Influence of GABAergic inhibition on concerted activity between the ganglion cells

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In this study, the spike discharges of one subtype of bullfrog retinal ganglion cells (dimming detectors) in response to repetitive full field light-OFF stimuli were recorded using multi-electrode arrays. Two different types of concerted activity (precise synchronization and correlated activity) could be distinguished. The nearby cells with overlapped receptive field areas often fired in synchrony, whereas the correlated activity was mainly observed from remote cell pairs with separated receptive fields. After the bicuculline application, the strength of the synchronized activity was increased whereas that of the correlated activity was decreased. These results suggest that the activation of $GABA_A$-receptor-mediated inhibitory pathways differentially modulates the concerted firing of the ganglion cells. *NeuroReport* **21:797–801 © 2010 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Introduction

Concerted activity is one of the important ways for retinal ganglion cells to efficiently organize and process visual information, with the concerted pattern dependent on a particular structure of the neuronal circuitry – the formation of synchronization (with time lags in cross-correlation function distributed within 1 ms) is attributed to gap junctions between the ganglion cells, whereas the correlated activity (with time lags in cross-correlation function distributed between 10 and 100 ms) results from common input from other neurons (amacrine/bipolar cells) [1,2]. To date, the contribution of inhibitory GABAergic input on the formation of concerted firing in ganglion cells remains unclear. Two possible changes of concerted activity may occur when the firing activity of ganglion cells is inhibited by the GABAergic input. One is that the strength of the concerted activity (quantified by the peak value of cross-correlation function between the pair-wise firing sequences) is weakened [2,3], the other is that the strength of concerted activity is increased while the neurons' uncorrelated firing is inhibited [4–6].

In the frog retina, ganglion cells can be classified into four subtypes based on their response properties: dimming detectors, sustained contrast detectors, net convexity detectors and moving-edge detectors [7]. Light-OFF stimuli evoke OFF-sustained spike discharges in the dimming detectors but elicit only a few transient spike discharges in other cell subtypes [8,9]. In this study, it was observed that nearby dimming detectors with overlapped receptive field areas often fired in synchrony, while remote cells were mainly occupied in correlated activity. Application of $GABA_A$-receptor antagonist bicuculline (BIC) significantly decreased the strength of correlated activity while the cells' firing rates were increased; but the strength of synchronized activity was increased.

Materials and methods

Electrophysiology recording

Extracellular recordings were made in isolated frog retina using the coplanar multi-electrode arrays (MEA, MMEP-4, CNNS UNT, USA) which consisted of 64 electrodes (8 μm in diameter) arranged in an 8×8 matrix (covering an area of $1.05 \times 1.05\,mm^2$) with 150 μm tip-to-tip distances between the nearest electrodes. All procedures strictly conformed to the humane treatment and use of animals as prescribed by the Association for Research in Vision and Ophthalmology. Frogs were dark adapted for at least 30 min before experiment. Under dim red light, the frog's eyes were enucleated and the retina was isolated. A small piece ($4 \times 4\,mm^2$) of retina was placed on the MEA with the ganglion cell side contacting the electrodes and superfused with oxygenated (95% O_2 and 5% CO_2) standard solution, which contained (in mM): NaCl 100.0, KCl 2.5, $MgCl_2$ 1.6, $CaCl_2$ 2.0, $NaHCO_3$ 25.0, glucose 10.0. In pharmacological experiments, 10 μM (–)-Bicuculline methiodide (BIC, Sigma, St. Louis, Missouri, USA) was applied with the Ringer's solution as desired.

Ganglion cells' firing activities were recorded by the MEA and the signals were amplified through a 64-channel amplifier (MEA workstation, Plexon Inc., Texas, USA). Signals from all available channels along with the stimulus were recorded with sampling rate of 40 kHz for each individual channel and stored. Spikes from individual neurons were sorted using the spike-sorting unit in the commercial software OfflineSorter (Plexon Inc.).

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Stimulus

Light stimulus was generated from a computer monitor (Iiyama, Vision Master Pro 456, Japan) and was focused to form a $1.1 \times 1.1\,mm^2$ image on the isolated retina through a lens system. Full-field sustained white light ($38.9\,nW/cm^2$) was given for 30 s before the stimulation protocols were applied, the purpose of which was to adjust the sensitivity of the ganglion cells to similar levels. The stimulation protocols were: (i) pseudo-random checker-board consisted of 16×16 sub-squares (frame refresh rate = 20 Hz), each sub-square covered an area of $66 \times 66\,\mu m^2$ on the retina and was assigned a value either '+1' (white light, $77.7\,nW/cm^2$) or '−1' (dark) following a pseudo-random binary sequence; (ii) repetitive full field light-OFF stimuli with duration of 9 s were given repeatedly for 30 times.

Measure the receptive field profiles of the ganglion cells

The spike triggered average algorithm was applied, and the profile of the spatial receptive field was fitted with a two-dimensional Gaussian distribution [10].

Cross-correlation analysis

The concerted firing between the neuron pairs were analyzed using cross-correlation function. To determine the concerted activity because of the wiring of neural circuitry, the shift predictor was calculated [6,11,12]. The concerted activity strength was quantified as the peak value of the cross-correlation function. The synchronized activity can be identified when the peak width is less than 1 ms. All values are presented as mean ± SD. Paired *t*-test was applied, and statistical significance was defined as $P < 0.05$.

Results

Relationship between inter-cellular distance and the concerted activity pattern

The distances between any two dimming detectors' receptive field centers were measured. Figures 1a and b are examples showing the receptive field profiles of the two neurons in one piece of retina. The distance between the receptive field mass centers was 334.6 μm.

Two types of concerted pattern among dimming detectors were identified. Figure 1c shows an example of synchronized activity between two adjacent cells with overlapped receptive fields (distance = 61.8 μm). Figure 1d shows an example of correlated activity between the two remote neurons with separated receptive fields (distance = 365.0 μm). Further analysis performed on 298 pairs of neurons recorded from two retinas showed that the concerted pattern was related to the distance between the neurons' receptive field centers. Figure 1e gives the summarized results. Among the neuron pairs under investigation, 90 pairs were with short inter-center distances (less than 250 μm), among which 62 pairs had overlapped receptive fields. For these closely neighbored pairs, synchronization was the major component. For other 208 cell pairs with longer inter-center distance (larger than 250 μm), only 17 pairs had overlapped receptive fields. For these remote neuron pairs, correlated activity was generated more frequently.

Effects of BIC on concerted activities

To investigate the role of $GABA_A$-receptor-mediated inhibitory pathways in the formation of concerted activity, we blocked $GABA_A$ receptors using 10 μM BIC. Figure 2A shows the recordings obtained from two adjacent ganglion cells (distance = 182.5 μm, with overlapped receptive fields) before, during and after BIC application. The firing rates of these two cells were low in normal Ringer's solution [Fig. 2A (a) and (b)]. The cross-correlation function given in Fig. 2A (c) represents synchronization (peak value = 0.065). When BIC was applied, the firing rates were increased [Fig. 2A (d) and (e)] and the strength of the synchronized activity were also increased [peak value = 0.12, Fig. 2A (f)]. After BIC wash-out, the neurons' responses were basically recovered [Fig. 2A (g) and (h)], and strength of the synchronization became weak again [peak value = 0.092, Fig. 2A (i)]. Figure 2B shows the recordings obtained from two remote ganglion cells (distance = 424.2 μm, with separated receptive fields) before, during and after BIC application. The firing rates of these two cells were low in normal Ringer's solution [Fig. 2B (a) and (b)]. These two neurons' activities were correlated [peak value = 0.040, Fig. 2B (c)]. BIC application increased the firing rates [Fig. 2B (d) and (e)], however, the correlation strength was decreased [peak value = 0.023, Fig. 2B (f)]. After BIC wash-out, the neurons' responses were basically recovered [Fig. 2B (g) and (h)], and the correlation strength was also recovered [peak value = 0.036, Fig. 2B (i)].

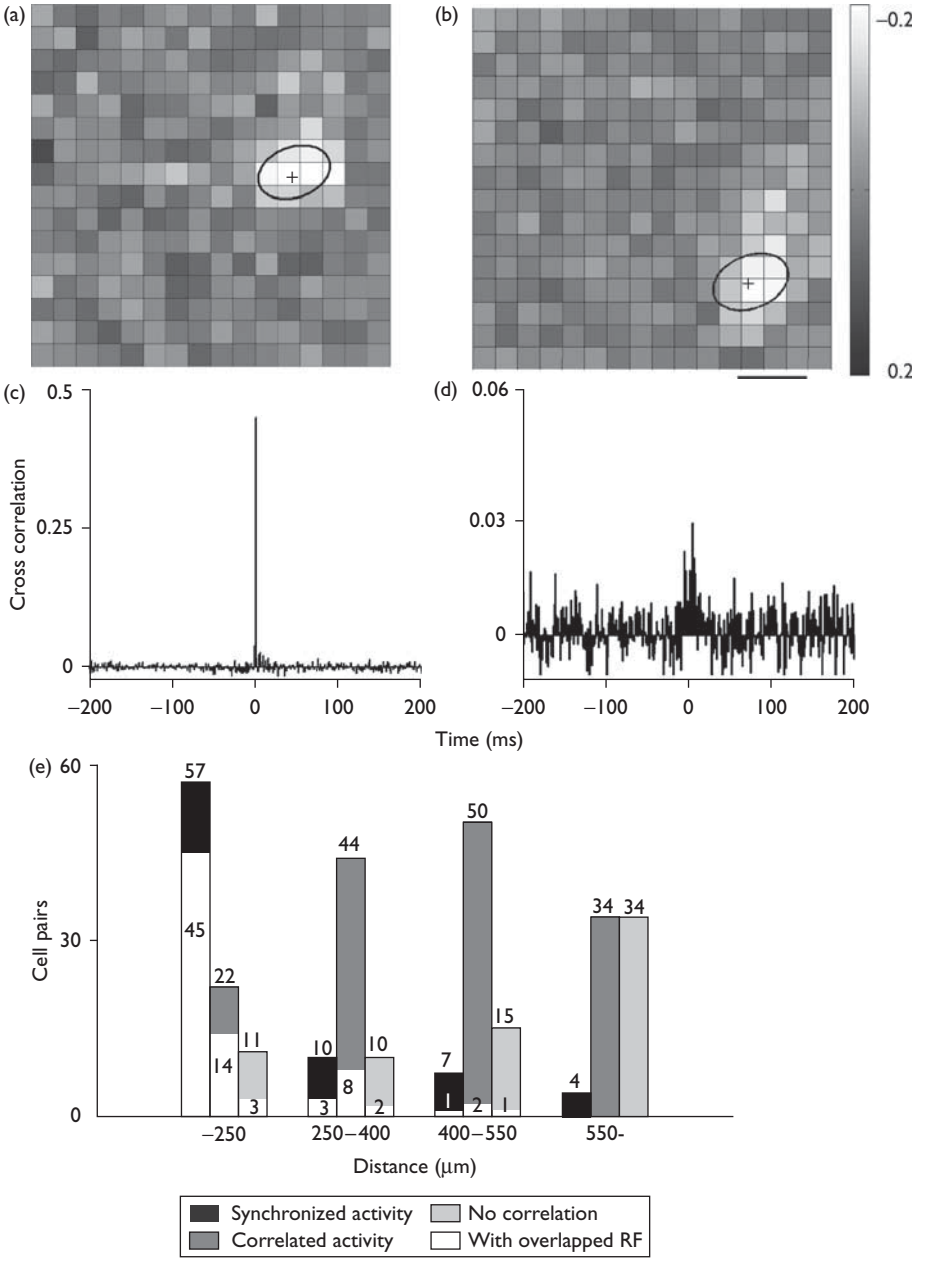
The histograms of the distributions of the correlation strength changes (including synchronized and correlated activity) during BIC application, as compared with control, are plotted in Fig. 3a and b. The results show that the distributions are both mono-modal. When BIC was applied, the BIC mainly resulted in increase of synchronization strength (133.5% ± 63.7%, $P < 0.05$, 78 pairs), while the BIC mainly resulted in decrease of correlated activity (74.2% ± 41.9%, $P < 0.05$, 150 pairs).

Taken together, these results reveal that (i) nearby neurons with overlapped receptive fields tended to fire in synchrony, whereas remote cells with separated receptive fields were more frequently occupied in correlated activity and (ii) during BIC treatment, the neurons' firing rates were increased, the strength of the synchronized activity was mainly increased, but that of correlated activity was mainly decreased.

Discussion

Our results show that in frog retina, nearby dimming detectors with overlapped receptive fields often fired in

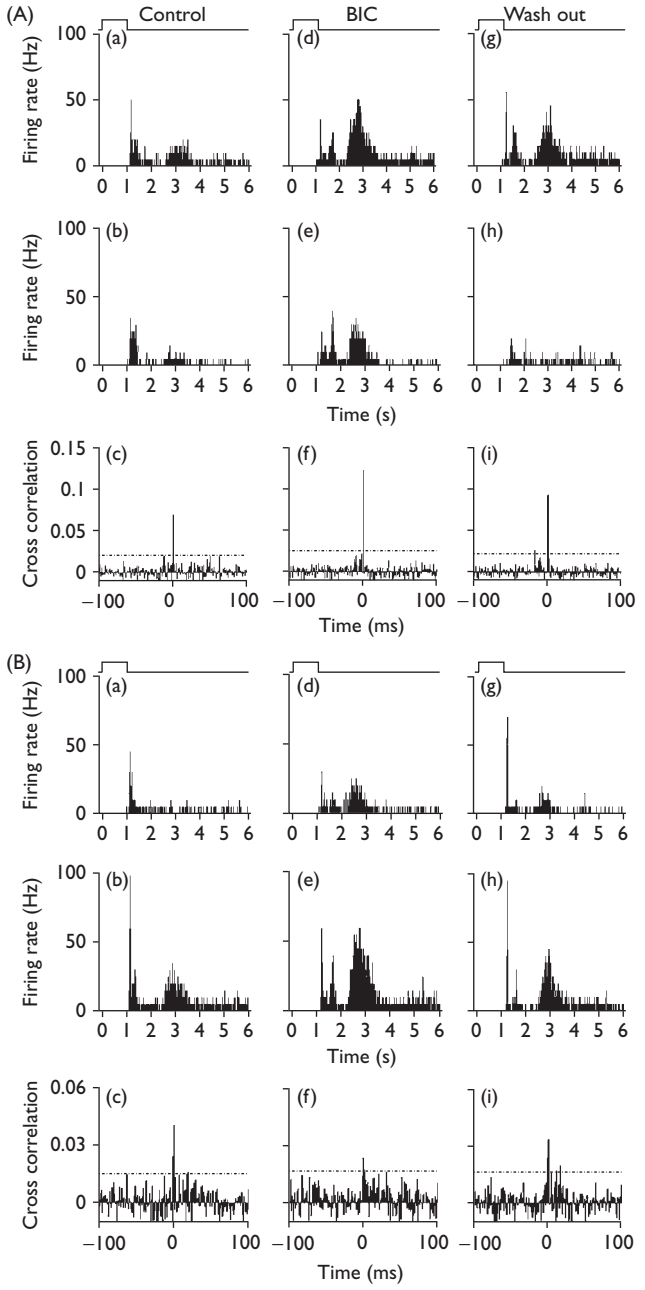
Fig. 1



(a and b) The receptive field (RF) profiles of two example dimming detectors. The cross indicates the mass center of the neuron's RF; 1-SD boundary is also indicated (scale bar, 200 μm). (c and d) Examples of synchronized and correlated activity respectively. (e) Relationship between the inter-center distance and the concerted patterns.

synchrony, whereas the correlated activity with separated receptive fields was predominantly recorded from remote cell pairs. In the retina, there are extensive gap junctions between ganglion cells, which can lead neurons to activate each other reciprocally. Such interactions were mainly observed from cell pairs whose dendritic fields overlapped [13,14], which supports that synchronized activity is generated through the gap junctions formed between dendrites of the two neurons. Our results confirmed that dimming detectors firing in synchrony often had overlapping receptive fields (among 78 synchronized pairs, 49 neuron pairs had overlapped receptive fields, see Fig. 1e). Although synchronization could sometimes form between the two remote neurons, this might be mediated by a third neuron located between the recorded neuron pair and providing them with excitatory signals through the gap junctions. Correlated pairs have been suggested to be driven by

Fig. 2



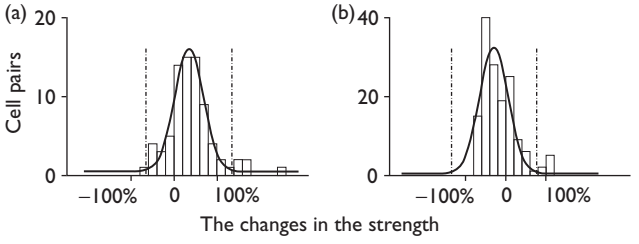
Bicuculline effect on the concerted activity. The PSTHs (A and B, top and middle rows, bin width 5 ms, 30 repeats) and cross-correlation functions (A and B, bottom rows, bin width 1 ms) were calculated before (A and B, left columns), during (A and B, middle columns) and after (A and B, right columns) BIC application. The dash lines show 'mean + 3SD' boundary (A and B, bottom rows). The upper trances in a, b and g (A and B) illustrate the time course of the light-OFF stimulus.

shared excitatory input from bipolar and/or amacrine cells [1,4], and modulated by common inhibitory input from amacrine cells [4–6]. Our data show that the correlated activity were basically observed from two remote neurons with separated receptive fields (only 24 pairs had overlapped receptive fields among 150 correlated pairs, see Fig. 1e), which is compatible with the previous reports that correlated activity were driven by common chemical input to the neuron pair.

In most neuron pairs invested in our experiment, the concerted activity could be effectively regulated by $GABA_A$ receptor antagonist BIC. During BIC application, the strength of the synchronized activity was mostly increased whereas the strength of correlated activity was basically down-regulated, which suggests that the activity of the GABAergic pathways regulates the synchronized activity and correlated activity through different mechanisms. Although there are quite a portion of neuron pairs showing opposite changing tendencies during BIC application, the histograms plotted in Fig. 3 shows that the distributions of the strength changes are both monomodal which could reflect a single dominant mechanism for each case. Figure 3 also shows that both the distributions have a longer tail at the right side. One explanation could be that while relative change is defined as $(y_1 - y_0)/y_0$, the increase of the strength could have a wide range, but the decrease range has a lower limitation at -100%, since in our case, $y \geq 0$.

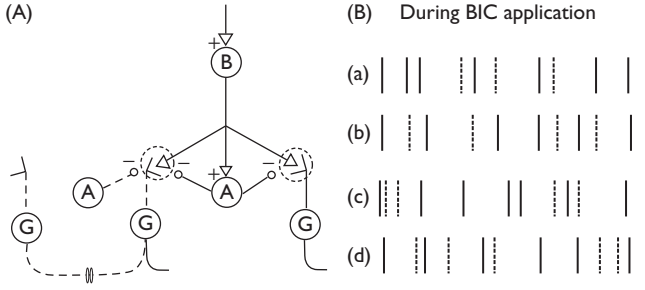
Neighboring ganglion cells' firings in synchrony are connected by gap junctions sensitive to the voltage difference across the junctional membrane [14–16]. Activation of the GABAergic pathways decreases the neuron's firing activity, and thus decreases the gap junction permeability, which results in a reduction in synchronized activity (Fig. 4A, the part indicated by dash lines). In our experiment, the neurons' firing rates were increased during BIC application. Such excitatory changes were propagated to the neighboring neurons through the gap junctions, which resulted in an increase in the strength of the synchronized activity [Fig. 4B (a) and (b)]. On the other hand, the correlated pairs share common input from bipolar and/or amacrine cells. During BIC application, the neurons firing rates increased, but the strength of correlated activity was significantly decreased (Fig. 4A, indicated by solid lines). It might suggest that the

Fig. 3



The changes in the strength of concerted activity during BIC application as compared to control. (a and b) Synchronized and correlated activity, respectively. Solid curves are Gaussian fittings to the histograms. The vertical dashed lines in (a) and (b) show 'mean ± 3SD' boundaries.

Fig. 4



$GABA_AR$-mediated modulatory effect on concerted activity. (A) Activation of the GABAergic pathways decreases the synchronized activity and increases the correlated activity. (B) Possible firing changes in synchronized activity (a and b), and correlated activity (c and d), during control and BIC application. Solid bars represent the firing activities during control and dashed bars represent the increased spikes during BIC application.

activation of $GABA_A$ receptors could suppress uncorrelated and comparatively weak signals from the common input [Fig. 4B (c) and (d)]. Earlier studies of our laboratory show that the GABAergic input exerts significant inhibitory effect on the uncorrelated firing activity in chicken ganglion cells; however, the correlated spikes remain less affected [5,6].

In frog retina, the activation of $GABA_A$-receptor-mediated inhibitory pathways suppresses the synchronized spikes formed through the gap junction. Such regulation might serve to prevent the network from being saturated. Meanwhile, it reduces the uncorrelated activity but allows for the correlated spikes. Such regulation makes the network efficient and energy-saving.

Conclusion

In frog retina, nearby ganglion cells often fired in synchrony, while correlated activity were mainly recorded from remote pairs. Concerted activity could provide higher transmission efficiency with the modulation of $GABA_A$-receptor-mediated inhibitory pathways.

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References

1 Brivanlou IH, Warland DK, Meister M. Mechanisms of concerted firing among retinal ganglion cells. *Neuron* 1998; **20**:527–539.
2 Meister M, Lagnado L, Baylor DA. Concerted signaling by retinal ganglion cells. *Science* 1995; **270**:1207–1210.
3 Amthor FR, Tootle JS, Grzywacz NM. Stimulus-dependent correlated firing in directionally selective retinal ganglion cells. *Vis Neurosci* 2005; **22**:769–787.
4 DeVries SH. Correlated firing in rabbit retinal ganglion cells. *J Neurophysiol* 1999; **81**:908–920.
5 Liu X, Zhang YY, Gong HQ, Liang PJ. The GABA(A) receptor-mediated inhibitory pathway increases the correlated activities in retinal ganglion cells. *Sheng Li Xue Bao* 2009; **61**:99–107.
6 Liu X, Zhou Y, Gong HQ, Liang PJ. Contribution of the GABAergic pathway(s) to the correlated activities of chicken retinal ganglion cells. *Brain Res* 2007; **1177**:37–46.
7 Lettvin JY, Maturana HR, McCulloch WS, Pitts WH. What the frog's eye tells the frog's brain. *Proc Inst Radio Eng* 1959; **47**:1940–1951.
8 Ishikane H, Gangi M, Honda S, Tachibana M. Synchronized retinal oscillations encode essential information for escape behavior in frogs. *Nat Neurosci* 2005; **8**:1087–1095.
9 Ishikane H, Kawana A, Tachibana M. Short- and long-range synchronous activities in dimming detectors of the frog retina. *Vis Neurosci* 1999; **16**:1001–1014.
10 Devries SH, Baylor DA. Mosaic arrangement of ganglion cell receptive fields in rabbit retina. *J Neurophysiol* 1997; **78**:2048–2060.
11 Perkel DH, Gerstein GL, Moore GP. Neuronal spike trains and stochastic point processes. II. Simultaneous spike trains. *Biophys J* 1967; **7**:419–440.
12 Pauluis Q, Baker SN, Olivier E. Precise burst synchrony in the superior colliculus of the awake cat during moving stimulus presentation. *J Neurosci* 2001; **21**:615–627.
13 Hidaka S, Akahori Y, Kurosawa Y. Dendrodendritic electrical synapses between mammalian retinal ganglion cells. *J Neurosci* 2004; **24**:10553–10567.
14 Bloomfield SA, Völgyi B. The diverse functional roles and regulation of neuronal gap junctions in the retina. *Nat Rev Neurosci* 2009; **10**:495–506.
15 Srinivas M, Costa M, Gao Y, Fort A, Fishman GI, Spray DC. Voltage dependence of macroscopic and unitary currents of gap junction channels formed by mouse connexin50 expressed in rat neuroblastoma cells. *J Physiol* 1999; **517**:673–689.
16 Spray DC, Harris AL, Bennett MV. Voltage dependence of junctional conductance in early amphibian embryos. *Science* 1979; **204**:432–434.