ARTICLE

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Non-synaptic inhibition between grouped neurons in an olfactory circuit

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Diverse sensory organs, including mammalian taste buds and insect chemosensory sensilla, show a marked compartmentalization of receptor cells; however, the functional impact of this organization remains unclear. Here we show that compartmentalized *Drosophila* olfactory receptor neurons (ORNs) communicate with each other directly. The sustained response of one ORN is inhibited by the transient activation of a neighbouring ORN. Mechanistically, such lateral inhibition does not depend on synapses and is probably mediated by ephaptic coupling. Moreover, lateral inhibition in the periphery can modulate olfactory behaviour. Together, the results show that integration of olfactory information can occur via lateral interactions between ORNs. Inhibition of a sustained response by a transient response may provide a means of encoding salience. Finally, a CO_2-sensitive ORN in the malaria mosquito *Anopheles* can also be inhibited by excitation of an adjacent ORN, suggesting a broad occurrence of lateral inhibition in insects and possible applications in insect control.

An intriguing feature of a number of sensory systems is the compartmentalization of their primary sensory cells. These cells are housed together in specialized structures such as the taste buds of vertebrates and the chemosensory sensilla of invertebrates. The compartmentalized primary sensory cells often respond to diverse stimuli. The functional consequence of such organization is unknown.

Olfactory receptor neurons (ORNs) are the primary units of odour perception[1]. ORNs are widely believed to function as autonomous units, each responding to odorants independent of other ORNs. In some organisms, such as insects, ORNs are compartmentalized into sensilla (Fig. 1a). An individual sensillum encapsulates the dendrites of neurons[2–4]. The neighbouring ORNs exhibit differing spike amplitudes and odorant sensitivities[5]. In *Drosophila melanogaster*, each ORN is assigned a designation indicating the type of sensillum in which it is housed and its relative spike amplitude among the ORNs of the sensillum. Thus, the ab3A neuron is located in antennal basiconic sensilla of type 3, and the 'A' indicates that its spike amplitude is greater than that of the neighbouring 'B' neuron. In fruitflies, moths and mosquitoes, ORNs are grouped in stereotyped combinations[5–9].

The functional significance of this widespread pattern of ORN organization is unknown. In *Drosophila*, neighbouring ORNs do not have obvious functional relationships[10], and they do not project to adjacent regions in the brain[11]. In certain sensilla of flies, moths and beetles, the activation of neighbouring ORNs elicits opposing behaviours[6,8,9,12–16]. There are theoretical predictions based on electrical circuit modelling that the transient activation of one ORN may interfere with the signalling of a neighbouring ORN[17], and there is precedent for olfactory stimuli that activate one neuron and inhibit its neighbour[15,16], but in the absence of molecular genetic analysis it is difficult to determine whether such stimuli act uniquely on one ORN or directly on both. Similar examples can also be found in insect taste sensilla[18–22], but in *Drosophila* some bitter compounds have been shown to act directly both on a sugar neuron and on a bitter neuron, inhibiting one and exciting the other[23].

Here we use the molecular genetics of *Drosophila* to examine the coding of pairs of odours by the ORNs of olfactory sensilla. We find that the prolonged activation of one ORN is inhibited by the transient excitation of its neighbour. This lateral inhibition is observed within diverse types of *Drosophila* sensilla, and the activation of a mosquito ORN laterally inhibits the response of a neighbouring ORN to CO_2, a key cue used by mosquitoes to find their human hosts. The communication between neurons does not require a synapse, and probably proceeds via ephaptic coupling. Finally, we find that this lateral inhibition at the periphery of the olfactory circuit can modulate olfactory behaviour. Together, our results indicate that ORNs do not signal cell-autonomously in all circumstances, but rather their responses can be regulated by the activity of their ORN neighbours in a sensillum.

Activation of an ORN inhibits its neighbour

To analyse the relationship between two ORNs in a sensillum, we used a paradigm that allows us to deliver two odours, one for each neuron (Fig. 1b, c). One odorant, the 'background odorant', is provided continuously via an airstream and elicits the sustained firing of one ORN, the A neuron in most experiments. Superimposed on this background stimulus, a short pulse of a second odorant is delivered to activate the other ORN, usually the B neuron. This paradigm of odour presentation is distinct from the single-odorant paradigm used commonly in many studies[5,10,24], but it simulates a coding problem that the system encounters in its natural environment, for example when a fly receiving sustained olfactory input from a local source receives a superimposed, transient stimulus from a distant source delivered by a gust of wind.

When the ab3 sensillum is stimulated with a prolonged dose of methyl hexanoate, the ab3A neuron responds with a sustained train of action potentials (large action potentials in Fig. 1d). When a pulse of 2-heptanone is superimposed on this background, not only does ab3B fire (small action potentials) but there is a marked reduction in the firing of ab3A (Fig. 1d).

This inhibitory effect could, in principle, be due to direct inhibition of OR22A, the receptor of ab3A, by 2-heptanone. However, ablation of ab3B by expression of the cell death gene *reaper* (*rpr*) completely abolished the inhibition of ab3A (Fig. 1d, bottom). This result indicates that the inhibition of the A neuron depends on the excitation of the B neuron.

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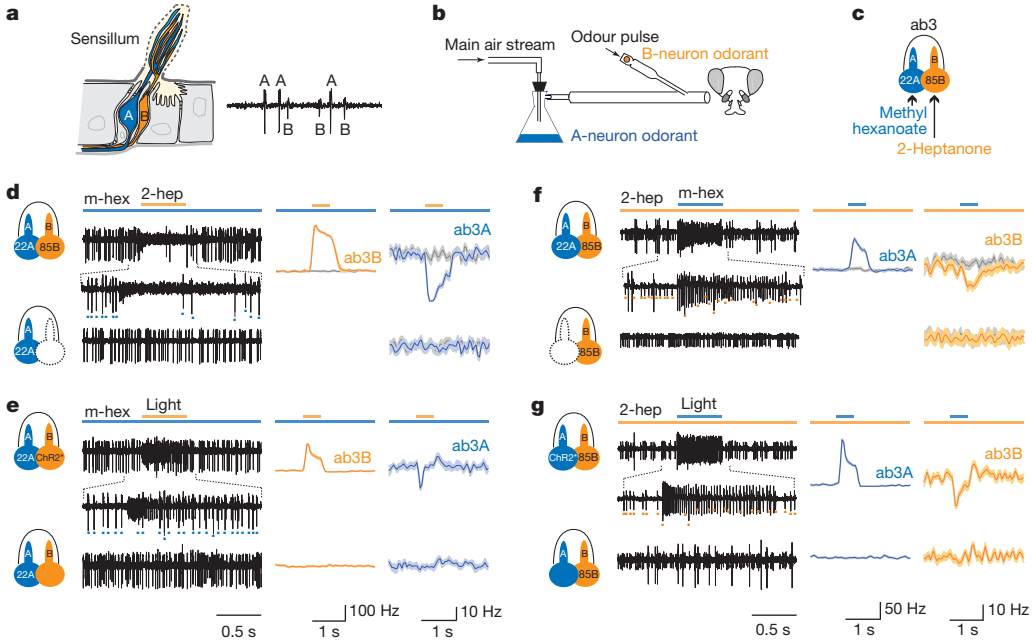

Figure 1 | Lateral inhibition of ORNs. **a**, An olfactory sensillum that houses two ORNs, A and B. Inset: a single-unit recording. 'A' has a larger spike amplitude than 'B'. **b**, The two-odour paradigm. **c**, The ab3 sensillum, the ORNs of which express the OR22A and OR85B receptors, which are sensitive to the indicated odorants. **d**, Top: a sustained stimulus of methyl hexanoate (m-hex, 10^{-7} dilution, long blue bar) elicits a response from ab3A (large spikes, ~37 spikes s^{-1}). A 500-ms pulse of 2-heptanone (2-hep, 10^{-4} dilution, orange bar) activates ab3B (small spikes). The response of ab3A is inhibited by the 2-heptanone stimulus. Right, averaged responses. Grey traces indicate responses when a pulse of diluent is delivered instead of 2-heptanone. Shaded areas represent s.e.m. Inset: blue dots indicate ab3A spikes. Bottom: genetic ablation of ab3B prevented inhibition. **e**, In flies expressing ChR2* in ab3B (top), a 500-ms pulse of blue light (473 nm, ~10 mW mm^{-2}) excited ab3B, which inhibited the response of ab3A to methyl hexanoate (~32 spikes s^{-1}, 10^{-6}). The more phasic inhibition is probably due to the kinetics of ChR2-dependent activation. Bottom: flies without ChR2*. **f**, Top: activation of ab3A by a pulse of methyl hexanoate (10^{-6}) inhibited the response of ab3B to 2-heptanone (~38 spikes s^{-1}, 5×10^{-7}). Bottom: genetic ablation of ab3A prevented inhibition. Inset: orange dots indicate ab3B spikes. Very large spikes represent the coincidence of A and B spikes. **g**, ChR2* expressed in ab3A. A pulse of blue light (~25 mW mm^{-2}) excited ab3A, inhibiting the response of ab3B to 2-heptanone (~35 spikes s^{-1}, 5×10^{-7}). $n = 12$ in **d**–**g**.

To test further the possibility that activation of the ab3B neuron can inhibit the ab3A neuron, we expressed Channelrhodopsin2 (H134R-ChR2)[25] in ab3B. As expected, blue light elicited an excitatory response in ab3B of these engineered flies (Fig. 1e). Activation of ab3B by light also inhibited the tonic firing of ab3A elicited by methyl hexanoate. Blue light had no effect on ab3A firing in control flies lacking H134R-ChR2 (Fig. 1e, bottom), indicating that it does not inhibit ab3A directly. The simplest interpretation of these results is that activation of ab3B inhibits the firing of ab3A.

We next asked whether activation of ab3A can inhibit ab3B. We first elevated ab3B activity by delivering 2-heptanone as the background odorant and then presented a pulse of methyl hexanoate to activate ab3A. Indeed, the pulse of methyl hexanoate inhibited the activity of ab3B (Fig. 1f, top). Genetic ablation of ab3A demonstrated that this inhibition depended on ab3A (Fig. 1f, bottom). Similarly, when H134R-ChR2 was expressed in ab3A, a blue-light stimulus activated ab3A and inhibited the tonic firing of ab3B (Fig. 1g).

Lateral inhibition in other sensilla

There are four morphological types of antennal sensilla: large basiconic sensilla, small basiconic sensilla, coeloconic sensilla and trichoid sensilla[1,26,27]. ab3 is a large basiconic sensillum containing two ORNs. We analysed four other sensilla, chosen for their morphological diversity and their functional specificities. Their ORNs express receptors that have been functionally characterized, and odorants have been identified that at certain concentrations selectively activate the receptor of only one ORN in each sensillum[10,24].

Lateral inhibition between ORNs was observed in all sensillar types examined: a large basiconic sensillum containing four ORNs (ab1); a large basiconic sensillum with two ORNs (ab2); a small basiconic sensillum (ab5); and a coeloconic sensillum (ac3). In each case, a short odorant pulse that activated one target ORN inhibited the tonic firing of a neighbouring ORN (Fig. 2a–d). When the targeted ORN was ablated or non-functional, the short odorant pulse showed no inhibition of the neighbouring ORN (Supplementary Fig. 1). We note also that the pulsed odorant alone did not directly inhibit the spontaneous firing of the A neuron (Supplementary Fig. 2). These results indicate that lateral inhibition is observed broadly in the *Drosophila* antenna.

Lateral inhibition in a mosquito sensillum

ORNs are compartmentalized in sensilla in a wide variety of insects. We examined a sensillum of the malaria vector *Anopheles gambiae* that responds to CO_2 (ref. 7), a human volatile that attracts many mosquito species[28]. This sensillum contains an ORN, cpA, that responds to CO_2, and a neighbouring ORN, cpB, that is excited by 1-octen-3-ol (ref. 7).

We used a prolonged CO_2 stimulus to elicit a sustained response from cpA. When a short pulse of 1-octen-3-ol was superimposed, the cpB neuron was excited and cpA was robustly inhibited (Fig. 2e). We note that when 1-octen-3-ol was delivered in the absence of CO_2, it did not inhibit the spontaneous firing of the CO_2-responsive cpA neuron directly (Supplementary Fig. 2d), consistent with previous results[7].

Taken together, our results show that lateral inhibition occurs in olfactory sensilla of multiple insect species, in sensilla of radically different morphology, and in sensilla containing two, three or four ORNs.

Inhibition is dose-dependent

When ab3A was tonically excited with a constant concentration of methyl hexanoate, increasing doses of 2-heptanone produced increasing excitation of ab3B and increasing inhibition of ab3A (Fig. 3a, b). When the scales of the firing ranges are adjusted (Fig. 3b), the dose–response functions seem to be symmetrical.

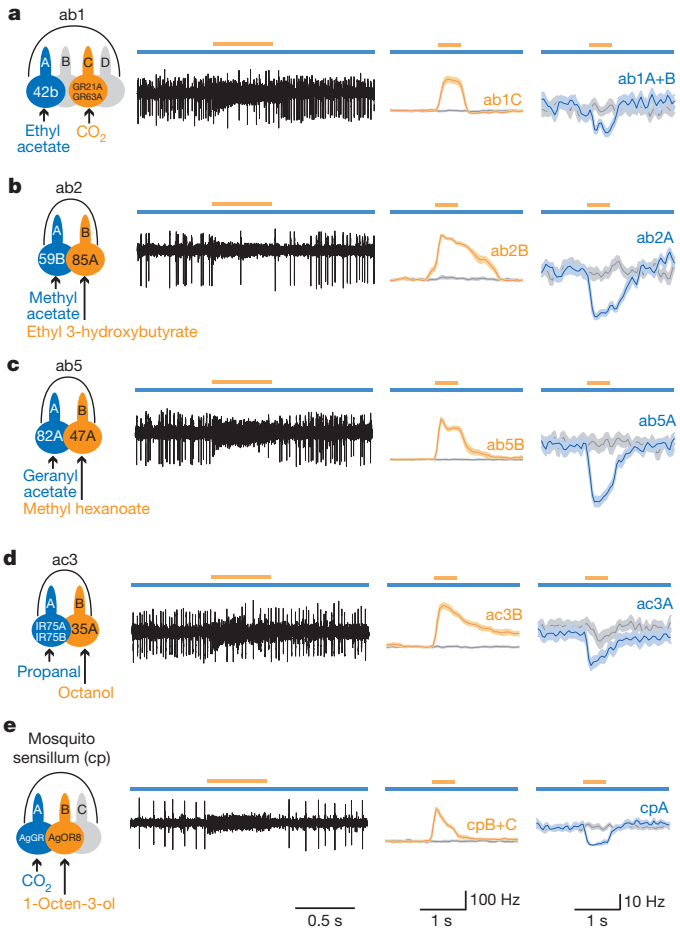

Figure 2 | Lateral inhibition in diverse sensilla. Odorants at the tested concentrations activate only one ORN in each sensillum. **a**–**d**, *Drosophila* sensilla. Activation of the target ORN (orange) inhibited the response of the neighbouring ORN (blue) to the background odorant. In **a**, ab1A and ab1B spikes could not be sorted reliably and were grouped. **e**, In the capitate-peg sensillum of *Anopheles*, activation of the cpB neuron by 1-octen-3-ol (10^{-4}) inhibited the response of cpA to CO_2. cpB and cpC spikes were combined. $n = 11$–13. Odour dilutions and A neuron basal activities are in Supplementary Table 2.

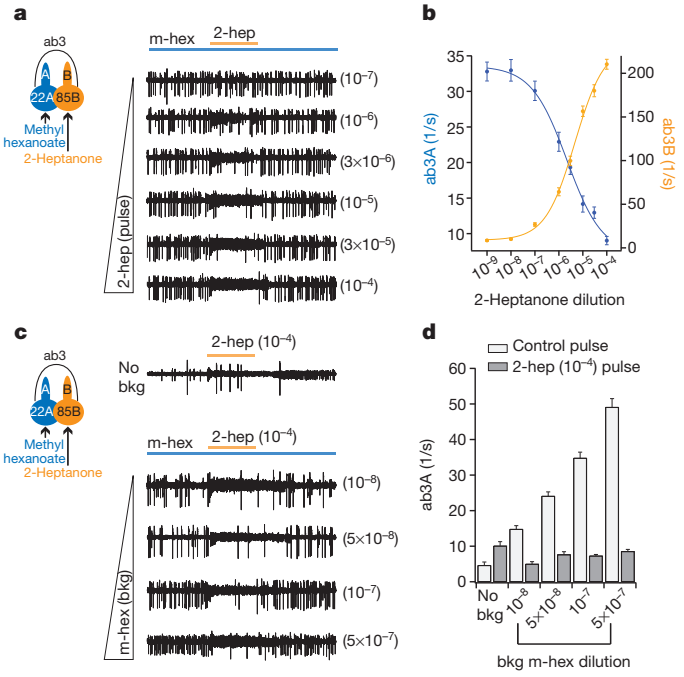

Figure 3 | Lateral inhibition is dose-dependent. **a**, Responses of ab3A and ab3B to a 500-ms pulse of 2-heptanone (orange) superimposed on a background odorant, methyl hexanoate (10^{-7} dilution; ~37 spikes s^{-1}). At these low concentrations, methyl hexanoate and 2-heptanone selectively activate ab3A and ab3B, respectively. 2-Heptanone dilutions are at the right of the panel. **b**, Activities of ab3A and ab3B during 2-heptanone pulses. Fit is with the Hill equation; $n = 12$. **c**, Responses to a pulse of 2-heptanone (10^{-4}) in the presence of varying levels of methyl hexanoate, indicated at the right of the panel. **d**, Responses of ab3A during 500-ms exposures to paraffin oil (control) or 2-heptanone with varying concentrations of background methyl hexanoate; $n = 12$. In the absence of sustained stimulation of the A neuron ('no bkg'), strong activation of the B neuron elicited a small increase in the firing of A, which may represent passive depolarization of A resulting from close apposition of the neuronal membranes[43,44]. This effect seems to be overwhelmed by the passive hyperpolarization produced by ephaptic interactions (discussed below) when B is activated during sustained stimulation of A. Differences are significant in all conditions ($P < 0.002$, paired *t*-test); $n = 12$.

When the background odorant, methyl hexanoate, was delivered at increasing concentrations, the rate of ab3A tonic firing increased across a range of ~15 spikes s^{-1} to ~50 spikes s^{-1} (Fig. 3c, d, and Supplementary Table 2). Inhibition by a strong ab3B stimulus was potent across all these concentrations; in all of these cases the rate of firing was reduced to approximately the same level. A genetic ablation experiment confirmed that these reductions depended on ab3B (Supplementary Fig. 3). We note that 2-heptanone alone did not directly inhibit ab3A spontaneous activity (Fig. 3c, d, 'no bkg').

Transmission without a synapse

Next we asked whether the intrasensillar communication is mediated by synapses. First we used tetanus toxin (TNT)[29] to block synaptic transmission. We expressed TNT in ORNs using the *Orco* promoter and the *GAL4*/*UAS* system, which is expected to drive expression in all basiconic ORNs[30] except the CO_2-sensitive ab1C neuron[31,32]. Activation of ab3B inhibited the tonic excitation of ab3A in these TNT-expressing flies (Fig. 4a, top). Moreover, the degree of inhibition was comparable to that in control flies (Fig. 4a, bottom). T-maze behavioural tests confirmed that synaptic transmission was blocked in the targeted ORNs (Fig. 4b).

Second, we performed single-unit recordings from isolated antennae, severed from the heads of flies. Activation of ab3B again inhibited the tonic excitation of ab3A (Fig. 4c), supporting the conclusion that lateral inhibition between neighbouring ORNs occurs in the periphery without involvement of central synapses.

Third, we tested the possibility of axo-axonic synapses between ORNs with a cross-correlation analysis[33]. Analysis of ab3A and ab3B spontaneous spikes did not reveal coordinated spiking patterns and thus provided no evidence for axo-axonic synaptic interactions (Fig. 4d), similar to what has been found between homotypic ORNs in *Drosophila*[34].

Finally, we used Cd^{2+} to block synaptic neurotransmission[35]. We included a high concentration of Cd^{2+} in the recording pipette so as to allow Cd^{2+} to diffuse into the sensillum lymph and block any peripheral dendro-dendritic synapses in sensilla of *Orco-GAL4; UAS-TNT* flies. We observed little if any effect on the inhibition of ab3A firing after ab3B excitation (Fig. 4e; compare with Fig. 4a). To verify the efficacy of our drug delivery method, we applied the Orco agonist VUAA1 (ref. 36) via the recording pipette and observed elevated ORN spike activities, as expected (Fig. 4f). Together, these results indicate that lateral inhibition does not depend on chemical synapses.

Intrasensillar communication could, in principle, be mediated via gap junctions; however, the activation of one ORN would then probably lead to the activation, rather than the inhibition, of its neighbour. Moreover, we found that nitric oxide signalling inhibitors had

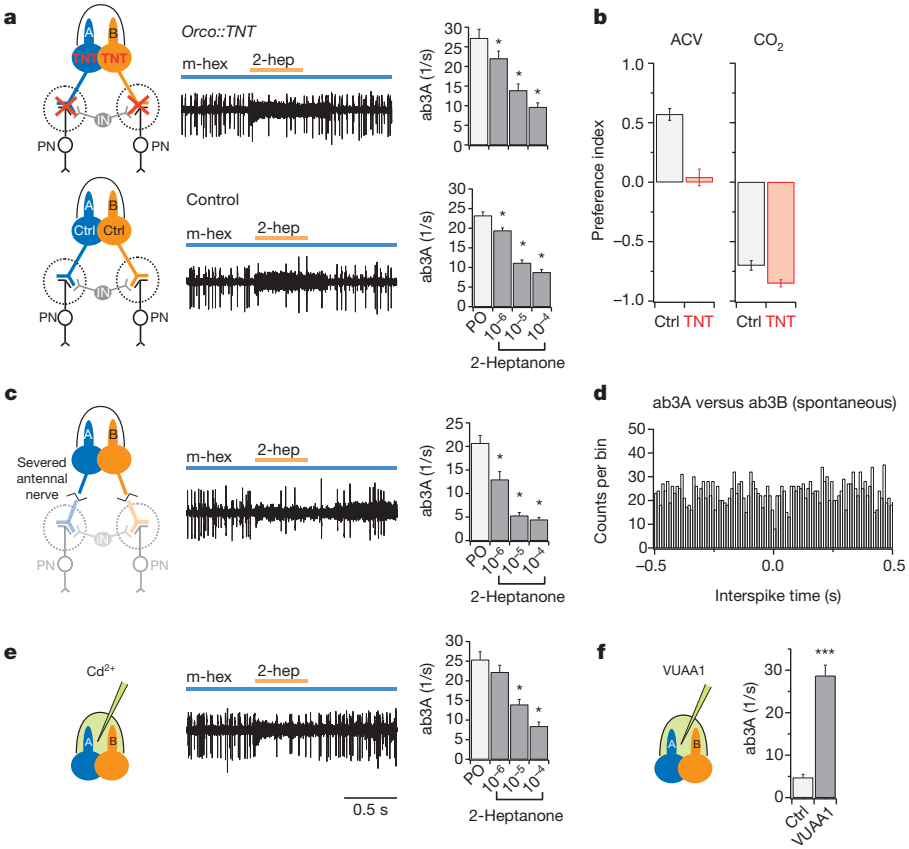

Figure 4 | Lateral inhibition does not require synapses. **a**, ab3 sensilla in flies expressing TNT in ORNs via the *Orco* promoter. Neurons were exposed to a 500-ms pulse of the ab3B odorant, 2-heptanone (orange, 10^{-4}), superimposed on the background ab3A odorant, methyl hexanoate (blue, 10^{-7}). IN, representative interneuron. Right: ab3A activity during a 500-ms exposure to paraffin oil (PO) or 2-heptanone in the presence of methyl hexanoate. Error bars indicate s.e.m.; $*P < 0.05$, one-way repeated measures ANOVA, multiple comparison versus control group (PO) with Dunnett's method ($n = 12$). **b**, T-maze choice between water and 25% ACV or between air and 0.67% CO_2. CO_2 neurons do not express *Or* genes. ($n = 9$). **c**, Recordings were performed as in **a** except that the antenna was severed from the head ($n = 7$). **d**, Cross-correlation analysis of spontaneous spikes from an ab3 sensillum, showing intervals between ab3A spikes and ab3B spikes, binned in 10-ms increments. Each ab3B spike is used as a reference. Another ab3 sensillum gave similar results. **e**, Recordings made 15 min after introduction of Cd^{2+} ($n = 12$). **f**, VUAA1 (1 mM) or vehicle (1% dimethylsulphoxide (DMSO)) was delivered via the recording electrode[36]. ab3A responses were recorded for 10 s. $***P < 0.001$, *t*-test ($n = 12$).

no effect on lateral inhibition (not shown). In summary, conventional forms of neuronal communication are unlikely to mediate lateral inhibition in a sensillum.

Lateral inhibition modulates behaviour

To determine whether intra-sensillar neuronal inhibition can modulate olfactory behaviour, we examined a pair of neighbouring ORNs, the activation of which leads to opposing behavioural outputs (Fig. 5). ab1A mediates attraction to apple cider vinegar (ACV)[13], whereas its neighbour ab1C mediates aversion to low concentrations of CO_2 (refs 12, 32, 37, 38). We confirmed that in a T-maze assay, when given a choice between CO_2 and air alone, flies avoid CO_2, whereas when faced with a choice between ACV and water, they are attracted to ACV (Fig. 5a, black bars).

We then tested whether the two behavioural pathways interact. When both arms of the T-maze contained CO_2, the flies showed no preference (Fig. 5a). When ACV was added to one of the CO_2-containing arms, the flies preferred that arm. The preference for the arm containing both CO_2 and ACV could have two sources: the attraction to its ACV that is mediated by ab1A, and a reduction in the avoidance of its CO_2 that is mediated by ab1C.

To evaluate the contributions of these sources, we used *Orco-GAL4;UAS-TNT*, which blocks synaptic transmission from ab1A but not ab1C. Consistent with the expected specificity of this block, these flies did not respond to ACV but avoided CO_2 (Fig. 5b, black bars). We note that in these flies, physiological recordings confirmed that ab1A neurons respond to ACV (not shown). When given a choice between two arms, one with CO_2 and one with CO_2 and ACV, these flies preferred the arm with ACV (Fig. 5b). Because synaptic transmission from ab1A neurons is blocked and the flies have no attraction to ACV, the simplest interpretation of these results is that activation of ab1A attenuated the response of ab1C to CO_2 via lateral inhibition: the reduced CO_2 response decreased the avoidance of the arm containing CO_2 and ACV relative to the arm containing CO_2 alone, and this decreased avoidance is seen as an attraction to the arm containing CO_2 and ACV.

If this interpretation is correct, and the preference for the arm containing CO_2 and ACV depends on the activation of ab1A, then the preference should be abolished in *Orco* mutants, which lack a co-receptor required for the response of ab1A but not ab1C. Consistent with this prediction, *Orco* mutants showed no preference between the arm containing CO_2 and the arm containing CO_2 and ACV (Fig. 5c). We note that ACV does not inhibit ab1C directly (Supplementary Fig. 4). Taken together, these results provide evidence that lateral inhibition within a sensillum can modulate behaviour.

Discussion

Integration of olfactory information has long been known to occur in the CNS, and has more recently been shown to occur in individual ORNs[39]. We have demonstrated that integration also occurs at a third

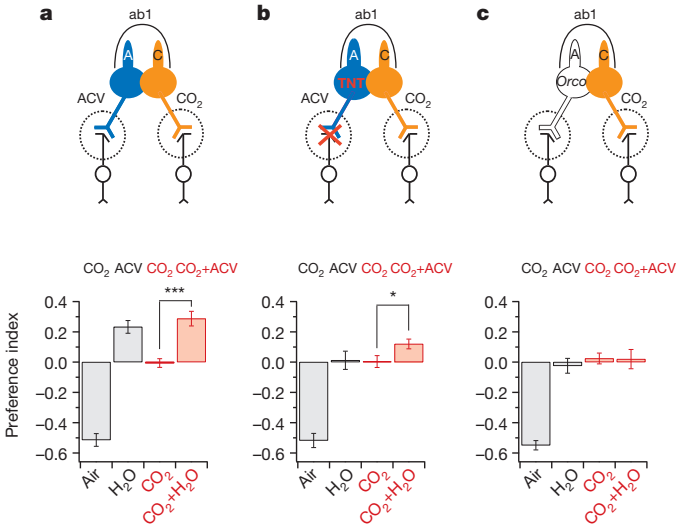

Figure 5 | Lateral inhibition modulates behaviour. a–c, Activation of ab1A mediates attraction to ACV; activation of ab1C mediates aversion to CO_2. Two of the four ORNs in ab1 are depicted. Preference indices of control (**a**), *Orco-GAL4; UAS-TNT* (**b**) and *Orco* (**c**) are shown (mean ± s.e.m.). The ab1A neurons of the TNT-expressing flies respond to ACV but are expected not to transmit information to postsynaptic neurons, whereas ab1A neurons in *Orco* are expected not to respond to ACV. In each T-maze assay, ~50 flies were allowed 2 min to choose. In single-odour experiments (black bars) the test arm contained either CO_2 or ACV. $^*P < 0.05$; $^{***}P < 0.001$, *t*-test ($n = 16$). CO_2 was 0.13%; ACV was 100%, pH 7.5. In physiological recordings from *Orco* flies, ACV did not inhibit the spontaneous firing of the CO_2 neuron.

level, the sensillum, via lateral inhibition between ORNs responding to different components of a mixture. The sensillum thus acts as a processing unit in olfactory computation.

Lateral inhibition of a prolonged signal by a transient signal may provide a neural representation of the salience of an odour that has recently reached the fly[40]. Sustained responses were inhibited more strongly by stronger transient pulses. This graded pattern of lateral inhibition may give rise to a potent form of contrast enhancement in which the output of a sensillum is dominated by a pulse of a strong odour. Graded lateral inhibition may provide a peripheral mechanism for evaluating countervailing signals and allowing one to prevail. We note that in *Drosophila*, an ORN that responds to a pheromone[41,42] is the only ORN that does not have a neighbour, as if to ensure that its sustained response is not inhibited by a pulse of any other odorant.

Our finding that lateral inhibition does not require synapses is consistent with anatomical data. Electron microscopy in *Drosophila* has not revealed synaptic structures or gap junctions between ORNs housed in the same sensillum[2,3]. Rather, as detailed below, the physiological features of olfactory sensilla suggest another mechanism of lateral information flow: ephaptic transmission, which refers to non-synaptic communication between adjacent neurons through an extracellular electrical field[43,44]. The ability of either neuron in a two-neuron sensillum to inhibit the other, as well as the grossly similar temporal dynamics of activation and lateral inhibition (Supplementary Fig. 5), are consistent with ephaptic transmission.

In insect olfactory sensilla, a substantial electrical potential exists between two isolated compartments: the sensillum lymph, which bathes the dendrites, and the haemolymph, which surrounds the somata (Fig. 1a and Supplementary Fig. 6). This 'transepithelial' potential serves as the primary driving force for odorant-induced transduction currents of the ORNs[17,45]. Elaboration of an established electrical circuit model[17,45] based on these physiological features predicts that strong activation of one ORN will hyperpolarize the soma of a co-compartmentalized ORN (Supplementary Fig. 6), resulting in a reduced firing rate. This prediction is consistent with the results of our molecular genetic analysis and with our interpretation that lateral inhibition is due to ephaptic interactions.

The model further predicts that the magnitude of the hyperpolarization of the neighbouring neuron, and hence its reduction in firing rate, is reflected by the change in the transepithelial potential (V_A) (Supplementary Fig. 6), measured experimentally as a local field potential (LFP) (Supplementary Fig. 7a). Although strong activation of an ORN can influence the LFP in a neighbouring sensillum[46], we found that the magnitude of the LFP change in nearby unstimulated sensilla is small (Supplementary Fig. 7). Consistent with this observation, lateral inhibition does not spread among homotypic sensilla that are in close proximity to one another (Supplementary Fig. 8). These results further support the conclusion that the lateral inhibition is due to local electrical interactions between neighbouring ORNs within a sensillum.

The two-odour paradigm used in this analysis, in which a transient odour is superimposed upon a sustained odour, differs from the classic one-odour paradigm in which a transient pulse of a single odour is delivered. A priori one might expect to observe ephaptic effects in the one-odour paradigm if one ORN were excited sufficiently strongly, but the effects may be expected to be less pronounced than in the two-odour paradigm. ORN spike frequency is determined not only by the somatic transmembrane potential V_m, but also by its rate of change, dV_m/dt (ref. 46). According to the model, transient activation of ORN_2 reduces the depolarizing current of ORN_1 (Supplementary Fig. 6). In the two-odour paradigm, activation of ORN_2 has a marked effect on the value of dV_{m1}/dt, which changes from 0 to a negative value ($dV_{m1}/dt \ll 0$; Supplementary Fig. 6). By contrast, in the one-odour paradigm, the activation of ORN_2 has a more subtle effect on dV_{m1}/dt when the sensillum is stimulated with an odour that activates both neurons: dV_{m1}/dt is positive either in the presence or absence of ORN_2 activation, only somewhat less positive when ORN_2 is activated. The more subtle influence of ORN_2 activation on dV_{m1}/dt in the one-odour paradigm may explain why in the one-odour paradigm, the excitatory responses of an ORN containing an ectopically expressed receptor were markedly similar to those of the ORN that endogenously expresses the same receptor[24], despite major differences in the response profiles of their neighbours.

We note finally that our results indicate the possibility of a new approach to insect control: the inhibition of key insect ORNs by activation of their neighbours with odorants.

METHODS SUMMARY

Fly antennal preparations and single-unit recordings were performed essentially as described[24], except for the isolated antennal preparation in which the stabilized antenna was severed from the head using the broken tip of a tapered glass microcapillary tube. Recordings were performed on adult female flies 5–7 days after eclosion, except that flies 24–36 h after eclosion were used in *UAS-TNT* experiments because TNT-expressing ORNs began to lose spike activities in older flies. Supplementary Table 1 lists fly genotypes for all experiments. Female *Anopheles gambiae* mosquitoes were used ~4 days after eclosion. Extracellular recordings from the capitate-peg sensilla on the maxillary palp were performed as described[7,47]. AC signals (300–2,000 Hz) were recorded, except for local field potential recordings where DC signals (low-pass filtered at 2 kHz) were recorded. ORN spikes were detected and sorted based on spike amplitude using routines in Igor Pro 6.01 and binned at 50-ms intervals.

For optogenetic experiments, flies were reared in constant darkness on fly food supplemented with ~100 μM all trans-retinal[37]. Recordings were performed on adult females 7 days after eclosion using an established optics set-up[48]. For pharmacological experiments, chemicals were delivered inside the sensillum via the recording glass electrode.

T-maze behavioural tests were performed essentially as described[12]. For experiments shown in Fig. 4b, flies were given 1 min to choose between the two arms: air versus CO_2 (0.67%) or H_2O versus ACV (25%). For experiments shown in Fig. 5, four experimental conditions were used: (1) air versus CO_2 (0.13%); (2) H_2O versus ACV (100%, pH 7.5); (3) CO_2 (0.13%) versus CO_2 (0.13%); (4) CO_2 (0.13%) plus H_2O versus CO_2 (0.13%) plus ACV (100%, pH 7.5). Preference

index was calculated as the fraction of flies entering the test arm minus the fraction of flies entering the control arm.

Full Methods and any associated references are available in the online version of the paper.

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Supplementary Information is available in the online version of the paper.

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Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to J.R.C. (john.carlson@yale.edu).

METHODS

***Drosophila* stocks.** Recordings were performed on adult female flies 5 days after eclosion, except that 7-day flies were used in *UAS-rpr* experiments, and flies 24–36 h after eclosion were used in *UAS-TNT* experiments because TNT-expressing ORNs began to lose spike activities in older flies. Flies were reared at 25 °C in an incubator with a 12-h light/dark cycle. The following fly stocks were used: (1) *UAS-rpr* (ref. 49), (2) w^{1118} and *PBac[WH]Or35a*^{f02057}, (3) *UAS-TNT* (ref. 29), (4) *UAS-H134R-ChR2* (ref. 25), (5) *Or-GAL4* lines (Bloomington stock centre), (6) *Gr21a-GAL4* (ref. 31). Supplementary Table 1 lists genotypes for all experiments.

Mosquitoes. Female *Anopheles gambiae* mosquitoes were used ~4 days after eclosion. Extracellular recordings from the capitate-peg sensilla on the maxillary palp were performed as described[7,47].

Electrophysiology and data analysis. For the standard antennal preparation, a fly was wedged into the narrow end of a truncated plastic pipette tip to expose the antenna, which was subsequently stabilized between a tapered glass microcapillary tube and a coverslip covered with double-sided type. For the isolated antennal preparation, a standard antennal preparation was made first and the stabilized antenna was gently severed from the head using the broken tip of a tapered glass microcapillary tube. Extracellular single-unit recordings were performed essentially as described[24]. Briefly, electrical activity of the ORNs was recorded extracellularly by placing a sharp electrode filled with Ringer solution[24] into a sensillum and the reference electrode filled with the same Ringer solution was placed in the eye (standard antennal preparation) or in the first antennal segment (severed antennal preparation). No more than four sensilla from the same antenna were recorded in the standard preparation, and no more than two sensilla from the same antenna were recorded in the severed preparation. For each sensillum, one trial of each odorant concentration was presented. AC signals (300–2,000 Hz) were recorded on an Iso-DAM amplifier (World Precision Instruments), except for local field potential recordings where DC signals (low-pass-filtered at 2 kHz) were recorded and digitized at 5 kHz with Axoscope 10.2 (Molecular Devices). ORN spikes were detected and sorted based on spike amplitude using routines in Igor Pro 6.01 (Wavemetrics). Peri-stimulus time histograms (PSTHs) were obtained by averaging spike activities in 50-ms bins and smoothed using a binomial algorithm (Igor Pro 6.01, Wavemetrics).

Odour stimuli. Odorants were diluted in paraffin oil (v/v). For short odour pulses, odour stimuli (50 µl applied to a filter disc) were delivered from a Pasteur pipette via a pulse of air (200 ml min^{-1}) into the main air stream (2,000 ml min^{-1}) as described previously[24]. In Fig. 2a, stimulation with CO_2 was by filling the Pasteur pipette with pure CO_2, which was subsequently puffed into the main air stream. On the basis of the published dose–response relationships of ab1A to CO_2 (refs 31, 50), the concentration of CO_2 was estimated to be ~1% (mean ab1C response shown in Fig. 2a: 163 Hz). Background odour stimuli were delivered from a 125-ml flask containing 3 ml of odour dilutions (or 25 ml of carbonated water for background CO_2) directly downstream of the main air stream (2,000 ml min^{-1}).

Optogenetic stimulation. Flies expressing H134R-ChR2 in targeted ORNs and control flies (*UAS-H134R-ChR2; +*) were reared in constant darkness on fly food supplemented with ~100 µM all trans-retinal (Sigma) as described[37]. Recordings were performed on adult females 7 days after eclosion using an established optics setup[48]. Briefly, a light stimulus was generated via a blue laser (MBL-III-473/30 mW, Opto Engine LLC) and delivered by an optical fibre (200-µm core diameter, BFH22-200, Thorlabs). The tip of the optical fibre was positioned above the antenna. Light pulses (500-ms duration) were controlled by an isolated pulse stimulator (Model 2100, A-M Systems). Light output at the tip of the optical fibre was measured with an optical power meter (Model 1916-C, Newport).

Cross-correlation analysis. The basal spike activity was investigated using 30 sweeps of 10-s duration. Action potentials of the ab3A and ab3B neuron were identified based on size and their triphasic (ab3A neuron) or more biphasic (ab3B neuron) shape using Origin software (OriginLab Corporation). Spike times of ab3A and ab3B neurons of individual sweeps were cross-correlated using Matlab software (MathWorks). Interspike times were accumulated across all recorded sweeps and binned in 10-ms intervals. Such an analysis can reveal coordinated spiking patterns and was used to identify axo-axonic synapses between neighbouring scorpion ORNs[33].

Pharmacology. Drugs were prepared as concentrated stock solutions and diluted in Ringer solution before experiments. Chemicals were delivered inside the sensillum via the recording glass electrode. Recordings were performed in flies expressing TNT in the ORNs ~15 min after drug introduction, except for the experiments with VUAA1, where recordings were performed within minutes after electrode insertion. The electrode stayed inside the sensillum throughout the 15-min period. VUAA1 (Chemical Diversity Research Institute, Joint Stock Company) was used at 1 mM (stock: 100 mM in DMSO). $CdCl_2$ (Aldrich) was used at 1 mM (stock: 100 mM in Ringer solution).

T-maze assay. Flies were collected within ~8 h after eclosion without using CO_2 anaesthesia. Flies were tested 24–32 h after eclosion after ~24 h starvation. For starvation, flies were gently tapped into empty vials with moistened foam plugs and kept at 25 °C in an incubator.

Behavioural tests were performed as described previously[12] at room temperature in a dark room. About 40–60 flies were transferred by an aspirator into a 15-ml centrifuge tube (Corning 430791), which was subsequently connected to the sliding chamber (elevator) of the T-maze apparatus. Flies were gently tapped into the elevator, which was then lowered to the opening where the test arm and the control arm were connected. A 16-inch 15-W fluorescent bulb was placed horizontally behind the test and control arms, and the light was on only for the duration of the assay. Phototaxis drew flies out of the elevator. Flies were given 1 min to choose between the two arms, after which the elevator was partially lifted to block any further choices. Preference index was calculated as the fraction of the flies entering the test arm minus the fraction of the flies entering the control arm. The total number of flies used in calculation of the preference index included flies in both arms and in the elevator.

For the experiment shown in Fig. 4b, 10 µl of apple cider vinegar solution or 10 µl of water was added to a Whatman filter disc (1/2 inch diameter) that was positioned around the 1.5-ml mark of the 15-ml centrifuge tube. Twenty-five per cent apple cider vinegar was used because it attracted flies in a T-maze assay without triggering the acid-mediated avoidance pathway[38]. Ten minutes of equilibrium time was allowed before the tubes were connected to the T-maze apparatus immediately before the assay. For the experiment in Fig. 4b using CO_2, 0.1 ml of pure CO_2 (UN1013, Airgas) was injected into the tube immediately before the assay. The positions of the test and control tubes were alternated for each trial. New groups of flies and new tubes were used for each test. The air inside the 15-ml tube was equilibrated with the air in the room for at least 4 h before use.

For experiments shown in Fig. 5 to address the behavioural relevance of lateral inhibition, we used four experimental conditions: (1) air versus CO_2; (2) H_2O versus ACV; (3) CO_2 versus CO_2; and (4) CO_2 plus H_2O versus CO_2 plus ACV. Thirty microlitres of neutralized apple cider vinegar (100%, pH 7.5) or water was added to a Whatman filter disc that was positioned horizontally via permanent double-sided tape (Scotch, 3M) around the 10-ml mark of the centrifuge tube. When CO_2 was used, 0.1 ml of 20% CO_2 was injected into the tube(s) (near the 5-ml mark) immediately before the assay. When CO_2 was used in both arms, the CO_2 was injected, the two tubes were connected to the T-maze apparatus, and then the apparatus was inverted gently ~10 times and allowed to equilibrate for an additional minute to ensure that CO_2 was distributed evenly between the two arms. The elevator was then lowered to release the flies. The positions of the test and control tubes were alternated for each trial.

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