Population activity changes during a trial-to-trial adaptation of bullfrog retinal ganglion cells

Wei Ding, Lei Xiao, Wei Jing, Pu-Ming Zhang and Pei-Ji Liang

A 'trial-to-trial adaptation' of bullfrog retinal ganglion cells in response to a repetitive light stimulus was investigated in the present study. Using the multielectrode recording technique, we studied the trial-to-trial adaptive properties of ganglion cells and explored the activity of population neurons during this adaptation process. It was found that the ganglion cells adapted with different degrees: their firing rates were decreased in different extents from early-adaptation to late-adaptation stage, and this was accompanied by a decrease in cross-correlation strength. In addition, adaptation behavior was different for ON-response and OFF-response, which implied that the mechanism of the trial-to-trial adaptation might involve bipolar cells and/or their synapses with other neurons and the stronger adaptation in the ganglion cells' OFF-responses might reflect the requirement to avoid possible saturation in the OFF circuit. *NeuroReport* **25:801–805 © 2014 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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School of Biomedical Engineering, Shanghai Jiao Tong University, Shanghai, China

Correspondence to Pei-Ji Liang, PhD, School of Biomedical Engineering, Shanghai Jiao Tong University, 800 Dong-Chuan Road, Shanghai 200240, China Tel: +86 21 34204015; fax: +86 21 34204016; e-mail: pjliang@sjtu.edu.cn

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Introduction

In vertebrates, the retina is the first stage of visual neural information processing. Retinal ganglion cells (RGCs), the output units of retina, convey the neural signals to the central visual system through action potentials. To deal with the wide range of ambient light conditions with the relatively narrow neural spiking range, the visual neurons in the retina and other neural stages adapt dynamically in response to sustained stimulation [1,2]. Retinal visual adaptation, such as luminance adaptation and contrast adaptation, has been studied intensively [3,4]. These adaptive processes are similar in a sense that when the retina is exposed to prolonged luminance or contrast stimulations, the firing activities of ganglion cells are gradually decreased after an abrupt increase at the onset of the stimulus. In the present study, another type of adaption process was observed in bullfrog RGCs: discharging activities were gradually attenuated when the light stimulus was repeatedly presented. Such 'trial-to-trial adaptation' has been reported in other neural systems [5,6], and it was shown that in rat vibrissal pathway, a trial-to-trial adaptation in cortical neurons to periodic tactile inputs served to enhance the discrimination of stimuli [6]. However, in the retina, knowledge of how such trial-to-trial adaptation behaves and what effects it exerts on visual information processing is still lacking.

In addition to adaptive behavior, population neurons' activity is also considered to play an essential role in neural information processing [7,8], although some others also debated that correlation can be ignored in the neural coding [9,10]. Using the multielectrode recording technique, we investigated how the population activity changed during the trial-to-trial adaptation. It was found that the adaptation process of single neurons' activity was accompanied by adaptive changes of cross-correlation strength between cells. Moreover, analysis of RGCs' ON-responses and OFF-responses indicated that the ON-pathway and OFF-pathway adapted differently to the repetitive stimulus, which implies that possible mechanisms controlling the trial-to-trial adaptation might involve bipolar cells and/or their synapses with other neurons. Moreover, the stronger adaptation in OFF-response might reflect the requirement to avoid possible saturation in the OFF-circuit and to adjust the sensitivity of the more responsive OFF-pathway.

Methods

Experiments

The surgery procedures and multielectrode recordings followed the protocol as described previously [11]. All the experimental procedures were approved by the Ethic Committee, School of Biomedical Engineering, Shanghai Jiao Tong University.

Stimulus and ganglion cell classification

The light stimulus was generated from a computer monitor (Iiyama, Vision Master Pro 456; Iiyama Corporation, Japan) and was focused to form a 1.1×1.1 mm^2 image on the isolated retina through a lens system. A stimulus consisting of full-field white light (77.7 nW/cm^2) with a duration of 1 s and a dark interval of 9 s was administered repeatedly for 30 times. Before this, a full-field white light (38.9 nW/cm^2) was administered

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for 30 s to adjust the sensitivity of the ganglion cells to a similar level [11].

Ganglion cells with ON-responses, OFF-responses, or ON–OFF responses have been described previously in the frog retina [12]. Most RGCs recorded in our experiments responded to both light-ON and light-OFF stimulation. In the present study, ON–OFF cells with long-lasting firings in OFF-responses (133 from a total number of 176 ganglion cells recorded from five retinas), which can be classified as dimming detectors [13], were selected for further analyses.

Adaptation index

Adaptation index (AI) was calculated to quantify the adaptation degree of the RGC's firing activity in response to the repetitive stimulus. First, given that the discharging of neurons decreased gradually in the first several trials and fluctuated slightly in the later trials, we defined the 'early-adaptation part' as the 1st–10th trials of the whole response and 'late-adaptation part' for the 21st–30th trials. Then, the AI was defined as the ratio between the averaged firing rates in the early-adaptation part (S_e) and that of the late-adaptation part (S_l).

Cross-correlation function

The cross-correlation function (CCF) between neurons was calculated to measure the concerted activity among neuron population as follows [11,14]:

$$c_{xy}(m) = \begin{cases} \dfrac{\sum_{n=0}^{N-|m|-1} x_n y_{n+m}}{R} & m \geq 0 \text{, in which } R \\ c_{yx}(-m) & m < 0 \end{cases} = \sqrt{\sum_{i=1}^{N} x_i^2 \sum_{i=1}^{N} y_i^2}, \quad (1)$$

where $c_{xy}(m)$ is the CCF for the spike train of two cells x and y with a time-lag of m, x_n and y_{n+m} are the values of sequence x at the moment n and that of y at $n+m$, respectively, N is the length of the sequence, and R is the normalization term. Here, bin size was set as 1 ms and we calculated the $c_{xy}(m)$ for the set of $-200 < m < 200$ (ms) and took the maximum in this set to quantify the strength of correlation (C). Then, if the value of C for a neuron pair did not exceed the mean + 3 SD of the $-200 < m < 200$ (ms) set, this pair were excluded from analysis for that their flat CCF indicated only correlation of coincidence by chance.

Results

Response features of bullfrog retinal ganglion cells during repetitive stimulation

On exposure to the periodic 1 s-ON/9 s-OFF light stimulation, RGCs generated action potentials, but the firing activities of RGCs in each repeated trial were not always identical. When some neurons' discharging fluctuated within a small range throughout all 30 trials, some others showed progressive reduction, which showed the property of trial-to-trial adaptation.

Firing activities of two representative cells are shown in Fig. 1a and b. In each plot, the upper panel shows the raster plots of spikes in repeated trials and the lower panel shows the firing rates (1-s bin) with time. The neuron presented in Fig. 1a showed remarkable trial-to-trial adaptation, with both firing rate and response duration being decreased when the stimulus was repeated. After firing most intensely in the early trials, the cell's discharging activity decreased exponentially to a lower level during the successive trials. However, the neuron in Fig. 1b did not show such an obvious adaptation. The adaptation indices (see the Methods section) of the cells in Fig. 1a and b were 2.86 and 1.22, respectively. Generally, the 133 neurons from five retinas in our experiment showed variation in adaptation degrees (Fig. 1c). Some cells' firing activities decreased markedly, corresponding to the dots distributed far away from the diagonal line, whereas some others adapted slightly, with their adaptation indices being close to '1'. In sum, the adaptation indices (1.4678 ± 0.0363, mean ± SE) were statistically higher than 1.0 (Fig. 1d, Wilcoxon signed-rank test, $P < 0.05$), implying a significant decrease in the firing rate from early to late adaptation.

Population activity changes during the trial-to-trial adaptation

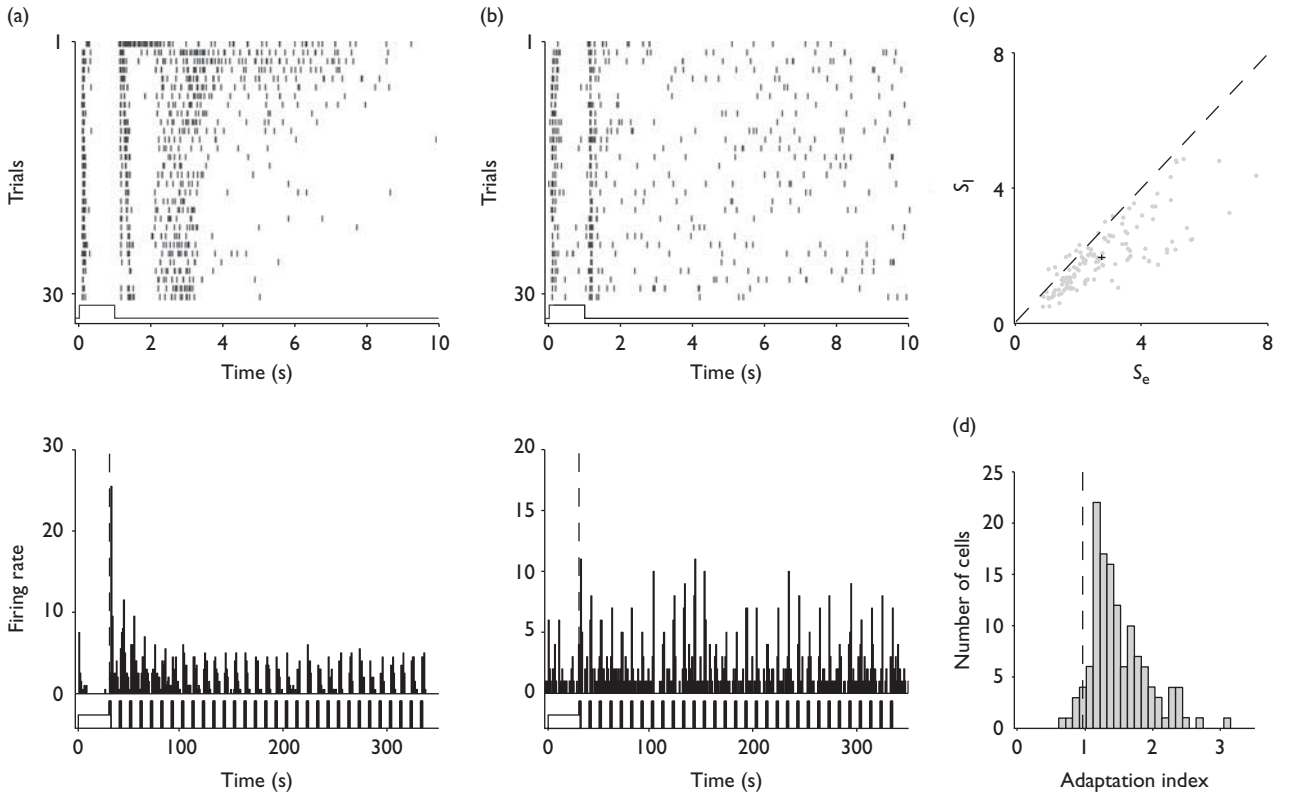
It has been suggested that population activity pattern of bullfrog RGCs was changed in response to repetitive stimulation [15]. In the present study, RGCs showed adaptation to the repeated stimulation; thus, we speculated that the multineuronal pattern might also be changed during this process.

We computed the CCF between cells for the early-adaptation part (C_e) and the late-adaptation part (C_l), and the comparison between the two showed a significant reduction. For one example, the retina in which 133 neuron pairs were formed by 26 RGCs, the CCF strength was reduced from 0.2398 ± 0.0139 in the early part to 0.2138 ± 0.0115 in the late part (Fig. 2a). This decreasing tendency was also observed in the other four retinas. All together for 909 pairs on the five retinas, the C_e/C_l ratio, 1.1832 ± 0.0155 (mean ± SE), was found to be significantly greater than 1.0 (Wilcoxon signed-rank test, $P < 0.05$, Fig. 2b).

The different performances of ON-responses and OFF-responses during the trial-to-trial adaptation

It has been reported that the ON-responses and OFF-responses of vertebrate RGCs are mediated through distinct neural pathways [16]. Here, we investigated the light-ON and light-OFF responses during the trial-to-trial adaptation, and observed more pronounced

Fig. 1



Firing activities of bullfrog RGCs in response to periodic 1 s-ON/9 s-OFF stimulus. (a, b) Responses of two representative cells. Upper panels show raster plots of firing activities. Lower panels show histograms of firing rates computed with a 1-s time bin, with the dashed line marking the beginning of the first trial (response to the 30-s before stimulus before the dashed line was not included in the analysis). The traces below each panel show the time course of stimulus. (c) S_e versus S_I for the 133 RGCs from five retinas. Each symbol represents one cell. The error bars indicate the mean ± SE (S_e: 2.758 ± 0.118, S_I: 1.949 ± 0.082). (d) Histogram of adaptation indices for the 133 cells (bin width = 0.1). The statistical test shows that AI significantly fell into the positive side of 1.0, which is represented by the vertical dashed line (1.4678 ± 0.0363, mean ± SE, Wilcoxon signed-rank test, $P < 0.05$). AI, adaptation index; RGCs, retinal ganglion cells.

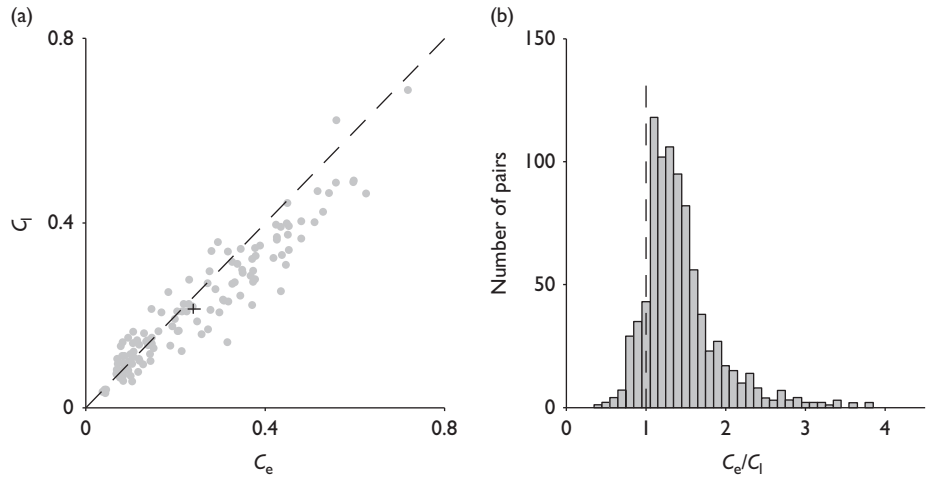
adaptation in the cells' OFF-responses than ON-responses. Figure 3a shows the firing rates measured during light-ON and light-OFF periods from the example retina presented in Fig. 1 (each symbol is the value averaged across the 26 RGCs). Adaptation indices for these 26 cells' ON-responses and OFF-responses are compared in Fig. 3b, which also showed a more pronounced adaptation in the OFF-response. Similar results were obtained from the other four retinas. We further studied the CCF strength changes (C_e/C_l, the ratio between CCF strength for early-adaptation and late-adaptation part) for the ON-responses and OFF-responses separately, and found that the decrease in correlation in light-OFF responses prevalently surpassed their counterparts in light-ON responses [statistical results for all five retinas show significance: R1: 1.036 ± 0.026 vs. 0.974 ± 0.027 ($n = 152$); R2: 1.225 ± 0.040 vs. 1.037 ± 0.027 ($n = 133$); R3: 1.272 ± 0.031 vs. 1.039 ± 0.028 ($n = 283$); R4: 1.153 ± 0.074 vs. 1.065 ± 0.078 ($n = 35$); R5: 1.154 ± 0.022 vs. 1.109 ± 0.026 ($n = 298$), Wilcoxon signed-rank test, $P < 0.05$, Fig. 3c], which implied the different change of neuronal connections for ON-pathway and OFF-pathway during the adaptation.

Discussion

Trial-to-trial adaptation involves the modulation of lateral connection in the retinal neuronal network

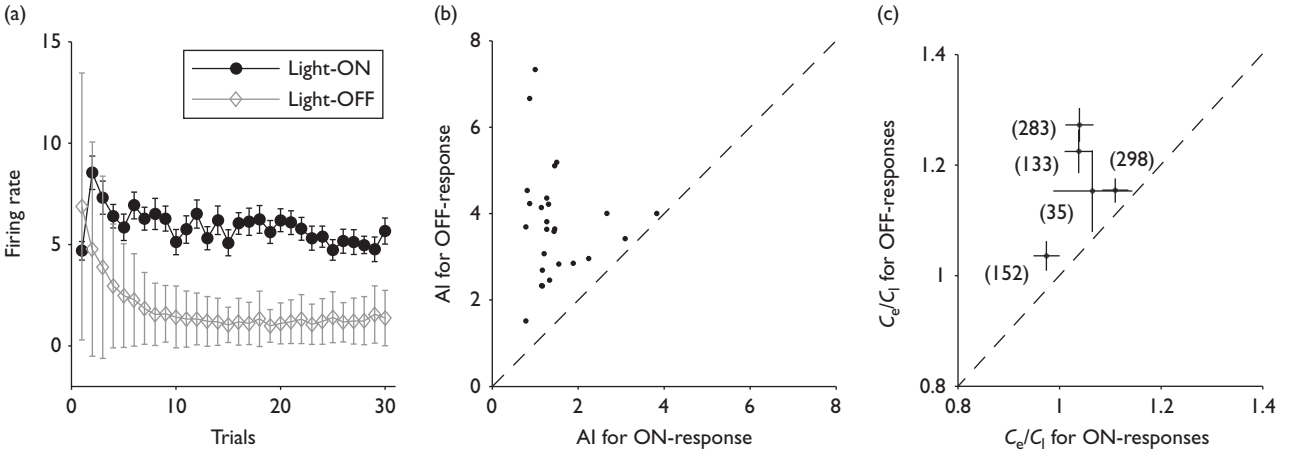
Our results showed that the population activity was decreased during the trial-to-trial adaptation (Fig. 2). Multineuronal activity contributes toward information processing and affects the information transmission of downstream neurons [7]. Stronger concerted activity is related to stronger and more efficient input to post-synaptic neurons [17]. In this way, the general weakening of concerted activities could suggest an adaptation in population coding in the visual system: less synchronous inputs to the postsynaptic neurons lead to a weakening in spatial integration, and hence the sensitivity to the repetitive stimulus can be adjusted.

Fig. 2



(a) Change in correlation strength during trial-to-trial adaptation. CCF strength for the late-adaptation part (C_l) versus early-adaptation part (C_e) for 26 RGCs from the example retina. Each symbol represents one neuron pair. The error bar shows the statistical result (C_e: 0.2398 ± 0.0139, C_l: 0.2138 ± 0.0115, mean ± SE, $n = 133$). (b) Histogram of C_e/C_l ratios for all the five retinas investigated (bin width = 0.1). The vertical dashed line represents the C_e/C_l ratio = 1.0. The statistical test shows C_e/C_l significantly larger than 1.0 (1.1832 ± 0.0155, mean ± SE, Wilcoxon signed-rank test, $P < 0.05$). CCF, cross-correlation function; RGCs, retinal ganglion cells.

Fig. 3



(a) Firing rates (bin = 1 ms) averaged across the 26 RGCs' activity from one retina. The line with solid circles represents the firing rates under light-ON stimulus, whereas the diamond line represents that for the ON-part. Error bars are mean ± SE. (b) Adaptation indices for ON-responses and OFF-responses of the same 26 cells. (c) C_e/C_l for ON-responses and OFF-responses in all five retinas. Each cross symbol represents the statistical result obtained from one retina (mean ± SE, Wilcoxon signed-rank test, $P < 0.05$, numbers of neuron pairs on each retina are marked near each symbol). AI, adaptation index; RGCs, retinal ganglion cells.

More specifically, the neuronal connections in the retina can be classified into two subtypes on the basis of the peak width of their CCF: one shows a narrow peak as fine as about 1 ms, being considered a result of electrical coupling between RGCs, and the other has a wide peak extending tens of milliseconds, which reflects the common inputs from presynaptic neurons through gap junction [18]. These two types of correlation showed different changing tendencies during the adaptation (data not shown). Narrow correlations decreased significantly, whereas wide correlations did not show a significant change and sometimes even increased, which suggested that the electrical synapses at different sites in the retinal network underwent differentiated modulation during the trial-to-trial adaptation.

Given that the neurons' correlation is somewhat dependent on the neurons' firing rates, a normalized cross-correlation method was applied in our present study. The firing rate-dependent changes in the neurons' correlation

can be attenuated effectively using this normalized cross-correlation method, although it is not completely eliminated.

Asymmetry in ON-responses and OFF-responses during the trial-to-trial adaptation

In the presence of the repetitive stimulus, strong trial-to-trial adaptation was observed in the OFF-responses of bullfrog RGCs, but much less was shown in the ON-responses. The RGCs' ON-responses and OFF-responses are mediated by parallel ON-neuronal and OFF-neuronal pathways in the retina [19], and the ON–OFF RGC converges inputs from both ON-center and OFF-center bipolar cells that express different classes of neurotransmitter receptors and have their axon terminals stratified into different sublaminae in the inner plexiform layer; thus, the kinetics and sensitivity of the ON-response and OFF-response differ [20]. It has been suggested that the ON-pathways and OFF-pathways do not have simply equal and opposite response properties, but are functionally asymmetric [21]. Further, some studies have reported differences of contrast adaptation between ON-ganglion and OFF-ganglion cells and pathways, suggesting differences in the inputs reaching RGCs or intrinsically in the ganglion cells' spike generation [22,23]. However, given that the trial-to-trial adaptation in our present study was the ON-responses and OFF-responses of the same ON–OFF ganglion cells, it is likely that the mechanisms governing the trial-to-trial adaptation are with the bipolar cells and/or the synaptic connections with their preneurons and postneurons, but where the exact location is remains to be studied. In addition to the firing rate adaptation, the correlation strength changes in the ON-response and OFF-response also differed, verifying that the modulation of lateral connection in the ON-circuit and OFF-circuit during the trial-to-trial adaptation should differ. The discharging at light offset was much stronger than that at onset (as can be seen in Fig. 1, 1-s bin) in the initial trials. This asymmetry was verified by our unpublished results that for the type of dimming detector RGCs we investigated, their transient ON-response pattern would not be prolonged even under a longer light-ON (such as 5 s-ON/5 s-OFF) stimulus. Consequently, a greater potential for saturation of the OFF-response was presented. Thus, a more significant adaptation to avoid the saturation and adjust the sensitivity of the OFF-pathway might be required, which is similar to the results reported on contrast adaptation in parallel retinal pathways [24,25].

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Conflicts of interest

There are no conflicts of interest.

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