Supporting Information

Yamaguchi et al. 10.1073/pnas.0809398107

SI Materials and Methods

Fly Stocks. Fly stocks were raised on standard medium under a constant light and dark cycle (14 h/10 h). Flies were raised at 25 °C, 60% relative humidity, unless described otherwise. Mutant lines used are as follows: $rh5^2$ (1), $rh6^1$ (2), sev^{LY3}, UAS-$lacZ^{[melt]}$ (*meltGOF*)(3), *ninaE17* (Berlin background), and *ninaE P[rh1>3]* (4). Gal4 driver and effector lines used were *panR7*-Gal4 driver (3), *rh1*-Gal4 driver (5), and UAS-$shibire^{ts1}$ (6, 7). Wild-type Canton-S (CS) and Berlin were used, and no significant difference was observed between the two wild types in the assays. Mutants are in a CS background unless stated otherwise. All flies are wild type for eye color.

Behavioral Assays. Preference index (PI) was calculated as described in *Results*. The PI ranged from −1 to 1; when all flies preferred longer wavelength (blue > UV or green > blue), PI was 1. When there was no preference (i.e., flies distribute 50:50), PI was 0, and when all flies preferred the shorter wavelength, PI was −1.

In the "light vs. dark" experiments, flies were placed in a T-maze with two tubes, with a light on one side and no light on the other. After the flies were placed at the choice point of the T-maze, they moved toward the illuminated tube when the light intensity was above the phototaxis threshold.

shibire experiments were performed as follows: UAS-shi^{ts} flies (6, 7) were raised at 18 °C until adulthood. Flies were incubated at 34–36 °C for 30 min before the experiments. Experiments were performed at 34–36 °C.

LEDs and Spectroradiometry. An OceanOptics USB2000 spectroradiometer was used. The relative quantal emission and spectral curves of the LEDs were measured with the spectroradiometer, as described previously (1). The relative quantum capture of each subclass of photoreceptors is shown in Fig. 1*C* for UV/B (*Upper*) or for B/G (*Lower*). The spectroradiometer was calibrated using a standard calibration lamp (LS-1-CAL; OceanOptics) for measuring absolute intensities of visible (blue and green) stimuli. Because the calibration underestimates the irradiance of UV light, a photomultiplier (international light, PM270/IL700) was additionally used for the correction of UV irradiance. The irradiance ratio between UV and blue LEDs was measured by the photomultiplier, and the spectral curves measured by the spectroradiometer were corrected accordingly.The relative sensitivity of each subclass of photoreceptors is shown in Fig. 1*C*, which indicates the relative number of quanta captured by each photoreceptor subtype for UV/B (*Upper*) or for B/G (*Lower*). It was calculated as follows: the spectral curves of LEDs measured by spectroradiometer were corrected by the photomultiplier to estimate the precise irradiance ratio between UV/B LEDs (Fig. 1*B*, *Upper*) and B/G LEDs (Fig. 1*B*, *Lower*). The relative sensitivity of each photoreceptor subtype in Fig. 1*A* was taken from refs. 8 and 9 and R. Hardie (unpublished data). For each PR subtype, the PR sensitivity was multiplied by the relative irradiance at each wavelength for each LED, and the value was integrated over all wavelengths.

Intensities of the LEDs were adjusted electronically. The intensities used are as follows: UV—2.1×10^{12} quanta·cm^{-2}·s^{-1}; blue—1.2×10^{14} quanta·cm^{-2}·s^{-1} for UV/B experiments; blue—1.4×10^{12} quanta·cm^{-2}·s^{-1}; and green—1.1×10^{13} quanta·cm^{-2}·s^{-1} for B/G experiments.

Statistical Analyses. For comparison between genotypes, Kruskal-Wallis test was performed to detect overall significance. For pairwise comparisons, Mann-Whitney U test was performed. For all multiple comparisons, Bonferroni correction was applied. In all figures, one, two, and three asteriks indicate an a-level of 0.05, 0.01 and 0.001, respectively. The significance before Bonferroni correction is indicated in parentheses.

Calculating an Additive Model.

For each genotype, the expected relative sensitivities for UV, blue, and green can be calculated by assuming additive contributions and by choosing appropriate weight factors for the photoreceptor types. Taking into account the 30:70 p:y ratios in R7 and R8 as well as the ratio of inner-to-outer photoreceptors (1:6), one would arrive at a sensitivity of S_{WT} = [a*R7p*0.3 + b*R7y*0.7 + c*R8p*0.3 + d*R8y*0.7 + e*(R1–R6)*6] for wild type and S_{melt} = [a*R7p*0.3 + b*R7y*0.7 + c*R8p*1 + e*(R1–R6)*6] for $melt^{GOF}$ flies. The *R* values (e.g., R7p) would represent the sensitivities of the photoreceptor types as shown in Fig. 1*C*, the factors (a–e) would give their relative weights, and the numbers at the end of each term would give their relative abundance in the eye.

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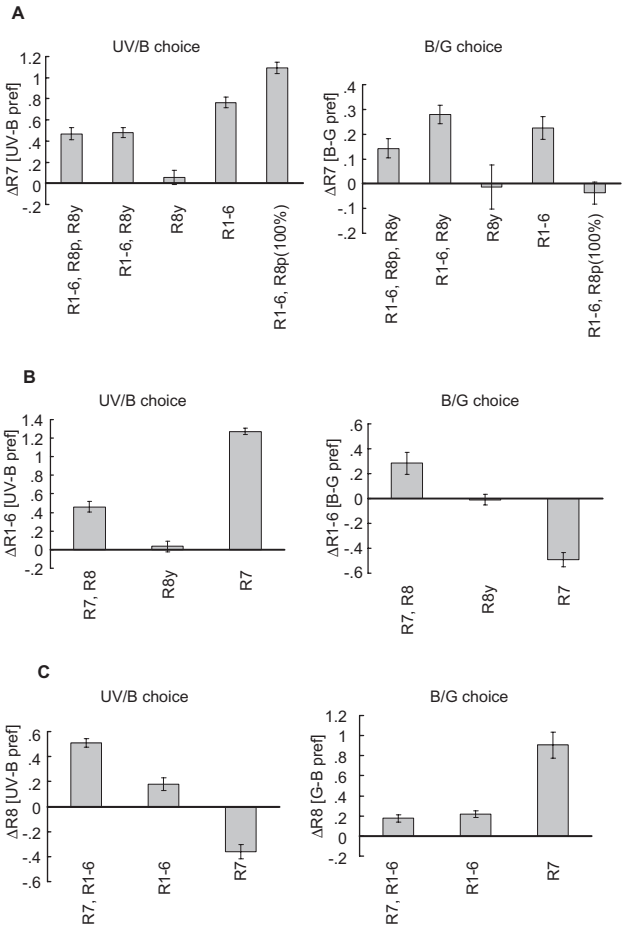

Fig. S1. Effects of adding photoreceptor subsystems on spectral preference in differential phototaxis were calculated by subtracting the score of one mutant from that of another. The other photoreceptor types present in the respective flies are indicated beneath the columns. (*A*) Effects of adding R7 photoreceptors. (*B*) Adding R1–R6. (*C*) Adding R8.