Supplemental Data

Light Activation of an Innate Olfactory Avoidance Response in *Drosophila*

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Supplemental Experimental Procedures

Fly Genetics and Rearing

All transgenic lines were backcrossed for at least five generations into a Canton S (CS) background. The flies used in these experiments contained a single copy of the *Gr21a-Gal4* driver [S1], and two copies of the *UAS-ChR2* responder [S2] transgenes. Genetic controls were bred in parallel and carried either the driver alone or the responder alone. The fly-food recipe used here is composed (per 20 l final volume) of the following: agar, 136 g; cornmeal, 1335 g; yeast, 540 g; sucrose, 320 g; molasses, 1.64 L; $CaCl_2 \bullet 2H_2O$, 12.5 g; Na Tartrate $\bullet$ $4H_2O$, 150 g; Tegosept, 18.5 g; 95% EtOH, 153.5 ml; and propionic acid, 91.5 ml. We supplemented vials containing fly food with ~10 μM all-*trans*-retinal (Sigma) by melting ca. 20 ml of solidified fly food in a microwave oven (full power, ~15 s), allowing it to cool to ~52°C, and adding 100 μl of 20 mM all-*trans*-retinal in 95% EtOH and then rapidly stirring with a spatula, plunging it into an ice bucket, and allowing it to solidify for 5 min. Excess condensation was wiped from inside the tube, and the tube stoppered with a foam plug and stored at room temperature in the dark. These were typically used within 2–3 days of preparation.

Fly crosses were set up in retinal-containing food (or non-retinal-containing, as indicated) and maintained in the dark at room temperature. One to two days after eclosion, mixed male and female flies were transferred to fresh retinal food vials without anesthesia and allowed to age in the dark for 4–6 days prior to use. Flies were transferred to fresh vials after 2 days to minimize attachment of larval-churned food to the wings because this attachment interferes with performance in the T maze. Vials containing obviously "sticky" flies were not used.

Electrophysiology

Flies were mounted on a glass slide as described by Clyne et al. [S3] and viewed with an Olympus BX51WI upright microscope at 1000× magnification. Recordings were performed by insertion of a tungsten electrode into the base of an individual ab1 sensillum in the antenna, with a reference electrode in the eye. The tungsten wire (0.125 mm diameter, Small Parts) was electrolytically sharpened in a 10% $NaNO_2$ solution [S3]. Low concentrations of CO_2 were delivered to the fly antenna with filtered house air mixed with pure CO_2. Electrical signals were recorded with a MultiClamp 700B amplifier, with Digidata 1322A and stored on a PC with pClamp 9.2 software (Molecular Devices). Signals were sampled at 10 kHz and filtered by low-pass (cutoff 2 KHz) and high-pass (cutoff 0.2 kHz) filters with Clampex data-acquisition software. Analysis and plotting of the recording traces were done with Clampfit 9.2 and SigmaPlot 9 software. Spike sorting was performed by hand, with the characteristic spike heights to distinguish the ab1c from the ab1(a + b) responses [S4]; ab1d spikes were too small to count reliably. However, those neurons are known not to respond to CO_2 [S5]. Raster plots for spikes induced by CO_2, and blue lights were generated with MATLAB software.

T Maze Apparatus and Behavioral Testing

All behavioral experiments were carried out in a darkroom under a red-filter safelight. Polycarbonate test tubes (Falcon # 149598) were inserted into a standard T-maze [S6] and fitted with plexiglass sleeves, to which four blue-emitting LEDs (Luxeon, LHXL-LB5C, 30 mW at maximum power) were attached via water-cooled aluminum plates (Figure 3). The control arms were similarly outfitted, but the diodes were not illuminated during the test. Details of construction are available on request. An additional LED attached to

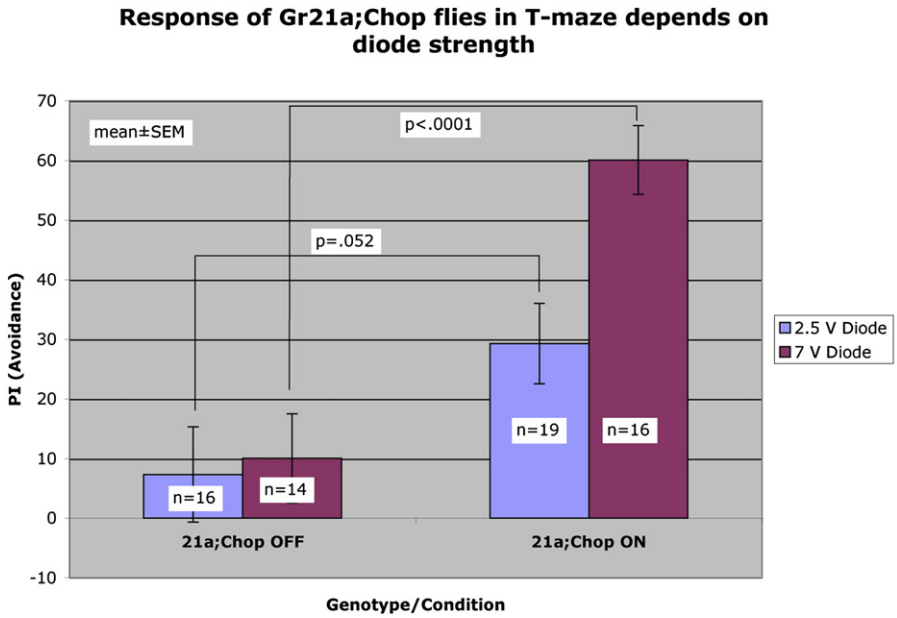

Figure S1. Avoidance of Blue Light Depends on Diode Strength

Flies were tested in a modified T maze equipped with either 2.5 V (blue bars) or 7 V (violet bars) diodes, run at 330 and 700 mA, respectively. A significantly greater level of avoidance was obtained with the stronger diode ($p < 0.0001$). Flies used in these experiments contained a single copy of the UAS-ChR2 responder gene. "21a;ChR2 OFF" and "21a;ChR2 ON" indicate data obtained in the absence or presence of diode illumination, respectively.

an aluminum plate was held in place by hand next to the decision point of the T maze during the test (Figure 3D). Flies (~20–30 per run) were introduced into the elevator of the T maze in the dark and then moved down to the choice position with the diodes illuminated at maximum power (730 mA) for 30–60 s. A separate power supply was used to provide pulsed light to the plate-attached diode during the test period. After the test, flies were collected in the test tubes, anesthetized with CO_2, and counted. The performance index (PI) is calculated as the percentage of flies in the control arm subtracted by the percentage of flies in the test arm.

Wild-type CS flies placed in the T maze exhibited strong attraction to the blue diodes (data not shown). However, control genotypes (Gr21a-Gal4;+ or +;UAS-ChR2), with or without retinal in the food, did not show attraction to the LEDs. The reason for this difference is not clear but may reflect the fact that the levels of *white* expression in these transgenic flies (which express a P{*w*} mini-gene in a w^- background), as assessed by eye color, are substantially lower than in w^+ CS flies and that *w* may be required for efficient phototaxis in the transparent plastic tubes of the T maze because of its role in filtering out offaxis illumination (R. Wolf, personal communication). Consistent with this interpretation, control flies containing P{*w*} minigenes in a w^- background showed stronger phototaxis toward blue light in prototype T mazes in which the arms were opaque.

Supplemental References

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