RESEARCH ARTICLE

Honeybees consolidate navigation memory during sleep

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SUMMARY

Sleep is known to support memory consolidation in animals, including humans. Here we ask whether consolidation of novel navigation memory in honeybees depends on sleep. Foragers were exposed to a forced navigation task in which they learned to home more efficiently from an unexpected release site by acquiring navigational memory during the successful homing flight. This task was quantified using harmonic radar tracking and applied to bees that were equipped with a radio frequency identification device (RFID). The RFID was used to record their outbound and inbound flights and continuously monitor their behavior inside the colony, including their rest during the day and sleep at night. Bees marked with the RFID behaved normally inside and outside the hive. Bees slept longer during the night following forced navigation tasks, but foraging flights of different lengths did not lead to different rest times during the day or total sleep time during the night. Sleep deprivation before the forced navigation task did not alter learning and memory acquired during the task. However, sleep deprivation during the night after forced navigation learning reduced the probability of returning successfully to the hive from the same release site. It is concluded that consolidation of novel navigation memory is facilitated by night sleep in bees.

Key words: honeybee, navigation, sleep, memory consolidation, sleep deprivation.

INTRODUCTION

The beneficial role of sleep in memory consolidation is well documented for humans (Born et al., 2006; Marshall and Born, 2007; Born, 2010; Diekelmann and Born, 2010), mammals (Siegel, 2005; Capellini et al., 2009) and birds (Jackson et al., 2008). Different stages of sleep appear to be involved in specific forms of memory consolidation, e.g. slow-wave sleep in hippocampus-dependent declarative forms of memory, and rapid eye movement (REM) sleep in memory for motor performance. The processes involved for selective memory consolidation in different sleep phases are, however, not understood. Rest states and sleep have also been documented in insects, for example in the fruit fly *Drosophila* (Hendricks et al., 2000; Greenspan et al., 2001; Joiner et al., 2006). A link to memory processing in *Drosophila* is indicated by molecular genetic manipulations of the mushroom body, a structure well known to be involved in memory formation and retrieval in the insect brain (Menzel and Müller, 1996; Heisenberg, 2003; Davis, 2011), but different forms of memory have not been tested for their sleep dependence. In honeybees (*Apis mellifera*), sleep is characterized by a characteristic body position during resting, relaxed muscle tonus, lower sensitive to sensory stimuli and a rebound effect after sleep deprivation (Kaiser and Jander, 1988; Kaiser and Steiner-Kaiser, 1988; Kaiser, 2002; Sauer et al., 2003). In a rebound of sleep, animals and humans extend their sleep periods during the night following sleep deprivation. Deprivation of sleep in bees leads to reduced performance in the waggle dance behavior, particularly with respect to the precision of directional coding of the indicated outbound flight (Klein et al., 2010). A link to memory consolidation in bees was established by the finding that bees remember a learned extinction task less well if they were sleep-deprived the night after extinction learning (Hussaini et al., 2009). In such a learning

paradigm, the bee first learned to respond to an odor associated with reward. This form of learning, called acquisition learning, was not adversely affected by sleep deprivation. After acquisition learning, the bee was exposed to extinction trials, i.e. stimulations with the learned odor without reward, and formation of extinction memory was compromised after sleep deprivation. Because reduced extinction memory after sleep deprivation induces higher responses to the stimulus acquired during acquisition learning, unspecific effects leading to a failure to respond could be excluded as an explanation because animals perform better in reference to the original memory. Extinction memory resembles characteristic features of the modulation of an existing memory trace by novel experience [e.g. spontaneous recovery from extinction and reconsolidation (Stollhoff et al., 2005; Eisenhardt and Menzel, 2007)]. It is therefore possible that in bees, as in mammals and humans, particular forms of memory consolidation may depend on sleep and others may be independent of sleep.

We previously hypothesized (Hussaini et al., 2009) that those forms of learning that require a change or modification of a memory trace established under similar context conditions may require sleep-dependent processing. Here we address the question of whether learning during navigation leads to such sleep-dependent memory consolidation. We developed a forced navigation task using harmonic radar to quantify the learning effect. Test bees were exposed to such a forced navigation task and their sleep behavior was recorded under natural conditions inside the hive. This procedure also allowed us to investigate whether flight activity and the manipulations during the forced navigation learning lead to prolonged sleep. Then we asked whether the bees' navigation performance as tested on the next day is reduced if the bees were deprived of sleep during the night. We found that sleep-deprived

bees suffer from reduced navigation memory for the acquired information during the forced navigation task, providing evidence for a supportive function of sleep in the consolidation of newly established navigation memory.

MATERIALS AND METHODS
Experiment 1: flight tracking with harmonic radar and quantifying forced navigation learning

A group of honeybees (*Apis mellifera carnica* Pollman 1879) was trained to a feeder 200 m to the east of the hive on a flat pastureland near Klein Lüben (Brandenburg, Germany) that was free from obstacles which would have obscured the radar's field of view, but included radar-transparent colored tents (for details, see Menzel et al., 2005; Menzel et al., 2011). A test bee was caught at the feeder while preparing to fly back to the hive after sucking its fill, transported to a release site several hundred meters away from the hive, and equipped with a transponder to track its flight as described previously (Riley et al., 2005). In brief, the transponder returns a signal to the radar at twice the original transmitted frequency, which can be reliably distinguished from all other signals and noise. The test bees were individually tagged with colored number tags glued to the thorax so they could be identified in repeated catch-and-release experiments.

For displacement, single test bees were caught at the training feeder after they had sucked their fill and transported in a small container to one of the release sites, 300 to 450 m away from the hive and located in different directions from the hive. The radar recorded the flight trajectories of the same animal in repeated releases from the same release site, and the length of flight, the time of homing behavior and the homing rate were evaluated. Here we used the reduction of flight length after the second release as compared with the first release expressed in percentage of change to quantify the learning effect (LE).

Experiment 2: recording rest and sleep behavior with RFID inside the hive

The second experiment was performed in an urban area around the institute in Berlin-Dahlem and consisted of the following steps: training a group of bees carrying radio frequency identification devices (RFIDs) (RFIDs von der GIS-mbh, Lenningen, Germany) to a feeding station, monitoring their inbound and outbound flights at the hive entrance, which allowed us to monitor their movements inside of the hive, and exposing them to a forced navigation task.

A bee colony with ~500 workers was kept in a single-frame, one-sided observation hive allowing observation of the bees through a glass window. The colony was housed in a hut behind our laboratory in Berlin. The bees entered the colony through a 30 cm long tube that was fitted with two antennae for detecting the RFIDs attached to the thorax of the experimental bees (Fig. 1). The sequence of RFID signals recorded by the two antennae allowed us to distinguish outbound from inbound flights. The experimental bees were trained to a feeder 50 m from the colony marked with a color signal. The RFID (Microsensys, Erfurt, Germany, www.microsensys.com) was glued to the thorax of the bee with cyanoacryl super glue (Sekunden Kleber, Conrad, Hirschau/Oberpfalz, Bayern, Germany). In addition, colored dots on the bees were used to visually identify the test bee.

The signals from the RFIDs of several bees (up to 15) were detected inside the colony by an antenna affixed to the moving part of a digital X/Y plotter (Roland Digital Group DXY-1300, Hamamatsu, Japan) that scanned the full frame. The spatial

Fig. 1. Radio frequency identification device (RFID) glued to the thorax of an experimental bee, *Apis mellifera carnica*. This type of RFID was chosen because it can be read at distances of several centimeters.

resolution of the RFID antennae was 1.5 cm on the level of the comb. A personal computer controlled the movement of the plotter (one full meandering scan across the whole frame every minute) and recorded the signals of the RFIDs. At the entrance to the hive, a custom program extracted the flight data of the bees and stored their position on the frame at intervals of 1 min. We visually observed and video recorded the marked bees at night. We found that rest times >5 min were reliably connected with relaxed and downward-pointing antennae, indicating sleep (Kaiser, 1988). Therefore, we assigned any rest at the same location at night for >5 min as sleep. During the day, rest times could not be so reliably assigned as sleep; therefore, we refer to rest rather than sleep during the day (test bees detected at the same location for >3 min). Note that it was not possible to glue both an RFID and a radar transponder to an animal. As a result, we performed the experiments using the RFID for flight and sleep behavior without radar tracking.

For displacement, bees equipped with an RFID were caught at the feeder after they had sucked their fill and transported to a release site 600 m north-northwest of the hive. These experiments were performed in an urban area (Berlin-Dahlem, Germany). The areas around the feeder and the release sites were very different. The feeder was positioned between houses on a parking lot, whereas the release sites were located along roads with different structures nearby (e.g. trees, houses).

Sleep deprivation

Test animals were collected at the training feeder and transferred into a small box. The box was placed on a vortex that was gently activated for 8 h during the night, preventing animals falling asleep, as described in Hussaini et al. (Hussaini et al., 2009). Because we observed that bees isolated from the colony tended to fall asleep already during the day, we deprived sleep only during the night.

Statistical analysis

The Mann–Whitney U-test, one-way ANOVA and Fisher's exact test were applied to the data as described in the respective figures. Correlations were analyzed using a χ^2 independence test.
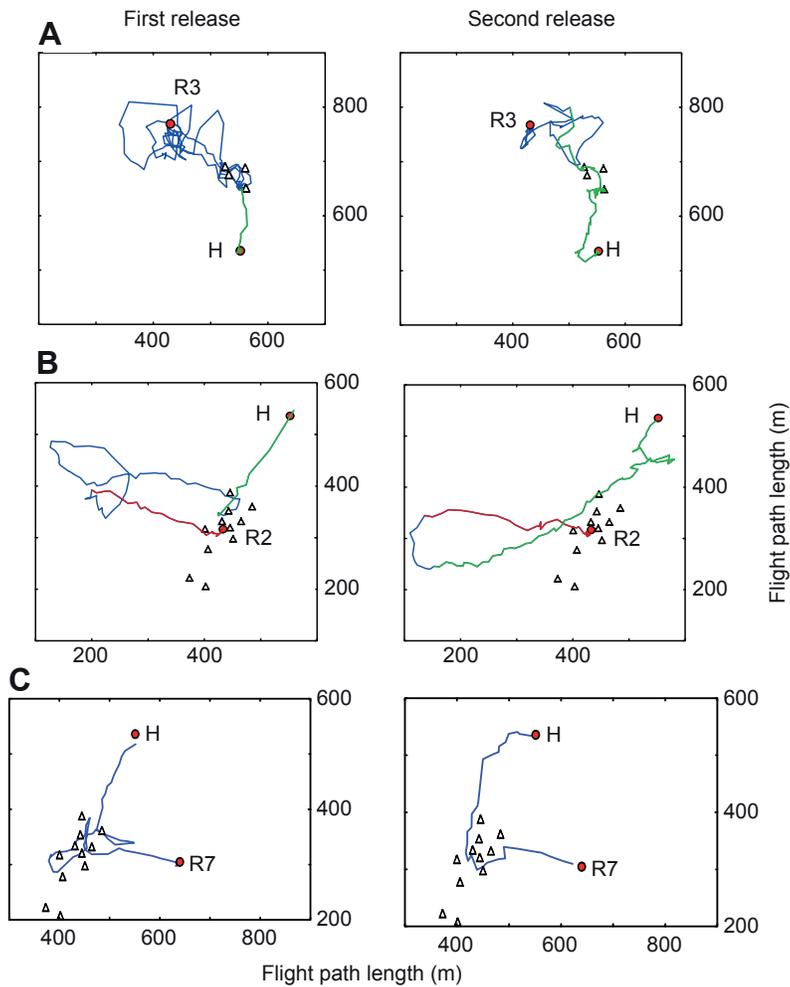
Fig. 2. Three examples of radar tracks of homing flights in catch-and-release experiments. Each of the three bees was collected at a feeder, after it had sucked its fill, transported to the release site (R2, R3 and R7), equipped with a radar transponder and then released. The left column shows the flight trajectories after the first release, the right column those after the second release. The bee in A was trained to a feeder close to the hive (distance 10 m), which rotated around the hive (two to four revolutions per day), a training procedure that prohibits learning of a flight vector by the bee. The two bees in B and C were trained to a feeder 200 m east of the hive. The small triangles indicate colored tents serving as radar-transparent landmarks. The learning effect (LE) is the ratio of the flight path length during the second release to that during the first release. The LE for flight path A is 0.47, for B is 0.72 and for C is 0.56 (mean ± s.d. LE for 14 bees=0.63±0.17).

RESULTS
Experiment 1: the forced navigation learning task

In the catch-and-release procedure, a foraging bee was collected when leaving the hive or a feeding site, and then transported to an unexpected release site within the range of the explored area around the hive (300–600 m radius around the hive). Under such conditions, the bee first applies the vector memory of the flight it would have performed if it were not transported (outbound or inbound to the hive), and then it switches to search flights before returning back to the hive along straight and fast flights [homing behavior (Menzel et al., 2000)]. Fig. 2 gives three representative examples, showing that bees released the second time at the same site performed shorter search flights and more directed homing flights. The LE during the first homing flight is calculated as the ratio of the flight path length during the second release to the flight path length during the first release. The mean ± s.d. LE measured for 14 bees was 0.63±0.17. This means that the bees learned landmark constellations on their first homing trip that they used to perform more effective homing flights later.

The catch-and-release procedure combined with gluing the transponder to the test bee at the release site did not stress the bee in any obvious way. Their flight speed was not different from bees without a transponder. Even animals recruited by the waggle dance and caught at the hive entrance when leaving the hive perform route flight according to the information received from the dancing bee (Riley et al., 2005, Menzel et al., 2011).

Experiment 2: bees do not rest or sleep longer after prolonged foraging trips

The navigation task we exposed the bees to during their first release will lead to longer flight times than when navigating along the learned direct route between hive and feeder because they first need to localize themselves and then find the way back to the hive. The strategies applied and the memory retrieved under these conditions were discussed previously (Menzel et al., 2005). Animals first apply the vector component which would have brought them back to the hive if not transported to an unexpected release site, then they need to localize themselves, recruiting memory from their exploratory orientation flight early in their life as foragers, and finally they have to make decisions according to the selected goal, the hive or the feeder. These cognitive processes are performed during the search flight mode and involve novel learning with respect to the navigational cues experienced during the homing flight. These extended flights may themselves lead to longer rest or sleep time afterwards. Therefore, we first asked whether longer flights during natural foraging behavior along well-learned routes lead to longer rest and sleep times. Bees marked with a transponder foraged either at the experimental feeding site close to the hive (50 m) or at unknown natural feeding sites. Time intervals between outbound and inbound crossings of the RFID detectors at the hive entrance (flight time) were <10 min for the bees foraging at the close feeder (mean ± s.d.=7.4±1.2 min) and >10 min for most of those foraging at unknown sites (mean ± s.d.=24.6±19.7 min). We first calculated the cumulative flight times
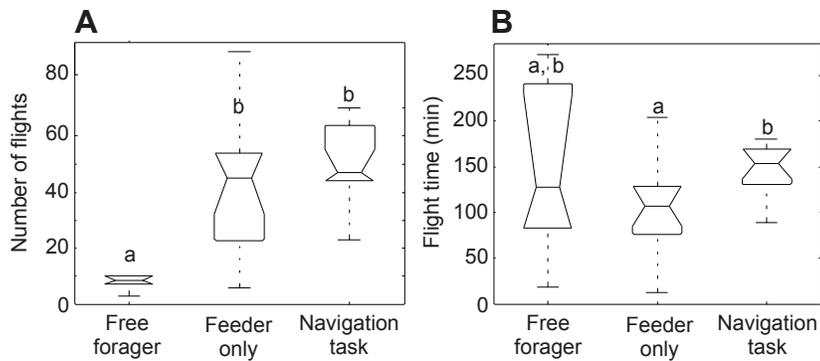
Fig. 3. Number of foraging flights (A) and flight time (B) of three groups of bees: free foragers (foragers at unknown natural food sources, $N=6$), feeder-only bees (bees foraging at the training site without interruption, $N=15$) and navigation-task bees (bees foraging at the training site after they had been exposed to a forced navigation task, $N=16$). The forced navigation task did not reduce the number of foraging trips to the trained feeder but prolonged the flight time (Mann–Whitney U-test, $P<0.05$). Shown are medians and interquartiles; different letters indicate significantly different values between groups ($P<0.05$).

of 52 bees and correlated them with their cumulative sleep times during the following night. No correlation was found (χ^2 independence test, $N=52$, $R^2=0.0016$). We then observed single bees that performed very different flight times (between 2 and 29 min, $N=49$) between the hive and unknown natural food sources, and correlated their flight times with their cumulative rest times immediately following the respective flight until the next departure from the hive. No correlation was found (χ^2 independence test, $N=49$, $R^2=0.0016$). Similarly, average cumulative rest times for flight durations greater than 19 min or less than 10 min did not differ. We therefore conclude that rest times during the day were independent of the last flight duration, suggesting that rest is not related to energy consumption during flight, or that flight times in natural foraging do not stress the bee with respect to energy consumption.

Navigation learning does not interfere with trained foraging but leads to longer foraging times at the trained site

The catch-and-release procedure applied in the forced navigation task may have a demotivating effect on the test bees, possibly leading to longer periods inside the hive and less foraging activity at the trained feeding site. Therefore, we first examined whether test bees reduced their foraging activity after they were exposed to the forced navigation task. Fig. 3 gives the results for two measures, the number of outbound flights toward the feeder during 1 day and flight time (interval between outbound and inbound at the hive entrance detector). Three groups were compared: bees foraging at unknown natural sites (free foragers), bees foraging at the training feeder (feeder only) and bees foraging at the same training feeder after they had been exposed to the forced navigation task (navigation task). No significant differences were found in the number of foraging trips to the trained feeder for feeder-only bees or navigation-task bees (Fig. 3A). However, the flight time of navigation-task bees was significantly longer than that of feeder-only bees (Fig. 3B). This result indicates that bees are not demotivated to forage at the trained feeder and do not extend their time inside the hive after a forced navigation task, but they extend their trips to the trained feeder, possibly by additional exploratory behavior. Bees foraging at unknown natural sites perform significantly fewer trips per day, and although the average flight time
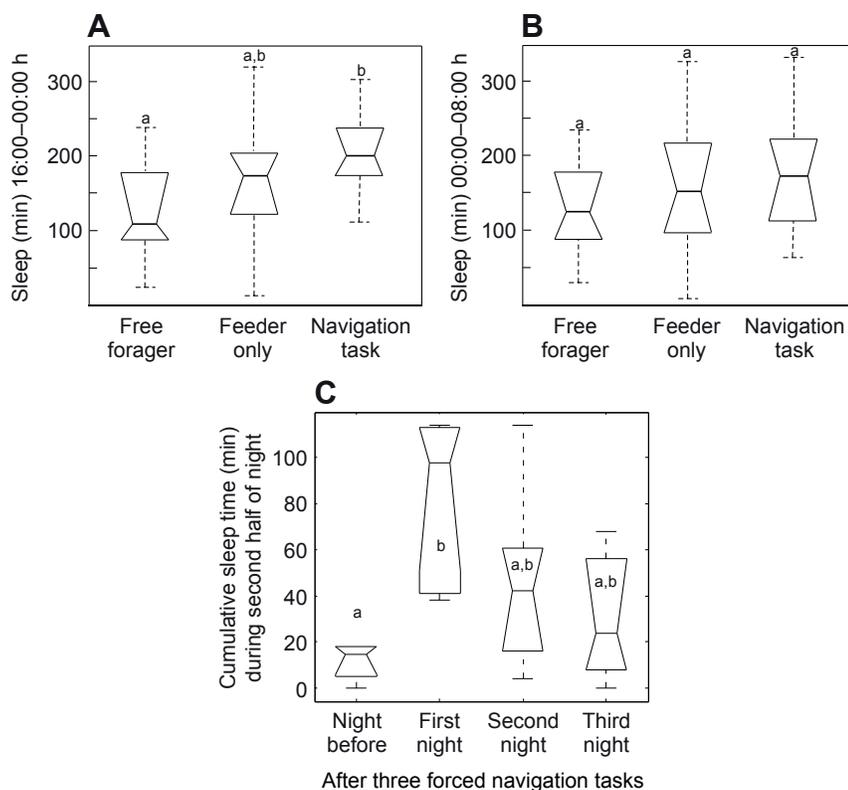

Fig. 4. Cumulative sleep times during the first (A) and second (B) part of the night in three groups of bees: free foragers ($N=8$), feeder-only bees ($N=33$), and navigation-task bees ($N=14$). Freely foraging bees slept less during the first part of the night (16:00–00:00 h) than navigation-task bees (one-way ANOVA, $P<0.05$). No significant differences were found between the three groups during the second half of the night (00:00–08:00 h). (C) Cumulative sleep time before and after exposing bees to three forced navigation tasks in which they had to home from a distance of 300 m but from three different locations. Shown are medians and interquartiles; different letters indicate significantly different values between groups (one-way ANOVA, $P<0.01$).
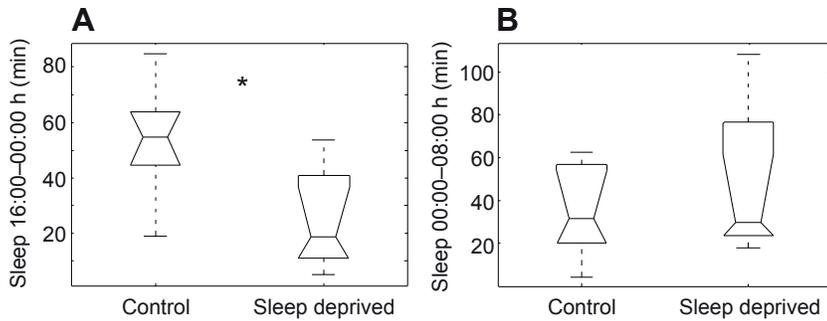
A

B

Fig. 5. Cumulative sleep times for control and sleep-deprived bees. (A) Sleep time during the first part of the night (16:00–00:00 h). Sleep-deprived-bees showed significantly less sleep time during the first part of the night (Mann–Whitney U-test, $P<0.05$; N=7 for control bees and 7 for sleep-deprived bees). (B) Sleep time during the second part of the night (00:00–08:00 h). No significant difference was found between control bees and sleep-deprived bees (Mann–Whitney U-test).

was not longer than in the other two groups of bees, the distribution was skewed to longer flight times.

Forced navigation learning may lead to increased sleep in the first part of the following night

We observed the sleep behavior at night of three groups of bees: free foragers, feeder-only bees and navigation-task bees (Fig. 4). Average cumulative sleep times were highest for navigation-task bees, but the difference was significant only between free foragers and navigation task bees during the first half of the night (Fig. 4A). A similar trend was seen for the second half of the night, but here no significant differences were found. Thus forced navigation learning may lead to increased sleep during the first part of the night. As we did not find a correlation between cumulative sleep time and duration of foraging flight (see above), this trend may reflect an effect of learning and memory formation after the forced navigation task.

To test whether the trend of prolonged sleep after displacement may become more pronounced after multiple releases, we performed an experiment in which the same bees were released three times at a distance of 300 m from the hive but at different locations around the hive. Fig. 4C shows that indeed cumulative sleep time was significantly longer during the night after these multiple releases but returned to baseline two and three nights later.

Sleep deprivation does not lead to sleep rebound

Preventing bees from sleeping at night induces prolonged sleep during the following night (Hussaini et al., 2009; Klein et al., 2010). We asked whether this also applies to our test conditions in which animals were removed from the hive for one night, deprived of sleep by shaking (see Materials and methods), and then returned to the hive the next morning. These animals resumed foraging at the trained feeding site without any noticeable reduction in foraging activity or delayed start of foraging in the morning. In addition, the cumulative rest time of sleep-deprived and control bees during the day after sleep deprivation did not differ before the first foraging flight or throughout the day (data not shown). When the bees were monitored for sleep on the following night, we observed reduced cumulative sleep phases instead of a rebound of sleep (Fig. 5). Because bees may respond differently to sleep deprivation during the first and the second half of the night, we evaluated cumulative sleep phases separately for the two parts of the night. Significantly less sleep was found for the first part of the night, while no difference was seen for the second part.

Sleep deprivation does not compromise forced navigation learning

One explanation for the failure of a rebound effect after sleep deprivation may be that the animals were aroused by removal from the hive and the shaking procedure. Although we did not see any change in their foraging behavior at the training site or prolonged rest

times inside the hive, we asked whether their navigation learning during the forced navigation task was compromised. The sleep-deprived bees were exposed to the same forced navigation task as a group of control bees, and their flight time after the first and the second release at the release site was determined. Fig. 6 shows that control bees and sleep-deprived bees did not differ with respect to their flight times when returning from the unexpected release site to the hive. Furthermore, we evaluated the probability of bees returning from the release site to the hive and found no difference in the return rate between these two groups. These results indicate that neither motivation to navigate back to the hive nor navigational performance is compromised after sleep deprivation the night before.

Sleep deprivation impairs consolidation of novel navigation memory

Finally, we asked whether sleep deprivation during the night following forced navigation learning impairs consolidation of memory acquired during the successful return flight from the release site to the hive. Both groups of bees performed one forced navigation trial and afterwards resumed foraging at the training site. Approximately half of these bees were collected in the evening when heading back to the hive from the feeder and were sleep-deprived during the night; the other half were kept in the hive. The next morning, the first group was put back into the hive. Similar to the control bees, these bees resumed foraging at the trained feeder. Single animals of the control and the sleep-deprived bees were then transported to the same release site as the day before, and the success rate and the flight times of these second return flights were determined. As Fig. 7 shows, control bees were significantly more successful in returning to the hive during the second homing flight than during the first homing flight. Approximately 42% of the bees were lost on the first homing flight, and ~17% during the second homing flight, indicating that they used a navigation memory
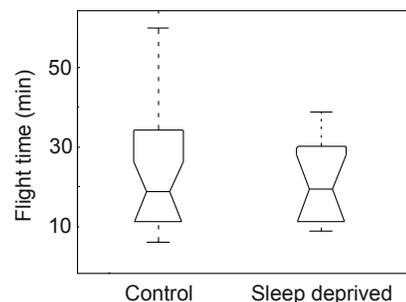

Fig. 6. Flight times of control bees and sleep-deprived bees from the release site to the hive. Shown are medians and interquartile ranges. Mann–Whitney U-test, n.s.; N=7 for control bees and 7 for sleep-deprived bees.
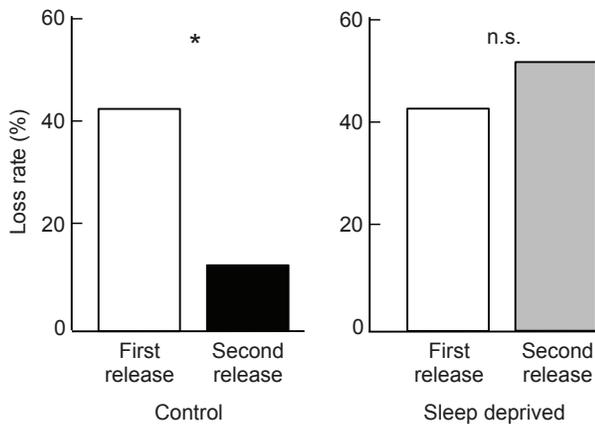
Fig. 7. Loss rates after displacement of control bees (left two columns) and sleep-deprived bees (right two columns). Control bees: first release *versus* second release, Fisher's exact test, *P<0.05. Sleep-deprived bees: first release *versus* second release, Fisher's exact test, n.s. *N*=55 total bees tested on the first day, and 23 control bees and 13 sleep-deprived bees on the second day.

acquired during the first homing flight as shown in the radar-tracking experiments. Sleep-deprived bees, however, were significantly less effective, and about the same loss rate was found on the second homing flight as on the first homing flight. This result shows that sleep deprivation leads to reduced navigation memory. Flight time of the successfully homing animals did not differ between the two groups for the first or second homing flights (data not shown). This result indicates that sleep deprivation did not disturb the animals' ability to access the memory they used during the first homing flight but rather interfered with the consolidation of the newly acquired navigation memory.

DISCUSSION

Sleep, as defined by a state of rest, reduced sensory sensitivity, relaxed muscle tonus and altered neural activity, is well known across the animal kingdom from mollusks (Stephenson and Lewis, 2011) to humans. The role of sleep is likely to be manifold, but one particular property appears to exist across animal species – the supportive effect on memory consolidation. In humans, consolidation of different forms of memory appears to be supported by different sleep phases, e.g. perceptual memory (Mednick et al., 2003), sensory-motor memory (Brawn et al., 2008) and motor skill memory (Walker et al., 2002) by REM sleep, and declarative memory by slow wave sleep (Diekelmann and Born, 2010). Insects are known to go through rest phases that resemble key features of sleep [*Drosophila* (Hendricks et al., 2000; Shaw et al., 2000); honeybee (Kaiser, 1988)]. In *Drosophila*, the genes and signaling cascades that control sleep are already well understood (Sehgal and Mignot, 2011), and some of these regulatory systems can be related to synaptic plasticity and memory consolidation (Foltenyi et al., 2007; Bushey et al., 2011; Seugnet et al., 2011; Wu and Raizen, 2011). The neural networks involved in these cellular processes highlight the role of the mushroom body (Joiner et al., 2006; Guo et al., 2011) and an identified protocerebral cell group, the octopamine-containing neurons in the pars intercerebralis (Crocker et al., 2010), possibly also involved in synaptic plasticity and memory consolidation. Because the mushroom body in insects is related to learning and memory formation (Menzel and Müller, 1996; Heisenberg, 2003; Davis, 2011), it is likely that sleep in insects may also serve a function in memory consolidation.

Indeed, Hussaini et al. (Hussaini et al., 2009) reported a selective sleep deprivation effect on the consolidation of extinction memory in honeybees, and Klein et al. (Klein et al., 2010) found that the precision of social communication by the waggle dance is reduced after sleep deprivation. Hussaini et al. (Hussaini et al., 2009) hypothesized that consolidation is served specifically by memory processes that integrate new and old memories. This hypothesis motivated us to look into the role of sleep in novel navigation memory formation, a form of memory that needs to be incorporated into existing forms of navigation memories.

Learning to navigate is a stepwise process in the life of a honeybee (von Frisch, 1967). First, young foragers perform exploratory orientation flights learning about the local solar azimuth–time function, the appearance of the hive entrance and the spatial relationships between landmarks in the further surroundings. Later, foragers learn the routes between feeding sites and the hive, and they learn from each other the distance and direction to feedings sites and nest sites using the waggle dance for communication. Acquiring a new flight route therefore involves updating of the existing navigational memory and the incorporation of novel experiences into the existing navigation memory. The task the bees had to solve in our catch-and-release tests was rather demanding as the displaced bees had to find their way back over a distance up to 600 m in a highly structured urban environment. On average, approximately half of the bees mastered this task after the first release and close to 100% after the second release. Following the flight paths of displaced bees with harmonic radar in a more even environment and over shorter distances (Fig. 2) indicated more direct homing flights during the second release, proving that a successful homing flight leads to an updating of navigation memory. It is noteworthy that the more homeward-directed flights during the second release shortened the initial vector components and also reduced the search component. These changes indicate that the bees had recalled their successful homing flight after the first release. This kind of memory updating is, however, less well understood. It may involve recognition of landmarks and their spatial relationships to the hive within the reference frame of the sun compass as well as a devaluation of the vector memory (Menzel et al., 2005; Menzel et al., 2011).

The rest and sleep behavior of bees was automatically monitored using RFIDs to localize individually identifiable bees on the surface of a comb in an observation hive. Bees equipped with the rather bulky RFID (Fig. 1) showed rest and sleep behavior similar to that of bees without an RFID. The body of a sleeping bee is closer to the wax surface, the antennae are pointing downwards and motionless, and they do not react to other bees bumping into them or crawling over them. We used a criterion of ≥5 min of motionlessness to define sleep at night. Bees carrying an RFID could not enter a wax cell with the thorax. Although the antennae were not visible when the head of the bee was inside a wax cell, movements of the abdomen were clearly visible. The discontinuity of such movements indicates sleep in bees (Sauer et al., 2003; Klein and Seeley, 2011). Thus the lack of such movements for >5 min can be conservatively interpreted as sleep.

During the day, rest times were more fragmented, and bees did not always meet the criteria of sleep when motionless. Therefore, we assign motionlessness of ≥3 min as rest rather than sleep. Foragers slept mostly at night and young bees slept about equally during the day and night, corroborating reports by Eban-Rothschild and Bloch (Eban-Rothschild and Bloch, 2008) and Klein and Seeley (Klein and Seeley, 2011). Some of the bees appeared to choose the same region on the comb for sleep during different nights, but this behavior was not analyzed further. The RFID did not interfere with the bees' foraging behavior, neither when foraging at unknown

natural sites nor when visiting a feeder. Bees with and without an RFID behaved similarly when displaced. These results indicate that the RFID did not change the bees' behavior either inside or outside the hive.

Navigation learning as induced by our forced navigation task leads to prolonged sleep during the night after learning. A single forced task led only to a trend, but three forced homing flights from different locations led to a significant increase during the night following learning. As we have not seen any dependence of sleep time on flight duration, this result is consistent with the hypothesis that memory from novel navigational learning may require consolidation during sleep. One way of testing this hypothesis is to interfere with sleep during the night after navigation learning.

The procedure used here for sleep deprivation required removal of the bees from the colony and shaking them at intervals throughout the night. We carefully monitored whether their behavior was altered with respect to their motivation to forage and their ability to acquire novel navigation memory. In both behavioral components we found no effect (Fig. 6). The finding of reduced sleep during the first half of the night following sleep deprivation the night before (Fig. 5) may indicate a lasting arousal effect. At least sleep-deprived bees did not compensate for loss of sleep the night before (known as rebound), as seen in harnessed bees (Hussaini et al., 2009) and in colony-housed bees (Klein et al., 2010).

Sleep deprivation during the night following novel navigation learning reduces the probability of the test bees returning from the same release site on the next day (Fig. 7). This suggests that the test bees did not gain from the experience the day before as the control bees did. The flight lengths of test bees that performed homing flights successfully was not different from that of control bees, indicating that their flight performance was not compromised. We thus conclude that night sleep following novel navigation learning facilitates memory formation. Although we think that sleep deprivation caused the deficits in navigation on the second day, it is conceivable that other effects resulting from the sleep deprivation manipulation also played a causal role. These include isolation from hive mates and arousal. The separation between memory consolidation and memory retrieval effects is problematic in general and does not apply to sleep research specifically. It will be important for future research to include additional tests, e.g. retrieval tests after longer intervals.

As pointed out above, sleep in humans is characterized by different phases (e.g. REM sleep, slow wave sleep), and different forms of memory consolidation appear to be related to different sleep phases. It is unknown whether sleep in insects is discernible into different forms or phases. Kaiser (Kaiser, 1988) assigned deep sleep to resting bees that did not react to other bees. Klein and Seeley (Klein and Seeley, 2011) speak of deep sleep in bees both during the day and at night, and refer to day sleeping as 'napping', appearing either in the morning or in the afternoon depending on when the respective bees were trained. We distinguished between rest and sleep, the latter one characteristic of sleep at night. Harnessed bees show all signs of sleep, and antennal movements are good indicators of sleep (Hussaini et al., 2009). Interestingly, prolonged sleep phases at night are interrupted by short bursts of symmetrical antennal movements, possibly indicating a different form of sleep. It will be important to further characterize different forms of sleep in insects and try to interfere selectively with these forms in order to elucidate whether memory consolidation is facilitated by particular forms of sleep phases. The concept behind such an approach relates different sleep phases to different neural replay phenomena activating recently acquired memory traces. Because insect brains allow access to learning-related

plasticity at the level of single neurons and small neural networks, it may be possible to unravel neural processes in sleep-dependent memory consolidation.

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