ELECTRICAL FEEDBACK IN THE CONE PEDICLE:
A COMPUTATIONAL ANALYSIS.

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ABSTRACT

One of the fundamental principles of neuroscience is that direct electrical interactions between neurons are not possible without specialized electrical contacts, gap junctions, because the transmembrane resistance of neurons is typically much higher than the resistance of the adjacent extracellular space. However it has been proposed that in the retina direct electrical interactions between cones and second order neurons occur due to the specific morphology of the cone synaptic terminal. This electrical mechanism could potentially explain the phenomenon of “negative feedback” from horizontal cells to cones, and the recent finding that the tips of horizontal cell dendrites contain hemichannels has rekindled interest in the idea. We quantitatively evaluated the possibility that hemichannels and/or glutamate channels mediate electrical feedback from horizontal cells to cones. The calculations show that it is unlikely that an electrical mechanism plays a significant functional role because 1) the necessity of preserving adequate cone to horizontal cell synaptic transmission limits the extracellular space resistance and the horizontal cell dendritic transmembrane resistances to values at which the effectiveness of electrical feedback is very low and its electrical effect on the cone presynaptic membrane is negligible, 2) electrical feedback is most effective in the dark and weaker during light adaptation, which contradicts the experimental data, and 3) electrical negative feedback is associated with much stronger electrical positive feedback from horizontal cells to cones, a phenomenon that has never been reported. Therefore, it is likely that negative feedback from horizontal cells to cones is chemical in nature.
INTRODUCTION

A fundamental principle of neuroscience states that all neurons are electrically independent and specialized contacts called gap junctions must be utilized for electrical current to pass directly from one cell to another. Yet hypothetically, a direct electrical interconnection between neurons that does not utilize gap junctions could be possible if certain conditions were present, namely, if the local transmembrane resistance were much lower and the adjacent extracellular resistance were much greater than is typical. Byzov first pointed out that this might be the case in the highly specialized synaptic structure in the vertebrate retina called the cone pedicle, where cones contact second-order neurons called bipolar cells and horizontal cells (Byzov, 1977; Byzov and Shura-Bura, 1986).

Horizontal cells (HCs) also provide a signal back to cone photoreceptor cells (Baylor et al, 1971). Cones hyperpolarize to light with graded potentials, but when they are constantly stimulated with a central spot of light, they depolarize in response to annular stimulation. This phenomenon was called “negative feedback,” and for many years, it was assumed that this feedback depolarization is due to some chemical inhibitory signal from HCs to cones (see Burkhardt, 1993; Kamermans and Spekreijse, 1999). However, this chemical hypothesis has still not been proven, and an alternative, electrical mechanism of negative feedback has also been proposed (Byzov, 1977; Byzov and Shura-Bura, 1986; Kamermans et al, 2001). The most intriguing feature of this electrical mechanism of negative feedback is the claim that HCs transfer electrical signals to cones in the absence of electrical synapses, since there are no gap junctions between HCs and cones. In fact, this mechanism, in contrast to conventional electrical communication through gap junctions, has been proposed as unilateral (from HC to cone) and sign-reversing (HC hyperpolarization produces cone depolarization).
Byzov proposed that “electrical feedback is an intrinsic property of any chemical synapse,” because the presynaptic membrane possesses a large local transmembrane conductivity. The unique architecture of the cone synaptic terminal might facilitate the generation of electrical feedback. In the cone pedicle, the dendrites of HCs, together with the dendrites of bipolar cells, invaginate approximately 1 µm inside the cone presynaptic terminal. As a result, the resistance of the restricted extracellular space for the current running from the entrance of the invagination to the tip of the horizontal cell dendrite might be much larger than the resistance of the extracellular space outside the synaptic region.

An electrical circuit representation of Byzov’s electrical feedback model is shown in Figure 1. The glutamate-gated cation channels are located on the HC postsynaptic membrane and determine the resistance of the HC dendrite (Rd, Fig. 1). The reversal potential of the glutamate channels is close to 0 mV, and the only variable current-generating element in the circuit is the battery of the membrane potential of the HC body (Eb, Fig. 1). Since the resistance of the synaptic cleft inside the invagination of the cone pedicle (Rf, Fig. 1) is a common element for both the HC and the cone, the potential generated across this resistance by the current caused by Eb influences the local membrane potential in the presynaptic region of the cone. This extracellular potential inside the cone pedicle is, in essence, the “feedback” potential (Vf), because it is equal to the value at which the cone presynaptic membrane potential (between points 3 and 2 in Fig. 1) is different from the membrane potential of the rest of the cone (between points 3 and 1 in Fig.1, where point 1 is extracellular “ground”). An increase of Eb, which corresponds to a hyperpolarization of the HC, enhances the extracellular current through Rf, which in turn increases the feedback potential. The membrane potential of the cone measured between points 1 (ground) and 3 does not appreciably change (because the transmembrane
resistance of the cone presynaptic membrane, Rs, is by far the largest resistance in the circuit), and consequently, the increase in the negative potential between point 2 and ground is associated with a decrease in the negative potential between points 2 and 3, i.e. a local depolarization of the cone presynaptic membrane. Formally, Rs is a variable resistor, because it includes Ca$^{2+}$ voltage gated channels. However, in the context of the electrical feedback hypothesis, the opening and closing of Ca$^{2+}$ channels in the cone presynaptic membrane must have a negligible effect on the resistance of the cone presynaptic membrane, Rs. In fact, the very high resistance of the cone presynaptic membrane, and accordingly, the very small conductance of the Ca$^{2+}$ channels in the cone presynaptic membrane, is an important condition for the electrical mechanism of negative feedback. Characteristically, in a recent version of the hemichannel-mediated electrical feedback hypothesis (see below), the Ca$^{2+}$ channels on the cone presynaptic membrane are presented as voltmeters (Kamermans and Fahrenfort, 2004), i.e. a device with very high resistance.

It should be emphasized that this above electrical feedback mechanism can only produce a local voltage drop of the cone presynaptic membrane, and not a depolarization of the whole cone. Thus, the electrical mechanism is not able to reproduce the phenomenon of negative feedback, which is the depolarization of the cone when its neighboring cones are illuminated. But, as Byzov pointed out, the electrical mechanism can potentially influence the result of negative feedback, which is an increase in glutamate release, because electrical feedback could locally depolarize the cone presynaptic membrane, at which voltage-sensitive Ca$^{2+}$ channels regulate glutamate release. Nevertheless, the crucial point of the model, namely, the near equality of the resistance of the postsynaptic membrane of the HC dendrite and the feedback resistance (Rd ≈ Rf) has never been demonstrated.
The debate over the possibility of electrical feedback from HCs to cones was recently reinvigorated by the immunohistochemical demonstration that the tips of HC dendrites inside the cone pedicles of fish and turtle retinas express hemichannels (Janssen-Bienhold et al, 2001a, b). A hemichannel is half of a gap junction localized on the membrane of a cell without a counterpart on an adjacent cell. As a result, a hemichannel is open into the extracellular space. According to Kamermans and his colleagues (2001; for review see Kamermans and Fahrenfort, 2004), hemichannels could provide a sink for extracellular current, and thus play the same role in electrical feedback as the glutamate-mediated current in Byzov’s model. An advantage of hemichannel-mediated electrical feedback over the glutamate-mediated version is that the former would presumably not be modulated by light stimulation, at least over a short time scale. Again, the critical point of the Kamermans et al. (2001) model is the ratio between the transmembrane and extracellular resistances, that is, the ratio between the transmembrane resistance of the hemichannels on the HC dendrite and the extracellular feedback resistance. Kamermans and coworkers suggested that the ratio could be as low as 4:1, but they have likely significantly underestimated the hemichannel resistance (see below).

The aim of this article is to investigate through computational means whether an electrical mechanism can provide the feedback signal from HCs to cones. The electrical model we used to evaluate electrical feedback from a HC to a cone is presented in Figure 2 (for details see Methods). The circuit calculations were made using Kirchhoff’s laws, which are generalized extensions of Ohm’s law employed in network analysis. A computer program was custom-made to perform the calculations.
METHODS

Electrical Model of the Synaptic Interactions between Cones and Horizontal Cells

We used a “parallel shunt” model (Fig. 2) to evaluate electrical feedback from a HC to a cone. The only battery in the circuit is the battery of the potassium equilibrium potential (or reversal potential of the potassium channels - \( E_k \)), which is in series with the transmembrane potassium resistance, \( R_k \). This potential-resistance element is localized in the HC body and shunted by other transmembrane resistances in the HC dendrites. Each dendrite has two transmembrane resistances arranged in parallel, the resistance of the glutamate-regulated channels, \( R_g \), and the resistance of the hemicannels, \( R_h \). There are no batteries for these transmembrane resistances because we accept following Byzov and Shura-Bura (1986) and Kamermans and colleagues (2001) that the reversal potential for both is 0 mV. Also each dendrite has two longitudinal resistances that are in series with the transmembrane resistances. One of these, the feedback resistance, \( R_f \), represents the extracellular resistance of the invagination within the cone pedicle. The drop of potential across this resistance, \( V_f \), is the main focus of our simulations because it is the feedback potential that is equal to the difference between the cone membrane potential in the presynaptic region and in the rest of the cone. The other radial resistance, \( R_r \), represents the sum of the extracellular and intracellular resistances that the current meets on its way from the cell body to the invagination through the extracellular space and back through the cytoplasm, excluding the resistance of the extracellular space inside the invagination, which was defined above as \( R_f \). As we will show, the value of \( R_r \) is likely to be comparable to \( R_f \).

\( R_g \) is a variable resistance (indicated with an arrow) because the opening of glutamate-gated channels on the HCs is regulated by the light-induced release of glutamate from cones (for
review see Dowling, 1987; Massey, 1990). The effects of light stimulation are simulated in the model by changes in $R_g$, which is the only variable element in the circuit; all other elements are constant. Although changes in membrane potential can alter the conductance of many types of hemichannel, the voltage-dependent modulation of hemichannel conductance is relatively small; a 50 mV change in voltage under physiological conditions almost always changes hemichannel conductance by less than two-fold (for review see Harris, 2001). Moreover, connexin26 (Cx26), which is located in fish HC dendrites (Janssen-Bienhold et al., 2001a, b), has a very low sensitivity to voltage. The steady-state junctional conductance of Cx26-containing hemichannels decreases by only 10% when a cell is depolarized from -100 mV to 0mV (Barrio et al., 2000). We have therefore not included a voltage-dependent modulation of hemichannel conductance in our analysis, assuming instead that the hemichannels in HC dendrites remain as open in the depolarized (dark adapted) state as they are in a maximum hyperpolarized (saturated light) state, a condition that favors the hemichannel-mediated electrical feedback hypothesis.

For simplicity we have ignored a nonlinearity in $R_k$. In addition, since we were interested in the final amplitude of the potential, but not in its dynamics, membrane capacitance was not included in the circuit. We have also not included the conductances and batteries for other ions besides $K^+$ in the cell body. It is known that in the absence of glutamate the membrane potential of HCs is determined almost exclusively by $K^+$ (Tachibana, 1981). Taking into account other ions would decrease the transmembrane resistance of the soma and reduce the potential of the battery, thus decreasing the possible electrical feedback potential. The inclusion of only the $K^+$ battery in the analysis therefore maximizes the effect of electrical feedback. For the same reason our idealized “horizontal cell” lacks an axon terminal.
Finally, electrical coupling between HCs was also not included in the analysis, since we analyzed feedback from an individual HC to a cone. Our virtual “illumination” is always uniform and our modeled horizontal cell responds to light with its maximum possible hyperpolarization. Thus coupling cannot affect this response.

The above described electrical circuit is an appropriate model of the cone-HC synapse because 1) it consists of the most essential elements, 2) the electrical values of its elements can be estimated reasonably well, and 3) the circuit is able to reproduce HC light-induced activity and electrical feedback to cones. In darkness, when cones release glutamate, the glutamate-gated channels on HCs are open. As a result, $R_g$ is minimal, the shunt of the HC membrane is maximal, and the transmembrane potential of the HC body (between points 1 and 4 in Fig. 2) is minimal. The HC is thus depolarized. When cones are illuminated, $R_g$ in the HC dendrites that contact them increases, the shunt of the HC membrane decreases, the current across $R_k$ diminishes, and the HC membrane potential shifts closer to $E_k$, i.e. the HC hyperpolarizes. This hyperpolarization increases the current across the dendrites that contact “nonilluminated” cones, and consequently, the feedback potential $V_f$ across the extracellular resistance inside the invagination in the cone pedicle, $R_f$, increases. The increase in $V_f$ is equal to the local depolarization of the cone presynaptic membrane. Thus the model reproduces electrical feedback from HCs to cones. The principal question is whether the feedback electrical signal is large enough to produce a significant effect on the cone presynaptic membrane.
RESULTS

Derivation of Values for the Circuit Elements

The values of the circuit elements can be estimated from the extant literature and especially from data provided by Kamermans and coauthors (2001, note 17). According to their measurements, when both glutamate channels and hemichannels were blocked, the HC membrane potential was equal to -82.7 mV. In this condition the shunting conductance in the HC dendrites was eliminated, and the HC membrane potential was equal to the battery in the cell body, e.g. $E_k = -82.7$ mV. Because the average resting potential of HCs in the dark (i.e. when glutamate channels are open) is -34.7 mV, and blocking the glutamate-gated conductance leads to a hyperpolarization of -36.7 mV (to -71.4 mV) (Kamermans et al, 2001), we can conclude, based on Ohm’s law, that the contributions of potassium, glutamate-gated, and hemichannel conductances to the total cell conductance are 42.0 %, 51.4 %, and 6.6 %, respectively. The fact that the potassium and glutamate-gated cationic conductances are approximately equal and together provide most of the total HC membrane conductance is well known and enables the HC membrane potential to change from approximately -40 mV in the dark, when the glutamate channels are open, to about -80 mV in the light, when the glutamate channels are closed. It is also unlikely that the contribution of the hemichannels could be much larger than 6.6 %. Because they are located at the tip of HC dendrites, the hemichannels provide a constant shunt that interferes with the postsynaptic current through the glutamate-gated channels. The larger the relative conductance of the hemichannels, the smaller the postsynaptic signal that can be generated in the HC. Figure 3A illustrates the relationship between the relative conductance of the hemichannels, $G_h$, shown as a percentage of the total HC transmembrane conductance and the amplitude of the changes in the membrane potential of the HC body, $\Delta V_b$, (% of maximum) which are produced
by modulation of the postsynaptic glutamate-gated conductance. The conductance of the potassium channels is fixed at 42%, and the conductance of the hemichannels increases at the expense of the glutamate conductance. The response of the HC when the relative conductance of the hemichannels equals 0 is defined as 100 % and even a small constant shunt from the hemichannels significantly reduces $\Delta V_b$. If $G_h = 6.6 \%$, the glutamate-dependent change of the HC membrane potential is approximately 75 % of its maximum value, and if $G_h = 18 \%$, only half of the maximal $\Delta V_b$ can be evoked. Thus, it is unlikely that the hemichannels contribute more to the total membrane conductance than the estimated value of 6.6 %. Otherwise, it would be in direct conflict with the main function of the synapse, which is to transfer a signal from the cone to the HC.

If the relative conductances of potassium channels, glutamate-gated channels, and hemichannels are known, one can estimate the values of individual resistors in the circuit (Fig. 2). The potassium conductance is represented in the circuit by one resistor, $R_k$, while each HC dendrite has its own glutamate-gated and hemichannel conductances. In the scheme in Figure 2 only three “dendrites” are shown, but 20 dendrites have been used in the calculations, because each goldfish cone HC connects with approximately 20 cones (23 for H1 type, and 17 for H2 type - Stell and Lightfoot, 1975). As a result, the portions of the total HC conductance provided by $R_k$ and each individual $R_g$ and $R_h$ are 42%, 2.57 % and 0.33 %, respectively. The absolute values of the resistors depend on the total resistance of the HC membrane, or the input resistance of the HC. For most calculations we have used an input resistance, $R_{in}$, equal to 50 M$\Omega$. The input resistance is the total resistance of all the transmembrane resistors, consisting of $R_k$, 20 $R_g$, and 20 $R_h$, connected in parallel. As a result, $R_k$, $R_g$ and $R_h$ have values of 119.05, 1945.53 and 15151.5 M$\Omega$, respectively. We have used these values in our calculations because a lower input
resistance favors electrical feedback. In fact, the input resistance of goldfish HCs is likely not less than 100 MΩ. Measurements of the input resistance of freshly isolated goldfish HCs obtained in our laboratory (Gavrikov and Mangel, unpublished observations) were always greater than 200 MΩ, which is similar to the calculated value of 307 MΩ (the surface area of goldfish HC soma is 1300 µm² - Yagi and Kaneko, 1988; the specific membrane resistance is 4000 Ω*cm²).

The most important element of the model with respect to electrical feedback is the feedback resistance of the extracellular space within the invagination of the cone pedicle, Rf. Kamermans and colleagues (2001, note 20) estimated its value as 6 to 60 MΩ. This estimate was based on morphometry data from goldfish retina (Vandenbranden et al, 1996) and seems high, but reasonable. For the calculations here we have used the highest value (60 MΩ) estimated by Kamermans and colleagues in order to favor the possibility of electrical feedback. It should be noted, however, that if Rf were greater than 60 MΩ, it would have negative effects on transmission of the synaptic signal from cone to HC. Figure 3B shows the relationship between the resistance of the extracellular space within the invagination of the cone pedicle (Rf, MΩ) and the amplitude of the maximum “light-induced” change in HC membrane potential (ΔVb, %). Increasing Rf to ~ 100 MΩ has little or no effect on the HC response, but when Rf increases to higher values, these resistors begin to isolate the dendrites from the body and ΔVb is dramatically reduced.

The resistance of the extracellular space outside the invagination of the cone pedicle is much lower compared to Rf if it is calculated per 1 µm of dendritic length, but has a larger, significant value when the entire length of the dendrite is considered. This extracellular resistance can be approximated from the geometry of the HC dendritic tree (diameter of the
The dendritic field is 50 µm, diameter of the cell body is 10 µm (Stell and Lightfoot, 1975), the thickness of the outer plexiform layer (5 µm), and the extracellular space volume fraction in the outer plexiform layer (11% - Karwoski et al, 1985). Because our modeled “cell” has 20 dendrites to share the extracellular space and assuming the specific resistance of the extracellular fluid is the same as that which was used for the estimation of Rf, the total extracellular resistance per 1 dendrite of 20 µm length is about 6 MΩ. The intracellular resistance of the dendrite should be larger simply because the dendritic end is very thin. If the diameter of the dendrite at the entrance of the cone pedicle is 0.3 µm, it has a resistance of 8.5 MΩ per 1 µm of length, assuming that the mobility of ions in the cytoplasm is the same as in the Ringer (It should be less because most intracellular anions are macromolecules with low mobility, the cytoplasm is filled with organelles, and the colloid density of the cytoplasm is apparently higher than that of the Ringer). Thus, several µm of the most distal part of the HC dendrite can have a resistance that is comparable to the feedback resistance. We define the value of Rr, which represents the extracellular resistance outside the invagination of the cone pedicle and all the intracellular resistance, as 30 MΩ. In fact, Rr is the element that is the most difficult to estimate, but as it apparent from Figures 4 and 5, Rr has a very small impact on the results of the calculations.

**The Feedback Resistance inside the Cone Pedicle and the Input Resistance of HC determine the Value of the Feedback Potential**

The feedback potential (Vf), which represents the extent that the potential of the cone presynaptic membrane is different from the membrane potential of the rest of the cone, depends on the resistance of the extracellular space within the invagination of the cone pedicle, which is defined here as the feedback resistance, Rf. Figure 4 demonstrates the relationship between
changes in the feedback potential ($\Delta V_f$) and the feedback resistance $R_f$. Kamermans and colleagues believed that $\Delta V_f$ could be as large as 10 mV (Kamermans et al., 2001, note 20). However according to our calculations a value of 10 mV can be reached only if $R_f$ is about 2000 MΩ (solid line, Fig. 4). A larger value of $R_f$ interferes with the ability of HCs to respond to light-induced modulation of glutamate-gated channels (see Fig. 3B), and the feedback potential $V_f$ decreases. Kamermans and his colleagues estimated that $R_f$ was between 6 and 60 MΩ (Kamermans et al., 2001), which is in agreement with our estimations, and in this range of $R_f$, the feedback potential $V_f$ is between 0.1 and 1 mV (see insert in Fig. 4).

It is important to state that all of the calculations here were performed using the largest possible changes of the HC membrane potential. The largest changes of the membrane potential of our modeled HC occur when the glutamate channel resistances $R_g$ of all but one dendrite - that which ends inside the cone targeted for feedback - increase from their standard “dark” value to infinity. This mimics the physiological condition in which the dark adapted retina is stimulated in such a manner that the cones connected with the 19 dendrites of the HC are illuminated by a saturating light, but the cone targeted for feedback is not illuminated at all. Obviously, this is a purely theoretical situation that rarely, if ever, occurs in a real visual environment.

This result was obtained with $R_r$ equal to 30 MΩ, but did not change much when $R_r = 300$ MΩ (dotted line, Fig. 4) and almost did not change when $R_r = 0$ MΩ (dashed line, Fig. 4). Thus, the model is stable with respect to $R_r$, the intra- and extracellular resistance along the HC dendrite, the least reliably established parameter.

In the calculations above, the glutamate channels in the HC dendrite inside the pedicle of the cone targeted for feedback were open (i.e. the cone was “in darkness”) and served as the main sink for the current that was responsible for $V_f$. When these glutamate channels were closed (the
cone targeted for feedback was “illuminated”), only the hemichannels provided a sink for the current. The conductance of the hemichannels is eight times less than the conductance of the glutamate channels (Kamermans et al., 2001, note 17), so changes in Vf were much smaller under these conditions (line with alternating dots and dashes, Fig. 4). At Rf = 60 MΩ, the ΔVf was slightly more than 0.1 mV (see insert on Fig. 4).

The above-described results were obtained when the input resistance (Rin) of the “cell” was 50 MΩ. In Figure 5, ΔVf was plotted against Rin. Here and in all other calculations Rf is constant and equal to 60 MΩ. As was expected, lowering Rin increased ΔVf. However the changes never exceeded 7 mV in calculations that took into account the intracellular and extracellular resistances (Rr = 30 MΩ, solid line, Fig. 5). When all the intracellular and extracellular (except inside the synaptic invagination, Rf) resistances were ignored (Rr = 0), ΔVf could reach a value of 10 mV, but only when Rin was as low as 2 MΩ (dashed line, Fig. 5), a value which is two orders of magnitude smaller than the input resistance of HCs that has been measured experimentally (Tachibana, 1981). When Rin = 50 MΩ, which is probably its lowest reasonable value, the feedback signal ΔVf was about 1 mV. Again, if the glutamate-gated channels were blocked and only the hemichannels supported electrical feedback, as would occur during light illumination, ΔVf was negligibly small (line with alternating dots and dashes, Fig. 5).

The results of the calculations presented in Figs. 3 and 4 suggest that when reasonable electrical parameters are used, individual HCs are able to produce only a very small electrical feedback potential, which can hardly serve as an effective feedback signal to the cone. Yet, it has been noted that not one, but several HC dendrites invaginate in the cone pedicle, and that the extracellular currents generated by each of the dendrites could have an additive effect on the local
membrane potential of the presynaptic area (Kamermans et al, 2001, note 20). However, in order to sum the feedback currents from several HCs, their dendrites have to share the same extracellular resistance, i.e. these dendrites have to end in the same invagination, not just in the same pedicle. It is well known that only two HC dendrites are typically located in one invagination in a cone pedicle (Dowling, 1987), constituting together with the bipolar cell dendrite a structure that is called the triad. Thus, two HC dendrites in one invagination would double the size of the feedback current across $R_f$, generating a 2 mV feedback potential $V_f$, instead of the 1 mV feedback potential that one HC can produce under the most favorable ‘light stimulation” conditions, when the most favorable assumptions for the electrical characteristics of the system are used.

**Light/Dark Adaptation State Greatly Influences Electrical Feedback**

The conductance of the glutamate-gated channels is significantly larger than the conductance of the hemichannels (Kamermans et al, 2001, note 17; see also Fig. 3A), and accordingly, the glutamate channels could support a larger feedback current than the hemichannels. But light reduces the glutamate conductance and the glutamate-mediated electrical feedback should therefore be most effective in darkness. To characterize the effectiveness of electrical feedback we have used the ratio $\Delta V_f / \Delta V_b$, where $\Delta V_f$ is the change in the feedback potential and $\Delta V_b$ is the change in the membrane potential of the HC body. This ratio shows how large the electrical feedback signal is per 1 mV change in the membrane potential of the cell body. The effectiveness of negative feedback was calculated separately for glutamate-mediated (solid line in Fig. 6) and hemichannel-mediated (dots and dashed line in Fig. 6) mechanisms, and was plotted against the reduction of the light-regulated glutamate conductance, which mimicked
the increase in “background illumination.” As expected, Byzov’s glutamate-mediated mechanism is most effective in “darkness” when all of the glutamate channels of the HC dendrite are open. In this situation each 1 mV hyperpolarization of the HC body will locally depolarize the cone presynaptic membrane by 0.029 mV. The effectiveness of glutamate-mediated electrical feedback is reduced when the glutamate conductance is blocked by light. When 90% of the glutamate conductance is blocked, the glutamate-mediated mechanism is less effective than hemichannel-mediated electrical feedback, which is independent of light but able to produce only about 0.004 mV ΔVf with 1 mV of membrane potential change in the HC body. These calculations indicate that electrical feedback is greatest under dark-adapted conditions. Consequently, the well known experimental finding that negative feedback is most effective under light-adapted conditions (Baylor et al, 1971; Kamermans et al, 2001) cannot be explained by the hypothesized electrical mechanism.

**Positive Electrical Feedback**

The electrical parameters that generate electrical negative feedback inevitably also produce *positive* electrical feedback (Byzov and Shura-Bura, 1986; Kamermans et al, 2001). In the case of *negative* feedback, the feedback potential Vf increases because hyperpolarization of the HC membrane outside of the invagination enhances the current through the dendrite in which the transmembrane resistance does not change. It mimics the physiological condition during which the cone that is targeted for feedback does not experience changes in illumination, but the cones around it are illuminated. When the cone targeted for feedback is illuminated, it decreases its release of glutamate, and the resistance of the glutamate channel in the HC dendrites postsynaptic to this cone (Rg) increases. Reduction of the transmembrane shunt leads to
hyperpolarization of the HC. But in this case, the current through the dendrite, and consequently the feedback potential $V_f$ decreases. The decrease in $V_f$ produces a more positive potential on the outside of the cone terminal membrane, which is equivalent to a local hyperpolarization of the cone presynaptic membrane (see Fig. 1). Thus, a light-induced hyperpolarization of the cone leads to a hyperpolarization of the HC, which in turn produces an additional hyperpolarization of the cone, i.e. positive electrical feedback is established.

One important feature of electrical positive feedback in the cone pedicle is that its effectiveness should always be higher than the effectiveness of electrical negative feedback because of a simple electrophysiological reason. For both negative and positive feedback the key element that defines the amplitude of the feedback potential is the resistance of the extracellular space ($R_f$) inside the synaptic invagination within the cone pedicle. But the other resistor with which $R_f$ divides the potentials is different for negative and positive feedback. As was explained earlier, the negative feedback potential depends on the ratio of $R_f$ and the transmembrane resistance, $R_d$, of the tip of the HC dendrite, which consists of the resistance of glutamate-gated channels, $R_g$, and the resistance of the hemichannels, $R_h$, connected in parallel (see Figs 1 and 2). In contrast, positive feedback depends on the ratio of $R_f$ and the resistance of the entire HC membrane, except for the one synaptic terminal that generates the changes in current. This resistance, $R_b$ (see Fig. 2), is obviously lower than the resistance of one dendrite $R_d$, because it consists of the resistances of other dendrites and the resistance of the HC body connected in parallel. Consequently, the ratio $R_f/R_b$, which determines the extent of positive feedback, is larger than the ratio $R_f/R_d$, which determines the extent of negative feedback. As a result, the negative feedback potential generated by illumination of all the cones connected with the HC, except for the cone targeted for feedback (dashed line in Fig. 7), is about the same or even
smaller (with Rh > 100 MΩ) than the positive feedback potential generated by illumination of only one cone (dotted line in Fig. 7). In addition, the light-induced decrease in glutamate conductance reduces the effectiveness of negative feedback (see Fig. 6). When all of the cones that contact the HC are equally illuminated, the extent of positive feedback greatly exceeds that of negative feedback and the total electrical effect on the cone presynaptic membrane is a hyperpolarization (solid line in Fig. 7).

**DISCUSSION**

Using a realistic model of the cone-HC synapse that investigated a wide range of values for electrical parameters (e.g. Rf: 1-10,000 MΩ; HC input resistance: 1-1,000 MΩ) and that incorporated values for these electrical parameters that favor electrical negative feedback, our calculations were performed to quantitatively determine whether an electrical mechanism could be effective enough to account for negative feedback from HCs to cones. An effective electrical feedback mechanism in any of its variations is possible only if one condition is met: the extracellular resistance inside the synaptic invagination in the cone pedicle (Rf) must be comparable in value to the transmembrane postsynaptic resistance (the total resistance of the glutamate channels in one synaptic terminal of the horizontal cell (Rg) in Byzov’s model (Byzov, 1977; Byzov and Shura-Bura, 1986) or the total resistance of the hemichannels (Rh) in one synaptic terminal of the horizontal cell in the model of Kamermans et al. (2001)). If Rf is small compared to Rg or Rh, the electrical mechanism is not effective. Although one might speculate that Rf is relatively large and/or that Rg or Rh are relatively small, our analysis shows that this is unlikely. In addition, our calculations show that the values of the extracellular and transmembrane resistances must be within specific narrow ranges, because they are limited by
the necessity of preserving adequate cone to HC signal transfer. The conditions under which
electrical negative feedback could and could not occur are discussed below.

The key element of an electrical feedback model is the resistance of the extracellular
space inside the synaptic invagination in the cone pedicle (Rf). We show in Fig. 4 that Rf must
be very large (~ 3 GΩ) to support an electrical mechanism that produces significant (10 mV or
larger) negative feedback, and that this occurs only under dark-adapted conditions when the
 glutamate-gated conductance is at its maximum. Under bright illumination when the glutamate-
gated conductance is blocked and the hemichannel conductance, which we assume is not
suppressed by light, dominates the HC dendritic tip, electrical negative feedback is small in size
(<3 mV) even when Rf is very large (3-10 GΩ). However, it is very unlikely, that Rf is as large
as 3 GΩ. The value of Rf can be estimated with confidence based on the well known morphology
of the cone pedicle. The electrical resistance of the extracellular space has routinely been
estimated elsewhere in the CNS based on morphology and these estimates have agreed with
direct electrical measurements and with measurements that used diffusible extracellular space
markers (see Nicholson and Sykova, 1998). In the retina, measurements of electrical resistance
were used to determine the volume fraction of the extracellular space in different retinal layers
(Karwoski et al., 1985). Our calculations indicate that the extracellular space inside the
invagination in the cone pedicle has a resistance of about 10 MΩ, which is close to Byzov’s
estimation (15 MΩ, Byzov and Shura-Bura, 1986), and is in the range (6-60 MΩ) suggested by
Kamermans and his colleagues (Kamermans et al., 2001). A much larger Rf would occur only if
the extracellular space inside the invagination in the cone pedicle included a special structure
such as tight junctions, but such structures have never been observed in the cone pedicle of any
species. More importantly, our calculations show that if Rf exceeded 100 MΩ, it would produce
a significant obstacle for current, leading to electrical isolation of the HC dendritic tip and a compromise in signal transfer from cone to HC (Fig. 3b).

In the hemichannel-mediated variant of the electrical hypothesis, the effectiveness of feedback depends on the ratio $R_f/R_h$, where $R_h$ is the resistance of the hemichannels in one dendritic tip. Kamermans and coauthors have estimated $R_h$ as $24 - 240 \ \text{M} \Omega$ (Kamermans et al, 2001, note 20), but if this were the case, the HC input resistance would be improbably small. That is, if the resistance of the hemichannels is $240 \ \text{M} \Omega$, then the resistance of the glutamate channels in the same HC dendrite would be $\sim 30 \ \text{M} \Omega$, because the resistance of the glutamate channels is about 8 times less than the resistance of the hemichannels. Consequently, the total resistance of the glutamate channels in 20 HC dendrites would be $\sim 1.5 \ \text{M} \Omega$. Because the glutamate channels provide about half of the total HC transmembrane conductance (see Derivation of Values for the Circuit Elements in RESULTS), the input resistance of the HC would be less than $1 \ \text{M} \Omega$, which is more than two orders of magnitude lower than the input resistance that has been measured experimentally (Tachibana, 1981). In our analysis, $R_h$ was calculated based on the following properties of the HCs: 1) the input resistance of the horizontal cell is $50 \ \text{M} \Omega$, a value that is several times smaller than has been experimentally measured (Tachibana, 1981), but one that is more favorable for effective electrical feedback; 2) the hemichannels are responsible for $6.6 \%$ of the total transmembrane conductance of the cell (see Derivation of Values for the Circuit Elements in RESULTS); and 3) the hemichannel-mediated conductance is distributed between 20 HC dendrites. Using these three properties, a realistic estimate of $R_h$ is about $15,000 \ \text{M} \Omega$, and as a result, the ratio $R_f/R_h$ is too small to generate a significant electrical feedback signal, as suggested previously (Schwartz, 2002). In fact, this resistance corresponds to a conductance of $66 \ \text{pS}$, suggesting that the hemichannels are mostly in
a closed state. Moreover, it should be noted that even in this low conductive state the hemichannels represent a significant transmembrane shunt that diminishes the glutamate-mediated response of HCs by 25% (Fig. 3a). An increase in the hemichannel conductance would further compromise cone to HC synaptic transfer. For example, reduction of Rh by even a factor of four from 15,000 $\text{M} \Omega$ to 3,750 $\text{M} \Omega$, which corresponds to a conductance of about 260 pS, would reduce the size of HC light responses to one third of their maximum value.

Thus, our calculations show that the hemichannels that are located at the tips of HC dendrites likely do not generate significant electrical negative feedback. Even if it is assumed that the resistance of the extracellular space in the synaptic invagination within the cone pedicle is as large as 60 $\text{M} \Omega$ and the input resistance of HCs is as small as 50 $\text{M} \Omega$, the feedback current through the sink provided by the hemichannels can locally depolarize the presynaptic membrane of the cone by only 0.10 – 0.15 mV (Fig. 4, insert) when the HC is maximally hyperpolarized by saturating illumination of all cones connected with it except for the cone targeted for the feedback.

The glutamate-gated channels in the dendritic tips of HCs can support a larger current for the generation of a negative feedback potential, compared to the hemichannels, because the glutamate channels have about 8 times smaller resistance than the hemichannels (Kamermans et al, 2001). However, using realistic electrical values (see above), the effectiveness of the feedback mechanism mediated by the glutamate channels would still be only $\sim$ 3%, e.g. a 30 mV HC hyperpolarization would depolarize the local cone presynaptic membrane by only $\sim$1 mV. Moreover, glutamate-mediated electrical negative feedback would be most effective under dark-adapted conditions when the glutamate-gated channels are open and their resistance is lowest (Fig. 6). This feature of glutamate-mediated electrical negative feedback directly contradicts the
experimental data that show that feedback from HCs to cones is greatest under light adapted conditions (Baylor et al, 1971; Kamermans et al, 2001).

Finally, the electrical circuitry characteristics that generate negative feedback in the cone-HC synapse, namely, the very high resistance of the extracellular space inside the synaptic invagination within the cone pedicle and the relatively low transmembrane resistance of the HC dendritic tips, also produce positive feedback from HCs to cones. Moreover, because the positive feedback at the cone-HC synapse appears to be much more effective than the negative feedback, it would manifest itself more prominently than the negative feedback. In other words, the electrical characteristics of the cellular elements in the cone pedicle necessitate that any increase in the extracellular space resistance will increase positive feedback more than negative feedback. It should also be noted that in contrast to negative feedback, which utilizes both glutamate-gated channels and hemichannels proportionally to their conductances, positive feedback relies only on glutamate-gated channels. Potentially, hemichannels could indirectly diminish positive feedback because their presence in the postsynaptic membrane of HC dendritic tips produces a constant electrical shunt that diminishes HC light-induced responses. Thus if such hemichannel-mediated reduction of positive feedback occurs, it would only be at the expense of reducing HC light-induced activity, and negative feedback would also be reduced.

The presence of strong positive feedback is a feature that is difficult to associate with the gradual character of cone and HC light responses. When positive feedback occurs, a system tends to respond in an “all-or-nothing” manner. Thus, if positive feedback were present, a small light-induced cone response would generate a maximal response in the HC dendrite. However, the experimental data show that there is no such positive feedback in the cone pedicle. The probable explanation of the absence of positive feedback in the cone-HC synapse is that the actual
electrical characteristics of the circuit are not as favorable for electrical feedback as they were in our calculations. For example, if $R_f$ has a value of 10 MΩ, rather than 60 MΩ as used in our calculations, the positive feedback potential would not exceed 0.1 mV when the glutamate-gated conductance maximally changed. Accordingly, the negative feedback potential is probably even smaller than was estimated in our calculations.

Thus, according to the computational analysis performed here, it seems doubtful that an electrical mechanism could be responsible for negative feedback from HCs to cones. Hemichannel-mediated, compared to glutamate-mediated, electrical feedback appears to be especially problematic, and experimental data offered in support of the hemichannel hypothesis are not convincing. As has been noted, in the best case an electrical feedback mechanism can only produce a local depolarization of the cone presynaptic membrane, a phenomenon that cannot be measured when an intracellular electrode monitors the cell body of the cone. Thus the effects of the gap junction blocker carbenoxolone on the depolarizing response of a cone evoked by surround illumination (Kamermans et al, 2001) cannot be used as evidence for or against the electrical model. Moreover, carbenoxolone produces several other effects in addition to blocking gap junctions. For instance, carbenoxolone inhibits the $Na^+$-$K^+$-ATPase (Zhou et al, 1996), and in retina, it has been shown that carbenoxolone reduces the light-evoked responses of photoreceptors (Verweij et al, 2003). Most importantly, inhibitory effects of the drug on Ca channels in photoreceptors have recently been demonstrated (Vessey et al, 2004). The carbenoxolone-induced block of the Ca channels in the photoreceptor presynaptic terminal results in the cessation of glutamate release and may explain why the drug hyperpolarizes HCs and significantly reduces their light-evoked responses (Kamermans et al, 2001; Vessey et al, 2003; Pottek et al, 2003). When HC light-evoked responses are reduced, negative feedback from HCs
to cones should also be decreased. Hirasawa and Kaneko have also pointed out that changes in the I-V relationship of the cone calcium current evoked by surround illumination are inconsistent with the purely parallel shift toward a negative potential that is predicted by the electrical hypothesis (Hirasawa and Kaneko, 2003). Finally, it should also be noted that the presence of hemichannels in the HC dendritic tip might produce additional problems because of leakage of various molecules, including neurotransmitters, directly into the synaptic cleft (Schwartz, 2002).

To summarize, it is unlikely that an electrical mechanism plays a significant role in negative feedback from HCs to cones. The nature of the negative feedback mechanism thus still remains to be defined. One possibility, which recently received strong experimental support (Hirasawa and Kaneko, 2003; Vessey et al., 2005), is that protons mediate negative feedback from HCs to cones. Consistent with this idea is the finding that the circadian clock in the retina decreases retinal pH to its lowest level at night, when negative feedback from HCs to cones is weakest (Dmitriev and Mangel, 2000, 2001).

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REFERENCES


**FIGURE LEGENDS**

**Figure 1** Basic features of electrical feedback from HCs to cones.

Eb – *variable* battery of HC membrane potential; Ec – battery of cone membrane potential; Es – battery of the cone presynaptic membrane; Rb – transmembrane resistance of HC outside the cone pedicle; Rd – resistance of the tip of the HC dendrite within the cone pedicle; Rs – resistance of the cone presynaptic membrane; Rc – resistance of remaining part of the cone membrane; Rf – resistance of the extracellular space inside the invagination of the cone pedicle.

**Figure 2** The “parallel shunt” model of an individual HC.

Ek – K\(^+\) equilibrium potential; Rk – transmembrane resistance of the HC (primarily determined by K\(^+\)); Rg – *variable* resistance of the glutamate-gated channels of the HC dendrites; Rh – resistance of the hemichannels on the HC dendrites; Rr – extracellular resistance outside the cone pedicle plus intracellular resistance for each HC dendrite; Rf – resistance of the extracellular space inside the invagination of the cone pedicle.

**Figure 3** Synaptic transmission from the cone to the HC is compromised by the high relative conductance of the hemichannels (A) and by the high “electrical feedback” resistance (B).

A – Dependence of the amplitude of HC “light-induced” responses (ΔVb, in % of maximum) on relative conductance of the hemichannels (G\(h\), in % of total HC conductance). The estimated value of the hemichannel contribution to the total conductance of the HC (6.6%) is indicated with a vertical dashed line. The presence of hemichannels in the HC dendritic tip compromises the
main function of the synaptic terminal, which is to transfer a signal from the cone to the HC. \( R_{in} = 50 \, \text{M}\Omega, R_f = 60 \, \text{M}\Omega. \)

B - Dependence of the amplitude of HC “light-induced” responses (\( \Delta V_b \), in % of maximum) on the extracellular resistance within the invagination of the cone pedicle (\( R_f \)). A large value for \( R_f \) decreases synaptic currents and disturbs transfer of the postsynaptic potential to the HC body. \( R_{in} = 50 \, \text{M}\Omega. \)

**Figure 4** Relationship between the feedback potential (\( \Delta V_f \), in mV) and the extracellular resistance within the invagination of the cone pedicle (\( R_f \), in M\( \Omega \)).

Solid line - glutamate channels open (“dark”), \( R_r = 30 \, \text{M}\Omega \); dashed line - glutamate channels open (“dark”), \( R_r = 0 \, \text{M}\Omega \); dotted line - glutamate channels open (“dark”), \( R_r = 300 \, \text{M}\Omega \); the line with alternating dashes and dots – only hemichannels open (“saturated light”), \( R_r = 30 \, \text{M}\Omega \). The insert depicts the portion of the graph near the expected value of \( R_f \) (linear scale). The largest expected value of \( R_f \) was estimated as 60 M\( \Omega \). Even when \( R_f \) was this large, the change in feedback potential slightly exceeded 1 mV under the most favorable light stimulation conditions. \( R_{in} = 50 \, \text{M}\Omega. \)

**Figure 5** Relationship between the feedback potential (\( \Delta V_f \), in mV) and the input resistance of HCs (\( R_{in} \), in M\( \Omega \)).

Solid line - glutamate channels open (“dark”), \( R_r = 30 \, \text{M}\Omega \); dotted line - glutamate channels open (“dark”), \( R_r = 0 \, \text{M}\Omega \); line with alternating dashes and dots - hemichannels only (“saturated light”), \( R_r = 30 \, \text{M}\Omega \). The insert depicts the portion of the graph near the expected value of \( R_{in} \).
(from 50 to 100 MΩ). In order to generate a change in feedback potential of 5 mV, the HC input resistance must be lower than 7 MΩ. Rf = 60 MΩ.

**Figure 6** Relationship between the effectiveness of electrical feedback (ΔVf/ΔVb) and the intensity of “illumination” (in % of glutamate-gated conductance suppressed by light). Solid line - negative feedback through glutamate channels; line with alternating dashes and dots - negative feedback through hemichannels. Glutamate-gated channels are potentially more effective than hemichannels in supporting a feedback current. However, when glutamate-gated channels mediate feedback, the feedback from HCs to cones is most prominent under dark-adapted conditions. Rin = 50 MΩ, Rf = 60 MΩ.

**Figure 7** Relationship between the potentials generated by positive and negative feedback (ΔVf, im mV) and the extracellular resistance within the invagination of the cone pedicle (Rf, in MΩ). Dashed line - negative feedback; dotted line - positive feedback; solid line - both negative and positive feedback. When all cones are illuminated and both positive and negative feedbacks are present, positive feedback dominates negative feedback, especially at high values of Rf. Rin = 50 MΩ.
**Figure 1** Basic features of electrical feedback from HCs to cones.
Figure 2 The “parallel shunt” model of an individual HC.
**Figure 3** Synaptic transmission from the cone to the HC is compromised by the high relative conductance of the hemichannels (A) and by the high “electrical feedback” resistance (B).
**Figure 4** Relationship between the feedback potential ($\Delta V_f$, in mV) and the extracellular resistance within the invagination of the cone pedicle ($R_f$, in MΩ).
Figure 5 Relationship between the feedback potential ($\Delta V_f$, in mV) and the input resistance of HCs ($R_{in}$, in MΩ).
Figure 6 Relationship between the effectiveness of electrical feedback ($\Delta V_f / \Delta V_b$) and the intensity of “illumination” (in % of glutamate-gated conductance suppressed by light).
**Figure 7** Relationship between the potentials generated by positive and negative feedback ($\Delta V_f$, in mV) and the extracellular resistance within the invagination of the cone pedicle (R_f, in MΩ).