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Supplemental Information

Response competition between neurons

and antineurons in the mushroom body

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Figure S1. Three types of core-innervating MBON, related to Figure 1

(A) Biocytin fill of MBON- γ 4> γ 1 γ 2.

(B) Example voltage traces (top) and spike rasters (bottom) of MBON- γ 4> γ 1 γ 2 during optogenetic activation of $\alpha\beta_c$ KCs for the indicated durations.

(C) Average spike rates before and during optogenetic activation of $\alpha\beta_c$ KCs. Illumination left the spike rate of MBON- γ 4> γ 1 γ 2 unchanged (P = 0.2500; Wilcoxon test). n, number of cells.

(D) Biocytin fill of MBON- α 2sc (reproduced from Figure 1A).

(E) Example voltage trace (top) and spike rasters (bottom) of MBON- α 2sc during optogenetic activation of $\alpha\beta_c$ KCs for the indicated duration.

(F) Voltage traces of MBON- $\alpha 2sc$ during optogenetic activation of $\alpha\beta_c$ KCs in the presence of

TTX (black, average; gray, individual trials), followed by the addition of mecamylamine (dark red, average; light red, individual trials).



Figure S2. Long odor pulses facilitate the detection of off responses, related to Figure 2 (A) Example voltage traces (top) and spike rasters (bottom) of MBON- α 2sc during odor pulses of the indicated durations.

(B) Off responses increased as a function of odor pulse duration (Pearson r = 0.9928, P = 0.0072).



Figure S3. Disinhibition of odor responses in PPL1 neurons, related to Figure 4

(A) Odor-evoked changes in the average spike rates of PPL1 neurons before and after the addition of picrotoxin (P = 0.0020; Wilcoxon test).

(B) Odor-evoked changes in the average spike rates of PPL1- γ 1pedc in wild-type flies or flies expressing *MB320C-GAL4*-driven *Rdl*^{RNAi} (P = 0.0075; Mann-Whitney test). *P < 0.05. Data are means ± SEM; n, number of cells.



Figure S4. Reciprocal plasticity of on and off responses, related to Figure 5

(A) Example voltage traces of MBON- γ 1pedc> $\alpha\beta$ during 15-s pulses of odor (gray shading indicates measured concentration time courses) with electric shock reinforcement (magenta bars). The traces depict the evolution of the neuron's on and off responses from the untrained (top) to the trained state (bottom). The same neuron is shown in Figure 5C. (B) Example voltage traces of MBON- γ 1pedc> $\alpha\beta$ during 15-s pulses of odor (gray shading

indicates measured concentration time courses) with optogenetic activation of PPL1- γ 1pedc (crimson bars). The traces depict the evolution of the neuron's on and off responses from the untrained (top) to the trained state (bottom). The same neuron is shown in Figure S5B.



Figure S5. Reciprocal plasticity of on and off responses, related to Figure 5

(A) Responses of MBON- α 2sc during 15-s pulses (gray shading indicates measured concentration time courses) of control (CS⁻, left) or reinforced odor (CS⁺, right), before and after the optogenetic activation of PPL1- α '2 α 2 (light and dark colors). Top to bottom, example voltage traces, spike rate averages (± SEM), and spike rate changes caused by odor on- and offset. The on and off responses to CS⁺ were depressed and potentiated, respectively (P = 0.0312 and P = 0.0312; Wilcoxon tests), while the on and off responses to CS⁻ were unchanged (P = 0.0938 and P = 0.4375; Wilcoxon tests).

(B) Responses of MBON- γ 1pedc> $\alpha\beta$ during 15-s pulses (gray shading indicates measured concentration time courses) of control (CS⁻, left) or reinforced odor (CS⁺, right), before and after the optogenetic activation of PPL1- α '2 α 2 (light and dark colors). Top to bottom, example voltage traces, spike rate averages (± SEM), and spike rate changes caused by odor on- and offset. The on and off responses to CS⁺ were depressed and unchanged, respectively (P = 0.0312 and P = 0.6875; Wilcoxon tests), while the on and off responses to CS⁻ were unchanged (P = 0.1250 and P = 0.0625; Wilcoxon tests). *P < 0.05. Data are means ± SEM; n, number of cells.



Figure S6. Optogenetic stimulation of $\alpha\beta_c$ KCs, related to Figure 7

Example voltage traces of a CsChrimson-positive $\alpha\beta_c$ KC during light pulses of the indicated durations.