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A non-canonical pathway for mammalian blue-green color vision

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Abstract

The dynamic range of visual coding is extended by having separate ganglion cell types that respond to light increments and decrements. While the primordial color vision system in mammals contains a well-characterized S-On center ganglion cell, less is known about S-Off counterparts. We identified a regular mosaic of S-Off ganglion cells in the ground squirrel. Contrary to convention, S-Off center responses come from an S-On bipolar and inverting amacrine cell.

The primordial color vision system in mammals antagonistically combines the signals in S-cones with a combination of medium (M) and long (L) wavelength-sensitive cones to enable blue-yellow discrimination in trichromatic species and blue-green discrimination in dichromats[1]. An S-On/(L+M)-Off ganglion cell whose receptive fields tile the retina is well-described in the primate, and corresponds to the anatomical small bistratified cell that receives its S-cone input via an S-On bipolar cell[2,3]. An S-Off counterpart with similar spatiotemporal properties is expected, but instead, S-Off responses recorded from the retina[1,4–6], optic nerve[7,8], and lateral geniculate nucleus[9,10] demonstrate diverse properties. In addition, the retinal circuitry leading to S-Off ganglion cell responses is unknown. While Off ganglion cell center cone responses are mediated by Off bipolar cells, mammalian retinas do not appear to contain an Off bipolar cell type that exclusively contacts S-cones. S-Off ganglion cells could receive their input via an S-On bipolar cell if signals were inverted by an inhibitory amacrine cell (Supplementary Fig. 1). This novel pathway for producing center responses has not been tested, since S-Off ganglion cell responses are rarely recorded in the isolated retina where straightforward pharmacological manipulations are possible.

Recordings from the ganglion cell axons in the ground squirrel optic nerve show an abundance of S-Off responses[8]. Thus, we used a 512-electrode array[11] to record the spiking activity of hundreds of ganglion cells in the isolated ground squirrel (*Ictidomys tridecemlineatus*) retina. Approximately 90% of ground squirrel photoreceptors are cones, of which 7% are S-cones[12]. The S-cones contact S-On bipolar cells[13]. We classified ganglion

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Author Contributions

The authors contributed equally to this work.

cells according to their spike-triggered average (STA) response to a spatiotemporal white noise stimulus in which the red, green, and blue monitor primaries were independently modulated. We then used the relative STA response to the monitor primaries to separate the cells receiving mostly M-cone input from those selectively sampling S-cones. The former were broadly divided into On- and Off-center types. The latter consisted of just two distinct ganglion cell types: S-Off/M-On and S-On/M-Off (Supplementary Fig. 2). The evidence that S-Off/M-On cells constituted a distinct type comes from the tight grouping of their STA time-courses during simultaneous recordings (Fig. 1a) and a regular tiling of their receptive fields (Fig. 1c; Supplementary Fig. 3). S-On/M-Off cells also comprised a type by the same criteria (Fig. 1b,d). While the polarities of S-On/M-Off and S-Off/M-On cell responses were opposite, other spatiotemporal properties were similar including the time-courses of their S-cone STA responses (Fig. 1a,b,e) and S-cone driven receptive field diameters, which were only ~20% larger in S-On/M-Off cells (Fig. 1c,d). In addition, the component S-Off and M-On fields of S-Off/M-On cells were coextensive (Fig. 1f; Supplementary Fig. 4a), a property shared with primate S-On/(L+M)-Off ganglion cell responses obtained during intracellular recordings[14]. The main difference between the two types involved the M-cone response, which peaked earlier than the S-cone response in S-Off/M-On cells and later than the S-cone response in S-On/M-Off cells (Fig. 1e; Supplementary Fig. 1).

We used pharmacology to identify the pathways that supply signals to S-Off/M-On cells. A combination of metabotropic glutamate receptor antagonist (LY341495, 75–150 μM) and agonist (L-AP4, 50–100 μM) blocks On bipolar cell responses while minimally changing membrane voltage (see Methods). LY341495/AP4 (n=2 retinas) reversibly blocked the STA responses of S-Off/M-On cells, consistent with the center response being mediated by On bipolar cells (Fig. 2). As a control, LY341495/AP4 produced the expected block of M-cone dominated On-center and S-On/M-Off ganglion cell[3] (see Supplementary Fig. 1) responses, while only slightly affecting the responses of M-cone dominated Off-center ganglion cells (consistent with a blockade of crossover inhibition from On circuits). L-AP4 alone (n=2) produced similar results. If S-Off/M-On ganglion cells receive their center signal from an S-On bipolar cell, then the circuit must contain an inverting amacrine cell. Consistent with a role for a glycinergic amacrine cell, strychnine blocked the S-Off but not the M-On responses in S-Off/M-On cells, while minimally effecting the receptive field centers of other ganglion cells. Picrotoxin, a GABA receptor antagonist, had no effect on S-Off/M-On ganglion cells responses (n=2 retinas).

Our results show that a mammalian retina contains S-On and S-Off center ganglion cells with similar spatiotemporal properties and receptive field tiling useful for color vision. S-cone signals are communicated by S-On bipolar cells to S-On/M-Off ganglion cells at sign-conserving glutamatergic synapses[3,14] (Supplementary Fig. 1). We infer that the S-On bipolar cell also signals to a glycinergic amacrine cell, which is predicted to have an S-On response. The S-On amacrine cell makes a sign-inverting synapse either directly onto S-Off/M-On ganglion cells or presynaptically onto On bipolar cells that receive input from M- and possibly S-cones, and which excite S-Off/M-On ganglion cells. Extracellular recordings do not reveal the anatomy of the S-Off/M-On cell, and thus we cannot exclude that it is a type of intrinsically photosensitive retinal ganglion cell (ipRGC), which in the primate carries an S-Off/(M+L)-On signal under photopic conditions[5,6]. However, this is unlikely

since ipRGCs are among the largest ganglion cells in the retina[5], whereas the receptive fields of ground squirrel S-Off/M-On cells were of average size (Supplementary Fig. 4b). This primordial color vision circuit shares features with the sensitive rod pathway, which also contains a single On bipolar cell type. Like the rod pathway, the S-cone pathway may use the amplification provided by On bipolar cell transduction. In the case of S-cone circuits, amplification may serve to compensate for the high M- to S-cone ratio in mammalian retinas[1]. Also, like the glycinergic AII amacrine cell in the rod pathway, the glycinergic interneuron in the S-Off pathway mediates a form of crossover inhibition between an On bipolar cell and either an Off bipolar or ganglion cell[15].

Methods

Tissue preparation and recording conditions

We have previously described the procedures for obtaining pieces of ground squirrel retina[16]. In brief, after euthanasia, eyes were enucleated under dim red light and hemisected around the anterior pole. Following vitrectomy, a piece of superior retina with attached pigmented epithelium was separated from the sclera and placed on the electrode array ganglion cell side down[3]. Experimental use of animals was approved by the Institutional Animal Care and Use Committees at the University of California, Santa Cruz. During the experiment, the retina was superfused with carboxygenated Ames' solution (Sigma-Aldrich, St. Louis, MO) at 30–32 °C. Strychnine and picrotoxin were obtained from Sigma-Aldrich. L-AP4 and LY341495 were obtained from Tocris Bioscience (Minneapolis, MN). Application of the LY341495/L-AP4 mixture resulted in a roughly 40% drop in spike rate in the M-dominated On-center cells in the presence of the visual stimulus, consistent with either no change or a small hyperpolarization in the membrane potential of the On-bipolar cells[17]. For comparison, application of L-AP4 alone led to almost complete silencing of these cells, consistent with a strong hyperpolarization of On-bipolar cells.

Light stimulation and data analysis

The image from a computer controlled color CRT monitor was focused on the photoreceptor layer through the transparent electrode array and the retina. Spatiotemporal white noise, in which the three monitor primaries varied independently, had a 90×90 μm^2 pixel size and a refresh rate of 60 Hz. The mean photon absorption rate by S(M)-cones was equivalent to the one caused by a monochromatic light of 440(500) nm wavelength and 5400(10000) photons-μm^{-2}-s^{-1}. The recorded voltage waveforms were used to sort spikes belonging to individual ganglion cells. STA responses to the spatiotemporal white noise stimulus were calculated for each detected cell. Transformation from the measured response to the green and blue monitor primaries into the S-cone and M-cone responses was calculated using the emission spectrum of the monitor phosphors and S-cone and M-cone spectral sensitivity curves[18] (Supplementary Fig. 2). The response to the red monitor primary color was negligible and was not taken into account in the cone response calculations. The transformation resulted in cone responses consistent with the ones obtained through S- and M-cone isolating stimulus (verified in one preparation). The time-course of a ganglion cell's STA response was calculated from regions within the receptive field center that exceeded background intensity at any time during a response by more than 3 standard deviations. STA

response amplitudes (Fig. 2b) were obtained from the first (closest to the spike) peak in the STA time-course. A two dimensional Gaussian surface was fitted to the STA frame that contained the peak response. Receptive field size was calculated as the diameter of a circle with the area equivalent to the area of the 1−σ contour. The center point of the Gaussian fit was used to calculate nearest neighbor distance (NND). A theoretical prediction for the NND distribution arising from random cell placement was calculated according to the probability function $p(r) = 2\pi\lambda r \exp(-\lambda\pi r^2)$, where r is the distance between cells and λ is the measured cell density[19]. We used a single point Kolmogorov-Smirnov test to assess the difference between the observed and random distributions.

The Electrophysiological Image (EI) of a ganglion cell

We used the unique pattern of electrical activity detected on the electrode array as each ganglion cell produces a spike[20] to track each ganglion cell through different conditions (Supplementary Fig. 5). The EI of a retinal ganglion cell consisted of voltage traces recorded on all 512 electrodes at the time of the cell's spike, and then averaged over all the spikes produced by that retinal ganglion cell in the recording. A typical EI contains large amplitude somatic signals on electrodes close to cell body location and smaller, time delayed, axonal signals on electrodes along the axonal trajectory of the neuron[20]. In EI plots, a filled circle assigned to the location of each electrode in the array corresponds in diameter to the relative amplitude of the signal at that electrode.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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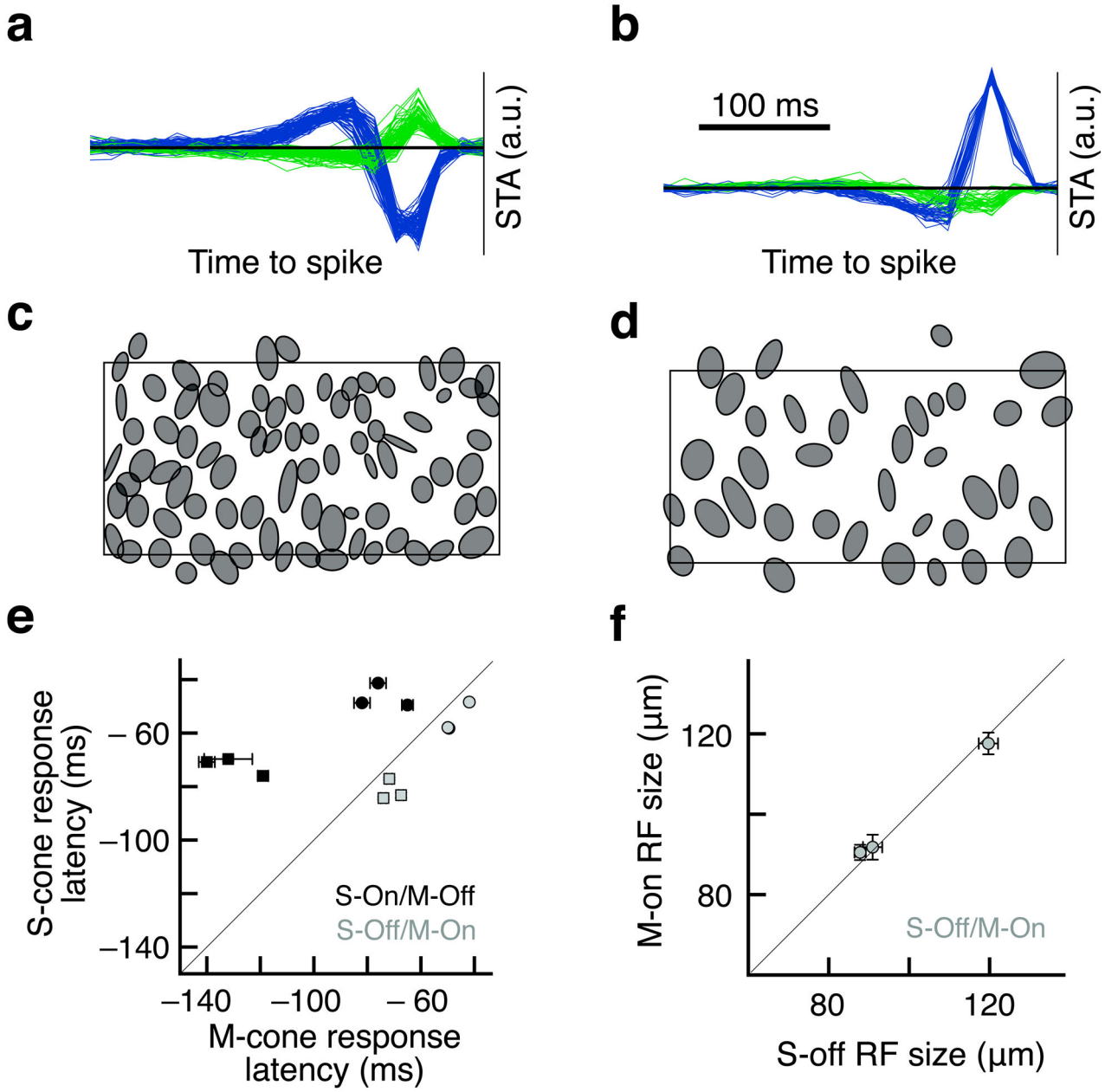

Figure 1.

Ground squirrel retina contains an S-Off/M-On ganglion cell type. Superimposed time-courses of S (blue lines) and M (green lines) cone inputs to S-Off/M-On (**a**) and S-On/M-Off (**b**) ganglion cells in a single retina. 1−σ contours of two-dimensional Gaussian fits to the receptive fields of the same S-Off/M-On (**c**) and S-On/M-Off (**d**) cells. The rectangle is the outline of the electrode array (1800×900 µm^2). **e**) Latencies of S- and M-cone responses in S-On/M-Off and S-Off/M-On ganglion cells. Squares show the time to first zero-crossing and circles the time to the first peak in the STA time-course (plotted results from 3 retinas with a total of 232 S-Off/M-On and 108 S-On/M-Off cells). **f**) Comparison of S-Off and M-

On receptive field sizes of S-Off/M-On cells in the three preparations. Mean ± standard error of the mean.

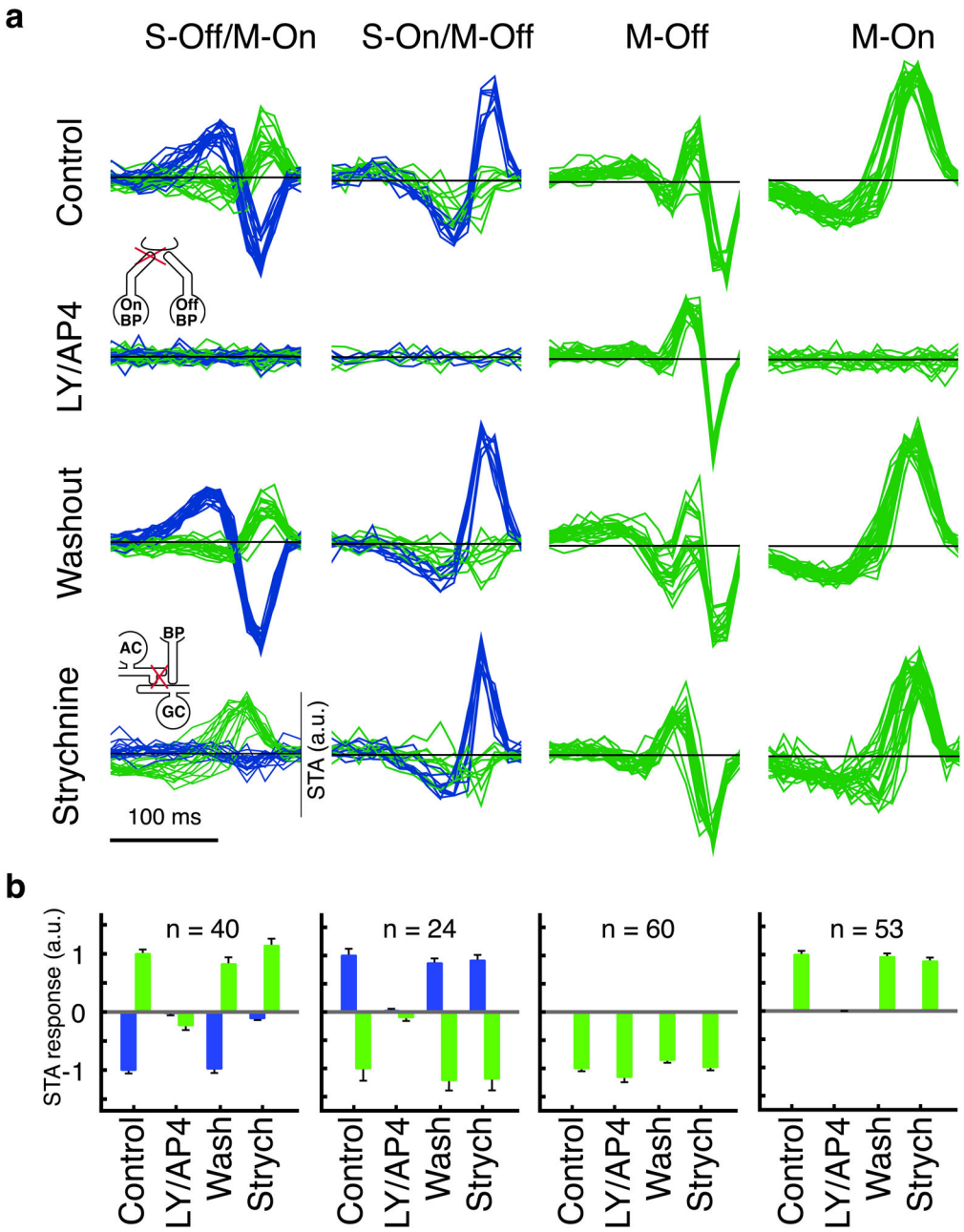

Figure 2.

Pharmacological dissection of S-Off/M-On ganglion cell responses. (**a**) Superimposed time-courses of S-Off/M-On, S-On/M-Off, M-cone dominated Off-, and M-cone dominated On-center ganglion cells simultaneously recorded in a single retina. Responses were obtained in a sequence of four solutions: control, 50 μM L-AP4/75 μM LY341495 (LY/AP4), washout, and 100 μM strychnine. Sites of drug action are diagramed in insets. High drug concentrations were required because the attached pigment epithelium occluded direct access to the retina. The effects of strychnine did not wash out. (**b**) Average amplitudes of S-cone (blue) and M-cone (green) responses for the four ganglion cell groups in the four solutions (data from 3 retinas). ‘n’ indicates the number of ganglion cells in each group.

Response amplitudes were normalized to the control. Only M-cone responses are shown for the M-cone dominated cells. Mean ± standard error of the mean.