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Prediction and Memory Mechanisms for Dynamic Stimuli in the Retina
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Abstract
"Visual memory" is a phenomenon that occurs when the visual system receives periodic stimuli, and the synchronized activity can be preserved even after the stimulations are turned off. Such kind of behavior in the sensory system might be similar to the working memory in more complex neural networks. However, basic understandings for these phenomena are still lacking. In this project, we setup a multi-electrode system to study visual memory phenomenon in the retina of bullfrogs, and light stimuli with alternative dynamics were projected to the photoreceptors. In result, an omitted stimulus response (OSR) was observed after the periodic stimuli ends, and the latency of OSR corresponds to the period of the stimuli. In addition, temporal organizations of two opponent color stimuli change the synchronization behavior of retina, and spatial dynamics might also affect the OSR behavior. By analyzing the complex responses from the ganglion cells in the retina, we come up with corresponding models to simulate the behaviors of the adaptive dynamics. Further investigations on short-term synaptic plasticity and responses to spatial dynamics may help understanding the biophysical mechanisms and information coding in the retina.

Introduction
Phase synchronization in dynamic systems to the input signal is a generic phenomenon [1]. A similar period to the periodic input signal can be observed in entrained system under certain coupling to the stimuli [2]. For instance, the adaptation to daily period in circadian would be a significant example in biological systems [3]. However, different from physical systems, rather than moving back to its initial period when external drive removed, biological systems are able to retain memory in the dynamic of the un-driven system. For example, in the phenomenon of visual memories, it has been shown that dynamics of external light stimuli can be remembered by the driven sensory system.
In the neural system, there are a number of researches on the relations between synaptic connections and long-term memory [4]. In contrast, we have less understanding in the underlying mechanisms of short-term memory. Sensory memory, a kind of short-term memory retained in the sensory organs, is an appropriate experiment platform for us to understand the properties and mechanisms of short-term memory and neural computation. Previous references used either in vivo or in vitro experiments on auditory and visual systems to demonstrate similar abilities for maintaining synchronized periodic activities to its stimuli, even after the source of stimuli has been removed for a short duration [5-8].

In addition, a number of biophysical researches provide conceptual models for short-term synaptic plasticity for short-term memory in random networks [9, 10]. However, as we know from experiments in sensory neuroscience, particular organizations such as the parallel process and receptive field structure should be considered in the sensory signal process [11]. In classical psychophysical experiments, illusions such as “flash lag effect” imply underlying dynamics in the visual system [12, 13]. Numerous models such as latency differential and extrapolation mechanisms were raised to explain the accurate prediction in moving objects [14-17]. Moreover, according to electrophysiological experiments, such sophisticated behavior seems to start from the primary visual system—the retina [15].

To date, experiments in the primary visual system indicate abilities such as short-term memory, prediction in firing timing, and anticipation in spatial domain. It is possible to utilize behaviors in retina to explain well-known illusions [18-24]. However, detail synaptic or circuitry mechanisms for these behaviors are still incompletely understood clear yet, and considerable interesting nonlinear behaviors in the sensory system are still undiscovered. Herein, by extending experiments on dynamic stimuli in a retina, we observed new behaviors in the retina under certain light entrainments. These results may indicate the underlying mechanisms of the adaptive dynamics in the retina.

Materials and Methods
Animal preparation: Bullfrogs (Rana catesbeiana) were used for retinal electrophysiology experiments in accordance to the guidelines for the care and use of animals. Before each experiment, bullfrog was dark adapted for 30 min.
After adaptation, bullfrog was double pithed and eyes were enucleated under dim red light. The retina tissue was isolated from the pigment epithelium and perfused in Ringer’s solution (100.0 mM NaCl, 2.5 KCl, 1.6 MgCl₂, 1.0 CaCl₂, 18.0 NaHCO₃ and 10.0 D-glucose), bubbled with 95% oxygen, and pH = 7.2-7.4 under room temperature [25, 26].

Recording: A 60 channel multi-electrode array (MEA) with electrodes with 100 μm in diameter (50 kΩ) and 500μm separation between electrodes. The dissected retina tissue was fixed on the MEA by nylon web with the ganglion cell side touching the electrodes. Electric signals were amplified and recorded through 20 kHz sampling rate through the multi-channel system [26, 27].

Stimulation: Light stimuli were generated through either LED or a computer monitor and focused to project onto the retina. For experiments with color lights, LED lights were filtered through chromatic filter, producing 540 nm or 415 nm wavelengths, and the temporal dynamics controlled by MC_Stimuli. For experiments done with the monitor, light pulses with spatiotemporal properties were programmed through Matlab. Each designed stimuli were presented multiple times (at least 10 times) with 5 sec separation between trials on the same retina, and over three different bullfrogs were used to receive each stimuli. Combining the recording system, the experimental setup was kept on an optical table to avoid external disturbance (Fig. 1).

Figure 1: Experimental setup for retina light stimulation and MEA recordings.

Analysis: Signals were recorded by MC_Rack software. The raw electrode signals were filtered through low pass 200 Hz filter and spikes were detected by analyzing amplitudes higher than 5 standard deviation of the signal within 1 ms.
Afterwards, spike counts (bin time = 0.5 ms) and timings were analyzed by Matlab. We compare firing rate and spike times with the stimuli, between trials, and among different channels. In addition, Offline Sorter software was used to verify the number of cells recorded by a channel by classifying the waveforms.

Results

Electrophysiology of retina

Electrophysiological recordings for bullfrog retina present -50~100 microvolt in amplitude. Spontaneous firing rates were less than 5 Hz under dark adaptation, while firing rate was significant over ten spikes per second approximately 50 ms after a single light flash (Fig. 2).

![Figure 2: Raw data from the MEA recordings, 16 channels were selected for demonstration. (A) Under dark environment. (B) When light stimuli provided.](image)

Omitted stimulus response

Retina produces an omitted stimulus response (OSR) after a certain periodic light stimulus. The range of the period to produce was around 50-200 ms to produce the OSR, and the latency of OSR to the last light stimuli correlates with the period of entrainment (Fig. 3 A-D, H).

Moreover, there are different types of reaction during the entrainment of periodic light stimuli, and also some in lack of OSR or showing different amplitude in firing rate (Fig. 3 E-G). If we trace the activity in certain channels (which may correspond to single ganglion cells), the behaviors may even change through different frequency of stimuli.
Figure 3: OSR phenomenon. (A)-(D) Activities under different periodic stimuli. 60 channels are expanding on the vertical axis, while the firing rates are presented by color code through the horizontal time line. Stimuli are shown with blue lines (width = 50 ms), and the red arrow indicates positions of the omitted stimulus. All plots are composition of multiple identical trials. (E-G) Diversity of
activities recorded in three distinct channels under identical stimuli. (H) A positive correlation between interval of periodic light stimuli and the measured OSR latency. Standard deviations for measurements through 60 channels were less than 5 ms.

In addition, the OSR phenomenon is observed in different length of the entrainment, durations of light stimuli (fig. 4A), or intensity of the light source (fig. 4B). Also, similar synchronization and OSR behaviors are shown when we change the base line into light exposure and giving “dark stimuli” in the same temporal dynamic. Therefore, OSR should not be a specific off response to the periodic stimuli, but encoding the temporal information robustly.

![Figure 4. OSR under different conditions with the same periodic stimuli. (A) After 20 pulses with 200 ms period, the OSR still occurred. (B) When light flashes decreased to 5 ms, OSR pattern remained but with lower intensity.](image)

Different from the original response under periodic signals, retina produced multiple OSR-like activities under irregular signals, which misses some stimuli during the synchronization process. Interestingly, in average, the latency of OSR tends to shift towards the length of latency under regular stimuli when the there’re more stimuli added (Fig. 5). The fourth average latency in Fig. 5-B was around 240 ms, which corresponds to the latency under regular stimuli with approximately 150 ms period (Fig. 3H).
Figure 5: Missing stimuli in the periodic trials. (A) One flash missed every two flashes presented. (B) One flash missed every four flashes presented.

Difference in chromatic stimuli

A simple model with two types of bipolar cells in the upstream of ganglion cells was able to explain certain behaviors of OSR in the reference [20]. ON and OFF bipolar cells that respond to stimuli with different dynamics are couple and provide resonant signals to the ganglion cell. Due to the dynamic of calcium in the ON type bipolar cell axon terminal, the ganglion cell may be able to produce an OSR without being canceled by the OFF type bipolar cell [23, 24]. We came up with a method using opponent color stimuli to test the hypothesis, but the interactions and variety were more complex than assumed.

According to the organization of receptive field to produce color opponency, two colors may provide opposite signals to the downstream ganglion cell [28]. In bullfrogs, the dichromatic visual system contains blue and green color perception. Under most circumstances, the activities under two colors of periodic stimuli differ from each other. Both reacting to a single light stimulus, but one without OSR nor synchronization behaviors (data not shown). This indicates the asymmetrical connections or synaptic weights to the recorded ganglion cells between two parallel color vision pathways.

In some rare cases, the differences of activities between both colors are negligible (Fig. 6 A, B). The intermeshed series of color stimuli were then tested (Fig. 6 C-F). Interesting interaction between two signaling pathways under the dichromatic stimuli was observed. Retina seems to follow a different characteristic period when two colors are intermeshed. Possible explanations for the purpose and mechanisms of this phenomenon are in discussion section.
Figure 6: Dichromatic stimuli. Activities of 60 channels are summed and plotted for better temporal comparison with the analog stimuli signal. Vertical axis shows firing rate (spikes/bin), while horizontal axis shows time (seconds). (A-B) Activities under two different colors are similar. (C-F) Firing rate constantly align to only one color when two color stimuli are intermeshed. The aligned color is independent to neither the starting stimulus nor certain retina sample.

Discussion

Omitted stimulus response

We were able to reproduce most of the experiments from the reference [5, 20] and received similar patterns in results. Signals recorded from retinal ganglion cells can synchronize to the applied periodic light stimuli, and an OSR with the firing rate higher than those during the synchronization process would show up after the last light flash. In addition, the latency of OSR is correlated to the period of stimuli driving the activity but not simply some off effects observed under a single flash stimulus. Expanding all channels by the Raster plot, we observe the diversity of activity during the synchronization process, but the OSR
still appears in over half of the population of ganglion cells we recorded.

In fact, a number of additional phenomena were seen in the results of OSR experiments. Activities may vary through different periods of stimuli rather than maintaining a certain type of synchrony or OSR behavior. This differs from the original research that categorized different activity types of ganglion cells with a standard frequency. It also indicates a range of “drivable” frequency of light to produce OSR or for retina to be entrained through time.

Another interesting observation is in the latency of OSR. Though OSR do positively correlate to the driving light period, latencies of OSR are sometimes shorter than the latencies during the synchronization state! It is intuitive for OSR to correlate with the period of the applied light stimuli, since we claim to view OSR as a kind of visual memory maintained in the system to encode the prior information. However, a latency shorter than the synchronization state turns out to indicate the prediction of missing stimulus before the retina even perceive the real missing of light (Fig. 7). Therefore, this reveals that under some circumstances, after the activity synchronizes to the stimuli, the firing time depends on both the original stimulus and the other following stimulus. So we cannot predict the latency of OSR by simply calculating the period plus the latency during the synchronized state. The properties under dynamic stimuli for a certain type of ganglion cell should be considered in a model of OSR.

Figure 7: There are two firing peaks after the stimuli turned off. The first smaller peak might be simply the light response to the last stimulus. However, the second peak, which is the OSR (indicated by a red arrow), is just beside the last peak, producing latency shorter than those in the steady state. Vertical axis shows firing rate (spikes/bin), while horizontal axis shows time (seconds). The low panel indicates the periodic light pulses.
An adaptive dynamic view on OSR

FitzHugh-Nagumo (FHN) model is a well-known excitable system to simulate spikes generated by a neuron. It includes the characters of action potentials and could be tuned by certain parameters. Recently, an adaptive dynamic behavior added in the term that controls the period of oscillation forms an adaptive FHN model [29]. The behavior of this modified system mimics the synchronous state and also the OSR we record in the experiment. This model provides a theoretical explanation for some results in the experiment.

The dynamic of variable ‘a’ is added to the original FHN model, which contains two variable ‘v’ and ‘w’ in the differential equations:

\[
\frac{dv}{dt} = v - \frac{v^3}{3} - w + I_{ext}(A, T_s)
\]

\[
\frac{dw}{dt} = \frac{1}{\tau} (v + a)
\]

\[
\frac{da}{dt} = \frac{\left(\left(1 - p\right)a_0 + p\frac{a^3}{3}\right) - pw}{\tau_a} - a
\]

Variable ‘v’ mimics the membrane potential of a neuron and receives the input function ‘I’, which is periodic in our experiment. Different from the original FHN model, after the periodic input to the system ends, the ‘v’ variable maintains its oscillation with a period near the synchronous state due to the adaptive term ‘a’ that turns back much slower to its initial state. Therefore, we can intuitively see the OSR as an additional oscillation in the phase space before ‘a’ reaches the steady state, and the phenomenon in figure 7 may be due to a specific condition that makes the limit cycle in the phase space depend on both the prior and successive periodic stimuli.

According to such simulation, a single synapse within the retina tissue is able to produce complex behaviors like OSR in theory. Further investigation through pharmacological methods to control parameters for short-term synaptic plasticity in the retinal synapse may verify the hypothetical viewpoint.

Coupling between two chromatic pathways

According to our experiment with dichromatic stimuli, we observed an interesting interaction during the synchronization process. Retina selectively
synchronizes to one of the two colors and the latency of the OSR behaves as if the other color stimuli did not participate. In an information sense, the selection of only one color under such periodic stimuli is able to characterize the periodic presentation of intermeshed blue and green color, while conserving energy rather than firing on each stimulus. For a mechanistic explanation, we may postulate a reciprocal inhibition between two signaling pathways. Either the dynamic property or a structural organization may cause an asymmetric coupling and produce the behavior in our recordings.

In previous reports [28], blue and green color pathways were hypothesized to form in an asymmetrical way. There is no “blue OFF” bipolar cell, so such blue off signal might pass to ganglion cell through coupling “blue On” bipolar cells to an amacrine cell, which results in an inhibitory input to ganglion cells under blue light.

However, as shown in figure 6, in terms of activity under light stimuli, blue and green light induces symmetrical activity. Is it possible to produce asymmetrical behavior while two components are actually identical? The possible answer leads to a simple model for “binocular rivalry”, an important illusion in psychophysical experiments [30]. When two eyes viewing different patterns, a person reports perceiving either one of the patterns, and the patterns gradually switch between two on another. Here, after rescaling the inhibitory strength and the time scale, the output activity for two sides, which means two parallel opponent color pathways, may be similar to the recorded activity in figure 6 (fig. 8). There are periods with only one color signal dominated, and two colors are switchable through time. Further investigation for the connections between two types of bipolar cells and the time scale of rivalry between two colors are needed for a solid conclusion for this hypothesis, and may also provide detail for how retina synchronizes to stimuli and produce OSR in the end.
Figure 8: Simulation through a simplified binocular rivalry model. As the stimuli for two channels (presented in blue and green color code) presented intermeshed through time in the lower panel, the activity shown in the upper panel is dominated by only one channel within a time interval, but gradually flips over through time.

Future works

An important task is to sort different functional cell types in MEA for sophisticate analysis. This will not only decrease the deviation between analyzed OSR activities but also reveal the diversity of visual information coding in the primary system. In addition, tuning the concentration of calcium in the buffer brings significant change in the synchronize ability and OSR behavior in retina. This suggests the dynamics of specific synaptic mechanisms that govern OSR, which we should further investigate [31].

Furthermore, we developed an explanation for the behavior of OSR according to research on microsaccade [32]. In a number of psychophysical researches, we know that images do not project on a constant position on retina, but actually move in a “tremor” motion due to eye movement in some animals. We hypothesize synchronization of retina activities to light stimuli under the time scale around a hundred milliseconds do happen under natural vision, and synchronization and OSR are important information that encodes visual cues to the higher level in visual system. Further investigations on OSR in the spatial domain may integrate the organization of receptive fields and also concepts for dynamic information coding in the sensory system. Some preliminary results from presenting stimuli with simple spatial patterns have indicated heterogeneity of OSR property in space (fig. 9).
Figure 9: A sample unable to generate OSR while stimuli presented on the whole retina (A). However, OSR with low intensity emerge when the stimuli are only presented on half of the electrodes (B, the red circle indicating the OSR behaviors).

References