Supplementary Materials

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Supplemental methods

Rhodopsin bleaching assay

Purified rhodopsin was prepared in 2 ml of solubilizing buffer containing 0.02% SM and 20 mM hydroxylamine. The final concentration of rhodopsin was 1 μM. Four hundred micromolar vitamin A and 40 μM vitamin E in 10 μl of ethanol were added to half of the preparations. Preparations were irradiated with light from a 75 W xenon arc lamp that passed through a 331 nm or a 500 nm six-cavity interference filter (10-nm bandwidth at half-maximal transmission; Omega Optical), while open to the air at room temperature (20–22°C). For 500 nm light, quartz neutral density filters (Omega Optical) attenuated the intensity. After each irradiation period, the absorption spectrum was measured to quantify the amount of rhodopsin remaining. In control experiments, negligible bleaching of rhodopsin occurred during incubation with or without vitamin A in the presence of hydroxylamine and vitamin E in darkness for 1 h. The light intensity was calibrated with the photometer (UDT 350).

Single-cell recording and MSP with vitamin A degradation product

Both experiments were carried out according to methods for vitamin A (see Materials and methods) except that vitamin E was omitted. We confirmed that the flash responses of GS rods were unaffected by vitamin E, added after formation of the degradation product.

Fig. S1. Stabilization of vitamin A with vitamin E. Absorption spectra of 10 μM vitamin A in Ringer's containing 1 μM of BSA without (**A**) or with 1 μM vitamin E (**B**) measured 1 min after preparation (black line) or 3 h later (gray line). Ringer's containing BSA was used for baseline measurements. Solutions were kept at room temperature (20–22°C). Initially, the spectral maximum was 327–328 nm. In the absence of vitamin E, the maximum shifted to 289 nm.

Fig. S2. Greater effects of vitamin A + degradation product(s) on flash response properties of a GS rod. Averaged responses to 500 nm flashes before treatment (**A**), during perfusion with vitamin A + degradation product(s) (**B**), and after washing with Ringer's containing 0.8 μM of BSA (**C**). Flashes were given at time = 0 s. Flash strengths were 0.17, 0.71, 2.6, and 41 photons/μm^2 for the untreated and washed families and 11, 41, 98, 360, and 640 photons/μm^2 for the treated family. The maximal responses were 21.5, 8.6, and 21.2 pA for the untreated rod, the treated rod, and the washed rod, respectively. Each trace is an average of 6–11 trials. (**D**) Dim flash response kinetics for the untreated rod (black trace), the treated rod (gray trace), and the washed rod (dashed black trace). (**E**) Stimulus–response relations before treatment (open circles), during treatment (gray circles), and after washing (open triangles) for the rod whose responses are shown in (A–C). Lines show the fits of the Hill equation [eqn. (1)]. For the untreated rod, $i_{0.5}$ = 2.6 photons/μm^2, H = 1.2. For the treated rod, $i_{0.5}$ = 59 photons/μm^2, H = 1.5. For the washed rod,

$i_{0.5}$ = 2.7 photons/μm^2, H = 1.2, indicating full recovery of sensitivity. (**F**) Difference spectra (black lines), estimated from measured spectra shown in Inset, were fit with Gaussian functions (dashed red lines). The average absorption maximum was 313 nm, reflecting the uptake of vitamin A and a degradation product absorbing at shorter wavelength. Inset: Absorption spectra from the outer segment of a GS rod before (black line) and during treatment for 12, 52, and 83 min with vitamin A whose concentration at the start of the experiment was 8 μM (gray lines). MSP and single-cell recording were carried out on different cells.

Fig. S3. Relative changes in flash response parameters of rods and cones caused by 8–10 μM vitamin A. Maximal response amplitude, time to peak, integration time, and $i_{0.5}$ are shown in (**A**–**D**), respectively. Each value is given as a mean ± S.E.M. for the ratio of the parameter during treatment to that prior to treatment (GS rod, n = 5; BS rod, n = 2; RS cone, n = 1; BS cone, n = 2). The time to peak and integration time refer to dim flash responses, whose kinetics match those of the single photon response. The $i_{0.5}$ values of GS rods, BS rods and cones, and RS cone are given for wavelengths near their spectral maxima: 500, 434, and 626 nm flashes, respectively.

Fig. S4. UV photosensitivity of bovine rhodopsin in solution was enhanced by vitamin A. The amount of rhodopsin was plotted on a logarithmic scale as a function of the duration of exposure to 331 nm light in the absence (open black circles) or presence of a predicted [vitamin A] = 4 μM (filled black circles) or to 500 nm light in the absence (open gray triangles) or presence of vitamin A (filled gray triangles). Equivalent exposure durations were calculated by multiplying the actual duration for 500 nm by the ratio of the light intensity at 500 nm to that at 331 nm. Error bars show S.E.M. (n = 4). Lines (continuous lines for presence of vitamin A, dashed lines for absence of vitamin A) show the fits with the single exponential equation: fraction of remaining pigment = $\exp^{(-t/\tau)}$, where t is light exposure duration and τ is the time constant. For 331-nm light bleaching, τ was 26 min without vitamin A and 6.3 min with vitamin A. For 500 nm, τ = 6.7 min without vitamin A and τ = 6.5 min with vitamin A.