

## Chicken retinal ganglion cells response characteristics: multi-channel electrode recording study

CHEN Aihua (陈爱华)<sup>1</sup>, ZHOU Yi (周 艺)<sup>2</sup>, GONG Haiqing (龚海庆)<sup>2</sup>  
& LIANG Peiji (梁培基)<sup>2</sup>

1. Shanghai Institute of Physiology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, China;

2. Department of Biomedical Engineering, Shanghai Jiaotong University, Shanghai 200030, China

Correspondence should be addressed to Liang Peiji (email: pjliang@sjtu.edu.cn)

Received October 5, 2002

**Abstract** The first stage of visual processing occurs in the retina, the function of which is to process the raw information obtained from the outside world. In the present study, the electrical activities of a group of retinal ganglion cells were recorded from a small functioning piece of retina, using multi-electrode array (MEA), and the action potentials were detected by applying nonlinear algorithm. By analyzing the ensemble retinal ganglion output characteristics, it is revealed that both firing rates and correlated activity between adjacent neurons in the retina contribute to visual information encoding.

**Keywords:** retina, ganglion cells, multi-unit recording.

**DOI:** 10.1360/02yc0053

A fundamental question in neuroscience is how the information relevant to behavior is presented in the activity of neurons<sup>[1]</sup>. The visual system, especially the retina, offers some advantage to explore the neural code owing to its explicitly layered structure and relatively simple neuron types<sup>[2]</sup>.

However, most of what we know about retinal signaling is derived from single neuron recordings<sup>[2,3]</sup>. The assumptions underlying this approach are that individual neuron acts as a unique element dedicated to a unique process, and the firing of each type of neuron in the population depends only on the stimulus, but not necessarily the activity of other neurons, so the neural coding can be understood from the analysis of single cell responses. Although the classical methods have been very helpful for understanding the cellular and molecular mechanisms underlying relevant processes, it recently became clear that information processing in the brain is based on cooperation and integration of relevant neurons<sup>[4]</sup>. A series of studies carried out by Meister et al. suggested that the nerve impulses in the population of retinal ganglion cells collectively encode the visual stimulus<sup>[5-9]</sup>. It has also been suggested that correlated firing of the retinal ganglion cells may play a crucial role in forming appropriate, ordered connections to a target structure during development<sup>[10]</sup>. Moreover, the correlation was found as having quickly changing properties during information processing. Neurons in the retina and the lateral geniculate nucleus (LGN) can associate rapidly into different functional groups in response to various

stimulus conditions<sup>[11]</sup>. Thus, in order to understand coding, decoding and information processing that occur in the retina, recently developed techniques, such as multi-site extracellular electrode arrays, have been employed<sup>[7-9]</sup>. In the present study, a multi-electrode data acquisition system was employed to make simultaneous recording from the retinal ganglion cells. Relevant spike sorting algorithm was explored, so as to detect the neural activity, while the background noise is relatively high. Correlated activities between adjacent neurons were analyzed to compare the activity pattern during spontaneous firing and light elicited firing of the neurons investigated.

## 1 Methods

### 1.1 Experimental procedure

Extracellular ganglion cell recording was made in the isolated newly hatched (about 2—4 days) chicken retina with multi-electrode array (MEA, Multi Channel Systems MCS GmbH, German) (fig. 1(a)), which consisted of a 5 cm × 5 cm transparent quartz glass plate with 60 substrate integrated and insulated golden connection lanes running from connector pads at the edge of the plate to the electrode matrix at the center. The tips of these conductors serve as the electrodes (10 μm in diameter) and are arranged in an 8 × 8 matrix with 100 μm distance from tip to tip (fig. 1(b))<sup>[12,13]</sup>. The patterned area of microelectrodes is enclosed in a glass ring forming the tissue perfusion chamber.

After the enucleation of the eye, the eyeball was hemisected with fine scissors or a razor blade, separating the cornea and lens from the posterior half. The vitreous that adhered to the retinal surface was removed with tweezers. The retina was dissected into 4 mm × 4 mm squares. Finally, retinal segments were attached with the ganglion cell side to the surface of multi-electrode array. For improving the adhesion, the MEA was initially covered with 3 μL dissolved cellulose nitrate (1 cm<sup>2</sup> of Sartorius cellulose nitrate filter in 10 mL methanol) and dried in air. The preparation was kept in standard perfusate containing (in mmol/L): 100 NaCl, 5 KCl, 3 MgSO<sub>4</sub>, 1.8 CaCl<sub>2</sub>, 25 NaHCO<sub>3</sub>, 25 glucose, and bubbled with a mixed gas of 95% O<sub>2</sub> and 5% CO<sub>2</sub> with a pH value of 7.5 ± 0.2. The perfusion was delivered at a rate of 4.5 mL/min and the tissue was kept at 37 °C with a temperature control unit (Thermostat HC-X, Multi Channel Systems MCS GmbH, German). An Ag/AgCl pellet, which was dipped into the bath solution, acted as the reference electrode.

Light stimulus was generated using a computer monitor, and was focused into a 0.7 mm × 0.7

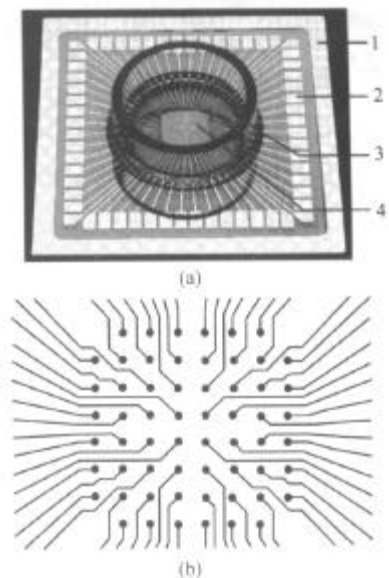


Fig. 1. The multi-electrode array. (a) The microelectrode array: 1, the quartz glass; 2, the connecting pads; 3, the glass ring; 4, the patterned area containing the electrodes. (b) The detailed arrangement of electrode in the array.

mm image on the isolated retina via a lens system. The stimuli in this experiment consisted of 500 ms full field, uniformly illuminated white flashes followed by 500 ms ‘light-off’ periods. The intensity of white light was  $0.139 \mu\text{W}/\text{m}^2$ .

Multi-unit photoresponses were simultaneously recorded from all 60 electrodes, which were amplified with a 60-channel amplifier (single-ended amplifiers, bandwidth 10 Hz–3.4 kHz, amplification  $1200 \times$ , amplifier input impedance  $> 10^{10} \Omega$ , output impedance  $330 \Omega$ )<sup>[14]</sup>. The selected channels of recording along with one stimulus signal were digitized with a commercial multiplexed data acquisition system (MCRack) and stored in a Pentium-based computer. MCRack sampled the incoming data at 10 kHz, plotted the waveforms on the screen, and stored the record for off line analysis.

## 1.2 Signal analysis

Usually, the electrophysiological recordings with planar MEAs exhibit a relatively low signal amplitude (between 0.01 and 0.1 mV) and low signal-to-noise ratio<sup>[15]</sup>. Moreover, the spectral characteristics of the noise and the signal are so similar with each other that application of conventional bandpass filtering is not able to separate the spikes from the noise.

In this study, single unit classification was accomplished by performing a nonlinear algorithm to the data<sup>[16]</sup>, and utilizes the instantaneous frequency and amplitude information simultaneously for the detection of action potential, which results in an enhanced signal-to-noise ratio and thus highlights the action potential peak. In the present study, the nonlinear energy operator,  $\mathbf{j}$ , is defined as

$$\mathbf{j}(x(t)) = \left( \frac{dx(t)}{dt} \right)^2 - x(t) \left( \frac{d^2x(t)}{dt^2} \right),$$

where  $x(t)$  is the original electrode signal at time  $t$  and  $\mathbf{j}(x(t))$  is the output of the nonlinear operator. By examining the times when peak values occur in sequence  $\mathbf{j}(x(t))$ , we are able to determine when spikes occur in record  $x(t)$ . Detected action potentials were then taken as identical, only the precise times of occurrence were constructed, and a discrete series of time events characterizing the spike train of each electrode was obtained.

While the spike train is important for the temporal code of the ganglion cells on each electrode, the correlation between the relevant electrodes provides necessary information for the interaction of the ganglion cells simultaneously recorded<sup>[7,10,17]</sup>. The patterns of interactions between pairs of neurons in chicken retinal ganglion cells were studied by examining their cross-correlation function, which reflect the mean firing rate of one cell as a result of the activity of another. Generally, the cross-correlation is defined as

$$c_{xy}(m) = \begin{cases} \sum_{n=0}^{N-|m|-1} x_n y_{n+m}, & m \geq 0, \\ c_{yx}(-m), & m < 0, \end{cases}$$

where  $x_n$  denotes the value of sequence  $x$  at moment  $n$ ;  $y_{n+m}$  is the value of sequence  $y$  at moment  $n+m$ ;  $C_{xy}(m)$ , by definition, represents the cross-correlation between sequences  $x$  and  $y$  with a lag of  $m$ , which reflect the effect signal  $x$  exerts on signal  $y$  with a time delay  $m$ .

## 2 Result

### 2.1 Spike detection

The original electrode recording made from the chicken retina is processed using the nonlinear algorithm described in the method section. Spike trains were sorted for the recording made during the retinal ganglion cells spontaneous activities and that elicited by full field white light illumination, as plotted by fig. 2(a) and fig. 3(a) respectively.

### 2.2 Spontaneous activities of retinal ganglion cells

Retinal ganglion cells often show some spontaneous activity during darkness. Fig. 2(a) gives an example. The retinal ganglion cells activities were simultaneously recorded from 9 electrodes when no light was applied. Spike trains detected during 6 s, recording period were plotted for each channel. It is shown that the neurons recorded fired randomly and sparsely. The autocorrelation within the data sequences and the cross-correlation between the records were calculated. As plotted in fig. 2(b), the central panel gives the autocorrelation estimated for channel 65, with the rest panels giving the cross-correlation between channel 65 and each of its adjacent channels as arranged in fig. 2(a). The flat autocorrelation function calculated for channel 65 suggests that the neuron fired spikes on a random basis (this is also the case for the signals recorded from other electrodes). At the mean time, the cross-correlation functions are also flat, which suggests that the neurons activities were independent to each other, when fired spontaneously.

### 2.3 Ganglion cells activities in response to full field white light flash

Same neurons were recorded when full field white light flash was performed. The results showed that the neurons activities were remarkably changed. As demonstrated in fig. 3(a), most spikes were detected immediately after the on- and off-transient of light stimulus. As a result, autocorrelation shows some positive interaction between the successive firings detected from one electrode (channel 65 as plotted in fig. 3(b)), although modest. At the mean time, estimated cross-correlation between channel 65 and most of its adjacent channels all shows a pronounced peak at or near zero-lag, which might suggest some tendency of synchronous firing among the neurons and the neuron activities are not independent to each other in exposure to the repeated white flashes. However, cross-correlation function between channel 65 and 55 is estimated at a low level, as compared to other channels (fig. 3(b)), which might suggest that the signals from channels 65 and 55 were independent to each other. Actually, the correlation patterns are somewhat diversified: a negative lag reflecting the signal detected from channel 65 is prior to that from channel 74 and 76, a positive lag suggests the signal detected from channel 65 might result from the activity of the channel 54, 56 and 64, and a zero lag probably represents the synchronization between channel 65 and channels 66 and 75<sup>[17]</sup>.

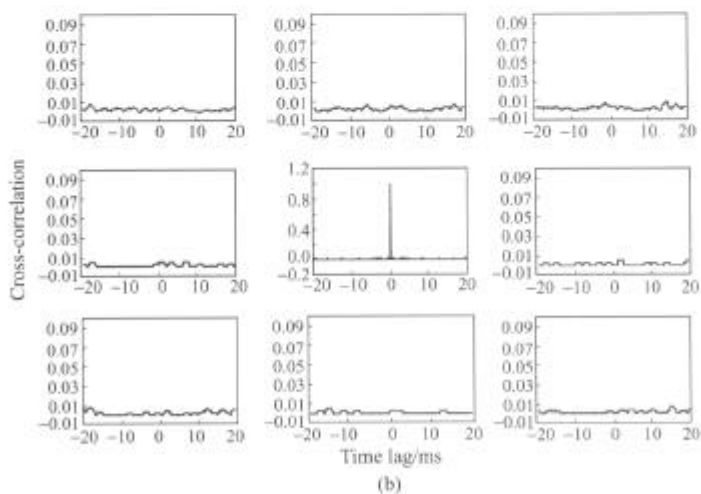
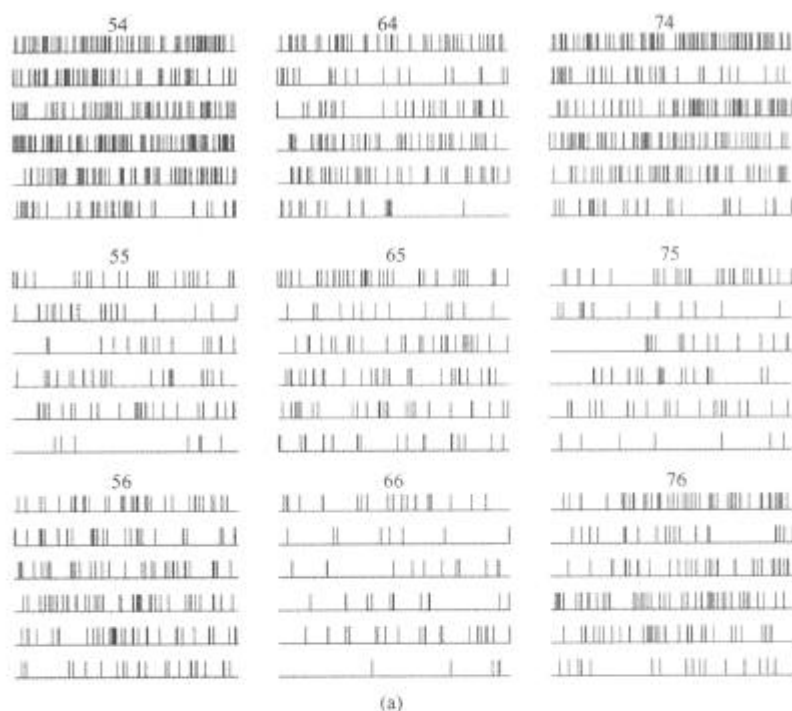


Fig. 2. Responses of the chicken retinal ganglion cells recorded from 9 electrodes in darkness. (a) The spontaneous activities of the ganglion cells simultaneously recorded from 9 electrodes. The recording lasted for 6 s, with each trace representing 1 s recording. (b) The correlation of the spike trains: the central panel is the auto-correlation function for channel 65, and the other panels are the cross-correlation functions between channel 65 and relevant channels as arranged in panel (a). Abscissa: time lag (ms); Ordinate: correlation functions.

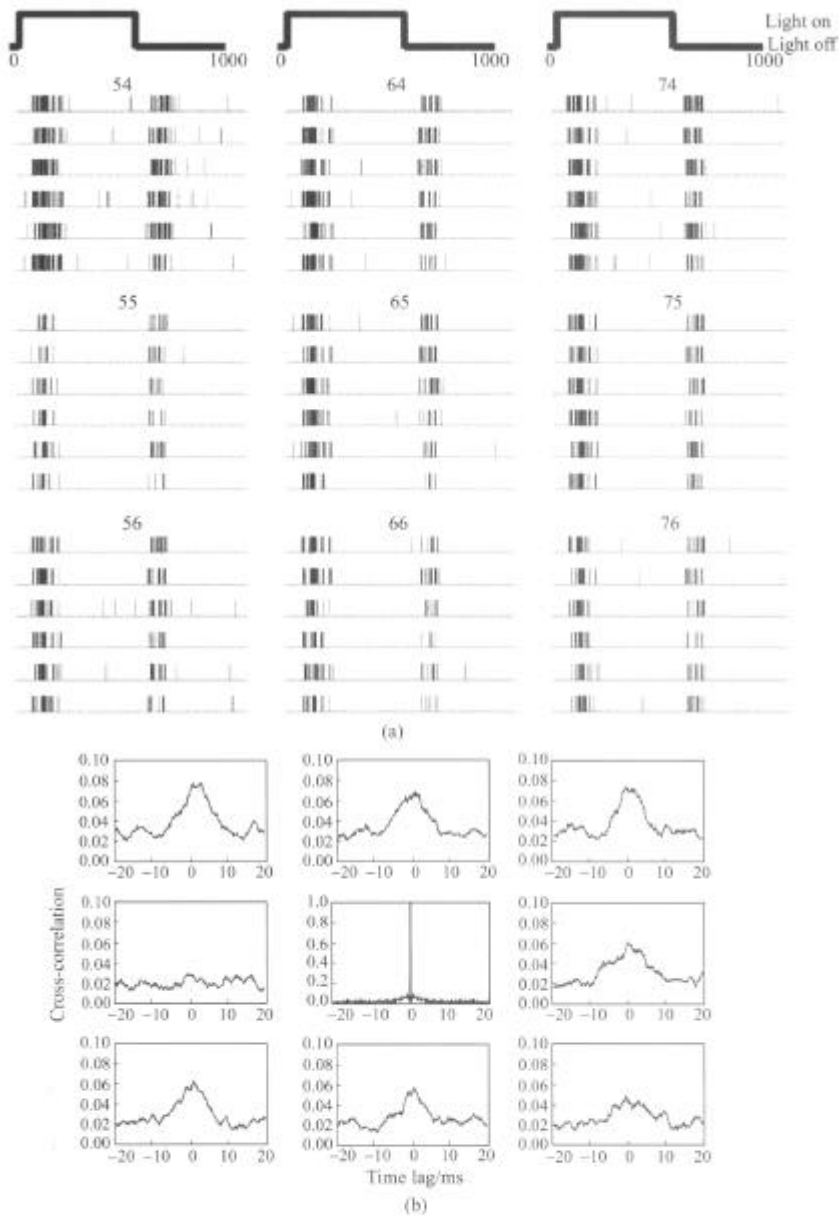


Fig. 3. Responses of the chicken retinal ganglion cells recorded from 9 electrodes to periodic flashes, which consisted of 500 ms full field, uniformly illuminated white flashes followed by 500 ms "light-off" period. The stimulus was repeated for 6 times. (a) Firing sequences recorded from a group of electrodes, the same as that given in fig. 2. Top traces are for the stimulus signal, and the rest ones are the cell spike trains detected for 6 s successive recordings. (b) The correlation function of spike trains during full-field light flashes: the central panel is the auto-correlation function for channel 65, and the other panels are the cross-correlation function between channel 65 and relevant channels as arranged in panel (a).

These correlations suggest that cells may respond in synchrony to strong signal inputs and that, in some cases, ganglion cells may even have influence on one another. Observations of correlated firing can provide more information of retinal connections and signal processing.

### 3 Discussion

#### 3.1 Single neuron recording

The use of microelectrode-based electrophysiology in understanding the function of single neurons has a long and productive history. In visual study, people often define a simple test stimulus, such as a flashing spot or a traveling grating, repeats the stimulus many times, and determines the ganglion cell's response. One can clearly distinguish different functional types in the ganglion cell population and characterize the ganglion cell response depending on the temporal, spatial, and spectral composition of the light stimulus. However, close inspection of the firing patterns to repeated identical stimuli obtained from the present study showed some degree of variability as given in figs. 2(a) and 3(a). All these data were collected from 20 pieces of retina, and there was no obvious difference among these results. Thus, it seems very unlikely that the features of a visual stimulus can be derived exclusively from the activity of single ganglion cells. This suggests that the concerted activity of many cells rather than individual activity of single neurons is encoding visual stimulus features<sup>[5]</sup>. On the other hand, little is known about how a population of these neurons collectively represents a complex visual scene and how subsequent stages of the visual system might extract features of the scene from this population activity. Part of the reason is certainly the complexity of the many feedforward and feedback neural pathways. Other difficulties contribute, involving collecting and analyzing a large number of responses, and extracting from these responses the meaningful information that pertains to the stimuli.

#### 3.2 Multi-electrode recording and population coding

With parallel recording from 60 sites of a multi-electrode array, it is possible to monitor the neural activities simultaneously, which should be helpful to analyzing how retinal neurons collectively process and encode visual information.

In the present study, spikes were detected by applying a nonlinear algorithm which enhanced the signal-to-noise ratio and enabled the spike detection effective even when the background noise level was comparatively high. Neuron activities recorded from adjacent electrodes were analyzed by looking into the auto- and cross-correlation of the spike trains. Generally, the correlations can be distinguished by the temporal details, indicating that there are several types of neural circuits among these neurons<sup>[10]</sup>. According to the definition of the synchrony<sup>[18]</sup>, the precise synchronous firing suggests that the two cells might share excitatory input from a third neuron. A positive or a negative lag suggests that one cell might excite the other, for example, the postsynaptic cell would fire systematically with a short delay after the presynaptic spike. Although the functional significance and importance of retinal synchrony is yet to be clear, it might be speculated that the cooperation and integration among neurons play an important role in visual information

processing.

In the present study, various correlation patterns have been detected for the neuron pairs under investigation. Variation even occurred for the same neuron pairs, under different conditions. For example, the spike activities on the channel 65 and the channel 54 under spontaneous activities are independent while their activities are highly correlated under the white full field light. These observations further emphasize the concept of the contextual encoding of stimulus features by ganglion cell ensemble.

Taken together, the response properties observed via multi-electrode recordings and data analysis suggest that stimulus features can hardly be deduced from the responses of individual ganglion cells. At the mean time, the analysis of correlation pattern of neighboring neurons yields more information about the neural activity during various experimental conditions. The experiments described in this paper were intended to explore the contextual encoding of stimulus features by ganglion-cell ensembles. And we look forward to applying this technique to study other features of simple and more natural visual stimuli.

**Acknowledgements** This work was supported by the grants from Shanghai Science and Technology Development Funding (012A14073, 02JC14008) and the National Basic Research Program of China (G1999054000).

## References

1. Warland, D. K., Reinagel, P., Meister, M., Decoding visual information from a population of retinal ganglion cells, *J. Neurophysiol.*, 1997, 78: 2336.
2. Dacey, D. M., Circuitry for color coding in the primate retina, *Proc. Natl. Acad. Sci. USA*, 1996, 93: 582.
3. Kuffler, S. W., Discharge patterns and functional organization of mammalian retina, *J. Neurophysiol.*, 1953, 16: 37.
4. Nirenberg, S., Latham, P. E., Population coding in the retina, *Current Opinion in Neurobiology*, 1998, 8: 488.
5. Meister, M., Multineuronal codes in retinal signaling, *Proc. Natl. Acad. Sci. USA*, 1996, 23: 609.
6. Meister, M., Berry, M. J., The neural code of the retina, *Neuron*, 1999, 22: 435.
7. Meister, M., Lagnado, L., Baylor, D. A., Concerted signaling by retinal ganglion cells, *Science*, 1995, 270: 1207.
8. Meister, M., Pine, J., Baylor, D. A., Multi-neuronal signals from the retina: Acquisition and analysis, *J. Neurosci. Methods*, 1994, 51: 95.
9. Meister, M., Wong, R. O. L., Baylor, D. A. et al., Synchronous bursts of action potentials in ganglion cells of the developing mammalian retina, *Science*, 1991, 252: 939.
10. Mastrorarde, D. N., Correlated firing of retinal ganglion cells, *TINS*, 1989, 12: 75.
11. Neuenschwander, S., Singer, W., Long-range synchronization of oscillatory light responses in geniculate nucleus, *Nature*, 1996, 379: 728.
12. Hämmerle, H., Egert, U., Mohr, A. et al., Extracellular recording in neuronal networks with substrate integrated microelectrode arrays, *Biosensors & Bioelectronics*, 1994, 9: 691.
13. Nisch, W., Böck, J., Egert, U. et al., A thin film microelectrode array for monitoring extracellular neuronal activity *in vitro*, *Biosensors & Bioelectronics*, 1994, 9: 737.
14. Egert, U., Schlosshauer, B., Fennrich, S. et al., A novel organotypic long-term culture of the rat hippocampus on substrate-integrate multi-electrode arrays, *Brain Res.*, 1998, 2: 229.
15. Thiébaud, P., Beuret, C., Koudelka-Hep, M. et al., An array of Pt-tip microelectrodes for extracellular monitoring of activity of brain slices, *Biosensors & Bioelectronics*, 1994, 14: 61.
16. Kim, K. H., Kim, S. J., Neural spike sorting under nearly 0 dB signal-to-noise using nonlinear energy operator and artificial neural network classifier, *IEEE Trans. Biomed. Eng.*, 2000, 47: 1406.
17. Brivanlou, I. H., Warland, D. K., Meister, M., Mechanisms of concerted firing among retinal ganglion cells, *Neuron*, 1998, 20: 527.
18. Usrey, W. M., Reid, R. C., Synchronous activity in the visual system, *Annu. Rev. Physiol.*, 1999, 61: 435.