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3 Odor-evoked inhibition of olfactory sensory neurons drives  
4 olfactory perception in *Drosophila*

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35 **ABSTRACT**

36 **Inhibitory response occurs throughout the nervous system, including the peripheral**  
37 **olfactory system. While odor-evoked excitation in peripheral olfactory cells is**  
38 **known to encode odor information, the molecular mechanism and functional roles**  
39 **of odor-evoked inhibition remain largely unknown. Here, we examined *Drosophila***  
40 **olfactory sensory neurons (OSNs) and found that inhibitory odors triggered**  
41 **outward receptor currents by reducing the constitutive activities of odorant**  
42 **receptors, inhibiting the basal spike firing in OSNs. Remarkably, this odor-evoked**  
43 **inhibition of OSNs elicited by itself a full range of olfactory behaviors from**  
44 **attraction to avoidance, as did odor-evoked OSN excitation. These results indicated**  
45 **that peripheral inhibition is comparable to excitation in encoding sensory signals**  
46 **rather than merely regulating excitation. Furthermore, we demonstrated that a**  
47 **bidirectional code with both odor-evoked inhibition and excitation in single OSNs**  
48 **increases the odor-coding capacity, providing a means of efficient sensory-encoding.**  
49

50 **INTRODUCTION**

51 Primary sensory receptor cells of most modalities exhibit spontaneous activities<sup>1-6</sup>,  
52 enabling their stimulus-induced responses with an activity decrease from the baseline by  
53 sound, temperature, or chemicals. For example, in addition to exciting olfactory sensory  
54 neurons (OSNs) by increasing the action-potential firing, odors have been found to inhibit  
55 the basal firing of OSNs from insects to mammals<sup>7-15</sup>. While excitation is known to  
56 encode sensory information, the functional roles of inhibition in peripheral sensory  
57 receptor cells remain unsolved. Does the stimulus-evoked inhibition simply regulate the  
58 excitability of primary sensory cells<sup>16</sup>, or does it act as a sensory code<sup>17</sup>? If the latter is  
59 true, what stimulus information does it encode, and how does it improve sensory coding?

60 To address these questions, we examined olfaction in *Drosophila*, a well-  
61 established and genetically tractable model system<sup>18-20</sup>. Olfaction begins with odor  
62 detection by odorant receptors (ORs) expressed in the OSNs. In adult *Drosophila*, there  
63 are ~50 types of ORs; the OSNs expressing a given OR converge their axons to one of  
64 ~50 glomeruli in the antennal lobe. This olfactory architecture is conserved from insects  
65 to mammals, suggesting a common solution to olfaction<sup>18</sup>. Odor-evoked inhibition exists  
66 in most *Drosophila* OSNs<sup>15, 21, 22</sup>, providing an opportunity to investigate its molecular  
67 origin, physiological functions, and computational roles in shaping odor perception.

68 By combining molecular genetics, electrophysiology, two-photon calcium  
69 imaging, optogenetics, behavioral studies and computations, we report for the first time  
70 that odor-evoked inhibition of *Drosophila* OSNs directly encodes odor identity and drives  
71 both attraction and avoidance behaviors. A single type of OSNs with odor-evoked  
72 inhibition and activation can drive two opposing behaviors and can also effectively

73 discriminate odor mixtures. Notably, the blockage of synaptic transmission of odor-  
74 evoked inhibition can result in a complete switch of olfactory behaviors. Mechanistically,  
75 such inhibition is caused by a direct odor inhibition of the constitutively activated ORs. A  
76 bidirectional odor response with both odor-evoked inhibition and activation in the same  
77 OSNs increases odor-coding capacity by reducing response saturation and decorrelating  
78 odor representation. Taken together, our work demonstrates that odor-evoked inhibition of  
79 OSNs is comparable to odor-evoked activation in encoding odor information for behavior  
80 and perception in *Drosophila*.

81

## 82 RESULTS

### 83 An outward receptor current underlies odor-evoked inhibition of OSNs

84 In *Drosophila* OSNs, both the levels of basal activities and the modes of odor-evoked  
85 responses (activation vs. inhibition) are determined by the expressed ORs<sup>21,22</sup>. However,  
86 a mechanistic understanding of the connections between these two phenomena has been  
87 hampered by difficulties in recording intracellularly from OSNs. We used our recent  
88 technical advances<sup>23</sup> to perform patch-clamp recordings on *Drosophila* OSNs and  
89 investigate the molecular origin of odor-evoked inhibition. The *Or85a*-expressing OSNs  
90 exhibited spontaneous firing in the absence of stimuli, and this effect was reversibly  
91 abolished by acetophenone (Fig. 1a, top). Correspondingly, an outward receptor current  
92 was induced by acetophenone under a voltage-clamped configuration (Fig. 1a, bottom).  
93 This acetophenone-induced inhibition was a direct effect on *Or85a*-OSNs rather than an  
94 ephaptic inhibition<sup>24</sup> because similar results were obtained in isolated *Or85a*-OSNs  
95 (Supplementary Fig. 1a). This conclusion was further supported by a similar inhibition in  
96 *Or85a*-OSNs of *Orco*<sup>-/-</sup> flies<sup>25</sup> with *Orco* restored only to *Or85a*-OSNs (Supplementary  
97 Fig. 1b).

98 In the absence of odor stimuli, we recorded a basal inward current of  $-18.2 \pm 14.4$   
99 pA (n = 42, mean  $\pm$  SD) in *Or85a*-OSNs at 23 °C, suggesting the existence of ion  
100 channels that are constitutively open. Acetophenone evoked outward receptor currents in  
101 a dose-dependent manner (Fig. 1b and Supplementary Table 1), with the same amplitude  
102 of its maximal responses as the basal inward current. These results imply that the  
103 acetophenone-induced outward receptor current is a reduction of the basal inward current.  
104 We found that the basal inward current was completely eliminated after ablation of *Orco*

105 (Supplementary Fig. 1c,d), indicating its origin from the constitutive activity of  
106 OR/ORCO complex. We further found that the basal inward current was temperature  
107 dependent, increasing at higher temperatures and decreasing at lower temperatures (Fig.  
108 1c and Supplementary Fig. 1e). Similarly, the spontaneous firing in *Or85a*-OSNs also  
109 depended on temperature and the presence of *Orco* (Fig. 1d and Supplementary Fig. 1f,g),  
110 suggesting that the spontaneous firing in *Or85a*-OSNs was mainly driven by the basal  
111 activity of ORs.

112         The outward receptor current that underlies acetophenone-evoked inhibition could  
113 be produced by distinct mechanisms. One possibility is that acetophenone binds to *Or85a*  
114 receptor proteins to open ion channels with a reversal potential below the resting potential  
115 of OSNs, exemplified by the opening of potassium channels that mediate odor-evoked  
116 inhibition in lobster and toad OSNs<sup>11,26</sup>. Alternatively, acetophenone may interact with  
117 *Or85a* receptors to close ion channels that are constitutively open and have a reversal  
118 potential above the resting potential of OSNs. To differentiate between the two  
119 possibilities, we measured changes in membrane conductance to determine whether the  
120 odor-evoked inhibition was caused by the opening or closing of ion channels<sup>10</sup>. We found  
121 that acetophenone dramatically decreased the membrane conductance when probed with  
122 pulses of hyperpolarizing voltage (Fig. 1e), thus demonstrating the closure of ion  
123 channels that are constitutively open. In contrast, ethyl 3-hydroxybutyrate, an excitatory  
124 odor for *Or85a*-OSNs, increased the membrane conductance of the same OSNs and  
125 produced an inward receptor current (Fig. 1e), indicating the opening of ion channels.

126         Together, our results demonstrated that odor-evoked OSN inhibition is produced  
127 by the closure of ion channels that are opened by constitutively/thermally activated ORs,

128 thus yielding an outward receptor current. Our findings support the hypothesis that OR  
129 molecules fluctuate between active and inactive states in a temperature dependent manner,  
130 with inhibitory odors stabilizing their inactive states<sup>21</sup>. However, our results differ from  
131 previous findings in other species in which odor-evoked inhibition of OSNs is mediated  
132 via the opening of potassium channels<sup>11,26</sup>.

### 133 **Spontaneously activated and odor-activated ORs share similar signaling properties**

134 To investigate the signaling properties of constitutively activated ORs, we recorded the  
135 basal inward current and receptor currents evoked in *Or85a*-OSNs by the inhibitory odor  
136 acetophenone and excitatory odor ethyl 3-hydroxybutyrate (Fig. 2a). Power spectral  
137 analysis revealed similar waveforms between the basal inward current and odor-evoked  
138 responses (Fig. 2b,c), indicating that constitutively activated ORs and odor-activated ORs  
139 have similar signaling dynamics.

140 The finding that acetophenone-evoked inhibitory responses are a direct reduction  
141 of the basal inward current allows us to study the basal inward current by examining the  
142 inhibitory responses. Next, we examined the current-voltage relationship of  
143 acetophenone-evoked inhibitory responses and ethyl 3-hydroxybutyrate-evoked  
144 excitatory responses in the same *Or85a*-OSN. We found that inhibitory responses  
145 exhibited similar, although opposite in direction, current-voltage relationship with an  
146 identical reversal potential (Fig. 2d), indicating that the odor-evoked inhibitory and  
147 excitatory responses were mediated by similar or even identical ion channels.

148 Previously, we have showed that odor-evoked excitatory responses in *Drosophila*  
149 OSNs are modulated by calcium<sup>23</sup>. We reasoned that the basal inward current and odor-

150 evoked inhibitory responses would show similar modulatory effects to calcium, if they  
151 share similar signaling pathways with the excitatory responses. Consistent with our prior  
152 findings<sup>23</sup>, the removal of extracellular calcium increased the ethyl 3-hydroxybutyrate-  
153 evoked excitatory responses in *Or85a*-OSNs (Fig. 2e,f). At the same time, the removal of  
154 extracellular calcium also increased the inhibitory odor responses and basal inward  
155 current (Fig. 2e,f). These results suggest that constitutively activated ORs are likely to  
156 share a similar signaling mechanism with odor-activated ORs in generating inward  
157 receptor currents.

#### 158 **Interaction between odor-evoked inhibition and activation in the same OSN**

159 The coexistence of odor-evoked inhibitory and excitatory responses in the same OSN  
160 raises a possibility that the two responses may interact with each other. Next, we  
161 examined whether acetophenone could inhibit the ethyl 3-hydroxybutyrate-induced  
162 excitatory responses in *Or85a*-OSNs. In the presence of a background ethyl 3-  
163 hydroxybutyrate, a larger acetophenone-induced outward receptor current was obtained  
164 (Fig. 3a), suggesting that acetophenone could inhibit the basal inward current and odor-  
165 evoked excitatory responses. Acetophenone inhibited the inward receptor currents  
166 triggered by ethyl 3-hydroxybutyrate in a dose-dependent manner (Fig. 3b), but it did not  
167 inhibit the inward currents mediated, for example, by the exogenously expressed ATP-  
168 gated P2X<sub>2</sub> cation channels (Fig. 3c and Supplementary Fig. 2a, see also Methods) or by  
169 the light-gated channelrhodopsin ChR2 (Fig. 3d and Supplementary Fig. 2b,c, see also  
170 Methods). Therefore, in *Or85a*-OSNs, acetophenone specifically inhibited the activity of  
171 *Or85a* receptors.

172 To further explore the property of acetophenone-induced inhibition, we examined  
173 the dose-response relationship of the excitatory responses to ethyl 3-hydroxybutyrate in  
174 the presence of a background acetophenone. We found that acetophenone shifted the  
175 dose-response relationship of excitatory responses by significantly increasing the half-  
176 saturating odor concentration (Fig. 3e), while maintaining the amplitude of the maximal  
177 excitatory responses and the kinetics and shape of the responses (Fig. 3e and  
178 Supplementary Table 1). These results indicate a competitive inhibition of ethyl 3-  
179 hydroxybutyrate-induced excitatory responses by acetophenone.

### 180 **Odor-evoked OSN inhibition independently drives olfactory behaviors**

181 To investigate the physiological functions of odor-evoked inhibition in the OSNs, we  
182 examined whether this inhibition by itself could elicit olfactory behaviors (Fig. 4a). A  
183 single odor simultaneously excites and inhibits different OSNs<sup>21,22</sup>. To exclude the  
184 confounding effects from activation of OSNs expressing other ORs (Fig. 4b), we  
185 generated different flies in which *Orco* was restored to a single type of OSNs that  
186 expressed *Or10a*, *Or42b*, *Or43a*, *Or49b*, *Or56a*, *Or82a*, *Or85a*, or *Or92a* in an *Orco*<sup>-/-</sup>  
187 background<sup>27</sup>. To further limit contributions from the *Orco*-independent antennal  
188 chemosensitive neurons expressing either ionotropic or gustatory receptors<sup>28-31</sup>, we  
189 focused on odors that did not evoke chemotaxis in flies of an *Orco*<sup>-/-</sup> background  
190 (Supplementary Fig. 3).

191 We used cell-attached recordings to identify odors that inhibited the basal firing of  
192 the *Orco*-restored OSNs in the flies generated above (Fig. 4c,d). The odor-evoked  
193 inhibition was further confirmed in the glomeruli by using two-photon or confocal

194 imaging of GCaMP6m fluorescence expressed in the *Orco*-restored OSNs (Fig. 4c,d and  
195 Supplementary Fig. 4).

196 Unexpectedly, linalool, an odor that inhibits the basal firing and calcium signals  
197 of *Or56a*-OSNs and that does not elicit chemotaxis in *Orco*<sup>-/-</sup> flies, attracted flies with  
198 *Orco* restored to *Or56a*-OSNs (Fig. 4c). Similar inhibition-elicited attraction behaviors  
199 were observed in flies with *Orco* restored to either *Or82a*-OSNs or *Or92a*-OSNs (Fig.  
200 4c). In contrast, geraniol, an odor that inhibits *Or10a*-OSNs, repelled flies with *Orco*  
201 restored to *Or10a*-OSNs (Fig. 4d). Similar avoidance behaviors elicited by inhibition  
202 were also observed in flies with *Orco* restored to *Or42b*-OSNs or *Or85a*-OSNs (Fig. 4d).  
203 On the other hand, odor-evoked activation alone also elicited attraction (Fig. 4e) and  
204 avoidance (Fig. 4f) behaviors depending on the type of OSN activated. These gain-of-  
205 function results demonstrated that similar to odor-evoked activation of OSNs, odor-  
206 evoked inhibition of basal activities in the OSNs also encodes odor information for  
207 perception and behaviors.

### 208 **Discriminating odor mixtures by using a single type of OSN**

209 The above results also show that a single type of OSN could drive two opposing  
210 behaviors when inhibition and activation coexist. For example, in flies with *Orco* restored  
211 to *Or85a*-OSNs, the inhibitory and excitatory odors elicited opposing behaviors, i.e.,  
212 avoidance in response to acetophenone (Fig. 4d) and attraction to ethyl 3-  
213 hydroxybutyrate (Fig. 4e). These results indicate that flies with *Orco* restored to *Or85a*-  
214 OSNs may have an ability to discriminate among the mixtures of inhibitory and  
215 excitatory odors. By examining chemotaxis of these flies to such mixtures, we found that

216 a full range of behaviors from attraction to avoidance were elicited by mixtures at  
217 different ratios (Fig. 5a). A strong attraction was elicited at a high ratio of ethyl 3-  
218 hydroxybutyrate to acetophenone (Fig. 5a). However, attraction gradually decreased as  
219 the ratio was lowered, and avoidance was eventually elicited (Fig. 5a).

220 Similarly, flies with *Orco* restored to the *Or10a*-OSNs were also able to  
221 discriminate mixtures of acetophenone (activating *Or10a*-OSNs and eliciting attraction  
222 behaviors, Fig. 4e) and geraniol (inhibiting *Or10a*-OSNs and eliciting avoidance  
223 behaviors, Fig. 4d). A strong attraction was elicited at a high ratio of acetophenone to  
224 geraniol (Fig. 5b). Attraction gradually decreased as the ratio was lowered, and avoidance  
225 was eventually elicited (Fig. 5b). Our spike recordings on *Or10a*-OSNs in these  
226 transgenic flies revealed a gradual transition from an increase of spike firing to a decrease  
227 of spike firing relative to the baseline level when the mixture ratio was lowered  
228 (Supplementary Fig. 5).

229 Therefore, we demonstrate that odor-evoked bidirectional responses in the same  
230 OSN enable olfactory computations at the level of single OSNs, which can be used to  
231 effectively discriminate odor mixtures.

### 232 **Odor-evoked inhibition contributes to odor coding in Wildtype (WT) flies**

233 In adult *Drosophila*, odor recognition is based on the activity patterns of ~50 ORs. Our  
234 gain-of-function studies have revealed that odor-evoked OSN inhibition encodes odor  
235 information in flies that have only one type of functional OR. A question is whether such  
236 inhibition could contribute to odor coding when many ORs are functional. To address this  
237 question, we expressed tetanus toxin (TNT) in *Or85a*-OSNs to block their synaptic

238 transmission to the antennal lobe (see Methods). We found that *Or85a*-TNT flies were  
239 attracted to the inhibitory odor acetophenone, which normally does not elicit chemotaxis  
240 in WT flies (Fig. 6a). In contrast, flies expressing an inactive TNT exhibited no  
241 chemotaxis to acetophenone (Fig. 6a). Knockout of *Or85a* receptor elicited attraction  
242 behaviors to acetophenone (Fig. 6a, see also Methods). These loss-of-function results  
243 indicate that olfactory system integrates an avoidance signal from *Or85a* and an  
244 attraction signal collectively from other chemoreceptors, thereby leading to a non-  
245 chemotactic behavior to acetophenone in WT flies.

246           Similar results were obtained by disrupting the signaling of odor-evoked  
247 inhibition in *Or10a*-OSNs. Both WT flies and transgenic flies with inactive TNT  
248 expressed in *Or10a*-OSNs exhibited no chemotaxis to geraniol (Fig. 6a), an inhibitory  
249 odor for *Or10a*-OSNs. In contrast, *Or10a*-TNT flies were attracted to geraniol (Fig. 6b).  
250 Knockout of *Or10a* receptor also elicited attraction behaviors to geraniol (Fig. 6b, see  
251 also Methods). Thus, geraniol-evoked inhibition in *Or10a*-OSNs contributes to the  
252 integration of geraniol-elicited behaviors in WT flies.

253           To test the generality of the above findings, we also examined flies with a  
254 disruption of odor-evoked inhibition in *Or49b*-OSNs. Methyl salicylate, an inhibitory  
255 odor to *Or49b*-OSNs (Supplementary Fig. 6), elicited attraction in WT flies (Fig. 6c), but  
256 the attraction was lost in transgenic flies with TNT expressed in *Or49b*-OSNs (Fig. 6c).  
257 These results imply that methyl salicylate-evoked inhibition in *Or49b*-OSNs is a  
258 dominant drive for the methyl salicylate-elicited attraction in WT flies. This conclusion is  
259 supported by our finding that *Orco*<sup>-/-</sup> flies with *Orco* restored to *Or49b*-OSNs was  
260 attracted by methyl salicylate (Fig. 6c).

261 Taken together, these results imply that the odor-evoked inhibition of basal  
262 activities in OSNs plays an important role in odor coding in WT flies. However, one  
263 caveat of these experiments is that, in addition to disrupting odor-evoked OSN inhibition,  
264 these experimental manipulations also eliminate the basal firing of OSNs, which may  
265 affect the sensitivity of the downstream olfactory neurons<sup>3</sup>.

266 **Odor-evoked inhibition reduces response saturation and decorrelates odor**  
267 **representation**

268 The above gain-of-function and loss-of-function studies reveal that odor-evoked OSN  
269 inhibition is comparable to odor-evoked activation as a primary odor code. Therefore, the  
270 combinatorial odor coding in the olfactory periphery is based on not only excitatory<sup>27,32-35</sup>  
271 but also inhibitory responses across the OSNs. To explore the effects of this bidirectional  
272 odor coding scheme, we developed a computational model based on the measured odor-  
273 OSN response matrix<sup>22</sup> to investigate whether and how the implementation of both odor-  
274 evoked inhibition and activation in single OSNs improves odor coding.

275 Suppose that there are N odors in a mixture with the concentration of odor i (= 1, 2,  
276 3...N) given by  $C_i$  and there are M OSNs with odor-evoked responses of OSN j (=1, 2,  
277 3... M) denoted by  $R_j(\vec{C})$ , where  $\vec{C} = (C_1, C_2, C_3, \dots, C_N)$  is the N-dimensional  
278 concentration vector. To determine the odor-evoked response  $R_j(\vec{C})$ , we developed a  
279 simple model in which ORs have two states: an inactive state that produces no OSN  
280 activity and an active state that produces a maximum OSN activity  $R_{max}$ . Odor i binds to  
281 OSN j with a dissociation constant  $K_{ij}$  and modulates the transition between the two OR

282 states: excitatory odors stabilize the active states and inhibitory odors stabilizes the  
283 inactive states. As explained in detail in the Methods section, we obtain

$$R_j = R_{max} [1 + \alpha_j \prod_{i=1}^N (1 + \frac{C_i}{K_{ij}})^{-w_{ij}}]^{-1} \quad (1)$$

284 where  $w_{ij} = \{1, -1, 0\}$  for excitatory, inhibitory and null responses, respectively. The  
285 parameter  $\alpha_j$  determines a basal activity  $R_{j0} = R_{max}/(1 + \alpha_j)$ . Here, we assume that  
286 different odor molecules bind with different sites on the OR receptor. However, similar  
287 results were obtained for odor molecules competing for the same binding site (see  
288 Methods for details).

289 The transformation from an odor mixture characterized by the concentration  
290 vector  $\vec{C}$  to the OSN response vector  $\vec{R} = (R_1, R_2, R_3, \dots, R_M)$  determines how odors are  
291 represented (coded) by the OSNs (Fig. 7a). As shown explicitly in Eq. (1), the odor  
292 coding scheme is specified by the odor-OSN interaction matrix  $(\omega_{ij}, K_{ij})$ . The capacity  
293 of a coding scheme (Fig. 7a, middle) can be evaluated by its ability to separate odors with  
294 similar odor space (Fig. 7a, top) in the corresponding OSN response space (Fig. 7a,  
295 bottom). Here, we try to address whether and how the inclusion of inhibitory OSN  
296 responses in addition to the excitatory ones can enhance the coding capacity.

297 To answer these questions, we used Eq. (1) with the parameters  
298  $\{R_{max}, \alpha_j, \omega_{ij}, K_{ij}\}$  determined from the experimentally measured responses of  $M = 24$   
299 ORs to  $N = 110$  odors by Hallem and Carlson<sup>22</sup> (see Methods for details). For a large  
300 ensemble of random odor mixtures with the odor number varying from 5 to 60, we  
301 computed the coding capacity in terms of information entropy for each OSN and the

302 principal component spectrum of all the OSN responses for cases with and without odor-  
303 evoked inhibitory responses.

304           As shown in Fig. 7b, the inclusion of odor-evoked inhibition in OSNs increases  
305 the information entropy of most OSNs and the total entropy of the whole system for all  
306 odor number tested (Fig. 7b, inset). Mechanistically, the inclusion of OSN inhibition  
307 increases the coding capacity of OSNs by preventing response saturation (Supplementary  
308 Fig. 7) and making the responses more uniformly distributed within the response dynamic  
309 range (Supplementary Fig. 8). The correlations in the OSN responses were studied by the  
310 principle component analysis. A given principle component (PC) can be used for coding  
311 if its variance determined by its eigenvalue is larger than a detection (coding) threshold  
312 set by the noise level in the system. The number of PCs whose eigenvalue is above the  
313 coding threshold (set to be 1 in this study) defines an effective (independent) dimension  
314 for coding. As shown in Fig. 7c, the inclusion of OSN inhibition decorrelates odor-  
315 evoked responses across OSNs, thus increasing the independent dimensions of odor  
316 coding for all odor number tested. Mechanistically, an OSN with both odor-evoked  
317 inhibitory and excitatory responses can compute and amplify the difference between  
318 similar odor mixtures as illustrated in Fig. 7a.

319

320 **DISCUSSION**

321 A central question in olfaction is how odors are recognized by the olfactory system.  
322 Research over the past has focused predominantly on odor-evoked activation of OSNs  
323 and established that odors are represented by the combinatorial activation of OSNs<sup>27,32-35</sup>.  
324 Here, we demonstrate that odor-evoked inhibition of *Drosophila* OSNs can directly  
325 encode odor identity and drive olfactory perception, and that odors are encoded by both  
326 odor-evoked inhibition and activation of OSNs. We report four major findings. First, in  
327 flies with only one type of functional OR, odor-evoked inhibition can drive olfactory  
328 behaviors, just as odor-evoked activation does. Second, a single type of OSN can drive  
329 two opposing behaviors and discriminate odor mixtures if inhibition and activation  
330 coexist. Third, genetic disruption of odor-evoked inhibition induces a switch of olfactory  
331 behaviors. Fourth, odor-evoked inhibition of OSNs increases odor-coding capacity by  
332 reducing response saturation and decorrelating odor representation. These findings  
333 establish that odor-evoked inhibition of OSNs is a primary odor code and that a  
334 bidirectional code with both inhibition and activation in the same OSN is an efficient  
335 coding strategy.

336 Inhibition of the OSNs can result from diverse mechanisms such as odor blockage  
337 of transduction channels<sup>36</sup>, competitive binding between odors in a mixture<sup>37</sup>, or an  
338 inhibitory signaling distinct from odor excitation<sup>17</sup>. Here, we focused on the odor-evoked  
339 inhibition of the basal activity in the OSNs. Two alternative mechanisms may drive such  
340 an odor- and OR-specific inhibition<sup>17</sup>. Odors might bind to OR proteins, leading to the  
341 opening of an inhibitory conductance such as potassium channels<sup>11,26</sup> to counteract the

342 basal activity. Alternatively, odors might bind to ORs and stabilize them in inactive  
343 state<sup>21</sup>, thus directly reducing the basal activity of ORs.

344 In the absence of odor stimulation, we observed a basal inward current in the  
345 voltage-clamped OSNs, which increased at higher temperatures but was abolished in the  
346 absence of OR/ORCO complex. These results suggest that ORs can be  
347 constitutively/thermally activated, producing a basal inward current. This basal current is  
348 directly inhibited by inhibitory odors, demonstrated by our finding that inhibitory odors  
349 reduce the basal membrane conductance in the voltage-clamped OSNs. A direct  
350 inhibition of the OR basal activity is further supported by a lack of odor inhibition of the  
351 inward currents mediated by P2X<sub>2</sub> channels or ChR2 in the same OSN. In addition, we  
352 found that inhibitory odors also inhibit the responses to excitatory odors. Therefore,  
353 inhibitory odors can inhibit both spontaneous OR activity and excitatory odor-induced  
354 OR activity.

355 At the functional level, we demonstrated that, similar to odor-evoked activation,  
356 odor-evoked inhibition of *Drosophila* OSNs directly encodes perceptual signals and  
357 drives olfactory behaviors of both attraction and avoidance. The inclusion of odor-evoked  
358 inhibition and activation in the same *Drosophila* OSNs increases odor-coding capacity  
359 and also allows neural computation at the level of OSNs before odor information is  
360 transferred to the downstream olfactory networks.

361 Efficient coding with limited sensory channels of limited capacity is a general  
362 task for sensory systems<sup>38</sup>. In olfaction, animals use a large repertoire of ORs to  
363 discriminate odors<sup>18-20,39</sup>. A common strategy in olfaction is the use of a combinatorial

364 odor coding based on the activation patterns of ORs<sup>27,32-35</sup>, allowing the discrimination of  
365 more odors than the number of OR types. Our experiments reveal another strategy for  
366 efficient odor coding by compacting two modes of neuronal responses (i.e., inhibition and  
367 activation) in the same OR, hence effectively increasing the number of ORs because the  
368 two OR modes can drive opposing behaviors. The existence of odor-evoked inhibition in  
369 OSNs from insects to mammals<sup>7-15</sup> suggests that this dual odor coding may not be unique  
370 to *Drosophila* olfaction. However, this strategy may be particularly important for  
371 *Drosophila* because its array of ORs is much smaller than that of vertebrates<sup>18-20,39</sup>.

372           Our finding that odor-evoked inhibition in the OSNs can drive olfactory  
373 behaviors raises a question of how the brain processes odor-evoked inhibition. Odor-  
374 evoked inhibitory responses have been observed in *Drosophila* OSNs<sup>21,22</sup> and projection  
375 neurons of the antennal lobe<sup>40</sup>, but they have not been observed in Kenyon cells of the  
376 mushroom body<sup>41</sup>. One possibility is that the inhibitory responses may have been  
377 converted into excitatory responses via feedback<sup>42</sup> or feedforward inhibition<sup>43-45</sup>.  
378 Alternatively, odor-evoked inhibition may be transmitted through other circuits because  
379 the higher olfactory centers in *Drosophila* have not yet been fully characterized<sup>46</sup>.

380           Although spontaneous activity is known to be important in early neural  
381 development<sup>47,48</sup>, its widespread existence in adults has remained puzzling<sup>3,9</sup>. Basal  
382 activity could desensitize the excitatory responses<sup>22</sup>, but this effect is only modest in  
383 *Drosophila* OSNs because it occupies less than 20% of the OSN response range  
384 (Supplementary Table 1). A similar amount of basal activity has also been reported in  
385 auditory hair cells<sup>4</sup>. Our results show that such a level of basal activity enables odor-  
386 evoked bidirectional responses in single OSNs, which greatly increase the odor-coding

387 capacity. Our findings thus highlight the importance of spontaneous basal activity in  
388 sensory coding and perception.

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400

401

402

403 **AUTHOR CONTRIBUTIONS**

404 L.H.C. and D.G.L. conceived and designed the study. L.H.C., D.Y., B.Y.J., M.T.L. and  
405 D.G.L. performed electrophysiological recordings. X.Z. and D.G.L. performed fly  
406 genetics. W.W. performed behavioral experiments. B.Y.J. and D.G. L. performed  
407 calcium imaging. Y.T., S.Q., C.T. and D.G.L. performed computation analyses. L.H.C.,  
408 Y.T., X.Z. and D.G.L. wrote the manuscript.

409

**410 METHODS**

411

**412 Animals**

413 All flies were raised on standard cornmeal agar medium, under 60% humidity and a 12-h  
414 light/12-h dark cycle at 25 °C. The *Or10a-Gal4*, *Or42b-Gal4*, *Or43a-Gal4*, *Or49b-Gal4*,  
415 *Or56a-Gal4*, *Or82a-Gal4*, *Or85a-Gal4*, *Or92a-Gal4*, *UAS-Orco*, *Orco*<sup>1</sup>, *Orco*<sup>2</sup>, *UAS-*  
416 *mCD8-GFP*, *UAS-TNT*, *UAS-TNT* (inactive), *UAS-GCaMP6m*, and *UAS-H134R-ChR2*  
417 flies were from the Bloomington Stock Center. *UAS-P2X<sub>2</sub>* was a gift from Dr. Zuoren  
418 Wang at the Institute of Neuroscience, China. The flies have been backcrossed for seven  
419 generations to a laboratory *w<sup>1118</sup>* strain.

420

**421 Patch-clamp recordings**

422 Antennal slices were prepared as previously described<sup>23</sup> Briefly, adult flies were  
423 immobilized on ice. The isolated third antennal segment was cut into transverse slices.  
424 Slices were stabilized and perfused with 95% O<sub>2</sub>/5% CO<sub>2</sub>-bubbled *Drosophila* saline (in  
425 mM): 158 NaCl, 3 KCl, 4 MgCl<sub>2</sub>, 1.5 CaCl<sub>2</sub>, 26 NaHCO<sub>3</sub>, 1 NaH<sub>2</sub>PO<sub>4</sub>, 5 N-tri-  
426 (hydroxymethyl)-methyl-2-aminoethane-sulfonic acid (TES), 10 D-glucose, 17 sucrose  
427 and 5 trehalose (pH 7.4). The dissection solution was prepared by replacing NaHCO<sub>3</sub>,  
428 NaH<sub>2</sub>PO<sub>4</sub>, and TES with 5 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid  
429 (HEPES) and 27 mM NaCl (pH 7.4, adjusted with NaOH, bubbled with O<sub>2</sub>). All  
430 chemicals were from Sigma-Aldrich.

431 GFP-labeled OSNs were visualized on an upright microscope with an IR-LED  
432 (>850 nm) and infrared-differential interference contrast optics. Patch-clamp recordings  
433 were performed using MultiClamp 700B. Patch electrodes were filled with intracellular  
434 saline (in mM: 185 K-gluconate, 5 NaCl, 2 MgCl<sub>2</sub>, 0.1 CaCl<sub>2</sub>, 1 EGTA, 10 HEPES; pH  
435 7.4; approximately 390 mOsm). For perforated patch-clamp recordings, 200 µg/ml  
436 amphotericin B was back-filled into the recording pipette. For the I-V relationship, a  
437 cocktail of TTX (50 nM) and TEA (10 mM) was used to block voltage-gated channels.  
438 For cell-attached recordings, the recording pipettes were filled with the dissection  
439 solution. Signals were digitized and recorded with a Digidata 1440A and pClamp 10.2,

440 filtered at 2 kHz and sampled at 5 kHz. Measured voltages were corrected for a liquid  
441 junction potential.

#### 442 **Odor stimulation for patch-clamp recordings**

443 Rapid solution changes were effected by translating the laminar flow between two  
444 solution streams across the recorded OSN with an electronic SF-77B stepper (Warner  
445 Instruments). The solution flow was driven by gravity. Odors were freshly dissolved in  
446 *Drosophila* saline daily.

447

#### 448 **Optogenetic stimulation**

449 Flies expressing ChR2<sup>49</sup> in *Or85a*-OSNs were raised in complete darkness on standard  
450 medium supplemented with 100  $\mu$ M all-trans retinal. Antennal slices were prepared as  
451 usual. The patch-clamp recordings were conducted in a light-proof Faraday cage. A 480-  
452 nm LED (Sutter Instrument) coupled to the epifluorescence port of the Slicescope Pro  
453 6000 (Scientifica) was used to activate ChR2.

454

#### 455 **Pharmacogenetic stimulation**

456 Flies expressing P2X<sub>2</sub><sup>50</sup> in *Or85a*-OSNs were raised on standard medium under regular  
457 conditions. P2X<sub>2</sub> was activated by the application of ATP (1 mM, dissolved in  
458 *Drosophila* saline) through the odor-deliver system.

459

#### 460 **Temperature change**

461 Temperature was controlled using a Warner CL-100 (Warner Instruments). The heater  
462 and cooler were positioned close to the inlet of the recording chamber, and the perfusion  
463 solution flowed through the heater/cooler. A temperature sensor was positioned  
464 approximately 50  $\mu$ m from the recorded OSNs for measurement of local temperature  
465 around the recorded cells.

466

#### 467 **Power spectral analysis**

468 Under voltage-clamped configuration, continuous recordings lasting minutes were made  
469 in the targeted OSNs. The average power density spectrum was calculated in 30-s  
470 segments with 50% overlap. The difference between the two spectra of the basal current

471 and the inward current induced by a short-pulse of ethyl 3-hydroxybutyrate represented  
472 the power spectrum of excitatory responses. The power spectrum of the basal current was  
473 calculated as the difference between the spectrum of the basal current and the outward  
474 current induced by acetophenone. The power spectrum of the excitatory responses to a  
475 long-step of ethyl 3-hydroxybutyrate was calculated from the two spectra of the basal  
476 current and the steady inward current in responding to a long-step of ethyl 3-  
477 hydroxybutyrate.

478

### 479 **Calcium imaging**

480 Adult flies of 1-2 day after eclosion were immobilized on ice, and then stabilized on a  
481 piece of 3 M tape (0.6×0.6 cm) with the wings and dorsal head glued to the tape. To  
482 reduce the brain movement, the proboscis and legs were further stabilized with small  
483 stripes of tape. The tape with a stabilized fly was transferred to a recording chamber and  
484 the tape was used to seal a square opening (0.4×0.4 cm) at the bottom of the chamber  
485 (Supplementary Fig. 4). The fly was placed in middle of the chamber opening, facing  
486 down the chamber. A small window was opened in the tape with sharp blades to expose  
487 the fly head. The leak between the tape and the head was sealed with vacuum grease.  
488 Adult-like hemolymph (ALH) without  $\text{Ca}^{2+}$  was then added to the recording chamber.  
489 The regular ALH is composed of (mM): 108 NaCl, 5 KCl, 2  $\text{CaCl}_2$ , 4  $\text{MgCl}_2$ , 26  
490  $\text{NaHCO}_3$ , 1  $\text{NaH}_2\text{PO}_4$ , 5 HEPES, 5 trehalose, 5 sucrose, 17 glucose, bubbled with 95%  
491  $\text{O}_2/5\% \text{CO}_2$ . The cuticle and sac covering the antennal lobe were removed with sharp  
492 forceps. The recording chamber was transferred to the microscope stage and perfused  
493 with regular ALH for either two-photon, or confocal, or wide-field fluorescence calcium  
494 imaging.

495 For odor stimulation, air-phase odors were delivered to the antennae, which  
496 positioned under the recording chamber. The tube for a background humidified air flow  
497 at a rate of 500 ml/min was positioned ~1 cm away from the fly antennae. A filter paper  
498 absorbed 100  $\mu\text{l}$  liquid odor was placed inside a glass tube. An solenoid valve-controlled  
499 air flow of 50 ml/min passed through the glass tube and was then mixed with the  
500 background air flow.

501 Calcium imaging was performed with an A1 R Multi-Photon Laser-Scanning  
502 Confocal Microscope (Nikon) with a 60×, NA 1.0 water-immersion objective (Nikon),  
503 high-sensitivity non-descanned detectors, and a Mai Tai DeepSee ultrafast laser (Spectra-  
504 Physics). Time-lapse imaging series of GCaMP6m from a single z plane of the targeted  
505 glomerulus were acquired at ~7 frames per second with a resolution of 512×128 pixels. In  
506 some cases, imaging was performed under the confocal mode of the same A1 microscope  
507 with a sapphire laser of 488 nm (Coherent), or performed under wide-field fluorescence  
508 imaging with a slicescope Pro 6000 (Scientifica) with a 60×, NA 1.0 water-immersion  
509 objective (Olympus) and a Zyla sCMOS camera (Andor).

510 The GCaMP6m fluorescence images<sup>51</sup> were analyzed with the software Nikon  
511 NIS-Elements. A mean background was subtracted from the targeted glomerulus. We  
512 calculated the odor-evoked fluorescence intensity changes as  $\Delta F/F$ , where F is the  
513 maximal fluorescence intensity,  $\Delta F$  is the fluorescence changes from the baseline.

#### 514 **Behavioral assays**

515 Adult male and female flies were collected within 6 hr after eclosion. After 24-hr  
516 starvation in vials with water-absorbed filter strips, approximately 70 flies were  
517 transferred into the sliding chamber of a T-maze (4M Instrument & Tool LLC). The  
518 slider was then lowered, enabling the flies to face the opening of the choice tubes  
519 connected. The test tube contained a filter paper strip loaded with 10  $\mu$ l of test liquid odor  
520 (diluted in either paraffin oil or water), unless stated otherwise. The control tube  
521 contained a strip loaded with 10  $\mu$ l of odor solvent. The tubes were prepared 30 min  
522 before the behavioral test, allowing odor and solvent partition to equilibrate. The  
523 positions of the test and control tubes were alternated for each trial. New flies and tubes  
524 were used for each trial. The flies were allowed to choose between the tubes for two  
525 minutes in complete darkness and then counted. More than eight trials were repeated for  
526 each behavioral test. The preference index, PI, was calculated as the difference between  
527 the fly numbers in the test and control tubes divided by their sum. PI = 0 indicates an  
528 equal distribution of flies between the two tubes; PI = 1 indicates that all flies were  
529 attracted to the test tube; PI = -1 indicates that all flies avoided the test tube. All the  
530 behavioral experiments were performed at the circadian time of CT5-CT9.

531

532 **Generation of *Or10a* knock-out and *Or85a*<sup>Gal4</sup> knock-in flies**

533 The flies were generated using Cas9 mediated gene editing methods described before<sup>52,53</sup>.

534 For *Or10a* mutant, the two following guide RNAs were used:

535 *Or10a*-sg1 GACATAATGGGCTATTGGCCGGG

536 *Or10a*-sg2 GGTGGCCACGCCAATGGCCAGG

537 To generate *Or85a* Gal4-knock-in donor construct, the left homology arm was  
538 amplified with primer *Or85a*-5arm-F and *Or85a*-5arm-R, and the right homology arm  
539 was amplified with primer *Or85a*-3arm-F and *Or85a*-3arm-R. The amplified backbone  
540 and the homology arms were first linked together with Gibson Assembly Kit as pBS-  
541 85aLA-85aRA. The 2A-Gal4-loxP-3×P3 RFP-loxP cassette was cut with restriction  
542 enzyme Not 1 and Asc 1. 85aLA-pBS-85aRA linear DNA was cut from pBS-85aLA-  
543 85aRA vector, and combined with the cassette with Gibson Assembly Kit, producing the  
544 final donor construct pBS-85aLA-2A-Gal4-loxP-3×P3 RFP-loxP-85aRA.

545 Primers:

546 pBF 5' -TGGCGTAATCATGGTCATAGC -3'

547 pBR 5' -CTGGCGTAATAGCGAAGAGG -3'

548 *Or85a*-5arm-F 5' -CCTCTTCGCTATTACGCCAGACGGCTGGTAGATGGAGTTG -3'

549 *Or85a*-5arm-R 5' -GGCGCGCCATAAGAATGCGGCCGCAAGGACTGGCTCTTGAATGTACT-3'

550 *Or85a*-3arm-F 5' -GCGGCCGATTCTTATGGCGCGCCTTCCACCACAGCAACTCCAAG -3'

551 *Or85a*-3arm-R 5' -GCTATGACCATGATTACGCCATGAGAACCGCACAGATTTATGG -3'

552 Below are the two sgRNAs designed to target the region about 20-300 bp  
553 downstream of start codon of *Or85a*, and the third sgRNA targeted the site about 1.6 kb  
554 downstream of start codon. The sgRNAs' sequences were as follows.

555 *Or85a*-sg1 GGATCCTTATTTTCGATCCCGGG

556 *Or85a*-sg2 GTTCAAGAACTTCACGACCACGG

557 *Or85a*-sg3 GCCCGTCTGAAACTGCCGTCCGG

558 Both the *Or10a* mutant and *Or85a*<sup>Gal4</sup> knock-in flies were validated by  
559 sequencing.

560

561 **Computation modeling and analysis**

562 Suppose that there are  $N$  odors in a mixture, with the concentration of odor  $i$  ( $= 1, 2,$   
 563  $3 \dots N$ ) given by  $C_i$ . The odor-evoked responses in OSNs are determined by the odor-OR  
 564 interactions<sup>21,22</sup>. Because the olfactory transduction in *Drosophila* OSNs remains  
 565 controversial<sup>54</sup>, we used a simple two-state model to describe the odor-evoked response  
 566 in OSNs (Supplementary Fig. 9). In this model, ORs have two states: an inactive state  
 567 that leads to no OSN activity and an active state that leads to a maximum activity  $R_{max}$  in  
 568 the OSNs. Odor molecules bind to ORs and modulate the transition rates between these  
 569 two OR states, with excitatory odors stabilizing the active states and inhibitory odors  
 570 stabilizing the inactive states. We set two binding constants for each odor-OR/OSN pair  
 571 ( $i, j$ ):  $K_{I,ij}$  is the dissociation constant for the inactive OR state and  $K_{A,ij}$  is the  
 572 dissociation constant for the active state. We modeled the responses of OSNs to odor  
 573 mixtures by using two modes of odor-OR/OSN interactions: multiple binding sites, in  
 574 which different odors bind to different sites, and competitive binding, in which different  
 575 odors compete for the same binding sites. We show below that these two cases produce  
 576 similar results.

577

578 **Multiple binding sites.** We constructed the model based on transition kinetics between  
 579 the two functional states (active and inactive) and the different odor binding states (bound  
 580 and unbound). For steady-state properties, the effective “free energy” difference between  
 581 the inactive and active states of the OR/OSN $_j$ ,  $\Delta F_j$ , depends on the odor concentrations:

$$\Delta F_j \equiv F_{j,I} - F_{j,A} = E_{0,j} + \sum_i \left[ \ln \left( 1 + \frac{C_i}{K_{A,ij}} \right) - \ln \left( 1 + \frac{C_i}{K_{I,ij}} \right) \right] \quad (1)$$

582 where  $E_{0,j}$  is the free energy difference in the absence of any odors, and the other terms  
 583 correspond to the entropic contributions because OR can be either vacant or bound by an  
 584 odor molecule. The average activity of the OR/OSN $_j$ ,  $R_j$ , can then be written as

$$R_j = \frac{R_{max}}{1 + \exp(-\Delta F_j)} = R_{max} \left[ 1 + \alpha_j \prod_i \frac{1 + C_i/K_{I,ij}}{1 + C_i/K_{A,ij}} \right]^{-1} \quad (2)$$

585 where  $\alpha_j = \exp(-E_{0,j})$ , and the baseline activity in the absence of any stimulus is  
 586  $R_0 = R_{max}/(1 + \alpha_j)$ . This energetic approach gives the same results as solving the  
 587 steady state of the kinetic equations, as shown below.

588 For excitatory odors, we have  $K_{A,ij} < K_{I,ij}$ , which indicates that they bind to the  
 589 active OR state with a higher affinity. Inhibitory odors stabilize the inactive OR state, i.e.,  
 590  $K_{I,ij} < K_{A,ij}$ . For simplicity, we assume the excitatory odor only binds to the active OR  
 591 state and the inhibitory odor to the inactive OR state. That is,  $K_{I,ij} = \infty$  for excitatory  
 592 odors, and  $K_{A,ij} = \infty$  for inhibitory odors. Then, we have

$$R_j = R_{max} \left[ 1 + \alpha_j \prod_{i=1}^N \left( 1 + \frac{C_i}{K_{ij}} \right)^{-w_{ij}} \right]^{-1} \quad (3)$$

593 where  $w_{ij} = \{1, -1, 0\}$  for excitatory, inhibitory and null responses, respectively;  $K_{ij}$   
 594 represents the finite dissociation constant  $K_{I,ij}$  for inhibitory odors and  $K_{A,ij}$  for excitatory  
 595 odors.

596 **Competitive binding.** The free energy difference between the inactive and the active  
 597 states of the OR/OSN<sub>j</sub> is

$$\Delta F_j \equiv F_{j,I} - F_{j,A} = E_{0,j} + \ln \left( 1 + \sum_p \frac{C_p}{K_{pj}} \right) - \ln \left( 1 + \sum_q \frac{C_q}{K_{qj}} \right) \quad (4)$$

598 where p and q represent the inhibitory and excitatory odors, respectively. The activity of  
 599 OR/OSN<sub>j</sub> corresponding to Eq. 3 is

$$R_j = R_{max} \left[ 1 + \alpha_j \frac{1 + \sum_{p=1}^{n_i} C_p / K_{pj}}{1 + \sum_{q=1}^{n_e} C_q / K_{qj}} \right]^{-1} \quad (5)$$

600 where  $n_e$  and  $n_i$  are the numbers of excitatory and inhibitory odors, respectively.

601 Most of the results presented in the main text were obtained using the model of  
 602 multiple binding sites. However, the model with competitive binding as described in Eq.  
 603 5 generates qualitatively similar results (see below).

604 **Advantages of encoding with bidirectional odor-evoked responses.** Odors in the  
 605 natural environment vary in both their frequencies of appearance and their concentrations.  
 606 In addition, some odors may appear together (or correlated). An optimal olfactory system  
 607 should be able to make two chemical mixtures or two vectors in the odor space ( $\vec{C}_1$  and  
 608  $\vec{C}_2$ ) distinguishable in the OSN response space, i.e., make the OSN activity vectors  
 609 ( $\vec{R}_1$  and  $\vec{R}_2$ ) separable. Essentially, for the distribution of points in the odor space  $P(\vec{C})$ ,  
 610 the coding transforms it to the distribution of OSN activity  $P_n(\vec{R})$ . When  $P_n(\vec{R})$  is  
 611 uniform, it encodes the maximum amount of information.

612 We compared the coding capacity (in terms of entropy) and de-correlation (in  
 613 terms of the principal component spectrum) of OSN responses for cases with and without  
 614 odor-evoked inhibitory responses. The distribution of odor mixtures in the fly's natural  
 615 environment is unknown. Here, we used the ensemble of odors that have been  
 616 comprehensively studied in the literature by the Carlson lab<sup>22</sup> to compose the odor  
 617 mixtures.

618 **Setting the parameters based on experimental data.** All the parameters  
 619  $\{R_{max}, \alpha, \omega_{ij}, K_{ij}\}$  are determined according to the measured responses of 24 ORs to 110  
 620 odors<sup>22</sup>, where responses were coarse-grained (digitalized) to 6 levels:  $\Delta R =$   
 621  $\{-1, 0, 1, 2, 3, 4\}$ , in which -1 denotes inhibition and positive numbers represent various  
 622 degrees of excitation. We ignore the 3 odors that elicited no OR/OSN responses, and the  
 623 3 ORs/OSNs that showed only inhibitory or no responses. To calculate the OR/OSN  
 624 responses to odor mixtures according to Eq. 3 or Eq. 5, we defined the discrete response,  
 625  $\Delta R$ , based on the spiking rates  $R_{spike}$  according to the criteria used by others<sup>22</sup>.

$$626 \quad \Delta R = \begin{cases} -1, & R_{spike} \leq 15 \\ 0, & 15 < R_{spike} < 50 \\ 1, & 50 \leq R_{spike} < 100 \\ 2, & 100 \leq R_{spike} < 150 \\ 3, & 150 \leq R_{spike} < 200 \\ 4, & R_{spike} \geq 200 \end{cases} \quad (6)$$

627 For simplicity, we set the baseline firing rate of all the ORs/OSNs as  $R_0 = 30$   
 628 spikes/s (and the maximum firing rate of 250 spikes/s), which leads to a constant  $\alpha =$

629  $\frac{R_{max}}{R_0} - 1 = \frac{22}{3}$ . The concentration was set as 1 in an arbitrary unit. For excitatory odors,  
 630 we set the  $\frac{1}{K_{ij}} + 1 = \frac{1}{4}\alpha, \frac{2}{3}\alpha, \frac{3}{2}\alpha, 4\alpha$ , corresponding to the response states 1, 2, 3, and 4,  
 631 respectively. For inhibitory odors, we selected the inhibition strength such that the  
 632 average effect from excitatory odors is roughly balanced by that from inhibitory odors.  
 633 For simplicity, we assume  $K_{ij} = K_j$ ; that is, the inhibitory strength is the same for any  
 634 inhibitory odor-OSN<sub>j</sub> pair, where  $N_{0j}$  is the number of inhibitory odors for OSN<sub>j</sub>. The  
 635 value of  $K_j$  is then set by requiring a rough balance of inhibitory and excitatory stimuli on  
 636 average:

$$N_{0j} \ln \left( 1 + \frac{1}{K_j} \right) - \sum_{q=1}^{N_{1j}} \ln \left( 1 + \frac{1}{K_{qj}} \right) = -\ln(\beta_j) \quad (7)$$

637 where  $N_{1j}$  is the number of excitatory odors of the OSN<sub>j</sub> and  $\beta_j$  is a constant. For a larger  
 638  $\beta_j$ , the average response becomes larger. In the following simulations, we set it as 1.  
 639 Other values were also used without changing the general results.

640 **The coding capacity for odor mixtures.** We used two methods to compose the odor  
 641 mixture: the sampling method and the enumeration method. With the sampling method,  
 642 we fixed the number of odors in the mixtures and randomly sample from the 107 odors  
 643 100,000 times. The concentration of each chosen odor was set as 1, and other  
 644 concentrations were also used without changing the results qualitatively. For each odor  
 645 mixture, we calculated the responses of the 21 ORs/OSNs. The Shannon entropy of each  
 646 OR/OSN is computed by  $H_j = \int_{R_{min}}^{R_{max}} P_j(R) [\log_2 P_j(R)]$ , where  $P_j(R)$  is the distribution  
 647 function of the response  $R_j$  for the OSN<sub>j</sub>. In our calculation, this probability is  
 648 approximated by dividing the whole response range into bins and counting the numbers  
 649 of the responses that fall into each bin of 1 spike/s. The integration was substituted by  
 650 summation. The maximum entropy per OR/OSN is  $H_{max} = \log_2 (R_{max} - R_{min}) \approx 8$  bit.

651 For the enumeration method, we assume that any given odor has a probability  $p$  to  
 652 appear in the mixture. For example,  $p = 0.2$  leads to roughly  $N_{ave} \sim 21$  odors in the  
 653 mixture. For a given neuron, we denote  $N_0$  as the total number of inhibitory odors with

654 dissociation constant  $K_0$  and  $N_l$  as the total number of “type- $l$ ” excitatory odors with  
 655 dissociation constant  $K_l$  for  $l = 1, 2, 3, 4$  in the odor repertoire. Only a subset of these  
 656 odors are present in a given mixture. For a mixture with  $N_0$  inhibitory odors and  $N_l$   
 657 “type- $l$ ” excitatory odors, the response activity is

$$R(\vec{n}) = R_{max} \left[ 1 + \alpha \frac{(1 + C_0/K_0)^{n_0}}{\prod_{l=1}^4 (1 + C_0/K_l)^{n_l}} \right]^{-1} \quad (8)$$

658 where  $\vec{n} = (n_0, n_1, n_2, n_3, n_4)$  characterizes the odor mixture. The probability of this  
 659 random mixture characterized by  $\vec{n}$  is given by

$$P(\vec{n}) = \prod_{l=0}^4 \binom{N_l}{n_l} p^{n_l} (1 - p)^{N_l - n_l} \quad (9)$$

660 where the range of  $n_l$  is from 0 to  $N_l$ . For each choice of  $\vec{n}$ , we computed the  
 661 corresponding  $R_{\vec{n}}$  and  $P(\vec{n})$ , from which we get the distribution  $P(R)$  for this neuron  
 662 exactly without sampling. The summation of all the entropy was computed for cases with  
 663 or without odor-evoked inhibitory responses. The entropy of each OR/OSN and the total  
 664 entropy for all OR/OSNs is much larger when including odor-evoked inhibitory  
 665 responses (Supplementary Figs. 7 and 8). Including inhibitory response also reduced the  
 666 average response, thus avoiding OR/OSN saturation (Supplementary Fig. 7) and making  
 667 the OR/OSN responses more uniform (Supplementary Fig. 8). These two effects both  
 668 increase the coding capacity of OR/OSNs.

669 Results from the sampling method (Supplementary Fig. 10a,b) were consistent  
 670 with the enumeration method (Supplementary Fig. 10c,d). We also computed the total  
 671 entropy of all the OR/OSNs for the competitive binding case (Supplementary Fig. 10e-g),  
 672 which gave results similar to those in Fig. 7b.

673 **Principal component analysis (PCA).** For the 100,000 randomly sampled odor mixtures,  
 674 we first transformed the response of OR/OSN into discrete states according to Eq. 6,  
 675 producing 100,000 points in a 21-dimension space. We then performed the principal  
 676 component analysis by constructing the correlation matrix of the 21 OR/OSNs. The  
 677 eigenvalue of a given principal component (PC) characterizes the variation of OSN

678 responses along that PC direction. The higher the eigenvalue, the more information can  
 679 be coded in that PC. A PC can be used effectively for coding when its eigenvalue is  
 680 larger than a threshold determined by noise. The number of PCs with eigenvalues above  
 681 the noise threshold is defined as the effective coding dimensions. A higher effective  
 682 coding dimensions corresponds to more independent directions for odor representation by  
 683 OR/OSNs. The noise threshold is set to be 1 in our study. The PCA results using  
 684 competitive binding (Supplementary Fig. 10f) were similar to those using multiple  
 685 binding sites (Fig. 7c).

686 **Equivalence of the kinetic approach and the energetic approach.** Both the kinetic and  
 687 the energetic approaches can be used to describe the odor responses in OSNs. We used  
 688 the energetic approach in the main text because it is easier to generalize to odor mixtures.  
 689 The kinetic approach (Supplementary Fig. 9) can yield additional time-dependent  
 690 information. However, this information is not considered in this study. In the following  
 691 section, we determine the steady-state response by solving the kinetic equations. We first  
 692 consider the simple situation with only one odor. As shown in Supplementary Fig. 9, an  
 693 OR/OSN<sub>j</sub> can exist in two functional states: the active and the inactive states labeled by  
 694  $R_j^*$  and  $R_j$  respectively. In addition, a receptor can be either bound or unbound by odor  
 695 molecules  $L_i$ . Therefore, there are four microscopic states:  $R_j$ ,  $L_i \cdot R_j$ ,  $L_i \cdot R_j^*$ ,  $R_j^*$ ,  
 696 denoted as 1, 2, 3, and 4, respectively, for simplicity. The ligand binding/unbinding  
 697 kinetics and the active/inactive kinetics among the four states are illustrated in  
 698 Supplementary Fig. 9 with their rates specified. The kinetics of the four microscopic  
 699 states can be described by the following rate equations:

$$\frac{dP_1}{dt} = k'_{off}P_2 - k'_{off}\frac{C_i}{K_{I,ij}}P_1 - \omega P_1 + \omega\alpha P_4 \quad (10)$$

$$\frac{dP_2}{dt} = k'_{off}\frac{C_i}{K_{I,ij}}P_1 - k'_{off}P_2 - \omega'P_2 + \omega'\alpha'P_3 \quad (11)$$

$$\frac{dP_3}{dt} = k_{off}\frac{C_i}{K_{A,ij}}P_4 - k_{off}P_3 - \omega'\alpha'P_3 + \omega'P_2 \quad (12)$$

$$\frac{dP_4}{dt} = k_{off}P_3 - k_{off}\frac{C_i}{K_{A,ij}}P_4 - \omega\alpha P_4 + \omega P_1 \quad (13)$$

700 where  $p_n(t)$  is the probability in a given state  $n(=1,2,3,4)$  at time  $t$  with the sum  $P_1 +$   
701  $P_2 + P_3 + P_4 = 1$ .

702 Under steady-state conditions, i.e.,  $dP_n/dt = 0$  ( $n=1,2,3,4$ ), and with the detailed  
703 balance condition  $\alpha'/K_{A,ij} = \alpha/K_{I,ij}$  satisfied, the steady-state probabilities of the four  
704 microscopic states can be obtained by requiring that the forward and backward fluxes be  
705 equal for each transition pair:

$$k'_{off}P_2 = k'_{off}\frac{C_i}{K_{I,ij}}P_1 \quad (14)$$

$$\omega'P_2 = \omega'\alpha'P_3 \quad (15)$$

$$k_{off}\frac{C_i}{K_{A,ij}}P_4 = k_{off}P_3 \quad (16)$$

$$\omega\alpha P_4 = \omega P_1 \quad (17)$$

706 By solving the above equations, we obtain  $P_1, P_2, P_3, P_4$ . The total probability of  
707 receptors being in the active state (both ligand bound and unbound) is

$$P_{active} = P_3 + P_4 = \frac{1 + \frac{C_i}{K_{A,ij}}}{\alpha\left(1 + \frac{C_i}{K_{I,ij}}\right) + \left(1 + \frac{C_i}{K_{A,ij}}\right)} = \left[1 + \alpha\frac{1 + \frac{C_i}{K_{I,ij}}}{1 + \frac{C_i}{K_{A,ij}}}\right]^{-1} \quad (18)$$

708 which makes the average activity of the OR/OSN

$$R_j = R_{max}P_{active} = R_{max}\left[1 + \alpha\frac{1 + \frac{C_i}{K_{I,ij}}}{1 + \frac{C_i}{K_{A,ij}}}\right]^{-1} \quad (19)$$

709 The above result is the same as Eq. 2 obtained from the effective free energy  
710 difference given by Eq. 1. To generalize to the case with multiple odors acting on the  
711 same OR/OSN, the energetic approach is much easier to use because the free energy

712 difference is additive, see Eq. 1. Of course, the same result can be obtained from solving  
 713 the steady state of the kinetic equations. Here, we briefly show the case for two odors  
 714 (indexed as i,k), where there are four different active states due to the bound/unbound  
 715 states of the two ligands. The total probability of being active is then

$$\begin{aligned}
 P_{active} &= \frac{1}{\alpha} \left( 1 + \frac{C_i}{K_{A,ij}} + \frac{C_k}{K_{A,kj}} + \frac{C_i}{K_{A,ij}} \frac{C_k}{K_{A,kj}} \right) P_1 \\
 &= \frac{\frac{1}{\alpha} \left( 1 + \frac{C_i}{K_{A,ij}} \right) \left( 1 + \frac{C_k}{K_{A,kj}} \right)}{\frac{1}{\alpha} \left( 1 + \frac{C_i}{K_{A,ij}} \right) \left( 1 + \frac{C_k}{K_{A,kj}} \right) + \left( 1 + \frac{C_i}{K_{I,ij}} \right) \left( 1 + \frac{C_k}{K_{I,kj}} \right)} \\
 &= \left[ 1 + \alpha \frac{\left( 1 + C_i/K_{I,ij} \right) \left( 1 + C_k/K_{I,kj} \right)}{\left( 1 + C_i/K_{A,ij} \right) \left( 1 + C_k/K_{A,kj} \right)} \right]^{-1} \quad (20)
 \end{aligned}$$

716 where  $P_1$  is the probability of the inactive state without any odor binding. The above  
 717 equation can be generalized to N odors, with  $2^N$  different odor (ligand) binding  
 718 combinations for the active state of the receptor. The corresponding active probability is

$$\begin{aligned}
 P_{active} &= \frac{1}{\alpha} \prod_{i=1}^N \left( 1 + \frac{C_i}{K_{A,ij}} \right) P_1 = \frac{\frac{1}{\alpha} \prod_{i=1}^N \left( 1 + \frac{C_i}{K_{A,ij}} \right)}{\frac{1}{\alpha} \prod_{i=1}^N \left( 1 + \frac{C_i}{K_{A,ij}} \right) + \prod_{i=1}^N \left( 1 + \frac{C_i}{K_{I,ij}} \right)} \\
 &= \left[ 1 + \alpha \prod_{i=1}^N \frac{\left( 1 + C_i/K_{I,ij} \right)}{\left( 1 + C_i/K_{A,ij} \right)} \right]^{-1} \quad (21)
 \end{aligned}$$

719 and the average activity of the OSN<sub>j</sub> is

$$R_j = R_{max} \left[ 1 + \alpha \prod_{i=1}^N \frac{\left( 1 + C_i/K_{I,ij} \right)}{\left( 1 + C_i/K_{A,ij} \right)} \right]^{-1} \quad (22)$$

720 which is exactly Eq. 2.

721 For the competitive binding interaction, i.e., all ligands compete for the same  
 722 binding sites, it is easy to follow the same analysis as above. Given N odors, there are  
 723  $2(N + 1)$  microstates, and half of them correspond to active state. The corresponding  
 724 active probability is

$$\begin{aligned}
P_{active} &= \frac{1}{\alpha} \left( 1 + \sum_{i=1}^N \frac{C_i}{K_{A,ij}} \right) P_1 = \frac{\frac{1}{\alpha} (1 + \sum_{i=1}^N C_i/K_{A,ij})}{\frac{1}{\alpha} (1 + \sum_{i=1}^N C_i/K_{A,ij}) + (1 + \sum_{i=1}^N C_i/K_{I,ij})} \\
&= \left[ 1 + \alpha \frac{1 + \sum_{i=1}^N C_i/K_{I,ij}}{1 + \sum_{i=1}^N C_i/K_{A,ij}} \right]^{-1} \quad (23)
\end{aligned}$$

725 and the average activity of the OSN<sub>j</sub> is

$$R_j = R_{max} P_{active} = R_{max} \left[ 1 + \alpha \frac{1 + \sum_{i=1}^N C_i/K_{I,ij}}{1 + \sum_{i=1}^N C_i/K_{A,ij}} \right]^{-1} \quad (24)$$

726 which is exactly Eq. 5. Therefore, the energetic approach and the kinetic approach lead to  
727 the same results.

728

## 729 **Statistics**

730 All experiments were performed with experimental and control groups in parallel. Sample  
731 size was determined based upon preliminary experiments. Data were analysed  
732 statistically using one-way ANOVA t-test, presented as mean  $\pm$  s.e.m, unless otherwise  
733 stated.

734

## 735 **Data Availability**

736 All relevant data supporting the findings of this study are available from the  
737 corresponding author on request.

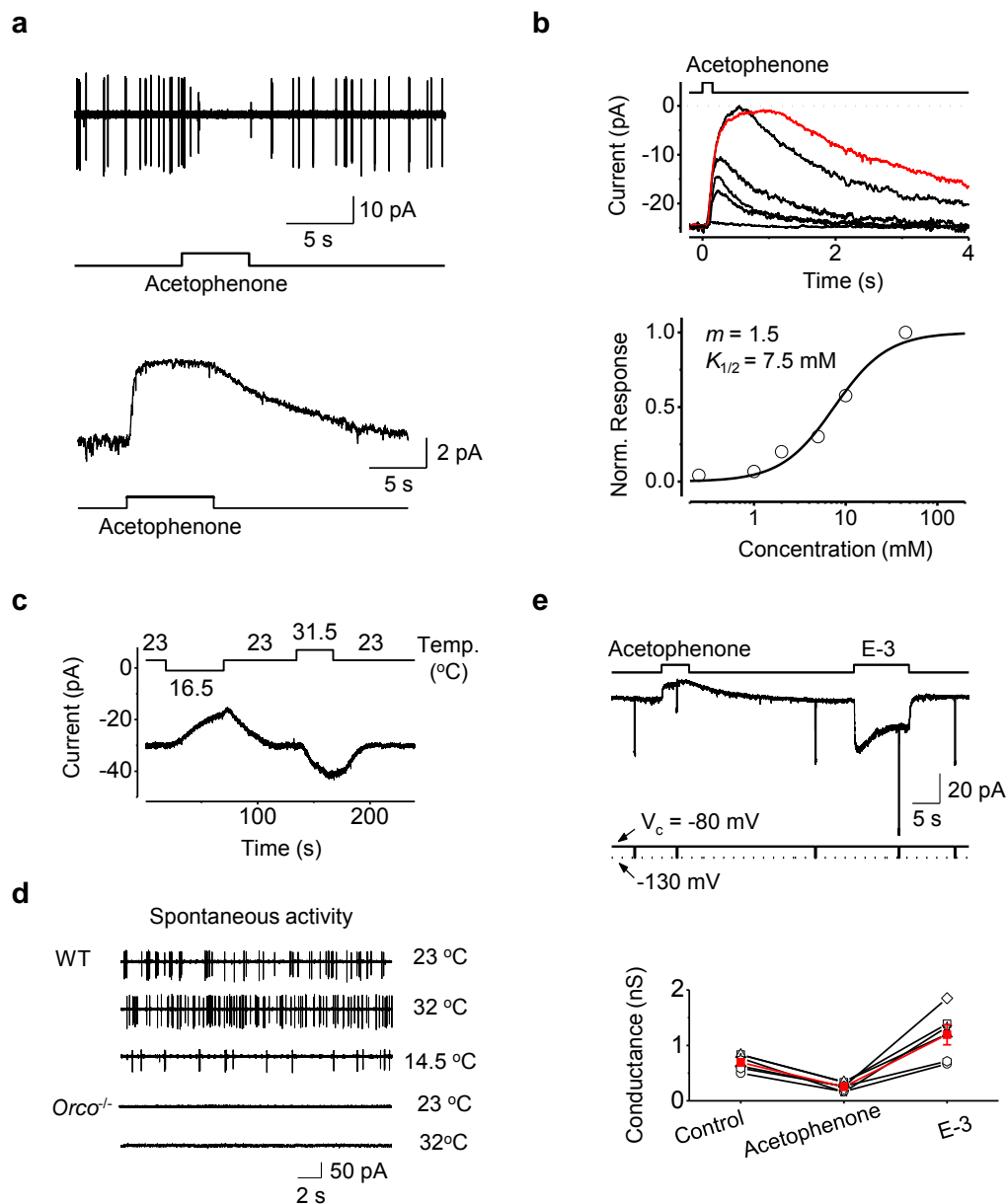
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868

869 **Figure 1** An outward receptor current underlies odor-evoked inhibition in *Or85a*-  
 870 expressing OSNs

871 (a) Acetophenone (10 mM) abolishes the spontaneous firing (cell-attached recording, top)

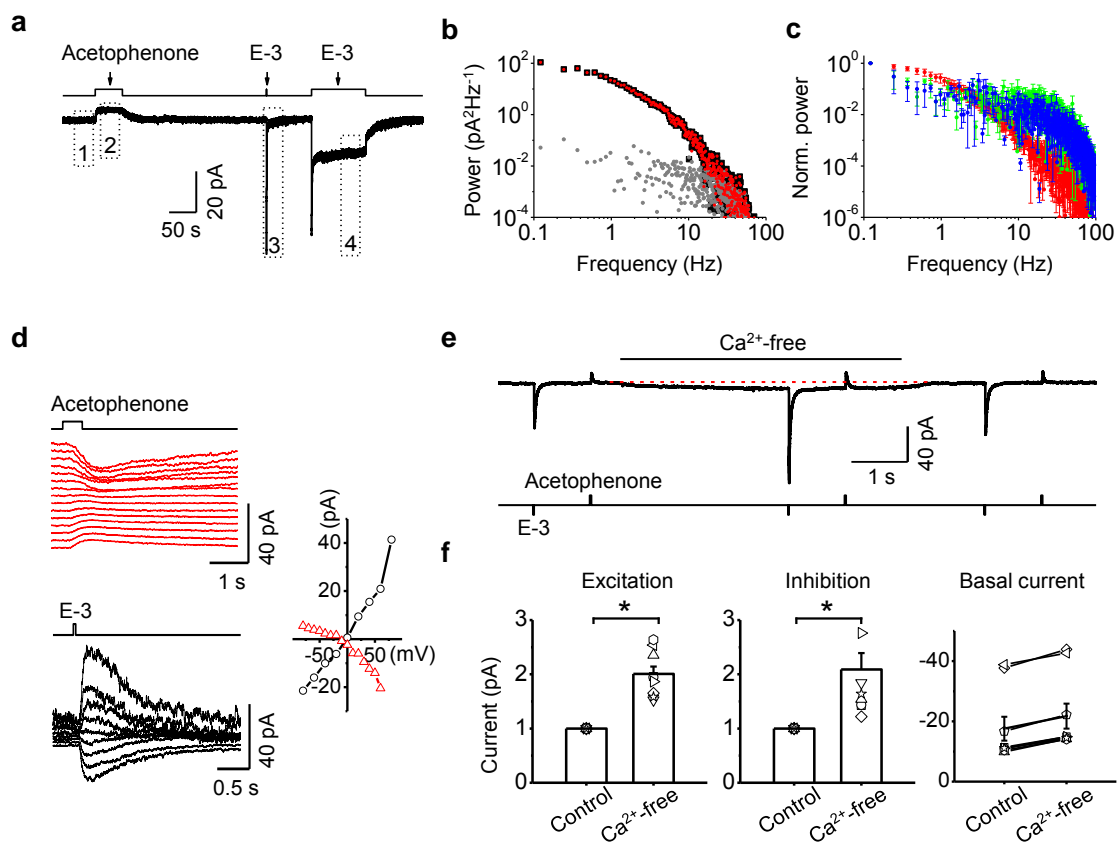
872 and triggers an outward receptor current (perforated patch-clamp recording, voltage-

873 clamped at -80 mV, bottom). The timing of odor application is indicated. (b) Dose-

874 response relationship of the odor-evoked inhibition. Top, superimposed traces of

875 responses to 150-ms pulses of acetophenone at 0.25, 1.25, 5, 10, and 45 mM. The

876 inhibitory responses to acetophenone at 45 mM (maximal water solubility) with durations  
877 of 150 ms and 500 ms (red trace) exhibit the same peak amplitude and reduce the basal  
878 inward current to 0 pA. Each trace is the average of five to ten trials. Bottom, the  
879 normalized dose-response relationship. The fit is the Hill equation,  $R/R_{max} = C^m / (C^m$   
880  $+ K_{1/2}^m)$ , where  $R$  is the peak-response amplitude,  $R_{max}$  is the saturated peak response,  $C$  is  
881 the odor concentration,  $K_{1/2}$  is the odor concentration that half-saturates the response, and  
882  $m$  is the Hill coefficient. In this experiment,  $K_{1/2} = 7.5$  mM, and  $m = 1.5$ . Collective  
883 results ( $n = 4$ ):  $K_{1/2} = 27 \pm 13$  mM, and  $m = 1.4 \pm 0.2$ . (c) Temperature dependence of  
884 the basal inward current in WT flies. (d) Temperature dependence of spontaneous firing  
885 in WT flies (three top traces) and *Orco*<sup>-/-</sup> flies (two bottom traces). (e) Odor-evoked  
886 inhibition decreases membrane conductance. Top, membrane conductance was monitored  
887 using 10-ms voltage pulses (stepping from the holding voltage of -80 mV to -130 mV)  
888 before, during, and after the application of acetophenone (10 mM) and ethyl 3-  
889 hydroxybutyrate (E-3) (1 mM). The timing of odor stimulation and voltage pulses is  
890 indicated above and below the response trace, respectively. Bottom, collective data of  
891 membrane-conductance changes, with average indicated in red.



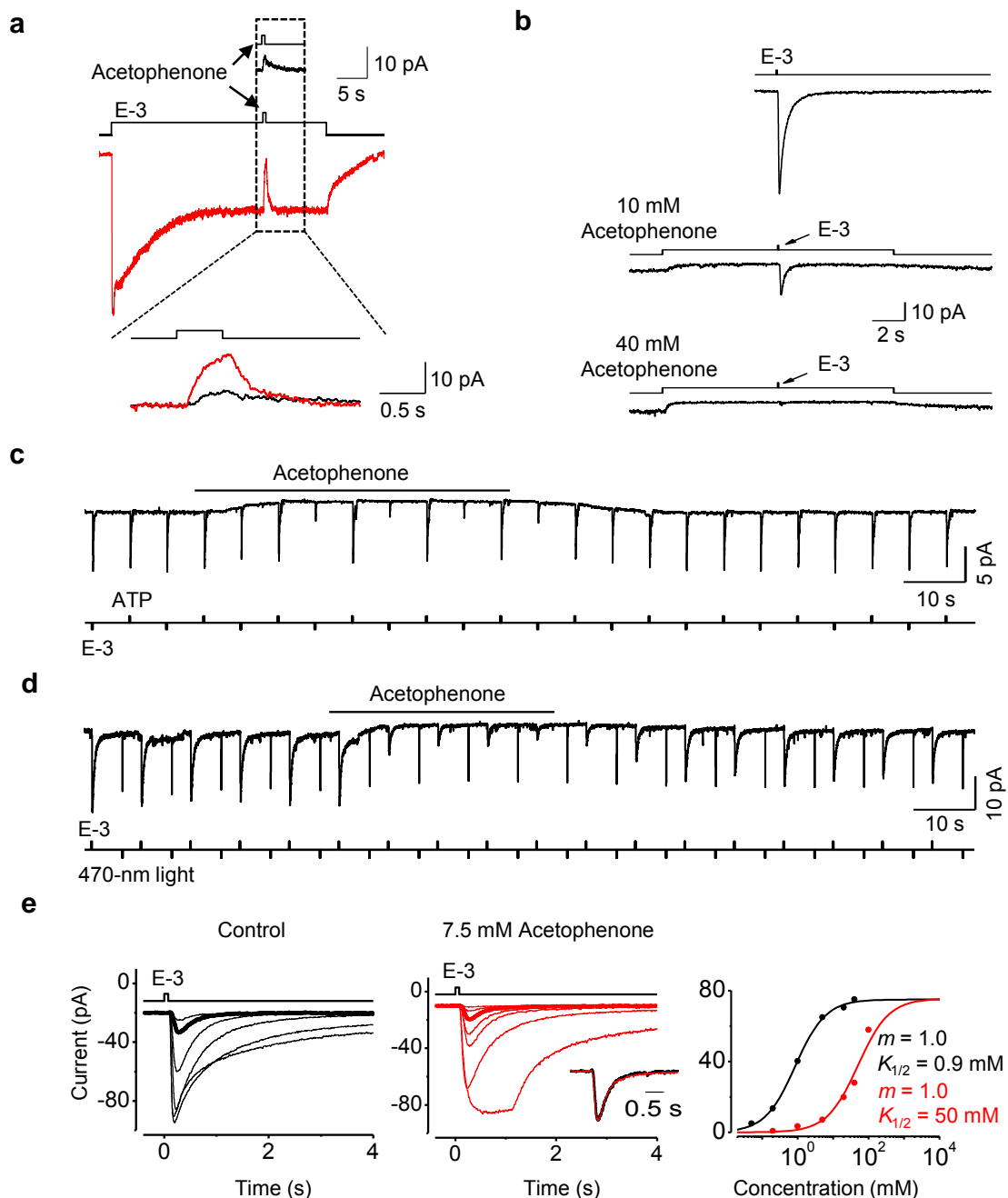
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894 **Figure 2** Spontaneously activated and odor-activated ORs share similar signaling  
 895 (a) The basal inward current and odor-evoked receptor currents to acetophenone (20 mM;  
 896 30 s) and E-3 (1 mM; 35 ms and 60 s). (b) Power spectrum of excitatory responses. Gray  
 897 and black represent the power spectra of segments 1 and 3 in A, respectively; red  
 898 represents the difference spectrum of segments 3 minus 1. (c) Scaled power spectra of the  
 899 basal activities (segments 1 minus 2, blue) and the excitatory response to a pulse  
 900 (segments 3 minus 1, red) and a step (segments 4 minus 1, green) of E-3.  $N = 3$ . (d) I-V  
 901 relationships. Voltage dependence of the receptor currents induced by inhibitory (left, top)  
 902 and excitatory (left, bottom) odors. Current-voltage relationships of inhibitory and  
 903 excitatory responses (right). The reversal potentials of inhibitory and excitatory responses  
 904 are  $-5.3 \pm 2.0$  mV and  $-2.5 \pm 2.0$  mV ( $n = 5$ ), respectively. Acetophenone: 20 mM; E-3:  
 905 2 mM. (e) Calcium modulation of the basal inward current and odor responses. Removal  
 906 of extracellular calcium increases the basal current, outward receptor current to  
 907 acetophenone (20 mM, 150 ms), and inward receptor current to E-3 (1 mM, 35 ms). (f)  
 908 Removal of extracellular calcium increases the excitatory (left), inhibitory (middle)  
 909 responses, and basal current (right).  $N = 9$ , asterisk,  $P < 0.05$ .

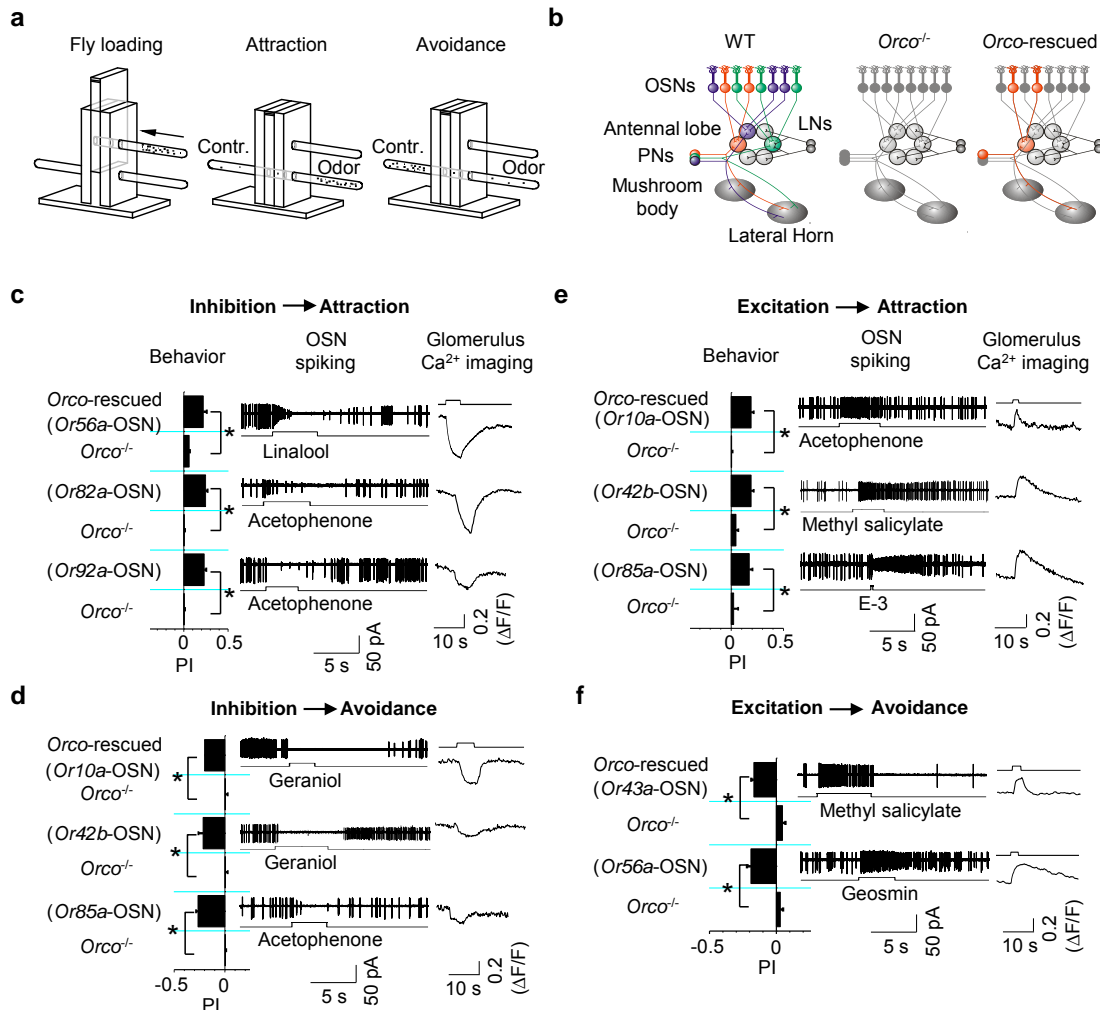
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912  
 913 **Figure 3** Interactions between odor-evoked inhibition and activation in the same OSNs  
 914 (a) Acetophenone reduces the basal current and odor-evoked inward current. Top, the  
 915 acetophenone (10 mM)-induced response; middle, the response induced by acetophenone  
 916 (10 mM) in the presence of E-3 (4 mM); bottom, the overlay of acetophenone-induced  
 917 responses with (red) and without (black) the presence of E-3. E-3 increases  
 918 acetophenone-induced responses from  $3.0 \pm 0.9$  pA to  $15 \pm 2$  pA ( $n = 6$ ;  $P < 0.001$ ). (b)  
 919 Dose-dependence of acetophenone-induced reduction of excitatory responses. The inward  
 920 receptor current induced by E-3 (4 mM, 35 ms) (top) is inhibited by background  
 921 application of 10 mM (middle) or 40 mM (bottom) acetophenone. Acetophenone

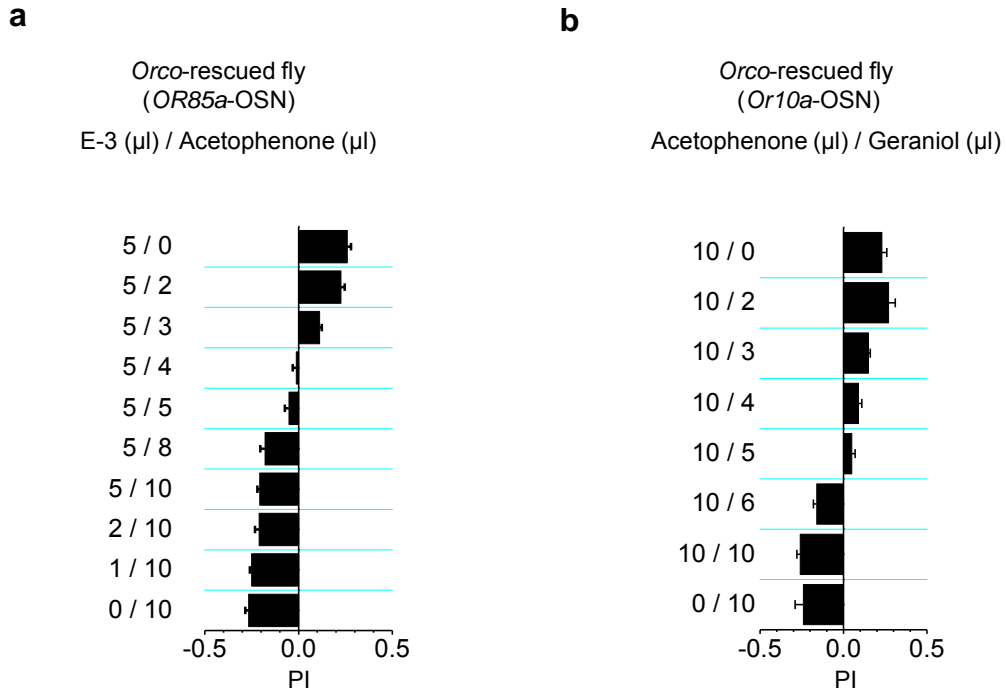
922 decreases E-3-induced responses from  $51.3 \pm 16.0$  pA (control,  $n = 5$ ) to  $23.0 \pm 8.0$  pA  
923 (10 mM acetophenone) to  $6.0 \pm 4.0$  pA (40 mM acetophenone). (c) Acetophenone does  
924 not inhibit P2X<sub>2</sub>-mediated responses. The *Or85a*-OSNs express exogenous ATP-gated  
925 P2X<sub>2</sub> cation channels. The inward current triggered by 1 mM E-3 is decreased to  $23 \pm 3\%$   
926 ( $n = 22$ ) by 5 mM acetophenone, but the inward current triggered by 1 mM ATP is  
927 unaffected by acetophenone. Note the reduction of the basal inward current by  
928 acetophenone. (d) Acetophenone does not inhibit ChR2-mediated inward current, but  
929 decreased the E-3-induced responses to  $13 \pm 3\%$  ( $n = 32$ ). Acetophenone: 5 mM; E-3: 1  
930 mM, 35 ms, light: 10 ms. (e) Competitive inhibition of the excitatory responses by  
931 acetophenone. Superimposed traces showing responses to E-3 (35 ms) in the absence  
932 (black, left) and presence (red, middle) of 7.5 mM acetophenone. The red trace of the  
933 largest amplitude was obtained with a 1-s pulse of 70 mM E-3. The normalized responses  
934 of the two thicker traces with similar small response amplitudes have identical response  
935 shape and kinetics (inset, middle). Dose-response relationships (right) for the data with  
936 and without the presence of acetophenone are shown in red and black, respectively. 7.5  
937 mM acetophenone decreases the sensitivity to E-3 from a  $K_{1/2}$  of  $1.1 \pm 0.2$  mM to  $40 \pm 10$   
938 mM ( $n = 3$ ;  $P < 0.001$ ).  
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942 **Figure 4** Odor-evoked OSN inhibition drives olfactory behaviors

943 (a) Schematic T-maze behavioral assay. Fly loading to the T-maze (left); attraction by  
 944 odors (middle); repellence by odors (right). (b) Olfactory neural pathways in *Drosophila*.  
 945 There are approximately 50 types of ORs in the adult antennae, with OSNs expressing the  
 946 same type of OR (marked by one color) converging onto a glomerulus in the antennal  
 947 lobe (left). In *Orco*<sup>-/-</sup> flies (middle), the ORs are not functional because of their  
 948 dependence on *Orco*<sup>25</sup>; in *Orco*-rescued flies (right), *Orco* is restored to the OSNs  
 949 expressing a specific type of OR (marked in orange) in an *Orco*<sup>-/-</sup> background, thus  
 950 restoring the odor-sensing ability in the targeted OSNs. PNs: projection neurons; LNs:  
 951 local neurons. (c) Odor-evoked inhibition elicits attraction. Odors inhibiting the basal  
 952 firing of OSNs (middle) and calcium signals in corresponding glomeruli (right) attract  
 953 flies (left) with *Orco* restored to OSNs expressing *Or56a*, *Or82a*, or *Or92a*, as indicated.  
 954 (d) Odor-evoked inhibition elicits avoidance. (e) Odor-evoked activation elicits attraction.  
 955 (f) Odor-evoked activation elicits avoidance. Odor concentrations: acetophenone, linalool,  
 956 and methyl salicylate at a dilution of 10<sup>-3</sup> (vol/vol); geraniol at 10<sup>-4</sup>; E-3 at 10<sup>-2</sup>; and  
 957 geosmin at 10<sup>-5</sup>. N = 8-20, asterisk, *P* < 0.01.  
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961 **Figure 5** Discriminating odor mixtures by a single type of OSN962 **(a)** Behaviors of the flies with *Orco* restored to *Or85a*-OSNs to mixtures of E-3

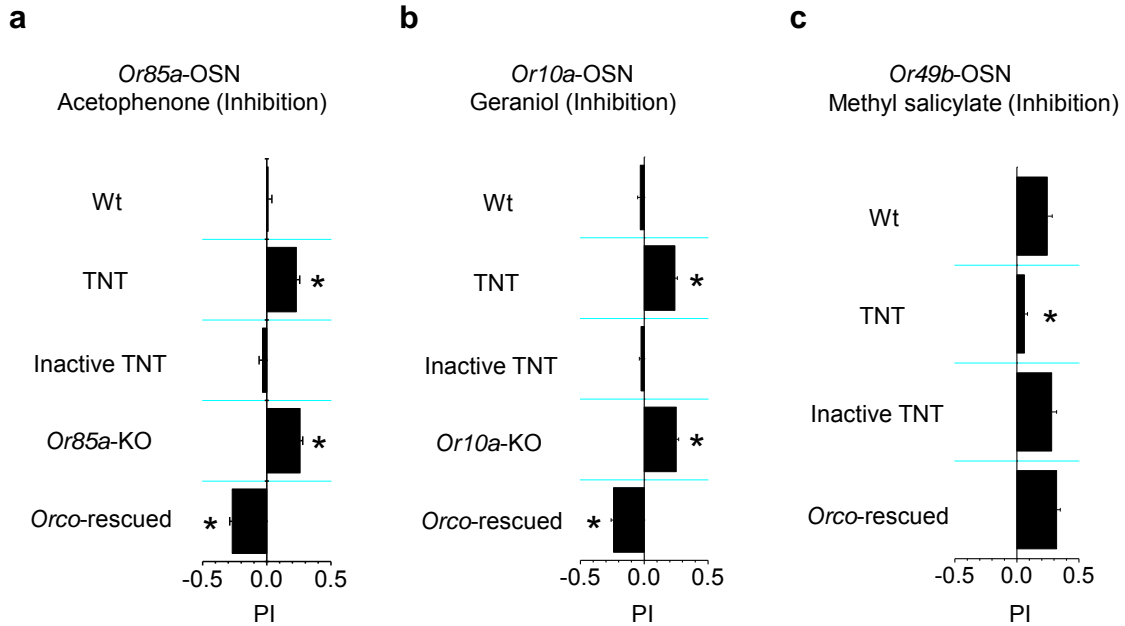
963 (increasing firing) and acetophenone (reducing basal firing) at different ratios, as

964 indicated. **(b)** Behaviors of the flies with *Orco* restored to *Or10a*-OSNs to mixtures of

965 acetophenone (increasing firing) and geraniol (reducing basal firing) at different ratios.

966 N=8-12.

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**Figure 6** Odor-evoked OSN inhibition contributes to the combinatorial odor coding in WT flies

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(a) Acetophenone-elicited behaviors in WT flies, flies expressing *TNT* in *Or85a*-OSNs,

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flies expressing inactive *TNT* in *Or85a*-OSNs, mutant flies of knocking out *Or85a*, and

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flies with *Orco* restored to *Or85a*-OSNs in an *Orco*<sup>-/-</sup> background. (b) Geraniol-elicited

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behaviors in WT flies, flies expressing *TNT* in *Or10a*-OSNs, flies expressing inactive

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*TNT* in *Or10a*-OSNs, mutant flies of knocking out *Or10a*, and flies with *Orco* restored to

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*Or10a*-OSNs in an *Orco*<sup>-/-</sup> background. (c) Methyl salicylate-elicited behaviors in WT

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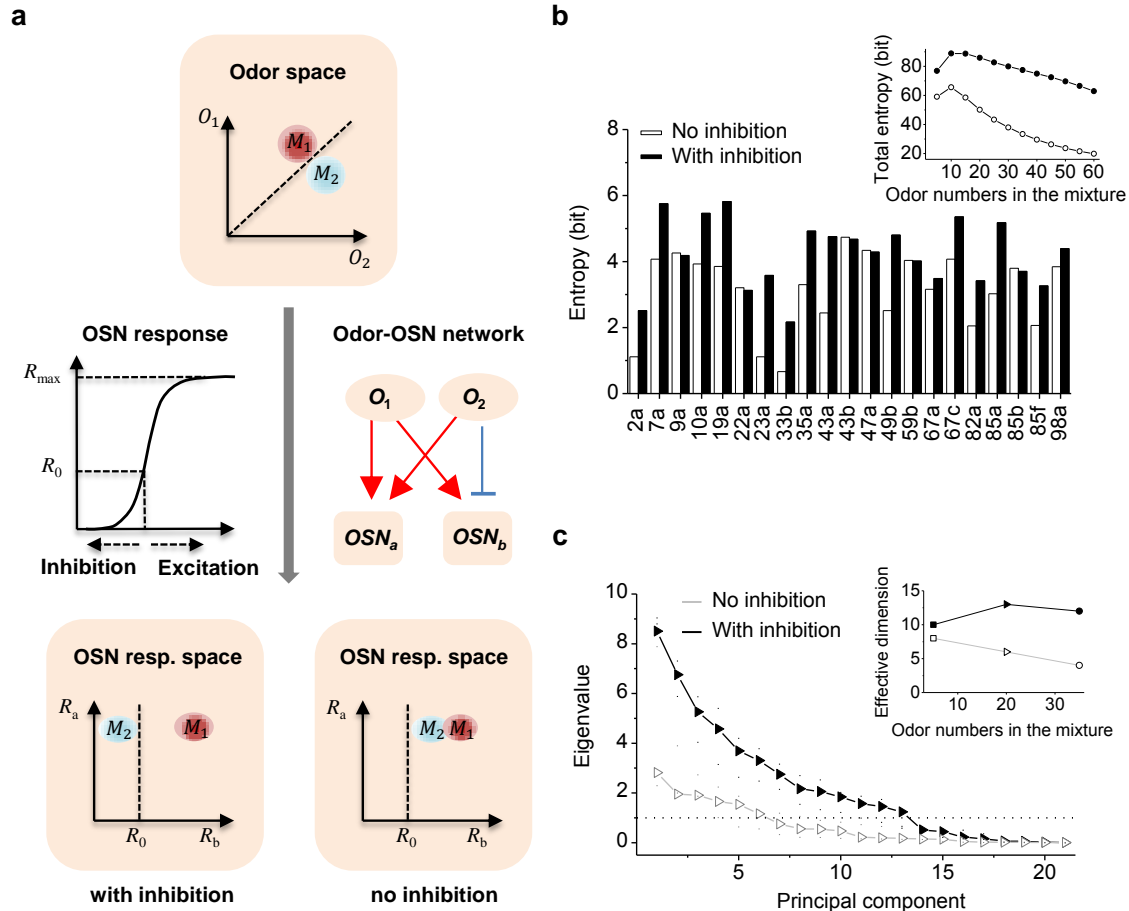
flies, flies expressing *TNT* in *Or49b*-OSNs, flies expressing inactive *TNT* in *Or49a*-OSNs,

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and flies with *Orco* restored to *Or49b*-OSNs in an *Orco*<sup>-/-</sup> background. N=8-12.

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982 **Figure 7** Dual odor coding in OSNs increases odor-coding capacity

983 (a) Schematic odor representation by OSNs. Two similar odor mixtures,  $M_1$  and  $M_2$ , are  
 984 composed of two odors,  $O_1$  and  $O_2$  (top). The dashed line corresponds to equal  
 985 concentrations of  $O_1$  and  $O_2$ . After signal transformation, the two mixtures are  
 986 represented in the OSN response space (bottom). The first transformation is achieved by  
 987 the OSN response properties (left, middle), including response modes and sensitivity. The  
 988 second transformation is effected by the odor-OSN network, with red arrows representing  
 989 activation and blue representing inhibition (right, middle).  $OSN_a$  is excited by  $O_1$  and  $O_2$ ;  
 990  $OSN_b$  is excited by  $O_1$  but inhibited by  $O_2$ . The responses of  $OSN_a$  to each of the two  
 991 mixtures likely approach saturation after summing the excitatory responses to individual  
 992 odors, and  $M_1$  and  $M_2$  are not well separated by  $OSN_a$ . In contrast,  $M_1$  and  $M_2$  are better  
 993 separated by  $OSN_b$  because the inclusion of inhibition yields a dominant inhibition for  $M_2$   
 994 (with the concentration of inhibitory odor  $O_2$  being higher than excitatory odor  $O_1$ ) but a  
 995 dominant activation for  $M_1$  (left, bottom). In the absence of inhibition, the separation  
 996 between  $M_1$  and  $M_2$  is smaller (right, bottom).  $R_{max}$  and  $R_0$  are the maximal responses and  
 997 the basal activity of OSNs, respectively;  $R_a$  and  $R_b$  are odor-evoked responses of  $OSN_a$   
 998 and  $OSN_b$ , respectively. (b) Odor-evoked inhibition increases the capacity of odor  
 999 encoding by OSNs. The coding capacity quantified by the entropy of individual  
 1000 OR/OSNs is computed (see Methods) based on the OR-response matrix<sup>24</sup>. The inclusion  
 1001 of inhibition increases the entropy in most OSNs for mixtures containing 10 odors. The

1002 inset is the total entropy summed over all ORs/OSNs responding to mixtures of varying  
1003 odor numbers. (c) Odor-evoked inhibition decorrelates odor representation. Principal  
1004 component analysis of the OSN responses to 20-odor mixtures (see Methods) based on  
1005 the OR-response matrix<sup>24</sup> showed that the inclusion of inhibition increases eigenvalues of  
1006 principal components and decorrelates odor representation. Consequently, as shown in  
1007 the inset, the inclusion of inhibition increases the number of effective coding dimensions  
1008 that have eigenvalues above a noise threshold (dotted line).

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1010 **Supplementary Text and Figures**

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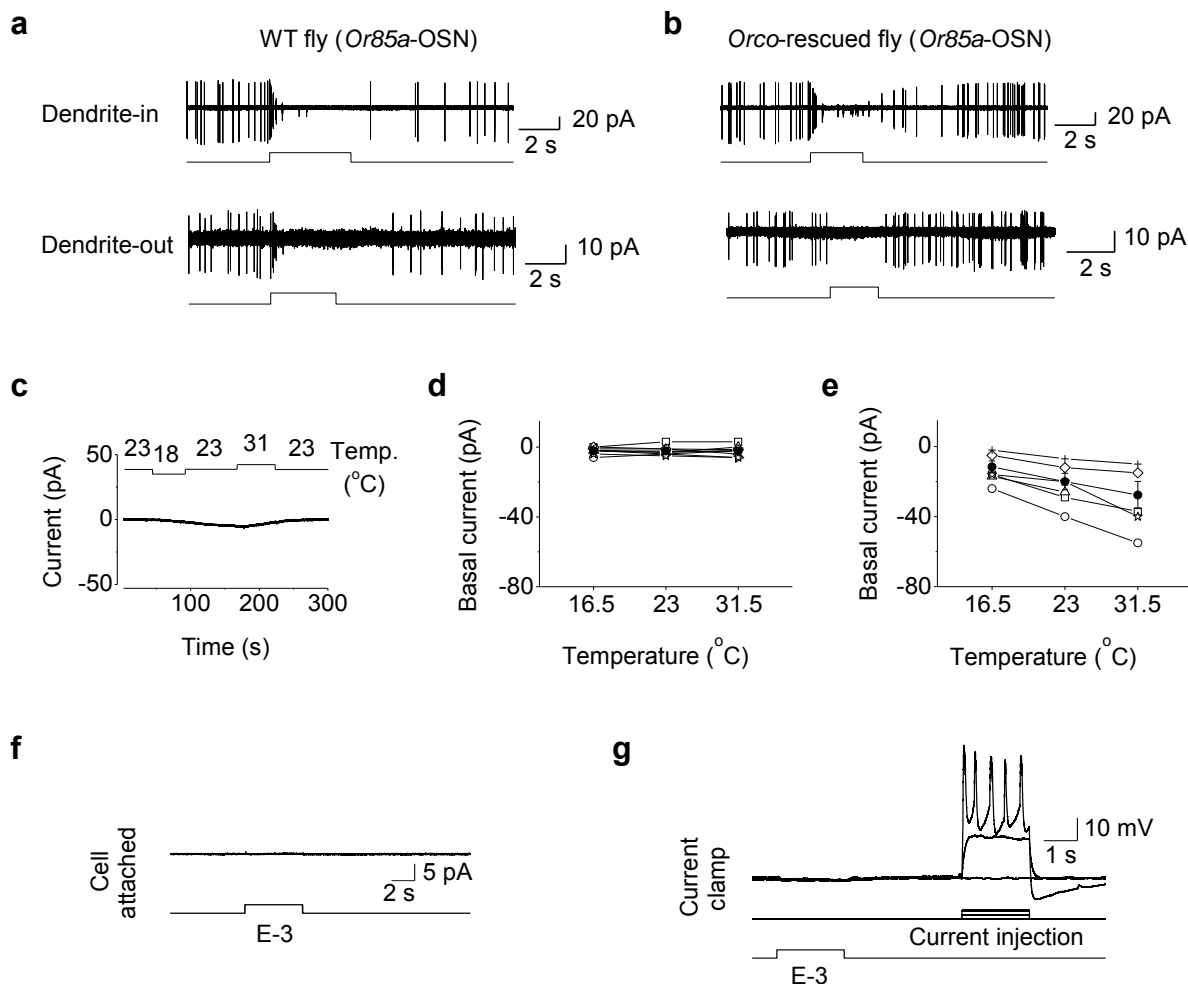
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1014 Odor-evoked inhibition of olfactory sensory neurons drives  
1015 olfactory perception in *Drosophila*

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1017 Li-Hui Cao, Dong Yang, Wei Wu, Xiankun Zeng, Bi-Yang Jing, Meng-Tong Li,  
1018 Shanshan Qin, Chao Tang, Yuhai Tu, Dong-Gen Luo

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1021 **Supplementary Figure 1**

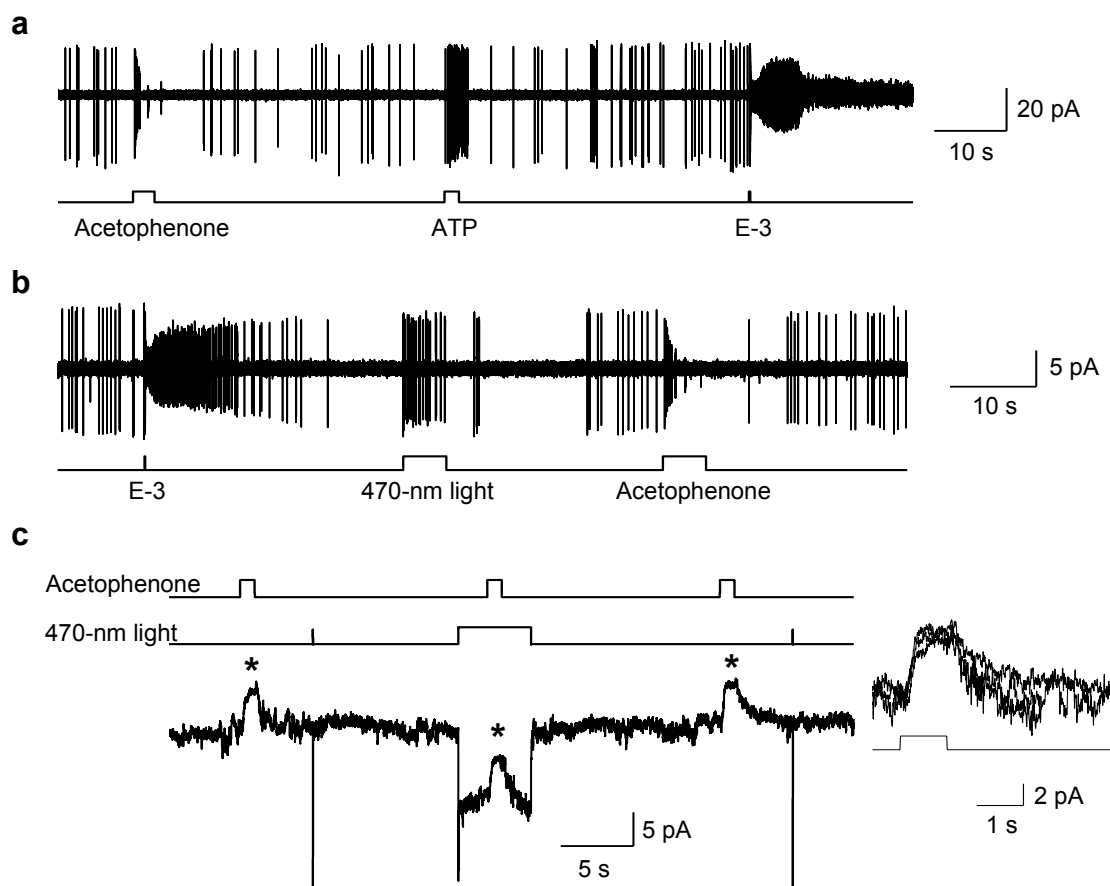
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1023 **Direct odor inhibition, temperature and *Orco* dependence of basal receptor current**  
 1024 **in *Or85a*-OSNs**

1025

1026 (a) Cell-attached recordings from *Or85a*-OSNs in WT flies. Top, recording from an  
 1027 *Or85a*-OSN with its sensory dendrite remaining intact in the sensillum (Dendrite-in)<sup>25</sup>.  
 1028 Bottom, recording from an isolated *Or85a*-OSN with its dendrite pulled out from the  
 1029 sensillum socket and directly exposed to the bath perfusion (Dendrite-out)<sup>25</sup>, which  
 1030 eliminates ephaptic inhibition from the neighboring OSN dendrites in the same  
 1031 sensillum<sup>26</sup>. Timing of the odor stimulation is indicated at the bottom. Odor concentration:  
 1032 10 mM acetophenone. (b) Cell-attached recordings from *OR85a*-OSNs in the flies with  
 1033 *Orco* restored in *Orco*<sup>-/-</sup> background. Top, recording from an *Or85a*-OSN in the  
 1034 Dendrite-in configuration. Bottom, recording from an *Or85a*-OSN in the Dendrite-out  
 1035 configuration. Odor concentration: 10 mM acetophenone. (c) No change in basal current  
 1036 by temperature in *Or85a*-OSNs of *Orco*<sup>-/-</sup> flies. Temperature changes are indicated above  
 1037 the recording trace. (d) Collective data for the basal current at different temperatures in  
 1038 *Or85a*-OSNs of *Orco*<sup>-/-</sup> flies, with average indicated in black dots. (e) The collective data

1039 of temperature-dependence of the basal current in *Or85a*-OSNs of WT flies, with average  
1040 indicated in black dots. (f) Loss of spontaneous activity and odor-evoked responses in  
1041 *Or85a*-OSNs of *Orco*<sup>-/-</sup> flies. Under the cell-attached configuration, strong odor  
1042 stimulation with E-3 (10 mM, 5 s) did not trigger any action-potential firing. (g) The  
1043 dependence of odor-evoked depolarization on the presence of *Orco*. Under the current  
1044 clamp, odor stimulation as in (a) did not induce any depolarization in the perforated-  
1045 patch-clamped *Or85a*-OSNs of the *Orco*<sup>-/-</sup> flies. Injections of inward currents (3 and 5 pA;  
1046 5 s) depolarizes the OSN and triggers bursts of firing, indicating that the OSN is  
1047 electrically excitable.  
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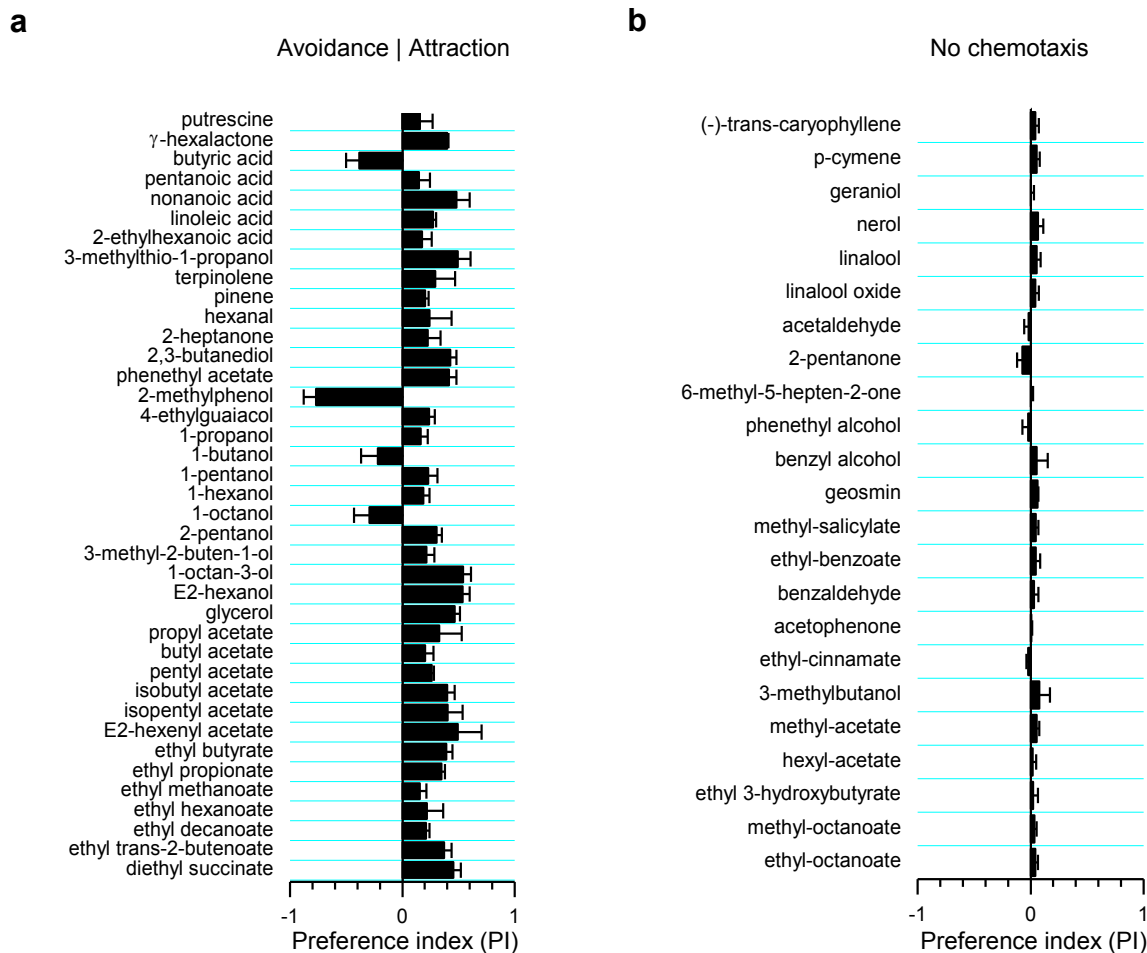
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## Supplementary Figure 2

### ATP and light-induced excitatory responses in *Or85a*-OSNs

(a) Under the cell-attached configuration, *Or85a*-OSNs expressing the ATP-gated P2X<sub>2</sub> cation channel were stimulated by acetophenone (20 mM, 3 s), ATP (1 mM, 2 s), and E-3 (1 mM, 35 ms). (b) Spike firing induced by both odor and light in *Or85a*-OSNs. Under the cell-attached configuration, the OSN expressing ChR2 was stimulated by E-3 (1 mM, 35 ms), acetophenone (20 mM, 5 s), and 470 nm light (5 s). The timing of odor and light stimulation is indicated at the bottom. (c) Acetophenone inhibited the basal current but not the ChR2-induced current. Inset, overlap of the acetophenone-induced outward currents in the absence or presence of light-induced inward current. The timing of the application of acetophenone and 470-nm light (10 ms) is indicated on the top.

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1065 **Supplementary Figure 3**

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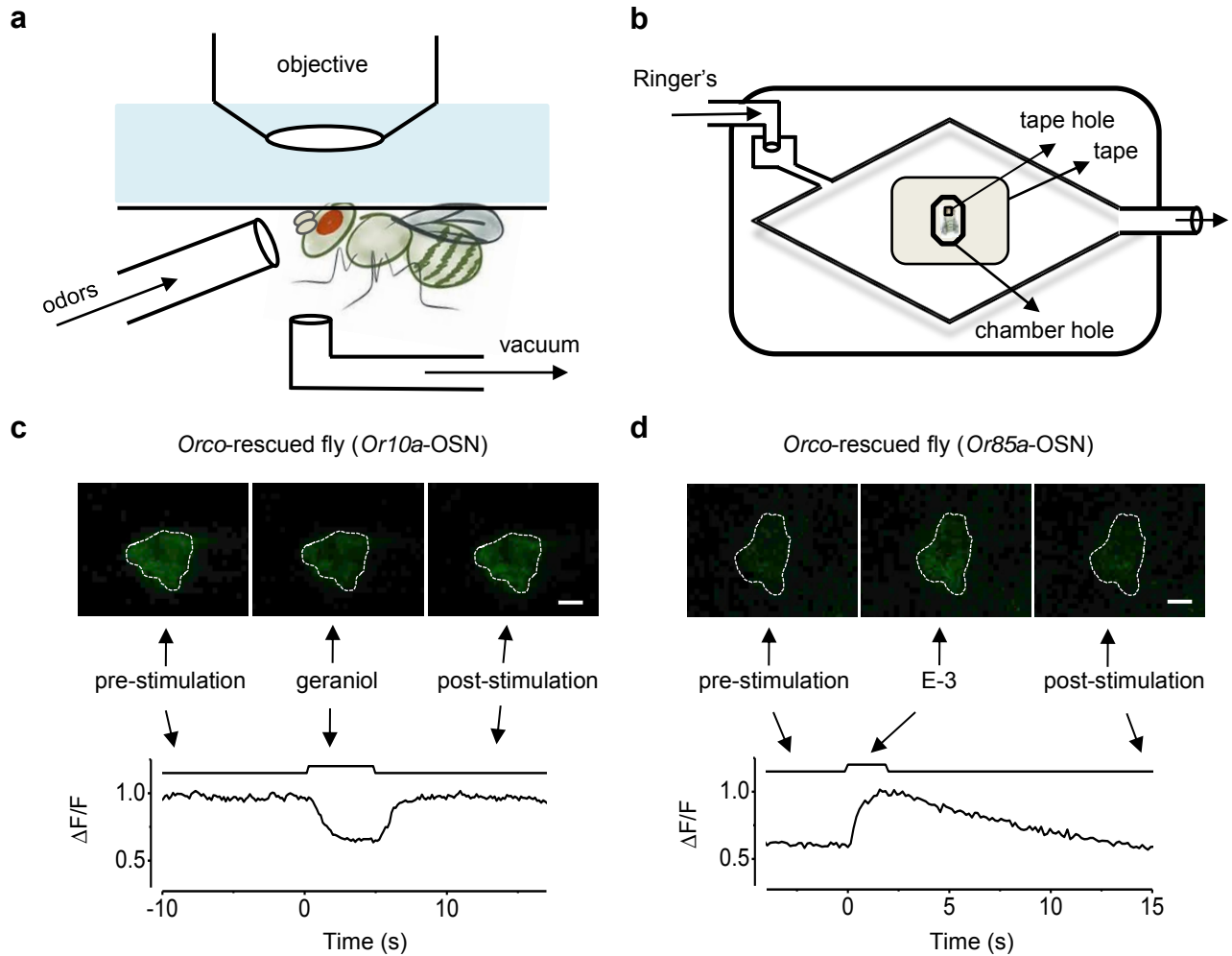
1067 **Chemotaxis of *Orco*<sup>-/-</sup> flies to odors**

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1069 (a) Avoidance and attraction by different odors, which are probably mediated by other  
 1070 chemoreceptors, such as ionotropic receptors and gustatory receptors. Odors as indicated  
 1071 (dissolved in mineral oil or water). (b) Odors did not trigger obvious chemotaxis in  
 1072 *Orco*<sup>-/-</sup> flies.

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1076 **Supplementary Figure 4**

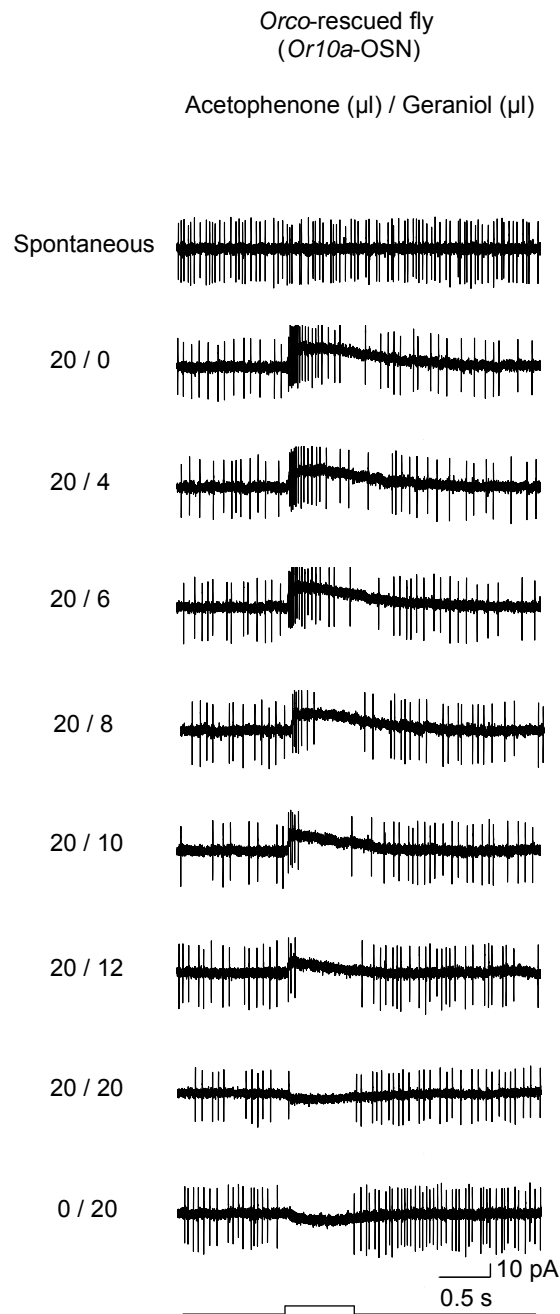
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1078 **Calcium imaging of the antennal lobe in a live fly**

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1080 (a) Schematic of the calcium imaging and odor stimulation. (b) Schematic of the  
1081 recording chamber. (c) Odor-evoked inhibitory responses. (d) Odor-evoked excitatory  
1082 responses. Scale bar: 10  $\mu$ m.

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**Supplementary Figure 5**

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**Spike firing of *Or10a*-OSNs in response to odor mixtures**

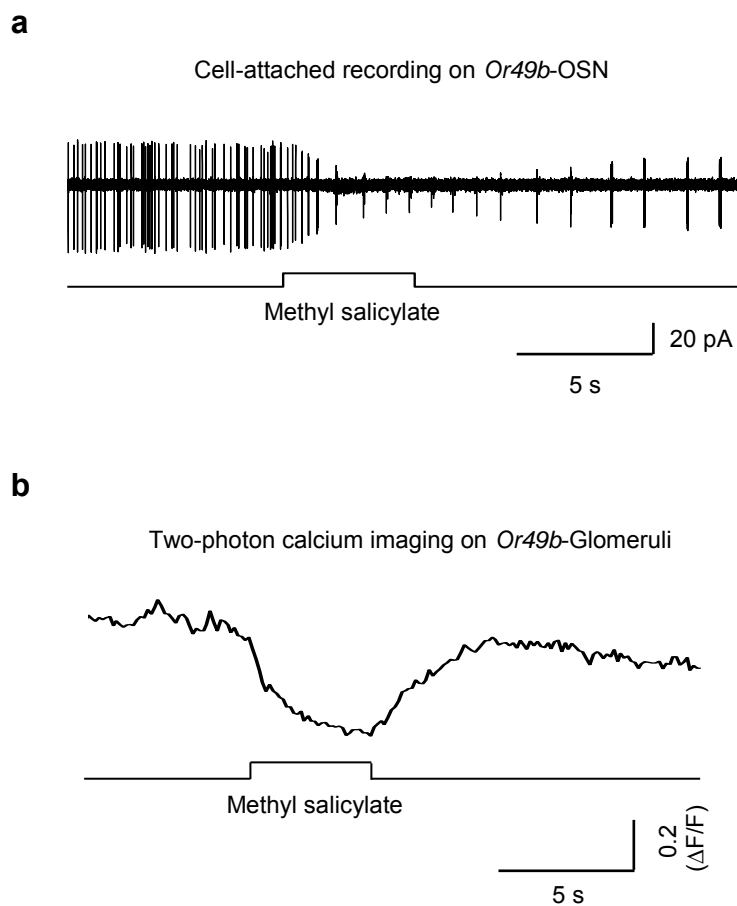
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1089 Single-sensillum recordings were performed on ab1 sensilla of *Orco*<sup>-/-</sup> flies with *Orco*1090 restored to *Or10a*-OSN. Odor stimulations were mixtures of acetophenone ( $10^{-2}$  dilution)1091 and geraniol ( $10^{-2}$  dilution) as indicated. Note: the odor stimulation would be further

1092 diluted before reaching the recorded OSNs; these results thus do not directly match the

1093 corresponding behavioral conditions.

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**Supplementary Figure 6**

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**Methyl salicylate-evoked inhibitory responses in *Or49b*-OSNs**

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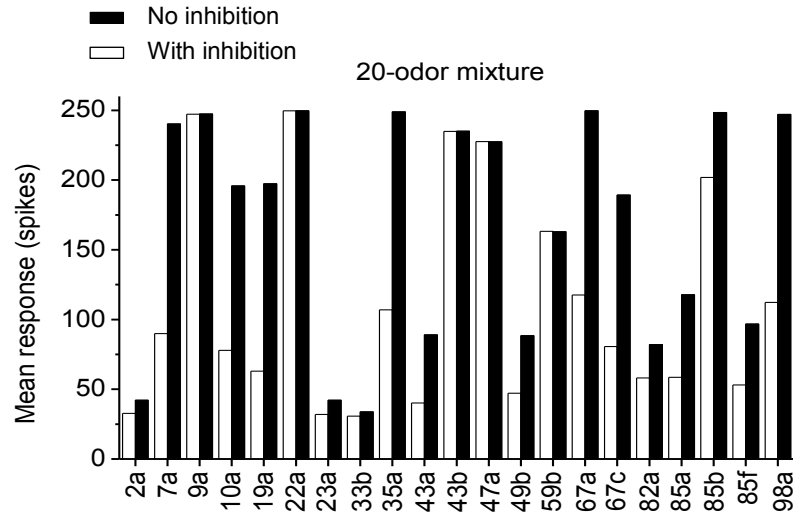
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(a) Cell-attached recordings on *Or49b*-OSN. Odor stimulation: 3 mM methyl salicylate, applied for 5 s. (b) Two-photon calcium imaging on the glomeruli labeled by GCaMP6m expressing in *Or49b*-OSNs.

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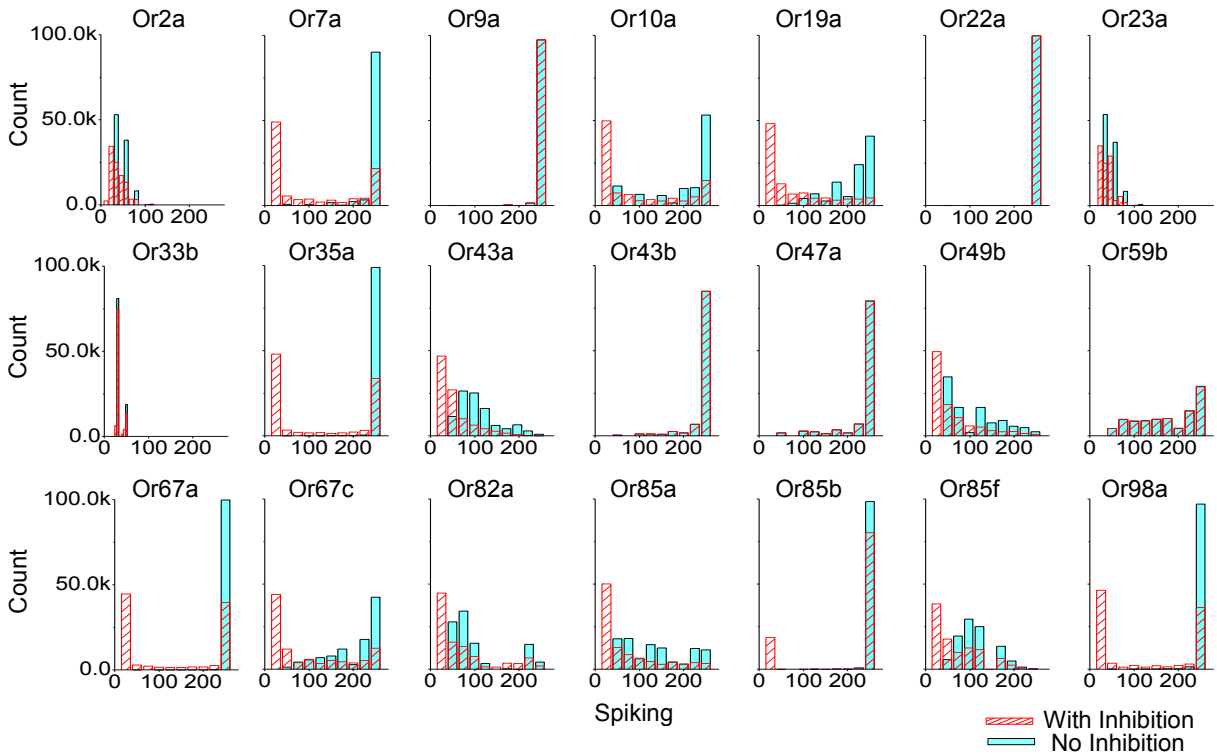
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**Supplementary Figure 7**

**Reducing response saturation by inhibition**

The average activity (spiking rate) of each OR/OSN in response to odor mixtures containing 20 odors. The random-sampling method with a total of 100,000 odor mixtures was used. The inclusion of inhibition reduces the response saturation of many ORs/OSNs.

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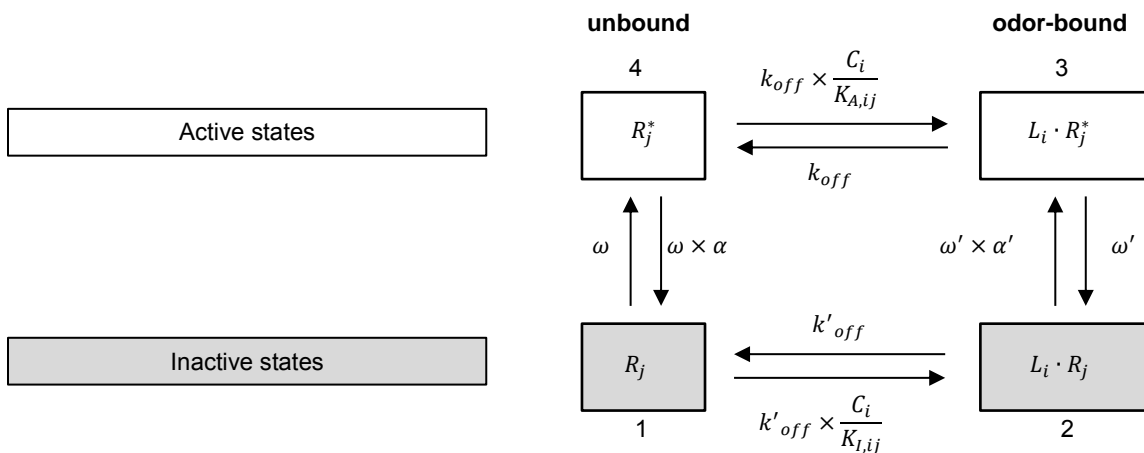
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### Supplementary Figure 8

#### Decorrelation of odor responses by inhibition

Including the inhibitory response avoids saturation and makes the response more uniform.  
The number of odors in the mixture was 20. The sample size was 100,000 odor mixtures.

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1133 **Supplementary Figure 9**

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1135 **Illustration of the different microscopic states of ORs**

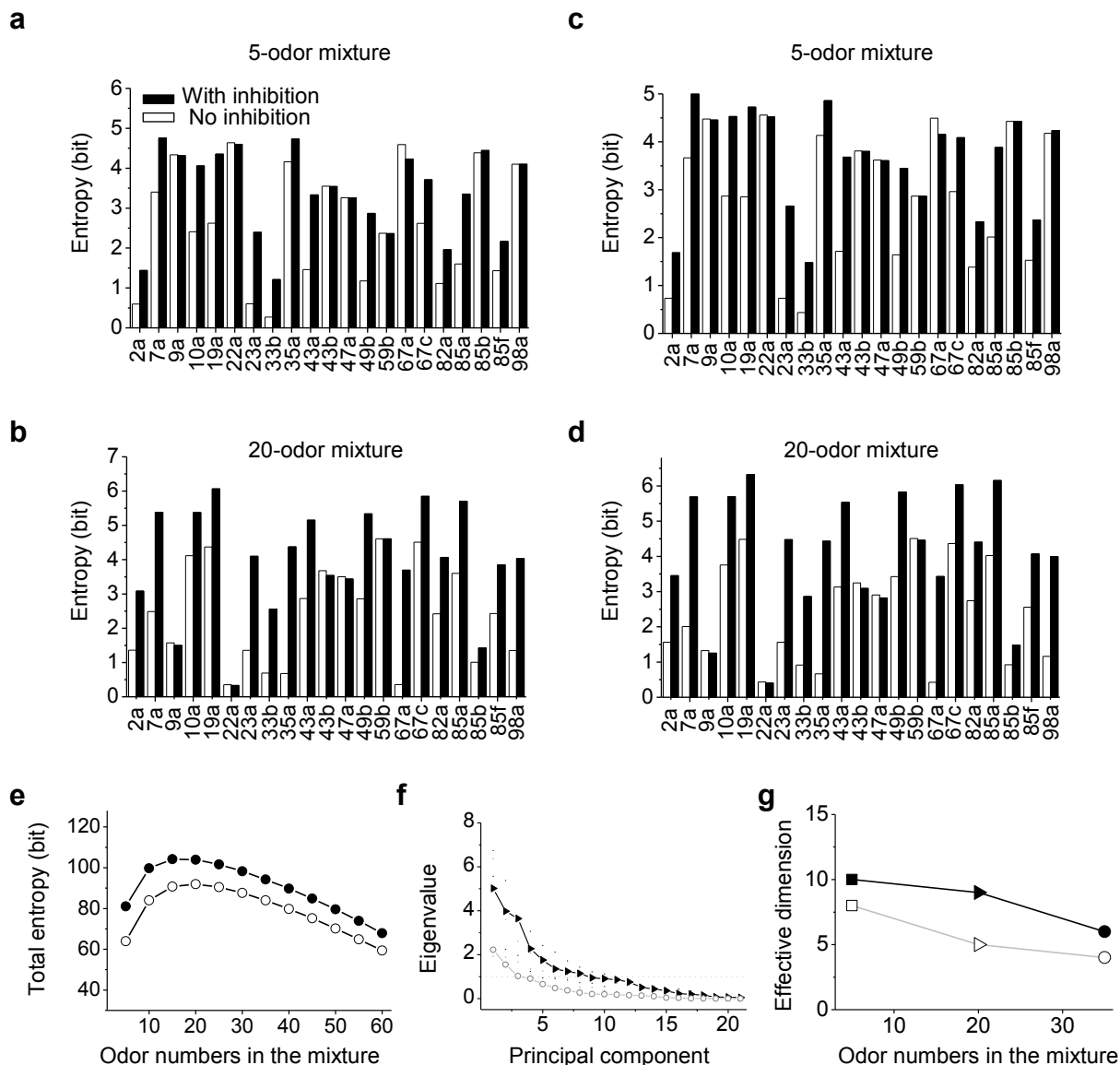
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1137 The active and inactive forms of the OR/OSN<sub>j</sub> are represented by  $R_j^*$  and  $R_j$  respectively.

1138 The transition rates are given next to the corresponding arrows. The odor is represented

1139 by  $L_i$ ,  $k_{off}$ ,  $k'_{off}$ ,  $\omega$ ,  $\omega'$  are kinetic rates,  $\alpha'$  is a ratio of kinetic rates.

1140



1141 **Supplementary Figure 10**

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1143 **Including inhibition increased the information entropy of each OR/OSN**

1144

1145 (a) The entropy was computed for 100,000 randomly sampled mixtures containing 5  
 1146 odors. (b) The entropy was computed for 100,000 randomly sampled mixtures containing  
 1147 20 odors. (c) The entropy was computed with the enumeration method for mixtures  
 1148 containing 5 odors. (d) The entropy was computed with the enumeration method for  
 1149 mixtures containing 20 odors. (e) The entropy was calculated for mixtures containing  
 1150 different numbers of odors with competitive binding using the enumeration method.  
 1151 Including inhibition increased the total entropy. (f) The eigenvalues of principal  
 1152 components increase by including inhibition. The mixture contained 20 odors. (g) The  
 1153 effective coding dimension, defined as the number of principal components whose  
 1154 eigenvalues are larger than 1 (dashed horizontal line in f).

1155 **Supplementary Table 1**1156 **Properties of acetophenone-evoked inhibition of basal activities and E-3-evoked**1157 **excitatory responses in *Or85a*-OSNs**

Odor stimuli	$R_{max}$ (pA)	$t_{peak}$ (ms)	$K_{1/2}$ (mM)
Acetophenone	18.2 ± 2.2 (n = 42)	275 ± 25 (n = 4)	35 ± 11 (n = 4)
E-3	92.6 ± 24.4 (n = 5)	192 ± 26 (n = 5)	1.1 ± 0.1 (n = 5)
E-3 (in the presence of 7.5 mM acetophenone)	92.8 ± 24.6 (n = 5)	192 ± 26 (n = 5)	26.8 ± 12.5 (n = 5)

1158  $T_{peak}$ , or time to peak, is defined as the time duration from odor arrival to the transient

1159 peak of odor-evoked responses.

1160