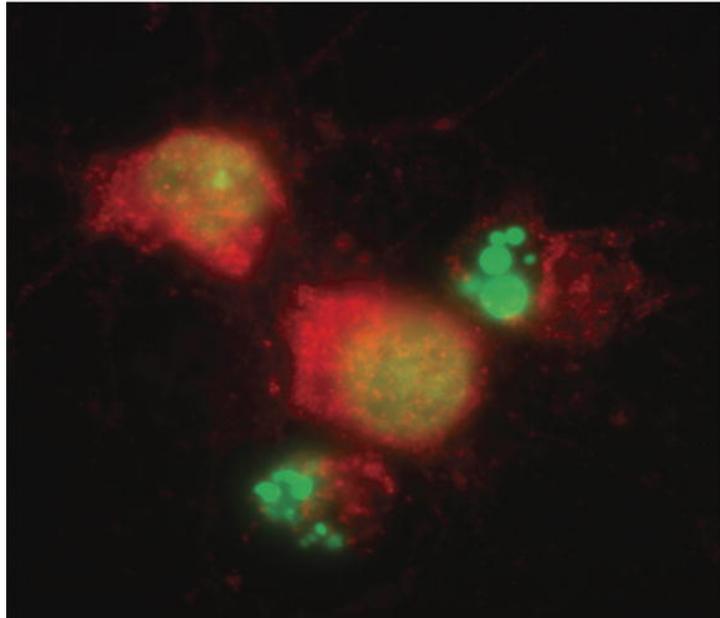


Brain Research



MARCH 23, 2007 | VOLUME 1138
ISSN 0006-8959

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**BRAIN
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Research Report

The dual-peak light response of ganglion cells in chicken retina

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ARTICLE INFO

Article history:

Accepted 21 December 2006

Available online 29 December 2006

Keywords:

Multi-unit recording

Response pattern

Adaptation

GABA

ABSTRACT

In the present study, a particular temporal pattern of the ganglion cells' light response specified as "dual-peak" was observed. In the chicken retina ($N=15$), about 37.5% (174 out of 464) of the ganglion cells showed such special temporal property in response to the onset of light flash. These neurons responded to light stimulus with two successive components: a transient increase of firing rate which lasted for less than 100 ms, and another prolonged light response appeared in about 100 ms after the first transient response. Moreover, our data demonstrated a temporal adaptation process in the later phase of firing activities when repeated flashes were applied. Meanwhile, the earlier phase had a more stable latency in response to the stimulus. Application of picrotoxin could evoke the dual-peak responses in transient ganglion cells. These results suggest that the origination of the two response components might be distinct and the later one is likely related to GABAergic pathways.

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1. Introduction

Ganglion cells are the output neurons in the vertebrate retina. Different from the graded neurons in the retina, ganglion cells respond to light stimulation with action potentials (spikes). In the retina, visual information is finally encoded into spike trains by ganglion cells and transmitted to LGN via optic nerve (Masland, 2001; Meister and Berry, 1999).

According to the duration of firing activities, the light responses of ganglion cells can be classified into transient and sustained subtypes (Garcieri et al., 2003). In the present study, a special response pattern which differs from traditionally defined transient or sustained response was observed in the chicken retinal ganglion cells.

In our previous work, it was observed that for some non-color-opponent ganglion cells, the spatial-temporal patterns

of the cells' light responses could be related to the chromatic configuration of the light stimulation (Zhou et al., 2005). Further analysis revealed that the temporal structure of these neurons' firing activity was quite diversified and was color related. Typical transient, sustained and "dual-peak" response were all observed at the onset of light stimulation. The dual-peak response consisted of two separated peaks in firing rate: one occurred instantaneously in response to the stimulus onset, the other appeared following the first one with a short interval where the firing rate was quite low.

In the present study, the dual-peak pattern of the chicken retinal ganglion cells was investigated in details. The time latencies of the two peaks were measured and compared. Moreover, by analyzing their light response characters during repeated flashes, an adaptation process in the response

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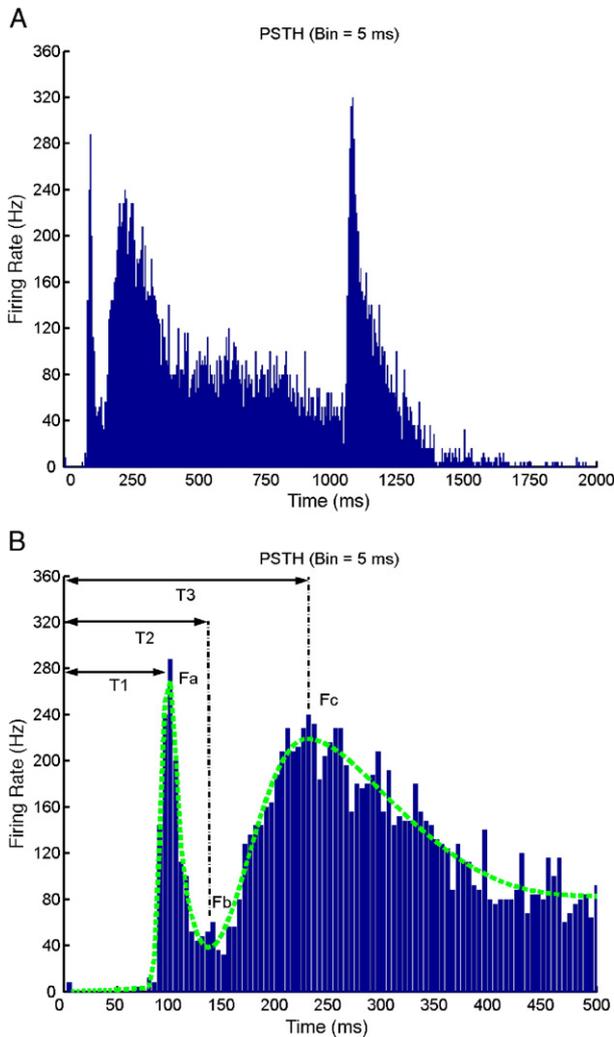


Fig. 1 – (A) An example of retinal ganglion cell’s dual-peak response (50 repeats, bin = 5 ms). Light stimulus was given at 0 ms and ceased at 1000 ms. (B) An expansion of panel A at the onset part (0–500 ms). Dashed line indicates the smoothed curve generated by the BARS method. F_a , F_b and F_c are the firing rates measured at the first peak, the valley and the second peak, respectively. T_1 , T_2 and T_3 indicate the response latencies of the first peak, the valley and the second peak to the stimulus, respectively.

pattern was found. While the delay time of the first peak was mostly kept unaltered during the experiment, the latency of the second peak changed along with flash repeats. Such difference suggests that the origination of the two phases of light response might be distinct.

2. Results

2.1. Identification criterion of dual-peak response

Fig. 1A gives an example of the ganglion cell’s dual-peak response. To identify the dual-peak pattern from the

traditional transient or sustained ones, a set of parameters are required to describe the characteristics of this particular response pattern. As illustrated in Fig. 1B, the earlier phase of light response is similar to transient response and can be detected first. The peak level of this quick response is defined as F_a . The latency of this peak T_1 is usually shorter than 125 ms ($T_1 < 125$ ms). This first peak is transient with the firing rate descending quickly and reaching a minimal level F_b at T_2 ($F_b < 50\% F_a$). If a second peak raises again with a firing rate 50% higher than the valley value ($F_c > 150\% F_b$) and a peak latency not longer than 500 ms after the stimulus onset ($T_3 \leq 500$ ms), a dual-peak light response can be identified.

2.2. Temporal characteristics of the transient and prolonged components

Among the ganglion cells investigated (464 cells from 15 chicken retinas), 37.5% of them (174 from 13 retinas) showed the dual-peak light response. The temporal properties of these two peaks can be characterized by parameter T_1 , T_2 and T_3 as described. In our experiments, the averaged response latencies of normal transient cells and sustained cells are 66.9 ± 18.1 ms ($N=118$) and 74.0 ± 16.3 ms ($N=20$), respectively, in response to full-field white light stimulus. Meanwhile, the latencies of the first peak (T_1) and second peak (T_3) of the dual-peak neurons are 79.0 ± 15.3 ms and 223.6 ± 76.3 ms respectively (white light, $N=148$) (see Table 1). This T_3 value is significantly larger than the response latency of the traditional sustained neurons ($p < 0.05$, Student’s t-test). The large value of T_3 suggests that the dual-peak response should not be a simple combination of the activities from a transient ganglion cell and a sustained ganglion cell. In addition, the variation range for T_1 (SD/mean ratio, around 16%) is much smaller than that of T_3 (around 32%), while the situation is moderate for T_2 (around 24%).

2.3. Adaptation process in the prolonged period

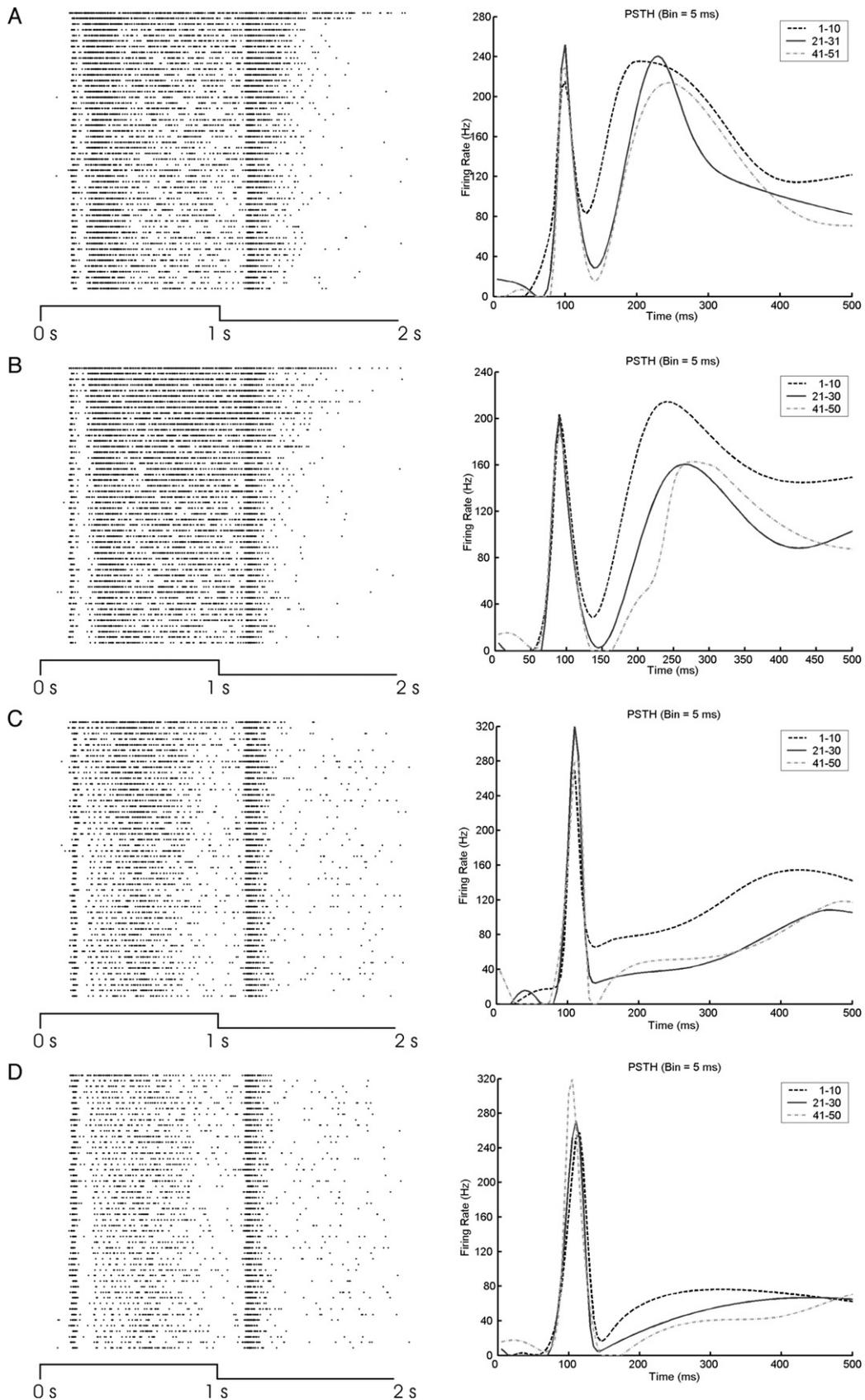
To obtain a better understanding of the dual-peak response, we tried to look into the detailed raster plot of the cells’ response. Fig. 2A gives an example of a neuron’s response to repeated full-field white flashes (50 trials). The adaptation process of the prolonged period in the dual-peak pattern is apparent (Fig. 2A, left panels). While the neuron’s response to the first few flashes showed a sustained response property, the dual-peak pattern gradually appeared in the neuron’s firing response to the later trials, which resulted in a “gap” (silent period) between the two response phases in the raster plot.

Table 1 – Statistical results (mean \pm SD) for T_1 , T_2 and T_3 under different stimulus protocols

	Red (N=29)	Green (N=103)	Yellow (N=55)	White (N=148)
T_1 (ms)	89.1 ± 12.2	95.8 ± 13.0	94.2 ± 15.3	79.0 ± 15.3
T_2 (ms)	181.7 ± 40.2	136.4 ± 29.4	198.0 ± 60.1	129.2 ± 28.4
T_3 (ms)	216.7 ± 81.0	212.8 ± 67.5	235.8 ± 57.8	223.6 ± 76.3

Although such kind of changes in neuronal responses could reflect an adaptation process, it might also be caused by damage of the retina preparation. To test the stability of the

neuronal responses, the stimulus protocols were repeated for at least twice, for each neuron (see Experimental procedures for details). The left panels of Fig. 2A and B illustrate two 50-



trial recordings of an example neuron's responses during repeated stimulation respectively. The recovery interval between these two experiments was more than 10 min. Although some variance between the two 50-trial recordings is observable, the adaptation process is apparent for both.

The right panels of Figs. 2A and B illustrate the corresponding smoothed PSTH curves. The smoothed PSTHs obtained from the early period (1st–10th trial), the middle period (21st–30th) and the late period (41st–50th) are plotted. It is clear that the latency time and shape of the first peak did not change much during the repeated trials. At the mean time, the temporal feature of the later component (T2 and T3) changed gradually, apart from the variations in firing rate.

The stability of T1 and variability of T2 and T3 are prevalent in the neurons that responded to light stimulation with dual-peak response pattern. Similar results obtained from another ganglion cell are given in Figs. 2C and D.

However, it is still difficult to present a statistical result about the adaptation process although it is commonly observed in the raster plots of many dual-peak ganglion cells. As we described before, T3 changes quite a lot among different ganglion cells (Table 1). For some cells, we are able to calculate the variance of T2 and T3 between different stimulation stages (e.g. right panels of Figs. 2A and B). For some other cells, the adaptation process was very quick that it could be completed even within 10 trials. If we only adopt the first few trials to describe the early situation reflected by the raster plots, the PSTHs would be too noisy to obtain a reliable temporal character.

2.4. Dual-peak property and On–Off response

Although the dual-peak response pattern was mostly seen at the light onset, it has also been observed at the offset part. The occurrence of the second phase of firings in response to light offset was much less as compared to the onset part. In our experiment, only 51 neurons from 9 retinas showed the dual-peak pattern at the offset part in response to white flashes. Moreover, it seems that the occurrence of dual-peak pattern in response to light offset did not depend on that at the onset part—the ganglion cells can have the dual-peak activity at either light onset or offset, or both. Fig. 3 shows some examples of these situations recorded from different retinas.

2.5. Application of picrotoxin (PTX) could evoke dual-peak response

In previous reports, it was found that the application of picrotoxin (PTX), a GABA_A and GABA_C receptor antagonist, could evoke similar dual-peak responses in transient ganglion

cells (Dong and Werblin, 1998; Nirenberg and Meister, 1997; Popova et al., 2003). In our present study, it was also found that dual-peak responses could be induced from normal transient ganglion cells when exogenous picrotoxin (50 μM) was applied (Fig. 4). Such changes were observed from 3 out of 7 ganglion cells investigated in which the pharmacological effect of PTX could be washed out. This suggests that the dual-peak responses might be related to the activation of GABA receptors.

3. Discussion

3.1. Possible mechanism

Different from the conventional transient or sustained response, the dual-peak pattern consists of two components in the neuron's response to light stimulation. While the first part of dual-peak response is very similar to the traditionally defined transient response, the later prolonged part shows an interesting property of adaptation process. Similar responses were sometimes classified as sustained or "rebound" type in previous reports (Dong and Werblin, 1998; Segev et al., 2006).

Considering the two peaks in PSTH, two possible mechanisms can be proposed. One possible mechanism might be related to certain inhibitory input which inhibits the neuron's sustained response at a certain stage and thus separates the PSTH into two distinct parts. If this is the case, T2 should be the time when this inhibition reaches a maximum level. However, according to the recorded data, the firing rate of the second peak was sometimes significantly higher than that of the first one (for an example, see Fig. 3A). This suggests that the formation of the dual-peak pattern should not be caused by a simple combination of a sustained response and an inhibitory input.

Another possible mechanism is that the originations of the two peaks are separated. Since the first peak has a short latency after the stimulus onset, it is likely to be generated through a direct pathway. Secondly, the latency of the first peak is stable (Table 1), which suggests that this pathway should not be complicated. These aspects suggest that the first part of the neuronal activity (first peak in PSTH) might reflect the direct visual information processes via the photoreceptors–bipolar–ganglion cells pathway.

Compared to the first peak, the second peak is more complicated. First, it has a much longer latency with large variation. The response latency of the second peak is usually between 150 and 350 ms (white light, mean±SD=223.6±76.3 ms). Meanwhile, the shapes of the second peak were quite variable among different cells (right panels of Figs. 2A–D and 3). These characters suggest that the origination of the

Fig. 2 – (A) Left panel shows the raster plot of one dual-peak ganglion cell's firing activity in response to repeated full-field white light flashes (50 trials). Right panel shows the smoothed PSTHs obtained during different stimulation stages based on the data presented in the left panel. (B) Another 50-trial recording made from the same neuron as presented in panel A (same protocol). The interval between the two recordings presented in panels A and B was more than 10 min. Panels C and D are similar to panels A and B but recorded from another ganglion cell from a different retina.

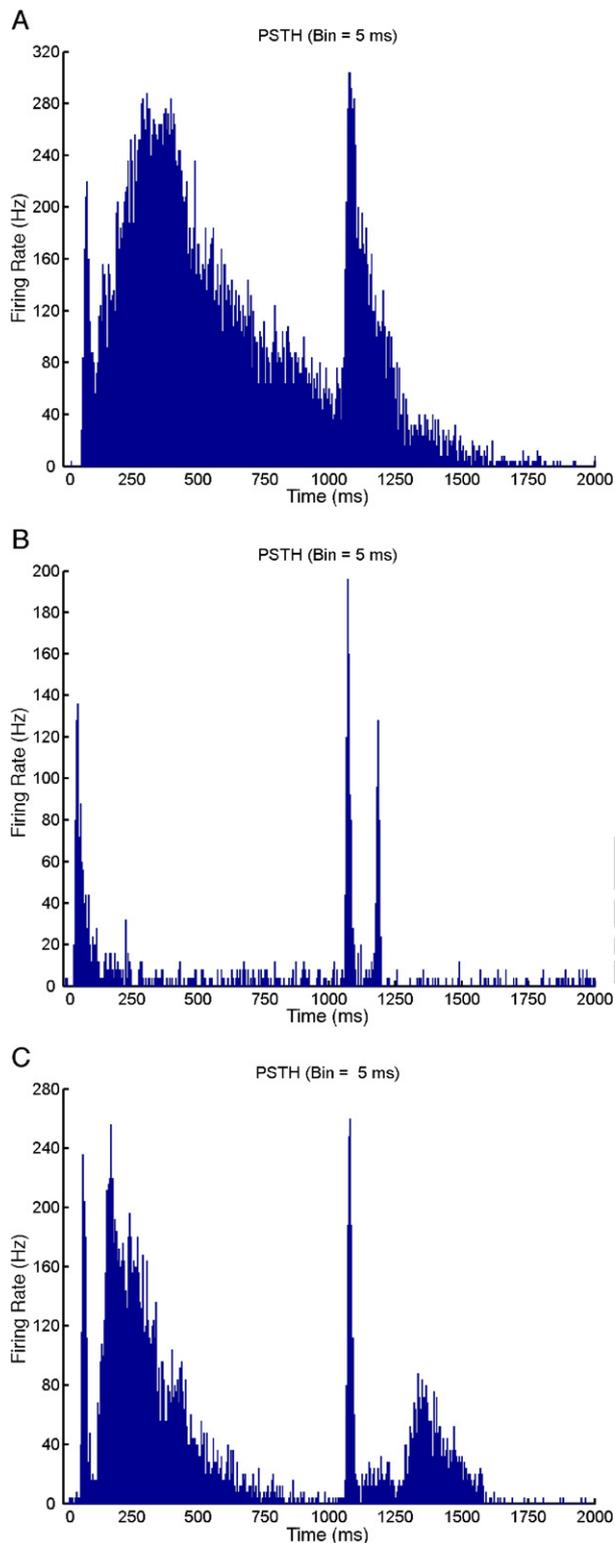


Fig. 3 – (A) An example ganglion cell's light response with the dual-peak pattern occurring at the light onset. (B) An example showing the dual-peak pattern occurring at the light offset. (C) An example showing the situation where dual-peak responses observed both at the light onset and offset.

later firings might be related to a more complicated neuronal circuitry.

According to previous research, similar dual-peak responses were found in isolated mice retina in which GABA_A and GABA_C receptors were blocked. It was reported by Nirenberg and Meister (1997) that when picrotoxin was applied, "... most retinal ganglion cells (17/23) produced a transient burst of spikes after light onset, but this was followed by a pause of 50–100 ms, and then a second, more extended response phase". Similar results were also found in picrotoxin-treated frog retina (Popova et al., 2003) and salamander retina (Dong and Werblin, 1998).

Since picrotoxin was involved in the formation of the dual-peak response as observed in our experiment (Fig. 4) and other works (Dong and Werblin, 1998; Nirenberg and Meister, 1997; Popova et al., 2003), GABAergic pathway should play an important role. We suppose that there are two different pathways which generate the first peak and the later peak of the dual-peak cell's responses respectively. However, if the pathways related to the later peak are inhibited by GABAergic activities, the neuron will demonstrate a transient response to light stimulation—this is why dual-peak response can be evoked by the application of exogenous picrotoxin.

Because GABA receptors are widely expressed in the retina (Yang, 2004), it is difficult to specify the exact site of this GABAergic pathway in the intact retina. However, the large latency of the later peak in response to light stimulus suggests that the formation of the later peak should be related to a complicated neuronal circuitry. Hence, the activity of horizontal and/or amacrine cells is likely involved.

However, the formation of the adaptation process of the later peak due to the complexity of the GABA related circuitry is still unclear. The synaptic plasticity within the amacrine cell network might play a role.

3.2. Possible physiological significance

Since the early response phase has a short and stable latency related to the stimulus, the information about the occurrence of the stimulus can be transmitted instantaneously. At the mean time, the later firings could provide more intensified synchronization between nearby neurons than the earlier part (Zhou et al., 2005). Similar situations can be found in many (but not all) nearby neuron pairs. This synchronization might also be coupled with amacrine cell-dependent mechanism(s) because a single amacrine cell can influence several ganglion cells simultaneously and hence affect their correlated firings.

4. Experimental procedures

4.1. Methods

Fifteen retinas obtained from neonatal chicken (3–8 days post hatching) were investigated in this research. Similar experimental operations can be found in our previous report (Chen et al., 2004). All procedures strictly conformed to the humane treatment and use of animals as prescribed by the

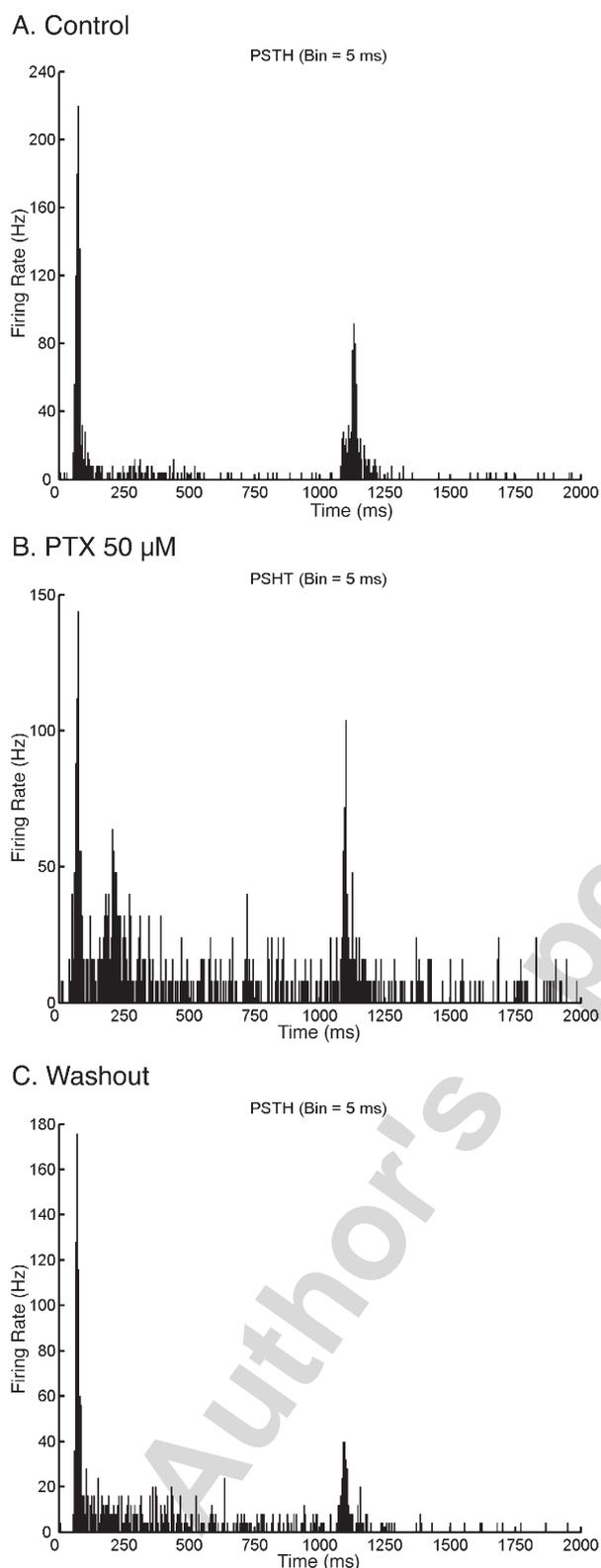


Fig. 4 – (A) Transient response recorded from a ganglion cell in normal solution. (B) When PTX (50 μ M) was applied (more than 5 min), the light response recorded from the same cell showed a dual-peak pattern at the light onset. (C) Transient response can be observed again after PTX was washed out.

Association for Research in Vision and Ophthalmology. Chickens were kept in the dark for 30 min before experiment to make it easier to remove the sclera from the retina. After decapitation and enucleation of the eye, the eyeball was hemisected with a fine razor blade. The vitreous body and sclera were removed carefully. To record the spike trains from the retinal ganglion cells, a small piece (4 mm \times 4 mm square) of the isolated retina was placed on a flat array containing 60 microelectrodes (MEA60, MCS GmbH, Germany) with the ganglion cell side facing the electrodes. A small drop (3.0 μ l) of nitrate cellulose solution (1.0 mg Sartorius cellulose nitrate dissolved in 10.0 ml methanol) was smeared on the electrode array as electric glue to ensure a better contact with the retina. The preparation was perfused in oxygenated (95% O₂ and 5% CO₂) Ringer's solution (contained in mM: 120.0 NaCl, 5.0 KCl, 3.0 MgCl₂, 1.8 CaCl₂, 25.0 NaHCO₃, 25.0 glucose) with a pH value of 7.5 \pm 0.2. The tissue and perfusate were kept at 38 $^{\circ}$ C by a temperature control unit (Thermostat HC-X, MCS GmbH, Germany). A small Ag/AgCl pellet with wire was immersed into the bath solution and acted as the reference electrode.

The neuronal photoresponses were recorded simultaneously by the multi-electrode array, and the signals were amplified through a 60-channel amplifier (single-ended amplifier, amplification 1200 \times , amplifier input impedance >10¹⁰ Ω , output impedance 330 Ω). Signals from the selected channels along with the stimulus were sampled at the rate of 20 kHz (MCRack) and stored in a Pentium IV based computer.

After 10–20 min adaptation to the solution environment, the light response of the retinal ganglion cells would go stable and the stability could last for 3–4 h in our experiments. All the data analyzed in this study were obtained from the stable period.

4.2. Stimulus

Spatially uniform light (white, yellow, red, and green) was generated from a computer monitor and projected onto the retinal surface via a lens focus system, at certain photonic mean intensities (in nW/cm²: white 12.18, red 4.00, green 6.52, yellow 10.87, measured using a photometer IL1400, USA). In order to relief the possible dark inhibition effect

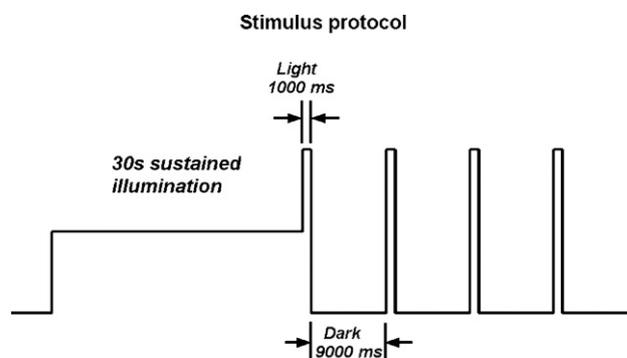


Fig. 5 – Light stimuli consisted of a 30-s background illumination and flashes with 1-s light-on duration and 9-s light-off interval.

caused by the pre-surgical dark environment, a full-field sustained light with medium intensity (White 6.09 nW/cm²) was projected onto the retina for 30 s before the application of experimental protocols. Flash stimulation (with 1-s On duration and 9-s Off intervals) was given repeatedly for 50 times in each experimental protocol (Fig. 5). Various color protocols were applied on a random basis, and each color protocol was applied for at least twice on each retina with the inter-protocol intervals being 5 min. Only data recorded from a single retina which showed stable responses during two identical experiments were chosen for further analysis. More details can be found in previous reports (Chen et al., 2005; Zhou et al., 2005).

4.3. Spike detection and spike sorting

Before spike detection, the field potentials were wiped off through a band pass filter (100–3000 Hz). Since the extracellular measurement conditions attenuated the signal by a factor of about 1000 (Watt et al., 1988), the signal-to-noise ratio (SNR) for extracellular recording was normally quite low, and it was difficult to set a fixed threshold to detect the spike events. In our study, the threshold for spike detection was set to be 4 times of the standard deviation (SD) of the measured signal for each individual electrode (Segev et al., 2004).

Spike sorting is a necessary and important procedure for the analysis of data from extracellular recording (Brown et al., 2004; Fee et al., 1996; Lewicki, 1998). Spike events recorded from each electrode were classified into neuronal activities based on principal component analysis (PCA) (Offline Sorter, Plexon Inc, USA). A total number of 411 electrode signals obtained from 15 retinas were analyzed and sorted into 464 neuron firing sequences for further analysis. The interspike intervals within the spike trains were also measured to countercheck the sorting results (Segev et al., 2004).

4.4. Curve fitting

Since it was difficult to define the precise latency of the peak activities in the raw PSTH (Ventura et al., 2002), a non-parametric regression method called Bayesian Adaptive Regression Splines (BARS) was used to generate a smooth curve to describe the time-dependent changes in the PSTH (Kass et al., 2003; Ventura et al., 2002). All the parameters used to describe the PSTH were based on the fitted curve.

Foundation of Natural Science of China (No. 30670519) and the Ministry of Education (No. 20040248062).

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Acknowledgments

This work was supported by grants from the State Key Basic Research and Development Plan (No. 2005CB724301), National