J Comput Neurosci (2014) 37:149–160
DOI 10.1007/s10827-013-0492-2

Response dynamics of bullfrog ON-OFF RGCs to different stimulus durations

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Received: 3 May 2013 / Revised: 31 October 2013 / Accepted: 19 December 2013 / Published online: 4 January 2014

Abstract Stimulus duration is an important feature of visual stimulation. In the present study, response properties of bullfrog ON-OFF retinal ganglion cells (RGCs) in exposure to different visual stimulus durations were studied. By using a multi-electrode recording system, spike discharges from ON-OFF RGCs were simultaneously recorded, and the cells' ON and OFF responses were analyzed. It was found that the ON response characteristics, including response latency, spike count, as well as correlated activity and relative latency between pair-wise cells, were modulated by different light OFF intervals, while the OFF response characteristics were modulated by different light ON durations. Stimulus information carried by the ON and OFF responses was then analyzed, and it was found that information about different light ON durations was more carried by transient OFF response, whereas information about different light OFF intervals were more carried by transient ON response. Meanwhile, more than 80 % information about stimulus durations was carried by firing rate. These results suggest that ON-OFF RGCs are sensitive to different stimulus durations, and they can efficiently encode the information about visual stimulus duration by firing rate.

Action Editor: Tim Gollisch

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Keywords Retinal ganglion cells · Stimulus duration · Response latency · Firing rate · Correlation strength · Information coding

1 Introduction

In vertebrates, visual information is initially processed in the retina. The retinal neuronal network consists of parallel ON and OFF pathways, which are segregated to signal light increment and decrement, respectively (Wassle 2004; Oesch et al. 2011). ON-OFF retinal ganglion cells (RGCs), dendrites of which are branched throughout the inner plexiform layer (IPL), are one physiological class of RGCs and can respond to both light increment and decrement (Bloomfield and Miller 1986). In the frog retina, RGCs can be generally classified into four subtypes based on their response properties: sustained edge detector, convexity edge detector, changing contrast detector, and dimming detector (Maturana et al. 1960; Ishikane et al. 2005). Of these cell classes, "changing contrast detector" is characterized as one kind of ON-OFF RGCs, which is highly sensitive to both increase and decrease in illumination (Maturana et al. 1960). Frog RGCs can also be classified into about 12 distinct cell types based on cells' morphology, which is similar to classification in many other species (Masland 2012; Straznicky and Straznicky 1988). For frog RGCs, The classification based on morphology can be correlated well with the classification based on response properties, e.g., large size RGCs are related to dimming detectors, medium size cells are related to changing contrast detectors, and small size cells are related to the other two types (Straznicky and Straznicky 1988).

Response latency and firing rate are most common indices for describing neuronal activity in response to different stimuli, and they are also suggested to efficiently carry information about stimulus features, such as stimulus contrast, location, moving speed and direction, etc. (Risner et al. 2010; Gollisch and Meister 2008; Thiel et al. 2007; Nowak et al. 2011; Panzeri et al. 2001). On the other hand, RGCs' activities are not independent to each other, concerted activities in retina have been extensively documented (Xiao et al. 2011; Shlens et al. 2008; Usrey and Reid 1999). At the mean time, concerted activities between RGCs are suggested to be dynamically changing with different stimuli (Jing et al. 2010b; Schnitzer and Meister 2003), and they are also suggested to carry visual information (Pillow et al. 2008; Schneidman et al. 2006).

In the present study, the roles of the "changing contrast detector" in processing different stimulus durations were studied. Using a multi-electrode recording system, response activities of the transient ON-OFF RGCs in exposure to different stimulus durations were investigated. It was found that the ON and OFF response characteristics, including response latency, firing rate, correlation strength and relative latency, were affected by different light OFF and ON durations, respectively. Information analysis showed that the mutual information about different light ON durations was more carried by the OFF response, while the ON response carried more information about different light OFF intervals. Though response properties of single neuron and pair-wise neurons were both changed with stimulus durations, more than 80 % information about stimulus durations was carried by firing rate. These results suggest that the ON-OFF RGCs are sensitive to different stimulus durations, and they can efficiently encode the information about stimulus duration by firing rate.

2 Materials and methods

2.1 Retinal recording

Experiments were performed on isolated bullfrog retinas at room temperature (22–26 °C) (Jing et al. 2010a; Li et al. 2012). Bullfrogs were dark adapted for about 30 min prior to experiments. A piece of retina (about 4×4 mm^2) was placed on multi-electrode arrays (MEA, MMEP-4, CNNS UNT, USA) with the ganglion cell side contacting the electrodes. The retina was perfused with oxygenated Ringer's solution (95 % O_2 and 5 % CO_2) containing (in mM): NaCl 100.0, KCl 2.5, $MgCl_2$ 1.6, $CaCl_2$ 2.0, $NaHCO_3$ 25.0, glucose 10.0. The activities of neurons were recorded by the MEA consisting of 64 electrodes (8 μm in diameter) which were arranged in an 8×8 matrix with 150 μm tip-to-tip distance, horizontally and vertically. Signals were amplified by a 64-channel amplifier (MEA workstation, Plexon Inc. Texas, USA), with each channel being sampled at a rate of 40 kHz (along with the stimulus). Well isolated action potentials were sorted by the commercial software OfflineSorter (Plexon Inc. Texas, USA). All procedures strictly conformed to the humane treatment and use of animals as prescribed by the Association for Research in Vision and Ophthalmology, and were approved by the Ethic Committee, School of Biomedical Engineering, Shanghai Jiao Tong University.

2.2 Stimulus protocols

Light stimuli were generated from a computer monitor (Iiyama, Vision Master Pro 456, Japan) and were focused on the isolated retina via a lens system. To adjust the sensitivity of ganglion cells to similar levels, full-field sustained dim white light (38.9 nW/cm^2) was given for 30 s before stimulus protocols were applied (Jing et al. 2010a). In the present study, two stimulus protocols were applied: (1) Stimulation with different light ON durations for investigating the influence of the ON pathway activity on the OFF response. In this protocol, each experimental trial contained randomly presented light ON (77.7 nW/cm^2) stimuli with duration of 1 s, 5 s and 9 s which were separated by 1-s full-field light OFF (about 0.0015 nW/cm^2) intervals. Each experiment contained 30 trials (Fig. 1a). (2) Stimulation with different light OFF intervals for studying the influence of the OFF pathway activity on the ON response, in which light OFF intervals of 1 s, 5 s and 9 s were presented randomly in each trial and separated by full-filed 1-s light ON stimuli, with 30 trials (Fig. 1b).

Totally 21 retinas from 18 bullfrogs were used in the present study. Among them, ten retinas were used for studying the neuronal responses in exposure to different light ON durations, eight retinas for different light OFF intervals, and

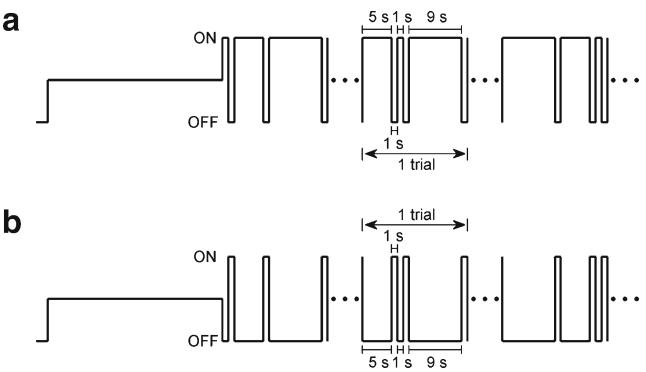

Fig. 1 Stimulus protocols. **a** Stimulus protocol with different light ON durations, in which 1-s, 5-s and 9-s light ON were given randomly and separated by 1-s light OFF intervals in each trial. **b** Stimulus protocol with different light OFF intervals, in which 1-s, 5-s and 9-s light OFF intervals were presented randomly in each trial and were separated by 1-s light ON. Full-field sustained dim white light was given for 30 s before each stimulus protocol

the remaining three retinas for both experimental protocols. Meanwhile, 262 RGCs and 179 RGCs were respectively recorded for testing the cells' response characteristics in exposure to different light ON and OFF durations.

2.3 Correlation strength

To quantitatively analyze the characteristics of the neuronal responses, spike trains were discretized into "0-1" sequences using 1-ms time bins, where $r_i(n)=1$ means that the cell i fired in the nth time bin, and "0" means that it did not fire. The cross-correlation function between RGCs' firing sequences was calculated by (X. Liu et al. 2007):

$$c_{ij}(m) = \sum_{n=1}^{N-|m|-1} r_i(n) r_j(n+m) \Big/ \sqrt{\sum_{n=1}^{N} r_i(n)^2 \sum_{n=1}^{N} r_j(n)^2}, \quad (1)$$

where $r_i(n)$ denotes the value of spike train r_i at moment n, and $r_j(n+m)$ the value of spike train r_j at moment $n+m$. Cross-correlation function was fitted by a Gaussian function, and the correlation strength between neurons' activities was quantified as the peak value of the fitted curve.

Correlated activity between RGCs induced by common inputs from bipolar/amacrine cells has been widely observed (Brivanlou et al. 1998; DeVries 1999). In the present study, cross-correlation function was calculated for all the neuron pairs of ON-OFF RGCs being recorded in each retina, and correlate activity between RGCs can be easily identified based on widely distributed time-lags (between 20 and 100 ms) of the cross-correlation function (Jing et al. 2010a). Because many RGCs can receive common inputs from bipolar/amacrine cells, and will form correlated activity with each other, therefore one RGC can have correlated activity with several other RGCs.

2.4 Mutual information

To perform quantitative analysis, the conditional probability of observing the neural response $\mathbf{r}$ given the stimulus s is defined as $p(\mathbf{r}|s)$. For N neurons, $\mathbf{r} = \{r_i\}$, with $i=1,\ldots,N$. In the present study, three stimulation patterns occurred with equal probability in each protocol, i.e., $p(s)=1/3$. The total amount of stimulus information that can be extracted from the neuronal data is given by the mutual information:

$$I_{MI} = -\int d\mathbf{r}\, p(\mathbf{r}) \log_2 p(\mathbf{r}) + \int d\mathbf{r} \sum_s p(s) p(\mathbf{r}|s) \log_2 p(\mathbf{r}|s). \quad (2)$$

2.5 Decomposing the stimulus information

The mutual information contained by spike trains can be decomposed into information encoded by firing rate and correlated activity between pair-wise cells (Xiao et al. 2013a). Without loss of generality, information measure I^*, which is directly linked to the decoding error of maximum likelihood inference based on a mismatched model (Wu et al. 2001; Oizumi et al. 2010), was applied for decomposing the stimulus information. I^* quantifies the information gain when a mismatched neural encoding model $q(\mathbf{r}|s)$ is applied, and is calculated as (Merhav 1994):

$$I^*(q) = \max_{\beta} \widetilde{I}(q,\beta), \quad (3)$$

$$\widetilde{I}(q,\beta) = -\int d\mathbf{r} p(\mathbf{r}) \log_2 \sum_s p(s) q(\mathbf{r}|s)^{\beta} + \int d\mathbf{r} \sum_s p(s) p(\mathbf{r}|s) \log_2 q(\mathbf{r}|s)^{\beta}, \quad (4)$$

where β is a constant to be optimized. This information measure has been applied recently for studying neural coding (Oizumi et al. 2010). By choosing the form of $q(\mathbf{r}|s)$ properly, the amount of stimulus information contained in different orders of neural correlation can be computed.

When considering two binary sequences, their joint probability can be written as (Amari 2001):

$$p(\mathbf{r}|s) = \frac{1}{Z} \exp\left[\sum_i \theta_i^1 r_i + \sum_{i<j} \theta_{ij}^2 r_i r_j\right], \quad (5)$$

where Z is the normalization factor, and θ_i^1 is related to the 1st-order correlation, i.e., the firing rate of the ith neuron, and θ_{ij}^2 is the 2nd-order correlation between ith and jth neurons. The values of θ can be uniquely determined by matching $p(\mathbf{r}|s)$ with the real distribution of neural firing activities.

Suppose $q_1(\mathbf{r}|s)$ is chosen to be a probability distribution which has the same firing rates as $p(\mathbf{r}|s)$ but with correlation between neurons vanishing. It is given by:

$$q_1(\mathbf{r}|s) = \frac{1}{Z_1} \exp\left[\sum_i \overline{\theta}_i^1 r_i\right], \quad (6)$$

where the parameter $\overline{\theta}_i^1$ is determined by the condition $\langle r_i \rangle_{q_1} = \langle r_i \rangle_p$, with the symbol $\langle . \rangle_p$ denoting average over the distribution p. Then the corresponding $I^*_{(q_1)}$, referred to I^*_1,

is the amount of stimulus information contained in the firing rate. Information contained by correlated activity can be obtained by the difference between I_{MI} and I_1^*.

2.6 Reliability of limited sampling

Estimating the mutual information using Eq. (2) with limited trials of data may cause a sampling bias problem (Panzeri and Treves 1996). To check this issue, the upper and lower bounds were checked for the mutual information calculated using the experimental data. The result obtained by Eq. (2), I_{MI}, is the upper bound of the true mutual information. A lower bound of the mutual information can be calculated by (Montemurro et al. 2007):

$$I_{sh} = I_{LB} + \Delta I_{sh}, \tag{7}$$

$$I_{LB} = -\sum_{\mathbf{r}} p(\mathbf{r}) \log_2 \sum_{s} p(s) q_1(\mathbf{r}|s) + \sum_{\mathbf{r}} \sum_{s} p(s) q_1(\mathbf{r}|s) \log_2 q_1(\mathbf{r}|s), \tag{8}$$

$$\Delta I_{sh} = I_{MI} + \sum_{\mathbf{r}} p(\mathbf{r}) \log_2 \sum_{s} p(s) q_1(\mathbf{r}|s) - \sum_{\mathbf{r}} \sum_{s} p(s) p_{sh}(\mathbf{r}|s) \log_2 p_{sh}(\mathbf{r}|s), \tag{9}$$

where $p_{sh}(\mathbf{r}|s)$ is the distribution of the shuffled neural responses across trials.

3 Results

Experiments were performed on isolated bullfrog retinas. In our experiments, transient ON-OFF cells (which can be classified as changing contrast detectors) were identified based on neuronal response properties during light ON and OFF transients (Maturana et al. 1960). In the present study, more than 90 % RGCs recorded were changing contrast detectors. Since these ON-OFF RGCs almost only fired in the first 200 ms of light ON and OFF transients, hereafter only the first 200-ms responses to light ON and OFF stimulations were taken for further analyses.

3.1 Single neuronal activity during exposure to different stimulus durations

We first examined the latencies of the ON and OFF response when the light OFF interval was fixed but light ON duration was changed (following protocol presented in Fig. 1a). A typical cell's activity during exposure to different light ON durations is shown in Fig. 2a. We defined the timing of the first spike after stimulation switch as the response latency in each trial (Gollisch and Meister 2008; Greschner et al. 2006). In the example neuron, the ON response latency was most of the time longer than that of the OFF response in all the three stimulus patterns (Fig. 2b). The average latencies of the ON and OFF responses of this neuron in 30 trials are listed in Table 1, and it is shown that only the OFF response latency tended to be shortened with the light ON duration. Statistical results about the latencies from 241 neurons in ten retinas suggested that only the OFF response latency, not the ON response latency, was decreased significantly when the light ON duration was increased (Fig. 2c; paired t-test, $p<0.05$).

It was reported that firing rate of neuron was often negatively correlated with response latency, i.e., firing rate was increased when response latency was decreased (Risner et al. 2010; Gawne et al. 1996; Gollisch and Meister 2008). In our present study, neuronal firing count of the OFF response was increased significantly when the light ON duration was increased (Table 1 and Fig. 2d; paired t-test, $p<0.05$), which was also opposite to the change of the response latency (Fig. 2c).

Previous studies have suggested that stimuli with various exposure durations can affect the response properties of neurons in sensory systems (Chen 1998; Mirpour and Esteky 2009). In the present study, different light ON durations could significantly affect response latency and firing rate of the cells' OFF responses (Fig. 2). At the mean time, we further looked whether different light OFF intervals would also induce any changes in the cells' ON responses.

It was observed that when protocol 2 (Fig. 1b) was performed, the ON response latency of an example neuron was obviously shortened when the light OFF interval was increased (Fig. 3a, b and Table 2). Statistical results about the latency from 159 transient ON-OFF RGCs in eight retinas showed that only the ON response latency was significantly decreased when the light OFF interval was increased (Fig. 3c; paired t-test, $p<0.05$). Meanwhile, neuronal firing count of the ON response was also significantly increased with longer light OFF intervals (Table 2 and Fig. 3d; paired t-test, $p<0.05$). Latency and firing count of the OFF response were not changed significantly (Fig. 3c and d).

3.2 Correlated activities and relative latency between RGCs during exposure to different stimulus durations

Response latency and firing rate of the OFF response were sensitive to different light ON durations and *vice versa*, which suggested that single ON-OFF RGC's activity can be modulated by the preceding stimulus. Concerted activity between

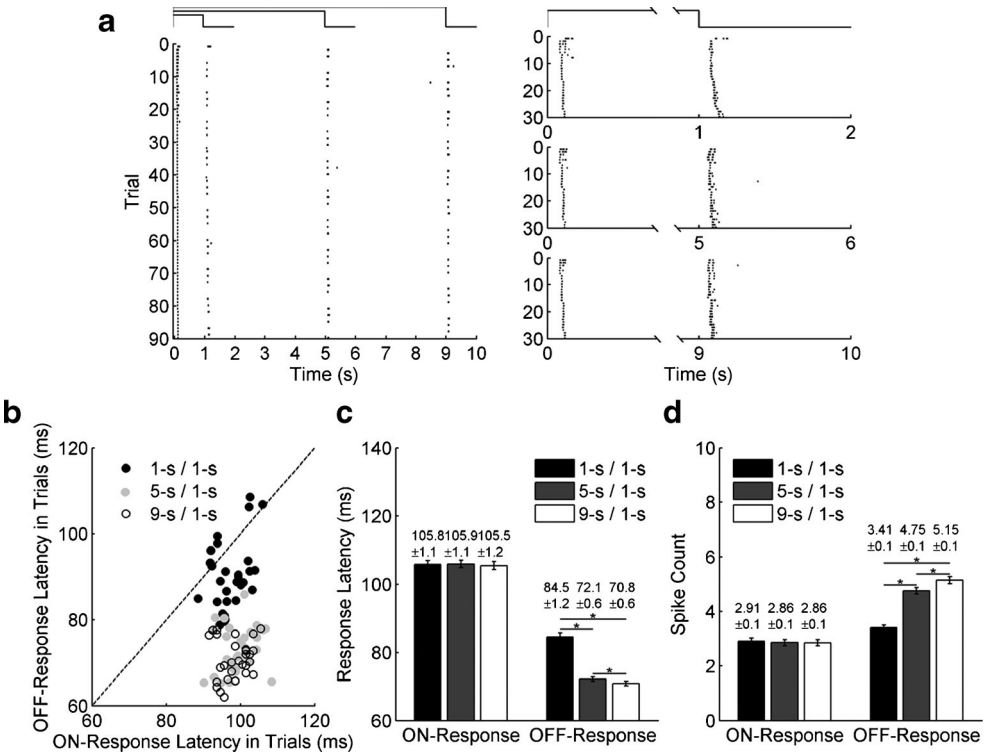

Fig. 2 Changes in latency and spike count when light ON duration was varied (protocol 1). **a** A typical cell's response to the stimulus protocol plotted in Fig. 1a. The *left panel* shows the cell's firing activities in all the 30 trials, the occurrence of each spike is represented by a *dot*; the *right panels* show the cell's response latencies during 1-s/1-s, 5-s/1-s and 9-s/1-s (ON/OFF) stimulus patterns. **b** Scatter plots of the ON and OFF response latencies of the example neuron during exposure to different light ON durations (30 trials). **c** and **d** Statistical results of the latencies and spike counts for the ON and OFF response during exposure to different light ON durations. n=241 cells from ten retinas, *error bars* indicate ± SEM and *p<0.05, paired t-test

neurons is also suggested to be important in carrying stimulus information (Gollisch and Meister 2008; Meister et al. 1995; Shlens et al. 2008). Therefore, the concerted activities of the cells transient ON and OFF responses in exposure to different stimulus durations were further analyzed.

In the present study, cross-correlation function between RGCs was computed separately for the cells' ON and OFF responses following Eq. (1) and then fitted by a Gaussian function (Fig. 4a and c), with correlation strength being quantified as the peak value of the fitted curve. For correlated activity, it was observed that the correlation strength of the OFF responses was increased significantly when the light ON duration was increased (Fig. 4a and b; n=1,342 neuron pairs from ten retinas; paired t-test, p<0.05), while the correlation strength of the ON responses was decreased along with the increment of the light OFF interval (Fig. 4c and d; n=989 neuron pairs from eight retinas; paired t-test, p<0.05).

These results suggest that correlated activity between neurons was also modulated by the preceding stimulus, but the influence of light ON and OFF durations on correlation strength between neurons was opposite, which might be due to the effect from both firing activity and network connectivity (Liu et al. 2007; de la Rocha et al. 2007).

In addition to correlated firings, it was also suggested that relative latency between neurons was an efficient way to transfer stimulus information to the brain (Gollisch and Meister 2008). The change of relative latency between correlated cell pairs with stimulus duration was further analyzed, with relative latency being defined as the difference in response latencies between neuronal pairs. For the correlated neuronal pairs, response latencies of one neuron are always longer than the other one when in exposure to different stimulus durations, so we used the neuron with longer latencies as the reference.

In exposure to different light ON durations, relative latencies between OFF responses of cell pairs with correlated activity were decreased significantly when the light ON duration was increased (p<0.05; n=1,342 cell pairs), while that of the ON responses were not changed obviously

Table 1 Response latency and spike count of the example neuron (Fig. 2a) in response to different light-ON durations (mean ± SEM, 30 trials)

Stimulation (ON/OFF)		1-s/1-s	5-s/1-s	9-s/1-s
Response latency (ms)	ON-Response	98.05±1.43	98.87±0.71	97.92±0.72
	OFF-Response	**91.04±2.14**	**72.87±0.92**	**70.15±0.91**
Spike count	ON-Response	2.07±0.16	1.99±0.13	1.98±0.09
	OFF-Response	**2.79±0.14**	**3.08±0.22**	**3.26±0.16**

Bold entries indicate response latency/spike count were significant changed with light-ON durations

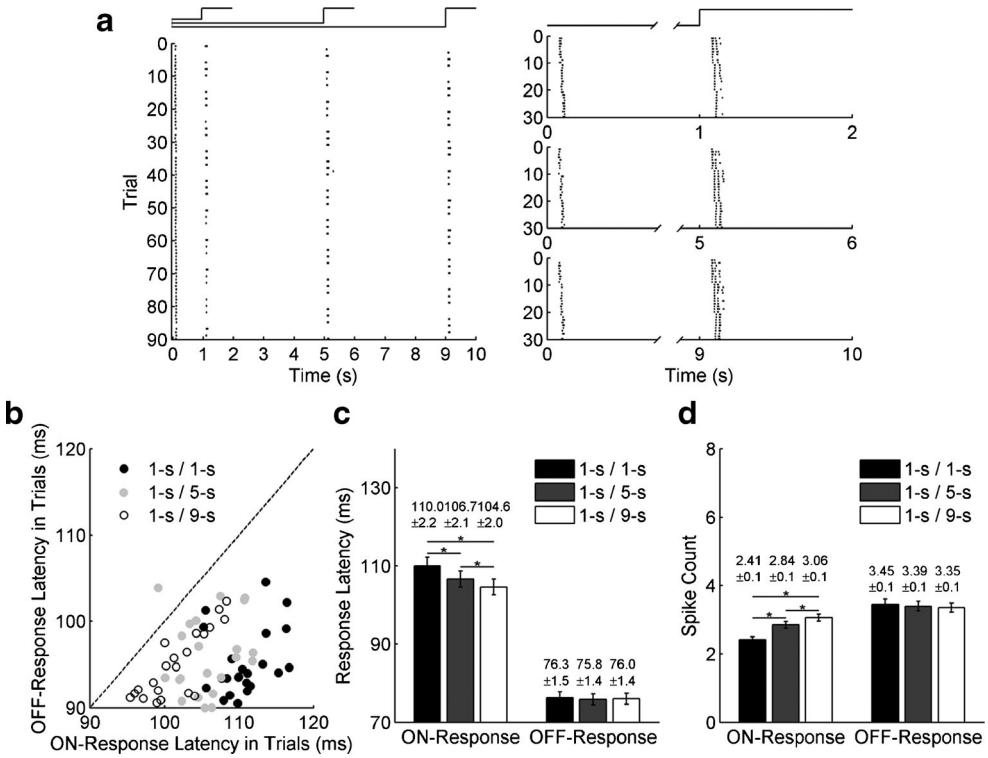

Fig. 3 Changes in response latency and firing count when light OFF interval was varied (protocol 2). **a** A typical cell's response to the stimulus protocol plotted in Fig. 1b. The *left panel* shows the neuronal activities in all the 30 trials, the occurrence of each spike is represented by a *dot*; the *right panels* show the cell's response latencies during 1-s/1-s, 1-s/5-s and 1-s/9-s (ON/OFF) stimulus patterns. **b** Scatter plots of the ON and OFF response latencies of the example neuron during exposure to different light OFF intervals (30 trials). **c** and **d** Statistical results of the response latencies and spike counts for the ON and OFF responses during exposure to different intervals of light OFF. n=159 cells from eight retinas, *error bars* indicate ± SEM, *p<0.05, paired t-test

(Fig. 5a). Meanwhile, when in exposure to different light OFF intervals, relative latencies between the ON responses of cell pairs with correlated activity were increased significantly when the light OFF interval was increased (Fig. 5b; p<0.05; n=989 cell pairs).

The change of relative latency of the OFF responses with light ON duration (Fig. 5a) was negatively correlated with the change of the correlation strength of the OFF responses (Fig. 4b), and similar results for the ON responses were observed in exposure to different light OFF intervals (Figs. 4d and 5a). These results suggest that pair-wise neuronal activity, including correlation strength and relative latency, can be modulated by the preceding stimulus and correlation strength is related with the relative latency between neurons.

3.3 Information encoded by ON and OFF responses

Given that RGCs' ON and OFF responses were separately modulated by the preceding light OFF and ON stimulations, with single neuronal activity and pair-wise neuronal response being modulated, we further considered about how much information the neural responses carry about different stimulus durations, which could be quantitatively measured by mutual information (Borst and Theunissen 1999).

Mutual information, including both information carried by firing rate and correlated activities, contained by the ON and OFF responses in exposure to different stimulus durations were computed based on the data recorded during the first 200-ms responses of the light ON and OFF transients of all the repeated trials (Eq. (2)). In exposure to different light ON durations, mutual information carried by the OFF response was significantly higher than that carried by ON response (Fig. 6a and b); at the mean time, mutual information carried by ON response was significantly higher than that carried by the OFF response in exposure to different light OFF intervals (Fig. 6c and d).

Neurons can carry stimulus information only if response variability is correlated with the stimulation parameters (Borst and Theunissen 1999). When elicited by stimulus with different

Table 2 Response latency and spike count of the example neuron (Fig. 3a) in response to different light-OFF intervals (mean ± SEM, 30 trials)

Stimulation (ON/OFF)		1-s/1-s	1-s/5-s	1-s/9-s
Response latency (ms)	ON-Response	**110.7±0.7**	**106.5±0.7**	**102.5±0.9**
	OFF-Response	96.6±0.9	96.1±0.9	95.8±0.8
Spike count	ON-Response	**2.55±0.23**	**3.07±0.15**	**3.59±0.18**
	OFF-Response	2.67±0.18	2.57±0.17	2.58±0.19

Bold entries indicate response latency/spike count were significant changed with light-OFF intervals

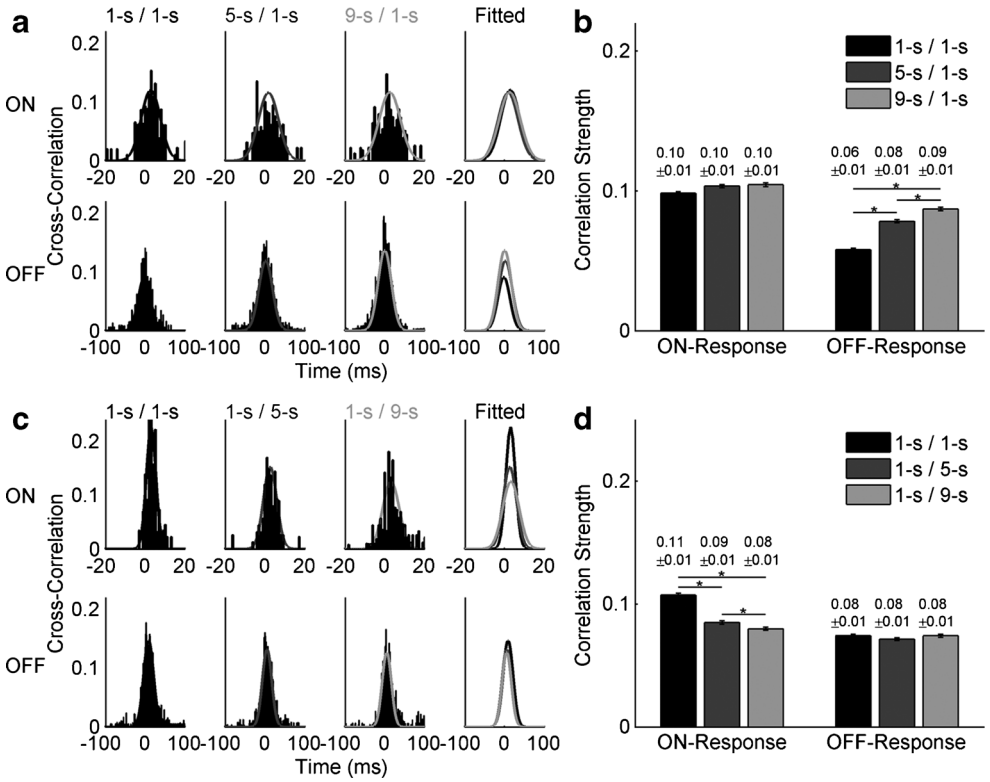

Fig. 4 Changes in correlation strengths during the cells' responses to different stimulus durations. **a** Correlation strengths of the ON and OFF responses of an example neuron pair during exposure to different light ON durations. **b** Statistical results of correlation strength in response to different light ON durations. n=1,342 neuron pairs from ten retinas. **c** Correlation strengths of the ON and OFF responses of an example neuron pair during exposure to different light OFF intervals. **d** Statistical results of correlation strengths for ON and OFF responses during exposure to different intervals of light OFF. n=989 cell neuron pairs from eight retinas. *Error bars* indicate ± SEM, *p<0.05, paired t-test

light ON durations, neuronal response characteristics were only changed significantly in the cells' OFF responses (Figs. 2 and 4), which was consistent with the result that the OFF responses significantly contained more mutual information; at the mean time, during exposure to different light OFF intervals, neuronal response characteristics were only changed significantly in the cells' ON responses (Figs. 3 and 4), and the information was more carried by the cells' ON responses.

3.4 Information carried by the firing rate and correlated activity

It was suggested that both single neuronal activity, such as spike count, and population activity, such as correlation strength, contained stimulus information (Averbeck et al. 2006; Pillow et al. 2008). In our present study, it was observed that both single neuron's activity and pair-wise neuronal activity were changed with stimulus duration, so we further calculated how much information is respectively carried by firing rate and correlated activity.

Based on a mismatch decoder model (Oizumi et al. 2010; Merhav 1994), mutual information was decomposed into the parts contained by firing rate and correlated activity between neurons (Eqs. (3–6)) (Xiao et al. 2013a).

In exposure to different light ON durations, the firing rate (Rate) and correlation (Cor) of the OFF response carried more information than the ON response (Fig. 7a and b). For the OFF response, the firing rate induced by different light ON durations contained more than 80 % (84.48±0.48 %, mean ± SEM) information about this stimulus property.

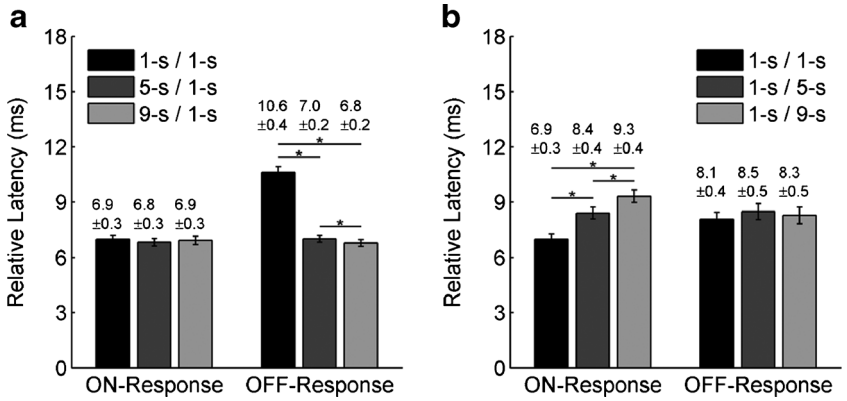

Fig. 5 Relative latencies between correlated cell pairs in exposure to different light ON durations (**a**) and different light OFF intervals (**b**). n=1,342 cell pairs from ten retinas when in exposure to different light ON durations and n=989 cell pairs from eight retinas when in exposure to different light OFF intervals. *Error bars* indicate ± SEM, *p<0.05, paired t-test

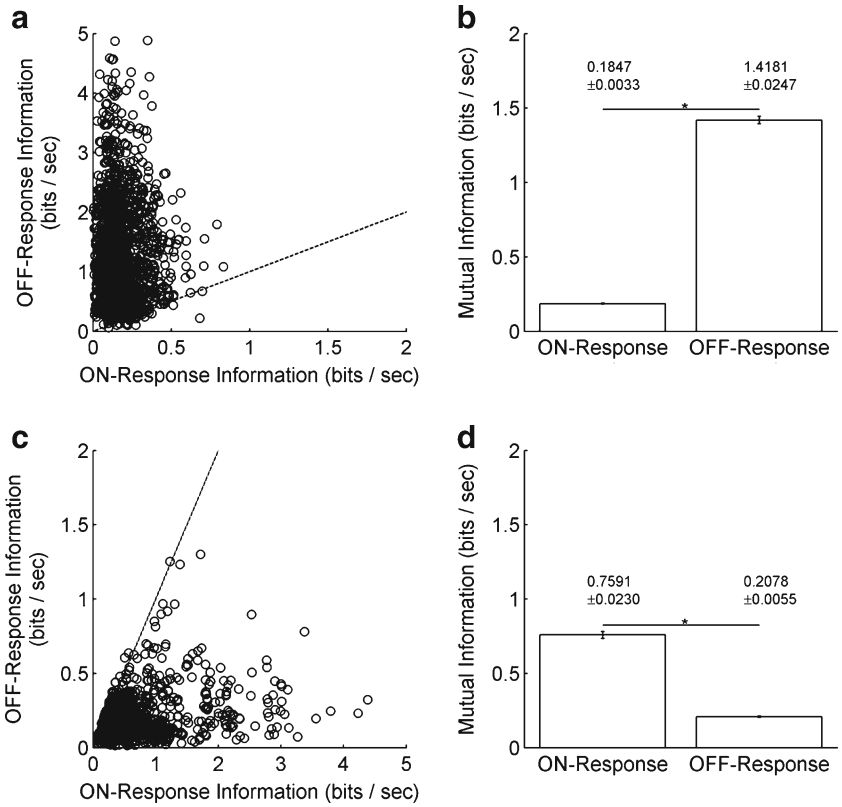

Fig. 6 Mutual information contained by the ON and OFF responses in exposure to different stimulus durations. **a** Scatter plots of mutual information contained by the ON and OFF responses in different light ON durations for 1,342 neuron pairs from ten retinas. **b** The average mutual information contained by the ON and OFF response in different light ON durations. *Error bars* indicate ± SEM, *$p<0.05$, paired *t*-test. **c** and **d** Scatter plots and average mutual information contained by the ON and OFF response in different light OFF intervals. $n=989$ cell neuron pairs from eight retinas, *error bars* indicate ± SEM, *$p<0.05$, paired *t*-test

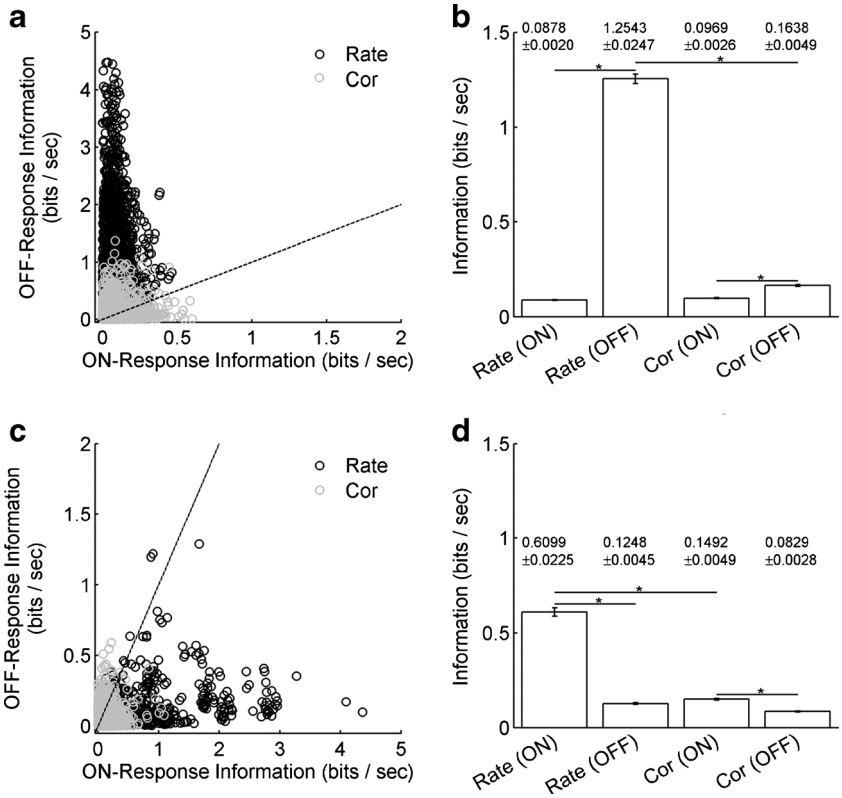

Fig. 7 Information contained by firing rate (Rate) and correlation (Cor) of the ON and OFF response in exposure to different stimulus durations. **a** Scatter plots of information contained by firing rate and correlated activity of the ON and OFF response in different light ON durations for 1,342 neuron pairs from ten retinas. **b** Statistical results of information contained by firing rate and correlation of the ON and OFF response in response to different light ON durations. **c** and **d** Scatter plots and statistical results of information contained by the ON and OFF response in response to different light OFF intervals. $n=989$ cell neuron pairs from eight retinas. *Error bars* indicate ± SEM, *$p<0.05$, paired *t*-test

At the mean time, in exposure to different light OFF intervals, the firing rate and correlation of the ON response carried more information (Fig. 7c and d). For the ON response, the firing rate also contained more than 80 % (80.26±1.79 %, mean±SEM) information about different light OFF intervals.

4 Discussions

In the present study, response latency, spike count, correlated activity, and relative latency of the ON and OFF responses during exposure to different stimulus durations were investigated in "changing contrast detectors" of the bullfrog retina. Our results suggested that the cells' OFF response characteristics were only modulated by the light ON duration (Figs. 2, 4, and 5), whereas the ON response characteristics were modulated by the light OFF interval (Figs. 3, 4, and 5). These results imply that ON-OFF RGCs are sensitive to different stimulus durations and RGCs' activities are affected by the preceding stimulus. Our results showed that information about different light ON durations was more carried by the OFF response and *vice versa* (Fig. 6). The contributions of firing rate and correlated activity in encoding different stimulus durations were further analyzed. The results showed that both of them contained information, but more than 80 % information was carried by the firing rate (Fig. 7), which indicates that RGCs may adopt rate coding to encode and transmit information about different stimulus durations.

4.1 The response dynamics of ON-OFF RGCs to different stimulus durations

Stimulus duration is an important index for almost all the sensory systems. In both auditory and olfactory systems, it has been reported that neuronal response latency and firing rate were modulated by different stimulus durations (Tanaka et al. 1992; Chen 1998; Firestein et al. 1993; Mirpour and Esteky 2009; Sachdev and Catania 2002). In our present study, it was also observed that response latency and firing rate of ON-OFF RGCs were influenced by different stimulus durations. Response latency and firing rate are basic response characteristics of single neurons, which are related with the neuronal sensitivity. RGC's response latency would be shortened and the firing rate would be increased when its sensitivity is increased. Prolonged light ON and OFF durations might increase the sensitivity of RGCs, which will elevate the responsiveness of RGCs. Since our present experiments were conducted on transient ON-OFF RGCs, all features of the ON response should be independent on the following light ON duration (and same should be true for the OFF response). Therefore, the ON response property measured during different ON duration should be taken as control (and same for the OFF response). We also analyzed the long-term effects of stimulus duration on RGCs' response properties, and found that different light ON durations had no obviously effect on the next ON response following the 1 s light OFF interval, and similar results were obtained when in exposure to different light OFF intervals (data not shown).

Concerted activity has been widely observed in sensory systems (Usrey and Reid 1999; Xiao et al. 2011; Shlens et al. 2008). Previous studies have revealed that in RGCs, concerted activity patterns were dynamically modulated by the activation status of the retinal neural network (Li et al. 2012; Schnitzer and Meister 2003). Generally, correlated activity between neuron pairs may arise from two sources, with one due to functional wiring of neural network, and another due to the co-variation of firing rates of neurons (Pauluis et al. 2001). In retinas, correlated activity due to functional wiring of neural network can be modulated by neuro-active chemicals, such as GABA and dopamine (Liu et al. 2007; Jing et al. 2010a; Li et al. 2012). A study on *in vitro* cortical neurons and theoretical analysis has also shown that correlated activity due to the co-variation of firing rates was strengthened with firing rate (de la Rocha et al. 2007).

In our present study, the correlated activity between RGCs' ON responses was weakened when the light OFF interval was extended, but the correlated activity between RGCs' OFF responses was strengthened when the light ON duration was prolonged (Fig. 4). Since firing rates of the ON response and responses were increased with the light OFF interval and ON durations respectively, we infer that the correlated activity of the OFF response may be primarily due to the co-variation of firing rates and the correlated activity of the ON responses may due to the functional wiring of neural network. This is in accordance with the notion that concerted activity between ON and OFF RGCs may be induced by different mechanisms (DeVries 1999; Xiao et al. 2011; Trong and Rieke 2008).

In cat visual cortex, it was suggested that correlation strength of pair-wise neurons was negatively correlated with the relative latency (Samonds and Bonds 2005). In bullfrog retina, it was also observed that cell pairs exhibited stronger correlation when their relative latencies were small. Comparing ON response with OFF response, it was found in the present study that correlation strength of the ON response was obviously stronger than that of the OFF response, and relative latency of the ON response was also shorter. These results further suggested that neuronal communications in ON and OFF pathway are asymmetric.

It was suggested that correlated activity due to the common inputs from bipolar cells via chemical synapses could be reflected by a cross-correlation function with a broader peak (40–100 ms) than that due to the common inputs from amacrine cells via electrical junctions (10–50 ms) (Brivanlou et al. 1998). In our present study, the time-lag distribution of the cross-correlation functions between the ON responses (about 20 ms; Fig. 4) was similar to that due to the common inputs from amacrine cells, and that between the OFF responses (more than

50 ms; Fig. 4) was similar to that due to the common inputs from bipolar cells. Signal transmission via electrical junctions is faster than via chemical synapses, which may make relative latency of the ON response shorter than that of the OFF response.

4.2 Single cell's firing rate and correlated activity in information encoding

Since mutual information measured using limited trials of data may cause a sampling bias problem (Panzeri and Treves 1996), in our present study, we compared the upper and lower bounds of the mutual information calculated using the experimental data (see Section 2, Eqs. (7–9)), and found that the discrepancy between upper and lower bounds was small (less than 5 %, data not shown), which confirmed the reliability of our data set for measuring mutual information.

In the present study, a mismatched model method (equivalent to a pair-wise interaction model when only two neurons are considered) was applied to assess the role of correlation on encoding information (Merhav 1994). As comparison, we also computed the information carried by correlation based on synergy information, and obtained similar results that more than 80 % information were carried by firing rate (data not shown). There is a standing debate on which information measure is more appropriate to quantify the information carried by correlation (Latham and Nirenberg 2005; Schneidman et al. 2003). Nevertheless, our analyses showed that these different measures give qualitatively similar results for the issues explored in this study.

Firing rate and correlated activity are important aspects of neuronal responses (Xiao et al. 2013a). However, their contribution for information coding is still under debating. For retinal information coding, some studies suggested that RGCs are almost independent to each other in encoding and transmitting information, with more than 90 % information carried by firing rates of single neurons (Nirenberg et al. 2001; Oizumi et al. 2010). On the other hand, concerted activity has been widely observed among visual neurons, and it was also reported that concerted activity encoded about 20 % stimulus information, in addition to the information encoded by firing rates of single neurons (Pillow et al. 2008; Schnitzer and Meister 2003).

In our present study, both firing rate and correlated activity were dynamically modulated by different stimulus durations, which may suggest that both aspects could contribute to the RGCs information coding. By decomposing the stimulus information into the parts carried by firing rate and correlated activity, the results showed that firing rate carried more than 80 % information about the external stimuli. These results suggested that firing rate of single RGC' activity played crucial role in encoding different stimulus durations, although correlated activity also contributed to information encoding.

Many experimental studies have also suggested that single and population neuronal activities can encode different stimulus patterns. A recent study on rabbit RGCs has shown that firing rate would decrease when stimulus edge contrast was decreased, and the change of latency was opposite (Risner et al. 2010). In turtle retina, ON response latency of ON-OFF RGCs was decreased with light intensity and the change of OFF response latency was opposite (Thiel et al. 2006), and the instantaneous firing rates of RGCs were also demonstrated to encode multiple aspects of visual motion, such as speed and direction (Thiel et al. 2007). On the other hand, some studies have revealed that population activities, such as relative latency between neurons and concerted activity, are more effective in encoding spatial structure of stimuli (Jing et al. 2010b; Gollisch and Meister 2008). Our recent study showed that the encoding strategy of RGCs was shifted during the luminance adaptation: from firing rate to neural correlation (Xiao et al. 2013b). So we infer that RGCs' encoding strategy is dynamically changed with external conditions. At the mean time, single neuronal activity and population neuronal activity are not independent of each other, and they can mutually modulated which can to some extent improve information coding (Liu et al. 2011).

In natural environments, visual stimuli are complex and usually contain both spatial and temporal variables (Lesica et al. 2008). Because artificial stimuli are easy to design and control, in laboratory the response properties of neurons are usually studied using simple artificial stimuli such as full-filed flashes, bars, and sinusoidal gratings, etc. (Jing et al. 2010a; Ishikane et al. 2005; Gollisch and Meister 2008). Neuronal activity in response to artificial stimulation is not completely equal to that in natural environment, but some basic response characteristics of neurons can be studied based on the artificial stimuli (Mante et al. 2008; Rieke and Rudd 2009). In the present study, we focused on the encoding strategy of transient ON-OFF RGCs to stimulus duration, and our results may be served as a reference when studying the coding strategy in natural environments.

5 Conclusion

In bullfrog retina, ON-OFF RGCs' activities could be modulated by the preceding stimulus, i.e., the cells' ON response characteristics were modulated by different light OFF intervals and *vice versa*. These results imply that ON-OFF RGCs are sensitive to different stimulus durations and RGCs' activities are affected by the preceding stimulus. Our study also suggests that RGC's firing rate plays a crucial role in encoding stimulus durations.

Acknowledgments We thank Mr. Xin-Wei Gong and Hai-Qing Gong for important technical assitance. This work was supported by grants from National Foundation of Natural Science of China (No. 61075108,

P.J.L.; No. 61375114, P.M.Z; No. 91132702, S.W.) and Graduate Student Innovation Ability Training Special Fund of Shanghai Jiao Tong University (L.X.).

Conflict of interest The authors declare that they have no conflict of interest.

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