

Figure S1. Major steps in the behavioral module classification algorithm.

Flow chart showing the order and major criteria for behavioral categorization. First, Probing behavior was marked (speed < 2 pixels per frame for at least 5 frames). Second, Sinusoidal behavior was marked (two head-to-trail distance peaks within one second). Third, Off Trail was marked (head-to-trail distance greater than two-thirds of a body size based unit, or segment). Fourth, we identified Trail Following (head-to-trail distance less than one-third of a segment for at least 5 frames). Any remaining unclassified frames were marked as Other.

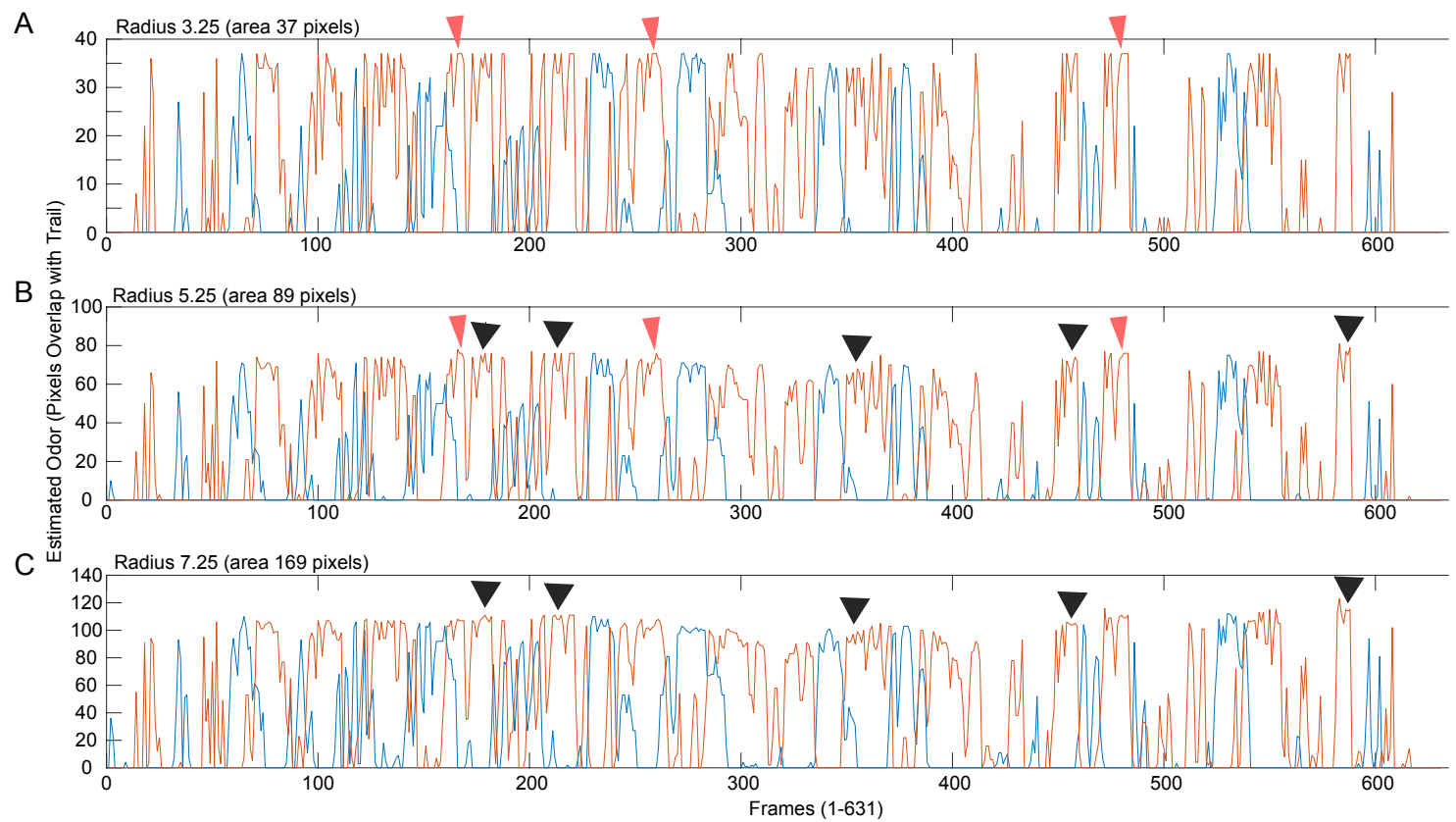


Figure S2. Effect of radius size on 'trail overlap' values.

For a given ant trial (631 frames), we plotted 'trail overlap' over time for circles of radius 3.25 (top), 5.25 (middle), and 7.25 (bottom). Minor differences in the shape of the curves are apparent (right antenna: orange line, left antenna: blue line). Black arrowheads mark differences (information loss) occurring at the larger radius. Crimson arrowheads mark differences (information loss) occurring at the smaller radius.

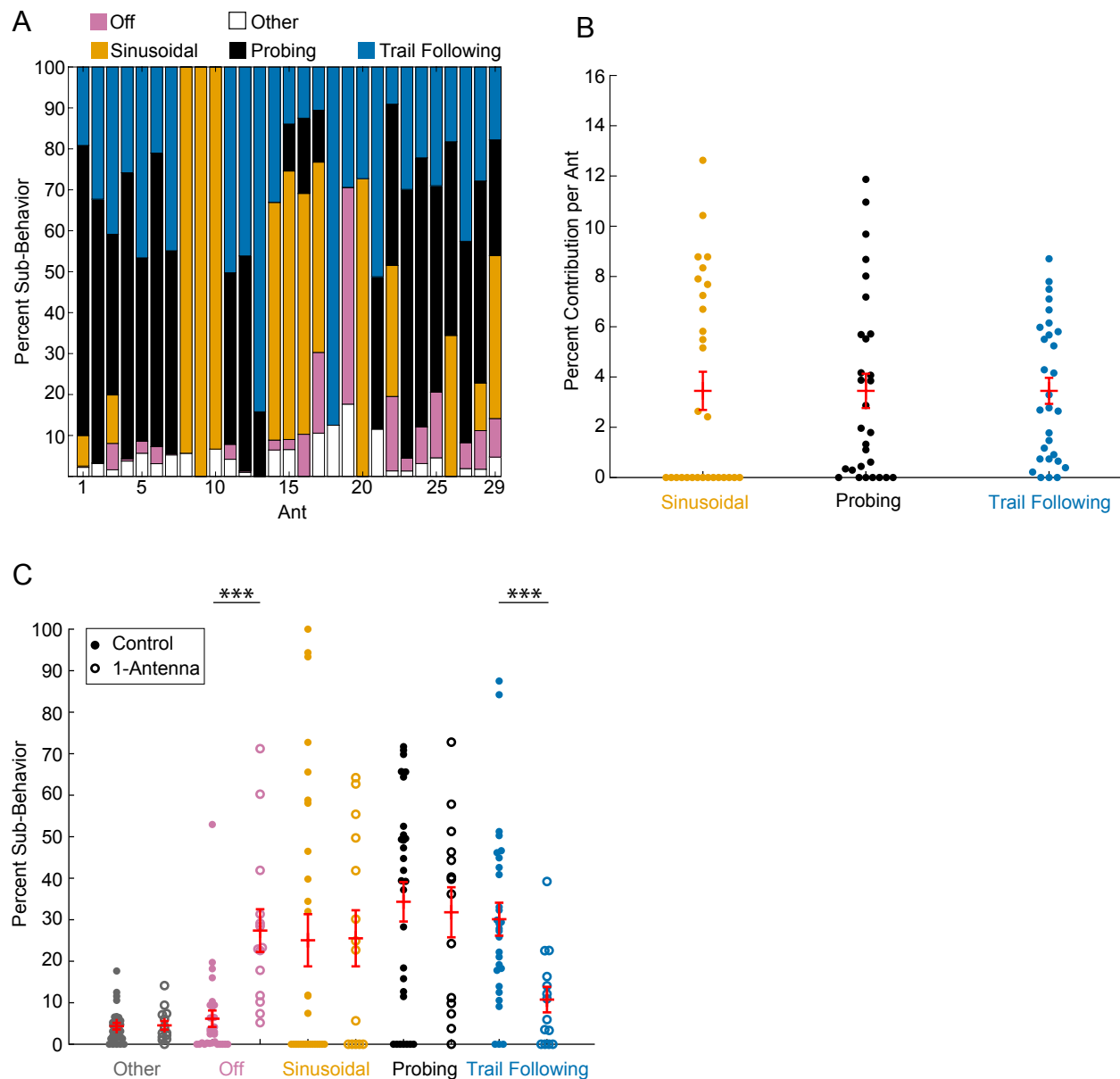


Figure S3. Percentage of behavioral modules per ant trial.

A: Bar plot showing the percent of frames in a given trail-tracking trial that the ant spent in each behavioral module (Probing, Sinusoidal, Trail Following, Off Trail, and Other). Most ants (24 of 29) exhibited two or more of the specific trail tracking behavioral modules (Sinusoidal, Probing, or Trail Following).

B: Plot showing the percent of frames each trail tracking ant ($n=29$) contributed to the total data analyzed for each behavioral module. As shown, each ant contributes a fraction of the data (mean 3.5 percent). Mean and SEM for each group is shown in red.

C: Plot showing a comparison of the percent of frames trail tracking control ants ($n=29$) and single antenna ants ($n=14$) spent in each behavioral module. Mean and SEM for each group is shown in red. Wilcoxon Rank-Sum, one-tailed. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

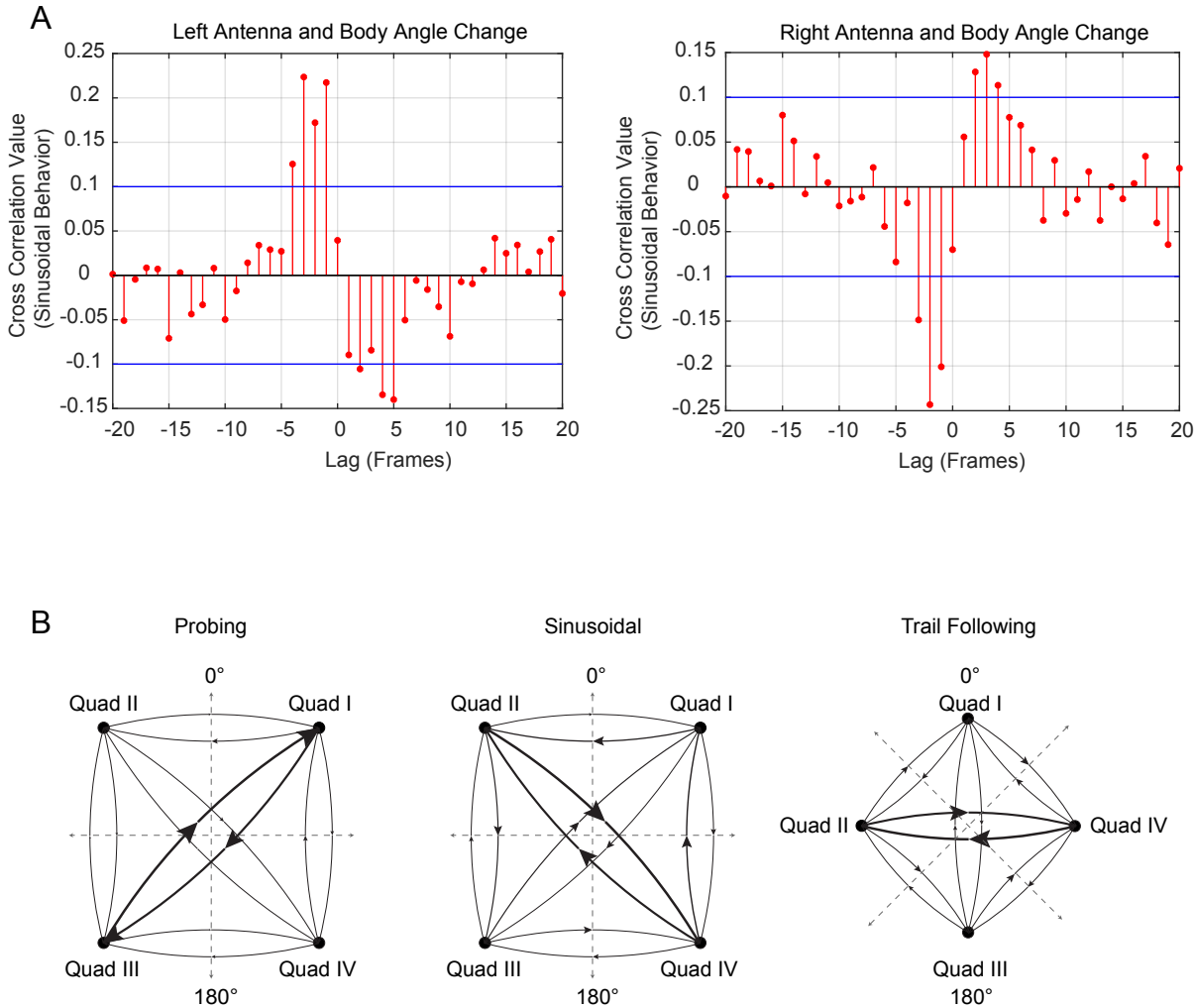


Figure S4. Antennae Movements

A: Correlation values between the change in the body angle (Figure 5E) and the change in the angle of antennae (see Figure 3B, Methods) for Sinusoidal Behavior. Based on the sign convention for the two angles, these correlations show that the left antenna moves in towards the body before the body axis turns right, and vice versa. The reverse holds for the right antenna.

B: Vectors of relative antennae tip movement from Fig. 4A were categorized based on the coordinate plane quadrant in which they reside. To evaluate how ants move their antennae over time, we quantified the transitions between these antennae movements binned by quadrant. The distribution of these transitions in movement is shown as relative line thickness in a connectivity diagram. The plots are dominated by a back and forth motion in two main directions for each behavior. Note that for Trail Following, axes were rotated by 45 degrees before binning the movement vectors to highlight connectivity between movements at 90 and 270 degrees (quadrants II and IV).

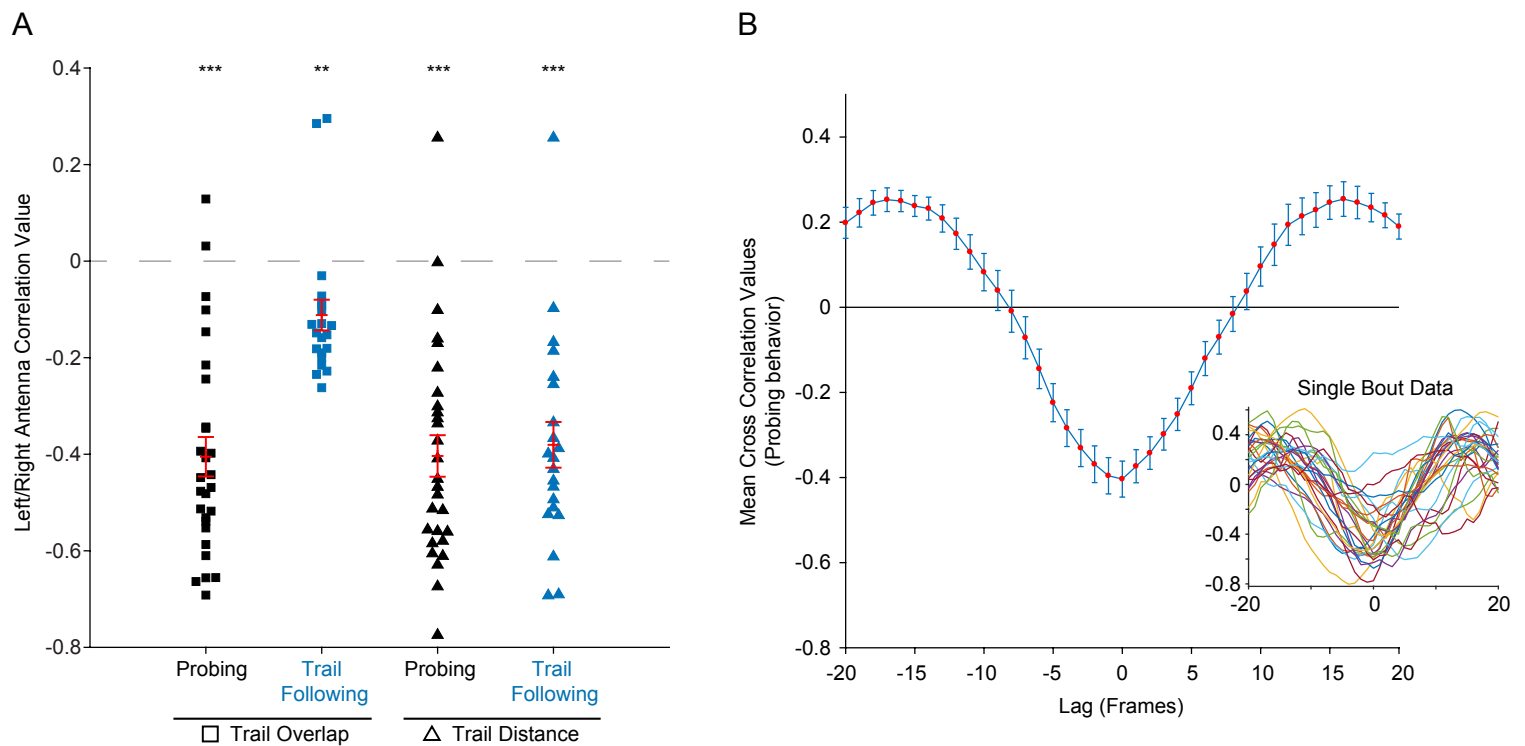


Figure S5. Cross correlations between antennae

A: Correlation values for left and right antennae trail overlap during Probing and Trail Following behaviors (squares, t-test, two-tailed, $p < 0.0001$ and $p = 0.0025$, respectively), and distance of the antennae to the trail for the same behaviors (triangles, t-test, two-tailed, $p < 0.0001$). Each point represents a behavioral 'bout' of at least 1 second (Probing bouts=28, Trail Following bouts=22). Statistical significance was evaluated using a t-test after Fisher's z-transformation. Mean and SEM for each distribution is shown in red. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

B: Correlation comparing the left and right antennae distance to the trail during Probing for lags of ± 20 frames. The inset shows the correlation results for each behavioral bout ($n=28$) and the large plot shows the mean correlation at each lag. Error bars represent SEM.

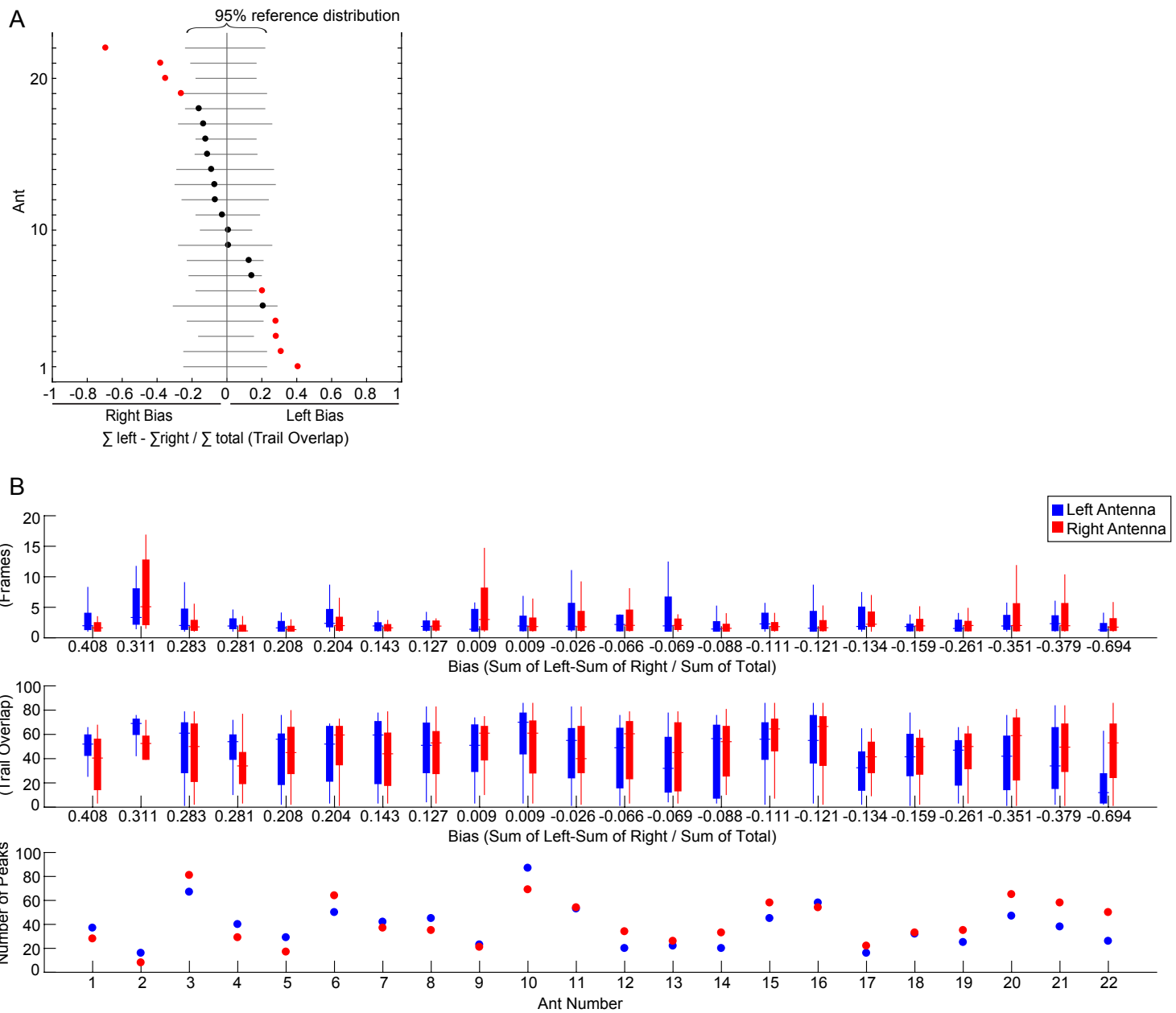
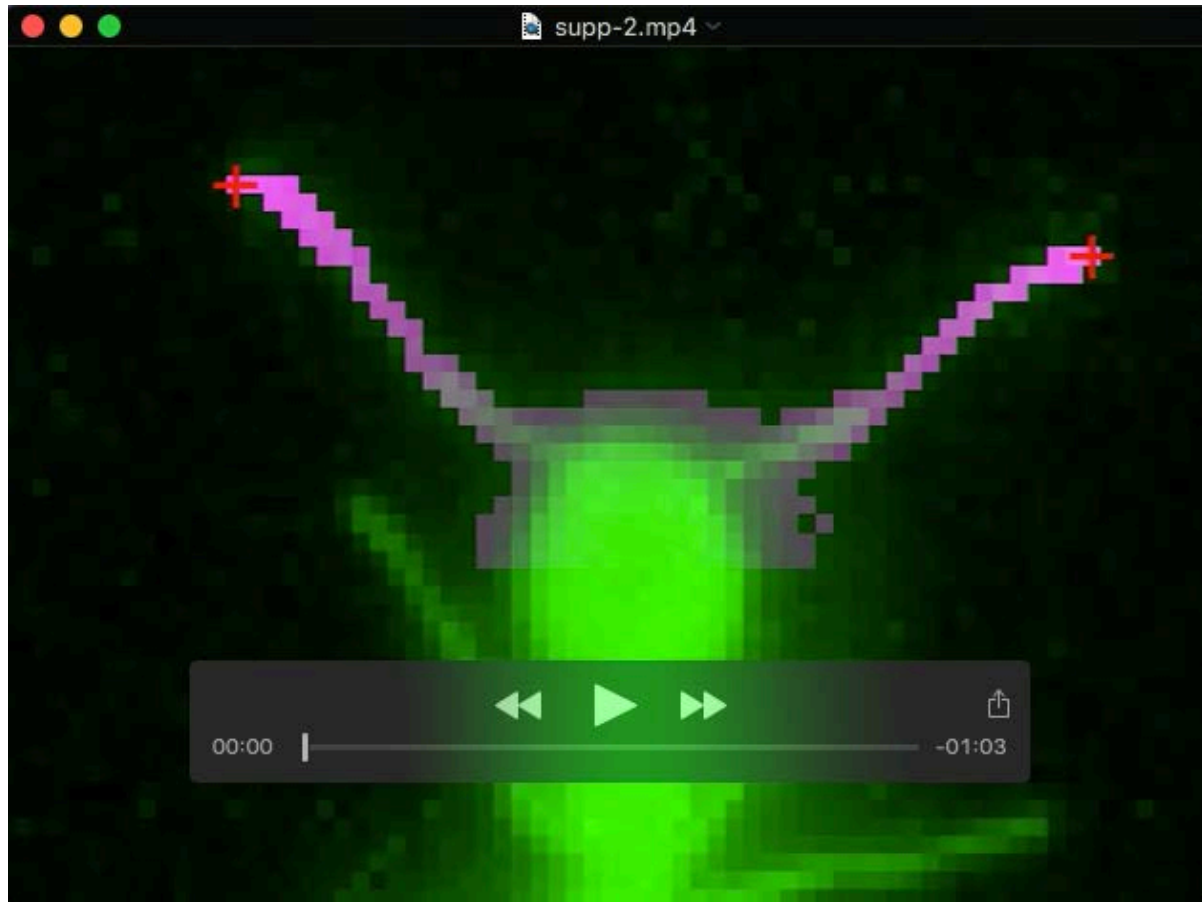


Figure S6. Analysis of left-right bias among antenna pairs

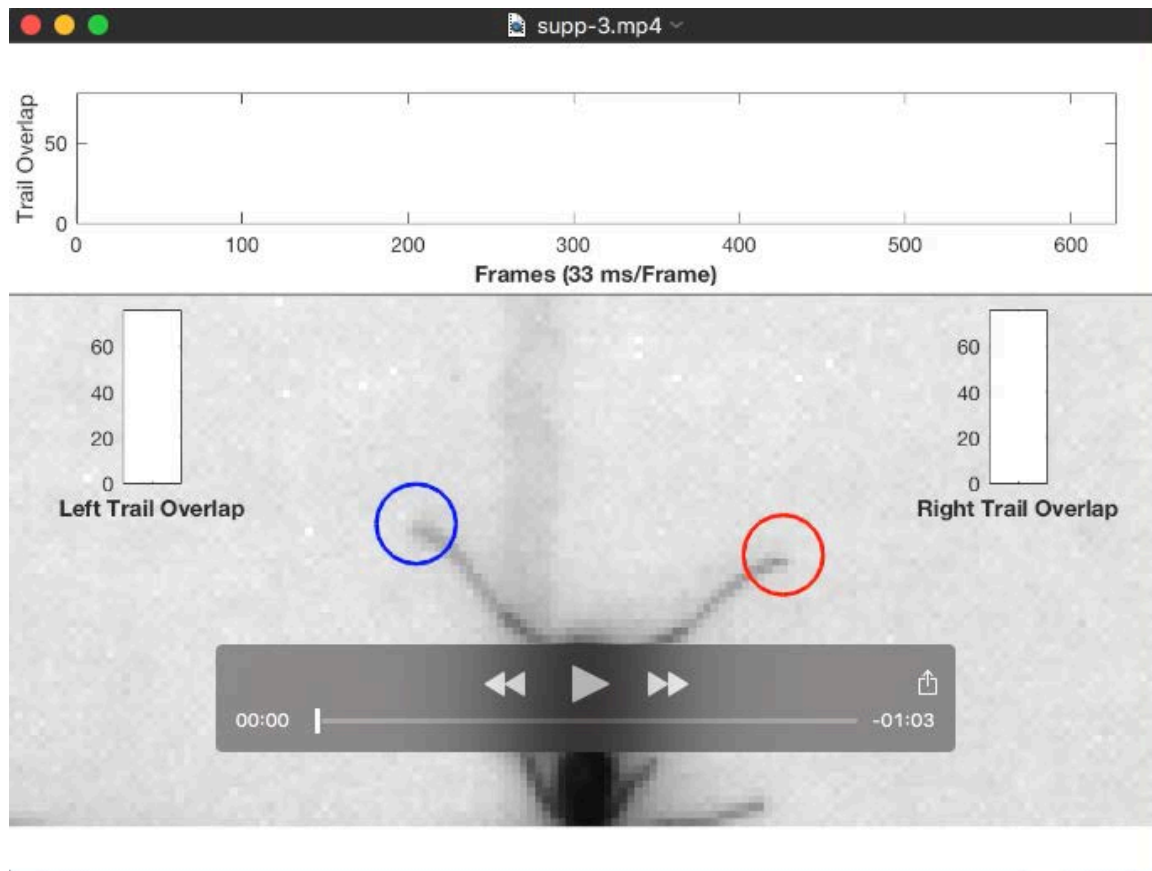
A: A bias index was defined as the relative difference of total trail overlap values between the left and right antenna of a single ant after tracking a straight-line trail ($n=22$ ants). Solid grey lines show the expected distribution of left-right bias for each ant that results from randomly distributing the sum of all trail overlap values (left and right) in blocks equal to the total number of discrete peaks in the ant trial (see Methods; 100,000 iterations per ant). The observed bias is shown as black and red dots (not significant and significant, respectively). 9 ants showed a dominant antenna ($p<0.05$, two-tailed, bootstrap distribution), with 5 and 4 ants showing a significant left and right bias, respectively.

B: The peak widths (top), heights (middle) and number (bottom) from left (blue) and right (red) antennae trail overlap data ($n=22$ ants). Ants differ in how each of these variables contribute to the total trail overlap values of the left and right antennae.



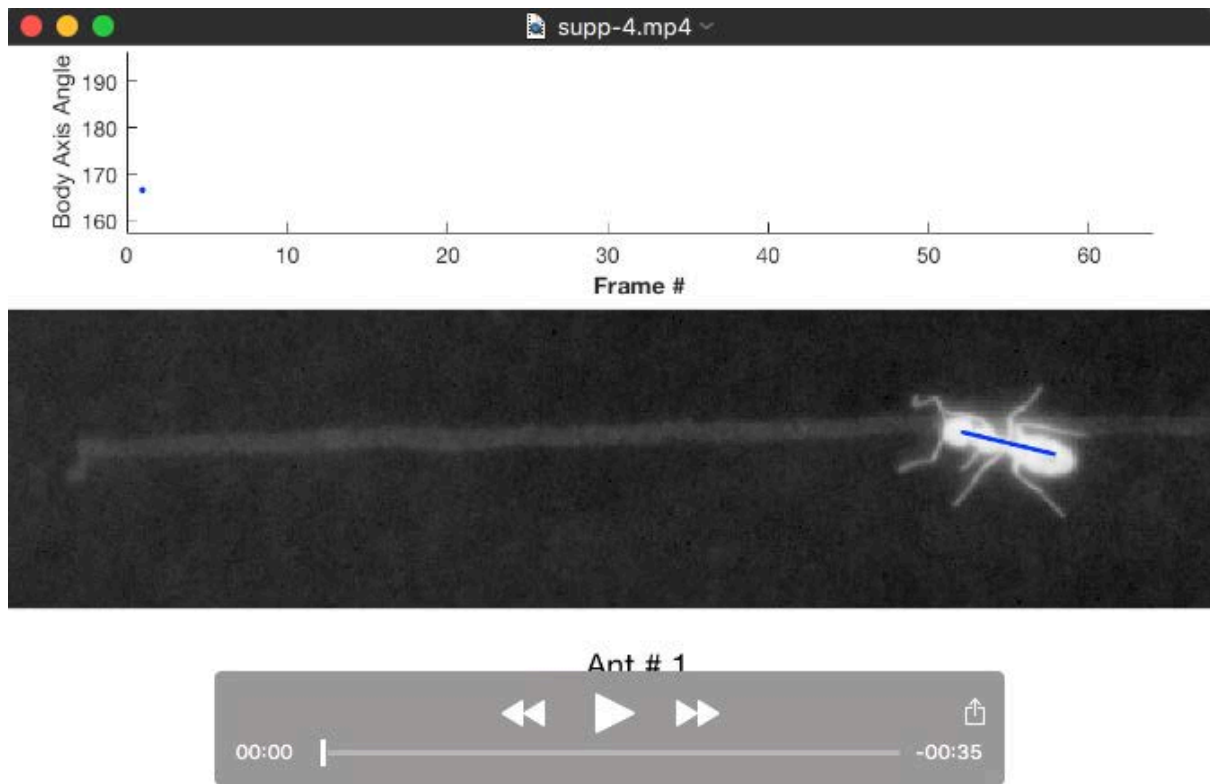
Movie 1. Antennae tip detection and marking.

A tracking trial (631 frames) was background subtracted (green), cropped, and aligned. A search window was defined in the area above the head centroid and limited to pixels with values greater than a dynamic threshold (purple). Within the search area, the longest paths from the head point were used to find the antenna tips (crosses). Data were checked for errors and manually corrected.



Movie 2. 'Trail overlap' during tracking.

A tracking trial was cropped and aligned. Blue (left) and red (right) circles display the approximate area used for 'trail overlap' measurement. Bar plots show the 'trail overlap' values per frame, and a line plot of these values over time is shown above.



Movie 3. Changes in body axis angle during Trail Following

Video clips (inverted for clarity) showing four ants during Trail Following Behavior. Periodic changes in the body axis towards and away from the trail can be seen. Body angles were plotted and color-mapped to the maximum (red) and minimum (blue) angles during each bout.