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ON–OFF retinal ganglion cells temporally encode OFF/ON sequence

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Abstract

While the functions of ON and OFF retinal ganglion cells have been intensively investigated, that of ON–OFF cells has not. In the present study, the temporal properties of spike trains emitted from ON-OFF cells in response to randomly flickering or multiphase ramp stimuli were examined in the Japanese quail. The results indicate that the firing of ON-spikes was influenced by the recent firing of OFF-spikes, and vice versa. As a result of this interaction, OFF/ON sequence of light intensity change was encoded with a spike pair with an interval of 20 ms, indicating that temporal coding is utilized in the vertebrate visual system as early as the retina. Thus, the present results suggest that retinal neuronal circuits may detect specific sequential features of stimuli.

Keywords: ON–OFF retinal ganglion cells; Spike train; Stimulus sequence; Temporal coding; Retina; Optic nerve; Quail

1. Introduction

Since Hartline (1938) first identified three response types (ON, OFF, and ON–OFF) of vertebrate retinal ganglion cell responses, the properties of ON and OFF cells have been well characterized (Enroth-Cugell & Robson, 1966; Kuffler, 1953; Rodieck & Stone, 1965) and their function has been investigated (Schiller, 1992). On the other hand, while it is known that ON–OFF cells respond transiently to both onset and offset of light, it is not known what purpose they serve to animals. If spikes are evoked evenly and independently by intensity increases (ON) and decreases (OFF) in the ON–OFF cells, the resulted spike trains may convey only information about the timing of intensity changes of either direction, whereas more complex temporal features could be encoded if there is an asymmetrical interaction between ON- and OFF-responses. In the present study, we analyzed the temporal properties of spike trains emitted from the ON–OFF cells in response to randomly flickering or multiphase ramp stimuli in the Japanese quail.

2. Materials and methods

Japanese quail (*Coturnix japonica*) of both sexes were used in the present study. Animals were treated in accordance with the animal usage guideline of the Society for Neuroscience. Surgical procedures were performed as described previously (Uchiyama & Barlow, 1994; Uchiyama, Kanaya & Sonohata, 2000). A tungsten electrode (12 MΩ; A-M Systems, Everett, WA) was inserted stereotactically into the optic chiasm through a hole in the skull. Spikes were detected using a time-window discriminator. Upon a successful isolation of a single optic nerve fiber, the receptive field was mapped on a monitor scope (608; Tektronix, OR). Stimuli for mapping were generated using an image synthesizer (Picasso; Innisfree, UK). A light-emitting diode (LED; $\lambda p = 590$ nm) was positioned in the center of the receptive fields at a distance of 20–40 cm from the cornea. The mean light level near the cornea was 1.5–3.0 mW/m^2. Voltage applied to the LED was modulated by a computer-generated multiphase ramp and a Gaussian white noise (cut-off frequency, 50 Hz) generator (WG-721A; NF Electronic Instruments, Japan). Stimulus waves were sampled at 250 or 1000 Hz using a photodiode (S2281-01, C2719; Hamamatsu Photonics, Japan) and/or directly from the stimulators. The spike time series and stimulus waves went simultaneously to a personal computer through an intelligent interface (1401plus, Cambridge Electronic Design, UK). Spike-triggered analysis of the stimulus waves and correlation analyses were performed off-line on a personal computer using MATLAB (Mathworks, MA). Spike-triggered analysis was conducted by extracting the stimulus waveform preceding each spike, and then

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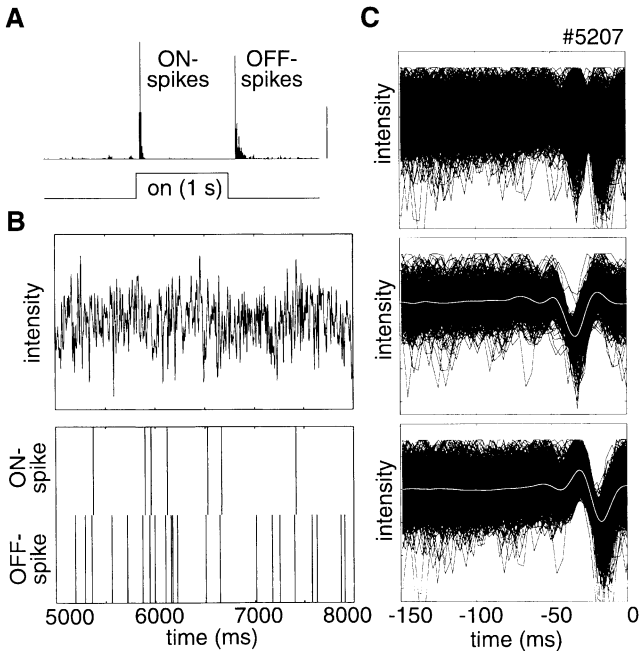

Fig. 1. A. Response to a stationary spot of light in a quail ON–OFF retinal ganglion cell (#5207). Peristimulus time histogram. Thirty sweeps. Calibration bar = 200 spikes/s. B. Randomly flickering stimulus (upper) and evoked spikes (lower) in the same ON–OFF cell. Evoked spikes were identified as ON- or OFF-spikes, based on the slope of the stimulus wave approximately 20–40 ms prior to the spike as analyzed in C. Stimulus and responses for 3 s in a 300 s session. C. Analysis of spike-evoking stimulus waves. The stimulus waves preceding each spike were extracted and overwritten. All the spike evoking waves (top; $n = 1613$) were sorted into ON-spike-evoking (middle; $n = 410$) and OFF-spike-evoking (bottom; $n = 1195$) waves. Time 0 corresponds to the spike discharge. Eight (0.5%) waves were unclassified in this case. White lines (middle, bottom) indicate average waves.

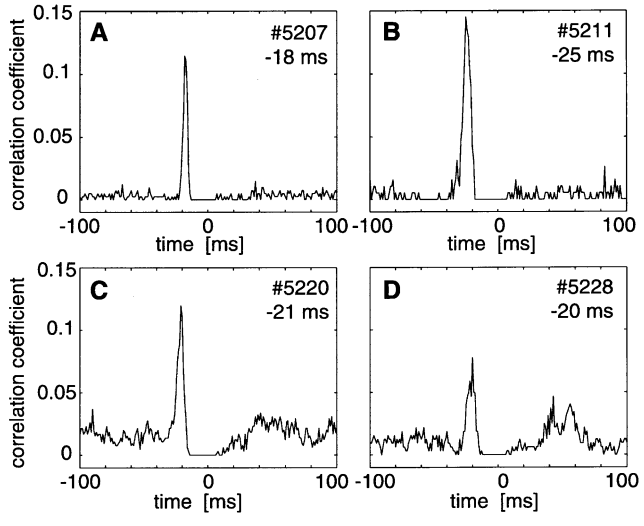

Fig. 2. Cross correlation functions between ON- and OFF-spikes in four quail ON–OFF retinal ganglion cells that exhibited highly correlated OFF/ON responses (#5207, 5211, 5220, and 5228). Time 0 corresponds to ON-spikes, and the correlation coefficients indicate the incidence probability of OFF-spikes normalized to the number of ON-spikes. Coincidence bin is 1 ms.

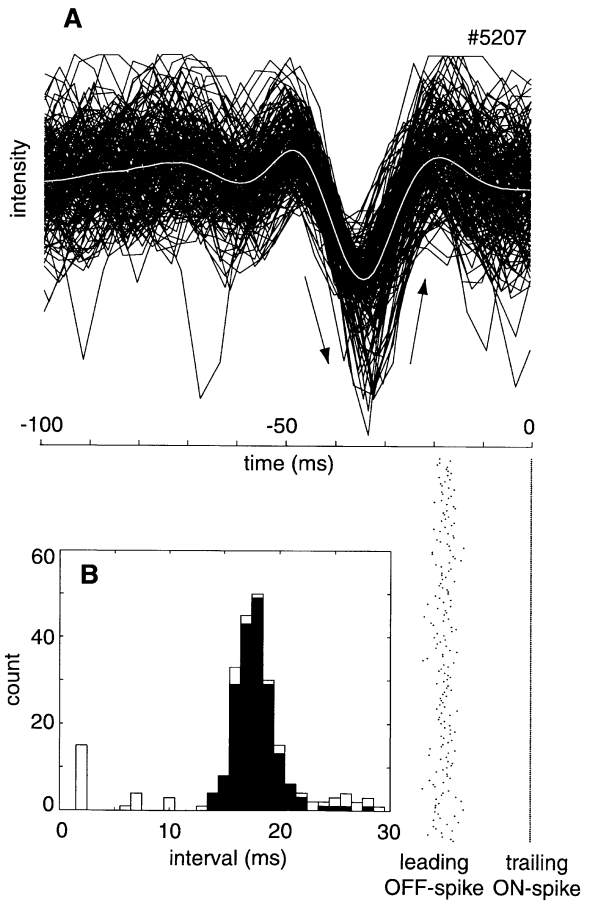

Fig. 3. A. Analysis of 184 stimulus waves evoking OFF/ON spike pairs with 14–22 ms interval in a quail ON–OFF cell (#5207). OFF/ON spike pairs are shown in a raster. Stimulus waves and raster were triggered by ON-spikes. The white line indicates the average. B. Interspike interval histogram. Filled areas indicate OFF/ON intervals, and open areas indicate non-OFF/ON (mainly OFF/OFF) intervals.

sorting all of the waveforms into two groups based on their slope. Finally, individual evoked spikes were identified as ON- or OFF-spikes, based on the slope of the stimulus waves.

3. Results

A total of 38 ON–OFF retinal ganglion cells from 27 quails were analyzed (Fig. 1A). The ON–OFF cells responded vigorously to randomly flickering light stimuli modulated with a Gaussian white noise (Fig. 1B). The average firing rate of the 38 cells during flickering stimulation was 9.4 ± 6.1 Hz. Most ON–OFF cells dynamically adapted to stimulus amplitudes within a 20–30 dB range, and responded with similar strength. Spike-triggered analysis of stimulus waves revealed that spikes were evoked 15–50 ms after an increase or decrease in intensity (Fig. 1C). Because the spike-triggered stimulus waves have steep positive or negative slopes, almost all spikes could be clearly

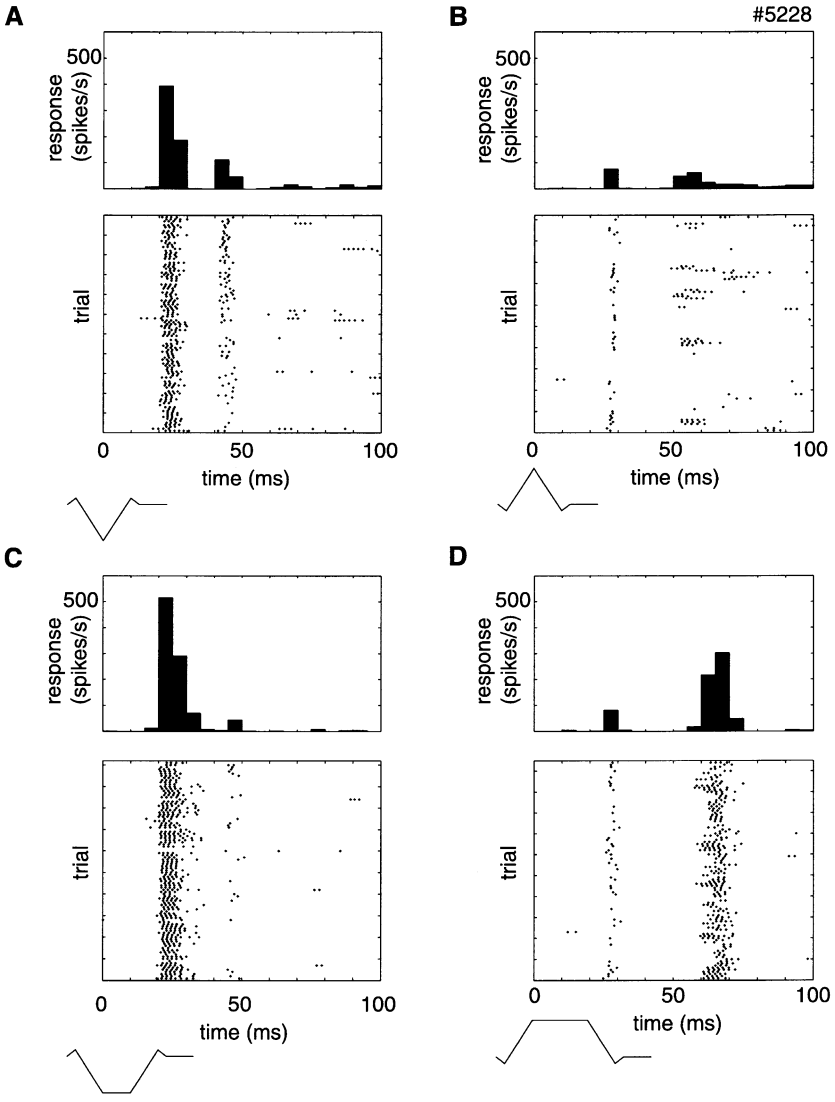

Fig. 4. Peristimulus time histogram (PSTH, top) and raster (middle) representations of responses to multiphase ramp stimuli (bottom) recorded from a quail ON–OFF retinal ganglion cell (#5228). Ten different shapes of ramp stimuli were presented in a random order with a random interval, and each stimulus was presented approximately 100 times. Each stimulus consisted of 3-, 10-, 10-, and 3-ms ramp waves with an interval (0 (A, B), 5 (not shown), 10 (C), 15 (not shown), or 20 (D) ms) between the two 10-ms ramp phases. The slope of the first and last 3-ms waves was opposite and half of that of the 10-ms waves. PSTHs and rasters were triggered at the beginning of the third phase of each stimulus. Time bins of PSTHs are 5 ms.

assigned as ON- or OFF-spikes (Fig. 1B). The response latency varied from cell to cell. In the cell in Fig. 1, ON-spikes were evoked 20–34 ms after an intensity increase and OFF-spikes were evoked 17–32 ms after an intensity decrease. Although the ON-spike/OFF-spike ratio varied from cell to cell, the cells that exhibited highly correlated responses to an OFF/ON sequence of stimuli (described below), predominantly fired OFF-spikes.

Crosscorrelation analyses for identified ON- and OFF-spikes revealed that ON- and OFF-spikes were correlated in most ON–OFF ganglion cells (23 out of 38 cells), and that the correlation was highly asymmetrical. There was a significant number of ON-spikes occurring approximately 20 ms after an OFF-spike, and the crosscorrelograms showed a sharp single peak at this interval (Fig. 2). The correlational strength and the exact peak interval varied from cell to cell. The average peak interval was 23 ± 5 ms in the 7 ON–OFF cells exhibiting the most distinct OFF/ON spike correlation. Crosscorrelograms also showed that no OFF-spikes were fired 0–15 ms prior to an ON-spike (Fig. 2). Conversely, ON-spikes were complexly inhibited for 15 ms after an OFF-spike. Thus, the ON–OFF ganglion cells fire OFF/ON spike pairs with an interval of approximately 20 ms. The reverse (ON/OFF) sequence was not significantly correlated in any of the cells examined. Autocorrelograms of ON-spikes alone or OFF-spikes alone did not have any significant peaks at or near 20 ms.

Spike-triggered analysis of stimulus waves confirmed that OFF/ON spike pairs with intervals of approximately

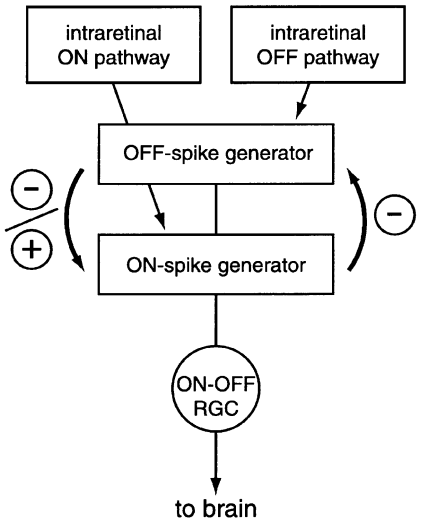

Fig. 5. A model of ON- and OFF-spike generation in the ON–OFF retinal ganglion cell. (−)/(+) indicates biphasic suppressive/facilitative effect, and (−) indicates monophasic suppressive effect.

20 ms were evoked by a successive decrease and increase in stimulus intensity (OFF/ON stimulus sequence) within approximately 30 ms (Fig. 3A). For the ON–OFF cell in Figs. 1 and 3, 94% of spike pairs with intervals of 14–22 ms were evoked by an OFF/ON stimulus sequence (Fig. 3B), and overall firing rate of the OFF/ON spike pairs was 0.6 Hz. Multiphase ramp stimuli were used to confirm that the OFF/ON stimulus sequence specifically evoked OFF/ON spike pairs (Fig. 4A). The reverse (ON/OFF) stimulus sequence evoked fewer ON spikes and very few trailing OFF spikes (Fig. 4B). An OFF/ON stimulus sequence with a 10-ms interval evoked much fewer ON spikes than an OFF/ON stimulus sequence without any interval (Fig. 4C). An ON/OFF stimulus sequence with a 20-ms interval evoked OFF-spikes (Fig. 4D), probably because inhibition by leading ON-spikes against OFF-spikes may become less effective. Thus, OFF-spike-evoking events may first decrease for 15 ms and then increase the firing probability of trailing ON-spikes, while ON-spike-evoking events may decrease the firing probability of trailing OFF-spikes. Such asymmetrical interactions between ON- and OFF-spike generation may underlie the asymmetrical response to the OFF/ON stimulus sequence. Thus, ON–OFF cells may temporally encode OFF/ON sequential changes in light intensity within a range of 20–30 ms with a pair of leading OFF- and trailing ON-spikes with a 20-ms interval. Leading OFF-spikes consisted of a burst of 2–4 spikes in some cells (Fig. 4).

4. Discussion

Retinal ganglion cells receive inputs from bipolar and amacrine cells in the inner plexiform layer of the retina. The inner plexiform layer is functionally segregated into distal OFF and proximal ON sublayers (sublaminae a and b, respectively), where OFF and ON bipolar cell terminals are located, respectively (Famiglietti & Kolb, 1976). Avian ON–OFF cell dendrites ramify in both the OFF and ON sublayers (Ramón y Cajal, 1972). The results of the present study indicate that ON–OFF cells generate OFF- and ON-spikes independently, and that the OFF- and ON-spike generators influence each other asymmetrically and transiently within a fixed time window (Fig. 5). Sakai, Machuca, Korenberg and Naka (1997) reported that spikes are enhanced by a specific green/red sequential change with an interval of approximately 20 ms in an unclassified ganglion cell of a teleost retina. Taken together, these results suggest that retinal neuronal circuits may detect specific sequential features of stimuli.

Barlow (1953) and Lettvin, Maturana, McCulloch and Pitts (1959) consider frog ON–OFF cells to be ‘fly detectors’ or ‘moving-edge detectors’, respectively, based primarily on the high sensitivity of these cells to movement. Also, Pearlman and Hughes (1976) classify a portion of pigeon ON–OFF cells as ‘motion sensitive units’. The results of the present study suggest that ON–OFF cells encode more specific stimuli than simply general movement, and that temporal coding is utilized in very early stages of the vertebrate visual system, as Berry and Meister (1998) and Berry, Warland and Meister (1997) predicted from the precise timing of the spike trains of the retinal ganglion cells. This temporal coding mechanism at the level of the retina may facilitate the transmission rate of environmentally relevant information to higher areas of the visual system (Softky, 1996). This idea is supported by the finding that the interval of the spike pairs and the duration of the OFF/ON sequencial change occurs on an ecologically relevant time scale (approximately 30 ms) as has been observed in other systems (Buracas & Albright, 1999; Rieke, Warland, de Ruyter van Steveninck & Bialek, 1997).

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