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Synchronized Firings in Retinal Ganglion Cells in Response to Natural Stimulation *

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The response of synchronously firing groups of population retinal ganglion cells (RGCs) to natural movies (NMs) and pseudo-random white-noise checker-board flickering (CB, as control) are investigated using an information-theoretic algorithm. The main results are: (1) the population RGCs tend to fire in synchrony far more frequently than expected by chance during both NM and CB stimulation; (2) more synchronous groups could be formed and each group contains more neurons under NM than CB stimulation; (3) the individual neurons also participate in more groups and have more distinct partners in NM than CB stimulation. All these results suggest that the synchronized firings in RGCs are more extensive and diverse, which may account for more effective information processing in representing the natural visual environment.

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Retinal function involves organizing visual information via various patterns of RGC action potentials, which are then sent to the central visual system to form visual perception. However, most of what we know about retinal information processing is obtained from studies performed on single neurons and using simple stimulation patterns, such as light spots or light bars. However, it is clear that the nervous system does its job by generating coordinated patterns of population neuron responses, and the retina is settled in a complex visual environment and should naturally be optimized to process natural stimuli. Thus understanding the coding strategy of population RGCs' in natural visual information processing and transmission is instructive for exploring the neuronal system. Population activities of dynamic groups and synchronization among a group of neurons are indicative aspects to show how the neurons work together to encode stimulus features.[1–5] An important technique in neuroscience research, i.e. a multi-channel recording system, has been developed to measure the activity of a group of neurons simultaneously, which offers a window to explore how neurons work in concert to encode specific information.[6–8] A combination of multi-neuronal activities results in plentiful population firing patterns, which can carry more information. An algorithm based on information theory has been developed to detect the concerted activities of arbitrarily large groups of neurons, which breaks through the limitation caused by the traditional pair-wise comparisons among neurons.[9] In the present study, various aspects of the synchronous spiking groups under NMs and CB stimulation are investigated, and the results show that there are more dynamic groups and that the combination patterns of synchronous spiking neurons are more complex and diverse in the NM-responses.

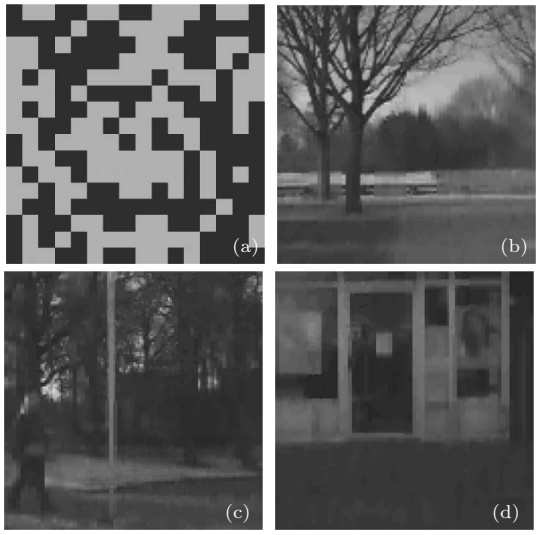

Fig. 1. Example frames of CB (a) and NMs (b–d).

Details of the extracellular-recording procedure have been described in our previous reports.[10,11] All procedures are strictly conformed to the humane treatment and use of animals as prescribed by the Association for Research in Vision and Ophthalmology. Briefly, retinas from chicks (about 1–3 weeks post hatching) were investigated. Action potentials fired by RGCs were recorded using an MEA system (8×8, MEA60, MCS GmbH, Germany) via a commercial multiplexed data acquisition system with a

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sampling rate of 20 kHz. Stimuli were generated using programs written in VC^{++} and DirectX9 (unless otherwise specified), displayed on a computer monitor (796 FD Ⅱ, MAG) and projected onto the isolated retina via a lens system. The following stimulation protocols were applied: (1) CB (1920 frames, 16×16 grid, frame rate 9.05 Hz, lasted for 221 s), (2) NMs (download from the website of van Hateren's lab, http://hlab.phys.rug.nl/vidlib/index.html,[12] 1920 frames, 128×128 pixels, frame rate 10 Hz, lasted for 192 s). Example frames of CB and three NMs are shown in Figs. 1(a)–1(d).

To identify the synchronized neuronal groups, the following information-theoretic algorithm based on entropy analysis is applied. Firstly, the spike trains are binned into 0 and 1 with a time bin of 50 ms, where 1 represents that there were spikes ($\geqq 1$ spikes) in the time bin and 0 represents that no spike is fired in the time bin. Given two neurons A and B, a new symbolic neuron AB can be defined such that

$$r_j^{(AB)} = r_j^{(A)} r_j^{(B)} = \begin{cases} 1, & \text{if the neuron } A \text{ and neuron } B, \\ & \text{fired in time bin } j, \\ 0, & \text{otherwise.} \end{cases} \quad (1)$$

Secondly, to see whether the neurons A and B are concertedly activated, the entropy is computed,

$$H_i = -[P_i \log_2 P_i + (1 - P_i) \log_2 (1 - P_i)], \quad (2)$$

where P_i is the probability that symbolic neuron i has a spike in the time bin ($P_i = \frac{1}{N} \sum_{i=1}^{N} r_j^{(i)}$, N is the number of time bins in the data set). As usual, only a small fraction of each neuron's spikes fire in synchrony with others, the net reduction in entropy can be calculated by[9]

$$\Delta H_{AB} = H_A + H_B - H_{AB} \approx P_{AB} \log_2 (P_{AB}/P_A P_B). \quad (3)$$

The identification of concerted neuron groups starts with computing ΔH for all the cell pairs. If the largest ΔH value is greater than a predetermined threshold (defined by shifting all the spike trains by a randomly chosen time delay without changing the structure of the sequences and calculating the largest ΔH in the shifted data), we regard these two neurons as a concerted group, and the synchronous spikes of this neuron pair form the spike sequence of the symbol neuron AB. We then search for other synchronous neuron pairs or synchronous groups containing more cells by repeating this process. In each round, each symbol neuron defined by the previous iterations is treated equally as a real neuron. The process is repeated until the largest ΔH falls below the predetermined threshold.

Correlation index is the ratio between the observed frequency of synchronous activities and the frequency expected by chance,[13] which is used to estimate the significance of the synchronous firings. The correlation index is measured by calculating the frequency of synchronous firings among M cells:

$$P_{1 \cdots M} = \frac{1}{N} \sum_{j=1}^{N} \prod_{i=1}^{M} r_j^{(i)}, \quad (4)$$

and the frequency of synchronous firings expected by chance:

$$P_1 \cdots P_M = \prod_{i=1}^{M} \frac{1}{N} \sum_{j=1}^{N} r_j^{(i)}. \quad (5)$$

Then the correlation index can be computed by

$$C_{1 \cdots M} = P_{1 \cdots M} \Big/ \prod_{i=1}^{M} P_i. \quad (6)$$

The correlation index defines the occurrence probability of the identified synchronous group compared with that expected by chance.

A total number of 44 neurons' activities from one retina are simultaneously recorded during the retinal responses elicited by CB and NMs (NM1–3). The overall mean firing rates of all the neurons' CB-responses are 3.13 Hz, and those of the responses during the three NMs are 3.53, 3.27 and 2.85 Hz, respectively. It is clear that the total amounts of the population neurons' spikes fired during NM2 are very close to those during CB, while the firing rates in response to the other two movies are slightly different (either higher or lower than the CB-responses). However, the concerted spiking among the population RGCs' spike trains in response to these two kinds of stimuli varied significantly in spite of the mean firing rates.

The correlation index, which is the ratio between the observed frequency of synchronized firings and the frequency expected by chance, of the identified concerted firing neurons is calculated. Figure 2 shows the cumulative frequency (defined as the cumulative distribution function) of the correlation index values of CB-responses and NM-responses for synchronous neuronal pairs, triplets and quadruplets. The results clearly show that the identified synchronous groups under all the stimuli occur more frequently than expectated. The correlation index of the synchronous groups increases with the group size. It is also shown that with the group size of 4 neurons, the difference of the correlation index between the CB-responses and the NM-responses is remarkable.

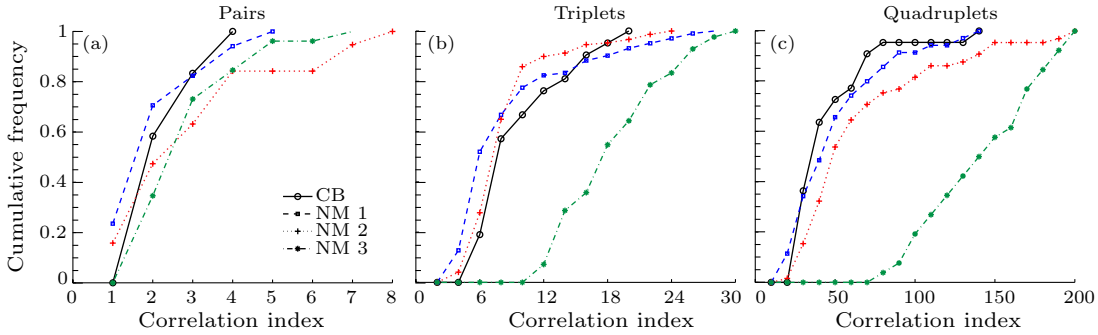

Fig. 2. The cumulative frequency of the correlation index in spike pairs, triplets and quadruplets from the recorded RGCs in response to CB and NMs.

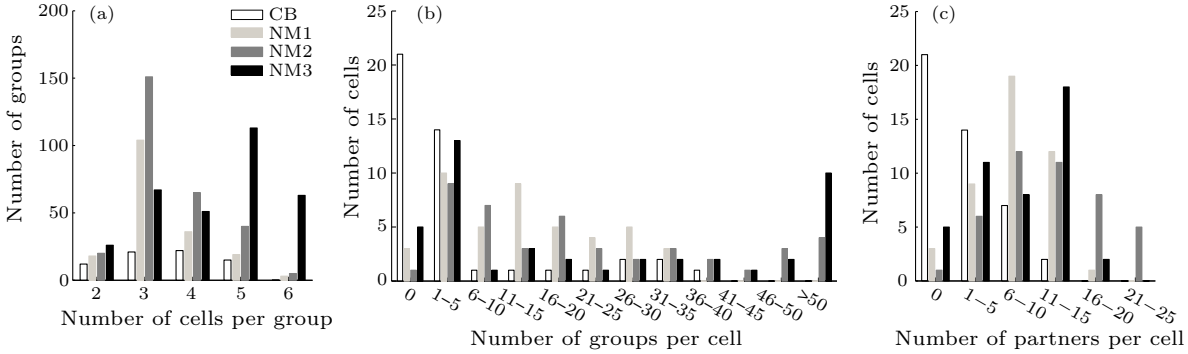

Fig. 3. Statistics of synchronous aspects in the population RGCs. (a) Histogram of group size during CB-responses (hollow bars) and various NMs-responses (filled bars in different gray scales). (b) Histogram of group numbers that each neuron participates in during CB-responses and NMs-responses. (c) Histogram of partners that each neuron has in CB and under NM stimulation.

Furthermore, the size of the synchronous spiking groups and the number of distinct partners each neuron engages are investigated. The results are presented in Fig. 3. There are 70, 180, 281 and 300 synchronous groups identified during the neuronal responses to CB and NM1–3, respectively. As shown in Fig. 3(a), pair-wise groups account for 17% of the synchronously firing patterns in the CB-responses, while in the NM-responses the synchronous pairs only account for 10% or less (10%, 7% and 8% for NM1–3, respectively), and the synchronized triplets and quadruplets occur relatively more frequently in CB, NM1 and NM2. In NM3, the synchronous groups of sizes 5 and 6 occur more frequently. However, what does persist is that the group numbers in NM-responses are always larger than those in CB-responses in spite of the group sizes and the mean firing rates of the responses.

Given that the number of synchronous neuron groups dramatically exceeds the number of neurons recorded in the population, individual neurons must participate in more than one concerted firing pattern. As shown in Fig. 3(b), in CB-responses, 21 neurons out of the 44 neurons under investigation do not participate in any groups and only 9 neurons participated in more than 5 groups. While in NM-responses, less than 5 neurons persistently fire independently and there are 21, 34 and 16 neurons participated in more than 5 groups in response to NM1–3, respectively. Especially during NM3, 11 neurons participated in more than 50 groups. Figure 3(c) shows that an individual neuron could have up to more than 16 distinct partners in forming different concerted firing groups during NM-responses. However, during CB-responses the grouping of concerted firing neurons has a smaller range; a single neuron could have only up to 13 distinct partners.

In our experiment, the multi-electrode arrays are only able to record a small fraction of the RGCs in its vicinity, with the rest of their partners not being recorded. Furthermore, the search algorithm we adopted is not exhaustive[9] and may have missed some spiking groups even among the cells whose activity are recorded. Thus the present results are actually under-estimations.

The NM stimulations are more correlated both in spatial and temporal domains as compared to CB stimulation.[14] These differences can be detected by the retinal photoreceptors, which are directly sensitive to the light of the visual stimulation, and then the neural signals from the photoreceptors further processed by the neural network in the retina involving bipolar cells, horizontal cells and amacrine cells before they reach the RGCs. The action of the lateral networks can greatly influence the behaviors of the RGCs. The spatial and temporal correlation of the NM stimulation could be more effective in inducing the lateral

interaction of the retinal neural network than the random CB flickering. The larger correlation indices indicate stronger internal links of the neurons' activities within the group. Figure 2 shows that the synchronous groups of different sizes in NM-responses and CB-responses all occur more frequently than expected by chance and the correlation indices are greater in NM-responses. Thus the continuous spatiotemporal extent of the NMs makes more neurons fire in synchrony more frequently by involving them in more extended lateral interactions of the neural network. Therefore, as the group grows bigger, the difference of the correlation index between the CB-responses and the NM-responses becomes more remarkable (Fig. 2).

These results seem to suggest that an individual ganglion cell needs to cooperate with other neurons to form dynamic synchronous groups to transmit more distinct visual messages in NM-responses, which can be confirmed by the result presented in Fig. 3(b). Furthermore, the number and size of the identified synchronous groups are not in direct proportion to the mean firing rates of the responses but related to the nature of the stimulation for the diversity of the overall mean firing rates of the CB- and NMs-responses and the consistency in the differences of the properties of the synchronous groups between CB- and NMs-responses.

Neurons communicate with each other via synapses to encode the information of the stimuli in temporal and spatial domains.[15] Temporal summation and spatial summation are two essential ways for a postsynaptic neuron to process and convey input from its presynaptic neurons. Spatial summation of the input neurons is more efficient as it can enhance the firing probability of the target postsynaptic neuron.[16] The synchronous firing of presynaptic neurons forms spatial summation, which contributes to efficient information transmission. Efficient coding of the visual system in representing information about the natural world has been proposed and studied for years (see Ref. [17] for review) and this is further supported by the more extensive and dynamic synchronous activities of the population RGCs in response to the natural stimuli in the present study.

In summary, the population RGCs in response to both CB and NMs tend to fire synchronously far more frequently than expected by chance. The synchronous neuronal groups can include up to six neurons under our experimental conditions, some individual neurons could participate in more than fifty different groups and some could have up to twenty or more different partners during NMs. Under CB the size of the groups is much smaller and each individual neuron only has fewer partners. The results confirm the inference that population neurons act in more dynamic synchronous grouping manner to represent more complex stimuli such as NM stimulation used in the present study and the real natural visual stimuli in daily life.

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