

Supplementary Figure 1: Odor-evoked changes in wingbeat frequency and amplitude are strain-dependent.

(A) Wild flies respond to mango odor (undiluted) with a large increase in WBF. Top: each trace is the trial-averaged response of an individual fly (typically 4-6 trials). Bottom: mean \pm SEM, averaged across flies ($n=12$ wild flies). Experiments were performed in the fixed-tether apparatus.

(B) In w^{1118} flies, the same stimulus elicits a smaller change in WBF than in the wild flies ($n=8$ w^{1118} flies, $p<0.05$, t -test).

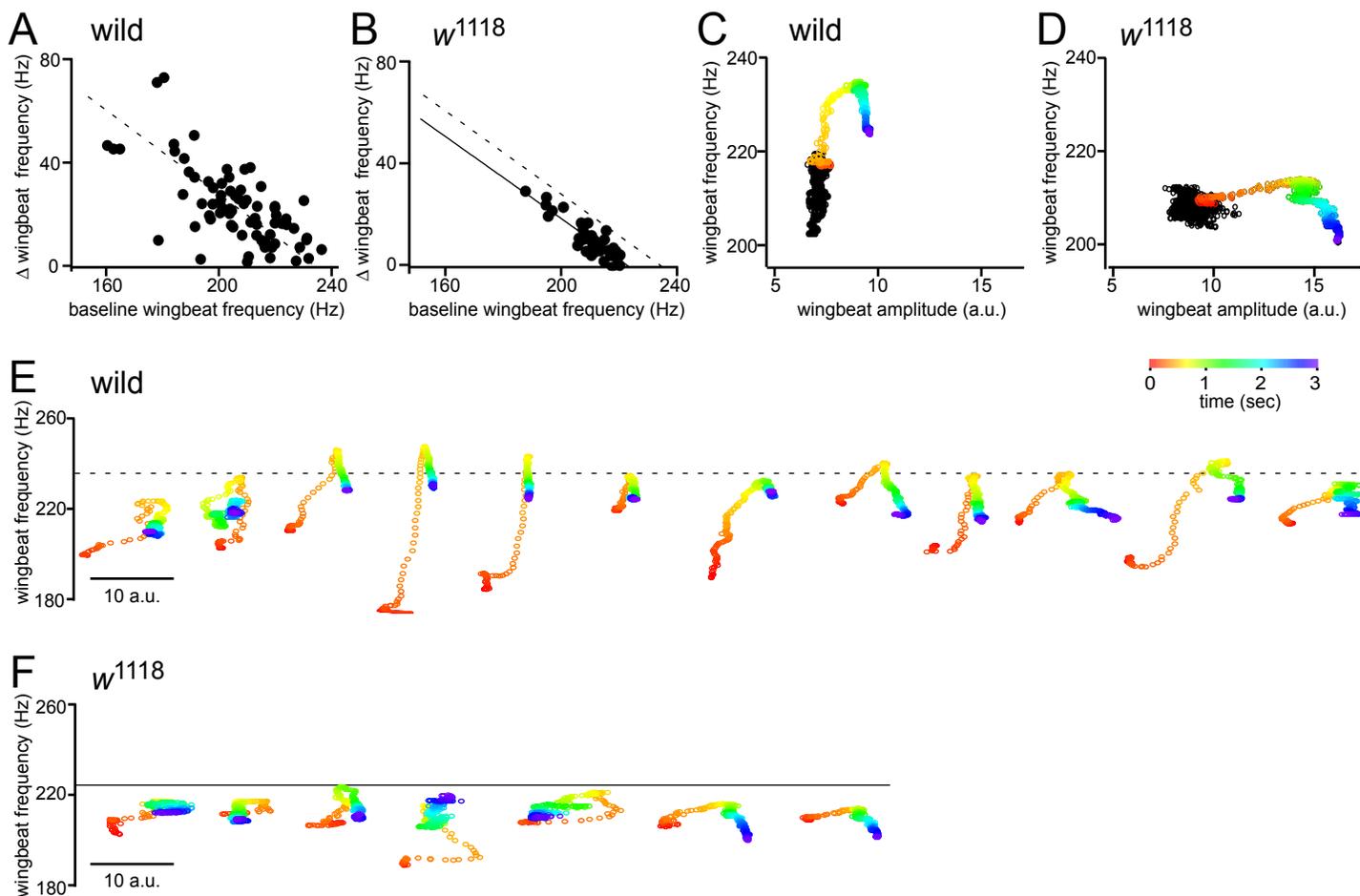
(C-D) Like their parental strain (w^{1118}), the $Or83b^{+/+}$ flies show a significantly smaller WBF response than wild flies ($p<0.01$, t -test). Here, the odor is mango, 0.1 dilution. Note that $Or83b^{+/+}$ flies carry a w^+ minigene, meaning that the WBF response defect in the w^{1118} flies is probably not due to the mutation in the *white* gene, but rather reflects some other aspect of this genetic background.

(E) In wild flies, mango odor (undiluted) evokes a robust increase in WBA.

(F) In w^{1118} flies, the odor-evoked change in WBA is not significantly different from wild files.

(G-H) The $Or83b^{+/+}$ flies show a WBA response which is similar to that of the wild flies.

The strain-dependent difference in the odor-evoked wingbeat frequency illustrated here suggests that w^{1118} flies (and stocks derived from w^{1118} , like $Or83b^{+/+}$) might not be able to reach the same maximum WBF that wild flies can achieve. If in the absence of odors a typical w^{1118} fly is flying closer to its maximum WBF than a typical wild fly, then its response to odor may be more tightly constrained by that ceiling. In Supplementary Figure 2 we investigate this idea.



Supplementary Figure 2: Strains differ in their maximum wingbeat frequency.

(A) In wild flies, the odor-evoked increase in wingbeat frequency is negatively correlated with baseline WBF on a trial-to-trial basis. Each point represents different trial (pooled trials from 12 flies, 78 trials total, $r=-0.81$, $p<0.05$). Odor is mango (undiluted). Experiments were performed in the fixed-tether apparatus.

(B) Similarly, in w^{1118} flies, the odor-evoked increase in wingbeat frequency is also negatively correlated with the baseline wingbeat frequency (pooled trials from 8 flies, 42 trials total, $r=-0.89$, $p<0.05$). However, the linear relationship (solid line) is shifted down as compared to the wild strain (compare dashed line), suggesting that w^{1118} flies are constrained by a lower maximum stroke frequency.

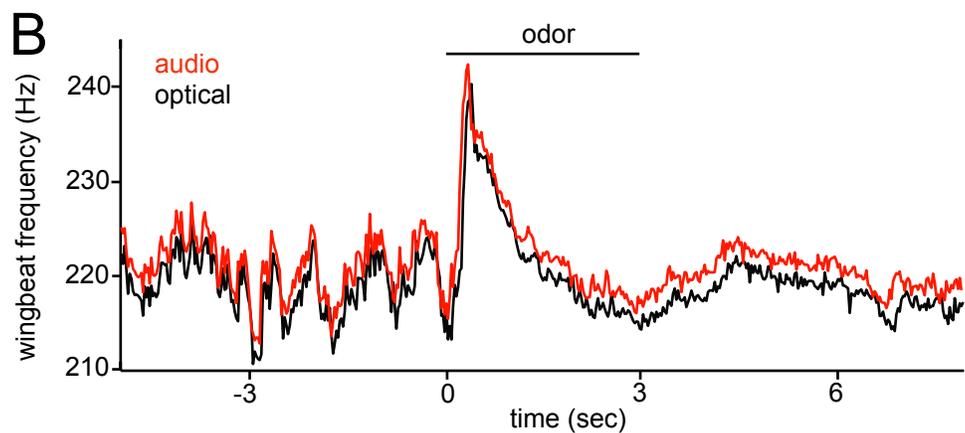
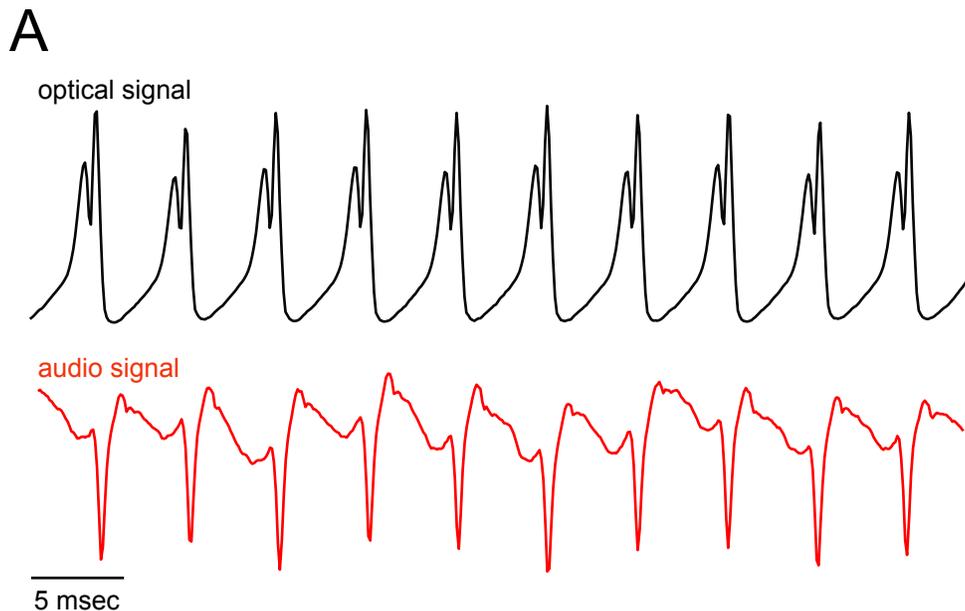
(C) Instantaneous wingbeat frequency is plotted against instantaneous wingbeat amplitude for a single typical trial in a wild fly (baseline period in black, time after odor onset encoded in color). The odor-evoked surge begins with a large increase in wingbeat frequency, accompanied by an increase in wingbeat amplitude. Wingbeat frequency then reaches a maximum, after which the fly may be obtaining further increases in flight force by trading wingbeat frequency for wingbeat amplitude.

(D) In a typical w^{1118} fly, the odor-evoked surge in wingbeat frequency reaches a smaller maximum value as compared to the wild fly.

(E) Analogous plots for each wild fly show the same trends as in (C). Dashed line indicates the x-intercept of the fitted line in (A).

(F) Same as (E) but for each w^{1118} fly. Solid line indicates the x-intercept of the fitted line in (B).

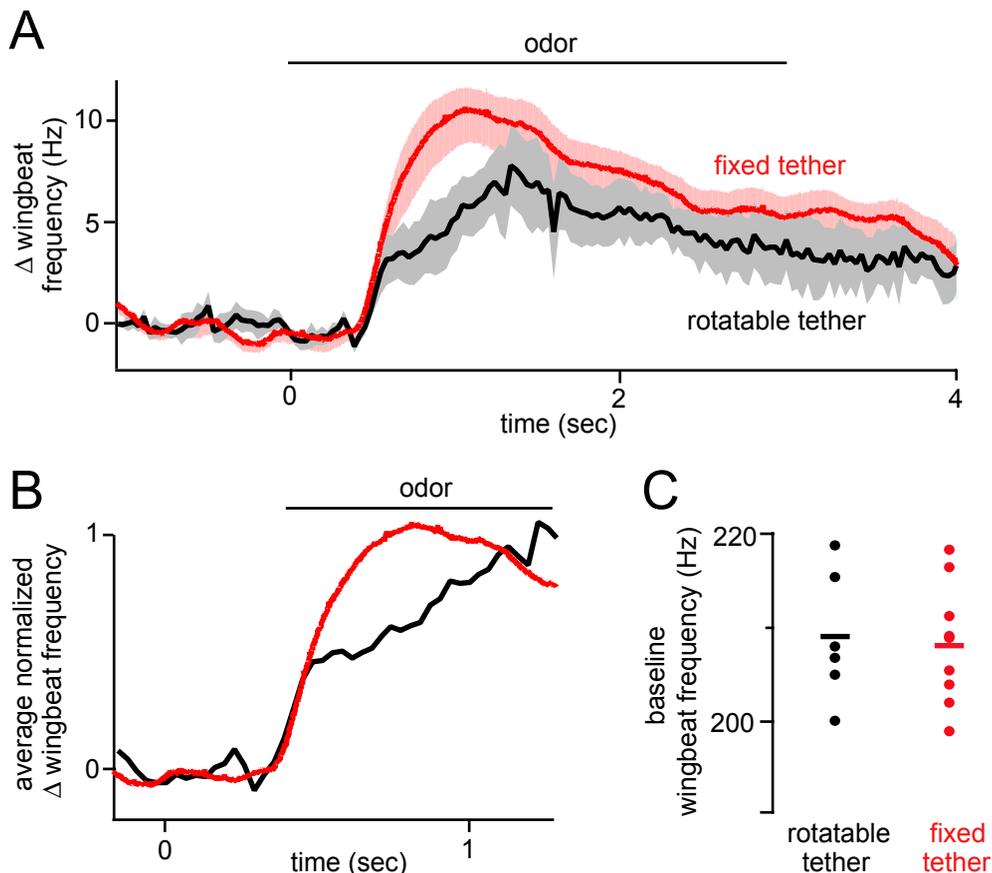
Taken together, these results imply that different fly strains have different available kinematic ranges for flight modulation.



Supplementary Figure 3: Comparison of wingbeat frequency measurements obtained with a photodiode and with a microphone in the rotatable-tether apparatus.

(A) Top trace shows the optical signals obtained from a photosensitive detector beneath the fly (see cartoon in Fig. 1A, right detector only), while the bottom trace shows the audio signals obtained from a microphone positioned next to the fly (see cartoon in Fig. 5A). Both signals show the same periodicity.

(B) Measurements obtained simultaneously using these two methods are in good agreement.

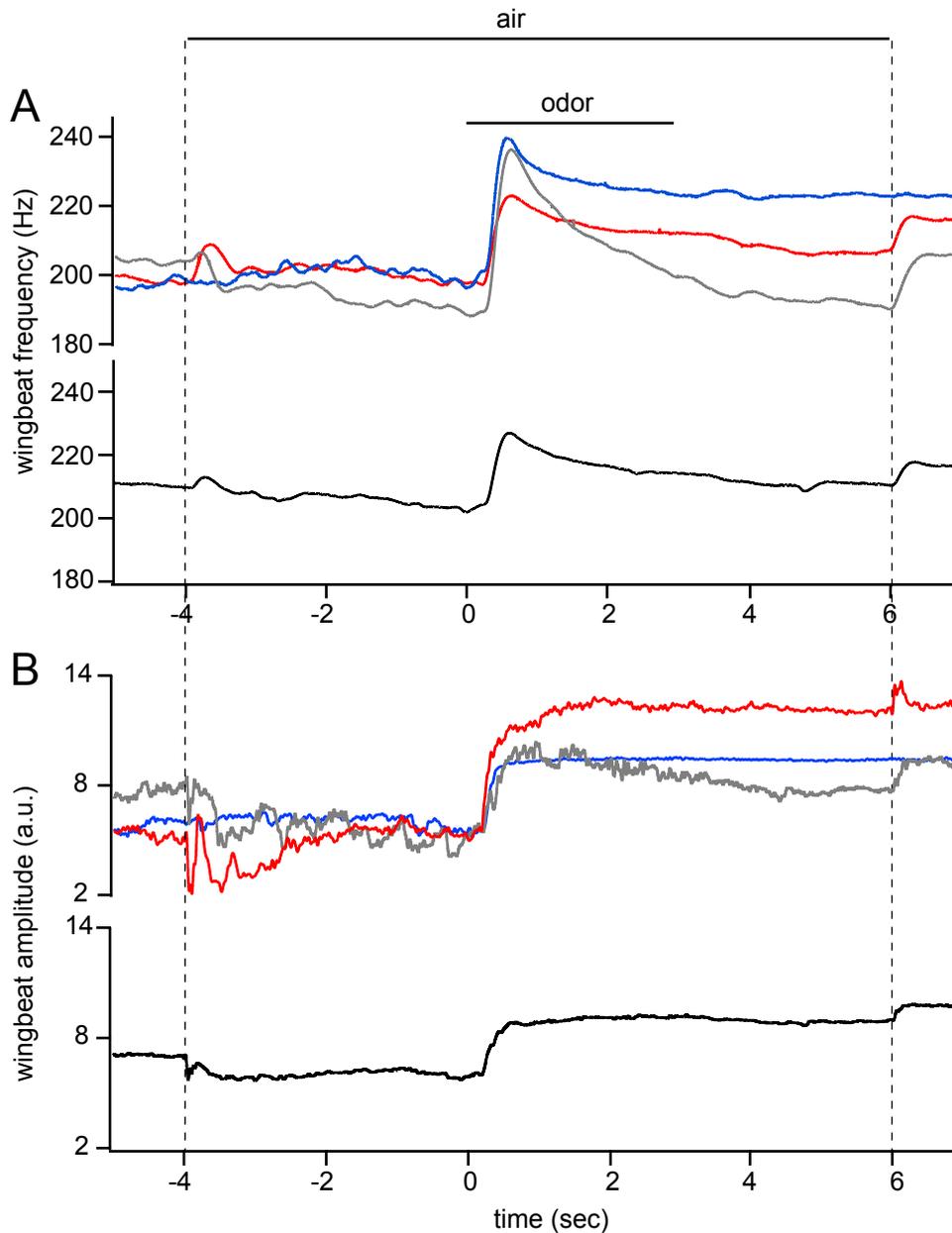


Supplementary Figure 4: The odor-evoked increase in wingbeat frequency is similar in the fixed- and rotatable-tether apparatus.

(A) Odors produced a similar change in WBF in the fixed- and rotatable-tether conditions. Differences in response peak were not statistically significant ($n=8$ fixed-tether, 6 rotatable-tether, $p>0.2$, t -test). Values are \pm SEM, averaged across flies. Odor is mango (undiluted), flow rate is 550 mL/min for both the fixed- and rotatable-tether setups.

(B) A comparison between the mean time course of WBF responses in the two setups. Each fly's response was normalized to the same maximum before averaging across flies.

(C) Baseline WBF was similar in the two setups. Each point is a different fly, horizontal lines are averages across flies.



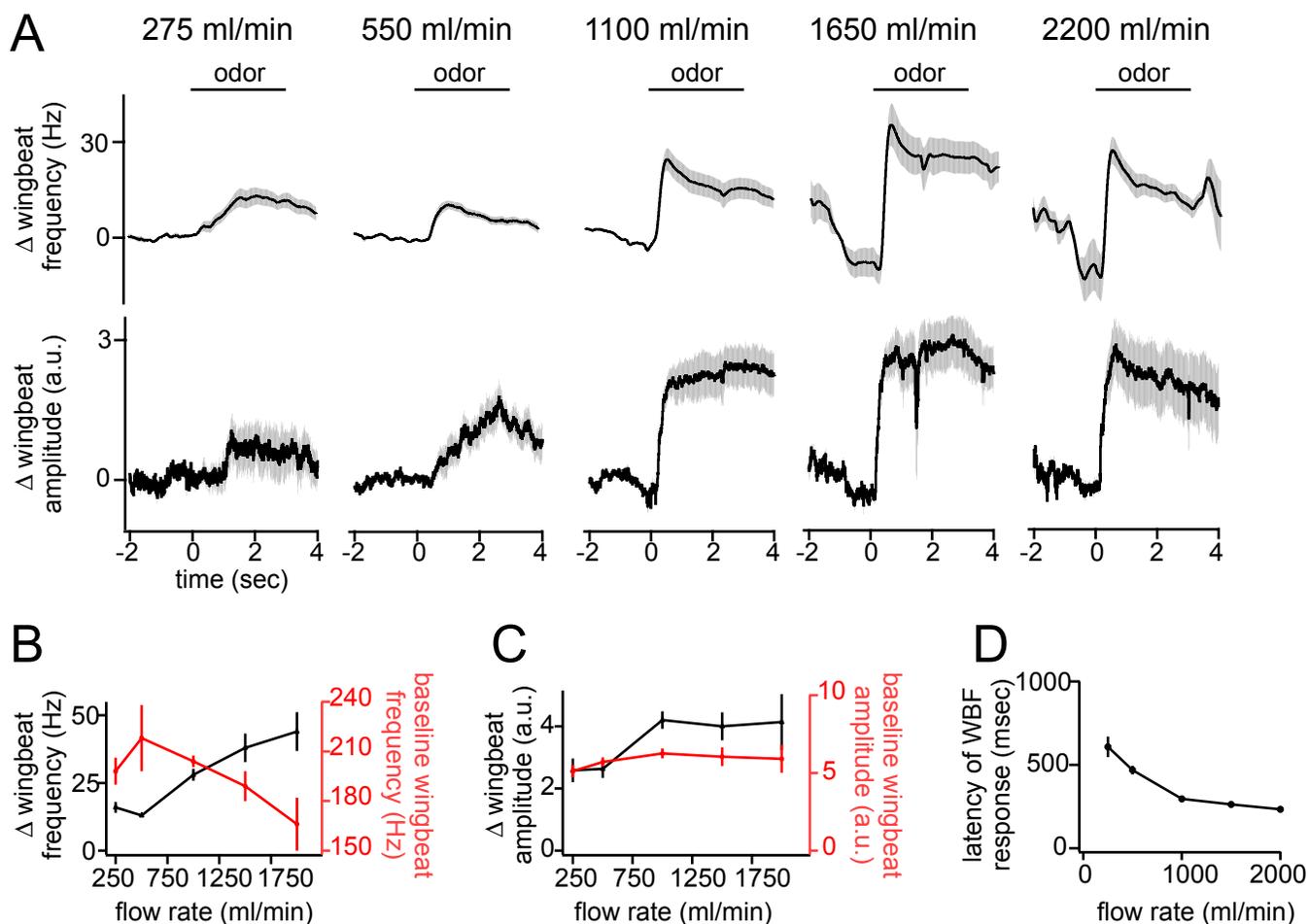
Supplementary Figure 5: Effect of the air stream on wingbeat frequency and amplitude.

These representative traces show the period of air stream onset and offset surrounding the odor stimulus. Flow rate is 1100 ml/min.

(A) Mean wingbeat frequency in three individual flies (top) and averaged across all flies (bottom).

(B) Mean wingbeat amplitude in three individual flies (top) and averaged across all flies (bottom).

Note that wingbeat frequency tends to increase transiently at air-on and air-off. Wingbeat amplitude tends to decrease at air-on and increase at air-off.



Supplementary Figure 6: Effect of flow rate on the odor-evoked flight surge.

(A) Higher flow rates (corresponding to higher air speeds) produce larger odor-evoked changes in WBF and WBA. Also note that within each trial at high flow rates, the fly's baseline (pre-odor) flight force decreases steadily after the air flow is turned on at $t = -4$ sec. (The dip in WBF/WBA halfway through the odor pulse was a consistent finding at 1650 ml/min, but the reason for this is not clear.)

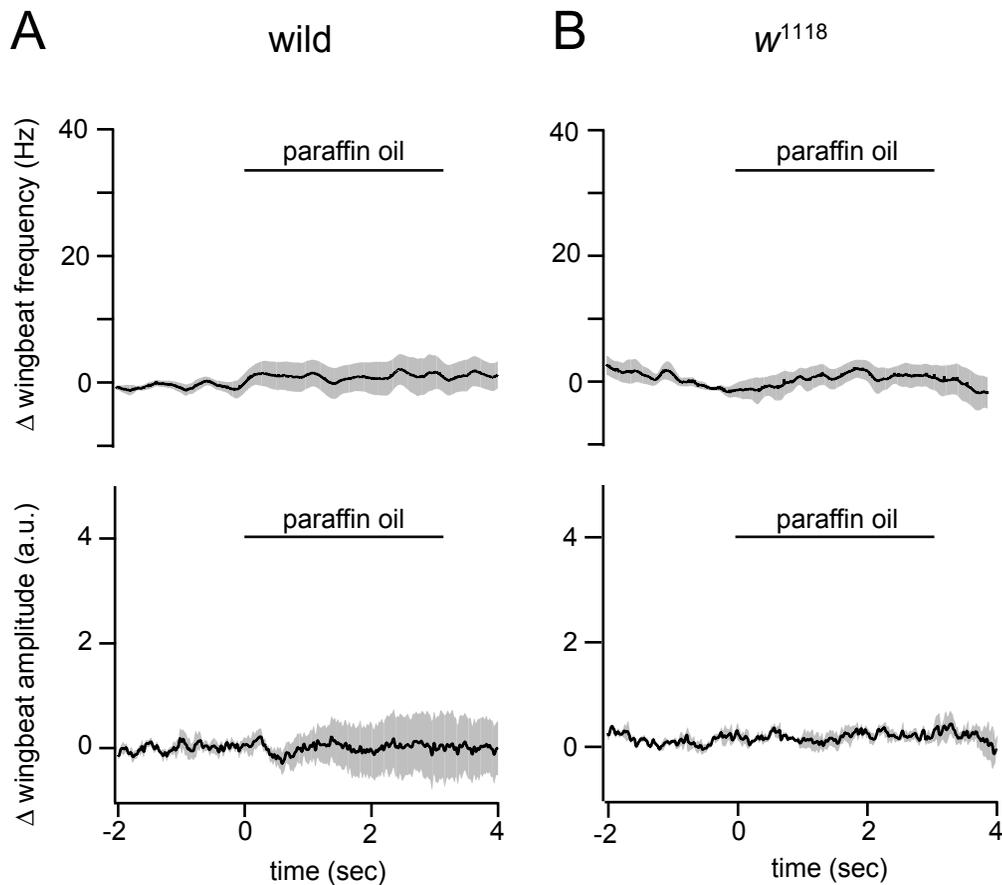
(B) Increasing flow rate increases Δ WBF but decreases baseline (pre-odor) WBF.

(C) Increasing flow rate increases odor-evoked Δ WBA, but has little effect on baseline WBA.

(D) The latency of the WBF response is strongly influenced by the flow rate.

Odor is mango (undiluted), flies are wild strain. All values are mean \pm SEM, averaged across flies.

High flow rates are useful in olfaction experiments because they produce good trial-to-trial consistency in the dynamics of the odor stimulus. However, these results suggest that an intermediate flow rate (\sim 1100 ml/min) is optimal for these experiments. At this flow rate, baseline WBF and WBA are relatively steady, yet odor-evoked flight modulations are also crisp.

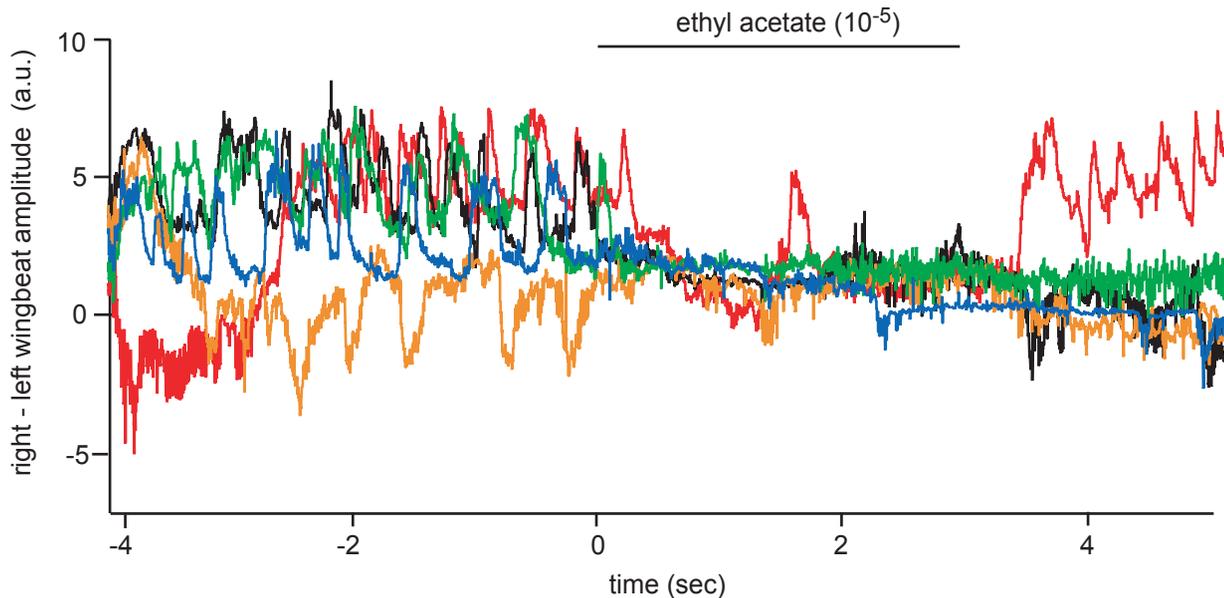


Supplementary Figure 7: The odor solvent alone does not affect wingbeat frequency or amplitude.

(A) The odor solvent alone (paraffin oil, J.T. Baker, VWR #JTS894) has no effect on the wingbeat frequency or amplitude of the wild strain flies ($n=7$).

(B) Similar negative results were obtained for w^{1118} flies ($n=6$).

Data in this figure come from the fixed-tether apparatus.



Supplementary Figure 8: Ethyl acetate can evoke fictive turning in the fixed-tether apparatus.

In the fixed-tether apparatus, the difference between right and left wingbeat amplitude values is a proxy for the fly's yaw torque. This plot shows the (right - left) difference plotted over time in a series of trials from a representative fly, with each trial displayed in a different color. Note that during the baseline period, the fly executes fast changes in the relative amplitude of the right and left wingbeats (so-called "body saccades"). The odor ethyl acetate (10^{-5}) decreases this saccade rate, indicating a more steady fictive upwind orientation. Similar results were observed with other flies.

These results show that although this odor stimulus does not elicit a change in wingbeat frequency or amplitude in wild flies in the fixed-tether setup (Fig. 3), it does elicit other flight behaviors. This demonstrates that the fly can perceive the stimulus, consistent with the fact that it produces a large field potential response in the antennae and palps.