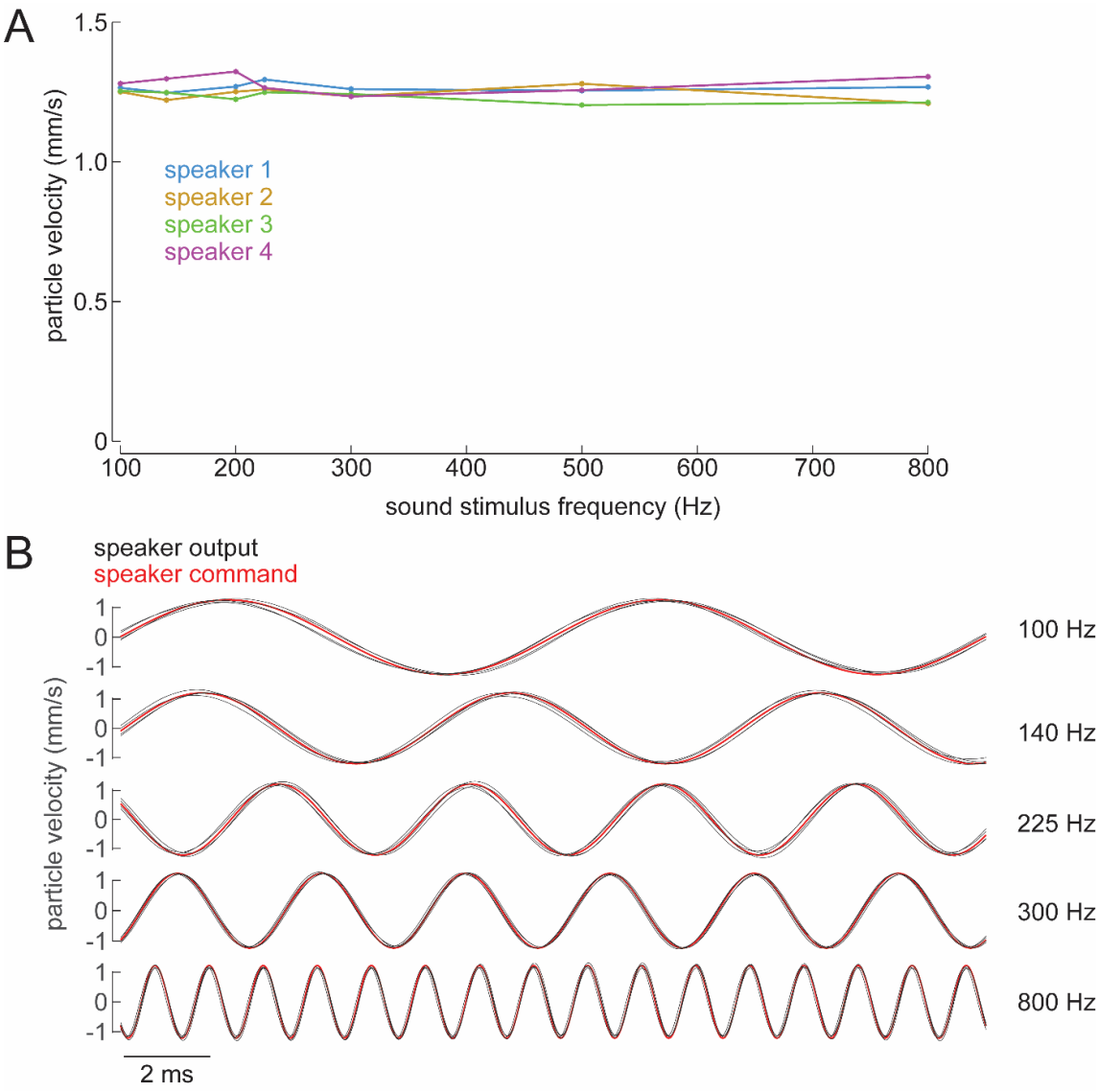

Figure S1: Speaker calibrations.

(A) Each point is the mean of the peak particle velocity, measured at the fly's position, averaged across 10 repetitions of the stimulus; these values are shown for all four speakers and each sound stimulus frequency used in this study. Note that, for all speakers, particle velocity was essentially constant for all sound stimulus frequencies. We adjusted the amplitude of the sound stimulus command waveform to achieve this outcome.

(B) Black lines are particle velocity versus time (one on each panel for each of the four speakers). Red lines are the sine-wave voltage commands sent to the speaker amplifier, phase-shifted to match the phase of the recorded particle velocities. The similarity in shape of the black and red waveforms indicates that harmonic distortions are absent.

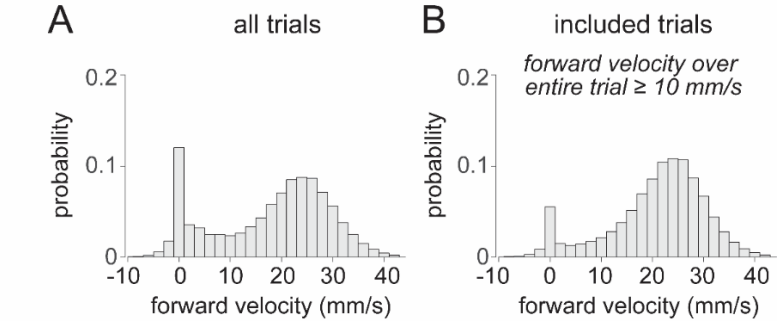

Figure S2: Distribution of forward velocities.

(A) Histogram showing the normalized distribution of forward velocities in a set of typical experiments. Forward velocities were measured over time windows 10 ms in duration. This histogram was generated using data from the five flies in Fig. 3A; histograms were first generated for each fly, and then were averaged together after normalizing the area under each histogram to one. This bimodal distribution is typical of flies that run well on a spherical treadmill (Gaudry et al., 2013).

(B) Same as (A) but excluding trials where the forward velocity was below threshold. The trials where the average forward velocity during the pre-stimulus period was > 10 mm/s were the trials included in data analysis.

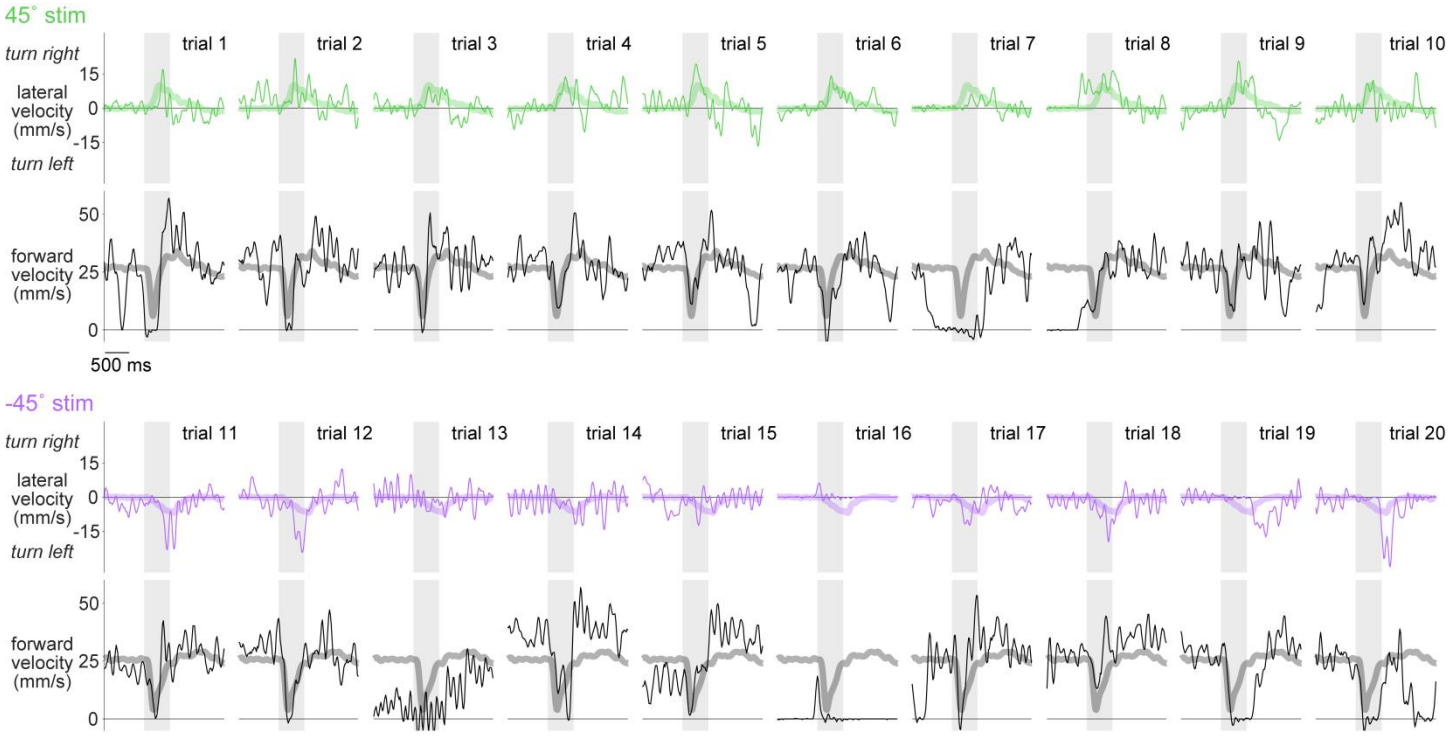

Figure S3: Additional single-trial examples of phonotaxis and acoustic startle behavior.

Additional examples of one typical fly's responses to sounds from the right (45°, green) or left (-45°, purple). Periodic fluctuations in lateral and forward velocity correspond to individual strides. Thick pastel lines are the trial-averaged data for this fly. These trials and those shown in Fig. 3B are randomly selected from the same fly. Trials are numbered here in the order shown, but were in fact presented in a pseudo-random order that interleaved trials from the two speakers. Note that the fly typically briefly stops just after sound onset, and then resumes walking, often turning toward the sound as walking resumes.

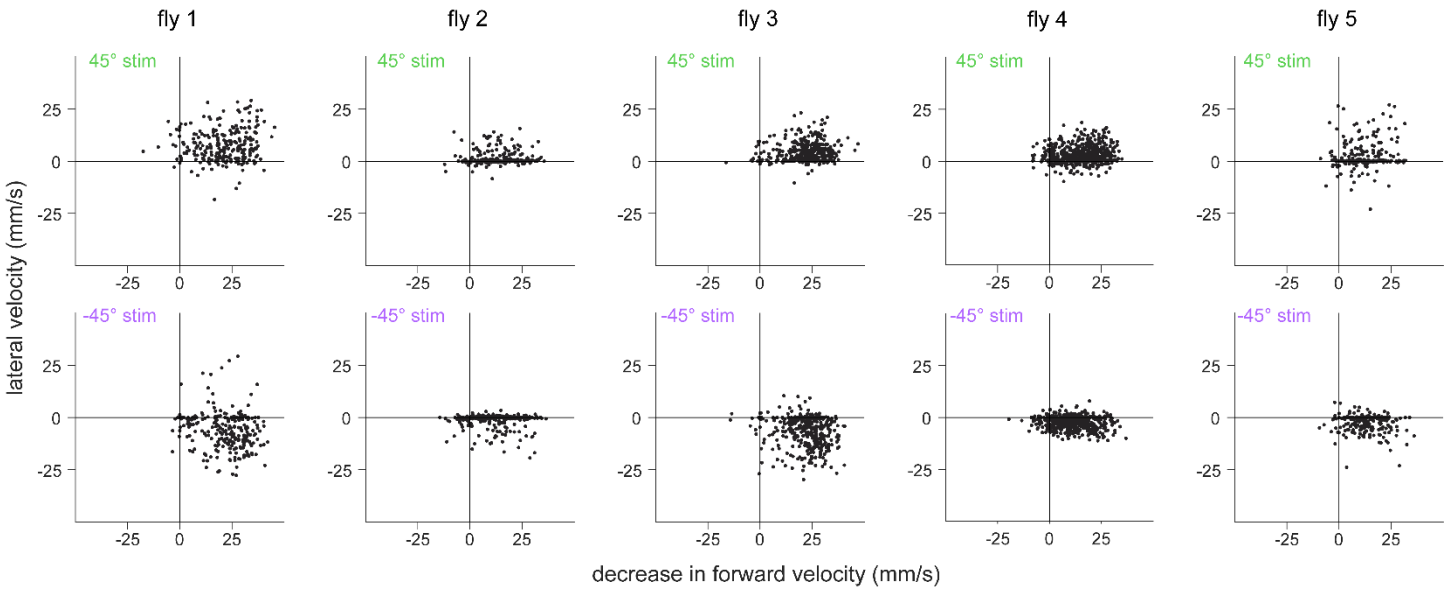

Figure S4: Trial-to-trial variation in forward and lateral velocity are not strongly correlated.

These five columns show data for five typical flies (the five flies in Fig. 3A). In each plot, the fly's sound-evoked lateral velocity in a single trial is plotted versus the fly's sound-evoked decrease in forward velocity in the same trial. Note that these values are not strongly correlated on a single-trial basis. This indicates that stopping and turning are fairly independent behaviors. The lateral velocity shown here is the lateral velocity measured at stimulus offset. The decrease in forward velocity shown here was computed as the forward velocity just before stimulus onset, minus the forward velocity 120 ms after stimulus onset. We measured the lateral velocity and decrease in forward velocity at the same time points for the stripcharts shown throughout the paper (e.g. Fig. 4C,D).