LIGHT ADAPTATION INCREASES RESPONSE LATENCY OF ALPHA GANGLION CELLS VIA A THRESHOLD-LIKE NONLINEARITY

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Abstract—Adaptation is an important process of sensory systems to adjust sensitivity to ensure the appropriate information encoding. Sensitivity and kinetics of retinal ganglion cell (RGC) responses have been studied extensively using a brief flash superimposed on different but steady backgrounds. However, it is still unclear if light adaptation exerts any effect on more complex response properties, such as response nonlinearity. In this study, we found that the latency of spike responses to a repeated flashing spot stimulation increased by 30 ms in the mouse ON a latency of spike responses to a repeated flashing spot (An ON-type RGC is excited when a spot is turned on in the center of its receptive field). A single dimming event preceding the test flash on a steady adapting background could also produce similar effect in increasing latency of light responses. A simple computational model with a linear transformation of the light stimulus and a threshold-like nonlinearity could account for the experimental data. Moreover, the strength of the measured nonlinearity and the response latency were affected by the duration of light adaptation. The possible biological processes underlying this nonlinearity were explored. Voltage clamp recording revealed the presence of the increase in latency and threshold-like nonlinearity in the excitatory input of RGCs. However, no comparable nonlinearity was observed in the light responses of the ON cone bipolar cells. We further excluded GABAergic and glycnergic inhibition, N-methyl-D-aspartate receptor rectification and voltage-gated Na+ channels as potential sources of this nonlinearity by pharmacological experiments. Our results indicate the bipolar cell terminals as the potential site of nonlinearity. Computational modeling constrained by experimental data supports that conclusion and suggests the voltage-sensitive Ca2+-dependent vesicle release in the bipolar cell terminals as mechanistic basis. © 2013 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: alpha retinal ganglion cell, mouse, light adaptation, nonlinearity, response latency.

INTRODUCTION

Our visual system operates over an enormous range of light intensities, much larger than its coding range. Light adaptation maintains the efficiency of coding by continuously altering the rules by which a neuron responds to input. Many previous studies investigated changes in two aspects of neural responses: sensitivity and kinetics, using stimulus protocols such as a brief incremental flash superimposed on a steady background of different intensities. Backgrounds with higher light intensities were shown to reduce the sensitivity and to accelerate the kinetics of light responses (Dowling, 1967; Dunn et al., 2006, 2007; for review see Shapley and Enroth-Cugell, 1984).

However, besides sensitivity and kinetics, there are other factors that contribute to the response dynamics of retinal neurons, including response nonlinearity. For example, one of the earliest reports of nonlinear processing in the retina is the nonlinear spatial summation in the Y-type retinal ganglion cells (RGCs) (Enroth-Cugell and Robson, 1966). This nonlinearity is present in almost all vertebrate species studied (cat: Enroth-Cugell and Robson, 1966; guinea pig: Demb et al., 1999; salamander: Ólveczky et al., 2003; primate: Petrusca et al., 2007). A recent study demonstrated that this type of nonlinearity also exists in mouse ON α RGCs and enables the neurons to discriminate much finer spatial structure than its receptive field (Schwartz et al., 2012).

Despite the importance of both light adaptation and nonlinearity for retinal processing (for review see Rieke and Rudd, 2009; Golisch and Meister, 2010; Baden et al., 2013b), the relationship between these two has not been well studied, e.g. how is the nonlinearity modulated by adaptation? This question was explored in the context of adaptation to temporal contrast (Baccus and Meister, 2002). The authors presented white noise stimulus with different contrasts, and used linear–nonlinear (LN) model to analyze the responses of retinal neurons (Chichilnisky, 2001). They found the...
nonlinearity in the LN model is changed during contrast adaptation. However, the biological mechanism of the nonlinearity was not revealed in their study. On the other hand, although a number of studies explored the mechanisms of nonlinear processing in the retina (e.g. Demb et al., 2001; Sampath and Rieke, 2004; Baccus et al., 2008; Jarsky et al., 2010; Liang and Freed, 2010; Baden et al., 2011; Borghuis et al., 2013), the relationship between nonlinearity and adaptation was not investigated.

In the present study, we report that light adaptation enhances a threshold-like nonlinearity to increase the latency of light responses in α RGCs in the mouse retina. To pinpoint the site of this nonlinearity, we ruled out synapses between photoreceptors and bipolar cells, inhibitory inputs, N-methyl-D-aspartate (NMDA) receptor, voltage-gated Na⁺ channel-mediated responses and the threshold for spike generation in RGCs. With data showing the nonlinearity present in the excitatory input to RGCs, our results indicate that the nonlinearity mediates the increase in latency resides in the bipolar cell terminals.

**EXPERIMENTAL PROCEDURES**

**Whole-mount retina preparation**

The C57BL/6 mice older than 30 postnatal days were used in this study. Use and handling of animals were strictly in accordance with the guidelines of the Institute of Biophysics Chinese Academy of Sciences and with the Society for Neuroscience’s policies on the use of animals and human subjects in neuroscience research [Approval No. SYXK (SPF) 2007-119]. All experimental procedures have been previously described (Weng et al., 2005) and are briefly summarized here. Animals used to record light responses were dark adapted for at least 1 h, deeply anesthetized with an i.p. injection of ketamine (50 mg kg⁻¹) and xylazine (10 mg kg⁻¹) (Sigma, St Louis, MO, USA) and decapitated. The eyes were cut temporally to preserve the retina’s orientation and were enucleated and transferred to a Petri dish containing Ames’ medium equilibrated with 95% O₂ and 5% CO₂. The retina was carefully dissected from the pigment epithelium, and attached, ganglion cell side up, to a piece of black Millipore filter paper (AABP02500, Millipore, Billerica, MA, USA) with a 2-mm-diameter hole in the center for adequate infrared illumination and visual stimulation. The whole-mount retinal preparation was then transferred into a recording chamber (0.5 ml in volume) on the fixed stage of an upright microscope (E600FN, Nikon, Tokyo, Japan) equipped with epifluorescence and a 40× water-immersion objective (N.A. 0.8) configured for differential interference contrast (DIC). The preparation was continuously superfused with oxygenated bicarbonate-buffered Ames’ medium at 32–35 °C.

For bipolar cell recordings, acute retinal slices were prepared. After the retina was mounted on the filter paper, vertical slices (200 μm) were cut by using a tissue chopper (Narishige, Tokyo, Japan). Slices with the filter paper attached were stabilized using vacuum grease (Dow Corning, Midland, MI, USA) on glass cover slips. This allowed storing slices in a holding chamber with carboxygenated Ames’ medium at room temperature before they were placed under the microscope.

**Patch clamp recording**

Micropipettes were manufactured from thick-walled borosilicate filament glass tubing (1.5 mm outer and 0.86 mm inner diameter; Sutter Instruments Inc., San Rafael, CA, USA) using a Flaming–Brown P97 puller (Sutter Instruments Inc.). Under infrared illumination and visual control using a cooled CCD camera (Sensicam, Cooke, Auburn Hills, MI, USA), a pipette was advanced to the retina using an MP 285 micromanipulator (Sutter Instruments Inc.), and the inner limiting membrane was dissected to expose the somata of several RGCs. RGCs with the largest soma size (≈20 μm) were targeted and their spike activities recorded in loose-patch mode with a pipette (2–4 MΩ) filled with Ames’ medium. Using a flashing spot the ON-type ganglion cells were selected for further recordings. For whole-cell voltage clamp recording, the extracellular pipette was replaced with a patch pipette with 4–7-MΩ resistance filled with intracellular solution (mM): 105 cesium methanosulfonate, 10 tetra-ethylammonium chloride (TEA-Cl), 20 HEPES, 10 EGTA, 5 Mg-ATP, 0.5 Tris-GTP and 2 Lidoacine N-ethyl bromide (pH 7.3 with CsOH, 280 mOsm). In addition 0.5% Neurobiotin (Molecular Probes, Eugene, OR, USA) and 0.1% Lucifer Yellow (Sigma) were added to reveal the dendritic morphology of the recorded cells. The whole-cell configuration was established when the seal resistance was >1 GΩ. The liquid junction potential of 10 mV was always corrected. For whole-cell current clamp recording, pipette solution consisted of (mM): 120 K-glucosone, 5 NaCl, 10 KCl, 1 MgCl₂, 1 EGTA, 10 HEPES, 2 Mg-ATP, and 0.5 Tris-GTP, adjusted to pH 7.2 using 1 M KOH. The liquid junction potential of 14 mV was corrected. The same intracellular solutions and recording procedures were used for voltage/current clamp recordings in cone bipolar cells. Data acquired from the Axopatch 200B amplifier were low-pass filtered at 2 kHz, digitized simultaneously with an A/D converter (Digidata 1320A, Axon Instruments, Union City, CA, USA) and stored on a personal computer. Offline data analysis was done using Clampfit (Axon Instruments) and Matlab (Mathworks, Natick, MA, USA).

**Light stimulation**

Light stimuli were displayed on a computer monitor, driven by a program written in C++, and focused on the retina through a microscope condenser. The display intensity was measured and linearized using a photometer (IL1400A, International Light Technologies, Peabody, MA, USA). The mean intensity was about 15 mW m⁻² at the retina, in the regime of photopic vision (Keat et al., 2001).

Four types of stimuli were presented:
(1) 6-Hz square-wave flicker stimulus with 200-µm diameter.
(2) 50-ms light flash with 200-µm diameter (test stimulus) presented on a constant background (adapting stimulus (AS)).
(3) Same as (2), but the test and the preceding AS were separated by a dimming event with varying durations (see Section ‘A model of delayed responses and the parameters affecting threshold-like nonlinearity’ for details).
(4) Same as (3), but with shorter AS (300 ms).

For protocols (2–4), the AS was either a spot with the same size as the test stimulus (200-µm diameter) or a full-field stimulus (>1 mm). Data collected in both paradigms showed no significant difference (t-test, p > 0.05) and were pooled together.

Since the computer monitor cannot activate the UV-sensitive S-opsins in the mouse retina very efficiently (Wang et al., 2011), we mainly recorded RGCs and cone bipolar cells in the dorsal retina where M-opsins are dominantly expressed in cones (Röhlich et al., 1994; Haverkamp et al., 2005; Breuninger et al., 2011; Wang et al., 2011; Chang et al., 2013). Due to the broad emission spectra of the computer monitor, we did not calculate the corresponding photo-isomerization rates for cones/rods generated by this stimulator.

Computational modeling and data analysis

The basic framework of our model was based on a computational study published previously (Sikora et al., 2005). The model started from an 8-compartment model of a cone bipolar cell. Capacitance and resistance for each compartment are based on the morphology of type 6 cone bipolar cells (Ghosh et al., 2004) which provide excitatory input to a cells (Morgan et al., 2011) and an earlier estimation of the electrical properties of bipolar cell membranes (Olstedal et al., 2007). The input of the model is the conductance change of nonspecific cation channels in the first compartment (dendrite + soma), and is assumed to be a linear transformation of the light stimulus (based on Fig. 6). Therefore we calculated the input as the convolution of the stimulus and the impulse response function of cone bipolar cell, which is estimated using the recorded response to a short light flash (Fig. 2E, inset). In each compartment representing axon terminal of the cone bipolar cell there are three active zones where L-type calcium channels and synaptic vesicles are located. Calcium current through L-type calcium channels is calculated as follows:

$$\text{I}_{\text{Ca}} = g_{\text{Ca}} m^2 (V - E_{\text{Ca}}),$$

with $g_{\text{Ca}}$ the maximal conductance of the channels, $m$ the gating variable, and $E_{\text{Ca}}$ the reversal potential of Ca$^{2+}$. The gating functions for $m$ are estimated by fitting the previous measurement of the voltage dependence of L-type calcium channel current in bipolar cells (Burrono and Lagnado, 2000):

$$\alpha_m(V) = 0.061 \frac{V + 26}{1 - e^{-0.12(V - 26)}},$$
$$\beta_m(V) = 0.058 e^{-0.5(V - 19)},$$
with $V$ the membrane potential of the “terminal” compartment in mV.

Finally, $m$ is determined by the following equation:

$$\frac{dm}{dt} = \alpha_m(V) \cdot (1 - m) - \beta_m(V) \cdot m.$$

Besides L-type calcium channels, we also included calcium-dependent potassium channels in the “terminal” compartments (Turrigiano et al., 1995; Burrone et al., 2002), with potassium current:

$$I_K = g_K c^2(V - E_K),$$

where $g_K$ is the maximal conductance of the channels, $c$ the gating variable, and $E_K$ the reversal potential of $K^+$. The gating equation for $c$:

$$\tau_c(V) \frac{dc}{dt} = c_m(V) - c,$$

where

$$c_m(V) = \frac{10^{10.2 - \frac{V - E_K}{28}}}{1 + 10^{10.2 - \frac{V - E_K}{28}}}$$

and

$$\tau_c(V) = 90.3 - \frac{75.1}{1 + 10^{3.5 - \frac{V}{28}}},$$

with $V$ in units of mV and $\tau_c$ in ms.

In addition to ligand-gated and voltage-gated channels, a leak current is included in the model to

Fig. 2. Dimming event after light adaptation reproduces the deceleration of RGC response latency. (A) Three stimulus paradigms. (B) Peristimulus time histogram of the spike responses recorded from an RGC to three types of stimuli with corresponding colors, aligned to the onset of the incremental flash (arrowhead in A). (C) Excitatory synaptic inputs to an RGC evoked by the corresponding stimuli. Holding potential = -75 mV. (D) Response latencies, quantified as the difference between onset of test stimulus and the time point when 20% of peak response is reached, $n = 6$. Student’s t-test for paired samples: $p = 0.0089$ between conditions (a) and (b); $p = 0.00001$ between conditions (b) and (c). Squares and error bars indicate mean and standard errors, respectively. (E) Simulated linear responses (bottom) to stimuli with varying durations of the dimming event (top, shown with different colors), calculated by the convolution of the stimulus and the impulse response function of a cone bipolar cell, which is estimated using the recorded response to a short light flash (see inset, scale bar = 50 ms). Dashed line represents an arbitrary threshold. With a longer dimming event, the latency of the rectified response (arrowheads) becomes longer. (F) Response latencies plotted against the duration of the dimming event (interval). Statistical significance between latencies at neighboring intervals was determined using paired t-test: from short to long intervals, $p = 0.009, 0.017, 0.166, 0.476$ and 0.037, respectively.
approximate passive electrical properties of the bipolar cell:

\[ I_L = g_L(V - E_L) \]

where \( g_L \) is the leak conductance, and \( E_L \) the reversal potential for this current. \( E_L \) is also the steady-state membrane potential of the bipolar cell without any current input from ligand-gated or voltage-gated channels.

Calcium concentration in the bipolar cell terminal is determined by calcium influx via calcium channels, calcium buffer in the terminal, calcium extrusion pump and calcium diffusion (Sala and Hernandez-Cruz, 1990; Fig. 3. Threshold-like nonlinearity in excitatory synaptic inputs to RGCs. (A) Flashes of three different intensities (0%, 100% and 200% of adapting background) used to probe nonlinearity after varying dimming intervals (top). *Middle*: modeled linear responses to the stimuli (top). Dashed line shows an arbitrary threshold and response after rectification is shown below (cf. Fig. 2E. Note that the response polarity is reversed to match the recorded current inputs to α cells). (B) Recorded excitatory inputs to an RGC elicited by the stimuli in (A). Holding potential = −75 mV. (C) Relationship between the nonlinearity, expressed as linearity index (\( Li = 2R_{AS}/R_{2AS} \), where \( AS \) is the intensity of adapting stimulus and \( R \) is the response amplitude), and the duration of the dimming event. Statistical significance between \( Li \) at neighboring intervals was determined using paired \( t \)-test: from short to long intervals, \( p = 0.010, 0.029, 0.584, 0.242 \) and \( 0.008 \), respectively. (D) The same stimuli as in (A), but with five different intensities and one single interval (100 ms). *Middle and Bottom*: modeled linear responses (middle) and rectified responses (bottom) to the stimuli. (E) Recorded excitatory synaptic inputs to an RGC. Two gray traces represent responses to intermediate intensity levels. *Middle and Bottom*: modeled linear responses (middle) and rectified responses (bottom) to the stimuli. (E) Recorded excitatory synaptic inputs to an RGC. Two gray traces represent responses to intermediate intensity levels. (F) Response amplitudes plotted against intensities of flash stimuli. Two different sizes of adapting stimulus were used: 200 μm (filled circle, solid line) and >1 mm (open circle, dash-dot line). All the data are normalized by the amplitude of response to maximum intensity. Intensity–response function measured under dark adaptation condition is shown as control (open square, dashed line). Statistical significance between dark and light adaptation conditions (for all \( n = 17 \) cells) at different flash intensities were determined using paired \( t \)-test: from low to high intensities, \( p = 1.4 \times 10^{-15}, 4.8 \times 10^{-15}, 1.3 \times 10^{-17} \) and \( 2.7 \times 10^{-4} \), respectively. Error bars represent standard errors.
Nowycky and Pinter, 1993; Wu et al., 1996; Burrone et al., 2002; McHugh and Kenyon, 2004; Schmidt and Eilers, 2009.

The total concentration of calcium buffer in the terminal is 1.6 mM. The chemical equation for calcium buffering:

\[ \text{Ca}^{++} + \text{Buf} \rightarrow \text{CaBuf} \]

with a forward rate for binding calcium of 20 \( \mu \text{M} \text{s}^{-1} \) and a backward rate of 44 \( \text{s}^{-1} \). The calcium extrusion pumps are calcium dependent:

\[ \text{Ca}^{++} \text{extrusion} = \frac{P_{\text{max}}}{K_{P} + \frac{[\text{Ca}^{++}]}{C_{1}}} \]

with \( K_{P} = 10 \mu \text{M} \) the dissociation constant of the calcium pump, and \( P_{\text{max}} = 1.5 \times 10^{4} \mu \text{M} \text{ m}^{2} \text{s}^{-1} \) the maximal extrusion rate.

The diffusion coefficients for \( \text{Ca}^{++} \) and calcium buffer are 220 and 20 \( \mu \text{m}^{2} \text{s}^{-1} \), respectively. For computational convenience, we split bipolar cell terminal into eight discrete regions according to their distances to the calcium channels and assumed there is negligible variation of calcium concentration across a single region (Fig. 9A right). The smallest two regions are within 25

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**Fig. 4.** Threshold-like nonlinearity and response latency is dependent on duration of light adaptation. (A) Excitatory inputs to an RGC evoked by a family of flashes as in Fig. 3D, but with shorter duration of light adaptation (300 ms, top), aligned to the onset of the flash (arrowhead). (B) Intensity–response functions measured under dark adaptation, 300-ms light adaptation (A), and constant light adaptation (\( >3 \text{s} \), Fig. 3D), \( n = 8 \). (C) Response latencies to the strongest test stimuli under three different adaptation conditions. Student’s \( t \)-test: \( p = 0.0009 \) between dark and 300-ms light adaptation conditions; \( p = 0.0046 \) between 300-ms and \( >3 \text{s} \) light adaptation conditions. Error bars represent standard errors.

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**Fig. 5.** Spike generation mechanism of \( \alpha \) cells cannot account for the delayed response. (A) Voltage responses of an \( \alpha \) cell to 20 cycles of square wave current pulses at 6 Hz. (B) Peristimulus time histogram of the spike responses (10-ms time bin) to the 1st pulse and last 5 pulses (average), aligned to the onset of the current pulse. (C) The voltage responses to repeated injection of a previously recorded current response evoked by a light flash in an \( \alpha \) RGC. (D) Peristimulus time histogram of the spike responses (10-ms time bin) in (C) to the 1st pulse and last 5 pulses, aligned to the onset of rising phase (4% of the peak) of the injected current. (E) Latency difference between the 1st pulse and last 5 pulses, measured with the two current injection protocols and with light flashes (cf. Fig. 1D). Boxes and error bars indicate mean and standard errors, respectively. Student’s \( t \)-test: \( p = 0.0002 \) between the current injection protocol in (A) and light stimulation; \( p = 0.0013 \) between the current injection protocol in (C) and light stimulation.
and 50 nm from the calcium channel, and we used [Ca\(^{++}\)] in these two regions for estimation of vesicle release from ultrafast pool and sustained pool, respectively (Sikora et al., 2005).

The dependence of vesicle release on calcium concentration is estimated by a Hill function:

\[
\text{Release rate} = \frac{R_{\text{max}} \cdot [\text{Ca}^{++}]^h}{[\text{Ca}^{++}]^h + K_{\text{Ca}}^h},
\]

with \( h = 3.24 \) reflecting the co-operativity of calcium’s action on exocytosis (Heidelberger et al., 1994; Sikora et al., 2005). It is worth noting that a recent study reported a stronger co-operativity \((h \approx 5)\) in mammalian rod bipolar cells (Jarsky et al., 2010).

After vesicle fusion, the vesicle pool at the release site is replenished with new vesicles at a rate calculated as following (Sikora et al., 2005):

\[
\text{Rate} = (R_{\text{VP}}_{\text{max}} - R_{\text{VP}}(t)) \cdot r_{\text{RC}},
\]

where \( R_{\text{VP}}_{\text{max}} \) and \( R_{\text{VP}}(t) \) are the maximal and current sizes of the release site vesicle pool, respectively, and \( r_{\text{RC}} \) is the replenishment rate constant.

The time-dependent transmitter (glutamate) concentration after a vesicle release event at the position of postsynaptic receptors is estimated using a log normal function (Sikora et al., 2005):

\[
[\text{glutamate}](t) = \left( \frac{A}{(\sqrt{2\pi}w)} \right) \cdot e^{-\left(\ln(t)/tp\right)^2/2w^2},
\]

with \( A = 0.124 \text{ mM/ms}, \) \( tp = 0.135 \text{ ms} \) and \( w = 0.672 \).

Finally, we used a 6-state model of AMPA receptor (Partin et al., 1996) to convert the transmitter concentration into excitatory input to the postsynaptic RGC. This AMPA receptor model starts with a glutamate free state \((R)\), and binding of two molecules of glutamate (intermediate states: \(RG\) and \(RG_2\), \(G\) is glutamate) leads to a conformational transition to a single open state. In the continuous presence of glutamate, receptor can undergo transitions to non-conducting, agonist-bound desensitized states \((R_dG\) and \(R_dG_2\)).

For fitting the model to our data, four free parameters were adjusted, including: the reversal potential of the leak current of bipolar cell; the rate constant for replenishment of the tethered vesicle pool at the bipolar terminal; the rate
constants of glutamate-binding and unbinding for AMPA receptors. To quantify the goodness of fit, we calculated the coefficient of determination ($R^2$),

$$R^2 = 1 - \frac{SS_{res}}{SS_{tot}},$$

where $SS_{tot}$ is the sum of the squares of the difference between the data and its mean, and $SS_{res}$ the sum of the squares of the difference between the data and the model. The better the model fits the data, the closer the value of $R^2$ is to 1.

Furthermore, a simple computational model was constructed to illustrate some basic features of RGCs' nonlinear responses. This model was similar in structure to the linear–nonlinear cascade model (LN model) used for interpreting RGCs' responses to white noise stimulus (Chichilnisky, 2001; Kim and Rieke, 2001; Baccus and Meister, 2002; Wang et al., 2011) and contained a linear transformation and a nonlinearity. For the former, stimulus was convolved with the impulse response function of cone bipolar cell (Fig. 2E, inset) to generate the linearly transformed response; for the latter, a simple threshold was used to rectify the linear response.

To quantify the strength of nonlinearity in $\alpha$ RGCs, the responses to various flash intensities were fitted with the Hill function:

$$R_l = R_{\text{max}} \cdot \frac{I}{I + I_{1/2}},$$

with $R_l$ the amplitude of RGC's response to the stimulus with intensity $I$, $R_{\text{max}}$ the maximal response amplitude, $I$ the intensity of the stimulus, $I_{1/2}$ the intensity of the stimulus eliciting half maximal response, and $h$, the Hill exponent, reflecting the strength of nonlinearity in the intensity–response ($I-R$) function.

Student's $t$-test was used to determine statistical significance between different conditions, with "*" $p < 0.01$ and "*" $p < 0.05$.

**RESULTS**

Repeated flashes increase response latency of ON $\alpha$ cells

RGCs with largest soma size (≈20 μm) and ON-type light response were targeted in the dark-adapted mouse retina. The dendritic morphology revealed by intracellular infusion of Neurobiotin confirmed the A-type cell identity ("$\alpha$-like" cells, Fig. 1A) (Sun et al., 2002). Light responses of RGCs to a 200-μm spot modulated by a 6-Hz square-wave were recorded (Fig. 1). At the onset of the stimulus, responses of RGCs were sustained and unable to phase-lock with the stimulus (Fig. 1B). This is consistent with response properties of the mouse ON-type $\alpha$ cells from a previous report (Pang et al., 2003). After about 1-s stimulation, RGCs followed each stimulus reliably (Fig. 1B). When we examined the temporal kinetics of the RGC spike response, we found that at the end of a 3.3-s stimulation, the RGC stopped firing at the earlier half of each stimulus cycle and the 1st spike latency was about 30 ms longer than that to the 1st flicker (mean ± SEM: 29.7 ± 4.1 ms; $n = 8$) (Fig. 1C, D).

Our finding differs from many earlier studies where faster kinetics was observed with a brighter background (Baylor and Hodgkin, 1974; Enroth-Cugell and Lennie, 1975; Dunn et al., 2007). However, the stimulus used in these studies is an incremental test flash superimposed on a steady background. Therefore, we performed some experiments using identical stimuli (Fig. 2A, stimuli (a) and (b)). Consistent with previous reports, a constant light adaptation did not slow down the response kinetics, showing nearly overlapping rising phase and shorter response duration compared with that recorded under dark adaptation (Fig. 2B–D). To mimic the flicker condition, and at the same time to facilitate comparison with previous stimulus protocol, we added a dimming event of 100 ms before the test flash (Fig. 2A, stimulus (c)). We found responses were delayed to a similar degree as observed with the flicker stimulus (Fig. 2B). Furthermore, the temporal delay exhibited in the spike response was also clearly present in the excitatory current input to ganglion cells measured under voltage clamp mode (Fig. 2C, D).

A model of delayed responses and the parameters affecting threshold-like nonlinearity

To interpret our data, we used a model similar in structure to the LN model, which is able to capture the basic characteristics of RGC responses to time-varying stimuli, such as white noise (Chichilnisky, 2001; Kim and Rieke, 2001; Baccus and Meister, 2002; Wang et al., 2011). The LN model consists of a linear filter that determines the cell's temporal and spatial sensitivity, as well as a static nonlinearity that converts the filtered stimulus into a firing rate. Similarly, our model also contained a linear transformation and a nonlinearity (see Section 'Computational modeling and data analysis'). However, since we were not using white noise stimuli to estimate the model parameters, we referred to this model as generalized LN model, so as to distinguish it from the conventionally used LN model. In our case, the simplest interpretation for the latency increase induced by the dimming event (Fig. 2A, B) only requires a threshold-like nonlinearity: the 100-ms dimming event could either lower the linearly transformed response below threshold (see the model in Fig. 2E) or raise the threshold, to prolong the latency of RGC responses.

We systematically varied duration of the dimming event preceding the test flash, and measured latency of the rising phase of RGC responses (Fig. 2F). The latency increased with lengthening interval to about 100 ms, then began to decrease, as if returning to the dark-adapted condition. Since our model suggested response latency is largely determined by a threshold-like nonlinearity, we went on to test how nonlinearity varied with the interval (Fig. 3A). To quantify the linearity of the responses, we defined a linearity index ($L_i = 2R_{AS}/R_{0,AS}$), where $R_{AS}$ is response amplitude to a flash with the intensity of AS (100% intensity in Figs. 3 and 4). In a linear system, the response should be twice as strong to a flash twice as bright, and $L_i$ should be 1. Consistent with the change of latency, we found the threshold-like behavior of the nonlinearity became...
stronger with increasing interval, reaching the peak at about 50 ms and then gradually decreased after 200 ms (Fig. 3B, C). Both the nonlinearity and latency co-varied with the duration of the dimming interval (Fig. 2E, F): with intermediate interval (50–200 ms) producing strongest threshold-like nonlinearity (Fig. 3C) and longest latency (Fig. 2F).

To better characterize the threshold-like nonlinearity, we measured I–R function at 100-ms dimming interval where the strongest nonlinearity and largest delay in response were measured. Flashes of five different intensities were presented (Fig. 3D). We found the amplitude of the response to AS is only 10% of that to the 2AS, and the 0.5AS flash evoked a sub-threshold response (Fig. 3E). Our data could be accounted for by the generalized LN model with a threshold as the nonlinearity (Fig. 3D). In addition, we examined the dependence of nonlinearity on the size of the adapting stimuli. Two stimulus sizes were used in the above experiment: 200-μm diameter and >1-mm diameter (Fig. 3F). We found the nonlinearity for two conditions were very similar (p = 0.26; t-test): Li = 0.26 ± 0.15 for 200-μm stimulus (n = 7); Li = 0.33 ± 0.13 for >1-mm stimulus (n = 10).

Since the linear filter of RGCs usually extend for about 400 ms (Baccus and Meister, 2002), the difference in responses to a test flash under dark adaptation and a >3-s constant light adaptation followed by a dimming event (Fig. 3E, F) may reflect the difference in linear responses. Therefore, we added another stimulus paradigm with a 300-ms light adaptation. The stimulus within 400-ms time window before test flash is identical between this 300-ms and the >3-s light adaptation. We found RGCs responded to this 300-ms adaptation (Fig. 4B, dash-dot line) with a much weaker nonlinearity (Li = 1.213 ± 0.174 (mean ± SEM)) than that to a >3-s adaptation (Li = 0.215 ± 0.070) (Fig. 4B, solid line; p = 0.0012, paired t-test). In addition to linearity index, we used another measure of response nonlinearity, similar to a previous study on nonlinear retinal processing (Sampath and Rieke, 2004): Hill exponent (h) was extracted after the I–R function was fitted with Hill function (see Section ‘Computational modeling and data analysis’), with higher h indicating stronger threshold-like nonlinearity. Similar to the linearity index, the Hill exponent also suggests that longer light adaptation results in stronger nonlinearity (dark adaptation: h = 1.053 ± 0.237; 300-ms light adaptation: h = 4.187 ± 0.589; >3-s light adaptation: h = 6.514 ± 0.766; paired t-test: p = 0.0038 between dark and 300-ms light; p = 0.0122 between 300-ms and >3-s light). Furthermore, we analyzed response latency to the test flash with strongest intensity under the dark adaptation, the 300-ms and >3-s light adaptation (Fig. 4C). We found that the protocols eliciting stronger nonlinearity caused a larger delay in latency, consistent with the output of the generalized LN model.

Our finding that response nonlinearity depends on adaptation state is in line with a previous study on contrast adaptation in the retina (Baccus and Meister, 2002). In addition, we are also interested in the biological processes underlying this change in nonlinearity. Most importantly, which cellular/molecular mechanisms are responsible for the threshold-like nonlinearity?

**Biological mechanisms of the nonlinearity**

We first asked whether changes in intrinsic properties of RGCs, such as threshold of spike generation, could account for the observed delay in RGC’s response (Kim and Rieke, 2001; O’Brien et al., 2002; Weick and Demb, 2011). To test this possibility, 6-Hz square-wave current pulses were injected into α cells and its voltage responses were measured in whole-cell current clamp mode (Fig. 5A). With repeated stimulation, spike rate of ON-type α cells was reduced, and latency slightly prolonged (Fig. 5B), however, the change in latency was much less dramatic compared with that induced by light flickers (Fig. 5E). The latency difference between the 1st pulse and average of last 5 pulses was 1.0 ± 0.2 ms; whereas the difference between the 1st flash and last 5 was 29.7 ± 4.1 ms. To avoid a potential artifact caused by the fast rising phase of square wave pulses, we injected a previously recorded current response evoked by a flash in an ON α RGC (Fig. 5C). Results similar to square wave current injection were obtained, the difference in latency was 3.3 ± 1.0 ms (Fig. 5D, E).

In addition, as shown previously, under voltage clamp mode and with intracellular voltage-gated sodium channel blocker Lidocaine N-ethyl bromide (QX314-Br), we observed the latency difference in the excitatory input to RGCs just as in the spike responses (Fig. 2C, D). These two experiments indicate the increase in latency is not due to intrinsic properties of RGCs, but takes place presynaptic to RGCs. The same holds true for the threshold-like nonlinearity (Fig. 3E).

Since we found the nonlinearity exists in the excitatory input to RGCs (Fig. 3), we went on to examine whether bipolar cells already receive nonlinear input. We recorded excitatory input to ON cone bipolar cells using the same stimulus paradigm with a dimming event. In contrast to ON α cells, ON cone bipolar cells responded approximately linearly to the stimulus (Fig. 6A). None of the recorded ON bipolar cells (n = 9) reached a comparable level of nonlinearity with RGCs (n = 17; Fig. 6B). Recently, type 6 cone bipolar cells were reported to provide excitatory input to α ganglion cells (Morgan et al., 2011). Unfortunately, we did not identify the morphological subtypes of recorded bipolar cells in this study. Instead, we analyzed the stratification of the bipolar cell axon terminal within the inner-plexiform layer by superimposing the fluorescent image of the recorded cell on the infrared transmission image of the retinal slice. We did not see any difference in response nonlinearity (data not shown). For the bipolar cells most likely to contact α ganglion cells (stratification between 70% and 80%), the linearity index = 1.08 ± 0.18 (mean ± SD, n = 3). This finding demonstrated that the nonlinearity did not arise prior to bipolar cells (Field and Rieke, 2002; Sampath and Rieke, 2004), excluding synapse between photoreceptors and bipolar cells and...
feedback of horizontal cells (Kamermans and Spekreijse, 1999; Kamermans et al., 2001).

Bipolar cells are known to express voltage-gated Na$^+$ channels (Ichinose et al., 2005; Saszik and DeVries, 2012), it is also possible that activation of voltage-gated Na$^+$ channels mediate the nonlinear responses we observed in α RGCs. To examine this possibility, we added voltage-gated Na$^+$ channel blocker, TTX in perfusion medium. The nonlinearity induced by the dimming event was not affected by addition of TTX (Fig. 6 C), ruling out the possibility that the nonlinearity originates from voltage-gated Na$^+$ channel expression in bipolar cells.

Next we asked whether nonlinearity we observed depends on inhibitory synaptic interactions in the inner plexiform layer, where lateral input from various types of amacrine cells give rise to diverse retinal computations (Fried et al., 2002; Ölveczky et al., 2003; Münch et al., 2009). To examine the role of inhibition for this nonlinearity, nonspecific GABA receptor antagonist picrotoxin and glycine receptor blocker strychnine were applied simultaneously through superfusion. We found that removing inhibition from the retinal circuit enhanced the threshold-like nonlinearity (Fig. 7 A, B), if anything, suggesting this nonlinearity does not require inhibitory synaptic interactions (Demb et al., 2001).

Finally, we asked whether the threshold-like nonlinearity is produced by NMDA receptors since NMDA receptors require depolarization to be activated and therefore could function as a threshold (Dingledine et al., 1999; Erreger et al., 2004; Manookin et al., 2010). We blocked NMDA receptors by application of 2-amino-5-phosphonopentanoic acid (AP-5). The threshold-like nonlinearity was not weakened (Fig. 8 A, B).

Our results indicate that the threshold-like nonlinearity arises in the axon terminals of bipolar cells.

**Computational modeling of threshold-like nonlinearity**

To better understand the mechanisms of the nonlinearity explored here, we constructed a biophysical model of bipolar–ganglion synapse constrained by our experimental data. The framework of our model is based on an earlier computational study (Sikora et al., 2005), with a few modifications to make the model more realistic (Fig. 9 A; details see Section ‘Computational modeling and data analysis’). In brief, we simulated a single bipolar cell with 8 iso-potential compartments (1-dendrite + soma; 1-axon branching point; 6-axon terminals) connected by resistors and used the conductance change of nonspecific cation channels in the first compartment (dendrite + soma) as input. Depending on the voltage of the axon terminal compartment, calcium concentration is estimated with realistic calcium buffer, extrusion and diffusion processes reported previously (Sala and Hernandez-Cruz, 1990; Nowycky and Pinter, 1993; Wu et al., 1996; Burrone et al., 2002; McHugh and Kenyon, 2004; Schmidt and Eilers, 2009). Transmitter release rate is nonlinearly dependent on calcium concentration close to the release site (Heidelberger et al., 1994; Sikora et al., 2005). Finally, a 6-state model of AMPA receptor (Partin et al., 1996) was used to convert the transmitter concentration into excitatory current input to ganglion cells.

To constrain our model, parameters were adjusted to fit the RGC excitatory current input measured in our experiments. Exemplary RGC responses to the two stimulus protocols that generate a large difference in response latency were chosen for the fit (Fig. 2 A, gray and red lines)$. Indeed, our model is able to reproduce the rising phases of the recording traces (Fig. 9 B). Moreover, responses of the same model to light flashes with different intensities (Fig. 3 D) displayed clear threshold-like nonlinearity (Fig. 9 D). Finally, the model also replicated the effect of dimming interval on response latency and nonlinearity (Fig. 9 E, F).

To look into possible biological processes underlying this nonlinearity, we compared the I–R relationship for different retinal processes in the model (Fig. 10 A), including: (1) the cation channel conductance in the bipolar cell dendrite (input of the model); (2) voltage response in the bipolar cell terminals; (3) calcium concentration in the bipolar cell dendrite; (4) voltage response in the bipolar cell terminals; (5) calcium concentration in the bipolar cell dendrite; (6) voltage response in the bipolar cell terminals. 

$^1$ For interpretation of color in Fig. 2, the reader is referred to the web version of this article.
Fig. 9. Computational model of threshold-like nonlinearity. (A) The model consists of an eight-compartment bipolar cell (left) with calcium concentration estimated for each “terminal” compartment (right, coded in red; only one “terminal” is shown for simplicity) and postsynaptic sites on the RGCs with only AMPA receptors (see Section ‘Computational modeling and data analysis’ for details). For clarity of this illustration, vesicle release machinery in BC terminals is not depicted. (B) Recording traces of RGCs to two stimulus conditions generating the strongest latency difference (blue and green, see also Fig. 2), superimposed by the model fit (red). (C) Simulated voltage responses of the bipolar cell to two stimulus conditions in (B). The line in gray indicates the threshold of voltage-gated calcium channel (≈−41.1 mV; 5% maximal activation). (D) Simulated responses to a family of flashes with five different intensities as presented in Fig. 3D. (E) Simulated responses to flashes with different intensities after varying dimming intervals (Fig. 3A, B). (F) Response latency (time to 20% peak response) and linearity index plotted against the dimming interval (cf. Figs. 2F and 3C). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
concentration within 25 nm from the active zone in the terminal; (4) glutamate concentration in the synaptic cleft; (5) excitatory current input to RGCs (output of the model, see also Fig. 9D). We found the threshold-like behavior of \( I_{\text{R}} \) relationship was first clearly observed in the variation of calcium concentration, and further enhanced in glutamate release, and finally with a small increase in the excitatory inputs to RGCs. Therefore, our model indicates threshold-like nonlinearity results from the combined effects of the threshold of voltage-gated calcium channels in the bipolar cell terminals and the high co-operativity of the calcium dependence of vesicle release. For the latter, it has been reported that \( \geq 3 \) calcium ions are required to activate the fusion of a single synaptic vesicle (Heidelberger et al., 1994; Sikora et al., 2005; Jarsky et al., 2010). Such superlinear relationship between calcium and vesicle fusion could account for the threshold-like nonlinearity we observed in \( \kappa \) RGCs in this study.

For fitting the model to our data, four free parameters were adjusted (see Section 'Computational modeling of threshold-like nonlinearity'). The questions arise as to whether the parameters we found were unique and whether our results concerning the mechanism of nonlinearity were reliable. To answer these questions, the following analyses were performed: (1) We fixed two of our free parameters, namely, the reversal potential of the bipolar leak current and the rate constant of glutamate-binding for AMPA receptors, and then adjusted the other two parameters to fit the recording traces. The relationship between goodness of fit (coefficient of determination, \( R^2 \)) and the two fixed parameters is plotted in Fig. 10B. We found good fits could be achieved with different sets of parameters (red dots, coefficient of determination > 0.98). We went on to test whether our results concerning the mechanism of nonlinearity (Fig. 10A) changed across different parameters. The results turned out to be rather consistent as long as the quality of fit was good (for population data see Fig. 10C). (2) The parameters of the model were adjusted to fit the recording traces of five other cells. We found similar results for different cells (for population data see Fig. 10D).

**DISCUSSION**

**Localization of the threshold-like nonlinearity**

The origin of \( \kappa \) RGCs’ response nonlinearity in bipolar cells and its threshold-like profile are reminiscent of nonlinear processing in Y-type RGCs, the mechanism of which has been explored extensively since its discovery (Enroth-Cugell and Robson, 1966). The receptive fields of these RGCs were shown to contain small nonlinear subunits and amacrine cells were thought to play the most pivotal role initially because neither bipolar nor horizontal cells were shown to possess nonlinear responses then (Hochstein and Shapley, 1976).
Pharmacological blockade of inhibition in the guinea-pig retina did not affect nonlinearity, indicating bipolar cells as the possible site of nonlinearity (Demb et al., 2001). A recent study on nonlinear receptive field of α cells in the mouse retina demonstrated that superlinear summation took place when two light spots stimulated a single bipolar cell (Schwartz et al., 2012), again indicating bipolar cells as the site of the nonlinear processing. However, direct measurements of nonlinear responses in bipolar cells were not attempted in these studies.

The experiments directly comparing response nonlinearity between bipolar cells and ganglion cells were performed later. In the salamander retina, a threshold-like nonlinearity might account for the phase-independent responses observed in the OFF motion-sensitive RGC to the jittering gratings, a circuit thought to segregate moving objects from background motion (Olveczky et al., 2003). It is reported that an optimal fit of RGC responses can be achieved using recorded bipolar cell responses by simply placing a nonlinearity between bipolar and RGCs (Baccus et al., 2008).

Pharmacological experiments were not performed in this study, therefore, involvement of inhibition was not ruled out. In a recent study directly characterizing transmission from bipolar cells to RGCs (Asari and Meister, 2012), a large proportion of transmission displayed threshold-like nonlinearity and it was unaffected by blocking inhibitory connections. This finding is consistent with that reported in the guinea-pig retina (Demb et al., 2001). However, the spike responses were used to represent RGC activity, therefore the nonlinearity reported might also reflect threshold of spike generation mechanism in the RGC.

In the present study, we directly recorded from ON cone bipolar cells and therefore ruled out the possibility that the nonlinear processing originated from the synapse between photoreceptors and bipolar cells, as well as feedback of horizontal cells. Application of TTX in the perfusion medium ruled out voltage-gated Na⁺ channels in bipolar, amacrine and ganglion cells. Current injections into RGCs ruled out intrinsic membrane threshold. Pharmacological experiments to block NMDA receptors and inhibitory receptors ruled out rectification of glutamatergic receptors, and the involvement of GABA and glycine in producing the nonlinearity. It is worth noting that, due to the long and thin axons of cone bipolar cells, the recorded responses from the bipolar somas may not reflect the current/voltage changes in axon terminals of the bipolar cells (Baden et al., 2011). Therefore, with our experiment on bipolar cells (Fig. 6), we cannot exclude other mechanisms in cone bipolar cells, e.g. spikes in bipolar cells largely originating from the axon terminals, as the potential source of nonlinearity (for review see Baden et al., 2013b). Taken together, our results suggested a threshold-like nonlinearity at bipolar cell terminals.

Interestingly, previous studies also indicated the bipolar cell terminal as the site for other important retinal computations, e.g. adaptation (Jarsky et al., 2011; Oesch and Diamond, 2011; Bolinger and Gollisch, 2012; Ozysal and Baccus, 2012; Garvert and Gollisch, 2013; Nikolaev et al., 2013). The next interesting question will be how different retinal mechanisms work in cooperation to generate the full response dynamics of RGCs.

Light adaptation affected the threshold-like nonlinearity

While past studies investigating adaptation to average intensity showed that brighter background reduced sensitivity and accelerated kinetics of light responses of the RGCs (Dowling, 1967; Dunn et al., 2006, 2007), effects of adaptation on response nonlinearity have not been systematically investigated (but see Baccus and Meister, 2002). Our study demonstrated that the duration of adapting light strongly affected the strength of nonlinearity (Fig. 4).

Our findings can be understood with a threshold placed on bipolar cell responses. The membrane potential of ON cone bipolar cells first depolarized and then gradually hyperpolarized to sustained light stimulus; at the termination of the stimulus, membrane potential hyperpolarized to a level lower than resting potential (after-hyperpolarization) and then gradually recovered (Fig. 11B, see also Awatramani and Slaughter, 2000; Euler and Masland, 2000; Breuninger et al., 2011). We simulated the responses to the flash after different lengths of adaptation (Fig. 11C). Comparing case 1 (300-ms light adaptation) and 2 (>2-s light adaptation), with a longer adaptation in case 2, hyperpolarization is much more dramatic, yielding a much weaker response with a low-intensity stimulus. When comparing cases 2 and 3 (dark adaptation), with a longer recovery in case 3, the membrane potential largely returned to the resting level, therefore the threshold did not have a pronounced effect. As we have demonstrated, this threshold likely arises in bipolar cell terminal. However, the mechanism of adaptation in bipolar cell is still unclear. It could be due to properties of cone photoreceptors (Soo et al., 2008) or bipolar cell dendrites (Awatramani and Slaughter, 2000).

Comparisons with other threshold-like nonlinearities in retina

A threshold-like nonlinearity at a very early stage of retina processing was elegantly demonstrated before (Field and Rieke, 2002; Sampath and Rieke, 2004). This nonlinearity is located at rod–rod bipolar synapse and dramatically improves absolute sensitivity of rod signals. Based on pharmacological experiments, it was demonstrated that this nonlinearity exhibit a postsynaptic origin. The antagonist of metabotropic glutamate receptors (mGluR6), LY341495, reduced nonlinearity in bipolar cell responses, whereas mGluR6 agonist 2-amino-4-phosphonobutyrate (APB) increased the nonlinearity. In contrast to previous work, we did not observe clear nonlinearity in the ON cone bipolar cells under our stimulus conditions. This discrepancy could reflect either the difference in test stimulus intensity (photopic vs.
Another interesting study explored synaptic transmission from rod bipolar cell to All amacrine cell in the mouse retina by recording in both neurons simultaneously (Jarsky et al., 2011). While stimulating a rod bipolar cell with randomly fluctuating voltage command, excitatory current responses of the postsynaptic All were recorded. Using the LN model, the authors identified a clear nonlinearity in the synaptic transmission between these two cells. By recording calcium current directly in rod bipolar cells, they concluded that the nonlinearity arises downstream of voltage-gated calcium channels. However, since the authors used only electrical stimulation to activate the retinal neurons, it is unclear whether this conclusion can be generalized also for light stimulation.

The most important threshold in the retina is probably the threshold of spike generation in RGCs, which is considered as the central step in turning an analog code into a digital code in visual system. However, this view was challenged recently by direct recordings of spikes in bipolar cells of the vertebrate retina (Burrone and Lagnado, 1997; Protti et al., 2000; Baden et al., 2011, 2013a; Dreosti et al., 2011; Saszik and DeVries, 2012).

Our experiment revealed another possible mechanism that the signal could be rectified by the combination of voltage-gated calcium channels and calcium dependence of vesicle release at the terminals of bipolar cells (see also Jarsky et al., 2011).

### Origin of temporal delay in light responses of α RGCs

One of the most surprising findings is that a dimming event induced a significant delay in RGCs’ response latency. Our model (Fig. 9) offers some insights into the origin of this temporal delay. Bipolar membrane potential in response to light stimuli generating the longest latency delay is plotted in Fig. 9C. In the model: the bipolar cell strongly hyperpolarized at the termination of AS, and when a 50-ms light flash was presented, the bipolar cell gradually depolarized to the threshold of vesicle release. The stronger the hyperpolarization of the membrane potential is, the greater the difference between membrane potential and the threshold will be, therefore stronger stimulus is needed to elevate bipolar membrane potential to the threshold level (stronger threshold-like nonlinearity); and for the same stimulus, a longer time is needed for the rising phase of bipolar response to reach the threshold (longer latency). Any...

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**Fig. 11.** Working model for adaptation state-dependent nonlinearity. (A) ON-type α cell receives excitatory input from ON cone bipolar cells (CBCs) via nonlinear synaptic transmission (red). (B) Voltage response of cone bipolar cells to a 2-s light flash recorded under whole-cell current clamp mode (n = 4 cells, average response is shown in black, and the standard error is indicated with the gray area). Dashed line indicates the resting potential. The light intensity is the same as the adaptation background used in previous experiments. (C) The nonlinearity in A can largely be accounted for by calcium-dependent vesicle release driven by changes in the bipolar cell membrane potential. To the adaptation background, bipolar cells initially depolarized, then the depolarization declined toward resting potential. At the termination, bipolar cells hyperpolarized, longer adaptation induced larger hyperpolarization (middle; gray-dashed line shows a threshold of bipolar cell potential for vesicle release). The responses of RGCs (bottom) evoked by test flashes were more affected by the threshold-like nonlinearity with a longer adapting duration (compare case 1 with case 2), due to larger hyperpolarization of bipolar cells with a longer adaptation. The membrane potential recovered toward resting potential with increasing duration of the dimming event, therefore, the current responses of RGCs was less affected by the threshold-like nonlinearity with a longer duration of the dimming event (compare case 2 with case 3). In this simulation, the time constants for the slow decay phase after light onset and the recovery phase after light offset are 930 and 430 ms, respectively, based on the recorded response of cone bipolar cell to a 2-s light flash (B). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
change in the membrane potential of cone bipolar cell induced by light stimulus pattern will change both the measured nonlinearity and the latency of ganglion cell response. For example, a longer dimming event between adapting and test stimulus (but \( < 100 \text{ ms} \)) results in a lower membrane potential of the bipolar cell, and therefore, stronger nonlinearity and longer response latency (Fig. 5F). Direct recording of cone bipolar cells revealed that at the termination of light stimulus cone bipolar membrane potential strongly hyperpolarized (9.1 \( \pm \) 2.4 mV lower than their resting potential under darkness; Fig. 11B), supporting our idea that strong nonlinearity and long response latency in ganglion cell at light offset are the results of hyperpolarized membrane potential of cone bipolar cells. The reader might be surprised by the length of the delay (\( = 38.7 \pm 5.6 \text{ ms} \) between condition b and c in Fig. 2D) because the responses of ganglion cells were considerably faster (the duration from 10\% to 90\% of peak response \( = 26.8 \pm 3.2 \text{ ms} \) and 18.4 \( \pm \) 3.4 ms for conditions b and c in Fig. 2C), but we have explained, what determined the delay in latency was not the kinetics of ganglion cells responses, but that of bipolar cells (\( t_{10-90} = 52.5 \pm 7.2 \text{ ms} \); Fig. 6A).

### Functional implication of threshold-like nonlinearity on temporal coding

As we have shown, threshold-like nonlinearity plays an important role in producing the delayed responses of \( \alpha \) RGCs. Since the delay is dependent on the duration of dimming stimulus, the information about this duration is carried by the latency of response (Fig. 2F) (Gollisch and Meister, 2008). A stronger nonlinearity can result in a larger dynamic range of response latency and, given a constant noise level, a better temporal coding quality. This is in line with a recent paper reporting that nonlinearity in retinal processing strongly contributes to decorrelation and efficient coding in RGCs (Pitkow and Meister, 2012) and the aforementioned study on nonlinear signal transfer in the rod system (Field and Rieke, 2002).

### CONFLICT OF INTEREST

The authors declare no competing financial interests.

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