Supporting Information

Do and Yau 10.1073/pnas.1304039110

SI Text

We calculated the number of photoactivated melanopsin molecules in the following way. Previously, we found that the light intensity required to produce one single-photon response, I_ϕ, upon illuminating the soma of an intrinsically photosensitive retinal ganglion cell (ipRGC) is $\sim 4 \times 10^5$ photons per square micron (1). Illumination of both the soma and dendrites would increase sensitivity by approximately 10-fold, giving $I_\phi = \sim 4 \times 10^4$ photons per square micron (1). To calculate the number of photoactivated melanopsin molecules during a stimulus, we simply divide the stimulus intensity by the appropriate I_ϕ. Because we observed a large dispersion in I_ϕ values ($1.2 \times 10^4 - 2.7 \times 10^6$ photons per square micron for 17 cells) (1), we consider this a broad estimate.

1. Do MTH, et al. (2009) Photon capture and signalling by melanopsin retinal ganglion cells. *Nature* 457(7227):281–287.

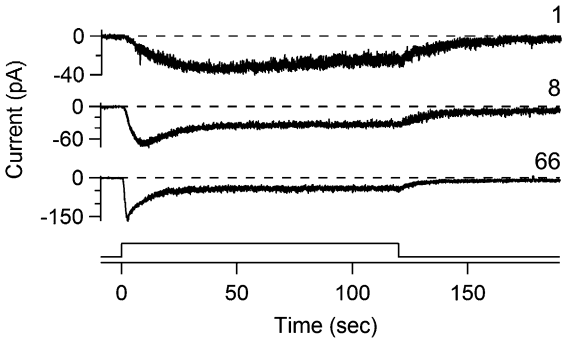

Fig. S1. Responses of an ipRGC to 3-min steps of light. Diffuse illumination with 480-nm light of, from top to bottom, 13,400, 108,000, and 890,000 photons per square micron per second. Relative intensities are indicated on the right. Note the different scalings of ordinates. Perforated-patch, voltage-clamp recording (−80 mV) was obtained in flat-mount retina at 23 °C. Bath solution was Ames medium with blockers of fast-synaptic transmission.

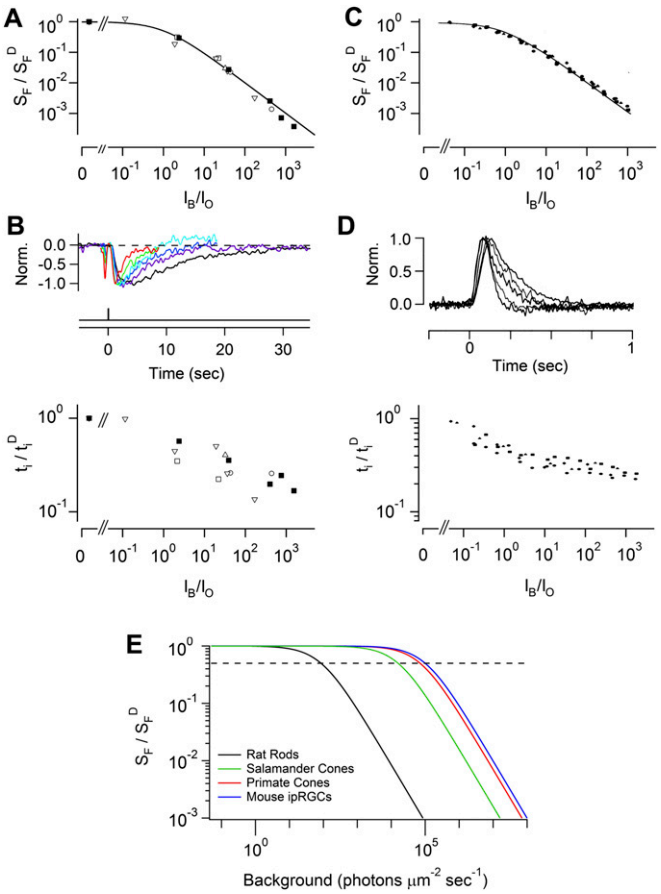

Fig. S2. Comparison of adaptation between ipRGCs and rods and cones. (*A* and *B*) Data on ipRGCs from Fig. 2 *A* and *B* are reproduced here. *C* and *D* are adapted and reproduced with permission from ref. 1, plotted on modified axes for comparison with *A* and *B*. Normalized flash sensitivity (S_F/S_F^D; *Results*) was plotted against normalized background-light intensity (I_B/I_O; *Results*). Fit (solid black line) is the Weber–Fechner relation, $S_F/S_F^D = 1/[1 + (I_B/I_O)]$ (*Results*). (*E*) Weber–Fechner fits to adaptation data for mouse ipRGCs ($I_O = 1.1 \times 10^5$ photons per square micron per second), rat rods ($I_O = 85$ photons per square micron per second) (2), salamander cones ($I_O = 1.6 \times 10^4$ photons per square micron per second) (1), and primate cones ($I_O = 7.1 \times 10^4$ photons per square micron per second) (3) are plotted together for comparison. Background-light intensities are reported in terms of wavelengths at or near the λ_{max} of each photoreceptor. The dashed line corresponds to $S_F/S_F^D = 0.5$, thus giving the respective I_O values. IpRGC data were obtained at 23 °C. At near-physiological temperature, the speed and sensitivity of ipRGCs are higher by approximately threefold (4).

1. Matthews HR, Fain GL, Murphy RL, Lamb TD (1990) Light adaptation in cone photoreceptors of the salamander: A role for cytoplasmic calcium. *J Physiol* 420:447–469.
2. Nakatani K, Tamura T, Yau KW (1991) Light adaptation in retinal rods of the rabbit and two other nonprimate mammals. *J Gen Physiol* 97(3):413–435.
3. Schnapf JL, Nunn BJ, Meister M, Baylor DA (1990) Visual transduction in cones of the monkey Macaca fascicularis. *J Physiol* 427:681–713.
4. Do MTH, et al. (2009) Photon capture and signalling by melanopsin retinal ganglion cells. *Nature* 457(7227):281–287.

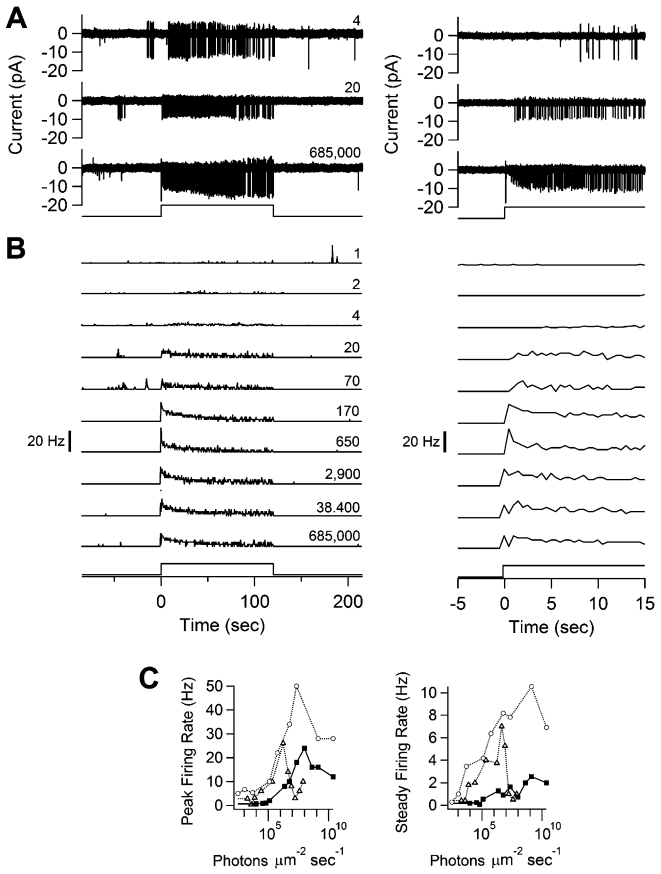

Fig. S3. Spiking of ipRGCs during steps of light at 35 °C. Capacitive/extracellular currents associated with spikes were recorded in loose-patch configuration (V_{hold} = 0 mV; Hepes-buffered Ames intrapipette solution). (*A*, *Left*) Sample traces from an ipRGC, with increasing intensity from top to bottom (diffuse, 480 nm or white expressed as equivalent 480-nm photons, ranging from 3.4×10^4 to 2.4×10^{10} photons per square micron per second). Light monitor is shown at the bottom, with relative intensities marked on the right. (*A*, *Right*) Same traces shown on an expanded time base at light onset. (*B*, *Left*) Peristimulus time histograms (PSTHs) of spike activity for the same cell as in *A*, with relative intensity increasing from top to bottom. PSTHs are derived from single or multiple sweeps, with a bin width of 500 ms. (*B*, *Right*) Same PSTHs shown on an expanded time base at light onset. The spike rate seemingly increases before the stimulus, simply because the stimulus monitor trace has not been binned. (*C*) Plots of peak (*Left*) and steady (*Right*) firing rates versus light-step intensity. The cell from *A* and *B* is indicated with black squares (with some points being unrepresented in *B*); other cells are indicated with other symbols. Peak spike rates were measured at the first transient peak of the PSTH or at maximum if there is no peak. Steady spike rates were measured at the end of the light step. IpRGCs were recorded in flat-mount retina with superfused Ames medium and fast-synaptic transmission blocked pharmacologically (*Materials and Methods*).

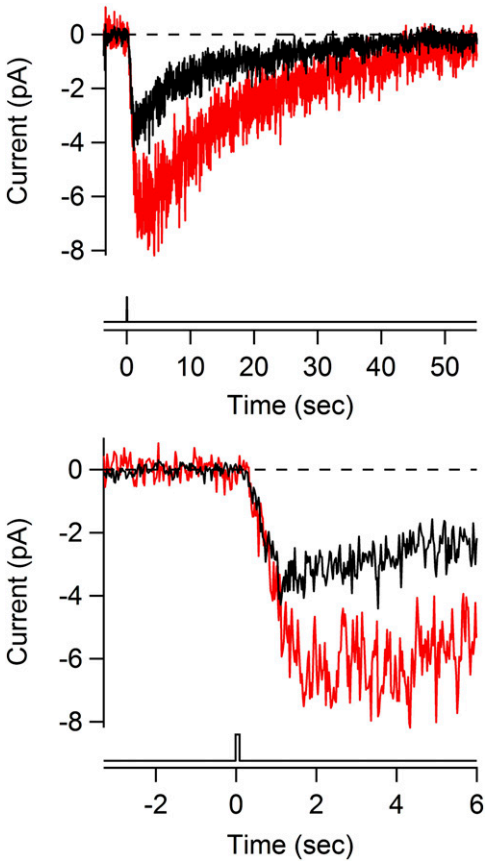

Fig. S4. Effect of Ca^{2+} influx on light responses of dissociated ipRGCs. (*Upper*) Dim-flash response of an acutely dissociated ipRGC in control solution containing 1.5 mM $CaCl_2$ (black trace) versus a solution with $CaCl_2$ replaced by $MgCl_2$ (no EGTA; red trace). (*Lower*) Same responses plotted on an expanded time base for resolution of the rising phase. Stimulus was 50-ms flash of diffuse, 480-nm light delivering 1.1×10^8 photons per square micron. Perforated-patch recording in voltage clamp (−80 mV) was obtained at 23 °C. The current is small (and, therefore, noisy) because the flash intensity is at the low end of this cell's intensity–response relation.