

Way-finding in displaced clock-shifted bees proves bees use a cognitive map

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Mammals navigate by means of a metric cognitive map. Insects, most notably bees and ants, are also impressive navigators. The question whether they, too, have a metric cognitive map is important to cognitive science and neuroscience. Experimentally captured and displaced bees often depart from the release site in the compass direction they were bent on before their capture, even though this no longer heads them toward their goal. When they discover their error, however, the bees set off more or less directly toward their goal. This ability to orient toward a goal from an arbitrary point in the familiar environment is evidence that they have an integrated metric map of the experienced environment. We report a test of an alternative hypothesis, which is that all the bees have in memory is a collection of snapshots that enable them to recognize different landmarks and, associated with each such snapshot, a sun-compass-referenced home vector derived from dead reckoning done before and after previous visits to the landmark. We show that a large shift in the sun-compass rapidly induced by general anesthesia does not alter the accuracy or speed of the homeward-oriented flight made after the bees discover the error in their initial postrelease flight. This result rules out the sun-referenced home-vector hypothesis, further strengthening the now extensive evidence for a metric cognitive map in bees.

navigation | course-setting | shortcuts | terrain map | circadian

A metric cognitive map enables an animal to locate itself in space. In recent decades, accumulating behavioral and neurobiological evidence has established a broad consensus that the brains of mammals, and perhaps even all vertebrates, compute a metric cognitive map of the experienced environment on which they maintain a continuously updated representation of the animal's position (1–7). Because a metric map is far removed from the elementary sense data from which it must be computed, and because a map is a mathematical construction carried in a symbolic memory, the conclusion that the vertebrate brain computes a metric cognitive map of the environment is a strong argument for the computational theory of mind, which is a fundamental concept of cognitive science.

The mammalian hippocampus and its putative homologs in nonmammalian vertebrates appear to play a central role in the requisite computations in vertebrates (3). Insofar as the brain's computational capacities are thought to derive from the structure of its circuits, this theory suggests that understanding the circuitry unique to the hippocampus and its homologs might be a key to constructing a neurobiologically anchored model of these computations. Before this line of thought is pursued further, it is important to know whether the construction of a metric cognitive map is limited to vertebrates. Invertebrates, particularly the social insects, whose brains are minuscule in comparison with the vertebrate brain and lacking in a homolog of the mammalian hippocampus, are nonetheless known to possess impressive navigational abilities (8–10). It is, however, a matter of long-standing controversy

whether these abilities rest on the construction and use of a metric cognitive map (11–18).

Way-finding, the ability to set a course from one familiar but otherwise arbitrarily chosen location to another location that is not perceptible from the first, is the signature of a metric cognitive map. Displacing an animal from its current location to an experimenter-chosen location within the territory the animal is presumed to be familiar with, and then observing its subsequent goal-seeking behavior, is a common test of way-finding. To set a course from the release site to the unseen goal, the navigator must read the coordinates of the release site from its map and compute the difference between those coordinates and the goal coordinates to obtain the difference vector specifying the direction and distance to move from its current location to its goal.

In most early displacement experiments with honey bees, the released bee took off on the goal vector from the capture site (17, 19), not the goal vector from the release site, suggesting that the bee lacks a true cognitive map. This conclusion came into question after the recent development of systems for tracking the flights of the released bees using harmonic radar (20, 21). When no extended landmarks and no structured skyline of the horizon were available at the release site, bees flew first on the goal vector from the capture site, as was expected from the earlier work. The bees were seemingly unaware that this initial direction was wrong and would not lead them to their intended goal. However, they were not lost; after the initial misoriented component of their flight, the bees flew toward the intended goal along novel short-cuts (18, 20). The bees seemed to become

Significance

The question of the computational capacities of the brains of widely separated genera of animals is of interest to behavioral biologists, comparative psychologists, computational neuroscientists, philosophers of mind, and—we believe—much of the scientific community. Half a century ago, the claim that any nonhuman animal had a cognitive map was deeply controversial. If true, it greatly favored a computational theory of mind, as opposed to an antirepresentational behaviorist theory. Now that it is well established by behavioral and neurobiological evidence that rodents have a metric cognitive map, the question of whether insects do is a frontier question, the answer to which has broad implications in several disciplines.

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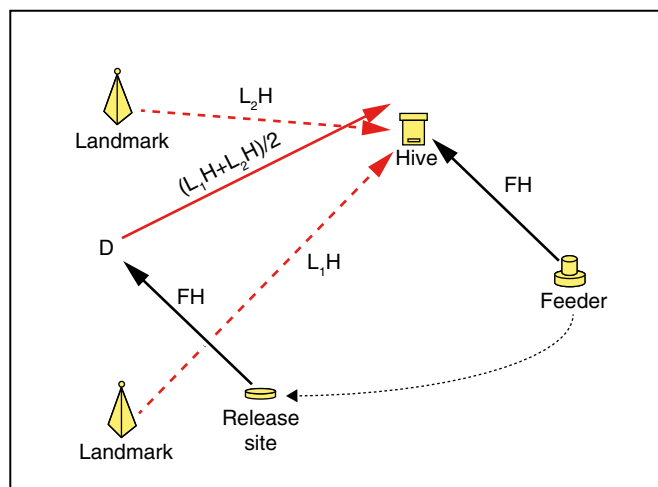


Fig. 1. The average-vector hypothesis. Bee is captured at the feeder F bound for the hive H on vector FH and released at a release site R. It flies the vector FH from R, discovers its error at D, recognizes the landmarks L_1 and L_2 , and flies the average of the associated home vectors, which is the vector $(L_1H + L_2H)/2$.

aware of the displacement only when flying the capture-site goal vector failed to bring them to the goal, at which point they were able to set the correct course to the goal from a point just as arbitrary as the experimenter-chosen release site.

These experiments did not, however, rule out an alternative account. In this account, proposed by Collett et al. (12) and formalized by Cruse and Wehner (13), the bee never constructs an integrated cognitive map; rather, it associates with each snapshot stored in memory the home vector from that landmark. When the bee discovers that it has not arrived home, it identifies nearby landmarks, averages the associated home vectors, and flies the resulting vector (Fig. 1).

An extension of this account is the proposal that multiple sequentially learned and retrieved views are integrated in a common memory replacing the notion of discreet views (22). All of these proposals rely on the assumption that learned views are related to each other by reference into a common reference system, the sun-compass, and lead to computations of vectors embedded in the sun-compass. The attraction of this alternative account is, first, that it relies on the well-established fact that bees and ants do store the home vectors from familiar landmarks and, second, that it avoids the assumption that the bee has an integrated readable cognitive map. However, the home vectors from landmarks are known to be compass-referenced. These vectors are equivalent to an instruction of the form: "fly northeast 100 m." For bees and many other animals, the compass direction of vectors derived from their dead reckoning is determined by reference to the sun. In contrast, vectors computed from differences in location coordinates are terrain-referenced. Flying these vectors requires continued attention to the mapped terrain because it requires maintaining appropriate angles with respect to terrain features. The appropriate angles and the terrain features change during the course of a flight, and the angle appropriate to a given terrain feature and to the current location of the navigator, can be determined only from the map. Holding to a terrain-referenced direction is called "carrying the parallel" because, conceptually, a direction on a map is an uncountable infinite set of parallel lines in a vector space (some vectors of which reference experienced locations).

The difference in the directional referents for the two kinds of vectors makes possible a decisive test: The sun-compass is time-compensated, because the compass direction of the sun changes

dramatically during the day. The time compensation depends on a time-of-day signal from the bee's circadian clock. Therefore, a large (several hours) shift in the phase of the circadian clock introduces a large shift in the sun compass (23, 24). If the course of the flight made after discovery of the initial error (at point D in Fig. 1) depends on the sun-referenced home vectors known to be associated with familiar landmarks, a large clock-shift will introduce a large error in the postdiscovery flights (DH vector in Fig. 1), as well as in the predisccovery portions of those flights. Consequently, the clock-shifted bees should become even more lost than they already are. The proportion making it to the hive should be greatly reduced, and the times taken to make it there should be greatly increased. In contrast, if the course after point D depends on a terrain-referenced vector obtained by reading the map, clock-shifting will produce no systematic error in the postdiscovery flight. The course should be as accurate in clock-shifted bees as in unshifted control bees, in which case the clock-shifted bees should make it to the hive in the same proportions and with the same flight times as the unshifted control bees.

We ran this test under two different environmental conditions, using general anesthesia to rapidly induce clock-shifts in some groups of the bees (24). In the first experiment, sky-line cues were not available to the bees throughout their vector and homing flights because all flights were performed within an area characterized by $\leq 2^\circ$ visual angle of the skyline but with panorama cues as seen by the flying bee (pattern of local structures on the ground were available) (Fig. 2A and Figs. S1 and S2). Visual resolution of the bees' compound eye is in the range of 2° (25, 26). Thus, modulation of the skyline $\leq 2^\circ$ will not be resolved. In the second experiment, there were salient skyline and panorama cues (a line of bushes) (Fig. 2B and Fig. S2) visible during both flight phases and during the initial training of the bees from the hive to the feeder. In addition, the conditions during the homing phase in the first experiment were made relatively more difficult

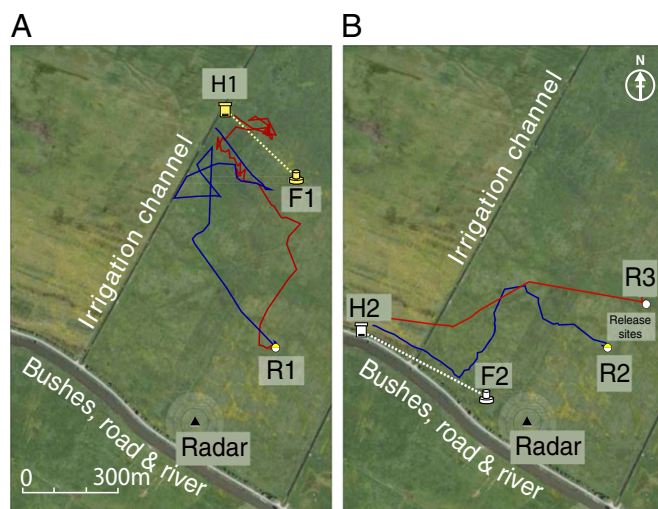


Fig. 2. Layout of the experimental site. A large flat open field bordered by a row of bushes, a road and a river. Perpendicular to the bushes ran two irrigation channels. Representative bee flights shown. (A) In Exp. 1 bees were trained from hive H1 to the feeder F1 along the yellow dotted line. (B) In Exp. 2 bees were trained from hive H2 to feeder F2 along the white dotted line. The release site in Exp. 1 was R1. In Exp. 2 two release sites were used, R2 and R3. The radar (black triangle at the origin) was positioned ~ 60 m from the row of bushes stretching along a small road and a river. H1 is located at the upper part of the western irrigation channel. H2 is located on the edge of the bushes to the south. The blue lines in the figures show representative flight paths from control bees, the red lines from clock-shifted bees.

for the clock-shifted bees by allowing the control bees to refer to an extended landmark when they arrived at the point of error discovery (an irrigation channel) (Fig. 2A), which offered a salient feature for home-directed guidance, whereas the clock-shifted experimental bees arrived at the point of error discovery in a rather low feature landscape: an open, flat grass pasture. If the experimental bees would perform their homing flights equally as well as the control bees, the spatial relations of ground structures, as learned during the orientations flights or later during foraging flights, must be sufficient for homing, indicating a terrain-based spatial reference system independent of the sun-compass.

Results

In Exp. 1, under conditions where there were no salient landmarks at the release point (R1 in Fig. 2A), bees that were not clock-shifted departed initially on the vector (denoted FH in Fig. 1), as expected (blue vectors in Fig. 3A), whereas the clock-shifted bees departed on a vector shifted by a large amount in the direction expected from the clock shift (red vectors in Fig. 3A; see also Fig. S3A). Thus, the clock-shifted and non-clock-shifted bees arrived at different error discovery locations, as illustrated by the examples in Fig. 2A, and as shown by the representations of the aggregated flight data in Fig. 4A and B. However, the bees' flights after they discovered their error were equally accurately directed toward the hive (Figs. 2A and 4B) and brought them to it in the same proportions and in the same flight times as the bees that were not clock-shifted. The individual flights of the clock-shifted and control bees for both experiments are shown in Figs. S4 and S5 for Exp. 1 and Figs. S6 and S7 for Exp. 2.

If the postdiscovery flights of the clock-shifted bees had been made using sun-referenced vectors, they should have shown the systematic error seen in the prediscovery portions of the flights, and they should have become even more lost. No matter what familiar landmarks the bees encountered as they searched for the way home, the home vectors associated with those landmarks should have headed the clock-shifted bees in the wrong direction. Notice that the former feeding site F1 (Figs. S4 and S5) was not marked in any way (the feeding equipment was removed and no trained bees were flying).

In support of the conclusion that the clock-shift did not impair the postdiscovery home-bound flights, we note the following statistical results: No evidence of a difference was found between the homing flights of control and experimental animals in Exp. 1 with respect to the rate of success in getting back to the hive ($P = 0.63$, logistic regression). Of the 24 bees in the

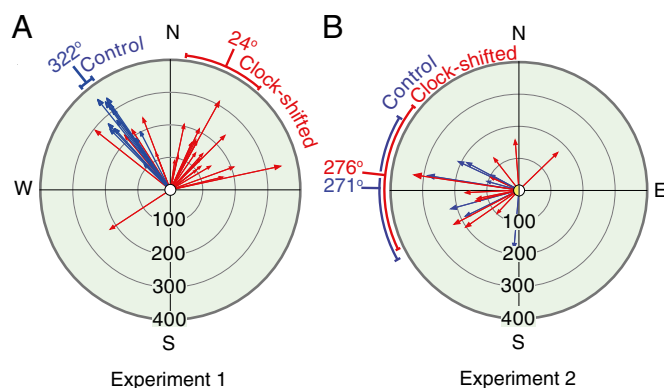


Fig. 3. Initial vector flight components (vector flights) of bees in Exps. 1 and 2. Clock-shifted bees shown in red and control bees shown in blue. (A) Exp. 1: clock-shifted mean vector angle 24°, (95% CI 7°, 41°); control mean vector angle 322°, (95% CI 319°, 325°). (B) Exp. 2: clock-shifted mean angle 276°, (95% CI 244°, 308°); control mean vector angle 271°, (95% CI 240°, 301°). Scale in meters.

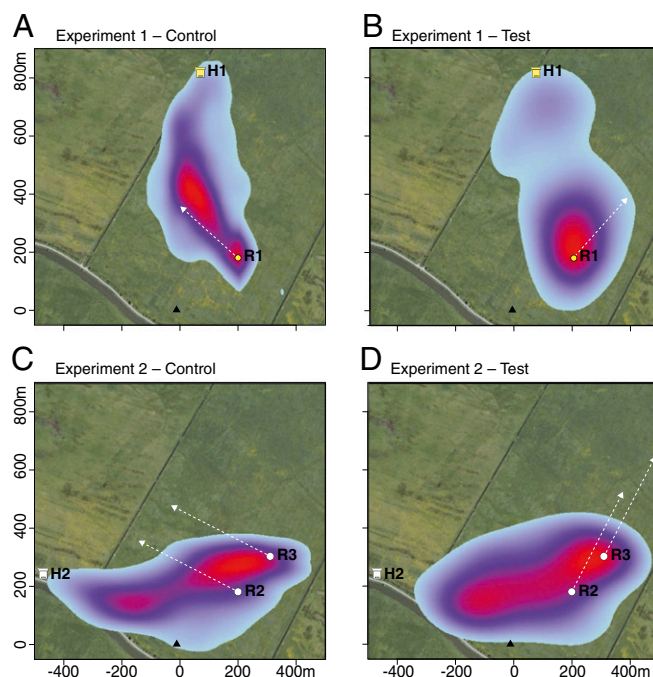


Fig. 4. Kernel density (kde) plots overlaid on a Google map showing the homing behavior of animals from the point of release in Exps. 1 and 2. (A) Exp. 1 control bees ($n = 13$); (B) Exp. 1 clock-shifted bees ($n = 24$); (C) Exp. 2 control bees ($n = 8$); (D) Exp. 2 clock-shifted bees ($n = 12$). The color maps indicate the probability of a bee being at a particular coordinate with blue indicating low and red indicating higher probability of occurrence. The white dashed lines indicate the expected sun-compass orientation angle under each experimental condition. Scale in meters from the radar.

experimental group, 19 returned to the hive compared with 11 of 13 in the control group. Because the predictions from the map hypothesis for this test are null predictions (no effect), we computed Bayes Factors (BFs) to complement the insignificant P values obtained from the more conventional null-hypothesis significance tests. Unlike the conventional tests, a Bayesian model-selection test requires that we compare the predictions of the null to the predictions of an alternative to it. Typically, the alternative specifies a range of possible experimental effects (whereas the null specifies 0 effect). If one assumes as an alternative hypothesis in which the effect of the 6 h of 2% isoflurane anesthetic might range anywhere from no effect to a devastating effect on the proportion of experimental bees finding their way home, then the BF is 3.54 in favor of the null (Fig. S34). A BF of 3 or greater is roughly equivalent to a P value of 0.05 or less; it signifies substantial support from the data for a conclusion. In this case, the conclusion is that the proportion of clock-shifted bees making it home is no different from the proportion of control bees making it home. Even when one assumes that the experimental effect can be at most such as to lower the true proportion making it home by no more than 20%, the BF of 1.05 still slightly favors the null hypothesis. When measuring homing components of those bees in both groups that did return home, there was no evidence of a difference in postvector flight time ($P = 0.25$) or postvector flight distance ($P = 0.40$) (Table 1). If one takes as an alternative to the null hypothesis the hypothesis that the effect of the clock-shift on homing time or flight distance might range anywhere from 0 to 1 σ , then the BF favors the null by greater than 3 (in other words, substantial support for the null relative to an alternative that posits some small effect) (Fig. S34). Taken together, the results of Exp. 1 clearly show that clock-shifted animals were not compromised in returning home, although they initially referred to an incorrect

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Supporting Information

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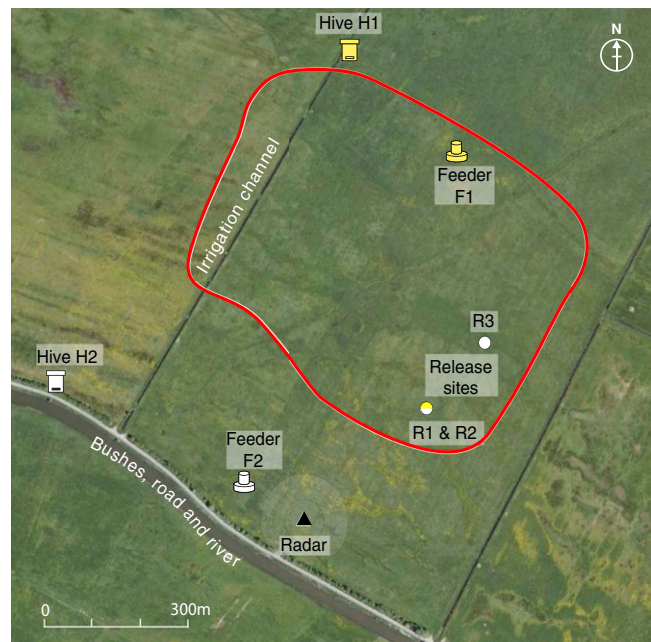


Fig. S1. Map of the study area showing the limits of the influence of the skyline. Inside the red boundary, no skyline cues are greater than 2° above the horizon. Release site R1 was selected for the first experiment to keep flights of control bees and time-shifted bees within this area, preventing their referring to the structure of the skyline.



Fig. S2. Two views from release site R1: (A) toward north; (B) toward south.

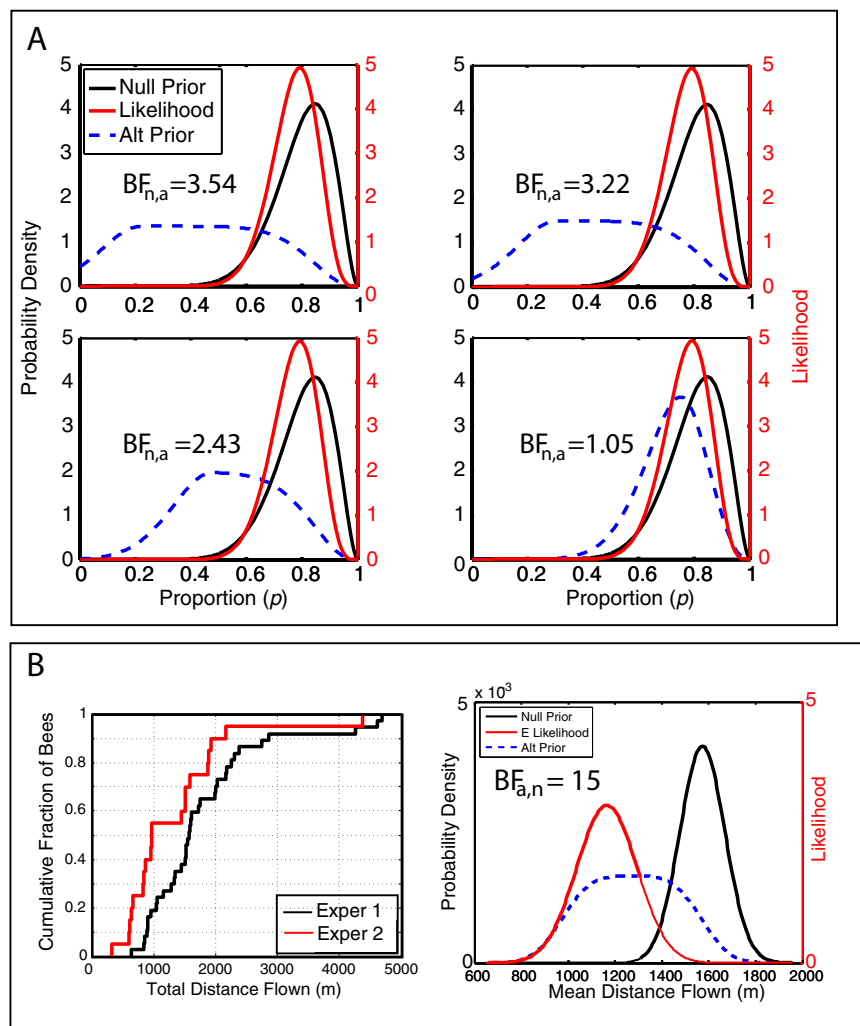
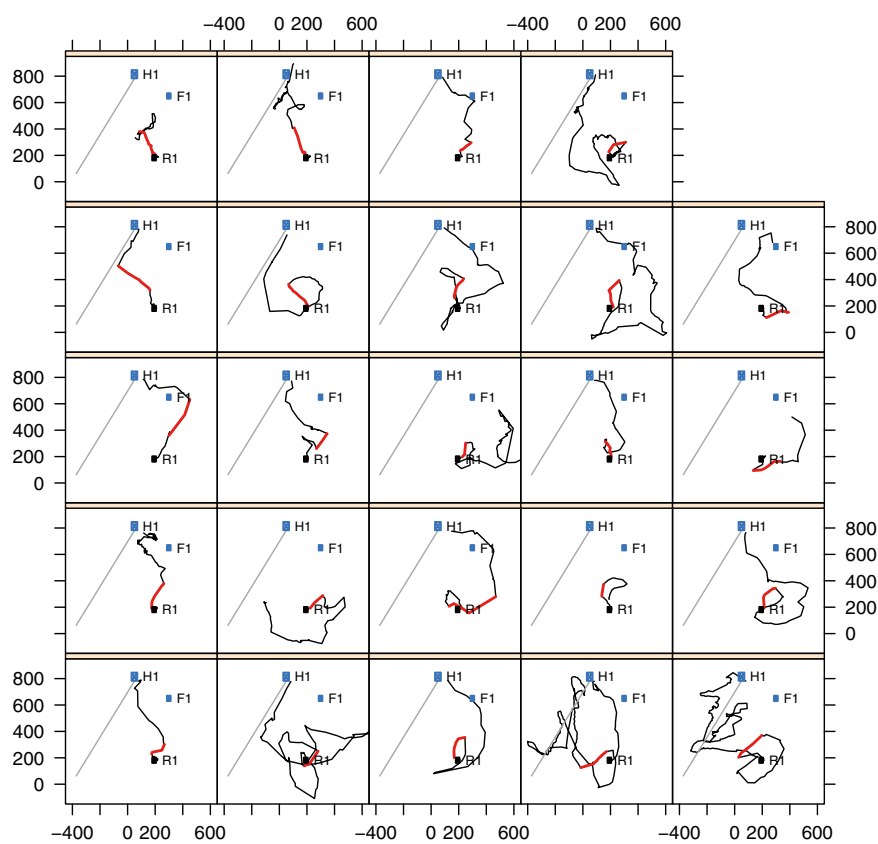


Fig. S3. (A) Graphic illustration of the computation of Bayes Factors for the null hypothesis showing that there is no difference between the proportions of bees returning to the hive in the control and clock-shifted groups. The null hypothesis (no difference in the proportions) is represented by the black probability distribution, which is the same from panel to panel. This distribution is the normalized likelihood function, given the control data. The alternative hypotheses, which suggest that there is a range of possible differences, are represented by the dashed blue probability distributions, which vary from panel to panel. The prior probability distributions, representing the competing hypotheses (Null and Alt Prior) are plotted against the left Probability Density axis. The red curve, which is the same from panel to panel, is the likelihood function given the clock-shifted data. It is plotted against the right axis (Likelihood). The two posterior likelihood functions for a given panel are the products of the likelihood function with each of the competing probability distributions. The marginal likelihoods of the competing hypotheses are the integrals of the posterior likelihood functions. The Bayes Factors ($BF_{n,a}$) are the ratios of the two marginal likelihoods. (The subscript “n,a” indicates that the marginal posterior likelihood of the null is the numerator and the marginal posterior likelihood of the alternative the denominator.) The probability distribution that puts more prior probability under the likelihood function has the greater marginal likelihood; hence, the Bayes Factor favors that that hypothesis (that prior). It is generally graphically obvious which prior puts more probability under the likelihood function. In this sequence, we see that no matter how limited the range of the alternative to the null is taken to be (no matter how narrow the dashed blue distribution), the black distribution puts more probability under the likelihood function (the red curve); hence, the Bayes Factor always favors the null. Of course, the extent to which it favors the null gets smaller as the alternative to the null becomes less and less distinguishable from the null. (B) The cumulative distributions of total distances flown by the bees in Exps. 1 and 2. The values $>3,000$ m appear to be outliers and were removed from the data before computing the Bayes Factor for the effect of the manipulation that distinguished Exp. 2 from Exp. 1. One sees that the distribution of distances flown in Exp. 2 (red plot) lies substantially to the left of the distribution of distances flown in Exp. 1 (black plot); that is, the bees in Exp. 2 did not fly as far in the course of getting home, even though they were released farther from the hive than were the bees in Exp. 1. (Right) Graphic illustration of the computation of the Bayes Factor. Because the alternative hypothesis (some effect, dashed blue distribution, plotted against left axis) places much more probability mass under the likelihood function (red curve, plotted against right axis) than does the null hypothesis (black distribution, plotted against the left axis), the Bayes Factor ($BF_{a,n}$) strongly favors the hypothesis that the manipulation did affect the distance flown.



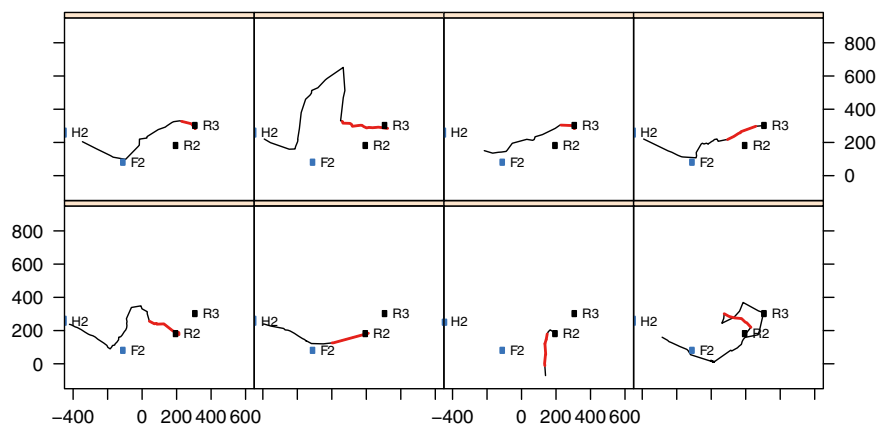


Fig. S6. Individual flight paths of the control bees in Exp. 2. The red line indicates the initial vector flight component. The hive, feeder, and release sites are labeled H2, F2, R2, and R3, respectively.

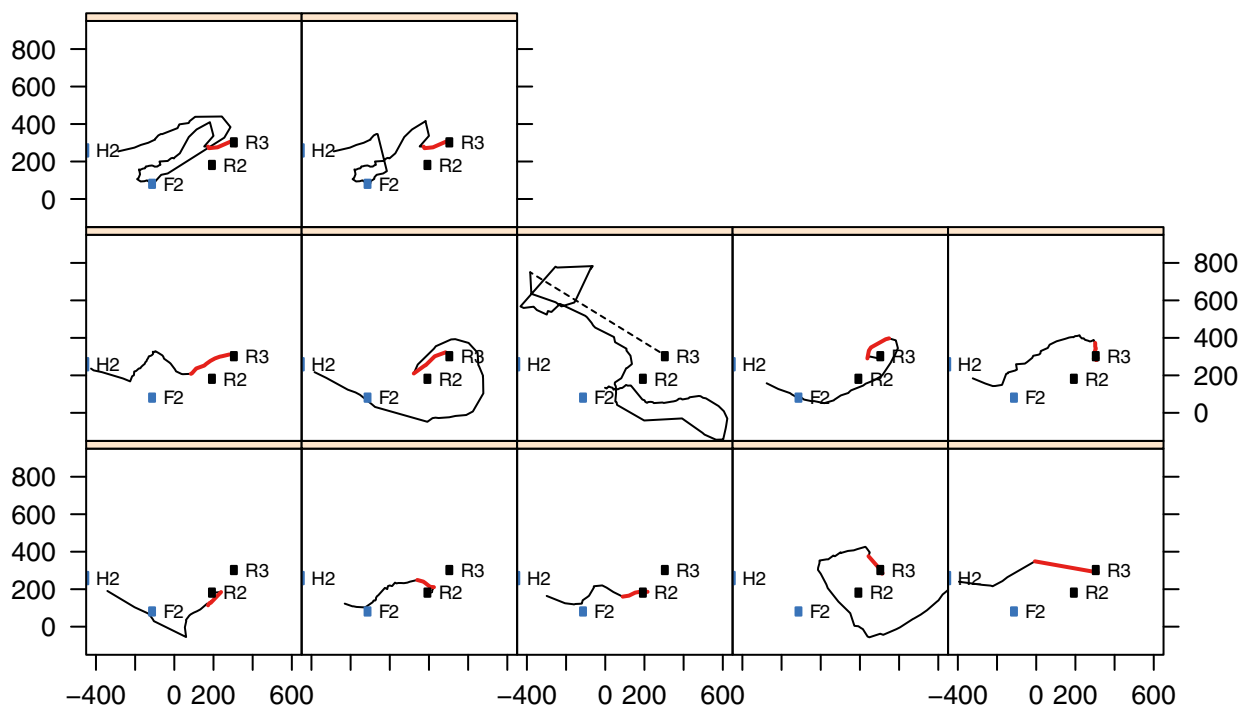


Fig. S7. Individual flight paths of the time-shifted bees in Exp. 2. In one animal (center row, center columns) the dashed line indicates missing data interpolated from the release site. For this animal, no vector flight was defined. The hive, feeder, and release sites (1 and 2) are labeled H2, F2, and R2, respectively.

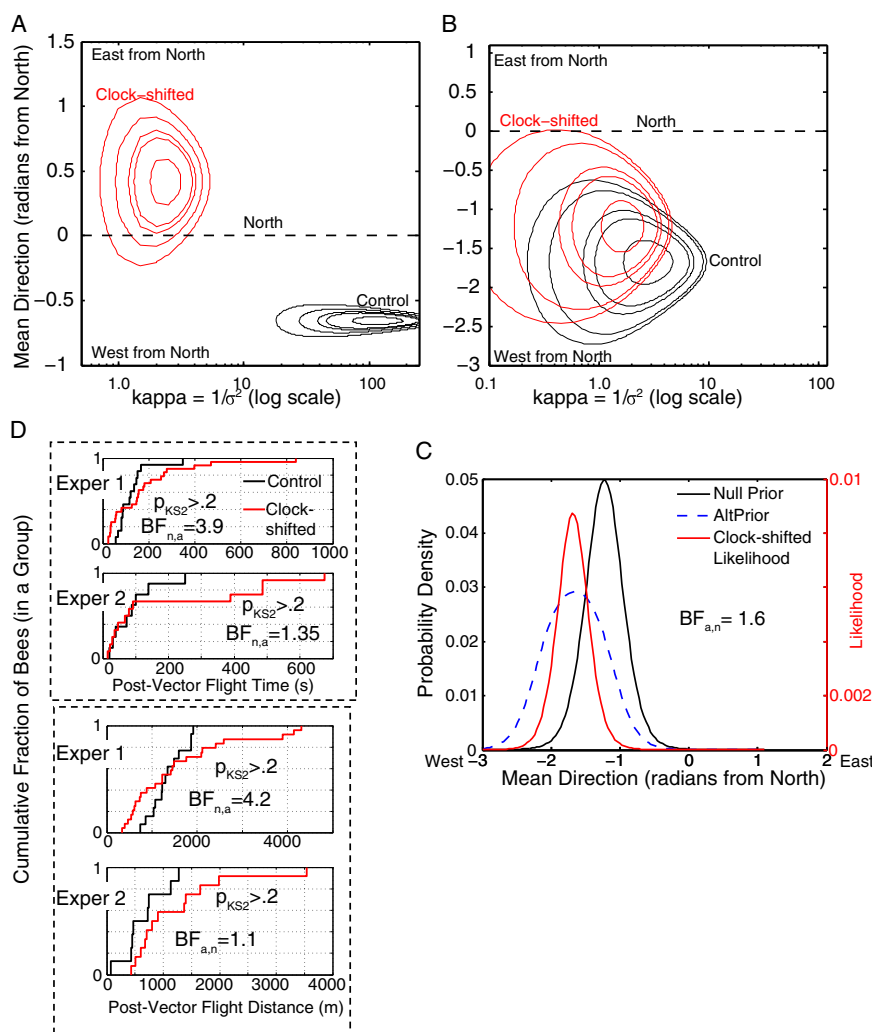


Fig. 58. (A) Contour plots of the directional likelihood functions for the control and clock-shifted bees in Exp. 1. The outer contours are for a likelihood $1/1,000$ th of the maximum likelihood. Because the likelihood functions do not overlap (either in their means or in their precisions (κ), either a null-hypothesis significance test or a Bayes Factor will show overwhelming support for the existence of an experiment effect. (B) The directional likelihood functions in Exp. 2. These overlap strongly. (C) Computation of the Bayes Factor for difference in the mean directions in Exp. 2. The Null Prior (black distribution, plotted against left axis, Probability Density) is the normalized marginal likelihood function for the mean direction of the control bees (partial integral of the black likelihood function in B). The red function is the (unnormalized) marginal likelihood function for mean direction of the clock-shifted bees (the partial integral of the red likelihood function in B). It is plotted against the right axis (Likelihood). The dashed blue prior probability distribution (plotted against the left axis) is for the hypothesis that the directional shift in this experiment is somewhere within the range between 0 (no shift) and the shift observed in Exp. 1. The alternative prior (some shift in direction) puts slightly more probability mass under the likelihood function. However, the BF of 1.6 is far from the 3.0 level generally taken to indicate nonnegligible evidence for an experimental effect. (D) Cumulative distributions of postvector flight times (Upper) and flight distances (Lower) together with Kolmogorov-Smirnov P values and Bayes Factors. By either assessment, there are no within-experiment effects on flight time and distance flown: that is, the clock-shifted bees took no longer to get to the hive and flew no further in doing so than did the control bees. There is, however, a highly significant difference in flight distances between the two experiments (see Fig. S3B).