SUPPLEMENTAL MOVIE CAPTIONS

Movie S1. Pseudocolored odor responses in the β and γ lobes before (Pre; left) and after (Post; center) thermogenetic stimulation of TH-GAL4+ neurons. The change in response is shown as the Post / Pre response ratio in the right panel. Images were collected at 10 Hz, and the frame count is shown in the top right. The odor was presented at frame 27, indicated by orange text at the bottom. These data are from the same fly shown in the top left panel of Figure S2. Note the localization of the calcium response plasticity to the γ lobe (see the top left panel of Figure S2 for ROIs and static images at the peak of the odor response).

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Fly strains. Flies were cultured according to standard methods. Imaging was performed using the following genetically-encoded reporters: GCaMP3 and GCaMP6f (Ca^{2+}; [S1, S2]), epac-camps and $^{T}epac^{VV}$ (cAMP; [S3-S5]), and AKAR3 (PKA; [S6]). The reporters were expressed in specific neuronal populations by either driving directly in mushroom bodies (MB-$^{T}epac^{VV}$ and MB-GCaMP3) using a 247-bp fragment of the dMef2 enhancer [S7] or using the GAL4-UAS system. The MB-$^{T}epac^{VV}$ and MB-GCaMP3 constructs were generated by cloning the reporter constructs into the pMef247 plasmid, and flies were generated with standard P element transgenesis. The c739- and 238Y-GAL4 drivers were chosen for their robust expression in MB neurons [S8-S13] Various GAL4 lines were used to drive expression of GAL4 in subsets of dopaminergic neurons, using a MB specific GAL80 repressor (MB-GAL80) to remove GAL4 expression in the MB when necessary. Driver lines were as follows: TH-GAL4 [S14], w;MB-GAL80;C150 [S15], NP7198;MB-GAL80 [S16], C061;MB-GAL80 [S17] (fly-trap.org), NP2755/CyO [S16], Ddc(4.36)-GAL4 [S18], and R58E02-GAL4 [S19]. The MB-GAL80 stock was constructed by Hiromu Tanimoto and characterized by Krashes et al. [S20]. All fly lines were backcrossed ≥6 generations into the reference strain w^{CS10}.

Functional imaging. Isolated brain preparations were performed as previously described [S21]. Flies were anesthetized on ice, and brains were dissected and maintained in *Drosophila* saline with 1 ml/min continuous bath perfusion. Brains are viable in this preparation for ≥4 hours, but we restricted recordings to within one hour of the dissection. Dopamine was applied and washed out by switching the source of the bath perfusion solution for 30 s. The total concentration of drug in the chamber was measured (in separate calibration experiments) with fluorescein as a tracer, and found to peak at ~30% of the concentration applied to the inlet of the chamber over the 30 s application (data not shown). Nonetheless, given the difficulty of

measuring the amount that penetrates into the brain, concentrations are reported here are uncorrected. SCH-23390 (Tocris Bioscience) was applied in the bath for 10 min prior to recording dopamine-evoked responses, and washed out for 20 min prior to the final recording. *In vivo* functional imaging was performed as described previously [S22]. Flies were immobilized in a custom-machined polycarbonate recording chamber that allows saline to flow across the dorsal head and thorax, while keeping the rest of the fly dry. A small window was opened in the cuticle with a syringe needle to allow optical access to the brain, and saline was perfused at 2 ml/min. The odorant ethyl butyrate (Sigma-Aldrich) was delivered through a stainless steel pipette mounted 1 cm anterior to the fly's head, and presented for either 3 s (imaging) or 30 s (training, in combination with heating or application of drug for 30 s). Air flow was 20-60 ml/min, empirically adjusted for each experimental setup and brain region. The odorant was presented by switching the odor stream between scintillation vials containing either 1 μl of odorant on filter paper or filter paper alone using solenoid valves (The Lee Co) controlled by a programmable logic controller (Omron). The odor/air streams were directed through PTFE (Teflon) tubing.

Baseline temperature in the recording chamber was held at 22 °C in experiments involving TRPA1 and 24 °C otherwise. Temperature was controlled with an inline Peltier element (Warner Instruments), monitored with a thermistor placed in the recording chamber adjacent to the fly's head, and digitized (Axon Instruments). In forward conditioning experiments, where TRPA stimulation was paired with odor delivery, we ramped the temperature up from 22 °C. When the bath temperature reached 24 °C, the solenoid controlling 30-s odor delivery was activated to begin odor delivery immediately before the TRPA activation threshold (27 °C) [S23] was reached. In backward conditioning experiments, the temperature was shifted to 32 °C and back, and the solenoid controlling odor delivery was activated when the temperature fell below 24 °C.

Forskolin and 1,9-dideoxyforskolin (Santa Cruz Biotechnology, Inc.) were diluted into saline from 100 mM stock solutions in DMSO and applied for 30s in the bath solution.

Imaging and statistical analysis. Optical reporters were imaged with confocal microscopy using appropriate laser lines and emission filter settings on Leica TCS SP5 and SP8 confocal microscopes at 256 x 256. For Ca^{2+} imaging with GCaMP, 488 nm excitation was paired with 500-600 nm bandpass emission filtration, acquired at 10 Hz. For CFP/YFP FRET imaging (epac-camps, TepacVV, and AKAR3), 442 nm excitation was paired with 465-505 nm [CFP] / 525-600 nm [YFP] bandpass emission filtration, acquired at 1 Hz. Responses were plotted as the baseline-normalized change in GCaMP fluorescence ($\Delta F/F_0$), FRET ratio (YFP/CFP, $\Delta R/R_0$; AKAR3), or inverse FRET ratio (CFP/YFP, $\Delta R/R_0$; epac-camps, $^{T}epac^{VV}$), averaged across a circumscribed region of interest. Dose-response curves were analyzed by fitting a variable-slope model to the normalized responses and calculating the logEC50. Pseudocolor images were generated in Matlab (Mathworks). Images were Kalman filtered, masked to remove pixels containing no fluorescent signal, and the $\Delta F/F_0$ was calculated for each pixel as the ratio from the frame at the peak odor response relative to basal fluorescence (1 s before odor delivery). Statistical analysis was performed in Matlab and Prism (Graphpad). Normality was tested with the Shapiro-Wilk test, and statistical significance (omnibus/post-hoc) was determined using ANOVA/Tukey HSD (one-way parametric), ANOVA/Sidak (two-way parametric), Kruskal-Wallis/Mann-Whitney U tests (one-way nonparametric), or Friedman/Wilcoxon signed-rank test (two-way nonparametric). Wilcoxon signed-rank tests were used to compare forskolin-treated with control groups. Multiple comparisons were conducted with Bonferroni-adjusted α values, using the family-wise error rate $\alpha_{FW} = 0.05$.

SUPPLEMENTAL REFERENCES

S1. Tian, L., Hires, S.A., Mao, T., Huber, D., Chiappe, M.E., Chalasani, S.H., Petreanu, L., Akerboom, J., McKinney, S.A., Schreiter, E.R., et al. (2009). Imaging neural activity in worms, flies and mice with improved GCaMP calcium indicators. Nat Methods *6*, 875-881.

S2. Chen, T.W., Wardill, T.J., Sun, Y., Pulver, S.R., Renninger, S.L., Baohan, A., Schreiter, E.R., Kerr, R.A., Orger, M.B., Jayaraman, V., et al. (2013). Ultrasensitive fluorescent proteins for imaging neuronal activity. Nature *499*, 295-300.

S3. Nikolaev, V.O., Bunemann, M., Hein, L., Hannawacker, A., and Lohse, M.J. (2004). Novel single chain cAMP sensors for receptor-induced signal propagation. J. Biol. Chem. *279*, 37215-37218.

S4. Shafer, O.T., Kim, D.J., Dunbar-Yaffe, R., Nikolaev, V.O., Lohse, M.J., and Taghert, P.H. (2008). Widespread receptivity to neuropeptide PDF throughout the neuronal circadian clock network of Drosophila revealed by real-time cyclic AMP imaging. Neuron *58*, 223-237.

S5. Klarenbeek, J.B., Goedhart, J., Hink, M.A., Gadella, T.W., and Jalink, K. (2011). A mTurquoise-based cAMP sensor for both FLIM and ratiometric read-out has improved dynamic range. PLoS One *6*, e19170.

S6. Allen, M.D., and Zhang, J. (2006). Subcellular dynamics of protein kinase A activity visualized by FRET-based reporters. Biochem. Biophys. Res. Commun. *348*, 716-721.

S7. Schulz, R.A., Chromey, C., Lu, M.F., Zhao, B., and Olson, E.N. (1996). Expression of the D-MEF2 transcription in the Drosophila brain suggests a role in neuronal cell differentiation. Oncogene *12*, 1827-1831.

S8. Yang, M.Y., Armstrong, J.D., Vilinsky, I., Strausfeld, N.J., and Kaiser, K. (1995). Subdivision of the Drosophila mushroom bodies by enhancer-trap expression patterns. Neuron *15*, 45-54.

S9. Zars, T., Fischer, M., Schulz, R., and Heisenberg, M. (2000). Localization of a short-term memory in Drosophila. Science *288*, 672-675.

S10. Dubnau, J., Grady, L., Kitamoto, T., and Tully, T. (2001). Disruption of neurotransmission in Drosophila mushroom body blocks retrieval but not acquisition of memory. Nature *411*, 476-480.

S11. McGuire, S.E., Le, P.T., Osborn, A.J., Matsumoto, K., and Davis, R.L. (2003). Spatiotemporal rescue of memory dysfunction in Drosophila. Science *302*, 1765-1768.

S12. McGuire, S.E., Le, P.T., and Davis, R.L. (2001). The role of Drosophila mushroom body signaling in olfactory memory. Science *293*, 1330-1333.

S13. Yu, D., Akalal, D.B., and Davis, R.L. (2006). Drosophila alpha/beta mushroom body neurons form a branch-specific, long-term cellular memory trace after spaced olfactory conditioning. Neuron *52*, 845-855.

S14. Friggi-Grelin, F., Iche, M., and Birman, S. (2003). Tissue-specific developmental requirements of Drosophila tyrosine hydroxylase isoforms. Genesis *35*, 260-269.

S15. Dubnau, J., Chiang, A.S., Grady, L., Barditch, J., Gossweiler, S., McNeil, J., Smith, P., Buldoc, F., Scott, R., Certa, U., et al. (2003). The staufen/pumilio pathway is involved in Drosophila long-term memory. Curr. Biol. *13*, 286-296.

S16. Tanaka, N.K., Tanimoto, H., and Ito, K. (2008). Neuronal assemblies of the Drosophila mushroom body. J. Comp. Neurol. *508*, 711-755.

S17. Krashes, M.J., DasGupta, S., Vreede, A., White, B., Armstrong, J.D., and Waddell, S. (2009). A neural circuit mechanism integrating motivational state with memory expression in Drosophila. Cell *139*, 416-427.

S18. Li, H., Chaney, S., Roberts, I.J., Forte, M., and Hirsh, J. (2000). Ectopic G-protein expression in dopamine and serotonin neurons blocks cocaine sensitization in Drosophila melanogaster. Curr. Biol. *10*, 211-214.

S19. Liu, C., Placais, P.Y., Yamagata, N., Pfeiffer, B.D., Aso, Y., Friedrich, A.B., Siwanowicz, I., Rubin, G.M., Preat, T., and Tanimoto, H. (2012). A subset of dopamine neurons signals reward for odour memory in Drosophila. Nature *488*, 512-516.

S20. Krashes, M.J., Keene, A.C., Leung, B., Armstrong, J.D., and Waddell, S. (2007). Sequential use of mushroom body neuron subsets during drosophila odor memory processing. Neuron *53*, 103-115.

S21. Tomchik, S.M., and Davis, R.L. (2009). Dynamics of learning-related cAMP signaling and stimulus integration in the Drosophila olfactory pathway. Neuron *64*, 510-521.

S22. Tomchik, S.M. (2013). Dopaminergic neurons encode a distributed, asymmetric representation of temperature in Drosophila. J. Neurosci. *33*, 2166-2176a.

S23. Viswanath, V., Story, G.M., Peier, A.M., Petrus, M.J., Lee, V.M., Hwang, S.W., Patapoutian, A., and Jegla, T. (2003). Opposite thermosensor in fruitfly and mouse. Nature *423*, 822-823.