The Nature of Surround-induced Depolarizing Responses in Goldfish Cones

D.A. Kraaij, H. Spekreijse, and M. Kamermans

From the Graduate School Neurosciences Amsterdam, The Netherlands Ophthalmic Research Institute, 1105 BA Amsterdam, The Netherlands

abstract Cones in the vertebrate retina project to horizontal and bipolar cells and the horizontal cells feedback negatively to cones. This organization forms the basis for the center/surround organization of the bipolar cells, a fundamental step in the visual signal processing. Although the surround responses of bipolar cells have been recorded on many occasions, surprisingly, the underlying surround-induced responses in cones are not easily detected. In this paper, the nature of the surround-induced responses in cones is studied. Horizontal cells feed back to cones by shifting the activation function of the calcium current in cones to more negative potentials. This shift increases the calcium influx, which increases the neurotransmitter release of the cone. In this paper, we will show that under certain conditions, in addition to this increase of neurotransmitter release, a calcium-dependent chloride current will be activated, which polarizes the cone membrane potential. The question is, whether the modulation of the calcium current or the polarization of the cone membrane potential is the major determinant for feedback-mediated responses in second-order neurons. Depolarizing light responses of biphasic horizontal cells are generated by feedback from monophasic horizontal cells to cones. It was found that niflumic acid blocks the feedback-induced depolarizing responses in cones, while the shift of the calcium current activation function and the depolarizing biphasic horizontal cell responses remain intact. This shows that horizontal cells can feed back to cones, without inducing major changes in the cone membrane potential. This makes the feedback synapse from horizontal cells to cones a unique synapse. Polarization of the presynaptic (horizontal) cell leads to calcium influx in the postsynaptic cell (cone), but due to the combined activity of the calcium current and the calcium-dependent chloride current, the membrane potential of the postsynaptic cell will be hardly modulated, whereas the output of the postsynaptic cell will be strongly modulated. Since no polarization of the postsynaptic cell is needed for these feedback-mediated responses, this mechanism of synaptic transmission can modulate the neurotransmitter release in single synaptic terminals without affecting the membrane potential of the entire cell.

key words: retina • negative feedback • calcium-dependent chloride current • lateral inhibition

INTRODUCTION

The photoreceptors (rods and cones) in the vertebrate retina respond with sustained hyperpolarizing responses to stimulation with small spots of light. They have small receptive fields and feed into a network of horizontal cells (HCs)[1] and bipolar cells (BCs). The HCs are strongly electrically coupled, have large receptive fields (Naka and Rushton, 1967; Norton et al., 1968; Baylor et al., 1971) and feed back negatively to the cones (O'Bryan, 1973; Verweij et al., 1996; Kraaij et al., 1998). This feedback pathway forms the basis of the spectral coding of HCs (Fuortes and Simon, 1974; Stell and Lightfoot, 1975; Kamermans et al., 1991) and of the surround responses of BCs (Kaneko, 1970; Kaneko and Tachibana, 1981; Toyoda and Kujiraoka, 1982). Due to the spatial extension of the HC-receptive fields, stimulation in the surround of a cone can induce depolarizing responses in cones (Baylor et al., 1971; O'Bryan, 1973; Lasansky, 1981). These surround-induced depolarizing responses are mainly carried by Cl^- (Lasansky, 1981; Thoreson and Burkhardt, 1991; Barnes and Deschenes, 1992).

On the other hand, it has recently been shown that HCs feedback to cones by modulating the calcium current (I_{Ca}) in cones in a γ-aminobutyric acid (GABA)–independent way (Verweij et al., 1996). Unfortunately, the neurotransmitter mediating this feedback signal from HCs to cones is presently unknown. Consistent with this finding is the observation that the surround responses in BCs do not seem to be GABA mediated (Hare and Owen, 1996). Hyperpolarization of the HCs shifts the activation function of I_{Ca} in cones to more negative potentials, which leads to an increased calcium influx. This finding seems in conflict with the well-established Cl dependence of the surround-induced responses in cones. Several authors (Maricq and Koren-

Address correspondence to M. Kamermans, Graduate School Neurosciences Amsterdam, The Netherlands Ophthalmic Research Institute, Meibergdreef 47, 1105 BA Amsterdam, The Netherlands. Fax: 31 20 566 6121; E-mail: m.kamermans@ioi.knaw.nl

[1]*Abbreviations used in this paper:* BC, bipolar cell; BHC, biphasic horizontal cell; E_{Cl}, chloride equilibrium potential; GABA, γ-aminobutyric acid; HC, horizontal cell; MHC, monophasic horizontal cell.

J. Gen. Physiol. © The Rockefeller University Press • 0022-1295/2000/01/3/13 $5.00
Volume 115 January 2000 3–15
Released online 13 December 1999
http://www.jgp.org

brot, 1988; Thoreson and Burkhardt, 1991; Barnes and Deschenes, 1992) have suggested that the surround-induced depolarizing responses in cones are mediated by a calcium-dependent chloride current ($I_{Cl(Ca)}$), which can be activated by a feedback-induced calcium influx. They suggested that such a pathway could become, under certain conditions, regenerative, leading to the observed feedback-induced polarization of the cone membrane potential.

Depending on the intracellular chloride concentration ($[Cl]_i$) in cones [i.e., different chloride equilibrium potentials (E_{Cl})], surround stimulation would generate either depolarizing or hyperpolarizing responses in cones. This suggests that with values of E_{Cl} more positive than the resting membrane potential, feedback from HCs would be negative, whereas with E_{Cl} more negative than the resting membrane potential, feedback from HCs would be positive. Estimates of E_{Cl} in cones, reported in literature, range from values more positive than −40 mV (Thoreson and Burkhardt, 1991) to values more negative than −50 mV (Kaneko and Tachibana, 1986). Since the cone dark resting membrane potential is approximately −45 mV, the estimates of E_{Cl}, reported in literature, suggest that feedback from HCs can be either positive or negative, which is not consistent with the well-established antagonistic nature of the bipolar cell surround.

The question now arising is whether the modulation of I_{Ca} alone is enough to induce a feedback response in second-order neurons or whether depolarization of the cone is essential for transmission of the feedback signal to second-order neurons. In this study, the role of Cl^- and $I_{Cl(Ca)}$ in the negative feedback pathway from HCs to cones is studied and the relative contribution of the cone depolarization and the modulation of I_{Ca} on the feedback-mediated responses in second-order neurons is estimated.

It will be shown that the surround-induced responses are initiated by a feedback-induced calcium influx in the cones, which can activate $I_{Cl(Ca)}$. Since the conductance of the Ca channels is relatively small compared with the total conductance of the cell and $I_{Cl(Ca)}$ is hardly activated in the dark, the membrane potential of the cone is nearly independent of feedback, whereas the cone neurotransmitter release is strongly modulated by feedback. The consequence is that, as will be shown, HCs can have depolarizing responses even in conditions where cones do not depolarize, which means that the feedback pathway from HCs to cones is electrically almost silent.

MATERIALS AND METHODS

Preparation

Goldfish, *Carassius Auratus*, (12–16-cm standard body length) 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 8 ± 1 min, decapitated, and an eye was enucleated. 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 under infrared light illumination (λ = 920 nm) using infrared viewers (Edmund Scientific).

Recording Procedure

Whole cell patch clamp recordings. The superfusion chamber was mounted on a microscope (Optiphot-X2; Nikon, Inc.). The preparation was illuminated with infrared light ($\lambda > 850$ nm, wratten filter 87c; Eastman-Kodak Co.) and viewed with a 40× water immersion objective (numeric aperture, NA = 0.55; Nikon, Inc.), Hoffman modulation contrast optics and a video camera (Philips). Electrodes were mounted on an MP-85 Huxley/Wall-type micro-manipulator (Sutter Instruments Co.) and connected to an integrating patch clamp (3900A; Dagan Corp.). For the data acquisition, control of the patch clamp and optical stimulator (CED 1401 AD/DA convertor; Cambridge Electronic Design Ltd.) and an MS-DOS based computer system was used.

Intracellular recordings. For the intracellular recordings from HCs, the retina was mounted on an inverted microscope (IMT-2; Olympus Corp.), illuminated with an infra red light emitting diode (LED SFH 484-II; Telefunken), and viewed through a 2× objective of the microscope and a video camera (Philips). The recordings were made with a S7000A microelectrode amplifier with a S7071A electrometer module (World Precision Instruments, Inc.).

Data acquisition, control of the microelectrode amplifier, and of the optical stimulator were done with a CED 1401 AD/DA convertor with a sample frequency of 1.0 kHz (Cambridge Electronic Design Ltd.) and an MS-DOS based computer system.

Optical Stimulator

Whole cell patch clamp recordings. A 450-W xenon-lamp supplied two beams of light. Both beams were projected through Uniblitz VS14 shutters (Vincent Associates), neutral density filters (NG Schott), interference filters with a bandwidth of 8 ± 3 nm (Ealing Electro-Optics, Inc.), lenses, and apertures. The 65-μm diameter spots were projected through the 40× water immersion objective and the 3,000-μm diameter spots were projected through the condenser (N.A. = 1.25) of the microscope.

Intracellular recordings. The optical stimulator consisted of two beams from a 450-W xenon light source, projected through a monochromator (Ebert), or interference filters with a bandwidth of 8 ± 3 nm (Ealing Electro-Optics, Inc.) and a pair of circular neutral density filters (Barr & Strout). The full-field light stimuli were projected onto the retina through a 2× objective lens (N.A. = 0.08) of an inverted microscope (IMT-2; Olympus Corp.). The photon flux density of 4.0×10^8 photons $\mu m^{-2}\ s^{-1}$ corresponds to an intensity of 0 log.

Patch Electrodes and Pipette Medium

Whole cell patch clamp recordings. The patch pipettes were pulled from borosilicate glass (GC150TF-10; Clark) with a micropipette puller (P-87; Sutter Instruments Co.) and had impedances between 5 and 10 MΩ when filled with standard patch pipette medium and measured in Ringer's solution. The series resistance during the whole cell recording was between 10 and 20 MΩ.

The standard patch pipette medium contained (mM): 20.0 KCL, 70.0 d-gluconic-K, 5.0 KF, 1.0 $MgCl_2$, 0.1 $CaCl_2$, 1.0 EGTA, 5.0 HEPES, 4.0 ATP-Na_2, 1.0 GTP-Na_3, 0.2 3′:5′-cGMP-Na, 20 phosphocreatine-Na_2, 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. All chemicals were obtained from Sigma-Aldrich.

Intracellular recordings. Microelectrodes were pulled on a micropipette puller (P-80-PC; Sutter Instruments Co.) using aluminosilicate glass (o.d. = 1.0 mm, i.d. = 0.5 mm; Clark), and had impedances ranging 100–200 MΩ when filled with 4 M KAc. The intracellular voltages were amplified (WPI S7000A; World Precision Instruments, Inc.), recorded on paper (Graphtec Linearcorder), and sampled using an MS-DOS–based computer system and an AD/DA converter (CED 1401; Cambridge Electronic Design).

Liquid junction potential. The liquid junction potential was measured with a patch electrode, filled with pipette medium, and positioned in a pipette medium containing bath. The reference electrode was a patch electrode 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 and all data were corrected accordingly. All results presented in this paper are obtained ~15 min after whole cell configuration was achieved, unless otherwise indicated.

Classification

Cones were selected visually and the voltage light responses to 500-ms flashes of 65 μm spots with seven different wavelengths and various intensities were recorded. Cells with a maximal response of ~450, 550, and 650 nm were classified as short, middle, and long wavelength–sensitive cones, respectively. Upon classification, only white stimuli of either 65 or 3000 μm were used.

Horizontal cells were searched by penetrating the retina with a microelectrode, while stimulating the retina with alternating 550- and 650-nm full-field stimuli. Subsequently, the HCs were classified based on their spectral properties formulated by Norton et al. (1968).

Ringer's Solution

The Ringer's solution contained (mM): 102.0 NaCl, 2.6 KCl, 1.0 $MgCl_2$, 1.0 $CaCl_2$, 28.0 $NaHCO_3$, 5.0 glucose, and in some cases 0.1 niflumic acid, and was continuously gassed with ~2.5% CO_2 and 97.5% O_2, yielding a pH of 7.8. All chemicals were obtained from Sigma-Aldrich.

RESULTS

Cl Dependence of the Surround-induced Response in Cones

Many of the surround-induced responses in cones reported in literature were measured with intracellular electrodes. Those responses were depolarizing and seemed to depend on the intracellular Cl concentration. Since in those experiments the intracellular Cl concentration could only be controlled to a limited extent, the first set of experiments in this study deals with the Cl dependence of the surround-induced responses in cones.

Feedback-induced voltage responses can be measured when cones are saturated with a bright 65-μm spot and stimulated with a full-field stimulus (3,000 μm). Since feedback is most efficient at potentials between −55 and −45 mV (Verweij et al., 1996) and the bright saturating spot hyperpolarized the cones further than that potential range, a small inward current was injected into the cones such that they depolarized again to their resting membrane potential between −55 and −45 mV. By recording from a large number of cones with different intracellular Cl concentration, the dependence of the surround-induced responses on Cl was determined without interference of the strongly nonlinear behavior and voltage dependence of the feedback-induced currents.

Fig. 1 (top) shows the surround-induced response of a cone with a $[Cl]_i$ of 50 mM. In this condition, surround stimulation induces a large depolarizing response. Fig. 1 (bottom) shows the response of a cone with $[Cl]_i$ of 7 mM. In that condition, the surround-

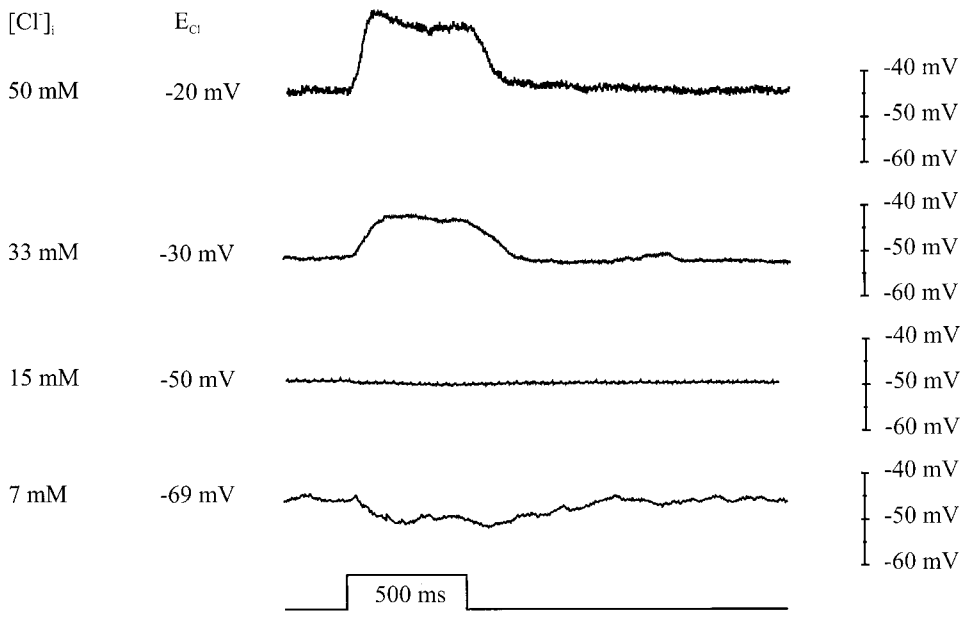

Figure 1. Voltage light responses of cones, with various $[Cl]_i$, to 550-nm, 500-ms flashes of 3,000-μm surround fields. Hyperpolarizing responses are found with E_{Cl} above −50 mV and depolarizing responses are found with E_{Cl} below −50 mV. The scaling, timing, $[Cl]_i$, and calculated E_{Cl} are indicated in the figure.

induced response is a small hyperpolarizing response. If this response was generated by a residual light sensitivity of the cone, the response should become larger with hyperpolarization, but at −60 mV surround-induced responses were absent (not shown), indicating that these hyperpolarizing responses were due to feedback. The two traces in between show the surround-induced responses of a cone with intermediate $[Cl]_i$. This figure illustrates that surround stimulation of cones with high $[Cl]_i$ evokes depolarizing responses, whereas hyperpolarizing responses are generated in cones with low $[Cl]_i$. With $[Cl]_i$ of 15 mM, surround stimulation induces no polarization. This $[Cl]_i$ yields a calculated E_{Cl} of −50 mV.

Fig. 2 shows the mean amplitude of the surround responses of 34 cones as function of E_{Cl}. As can be seen, the response amplitude becomes zero with E_{Cl} around −50 mV, which is slightly more negative than the mean resting membrane potential, which was −46.3 ± 8.3 mV (mean ± SD), under these experimental conditions. At E_{Cl} values more negative than −50 mV the surround responses are hyperpolarizing, and at E_{Cl} values more positive than −50 mV the surround responses are depolarizing. This shows that the amplitude and polarity of the surround-induced responses varies with E_{Cl} in a manner suggesting that these responses are mediated mainly by chloride ions.

On some particular occasions, surround-induced responses can become regenerative. Fig. 3 shows the surround-induced responses of a cone with E_{Cl} at −20 mV. Sometimes the cone membrane potential did not hyperpolarize after the surround stimulus was turned off (arrow). In this continued depolarized condition, surround stimulation did not induce a significant further depolarization (star). Similar results are obtained in 14 cones as long as E_{Cl} is more positive than −30 mV, indicating that in those conditions surround simulation can trigger a regenerative process, which depolarizes the cones to the reversal potential of the current underlying the surround-induced response; i.e., the Cl current. These spontaneous depolarizing responses were never found immediately after achieving whole cell configuration, indicating that under physiological conditions E_{Cl} is more negative than −30 mV. It has been suggested that a possible source of this regenerative behavior of the surround-induced responses is the combined activity of I_{Ca} and $I_{Cl(Ca)}$ (Thoreson and Burkhardt, 1991; Barnes and Deschenes, 1992). To test this, the currents induced by surround stimulation were studied.

Feedback-induced Current in Cones

Surround stimulation induces an initial inward current, usually followed by a slowly developing current. The I-V relation of a cone without and with the full-field stimulus is shown in Fig. 4 A. Surround stimulation only induces significant changes in the I-V relation in the range between −20 and −60 mV. Previously it was shown that this change in I-V relation is due to a shift in the activation function of the calcium current to more negative potentials (Verweij et al., 1996). In our experimental conditions, the surround-induced I_{Ca} was ∼10 pA at −46 mV, while the light-modulated current was ∼100 pA, showing that, at that potential, the feedback-induced changes in I_{Ca} are relatively small. The initial surround-induced current triggers a slowly developing current. The

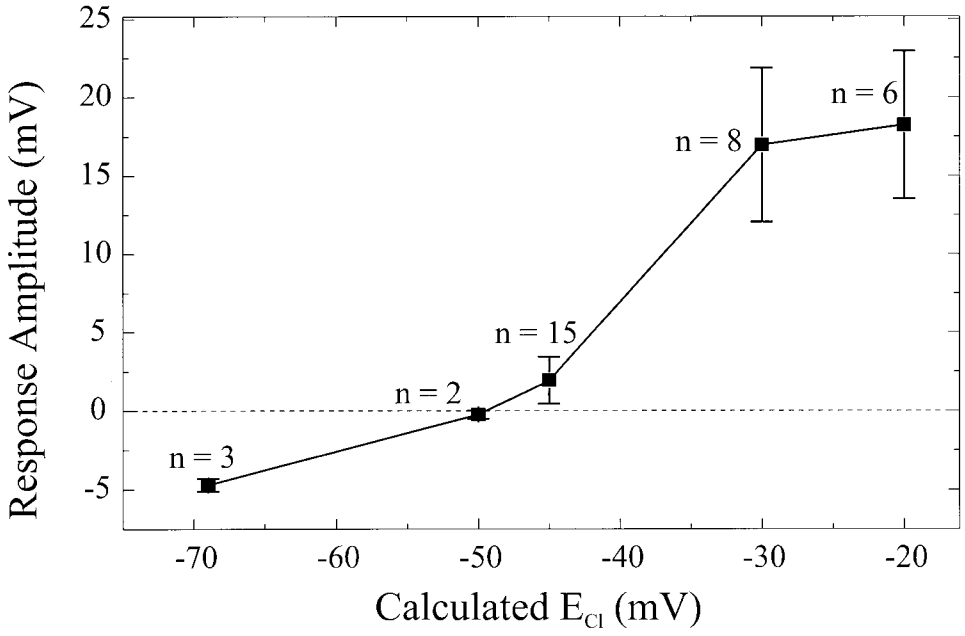

Figure 2. Mean sustained response amplitudes of surround-induced light responses in cones as a function of the calculated E_{Cl}. The error bars indicate the SEM. The number of cells used for each data point is indicated near each data point.

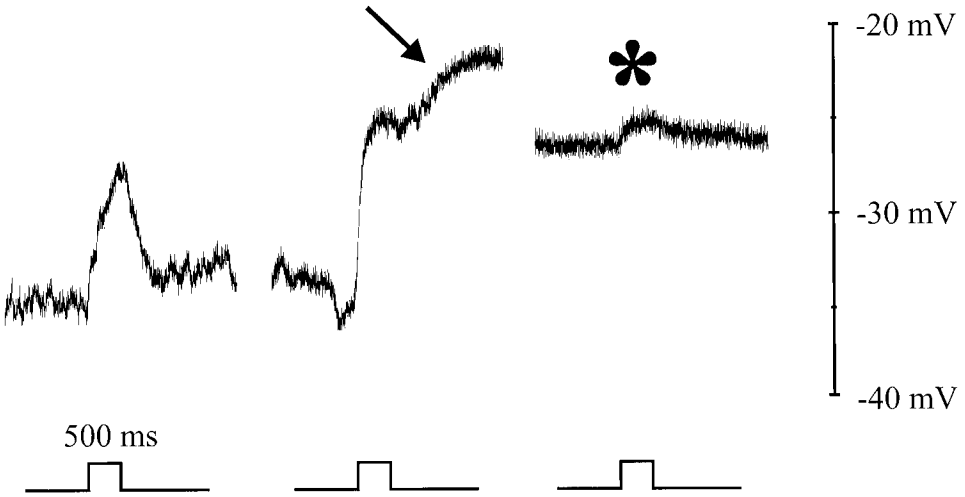

Figure 3. Surround-induced depolarizing responses in a cone with $[Cl]_i$ = 50 mM. Occasionally the cone depolarized spontaneously or remained depolarized for a long time (arrow). In this depolarized condition, only small surround-induced responses could be measured (*). The scaling and surround stimulus are indicated in the figure.

sign of this slowly developing current depends strongly on E_{Cl}. This current is outward when E_{Cl} is set at −40 mV (Fig. 4 B) and inward when E_{Cl} is set at −20 mV (Fig. 4 C). 100 μM niflumic acid, a blocker of the $I_{Cl(Ca)}$, blocks the slowly developing current (Fig. 4 D), while the initial inward current remained.

To exclude that niflumic acid might have affected the retinal network by affecting calcium and/or potassium channels in a nonspecific way, we tested whether the synaptic transmission between cones and HCs was still intact. Fig. 5 shows the responses of a monophasic horizontal cell (MHC) in the isolated goldfish retina to full field white light stimuli of various intensities before (left), during (middle), and after (right) application of 100 μM niflumic acid. Fig. 5 shows that, although the surround-induced slowly developing current in cones is

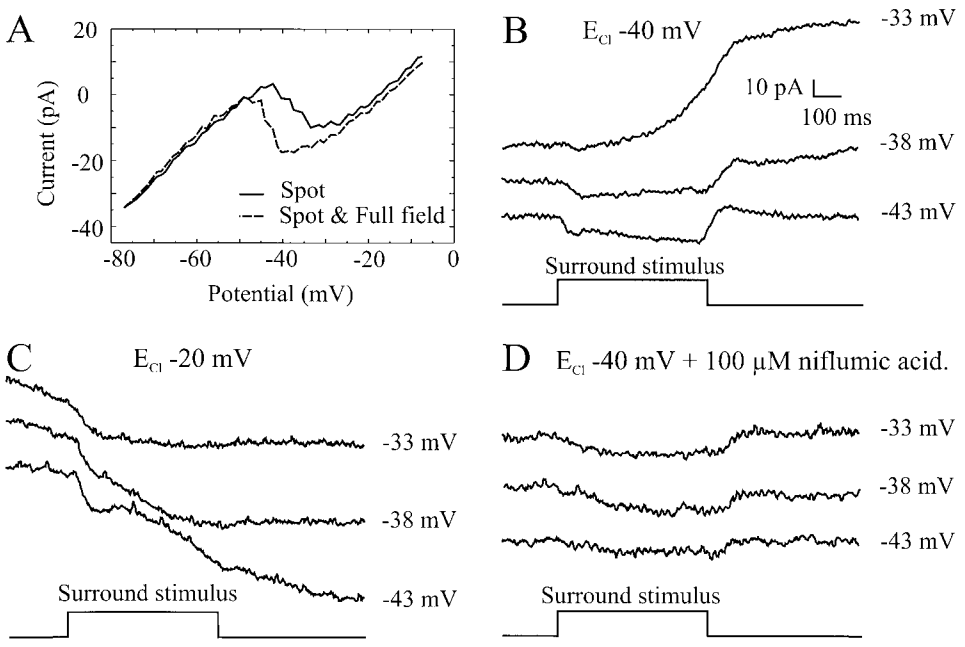

Figure 4. (A) Cone I-V relations with and without surround stimulation. The cone was held at −77 mV and ramped in 500 ms to −7 mV. The I-V relation depicted with the solid line was determined when the cone was illuminated with a 20 μm saturating white spot and the I-V curve depicted with the dotted line was determined when the cone was illuminated with a 20-μm saturating white spot plus a 3,000 μm white spot stimulating the cone surround. (B) Current traces of a voltage clamped cone, clamped at −58 mV and stepped for 2,000 ms to −43, −38, and −33 mV. During this protocol, the cone was continuously saturated with an intense white spot, while a 3,000-μm full field of 550 nm was illuminating the cone surrounding for 500 ms. In this cone, the $[Cl]_i$ was 22 mM, which results in a calculated E_{Cl} of −40 mV. Surround stimulation induced an inward current, followed by a secondary slowly developing outward current above −38 mV. (C) Current traces of a voltage-clamped cone, clamped at −58 mV and stepped for 2,000 ms to −43, −38, and −33 mV. During this protocol, the cone was continuously saturated with an intense white spot, while a 3000-μm full field of 550 nm was illuminating the cone surrounding for 500 ms. In this cone, the $[Cl]_i$ was 50 mM, which results in a calculated E_{Cl} of −20 mV. Surround stimulation induced an inward current, followed by a secondary slowly developing inward current above −40 mV. (D) Current traces of a voltage-clamped cone, clamped at −58 mV and stepped for 2, 000 ms to −43, −38, and −33 mV. During this protocol, the cone was continuously saturated with an intense white spot, while a 3000-μm full field of 550 nm was illuminating the cone surrounding for 500 ms. In this cone, the $[Cl]_i$ was 22 mM, which results in a calculated E_{Cl} of −40 mV, and 100 μM niflumic acid was added to the Ringers solution. Surround stimulation induced only an initial inward current, without a slowly developing secondary current. The scaling and timing are shown in the figure.

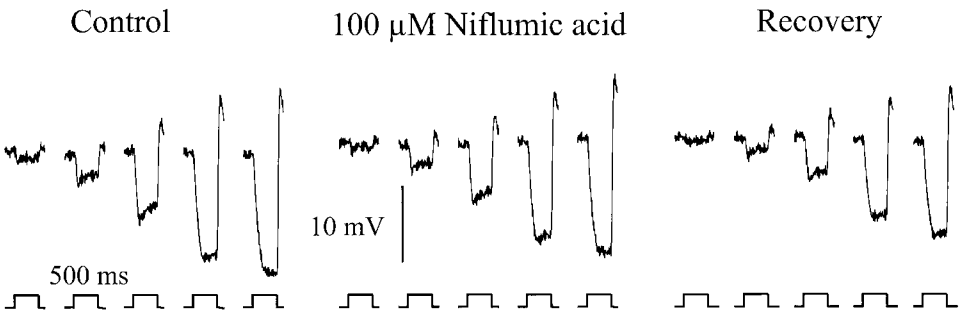

Figure 5. Monophasic horizontal cell responses to 500 ms lasting full-field white light stimuli of five intensities. (Left) Responses in control Ringers solution before application of niflumic acid. (Middle) Responses are recorded in a 100-μM niflumic acid containing Ringers solution. (Right) Responses again in control Ringers solution after application of niflumic acid.

blocked by niflumic acid, the MHC resting membrane potential and the MHC light responses are hardly affected ($n = 4$).

Summarizing: surround stimulation generates a fast inward current (I_{Ca}) and, secondary to this, a slowly developing current whose presence depends on the activation of the calcium current. Furthermore, this secondary current can be blocked by niflumic acid and its sign depends on $[Cl]_i$, features that are characteristic for $I_{Cl(Ca)}$. Thus the results presented so far suggest that the surround-induced voltage responses of cones are carried by $I_{Cl(Ca)}$. If this is indeed the case, then it should be possible to block the surround-induced voltage responses by niflumic acid. Fig. 6 shows the surround-induced voltage responses of a cone with E_{Cl} at −30 mV before (left) during (middle), and after (right) application of 100 μM niflumic acid. Niflumic acid had no large effect on the resting membrane potential of the cones, indicating that $I_{Cl(Ca)}$ is only slightly activated at physiological membrane potentials. Before application of niflumic acid the cone depolarized ~15 mV in response to surround stimulation. This response could be blocked completely by niflumic acid and recovered slightly. Similar results were obtained in all nine cells tested this way.

The results presented so far show that niflumic acid can effectively block the surround-induced depolarizations in cones, without affecting the feedback-induced modulation of I_{Ca}. The question now arising is whether the cone depolarization or the modulation of I_{Ca} is most important for feedback-induced response in second-order neurons, such as the biphasic horizontal cells (BHCs). The depolarizing responses of the BHCs, due to red light stimulation, are thought to be generated by feedback from the MHCs to the middle wavelength–sensitive cones (Fuortes and Simon, 1974; Stell et al., 1975; Stell, 1976; Kamermans et al., 1991). Fig. 7 A shows the responses of a BHC to flashes of 500 ms with wavelength ranging from 500 to 700 nm in 50-nm steps. The neutral point, the wavelength where the hyperpolarizing response changes into a depolarizing response, is close to 650 nm. If niflumic acid blocks feedback, then the depolarizing response to 700 nm should be blocked and the neutral point should shift to longer wavelength. Fig. 7 B shows the responses to 650 and 700 nm before and during niflumic acid application.

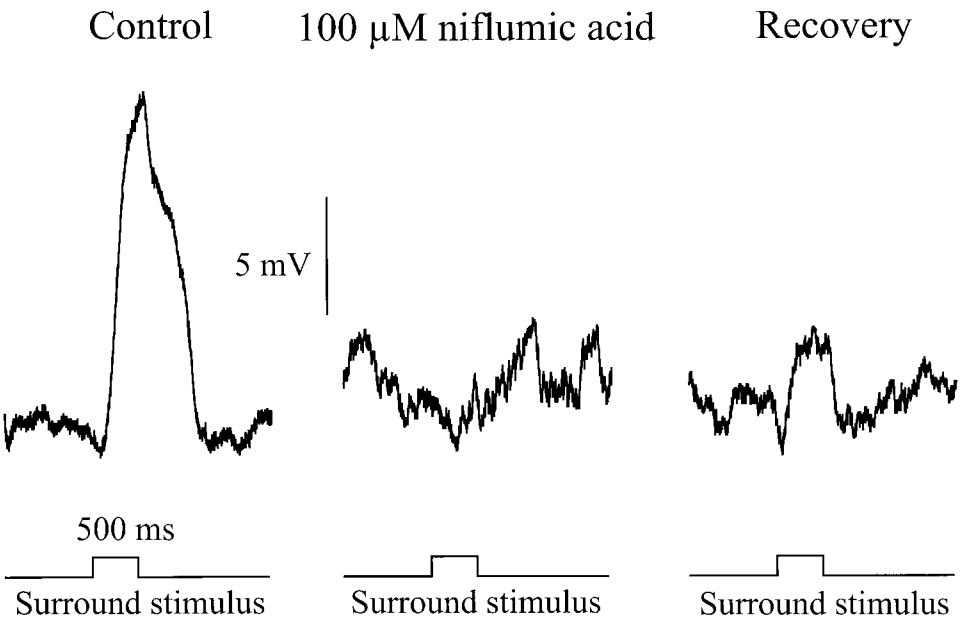

Figure 6. Surround-induced light responses of current-clamped cones to 500-ms flashes of a 3,000-μm surround field ($E_{Cl} = -20$ mV). In control conditions (left), surround stimulation induced depolarizing responses, in 100 μM niflumic acid these responses were absent (middle), and after wash the depolarizing responses recovered slightly (right).

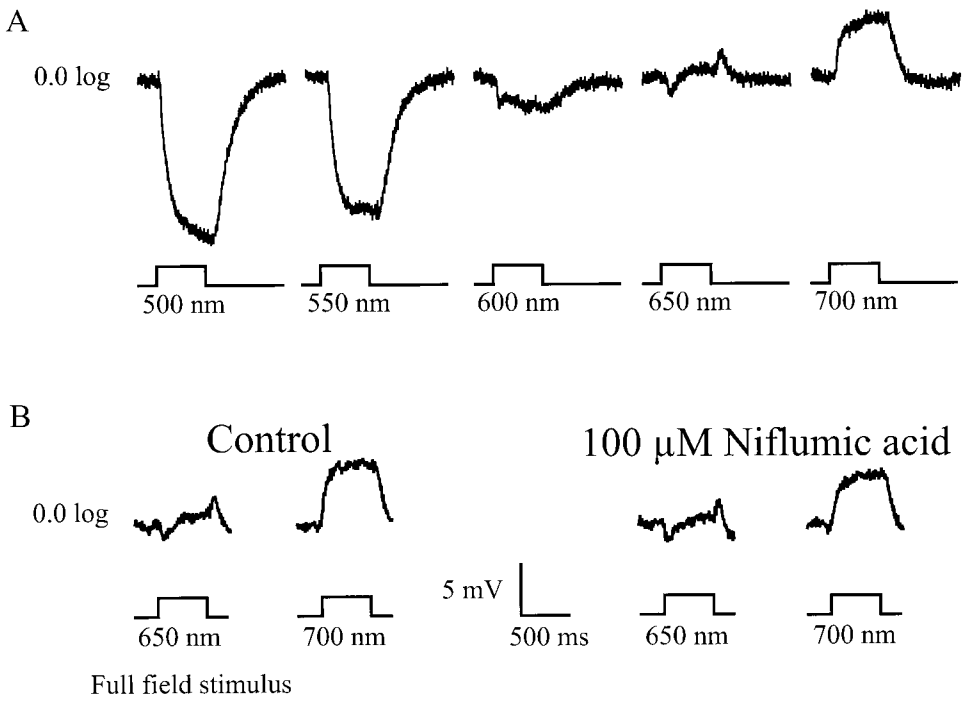

Figure 7. (A) Light responses of a biphasic horizontal cell, in normal Ringers solution, to 500-ms lasting full-field light stimuli with wavelengths ranging from 500 to 700 nm in 50-nm steps. (B) Biphasic horizontal cell responses to 500-ms lasting full-field light stimuli of 600, 650, and 700 nm. (Left) Depolarizing light responses in control Ringers solution. (Right) Depolarizing light responses recorded in a 100-μM niflumic acid containing Ringers solution.

As is clear from this figure, niflumic acid does not block the depolarizing light responses and did not shift the neutral point of the BHC. Although minor changes in the response amplitude were seen occasionally, the feedback-induced responses in BHCs were never blocked in all cells tested (n = 5), whereas the feedback-induced depolarizations in cones were always blocked completely. This experiment shows that depolarization of the cones is not essential for the transmission of a feedback response to second-order neurons.

Estimation of the Cl Equilibrium Potential under Physiological Conditions

So far we have shown: (a) that the size and sign of the surround-induced responses in cones depend on $[Cl]_i$, (b) that these responses are carried by $I_{Cl(Ca)}$, and (c) that the feedback-induced depolarizing responses in cones are not essential for depolarizing responses in BHCs. To quantify the role of the $I_{Cl(Ca)}$ in this synapse further, it is essential to know the value of E_{Cl} under physiological conditions. With E_{Cl} more positive than the resting membrane potential, cones will depolarize due to surround stimulation. This depolarization might amplify the effect of the feedback signal received by the cones on the cone output. On the other hand with E_{Cl} more negative than the resting membrane potential, cones will hyperpolarize due to surround stimulation, which might lead to a reduction of the effect of the feedback signal received by the cones on the second-order neurons.

In voltage-clamp experiments, the properties of $I_{Cl(Ca)}$ can be examined rather easily since $I_{Cl(Ca)}$ has three distinct properties: (a) it is a slowly developing current that depends on E_{Cl}, (b) it can generate slow tail currents, and (c) it can be blocked by niflumic acid (Barnes and Deschenes, 1992). In Fig. 8, the current traces of two voltage-clamped cones are shown. These cones were clamped at −77 mV and stepped for 2,000 ms to −25, −15, and −5 mV. In Fig. 8, left, E_{Cl} was −50 mV. Stepping the cone membrane potential to the depolarized potentials yields slowly developing outward currents, preceded by an inward current. Small tail currents are visible when the membrane potential is hyperpolarized back to −77 mV. Similar results were found in 20 cones. With E_{Cl} at −20 mV (middle), the properties of the slowly developing current change strongly. Now stepping from −77 to −25 mV yields a slowly developing inward current and large tail current after returning to −77 mV. This was found in all 15 cones tested in this way. This slowly developing current could be blocked by 100 μM niflumic acid (right). Note that in this condition both the slowly developing current and the tail currents are absent except for a small transient tail current in the first 50 ms. All four cells tested this way behaved similarly. These experiments show that goldfish cones have a slowly developing current that depends on E_{Cl} and can generate tail currents. This current can be used for the estimation of the physiological value of E_{Cl}.

The rationale for the next set of experiments is that, immediately after achieving whole cell configuration,

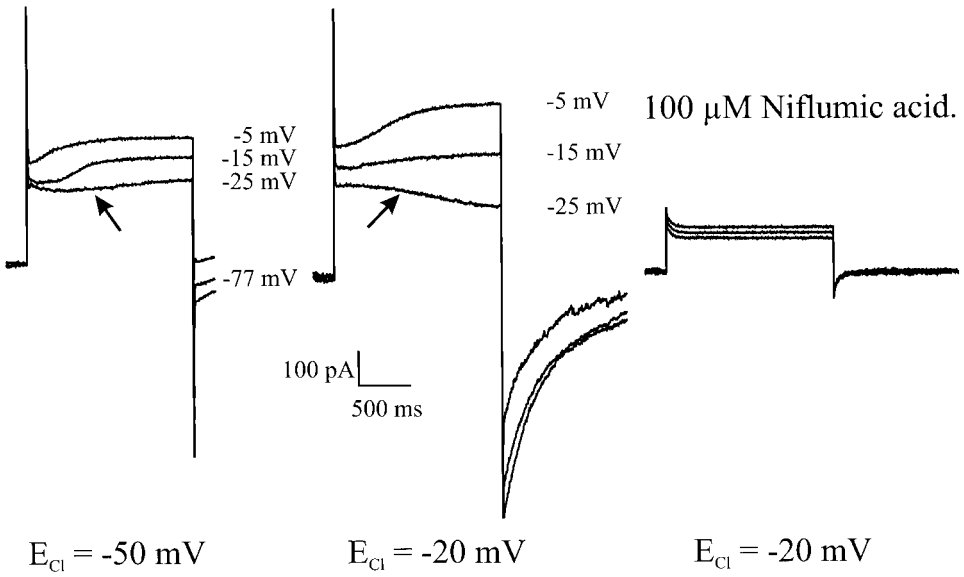

Figure 8. Current traces of two voltage-clamped cones, clamped at −77 mV and stepped for 2,000 ms to −25, −15, and −5 mV. (Left) E_{Cl} was −50 mV. Above −25 mV, an outward current is present that generates a tail current. (Middle) E_{Cl} was −20 mV. In this condition, at −25 mV, a slowly activating inward current developed. At −15 mV, this current reverses and becomes outward and generates large tail currents. (Right) Current traces of the same cone are presented, but now with 100 μM niflumic acid added to the Ringer's solution. Both the slowly developing current and tail currents are completely blocked with niflumic acid.

the pipette solution will not yet have diffused into the cell and at that moment E_{Cl} will still have a value close to the physiological value, whereas after 20 min the cell interior has been perfused with the pipette solution and thus E_{Cl} in the cones will have the value of E_{Cl} in the pipette. As shown in Fig. 8, the sign of $I_{Cl(Ca)}$ depends strongly on the value of E_{Cl}, and thus could be used to estimate the physiological E_{Cl}. When $I_{Cl(Ca)}$ is outward, E_{Cl} is more negative than the clamp potential, and when $I_{Cl(Ca)}$ is inward, E_{Cl} is more positive than the clamp potential. Due to the nature of $I_{Cl(Ca)}$, this method is limited to conditions where the intracellular calcium concentration ($[Ca]_i$) is sufficiently high to activate $I_{Cl(Ca)}$. This happens at potentials above −40 mV, which means that we can only determine E_{Cl} exactly when it is more positive than −40 mV.

Fig. 9 shows the results of two experiments in which the effect of $[Cl]_i$ on the sign of $I_{Cl(Ca)}$ was studied as a function of time. Since the size of $I_{Cl(Ca)}$ varies between the various cones, the cells may not be compared with each other, but changes in $I_{Cl(Ca)}$ with time in one individual cell can be used to estimate E_{Cl}. Fig. 9 (top) shows the current traces of a cone clamped at various potentials with E_{Cl} at −50 mV at 2 and 22 min after achieving whole-cell configuration. In this condition, at both moments in time, a slowly developing outward current can be seen at potentials of −40 mV and above. In both conditions, small tail currents are present. The overall response to the voltage steps does not seem to change dramatically over time. This behavior was observed in all 29 cells tested this way. With E_{Cl} at −20 mV, on the other hand, the responses to the same voltage steps strongly change with time (Fig. 9, bottom). At 2 min after achieving whole-cell configuration, only a very small slowly developing outward cur-

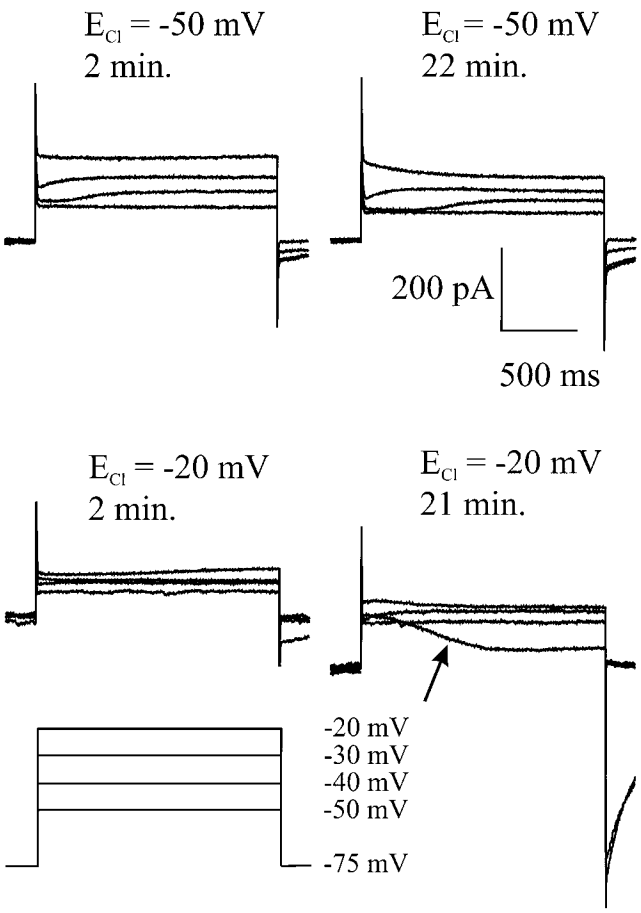

Figure 9. Time dependence of $I_{Cl(Ca)}$ in cones with two different $[Cl]_i$. The cones were held at −75 mV and stepped to −50, −40, −30, and −20 mV. For both values of E_{Cl}, the current responses are given at 2 min and >20 min after whole-cell configuration was achieved. With E_{Cl} at −20 mV, the slowly developing current became inward after an ∼21-min whole-cell configuration (arrow), while it remained outward when E_{Cl} was −50 mV.

rent can be observed and the tail currents are very small. However, at 21 min, a voltage step to −40 mV induces a large slowly developing inward current and returning back to −75 mV generates a very large tail current. This indicates that E_{Cl} has changed strongly during the 20-min whole cell configuration. In all 55 cones tested directly after achieving whole cell configuration, voltage steps to −40 mV never induced a slowly developing inward current and mostly induced a slowly developing outward current. These results indicate that the physiological E_{Cl} is about or more negative than −40 mV.

Since this method to estimate E_{Cl} has a limited resolution and works only in the range where $I_{Cl(Ca)}$ is activated, a second method for the determination of E_{Cl} is needed. Therefore, a second estimate of E_{Cl} was made using the tail currents. The experiments of Fig. 8 show that the tail currents can be almost completely blocked by 100 μM niflumic acid, indicating that these currents are carried by $I_{Cl(Ca)}$. Fig. 10 shows the current traces of a cone filled with a 33 mM Cl-containing pipette solution 8 min after the whole-cell configuration was achieved. The cone was clamped at −77 mV, stepped to −20 mV for 500 ms and successively clamped for 400 ms to various potentials more negative than −50 mV. The step to −20 mV activates I_{Ca} and $I_{Cl(Ca)}$, and the subsequent step to negative potentials induces the $I_{Cl(Ca)}$-dependent tail current. For the estimation of the reversal potential of the tail current, no potentials above −50 mV were used because at these potentials the activation of the calcium current interferes with the measurements. To correct for a possible remaining leak current that might be present in the potential range between −100 and −50 mV, the difference between the tail current measured 50 and 300 ms after the step from −20 mV to the negative potentials (gray bars), was used to determine the reversal potential of the tail current. In this way, an estimate of the size of the tail current was obtained for various potentials. In the insert, this estimate is plotted as a function of the potential. The dotted line is a linear curve fitted through the data points using the linear regression algorithm. The intersection with the x axis gives the reversal potential of the tail currents. Since the tail currents are mainly carried by Cl, this value is an estimate of E_{Cl}.

Fig. 10 B shows the estimates of E_{Cl} for 11 cells with the [Cl] in the pipette being 33 mM (●) and 2 cells with [Cl] in the pipette being 7 mM (○) as function of time. At 2 min, the estimate yields values of −55 ± 7 mV (n = 5). This value is independent of the [Cl] in the pipette. For low [Cl] (○), E_{Cl} does not change very much, whereas for high [Cl] (●), E_{Cl} becomes −33 ± 4 mV (n = 5) after 30 min and approaches its calculated E_{Cl} value of −30 mV. This experiment shows that the physiological value of E_{Cl} is approximately −55 mV and that E_{Cl} shifts in time with the [Cl] in the pipette. With E_{Cl} around −55 mV, surround stimulation will not induce a substantial polarization of the cone membrane potential, as indicated in Fig. 1.

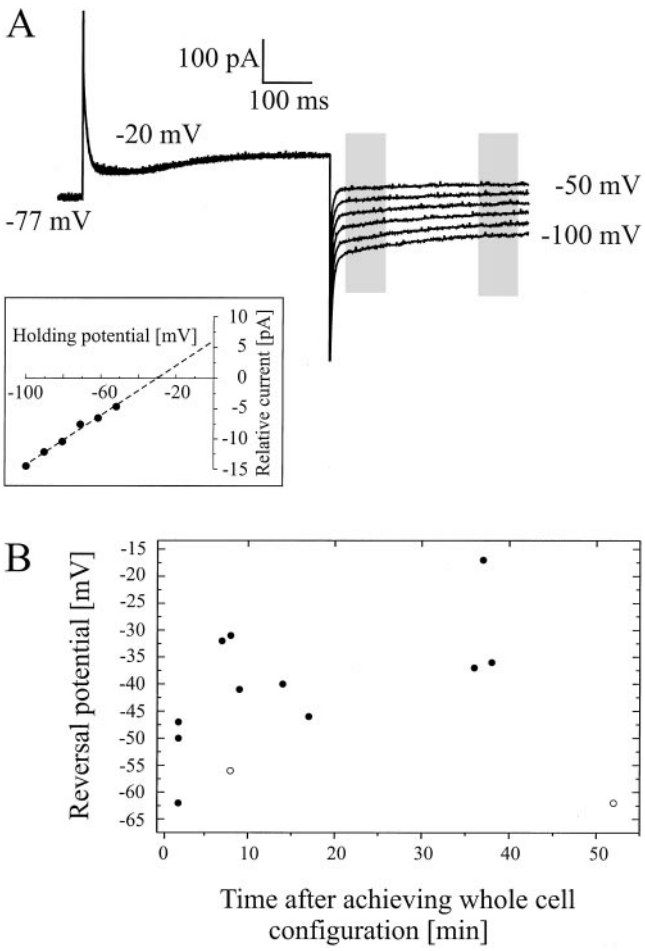

Figure 10. (A) An example of a tail current measurement, 8 min after achieving whole-cell configuration. The cone was recorded with a 33-mM Cl^--containing patch pipette, clamped at −77 mV, stepped to −20 mV for 500 ms, and successively clamped for 400 ms to various potentials. The step to −20 mV activates the $I_{Cl(Ca)}$, and the step to the various potentials induces the Ca-dependent tail current. For the estimation of the reversal potential of the tail current, no potentials above −50 mV were used, because at these potentials the activation of the Ca current interferes with the measurements. The difference between the mean current for 50 ms, measured 50 and 300 ms after the step from −20 mV to the various clamp potentials, ranging from −100 up to −50 mV, was used to determine E_{Cl} by assuming that the tail current reverses at E_{Cl}; i.e., in that condition the difference between the mean current at 50 and 300 ms is zero. (B) Estimation of E_{Cl} using the tail-current measurements. The measured E_{Cl}'s are shown for 11 cells with [Cl] in the pipette (33 mM, ●) and for 2 cells with [Cl] in the pipette (7 mM, ○), measured at various moments after achieving the whole-cell configuration. At 2 min, the estimated E_{Cl} yields values of approximately ∼55 mV for both values of [Cl]. For the low [Cl], E_{Cl} becomes more negative with time, and, for the high [Cl], E_{Cl} becomes more positive with time.

DISCUSSION

In this paper we have shown that: (a) surround-induced polarizations in cones are due to the activation of I_{Ca} and $I_{Cl(Ca)}$, (b) E_{Cl} is approximately −55 mV under physiological conditions, and (c) the effect of negative feedback from HCs to cones on second-order neurons is still present even when the cones do not depolarize during surround stimulation.

The Feedback Pathway from Horizontal Cells to Cones

Surround stimulation hyperpolarizes the HCs, which in turn shifts the I_{Ca} activation function in cones to more negative potentials (Verweij et al., 1996). This shift will increase the Ca-influx leading to an increase in $[Ca]_i$ and finally to activation of $I_{Cl(Ca)}$. Depending on E_{Cl}, this will lead to either a hyperpolarizing or depolarizing response of the cones. Since HCs feed back to cones negatively, one could argue that cones should depolarize to surround stimulation. Therefore, E_{Cl} should be more positive than the resting membrane potential. Indeed, for turtle cones, some evidence exists that E_{Cl} might be more positive than the resting membrane potential (Thoreson and Burkhardt, 1991). However, others found in turtle that E_{Cl} is more negative than the resting membrane potential (Tachibana and Kaneko, 1984; Kaneko and Tachibana, 1986). This negative value of E_{Cl} was also found in other species (Miller and Dacheux, 1983; Wu, 1986) and is in complete agreement with the findings presented in this paper. The question addressed in this paper is whether cone polarizations are essential for feedback-induced responses in second-order neurons. We showed that, when the modulation of $I_{Cl(Ca)}$ in cones is blocked by niflumic acid, negative feedback-mediated responses in second-order neurons are still present. This means that the polarization of the cone by negative feedback is of lesser importance than the direct modulation of the Ca current.

This makes the feedback synapse from HCs to cones a very unique synapse. Polarization of the presynaptic cell (HC) leads to Ca influx in the postsynaptic cell (cone). Since the Ca conductance is small relative to the total conductance of the cone, modulation of I_{Ca} will hardly polarize the cone. Moreover, the depolarizing effect of I_{Ca} on the cone membrane potential will be counteracted by the activation of $I_{Cl(Ca)}$, which tend to hyperpolarize the cone. As a result, the membrane potential of the postsynaptic cell is hardly modulated, whereas the output of the postsynaptic cell is strongly modulated. This mechanism of synaptic transmission is restricted to one synaptic terminal and does not integrate the input to the whole neuron. It is an open but very intriguing question whether such synapses are also present in the central nervous system.

Consequences for Surround Responses in Bipolar Cells

The consequence of this type of synaptic transmission is that, in the cones, the information about the stimulus within its receptive field is visible in the membrane potential, whereas the information about the stimulus outside the receptive field of the cones, but inside the receptive field of the HCs expresses itself mainly in changes in $[Ca]_i$ in the synaptic terminal of the cone, without affecting the cone membrane potential. Consistent with this finding is the work of Hare and Owen (1996), who studied the effect of the GABA analogue d-aminovaleric acid (AVA) on the surround responses of BCs in tiger salamander. They found that AVA blocked the surround responses of BCs without any obvious effect on the cone membrane potential. This result could mean two things: (a) HCs feed directly into BCs via an AVA-sensitive pathway or (b) feedback from HCs to cones does not modulate the cone membrane potential (Hare and Owen, 1996). In view of the results presented in this paper, the later interpretation seems more likely.

Taken together, it seems that the polarization of the cone membrane potential is not a good estimate of the output of the cones. Therefore, determining the transfer function of this synapse by describing the postsynaptic potential changes as a function of the presynaptic potential will not yield the function governing the complete signal flow between cones and HCs. This calls for a re-evaluation of the data concerning the transfer functions of cones to HCs and BCs.

Functional Role of the Ca-dependent Cl Current

The results presented in this paper indicate that $I_{Cl(Ca)}$ can have a large effect on the cone membrane potential when E_{Cl} is more positive than −30 mV. However, under physiological conditions, E_{Cl} was estimated to be about −55 mV. As indicated by Fig. 1, with E_{Cl} at this potential, surround stimulation hardly modulates the cone membrane potential. Furthermore, the niflumic acid experiments have shown that blocking $I_{Cl(Ca)}$ hardly affects the cone and HC resting membrane potential or the cone and HC light responses. This illustrates that in the dark $I_{Cl(Ca)}$ is hardly activated. Since light stimulation will hyperpolarize the cones, this suggests that $I_{Cl(Ca)}$ will never be activated substantially. This seems counterintuitive because in the dark the Ca influx is large. However, there are conditions in which $I_{Cl(Ca)}$ could be activated. First one has to realize that, although the Ca-influx is large, in the dark it is not maximal. Depolarizing a cone further than the dark membrane potential will substantially increase the Ca influx and might activate $I_{Cl(Ca)}$. Such depolarizations might occur at light offset when cones can generate depolarizing light-off transients. In that condition, $I_{Cl(Ca)}$ might be activated and will yield a

hyperpolarizing influence on the cone membrane potential that terminates the depolarizing light-off transients. Therefore, the role of $I_{Cl(Ca)}$ seems to be to prevent the cone from large depolarizations.

Another condition in which $I_{Cl(Ca)}$ could be activated is when only the surround of a cone is stimulated with, for instance, an annular stimulus. In that condition, the central cone will remain at its dark-membrane potential, but the activation function of I_{Ca} will be shifted to more negative potentials. This will induce a larger Ca influx that might become large enough to activate $I_{Cl(Ca)}$. For this cone, the increased I_{Ca} tends to depolarize the cone, whereas the increased $I_{Cl(Ca)}$ has the opposite effect on the cone. The overall result might be that the cone membrane potential in that condition is hardly modulated by feedback, whereas the glutamate release is increased. The functional consequence is that the driving force for the modulation of the cGMP-gated channel in the outer segment is not affected by negative feedback, which makes the gain of the light response of the cones independent of surround stimulation. Similar effects can be found in the spectral domain as well. For instance, short wavelength–sensitive cones when stimulated with a red background do not respond to that background, but will receive a feedback signal from the HCs (Kraaij et al., 1998). Also, in this condition the activation function of I_{Ca} in the cones shifts to more negative potentials, leading to an increase in the Ca influx, which tend to depolarize the cone, whereas the activation of $I_{Cl(Ca)}$ counteracts this depolarization.

In turtle, on the other hand, it has been suggested that E_{Cl} is more positive than the dark resting membrane potential. Although this a controversial point that is not settled at all, next the effect of a positive value of E_{Cl} on the feedback pathway will be discussed. When E_{Cl} is positive, activation of $I_{Cl(Ca)}$ will depolarize the cones, which in turn activates the voltage-dependent Ca channels and amplify the feedback signal in the cones. This makes $[Cl]_i$, at least in these species, an effective tool to modulate the efficiency of the feedback signal. In this respect, it would be very interesting to see whether E_{Cl} in goldfish cones can vary with, for instance, the adaptational state of the retina.

Possible Other Cl-dependent Processes in the Cone Terminal Involved in Feedback

Niflumic acid is known to be a rather nonspecific $I_{Cl(Ca)}$ blocker. Could this nonspecific action of niflumic acid have interfered with our analysis? A direct action of niflumic acid on I_{Ca} can be ruled out, because the synaptic transmission between cones and HCs remains intact during niflumic acid application, as shown in Fig. 7. Various reports have shown that GABA-gated Cl channels are located in the cones (Tachibana and Kaneko, 1984; Kaneko and Tachibana, 1986; Yazulla et al., 1989). Can our results be explained by the assumption that niflumic acid blocks the GABA-gated Cl channels in cones? It has been shown that surround stimulation did not modulate the GABA-gated chloride channels in cones (Verweij et al., 1996). Since GABA is not involved in the generation of the surround-induced depolarizing responses in cones, a possible block of the GABA-gated channels by niflumic acid cannot account for the present results.

Another possible mechanism by which feedback could activate a chloride current is by activating the glutamate transporter, which is associated with a chloride conductance (Eliasof and Werblin, 1993; Picaud et al., 1995; Arriza et al., 1997). Because glutamate is released in a Ca-dependent manner, an increase of I_{Ca} in cones would increase the release of glutamate. Picaud et al. (1995) showed that the glutamate released by the cones can increase the Cl conductance of the glutamate transporters in that cone. If the surround-induced Cl current is mediated by the glutamate transporter, then niflumic acid should block the glutamate transporter. This is not the case because niflumic acid did not affect the HC responses significantly, whereas blocking the transporter with dihydrokainate or dl-threo-β-hydroxyaspartate induces a considerable change in HC response dynamics (Eliasof and Werblin, 1993; Vandenbranden et al., 1996).

In summary, we conclude that the underlying current for the surround-induced depolarizing responses is most likely $I_{Cl(Ca)}$. We based this conclusion on mainly four findings: (a) the surround-induced currents depend strongly on E_{Cl}, (b) these Cl currents only activate after sufficient activation of the Ca current, (c) these currents generate slow tail currents, and (d) the slowly developing surround-induced currents in cones can be blocked by niflumic acid. Finally, in the literature, a number of papers appeared that show that in various species cones have an $I_{Cl(Ca)}$ with similar properties as shown in this paper (Maricq and Korenbrot, 1988; Barnes and Hille, 1989; Barnes and Deschenes, 1992; Yagi and MacLeish, 1994).

Depolarizing Cone Responses Due to Full-Field Stimulation

Sometimes, cones also depolarize during full-field stimulation (Baylor and Fuortes, 1970; Lasansky, 1981; Burkhardt et al., 1988; Piccolino, 1995). The responses were mostly recorded with KCl- or KAc-filled microelectrodes and are identified as responses generated via feedback from HCs to cones. These results are confusing because full-field stimulation will hyperpolarize all cones, and consequently all HCs should hyperpolarize. The depolarizing responses of cones during full-field stimulation would imply that the effect of the feedback signal on the cone membrane potential has become

stronger than the initial cone response. This could not be true for all the cones, because under such a condition the HCs would not remain hyperpolarized. To account for these results, one has to consider the effect of Cl^- on the various response components. The depolarizing responses of cones due to full-field stimulation were mostly recorded with Cl-containing electrodes. In this condition, the feedback responses in the cones are enhanced due to the positive value of E_{Cl}. Furthermore, we found that the direct light responses of the cones reduce over time with E_{Cl} more positive than −30 mV, suggesting that high Cl^- in the cones inhibits the phototransduction pathway. Similar observations have been made by Lasansky (1981) with KCl-filled electrodes and by Attwell et al. (1984) with KAc-filled electrodes. In conclusion, the depolarizing responses of cones due to full-field stimulation seems to be due to artificially high anion concentrations in the cones recorded from, which enhances the feedback-induced membrane potential changes and reduces the direct-light responses.

Conclusion

The sequence of events during surround stimulation can be summarized as follows. Surround stimulation leads to hyperpolarization of HCs, which shifts the Ca current-activation function of the cones to more negative potentials. This induces a Ca influx, which leads to an increase in glutamate release. Since the Ca conductance is relatively small compared with the total membrane conductance of the cone, this Ca influx will only slightly depolarize the cone. Under physiological conditions, this modulation of the Ca influx will be too small to activate $I_{Cl(Ca)}$ substantially. The result is that the cone membrane potential is hardly modulated by feedback, while the cone's glutamate release is strongly modulated by feedback. Thus, feedback-mediated responses such as the depolarizing responses in the BHC, due to red light stimulation and the surround responses of BCs, remain present in the absence of depolarizing cone responses.

This work was supported by the Human Frontier Science Program (Private Investigator, H. Spekreijse) and the Netherlands Organization for Scientific Research (NWO).

Submitted: 10 June 1999
Revised: 5 November 1999
Accepted: 16 November 1999
Released online: 13 December 1999

REFERENCES

Arriza, J.L., S. Eliasof, M.P. Kavanaugh, and S.G. Amara. 1997. Excitatory amino acid transporter 5, a retinal glutamate transporter coupled to a chloride conductance. *Proc. Natl. Acad. Sci. USA.* 94: 4155–4160.

Attwell, D., M. Wilson, and S.M. Wu. 1984. A quantitative analysis of interactions between photoreceptors in the salamander (*Ambystoma*) retina. *J. Physiol.* 352:703–737.

Barnes, S., and M.C. Deschenes. 1992. Contribution of Ca and Ca-activated Cl channels to regenerative depolarization and membrane bistability of cone photoreceptors. *J. Neurophysiol.* 68:745–755.

Barnes, S., and B. Hille. 1989. Ionic channels of the inner segment of tiger salamander cone photoreceptors. *J. Gen. Physiol.* 94:719–743.

Baylor, D.A., and M.G.F. Fuortes. 1970. Electrical responses of single cones in the retina of turtle. *J. Physiol.* 207:77–92.

Baylor, D.A., M.G.F. Fuortes, and P.M. O'Bryan. 1971. Receptive fields of cones in the retina of the turtle. *J. Physiol.* 214:265–294.

Burkhardt, D.A., J. Gottesman, and W.B. Thoreson. 1988. Prolonged depolarization in turtle cones evoked by current injection and stimulation of the receptive field surround. *J. Physiol.* 407:329–348.

Eliasof, S., and F.S. Werblin. 1993. Characterization of the glutamate transport in the retinal cones of the tiger salamander. *J. Neurosci.* 13:402–411.

Fuortes, M.G.F., and E.J. Simon. 1974. Interactions leading to horizontal cell responses in the turtle retina. *J. Physiol.* 240:177–198.

Hare, W.A., and W.G. Owen. 1996. Receptive field of the retinal bipolar cell: a pharmacological study in the tiger salamander. *J. Neurophysiol.* 76:2005–2019.

Kamermans, M., B.W. Van Dijk, and H. Spekreijse. 1991. Color opponency in cone-driven horizontal cells in carp retina. Aspecific pathways between cones and horizontal cells. *J. Gen. Physiol.* 97: 819–843.

Kaneko, A. 1970. Physiological and morphological identification of horizontal, bipolar and amacrine cells in goldfish retina. *J. Physiol.* 207:623–633.

Kaneko, A., and M. Tachibana. 1981. Retinal bipolar cells with double colour-opponent receptive fields. *Nature.* 293:220–222.

Kaneko, A., and M. Tachibana. 1986. Effects of gamma-aminobutyric acid on isolated cone photoreceptors of the turtle retina. *J. Physiol.* 373:443–461.

Kraaij, D.A., M. Kamermans, and H. Spekreijse. 1998. Spectral sensitivity of the feedback signal from horizontal cells to cones in goldfish retina. *Vis. Neurosci.* 15:799–808.

Lasansky, A. 1981. Synaptic action mediating cone responses to annular illumination in the retina of the larval tiger salamander. *J. Physiol.* 310:205–214.

Maricq, A.V., and J.I. Korenbrot. 1988. Calcium and calcium-dependent chloride currents generate action potentials in solitary cone photoreceptors. *Neuron.* 1:503–515.

Miller, R.F., and R.F. Dacheux. 1983. Intracellular chloride in retinal neurons: measurement and meaning. *Vision Res.* 23:399–411.

Naka, K.I., and W.A.H. Rushton. 1967. The generation and spread of S-potentials in fish (cyprinidae). *J. Physiol.* 192:437–461.

Norton, A.L., H. Spekreijse, M.L. Wolbarsht, and H.G. Wagner. 1968. Receptive field organization of the S-potential. *Science.* 160: 1021–1022.

O'Bryan, P.M. 1973. Properties of the depolarizing synaptic potential evoked by peripheral illumination in cones of the turtle retina. *J. Physiol.* 235:207–223.

Picaud, S.A., H.P. Larsson, D.P. Wellis, H. Lecar, and F.S. Werblin. 1995. Cone photoreceptors respond to their own glutamate release in the tiger salamander. *Proc. Natl. Acad. Sci. USA.* 92:9417–9421.

Piccolino, M. 1995. The feedback synapse from horizontal cells to cone photoreceptors in the vertebrate retina. *Prog. Retin. Eye Res.* 14:141–196.

Stell, W.K. 1976. Functional polarization of horizontal cell dendrites in goldfish retina. *Invest. Ophthalmol.* 15:895–908.

Stell, W.K., and D.O. Lightfoot. 1975. Colour-specific interconnec-

tions of cones and horizontal cells in the retina of the goldfish. *J. Comp. Neurol.* 159:473–502.

Stell, W.K., D.O. Lightfoot, T.G. Wheeler, and H.F. Leeper. 1975. Goldfish retina: functional polarization of cone horizontal cell dendrites and synapses. *Science.* 190:989–990.

Tachibana, M., and A. Kaneko. 1984. Gamma-aminobutyric acid acts at axon terminals of turtle photoreceptors: difference in sensitivity among cell types. *Proc. Natl. Acad. Sci. USA.* 81:7961–7964.

Thoreson, W.B., and D.A. Burkhardt. 1991. Ionic influences on the prolonged depolarization of turtle cones in situ. *J. Neurophysiol.* 65:96–110.

Toyoda, J.I., and T. Kujiraoka. 1982. Analyses of bipolar cell responses elicited by polarization of horizontal cells. *J. Gen. Physiol.* 79:131–145.

Vandenbranden, C.A.V., J. Verweij, M. Kamermans, L.J. Muller, J.M. Ruijter, C.F.J.M. Vrensen, and H. Spekreijse. 1996. Diffusion of neurotransmitters in the cone synaptic terminals of goldfish retina. *Vision Res.* 36:3859–3875.

Verweij, J., M. Kamermans, and H. Spekreijse. 1996. Horizontal cells feed back to cones by shifting the cone calcium-current activation range. *Vision Res.* 36:3943–3953.

Wu, S.M. 1986. Effects of gamma-aminobutyric acid on cones and bipolar cells of the tiger salamander retina. *Brain Res.* 365:70–77.

Yagi, T., and P.R. MacLeish. 1994. Ionic conductances of monkey solitary cone inner segments. *J. Neurophysiol.* 71:656–665.

Yazulla, S., K.M. Studholme, J. Vitorica, and A.L. De Blas. 1989. Immunocytochemical localization of GABA a receptors in goldfish and chicken retinas. *J. Comp. Neurol.* 280:15–26.