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The feedforward component in depolarizing red responses of R/G horizontal cells in carp retina

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Abstract

Light responses of R/G chromaticity-type horizontal cells (R/G HCs) and luminosity-type horizontal cells (LHCs) were intracellularly recorded in isolated superfused carp retina, and the response dynamics analyzed. The results revealed that (1) No significant difference in delay was detected between R/G HC red and green responses; (2) The rising speed was quicker for R/G HC depolarizing red responses compared to that of its hyperpolarizing green responses; and (3) Dynamic characteristics of R/G HC red responses and its changes caused by green background illumination did not follow that of LHC red response. All these results suggest that the depolarizing response of the R/G HCs cannot be entirely mediated by the negative feedback pathway from LHCs onto cones. A direct inhibitory input from red cones to R/G HCs may exist.

Keywords: R/G horizontal cell (R/G HC), Carp retina, Color opponency, Dynamic characteristics

Introduction

In cyprinid retinas, cone-driven horizontal cells (HCs) are classified into luminosity and chromaticity types, with the former hyperpolarizing over the whole spectrum and the latter varying response polarity depending on the stimulus wavelength. For instance, R/G type HCs hyperpolarize to short-wavelength stimuli, but depolarize to long-wavelength stimuli. According to the hypothesis proposed by Stell and Lightfoot (1975), mainly based on anatomical observations, the hyperpolarizing response of the R/G HCs is driven by input directly from green cones, whereas the depolarizing response to red light is induced by the cascade signal of green cones fed back from luminosity HCs (LHCs). This was consistent with the knowledge that in the outer retina, glutamate was continuously released from photoreceptors in the dark and the release was reduced by light (Dowling & Ripps, 1973). LHC was depolarized by glutamate and, in turn, released inhibitory neurotransmitter GABA upon depolarization to mediate a feedback pathway from HCs to cones (Lam & Steinman, 1971; Lam, 1975).

While a lot of morphological and physiological evidence support the Stell's cascade model (Murakami et al., 1982*a*,*b*; Toyoda & Fujimoto, 1983), numerous recent findings have challenged it. Among others, it was recently demonstrated in turtle retina that R/G HCs' responses to red illumination were quicker than those of LHCs (Asi & Perlman, 1998), which suggests that some other signal pathway may be involved in the formation of the color opponency of R/G HCs.

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In the present study, we analyzed and compared the dynamic characteristics of light responses of R/G HCs and LHCs under various experimental conditions. Our results suggest that a direct sign-inverting input from red cones to R/G HCs may exist, which contributes to the depolarizing red response of these cells.

Methods

Experimental procedures and data collecting

Experiments were performed following the procedure described in a previous report (Zhang & Yang, 1997). Briefly, the eye of crucian carp (*Carassius auratus*, body length 15–20 cm) was enucleated after dark adapted for 20 min and the retina was isolated under dim red light. The retina was placed with the photoreceptor-side-up in a chamber with a volume of 1.4 ml and continuously perfused at a flow rate of 1.8 ml/min with oxygenated (95% O_2+5% CO_2) Ringer solution containing (in mM): NaCl 116.0, KCl 2.4, $CaCl_2$ 1.2, $MgCl_2$ 1.0, NaH_2PO_4 1.0, $NaHCO_3$ 30.0, and Glucose 10.0 (pH 7.7).

The photostimulator consisted of two identical optical beams generated from a 100-W tungsten halogen light source (Osram, Germany). Both beams were set to present coincided 8-mm-diameter diffuse light spots on the retina, used for test flash and background light, respectively. The duration of light stimuli was controlled by a magnetic shutter (MS-101, Hirogo, Japan) and set at 500 ms for all test flashes, with the off intervals being 5.5 s, in the present study. The wavelength and intensity of the stimulus were selected by interference filters and neutral density filters, respectively. The light intensities were measured using a photodetector (UDT-114A, United Detector Technology, Hawthorne, CA).

The unattenuated 703-nm light ($\log I = 0$) was 6.11×10^{13} photons$\cdot$cm$^{-2}\cdot$s^{-1}. All stimulus intensities are presented in relative log units in this paper. The microelectrode was pulled using a microelectrode puller (PD-5, Narishige, Japan) and had a resistance around 100 MΩ measured in Ringer solution when filled with 4 M potassium acetate. A micromanipulator (PF-1, Narishige, Japan) was used to advance the electrode into the retina. Responses were measured intracellularly using an amplifier system (MEZ-8201, Nihon Kohden, Japan) and displayed on an oscilloscope (DSS-6521, Kikusui, Japan). Raw data were sampled *via* an A/D interface (AD8111, Adlink Technology, Inc., Taiwan) at a rate of 100 Hz for both stimulus signals and cells' responses, and stored in an IBM-compatible PC for off-line analysis.

In the current study, the experiments started immediately after the isolation of retina, and no further dark adaptation was performed. The test light was given in the dark, unless otherwise stated. For those experiments with a dim green (501 nm, $\log I = -4.4$) background light, the background light was switched on 2 ~ 3 s before the test light was given.

Dynamic analysis and parameter comparisons

To analyze the dynamic characteristics of light response of relevant types of horizontal cells during relevant light stimuli, two parameters, that is, response delay and the speed of the rising phase, were estimated and statistically compared using Student's *t* test. In the current study, response delay δ was defined as the difference between the stimulus onset and the time by when the cell's membrane potential started to change. Moving average algorithm was applied to minimize the noise effect when δ was determined. The speed of rising phase was estimated using the curve-fitting technique. To avoid possible bias of estimate caused by over simplification, a two-component exponential function was applied.

$$V_{HC}(t) = V_{dark} + G_1(1 - e^{-t/\tau_1}) + G_2(1 - e^{-t/\tau_2}),$$

where V_{HC} and V_{dark} denote the HC's membrane potential measured during light response and in the dark, respectively. G and τ represent the gain term and the time constant for each exponential term, respectively. The fitting was performed *via* quasi-Newton method, and the time constant τ_1 of the dominant component (with a dominating gain term G_1) was taken as the measure of rising speed of the cell's light response, for statistical analysis.

Results

Dynamic comparison between red and green responses of R/G HCs

For dynamic analysis, we first compared rising phases of hyperpolarizing and depolarizing responses of R/G HCs. Fig. 1A shows the depolarizing response to a red flash (703 nm, $\log I = -2.97$) and the hyperpolarizing response to a green flash (501 nm, $\log I = -3.58$) of an R/G HC. To facilitate the comparison and avoid possible significant difference in noise level due to the difference in scaling factors of the curves, the stimulus intensities were chosen for the two wavelengths so as to yield the responses of similar amplitudes. When the comparison was made, the depolarizing response was inverted in polarity and both the responses were normalized (Fig. 1B). It was clear that there was no obvious difference

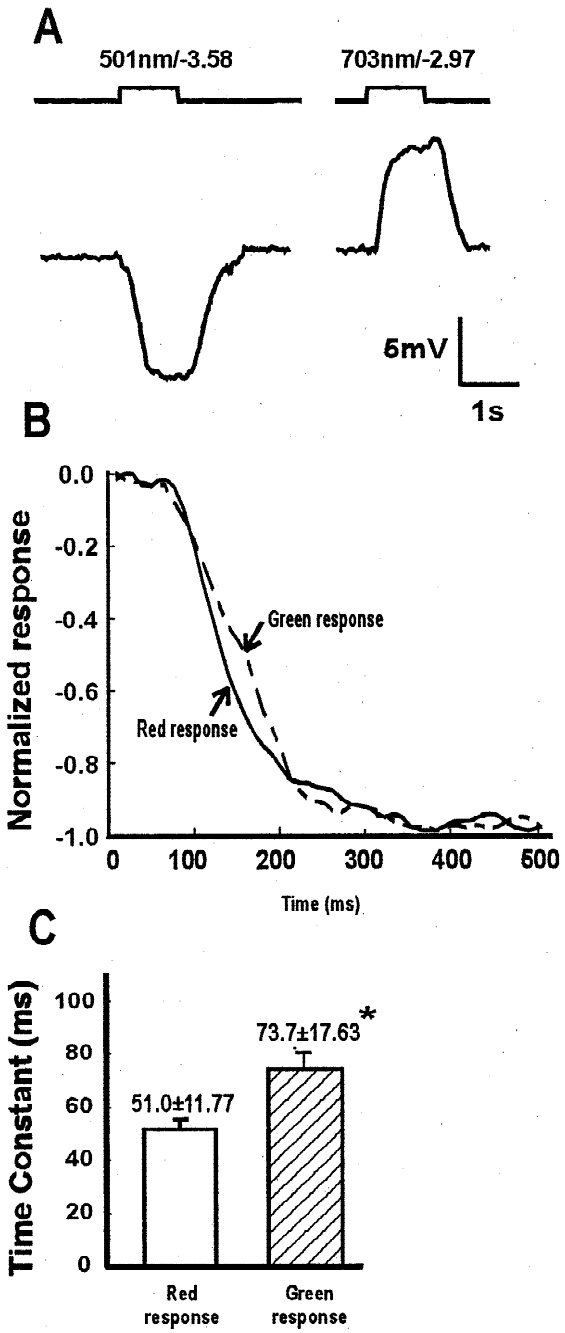

Fig. 1. Dynamic comparison of R/G HC red and green responses. (A) Light responses of an R/G HC to green (501 nm, −3.58) and red (703 nm, −2.97) flashes. (B) Normalized rising phases of the cell red (inverted) and green responses (same as A). The dynamic of the red response was clearly faster. (C) Statistics of the time constant τ_1 for R/G HC red and green responses (∗, $P < 0.05$, paired *t* test, $n = 8$). Mean ± S.D. values are given with bars indicating standard errors.

in δ between the two responses (65.0 ms *vs.* 60.0 ms for the cell's red and green responses, respectively). Statistics from 8 cells resulted in δ values (mean ± S.D.) of 61.3 ± 12.46 ms for the red response and 63.8 ± 11.88 ms for the green response. No significant difference was tested ($P > 0.05$, paired *t* test). On the other hand, the dynamics of the red response was faster than that of the green response, the values of τ_1 were estimated as 61.4 ms and

73.3 ms for the red and green responses respectively, in this case. Data from eight cells showed that the difference in values of τ_1 for the red response (51.0 ± 11.77 ms) and the green response (73.7 ± 17.63 ms) was significant (Fig. 1C, $P < 0.05$, paired *t* test).

Dynamic comparison between red responses of R/G HCs and LHCs

The dynamic characteristics of the depolarizing responses of R/G HCs were further compared with the hyperpolarizing responses of LHCs elicited by red flashes of same intensity (703 nm, $\log I = -2.97$). A pair of representative responses recorded from an R/G HC and an LHC in the same preparation under the same adaptation state are illustrated in Fig. 2A. The rising phases of the normalized responses are compared in Fig. 2B, with the R/G HC response trace being inverted. It was obvious that the rising phase of the R/G HC response did not follow that of the LHC response. Although R/G HC response started with a longer delay, that is, 55.0 ms as compared to 35.0 ms for LHC (with the mean ± S.D. of δ values for eight R/G HCs and six LHCs being 61.3 ± 12.46 ms and 30.0 ± 6.32 ms, respectively, $P < 0.05$, unpaired *t* test), it ran much faster than the LHC response. The values of τ_1 were estimated as 55.1 ms and 66.5 ms for the R/G HC and LHC responses, respectively, in this case. Comparison between data collected from eight R/G HCs and six LHCs gave evidence that the R/G HC response has a steeper rising phase as compared to that of the LHC response. The mean ± S.D. of τ_1 was 51.0 ± 11.77 ms for the R/G HC response which was significantly shorter than that for the LHC response (66.4 ± 12.41 ms) (Fig. 2C, $P < 0.05$, unpaired *t* test).

Chromatic adaptation differentially altered the dynamics of red responses of R/G HCs and LHCs

The influence of background illumination on the dynamics of R/G HC and LHC red responses was further investigated. In Fig. 3A, the effect of a dim green background light (501 nm, $\log I = -4.40$) on the response of an LHC to a red flash (703 nm, $\log I = -2.97$) is shown. The background light hyperpolarized the cell by 2.3 mV, and slightly increased the response from 6.3 mV in the dark to 8.8 mV. When both the responses were normalized and compared (Fig. 3B), the rising phase of the response in the presence of the background light was shown to be steeper and with a shorter value of τ_1 (49.8 ms during background illumination *vs.* 65.7 ms in the dark). Statistics from six LHCs resulted in significant difference ($P < 0.05$, paired *t* test) in τ_1 values obtained in the dark and in the presence of the green background light (66.4 ± 12.41 ms and 52.5 ± 14.91 ms, respectively, Fig. 3C). Fig. 3D shows the effects of the same green background light on the depolarizing response of an R/G HC to the same red flash. The background light hyperpolarized the cell by 17.0 mV, and enhanced the response from 6.8 mV in the dark to 10.0 mV. Interestingly, the rising phase of the response ran much slower in the presence of the background light (τ_1 being 66.7 ms during background illumination *vs.* 55.1 ms during in the dark, Fig. 3E). Statistics from six R/G HCs showed that τ_1 was 53.4 ± 12.86 ms for the responses recorded in the dark and 64.3 ± 8.55 ms for those obtained with the background light (Fig. 3F). The difference was significant ($P < 0.05$, paired *t* test).

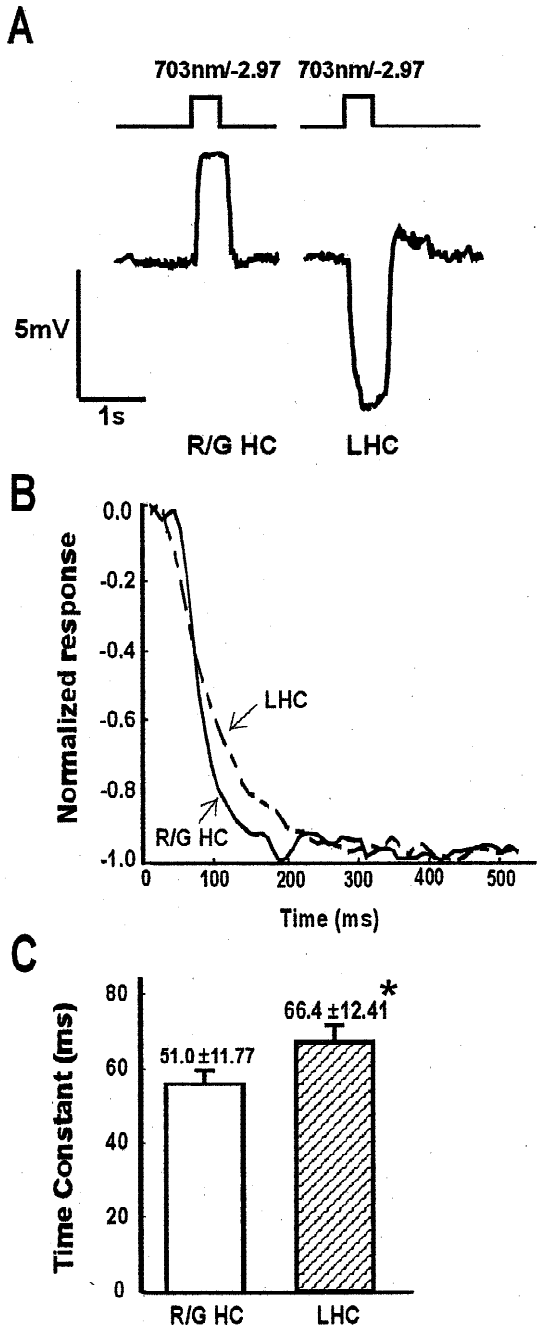

Fig. 2. Comparison between the red responses of R/G HCs and LHCs. (A) Red stimulus (703 nm, −2.97) elicited responses recorded from an R/G HC and an LHC. (B) Normalized rising phases of the R/G HC (inverted) and LHC responses (same as A). The R/G HC response has a longer response delay but a steeper rising phase. (C) Comparison between the time constant τ_1 of R/G HC and LHC responses (*, $P < 0.05$, unpaired *t* test, 8 R/G HCs *vs.* 6 LHCs). Mean ± S.D. values are given with bars indicating standard errors.

Discussion

The mechanism underlying color opponency of chromaticity horizontal cells (CHCs) has been extensively studied, but still remains ambiguous. The cascade model proposed on the basis of anatomical observations made in goldfish retina (Stell & Lightfoot, 1975) has been supported by a lot of evidence, mainly collected from the carp retina. For instance, the time delay of R/G HC red responses was reported to be significantly longer than that of its green responses (Spekreijse & Norton, 1970; Yamada et al., 1985). It was

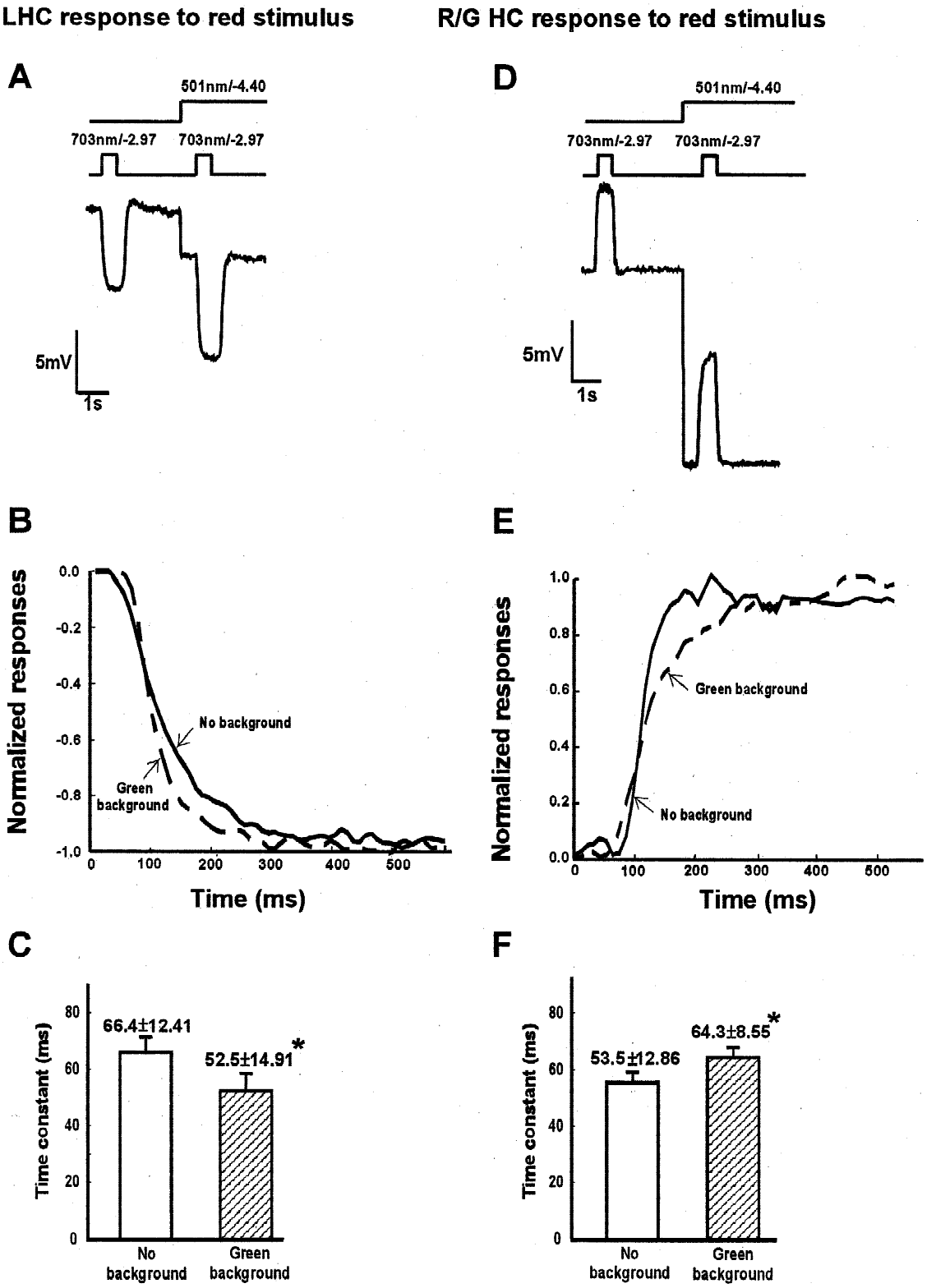

Fig. 3. Effects of green background illumination on the dynamics of LHC and R/G HC red responses. (A) An LHC red (703 nm, −2.97) response was enhanced by a green background light (501 nm, −4.4), from 6.3 mV to 8.8 mV. (B) Normalized rising phases of the cell (same as A) response dynamics. It was faster when the background light was applied. (C) Comparison between the τ_1 values of LHC responses during different chromatic adaptation states (∗, $P < 0.05$, paired t test, $n = 6$). (D) A green background light (501 nm, −4.4) hyperpolarized an R/G HC and potentiated its response to red stimulus (703 nm, −2.97) from 6.8 mV to 10.0 mV. (E) Normalized rising phases show that the cell (same as D) response dynamics was slowed down during the background illumination. (F) Comparison between the τ_1 values of the R/G HC responses during different chromatic adaptation states (∗, $P < 0.05$, paired t test, $n = 6$). Mean ± S.D. values are given with bars indicating standard errors.

also found that a hyperpolarizing current injected into LHCs could result in a depolarizing response in adjacent R/G HCs (Toyoda & Fujimoto, 1983).

On the other hand, this cascade model has been challenged. Evidence from fish and turtle retinas showed that the color opponency of R/G HCs is not necessarily related to the feedback-induced depolarization in cones. It was reported that the feedback-induced depolarization in cones was not observable when the stimulus light diameter was smaller than 0.25 mm, while the color opponency persisted (Burkhardt & Hassin, 1978; Teranishi et al., 1982; Stone et al., 1990). It was further demonstrated in turtle and bowfin retinas that when red stimulation was given, the dynamics of R/G HC depolarizing responses was quicker than that of LHC hyperpolarizing responses (Gottesman & Burkhardt, 1987; Asi & Perlman, 1998). These results stand against the cascade model and suggest that some pathway other than the feedback one might be involved in forming the color opponency of R/G HCs.

Following the cascade model, the signal mediating R/G HC depolarizing red responses would have to pass two more synapses, as compared to that of its hyperpolarizing green responses, which would have resulted in a longer response delay and a slower rising phase of the former. In this study, however, the comparison between the R/G HC red and green responses of similar amplitudes indicated no significant difference in the response delay. This result is inconsistent with the previous reports in carp that the response latency of R/G HC red responses was longer than that of the green responses (Spekreijse & Norton, 1970; Yamada et al., 1985). The estimate made in these previous work were based on frequency-domain analysis, that is, it was calculated from the cells' response elicited by white noise stimuli. However, the measurement reported in this study was taken directly from the time-domain data of the cells' responses to step stimuli. Since there is possible nonlinearity existing in the cells' response dynamics, it is not surprising that the latency estimated from the cells' responses to noise input differs from that measured from the cell's response to step input.

Our results further showed that the rising speed of the R/G HC depolarizing red response was significantly quicker than that of the hyperpolarizing green response, that is, the red response was with a shorter time constant. One possible problem with this approach is that when the R/G HCs red and green responses of similar amplitude are elicited (as performed in this study), the intensity of the red stimulus should be considerably stronger than that of the green stimulus, and the response elicited by an intense stimulus usually has a steeper rising phase. However, in our experiments, the comparison between the R/G HCs green responses evoked by different stimulus intensities revealed that a stronger stimulus elicited a response with a steeper slope, but not necessarily a shorter time constant (data not shown). This seems to suggest that the difference in the R/G HCs red and green response dynamics is not a stimulus-intensity-dependent artifact. Our results are consistent with the findings made in bowfin (Gottesman & Burkhardt, 1987) and against the cascade hypothesis.

When comparison was made between the red response of R/G HCs and LHCs, it was found that the rising speed of the R/G HC red response was significantly quicker than that of the LHC red response, which was similar to the observation made in the turtle retina (Asi & Perlman, 1998), suggesting that the former is not necessarily dependent on the latter. On the other hand, the observation that R/G HC response delay was considerably longer as compared to that of LHC. This might reflect different postsynaptic receptor mechanisms, although there is no direct evidence from this study. However, previous work on dual whole-cell recording from photoreceptor–bipolar cell pairs in mudpuppy retina showed that the response delay of ON-bipolar cell to current injection onto the photoreceptor was about 50 ms longer than that observed from OFF-bipolar cell (Kim & Miller, 1993). Since ON- and OFF-bipolar cells possess metabotropic and ionotropic glutamate receptors, respectively, such difference in response delays was attributed to the differences in the postsynaptic receptors activation dynamics (Shiells & Falk, 1994).

A most interesting finding in the present work was that green background illumination differentially modulated the dynamics of the LHC and R/G HC red responses. When the green background light accelerated the response dynamics of LHC response, it slowed down the dynamics of the R/G HC red responses. This would have not occurred if the R/G HC depolarizing responses were entirely mediated through the feedback pathway from LHC to cones.

One possible argument for this phenomenon is that the green background illumination hyperpolarized R/G HC to a greater degree as compared to LHC, and activation of voltage-dependent conductances may change the dynamics of the responses. However, in our experiment, whether or not a background illumination was given, the resting membrane potential of R/G HC and LHC were both within the range between $-40 \sim -20$ mV. As reviewed by Lasater, the overall *I–V* relationship recorded from the horizontal cell was reasonably flat within this range (Lasater, 1992, Figs. 15 and 16), which seems to suggest that the effects of voltage-gated current may have little effect in this case.

In vertebrate retina, light stimulus causes a decrease of glutamate concentration in the cone–HC synaptic cleft, which includes two parallel processes—a slow decrease of glutamate release and fast dynamics of uptake *via* glutamate transporters located on the cone terminals (Vanderbranden et al., 1996; Gaal et al., 1998). Recent observations made in our laboratory actually revealed that the green-background-induced enhancement in LHC red response might be due to an activation of the glutamate transporter system (Liang, 1999), that is, the response enhancement was related to a presynaptic mechanism, which increased the response amplitude as well as accelerated the dynamics. On the other hand, a green background light hyperpolarized R/G HC, which in turn increased the depolarizing driving force for the cell's response to red stimulus. Thus the enhancement in response amplitude might be attributed, at least partly, to the change of the R/G HC membrane potential which involves a postsynaptic mechanism.

Taken together, the results reported here basically stand against the cascade hypothesis and suggest that, in addition to the feedback pathway, a direct sign-inverting input from red cones to R/G HC likely exists and contributes to the R/G HC depolarizing response in the carp retina.

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