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Research Report

Color information encoded by the spatiotemporal patterns of light response in ganglion cells of chick retina

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Abstract

In the present study, the light responses of ganglion cells to chromatic stimulus were recorded from isolated retina of neonatal chick. It was found that for some non-color-opponent ganglion cells, the spatiotemporal patterns of the cells' light responses were related to the chromatic information that they received. When stimulus with some chromatic component was applied, some ganglion cells would generate distinguishable temporal patterns of light responses although these cells can be classified as non-color-opponent according to their light responses. The results suggest that in chick retina, the color information might be encoded not only by the color opponent ganglion cells, but also the spatiotemporal patterns of some ganglion cells that are traditionally classified as non-color-opponent subtype.

Theme: Sensory system
Topic: Retina and photoreceptors

Keywords: Retina; Ganglion cell; Multi-unit recording; Color information processing; Correlated activity

1. Introduction

It is well known that retinal ganglion cells, the final output neurons of the vertebrate retina, play an important role in visual information processing [6,15]. Various light stimuli are converted into spikes in the retinal ganglion cells and the encoded information is transmitted to the lateral geniculate nucleus (LGN) via optic nerve fibers [3,13]. Among the many features of ganglion cells, one widely accepted concept is that during retinal processing, color information is processed by color-opponent pathways, with relevant neuronal activities changing in opposite directions in response to opponent color stimulations [7,17]. Nevertheless, according to some recent observations made in this laboratory, non-color-opponent ganglion cells might also partly participate in color information processing, with the red–green pathway inputs influencing each other [4]. It was found that not only the firing rates, but also the correlation pattern of firing would contribute to the retinal information processing. For some ON–OFF ganglion cells, the neurons that spatially close to each other would fire in synchrony in response to red or green light, but the synchronization was broken when yellow or white light was applied [4].

Since any changes in synchronization should be originated from the temporal pattern of the relevant spike trains and the cross-correlation analysis could hardly draw the temporal details of the neuronal activity, we tried to use joint peri-stimulus time histogram (JPSTH) [1,11] for a better understanding of the neuronal interrelationship. JPSTH is a widely used method for investigating the dynamics of the interdependence of spike events between pairs of cells. Its results are often taken as an estimate of interaction strength between cells, independent on cells' firing rates [11]. In our experiments, spatially uniform chromatic light stimulus was given repeatedly. Interestingly, it was

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found that for a considerable portion of pair-wise adjacent non-color-opponent ganglion cells, two distinct peaks of synchronization could be identified in JPSTH when chromatic stimulus was given. Furthermore, it was noticed that for some non-color-opponent ganglion cells, there were diversified temporal patterns of responses under various light stimuli, which might be the origination of the temporal pattern found in JPSTH. The occurrence probability of this "double-peak" light response under different wavelength light stimulus was analyzed. Moreover, by analyzing peri-stimulus time histogram (PSTH) of single neurons and JPSTH between pair-wise adjacent neurons, we discussed the possible relationship between spectral configuration of light stimulus and the temporal pattern of neuronal response.

2. Materials and methods

2.1. Experimental procedure

Four retinas from neonatal chicks (3–8 days post hatching) were investigated in this research. Similar experimental operations can be found in previous reports [4,5]. All procedures strictly conformed to the humane treatment and use of animals as prescribed by the Association for Research in Vision and Ophthalmology. After decapitation and enucleation of the eye, the eyeball was hemisected with a fine razor blade. The vitreous body and cornea were removed carefully. To record the spike trains of retinal ganglion cells, a small piece (4 mm × 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 quantity (3 μl) of nitrate cellulose solution (1.0 mg Sartorius cellulose nitrate dissolved in 10.0 ml methanol) was smeared onto the electrode array as electric glue to make a better contact between the array and the retina. The preparation was perfused in oxygenated (95% O_2 and 5% CO_2) Ringer's solution (containing in mM: 100.0 NaCl, 5.0 KCl, 3.0 $MgCl_2$, 1.8 $CaCl_2$, 25.0 $NaHCO_3$, 25.0 glucose) with a pH value of 7.5 ± 0.2. The tissue and perfusate were kept at 38 °C by a temperature control unit (Thermostat HC-X, MCS GmbH, Germany). A small Ag/AgCl pellet with wire was immerged 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×, amplifier input impedance $>10^{10}$ Ω, output impedance 330 Ω). Signals from the selected channels along with the stimulus were sampled at a rate of 20 kHz (MCRack) and stored in a Pentium IV-based computer.

After 10–20 min adaptation to the perfusion environment, the light response of the retina would go stable and its stability would last for 7–8 h in our experiments. In this study, all the data analyzed were recorded from the stable period.

2.2. Stimulus

Spatially uniform light (red, green and yellow) was generated from a video monitor and was projected onto the retina via a lens focus system at a certain photonic mean intensity (IL1400, USA) (Table 1). Full-field sustained light with medium intensity was given for 30 s before the repeated stimulus in order to adjust the sensitivity of the ganglion cells to a similar level. Stimulus consisting of light duration of 1000 ms and dark interval of 9000 ms was given repeatedly for 50 times after the adaptation was completed (as shown in Fig. 1). For each individual retina, four color stimulus protocols were applied in random order, and the interval between successive color protocols was 5 min. To assure the stability of retinal responses, any kind of stimulus pattern were repeated at least twice for each retina, in random order. The experimental data were used for further analysis only if the neuron's firing activity was repeatable in response to identical stimulation.

2.3. 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 intracellular potentials by a factor of about 1000 [21], the signal-to-noise ratio (SNR) for extracellular recording was usually not high, and it could hardly set a fixed threshold to select the spike signals. In our study, the threshold for detection of spike events was set to be 4 times the standard deviation (s.d.) of the voltage for each electrode independently [18].

Spike sorting is a necessary and important procedure for the analysis of data from extracellular recording [2,10,12]. Spike events recorded from each electrode were classified into neuronal activities based on principal component analysis (PCA), as described in previous reports [4,22]. In the present study, a total number of 91 electrode signals from 4 individual retinas were analyzed and sorted into 108 neuron activities for further analyses. The interspike

Table 1
Composition and intensity of the chromatic stimulations

Stimulation	White	Red	Green	Yellow
Red gun index	255 (127)	255 (127)	0	255 (127)
Green gun index	255 (127)	0	255 (127)	255 (127)
Blue gun index	255 (127)	0	0	0
Intensity (nW/cm^2)	12.18 (6.09)	4.00 (2.00)	6.52 (3.26)	10.87 (5.44)

Data of the background luminance are presented in parentheses.

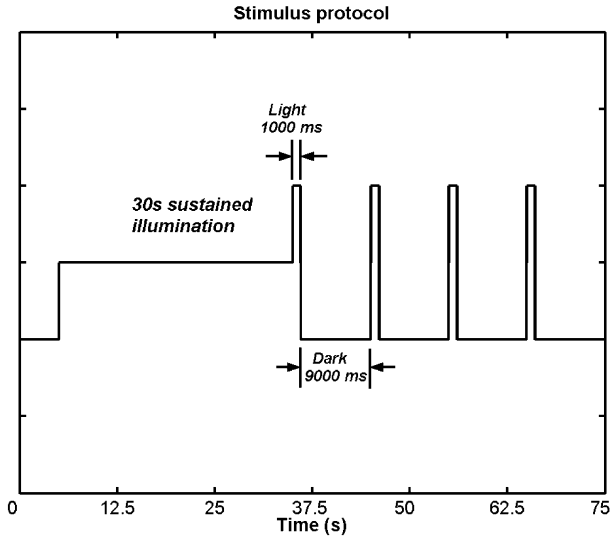

Fig. 1. The stimuli in the experiment consisted of a 30 s background illumination and repeated flashes with 1000 ms light-on duration and 9000 ms light-off interval.

intervals of the spike trains were also calculated to countercheck the sorting results [18].

3. Results

JPSTH is a two-dimensional histogram that illustrates joint spike count per unit time at each time u for one neuron and time v for another. The main diagonal of JPSTH (PST coincidence histogram) represents the observed rate of synchronized firings as a function of time t [5]. Fig. 2 is an example for JPSTH between a pair of adjacent ganglion cells (Channel 27 and 37) under various color stimuli. The intensity of synchronization and its varying dynamics clearly reveals that the synchronization under red light was unique from the other patterns: only one main peak can be observed in the JPSTH during the neurons' response to the light-ON transient of red stimulus, while two peaks could be elicited when green and yellow stimuli were applied.

The corresponding PSTH of each relevant neuron are presented near the X and Y coordinates of JPSTH in Fig. 2. It can be found that the diversity in JPSTH observed during various color stimulations might be caused by the difference in the temporal pattern of the neuronal activity in response to distinct stimuli. While the neurons would respond with two peaks in PSTH under green, yellow and white light, only one peak can be observed under red light stimulus. Fig. 3A is a typical example of this special "double-peak" light response observed from a non-color-opponent ganglion cell. From the PSTH (Fig. 3A), it can be found that the first peak of light response was elicited with a short delay (peak centered around 100 ms) after the light-ON transient. Following this period, there was a rapid drop in firing rate. However, different from the usual transient or sustained response (as illustrated in Figs. 3C and D) [3,9], a second peak appeared with a lag of about 150 ms after the first peak of light response (Fig. 3A). Detailed responses for each stimulus trial are also given in Fig. 3B.

Although this special temporal pattern of light response is noticeable, it is necessary to set a criterion to identify this special activity from the traditionally defined "transient" or "sustained" response for statistical analysis. In the example given in Fig. 3A, the primal light response which is similar to transient response (as presented in Fig. 3C) can be detected first [3]. After that, the firing rate descends quickly

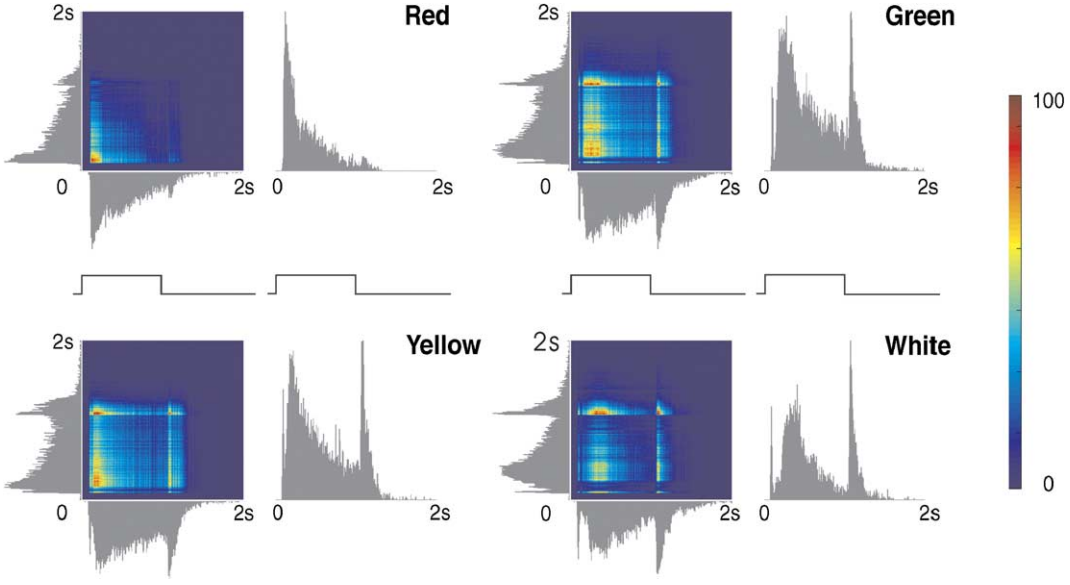

Fig. 2. Comparison of JPSTH of adjacent ganglion cells under various light stimulus. Bright area indicates higher probability of statistical synchrony, and dim area means the opposite. The PSTH of corresponding neurons are presented near the X and Y coordinates of the JPSTH. The main diagonal of the matrix is presented at the right side of each panel (x axis: Channel 27, y axis: Channel 37, Bin = 5 ms).

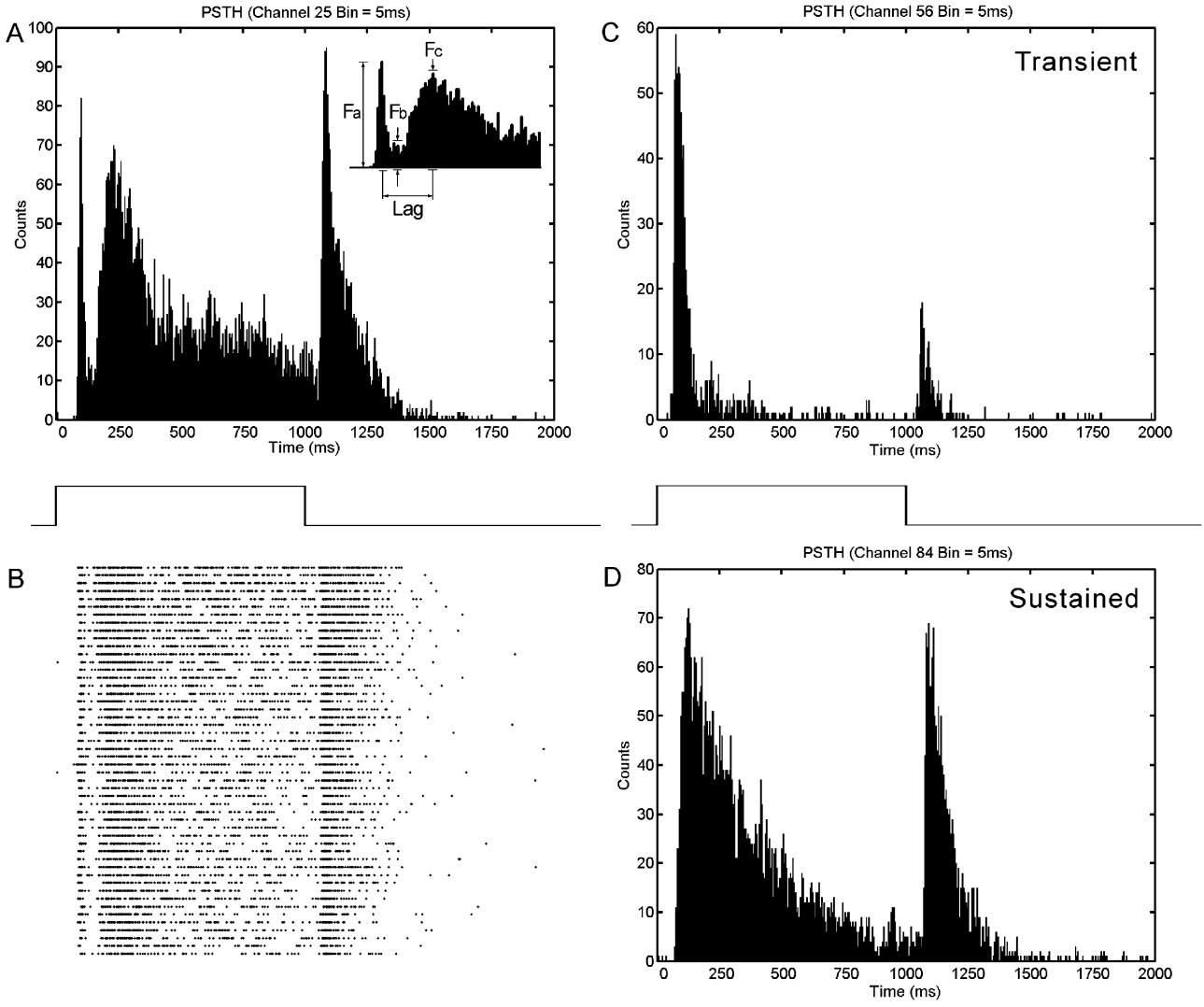

Fig. 3. (A) The PSTH of an example neuron which has a double-peak response to light-on transient. The insertions explains the criterion in identifying the double-peak response discussed in this work. F_a, F_b and F_c are the firing rate values of the first peak, the valley, and the second peak, respectively. Lag indicates the delay between the first peak and the second peak in response to light-on transient. (B) The raster plot of the neuron's firing activities recorded in 50 trails, with the occurrence of each spike presented by a dot. C and D. Examples for transient and sustained ganglion cells, respectively. (Transinet: Channel = 56, Sustained: Channel = 84, Bin = 5 ms).

and reaches a much lower level (Fig. 3A insertion, F_b < 50% F_a). A double-peak light response pattern can be identified if a second peak raises with a firing rate 50% higher than the valley value (Fig. 3A insertion, F_c > 150% F_b), within a lag shorter than 300 μs after the first peak.

Three kinds of chromatic light were performed to test the diversity of the neuronal activity under various color stimuli (green, red and yellow). Only a small number of ganglion cells acted with the temporal characteristic which was independent on the color of light stimuli. According to our definition and statistical data, 81 (75.0%) among all the 108 neurons had the second order light response under specific color stimulus. When green light was given, 66 (61.1%) neurons had the second order light response while the number for red and yellow light stimulus were 35 (32.4%) and 47 (43.5%), respectively. The detailed situation is given in Table 2 and the response characteristics under three kinds of chromatic light were considered: some ganglion cells would be evoked into the "double-peak" pattern by any chromatic stimuli while others would be "sensitive" to only one or two specific color. However, it is apparent that the green light stimulus had an advantage in evoking the "double-peak" pattern of ganglion cells than other chromatic lights (Table 2). Meanwhile, 44 (40.7%) neurons had an identical pattern (all "single-peak" or all "double-peak") in response to various chromatic stimuli while for 64 (59.3%) ganglion cells the color-dependent differences in temporal responses were distinguishable. In other words, most ganglion cells investigated were able to describe the diversity between different color lights by their temporal activities.

Another thing needs attention is the asymmetry between the temporal features under opponent color green and red.

Table 2
Statistical result of influence by opponent color stimulus (108 ganglion cells total)

Stimulation	Green (+)	Red (+)	Yellow (+)
Green (+)	Yellow (−) Red (−) 19 (17.6%)	Yellow (+) 17 (15.7%) Yellow (−) 10 (9.3%)	Red (−) 20 (18.6%)
Red (+)		Green (−) Yellow (−) 5 (4.6%)	Green (−) 3 (2.8%)
Yellow (+)			Green (−) Red (−) 7 (6.5%)

(+) means ganglion cell that has the two order responses to the specific color, (−) means the opposite.

Although yellow light was generated as a combination of green and red lights in this study (Table 1), the neuronal response to the yellow light was not a simple summation (Table 2). Specifically, the temporal structure of ganglion cells in response to yellow light was more likely to follow the characteristics under green light stimulation rather than that during red light stimulation: twenty (18.6%) neurons shared the "double-peak" pattern under green and yellow light where only seven (6.5%) neurons had that pattern under red and yellow light (Table 2).

4. Discussion

Because the time-dependent variability of the neuronal response was significant and inevitable, JPSTH and PSTH were used to present the spatiotemporal and temporal pattern of the neuronal responses in this study. Raster plots of spike trains are also illustrated to testify the stability of light responses. As an example, it can be found in Fig. 3B that there were only little changes between different trials of light responses. In that case, the (J)PSTH can reliably describe the time-dependent dynamics of light response and dynamic relationship between adjacent neurons.

Intuitively, the bin size for (J)PSTH might be important for the results. In the present study, preliminary analyses were made with bin size being 1 ms, 2 ms, 5 ms and 10 ms, respectively, and the results were compared. The results showed that the double-peak pattern in neuronal activities can be well identified, in spite of the difference in parameters. This suggests that the observed phenomenon should be attributed to the temporal pattern of the neuronal light responses rather than a biased estimate caused by improper parameter settings. Therefore, the time bin was set to be 5 ms in this study for the rest analyses. The firing rate of neuron pairs have been normalized in JPSTH.

Ganglion cells can be classified into several subtypes [3,9] according to their characterized light response properties. In the present study, the relationship between the temporal pattern of light response and the chromatic configuration of light stimulus was studied in the chick retina. Most of the ganglion cells being recorded were of ON–OFF subtype. The "double-peak" pattern response was more likely to occur at the ON-transient, only a small number of neurons showed such temporal pattern at the OFF-transient. It is interesting to find that: (1) the specific temporal pattern was related to the color configuration of light stimulus, (2) the two different components of light response might contribute to distinct aspects of neural information processing.

It has been previously suggested that changes in temporal structure of the spike train can efficiently convey the information to be processed, while the rate codes are less efficient in fast information transmission [19,20]. Although the ganglion cells under investigation are of non-color-opponent subtype, neuronal activities elicited by different chromatic stimuli are distinct in temporal pattern according to the results shown in Table 2. Hence, the specific temporal pattern might be related to the color information coding.

Color discrimination is more complex than intensity discrimination since intensity cannot be kept exactly constant for different wavelengths. Besides, when the intensity of a monochromatic stimulus is changed, the differential excitation of the three classes of cone photoreceptors should also be changed accordingly. In our experiments, yellow light was generated as a combination of green and red lights. Although the intensity for green flashes was comparatively weaker as compared to white or yellow light, green stimulus seems to be more likely to evoke the "double-peak" response pattern in ganglion cells. This phenomenon was not dependent on the intensity, but rather the spectral configuration of the light stimulation—the temporal pattern of the ganglion cells' response might be related to the combined activation pattern of various cone pathways.

From the result shown in Figs. 2 and 3, it is clear that there are two components of light responses at the ON-transient of specific chromatic stimulus. The first peak of firing activity appeared with a short latency, thus it might be generated to encode the arrival time of the stimulus. It is not surprising that the "double-peak" of single neuronal response might be the origination of the temporal pattern in JPSTH. However, it seems puzzling why should the ganglion cells generate a second peak, and what functional role of the latter firings play in neuronal processing.

A possible explanation to the role of the second peak is related to the interaction between neurons. Concerted firings of neurons has been suggested to contribute significantly during information processing in the visual system [8,14,16]. To investigate the dynamics of correlated activities of nearby ganglion cells, the JPSTH of 85 neuron pairs were calculated. Since JPSTH calculates the synchronization dynamics between neurons, it provides a spatio-temporal pattern which is a three-dimensional variable and is very changeable across neuron pairs due to the color-

related temporal sensitivity and spatial positions of the relevant neurons, it is therefore very difficult to define a statistics to show its significance. However, considering about the example shown in Fig. 2, the intensity of synchronization was stronger in the latter part than the primal part when ON stimulus was given. Similar situations can be found in many (but not all) adjacent neuron pairs. This suggested that the second peak of light response might play a more important role than the first response in the neuronal population coding. Specifically, the functional roles of the two parts of light responses might be different where the first activity was possibly generated to indicate the arrival of the stimulus, and the second part might play a more important role in the more complex neuronal communication and population coding.

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