Supporting Information

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SI Materials and Methods

Experimental Procedures—Patch-Clamp Recordings. Measurements of the outer segment dark current from mouse rods were made from 200-μm-thick, dark-adapted retinal slices with patch electrodes as detailed in ref. 1. Briefly, mice were dark adapted overnight and euthanized according to guidelines set by the Institutional Animal Care and Use Committee of the University of Southern California. Under infrared illumination a small piece of retina was embedded in Agar and cut with a vibrating microtome. The resulting slices were transferred into a recording chamber and were superfused with Ames' medium at a rate of 5 mL/min, equilibrated with 5% CO_2/95% O_2 (vol/vol), and maintained at 35–37 °C. Whole-cell voltage-clamp recordings ($V_m = -40$ mV) were used to measure dark current. The normal pipette internal solution for whole-cell recordings consisted of 125 mM K-Aspartate, 10 mM KCl, 10 mM Hepes, 5 mM *N*-methyl glucamine-HEDTA, 0.5 mM $CaCl_2$, 1 mM ATP-Mg, and 0.2 mM GTP-Mg; pH was adjusted to 7.2 with *N*-methyl glucamine hydroxide. Recordings of dark noise were filtered at 300 Hz and sampled at 10 kHz. Further filtering was performed offline as indicated.

Experimental Procedures—Suction-Electrode Recordings. Wild-type (C57BL/6) mice between 2 mo and 6 mo of age were dark adapted 3–5 h in a well-ventilated light-tight plastic box and euthanized according to guidelines set by the Institutional Animal Care and Use Committee of the University of California, Los Angeles. Rods were perfused with DMEM (D-2902; Sigma Chemicals), supplemented with 15 mM $NaHCO_3$, 2 mM Na succinate, 0.5 mM Na glutamate, 2 mM Na gluconate, and 5 mM NaCl, pH 7.4, bubbled with 95% O_2/5% CO_2 at 37–39 °C. This solution had somewhat more $CaCl_2$ and less $MgSO_4$ than the solution used for the dark noise measurements, but these differences are unlikely to have had any significant effect on our conclusions. Recordings were made with suction pipettes as described previously (2). For suction-electrode recordings of wild-type rod single-photon responses, flashes were given at 2-s intervals at a strength that bleached on average $0.7R^*$. Data were acquired at 100 Hz and filtered at 20 Hz (eight-pole Bessel). For the rod in Fig. 4*A*, from a total of 59 flashes there were 27 responses that were identified to be responses to single photons from the first nonzero peak of the amplitude histogram (3), in approximate agreement with the Poisson equation. Similar results were obtained from seven additional rods.

Simulation Protocol. To model the dark noise we generated for each compartment the time course of spontaneously activated PDE $P^*_{sp}(\mathrm{n},\mathrm{t})$, using the Gillespie algorithm (4). To model the single-photon response, we simulated the stochastic number of light-activated PDEs $P^*_{li}(\mathrm{t})$ in the compartment where the photon is absorbed (we assumed absorption at the center of the OS). Finally, with $P^*_{sp}(\mathrm{n},\mathrm{t})$ and $P^*_{li}(\mathrm{t})$ as input functions, we integrated the system of equations for calcium and cGMP (Eq. **3**) and then computed $\hat{I}(n,t)$ and $\hat{I}_{os}(t)$ from Eq. **4**. All simulations were run with Matlab.

1. Okawa H, et al. (2010) Optimal processing of photoreceptor signals is required to maximize behavioural sensitivity. *J Physiol* 588(Pt 11):1947–1960.
2. Woodruff ML, et al. (2008) Modulation of phosphodiesterase6 turnoff during background illumination in mouse rod photoreceptors. *J Neurosci* 28(9):2064–2074.
3. Field GD, Rieke F (2002) Mechanisms regulating variability of the single photon responses of mammalian rod photoreceptors. *Neuron* 35(4):733–747.
4. Gillespie DT (1976) General method for numerically simulating stochastic time evolution of coupled chemical-reactions. *J Comput Phys* 22:403–434.

Other Supporting Information Files

SI Appendix (PDF)