Supplementary Figures S1-S3

**Figure S1: Expression pattern of the various Gal4-strains used for behavioural experiments.** Three-dimensional reconstructions of anti-GFP immunoreactivity (green) of whole-mount larval third-instar brains using the ImageJ 3D Viewer. (A) Dorsal view with the major brain regions reconstructed. The inset shows a magnified view of the MB. (B-D) Brain-wide expression of GFP using *elav*-Gal4. (B) Whole brain. (C, D) Details of the brain seen in B. (E-H) Mushroom body expression of GFP using *mb247*-Gal4 (E, F) with whole brain (E) and a magnified view of the mushroom body (F). (G, H) Mushroom body expression of GFP using *D52H*-Gal4 showing (G) both mushroom bodies and (H) a magnified view of a single mushroom body. (I-O) Projection neuron expression of GFP in whole mounts using (I-L) *GH146*-Gal4 or (M-O) NP225-Gal4 as drivers for GFP expression. Additionally to projection neuron staining, a mushroom body extrinsic neuron (►) shows strong GFP immunoreactivity as well. (I, M) Whole brain. (K, L and N, O) Magnification of projection neurons and extrinsic mushroom body neurons. Optic lobe Anlagen (*), antennal lobe (AL), inner antennocerebral tract (iACT), projection neuron (PN), mushroom body (MB), calyx (Cx), peduncle (P), medial lobe (ML)vertical lobe (VL), lateral horn (LH), ventral nerve cord (VNC). Scale bars: 50µm in B, E, I, M; 25µm in C, D, F-H, K, L, N, O.

**Figure S2: Blocking synaptic output from projection neurons massively reduces odour preferences.** The following genotypes were generated: for the experimental group we crossed NP225-Gal4 to UAS-*shit* 

$^{stl}$, yielding double heterozygous larvae ($; NP225$-Gal4/ +; UAS- *shit* 

$^{stl}$/ +); for the driver control we crossed NP225-Gal4 to no-transgene carrying flies yielding single-heterozygous ($; NP225$-Gal4/ +); for the effector control we crossed UAS-*shit* 

$^{stl}$ to no-.
transgene carrying flies yielding \( ; ; \) UAS-\( shi^{as} \)/ + animals. Experimentally naive larvae were incubated in their food vials for 30 min on 37 °C in a water bath. To test their ability to detect odours, we determined their PREF values when given a choice between either paraffin-diluted AM versus paraffin, or between undiluted OCT versus an empty container. These odour preference tests were performed either at 34 °C (restrictive temperature) or at room temperature (22 °C). \( NP225\)-Gal4/ UAS-\( shi^{as} \) larvae do not differ from controls when AM Preference (A; KW-test: P= 0.58; H= 1.94; df= 3; N= 16 for all genotypes) or OCT Preference (C; KW-test: P= 0.57; H= 2.00; df= 3; N= 16 for all genotypes) was measured at 22 °C. However, when synaptic output of projection neurons is blocked at restrictive temperature, odour preferences of \( NP225\)-Gal4/ UAS-\( shi^{as} \) are significantly lower than of control larvae, both for AM (B; KW-test: P< 0.05; H= 28.36; df= 3; N= 20 for all genotypes; \( NP225\)-Gal4/ UAS-\( shi^{as} \) versus wild-type CS: MW: P< 0.05/ 3; U= 29; N= sample size as above ; \( NP225\)-Gal4/ UAS-\( shi^{as} \) versus projection-neuron DRIVER control: MW: P< 0.05/ 3; U= 53; sample size as above; \( NP225\)-Gal4/ UAS-\( shi^{as} \) versus EFFECTOR control: MW: P< 0.05/ 3; U= 45; sample sizes as above) and for OCT (D; KW-test: P< 0.05; H= 27.45; df= 3; N= 20 for all genotypes; \( NP225\)-Gal4/ UAS-\( shi^{as} \) versus wild-type CS: MW: P< 0.05/ 3; U= 37; sample size as above; \( NP225\)-Gal4/ UAS-\( shi^{as} \) versus projection-neuron DRIVER control: MW: P< 0.05/ 3; U= 50; sample size as above; \( NP225\)-Gal4/ UAS-\( shi^{as} \) versus EFFECTOR control: MW: P< 0.05/ 3; U= 46; sample sizes as above).

All other details as in the legend of Fig. 1.

**Figure S3: Odour preferences, separated by training regimen.** For documentation, we present the AM preferences from the reciprocally trained groups, i.e. the PREF\( _{AM} \) scores after either AM had been rewarded during training (AM+, gray boxes) or after OCT had been rewarded during training (OCT+, white boxes) for all behavioural experiments reported in the body text. Overall, genotypes show a slant towards AM, independent of the rewarded odour.
The effect of associative training consists in the observation that $PREF_{AM}$ scores are increased after AM+ training, and decreased after OCT+ training. In the one odour paradigm $PREF_{AM}$ scores or $PREF_{OCT}$ scores are presented after either AM or OCT, respectively, had been rewarded during training (AM+ or OCT+, gray boxes) or after EM had been rewarded during training (EM+, white boxes). D-ctrl: driver-control in the $w^{1118}; syn^{97}$ background, E-ctrl: effector-control in the $w^{1118}; syn^{97}$ background, Ex: experimental group in the $w^{1118}; syn^{97}$ background, D$^{w1118}$: driver-control in the $w^{1118}$ background, E$^{w1118}$: effector-control in the $w^{1118}$ background.

**Supplementary Movies S1-S6**

All movies are three-dimensional reconstructions of anti-GFP immunoreactivity of whole-mount larval third-instar brains.

**Movie S1: *Drosophila* larval brain with the major brain regions reconstructed.** Shown are antennal lobes (green), projection neurons (white), the mushroom bodies (yellow), and the Kennyon cell bodies (blue). The light grey shade sketches the rest of the larval brain. Based on a brain from a larva obtained by crossing GH146-Gal4; mb247-Gal4 to UAS-GFP. The 3D representation was obtained from 1 micron confocal serial sections using ImageJ software.

**Movie S2: Gal4 expressing cells in elav-Gal4 monitored by UAS-GFP (green).** The larval brain shows GFP expression throughout all neuropil regions, with strong expression in the mushroom bodies.

**Movie S3: Gal4 expressing cells in mb247-Gal4 monitored by UAS-GFP (green).** View on the larval mushroom body. In terms of expression pattern, mb247-Gal4 leads to GFP-
expression in all basic compartments of the larval mushroom body, i.e. in calyx, peduncle and lobes.

**Movie S4: Gal4 expressing cells in D52H-Gal4 monitored by UAS-GFP (green).** View on a single mushroom body. Expression is found in only very few mushroom body neurons (~7 mushroom body neurons per brain hemisphere). Notably, although only so few mushroom body neurons are covered, GFP expression reveals all basic compartments of the larval mushroom bodies; in particular the mushroom body input regions (the calyx) seems to be covered fairly well.

**Movie S5: Gal4 expressing cells in GH146-Gal4 monitored by UAS-GFP (green).** View on the projection neurons in the larval brain. When the GH146-Gal4 driver is used to express GFP, additionally to the expression in the projection neurons, a single mushroom body-extrinsic neuron per hemisphere is GFP-positive.

**Movie S6: Gal4 expressing cells in NP225-Gal4 monitored by UAS-GFP (green).** Same as Movie S5 but using NP225-Gal4 as another projection-neuron Gal4 strain.