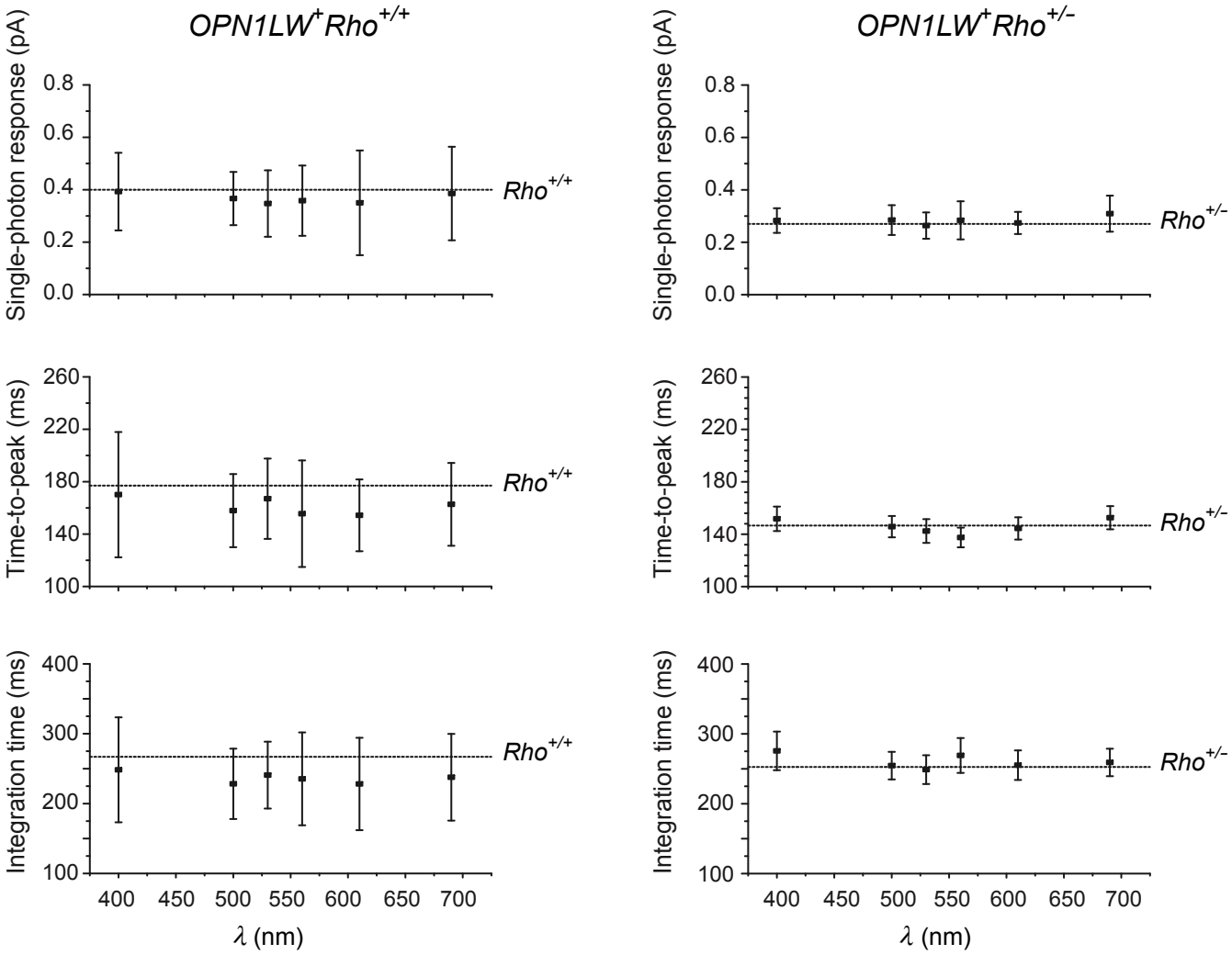
OPN1LW+Rho+/+
OPN1LW+Rho+/-
Single-photon response (pA)
Time-to-peak (ms)
Integration time (ms)
Rho+/+
Rho+/-
λ (nm)

Supplementary Fig. 1. Transgenic *OPN1LW*$^{+}$*Rho*$^{+/+}$ (left column) and *OPN1LW*$^{+}$*Rho*$^{+/–}$ (right column) rods showed invariance in single-photon-response amplitude and dim-flash-response kinetics with stimulating wavelength at 400-690 nm. Repeated dim flashes delivered 65 (400 nm), 24 (500 nm), 32 (530 nm), 152 (560 nm), 3,466 (610 nm) and 634,424 (690 nm) photons μm^{-2}, respectively. Averaged data from 10 *OPN1LW*$^{+}$*Rho*$^{+/+}$ and 11 *OPN1LW*$^{+}$*Rho*$^{+/–}$ rods. Error bars give SEM. According to the spectral templates, light at 610 nm and 690 nm should discriminate for one or the other pigment by 36- and 245-fold, respectively. Based on the expression level of red cone pigment (~0.25% for *OPN1LW*$^{+}$*Rho*$^{+/+}$ rods and ~0.5% for *OPN1LW*$^{+}$*Rho*$^{+/–}$ rods), however, this pigment should hardly be excited at $\lambda < 610$ nm. At 610 nm and 690 nm, 8% and 38%, respectively, of the flash responses should have been triggered by the red cone pigment for *OPN1LW*$^{+}$*Rho*$^{+/+}$ rods, and 15% and 54%, respectively, for *OPN1LW*$^{+}$*Rho*$^{+/–}$ rods. Dashed lines indicate the parameter value for control *Rho*$^{+/+}$ (wild-type) rods (n=10, left) and *Rho*$^{+/–}$ rods (n=11, right).

Comparison of signaling by endogenous rhodopsin and by transgenic cone pigment in *OPN1LW*$^{+}$*Rho*$^{+/+}$ and *OPN1LW*$^{+}$*Rho*$^{+/–}$ rods

Neither the single-photon response amplitude (estimated from variance analysis) nor the kinetics (time-to-peak and integration time) of the dim-flash response of *OPN1LW*$^{+}$*Rho*$^{+/+}$ rods varied with the stimulating wavelength (**Supplementary Fig. 1**, left column), both being similar to *Rho*$^{+/+}$ (dashed lines). The same was found for *OPN1LW*$^{+}$*Rho*$^{+/–}$ rods, which expressed half the normal amount of rhodopsin[1] (**Supplementary Fig. 1**, right column). These results suggested that rhodopsin and red cone pigment signaled identically in a given rod. Other supporting evidence is provided in the main text.

Effect of dark quantal noise on cone sensitivity

In amphibians such as salamander, green rods and blue cones share the same blue pigment[2] and also show similar single-photon-response amplitudes and kinetics[2–4], suggesting that the visual pigment may dictate the response properties of rods and cones. Based on this reasoning, the rather high spontaneous activity of the A_2 pigment in salamander red cones[3,5] is expected to account for the bulk of the sensitivity difference between these cells and salamander red rods under dark-adapted conditions[5]. If A_1 and A_2 red cone pigments were to have similar spontaneous isomerization rates, the same reasoning[5] would suggest ~10-fold out of an overall~100-fold difference in sensitivity (or ~half, because the numbers-of-fold multiply with each other) between primate rods and primate red cones being caused by red cone pigment activity, based on the background-adaptation curve of primate rods[6]. However, given our present finding that A_1 red cone pigment is in fact ~40-fold less spontaneously active than the A_2 counterpart, the dark quantal rate in primate red cones should – even if the above reasoning is

adopted – account negligibly (less than 2-fold) for the overall ~100-fold difference in sensitivity between these cells and primate rods.

From a different perspective, however, we have previously pointed out[7] that the situation with salamander green rods/blue cones may be rather unusual, so the above reasoning based on the comparison of these particular rod/cone types may not be generally valid. Instead, a lower amplification is probably built into each of the phototransduction stages in cones[8], in conjunction with quantitatively different Ca^{2+}-feedbacks[9,10]. If so, regardless of whether the spontaneous isomerization rate of a cone pigment is high or low, a much stronger background light will still be required to produce the same desensitization in cones as in rods; in other words, rods and cones do not operate on the same basic Weber-Fechner adaptation curve (see detailed Discussion in Ref. 7). Applying this alternative viewpoint to primate cones, one can start with the Weber-Fechner relation for background adaptation measured in these cells[11]:

$$S_F = S_F^D \, I_o/(I_B + I_o)$$

where S_F^D is the flash sensitivity in the absence of background light, S_F is the flash sensitivity in the presence of a background light I_B, and I_o is a constant that designates the background intensity required to reduce the cone sensitivity to half of its dark value. For primate red cones, $I_o \sim 26{,}000$ R* sec^{-1} (Ref. 11). In other words, it would require a very large number of R* sec^{-1} in order to decrease the sensitivity of a primate red cone by half; thus, removing an equivalent background light of a mere 8.8 R* sec^{-1} as we obtained (see main text) would hardly increase its sensitivity. This can explain why primate red, green and blue cones all have similar sensitivities irrespective of their difference in visual pigments[11].

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