Supplemental Data

Learned Odor Discrimination in *Drosophila* without Combinatorial Odor Maps in the Antennal Lobe

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Supplemental Experimental Procedures

Fly strains

Fly stocks were raised on standard cornmeal food at 25°C and 40-50% relative humidity. The wild-type *Drosophila* strain is Canton-S. The uas-*n-syb::GFP* flies are described (*1*). *Or83b*^{2} mutant flies, *Or98a*-GAL4 (*2*), and uas-*Or83b* transgenic flies were obtained from L. Vosshall (Rockefeller University). *Or67a*-GAL4 transgenic flies (*3*) were obtained from B. Dickson (IMP, Vienna) and Or46a-GAL4 (*2*); *Or83b*^{2} flies were obtained from R. Wilson (Harvard University). All transgenic insertions are on the second chromosome. We generated *OrX*-GAL4/uas-*Or83b*; *Or83b*^{2} flies by crossing *OrX*-GAL4; *Or83b*^{2} flies to uas-*Or83b*; *Or83b*^{2} flies. Heterozygous uas-*Or83b*/+; *Or83b*^{2} and *OrX*-GAL4/+; *Or83b*^{2} flies were generated by crossing uas-*Or83b*; *Or83b*^{2} and *OrX*-GAL4; *Or83b*^{2} flies to *Or83b*^{2} flies. Mixed sex populations of flies, heterozygous for the listed transgenes, were tested for olfactory learning.

Behavioral analysis

The olfactory avoidance paradigm was essentially performed as described

previously (*4*). The Performance Index (PI) was calculated as the number of flies avoiding the conditioned odor minus the number of flies avoiding the unconditioned odor divided by the total number of flies in the experiment. A single PI value is the average score from flies of the identical genotype trained with each odor. For training, all odors were diluted 1:1000 in mineral oil (unless stated otherwise). For testing, all odors were diluted 1:1000 in mineral oil and adjusted so that naïve flies distributed equally between the odorant pair.

For olfactory adaptation experiments naïve flies or flies exposed to odorant A for 30min in the training chamber of the T-maze were tested for their preference between tubes with either odorant A versus air or for a different odorant versus air. Odorants were diluted approximately 1:1000 in mineral oil. Avoidance Index was calculated as the number of flies avoiding the odorant minus the number of flies avoiding the air divided by the total number of flies in the experiment.

To reduce variation within experiments, all genotypes were tested in each experimental session. Statistical analyses were performed using KaleidaGraph (Synergy Software). Overall analyses of variance (ANOVA) were followed by planned pairwise comparisons between the relevant groups with a Tukey HSD post-hoc test. Statistical significance from zero was determined using the Mann-Whitney U-test. All experiments are $n \geq 8$.

Methyl benzoate, isoamyl acetate, pentyl acetate, 6-methyl-5-hepten-2-one, 4-methyl phenol, methyl salicylate, methyl hexanoate, di-ethyl succinate, and ethyl acetate were obtained from Sigma and geranyl acetate from Fluka.

Immunohistochemistry

Adult brains expressing transgenic uas-*n-syb::GFP* (*5*) were removed from the head capsule and fixed in 4% paraformaldehyde in Phosphate Buffered Saline (PBS), [1.86mM NaH_2PO_4, 8.41mM Na_2HPO_4, 175mM NaCl] for 15 min, and rinsed in PBS-T (PBS containing 0.25% Triton X-100). Brains were incubated with 1:200 mAb anti-GFP (Invitrogen) and 1:100 mAb nc82 (Hybridoma Bank, University of Iowa) followed by the appropriate fluorescent secondary antibodies (Jackson Laboratories). Confocal analysis was performed on a Zeiss LSM 5 Pascal confocal microscope. Confocal stacks were processed using Amira, ImageJ and Adobe Photoshop.

Supplemental Figures

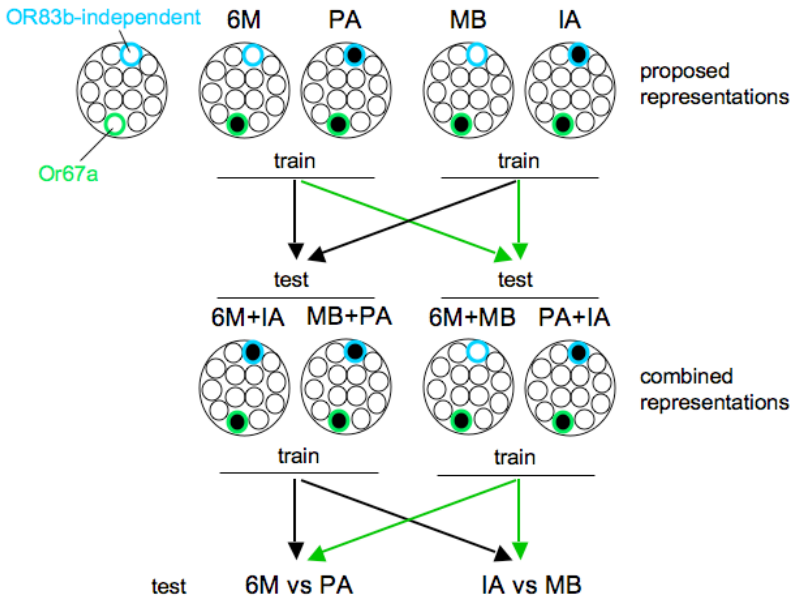

Figure S1. Results from odor mixture experiments cannot be explained by the OR67a restored flies using spatial coding with OR67a neurons and

another class of *Or83b*-independent neurons. In this model a black-filled circle represents an activated glomerulus. Green arrows represent predicted successful experiments and black arrows are predicted failure. OR67a restored flies can discriminate between 6-methyl-5-hepten-2-one (6M) and pentyl acetate (PA) (Fig. 1E) and between methyl benzoate (MB) and isoamyl acetate (IA) (Fig. 1D). Since all of these odorants activate OR67a neurons (green-edged glomerulus), a spatial coding model requires that only one of each discriminable odorant pair activates the other class of *Or83b*-independent neurons.

Irrespective of the odorant chosen to activate the *Or83b*-independent neurons, combining discriminable pairs into mixtures raises a scenario where two of the three mixtures generate identical spatial patterns of activation, and one is unique. Such a spatial coding model predicts flies will be able to discriminate appropriately when trained with components and tested with 6M+MB versus PA+IA odor blends but that when trained with components and tested 6M+IA versus MB+PA there will be no evidence of learning. Our results do not fit this model because OR67a restored flies show learned discrimination for one of the odorant pairs when tested with either odorant mixture.

Similarly, our results from experiments training flies with mixtures and testing with components do not support a spatial model employing OR67a neurons and another class of *Or83b*-independent neurons. The spatial model predicts that training with the odorant mixtures will not produce component learning in one case and will produce learning for both components in the other

instance. However, OR67a restored flies show learned discrimination for one odorant pair in each experiment.

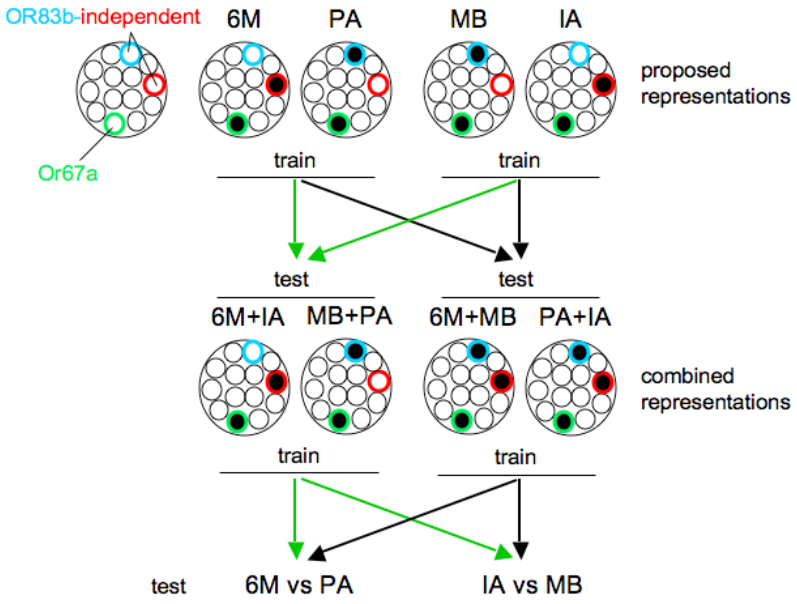

Figure S2. Results from odor mixture experiments cannot be explained by the OR67a restored flies using spatial coding with OR67a neurons and 2 additional classes of *Or83b*-independent neurons. Our results show that OR67a restored flies learn one odor from a binary mixture and these results also cannot be explained by increasing the number of available *Or83b*-independent neurons to two that are activated in a non-overlapping manner. Even that scenario produces mixtures that cannot be discriminated using purely spatial encoding. One could continue to increase the number of putative *Or83b*-independent OSNs and eventually the flies would be predicted to encode both components of the mixture. However, OR67a restored flies encode one component of a binary mixture. Therefore the most parsimonious explanation is that they utilize a single class of *Or83b*-dependent OSNs in our experiments.

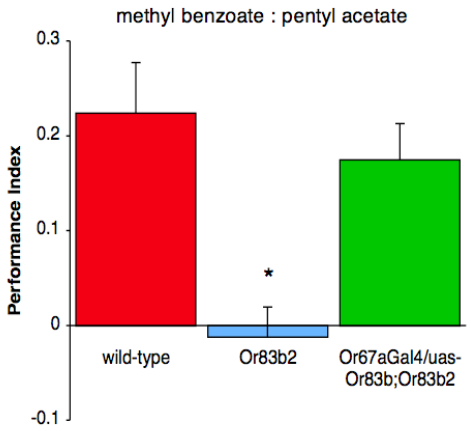

Figure S3. Flies with only functional OR67a neurons can learn to discriminate between methyl benzoate and pentyl acetate. Asterisk indicates significant difference (P<0.005, ANOVA) between the marked group and the others. Data are mean ± s.e.m.

Supplemental References

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