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Responses and receptive fields of amacrine cells and ganglion cells in the salamander retina

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ABSTRACT

Retinal amacrine cells (ACs) and ganglion cells (GCs) have been shown to display large morphological diversity, and here we show that four types of ACs and three types of GCs exhibit physiologically-distinguishable properties. They are the sustained ON ACs; sustained OFF ACs; transient ON–OFF ACs; transient ON–OFF ACs with wide receptive fields; sustained ON-center/OFF-surround GCs; sustained OFF-center/ON-surround GCs and transient ON–OFF GCs. By comparing response waveforms, receptive fields and relative rod/cone inputs of ACs and GCs with the corresponding parameters of various types of the presynaptic bipolar cells (BCs), we analyze how different types of BCs mediate synaptic inputs to various ACs and GCs. Although more types of third-order retinal neurons may be identified by more refined classification criteria, our observations suggest that many morphologically-distinct ACs and GCs share very similar physiological responses.

1. Introduction

In the vertebrate retina, the primary information channels are the photoreceptors-bipolar cells (BCs)–ganglion cells (GCs) synaptic pathways, which convey rod and cone signals to the brain (Dowling, 1987). The lateral channels include the cone-horizontal cell (HC) feedback synapse, the HC–BC feedforward synapse, the amacrine cell (AC)–BC feedback synapse and the AC–GC feedforward synapse (Wu, 1994; Zhang & Wu, 2009a). Cones, BCs and GCs exhibit center–surround antagonistic receptive field (CSARF) organization (Kaneko, 1970; Kuffler, 1953; Werblin & Dowling, 1969), the basic template for spatial information processing in the visual system (Hubel & Wiesel, 1962). Center responses are mediated by the photoreceptor-BC–GC channels, whereas the surround responses are mediated by the lateral HC and AC feedback and feedforward synapses (Werblin & Dowling, 1969).

In earlier studies, we characterized the response waveforms, relative rod/cone input, receptive field properties and patterns of dye coupling of the A- and B-type HCs (Zhang, Zhang, & Wu, 2006a; Zhang, Zhang, & Wu, 2006b) and six types of BCs (Zhang & Wu, 2009a) in the salamander outer retina. Here we continue our studies by correlating response, receptive field and morphological properties of various types of ACs and GCs. We also compare results obtained with cell morphology and light response characteristics of ACs and GCs under voltage clamp recorded in living retinal slices, in which the cells' receptive fields could not be measured (Pang, Gao, & Wu, 2002a, 2002b). Since ACs and GCs are postsynaptic to BCs, by comparing response waveforms, receptive fields and relative rod/cone inputs of ACs and GCs with the corresponding parameters of BCs, we are able to postulate how various types of BCs mediate synaptic inputs to different types of ACs and GCs.

It has been shown that vertebrate retinal ACs and GCs exhibit large morphological diversity (Cleland, Levick, & Wassle, 1975; MacNeil & Masland, 1998; Sun, Li, & He, 2002). However, it is not clear whether each of the morphologically-distinguishable ACs and GCs represents a physiologically-distinct type of neuron. In this study, we examined the response waveforms, receptive field properties, relative rod/cone inputs and morphology of 43 ACs and 40 GCs in dark-adapted flat-mounted salamander retinas, and found that the numbers of physiologically-distinguishable AC and GC types are significantly less than the numbers of morphological types, suggesting that many of the morphological types of ACs and GCs may share similar physiological responses.

2. Methods

Flat-mounted, isolated retinas of larval tiger salamanders (*Ambystoma tigrinum*) purchased from Charles E. Sullivan, Co. (Nashville, TN) and KON's Scientific Co., Inc. (Germantown, WI) were used in this study. Animals were handled in accordance with the policies on treatment of laboratory animals of Baylor College of

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Medicine and the National Institutes of Health. Detailed experimental procedures were described in previous publications (Yang & Wu, 1989; Zhang et al., 2006a). Prior to an experiment, the animal was dark-adapted for at least one hour and then decapitated and dissected under infrared illumination with a dual-unit Nitemare (BE Meyers, Redmond, WA). Oxygenated Ringer's solution was introduced to the superfusion chamber at a rate of about 5 ml/min, so that the retina was immersed totally under solution. The control Ringer's contained 108 mM NaCl, 2.5 mM KCl, 1.2 mM $MgCl_2$, 2 mM $CaCl_2$, 5 mM Hepes (adjusted at pH 7.7).

Intracellular recordings were made with micropipettes drawn out on a modified Livingstone puller or Sutter microelectrode puller with single barrel omega dot tubing. The pipettes were filled with 2 M potassium acetate and have resistance, measured in Ringer's solution of 100–600 MΩ. Amacrine cells and ganglion cells were recorded with a microelectrode amplifier (MEZ-8300, Nihon Kohden). For cell morphology and dye coupling studies, microelectrode tips were filled with 3% Neurobiotin in 50 mM Tris and backfilled with 3 M lithium chloride. After physiological experiments, dyes were injected with positive and negative currents (1–5 nA, 3 Hz, 30 min). Then the tissues were fixed with 4% paraformaldehyde for 2 h and were subsequently immunolabeled with streptavidin conjugated Cy-3. Cell morphology and patterns of dye coupling were visualized with a confocal microscope (Zeiss 510).

A new computer-controlled, dual-beam light stimulator with an automated projector head was constructed for experiments that require center and surround light stimuli in flat-mounted retinas. Both light beams pass through interference filters, neutral density filters and apertures of various configurations mounted on motorized wheels controlled by the computer. The receptive field of a given cell was mapped by a moving light bar through the automated projector head in two orthogonal directions, and the cell's receptive field center was determined by the intersecting point of the maximum responses to the light bar in the two directions. The receptive field diameter was estimated by the distance between the light bar positions that generate 5% of the cell's maximum responses. The center light spot (with various diameters) and a concentric surround light annulus (with various inner and outer diameters) were projected to the retina. The intensity of unattenuated 500 nm light (Log I = 0) is 2.05×10^7 photons $\mu m^{-2}\ s^{-1}$.

3. Results

3.1. Response waveforms, morphology, receptive field properties of four types of amacrine cells

Forty-three ACs in the dark-adapted flat-mounted tiger salamander retina were recorded with microelectrodes. ACs are identified by their soma depth (in the proximal half of the inner nuclear layer), dendritic morphology and that they do not bear axons, when viewed after Neurobiotin filling. Four major AC types were distinguished, according to their response waveform, morphology and receptive field properties. The first type is the sustained ON ACs (sON-ACs, $N = 10$), as shown in Fig. 1, which illustrates the voltage responses to a whole-field light step and center/surround illuminations (A), morphology (B), responses to a stepwise moving light bar (C) and to a continuous moving bar in two opposite directions (D). The response–intensity (V–Log I) curves of the responses to 500 nm and 700 nm lights are shown in (E), which give a spectral difference (ΔS, defined as $S_{700} - S_{500}$ (where S_{700} and S_{500} are intensities of 700 nm and 500 nm light eliciting responses of the same amplitude) (Yang & Wu, 1990) of 0.91 (average = 1.15 ± 0.69), indicative of a cone-dominated or rod/cone mixed light input. Neurobiotin staining reveals a dendritic field diameter (DFD) of 420 μm (average = 428 ± 197 μm), and no indications of dye coupling with adjacent cells. These cells exhibited an average

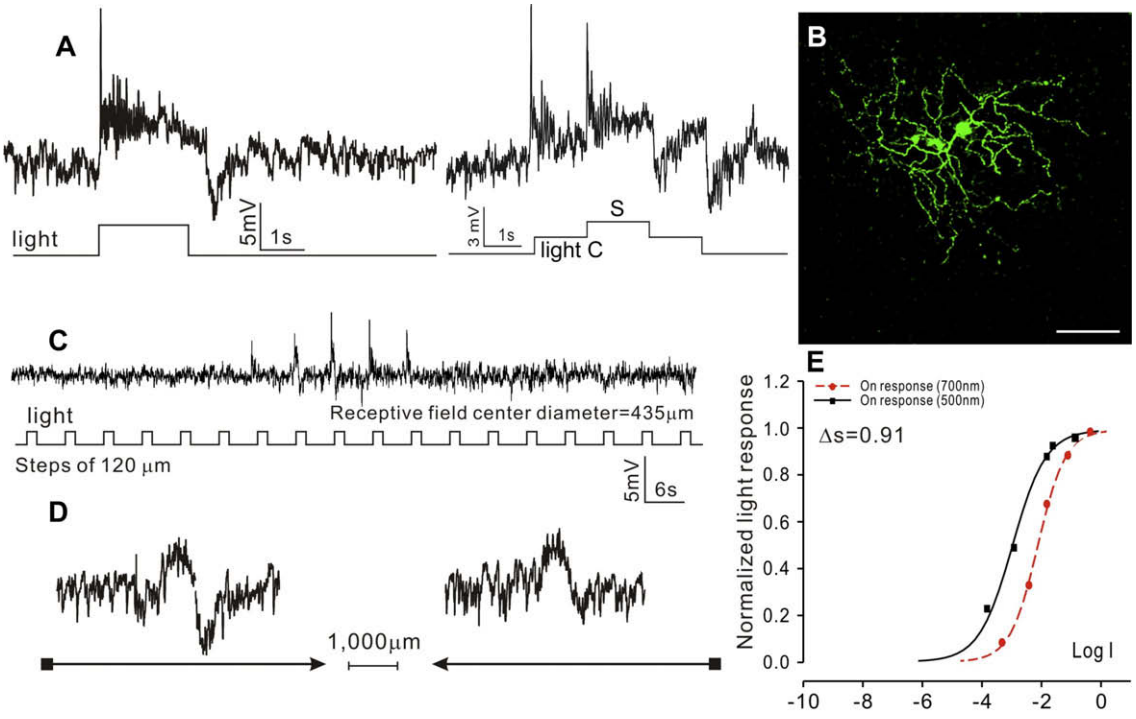

Fig. 1. (A) Voltage responses of a sustained ON AC (sON-AC) elicited by whole-field light step (−3, 500 nm) and by a center light spot (300 μm) and a surround light annulus (700 μm inner diameter, 2000 μm outer diameter) recorded from dark-adapted salamander flat-mounted retinas. ΔS of this AC is 0.91. (B) Fluorescent micrographs of the AC soma and dendrites (confocal images of the inner INL and IPL) stained with Neurobiotin (green) by 10-min dye injection. Dendritic field diameter (DFD) is 420 μm. Calibration bar: 100 μm. (C) Voltage responses of the AC to a light bar moving stepwise (with 120 μm step increments) across the receptive field. The receptive field diameter (RFD) is 435 μm. (D) Voltage responses to a moving light bar in the x and $-x$ directions. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)