Dynamic antennal positioning allows honeybee followers to decode the dance

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Report

Dynamic antennal positioning allows honeybee followers to decode the dance

Highlights
- Nestmates follow dancers from varied and changing positions on the comb
- Their antennal positioning correlates with their angle to the dancer
- This can compensate for their changing relative orientation
- A circuit in nestmates can use their antennal positions to recover the dance vector

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In brief
On a vertical comb in a dark nest, bees perform the waggle dance to signal a flight vector to food, informing the direction and distance from the hive. Hadjitofi and Webb show how a neural circuit in nestmates can interpret the dance vector from any position around the dancer, using the angle of their antennae to detect their relative orientation.

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Dynamic antennal positioning allows honeybee followers to decode the dance

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REPORT

The honeybee waggle dance has been widely studied as a communication system, yet we know little about how nestmates assimilate the information needed to navigate toward the signaled resource. They are required to detect the dancer’s orientation relative to gravity and duration of the waggle phase and translate this into a flight vector with a direction relative to the sun and distance from the hive. Moreover, they appear capable of doing so from varied, dynamically changing positions around the dancer. Using high-speed, high-resolution video, we have uncovered a previously unremarked correlation between antennal position and the relative body axes of dancer and follower bees. Combined with new information about antennal inputs and spatial encoding in the insect central complex, we show how a neural circuit first proposed to underlie path integration could be adapted to decoding the dance and acquiring the signaled information as a flight vector that can be followed to the resource. This provides the first plausible account of how the bee brain could support the interpretation of its dance language.

RESULTS

Dynamic variation of relative body axes during following

Von Frisch originally termed nestmates around the dancer “followers” due to their apparent motivation to follow behind the general path of the dancer. An early assumption was that nestmates could determine the signaled direction by aligning their body to be parallel with the dancer’s. This “follow-behind” idea is consistent with the behavior of primitive bee species: experienced stingless bees lead the inexperienced to the resource, and horizontal open-nesting honeybee species (Apis florea) dance directly toward the resource with respect to the sun. Cavity-nesting species instead dance relative to gravity, such that the angle on the vertical comb correlates with the direction relative to the sun upon leaving the hive. Clear evidence has since emerged that nestmates can also assimilate the information from apparently arbitrary positions around the dancer. We filmed native Scottish black bees, A. mellifera mellifera, to study the positioning of follower bees around dancers in the hive (Figure 1A). Nestmates were defined as following a dance if facing toward the dancer and within one bee’s length. We observed that follower bees were positioned all around the dancer. Most exhibited significant changes in the angle of their body axis relative to the dancer throughout a waggle phase (Figure 1B). Plotting this angle over time as a path, straightness values (straight-line distance divided by path length, i.e., consistency in maintaining the same angle to the dancer) were observed across almost the entire range of Batschelet’s straightness index (Figure 1B). Nestmates that started to follow from particular angles (e.g., behind the dancer) were not any more consistent than those at other angles. Few nestmates appeared truly consistent at maintaining an angle and these tended to follow for a short time (Figure 1C).

It has been proposed that mechanosensory input via the antennae allows nestmates to assimilate the dance vector information when not aligned behind the dancer, a possibility known as the tactile hypothesis. Nestmates appear to position themselves at a distance where their antennae receive repeated contact with the dancer as it waggles by. Actual contact appears passive; that is, nestmates do not actively touch the dancers with their antennae but instead are simply hit by the body moving past and exhibit no signs of collision avoidance. Recent work has also shown that genes are upregulated in the antennae of bees that use dance information compared with private information of route memories acquired during previous foraging trips. Using the antennae to obtain the information when positioned at any angle to the dancer should provide several advantages. It alleviates the constraint of limited space around and behind the dancer. It does not rely on visual input, allowing communication in the darkness of a cavity nest, and removes any need for accurate positioning capabilities of a follower bee in relation to a dance. But how could antennae be used to detect the actual angle of the dancer relative to gravity from an arbitrary—and changing—relative angle between dancer and follower?

A relationship between antennal positioning and angle to the dancer during waggling

We first tested whether antennal positioning could be used by nestmates to distinguish their angle relative to the dancer by tracking the antennae positions of followers in high-speed, high-resolution video. Compared with their movement
Figure 1. Positioning of follower bees and their antennae when following waggle phases

Data from 47 bees across 59 total phases.

(A) Measuring the angle of follower bees’ relative body axes (blue) with respect to the dancer (gray) when following the waggle phase.

(B) Batschelet’s straightness index18 of bees’ angles to the dancer treated as a path over time. A constant angle would have a value of 1.

(C) Stable (index > 0.75) and changing (index < 0.5) example paths colored by their straightness index.

(D) Measuring the angle of a bee’s left (green) and right (pink) antenna relative to its body axis (blue). Angles of the left antenna are denoted as positive angles and the right antenna denoted as negative.

(E) Angle of nestmates’ antennae when positioned around the dancer. As indicated by the axis to the left, the distance from the origin indicates the antennal angle at an angle to the dancer. The two circular boundaries indicate antennal angles of ± 45° and ± 90°. Dots indicate the circular mean of left and right antennae angles computed across nestmates in 5° bins of angles to the dancer; the shaded area represents mean ± SD. Dancers’ directions have been normalized to 0°.

(F) The mean midpoint of nestmates’ antennae (gray) along with the same data as in (E). Data computed in 15° bins of angle to dancer; shaded area represents mean ± SD. Midpoint data circular correlation coefficient of −0.56 (F = −56.76, p < 0.001).

(G) Real nestmates’ antennae when positioned at 45° (left), 180° (middle), and −45° (right) to the dancer.

See also Figures S2 and S1.

elsewhere in the hive, when nestmates approach a dancer, they exhibit a notably consistent posture, with antennae evenly outstretched from their midline and their roll and pitch head orientation aligned with the plane of the comb (Figure S1). The angle of the antennae could hence be estimated as the angle from base to tip (or to its halfway point, see Figure S2). We identified that the position of nestmates’ antennae relative to their midline followed a unique relationship according to their positional angle to the dancer (Figures 1D–1G). When positioned on the left side of the dancer (e.g., between 0° and +90°), the left antenna (green) is angled further away from the nestmates’ midline, whereas the right antenna (pink) is angled much closer (Figure 1D). A similar but opposite effect is seen when on the right of the dancer (e.g., between 0° and −90°), with a smooth transition in between. We also found a statistically significant circular correlation coefficient of −0.56 for the relationship between the midpoint between nestmates’ antennae and their angle to the dancer (p < 0.001, Figure 1F). Similar results were observed if the angle was estimated using the halfway point along an antenna instead of its full length (Figure S2) and is consistent with early experiments showing that clipping the tips of both antennae did not reduce the success of recruited follower bees. Previous work19 recorded the angle formed between the two antennae (rather than the angle of each relative to the head) at the end of each waggling movement but did not identify a consistent relationship of this angle to the follower’s angle to the dancer. Our recordings at 200 frames per s (fps) captured around eight frames per waggle of the dancer’s abdomen and indicated that the change in antennal position with the angle to the dancer is continual across waggling movements.

Proposed dance vector assimilation by the central complex

This antennal relationship could allow the nestmate to detect its orientation relative to the dancer, but how could this be transformed into a flight vector toward food? Recent research6,7 has shown that the insect central complex contains circuitry that supports the transformation of angular variables from egocentric (body-centered) to allocentric (world-centered) coordinates. Optic flow-sensitive neurons (noduli tangential neurons [TNs] in the fruit fly6,7) fire proportionally to the velocity component in directions of ± 45° and ± 135° around the insect. This motion appears to modulate the relative amplitudes of left and right (for front and back) sinusoidal bumps of activity in the brain that are otherwise yoked to the insect’s head direction. Sinusoidal activity across a neural population can represent a flight vector, where the phase encodes the angle of the vector and the amplitude represents its length. The projection pattern of these neurons to the fan-shaped body in the central complex supports
Feeding real antennae data to the assimilation circuit

We used the real set of antennae positions from each follower bee as input to the assimilation circuit to measure the directional accuracy of the assimilated foodward vectors (Figure 4; see also Video S1). We assume that the integration starts on the first frame that the nestmate met the follower criteria and the dancer was waggling and ends on the last frame they met the criteria or the dancer stopped waggling. Note that this inherently produces a correlation between the length of the integrated vector and the waggle duration, implicitly encoding distance. In the bee, such gating of the integration could occur in the central complex. We suggest that the input could come from an auditory interneuron that has been observed to have stopwatch-like behavior encoding sounds emitted by the dancer during the waggle phase, the duration of which signals distance to the resource. Lacking information about this sound signal in our recordings, and also lacking “ground truth” for the intended distance communication, our analysis focuses on the
direction of the vector estimate rather than the length. However, we note that our inclusive estimate of the waggle could also contribute to error in the direction that could be reduced by more precise gating of the integration period.

We compared the performance of the circuit with the performance predicted by the simple follow-behind hypothesis by also calculating the vectors that would be assimilated if follower bees solely used their heading relative to gravity to assimilate the vector (i.e., no antennal modulation). We found that simulated nestmates’ estimated the average direction toward food more accurately when using antennal information than when estimating based on their own orientation (p < 0.005, Figure 4A). Accuracy was similar, if we used information from both antennae, the midpoint of the antennae, or just the left or right antenna. Angular error was largest for nestmates positioned anterior to the dancer and lowest for those positioned behind (Figure 4B). Although some bees appear motivated to more actively pursue the dancer around the comb and others do not, the accuracy of assimilation when using antennal information was independent of whether nestmates exhibited a changing or stable angle relative to the dancer during following (Figure 4C). Moreover, the nestmates’ angles to gravity also gave no apparent advantage to assimilating the foodward direction (Figure S4A). The angular error of each nestmates’ vector estimates successively throughout following a waggle phase are shown in Figure 4D. Utilizing antennal positioning noticeably reduced the overall spread of recruits’ flight vectors and results in search flights that would be centered on the food source and fanned out in directions across the correct hemisphere containing the food (Figure 4D, right).

Figure 3. A central complex circuit to recover the allocentric dance angle
(A) Relationship of the angle of a nestmate’s antennae to their angle to the dancer used in testing the circuit.
(B) Frontal view of a 3D reconstruction of the bumblebee Bombus terrestris brain, with central complex neuropils illustrated in green (obtained from the insect brain database, www.insectbraindb.org; data from Rother et al.34).
(C) Example cell activations in the assimilation circuit for a nestmate positioned at 147° relative to the dancer. Cells in the left and right sides of the protocerebral bridge (PB) (green and purple, for front and back) receive inputs from head direction cells in the ellipsoid body (EB) (blue) of the central complex, which are multiplied by mapped antennal input from the noduli (NO) (orange), resulting in amplitude modulation. Summing the four PB cell populations results in a vector that represents the signaled foodward direction relative to gravity in the fan-shaped body (FB) (yellow). A schematic illustration of the central complex neuropils is shown in the top left. Circuitry adapted and inspired by Lyu et al.7 Connections between the EB and PB backward-sensitive cell populations are not shown for brevity.
(D) Example antennal positions mapped onto noduli cell activity, as a result of different orientations of the follower bee relative to the dancer.
(E) Example activity patterns of cell populations in (C) simulated for a nestmate (dark gray bee) changing orientation while following a dance (light gray bee). Dashed line indicates the target allocentric orientation of the dancer.
(F) Angular error of accumulated foodward vectors (n = 25) resulting from (left) perfect simulation of relationship and (right) simulation with noise added to antennae at each time step, drawn from von Mises distribution on right (μ = 0 and κ = 10).
See also Figures S3 and Video S1.
Previous work suggests that recruited nestmates fly in a direction that more closely resembles an average of many waggle phases, rather than any single phase.\textsuperscript{40} In line with this, while the length of time that nestmates followed an individual phase showed no effect on accuracy (Figure S4B), we found that averaging the final vectors that nestmates assimilated at the end of consecutive waggle phases led to a significant reduction in the error ($\rho < 0.05; \ast$ if $\rho < 0.01; \ast\ast$ if $\rho < 0.001$). (B) Mean absolute angular error according to nestmates’ angles to the dancer computed for 15° bins when using midpoint feature; shaded area represents mean ± SD. (C) Absolute angular error according to nestmates that have more stable (straightness index > 0.5, $n = 22$) or changing (index < 0.5, $n = 37$) angles to the dancer. (D) Angular error of nestmates’ vectors shown for each time step against the foodward direction when using gravity heading (left) or midpoint feature (right). (E) Absolute angular error of the final vector for nestmates that only followed one waggle phase (“first,” $n = 38$) or when the final vector is averaged for nestmates that followed more than one consecutive phase (“averaged,” $n = 12$). (F) The probability of nestmates ($n = 27$) changing sides of the dancer when transitioning from “this” to the “next” waggle phase. L and R indicate left and right sides of the dancer, respectively; $\chi^2$ test of independence: $p = 0.001$. (G) Foodward vectors from a nestmate following three consecutive waggle phases. Thin lines indicate the error at each time step; thick lines indicate the final vector for that phase; blue is the vector that arises if averaging across waggle phases. See also Figure S4 and Video S1.

**DISCUSSION**

We have reported a novel feature of the antennal positioning of bees following a dance: that it correlates to their angle to the dancer. Combining this with recent insights into the vector-processing circuitry of the central complex, we proposed a plausible mechanism by which the follower could assimilate a vector indicating the resource being advertised. Specifically, supported by recent characterization of pathways between antennal input and the central complex,\textsuperscript{4,5,33} we suggested antennal positioning during the dance maps to an orthogonal basis vector representation known to exist for other cues: wind\textsuperscript{4} (also sensed via the antennae) and optic flow.\textsuperscript{7} Then, following the previously proposed principle for using such a basis to map head direction to allocentric ground velocity and integrate this into a home vector,\textsuperscript{23} we showed through modeling that, in principle, the same neural circuit could be used to integrate the dancer’s direction relative to gravity, forming a food vector.

In this work, we have tacitly assumed that the nestmate’s antennal position is a consequence of its angle to the dancer, e.g., the antennae are passively deflected further by the dancer’s
body when at more extreme angles to it. However, we cannot rule out that some other sensory cue provides information about this angle and leads the nestmate to actively adopt different antennae positions. Indeed, it seems likely that the mechanosensory experience of the bee, either through other antennal cues such as the force exerted on them, or other tactile, wind, or electric-field-sensing systems, is substantially richer than the one simple kinematic measurement we have made. The integration of such cues (although difficult to measure) could significantly improve the estimate of the angle, and consequently the accuracy of the vector. There are many additional sensory cues emitted by the dancer that likely play a role in communication, including olfactory and chemical cues, temperature, and comb vibrations. These may assist nestmates in identifying, localizing, and broadly orientating toward dancers within an area of the hive but seem unlikely to provide accurate information about relative angles. We note that gravity is most likely detected through mechanosensors (e.g., antennae in Drosophila, and sensory neurons of the neck hair plates in bees). We would hypothesize that in the central complex, the mechanosensory pathway via the ring neurons would subserve the formation of an egocentric gravity bump whereas the pathway via the noduli to the fan-shaped body would subserve the orthogonal basis representation that transforms this to an allocentric direction estimate.

It is difficult to ascertain what accuracy should be expected for the nestmate’s assimilation of the vector. The unpredictability of the dispersal and quality of foraging patches for bee colonies may reduce the evolutionary pressure to minimize communication errors. Determining the true nature of the information transfer requires a sophisticated setup: (1) the foraging of prospective dancers constrained to a known location, (2) the identity and behavior of both the dancers and naive followers recorded in detail in the hive, and (3) accurate tracking of every follower bee’s immediate subsequent search flight, ideally after displacement so that only vector information can be used to direct their path. Historically there have been several attempts to characterize the accuracy of information transfer, e.g., from the distribution of recruited nestmates on an arc of feeders surrounding the one to which the dancer was trained.

Alternatively, harmonic radar has been used for tracking flight behavior. We have plotted open-source data from Wang et al. showing recruits’ final vector search positions obtained from the dancer to a previously unknown feeder (Figure S4E). We note that the recruits are scattered in varied directions (and distances) centered on the feeder, and we believe that it is plausible that such a distribution could arise from the base model described in this paper with appropriate vector-averaging over consecutive waggle phases or augmented with additional mechanosensory cues (Figure S4E). Moreover, to date, studies have not linked search accuracy to what an individual bee experienced in the hive or to the experience of followers that find the resource versus those that do not.

We have not addressed the other side of this communication, i.e., how the dancer produces a waggle in the appropriate direction and of appropriate duration. A working hypothesis is that this results from a replay in miniature (and in the gravity reference frame) of a return flight to food. We have previously described how such return flights could be produced by the central complex steering circuity if the bee can store, and selectively recall, a “vector memory,” in the form of a snapshot of the state of its path integrator when food is encountered. The same mechanism could be used with a food vector acquired from dance following (as described in this paper) to allow the nestmate to fly to the food for the first time.

This work only provides proof of principle: given the follower’s antennal positions as input, a model of the neural circuitry of the central complex could recover the vector signaled by the dancer. It is currently beyond the state of the art to directly measure neural activity in actively dancing bees or their nestmates at the level of detail needed to test our hypothesis for assimilation of the vector. However, some new developments are bringing this possibility closer and our model predicts a particular convergence of sensory pathways in the central complex. Experiments in other insects, such as Drosophila, might meanwhile provide evidence for (or against) some of our key predictions: that a vertically oriented insect experiences a gravity orientation bump in the ellipsoid body and protocerebral bridge and that antennal inputs can modulate the processing of an orientation bump in an equivalent co-ordinate transform process as seen for optic flow inputs to the fan-shaped body.

STAR METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - Lead contact
  - Materials availability
  - Data and code availability
- EXPERIMENTAL MODEL AND SUBJECT DETAILS
- METHOD DETAILS
  - Honeybee data
  - Rate-based assimilation model
- QUANTIFICATION AND STATISTICAL ANALYSIS
  - Analyzing changes in body orientation
  - Multivariate Gaussian Process regression
  - Model performance
  - Transition counts
  - Analysis software
  - Statistical tests

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.cub.2024.02.045.

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AUTHOR CONTRIBUTIONS

Conceptualization, data collection, data curation, formal analysis, funding acquisition, methodology, software, visualization, and writing, A.H.; conceptualization, funding acquisition, project administration, supervision, and writing, B.W.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES


STAR METHODS

KEY RESOURCES TABLE

<table>
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<th>REAGENT or RESOURCE</th>
<th>SOURCE</th>
<th>IDENTIFIER</th>
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</thead>
<tbody>
<tr>
<td>Deposited data</td>
<td></td>
<td></td>
</tr>
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<td>Raw and analyzed data files</td>
<td>This paper</td>
<td>Figshare: <a href="https://doi.org/10.6084/m9.figshare.24715977">https://doi.org/10.6084/m9.figshare.24715977</a></td>
</tr>
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<td>Wang et al.52 open source harmonic radar data (see Figure S4)</td>
<td>Cited paper: Wang et al.52</td>
<td>Gallistel and Beetz57</td>
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Experimental models: Organisms/strains

| Apis mellifera | Wild honeybees, scotland | RRID: NCBITaxon_7460 |

Software and algorithms

| Custom scripts for data processing and model simulation | This paper | [https://github.com/annahadji/dance2vec](https://github.com/annahadji/dance2vec) |

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Anna Hadjitofi (a.hadjitofi@ed.ac.uk).

Materials availability

This study did not generate any unique reagents.

Data and code availability

- Bee antennal data is deposited at Figshare68 and publicly available as of the date of publication.
- Original code will be publicly available on GitHub ([https://github.com/annahadji/dance2vec](https://github.com/annahadji/dance2vec)) as of the date of publication. The code contains the necessary elements reproduce results in this paper (e.g. model simulation, processing of raw antennal data).
- Any additional information required to reanalyze the data reported in this paper is available from the corresponding author upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Dance communication behaviour was studied in A. mellifera mellifera honeybee workers (female) kept in a three-frame observation hive.

METHOD DETAILS

Honeybee data

Data collection and labelling

Honeybee data was collected during late summer (August 2022) and complied with the ‘Principles of Animal Care’, publication No. 86-23, revised 1985 by the National Institute of Health, and with the current laws of the country in which the bees were kept. We filmed a colony of A. mellifera mellifera bees housed in a three-frame observation hive. Waggle dances of bees foraging at natural food sources were filmed at 200 fps under infra red illumination (690 nm), using a Basler acA1440-220UM ace camera and a 6 mm lens. The beginning and end of the waggle phase was determined by the first and last video frames where the dancer’s abdomen had swung to one side relative to its head direction (this is also accompanied by blurring or unblurring of the dancer’s wings, respectively). In each frame of the waggle phase, we extracted the angle of each follower bee relative to the dancer as well as the angle of their antennae relative to their body axis angle (also called “midline”). This was done by manually annotating key body parts using the DeepLabCut interface.59,60 A honeybee antenna is divided into three main sections: the scape, pedicel and flagellum. The scape is the longest segment and rises from the antennal socket. Attached to the end of the scape is the pedicel, a much shorter segment that forms an elbow-like joint that allows rotation in many directions. When visible, we labelled the centre point of a bee’s thorax as well as the base, halfway point and tip of their left and right antennae. In cases where there was a bend in the antenna (i.e. between scape and pedicel), this point was labelled instead of the halfway point.
Extracting angles

From labelled body parts, we determined the angle of each follower bee relative to the dancer, where the angle of a bee was defined using the straight line stemming from their thorax to the midpoint between the bases of the left and right antenna. The angle of an antenna relative to the midline was determined using the straight line stemming from the base to the tip of the antenna, or the base to the halfway point (or bend) in the case the tip was not visible. We depict these using negative angles for a clockwise rotation (in degrees). For example, 0° corresponds to the situation in which the follower faces towards the dancer’s head in the case of body axis orientation, or an antenna being directly aligned with the follower bee’s own body axis. 90° refers to the follower facing eastward towards the dancer or an antenna being positioned 90° to the left relative to their body axis (see Figure S2A for an example of how we compute and present these angles on a polar plot). The bees’ heading relative to gravity is also determined from the angle of their body axis relative to the vertical axis (both the hive and filming rig were aligned beforehand using a spirit level and weighted string). Follower bees were also tracked over as many consecutive waggle phases that they followed, where their angles, identity and the side they followed from were recorded. The final dataset included the data of a total of 47 follower bees as they followed waggle phases of a dance. Each follower was measured from the start to end of their time following each waggle phase, with the minimum being 0.3 s and the maximum being 2 s (the full length of a waggle phase in the dataset).

Rate-based assimilation model

Antennal input

The angles of the left and right antennae (θL and θR) are first determined with respect to the nestmate’s heading (described above in extracting angles section) and clipped between a minimum and maximum angular position determined by the data. In our case, this is between 0° and ±90°,

\[ 0 \leq \theta_L \leq \frac{\pi}{2} - \frac{\pi}{2} \leq \theta_R \leq 0 \]  
(Equation 1)

The model uses the relative positions of the antennae whilst following the waggle phase to differentially modulate four sinusoidal gravity bumps to allow nestmates to charge their flight vector in the foodward direction (Figure 3). To determine the modulation, features of antennal position are mapped onto the activity of four neurons (two in each hemisphere), corresponding to peak sensitivity at 45°, −45°, 135° and −135°, respectively. These are based on the optic flow sensing cells in the noduli – TN cells in the sweat bee23; LNO in the fruit fly6,7 – which fire proportionally to the velocity component in their preferred directions, modelled as a dot product. The antenna-induced output (AIO) of these cells is defined by,

\[ f(x) = [\cos x, \sin x] \]  
(Equation 2)

\[ g(\theta_{\text{pref}}) = \begin{bmatrix} f(\theta_{\text{heading}} - \theta_{\text{pref}}) f(\theta_{\text{heading}} + \theta_{\text{pref}}) \end{bmatrix} \]  
(Equation 3)

\[ \text{AIO}_{\text{front}} = g \left( \frac{\pi}{4} \right) \quad \text{AIO}_{\text{back}} = g \left( \frac{3\pi}{4} \right) \]  
(Equation 4)

where \( \theta_{\text{pref}} = \frac{\pi}{4} \) and \( \theta_{\text{pref}} = \frac{3\pi}{4} \) are the offset preference angles for each pair of front (±45°) and back (±135°) sensitive neurons respectively, and \( \phi_L \) and \( \phi_R \) are antennal features (described below) for the left and right sides. To test whether the accuracy of vector assimilation varied when different features were used (Figure 4A), the activity of these neurons was determined using the midpoint of the antennae, where we set \( \phi_L = \phi_R = s(\mu) \); information from the left antenna only, \( \phi_L = \phi_R = s(\theta_L) \); right antenna only, \( \phi_L = \phi_R = s(\theta_R) \); and information from both, \( \phi_L = s(\theta_L), \phi_R = s(\theta_R) \).

\[ \mu = \text{atan} 2 \left( \sum_{i \in \{L,R\}} \sin \theta_i, \sum_{i \in \{L,R\}} \cos \theta_i \right) \]  
(Equation 5)

\[ s(x) = \theta_{\text{heading}} + \lambda x \]  
(Equation 6)

where \( s \) is a mapping function to scale the range of possible antennae positions to a range of angles relative to the dancer. We set \( \lambda = 4 \) to map to a 360° range of possible angles (shown as ±180°). We also investigated other maximum antennae angles, e.g. down to ±60°, to test the limits of the relationship; these led to more variable vector estimates, in particular when using information from the left or right antenna only, or both (Figure S4C).

Gravity bump

The head direction is calculated relative to gravity (described above in extracting angles section) and represented as a sinusoidal bump of activity, akin to the head direction bump seen in the ellipsoid body and known to be influenced by celestial,27 visual28 and proprioceptive29 cues. At each time step, the model creates a sinusoidal encoding of the heading (\( \text{Bump}_{\text{gravity}} \in \mathbb{R}^8 \)) across a population of eight neurons representing eight cardinal directions relative to gravity,

\[ \text{Bump}_{\text{gravity}} = \frac{1 + \cos (\theta_{\text{cardinal}} + \theta_{\text{heading}})}{2} \]  
(Equation 7)
Sinusoid modulation

Four copies of the $Bump_{gravity}$ are replicated in the protocerebral bridge. The amplitude of each is modulated proportionally by the corresponding projection of the angle to the dancer, inferred from the antennal input, onto the four $AIO$ projection axes. Hence there are modulated copies on the left and right sides of the bridge for both front ($Bump_{mod_{front}} \in \mathbb{R}^{16}$) and back ($Bump_{mod_{back}} \in \mathbb{R}^{16}$) $AIO$ axes,

$$Bump_{mod_{front}} = (AIO_{front} W_{AIO} \rightarrow Bump_{mod}) \odot (Bump_{gravity} W_{Bump_{gravity}} \rightarrow Bump_{mod})$$

(Equation 8)

$$Bump_{mod_{back}} = (AIO_{back} W_{AIO} \rightarrow Bump_{mod}) \odot (Bump_{gravity} W_{Bump_{gravity}} \rightarrow Bump_{mod})$$

(Equation 9)

where $\odot$ is element-wise multiplication, and $Bump_{gravity} W_{Bump_{gravity}} \rightarrow Bump_{mod}$ is the matrix representing the connectivity pattern between the $Bump_{gravity}$ and $Bump_{mod}$. See Figure S3 for connectivity matrices. These neurons are based on the populations of protocerebral bridge-fan-shaped body-noduli (PFN) neurons, PFN$_d$ and PFN$_r$, in the fruit fly. 7

Allocentric orientation of the dancer

Following observations by Lyu et al. 7 the projections of the $Bump_{mod}$ neuron populations from the bridge to neurons in the fan-shaped body result in anatomically shifted inputs that implement a vector sum, where the amplitudes and phases of the sinusoids correspond to the length and angles, respectively (Figure S3; see Lyu et al. 7 for a detailed description of the projection anatomy). This promotes sinusoidal activity across eight neurons that represent the allocentric bump relative to the dancer’s orientation ($Bump_{dancer}$), which accumulates in short-term memory ($Memory_{Bump_{dancer}}$) with a gain (gain, set to 0.005),

$$Bump_{dancer} = Bump_{mod_{front}} W_{Bump_{mod_{front}} \rightarrow Bump_{dancer}} + Bump_{mod_{back}} W_{Bump_{mod_{back}} \rightarrow Bump_{dancer}}$$

(Equation 10)

$$Memory_{Bump_{dancer}}^{t+1} = Memory_{Bump_{dancer}}^t + \text{gain} Bump_{dancer}$$

(Equation 11)

This bump is then decoded at each timestep $t$ (Figure 4D) using the fast fourier transform 61 and the error between the angle of the decoded vector and target vector is calculated. A positive error angle indicates that the assimilated vector is deviated to the left of the target angle (counterclockwise rotation) and a negative error indicates it deviates to the right (clockwise).

Quantification and Statistical Analysis

Analyzing changes in body orientation

To determine whether nestmates maintain a particular body orientation whilst following a dance, we constructed trajectories composed of their angles to the dancer along constant step-size points in time (i.e. viewing a trajectory as a stream of body axis angles for a bee). The Batchelet’s straightness index 18 was then computed as $D/L$, where $D$ is the straight line distance between the first and last points in the trajectory, and $L$ is the path length travelled (Figure 1B). The index ranges between 0 and 1, where 1 indicates a constant angle. The raw body axis angles of nestmates show a small oscillation due to the dancers’ waggles and the angle of nestmates being recorded relative to the dancer in each frame. We smoothed these oscillations in this part of the analysis by applying a Savitzky-Golay filter using the traja 62 Python library prior to computing the straightness index. The values of each nestmates’ index were later used to categorise them into stable and changing orientations using a threshold of 0.5 when evaluating the circuit’s performance (Figure 4C).

Multivariate Gaussian Process regression

We fitted a multivariate Gaussian Process regression model (see Schulz et al. 63) to see how well pairs of left and right antennae angles could be used to predict the angle of the nestmate relative to the dancer (Figure S2E). The cosine and sine transformations of the left and right antennae angles were used as features for the model. The data was divided into training and test sets using a 70 / 30 split, with individual nestmates featuring in only one set. Model fitting and optimisation of parameters was performed using the mgpr R library, 64 and predictions on the unseen test data were obtained. Pearson’s $r$ coefficients were calculated to evaluate the predictions.

Model performance

The neural model was evaluated based on the angular error of the decoded vectors relative to the signalled food (normalized to 0° across dancers) when using antennal positioning information or heading orientation relative to gravity as input (Figures 4 and S4). The latter assumes that follower bees assimilate the vector from their heading orientation only, as suggested by early hypotheses that nestmates vie for a position directly behind the dancer, 1 where their angle to the gravity equals the waggle phase foodward angle. For each nestmate, the mean absolute vector error was computed with respect to the mean orientation of the dancer over the period that the nestmate had followed for (Figures 4A–4C, 4E, S4A–S4C, and S4E). Figure 4D shows the signed error of the vector estimate for each time step. The mean error for a nestmate was determined by either using all intermediary vectors accumulated successively throughout following a waggle phase (Figures 4A–4E, 4G, and S4A–S4C) or only the final vector accumulated by the end of following a waggle phase (the vector stored in long-term memory) (Figures 4E, 4G, S4D, and S4E). The best vector for each follower bee is also
shown in Figure S4D (bottom) and taken to be the most accurate vector at any point during following a phase. The mean error of vectors averaged over consecutive waggle phases was computed as the circular mean of the final vectors assimilated from consecutive waggle phases (Figures 4E and S4E). For brevity, proceeding Figure 4A model results are only reported for the antennal midpoint feature along with the condition without antennal modulation.

**Transition counts**

The tendency for nestmates to switch sides of the dancer for consecutive waggle phases that they followed was determined by calculating the first-order Markov transition probabilities (Figure 4F). For example, the transition probability (TP) of following the dancer on her right side after just following on her left side (LR) was calculated as, \( TP_{LR} = LR / (LL + LR) \), where LR indicates the corresponding frequency counts for that sequence across the data and so on. The observed counts were evaluated using a chi-square test of independence, \( \chi^2 = 10.359, P = 0.001 \).

**Analysis software**

Data analysis and modelling was carried out using Python or R code, relying mainly on Numpy, Scipy, and Pandas libraries. Seaborn and Matplotlib were used for plots.

**Statistical tests**

All plots show mean ± SD unless stated otherwise. Circular equivalents were used when appropriate. Groups were compared using two-sided statistical tests, including Kruskal–Wallis \( H \) test (as data non-normal, tested by Levene’s test for equal variance and Shapiro-Wilk normality test) with post hoc Dunn’s testing for pairwise comparisons, and chi-square test of independence. The Wilcoxon signed-rank \( W \) test was used to compare groups of paired data (i.e. different antennal features or modulation conditions across the same bees), and for comparing more than two paired groups, the Friedman test was used with Bonferroni adjusted post hoc tests. Pearson’s correlations were used to investigate data and model fit relationships. \( P \) value ranges are reported in the figure legends, and we provide the full list of statistics here.

The following \( P \) values are associated with Figure 4A, comparing the angular errors obtained when no antennal information (here termed ‘without modulation’) against different features of antennal position (Friedman test \( \chi^2 = 32.66, P = 0.000014 \)): without modulation versus both antennae, \( P = 0.00011 \); without modulation versus midpoint, \( P = 0.00011 \); without modulation versus left antenna only, \( P = 0.0021 \); and without modulation versus right antenna only, \( P = 0.0028 \). The following \( P \) values are associated with Figure 4C, comparing the angular errors obtained for nestmates that exhibited more changing versus stable angles relative to the dancer for the midpoint antennal feature and without modulation: changing-midpoint versus changing-without modulation, \( W = 66.0, P = 0.000017 \); stable-midpoint versus changing-without modulation, \( H = 5.60, P = 0.018 \); and stable-midpoint versus stable-without modulation, \( W = 45.0, P = 0.0066 \). The following \( P \) values are associated with Figure 4E, comparing the angular errors calculated of bees that followed only one waggle phase versus the average final vectors from bees that followed consecutive waggle phases followed for the midpoint antennal feature and without modulation: first phase-midpoint versus average-without modulation, \( H = 5.47, P = 0.019 \); first phase-midpoint versus first phase-without modulation, \( W = 88.0, P = 0.000042 \); first phase-midpoint versus average-midpoint, \( H = 5.28, P = 0.022 \); average-midpoint versus first phase-without modulation, \( H = 7.61, P = 0.0058 \); and average-midpoint versus average-without modulation, \( W = 2.0, P = 0.012 \). All other comparisons were non-significant.