Supporting Information

Franco et al. 10.1073/pnas.1012293108

SI Materials and Methods

Discrimination. Groups of flies were lowered to the choice point of the maze and allowed to choose for 90 s between its two arms scented with odorants as indicated. Flies in each arm were trapped and counted and their distribution relative to the total number of flies (i.e., percent excess flies) was calculated by subtracting the number of flies in one arm from those in the opposite arm and dividing by the total number per experiment. The resultant measure is an indication of the relative distribution of the flies in the maze arms at the end of the choice period. Therefore, equally aversive amounts of odorants result in nearly equal number of flies in each arm and yield a distribution near zero (i.e., balanced distribution). These experimentally determined equivalent odorant amounts were used in the testing phase of subsequent associative learning experiments.

Olfactory Learning. *Drosophila* were trained and tested as described before (1, 2) with the following modifications. First, to minimize equipment-dependent variability, a single maze was used in all experiments. The amounts of each odorant used in the learning and testing phases of learning experiments were those that gave a balanced distribution in the odor discrimination experiments. When different odors were used for training and testing—for example, training with benzaldehyde-d_5 and benzaldehyde but testing with d_{17}-1-octanol and 1-octanol—balancing was required of training and testing odor pair. If balancing was not possible, conditioning experiments did not commence and the experimental setup was disassembled. The experiment was attempted again at another time and only after establishing balanced maze conditions.

Groups of *Drosophila* were placed into the training arm lined with an electrifiable grid and exposed sequentially to two odors carried in the air current. During the 1-min exposure to the first odor, flies were given 12 electric foot shocks of 90 V DC each, lasting 1.2 s, followed by 1 min of room air to purge the tube of odor. This was followed by 1 min of the second odor without foot shocks, followed by another 60 s of room air. *Drosophila* were then gently lowered to the choice point of the T-maze where they were allowed to choose for 90 s between the two converging air streams scented with the odors used for training or different ones as indicated. After the test, flies were trapped in the two arms, collected, and counted. The distribution of the flies in the arms relative to the odor they were trained to selectively avoid was calculated as the number of flies avoiding the shock-associated odor minus those that do not, divided by the total. Between each cycle of training, the maze was purged of potential lingering odors by passing room air through both arms for at least 2 min. Another group of flies were then reciprocally trained such that the punished odor from the first experiment became the non-punished one in the second experiment. Although the performance of the two reciprocally trained groups are typically averaged (3), we report them separately as better indicators of behavior in the two independently differentially trained groups.

Results from the behavioral tests were normally distributed and therefore analyzed parametrically with the JMP statistical package by using the statistical tests indicated in the legends to Figs. 1–4.

1. Pavlopoulos E, Anezaki M, Skoulakis EMC (2008) Neuralized is expressed in the α/β lobes of adult Drosophila mushroom bodies and facilitates olfactory long-term memory formation. *Proc Natl Acad Sci USA* 105:14674–14679.
2. Moressis A, Friedrich AR, Pavlopoulos E, Davis RL, Skoulakis EMC (2009) A dual role for the adaptor protein DRK in Drosophila olfactory learning and memory. *J Neurosci* 29: 2611–2625.
3. Tully T, Quinn WG (1985) Classical conditioning and retention in normal and mutant Drosophila melanogaster. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol* 157: 263–277.

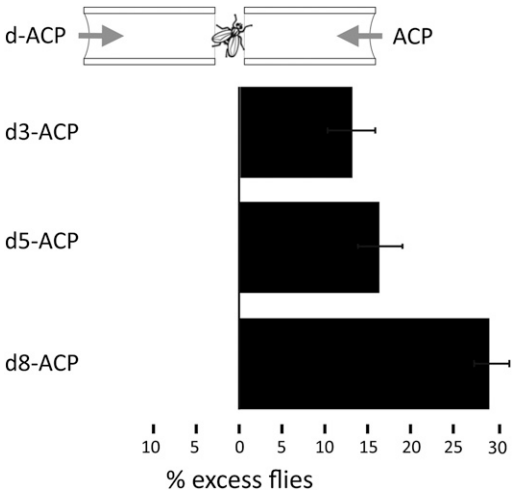

Fig. S1. Deuteration transforms ACP to an aversive odorant The mean relative distribution of flies in the arms of the maze (% excess flies) carrying the indicated odorants ± SEM is shown in all graphs. Flies avoided all three deuterated versions of the odorant distributing preferentially in the h-ACP–carrying arm of the maze. Avoidance of d_8-ACP was significantly different ($P < 0.001$) from that of d_3- and d_5-ACP. Avoidance of d_3-ACP was not significantly different from that of d_5-ACP but both were significantly different from zero ($n \geq 6$ for all groups; total flies per group > 445).

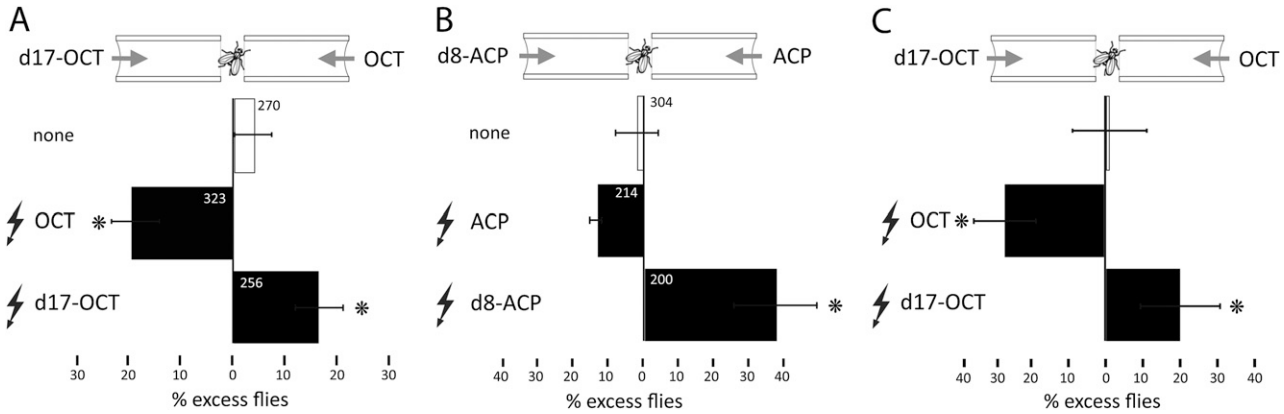

Fig. S2. Conditioned avoidance in Canton-S flies and reverse conditioning in the w^{1118} strain. The mean relative distribution of flies in the arms of the maze (% excess flies) carrying the indicated odorants ± SEM is shown and the total number of flies in each group is denoted. (*A*) Canton-S strain *Drosophila* were trained by using the negatively reinforced conditioned olfactory avoidance paradigm to selectively avoid octanol or d_{17}-1-octanol. Dunnett tests revealed highly significant ($P < 0.0001$) differences in the performance of both groups of conditioned animals from that of naive ones (open bars; $n \geq 5$). (*B*) Conditioned avoidance of normal or deuterated ACP in Canton-S *Drosophila*. Subsequent Dunnett tests demonstrated that *Drosophila* trained to avoid d_8-ACP chose ACP upon testing significantly more than naive *Drosophila* ($P < 0.001$), whereas the behavior of *Drosophila* trained to avoid ACP in choosing the deuterated ACP side was significantly different from that of naive at the $P < 0.01$ level. (*C*) w^{1118} control flies were conditioned to selectively avoid octanol or d_{17}-1-octanol. However, delivery of the control odor, not associated with the electric foot shock, preceded presentation of the punished odor (i.e., reverse-order training). However, this reversal of the training scheme did not alter the preferential avoidance of the punished odor upon testing, as the performance of both trained groups was significantly different ($P < 0.001$) from that of naive animals ($n \geq 5$).

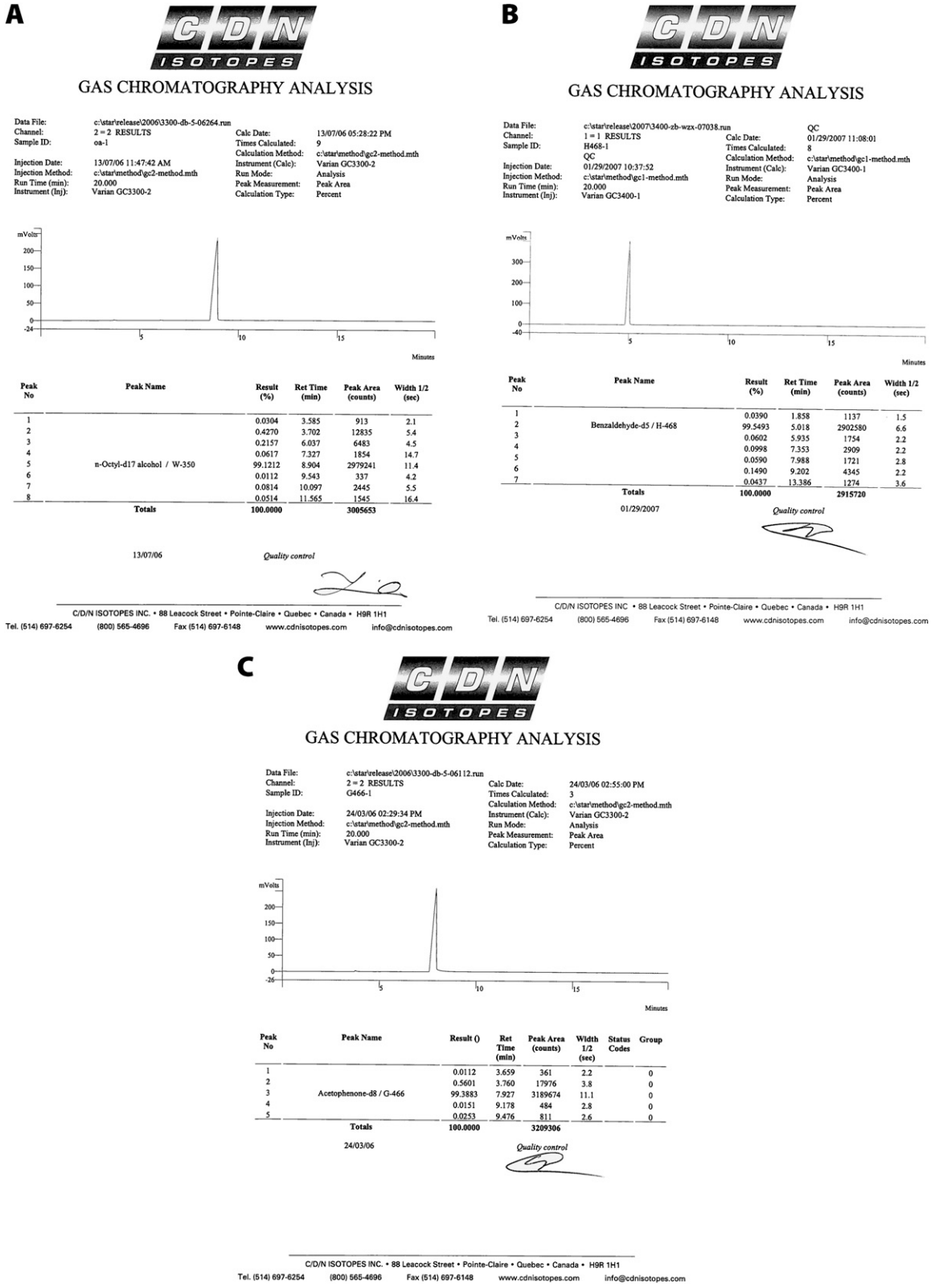

Fig. S3. Gas chromatographs of the three perdeuterated odorants used in the behavioral experiments. Per the supplier (CDN), d_{17}-1-octanol alcohol (chromatogram A) was prepared by reduction of octanoic-d_{15} acid. Benzaldehyde-d_6 (chromatogram b) was prepared by a Grignard reaction by using bromobenzene-d_5 and *N*,*N*-dimethylformamide-d_1. ACP-d_8 (chromatogram C) was prepared by a Friedel–Crafts reaction by using benzene-d_6 and acetyl chloride.

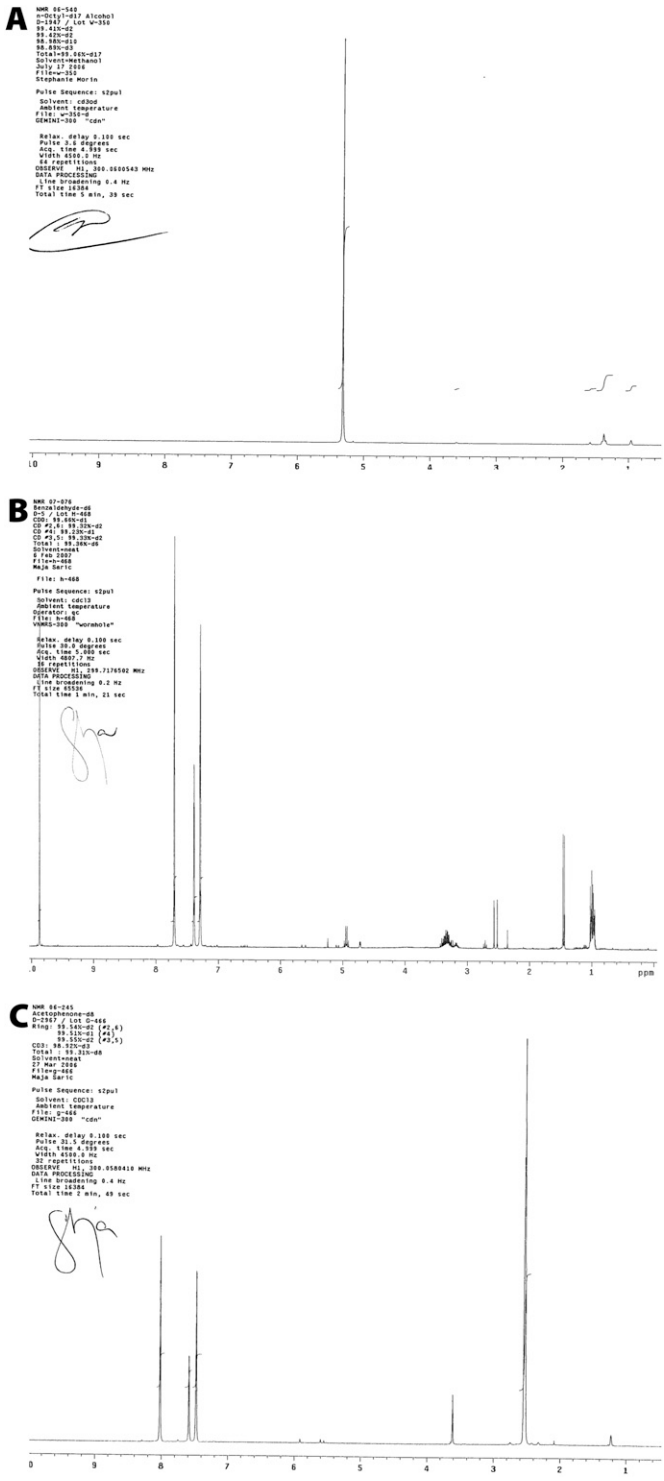

Fig. S4. NMR spectra of the three deuterated odorants used in the behavioral experiments. (*A*) 1-octanol-d_{17}; (*B*) BNZ-d_6; (*C*) ACP-d_8.