Spectral and dynamic properties of cone/horizontal cell interaction in goldfish retina

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Chapter 4

The Dynamic Characteristics of the Feedback Signal from Horizontal Cells to Cones in the Goldfish Retina

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Abstract

We examined the dynamic properties of the microcircuitry formed by the cones and the horizontal cells in the goldfish retina. Cones project to horizontal cells and horizontal cells feed back to cones via a relatively slow negative feedback pathway. We determined the timeconstant of the feedback signal and of the feedback-induced responses of second order neurons. We found that the feedback signal had a timeconstant of around 80 ms, whereas the timeconstants of the feedback-induced responses of the second order neurons ranged from 36 to 116 ms. This range of timeconstants can be accounted for by the non-linearity of the calcium current in the cones. For depolarized cones the feedback-mediated response has about the same timeconstant as the cone direct light response, whereas for hyperpolarized cones the timeconstant of the feedback-mediated response is considerably larger. Furthermore, we show for the first time that there is no delay in the feedback pathway. Due to the interaction of the direct light response and the feedback response of the cone, an apparent delay can occur in the horizontal cell responses. These results illustrate that, due to the non-linearity of synaptic processes, responses generated via longer pathways can appear to be equally fast or even faster than the responses generated via a shorter pathway.

Introduction

A spot projected onto the vertebrate retina generates two types of signals: (1) a center signal, that is transferred from cones to ganglion cells via bipolar cells and finally to the rest of the visual system, and (2) an opposite surround signal, generated via a negative feedback pathway from horizontal cells (HCs) to cones. This organization plays a prominent role in contrast enhancement (Dowling, 1987) and color constancy (Kamermans et al., 1998). The feedback pathway is assumed to be much slower than the feedforward pathway (Piccolino et al., 1981; Wu, 1994; Kamermans et al., 1996; Kamermans and Spekreijse, 1999; Fahrenfort et al., 1999) and it has been suggested that this signal pathway contains a pure delay of 25 ms (Spekreijse and Norton 1970). Since no direct measurements of the dynamic features of the feedback signal from HCs to cones are available in literature, the aim of this study is to generate these data for goldfish.
The events taking place in this first synapse can be summarized as follows. Cones project to HCs via a calcium-dependent, glutamatergic pathway. Light stimulation hyperpolarizes cones, leading to a hyperpolarization of HCs. HCs feed back to cones by modulating the calcium current \( I_{\text{Ca}} \) in cones via a unique electrical feedback mechanism (Verweij et al. 1996; Kamermans et al. 2000). This modulation of the \( I_{\text{Ca}} \) can be measured directly in the cones and results in an increase of the glutamate release. This feedback mechanism forms the basis for the surround responses of the bipolar cells (BCs) and the spectral coding of the HCs. The effect of the feedback-induced increased glutamate release can be recorded most accurately in HCs. For instance in the monophasic horizontal cell (MHC) (Spekreijse and Norton 1970; Stell et al. 1975; Kamermans et al. 1991) response, a depolarizing rollback in the sustained light response is present, which can be attributed to negative feedback from HCs to cones (Piccolino et al. 1981; Wu 1994; Kamermans and Spekreijse 1999; Fahrenfort et al. 1999). Furthermore, the depolarizing response of the biphasic HCs (BHC) to red light stimulation is due to negative feedback from HCs to cones (Stell and Lightfoot 1975; Kamermans et al. 1991). Since in this neural network the response properties of the cones, the feedback signal in the cones and the resulting change in the cone output can be measured directly, we can obtain a description of the dynamic properties of both the feedforward and feedback signals flowing across the first synapse of the visual system. We determined (1) the timeconstant of the feedback pathway and (2) the delay in the feedback pathway in order to estimate the relative timing of the feedforward- and the feedback-mediated responses in the outer retina.

**Material and Methods**

**Preparation**

Goldfish, *Carassius auratus*, (12-16 cm standard body length) were kept at 18 °C under a 12 hour dark, 12 hour light regime. Before the experiment, the fish was kept in the dark for 8 ± 1 min to facilitate the isolation of the retina from the pigment epithelium, while keeping the retina still light adapted. Under infra red illumination (\( \lambda = 920 \) nm) the fish was decapitated, and one 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 Ringers solution (pH 7.8, 18 °C).

For the whole-cell recordings of the photoreceptors, the superfusion chamber was mounted on a microscope (Optiphot-X2 microscope; Nikon, Inc.). The preparation was illuminated with infrared light (\( \lambda > 850 \) nm, wratten filter 87c; Eastman-Kodak Co.) and viewed with a Nikon 40x water immersion objective (numeric aperture, \( NA = 0.55 \); Nikon, Inc.), Hoffman modulation contrast optics and a video camera (Philips). Electrodes were mounted on a MP-85 Huxley/Wall-type micro-manipulator (Sutter Instruments Co.) and connected to an integrating patch clamp (3900A; Dagan Corp.).

For the intracellular recordings from HCs, the retina was illuminated with an infra red light emitting diode (LED SFH 484-II, Telefunken) and viewed through a 2x objective of an inverted microscope (IMT-2; Olympus, Corp.) 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 patch clamp, the microelectrode amplifier, and of the optical stimulator were done with a CED 1401 AD/DA converter with a sample frequency of 1.0 kHz (Cambridge Electronic Design Ltd.) and an MS-DOS based computer system.

**Optical stimulator**

The optical stimulator with the Nikon microscope consisted of a 450-W xenon-lamp (Osram) which supplied two beams of light. These were projected through Uniblitz VS14 shutters (Vincent associates), neutral density filters (NG Schott), bandpass interference filters with a bandwidth of 8 ± 3 nm (Ealing Electro-Optics Inc.), lenses and apertures. The 20-, 65- and 250-μm spots were projected through the 40x objective of the microscope and light stimuli of 3,000-μm were projected through the condenser (NA = 1.25). Throughout this chapter, for monochromatic light stimuli, an intensity of 0 log corresponds to a photon flux density of 1.0 * 10^9 photons μm^2 s^-1, and for white light stimuli, an intensity of 0 log corresponds to a photon flux density of 4.0 * 10^3 candela m^-2 s^-1. The optical stimulator with the Olympus microscope consisted of two light beams from a 450-W xenon light-source (Osram). These were used to project light spots of various sizes, wavelengths and intensities onto the retina. In one stimulus channel the wavelength was controlled by a monochromator (Ebert) and in the other one by interference filters (Ealing Electro-Optics Inc.). The intensity of each channel was controlled by a pair of circular neutral density filters (CND3, Barr and Strout). The light stimuli were projected onto the retina through the epifluorescence channel of the microscope.

**Patch electrodes and pipette medium**

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 - 10 MΩ when filled with standard patch pipette medium and measured in Ringers solution. The series resistance during the whole-cell recording was between 10 - 20 MΩ.

The standard patch pipette medium contained (mM): 20.0 KCl, 70.0 D-gluconic-K, 5.0 KF, 1.0 MgCl₂, 0.1 CaCl₂, 1.0 EGTA, 5.0 HEPES, 4.0 ATP-Na₂, 1.0 GTP-Na₃, 0.2 3':5'-cGMP-Na, 20 phosphocreatine-Na₂, 50 U/ml creatine phosphokinase. The pH of the pipette medium was adjusted to 7.25 with KOH. All chemicals were obtained from Sigma-Aldrich.

**Microelectrodes**

Intracellular 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 from 80 to 200 MΩ when filled with 4 M KAc.

**Liquid junction potential**

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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 solution. The resulting potential change was considered to be the junction potential and all data were corrected accordingly. All results presented in this chapter were obtained ~15 min after whole-cell configuration was achieved.

**Estimating the timeconstant**

An exponential function (eq. 4.1.) was fitted through the responses using Levenberg-Marquardt iterations. These calculations were done with Origin 5.0.

\[
R(t) = R(t_0) + R_m (1 - \exp(-\frac{(t-t_0)}{\tau}))
\]

(4.1.)

Where:
- \(R(t)\) = Response amplitude (mV or pA)
- \(R(t_0)\) = Membrane potential when the response starts (mV or pA)
- \(R_m\) = Maximal response amplitude (mV or pA)
- \(t\) = Time (ms)
- \(t_0\) = Time at which the light response starts (ms)
- \(\tau\) = Timeconstant (ms)

**Results**

First, the various signals studied will be defined. The feedback signal is the feedback-induced shift in the activation function of the \(I_{Ca}\) of the cones. The feedback response in a cone is defined as the feedback-induced change in the \(I_{Ca}\) in a voltage-clamped cone. The feedback-induced response in HCs is the change in HC membrane potential which can be attributed to the feedback-induced change in \(I_{Ca}\) in cones.
The timeconstant of the feedback-induced responses in HCs

The effect of negative feedback from HCs to cones can be measured in HCs. Figure 4.1. shows two examples of these feedback-induced responses. (A) gives the response of an MHC to a 550 nm full-field stimulation. The response consists of a fast hyperpolarizing phase and a slower feedback-induced rollback (arrow). In the 20 cells analyzed this way the mean timeconstant was 116 ± 6 ms, indicating that feedback is slow indeed. On the other hand, (B) shows that the depolarizing response of a BHC to 700 nm full-field stimulation is fast. This stimulus activates the long wavelength cones (L-cones) strongly, whereas it hardly stimulates the middle wavelength sensitive cones (M-cones). This depolarizing response is generated via negative feedback from the MHCs to the M-cones and is subsequently forwarded to the BHCs. In the 6 BHCs analyzed this way, the mean timeconstant was 36 ± 8 ms (n=6). Therefore these experiments show that feedback is fast.

Dynamic characteristics of the feedback signal...
Although the number of synaptic transitions is equal for both feedback-mediated HC responses (cone → MHC → cone → MHC versus cone → MHC → cone → BHC), the difference in time constant of the resulting response is considerable (116 versus 36 ms). To account for this difference, one has to realize that: (1) the stimulus wavelength was 700 nm for the BHC and 550 nm for the MHC response and (2) the cones receiving the feedback signal were hyperpolarized in the case of figure 4.1.(A) and relatively depolarized in the case of figure 4.1.(B). One could argue that the time constant of the feedback response of the cones could be either wavelength or voltage dependent. These two possibilities will be tested.

Figure 4.2. (A) Feedback-induced response of an M-cone clamped at -45 mV, which was continuously saturated with an intense white spot of 65 μm and stimulated for 500 ms with a 3,000-μm white spot. The scaling and timing are shown in the figure. (B) Mean time constants of the feedback response of 7 cones clamped at -47 mV, which were continuously saturated with an intense white spot of 65 μm and in addition stimulated for 500 ms with a 3,000-μm 550 nm spot of increasing intensity. (C) Mean time constants of the feedback responses of 5 cones clamped at -47 mV, which were continuously saturated with an intense white spot of 65 μm and in addition stimulated for 500 ms with a 3,000-μm 550 nm spot. The clamp potentials of the cone are given in the figure. An exponential function was fitted through these responses for 6 cones and the mean time constants are plotted as function of the holding potential in (E).

in addition stimulated for 500 ms with a 3,000-μm spot of various wavelengths with the same intensity. (D) Feedback responses of a voltage-clamped M-cone, which was continuously saturated with an intense white spot of 65 μm and stimulated for 500 ms with a 3,000-μm 550 nm spot. The clamp potentials of the cone are given in the figure. An exponential function was fitted through these responses for 6 cones and the mean time constants are plotted as function of the holding potential in (E).
The timeconstant of the feedback-induced change in the $I_{\text{Ca}}$ in cones

Feedback can be measured in cones most effectively when a cone is voltage-clamped around -45 mV, saturated with a small bright spot (65 μm) and stimulated with a full-field stimulus. This induces a small inward current, figure 4.2.(A) with a timeconstant of about 90 ms, which has been identified as an increased $I_{\text{Ca}}$ (Verweij et al. 1996; Kamermans and Spekreijse 1999; Kraaijeveld et al. 2000a). The mean timeconstant of this response is $87 \pm 4$ ms ($n=18$). By changing the intensity or wavelength of the full-field stimulus, the dependence of the timeconstant on the stimulus intensity and wavelength was studied. Figure 4.2.(B) shows the intensity dependence of the timeconstant and illustrates that it is independent of the stimulus intensity. Likewise figure 4.2.(C) shows that the timeconstant of the feedback signal is also rather independent of the stimulus wavelength. These two experiments rule out that the difference in feedback responses in HCs is caused by something else. Figure 4.2.(D) gives the (normalized) feedback response in a cone clamped at various potentials. At -37 mV the timeconstant is about 30 ms, whereas at -52 mV the timeconstant is 110 ms. Figure 4.2.(E) presents the relation between the timeconstant and the holding potential of six cones and illustrates that the timeconstant increases with hyperpolarization.

**Figure 4.3.** (A) Simulated I-V relation of the $I_{\text{Ca}}$ (eq. 4.2.) in a cone during stimulation with an intense 65-μm white spot (solid line) and during stimulation with a 3000 μm spot in addition (dotted line). Negative feedback from HCs to cones results in a shift of the calcium current activation function to more negative potentials (arrow 1). This shift will induce an increase of the $I_{\text{Ca}}$ (arrow 2). With a timeconstant of the feedback signal of 80 ms, and holding potentials ranging from -60 mV up to -30 mV, feedback-induced cone responses with timeconstants ranging from ~140 to ~30 ms could be simulated (B). The voltage dependence of the timeconstants from these simulated responses (open circles) are compared with the timeconstants of the measured feedback-induced cone responses from figure 4.2. (closed symbols) (C).
How can we account for the potential dependence of the timeconstant of the feedback response? Negative feedback modulates the activation function of the $I_{Ca}$ in cones (the feedback signal). This induces a change in the $I_{Ca}$, which can be measured as a feedback-induced inward current in the cones (the feedback response). The question now arises whether the timeconstants of the various feedback responses in the cone can be accounted for with one timeconstant of the feedback signal. The relation between these two timeconstants was studied in a very simple model, consisting of only a $I_{Ca}$ (eq. 4.2.). The parameters for the $I_{Ca}$ used for this simulation are given in table 1. These values are in the same range as the values determined and used by Verweij et al. (1996), Fahrenfort et al. (1999) and Kraaij et al. (2000b). Kraaij et al. (2000b) showed that the half-maximal activation of the $I_{Ca}$ ($K$) depends linearly on the HC membrane potential. Figure 4.3.(A) gives the, with eq. 2, simulated $I_{Ca}$ without (solid) and with (dotted) feedback. Surround stimulation results in a shift of the calcium current activation curve to negative potentials (arrow 1) and thus in an increase in the $I_{Ca}$ (arrow 2). With a timeconstant of 80 ms for the feedback signal, feedback responses could be simulated with timeconstants ranging from 30 ms for a holding potential of -30 mV to 150 ms for a holding potential of -60 mV, figure 4.3.(B). Figure 4.3.(C) gives the relation between the holding potential and the timeconstant of the feedback response for the simulations (open dots) and the experiments, filled dots; taken from figure 4.2.(E). Since the two curves overlap nicely, this simulation illustrates the way by which the non-linearity of the $I_{Ca}$ in the cones can account for the observed range of timeconstants of the feedback responses in cones and thus eventually of the feedback-induced responses in HCs.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_{Ca}$ without feedback</td>
<td>-36 mV</td>
</tr>
<tr>
<td>$K_{Ca}$ with feedback</td>
<td>-48 mV</td>
</tr>
<tr>
<td>$n_{Ca}$</td>
<td>3.7 mV</td>
</tr>
<tr>
<td>$g_{Ca}$</td>
<td>1 nS</td>
</tr>
<tr>
<td>$E_{Ca}$</td>
<td>50 mV</td>
</tr>
</tbody>
</table>

Table 1. Fitting parameters used for the simulation of the $I_{Ca}$, the feedback-induced cone responses, and the potential dependence of the feedback timeconstant, shown in figure 4.3.(A), (B), and (C) respectively. $K_{Ca}$ is the potential for half-maximal activation of the calcium current activation function.
\[ I_{Ca} = (V_m - E_{Ca}) \cdot g_{Ca} \cdot \frac{1}{1 + \exp\left(\frac{(V_m - E_{Ca})}{\theta_{Ca}}\right)} \]  

(4.2)

Where:

- \( I_{Ca} \): Calcium current in cones (pA)
- \( V_m \): Holding potential (mV)
- \( E_{Ca} \): Reversal potential of the calcium current (mV)
- \( g_{Ca} \): Maximal conductance of the calcium channels (nS)
- \( K_{Ca} \): Potential for half-maximal activation of the calcium current activation function (mV)
- \( n_{Ca} \): Slope factor of the calcium current activation function (mV)

Evidence for a potential-dependent feedback response can also be found around the neutral point (i.e., the wavelength where the BHC response changes from a hyperpolarizing into a depolarizing response). Figure 4.4. shows the responses of a BHC to 600, 650 and 700 nm stimuli for three stimulus intensities. It is obvious from this figure that the responses to 650 nm
become more and more transient with increasing intensity and that the hyperpolarizing part of the response is always earlier than the depolarizing part of the response. For higher intensities the hyperpolarizing part of the response grows faster than the depolarizing part. To account for this one has to take three factors into account: (1) hyperpolarization of a cone slows down the feedback response of the cone, (2) the efficiency of the feedback will become smaller at hyperpolarized cone potentials, and (3) increasing the stimulus intensity will speed up the cone response (Kraaij et al. 2000b). The increase in timeconstant can be seen in figure 4.4, where an exponential function is fitted through the depolarizing component of the response of a BHC to 650 nm light for the three intensities used. The timeconstant of this component increases from 47 ms to 101 ms. The combined effect of these three factors will be that with increasing intensity the hyperpolarizing component of the response will become faster and that the depolarizing component will become slower and smaller.

Figure 4.5. (A) Light responses of a BHC to stimuli of three intensities and six wavelengths. Short- and middle-wavelength stimuli evoked hyperpolarizing responses, whereas long wavelength stimuli induced depolarizing responses. (B) Light responses of a BHC to 600, 650 and 700 nm full-field light stimuli (gray), compared to the 500 nm wavelength-induced light responses of the same BHC (black). The responses are scaled and inverted such that they overlap the 500 nm light-responses. (C) Redraw of light onset responses of (B) on an expanded time scale.
Delay in the feedback responses in cones and the feedback-induced responses in HCs

Next, it was studied whether or not a pure delay of about 25 ms exists in the negative feedback pathway from HCs to cones as estimated by Spekreijse and Norton (1970). First, the timing of the feedback-mediated responses in HCs was studied. The hyperpolarizing responses of BHCs in the short and middle wavelength part of the spectrum are due to direct input from the cones, whereas the depolarizing responses to long and middle wavelengths are feedback-mediated. Figure 4.5.(A) gives the responses of a BHC for various wavelengths at three intensities. To determine whether a pure delay exists between the direct (hyperpolarizing) and the feedback-mediated (depolarizing) responses, the depolarizing responses of the BHC due to 600, 650, and 700 nm stimulation were inverted and scaled such that they overlapped the hyperpolarizing responses to 500 nm stimulation, figure 4.5.(B). Expansion of the time axis, figure 4.5.(C) shows that indeed a pure delay of about 25 ms between the 500 nm responses and the 650 nm responses exists, as estimated by Spekreijse and Norton (1970). This delay reduces for longer and shorter wavelengths. This was found in all 6 cells analyzed this way.

Next, the delay of the feedback response in the cones was studied, and surprisingly no delay was found between the light response of a cone (figure 4.6., solid trace) and the feedback response of the same cone (figure 4.6., dotted trace). This was found in all 15 cells tested. The results of figure 4.5.(C) and 4.6. seem to contradict each other. However, the main difference between these two experiments is that during the experiment of figure 4.6. the cone recorded from was voltage-clamped, while during the experiment of figure 4.5. it was not. The depolarizing responses of a BHC due to 650 and 700 nm stimulation are generated by feedback from MHCs to M-cones. However, M-cones are still sensitive to 650 nm light, figure 4.7.(A). Although their responses are small for 665 nm stimulation, they still respond to long wavelength stimuli. Thus for 650 nm stimuli M-cones hyperpolarize a few mV and the activation function of
the cone $I_{\text{C}}$ shifts to more negative potentials. Both processes have different amplitudes and timeconstants.

The combined effect of these two processes is simulated in figure 4.7.(B). With a timeconstant of 80 ms for the feedback signal and of 30 ms for the M-cone light response (Kraaij et al. 1998) and with the same parameters as used in the simulations of figure 4.3., the feedback responses in the M-cone for various M-cone response amplitudes were simulated, figure 4.7.(B) and (C). During these simulations $K_2$ was -44 mV and the dark-resting membrane potential of the model cone was -45 mV. The solid traces in figure 4.7.(C) show the response without hyperpolarization of the M-cone. The dotted traces show the feedback-induced responses for different M-cone hyperpolarizations (indicated at the right of the figure). During about the first 25 ms, the feedback response and the cone response tend to compensate each other and both the Ca influx and the glutamate release hardly change. However, due to the differences in amplitudes and timeconstants of these two processes, the feedback-induced shift of the activation function will become the major effect after about 25 ms. The result is that the output of the cone will only change significantly after about 25 ms, which induces an apparent delayed depolarizing BHC response. This analysis confirms that the delay reduces for longer wavelengths, because stimulation of the M-cone by 700 nm light is less effective than for 650 nm, and that for shorter wavelengths the direct light response will become dominating, resulting in a hyperpolarizing transient response at light onset (figure 4.4.). This simulation illustrates how an apparent delay can occur in the cone output, without a genuine delay in the feedback input.
Figure 4.7. (A) Responses of an L- (top) and an M-cone (bottom) to 65-μm stimuli of various wavelength and intensities. (B) Simulation of the feedback response in an M-cone for long wavelength stimuli. The time constant of the feedback signal in the cone is 80 ms and the time constant of direct light-response is 30 ms. The solid trace is the change in the $I_{Ca}$ when the M-cone does not respond due to direct light stimulation (i.e., stimulation with deep red light). The dotted traces indicate the changes in the $I_{Ca}$ when the M-cone responses have the amplitude as indicated on the right side of the figure. (C) The onset of the change in the $I_{Ca}$ on an expanded time scale.
Discussion

In this study we showed that negative feedback from HCs to cones is strongly potential-dependent. Feedback to depolarized cones can be almost as fast as the cone responses themselves. Secondly, we have shown that no pure delay of 25 ms in the negative feedback pathway from HCs to cones exists. This is in seemingly contrast to the depolarizing responses of BHCs to red light stimulation, which are delayed relative to the hyperpolarizing responses. With a simple model we could account for both the potential dependence of the feedback signal in cones and the apparent delay in the BHC response to red light.

Feedback or feedforward inhibition?

It is generally assumed that depolarizing responses of the BHCs are generated via negative feedback to M-cones (see for instance Kamermans and Spekreijse 1995). It was argued that due to this feedback the depolarizing responses are expected to develop at a slower rate than to the hyperpolarizing responses. As it becomes obvious from figure 4.1, this is not the case. Similar results have been obtained in turtle retina. Asi and Perlman (1998) argued that since the depolarizing responses of the BHCs are too fast compared to the hyperpolarizing responses, the depolarizing responses of BHCs can not be induced by feedback, but are due to a direct inhibitory input from the cones to the HCs. In this study we show that there is no need to dismiss the feedback hypothesis. The non-linearity of the \( I_{Ca} \), together with the unique electrical feedback mechanism from HCs to cones (Kamermans et al. 2000) can adequately account of the different dynamic features of the hyperpolarizing and the depolarizing responses of the BHCs.

Center surround organization of the bipolar cells

The receptive fields of almost all neurons in the visual system have a center/surround organization. Lateral inhibitory interactions are essential for this organization. One of the places where such lateral interactions take place is the outer retina. There, the HCs integrate the visual stimulus over a large area and feed back negatively to the cones. It was generally assumed that this feedback signal is slower than the feedforward signal and has a delay relative to the feedforward signal. If that was true, the center/surround organization would be compromised when a moving spot is presented (Werblin 1991). At the leading edge, the surround would be compressed and at the trailing edge the surround would be extended. This would suggest a difference in contrast enhancement between the leading and the trailing edge of a moving spot. In other words, the spot would subjectively change its shape when it is moving. To the best of our knowledge there are no psychophysical data supporting this prediction (Barbur et al. 1986; Burr 1980).

In this chapter we have shown that due to the non-linearity of the \( I_{Ca} \), the timeconstant of the surround response can be of the same order as that of the center response. The result is that the surround response of BCs will not lag the center response for a moving spot, retaining the integrity of the center/surround organization of their receptive fields. This finding is consistent with the results of Schellart and Spekreijse (1972) who showed that, in goldfish, the center and
the surround responses of ganglion cells have equal dynamic properties.

The results presented in this chapter illustrate why one should be careful in using the dynamic features of neuronal responses as a measure for the pathway via which a response is generated. Due to the non-linearity of the synaptic processes involved, responses generated via longer pathways can be equally fast or even faster than responses generated via a shorter pathway.