# Horizontal Cell Dynamics: What are the Main Factors?

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The factors potentially determining the dynamics of horizontal cell (HC) responses are: (1) the rate of transmitter release (including its transient component) and removal; (2) the voltage non-linearity of HC non-synaptic membrane combined with its capacitance; and (3) the dynamics of feedback from HCs to photoreceptors. Using, in consecutive order, the models of an isolated HC, a HC with one or two synaptic inputs and a HC of chromatic type, we have analysed the relative importance of three factors in shaping HC responses to the light and electrical current. The most prominent effect on the shape of HC ON responses derives from the voltage-dependency of the non-synaptic membrane. The dynamics of synaptic transmission plays a leading role in shaping the OFF light responses. For depolarizing responses of C-type HCs, the key factor is the electrical feedback from L-type HCs, which provides not only the response of opposite polarity (to red light), but also the typical feedback delay. Copyright © 1996 Elsevier Science Ltd.

Horizontal cells    Model    Electrical feedback    Non-linear membrane

## INTRODUCTION

Starting from the first recordings in the 1950s of the responses of horizontal cells (HC) in the vertebrate retina (Svaetichin, 1953; Grüsser, 1957; MacNichol & Svaetichin, 1958) these cells are the subject of special attention. One main reason for the interest is that all neurons of the distal retina, including photoreceptors, generate only slow gradual light responses with different, sometimes very poorly understood, dynamics. It seems obvious that the shape of the response somehow reflects important parameters of the image. What are the factors determining the dynamic properties of the responses?

There are several well documented factors. The first is the rate of transmitter release from photoreceptor terminals and its removal from the synaptic gap. It is known that the transmitter is excitatory (glutamate), is released in darkness when photoreceptors are depolarized, and that the release is decreased in light (Trifonov, 1968). Therefore, the leading edge of the hyperpolarizing light response seems to be determined by the decrease of the transmitter concentration due to its removal from the synapse. However, as is known from experiments (e.g. Kamermans & Werblin, 1992) and will be shown later, the shape of the leading edge of the light response is strongly variable and evidently depends not only on the rate of transmitter removal, but other factors as well.

The release of transmitter is governed by the potential

changes across the photoreceptor presynaptic membrane. Considering the dynamics of this process one should take into account its strong asymmetry, observed in old experiments of Byzov and Trifonov (1968). The short depolarizing pulses of current passed through the photoreceptor presynaptic membrane evoked depolarizing responses in HCs [Fig. 1(A)], while similar hyperpolarizing pulses never evoked hyperpolarization.

The second important factor which strongly influences the dynamics of HC responses is the properties of the HC non-synaptic membrane. As shown previously (Trifonov *et al.*, 1974; Byzov *et al.*, 1977), the conductance of the non-synaptic membrane of fish and turtle HCs is strongly non-linear: its resistance is low under hyperpolarization and rises significantly under depolarization in the physiological range of membrane potentials. Without considering the ionic mechanisms of this voltage-dependency (Trifonov *et al.*, 1981; Byzov & Trifonov, 1981), we want to emphasize that this non-linearity is very strong: in the working range of potentials the *I–V* curve can be almost horizontal, i.e. the differential (slope) resistance is almost infinitely large or even negative (see Fig. 2). All these results were confirmed, at least qualitatively, on enzymatically isolated HCs of fish retina (Dowling *et al.*, 1983; Tachibana, 1983; Perlman *et al.*, 1992; and others). However, surprisingly enough, little has been done to estimate the importance of this factor for the dynamics of HC responses. In the meantime it is *a priori* evident that due to the high slope resistance of the HC non-synaptic membrane, its time constant can increase strongly, inevitably influencing the dynamics of HC responses. As will be shown later, the non-linearity of
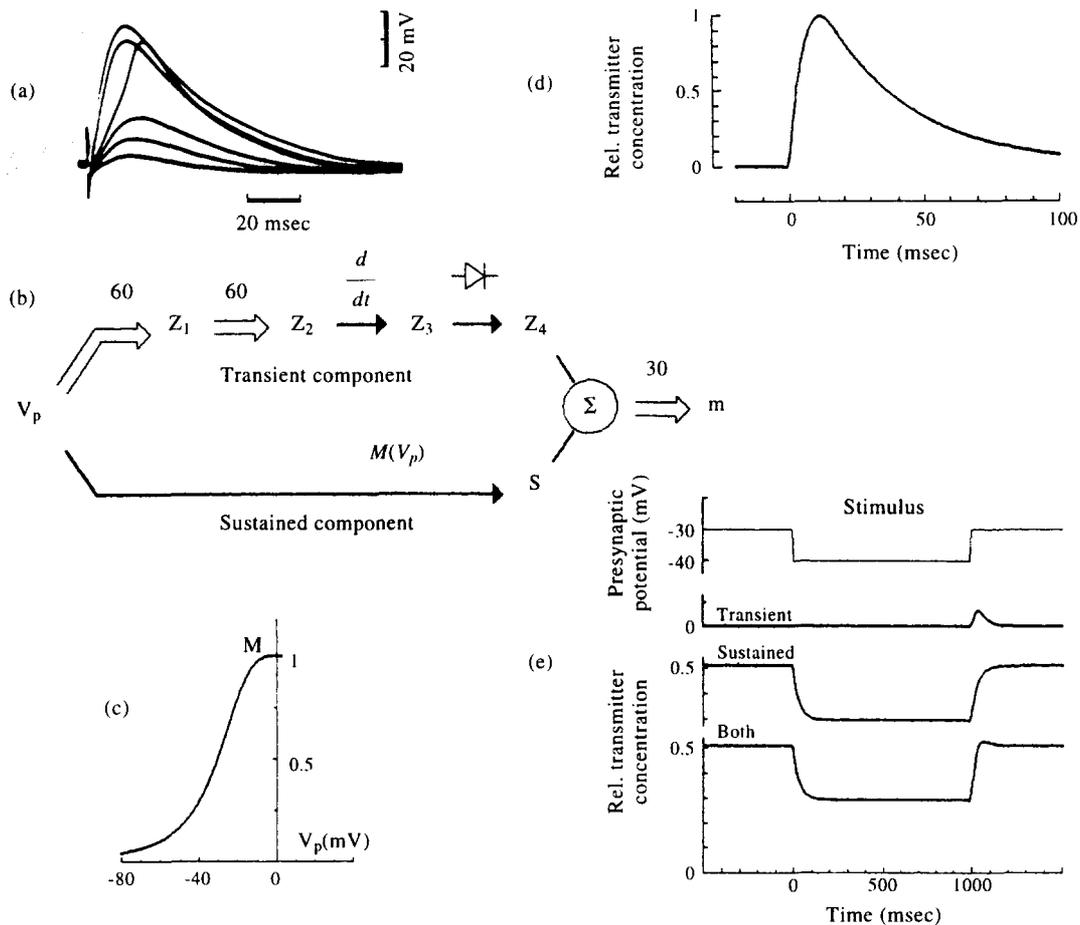
FIGURE 1. Dynamics of photoreceptor transmitter concentration in the synaptic gap, $M$, in the model. (a) The responses of carp horizontal cell (HC) to short (1–3 msec) sclera-positive transretinal current pulses of different intensities (Byzov & Trifonov, 1968). (b) Diagram illustrating two components of transmitter release in response to polarization of presynaptic membrane $V_p$. Sustained component—the rate of transmitter release as a function of $V_p$: $M(V_p)$ is shown in (c) and is the same as in our previous stationary model (Byzov et al., 1992). The transient component is a sequence of two smoothing low-pass filters, shown by white arrows (with rate constants in $\sec^{-1}$, indicated above), differentiating and rectifying stages. After summation of both releasing components the transmitter is removed from the gap (the right-hand white arrow) with the rate constant $30\ \sec^{-1}$ to give the current concentration, $M$ [modified from Byzov & Shura-Bura (1992)]. (d) Changes in the model in relative transmitter concentration in the synaptic gap following an impulse depolarization of the presynaptic membrane [compare with experimental responses in a)]. (e) Dynamics of transmitter concentration in the synaptic gap produced separately by transient and sustained components and their sum.

the HC non-synaptic membrane seems to be the main factor, at least in some experimental conditions, which determines many of the dynamic properties of HC light responses.

The third factor important in shaping the second-order neuron response is the dynamic properties of the feedback mechanism from HCs to photoreceptors. This mechanism accounts for the opponency of the receptive field periphery to its centre in bipolar cells, as well as for the depolarizing responses of C-type HCs (Stell, 1975; Fuortes, 1976; Piccolino et al., 1980). Without discussing the physiological mechanism of the feedback (see Byzov & Shura-Bura, 1986), we will show that the electrical mechanism, despite the "instant action" of electrical current, produces a typical delay of the depolarizing response to red light in R/G cells as compared to their hyperpolarizing response to blue light (Spekreijse & Norton, 1970).

## MODELS

The programming environment for simulation was arranged in such a way that it allowed us to construct different models from standard preprogrammed modules. These modules are: (1) the invaginated synapse of the photoreceptor; and (2) the second-order neuron. Several second-order neurons of different types could invaginate the same photoreceptor terminal, and one neuron could receive synaptic inputs from several cones of different types. The parameters of the synapses and neurons can be changed in an interactive mode to observe immediately the effect on the dynamically monitored responses of the model.
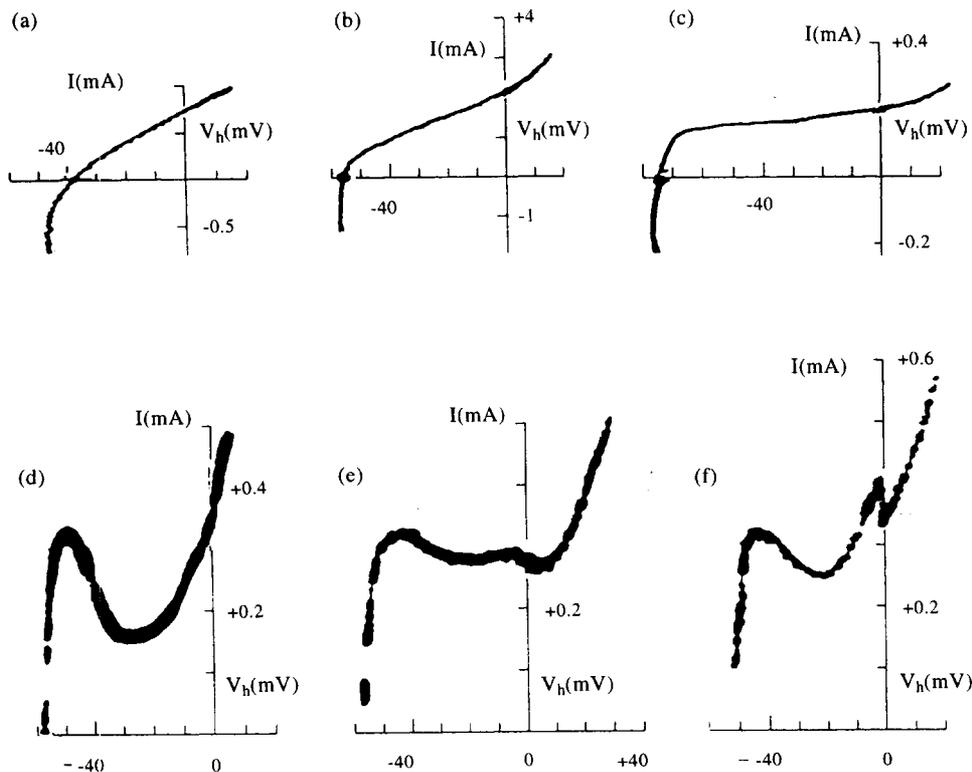
FIGURE 2. Examples of *I–V* curves for the HC non-synaptic membrane, measured experimentally in fish and turtle retinas. (a) Turtle *Emis orbicularis* (Byzov & Trifonov, 1973); (b) pike *Esox lucius* (Byzov et al., 1975); (c) pike (Byzov & Trifonov, 1982); (d–f) ide *Leuciscus idus* (Byzov et al., 1977). All curves were taken with ramp technique: (a–c) in the current-clamp mode, (d–f) in the voltage-clamp mode. The last technique was developed by K.V. Golubtzov (Trifonov et al., 1977) for polarization of the HC syncytium *in situ* by means of the extracellular electrical field of special configuration (see Byzov et al., 1977). Very high intensities of applied current are the reasons for noisy traces in (d–f).

## Synapse

The model of the synapse was constructed based on the aforesaid in the Introduction. We proceed from the previous models (Byzov & Shura-Bura, 1986, 1992). The parameters of the model were selected empirically to reproduce qualitatively the experimental results. The conductance of the HC subsynaptic membrane $G_h$ controlled by the transmitter concentration $m$, resulted from the transmitter release by photoreceptor and its removal from the synaptic gap [Fig. 1(b)]. The transmitter release is controlled by the potential $V_p$ at the presynaptic membrane and is the sum of two parts: the sustained component which is a function of the presynaptic potential only (Fig. 1c)], and a transient component that depends on the rate and direction of $V_p$ change. The process of transmitter removal is assumed to be exponential and is characterised by the rate constant $30 \sec^{-1}$ (see Byzov & Shura-Bura, 1992). The component processes and the changes of transmitter concentration in the gap, $m$, in response to a light stimulus are shown in Fig. 1(e). A more detailed description of the transmitter dynamics is given in the legend to Fig. 1.

The electrical circuit of the synapse is represented by three resistors [upper part in Fig. 6(c)]. The resistance of the cone presynaptic membrane $R_p$ whose value is not important is taken as infinite. The conductance of the HC subsynaptic membrane $G_s$ controlled by the concentra-

tion of transmitter $m$ in the synaptic gap; $G_s$ is taken proportional to $M$:

$$G_s = k \cdot m.$$

The synaptic efficiency $k$ could vary. Usually it was chosen so that in darkness the HC would be sufficiently depolarized. Introduction of the synaptic gap resistance $R_g$ common to both HC and photoreceptor circuits provides electrical interaction between them named "electrical feedback" (Byzov & Shura-Bura, 1986).

## Horizontal cell

The model of the HC (without synapse) is a simple electrical circuit [Fig. 3(a)] consisting of a battery $E_h$, membrane capacitance $C_h$, and membrane resistance $R_h$. The latter is voltage-dependent and the function $R_h(V_h)$ can be set arbitrarily (see later). The voltage-dependency is assumed to be time-independent. HCs were considered as a syncytium, therefore $R_h$, $C_h$, $G_s$ and corresponding currents are expressed in specific units (per cm$^2$). The only fixed parameters are $E_h = -80$ mV and $C_h = 1$ $\mu$F/cm$^2$.

Below we consider different models in order of their complexity.
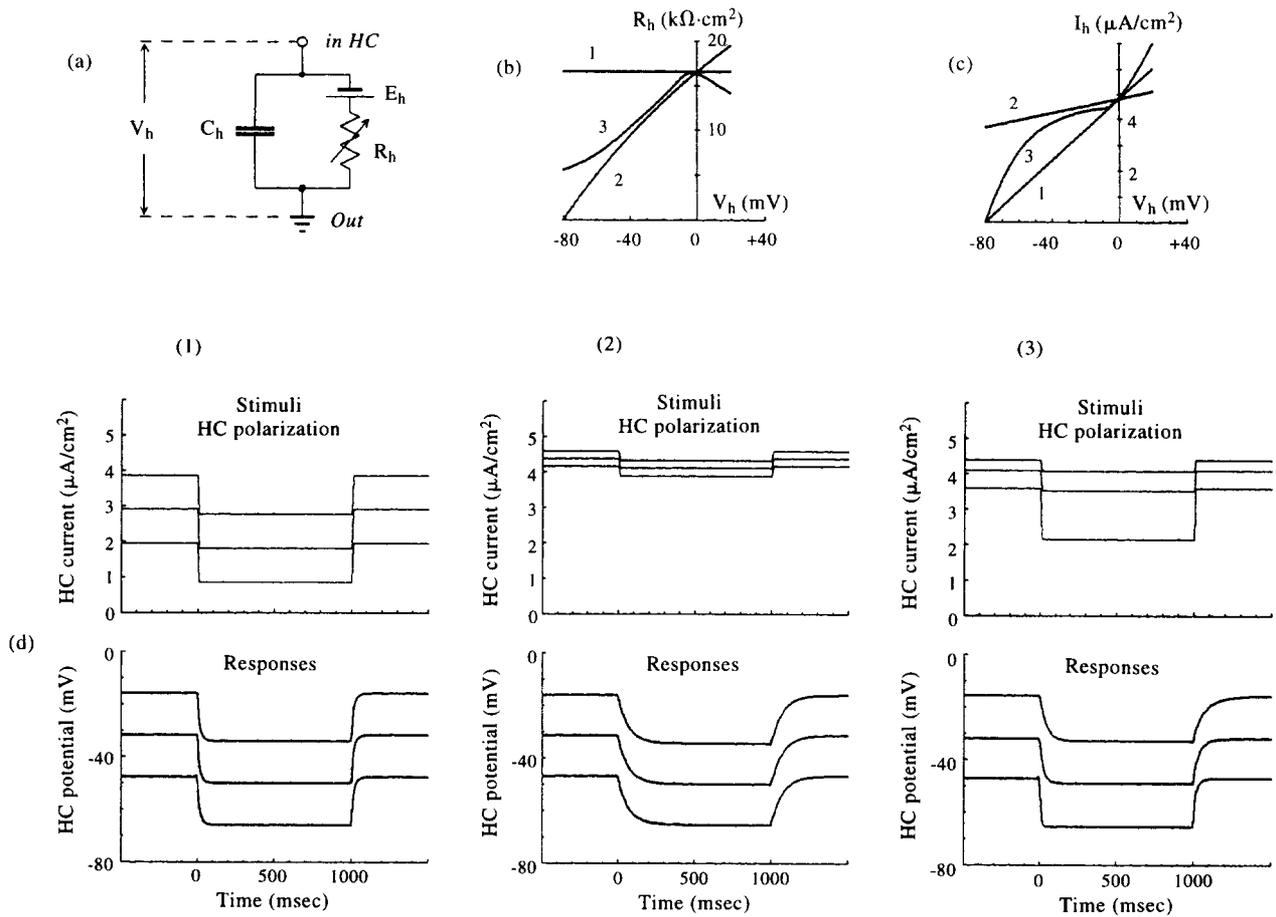
FIGURE 3. Dynamics of membrane potential changes evoked by current steps in an isolated HC without synaptic input. (a) Electrical circuit of the HC membrane; $E_h$ and $R_h$ are the battery and internal resistance (non-linear) of the resting membrane, $C_h$ is the membrane capacitance. (b) Three types of HC non-linearity $R_h(V_h)$ used for calculations and (c) the corresponding $I$–$V$ curves (see text). (d) Voltage responses of the model (bottom traces) to hyperpolarizing current steps (top traces) using three types of $R_h(V_h)$: (1), (2) and (3). For more details see the text.

## NON-LINEAR PROPERTIES OF HC NON-SYNAPTIC MEMBRANE AND DYNAMICS OF "ISOLATED" HCS

We start with the simplest situation to analyse the dynamic electrical properties of the HC itself without synaptic inputs. This situation corresponds to the isolated HC. Our goal was to compare the actual membrane potentials in response to current inputs to those from the simulated models. Figure 3(a) shows the equivalent circuit of such a HC. The membrane potential changes evoked by the polarizing current $I_h$ are described by the equation:

$$C_h \frac{dV_h}{dt} = \frac{E_h - V_h}{R_h} + I_h. \qquad (1)$$

The steady state solution $\left(\frac{dV_h}{dt} = 0\right)$ of this equation describes the $I$–$V$ curve:

$$I_h = \frac{V_h - E_h}{R_h}.$$

Figure 3 shows the result of the solution of Eq. (1) for three different functions $R_h(V_h)$. Three types of $R_h(V_h)$ are illustrated in two ways: $R_h$ as a function of $V_h$ (b) and

$I$–$V$ curves (c). The curves labelled by 1 correspond to the unrealistic case when the resistance of the non-synaptic membrane is constant: $R_h = \text{Const} = 17$ k$\Omega$/cm$^2$. The voltage-dependency of $R_h$ for the curves labelled by 2 was chosen so that the corresponding $I$–$V$ curve would also be linear but with a lower slope, i.e. with higher (and still constant) slope resistance. The curves labelled by 3 resemble a more realistic situation when the $I$–$V$ relationship has a curved shape, like in HCs [Fig. 2(a and b)]. A constant depolarizing current was injected in the HC imitating the depolarization in the darkness. Hyperpolarizing current pulses of 1 sec duration were applied superimposed on different levels of membrane potential, covering almost the whole range of $V_h$ [top traces in Fig. 3(d)].

When $R_h = \text{Const}$ [curves 1 in Fig. 3(b and c)] the shape of transients in the whole range of potentials is determined entirely by $\tau = R_h C_h = 17$ msec of HC membrane [Fig. 3(d), (1)]. The voltage-dependency of the second type (curves 2) results in three main changes [Fig. 3(d), (2)]. First, to reach the same initial membrane potential level in a HC one needs a stronger "back-
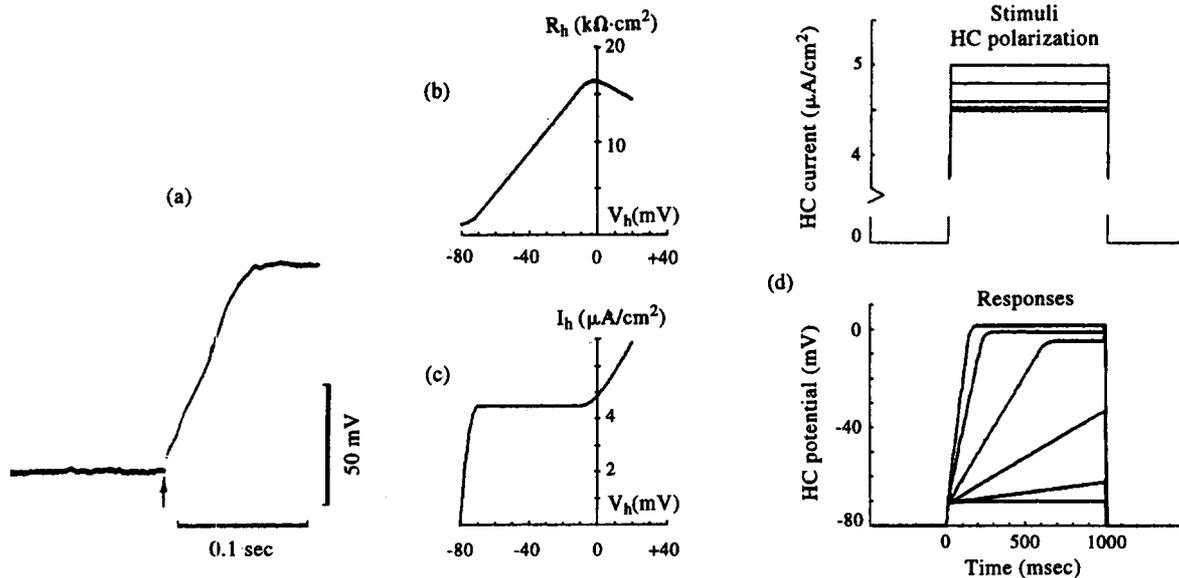
FIGURE 4. Dynamics of membrane potential shifts in a HC with infinite slope resistance. (a) Slowly developing depolarization in a HC of the ide *Leuciscus idus* retina in response to a rectangular depolarizing current step; arrow indicates the beginning of the current (Trifonov *et al.*, 1977). (b) and (c) parameters of the HC non-synaptic membrane as used in the model: $R_h(V_h)$ and $I$–$V$ curve with zero slope conductance in the range $-10$ to $-70$ mV. (d) The responses of the model (bottom traces) to depolarizing current steps of different intensities (top traces).

ground" current because the membrane resistance $R_h$ in the whole range of $V_h$ is lower [Fig. 3(b)]. On the other hand, the same changes of the membrane potential are evoked by much smaller current steps [Fig. 3(d), (2), top traces] because the slope resistance of the HC is higher. Because of this, the non-synaptic HC membrane operates as an amplifier of graded potentials (Byzov *et al.*, 1977). Finally, the noticeable slowing of transients is observed ($\tau = 70$ msec) as compared with the case $R_h = $ Const, as a result of the higher slope resistance [Fig. 3(d), (2), bottom traces]. This slowing is the same in the whole range of potentials because the slope resistance was made constant in this range.

More realistic and more complicated results were obtained with the non-linearity of type 3 in Fig. 3. As seen in Fig. 3(b), (3), the slope resistance is high at low membrane potentials and drops with steady hyperpolarization. Therefore, to evoke the same potential changes one should apply weaker current pulses when $V_h$ is low and stronger pulses when $V_h$ is high. Moreover, the transients are slower ($\tau = 42$ msec) with steady depolarization and faster ($\tau = 8$ msec) with steady hyperpolarization [Fig. 3(d), (3), bottom traces]. As will be shown later, this result is typical also for HCs with synaptic inputs.

Of special interest is the case where $R_h$ is proportional to $V_h$–$E_h$ in some range of potentials [Fig. 4(b)]. In this case Eq. (1) is simplified. Indeed, let $R_h = (V_h - E_h)/I_0$, where $I_0$ is some constant, then:

$$C_h \frac{dV_h}{dt} = \frac{E_h - V_h}{R_h} + I_h = \frac{E_h - V_h}{V_h - E_h} \cdot I_0 + I_h = -I_0 + I_h.$$

(2)

The steady state solution gives: $I_h = I_0$, that is the

corresponding $I$–$V$ curve is horizontal in this range and the slope resistance is infinite [Fig. 4(c)]. $I$–$V$ curves of this type are rather common for the fish retina [see Fig. 2(c)]. If to apply the constant current $I_h$ to the cell, then, according to Eq. (2), the voltage $V_h$ will change in time not as exponential but as linear, in the given range of potentials. In Fig. 4(d) the HC membrane potential was computed in response to depolarizing rectangular current pulses of slightly different intensities [see top traces in Fig. 4(d)]. The potential changed linearly in time until it reached a new steady level. The rate of these changes increased with the increase of the current.

This model result was actually obtained [Fig. 4(a)] in an old experiment on the ide retina (Trifonov *et al.*, 1977). The initial membrane potential level corresponded to bright light (due to the deterioration of synaptic transmission from photoreceptors), as if the synapses did not exist, like in the equivalent circuit in Fig. 3(a). The current was passed through the horizontal cell membrane by means of a special method of polarization with extracellular electric fields [for details see: Trifonov *et al.* (1974); Trifonov & Chailahian (1975); Byzov *et al.* (1977)]. The depolarizing current step evoked an almost linearly increasing depolarization that reached a new steady level in about 65 msec. In this experiment of Fig. 4(a) we did not try to apply currents of different intensities, but this can be easily done on isolated HCs.

An even more interesting simulation result was obtained when the $I$–$V$ curve had a region of negative slope resistance in the working range of potentials [see Fig. 2(d)]. An example of calculation is shown in Fig. 5(b–d). With gradual increase of depolarizing current pulses the HC potential at first slows down and then, in a
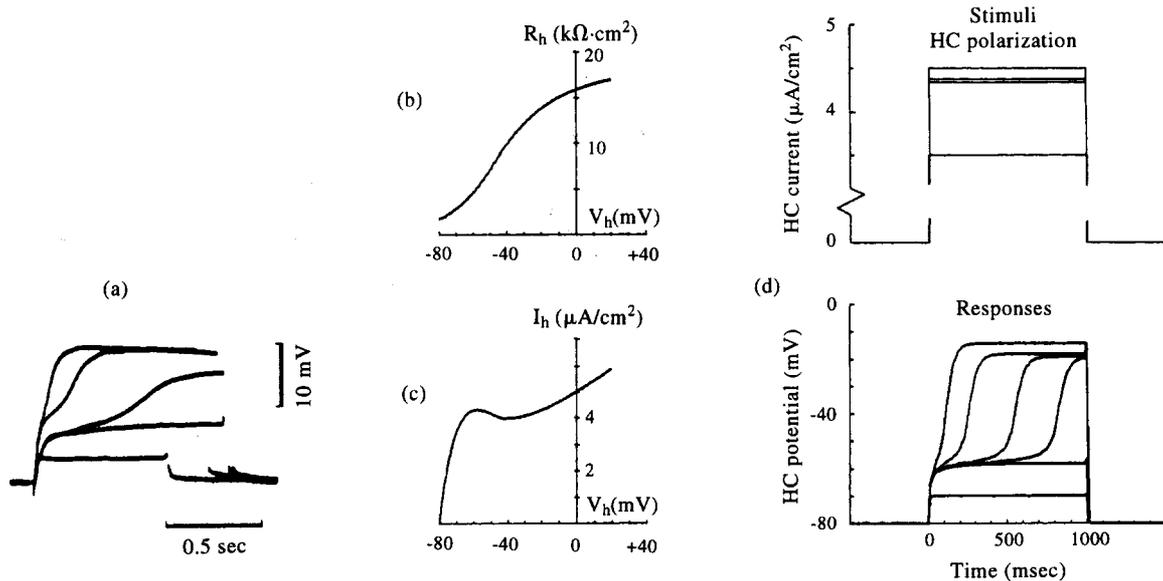
FIGURE 5. Dynamics of a HC potential shift from one stable level to another in the case of an $I-V$ curve with negative slope resistance. (a) The shifts of HC membrane potential in the pike retina evoked by depolarizing constant current steps of different intensities. Synaptic transmission from photoreceptors was blocked (Trifonov et al., 1971). (b) and (c) parameters of the HC non-synaptic membrane: $R_h(V_h)$ and $I-V$ curve. (d) The responses of the model (bottom traces) to depolarizing current steps of different intensities (top traces).

very narrow range of current intensities, shifts to a new stable level in a wave-like manner with different dynamics [Fig. 5(d), bottom traces]. The range of these potential changes exactly corresponds to the negative slope resistance region in the $I-V$ curve, and the rate of potential changes is determined by the minor alterations of current intensity. This result very closely repeats the observation of Tachibana (1981) on the isolated goldfish HCs [see his Fig. 1(b)] as well as our old experiment on HCs of pike retina (Trifonov et al., 1971) [Fig. 5(a)]. In this experiment the synaptic transmission from photoreceptors was blocked completely, therefore the model of Fig. 3(a) seems to be adequate.

## HORIZONTAL CELLS WITH SYNAPTIC INPUTS

HCs in situ are able to respond to light, having synaptic inputs from photoreceptors. Therefore the shape of ON transients should be influenced by the rate of transmitter removal. According to the model of Byzov and Shura-Bura (1992) for the turtle retina and to the scheme in Fig. 1(b), its time constant is 33 msec (rate constant $30 \sec^{-1}$). To reproduce the slow responses of pike retina it even had to be doubled. The equivalent circuit of the model has to be modified [Fig. 6(c)] by the addition of synaptic conductance $G_s$, which is controlled by the transmitter released from photoreceptor terminals. For the current passed through the HC membrane, this conductance shunts the non-synaptic membrane and therefore influences the electrical properties of the circuit. In the following examples we will show that even under these physiologically more relevant conditions the shaping of HC light responses is mainly

determined by voltage-dependent properties of the non-synaptic membrane.

Figure 6(a) illustrates an experiment on HC of the pike retina (Maximova & Maximov, 1971). Weak light evoked a slowly developing ON response and a faster OFF response. However, when the cell was hyperpolarized by an extrinsic current passed through a second intracellular microelectrode, the same light evoked a much faster ON response. The result resembles that obtained in the model of an "isolated" HC [Fig. 3(d), (3), bottom traces] and indicates that the non-linearity of the non-synaptic membrane may be the cause of this speeding up of the ON response.

This assumption was tested in the model of a HC with synaptic input. The chosen non-linearity of the non-synaptic membrane is shown in Fig. 6(d) for $R_h(V_h)$ and in Fig. 6(e) for the $I-V$ curve. The last one has a region with a negative slope resistance, which is a common property of fish HCs (Byzov et al., 1977). The input signal was a stepwise hyperpolarization of the cone by 10 mV [Fig. 6(f), top trace] imitating the light stimulus. Bottom traces in Fig. 6(f) show the model responses of a HC without extrinsic current and during a HC hyperpolarization by a current of $2~\mu A/cm^2$. One can see [Fig. 6(f)] that the model reproduces the speeding up of the HC ON response by extrinsic hyperpolarization quite well.

The HC membrane potential can also be shifted synaptically by polarizing the photoreceptor presynaptic membrane. Figure 7(a) shows such an experiment in the turtle retina (Byzov, 1988). The retina was moderately stimulated by light, and the membrane potential of the HC was shifted using a constant current passed radially through the retina. This current evoked depolarization (+)
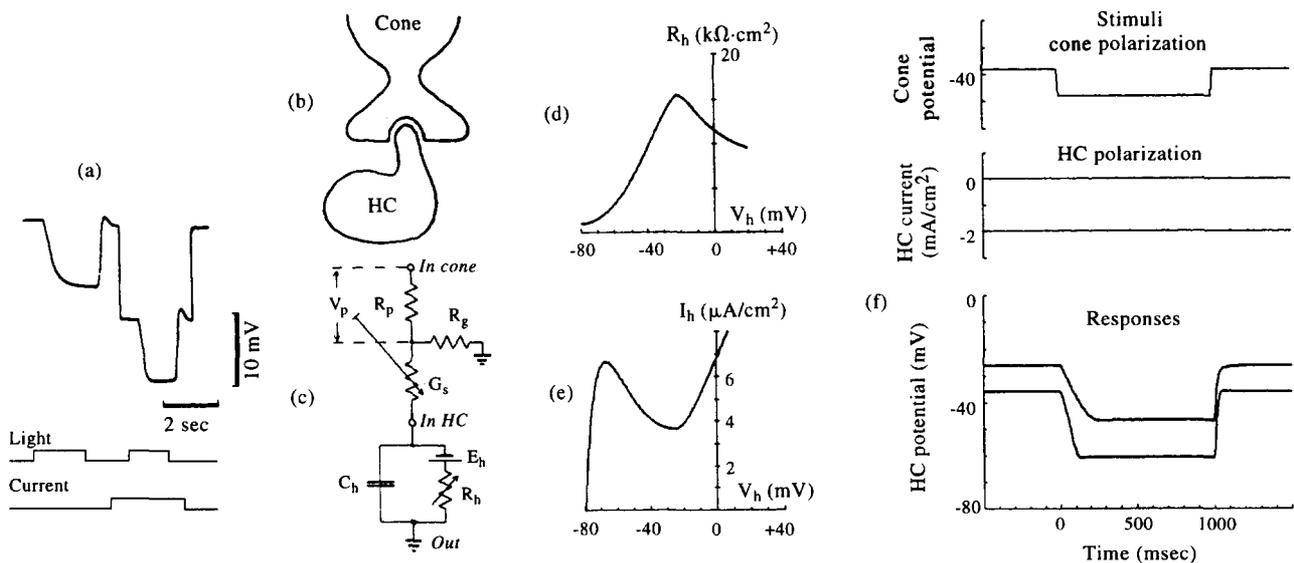
FIGURE 6. The effect of HC polarization on the dynamics of its light responses. (a) Experiment on the pike retina: extrinsic hyperpolarization of the HC speeds up its light response (Maximova & Maximov, 1971). (b) and (c) Schematic representation and equivalent circuit of the model HC with synaptic input; $G_s$, conductance of the subsynaptic HC membrane that is taken proportional to the concentration of transmitter in the gap, $m$; $V_p$, the potential difference across the presynaptic membrane that controls subsynaptic conductance $G_s$; $R_g$, the longitudinal resistance of the synaptic gap that introduces the electrical feedback in the circuit. (d and e) Parameters of the HC non-linearity. (f) Model results: hyperpolarization of the HC by current speeds up the light response.

or hyperpolarization (−) in the HC due to a tonic activation or deactivation of transmitter release from photoreceptor terminals [for other details see Byzov (1988)]. In almost all experiments depolarization of HCs was accompanied by slowing down and hyperpolarization—by a speeding up of the ON response.

Almost the same model of the fish HC with a synapse [Fig. 6(b and c)] was applied to reproduce this result. To imitate the faster HC responses of the turtle retina (in comparison with fish), we used a higher rate of transmitter removal [rate constant $30 \, \text{sec}^{-1}$ as in Fig. 1(b)] than for the pike HCs. Also, the less pronounced non-linearity of $R_h$ as shown in Fig. 5(b, c) was used, since it seemed to be more characteristic for turtle HCs. Depolarization or hyperpolarization of the photoreceptor synaptic terminals by radial current was simulated simply by changes in the initial level of the cone potential $V_c$. Light responses of the cone were mimicked by hyperpolarizing current pulses to the cone $-V_c(t)$. The result is shown in Fig. 7(b). Slowing of the ON response under depolarizing radial current and its speeding up under hyperpolarizing current were readily reproduced. Quantitatively, the time constant of the leading edges of the model's ON responses approximated by exponents, were 43 msec with a depolarizing current and 32 msec with a hyperpolarizing current. This result qualitatively corresponds to the actual retinal responses in Fig. 7(a).

Thus, it is evident that the shape of HC ON responses is strongly influenced not only by the rate of transmitter removal but also by the non-linear properties of the non-synaptic membrane. One can expect that any shift of HC membrane potential irrespective of its cause would

produce similar changes of the ON response shape. This was confirmed in experiments on L-type HCs of the pike retina, where the interaction of the input signals from red and green cones was examined (Maximova & Maximov, 1971). The response to the same red light [Fig. 8(a)] was slow when evoked in darkness and fast, even accompanied by an ON peak, when evoked on a blue background.

A model capable of reproducing this result [Fig. 8(b and c)] was produced from the previous one [Fig. 6(b and c)] by simple dividing the synapse into two equal parts: one from the red (r) and one from the green (g) cones. All other parameters were preserved. The result is shown in Fig. 8(d). In general, the phenomenology is reproduced quite well: the ON response to the red on a blue background is much faster (even with the ON peak) than in darkness. A two-fold increase of the amplitude of the red response on a blue background reproduces the "enhancement" phenomenon (Maximova et al., 1966). It results mainly from amplifying properties of the HC non-synaptic membrane (Byzov et al., 1977).

The most striking effect of HC membrane non-linearity on the dynamics of their light response is shown in Fig. 9. The experimental recordings illustrated in (a) were obtained from the pike retina (Byzov et al., 1977) under conditions when synaptic transmission was partially deteriorated, so that the dark potential was somewhat hyperpolarized and the light response decreased. Depolarization of the HC by extrinsic current [uniform polarization of the HC layer by an extracellular electrical field of special form—see Byzov et al. (1977)] was accompanied, in a certain range of potentials, by a strong
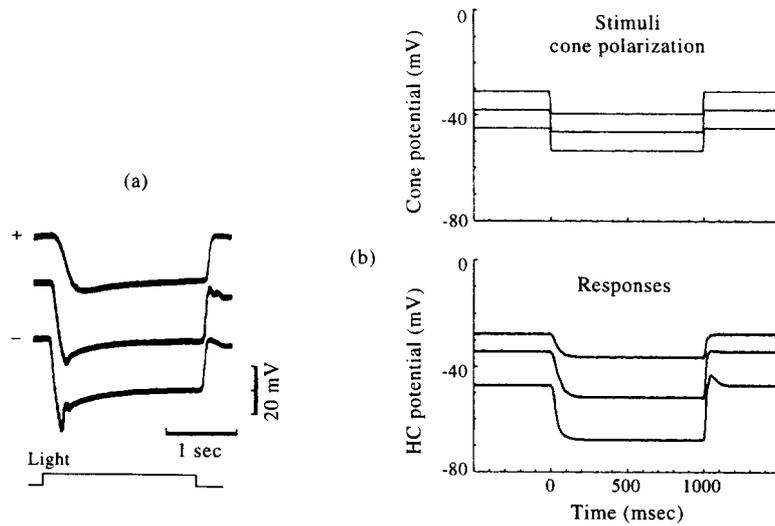
FIGURE 7. The dynamics of HC ON light responses in the turtle retina under different levels of initial membrane potential. In this case the membrane potential was controlled by constant polarization of the photoreceptor presynaptic membrane thus activating or inactivating transmitter release. (a) Experiment on the turtle retina: depolarization (+) and hyperpolarization (−) was evoked by constant radial current of opposite directions (Byzov, 1988). (b) Model results: the HC non-linearity parameters were taken similar to those in Fig. 5(b and c). The time constant of the leading edge of the upper response is 43 msec, of the lower response 32 msec.

slowing of the ON light response. Further depolarization resulted in a decrease and reversal of the response. This phenomenon looks similar to the recordings demonstrated by Kamermans and Werblin (1992). It also resembles the slow changes of membrane potential (but in opposite direction) in the models of "isolated" HCs (Figs 4 and 5). The model of Fig. 6 could immediately reproduce the phenomenon [Fig. 9(b)]. The only adjustment needed was to reduce the synaptic input (to decrease

synaptic efficiency $k$ by 1.5 times) thus simulating the deterioration of synaptic transmission.

## DYNAMICS OF C-TYPE HORIZONTAL CELL RESPONSES

In all above examples of HC light response simulation the electrical feedback from HCs to photoreceptors was incorporated in the model due to the existence of gap
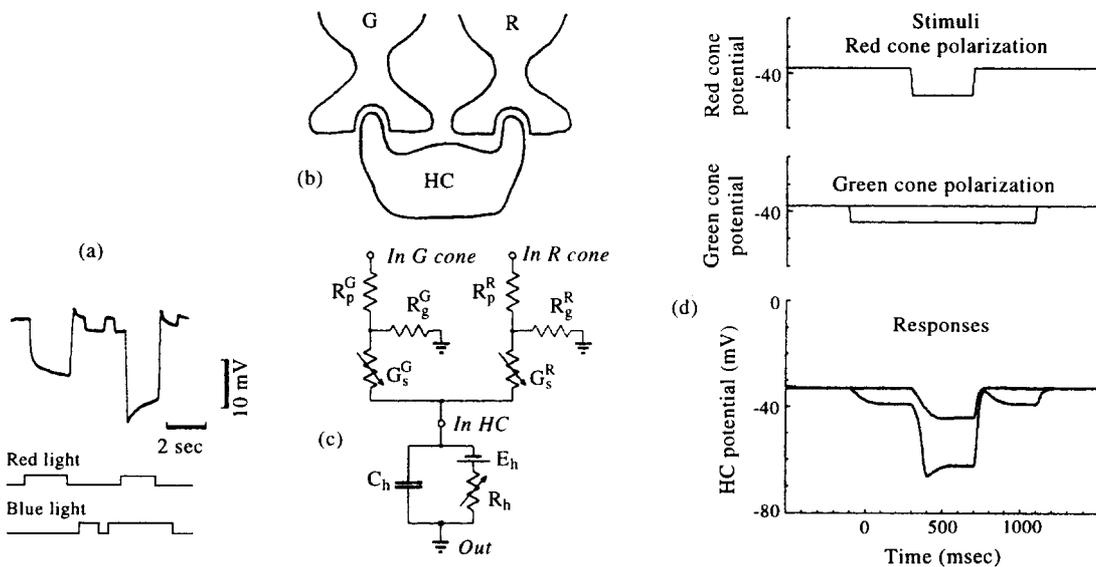


FIGURE 8. Interaction of red and green cone inputs to a HC of the L-type. (a) Experiment on the pike retina: the same intensity red light evokes a much higher and faster response on the blue background than in darkness (Maximova & Maximov, 1971). (b) and (c) Schematic representation and the equivalent circuit of the model. The model is the same as that in Fig. 6(c) except that it has two equal synaptic inputs from R and G cones. (d) The model reproduces both the speeding up and the enhancement of the red response by a blue background.
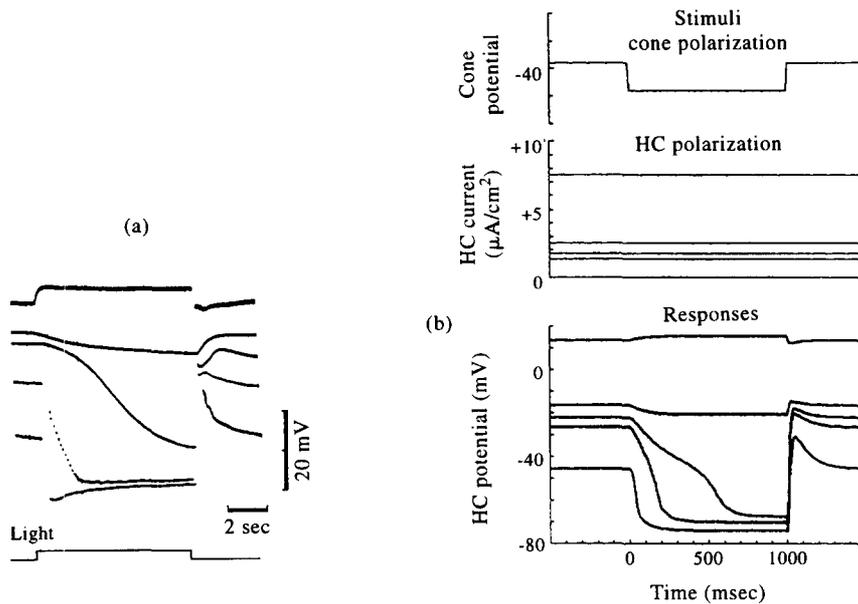
FIGURE 9. Extremely slow light responses in a HC. (a) Experiment on the pike retina (Byzov *et al.*, 1977). The lower trace is a light response without the current: the initial dark potential is somewhat hyperpolarized due to a partial deterioration of synaptic transmission; other curves—light responses to the same light during depolarization of the HC by currents of successively increasing intensity. (b) Reproducing the effect on the model of Fig. 6 with synaptic efficacy *k* decreased by 1.5 times.

resistance $R_g$ (Figs 6–8). However, its effect was obscured by a more powerful factor—the non-linearity of the HC non-synaptic membrane. For C-type HCs the feedback mechanism is obviously the most important factor. To reproduce the depolarizing response to red light in R/G-cell the equivalent circuit was changed in accordance with the basic idea of Stell (1975) and Fuortes (1976) that was already applied in our previous stationary model of C-type HCs (Byzov *et al.*, 1992) [Fig. 10(b and c)]. The model contains cones of two types: red (R) and green (G); and two HCs: one with inputs from red and green cones (L-cell) and the other with input from green cones only (R/G-cell). The L-cell is identical in all respects to that in the previous model [Fig. 8(b and c)], so only the R/G-cell is a new element of the circuit. L- and R/G-cell non-linearities are the same as in Fig. 5(b and c). The hyperpolarizing response of an R/G-cell to blue light is similar to that in L-cells, but the depolarizing response to red light is the result of electrical feedback from L-cell to the green cone's presynaptic membrane. One can see from Fig. 10(d) that the model is able to reproduce not only the depolarizing response to stimulation of the red cone, but also the typical delay (30–50 msec) between the responses to blue and red stimuli. This delay of the red response is easily explained in the model. The same delay already exists on the presynaptic membrane of the green cone because it reflects exactly, but with reversed sign, the response of L-cell to red light. The leading edge of the last one is slowed by: (1) the dynamics of transmitter removal from the synaptic gap of the red cone; and (2) the time constant of the L-cell itself. In Fig. 10(a) the experimental responses of an R/G-cell in the turtle retina are shown for comparison.

## DISCUSSION

The results described clearly demonstrate the importance of the HC membrane non-linearity in shaping their light responses. However, the role of this factor is not always evident. The more complex the model, the more factors influence its performance. Connection of a synaptic input to the HC results in two complications. The first is that high conductance of the subsynaptic membrane in darkness shunts the non-synaptic membrane, thus reducing the total time constant of the HC. As seen from Figs 6–9, this factor does not prevent the observance of non-linear effects but makes it impossible to reveal the negative slope resistance of the HC membrane when the subsynaptic conductance is activated, even partially, by the transmitter.

The second complication results from the dynamics of synaptic transmission. It can be evidenced when considering the OFF responses to light stimuli. In experimental and model recordings (Figs 5–9) the OFF transients in HCs are fast and their dynamics do not depend appreciably on the non-linearity of the HC membrane. We postulate this happens because there is a transient component of transmitter release. This component is activated only by depolarization of the presynaptic membrane, that is by the OFF response in photoreceptors.

The existence of the transient component of synaptic transmission can be the subject of a separate discussion. Introducing this component to the model allowed us to reproduce not only OFF responses in HCs, but also the dynamics of transition from the hyperpolarizing ON peak to the plateau. The parameters of the transient component of synaptic transmission [Fig. 1(b)] were adjusted to reproduce as close as possible the impulse response of the
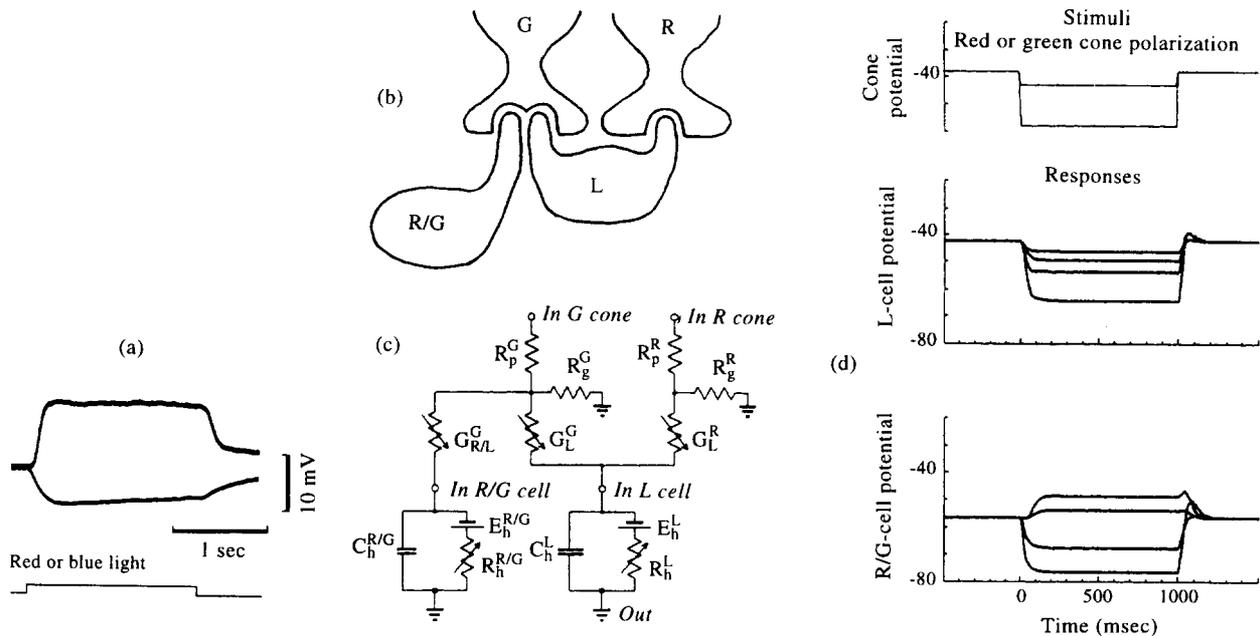
FIGURE 10. Simulation of R/G HC responses. (a) HC responses to blue and red lights in the turtle retina. (b) and (c) scheme and equivalent circuit of the model. The double indexes for subsynaptic conductances $G$ mark the cone-type (R or G) of invaginations (upper) and the H(C) type to which the processes belong (lower)-modified from Byzov *et al.* (1992). (d) Results of the model study of L- and R/G-cell responses (middle and bottom traces) to stimulation of red and green cones-hyperpolarizing potential steps of two intensities (top traces).

HC: its response to a very short depolarizing current pulse [*cf* Fig. 1(c) and (a)]. Certainly, this response is the result of several consecutive processes: the dynamics of transmitter release, activation and possible desensitization of postsynaptic receptors to the transmitter, involvement of voltage-dependent currents in HC non-synaptic membrane. The last component can be largely excluded by the fact that in the voltage-clamp experiments in fish HCs *in situ* ionic currents were stabilized at least in 15 msec after the depolarizing voltage step (Trifonov *et al.*, 1977). Fast activation of $Ca^{2+}$ current and $K^+$ current of anomalous rectification-type was observed also in isolated fish HCs (Tachibana, 1983). Therefore the non-linearity of HC non-synaptic membrane was taken in our model as instant. However, in the late slow phase of the impulse response the non-linearity of the HC membrane in combination with its capacitance can modify the response shape.

As to the desensitization of postsynaptic glutamate receptors, there is no experimental evidence that it really takes place. The extracellular application of glutamate or its agonists to isolated HCs showed no sign of desensitization, at least for a few seconds after application (Tachibana, 1985). Nevertheless, we cannot exclude completely the involvement of some non-presynaptic processes in our model description of transient components of transmitter release.

Finally, the mechanism of electrical feedback from HCs to photoreceptors, in combination with synaptic transmission dynamics, not only produces the depolarizing response in R/G-cells to red light, but delays its onset

by 30–50 msec in respect to the latency of a response to blue light. This almost exactly coincides with what was measured by Spekreijse and Norton (1978)—around 30 msec. Interestingly, this delay was relatively insensitive to any variations of the model parameters.

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