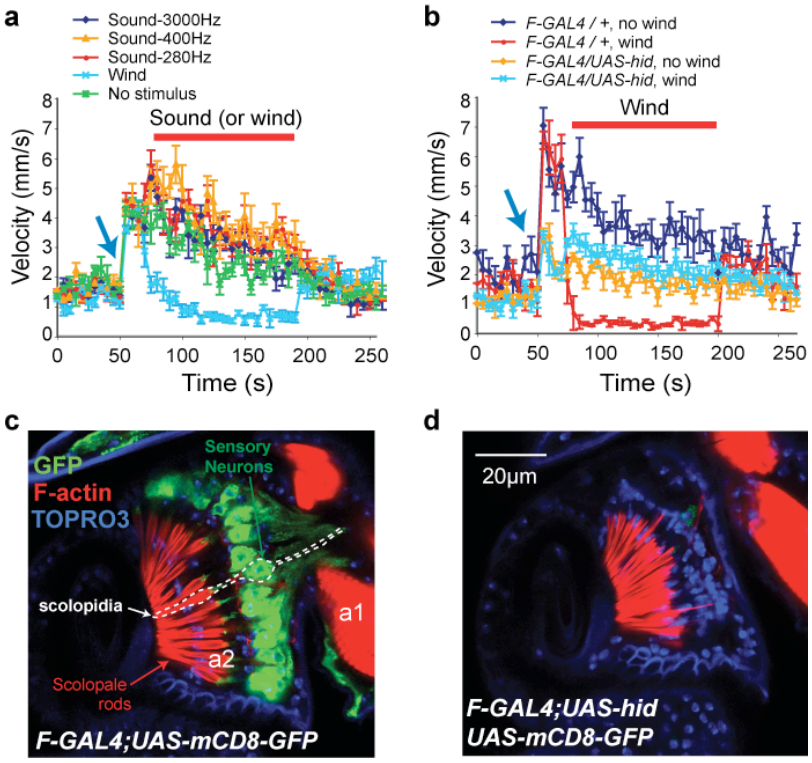
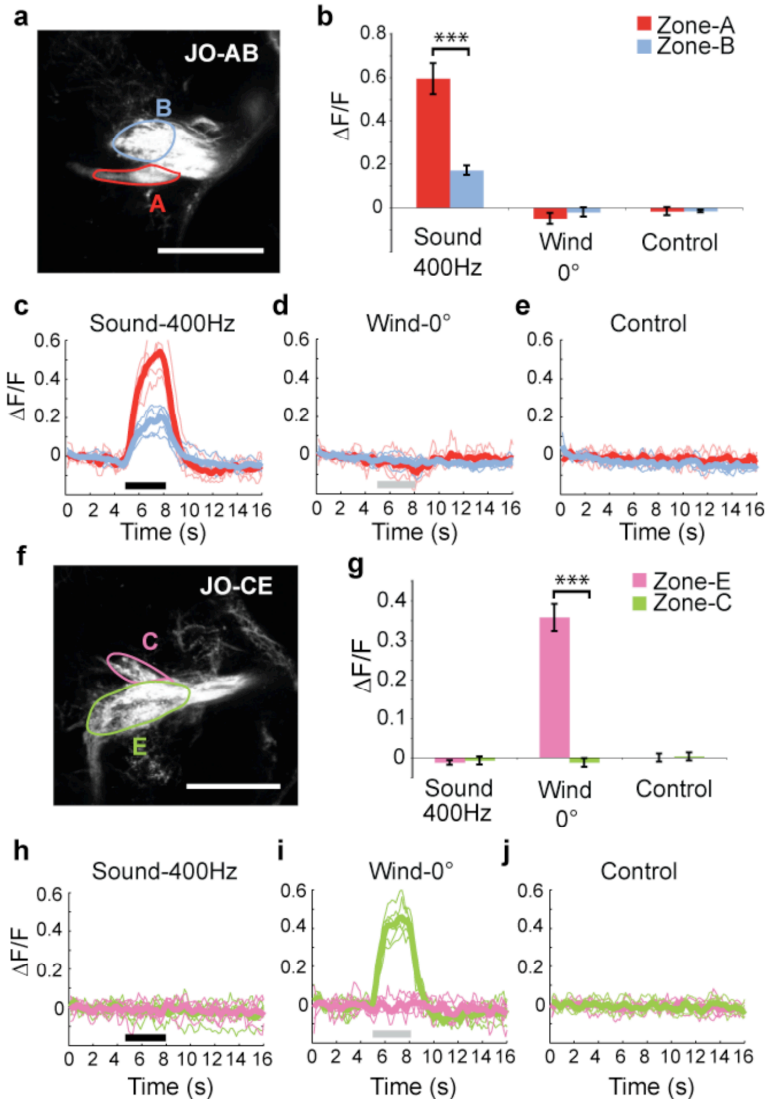


Supplemental Figure S1. WISL is not elicited by courtship song and is dependent on chordotonal mechanosensory neurons.

(a) Average locomotor velocity vs. time plots for wild-type CS flies exposed to recordings of peak frequency-modified (see Supplemental Methods) pulse song derived from *D. melanogaster* courtship song[23], presented at 90 dB (SPL) at the antenna. Blue arrows indicate brief mechanical startle; red line indicates duration of wind or sound exposure. Note that only wind causes locomotor suppression (light blue curve). "Wind" vs. "No stimulus" curves, p=0.0002 (Kruskal-Wallis ANOVA); p=0.0013 for comparison

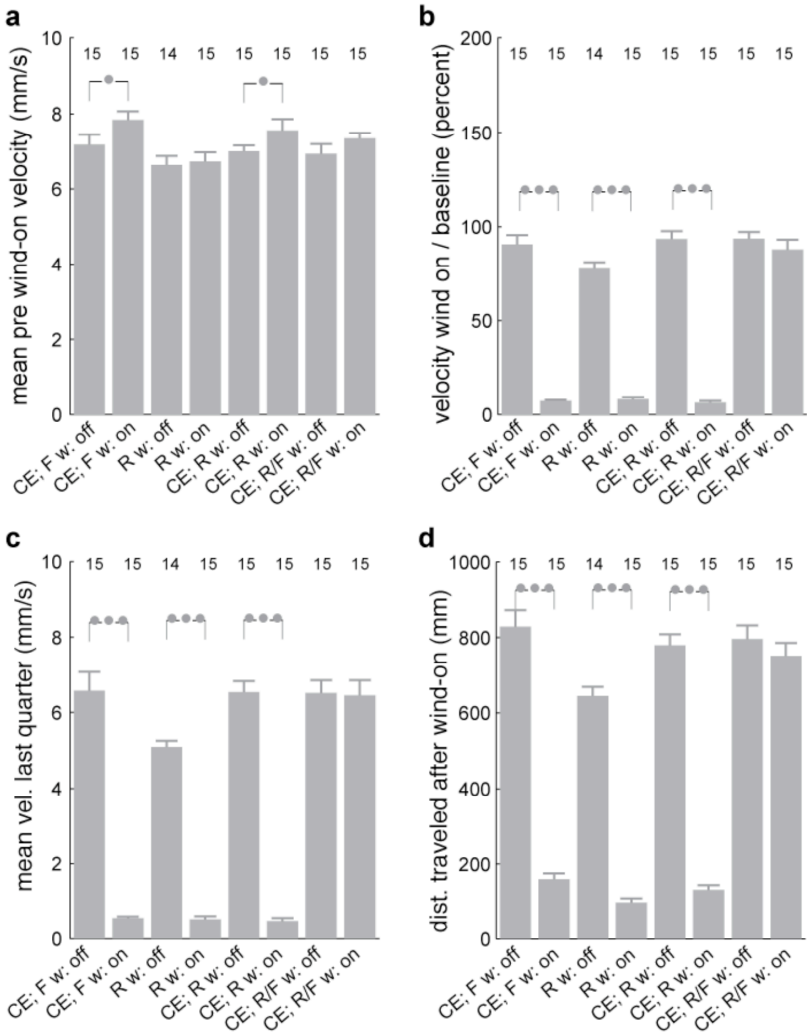
of average velocity during the wind ON period of "Wind" vs. "No stimulus" conditions (Mann-Whitney U-test). Data are mean ± SEM, n = 10 (20 flies/assay). (b) WISL behavior is lost in flies in which JO neurons are killed using *nanchung (F)-Gal4* and *UAS-hid.* The light blue (wind) and orange (no wind) curves of *F-Gal4; UAS-hid* flies are not significantly different ($p>0.05$, Kruskal-Wallis ANOVA). Control *F-Gal4/+* flies show a clear WISL effect: wind vs. no wind, $p<0.0001$ (Kruskal-Wallis ANOVA); $p=0.0006$ for comparison of average velocity during the "Wind ON" period of "no wind" vs "wind" conditions (Mann-Whitney U-test). Control *UAS-hid/+* flies also show a robust WISL effect; $p<0.0001$ (Kruskal-Wallis ANOVA; data not shown). (c, d) Confirmation that JO neurons in the antenna are ablated by *F-Gal4; UAS-hid.* (c) Control flies with *F-Gal4; UAS-mCD8GFP.* A scolopidium, the sensory organ unit of JO, is outlined (white dashed lines). Sensory neurons are green (GFP^+). Scolopale rods are labeled by rhodamine F-actin (red). TOPRO3 is a nuclear counter-stain. (d) Similar view of JO from *F-Gal4; UAS-hid/UAS-mCD8GFP* flies. Note loss of sensory neurons. The scolopale rods remain because they are synthesized by scolopale cells which do not express the *F-Gal4* driver and are therefore spared. Scale bar, 20 μm.



Supplemental Figure S2. Different subpopulations of JONs are activated by wind and sound.

Flies were exposed to peak frequency-modified pulse song (see Supplementary Methods) at 400 Hz and 90 dB (as well as at 75 and 100 dB; not shown), or to wind (0.9 m/sec; also 0.01m/s, data not shown) delivered from the anterior (0^o), in flies expressing GCaMP in either zones A and B (a-e), or in zones C and E (f-j). Zones A and B were activated by sound (b, c) but not wind (b, d), while zone E was activated by wind (g, i)

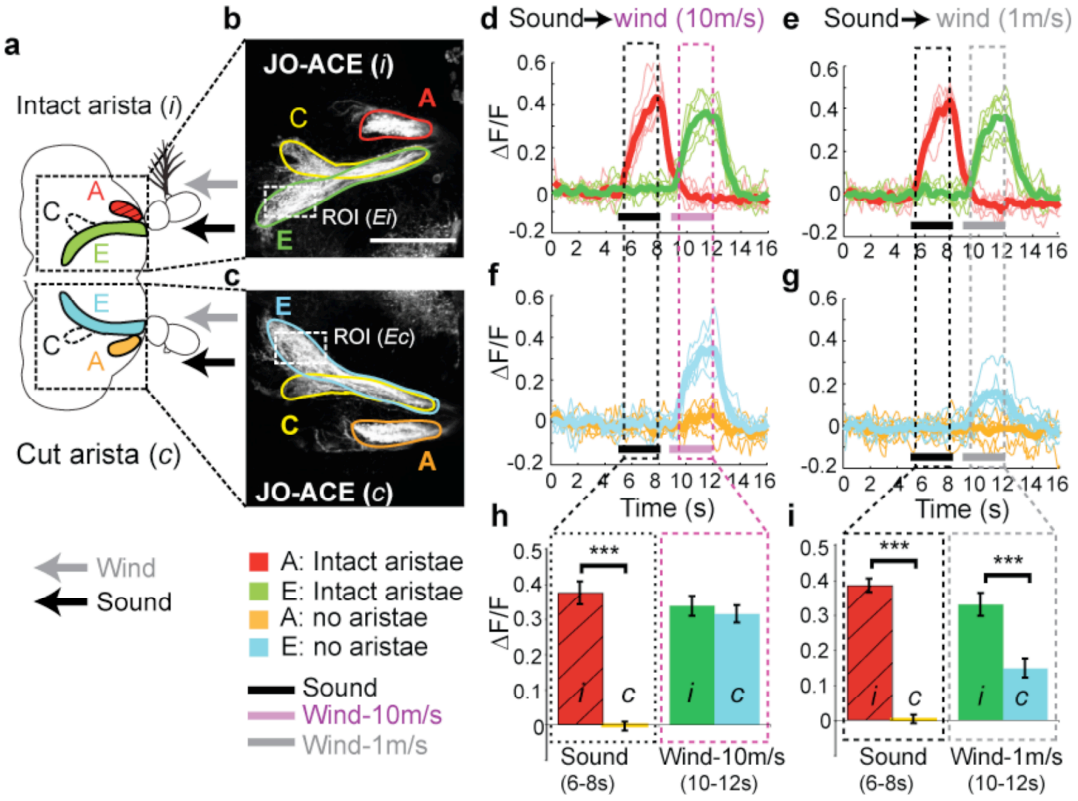
but not by sound (g, h). Zone C was also activated by wind, but only if presented from the posterior (180^{o}; see Fig. 4). Data are mean ± SEM, n=6 experiments. Scale bars, 50μm. ***, $p<0.001$ (Repeated measure ANOVA and Bonferroni multiple comparisons).



Supplemental Figure S3. Quantification of WISL behavior in flies lacking JO-CE neurons.

The parameters shown are calculated from the data in Fig. 3 (d-g). (a) Mean velocity prior to wind exposure. (b) Mean velocity during wind exposure, as a percentage of pre-wind baseline velocity. (c) Mean velocity during the last quarter of the wind exposure period. (d) Distance traveled following the onset of wind exposure. •, $p<0.05$; •••, $p<.001$ (Kruskal-Wallis and Mann-Whitney U-test). Numbers above the bars indicate number of assays performed for each condition (20 flies/assay). Genotype

abbreviations: *"CE; F" = JO-CEGal4; eye-FLP; "R" = UAS-FRT-STOP-FRT-Ricin; "CE; R" = JO-CEGal4; UAS-FRT-STOP-FRT-Ricin; "CE; R/F" = JO-CEGal4; UAS-FRT-STOP-FRT-Ricin/eye-FLP.* "w: off" vs. "w: on" indicate no wind applied during the experiment, vs. wind-applied during the interval indicated by the gray bar in Fig. 3 d-g, for each genotype.



Supplemental Figure S4. Effect of arista ablation on wind and sound sensitivity.

(a) Schematic illustrating arista ablation experiment: The arista was ablated on one side and the contralateral arista was left intact and served as an internal control. (b,c) Expression of UAS-mCD8-GFP in zones A, C, and E on the arista-intact side (b) and arista-ablated side (c). ROI, region of interest for ΔF/F measurements in zone E, on either the arista-intact ("*Ei*") or arista-cut ("*Ec*") hemi-brains. Scale bar, 50μm. (d, f, h) Responses to sound (red and orange traces in dashed black box) and 10m/sec wind (green and blue traces in dashed purple box) in control (d) vs. arista-ablated (f) hemi-brains. (h) Comparison of integrated ΔF/F values in zone A vs. zone E in intact ("*i*") vs. cut ("*c*") hemi-brains during the stimulus periods (dashed black and purple box). Zone C is not activated because the wind is delivered from the front (0^o). ***, $p<0.001$ (Repeated

measure ANOVA and Bonferroni multiple comparisons). (e,g,i) As in (d, f, h), except that wind was delivered at 1 m/s. Note that detection of airflow is less sensitive than that of sound to removal of the aristae. This could be because high velocity airflow (10m/s) can still move a3 relative to a2 in the absence of an arista, thus activating the JO-CE neurons.

Supplementary Footnote S1

J.S. Johnston and colleagues have measured wind speeds in the habitats of several wild-caught *Drosophila* species[1, 2] including both tropical and desert environments. In Kamuela, Hawaii, for example, *D. mercatorum* and *D. hydei* inhabit environments where trade winds blow in the range of 5-25 km/hr (1.4 m/s – 6.9 m/s), with an average velocity of 15 km/hr (4.17 m/sec); gusts over 35 km/hr (9.72 m/s) are not uncommon[1]. Wind speeds in the range of 0.46 – 4.64 m/s have been measured in the Arizona desert, the habitat of *D. nigrospiracula* [2]. We observed wind-induced suppression of walking in *D. melanogaster* between 0.7-1.6 m/s; these velocities are therefore well within the range of wind speeds measured in several *Drosophila* natural habitats. Wild-caught *D. mercatorum* and *D. hydei* exhibited locomotor arrest in the laboratory at wind speeds of 10 km/hr (2.8 m/sec) and greater[1], while wild-caught *D. mimica*, another Hawaiian species, exhibited locomotor arrest at air speeds between 6 and 7 km/hr (1.67 – 1.94 m/s)[4]. Anecdotal evidence that wind suppresses *Drosophila* locomotor activity in the wild derives from the observation that during occasional days in Hawaii when the trade winds stop, called "Kona" weather[1], *Drosophila* in flight are abundant, while during the trade winds very few *Drosophila* are observed in flight because most of them are immobilized on their *Opuntia* substrate (J.S. Johnston, personal communication). These data suggest that WISL is a naturally occurring behavior exhibited by *Drosophila* at wind speeds normally encountered in their wild ecological habitat. Johnston and colleagues speculate that this behavior may be the dominant environmental influence (rather than, e.g., temperature and humidity) affecting the dispersal of wild *Drosophila* populations,

and thereby an important determinant of their "genoclines," geographic gradients in gene frequencies[1, 4].

Supplementary Footnote S2

Our detection of distinct sound- and wind-evoked spiking responses in antennal nerve electrophysiological recordings (Fig. 1e-l) argues that the differential activation of sound- vs. wind-sensitive axons observed by GCaMP imaging is unlikely to be explained by local circuit interactions within the AMMC. Electrophysiological recordings from sound-selective locations in the antennal nerve usually revealed 1 or 2 spikes at the onset and offset of the wind stimulus (Fig. 1i, j). These brief spiking responses probably reflect the fact that phasically responsive JO neurons can be transiently activated by deflections of the aristae caused by wind (see Fig. 5).

Supplementary Footnote S3

Our calcium imaging experiments indicate that arista displacements triggered by the mechanical probe activate both wind- and sound-sensitive neurons (Fig. 5m, n), while the natural stimuli (wind and sound) activate these neurons in a mutually exclusive manner (Fig. 5f, g). Wind-sensitive neurons may not be activated by sound stimuli, because the magnitude of the antennal displacements produced by courtship song may be too small to evoke a detectable response (see also Supplementary Footnote S4). This hypothesis is supported by the fact that short-distance mechanical displacements of the aristae activate sound- but not wind-sensitive neurons (Fig. 5e, 0.01mm). Why, then, are sound-sensitive neurons not also activated by wind? In fact, our electrophysiological data indicate that they *are* activated, albeit very transiently: brief spiking responses are observed in sound-sensitive JO neurons at the onset and offset of the wind stimulus (Fig. 1j, blue traces). These transient spiking epochs are unlikely to produce sufficient accumulations of intracellular calcium to yield detectable GCaMP signals[24]. In contrast, the GCaMP signals elicited in sound-sensitive JO neurons by controlled mechanical displacements (Fig 5l-n, red lines) may reflect more extended spiking responses caused by damped oscillatory vibrations of the probe as it pushes against the arista. Finally, it is possible that wind- and sound-selective neurons differ in their sensitivity to the position, velocity or acceleration of the arista displacement caused by these different stimuli, as shown for limb chordotonal organs in the stick insect[25, 26]. The mechanical probe may not faithfully mimic these natural stimulus-specific differences in arista displacements.

Supplementary Footnote S4

We did not observe activation of zone C/E JO neurons by arista displacements below ~20 μm, while Kamikouchi et al.[22] observed activation with deflections as small as 1 μm, an estimated magnitude of the extent of deflection of the aristae that could be caused by the earth's gravitational field acting on the mass of the antenna. This difference is probably due to differences in the calcium imaging methods used in the two studies. Our approach measures activity in JO neuron axon terminals (using a GCaMP-1.3), which most likely reflects influx of extracellular Ca^{2+} due to spike firing. In contrast, Kamikouchi et al.[22] measure activity in JO cell bodies (using Cam2.1), which may reflect both Ca^{2+} influx and release from intracellular stores. In addition, the kinetics of the decay of Cam2.1 signal in response to transient Ca^{2+} increases is much slower (~2,000 ms) than that of GCaMP-1.3 (330 ms)[27], so that the method employed by Kamikouchi et al.[22] might integrates small changes in $[Ca^{2+}]_{in}$ and extend Cam2.1 responses over a longer period of time from the onset of the stimulus, than would GCaMP-1.3.

SUPPLEMENTARY MOVIE LEGENDS

Supplementary Movie 1

WISL behavior in *Drosophila melanogaster*. The movie shows the flies' baseline locomotor activity, followed by a brief mechanical startle, and finally wind-induced suppression of locomotion. The onset of mechanical startle and the onset and duration of wind treatment are indicated during the movie footage. 2x normal speed. (QuickTime (880KB).

Supplementary Movie 2 (a-e)

These movies show the responses of JO neurons to sound and wind stimuli in zones A, C and E, using the Gal4 line JO-ACE to drive expression of UAS-GCaMP. All movies are pseudo-colored to illustrate the magnitude of the calcium responses (ΔF) (red, green, and blue indicates the maximum, intermediate, and minimum, respectively). All movies are 3x normal speed. (a) Response of zone A neurons during modified courtship song presentation. Note that the zones C and E show no responses to song. (b) Response of zone E neurons during wind (0^o) stimulation. Zone C is not active because it responds to wind from 180^o (see Supplementary movie 3). Note that zone A is not activated during wind stimulation. (c) Response of zone A and E neurons to sequential presentation of courtship song and wind (0^o), respectively. (d) Response of zone A and E neurons to simultaneous presentation of courtship song and wind. (e) Control (neither courtship song nor wind presented). QuickTime (1.2MB/ each).

Supplementary Movie 3 (a-e)

These movies show the responses of zone C and E neurons to wind delivered from different directions, using the Gal4 line JO-CE to drive expression of GCaMP. See Supplementary Movie 2 legend for details. (a) Response during wind (0^o) presentation. Note that zone E is activated in both hemi-brains. (b) Response during wind (45^o) presentation. Zone E is activated in both hemi-brains, similar to the wind (0^o) responses. (c) Response during wind (180^o) presentation. Note that zone C is activated in both hemi-brains. (d) Response during wind (90^o) presentation. Note that zone C and E neurons are activated in the ipsi- and contra-lateral sides, respectively. (e) No stimulus control. QuickTime (2.7MB/ each).

Supplementary Movie 4 (a-e)

These movies were made to show the direction of aristae deflection during the presentation of wind stimuli, under the same conditions used to obtain the data illustrated in Supplementary Movie 3. The movies are played at actual (real-time) speed. (a) Both aristae move anteriorly when wind is presented from the posterior (180^o). (b) Both aristae move posteriorly when wind is presented from the front (0^o). (c) Both aristae move posteriorly when wind is presented at a 45^o angle. (d) The aristae move anteriorly on the hemi-brain ipsi-lateral to the stimulus and posteriorly on the contra-lateral side, when wind is presented at a 90^o angle (in this case from the right). (e) Control condition in which no wind is presented. QuickTime (450KB/each).