

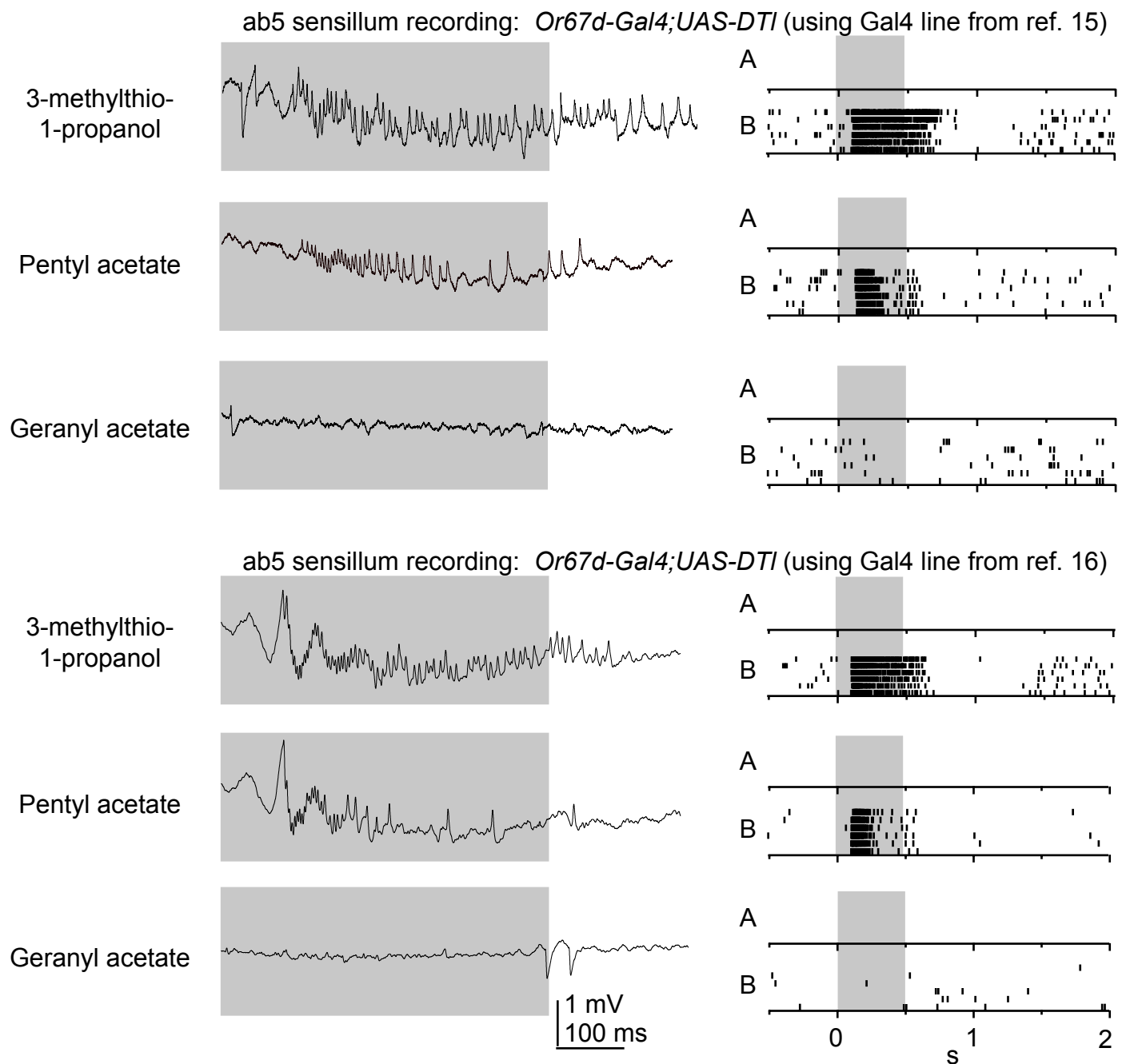
Supplementary Table 1:

This table lists the order of stimuli along the x -axis of each tuning curve. Stimuli are listed in order from left to right, along with the number (n) of independent experiments testing each odor/cell-type combination. Each experiment represents a different fly. The same n values apply to the PSTHs in Figs. 4 and 5. Also listed are the n values for the 3 control stimuli (shaded); these control stimuli are not included in tuning curves.

Figure 1b		Figure 4c	
DA1 ORNs	n	DA1 PNs	n
cis-3-hexen-1-ol	4	4-methyl phenol	6
octanal	4	octanal	6
isoamyl acetate	5	propionic acid	10
pyrrolidine	4	benzaldehyde	6
methyl salicylate	4	cyclohexanone	5
propionic acid	4	1-butanol	7
3-methylthio-1-propanol	4	2-octanone	10
geranyl acetate	5	trans-2-hexenal	10
butyric acid	4	isoamyl acetate	10
cis-vaccenyl acetate	7	cis-vaccenyl acetate	12
4-methyl phenol	5	pentyl acetate	11
cyclohexanone	5	ethyl butyrate	11
γ -valerolactone	4	3-methylthio-1-propanol	7
pentyl acetate	4	pyrrolidine	5
1-butanol	4	γ -valerolactone	7
trans-2-hexenal	6	geranyl acetate	8
benzaldehyde	4	butyric acid	7
ethyl butyrate	4	methyl salicylate	6
2-octanone	4	cis-3-hexen-1-ol	7
paraffin oil	4	paraffin oil	8
water	4	water	7
empty vial	4	empty vial	8
Figure 1e		Figure 5c	
VA6 ORNs	n	VA6 PNs	n
ethyl butyrate	8	octanal	6
γ -valerolactone	6	cis-vaccenyl acetate	5
4-methyl phenol	5	trans-2-hexenal	6
butyric acid	8	γ -valerolactone	5
isoamyl acetate	14	1-butanol	8
pentyl acetate	19	cis-3-hexen-1-ol	6
cis-vaccenyl acetate	5	isoamyl acetate	6
cis-3-hexen-1-ol	7	2-octanone	7
3-methylthio-1-propanol	16	geranyl acetate	13
geranyl acetate	30	pyrrolidine	4
pyrrolidine	5	pentyl acetate	10
octanal	7	3-methylthio-1-propanol	7
1-butanol	10	cyclohexanone	7
methyl salicylate	5	propionic acid	5
propionic acid	5	ethyl butyrate	8
2-octanone	11	4-methyl phenol	6
benzaldehyde	8	benzaldehyde	8
trans-2-hexenal	5	methyl salicylate	7
cyclohexanone	6	butyric acid	7
paraffin oil	5	paraffin oil	9
water	5	water	4
empty vial	5	empty vial	7

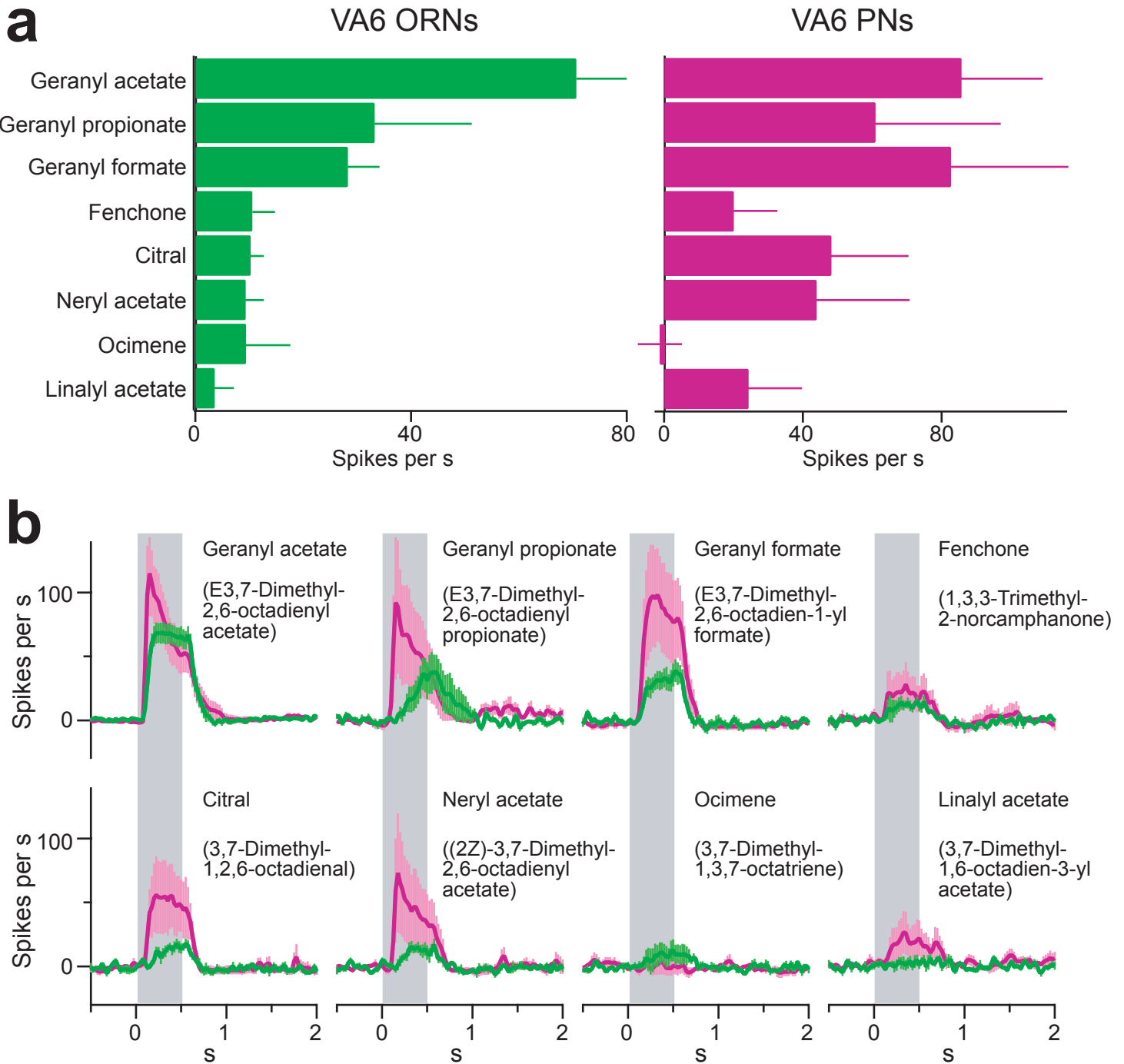
Supplementary Figure 1. In *Or67d-Gal4* flies, Gal4 is expressed ectopically in ab5A ORNs.

Two independent *Or67d-Gal4* lines label ORN projections in both the DA1 and VA6 glomeruli^{15,16}. This could be the result of either the *Or67d*-expressing ORNs unexpectedly projecting to both glomeruli, or of ectopic *Gal4* expression in the only ORNs that are currently known to project to VA6 (the ab5A ORNs). The ab5A ORNs do not express *Or67d*^{15,16}. If *Or67d-Gal4* drives ectopic expression of *Gal4* in the ab5A ORNs, then we would predict that driving *UAS-DTI* with *Or67d-Gal4* would kill the ab5A ORNs. However, if the explanation is that *Or67d*-expressing ORNs project to both DA1 and VA6, then we would predict that driving expression of *UAS-DTI* with *Or67d-Gal4* would not have any effect on the ab5A ORNs. We drove diphtheria toxin expression using both of these *Gal4* lines (*Or67d-Gal4;UAS-DTI*) and recorded from ab5 sensilla, which house both the ab5A ORNs and another ORN type (ab5B). Of this pair, the ab5A ORN always produces larger action potentials and responds strongly to geranyl acetate, whereas the “B” neuron responds strongly to 3-methylthio-1-propanol and pentyl acetate. In both lines, 5 of 5 ab5 sensilla we tested lacked the “A” ORN. We conclude that both *Or67d-Gal4* lines ectopically express Gal4 in the ab5A ORNs. ORN recordings from *Or67d-Gal4;UAS-DTI* flies are shown on the left. Rasters (right) show that all the spikes are “B” spikes. Compare geranyl acetate responses to controls in Fig. 1d.



Supplementary Figure 2. VA6 ORNs respond more strongly to geranyl acetate than to other highly related odors.

VA6 ORNs and PNs were tested with a panel of 7 odors that are very similar in structure to geranyl acetate. Tuning curves (a) and PSTHs (b) show that none of these odors elicited a response larger than geranyl acetate. In addition, Hallem and Carlson¹⁹ tested 15 more odors similar to geranyl acetate, and also found that none of these odors elicited a larger response. We cannot exclude the possibility that VA6 ORNs have evolved to detect yet another terpenoid which has never been tested in these studies, and which might elicit a larger response. Note that the VA6 PNs are more broadly tuned than their presynaptic ORNs with respect to this small odor set, as for our larger odor set.



Supplementary Methods

As in all the electrophysiology experiments, all flies were females aged 3-8 days. A modified Y-maze was constructed by connecting two 2L Pyrex filtration flasks. The filtration nozzle of each flask was removed by scoring with a diamond-tipped knife. The flasks were connected with a narrow tube of paper that fit snugly into the nozzle hole. This paper tube had a hole at the midpoint which matched the size of the hole in the fly loading tube. Prior to behavioral assays, flies were housed 36 hours at room temperature in small groups (20-40 flies) inside a 50-ml plastic conical tube containing a Kimwipe folded over 4 times and cut to fit the cap exactly, and wetted with 250 μ L of purified H₂O. A hole was cut in the bottom of the conical tube and covered with Parafilm. An assay was begun by placing 500 μ L of the control and experimental odors (diluted in H₂O) on folded Kimwipes, and then placing these Kimwipes into the flasks and sealing both flasks with Parafilm for 5 minutes (with the paper tube removed). The paper tube was then connected for 30 seconds prior to loading the flies. Flies were loaded by removing the Parafilm from the bottom of the conical tube, and then raising the conical tube “bottom-side up” to the hole at the midpoint of the paper tube connecting the two flasks. A flashlight was held about 1cm above the tube and flashed on for 2 seconds. Phototaxis and gravitaxis drew flies out of the conical and up into the tube. Flies were allowed a total of 3 minutes to enter the tube and an additional 3 minutes to walk into one of the flasks (total run time = 6 minutes). Flies walked back and forth in the tube several times before entering a flask; 75-100% of flies that entered the choice tube chose a flask by the end of 6 min. Flies were not observed to leave a flask after entering. Assays were conducted in the dark under red illumination. Between runs, flasks were washed with ethanol and detergent and thoroughly dried, and the identity of the odor and control flasks was reversed. The flies’ behavior was quantified as:

$$\text{response index} = 100 \cdot (\# \text{ of flies in odor flask}) / (\# \text{ of flies in odor flask} + \# \text{ in control flask})$$

Results in Figs. 2-3 are mean \pm SEM averaged across at least 6 independent runs with different flies.

Because flies moved freely in the maze, they sampled a range of odor concentrations.