The Journal of Experimental Biology 208, 3885-3894
Published by The Company of Biologists 2005
doi:10.1242/jeb.01832

Encoding spatial information in the waggle dance

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Accepted 11 August 2005

Summary

Apis mellifera **bees execute waggle dances to recruit other bees to desirable food sources. Several components of the waggle dance are correlated with the direction of and the distance to food. Moreover, recruits use the spatial information encoded in the dance to locate the signalled food. However, although dance communication has been studied extensively, little is known about how the dancers combine the compass (direction) and the odometric (distance) information they acquire during the foraging flight. In the present study, we analysed the encoding of spatial information in the waggle dance by manipulating the navigational information provided to dancing bees. To this end, we took advantage of the bees' visually driven odometer. We found that the waggle dance basically encodes information on the distance gauged during the outbound (hive-to-food) flight. However, it does not necessarily refer to a global vector based on path integration of the outbound flight. Whenever the direction connecting the subjective food location and the hive does not match the direction of the global vector, dancers refer to a direction close to that of the shortcut connecting the actual food location and the hive. Moreover, in our experiments, this direction was close to that of the inbound (food-to-hive) flight, indicating that landmark-based information is computed during the inbound flight and that it may strongly affect the encoding of directional information in the waggle dance. Moreover, we found that the bees' experience of the terrain modulates the encoding of spatial information in the waggle dance, suggesting that interactions between path integration and visual landmarks are computed in the context of dance communication.**

Key words: *Apis mellifera*, waggle dance, spatial information, visual landmark, path integration.

Introduction

In the waggle dance, a dancing bee (*Apis mellifera*) executes fast and short forward movements straight ahead on the comb surface, returns in a semicircle in the opposite direction and starts the cycle again in regular alternation (each waggle dance involves several of these cycles; von Frisch, 1965). The straight portion of this course, called a waggle-run, consists of a single stride (Tautz et al., 1996) emphasized by lateral waggling motions of the abdomen. The length of the individual waggle-runs increases with the distance flown to reach the source (von Frisch and Jander, 1957; von Frisch, 1965), and their angles relative to gravity correlate with the direction of the foraging flights relative to the sun's azimuth in the field and sun-linked patterns of polarized skylight (von Frisch, 1965). Thus, by encoding the visually measured distance (Esch and Burns, 1995, 2001; Srinivasan et al., 2000; Tautz et al., 2004) and the direction towards the goal (Lindauer, 1963; von Frisch, 1965; Gould and Gould, 1988), the waggle dance allows colony members to share information about the location of desirable food sources (von Frisch, 1965; Seeley, 1995; Dyer, 2002). Moreover, based on these correlations, the waggle dance also allows analysis of internal representations of space (Lindauer, 1963; von Frisch, 1965; Srinivasan et al., 2000; Esch et al., 2001; Tautz et al., 2004).

Honeybees are remarkable navigators that display circuitous flight paths on the way to their goals as well as conspicuous shortcuts on the way back to their colonies (von Frisch, 1965; Wehner and Menzel, 1990; Dyer, 1998; Collett and Collett, 2002; Wehner and Srinivasan, 2003; Menzel et al., 2005). Like other central-place foragers (Müller and Wehner, 1988), they navigate by means of a celestial compass and landmark-based information (von Frisch, 1965; Dyer, 1998; Collett and Collett, 2002; Wehner and Srinivasan, 2003; Menzel et al., 1998, 2000, 2005). Certainly, bees recruited through the waggle dance fly the distance and direction encoded in the dance (Riley et al., 2005), and it is often concluded that a dancing bee communicates a flight vector pointing towards the indicated location. Nevertheless, little is known about how the compass (direction) and the odometric (distance) information acquired during the foraging flight are finally combined in the waggle dance.

First, an accurate indication of distance in the waggle dance presupposes that the dancer has correctly measured the flight length. We know that the retinal image flow that honeybees experience while flying provides them with some means of monitoring the distance travelled (Esch and Burns, 1995, 1996; Srinivasan et al., 1996, 1997, 1998, 2000; Esch et al., 2001; Tautz et al., 2004). However, although the flown distance seems to be primarily gauged on the way to a food source (Heran and Wanke, 1952; Heran, 1956; Otto, 1959; von Frisch, 1965; Srinivasan et al., 1997, 1998), honeybees also monitor distance information on the way back to their colonies (Otto, 1959; von Frisch, 1965; Srinivasan et al., 1997, 1998). Hence, evaluating the encoding of the visually measured distance in the waggle dance first requires analysing how the distance information available both on the way to the goal and on the way back to the colony is finally represented in the length of the waggle-run. Such analysis does not yet exist.

Furthermore, von Frisch (1965) designed some remarkable 'detour' experiments to analyse how the waggle dance encodes both compass and odometric information. In one of his experiments (reviewed in von Frisch, 1965), for instance, a group of foragers was guided around a jutting mounting ridge separating the food source from the colony. In their dances, the foragers indicated the straight line towards the goal (even when they followed the detour on the way back to the colony). Similar results were reported for bees flying indirect paths around a large building or a forest edge to finally reach an artificial source of food, i.e. they computed the direct line of the flight from the two legs of the detour, and this computed 'global vector' corresponded to the direction indicated in the waggle dance (von Frisch, 1965). Moreover, under these circumstances, the bees indicated the actual flown distances in their dances and not the distance of the shortcut towards the goal (i.e. a straight segment connecting the food source and the nest's entrance; von Frisch, 1965). Hence, dancing bees flying detour paths communicate the direction of a non-experienced shortcut together with the actual flown distance, thus reporting a subjective performance-dependent, but not a real, location. Since the bee's perception of the distance flown is scene dependent (Esch et al., 2001; Tautz et al., 2004), this subjective location can only be found if the recruited bees were to follow more or less the same flight path as the dancer. The simplest explanation about how a forager may indicate a non-experienced shortcut in its waggle dance relies on path integration, i.e. an internal process by which an animal continuously measures the distances and directions from a particular point and computes its current position in the environment (Mittelstaedt and Mittelstaedt, 1982; Wehner, 1992; Collett and Collett, 2002; Wehner and Srinivasan, 2003). Using path integration, for instance, *Cataglyphis* ants are able to establish global vectors at the end of their outbound (nest-to-food) paths, which allow them to follow straight trajectories of the appropriate distance and direction during their inbound (food-to-nest) paths (Wehner and Srinivasan, 1981; Müller and Wehner, 1988). Indeed, desert ants also use global vectors to follow straight trajectories from the nest back to a previously visited food site (Schmid-Hempel, 1984; Collett et al., 1999; Wolf and Wehner, 2000). In von Frisch's detour experiments described above, the direction of the global vector computed by path integration of either the outbound or the inbound flight always corresponded to the 'subjective' direction connecting the food site and the hive (von Frisch, 1965). Hence, experiments must still be performed to test the integration of the compass and the odometric information available throughout the different flight segments of the foraging bout (e.g. the outbound and the inbound flight) on the encoding of spatial information in the waggle dance.

In the present study, we took advantage of the bees' visually driven odometer (Srinivasan et al., 2000; Esch et al., 2001; Tautz et al., 2004) to manipulate the navigational information provided to a dancing bee. We then addressed two important questions: namely, (1) how the waggle dance encodes the subjective distance experienced during either the outbound or the inbound flight and (2) whether the encoding of directional information in the waggle dance is based on path integration of the outbound flight, i.e. what direction do the bees indicate in their dances if they experience a mismatch between the subjective food location, computed by path integration, and the actual location of the food site? Furthermore, since (1) honeybees navigate according to a map-like spatial memory (Menzel et al., 2005) and use both path integration and visual landmarks to solve complex navigational tasks (Dyer, 1998; Menzel et al., 1998, 2000, 2005; Wehner and Srinivasan, 2003) and (2) the simultaneous processing of path integration and visual landmarks may lead to changes in path integration coordinates depending on the animal's experience of the terrain (Etienne et al., 2004; Collett and Graham, 2004), we also analysed whether the bees' experience of the foraging area modifies the encoding of spatial information in the waggle dance.

Materials and methods

General methods

Honeybees (*Apis mellifera* L.) gauge the distance flown by means of the retinal image flow experienced while flying (Esch and Burns, 1995; Esch et al., 2001; Srinivasan et al., 1996, 1997, 1998, 2000; Tautz et al., 2004). When bees fly through narrow tunnels, the walls of which are decorated with random visual textures, they indicate a distance through their waggle dances that is much greater than that actually flown (Srinivasan et al., 2000; Esch et al., 2001). We trained bees to fly through such tunnels arranged outdoors in various configurations and monitored their dance behaviour. To this end, in a series of different experiments, individually marked bees were trained to forage at a feeder (offering unscented 1.8 mol l^{-1} sucrose solution) placed inside a 6 m-long, 30 cm-wide and 30 cm-high metal tunnel. The tunnel was installed outdoors, with its near end placed 3° NE and 129 m from the hive. Conspicuous landmarks were present in the study area. The walls and floor of this tunnel were decorated with a random visual texture (i.e. a random black-and-white Julesz pattern of pixel size 1 cm by 1 cm, as described by Srinivasan et al., 2000). The top of the tunnel was covered with UV-transmitting Plexiglas, which presented the bees with a view of the sky. A colony of *Apis* *mellifera carnica* bees was placed indoors in a two-frame observation hive with transparent walls. At the observation hive, the dances performed by the marked bees returning to the hive were video-filmed at 25 frames s^{-1}. Data were obtained by analysing the video recordings in slow motion. The hive was illuminated with diffuse light in order to not disturb the direction of the dances. For each waggle-run, we measured the duration of the waggle-phase (Tautz et al., 1996) in terms of the number of frames during which it occurred (Srinivasan et al., 2000). When the directional information encoded in the dance was analysed, the angle of the waggle axis relative to vertical (in degrees) was measured in parallel to the waggle-phase duration by using a protractor similar to that described by von Frisch (1965). The corresponding corrections according to changes in the sun's azimuth (von Frisch, 1965) were performed for the sake of comparisons. Data were analysed by means of linear regressions, one-way analyses of variance (ANOVAs), *t*-test for independent samples, Tukey comparisons, Raleigh tests and Watson *F*-tests for two circular means (Batschelet, 1981; Zar, 1984).

In each experiment, 6–10 bees were allowed to forage on a feeder placed ~2° NE and 125 m from the hive's entrance (close to the foraging area). The feeder offered unscented 1.8 mol l^{-1} solution for ~30 min. Next, 15–20 newly recruited bees were marked with numbered tags so that they could be individually identified (unmarked bees, as well as those marked bees whose behaviours had been previously recorded, were caged and released at the end of the experiment). Thus, in all the series described below (unless specified otherwise), animals were marked and recorded on the same day and lacked experience of flying inside the tunnel. The tunnel was absent in all series before the beginning of the single experimental sessions. In each of these sessions, it was set up (with its floor positioned 50 cm above the ground) only after a group of newly recruited bees was established. A second feeder (similar to the training feeder) was then placed inside the tunnel, 30 cm away from its entrance. Whenever the feeder was established at the near end of the tunnel (see below), it was stepped regularly from its initial position (at the near entrance) to its final position (at the very end of the tunnel). Recordings started approximately 90 min after the bees were presented with the tunnel. Experiments were conducted with an open view of the sky (i.e. no experiments were conducted when the sky was cloudy). Each recording session lasted approximately 3 h, and single marked bees performed a maximum of 30 foraging trips to the feeder per session.

Calibration of the odometer

Since the bee's perception of the flown distance is scene dependent (Esch et al., 2001; Tautz et al., 2004), we first recorded the waggle dances of returning bees foraging on outdoor feeders (offering unscented 1.8 mol l^{-1} sucrose solution) located 135, 248 and 360 m from the hive. We measured the durations of the waggle phases of the dances for each of these feeder distances. The results of these experiments provided us with information about the subjective distances experienced by the trained bees in our particular outdoor landscape.

The encoding of odometric information provided by either the outbound or the inbound flight

Two different situations were established in this series. In both situations, measurements were made with the tunnel oriented at 0°, with respect to the direct line connecting the near end of the tunnel and the hive's entrance. In the first situation, bees were trained to enter the tunnel at its near end and to leave it only *via* a small Plexiglas sliding door located at the top of the far end of the tunnel. Hence, they followed different flight paths during the outbound and the inbound component of the foraging bout, i.e. they flew inside the tunnel during their outbound flights but not during their inbound flights. By contrast, in the second situation, the bees were trained to enter the tunnel at its far end (where the feeder was located) and to leave it *via* its near end. Hence, they also followed different flight paths during the outbound and the inbound flight. However, in this case, the bees flew inside the tunnel only during their inbound flights. Comparisons between the duration of the waggle phase of the dances recorded for each of these two different situations were made on the basis of the subjective distances experienced in our particular landscape.

The encoding of compass information when a mismatch occurs between the subjective and the actual direction towards the goal

In this series, the tunnel was rotated 90°, either to the left or to the right, with respect to the direct line connecting the near end of the tunnel and the hive's entrance (Fig. 2). The feeder was then presented at the far end of the tunnel and the marked bees were trained to enter the tunnel at its near end. Two different experimental situations were then assayed for each orientation (90° to the left and 90° to the right). First, bees were allowed to leave the tunnel *via* the sliding door located at the top of its very end (see above), thus following different flight paths during their outbound and inbound flights. A mismatch was then established between the subjective location of the feeder, computed by path integration of the outbound flight, and its actual location. The angles of the waggle axes (relative to vertical) were measured in parallel to the waggle-phase durations. Second, attempts were made (see Results) to train the bees to leave the tunnel *via* its near end, thus following similar flight paths during their outbound and inbound flights.

The bees' experience of the terrain and the encoding of spatial information

Since trained bees were individually marked, their dance behaviour could also be evaluated according to their previous foraging experience in the tunnel. Hence, a comparison was made between the directions indicated in the dances of two different groups of bees flying through the 90°-oriented tunnel: (1) animals that lacked experience of flying regularly inside the tunnel and (2) animals that had previously foraged inside the

0°-oriented tunnel, ~24 h prior to the beginning of the recording session.

Results

Calibration of the odometer

Since the bee's perception of the distance flown is scene dependent (Esch et al., 2001; Tautz et al., 2004), we first evaluated the subjective distances experienced by trained bees in our particular outdoor landscape. Fig. 1A,C (open circles) shows the mean duration of the waggle phases of the dances for each of the outdoor feeders established during the preliminary phase of the experiment (see Materials and methods): the waggle-phase duration increased linearly with the distance flown. Linear regression of the data gave a correlation coefficient of 0.99 ($P<0.012$), and the slope of the regression line was 1.58 ms of waggle duration per metre of distance travelled. These results are in agreement with published data for comparable flight distances (von Frisch, 1965; Srinivasan et al., 2000; Esch et al., 2001; Tautz et al., 2004).

The encoding of odometric information provided by either the outbound or the inbound flight

Next, we evaluated the encoding of distance information provided by either the outbound or the inbound flight. Bees flying through the 0°-oriented tunnel during their outbound flights showed waggle-phase durations that lasted significantly longer than those performed by the bees that did not fly through the tunnel (Fig. 1A, black circle; $P<0.01$, Tukey comparisons; Fig. 2C, striped bar). The 6-m flight in the tunnel during the outbound flight was thus perceived as equivalent to an outdoor flight of 141 m (Fig. 1A, black circle; Fig. 2C, striped *vs* grey bar). This result is in close agreement with published data (Srinivasan et al., 2000; Esch et al., 2001). By contrast, bees did not overestimate the travelled distance when they flew

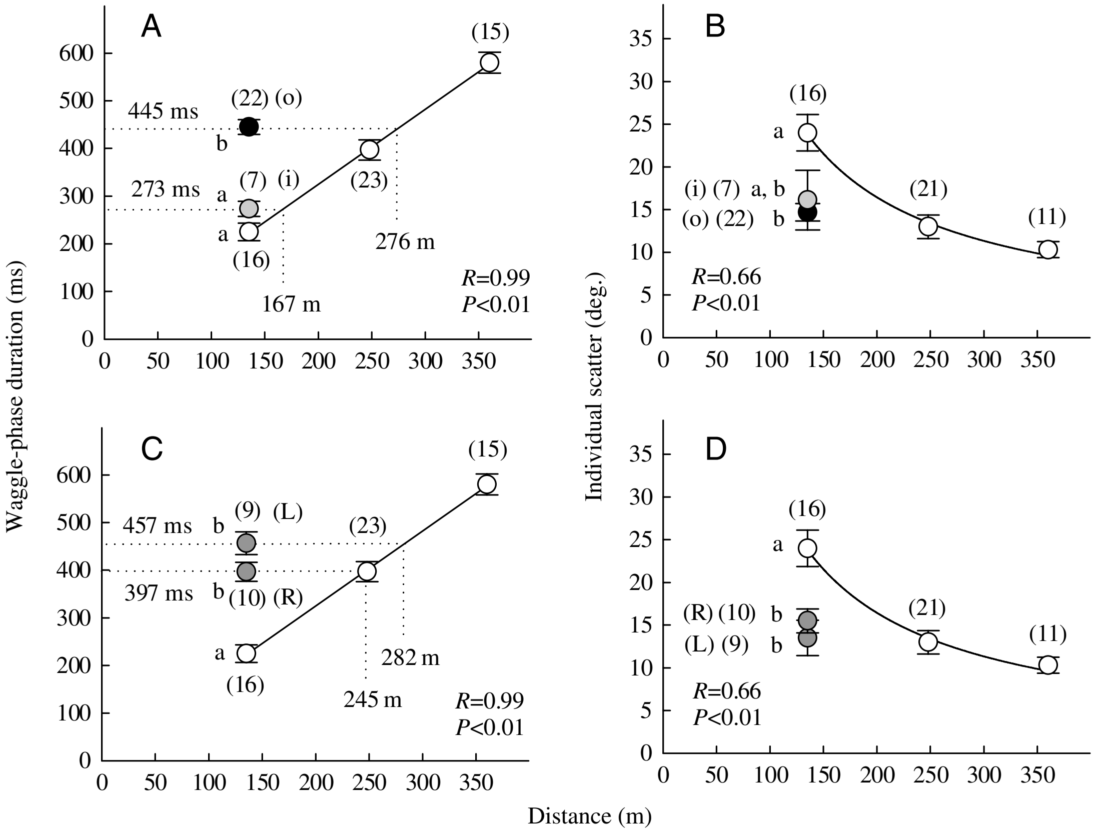

Fig. 1. (A,C) Mean waggle-phase durations (± S.E.M.) of dances elicited by outdoor feeders at three different distances (open circles). The straight line is a linear regression on the data ($R=0.99$, $P<0.01$), defined as: waggle phase duration=1.58×distance travelled+9.8. (A) Mean waggle phase durations (± S.E.M.) recorded in the experiments with the 0°-oriented tunnel (see Materials and methods and Results for details; black circle (o), outbound flight; grey circle (i), inbound flight). (C) Mean waggle-phase durations (± S.E.M.) recorded for the 90°-oriented tunnel (grey circles, (L), to the left; (R), to the right). Also shown are the mean waggle-phase durations measured in the tunnel experiments and their equivalent outdoor flight distances as read off from the regression line. (B,D) Individual scatter (in degrees) of successive waggle-runs measured throughout single waggle dances (mean ± S.E.M.) elicited by outdoor feeders at three different distances (open circles). Data fit an inverse polynomial function ($R=0.66$, $P<0.01$), defined as: individual scatter=1.1+3062.4×(distance)$^{-1}$. (B) Mean values (± S.E.M.) recorded in the experiments with the 0°-oriented tunnel (black circle (o), outbound flight; grey circle (i), inbound flight). (D) Mean values (± S.E.M.) recorded in the experiments with the 90°-oriented tunnel (grey circles, (L), to the left; (R), to the right). In all cases, different letters indicate statistical differences between groups (see Results for details). The number of animals analysed is shown in parentheses.

through the tunnel during their inbound flights, although a tendency was observed that indicated a slightly longer flown distance (Fig. 1A, grey circle; P=0.68, Tukey comparisons).

The encoding of odometric information in the 90°-oriented tunnel

Next, we evaluated the encoding of distance information when the tunnel was rotated 90°, either to the left or to the right, with respect to the direct line connecting the near end of the tunnel and the hive's entrance. Comparisons were made based on data from bees that entered the tunnel at its near end and left it *via* the sliding door located at the top of the very end of the tunnel, thus following different flight paths during their outbound and inbound flights. Under these circumstances, we found that the bees' subjective flight distance travelled inside the tunnel did not change statistically (Fig. 1C, grey circles; P=0.36, Tukey comparisons; Fig. 2F,I, striped bars). Bees flying through the 90°-oriented tunnel showed waggle-phase durations that lasted significantly longer than those performed by bees that did not fly through the tunnel, irrespective of the tunnel orientation (either to the left or to the right of the reference line; Fig. 1C, grey circles; P<0.01, Tukey comparisons). Moreover, the subjective flight distances recorded for both orientations of the tunnel did not differ statistically from those recorded previously for the 0°-oriented tunnel (Fig. 1A,C, black circle *vs* grey circles; 0° *vs* 90°-Left, P=0.99; 0° *vs* 90°-Right, P=0.52, Tukey comparisons; Fig. 2C,F,I, striped bars). Averaging the results obtained for the two different orientations showed that the 6-m flight in the tunnel was equivalent to an outdoor flight of 129 m (Fig. 1C, grey circles).

The directional scatter of the waggle-runs

A feature of the waggle dance is that consecutive waggle-runs are performed with some directional scatter, i.e. the directions of single waggle-runs usually diverge from each other, erring alternately to the right and left of the main vector (von Frisch, 1965). Early works demonstrated that the distance flown to reach the food source affects these divergences (von Frisch, 1965). Fig. 1B,D (open circles) shows the effects of the distance travelled in our particular landscape on the individual directional scatter obtained from the waggle dances for each of the recorded feeder distances. Data fit an inverse polynomial function (R=0.66, P<0.001) whose corresponding equation is $Y=Y_0+a(x)^{-1}$, in which Y corresponds to the individual directional scatter, x corresponds to the distance travelled, and Y_0 (1.1) and a (3062.4; P<0.001) are constants. Bees flying through the 0°-oriented tunnel during their outbound flights showed a directional scatter smaller than that corresponding to the same absolute distance travelled in the absence of the tunnel (Fig. 1B, black circle; P<0.002, Tukey comparisons). By contrast, the bees that flew through the 0°-oriented tunnel during their inbound flights showed a directional scatter that did not differ statistically from that recorded for the same absolute distance travelled in the absence of the tunnel (Fig. 1B, grey circle, P=0.18, Tukey comparisons). As in the case of the 0°-oriented tunnel, the bees that flew through the 90°-oriented tunnel (rotated either to the left or the right) also showed a directional scatter clearly smaller than that recorded for the same absolute distance travelled outdoor (Fig. 1D, grey circles, Left, P=0.01; Right, P<0.05, Tukey comparisons).

The encoding of compass information when a mismatch occurs between the subjective and the actual direction towards the goal

The global vectors (pointing toward subjective locations) computed by path integration of the outbound flight have different directions at the end of the different flight paths recorded throughout the experiments, i.e. those assayed with the tunnel oriented at 0°, 90° to the left and 90° to the right (see Materials and methods). According to early detour experiments by von Frisch (1965), these global vectors might provide the bees with the directional information encoded in the waggle dance. If that were the case, the expected directions to be signalled by dancers flying inside the 90°-rotated tunnel can be easily calculated by considering the actual food location and the subjective flight distance recorded for the 0°-oriented tunnel. These expected directions are 48.7°, either to the left or to the right, with respect to the direct line connecting the near end of the tunnel and the hive's entrance (Fig. 2E,H,K, open arrowhead). However, the directions actually signalled in the dances clearly differed from those pointing towards the virtual locations after path integration of the outbound flight (Fig. 2E,H, black bars *vs* open arrowheads): dances indicated directions of only 3.9±2.9° (mean ± S.E.M., P<0.001, Rayleigh test) to the left (for the tunnel oriented 90° to the left) and only 6.8±4.0° (mean ± S.E.M., P<0.001, Rayleigh test) to the right (for the tunnel oriented 90° to the right), regarding the linear distance connecting the near end of the tunnel and the hive's entrance. Each of these directions (Fig. 2E,H, black bars) was not significantly different from the direction (Fig. 2B, black bars; 1.3°±1.5°, mean ± S.E.M., P<0.001, Rayleigh test) recorded for the 0°-oriented tunnel (Fig. 2B,E,H; 90°-Left *vs* 0°, $F_{1,29}$=3.3, P=0.08; 90°-Right *vs* 0°, $F_{1,30}$=2.7, P=0.11, Watson F-test), although they differed from each other (Fig. 2E,H; $F_{1,17}$=5.6, P<0.001, Watson F-test).

We also compared the mean duration of the foraging cycle, i.e. the time interval between two successive departures from the nest to the feeder, recorded from four different groups of animals: bees that foraged on the outdoor feeder placed 135 away from the hive's entrance (i.e. in the absence of the tunnel), bees that flew through the 0°-oriented tunnel during their outbound flights, and bees flying through the 90°-rotated tunnel, either to the left or to the right, during their outbound flights. No differences in the mean duration of the foraging cycle were found among these groups (mean ± S.E.M.; 135 m, 6.5±0.56 min, N=6; 0°, 7.92±0.96 min, N=13; 90°-Left, 7.91±0.97 min, N=11; 90°-Right, 7.89±0.89 min, N=6; $F_{3,35}$=0.37, P=0.77, one-way ANOVA).

Next, we asked what directions the bees indicate in their dances if they leave the tunnel using the same route they followed to reach the feeder, i.e. if they fly through the tunnel

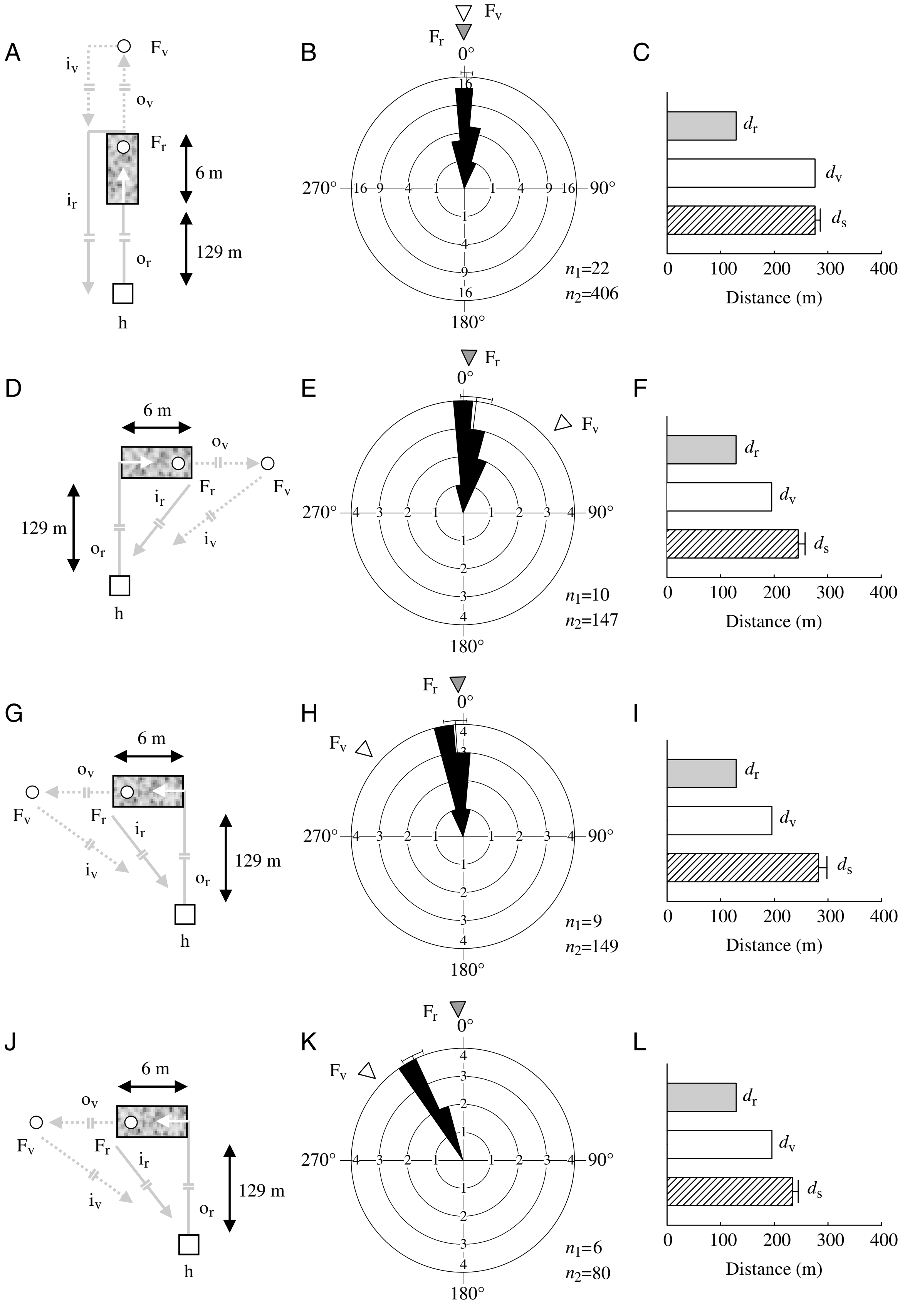

Fig. 2. See next page for legend.

during both the outbound and the inbound flights. Hence, a second experimental situation was assayed with the tunnel rotated 90°, either to the left or to the right: attempts were made to train the bees to enter and leave the tunnel *via* its near end. Surprisingly, the marked bees could not be trained to fly back through the tunnel. They usually covered approximately 1.5 m of their inbound flights (performing circuitous flights inside the tunnel) and then returned to the far end of the tunnel, where the feeder was located. These circuitous displacements could be repeated several times before the bees stopped flying and finally stayed on the walls at the far end of the tunnel. Presumably, the information gathered during the outbound flight with respect to distance and direction prevented them from flying back through the tunnel, thus ignoring the shortcut flight computed by path integration. Furthermore, whenever the bees were flying through the 0°-oriented tunnel, they could not be trained to fly through a 90°-oriented tunnel immediately afterwards. Under these circumstances (i.e. immediately after the tunnel was rotated 90°, either to the left or to the right), all the animals that entered the tunnel displayed repetitive short flights in the former direction (0°), within the first 1–2 m of the tunnel. Somehow, the directional information available inside the tunnel, which suddenly changed by 90° with respect to the direction recently experienced, compelled the bees to fly no more than a short distance inside the re-oriented tunnel. Throughout several trials performed on different days (which involved different groups of animals), only two marked bees (out of 40) finally reached the feeder after several minutes of circuitous flights in which most of the straight components were also displayed in the former direction. Once returned to the hive, these two animals did not dance and stopped foraging.

The bees' experience of the terrain and the encoding of spatial information in the waggle dance

A comparison was made between the directions indicated in the dances of two different groups of bees flying through the 90°-oriented tunnel: (1) bees that lacked experience of flying inside the tunnel (henceforth termed 'naïve' bees) and (2) bees that had previously foraged inside the 0°-oriented tunnel, ~24 h prior to the beginning of the experiment (henceforth termed 'experienced' bees). We found that the experienced bees (Fig. 2K) indicated a mean direction (± S.E.M.) of 26±2.9° ($P<0.01$, Rayleigh test), which was different ($F_{1,13}=34.9$, $P<0.001$, Watson F-test) from that indicated by naïve bees (Fig. 2H, see above). Moreover, they also indicated a shorter distance in their dances (Fig. 2L *vs* Fig. 2I, striped bars, $t_{13}=2.4$, $P=0.032$).

Fig. 2. Experimental layout and results to investigate the encoding of spatial information in the waggle dance. Individually marked bees were trained to forage on a feeder placed at the far end of a 6 m-long, 30 cm-wide and 30 cm-high tunnel. The tunnel's entrance was located 129 m away from the hive, and its walls and floor were decorated with a random visual texture (see Materials and methods for details). (A) Experimental arrangements with the tunnel oriented at 0° with respect to the direct line connecting its near end and the hive (h). The bees flew through the tunnel during their outbound flights (o_r) but not during their inbound flights (i_r). F_r and F_v correspond to the real and the virtual location of the feeder (open circles), respectively, whereas o_v and i_v correspond to the virtual outbound and inbound flights, respectively, as derived from the overestimated distance flown inside the tunnel (see Materials and methods). (B) Distribution of the individual mean directions signalled in the waggle dances recorded in the tunnel experiment described in A, mean vector direction $\mu=1.33°$, $r=0.99$, $P<0.001$, n_1 (number of animals analysed)=22, n_2 (number of waggle-runs analysed)=406. The frequencies within 10° class ranges are shown as the areas of the dark wedges. The dark spoke and segment indicate μ and 95% confident interval, respectively. The grey and open arrowheads indicate the directions towards the real (F_r) and the virtual (F_v) feeders shown in A, respectively. (C) The flown distance (mean ± S.E.M.) indicated in the waggle dances recorded in the tunnel experiment described in A (d_s, striped bar), the distance to the virtual feeder (d_v, open bar, in this case equivalent to the indicated distance) and the real distance from the hive to the food site (d_r, grey bar). (D–F) Experimental arrangements and results as in A–C with the tunnel rotated 90° to the right. The distance flown inside the tunnel oriented at 0° (C) was used to compute the location to be encoded (F_v, direction; d_v, distance) if the global vector computed by path integration of the outbound flight provides the dancers with the spatial information encoded in the waggle dance. In E, $\mu=6.77°$, $r=0.98$, $P<0.001$, $n_1=10$, $n_2=147$. (G–I) Experimental arrangements and results as in D–F with the tunnel rotated 90° to the left. In H, $\mu=356.1°$, $r=0.99$, $P<0.001$, $n_1=9$, $n_2=149$. (J–L) Experimental arrangements and results as in G–I, obtained with experienced bees (see Materials and methods). In K, $\mu=333.99°$, $r=0.99$, $P<0.001$, $n_1=6$, $n_2=80$. See Results for details on comparisons.

Discussion

The encoding of odometric information provided by either the outbound or the inbound flight

Early studies suggested that bees estimate the distance travelled by gauging the amount of energy they expend during their outbound flights (Heran and Wanke, 1952; Heran, 1956; Bisetzky, 1957; Scholze et al., 1964; von Frisch, 1965). However, the results of these studies appeared to be controversial (Neese, 1988; Esch and Burns, 1996). Moreover, experiments by Otto (1959) indicated that bees use both their outbound and inbound flights to gauge the distance encoded in the waggle dance. A more recent line of evidence describes distance estimation on the basis of the amount of image motion experienced while flying (Esch et al., 1994, 2001; Esch and Burns, 1995, 1996; Srinivasan et al., 1996, 1997, 1998, 2000; Si et al., 2003; Chittka and Tautz, 2003; Tautz et al., 2004). Results obtained in experiments conducted to test the so-called 'optic flow' hypothesis also indicate that information on the distance to food is acquired primarily during the outbound flight, although bees need and use distance information to navigate their inbound flights (Srinivasan et al., 1997, 1998). So far, however, the encoding of the visually measured distance provided by either the outbound or the inbound flight has not been addressed within the context of the waggle dance. In the present study, we found that bees execute longer waggle-runs and show a smaller directional scatter only when the magnitude of the experienced optic flow is amplified during their outbound flights (Fig. 1A,B), indicating that the waggle dance basically refers to the distance gauged on the way to the goal.

The direction of the global vector and the encoding of compass information

Bees benefit from path integration to solve complex navigational tasks (von Frisch, 1965; Dyer, 1998; Collett and Collett, 2002; Wehner and Srinivasan, 2003; Menzel et al., 2005). This means that they appear to keep continuously updated records of their current directions and distances from some reference point as they move away from that place, which allows them to establish global vectors encoding the appropriate distances and directions of the straight trajectories connecting their current locations and the reference point (Mittelstaedt and Mittelstaedt, 1982). Early detour experiments by von Frisch (1965) suggested that the directions of these global vectors provide the bees with the directional information encoded in the waggle dance. But does the waggle dance refer only to the direction of the global vector computed by path integration of the outbound flight? If that were the case, the directions signalled in some of our experiments (Fig. 2E,H,K, black bars) should have matched those of the global vectors computed at the end of the different outbound flights (Fig. 2E,H,K, open arrowheads). However, when dancers were presented with conflicting navigational information at the end of their outbound flights (Fig. 2D,G,J), their waggle dances did not refer to the directions of the global vectors (Fig. 2E,H,K, black bars). In the case of naïve bees (see Materials and methods), the directions encoded in the dances (Fig. 2E,H, black bars) were close to the direct line connecting the actual locations of the feeder and the hive's entrance (Fig. 2E,H, grey arrowheads). Each of these two directions (recorded when the tunnel was rotated 90°, either to the left or to the right) was not significantly different from the mean direction recorded for the 0°-oriented tunnel (Fig. 2B *vs* Fig. 2E,H, black bars; also see Results). The simplest hypothesis that would presumably explain the signalled directions is that the dances referred to the entrance of the visually patterned tunnel, thus ignoring the directional information available inside the tunnel. However, the signalled directions recorded for both orientations of the 90°-rotated tunnel, either to the left or to the right of the direct line connecting the tunnel's entrance and the hive (Fig. 2E,H), actually differed from each other (see Results). Moreover, results from experienced bees (see below) show that directional information is being computed in the 90°-oriented tunnel. This means both that bees (Fig. 2E,H) did not ignore the directional information available inside the tunnel and that their waggle dances did not refer to the tunnel's entrance.

The visually driven odometer is primarily decoupled from the processing of directional information

Thus, we found that the waggle dance does not necessarily refer to the direction of the global vector computed by path integration of the outbound flight (Fig. 2E,H). In addition, dances of naïve bees flying through the 90°-oriented tunnel signalled similar distances, irrespective of the tunnel's orientation, either to the left or to the right of the reference line (Fig. 1C, grey circle; Fig. 2F,I, striped bars). These distances, in turn, were longer than those expected according to the length of the global vectors computed by path integration of the outbound flights (Fig. 2F,I, striped *vs* open bars). Moreover, in these cases, neither the signalled distances nor the directional scatter of the successive waggle-runs were different from the corresponding values recorded for the 0°-oriented tunnel (Fig. 1A–D, black and grey circles; Fig. 2C,F,I, striped bars; see also Results). Hence, our results also demonstrate that the bees' visually driven odometer is primarily decoupled from the processing of directional information, at least in the context of the waggle dance. This finding is in close agreement with published data (von Frisch, 1965).

The inbound flight and the encoding of compass information

One might ask whether or not the waggle dance encodes directional information computed during the inbound flight. Indeed, early detour experiments addressed the question of whether bees use their inbound vectors for the purpose of directional indication, for instance, by 'neurally' switching them by 180° (Otto, 1959; Lindauer, 1963; von Frisch, 1965). If that were the case, the directions signalled in our experiments with the 90°-oriented tunnel might have been based on path integration of the inbound flight. That is, since the bees flew a two-legged journey to reach the feeder (Fig. 2D,G), travelling relatively equivalent subjective distances during each of the two flight segments (Fig. 2F,I; see differences between grey and striped bars), they might have initially directed their homing flights in the direction of the global vectors (Fig. 2E,H, open arrowheads) and then corrected their subsequent displacements to reach the location of the hive. The direction computed by path integration of these corrected inbound flights might have been finally used for the purpose of directional indication in the waggle dance. In this case, differences must be expected in the mean duration of the foraging cycle when the results from different experiments are compared due to the time involved in the erroneous inbound headings and the subsequent orientation flights. However, we found that bees invested similar amounts of time in their successive foraging cycles, irrespective of the tunnel's orientation (see Results). Moreover, the duration of the foraging cycle recorded for each of the three different orientations of the tunnel (0°, 90° to the left and 90° to the right) did not statistically differ from that recorded for the same absolute distance travelled in the absence of the tunnel (see Results).

Hence, when the subjective direction towards the goal did not match the actual one, the naïve bees (see Materials and methods) referred in their dances to a direction (Fig. 2E,H, black bars) that was close to both the direction pointing towards the actual location of the feeder (Fig. 2E,H, grey arrowheads) and that of the inbound flight (see above). This means that, at the beginning of the inbound flight, the dancer's nervous system had to correct the directional information provided by the global vector in order to establish the signalled direction, suggesting that the encoding of spatial information in the waggle dance includes a component that detects the deviation between subjective measures of distance and direction and landmark-based information defining the shortcut between the goal and the hive. Our results can only be explained if we assume that landmark-based information is computed during the inbound flight and that it may strongly affect the encoding of directional information in the waggle dance. It would then be interesting to present the dancers with different inbound flights to further evaluate their role in the encoding of spatial information (for instance, by displacing them to unfamiliar environments after the travelled outbound flights in which navigational information is manipulated).

But how do bees establish the direction of the shortcut when they experience a mismatch between visually defined coordinates and the coordinates provided by the global vectors? *Cataglyphis* ants, a walking central-place foraging insect, also navigate by means of path integration (Collett and Collett, 2000; Wehner and Srinivasan, 2003) and landmark-based information (Collet et al., 1992; Wehner et al., 1996; Collett and Collett, 2002). Their global vectors, unlike their local vectors (nestward-directed vector memories), seem not to be recalled by familiar landmarks (Collett et al., 1998). However, if the information from path integration is experimentally eliminated, desert ants associate local vectors with landmarks present during their inbound paths (Bisch-Knaden and Wehner, 2003), indicating that the inbound path constitutes a temporal stage at which the ants acquire critical navigational information. Both bees and ants acquire memories of landmarks and of actions associated with those landmarks to navigate between their nests and a foraging area (Collett and Collett, 2002; Menzel et al., 1998, 2000, 2005). It is often suggested that, within familiar terrain, the inevitable errors associated with path integration (Benhamou et al., 1990) can be reduced through landmark-based information (Srinivasan et al., 1997; Collett and Graham, 2004), and even that path integration controls navigation only as long as the animal combines it with learned strategies and environmental cues (Etienne et al., 2004). In our experiments, naïve bees might have linked the visual landmarks perceived at the beginning of their inbound flights with their best estimate of the path integration coordinates of that place (Collett and Graham, 2004). When provided with conflicting navigational information, namely a strong mismatch between their current path integration coordinates (computed during the outbound flight) and a set of different path integration coordinates previously associated with visual landmarks, they might have used the later source of navigational information to define the direction of the shortcut. Either the same coordinates or the compass information acquired during the inbound flight might be used to indicate direction in the waggle dance.

The bees' experience of the terrain and the encoding of spatial information

The resetting of an integrator using visual landmarks implies that an animal is able to link the landmarks associated with a particular location with its best estimate of the path integration coordinates of that location. For visual resetting to occur, the stored path integration coordinates of a visually defined location should come from the average path integration coordinates experienced in that particular location, and thus would change with the animal's increasing experience of the terrain (Collett and Graham, 2004). Intriguingly, we found that the bees' experience of the two-legged journey changed the encoding of spatial information in the waggle dance, i.e. bees flying inside the 90°-oriented tunnel modified the encoding of both direction and distance based on whether (or not) they had foraged inside the 0°-oriented tunnel, approximately 24 h before the experiments (Fig. 2H,I *vs* Fig. 2K,L; also see Results). Most likely, the increasing experience of the visual landmarks present in the foraging area and of the two different types of landscapes available during both segments of the two-legged outbound flight (but not of a particular route defined by subjective measures of both distances and directions) modulated the encoding of spatial information in the waggle dance. Indeed, the directional information perceived inside the tunnel was processed differentially in the context of the waggle dance only after experiencing the prospective path integration coordinates and visual cues throughout two successive days (Fig. 2K,L). This means that the difference observed in the dances of both experienced and naïve bees might be explained on the basis of the magnitude of the mismatch experienced at the beginning of the inbound flight between the current and stored path integration coordinates (see above); particularly since the bees were always exposed to the same landmark views at the beginning of their inbound flights. In this context, the higher the number of flights in which the bees are exposed to the two different types of landscapes available *en route* to the goal (i.e. those provided by the successive outdoor- and tunnel-flights) the lower the magnitude of the experienced mismatch. It is under these circumstances that the path integration information computed inside the 90° tunnel becomes more heavily weighted in the context of the waggle dance; which, in turn, involves a robust interaction between landmark-based and path integration information. Early experiments by Otto (1959) also indicated that the directional information available during the entire foraging flight may be combined in the waggle dance only after increasing experience of the terrain. Interestingly, our results suggest that the waggle dance may benefit from the computation of stored path integration coordinates of visually defined locations. Since honeybees navigate according to a map-like spatial memory (Menzel et al., 2005), further research on dance communication would benefit from the possible interactions between path integration and landmark-based information, which, in turn, might allow landmark views to be assigned with metric coordinates.

The authors thank U. Greggers and five anonymous referees for fruitful comments. R.D.M. gratefully acknowledges advice and helpful assistance from M. Gil. This study was supported by funds from the Deutscher Akademischer Austauschdienst (DAAD) to R.D.M. The present experiments comply with the *Principles of Animal Care* (publication No. 86-23, revised 1985) of the National Institute of Health and the corresponding current national laws.

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