Sleep deprivation impairs precision of waggle dance signaling in honey bees

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Sleep is essential for basic survival, and insufficient sleep leads to a variety of dysfunctions. In humans, one of the most profound consequences of sleep deprivation is imprecise or irrational communication, demonstrated by degradation in signaling as well as in receiving information. Communication in nonhuman animals may suffer analogous degradation of precision, perhaps with especially damaging consequences for social animals. However, society-specific consequences of sleep loss have rarely been explored, and no function of sleep has been ascribed to a truly social (eusocial) organism in the context of its society. Here we show that sleep-deprived honey bees (*Apis mellifera*) exhibit reduced precision when signaling direction information to food sources in their waggle dances. The deterioration of the honey bee's ability to communicate is expected to reduce the foraging efficiency of nestmates. This study demonstrates the impact of sleep deprivation on signaling in a eusocial animal. If the deterioration of signals made by sleep-deprived honey bees and humans is generalizable, then imprecise communication may be one detrimental effect of sleep loss shared by social organisms.

dance language | signal precision | recovery sleep | sleep rebound

Deprivation of sleep can result in dire consequences to health and to cognitive performance (1–3). When deprived of sleep, humans are susceptible to communication lapses, both when signaling (4, 5) and when receiving (6) information. Human speech performance, such as word fluency and intonation, declines (7). A speaker's voice is sensitive to fatigue (8), with both fundamental frequency and word duration differing in sleep-deprived subjects (9). Speech deterioration is so obvious after sleep deprivation that "rambling, incoherent speech for brief periods" features in a cognitive disorganization scale (10). Although the potential exists for sleep to impact communication in nonhuman animals, we are aware only of studies addressing the role of sleep in song learning in zebra finches (11, 12).

In the present study, we investigated a possible degradation of social function by testing the effect of sleep deprivation on the precision of signaling in European honey bees (*Apis mellifera* Linnaeus, 1758). Honey bees regularly inform nestmate workers about the distance and direction to desirable foraging and nest-site locations by performing waggle dances (Fig. 1*A*), in which the distance to the advertised destination is indicated by the duration of the waggle phase of the dance, and the destination's direction relative to the sun's azimuth is indicated by the angle of the dance relative to the vertical (i.e., dance angle) (Fig. 1*A*) (13). Imprecision in a bee's performance of the waggle dance could result in degraded transfer of information and a consequent decline in foraging efficiency for vital resources (14, 15).

We hypothesized that depriving honey bees of sleep would decrease the precision of their dance's direction and distance information. For directional precision, we predicted that the SD of a bee's dance angles would increase after sleep deprivation. For distance precision, we predicted that the coefficient of variation (CV) of a bee's waggle phase durations would increase after sleep deprivation. We discuss the relevance of these different measures in *Materials and Methods*.

To establish the effectiveness of sleep deprivation, we need to identify the features diagnostic of sleep. Honey bees exhibit various criteria that define behavioral sleep (16), including an increased threshold of response to disturbance and a specific posture during easily reversed bouts of relative immobility (17). A sleep-specific behavior in honey bees is discontinuous ventilation, consisting of several pumping motions of the abdomen (metasoma), followed by an extended pause in ventilation (18–20). Discontinuous ventilation co-occurs with increased antennal immobility and other indicators of sleep (20) and thus can be used as a proxy for detecting sleeping honey bees both inside and outside comb cells (21) (Fig. 1 *B* and *C*). During periods of reduced antennal mobility, bees exhibit increased response thresholds, and total antennal immobility is suggestive of a deeper sleep state (16). We used relative immobility combined with discontinuous ventilation as our indicators of sleep, and these conditions combined with antennal immobility to identify periods of deep sleep.

The present study addresses the impacts of sleep deprivation on specifically social phenomena under normal social conditions. Because honey bee foragers sleep primarily at night (16, 20–25), we disturbed foragers for 1 night by magnetically disturbing a select subset of bees in the hive using an "insominator" device (Fig. 2).

Results

Sleep is internally controlled (26), and an organism deprived of sleep is expected to exhibit a sleep rebound, manifested as increased total sleep or increased sleep intensity. We first evaluated the effectiveness of the insominator by recording compensatory responses indicative of recovery sleep after periods of sleep deprivation.

In our treatment bees, total sleep did not differ from that in control bees on any night before or after sleep deprivation. However, sleep deprivation had an activating effect during the beginning of the subsequent sleep period, followed by increased sleep in the middle of the night. The treatment bees slept less than the control bees during the first 3 h (22.6% ± 8.3% vs. 58.6% ± 8.6% of observations; $z = 2.69$; $P = 0.0127$; $n = 60$ observations of 16 bees), but slept more and proportionally more deeply than the control bees during the subsequent 2 h (pooled data: 72.2% ± 11.0% vs. 30.4% ± 9.7% of observations, $t = 2.85$, $P = 0.0070$, $n = 41$ observations of 13 bees; mixed effects: $z = 1.83$, $P = 0.1140$, $n = 34$ observations of 13 bees in deep sleep) (Fig. 3). We found no difference between the treatment and control bees during any period on any other night, except for an initial decrease in sleep on the night after daytime disturbance (DD) in the treatment bees.

Operation of the insominator jostled only the treatment bees. We conducted pilot studies in several observation hives in two

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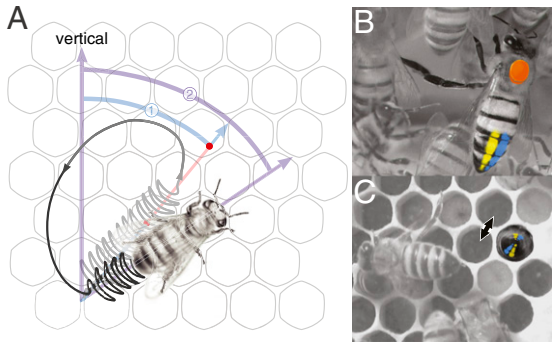

Fig. 1. Dancing and sleeping bees. (*A*) A dance consists of waggle phases which code information about a location (e.g., a food source). Pictured are two waggle phases, shown as sequences of jagged lines, connected by a long, curved path. The angle of a waggle phase relative to the vertical is the dance angle, and corresponds to the flight angle to a food source relative to the sun's azimuth. Here, 1 and 2 represent two consecutive dance angles. The duration of a waggle phase corresponds to the distance to a food source, and is the time taken for a dancer's head to traverse the distance of the waggle (red dots mark the extent of a waggle phase). SD of dance angles and CV of durations of waggle phases per dance were our precision measures of direction and distance information signaled by dancers. (*B*) A tagged and marked sleeping bee. (*C*) Bee sleeping inside a cell. Sleeping bees can be identified by their dorsoventral discontinuous ventilatory motions, represented by arrows. The images of sleeping bees have been modified to highlight sleeping individuals and display metal tag (orange circle on thorax) and paint markings (blue and yellow on abdomen).

apiaries before beginning our experiment. These pilot studies demonstrated that bees with steel tags were effectively jostled by magnets in an observation hive setting, whereas bees with copper tags were not jostled. Not all bees could be monitored for a response to the insominator at all times (e.g., bees inside cells or on the opposite side of the hive), but most treatment bees exhibiting immobility responded by moving their bodies and antennae, grooming, or walking immediately after the array of magnets swept by. The response to jostling induced by magnets varied among treatment bees, especially later at night.

Along with examining the effectiveness of the insominator (i.e., establishing recovery sleep by treatment bees), we investigated the precision of direction and distance information signaled by sleep-deprived foragers performing waggle dances. Directional precision was lower (i.e., greater SD of dance angles) in the treatment bees than in the control bees after a night of sleep deprivation compared with on the control day after DD ($z = 2.36$; one-tailed $P = 0.0499$; $n = 545$ observations of 17 bees). As predicted, this difference in directional precision between the treatment and control bees was not apparent on any other day compared with the control day after DD ($z = 2.37$ and 1.67; two-tailed $P = 0.098$ and 0.440 before nighttime disturbance (ND) and on the recovery night relative to post-DD, respectively). The results of t tests examining the averages of angles (one angle per bee per day) are consistent with this finding; treatment bees exhibited greater SDs in their dance angles than control bees on the day after sleep deprivation (16.49 ± 0.98 vs. 13.78 ± 0.91; $t = 2.02$; one-tailed $P = 0.0342$; $n = 6$ treatment bees and $n = 7$ control bees) (Fig. 4), but not on any other day (pre-ND: $t = 1.34$, two-tailed $P = 0.2174$, $n = 4$ treatment bees and $n = 6$ control bees; recovery: $t = 1.78$, two-tailed $P = 0.1003$, $n = 8$ treatment bees and $n = 6$ control bees; post-DD: $t = -1.61$, two-tailed $P = 0.1400$, $n = 6$ treatment bees and $n = 5$ control bees). Directional precision decreased in waggle phases performed specifically after left turns (post-ND relative to post-DD control day: $z = 2.81$; one-tailed $P = 0.01548$; $n = 543$ observations of 17 bees). For clear examples of dances by a treatment bee and control bee, see Movie S1 and Movie S2.

In contrast to directional precision, distance precision did not differ between treatment and control bees on the day after sleep deprivation relative to the control day after DD ($z = 0.96$; one-tailed $P = 0.434$; $n = 545$ observations of 17 bees), and also did not differ on any other day relative to the control day after DD ($z = 0.55$ and 0.68; two-tailed $P = 0.992$ and 0.973, pre-ND and recovery day relative to post-DD, respectively). Results were

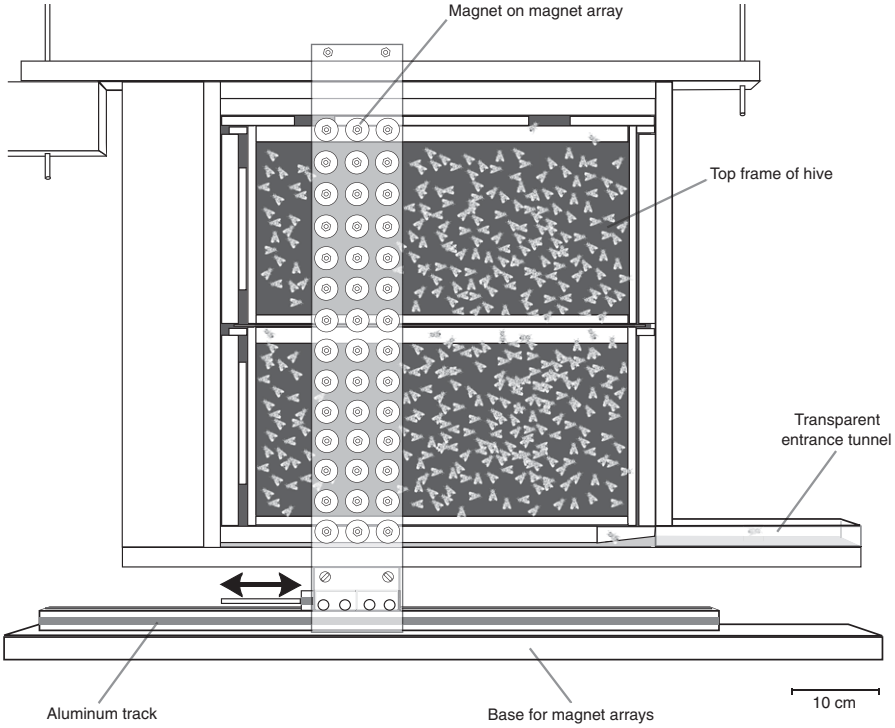

Fig. 2. Anterior view of a two-frame observation hive and insominator. The insominator, consisting of two magnetic arrays flanking the hive, a track, and a base supporting the track and arrays directly contacts the suspended observation hive at only one point on each side of the hive. Arrows represent directional movement of magnetic arrays on the anterior and posterior sides of the hive. Dark areas on the periphery of the hive represent passageways between the anterior and posterior sides of the hive. The magnets jostled treatment bees, but not control bees. Magnet arrays remained to the left or right of the hive between insominator movements.

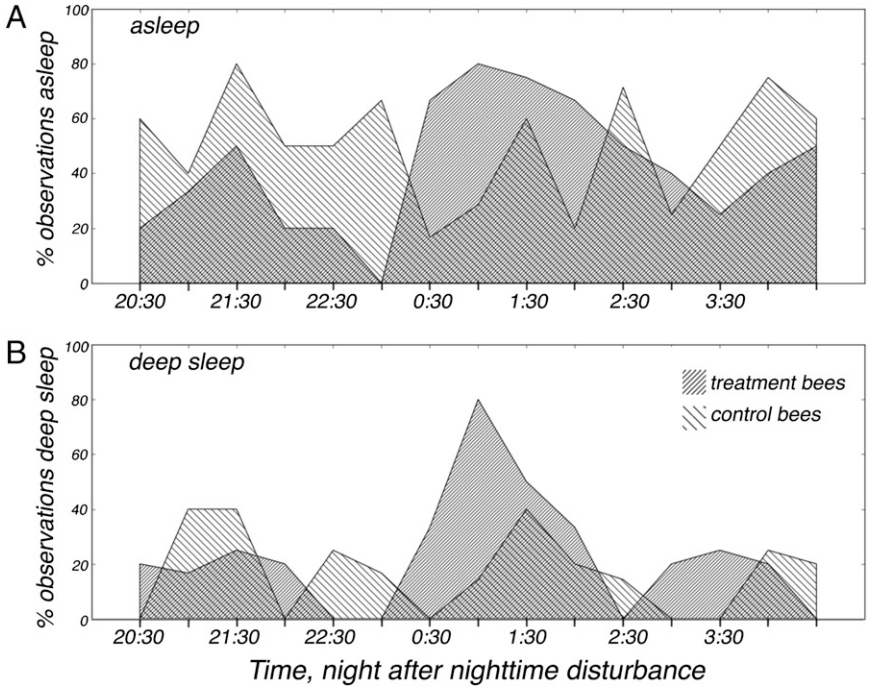

Fig. 3. Recovery sleep. Shown are the percentages of observations during the night post-ND in which bees were asleep (*A*) and exhibiting deep sleep (*B*). Treatment bees ($n = 9$) were more active at the beginning of the night, but experienced more sleep and more deep sleep than the control bees ($n = 7$) during the middle of the night. A bee's behavior was recorded once every 30 min.

consistent when analyzing only SDs. Measures unrelated to the waggle dance, such as feeder visitation, visitation rates, tendency to perform waggle dances, and waggle dance rates, also did not differ significantly between the treatment bees and control bees.

Nineteen of the 50 individually marked honey bee foragers survived and retained their metal tags, 11 with magnetic steel tags and 8 with nonmagnetic copper tags. One treatment bee and one control bee never danced and so could not be included in the dance analyses. Two treatment bees and two control bees could not be distinguished from one another at the feeder and so were excluded from the feeder analysis. The weather was stable throughout the study period (16.9 ± 1.1 °C, no precipitation), and bees foraged and danced every day.

Discussion

Sleep deprivation is the primary experimental approach for testing the importance of sleep in the functioning of an organism. In the present study, only sleep-deprived honey bees were affected by disturbances, both in terms of compensatory sleep indicative of a sleep rebound and in terms of an effect on the directional precision in their waggle dances.

Although recovery sleep would be expected to commence promptly at the beginning of a typical sleep period after sleep deprivation, our population of treatment bees exhibited an initial increase in nocturnal activity relative to the control bees. This initial activation was followed by an increase in sleep and deep sleep in the treatment bees. Previous studies also reported increased activity during typically "inactive" periods after sleep deprivation, occurring in conjunction with a period of increased sleep of some form (e.g., sleep stage or intensity) (27–31). It is likely that this period of increased sleep and deep sleep represents recovery sleep (Fig. 3); thus, we have evidence that the insominator caused sleep deprivation in honey bees in situ. In addition to this nighttime recovery sleep, it is conceivable that the treatment bees obtained recovery sleep during the day. One previous study that deprived isolated honey bees of sleep reported recovery sleep occurring only during the night after deprivation (22), whereas another reported recovery sleep during the day as well (32). We could not record daytime sleep (because we were recording dancing behavior), and could not record deep sleep obtained inside comb cells (*SI Discussion*).

Having obtained evidence of induction of sleep deprivation using the insominator, we tested for a negative impact of sleep deprivation on the precision of signal production in honey bees. Directional precision was impaired, but distance precision was not. Our measures of distance precision did not differ greatly in the treatment and control bees; differences might have been obscured by a range of factors that affect waggle phase duration, including the optic flow that an individual bee experiences (33). It is also possible that processing and signaling direction information is cognitively or physiologically more taxing than signaling distance information, and that distance precision across waggle phases is less sensitive to fatigue. Along with dance angles, we initially examined divergence angles (i.e., absolute value of the difference between dance angles performed after a left turn and a right turn) as a possible measure of directional precision (34–36). However, several recent studies have concluded that the divergence angle results from a physiological constraint limiting a bee's ability to measure body orientation during short waggle phases, and thus it might not be an adaptive feature of bee communication (37–39). In light of these findings, we report results only for the precision of the dance angle, which is not constrained like divergence angles and thus should provide a more direct, intuitive measure of precision in direction signaling by dancers and should be more likely to be sensitive to sleep deprivation.

Three previous studies have examined sleep-deprived honey bees. Two of these studies did so to establish that subjects isolated from their hives were actually sleeping and that sleep was controlled internally (22, 32). The third study examined isolated, tethered foragers to report an effect of sleep deprivation on extinction learning (40). Here we introduce a method to effec-

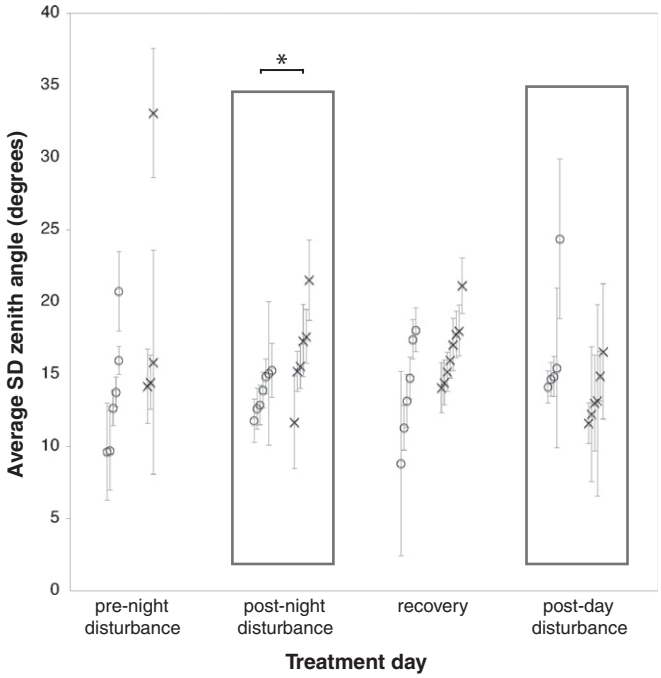

Fig. 4. Effect of sleep deprivation. Directional precision in treatment bees (X) versus control bees (O) on the day before ND, the day after ND, 2 d after ND (recovery), and the day after DD is shown. The asterisk highlights a significant difference in directional precision between treatment bees (n = 6) and control bees (n = 7), based on a mixed-effects model. Treatment bees exhibited lower precision (i.e., greater SD averages) than control bees in post-ND periods relative to post-DD periods (boxes). Symbols represent average values (± SEM) for each bee for each treatment day.

tively sleep-deprive populations of a social model organism in situ while controlling for nonspecific stressors, and we demonstrate an effect relevant to their social functioning.

Our findings show that sleep deprivation degrades the ability of European honey bees to precisely signal directional information via their waggle dances. The communication of direction information may suffer when either dancers or dance followers lack sleep, and this can be expected to result in less efficient foraging. Testing this hypothesis will be the next step in investigating the potential consequences of sleep deprivation on the fitness of a colony. Additional materials and updated information will be made publicly available via http://www.pupating.org.

Sleep may have evolved novel functions in species whose members live in societies, and thus sleep studies are needed in a range of social species. There is also a need for tractable model systems for studying sleep function in social species. Inventing a means to deprive honey bees of sleep inside their hive, and demonstrating that their signaling ability is thereby compromised are important steps forward in addressing these needs.

Materials and Methods

Study Site and Preparation of Subjects. On July 7. 2007, we suspended a two-frame observation hive of ≈4,200 European honey bees (*Apis mellifera ligustica*; queen breeder; C.F. Koehnen and Sons) from the ceiling of a wooden hut (41) at Cranberry Lake Biological Station in Adirondack State Park, NY (44°09″N, 74°48″W) and allowed the bees to forage freely for 2 wk before beginning the experiment. After this 2-wk period, we trained a group of 50 bees to visit a feeder with sucrose solution located 1 km away from the hive. We collected the bees in perforated Ziplock bags at the feeder, cooled them individually in a refrigerator, and then marked each bee with a unique combination of colors (using Sharpie oil-based pens), repeating marks on the dorsal and ventral sides of the abdomen (metasoma) to facilitate identification of a bee when in a cell (Fig. 1*C*) or when dorsally obscured (e.g., while clinging to the glass pane of the hive). We used shellac to adhere a magnetic disk (2.38 mm diameter, 0.25 mm thick) of cold rolled steel punched from shim stock (Lyon Industries) to the dorsal mesosoma of 25 of the foragers (42) (Fig. 1*B*). Handling the control bees identically, we adhered a piece of nonmagnetic copper of equal dimensions and mass to the dorsal mesosoma of the remaining 25 foragers. Metal disks were coated with orange paint to obscure the bees' status as treatment or control. The bees were fed and caged to allow the shellac to dry before being reintroduced into their hive.

Sleep Deprivation Device. B.A.K. constructed a manually operated sleep deprivation device, the insominator (Fig. 2), designed to jostle only bees with magnetic metal disks. The insominator consisted of two pieces of Plexiglas, each containing three columns of 14 neodymium rare earth magnets (nickel-plated NdFeB, grade N42, #RX054; K&J Magnetics), totaling 42 magnets per side, arranged 1 cm apart within an array and so that the magnetic polarities of the two arrays cancelled out each other. When viewed laterally, the facing Plexiglas pieces were parallel, setting up a consistent magnetic attraction throughout the hive. The magnetic arrays slid along an aluminum rail that was permanently affixed below the suspended hive, limiting the points of contact between the insominator and the hive to only one felt-lined magnet per side (Fig. 2). Unlike the automated sleep-deprivation devices previously used on isolated, caged honey bees (22, 32), our insominator selectively disrupted uncaged treatment bees while they were in their hive, to maintain normalcy of the social conditions and avoid any observable disruption of control bees.

Schedule. The hive was lit by day (08:30–20:30 hours) with a 15W incandescent light bulb and by night (20:30–08:30 hours) with a 52W incandescent bulb filtered by red acetate, to minimize the bees' vision. The feeder located 1 km from the hive was made available between 1000 hours and 1400 hours each day from July 21 to 26, 2007. Individual bees' visits to the feeder were recorded on a voice recorder (VN-400PC; Olympus), and all dances by marked bees were videotaped (AGDVC 30; Panasonic). On night 1 and day 1, we recorded normal behavior (sleep and dance, respectively) before sleep deprivation. On night 2, we operated the insominator for 12 h (2030 hours–0830 hours). For the next 24 h, we recorded the daytime dances and nighttime sleep behavior, and then repeated the recordings for an additional 24-h "recovery" period (Fig. S1). A growing number of sleep deprivation studies include a stress control, disturbing subjects during the period when they are primarily awake (22, 43, 44); thus, we also operated the insominator for 12 h during the day and recorded subsequent sleeping and dancing as a control for disturbance-induced stress (Fig. S1). During periods of disturbance, we moved the arrays of magnets a minimum of three times each minute across the hive, for 3 s per sweep. Late in the night (beginning at 0300 hours), we jostled bees when they were not responding to the insominator by shaking a single magnet in each bee's vicinity for several seconds between insominator sweeps. Audio notes were recorded during the nighttime disturbances, and the frequency of both types of disturbances was replicated as closely as possible during the DD period, guided by playback of the audio recording. To verify the impact and evidence of sleep deprivation, one of us (B.A.K.) observed marked bees with a red-filtered headlamp during the nights before and after the night of sleep deprivation, scanning both sides of the hive in a consistent manner across a grid superimposed on the observation hive, and recorded each bee's behavior every 30 min between 2030 hours and 0400 hours. Recording a bee's behavior required several seconds to several minutes, depending on visibility.

Behaviors Recorded. We identified a bee as asleep when she was in a relaxed state (i.e., body immobile, except for occasional twitches of a leg, tarsus, or extension of proboscis) and discontinuously ventilating. B.A.K. recorded sleep when individually marked bees were inside cells or when the bees were outside cells. When outside cells, bees' antennae were immobile, exhibited minute twitches, or exhibited larger, usually swaying, motions. A discontinuously ventilating bee with immobile antennae was reported to be in a deep sleep state. Identifying discontinuous ventilation required careful examination of a bee for a minimum of 10 uninterrupted seconds, a duration estimated from an analysis distinguishing respiratory rates of heating bees versus resting bees (19). Distinguishing the antennal state required an average minimum of 3–5 s. If a bee was obscured from view, then her behavioral state was not recorded. A bee sleeping inside a cell could not be distinguished as exhibiting deep sleep, because her antennae were hidden from view. For more information on identification of sleep in honey bees, see Klein et al. (21).

Transcription of Data. Videos of waggle dances were transcribed using QuickTime (v7, http://www.apple.com/quicktime). We measured the di-

rectional precision of the dance as the SD of dance angles and the distance precision as the CV of waggle phase durations. The CV was calculated to compare the precision of waggle phase durations, because this measure takes into account magnitudes of means. The CV could not be applied to the comparison of directional precision, because mean angles were often close to 0 or negative, making comparisons less meaningful. The time and place of each waggle phase start and end point were transcribed by playing the videos frame-by-frame beneath a transparent Web browser (aeroFox v1.0.5), and using the computer mouse to click on a single point of a dancing bee viewed through the transparent browser window (Fig. S2). Of the dancer's body regions, the head moves the least in the lateral direction (13); thus, a point located medially and immediately posterior to the head was selected to establish the start and end points of a waggle phase. A.K. wrote a computer program that calls the JavaScript libraries jQuery (1.2.1) (http://jquery.com/) and Walter Zorn's Vector Graphics Library (3.0.3) to store each pair of browser-based mouse-click coordinates in a MySQL database, and wrote a second program in Python (v2.6, http://www.python.org) to compute dance angles and waggle phase durations from the pairs of mouse clicks (*SI Appendix*). Data transcription was done blindly, and interobserver error for calculating angles was 1.06% ± 0.24% (3.33 ± 0.82 degrees).

Analyses. We fitted some of our foraging honey bees with copper tags as a control for any baseline disturbance caused by the insominator and for any environmental factors specific to the day of testing. We tested bees within the hive to maximize normalcy, increase the likelihood of bees dancing, and avoid confounders associated with testing isolated bees, which sleep less over consecutive days and die prematurely (16, 20, 22). Treatment effects were compared with results after a DD period to test for differences between sleep deprivation and general disturbance. The results are products of linear mixed-effects models, programmed in R (45), with bee population (treatment vs. control) and treatment day as fixed effects and bee identity as a random factor (i.e., observations were nested within a bee). Linear mixed-effects models were fitted using the lmer function in the lme4 package (46). The multcomp package was used to perform likelihood ratio tests to distinguish between competing models (47); the complicated (e.g., missing data, correlated covariates) and unbalanced nature of the data precluded the use of standard likelihood ratio tests (48). The resulting continuous, linear predictors are reported as mean ± SEM. To visualize the data (Figs. 3 and 4) and confirm the mixed-effects model results, we also calculated average values (one per bee) using JMP version 8 (SAS Institute). We set $\alpha = 0.05$ for all tests. The sleep deprivation literature overwhelmingly documents decrements in precision and efficiency; because sleep deprivation was not expected to induce greater precision, we report one-tailed *P* values when testing our predictions relating to the impact of sleep deprivation on dances on the day after nocturnal perturbations. We report two-tailed *P* values under all other conditions.

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Supporting Information

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SI Discussion

Adult worker honey bees spend less time sleeping inside cells as they age and change caste, but foragers slept considerably more inside cells during this study than previously documented (1) (treatment bees, 8.4% ± 4.4%; control bees, 9.3% ± 4.6% of total observations, averaging measures per bee). Treatment bees slept less inside cells during the night after sleep deprivation compared with control bees (5.7% ± 3.0% vs. 21.7% ± 8.0% of observations) relative to the night after DD ($z = -3.21$; $P = 0.0090$) and relative to the night before sleep deprivation, although this latter comparison was not statistically significant ($z = -2.22$; $P = 0.1400$). At present, how much deep sleep is obtained inside cells, and how sleep inside cells specifically contributes to recovery sleep, remain unclear.

1. Klein BA, Olzsowy KM, Klein A, Saunders KM, Seeley TD (2008) Caste-dependent sleep of worker honey bees. *J Exp Biol* 211:3028–3040.

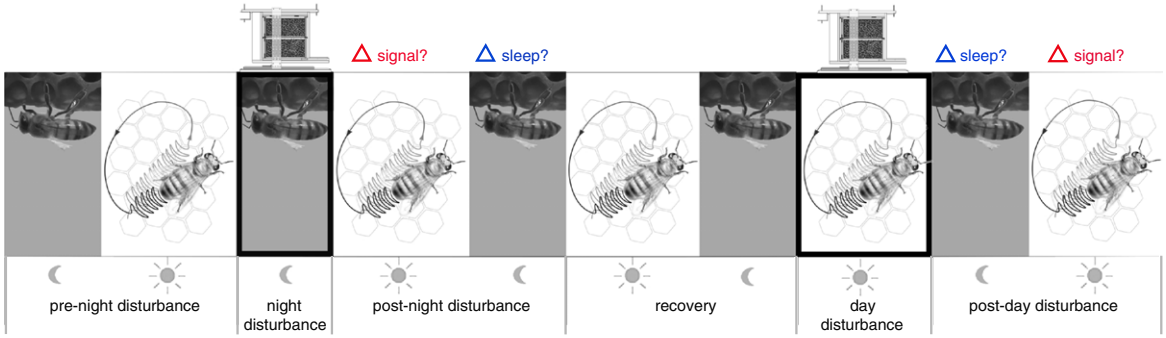


Fig. S1. Schedule of study. We examined bee sleep each night (upside-down bee with moon below), and waggle dances each day (dancer with sun below), except during periods of ND and DD (insominator above each boxed disturbance period). We tested for an effect of sleep deprivation on sleep the night after deprivation (Δ sleep?) and for an effect of sleep deprivation on signaling (Δ signal?) by comparing the results in treatment and control bees during post-ND and post-DD periods.

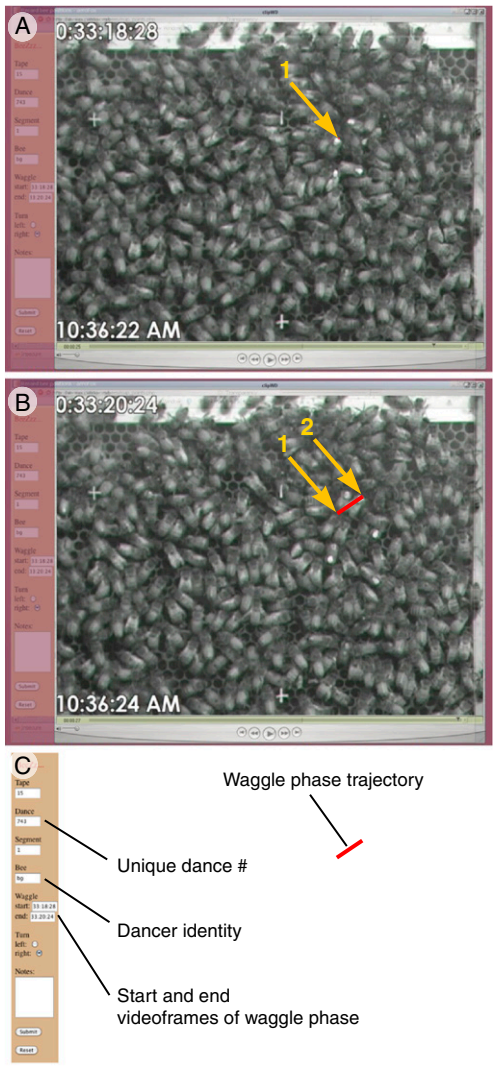
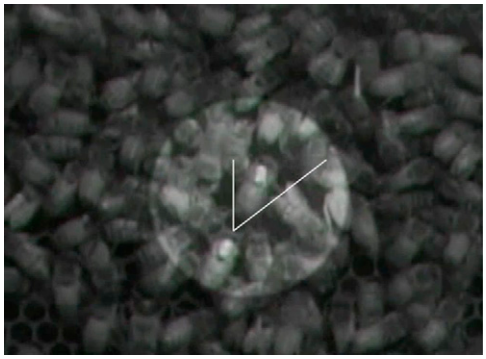
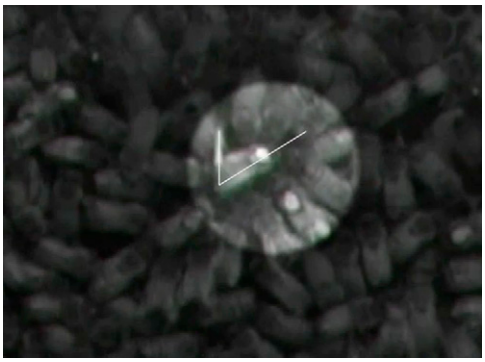


Fig. S2. Transcription of waggle dance direction and distance information. We selected the start point (1) and end point (2) for each waggle phase. (*A*) After a dancer turned and began her lateral body waggling, marked by a mouse click in the medial, anterior region of the mesosoma (white spot). (*B*) The final video frame of a dancer's waggling (at the end of her dance, or immediately before turning and beginning another waggle phase), marked by a second mouse click. (*C*) The line segment used to calculate dance angle for direction information. Start and end times (duration) were used for distance information.



Movie S1. Waggle dance performed by a sleep-deprived bee. This movie spotlights a clear example of one waggle dance performed by a forager that had been sleep-deprived the previous night. The average dance angle of this dance is superimposed over the dancer, and variance around this angle indicates imprecision of signaling direction information (Quicktime; 7.9 MB).

Movie S1



Movie S2. Waggle dance performed by a control bee. This movie spotlights one complete waggle dance performed within minutes of the dance performed by the sleep-deprived forager featured in Movie 1. This forager was tagged with a nonmagnetic metal (copper), and so was not perturbed by the insominator when it operated the previous night (Quicktime; 5.6 MB).

Movie S2

Other Supporting Information Files

SI Appendix (PDF)

Appendix

Computer code used for transcribing waggle dance information. The following computer program is the JavaScript used to record bee positions and orientations presented below a transparent browser. This program calls the JavaScript libraries jQuery (1.2.1) (http://jquery.com/), Walter Zorn's Vector Graphics Library (3.0.3), and the PHP program store_points.php to store the coordinates (X1,Y1) and (X2,Y2) of mouse clicks in a MySQL database.

```
<script type="text/javascript" src="scripts/jquery.js"></script>
<script type="text/javascript" src="scripts/wz_jsgraphics.js"></script>
<script type="text/javascript">
 $(document).ready(function(){
   var offsetX = 0;
   var offsetY = 0;
   var line_offsetX = offsetX
   var line_offsetY = offsetY
   jQuery(document).ready(function(){
     var X = [];
     var Y = [];
     $("#window").mousemove(function(e){
       $('#live').html((e.pageX-offsetX) +', '+ (e.pageY-offsetY));
     });
     $("#window").click(function(e){
       X[X.length] = e.pageX-offsetX;
       Y[Y.length] = e.pageY-offsetY;
       if (X.length==1) {
         $('#click1').html(X[0] +', '+ Y[0]);
       } else if (X.length==2) {
         jg.setColor("#ff0000");
         jg.setStroke(Stroke.DOTTED);
         jg.drawLine(X[1]+line_offsetX,Y[1]+line_offsetY,X[0]+line_offsetX,Y[0]+line_offsetY);
         jg.paint();
         $('#click2').html(X[1] +', '+ Y[1]);
         $.get("store_points.php", { X1:X[0],Y1:Y[0],X2:X[1],Y2:Y[1] });
         $("div.main_insert").find("p.warning:visible").slideUp("slow");
       }
     });
   })
 });
</script>
```