Supporting Information

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SI Methods

Odor Conditioning Media Preparation. The odor conditioning media were prepared using the ingredients listed in Table S3.

Each of the salt solutions (×100) were prepared separately and autoclaved. Agar was boiled in approximately 70 mL of distilled water. It was then cooled and sucrose was added to it. Yeast extract, Cas-amino acid, and tryptophan were dissolved in approximately 20 mL of distilled water separately. The above mentioned solution was added to the lukewarm agar and mixed until it was dissolved completely. One milliliter of each of the ×100 salt solutions was added. The volume was increased to 100 mL with distilled water and mixed well. The resultant solution was allowed to cool before pouring into the cut glass bottles (approximately 50 mL per bottle). In case of medium with odor, the required amount of odorant was added after making up the volume, which was mixed well and poured into the bottles. The bottles were cooled at 4 °C for 10 min and allowed to cool at room temperature before inoculating for 3 h. To avoid fungal growth on the medium, larvae were transferred to fresh media bottles every 2 d. It takes approximately 13 d for flies to emerge from the medium.

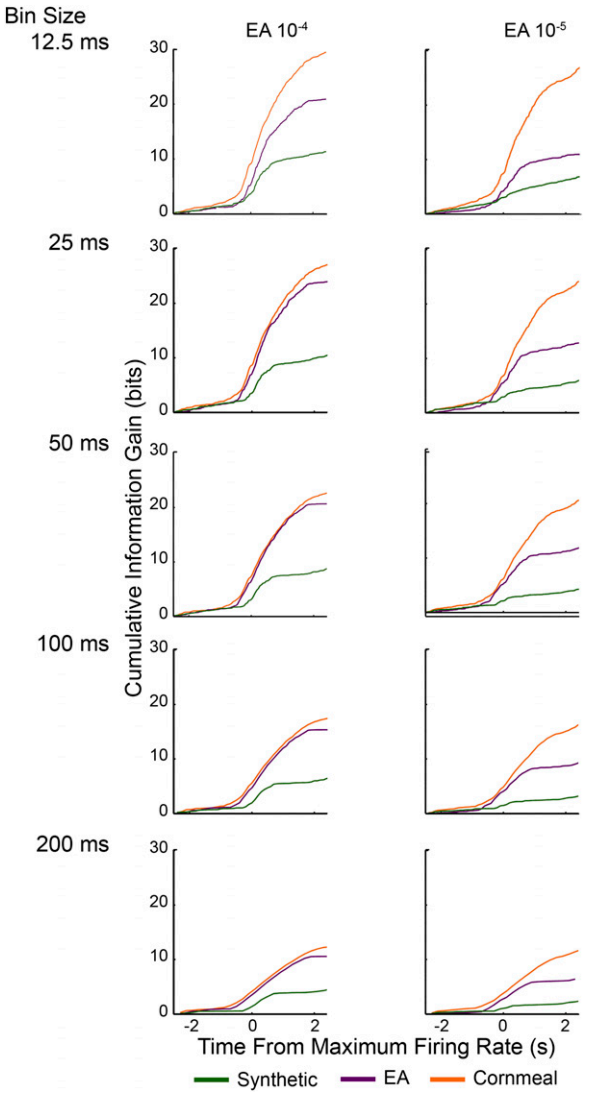

Fig. S1. Bin size and information gain. Cumulative plots of the information gain against SF for the responses to EA at dilutions of 10^{-4} and 10^{-5} using the indicated bin sizes (using the same color coding as in Fig. 2). Around the bin size of 50 ms, the relative information gain values do not change significantly in both responses except for the smallest bin size, 12.5 ms, which captures too few spikes per bin. However, the values of the information gain will decrease with larger bin sizes as a result of the reduced number of bins, although the values of the information gain will change slightly.

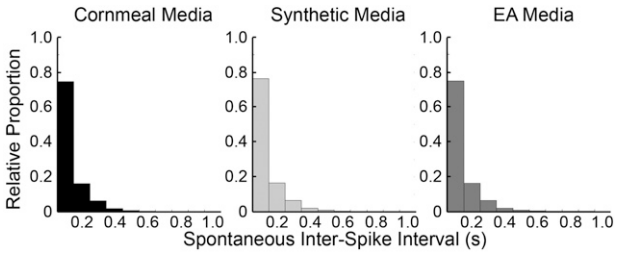

Fig. S2. SF of conditioned ORNs. Histograms of the interspike intervals of the SF in our imaginally conditioned flies (bin size, 0.2 s) indicate that the SF patterns are not significantly altered as a result of imaginal conditioning (χ^2 goodness of fit test, $P > 0.05$).

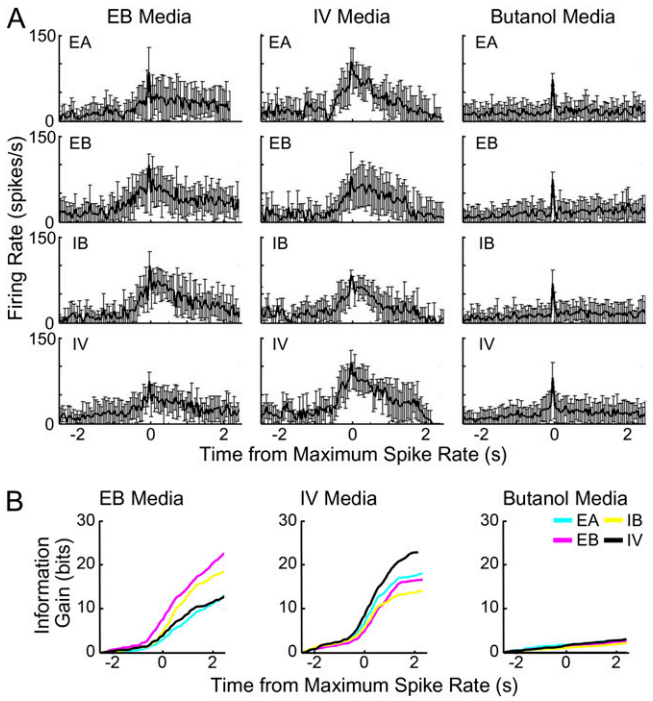

Fig. S3. Odor responses of EB-, IV-, and butanol-conditioned flies. (*A*) The firing frequency distributions of the responses to EA, EB, IB, and IV at 10^{-4} dilution for flies raised on EB-, IV-, and butanol-conditioning media, aligned by maximum firing frequency (mean represented by bold line, SD represented by error bars; $n = 9–19$). (*B*) Cumulative plots of the information gain (based on ΣD_{JS}) against SF of the responses to the odors (represented by line colors) in the respective conditioning media. Again imaginal conditioning by EB or IV increases sensitivity not only to the conditioning odor, but also to related esters. Conditioning by exposure to an alcohol suppresses responses to esters.

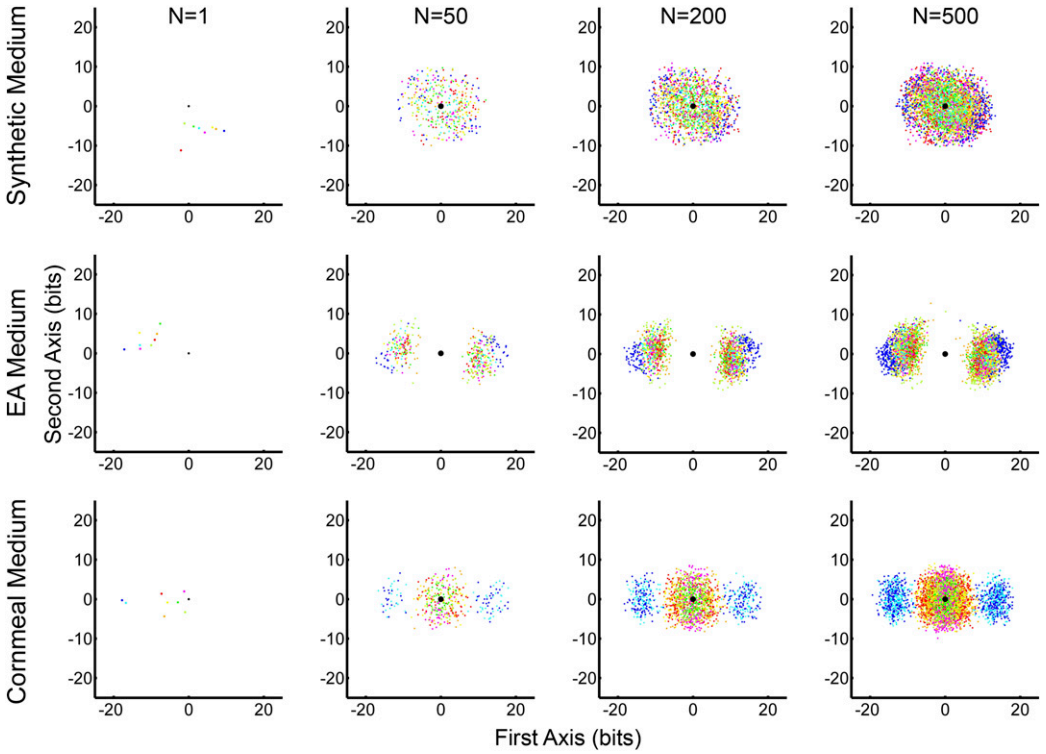

Fig. S4. ISOMAP with different number of bootstrap resampled points. These plots visualize the odor response space in our conditioned flies 1.25 s after the time of maximum spiking, using 1, 50, 200, and 500 bootstrap resampled matrices (N, see Methods in the main text for explanation). Although the number of matrices used in the visualization vary, the general characteristics of the plot do not change as long as a sufficient number of matrices are used. Fig. 6 in the main text uses 200 resampled matrices.

Table S1. ΣD_{JS} based on 1,000 bootstrap resamples between pairs of responses to EA dilutions against each other and against SF for flies conditioned on the three types of media

Mean (SD)	SF	EA 10^{-3}	EA 10^{-4}	EA 10^{-5}	EA 10^{-7}
Synthetic medium raised					
SF	—	23.3 (3.0)	11.0 (4.1)	4.5 (2.7)	2.2 (1.4)
EA 10^{-3}	23.3 (3.0)	—	17.1 (3.0)	22.2 (2.9)	28.7 (2.8)
EA 10^{-4}	11.0 (4.1)	17.1 (3.0)	—	7.6 (1.4)	12.7 (1.8)
EA 10^{-5}	4.5 (2.7)	22.2 (2.9)	7.6 (1.4)	—	4.1 (1.6)
EA 10^{-7}	2.2 (1.4)	28.7 (2.8)	12.7 (1.8)	4.1 (1.6)	—
EA medium raised					
SF	—	45.0 (3.3)	21.4 (1.7)	12.5 (1.3)	9.1 (2.1)
EA 10^{-3}	45.0 (3.3)	—	21.0 (4.4)	30.2 (4.8)	36.7 (5.2)
EA 10^{-4}	21.4 (1.7)	21.0 (4.4)	—	9.0 (1.9)	12.4 (3.4)
EA 10^{-5}	12.5 (1.3)	30.2 (4.8)	9.0 (1.9)	—	5.4 (1.4)
EA 10^{-7}	9.1 (2.1)	36.7 (5.2)	12.4 (3.4)	5.4 (1.4)	—
Cornmeal medium raised					
SF	—	29.4 (4.9)	23.6 (2.9)	21.8 (2.1)	4.8 (0.9)
EA 10^{-3}	29.4 (4.9)	—	5.1 (2.0)	5.7 (2.3)	28.0 (5.3)
EA 10^{-4}	23.6 (2.9)	5.1 (2.0)	—	3.5 (0.9)	22.9 (3.6)
EA 10^{-5}	21.8 (2.1)	5.7 (2.3)	3.5 (0.9)	—	20.9 (3.1)
EA 10^{-7}	4.8 (0.9)	28.0 (5.3)	22.9 (3.6)	20.9 (3.1)	—

The color intensity in **Fig. 3** was based on these values.

Table S2. Results of 1,000 bootstrap resamples of the final ΣD_{JS} values for pairwise total information gain calculations for each ester odor/dilution combination in flies conditioned on the three types of media

Mean (SD)	SF	EA 10^{-4}	EA 10^{-5}	EB 10^{-4}	EB 10^{-5}	IB 10^{-4}	IB 10^{-5}	IV 10^{-4}	IB 10^{-5}
Synthetic medium raised									
SF	—	11.0 (4.1)	4.5 (2.7)	7.02 (1.7)	4.1 (1.5)	5.0 (2.2)	3.9 (1.5)	6.1 (2.4)	4.5 (1.4)
EA 10^{-4}	11.0 (4.1)	—	7.6 (1.5)	5.4 (2.9)	7.9 (1.4)	6.6 (1.8)	6.4 (1.8)	5.6 (1.3)	6.7 (1.2)
EA 10^{-5}	4.5 (2.7)	7.6 (1.5)	—	4.4 (2.3)	3.4 (1.1)	3.1 (1.4)	2.9 (1.1)	3.1 (1.2)	3.0 (1.2)
EB 10^{-4}	7.02 (1.7)	5.4 (2.9)	4.4 (2.3)	—	4.6 (2.2)	4.5 (2.2)	4.3 (2.1)	4.9 (2.5)	5.1 (2.7)
EB 10^{-5}	4.1 (1.5)	7.9 (1.4)	3.4 (1.1)	4.6 (2.2)	—	3.6 (1.5)	4.1 (1.5)	3.5 (1.2)	3.1 (1.0)
IB 10^{-4}	5.0 (2.2)	6.6 (1.8)	3.1 (1.4)	4.5 (2.2)	3.6 (1.5)	—	3.4 (1.5)	3.2 (1.4)	3.4 (1.5)
IB 10^{-5}	3.9 (1.5)	6.4 (1.8)	2.9 (1.1)	4.3 (2.1)	4.1 (1.5)	3.4 (1.5)	—	3.8 (1.3)	3.6 (1.3)
IV 10^{-4}	6.1 (2.4)	5.6 (1.3)	3.1 (1.2)	4.9 (2.5)	3.5 (1.2)	3.2 (1.4)	3.8 (1.3)	—	2.9 (1.0)
IV 10^{-5}	4.5 (1.4)	6.7 (1.2)	3.0 (1.2)	5.1 (2.7)	3.1 (1.0)	3.4 (1.5)	3.6 (1.3)	2.9 (1.0)	—
EA medium raised									
SF	—	21.5 (1.7)	12.5 (1.3)	11.0 (1.1)	12.2 (1.5)	11.5 (1.4)	11.0 (1.9)	11.9 (2.1)	14.1 (2.1)
EA 10^{-4}	21.5 (1.7)	—	9.1 (1.8)	11.5 (2.0)	9.2 (2.3)	12.1 (3.1)	10.9 (2.1)	10.7 (2.6)	9.0 (2.4)
EA 10^{-5}	12.5 (1.3)	9.1 (1.8)	—	5.0 (0.9)	4.4 (0.9)	6.6 (1.7)	4.4 (0.9)	6.0 (1.2)	4.3 (1.0)
EB 10^{-4}	11.0 (1.1)	11.5 (2.0)	5.0 (0.9)	—	4.7 (1.1)	6.1 (1.5)	4.0 (0.9)	6.0 (1.1)	4.8 (1.3)
EB 10^{-5}	12.2 (1.5)	9.2 (2.3)	4.4 (0.9)	4.7 (1.1)	—	6.8 (1.8)	4.2 (1.0)	6.7 (1.3)	4.5 (1.1)
IB 10^{-4}	11.6 (1.4)	12.1 (3.1)	6.6 (1.7)	6.1 (1.5)	6.8 (1.8)	—	6.1 (1.6)	6.4 (1.8)	6.5 (1.9)
IB 10^{-5}	11.0 (1.9)	10.9 (2.1)	4.4 (0.9)	4.0 (0.8)	4.2 (1.0)	6.1 (1.6)	—	5.8 (1.3)	4.9 (1.5)
IV 10^{-4}	11.9 (2.1)	10.7 (2.6)	6.0 (1.2)	6.0 (1.1)	6.7 (1.3)	6.4 (1.8)	5.8 (1.3)	—	6.3 (1.6)
IV 10^{-5}	14.1 (2.1)	9.0 (2.4)	4.3 (1.0)	4.8 (1.3)	4.5 (1.1)	6.5 (1.9)	4.9 (1.5)	6.3 (1.6)	—
Cornmeal medium raised									
SF	—	23.5 (3.0)	21.8 (3.3)	6.6 (1.5)	5.2 (1.0)	3.6 (1.2)	2.5 (0.9)	4.8 (2.0)	3.6 (2.7)
EA 10^{-4}	23.5 (3.0)	—	3.5 (0.9)	14.4 (2.7)	19.1 (2.7)	18.7 (2.8)	20.1 (2.6)	16.9 (4.1)	15.8 (4.6)
EA 10^{-5}	21.8 (3.3)	3.5 (0.9)	—	13.6 (1.9)	17.6 (2.0)	17.2 (1.9)	18.2 (1.6)	15.8 (3.4)	14.8 (4.2)
EB 10^{-4}	6.6 (1.5)	14.4 (2.7)	13.6 (1.9)	—	7.2 (1.3)	5.9 (1.1)	7.5 (1.2)	6.5 (1.4)	6.3 (1.3)
EB 10^{-5}	5.2 (1.0)	19.1 (2.7)	17.6 (2.0)	7.2 (1.3)	—	5.9 (1.1)	5.4 (1.0)	3.5 (1.4)	3.3 (1.9)
IB 10^{-4}	3.6 (1.2)	18.7 (2.8)	17.2 (1.9)	5.9 (1.1)	5.9 (1.1)	—	3.1 (1.1)	3.0 (1.1)	3.0 (1.6)
IB 10^{-5}	2.5 (0.9)	20.1 (2.6)	18.2 (1.6)	7.5 (1.2)	5.4 (1.0)	3.1 (1.1)	—	3.5 (1.6)	3.5 (2.0)
IV 10^{-4}	4.8 (2.0)	16.9 (4.1)	15.8 (3.4)	6.5 (1.4)	3.5 (1.4)	3.0 (1.1)	3.5 (1.6)	—	2.8 (1.7)
IV 10^{-5}	3.6 (2.7)	15.8 (4.6)	14.8 (4.2)	6.3 (1.3)	3.3 (1.9)	3.0 (1.6)	3.5 (2.0)	2.8 (1.7)	—

Table S3. Ingredients of odor conditioning media

Ingredient	Amount, g/100 mL
Sodium bicarbonate (Himedia)	0.10
Potassium dihydrogen orthophosphate (Qualigens)	0.07
Di-potassium hydrogen orthophosphate (Qualigens)	0.39
Magnesium sulfate (Qualigens)	0.02
Agar-agar (Qualigens)	0.9
Sucrose (Qualigens)	1.0
Casein enzyme hydrolysate (Himedia)	0.1
Yeast extract (certified; Himedia)	0.05
L-tryptophan (Himedia)	0.005