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Short communication

Stimulus pattern related plasticity of synapses between cones and horizontal cells in carp retina

Jian-Feng Hu, Yu Liu, Pei-Ji Liang *

Key Laboratory of Neurobiology, Shanghai Institute of Physiology, Chinese Academy of Sciences, 320 Yue-Yang Road, Shanghai 200031, China

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Abstract

Stimulus pattern related synaptic plasticity in the luminosity-type horizontal cell (LHC) of isolated carp retina was investigated. The major findings were: (1) repetitive red flashes progressively strengthened the synaptic connection between red-cone and LHC, whereas weakened that between green-cone and LHC; (2) repetitive green flashes remarkably depressed the LHC's red response, but caused little changes in the cell's green response. A competitive depression between different cone signals is suggested.

Keywords: Horizontal cell; Stimulus pattern; Synaptic plasticity; Competition

In cyprinid retina, the luminosity type cone horizontal cell (LHC) receives input from both red- and green-sensitive cones, and the signals from different cone systems are not independent to each other. Early study showed that in goldfish retina, a leading green conditioning flash increased the LHC's response to the successive red test stimulus but suppressed the cell's response to green stimulus, while red conditioning light had no enhancement effect on the cell's responsiveness [11]. It was postulated that the green cone signal acted on the red cone signal that input to the LHC and caused the response enhancement. Recent work by Djamgoz et al. [2], however, revealed that in carp retina, application of prolonged red background illumination reduced the LHC's responsiveness to short-wavelength stimulus, whereas blue background illumination had little effect on the cell's spectral sensitivity. Evidence on morphological front further showed that the cone-HC synaptic connection was plastic, which was closely related to the pattern of the conditioning light [3,8].

In the current study, experiments were performed on LHC of isolated carp retina. Repetitive flash sequences with selected wavelengths (501 and 703 nm, referred to as green and red, respectively) were applied, and the consequent changes in the LHC's spectral sensitivity were investigated. The results showed that the cone-LHC synaptic efficacy was determined by the spectral characteristics and the time course of the stimulus. An interesting finding was that a competitive depression effect exists between red cone- and green cone-input fed forward to the LHC.

Experiments were performed on adult carp (*Carassius auratus*; body length: 15–20 cm) retina following the procedure described in a previous report [12]. In brief, the carp was kept in an aquarium on natural light/dark cycle. The eye was enucleated after the animal was kept in the dark for 20 min to allow for the isolation of the retina and avoid the retina to be deeply dark-adapted. The retina was isolated under dim red light, and was placed with the photoreceptor-side-up in a chamber with a volume of 1.4 ml and continuously perfused with oxygenated (95% O_2 + 5% CO_2) Ringer solution at a flow rate of 4.5 ml/min. The normal Ringer solution contained (in mM): NaCl 116.0, KCl 2.4, $CaCl_2$ 1.2, $MgCl_2$ 1.2, NaH_2PO_4 1.0, $NaHCO_3$ 30.0, glucose 10.0 and had a pH value of 7.7 in room temperature. Light stimuli were provided by a photo-stimulator using a 100-W tungsten halogen (Osram, Germany) light source. The light beam was set to deliver an 8-mm-diameter diffuse light spot projected onto the retina. The wavelength and intensity of the stimulus were selected by interference filters and neutral density filters, respectively. The unattenuated 703 nm light ($\log I = 0$) was 6.11×10^{13} photons/($cm^2 \cdot s$), measured using a UDT-114A photodetector (United Detector Technology, USA). All stimulus intensities will be presented in relative

* Corresponding author. Fax: +86-21-6433-2445; e-mail: pjliang@server.shcnc.ac.cn

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log units in this paper. The duration of light flash was controlled by a magnetic shutter (MS-101, Hirogo, Japan). The microelectrode was pulled on a microelectrode puller (PD-30, Narishige, Japan) and had a resistance around 100 MΩ when filled with 4 M KAc and measured in Ringer solution. The LHC's responses were recorded intracellularly using an amplifier system (MEZ-8300, Nihon Kohden, Japan) and monitored on an oscilloscope (DSS-6521, Kikusui, Japan). The cell's responses were sampled via an A/D interface (AD8111, Adlink Technology, Taiwan) at a rate of 200 Hz and stored on a Pentium PC for off-line analysis.

To study the plasticity of synapses between cones and LHC, repetitive flashes with light-on duration being 500 ms were given at a frequency of 1 Hz, and lasted for 20 s, otherwise stated. Green (501 nm, log $I = -2.21$) and red (703 nm, log $I = -1.13$) flickering sequences were used. The LHC's response to green and red flashes before and after the exposure to flickering lights were tested and compared. Student's *t*-test (paired) was performed for statistical analysis.

The application of repetitive red flashes caused changes in the LHC's chromatic sensitivity. Firstly, the application of repetitive red flashes remarkably inhibited the LHC's green response. As shown in Fig. 1A, the peak value of green flash (501 nm, −2.21) elicited response was 27.9 mV during control and the response amplitude was reduced to 22.5 mV (b and d, respectively) after application of the red flickering. Aside from a decrease in the LHC's green response, a progressive increase of the cell's red response occurred when the retina was exposed to the repetitive red flashes. The amplitude of the cell's red response was enhanced from 28.4 to 33.3 mV during the 20-s period (a and c, respectively). The spectral sensitivity change was reversible, and the cell's red- and green-response returned to the control level in 15 s after the light flickering had ceased (e,f). Waveforms are illustrated in Fig. 1B. Red response enhancement (22.6 ± 5.69 vs. 25.7 ± 5.77 mV, mean ± S.D., with relative enhancement being $116.1 \pm 4.97\%$) and green response depression (27.2 ± 9.76 vs. 24.0 ± 10.01 mV, with relative depression being $82.7 \pm 7.56\%$) induced by red flickering light were both significant ($P < 0.01$, paired *t*-test, $n = 20$), as illustrated in Fig. 1C.

One possible explanation for such phenomenon might be that the exposure to repetitive red flashes resembled the process of light adaptation, and light adaptation suppressed the sensitivity in short-middle wavelength region of the spectrum. To test whether or not this was the case, the influence of green flickering light was examined. Fig. 1D gives a representative cell's response. A red flash (703 nm, −1.13) evoked a response of 30.1 mV in the LHC during control (h). The response amplitude was reduced to 26.6 mV (j), when measured immediately after the exposure to the 20-s green flickering light. A recovery in the cell's red response was observed in about 15 s after the light flickering had ceased (l). The waveforms of the cell's responses during control, towards the end of flickering and after recovery are plotted in Fig. 1E. The change in the cell's response to green stimulus during the period was trivial (g,i,k). Similar results were obtained from a total number of 17 cells. The mean (± S.D.) values of the cells' response to red test flash during control and towards the end of green flickering were 21.8 ± 7.60 and 19.3 ± 6.87 mV, respectively (with relative depression being $88.1 \pm 5.97\%$, Fig. 1F). The suppression effect was significant ($P < 0.01$, paired *t*-test). These results indicated that the efficacy plasticity of synapses between cones and LHC was stimulus dependent and could not be explained by simple light adaptation effect.

To investigate the time dependence in the flickering induced auto-enhancement and mutual-chromatic suppression effects occurred, the LHCs' responses to red/green stimuli were sampled during control as well as during various stages of repetitive light flashes. The development of red flickering induced red response enhancement can be determined simply by measuring the changing response amplitude during the 20-s period of the red flickering. The relative response amplitude (mean ± S.E., normalized against control) is plotted in Fig. 2A, with sampling intervals being 5 s. To monitor the process of red flickering induced green response depression in LHC, the cells' responses to green flash were tested during control, as well as immediately after 5, 10, 15 and 20 red flashes were applied and relative responsiveness plotted in Fig. 2B. The same protocol was employed for estimating the development of green flickering induced red response depression (Fig. 2C). It is demonstrated by the plottings that the time course of mutual depression processes were similar and with a quicker speed than that of the auto-enhancement. The recovery speed of homo-wavelength enhancement (red) and hetero-wavelength depression effects were also de-

Fig. 1. Effects of flickering light on LHCs' chromatic plasticity. (A) An LHC's light response before, during and after a red (703 nm, log $I = -1.13$) flickering sequence (upper panel: LHC's response; lower panel: light flash). The repetitive red flashes increased the cell's red response (a,c) but depressed its green response (b,d). The cell's chromatic sensitivity change was reversible (e,f). (B) Waveform of the cell's red and green responses during control, towards the end of red flickering, and after recovery. (C) Response amplitude to red and green test flash before and immediately after exposure to red flickering light and relative changes in percentage. Mean ± S.D. values are given, bars show the standard error. (D) A typical LHC's light response before, during and after a green (501 nm, log $I = -2.21$) flickering sequence. No significant increase was observed in the cell's response to green light during the period of repetitive flashes (g,i). However, the cell's response to red flash (703 nm, log $I = -1.13$) was remarkably reduced (h,j). The depression effect was reversible when the retina was kept in the dark for about 15 s (l). (E) Waveform comparison for the cell's red- and green-response during various periods. (F) Statistics for the LHCs' response amplitude before and immediately after the green flickering light and relative changes in percentage. (* $P < 0.01$, paired *t*-test.)

tected. The cells' light responses were measured immediately, as well as 5, 10 and 15 s after the 20-s flickering stimulus had ceased, as plotted in Fig. 2D–F. The results showed that the red flickering induced red response enhancement was recovered in 10 s, whereas the recovery from hetero-wavelength depression took about 15 s.

It has been reported by numerous authors that repetitive stimulus could induce changes in synaptic efficacy in

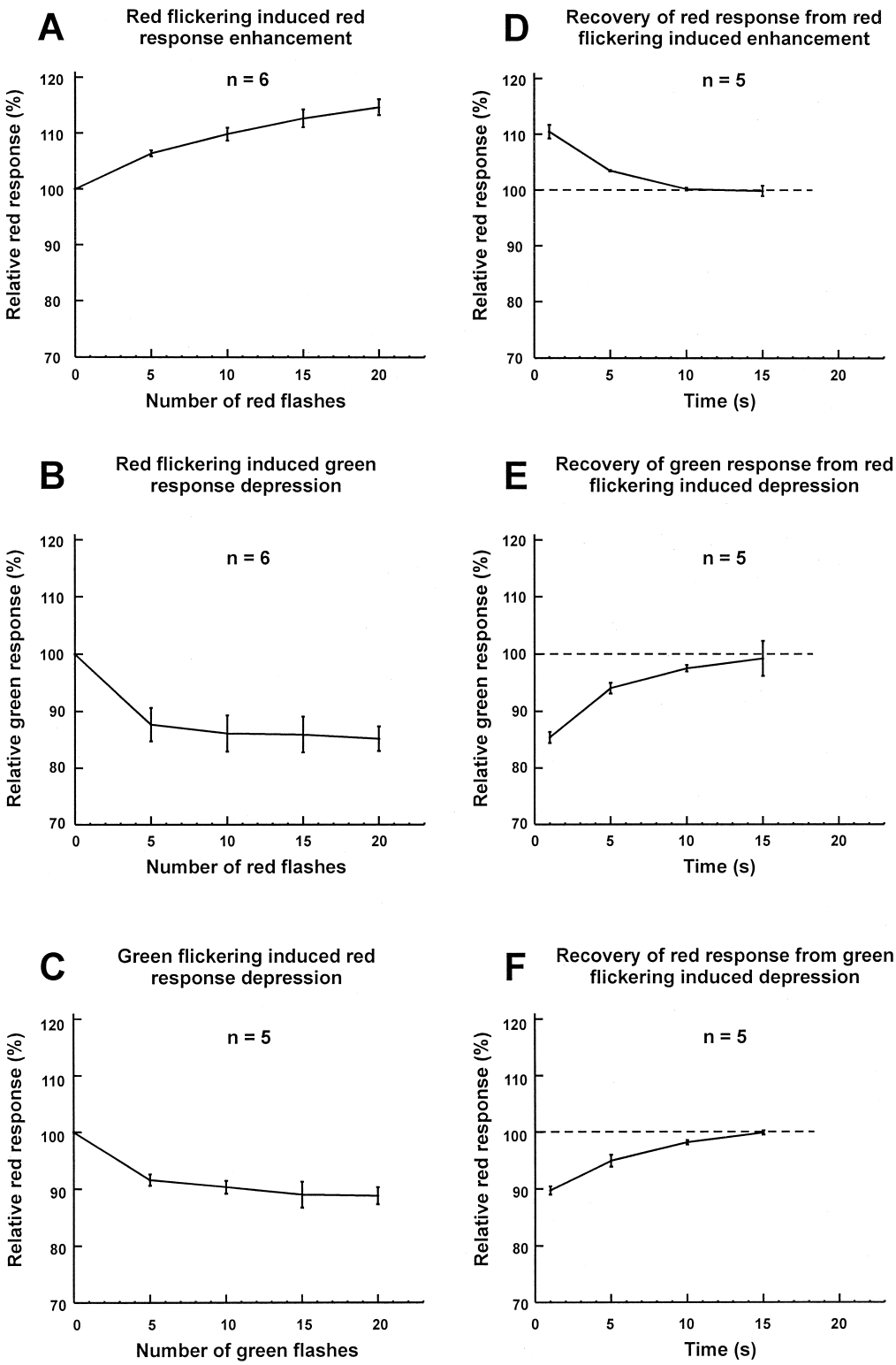

Fig. 2. The time course of development and recovery of flickering induced chromatic plasticity. (A) The development of red flickering induced red response enhancement. The response amplitude was sampled every 5 s, and normalized against the value measured during control (mean ± S.E.). (B) The process of red flickering induced green response depression. The cells green responses were measured during control, as well as immediately after 5, 10, 15, 20 red flashes were applied. Normalized data (mean ± S.E.) are given. (C) The process of green flickering induced red response depression. (D) The recovery of LHCs' red response from red flickering induced enhancement. The cells' red responses were measured immediately, as well as 5, 10, 15 s after the 20 s red flickering had ceased. The response amplitude is normalized against that measured before the flickering light was applied (mean ± S.E.). The response recovery was accomplished in 10 s. (E) The recovery of green response from red flickering induced depression was observed in 15 s after the flickering had ceased. (F) The red response was recovered from the green flickering induced depression in 15 s.

various parts of the nervous system, and the regulation of synaptic efficacy was dependent to the coincidence of electrical activity of the pre- and post-synaptic neurons and was closely related to the timing of the stimuli [1,4,13]. In the present study, the flickering sequences with various timing parameters were further applied and their modulatory effects on the LHC's spectral plasticity compared. Red sequences (20 flashes at a frequency of 1 Hz) were applied with on-flash duration being 100, 200, 300, 400 and 500 ms, separately. The flickering induced red response enhancement was observed in those groups with the light-on duration longer than 300 ms, but the green response depression occurred in all the groups, which seems to suggest that the processes of enhancement and depression were not necessarily modulated by the same mechanism. This is not surprising, given the results plotted in Fig. 2 that the processes of enhancement and depression follow different dynamics. Red flickering (20 flashes) with on-flash duration being 500 ms was also performed at frequencies of 1, 0.5, and 0.33 Hz, separately. The result was that only the sequence with frequency of 1 Hz was effective in inducing the spectral sensitivity change. These results show that the strength of synapses between cones and LHC is dependent to both the duration of on-flash and the off-interval between the successive light flashes of the flickering sequence.

The synapses between cones and LHC are plastic, and the modulatory effect is dependent to the properties, including spectral and timing characteristics, of the stimulus. Although it was reported that flickering light was effective in enhancing the LHC's responsiveness [9,10], the observation made in the current study was that aside from the homo-wavelength-signal enhancement, a competitive depression effect exists between hetero-wavelength-signals that input to the horizontal cell. Such depressive effect was also observable when long-lasting conditioning light (10 s) was applied (data not shown). This result is similar to that reported by Djamgoz et al. [2]. Although they observed only a red conditioning light caused depression in LHC's blue response but not any blue conditioning light induced change in the cell's red response, the difference may arise, according to the recovery effect observed in this study, from the time course they chose for the experiment. In their experiment, the interval between the red background light and the blue test flash was much shorter than that between the blue background light and the red test flash, which is probably why the depressive effect of blue light on the cell's red response was not observable.

The results of the current study also showed that the homo-wavelength-signal enhancement was apparent only when red flickering light was applied, while repetitive green flashes caused little change in the LHC's green response. This might be related to the spectral behavior of the red- and green-cones. Since LHC receives inputs from both red- and green-cones, and green-cones are around 2 log units less sensitive than red-cones to 703 nm stimulus [5], 703 nm flashes at intermediate intensity selectively activate red-cones. The repetitive red flashes actually form simultaneous stimuli on red cones and LHC via the red cone-LHC pathway, which result in a strengthened synaptic connection between the pre- and post-synaptic neurons. On the other hand, since the sensitivity of red-cones is only 0.5 log units lower than that of green-cones at 501 nm, the green flashes activate not only green-cones, but also red-cones to some extent. The modest enhancement of the LHC's green response during green flickering may reflect the mixed effects of homo-wavelength-signal enhancement and the competitive depression effect.

It has been well accepted that the efficacy of glutamatergic synapses is highly modifiable, and the degree and direction of the modifications are dependent on the correlation between pre- and post-synaptic activities [1,13]. Further, there are competition between different populations of synapses, the strengthening of some of the synapses may actually be accompanied by the weakening of the others [6,7]. These findings, although made from various parts of CNS, are fairly similar to the observations made in this study, and suggest that activity-dependent plasticity processes do neither expand nor contract the pool of synaptic resources, but simply allocate it.

Given the observations made in the present study, it is not able to answer, at this stage, whether pre- or post-synaptic mechanism(s) are responsible for flickering light induced cone-LHC synaptic efficacy change. However, having the results that the hetero-wavelength-signal depressive effect occurred even when the homo-wavelength-signal enhancement was not observable together with the difference in time course of the development/recovery of the response auto-enhancement and mutual depression, it seems to suggest that the two processes may involve different synaptic mechanisms.

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