Supporting Information

Shuai et al. 10.1073/pnas.1107489109

SI Materials and Methods

Odor Dissipation Measurement. Photoionization detector (PID) measurement was performed in a training tube equipped with a copper grid but with no fruit flies loaded. A hole of 3 mm in diameter was drilled at the midpoint of the side of the tube; a corresponding area of the copper grid was removed. The PID nozzle was connected to the hole via a short silicon tube. The air inlet of the PID sensor head was ~2 cm away from the outer surface of the training tube. Odors were brought to the training tube by clean air bubbling through the odor vials. In behavioral experiments, training tubes were attached to odor sources on one end and a suction pump on the other end; here, the open end of the training tube was blocked by a polystyrene board during PID measurement, allowing the pump on the PID to draw the air over the detector (~750 mL/min). During periods before and after odor delivery, clean air was bubbled through a control vial containing only heavy mineral oil to flush the training tube. PID data were acquired at 1 kHz using National Instruments hardware controlled via Matlab. The gain was set to 10× for 3-octanol (OCT) and 4-methycyclohexanol (MCH) and 5× for 1-butanol (BU) and ethyl acetate (EA). For determination of odor dilution curves, all components on the path of odor delivery were washed with 95% ethanol followed by double distilled (dd)H_2O and then dried. No odor contamination was evident because air current passing through odorless solvent did not generate a PID signal (Fig. S1*B*, B1 and B2). Odors were serially diluted, e.g., 10^{-2} odors were prepared by adding 100 μl of 10^{0} odors into 10 mL of heavy mineral oil and mixed by vortexing for 15 s. The control vial containing the solvent was also vortexed in case the shaking introduced an artifact. Measurements were performed serially, from low to high concentration. For ease of presenting in logarithmic scale, all of the data were adjusted by setting the average preodor baseline to 0.1 V.

Confocal Imaging. Female adult brains were dissected in cold PBS and then fixed in 4% paraformaldehyde in PBS for 30 min at room temperature. After three PBS washes of 10 min each, the samples were mounted in FocusClear (gift from Ann-Shyn Chiang, National Tsing Hua University, Hsinchu, Taiwan) and imaged on a Zeiss LSM710 laser-scanning confocal microscope. Image stack was obtained with Imaris 5.0 software (Bitplane) and adjusted in Adobe Photoshop.

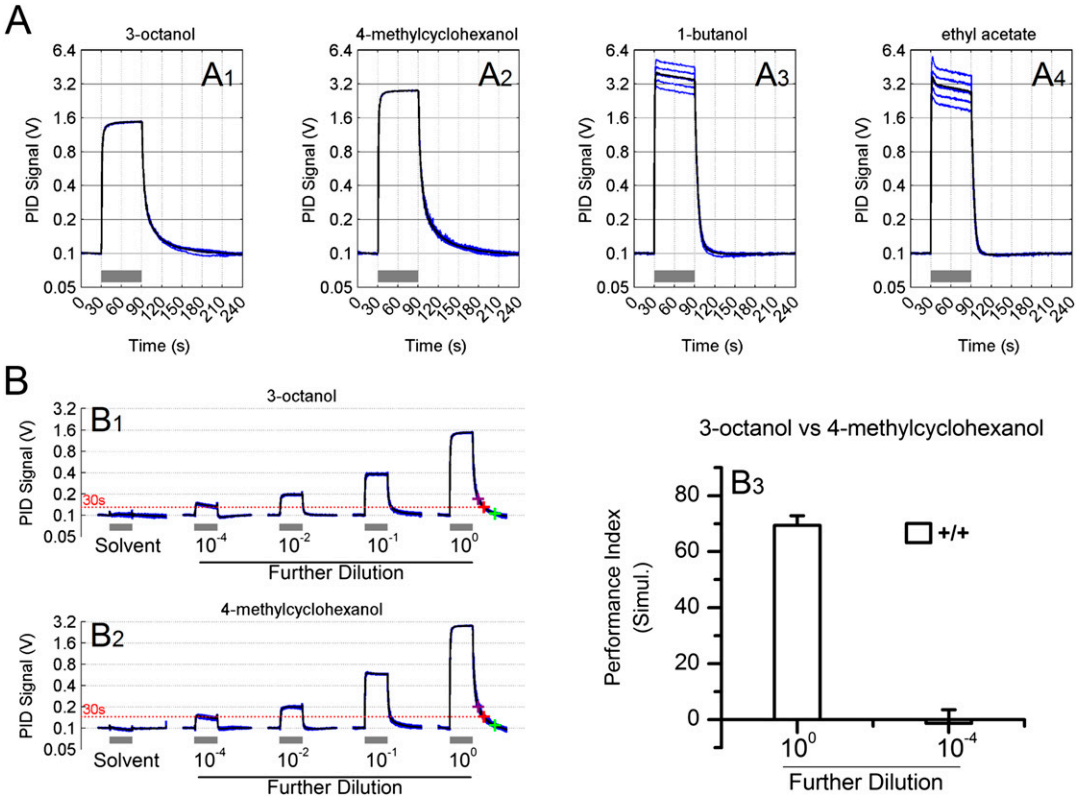

Fig. S1. Odor dissipation in the training tube. (*A*) Time courses of odor dissipation were monitored by a photoionization detector (PID) (Aurora Scientific) (*SI Materials and Methods*). Odor concentrations were the same as those in regular training [(vol/vol): 1.5×10^{-3} for 3-octanol (OCT); 1×10^{-3} for 4-methylcyclohexanol (MCH); 2×10^{-3} for 1-butanol (BU); and ethyl acetate (EA)]. PID response traces from five consecutive presentations were stacked (blue), averaged (black), and plotted on a logarithmic scale for better viewing of the decay phase. Gray bars indicate odor presentations for 1 min, which were followed by a clean air flush at 750 mL/min. Odor concentrations of BU and EA show notable decrements in the course of repetitive presentations. (*B*) Calibration of residual odors. (B1 and B2) OCT and MCH were diluted serially and measured for PID responses. The starting dilution 10^0 is the concentration used in regular training [(vol/vol): 1.5×10^{-3} and 1×10^{-3} for OCT and MCH, respectively]. 10^{-1}, 10^{-2}, and 10^{-4} represents 10^1-, 10^2-, and 10^4-fold of further dilution from this starting concentration. Data from five presentation trials are pooled. Each displayed trace includes a 30-s preodor baseline, 1-min odor presentation, and a 90-s postodor period. PID signal as a function of odor dilution is slightly nonlinear, probably due to a deviation from Raoult's law (1). Residual odor at 15, 30, and 60 s after termination of 10^0 odors is indicated by purple, red, and green +, respectively. The level of residual odor at 30 s is equivalent to that of the 10^{-4} dilution. (B3) Wild-type flies did not show measurable learning performance in simultaneous conditioning (Simul.) at 10^{-4} odors [final concentration in (vol/vol): 1.5×10^{-7} and 1×10^{-7} for OCT and MCH, respectively], suggesting that odors as low as these concentrations are insufficient to support associative learning. $n = 5$. Error bars indicate SEM. A separate study (2) used a slightly different training protocol (odor vs. solvent) but obtained similar results. In that report, flies failed to learn when odors were diluted to 1×10^{-5} (vol/vol) for OCT or 1×10^{-4} (vol/vol) for MCH.

1. Cometto-Muñiz JE, Cain WS, Abraham MH (2003) Quantification of chemical vapors in chemosensory research. *Chem Senses* 28:467–477.
2. Yarali A, Ehser S, Hapil FZ, Huang J, Gerber B (2009) Odour intensity learning in fruit flies. *Proc Biol Sci* 276 16723413–3420.

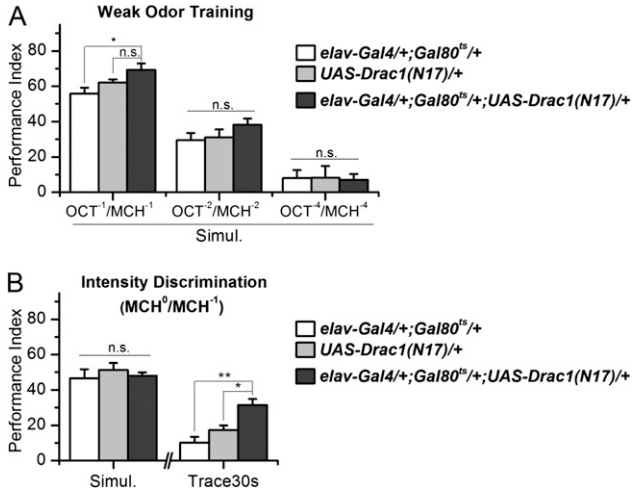

Fig. S2. Weak odor training and conditioned intensity discrimination. (*A*) Simultaneous conditioning with weakened odors. OCT^{-1}/MCH^{-1}, OCT^{-2}/MCH^{-2}, and OCT^{-4}/MCH^{-4} represented further dilution of 10^1-, 10^2-, and 10^4-fold from the regular concentration, respectively. Drac1(N17)-expressing flies show learning performance comparable to controls. The performance in OCT^{-1}/MCH^{-1} was slightly higher than one of the controls, *elav-Gal4/+; Gal80^{ts}/+* (ANOVA, $P = 0.02$), but no statistically significant differences were detected in any other comparison (ANOVA, $P > 0.3$). $n = 6$, except 5 for OCT^{-4}/MCH^{-4}. Error bars indicate SEM. (*B*) Conditioned discrimination of two different concentrations of the same odor. MCH^0 and MCH^{-1} represented no dilution and further dilution of 10-fold from the regular concentration of MCH, respectively. Drac1(N17)-expressing flies showed higher performance in the trace conditioning procedure (ANOVA, $P < 0.05$), but not in the simultaneous conditioning procedure (ANOVA, $P > 0.95$). $n = 5$. Error bars indicate SEM.

Table S1. Olfactory acuity of the hydrophilic odors

	Olfactory acuity	
Genotype	BU (2×10^{-3})	EA (2×10^{-3})
elav-Gal4/+; Gal80^{ts}/+; UAS-Drac1(N17)/+	63 ± 6	26 ± 2
elav-Gal4/+; Gal80^{ts}/+	66 ± 9	22 ± 3
UAS-Drac1(N17)/+	75 ± 6	34 ± 6

After exposure to 30 °C for 3 d, flies of the indicated genotypes were evaluated for olfactory acuity of 1-butanol (BU) and ethyl acetate (EA) at 2×10^{-3} dilution (vol/vol). The test was performed as described previously (1). Groups of ~100 untrained flies were allowed to make a choice between odor and fresh air in the T maze for 2 min. BU elicits stronger avoidance response than EA, but there were no statistically significant differences among genotypes in avoidance of each odor (ANOVA, $P > 0.1$). The data are shown as means ± SEM $n = 6$ for BU, $n = 7$ for EA.

1. Tully T, Preat T, Boynton SC, Del Vecchio M (1994) Genetic dissection of consolidated memory in Drosophila. *Cell* 79:35–47.