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NEURAL NETWORK CONTRIBUTION TO LIGHT ADAPTATION: FEEDBACK FROM HORIZONTAL CELLS TO CONES

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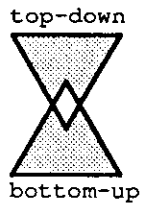

UNIVERSITY OF CALIFORNIA

LOS ANGELES

NEURAL NETWORK CONTRIBUTION TO LIGHT ADAPTATION: FEEDBACK FROM HORIZONTAL CELLS TO CONES

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SUMMARY

Vertebrate cones respond to a stepwise increase in localized light intensity with a graded potential change of corresponding amplitude. This S-shaped intensity-response (I-R) relation is limited to 3 log units of the stimulating light and yet, cone vision remains functional between twilight and the brightest time of day. This is in part due to light adaptation mechanism localized in the outer segment of a cone. The phenomenon of light adaptation can be described as a resetting of the system's response-generation mechanism to a new intensity domain that reflects the ambient illumination. In this project we examined spatial effects of annular illumination on resetting of I-R relation by measuring intracellular photoresponses in cones. Our results suggest that peripheral illumination contributes to the cellular mechanism of adaptation. This is done by a neural network involving feedback synapse from horizontal cell to cones. The effect is to unsaturate the membrane potential of a fully hyperpolarized cone, by "instantaneously" shifting cone's I-R curves along intensity axis to be in register with ambient light level of the periphery. An equivalent electrical circuit with three different transmembrane channels leakage, photocurrent and feedback was used to model static behavior of a cone. SPICE simulation showed that interactions between feedback synapse and the light sensitive conductance in the outer segment can shift the I-R curves along the intensity domain, provided that phototransduction mechanism is not saturated during maximally hyperpolarized light response.

INTRODUCTION

The phenomenon of light adaptation (LA) was shown to be localized in isolated photoreceptors (Nakatani & Yau, 88; Matthews et al. 88; Norman & Werblin, 74) Although the cellular mechanisms are known to be principal to light adaptation the effect of neural network interactions on this process remains unclear (see Witkovsky, 80) The mechanism for fast adjustment of sensitivity was localized to bipolar cells in the mudpuppy retina (Werblin, 74) and to isolated cones in the tiger salamander retina (Nakatani and Yau 1988). In this paper we present the evidence for the complementary mechanism of fast neural adaptation in cones that are embeded in the retinal network. It is based on the idea that in the tiger salamander retina, a neural network interactions involving feedback from horizontal cells to cones can help to reset cone's photoresponse generating mechanism.

Light response in cones

In the vertebrate retina, cones respond to a small spot of light with sustained hyperpolarization which is graded with the stimulus over three log units of intensity (Baylor & Fuortes, 70; Werblin & Dowling, 69). Mechanisms underlying this I-R relation was suggested to result from statistical superposition of invariant single-photon responses (Lamb, McNaughton & Yau, 81). In the dark an inward current flows through ionic channels in the outer segment of the receptor, resulting in depolarization of the cone membrane (Baylor et al., 74; Hagins et al., 70; Schnapf & McBurney, 80). Light reduces this current, presumably by using an intermediate agent that closes the channels (Fesenko et al. 85; Baylor et al., 74) thus hyperpolarizing the membrane. However, verification of this hypothesis was made difficult in the past, by the fact that the shape of the light-response is influenced by; 1) electrical coupling with neighboring photoreceptors (Detwiler & Hodgkin, 79), 2) antagonistic effect of feedback input from horizontal cells (Baylor et al., 71; O'Bryan, 73; Gerschenfeld et al., 80; Skrzypek & Werblin, 83) and 3) voltage and time dependent properties of the membrane itself (Baylor et. al., 74; Attwell et al, 82a; Lasansky & Marchiafava, 74).

Most of the recent evidence suggests that light-elicited hyperpolarization might result from sodium conductance changes that are gated by cyclic nucleotides (for review see Pugh & Altman, 88). Accordingly, ion channels in plasma membrane are maintained open by high levels of cyclic GMP in the dark. Light increases the hydrolysis of the nucleotides, resulting in closing of the channels. This blocks the entry of calcium into outer segment and with continuous activity of sodium-calcium pump, leads to local decline of internal calcium. Low internal calcium eventually leads to increase in cGMP and the opening of the channels.

The change in photoreceptors sensitivity can be monitored by recording I-R curves of the adapting cell. The I-R characteristics become compressed and are shifted to higher intensity levels that encompass background illumination (Normann & Werblin, 74; Normann & Pearlman,79). In rods, response compression resulting from the nonlinear relation between light and membrane potential is a dominating factor in sensitivity control (Matthews et al., 88; Penn & Hagins, 72; Normann & Werblin, 74;). Cones show minimal saturation effect, instead they shift their I-R curves to a new intensity domain (Normann & Werblin,74). And the range of light responses measured after adaptation remains unchanged (Normann & Pearlman, 79). Although it has been shown that key aspects of adaptation can be observed in isolated cones (Matthews et al., 88; Nakatani & Yau, 88; Normann & Werblin, 74), the effects of peripheral illumination on adaptation as related to feedback input from horizontal cells have not been examined.

The shape of the response measured in cones depends on the size of the stimulating spot of light. This is partially because of peripheral signals which are mediated by a negative feedback synapse from horizontal cells (O'Bryan, 73; Skrzypek, 79; Lasansky, 78). In other words, the hyperpolarizing response to the spot illumination in the central portion of the cone receptive field is antagonized by light in the surrounding periphery. Feedback to cones is responsible in part for center-surround antagonism in bipolar cells (Werblin & Dowling, 69) and horizontal cells (Skrzypek & Werblin, 83; Gerschenfeld, et al., 80; Piccolino, et al., 81). In addition, feedback was shown to be involved in color vision (Fuortes & Simon, 74). In all of these cases, cone membrane is under direct control of two antagonistic effects; 1) feedback, which is driven by peripheral illumination and 2) the light sensitive conductance, modulated by direct stimulation of the cone outer segment. Therefore, it is of interest to know the nature of interactions between feedback synapse and the response-generating mechanism in cones, particularly, after their membrane has been maximally hyperpolarized by light.

We report here a new finding which suggests that feedback from horizontal cells to cones can contribute to the neural component of light adaptation in cones. Specifically, peripheral signals mediated via feedback synapse reset the cone sensitivity by instantaneously shifting the I-R curves to a new intensity domain. The full range of light response potentials is preserved without noticeable compression.

MATERIALS AND METHODS

Preparation and the general experimental procedure were described previously in detail (Skrzypek, 84). Briefly, all experiments were performed using dry-eyecup preparation from the Tiger salamander. Intracellular recordings were made with single or double barrel micropipettes filled with 4M potassium acetate. For staining experiments, one of the two barrels was filled with 3% solution of Lucifer Yellow dissolved in 1M LiCl. Cones were identified on the basis of physiological criteria (Werblin & Dowling, 69; Kaneko,70; Lasansky, 81) and later confirmed by staining with Lucifer yellow (Stewart, 78). Light stimuli were generated by specially constructed double-beam photostimulator which could deliver concentric, circular and annular stimuli of white or monochromatic light. The flux density of the unattenuated light on the retina was about 15 mW/mm*mm.

Identification of cones

Several criteria were used to distinguish cones from other cells in the OPL. First of all, the depth of recording in the retina (Kaneko, 70) at which the cone was penetrated was correlated with histological evidence, as corresponding to the layer of cone inner segments. It also appears that at this depth, the only other cell bodies belong to rods (Lasansky, 73). After recording form a cone, further advancement of electrode sometimes resulted in penetration of a rod. On the other hand, after recording from bipolar cell, further advancement of electrode always produced impalements of horizontal cells followed by rods or at times cones. This sequence of penetrations concomitant with characteristic light responses was a very useful heuristic in differentiating cones from bipolars. One exception are cone pedicles, which could be penetrated and mistaken for bipolar cells, especially, if other impalements were obtained afterwords. Hence, it is important to emphasize that no single test alone could be used to differentiate cones from bipolar cells and only a sequence of tests followed by staining could give reasonable indication of the penetrated cell type. The identification of single cones was also based on spectral response curves. For the red cones, the only ones considered in this study, their peak in wavelength spectrum, fit well with absorption spectrum for red pigment (Hanani & Vallerga, 80; Attwell et al., 82a). The spectrum response curve for HC or bipolar cells were often flat or even peaked in the short wavelengths (Hanani & Vallerga, 81; Skrzypek, 79,84) Cones as compared to horizontal cells or bipolars, had smallest diameter for receptive field center (<75um) (Marshall & Werblin, 75). The average for bipolars was about 150 um and the smallest horizontal cells were found to be about 250um. Cones were also distinguished by the fastest time from dark potential to the peak of the light response (50 ms) as compared with bipolars (100 ms) or horizontal cells (>150 ms). One additional criterion was that the I-R curves for cones usually span 3 - 4 log units of intensity between 5% and 95% of the saturating membrane potential value. On the other hand the I-R curves in bipolars were found to span on the average 1.3 log units (Werblin, 77). These values represent averages derived from all intracellular recordings in 37 cones, 84 bipolar cells, more than 1000 horizontal cells, and more than 100 rods.

In some experiments the attempt was made to stain the physiologically identified cones with Lucipher yellow. A good staining was considered when the response of a cone did not deteriorate by more than 20%. Often, the center/surround antagonism would disappear or decrease significantly so that we could not complete the measurements of dynamic changes in I-R curves, but we could still record an unchanged center response. In most of these cases we attempted to fill the cone with the stain. The stained cells were viewed immediately after injections, under epifluorescent microscope, without going through conventional procedures of fixating and embedding tissue (Skrzypek, 79). While the stained HC and bipolar cells always showed extensive arborization of dendritic processes, protruding from the soma, this was not the case for cones which appeared as symmetrical spheres. In all 37 cones were identified by a combination of these criteria and partial results were obtained in most cases, including 19 attempts at staining. In six of these cells the experiments were completed but only in three of the six the staining was also successful.

RESULTS

Experimental procedure.

In order to validate the hypothesis that feedback contributes to resetting of the response-generating mechanism in the cone after its membrane potential has been hyperpolarized to some plateau level by the saturating, center-spot of light, we thought it sufficient to show that:

1. The effect of peripheral illumination, mediated by the negative feedback synapse from horizontal cells, can be measured in cones.

2. Annular illumination of proper dimension and intensity can restore the cone membrane potential to its resting level even after maximal hyperpolarization by the small spot of light.

3. Annulus of proper dimensions and intensity can shift the I-R curve of a cone to a new intensity domain

without compression or change of gain.

To test these criteria, in as short a time as possible, we have designed the following experiment. After successful identification of a cone, its I-R curve was recorded. Then, in a presence of center illumination (diameter = 100 um) which elicited maximal hyperpolarization from a cone, the periphery of the receptive field was stimulated with an annulus of inner diameter (ID) = 750 um and the outer diameter (OD) = 1500 um. The annular intensity was adjusted to elicit depolarization of the membrane back to the dark potential level. Finally, the center intensity was increased again in a stepwise manner to antagonize the effect of peripheral illumination, and this new I-R curve was recorded.

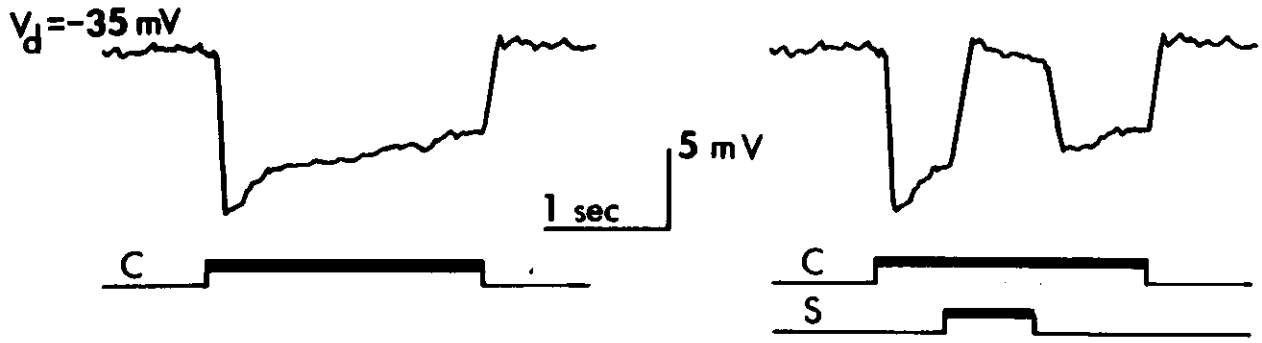

Fig. 1 Intracellular cone responses to a small spot of light (a) and to combined center and surround illumination (b). The diameter of the spot of light was approximately 300 um while the internal diameter of the annulus was set to 650 um. Intensity of the center was -3.0 log units and the surround illumination was set to -2.5 log units. White light was used it this and other experiments.

Annulus-elicited, depolarization in cones

Sustained illumination of a cone with a small spot of light, evokes a hyperpolarizing response, which after transient peak gradually repolarizes to some steady level (Fig. 1). When the periphery of the retina is illuminated with a ring of light in the presence of center spot, the antagonistic component of response can be recorded in a form of sustained depolarization. The peak of the center-light elicited response was about 10 mV in amplitude. This was completely antagonized by the surround-elicited response, which caused the membrane potential to return to resting level. Although light-scatter from the annulus to the center of the cone receptive field could not be completely avoided, its effect is only to underestimate the antagonistic effect of the depolarizing surround response. This scatter is perhaps partially responsible for the gradual hyperpolarization observed in the surround response after the transient, depolarizing peak (see discussion). Although we never observed a depolarizing response in cones when stimulated with an annulus alone (see also Lasansky & Vallerga, 75) such "sustained" response could be measured very clearly when the annulus was presented during the illumination with a small spot of light that first hyperpolarized the cone membrane. It has been argued previously that this type of response in cones is mediated via synaptic input from horizontal cells. In the tiger salamander cones, the sustained effects of feedback were previously reported by Lasansky & Vallerga (75); Skrzypek & Werblin, (78, 83); Lasansky (81). The significance of the feedback synaptic input from horizontal cells to cones has been also demonstrated in the turtle retina, by showing directly (Baylor et al.,71) that current injected into HC resulted in opposite polarity sustained response recorded intracellularly from a cone (see also O'Bryan, 73;). Assuming that the depolarizing response in cones is mediated by a feedback pathway from HC (see discussion), the result in fig.1. satisfies the first two criteria; the annulus can elicit depolarizing

response that completely antagonizes the center-elicited hyperpolarizing response by resetting the membrane potential back to the resting level.

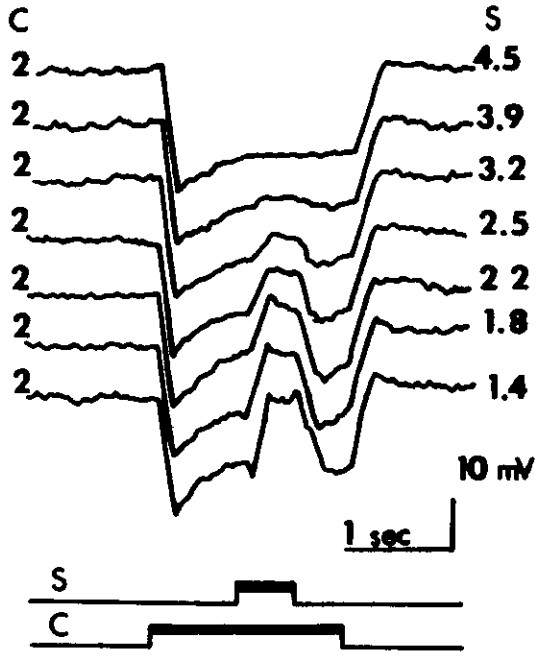

Fig. 2a. Series of responses to combined illumination of the central an d peripheral portion of the cone receptive field. Dimensions of the stimuli were set as follows: center spot diameter = 400 um; annulus, I. D. = 850 um, O.D. = 2 mm. Illumination of the center (C) was fixed at -2 l.u., while the intensity if the annulus (S) was increased as indicated by the numbers associated with each trace. Surround intensity of -1.4 l.u. could completely antagonize the hyperpolarizing response elicited by illumination of the center.

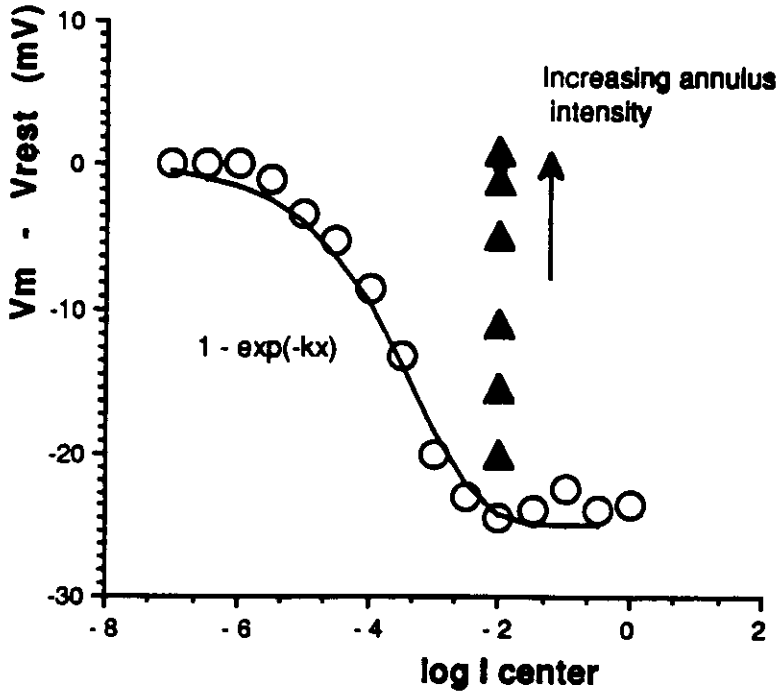

Fig. 2b. Amplitude of the response versus the logarithm of the central spot intensity (Log Ic) is shown

as filled circles in (b). Triangles represent the amplitudes of the depolarizing portion of the response in (a) at increasing annular intensities. The continuous curve was drawn according to expression 1-exp(-kx). Annular illumination repolarized the cone membrane to resting potential of -40 mV measured previously in the dark, although the central spot intensity was increased almost a thousand-fold.

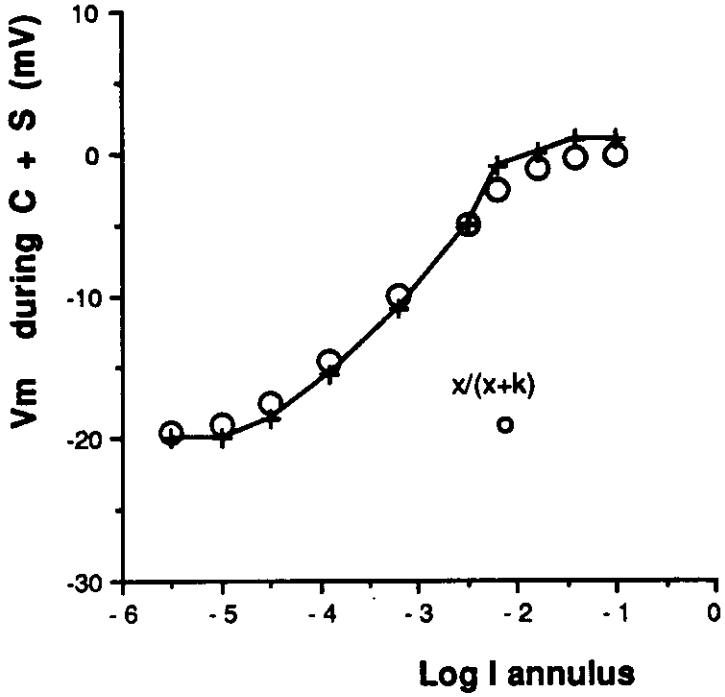

Fig. 2c. Plot of the surround-elicited depolarizing responses from (a) versus the logarithm of annular intensity (crosses connected by a continuous line). The curve spans about 3 log units of intensity and it fits reasonably well points (open circles) computed from equation x/(x+k). Light scatter form the surround to central portion of the receptive field could account for this difference.

Figure 2.a. shows series of traces representing actual responses to central and annular illumination. The intensity of the center spot of light was set to -2 log units. This elicited maximal peak response of approximately 24 mV, followed by repolarization to a sustained plateau of about -58 mV. Annulus-elicited response was immeasurably small when the intensity of peripheral illumination was less than -3.9 log units. Further increase of the annular intensity resulted in gradual increase of the depolarizing response. In the last trace annular intensity was sufficient to bring the membrane potential to the resting level normally measured in the dark. The plot of this data is shown in fig. 2.b. Circles represent the normal I-R curve recorded in this cone previously. The column of triangles represent measurments of membrane potential taken at the peak of the depolarizing response due to annular illumination. The significance of this result is that the resting membrane potential (-40 mV) for this cone can be reached at two drastically different intensities for a spot/annulus combinations. The result of the annular illumination is a fast depolarization of the membrane; the whole process is completed in a fraction of a second unlike the previous reports where the course of light-adaptation lasted for seconds or even minutes (for review see Witkovsky, 80). The I-R curve for the annulus-elicited responses, shown in Fig. 2.c. depicts amplitude of the depolarizing response as function of annular intensity. This S-shaped curve spans about three log units of intensity. The starting point of -20 mV (Vrest = -40 mV) corresponds to membrane potential of the cone during plateau of the response to central spot of light, measured near the end of the center stimulus. This result shows that peripheral illumination can bring the membrane potential of the light-hyperpolarized cone back to the resting level. Feedback action is graded with annular intensity and it depends on the balance between amount of light falling on the center and the surround of the cone receptive field. The continuous curve was drawn according to equation x/(x+k), suggesting presence of light adaptation.

Peripheral illumination shifts the I-R curve in cones

Fig. 3. shows results which address the third criterion of our hypothesis. The hyperpolarizing response increased in magnitude with increasing center light intensity over three log units (fig. 3.a). The peak of the response reached the maximal level of 25 mV at -2 log units of intensity. The same data is plotted as open circles in fig. 3.b. Initially, annulus presented during the central illumination did not produce a noticeable response. Its amplitude reached maximum when the center spot intensity was increased to 3 log units. Further increase of center intensity resulted in disappearance of the annulus-elicited depolarization. It is conceivable that feedback transmitter released from horizontal cells in the dark, opens channels to ions with reversal potential near -65 mV (Skrzypek & Werblin, 83). Hence, hyperpolarizing cone membrane by increasing center spot intensity would reduce the depolarizing feedback response as cone nears the battery of involved ions. In this respect, the result in Fig. 2 is interesting, because it shows that in a center-spot hyperpolarized cone, an increase in annular illumination that presumably further hyperpolarizes the HC', causes the depolarizing surround response in cones to increases again. In the discussion we argue that this additional increase can be explained as a nonlinear function of the balance between center and surround illumination. Additional increase in annular illumination, further reduces the feedback transmitter and the associated feedback conductance thus pushung cone's membrane potential away from the "feedback" battery. Eventually, at some values of the center intensity, cone membrane is so close to -65 mV that no change in feedback conductance can produce a depolarizing response. As a matter of fact, because the leakage battery is at -70 mV, a small reversal of feedback response is possible.

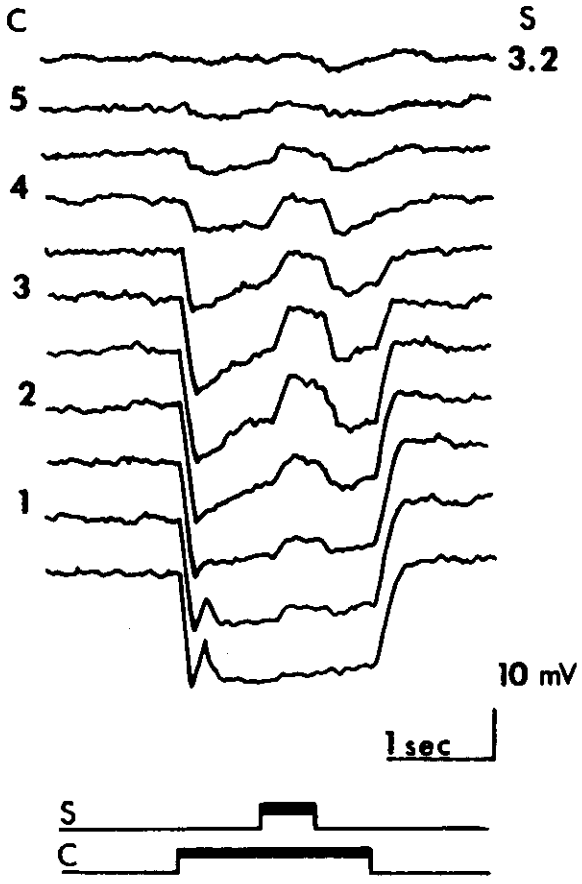

Fig. 3a. Series of responses to a combination of center spot and annulus. Dimensions of the stimuli as in fig. 2. Surround illumination (S) was fixed at -3.2 l.u. throughout the experiment. Center spot intensity (C) was increased in 0.5 l.u. steps as indicated by the numbers near each trace. In the dark (upper-most trace) surround illumination had no measurable effect on the cone membrane potential. Annulus-elicited depolarizing response increased with intensity in the center up to about -3 l.u. Further increase of the spot intensity diminished the surround response.

The change in cone's membrane potential, due to combined effects of central and annular illumination is plotted as filled circles in. fig. 3b. This new intensity-response curve is shifted along the intensity axis by approximately two log units. Both I-R curves span approximately three log units of intensity. The I-R curve due to combined center and surround illumination can be described by the function V/Vm = I/(I+k) (Naka & Rushton, 67) where Vm is a peak hyperpolarization and k is a constant intensity generating half-maximal response. This relationship was suggested to be an indication of the light adaptation (Matthews, et. al., 88). The I-R curve plotted using peak response values (open circles), fits a continuous line drawn according to equation (1-exp(-kx)). This has been argued previously to indicate absence of light adaptation (Matthews, et. al., 88; Nakatani & Yau, 88). There is little if any compression or change in gain after the shift of the cone operating point to some new domain of intensity. The maximum shift observed in the best of experiments was about 4 log units. The results described above confirm the third tests of our hypothesis; peripheral illumination can shift the center-spot elicited I-R curve of the cone. In the following discussion we argue that this shift in combination with results from Fig. 2. suggests a resetting of the response-generating mechanism in cones.

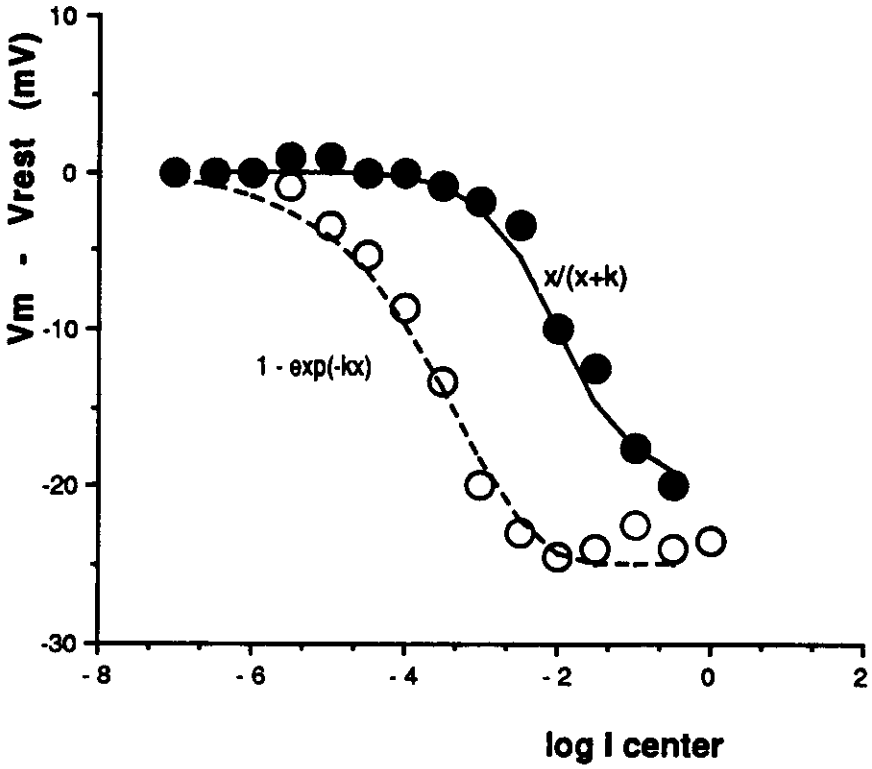

Fig. 3b. Plot of the peak hyperpolarizing response versus center spot intensity in log units in (open circles) fits the dashed curve drawn according to equation 1-exp(-kx). The curve indicated by filled circles represents the membrane potential measured in the middle of the depolarizing response. This data can be approximated by a continuous curve derived from x/(x+k). All membrane potential measurement are made with respect to the resting level in the dark (-40 mV). This result shows that in the presence of peripheral illumination, when the feedback is activated, membrane potential follows the intensity-response curve which is shifted along the Log I axis.

Simulation of a cone model.

The results presented in the previous sections seem to suggest that a level of maximal hyperpolarization for the cone membrane is not limited by the saturation in the phototransduction process alone. It seems reasonable to assume that such a limit may be in part determined by the batteries of involved ions. Furthermore, it appears that shifting I-R curves along the intensity domain is not dependent solely on the light adaptation mechanism localized to the outer segment of a cone. A simplified compartmental model of a cone was developed to test these hypothesis. First of all, we would like to verify that with peripheral illumination applied to a seemingly "saturated" cone it is still possible to generate a full light response. At the same time we would like to to find out whether change in Gin, Gl and Gfb required to get a response are within reasonable physiological limits. In this study we are not modeling details

of light adaptation or the dynamics of the light response.

We used the compartmental model for a cone represented by electrical circuit with three branches, each consisting of a conductance and battery (Fig 4). The model was exercised using SPICE developed at University of California Berkeley by Vladimirescu et al (1981). SPICE is capable of simulating linear and nonlinear compartmental models of neurons represented as equivalent electrical circuits. SPICE has been used extenssively to model passive and active neuronal membranes (Segev et. al., 85, Flack et. al., 87, Rall 87). There are some assumption, detailed in Rall (1988), that must be made when using SPICE to model neuronal cells. Briefly, since one dimensional cable theory, might not be applicable because of rectifying properties of the cone membrane (Skrzypek 79; Attwell, et al., 82) we use the compartmental approach where a set of ordinary differential equations describes currents flowing accross the cone's membrane within the compartment. This way any nonuniformity sych as diameter or membrane properties can be explained as being between compartments (Rall 64). Since we are interested in modeling only the shift of I-R curves without a detailed analysis of temporal aspects of light adaptation we can simplify our model further by assuming a cone to be one idelized compartment with uniform and passive, membrane. The extracellular medium is assumed to be isopotential and equal to ground.

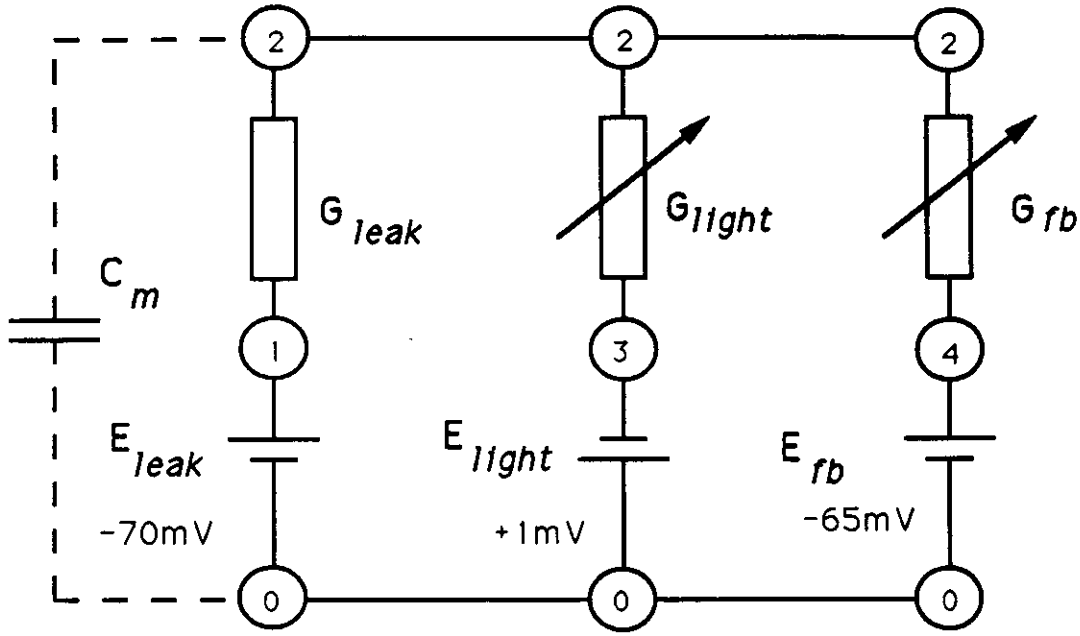

Fig. 4 Equivalent circuit model of a cone based on three different transmembrane channels. The ohmic leakage channel consists of a constant conductance Gleak in series with constant battery Eleak. Light sensitive channels are represented in the middle branch by Glight. Battery Elight, represents the reversal potential for light response at approximately 0mV. Feedback synapse is shown in the right-most branch as a series combination of Gfb and the battery Efb=-65 mV, representing reversal potential for annulus elicited, depolarizing response in a cone.

The equivalent circuit model is shown in Fig 4. The left most branch represents ohmic leakage channels (Gleak) which are associated with a constant battery Eleak. The middle brach represents the light sensitive conductance (Glight) in series with +1 mV ionic battery (Elight) (Attwell, et al. 82); an assumed reversal potential for Na ions flowing through cGMP channels. Light adaptation effects could be incorporated here by making Glight time varying and dependent on internal concentration of Calcium ions. In our preliminary studies we were only interested in examining whether the shift of I-R is possible and if it would explain the disappearance of depolarizing FB reponse with hyperpolarization by the center light. This can be done with passive measurements of membrane potential amplitude. The right-most branch represents ionic channels that are controlled by the feedback synapse. Here, Efb = -65 mV (Skrzypek & Werblin 83) is the battery representing equilibrium potential for ionic species involved in feedback synapse, and Gfb is a time and voltage independent feedback conductance. Using Kirchoff's current law, membrane current is im = cm(cV/dt) + Iionic. The ionic currents are specified by the batteries and conduc-

tances defined for each of the three branches. The leakage current is Ileak = Gleak(Vm - Eleak), the light sensitive current is Ilight = Glight * (Vm - Elight) and the feedback current is Ifb = Gfb * (Vm - Efb). Since we are not concerned with temporal properties of the phenomenon we can omit the membrane capacitance and further simplify our simulation.

The dynamic behavior of the model depends critically on the selection of its electrical components. Since we are not involved with temporal aspect of membrane properties, approximate component values should suffice. All parameter values are not too drastically different from the values reported in literature. We assumke that cell is in a resting state and electrical properties are uniform everywhere. The leakage battery is -70mV, and the battery associated with the light-sensitive channels is +1mv (Attwell, et al. 82). The feedback battery is -65mV (Skrzypek & Werblin 83). The input resistance of an isolated cone is taken to be near 500 Mohm (270 Mohm Attwell, et al., 82). Assuming specific membrane resistance of 5000 Ohm*cm*cm and that a cone is 40 microns long and has a 8 micron diameter at the base we get the leakage conductance Gleak = 1/(1Gohm). In our studies we assume Gleak to be linear altghouth there is evidence that cone membrane rectifies (Skrzypek, 79). The Glight and Gfb are assumed to be equal and add up to 1/(1Gohm). The Glight varies with light intensity in proportion of two to three log units of intensity for a tenfold change in conductance. This relation was derived empirically, by comparing intensity response data obtained from a cone {Vm = f(LogI)} to {Vm = f(LogGlight)} generated by the model. The changes in Gfb have not been calibrated to changes in light intensity of the annulus. However, we assume that Gfb can not undergo variation larger that Glight. The value of the lumped membrane cpacitance is approximately 100pF (Attwell et al, 82; Detwiler & Hodgkin, 79), assuming that specific membrane capacitnace is one microFarad per square centimeter. However, in our initial simulation studies we dont include membrane capacitance.

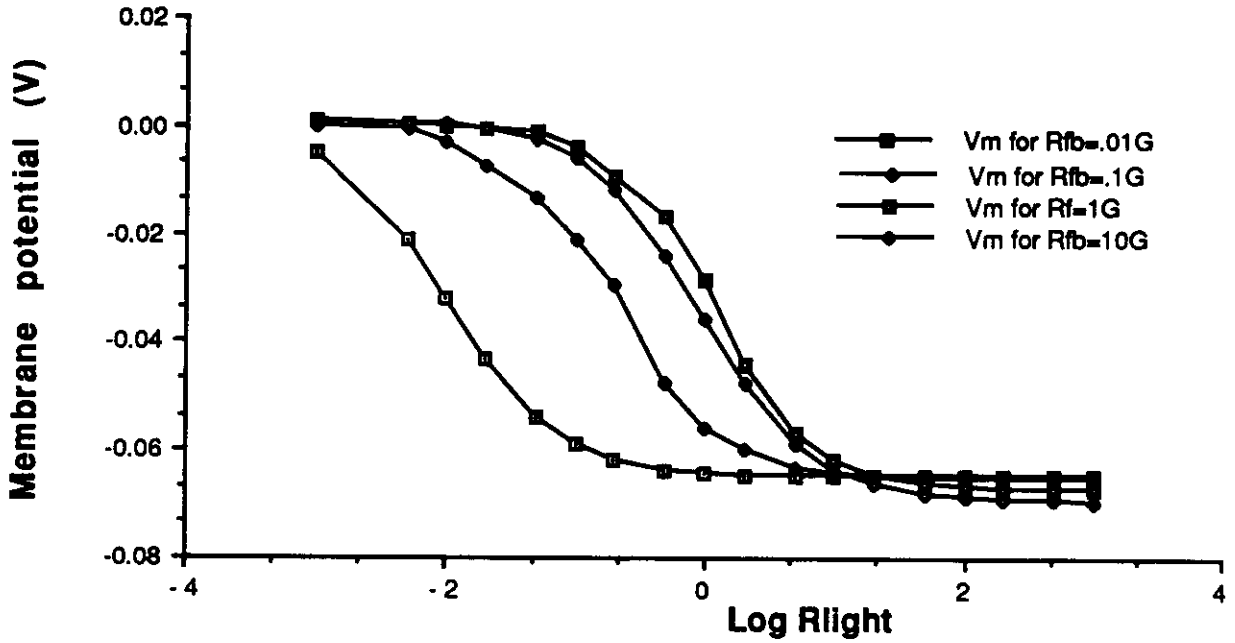

Fig. 5a. Plot of the membrane potential (a) versus the logarithm of light-sensitive resistance. The data was synthesized with the cone model simulated by SPICE. The curves can be fitted by x/(x+k) relation (not shown) at all different settings of Rfb indicated in the legend. The shift of the curves, measured at 1/2 maximal value (k=x) spans about two log units. With increasing settings of Rfb (10Gohms), curves begin to cross (Vm at -65mV) signifying decreasing contribution of "feedback" synapse.

Simulation results

The model used in this project is extremely simple and yet it is sufficient to demonstrate the shifts of I-R curves along the intensity domain, when the feedback synapse changes its activities. The model is also consistent with the physiological values of batteries and conductances measured in isolated cones or in cells embeded in the retinal network. For example, when the resting membrane potential is Vdark=-20mV and the input resistance Rin =.5Gohm (Skrzypek, 79; Attwell, et. al., 82), the model can generate 35mV light response, elicited by 3 log units change of stimulus intensity, with a change in input resistance Rin of only 20%. Interestingly the model predicts that this response requires a ten fold change in Glight. This might be an overestimate if the voltage and time dependent properties of the membrane are considered (Skrzypek, 79; Attwell, et. al., 82).

Figure 5a shows the membrane potential changes generated by the model plotted as a function of resistance associated with the light-sensitive channels, at different settings of the "feedback" resistance Rfb. Every response LogRlight curve spans approximately two log units. This would correspond to four to six log units for the I-R curves measured in cone. The difference could be accounted for in part by the rectifying properties of the real cone membrane. With increasing feedback resistance, there is a parallel shift along the abscissa without any changes in the shape of the curve. This is similar to data presented in Fig. 2 and Fig. 3. Increase in resistance associated with the light sensitive channels corresponds to increase in light intensity and the increasing magnitude of the light response from 0mV (Elight) all the way down to -65 mV (Efb). The increase in feedback resistance is associated with increasing intensity of the annular illumination, which causes additional hyperpolarization of the horizontal cell and consequently a decrease in "feedback" transmitter released from HC to cones. Since we assume the feedback battery in the cone to be at -65 mV, a more negative level than the normal resting membrane potential, a decrease in Gfb would cause a depolarizing response in the cone. This can be observed here as a shift of the curve along the abscissa. In our model, a hundred fold change in feedback resistance from 0.01Gohm to 1Gohm, resulted in shift of the "response-intensity" curve by approximately two log units along the abscissa. The relationship between changes in Rfb and the shift of the "response-intensity" curve is nonlinear and additional increases in Rfb from 1Gohm to 100Gohm results in decreasing shifts.

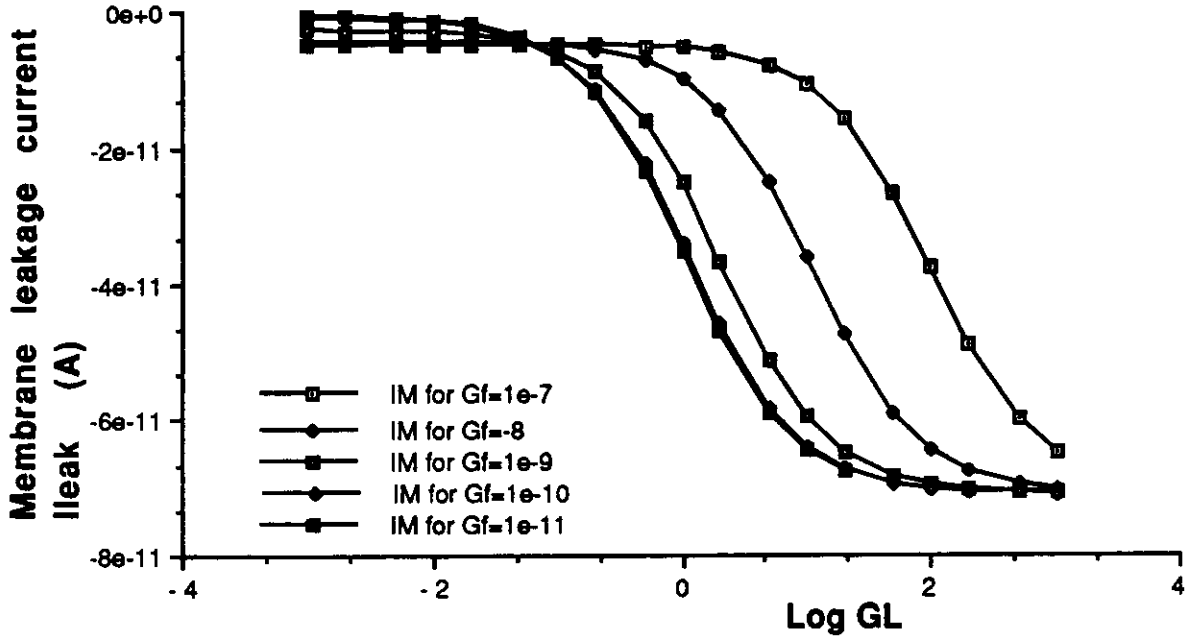

Fig. 5b. Plot of the leakage current versus the logarithm of light-sensitive conductance.

Membrane current undergoes similar parallel shift with changes in feedback conductance. Figure 5b. shows a plot of the membrane current (Ileak) generated by the model as a function of the light sensitive conductance expressed in logarithmic units. The data points can be fitted rather well to the function x/(x+k) but not to the exponential saturation function r = 1 - exp(-k/x) (Lamb, McNaughton & Yau, 81). It has been argued that in low calcium - no sodium solution when light adaptation is abolished, the response intensity relation fits the 1-exp(-kI) function

(Matthews, et al 88, Nakatani & Yau, 88). This suggests that our model approximates steady state light responses measured in a cone when light adaptation effects are clearly observable.

The photocurrent (Ilight) and the feedback current (Ifb), show only saturation with increasing Glight (Fig. 6a. and 6b). The limits of either Ilight or Ifb currents are defined by the batteries of the model. Since these currents are associated with batteries of opposite polarities, the difference between them at various settings of the feedback conductance Gfb determines the amount of shift for Ileak along the abscissa (Fig 6c). The compression in shift of "response intensity" curves at smaller values of Gfb results from smaller and smaller current flowing through the feedback branch of the circuit. Consequently, a smaller Gfb changes are required to get response in the dark than in the light.

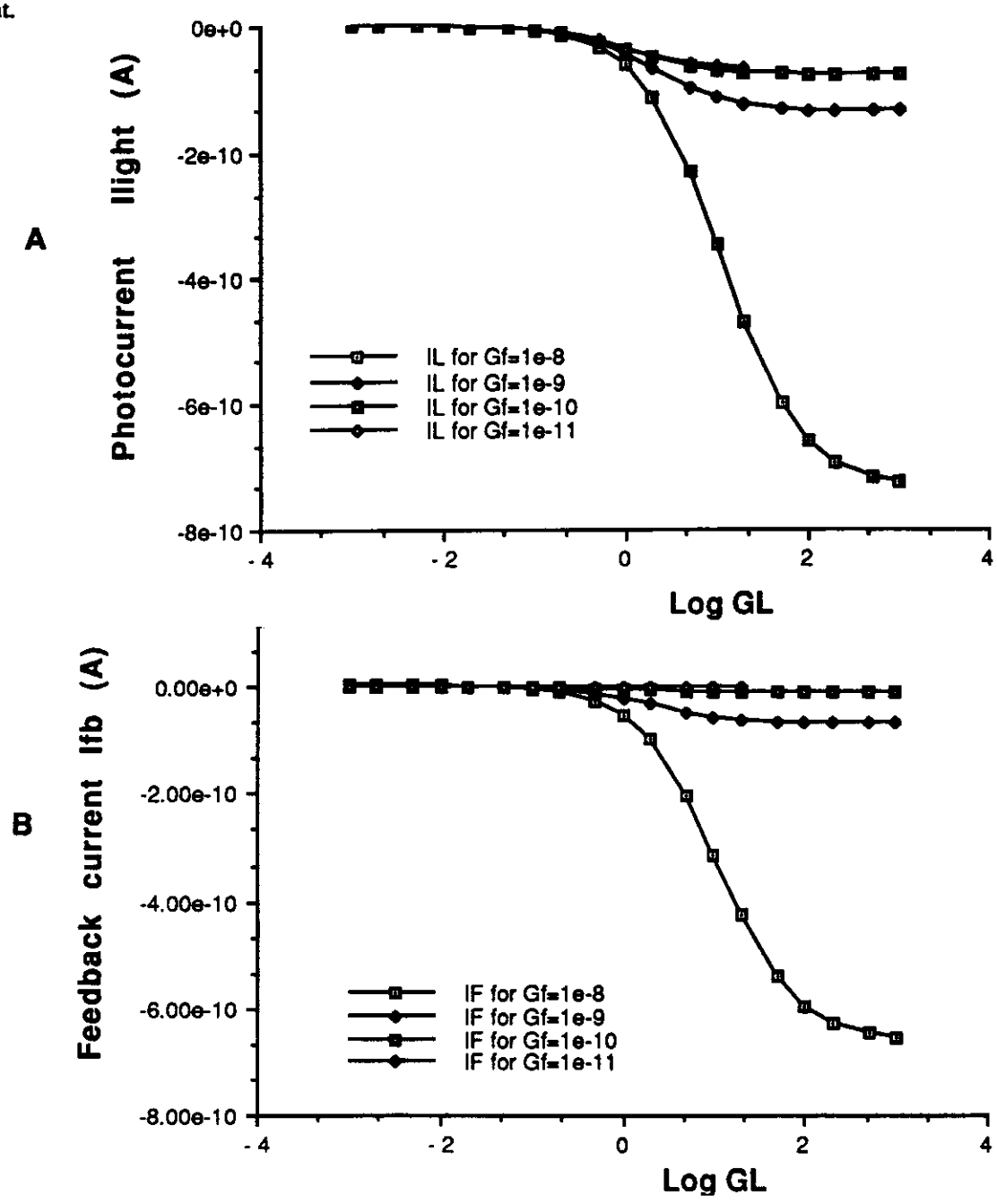

Fig. 6 Plot of the "photocurrent" - Ilight (a) and the "feedback" current - Ifb (b) as a function of the logarithm of Glight. Both currents can be fitted by the relation x/(x+k) but there is no shift along the

abscissa, only a compression in the amplitude value.

The shifting of the "response-intensity" curves generated by our model is not due to light adaptation as described by (Nakatani & Yau, 88; Matthews, et al., 88)although it is possible that feedback effects could be involved in modulating light-sensitive channels. Our model suggests that in order to generate additional light response after the membrane of a cone was fully hyperpolarized by light, it is insufficient to have a feedback effect alone that would depolarize the cone membrane. You still need to have light sensitive channels that were not previously closed (Attwell, et. al., 82).

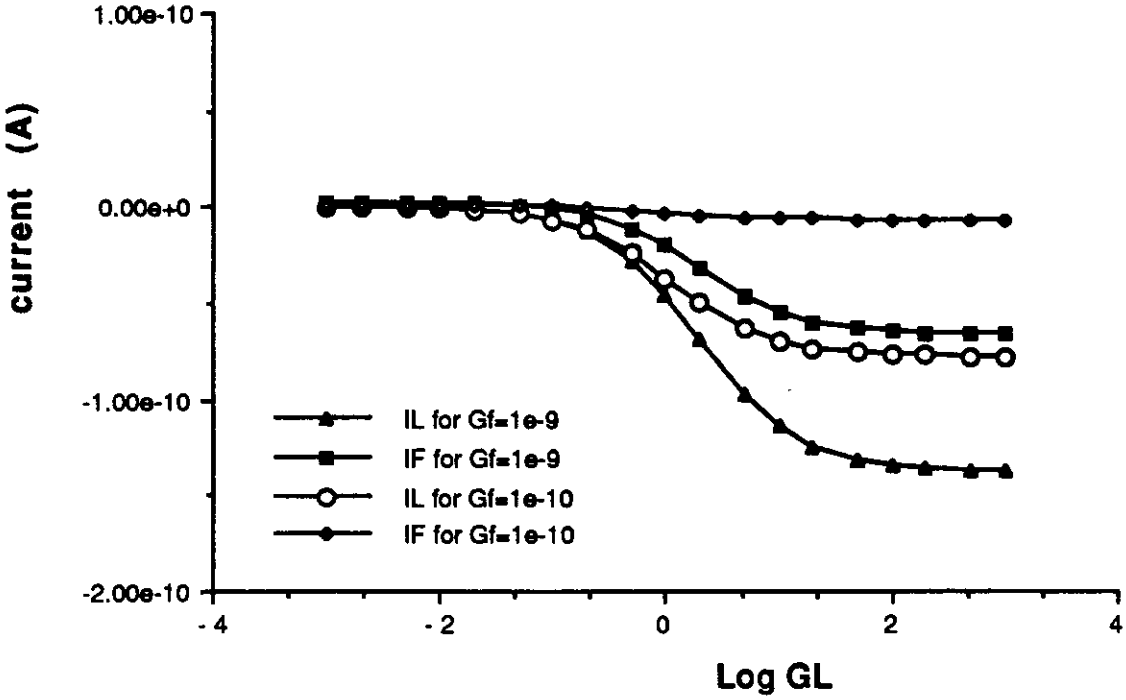

Fig. 6.c. A comparison of the two currents, Ilight (IL) and feedback (IF), at various settings of Glight and Gfb. For any specific Gfb, the photocurrent is larger in amplitude than the "feedback" current.

DISCUSSION

The results presented here suggest that synaptic feedback from horizontal cells to cones could contribute to the process of light adaptation at the photoreceptor level. This suggestion is supported by the following results: a) "sustained" effect of annular illumination can be measured in cones, b) peripheral illumination can depolarize the previously hyperpolarized cone membrane to its resting potential level in the dark and c) the cone can generate a full range of photoresponses in a presence of annular illumination. In other words, the results of fig. 2 and fig. 3 suggest that a maximally hyperpolarized cone and presumably insensitive to further increments of center spot intensity, can respond to additional increases in center light if the antagonistic periphery of its receptive field is stimulated.

A complete explanation of the underlying mechanism requires further studies but the results seem to suggest that depolarization of the cone membrane by a peripheral illumination, resets the response-generating process in the cone. This result can be explained withing the framework of the current hypothesis of the light adaptation, recently summarized by Pugh and Altman (88). Here, cGMP acts as an excitatory messenger that opens the Na channels in the outer segment. During the light response, the amount of cGMP decreases and the channels are closed. This prevents the calcium from leaking in. Meanwhile the calcium-sodium pump keeps extruding internal calcium. Lowered internal calcium activates guanylate cyclase. This increases cGMP and opens the Na channels.

Figure 3. shows that it is possible to get a new light response from the cone that has been previously hyperpolarized to its maximal level with a center spot of light. Several contributing mechanisms can be considered. During the maximal hyperpolarizing response, the cone membrane reaches a level of an ionic battery with a negative reversal potential but its transduction mechanism does not reach a saturation level (Attwell, et al., 82). Here, cellular mechanism underlying light adaptation keeps the light sensitive channels open. Thus, if the feedback effect can depolarize the cone membrane away from the battery level, the light sensitive conductance will be able to generate an additional response. Our SPICE based model of a cone verified the plausibility of this explanation.

We can not exclude the possibility that feedback could affect the ionic pump involved in the control of intracellular calcium (Yau & Nakatani, 84; Yau et al., 86). Two mechanism of transmembrane calcium flow are known; pumping out, via Na/Ca ion exchange and leaking in via the light sensitive channels. Presumably, the inward leak stops when the light response is generated by closing ionic channels. However, the pump might still be pumping out , eventually decreasing the internal calcium concentration. Here, feedback could affect the ionic balance maintained by the ionic exchanger and eventually increase the activity of cGMP, resulting in a new state of adaptation. It is not clear that this explanation could account for the speed with which the I-R curves are shifted along the intensity domain when the annular light is increased.

Another possibility is that all the light sensitive conductance is saturated during maximal hyperpolarization. It was shown that each single photon response results in a complete, regional closure of the light sensitive channels (Lamb, McNaughton & Yau, 81). In this case Fig 3., implies that feedback effect must somehow activate the light sensitive channels which were previously closed or keep them closed for a shorter time. This action can not be simply explained by direct depolarization of the cone membrane because the light sensitive channels were shown to be relatively independent of membrane potential (Attwell et al., 82a). Some intermediate agent is needed such as for example calcium. Furthermore, the agent must be able to modulate the degradation of the receptor-transmitter complexes (Baylor et al., 74) in order for the system to stay out of the saturation. This is consistent with the current hypothesis behind the mechanism of light adaptation (for review see Pugh and Altman, 88), but there is no evidence for a connections between the level of cGMP activity and the feedback synapse.

Finally, it is possible that feedback induced depolarization of the cone membrane has two components. One results from direct action of the feedback transmitter on the cone membrane; peripheral illumination hyperpolarizes HC's through lateral pathways thus reducing the effect of the feedback transmitter on the cone membrane. This decreases ionic current associated with reversal potential at -65 mV (Skrzypek & Werblin 83) leading to depolarizing feedback response. The second component associated with feedback action might be the decrease in internal calcium concentration. This in conjunction with calcium sequestering by internal mechanisms of the inner segment may participate in controlling action of cGMP on the light sensitive channels. This hypothesis could explain why it is difficult to measure intracellularly the reversal potential for the feedback response; the two components of the response are associated with opposing conductance changes that regulate ionic flows driven by batteries of opposite polarities.

Stray light

The effect of annular illumination measured in cones can not be considered to result from stray light falling into the center for the following reasons. We measured a depolarizing response, which is of opposite polarity to the expected effect of stray light exciting cone in the center of the annulus. In all experiments we were careful to set the dimension of the annulus so that inner diameter far exceed the measured extent of the receptive field center; annuls inner diameter usually was 750 um, while the RF center for the cone is on the average less than 100 um. In a separate experiments we attempted to quantify the amount of stray light by measuring I-R curves from the rods when stimulated with increasing intensity of the annulus (ID = 750um, OD = 1500um). This curve appear to be shifted in intensity domain by at least four log units with respect to the I-R curve obtained with center spot. Considering that rods are at least one log unit more sensitive than cones, this suggests that scatter from annulus to center for this ID is at least 1000 times less intense as compared to direct stimulation with center spot. In another test we attempted to measure the hyperpolarizing response from rods elicited by an annulus with ID =750 um. The minimum intensity that could elicit a depolarizing "surround" response in the cone did not scatter enough light to the center to elicit a measurable response from the rods. Considering that the diameter of RF-center for rods is about 200-300 um while for cones it is less than 100 um, there is less light scatter contributing to cone response. In view of all these arguments the assumption that a direct contribution of stray light is insignificant seems reasonable.

Effect of interreceptor coupling on the feedback response.

If the cone's intracellular responses to stimuli of varying diameter are matched for equal intensity, the peak of response is greater for larger stimuli. This implies the coupling to neighboring cones, which in turtle retina, was also confirmed by impaling two cones with separate electrodes; current injected into one cell caused a potential drop of the same polarity in the second cone (Baylor et al., 71; Attwell et al., 82b). Although the coupling between turtle

rods was shown to involve time-varying and voltage dependent behaviour, this was not found to be true for coupling between tiger salamander cones. (Copenhagen and Owen, 80; Attwell & Wilson, 80; Attwell, et al., 84). All reported connections are supposedly mediated via gap junctions (Raviola & Gilula, 73) and behave as electrical, noninverting synapses. Coupling between cones is not as extensive as between rods and only cones of identical spectral sensitivities are known to be coupled (Lamb & Simon, 76; Attwell et al., 84; Fain, 75). Could it be that a profile of potential distribution via gap junctions is responsible for observed effects? There is no evidence for the spread of adaptation between rods in the turtle retina (Copenhagen & Green, 85). It is known that rods have larger receptive fields and presumably stronger coupling between neighbors. Considering available physiological and anatomical evidence about the spatial extent of projections emanating from cones, and the properties of electrical synapses between them, it would be difficult to explain how an annulus with large internal diameter could lead to depolarizing surround response measured in cones. One possibility is that in electrically coupled network, where input resistance depends on length constant, the nonlinearities due to voltage-dependent behavior of the gap junction could result in reversed polarity response. However, such nonlinearities were not observed in cones (Attwell et al., 82b;84). And in rods where coupling is voltage dependent (Attwell & Wilson, 80), the antagonistic responses have never been measured. Another possibility is that lateral signals are carried by rod-cone pathway, but this coupling was shown to be very weak (Fain, 75; Hanani & Vallerga, 81; Attwell, et al., 82b;84).

Is depolarizing response in cones mediated by a feedback synapse from horizontal cells?

The surround-elicited, depolarizing responses, measured in cones are assumed to be mediated by the feedback synapse from horizontal cells. The functional significance of feedback synapse from HC to cones in the tiger salamander retina was previously reported by Lasansky & Vallerga, (75); Lasansky, (81) ; Skrzypek & Werblin (83). Measurement of I-V characteristics to discern the mechanism underlying feedback synapse were not very successful (Skrzypek, 79). In the turtle cones, O'Bryan (73) observed a feedback controlled depolarization associated with a conductance increase. He also reported time-varying secondary component of feedback input. Gerschenfeld et al. (80) suggested that feedback transmitter, when released in the dark, could modulate the potassium conductance as an intermediate step in controlling the voltage dependent calcium conductance. Furthermore, they observed that hyperpolarization of HC with injected current resulted in increased calcium conductance in a neighboring cones. These observations were used to argue that a feedback path from HC to cones mediates antagonistic responses.

The most direct evidence in favor of functional significance of the feedback pathway comes from the turtle retina (Baylor et al 71). They impaled simultaneously a horizontal cell and a nearby cone, and hyperpolarized the HC by injecting current while measuring depolarizing response from the cone. When the retina is perfused with aspartate or glutamate, so that HC response is abolished, the depolarizing component of response measured in cones, that is supposedly mediated by a feedback synapse from HC's is also abolished (Cervetto & McNichol, 72). This observation was further confirmed by blocking the synaptic transmission with cobalt (Cervetto & Piccolino, 74). Skrzypek & Werblin (1983) have shown that when cone membrane was polarized by light to reversal potential level for the feedback synapse, all antagonistic interactions in the postsynaptic cells have disappeared. The depolarizing response due to surround reverses at -60 mV. Assuming feedback transmitter is released from horizontal cells in the dark, it hyperpolarizes cone membrane. In this situation, light would cause the decrease in feedback transmitter and consequently the decrease in conductance to ions with reversal potential at -60 mV. In the goldfish retina Marc et al. (78) showed that feedback pathway probably involves GABA. This agrees with Lam's et al., (78) report that Bicuculine, a potent GABA blocker, modulates some feedback effects in cones. More recently, it was shown that GABA hyperpolarizes single cones (Kaneko & Tachibana, 86). Presumably, GABA released from HC in the dark, hyperpolarizes cone membrane, while the peripheral light, hyperpolarizes horizontal cells via electrical coupling thus reducing the release of GABA and consequently causing depolarization of a cone.

Although the mechanism underlying feedback synapse from horizontal cells to cones still remains unclear, the evidence presented here suggests that peripheral illumination, acting via lateral pathways between horizontal cells and through the feedback synapse to cones, can contribute to the light adaptation mechanism internal to cones.

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