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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 α RGCs (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 glycinergic 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 Ca^{++} channels and Ca^{++}-dependent vesicle release in the bipolar cell terminals as mechanistic basis.

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: Ölveczky 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; Gollisch 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

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Abbreviations: AP-5, 2-amino-5-phosphonopentanoic acid; AS, adapting stimulus; EGTA, ethylene glycol tetraacetic acid; HEPES, 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid; *I–R* function, intensity–response function; LN model, linear–nonlinear cascade model; mGluR6, metabotropic glutamate receptors; NMDA, *N*-methyl-D-aspartate; RGC, retinal ganglion cell.

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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 mediating 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 a mixture 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% O_2 and 5% CO_2. 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 Lidocaine *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-gluconate, 5 NaCl, 10 KCl, 1 $MgCl_2$, 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: