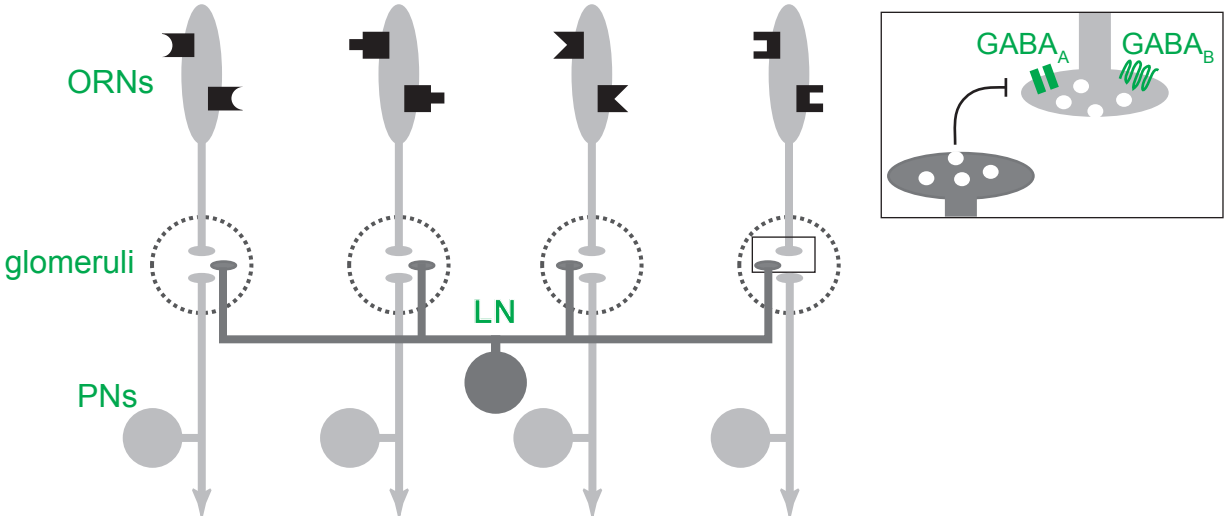
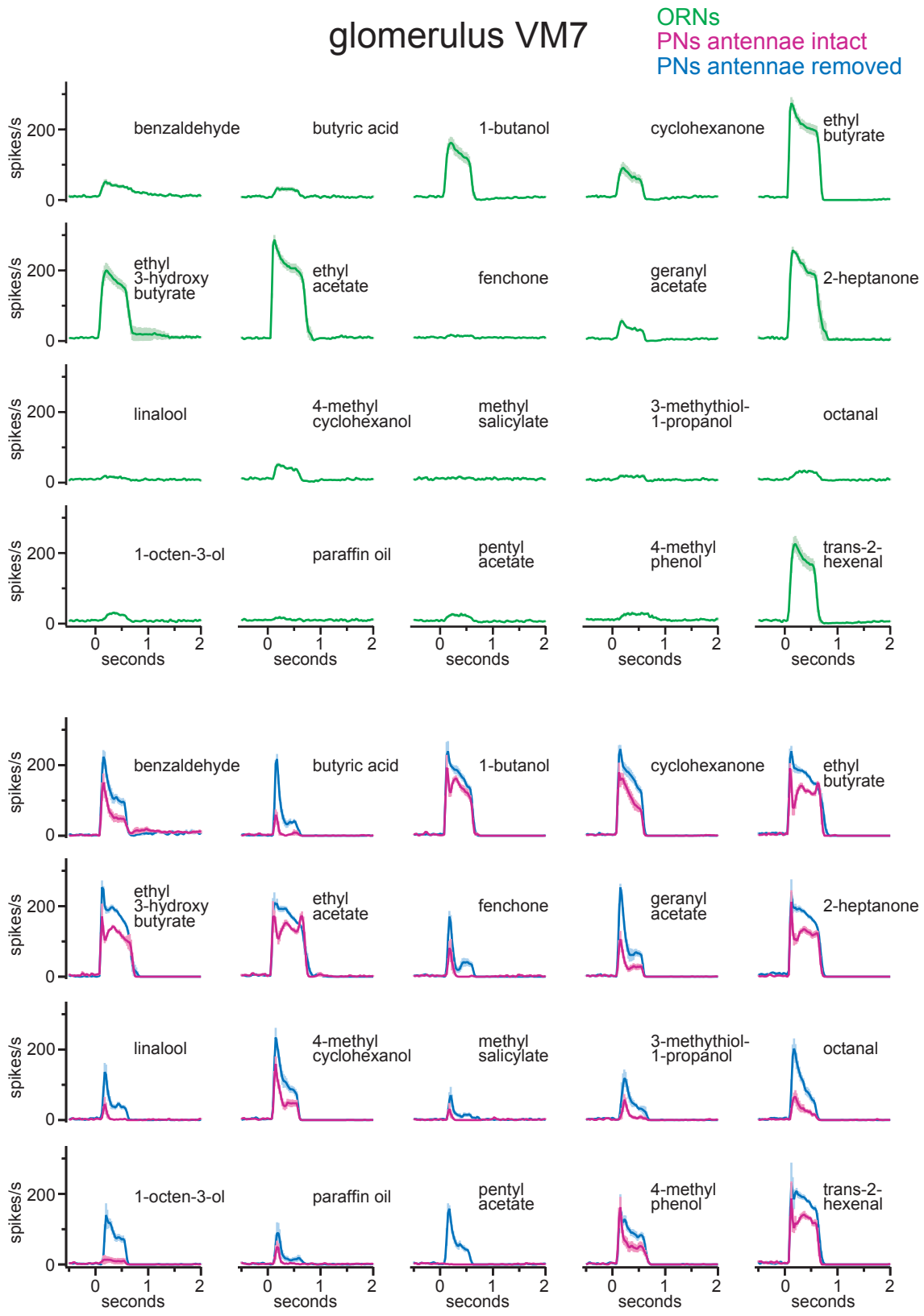
SUPPLEMENTARY INFORMATION

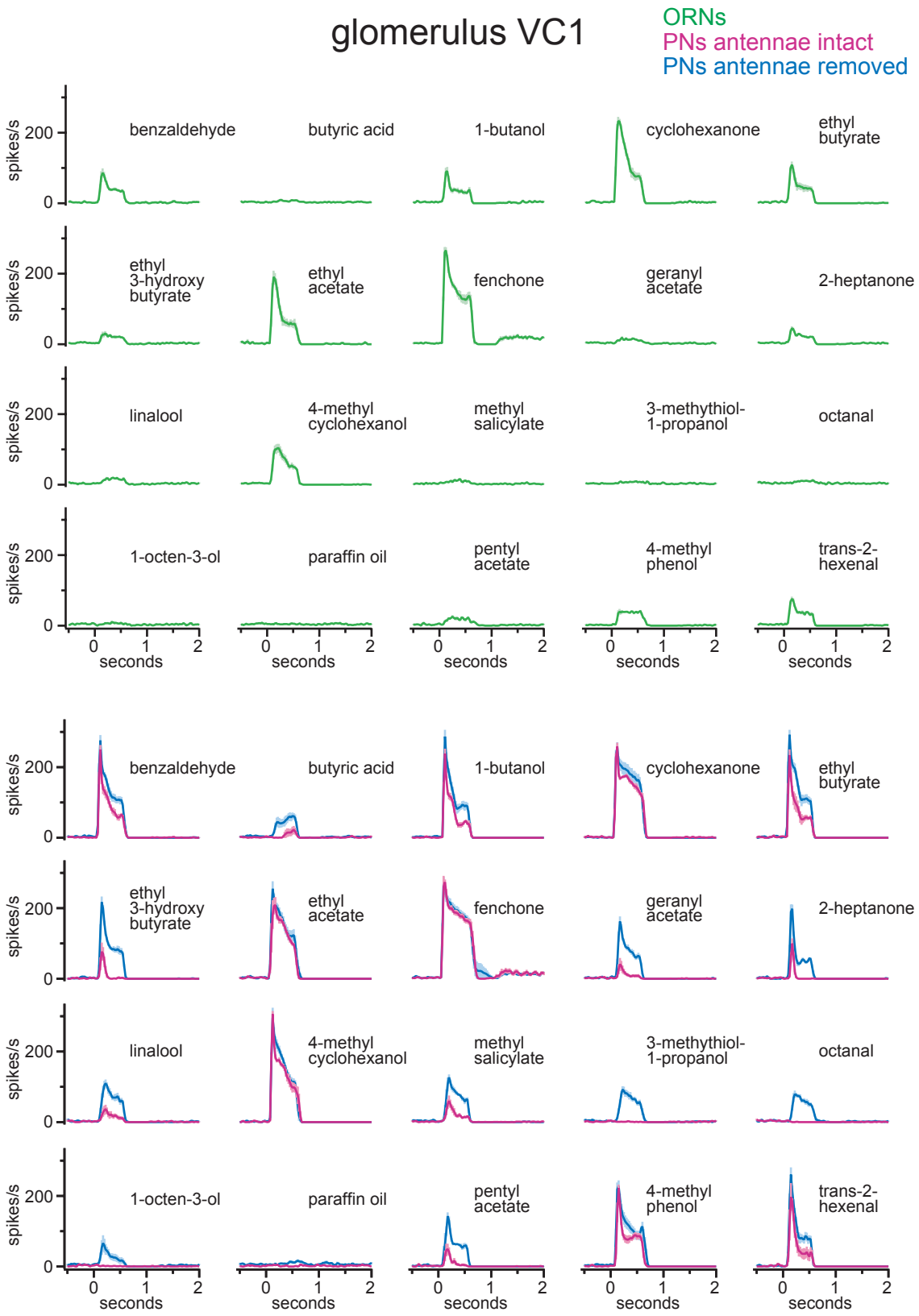


Supplementary Figure 1. Schematic illustrating major conclusions of this study.

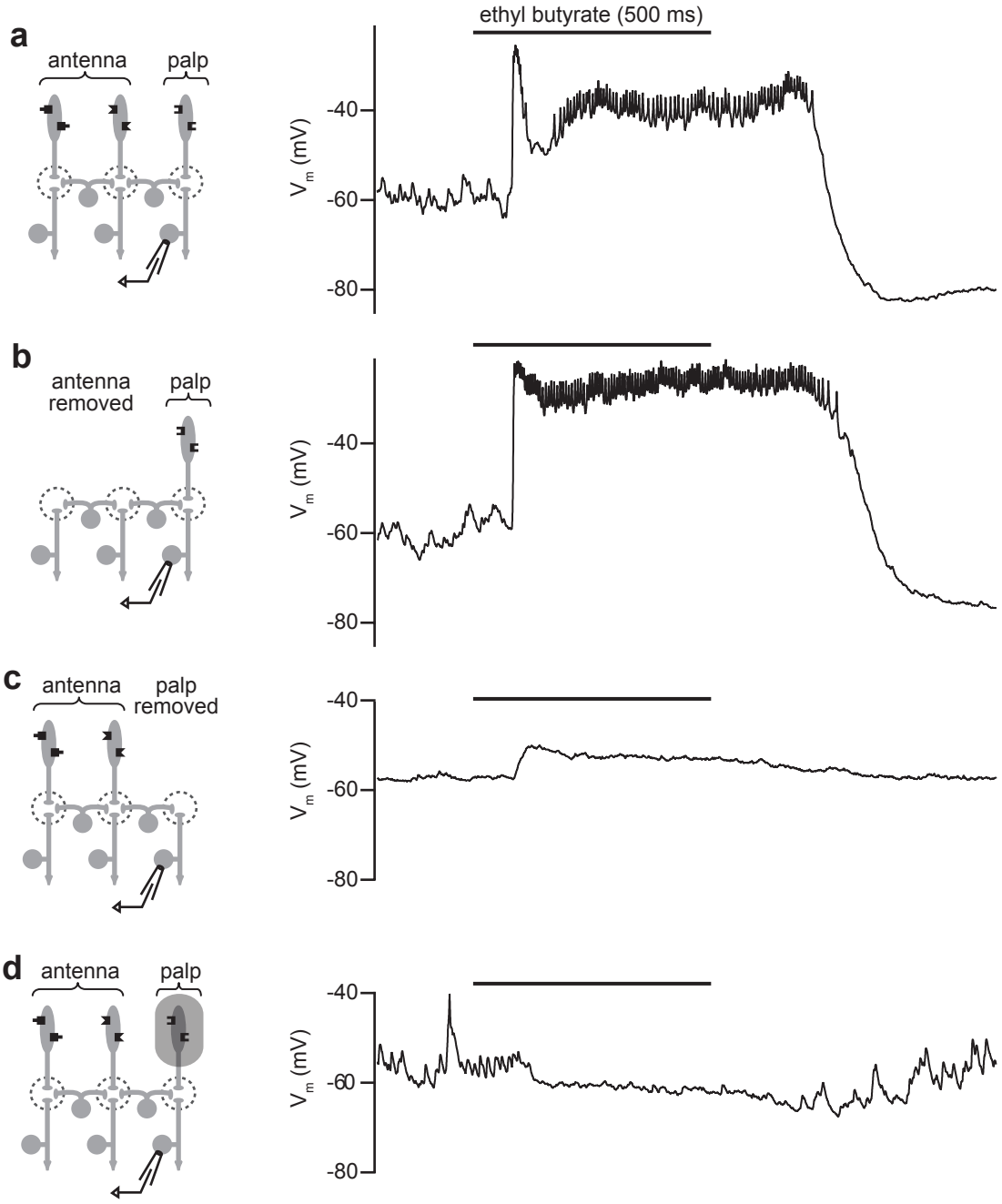
This study represents the most direct evidence to date of inhibitory interactions between olfactory glomeruli *in vivo*. Our results show that the odor responses of a *Drosophila* antennal lobe projection neuron (PN) can be substantially inhibited by activity in the rest of the antennal lobe. Much of this inhibition appears to occur at a presynaptic locus, via the suppression of neurotransmitter release from ORN axons. Our results suggest that both $GABA_A$ and $GABA_B$ receptors are present on the same presynaptic terminals. We find that the amount of lateral inhibition evoked by an odor stimulus is proportional to the total number of ORN spikes produced by that stimulus, suggesting that inhibition reflects pooled input from all ORN types. We propose that this form of spatially diffuse presynaptic lateral inhibition serves to control the gain of olfactory processing. When stimuli are weak, ORN-PN synapses are strong, but when stimuli are strong, ORN-PN synapses are weakened. This should prevent a stimulus from saturating the dynamic range of many PN types simultaneously. Because strong and redundant stimuli are preferentially suppressed, this may tend to decrease cross-correlations between the output of different glomeruli, and thus promote a more efficient neural code for odors.



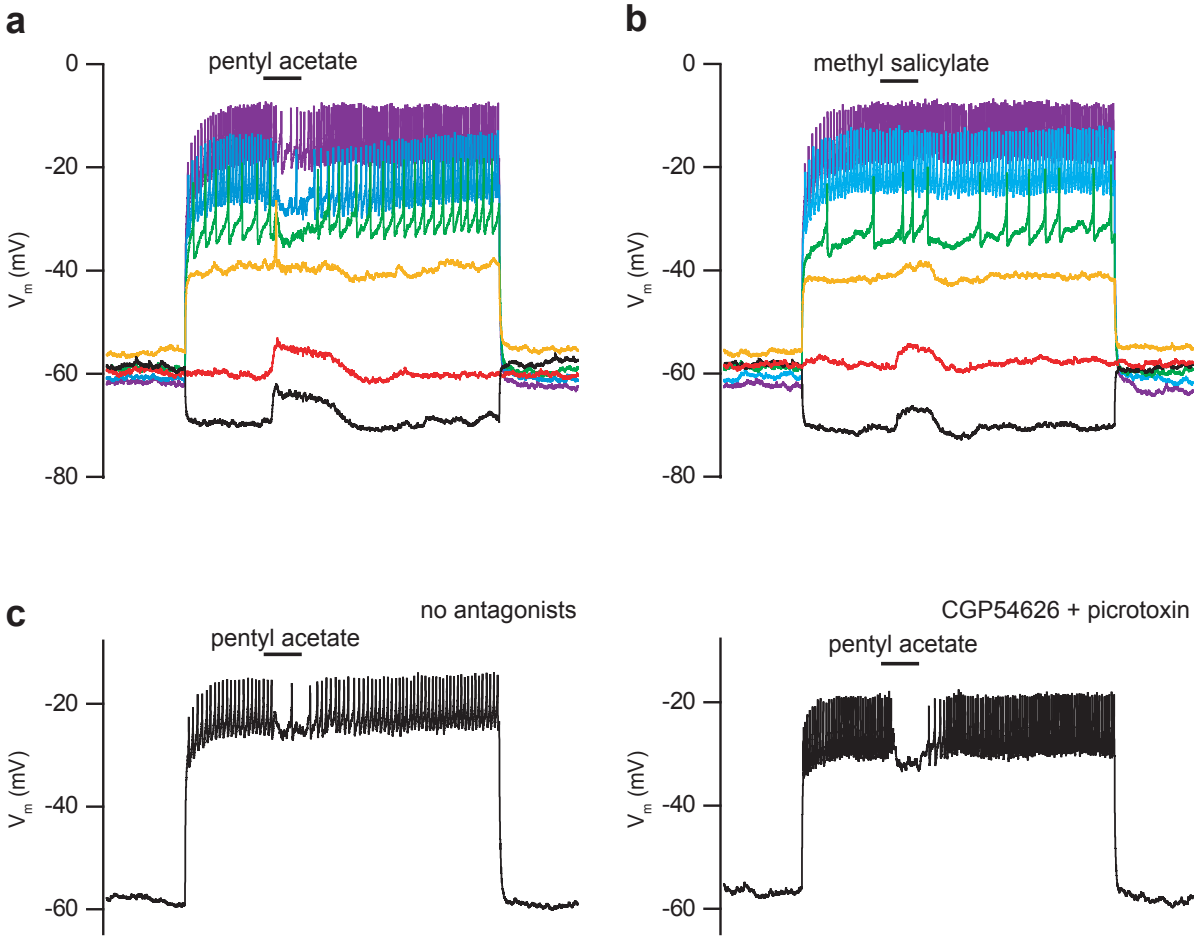
Supplementary Figure 2. Peri-stimulus time histograms for glomerulus VM7
PSTHs for ORNs (green) and PNs either with (magneta) or without (blue) antennal input. The odor stimulus in each panel is from 0 to 0.5 seconds. All panels use the same *x* and *y*-axes. All PSTHs are averaged across 4-12 experiments, ± s.e.m in pastel. In some PSTHs the pastel error bars are too small to be visible.



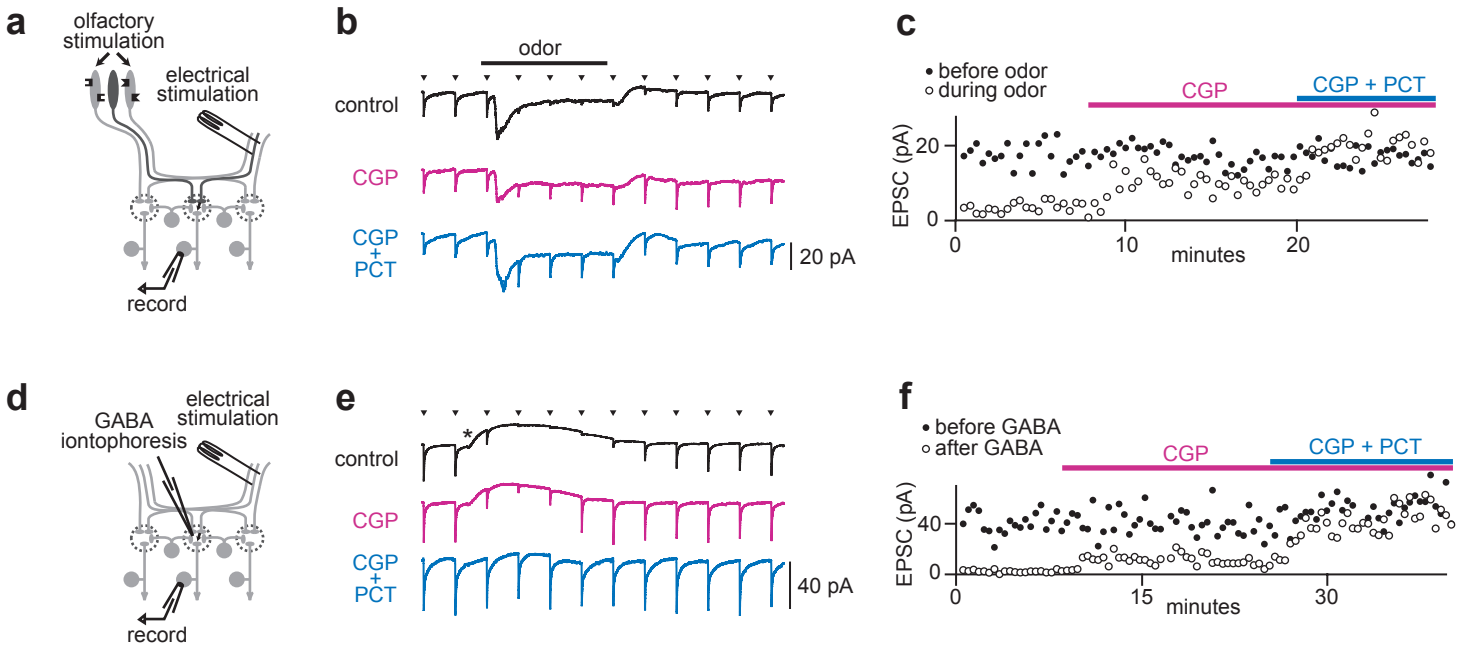
Supplementary Figure 3. Peri-stimulus time histograms for glomerulus VC1
PSTHs for ORNs (green) and PNs either with (magneta) or without (blue) antennal input. The odor stimulus in each panel is from 0 to 0.5 seconds. All panels use the same *x* and *y*-axes. All PSTHs are averaged across 4-12 experiments, ± s.e.m in pastel. In some PSTHs the pastel error bars are too small to be visible.



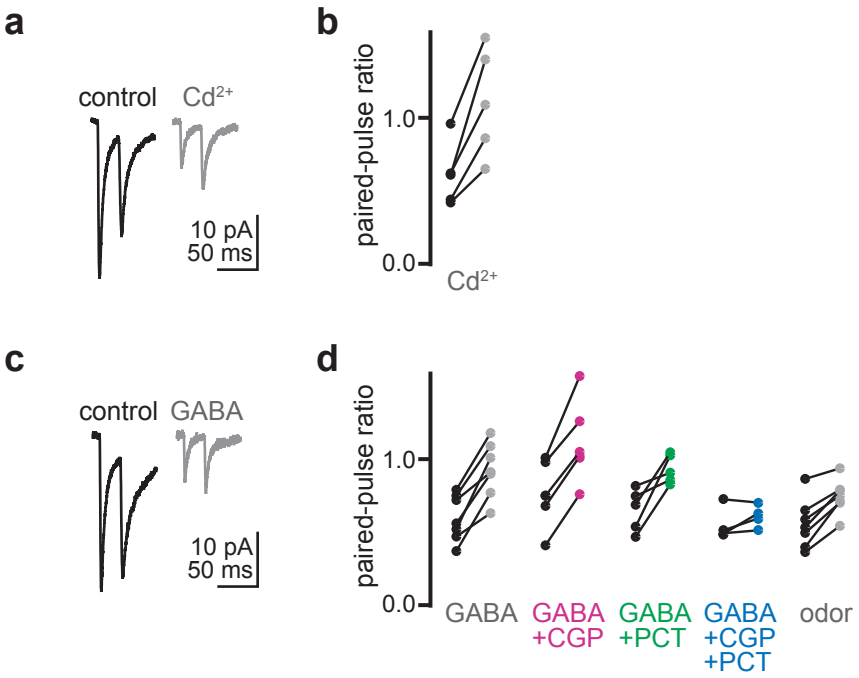
Supplementary Figure 4. Sample records for glomerulus VM7.
Odor stimulus for all records is ethyl butyrate (500 ms).
(a) A whole-cell patch clamp recording from a VM7 PN, recorded in the normal configuration (both antennae and palps attached, no shielding of palps). Note abundant spontaneous subthreshold input prior to odor stimulation. This mainly reflects spike-driven spontaneous EPSPs (sEPSPs) arising from ORN-PN synapses.
(b) Recording from a VM7 PN with antennae removed. Note that sEPSPs prior to odor stimulation are similar to above, as expected. Removing the antennae disinhibits the odor response, implying that antennal glomeruli are a source of lateral inhibition for this glomerulus.
(c) Recording from a VM7 PN with palps removed (same trace as in Fig. 2a). Note that sEPSPs are now absent, as expected. We and others[7,11,12] have interpreted the depolarizing odor response as lateral postsynaptic excitatory input from the antennae. Lateral postsynaptic inhibitory input may also be present, but during the odor response period it is evidently weaker than the lateral excitation.
(d) Recording from a VM7 PN with palps shielded (same trace as in Fig. 2b). Note that sEPSPs are visible again, implying that ORNs fire spontaneously even when they are shielded from odors. The shield is clearly preventing VM7 ORNs from sensing ethyl butyrate, because the strong excitation visible in **(b)** is absent. Now, lateral input is mainly hyperpolarizing. The only difference between **(c)** and **(d)** is the absence versus presence of spontaneous ORN-PN EPSPs, and this implies that the difference between these traces reflects lateral inhibition of ORN-PN synapses.



Supplementary Figure 5. Evidence for lateral postsynaptic inhibition.
In this experiment we tested whether the lateral postsynaptic input to a PN has an inhibitory component. To isolate the lateral postsynaptic input, the maxillary palps were removed, and recordings were made from PNs in the palp glomerulus VM7 (see Fig. 2a). Current pulses were applied to the cell so that odor responses could be recorded at different membrane potentials. Raw data from one experiment are shown here. Similar results were observed in other experiments (n = 5). The odor duration for all records is 500 ms.
(a) The odor pentyl acetate elicited depolarizations when the membrane potential was more hyperpolarized than about -40 mV. At potentials more depolarized than -40 mV, pentyl acetate produced a small hyperpolarization and an interruption of the spiking activity evoked by current injection. This result suggests that odors can elicit both lateral postsynaptic excitation and lateral postsynaptic inhibition. Moreover, the balance between excitation and inhibition depends on membrane potential: pentyl acetate could either evoke a spike (orange trace) or inhibit spikes (green, blue, purple traces).
(b) The odor methyl salicylate evoked postsynaptic excitation but did not hyperpolarize the cell at any membrane potential. This indicates that the balance of lateral postsynaptic excitation and inhibition is odor-dependent.
(c) Blocking $GABA_A$ and $GABA_B$ receptors with a combination of picrotoxin and CGP54626 did not eliminate the postsynaptic inhibition evoked by pentyl acetate. Because the postsynaptic response to GABA iontophoresis is completely blocked by picrotoxin and CGP54626 (ref. 9), this suggests that GABA may not mediate this postsynaptic inhibition. Moreover, this result indicates that the disinhibition produced by picrotoxin and CGP54626 in Fig. 5 is not due to the block of postsynaptic inhibition. Rather, the disinhibition in Fig. 5 is more consistent with the removal of presynaptic inhibition, which is shown in Figs. 3-4 to be completely sensitive to the combination of picrotoxin and CGP54626.



Supplementary Figure 6. GABA receptor antagonists do not block lateral postsynaptic excitation, but do block lateral inhibition.
(a) Experimental design for measuring odor-evoked lateral suppression of EPSCs.
(b) Voltage-clamp traces from the cell shown in Fig. 3(b). Antennal nerve stimulation (arrowheads) evokes EPSCs. Odor stimulation suppresses EPSCs and evokes an inward current. This inward current reflects odor-evoked lateral excitatory input. CGP54626 and picrotoxin block the EPSC suppression but do not block the odor-evoked inward current. This demonstrates that EPSC suppression is not due to postsynaptic shunting by lateral excitatory input.
(c) Time course for antagonist block of odor-evoked EPSC suppression. Symbols show the average EPSC amplitude immediately before odor stimulation (●) or during odor stimulation (○) for each trial.
(d) Experimental design for measuring GABA-evoked suppression of EPSCs.
(e) Voltage-clamp traces from the cell shown in Fig. 3(e). Antennal nerve stimulation (arrowheads) evokes EPSCs. GABA iontophoresis (indicated by asterisk) suppresses EPSCs and evokes an outward current. This outward current is due to the direct postsynaptic action of GABA (see ref. 9). CGP54626 and picrotoxin together block both the EPSC suppression and the GABA-evoked outward current.
(f) Time course for antagonist block of GABA-evoked EPSC suppression. Symbols show the average EPSC amplitude immediately before GABA iontophoresis (●) or following GABA iontophoresis (○) for each trial.



Supplementary Figure 7. Increased paired-pulse ratio indicates a presynaptic locus for EPSC inhibition.

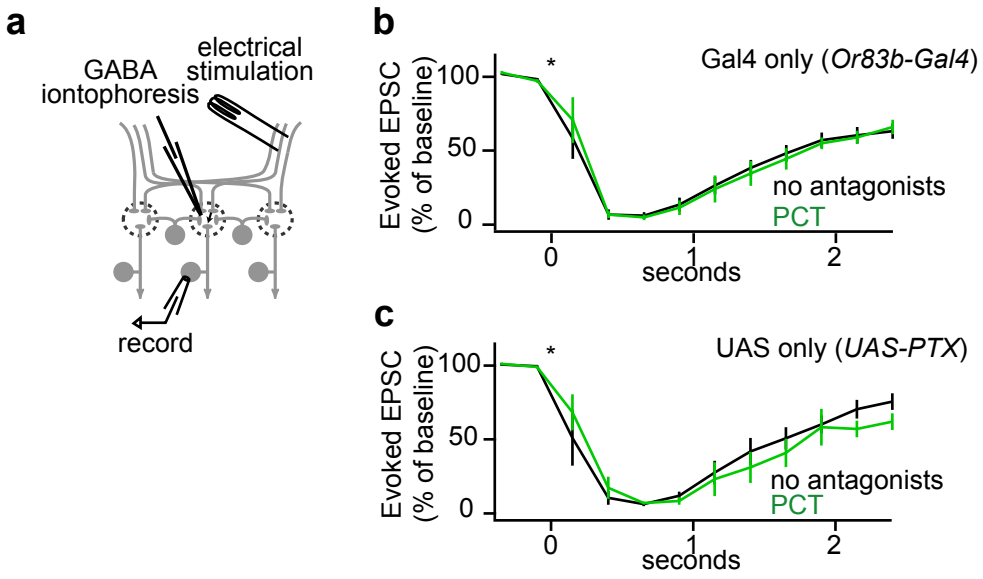
We performed paired-pulse experiments to test whether either $GABA_A$ and/or $GABA_B$ receptors mediate EPSC suppression at least partially presynaptically. Presynaptic inhibition generally increases the paired-pulse ratio (PPR), defined as the the amplitude of EPSC2/EPSC1. Reducing presynaptic release probability decreases vesicular depletion, so relatively more vesicles are available for release on the second stimulus. Although PPR changes generally indicate the involvement of a presynaptic mechanism, they do not exclude the involvement of additional postsynaptic mechanisms. As a positive control, we first verified that a purely presynaptic manipulation (inhibiting voltage-dependent calcium channels with Cd^{2+}) produces a detectable increase in PPR.

(a) Representative responses to paired-pulse stimulation before and after adding a sub-saturating dose of Cd^{2+} to the saline perfusate.

(b) Summary of PPR changes in five experiments measured in control saline (black) and after the addition of Cd^{2+} (gray) ($n = 5$, $p < 0.01$, paired t-test).

(c) Representative responses to paired-pulse stimulation before and after GABA iontophoresis.

(d) Summary of PPR changes during either GABA iontophoresis (see Fig. 3d) or olfactory stimulation of lateral input (see Fig. 3a). For GABA: $n = 7$ without antagonists, $p < 0.005$; $n = 5$ in CGP, $p < 0.005$; $n = 5$ in PCT, $p < 0.05$, paired t-tests. In the presence of both antagonists, GABA causes a very small suppression of EPSCs (see Fig. 3), associated with a small but non-significant change in PPR ($n = 4$, $p = 0.22$, paired t-test). This may reflect incomplete antagonism or a minor role for a pharmacologically distinct GABA receptor. For olfactory stimulation: $n = 7$, $p < 0.001$, paired t-test. The smaller PPR changes observed with odor compared to GABA likely reflects the smaller EPSC suppression produced by olfactory stimulation. GABA suppressed EPSC1 to 15 ± 2% of the control value, while odor suppressed EPSC1 to 46 ± 4% (mean ± s.e.m).



Supplementary Figure 8. Negative control for Gal4 driver and pertussis toxin transgene. In control flies GABA iontophoresis suppresses EPSCs, and this suppression is insensitive to picrotoxin (PCT) (Fig. 3f). In contrast, flies that express pertussis toxin specifically in ORNs display GABA-evoked EPSC suppression that is completely blocked by picrotoxin (Fig. 4c). Here we show that this phenotype requires both the Gal4 driver and toxin transgene. All panels show mean ± s.e.m averaged across experiments. Asterisk indicates GABA pulse (3-20 ms).
(a) Experimental configuration.
(b) Like control flies, flies with only the Gal4 driver (*Or83b-Gal4/Or83b-Gal4*) are insensitive to PCT (n = 5).
(c) Similarly, flies with only the UAS transgene (*UAS-PTX/+*) are also insensitive to PCT (n = 5).