Supplementary Information

Investigation of directional sensitivity of scolopidial neurons

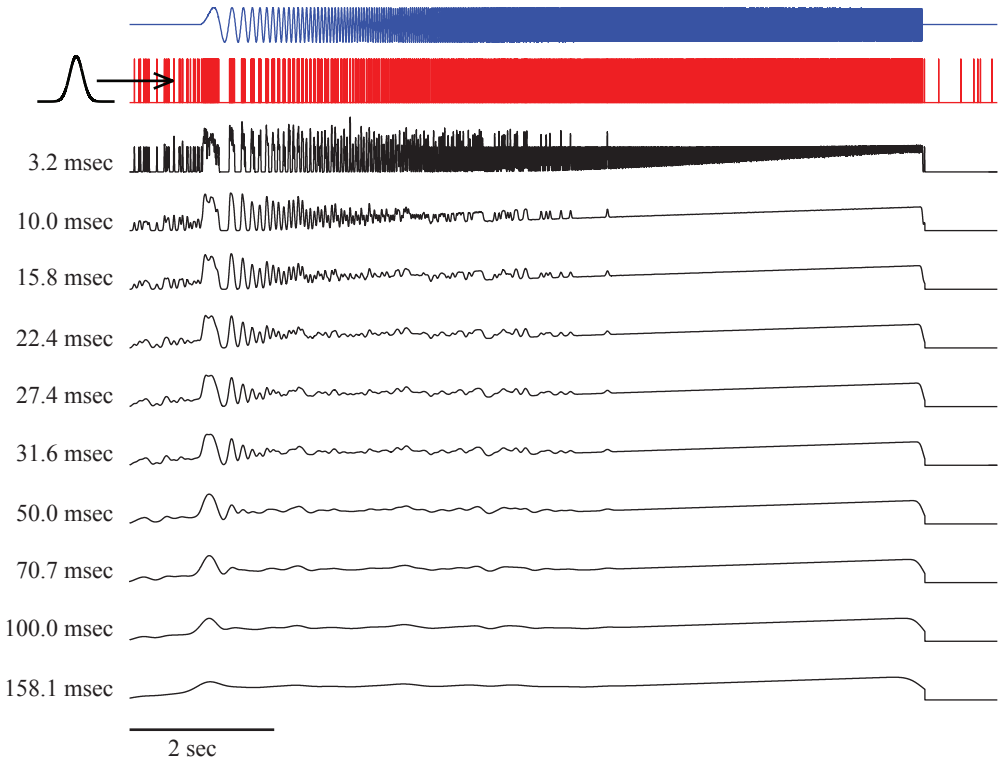
In the experiments described in the paper, the antennal oscillations occurred in a single plane unlike the typical natural motions of the antennae. In a few additional experiments, we used a smaller stimulation apparatus to oscillate the flagellum-pedicellar joints sequentially in both horizontal and vertical planes using sinusoidal stimuli (supplementary material Fig. S3A, black traces). While recording from a neuron, we first applied the stimulus in the dorsal/ventral (vertical) direction, and then rotated the apparatus by 90° and used the same stimulus in the rostral-caudal (horizontal) alignment. The GCFR amplitude modulation (supplementary material Fig. S3A, mean and standard deviation from the PSTH of 16 repeats displayed under) of a scolopidial neuron in response to 2 Hz sinusoidal oscillations showed a cell which was strongly responsive to the antenna moving towards the ventral direction (supplementary material Fig. S3A; red). The same neurons showed a much weaker response when the flagellum oscillated on the horizontal plane (supplementary material Fig. S3A, blue).

In another case, a scolopidial neuron from a different individual that responded to a sinusoidal frequency-sweep increasing linearly from 0 to 100 Hz in 10 sec show a robust response in the vertical direction with an increase in the GCFR that mimics the stimulus frequency (supplementary material Fig. S3B, red, especially above 60 Hz) whereas the response in the horizontal direction was much less correlated (supplementary material Fig. S3B, blue). In this setup, higher frequencies caused artifacts on both planes of oscillations. This could explain the response increase to the sweep at higher frequencies for the non-preferred direction. This selectivity for the vertical plane of oscillations was due to the way we designed the experiments by selecting neurons tuned in this orientation during our exploration phase (see methods). Although preliminary, these two examples nevertheless demonstrate that scolopidial neurons can be sensitive to the plane of antennal oscillations, and thus the Johnston's organ could map the motion of the flagellum in two-dimensional space. A more elaborate study is needed to map out the response of the neurons to precise angular ranges and frequencies of antennal motion.

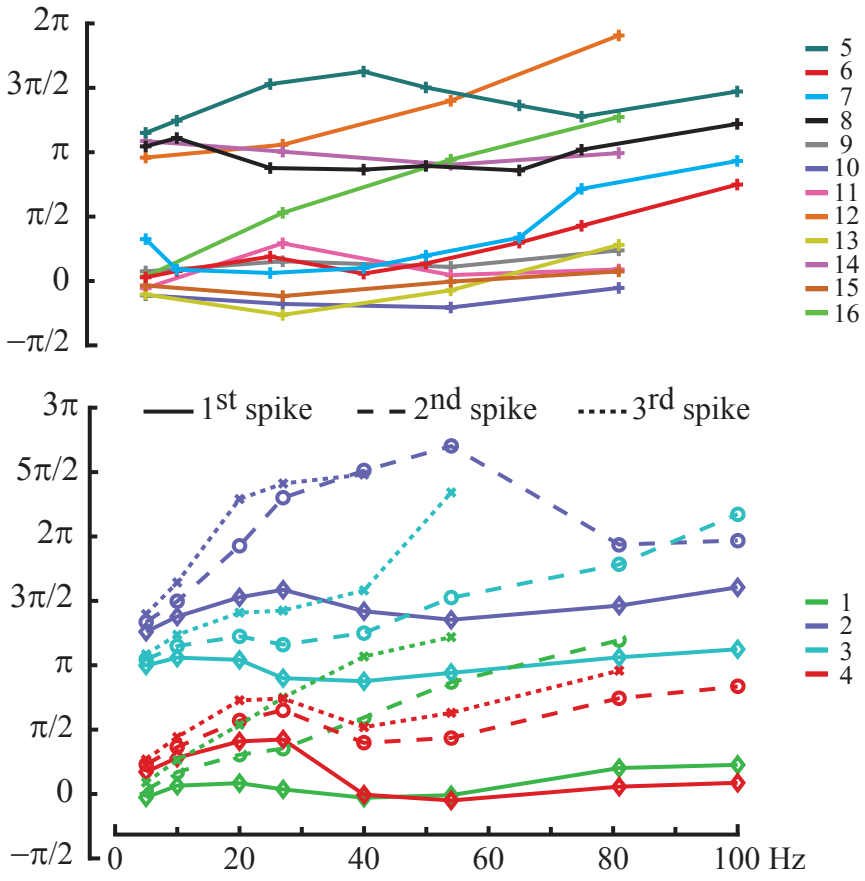
Fig. S1. Effect of the size of the Gaussian window on the computed Gaussian convolved firing rate (GCFR). We computed 10 GCFRs from the spike timings of a scolopidial neuron in response to a frequency-sweep from 0-100Hz increasing linearly in 10 sec. We varied the Gaussian window standard deviations from 3.2 to 158.1 ms. We have used 31.6 ms to compute the GCFR throughout the paper.

Fig. S2. The mean phase comparisons of 1st, 2nd and 3rd spikes in a burst response to mechanonsensory stimulus. The mean phase versus the sinusoidal motion frequency is plotted for 16 neurons (same cell numbers as Fig. 6A). The upper plot shows the mean phase of the first spike within a cycle of 12 cells while the lower plot shows the mean phase of the remaining 4 neurons for the first (solid lines), second (dashed lines), and third (dotted lines) spike within a cycle.

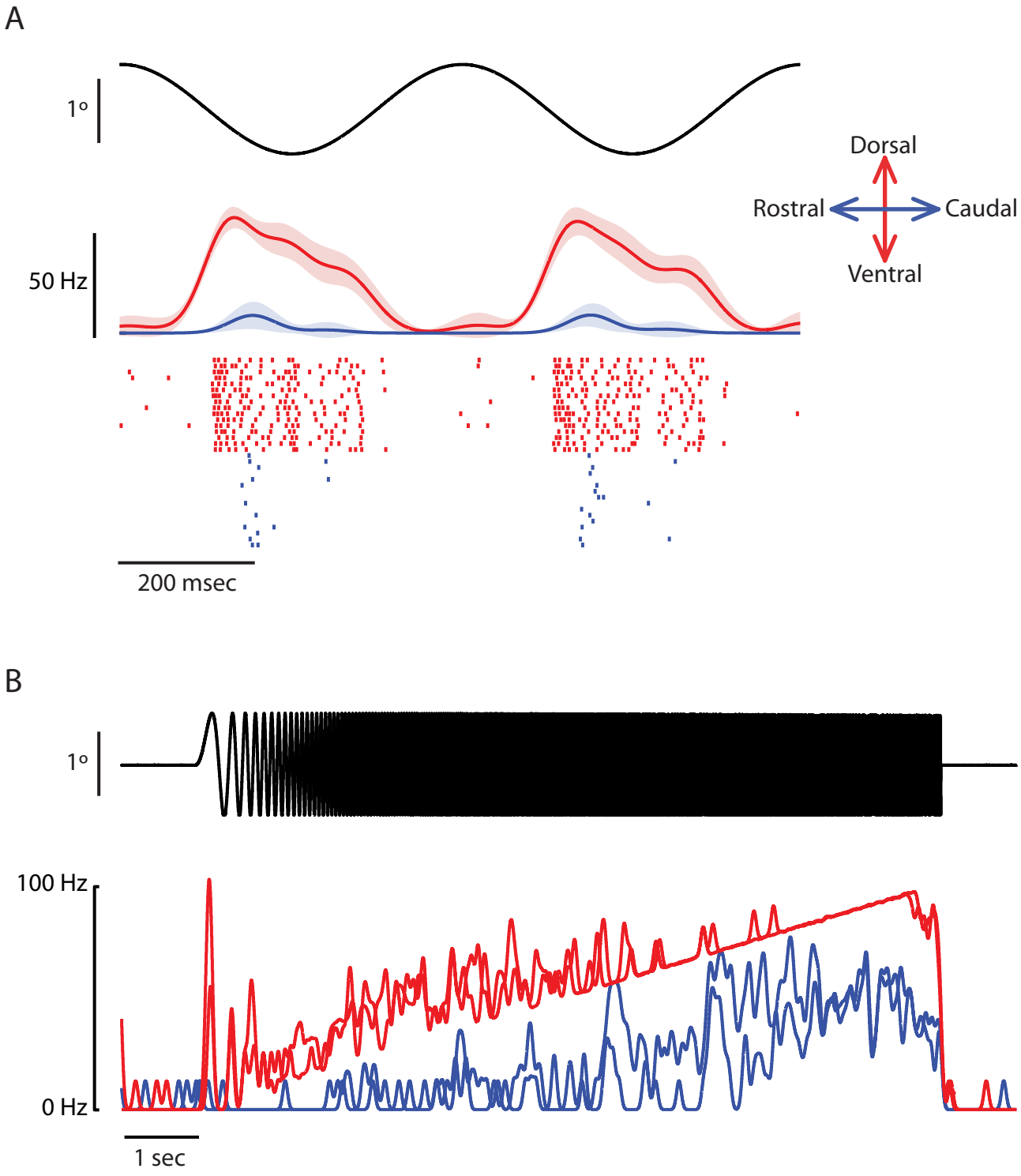
Fig. S3. Directional tuning of scolopidial neurons. (A) Response of a scolopidial neuron to a 2 Hz sinusoidal stimulus (black curve) applied to the left antenna along two orthogonal axis of motion. Red represents motion to the antenna applied along the dorsal/ventral (up/down arrow) axis and the blue for the rostral/caudal (left/right arrow) axis. The red and blue curves represent the average GCFRs (shaded areas are the standard deviations) computed from the 16 trials in each direction displayed beneath. Each line represents a trial and the tick marks the onset of the action potentials. (B) Response from another neuron to a sinusoidal frequency-sweep increasing linearly from 0 to 100 Hz in 10 sec (upper trace, black). We show the GCFR of two trials for the dorsal/ventral (red) and the rostral / caudal (blue) axes.



Supplementary Figure 1: Effect of the size of the gaussian window on the computed gaussian convolved firing rate (GCFR). We computed 10 GCFRs from the spike timings of a scolopidial neuron in response to a frequency sweep from 0-100Hz increasing linearly in 10 sec. We varied the gaussian window standard deviations from 3.2 to 158.1 msec. We used 31.6 msec to compute the GCFR throughout the paper.



Supplementary Figure 2: The mean phase versus the sinusoidal motion frequency is plotted for 16 neurons (same cell numbers as Figure 6A). The upper plot shows the mean phase of the first spike within a cycle of 12 cells while the lower plot shows the mean phase of the remaining 4 neurons for the first (solid lines), second (dashed lines), and third (dotted lines) spike within a cycle.


A
1°
50 Hz
Dorsal
Rostral
Caudal
Ventral
200 msec
B
1°
100 Hz
0 Hz
1 sec

Movie 1. 3D morphology of two scolopidial neurons in the Johnston's organ of the hawk moth, *Manduca sexta*. The neurons were iontophoretically filled with Lucifer yellow, imaged at 600X using a laser-scanning confocal microscope (Bio-Rad MRC 2000, Hercules, CA, USA), and reconstructed using NIH ImageJ software. The movie shows the soma of two neighbouring scolopidial neurons, with a cilium and its ciliary dilation. This ciliary extension connects the mechano-sensory scolopidium cell in the pedicel to the base of flagellum, and transduces minute deformations in pedicel-flagellum joint.