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INTERACTION OF CONE SIGNALS ON L2TYPE HORIZONTAL CELL IN CARP RETINA : EXPERIMENTS AND MODELING[3]

LIANG PEDJI

(Key Laboratory of Neurobiology , Shanghai Institute of Physiology , Chinese Academy of Sciences , 320 Yue2Yang Road , Shanghai 200031*)*

ABSTRACT Intracellular recordings were made from the luminosity type cone driven horizontal cells (LHCs) in the isolated carp retina and model analysis was performed to investigate possible mechanisms un2 derlying the interaction of different cone signals converging onto these cells. It was observed that a green background light enhanced the LHC's red response , and such enhancement was closely related to the activa2 tion of green cones. Model analysis showed that the activity of both glutamate2 and GABA2related pathways were potentiated during green background illumination. GABA application did not abolish the response en2 hancement. It is speculated that the extent of the LHC's response enhancement may be determined by the balance of the increased activity between the feedforward and feedback pathways.

Key words : isolated carp retina ; horizontal cell ; interaction ; model analysis

In lower vertebrate retina , luminosity type cone2driven horizontal cells (LHCs) are hyperpolar2 ized by light stimulus. It is well accepted that the hyperpolarization results mainly from a light2in2 duced decrease of the excitatory neurotransmitter (glutamate) released by photoreceptors[1～4]. The rapid decrease in concentration of glutamate in the synaptic cleft mediates a brief change in cation channel's conductance , and thus results in a swift dynamics of LHC's membrane hyperpolarization. This , in turn , causes a decrease in concentration of the inhibitory neurotransmitter γ2aminobutyric acid (GABA) released by horizontal cells , which contributes to regulate the LHC's response dynam2 ics by changing the activity of anion channel on the membrane[5,6].

It was previously reported that in teleost fish , red responses of LHC were enhanced by green background illumination , which was thought to be caused by an interaction of different cone signals converging onto LHC[7～12]. Since it was observed that a preceding green conditioning light enhanced the LHC's response to a subsequent red stimulus[7,8] , it was thus assumed that the input from green2 sensitive cones acts on the input from red2sensitive cones , which results in the enhancement in LHC's red responses. According to the models describing synaptic connection between horizontal cells and cones , the response enhancement is thought to be attributed to a change in the activity of the GABA

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mediated feedback pathway[8,10]. However, little has been done to examine the validity of the proposed models.

The current study aimed to investigate possible mechanisms underlying the signal interaction-induced response enhancement. The following aspects were investigated: (1) the relationship between the response enhancement and the activity of relevant neurons; and (2) the relationship between the enhancement and the activity of relevant neurotransmitters. Experiments were conducted under various conditions. A quantitative model was employed for the analysis, and parameters estimated for the glutamate and GABA related components were compared under different chromatic adaptation conditions.

1 MATERIALS AND METHODS

111 *Experimental aspects*

11111 *Preparation* Experiments were performed on isolated carp (*Carassius auratus*) retinas. Carp of 10～15 cm (body length) was kept in aerated tank on natural light/dark cycle and was put in the dark for 20 min prior to an experiment to allow for isolation of the retina. After an eye was removed under dim red light, the retina was separated from the pigment epithelium and then mounted onto a piece of filter paper with the photoreceptor side up. During the experiment, the preparation was placed in a superfusion chamber with a volume of 114 ml, being continuously superfused with Ringer solution at a rate of 118 ml/min.

11112 *Perfusate* Ringer solution perfusate contained (in mmol/L): 11610 NaCl, 214 KCl, 110 $MgCl_2$, 112 $CaCl_2$, 110 NaH_2PO_4, 3010 $NaHCO_3$ and 1010 glucose, and was oxygenated with a gas mixture of 95 % O_2 and 5 % CO_2 to keep a pH of 717.

11113 *Stimulating and recording systems* The photostimulator consisted of two identical 82mm2 diameter light beams generated from a 100 W tungsten halogen light source (Osram, Germany) to form coincided diffuse light spots, which were used as stimulus and background lights respectively. Interference filters were used to select the wavelength as desired. The intensities of the light beams were controlled by neutral density filters. Throughout the paper, intensity values will be expressed in relative log units. The unattenuated intensity of 703 nm was 6111×10^{13} photons/($cm^2 \cdot s$).

Intracellular recordings were made using an MEZ8201 micro-electrode amplifier (Nihon Kohden, Japan). The micro-electrode pulled from glass micropipettes on a PD25 puller (Narishige, Japan) was filled with 4 mol/L KAc, with a resistance around 100 MΩ measured in Ringer solution, and was mounted on a PF21 micromanipulater (Narishige, Japan). Cell responses were monitored by a DSS26521 oscilloscope (Kikusui, Japan), and data were collected and stored on a Pentium PC via an ADlink 8111 A/D interface (ADlink, Taiwan) for off-line analysis. Sample rate was set at 100 Hz.

11114 *Experimental protocol* HCs were classified according to the characteristics of the cells' light response. Only LHCs were investigated. Stimuli of 015 s with two selected wavelengths (501 nm and 703 nm, referred to as green and red, respectively) were given alternately at intervals of 215 s. An LHC was identified when the cell hyperpolarized to both red and green stimuli at medium intensi-

ty.

To investigate the influence of green cone activity on the LHC's red response, red (703 nm) flash was used as stimulus under various chromatic adaptation states.

112 *Model description* When a light flash is given, the change in cation channel's conductance induced by a reduction in glutamate concentration governs the LHC's response dynamics, and the change in anion channel's conductance induced by GABA further modulates the cell's response. Fol2 lowing the above idea, a simple model containing two parallel components was employed to describe the rising phase of the cell's responses, which can be equated as:

$$\tau_1 \frac{d\Delta V_1}{dt} + \Delta V_1 = G_1 \times I, \quad (1a)$$

$$\tau_2 \frac{d\Delta V_2}{dt} + \Delta V_2 = G_2 \times I, \quad (1b)$$

$$\Delta V_{HC} = \Delta V_{dark} + \Delta V_1 + \Delta V_2, \quad (1c)$$

where I denotes the intensity of a step light stimulus, V_{HC} is the membrane potential of the LHC dur2 ing light stimulus, which consists of a glutamate related hyperpolarizing component ΔV_1 and a GABA related depolarizing component ΔV_2 (V_{dark} reflects the cell's dark membrane potential). τ_1 and G_1 (negative) represent the time constant and the gain term for ΔV_1, τ_2 and G_2 (positive) represent the time constant and the gain term for ΔV_2.

Least2square fitting procedure using quasi2Newton method was applied for parameter estimate. Initial values of the parameters were chosen arbitrarily.

2 RESULTS

211 Experiments in normal Ringer's and model analysis

21111 *The activity of relevant neurons and the enhancement in LHC's red response* To investi2 gate the relationship between the activity of relevant neurons and the interaction induced response en2 hancement, experiments were performed under various chromatic adaptation states. Red responses of a cell recorded under these states are compared in Fig111 The test flash used was 703 nm, - 1199 log units. As illustrated in Fig11*A*, the red flash elicited the cell's response of 915 mV in the dark; a dim red background (703 nm, log I = - 510) hyperpolarized the cell by 114 mV, and the cell's response to the same test flash was reduced to 814 mV; when a moderate red background light (703 nm, log I = - 413) was given, the cell was hyperpolarized by 513 mV, and the cell's response was further reduced to 615 mV. The effect of green background lights on the same cell's red response is given in Fig11*B*. The cell was hyperpolarized by 211 mV when a dim green background light (501 nm/ - 414) was given, but the cell's red response was enhanced to 1019 mV; comparatively, a moderate green background light (501 nm/ - 318) hyperpolarized the cell by 515 mV and enhanced the cell's red response to 1413 mV. Similar results were observed from a total number of 8 cells, and statistical comparison of the cells' red response amplitude are shown in Fig11C and D. The cells' red response (819 ±1165 mV, mean ±S1D1, during control) was clearly reduced by the red background

lights (811 ±1128 and 513 ±1193 mV for the dim and moderate red backgrounds, respectively), but was considerably enhanced by the green background lights (1010 ±1100 and 1118 ±1147 mV for the dim and moderate green backgrounds, respectively). Paired *t*2test (control *vs* dim red back2 ground; control *vs* dim green background) indicated that the differences were significant ($P <$ 0105). The above results give evidence that the response enhancement relies basically on the activa2 tion of green cone system, and the change in the LHC′s membrane potential has little effect, which is consistent with the previous findings that signal from green cone system acted on signal from red cones that enhanced the LHC′s red response[7,8].

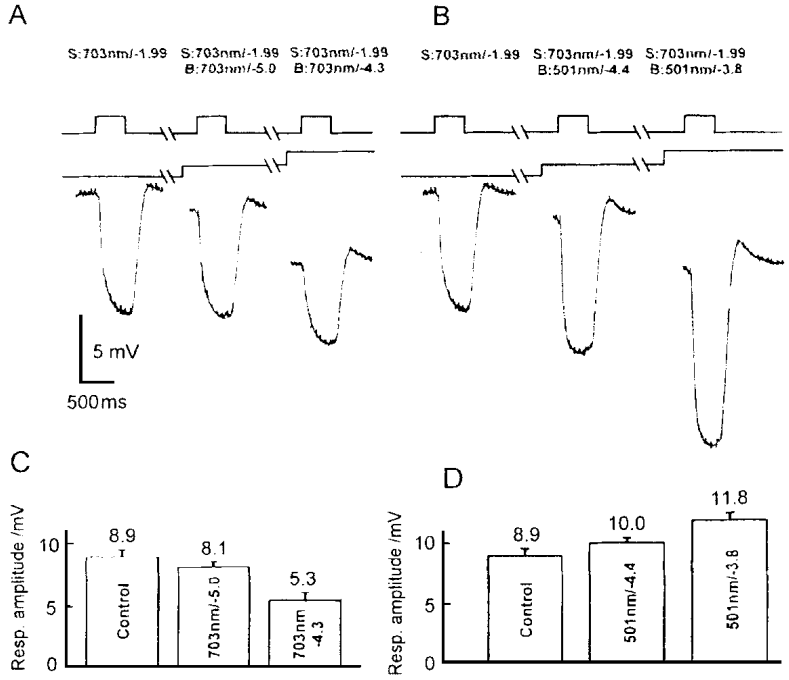

Fig11 LHC′s red responses under various chromatic adaptation states

A. Effects of dim and moderate red background lights (703 nm, - 510 and - 413, respectively) on an LHC′s response to a red flash (703 nm, - 1199). The background lights hyperpolarized the cell and re2 duced its red responses. *B*. Effects of dim and moderate green background lights (501 nm, - 414 and - 318, respectively) on the cell′s (same as *A*) red responses. The cell′s red response was enhanced when green background lights were given. *C* and *D*. Mean amplitudes (±S1E1) of 8 LHCs′ red re2 sponses measured in the dark and under various red/ green background lights accordingly.

21112 *Model analysis of LHC′s light response* Based on our model, the signal interaction induced response enhancement might be attributed to a decreased activity of the GABA mediated feedback pathway and/ or an increased activity of the glutamate mediated feedforward pathway. The upper panel of Fig12 gives an example for the model fitting, which was performed on the rising phase of the light response of a cell. It is clear that the model prediction (solid line) fits well to the experimental data (open circles). The glutamate2related and GABA2related components are plotted using dashed line and dotted line respectively. The residuals (differences between the model output and the experimen2

tal data, presented as closed circles) were very close to zero, which reveals a satisfactory fitting of the model to the data. The fitting was performed on data collected from 16 cells. The parameters were estimated for the rising phase of the cells' red responses recorded in control and in the presence of a green background light (501 nm, log I = - 414).

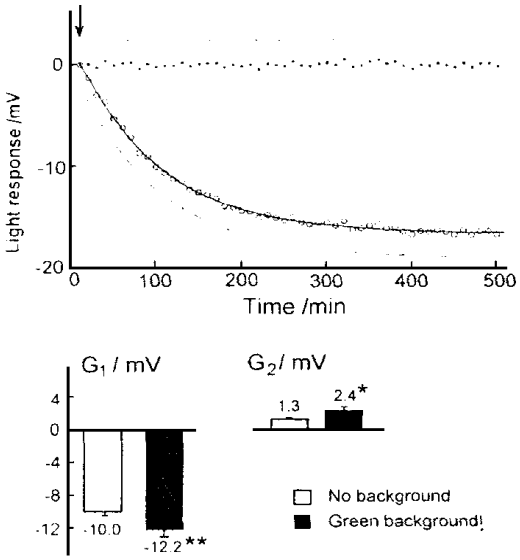

Fig12 LHCs' red responses and model analy2 sis

Upper panel, the rising phase of an LHC's red response and model fitting. Open circles, experimental data; solid line, model output; dashed and dotted lines, glutamate2 and GA2 BA2related components respectively; closed circles, residuals. The arrow indicates the re2 sponse starting point. Lower panels, gain terms of glutamate2 and GABA2related compo2 nents (G_1 and G_2 respectively) estimated from the dynamic model. Comparisons of the pa2 rameters were made between the data obtained in the dark and in the presence of a green (501 nm, - 414) background light (3, $P < 0105$, 3 3, $P < 0101$, paired t2test, $n = 16$). Mean values are given with bars indi2 cating standard errors.

As illustrated in the lower panel of Fig12, the gain terms of the glutamate2 and GABA2related components (G_1 and G_2 respectively) were - 1010 ±2110 and 113 ±0172 in control, and were en2 hanced to - 1212 ±3113 and 214 ±1148 in the presence of the green background light. It was note2 worthy that G_2 was, even though slightly, enlarged under green background illumination ($P < 0105$, paired t2test). It was thus unlikely that the response enhancement was due to a decreased activity of the feedback pathway. On the other hand, G_1 was significantly greater when green background was given, as compared to control ($P < 0101$, paired t2test), which implies that the change of gluta2 mate2mediated activity might be a determinant factor causing the response enhancement.

212 GABA effect and the interaction

Although the model analysis showed that the response enhancement was not likely caused by a weakening in the GABA mediated feedback, GABA activity was revealed to be altered during green background illumination. Further experiments were thus conducted to explore the GABA effect on LHC's red response enhancement. A total number of 9 LHCs were investigated using the same light stimulation protocol with the perfusate containing 215 mmol/L GABA. An example is given in the up2 per panel of Fig13. GABA effect was clear that it hyperpolarized the LHC by 215 mV and the cell's response to a red flash (703 nm, - 1199) was reduced (718 mV in normal Ringer's *vs* 417 mV dur2 ing GABA application). The effects of a green background light (501 nm/ - 414) on the cell's red response are compared in both normal Ringer's and in the presence of 215 mmol/L GABA. Given the red stimulus intensity as - 1199 log units, the cell's response was enhanced by the green background

light in normal Ringer's (from 718 to 1016 mV), but the enhancement disappeared during GABA perfusion. It is of interest to note that the response of this cell to a dimmer red flash (- 2197) could be enhanced by the same green background light (from 218 to 314 mV). Similar observations were made from 6 out of 9 cells. Statistics are given in the lower panels of Fig13.

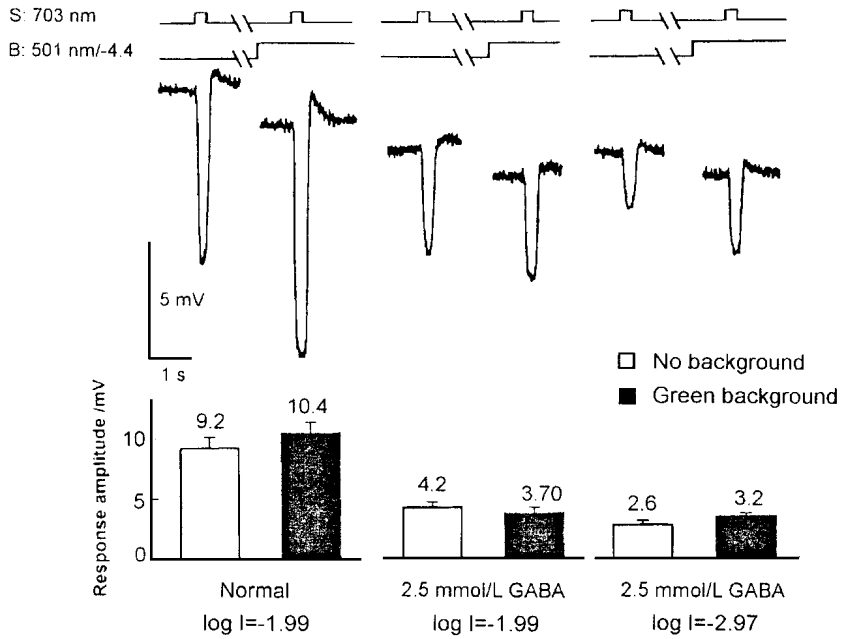

Fig13 Modification of the interaction induced enhancement by GABA

Upper panel, responses of an LHC to red flashes under various conditions. Presentation of a green backl2 ground light (501 nm, - 414) enhanced the cell's response to red flash (703 nm, - 1199). Application of 215 mmol/L GABA hyperpolarized the cell and reduced its light response. While the green background light failed to enhance the cell's response to the red flash (703 nm, - 1199), but the enhancement oc2 curred when a dimmer test flash (703 nm, - 2197) was given. Lower panel, mean amplitudes (±S. E.) of 9 LHCs' red responses measured under these conditions.

3 DISCUSSION

311 *LHC's response enhancement is related to the activity of green cones*

LHCs have been demonstrated to receive inputs from both red cones and green cones. According to the spectral characteristics, the sensitivity of red cones to 703 nm and 501 nm differs about 013 log unit. This means that the 703 nm background lights (- 510 and - 413) are nearly equivalent to the 501 nm background lights (- 414, - 318, respectively) in terms of the activation of red cones. As shown in Fig11, red responses of LHCs could only be enhanced by the green background lights, but not by the red2cone2equivalent red background lights. This fact suggests that the response enhance2 ment is related to the activity of green cones, a conclusion consistent with the previous findings[7,8].

312 *The excitatory interaction between cone signals*

Excitatory interaction between different cones of turtle retina was previously reported[13], based

on the red cone's response to red/green flash during various background illumination. A suggestion was made that the basal junction might be the synaptic site at which signal of one photoreceptor type is transmitted directly to an adjacent photoreceptor of different spectral types. However, spatial test performed on carp retina[11] also showed that the interaction on HCs persisted even when the test light and the background light were spatially separated to avoid cone2cone interaction, which suggested that the hypothesis of direct interaction between the cones was inadequate. Thus, in the current study, a dynamic model combining two parallel processes, *i.e.* a glutamate2mediated feedforward and a GABA2mediated feedback pathways between the cone systems and the horizontal cells, was structured and applied for analysis.

3.1.3 *The dynamics of LHC light response and the model structure*

To describe the relationship between the LHC's light response and the cleft clearance of glutamate, a simple first order dynamics was employed[14]. The model is somewhat simplified, since it has been reported that in horizontal cell's light response, the glutamate related dynamics should contain two processes, with one induced by a decrease in glutamate release depending on cone membrane hyperpolarization and the other related to removal of glutamate in the synaptic cleft via a voltage dependent transporter activity[14,15]. Since numerical analysis performed in this study failed to identify the parameters for these two parallel processes, a single first order structure was thus employed for approximation.

On the other hand, GABA2gated anion conductance dependent on HC membrane potential contributes to modify the dynamics of HC's light response[5,6]. A sign2inverting first order dynamics was incorporated into our model to describe the GABA2mediated process.

In the current study, the fitting result was satisfactory as shown by the comparison between the model output and the experimental observations (see Fig.12). This suggests that the model, although slightly simplified, can be applied for analysis as a reasonable approximation. The advantage is clear that it provides the possibility for a more detailed analysis of the parallel activity changes in the glutamate2 and GABA2related pathways, which might be interrelated when the cell's response change occurs under different physiological conditions.

3.1.4 *The response enhancement and the GABA effect hypothesis*

GABA mediated feedback pathway was previously suggested to be involved in the interaction between cone signals converging onto horizontal cell[8,10], *i.e.*, the cell's response enhancement might be caused by a reduced activity of GABA mediated feedback pathway.

However, the model analysis performed in the current study shows that this is unlikely the case. In contrast, the GABA related activity was even increased under a green background light (Fig.12). Since glutamate and GABA exert opposite effects on the LHC's membrane potential, thus the increased activity of the glutamate related pathway, in competition with the increased activity of the GABA related pathway, would determine whether or not the response amplitude is enhanced.

When exogenous GABA was applied, the activity of the GABA related pathway was enhanced and the background light2increased activity of the glutamate related pathway is relatively weak. These

changes may be why the response enhancement failed to be observed when an intense red stimulus (703 nm/21199) was given. However, the response enhancement persisted with dimmer stimulus intensity (703 nm/22197) during GABA perfusion. Although this may reflect a relatively more enhanced activity of the glutamate2mediated pathway, the underlying mechanism remains to be explored.

315 *Glutamate transporter and postsynaptic dynamics*

A number of authors[16,17] claimed that cone may possess a high2affinity glutamate transporter which is activated when the cell is exposed to light. Since the role of the transporter is to remove glutamate from the cleft, so the activation of the transporter system would result in an hyperpolarization of the horizontal cells.

Intracellular studies[14,15] actually showed that the transporter, by its voltage dependence on cone membrane potential, appeared to contribute significantly to the control of postsynaptic response dynamics as well as response amplitude. The model analysis performed in the current study shows that the positive interaction is likely attributed to the sensitivity enhancement of the glutamate related component. Since the glutamate transporter system of the cones is more active in the light, it is likely that a background induced response enhancement might be, partly at least, due to the activation of the glutamate transporter on the green cone membrane.

3 3 3

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鲫鱼视网膜亮度型水平细胞上不同视锥信号的相互作用：实验及模型[3]

梁培基
(中国科学院上海生理研究所神经生物学开放实验室，上海 200031**)**

摘　要

本文应用胞内记录和动态模型分析方法，研究了离体鲫鱼视网膜视锥驱动的亮度型水平细胞 (LHC) **上不同视锥信号的相互作用。实验表明，绿背景光的作用可以提高** LHC **的红光反应，这种增强作用与绿敏视锥的活动程度密切相关。模型分析表明，绿背景光的作用使谷氨酸介导的前馈性通路和** GABA **介导的反馈性通路活动同时得以增强。水平细胞对光反应的增强效应不能为外源性** GABA **所消除，而其程度为前馈性通路和反馈性通路活动增加的相对程度所决定。**

关键词: **鲫鱼视网膜；水平细胞；相互作用；模型分析**
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