Supplementary Information

Figure S1. Lateral inhibition in diverse sensilla requires activation of the target ORN. a-d, Odor stimuli and Drosophila sensillum types were identical to those shown in Fig. 2a-2d, respectively. When the target ORN was genetically ablated (a-c) or contained a receptor mutation (d), inhibition was not observed. Grey traces: control responses to diluent, paraffin oil. In a, ab1A and ab1B spikes could not be sorted reliably and were grouped. n=11~13. Odor dilutions and A neuron basal activities are in Table S2.

Figure S2. The pulsed odorant alone does not directly inhibit the A neuron. A 500-ms pulse of odorant (orange bar) was delivered via a constant stream of humidified air. a-c, Drosophila sensilla. Top: in all sensillar types examined, a pulse of odorant that activates the target ORN (orange neuron) did not directly inhibit the neighboring ORN (blue neuron). Activation of the target ORN led to a slight increase in the A neuron spike activity in ab1 (a) and ab2 (b) sensilla, and a slight decrease in ab5A spike activity (c). Bottom: when the target ORN was genetically ablated (a-c), no change in A neuron spike activity was observed by the pulsed odorant. In a, the activities of ab1A and ab1B were grouped together because their spike amplitudes were too similar to sort reliably. d, In the capitate-peg sensillum of Anopheles, a pulse of 1-octen-3-ol (10^-4) did not directly inhibit cpA spike activity (n=12).

Figure S3. ab3A inhibition by 2-heptanone depends on ab3B. A test of dose-dependency was carried out in a sensillum in which ab3B is genetically ablated. a, Olfactory responses of ab3A to a 500-ms pulse of 2-heptanone (orange bar, 10^-4 dilution) in the absence (top) or presence of varying levels of background methyl hexanoate (blue bar) as indicated to the right of each trace. b, Activities of ab3A during 500-ms exposures to paraffin oil (PO) or 2-heptanone (10^-4) with increasing concentrations of background methyl hexanoate. Application of 2-heptanone (10^-4) did not produce any significant change in ab3A responses, compared to ab3A responses to paraffin oil (paired t-test). Error bars indicate S.E.M. (n=12).

Figure S4. ACV does not inhibit ab1C directly. Single-unit recordings of ab1 sensilla were performed with a 500-ms pulse of neutralized apple cider vinegar (ACV, orange bar) as the odor stimulus. A representative trace (middle) and the averaged spike responses (right) are shown. To allow reliable spike sorting, recordings were conducted in orco mutant flies in which only one (ab1C) of the four ORNs is functional. ACV does not activate or inhibit ab1C directly, similar to the solvent control (water, grey trace). Shaded areas indicate S.E.M. (n=16).

Figure S5. Temporal features of lateral inhibition. Responses of two ORNs housed in the same sensillum, as shown in Fig. 1d and Fig. 2, were overlaid to demonstrate the temporal kinetics of the antagonism between neighboring ORNs. Responses of the A neuron are shown in blue and those of the B neuron (or the C neuron, in panel a) are in orange, except as indicated in cases in which spike sorting was difficult and spikes of the indicated neurons were combined. Averaged recordings (PSTHs) are binned in 50-ms increments.

Figure S6. Electrical interactions in olfactory sensilla. The figure depicts the electric circuit model, adapted here from the model of Vermeulen and Rospars 20041. The illustrations depict the ORNs and associated electrical currents in three different states: a) both ORN1 and ORN2 at
rest (unstimulated); b) ORN1 chronically activated by a background odorant (blue dots); c) ORN2 transiently activated by a pulse of odorant (orange dots) in the presence of a chronically activated ORN1. Insect olfactory sensilla are unusual in that at rest there is a large electric field, known as the transepithelial potential ($V_A$), between the sensillar lymph and the hemolymph, with the sensillar lymph 30 mV more positive $^{2,3}$. This potential is maintained by a tight barrier formed by the epithelial cell layer $^{4,5}$, which contains auxiliary cells that are thought to generate $E_A$ through an electrogenic $K^+$-pump $^{2,3}$. ORNs are spread between the two compartments; their dendrites lie in the sensillar lymph whereas their somata are embedded in the epithelial layer and are exposed to hemolymph. Also unusually, the ionic composition of the sensillar lymph is similar to that inside the ORNs (high $K^+$ and low $Na^+$), and consequently $V_A$ serves as the primary driving force for odorant-induced transduction currents instead of the typical ionic gradients found in neurons $^{1,2}$. The hemolymph surrounding the ORN somata has a typical ion composition and generates a typical neuron electrochemical ion gradient, depicted as $E_{RN}$ in the model. This unusual ionic distribution and external electrical potential is analogous to the hair cells of vertebrate cochlea and their endocochlear potential $^{6,7}$.

Each portion of the circuit has associated resistances: the epithelial layer ($R_A$), the ORN dendrites ($R_1$ and $R_2$), and the ORN somata ($R_{in}$). The transepithelial potential induces small currents, $I_1$ and $I_2$, through both ORNs and the epithelial layer ($I_A$) at rest (panel a). These physiological features form the basis of an electrical circuit model first developed over 30 years ago $^2$. Odorant activation of an ORN changes its dendritic resistances, and thereby alters the current flowing through each ORN (panels b and c). The thickness of the red arrows ($I_A$, $I_1$ and $I_2$) reflects the magnitude of the currents. As we detail in the text following the panels, further analysis of the model shows that the magnitude $I_1$ and $I_2$ determines the somatic transmembrane potentials of the ORNs and predicts that strong activation of one ORN passively hyperpolarizes the soma of its neighbor, the likely site of action potential generation $^1$. Similar to one of the most studied examples of ephaptic interactions, the goldfish Mauthner cell $^{8,9}$, the model also predicts that the transmembrane potential is differentially affected in different parts of the passive ORN, with the dendritic regions being depolarized (not derived here). Two notable differences between the classic Mauthner-cell system and insect sensilla, however, are that the electric field in the sensilla is generated by auxiliary cells at rest, and that the ORN somata are enwrapped by glial processes, which prevent direct ephaptic interactions between ORN somata.

**Figure S7. Recordings of local field potentials.** a, DC signals were recorded from ab3, ab2 and ab1 sensilla located in the same antenna to a 500-ms pulse of 2-heptanone (grey bar). At the concentrations tested ($10^{-6}$ to $10^{-4}$), 2-heptanone activates only the ab3B neuron. In the ab3 sensillum, the odor-evoked field potential change was large, ranging from $\sim 5$ to $\sim 15$ mV, roughly corresponding to $\sim 50$ to $\sim 250$ spikes/s in ab3B spike responses. Only a small fraction of the odor-evoked field potential ($< 3$ mV) was observed in ab2 and ab1 sensilla, despite their close proximity to ab3 sensilla in the antenna ($n=12$). b, Schematics of the distribution of ab3, ab2 and ab1 sensilla in an antenna, adapted from de Bruyne et al, 2001 $^{10}$.

**Figure S8. Examination of ephaptic effect across sensilla.** a, The cell death gene *reaper (rpr)*, under the control of a weak promoter, was expressed in a fraction of ab2B neurons in an antenna. The resulting fly has two populations of ab2 sensilla, one without and one with a functional ab2B neuron. In both types of ab2 sensilla, the A neurons are functional and are expected to project to the same glomerulus (dotted circle to left) in the antennal lobe. b, Single-unit recordings
performed in both populations of ab2 sensilla from the same antenna. A 500-ms pulse of ab2B odorant (ethyl 3-hydroxybutyrate, $3 \times 10^{-4}$, orange bar) was superimposed on a constant stream of background odorant (methyl acetate, $10^{-6}$, blue bar) that elevated the basal activity of the ab2A neuron (~40 spikes/s). Top: In a sensillum containing a functional ab2B cell, transient activation of ab2B inhibited the tonic response of ab2A to methyl acetate. Bottom: when ab2B was genetically ablated, inhibition of ab2A activity was not observed, despite the close proximity of the sensillum to other sensilla with a functional ab2B neuron (n=12). This result argues against the spread of field effects across sensilla and against involvement of antennal lobe neurons in lateral inhibition.
Table S1. Fly genotypes.

| Figure 1 | (d) w: UAS-rpr; + (top)  
|          | w: UAS-rpr; Or85b-GAL4 (bottom)  
|          | (e) w: UAS-H134R-Channelrhodopsin2; Or85b-GAL4 (top)  
|          | w: UAS-H134R-Channelrhodopsin2; + (bottom)  
|          | (f) w: UAS-rpr; + (top)  
|          | w: UAS-rpr; Or22a-GAL4 (bottom)  
|          | (g) w: UAS-H134R-Channelrhodopsin2; Or22a-GAL4 (top)  
|          | w: UAS-H134R-Channelrhodopsin2; + (bottom)  
| Figure 2 | (a-c) w: UAS-rpr; +  
|          | (d) w: UAS-rpr; +  
| Figure 3 | (a-d) w: UAS-rpr; +  
| Figure 4 | (a-b) w: UAS-TNT; Orco-GAL4 (Orco::TNT)  
|          | w: UAS-TNT; + (Control)  
|          | (c) w: UAS-TNT; +  
|          | (d-e) w: UAS-TNT; Orco-GAL4  
| Figure 5 | (a) w: UAS-TNT; +  
|          | (b) w: UAS-TNT; Orco-GAL4  
|          | (c) Orco\textsuperscript{[2]}  
| Figure S1 | (a) w: UAS-rpr; Gr21a-GAL4  
|          | (b) w: UAS-rpr; Gr43a-GAL4  
|          | (c) w: UAS-rpr/or47a-GAL4; +  
|          | (d) w: PBac(Wh)Or35a\textsuperscript{[2]}  
| Figure S2 | (a) w: UAS-rpr; + (top)  
|          | w: UAS-rpr; Gr21a-GAL4 (bottom)  
|          | (b) w: UAS-rpr; + (top)  
|          | w: UAS-rpr; Gr43a-GAL4 (bottom)  
|          | (c) w: UAS-rpr; + (top)  
|          | w: UAS-rpr/or47a-GAL4; + (bottom)  
| Figure S3 | (a-b) w: UAS-rpr; Or85b-GAL4  
| Figure S4 | Orco\textsuperscript{[2]}  
| Figure S5 | (a-d) w: UAS-rpr; +  
|          | (e) w:1118  
| Figure S7 | (a) w: UAS-TNT; +  
| Figure S8 | (b) w: UAS-rpr; Gr43a-GAL4  

<table>
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<th>Odor Dilutions</th>
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<th>Corresponding figure without background stimulation</th>
<th>A neuron spontaneous activities without background odor (spikes/s)</th>
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References


