Supplementary Figure 1: Image analysis method to estimate wingbeat amplitudes. (a) Still frame from a movie captured by the video camera below the fly during an experiment. (b) Image analysis regions, each with 12 sectors, overlaid on the still frame (see Methods). These analysis regions are schematized; they are not the ones used in the experiment. (c) Schematic background-subtracted intensity functions over the 12 sectors of each wing. Dotted lines indicate the wingbeat-amplitude estimates given these functions (see Methods).
Supplementary Figure 2: Responses of VS cells at high temporal resolution.

(a) Sample responses of a VS cell to down-right and up-left motion during flight and non-flight. These four traces were recorded from the example neuron in Figure 3. The traces have been shifted arbitrarily along the y-axis for clarity.

(b) Sample responses of a second VS cell to down-right and up-left motion during flight and non-flight. Traces have been shifted arbitrarily along the y-axis for clarity.
Supplementary Figure 3: The baseline depolarization and visual-response boost are clear during extended bouts of uninterrupted flight. (a) Mean responses to repeated presentations of downward motion (top) or upward motion (bottom) presented in the same format as in Figure 6. Only recordings where the fly flew continuously (with no air puffs) for at least 35 seconds at the end of the flight epoch are included in the average. Fewer cells/flies were available for downward motion because this stimulus leads to a drop in wingbeat amplitude and more cessations of flight. (b) Mean responses to repeated presentations of upward motion in cases where flies flew continuously (with no air puffs) for at least 90 seconds at the end of the flight epoch. Tiny downward glitches in the probability of flight trace during continuous flight represent occasional errors in the flight detection algorithm; we manually verified that all flies included here, and in panel ‘a’, did not stop flying during the indicated time window.
Supplementary Figure 4: Loose-patch recording of an unidentified spiking cell in the right visual lobe. (a) Raw traces of responses before and during flight. This cell was strongly direction selective to binocular leftward motion. In blowflies, H1 and H2 lobula-plate tangential cells increase their spike rate in response to back-to-front ipsilateral motion (Hausen, 1993); these cells are unresponsive to contralateral motion. Although our stimulus was binocular—and thus we could not definitely show that this cell’s receptive field was solely ipsilateral—the response profile of this neuron is consistent with that of H1 or H2 from blowflies. Responses from this cell were strongly boosted during flight. Traces were digitally bandpass filtered between 250-1000 Hz. (b) Close up of the response to up-left motion before flight and during flight. (c) Mean spike rate for the 4 blocks of eight stimuli before flight, during flight, and after flight. Note that spike rates were strongly boosted during flight, much like the graded membrane-voltage response of VS cells. This cell’s baseline spike rate remained at 0 Hz during flight, meaning that if any tonic depolarization of the resting potential took place this was not sufficient to elicit baseline spiking.