junction potential was measured with a patch pipette filled with the pipette medium, and positioned in a bath filled with pipette medium. The reference electrode was filled with 3 M KCl. After the potential was adjusted to zero, the bath solution was replaced with Ringer's solution. The resulting potential change was considered as the junction potential. The liquid junction potential was determined for the various pipette solutions and all data were corrected accordingly.

Dopamine Depletion of the Retina

Dopaminergic interplexiform cells were destroyed with intraocular injections of 5 μl Ringer solution containing 6-hydroxydopamine (5 μl) and paragyline (10 μl; both from Sigma-Aldrich Chemical Co.), administered on two successive days (Negishi et al., 1982; Lin and Yazulla, 1994). These fish were used 14–33 d after injection. All dopamine-depleted retinas used for intracellular recordings were tested afterwards for the presence of dopamine-containing cells using the tyrosine hydroxylase method (Yazulla and Studholme, 1997). Data were taken only from retinas that did not contain any tyrosine hydroxylase–positive cells.

Cell-type Classification

Cones were selected under visual control and spectrally classified (Kraaij et al., 1998). Only L, M, and S cones were found. HCs were classified on their spatial and spectral properties (Norton et al., 1968; Kaneko, 1970; Hashimoto et al., 1976; Kaneko and Stuart, 1984). Of the three classes of HCs, only monophasic HCs were used in the present study.

Stimulation Protocols

Presynaptic calcium current protocol. Since the large modulation of the light-sensitive conductance masks the much smaller presynaptic calcium current of the cone, it is not possible to measure

light-induced changes in presynaptic calcium currents of cones directly. To overcome this problem, previously recorded light responses of a cone to spot stimuli (20 μm diameter) of 500-ms durations were "played back" as command voltages in a condition where the light conductance of the cone is blocked with an intense (−0.15 log) white spot (20 μm diameter). In this way, "light-induced" changes in presynaptic calcium current can be measured without the interference of the light-sensitive conductance. Fig. 1 explains the voltage clamp protocol used to isolate light-induced changes in calcium current. The insert displays the original hyperpolarizing response of a cone to a 500-ms flash that was used to construct the voltage clamp protocol. Trace 1 consists of this hyperpolarizing response repeated nine times. Trace 2 is similar to trace 1, but now with a depolarization of 4 mV superimposed, applied in discrete 1-mV steps just before the second, third, fourth, and fifth hyperpolarizing responses. Because the 1-mV voltage steps were taken in between the "light flashes," the whole light response is shifted to a 1-mV more-depolarized level. During the last four hyperpolarizing steps, this final 4-mV depolarized level was maintained. For trace 3, a similar protocol as for trace 2 was generated, but the depolarizing steps were twice as large, yielding a total depolarization of 8 mV. The final amount of depolarization is indicated by the numbers to the right of the voltage traces. After the "flashes," a 100-ms step to −7 mV was applied, followed by a 1,500-ms step to −77 mV. Finally, the cone membrane potential was stepped back to the initial potential. This last part of the protocol was used to study the tail currents. During the step to −77 mV, a 50-ms step to −87 mV was applied. This step was used for leak subtraction to isolate the presynaptic currents of the cone. The part of the protocol used for determining the tail and leak currents are not shown in the figures.

Feedback protocol. To measure feedback from HCs in cones, the cone light responses were saturated with a spot of white light (−0.15 log) with a 20-μm diameter. Stimulating the cones with light in this saturated condition does not lead to a light response (not shown). Cones were clamped at the potential indicated in the text and figure legends and 10 white, full field flashes (intensity −1.0 log) of 500-ms durations were delivered to the retina.

RESULTS

First we will show that the kinetics of the HC light response changes during a train of repetitive flashes. After excluding all other possibilities, we will show that this can be accounted for by an adaptation-induced depolarization of the cone. Furthermore, we will show how this adaptive depolarization of the cones causes changes in the synaptic transmission between cones and HCs. Finally, a simple simulation will show that a small depolarization of the cone can account for the complex changes in HC response kinetics observed during light adaptation.

Definitions

HCs feed back to cones by shifting the calcium current activation function of the cones using an unknown neurotransmitter. Because we cannot measure the amount of this neurotransmitter, we will define the feedback signal as the size of the shift of the calcium current activation function of the cone. The resulting change in the calcium influx will be called the efficiency of the feedback signal.

HC Kinetics during the Flash Train

Fig. 2 A shows HC responses to a train of 10 white light flashes of 500-ms durations with an inter-stimulus interval of 200 ms for three different intensities (−2.0, −1.0, and 0.0 log). For the −2.0 log intensity, the responses are small and sustained. For the −1.0 log intensity, the responses are larger and show a pronounced secondary depolarization; i.e., the difference between peak and plateau value. This secondary depolarization has been shown to be the consequence of negative feedback from HCs to cones (Piccolino et al., 1981; Wu, 1994; Kamermans and Spekreijse, 1999; Kraaij et al., 1998).

The highest intensity shows round, saturated responses without the secondary depolarization. Of the three intensities, only −1.0 log shows a change in kinetics of the HC responses during the flash train. Comparison of the response to the first and last flashes reveals that the kinetics of the light response has changed considerably (Fig. 2 B). The gray bar in Fig. 2 B marks the size of the secondary depolarization in response to the first flash, and the dashed lines that to the last flash. Thus the secondary depolarization, which is due to negative feedback from HCs to cones, has increased during the flash train. This increase in the size[2] has a time constant of 2.7 ± 1.1 s ($n = 7$).

Changes in HC Kinetics Do Not Depend on Dopamine

It is known that dopamine modulates the feedback signal from HCs to cones (Kirsch et al., 1990). To test whether dopamine can account for the observed changes of HC light responses during the flash train, the experiment of Fig. 2 was repeated in dopamine-depleted animals. Fig. 3 A shows the first and last light responses of a HC from a retina, without dopaminergic interplexiform cells (IPCs). Like Fig. 2, the gray bar and the dashed lines indicate the amount of secondary depolarization of the first and last flash, respectively. The retina was isolated 14 d after intraocular injection of 6-hydroxy-dopamine, which is known to kill the IPCs. In these dopamine-depleted retinas, the changes in HC

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[2]To determine the amount of sustained depolarization during the flash train, we determined for each flash response the mean membrane potential in a time window of 225–700 ms after the onset of the flash. Through these mean membrane potentials, an exponential curve was fitted. The amplitude and time constant (τ) of this curve were determined and can be interpreted as the amplitude and time constant of the flash train–induced depolarization. The rational for this approach is that during the first light response the cone already starts to adapt, and thus starts to depolarize. Just determining the membrane potentials of the light response at the sustained potentials will in that way underestimate the change in cone membrane potential. Taking the depolarized levels as a measure for the cone membrane potential also yields an incorrect value because sometimes after the first flash an overshoot occurs that is of course absent at the onset of the first flash.
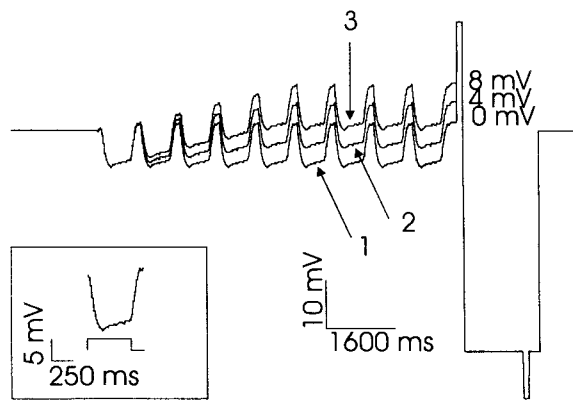
Figure 1. Voltage-clamp stimulation protocol, used to study changes in presynaptic calcium current during light-induced hyper-polarizations. The first trace shows a real light response (see inset) repeated nine times. In the second trace, a gradual depolarization of 4 mV during the first five steps, in 1-mV steps, is applied. For the third trace, a similar protocol as trace 1 was generated, but now all depolarizing steps are twice as large, yielding a total depolarization of 8 mV. The final amount of depolarization is indicated by the number to the right of the traces. After the flashes, a voltage protocol is made to study the tail currents, consisting of a 100-ms step to -7 mV, followed by a 1,500-ms step to -77 mV, after which it is stepped back to the resting membrane potential again. During the step to -77 mV, a 50-ms step to -87 mV is made to determine the leak conductance.

kinetics during the flash train remain present. This result was found in eight retinas that did not stain for tyrosine hydroxylase. Furthermore, blocking the D1 and D2 receptors in control retinas with the antagonist flupentixol (Fig. 3 B) did not influence the changes in response shape during the flash train.

These experiments show that dopamine, the main neuromodulator involved in light–dark adaptation, is not involved in the changes in HC kinetics during the flash train. The next step was to determine whether the changes of HC responses are due to changes in (a) the cones, (b) the HCs, or (c) the reciprocal cone/HC synapse.

Presynaptic Changes during the Flash Train

One possibility is that changes in the cone light response during the flash train can account for the effects observed in the HC response. Fig. 4 A shows the voltage responses of a cone under whole-cell configuration to the same flash train as used for the HCs. Cones hyperpolarize in response to repetitive stimulation, but, unlike HCs, the kinetics of the responses show a decrease in transientness (Fig. 4 B). This result was obtained for both full-field as well as small-spot stimulation (20 μm in diameter; not shown). However, the mean membrane potential of the cone depolarizes with the number of flashes. In 12 cells tested, the mean depolarization was 3.3 ± 1.4 mV (31% of the amplitude of the light response of each cone). The time constant of this depolarization was 3.3 ± 0.5 s, which is of the
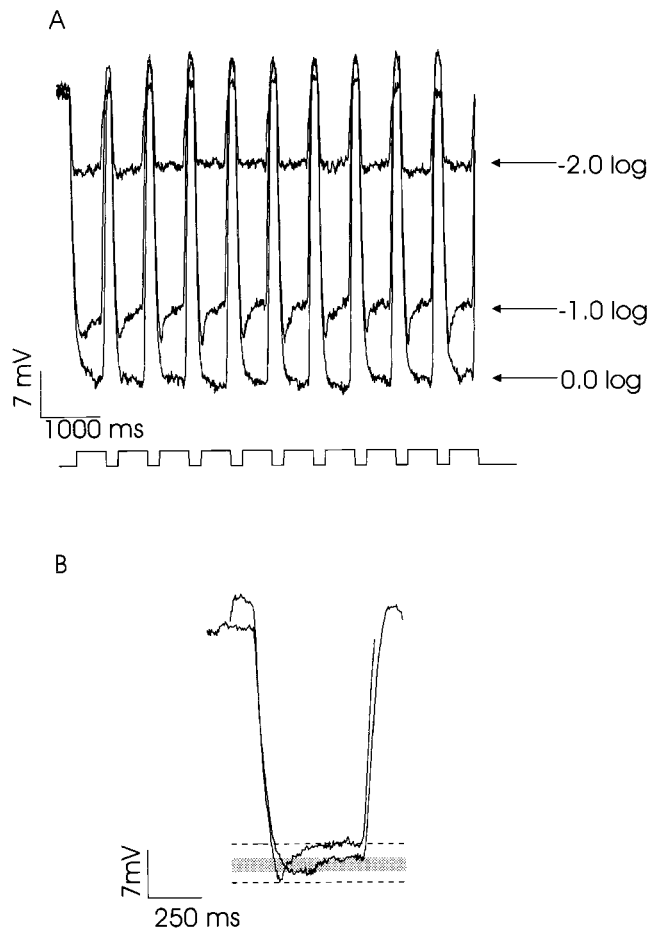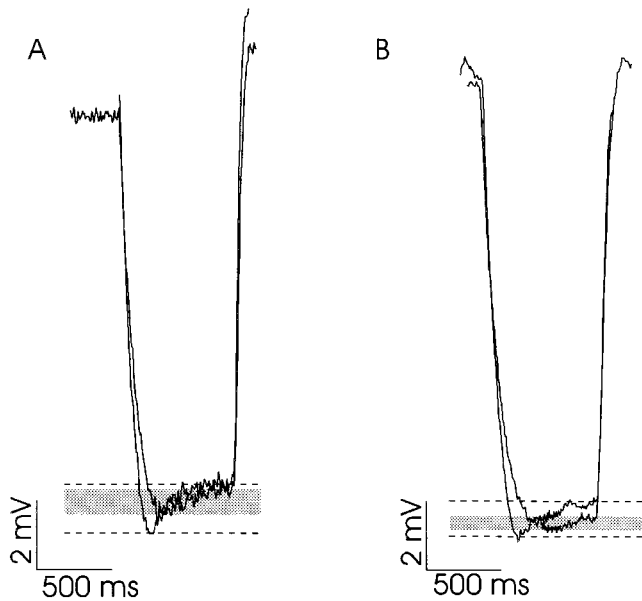

Figure 2. Changes in kinetics of HC light responses. (A) HC light responses to 10 white, 500-ms full field flashes of -2.0, -1.0, and 0.0 log. Mean resting membrane potential was -34.72 ± 2.27 mV ($\pm$ SEM; $n = 10$). Only the -1.0 log intensity responses are used in this study. There is a change in kinetics of HC light responses during the flash train. (B) An overlay of the first and last HC light response taken from A. The responses are shifted only along the horizontal axis. Timing and scaling are indicated. The gray area indicates the size of the secondary depolarization of the first flash and the area between the dashed lines indicates the size of the secondary depolarization of the last response.

same order as the time constant of the change of the secondary depolarization of the HCs.

Since the kinetics of the cone responses show a decrease in transientness, it cannot account for the increase in transientness of the HC light responses. However, during the stimulation protocol, the cone calcium current might have changed, leading to a change in the Ca-dependent glutamate release, and thus to a change in the synaptic output of the cone. The next step was therefore to measure changes in presynaptic currents of the cone during the flash train. Since the conductance modulated by light is so large that it completely masks the presynaptic currents of the cone, voltage light responses of a cone were recorded and used as a command voltage in a condition that the cone light re-

Figure 3. Changes in kinetics of HC light responses is independent of dopamine. (A) Overlay of the HC light responses to the first and last flash from 6-hydroxydopamine animals to the same flash train used in Fig. 2 A. Mean resting membrane potential was -34.6 ± 2.7 mV ($\pm$ SEM; $n = 8$). In dopamine-depleted animals, changes in kinetics are still present. (B) Overlay of the HC light responses to the first and last flash measured in control retinas perfused with flupentixol, a D_1, D_2 antagonist. The changes in HC kinetics persist in a situation where dopamine actions are blocked. The gray area and the dashed lines have the same meaning as in Fig. 2.

sponse was saturated with an intense small white spot (for details of the protocol, see materials and methods). Fig. 5 shows the presynaptic currents generated in a cone, clamped at its resting membrane potential, in response to a voltage protocol that simulates "light responses." Fig. 5 (top trace) shows that each hyperpolarizing light response causes an outward current in the cone, and this does not change with repetitive light responses. However, when the voltage protocol includes a stepwise depolarization of the cone (second and third current traces) the outward current elicited by each light response is increased in amplitude. The final amplitude of the step-wise (four steps) applied depolarization is indicated by the numbers to the right of the traces. The middle current trace (4-mV depolarization) mimics the physiological condition (Fig. 4). In that condition, the light-induced change in presynaptic currents of the cone increase with the number of flashes with a similar time constant as the increase in rollback in the HC response; i.e., the modulation depth of a light flash increases during the stimulation protocol. This increased modulation depth of a light flash could be due to two features of the command voltage protocol: (a) the repetitive light-induced hyperpolarizations or (b) the gradual "adaptation-induced" depolariza-
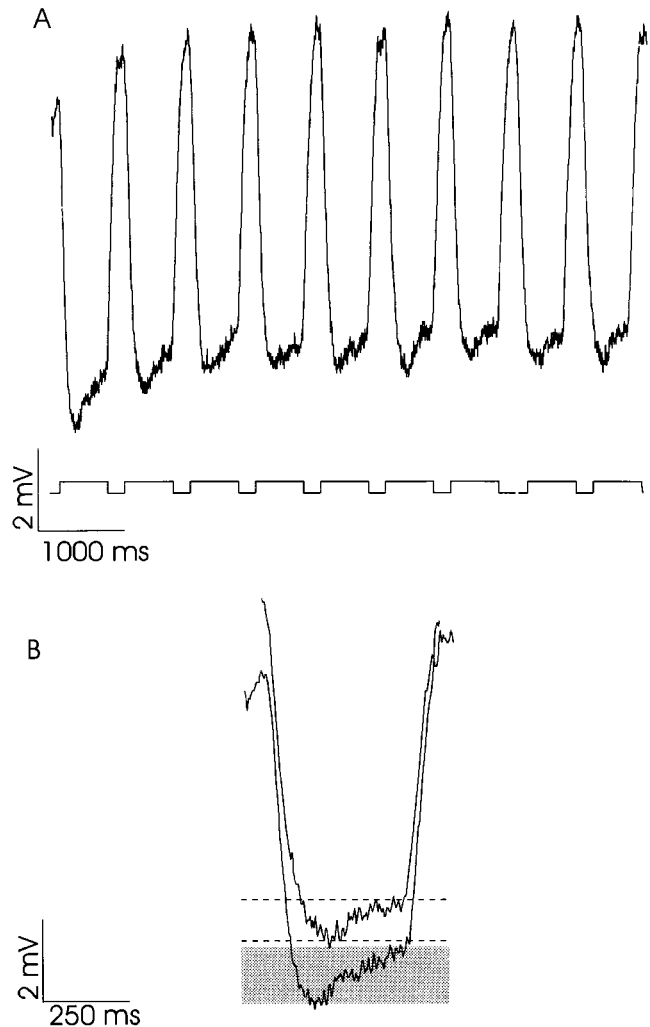
Figure 4. Changes in cone light responses during the flash train. (A) Cone light responses to the same flash train that induces changes in kinetics of HC light responses. The transientness of the light response decreases and the sustained cone membrane potential depolarizes slightly. (B) Overlay of the first and last cone light response. The responses are only shifted along the horizontal axis. The gray area and the dashed lines have the same meaning as in Fig. 2.

tion. In the upper trace (Fig. 5), the cone does not depolarize, although the same "flashes" are presented. In this condition, no changes in presynaptic currents of the cone are seen. This result suggests that the gradual depolarization, instead of the repetitive hyperpolarizations, is the basis of the increase in response size. Fig. 5 (bottom trace) confirms that the increase in presynaptic currents of the cone are due to the depolarization of the cone because an 8-mV depolarization increases the light-induced changes in the presynaptic currents even further than a 4-mV depolarization. These experiments ($n = 14$) show that the currents elicited in the absence of any depolarization (0 mV) do not change over the period of stimulation, whereas the presence of depolarization (4 or 8 mV) clearly potentiates the amplitude of
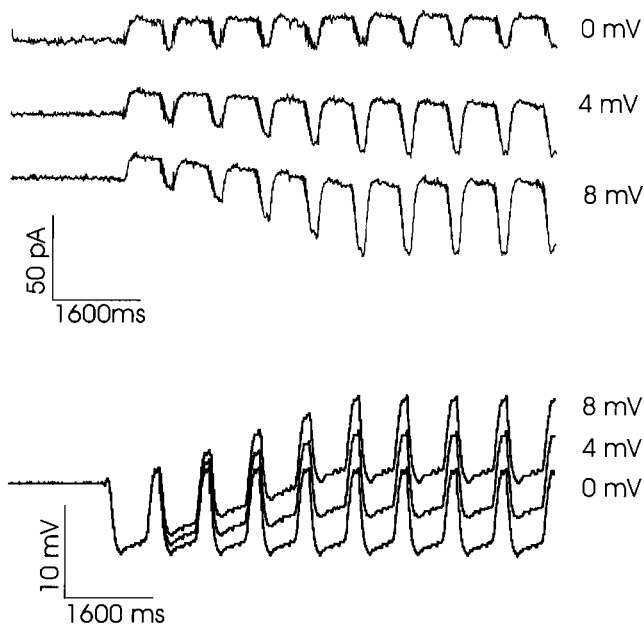
Figure 5. Modulation of light-induced changes in presynaptic calcium current due to depolarization of the cone. A voltage protocol was constructed, depicted at the bottom of the figure, that mimics cone responses to the flash train. Measurements were done at cone resting membrane potential -42.5 ± 1.4 mV ($\pm$ SEM; $n = 15$). The first trace shows the presynaptic currents due to such a protocol without depolarization of the cone (0 mV). The presynaptic currents stay unmodified. Second and third traces show presynaptic currents when cones are led to depolarize 4 and 8 mV, respectively. Values of the final depolarization level are indicated to the right of the current traces. There is an increase in "light-induced changes" of the presynaptic currents due to this depolarization. The increase is larger with larger depolarizations (8 mV).

the presynaptic currents of the cone. In the next section, we will show that these presynaptic outward currents elicited by hyperpolarizing light responses in the cone are due to reductions in inward calcium current, the presynaptic calcium current in the cone terminal.

The Nature of the Light-induced Presynaptic Currents of the Cone

Since the cone in Fig. 5 was voltage clamped at its resting membrane potential and the light-sensitive conductance is saturated, four different currents will be active (Hille, 1992); i.e., the inward current activated by hyperpolarization (I_h), the calcium current (I_{Ca}), the calcium-dependent chloride current (I_{ClCa}), and the delayed rectifier (I_{Kdr}). Blocking the I_{Kdr} with extracellular TEA and intracellular Cs^{2+} did not change the increase in current during the voltage protocol (not shown). This result excludes the possibility that I_{Kdr} is modulated during this protocol. The increase in the modulation depth of a light flash due to depolarization of the cone is not likely to be due to modulation of I_h because depolarization of the cone would then decrease the amount of current. The two remaining currents, I_{Ca}

and I_{ClCa}, are located in the terminal of the cone (Taylor and Morgans, 1998) and could therefore be involved in synaptic transmission.

It is known that large divalent cations like cobalt (Co^{2+}) block Ca-dependent synaptic transmission. To determine the contribution of I_{Ca} to the presynaptic currents, the effect of Co^{2+} on the experiments of Fig. 5 was studied. To check the effectiveness by which we could block I_{Ca}, we first determined the effect of Co^{2+} on the current–voltage relations of the cones. Fig. 6 (top) shows the leak-subtracted current responses to a voltage ramp protocol from -70 to 20 mV (drawn at the bottom of the figure) before (1), during (2), and after (3) cobalt. Co^{2+} shifts the calcium current activation function to more positive potentials ($n = 4$). This is a general feature of large divalent cations (Kostyuk et al., 1982; Hille, 1992; Piccolino et al., 1996). This shift will block the synaptic transmission from cones to HCs because I_{Ca} is shifted out of the operating range of the cone. Fig. 7 shows that the presynaptic currents are almost completely blocked with Co^{2+}. Fig. 7 (top, Control) shows the increase in light-induced changes in the presynaptic currents due to the voltage trace that depolarizes to 8 mV. This trace is similar to the 8-mV trace, presented in Fig. 5. Fig. 7 (middle) is the response of the same cell, but now in the presence of 2 mM Co^{2+}. Almost no modulation of the presynaptic currents is present ($n = 4$). After washing out the Co^{2+} (Fig. 7, bottom), the presynaptic currents reappear.

Co^{2+} shifted I_{Ca} to positive potentials. If the presynaptic currents are mainly carried by Ca, then depolarizing the cone by an equal amount as I_{Ca} has shifted to positive potentials would prevent Co^{2+} from block the presynaptic currents. This assumption is confirmed in Fig. 8 A. A current trace to the same 8-mV part of the protocol as Fig. 5 is shown in the presence of Co^{2+} when the cone is clamped at a potential 20-mV depolarized from the resting membrane potential. In the presence of Co^{2+}, almost no modulation of the presynaptic currents is present when the cone is clamped at its resting membrane potential, and gradually depolarizes to a level 8-mV more depolarized (see Fig. 7, top). Depolarizing the cone 20 mV (an equal amount as I_{Ca} has shifted; see Fig. 6) reveals the presynaptic currents again ($n = 3$). If I_{Ca} is the main source of the presynaptic currents, hyperpolarizing the cone would have an equal effect as the application of Co^{2+}. In Fig. 8 B, the response of a cone in control Ringer's solution to a similar protocol (only the 8-mV trace) as used in Fig. 5 is shown, but now at a clamp potential of -87 mV. At that potential, no light-induced changes in presynaptic currents are present. Together, these experiments show that I_{Ca} forms the basis of the presynaptic currents.

I_{ClCa} is known to generate slow tail currents. To investigate whether the I_{ClCa} also plays a role in the increase
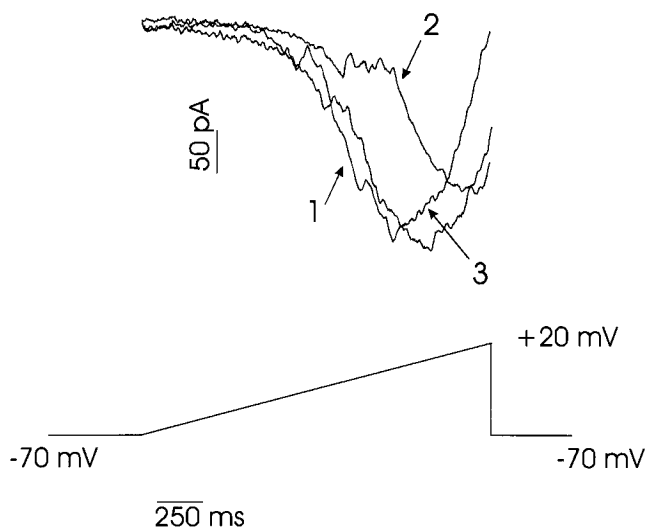
Figure 6. Current–voltage relations of a cone to the voltage ramp protocol depicted at the bottom of the figure, in control condition (arrow 1), in the presence of 2 mM cobalt (arrow 2), and after washout (arrow 3). Cobalt shifts the I_{Ca} to more positive potentials.

Figure 7. Cobalt blocks the light-induced presynaptic currents. The top current trace shows the voltage-dependent currents in control Ringer's solution to the part of the voltage protocol where the cone is let to depolarize 8 mV in 2-mV steps. The presence of 2 mM cobalt blocks the presynaptic currents (middle). After washout of cobalt, the presynaptic currents reappear. (Bottom) The synaptic current protocol is shown.

in presynaptic currents during the flash train protocol, tail currents were measured before and directly after the presynaptic current protocol. No changes in tail currents were found during these experiments (not shown), indicating that this current stays unmodulated during the flash train. In addition, we tested the role of I_{ClCa} by blocking this current with niflumic acid (Barnes and Deschenes, 1992). Fig. 9 B shows that application of 100 μM niflumic acid did not block the light-induced increase in synaptic currents ($n = 4$), whereas tail currents present in control (Fig. 9 A, 1) disappeared in the presence of niflumic acid (2). Furthermore, changing the calculated equilibrium potential for chloride from -47 to -28 mV did not change the depolarization-induced increase of the presynaptic currents (not shown). These experiments show that I_{ClCa} is not involved in the increase in presynaptic currents. Therefore, we can conclude that the increase in outward current, observed when the cone is allowed to depolarize is actually a decrease in the standing inward I_{Ca}.

Since I_{Ca} is directly related to the glutamate release, changes in I_{Ca} will result in changes in HC input. The next section will show that the change in HC response kinetics during the flash train is also due to changes in the cones.

Changes in HC Properties during the Flash Train

One of the striking changes in the HC response properties is the change in the secondary depolarization. This secondary depolarization is due to negative feedback from HCs to cones. If the changes in HC kinetics are due to changes in HC properties, one should expect that the output of the HCs would also change. Because we cannot measure the amount of neurotransmitter re-
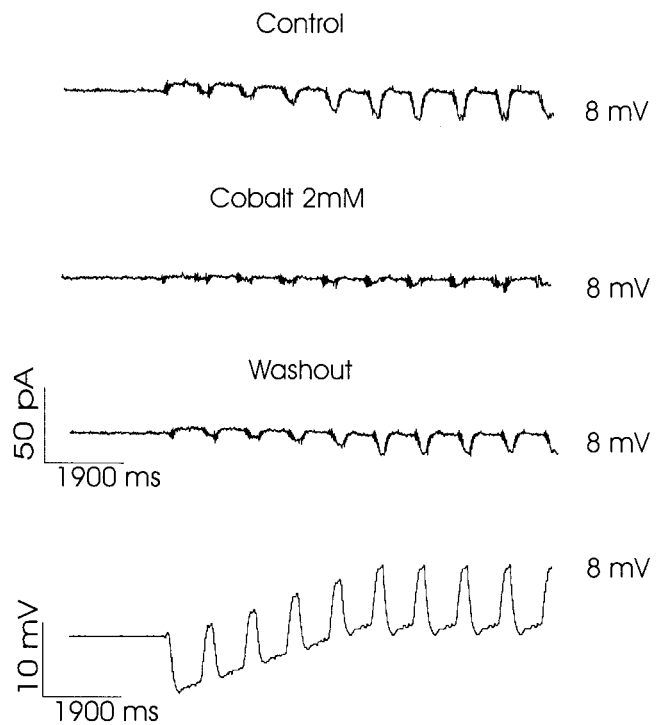
leased by the HCs, we looked at the effect of feedback on the cones; i.e., the change in calcium influx in the cone terminal. This effect of the feedback signal on the cone calcium current can be measured most effectively by clamping the cone at one potential, saturating the cone with a small spot and stimulating the retina with a full field flash stimulus (for details of the feedback protocol, see materials and methods). The result of this feedback protocol is that the cone recorded from responds only to the signal it receives from the HCs. In this way, the feedforward signal can be separated from the feedback signal, which cannot be done measuring HCs themselves. Fig. 10 shows a voltage-clamped cone stimulated with the feedback protocol. It is obvious from this figure that the feedback-induced inward current shows no pronounced changes during the flash train ($n = 4$). So, contrary to the increase in secondary depolarization, the feedback signal as measured in the cones has not increased during the flash train.

Thus, after a train of flashes, the secondary depolarization has increased. However, the feedback signal received by cones has remained equal. Presynaptic calcium currents also remained constant when the cone was clamped at one potential and showed only an in-

A   Cobalt 2 mM
Cone clamped at -20 mV

8 mV

8 mV

B   Control
Cone clamped at -87 mV

8 mV

8 mV
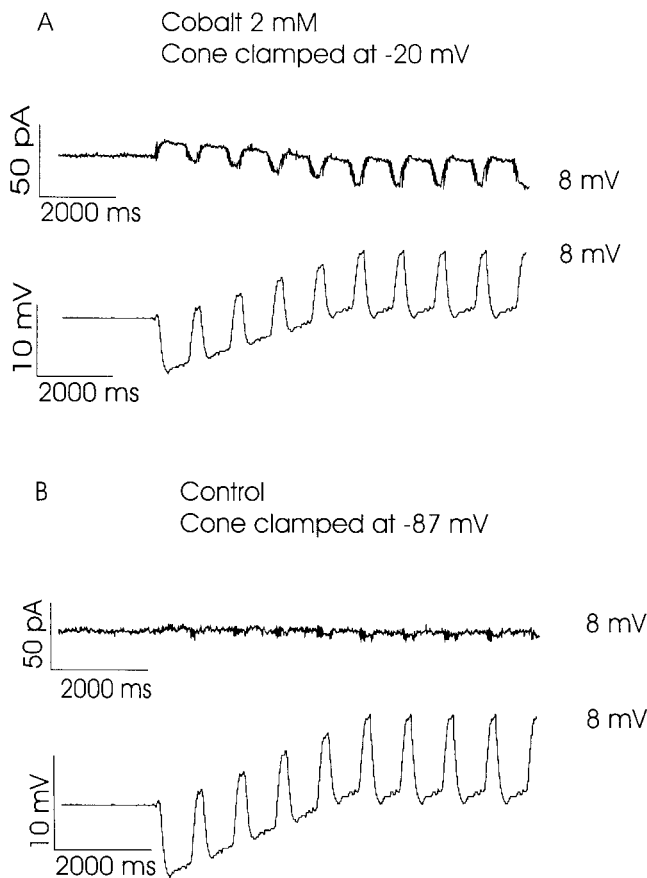
Figure 8. The relative position of the membrane potential to the activation function of the calcium current determines the light-induced modulation of the synaptic currents. (A) The modulation of the light-induced changes in presynaptic currents due to the stepwise depolarization of the cone (8 mV) are present in Co^{2+} when measured at depolarized levels. Clamping the cone at depolarized levels in the presence of cobalt makes the presynaptic currents reappear again. (Bottom) The voltage trace depicts the synaptic current protocol (8-mV trace) again. (B) Hyperpolarizing a cone to -87 mV blocks the presynaptic currents. The current trace is measured at a potential were I_{Ca} is not active. The increase in light-induced presynaptic currents due to the 8-mV depolarization is not present at these potentials.

crease when the cone was led to depolarize (Fig. 5). Therefore, it was tested whether a similar 4-mV depolarization of the cone increased the feedback-induced current in cones. Fig. 11 shows the feedback responses of a cone when it is depolarized, in four 1-mV steps during the protocol. Whereas the feedback-induced currents to the first three light flashes are small, the gradual depolarization of the cone results in a dramatic increase of the feedback-induced currents ($n = 5$). These experiments show that the depolarization of the cone makes feedback from the HCs to cones more efficient.

DISCUSSION

The main result of this paper is that stimulating the retina with a train of white light flashes can lead to a dou-

A

2

1

-7 mV

-77 mV   -36 mV

B

Control

8 mV

Niflumic acid

8 mV

8 mV
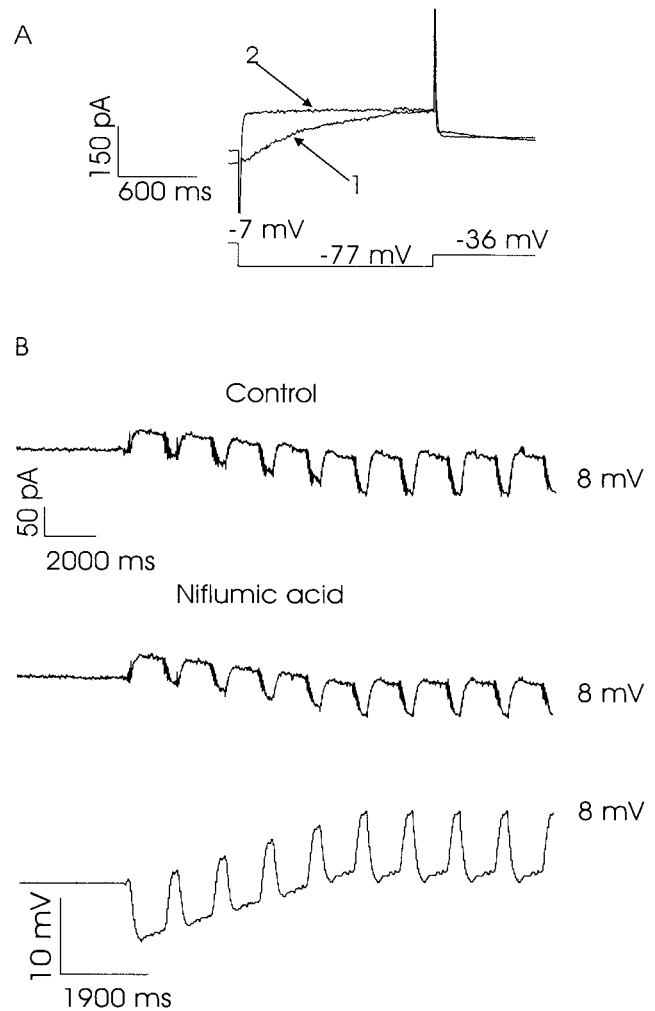
Figure 9. Effect of niflumic acid on the depolarization-induced increase in presynaptic currents. (A) Tail current in control Ringer's solution (1) and in 100 μM niflumic acid (2). Niflumic acid blocks the tail currents showing that niflumic acid did indeed block I_{ClCa}. (B) Synaptic current protocol (8-mV trace) in control Ringer's solution and in the presence of 100 μM niflumic acid. The increase in presynaptic currents due to depolarization is still present in niflumic acid.

bling or even a tripling of the amount of calcium flowing into the cone synaptic terminal due to the feedback signal from HCs to cones, while the feedback signal itself does not increase during the flash train. This increase in calcium influx is independent of dopamine and can be attributed to adaptation-induced depolarization of the cone membrane potential.

In the next sections, we will discuss this mechanism in detail and present results of a quantitative computer simulation showing that cone polarization alone is enough to generate the observed results. Finally, we will discuss the relevance of this mechanism in relation to the literature concerning adaptational changes in retinal processing.
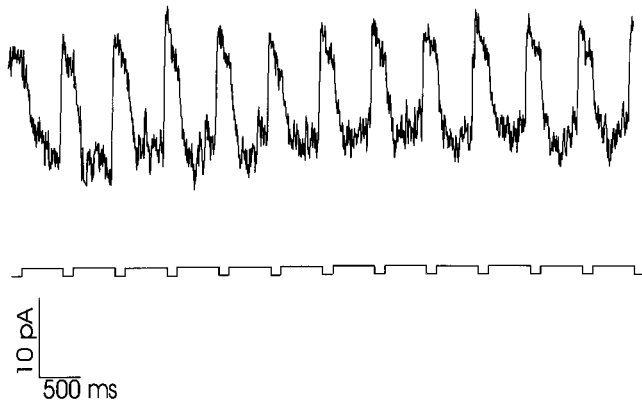
Figure 10. Changes in feedback signal from HC to cones during the flash train. Feedback-induced increases in I_{Ca} measured in cones using the same flash train that induces changes in the kinetics of HC responses. To measure feedback in a cone, the cone light response is saturated with a small spot (20-μm diameter). Feedback does not increase during the flash train.

Figure 11. Depolarization of the cone increases the effect of feedback on the increase of I_{Ca}. Cone were gradually depolarized during the first four flashes in 1-mV steps. In the beginning of the trace, the feedback-induced change in Ca current is small. Depolarization of the cone leads to an increase in the feedback-induced changes in I_{Ca}.

Changes Seen during the Adapting Flash Train

Two things happen with the HC responses during the adapting flash train used in this study that need to be accounted for: (a) the onset response becomes faster and (b) the secondary depolarization becomes larger.

The only significant change found in the cones during the flash train is that the mean cone membrane potential slightly depolarized during the flash train. Both the transient character of the first light response of the cone and the depolarization of the mean membrane potential are presumably due to the Ca-dependent feedback on the phototransduction pathway (Haynes and Yau, 1985a; Ripps and Pepperberg, 1987). The question now is, can the changes in HC kinetics during the flash train be accounted for by only a slight depolarization of the cone membrane potential?

Depolarization of the Cone Can Account for the Changes in HC Kinetics during the Train of Flashes

Fig. 12 A shows the current-voltage relation of the I_{Ca} of a cone. Since there is a linear relation between I_{Ca} of the cone and the amount of glutamate release, the I_{Ca} can be taken as the amount of glutamate release (Witkovsky et al., 1997). In the dark, cones rest at about -40 mV. At this potential, there is a certain amount of glutamate release (Fig. 12 A, 1). Hyperpolarization of the cone by light results in a reduction in I_{Ca}; i.e., in a decrease in glutamate release (Fig. 12 A, 2). Due to this decrease in glutamate release, HCs will hyperpolarize, leading to a change in feedback signal to the cones. By some yet unknown mechanism, the change in feedback shifts the calcium activation function to more negative potentials (dashed curve). This in turn causes an increase in I_{Ca} (Fig. 12 A, 3), which leads to an increased
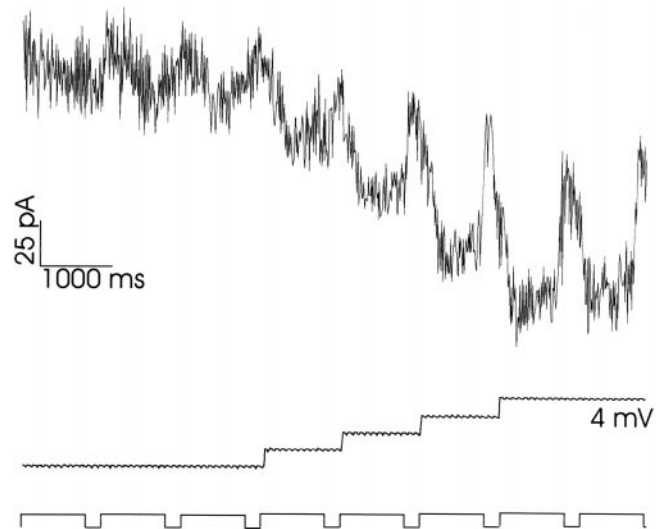
glutamate release, leading to the secondary depolarization of the HC responses.

The flash train gradually depolarizes the mean membrane potential of the cone by $\sim$4 mV, so the cone rests at -36 mV. Because the cone gradually becomes depolarized during the train of flashes, the range of its light response is shifted to a steeper part of the calcium activation function. Thus, the same hyperpolarizing cone light response will cause a larger decrease in calcium current, and a larger reduction in glutamate release, accounting for the increase in the feedforward HC response. The gradual depolarization of the cone during light adaptation can also explain the increase in the secondary depolarization in HCs. During the flash train (i.e., during light adaptation), the feedback signal from HCs to cones does not change. This means that in the light-adapted state the feedback from the HCs during each flash causes the same leftward shift of the calcium activation function as in the dark-adapted state. However, since the cone potential is in a steeper part of the calcium-activation function, this same leftward shift now causes a larger increase in presynaptic calcium current, a larger increase in glutamate release, and a larger secondary depolarization in HCs. So, now that we have discussed the increase in secondary depolarization of the HC light response due to the small depolarization of the cone, we can discuss how both the increase in feedforward signal and the increase in feedback efficiency can lead to a faster time to peak of the HC light response. The feedforward signal increases less than the increase in feedback efficiency (compare
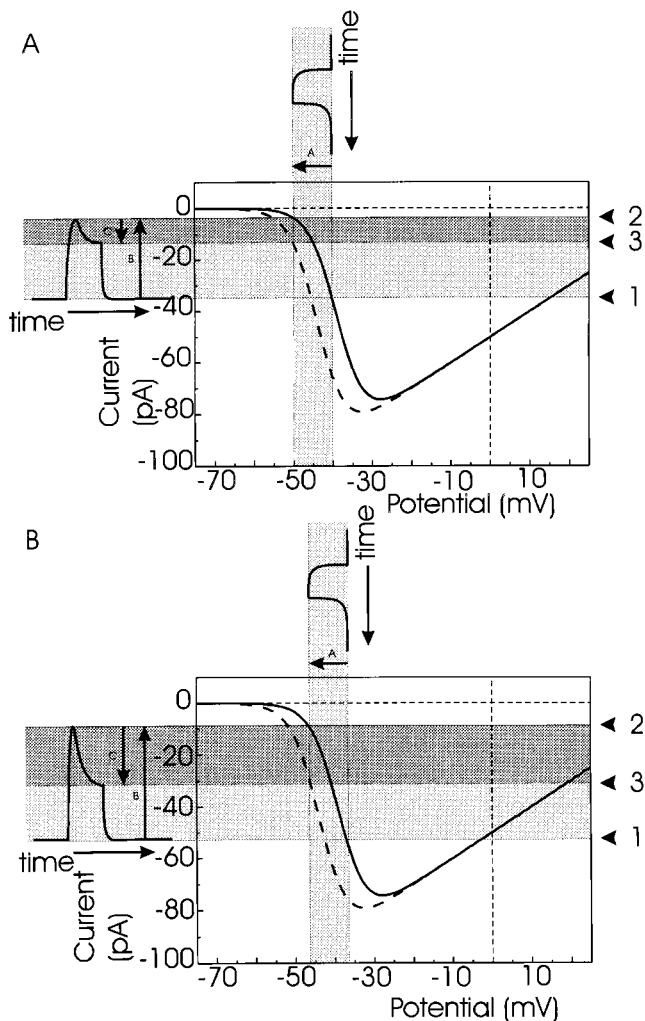
519   Fahrenfort et al.

Figure 12. Light-induced changes in presynaptic calcium current. (A) Situation at the beginning of the flash train. A schematic drawing of a cone light response and the resulting HC response are indicated at the top and on the left, respectively. (1) The I_{Ca} at the resting membrane potential of the cone. This determines the resting membrane potential of the HCs. Light stimulation hyperpolarizes the cone (arrow A), which leads to a decrease in I_{Ca} (2), and thus to hyperpolarization of the HC (arrow B). This hyperpolarization induces a shift of the activation function of the calcium current in the cones (dotted line), leading to an increase in I_{Ca} (3). The effect of this increase in I_{Ca} can be seen in the HC light response as the secondary depolarization (arrow C). (B) Situation at the end of the flash train. In this condition, cones are depolarized 4 mV due to photoreceptor light adaptation. Due to this depolarization of the cone, the size of the secondary depolarization in the HC response (arrow C) has increased relative to the hyperpolarizing part of the HC response (arrow B).

Figs. 5 and 11). The reason for this is that the 4-mV depolarization is a small depolarization relative to the cone light response, whereas it is a large depolarization relative to the feedback-induced shift in calcium activation function. Since feedback is slower than feedforward, and since the feedback efficiency increases more

than the feedforward signal, the result is that the time to peak of the HC responses decreases. Now the question arises why this decrease in time to peak of the HC response and the increase in rollback cannot be seen in the feedback signal received by the cones. Since feedback is slow, the peak of the HC light response might be cut off. Furthermore, the rollback is small, relative to the total response of the HCs, which actually generates the feedback signal. Together, they may explain why the changes in HC kinetics cannot be seen in the feedback signal received by the cones.

To test whether the effect of the depolarization on the I_{Ca} of the cone was enough to account for the observed changes in the kinetics due to this increase in synaptic efficiency, a quantitative model simulation was performed.

Simulation

The model consists of a cone and a HC. The cone projects via a Ca-dependent glutamatergic pathway to the HC and the HC feeds back to the cone via modulation of the Ca current by an unknown neurotransmitter (Verweij et al., 1996a). Cones are modelled using a simple resistive network consisting of a voltage-sensitive calcium conductance, the light conductance, and a passive potassium conductance. HCs are modelled with a glutamate conductance and a passive potassium conductance. A detailed description of the model is given in the appendix.

With the model, three simulations were performed. (a) The response of the model was studied using a similar flash train as used for the physiological experiments. To mimic the adaptation-induced cone depolarization as found experimentally, the cone membrane potential was depolarized by 1, 2, 3, and 4 mV during the second, third, fourth, and fifth and the rest of the flash train, respectively. The results are shown in Fig. 13 A. It is clear from this figure that the secondary depolarization increases with depolarization of the cone. This is a similar behavior as found in the physiological cone/HC system. Note that only the mean cone membrane potential is changed in the model during the adaptation by the flash train, showing that this small change is enough to induce the changes in kinetics of the HC response.

With the model behaving similarly as the physiological cone/HC network, the mechanism responsible for the changes in HC kinetics during the flash train can be evaluated. First the effect of the depolarization on the presynaptic calcium current was studied just as was done in Fig. 5. In this simulation, the HC membrane potential was held constant. This mimics the experimental condition where the cones were voltage clamped and polarized according to the prerecorded light responses. Due to the extensive electric coupling of HCs,
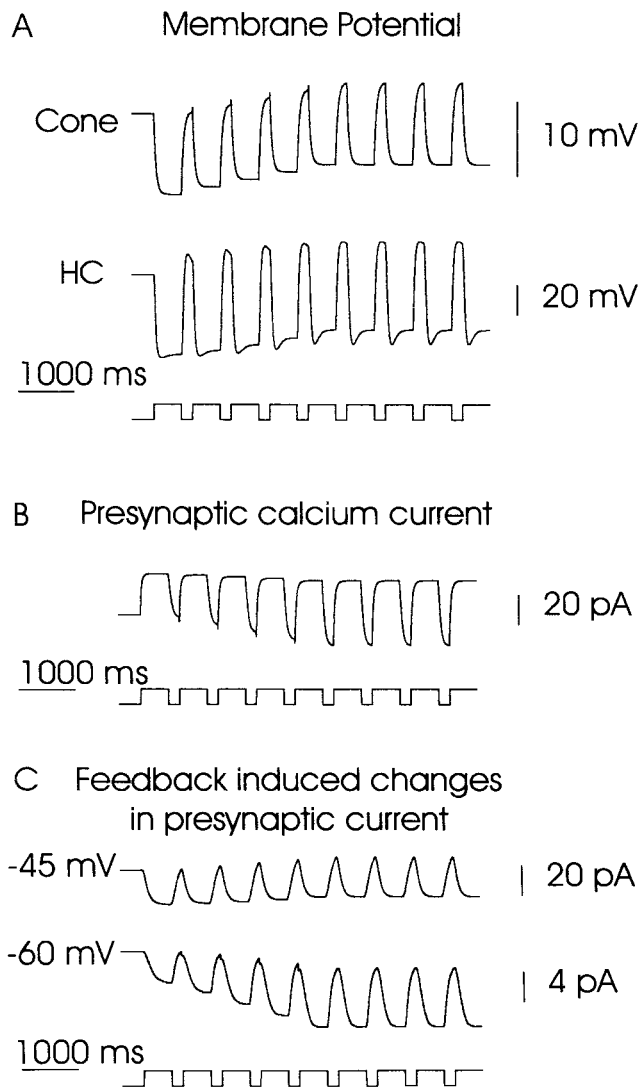
A   Membrane Potential

Cone | 10 mV

HC | 20 mV

1000 ms

B   Presynaptic calcium current

| 20 pA

1000 ms

C   Feedback induced changes
in presynaptic current

-45 mV | 20 pA

-60 mV | 4 pA

1000 ms

Figure 13. (A) Simulated cone and HC responses to the flash train. Cones rest at −46 mV and HCs at −32.2 mV in the dark. The horizontal cell response shows the characteristic increase of the secondary depolarization during this protocol. Cone adaptation is mimicked by a gradual depolarization of the cone by 4 mV during the first four flashes. (B) The changes in Ca current of the cone due to the flash stimulus protocol in the absence of feedback. (C) Increases in Ca current of the cone due to feedback from the HCs when the cone is clamped at −45 mV (top) and when the cone is initially clamped at −60 mV (bottom), and then gradually depolarized by 4 mV over the first five flashes. At −45 mV, the feedback-induced increase in the Ca current does not change, whereas gradual depolarization from −60 mV leads to a large increase in feedback-induced currents.

polarization of one cone does not lead to a change in HC membrane potential. Fig. 13 B shows that due to the polarization of the cones, the light-induced change of the presynaptic calcium current almost doubles. This result is similar to the result obtained with the physiological cone/HC system and is due to the fact that de-

polarization shifts the membrane potential of the cone to a steeper part of the Ca current.

Finally, the feedback signal in the model cones was studied (Fig. 13 C). This was done in two conditions: (a) when the model cone was clamped at −45 mV and (b) when the cone was initially clamped at −60 mV and subsequently depolarized during the first five flashes by a total of 4 mV. Fig. 13 C shows that when the cone is clamped at −45 mV, feedback-induced currents remain nearly equal in size throughout the protocol. This result is similar to the physiological result. However, when the cone is clamped at the light membrane potential (−60 mV) and is depolarized by a total of 4 mV, the feedback-induced currents are almost doubled at the end of the stimulus train, just as found physiologically.

These simulations show that a very simple model can account for the complex change in the HC response characteristics observed during adaptation to a flash train. Since this behavior can be generated with a model that only includes a Ca current and cone adaptation, it indicates that no other pathways or processes are involved. The relative position of the cone membrane potential to the Ca-current activation function is essential for the size and the kinetics of the HC response. Depolarization of the cone membrane potential due to adaptation of the cone leads to an increase in efficiency of the feedback pathway of the HCs to the cones.

Changes in HC/Cone Network Induced by Adaptation

Many papers have reported changes in the cone/HC network as a consequence of light adaptation. The mechanisms underlying these changes can be separated into two broad categories: "local" and "network" adaptive mechanisms. Local mechanisms are intrinsic to photoreceptors such as bleaching adaptation (Ripps and Pepperberg, 1987) and Ca-dependent feedback on the phototransduction cascade, which modulates the cGMP-gated channels in the cone outer segment (Haynes and Yau, 1985b). Both local mechanisms tend to depolarize the mean membrane potential of the cone (Burkhardt, 1994) when the retina becomes light adapted. The time constant of this process is between 0.1 and 100 s (Baylor and Hodgkin, 1974).

Network adaptation refers to mechanisms outside the photoreceptor, such as modulation of the electric coupling between HCs by dopamine (Mangel and Dowling, 1985; Tornqvist et al., 1988; Yang et al., 1988; Negishi et al., 1990), the change in sensitivity of the glutamate and $GABA_c$ receptors on the HCs (Knapp and Dowling, 1987; Dong and Werblin, 1994), and the change in the amount of GABA released by the HCs (Yazulla and Kleinschmidt, 1982). These dopaminergic processes function on a time scale of minutes to hours (Witkovsky and Shi, 1990). GABA also plays an important role in determining the network properties in the

outer retina (Yang and Wu, 1989; Kamermans and Werblin, 1992; Verweij et al., 1998) by modulating both the size and kinetics of the HC responses by means of a mechanism intrinsic to the HCs.

The present paper describes changes in HC kinetics without a change in the intrinsic HC properties, indicating that GABA does not play a role. The described mechanism is also present in dopamine-depleted animals, indicating that dopamine does not play a role in this short-term change of the HC kinetics. Other groups have also reported changes in HC kinetics due to background illumination or repetitive stimulation without the interference of GABA or dopamine. Akopian et al. (1991) reported changes in the responses of turtle HCs during repetitive stimulation with saturating white light flashes. These HCs show an increase of the Off overshoot, while the On response remained unaffected. Since it has been reported that the HC Off overshoot is mediated by feedback (Kamermans et al., 1989), the findings of Akopian et al. (1991) could be accounted for by the mechanism described in this paper. The absence of any effect on the On response in their experiments can be justified by the fact that they used saturating light stimuli. That condition might be similar to the HC responses to 0.0 log intensity light flashes shown in Fig. 2. For that intensity, we also did not find any effect on the On responses of HCs.

Normann and Perlman (1990) showed that background illumination reduces the sensitivity of the HC responses in turtle. High intensities of background light did not change the kinetics of the HC light response, whereas lower intensities lead to a reduction of the time to peak and an increase in secondary depolarization of the HC light response. Their lower intensities are comparable with the intensities in our experiments, eliciting the changes in HC response kinetics. Their results are similar to the results described in this paper despite their use of background light instead of a train of white light flashes. In their paper, no explanation was given why the time to peak of the HC response decreased when they used moderate light intensities. In light of our experiments, their results can be accounted for by the light adaptation–induced depolarization of the cone, which will make the synaptic efficiency between cones and HCs higher, leading to a reduction of the time to peak of the HC response.

The Possible Role of Cone Depolarization in Other Adaptive Processes

This study illustrates that the resting membrane potential of the cone can strongly modulate the size and kinetics of the light responses of neurons in the retina. Small changes in this potential can lead to drastic changes in synaptic transmission, and feedback efficiency in particular, influencing possibly all kinds of visual processing. As mentioned in the `introduction`, several papers have reported changes in the amount of feedback during light adaptation. Both the surround of ganglion cells and the spectral opponent responses of HCs disappear when a retina is dark adapted. Light adapting these retinae results in the reappearance of these feedback-mediated responses. Most of these changes during light–dark adaptation are assumed to be mediated by GABA or dopamine. Although the mechanism described in this paper is not GABA or dopamine mediated, it could very well play a role in the above-mentioned GABA- and dopamine-mediated processes. For instance, GABA is known to modulate the membrane potential of cones. GABA slightly hyperpolarize cones in goldfish, tiger salamander, and turtle by activating $GABA_A$ receptors (Kaneko and Tachibana, 1986; Kamermans and Werblin, 1992). By hyperpolarizing the resting membrane potential of the cone, GABA could decrease the feedback efficiency and thereby inhibit feedback-mediated responses. So, in conclusion, the mechanism to modulate the synaptic transmission between HCs and cones described in this paper can be driven by a variety of pathways. The effect will always be a strong modulation of feedback.

APPENDIX

This appendix presents a very simple quantitative model to account for the experimental findings presented in this paper with a minimum of components. The main question addressed is whether the characteristics of the Ca channel are sufficient to account for the observed changes in HC dynamics.

The model consists of a cone and a HC. The cone projects via a Ca-dependent glutamatergic pathway to the HC and the HC feeds back to the cone via modulation of the Ca current by an unknown neurotransmitter (Verweij et al., 1996a).

The Cone

The cone membrane potential (V_{cone}) depends on the light stimulus [$I(t)$]. The relation between the $I(t)$ and V_{cone} is given by Eq. 1:

$$V_{cone} = V_{rest} + I(t) \bullet V_{resp} - \tau_{cone} \frac{dV_{cone}}{dt}, \qquad (1)$$

where τ_{cone} is the time constant of the cone light response in milliseconds, V_{rest} is the cone resting membrane potential in millivolts, V_{resp} is the amplitude of the cone light response in millivolts, and $I(t)$ varies between 0 and 1. The timing of $I(t)$ is given in the figures.

V_{cone} modulates a voltage-dependent Ca channel (I_{Ca}). The relation between I_{Ca} and V_{cone} is taken from Verweij et al. (1996a) and given by Eq. 2:

$$I_{Ca} = g_{Ca}^{Max} \bullet (V_{cone} - E_{Ca}) \bullet 1/ \\ \{1 + \exp[-(V_{cone} - K_{Ca} - fb)/n_{Ca}]\}, \qquad (2)$$

where g_{Ca}^{Max} is the maximal conductance of the Ca current, E_{Ca} is the reversal potential of the Ca channel, K_{Ca} is the half-activation potential of the Ca-current activation function, fb is the shift of the Ca-current activation curve due to HC activity, and n_{Ca} is the slope factor of the Ca-activation curve.

The Horizontal Cells

Cones release glutamate in a Ca-dependent manner. The relation between the Ca current and the release of glutamate is linear (Schmitz and Witkovsky, 1997; ID: 3241). Glutamate activates an AMPA-type glutamate receptor on the HC dendrites. The Hill factor of the glutamate-induced currents in HCs is near 2 (O'Dell and Christensen, 1989; ID: 1472). The relation between I_{Ca} and the relative conductance of the AMPA receptor (g_{cat}) in the HC membrane is described by a Hill equation and is given in Eq. 3:

$$g_{cat} = g_{cat}^{Max} \bullet \frac{I_{Ca}^{n}}{I_{Ca}^{n} + K_{Glu}^{n}}, \qquad (3)$$

where g_{cat}^{Max} is the maximal conductance of the glutamate-gated channel, K_{Glu} is the Ca current needed to release the amount of glutamate that activates the AMPA-receptor half maximal, and n is the Hill-coefficient.

The relation between g_{cat} and the HC membrane potential (V_{HC}) is given by Eq. 4:

$$V_{HC} = \frac{g_{cat} \bullet E_{cat} + g_{K_{HC}} \bullet E_{K_{HC}}}{g_{cat} + g_{K_{HC}}} - \tau_{HC} \bullet \frac{dN_{HC}}{dt}, \qquad (4)$$

where τ_{HC} is the time constant of the HCs, E_{cat} is the equilibrium potential of the glutamate-gated channel, $g_{K_{HC}}$ is the relative passive potassium conductance in

the HC, and $E_{K_{HC}}$ is the equilibrium potential of the glutamate-gated current in the HC.

HCs feedback to cones by directly modulating the Ca-current activation range. HCs feedback to cones in an almost linear fashion (our unpublished observations). The time constant of the feedback signal is ~100 ms (Kraaij et al., 1998; ID: 2897). The relation between V_{HC} and the shift in the activation range (fb) is given by Eq. 5:

$$\tau_{fb} \bullet \frac{dfb}{dt} = A_{fb} + B_{fb} \bullet V_{HC} - fb, \qquad (5)$$

where τ_{fb} is the time constant of the feedback signal, and A_{fb} and B_{fb} are constants.

Parameters and Methods

The parameters used in the simulation are give in Table I. The differential equations were solved numerically on an MS windows–based computer system using the Runga Kutta method of order 4 (Press et al., 1992). dt was 0.1 ms in all simulations. Without stimulation, the cone membrane potential is -46 mV and the HC membrane potential is -37.8 mV.

TABLE I
Parameters Used in the Simulations

Cone		HC	
g_{Ca}^{Max}	0.8 nS	g_{cat}^{Max}	10
E_{Ca}	50 mV	E_{cat}	0 mV
K_{Ca}	-38.5 mV	n	2
n_{Ca}	-3.5 mV	τ_{HC}	20 ms
τ_{Cone}	40 ms	K_{Glu}	56 pA
		g_{KHC}	1
		E_{KHC}	-90 mV
		τ_{fb}	100 ms
		A_{fb}	0.5 mV
		B_{fb}	-0.01

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