Supporting Information

Goller and Altshuler 10.1073/pnas.1415975111

SI Results

Response to Forward-Backward, Lateral, and Vertical Visual Motion. Representative traces for feeding bouts in the presence of moving and stationary visual patterns (spirals and gratings) are presented in Figs. S1–S3. A bird's position and velocity were used to define two types of hovering behavior, which could each appear multiple times within a single feeding bout. Docked feeding segments are defined by the bird being within physical reach of the front of the feeder and having a net velocity less than 0.050 m/s. Undocked look-up segments are defined by the bird not being within physical reach of the front of the feeder but still having a net velocity less than 0.050 m/s. Only segments that lasted at least 0.25 s were included in analyses.

For each segment of docked feeding or undocked look-ups we calculated the average movement in six directions along three axes. The three axes (x, y, z) are defined globally with respect to the feeder, with x as forward-backward, y as left-right, and z as down-up. Movements in the forward, left, and down directions were defined as negative, and movements in the backward, right, and up directions were defined as positive.

We first calculated the derivative of the bird's position along each axis. These derivatives were then sorted by motion direction (positive vs. negative values within a single axis) and all values pertaining to a single motion direction were summed to get a total distance traveled in each of the six directions. We next normalized the summed movement by dividing it by the duration of the segment. Thus, movement in each direction is represented as movement (in centimeters) normalized to 1 s of flight.

Movement in each of the six directions was analyzed separately with six identical linear mixed-effects models with stimulus as a fixed effect and individual bird as a random effect. Stimulus was significant in all cases (all $P < 0.0001$) and Tukey post hoc tests were performed (glht in R) to examine pairwise comparisons of stimulus types (nine levels total). The results of all tests indicate that during undocked look-ups the hummingbirds exhibit an increase in movement in the direction of the stimulus pattern motion (Figs. S5–S7). During docked feeding, strong oscillatory movement is seen for looming spirals, but we were unable to measure changes in other stimulus levels. The design of our feeder limits the ability of a bird to move laterally and vertically, as well as forward, once docked.

We analyzed several additional measures to generally describe the behavior of the hummingbirds during stimulus trials. Feeding duration (time in docked position) and the frequency of look-ups (number of look-ups divided by total duration) in each trial were calculated and analyzed using linear mixed models and Tukey post hoc tests in R. There is considerable variation among individuals in the frequency of look-ups and in their feeding duration (Fig. S4). There is no consistent effect of stimulus treatment on look-up frequency, although one pairwise comparison, between looming and receding spiral treatments, was significant ($P < 0.001$) (Fig. S4*A*). Stimulus treatment did have a significant effect on feeding duration: birds fed for shorter durations in the presence of a receding spiral compared with most other stimulus treatments ($P < 0.01$), which were not different from each other (Fig. S4*C*).

Response to Offset Spiral Center. To examine how hummingbirds responded to the position of looming visual motion, we analyzed their movements in a spherical coordinate system. Every position was defined by a radius and two angles: azimuth and elevation. Movements toward the feeder were defined as negative, and movements away from the feeder were defined as positive. We first calculated the derivative of the radius and then extracted sequences of backward flight (positive values) that were at least 0.25 s in duration. By taking the start and end points of these segments, we calculated a single vector to describe the backward motion in three dimensions. The azimuth and elevation angles of the vector, which describe flight direction, are presented in Fig. 3. The radius (magnitude) of the backward flight segments is presented in Fig. S8.

The angles and radii were analyzed using linear mixed-effects models in R. Stimulus was treated as a fixed effect with individual bird as a random effect. Because the 10 trials per individual (two each of center, left, right, up, and down) were conducted in random order and we were not interested in an overall response magnitude, these models do not account for a possible response change over the course of the experiment within individuals. The radius length of backward-flight sequences in the off-center conditions was significantly shorter than the centered looming spiral condition ($F_{4,\ 1,197} = 170.75$, $P < 0.0001$) but were not different from each other (Tukey post hoc test, all $P > 0.395$). For the azimuthal angle the left and right positions were significantly different from all other positions (all $P < 0.001$) as predicted, and up and down did not differ from centered (all $P > 0.18$). For the elevation angle the down and up positions were significantly different from all other positions (all $P < 0.0001$), again as predicted. The left and right positions were different from the centered condition (all $P < 0.0001$) but were not different from each other ($P = 0.997$).

Response to Combined Stationary Pattern and Looming Spiral. To examine responses to looming motion in the presence of stationary features, we extracted only the docked feeding segments as defined above. We did not consider undocked look-ups because: (*i*) we only tested the response to looming which can be measured during docked feeding, and (*ii*) birds exhibit individual and day-to-day variation in how regularly undocked look-ups are performed but all birds consistently exhibit docked feeding. The motion in the backward direction was normalized for the length of the flight segment as above. The visual stimulus was described as a percentage of looming spiral pattern and stationary pattern, such that a totally stationary stimulus would have 0% looming and 100% stationary, but a looming spiral without a stationary pattern would be 100% and 0% looming and stationary, respectively. The backward response was also related to the order of the experimental trials because the response change experiment shows a decrease in response magnitude over time. These data describing response, stimulus composition, and trial order (1–14) were analyzed using nonlinear mixed models in R. Linear and exponential models did not fit the data well, so we used a logistic curve (function). Model parameters were estimated using individual birds as a random effect and then compared with models where trial order was additionally included as a fixed effect. This curve had the formula:

$$f(x) = i + \frac{a}{\left(1 + e^{-s*(x-c)}\right)}, \qquad \textbf{[S1]}$$

where a is the upper asymptote, s is a rate term, c is the center, and i is the lower asymptote. Curves without the lower asymptote were also fitted but these did not improve the model for increased looming ($P = 0.112$) and were significantly worse for increasing stationary background ($P < 0.0001$). Overall the best model for both datasets (looming: $P = 0.0467$, stationary: $P < 0.0001$)

included trial order as a fixed effect to estimate a. This finding suggests there is a decrease in the magnitude of responses with repeated exposure to the looming spiral. The parameter estimates for the best-fit models are shown in Table S1 and the curves are plotted with the raw data in Fig. 5 with trial order = 0. Otherwise, there would be 14 curves, one for each permutation of a, differing only slightly.

Response Change Over Time. We studied the change in flight response to a single stimulus, a looming spiral, using one camera placed directly above the feeder. This camera view provided 2D tracking data and we analyzed only the docked feeding segments, because these could reliably be obtained across many trials. We calculated three features of the flight response using two frames of reference: (*i*) the backward response in the x axis normalized to 1 s of feeding was calculated as above; (*ii*) the frequency of drifts was calculated in a circular coordinate system; (*iii*) the average speed of the drifts was also calculated in a circular coordinate system. Initially, we only analyzed segment that lasted longer than 0.25 s.

The three response features were analyzed separately using linear mixed-effects models in R with trial number (a proxy for time) and stimulus rotation frequency as fixed effects, and individual as a random effect. A significant "time" effect indicates a change in response over the course of the experiment. A decrease in response is interpreted as potential habituation, sensory adaptation, or fatigue. A significant stimulus rotation-frequency effect suggests that hummingbirds respond to the three treatment levels differently. In the case of significant "time" by stimulus type interaction, the analysis was split and each of the three rotation frequencies was considered separately. In addition, models were modified to account for autocorrelation with a lag of 1 over a series of trials using corAR1 to define potential correlation. These updated models were compared with the initial models where appropriate, and the better model was chosen using ANOVA. The better fit is reported here.

Looming spiral presentation produced significant increases in backward motion relative to nonrotating (control) spirals, and the frequency of backward flight (>0.1 s in length) even after 2 d (all three stimulus treatments $P < 0.0001$). The speed of these backward flights was not compared between looming and control spiral trials because very few control trials had qualifying flight segments. Control trials were subsequently removed from the dataset to analyze changes in the response to looming stimulus only. Backward motion exhibits autocorrelation ($\phi = 0.418$, model comparison $P < 0.001$) and decreases significantly over the course of subsequent trials (t value = −2.328, $P = 0.020$), but there is no effect of rotation frequency (t value = 0.449, $P = 0.659$) and the stimulus-by-time interaction is marginally insignificant (t value = −1.915, $P = 0.056$). Frequency of backward drifts during flight does not exhibit high autocorrelation ($\phi = 0.080$, model comparison $P = 0.060$) but again changes with trial sequence ($F_{1,\ 604} = 23.652$, $P < 0.001$). In the case of drift frequency, stimulus type ($F_{1,\ 16} = 8.651$, $P = 0.010$) and the interaction between time and stimulus type ($F_{1,\ 604} = 10.528$, $P = 0.001$) are also both significant. The mean frequencies of head oscillation during docked feeding are 0.60 Hz, 0.26 Hz, and 0.24 Hz for spiral rotation frequencies of 0.1 Hz, 0.5 Hz, and 0.9 Hz, respectively. The response frequency at 0.1 Hz was significantly higher than in the other two treatments, which did not significantly differ from each other. The change over time in the response frequencies was not consistent among treatments (Table S2, upper half). The speed of these backward flights is not autocorrelated ($\phi = 0.077$, model comparison $P = 0.125$) but changes over time ($F_{1,\ 444} = 20.838$, $P < 0.001$), although not with the rotation frequency of the spiral ($F_{1,\ 16} = 0.341$, $P = 0.568$). The interaction of trial sequence and stimulus type is significant, however ($F_{1,\ 604} = 7.626$, $P = 0.006$).

We split all response measures further to elucidate the changes within a stimulus group, even though total backward motion had a P value slightly greater than 0.05. We fit a linear mixed-effects model to the data for each treatment group and estimated the amount the initial looming response declined over the course of 2 d. The effect measure was calculated as the ratio between the predicted decline in response over 2 d (initial minus final model values) divided by the difference between the initial and control trials (measured values). The model estimates and effect sizes for the three variables are given in Table S2, lower half.

Because we imposed a time requirement of 0.25 s for analyzing a flight response, many of the trials (160 of 624) were not scored as having a drift. We decided to relax this requirement to 0.10 s to determine if that caused a change in our interpretation of drift frequency and drift speed. We did not retest backward motion because that measure did not use the circular coordinate system and all trials were already represented. In the set of analyses with less-stringent time requirements, very few trials (11 of 624) were not scored as having a drift. The model estimates and effect ratios for the reanalyzed variables are given in Table S2, lower half. This analysis also demonstrates a decline in response, but with different effect sizes.

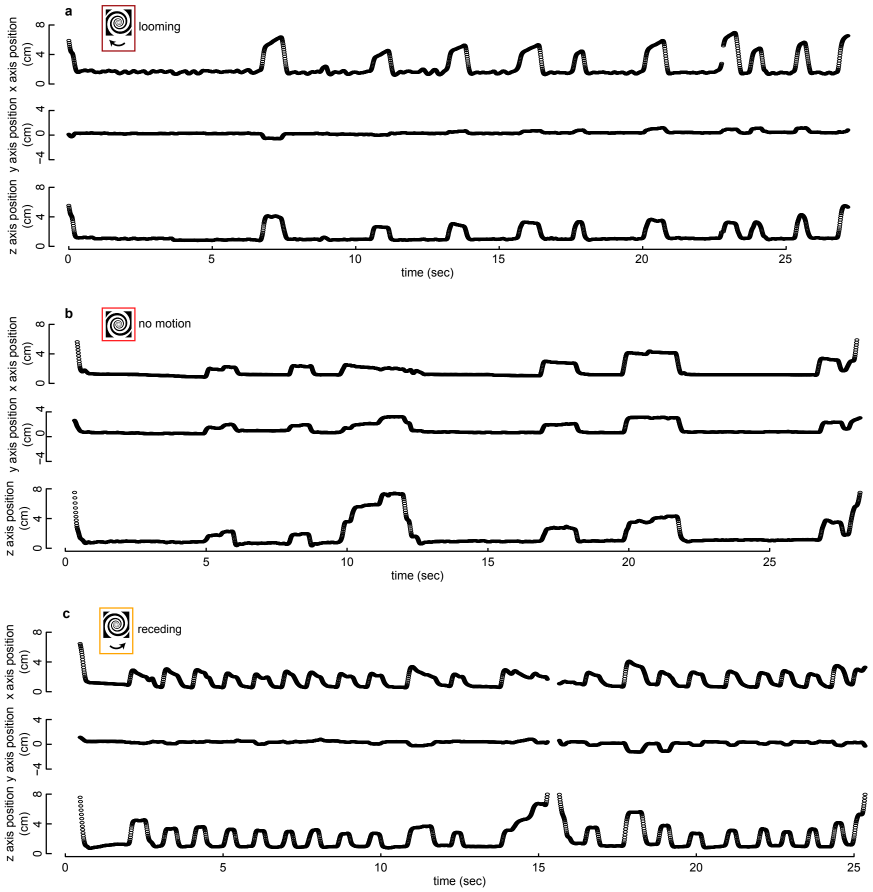

Fig. S1. Representative traces of hummingbird movement demonstrate how docked feeding and undocked look-ups differ according to spiral motion. Each feeding bout is represented by three traces corresponding to the *x*, *y*, and *z* axes. Positions are shown relative to the feeder, which is placed at ~0 in all directions. In the presence of looming motion (*A*), an individual hummingbird approached and docked at the feeder, and oscillated during feeding at less than 1-cm amplitude on the *x* axis. Periodic look-ups are evident where the bird flew backward (increasing *x*) and upward (increasing *z*), with these look-ups also exhibiting backward drifts on the *x* axis but little drift in the other axes. Look-ups are interspersed with short feeding events. In the presence of a nonmoving spiral (*B*), there were no oscillations during docked feeding or drifts during look-ups. In the presence of receding motion (*C*), there were no oscillations during docked feeding because the feeder physically restricts movement in the predicted direction. During undocked look-ups, however, hummingbirds drifted forward (decreasing *x*), as predicted. All representative traces (Figs. S1–S3) are from the same individual on the same day.

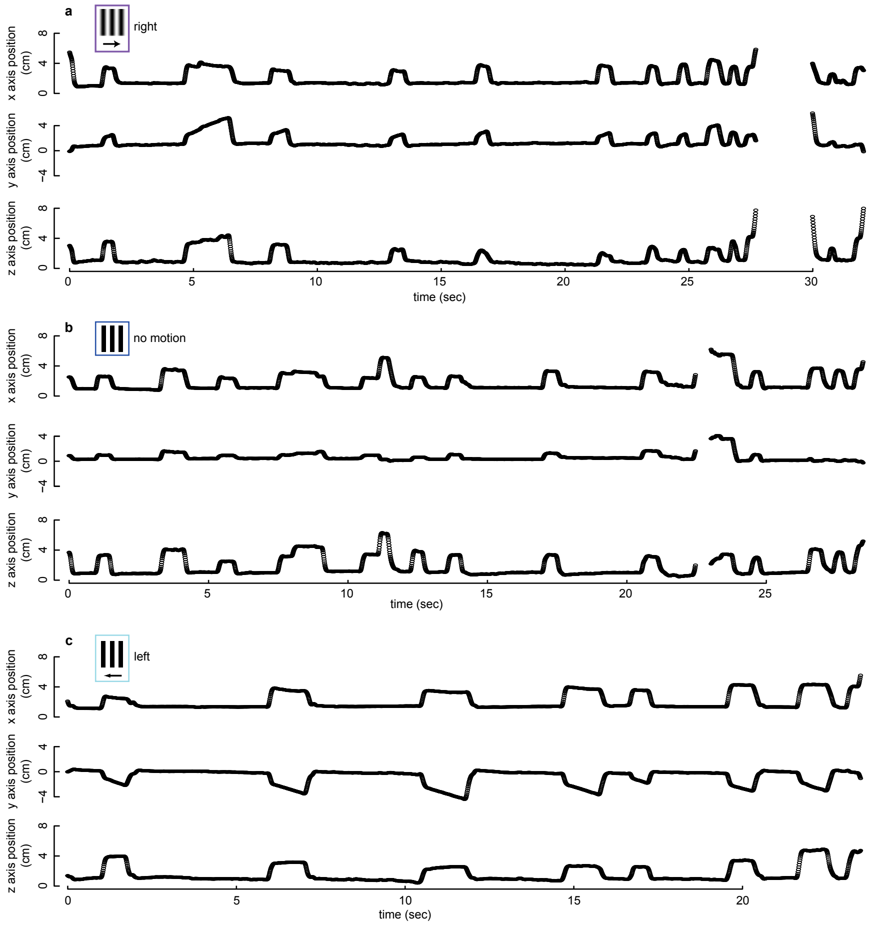

Fig. S2. Representative traces of hummingbird movement demonstrate how docked feeding and undocked look-ups differ according to motion of a vertical grating. In the presence of motion to the right (*A*), hummingbirds drifted to the right (increasing *y*) during undocked look-ups, with little drifting in the other axes. In the presence of a nonmoving vertical grating (*B*), there were no drifts during look-ups. In the presence of motion to the left (*C*), hummingbird drifted to the left (decreasing *y*), as predicted. There was little motion observed during docked feeding because of the physical restriction imposed by the feeder. All other details are as in Fig. S1.

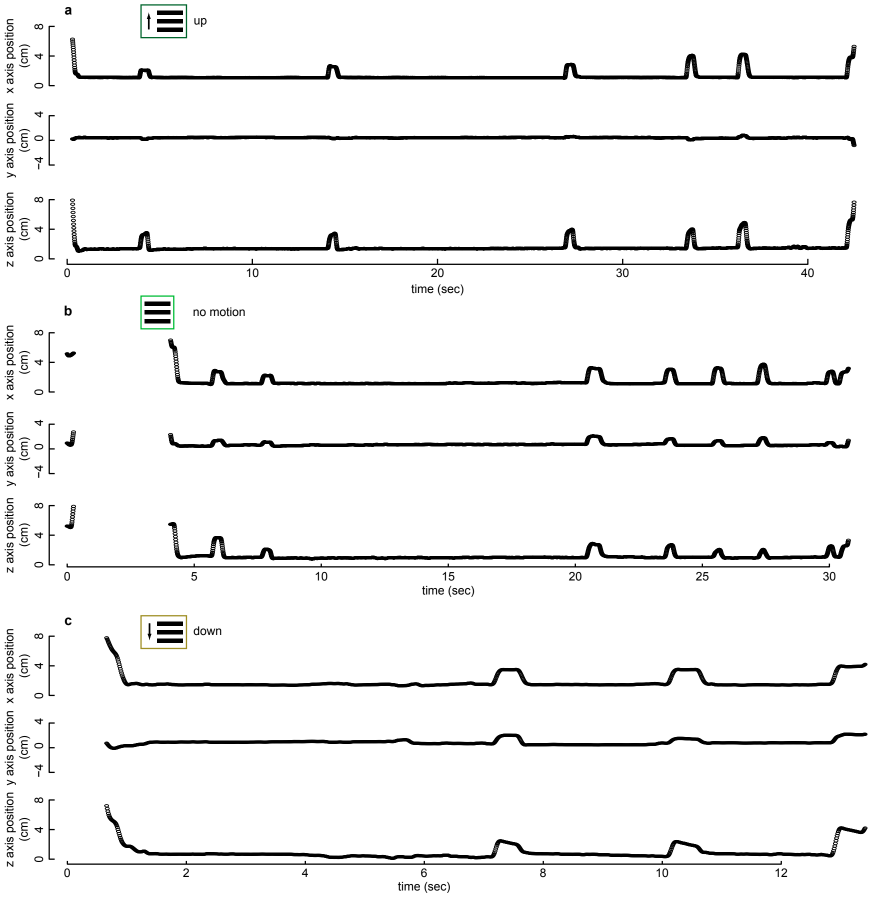

Fig. S3. Representative traces of hummingbird movement demonstrate how docked feeding and undocked look-ups differ according to motion of a horizontal grating. In the presence of upward motion (*A*), hummingbirds drifted upward (increasing *z*) during undocked look-ups with little drifting in the other axes. In the presence of a nonmoving horizontal grating (*B*), there were no drifts during look-ups. In the presence of downward motion (*C*), hummingbird drifted downward (decreasing *z*), as predicted. There was little motion observed during docked feeding because of the physical restriction imposed by the feeder. All other details are as in Fig. S1.

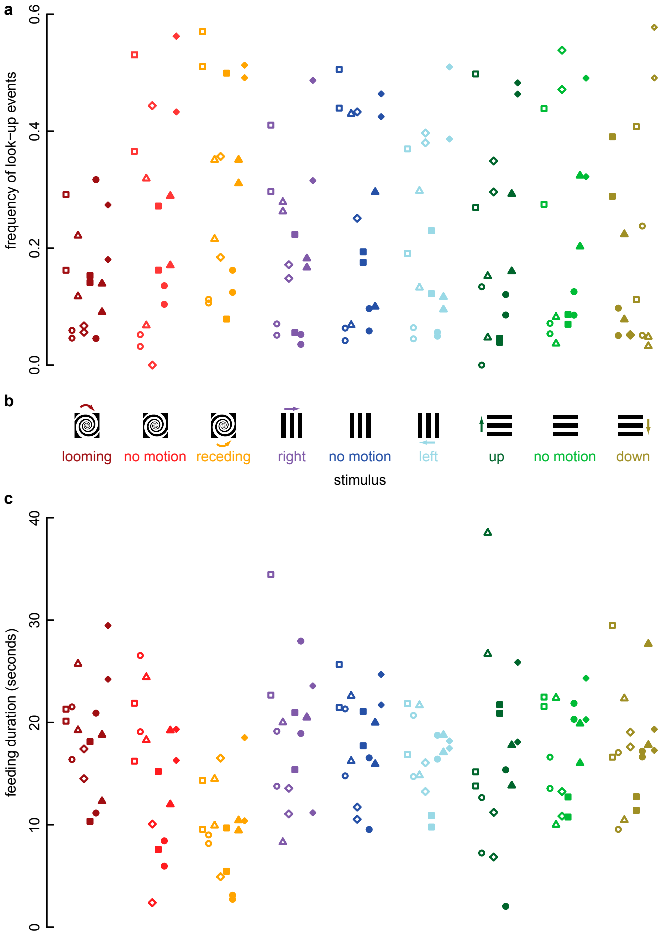

Fig. S4. Frequency of look-ups and feeding duration are similar among different stimulus treatments. The frequency of look-up events (*A*) does not vary consistently across the nine stimulus treatments (*B*). For feeding duration (*C*), there is a significant difference when receding spiral trials are compared with most other stimulus treatments. Other comparisons are not significant. Each column contains the data for a single background stimulus treatment and is colored to match Figs. 1 and 2, and Figs. S1–S3 and S5–S7. The two trials for each individual hummingbird within a single stimulus column are indicated by symbols and are slightly separated along the *x* axis. Individual identifications from left to right are 20–27.

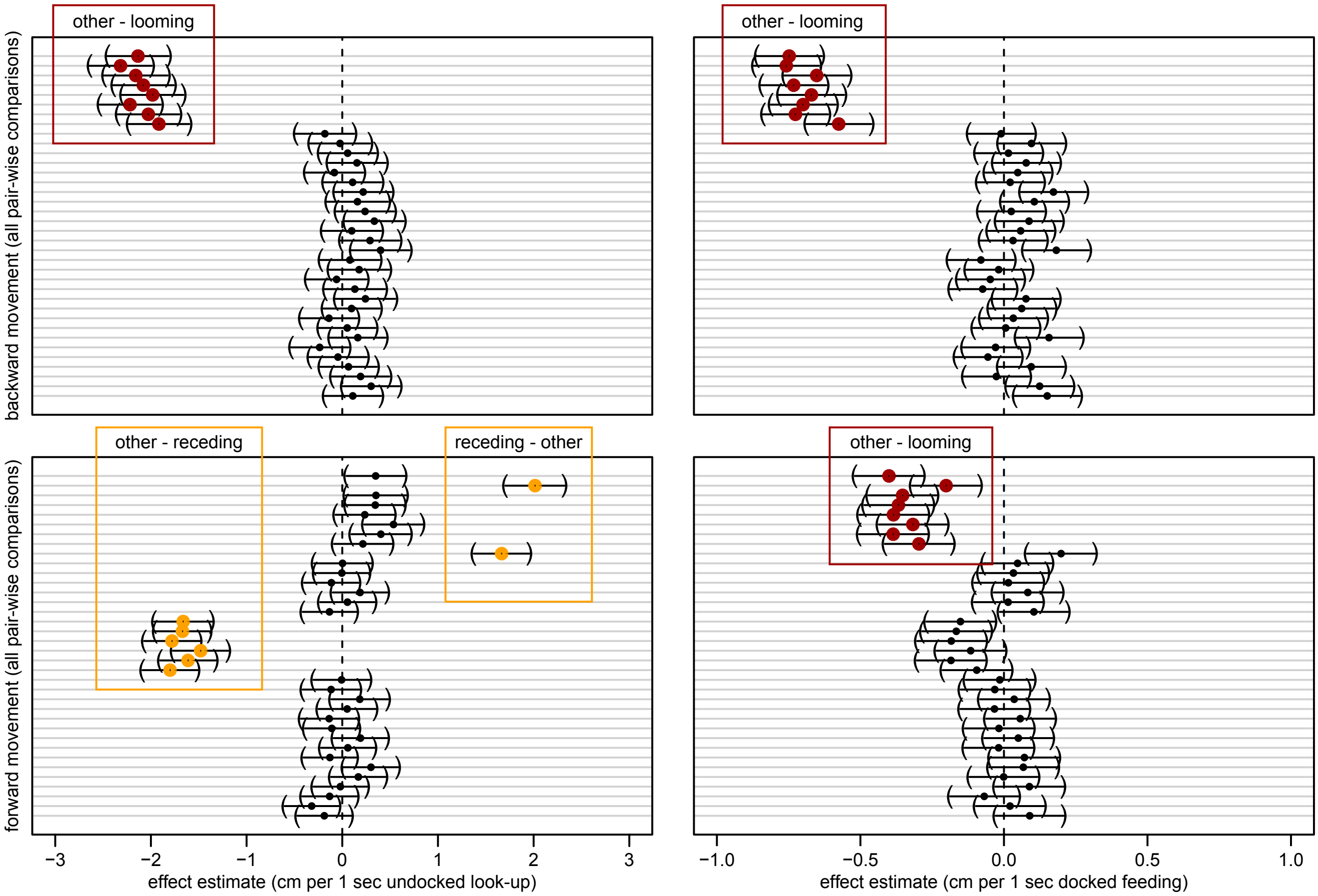

Fig. S5. Pairwise comparisons for movement along the *x* axis demonstrate that looming and receding motion elicit strong backward and forward flight responses. Each panel presents the post hoc test associated with a linear mixed-effects model. The upper panels correspond to post hoc analyses for backward movement during undocked look-ups (*Left*) and docked feeding (*Right*). The lower panels correspond to post hoc analyses for forward movement. Each panel contains 36 pairwise comparisons derived from nine stimulus levels. The stimulus levels (1–9) are assigned in the same order as the panels in Figs. S1–S3. For example, the top comparison (2-1) is the difference between a no motion spiral and a looming spiral. Black circles and brackets indicate the effect estimate and 95% confidence interval for comparisons where the stimulus level is not predicted to affect that type of movement. Colored circles and brackets indicate the same for comparisons where the stimulus is predicted to affect that type of movement. The colors correspond to movement direction in Figs. 1 and 2 (also Figs. S1–S4). Looming produces significantly more backward motion during both undocked look-up and docked feeding segments. Forward motion during undocked look-ups is similarly much higher in the receding spiral treatment. During docked feeding, however, the oscillations produced by looming are still evident. For all other stimulus levels, movement in the predicted direction is physically blocked by docking with the feeder.

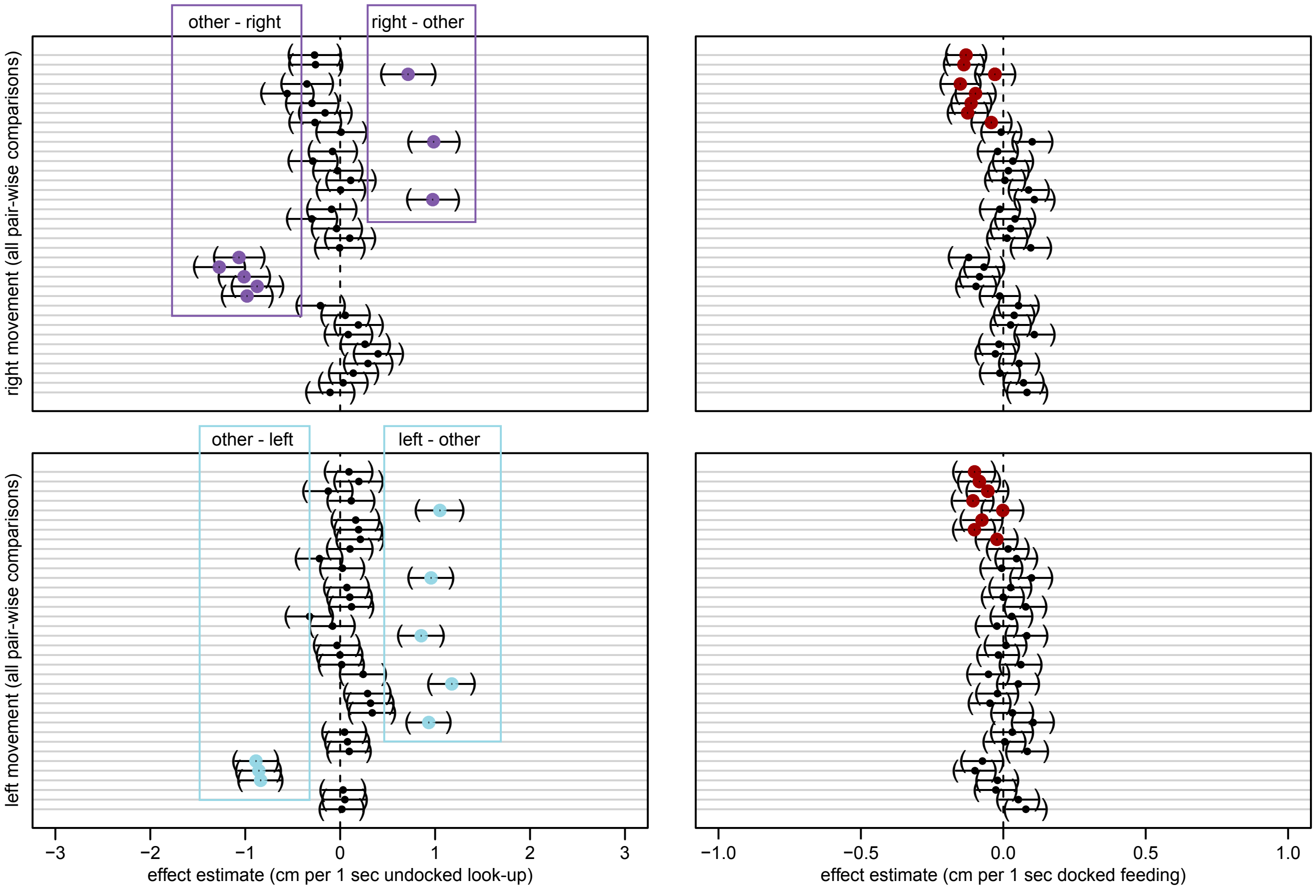

Fig. S6. Pairwise comparisons for movement along the *y* axis demonstrate that right and left visual motion elicit strong right and left flight responses. The upper panels correspond to post hoc analyses for movement to the right during undocked look-ups (*Left*) and docked feeding (*Right*). The lower panels correspond to post hoc analyses for movement to the left. Moving a vertical grating to the right produces significantly more flight to the right during look-ups compared with other stimulus levels. Moving a vertical grating to the left produces significantly more flight to the left during look-ups compared with other stimulus levels. During docked feeding (*Right*), the effect estimates were all close to zero, even for the oscillations produced by looming visual motion (maroon). All other details are as in Fig. S5.

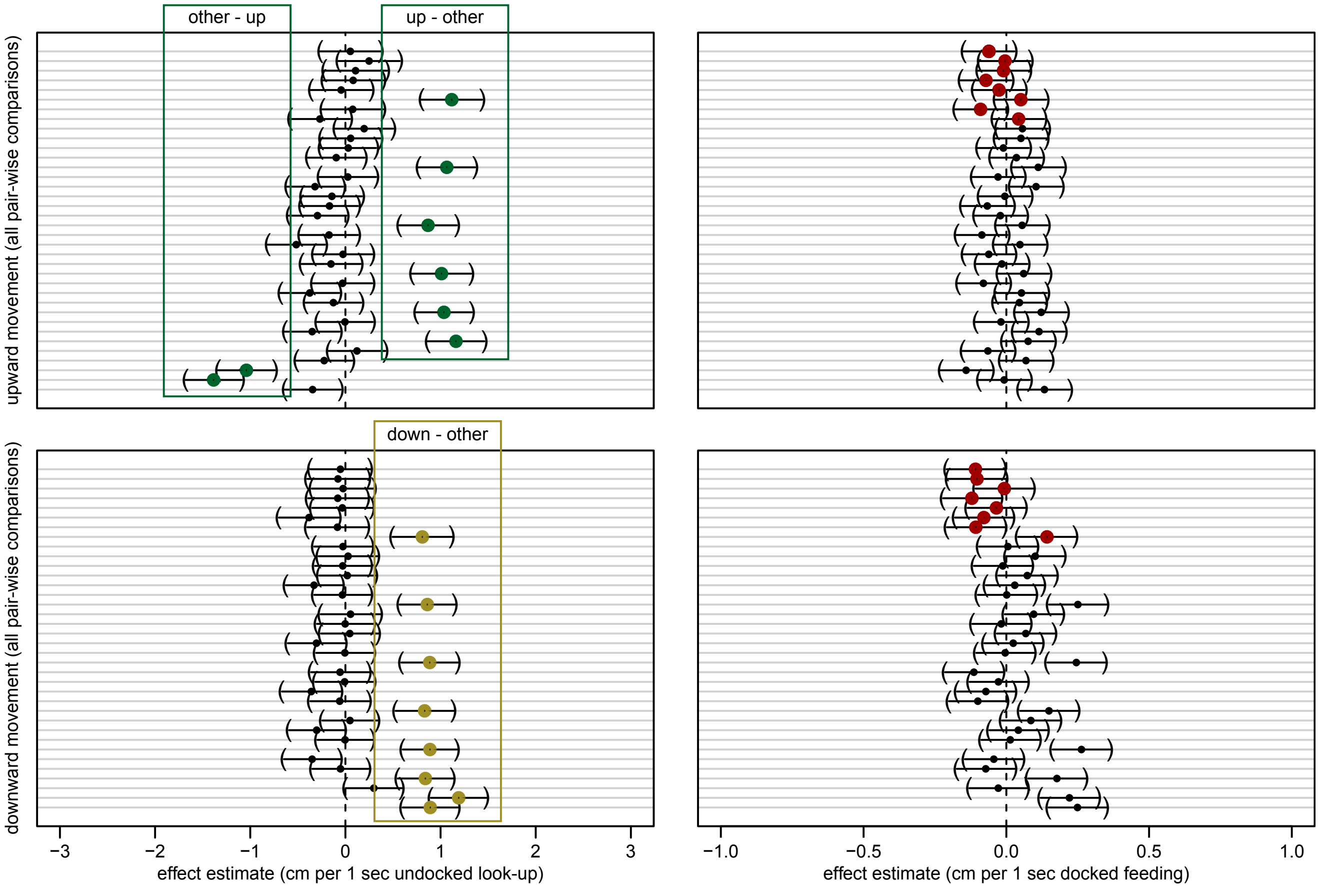

Fig. S7. Pairwise comparisons for movement along the *z* axis demonstrate that upward and downward visual motion elicit strong upward and downward flight responses. The upper panels correspond to post hoc analyses for upward movement during undocked look-ups (*Left*) and docked feeding (*Right*). The lower panels correspond to post hoc analyses for downward movement. Moving a horizontal grating upward produces significantly more upward flight during look-ups compared with other stimulus levels. Moving a horizontal grating downward produces significantly more downward flight during look-ups compared with other stimulus levels. During docked feeding (*Right*), the effect estimates were all close to zero, even for the oscillations produced by looming visual motion (maroon). All other details are as in Fig. S5.

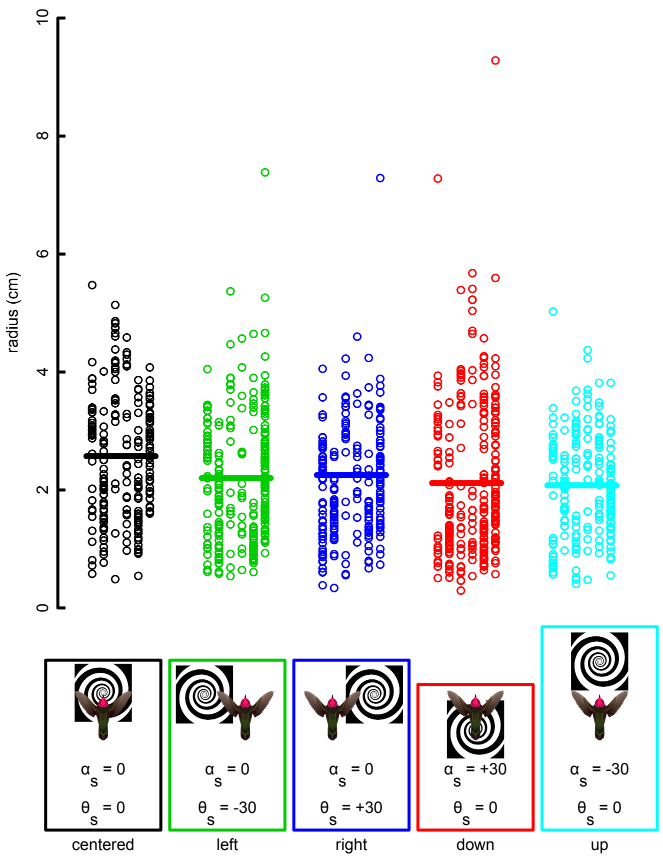

Fig. S8. Moving the looming spiral center left, right, up, or down causes the radius of the flight response to decrease relative to a centered spiral. Compared with the centered condition, hummingbirds exhibit significantly shorter backward avoidance flights when the center of the looming spiral is offset by 30°. None of the offset conditions were significantly different from each other. All other details are as in Fig. 3.

Table S1. Parameter estimates for series of combined patterns with either increasing looming spiral or stationary pattern

Parameter	Increasing looming spiral	Increasing stationary pattern
a	0.657–0.00652 × trial order	0.941–0.0280 × trial order
s	0.0704	−0.0656
c	56.694	42.051
i	0.0571	0.0685

Table S2. Model estimates and effect ratios

Spiral rotation frequency (Hz)	Backward motion (cm/s)	Backward motion effect	Drift frequency slope (Hz)	Drift frequency effect	Drift speed slope (cm/s)	Drift speed effect
Model estimates and effect ratios for drifts of minimum 0.25 s						
0.1	−0.00251	0.175	−0.00695	0.653	−0.00020	0.012
0.5	−0.00952	0.413	−0.00412	0.633	−0.01130	0.450
0.9	−0.00613	0.303	−0.00024	0.137	−0.00701	0.446
Model estimates and effect ratios for drifts of minimum 0.10 s						
0.1	No change	No change	−0.00060	0.021	−0.00341	0.275
0.5	No change	No change	−0.01160	0.276	−0.01284	0.543
0.9	No change	No change	−0.00728	0.159	−0.01189	0.391