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Supplemental Data

Excitatory Interactions

between Olfactory Processing Channels

in the *Drosophila* Antennal Lobe

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Table S1. Genotypes Used in Each Experiment

Fig. 1B	*UAS-CD8:GFP/+;Or83b-Gal4/+*
Fig. 1C-G	*w[1118]*
Fig. 2- 4	VM2: wild-type is *NP5103-Gal4,UAS-CD8:GFP*, mutant is *NP5103-Gal4,UAS-CD8:GFP;Or43b[1]* DL1: wild-type is *NP3529-Gal4,UAS-nlsGFP*, mutant is *Or10a[f03694];+/+;NP3529-Gal4,UAS-nlsGFP*
Fig. 5B	control (top): *Or33c-Gal4/+; UAS-DTl/+ or Or33c-Gal4/UAS-DTl*[c] mutant (middle): *Or83b[2]* rescued (bottom): *Or46-Gal4/UAS-Or83b;Or83b[2]*
Fig. 5C	top: *UAS-CD8:GFP/+;Or46a-Gal4/ UAS-Or83b;Or83b[2]* bottom: *Or46-Gal4/UAS-Or83b;Or83b[2]*
Fig. 5E	VM7 ORNs functional: *NP3481-Gal4,UAS-CD8:GFP;+/+;Δ85* VM7 ORNs non-functional: *Or42a[f04305];Δ85*
Fig. 6A,C	*Or46a-Gal4/UAS-Or83b;Or83b[2]*
Fig. 6B,D	*NP3481-Gal4,UAS-CD8:GFP;+/+;Δ85*
Fig. 7	*NP3481-Gal4,UAS-CD8:GFP;+/+;Δ85*
Fig. 8A-C	*Or46a-Gal4/UAS-Or83b;Or83b[2]*
Fig. 8D-F	*NP3481-Gal4,UAS-CD8:GFP;+/+;Δ85*
Fig. 9	*Or46a-Gal4/UAS-Or83b;Or83b[2]*
Fig. S1A	*Or10a[f03694];Or10a-Gal4;UAS-CD8:GFP*
Fig. S1C-D	VM2: wild-type is *GH146-Gal4,UAS-CD8:GFP* or *NP5103-Gal4,UAS-CD8:GFP*, mutant is *Or43b[1]* DL1: wild-type is *w[1118]* or *Or42b[EY14886]*[a] or *Or42b-Gal4/UAS-DiphTx;Or92a-Gal4/UAS-DiphTx*[b], mutant is *Or10a[f03694]*
Fig. S2	same as Fig. S1
Fig. S3	control: *w[1118]* mutant: *Or83b[2]*
Fig. S4	control: *Or33c-Gal4/+; UAS-DTl/+*[c] *or Or33c-Gal4/UAS-DTl*[c] rescued: *Or46-Gal4/UAS-Or83b;Or83b[2]*
Fig. S5	wild-type: *w[1118]* mutant: *Or42a[f04305];Δ85*
Fig. S6	see italicized labels in figure

[a] pBac insertion in the *Or42b* gene (Bloomington Stock Center). We have observed that this mutation abolishes odor responses in ab1A ORNs, allowing us to count more accurately the spikes from ab1D (DL1) and ab1B(VA2) ORNs in response to odors that normally drive the ab1A ORNs strongly.

[b] We killed ab1A and ab1B ORNs by expressing diphtheria toxin selectively in these cells. This allowed us to more accurately detect the small spikes of ab1D (DL1) ORNs in response to odors that normally drive both either the ab1A or ab1B ORNs strongly.

[c] We killed pb2A ORNs by expressing diphtheria toxin selectively in these cells, allowing us to count more accurately the spikes from pb2B (VA7l) ORNs.

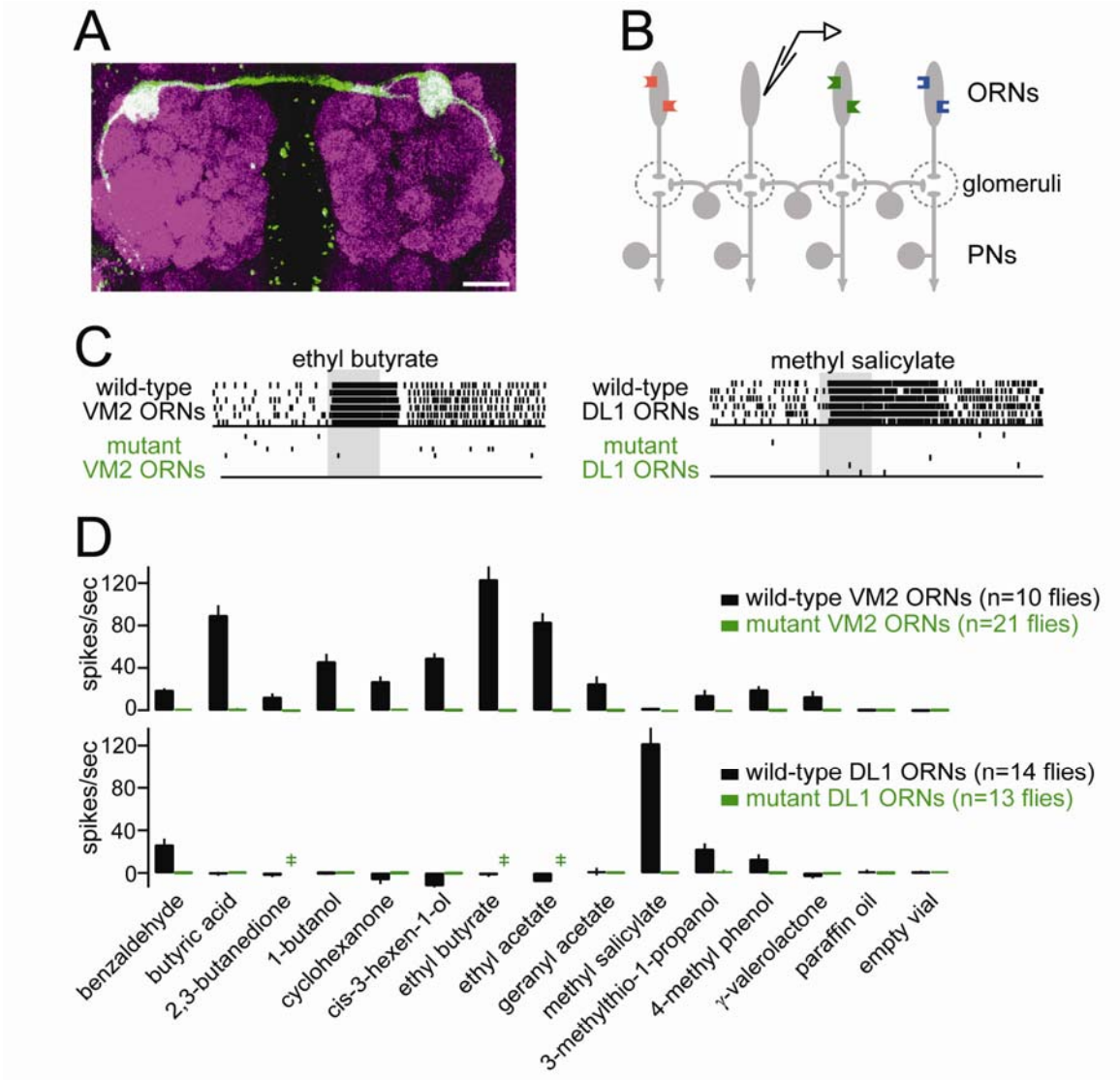


Fig. S1. Mutations in odorant receptor genes virtually abolish odor responses in ORNs.
(A) ORNs that normally express Or10a target glomerulus DL1 correctly in the *Or10a*^{f03694} mutant. Projection of a confocal stack through the antennal lobes (neuropil in magenta) shows ORN axons labeled with CD8:GFP (green). This result is consistent with a previous report that a functional odorant receptor is not required for correct ORN axon targeting in *Drosophila* (Dobritsa et al., 2003).
(B) Schematic of experiments in Fig. S1, panels C-D: extracellular recordings are performed from ORNs whose corresponding odorant receptor gene has been mutated.
(C) Rasters of odor-evoked responses from wild-type and mutant ORNs. Each row is a separate trial with the same odor. Gray bar = 500-ms period of odor delivery. Note that spontaneous activity is also reduced by these mutations.
(D) Average odor responses of wild-type and mutant ORNs. Each response is the mean ± SEM of 4-18 independent experiments in different flies. The firing rates for three odors (marked with ‡) could not be determined in the mutant due to the fact that other ORNs in the same sensillum as DL1 ORNs respond very strongly to these stimuli.

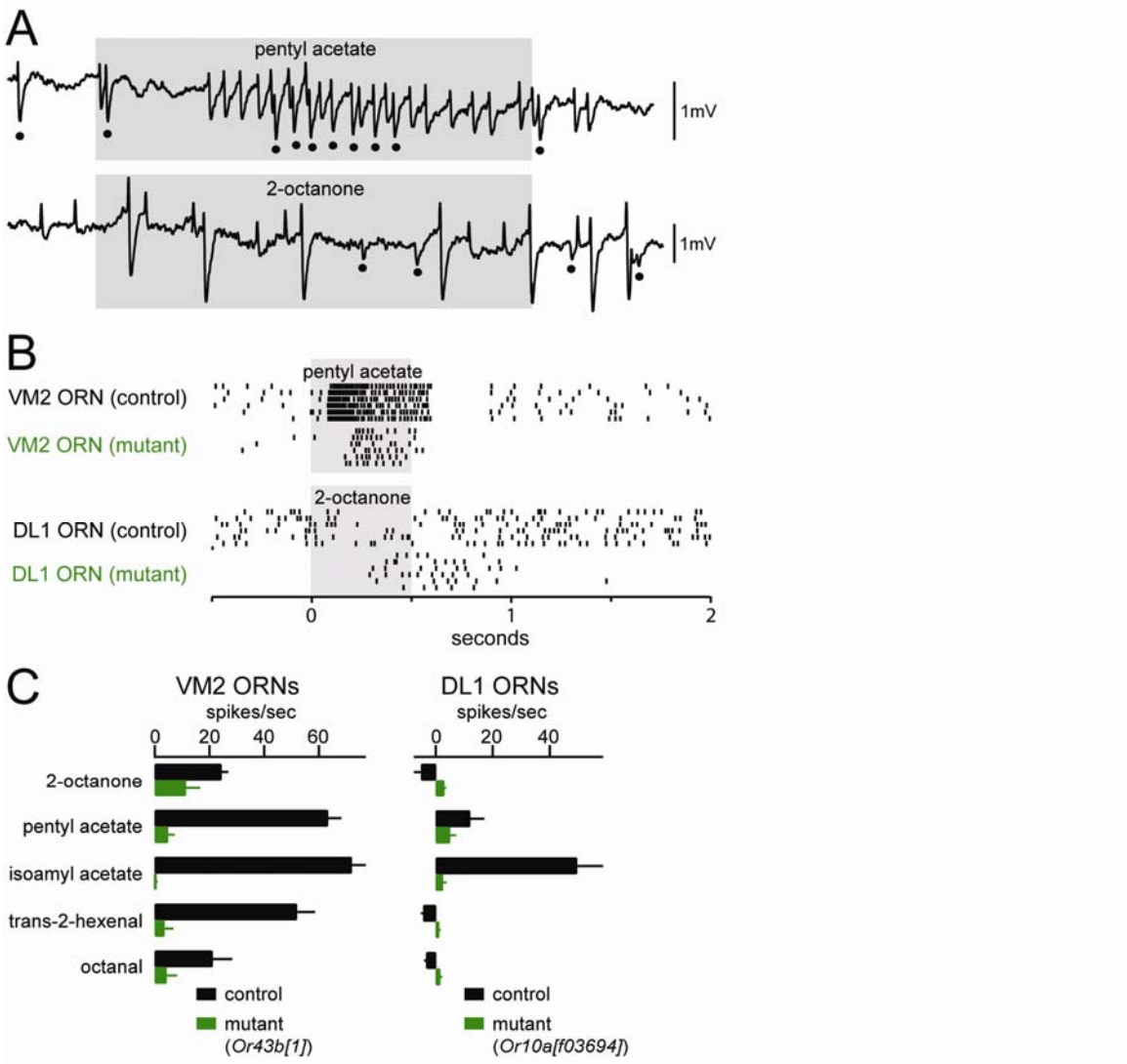


Fig. S2: Weak responses to a few odors persist in mutant ORNs.
(A) Single-sensillum recordings. Top trace is a recording from an ab8 sensillum in an *Or43b*^{1} fly. Symbols (●) indicate spikes originating from the neuron that normally expresses Or43b (the ab8A cell). Bottom trace is a recording from an ab1 sensillum in an *Or10a*^{f03694} fly. Symbols indicate spikes originating from the neuron that normally expresses Or10a (the ab1D cell). Although the odorant receptor gene corresponding to these ORNs has been mutated, they still show weak responses to a few odors.
(B) Rasters compare responses of control and mutant ORNs to the same odor. Mutant responses are very weak, but are consistent from trial to trial.
(C) Summary of odors eliciting a nonzero response in mutant ORNs. Mean response during the 500-msec odor stimulation period (±SEM), averaged over 4-12 experiments in different flies. Some of the same odors elicit nonzero responses in both types of mutant ORNs (especially 2-octanone and pentyl acetate). We have also observed very weak responses to some of these odors in Or22a mutant flies (*Δhalo*) while recording from ab3A ORNs. We have excluded all five odors shown in (C) from our analysis in Figs. 3-4 and Fig. S2.

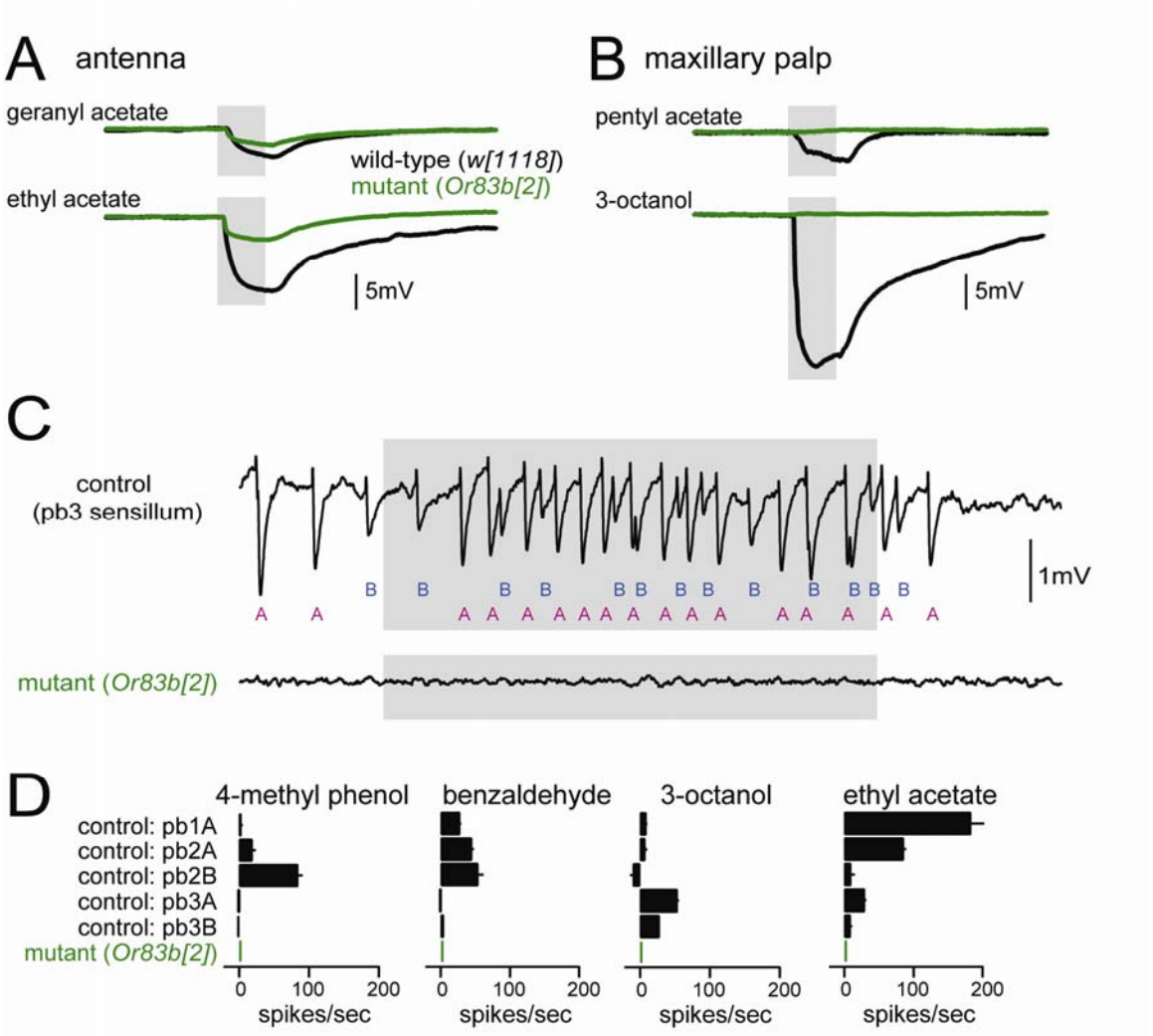


Fig. S3: The *Or83b²* mutation abolishes odor responses in the palps but not in the antennae.
(A) Odor-evoked local field potentials recorded from the antenna in wild-type (w^{1118}) and mutant ($Or83b^2$) flies. Gray bar indicates 500-ms odor stimulation period.
(B) Odor-evoked local field potentials recorded from the maxillary palp in w^{1118} and $Or83b^2$ flies.
(C) Extracellular recordings of spikes from single sensilla on the maxillary palp. All palp sensilla contain two ORNs, distinguishable by their spike shape and size. Top trace shows a recording from a palp basiconic type 3 sensillum (pb3). Large spikes come from the A neuron (pb3A, magenta) and small spikes from the B neuron (pb3B, blue). Lower trace shows a sensillum recording from an $Or83b^2$ mutant; both spontaneous and odor-evoked spikes are absent. Odor stimulus is ethyl acetate in both traces.
(D) Average firing rates evoked by four odors. These odors elicit ORN spikes in control sensilla, but no responses in $Or83b^2$ mutant sensilla (n = 3 sensilla of each type for control, n = 23 sensilla total for mutant). Responses are computed over the 500-msec odor stimulus period, minus baseline firing rate. Mean ± SEM, averaged across experiments.

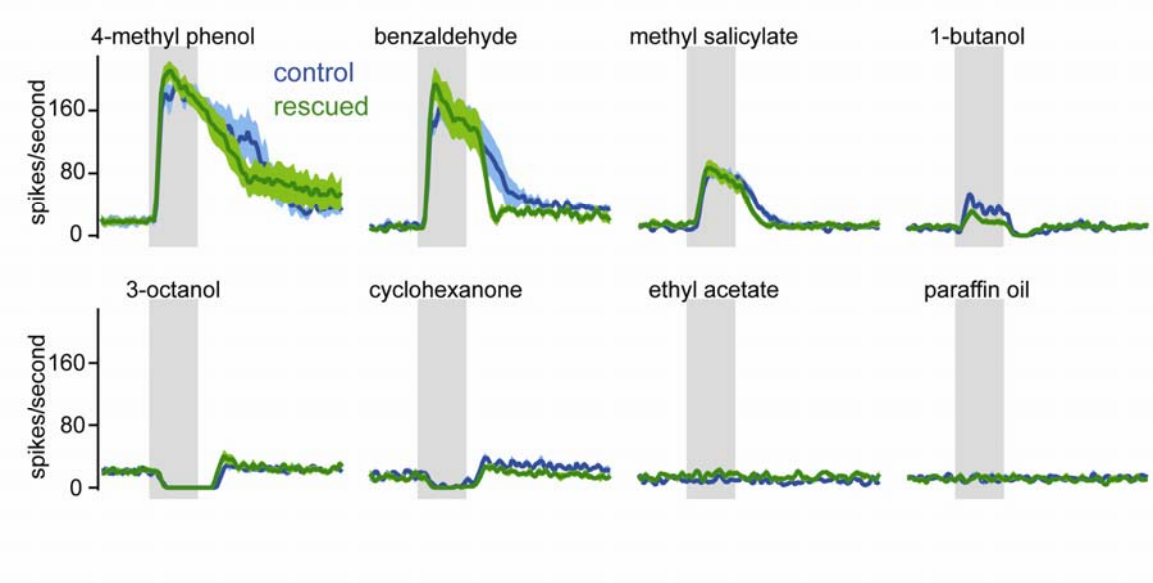


Fig. S4: Rescued VA7l ORNs recapitulate normal ORN odor responses.
Peristimulus-time histograms comparing odor responses in control versus "rescued" VA7l ORNs. Mean ± SEM, averaged across experiments, $n \geq 5$ for each trace. Gray bar = 500-ms period of odor stimulation. Genotype of control flies is *Or33c-Gal4/+;UAS-DTl/+* or *Or33c-Gal4/UAS-DTl.* (In order to count more accurately the spikes from VA7l ORNs in control sensilla, we expressed diphtheria toxin under the control of the *Or33c-Gal4* driver in order to kill the other ORN in the pb2 sensillum.) Genotype of "rescued" flies is *Or46-Gal4/UAS-Or83b;Or83b*^{2}.

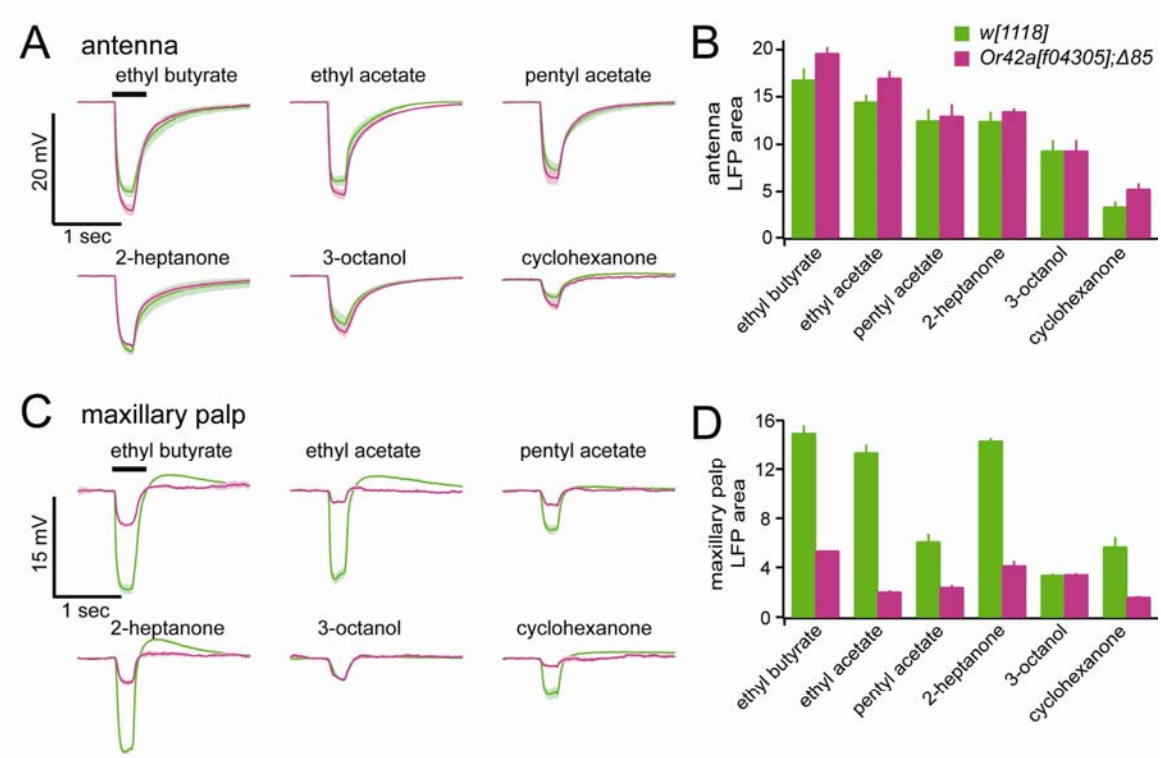
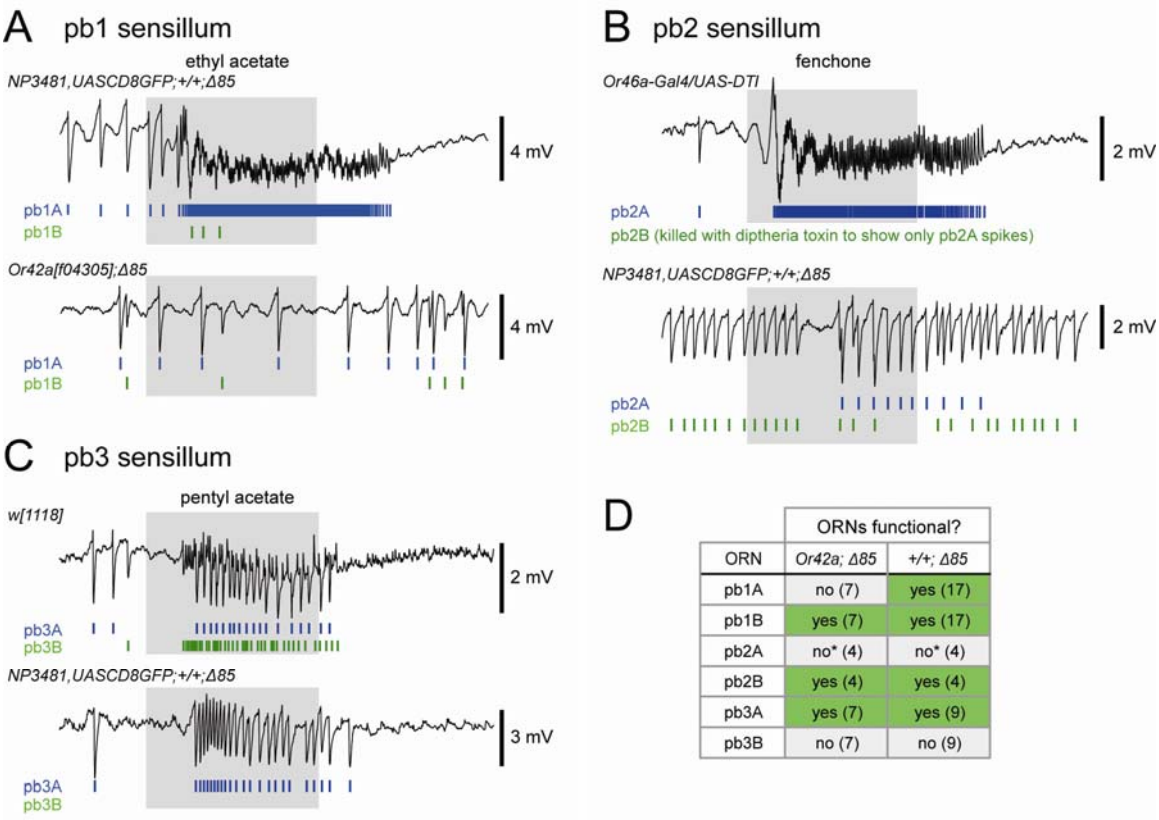


Fig. S5: *Or42a^{f04305};Δ85* flies have a deficit in some maxillary palp ORNs but not antennal ORNs.
(A) Antennal local field potential recordings from *w^{1118}* (green; n = 3) and *Or42a^{f04305};Δ85* (magenta; n=3). Mean ± SEM, averaged across experiments. Black bar = 500-ms period of odor stimulation.
(B) Quantification of field potential area shows that *Or42a^{f04305};Δ85* flies have normal odor responses in the antenna. This rules out a general olfactory deficit in these flies.
(C) Maxillary palp local field potential recordings from *w^{1118}* (green) and *Or42a^{f04305};Δ85* (magenta). Mean ± SEM, averaged across experiments. Black bar = odor.
(D) Quantification of maxillary palp field potential area shows that *Or42a^{f04305};Δ85* flies have reduced maxillary palp ORN responses to most odors, as compared to *w^{1118}*.



D

	ORNs functional?	
ORN	*Or42a; Δ85*	*+/+; Δ85*
pb1A	no (7)	yes (17)
pb1B	yes (7)	yes (17)
pb2A	no* (4)	no* (4)
pb2B	yes (4)	yes (4)
pb3A	yes (7)	yes (9)
pb3B	no (7)	no (9)

Fig. S6: Electrophysiological characterization of the *Δ85* and *Or42a^{f04305}* mutations.
(A) Extracellular recordings from pb1 sensilla showing that the *Or42a[f04305]* mutation abolishes odor-evoked activity in the pb1A neurons (these ORNs target the VM7 glomerulus). Each tick represents a spike from either the "A" (blue) or "B" (green) ORN in the pb1 sensillum. Odor stimulation is indicated by the gray bar (500 msec).
(B) Recordings from pb2 sensilla showing that the *Δ85* mutation severely reduces odor responses in the pb2A neuron. In the top trace, the pb2B neuron has been killed with diphtheria toxin to show only spikes from the pb2A neuron (*Or46a-Gal4/UAS-DTI*).
(C) Recordings from pb3 sensilla show that the *Δ85* mutation eliminates odor-evoked activity in pb3B neurons.
(D) Summary of single sensillum recordings in *Or42a^{f04305};Δ85* and *+/+;Δ85*. Numbers in parentheses indicate the number of recordings.
*Odor responses in pb2A are dramatically reduced, but not eliminated. This ORN expresses two receptors, Or33c and Or85e. We hypothesize that the *Δ85* mutation removes Or85e and that Or33c mediates the residual responses. This interpretation is consistent with the known odor tuning of Or33c and Or85e (Goldman et al, 2005).

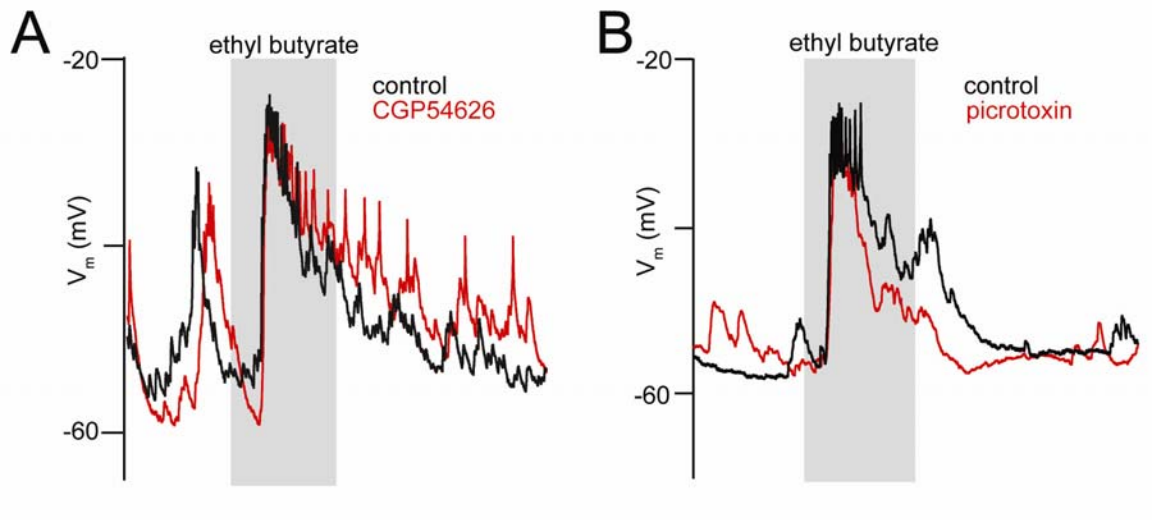


Fig. S7: GABA receptor antagonists do not abolish lateral excitatory inputs to PNs.
(A) Recording from a VM2 PN postsynaptic to non-functional ORNs (genotype *NP5103-Gal4,UAS-CD8:GFP;Or43b*^{1}). The lateral depolarization is unaffected by 50 μM CGP54626, a concentration which it is effective at blocking $GABA_B$ receptors in *Drosophila* PNs (Wilson & Laurent 2005). Similar results were observed in other experiments (*n*=3). Also, CGP54246 did not affect the magnitude of the lateral depolarization in antennal PNs in experiments where only VA7l ORNs were stimulated (*n*=2, experimental design same as in Figs. 6A and 8A).
(B) Recording from a VM2 PN postsynaptic to non-functional ORNs (genotype *NP5103-Gal4,UAS-CD8:GFP;Or43b*^{1}). The lateral depolarization is unaffected by 10 μM picrotoxin, a concentration which it is effective at blocking $GABA_A$ receptors in *Drosophila* PNs (Wilson & Laurent 2005, see Supplemental Fig. 3 of that paper). Similar results were observed in other experiments (*n*=3). Also, picrotoxin did not diminish the lateral depolarization in antennal PNs in experiments where only VA7l ORNs were stimulated (*n*=4, experimental design same as in Figs. 6A and 8A). In some experiments, picrotoxin speeded the kinetics of the lateral depolarization but did not diminish it.